USE OF SOS1 INHIBITORS TO TREAT MALIGNANCIES WITH SHP2 MUTATIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Ser. No. 63/074,045, filed September 3, 2020, the disclosure of which is hereby incorporated by reference as if set forth in its entirety. This application claims priority to U.S. Provisional Application Ser. No. 63/135,023, filed January 8, 2021, the disclosure of which is hereby incorporated by reference as if set forth in its entirety. This application claims priority to U.S. Provisional Application Ser. No. 63/172,791, filed April 9, 2021, the disclosure of which is hereby incorporated by reference as if set forth in its entirety.

FIELD OF THE DISCLOSURE

[0002] The field of the disclosure relates generally to cancer treatment, and more specifically to treatment of cancer associated with SHP2 mutations.

BACKGROUND OF THE DISCLOSURE

[0003] SHP2 is a non-receptor protein tyrosine phosphatase encoded by the PTPN11 gene that contributes to multiple cellular functions including proliferation, differentiation, cell cycle maintenance, and migration. SHP2 is involved in signaling through the RAS-mitogen-activated protein kinase (MAPK), the JAK-STAT pathway, and/or the phosphoinositol 3-kinase- AKT pathway.

[0004] SHP2 has two N-terminal Src homology 2 domains (N-SH2 and C-SH2), a catalytic domain (PTP), and a C-terminal tail. The two SH2 domains control the subcellular localization and functional regulation of SHP2. The molecule exists in an inactive, self - inhibited conformation stabilized by a binding network involving residues from both the N-SH2 and PTP domains. Stimulation by, for example, cytokines or growth factors acting through RTKs leads to exposure of the catalytic site resulting in enzymatic activation of SHP2.

[0005] Mutations in the PTPN11 gene and subsequently in SHP2 have been identified in several human developmental diseases, such as Noonan Syndrome and LEOPARD Syndrome, as well as human cancers, such as juvenile myelomonocytic

leukemias, neuroblastoma, melanoma, acute myeloid leukemia, and cancers of the breast, lung and colon. Some of these mutations destabilize the auto-inhibited conformation of SHP2 and promote auto-activation or enhanced growth factor-driven activation of SHP2.

[0006] Allosteric SHP2 inhibitors show reduced potency against clinically-relevant SHP2 mutants when the mutant SHP2 is in an activated state. See, e.g., Pádua et al., Nat Commun 9:4507 (2018); LaRochelle et al., Nat Commun 9:4508 (2018). Accordingly, a need exists for treating a disease or disorder associated with cells containing a mutant SHP2.

[0007] RAS proteins (KRAS, HRAS and NRAS) play an essential role in various human cancers and are therefore appropriate targets for anticancer therapy. Dysregulation of RAS proteins by activating mutations, overexpression or upstream activation is common in human tumors, and activating mutations in RAS are found in approximately 30% of human cancer. Of the RAS proteins, KRAS is the most frequently mutated and is therefore an important target for cancer therapy. RAS oscillates between GDP-bound "off" and GTP-bound "on" state, facilitated by interplay between a GEF protein (*e.g.*, SOS1), which loads RAS with GTP, and a GAP protein (*e.g.*, NF1), which hydrolyzes GTP, thereby inactivating RAS. Additionally, SHP2 associates with the receptor signaling apparatus and becomes active upon RTK activation, and then promotes RAS activation. Mutations in RAS proteins can lock the protein in the "on" state resulting in a constituitively active signaling pathway that leads to uncontrolled cell growth.

[0008] First-in-class covalent inhibitors of the "off" form of $KRAS^{G12C}$ have demonstrated promising anti-tumor activity in cancer patients with $KRAS^{G12C}$ mutations, albeit not in all. Further, therapeutic inhibition of the RAS pathway, although often initially efficacious, can ultimately prove ineffective as it may lead to over-activation of RAS pathway signaling *via* a number of mechanisms including, *e.g.*, reactivation of the pathway *via* relief of the negative feedback machineries that naturally operate in these pathways. For example, in various cancers, MEK inhibition results in increased ErbB signaling due to its relief of MEK / ERK-mediated feedback inhibition of RTK activation. As a result, cells that were initially sensitive to such inhibitors may become resistant. Thus, a need exists for methods of effectively inhibiting RAS pathway signaling without inducing activation of resistance mechanisms.

BRIEF SUMMARY

[0009] In some aspects, the present disclosure is directed to a method of treating a subject having a disease or disorder associated with cells having a SHP2 mutation. The method comprises administering to the subject a therapeutically effective amount of a SOS1 inhibitor alone or in combination with an additional therapeutic agent.

[0010] In some aspects, the SHP2 mutation induces an activated form of SHP2.

[0011] In some aspects, the subject expressed the SHP2 mutation after prior treatment with a SHP2 inhibitor. In some aspects, the subject expressed the SHP2 mutation after prior treatment with an allosteric SHP2 inhibitor.

[0012] In some aspects, the method further comprises administering to the subject a therapeutically effective amount of a RAS inhibitor selected from the group consisting of a RAS(ON) inhibitor, a RAS(OFF) inhibitor, and a combination thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIG. 1 is an illustration of the RAS-MAPK pathway. SHP2 activates SOS1, which, in turn, activates RAS, leading to signaling that drives cell growth and survival.

[0014] FIG. 2A is a graph depicting activating mutations in SHP2, which reduce sensitivity to inhibition with a SHP2 allosteric inhibitor, RMC-4550. As shown in the graph, cellular sensitivity correlates with energetic magnitude (ΔG_{op}) of SHP2 activating mutations. Lower ΔG_{op} indicates stronger activation. FIG. 2B is a table showing the IC_{50} concentrations of RMC-4550 sufficient to inhibit SHP2 proteins with activating mutations. As shown in the table, strongly activating mutations, such as G503V, require high concentrations of allosteric SHP2 inhibitor to achieve a response. These data were obtained according to the methods disclosed in Example 1.

[0015] FIGS. 3A and 3B are graphs showing that SOS1 inhibitors maintain sensitivity in isogenic HEK-293 cell lines carrying a SHP2 E76K mutant. These data were obtained according to the methods disclosed in Example 1.

[0016] FIGS. 4A through 4H are graphs showing that activating mutations induce resistance to allosteric SHP2 inhibition but maintain sensitivity to SOS1 inhibitors in

isogenic LN229 cell lines. These data were obtained according to the methods disclosed in Example 1.

[0017] FIGS. 5A through 5D show that activating mutation G503V induces resistance to allosteric SHP2 inhibition, but maintains sensitivity to SOS1 inhibitors in syngeneic mouse cell lines KLN205 (squamous cell carcinoma) and PAN02 (pancreatic cancer), by a) pERK Alphalisa® assay (FIGS. 5A through 5D graphs), and b) CellTiter-Glo® viability assay (associated Tables). These data were obtained according to the methods disclosed in Example 2.

[0018] FIG. 6 is a graph showing SOS1 inhibitors drive tumor growth inhibition *in vivo* in the context of activating SHP2 mutation G503V in syngeneic mouse cell line KLN205 (squamous cell carcinoma) in immunocompetent mice. These data were obtained according to the methods disclosed in Example 2.

[0019] FIG. 7 is a graph showing SOS1 inhibitors drive tumor growth inhibition *in vivo* in the context of activating SHP2 mutation G503V in syngeneic mouse cell line PAN02 in immunocompetent mice. These data were obtained according to the methods disclosed in Example 2.

[0020] FIGS. 8A through 8D are graphs showing LN229 cells with various SHP2 mutational variants confirmed to be sensitive to inhibition in non-targeting control with partial depth of inhibition, rescued from inhibition by BI-3406 by knockdown of SOS1, and sensitized by knockdown of SOS2 to restore full depth of inhibition. These data were obtained according to the methods disclosed in Example 3.

[0021] FIG. 9 is a graph showing that SHP2 mutants demonstrated significant reduction in basal pERK after SOS1 knockdown, and levels of pERK reduction correlate with biochemical potency for activated SHP2 variant.

[0022] FIG. 10 is a graph showing measured specific activity of SHP2 on DiFMUP as a function of [RMC-4550] and [SIRPA1]. These data were obtained according to the methods disclosed in Example 3.

[0023] FIG. 11 is a graph showing fit of data from FIG. 10 to the equation shown in Example 4 for wild type SHP2. These data were obtained according to the methods disclosed in Example 4.

[0024] FIG. 12 is a graph showing fit of data from FIG. 10 to the equation shown in Example 4 for mutant SHP2 A72S. These data were obtained according to the methods disclosed in Example 4.

[0025] FIG. 13 is a graph showing fit of data from FIG. 10 to the equation shown in Example 4 for mutant SHP2 E69K. These data were obtained according to the methods disclosed in Example 4.

[0026] FIG. 14 is a graph showing fit of data from FIG. 10 to the equation shown in Example 4 for mutant SHP2 G503V. These data were obtained according to the methods disclosed in Example 4.

[0027] FIG. 15 is a graph showing SOS1 inhibitors drive tumor growth inhibition *in vivo* in the context of activating SHP2 mutation G503V in syngeneic mouse cell line PAN02 in immunocompetent mice. These data were obtained according to the methods disclosed in Example 5.

[0028] FIG. 16 is a graph showing SOS1 inhibitors drive tumor growth inhibition *in vivo* in the context of activating SHP2 mutation A72S in syngeneic mouse cell line LN229 CDX in immunocompromised mice. These data were obtained according to the methods disclosed in Example 5.

[0029] FIGS. 17A and 17B are graphs showing the additive effect of SOS1 inhibitor Compound SOS1-(B) and RAS inhibitor Compound RAS-(E) in cell lines having both SHP2 and KRAS mutations. These data were obtained according to the methods disclosed in Example 6.

[0030] FIG. 18 is a Loewe 3D response surface plot showing the *in vitro* combination effect of SOS1 inhibitor Compound SOS1-(A) (also called RMC-0331) and RAS^{MULTI}(ON) inhibitor Compound RAS-(D) observed in Pan02 cells. A synergy score > 5 at any point on the plot indicates a positive interaction between the two compounds. These data were obtained according to the methods disclosed in Example 7.

[0031] FIG. 19 is a Loewe 3D response surface plot showing the *in vitro* combination effect of SOS1 inhibitor Compound SOS1-(A) (also called RMC-0331) and RAS^{MULTI}(ON) inhibitor Compound RAS-(D) observed in KLN205 cells (squamous cell carcinoma). A synergy score > 5 at any point on the plot indicates a positive interaction

between the two compounds. These data were obtained according to the methods disclosed in Example 7.

[0032] FIG. 20A is a graph depicting activating mutations in SHP2, which reduce sensitivity to inhibition with a SHP2 allosteric inhibitor (RMC-4550) but maintain sensitivity to a SOS1 inhibitor (Compound SOS1-(A)). Lower ΔG_{op} indicates stronger activation, and cellular sensitivity to SHP2 inhibition correlates with energetic magnitude (ΔG_{op}) of SHP2 activating mutations. In contrast, cellular sensitivity to SOS1 inhibition is maintained across all SHP2 mutations tested. FIG. 20B is a table showing the IC50 concentrations of RMC-4550 and Compound SOS1-(A) in cells with different SHP2 mutations. As shown in the table, strongly activating mutations, such as E76K, require high concentrations of allosteric SHP2 inhibitor to achieve a response, but relatively low concentrations of SOS1 inhibitor are required to achieve a response across all SHP2 mutations tested. These data were obtained according to the methods disclosed in Example 1.

[0033] FIG. 21 is a graph showing SOS1 inhibitors drive tumor growth inhibition *in vivo* in the context of activating SHP2 mutation E76K in syngeneic mouse cell line LN229.E76K in immunocompromised mice. These data were obtained according to the methods disclosed in Example 8.

[0034] FIG. 22 is a graph showing SOS1 inhibitors alone and in combination drive tumor growth inhibition *in vivo* in the context of activating SHP2 mutation G503V in syngeneic mouse cell line PAN02 in immunocompetent mice. These data were obtained according to the methods disclosed in Example 9.

[0035] FIG. 23 is a graph showing SOS1 inhibitors alone and in combination drive tumor growth inhibition *in vivo* in the context of activating SHP2 mutation G503V in syngeneic mouse cell line PAN02 in immunocompetent mice. These data were obtained according to the methods disclosed in Example 10.

[0036] FIG. 24 is a graph showing SOS1 inhibitors alone and in combination drive tumor growth inhibition *in vivo* in the context of activating SHP2 mutation E76K in syngeneic mouse cell line LN229.E76K in immunocompromised mice. These data were obtained according to the methods disclosed in Example 11.

[0037] FIG. 25 is a graph showing SOS1 inhibitors alone and in combination drive tumor growth inhibition *in vivo* in the context of activating SHP2 mutation G503V in syngeneic mouse cell line PAN02 in immunocompetent mice. These data were obtained according to the methods disclosed in Example 12.

[0038] FIGS. 26A, 26B, 26C, 26D, 26E, and 26F are graphs and tables showing SOS1 inhibitors alone and in combination drive tumor growth inhibition *in vivo* in the context of activating SHP2 mutation G503V in syngeneic mouse cell line PAN02 in immunocompetent mice. These data were obtained according to the methods disclosed in Example 13.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0039] The details of the present disclosure are set forth in the accompanying description below. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, illustrative methods and materials are now described. Other features, objects, and advantages of the present disclosure will be apparent from the description and from the claims. In the specification and the appended claims, the singular forms also include the plural unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the present disclosure belongs. All patents and publications cited in this specification are incorporated herein by reference in their entireties.

Terms

[0040] The articles “a” and “an” are used in this disclosure to refer to one or more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

[0041] The term “and/or” is used in this disclosure to mean either “and” or “or” unless indicated otherwise. The use of the term "or" is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternative are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or."

[0042] As used herein, the term “about” is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the

value. In certain embodiments, the term "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of a stated value, unless otherwise stated or otherwise evident from the context (e.g., where such number would exceed 100% of a possible value).

[0043] Pharmaceutically acceptable salts of compounds disclosed herein are contemplated by the present invention. Representative "pharmaceutically acceptable salts" include, e.g., water-soluble and water-insoluble salts, such as the acetate, amsonate (4,4-diaminostilbene-2,2-disulfonate), benzenesulfonate, benzonate, bicarbonate, bisulfate, bitartrate, borate, bromide, butyrate, calcium, calcium edetate, camsylate, carbonate, chloride, citrate, clavulariate, dihydrochloride, edetate, edisylate, estolate, esylate, fiunarate, gluceptate, gluconate, glutamate, glycollylarsanilate, hexafluorophosphate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, sethionate, lactate, lactobionate, laurate, magnesium, malate, maleate, mandelate, mesylate, methylbromide, methylnitrate, methylsulfate, mucate, napsylate, nitrate, N-methylglucamine ammonium salt, 3-hydroxy-2-naphthoate, oleate, oxalate, palmitate, pamoate (1,1-methene-bis-2-hydroxy-3-naphthoate, einbonate), pantothenate, phosphate/diphosphate, picrate, polygalacturonate, propionate, p-toluenesulfonate, salicylate, stearate, subacetate, succinate, sulfate, sulfosalicylate, suramate, tannate, tartrate, teoclate, tosylate, triethiodide, and valerate salts.

[0044] A "therapeutically effective amount" when used in connection with a compound is an amount effective for treating or preventing a disease in a subject as described herein.

[0045] The disclosure also includes pharmaceutical compositions comprising an effective amount of a disclosed compound and a pharmaceutically acceptable carrier. The term "carrier", as used in this disclosure, encompasses excipients and diluents and means a material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a pharmaceutical agent from one organ, or portion of the body, to another organ, or portion of the body of a subject.

[0046] The term "treating" with regard to a subject, refers to improving at least one symptom of the subject's disorder. Treating includes curing, improving, or at least partially ameliorating the disorder.

[0047] The term “prevent” or “preventing” with regard to a subject refers to keeping a disease or disorder from afflicting the subject. Preventing includes prophylactic treatment. For instance, preventing can include administering to the subject a compound disclosed herein before a subject is afflicted with a disease and the administration will keep the subject from being afflicted with the disease.

[0048] The terms “inhibiting” and “reducing,” or any variation of these terms, includes any measurable or complete inhibition to achieve a desired result. For example, there may be a decrease of about, at most about, or at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or more, or any range derivable therein, reduction of activity (e.g., SOS1:Ras-family protein binding activity) compared to normal.

[0049] The term “disorder” is used in this disclosure to mean, and is used interchangeably with, the terms disease, condition, or illness, unless otherwise indicated.

[0050] The term “administer”, “administering”, or “administration” as used in this disclosure refers to either directly administering a disclosed compound or pharmaceutically acceptable salt of the disclosed compound or a composition to a subject, or administering a prodrug derivative or analog of the compound or pharmaceutically acceptable salt of the compound or composition to the subject, which can form an equivalent amount of active compound within the subject’s body.

[0051] A "patient" or “subject” is a mammal, e.g., a human, mouse, rat, guinea pig, dog, cat, horse, cow, pig, or non-human primate, such as a monkey, chimpanzee, baboon or rhesus.

[0052] The term "sample" or "biological sample," as used herein, refers to a sample obtained from a subject, e.g., a human subject or a patient, which may be tested for a particular molecule, for example wild type . Samples may include, but are not limited to, biopsies, tissues, cells, buccal swab sample, body fluids, including blood, serum, plasma, urine, saliva, cerebral spinal fluid, tears, pleural fluid and the like.

[0053] As used herein, the term “inhibitor” refers to a compound that prevents a biomolecule, (e.g., a protein, nucleic acid) from completing or initiating a reaction. An inhibitor can inhibit a reaction by competitive, uncompetitive, or non-competitive means,

for example. With respect to its binding mechanism, an inhibitor may be an irreversible inhibitor or a reversible inhibitor. Exemplary inhibitors include, but are not limited to, nucleic acids, DNA, RNA, shRNA, siRNA, proteins, protein mimetics, peptides, peptidomimetics, antibodies, small molecules, chemicals, analogs that mimic the binding site of an enzyme, receptor, or other protein, e.g., that is involved in signal transduction, therapeutic agents, pharmaceutical compositions, drugs, and combinations of these. In some embodiments, the inhibitor is a small molecule, e.g., a low molecular weight organic compound, e.g., an organic compound having a molecular weight (MW) of less than 1200 Daltons (Da). In some embodiments, the MW is less than 1100 Da. In some embodiments, the MW is less than 1000 Da. In some embodiments, the MW is less than 900 Da. In some embodiments, the range of the MW of the small molecule is between 800 Da and 1200 Da. Small molecule inhibitors include cyclic and acyclic compounds. Small molecules inhibitors include natural products, derivatives, and analogs thereof. Small molecule inhibitors can include a covalent cross-linking group capable of forming a covalent cross-link, e.g., with an amino acid side-chain of a target protein. In some embodiments, the inhibitor can be nucleic acid molecules including, but not limited to, siRNA that reduce the amount of functional protein in a cell. Accordingly, compounds said to be “capable of inhibiting” a particular protein, e.g., SHP2 or SOS1, comprise any such inhibitor.

[0054] The term "mutation" as used herein indicates any modification of a nucleic acid and/or polypeptide which results in an altered nucleic acid or polypeptide. The term "mutation" may include, for example, point mutations, deletions of a single or multiple residues in a polynucleotide, or insertions of single or multiple residues in a polynucleotide, which includes alterations arising within a protein-encoding region of a gene as well as alterations in regions outside of a protein-encoding sequence, such as, but not limited to, regulatory or promoter sequences, as well as amplifications and/or chromosomal breaks or translocations.

[0055] The term “SHP2” means “Src Homology 2 domain-containing protein tyrosine phosphatase 2” and is also known as SH-PTP2, SH-PTP3, Syp, PTP1D, PTP2C, SAP-2 or PTPN11. SHP2 is a non-receptor protein tyrosine phosphatase encoded by the PTPN11 gene that contributes to multiple cellular functions including proliferation, differentiation, cell cycle maintenance and migration. SHP2 is involved in signaling through the RAS-mitogen-activated protein kinase (MAPK), the JAK-STAT and/or the

phosphoinositol 3-kinase-AKT pathways. SHP2 has two N-terminal Src homology 2 domains (N-SH2 and C-SH2), a catalytic domain (PTP), and a C-terminal tail. The two SH2 domains control the subcellular localization and functional regulation of SHP2. The molecule exists in an inactive, self-inhibited conformation stabilized by a binding network involving residues from both the N-SH2 and PTP domains. Stimulation by, for example, cytokines or growth factors acting through RTKs leads to exposure of the catalytic site resulting in enzymatic activation of SHP2.

[0056] The term "allosteric SHP2 inhibitor" means an agent (e.g., a small-molecule compound (e.g., less than 750 Da)) capable of inhibiting SHP2 through binding to SHP2 at a site other than the active site of the enzyme.

[0057] The term "inhibitor-resistant mutation" when used in reference to a SHP2 mutation, means a SHP2 mutation that renders a SHP2 polypeptide refractory or resistant to inhibition with a SHP2 inhibitor. Thus, in some embodiments, an inhibitor-resistant mutation in a SHP2 polypeptide decreases the inhibitory effect that a SHP2 inhibitor has on the SHP2 polypeptide as compared to the effect the inhibitor has on a similar SHP2 polypeptide differing only in the absence of the inhibitor-resistant mutation. Such activity may be measured using any suitable activity assay known in the art or disclosed herein. In some embodiments, an inhibitor-resistant mutation in a SHP2 polypeptide abolishes all detectable inhibitory effects that a SHP2 inhibitor has on the activity of the SHP2 polypeptide, wherein the inhibitor has detectable inhibitory efficacy on a similar SHP2 polypeptide differing only in the absence of the inhibitor-resistant mutation. Such inhibitor-resistant mutations include, without limitation, mutations that destabilize the auto-inhibited conformation of SHP2. An inhibitor-resistant mutation may be an allosteric inhibitor-resistant mutation.

[0058] As used herein, the term "activating SHP2 mutation" or "activated mutation of SHP2" or similar refers to a mutation of SHP2 that destabilizes the auto-inhibited conformation of SHP2, as measured by the free energy of opening (ΔG_{op}) of the mutation. Wild-type SHP2 has a ΔG_{op} of 2.8 kcal/mol. Values of ΔG_{op} below 2.8 in mutant SHP2 indicate activation, with lower values indicating stronger activation. A weakly activating SHP2 mutant is defined as one with a ΔG_{op} not more than 1.5 kcal/mol below wild type SHP2. A moderately activating SHP2 mutant has a ΔG_{op} between 1.5 kcal/mol and 2.24 kcal/mol below wild-type, and a strongly activating SHP2 mutation has

a ΔG_{op} more than 2.24 kcal/mol below wild type. Methods of measuring ΔG_{op} are provided in Example 4.

[0059] The term "allosteric inhibitor-resistant mutation" when used in reference to a SHP2 mutation, means a SHP2 mutation that renders a SHP2 polypeptide refractory or resistant to inhibition with a SHP2 allosteric inhibitor. Thus, in some embodiments, an allosteric inhibitor-resistant mutation in a SHP2 polypeptide decreases the inhibitory effect that a SHP2 allosteric inhibitor has on the SHP2 polypeptide as compared to the effect the inhibitor has on a similar SHP2 polypeptide differing only in the absence of the allosteric inhibitor-resistant mutation. Such activity may be measured using any suitable activity assay known in the art or disclosed herein. In some embodiments, an allosteric inhibitor-resistant mutation in a SHP2 polypeptide abolishes all detectable inhibitory effects that a SHP2 allosteric inhibitor has on the activity of the SHP2 polypeptide, wherein the inhibitor has detectable inhibitory efficacy on a similar SHP2 polypeptide differing only in the absence of the allosteric inhibitor-resistant mutation. Such allosteric inhibitor-resistant mutations include, without limitation, mutations that destabilize the auto-inhibited conformation of SHP2. In some embodiments, the allosteric inhibitor-resistant mutation is a SHP2 mutation is a mutation as described herein.

[0060] The term "SOS" (e.g., a "SOS mutation") refers to SOS genes, which are known in the art to include RAS guanine nucleotide exchange factor proteins that are activated by receptor tyrosine kinases to promote GTP loading of RAS and signaling. The term SOS includes all SOS homologs that promotes the exchange of Ras-bound GDP by GTP. In particular embodiments, SOS refers specifically to "son of sevenless homolog 1" ("SOS1"). SOS1 is critically involved in the activation of RAS-family protein signaling in cancer via mechanisms other than mutations in RAS-family proteins. SOS1 interacts with the adaptor protein Grb2 and the resulting SOS1/Grb2 complex binds to activated/phosphorylated Receptor Tyrosine Kinases (e.g., EGFR, ErbB2, ErbB3, ErbB4, PDGFR-A/B, FGFR1/2/3, IGF1 R, INSR, ALK, ROS, TrkA, TrkB, TrkC, RET, c-MET, VEGFR1/2/3, AXL) (Pierre et al., Biochem. Pharmacol., 2011, 82(9): 1049-56). SOS1 is also recruited to other phosphorylated cell surface receptors such as the T cell Receptor (TCR), B cell Receptor (BCR) and monocyte colony-stimulating factor receptor (Salojin et al., J. Biol. Chem. 2000, 275(8):5966-75). This localization of SOS1 to the plasma membrane, proximal to RAS-family proteins, enables SOS1 to promote RAS-family protein activation. SOS1-activation of RAS-family proteins can also be mediated by the

interaction of SOS1/Grb2 with the BCR-ABL oncoprotein commonly found in chronic myelogenous leukemia (Kardinal et al., 2001, Blood, 98:1773-81; Sini et al., Nat. Cell Biol., 2004, 6(3):268-74). SOS1 is also a GEF for the activation of the GTPases RAC1 (Ras-related C3 botulinum toxin substrate 1) (Innocenti et al., J. Cell Biol., 2002, 156(1):125-36). RAC1, like RAS-family proteins, is implicated in the pathogenesis of a variety of human cancers and other diseases (Bid et al., Mol. Cancer Ther. 2013, 12(10):1925-34). Son of sevenless 2 (SOS2), a homolog of SOS1 in mammalian cells, also acts as a GEF for the activation of RAS-family proteins (Pierre et al., Biochem. Pharmacol., 2011, 82(9): 1049-56; Buday et al., Biochim. Biophys. Acta., 2008, 1786(2):178-87). Published data from mouse knockout models suggests a redundant role for SOS1 and SOS2 in homeostasis in the adult mouse. Whilst germline knockout of SOS1 in mice results in lethality during mid-embryonic gestation (Qian et al., EMBO J., 2000, 19(4):642-54), systemic conditional SOS1 knockout adult mice are viable (Baltanas et al., Mol. Cell. Biol., 2013, 33(22):4562-78). SOS2 gene targeting did not result in any overt phenotype in mice (Esteban et al., Mol. Cell. Biol., 2000, 20(17):6410-3). In contrast, double SOS1 and SOS2 knockout leads to rapid lethality in adult mice (Baltanas et al., Mol. Cell. Biol., 2013, 33(22):4562-78). These published data suggest that selective targeting of individual SOS isoforms (e.g., selective SOS1 targeting) may be adequately tolerated to achieve a therapeutic index between SOS1/RAS-family protein driven cancers (or other SOS1/RAS-family protein pathologies) and normal cells and tissues. Selective pharmacological inhibition of the binding of the catalytic site of SOS1 to RAS-family proteins is expected to prevent SOS1-mediated activation of RAS-family proteins to the GTP-bound form. Such SOS1 inhibitor compounds are be expected to consequently inhibit signaling in cells downstream of RAS-family proteins (e.g., ERK phosphorylation). In cancer cells associated with dependence on RAS-family proteins (e.g., KRAS mutant cancer cell lines), SOS1 inhibitor compounds are be expected to deliver anti-cancer efficacy (e.g., inhibition of proliferation, survival, metastasis, etc.). High potency towards inhibition of SOS1:RAS-family protein binding (nanomolar level IC50 values) and ERK phosphorylation in cells (nanomolar level IC50 values) are desirable characteristics for a SOS1 inhibitor compound. Furthermore, a desirable characteristic of a SOS1 inhibitor compound would be the selective inhibition of SOS1 over SOS2. This conclusion is based on the viable phenotype of SOS1 knockout mice and lethality of SOS1/SOS2 double knockout mice, as described above.

[0061] As used herein, a "SOS1 inhibitor" refers to any agent, (e.g., a small molecule (e.g., less than 750 Da)) capable of inhibiting SOS1. SOS1 inhibitors can include selective SOS1 inhibitors and inhibitors that also inhibit other proteins. In some embodiments, SOS1 inhibitors may also inhibit SOS2, with a selectivity ratio less than 10-fold for inhibition of SOS1 relative to SOS2. In some embodiments, SOS1 inhibitors will selectively inhibit SOS1, with a selectivity ratio greater of at least about 10-fold, such as greater than at least about 30-fold, for inhibition of SOS1 relative to SOS2.

[0062] The terms "RAS pathway" and "RAS/MAPK pathway" are used interchangeably herein to refer to a signal transduction cascade downstream of various cell surface growth factor receptors in which activation of RAS (and its various isoforms and alleotypes) is a central event that drives a variety of cellular effector events that determine the proliferation, activation, differentiation, mobilization, and other functional properties of the cell. SHP2 conveys positive signals from growth factor receptors to the RAS activation/deactivation cycle, which is modulated by guanine nucleotide exchange factors (GEFs, such as SOS1) that load GTP onto RAS to produce functionally active GTP-bound RAS as well as GTP-accelerating proteins (GAPs, such as NF1) that facilitate termination of the signals by conversion of GTP to GDP. GTP-bound RAS produced by this cycle conveys essential positive signals to a series of serine/threonine kinases including RAF and MAP kinases, from which emanate additional signals to various cellular effector functions.

[0063] The terms "RAS inhibitor" and "inhibitor of [a] RAS" are used interchangeably to refer to any inhibitor that targets a RAS protein. In various embodiments, these terms include RAS(OFF) and RAS(ON) inhibitors such as, e.g., the KRAS(OFF) and KRAS(ON) inhibitors. A RAS inhibitor may be MRTX1133, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof. The term "RAS(OFF) inhibitor" refers to any inhibitor that binds to a RAS protein in its GDP-bound "OFF" position. The term "RAS(ON) inhibitor" refers to any inhibitor that binds to a RAS protein in its GTP-bound "ON" position.

[0064] As used herein, the term "RAS(ON) inhibitor" refers to an inhibitor that targets, that is, selectively binds to or inhibits, the GTP-bound, active state of RAS (e.g., selective over the GDP-bound, inactive state of RAS). Inhibition of the GTP-bound, active state of RAS includes, for example, the inhibition of oncogenic signaling from the GTP-bound, active state of RAS. In some embodiments, the RAS(ON) inhibitor is an inhibitor

that selectively binds to and inhibits the GTP-bound, active state of RAS. In certain embodiments, RAS(ON) inhibitors may also bind to or inhibit the GDP-bound, inactive state of RAS (e.g., with a lower affinity or inhibition constant than for the GTP-bound, active state of RAS). The term "KRAS(ON) inhibitor" refers to any inhibitor that binds to KRAS in its GTP-bound "ON" position.

[0065] As used herein, the term "RAS(OFF) inhibitor" refers to an inhibitor that targets, that is, selectively binds to or inhibits the GDP-bound, inactive state of RAS (e.g., selective over the GTP-bound, active state of RAS). Inhibition of the GDP-bound, inactive state of RAS includes, for example, sequestering the inactive state by inhibiting the exchange of GDP for GTP, thereby inhibiting RAS from adopting the active conformation. In certain embodiments, RAS(OFF) inhibitors may also bind to or inhibit the GTP-bound, active state of RAS (e.g., with a lower affinity or inhibition constant than for the GDP-bound, inactive state of RAS).

[0066] The term "KRAS(OFF) inhibitor" refers to any inhibitor that binds to KRAS in its GDP-bound "OFF" position. Reference to the term KRAS(OFF) inhibitor includes AMG 510 and MRTX849, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof. In some embodiments, reference to the term KRAS(OFF) inhibitor includes any such KRAS(OFF) inhibitor disclosed in any one of the following patent applications: WO 2020118066, WO 2020113071, WO 2020106647, WO 2020106640, WO 2020102730, WO 2020101736, WO 2020097537, WO 2020086739, WO 2020018282, WO 2020050890, WO 2020047192, WO 2020035031, WO 2020033413, WO 2020028706, WO 2019241157, WO 2019234405, WO 2019232419, WO 2019227040, WO 2019217933, WO 2019217691, WO 2019217307, WO 2019215203, WO 2019213526, WO 2019213516, WO 2019204442, WO 2019204449, WO 2019204505, WO 2019155399, WO 2019150305, WO 2019137985, WO 2019110751, WO 2019099524, WO 2019055540, WO 2019051291, WO 2018237084, WO 2018218070, WO 2018217651, WO 2018218071, WO 2018218069, WO 2018212774, WO 2018206539, WO 2018195439, WO 2018143315, WO 2018140600, WO 2018140599, WO 2018140598, WO 2018140514, WO 2018140513, WO 2018140512, WO 2018119183, WO 2018112420, WO 2018068017, WO 2018064510, WO 2018011351, WO 2018005678, WO 2017201161, WO 20171937370, WO 2017172979, WO 2017112777, WO 2017106520, WO 2017096045, WO 2017100546, WO 2017087528, WO 2017079864, WO 2017058807, WO

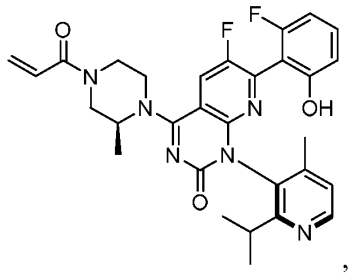
2017058805, WO 2017058728, WO 2017058902, WO 2017058792, WO 2017058768, WO 2017058915, WO 2017015562, WO 2016179558, WO 2016176338, WO 2016168540, WO 2016164675, WO 2016100546, WO 2016049568, WO 2016049524, WO 2015054572, WO 2014152588, WO 2014143659 and WO 2013155223, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof, each of which are incorporated herein by reference in its entirety.

[0067] As used herein, the term "RAS(ON)^{MULTI} inhibitor" refers to a RAS(ON) inhibitor of at least 3 RAS variants with missense mutations at one of the following positions: 12, 13, 59, 61, or 146. In some embodiments, a RAS(ON)^{MULTI} inhibitor refers to a RAS(ON) inhibitor of at least 3 RAS variants with missense mutations at one of the following positions: 12, 13, and 61.

[0068] In any embodiment herein regarding a RAS(OFF) inhibitor, such RAS(OFF) inhibitor may be substituted by a RAS inhibitor disclosed in the following patent publication: WO 2021041671, which is incorporated herein by reference in its entirety. In some embodiments, such a substituted RAS inhibitor is MRTX1133, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0069] Exemplary RAS(OFF) inhibitors include the following, without limitation:

[0070] AMG 510:



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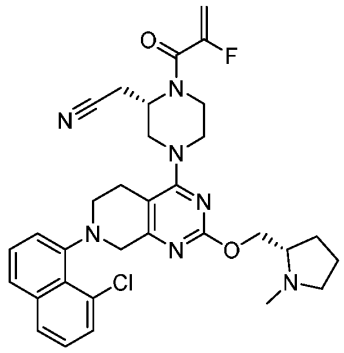
[0071] MRTX849:



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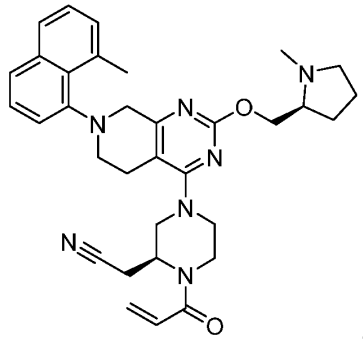
[0072] MRTX1257:



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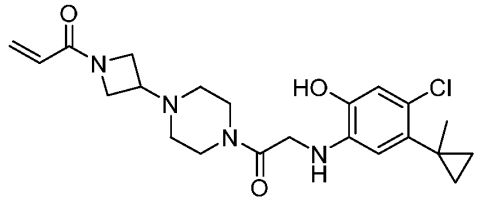
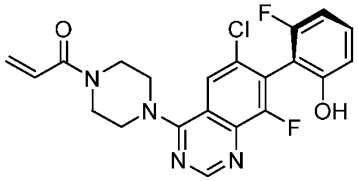
[0073] ARS-853:



, and

[0074] ARS-1620:



, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0075] Reference to a "subtype" of a cell (*e.g.*, a $KRAS^{G12C}$ subtype, a $KRAS^{G12S}$ subtype, a $KRAS^{G12D}$ subtype, a $KRAS^{G12V}$ subtype) means that the cell contains a gene mutation encoding a change in the protein of the type indicated. For example, a cell classified as a "$KRAS^{G12C}$ subtype" contains at least one KRAS allele that encodes an amino acid substitution of cysteine for glycine at position 12 (^{G12C}); and, similarly, other cells of a particular subtype (*e.g.*, $KRAS^{G12D}$, $KRAS^{G12S}$ and $KRAS^{G12V}$ subtypes) contain at least one allele with the indicated mutation (*e.g.*, a $KRAS^{G12D}$ mutation, a $KRAS^{G12S}$ mutation or a $KRAS^{G12V}$ mutation, respectively). Unless otherwise noted, all amino acid position substitutions referenced herein (such as, *e.g.*, "^{G12C}" in $KRAS^{G12C}$) correspond to substitutions in the human version of the referenced protein, *i.e.,* $KRAS^{G12C}$ refers to a G→C substitution in position 12 of human KRAS.

[0076] As used herein, "RASopathies" are a group of genetic conditions caused by changes in genes that are part of the RAS pathway. See, e.g., Rauen, Ann Rev Genomics Human Genetics, 14:355 (2013). Non-limiting examples include Neurofibromatosis type 1 (NF1), Noonan Syndrome (NS), Noonan Syndrome with Multiple Lentigines (NSML), Capillary Malformation-Arteriovenous Malformation Syndrome (CM-AVM), Costello Syndrome (CS), Cardio-Facio-Cutaneous Syndrome (CFC), Legius Syndrome, and Hereditary gingival fibromatosis.

[0077] The term "monotherapy" refers to a method of treatment comprising administering to a subject a single therapeutic agent, optionally as a pharmaceutical composition. For example, a monotherapy may comprise administration of a pharmaceutical composition comprising a therapeutic agent and one or more pharmaceutically acceptable carrier, excipient, diluent, and/or surfactant. The therapeutic agent may be administered in an effective amount. The therapeutic agent may be administered in a therapeutically effective amount.

[0078] The term "combination therapy" refers to a method of treatment comprising administering to a subject at least two therapeutic agents, optionally as one or more pharmaceutical compositions. For example, a combination therapy may comprise administration of a single pharmaceutical composition comprising at least two therapeutic agents and one or more pharmaceutically acceptable carrier, excipient, diluent, and/or surfactant. A combination therapy may comprise administration of two or more pharmaceutical compositions, each composition comprising one or more therapeutic agent

and one or more pharmaceutically acceptable carrier, excipient, diluent, and/or surfactant. In various embodiments, at least one of the therapeutic agents is a SOS1 inhibitor. In various embodiments, at least one of the therapeutic agents is a RAS inhibitor. The two agents may optionally be administered simultaneously (as a single or as separate compositions) or sequentially (as separate compositions). The therapeutic agents may be administered in an effective amount. The therapeutic agent may be administered in a therapeutically effective amount. In some embodiments, the effective amount of one or more of the therapeutic agents may be lower when used in a combination therapy than the therapeutic amount of the same therapeutic agent when it is used as a monotherapy, *e.g.*, due an additive or synergistic effect of combining the two or more therapeutics.

Methods of Treatment

[0079] Disruption of the RAS/MAPK signaling pathway is a common driver of abnormal growth and proliferation in many types of cancer and has also been implicated in developmental diseases such as Noonan Syndrome. Oncogenic hyper-activation of this pathway can occur through alterations in the levels of active GTP-bound RAS and inactive GDP-bound RAS, such as mutations resulting in disruption of RAS guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). SHP2 is a non-receptor protein tyrosine phosphatase encoded by the PTPN11 gene that functions upstream of RAS. SHP2 can regulate RAS signaling through activation of SOS1, a GEF that converts inactive RAS-GDP to RAS-GTP. The development of inhibitors targeting either SHP2 or SOS1 is an emerging and attractive approach toward treatment of RAS-driven cancers, and several such candidates are currently undergoing clinical trials.

[0080] According to some embodiments of the present invention, a subject having a disease or disorder associated with cells having a SHP2 mutation is treated by administering to the subject a therapeutically effective amount of a SOS1 inhibitor. In some embodiments, the SHP2 mutation induces an activated form of SHP2. In some embodiments, the subject expressed the SHP2 mutation after prior treatment with a SHP2 inhibitor. In some embodiments, the subject expressed the SHP2 mutation after prior treatment with an allosteric SHP2 inhibitor.

[0081] In some embodiments, the method further comprises administering to the subject a therapeutically effective amount of a RAS inhibitor selected from the group consisting of a RAS(ON) inhibitor, a RAS(OFF) inhibitor, MRTX1133, and a combination

thereof. In some embodiments, the RAS inhibitor targets a wild-type RAS protein. In some embodiments, the RAS protein is KRAS. In some embodiments, the RAS inhibitor targets a RAS protein mutation. In some embodiments, the RAS protein mutation is at a position selected from the group consisting of G12, G13, Q61, A146, K117, L19, Q22, V14, A59, and a combination thereof. In some embodiments, the mutation is at a position selected from the group consisting of G12, G13, and Q61. In some embodiments, the mutation is selected from the group consisting of G12C, G12D, G12A, G12S, G12V, G13C, G13D, Q61K, and Q61L.

SHP2 Mutations

[0082] Potent and selective allosteric SHP2 inhibitors, such as RMC-4550, have proven effective *in vitro* and *in vivo* across a wide range of histotypes and genotypes, at disrupting RAS-MAPK signaling, suppressing cell proliferation, and inducing tumor growth inhibition. Allosteric SHP2 inhibitors are effective in preclinical models of cancers driven by the RAS/MAPK signaling pathway, in part because they block activation of the RAS GEFs SOS1 and SOS2. See FIG. 1, which illustrates the RAS-MAPK pathway. However, SHP2 inhibitors, including allosteric SHP2 inhibitors, have previously been demonstrated to exhibit significantly reduced efficacy against a spectrum of specific clinically relevant mutations in SHP2 that induce an activated form of the SHP2 protein. Mutations can occur in SHP2 that, to varying degrees, activate signaling and reduce sensitivity to allosteric inhibitors. These mutations may arise during tumor development as drivers or be acquired as resistance mutations in response to treatment with allosteric SHP2 inhibitors. See FIGS. 2A and 2B, which depict several activating mutations in SHP2 proteins. In addition, patients treated with SHP2 inhibitors, including allosteric SHP2 inhibitors, may develop tumors with somatic SHP2 mutations, inducing pathway reactivation and drug resistance. Therefore, there exists an unmet medical need for alternative treatments for cancers associated with activating mutations of SHP2 protein.

[0083] Potent SOS1 inhibitors, including selective SOS1 inhibitors, have also been developed and have more recently shown preclinical promise as an alternative treatment for RAS/MAPK pathway-driven cancers. The first clinical trial involving a SOS1 inhibitor has recently begun. SOS1 inhibitors, which act directly downstream of SHP2, would represent an attractive alternative treatment in this regard if they maintain or show improved efficacy in the context of activated mutant SHP2. The present disclosure

provides evidence in support of this concept, showing that in contrast to SHP2 inhibitors, e.g., allosteric SHP2 inhibitors, SOS1 inhibition is efficacious *in vitro* and *in vivo* in cells or tissue with activating SHP2 mutations, even when SHP2 allosteric inhibitors are not effective. See FIGS. 3A, 3B, 4A through 4H, and 20A, and 20B.

[0084] Unexpectedly, genetic knockdown of SOS1 appears to induce a greater reduction in pathway activity in cells with activated SHP2, indicating that cancer cells harboring activating SHP2 mutations are uniquely dependent on SOS1. Experimental results showed that all cells are sensitive to simultaneous knockdown of SOS1 and SOS2, but unexpectedly, a trend is seen of greater effect of SOS1 knockdown in cells with more strongly activating SHP2 mutations. In contrast, SOS2 knockdown has only a small and similar effect in all cell lines. This suggests that strongly activating SHP2 mutations increase dependence on SOS1, and activating SHP2 mutations may therefore increase sensitivity to SOS1 inhibitors such as BI-3406, BI-1701963, and Compound SOS1-(A) (also called RMC-0331), or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof. This observation provides the basis for a therapeutic strategy whereby cancer patients with mutations in SHP2, e.g., activating SHP2 mutations, either present initially or arising in response to therapy with SHP2 inhibitors, e.g., allosteric SHP2 inhibitors, may be effectively treated with SOS1 inhibitors.

[0085] According to some embodiments of the present invention, a method is provided for treating a subject having a SHP2 mutation, e.g., a SHP2 mutation that induces an activated form of SHP2. These mutations act by destabilizing an auto-inhibited conformation of SHP2. Different activating mutations destabilize this conformation to different degrees, which can be expressed quantitatively as the free energy of opening (ΔG_{op}) of the mutation. Wild-type SHP2 has a ΔG_{op} of 2.8 kcal/mol. Values of ΔG_{op} below 2.8 in mutant SHP2 indicate activation, with lower values indicating stronger activation. A weakly activating mutant is defined as one with a ΔG_{op} not more than 1.5 kcal/mol below wild type SHP2. A moderately activating mutant has a ΔG_{op} between 1.5 kcal/mol and 2.24 kcal/mol below wild-type. A strongly activating mutation has a ΔG_{op} more than 2.24 kcal/mol below wild type. See FIG. 2A, which is a graph correlating the RMC-4550 pERK IC_{50} as a function of ΔG_{op}. FIG. 2B is a table showing the pERK IC_{50} values for RMC-4550 in a variety of activating mutations of SHP2 protein. The following Table 1 summarizes the model parameters for SHP2 and certain mutants.

Table 1: Summary of model parameters for SHP2 and mutants

Variant	ΔG_{op} (kcal/mol)	$\Delta G_{peptide}$ (kcal/mol)	ΔG_i (kcal/mol)	A_{op} (mRFU/s/nM)
wild-type	2.74	-10.78	-11.87	35
G60V	1.01	-11.48	-10.38	31.77
D61G	0.72	-10.55	-11.94	34.51
D61V	0.37	-11.39	-11.23	33.39
E69K	0.61	-11.54	-11.59	30.25
A72S	2.03	-11.07	-11.56	28.24
A72T	0.31	-10.72	-11.18	33.21
A72V	-0.01	-11.05	-10.62	31.84
T73I	1.76	-11.69	-11.56	38.4
E76A	0.41	-11.26	-11.51	32.1
E76G	0.53	-11.14	-11.45	35.64
E76K	-0.60	-10.91	-10.92	29.51
E76Q	1.24	-11.33	-11.91	30.83
S189A	3.22	-10.52	-11.95	35
L262R	2.00	-10.94	-11.85	37.72
F285S	1.97	-10.77	-11.94	19.81
N308D	1.89	-11.12	-11.96	34.42
T468M	3.63	-10.52	-11.11	5
P491S	2.06	-11.13	-9.13	35.67
G503V	-0.62	-10.57	-9.31	53.97
Q506P	0.67	-10.38	-11.19	2.679
T507K	1.97	-10.53	-11.23	30.3

[0086] As shown in FIG. 2A and Table 1, G503V, a mutation resulting in the lowest free energy of opening (ΔG_{op}), is a particularly activating mutation of SHP2. This is further shown, for example, by the pERK IC_{50} value of > 30,000 nM for G503V in FIG. 2B.

[0087] Accordingly, in some embodiments, a SHP2 mutation may occur at one or more of the following positions: G60, D61, E69, A72, T73, E76, S189, L262, F285, N308, T468, P491, S502, G503, Q506, T507, T253 or Q257. In some embodiments, a mutation is one or more of the following: G60V, D61G, D61V, E69K, A72S, A72T, A72V, T73I, E76A, E76G, E76K, E76Q, S189A, L262R, F285S, N308D, T468M, P491S, S502P, G503V, Q506P, T507K, T253M/Q257L, and a combination thereof.

[0088] Moreover, in some embodiments, a SHP2 mutation is at a position that occurs with a frequency in subjects greater than an alteration prevalence greater than 0.05%. In some embodiments, a SHP2 mutation is at a position selected from the group consisting of T52, I56, G60, D61, Y62, Y63, E69, K70, A72, T73, E76, E123, E139, Y197, S189, T253, Q257, L261, L262, R265, F285, N308, V428, A461, T468, P491, S502, G503, M504, Q506, Q510, T507, and a combination thereof. In some embodiments, a SHP2 mutation is at a position selected from the group consisting of A72, E76 and G503, and a combination thereof. In some embodiments, a SHP2 mutation is selected from the group consisting of T52I, I56V, G60V, D61G, D61V, D61Y, Y62D, Y63D, Y63C, E69K, E69Q, K70R, A72S, A72T, A72V, T73I, E76A, E76G, E76K, E76Q, E123D, E139D, S189A, T253M, Q257L, L261F, L261H, L262R, R265Q, F285S, N308D, V428M, A461T, A461G, T468M, P491S, S502L, S502P, G503A, G503V, M504V, Q506P, T507K, Q510P, Q510H, and a combination thereof.

[0089] In some embodiments, the SHP2 mutation is at a position selected from the group consisting of G60, D61, A72, E76, G503 and S502, and a combination thereof. In some embodiments, the SHP2 mutation is at a position selected from the group consisting of G60, D61, E69, A72, E123, Y197, N308, V428, A461, T468, S502, G503, T507, and a combination thereof. In some embodiments, the SHP2 mutation is selected from the group consisting of G60V, D61G, D61V, D61Y, E69K, E69Q, A72S, A72T, A72V, E123D, N308D, V428M, A461T, A461G, T468M, S502L, S502P, G503A, G503V, T507K, and a combination thereof. In some embodiments, the SHP2 mutation is at a position selected from the group consisting of T52, I56, Y62, Y63, E69, K70, E139, L261,

R265, N308, T468, M504, Q510, and a combination thereof. In some embodiments, the SHP2 mutation is selected from the group consisting of T52I, I56V, Y62D, Y63D, Y63C, E69K, E69Q, K70R, E139D, L261F, L261H, R265Q, N308D, T468M, M504V, Q510P, Q510H, and a combination thereof.

[0090] In some embodiments, the SHP2 mutation is expressed in a subject after a course of treatment with a SHP2 inhibitor. In some embodiments, the SHP2 mutation is expressed in a subject after a course of treatment with an allosteric SHP2 inhibitor. In some embodiments, the SHP2 mutation is expressed in a subject after a course of treatment with an active site SHP2 inhibitor. In some embodiments, the SHP2 mutation is expressed in a subject after a course of treatment with an allosteric SHP2 inhibitor. The SHP2 inhibitor, e.g., allosteric SHP2 inhibitor, is generally an inhibitor of wild-type SHP2 protein. SHP2 inhibitors, e.g., allosteric SHP2 inhibitors, may be selected from among those disclosed, without limitation, in WO 2021149817, WO 2021148010, WO 2021147879, WO 2021143823, WO 2021143701, WO 2021143680, WO2021121397, WO 2021119525, WO 2021115286, WO 2021110796, WO 2021088945, WO 2021073439, WO 2021061706, WO 2021061515, WO 2021043077, WO 2021033153, WO 2021028362, WO 2021033153, WO 2021028362, WO 2021018287, WO 2020259679, WO 2020249079, WO 2020210384, WO 2020201991, WO 2020181283, WO 2020177653, WO 2020165734, WO 2020165733, WO 2020165732, WO 2020156243, WO 2020156242, WO 2020108590, WO 2020104635, WO 2020094104, WO 2020094018, WO 2020081848, WO 2020073949, WO 2020073945, WO 2020072656, WO 2020065453, WO 2020065452, WO 2020063760, WO 2020061103, WO 2020061101, WO 2020033828, WO 2020033286, WO 2020022323, WO 2019233810, WO 2019213318, WO 2019183367, WO 2019183364, WO 2019182960, WO 2019167000, WO 2019165073, WO 2019158019, WO 2019152454, WO 2019051469, WO 2019051084, WO 2018218133, WO 2018172984, WO 2018160731, WO 2018136265, WO 2018136264, WO 2018130928, WO 2018129402, WO 2018081091, WO 2018057884, WO 2018013597, WO 2017216706, WO 2017211303, WO 2017210134, WO 2017156397, WO 2017100279, WO 2017079723, WO 2017078499, WO 2016203406, WO 2016203405, WO 2016203404, WO 2016196591, WO 2016191328, WO 2015107495, WO 2015107494, WO 2015107493, WO 2014176488, WO 2014113584, US 20210085677, US 10858359, US 10934302 and US 10954243, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer

thereof. A non-limiting list of exemplary such allosteric SHP2 inhibitors include ERAS-601, BBP-398, RLY-1971, JAB-3068, JAB-3312, TNO155, SHP099, RMC-4550, and RMC-4630, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof. In some embodiments, the SHP2 inhibitor is TNO155, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof. In some embodiments, the SHP2 inhibitor is RMC-4630, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof. A non-limiting list of exemplary active-site SHP2 inhibitors include NSC-87877, IIB-08, 11a-1, and GS-493, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof. In some embodiments, a course of treatment with these SHP2 inhibitors, including allosteric SHP2 inhibitors, active site SHP2 inhibitors, or other such inhibitors of wild type SHP2, may induce a mutation, e.g., an activating mutation, in SHP2. In some embodiments, the SHP2 mutation confers resistance to the SHP2 inhibitor. Alternatively, the resistance to the SHP2 inhibitor may occur due to a natural mutation in the SHP2 protein. In any event, the mutation may induce pathway reactivation and drug resistance to the SHP2 inhibitor.

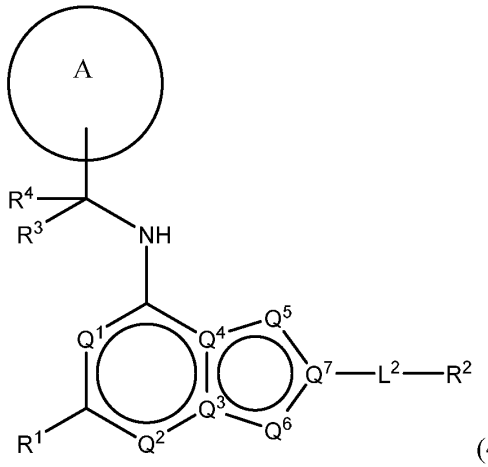
[0091] Accordingly, the present invention is directed to a method of treating a subject having a disease or disorder associated with cells having a mutation in SHP2 protein. In some embodiments, the mutation is an activating SHP2 mutation. According to embodiments of the present invention, the method comprises administering to the subject a therapeutically effective amount of a SOS1 inhibitor. In some embodiments, SOS1 inhibitors may also inhibit SOS2, i.e., the method comprises administering to the subject a therapeutically effective amount of a dual SOS1/SOS2 inhibitor. In some embodiments, such a SOS1 inhibitor is characterized by a selectivity ratio less than 10-fold for inhibition of SOS1 relative to SOS2. In some embodiments, the method comprises administering to the subject a therapeutically effective amount of a selective SOS1 inhibitor. In some embodiments, such a SOS1 inhibitor is characterized by a selectivity ratio greater of at least about 10-fold, such as greater than at least about 30-fold, for inhibition of SOS1 relative to SOS2.

SOS1 inhibitors

[0092] In some embodiments, the SOS1 inhibitor is selected from those disclosed in WO 2018/115380, WO 2018/172250, WO 2019/122129, and WO 2019/201848, or a

pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof, the disclosures of each of which are hereby incorporated by reference as if set forth in their entirety. In some embodiments, the SOS1 inhibitor is selected from those disclosed in WO 2021/092115, WO 2020/180768, and WO 2020/180770, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof, the disclosures of each of which are hereby incorporated by reference as if set forth in their entirety.

[0093] In some embodiments, the SOS1 inhibitor is a compound having the structure of Formula (41-I),



(41-I)

or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof, wherein:

Q^1 and Q^2 are independently CH or N;

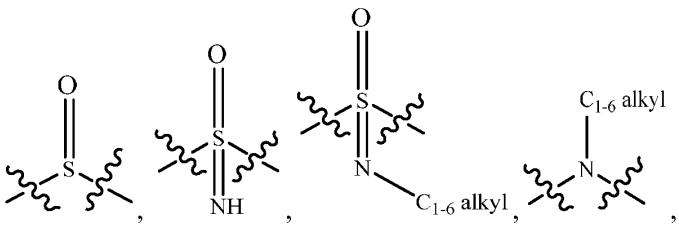
Q^3, Q^4, and Q^7 are independently C or N, wherein at least one of Q^3 and Q^4 is C and wherein Q^3, Q^4, and Q^7 are not all N;

Q^5 is CH, N, NH, O, or S;

Q^6 is CH, N, NH, N-C_{1-6} alkyl, N-C_{1-6} heteroalkyl, N-(3-7 membered cycloalkyl), N-(3-7 membered heterocyclyl), O, or S;

wherein at least one of Q^1, Q^2, Q^3, Q^4, Q^5, Q^6, and Q^7 is N, NH, O, or S;

R^1 is selected from the group consisting of H, C_{1-6} alkyl, halogen, -NHR^{1a}, –OR^{1a}, cyclopropyl, and –CN; wherein C_{1-6} alkyl is optionally substituted with halogen, -NHR^{1a}, or –OR^{1a}; wherein R^{1a} is H, C_{1-6} alkyl, 3-6 membered heterocyclyl, or C_{1-6} haloalkyl;

L^2 is selected from the group consisting of a bond, –C(O)–, –C(O)O–, –C(O)$NH(CH_2)_o$–, –$S(O)_2$–,  , –$C(O)(CH_2)_p$–, –$(CH_2)_p$–, and –O–; wherein o is 0, 1, or 2; and wherein p is a number from 1 to 6;

R^2 is selected from the group consisting of H, C_{1-6} alkyl, C_{2-6} alkenyl, -$NR^{2b}R^{2c}$, -OR^{2a}, 3-14 membered cycloalkyl, 3-14 membered cycloalkenyl, 3-14 membered heterocyclyl, 6-10 membered aryl, and 5-10 membered heteroaryl; wherein each C_{1-6} alkyl, C_{2-6} alkenyl, 3-14 membered cycloalkyl, 3-14 membered cycloalkenyl, 3-14 membered heterocyclyl, 6-10 membered aryl, and 5-10 membered heteroaryl are independently optionally substituted with C_{1-6} alkyl, C_{1-6} haloalkyl, –OH, -OR^{2a}, oxo, halogen, –C(O)R^{2a}, –C(O)OR^{2a}, –C(O)$NR^{2b}R^{2c}$, –CN, -$NR^{2b}R^{2c}$, 3-6 membered cycloalkyl, 3-7 membered heterocyclyl, 6-10 membered aryl, or 5-10 membered heteroaryl;

wherein R^{2a} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, 3-7 membered heterocyclyl, or –$(CH_2)_rOCH_3$, wherein r is 1, 2, or 3;

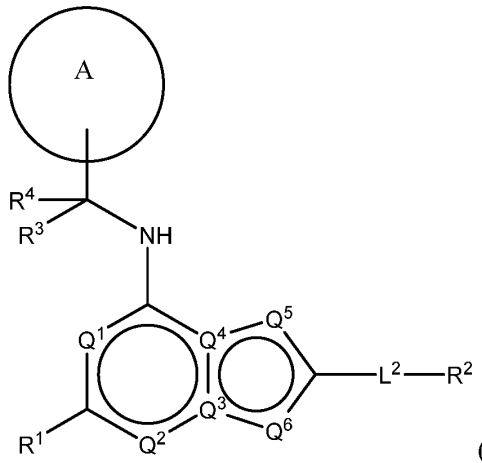
wherein R^{2b} is H or C_{1-6} alkyl;

wherein R^{2c} is H or C_{1-6} alkyl;

R^3 and R^4 are independently H or C_{1-6} alkyl optionally substituted with halo or -OH; wherein at least one of R^3 and R^4 is H or wherein R^3 and R^4 together with the atom to which they are attached combine to form a 3-6 membered cycloalkyl; and

A is an optionally substituted 6-membered aryl or an optionally substituted 5-6 membered heteroaryl.

[0094] In some embodiments, the SOS1 inhibitor is a compound having the structure of Formula (41-I-a),



(41-I-a)

or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof, wherein:

Q^1, Q^2, Q^5 and A are as defined in Formula (41-I);

Q^3 and Q^4 are independently C or N, wherein at least one of Q^3 and Q^4 is C;

Q^6 is CH, N, NH, O, or S;

wherein at least one of Q^1, Q^2, Q^3, Q^4, Q^5, and Q^6 is N, NH, O, or S;

R^1 is selected from the group consisting of H, halogen, C_{1-6} alkyl, cyclopropyl, –CN, and $–OR^{1a}$; wherein R^{1a} is H or C_{1-6} alkyl;

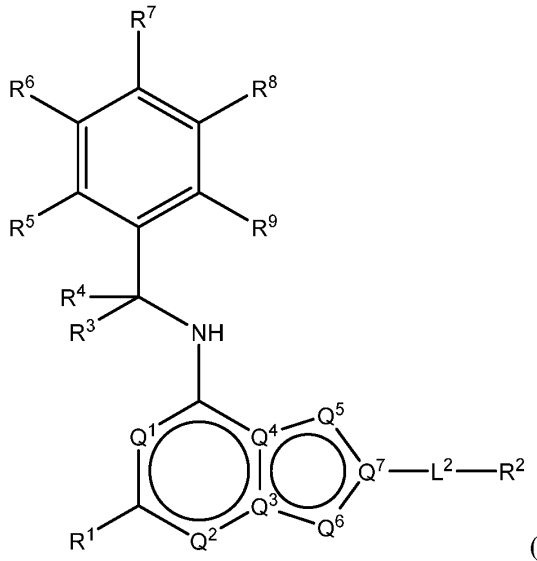
L^2 is selected from the group consisting of a bond, –C(O)–, –C(O)O–, $–C(O)NH(CH_2)_o–$, $–S(O)_2–$, $–C(O)(CH_2)_p–$, $–(CH_2)_p–$, and –O–; wherein o is 0, 1, or 2; and wherein p is a number from 1 to 6;

R^2 is selected from the group consisting of H, $–(CH_2)_qCH_3$, 3-14 membered cycloalkyl, 3-14 membered cycloalkenyl, 3-14 membered heterocyclyl, 6-10 membered aryl, and 5-10 membered heteroaryl; wherein q is a number from 1 to 5; wherein each 3-14 membered cycloalkyl, 3-14 membered cycloalkenyl, 3-14 membered heterocyclyl, 6-10 membered aryl, or 5-10 membered heteroaryl is optionally substituted with C_{1-6} alkyl, –OH,

halogen, –C(O)R^{2a}, or –C(O)$NR^{2b}R^{2c}$; wherein R^{2a} is C_{1-6} alkyl or –$(CH_2)_rOCH_3$, wherein r is 1, 2, or 3; wherein R^{2b} is H or C_{1-6} alkyl; and wherein R^{2c} is H or C_{1-6} alkyl; and

R^3 and R^4 are independently H or C_{1-6} alkyl; wherein at least one of R^3 and R^4 is not H; or R^3 and R^4 together with the atom to which they are attached combine to form a 3-6 membered cycloalkyl.

[0095] In some embodiments, the SOS1 inhibitor is a compound having the structure of Formula (41-II),



(41-II)

or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof, wherein:

L^2, Q^1, Q^2, Q^3, Q^4, Q^5, Q^6, Q^7, R^1, R^2, R^3 and R^4 are as defined in Formula (41-I);

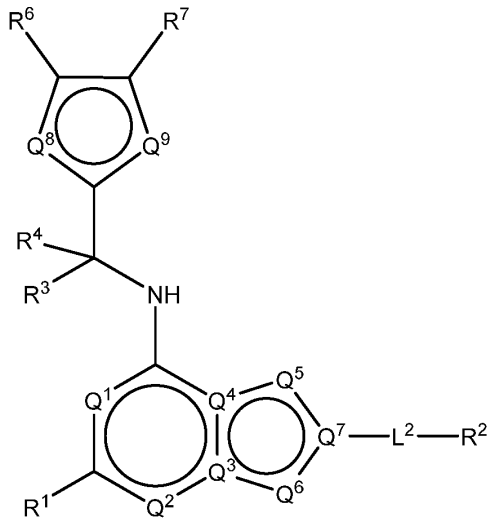
R^5, R^6, R^7, R^8, and R^9 are independently selected from the group consisting of H, D, C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, -OH, halogen, –NO_2, –CN, –$NR^{11}R^{12}$, –SR^{10}, –$S(O)_2NR^{11}R^{12}$, –$S(O)_2R^{10}$, –$NR^{10}S(O)_2NR^{11}R^{12}$, –$NR^{10}S(O)_2R^{11}$, –$S(O)NR^{11}R^{12}$, –$S(O)R^{10}$, –$NR^{10}S(O)NR^{11}R^{12}$, –$NR^{10}S(O)R^{11}$, –$C(O)R^{10}$, and –$CO_2R^{10}$, wherein each C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, and 3-8 membered cycloalkyl are independently optionally substituted with –OH, halogen, –NO_2, oxo, –CN, –R^{10}, –OR^{10}, –$NR^{11}R^{12}$, –SR^{10}, –$S(O)_2NR^{11}R^{12}$, –$S(O)_2R^{10}$, –$NR^{10}S(O)_2NR^{11}R^{12}$, –$NR^{10}S(O)_2R^{11}$, –$S(O)NR^{11}R^{12}$, –$S(O)R^{10}$,

$-NR^{10}S(O)NR^{11}R^{12}$, $-NR^{10}S(O)R^{11}$, 3-14 membered heterocyclyl, 6-10 membered aryl, or 5-10 membered heteroaryl;

R^{10}, R^{11}, and R^{12} are at each occurrence independently selected from H, D, C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, 3-14 membered heterocyclyl, $-OR^{13}$, $-SR^{13}$, halogen, $-NR^{13}R^{14}$, $-NO_2$, and –CN; and

R^{13} and R^{14} are at each occurrence independently selected from H, D, C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, and 3-14 membered heterocyclyl, wherein each C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, and 3-14 membered heterocyclyl are independently optionally substituted with –OH, –SH, $-NH_2$, $-NO_2$, or –CN.

[0096] In some embodiments, the SOS1 inhibitor is a compound having the structure of Formula (41-III),



(41-III)

or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof, wherein:

L^2, Q^1, Q^2, Q^3, Q^4, Q^5, Q^6, Q^7, R^1, R^2, R^3 and R^4 are as defined in Formula (41-I);

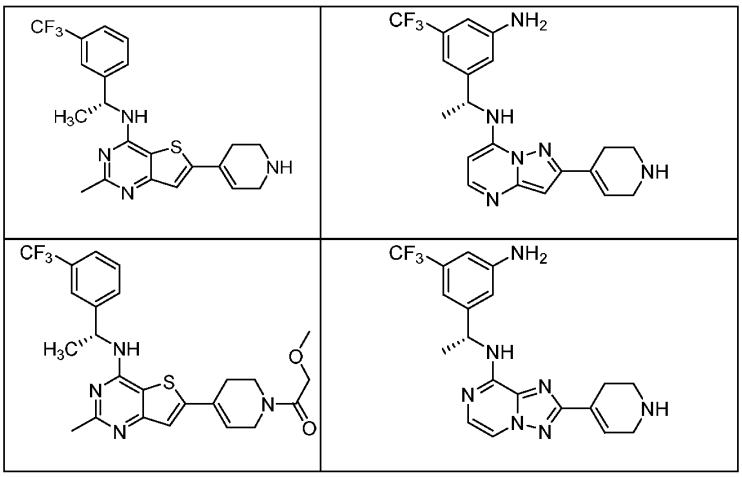
Q^8 and Q^9 are independently CH, N, NH, O, or S, provided at least one of Q^8 and Q^9 is N, NH, O, or S;

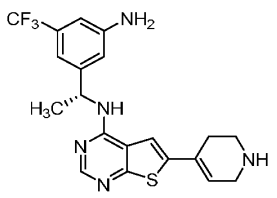
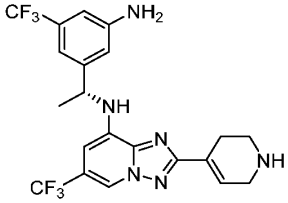
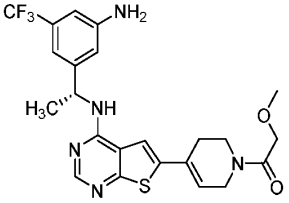
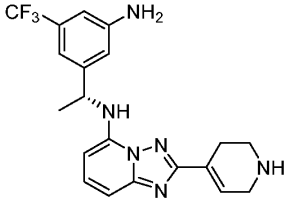
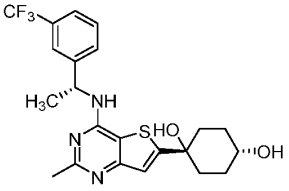
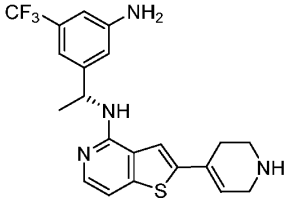
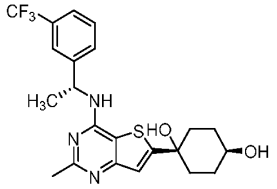
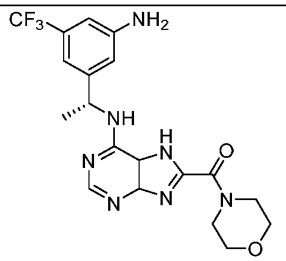
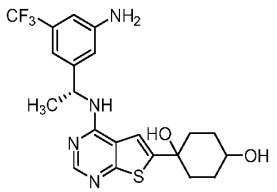
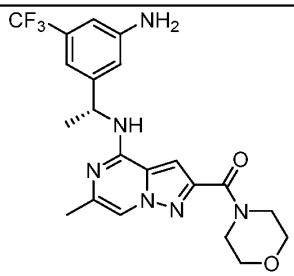
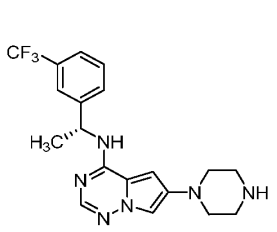
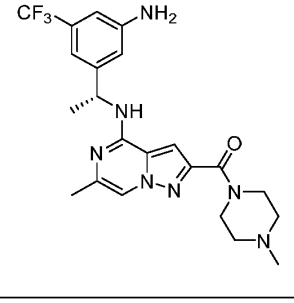
R^6 and R^7 are independently selected from the group consisting of H, D, C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, -OH, halogen, $–NO_2$, –CN, $–NR^{11}R^{12}$, $–SR^{10}$, $–S(O)_2NR^{11}R^{12}$, $–S(O)_2R^{10}$, $–NR^{10}S(O)_2NR^{11}R^{12}$, $–NR^{10}S(O)_2R^{11}$, $–S(O)NR^{11}R^{12}$, $–S(O)R^{10}$, $–NR^{10}S(O)NR^{11}R^{12}$, $–NR^{10}S(O)R^{11}$, $–C(O)R^{10}$, and $–CO_2R^{10}$, wherein each C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, and 3-8 membered cycloalkyl are independently optionally substituted with –OH, halogen, $–NO_2$, oxo, –CN, $–R^{10}$, $–OR^{10}$, $–NR^{11}R^{12}$, $–SR^{10}$, $–S(O)_2NR^{11}R^{12}$, $–S(O)_2R^{10}$, $–NR^{10}S(O)_2NR^{11}R^{12}$, $–NR^{10}S(O)_2R^{11}$, $–S(O)NR^{11}R^{12}$, $–S(O)R^{10}$, $–NR^{10}S(O)NR^{11}R^{12}$, $–NR^{10}S(O)R^{11}$, 3-14 membered heterocyclyl, 6-10 membered aryl, or 5-10 membered heteroaryl;

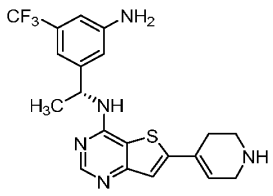
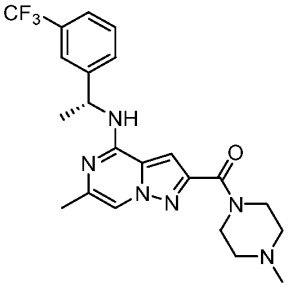
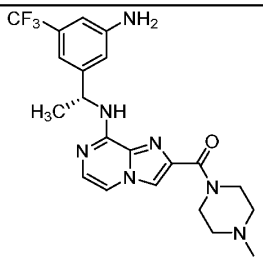
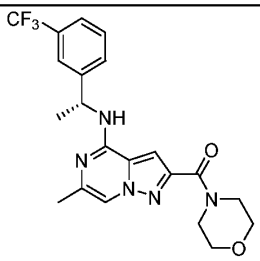
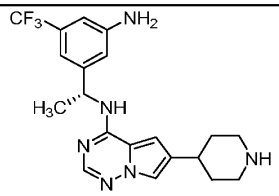
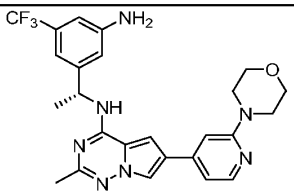
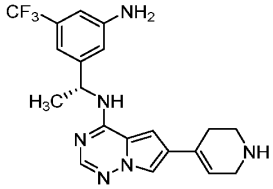
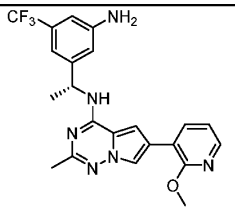
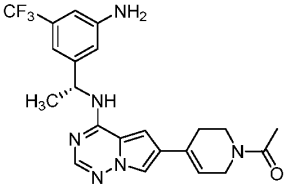
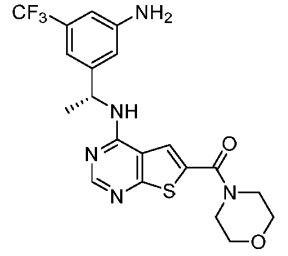
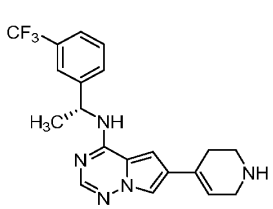
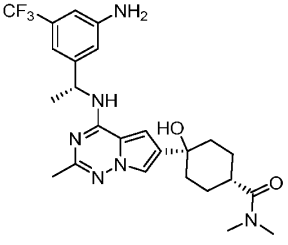
R^{10}, R^{11}, and R^{12} are at each occurrence independently selected from H, D, C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, 3-14 membered heterocyclyl, $–OR^{13}$, $–SR^{13}$, halogen, $–NR^{13}R^{14}$, $–NO_2$, or –CN; and

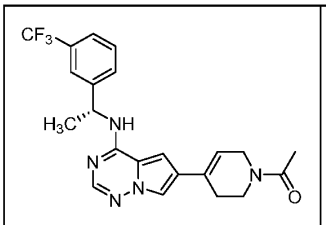
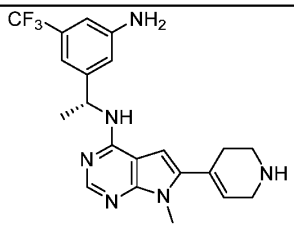
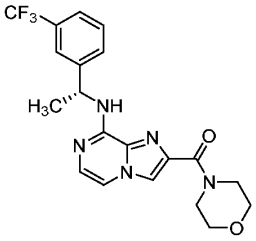
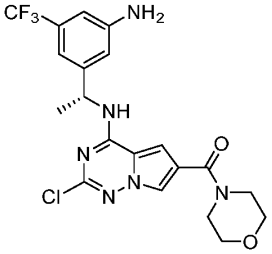
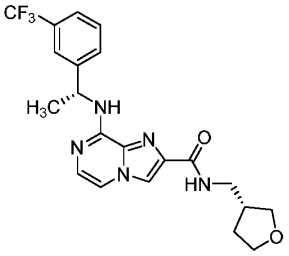
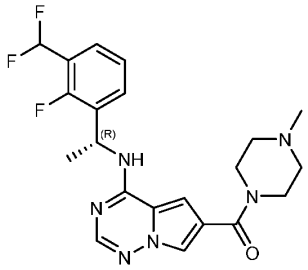
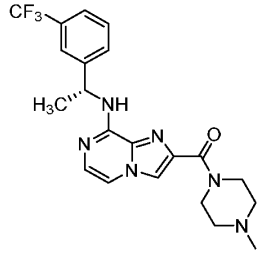
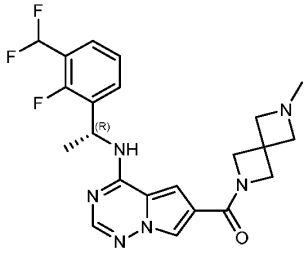
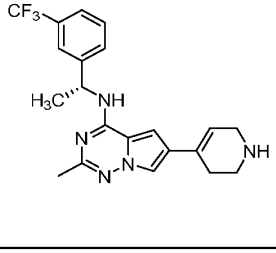
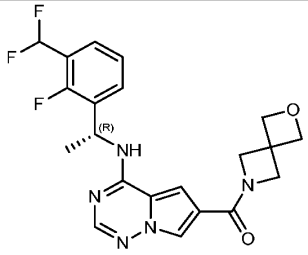
R^{13} and R^{14} are at each occurrence independently selected from H, D, C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, or 3-14 membered heterocyclyl, wherein each C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, and 3-14 membered heterocyclyl are independently optionally substituted with –OH, –SH, $–NH_2$, $–NO_2$, or –CN.

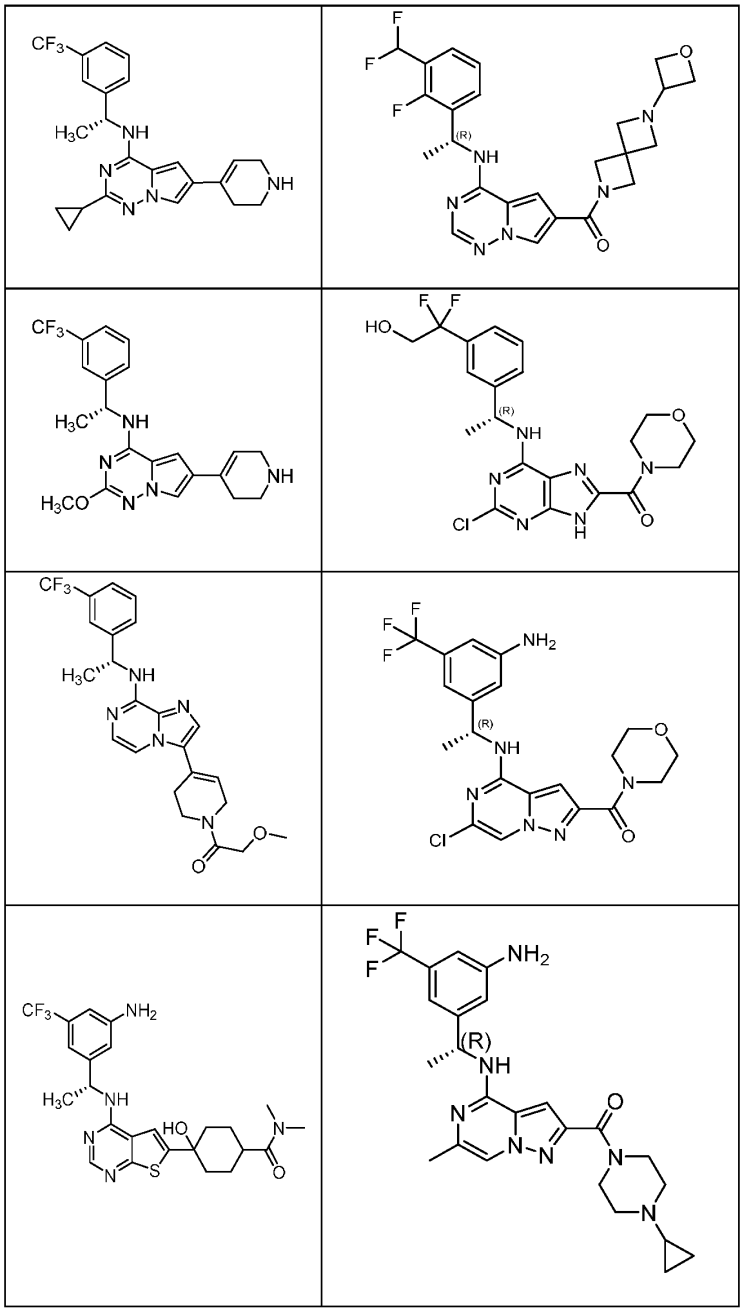
[0097] In some embodiments, the SOS1 inhibitor is a compound selected from the group consisting of the compounds in the following table, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof:

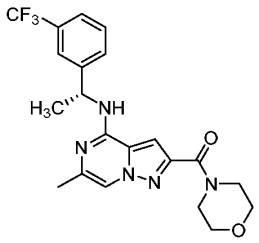
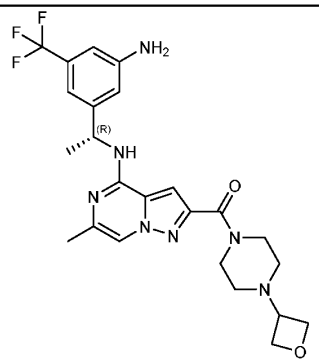
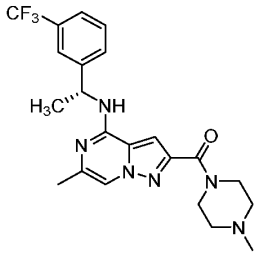
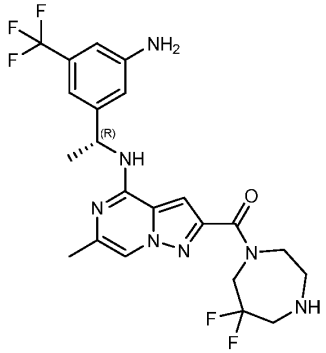
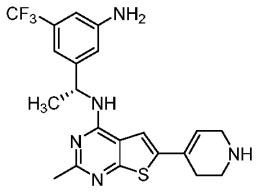
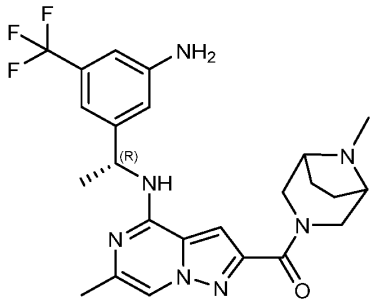
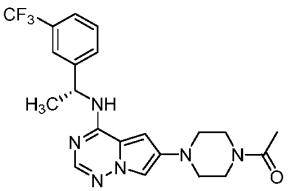
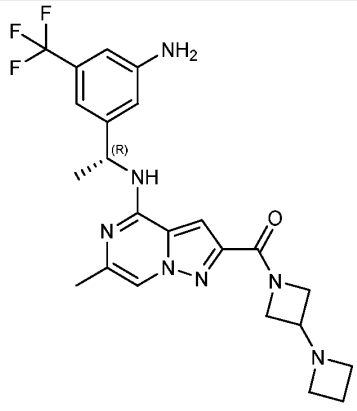


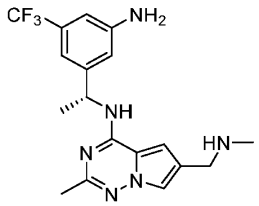
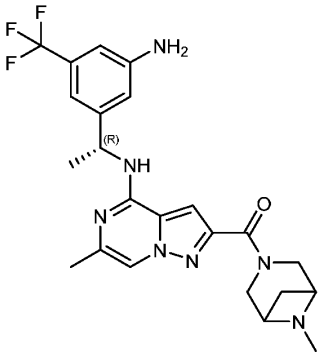
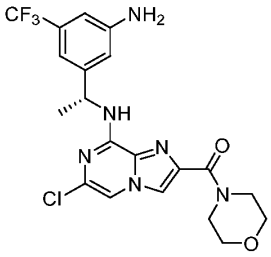
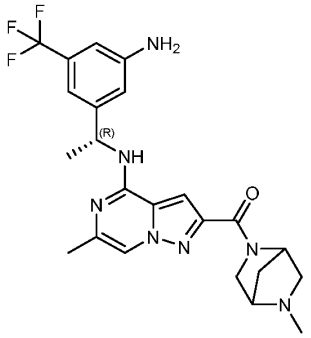
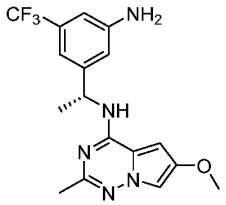
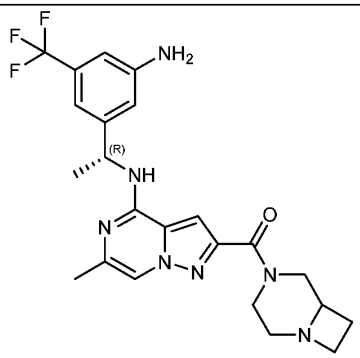
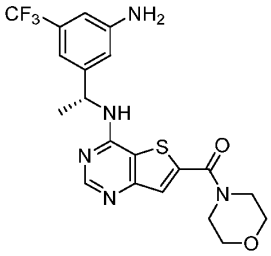
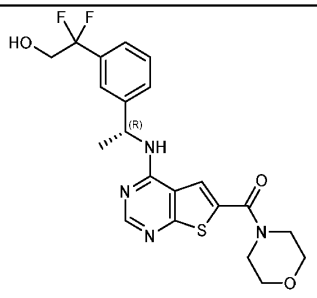
	
	
	
	
	
	

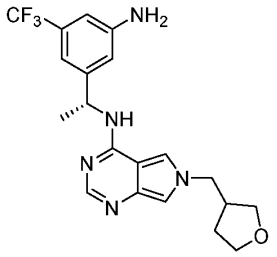
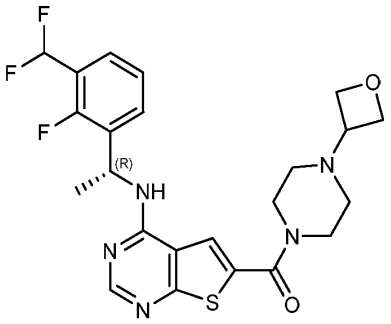
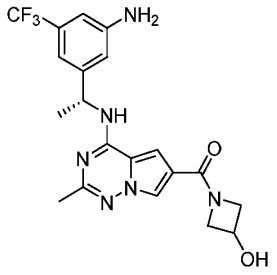
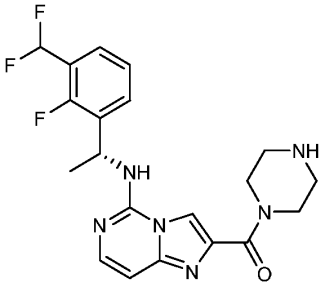
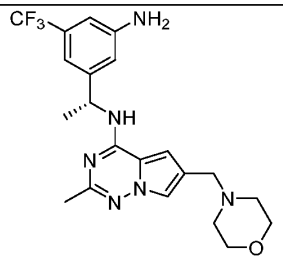
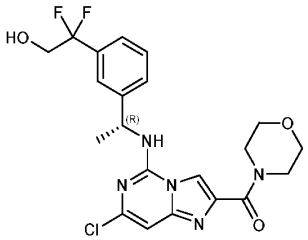
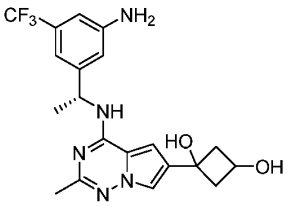
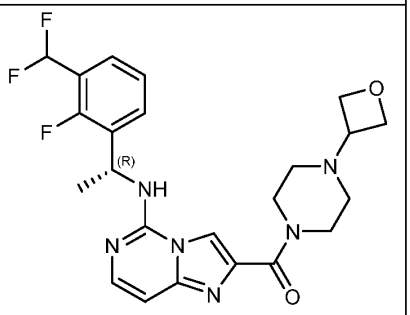
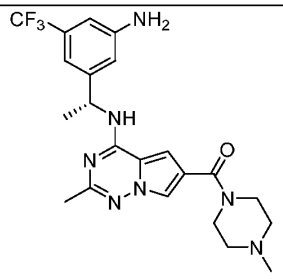
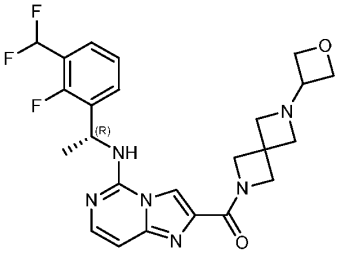
	
	
	
	
	
	

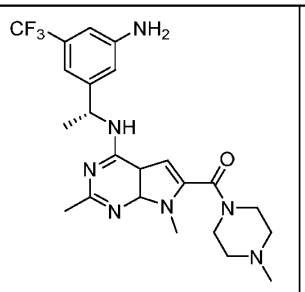
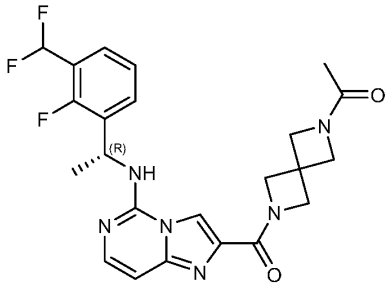
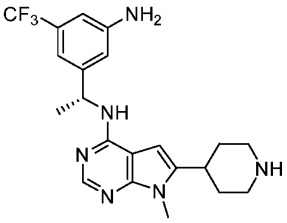
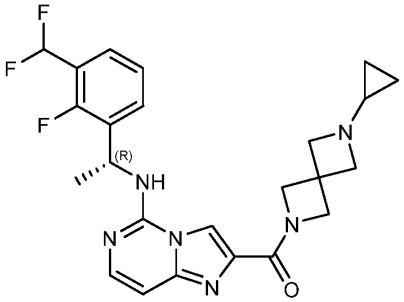
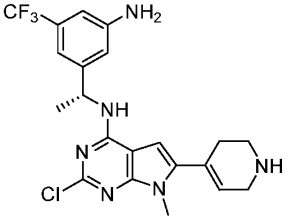
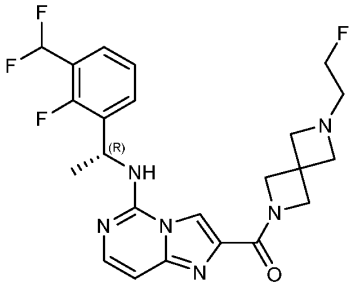
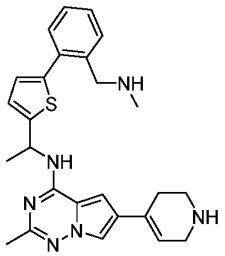
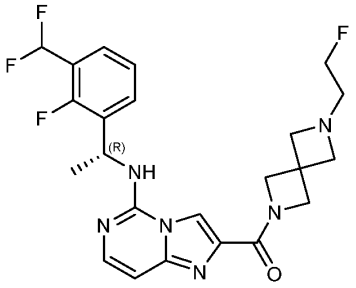
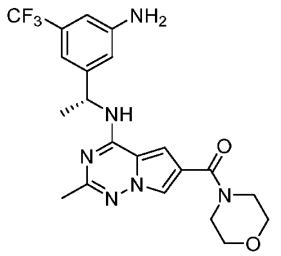
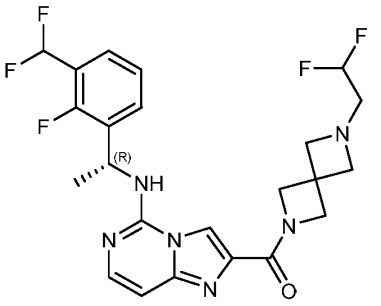
	
	
	
	
	

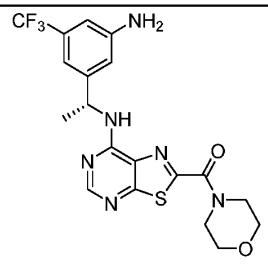
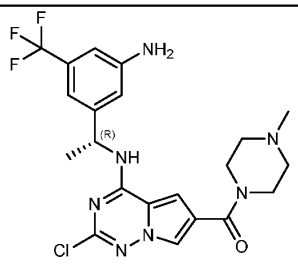
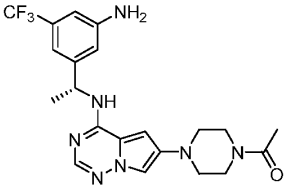
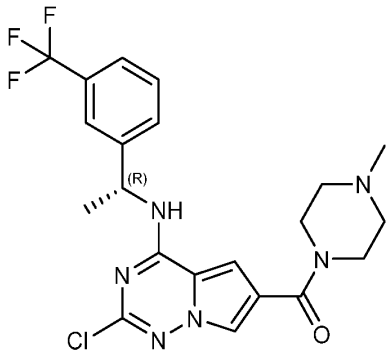
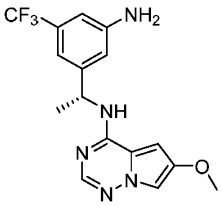
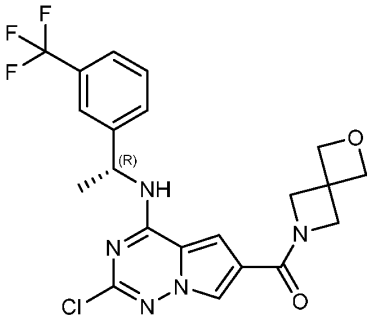
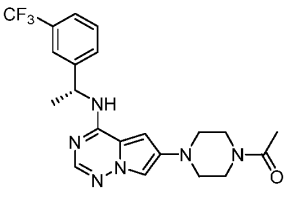
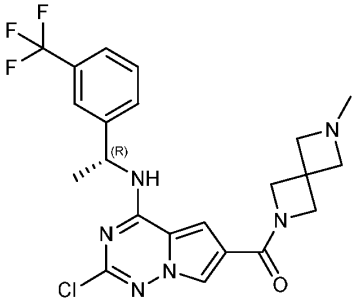


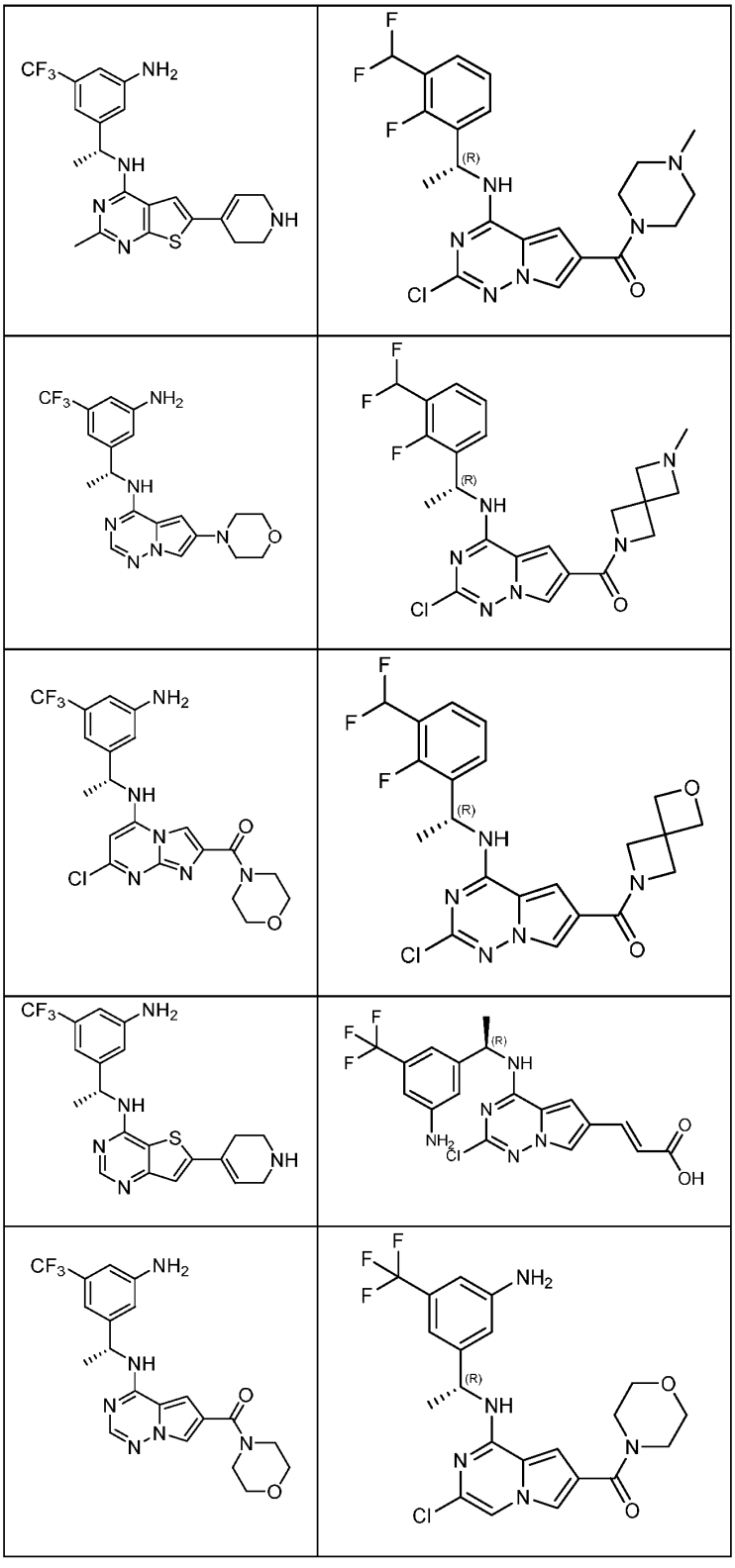
	
	
	
	

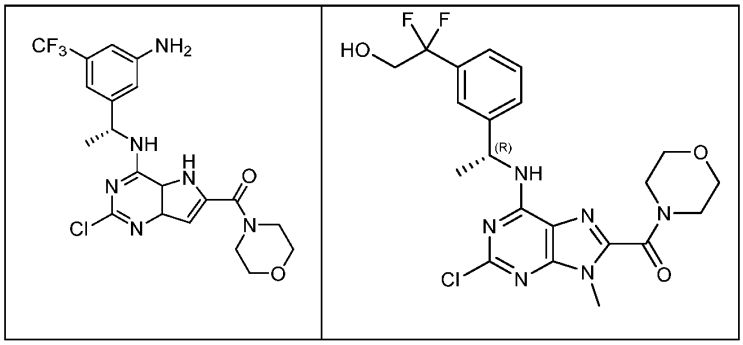
	
	
	
	

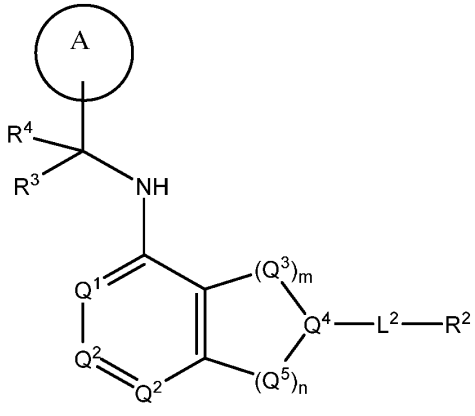
	
	
	
	
	





[0098] In some embodiments, the SOS1 inhibitor is a compound having the structure of Formula (42-I),



(42-I)

or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof, wherein:

Q^1 is CH or N;

Q^4 is CH, C, or N;

each Q^2 is independently C-R^1 or N, wherein one Q^2 is N and the other Q^2 is C-R^1;

each Q^3 and Q^5 are independently $C(R^{QC})_2$, NR^{QN}, CO, O, S, or SO_2, wherein each R^{QC} is independently H, F, Cl, Br, or 6-10 membered aryl, and wherein each R^{QN} is independently H, C_{1-6} alkyl, or 6-10 membered aryl;

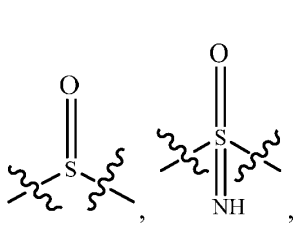
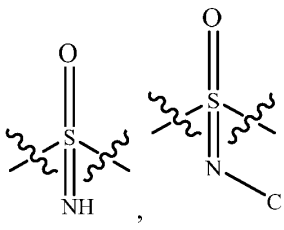
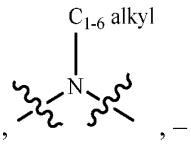
wherein at least one of Q^1, Q^2, Q^3, Q^4, and Q^5 is N, NR^{QN}, O, or SO_2;

m is 0, 1, 2, or 3;

n is 0, 1, 2, or 3;

wherein when m is 0, then n is not 0;

R^1 is selected from the group consisting of H, C_{1-6} alkyl, halogen, -$CONHR^{1a}$, -NHR^{1a}, –OR^{1a}, cyclopropyl, azetidinyl, and –CN; wherein each C_{1-6} alkyl and azetidinyl is optionally substituted with halogen, R^{1a}, -NHR^{1a}, or –OR^{1a}; wherein R^{1a} is H, C_{1-6} alkyl, cyclopropyl, 3-6 membered heterocyclyl, or C_{1-6} haloalkyl;

L^2 is selected from the group consisting of a bond, –C(O)–, –C(O)O–, –C(O)$NH(CH_2)_o$–, –$S(O)_2$–,  ,  ,  , –$C(O)(CH_2)_p$–, –$(CH_2)_p$–, and –O–; wherein o is 0, 1, or 2; and wherein p is a number from 1 to 6;

R^2 is selected from the group consisting of H, C_{1-6} alkyl, -$NR^{2b}R^{2c}$, -OR^{2a}, 3-14 membered cycloalkyl, 3-14 membered cycloalkenyl, 3-14 membered heterocyclyl, 6-10 membered aryl, and 5-10 membered heteroaryl; wherein each C_{1-6} alkyl, 3-14 membered cycloalkyl, 3-14 membered cycloalkenyl, 3-14 membered heterocyclyl, 6-10 membered aryl, and 5-10 membered heteroaryl are independently optionally substituted with C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} hydroxyalkyl, C_{1-6} methoxyalkyl, –OH, –OR^{2a}, oxo, =N, halogen, –$C(O)R^{2a}$, –$C(O)OR^{2a}$, –$C(O)NR^{2b}R^{2c}$, -SO_2R^{2a}, –CN, -$NR^{2b}R^{2c}$, 3-6 membered cycloalkyl, 3-7 membered heterocyclyl, 6-10 membered aryl, or 5-10 membered heteroaryl;

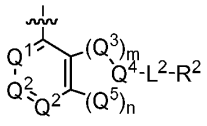
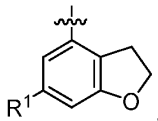
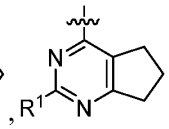
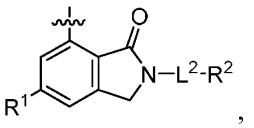
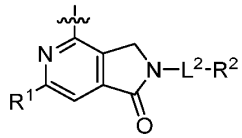
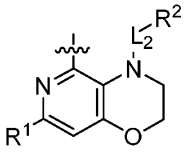
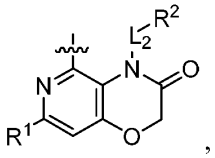
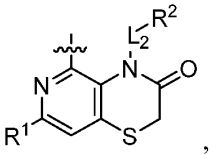
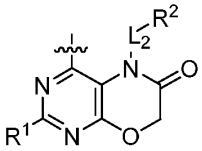
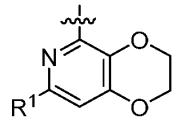
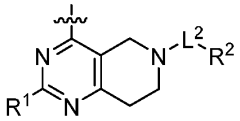
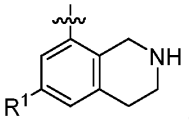
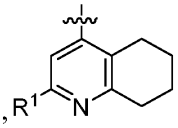
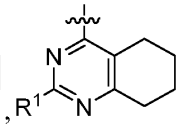
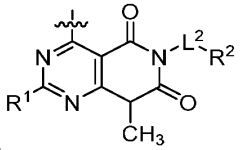
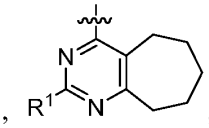
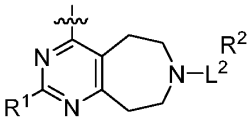
wherein R^{2a} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, 3-7 membered heterocyclyl, or –$(CH_2)_rOCH_3$, wherein r is 1, 2, or 3;

wherein R^{2b} is H or C_{1-6} alkyl;

wherein R^{2c} is H or C_{1-6} alkyl;

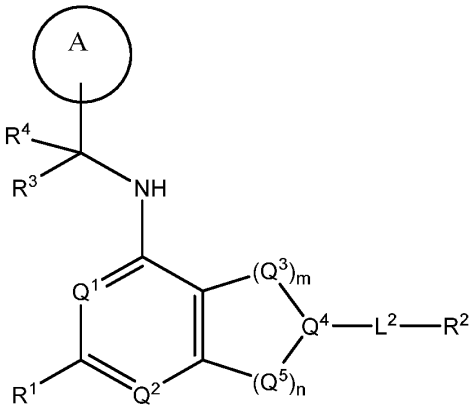
R^3 and R^4 are independently H or C_{1-6} alkyl optionally substituted with halo or -OH; wherein at least one of R^3 and R^4 is H or wherein R^3 and R^4 together with the atom to which they are attached combine to form a 3-6 membered cycloalkyl; and

A is an optionally substituted 6-membered aryl or an optionally substituted 5-6 membered heteroaryl;

with the proviso that when  is  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  , or  ;

then R^1 is not H.

[0099] In some embodiments, the SOS1 inhibitor is a compound having the structure of Formula (42-I-a),



(42-I-a)

or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof, wherein:

Q^1, Q^3, Q^4, Q^5, m, n and A are as defined in Formula (42-I);

Q^2 is CH or N;

wherein at least one of Q^1, Q^2, Q^3, Q^4, and Q^5 is N, NR^{QN}, O, or SO_2;

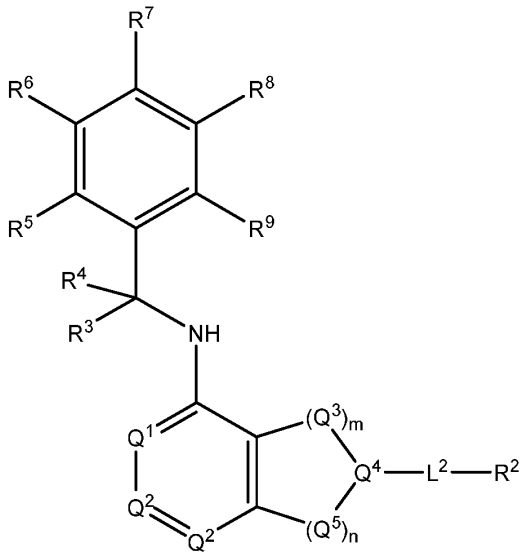
R^1 is selected from the group consisting of H, halogen, C_{1-6} alkyl, cyclopropyl, –CN, and $–OR^{1a}$; wherein R^{1a} is H or C_{1-6} alkyl;

L^2 is selected from the group consisting of a bond, –C(O)–, –C(O)O–, $–C(O)NH(CH_2)_o–$, $–S(O)_2–$, $–C(O)(CH_2)_p–$, $–(CH_2)_p–$, or –O–; wherein o is 0, 1, or 2; and wherein p is a number from 1 to 6;

R^2 is selected from the group consisting of H, $–(CH_2)_qCH_3$, 3-14 membered cycloalkyl, 3-14 membered cycloalkenyl, 3-14 membered heterocyclyl, 6-10 membered aryl, and 5-10 membered heteroaryl; wherein q is a number from 1 to 5; wherein each 3-14 membered cycloalkyl, 3-14 membered cycloalkenyl, 3-14 membered heterocyclyl, 6-10 membered aryl, or 5-10 membered heteroaryl is optionally substituted with C_{1-6} alkyl, –OH, halogen, $–C(O)R^{2a}$, or $–C(O)NR^{2b}R^{2c}$; wherein R^{2a} is C_{1-6} alkyl or $–(CH_2)_rOCH_3$, wherein r is 1, 2, or 3; wherein R^{2b} is H or C_{1-6} alkyl; and wherein R^{2c} is H or C_{1-6} alkyl; and

R^3 and R^4 are independently H or C_{1-6} alkyl; wherein at least one of R^3 and R^4 is not H; or R^3 and R^4 together with the atom to which they are attached combine to form a 3-6 membered cycloalkyl.

[0100] In some embodiments, the SOS1 inhibitor is a compound having the structure of Formula (42-V),



(42-V)

or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof, wherein:

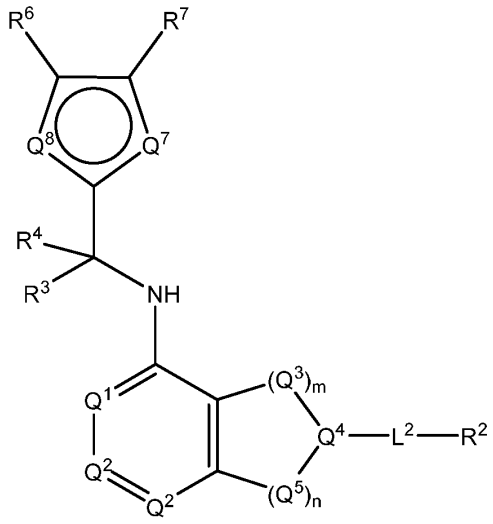
L^2, Q^1, Q^2, Q^3, Q^4, Q^5, m, n, R^1, R^2, R^3 and R^4 are as defined in Formula (42-I);

R^5, R^6, R^7, R^8, and R^9 are independently selected from the group consisting of H, D, C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, 3-14 membered heterocyclyl, -OH, halogen, $–NO_2$, –CN, $–NR^{11}R^{12}$, $–SR^{10}$, $–S(O)_2NR^{11}R^{12}$, $–S(O)_2R^{10}$, $–NR^{10}S(O)_2NR^{11}R^{12}$, $–NR^{10}S(O)_2R^{11}$, $–S(O)NR^{11}R^{12}$, $–S(O)R^{10}$, $–NR^{10}S(O)NR^{11}R^{12}$, $–NR^{10}S(O)R^{11}$, $–C(O)R^{10}$, and $–CO_2R^{10}$, wherein each C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, and 3-14 membered heterocyclyl are independently optionally substituted with –OH, halogen, $–NO_2$, oxo, –CN, $–R^{10}$, $–OR^{10}$, $–NR^{11}R^{12}$, $–SR^{10}$, $–S(O)_2NR^{11}R^{12}$, $–S(O)_2R^{10}$, $–NR^{10}S(O)_2NR^{11}R^{12}$, $–NR^{10}S(O)_2R^{11}$, $–S(O)NR^{11}R^{12}$, $–S(O)R^{10}$, $–NR^{10}S(O)NR^{11}R^{12}$, $–NR^{10}S(O)R^{11}$, 3-8 membered cycloalkyl, 3-14 membered heterocyclyl, 6-10 membered aryl, or 5-10 membered heteroaryl, or any two adjacent R^5, R^6, R^7, R^8, and R^9 forms a 3-14 membered fused ring;

R^{10}, R^{11}, and R^{12} are at each occurrence independently selected from H, D, C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, 3-14 membered heterocyclyl, $–OR^{13}$, $–SR^{13}$, halogen, $–NR^{13}R^{14}$, $–NO_2$, and –CN; and

R^{13} and R^{14} are at each occurrence independently selected from H, D, C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, and 3-14 membered heterocyclyl, wherein each C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, and 3-14 membered heterocyclyl are independently optionally substituted with –OH, –SH, $–NH_2$, $–NO_2$, or –CN.

[0101] In some embodiments, the SOS1 inhibitor is a compound having the structure of Formula (42-VI),



(42-VI)

or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof, wherein:

L^2, Q^1, Q^2, Q^3, Q^4, Q^5, m, n, R^1, R^2, R^3, and R^4 are as defined in Formula (42-I);

Q^7 and Q^8 are each independently CH, N, NH, O, or S, provided at least one of Q^7 and Q^8 is N, NH, O, or S;

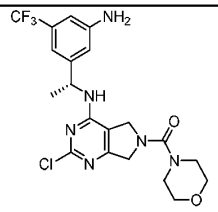
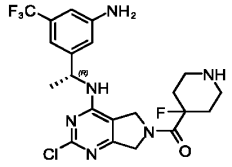
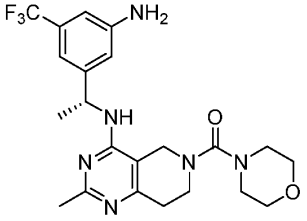
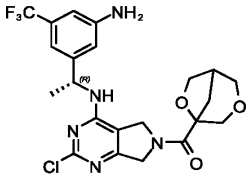
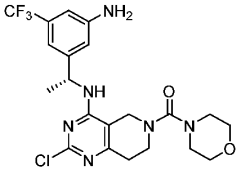
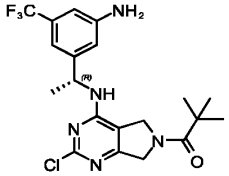
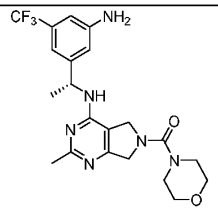
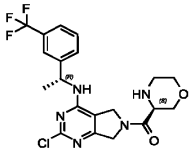
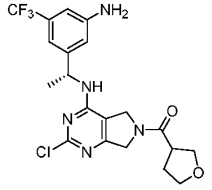
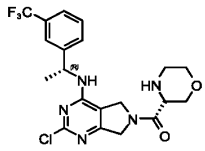
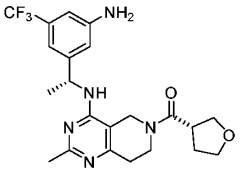
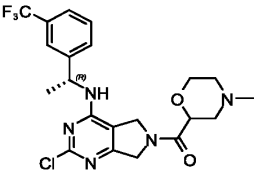
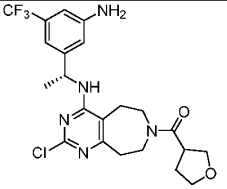
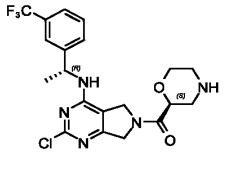
R^6 and R^7 are independently selected from the group consisting of H, D, C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, 3-14 membered heterocyclyl, -OH, halogen, $–NO_2$, –CN, $–NR^{11}R^{12}$, $–SR^{10}$, $–S(O)_2NR^{11}R^{12}$, $–S(O)_2R^{10}$, $–NR^{10}S(O)_2NR^{11}R^{12}$, $–NR^{10}S(O)_2R^{11}$, $–S(O)NR^{11}R^{12}$, $–S(O)R^{10}$, $–NR^{10}S(O)NR^{11}R^{12}$, $–NR^{10}S(O)R^{11}$, $–C(O)R^{10}$, and $–CO_2R^{10}$, wherein each C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, and 3-14 membered heterocyclyl are independently optionally substituted with –OH, halogen, $–NO_2$,

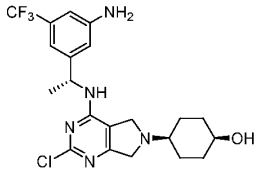
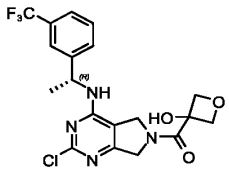
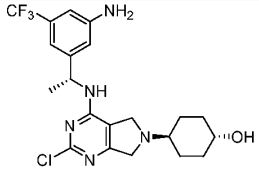
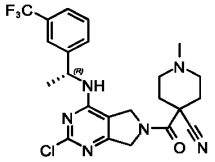
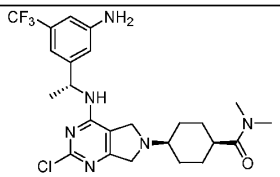
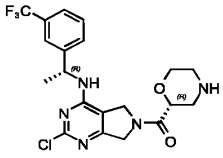
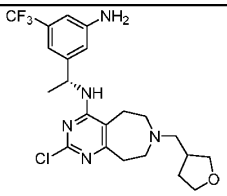
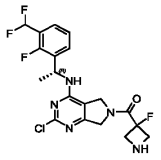
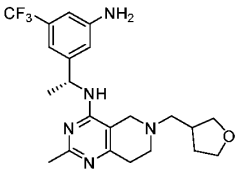
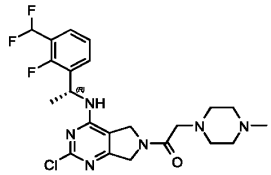
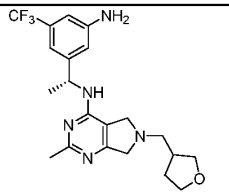
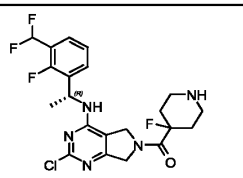
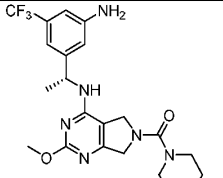
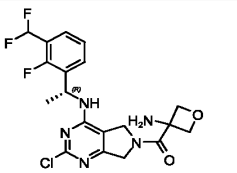
oxo, –CN, –R^{10}, –OR^{10}, –$NR^{11}R^{12}$, –SR^{10}, –$S(O)_2NR^{11}R^{12}$, –$S(O)_2R^{10}$, –$NR^{10}S(O)_2NR^{11}R^{12}$, –$NR^{10}S(O)_2R^{11}$, –$S(O)NR^{11}R^{12}$, –$S(O)R^{10}$, –$NR^{10}S(O)NR^{11}R^{12}$, –$NR^{10}S(O)R^{11}$, 3-8 membered cycloalkyl, 3-14 membered heterocyclyl, 6-10 membered aryl, or 5-10 membered heteroaryl,

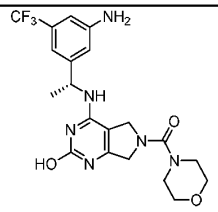
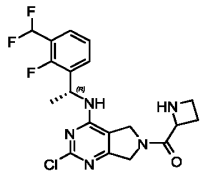
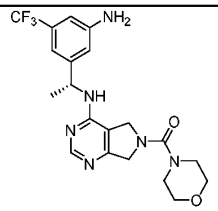
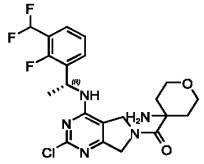
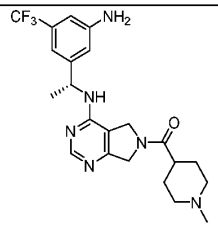
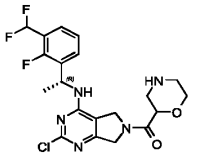
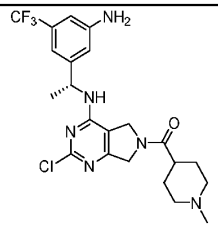
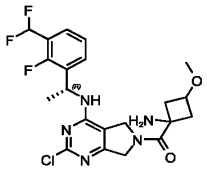
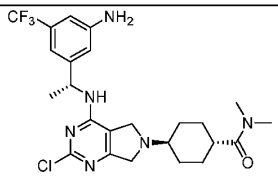
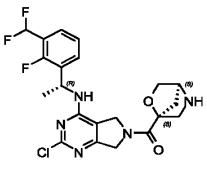
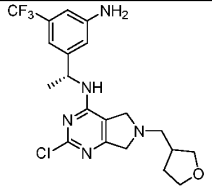
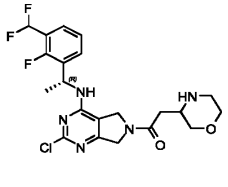
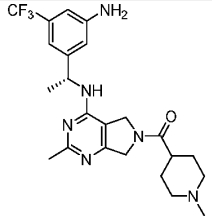
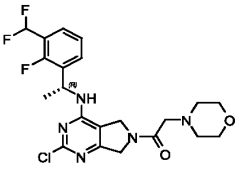
R^{10}, R^{11}, and R^{12} are at each occurrence independently selected from H, D, C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, 3-14 membered heterocyclyl, –OR^{13}, –SR^{13}, halogen, –$NR^{13}R^{14}$, –NO_2, and –CN; and

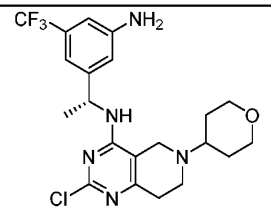
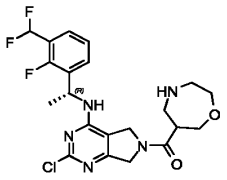
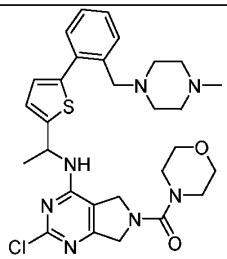
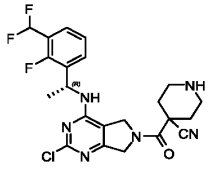
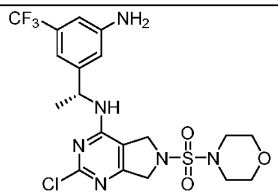
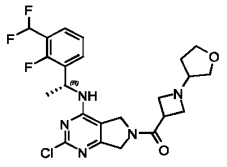
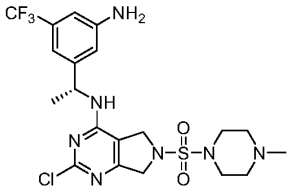
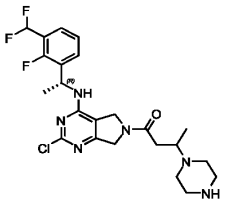
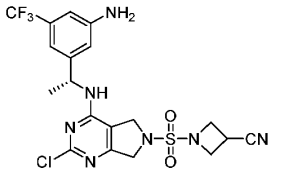
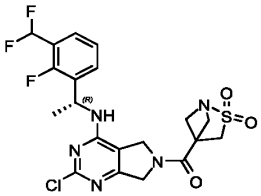
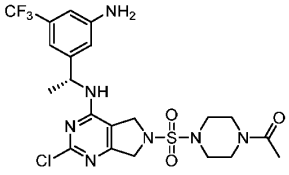
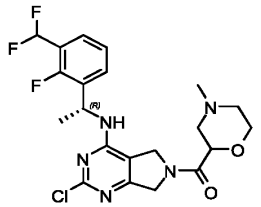
R^{13} and R^{14} are at each occurrence independently selected from H, D, C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, and 3-14 membered heterocyclyl, wherein each C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, and 3-14 membered heterocyclyl are independently optionally substituted with –OH, –SH, –NH_2, –NO_2, or –CN.

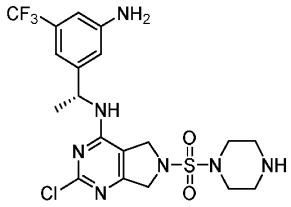
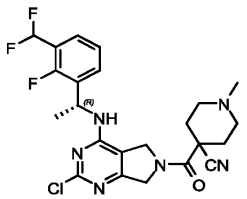
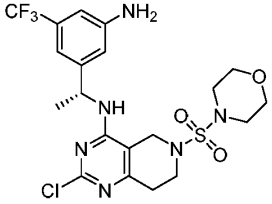
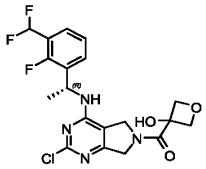
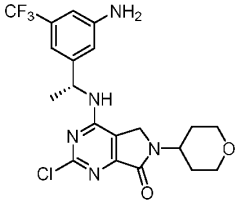
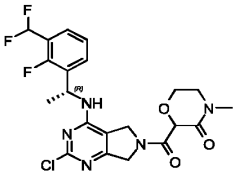
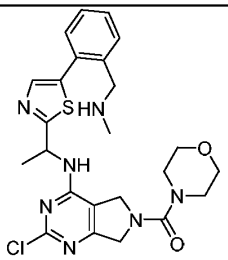
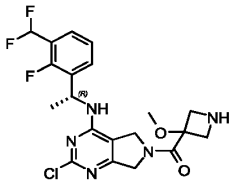
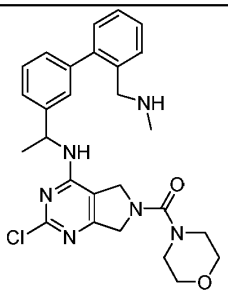
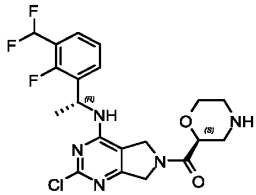
[0102] In some embodiments, the SOS1 inhibitor is a compound selected from the group consisting of the compounds in the following table, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof:

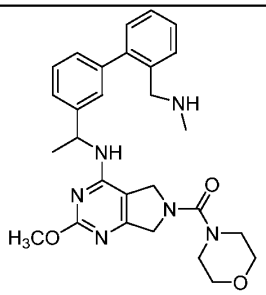
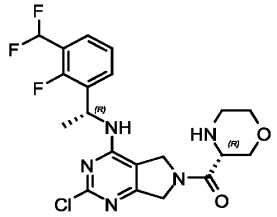
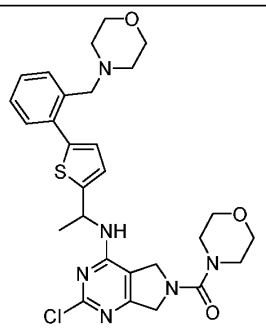
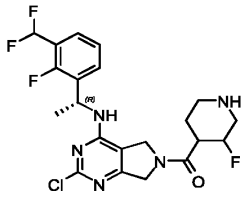
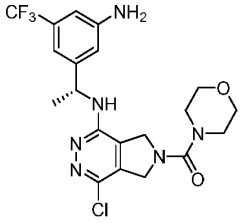
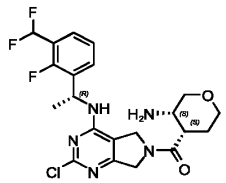
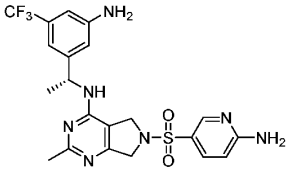
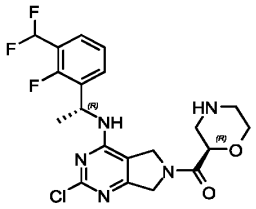
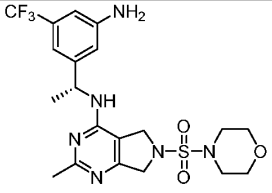
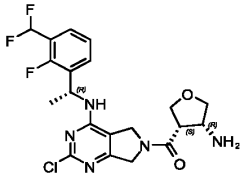
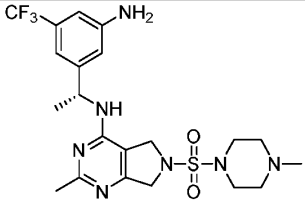
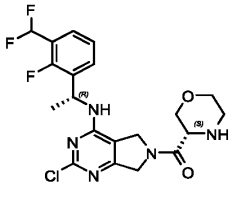
	
	
	
	
	
	
	

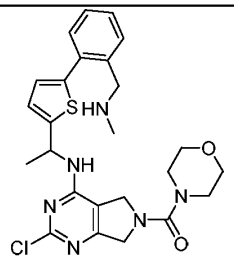
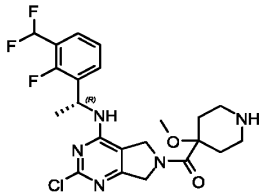
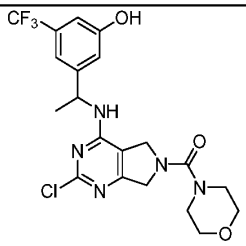
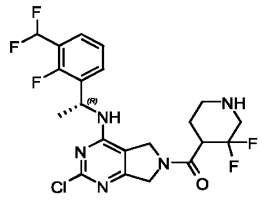
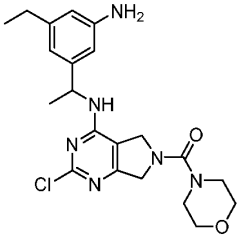
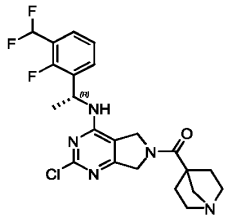
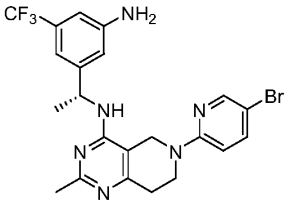
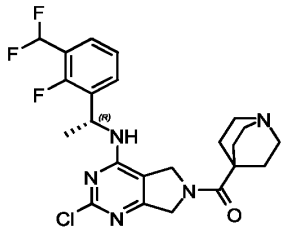
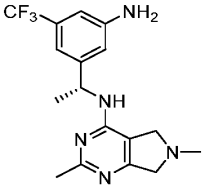
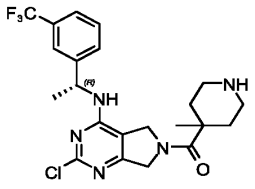
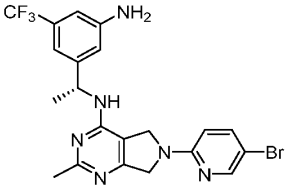
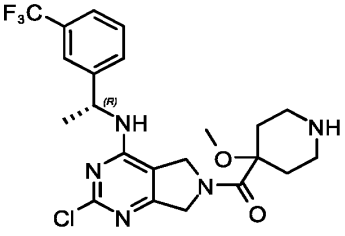
	
	
	
	
	
	
	

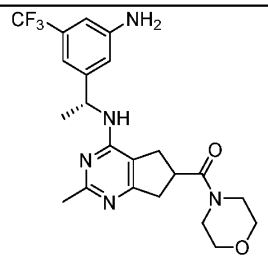
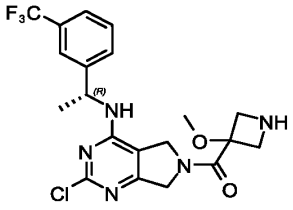
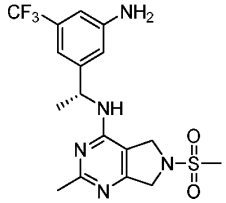
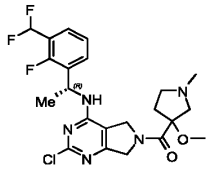
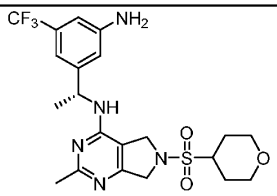
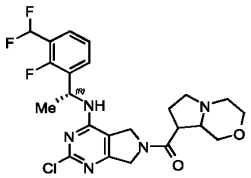
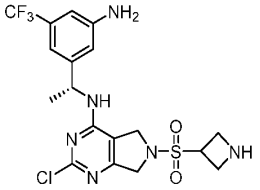
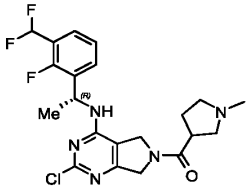
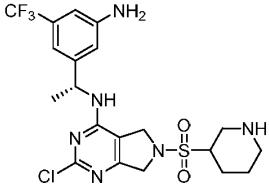
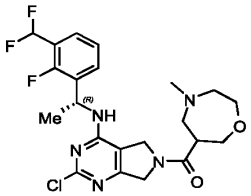
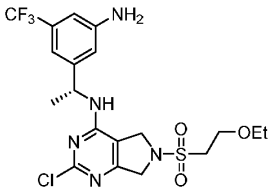
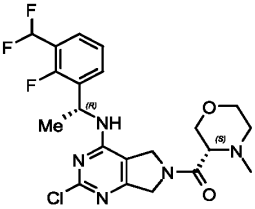
	
	
	
	
	
	
	

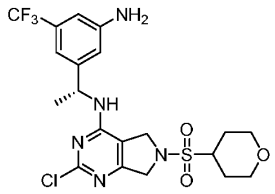
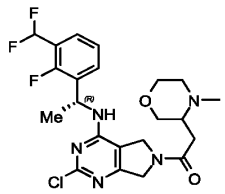
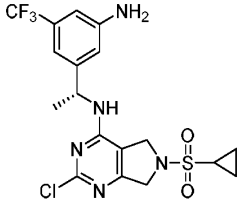
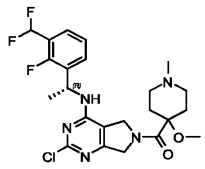
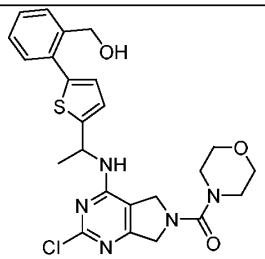
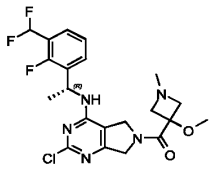
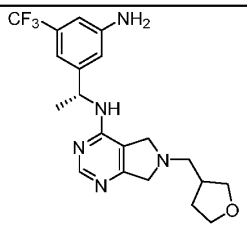
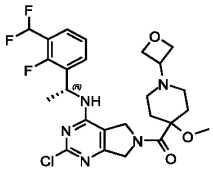
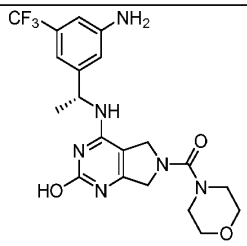
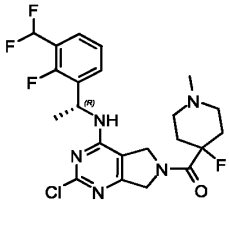
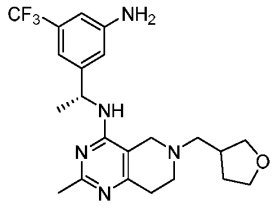
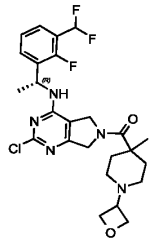
	
	
	
	
	
	

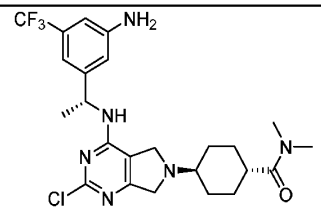
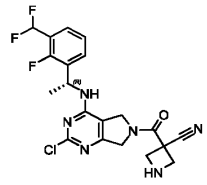
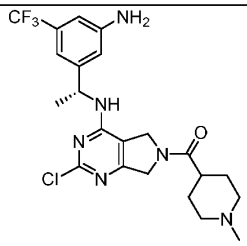
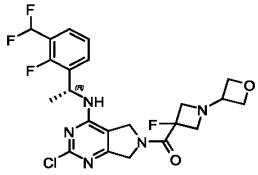
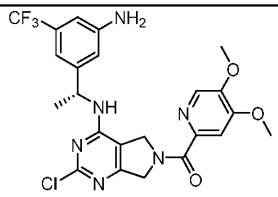
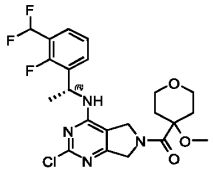
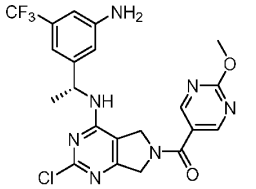
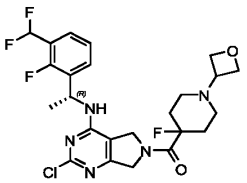
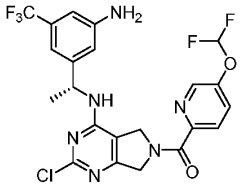
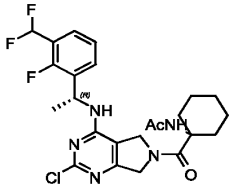
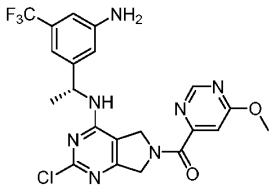
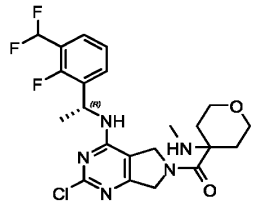
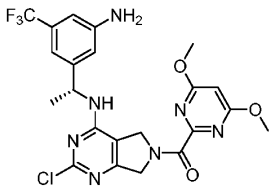
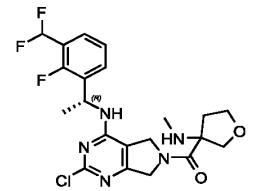
	
	
	
	
	

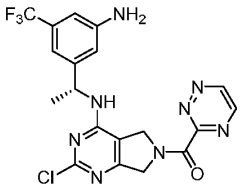
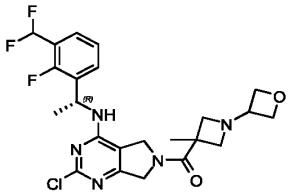
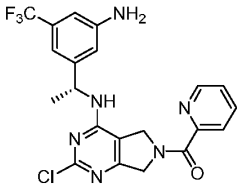
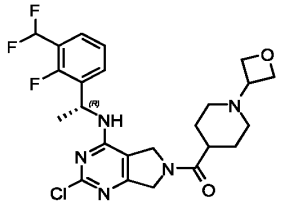
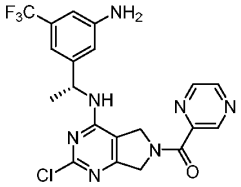
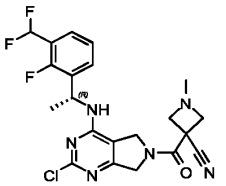
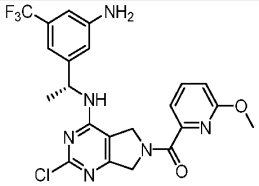
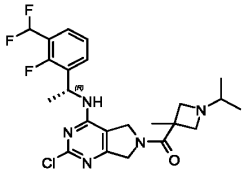
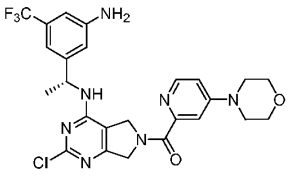
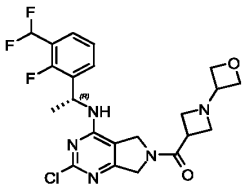
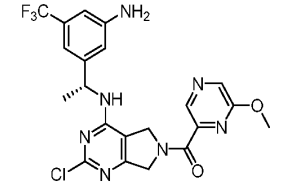
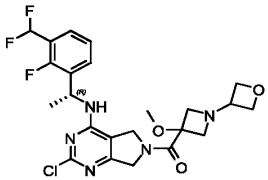
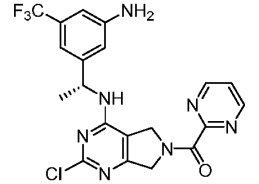
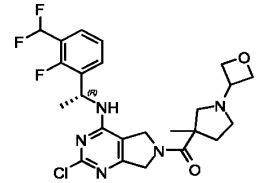
	
	
	
	
	
	

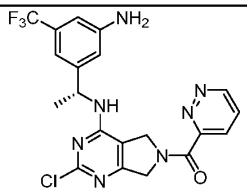
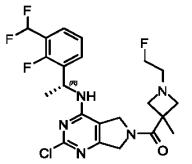
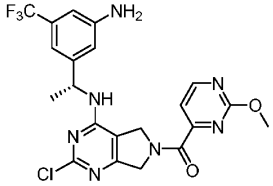
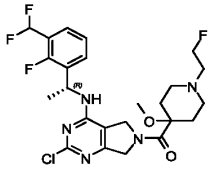
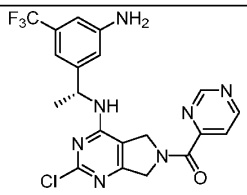
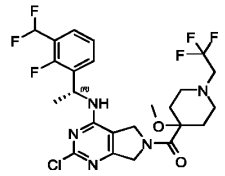
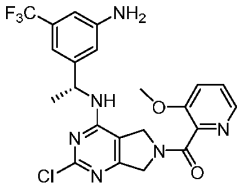
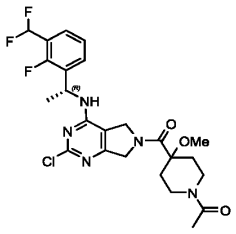
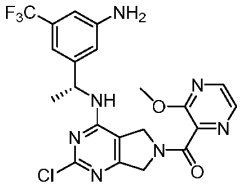
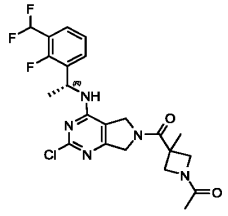
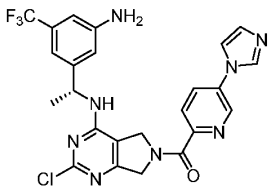
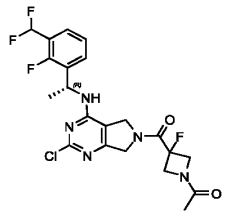
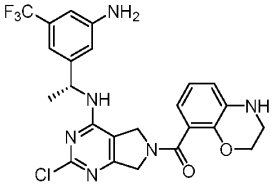
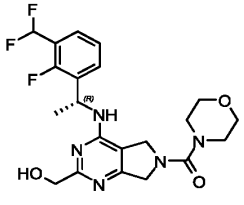
	
	
	
	
	
	

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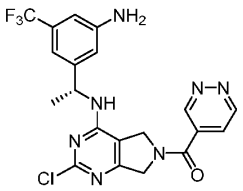	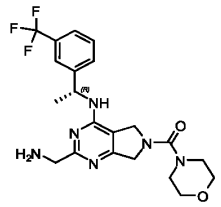
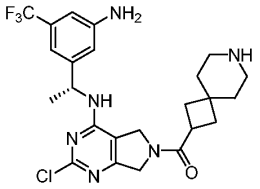	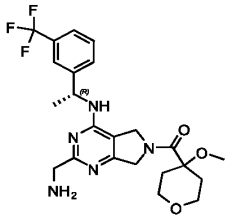
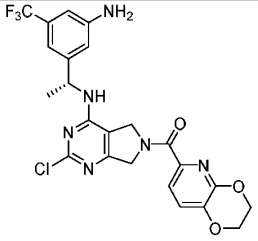	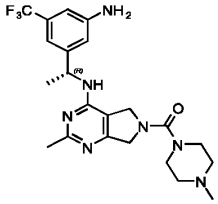
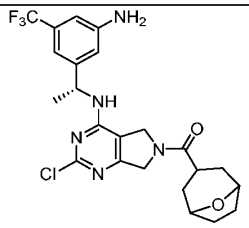	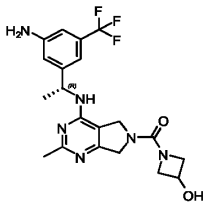
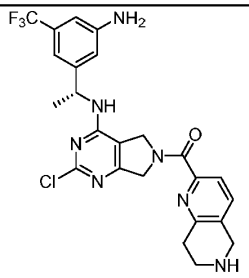	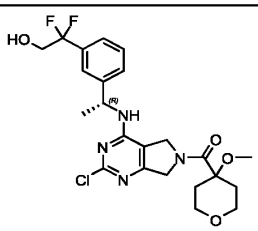
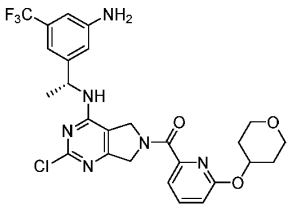	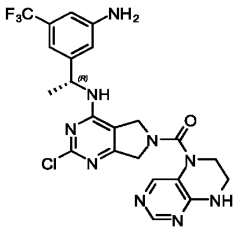

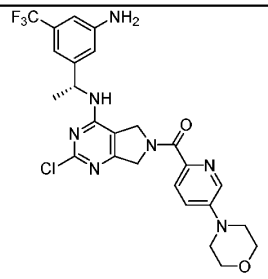	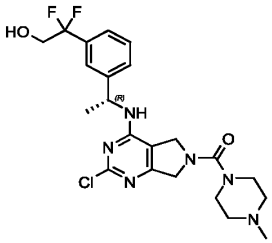
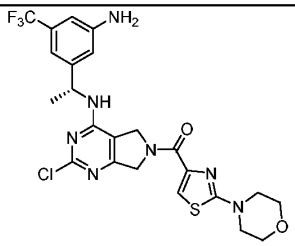	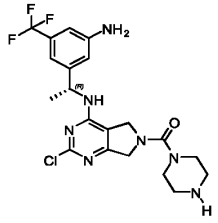
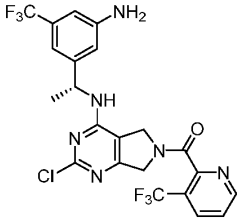	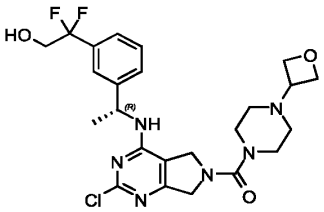
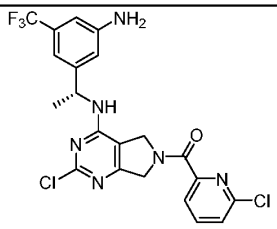	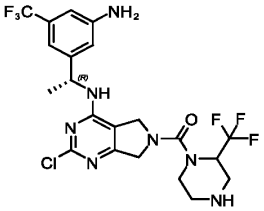
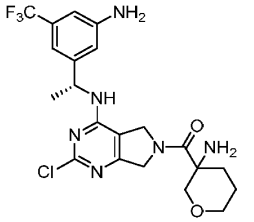	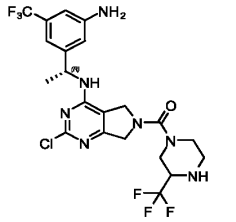
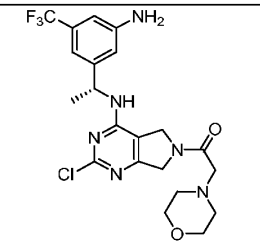	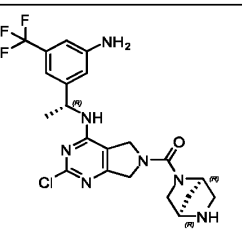

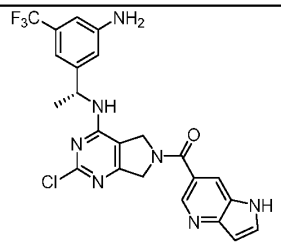	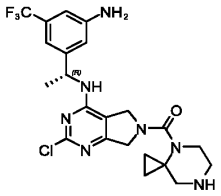
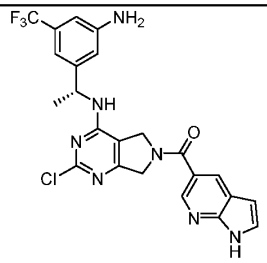	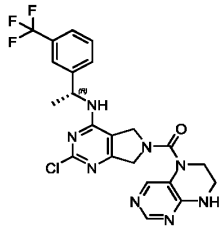
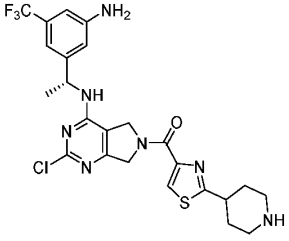	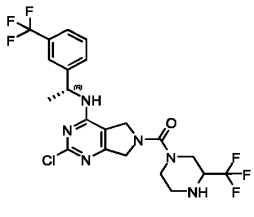
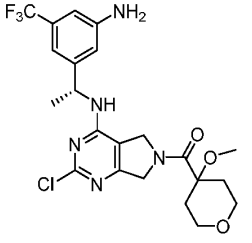	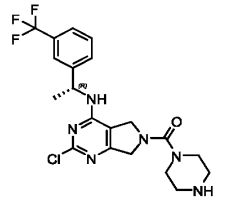
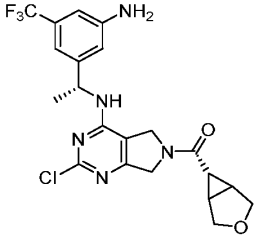	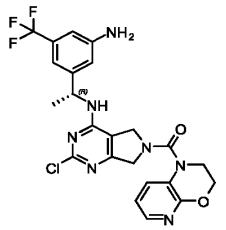
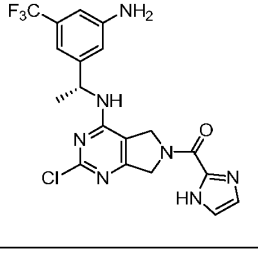	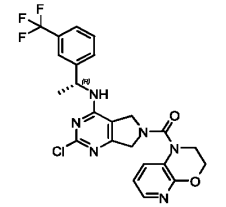

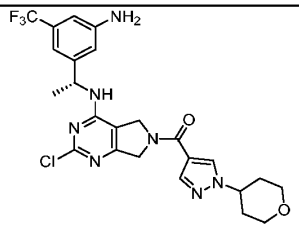	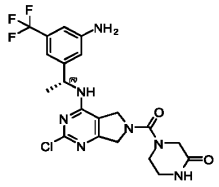
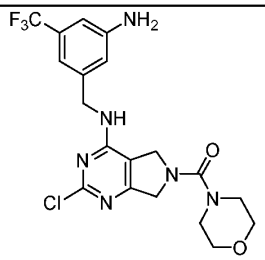	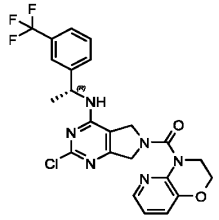
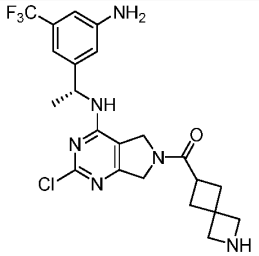	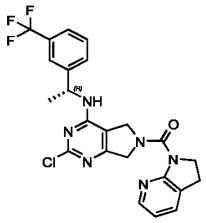
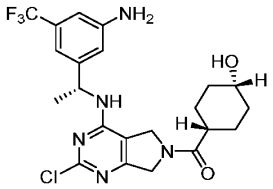	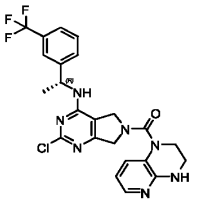
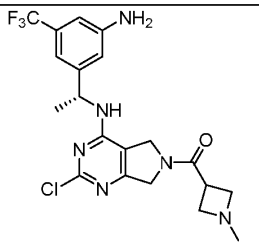	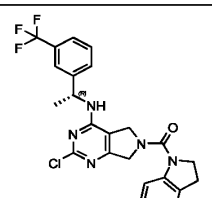
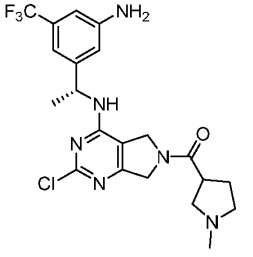	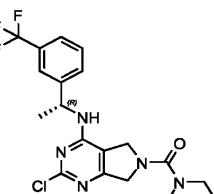

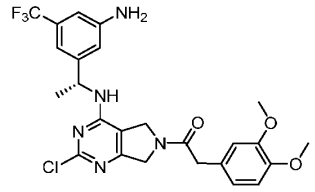	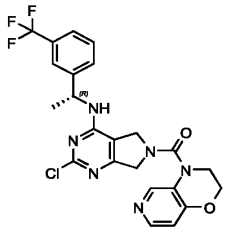
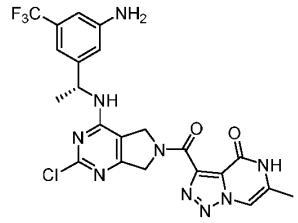	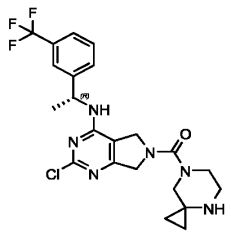
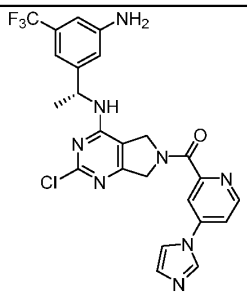	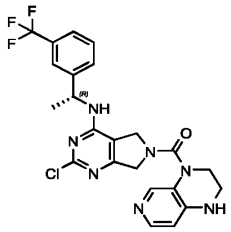
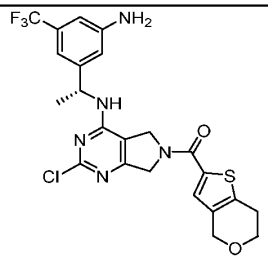	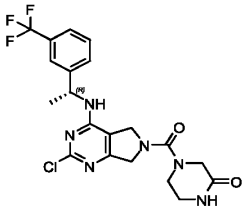
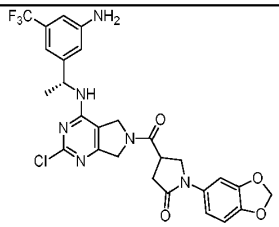	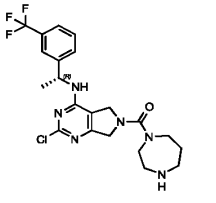
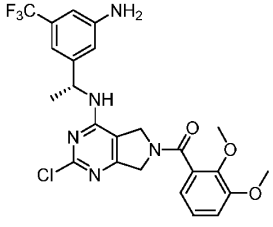	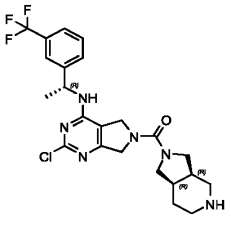

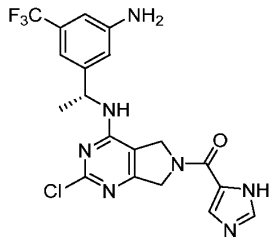	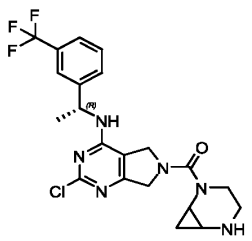
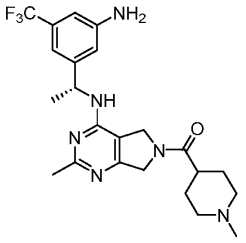	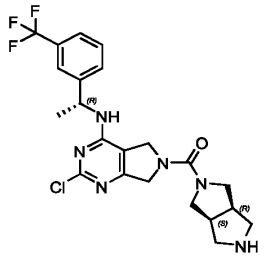
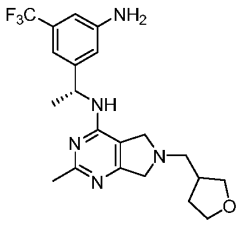	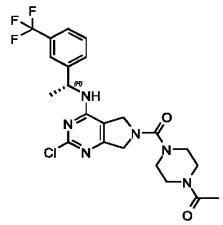
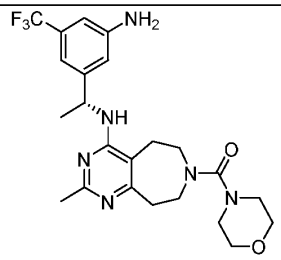	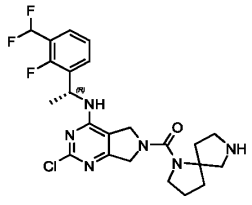
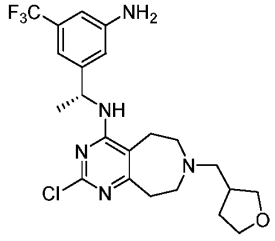	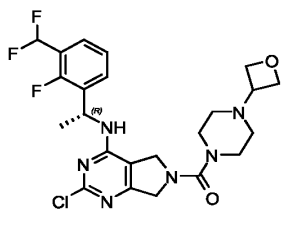

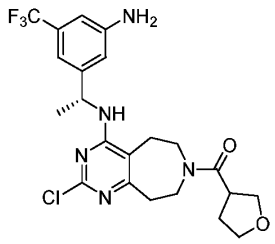	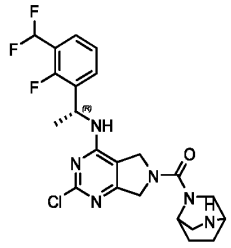
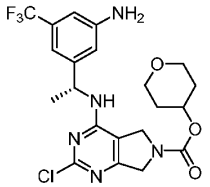	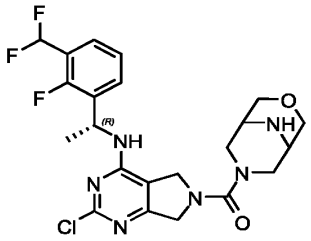
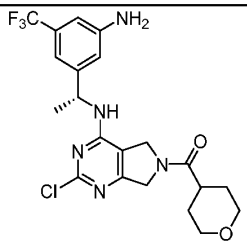	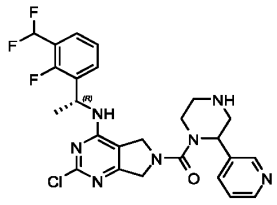
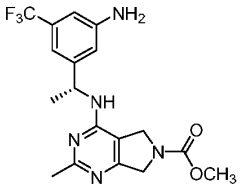	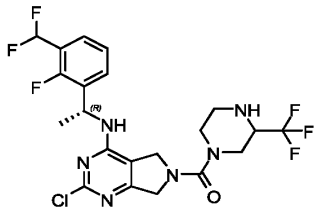
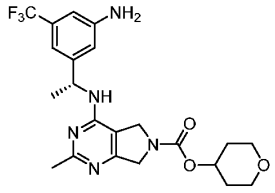	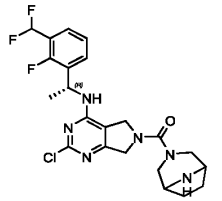
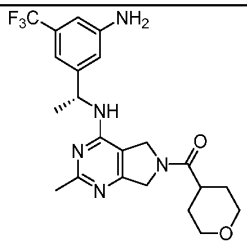	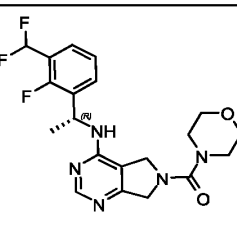

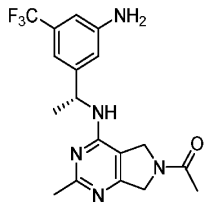	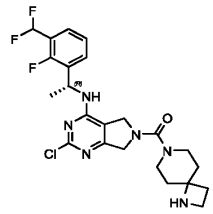
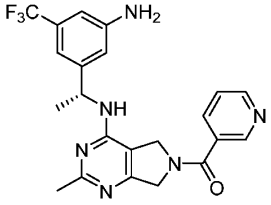	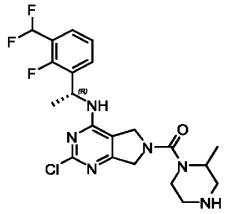
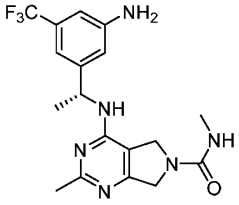	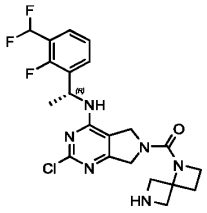
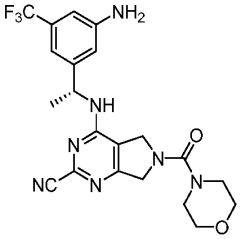	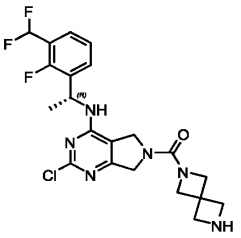
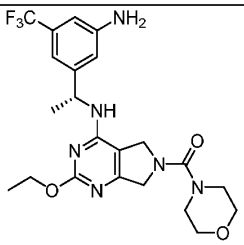	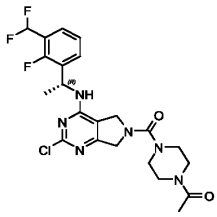
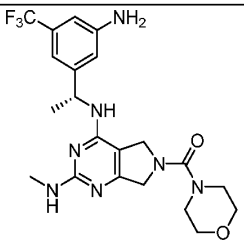	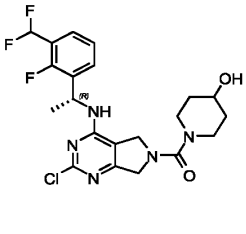

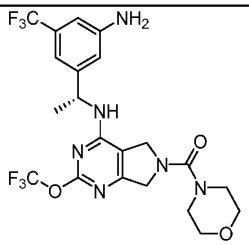	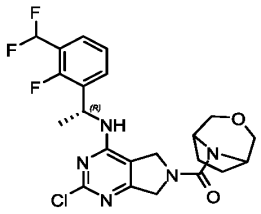
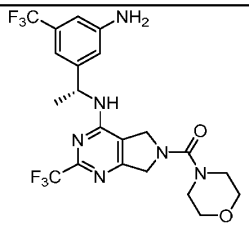	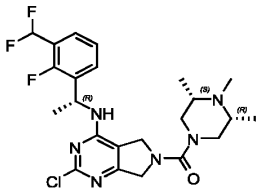
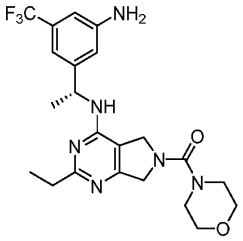	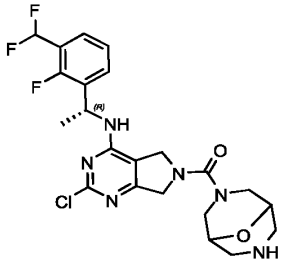
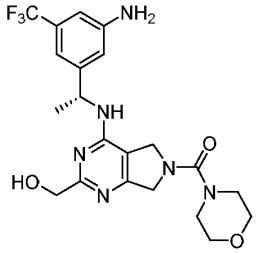	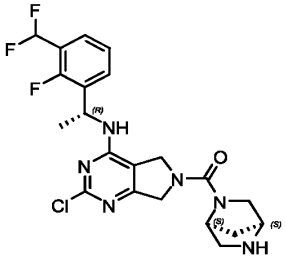
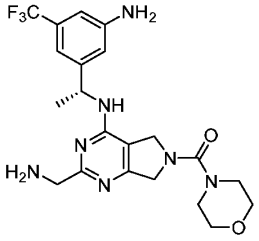	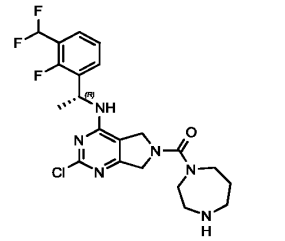

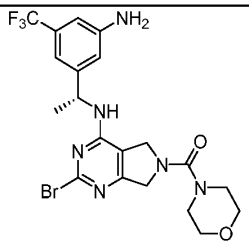	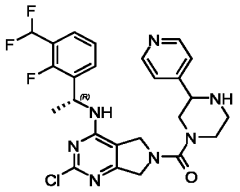
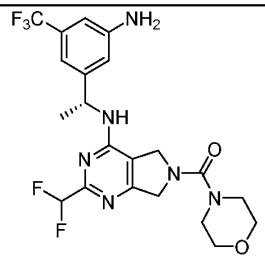	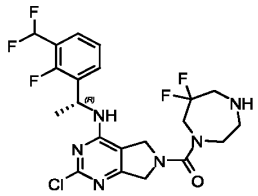
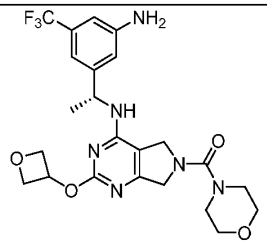	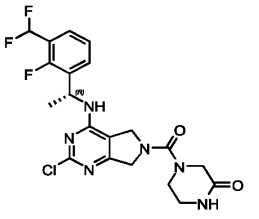
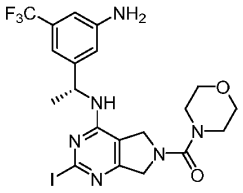	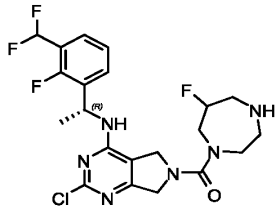
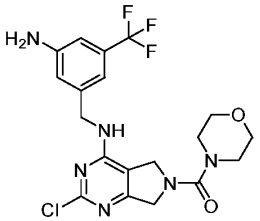	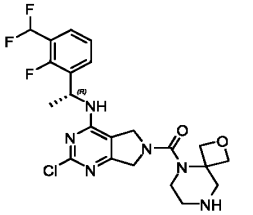
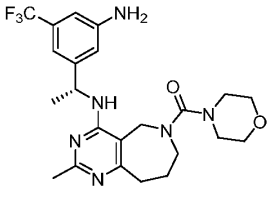	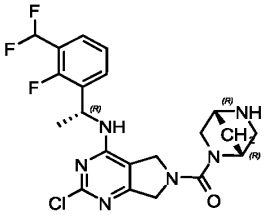

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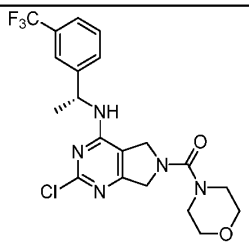
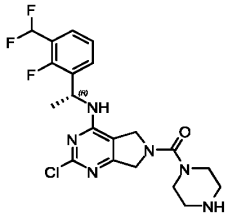
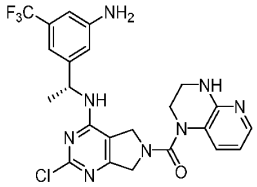
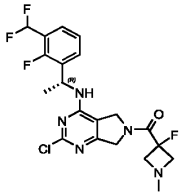
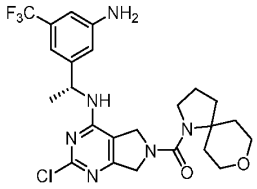
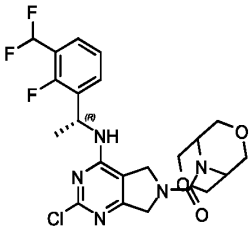
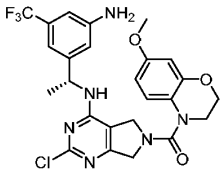
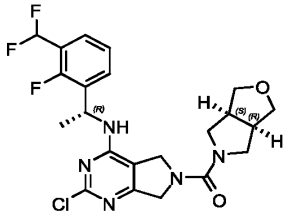
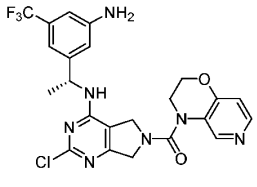
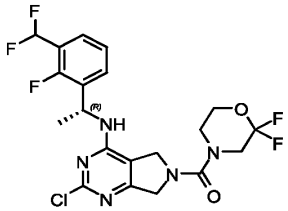
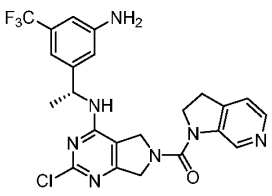
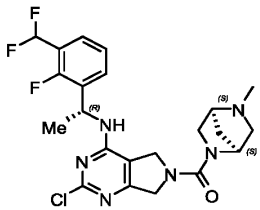
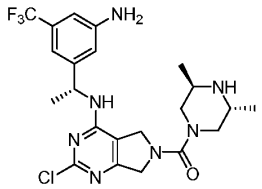
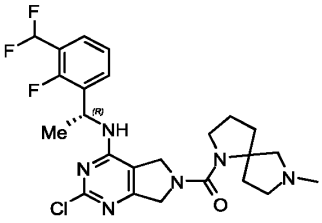
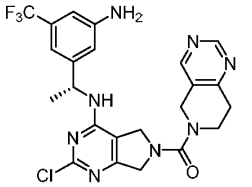
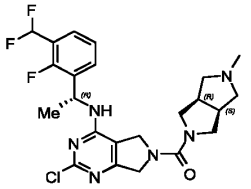
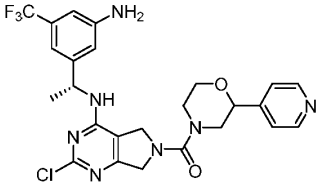
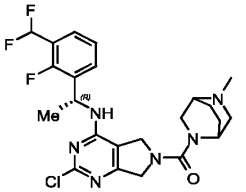
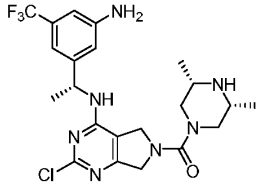
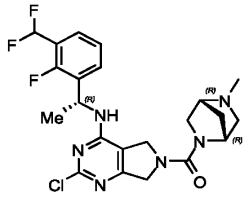
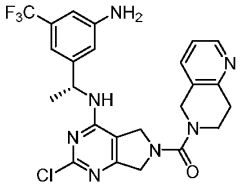
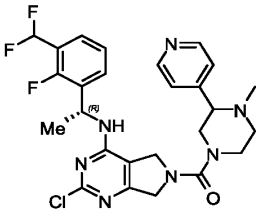
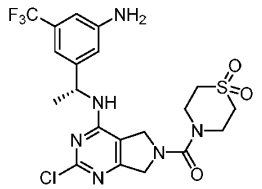
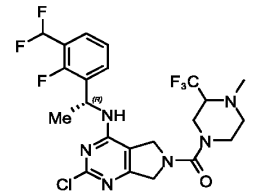
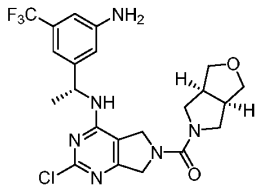
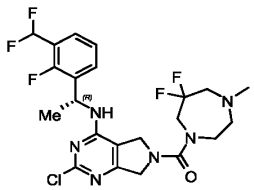
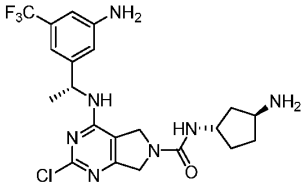
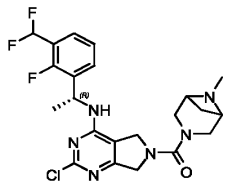
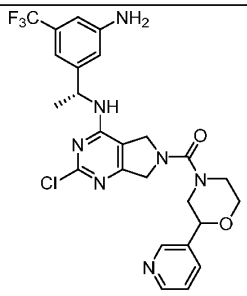
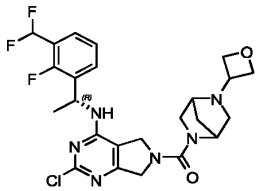
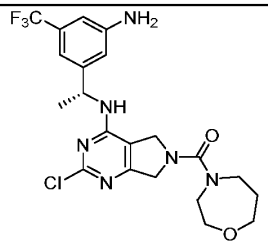
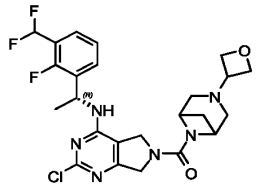
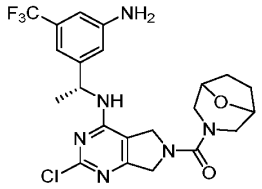
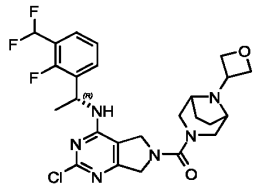
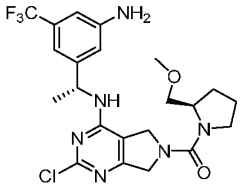
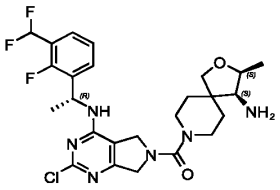
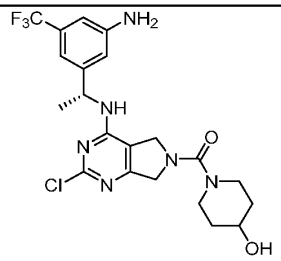
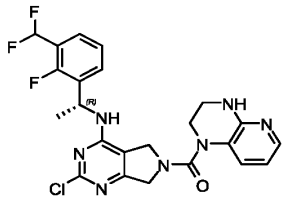
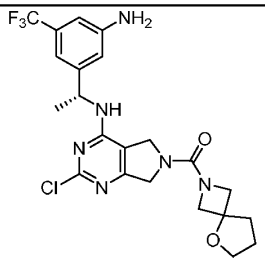
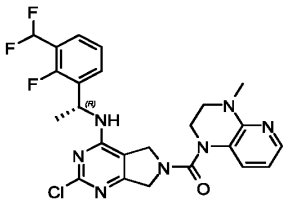
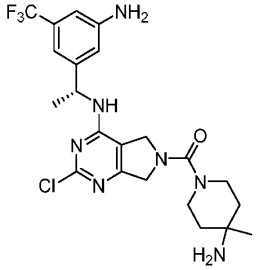
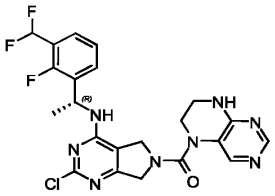
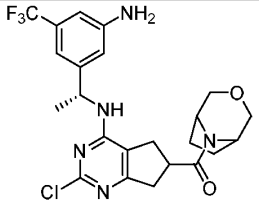
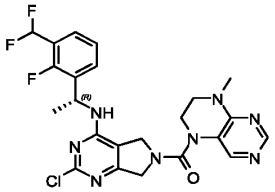
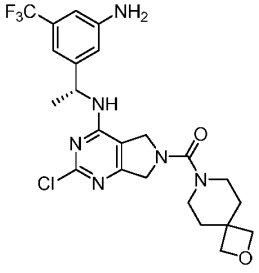
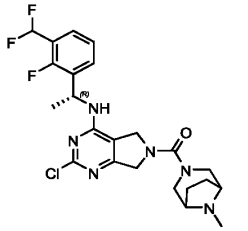
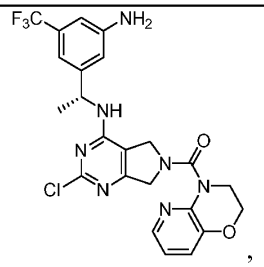
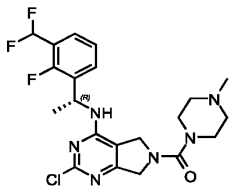
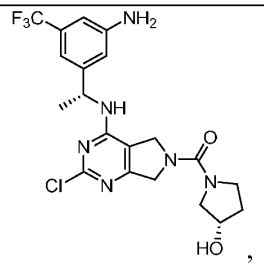
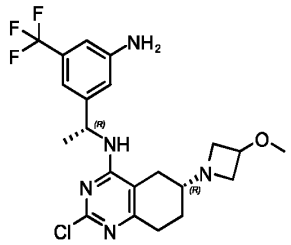
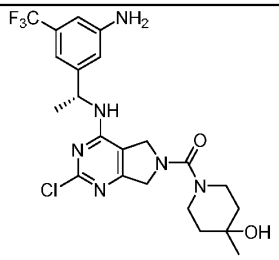
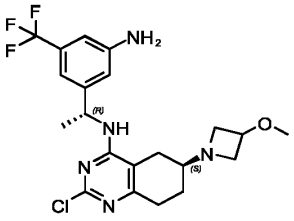
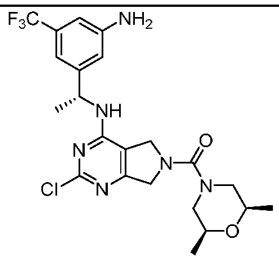
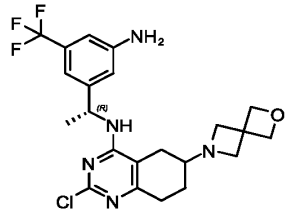
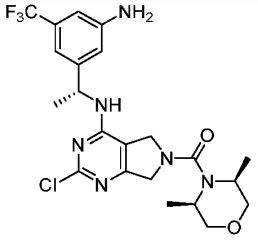
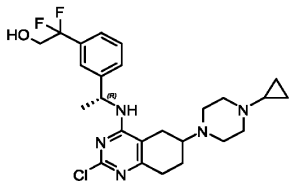
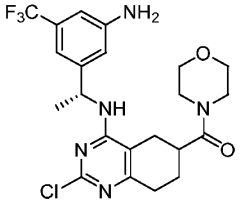
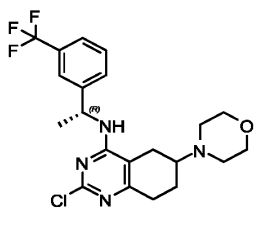
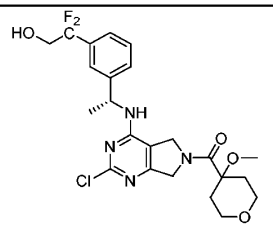
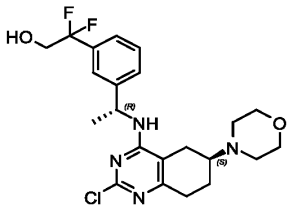
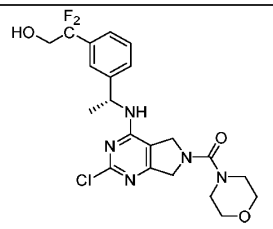
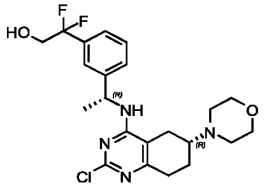
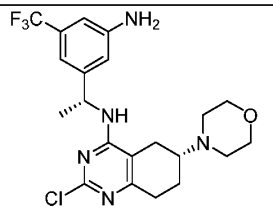
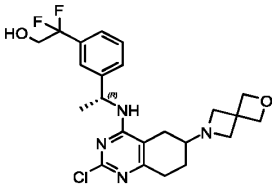
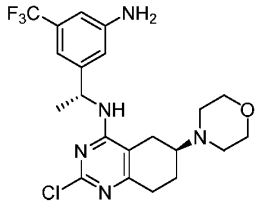
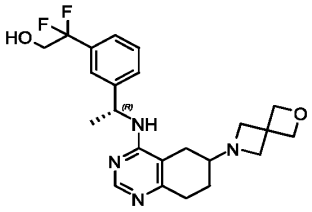
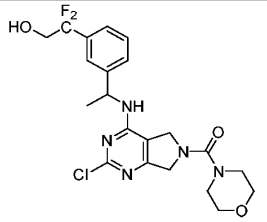
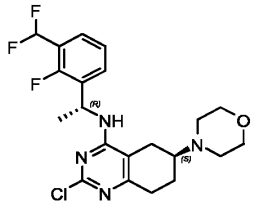
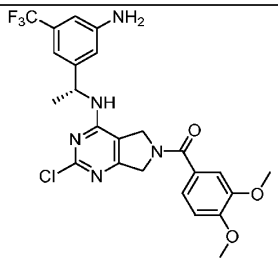
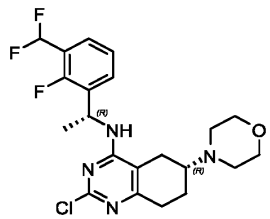
	
	
	
	
	
	

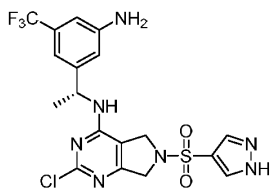
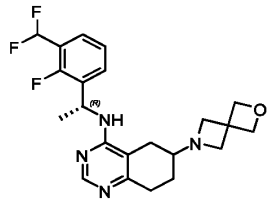
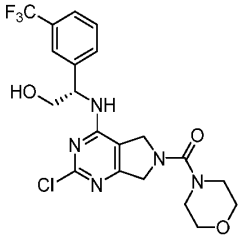
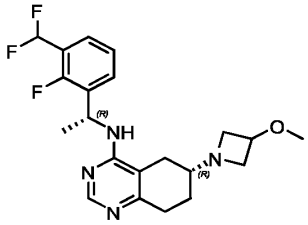
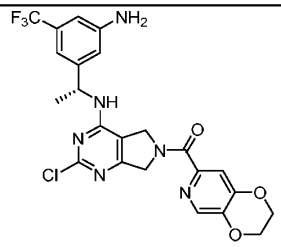
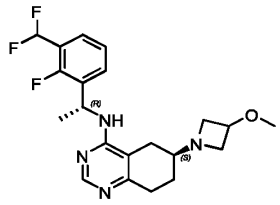
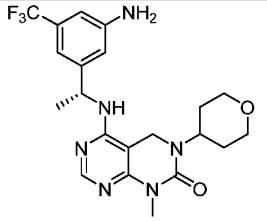
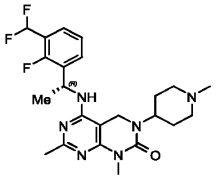
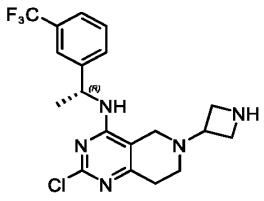
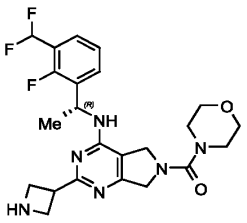
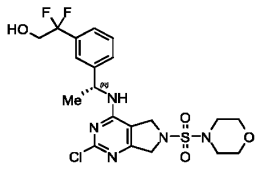
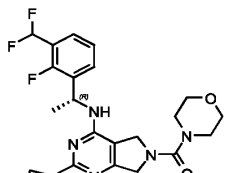
	
	
	
	
	
	

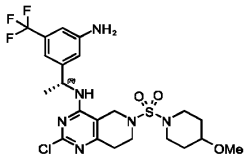
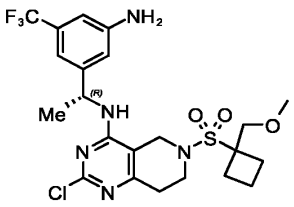
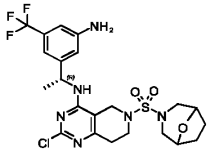
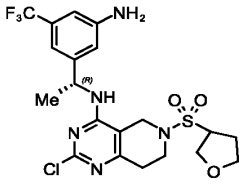
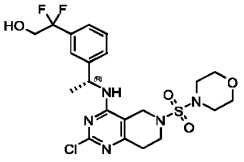
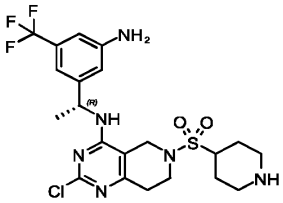
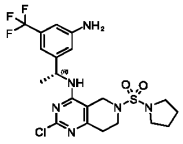
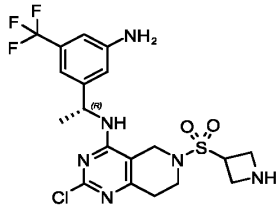
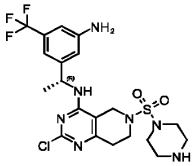
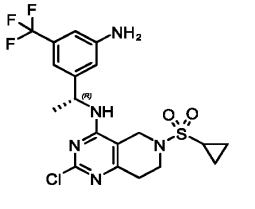
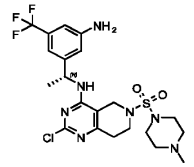
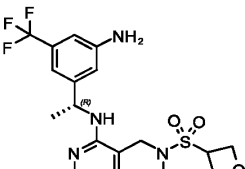
	
	
	
	
	
	

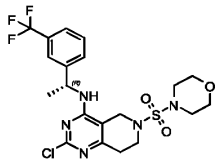
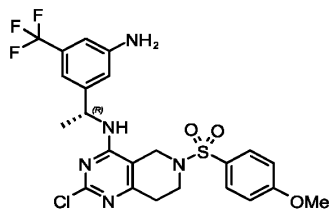
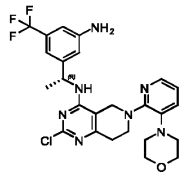
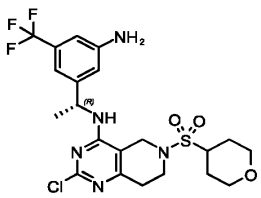
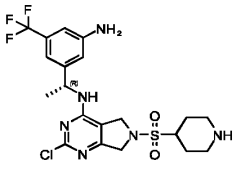
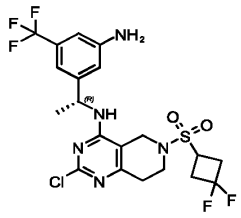
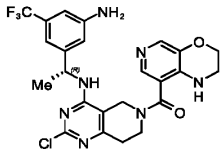
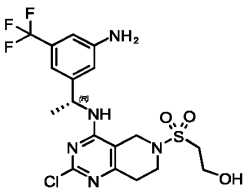
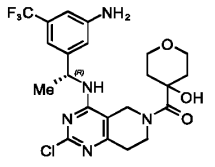
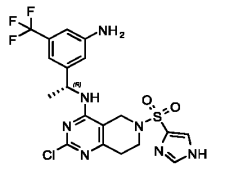
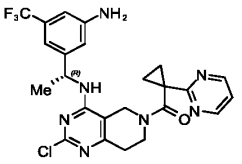
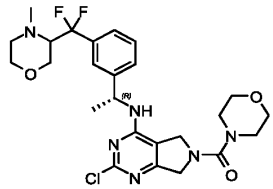
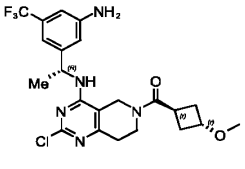
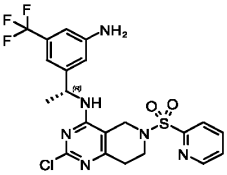
	
	
	
	
	

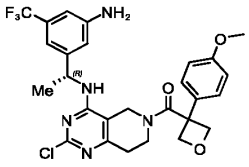
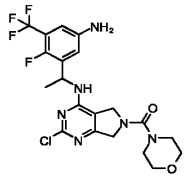
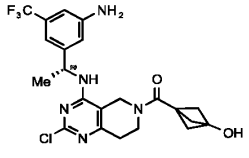
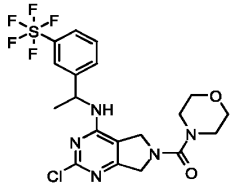
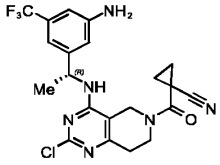
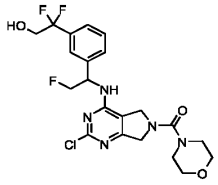
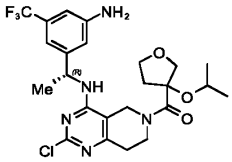
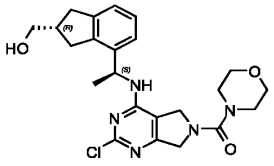
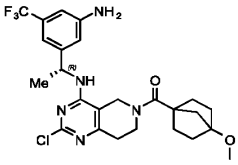
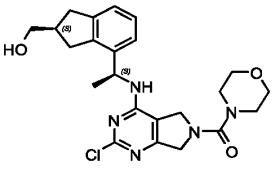
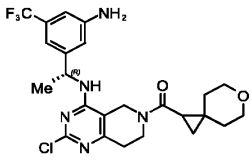
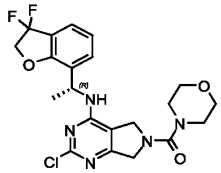
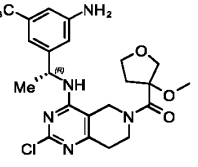
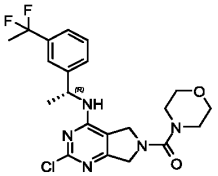
 ,	
 ,	
	
	
	
	

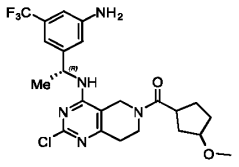
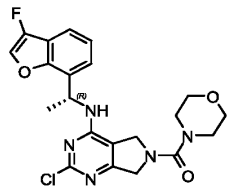
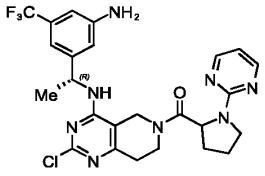
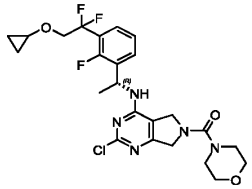
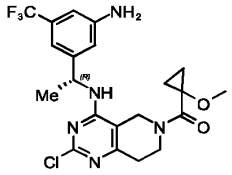
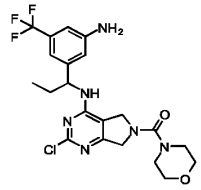
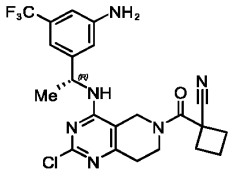
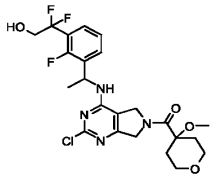
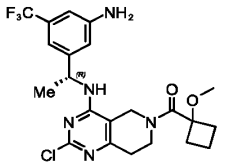
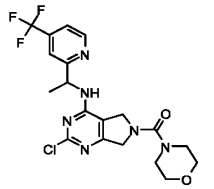
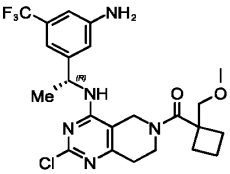
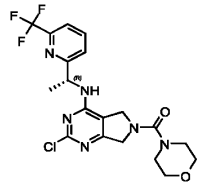
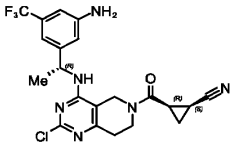
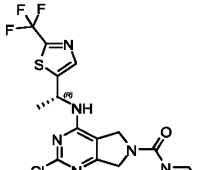
	
	
	
	
	
	

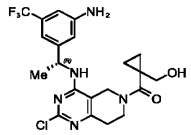
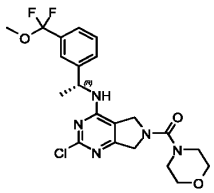
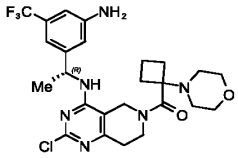
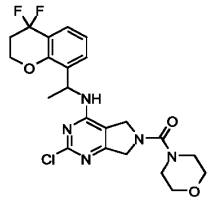
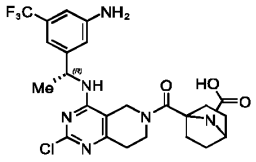
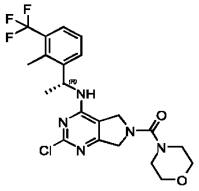
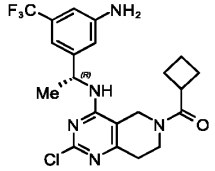
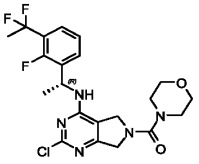
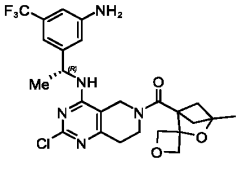
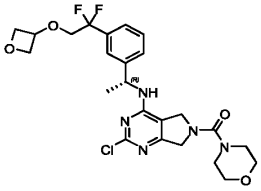
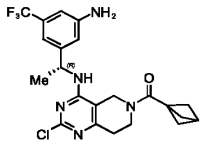
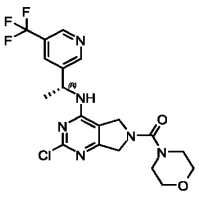
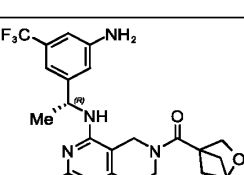
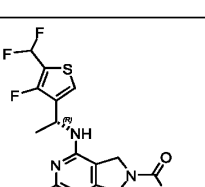
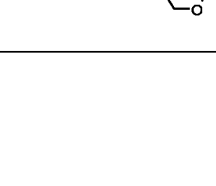
	
	
	
	
	
	

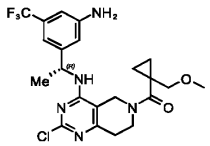
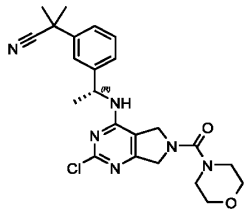
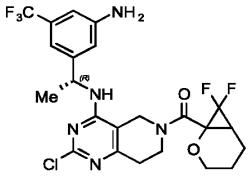
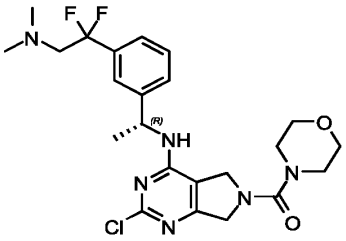
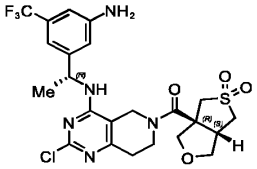
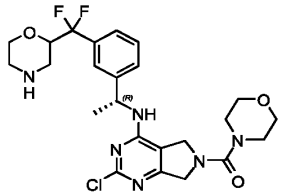
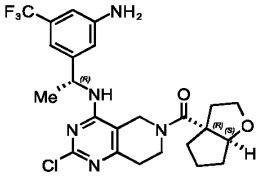
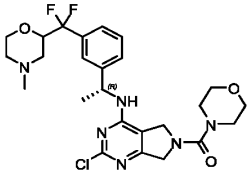
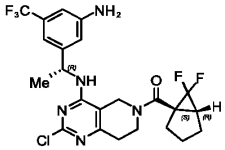
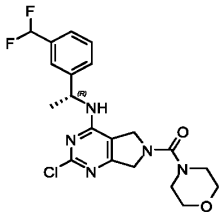
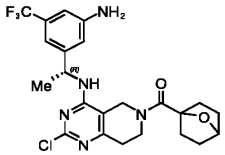
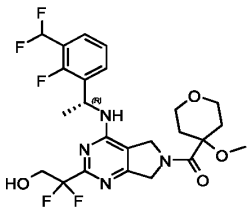
	
	
	
	
	
	

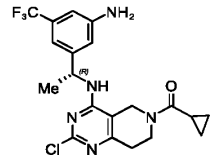
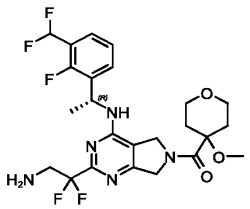
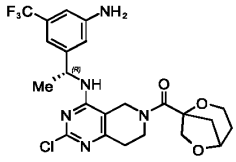
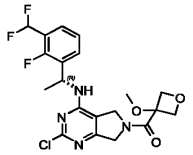
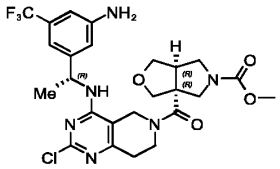
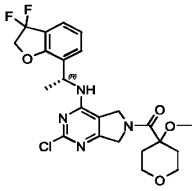
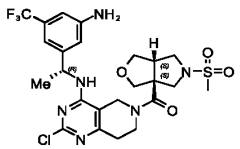
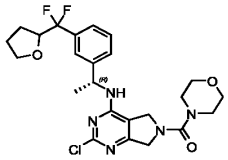
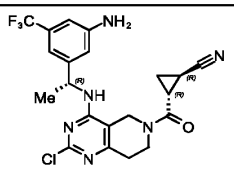
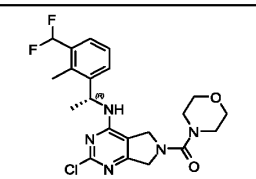
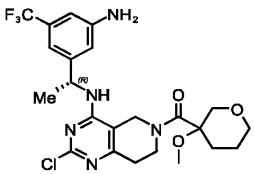
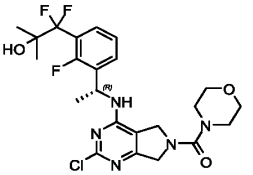
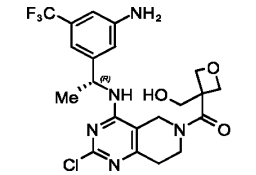
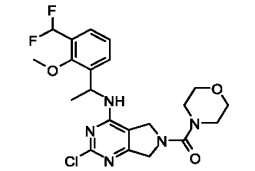
	
	
	
	
	
	
	

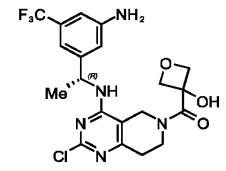
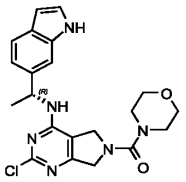
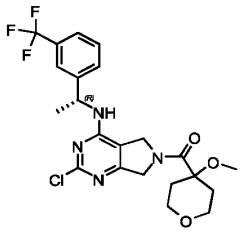
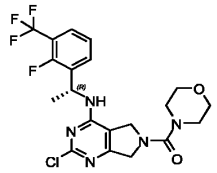
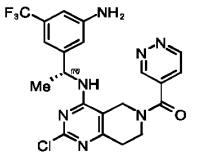
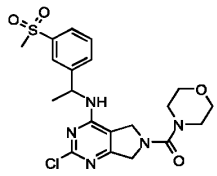
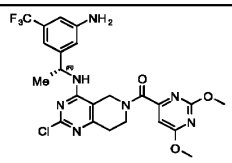
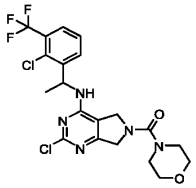
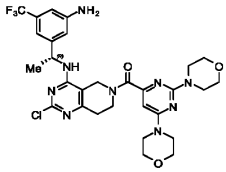
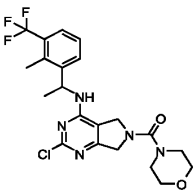
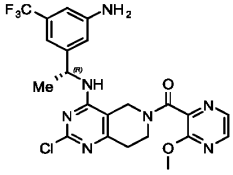
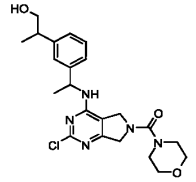
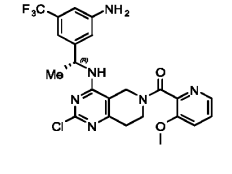
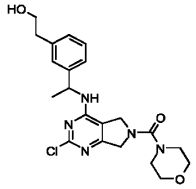
	
	
	
	
	
	
	

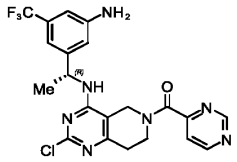
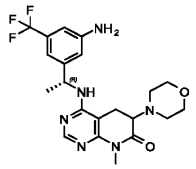
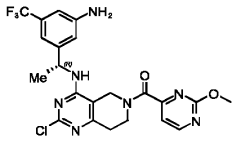
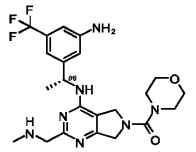
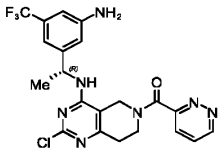
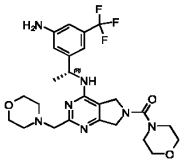
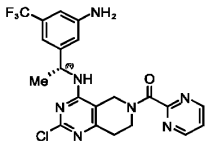
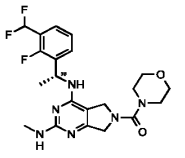
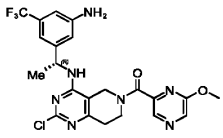
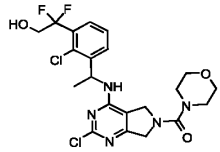
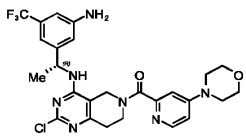
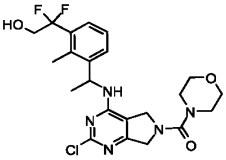
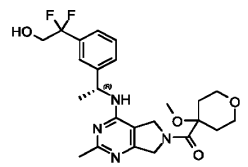
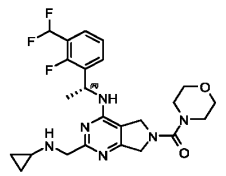
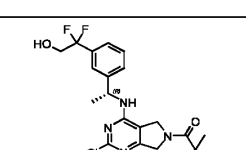
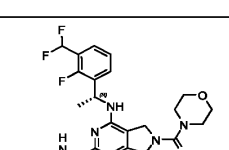
	
	
	
	
	
	
	

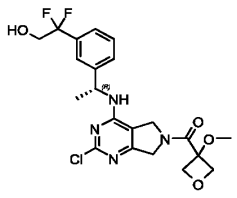
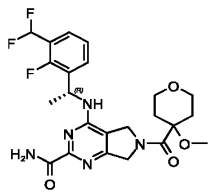
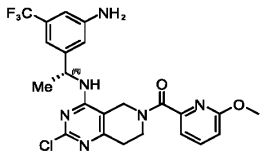
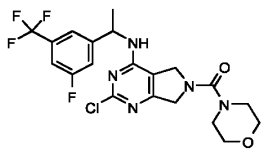
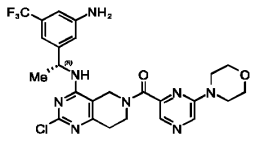
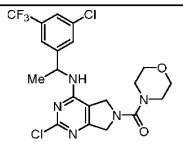
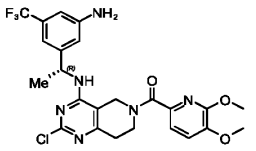
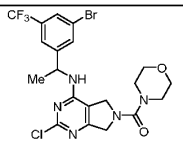
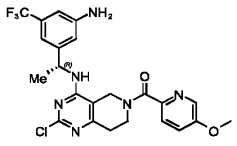
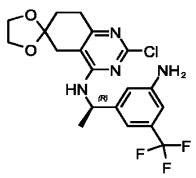
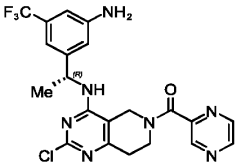
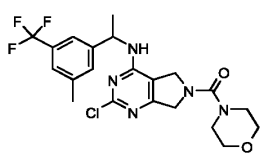
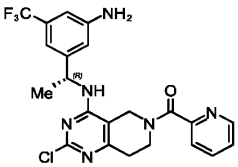
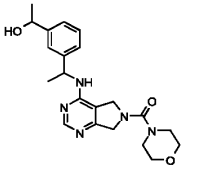
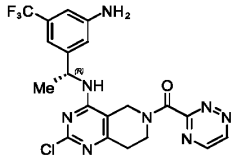
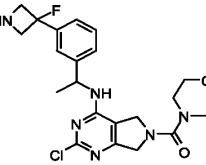
	
	
	
	
	
	
	
	

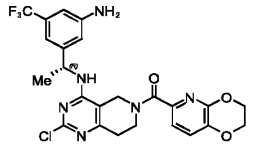
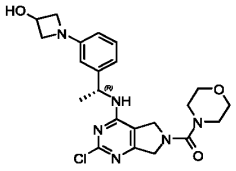
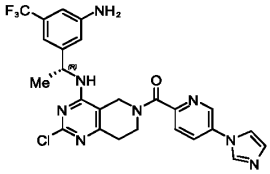
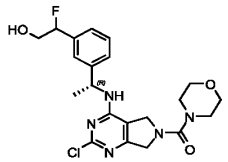
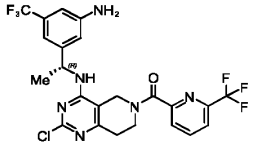
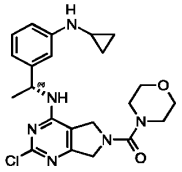
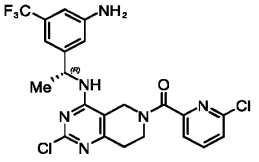
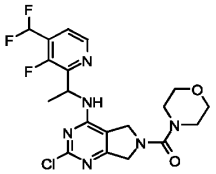
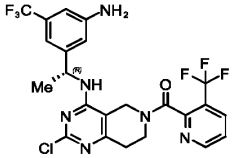
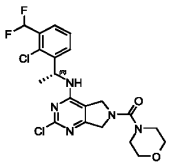
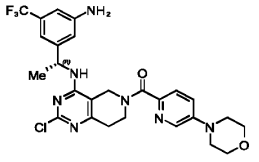
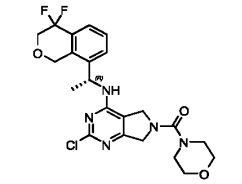
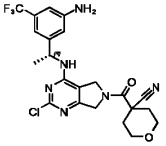
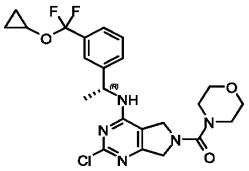
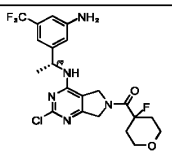
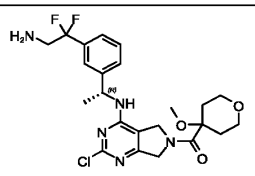
	
	
	
	
	
	

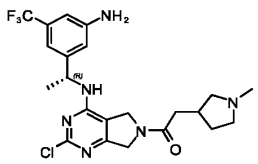
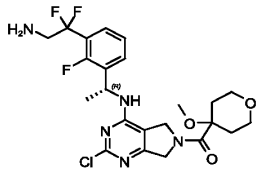
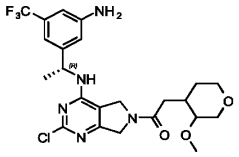
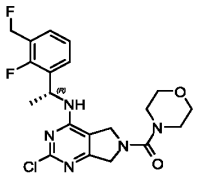
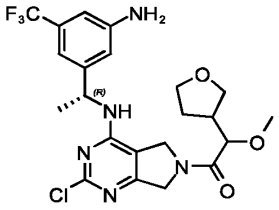
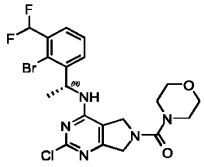
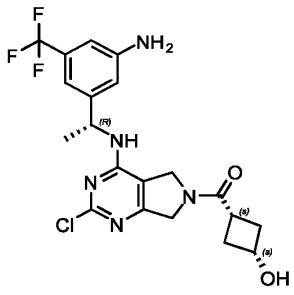
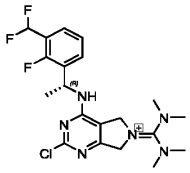
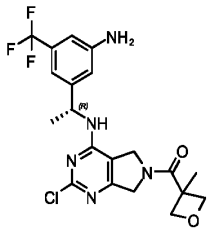
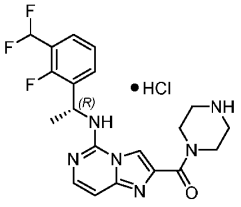
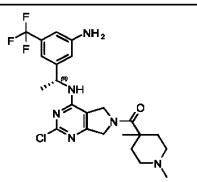
	
	
	
	
	
	
	

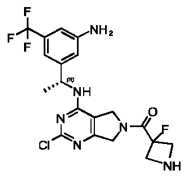
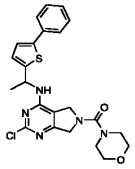
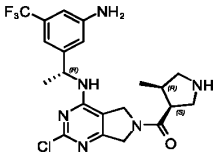
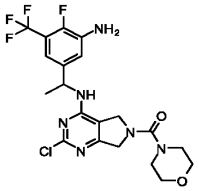
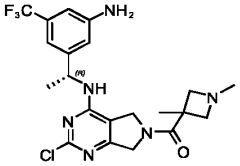
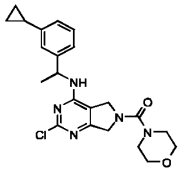
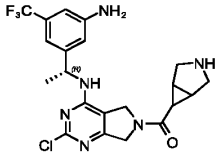
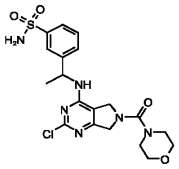
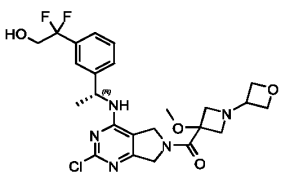
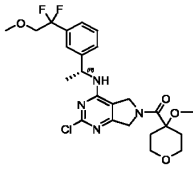
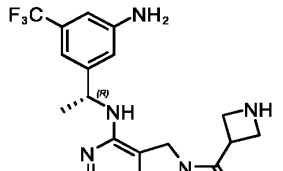
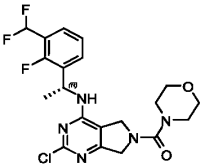
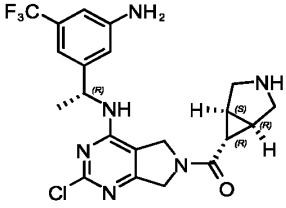
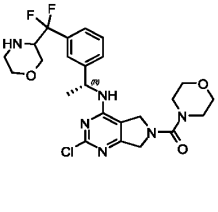
	
	
	
	
	
	
	

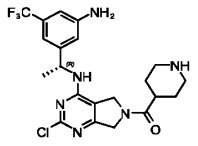
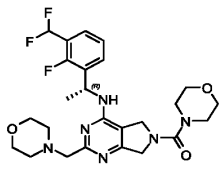
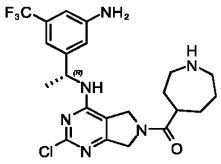
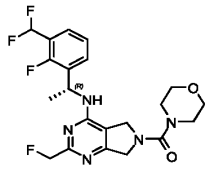
	
	
	
	
	
	
	
	

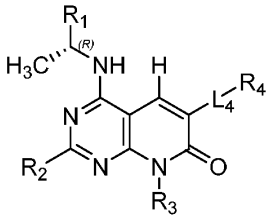
	
	
	
	
	
	
	
	

[0103] In some embodiments, the SOS1 inhibitor is a compound having the structure of Formula (48-I),



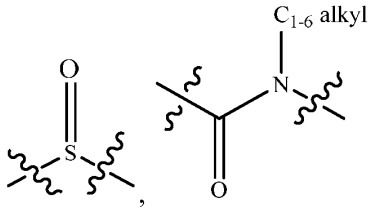
(48-I)

or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof, wherein:

R_1 is selected from the group consisting of optionally substituted 3-6 membered cycloalkyl, optionally substituted 3-6 membered heterocyclyl, optionally substituted 6-membered aryl, and optionally substituted 5-6 membered heteroaryl;

R_2 is selected from the group consisting of H, C_{1-6} alkyl, halogen, -NHR_{2a}, –OR_{2a}, cyclopropyl, and –CN; wherein C_{1-6} alkyl is optionally substituted with halogen, -NHR_{2a}, –OR_{2a}, or 5-6 membered heterocyclyl, and further wherein R_{2a} is selected from the group consisting of H, C_{1-6} alkyl, 3-6 membered heterocyclyl, and C_{1-6} haloalkyl;

R_3 is selected from the group consisting of H, C_{1-3} alkyl, –OR_{3a}, cyclopropyl, and 3-6 membered heterocyclyl, wherein each of C_{1-3} alkyl, cyclopropyl, and 3-6 membered heterocyclyl is optionally substituted with R_{3a}, and further wherein R_{3a} is selected from the group consisting of C_{1-3} alkyl, halogen, –OH, and –CN;

L_4 is selected from the group consisting of bond, –C(O)–, –C(O)O–, –C(O)NH$(CH_2)_o$–, –NH–, –S–, –S(O)$_2$–,  , –$(CH_2)_p$–, and –O–; wherein o is 0, 1, or 2; and wherein p is a number from 1 to 6; and

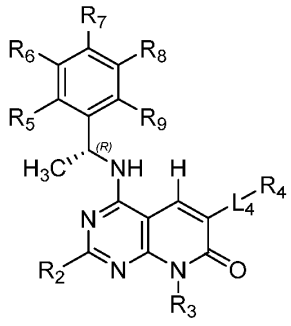
R_4 is selected from the group consisting of H, C_{1-6} alkyl, 3-14 membered cycloalkyl, 3-14 membered cycloalkenyl, 3-14 membered heterocyclyl, 6-10 membered aryl, and 5-10 membered heteroaryl; wherein each C_{1-6} alkyl, 3-14 membered cycloalkyl, 3-14 membered cycloalkenyl, 3-14 membered heterocyclyl, 6-10 membered aryl, and 5-10 membered heteroaryl is optionally substituted with C_{1-6} alkyl, –R_{4a}, –OR_{4a}, –O–C_{1-6} alkyl–R_{4a}, =O, halogen, –C(O)R_{4a}, –C(OO)R_{4a}, –C(O)$NR_{4b}R_{4c}$, –NR_{4b}C(O)R_{4c}, –CN, =NR_{4a}, –$NR_{4b}R_{4c}$, –SO_2R_{4a}, 3-6 membered cycloalkyl optionally substituted with R_{4a}, 3-7 membered heterocyclyl optionally substituted with R_{4a}, 6-10 membered aryl optionally substituted with R_{4a}, or 5-10 membered heteroaryl optionally substituted with R_{4a};

wherein R_{4a} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, –C(O)R_{4b}, –C(O)$NR_{4b}R_{4c}$, =O, 3-6 membered cycloalkyl, 6-10 membered aryl optionally substituted with –OR_{4b}, –CN, =N-3-6 membered cycloalkyl, 3-7 membered heterocyclyl, –$(CH_2)_rOCH_3$, or –$(CH_2)_rOH$, wherein r is 1, 2, or 3;

wherein each R_{4b} is independently H, C_{1-6} alkyl; and

wherein each R_{4c} is independently H or C_{1-6} alkyl.

[0104] In some embodiments, the SOS1 inhibitor is a compound having the structure of Formula (48-II),



(48-II)

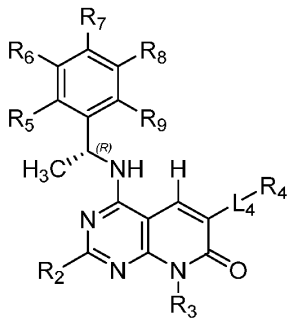
or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof, wherein R_2, R_3, L_4, and R_4 are as defined in Formula (48-I);

R_5, R_6, R_7, R_8, and R_9 are independently selected from the group consisting of H, D, C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, 3-14 membered heterocyclyl, –OH, halogen, $–NO_2$, –CN, $–NR_{11}R_{12}$, $–SR_{10}$, $–S(O)_2NR_{11}R_{12}$, $–S(O)_2R_{10}$, $–NR_{10}S(O)_2NR_{11}R_{12}$, $–NR_{10}S(O)_2R_{11}$, $–S(O)NR_{11}R_{12}$, $–S(O)R_{10}$, $–NR_{10}S(O)NR_{11}R_{12}$, $–NR_{10}S(O)R_{11}$, $–C(O)R_{10}$, $–CO_2R_{10}$, 6-10 membered aryl, and 5-10 membered heteroaryl, wherein each C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, 3-14 membered heterocyclyl, 6-10 membered aryl, and 5-10 membered heteroaryl is optionally substituted with –OH, C_{1-6} alkyl, halogen, $–NO_2$, oxo, –CN, $–R_{10}$, $–OR_{10}$, $–NR_{11}R_{12}$, $–SR_{10}$, $–S(O)_2NR_{11}R_{12}$, $–S(O)_2R_{10}$, $–NR_{10}S(O)_2NR_{11}R_{12}$, $–NR_{10}S(O)_2R_{11}$, $–S(O)NR_{11}R_{12}$, $–S(O)R_{10}$, $–NR_{10}S(O)NR_{11}R_{12}$, $–NR_{10}S(O)R_{11}$, 3-8 membered cycloalkyl, 3-14 membered heterocyclyl, 6-10 membered aryl, or 5-10 membered heteroaryl, or any two adjacent R^5, R^6, R^7, R^8, and R^9 forms an optionally substituted 3-14 membered fused ring;

R_{10}, R_{11}, and R_{12} are at each occurrence independently selected from H, D, C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, 3-14 membered heterocyclyl, $–OR_{13}$, $–SR_{13}$, halogen, $–NR_{13}R_{14}$, $–NO_2$, and –CN; and

R_{13} and R_{14} are at each occurrence independently selected from H, D, C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, and 3-14 membered heterocyclyl, wherein each C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, and 3-14 membered heterocyclyl are independently optionally substituted with –OH, –SH, $–NH_2$, $–NO_2$, or –CN.

[0105] In some embodiments, the SOS1 inhibitor is a compound having the structure of Formula (48-II),



(48-II)

or a pharmaceutically acceptable salt or a stereoisomer thereof, wherein:

R_2 is H;

R_3 is selected from the group consisting of H and C_{1-3} alkyl;

L_4 is a bond;

R_4 is selected from the group consisting of H, C_{1-6} alkyl, 3-14 membered cycloalkyl, 3-14 membered cycloalkenyl, 3-14 membered heterocyclyl, 6-10 membered aryl, and 5-10 membered heteroaryl; wherein each C_{1-6} alkyl, 3-14 membered cycloalkyl, 3-14 membered cycloalkenyl, 3-14 membered heterocyclyl, 6-10 membered aryl, and 5-10 membered heteroaryl is optionally substituted with C_{1-6} alkyl, $-R_{4a}$, $-OR_{4a}$, $-O-C_{1-6}$ alkyl$-R_{4a}$, =O, halogen, $-C(O)R_{4a}$, $-C(OO)R_{4a}$, $-C(O)NR_{4b}R_{4c}$, $-NR_{4b}C(O)R_{4c}$, $-CN$, $=NR_{4a}$, $-NR_{4b}R_{4c}$, $-SO_2R_{4a}$, 3-6 membered cycloalkyl optionally substituted with R_{4a}, 3-7 membered heterocyclyl optionally substituted with R_{4a}, 6-10 membered aryl optionally substituted with R_{4a}, or 5-10 membered heteroaryl optionally substituted with R_{4a};

wherein R_{4a} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, $-C(O)R_{4b}$, $-C(O)NR_{4b}R_{4c}$, =O, 3-6 membered cycloalkyl, 6-10 membered aryl optionally substituted with $-OR_{4b}$, $-CN$, =N-3-6 membered cycloalkyl, 3-7 membered heterocyclyl, $-(CH_2)_rOCH_3$, or $-(CH_2)_rOH$, wherein r is 1, 2, or 3;

wherein each R_{4b} is independently H, C_{1-6} alkyl;

wherein each R_{4c} is independently H or C_{1-6} alkyl;

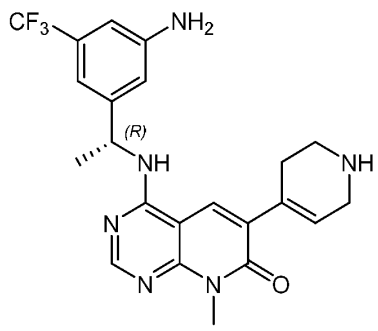
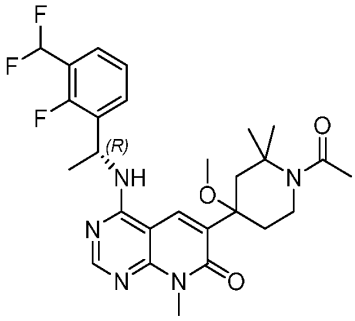
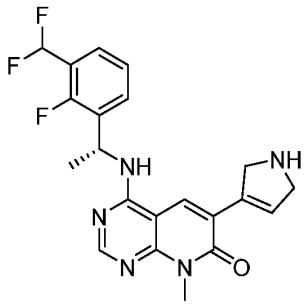
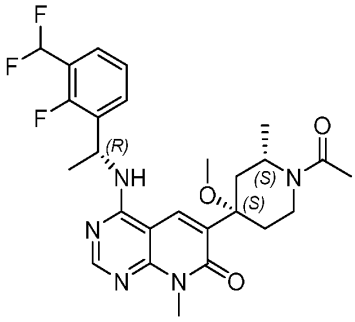
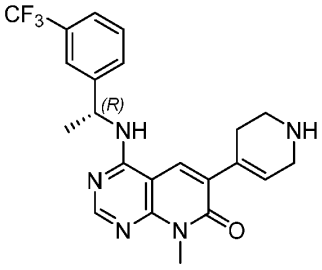
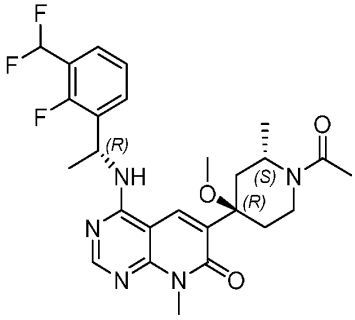
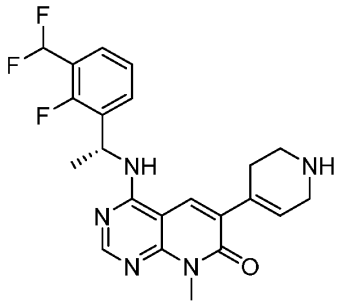
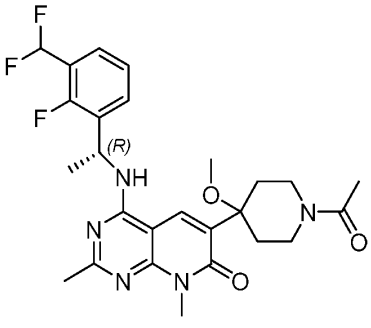
R_5, R_6, R_7, R_8, and R_9 are independently selected from the group consisting of H, D, C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, 3-14 membered heterocyclyl, –OH, halogen, $-NO_2$, $-CN$, $-NR_{11}R_{12}$, $-SR_{10}$, –

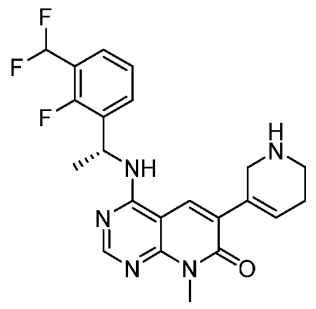
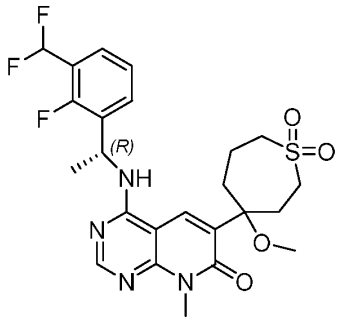
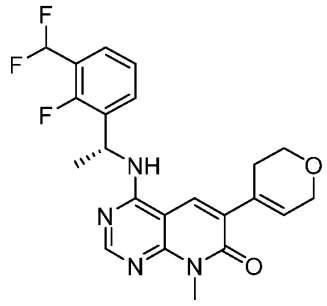
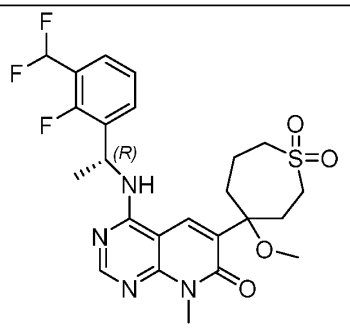
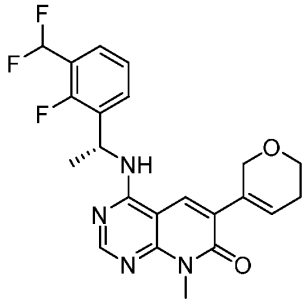
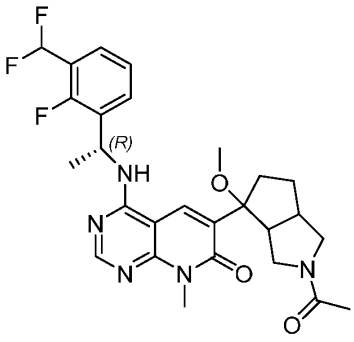
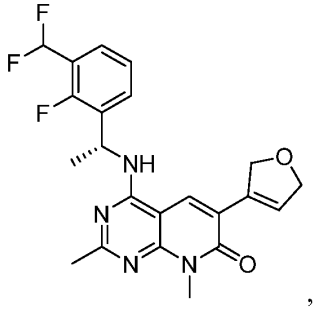
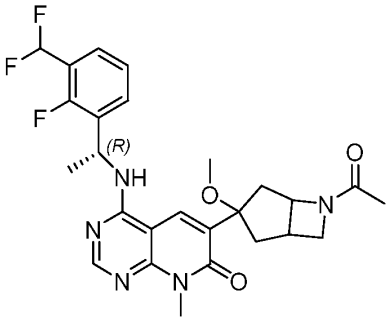
$S(O)_2NR_{11}R_{12}$, $–S(O)_2R_{10}$, $–NR_{10}S(O)_2NR_{11}R_{12}$, $–NR_{10}S(O)_2R_{11}$, $–S(O)NR_{11}R_{12}$, $–S(O)R_{10}$, $–NR_{10}S(O)NR_{11}R_{12}$, $–NR_{10}S(O)R_{11}$, $–C(O)R_{10}$, $–CO_2R_{10}$, 6-10 membered aryl, and 5-10 membered heteroaryl, wherein each C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, 3-14 membered heterocyclyl, 6-10 membered aryl, and 5-10 membered heteroaryl is optionally substituted with –OH, C_{1-6} alkyl optionally substituted with $-R_{10}$, halogen, $–NO_2$, =O, –CN, $−R_{10}$, $–OR_{10}$, $–NR_{11}R_{12}$, $−SR_{10}$, $–S(O)_2NR_{11}R_{12}$, $–S(O)_2R_{10}$, $–NR_{10}S(O)_2NR_{11}R_{12}$, $–NR_{10}S(O)_2R_{11}$, $–S(O)NR_{11}R_{12}$, $–S(O)R_{10}$, $–NR_{10}S(O)NR_{11}R_{12}$, $–NR_{10}S(O)R_{11}$, 3-8 membered cycloalkyl, 3-14 membered heterocyclyl optionally substituted with R_{10}, 6-10 membered aryl, or 5-10 membered heteroaryl;

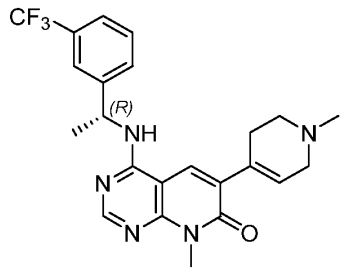
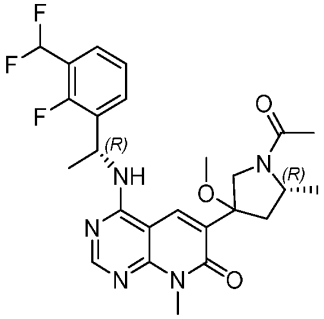
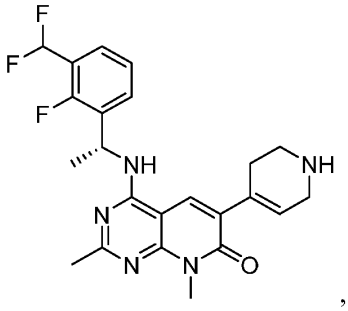
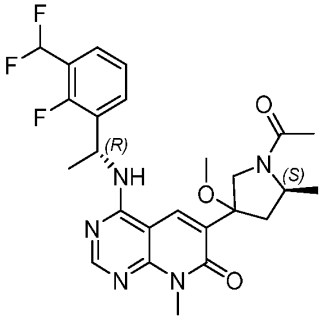
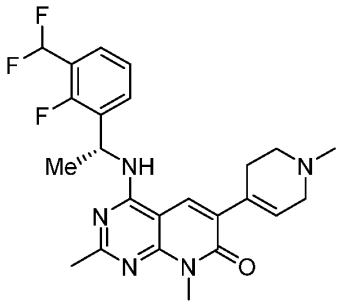
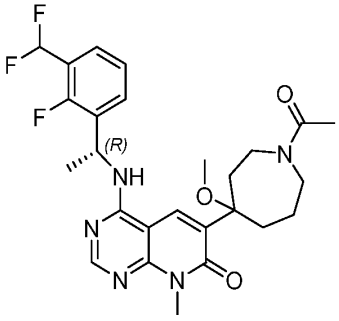
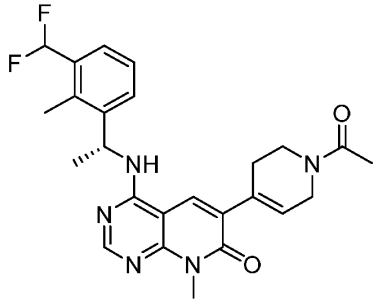
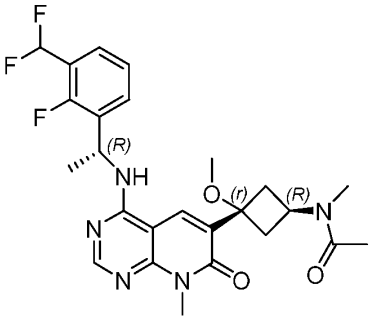
R_{10}, R_{11}, and R_{12} are at each occurrence independently selected from the group consisting of H, D, C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, 3-14 membered heterocyclyl, $–OR_{13}$, $–SR_{13}$, halogen, $–NR_{13}R_{14}$, $–NO_2$, and –CN; and

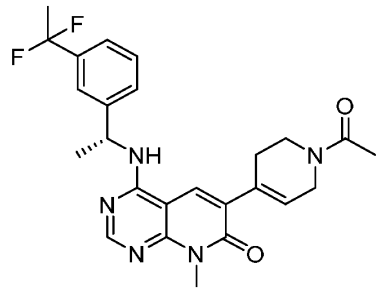
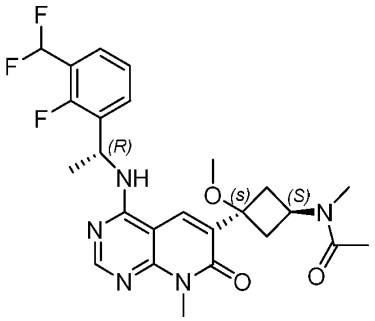
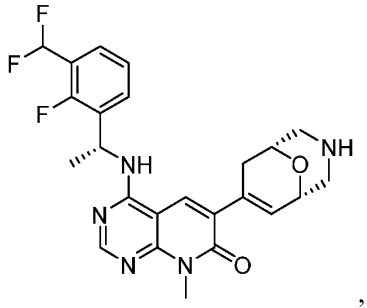
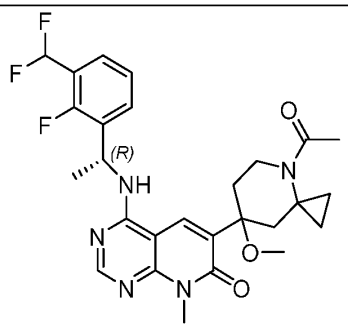
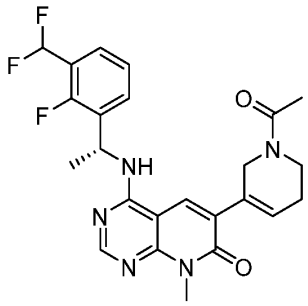
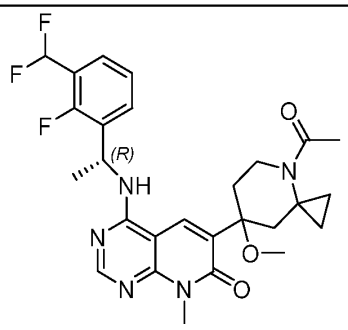
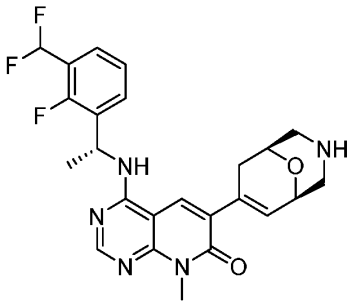
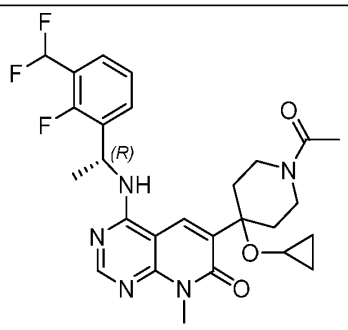
R_{13} and R_{14} are at each occurrence independently selected from the group consisting of H, D, C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, and 3-14 membered heterocyclyl, wherein each C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, and 3-14 membered heterocyclyl are independently optionally substituted with –OH, –SH, $–NH_2$, $–NO_2$, or –CN.

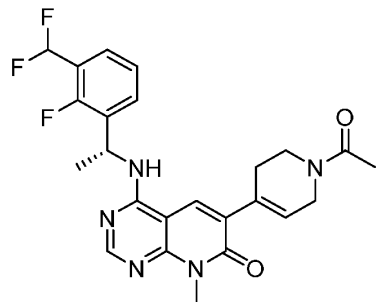
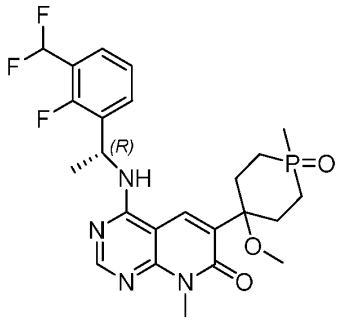
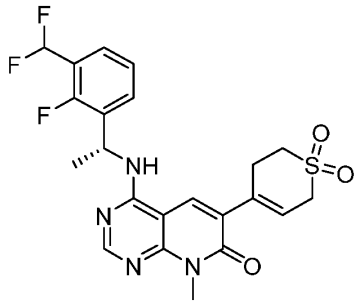
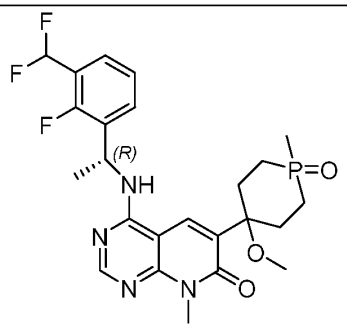
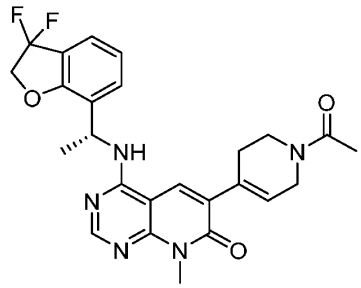
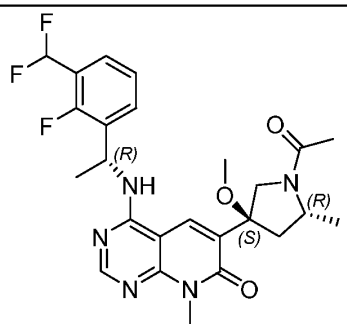
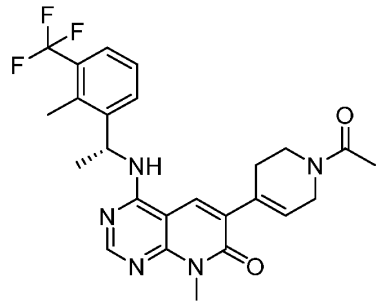
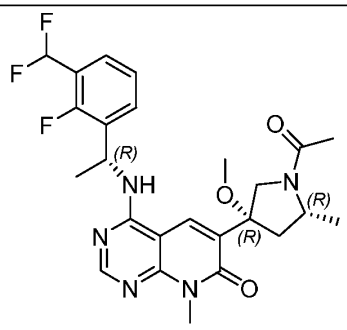
[0106] In some embodiments, the SOS1 inhibitor is a compound selected from the group consisting of the compounds in the following table, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof:

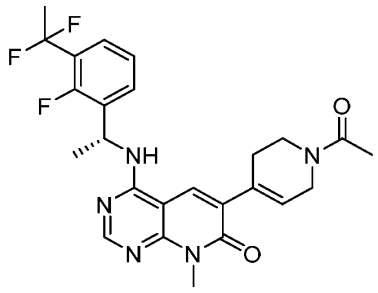
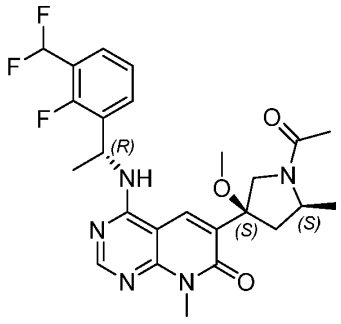
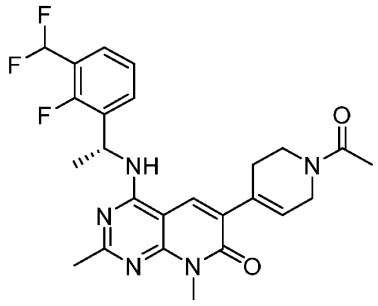
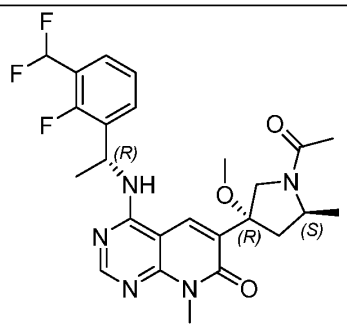
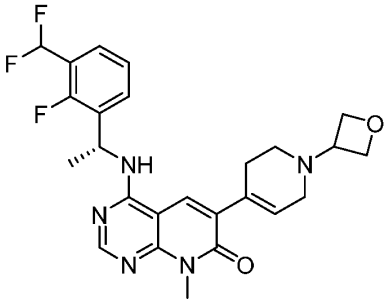
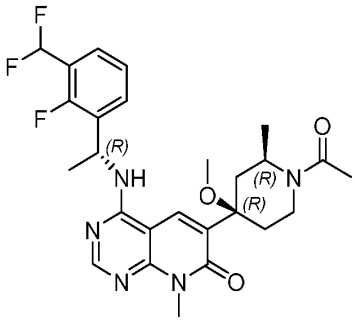
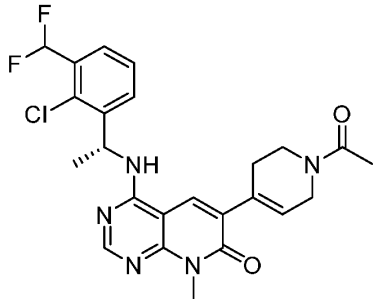
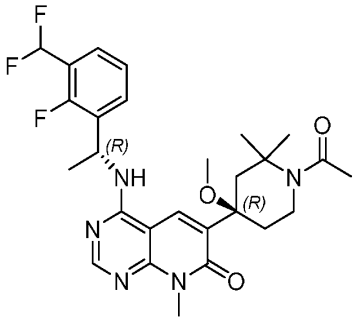
	
	
	
	

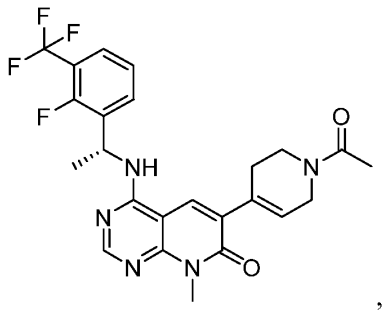
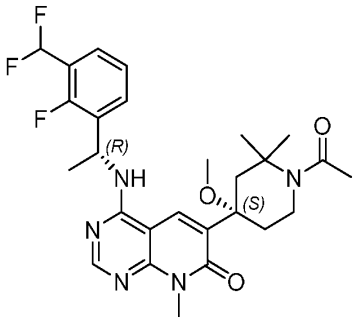
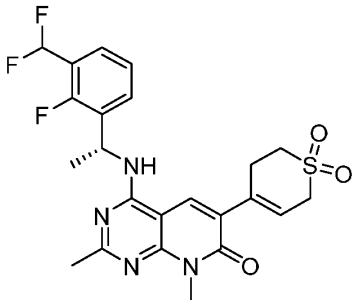
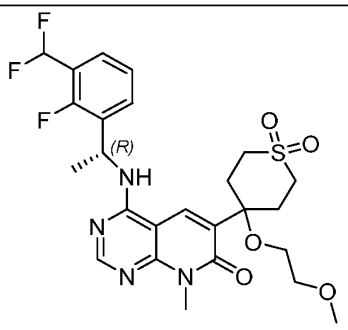
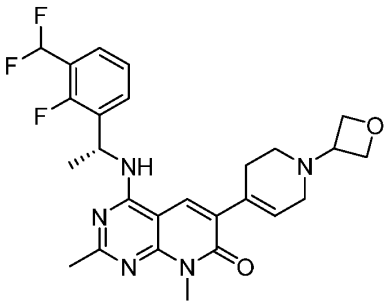
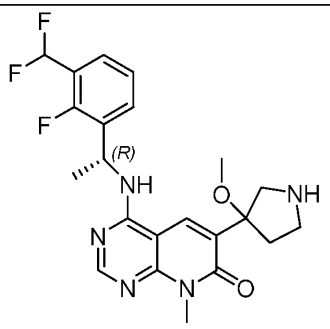
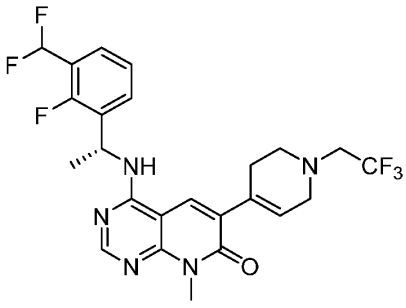
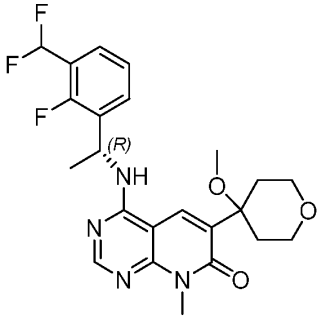
	
	
	
 ,	

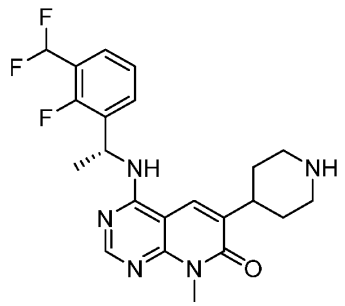
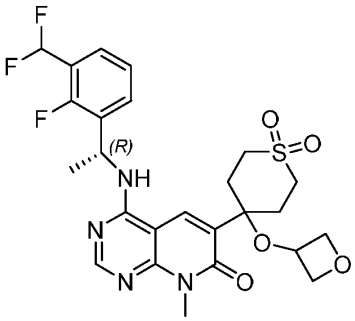
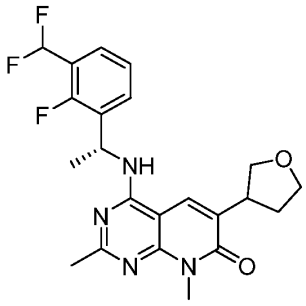
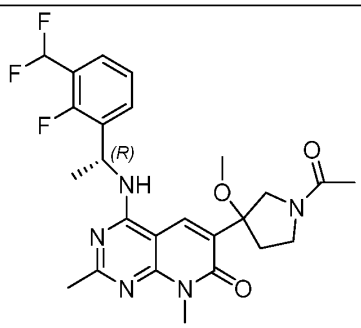
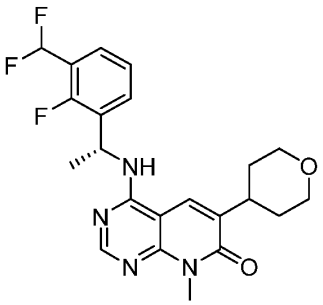
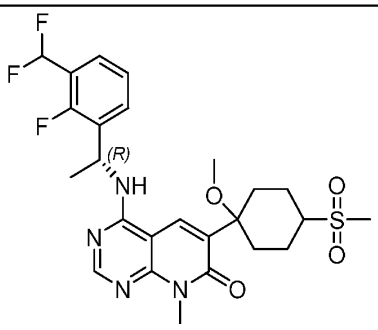
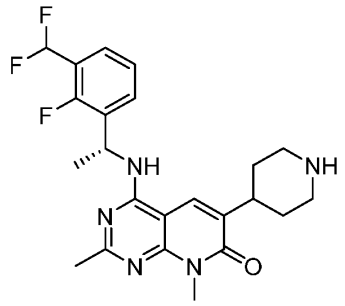
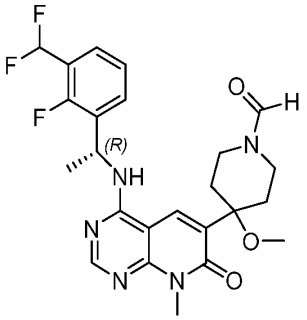
	
 ,	
	
	

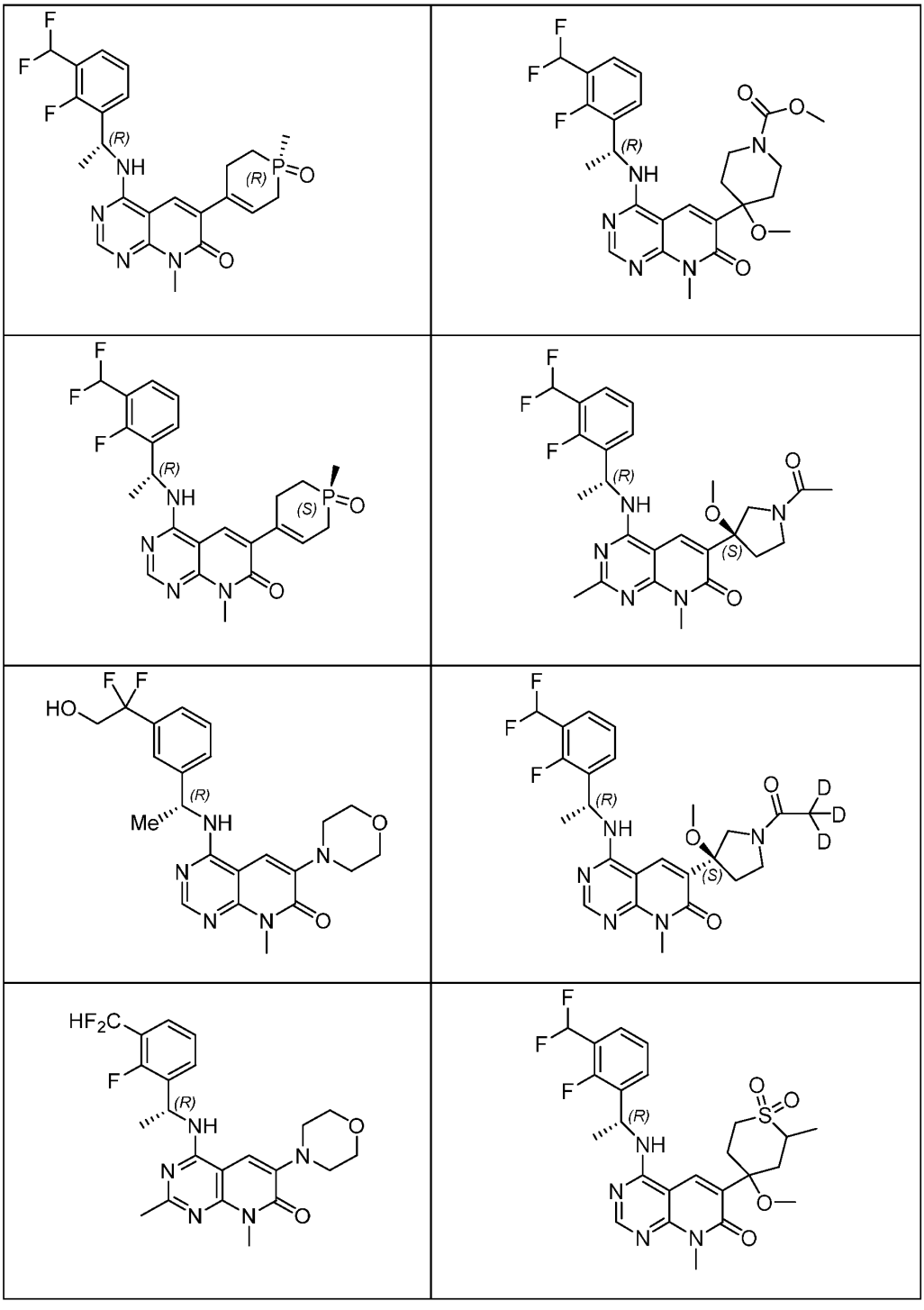
	
 ,	
	
	

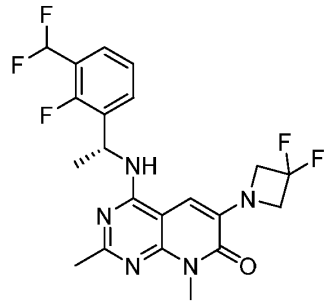
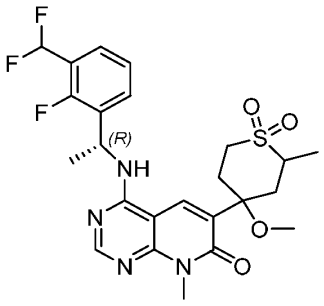
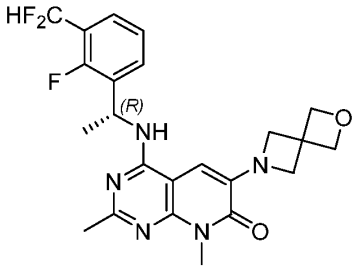
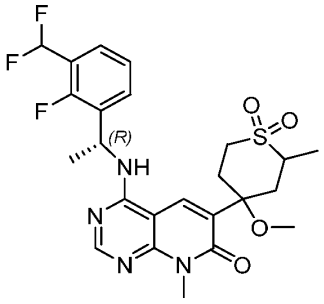
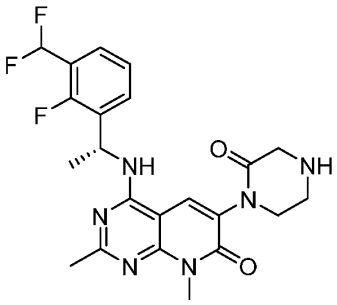
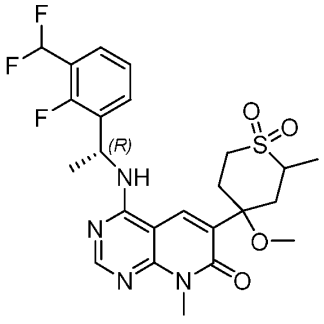
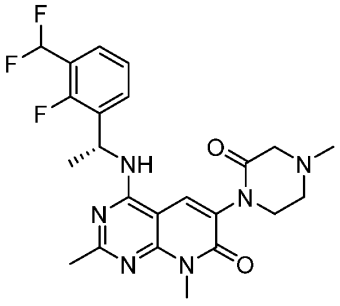
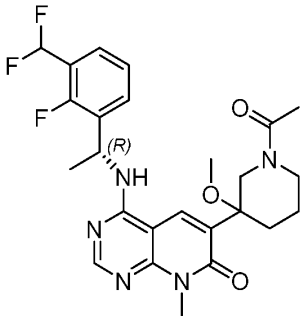
	
	
	
	

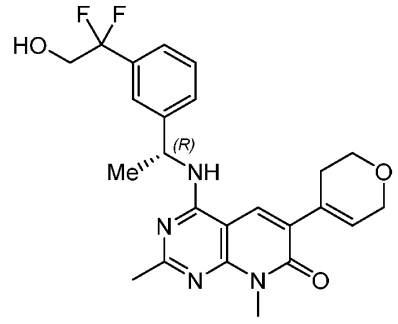
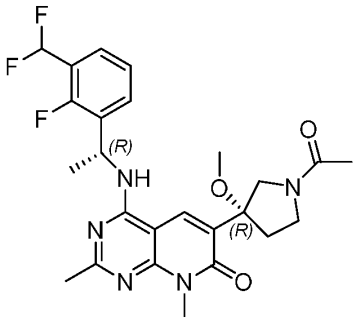
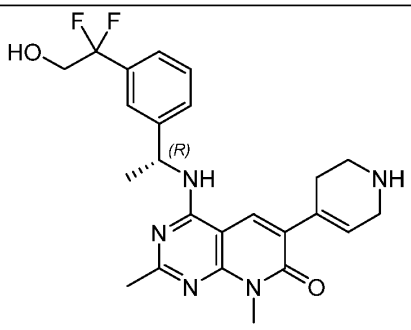
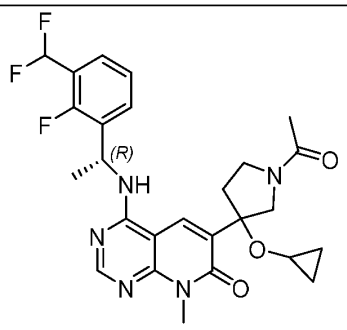
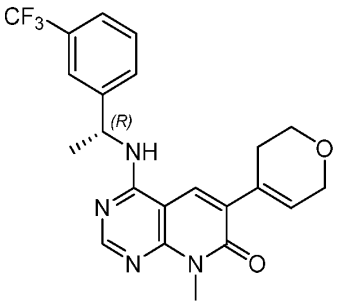
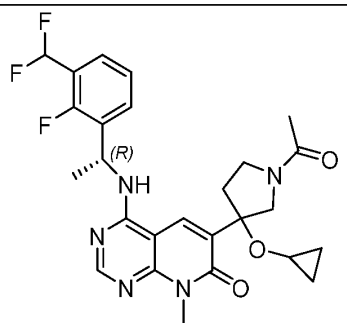
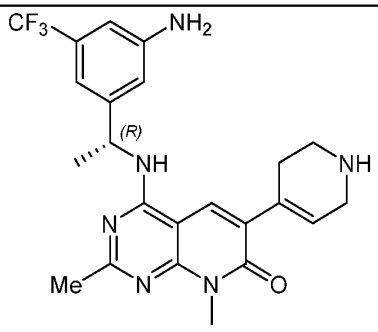
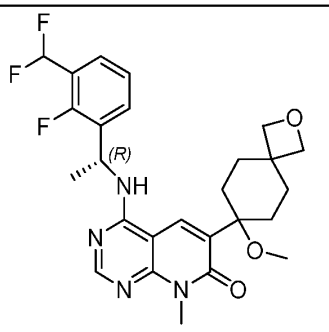
	
	
	
	

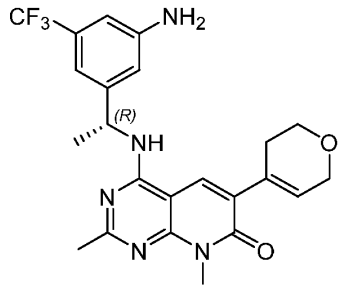
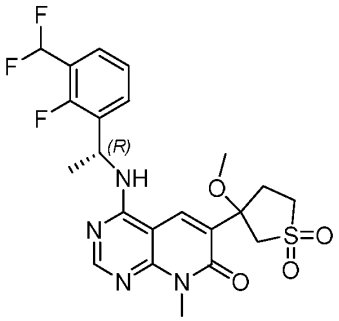
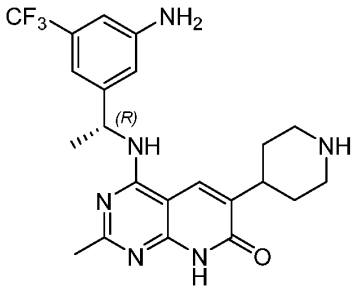
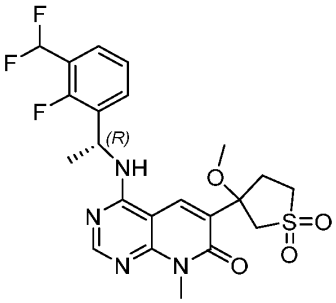
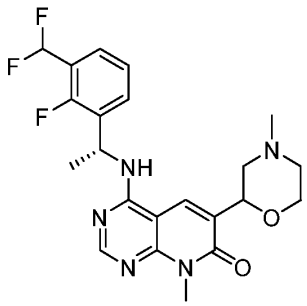
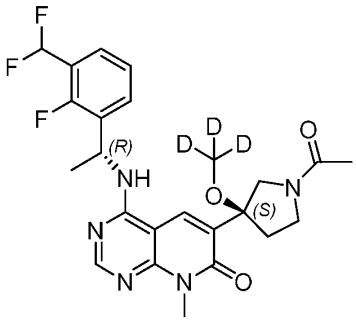
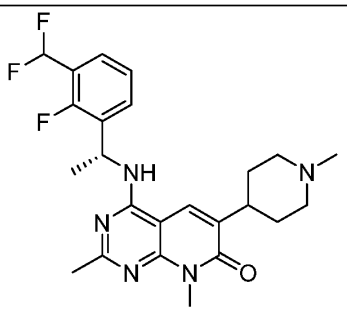
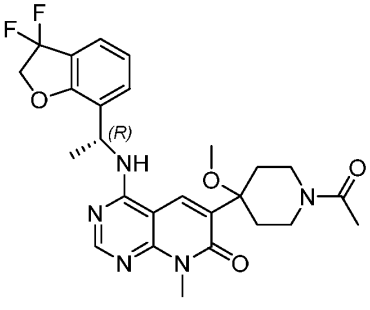
 ,	
	
	
	

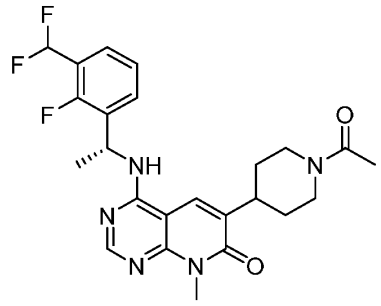
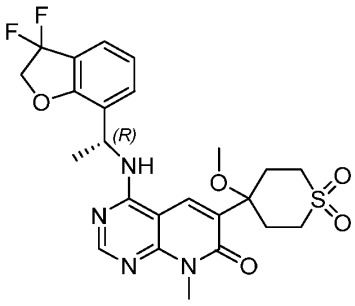
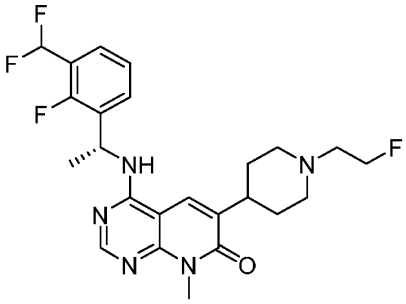
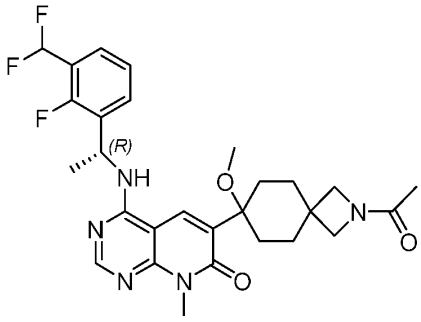
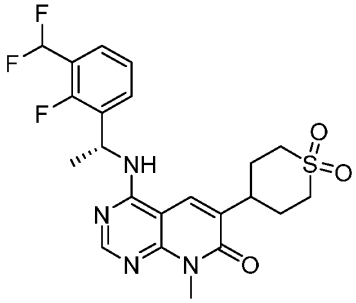
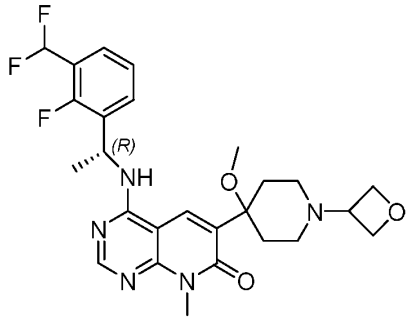
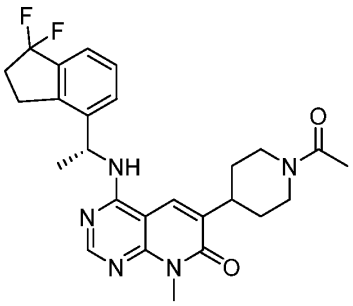
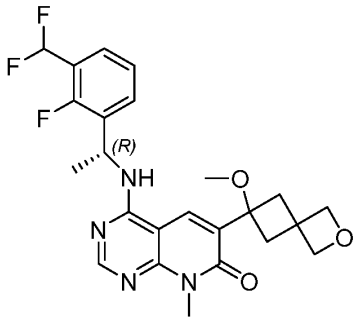
	
	
	
	

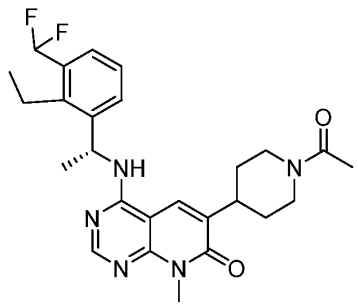
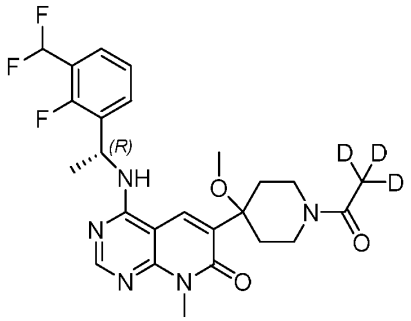
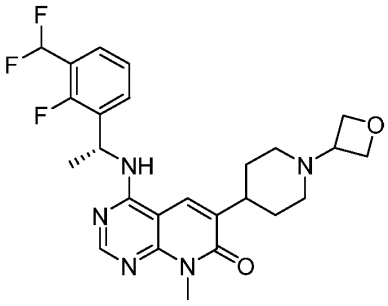
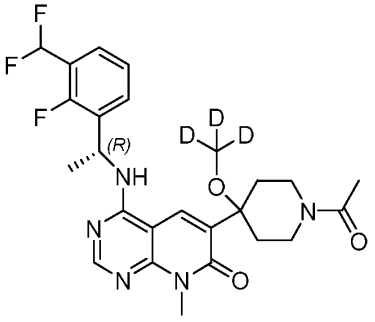
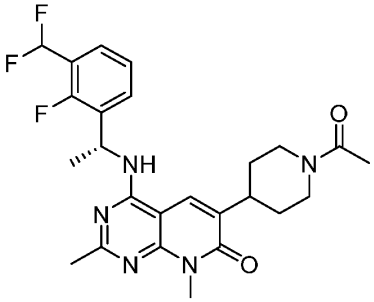
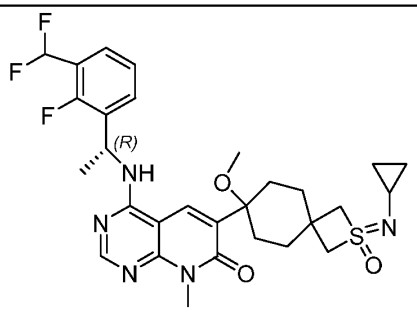
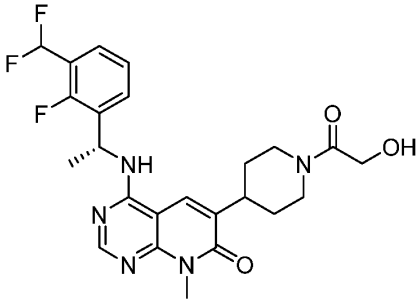
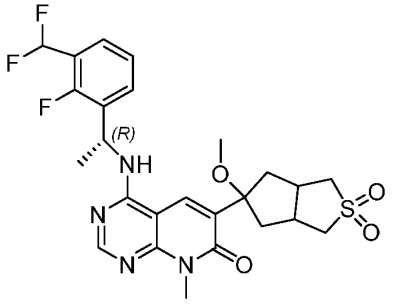


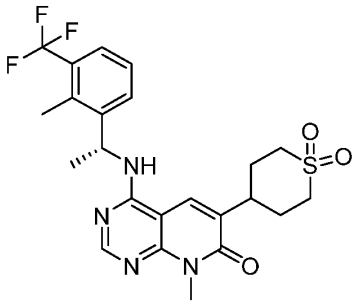
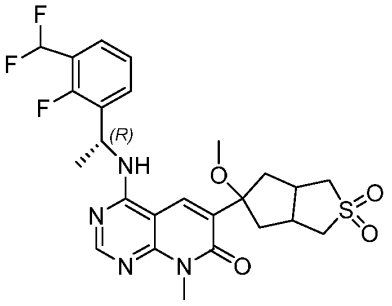
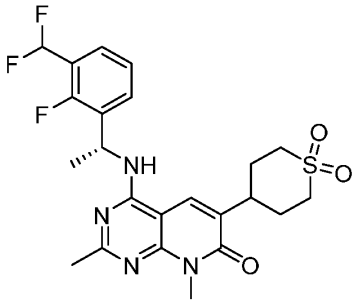
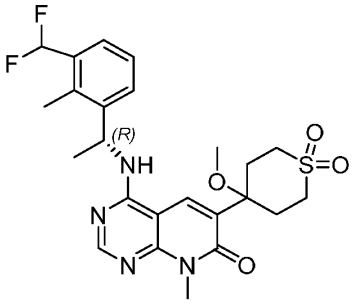
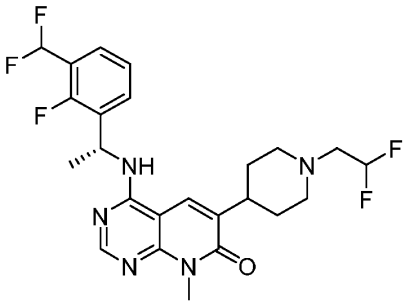
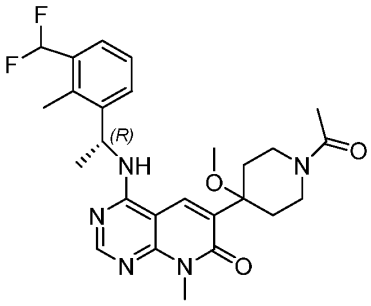
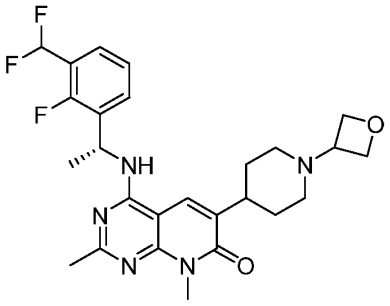
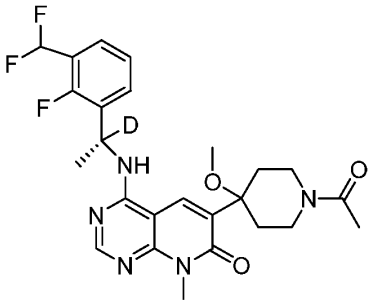
	
	
	
	

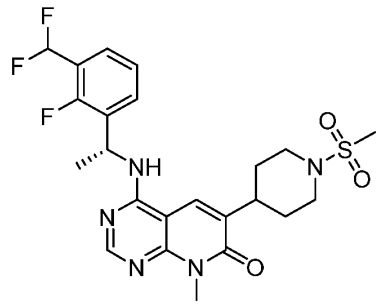
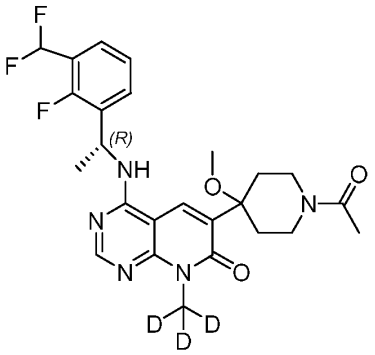
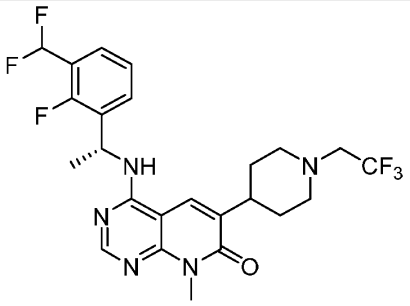
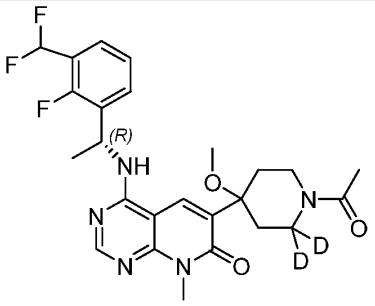
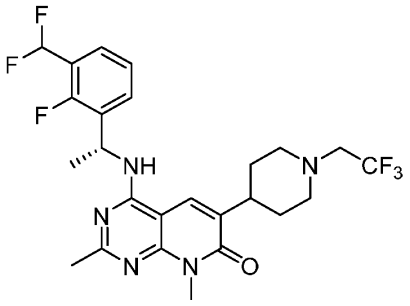
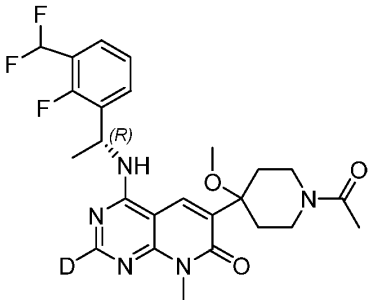
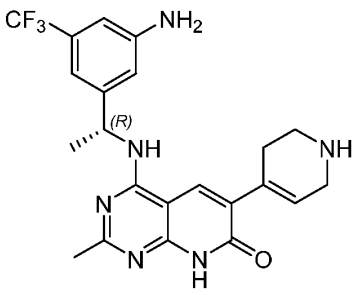
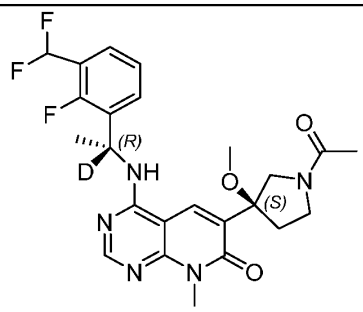
	
	
	
	

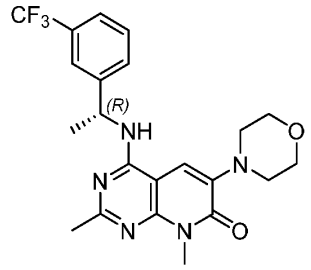
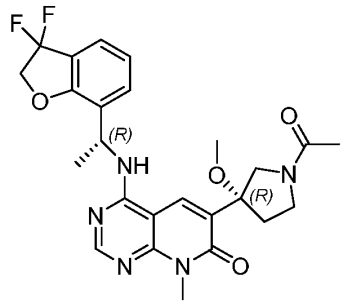
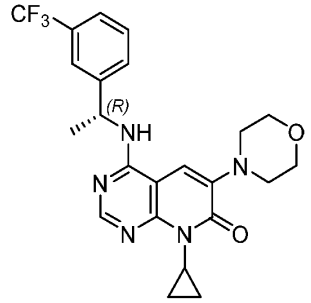
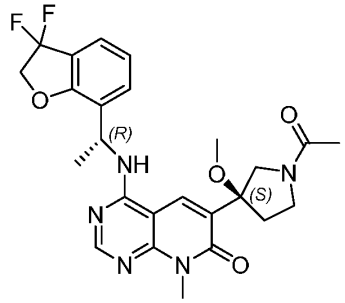
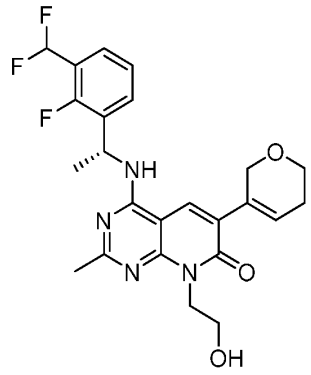
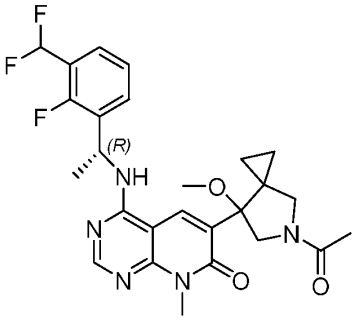
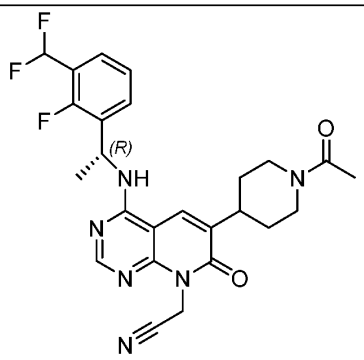
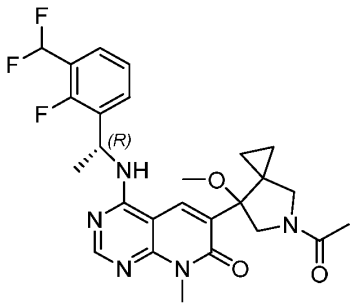
	
	
	
	

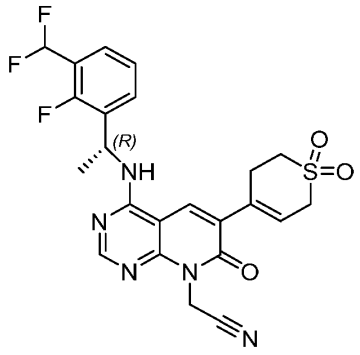
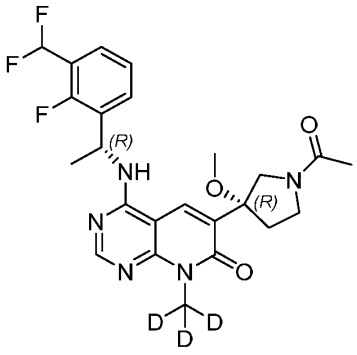
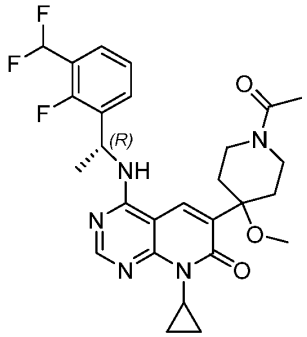
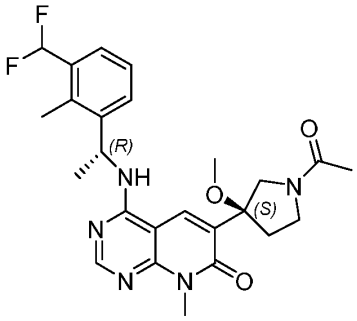
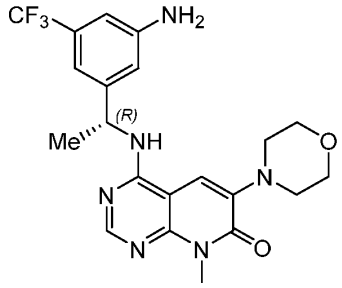
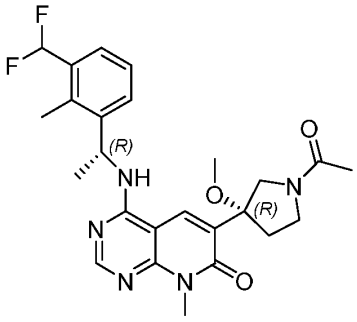
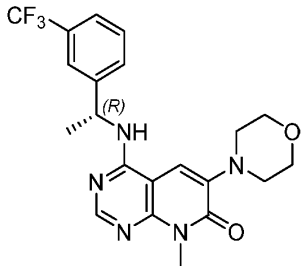
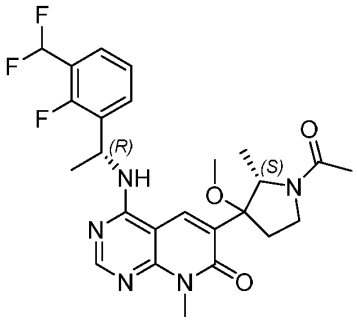
	
	
	
	

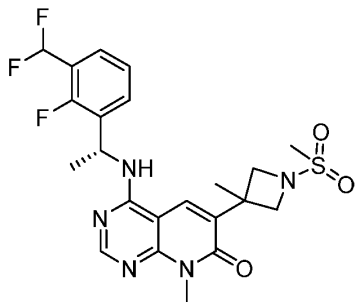
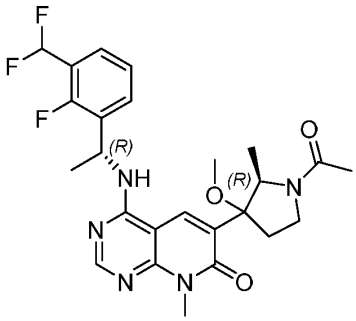
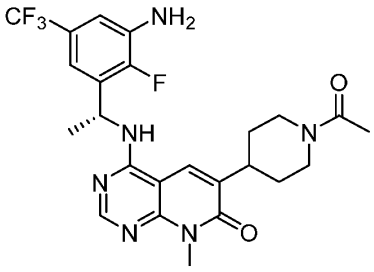
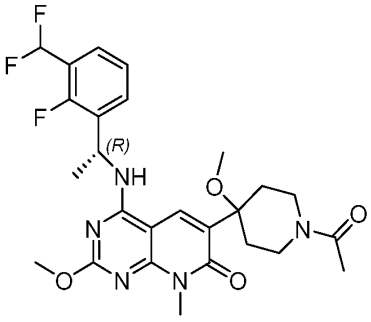
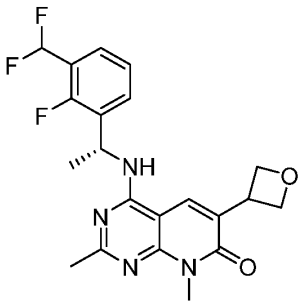
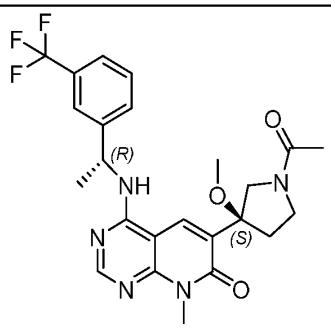
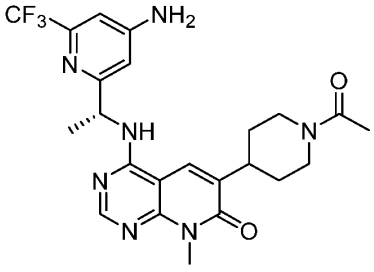
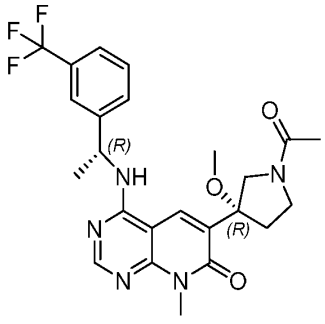
	
	
	
	

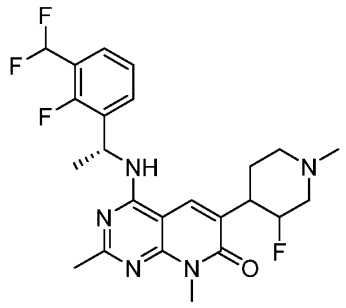
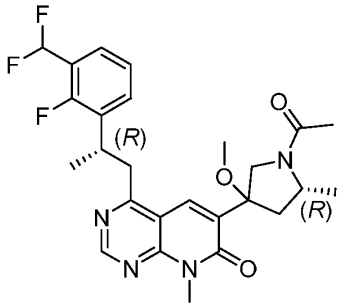
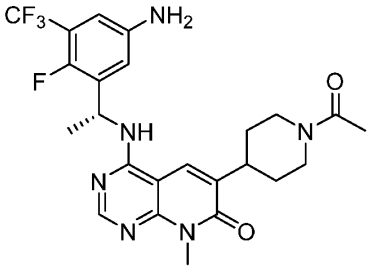
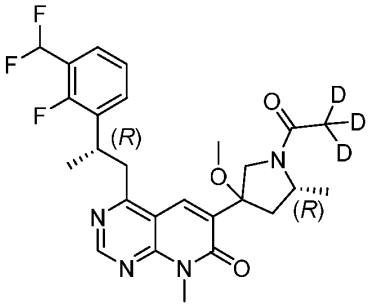
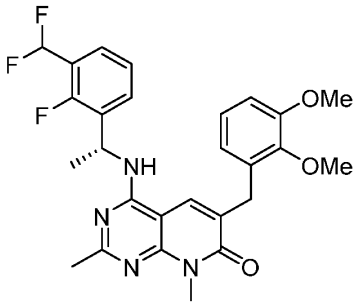
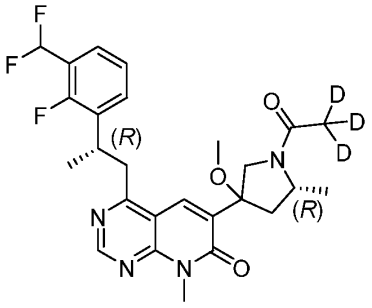
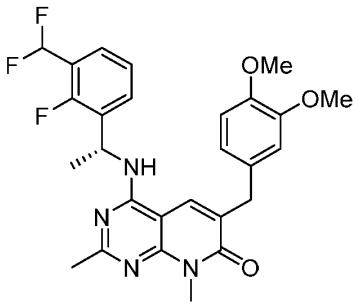
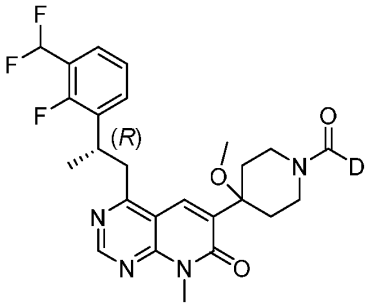
	
	
	
	

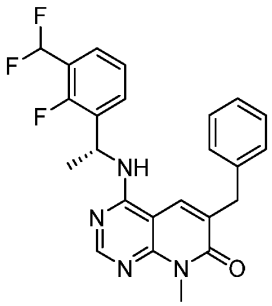
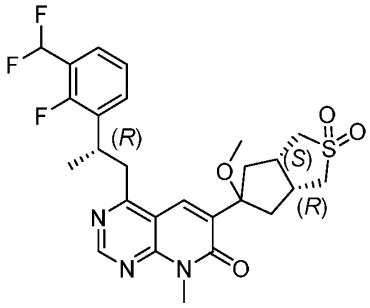
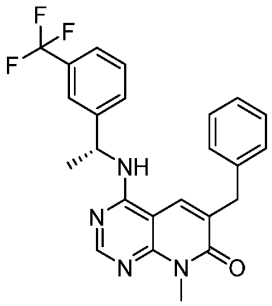
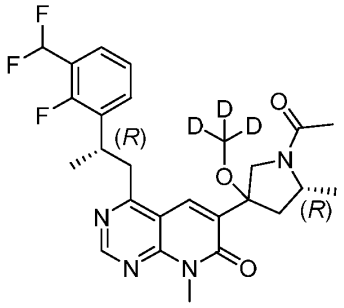
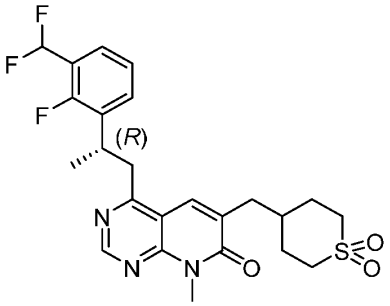
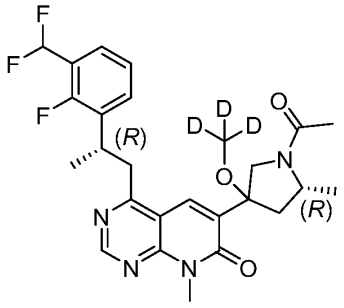
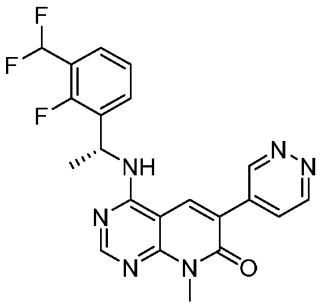
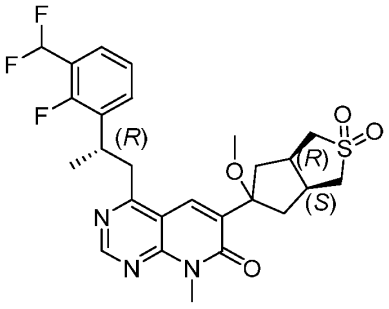
	
	
	
	

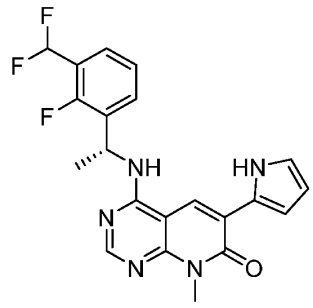
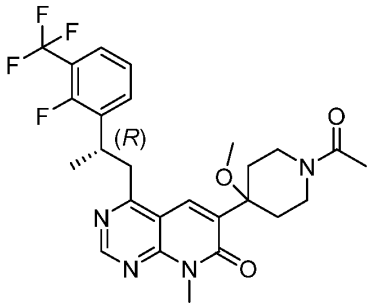
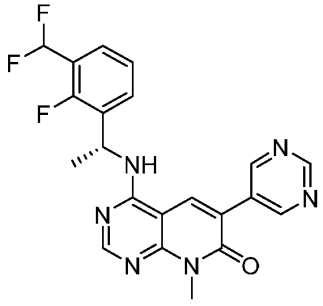
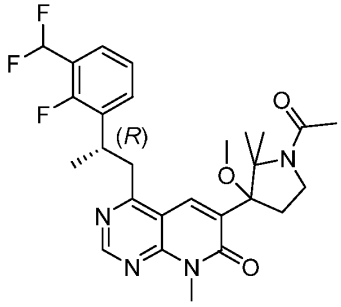
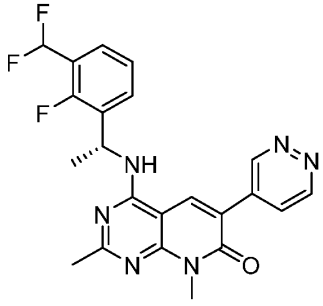
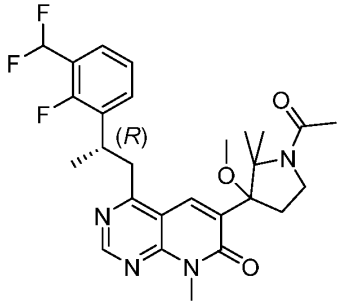
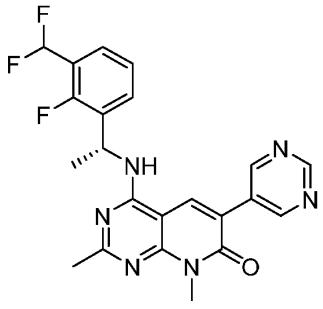
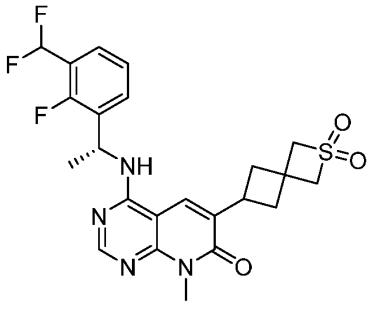
	
	
	
	

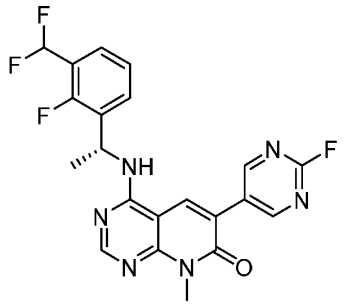
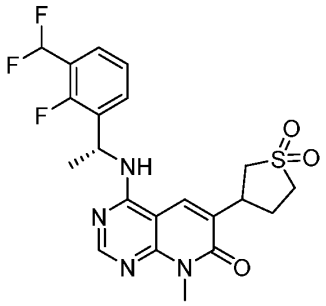
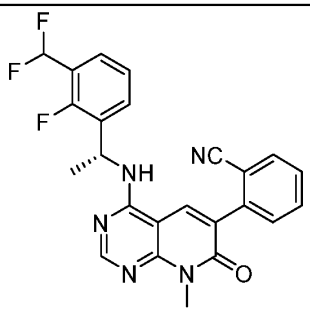
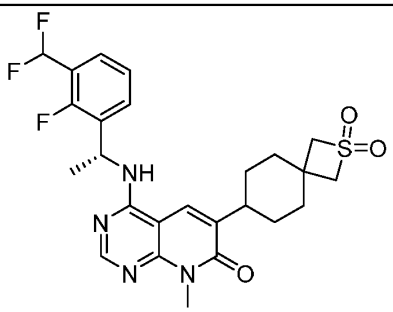
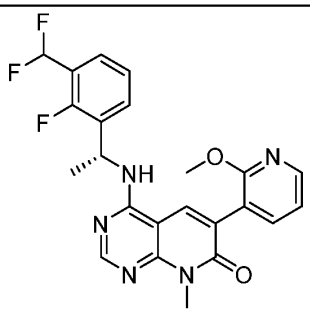
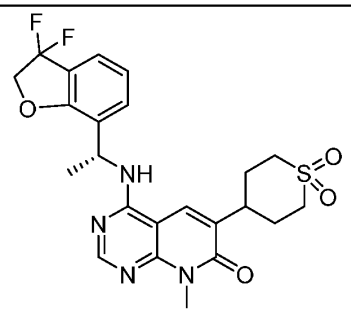
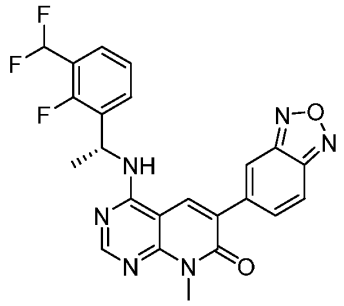
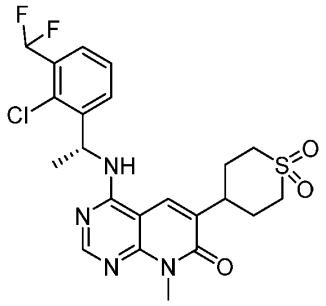
	
	
	
	

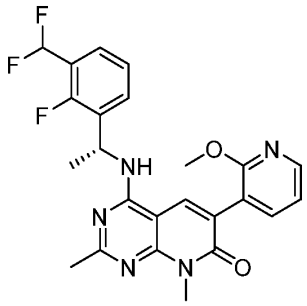
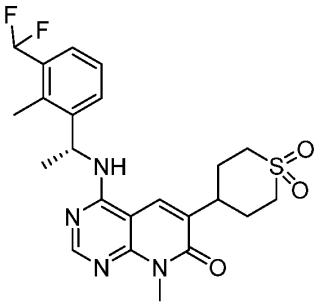
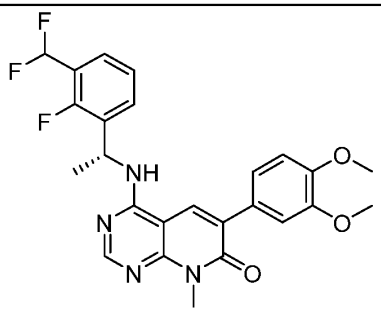
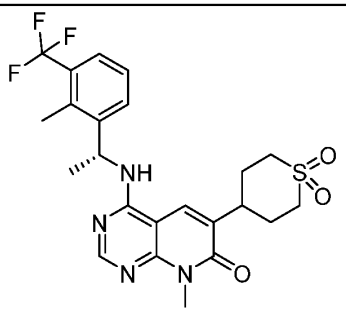
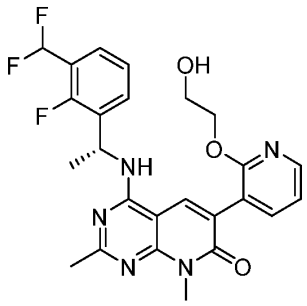
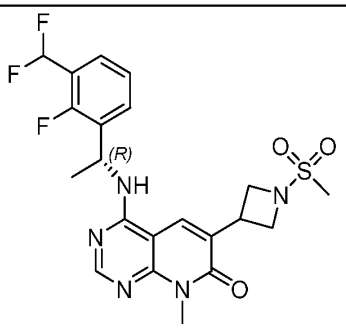
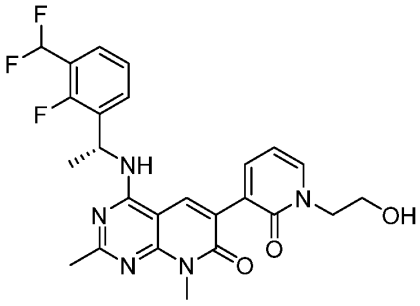
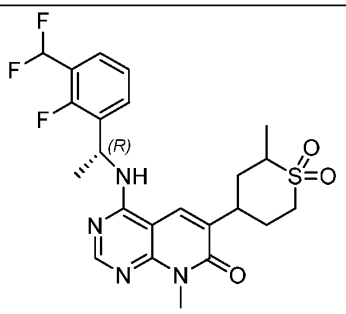
	
	
	
	

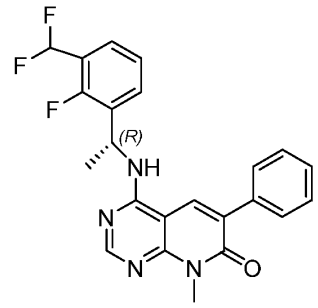
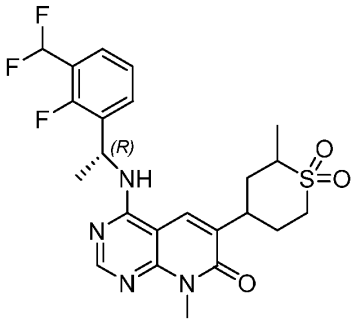
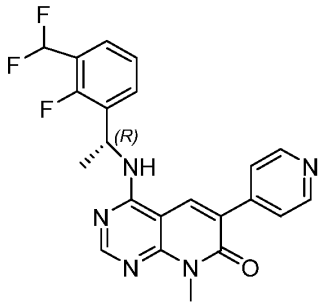
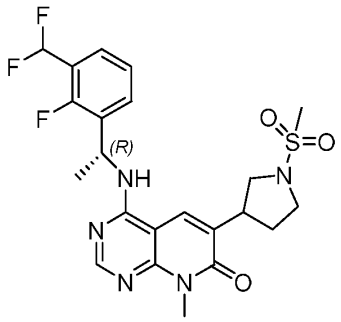
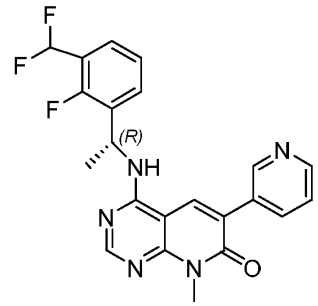
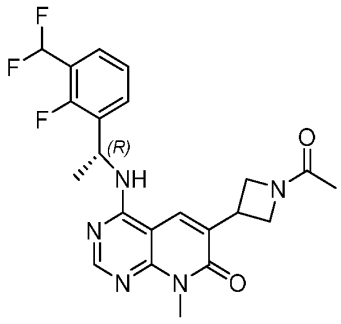
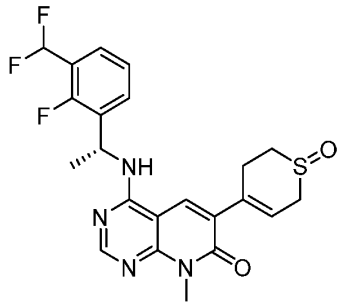
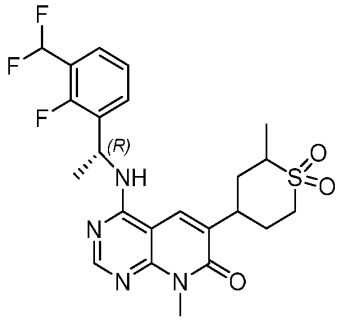
	
	
	
	

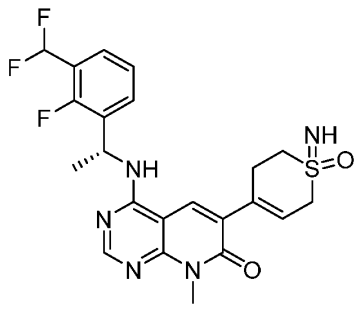
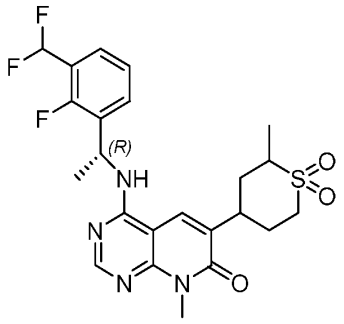
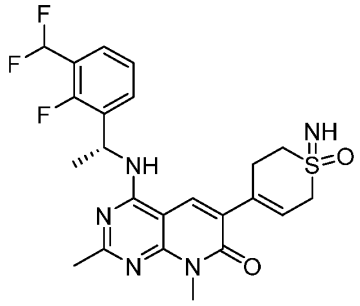
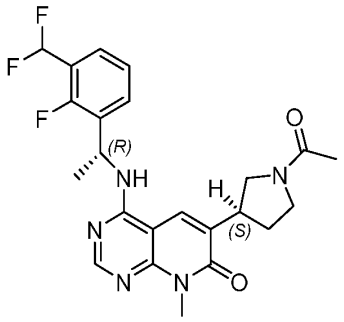
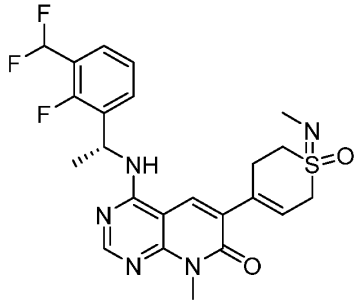
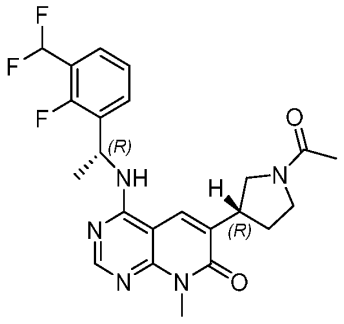
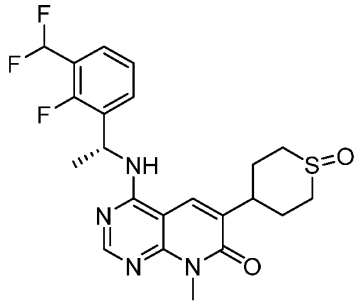
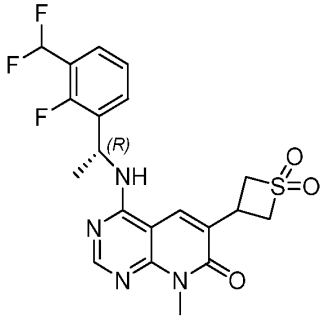
	
	
	
	

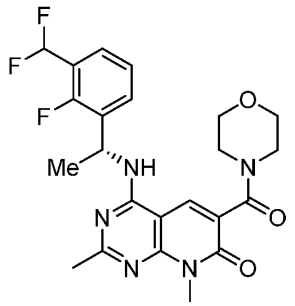
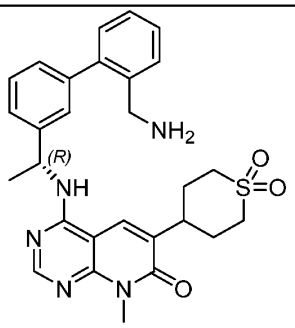
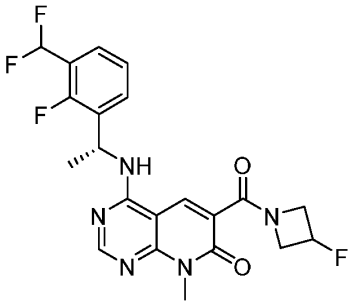
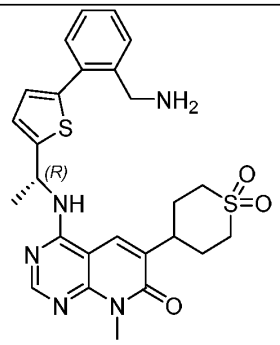
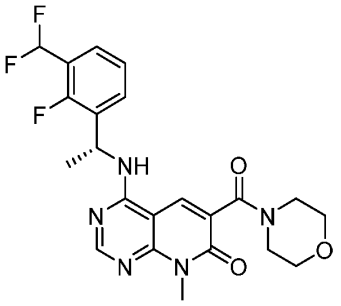
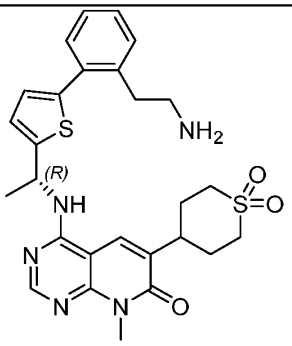
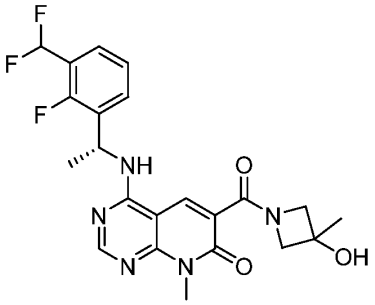
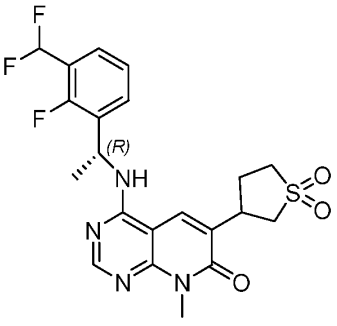
	
	
	
	

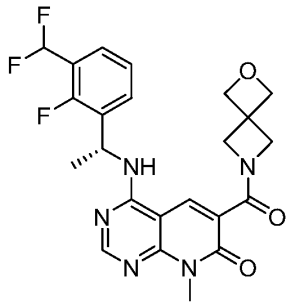
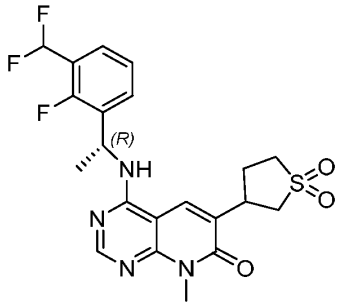
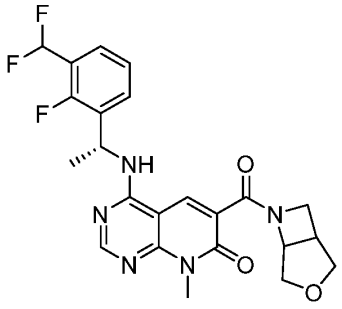
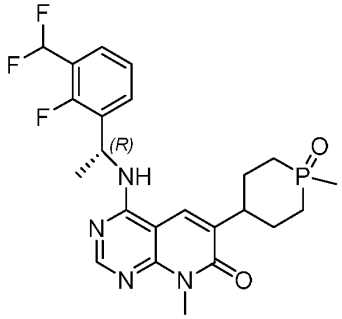
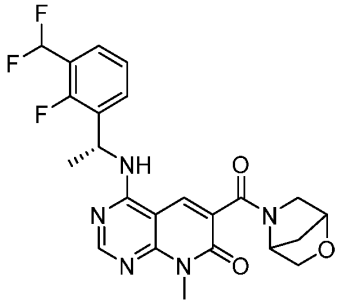
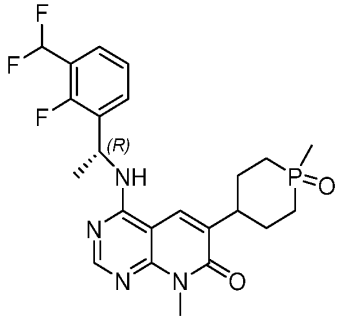
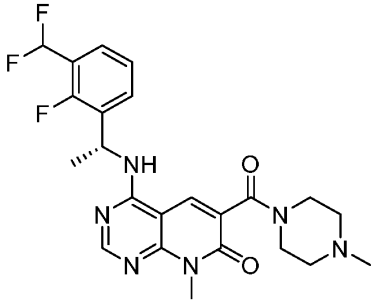
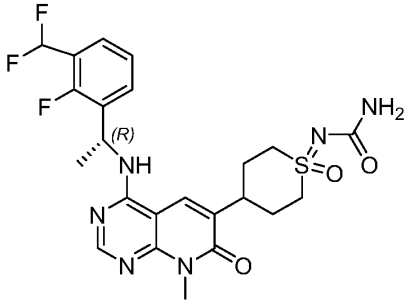
	
	
	
	

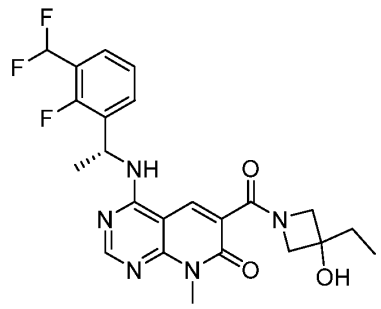
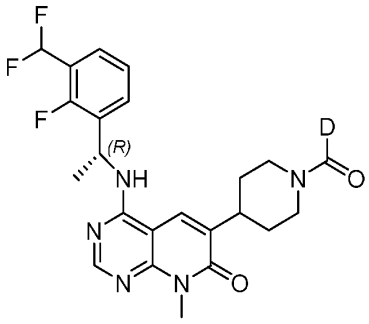
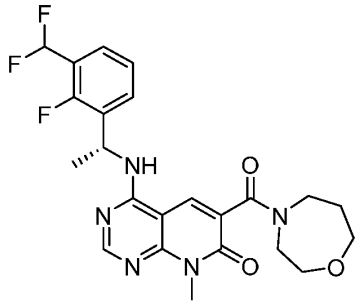
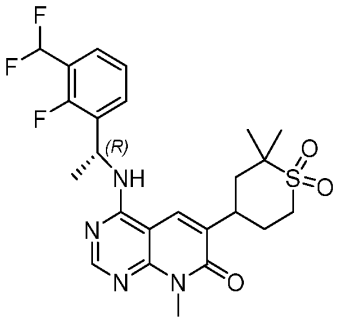
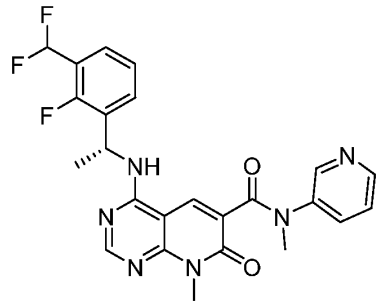
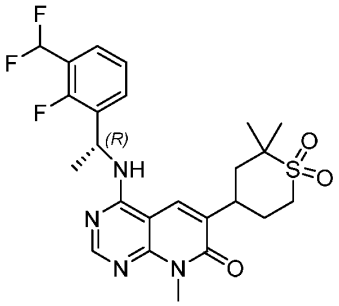
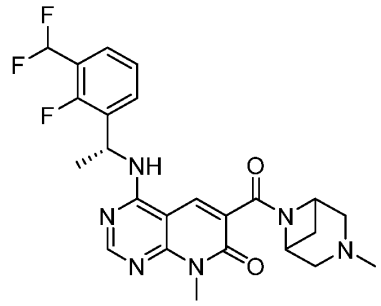
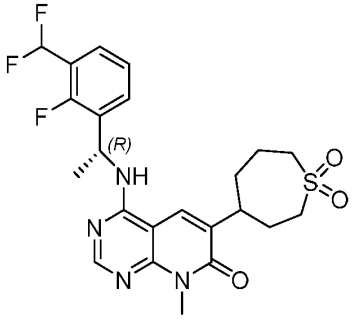
	
	
	
	

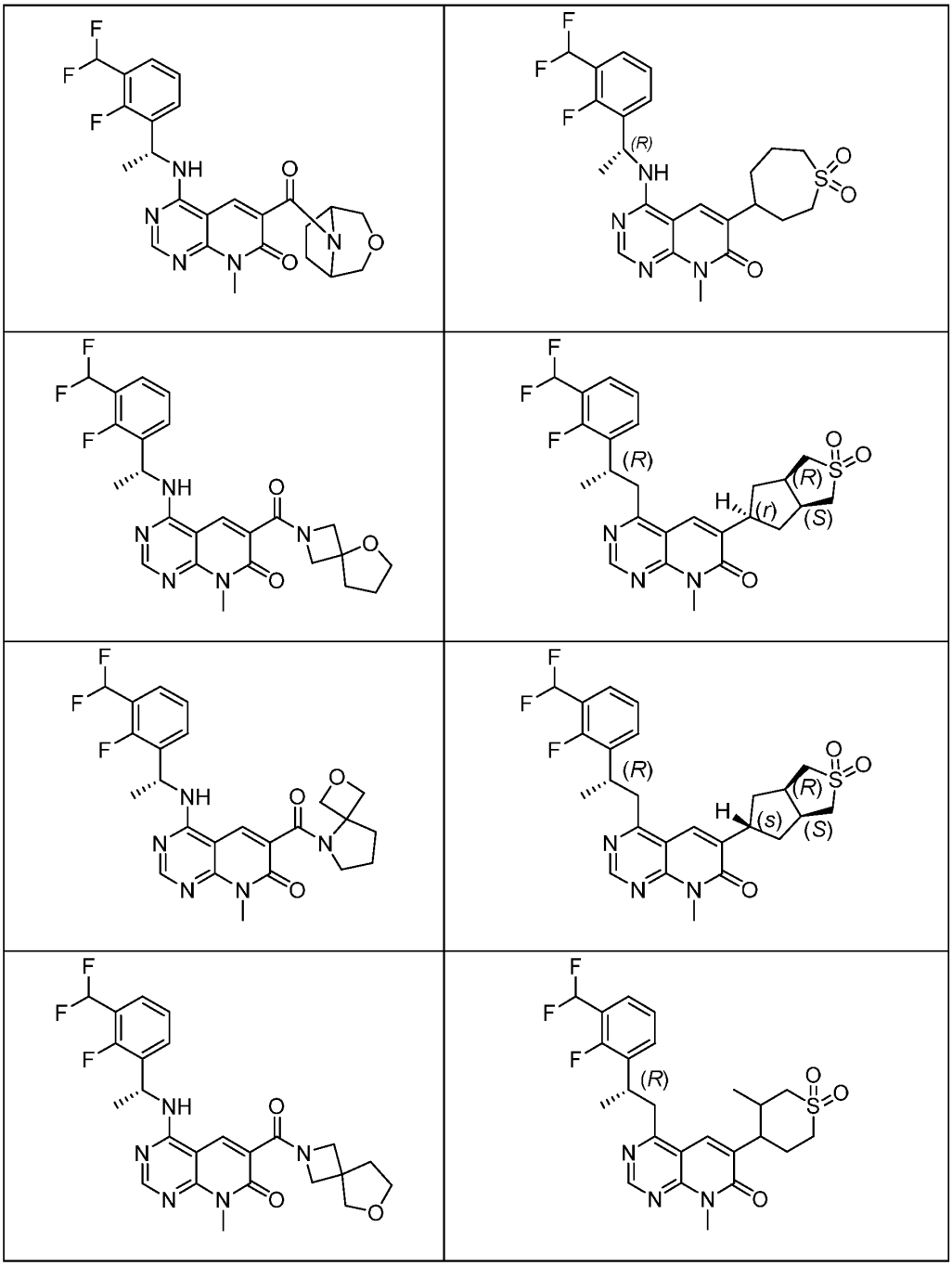
	
	
	
	

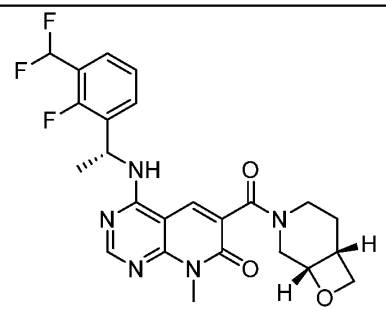
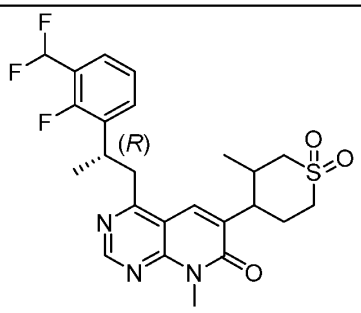
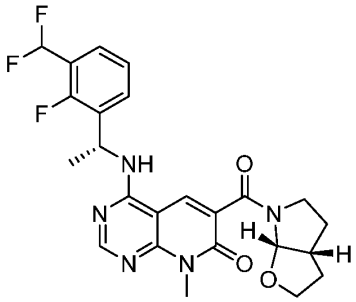
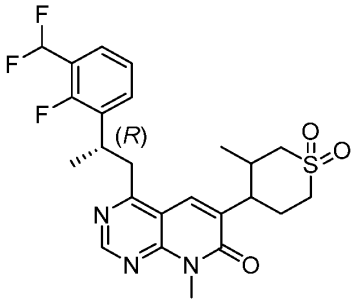
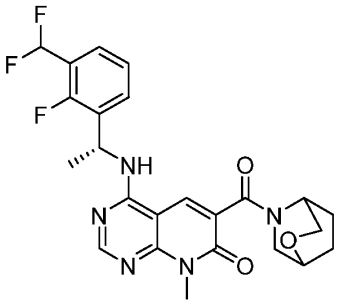
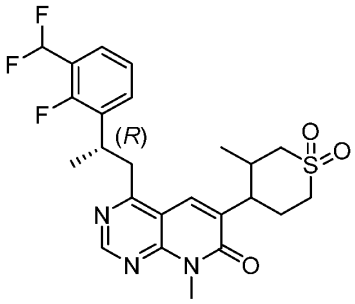
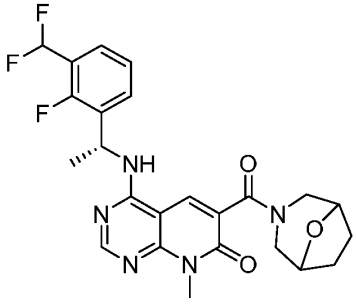
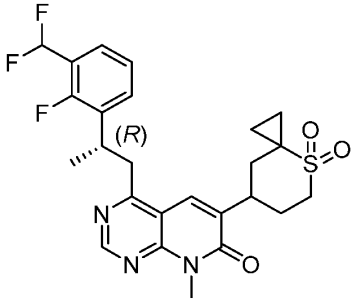
	
	
	
	

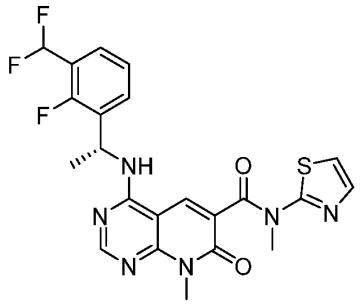
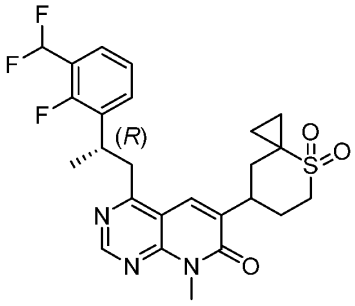
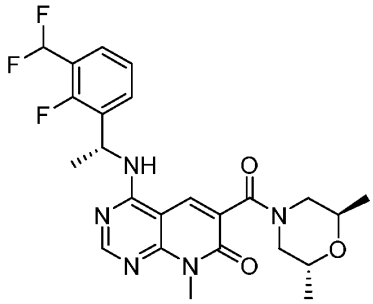
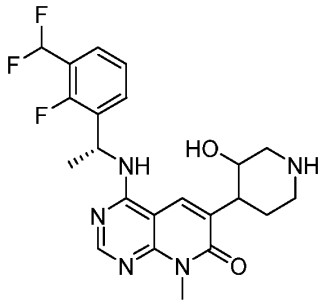
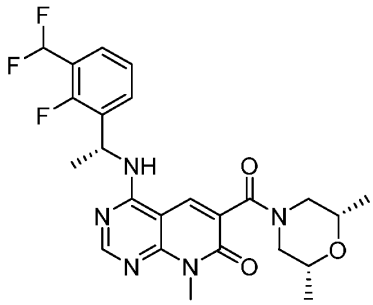
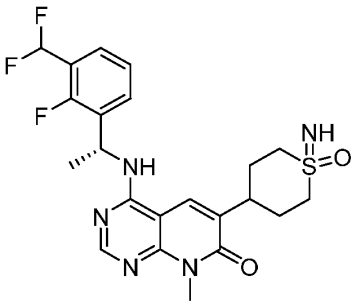
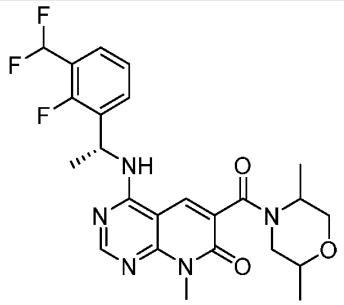
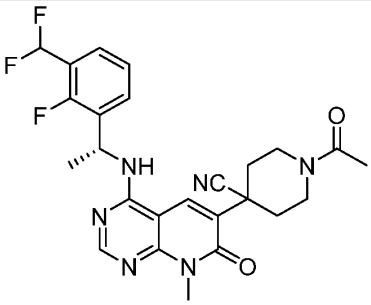
	
	
	
	

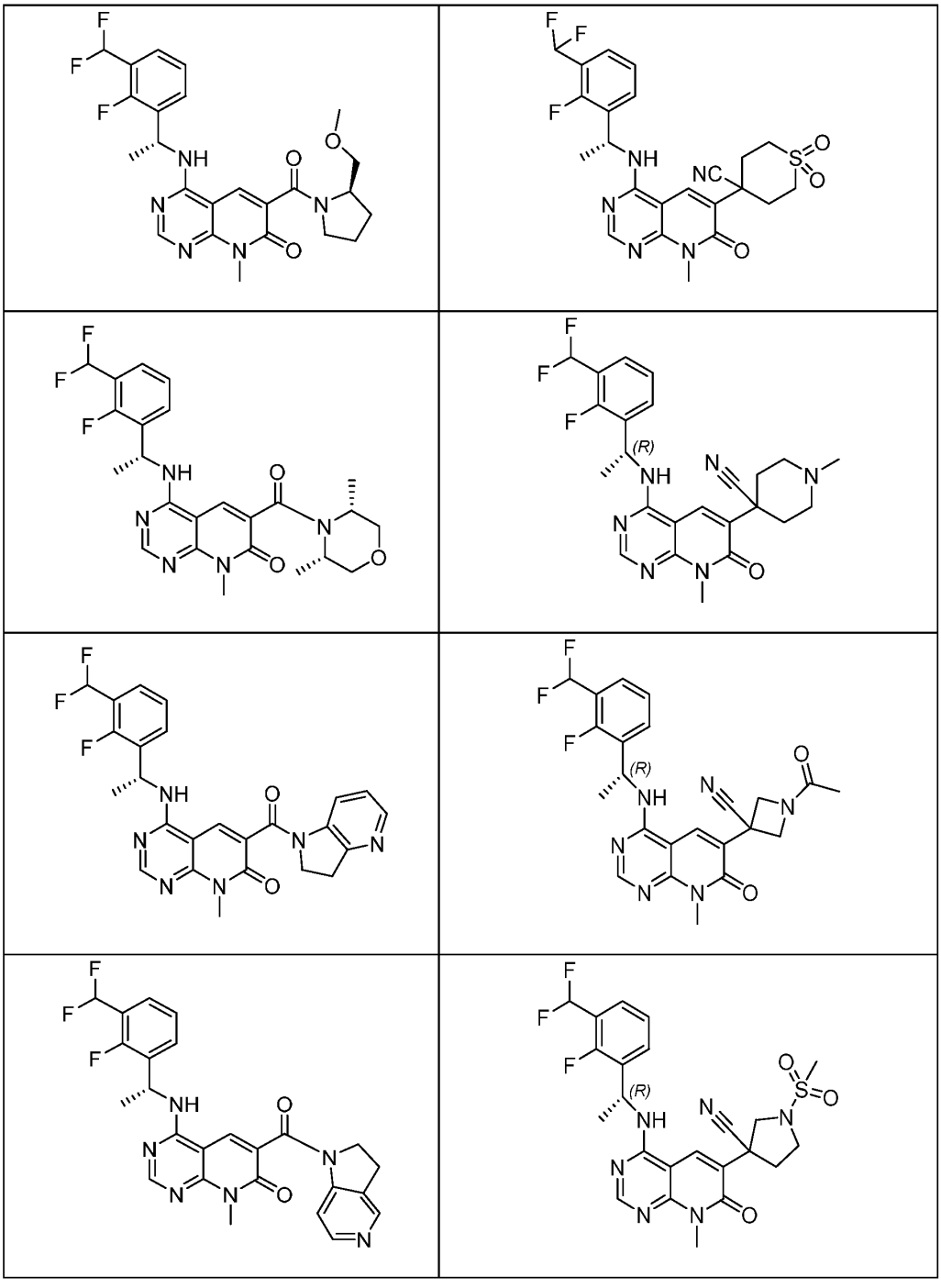
	
	
	
	

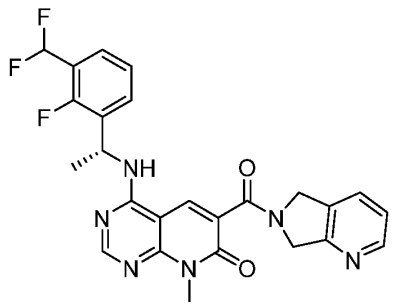
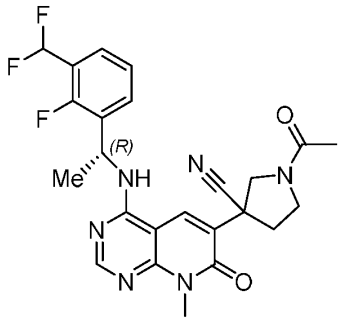
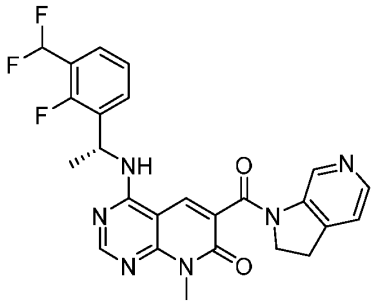
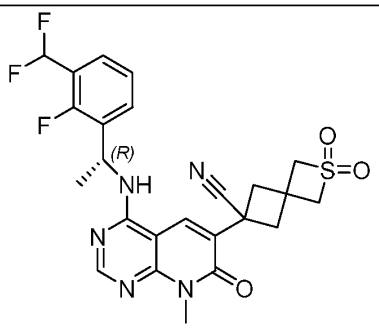
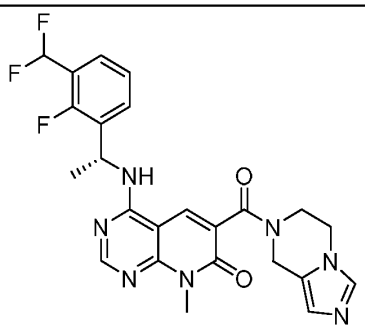
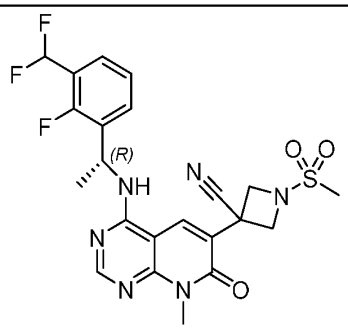
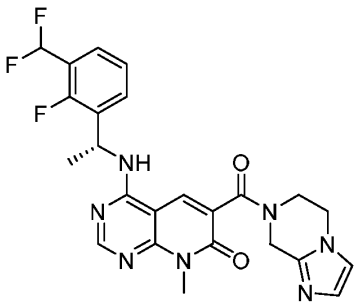
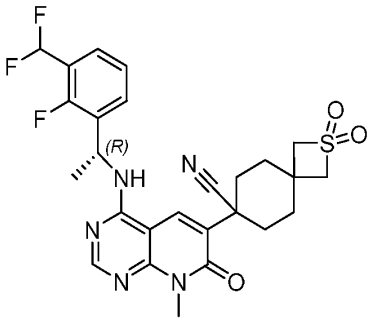
	
	
	
	

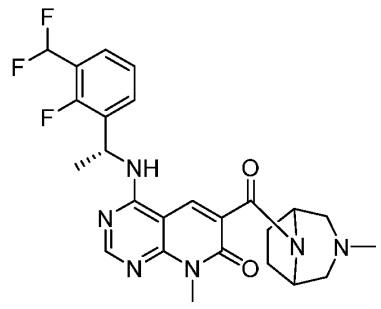
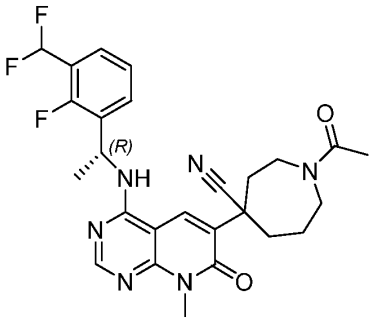
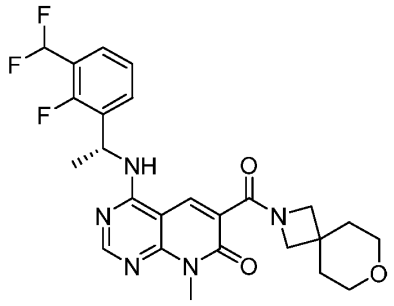
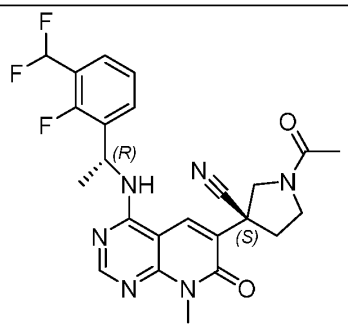
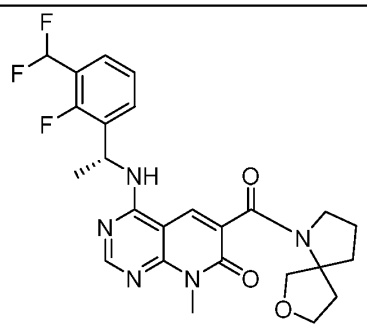
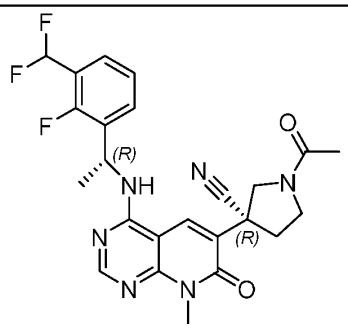
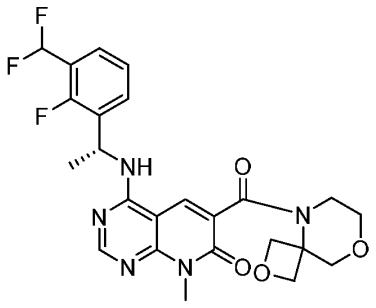
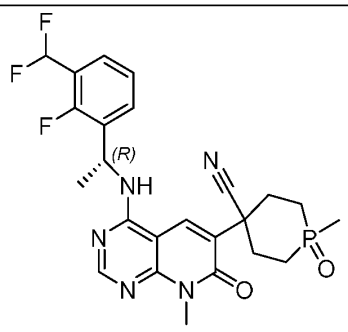


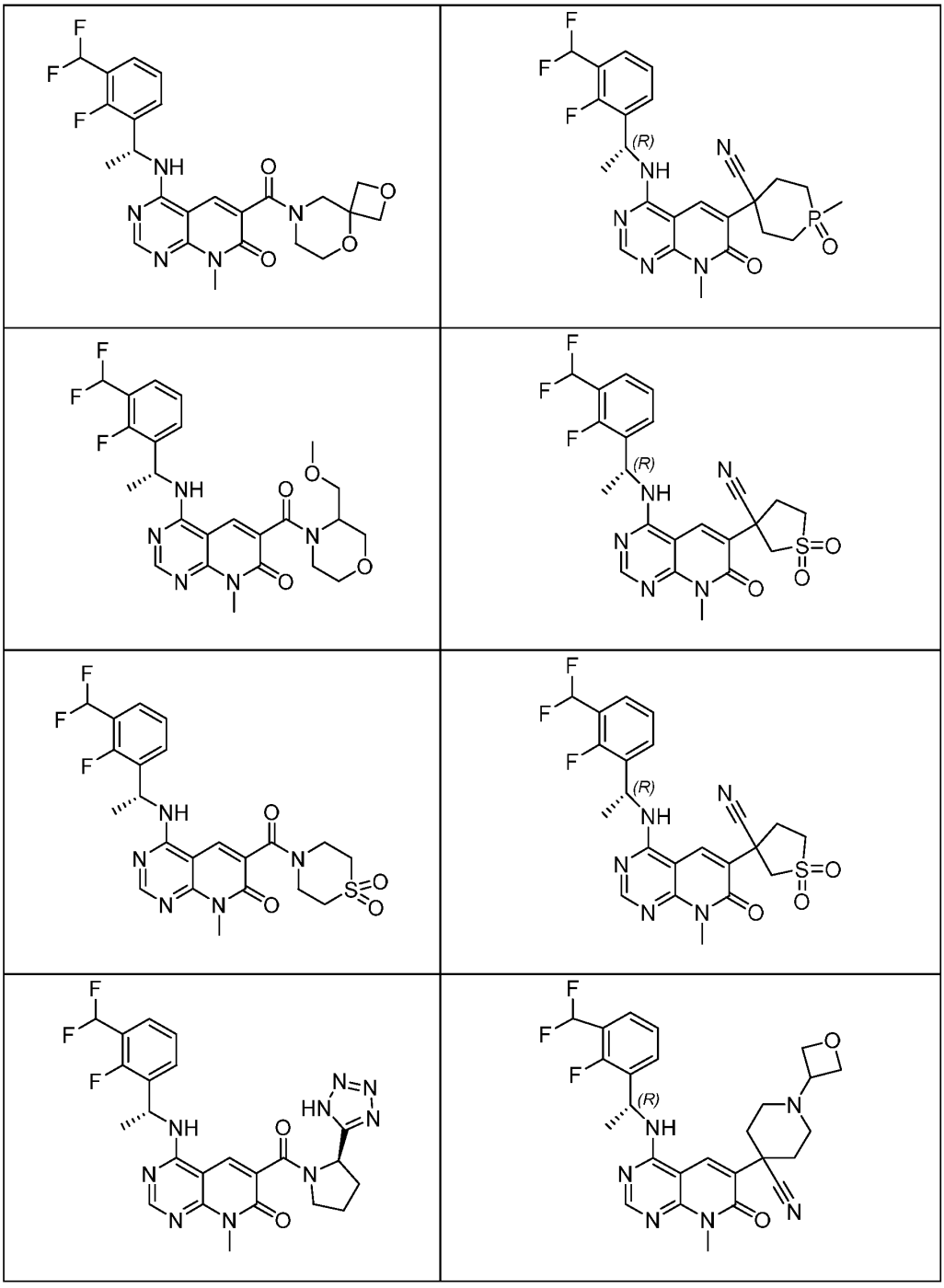
	
	
	
	

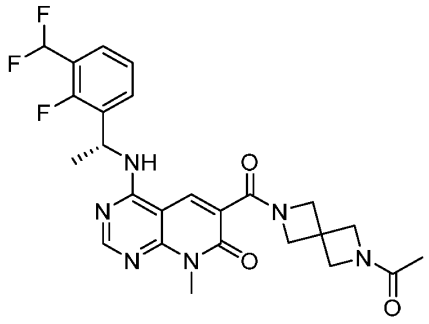
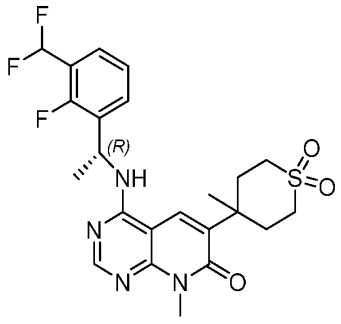
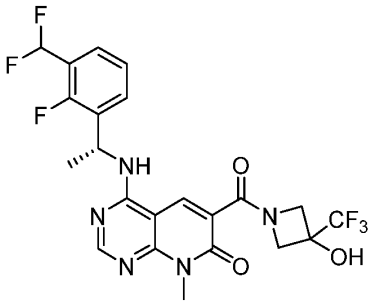
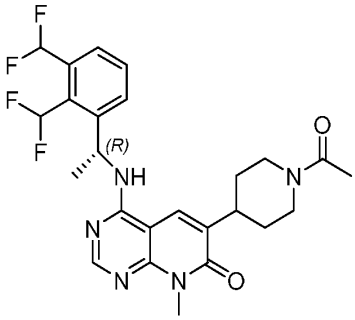
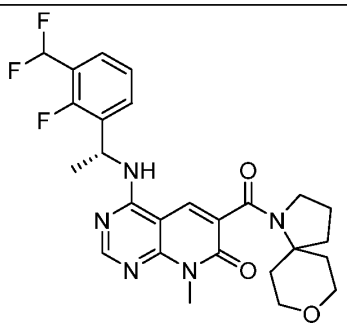
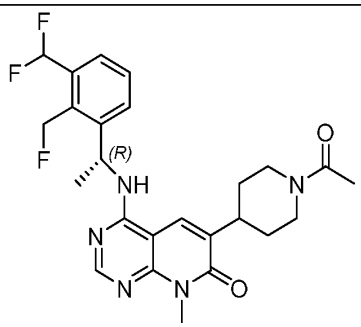
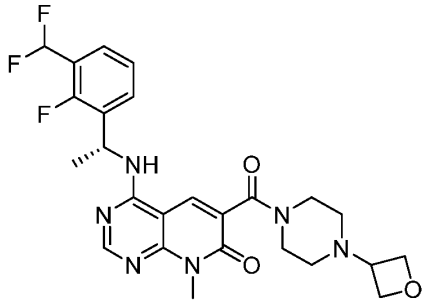
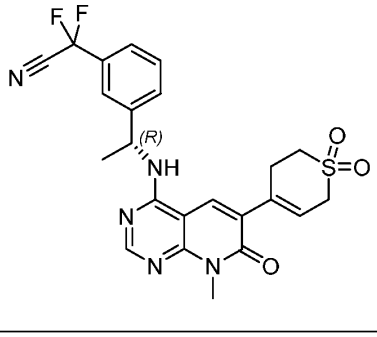
	
	
	
	

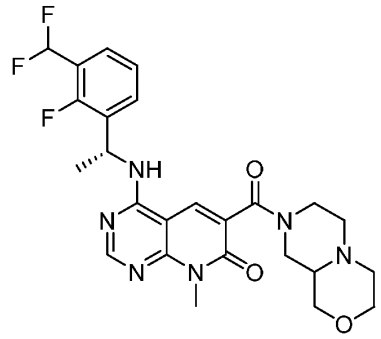
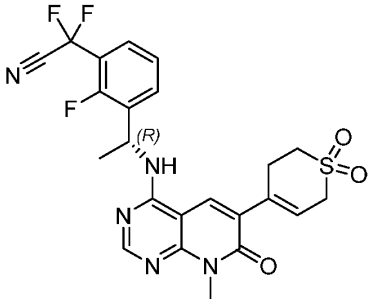
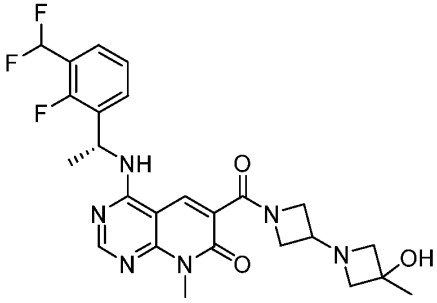
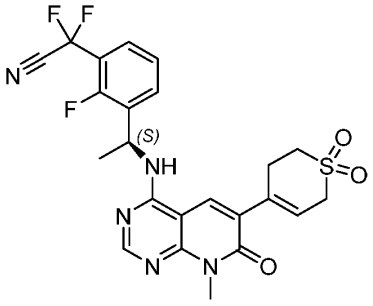
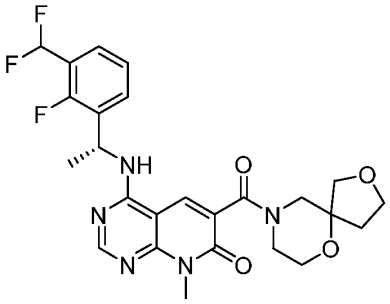
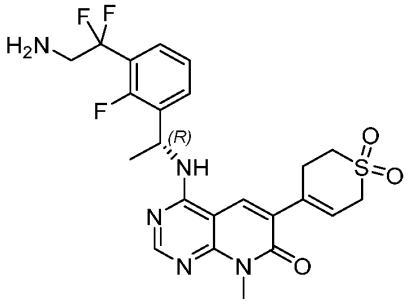
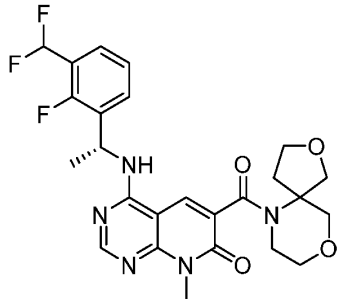
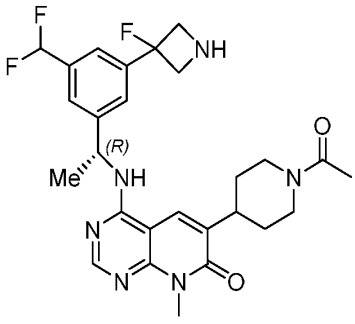


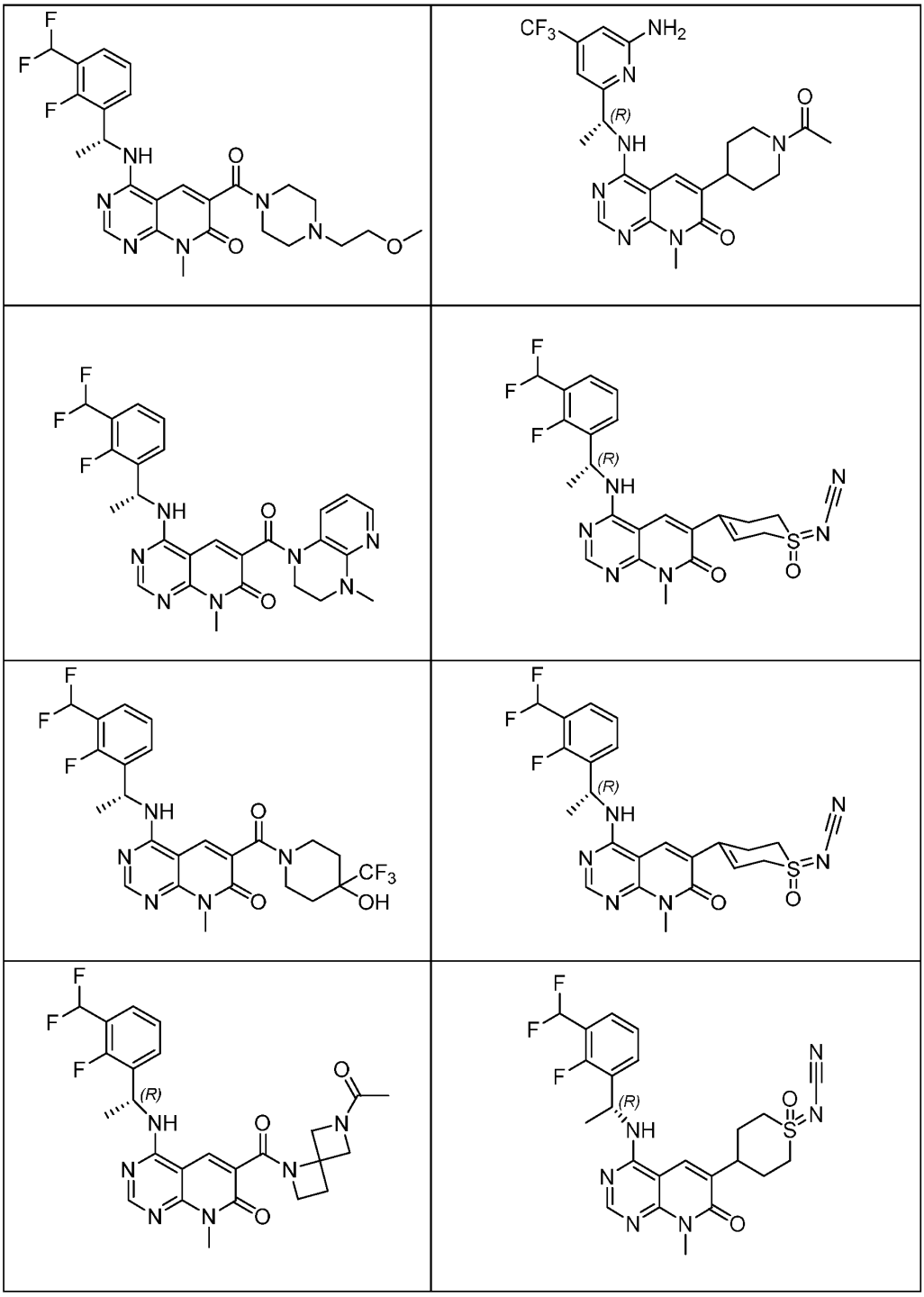
	
	
	
	

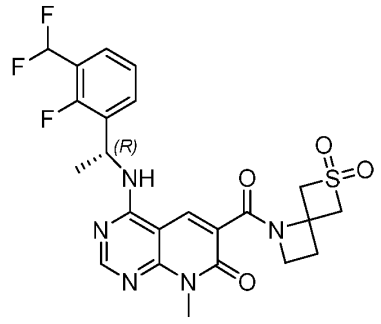
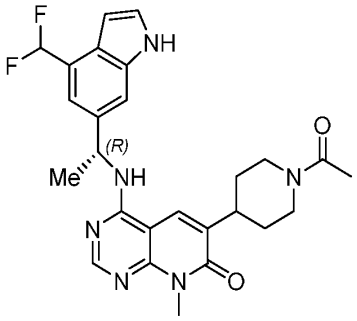
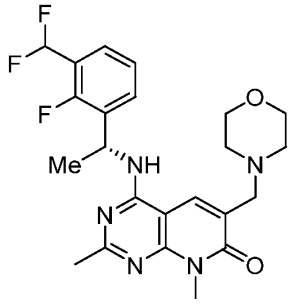
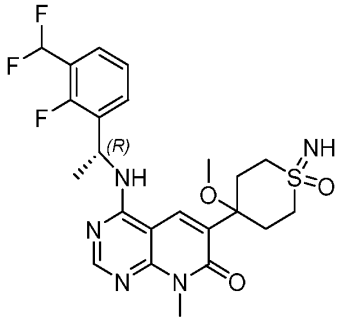
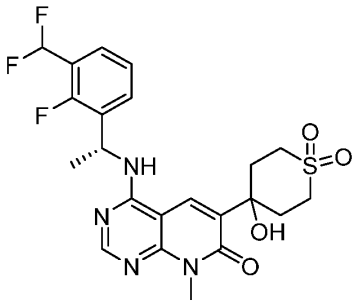
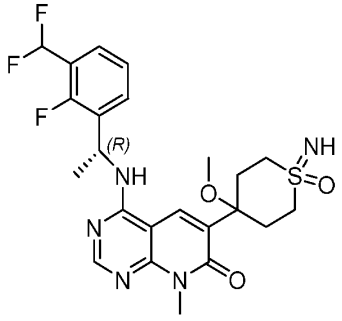
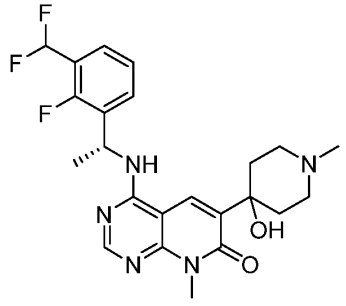
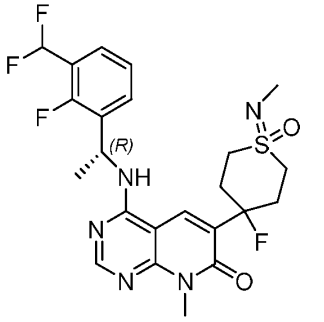
	
	
	
	

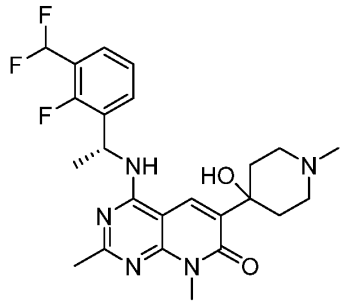
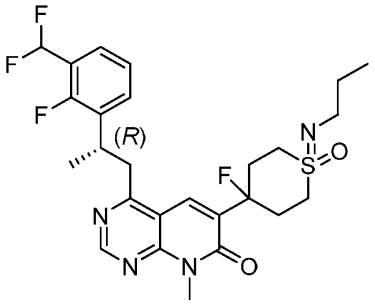
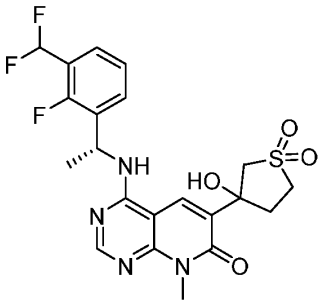
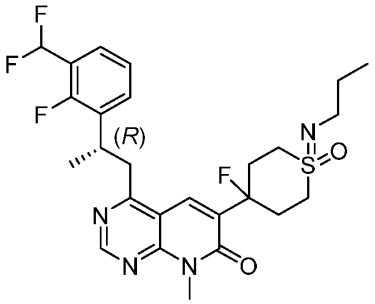
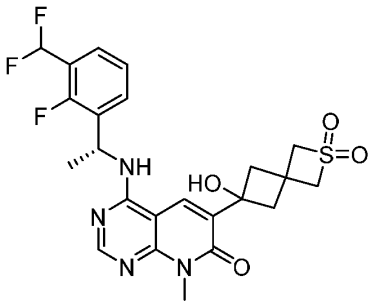
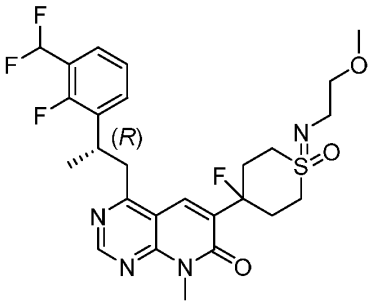
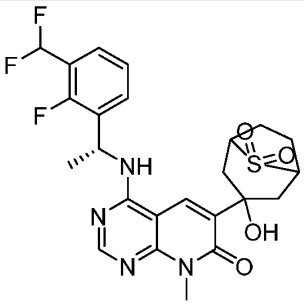
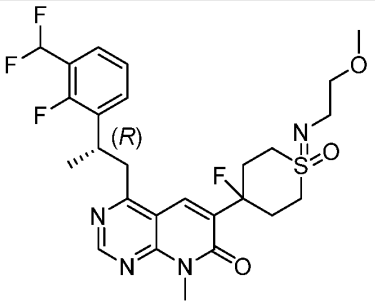


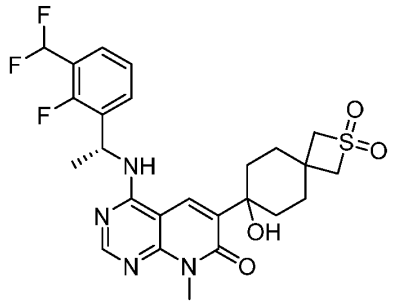
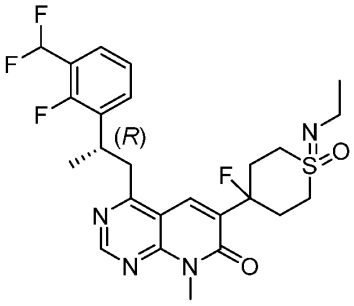
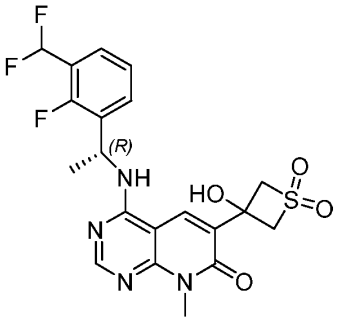
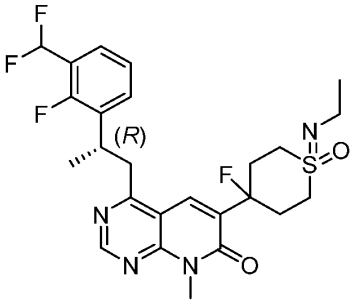
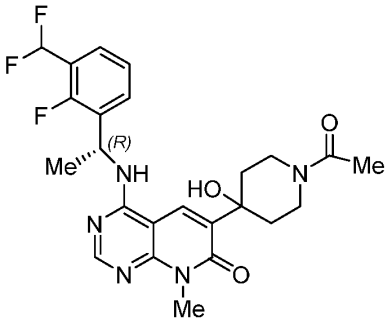
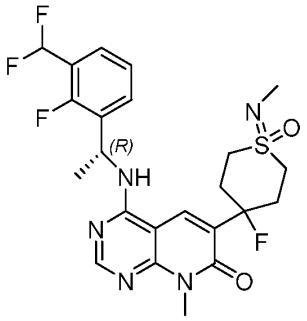
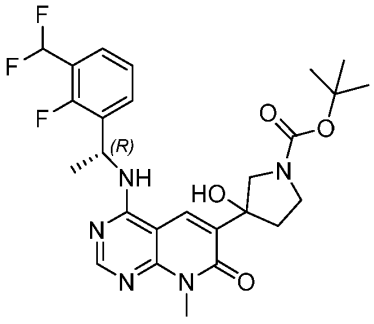
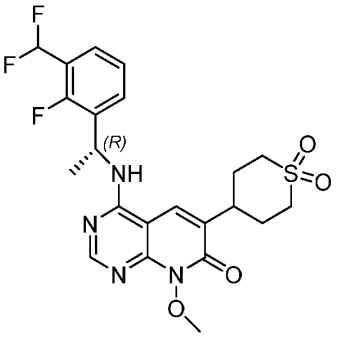
	
	
	
	

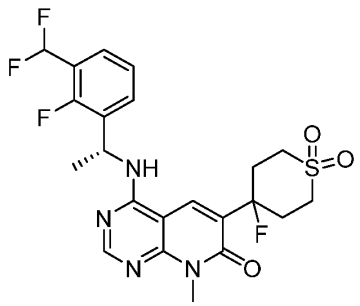
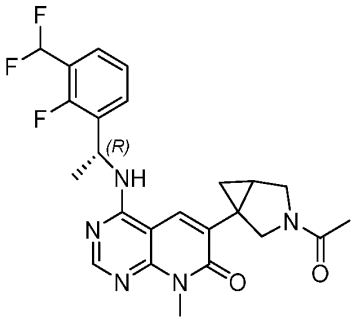
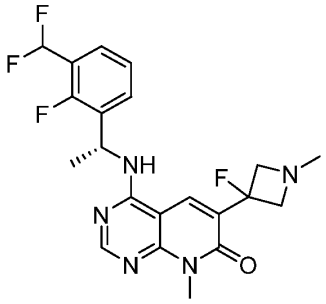
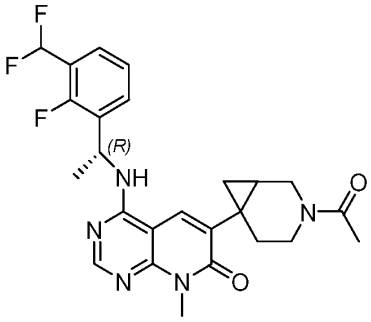
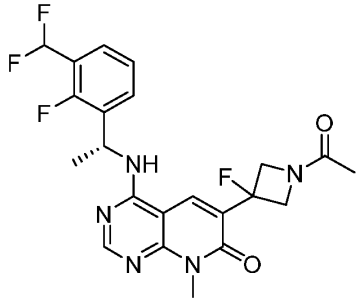
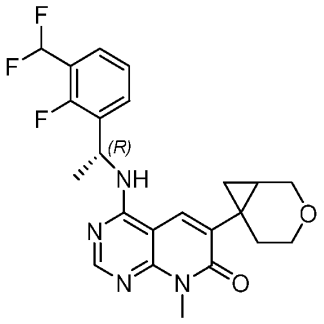
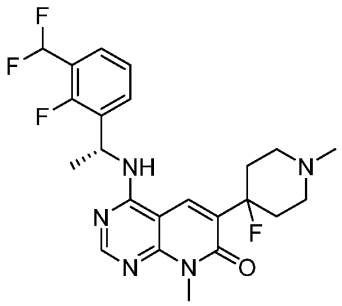
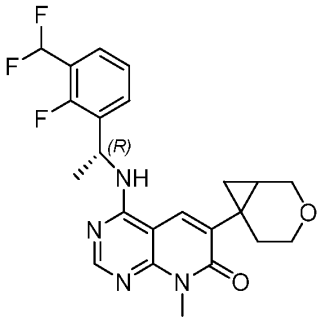
	
	
	
	

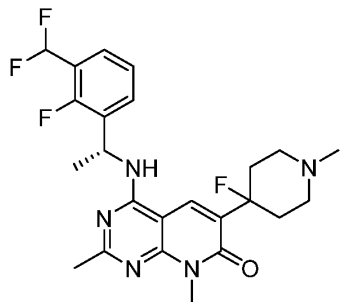
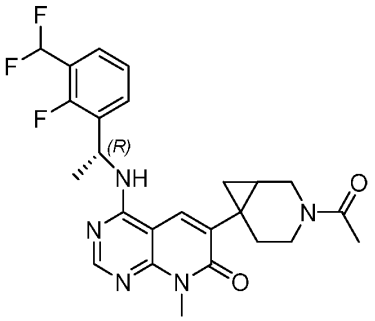
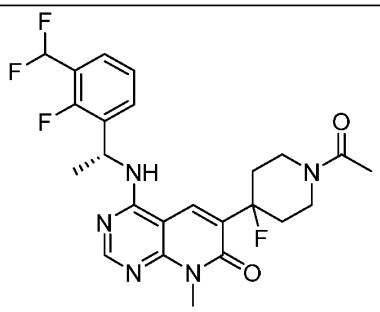
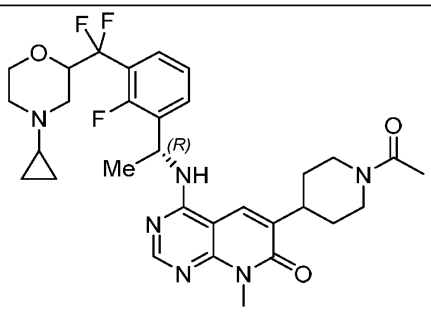
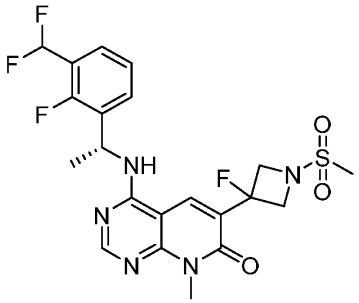
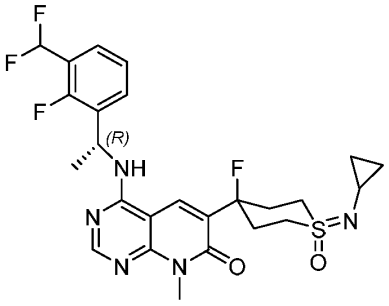
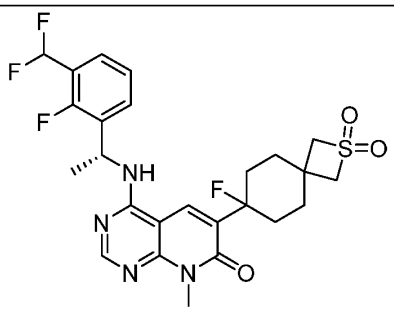
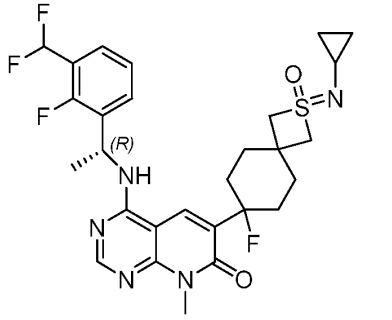


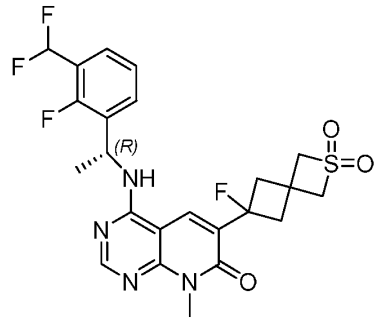
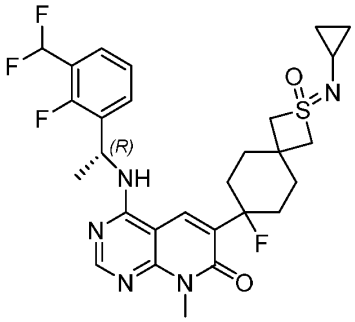
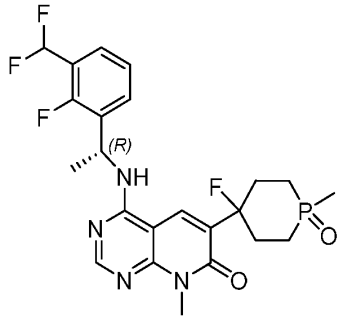
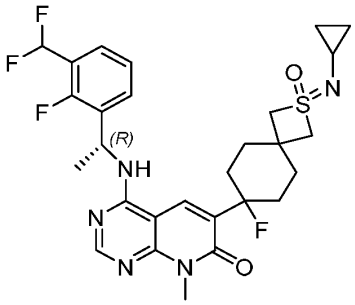
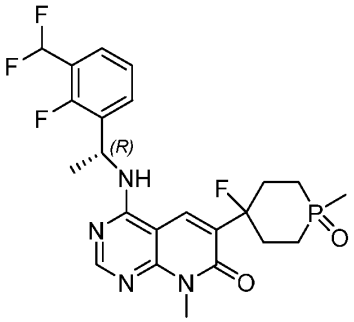
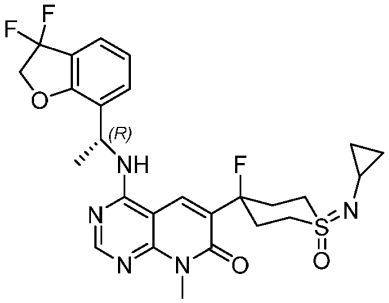
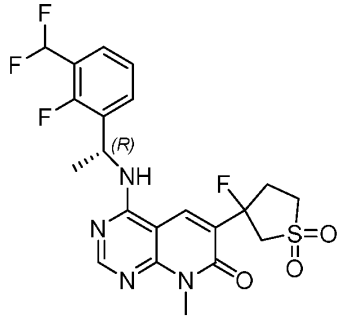
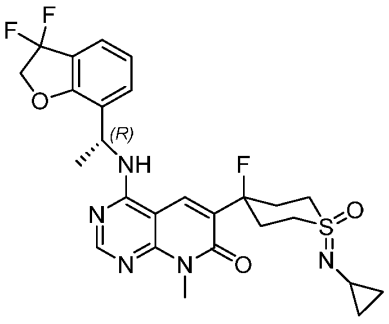
	
	
	
	

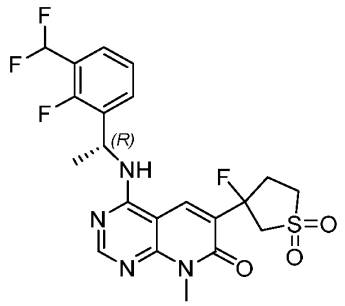
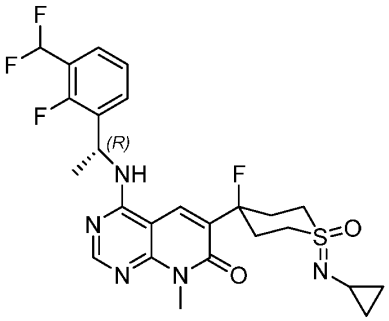
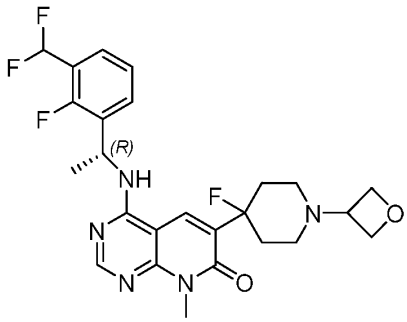
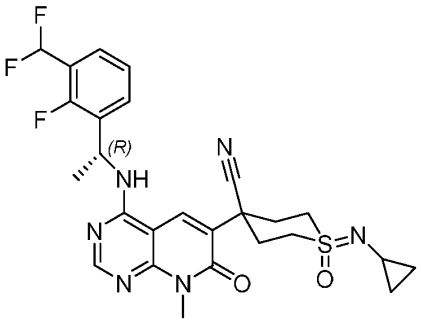
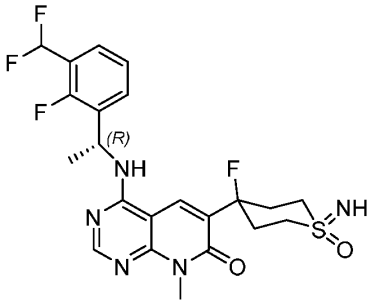
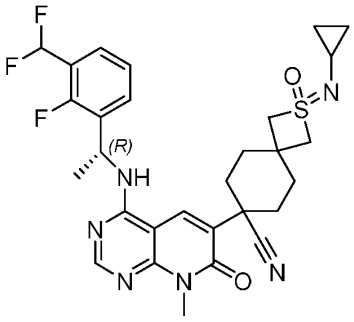
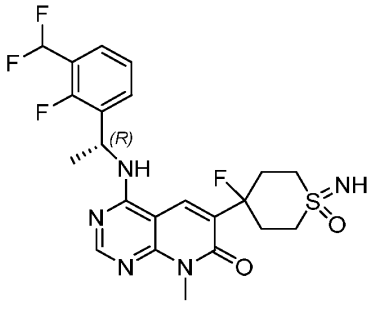
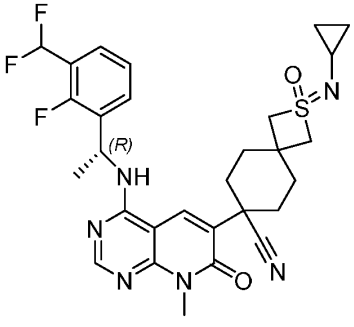
	
	
	
	

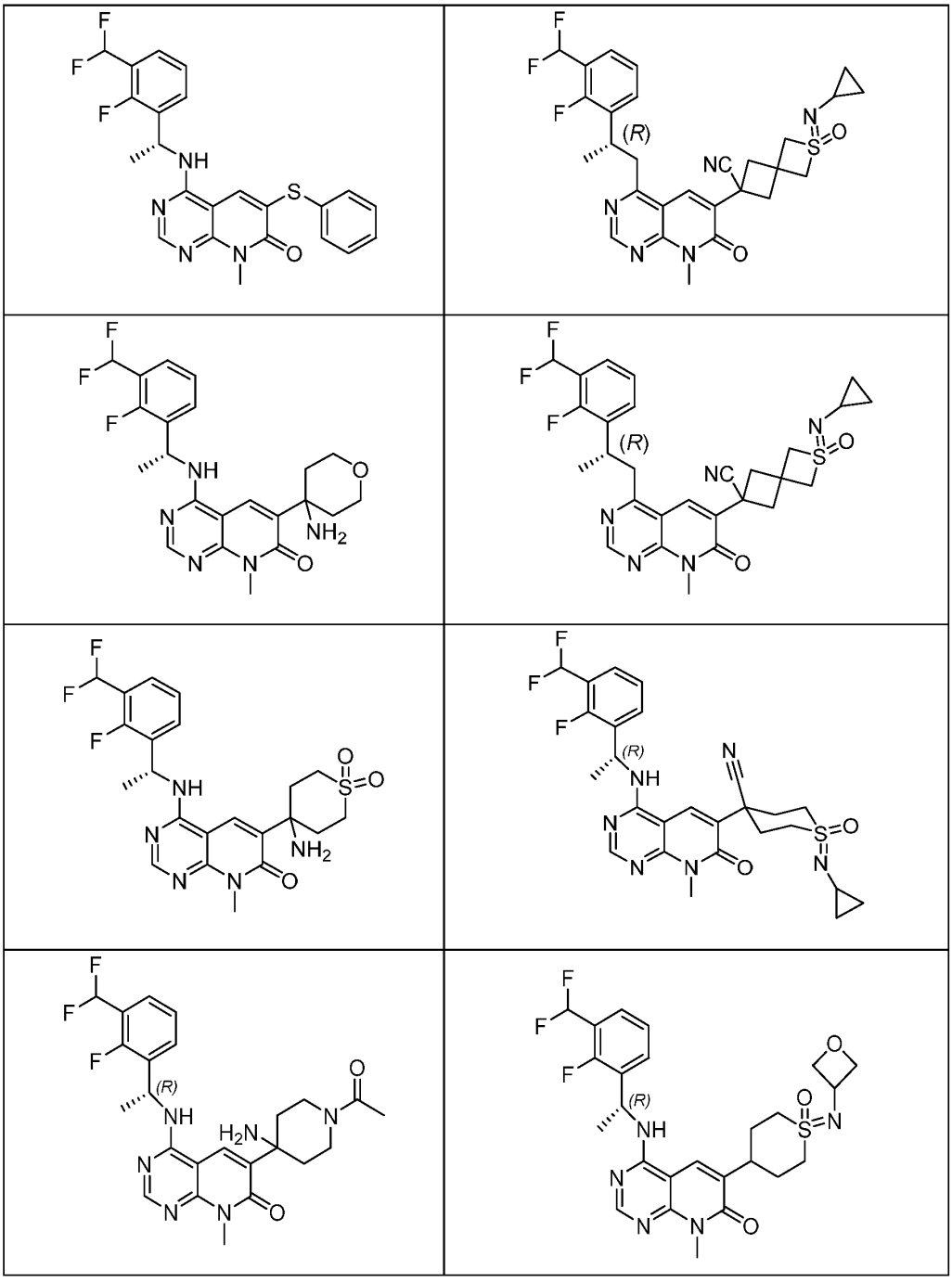
	
	
	
	

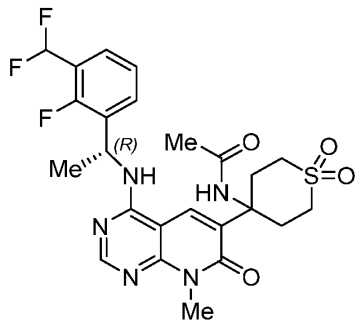
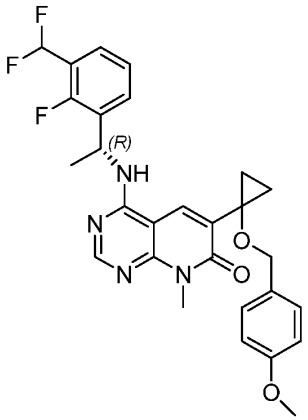
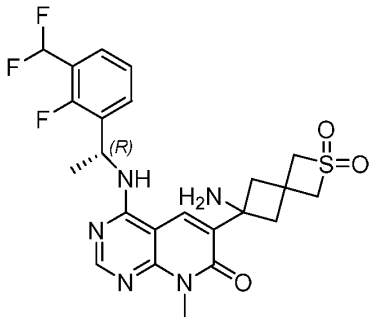
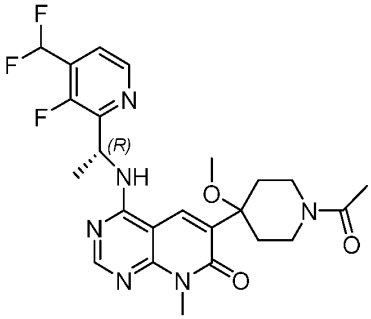
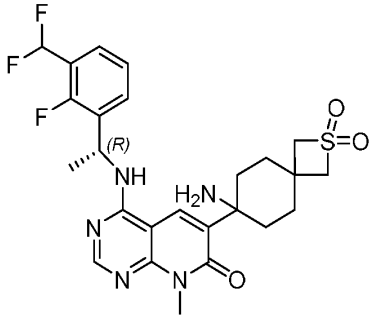
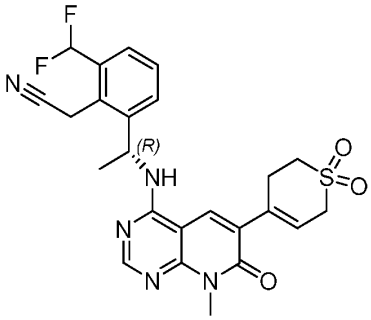
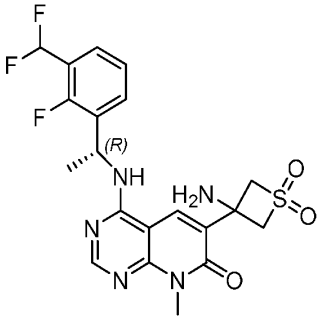
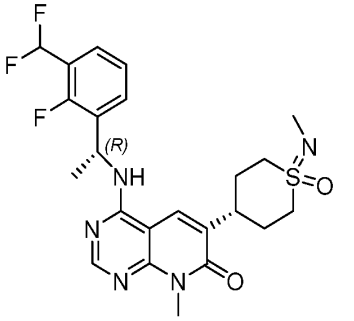
	
	
	
	

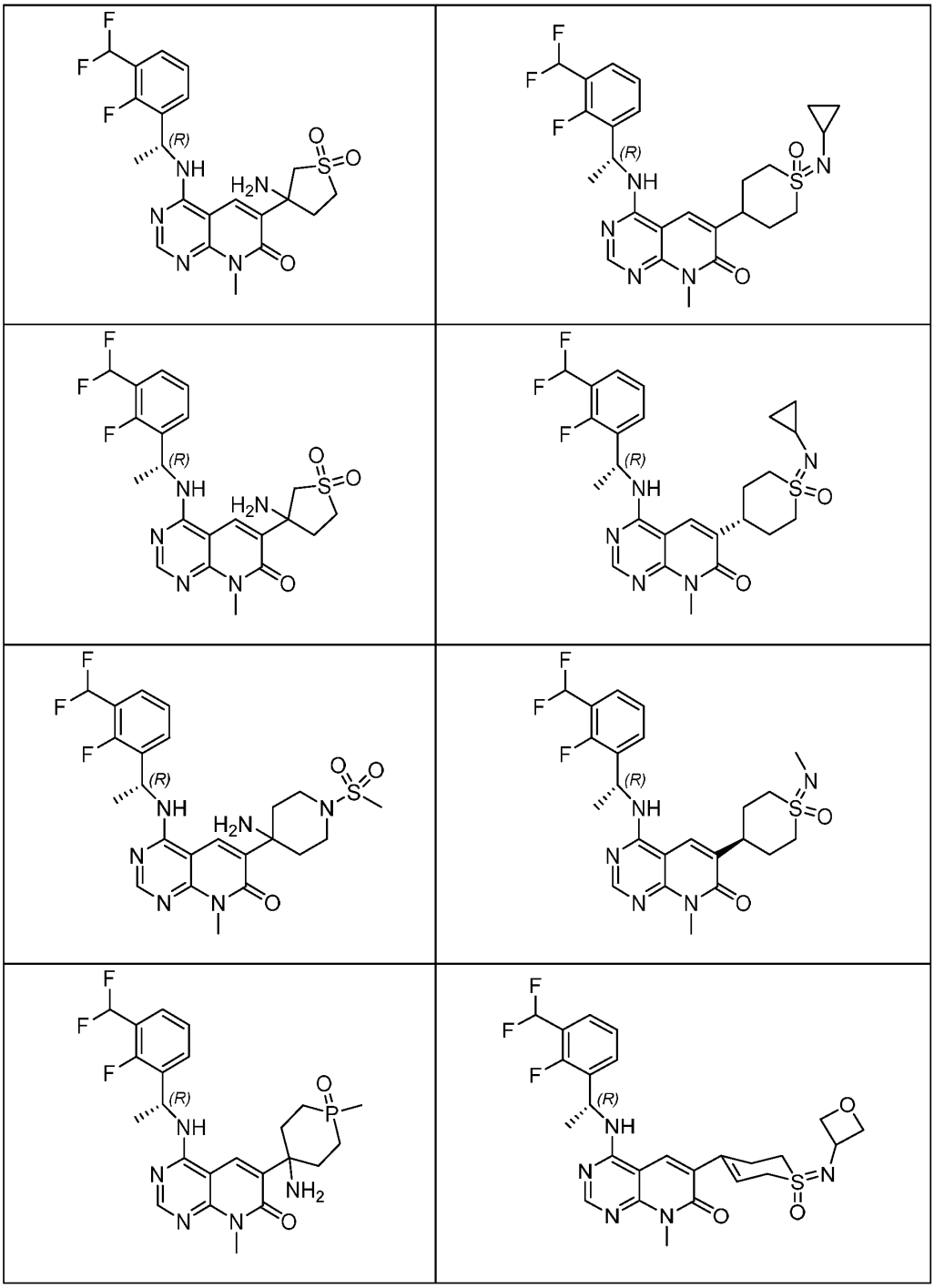
	
	
	
	

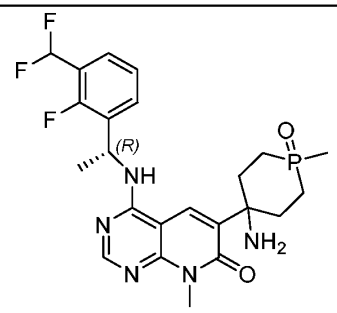
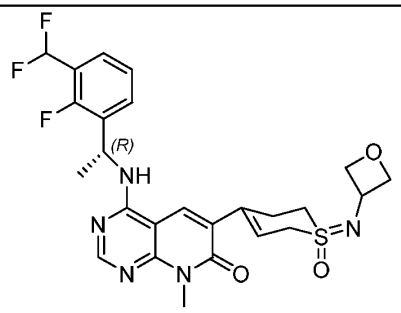
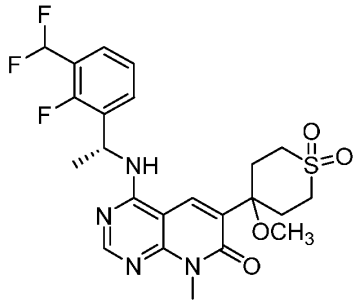
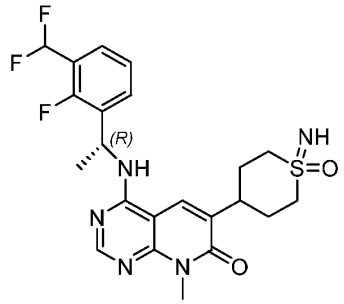
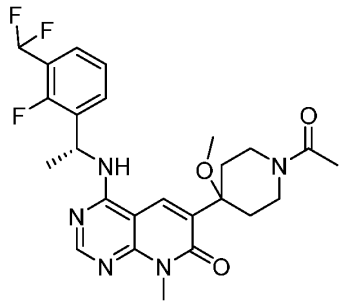
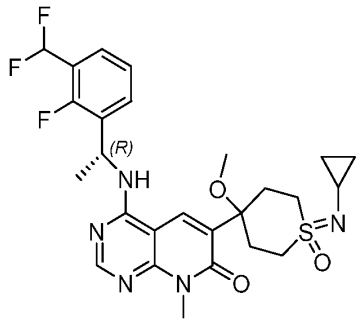
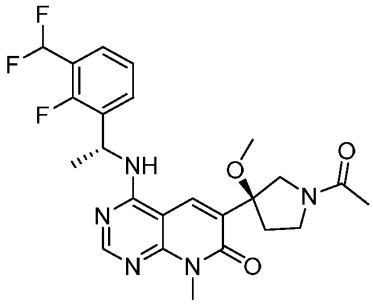
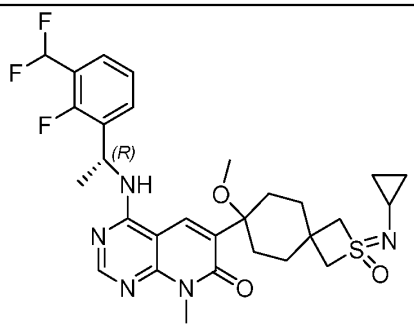
	
	
	
	

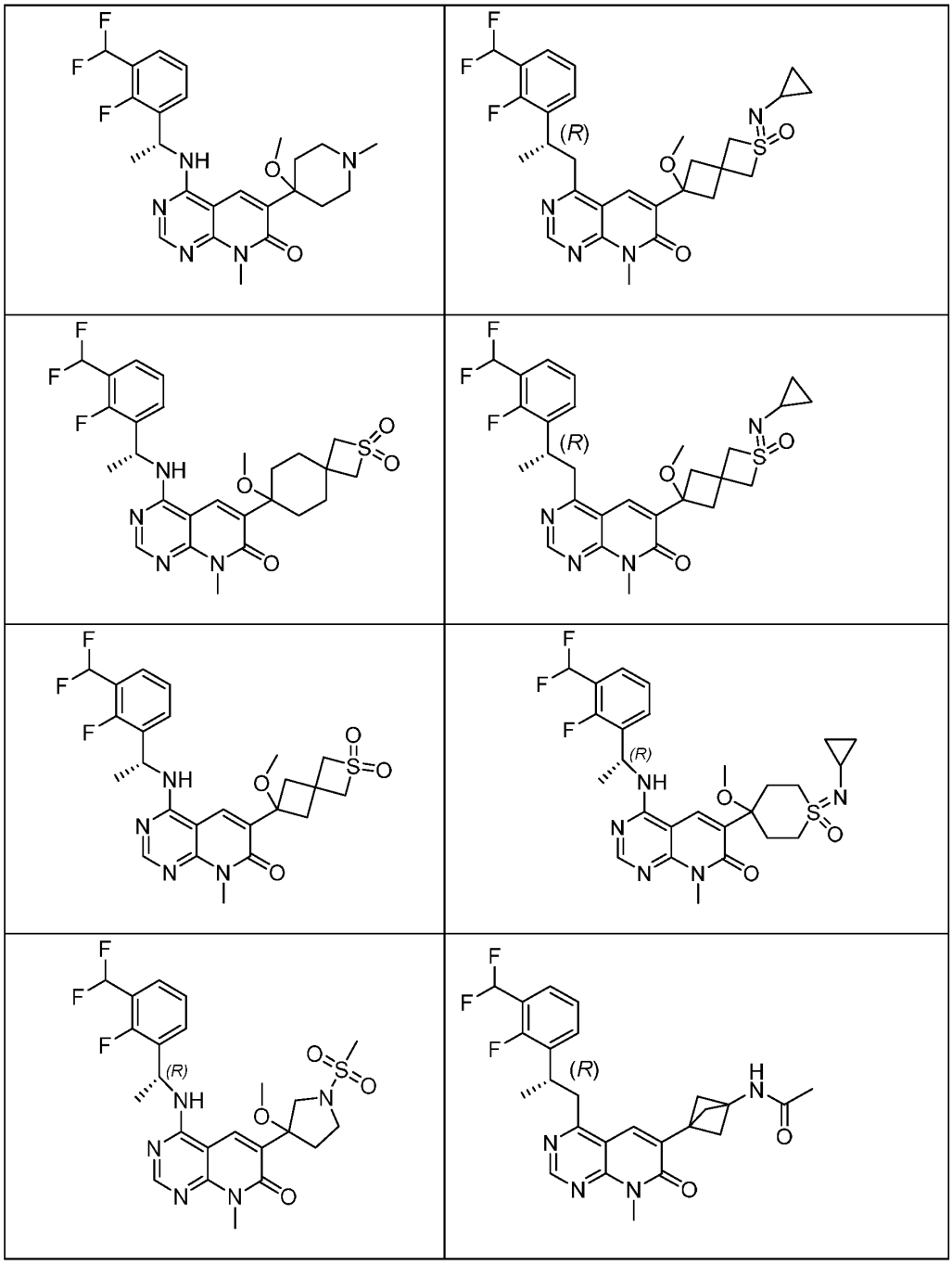
	
	
	
	

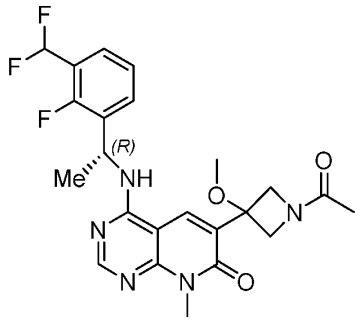
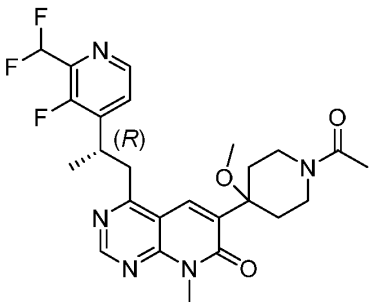
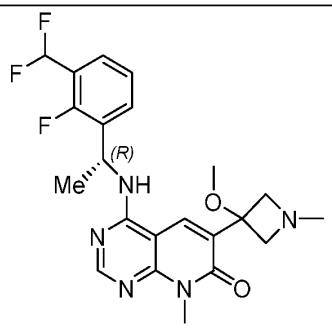
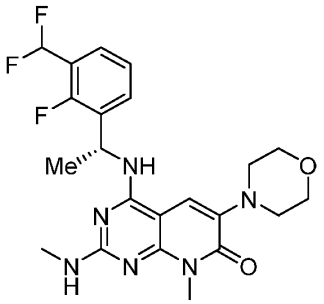
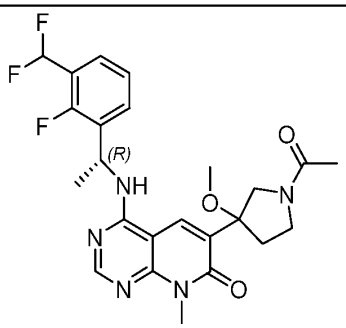
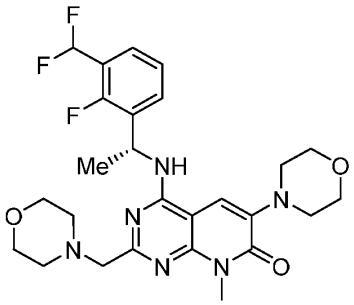
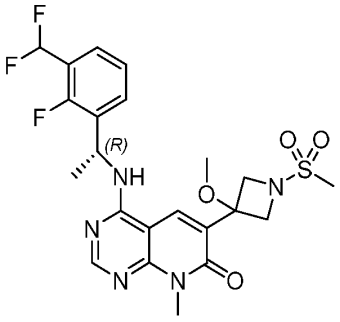
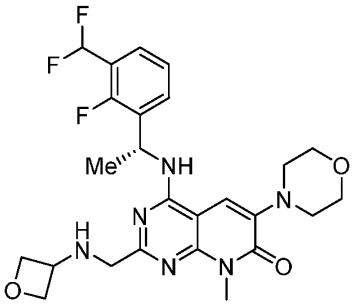


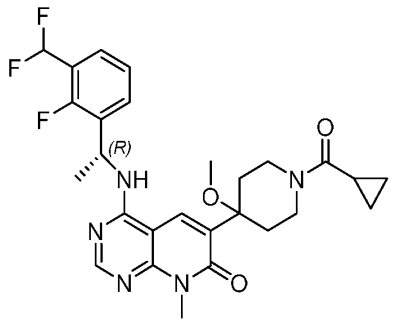
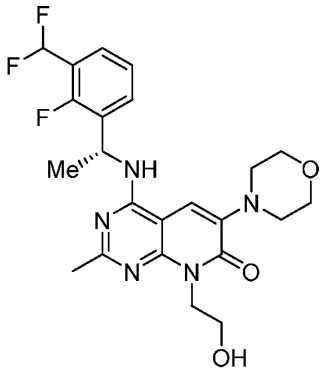
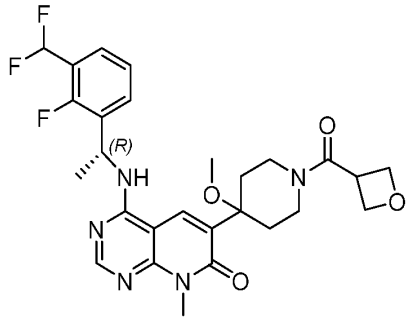
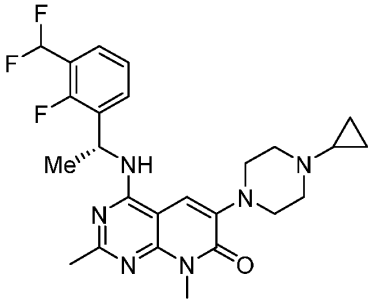
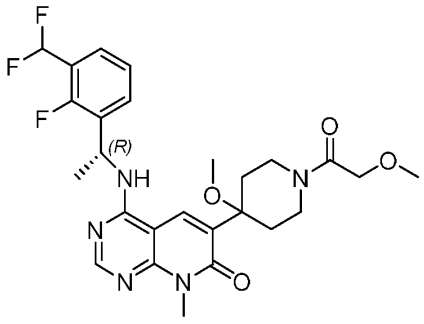
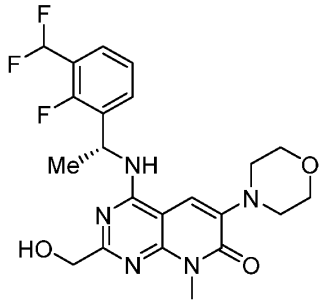
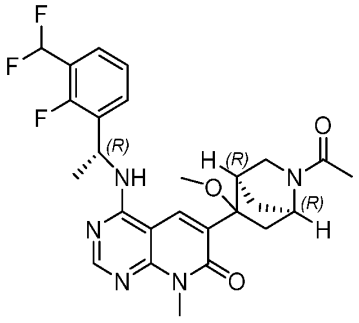
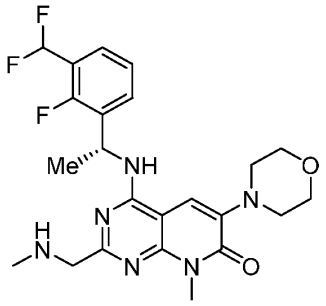
	
	
	
	

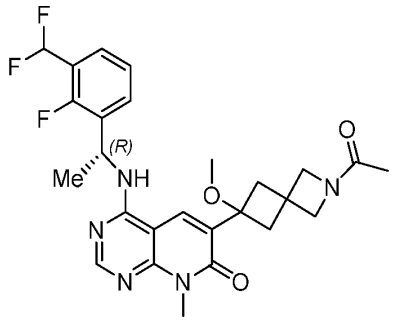
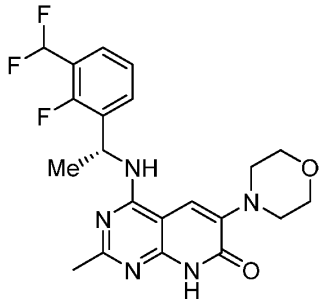
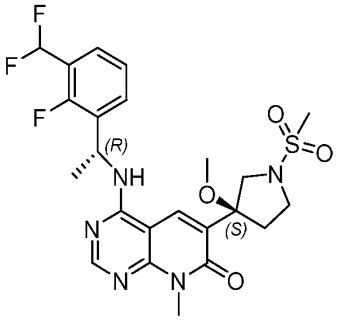
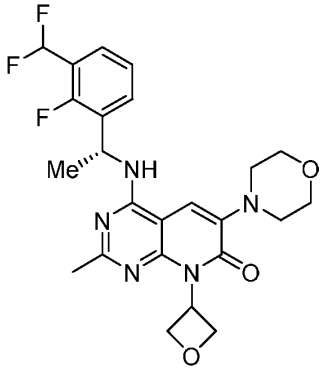
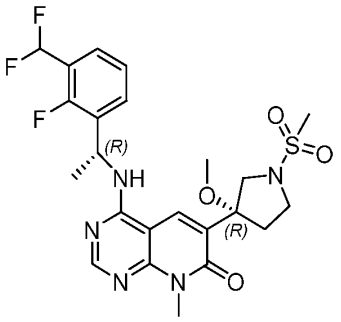
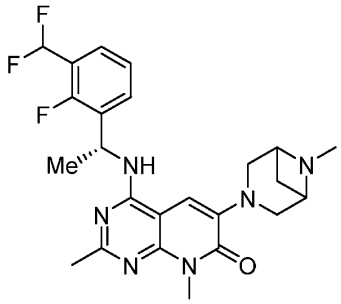
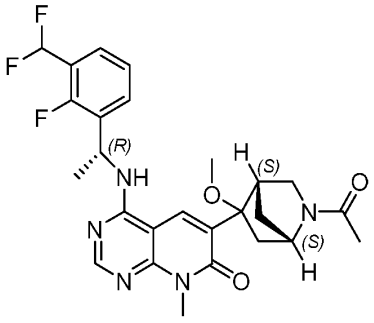
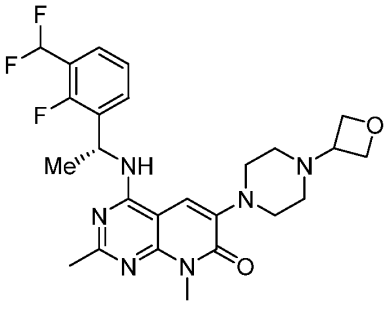


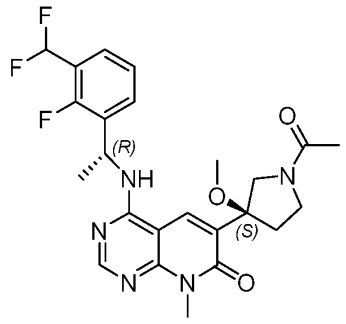
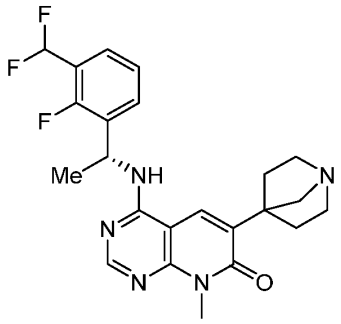
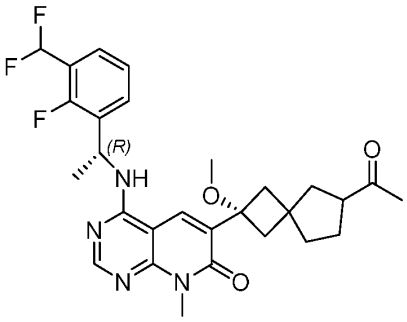
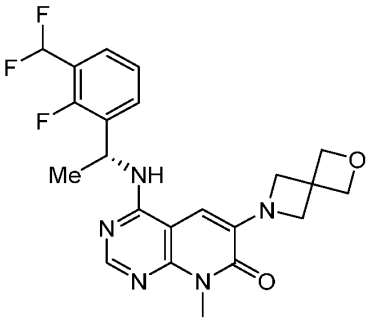
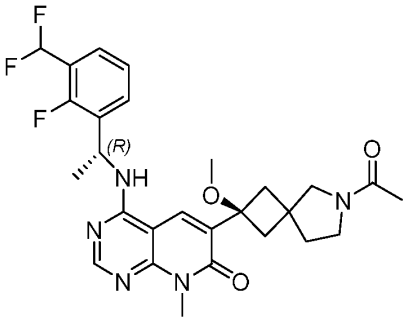
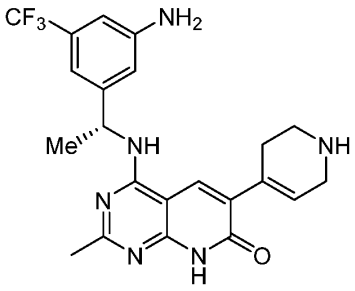
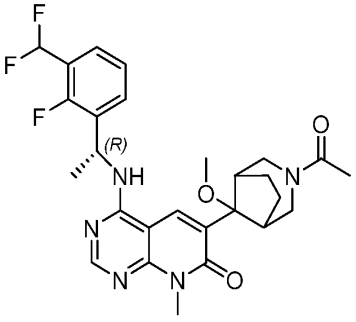
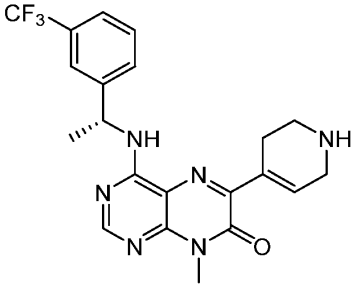
	
	
	
	

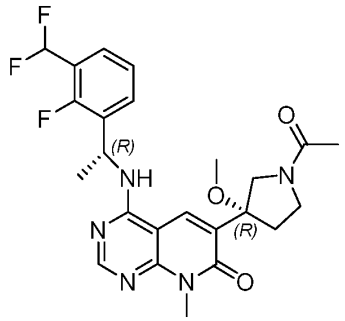
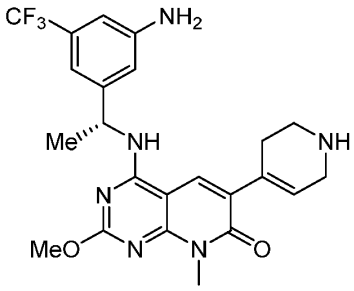
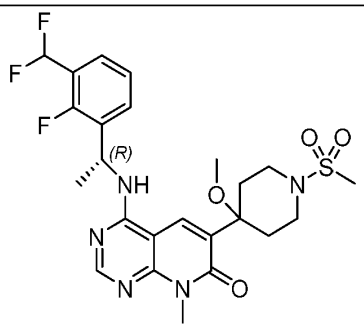
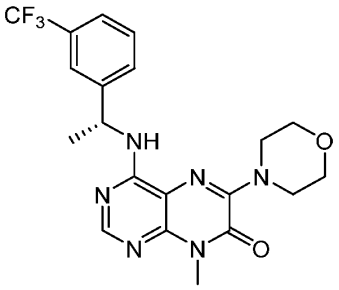
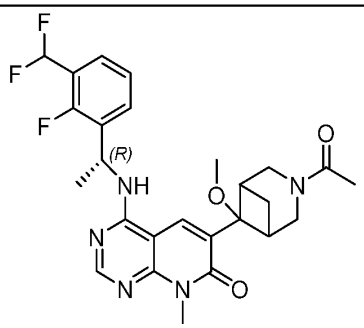
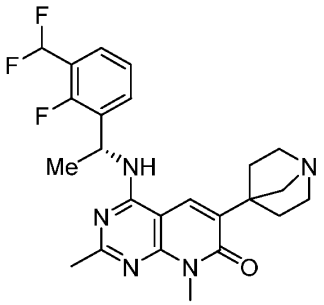
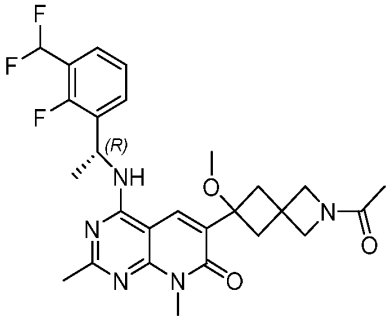
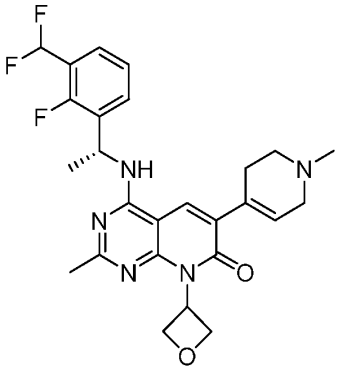


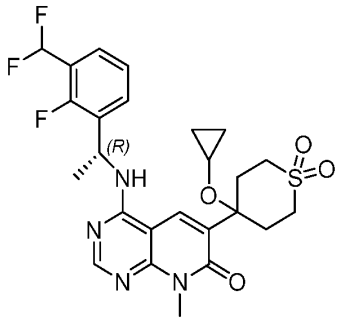
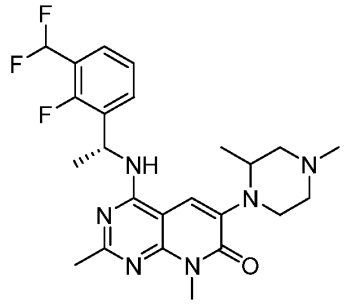
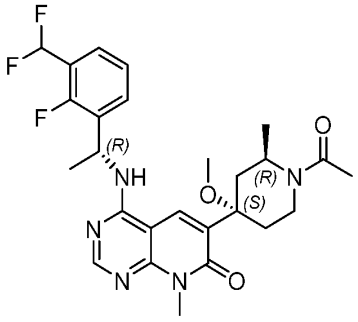
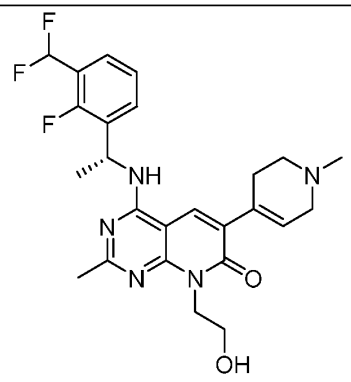
	
	
	
	

Note: any compound shown in the foregoing table may be a diastereomer, and the absolute stereochemistry of such diastereomer may not be known.

[0107] In some embodiments, the SOS1 inhibitor is selected from the group consisting of:

(*R*)-4-((1-(3-amino-5-(trifluoromethyl)phenyl)ethyl)amino)-8-methyl-6-(1,2,3,6-tetrahydropyridin-4-yl)pyrido[2,3-d]pyrimidin-7(8H)-one;

(R)-4-((1-(3-(1,1-difluoro-2-hydroxyethyl)phenyl)ethyl)amino)-8-methyl-6-morpholinopyrido[2,3-d]pyrimidin-7(8H)-one;

(*R*)-6-(3,6-dihydro-2H-pyran-4-yl)-8-methyl-4-((1-(3-(trifluoromethyl)phenyl)ethyl)amino)pyrido[2,3-d]pyrimidin-7(8H)-one;

4-{[(1*R*)-1-[3-amino-5-(trifluoromethyl)phenyl]ethyl]amino}-8-methyl-6-(morpholin-4-yl)-7H,8H-pyrido[2,3-d]pyrimidin-7-one;

8-methyl-6-(morpholin-4-yl)-4-{[(1*R*)-1-[3-(trifluoromethyl)phenyl]-ethyl]amino}-7H,8H-pyrido[2,3-d]pyrimidin-7-one;

4-{[(1*R*)-1-[3-(difluoromethyl)-2-fluorophenyl]ethyl]amino}-6-(1-methanesulfonyl-3-methylazetidin-3-yl)-8-methyl-7H,8H-pyrido[2,3-d]pyrimidin-7-one;

6-(1-acetyl-4- piperidyl)-4-[[(1*R*)-1-[3-amino-2-fluoro-5-(trifluoromethyl)phenyl]ethyl]amino]-8-methyl-pyrido[2,3-d]pyrimidin-7-one;

6-(1-acetyl-4-piperidyl)-4-[[(1*R*)-1-[5-amino-2-fluoro-3-(trifluoromethyl)phenyl]ethyl]amino]-8-methyl-pyrido[2,3-d]pyrimidin-7-one;

(*R*)-4-((1-(3-(difluoromethyl)-2-fluorophenyl)ethyl)amino)-8-methyl-6-(pyridazin-4-yl)pyrido[2,3-d]pyrimidin-7(8H)-one;

4-(((R)-1-(3-(difluoromethyl)-2-fluorophenyl)ethyl)amino)-8-methyl-6-(1-oxido-3,6-dihydro-2H-thiopyran-4-yl)pyrido[2,3-d]pyrimidin-7(8H)-one;

4-(((*R*)-1-(3-(difluoromethyl)-2-fluorophenyl)ethyl)amino)-6-(1-imino-1-oxido-1,2,3,6-tetrahydro-1λ^6-thiopyran-4-yl)-8-methylpyrido[2,3-d]pyrimidin-7(8H)-one;

4-(((*R*)-1-(3-(difluoromethyl)-2-fluorophenyl)ethyl)amino)-8-methyl-6-(1-(methylimino)-1-oxido-1,2,3,6-tetrahydro-1λ^6-thiopyran-4-yl)pyrido[2,3-d]pyrimidin-7(8H)-one;

(R)-4-((1-(3-(difluoromethyl)-2-fluorophenyl)ethyl)amino)-8-methyl-6-(1-oxidotetrahydro-2H-thiopyran-4-yl)pyrido[2,3-d]pyrimidin-7(8H)-one;

4-(4-{[(1*R*)-1-[3-(difluoromethyl)-2-fluorophenyl]-ethyl]amino}-8-methyl-7-oxo-7H,8H-pyrido[2,3-d]pyrimidin-6-yl)-4-hydroxy-1λ^6-thiane-1,1-dione;

4-(4-{[(1*R*)-1-[3-(difluoromethyl)-2-fluorophenyl]ethyl]amino}-8-methyl-7-oxo-7H,8H-pyrido[2,3-d]-pyrimidin-6-yl)-4-fluoro-1λ^6-thiane-1,1-dione;

4-{[(1*R*)-1-[3-(difluoromethyl)-2-fluorophenyl]ethyl]amino}-8-methyl-6-(phenylsulfanyl)-7H,8H-pyrido[2,3-d]pyrimidin-7-one;

6-(4-aminooxan-4-yl)-4-{[(1*R*)-1-[3-(difluoromethyl)-2-fluorophenyl]ethyl]amino}-8-methyl-7H,8H-pyrido-[2,3-d]pyrimidin-7-one;

4-(4-{[(1*R*)-1-[3-(difluoro-methyl)-2-fluorophenyl]ethyl]amino}-8-methyl-7-oxo-7H,8H-pyrido[2,3-d]pyrimidin-6-yl)-4-methoxy-1λ^6-thiane-1,1-dione;

6-(4-{[(1*R*)-1-[3-(difluoromethyl)-2-fluorophenyl]ethyl]amino}-8-methyl-7-oxo-7H,8H-pyrido[2,3-d]pyrimi-din-6-yl)-2λ⁶-thiaspiro[3.3]heptane-2,2-dione;

4-(((*R*)-1-(3-(difluoromethyl)-2-fluorophenyl)ethyl)amino)-6-(3-hydroxypiperidin-4-yl)-8-methylpyrido[2,3-d]pyrimidin-7(8H)-one;

(*R*)-4-((1-(3-(difluoromethyl)-2-fluorophenyl)ethyl)amino)-6-(1-imino-1-oxidohexahydro-1λ⁶-thiopyran-4-yl)-8-methylpyrido[2,3-d]pyrimidin-7(8H)-one;

1-acetyl-4-(4-{[(1*R*)-1-[3-(difluoromethyl)-2-fluorophenyl]ethyl]amino}-8-methyl-7-oxo-7H,8H-pyrido[2,3-d]pyrimidin-6-yl)piperidine-4-carbonitrile;

4-(4-{[(1*R*)-1-[3-(difluoromethyl)-2-fluorophenyl]ethyl]amino}-8-methyl-7-oxo-7H,8H-pyrido[2,3-d]pyrimidin-6-yl)-4-methyl-1λ⁶-thiane-1,1-dione;

6-(1-acetylpiperidin-4-yl)-4-{[(1*R*)-1-[2,3-bis(difluoromethyl)phenyl]ethyl]amino}-8-methyl-7H,8H-pyrido[2,3-d]pyrimidin-7-one;

6-(1-acetylpiperidin-4-yl)-4-{[(1*R*)-1-[3-(difluoromethyl)-2-(fluoromethyl)phenyl]ethyl]amino}-8-methyl-7H,8H-pyrido[2,3-d]pyrimidin-7-one;

2-{3-[(1*R*)-1-{[6-(1,1-dioxo-3,6-dihydro-2H-1λ⁶-thiopyran-4-yl)-8-methyl-7-oxo-7H,8H-pyrido[2,3-d]pyrimidin-4-yl]amino}ethyl]phenyl}-2,2-difluoroacetonitrile;

2-{3-[(1*R*)-1-{[6-(1,1-dioxo-3,6-dihydro-2H-1λ⁶-thiopyran-4-yl)-8-methyl-7-oxo-7H,8H-pyrido[2,3-d]pyrimidin-4-yl]amino}ethyl]-2-fluorophenyl}-2,2-difluoroacetonitrile;

4-(4-{[(1*R*)-1-[3-(2-amino-1,1-difluoroethyl)-2-fluorophenyl]ethyl]amino}-8-methyl-7-oxo-7H,8H-pyrido[2,3-d]pyrimidin-6-yl)-3,6-dihydro-2H-1λ⁶-thiopyran-1,1-dione;

6-(1-acetyl-4-piperidyl)-4-[[(1*R*)-1-[3-(difluoromethyl)-5-(3-fluoroazetidin -3-yl)phenyl]ethyl]amino]-8-methyl-pyrido[2,3-d]pyrimidin-7-one;

[4-[4-[[(1*R*)-1-[3-(difluoromethyl)-2-fluoro-phenyl]ethyl]amino]-8-methyl-7-oxo-pyrido[2,3-d]pyrimidin-6-yl]-1-oxo-3,6-dihydro-2H-thiopyran-1-ylidene]cyanamide;

[4-[4-[[(1*R*)-1-[3-(difluoromethyl)-2-fluoro- phenyl]ethyl]amino]-8-methyl-7-oxo-pyrido[2,3-d]pyrimidin-6-yl]-1-oxo-thian-1-ylidene]cyanamide;

4-(((*R*)-1-(3-(difluoromethyl)-2-fluorophenyl)ethyl)amino)-6-((1*S*,4*s*)-1-imino-4-methoxy-1-oxidohexahydro-1λ^6-thiopyran-4-yl)-8-methylpyrido[2,3-d]pyrimidin-7(8H)-one;

4-(((*R*)-1-(3-(difluoromethyl)-2-fluorophenyl)ethyl)amino)-6-((1*R*,4*r*)-1-imino-4-methoxy-1-oxidohexahydro-1λ^6-thiopyran-4-yl)-8-methylpyrido[2,3-d]pyrimidin-7(8H)-one;

4-(((*R*)-1-(3-(difluoromethyl)-2-fluorophenyl)ethyl)amino)-6-((1*S*,4*s*)-4-fluoro-1-(methylimino)-1-oxidohexahydro-1λ^6-thiopyran-4-yl)-8-methylpyrido[2,3-d]pyrimidin-7(8H)-one;

4-(((*R*)-1-(3-(difluoromethyl)-2-fluorophenyl)ethyl)amino)-6-((1*R*,4*r*)-4-fluoro-1-(methylimino)-1-oxidohexahydro-1λ^6-thiopyran-4-yl)-8-methylpyrido[2,3-d]pyrimidin-7(8H)-one;

6-{3-acetyl-3-azabicyclo[3.1.0]hexan-1-yl}-4-{[(1*R*)-1-[3-(difluoromethyl)-2-fluorophenyl]ethyl]amino}-8-methyl-7H,8H-pyrido[2,3-d]pyrimidin-7-one;

6-(1-acetyl-4-piperidyl)-4-[[(1*R*)-1-[3-[(4-cyclopropylmorpholin-2-yl)-difluoro-methyl]-2-fluoro-phenyl]ethyl]amino]-8-methyl-pyrido[2,3-d]pyrimidin-7-one;

6-((1*R*,4*r*)-1-(cyclopropylimino)-4-fluoro-1-oxidohexahydro-1λ^6-thiopyran-4-yl)-4-(((*R*)-1-(3-(difluoromethyl)-2-fluorophenyl)ethyl)amino)-8-methylpyrido[2,3-d]pyrimidin-7(8H)-one;

6-((1*S*,4*s*)-1-(cyclopropylimino)-4-fluoro-1-oxidohexahydro-1λ^6-thiopyran-4-yl)-4-(((*R*)-1-(3-(difluoromethyl)-2-fluorophenyl)ethyl)amino)-8-methylpyrido[2,3-d]pyrimidin-7(8H)-one;

(1*R*,4*r*)-1-(cyclopropylimino)-4-(4-(((*R*)-1-(3-(difluoromethyl)-2-fluorophenyl)ethyl)amino)-8-methyl-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidin-6-yl)hexahydro-1λ^6-thiopyran-4-carbonitrile 1-oxide;

(1*S*,4*s*)-1-(cyclopropylimino)-4-(4-(((*R*)-1-(3-(difluoromethyl)-2-fluorophenyl)ethyl)amino)-8-methyl-7-oxo-7,8-dihydropyrido[2,3-d]pyrimidin-6-yl)hexahydro-1λ^6-thiopyran-4-carbonitrile 1-oxide;

4-[[(1*R*)-1-[3-(difluoromethyl)-2-fluoro-phenyl]ethyl]amino]-8-methyl-6-[1-(oxetan-3-ylimino)-1-oxo-thian-4-yl]pyrido[2,3-d]pyrimidin-7-one;

4-[[(1*R*)-1-[3-(difluoromethyl)-2-fluoro-phenyl]ethyl]amino]-6-[1-[(4-methoxyphenyl)methoxy]cyclopropyl]-8-methyl-pyrido[2,3-d]pyrimidin-7-one;

2-[2-(difluoromethyl)-6-[(1*R*)-1-[[6-(1,1-dioxo-3,6-dihydro-2H-thiopyran-4-yl)-8-methyl-7-oxo-pyrido[2,3-d]pyrimidin-4-yl]amino]ethyl]phenyl]acetonitrile;

4-(((*R*)-1-(3-(difluoromethyl)-2-fluorophenyl)ethyl)amino)-8-methyl-6-((1*R*,4*r*)-1-(methylimino)-1-oxidohexahydro-1λ^6-thiopyran-4-yl)pyrido[2,3-d]pyrimidin-7(8H)-one;

4-(((*R*)-1-(3-(difluoromethyl)-2-fluorophenyl)ethyl)amino)-8-methyl-6-((*S*)-1-(oxetan-3-ylimino)-1-oxido-1,2,3,6-tetrahydro-1λ^6-thiopyran-4-yl)pyrido[2,3-d]pyrimidin-7(8H)-one;

4-(((*R*)-1-(3-(difluoromethyl)-2-fluorophenyl)ethyl)amino)-8-methyl-6-((*R*)-1-(oxetan-3-ylimino)-1-oxido-1,2,3,6-tetrahydro-1λ^6-thiopyran-4-yl)pyrido[2,3-d]pyrimidin-7(8H)-one;

4-[[(1*R*)-1-[3-(difluoromethyl)-2-fluoro-phenyl]ethyl]amino]-6-(1-imino-1-oxo-thian-4-yl)-8-methyl-pyrido[2,3-d]pyrimidin-7-one;

6-((1*R*,4*r*)-1-(cyclopropylimino)-4-methoxy-1-oxidohexahydro-1λ^6-thiopyran-4-yl)-4-(((*R*)-1-(3-(difluoromethyl)-2-fluorophenyl)ethyl)amino)-8-methylpyrido[2,3-d]pyrimidin-7(8H)-one;

6-((1*S*,4*s*)-1-(cyclopropylimino)-4-methoxy-1-oxidohexahydro-1λ^6-thiopyran-4-yl)-4-(((*R*)-1-(3-(difluoromethyl)-2-fluorophenyl)ethyl)amino)-8-methylpyrido[2,3-d]pyrimidin-7(8H)-one; and

N-[3-(4-{[(1*R*)-1-[3-(difluoromethyl)-2-fluorophenyl]ethyl]amino}-8-methyl-7-oxo-7H,8H-pyrido[2,3-d]pyrimidin-6-yl)bicyclo[1.1.1]pentan-1-yl]acetamide;

or a pharmaceutically acceptable salt or a stereoisomer thereof.

[0108] In some embodiments, the SOS1 inhibitor is a compound selected from the group consisting of the compounds in the following table, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof, wherein any

compound shown in brackets in the table indicates that the compound is a diastereomer, and the absolute stereochemistry of such diastereomer may not be known:

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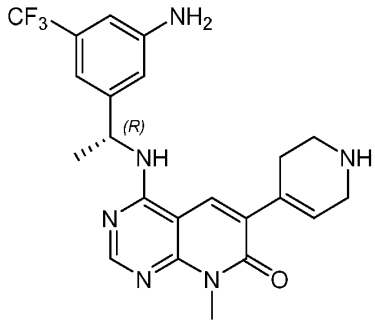
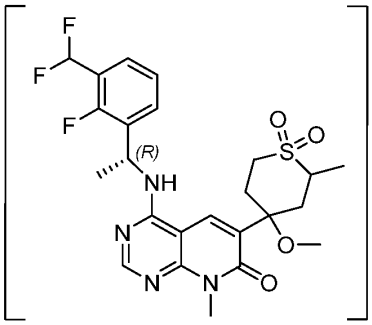
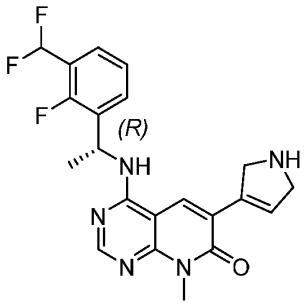
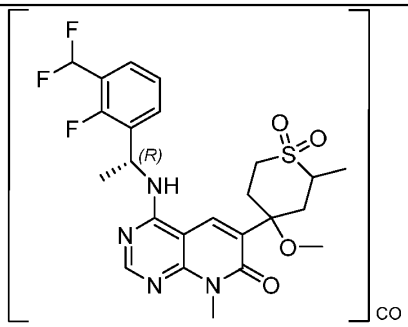
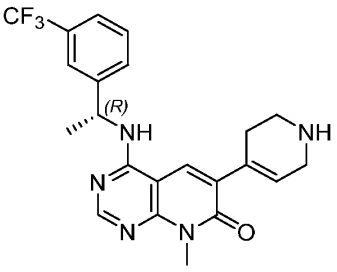
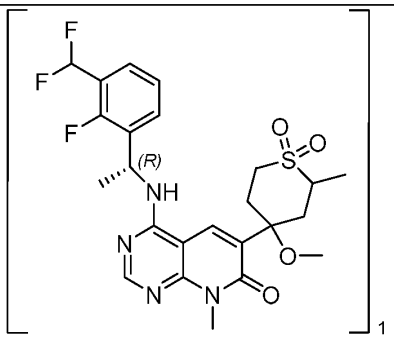
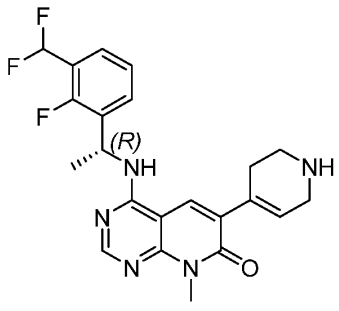
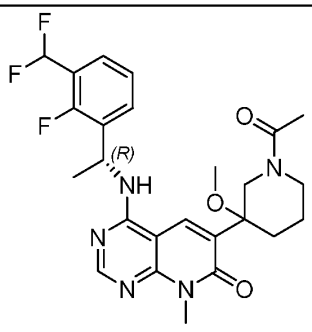
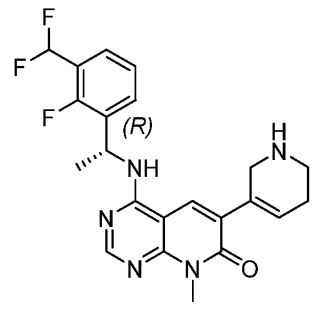
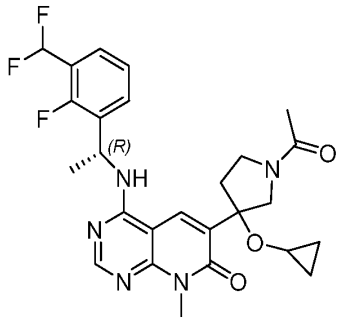
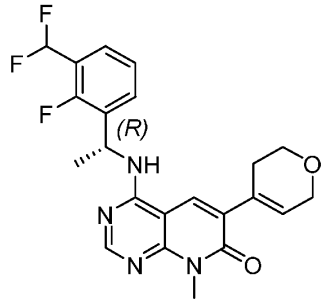
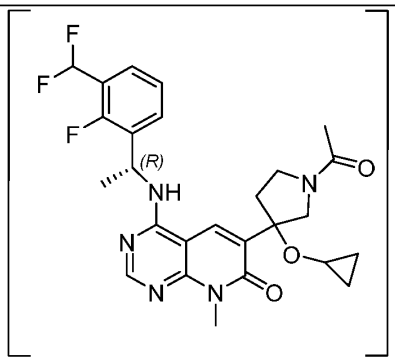
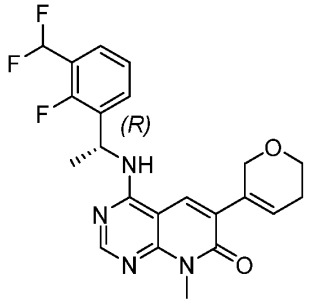
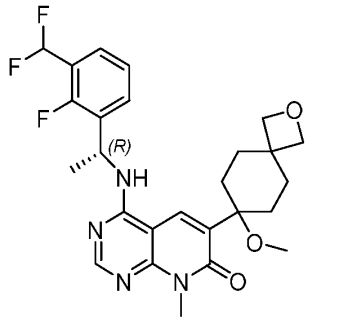
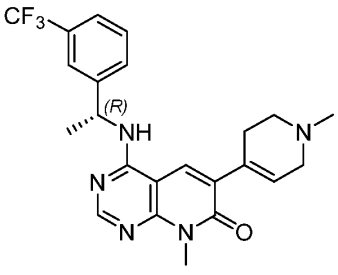
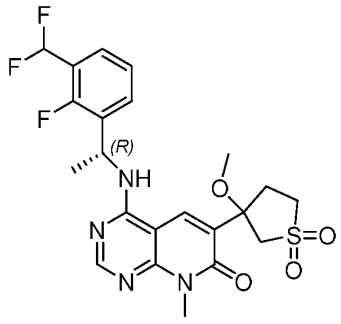
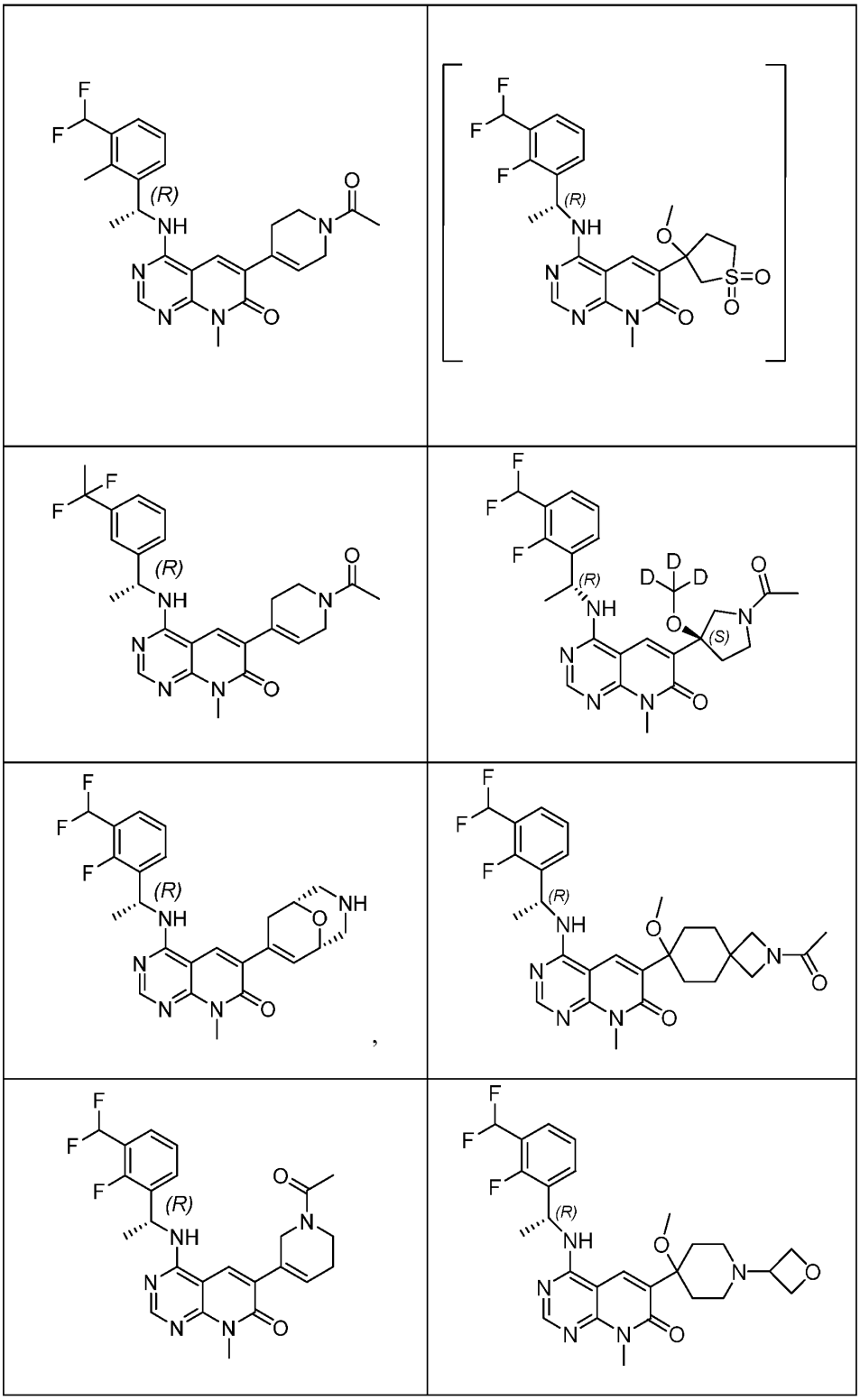
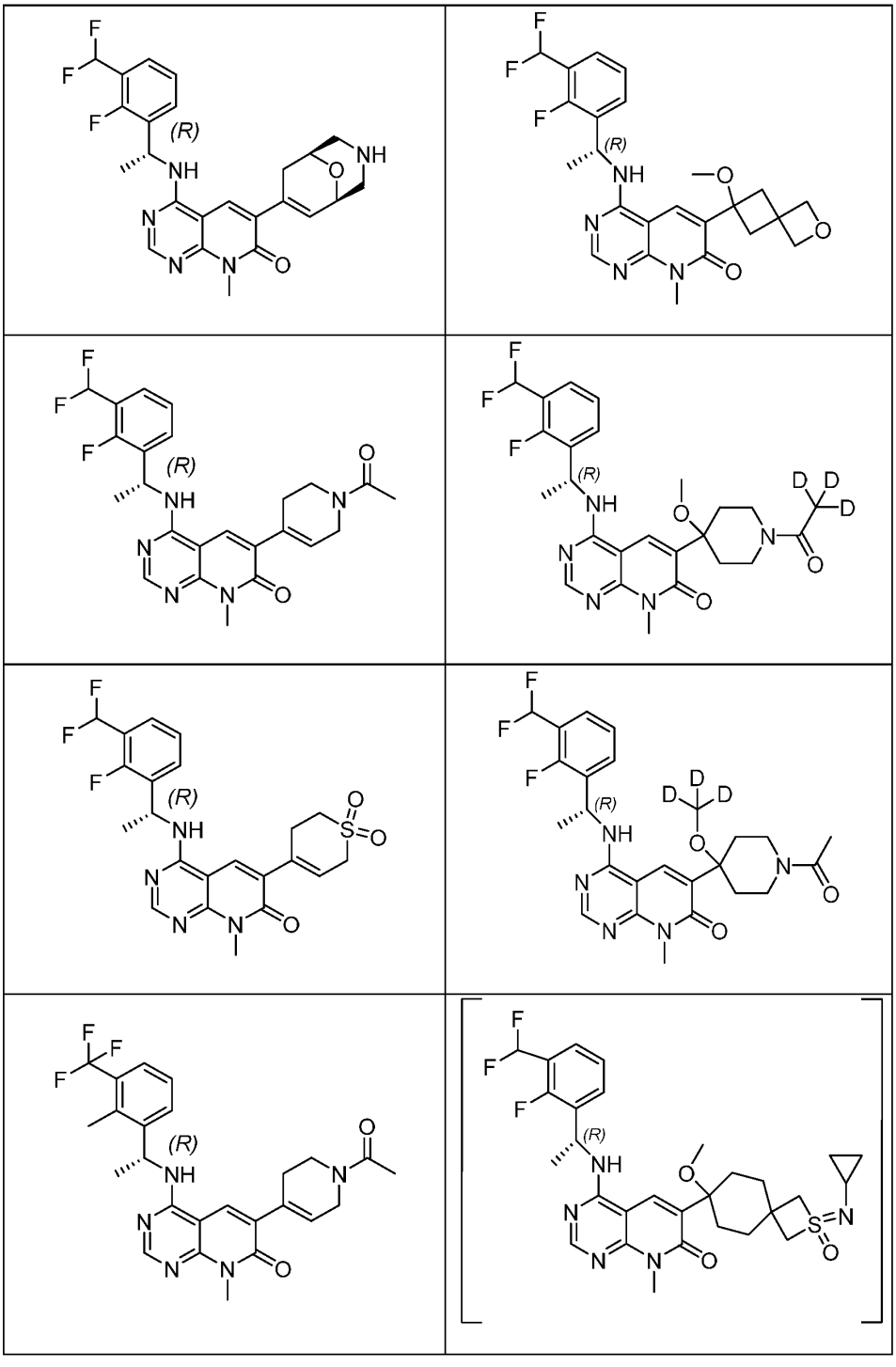
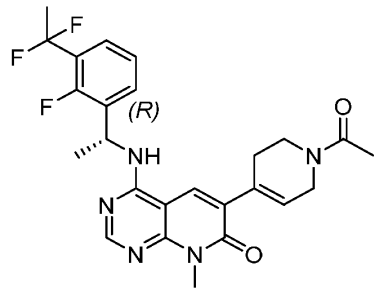
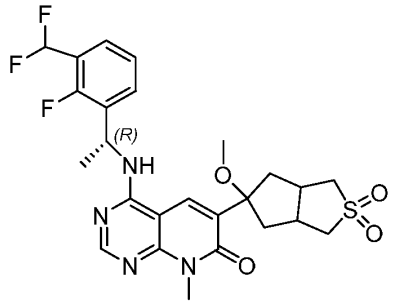
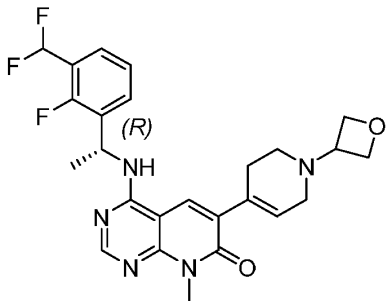
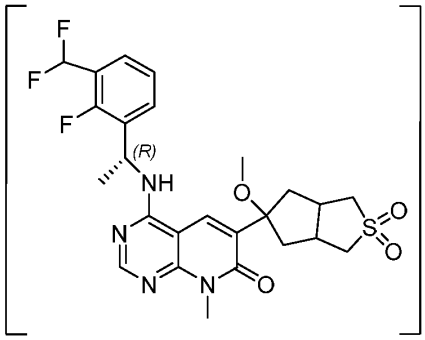
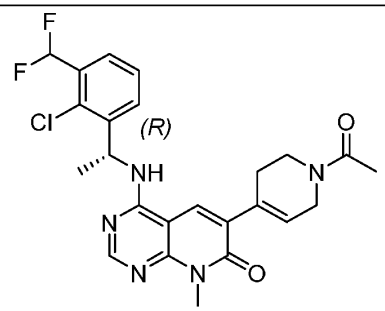
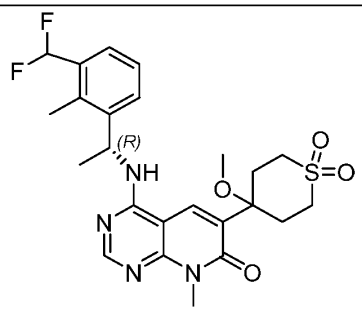
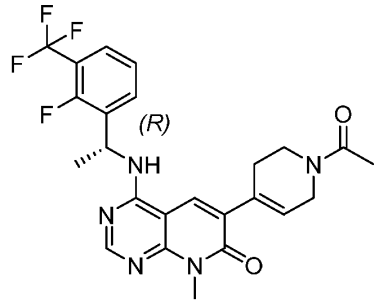
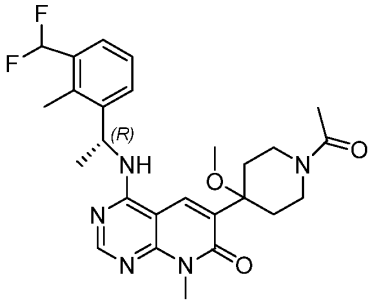
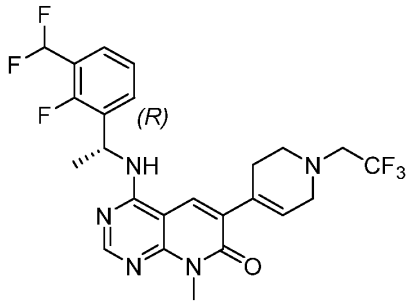
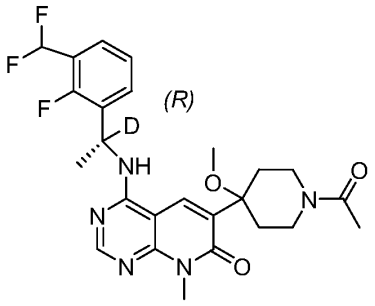
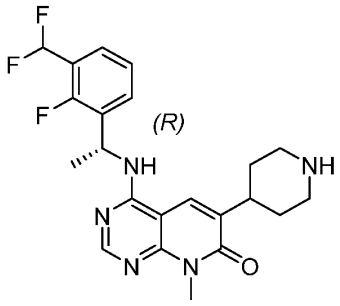
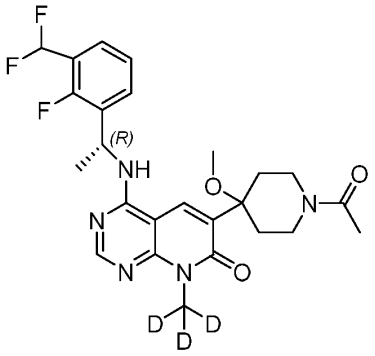
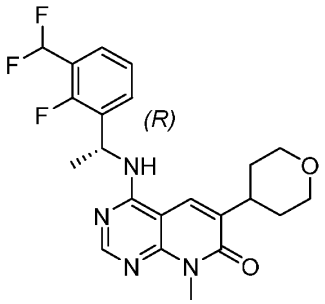
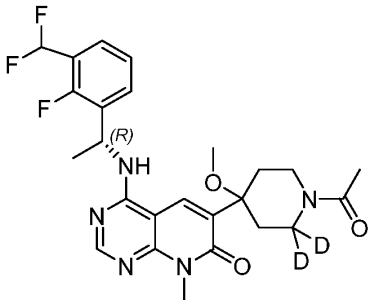
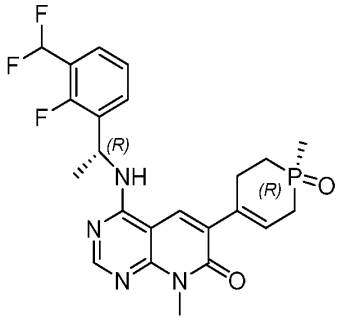
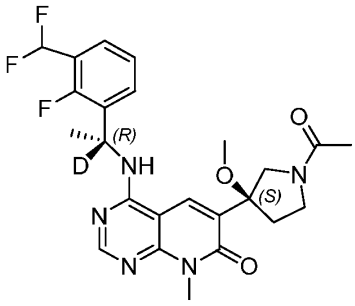
	
	
	
	

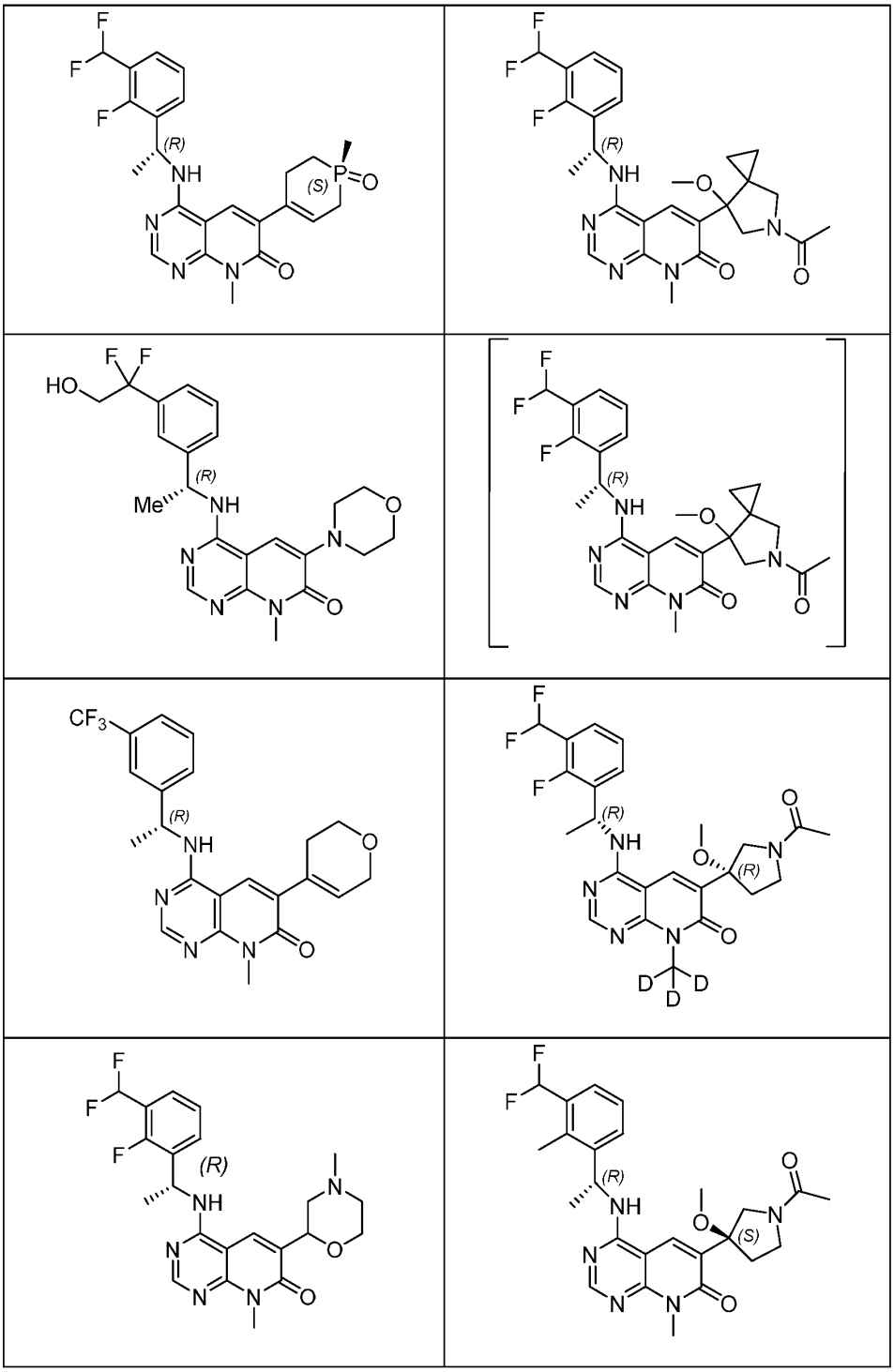
	
	
	
	

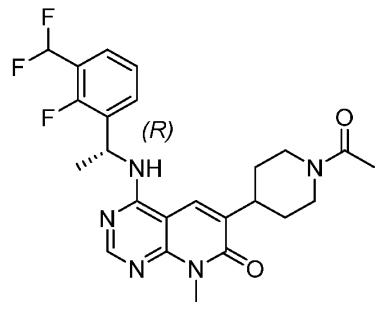
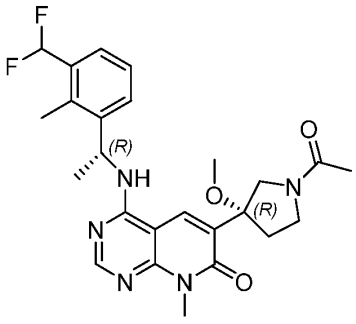
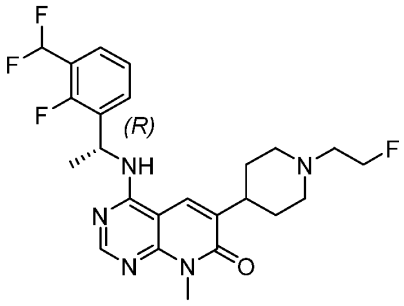
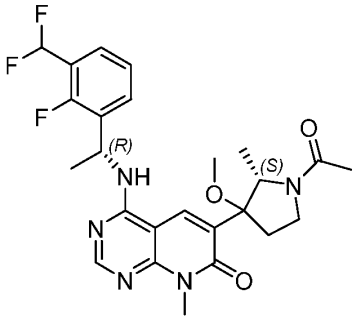
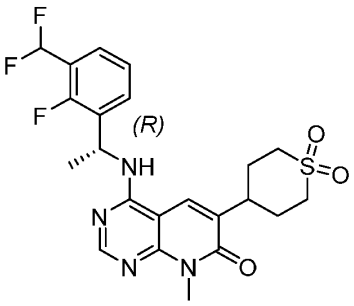
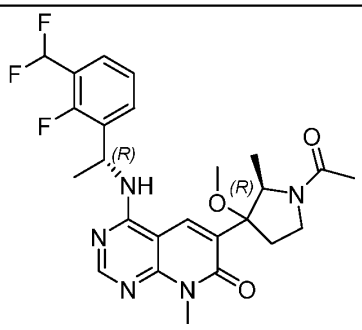
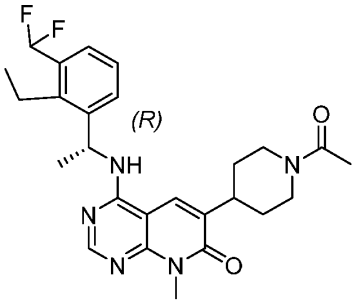
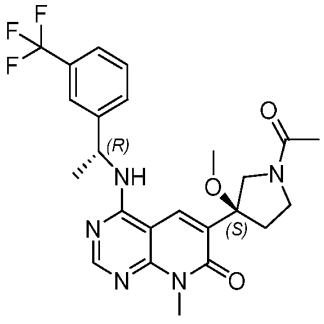


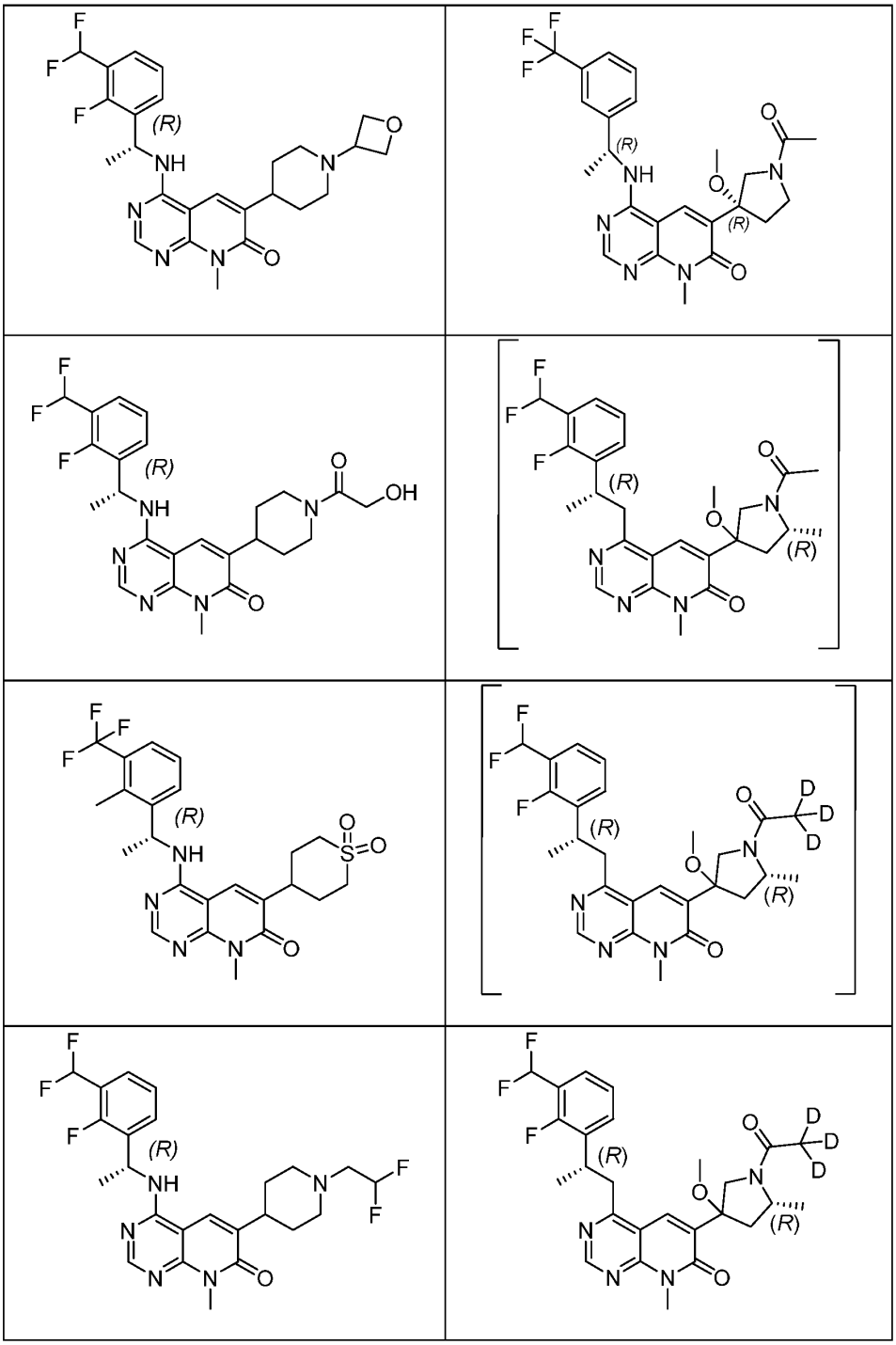


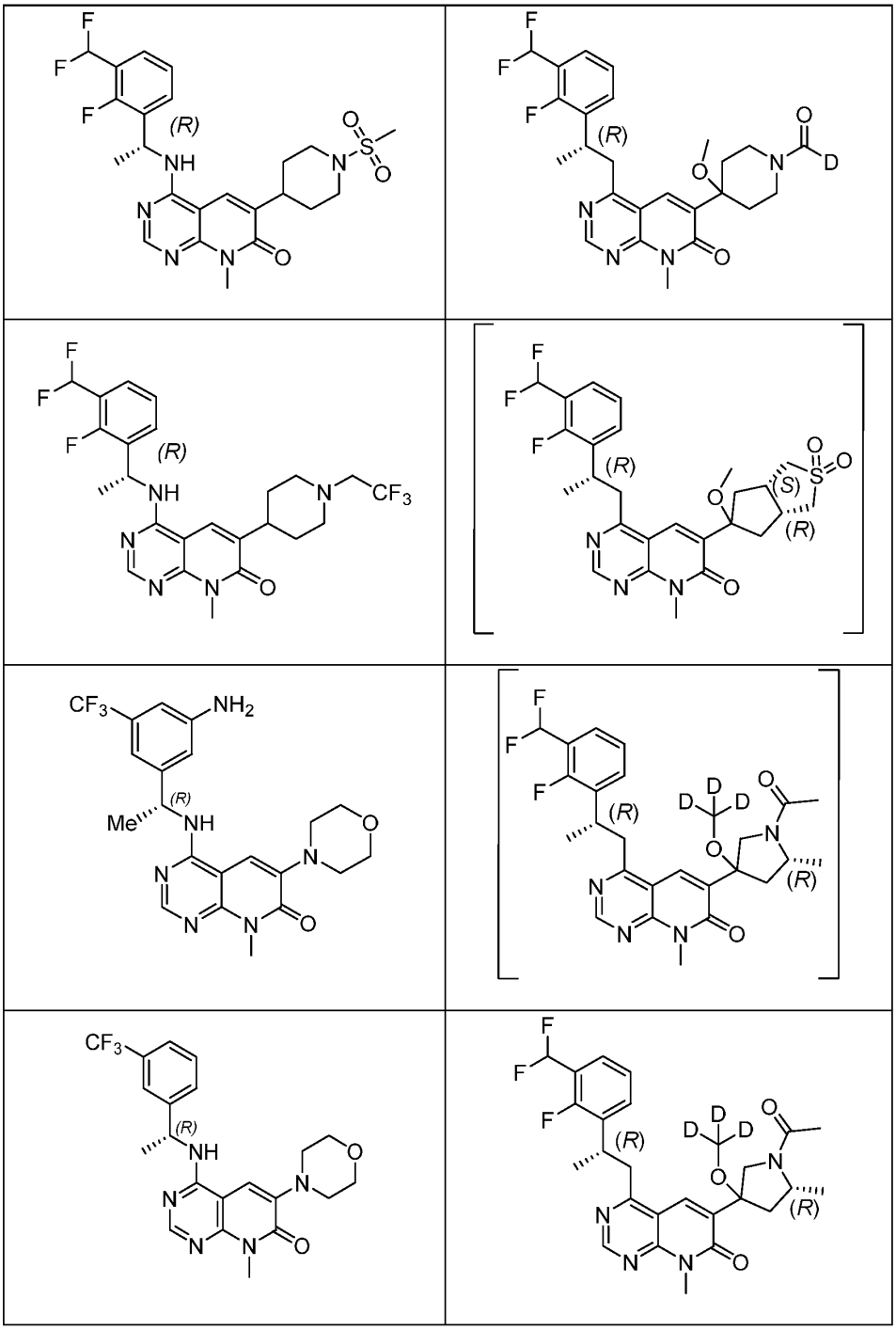
	
	
	
	

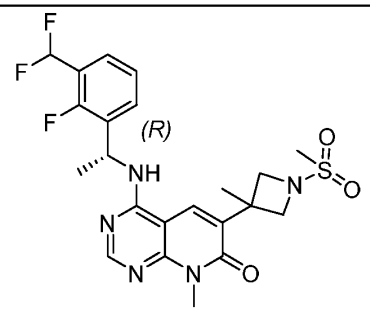
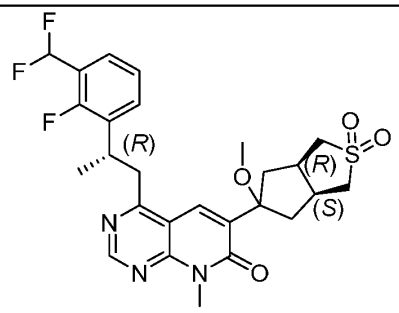
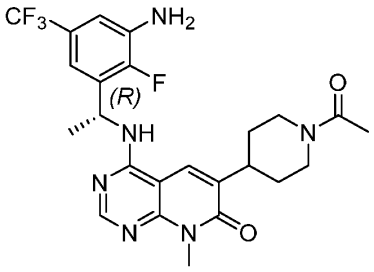
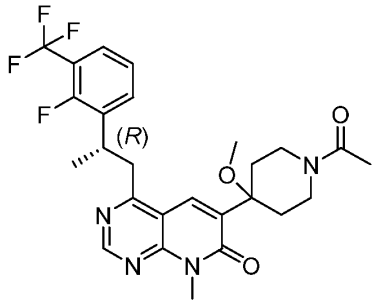
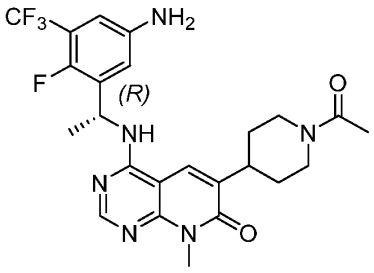
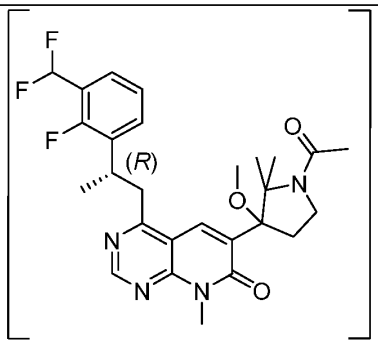
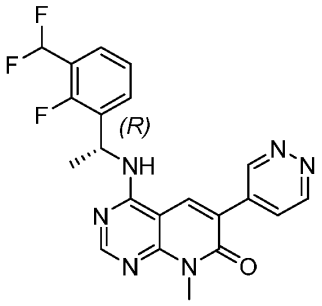
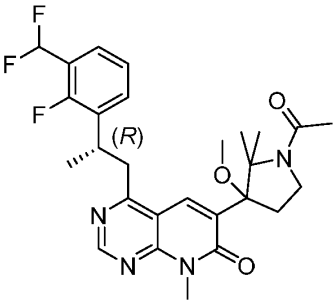
	
	
	
	

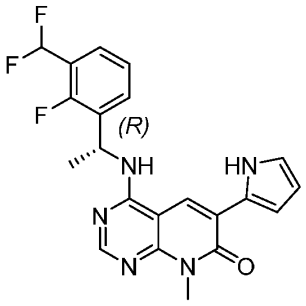
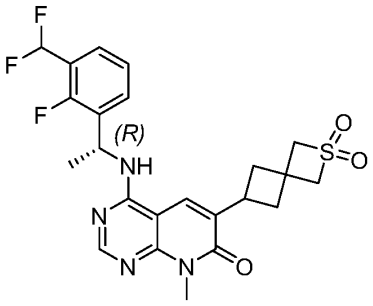
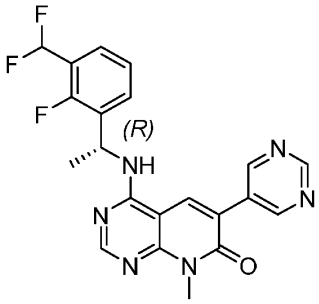
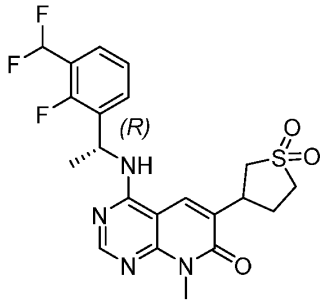
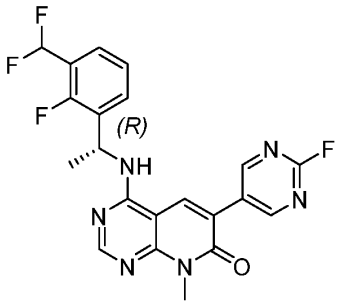
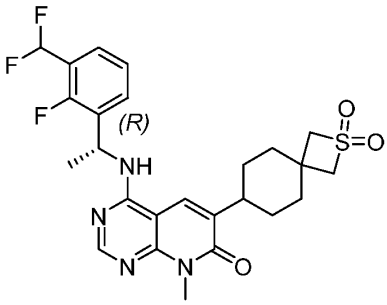
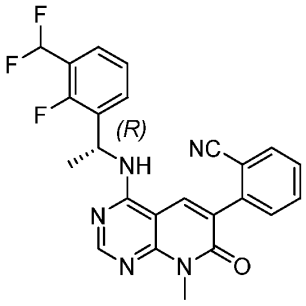
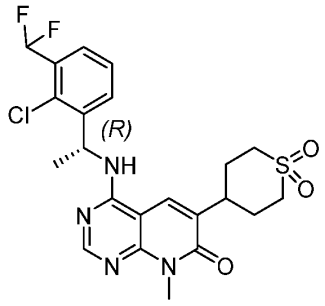


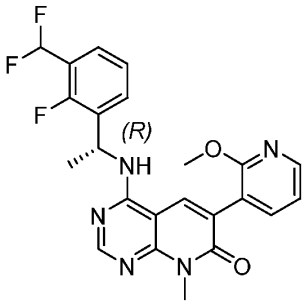
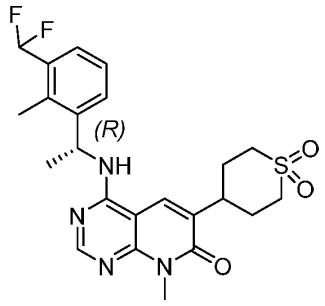
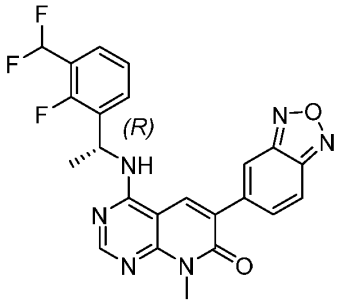
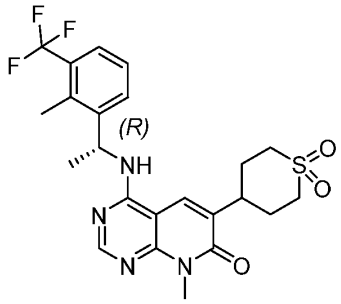
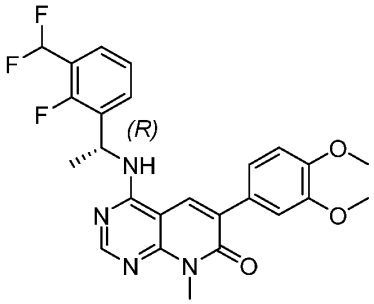
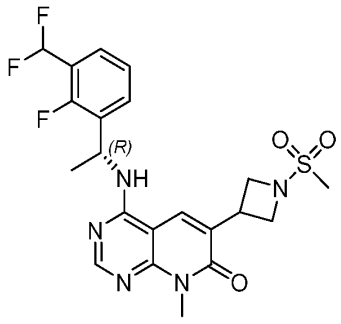
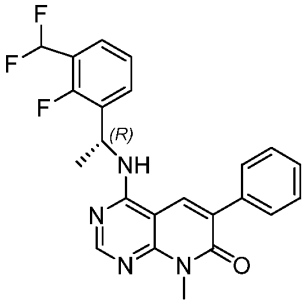
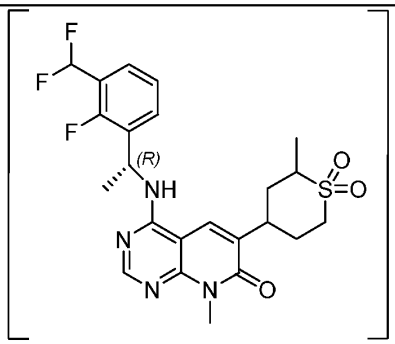
	
	
	
	

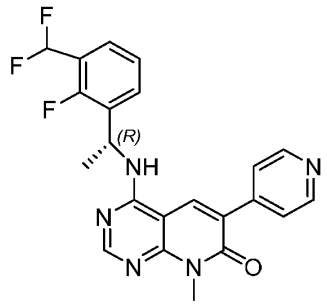
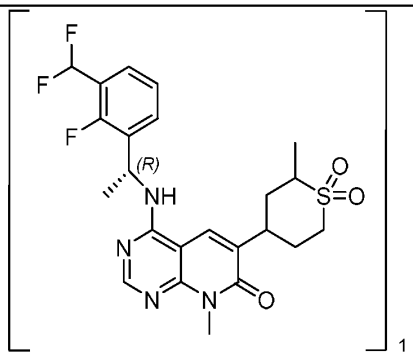
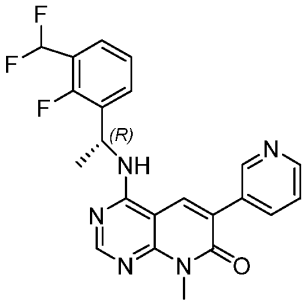
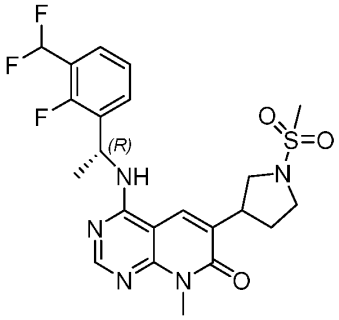
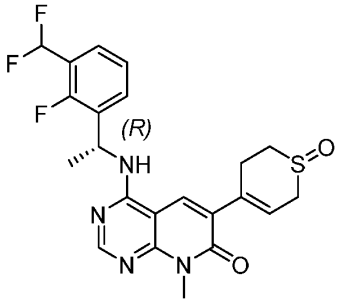
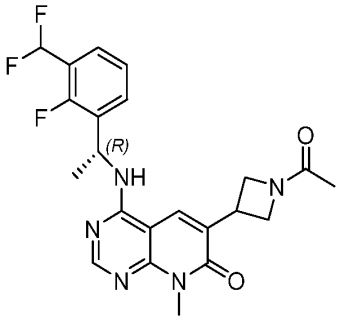
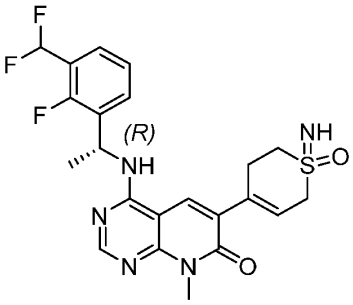
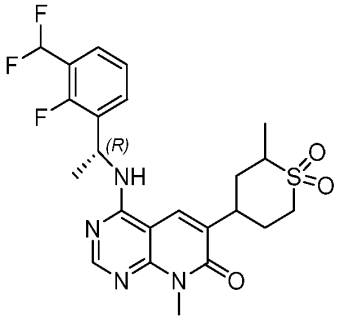


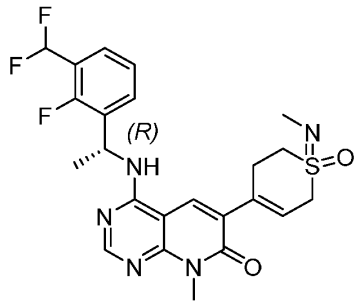
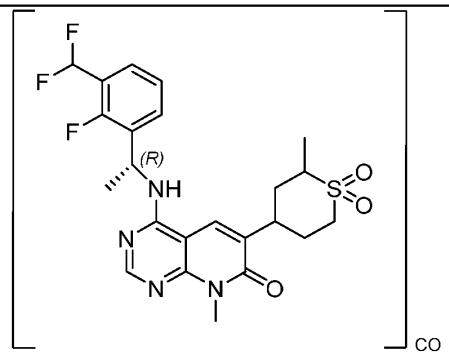
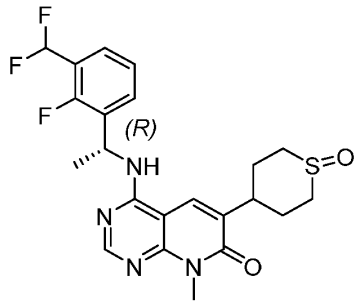
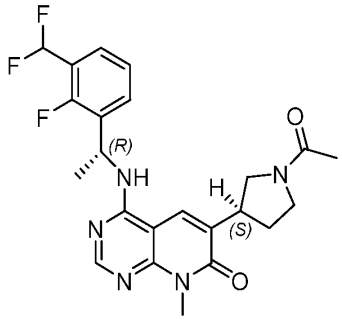
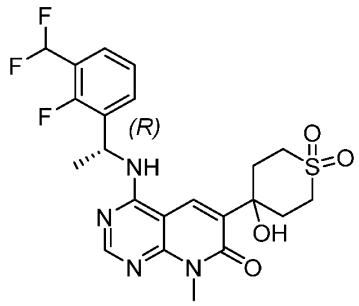
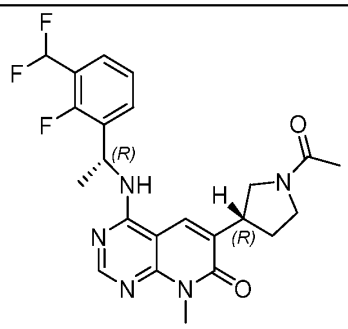
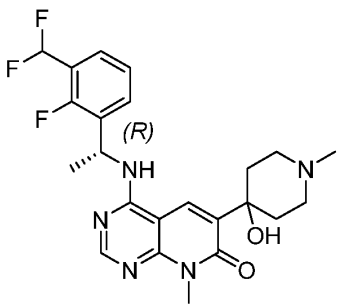
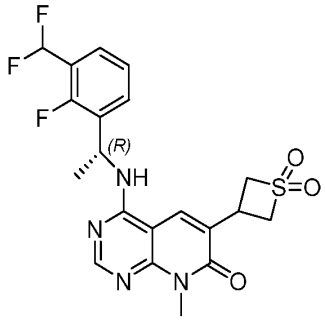


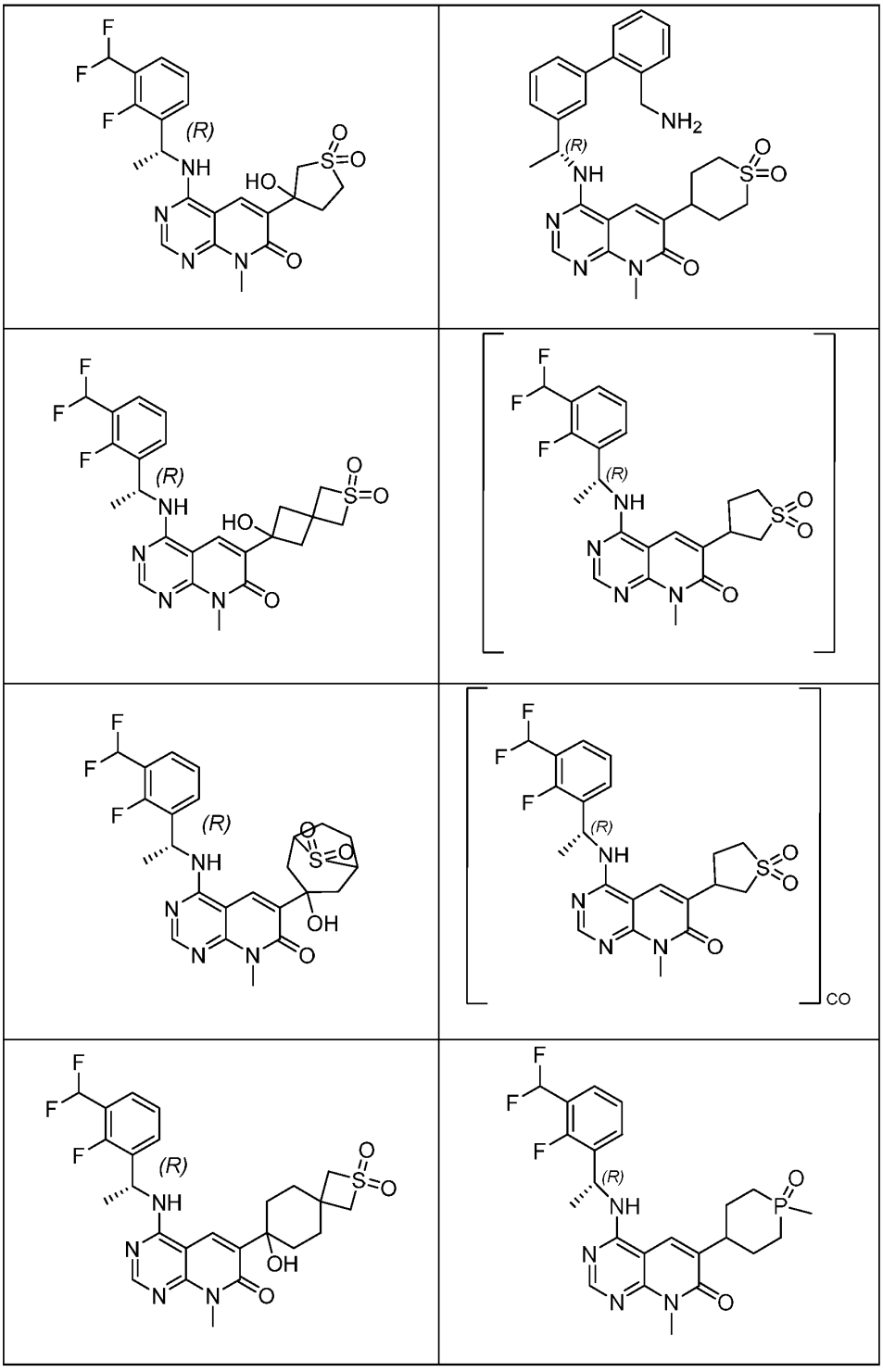
	
	
	
	

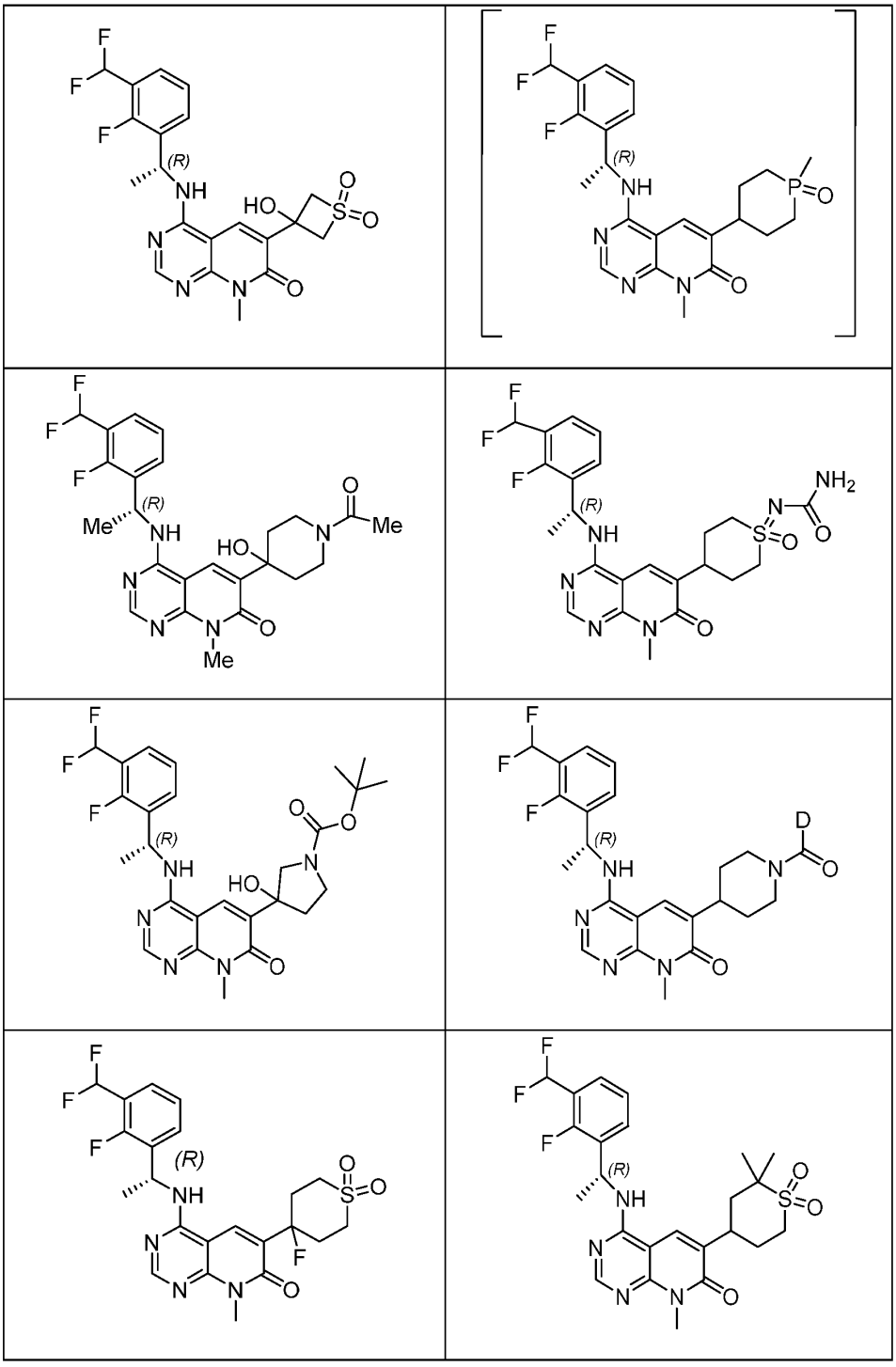
	
	
	
	

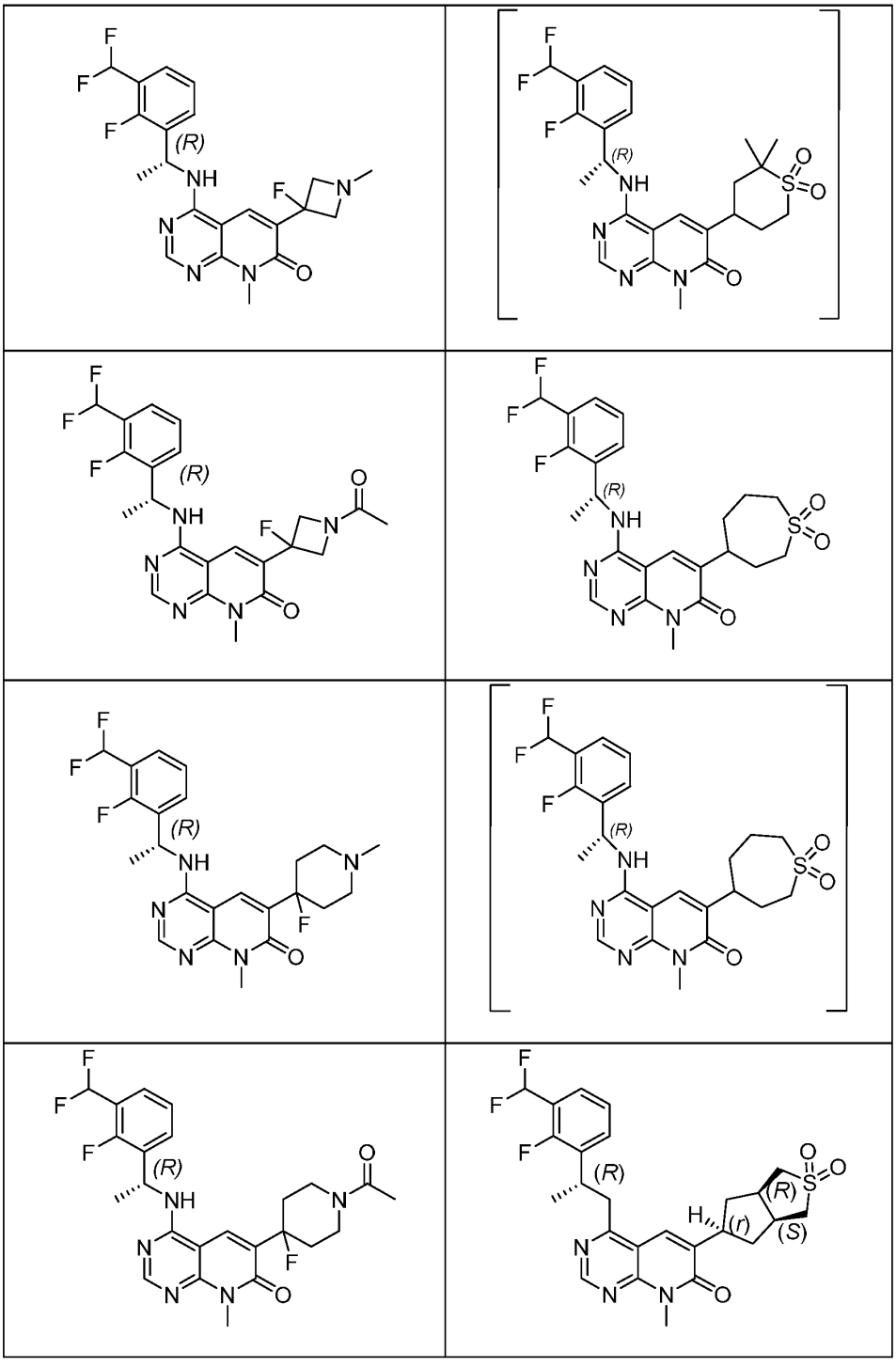
	
	
	
	

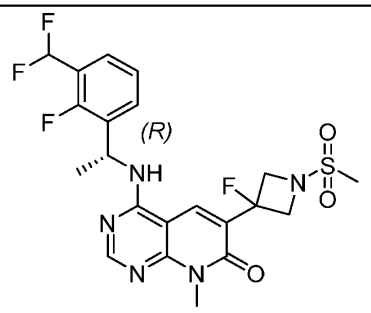
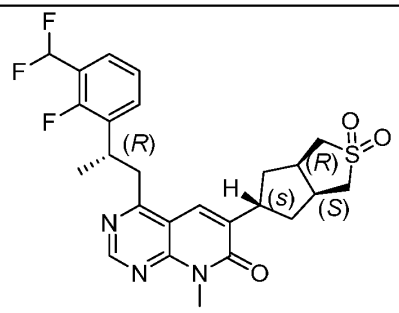
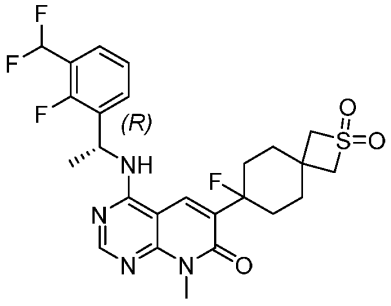
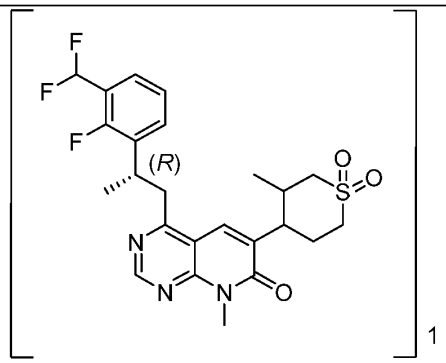
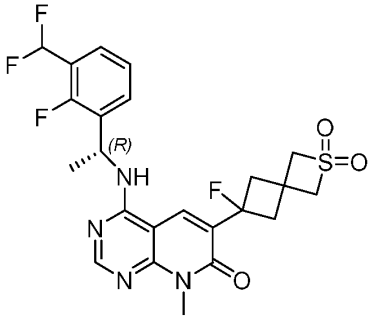
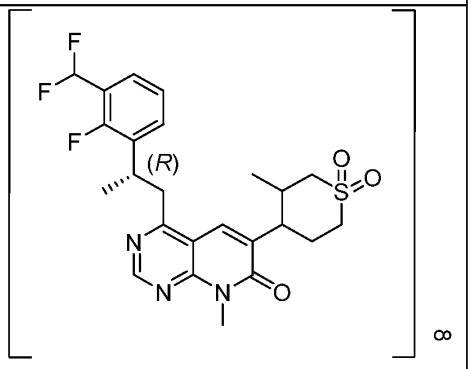
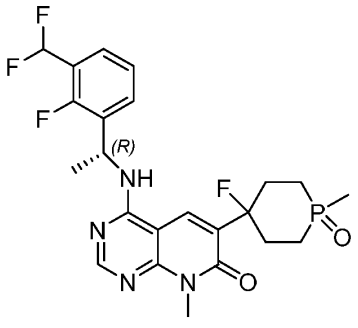
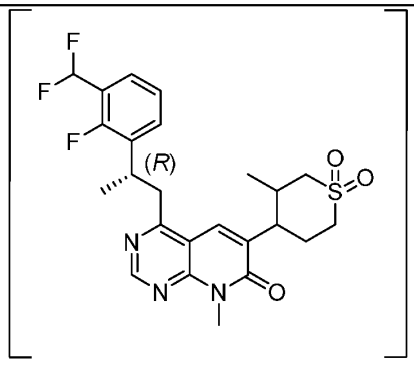
	
	
	
	

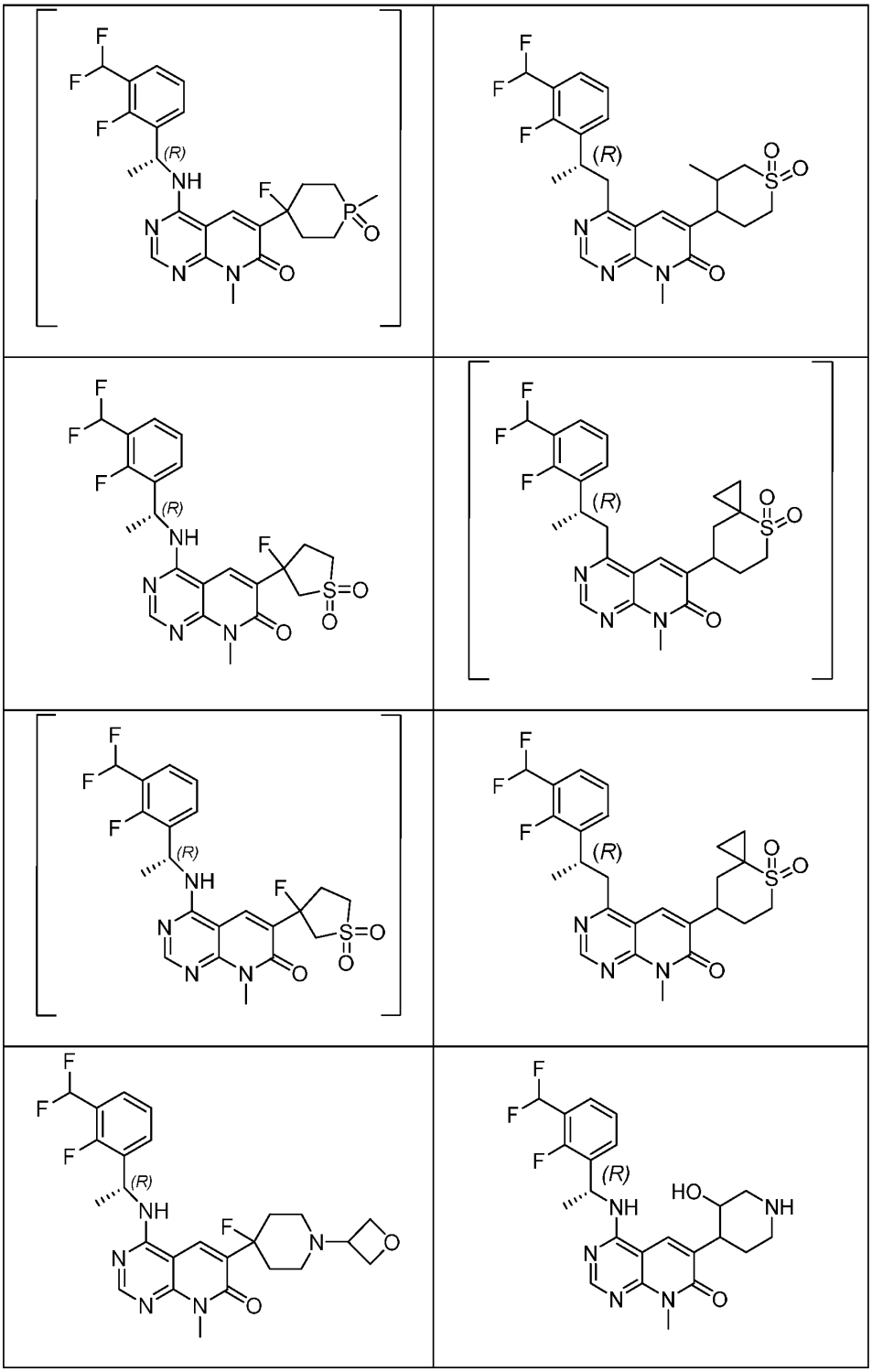
	
	
	
	

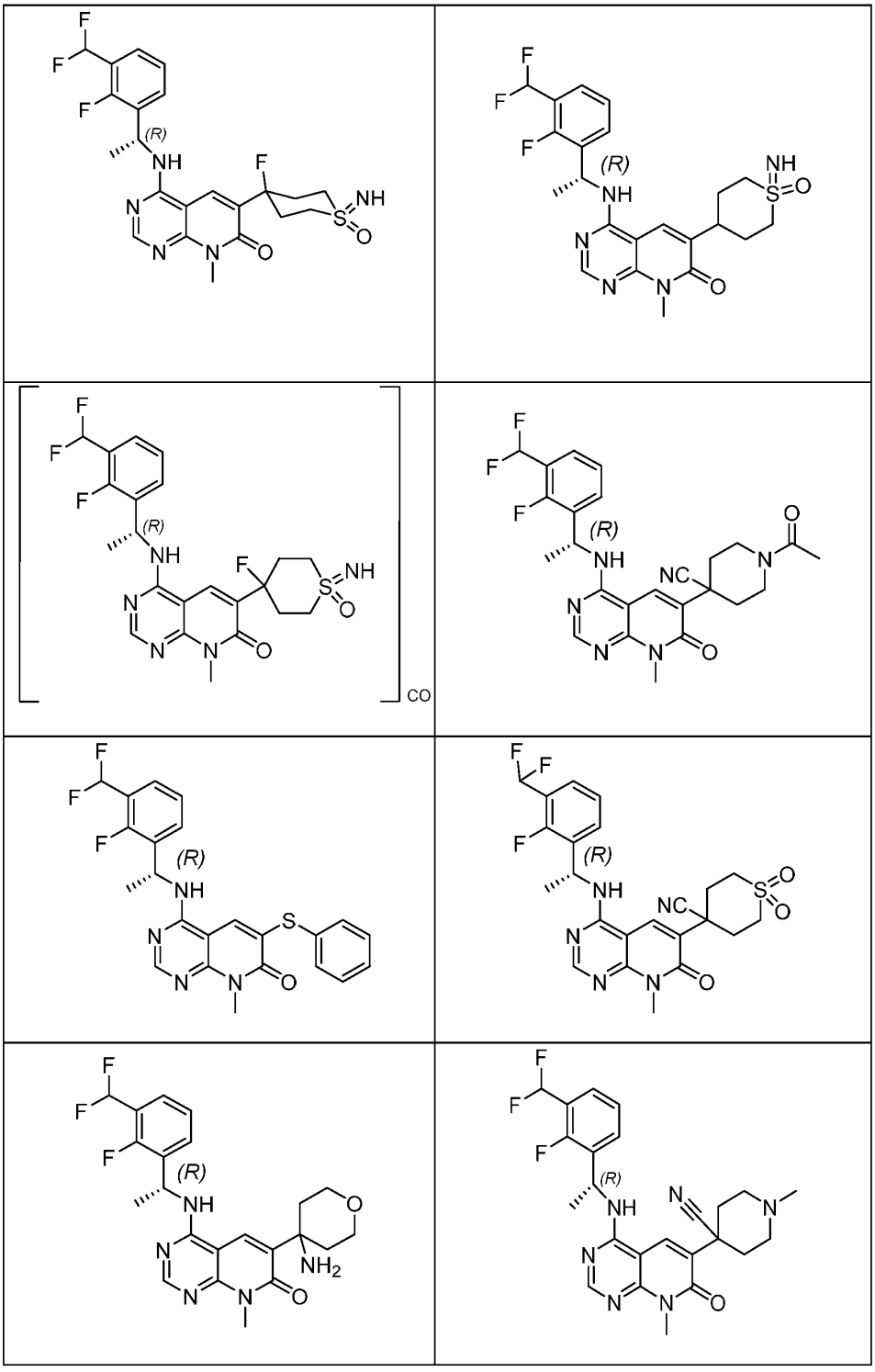


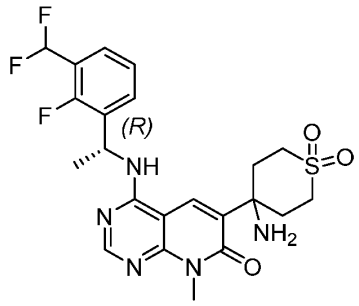
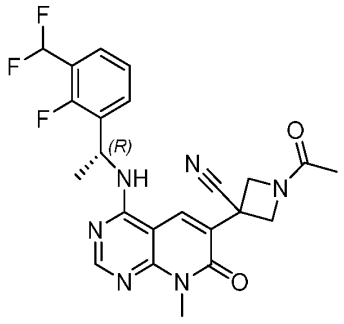
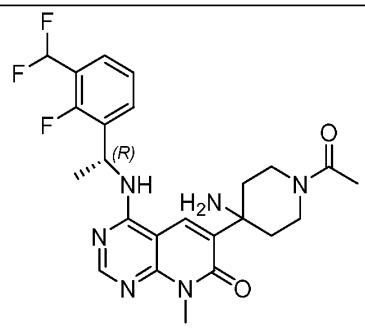
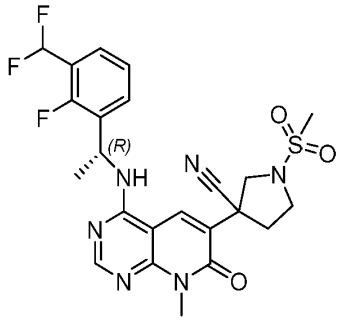
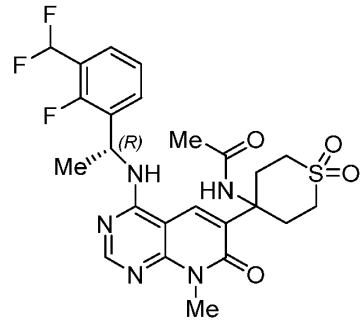
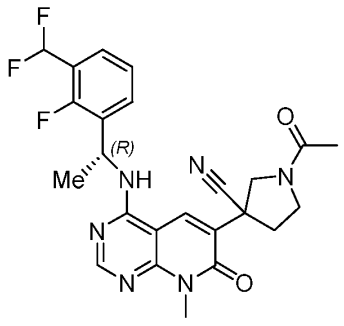
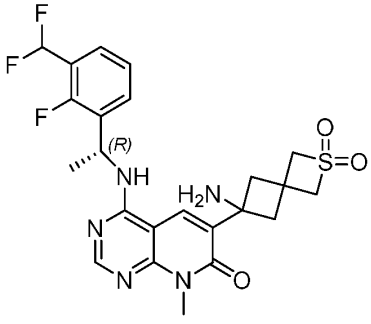
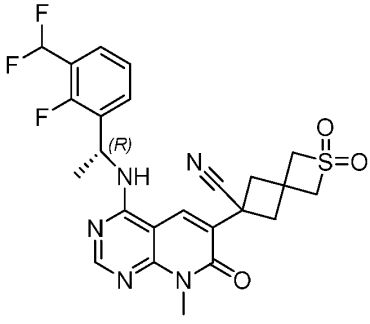


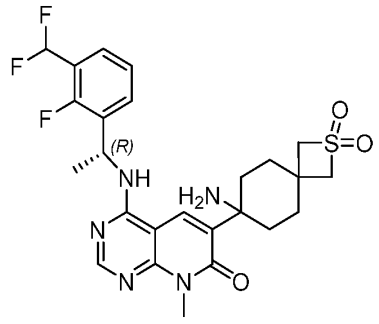
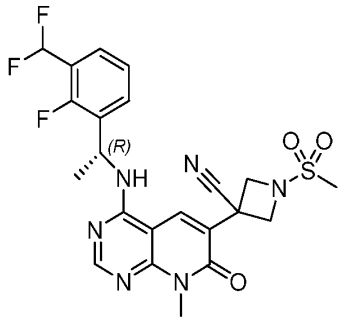
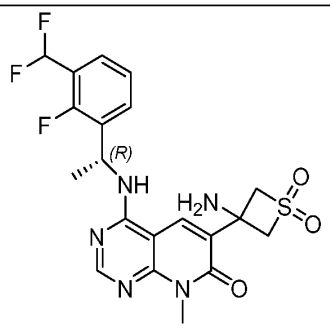
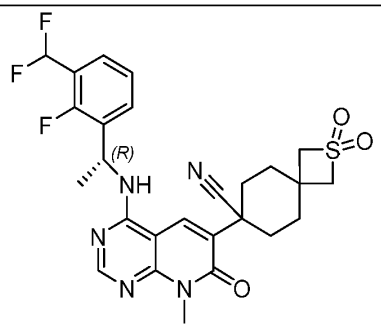
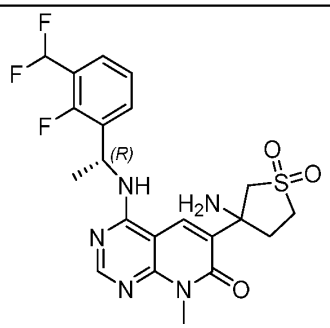
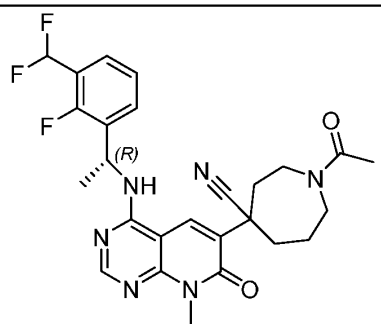
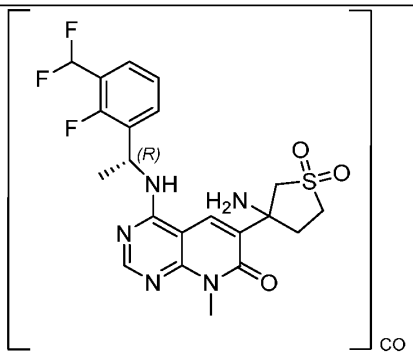
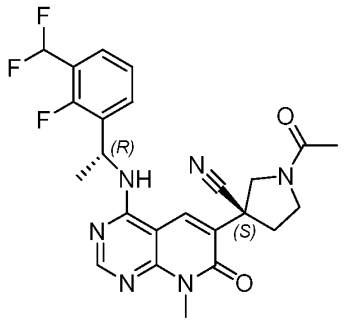


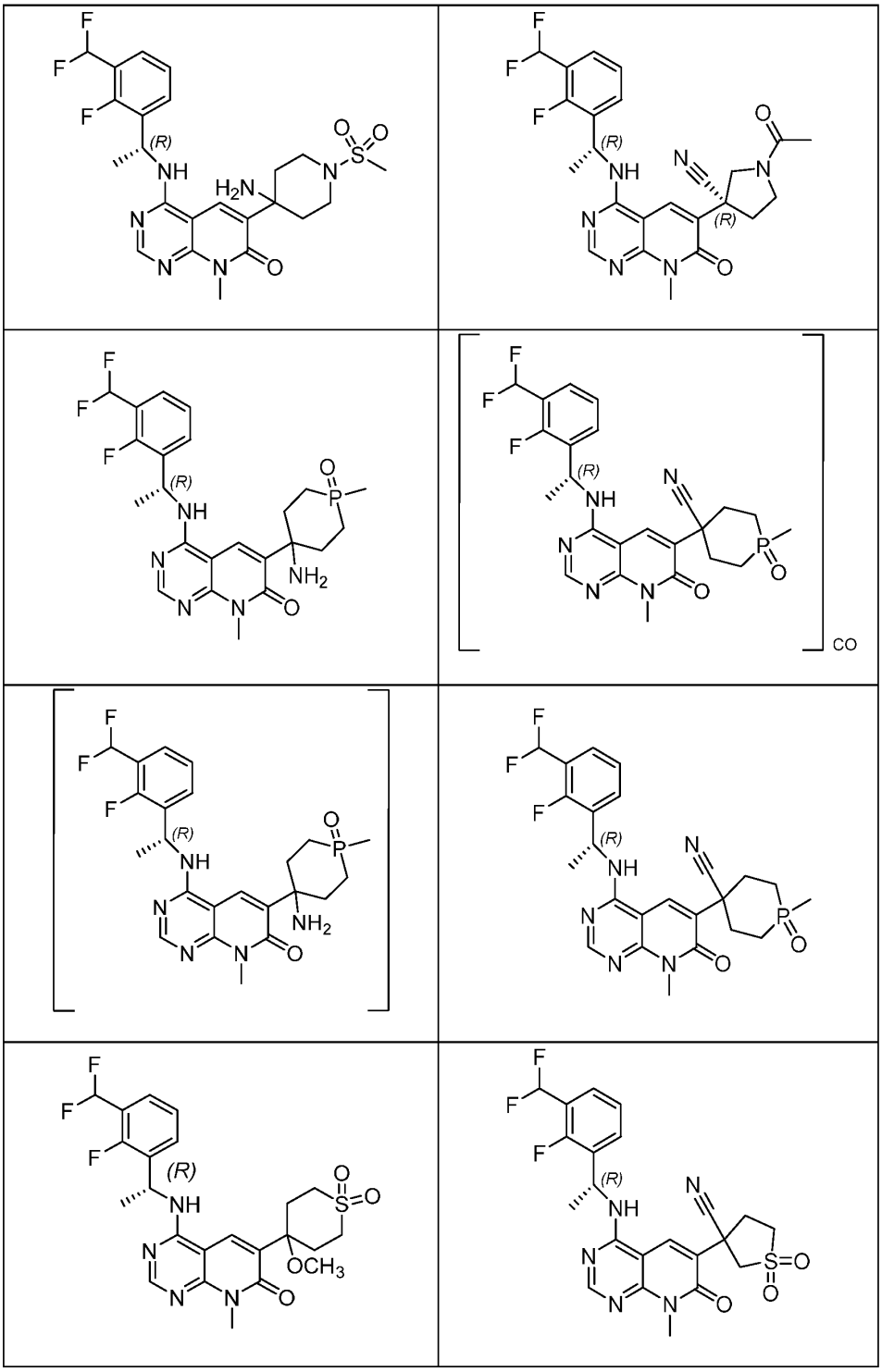
	
	 1
	 8
	

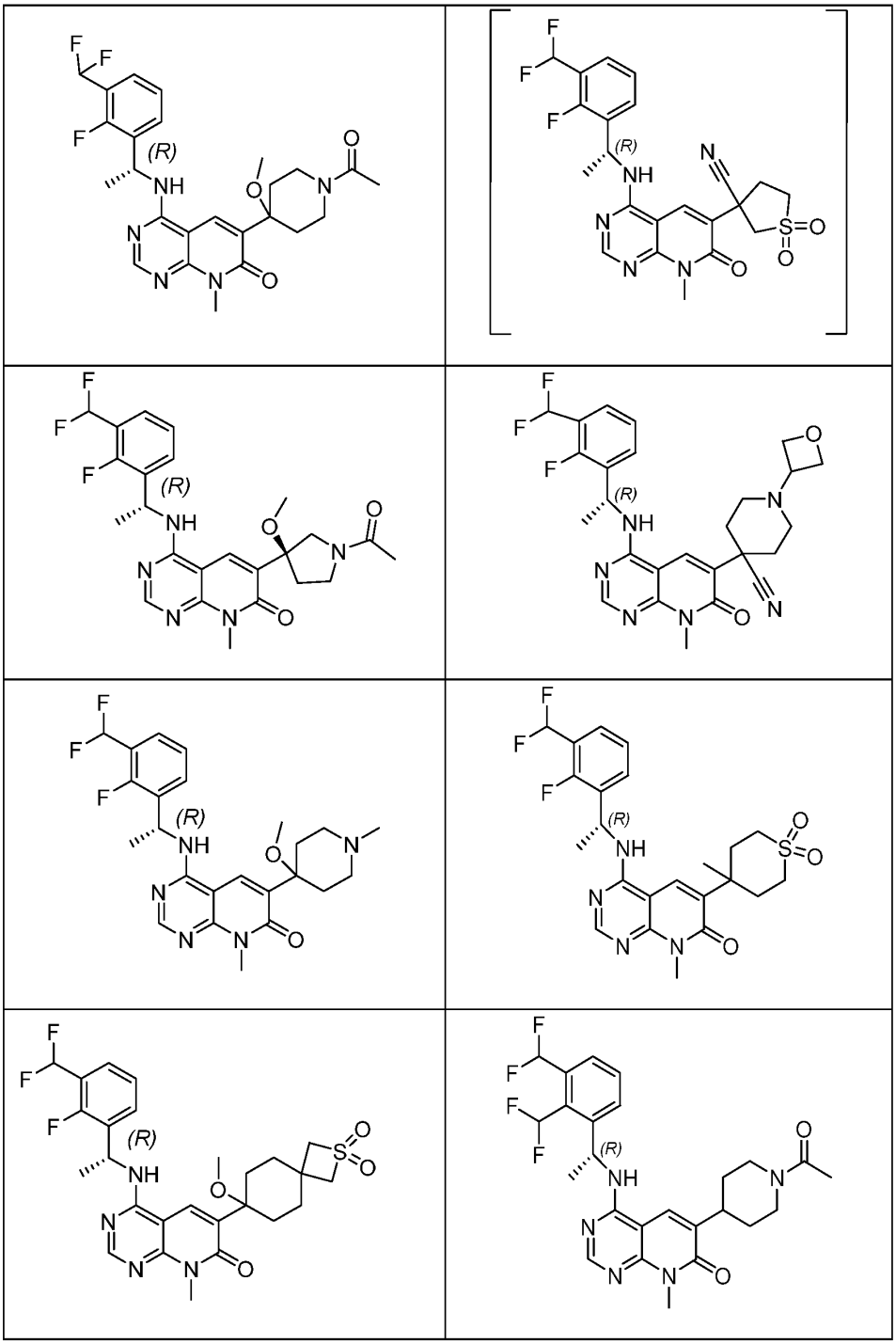


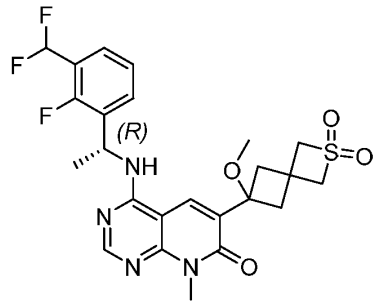
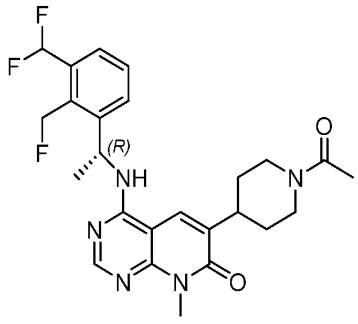
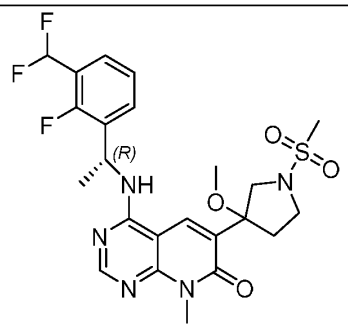
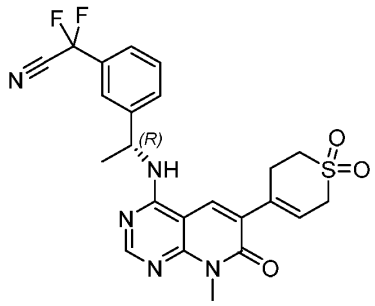
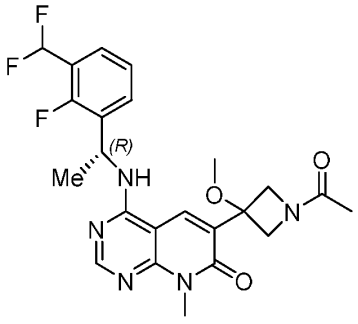
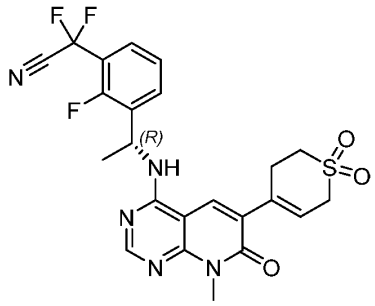
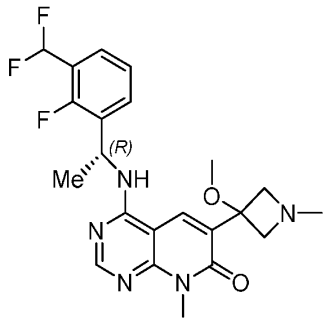
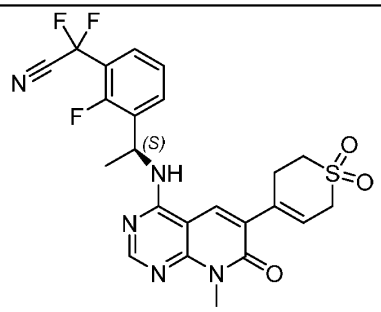


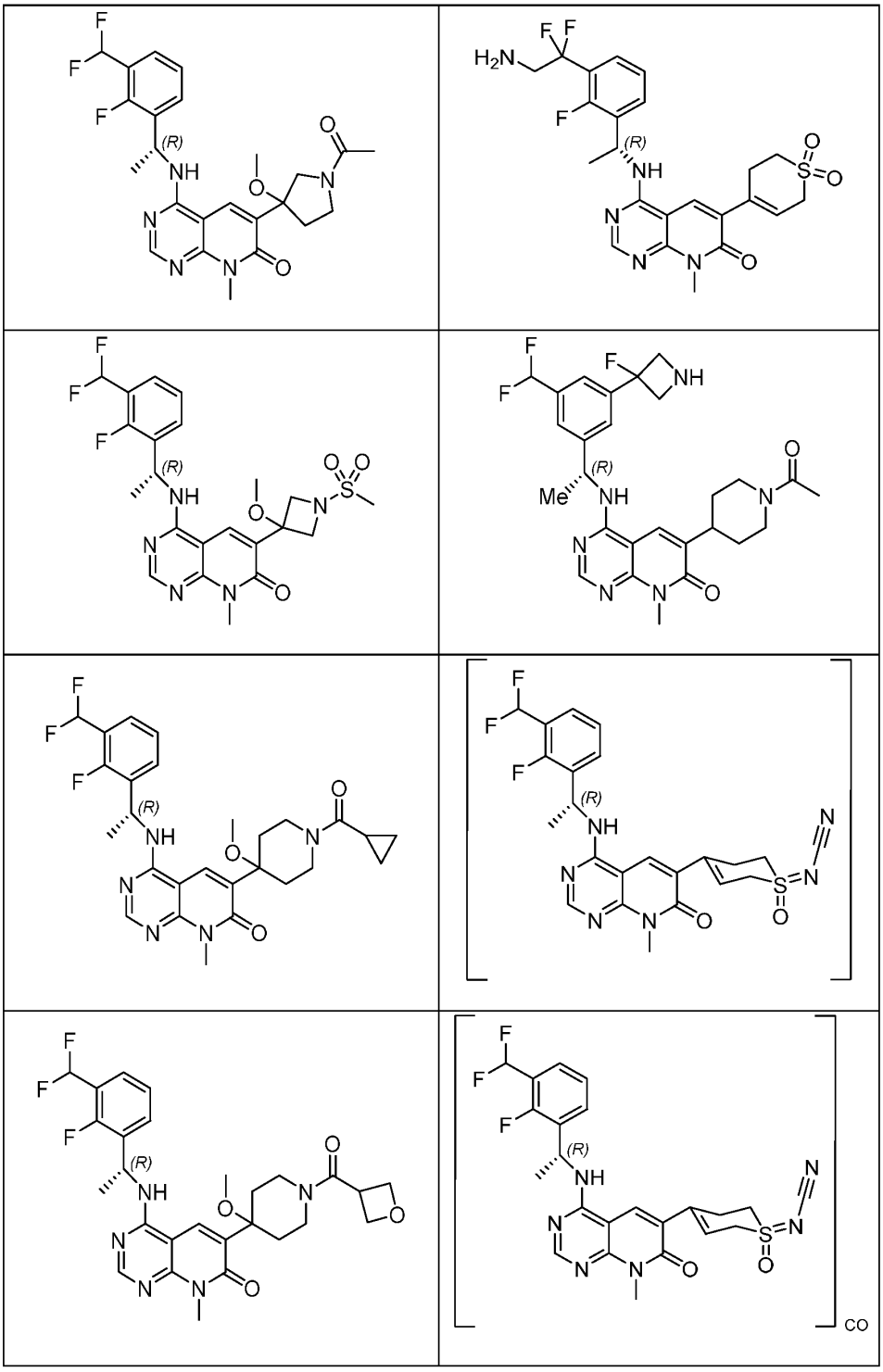
	
	
	
	

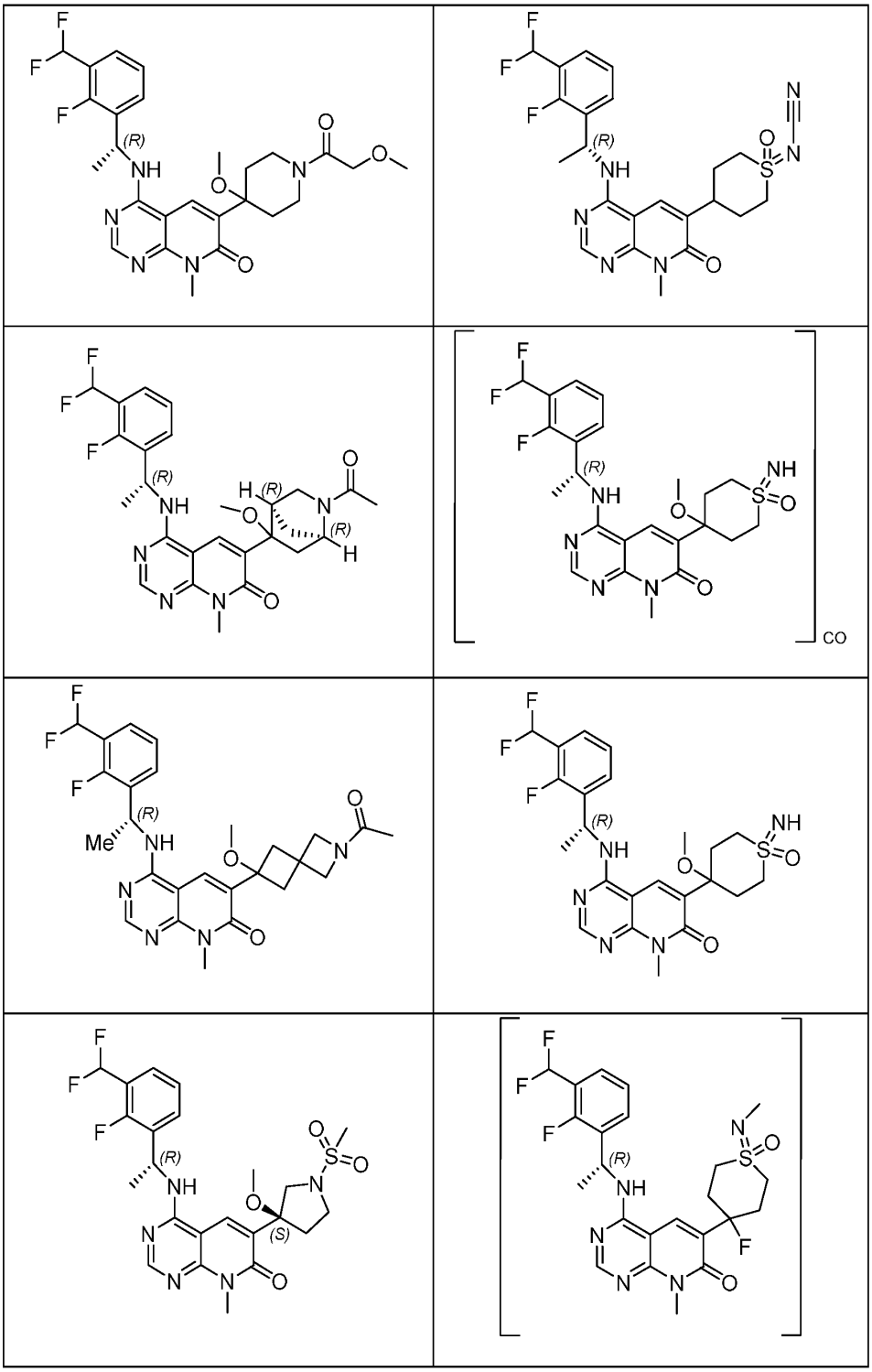
	
	
	
	

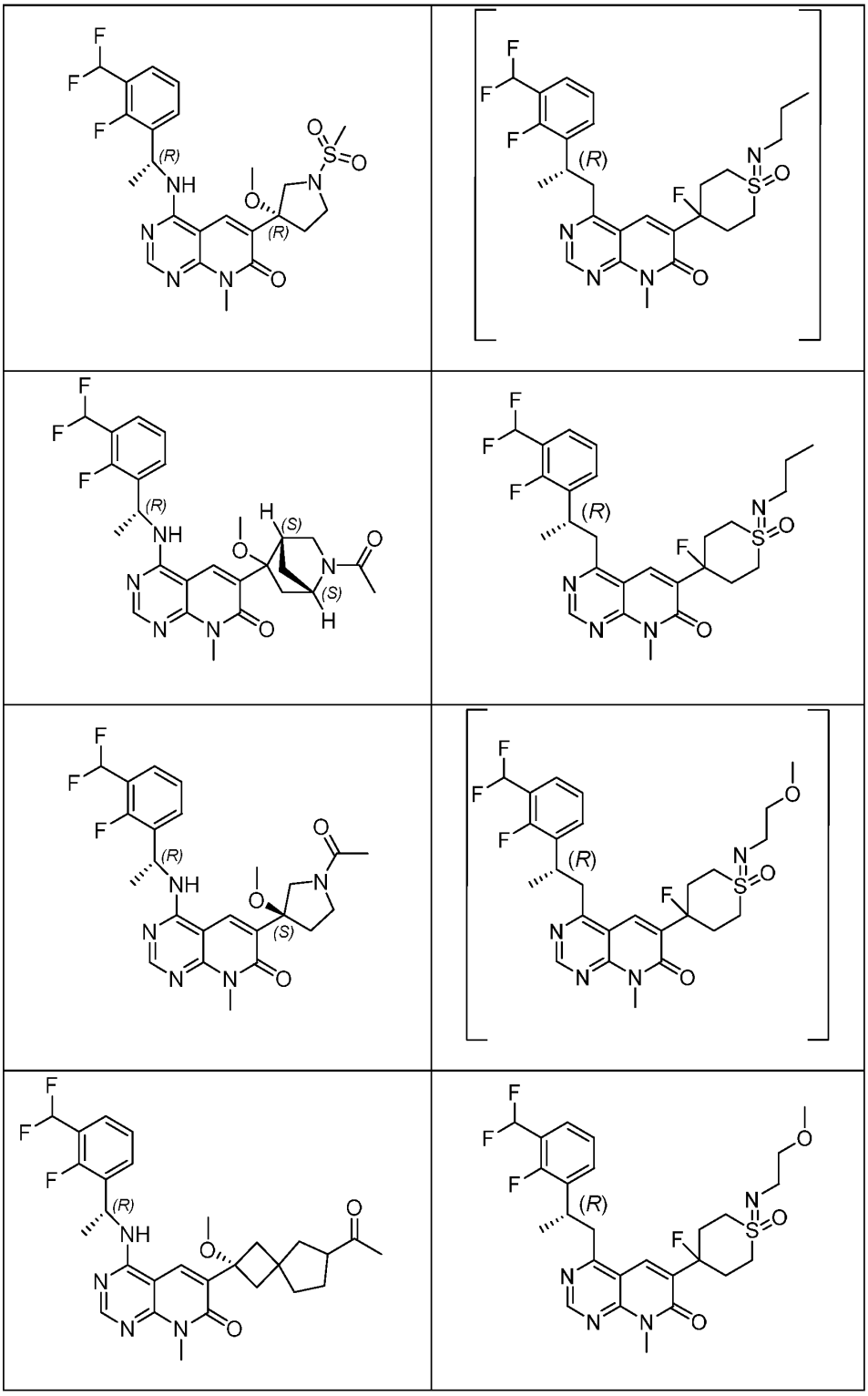


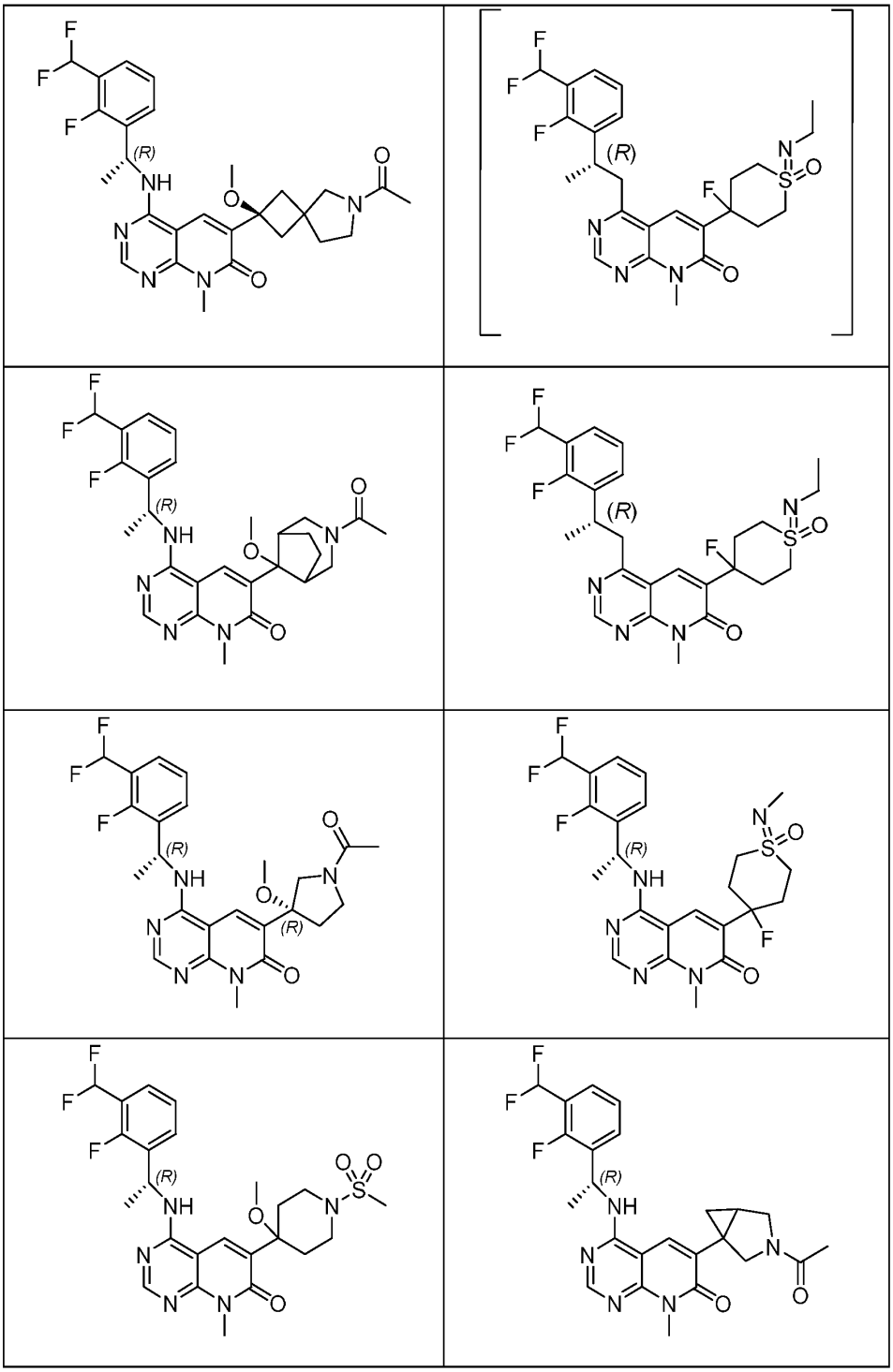


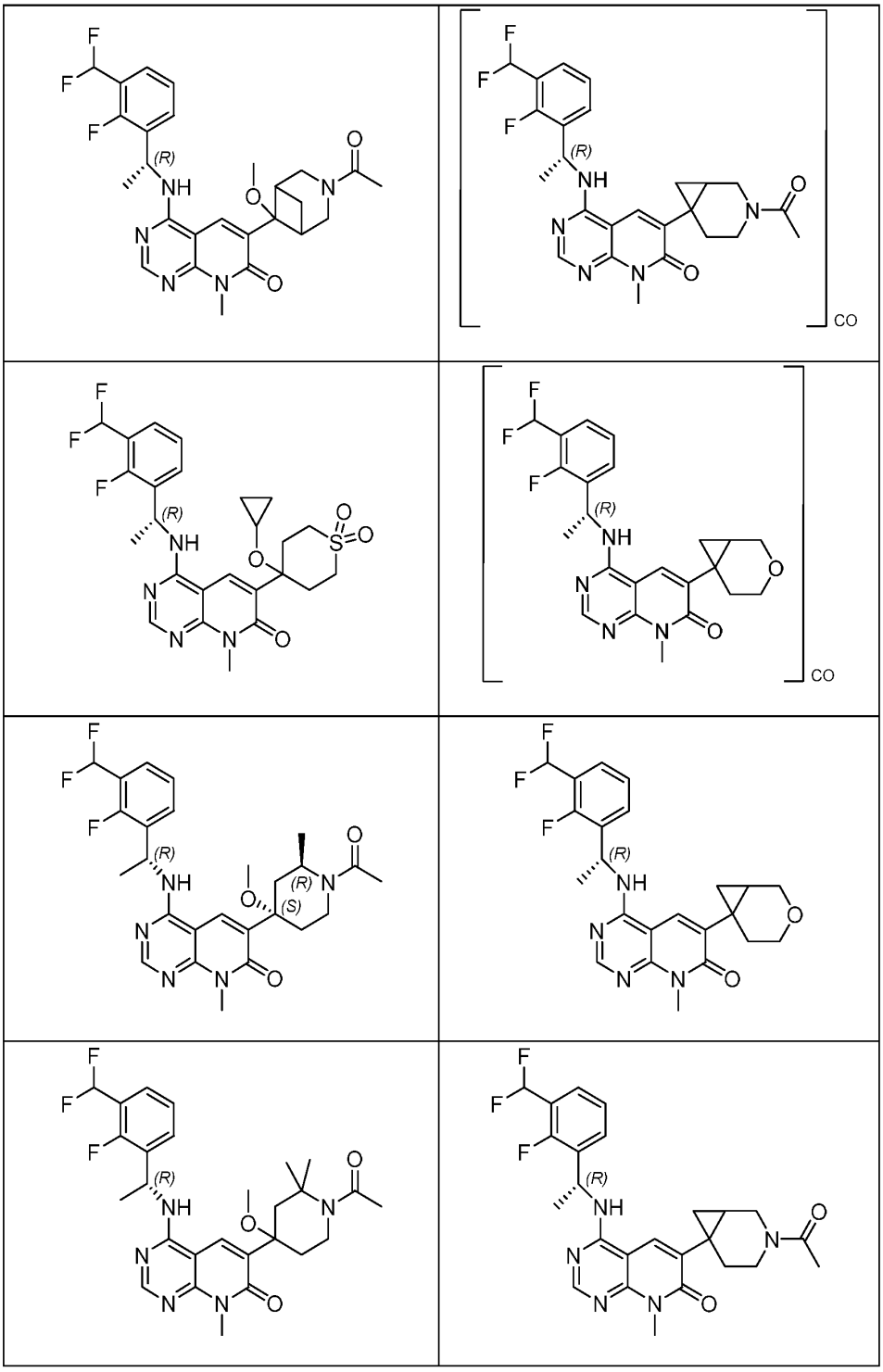
	
	
	
	

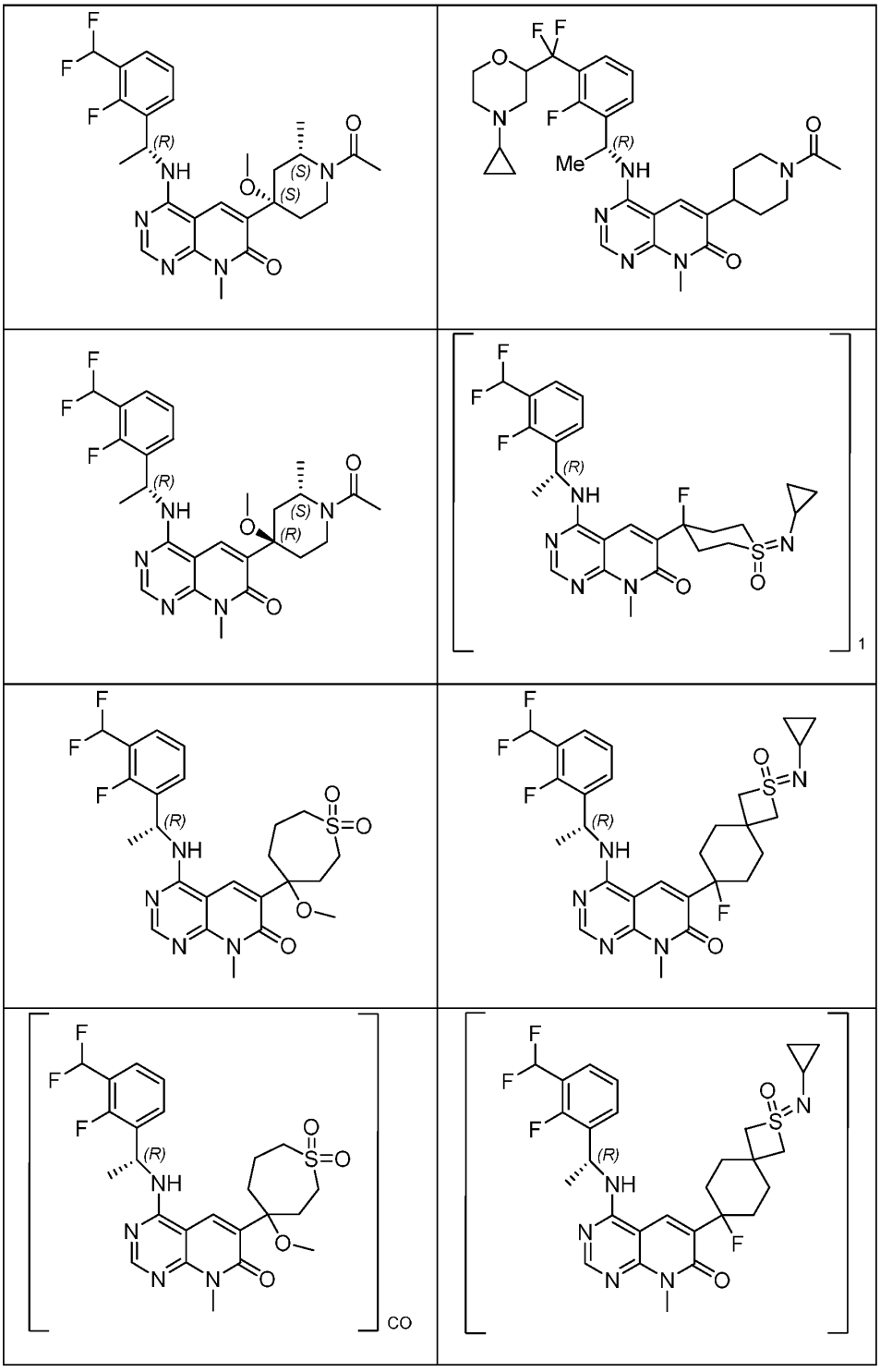


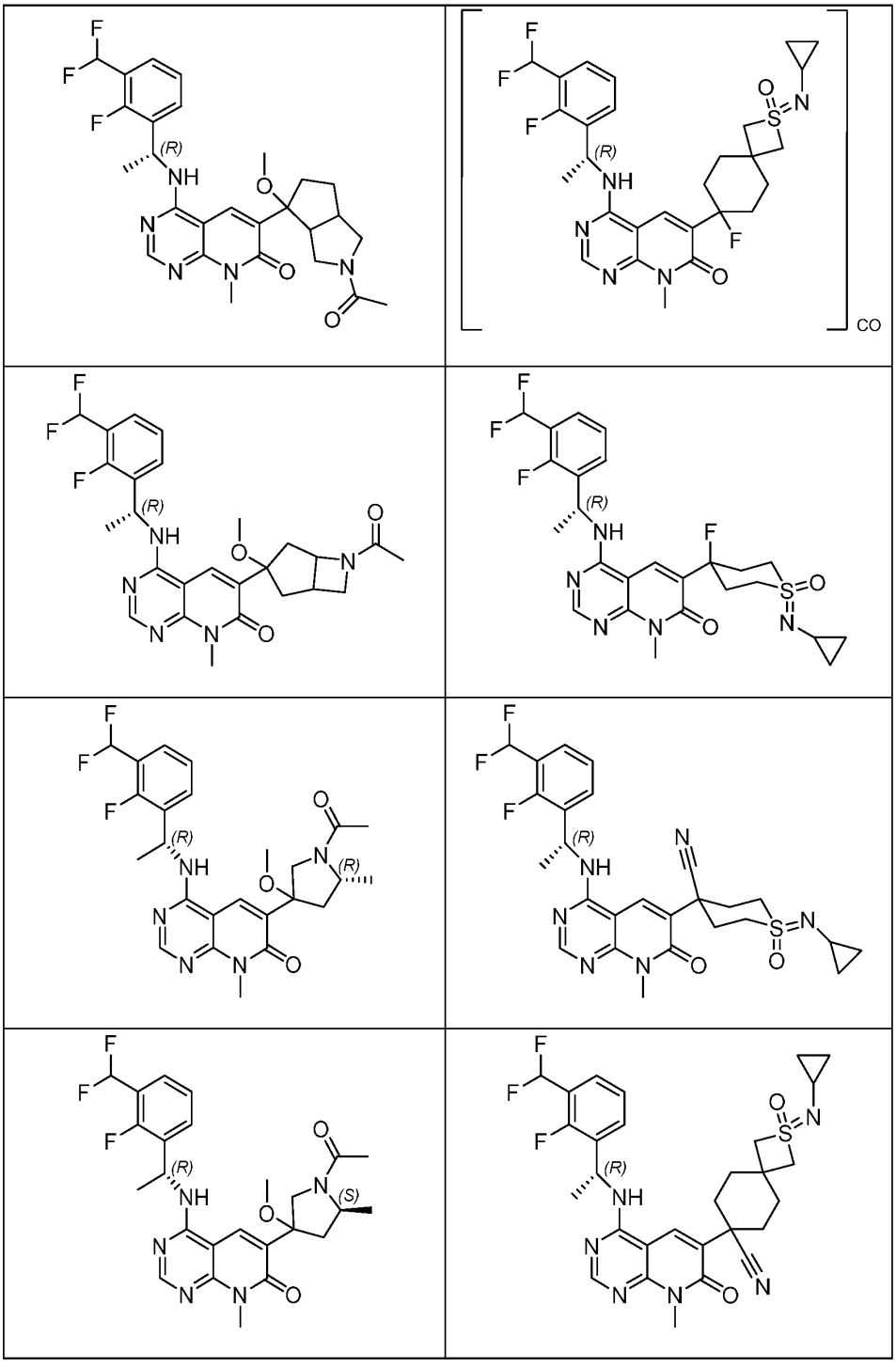


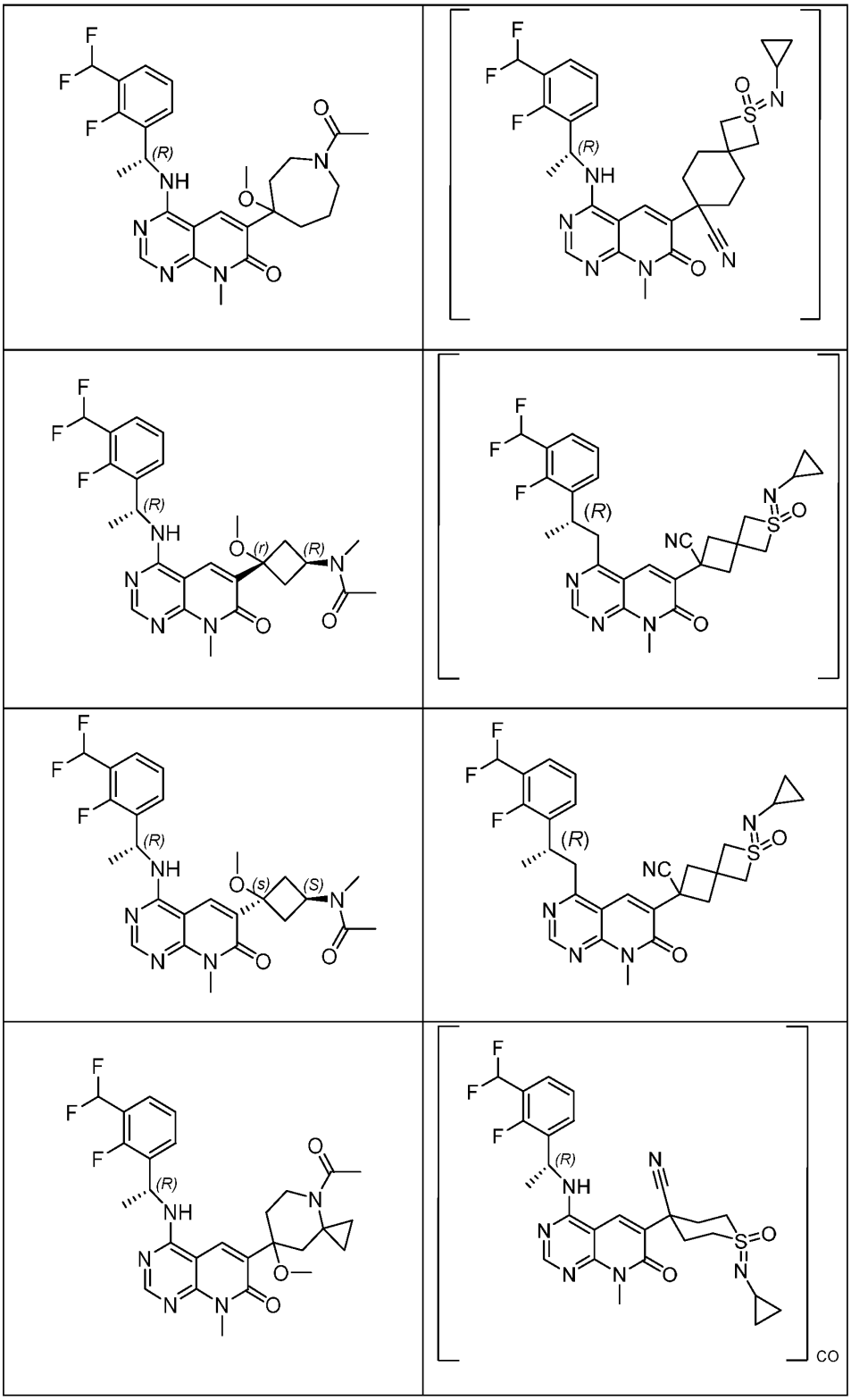


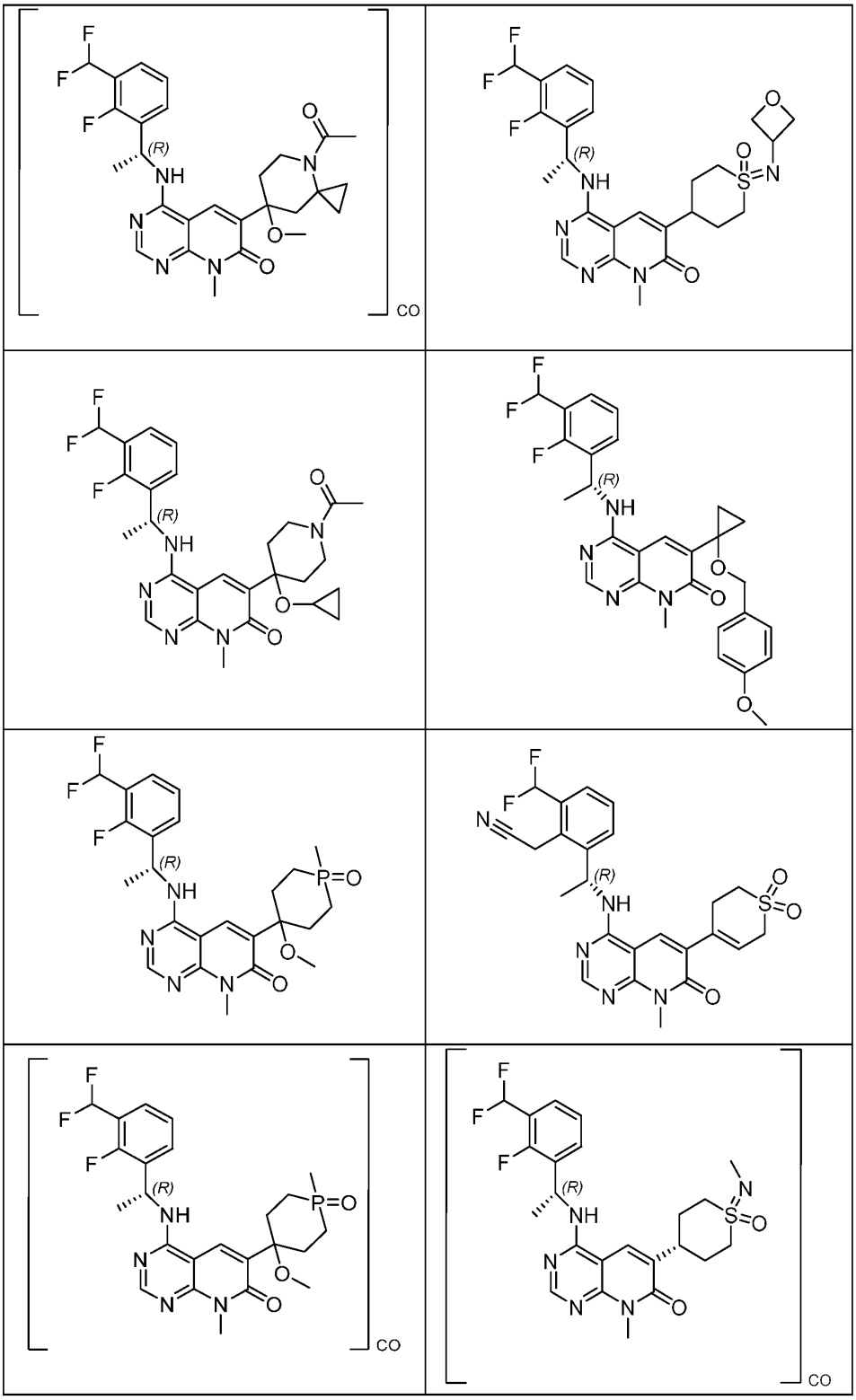


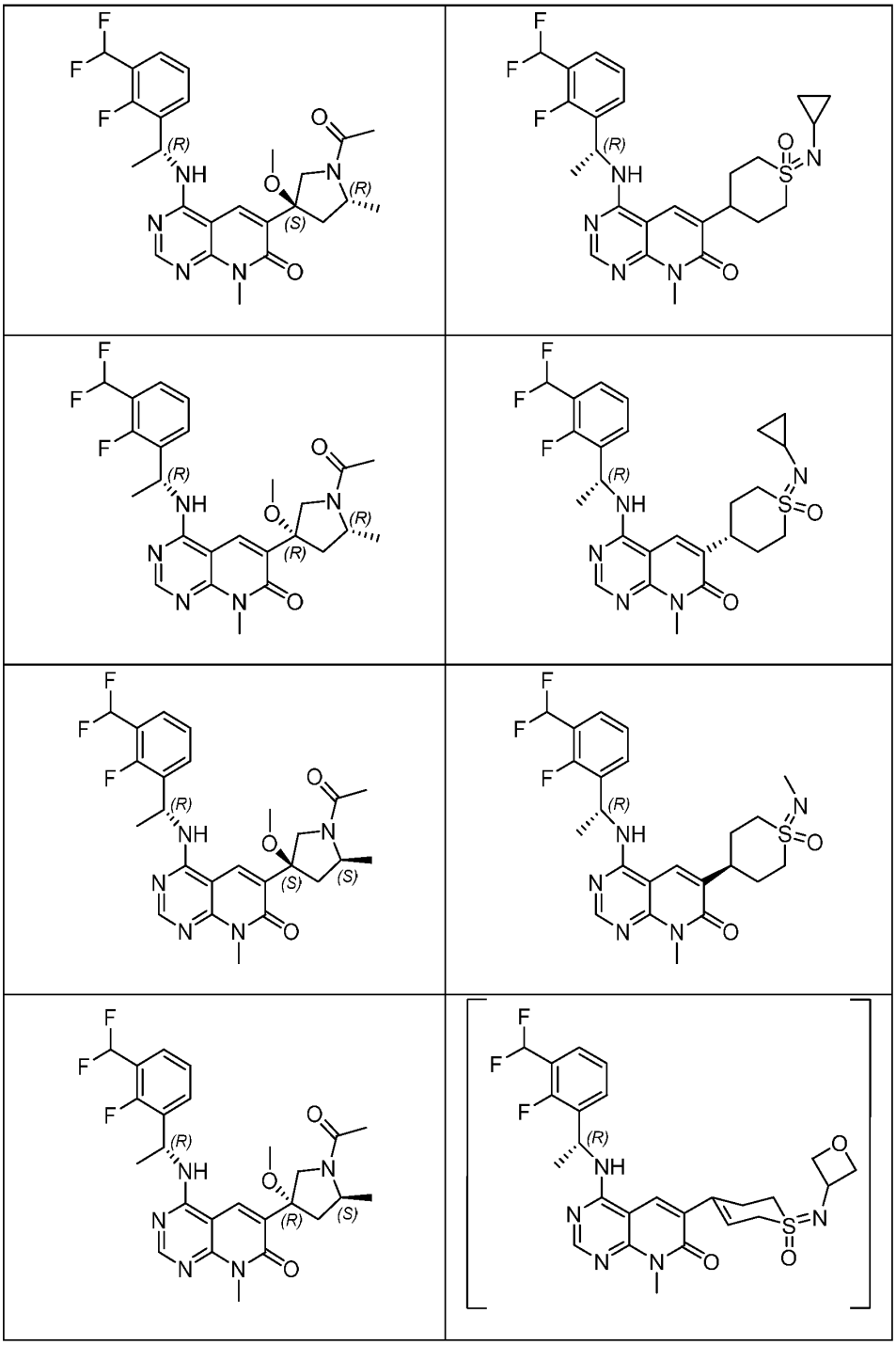


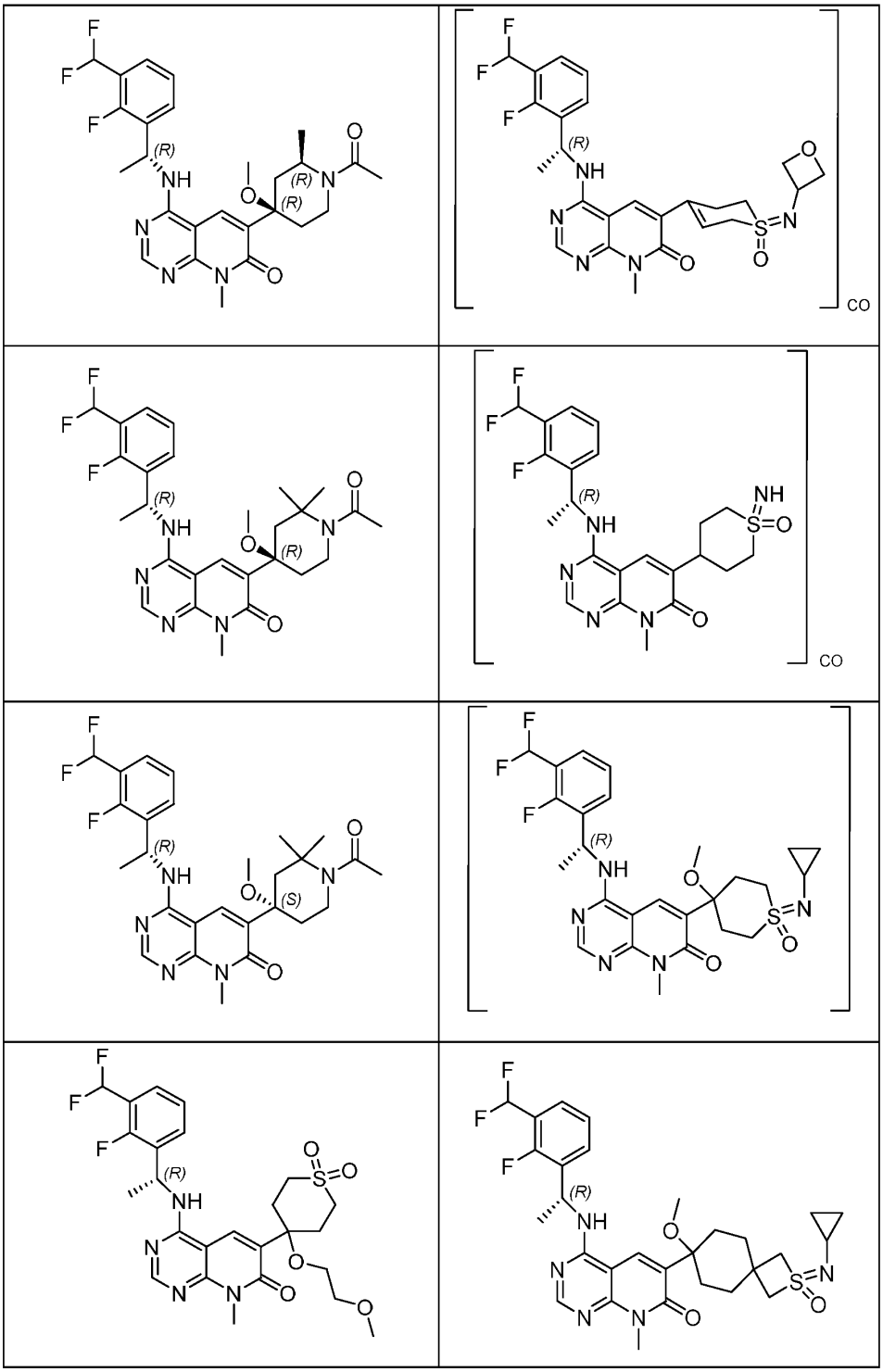


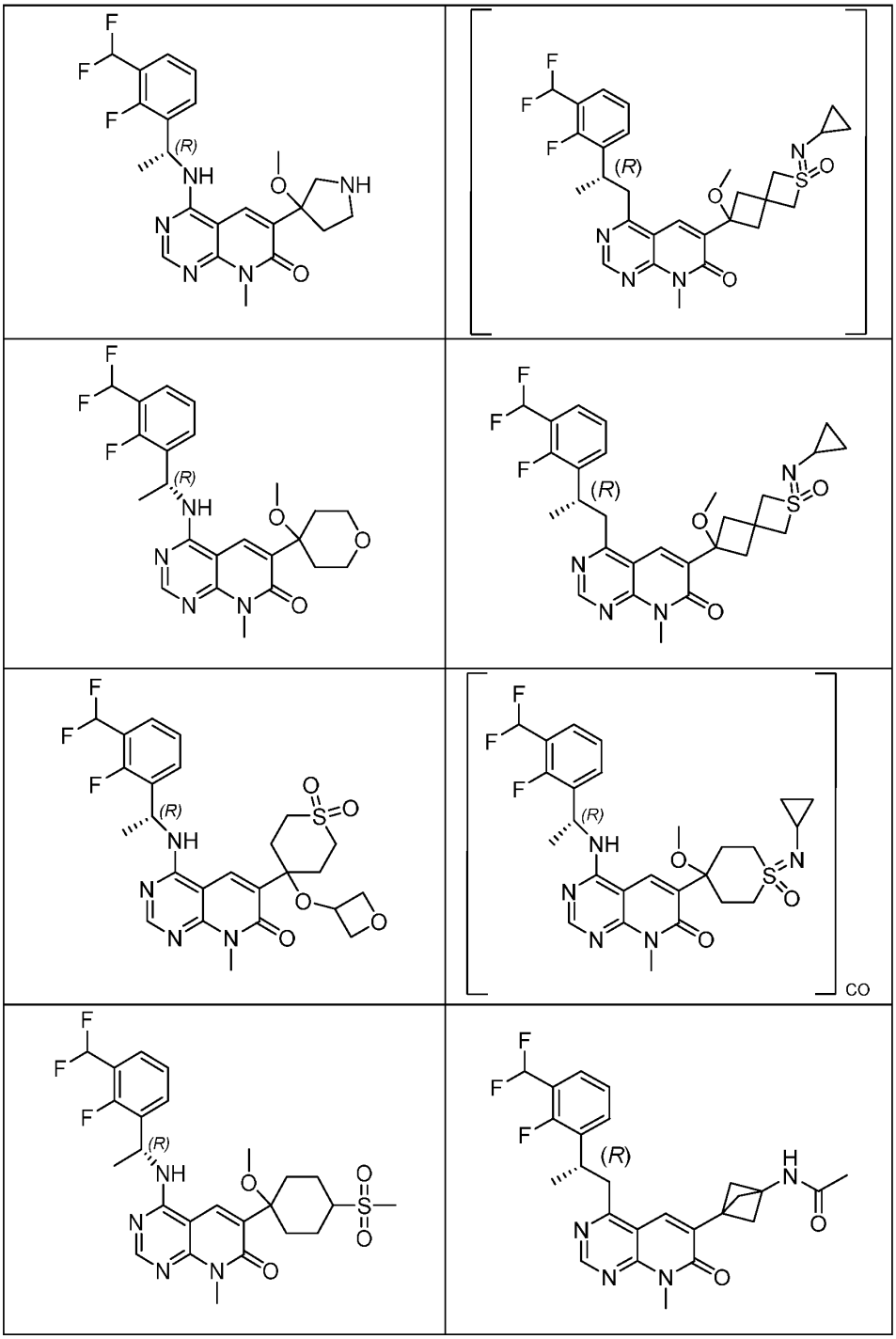


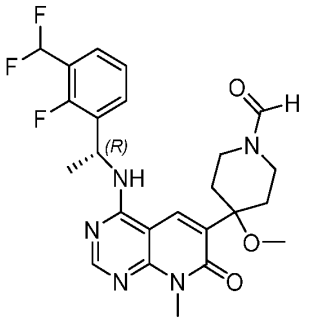
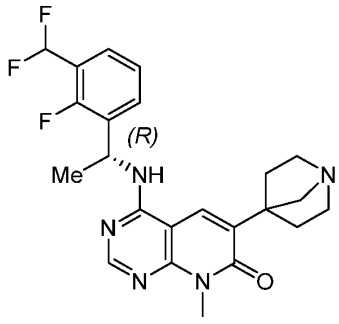
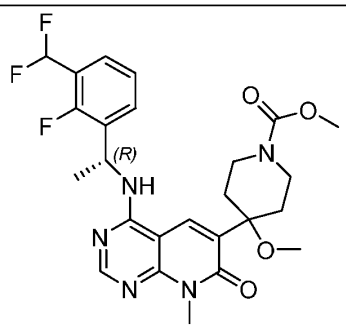
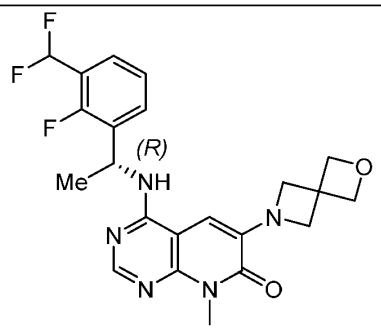
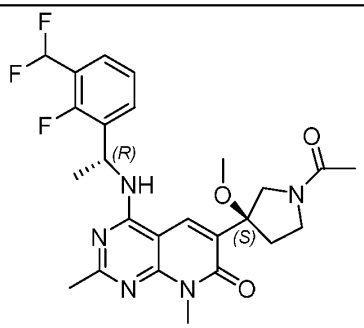
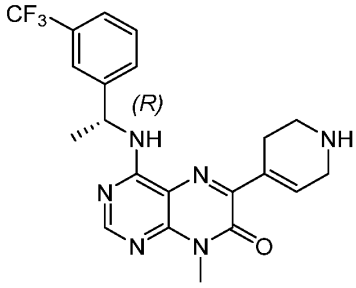
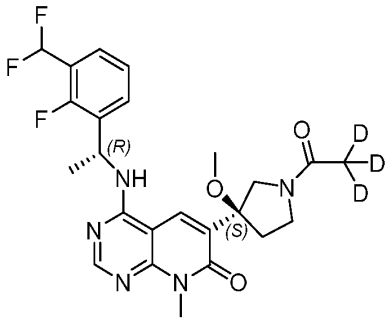
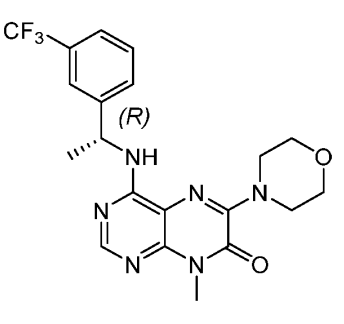


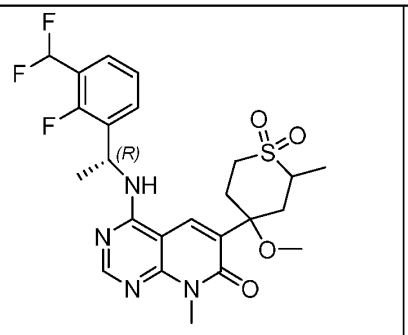






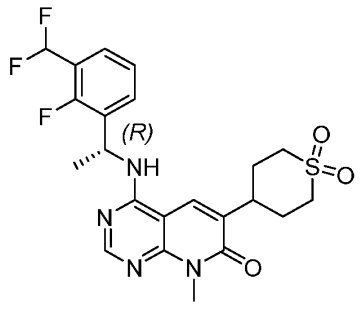
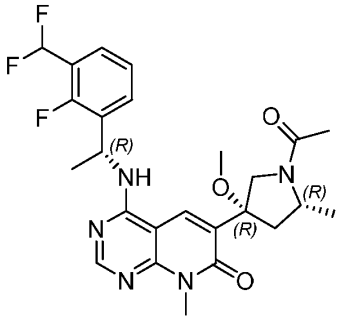
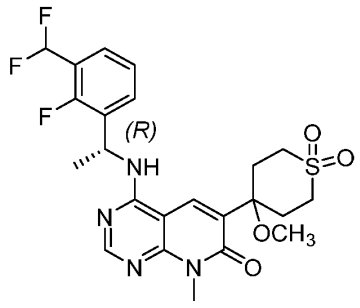
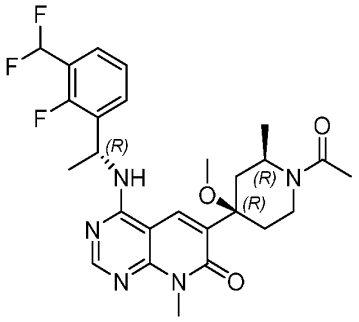
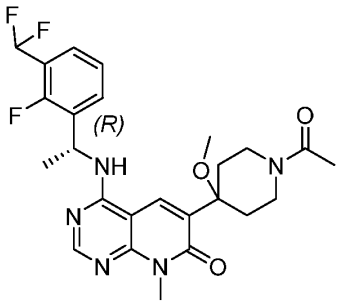
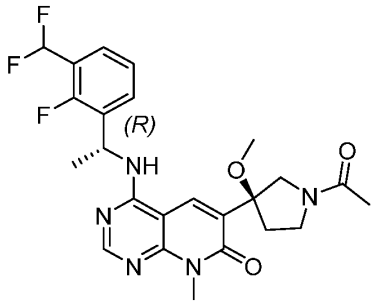
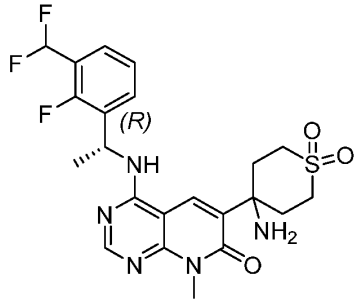


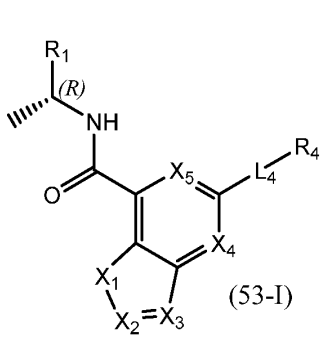
Note: any compound shown in the foregoing table may be a diastereomer, and the absolute stereochemistry of such diastereomer may not be known.

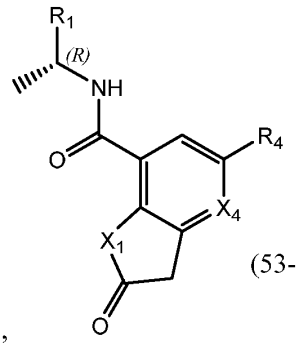
[0109] In some embodiments, the SOS1 inhibitor is a compound selected from the group consisting of the compounds in the following table, or a pharmaceutically acceptable salt or stereoisomer thereof:

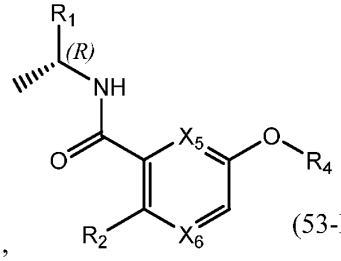
Structure	Structure
	
	
	
	

Note: any compound shown in the foregoing table may be a diastereomer, and the absolute stereochemistry of such diastereomer may not be known.

[0110] In some embodiments, the SOS1 inhibitor is a compound having the structure of Formula (53-I), (53-II), or (53-III):


(53-I)


(53-II)


(53-III)

or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof, wherein:

X_1 is NH or S;

X_2 is CH or N;

X_3 is CH or N;

X_4 is CR_3 or N;

X_5 is CH or N;

X_6 is CH or N;

R_1 is selected from the group consisting of optionally substituted 3-6 membered cycloalkyl, optionally substituted 3-6 membered heterocyclyl, optionally substituted 6-membered aryl, and optionally substituted 5-6 membered heteroaryl;

R_2 is selected from the group consisting of H, -NH-C_{1-6} alkyl, and $–NH_2$;

R_3 is selected from the group consisting of H, -O-C_{1-6} alkyl, and -O-C_{1-6} heteroalkyl;

L_4 is a bond or O; and

R_4 is selected from the group consisting of H, C_{1-6} alkyl, 3-14 membered cycloalkyl, 3-14 membered cycloalkenyl, 3-14 membered heterocyclyl, 6-10 membered aryl, and 5-10 membered heteroaryl; wherein each C_{1-6} alkyl, 3-14 membered cycloalkyl, 3-14 membered

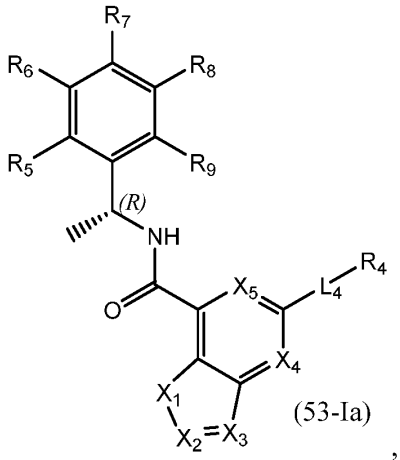
cycloalkenyl, 3-14 membered heterocyclyl, 6-10 membered aryl, and 5-10 membered heteroaryl is optionally substituted with one or more C_{1-6} alkyl, $–R_{4a}$, $–OR_{4a}$, $–O–C_{1-6}$ alkyl–R_{4a}, =O, halogen, $–C(O)R_{4a}$, $–C(O)OR_{4a}$, $–C(O)NR_{4b}R_{4c}$, $–NR_{4b}C(O)R_{4c}$, –CN, $=NR_{4a}$, $–NR_{4b}R_{4c}$, $–SO_2R_{4a}$, 3-6 membered cycloalkyl optionally substituted with R_{4a}, 3-7 membered heterocyclyl optionally substituted with R_{4a}, 6-10 membered aryl optionally substituted with R_{4a}, or 5-10 membered heteroaryl optionally substituted with R_{4a};

wherein R_{4a} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, $–C(O)R_{4b}$, $–C(O)NR_{4b}R_{4c}$, =O, 3-6 membered cycloalkyl, 6-10 membered aryl optionally substituted with $–OR_{4b}$, –CN, =N-3-6 membered cycloalkyl, 3-7 membered heterocyclyl, $–(CH_2)_rOCH_3$, or $–(CH_2)_rOH$, wherein r is 1, 2, or 3;

wherein each R_{4b} is independently H, C_{1-6} alkyl; and

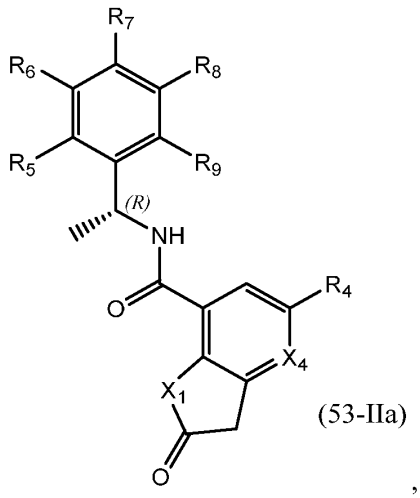
wherein each R_{4c} is independently H or C_{1-6} alkyl.

[0111] In some embodiments, the SOS1 inhibitor is a compound having the structure of Formula (53-Ia), (53-IIa), or (53-IIIa):



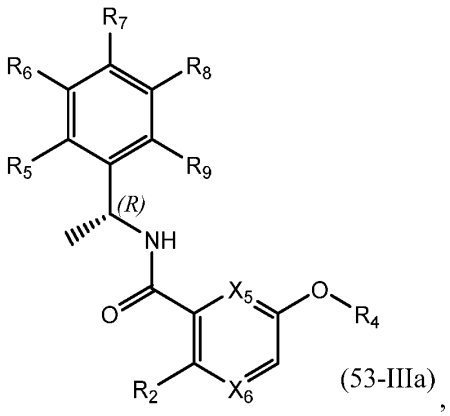
(53-Ia)

,



(53-IIa)

,



(53-IIIa)

,

or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof, wherein X_1, X_2, X_3, X_4, X_5, X_6, R_2, L_4, and R_4 are as defined in Formula (53-I), (53-II), or (53-III);

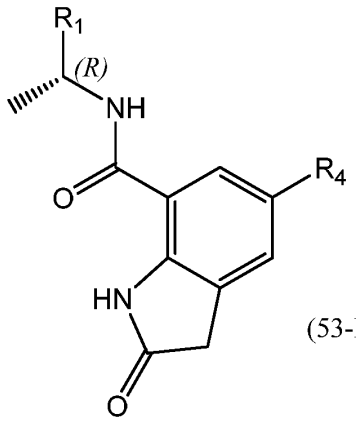
R_5, R_6, R_7, R_8, and R_9 are independently selected from the group consisting of H, D, C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, 3-14 membered heterocyclyl, –OH, halogen, $–NO_2$, –CN, $–NR_{11}R_{12}$, $–SR_{10}$, $–S(O)_2NR_{11}R_{12}$, $–S(O)_2R_{10}$, $–NR_{10}S(O)_2NR_{11}R_{12}$, $–NR_{10}S(O)_2R_{11}$, $–S(O)NR_{11}R_{12}$, $–S(O)R_{10}$, $–NR_{10}S(O)NR_{11}R_{12}$, $–NR_{10}S(O)R_{11}$, $–C(O)R_{10}$, $–CO_2R_{10}$, 6-10 membered aryl, and 5-10 membered heteroaryl, wherein each C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, 3-14 membered heterocyclyl, 6-10 membered aryl, and 5-10 membered heteroaryl is optionally substituted with –OH, C_{1-6} alkyl optionally substituted with $-R_{10}$, halogen, $–NO_2$, =O, –CN, $–R_{10}$, $–OR_{10}$, $–NR_{11}R_{12}$, $–SR_{10}$, $–S(O)_2NR_{11}R_{12}$, $–S(O)_2R_{10}$, $–NR_{10}S(O)_2NR_{11}R_{12}$, $–NR_{10}S(O)_2R_{11}$, $–S(O)NR_{11}R_{12}$, $–S(O)R_{10}$,

$-NR_{10}S(O)NR_{11}R_{12}$, $-NR_{10}S(O)R_{11}$, 3-8 membered cycloalkyl, 3-14 membered heterocyclyl optionally substituted with R_{10}, 6-10 membered aryl, or 5-10 membered heteroaryl, or any two adjacent R^5, R^6, R^7, R^8, and R^9 forms an optionally substituted 3-14 membered fused ring;

R_{10}, R_{11}, and R_{12} are at each occurrence independently selected from H, D, C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, 3-14 membered heterocyclyl, $-OR_{13}$, $-SR_{13}$, halogen, $-NR_{13}R_{14}$, $-NO_2$, and –CN; and

R_{13} and R_{14} are at each occurrence independently selected from H, D, C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, and 3-14 membered heterocyclyl, wherein each C_{1-6} alkyl, C_{2-6} alkenyl, 4-8 membered cycloalkenyl, C_{2-6} alkynyl, 3-8 membered cycloalkyl, and 3-14 membered heterocyclyl are independently optionally substituted with –OH, –SH, $-NH_2$, $-NO_2$, or –CN.

[0112] In some embodiments, the SOS1 inhibitor is a compound having the structure of Formula (53-II-1):

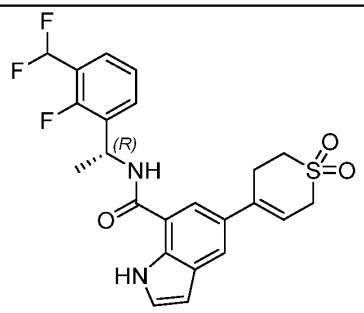
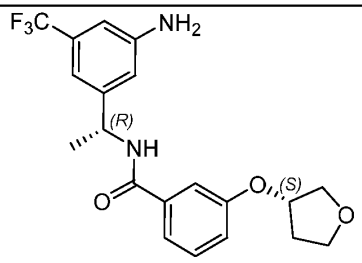
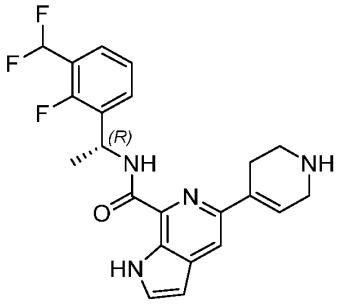
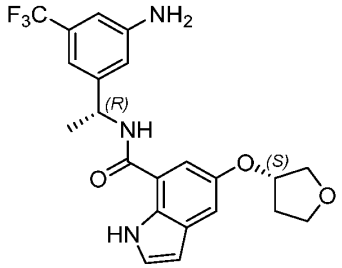
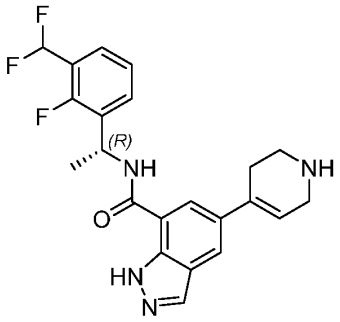
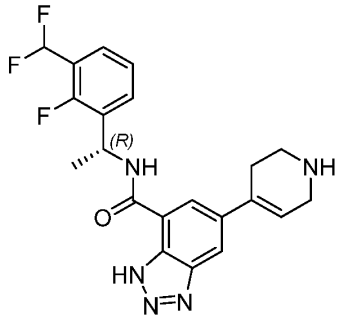
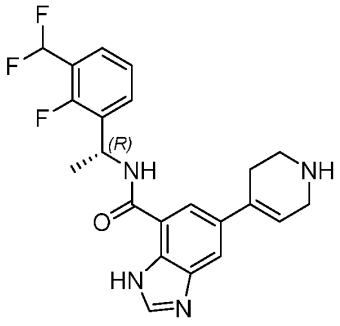
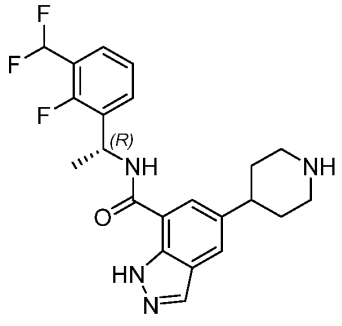


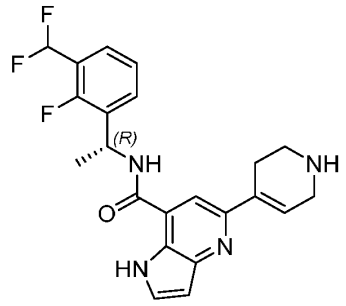
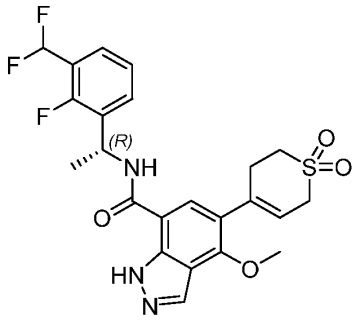
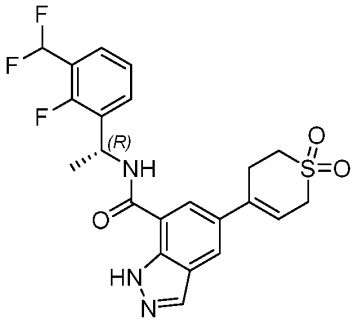
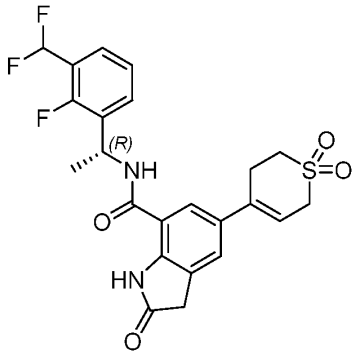
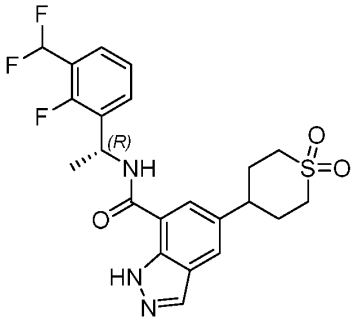
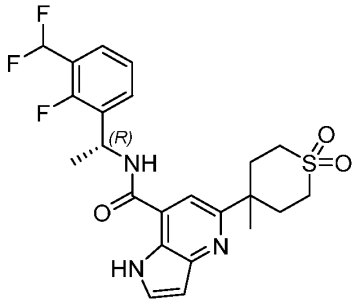
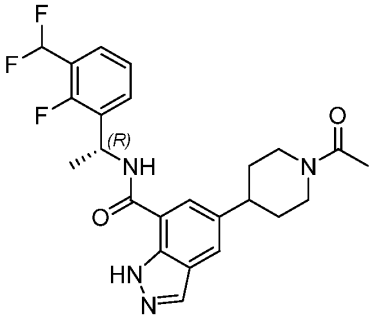
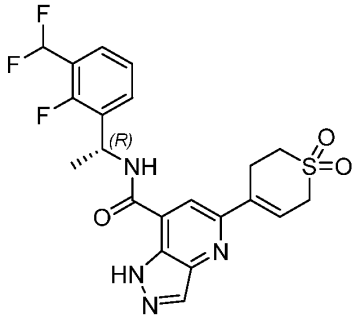
(53-II-1)

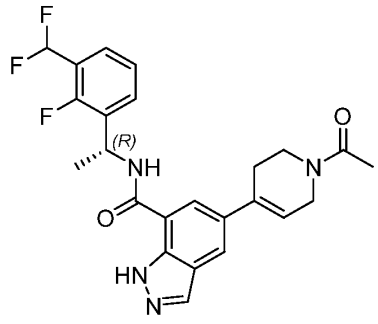
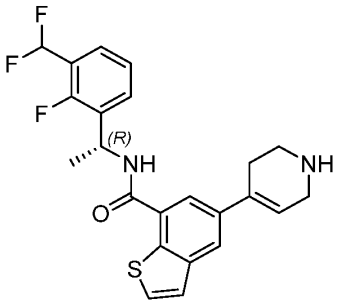
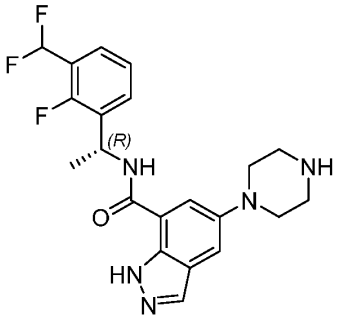
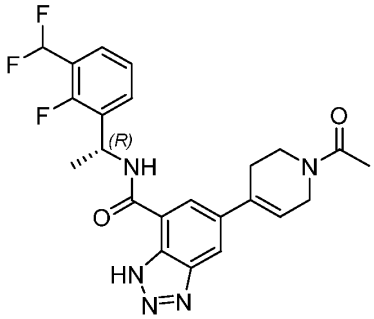
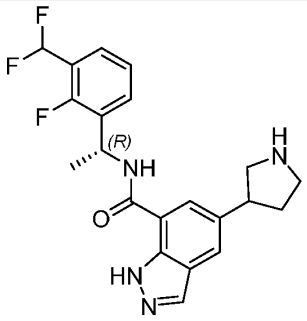
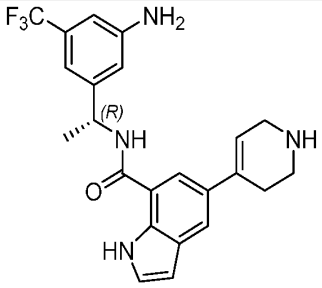
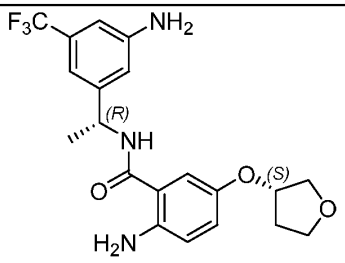
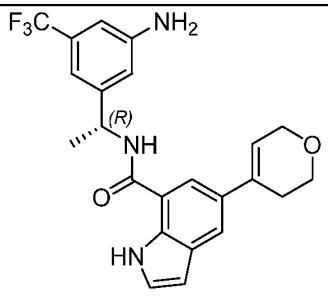
,

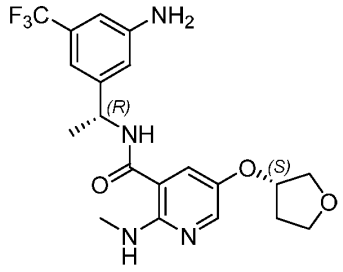
or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof, wherein R_1 and R_4 are as defined in Formula (II).

[0113] In some embodiments, the SOS1 inhibitor is a compound selected from the group consisting of the compounds in the following table, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof:

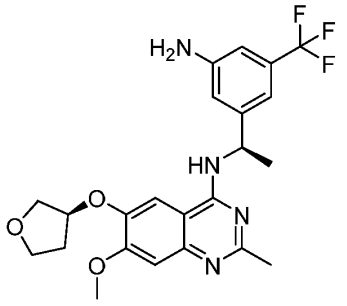
	
	
	
	

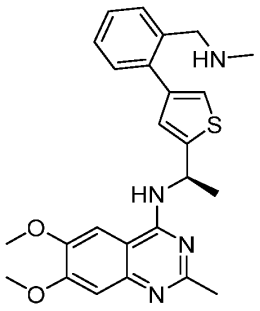
[0114] In some embodiments, the SOS1 inhibitor is BI-3406, having the structure:



or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0115] In some embodiments, the SOS1 inhibitor is BI-1701963 or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

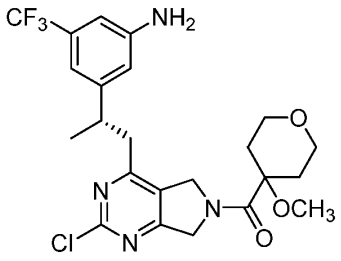
[0116] In some embodiments, the SOS1 inhibitor is BAY-293, having the structure:



or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0117] In some embodiments, the SOS1 inhibitor is SDGR5 or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0118] In some embodiments, the SOS1 inhibitor is Compound SOS1-(A) (also called RMC-0331), having the structure:



or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0119] In some embodiments, the SOS1 inhibitor is Compound SOS1-(B), or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0120] The SOS1 inhibitor dose may range from a dose sufficient to elicit a response to the maximum tolerated dose. Effective dosage amounts of the disclosed compounds, when used for the indicated effects, range from about 0.5 mg to about 5000 mg of the disclosed compound as needed to treat the condition. Compositions for *in vivo* or *in vitro* use can contain about 0.5, 5, 20, 50, 75, 100, 150, 250, 500, 750, 1000, 1250, 2500, 3500, or 5000 mg of the disclosed compound, or, in a range of from one amount to another amount in the list of doses, such as from 100 mg to 1300 mg, from 200 mg to 1300 mg, from 600 mg to 1300 mg, from 700 mg to 1200 mg, or from 800 mg to 1000 mg. In one embodiment, the compositions are in the form of a tablet that can be scored. The SOS1 inhibitor can be dosed once per day, twice per day, three times per day, or four times per day. In some aspects, SOS1 inhibitor is dosed once per day. In some aspects, SOS1 inhibitor is dosed twice per day. Dosing may be done with or without food. The dosing schedule may suitably be every day of a 28-day schedule, or 21 or more days of a 28-day schedule.

[0121] Mutations in SHP2, e.g., activating SHP2 mutations, may induce RAS/MAPK signaling pathway reactivation and drug resistance to a SHP2 inhibitor in a patient administered a SHP2 inhibitor, e.g., an allosteric SHP2 inhibitor, in the treatment of

a tumor or cancer. The present invention is suitable for the treatment of a patient who has a cancer characterized by a mutation of SHP2, e.g., an activating SHP2 mutation, and has therefore developed drug resistance to a SHP2 inhibitor, e.g., an allosteric SHP2 inhibitor. Empirical evidence to date suggests that SHP2 mutations, e.g., activating SHP2 mutations, increase dependence on SOS1 and may therefore increase sensitivity to SOS1 inhibitors such as BI-3406, BI-1701963, and Compound SOS1-(A) (also called RMC-0331), or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof. Accordingly, the therapeutic strategy according to the method of the present invention is directed to the effective treatment of cancer patients with activating mutations in SHP2 by the administration of a therapeutically effective amount of SOS1 inhibitor.

[0122] According to embodiments of the present invention, the method comprises administering to the subject a therapeutically effective amount of a SOS1 inhibitor in the treatment of a tumor or cancer. In some embodiments, the disease or disorder is selected from the group consisting of tumors of hematopoietic and lymphoid system; a myeloproliferative syndrome; a myelodysplastic syndrome; leukemia; acute myeloid leukemia; acute B-lymphoblastic leukemia-lymphoma; juvenile myelomonocytic leukemia; esophageal cancer; breast cancer; lung cancer; colon cancer; gastric cancer; neuroblastoma; bladder cancer; prostate cancer; glioblastoma; urothelial carcinoma; uterine carcinoma; adenoid and ovarian serous cystadenocarcinoma; paraganglioma; pheochromocytoma; pancreatic cancer; adrenocortical carcinoma; stomach adenocarcinoma; sarcoma; rhabdomyosarcoma; lymphoma; head and neck cancer; skin cancer; peritoneum cancer; intestinal cancer (e.g., small and/or large intestinal cancer); thyroid cancer; endometrial cancer; cancer of the biliary tract; soft tissue cancer; ovarian cancer; central nervous system cancer (e.g., primary CNS lymphoma); stomach cancer; pituitary cancer; genital tract cancer; urinary tract cancer; salivary gland cancer; cervical cancer; liver cancer; eye cancer; cancer of the adrenal gland; cancer of autonomic ganglia; cancer of the upper aerodigestive tract; bone cancer; testicular cancer; pleura cancer; kidney cancer; penis cancer; parathyroid cancer; cancer of the meninges; vulvar cancer; and melanoma. In some embodiments, the disease or disorder is selected from brain glioblastoma, lung adenocarcinoma, colon adenocarcinoma, bone marrow leukemia, acute myelocytic leukemia (AML), breast carcinoma, unknown primary melanoma, non-small cell lung carcinoma (NSCLC), skin melanoma, breast invasive ductal carcinoma, lung squamous cell carcinoma, unknown primary adenocarcinoma, bone marrow multiple myeloma,

gastroesophageal junction adenocarcinoma, bone marrow myelodysplastic syndrome, prostate acinar adenocarcinoma, bladder urothelial (transitional cell) carcinoma, uterus endometrial adenocarcinoma, bone marrow leukemia B cell acute, acute B-lymphoblastic leukemia-lymphoma, stomach adenocarcinoma, and unknown primary carcinoma. In some embodiments, the disease or disorder is selected from the group consisting of AML, lung adenocarcinoma, non-small cell lung carcinoma, brain glioblastoma, a myelodysplastic syndrome, skin melanoma, breast carcinoma, stomach adenocarcinoma, acute B-lymphoblastic leukemia-lymphoma, and colon adenocarcinoma.

[0123] In some embodiments, the disease or disorder is acute myelocytic leukemia (AML). In some embodiments, the disease or disorder is AML and the SHP2 mutation is at a position selected from the group consisting of G60, D61, A72, E76, G503 and S502, and a combination thereof, and the method optionally further comprises administering to a subject a therapeutically effective amount of a SHP2 inhibitor (e.g., RMC-4630 or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof) and a RAS(ON) inhibitor, such as a $RAS(ON)^{G12C}$ inhibitor of Appendix B (e.g., a compound of Table 1 or Table 2 therein) or a $RAS(ON)^{MULTI}$ inhibitor of Appendix A (e.g., a compound of Table 1 or Table 2 therein). See, e.g., Alfayez et al., Leukemia 35:691-700 (2021).

[0124] In some embodiments, the disease or disorder is selected from the group consisting of AML, lung adenocarcinoma, non-small cell lung carcinoma, brain glioblastoma, a myelodysplastic syndrome, skin melanoma, breast carcinoma, stomach adenocarcinoma, acute B-lymphoblastic leukemia-lymphoma, and colon adenocarcinoma, the SHP2 mutation is at a position selected from the group consisting of G60, D61, E69, A72, E123, Y197, N308, V428, A461, T468, S502, G503, T507 (e.g., A72, E76 or G503; or, e.g., G60V, D61G, D61V, D61Y, E69K, E69Q, A72S, A72T, A72V, E123D, N308D, V428M, A461T, A461G, T468M, S502L, S502P, G503A, G503V, T507K), and the method optionally further comprises administering to a subject a therapeutically effective amount of a SHP2 inhibitor (e.g., RMC-4630 or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof) and a RAS(ON) inhibitor, such as a $RAS(ON)^{G12C}$ inhibitor of Appendix B (e.g., a compound of Table 1 or Table 2 therein) or a $RAS(ON)^{MULTI}$ inhibitor of Appendix A (e.g., a compound of Table 1 or Table 2 therein).

[0125] Activating SHP2 mutations have been associated with developmental RASopathy pathologies such as Noonan syndrome and Leopard syndrome. SHP2 mutations have also been identified in other RASopathies. According to embodiments of the present invention, the method comprises administering to the subject a therapeutically effective amount of a SOS1 inhibitor in the treatment of a RASopathy. In some embodiments, the RASopathy is selected from the group consisting of Neurofibromatosis type 1, Noonan Syndrome, Noonan Syndrome with Multiple Lentigines, Capillary Malformation-Arteriovenous Malformation Syndrome, Costello Syndrome, Cardio-Facio-Cutaneous Syndrome, Legius Syndrome, and Hereditary gingival fibromatosis. In some embodiments, a RASopathy comprises a SHP2 mutation at a position selected from the group consisting of T52, I56, Y62, Y63, E69, K70, E139, L261, R265, N308, T468, M504, Q510 (e.g., T52I, I56V, Y62D, Y63D, Y63C, E69K, E69Q, K70R, E139D, L261F, L261H, R265Q, N308D, T468M, M504V, Q510P, Q510H). According to embodiments of the present invention, the method comprises administering to the subject a therapeutically effective amount of a SOS1 inhibitor in the treatment of Noonan syndrome or Leopard syndrome.

RAS and RAS Mutations

[0126] In some embodiments, the method of the present invention further comprises administering to the subject a therapeutically effective amount of a RAS inhibitor selected from the group consisting of a RAS(ON) inhibitor, a RAS(OFF) inhibitor, MRTX1133, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof, and a combination thereof. In some embodiments, the Ras protein is wild-type, and the RAS inhibitor targets a wild-type RAS protein. In some embodiments, the RAS inhibitor targets KRAS, NRAS, or HRAS. In some embodiments, the RAS inhibitor targets two or more of KRAS, NRAS, or HRAS.

[0127] In some embodiments, the RAS inhibitor targets a RAS protein having a mutation. In some embodiments, the RAS inhibitor is a RAS mutant specific inhibitor. In some embodiments, the RAS inhibitor targets a KRAS mutant, a NRAS mutant, or an HRAS mutant. In certain embodiments, RAS mutant is selected from:

[0128] (a) the following K-Ras mutants: G12D, G12V, G12C, G13D, G12R, G12A, Q61H, G12S, A146T, G13C, Q61L, Q61R, K117N, A146V, G12F, Q61K, L19F, Q22K, V14I, A59T, A146P, G13R, G12L, Y96D, or G13V, and combinations thereof;

[0129] (b) the following H-Ras mutants: Q61R, G13R, Q61K, G12S, Q61L, G12D, G13V, G13D, G12C, K117N, A59T, G12V, G13C, Q61H, G13S, A18V, D119N, G13N, A146T, A66T, G12A, A146V, G12N, or G12R, and combinations thereof; and

[0130] (c) the following N-Ras mutants: Q61R, Q61K, G12D, Q61L, Q61H, G13R, G13D, G12S, G12C, G12V, G12A, G13V, G12R, P185S, G13C, A146T, G60E, Q61P, A59D, E132K, E49K, T50I, A146V, or A59T, and combinations thereof;

[0131] or a combination of any of the foregoing (e.g., both K-Ras G12C and K-Ras G13C). In some embodiments, the cancer comprises a Ras mutation selected from the group consisting of G12C, G13C, G12A, G12D, G13D, G12S, G13S, G12V and G13V. In some embodiments, the cancer comprises at least two Ras mutations selected from the group consisting of G12C, G13C, G12A, G12D, G13D, G12S, G13S, G12V and G13V. In some embodiments, the cancer comprises at least a G12C mutation and a Y96D mutation. Mutations at these positions may result in RAS-driven tumors.

[0132] In some embodiments, the RAS inhibitor targets a wild-type RAS protein. In some embodiments, the Ras inhibitor targets RAS^{amp}. In some embodiments, the RAS protein is KRAS. In some embodiments, the RAS protein is NRAS. In some embodiments, a RAS inhibitor targets both a KRAS protein and an NRAS protein. In some embodiments, the RAS inhibitor targets a RAS protein mutation. In some embodiments, the RAS protein mutation is at a position selected from the group consisting of G12, G13, Q61, A146, K117, L19, Q22, V14, A59, and a combination thereof. In some embodiments, the mutation is at a position selected from the group consisting of G12, G13, and Q61. In some embodiments, the mutation is selected from the group consisting of G12C, G12D, G12A, G12S, G12V, G13C, G13D, Q61K, and Q61L.

RAS Inhibitors

[0133] According to some embodiments of the present disclosure, the method comprises treating a subject having a disease or disorder associated with cells having a SHP2 mutation by administering to the subject (a) a therapeutically effective amount of a SOS1 inhibitor or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof; and (b) a therapeutically effective amount of a RAS inhibitor selected from the group consisting of a RAS(ON) inhibitor, a RAS(OFF) inhibitor,

MRTX1133 or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof, and a combination thereof.

[0134] In some embodiments, the RAS inhibitor is a RAS(OFF) inhibitor known in the art or disclosed herein. The RAS(OFF) inhibitor may be any one or more of the RAS(OFF) inhibitors disclosed in any one of WO 2021168193, WO 2021158071, WO 2021155716, WO 2021152149, WO 2021150613, WO 2021147967, WO 2021147965, WO 2021143693, WO 2021142252, WO 2021141628, WO 2021139748, WO 2021139678, WO 2021129824, WO 2021129820, WO 2021127404, WO 2021126816, WO 2021126799, WO 2021124222, WO 2021121371, WO 2021121367, WO 2021121330, WO 2021120890, WO 2021120045, WO 2021119343, WO 2021118877, WO 2021113595, WO 2021107160, WO 2021106231, WO 2021106230, WO 2021104431, WO 2021088458, WO 2021086833, WO 2021085653, WO 2021084765, WO 2021081212, WO 2021058018, WO 2021057832, WO 2021055728, WO 2021041671, WO 2021031952, WO 2021027911, WO 2021023247, WO 2020259513, WO 2020259432, WO 2020234103, WO 2020233592, WO 2020216190, WO 2020178282, WO 2020146613, WO 2020118066, WO 2020113071, WO 2020106647, WO 2020106640, WO 2020102730, WO 2020101736, WO 2020097537, WO 2020086739, WO 2020018282, WO 2020050890, WO 2020047192, WO 2020035031, WO 2020033413, WO 2020028706, WO 2019241157, WO 2019234405, WO 2019232419, WO 2019227040, WO 2019217933, WO 2019217691, WO 2019217307, WO 2019215203, WO 2019213526, WO 2019213516, WO 2019204442, WO 2019204449, WO 2019204505, WO 2019155399, WO 2019150305, WO 2019137985, WO 2019110751, WO 2019099524, WO 2019055540, WO 2019051291, WO 2018237084, WO 2018218070, WO 2018217651, WO 2018218071, WO 2018218069, WO 2018212774, WO 2018206539, WO 2018195439, WO 2018143315, WO 2018140600, WO 2018140599, WO 2018140598, WO 2018140514, WO 2018140513, WO 2018140512, WO 2018119183, WO 2018112420, WO 2018068017, WO 2018064510, WO 2018011351, WO 2018005678, WO 2017201161, WO 20171937370, WO 2017172979, WO 2017112777, WO 2017106520, WO 2017096045, WO 2017100546, WO 2017087528, WO 2017079864, WO 2017058807, WO 2017058805, WO 2017058728, WO 2017058902, WO 2017058792, WO 2017058768, WO 2017058915, WO 2017015562, WO 2016179558, WO 2016176338, WO 2016168540, WO 2016164675, WO 2016100546, WO 2016049568, WO 2016049524, WO

2015054572, WO 2014152588, WO 2014143659 and WO 2013155223, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof, each of which is incorporated herein by reference in its entirety. In some embodiments, the RAS(OFF) inhibitor is selected from sotorasib (AMG 510), adagrasib (MRTX849), MRTX1257, JNJ-74699157 (ARS-3248), LY3537982, ARS-853, ARS-1620, GDC-6036, BPI-421286, JDQ443, and JAB-21000, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof. In some embodiments, the RAS(OFF) inhibitor selectively targets RAS G12C.

[0135] In some embodiments, the compositions and methods described herein utilize a RAS inhibitor that is a RAS(ON) inhibitor known in the art or disclosed herein. In some embodiments, the RAS inhibitor is a RAS(ON) inhibitor. In some embodiments, the RAS(ON) inhibitor is an inhibitor selective for RAS G12C, RAS G13D, or RAS G12D. In some embodiments, the RAS(ON) inhibitor is a RAS(ON)^{MULTI} inhibitor.

[0136] The RAS(ON) inhibitor may be any one or more of the RAS(ON) inhibitors disclosed in WO 2020/132597. or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof, or any one of Appendices A, B, C, or D, or a compound described by a Formula of any one of Appendices A, B, C, or D, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0137] In some embodiments, the RAS inhibitor is a compound disclosed in Appendix A. In some embodiments, the RAS(ON) inhibitor is a compound described by Formula I in Appendix A, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof. In some embodiments, the RAS(ON) inhibitor is selected from a compound of Table 1 or Table 2 of Appendix A, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0138] In some embodiments, the RAS inhibitor is a compound disclosed in Appendix B. In some embodiments, the RAS(ON) inhibitor is a compound described by Formula I in Appendix B, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof. In some embodiments, the RAS(ON) inhibitor is selected from a compound of Table 1 or Table 2 of Appendix B, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0139] In some embodiments, the RAS inhibitor is a compound disclosed in Appendix C. In some embodiments, the RAS(ON) inhibitor is a compound described by Formula I in Appendix C, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof. In some embodiments, the RAS(ON) inhibitor is selected from a compound of Table 1 or Table 2 of Appendix C, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0140] In some embodiments, the RAS inhibitor is a compound disclosed in Appendix D. In some embodiments, the RAS(ON) inhibitor is a compound described by Formula I in Appendix D, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof. In some embodiments, the RAS(ON) inhibitor is selected from a compound of Table 1 or Table 1-1 of Appendix D, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0141] In some embodiments, the RAS inhibitor is selected from the group consisting of Compound RAS-(A) (a $RAS(ON)^{G12C}$ inhibitor of Appendix B), Compound RAS-(B) (a $RAS(ON)^{G12C}$ inhibitor of Appendix B), Compound RAS-(C) (a $RAS(ON)^{G13C}$ inhibitor of Appendix B), Compound RAS-(D) (a $RAS(ON)^{MULTI}$ inhibitor of Appendix A), Compound RAS-(E) ($RAS(ON)^{MULTI}$ inhibitor of Appendix D), or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof, and a combination thereof. It is to be understood that any one of Compound RAS-(A), Compound RAS-(B), Compound RAS-(C), Compound RAS-(D), Compound RAS-(E) could be found in any one of Appendices A, B, C, and D. Accordingly, the letter reference to the RAS compound (e.g., RAS-(A)) should not be understood to necessarily indicate that the compound can be found in the corresponding Appendix (e.g., Appendix A).

[0142] In some embodiments, the RAS inhibitor is Compound RAS-(A), or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0143] In some embodiments, the RAS inhibitor is Compound RAS-(B), or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0144] In some embodiments, the RAS inhibitor is Compound RAS-(C), or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0145] In some embodiments, the RAS inhibitor is Compound RAS-(D), or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0146] In some embodiments, the RAS inhibitor is Compound RAS-(E), or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

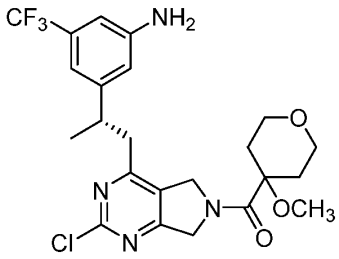
[0147] In some embodiments, the RAS inhibitor is selective for a mutation at position 12 or 13 of a RAS protein. In some embodiments, the RAS inhibitor selectively targets RAS G12D. In some embodiments, the RAS inhibitor is MRTX1133, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0148] The RAS inhibitor dose may range from a dose sufficient to elicit a response to the maximum tolerated dose. Effective dosage amounts of the disclosed compounds, when used for the indicated effects, range from about 0.5 mg to about 5000 mg of the disclosed compound as needed to treat the condition. Compositions for *in vivo* or *in vitro* use can contain about 0.5, 5, 20, 50, 75, 100, 150, 250, 500, 750, 1000, 1250, 2500, 3500, or 5000 mg of the disclosed compound, or, in a range of from one amount to another amount in the list of doses, such as from 100 mg to 1300 mg, from 200 mg to 1300 mg, from 600 mg to 1300 mg, from 700 mg to 1200 mg, or from 800 mg to 1000 mg. In one embodiment, the compositions are in the form of a tablet that can be scored. The RAS inhibitor can be dosed once per day, twice per day, three times per day, or four times per day. In some aspects, RAS inhibitor is dosed once per day. In some aspects, RAS inhibitor is dosed twice per day. Dosing may be done with or without food. The dosing schedule may suitably be every day of a 28-day schedule, or 21 or more days of a 28-day schedule.

Exemplary Combinations of RAS Inhibitors and SOS1 Inhibitors

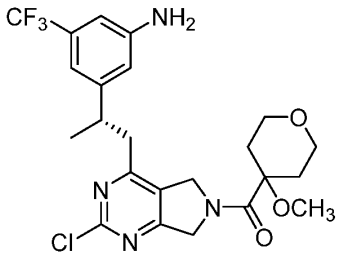
[0149] In some embodiments, the method comprises administering a combination of a RAS inhibitor and a SOS1 inhibitor. Exemplary, non-limiting combinations of such inhibitors include the following.

[0150] In one embodiment, (a) the SOS1 inhibitor is Compound SOS1-(A) (also called RMC-0331), having the structure:



or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof; and (b) the RAS inhibitor is Compound RAS-(C), or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0151] In one embodiment, (a) the SOS1 inhibitor is Compound SOS1-(A) (also called RMC-0331), having the structure:



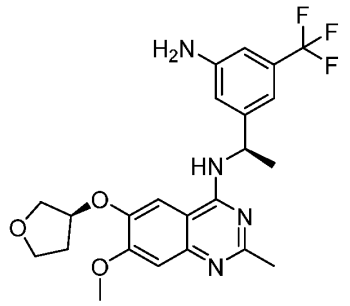
or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof; and (b) the RAS inhibitor is Compound RAS-(D), or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0152] In one embodiment, (a) the SOS1 inhibitor is Compound SOS1-(B), or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof; and (b) the RAS inhibitor is Compound RAS-(B), or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0153] In one embodiment, (a) the SOS1 inhibitor is Compound SOS1-(B), or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer

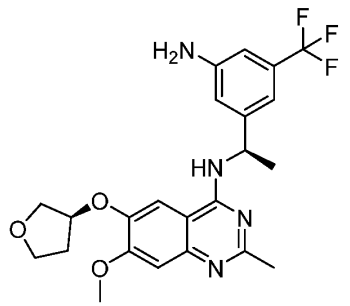
thereof; and (b) the RAS inhibitor is Compound RAS-(E), or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0154] In one embodiment, (a) the SOS1 inhibitor is BI-3406, having the structure:



or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof; and (b) the RAS inhibitor is Compound RAS-(A), or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0155] In one embodiment, (a) the SOS1 inhibitor is BI-3406, having the structure:



or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof; and (b) the RAS inhibitor is Compound RAS-(C), or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0156] In some embodiments, (a) the SOS1 inhibitor is BI-1701963 or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof; and (b) the RAS inhibitor is selected from the group consisting of Compound RAS-(A), Compound RAS-(B), Compound RAS-(C), Compound RAS-(D), Compound RAS-(E), and a combination thereof, or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer of any of the above.

[0157] In some embodiments, (a) the SOS1 inhibitor is BI-1701963 or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof; and (b) the RAS inhibitor is Compound RAS-(A), or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0158] In some embodiments, (a) the SOS1 inhibitor is BI-1701963 or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof; and (b) the RAS inhibitor is Compound RAS-(B), or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0159] In some embodiments, (a) the SOS1 inhibitor is BI-1701963 or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof; and (b) the RAS inhibitor is Compound RAS-(C), or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0160] In some embodiments, (a) the SOS1 inhibitor is BI-1701963 or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof; and (b) the RAS inhibitor is Compound RAS-(D), or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

[0161] In some embodiments, (a) the SOS1 inhibitor is BI-1701963 or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof; and (b) the RAS inhibitor is Compound RAS-(E), or a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof.

Additional Combination Therapies

[0162] In some embodiments, the subject is co-administered a therapeutically effective amount of an additional therapeutic agent. With respect to any compound in this Additional Combination Therapies section, it is to be understood that a pharmaceutically acceptable salt, solvate, isomer (e.g., stereoisomer), prodrug, or tautomer thereof is also contemplated.

[0163] For example, a therapeutic agent may be a steroid. Accordingly, in some embodiments, the one or more additional therapies includes a steroid. Suitable steroids may include, but are not limited to, 21-acetoxypregnenolone, alclometasone, algestone, amcinonide, beclomethasone, betamethasone, budesonide, chloroprednisone, clobetasol,

clocortolone, cloprednol, corticosterone, cortisone, cortivazol, deflazacort, desonide, desoximetasone, dexamethasone, diflorasone, diflucortolone, difuprednate, enoxolone, fluazacort, fiucloronide, flumethasone, flunisolide, fluocinolone acetonide, fluocinonide, fluocortin butyl, fluocortolone, fluorometholone, fluperolone acetate, fluprednidene acetate, fluprednisolone, flurandrenolide, fluticasone propionate, formocortal, halcinonide, halobetasol propionate, halometasone, hydrocortisone, loteprednol etabonate, mazipredone, medrysone, meprednisone, methylprednisolone, mometasone furoate, paramethasone, prednicarbate, prednisolone, prednisolone 25-diethylaminoacetate, prednisolone sodium phosphate, prednisone, prednival, prednylidene, rimexolone, tixocortol, triamcinolone, triamcinolone acetonide, triamcinolone benetonide, triamcinolone hexacetonide, and salts or derivatives thereof.

[0164] Further examples of therapeutic agents that may be used in combination therapy include compounds described in the following patents: U.S. Patent Nos. 6,258,812, 6,630,500, 6,515,004, 6,713,485, 5,521,184, 5,770,599, 5,747,498, 5,990,141, 6,235,764, and 8,623,885, and International Patent Applications WO01/37820, WO01/32651, WO02/68406, WO02/66470, WO02/55501, WO04/05279, WO04/07481, WO04/07458, WO04/09784, WO02/59110, WO99/45009, WO00/59509, WO99/61422, WO00/12089, and WO00/02871.

[0165] A therapeutic agent may be a biologic (e.g., cytokine (e.g., interferon or an interleukin such as IL-2)) used in treatment of cancer or symptoms associated therewith. In some embodiments, the biologic is an immunoglobulin-based biologic, e.g., a monoclonal antibody (e.g., a humanized antibody, a fully human antibody, an Fc fusion protein, or a functional fragment thereof) that agonizes a target to stimulate an anti-cancer response or antagonizes an antigen important for cancer. Also included are antibody-drug conjugates.

[0166] A therapeutic agent may be a checkpoint inhibitor. In one embodiment, the checkpoint inhibitor is an inhibitory antibody (e.g., a monospecific antibody such as a monoclonal antibody). The antibody may be, e.g., humanized or fully human. In some embodiments, the checkpoint inhibitor is a fusion protein, e.g., an Fc-receptor fusion protein. In some embodiments, the checkpoint inhibitor is an agent, such as an antibody, that interacts with a checkpoint protein. In some embodiments, the checkpoint inhibitor is an agent, such as an antibody, that interacts with the ligand of a checkpoint protein. In some embodiments, the checkpoint inhibitor is an inhibitor (e.g., an inhibitory antibody or

small molecule inhibitor) of CTLA-4 (e.g., an anti-CTLA-4 antibody or fusion a protein). In some embodiments, the checkpoint inhibitor is an inhibitor or antagonist (e.g., an inhibitory antibody or small molecule inhibitor) of PD-1. In some embodiments, the checkpoint inhibitor is an inhibitor or antagonist (e.g., an inhibitory antibody or small molecule inhibitor) of PDL-1. In some embodiments, the checkpoint inhibitor is an inhibitor or antagonist (e.g., an inhibitory antibody or Fc fusion or small molecule inhibitor) of PDL-2 (e.g., a PDL-2/Ig fusion protein). In some embodiments, the checkpoint inhibitor is an inhibitor or antagonist (e.g., an inhibitory antibody or small molecule inhibitor) of B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK 1, CHK2, A2aR, B-7 family ligands, or a combination thereof. In some embodiments, the checkpoint inhibitor is pembrolizumab, nivolumab, cemiplimab, PDR001 (NVS), REGN2810 (Sanofi/Regeneron), a PD-L1 antibody such as, *e.g.,* avelumab, durvalumab, atezolizumab, pidilizumab, JNJ-63723283 (JNJ), BGB-A317 (BeiGene & Celgene) or a checkpoint inhibitor disclosed in Preusser, M. et al. (2015) Nat. Rev. Neurol., including, without limitation, ipilimumab, tremelimumab, nivolumab, pembrolizumab, AMP224, AMP514, MEDI0680, BMS936559, MEDl4736, MPDL3280A, MSB0010718C, BMS986016, IMP321, lirilumab, IPH2101, 1-7F9, and KW-6002. In some embodiments, the PD-1 inhibitor may be JTX-4014, spartalizumab, camrelizumab, sintilimab, tislelizumab, toripalimab, dostarlimab, INCMGA00012, AMP-224 or AMP-514.

[0167] A therapeutic agent may be an agent that treats cancer or symptoms associated therewith (e.g., a cytotoxic agent, non-peptide small molecules, or other compound useful in the treatment of cancer or symptoms associated therewith, collectively, an “anti-cancer agent”). Anti-cancer agents can be, e.g., chemotherapeutics or targeted therapy agents.

[0168] Anti-cancer agents include mitotic inhibitors, intercalating antibiotics, growth factor inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, biological response modifiers, alkylating agents, antimetabolites, folic acid analogs, pyrimidine analogs, purine analogs and related inhibitors, vinca alkaloids, epipodopyyllotoxins, antibiotics, L-Asparaginase, topoisomerase inhibitors, interferons, platinum coordination complexes, anthracenedione substituted urea, methyl hydrazine derivatives, adrenocortical suppressant, adrenocorticosteroides, progestins, estrogens, antiestrogen, androgens, antiandrogen, and gonadotropin-releasing hormone analog.

Further anti-cancer agents include leucovorin (LV), irenotecan, oxaliplatin, capecitabine, paclitaxel, and doxetaxel. In some embodiments, the one or more additional therapies includes two or more anti-cancer agents. The two or more anti-cancer agents can be used in a cocktail to be administered in combination or administered separately. Suitable dosing regimens of combination anti-cancer agents are known in the art and described in, for example, Saltz et al., *Proc. Am. Soc. Clin. Oncol.* 18:233a (1999), and Douillard et al., *Lancet* 355(9209):1041-1047 (2000).

[0169] Other non-limiting examples of anti-cancer agents include Gleevec® (Imatinib Mesylate); Kyprolis® (carfilzomib); Velcade® (bortezomib); Casodex (bicalutamide); Iressa® (gefitinib); alkylating agents such as thiotepa and cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; sarcodictyin A; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, such as calicheamicin gammall and calicheamicin omegall (see, e.g., *Agnew, Chem. Intl. Ed Engl.* 33:183-186 (1994)); dynemicin such as dynemicin A; bisphosphonates such as clodronate; an esperamicin; neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores, aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, caminomycin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo- 5-oxo-L-norleucine, adriamycin (doxorubicin), morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, deoxydoxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin,

rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenishers such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfomithine; elliptinium acetate; an epothilone such as epothilone B; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes such as T- 2 toxin, verracurin A, roridin A and anguidine; urethane; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g., Taxol® (paclitaxel), Abraxane® (cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel), and Taxotere® (doxetaxel); chloranbucil; tamoxifen (Nolvadex™); raloxifene; aromatase inhibiting 4(5)-imidazoles; 4-hydroxytamoxifen; trioxifene; keoxifene; LY 117018; onapristone; toremifene (Fareston®); flutamide, nilutamide, bicalutamide, leuprolide, goserelin; chlorambucil; Gemzar® gemcitabine; 6-thioguanine; mercaptopurine; platinum coordination complexes such as cisplatin, oxaliplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; Navelbine® (vinorelbine); novantrone; teniposide; edatrexate; daunomycin; aminopterin; ibandronate; irinotecan (e.g., CPT-11); topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; esperamicins; capecitabine (e.g., Xeloda®); and pharmaceutically acceptable salts of any of the above.

[0170] Additional non-limiting examples of anti-cancer agents include trastuzumab (Herceptin®), bevacizumab (Avastin®), cetuximab (Erbitux®), rituximab (Rituxan®), Taxol®, Arimidex®, ABVD, avicine, abagovomab, acridine carboxamide, adecatumumab, 17-N-allylamino-17-demethoxygeldanamycin, alpharadin, alvocidib, 3-

aminopyridine-2-carboxaldehyde thiosemicarbazone, amonafide, anthracenedione, anti-CD22 immunotoxins, antineoplastics (e.g., cell-cycle nonspecific antineoplastic agents, and other antineoplastics described herein), antitumorigenic herbs, apaziquone, atiprimod, azathioprine, belotecan, bendamustine, BIBW 2992, biricodar, brostallicin, bryostatin, buthionine sulfoximine, CBV (chemotherapy), calyculin, dichloroacetic acid, discodermolide, elsamitrucin, enocitabine, eribulin, exatecan, exisulind, ferruginol, forodesine, fosfestrol, ICE chemotherapy regimen, IT-101, imexon, imiquimod, indolocarbazole, irofulven, laniquidar, larotaxel, lenalidomide, lucanthone, lurtotecan, mafosfamide, mitozolomide, nafoxidine, nedaplatin, olaparib, ortataxel, PAC-1, pawpaw, pixantrone, proteasome inhibitors, rebeccamycin, resiquimod, rubitecan, SN-38, salinosporamide A, sapacitabine, Stanford V, swainsonine, talaporfin, tariquidar, tegafur-uracil, temodar, tesetaxel, triplatin tetranitrate, tris(2-chloroethyl)amine, troxacitabine, uramustine, vadimezan, vinflunine, ZD6126, and zosuquidar.

[0171] Further non-limiting examples of anti-cancer agents include natural products such as vinca alkaloids (e.g., vinblastine, vincristine, and vinorelbine), epidipodophyllotoxins (e.g., etoposide and teniposide), antibiotics (e.g., dactinomycin (actinomycin D), daunorubicin, and idarubicin), anthracyclines, mitoxantrone, bleomycins, plicamycin (mithramycin), mitomycin, enzymes (e.g., L-asparaginase which systemically metabolizes L-asparagine and deprives cells which do not have the capacity to synthesize their own asparagine), antiplatelet agents, antiproliferative/antimitotic alkylating agents such as nitrogen mustards (e.g., mechlorethamine, cyclophosphamide and analogs, melphalan, and chlorambucil), ethylenimines and methylmelamines (e.g., hexaamethylmelaamine and thiotepa), CDK inhibitors (e.g., a CDK 4/6 inhibitor such as ribociclib, abemaciclib, or palbociclib), seliciclib, UCN-01, P1446A-05, PD-0332991, dinaciclib, P27-00, AT-7519, RGB286638, and SCH727965), alkyl sulfonates (e.g., busulfan), nitrosoureas (e.g., carmustine (BCNU) and analogs, and streptozocin), trazenes-dacarbazinine (DTIC), antiproliferative/antimitotic antimetabolites such as folic acid analogs, pyrimidine analogs (e.g., fluorouracil, floxuridine, and cytarabine), purine analogs and related inhibitors (e.g., mercaptopurine, thioguanine, pentostatin, and 2-chlorodeoxyadenosine), aromatase inhibitors (e.g., anastrozole, exemestane, and letrozole), and platinum coordination complexes (e.g., cisplatin and carboplatin), procarbazine, hydroxyurea, mitotane, aminoglutethimide, histone deacetylase (HDAC) inhibitors (e.g., trichostatin, sodium butyrate, apicidan, suberoyl anilide hydroamic acid, vorinostat, LBH

589, romidepsin, ACY-1215, and panobinostat), mTOR inhibitors (e.g., vistusertib, temsirolimus, everolimus, ridaforolimus, and sirolimus), KSP(Eg5) inhibitors (e.g., Array 520), DNA binding agents (e.g., Zalypsis®), PI3K inhibitors such as PI3K delta inhibitor (e.g., GS-1101 and TGR-1202), PI3K delta and gamma inhibitor (e.g., CAL-130), copanlisib, alpelisib and idelalisib; multi-kinase inhibitor (e.g., TG02 and sorafenib), hormones (e.g., estrogen) and hormone agonists such as leutinizing hormone releasing hormone (LHRH) agonists (e.g., goserelin, leuprolide and triptorelin), BAFF-neutralizing antibody (e.g., LY2127399), IKK inhibitors, p38MAPK inhibitors, anti-IL-6 (e.g., CNT0328), telomerase inhibitors (e.g., GRN 163L), aurora kinase inhibitors (e.g., MLN8237), cell surface monoclonal antibodies (e.g., anti-CD38 (HUMAX-CD38), anti-CSl (e.g., elotuzumab), HSP90 inhibitors (e.g., 17 AAG and KOS 953), P13K / Akt inhibitors (e.g., perifosine), Akt inhibitors (e.g., GSK-2141795), PKC inhibitors (e.g., enzastaurin), FTIs (e.g., Zarnestra™), anti-CD138 (e.g., BT062), Torcl/2 specific kinase inhibitors (e.g., INK128), ER/UPR targeting agents (e.g., MKC-3946), cFMS inhibitors (e.g., ARRY-382), JAK1/2 inhibitors (e.g., CYT387), PARP inhibitors (e.g., olaparib and veliparib (ABT-888)), and BCL-2 antagonists.

[0172] In some embodiments, the anti-cancer agent is a colony-stimulating factor 1 receptor (CSF1R) inhibitor. See, e.g., Cannarile et al., J ImmunoTherapy Cancer 5:53 (2017) and Xun et al., Curr Med Chem 27:3944 (2020).

[0173] In some embodiments, an anti-cancer agent is an anti-CD40 antibody, such as APX005M.

[0174] A therapeutic agent may be an anti-TIGIT antibody, such as MBSA43, BMS-986207, MK-7684, COM902, AB154, MTIG7192A or OMP-313M32 (etigilimab).

[0175] In some embodiments, an anti-cancer agent is selected from mechlorethamine, camptothecin, ifosfamide, tamoxifen, raloxifene, gemcitabine, Navelbine®, sorafenib, or any analog or derivative variant of the foregoing.

[0176] In some embodiments, an anti-cancer agent is an ALK inhibitor. Non-limiting examples of ALK inhibitors include ceritinib, TAE-684 (NVP-TAE694), PF02341066 (crizotinib or 1066), alectinib; brigatinib; entrectinib; ensartinib (X-396); lorlatinib; ASP3026; CEP-37440; 4SC-203; TL-398; PLB1003; TSR-011; CT-707; TPX-

0005, and AP26113. Additional examples of ALK kinase inhibitors are described in examples 3-39 of WO05016894.

[0177] In some embodiments, an anti-cancer agent is an inhibitor of a member downstream of a Receptor Tyrosine Kinase (RTK)/Growth Factor Receptor (e.g., a SHP2 inhibitor (e.g., SHP099, TNO155, RMC-4550, RMC-4630, JAB-3068, RLY-1971, ERAS-601), another SOS1 inhibitor (e.g., BI-1701963), a Raf inhibitor, a MEK inhibitor, an ERK inhibitor, a PI3K inhibitor, a PTEN inhibitor, an AKT inhibitor, or an mTOR inhibitor (e.g., mTORC1 inhibitor or mTORC2 inhibitor). In some embodiments, the anti-cancer agent is JAB-3312. In some embodiments, an anti-cancer agent is a Ras inhibitor (e.g., AMG 510, MRTX1257, JNJ-74699157 (ARS-3248), LY3537982, ARS-853, ARS-1620, GDC-6036, BPI-421286, JDQ443 or JAB-21000), or a Ras vaccine, or another therapeutic modality designed to directly or indirectly decrease the oncogenic activity of Ras.

[0178] In some embodiments, a therapeutic agent is an inhibitor of the MAP kinase (MAPK) pathway (or "MAPK inhibitor"). MAPK inhibitors include, but are not limited to, one or more MAPK inhibitor described in Cancers (Basel) 2015 Sep; 7(3): 1758–1784. For example, the MAPK inhibitor may be selected from one or more of trametinib, binimetinib, selumetinib, cobimetinib, LErafAON (NeoPharm), ISIS 5132; vemurafenib, pimasertib, TAK733, RO4987655 (CH4987655); CI-1040; PD-0325901; CH5126766; MAP855; AZD6244; refametinib (RDEA 119/BAY 86-9766); GDC-0973/XL581; AZD8330 (ARRY-424704/ARRY-704); RO5126766 (Roche, described in PLoS One. 2014 Nov 25;9(11)); and GSK1120212 (or JTP-74057, described in Clin Cancer Res. 2011 Mar 1;17(5):989-1000).

[0179] In some embodiments, an anti-cancer agent is a disrupter or inhibitor of the RAS-RAF-ERK or PI3K-AKT-TOR or PI3K-AKT signaling pathways. The PI3K/AKT inhibitor may include, but is not limited to, one or more PI3K/AKT inhibitor described in Cancers (Basel) 2015 Sep; 7(3): 1758–1784. For example, the PI3K/AKT inhibitor may be selected from one or more of NVP-BEZ235; BGT226; XL765/SAR245409; SF1126; GDC-0980; PI-103; PF-04691502; PKI-587; GSK2126458.

[0180] In some embodiments, an anti-cancer agent is a PD-1 or PD-L1 antagonist.

[0181] In some embodiments, additional therapeutic agents include EGFR inhibitors, IGF-1R inhibitors, MEK inhibitors, PI3K inhibitors, AKT inhibitors, TOR

inhibitors, MCL-1 inhibitors, BCL-2 inhibitors, SHP2 inhibitors, proteasome inhibitors, and immune therapies.

[0182] IGF-1R inhibitors include linsitinib, or a pharmaceutically acceptable salt thereof.

[0183] EGFR inhibitors include, but are not limited to, small molecule antagonists, antibody inhibitors, or specific antisense nucleotide or siRNA. Useful antibody inhibitors of EGFR include cetuximab (Erbitux®), panitumumab (Vectibix®), zalutumumab, nimotuzumab, and matuzumab. Further antibody-based EGFR inhibitors include any anti-EGFR antibody or antibody fragment that can partially or completely block EGFR activation by its natural ligand. Non-limiting examples of antibody-based EGFR inhibitors include those described in Modjtahedi et al., Br. J. Cancer 1993, 67:247-253; Teramoto et al., Cancer 1996, 77:639-645; Goldstein et al., Clin. Cancer Res. 1995, 1:1311-1318; Huang et al., 1999, Cancer Res. 15:59(8):1935-40; and Yang et al., Cancer Res.1999, 59:1236-1243. The EGFR inhibitor can be monoclonal antibody Mab E7.6.3 (Yang, 1999 supra), or Mab C225 (ATCC Accession No. HB-8508), or an antibody or antibody fragment having the binding specificity thereof.

[0184] Small molecule antagonists of EGFR include gefitinib (Iressa®), erlotinib (Tarceva®), and lapatinib (TykerB®). See, e.g., Yan et al., Pharmacogenetics and Pharmacogenomics In Oncology Therapeutic Antibody Development, BioTechniques 2005, 39(4):565-8; and Paez et al., EGFR Mutations In Lung Cancer Correlation With Clinical Response To Gefitinib Therapy, Science 2004, 304(5676):1497-500. Further non-limiting examples of small molecule EGFR inhibitors include any of the EGFR inhibitors described in the following patent publications, and all pharmaceutically acceptable salts of such EGFR inhibitors: EP 0520722; EP 0566226; WO96/33980; U.S. Pat. No. 5,747,498; WO96/30347; EP 0787772; WO97/30034; WO97/30044; WO97/38994; WO97/49688; EP 837063; WO98/02434; WO97/38983; WO95/19774; WO95/19970; WO97/13771; WO98/02437; WO98/02438; WO97/32881; DE 19629652; WO98/33798; WO97/32880; WO97/32880; EP 682027; WO97/02266; WO97/27199; WO98/07726; WO97/34895; WO96/31510; WO98/14449; WO98/14450; WO98/14451; WO95/09847; WO97/19065; WO98/17662; U.S. Pat. No. 5,789,427; U.S. Pat. No. 5,650,415; U.S. Pat. No. 5,656,643; WO99/35146; WO99/35132; WO99/07701; and WO92/20642. Additional non-limiting examples of small molecule EGFR inhibitors include any of the EGFR inhibitors described

in Traxler et al., Exp. Opin. Ther. Patents 1998, 8(12):1599-1625. In some embodiments, an EGFR inhibitor is osimertinib.

[0185] MEK inhibitors include, but are not limited to, pimasertib, selumetinib, cobimetinib (Cotellic®), trametinib (Mekinist®), and binimetinib (Mektovi®). In some embodiments, a MEK inhibitor targets a MEK mutation that is a Class I MEK1 mutation selected from D67N; P124L; P124S; and L177V. In some embodiments, the MEK mutation is a Class II MEK1 mutation selected from ΔE51-Q58; ΔF53-Q58; E203K; L177M; C121S; F53L; K57E; Q56P; and K57N.

[0186] PI3K inhibitors include, but are not limited to, wortmannin; 17-hydroxywortmannin analogs described in WO06/044453; 4-[2-(1H-Indazol-4-yl)-6-[[4-(methylsulfonyl)piperazin-1-yl]methyl]thieno[3,2-d]pyrimidin-4-yl]morpholine (also known as pictilisib or GDC-0941 and described in WO09/036082 and WO09/055730); 2-methyl-2-[4-[3-methyl-2-oxo-8-(quinolin-3-yl)-2,3-dihydroimidazo[4,5-c]quinolin-1-yl]phenyl]propionitrile (also known as BEZ 235 or NVP-BEZ 235, and described in WO06/122806); (S)-l-(4-((2-(2-aminopyrimidin-5-yl)-7-methyl-4-morpholinothieno[3,2-d]pyrimidin-6-yl)methyl)piperazin-1-yl)-2-hydroxypropan-1-one (described in WO08/070740); LY294002 (2-(4-morpholinyl)-8-phenyl-4H-l-benzopyran-4-one (available from Axon Medchem); PI 103 hydrochloride (3-[4-(4-morpholinylpyrido-[3',2':4,5]furo[3,2-d]pyrimidin-2-yl] phenol hydrochloride (available from Axon Medchem); PIK 75 (2-methyl-5-nitro-2-[(6-bromoimidazo[1,2-a]pyridin-3-yl)methylene]-1-methylhydrazide-benzenesulfonic acid, monohydrochloride) (available from Axon Medchem); PIK 90 (N-(7,8-dimethoxy-2,3-dihydro-imidazo[l,2-c]quinazolin-5-yl)-nicotinamide (available from Axon Medchem); AS-252424 (5-[l-[5-(4-fluoro-2-hydroxy-phenyl)-furan-2-yl]-meth-(Z)-ylidene]-thiazolidine-2,4-dione (available from Axon Medchem); TGX-221 (7-methyl-2-(4-morpholinyl)-9-[1-(phenylamino)ethyl]-4H-pyrido-[1,2-a]pyrirnidin-4-one (available from Axon Medchem); XL-765; and XL-147. Other PI3K inhibitors include demethoxyviridin, perifosine, CAL101, PX-866, BEZ235, SF1126, INK1117, IPI-145, BKM120, XL147, XL765, Palomid 529, GSK1059615, ZSTK474, PWT33597, IC87114, TGI 00-115, CAL263, PI-103, GNE-477, CUDC-907, and AEZS-136.

[0187] AKT inhibitors include, but are not limited to, Akt-1-1 (inhibits Aktl) (Barnett et al., Biochem. J. 2005, 385(Pt. 2): 399-408); Akt-1-1,2 (inhibits Akl and 2)

(Barnett et al., Biochem. J. 2005, 385(Pt. 2): 399-408); API-59CJ-Ome (e.g., Jin et al., Br. J. Cancer 2004, 91:1808-12); 1-H-imidazo[4,5-c]pyridinyl compounds (e.g., WO 05/011700); indole-3-carbinol and derivatives thereof (e.g., U.S. Pat. No. 6,656,963; Sarkar and Li J Nutr. 2004, 134(12 Suppl):3493S-3498S); perifosine (e.g., interferes with Akt membrane localization; Dasmahapatra et al. Clin. Cancer Res. 2004, 10(15):5242-52); phosphatidylinositol ether lipid analogues (e.g., Gills and Dennis Expert. Opin. Investig. Drugs 2004, 13:787-97); and triciribine (TCN or API-2 or NCI identifier: NSC 154020; Yang et al., Cancer Res. 2004, 64:4394-9).

[0188] mTOR inhibitors include, but are not limited to, ATP-competitive mTORC1/mTORC2 inhibitors, e.g., PI-103, PP242, PP30; Torin 1; FKBP12 enhancers; 4H-1-benzopyran-4-one derivatives; and rapamycin (also known as sirolimus) and derivatives thereof, including: temsirolimus (Torisel®); everolimus (Afinitor®; WO94/09010); ridaforolimus (also known as deforolimus or AP23573); rapalogs, e.g., as disclosed in WO98/02441 and WO01/14387, e.g., AP23464 and AP23841; 40-(2-hydroxyethyl)rapamycin; 40-[3-hydroxy(hydroxymethyl)methylpropanoate]-rapamycin (also known as CC1779); 40-epi-(tetrazolyt)-rapamycin (also called ABT578); 32-deoxorapamycin; 16-pentynyloxy-32(S)-dihydrorapanycin; derivatives disclosed in WO05/005434; derivatives disclosed in U.S. Patent Nos. 5,258,389, 5,118,677, 5,118,678, 5,100,883, 5,151,413, 5,120,842, and 5,256,790, and in WO94/090101, WO92/05179, WO93/111130, WO94/02136, WO94/02485, WO95/14023, WO94/02136, WO95/16691, WO96/41807, WO96/41807, and WO2018204416; and phosphorus-containing rapamycin derivatives (e.g., WO05/016252). In some embodiments, the mTOR inhibitor is a bisteric inhibitor (see, e.g., WO2018204416, WO2019212990 and WO2019212991), such as RMC-5552.

[0189] BRAF inhibitors that may be used in combination with compounds of the invention include, for example, vemurafenib, dabrafenib, and encorafenib. A BRAF may comprise a Class 3 BRAF mutation. In some embodiments, the Class 3 BRAF mutation is selected from one or more of the following amino acid substitutions in human BRAF: D287H; P367R; V459L; G466V; G466E; G466A; S467L; G469E; N581S; N581I; D594N; D594G; D594A; D594H; F595L; G596D; G596R and A762E.

[0190] Proteasome inhibitors include, but are not limited to, carfilzomib (Kyprolis®), bortezomib (Velcade®), and oprozomib.

[0191] Immune therapies include, but are not limited to, monoclonal antibodies, immunomodulatory imides (IMiDs), GITR agonists, genetically engineered T-cells (e.g., CAR-T cells), bispecific antibodies (e.g., BiTEs), and anti-PD-1, anti-PDL-1, anti-CTLA4, anti-LAGl, and anti-OX40 agents).

[0192] Immunomodulatory agents (IMiDs) are a class of immunomodulatory drugs (drugs that adjust immune responses) containing an imide group. The IMiD class includes thalidomide and its analogues (lenalidomide, pomalidomide, and apremilast).

[0193] Exemplary anti-PD-1 antibodies and methods for their use are described by Goldberg et al., Blood 2007, 110(1):186-192; Thompson et al., Clin. Cancer Res. 2007, 13(6):1757-1761; and WO06/121168 A1), as well as described elsewhere herein.

[0194] GITR agonists include, but are not limited to, GITR fusion proteins and anti-GITR antibodies (e.g., bivalent anti-GITR antibodies), such as, a GITR fusion protein described in U.S. Pat. No. 6,111,090, U.S. Pat. No. 8,586,023, WO2010/003118 and WO2011/090754; or an anti-GITR antibody described, e.g., in U.S. Pat. No. 7,025,962, EP 1947183, U.S. Pat. No. 7,812,135, U.S. Pat. No. 8,388,967, U.S. Pat. No. 8,591,886, U.S. Pat. No. 7,618,632, EP 1866339, and WO2011/028683, WO2013/039954, WO05/007190, WO07/133822, WO05/055808, WO99/40196, WO01/03720, WO99/20758, WO06/083289, WO05/115451, and WO2011/051726.

[0195] Another example of a therapeutic agent that may be used in combination with the compounds of the invention is an anti-angiogenic agent. Anti-angiogenic agents are inclusive of, but not limited to, in vitro synthetically prepared chemical compositions, antibodies, antigen binding regions, radionuclides, and combinations and conjugates thereof. An anti-angiogenic agent can be an agonist, antagonist, allosteric modulator, toxin or, more generally, may act to inhibit or stimulate its target (e.g., receptor or enzyme activation or inhibition), and thereby promote cell death or arrest cell growth. In some embodiments, the one or more additional therapies include an anti-angiogenic agent.

[0196] Anti-angiogenic agents can be MMP-2 (matrix-metalloproteinase 2) inhibitors, MMP-9 (matrix-metalloprotienase 9) inhibitors, and COX-II (cyclooxygenase 11) inhibitors. Non-limiting examples of anti-angiogenic agents include rapamycin, temsirolimus (CCI-779), everolimus (RAD001), sorafenib, sunitinib, and bevacizumab. Examples of useful COX-II inhibitors include alecoxib, valdecoxib, and rofecoxib.

Examples of useful matrix metalloproteinase inhibitors are described in WO96/33172, WO96/27583, WO98/07697, WO98/03516, WO98/34918, WO98/34915, WO98/33768, WO98/30566, WO90/05719, WO99/52910, WO99/52889, WO99/29667, WO99007675, EP0606046, EP0780386, EP1786785, EP1181017, EP0818442, EP1004578, and US20090012085, and U.S. Patent Nos. 5,863,949 and 5,861,510. Preferred MMP-2 and MMP-9 inhibitors are those that have little or no activity inhibiting MMP-1. More preferred, are those that selectively inhibit MMP-2 or AMP-9 relative to the other matrix-metalloproteinases (i.e., MAP-1, MMP-3, MMP-4, MMP-5, MMP-6, MMP- 7, MMP- 8, MMP-10, MMP-11, MMP-12, and MMP-13). Some specific examples of MMP inhibitors are AG-3340, RO 32-3555, and RS 13-0830.

[0197] Further exemplary anti-angiogenic agents include KDR (kinase domain receptor) inhibitory agents (e.g., antibodies and antigen binding regions that specifically bind to the kinase domain receptor), anti-VEGF agents (e.g., antibodies or antigen binding regions that specifically bind VEGF, or soluble VEGF receptors or a ligand binding region thereof) such as VEGF-TRAP™, and anti-VEGF receptor agents (e.g., antibodies or antigen binding regions that specifically bind thereto), EGFR inhibitory agents (e.g., antibodies or antigen binding regions that specifically bind thereto) such as Vectibix® (panitumumab), erlotinib (Tarceva®), anti-Angl and anti-Ang2 agents (e.g., antibodies or antigen binding regions specifically binding thereto or to their receptors, e.g., Tie2/Tek), and anti-Tie2 kinase inhibitory agents (e.g., antibodies or antigen binding regions that specifically bind thereto). Other anti-angiogenic agents include Campath, IL-8, B-FGF, Tek antagonists (US2003/0162712; US6,413,932), anti-TWEAK agents (e.g., specifically binding antibodies or antigen binding regions, or soluble TWEAK receptor antagonists; see US6,727,225), ADAM distintegrin domain to antagonize the binding of integrin to its ligands (US 2002/0042368), specifically binding anti-eph receptor or anti-ephrin antibodies or antigen binding regions (U.S. Patent Nos. 5,981,245; 5,728,813; 5,969,110; 6,596,852; 6,232,447; 6,057,124 and patent family members thereof), and anti-PDGF-BB antagonists (e.g., specifically binding antibodies or antigen binding regions) as well as antibodies or antigen binding regions specifically binding to PDGF-BB ligands, and PDGFR kinase inhibitory agents (e.g., antibodies or antigen binding regions that specifically bind thereto). Additional anti-angiogenic agents include: SD-7784 (Pfizer, USA); cilengitide (Merck KGaA, Germany, EPO 0770622); pegaptanib octasodium, (Gilead Sciences, USA); Alphastatin, (BioActa, UK); M-PGA, (Celgene, USA, US 5712291); ilomastat, (Arriva,

USA, US5892112); emaxanib, (Pfizer, USA, US 5792783); vatalanib, (Novartis, Switzerland); 2-methoxyestradiol (EntreMed, USA); TLC ELL-12 (Elan, Ireland); anecortave acetate (Alcon, USA); alpha-D148 Mab (Amgen, USA); CEP-7055 (Cephalon, USA); anti-Vn Mab (Crucell, Netherlands), DACantiangiogenic (ConjuChem, Canada); Angiocidin (InKine Pharmaceutical, USA); KM-2550 (Kyowa Hakko, Japan); SU-0879 (Pfizer, USA); CGP-79787 (Novartis, Switzerland, EP 0970070); ARGENT technology (Ariad, USA); YIGSR-Stealth (Johnson & Johnson, USA); fibrinogen-E fragment (BioActa, UK); angiogenic inhibitor (Trigen, UK); TBC-1635 (Encysive Pharmaceuticals, USA); SC-236 (Pfizer, USA); ABT-567 (Abbott, USA); Metastatin (EntreMed, USA); maspin (Sosei, Japan); 2-methoxyestradiol (Oncology Sciences Corporation, USA); ER-68203-00 (IV AX, USA); BeneFin (Lane Labs, USA); Tz-93 (Tsumura, Japan); TAN-1120 (Takeda, Japan); FR-111142 (Fujisawa, Japan, JP 02233610); platelet factor 4 (RepliGen, USA, EP 407122); vascular endothelial growth factor antagonist (Borean, Denmark); bevacizumab (pINN) (Genentech, USA); angiogenic inhibitors (SUGEN, USA); XL 784 (Exelixis, USA); XL 647 (Exelixis, USA); MAb, alpha5beta3 integrin, second generation (Applied Molecular Evolution, USA and Medlmmune, USA); enzastaurin hydrochloride (Lilly, USA); CEP 7055 (Cephalon, USA and Sanofi-Synthelabo, France); BC 1 (Genoa Institute of Cancer Research, Italy); rBPI 21 and BPI-derived antiangiogenic (XOMA, USA); PI 88 (Progen, Australia); cilengitide (Merck KGaA, German; Munich Technical University, Germany, Scripps Clinic and Research Foundation, USA); AVE 8062 (Ajinomoto, Japan); AS 1404 (Cancer Research Laboratory, New Zealand); SG 292, (Telios, USA); Endostatin (Boston Childrens Hospital, USA); ATN 161 (Attenuon, USA); 2-methoxyestradiol (Boston Childrens Hospital, USA); ZD 6474, (AstraZeneca, UK); ZD 6126, (Angiogene Pharmaceuticals, UK); PPI 2458, (Praecis, USA); AZD 9935, (AstraZeneca, UK); AZD 2171, (AstraZeneca, UK); vatalanib (pINN), (Novartis, Switzerland and Schering AG, Germany); tissue factor pathway inhibitors, (EntreMed, USA); pegaptanib (Pinn), (Gilead Sciences, USA); xanthorrhizol, (Yonsei University, South Korea); vaccine, gene-based, VEGF-2, (Scripps Clinic and Research Foundation, USA); SPV5.2, (Supratek, Canada); SDX 103, (University of California at San Diego, USA); PX 478, (ProlX, USA); METASTATIN, (EntreMed, USA); troponin I, (Harvard University, USA); SU 6668, (SUGEN, USA); OXI 4503, (OXiGENE, USA); o-guanidines, (Dimensional Pharmaceuticals, USA); motuporamine C, (British Columbia University, Canada); CDP 791, (Celltech Group, UK); atiprimod (pINN), (GlaxoSmithKline, UK); E 7820, (Eisai, Japan); CYC 381, (Harvard University, USA); AE 941, (Aeterna, Canada);

vaccine, angiogenic, (EntreMed, USA); urokinase plasminogen activator inhibitor, (Dendreon, USA); oglufanide (pINN), (Melmotte, USA); HIF-lalfa inhibitors, (Xenova, UK); CEP 5214, (Cephalon, USA); BAY RES 2622, (Bayer, Germany); Angiocidin, (InKine, USA); A6, (Angstrom, USA); KR 31372, (Korea Research Institute of Chemical Technology, South Korea); GW 2286, (GlaxoSmithKline, UK); EHT 0101, (ExonHit, France); CP 868596, (Pfizer, USA); CP 564959, (OSI, USA); CP 547632, (Pfizer, USA); 786034, (GlaxoSmithKline, UK); KRN 633, (Kirin Brewery, Japan); drug delivery system, intraocular, 2-methoxyestradiol; anginex (Maastricht University, Netherlands, and Minnesota University, USA); ABT 510 (Abbott, USA); AAL 993 (Novartis, Switzerland); VEGI (ProteomTech, USA); tumor necrosis factor-alpha inhibitors; SU 11248 (Pfizer, USA and SUGEN USA); ABT 518, (Abbott, USA); YH16 (Yantai Rongchang, China); S-3APG (Boston Childrens Hospital, USA and EntreMed, USA); MAb, KDR (ImClone Systems, USA); MAb, alpha5 beta (Protein Design, USA); KDR kinase inhibitor (Celltech Group, UK, and Johnson & Johnson, USA); GFB 116 (South Florida University, USA and Yale University, USA); CS 706 (Sankyo, Japan); combretastatin A4 prodrug (Arizona State University, USA); chondroitinase AC (IBEX, Canada); BAY RES 2690 (Bayer, Germany); AGM 1470 (Harvard University, USA, Takeda, Japan, and TAP, USA); AG 13925 (Agouron, USA); Tetrathiomolybdate (University of Michigan, USA); GCS 100 (Wayne State University, USA) CV 247 (Ivy Medical, UK); CKD 732 (Chong Kun Dang, South Korea); irsogladine, (Nippon Shinyaku, Japan); RG 13577 (Aventis, France); WX 360 (Wilex, Germany); squalamine, (Genaera, USA); RPI 4610 (Sirna, USA); heparanase inhibitors (InSight, Israel); KL 3106 (Kolon, South Korea); Honokiol (Emory University, USA); ZK CDK (Schering AG, Germany); ZK Angio (Schering AG, Germany); ZK 229561 (Novartis, Switzerland, and Schering AG, Germany); XMP 300 (XOMA, USA); VGA 1102 (Taisho, Japan); VE-cadherin-2 antagonists(ImClone Systems, USA); Vasostatin (National Institutes of Health, USA); Flk-1 (ImClone Systems, USA); TZ 93 (Tsumura, Japan); TumStatin (Beth Israel Hospital, USA); truncated soluble FLT 1 (vascular endothelial growth factor receptor 1) (Merck & Co, USA); Tie-2 ligands (Regeneron, USA); and thrombospondin 1 inhibitor (Allegheny Health, Education and Research Foundation, USA).

[0198] Further examples of therapeutic agents that may be used in combination with compounds of the invention include agents (e.g., antibodies, antigen binding regions, or soluble receptors) that specifically bind and inhibit the activity of growth factors, such as

antagonists of hepatocyte growth factor (HGF, also known as Scatter Factor), and antibodies or antigen binding regions that specifically bind its receptor, c-Met.

[0199] Another example of a therapeutic agent that may be used in combination with compounds of the invention is an autophagy inhibitor. Autophagy inhibitors include, but are not limited to chloroquine, 3-methyladenine, hydroxychloroquine (Plaquenil™), bafilomycin A1, 5-amino-4-imidazole carboxamide riboside (AICAR), okadaic acid, autophagy-suppressive algal toxins which inhibit protein phosphatases of type 2A or type 1, analogues of cAMP, and drugs which elevate cAMP levels such as adenosine, LY204002, N6-mercaptopurine riboside, and vinblastine. In addition, antisense or siRNA that inhibits expression of proteins including but not limited to ATG5 (which are implicated in autophagy), may also be used. In some embodiments, the one or more additional therapies include an autophagy inhibitor.

[0200] Another example of a therapeutic agent that may be used in combination with compounds of the invention is an anti-neoplastic agent. In some embodiments, the one or more additional therapies include an anti-neoplastic agent. Non-limiting examples of anti-neoplastic agents include acemannan, aclarubicin, aldesleukin, alemtuzumab, alitretinoin, altretamine, amifostine, aminolevulinic acid, amrubicin, amsacrine, anagrelide, anastrozole, ancer, ancestim, arglabin, arsenic trioxide, BAM-002 (Novelos), bexarotene, bicalutamide, broxuridine, capecitabine, celmoleukin, cetrorelix, cladribine, clotrimazole, cytarabine ocfosfate, DA 3030 (Dong-A), daclizumab, denileukin diftitox, deslorelin, dexrazoxane, dilazep, docetaxel, docosanol, doxercalciferol, doxifluridine, doxorubicin, bromocriptine, carmustine, cytarabine, fluorouracil, HIT diclofenac, interferon alfa, daunorubicin, doxorubicin, tretinoin, edelfosine, edrecolomab, eflornithine, emitefur, epirubicin, epoetin beta, etoposide phosphate, exemestane, exisulind, fadrozole, filgrastim, finasteride, fludarabine phosphate, formestane, fotemustine, gallium nitrate, gemcitabine, gemtuzumab zogamicin, gimeracil/oteracil/tegafur combination, glycopine, goserelin, heptaplatin, human chorionic gonadotropin, human fetal alpha fetoprotein, ibandronic acid, idarubicin, (imiquimod, interferon alfa, interferon alfa, natural, interferon alfa-2, interferon alfa-2a, interferon alfa-2b, interferon alfa-Nl, interferon alfa-n3, interferon alfacon-1, interferon alpha, natural, interferon beta, interferon beta-la, interferon beta-lb, interferon gamma, natural interferon gamma- la, interferon gamma-lb, interleukin-1 beta, iobenguane, irinotecan, irsogladine, lanreotide, LC 9018 (Yakult), leflunomide, lenograstim, lentinan sulfate, letrozole, leukocyte alpha interferon, leuprorelin, levamisole + fluorouracil,

liarozole, lobaplatin, lonidamine, lovastatin, masoprocol, melarsoprol, metoclopramide, mifepristone, miltefosine, mirimostim, mismatched double stranded RNA, mitoguazone, mitolactol, mitoxantrone, molgramostim, nafarelin, naloxone + pentazocine, nartograstim, nedaplatin, nilutamide, noscapine, novel erythropoiesis stimulating protein, NSC 631570 octreotide, oprelvekin, osaterone, oxaliplatin, paclitaxel, pamidronic acid, pegaspargase, peginterferon alfa-2b, pentosan polysulfate sodium, pentostatin, picibanil, pirarubicin, rabbit antithymocyte polyclonal antibody, polyethylene glycol interferon alfa-2a, porfimer sodium, raloxifene, raltitrexed, rasburiembodiment, rhenium Re 186 etidronate, RII retinamide, rituximab, romurtide, samarium (153 Sm) lexidronam, sargramostim, sizofiran, sobuzoxane, sonermin, strontium-89 chloride, suramin, tasonermin, tazarotene, tegafur, temoporfin, temozolomide, teniposide, tetrachlorodecaoxide, thalidomide, thymalfasin, thyrotropin alfa, topotecan, toremifene, tositumomab-iodine 131, trastuzumab, treosulfan, tretinoin, trilostane, trimetrexate, triptorelin, tumor necrosis factor alpha, natural, ubenimex, bladder cancer vaccine, Maruyama vaccine, melanoma lysate vaccine, valrubicin, verteporfin, vinorelbine, virulizin, zinostatin stimalamer, or zoledronic acid; abarelix; AE 941 (Aeterna), ambamustine, antisense oligonucleotide, bcl-2 (Genta), APC 8015 (Dendreon), decitabine, dexaminoglutethimide, diaziquone, EL 532 (Elan), EM 800 (Endorecherche), eniluracil, etanidazole, fenretinide, filgrastim SD01 (Amgen), fulvestrant, galocitabine, gastrin 17 immunogen, HLA-B7 gene therapy (Vical), granulocyte macrophage colony stimulating factor, histamine dihydrochloride, ibritumomab tiuxetan, ilomastat, IM 862 (Cytran), interleukin-2, iproxifene, LDI 200 (Milkhaus), leridistim, lintuzumab, CA 125 MAb (Biomira), cancer MAb (Japan Pharmaceutical Development), HER-2 and Fc MAb (Medarex), idiotypic 105AD7 MAb (CRC Technology), idiotypic CEA MAb (Trilex), LYM-1-iodine 131 MAb (Techni clone), polymorphic epithelial mucin-yttrium 90 MAb (Antisoma), marimastat, menogaril, mitumomab, motexafin gadolinium, MX 6 (Galderma), nelarabine, nolatrexed, P 30 protein, pegvisomant, pemetrexed, porfiromycin, prinomastat, RL 0903 (Shire), rubitecan, satraplatin, sodium phenylacetate, sparfosic acid, SRL 172 (SR Pharma), SU 5416 (SUGEN), TA 077 (Tanabe), tetrathiomolybdate, thaliblastine, thrombopoietin, tin ethyl etiopurpurin, tirapazamine, cancer vaccine (Biomira), melanoma vaccine (New York University), melanoma vaccine (Sloan Kettering Institute), melanoma oncolysate vaccine (New York Medical College), viral melanoma cell lysates vaccine (Royal Newcastle Hospital), or valspodar.

[0201] Additional examples of therapeutic agents that may be used in combination with compounds of the invention include ipilimumab (Yervoy®); tremelimumab; galiximab; nivolumab, also known as BMS-936558 (Opdivo®); pembrolizumab (Keytruda®); avelumab (Bavencio®); AMP224; BMS-936559; MPDL3280A, also known as RG7446; MEDI-570; AMG557; MGA271; IMP321; BMS-663513; PF-05082566; CDX-1127; anti-OX40 (Providence Health Services); huMAbOX40L; atacicept; CP-870893; lucatumumab; dacetuzumab; muromonab-CD3; ipilumumab; MEDI4736 (Imfinzi®); MSB0010718C; AMP 224; adalimumab (Humira®); ado-trastuzumab emtansine (Kadcyla®); aflibercept (Eylea®); alemtuzumab (Campath®); basiliximab (Simulect®); belimumab (Benlysta®); basiliximab (Simulect®); belimumab (Benlysta®); brentuximab vedotin (Adcetris®); canakinumab (Ilaris®); certolizumab pegol (Cimzia®); daclizumab (Zenapax®); daratumumab (Darzalex®); denosumab (Prolia®); eculizumab (Soliris®); efalizumab (Raptiva®); gemtuzumab ozogamicin (Mylotarg®); golimumab (Simponi®); ibritumomab tiuxetan (Zevalin®); infliximab (Remicade®); motavizumab (Numax®); natalizumab (Tysabri®); obinutuzumab (Gazyva®); ofatumumab (Arzerra®); omalizumab (Xolair®); palivizumab (Synagis®); pertuzumab (Perjeta®); pertuzumab (Perjeta®); ranibizumab (Lucentis®); raxibacumab (Abthrax®); tocilizumab (Actemra®); tositumomab; tositumomab-i-131; tositumomab and tositumomab-i-131 (Bexxar®); ustekinumab (Stelara®); AMG 102; AMG 386; AMG 479; AMG 655; AMG 706; AMG 745; and AMG 951.

[0202] In some embodiments, an additional compound is selected from the group consisting of a CDK4/6 inhibitor (e.g., abemaciclib, palbociclib, or ribociclib), a KRAS:GDP G12C inhibitor (e.g., AMG 510, MRTX 1257) or other mutant Ras:GDP inhibitor, a KRAS:GTP G12C inhibitor or other mutant Ras:GTP inhibitor, a MEK inhibitor (e.g., refametinib, selumetinib, trametinib, or cobimetinib), a SHP2 inhibitor (e.g., TNO155, RMC-4630), an ERK inhibitor, and an RTK inhibitor (e.g., an EGFR inhibitor). In some embodiments, a SOS1 inhibitor may be used in combination with a Ras inhibitor, a SHP2 inhibitor, or a MEK inhibitor. In some embodiments, a combination therapy includes a SOS1 inhibitor, a RAS inhibitor and a MEK inhibitor.

[0203] In some embodiments, an additional compound is selected from the group consisting of ABT-737, AT-7519, carfilzomib, cobimetinib, danusertib, dasatinib, doxorubicin, GSK-343, JQ1, MLN-7243, NVP-ADW742, paclitaxel, palbociclib and

volasertib. In some embodiments, an additional compound is selected from the group consisting of neratinib, acetinib and reversine.

[0204] MCL-1 inhibitors include, but are not limited to, AMG-176, MIK665, and S63845. The myeloid cell leukemia-1 (MCL-1) protein is one of the key anti-apoptotic members of the B-cell lymphoma-2 (BCL-2) protein family. Over-expression of MCL-1 has been closely related to tumor progression as well as to resistance, not only to traditional chemotherapies but also to targeted therapeutics including BCL-2 inhibitors such as ABT-263.

[0205] In the above, preferred additional therapeutic agents include MEK inhibitors, ERK inhibitors, pan-RAS(ON) inhibitors (that is, inhibitors that target the GTP-activated form of RAS), CDK4/6 inhibitors, mTORC1 inhibitors, HDAC inhibitors, BCL2 inhibitors, and PLK1 inhibitors.

[0206] In some embodiments, the method further comprises administering to the subject a therapeutically effective amount of an AML therapeutic agent. Such agents are known in the art, and may be selected from, e.g., cytarabine, an anthracycline drug (e.g., daunorubicin or idarubicin), midostaurin, gemtuzumab ozogamicin, cladribine, fludarabine, etoposide, azacytidine, decitabine, venetoclax, glasdegib, ivosidenib, or enasidenib, or a combination thereof.

[0207] In some embodiments, the present disclosure provides a method for patient stratification based upon the presence or absence of a SHP2 mutation, in particular an activating SHP2 mutation. As used herein, "patient stratification" means classifying one or more patient as having a disease or disorder (e.g., cancer) that is either likely or unlikely to be treatable with a SHP2 inhibitor, such as an allosteric SHP2 inhibitor. Patient stratification may comprise classifying a patient as having a tumor that is sensitive or resistant to treatment with a SHP2 inhibitor, such as an allosteric SHP2 inhibitor.

[0208] In some embodiments, the method of the present invention comprises identifying the subject as resistant to SHP2 inhibitor, such as an allosteric SHP2 inhibitor, by genotyping a biological sample from the subject for a SHP2 mutation, wherein the subject is identified as resistant to the SHP2 inhibitor if the SHP2 mutation comprises an inhibitor-resistant mutation, such as an allosteric inhibitor-resistant mutation.

[0209] For example, but not to be limited in anyway, in some aspects, a biological sample from a patient (e.g., a cell such as a tumor cell) may be genotyped using a hybridization detection method to determine whether the cell contains a SHP2 mutation, such as an activating SHP2 mutation, comprising an inhibitor-resistant mutation, such as an allosteric inhibitor-resistant mutation.

[0210] Hybridization detection methods are based on the formation of specific hybrids between complementary nucleic acid sequences that serve to detect nucleic acid sequence mutation(s). Such methods include, e.g., microarray analysis and real time PCR. Hybridization methods, such as Southern analysis, Northern analysis, or in situ hybridizations, may also be used (see Current Protocols in Molecular Biology, Ausubel et al., eds., John Wiley & Sons 2003, incorporated by reference in its entirety).

[0211] Other suitable methods for genotyping a cell (e.g., a tumor cell) include direct manual sequencing (Church and Gilbert, Proc. Natl. Acad. Sci. USA 81:1991-1995 (1988); Sanger et al., Proc. Natl. Acad. Sci. USA 74:5463-5467 (1977); Beavis et al. U.S. Pat. No. 5,288,644, each incorporated by reference in its entirety for all purposes); automated fluorescent sequencing; single-stranded conformation polymorphism assays (SSCP); clamped denaturing gel electrophoresis (CDGE); two-dimensional gel electrophoresis (2DGE or TDGE); conformational sensitive gel electrophoresis (CSGE); denaturing gradient gel electrophoresis (DGGE) (Sheffield et al., Proc. Natl. Acad. Sci. USA 86:232-236 (1989)), mobility shift analysis (Orita et al., Proc. Natl. Acad. Sci. USA 86:2766-2770 (1989), incorporated by reference in its entirety), restriction enzyme analysis (Flavell et al., Cell 15:25 (1978); Geever et al., Proc. Natl. Acad. Sci. USA 78:5081 (1981), incorporated by reference in its entirety); quantitative real-time PCR (Raca et al., Genet Test 8(4):387-94 (2004) , incorporated by reference in its entirety); heteroduplex analysis; chemical mismatch cleavage (CMC) (Cotton et al., Proc. Natl. Acad. Sci. USA 85:4397-4401 (1985), incorporated by reference in its entirety); RNase protection assays (Myers et al., Science 230:1242 (1985), incorporated by reference in its entirety); use of polypeptides that recognize nucleotide mismatches, e.g., E. coli mutS protein; allele-specific PCR, for example. See, e.g., U.S. Patent Publication No. 2004/0014095, which is incorporated herein by reference in its entirety.

[0212] In some embodiments, the method of the present invention comprises performing a diagnostic test to determine whether the subject has a SHP2 mutation that induces an activated form of SHP2.

EXAMPLES

[0213] The disclosure is further illustrated by the following examples, which are not to be construed as limiting this disclosure in scope or spirit to the specific procedures herein described. It is to be understood that the examples are provided to illustrate certain embodiments and that no limitation to the scope of the disclosure is intended thereby. It is to be further understood that resort may be had to various other embodiments, modifications, and equivalents thereof which may suggest themselves to those skilled in the art without departing from the spirit of the present disclosure and/or scope of the appended claims.

Methods Used in the Examples

[0214] **CrownSyn™ Methods (3-dimensional surface plots)**

[0215] Cells were grown in 3-dimensional culture in the appropriate growth medium containing 0.65% methylcellulose. On the day of cell seeding, the cells were harvested from 2-dimensional culture during the logarithmic growth period, mixed with appropriate cell media and centrifuged at 1000 rpm for 4 minutes. Cells were re-suspended and counted using CountStar. 3.5 mL of cell suspension was mixed with 6.5 mL of 1% methylcellulose, yielding 10 ml of cell suspension in 0.65% methylcellulose solution. 90 µL cell suspension was added to 96-well plates. Another plate prepared for T0 reading. Plates were incubated in humidified incubator at 37° C with 5% CO_2. Test articles were diluted using DMSO or culture medium to 10× working solution. 10µl each test article solution was dispensed separately to each well (triplicate for each concentration). Plates were cultured for 120hr in humidified incubator at 37°C with 5% CO2 or 100% air. For T0 reading, 10 µl culture medium was added to each well of T0 plate, and cell viability determined using CTG assay as described below. After 120 hours, plates were equilibrated at room temperature for approximately 30 minutes, prior to addition of 100µl of CellTiter-Glo® Reagent into each assay well. Contents mixed for 2 minutes on an orbital shaker to induce cell lysis. Plates were allowed to incubate at room temperature for 10 minutes to stabilize luminescent signal. Luminescence was recorded using EnVision MultiLabel

Reader. The software of GraphPad Prism used to calculate IC50. The graphical curves were fitted using a nonlinear regression model with a sigmoidal dose response. Two dimensional concentration-response surfaces were compared to two different additivity models, Bliss independence and Loewe additivity. Deviation from the prediction of either model was assessed in the form of a synergy score. Synergy scores greater than 5 with either model and at any point on the concentration response surface were interpreted as indicating a significant interaction between compounds. See Bliss C.I. (1939) The toxicity of poisons applied jointly. *Ann. Appl. Biol.*, 26, 585-615, and see Loewe S. (1953) The problem of synergism and antagonism of combined drugs. *ArzneimiettelForschung*, 3,2 86-290. The Bliss independence model is expected to hold true for non-interacting drugs that elicit their responses independently, e.g., by targeting separate pathways. Loewe additivity, in contrast, is more compatible with the cases where both drugs have similar modes of action on the same targets or pathways. As pointed out in the Saariselka agreement (Greco et al., 1992), and also by many others, neither Loewe additivity nor Bliss independence is necessarily reflecting the expected modes of action of a drug combination. Rather, Loewe and Bliss models should be used as data exploratory approaches, with a major purpose to identify potential synergistic drug combinations that warrant further mechanistic investigation, but not the other way around, i.e., using the mechanistic evidence to determine which reference model is more appropriate.

[0216] **2-dimensional potency shifts**

[0217] Cells were grown in 2-dimensional culture. Assay stocks were thawed and diluted in the recommended ATCC medium, supplemented with 10% serum and 1% pen/strep (final concentration) and dispensed in a 384-well plate. Depending on the cell line used, a cell density of 100 - 6400 cells per well in 45 µl medium was used. The margins of the plate were filled with phosphate-buffered saline. Plated cells were incubated in a humidified atmosphere of 5% CO2 at 37 ºC. After 24 hours, 5 µl of reference compound dilution, containing the single dose compound at indicated concentration was added to the plates and these were further incubated for 120hrs. At t=120 hours, 24 µl of ATPlite 1Step™ (PerkinElmer) solution was added to each well, and subsequently shaken for 2 minutes. After 5 minutes incubation in the dark, the luminescence was recorded on an Envision multilabel reader (PerkinElmer). On a parallel t=0 plate, 45 µl cells were dispensed and incubated in a humidified atmosphere of 5% CO2 at 37 ºC. After 24 hours 5 µl DMSO-containing Hepes buffer and 24 µl ATPlite 1Step™ solution were mixed, and

luminescence measured after 5 minutes incubation (= luminescence $_{t=0}$). Curves and IC_{50}s were calculated by non-linear regression using IDBS XLfit 5. The percentage growth after incubation until t=end (% growth) was calculated as follows: 100% x (luminescence $_{t=end}$ / luminescence$_{untreated,t=end}$). This was fitted to the ^{10}log compound concentration (conc) by a 4 parameter sigmoidal curve: *%-growth = bottom + (top – bottom) / (1+ 10(logIC$_{50}$ – conc)*hill))* where hill is the Hill-coefficient, and bottom and top the asymptotic minimum and maximum cell growth that the compound allows in that assay.

[0218] **Inhibition of phospho-ERK**

[0219] Phospho ERK in cells was determined using the MSD® platform. NCI-H1355 or TOV-21G cells were plated in clear flat-bottom 96-well tissue culture plates at 30,000 cells/well in 100 µL/well in complete media, and placed in incubator (37°C, 5% CO_2) overnight. All compounds were reconstituted in DMSO to reach 1000X of desired top concentration and arrayed in column 1 of a 96-well plate. 3- fold serial dilution performed in DMSO of all compounds across the 96-well plate, leaving column 11 with 100% DMSO and column 12 empty. Compounds were diluted in media 1:500 and mixed well. Dilution series at 2X final concentration were then mixed with 2X single dose compounds or DMSO vehicle. Cell media was aspirated from cell plates, and 100µL of 1X compound mixture was added. Plates were placed in incubator (37°C, 5% CO2) for 4 hours. MSD® lysis buffer was prepared immediately before time point by mixing 10 mL Tris Lysis Buffer (provided in MSD® kit), 1 tablet PhosSTOP EASYpack (Roche), 1 tablet cOmplete Mini, EDTA-free Protease Inhibitor Cocktail (Roche), 40 µL PMSF (provided in MSD® kit), 100 µL SDS (provided in MSD® kit), and kept on ice before use. After treatment time, media was aspirated from plates and 50 µL MSD® lysis buffer added to each well. Plates were sealed with foil adhesive and shaken for 5 minutes at 750 rpm at room temperature. Plates were then incubated on ice for 15 minutes, and stored at -80°C. MSD® Phospho(Thr202/Tyr204; Thr185/Tyr187)/Total ERK1/2 Assay performed according to manufacturer protocol.

Example 1. Cells with activating mutations in SHP2 have reduced sensitivity to allosteric inhibitors of SHP2 but retain sensitivity to SOS1

[0220] Aberrant activation of the RAS/MAPK signaling pathway (shown schematically in FIG.1) is a common driver of abnormal growth and proliferation in many types of cancer. SHP2 acts near the top of this pathway, responding to inputs from growth

factor receptors to recruit and activate the RAS GEFs SOS1 and SOS2. Allosteric inhibitors of SHP2 such as RMC-4550 can block pathway activation and cancer cell growth by interfering with this process. Mutations can occur in SHP2 that uncouple growth factor receptor signaling from SHP2 activation, leading to hyper-activation of pathway signaling. These mutations act by destabilizing an auto-inhibited conformation of SHP2. Different activating mutations destabilize this conformation to different degrees, which can be expressed quantitatively as the free energy of opening (ΔG_{op}) of the mutation. Wild-type SHP2 has a ΔG_{op} of 2.8 kcal/mol. Values of ΔG_{op} below 2.8 in mutant SHP2 indicate activation, with lower values indicating stronger activation. The strength of activation of a SHP2 mutation is correlated with reduced sensitivity to SHP2 allosteric inhibitors such as RMC-4550 in engineered HEK293 cells. See FIG. 2A, which is a graph correlating the RMC-4550 pERK IC_{50} as a function of ΔG_{op}. FIG. 2B is a table showing the pERK IC_{50} values for RMC-4550 in a variety of activating mutations of SHP2 protein.

[0221] Unlike allosteric SHP2 inhibitors, SOS1 inhibitors are effective in suppressing the RAS/MAPK signaling pathway activation in HEK293 cells with a variety of SHP2 mutations, even in cases where SHP2 inhibition is not effective. See FIGS. 3A,3B, 20A, and 20B, which show that SOS1 inhibitors maintain sensitivity across a broad panel of SHP2 mutant variant contexts in isogenic HEK-293 cell lines. Growth of HEK293 cells is not dependent on RAS/MAPK signaling pathway activation, but growth of LN-229 cells is. FIGS. 4A through 4H show that SOS1 inhibitor suppressed growth of LN229 cells with activating mutations in SHP2 more potently than the allosteric SHP2 inhibitor RMC-4550, with a greater benefit for SOS1 inhibition associated with more strongly activating SHP2 mutations.

[0222] The following methods were used to obtain the data shown in FIGS. 2A, 2B, 3A, 3B, 4A through 4H, 20A, and 20B.

Generation of isogenic SHP2 expression cell lines

[0223] An experimental system was created to test the activity of SHP2 mutants on an isogenic background. See FIGS. 2A, 2B, 3A, 3B, 20A, and 20B. The Flp-In T-REx-293 cell line was obtained from Gibco and cultivated in high glucose DMEM™ containing 2 mM L-glutamine (Hyclone), supplemented with 10% FBS (Hyclone), 1% penicillin/streptomycin (Gibco), 100 µg/mL Zeocin™ (Gibco), and 15 µg/mL blasticidin (Gibco) in a humidified cell culture incubator at 37°C, 5% CO_2.

[0224] Wild type or mutant SHP2 variants were synthesized and subcloned into the pcDNA5/FRT/TO vector (ThermoFisher). Plasmids were co-transfected with the pOG44 Flp recombinase expression plasmid (ThermoFisher) into Flp-In T-REx-293 cells using X-tremegene 9 DNA transfection reagent (Sigma), according to the manufacturer's instructions. Cells that underwent successful recombination were selected in high glucose DMEM containing 2 mM L-glutamine, supplemented with 10% FBS and, 1% penicillin/streptomycin, 200 μg/mL hygromycin B (Gibco), and 15 μg/mL blasticidin (Gibco) (recombinant selection media) in a humidified cell culture incubator at 37°C, 5% CO_2, until colonies were visually discernible. Colonies were expanded in recombinant selection media in a humidified cell culture incubator at 37°C, 5% CO_2 to establish isogenic SHP2 variant expression cell lines (T-REx-293-SHP2).

Determination of sensitivity to SOS Inhibitors

[0225] One day prior to compound treatment, T-REx-293-SHP2 cells for each tested variant were harvested and seeded in high glucose DMEM containing 2 mM L-glutamine, supplemented with 0.1% FBS and, 1% penicillin/streptomycin, 200 μg/mL hygromycin B, and 15 μg/mL blasticidin in 96-well assay plates at a density of 20,000 cells/well. Expression of SHP2 constructs was induced by the addition of doxycycline (final concentration = 0.1 μg/mL) (Sigma) for 24 hours.

[0226] On the day of the experiment, cells were incubated in quadruplicate wells in the presence of increasing concentrations of BI-3406, Compound SOS1-(A) (also called RMC-0331), RMC-4550 (0.17 nM to 10 μM final assay concentration), or vehicle (final assay concentration 0.1% DMSO) at 37°C, 5% CO_2 for 1 hour. For the final 5 minutes of drug treatment, cells were stimulated with 50 ng/mL Epidermal Growth Factor (Sigma). After this incubation was complete, media was aspirated and cellular lysates prepared using lysis buffer provided with the AlphaLISA® detection kit (PerkinElmer). ERK1/2 phosphorylation at Thr202/Tyr204 was assayed using the AlphaLISA® SureFire® Ultra™ HV pERK Assay Kit (Perkin Elmer) following the manufacturer's instructions. Samples were read using an EnVision® Multilabel Plate Reader (Perkin Elmer) using standard AlphaLISA® settings. Assay data was plotted and EC50 values were determined using four-parameter concentration-response model in GraphPad Prism 7. Data provided are mean +/- standard deviation of duplicate values from representative experiments.

Generation of isogenic LN229 SHP2 mutant cell lines

[0227] An experimental system was created to test the activity of SHP2 mutants on an isogenic background. See FIGS. 4A through 4H. SHP2 mutations were introduced with Synthego CRISPR editing techniques, and LN229 variant cell lines stably expressing these mutations were generated.

Determination of sensitivity to SOS1 and SHP2 inhibitors by pERK Alphalisa

[0228] One day prior to treatment, LN229 cell lines with each indicated mutation were harvested and seeded in high glucose DMEM containing 2 mM L-glutamine, supplemented with 10% FBS in 96-well assay plates at a density of 30,000 cells/well.

[0229] Cell medium was aspirated, and 50 µL standard cell culture medium was added (DMEM, 10% FBS). 50 µL of 2x stock of compounds prepared from DMSO dilution series with 0.2% DMSO added to yield final DMSO concentration of 0.1% (i.e. 1:1000 final dilution of DMSO stock in standard medium). Plate returned to incubator for 1 hours for pERK AlphaLISA®.

[0230] After this incubation was complete, media was aspirated and cellular lysates prepared using lysis buffer provided with the AlphaLISA® detection kit (PerkinElmer). ERK1/2 phosphorylation at Thr202/Tyr204 was assayed using the AlphaLISA® SureFire® Ultra™ HV pERK Assay Kit (Perkin Elmer) following the manufacturer's instructions. Samples were read using an EnVision® Multilabel Plate Reader (Perkin Elmer) using standard AlphaLISA® settings. Assay data was plotted and EC50 values were determined using four-parameter concentration- response model in GraphPad Prism 7. Data provided are mean +/- standard deviation of duplicate values from representative experiments.

Determination of sensitivity to SOS1 and SHP2 inhibitors by 3D CellTiter-Glo®

[0231] On day 0, cells seeded (100 µL per well for 96-well) at 5,000 cells/well in 96-well ultra-low attachment (ULA) plates (Corning #7007). Immediately after seeding, ULA plate centrifuged for 10 minutes, 1000 RPM, at room temperature, prior to incubation at 37˚C, 5% CO_2 overnight.

[0232] On day 1, wells visually inspected to confirm spheroid formation. Incubation continued until Day 3.

[0233] On day 3, ULA plate removed from the incubator and 10 µL of fresh culture media containing test compound at 11x the desired final concentration added. Plates placed in incubator at 37˚C, 5% CO2 until endpoint assay (Day 7-8).

[0234] On day 8, an ATP endpoint viability assay (CellTiter-Glo® 3D, Promega # G9681) performed, following the manufacturer's instructions. CellTiter-Glo® 3D reagent added at a volume equal to the volume of cell culture medium present in each well (100 µL). Plates were shaken for 5 minutes. Contents mixed by carefully pipetting up and down 10 times until spheroids are fully dissociated.

[0235] Lysates were transferred into solid white flat-bottom plates (Corning #3917), and incubated for an additional 25 minutes at room temperature. Luminescence measured on a SpectraMax Microplate Reader.

Results

[0236] Fifteen stable, isogenic cell lines in HEK-293 background expressing different SHP2 variants were created using the FRT/TO system. Cells were incubated with Compound SOS1-(A) (also called RMC-0331), BI-3406, or RMC-4550 prior to stimulation with epidermal growth factor (EGF) and measurement of cellular pERK levels by AlphaLISA®. See FIGS. 2A, 2B, 3A, 3B, 20A, and 20B. RMC-4550 potency for inhibition of mutants in cellular context correlated with biochemical potency for activated SHP2 variant. See FIGS. 2A and 2B.

[0237] Four isogenic LN229 cell lines expressing different stable SHP2 variants were generated. Activating mutations induce resistance to allosteric SHP2 inhibition, but maintain sensitivity to SOS1 inhibitors in isogenic LN229 cell lines. See FIGS. 4A through 4H.

Example 2. SOS1 inhibitors inhibit pathway signaling and cell growth in vitro, and tumor growth *in vivo* in tumor models bearing SHP2 activating mutations

[0238] In this example, two mouse tumor cell lines, KLN205 (lung squamous cell carcinoma) and PAN02 (pancreatic cancer), were characterized. The mouse tumor cell lines contained the highly activating G503V SHP2 mutation. In cell culture experiments *in vitro* the SHP2 allosteric inhibitor RMC-4550 showed low or moderate inhibition of pERK and of cell growth even at the highest test concentration of 10 µM, whereas the SOS1

inhibitor BI-3406 potently inhibited pERK and growth in both cell lines. See FIGS. 5A through 5D.

[0239] Consistent with in vitro observations, in both KLN205 tumor cell lines (See FIG. 6) and PAN02 tumor cell lines (See FIG. 7), BI-3406 treatment resulted in near complete inhibition of tumor growth in vivo, whereas the SHP2 allosteric inhibitor RMC-4550 resulted in less than 50% tumor growth inhibition.

[0240] The following methods were used to obtain the data shown in FIGS. 5A through 5D, 6, and 7.

Determination of sensitivity to SOS1 and SHP2 inhibitors in vivo

[0241] All studies were compliant with all relevant ethical regulations regarding animal research in accordance with approved institutional animal care and use committee IACUC protocols at HD Biosciences (San Diego, CA).

[0242] For KLN205 studies, female (6-8 weeks old) immunocompetent DBA/2 mice were implanted subcutaneously with 0.5E+06 KLN205 cells, Once tumors reached an average volume of 100 mm^3 administration of RMC-4550 (30 mg/kg, by daily oral administration), BI-3406 (50 mg/kg, by twice daily oral administration), or vehicle (2% HPMC in 50 mM sodium citrate buffer) was initiated.

[0243] For PAN02 studies, female (6-8 weeks old) C57/B6 were implanted subcutaneously with 5E+06 PAN02 cells, Once tumors reached an average volume of 100 mm^3 administration of RMC-4550 (40 mg/kg, by every 2 days oral administration), Compound SOS1-(A) (also called RMC-0331) (100 mg/kg, by daily oral administration), or vehicle (2% HPMC in 50 mM sodium citrate buffer) was initiated.

Results

[0244] Activating mutation G503V induces resistance to allosteric SHP2 inhibition, but maintains sensitivity to SOS1 inhibitors in syngeneic mouse cell lines KLN205 and PAN02. See FIGS. 5A through 5D, which are graphs depicting the pERK AlphaLISA® assay. The associated tables with FIGS. 5A through 5D depict the results of the CellTiter-Glo® viability assay.

[0245] In KLN205 models containing SHP2 strongly activating mutant G503V, SOS1 inhibition (as exemplified by BI-3406) yielded substantially higher levels of tumor growth inhibition (TGI) than SHP2 inhibition (RMC-4550) immunocompetent mice. See FIG. 6.

[0246] In PAN02 models containing SHP2 strongly activating mutant G503V, SOS1 inhibition (as exemplified by Compound SOS1-(A) (also called RMC-0331)) yielded substantially higher levels of tumor growth inhibition (TGI) than SHP2 inhibition (RMC-4550) in immunocompetent mice (FIG. 7).

Example 3. SHP2 activating mutations have differential effect on cellular dependence on SOS1 and SOS2.

[0247] SHP2 activates MAPK pathway signaling in part by recruiting and activating SOS RAS guanine nucleotide exchange factors. There are two isoforms of SOS, SOS1 and SOS2. The precise roles of SOS1 and SOS2 remain to be elucidated, but SHP2 is assumed to activate both isoforms, whereas SOS1 inhibitors such as BI-3406 and Compound SOS1-(A) (also called RMC-0331) inhibit only SOS1. This is shown in LN229 cells with four different SHP2 mutations in FIGS. 8A through 8D. In all cases, genetic knockdown of SOS2 resulted in more complete depth of inhibition of pERK after exposure to BI-3406 compared with knockdown with a non-targeting control siRNA, consistent with SOS2 having an overlapping role with SOS1 in pathway activation and not being targeted by BI-3406. In all cases knockdown of SOS1 completely abrogated the effects of BI-3406, validating SOS1 as the target of BI-3406 and demonstrating SOS2 can compensate for SOS1 loss.

[0248] FIG. 9 shows the effect of genetic knockdown of SOS1, SOS2, or both on the basal pERK level in LN229 cells with different activating mutations in SHP2. LN229 cells with more strongly activating SHP2 mutations (E76K and G503V) exhibit greater sensitivity to knockdown of SOS1 and/or SOS2, as shown by the magnitude of decrease in basal pERK upon knockdown (FIG. 9). All cells are sensitive to simultaneous knockdown of SOS1 and SOS2, but unexpectedly, a trend is seen of greater effect of SOS1 knockdown in cells with more strongly activating SHP2 mutations. In contrast, SOS2 knockdown has only a small and similar effect in all cell lines. This suggests that strongly activating SHP2

mutations increase dependence on SOS1, and may therefore increase sensitivity to SOS1 inhibitors such as BI-3406 and Compound SOS1-(A) (also called RMC-0331).

[0249] In view of these results, inhibition of SOS1 in combination with SOS2 by a dual inhibitor is contemplated by the method of the present invention. Selective SOS1 inhibitors are also useful in view of the greater effect of SOS1 knockdown in cells with more strongly activating SHP2 mutations. The following methods were used to obtain the data shown in FIGS. 8A through 8D and 9.

Generation of isogenic LN229 SHP2 mutant cell lines

[0250] An experimental system was created to test the activity of SHP2 mutants on an isogenic background. See FIGS. 4A through 4H, 8A through 8D, and 9. SHP2 mutations were introduced with Synthego CRISPR editing techniques, and LN229 variant cell lines stably expressing these mutations were generated.

Determination of sensitivity to SOS1 and SOS2 Knockdown

[0251] One day prior to transfection, LN229 cell lines with each indicated mutation were harvested and seeded in high glucose DMEM containing 2 mM L-glutamine, supplemented with 10% FBS in 96-well assay plates at a density of 10,000 cells/well. On the day of the transfection, transfection mixtures prepared according to Dharmafect I protocol. Per well, 1 μL of 5 μM siRNA in 10 μL OptiMEM, was combined with 0.2 μL Dharmafect I in 10 μL OptiMEM, plus 80 μL standard cell medium was added directly to cells after aspirating plating medium.

[0252] Per 100 wells in a single condition: 110 μL of 5 μM siRNA diluted with OptiMEM medium to 1.1 mL. Separately, 22 μL Dharmafect I diluted to 1.1 mL and each mixture incubated 5 minutes at room temperature. 1.1 mL siRNA mixture combined with 1.1 mL Dharmafect mixture, and incubated 20 minutes at room temperature. 2.2 mL siRNA/Dharmafect combined mixture diluted with standard cell culture medium (e.g. RPMI + 10% FBS, no p/s) to 11 mL total. Cell medium aspirated for all wells to be transfected, 100 μL transfection mixture added to each well. Cells incubated with transfection mixture for 72 hours.

[0253] Cell medium aspirated, 50 μL standard cell culture medium added (10% DMEM, 10% FBS). 50 μL of 2x stock of compounds prepared from DMSO dilution series

with 0.2% DMSO added to yield final DMSO concentration of 0.1% (i.e. 1:1000 final dilution of DMSO stock in standard medium). Plate returned to incubator for 3 hours.

[0254] After this incubation was complete, media was aspirated and cellular lysates prepared using lysis buffer provided with the AlphaLISA® detection kit (PerkinElmer). ERK1/2 phosphorylation at Thr202/Tyr204 was assayed using the AlphaLISA® SureFire® Ultra™ HV pERK Assay Kit (Perkin Elmer) following the manufacturer's instructions. Samples were read using an EnVision® Multilabel Plate Reader (Perkin Elmer) using standard AlphaLISA® settings. Assay data was plotted and EC50 values were determined using four-parameter concentration- response model in GraphPad Prism 7. Data provided are mean +/- standard deviation of duplicate values from representative experiments.

Results

[0255] Cells were transfected with SOS1 or SOS2 siRNA, and incubated with BI-3406 prior to measurement of cellular pERK levels by AlphaLISA®. Cell lines confirmed to be sensitive to inhibition in non-targeting control with partial depth of inhibition, rescued from inhibition by BI-3406 by knockdown of SOS1, and sensitized by knockdown of SOS2 to restore full depth of inhibition. See FIGS. 8A through 8D.

[0256] Overall, all SHP2 activating mutants demonstrated significant reduction in basal pERK after SOS1 knockdown, and levels of basal pERK reduction correlate with biochemical potency for activated SHP2 variant. See FIG. 9.

Example 4. Analysis of SHP2 Mutants.

Determining ΔG_{op} and the Strength of Activating Mutations in SHP2

[0257] In the absence of activating signals, SHP2 adopts an auto-inhibited ("Closed") conformation in which the N-SH2 domain binds over the active site, blocking access to substrates. Activation, by binding of the N- and C-SH2 domains to phosphotyrosine-containing sequences in effector proteins, causes the N-SH2 domain to move out of the active site, creating an active ("Open") conformation. Allosteric inhibitors of SHP2 bind exclusively to the Closed conformation with affinity K_i, and activating phosphopeptides (called "peptide" or "P" below) bind exclusively to the Open conformation with affinity K_d. The auto-inhibited conformation of SHP2 has an intrinsic

stability described by the opening equilibrium constant K_{op}. All of these equilibrium constants can also be expressed as Gibbs free energies (ΔG), according to the equation:

$$\Delta = - \quad ( )$$

[0258] where R is the ideal gas constant (0.00198588 kcal/mol*K used in all analysis) and T is the absolute temperature (298 K used in all analysis).

[0259] The K_{op} or ΔG_{op} governs the "activatability of SHP2, and is an intrinsic property of wild type SHP2, but can be changed by mutation. Some mutations decrease the ΔG_{op} , making SHP2 both more sensitive to activation by phosphotyrosine-containing peptides, and also less sensitive to allosteric inhibitors. The magnitude of this change varies with mutation, and can be small (weakly-activating mutations), moderate (moderately activating mutations), or large (strongly activating mutations).

[0260] The ΔG_{op} of wild type SHP2 or a mutant can be determined from a 2-dimensional concentration response experiment where the activity of SHP2 is measured as a function of varying concentrations of both an activating phosphopeptide (such as SIRPA1) and an allosteric inhibitor (such as RMC-4550). The results of such an experiment are shown in FIG. 10.

[0261] In order to estimate ΔG_{op} from this experiment, a model based on the following reaction scheme is fitted, which assumes affinity of inhibitor for open SHP2 and affinity of peptide for closed SHP2 are negligible.

$$\text{Closed-i} \overset{K_i}{\rightleftharpoons} \text{Closed} + \text{i} \overset{K_{op}}{\rightleftharpoons} \text{Open} + \text{P} \overset{K_d}{\rightleftharpoons} \text{Open-P}$$

[0262] The following equilibrium constants can be defined:

1. $= \frac{[\quad]}{[\quad]}$ (Stability of the closed conformation)

2. $= \frac{[\quad][\ ]}{[\quad \cdot \ ]}$ (Dissociation constant for peptide (P) and open conformation)

3. $= \frac{[\quad][\ ]}{[\quad \cdot \ ]}$ (Dissociation constant for inhibitor (i) and closed conformation)

[0263] The fraction of molecules in the open state (θ_{op}) is equal to

$$= \frac{[\quad]}{[\quad 2]} = \frac{[\quad] + [\quad \cdot \ ]}{[\quad] + [\quad] + [\quad \cdot \ ] + [\quad \cdot \ ]}$$

[0264] Dividing the numerator and denominator by [closed] gives

$$\frac{\frac{[\quad]}{[\quad]} + \frac{[\quad \cdot \ ]}{[\quad]}}{\frac{[\quad]}{[\quad]} + \frac{[\quad]}{[\quad]} + \frac{[\quad \cdot \ ]}{[\quad]} + \frac{[\quad \cdot \ ]}{[\quad]}}$$

[0265] Note that $\frac{[\quad \cdot \ ]}{[\quad]} = —[\ ]$ and other fractions are equal to the equilibrium constants defined above. Substituting these values results in the following equation:

$$= \frac{+ —[\ ]}{1 + \quad + —[\ ] + \frac{[\ ]}{}}$$

[0266] Assuming that the activity of the closed conformation is negligible, the apparent activity is the product of the specific activity of the open conformation and the fraction open:

$$= \quad \cdot$$

[0267] It is possible to fit this model to the experiment shown in FIG. 10 to estimate values of each parameter. Model fit curves are shown as lines in FIG. 11. The ΔG_{op} from this fit is 2.74 kcal/mol. Examples are also shown of a weakly activating mutant (A72S, 2.03 kcal/mol, FIG. 12), a moderately activating mutant (E69K, 0.61 kcal/mol, FIG. 13), and a strongly activating mutant (G503V, -0.62 kcal/mol, FIG. 14).

[0268] Model fit parameters for wild type SHP2 and 21 mutants are summarized in Table 1 above. $\Delta G_{peptide}$ corresponds to K_d, or affinity for activating phosphopeptide, and ΔG_i corresponds to Ki, or affinity of RMC-4550 for the closed conformation of SHP2.

[0269] A weakly activating mutant is defined as one with a ΔG_{op} not more than 1.5 kcal/mol below wild type SHP2. A moderately activating mutant has a ΔG_{op} between 1.5 kcal/mol and 2.24 kcal/mol below wild-type. A strongly activating mutation has a ΔG_{op} more than 2.24 kcal/mol below wild type.

Methods

[0270] The effect of RMC-4550 and SIRPA, a peptide, on the hydrolysis of the fluorogenic small molecule substrate 6,8-Difluoro-4-Methylumbelliferyl Phosphate (DiFMUP) was determined. Each SHP2 variant was assayed in triplicate 96-well plates, at 8 different [SIRPA1 peptide] and 12 different [RMC-4550].

[0271] SIRPA1 (Peptide sequence H_2N-IT[Y]ADLNLP[PEG8]HTE[Y]ASIQTSK-NH_2 (ThermoFisher Custom Peptides), where brackets indicate phosphotyrosine) was prepared at a stock concentration of 10 μM (20X max final) in 50 mM HEPES pH 7.2, 0.02% BSA. Six serial 3-fold dilutions were prepared and one well was prepared with dilution buffer alone. RMC-4550 was prepared in 50 mM HEPES pH 7.2, 0.02% BSA at a concentration of 2 μM (20X final) and 10 serial 3-fold dilutions were prepared. One well was prepared with buffer alone. 20X dilution series of SIRPA1 and RMC-4550 were mixed 1:1 in a matrix fashion in a 96-well plate to prepare a 10X peptide + compound plate, with decreasing [RMC-4550] in rows and decreasing [SIRPA1] in columns. Enzyme was prepared at 2X final concentration in 100 mM HEPES pH 7.2, 200 mM NaCl, 1 mM EDTA, 2 mM DTT, 0.002% Brij35. All mutants were assayed at a final enzyme concentration of 0.5 nM and wild-type was assayed at 1 nM.

[0272] 50 μl 2X enzyme stock was added to 96 well black polystyrene plates using an Agilent Bravo and mixed with 10 μl compound/peptide stock. Triplicate plates were prepared for each enzyme. Plates were incubated 20-40 minutes after preparation, and then 40 μl 50 μM DiFMUP in water was added to each well using a MultiDrop® Combi. Plates were shaken and read in a Spectramax® M5 plate reader in kinetic mode for five minutes, with excitation at 358 nm and emission at 450 nm. Linear signal vs. time curves were fit in SoftMax Pro and slopes were exported to Excel. Slopes from Softmax were converted to specific activity by dividing by the final enzyme concentration.

Example 5. SOS1 inhibitors shows Single Agent Anti-Tumor Activity *in vivo* in tumor models bearing SHP2 activating mutations

[0273] In this example, two mouse tumor cell lines, PAN02 (pancreatic cancer, immunocompetent) and LN229 CDX (glioma, immunocompromised), were characterized. The PAN02 mouse tumor cell line contained the highly activating G503V SHP2 mutation.

The LN229 CDX contained the highly activating A72S SHP2 mutation. In both PAN02 tumor cell lines (See FIG. 15; 100 mg/kg po qd and 250 mg/kg po qd) and LN229 CDX tumor cell lines (See FIG. 16; 100 mg/kg po qd), Compound SOS1-(A) (also called RMC-0331) treatment resulted in near complete inhibition of tumor growth in vivo.

[0274] The effect of the SOS1 inhibitor RMC-0331 on tumor cell growth in vivo was evaluated in (A)PAN02 Syngeneic model using female C57/BL6 mice and (B) LN229 xenograft model using female balb/c athymic nude mice (6-8 weeks old). Mice were implanted with (A) PAN02 tumor cells (1e6 cells/mouse) or (B) LN229 tumor cells in 50% matrigel (10e6 cells/mouse) subcutaneously in the flank. Once tumors reached an average size of ~200mm3 mice were randomized to treatment groups and administration of test article or vehicle (2% HPMC E-50, 0.5% Tween-80 in 50 mM Sodium Citrate Buffer, pH 4.0). Body weight and tumor volume (using digital calipers) were measured twice a week until study endpoints. Compounds were administered by oral gavage daily.

Example 6. SOS1 Inhibitor Shows Combination Benefits with RAS(ON) Inhibitors *in vitro*

[0275] In this example, two cell lines, SW837 (colorectal cancer, human) and PAN02 (pancreatic cancer, mouse), were characterized. The SW837 cell line contained the $KRAS^{G12C}$ mutation. The PAN02 mouse tumor cell line contained the highly activating G503V SHP2 mutation. The cell lines were treated with DMSO (vehicle) and a constant concentration of SOS1 inhibitor, Compound SOS1-(B). The cell lines were treated with varying concentration of a RAS inhibitor, Compound RAS-(E). FIGS. 17A (1 μM of SOS1 inhibitor; SW837 cell line; variable concentration or RAS inhibitor) and 17B (100 nM of SOS1 inhibitor; PAN02 cell line; variable concentration or RAS inhibitor) illustrate the viability of the cells as a function of RAS inhibitor, which depict the additive effect of SOS1 and RAS inhibition on cell viability. The data were obtained according to the 2-dimensional potency shifts experimental protocol.

Example 7. SOS1 Inhibitor Shows Combination Benefits with RAS(ON) Inhibitors *in vivo*

[0276] In this example, two mouse tumor cell lines, KLN205 (lung squamous cell carcinoma) and PAN02 (pancreatic cancer), were characterized. The mouse tumor cell lines contained the highly activating G503V SHP2 mutation. FIG. 18 is a Loewe 3D

response surface plot showing the *in vitro* combination effect of SOS1 inhibitor Compound SOS1-(A) (also called RMC-0331) and RAS^{MULTI}(ON) inhibitor Compound RAS-(D) observed in Pan02 cells. A synergy score > 5 at any point on the plot indicates a positive interaction between the two compounds. FIG. 19 is a Loewe 3D response surface plot showing the *in vitro* combination effect of SOS1 inhibitor Compound SOS1-(A) (also called RMC-0331) and RAS^{MULTI}(ON) inhibitor Compound RAS-(D) observed in KLN205 cells. A synergy score > 5 at any point on the plot indicates a positive interaction between the two compounds. These data were obtained according to the CrownSyn™ method.

Example 8. SOS1 inhibitors shows Single Agent Anti-Tumor Activity *in vivo* in tumor models bearing SHP2 activating mutations

[0277] Methods:

[0278] The anti-tumor efficacy of Compound SOS1-(C) was evaluated in comparison to RMC-4550 and Cobimetinib, in a human engineered model of PTPN11-mutant glioblastoma, LN229.E76K. 6-7 week athymic nude mice were implanted with LN229.E76K in 50% matirgel (10 x 106 cells/mouse) subcutaneously in the flank. Once tumors reached an average size of 200 mm^3, mice were randomized into treatment groups to start the administration of test articles or control. All treatments were administered daily by oral gavage. Body weight and tumor volume (using calipers) was measured twice weekly until study endpoint.

[0279] Results:

[0280] As shown in FIG. 21, single-agent Compound SOS1-(C) administered at 50 mg/kg PO daily led to a tumor growth inhibition (TGI) of 80%, single-agent RMC-4550 administered at 30 mg/kg PO daily led to a TGI of 7%, and cobimetinib administered at 2.5 mg/kg PO daily led to TGI of 41% in the LN229.E76K GMB CDX model with an engineered $PTPN11^{E76K}$ mutation. The anti-tumor activity of Compound SOS1-(C) and cobimetinib were statistically significant from the vehicle control group (****$p<0.0001$ and *$p<0.05$ respectively), while response to RMC-4550 treatment was not statistically significant, assessed by an ordinary one-way ANOVA of tumor volumes along with multiple comparisons via a post-hoc Tukey's test in GraphPad Prism software. All treatment arms were well tolerated.

Example 9. SOS1 Inhibitor Shows Combination Benefits *in vivo* in tumor models bearing SHP2 activating mutations

[0281] Methods:

[0282] The combinatorial effects of Compound SOS1-(C) with RAS-(E) on tumor cell growth in vivo were evaluated in the murine pancreatic ductal adenocarcinoma PAN02 $PTPN11^{G503V}$ syngeneic model using female C57BL/6J mice (6-7 weeks old). Mice were implanted with PAN02 tumor cells in PBS (5 $x10^6$cells/mouse) subcutaneously in the flank. Once tumors reached an average size of ~130 mm3, mice were randomized to treatment groups to start the administration of test articles or control. All test articles were administered by oral gavage daily. Body weight and tumor volume (using calipers) was measured twice weekly until study endpoints.

[0283] Results:

[0284] As shown in FIG. 22, single-agent Compound SOS1-(C) administered at 50 mg/kg PO daily led to a tumor growth inhibition (TGI) of 94%, single-agent RAS-(E) administered at 25 mg/kg PO daily led to a TGI of 64%, in the PAN02 murine PDAC CDX model with a $PTPN11^{G503V}$ mutation. However, the anti-tumor activity by the combination treatment led to tumor volume regressions of 54%. All treatment arms were statistically significant from the vehicle control group (**$p<0.01$, and ****$p<0.0001$, assessed by an ordinary one-way ANOVA of tumor volumes along with multiple comparisons via a post-hoc Tukey's test in GraphPad Prism software).

Example 10. SOS1 Inhibitor Shows Combination Benefits *in vivo* in tumor models bearing SHP2 activating mutations

[0285] Methods:

[0286] The combinatorial effects of Compund SOS1-(C) with cobimetinib on tumor cell growth in vivo were evaluated in the murine pancreatic ductal adenocarcinoma PAN02 $PTPN11^{G503V}$ syngeneic model using female C57BL/6J mice (6-7 weeks old). Mice were implanted with PAN02 tumor cells in PBS (5 $x10^6$cells/mouse) subcutaneously in the flank. Once tumors reached an average size of ~130 mm3, mice were randomized to treatment groups to start the administration of test articles or control. All test articles were

administered by oral gavage daily. Body weight and tumor volume (using calipers) was measured twice weekly until study endpoints.

[0287] Results:

[0288] As shown in FIG. 23, single-agent Compound SOS1-(C) administered at 100 mg/kg PO daily led to a tumor growth inhibition (TGI) of 94%, single-agent cobimetinib administered at 2.5 or 5 mg/kg PO daily led to a TGI of 38% and 49% respectively, in the PAN02 murine PDAC CDX model with a $PTPN11^{G503V}$ mutation. However, the anti-tumor activity by the combination treatment led to 42% tumor volume regressions and was statistically significant from the vehicle control group (****$p<0.0001$) and from the 5 mg/kg cobimetinib single agent group (*$p<0.05$), assessed by an ordinary one-way ANOVA of tumor volumes along with multiple comparisons via a post-hoc Tukey's test in GraphPad Prism software. All treatment arms were well tolerated.

Example 11. SOS1 Inhibitor Shows Combination Benefits *in vivo* in tumor models bearing SHP2 activating mutations

[0289] Methods:

[0290] The combinatorial effects of Compound SOS1-(B) with cobimetinib on tumor cell growth in vivo were evaluated in a human engineered model of PTPN11-mutant glioblastoma, LN229.E76K xenograft model using female athymic nude mice (6-7 weeks old). Mice were implanted with LN229.E76K tumor cells in 50% Matrigel (10 $x10^6$cells/mouse) subcutaneously in the flank. Once tumors reached an average size of ~200 mm3, mice were randomized to treatment groups to start the administration of test articles or control. All test articles were administered by oral gavage daily. Body weight and tumor volume (using calipers) was measured twice weekly until study endpoints.

[0291] Results:

[0292] As shown in FIG. 24, single-agent Compound SOS1-(B) administered at 50 mg/kg PO daily led to a tumor growth inhibition (TGI) of 85%, single-agent cobimetinib administered at 2.5 PO daily led to a TGI of 62% in the LN229.E76K GMB CDX model with an engineered $PTPN11^{E76K}$ mutation. The anti-tumor activity by the combination treatment led to TGI of 98% and was statistically significant from the vehicle control group (**$p<0.005$) assessed by an ordinary one-way ANOVA of tumor volumes

along with multiple comparisons via a post-hoc Tukey's test in GraphPad Prism software. All treatment arms were well tolerated. Single agent treatment with cobimetinib at 2.5 mg/kg was not statistically different from vehicle treatment. All treatment arms were well tolerated.

Example 12. SOS1 Inhibitor Shows Combination Benefits *in vivo* in tumor models bearing SHP2 activating mutations

[0293] Methods:

[0294] The combinatorial effects of Compound SOS1-(C) with Abemaciclib on tumor cell growth in vivo were evaluated in the murine pancreatic ductal adenocarcinoma PAN02 $PTPN11^{G503V}$ syngeneic model using female C57BL/6J mice (6-7 weeks old). Mice were implanted with PAN02 tumor cells in PBS (5 $x10^6$cells/mouse) subcutaneously in the flank. Once tumors reached an average size of ~130 mm3, mice were randomized to treatment groups to start the administration of test articles or control. All test articles were administered by oral gavage daily. Body weight and tumor volume (using calipers) was measured twice weekly until study endpoints.

[0295] Results:

[0296] As shown in FIG. 25, single-agent Compound SOS1-(C) led to tumor growth inhibition (TGI) of 78%, single-agent abemaciclib administered at 30 mg/kg PO daily led to a TGI of 52%, in the PAN02 murine PDAC CDX model with a $PTPN11^{G503V}$ mutation. Both single agent treatments were statistically significant from the vehicle control group (****$p<0.0001$, and ***$p<0.001$ respectively). However, the anti-tumor activity by the combination treatment led to 97% tumor volume regressions and was statistically significant from the vehicle control group (****$p<0.0001$) assessed by an ordinary one-way ANOVA of tumor volumes along with multiple comparisons via a post-hoc Tukey's test in GraphPad Prism software. All treatment arms were well tolerated. Compound SOS1-(C) was initially administered at 100 mg/kg PO daily for 7 days and then reduced to 50 mg/kg PO daily for the remainder of the study in both the single agent and combination treatment arms.

Example 13. SOS1 Inhibitor Shows Combination Benefits *in vivo* in tumor models bearing SHP2 activating mutations

[0297] Methods:

[0298] The combination effects of SOS1-(C) with anti-PD1 on tumor cell growth in vivo were evaluated in the mouse syngeneic pancreatic ductal adenocarcinoma line PAN02 carrying a $SHP2^{G503V}$ mutation using female C57BL/6 mice (6-8 weeks old). Mice were implanted with PAN02 tumors (5 x 106 cells/mouse) subcutaneously in the flank. Once tumors reached an average size of ~105 mm3, mice were randomized to treatment groups to start the administration of test articles or vehicle. Anti-PD1 (cloneRMP1-4) was administered by intraperitoneal injection twice weekly, and SOS1-(C) was administered by oral gavage daily. Body weight and tumor volume (using calipers) was measured twice weekly until study endpoints.

[0299] Results:

[0300] As shown in FIGS. 26A, 26B, 26C, 26D, 26E, and 26F, single-agent SOS1-(C) administered at 50 mg/kg PO daily led to a tumor growth inhibition (TGI) of 90.91%, and single-agent anti-PD1 administered at 10 mg/kg IP twice weekly led to a TGI of 26.75% in the PAN02 syngeneic mouse PDAC model with a $SHP2^{G503V}$ mutation. The combination led to a TGI of 93.96% and a complete tumor regression in one mouse in the PAN02 model. The anti-tumor activity of SOS1-(C) monotherapy was statistically significant with ***$p<0.001$ and the anti-tumor activity by the combination treatment was statistically significant from the vehicle control group, with ****$p<0.0001$, assessed by an ordinary One-way ANOVA of tumor volumes along with multiple comparisons via a post-hoc Tukey's test in GraphPad Prism software. Spaghetti plots show individual tumor responses. Waterfall plot shows individual tumor responses at the end of study, 1/10 tumors from the combination group showed complete regression. The combination treatment was well tolerated.

[0301] While the present invention has been described in conjunction with the specific embodiments set forth above, many alternatives, modifications and other variations thereof will be apparent to those of ordinary skill in the art. All such alternatives, modifications and variations are intended to fall within the spirit and scope of the present invention.

APPENDIX A

RAS INHIBITORS

Background

The vast majority of small molecule drugs act by binding a functionally important pocket on a target protein, thereby modulating the activity of that protein. For example, cholesterol-lowering drugs known as statins bind the enzyme active site of HMG-CoA reductase, thus preventing the enzyme from engaging with its substrates. The fact that many such drug/target interacting pairs are known may have misled some into believing that a small molecule modulator could be discovered for most, if not all, proteins provided a reasonable amount of time, effort, and resources. This is far from the case. Current estimates are that only about 10% of all human proteins are targetable by small molecules. Bojadzic and Buchwald, Curr Top Med Chem 18: 674-699 (2019). The other 90% are currently considered refractory or intractable toward above-mentioned small molecule drug discovery. Such targets are commonly referred to as "undruggable." These undruggable targets include a vast and largely untapped reservoir of medically important human proteins. Thus, there exists a great deal of interest in discovering new molecular modalities capable of modulating the function of such undruggable targets.

It has been well established in literature that Ras proteins (K-Ras, H-Ras and N-Ras) play an essential role in various human cancers and are therefore appropriate targets for anticancer therapy. Indeed, mutations in Ras proteins account for approximately 30% of all human cancers in the United States, many of which are fatal. Dysregulation of Ras proteins by activating mutations, overexpression or upstream activation is common in human tumors, and activating mutations in Ras are frequently found in human cancer. For example, activating mutations at codon 12 in Ras proteins function by inhibiting both GTPase-activating protein (GAP)-dependent and intrinsic hydrolysis rates of GTP, significantly skewing the population of Ras mutant proteins to the "on" (GTP-bound) state (Ras(ON)), leading to oncogenic MAPK signaling. Notably, Ras exhibits a picomolar affinity for GTP, enabling Ras to be activated even in the presence of low concentrations of this nucleotide. Mutations at codons 13 (e.g., G13D) and 61 (e.g., Q61K) of Ras are also responsible for oncogenic activity in some cancers.

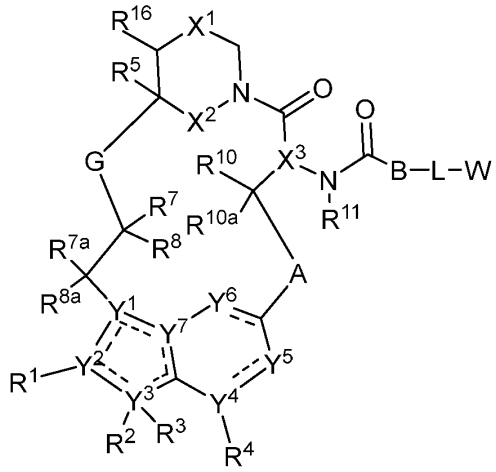
Despite extensive drug discovery efforts against Ras during the last several decades, a drug directly targeting Ras is still not approved. Additional efforts are needed to uncover additional medicines for cancers driven by the various Ras mutations.

Summary

Provided herein are Ras inhibitors. The approach described herein entails formation of a high affinity three-component complex between a synthetic ligand and two intracellular proteins which do not interact under normal physiological conditions: the target protein of interest (e.g., Ras), and a widely expressed cytosolic chaperone (presenter protein) in the cell (e.g., cyclophilin A). More specifically, in some embodiments, the inhibitors of Ras described herein induce a new binding pocket in Ras by driving formation of a high affinity tri-complex between the Ras protein

and the widely expressed cytosolic chaperone, cyclophilin A (CYPA). Without being bound by theory, the inventors believe that one way the inhibitory effect on Ras is effected by compounds of the invention and the complexes they form is by steric occlusion of the interaction site between Ras and downstream effector molecules, such as RAF and PI3K, which are required for propagating the oncogenic signal.

As such, in some embodiments, the disclosure features a compound, or pharmaceutically acceptable salt thereof, of structural Formula I:



Formula I

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 10-membered heteroarylene;

B is absent, -CH(R^9)-, or >C=C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is hydrogen, cyano, S(O)$_2$R', optionally substituted amino, optionally substituted amido, optionally substituted C_1-C_4 alkoxy, optionally substituted C_1-C_4 hydroxyalkyl, optionally substituted C_1-C_4 aminoalkyl, optionally substituted C_1-C_4 haloalkyl, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 guanidinoalkyl, C_0-C_4 alkyl optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 3 to 8-membered cycloalkyl, or optionally substituted 3 to 8-membered heteroaryl;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', $C(O)N(R')_2$, S(O)R', $S(O)_2R'$, or $S(O)_2N(R')_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl;

R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), $C=N(O\text{-}C_1\text{-}C_3$ alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is hydrogen, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo;

R^{11} is hydrogen or C_1-C_3 alkyl.Also provided are pharmaceutical compositions comprising a compound of Formula I, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient; and

R^{16} is hydrogen or C_1-C_3 alkyl (e.g., methyl).

It is specifically contemplated that any limitation discussed with respect to one embodiment of the invention may apply to any other embodiment of the invention. Furthermore, any compound or composition of the invention may be used in any method of the invention, and any method of the invention may be used to produce or to utilize any compound or composition of the invention.

Brief Description of the Figures

FIG. 1A: A compound of the present invention, Compound A, exhibits PK-dependent RAS pathway modulation in a Capan-2 CDX model (PDAC, KRAS G12V/WT). Single dose compared to twice administered PK/PD measurement of Compound A. Second dose of Compound A delivered 8 hours following first dose, depicted by black arrow. All dose levels well tolerated. Tumor DUSP6 mRNA expression as percent of control graphed as bars on left y-axis. Dotted line indicates return to control level of DUSP6. Unbound plasma PK (nM) graphed as lines, plotted in Log10 scale on right y-axis. N = 3/time point. Error bars represent standard error of the mean.

FIG. 1B: Combinatorial anti-tumor activity with a compound of the present invention, Compound A, and upstream SHP2 inhibition in a Capan-2 CDX model (PDAC, KRAS G12V/WT). Capan-2 cells were implanted in 50% Matrigel. Animals were randomized and treatment was initiated at average tumor volume of ~180mm3. Animals were dosed with SHP2 inhibitor RMC-4550 20 mg/kg po q2d, Compound A 100 mg/kg po bid, combination RMC-4550 and Compound A, or Control for 40 days. All dose levels were tolerated. n = 10/group (n = 9 in Combination arm). Ns = no significance; ***p<0.001 by one-way ANOVA.

Definitions and Chemical Terms

In this application, unless otherwise clear from context, (i) the term “a” means “one or more”; (ii) the term "or" is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternative are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or"; (iii) the terms “comprising” and “including” are understood to encompass itemized components or steps whether presented by themselves or together with one or more additional components or steps; and (iv) where ranges are provided, endpoints are included.

As used herein, the term “about” is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value. In certain embodiments, the term “about” refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of a stated value, unless otherwise stated or otherwise evident from the context (e.g., where such number would exceed 100% of a possible value).

As used herein, the term “adjacent” in the context of describing adjacent atoms refers to bivalent atoms that are directly connected by a covalent bond.

A “compound of the present invention” and similar terms as used herein, whether explicitly noted or not, refers to Ras inhibitors described herein, including compounds of Formula I and subformula thereof, and compounds of Table 1 and Table 2, as well as salts (e.g., pharmaceutically acceptable salts), solvates, hydrates, stereoisomers (including atropisomers), and tautomers thereof.

The term “wild-type” refers to an entity having a structure or activity as found in nature in a “normal” (as contrasted with mutant, diseased, altered, etc) state or context. Those of ordinary skill in the art will appreciate that wild-type genes and polypeptides often exist in multiple different forms (e.g., alleles).

Those skilled in the art will appreciate that certain compounds described herein can exist in one or more different isomeric (e.g., stereoisomers, geometric isomers, atropisomers, tautomers) or isotopic (e.g., in which one or more atoms has been substituted with a different isotope of the atom, such as hydrogen substituted for deuterium) forms. Unless otherwise indicated or clear from context, a depicted structure can be understood to represent any such isomeric or isotopic form, individually or in combination.

Compounds described herein can be asymmetric (e.g., having one or more stereocenters). All stereoisomers, such as enantiomers and diastereomers, are intended unless otherwise indicated. Compounds of the present disclosure that contain asymmetrically substituted carbon atoms can be isolated in optically active or racemic forms. Methods on how to prepare optically active forms from optically active starting materials are known in the art, such as by resolution of racemic mixtures or by stereoselective synthesis. Many geometric isomers of olefins, C=N double bonds, and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present disclosure. Cis and trans geometric isomers of the

compounds of the present disclosure are described and may be isolated as a mixture of isomers or as separated isomeric forms.

In some embodiments, one or more compounds depicted herein may exist in different tautomeric forms. As will be clear from context, unless explicitly excluded, references to such compounds encompass all such tautomeric forms. In some embodiments, tautomeric forms result from the swapping of a single bond with an adjacent double bond and the concomitant migration of a proton. In certain embodiments, a tautomeric form may be a prototropic tautomer, which is an isomeric protonation states having the same empirical formula and total charge as a reference form. Examples of moieties with prototropic tautomeric forms are ketone - enol pairs, amide - imidic acid pairs, lactam - lactim pairs, amide - imidic acid pairs, enamine - imine pairs, and annular forms where a proton can occupy two or more positions of a heterocyclic system, such as, 1H- and 3H-imidazole, 1H-, 2H- and 4H-1,2,4-triazole, 1H- and 2H- isoindole, and 1H- and 2H-pyrazole. In some embodiments, tautomeric forms can be in equilibrium or sterically locked into one form by appropriate substitution. In certain embodiments, tautomeric forms result from acetal interconversion.

Unless otherwise stated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. Exemplary isotopes that can be incorporated into compounds of the present invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, sulfur, fluorine, chlorine, and iodine, such as ^{2}H, ^{3}H, ^{11}C, ^{13}C, ^{14}C, ^{13}N, ^{15}N, ^{15}O, ^{17}O, ^{18}O, ^{32}P, ^{33}P, ^{35}S, ^{18}F, ^{36}Cl, ^{123}I and ^{125}I. Isotopically-labeled compounds (e.g., those labeled with ^{3}H and ^{14}C) can be useful in compound or substrate tissue distribution assays. Tritiated (i.e., ^{3}H) and carbon-14 (i.e., ^{14}C) isotopes can be useful for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium (i.e., ^{2}H) may afford certain therapeutic advantages resulting from greater metabolic stability (e.g., increased in vivo half-life or reduced dosage requirements). In some embodiments, one or more hydrogen atoms are replaced by ^{2}H or ^{3}H, or one or more carbon atoms are replaced by ^{13}C- or ^{14}C-enriched carbon. Positron emitting isotopes such as ^{15}O, ^{13}N, ^{11}C, and ^{18}F are useful for positron emission tomography (PET) studies to examine substrate receptor occupancy. Preparations of isotopically labelled compounds are known to those of skill in the art. For example, isotopically labeled compounds can generally be prepared by following procedures analogous to those disclosed for compounds of the present invention described herein, by substituting an isotopically labeled reagent for a non-isotopically labeled reagent.

As is known in the art, many chemical entities can adopt a variety of different solid forms such as, for example, amorphous forms or crystalline forms (e.g., polymorphs, hydrates, solvate). In some embodiments, compounds of the present invention may be utilized in any such form, including in any solid form. In some embodiments, compounds described or depicted herein may be provided or utilized in hydrate or solvate form.

At various places in the present specification, substituents of compounds of the present disclosure are disclosed in groups or in ranges. It is specifically intended that the present disclosure include each and every individual subcombination of the members of such groups and ranges. For example, the term "C_1-C_6 alkyl" is specifically intended to individually disclose methyl,

ethyl, C_3 alkyl, C_4 alkyl, C_5 alkyl, and C_6 alkyl. Furthermore, where a compound includes a plurality of positions at which substituents are disclosed in groups or in ranges, unless otherwise indicated, the present disclosure is intended to cover individual compounds and groups of compounds (e.g., genera and subgenera) containing each and every individual subcombination of members at each position.

The term "optionally substituted X" (e.g., "optionally substituted alkyl") is intended to be equivalent to "X, wherein X is optionally substituted" (e.g., "alkyl, wherein said alkyl is optionally substituted"). It is not intended to mean that the feature "X" (e.g., alkyl) *per se* is optional. As described herein, certain compounds of interest may contain one or more "optionally substituted" moieties. In general, the term "substituted", whether preceded by the term "optionally" or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent, e.g., any of the substituents or groups described herein. Unless otherwise indicated, an "optionally substituted" group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. For example, in the term "optionally substituted C_1-C_6 alkyl-C_2-C_9 heteroaryl," the alkyl portion, the heteroaryl portion, or both, may be optionally substituted. Combinations of substituents envisioned by the present disclosure are preferably those that result in the formation of stable or chemically feasible compounds. The term "stable", as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain embodiments, their recovery, purification, and use for one or more of the purposes disclosed herein.

Suitable monovalent substituents on a substitutable carbon atom of an "optionally substituted" group may be, independently, deuterium; halogen; $-(CH_2)_{0-4}R^\circ$; $-(CH_2)_{0-4}OR^\circ$; $-O(CH_2)_{0-4}R^\circ$; $-O-(CH_2)_{0-4}C(O)OR^\circ$; $-(CH_2)_{0-4}CH(OR^\circ)_2$; $-(CH_2)_{0-4}SR^\circ$; $-(CH_2)_{0-4}Ph$, which may be substituted with R°; $-(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ which may be substituted with R°; $-CH=CHPh$, which may be substituted with R°; $-(CH_2)_{0-4}O(CH_2)_{0-1}$-pyridyl which may be substituted with R°; 4-8 membered saturated or unsaturated heterocycloalkyl (e.g., pyridyl); 3-8 membered saturated or unsaturated cycloalkyl (e.g., cyclopropyl, cyclobutyl, or cyclopentyl); $-NO_2$; $-CN$; $-N_3$; $-(CH_2)_{0-4}N(R^\circ)_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)R^\circ$; $-N(R^\circ)C(S)R^\circ$; $-(CH_2)_{0-4}N(R^\circ)C(O)NR^\circ_2$; $-N(R^\circ)C(S)NR^\circ_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)OR^\circ$; $-N(R^\circ)N(R^\circ)C(O)R^\circ$; $-N(R^\circ)N(R^\circ)C(O)NR^\circ_2$; $-N(R^\circ)N(R^\circ)C(O)OR^\circ$; $-(CH_2)_{0-4}C(O)R^\circ$; $-C(S)R^\circ$; $-(CH_2)_{0-4}C(O)OR^\circ$; $-(CH_2)_{0-4}$-$C(O)$-$N(R^\circ)_2$; $-(CH_2)_{0-4}$-$C(O)$-$N(R^\circ)$-$S(O)_2$-R°; $-C(NCN)NR^\circ_2$; $-(CH_2)_{0-4}C(O)SR^\circ$; $-(CH_2)_{0-4}C(O)OSiR^\circ_3$; $-(CH_2)_{0-4}OC(O)R^\circ$; $-OC(O)(CH_2)_{0-4}SR^\circ$; $-SC(S)SR^\circ$; $-(CH_2)_{0-4}SC(O)R^\circ$; $-(CH_2)_{0-4}C(O)NR^\circ_2$; $-C(S)NR^\circ_2$; $-C(S)SR^\circ$; $-(CH_2)_{0-4}OC(O)NR^\circ_2$; $-C(O)N(OR^\circ)R^\circ$; $-C(O)C(O)R^\circ$; $-C(O)CH_2C(O)R^\circ$; $-C(NOR^\circ)R^\circ$; $-(CH_2)_{0-4}SSR^\circ$; $-(CH_2)_{0-4}S(O)_2R^\circ$; $-(CH_2)_{0-4}S(O)_2OR^\circ$; $-(CH_2)_{0-4}OS(O)_2R^\circ$; $-S(O)_2NR^\circ_2$; $-(CH_2)_{0-4}S(O)R^\circ$; $-N(R^\circ)S(O)_2NR^\circ_2$; $-N(R^\circ)S(O)_2R^\circ$; $-N(OR^\circ)R^\circ$; $-C(NOR^\circ)NR^\circ_2$; $-C(NH)NR^\circ_2$; $-P(O)_2R^\circ$; $-P(O)R^\circ_2$; $-P(O)(OR^\circ)_2$; $-OP(O)R^\circ_2$; $-OP(O)(OR^\circ)_2$; $-OP(O)(OR^\circ)R^\circ$, $-SiR^\circ_3$; $-(C_{1-4}$ straight or branched alkylene)O-$N(R^\circ)_2$; or $-(C_{1-4}$ straight or branched alkylene)$C(O)O$-$N(R^\circ)_2$, wherein each R° may be

substituted as defined below and is independently hydrogen, -C_{1-6} aliphatic, -CH_2Ph, -$O(CH_2)_{0-1}Ph$, -CH_2-(5-6 membered heteroaryl ring), or a 3-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of R°, taken together with their intervening atom(s), form a 3-12-membered saturated, partially unsaturated, or aryl mono- or bicyclic ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, which may be substituted as defined below.

Suitable monovalent substituents on R° (or the ring formed by taking two independent occurrences of R° together with their intervening atoms), may be, independently, halogen, -$(CH_2)_{0-2}R^•$, -(haloR•), -$(CH_2)_{0-2}OH$, -$(CH_2)_{0-2}OR^•$, -$(CH_2)_{0-2}CH(OR^•)_2$; -O(haloR•), -CN, -N_3, -$(CH_2)_{0-2}C(O)R^•$, -$(CH_2)_{0-2}C(O)OH$, -$(CH_2)_{0-2}C(O)OR^•$, -$(CH_2)_{0-2}SR^•$, -$(CH_2)_{0-2}SH$, -$(CH_2)_{0-2}NH_2$, -$(CH_2)_{0-2}NHR^•$, -$(CH_2)_{0-2}NR^•_2$, -NO_2, -$SiR^•_3$, -$OSiR^•_3$, -C(O)SR•, -(C_{1-4} straight or branched alkylene)C(O)OR•, or -SSR• wherein each R• is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently selected from C_{1-4} aliphatic, -CH_2Ph, -$O(CH_2)_{0-1}Ph$, or a 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents on a saturated carbon atom of R° include =O and =S.

Suitable divalent substituents on a saturated carbon atom of an "optionally substituted" group include the following: =O, =S, =NNR^*_2, =NNHC(O)R*, =NNHC(O)OR*, =$NNHS(O)_2R^*$, =NR*, =NOR*, -$O(C(R^*_2))_{2-3}O$-, or -$S(C(R^*_2))_{2-3}S$-, wherein each independent occurrence of R* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents that are bound to vicinal substitutable carbons of an "optionally substituted" group include: -$O(CR^*_2)_{2-3}O$-, wherein each independent occurrence of R* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

Suitable substituents on the aliphatic group of R* include halogen, -R•, -(haloR•), -OH, -OR•, -O(haloR•), -CN, -C(O)OH, -C(O)OR•, -NH_2, -NHR•, -$NR^•_2$, or -NO_2, wherein each R• is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, -CH_2Ph, -$O(CH_2)_{0-1}Ph$, or a 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

Suitable substituents on a substitutable nitrogen of an "optionally substituted" group include -R†, -$NR^†_2$, -C(O)R†, -C(O)OR†, -C(O)C(O)R†, -$C(O)CH_2C(O)R^†$, -$S(O)_2R^†$, -$S(O)_2NR^†_2$, -$C(S)NR^†_2$, -C(NH)$NR^†_2$, or -$N(R^†)S(O)_2R^†$; wherein each R† is independently hydrogen, C_{1-6} aliphatic which may be

substituted as defined below, unsubstituted -OPh, or an unsubstituted 3-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of $R^{\dagger}$, taken together with their intervening atom(s) form an unsubstituted 3-12-membered saturated, partially unsaturated, or aryl mono- or bicyclic ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

Suitable substituents on an aliphatic group of $R^{\dagger}$ are independently halogen, $-R^{\bullet}$, -(halo$R^{\bullet}$), -OH, $-OR^{\bullet}$, -O(halo$R^{\bullet}$), -CN, -C(O)OH, -C(O)O$R^{\bullet}$, $-NH_2$, -NH$R^{\bullet}$, -N$R^{\bullet}_2$, or $-NO_2$, wherein each $R^{\bullet}$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents on a saturated carbon atom of $R^{\dagger}$ include =O and =S.

The term "acetyl," as used herein, refers to the group $-C(O)CH_3$.

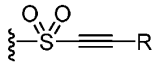
The term "alkoxy," as used herein, refers to a -O-C_1-C_{20} alkyl group, wherein the alkoxy group is attached to the remainder of the compound through an oxygen atom.

The term "alkyl," as used herein, refers to a saturated, straight or branched monovalent hydrocarbon group containing from 1 to 20 (e.g., from 1 to 10 or from 1 to 6) carbons. In some embodiments, an alkyl group is unbranched (i.e., is linear); in some embodiments, an alkyl group is branched. Alkyl groups are exemplified by, but not limited to, methyl, ethyl, *n*- and *iso*-propyl, *n*-, *sec*-, *iso*- and *tert*-butyl, and neopentyl.

The term "alkylene," as used herein, represents a saturated divalent hydrocarbon group derived from a straight or branched chain saturated hydrocarbon by the removal of two hydrogen atoms, and is exemplified by methylene, ethylene, isopropylene, and the like. The term "C_x-C_y alkylene" represents alkylene groups having between x and y carbons. Exemplary values for x are 1, 2, 3, 4, 5, and 6, and exemplary values for y are 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, or 20 (e.g., C_1-C_6, C_1-C_{10}, C_2-C_{20}, C_2-C_6, C_2-C_{10}, or C_2-C_{20} alkylene). In some embodiments, the alkylene can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein.

The term "alkenyl," as used herein, represents monovalent straight or branched chain groups of, unless otherwise specified, from 2 to 20 carbons (e.g., from 2 to 6 or from 2 to 10 carbons) containing one or more carbon-carbon double bonds and is exemplified by ethenyl, 1-propenyl, 2-propenyl, 2-methyl-1-propenyl, 1-butenyl, and 2-butenyl. Alkenyls include both cis and trans isomers. The term "alkenylene," as used herein, represents a divalent straight or branched chain groups of, unless otherwise specified, from 2 to 20 carbons (e.g., from 2 to 6 or from 2 to 10 carbons) containing one or more carbon-carbon double bonds.

The term "alkynyl," as used herein, represents monovalent straight or branched chain groups from 2 to 20 carbon atoms (e.g., from 2 to 4, from 2 to 6, or from 2 to 10 carbons) containing a carbon-carbon triple bond and is exemplified by ethynyl, and 1-propynyl.

The term "alkynyl sulfone," as used herein, represents a group comprising the structure  , wherein R is any chemically feasible substituent described herein.

The term “amino,” as used herein, represents -N(R^{1})$_2$, e.g., -NH_2 and -$N(CH_3)_2$.

The term “aminoalkyl,” as used herein, represents an alkyl moiety substituted on one or more carbon atoms with one or more amino moieties.

The term “amino acid,” as described herein, refers to a molecule having a side chain, an amino group, and an acid group (e.g., -CO_2H or -SO_3H), wherein the amino acid is attached to the parent molecular group by the side chain, amino group, or acid group (e.g., the side chain). As used herein, the term “amino acid” in its broadest sense, refers to any compound or substance that can be incorporated into a polypeptide chain, e.g., through formation of one or more peptide bonds. In some embodiments, an amino acid has the general structure H_2N-C(H)(R)-COOH. In some embodiments, an amino acid is a naturally-occurring amino acid. In some embodiments, an amino acid is a synthetic amino acid; in some embodiments, an amino acid is a D-amino acid; in some embodiments, an amino acid is an L-amino acid. “Standard amino acid” refers to any of the twenty standard L-amino acids commonly found in naturally occurring peptides. Exemplary amino acids include alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, optionally substituted hydroxylnorvaline, isoleucine, leucine, lysine, methionine, norvaline, ornithine, phenylalanine, proline, pyrrolysine, selenocysteine, serine, taurine, threonine, tryptophan, tyrosine, and valine.

The term “aryl,” as used herein, represents a monovalent monocyclic, bicyclic, or multicyclic ring system formed by carbon atoms, wherein the ring attached to the pendant group is aromatic. Examples of aryl groups are phenyl, naphthyl, phenanthrenyl, and anthracenyl. An aryl ring can be attached to its pendant group at any heteroatom or carbon ring atom that results in a stable structure and any of the ring atoms can be optionally substituted unless otherwise specified.

The term “C_0,” as used herein, represents a bond. For example, part of the term -N(C(O)-(C_0-C_5 alkylene-H)- includes -N(C(O)-(C_0 alkylene-H)-, which is also represented by -N(C(O)-H)-.

The terms “carbocyclic” and “carbocyclyl,” as used herein, refer to a monovalent, optionally substituted C_3-C_{12} monocyclic, bicyclic, or tricyclic ring structure, which may be bridged, fused or spirocyclic, in which all the rings are formed by carbon atoms and at least one ring is non-aromatic. Carbocyclic structures include cycloalkyl, cycloalkenyl, and cycloalkynyl groups. Examples of carbocyclyl groups are cyclohexyl, cyclohexenyl, cyclooctynyl, 1,2-dihydronaphthyl, 1,2,3,4-tetrahydronaphthyl, fluorenyl, indenyl, indanyl, decalinyl, and the like. A carbocyclic ring can be attached to its pendant group at any ring atom that results in a stable structure and any of the ring atoms can be optionally substituted unless otherwise specified.

The term “carbonyl,” as used herein, represents a C(O) group, which can also be represented as C=O.

The term “carboxyl,” as used herein, means -CO_2H, (C=O)(OH), COOH, or C(O)OH or the unprotonated counterparts.

The term “cyano,” as used herein, represents a -CN group.

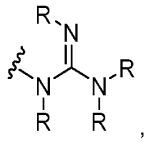
The term “cycloalkyl,” as used herein, represents a monovalent saturated cyclic hydrocarbon group, which may be bridged, fused or spirocyclic having from three to eight ring

carbons, unless otherwise specified, and is exemplified by cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cycloheptyl.

The term "cycloalkenyl," as used herein, represents a monovalent, non-aromatic, saturated cyclic hydrocarbon group, which may be bridged, fused or spirocyclic having from three to eight ring carbons, unless otherwise specified, and containing one or more carbon-carbon double bonds.

The term "diastereomer," as used herein, means stereoisomers that are not mirror images of one another and are non-superimposable on one another.

The term "enantiomer," as used herein, means each individual optically active form of a compound of the invention, having an optical purity or enantiomeric excess (as determined by methods standard in the art) of at least 80% (i.e., at least 90% of one enantiomer and at most 10% of the other enantiomer), preferably at least 90% and more preferably at least 98%.

The term "guanidinyl," refers to a group having the structure:  , wherein each R is, independently, any any chemically feasible substituent described herein.

The term "guanidinoalkyl alkyl," as used herein, represents an alkyl moiety substituted on one or more carbon atoms with one or more guanidinyl moieties.

The term "haloacetyl," as used herein, refers to an acetyl group wherein at least one of the hydrogens has been replaced by a halogen.

The term "haloalkyl," as used herein, represents an alkyl moiety substituted on one or more carbon atoms with one or more of the same of different halogen moieties.

The term "halogen," as used herein, represents a halogen selected from bromine, chlorine, iodine, or fluorine.

The term "heteroalkyl," as used herein, refers to an "alkyl" group, as defined herein, in which at least one carbon atom has been replaced with a heteroatom (e.g., an O, N, or S atom). The heteroatom may appear in the middle or at the end of the radical.

The term "heteroaryl," as used herein, represents a monovalent, monocyclic or polycyclic ring structure that contains at least one fully aromatic ring: i.e., they contain $4n+2$ pi electrons within the monocyclic or polycyclic ring system and contains at least one ring heteroatom selected from N, O, or S in that aromatic ring. Exemplary unsubstituted heteroaryl groups are of 1 to 12 (e.g., 1 to 11, 1 to 10, 1 to 9, 2 to 12, 2 to 11, 2 to 10, or 2 to 9) carbons. The term "heteroaryl" includes bicyclic, tricyclic, and tetracyclic groups in which any of the above heteroaromatic rings is fused to one or more, aryl or carbocyclic rings, e.g., a phenyl ring, or a cyclohexane ring. Examples of heteroaryl groups include, but are not limited to, pyridyl, pyrazolyl, benzooxazolyl, benzoimidazolyl, benzothiazolyl, imidazolyl, thiazolyl, quinolinyl, tetrahydroquinolinyl, and 4-azaindolyl. A heteroaryl ring can be attached to its pendant group at any ring atom that results in a stable structure and any of the ring atoms can be optionally substituted unless otherwise specified. In some embodiment, the heteroaryl is substituted with 1, 2, 3, or 4 substituents groups.

The term "heterocycloalkyl," as used herein, represents a monovalent monocyclic, bicyclic or polycyclic ring system, which may be bridged, fused or spirocyclic, wherein at least one ring is

non-aromatic and wherein the non-aromatic ring contains one, two, three, or four heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur. The 5-membered ring has zero to two double bonds, and the 6- and 7-membered rings have zero to three double bonds. Exemplary unsubstituted heterocycloalkyl groups are of 1 to 12 (e.g., 1 to 11, 1 to 10, 1 to 9, 2 to 12, 2 to 11, 2 to 10, or 2 to 9) carbons. The term "heterocycloalkyl" also represents a heterocyclic compound having a bridged multicyclic structure in which one or more carbons or heteroatoms bridges two non-adjacent members of a monocyclic ring, e.g., a quinuclidinyl group. The term "heterocycloalkyl" includes bicyclic, tricyclic, and tetracyclic groups in which any of the above heterocyclic rings is fused to one or more aromatic, carbocyclic, heteroaromatic, or heterocyclic rings, e.g., an aryl ring, a cyclohexane ring, a cyclohexene ring, a cyclopentane ring, a cyclopentene ring, a pyridine ring, or a pyrrolidine ring. Examples of heterocycloalkyl groups are pyrrolidinyl, piperidinyl, 1,2,3,4-tetrahydroquinolinyl, decahydroquinolinyl, dihydropyrrolopyridine, and decahydronapthyridinyl. A heterocycloalkyl ring can be attached to its pendant group at any ring atom that results in a stable structure and any of the ring atoms can be optionally substituted unless otherwise specified.

The term "hydroxy," as used herein, represents a -OH group.

The term "hydroxyalkyl," as used herein, represents an alkyl moiety substituted on one or more carbon atoms with one or more -OH moieties.

The term "isomer," as used herein, means any tautomer, stereoisomer, atropiosmer, enantiomer, or diastereomer of any compound of the invention. It is recognized that the compounds of the invention can have one or more chiral centers or double bonds and, therefore, exist as stereoisomers, such as double-bond isomers (i.e., geometric E/Z isomers) or diastereomers (e.g., enantiomers (i.e., (+) or (-)) or cis/trans isomers). According to the invention, the chemical structures depicted herein, and therefore the compounds of the invention, encompass all the corresponding stereoisomers, that is, both the stereomerically pure form (e.g., geometrically pure, enantiomerically pure, or diastereomerically pure) and enantiomeric and stereoisomeric mixtures, e.g., racemates. Enantiomeric and stereoisomeric mixtures of compounds of the invention can typically be resolved into their component enantiomers or stereoisomers by well-known methods, such as chiral-phase gas chromatography, chiral-phase high performance liquid chromatography, crystallizing the compound as a chiral salt complex, or crystallizing the compound in a chiral solvent. Enantiomers and stereoisomers can also be obtained from stereomerically or enantiomerically pure intermediates, reagents, and catalysts by well-known asymmetric synthetic methods.

As used herein, the term "linker" refers to a divalent organic moiety connecting moiety B to moiety W in a compound of Formula I, such that the resulting compound is capable of achieving an IC50 of 2 uM or less in the Ras-RAF disruption assay protocol provided in the Examples below, and provided here:

The purpose of this biochemical assay is to measure the ability of test compounds to facilitate ternary complex formation between a nucleotide-loaded Ras isoform and

cyclophilin A; the resulting ternary complex disrupts binding to a $BRAF^{RBD}$ construct, inhibiting Ras signaling through a RAF effector.

In assay buffer containing 25 mM HEPES pH 7.3, 0.002% Tween20, 0.1% BSA, 100 mM NaCl and 5 mM $MgCl_2$, tagless Cyclophilin A, His6-K-Ras-GMPPNP (or other Ras variant), and GST-$BRAF^{RBD}$ are combined in a 384-well assay plate at final concentrations of 25 µM, 12.5 nM and 50 nM, respectively. Compound is present in plate wells as a 10-point 3-fold dilution series starting at a final concentration of 30 µM. After incubation at 25°C for 3 hours, a mixture of Anti-His Eu-W1024 and anti-GST allophycocyanin is then added to assay sample wells at final concentrations of 10 nM and 50 nM, respectively, and the reaction incubated for an additional 1.5 hours. TR-FRET signal is read on a microplate reader (Ex 320 nm, Em 665/615 nm). Compounds that facilitate disruption of a Ras:RAF complex are identified as those eliciting a decrease in the TR-FRET ratio relative to DMSO control wells.

In some embodiments, the linker comprises 20 or fewer linear atoms. In some embodiments, the linker comprises 15 or fewer linear atoms. In some embodiments, the linker comprises 10 or fewer linear atoms. In some embodiments, the linker has a molecular weight of under 500 g/mol. In some embodiments, the linker has a molecular weight of under 400 g/mol. In some embodiments, the linker has a molecular weight of under 300 g/mol. In some embodiments, the linker has a molecular weight of under 200 g/mol. In some embodiments, the linker has a molecular weight of under 100 g/mol. In some embodiments, the linker has a molecular weight of under 50 g/mol.

As used herein, a “monovalent organic moiety” is less than 500 kDa. In some embodiments, a “monovalent organic moiety” is less than 400 kDa. In some embodiments, a “monovalent organic moiety” is less than 300 kDa. In some embodiments, a “monovalent organic moiety” is less than 200 kDa. In some embodiments, a “monovalent organic moiety” is less than 100 kDa. In some embodiments, a “monovalent organic moiety” is less than 50 kDa. In some embodiments, a “monovalent organic moiety” is less than 25 kDa. In some embodiments, a “monovalent organic moiety” is less than 20 kDa. In some embodiments, a “monovalent organic moiety” is less than 15 kDa. In some embodiments, a “monovalent organic moiety” is less than 10 kDa. In some embodiments, a “monovalent organic moiety” is less than 1 kDa. In some embodiments, a “monovalent organic moiety” is less than 500 g/mol. In some embodiments, a “monovalent organic moiety” ranges between 500 g/mol and 500 kDa.

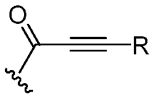
The term “stereoisomer,” as used herein, refers to all possible different isomeric as well as conformational forms which a compound may possess (e.g., a compound of any formula described herein), in particular all possible stereochemically and conformationally isomeric forms, all diastereomers, enantiomers or conformers of the basic molecular structure, including atropisomers. Some compounds of the present invention may exist in different tautomeric forms, all of the latter being included within the scope of the present invention.

The term “sulfonyl,” as used herein, represents an -$S(O)_2$- group.

The term “thiocarbonyl,” as used herein, refers to a -C(S)- group.

The term "vinyl ketone," as used herein, refers to a group comprising a carbonyl group directly connected to a carbon-carbon double bond.

The term "vinyl sulfone," as used herein, refers to a group comprising a sulfonyl group directed connected to a carbon-carbon double bond.

The term "ynone," as used herein, refers to a group comprising the structure  , wherein R is any any chemically feasible substituent described herein.

Those of ordinary skill in the art, reading the present disclosure, will appreciate that certain compounds described herein may be provided or utilized in any of a variety of forms such as, for example, salt forms, protected forms, pro-drug forms, ester forms, isomeric forms (e.g., optical or structural isomers), isotopic forms, etc. In some embodiments, reference to a particular compound may relate to a specific form of that compound. In some embodiments, reference to a particular compound may relate to that compound in any form. In some embodiments, for example, a preparation of a single stereoisomer of a compound may be considered to be a different form of the compound than a racemic mixture of the compound; a particular salt of a compound may be considered to be a different form from another salt form of the compound; a preparation containing one conformational isomer ((Z) or (E)) of a double bond may be considered to be a different form from one containing the other conformational isomer ((E) or (Z)) of the double bond; a preparation in which one or more atoms is a different isotope than is present in a reference preparation may be considered to be a different form.

Detailed Description

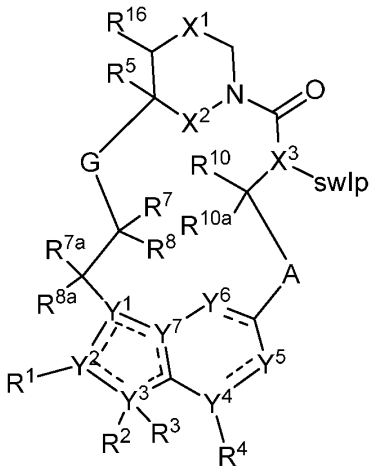
Compounds

Provided herein are Ras inhibitors. The approach described herein entails formation of a high affinity three-component complex between a synthetic ligand and two intracellular proteins which do not interact under normal physiological conditions: the target protein of interest (e.g., Ras), and a widely expressed cytosolic chaperone (presenter protein) in the cell (e.g., cyclophilin A). More specifically, in some embodiments, the inhibitors of Ras described herein induce a new binding pocket in Ras by driving formation of a high affinity tri-complex between the Ras protein and the widely expressed cytosolic chaperone, cyclophilin A (CYPA). Without being bound by theory, the inventors believe that one way the inhibitory effect on Ras is effected by compounds of the invention and the complexes they form is by steric occlusion of the interaction site between Ras and downstream effector molecules, such as RAF, which are required for propagating the oncogenic signal.

Without being bound by theory, the inventors postulate that non-covalent interactions of a compound of the present invention with Ras and the chaperone protein (e.g., cyclophilin A) may contribute to the inhibition of Ras activity. For example, van der Waals, hydrophobic, hydrophilic and hydrogen bond interactions, and combinations thereof, may contribute to the ability of the compounds of the present invention to form complexes and act as Ras inhibitors. Accordingly, a variety of Ras proteins may be inhibited by compounds of the present invention (e.g., K-Ras, N-

Ras, H-Ras, and mutants thereof at positions 12, 13 and 61, such as G12C, G12D, G12V, G12S, G13C, G13D, and Q61L, and others described herein).

Accordingly, provided herein is a compound, or pharmaceutically acceptable salt thereof, having the structure of Formula 00:



Formula 00

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 10-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

swIp (Switch I/P-loop) refers to an organic moiety that non-covalently binds to both the Switch I binding pocket and residues 12 or 13 of the P-loop of a Ras protein (see, e.g., Johnson et al., 292:12981-12993 (2017), incorporated herein by reference);

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl;

R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

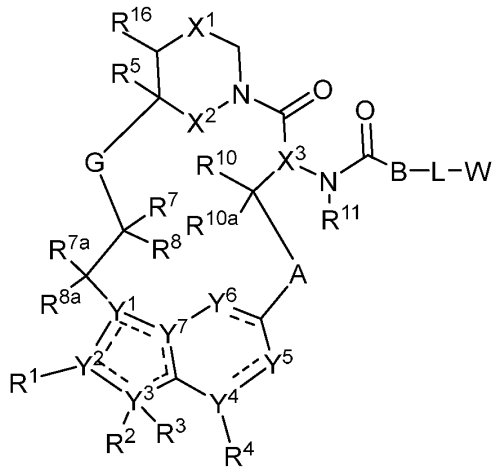
$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo; and

R^{16} is hydrogen or C_1-C_3 alkyl (e.g., methyl). In some embodiments, the resulting compound is capable of achieving an IC50 of 2 uM or less (e.g., 1.5 uM, 1 uM, 500 nM, or 100 nM or less) in the Ras-RAF disruption assay protocol described herein.

Accordingly, provided herein is a compound, or pharmaceutically acceptable salt thereof, having the structure of Formula I:



Formula I

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 10-membered heteroarylene;

B is absent, -CH(R^9)-, or >C=C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is hydrogen, cyano, S(O)$_2$R', optionally substituted amino, optionally substituted amido, optionally substituted C_1-C_4 alkoxy, optionally substituted C_1-C_4 hydroxyalkyl, optionally substituted C_1-C_4 aminoalkyl, optionally substituted C_1-C_4 haloalkyl, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 guanidinoalkyl, C_0-C_4 alkyl optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 3 to 8-membered cycloalkyl, or optionally substituted 3 to 8-membered heteroaryl;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', $C(O)N(R')_2$, S(O)R', $S(O)_2R'$, or $S(O)_2N(R')_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl;

R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), $C=N(O\text{-}C_1\text{-}C_3$ alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is hydrogen, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl;

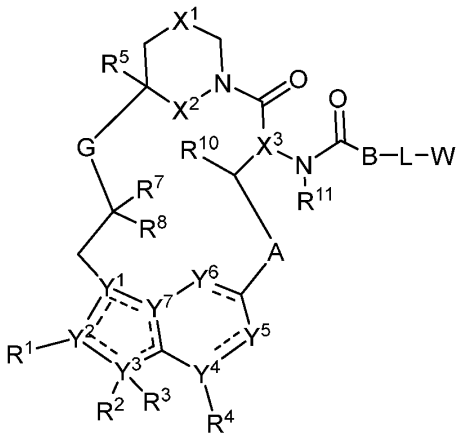
R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo;

R^{11} is hydrogen or C_1-C_3 alkyl;

R^{16} is hydrogen or C_1-C_3 alkyl (e.g., methyl).

In some embodiments, the disclosure features a compound, or pharmaceutically acceptable salt thereof, of structural Formula Ia:



Formula Ia

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 10-membered heteroarylene;

B is -CH(R^9)- or >C=C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is hydrogen, optionally substituted amino, optionally substituted C_1-C_4 alkoxy, optionally substituted C_1-C_4 hydroxyalkyl, optionally substituted C_1-C_4 aminoalkyl, optionally substituted C_1-C_4 haloalkyl, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 guanidinoalkyl, C_0-C_4 alkyl optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 3 to 8-membered cycloalkyl, or optionally substituted 3 to 8-membered heteroaryl;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or S(O)$_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl;

R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

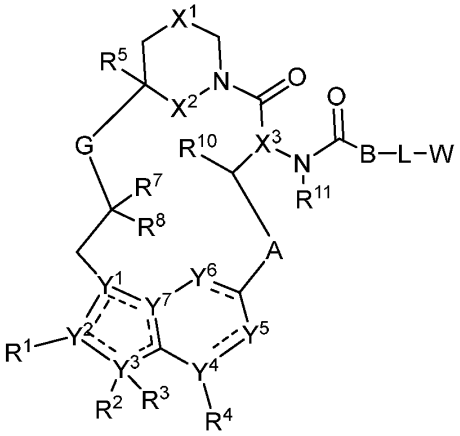
$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo; and

R^{11} is hydrogen or C_1-C_3 alkyl.

In some embodiments, the disclosure features a compound, or pharmaceutically acceptable salt thereof, of structural Formula Ib:



Formula Ib

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is hydrogen, optionally substituted amino, optionally substituted C_1-C_4 alkoxy, optionally substituted C_1-C_4 hydroxyalkyl, optionally substituted C_1-C_4 aminoalkyl, optionally substituted C_1-C_4 haloalkyl, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 guanidinoalkyl, C_0-C_4 alkyl optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 3 to 8-membered cycloalkyl, or optionally substituted 3 to 8-membered heteroaryl;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each $R^{'}$ is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

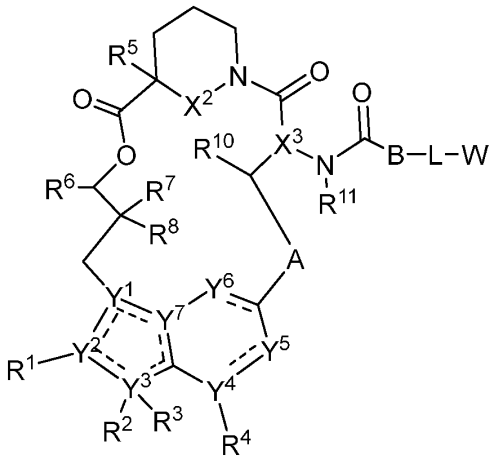
R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl; and

R^{11} is hydrogen or C_1-C_3 alkyl.

In some embodiments of compounds of the present invention, G is optionally substituted C_1-C_4 heteroalkylene.

In some embodiments, a compound of the present invention has the structure of Formula Ic, or a pharmaceutically acceptable salt thereof:



Formula Ic

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -$CH(R^{10})$-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -$CH(R^9)$- where the carbon is bound to the carbonyl carbon of -$N(R^{11})C(O)$-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is hydrogen, optionally substituted amino, optionally substituted C_1-C_4 alkoxy, optionally substituted C_1-C_4 hydroxyalkyl, optionally substituted C_1-C_4 aminoalkyl, optionally substituted C_1-C_4 haloalkyl, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 guanidinoalkyl, C_0-C_4 alkyl optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 3 to 8-membered cycloalkyl, or optionally substituted 3 to 8-membered heteroaryl;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', $C(O)N(R')_2$, S(O)R', $S(O)_2R'$, or $S(O)_2N(R')_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl;

R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

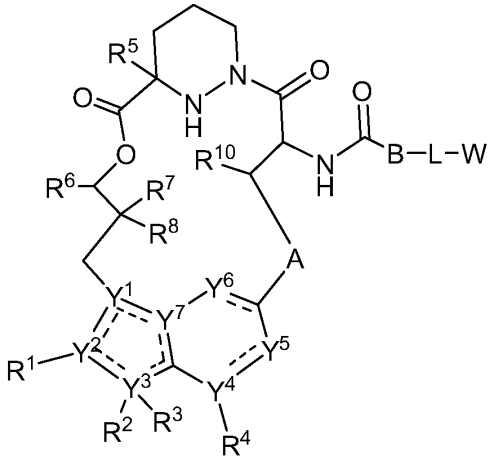
R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl; and

R^{11} is hydrogen or C_1-C_3 alkyl.

In some embodiments of compounds of the present invention, X^2 is NH. In some embodiments, X^3 is CH.

In some embodiments of compounds of the present invention, R^{11} is hydrogen. In some embodiments, R^{11} is C_1-C_3 alkyl. In some embodiments, R^{11} is methyl.

In some embodiments, a compound of the present invention has the structure of Formula Id, or a pharmaceutically acceptable salt thereof:



Formula Id

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is hydrogen, optionally substituted amino, optionally substituted C_1-C_4 alkoxy, optionally substituted C_1-C_4 hydroxyalkyl, optionally substituted C_1-C_4 aminoalkyl, optionally substituted C_1-C_4 haloalkyl, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 guanidinoalkyl, C_0-C_4 alkyl optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 3 to 8-membered cycloalkyl, or optionally substituted 3 to 8-membered heteroaryl;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; and

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl.

In some embodiments of compounds of the present invention, X^1 is optionally substituted C_1-C_2 alkylene. In some embodiments, X^1 is methylene. In some embodiments, X^1 is methylene

substituted with a C_1-C_6 alkyl group or a halogen. In some embodiments, X^1 is -CH(Br)-. In some embodiments, X^1 is -CH(CH_3)-.

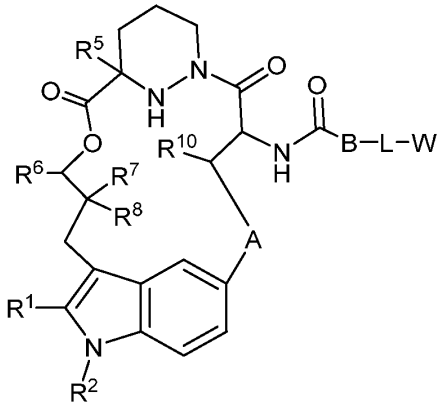
In some embodiments of compounds of the present invention, R^3 is absent.

In some embodiments of compounds of the present invention, R^4 is hydrogen.

In some embodiments of compounds of the present invention, R^5 is hydrogen. In some embodiments, R^5 is C_1-C_4 alkyl optionally substituted with halogen. In some embodiments, R^5 is methyl.

In some embodiments of compounds of the present invention, Y^4 is C. In some embodiments, Y^5 is CH. In some embodiments, Y^6 is CH. In some embodiments, Y^1 is C. In some embodiments, Y^2 is C. In some embodiments, Y^3 is N. In some embodiments, Y^7 is C.

In some embodiments, a compound of the present invention has the structure of Formula Ie, or a pharmaceutically acceptable salt thereof:



Formula Ie

wherein A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is hydrogen, optionally substituted amino, optionally substituted C_1-C_4 alkoxy, optionally substituted C_1-C_4 hydroxyalkyl, optionally substituted C_1-C_4 aminoalkyl, optionally substituted C_1-C_4 haloalkyl, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 guanidinoalkyl, C_0-C_4 alkyl optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 3 to 8-membered cycloalkyl, or optionally substituted 3 to 8-membered heteroaryl;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered

cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; and

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl.

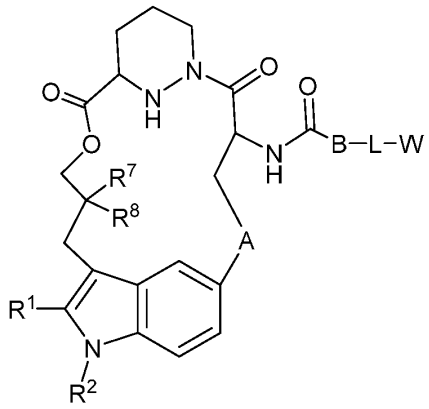
In some embodiments of compounds of the present invention, R^6 is hydrogen.

In some embodiments of compounds of the present invention, R^2 is hydrogen, cyano, optionally substituted C_1-C_6 alkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 6-membered heterocycloalkyl. In some embodiments, R^2 is optionally substituted C_1-C_6 alkyl, such as ethyl. In some embodiments, R^2 is fluoro C_1-C_6 alkyl, such as -CH_2CH_2F, -CH_2CHF_2, or -CH_2CF_3.

In some embodiments of compounds of the present invention, R^7 is optionally substituted C_1-C_3 alkyl. In some embodiments, R^7 is C_1-C_3 alkyl.

In some embodiments of compounds of the present invention, R^8 is optionally substituted C_1-C_3 alkyl. In some embodiments, R^8 is C_1-C_3 alkyl, such as methyl.

In some embodiments, a compound of the present invention has the structure of Formula If, or a pharmaceutically acceptable salt thereof:



Formula If

wherein A optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is hydrogen, optionally substituted amino, optionally substituted C_1-C_4 alkoxy, optionally substituted C_1-C_4 hydroxyalkyl, optionally substituted C_1-C_4 aminoalkyl, optionally substituted C_1-C_4 haloalkyl, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 guanidinoalkyl, C_0-C_4 alkyl optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 3 to 8-membered cycloalkyl, or optionally substituted 3 to 8-membered heteroaryl;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

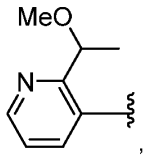
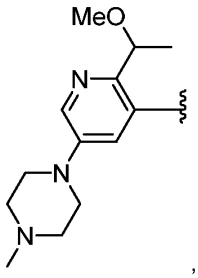
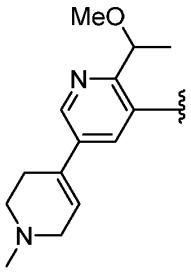
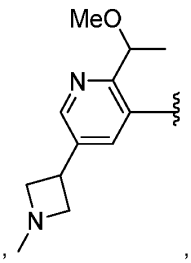
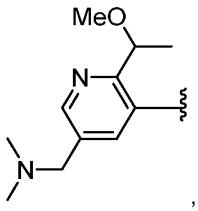
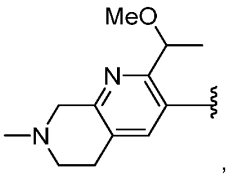
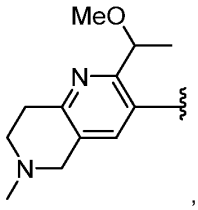
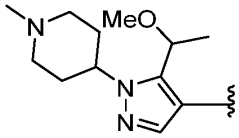
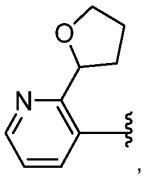
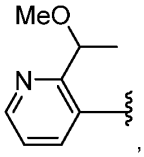
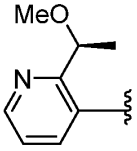
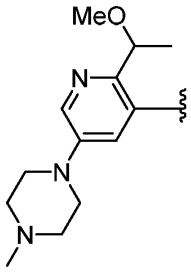
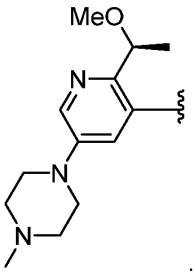
R^2 is C_1-C_6 alkyl or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

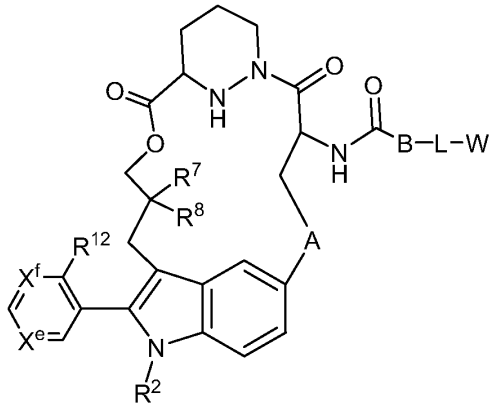
R^8 is C_1-C_3 alkyl; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

In some embodiments of compounds of the present invention, R^1 is 5 to 10-membered heteroaryl. In some embodiments, R^1 is optionally substituted 6-membered aryl or optionally substituted 6-membered heteroaryl.

In some embodiments of compounds of the present invention, R_1 is  ,  ,  ,  ,  ,  ,  ,  , or  , or a stereoisomer thereof. In some embodiments, R_1 is  , or a stereoisomer thereof. In some embodiments, R_1 is  . In some embodiments, R_1 is  , or a stereoisomer thereof. In some embodiments, R_1 is  .

In some embodiments, a compound of the present invention has the structure of Formula Ig, or a pharmaceutically acceptable salt thereof:



Formula Ig

wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is hydrogen, optionally substituted amino, optionally substituted C_1-C_4 alkoxy, optionally substituted C_1-C_4 hydroxyalkyl, optionally substituted C_1-C_4 aminoalkyl, optionally substituted C_1-C_4 haloalkyl, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 guanidinoalkyl, C_0-C_4 alkyl optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 3 to 8-membered cycloalkyl, or optionally substituted 3 to 8-membered heteroaryl;

R^2 is C_1-C_6 alkyl or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

R^8 is C_1-C_3 alkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

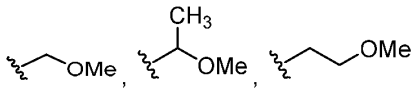
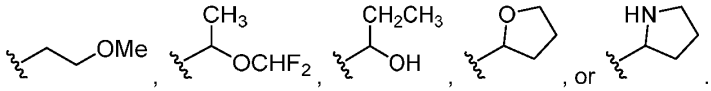
X^e is N, CH, or CR^{17};

X^f is N or CH;

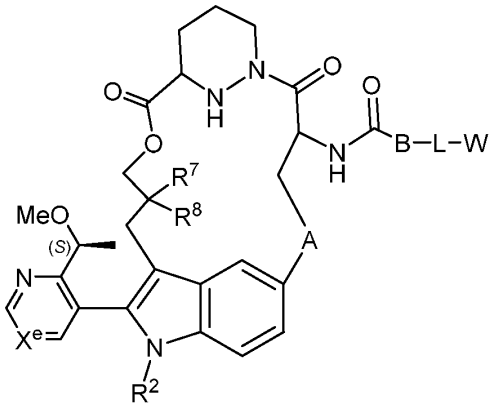
R^{12} is optionally substituted C_1-C_6 alkyl or optionally substituted C_1-C_6 heteroalkyl; and

R^{17} is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

In some embodiments of compounds of the present invention, X^e is N and X^f is CH. In some embodiments, X^e is CH and X^f is N. In some embodiments, X^e is CR^{17} and X^f is N.

In some embodiments of compounds of the present invention, R^{12} is optionally substituted C_1-C_6 heteroalkyl. In some embodiments, R^{12} is  ,  , , , , , or .

In some embodiments, a compound of the present invention has the structure of Formula Ih, or a pharmaceutically acceptable salt thereof:



Formula Ih

wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is hydrogen, optionally substituted amino, optionally substituted C_1-C_4 alkoxy, optionally substituted C_1-C_4 hydroxyalkyl, optionally substituted C_1-C_4 aminoalkyl, optionally substituted C_1-C_4 haloalkyl, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 guanidinoalkyl, C_0-C_4 alkyl optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 3 to 8-membered cycloalkyl, or optionally substituted 3 to 8-membered heteroaryl;

R^2 is C_1-C_6 alkyl or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

R^8 is C_1-C_3 alkyl;

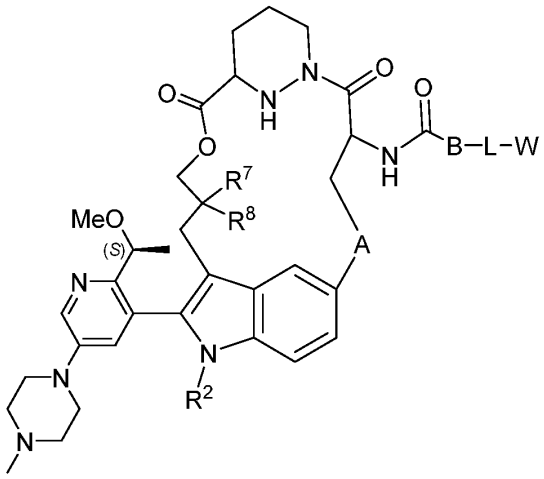
R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

X^e is CH, or CR^{17}; and

R^{17} is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl,

optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

In some embodiments, a compound of the present invention has the structure of Formula Ii, or a pharmaceutically acceptable salt thereof:



Formula Ii

wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is hydrogen, optionally substituted amino, optionally substituted C_1-C_4 alkoxy, optionally substituted C_1-C_4 hydroxyalkyl, optionally substituted C_1-C_4 aminoalkyl, optionally substituted C_1-C_4 haloalkyl, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 guanidinoalkyl, C_0-C_4 alkyl optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 3 to 8-membered cycloalkyl, or optionally substituted 3 to 8-membered heteroaryl;

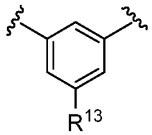
R^2 is C_1-C_6 alkyl or 3 to 6-membered cycloalkyl;

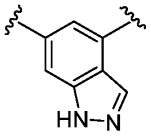
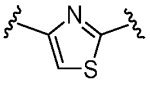
R^7 is C_1-C_3 alkyl;

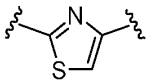
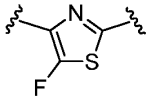
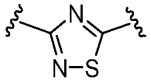
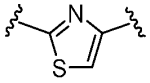
R^8 is C_1-C_3 alkyl; and

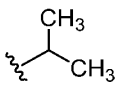
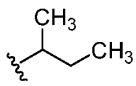
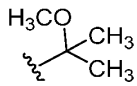
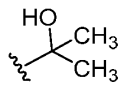
R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

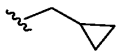
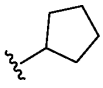
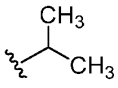
In some embodiments of compounds of the present invention, A is optionally substituted 6-membered arylene. In some embodiments, A has the structure:



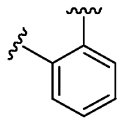
wherein R^{13} is hydrogen, hydroxy, amino, cyano, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl. In some embodiments, R^{13} is hydrogen. In some embodiments, R^{13} is hydroxy. In some embodiments, A is an optionally substituted 5 to 10-membered heteroarylene. In some embodiments, A is:  . In some embodiments, A is optionally substituted 5 to 6-membered heteroarylene. In some embodiments, A is: 

 ,  , or  . In some embodiments, A is  .

In some embodiments of compounds of the present invention, B is -CHR^9-. In some embodiments, R^9 is optionally substituted C_1-C_6 alkyl or optionally substituted 3 to 6-membered cycloalkyl. In some embodiments, R^9 is:  ,  ,  ,  ,

 or  . In some embodiments, R^9 is:  . In some embodiments, R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

In some embodiments, B is optionally substituted 6-membered arylene.

In some embodiments, B is 6-membered arylene. In some embodiments, B is:  . In some embodiments B is absent.

In some embodiments of compounds of the present invention, R^7 is methyl.

In some embodiments of compounds of the present invention, R^8 is methyl.

In some embodiments of compounds of the present invention, R^{16} is hydrogen.

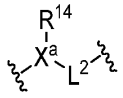
In some embodiments of compounds of the present invention, the linker is the structure of Formula II:

$$A^1\text{-}(B^1)_f\text{-}(C^1)_g\text{-}(B^2)_h\text{-}(D^1)\text{-}(B^3)_i\text{-}(C^2)_j\text{-}(B^4)_k\text{–}A^2$$

Formula II

where A^1 is a bond between the linker and B; A^2 is a bond between W and the linker; B^1, B^2, B^3, and B^4 each, independently, is selected from optionally substituted C_1-C_2 alkylene, optionally substituted C_1-C_3 heteroalkylene, O, S, and NR^N; R^N is hydrogen, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_3 cycloalkyl, optionally substituted $C_{2\text{-}C4}$ alkenyl, optionally substituted C_2-C_4 alkynyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted C_1-C_7 heteroalkyl; C^1 and C^2 are each, independently, selected from carbonyl, thiocarbonyl, sulphonyl, or phosphoryl; f, g, h, i, j, and k are each,

independently, 0 or 1; and D^1 is optionally substituted C_1-C_{10} alkylene, optionally substituted C_2-C_{10} alkenylene, optionally substituted C_2-C_{10} alkynylene, optionally substituted 3 to 14-membered heterocycloalkylene, optionally substituted 5 to 10-membered heteroarylene, optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 6 to 10-membered arylene, optionally substituted C_2-C_{10} polyethylene glycolene, or optionally substituted C_1-C_{10} heteroalkylene, or a chemical bond linking A^1-$(B^1)_f$-$(C^1)_g$-$(B^2)_h$- to -$(B^3)_i$-$(C^2)_j$-$(B^4)_k$–A^2. In some embodiments, the linker is acyclic. In some embodiments, the linker has the structure of Formula IIa:

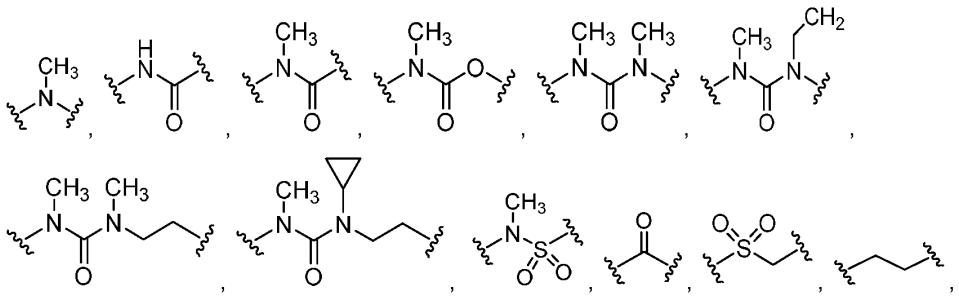


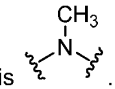
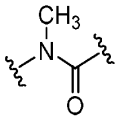
Formula IIa

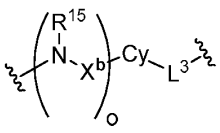
wherein X^a is absent or N;

R^{14} is absent, hydrogen or optionally substituted C_1-C_6 alkyl or optionally substituted C_1-C_3 cycloalkyl; and

L^2 is absent, -C(O)-, -SO_2-, optionally substituted C_1-C_4 alkylene or optionally substituted C_1-C_4 heteroalkylene, wherein at least one of X^a, R^{14}, or L^2 is present. In some embodiments, the linker has the structure:



. In some embodiments, L is  . In some embodiments, L is  . In some embodiments, linker is or comprises a cyclic group. In some embodiments, linker has the structure of Formula IIb:



Formula IIb

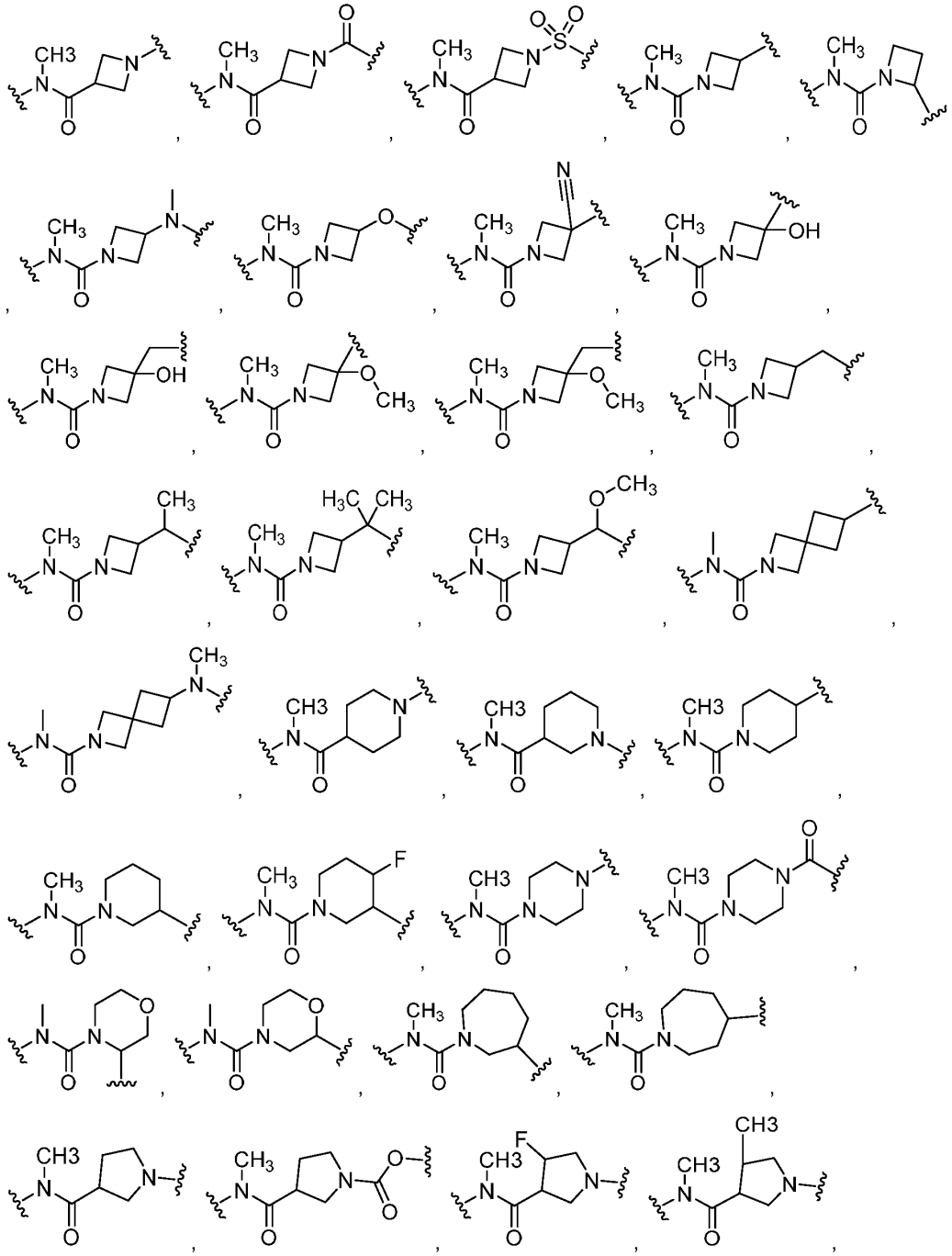
wherein o is 0 or 1;

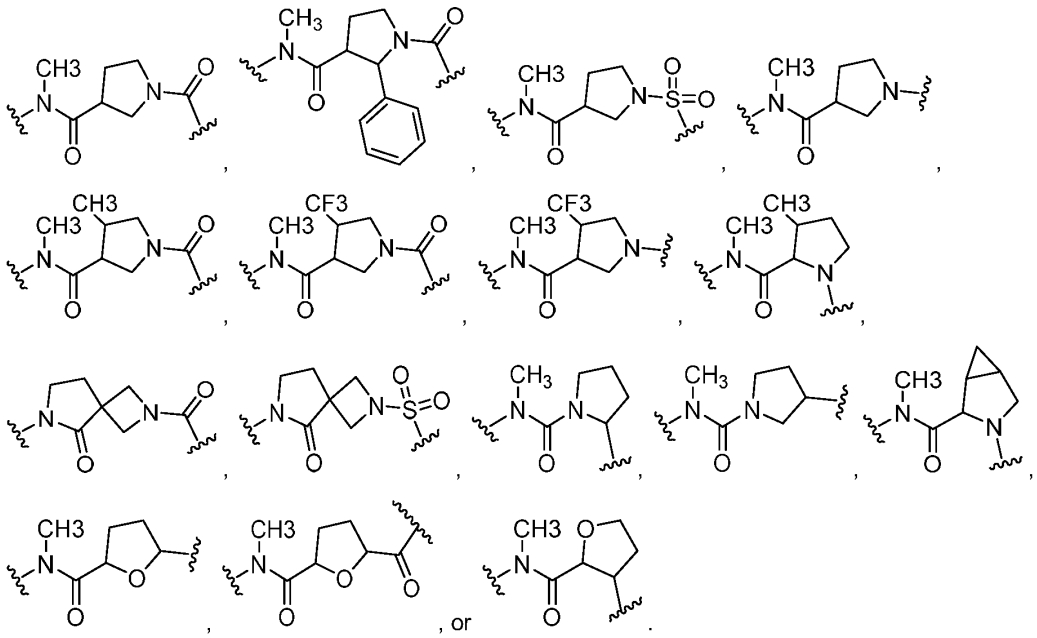
X^b is C(O) or SO_2;

R^{15} is hydrogen or optionally substituted C_1-C_6 alkyl;

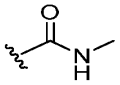
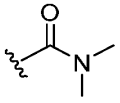
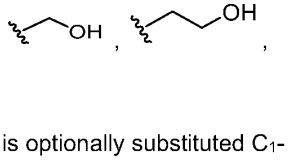
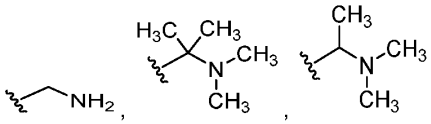
Cy is optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 3 to 8-membered heterocycloalkylene, optionally substituted 6-10 membered arylene, or optionally substituted 5 to 10-membered heteroarylene; and

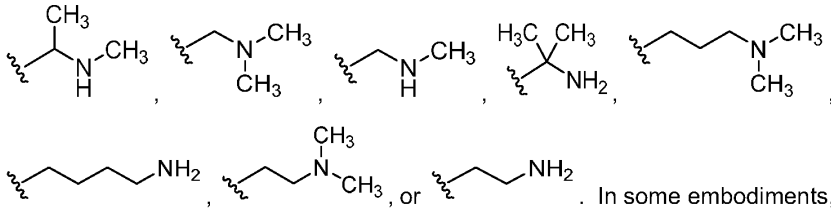
L^3 is absent, -C(O)-, -SO_2-, optionally substituted C_1-C_4 alkylene or optionally substituted C_1-C_4 heteroalkylene. In some embodiments, linker has the structure:

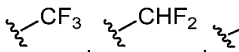
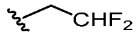
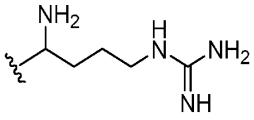
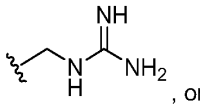
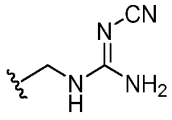
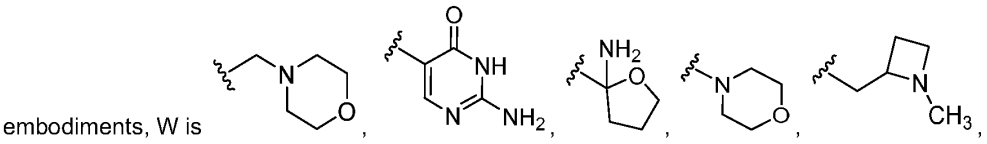
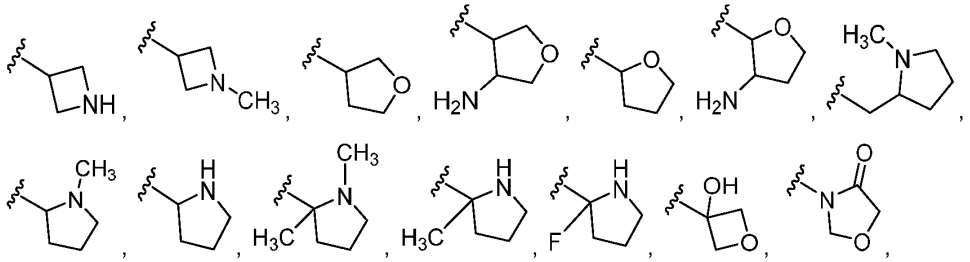
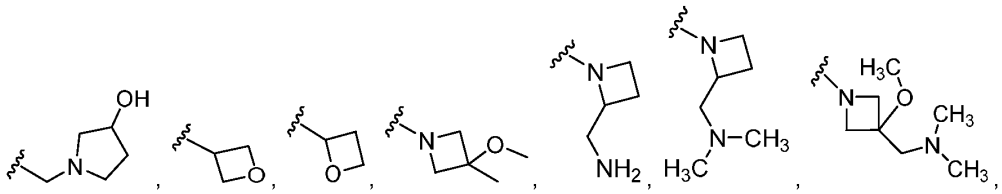
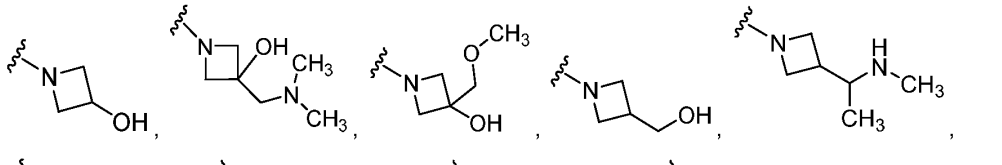
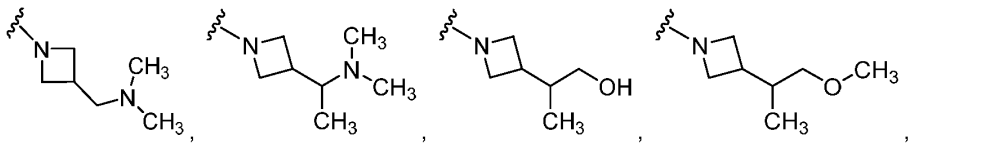
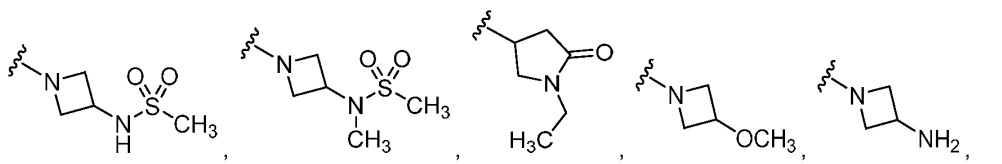


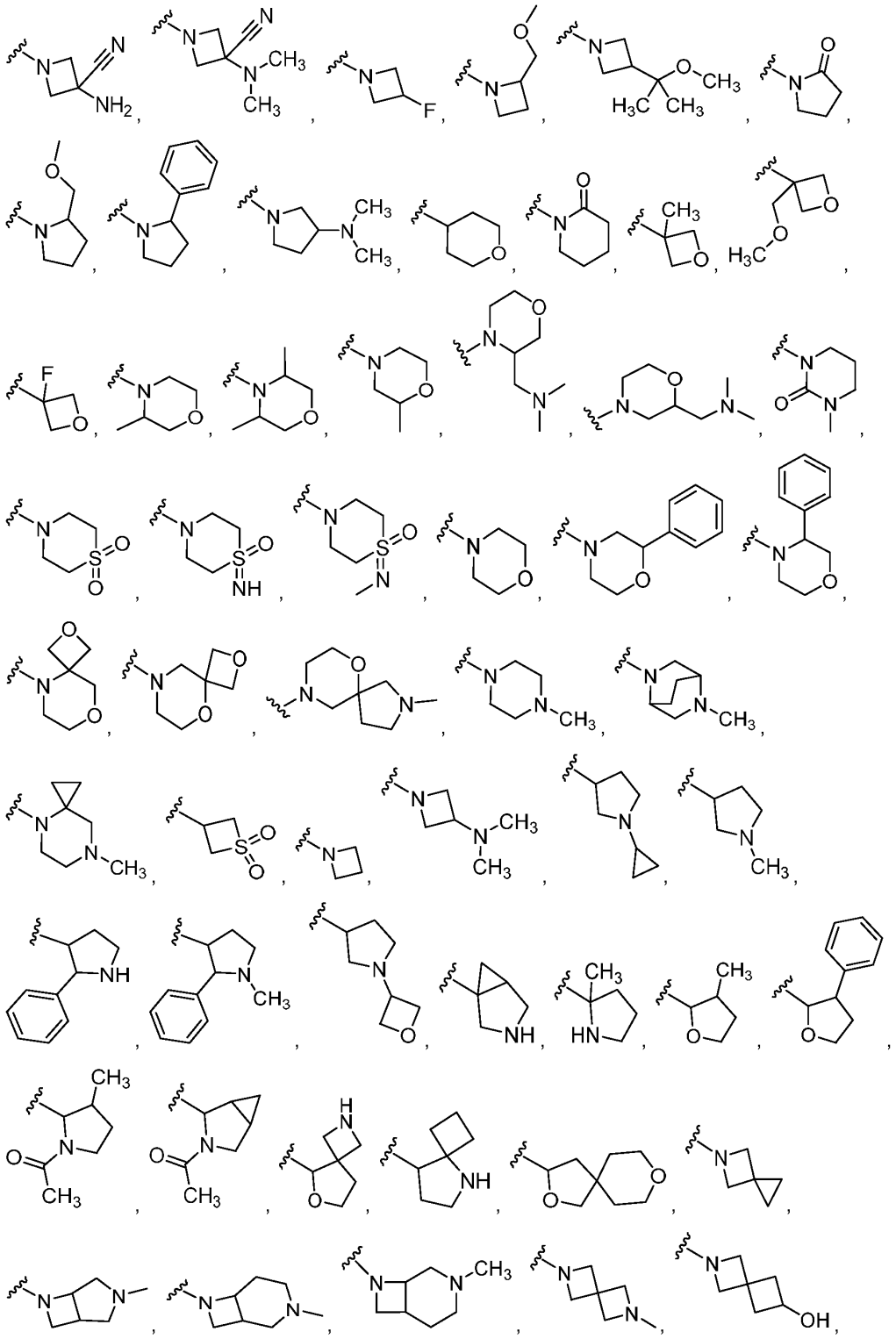


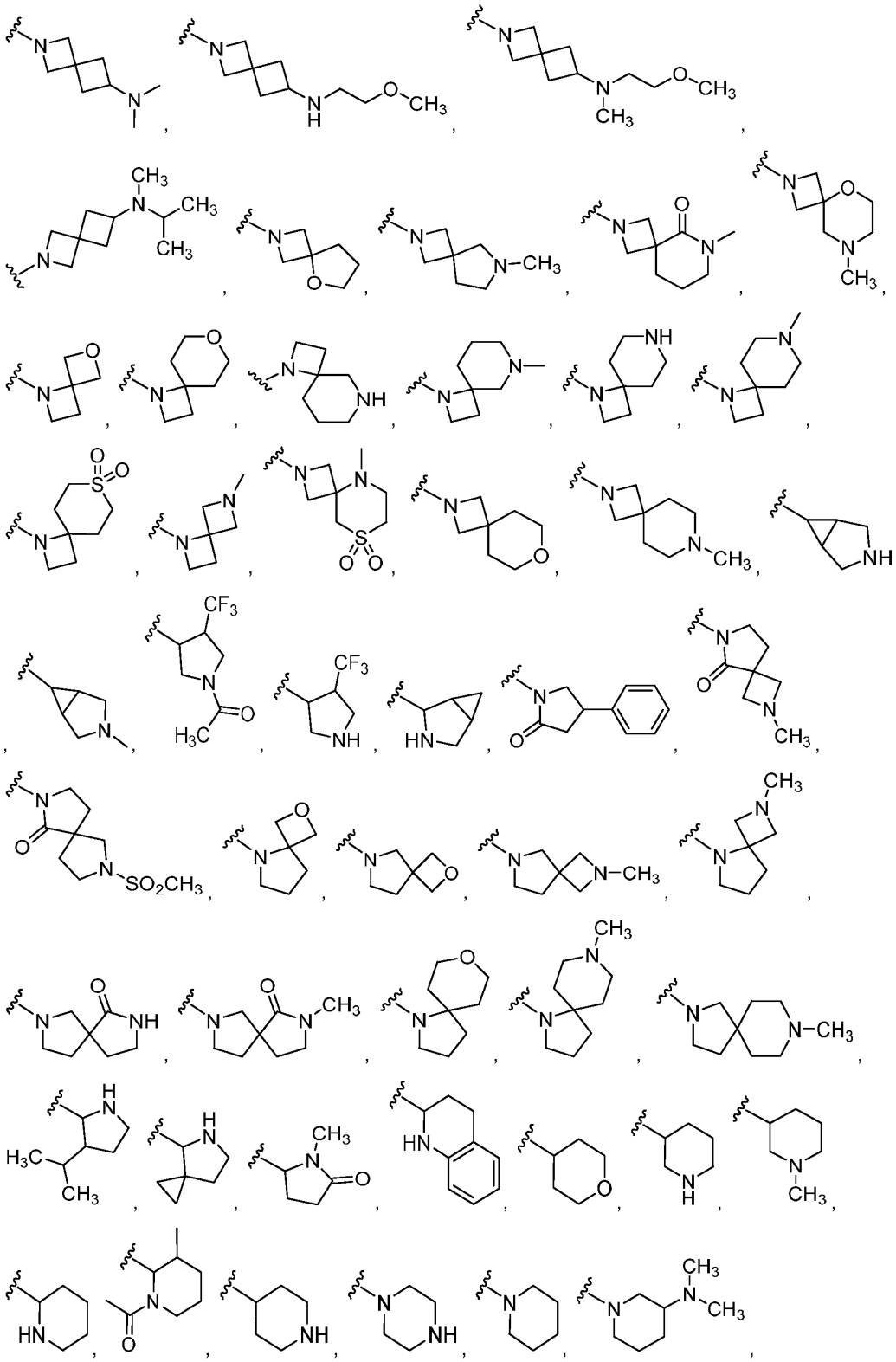
In some embodiments of compounds of the present invention, W is hydrogen, optionally substituted amino, optionally substituted C_1-C_4 alkoxy, optionally substituted C_1-C_4 hydroxyalkyl, optionally substituted C_1-C_4 aminoalkyl, optionally substituted C_1-C_4 haloalkyl, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 guanidinoalkyl, C_0-C_4 alkyl optionally substituted 3 to 8-membered heterocycloalkyl, optionally substituted 3 to 8-membered cycloalkyl, or 3 to 8-membered heteroaryl.

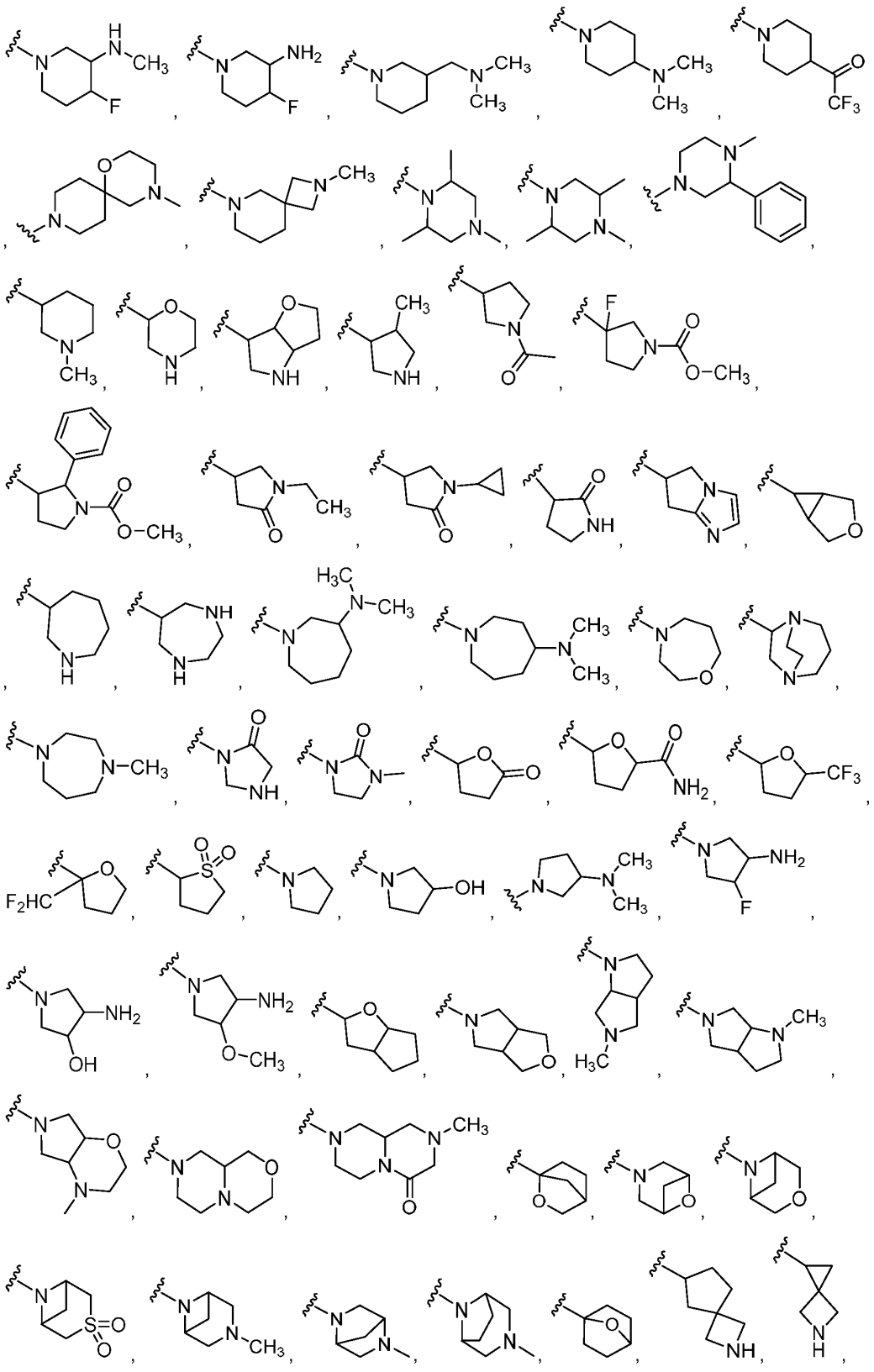
In some embodiments of compounds of the present invention, W is hydrogen. In some embodiments, W is optionally substituted amino. In some embodiments, W is $-NHCH_3$ or $-N(CH_3)_2$. In some embodiments, W is optionally substituted C_1-C_4 alkoxy. In some embodiments, W is methoxy or iso-propoxy. In some embodiments, W is optionally substituted C_1-C_4 alkyl. In some embodiments, W is methyl, ethyl, iso-propyl, tert-butyl, or benzyl. In some embodiments, W is optionally substituted amido. In some embodiments, W is  . In some embodiments, W is optionally substituted amido. In some embodiments, W is  . In some embodiments, W is optionally substituted C_1-C_4 hydroxyalkyl. In some embodiments, W is  . In some embodiments, W is optionally substituted C_1-C_4 aminoalkyl. In some embodiments, W is  .

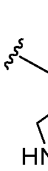
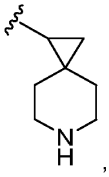
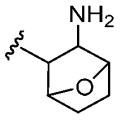
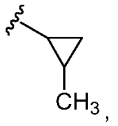
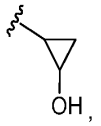
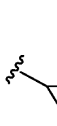


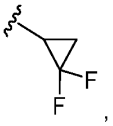
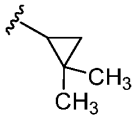
. In some embodiments, W is optionally substituted C_1-C_4 haloalkyl. In some embodiments, W is 

. In some embodiments, W is optionally substituted C_1-C_4 guanidinoalkyl. In some embodiments, W is 


. In some embodiments, W is C_0-C_4 alkyl optionally substituted 3 to 11-membered heterocycloalkyl. In some embodiments, W is 






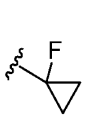
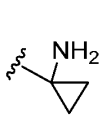
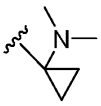
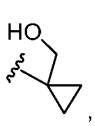
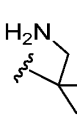
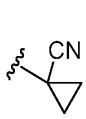
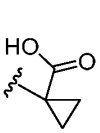
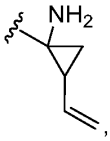
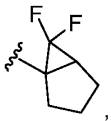
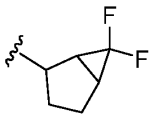
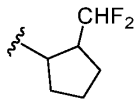
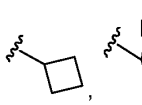


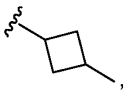
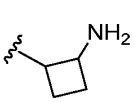
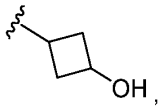
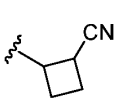
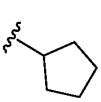
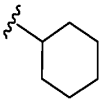
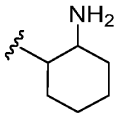
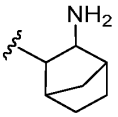


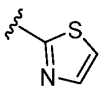
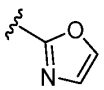
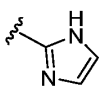
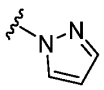
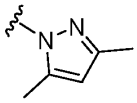
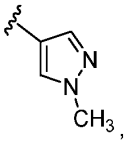
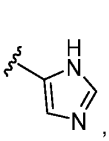
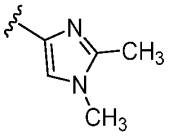
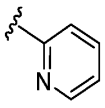
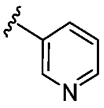
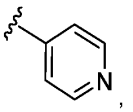
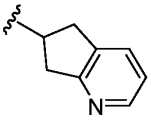
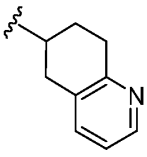





. In some embodiments, W is optionally substituted 3 to 8-membered cycloalkyl. In some embodiments, W is




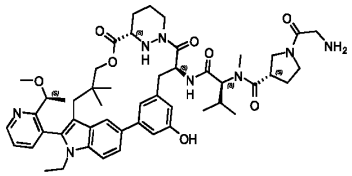
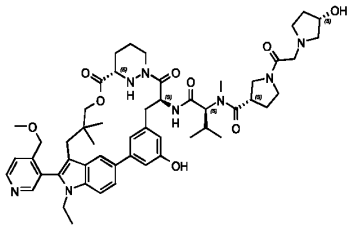
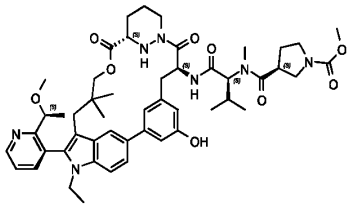
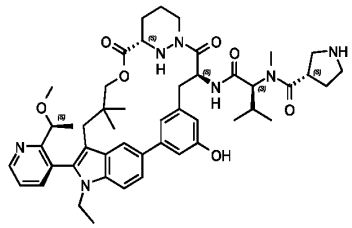
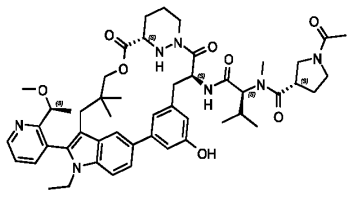








, or

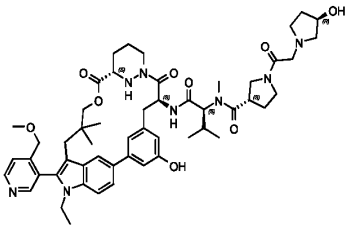
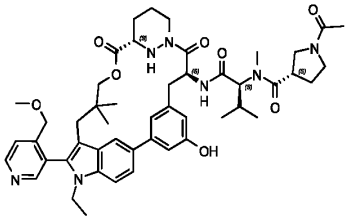
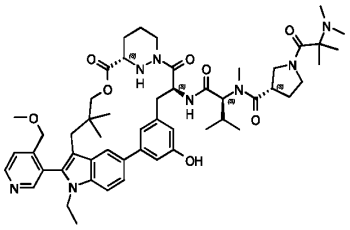
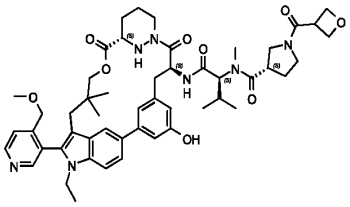
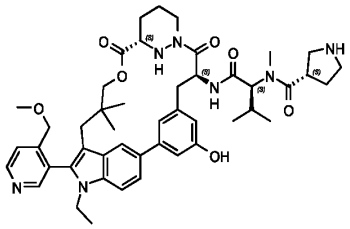
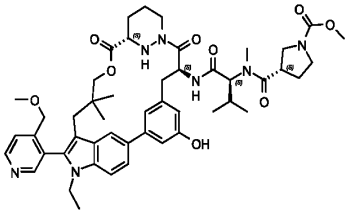
. In some embodiments, W is optionally substituted 3 to 8-membered heteroaryl. In some embodiments, W is













, or

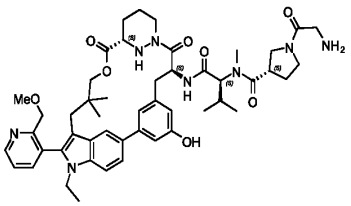
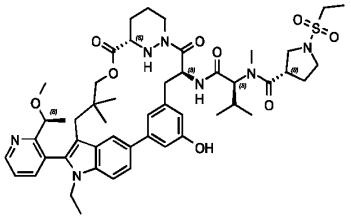
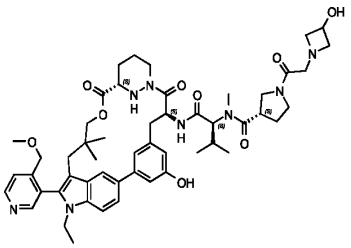
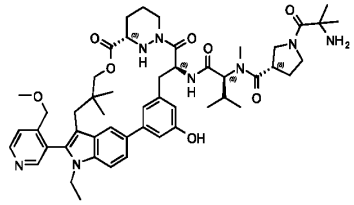
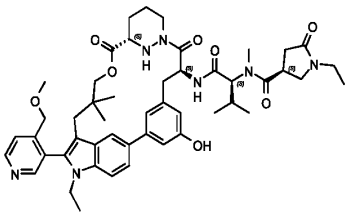
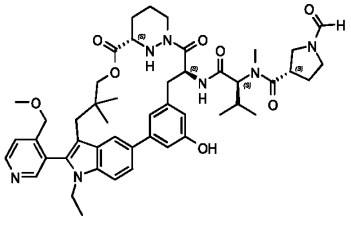
. In some embodiments, W is optionally substituted 6- to 10-membered aryl (e.g., phenyl, 4-hydroxy-phenyl, or 2,4-methoxy-phenyl).

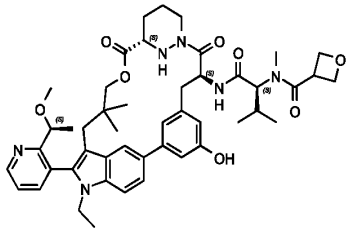
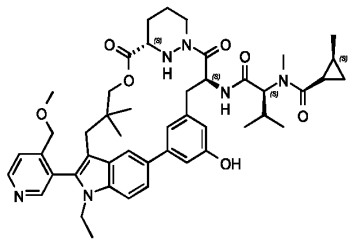
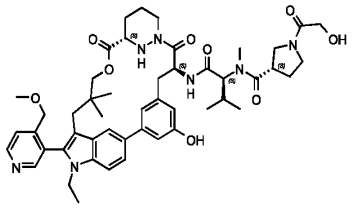
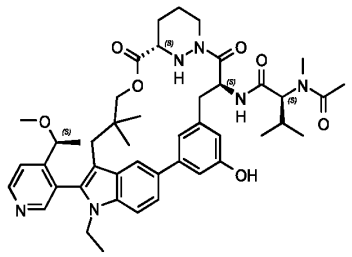
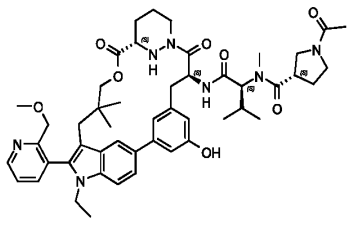
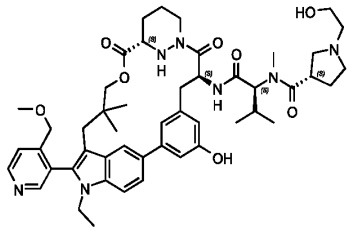
In some embodiments, a compound of the present invention is selected from Table 1, or a pharmaceutically acceptable salt or stereoisomer thereof. In some embodiments, a compound of the present invention is selected from Table 1, or a pharmaceutically acceptable salt or atropisomer thereof.

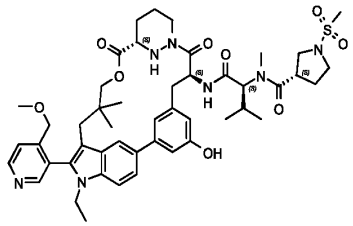
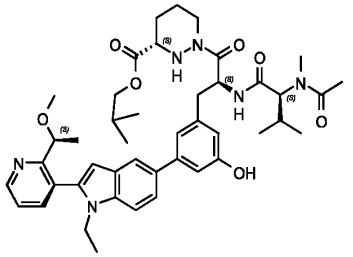
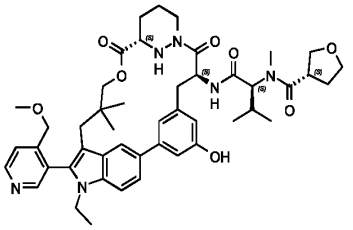
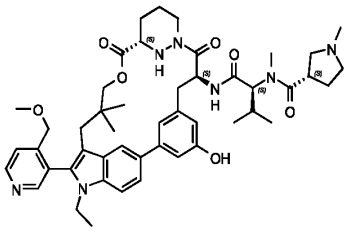
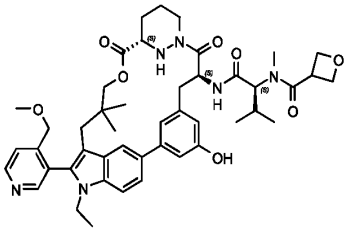
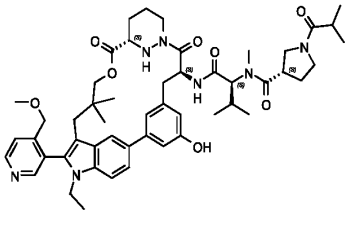
Table 1: Certain Compounds of the Present Invention

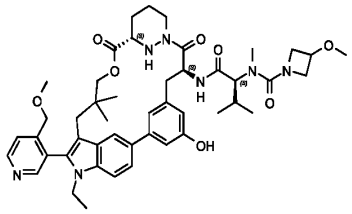
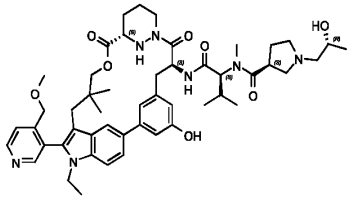
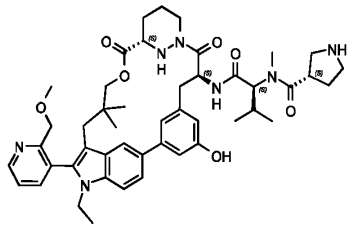
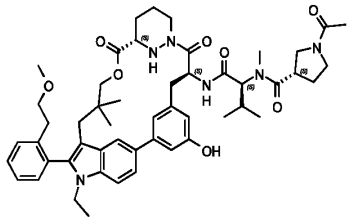
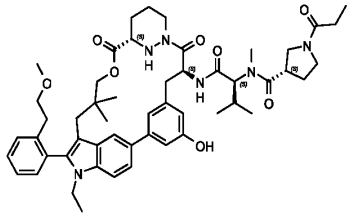
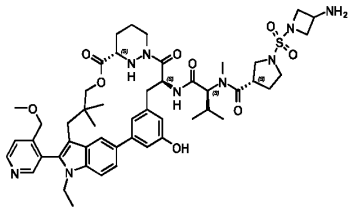
Ex#	Structure
A1	
A2	
A3	
A4	
A5	

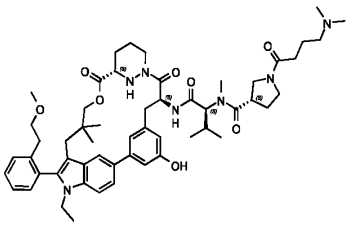
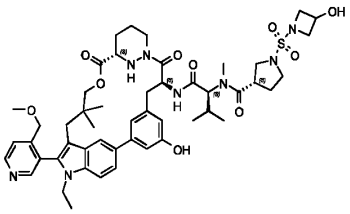
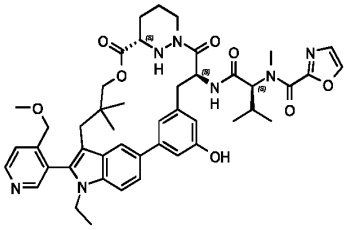
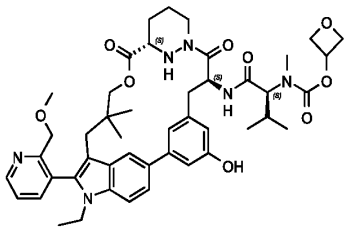
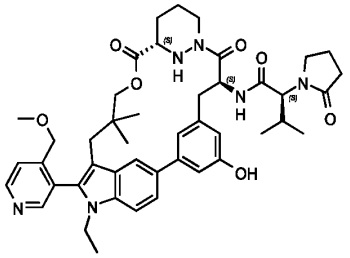
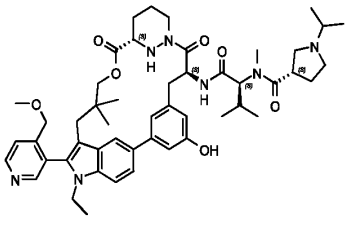
A6	
A7	
A8	
A9	
A10	
A11	

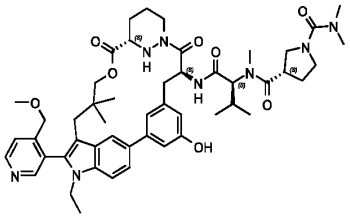
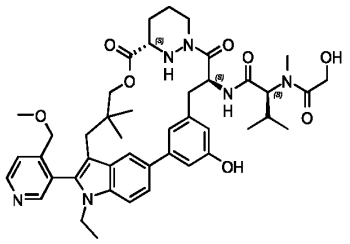
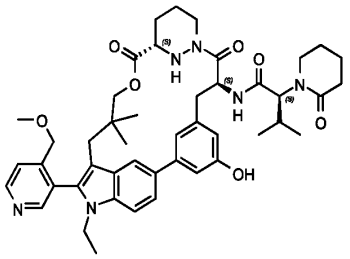
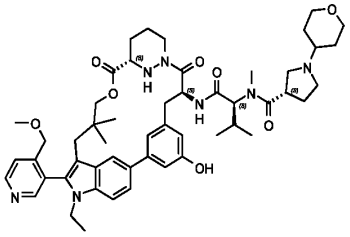
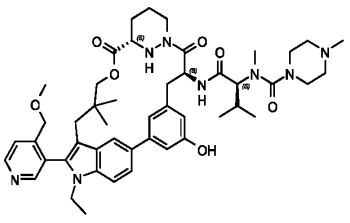
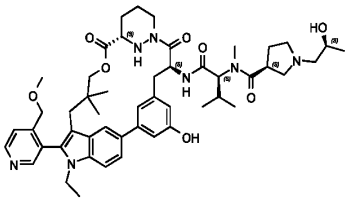
A12	
A13	
A14	
A15	
A16	
A17	

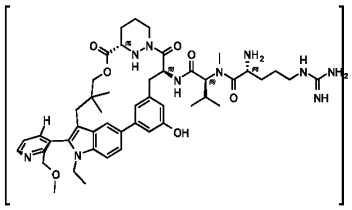
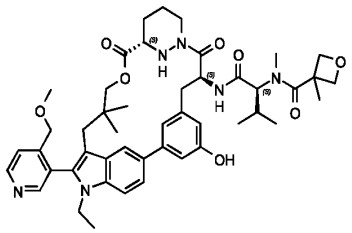
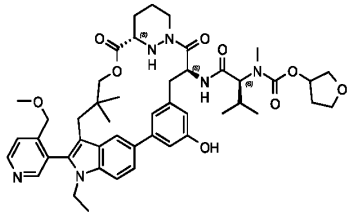
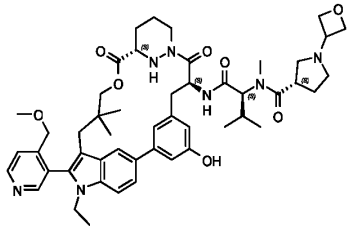
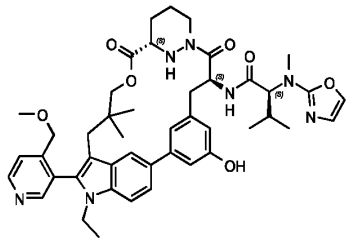
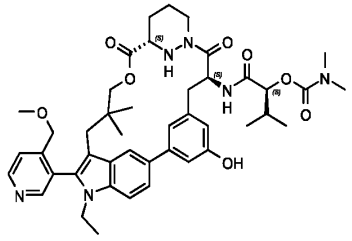
A18	
A19	
A20	
A21	
A22	
A23	

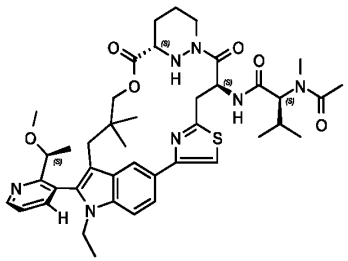
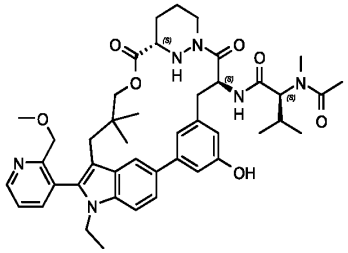
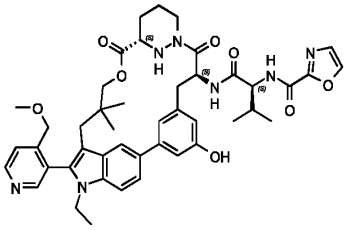
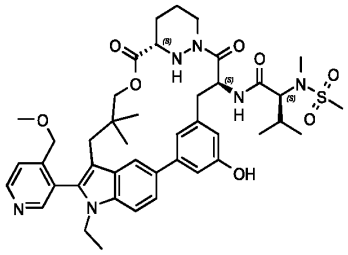
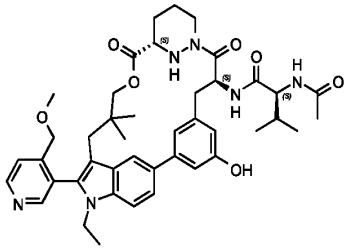
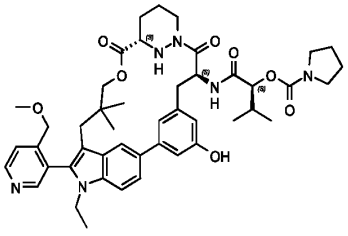
A24	
A25	
A26	
A27	
A28	
A29	

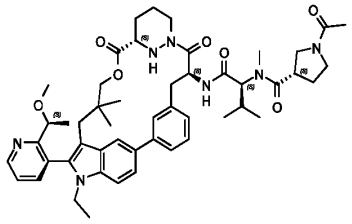
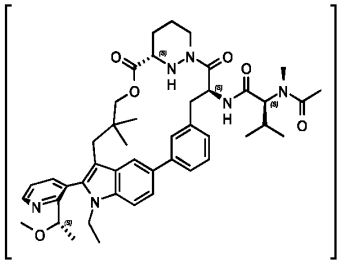
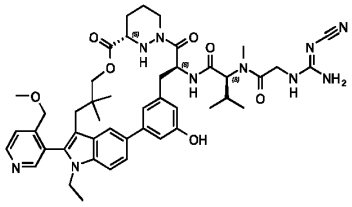
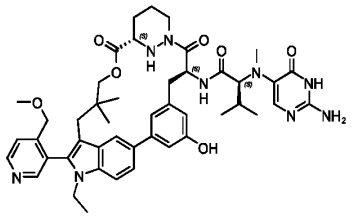
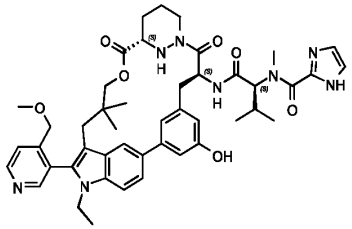
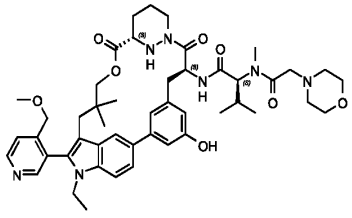
A30	
A31	
A32	
A33	
A34	
A35	

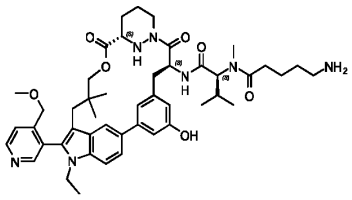
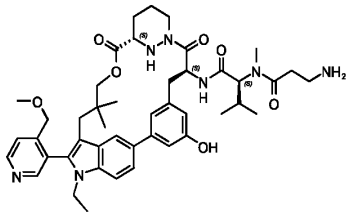
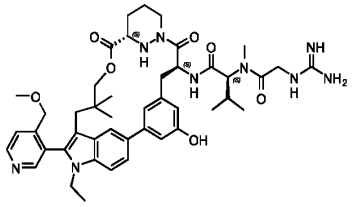
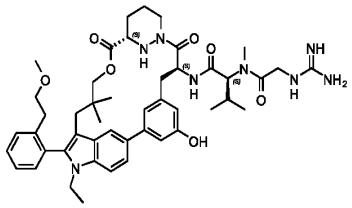
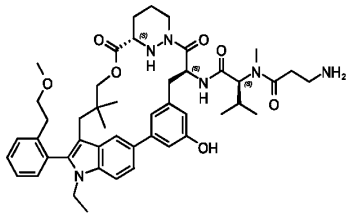
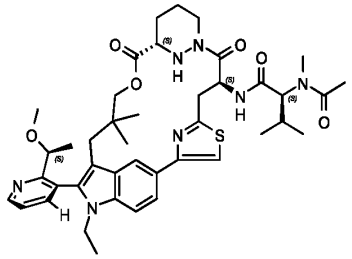
A36	
A37	
A38	
A39	
A40	
A41	

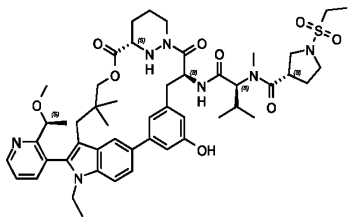
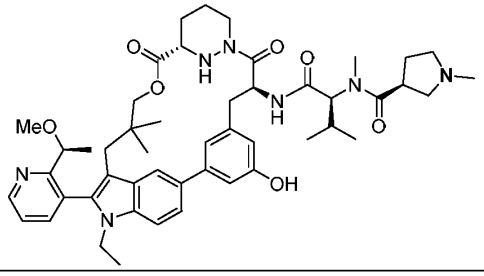
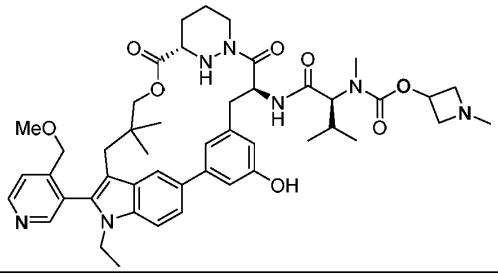
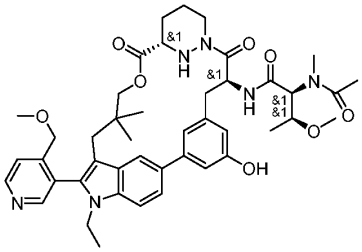
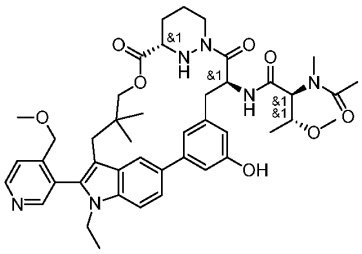
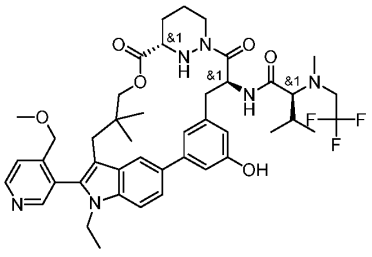
A42	
A43	
A44	
A45	
A46	
A47	

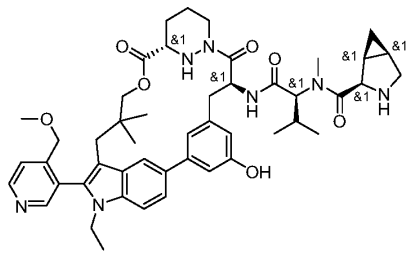
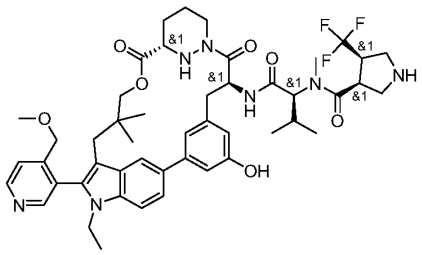
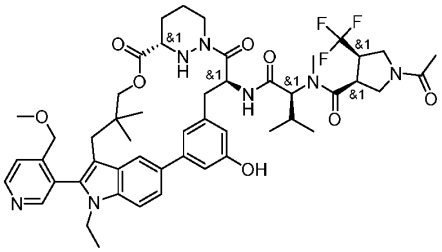
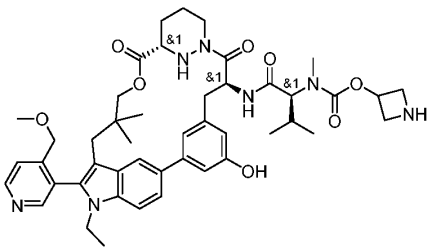
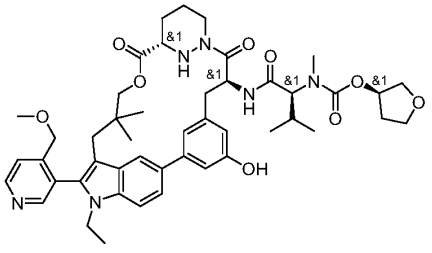
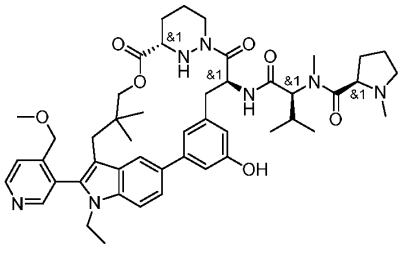
A48	
A49	
A50	
A51	
A52	
A53	

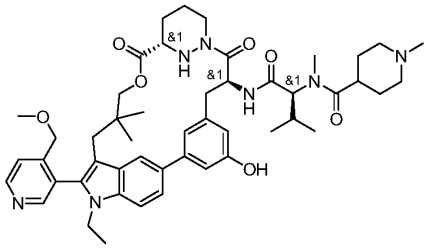
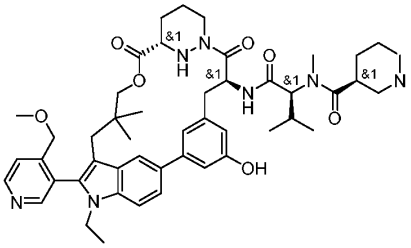
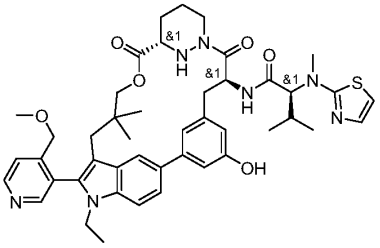
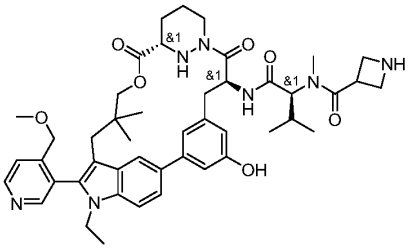
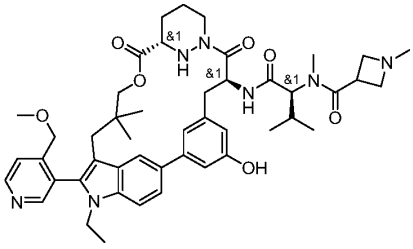
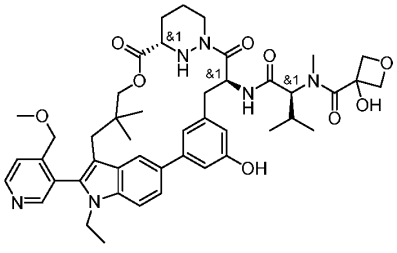
A54	
A55	
A56	
A57	
A58	
A59	

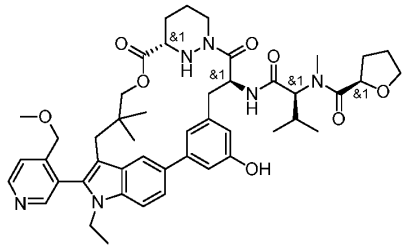
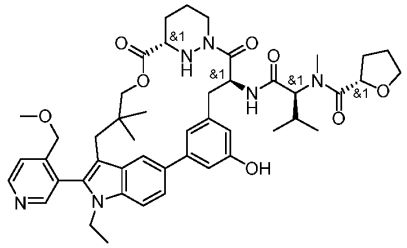
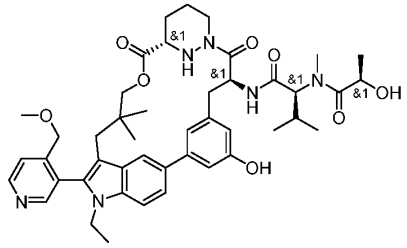
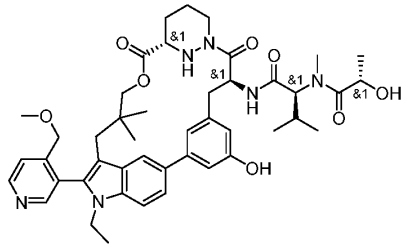
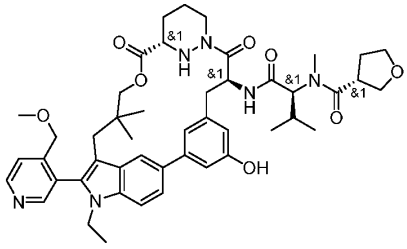
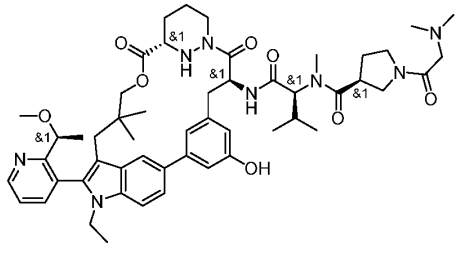
A60	
A61	
A62	
A63	
A64	
A65	

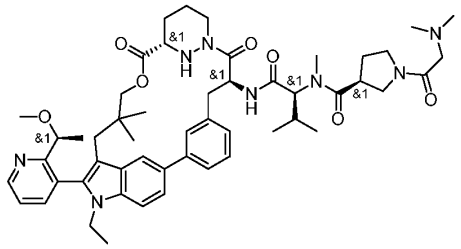
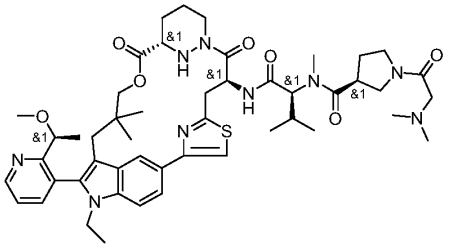
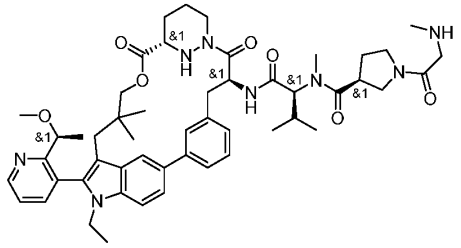
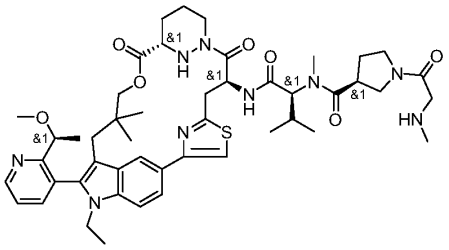
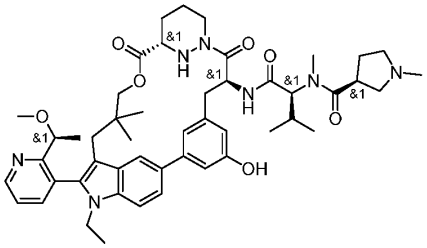
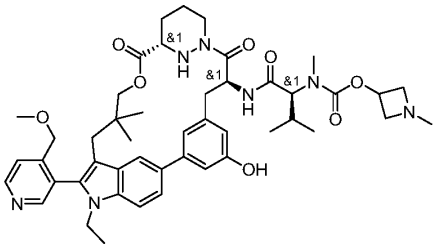
A66	
A67	
A68	
A69	
A70	
A71	

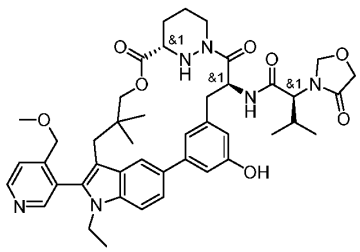
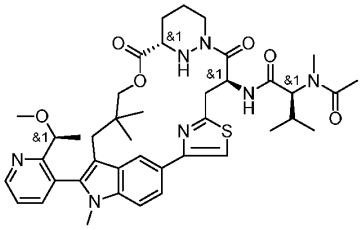
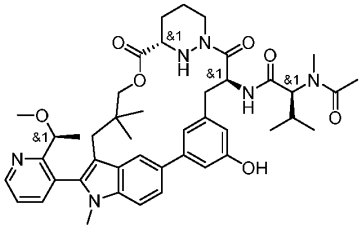
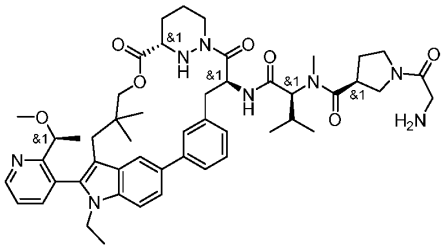
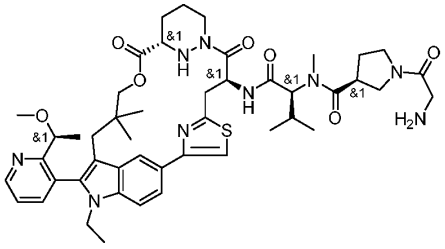
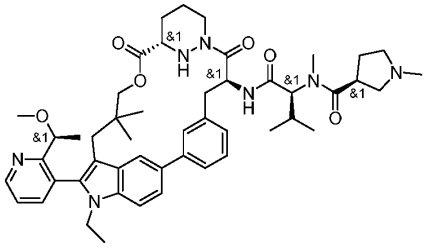
A72	
A73	
A74	
A75	
A76	
A77	

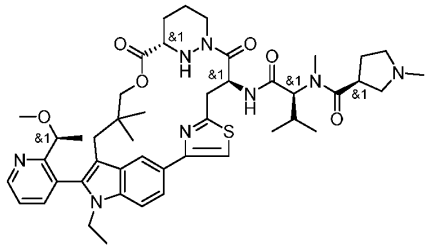
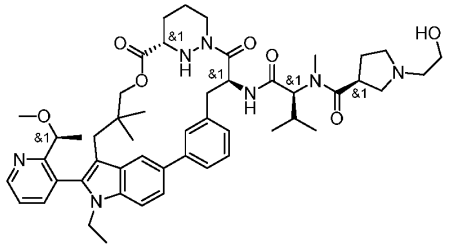
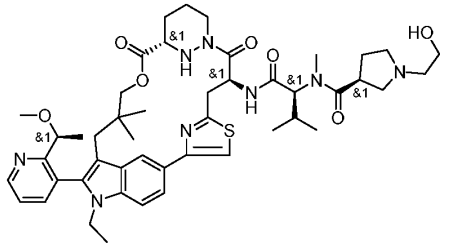
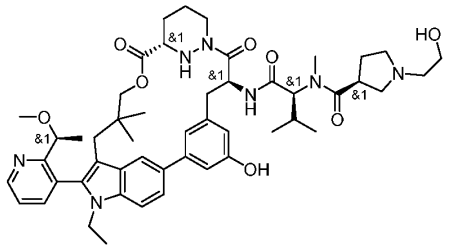
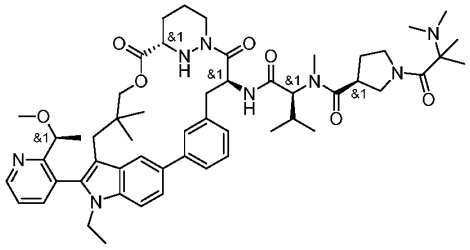
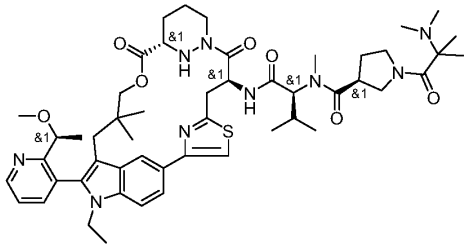
A78	
A79	
A80	
A81	
A82	
A83	

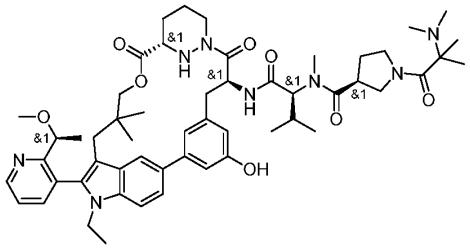
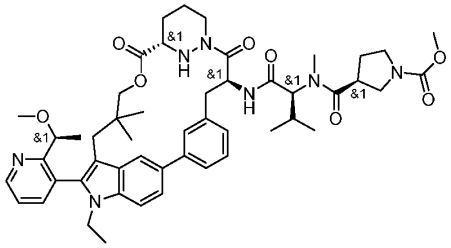
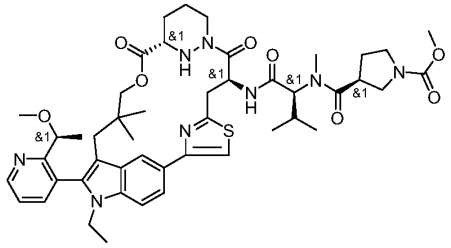
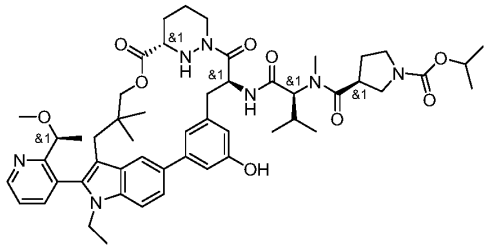
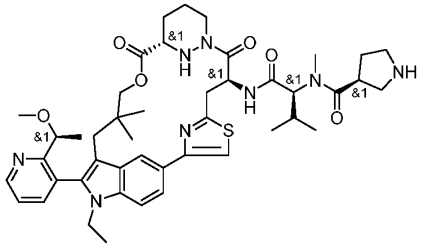
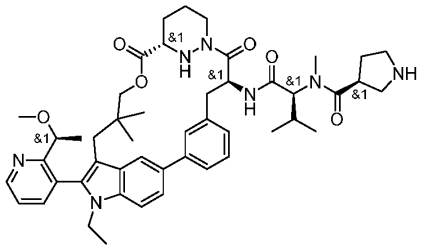
A84	
A85	
A86	
A87	
A88	
A89	

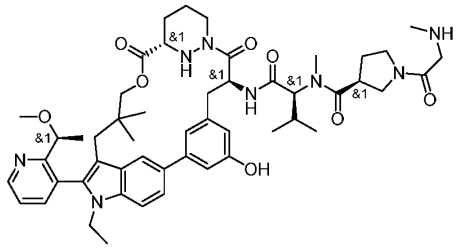
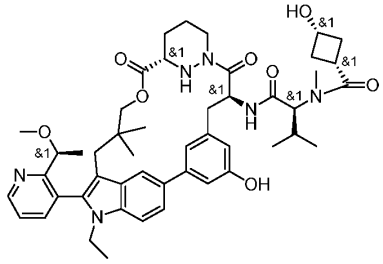
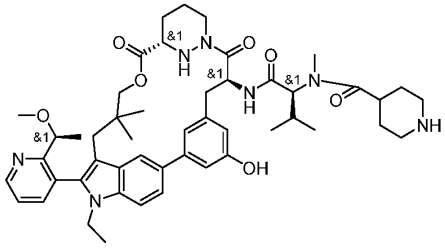
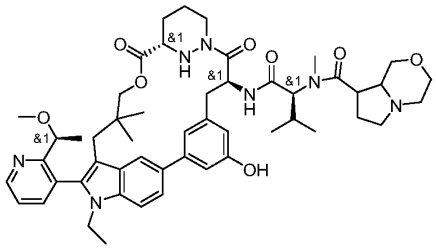
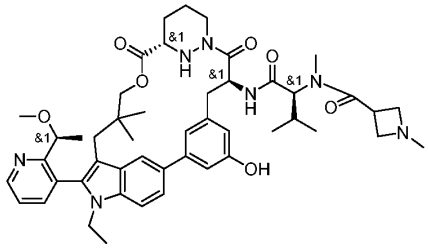
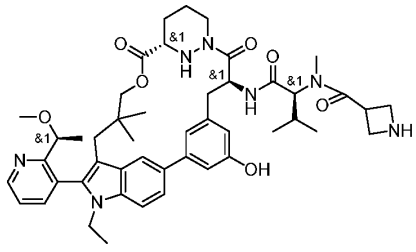
A90	
A91	
A92	
A93	
A94	
A95	

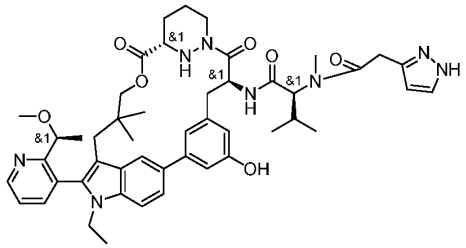
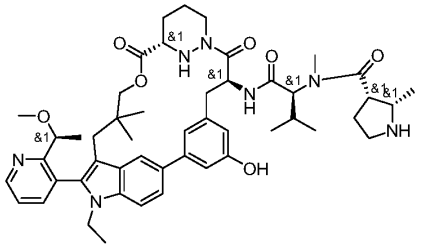
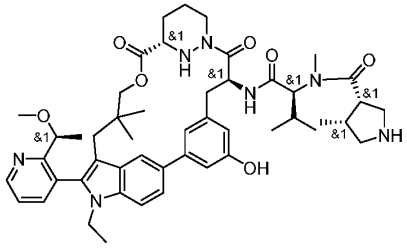
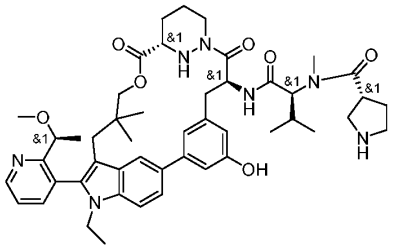
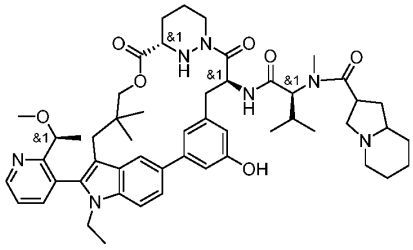
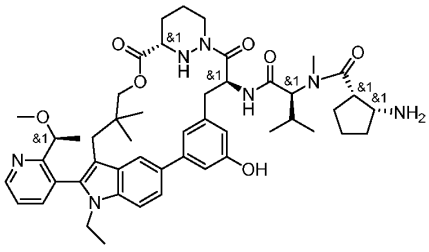
A96	
A97	
A98	
A99	
A100	
A101	

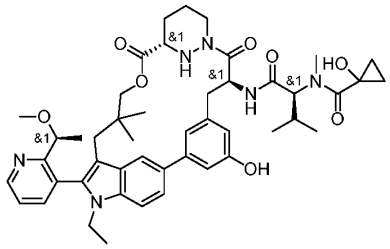
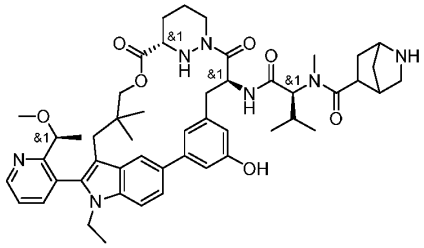
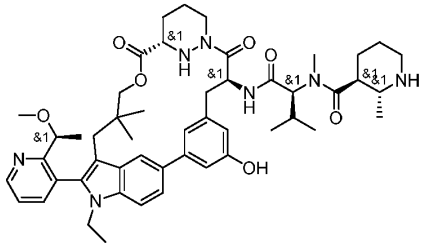
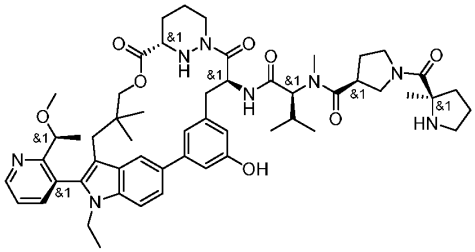
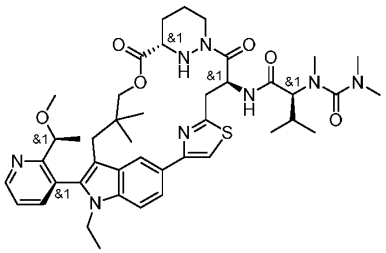
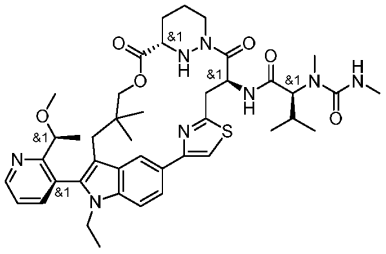
A102	
A103	
A104	
A105	
A106	
A107	

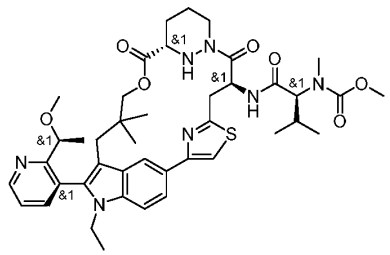
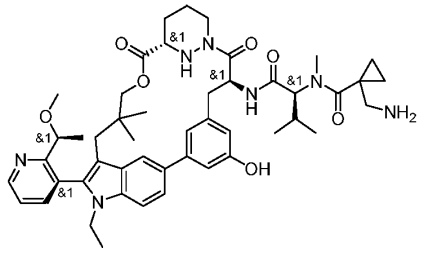
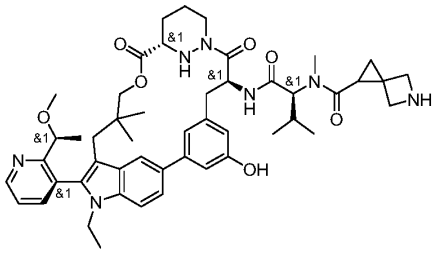
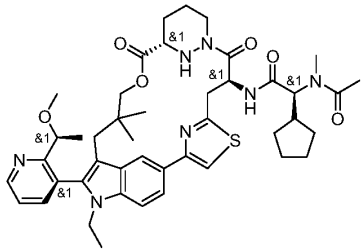
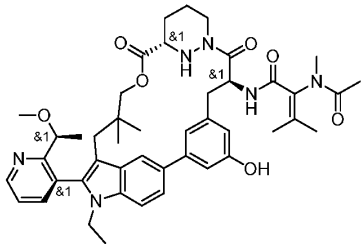
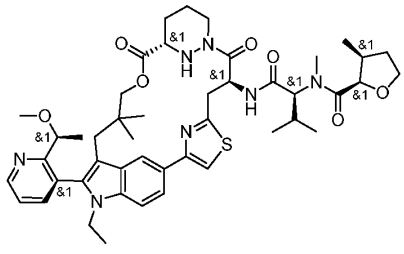
A108	
A109	
A110	
A111	
A112	
A113	

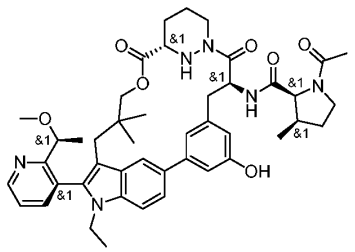
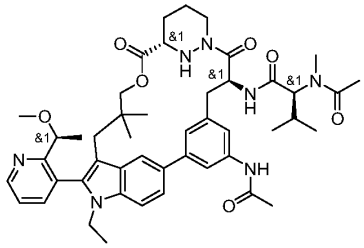
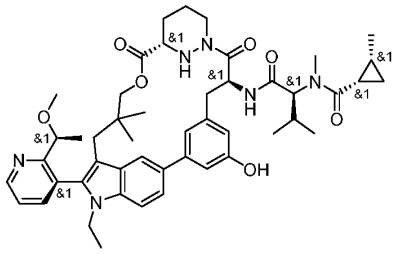
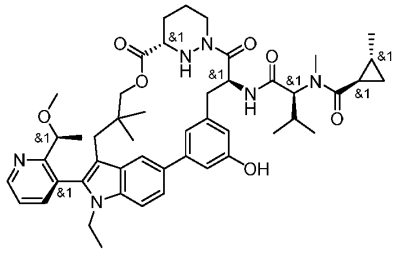
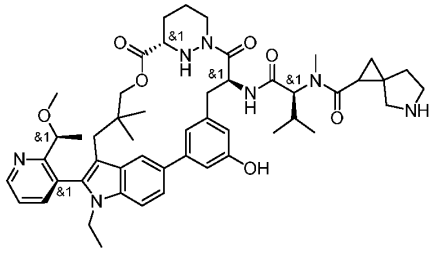
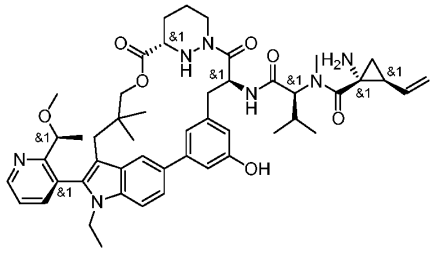
A114	
A115	
A116	
A117	
A118	
A119	

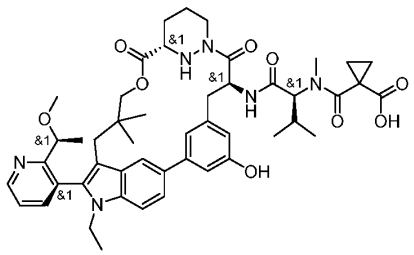
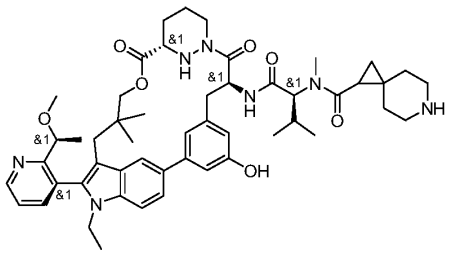
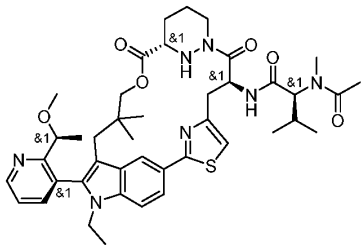
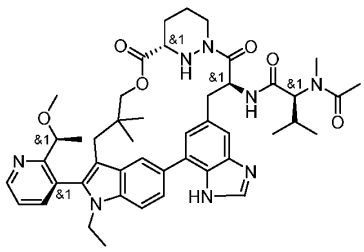
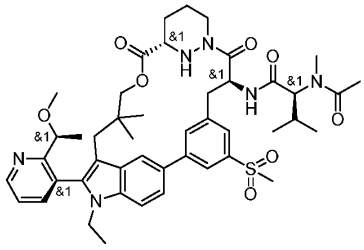
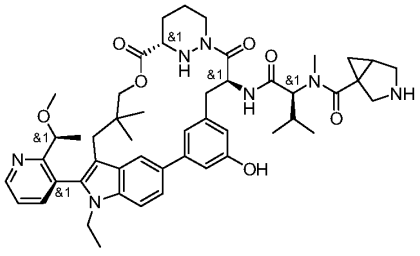
A120	
A121	
A122	
A123	
A124	
A125	

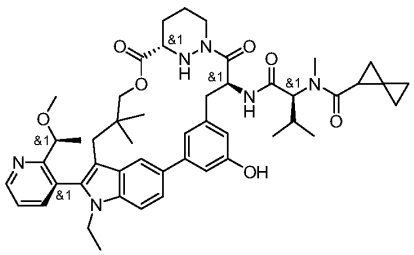
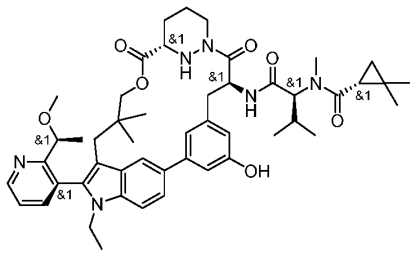
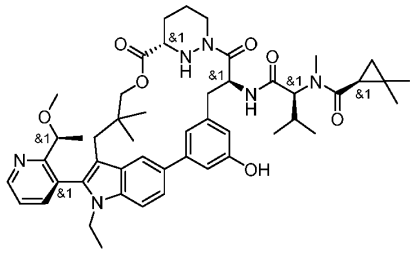
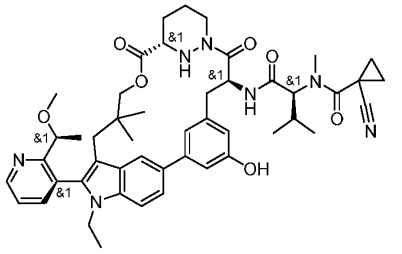
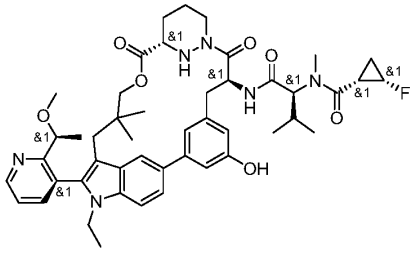
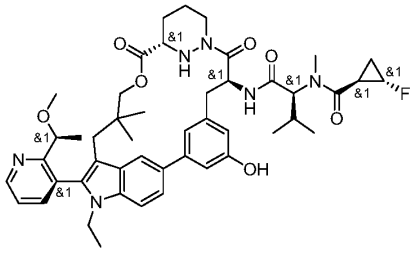
A126	
A127	
A128	
A129	
A130	
A131	

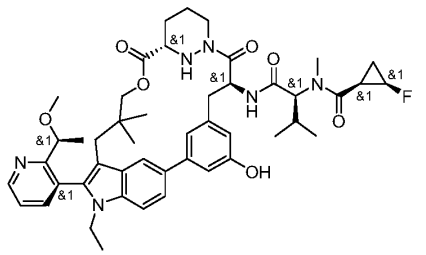
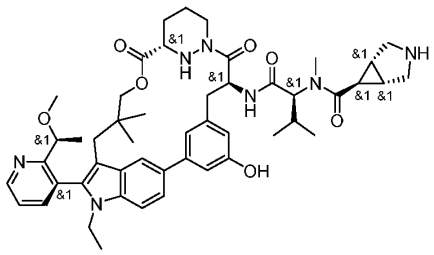
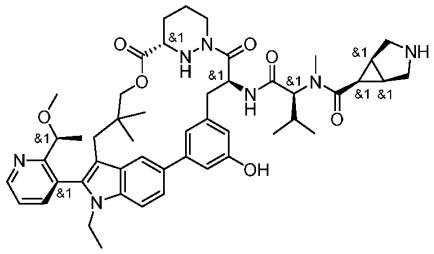
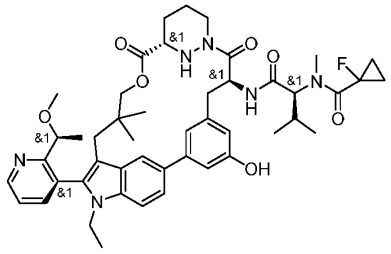
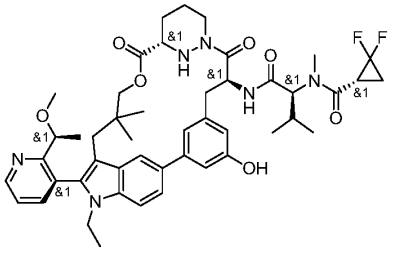
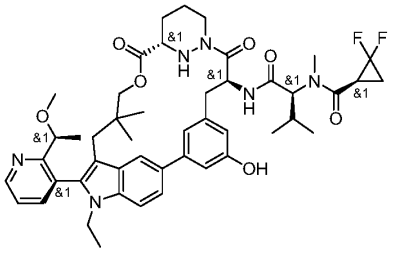
A132	
A133	
A134	
A135	
A136	
A137	

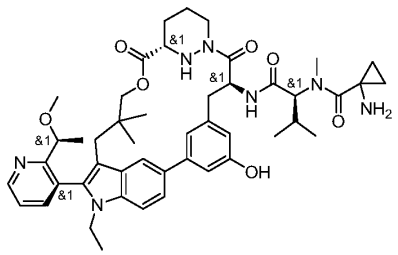
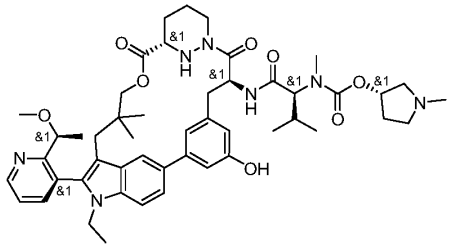
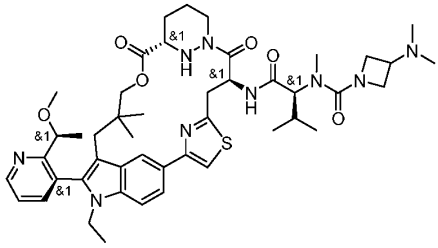
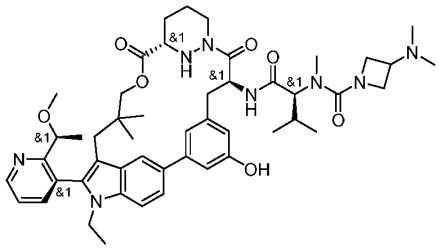
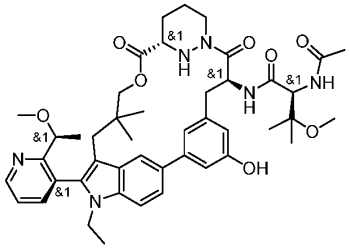
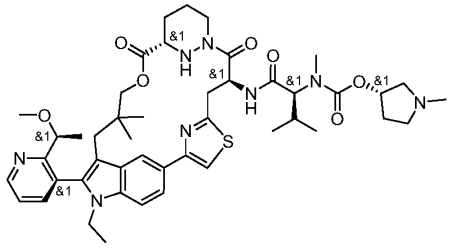
A138	
A139	
A140	
A141	
A142	
A143	

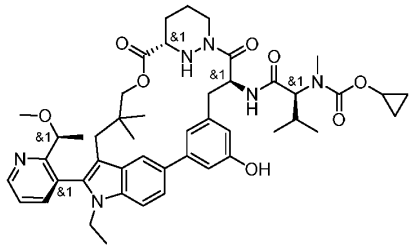
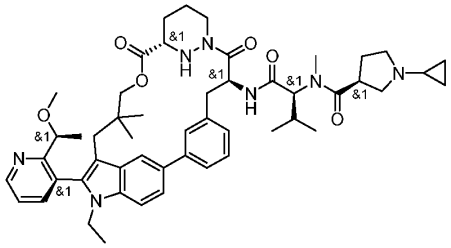
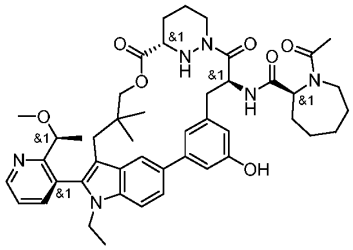
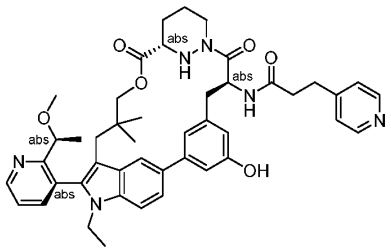
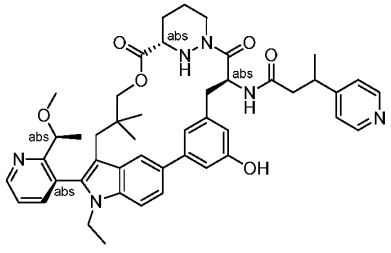
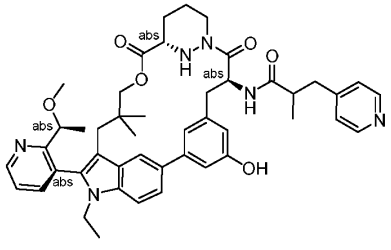
A144	
A145	
A146	
A147	
A148	
A149	

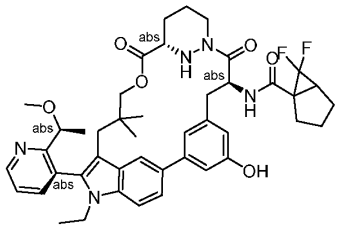
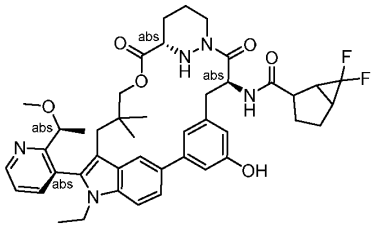
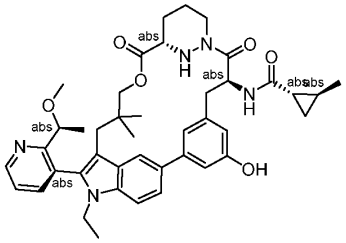
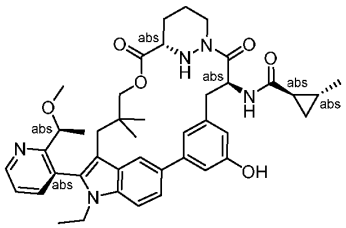
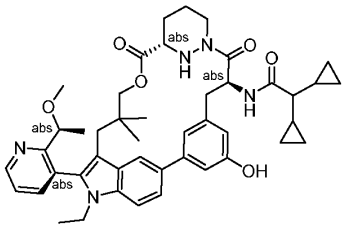
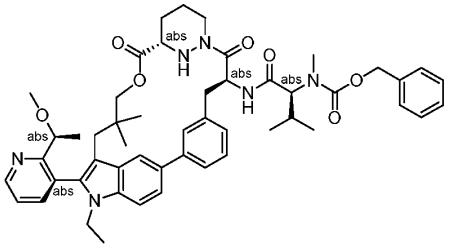
A150	
A151	
A152	
A153	
A154	
A155	

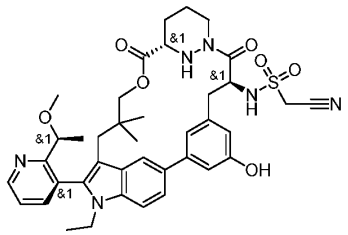
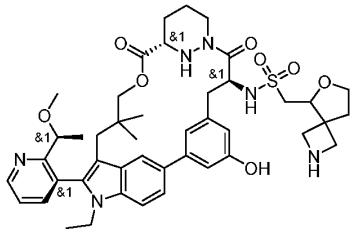
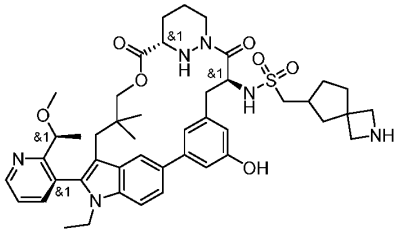
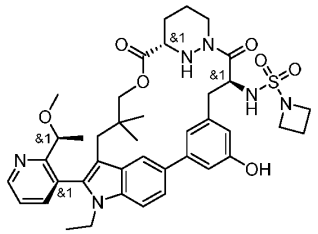
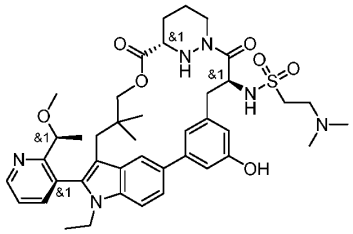
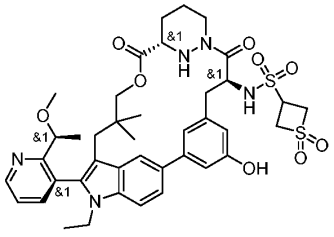
A156	
A157	
A158	
A159	
A160	
A161	

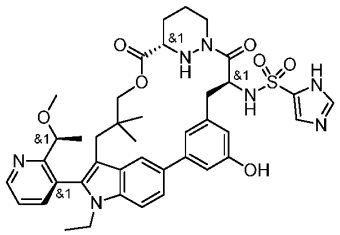
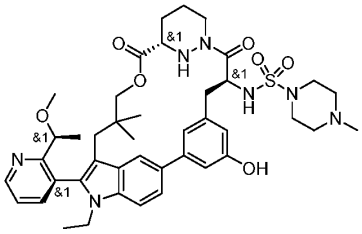
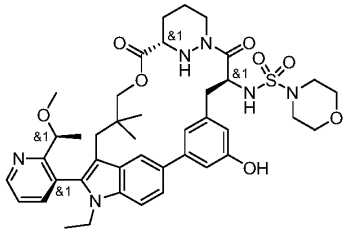
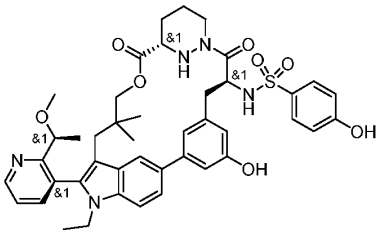
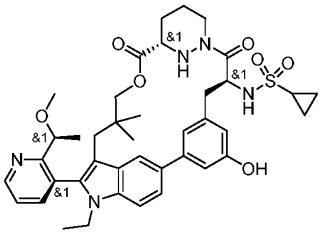
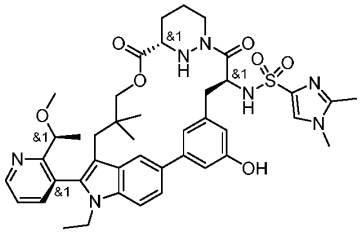
A162	
A163	
A164	
A165	
A166	
A167	

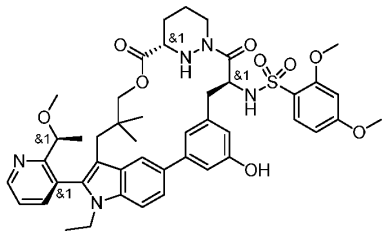
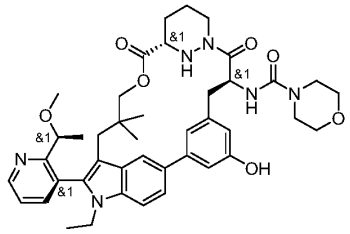
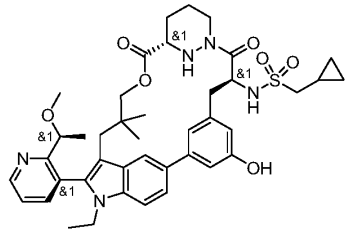
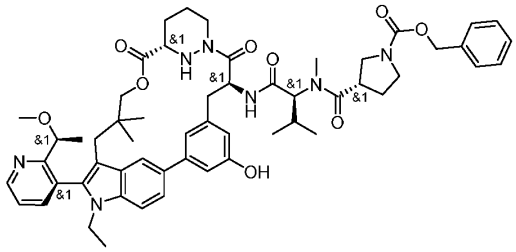
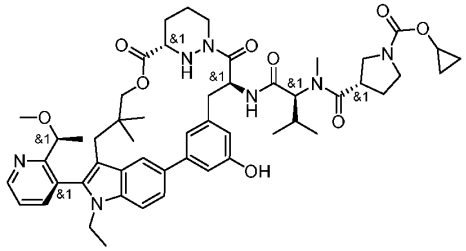
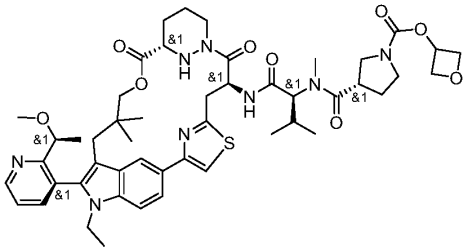
A168	
A169	
A170	
A171	
A172	
A173	

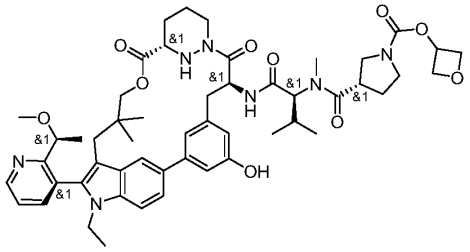
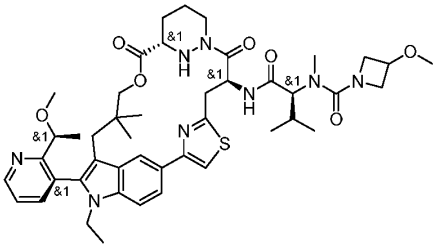
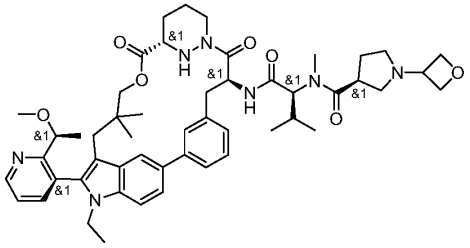
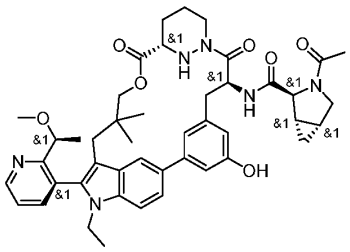
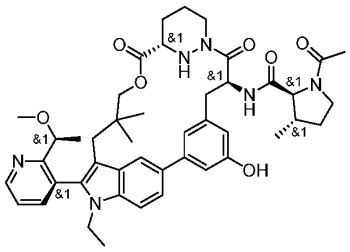
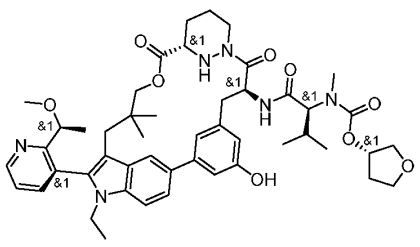
A174	
A175	
A176	
A177	
A178	
A179	

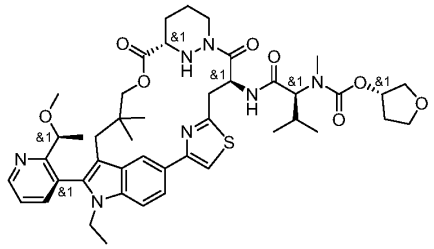
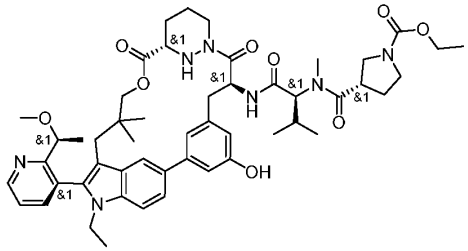
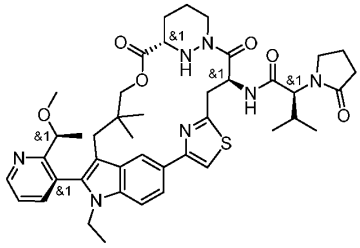
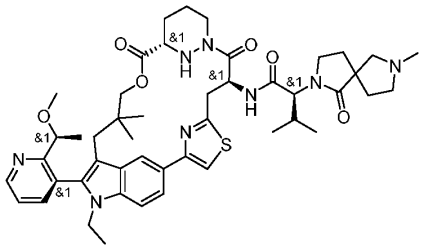
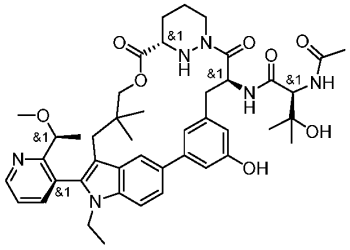
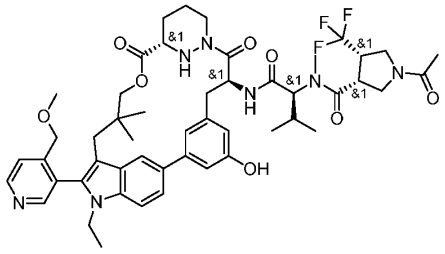
A180	
A181	
A182	
A183	
A184	
A185	

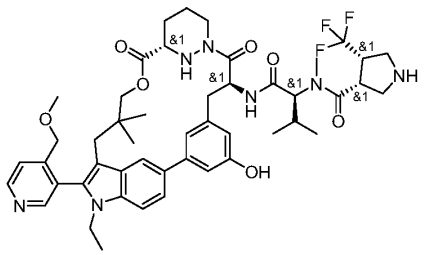
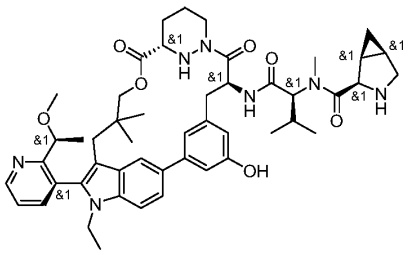
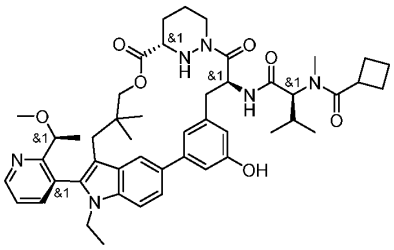
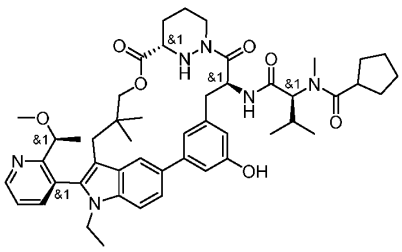
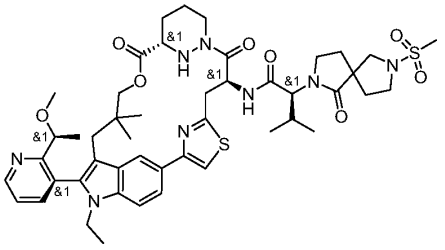
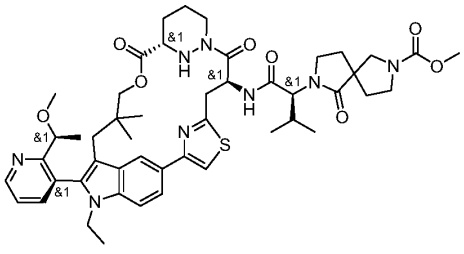
A186	
A187	
A188	
A189	
A190	
A191	

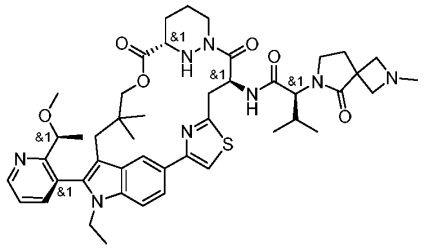
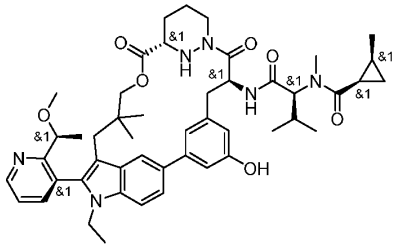
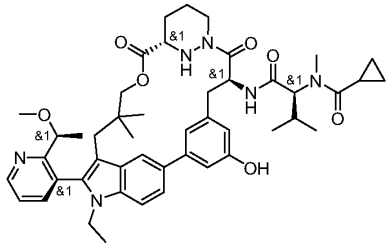
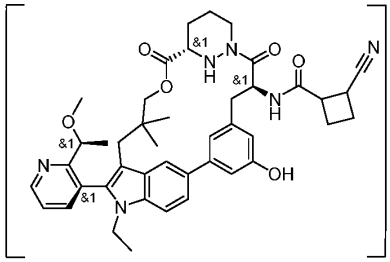
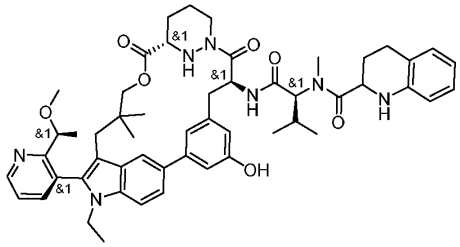
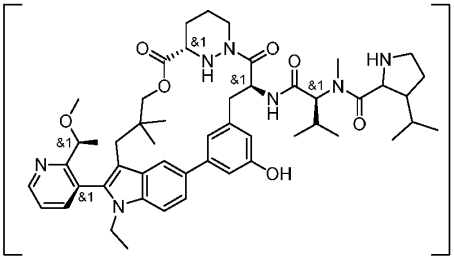
A192	
A193	
A194	
A195	
A196	
A197	

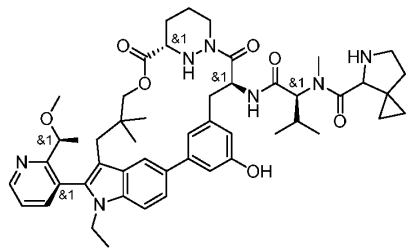
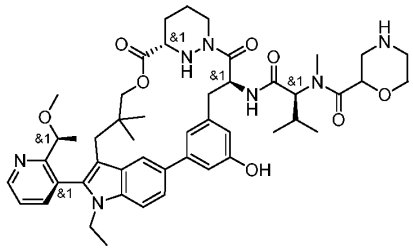
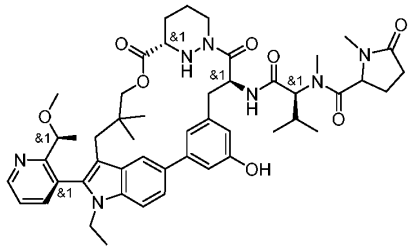
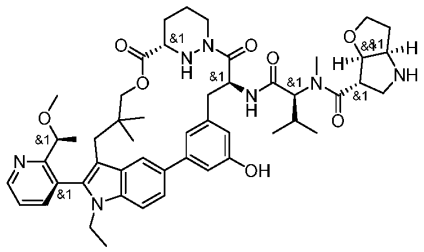
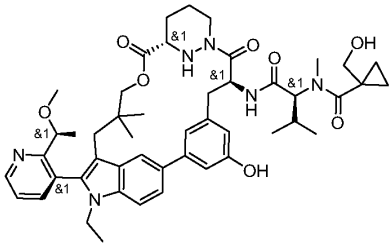
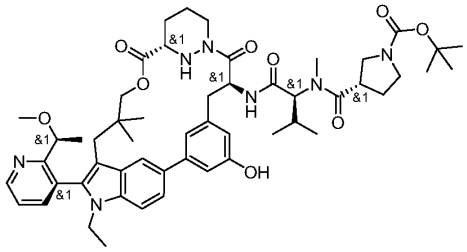
A198	
A199	
A200	
A201	
A202	
A203	

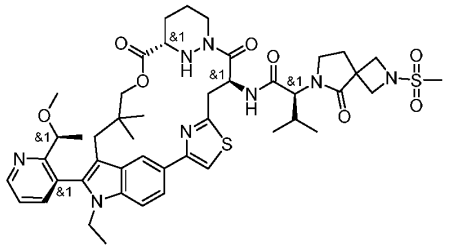
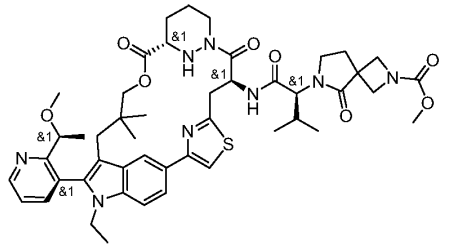
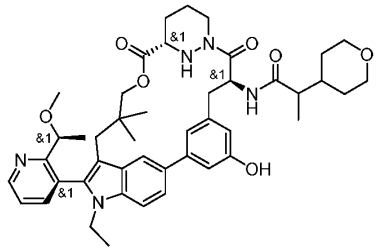
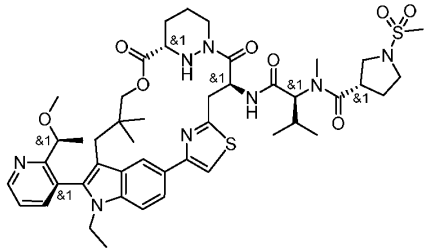
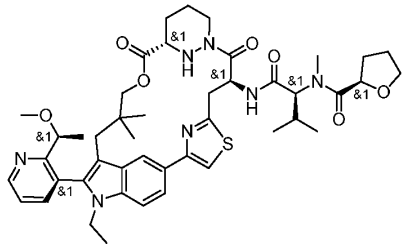
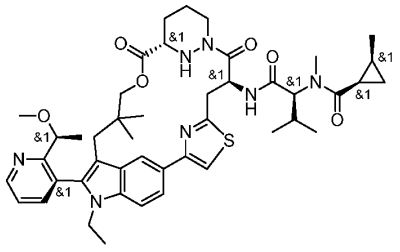
A204	
A205	
A206	
A207	
A208	
A209	

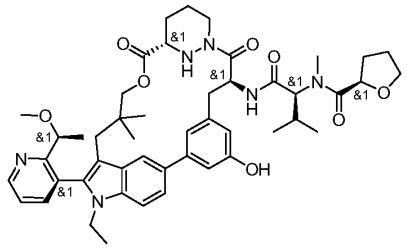
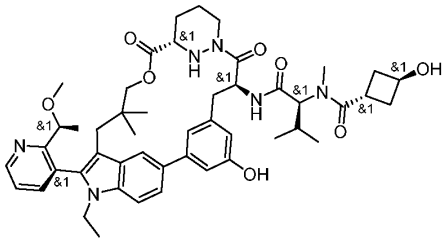
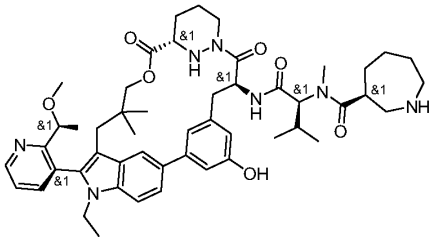
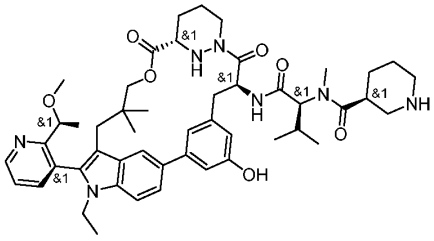
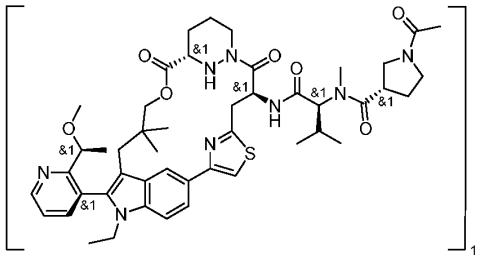
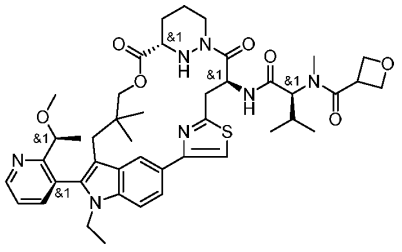
A210	
A211	
A212	
A213	
A214	
A215	

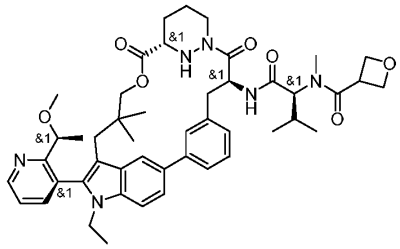
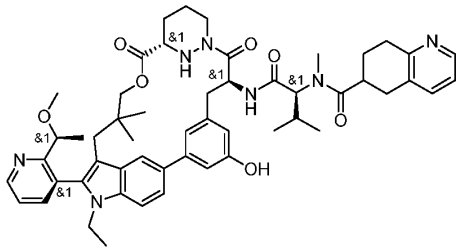
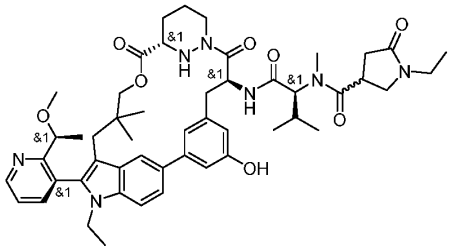
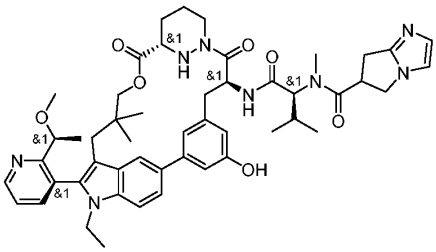
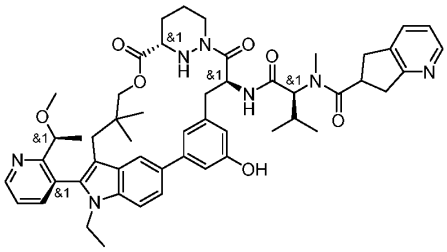
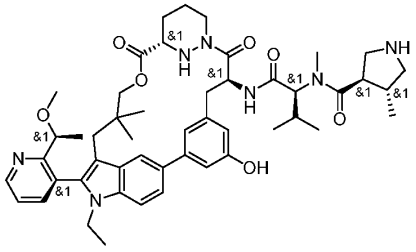
A216	
A217	
A218	
A219	
A220	
A221	

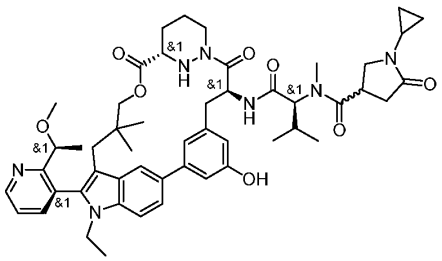
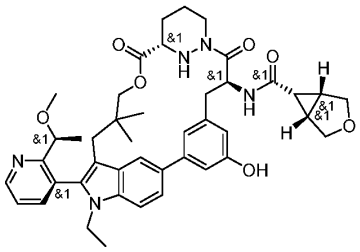
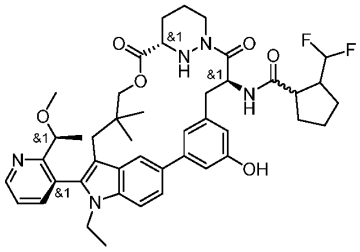
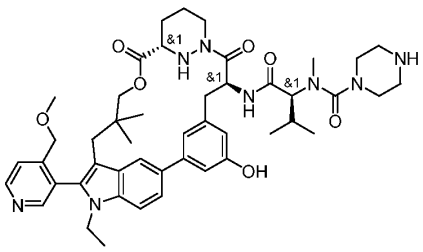
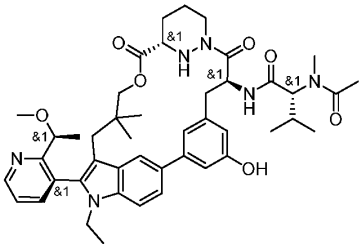
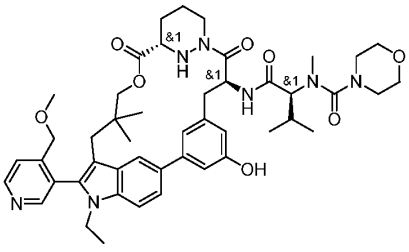
A222	
A223	
A224	
A225	
A226	
A227	

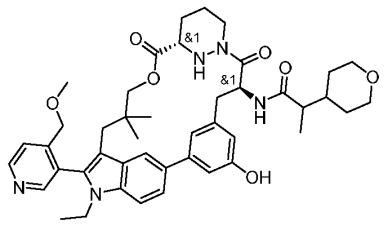
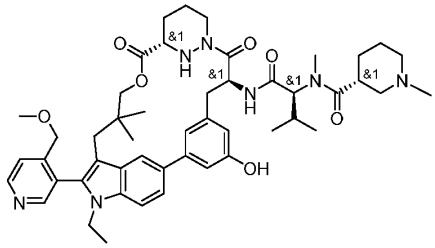
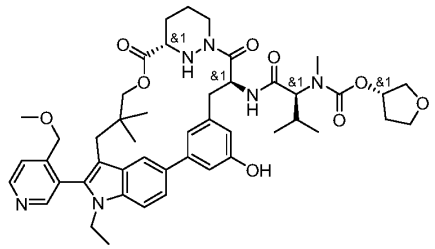
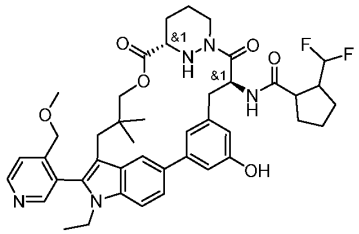
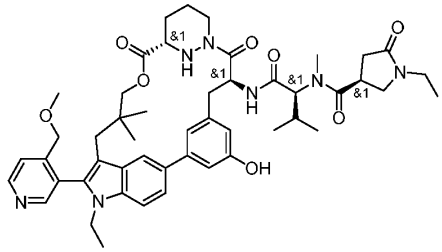
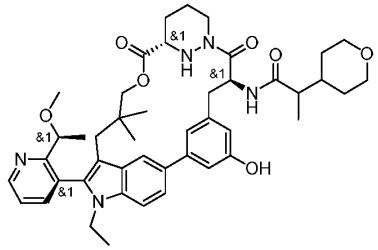
A228	
A229	
A230	
A231	
A232	
A233	

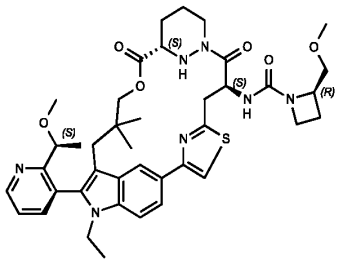
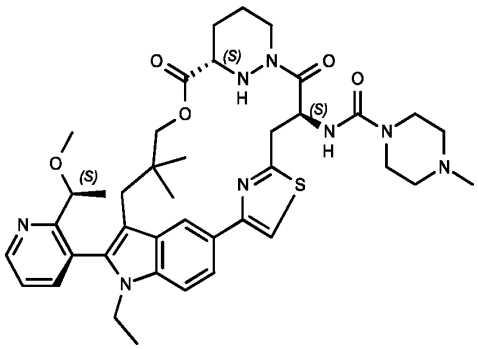
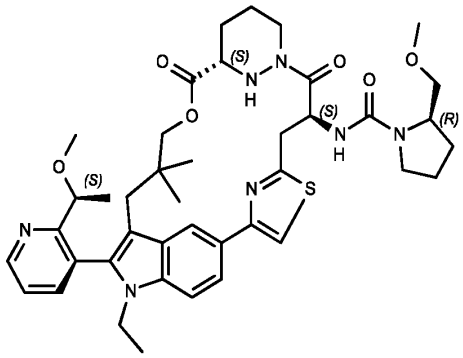
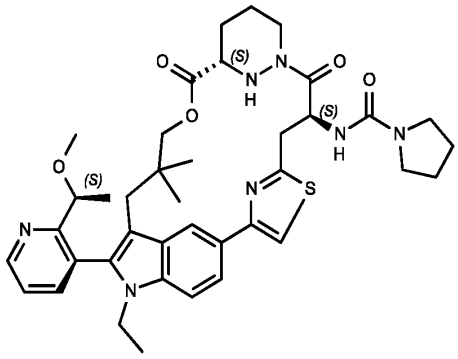
A234	
A235	
A236	
A237	
A238	
A239	

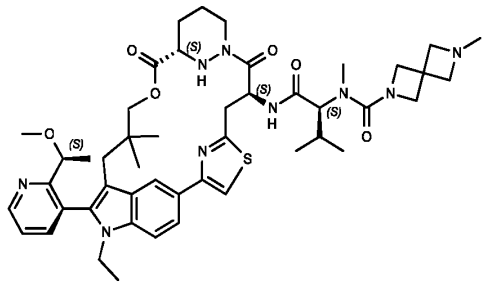
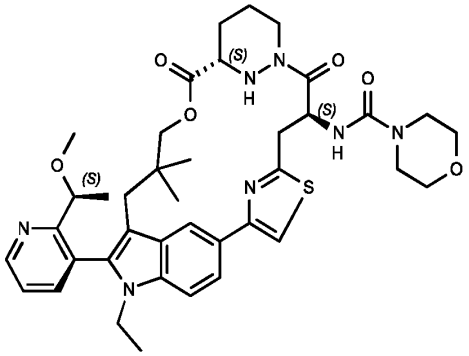
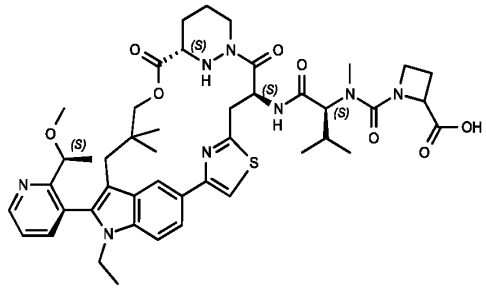
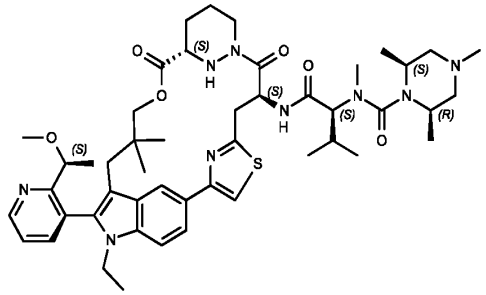
A240	
A241	
A242	
A243	
A244	
A245	

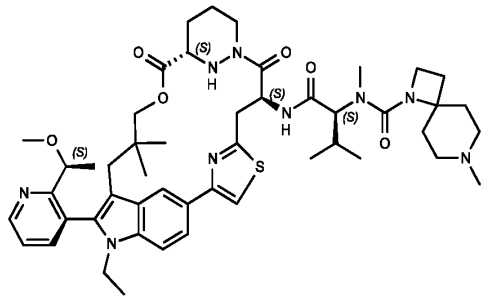
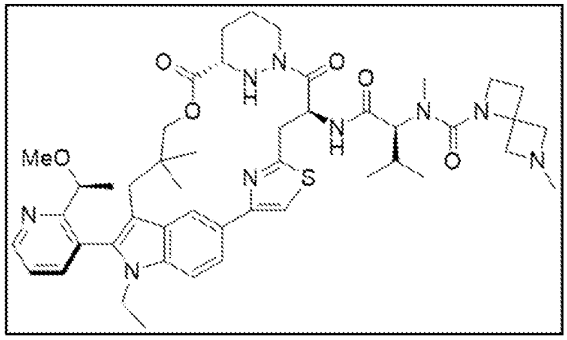
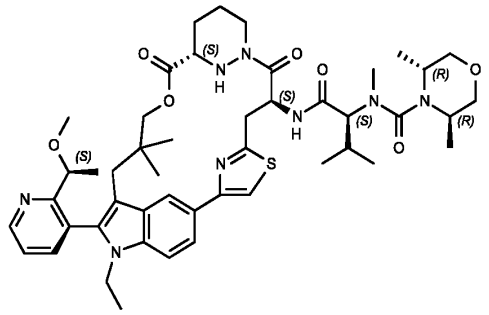
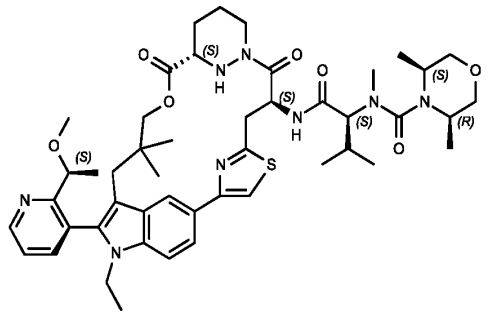
A246	
A247	
A248	
A249	
A250	
A251	

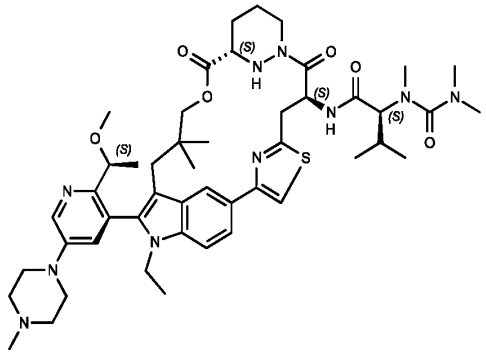
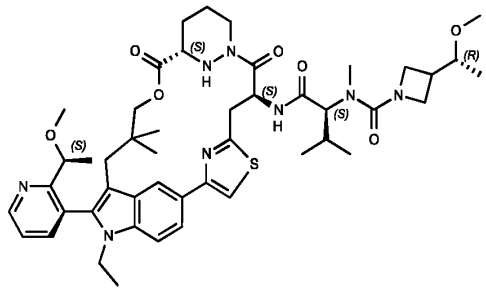
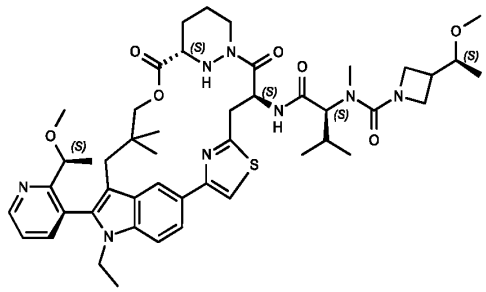
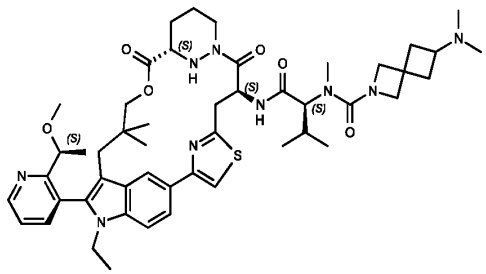
A252	
A253	
A254	
A255	
A256	
A257	

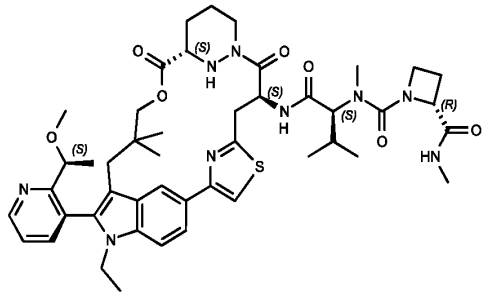
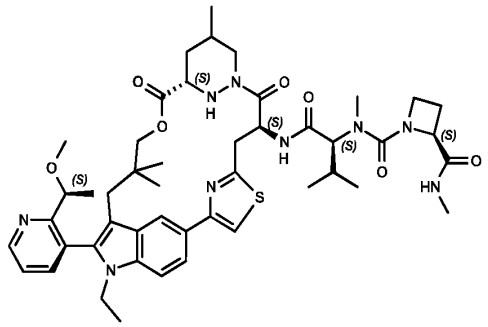
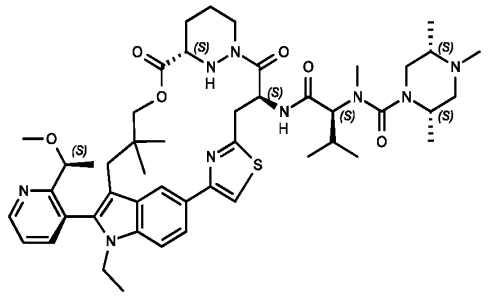
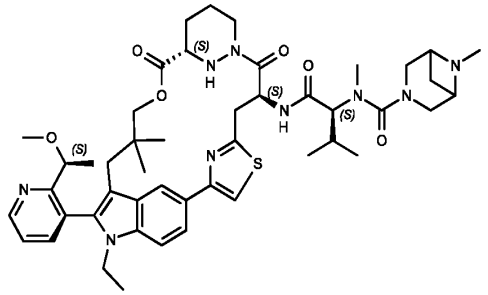
A258	
A259	
A260	
A261	
A262	
A263	

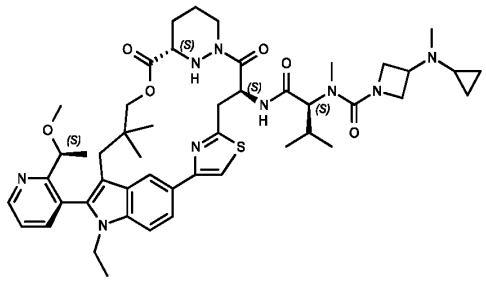
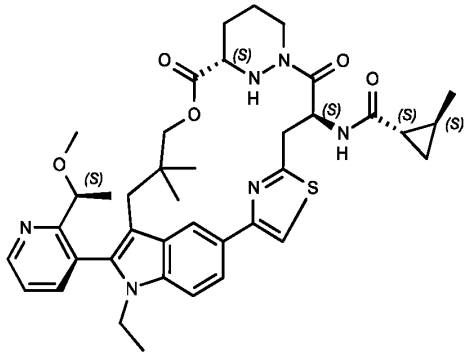
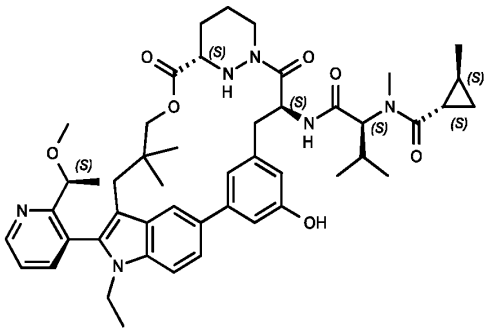
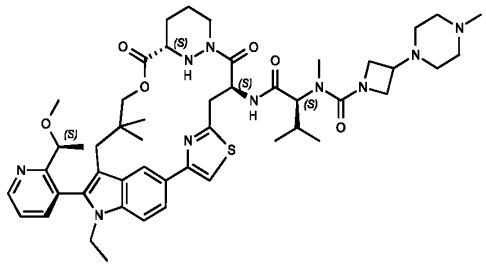
A264	
A265	
A266	
A267	

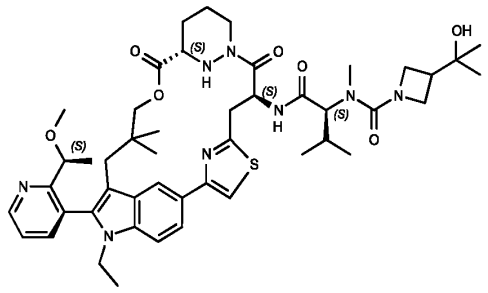
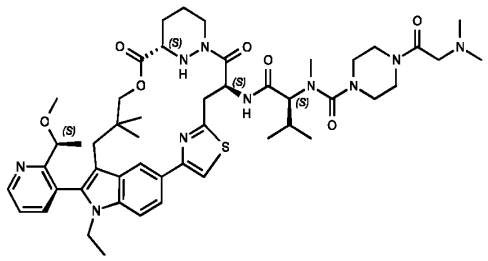
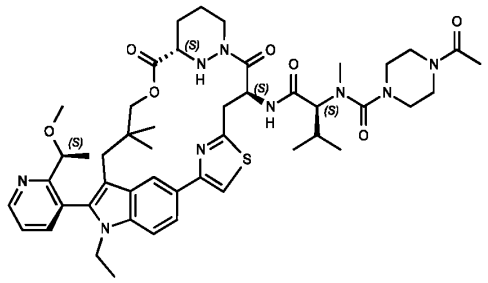
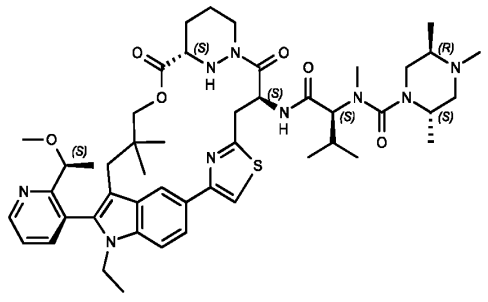
A268	
A270	
A271	
A272	

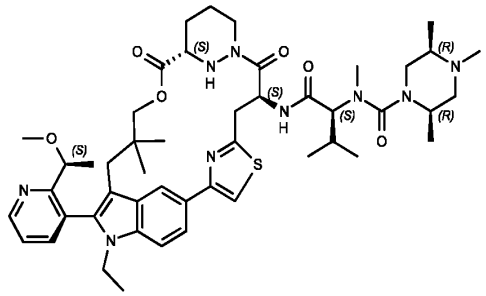
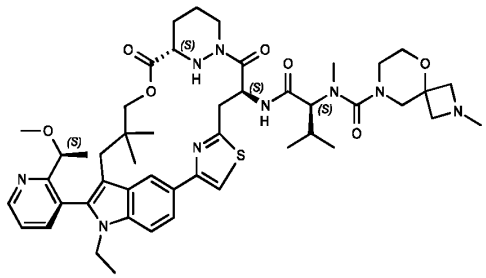
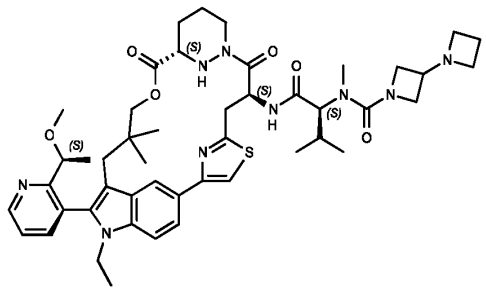
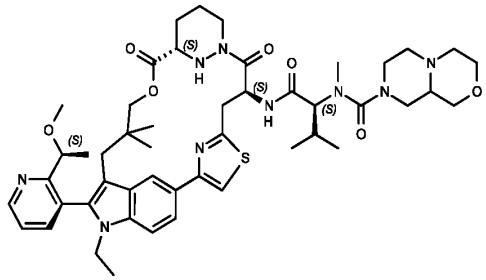
A273	
A274	
A275	
A276	

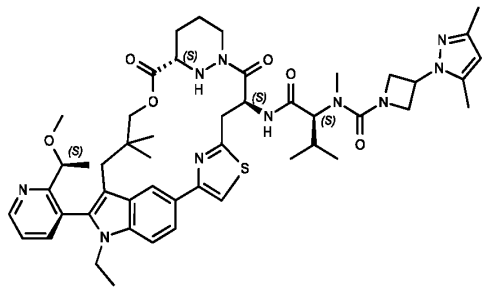
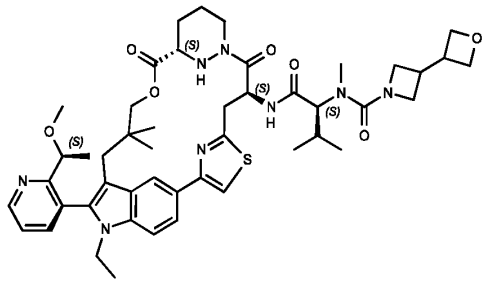
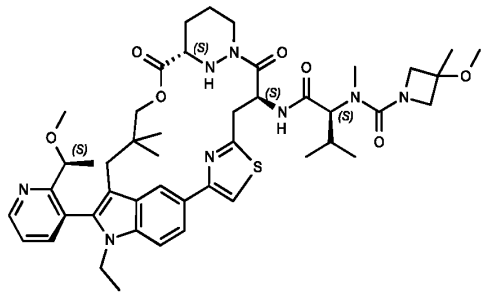
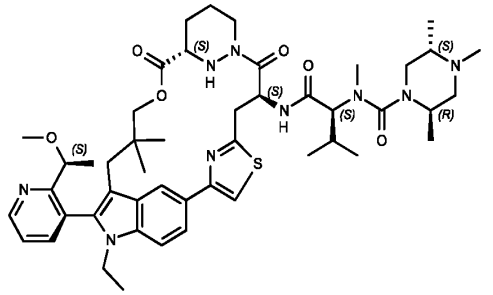
A277	
A278	
A279	
A280	

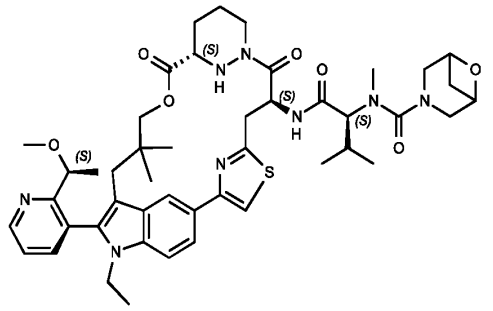
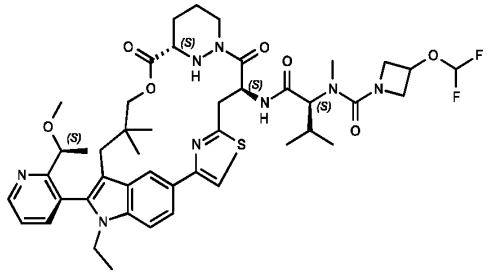
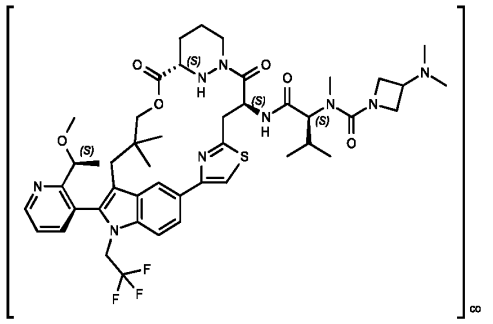
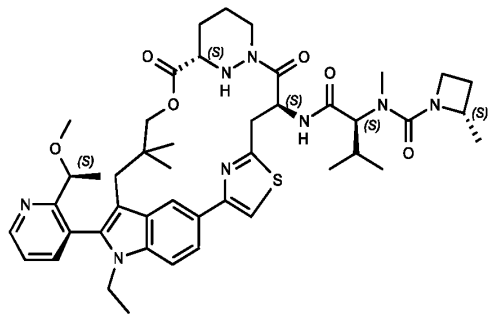
A281	
A282	
A283	
A284	

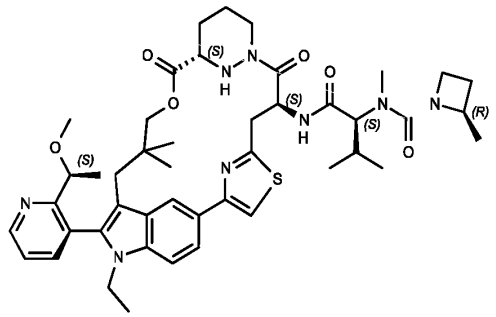
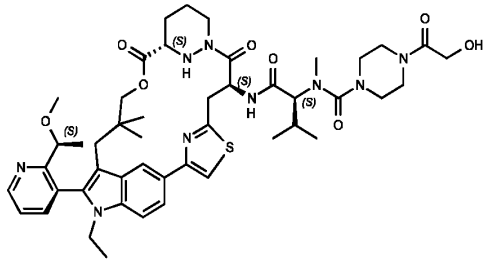
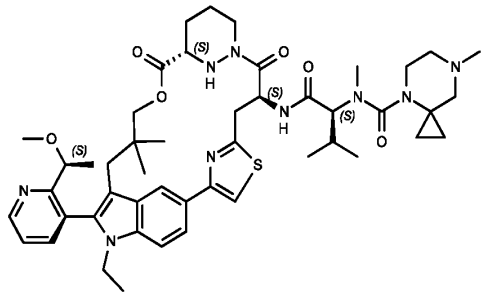
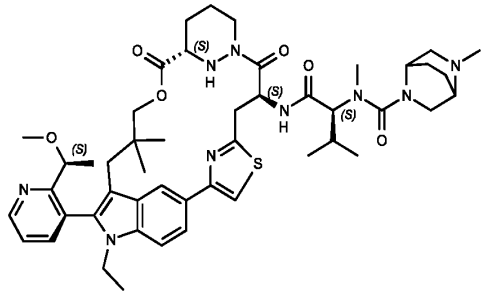
A285	
A286	
A287	
A288	

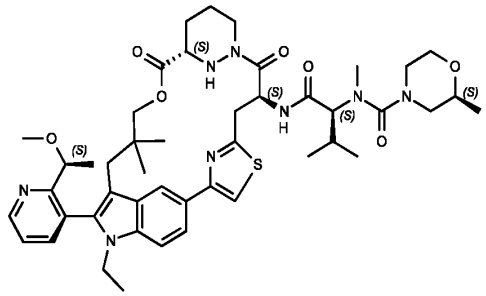
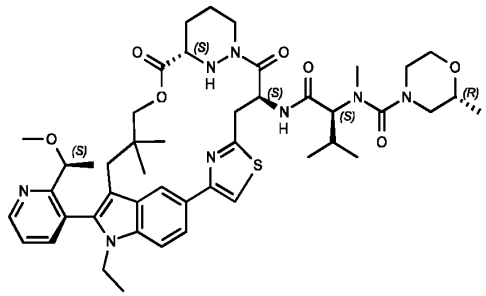
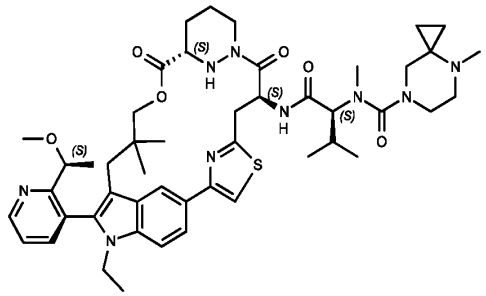
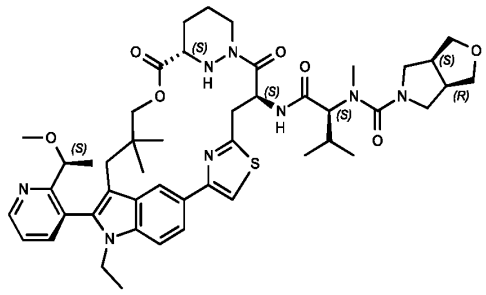
A289	
A290	
A291	
A292	

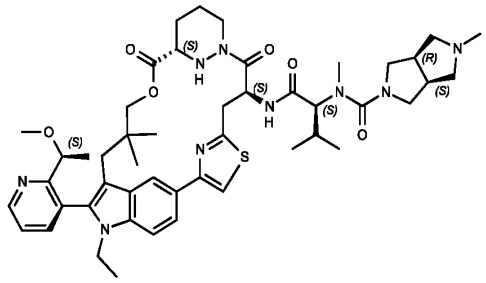
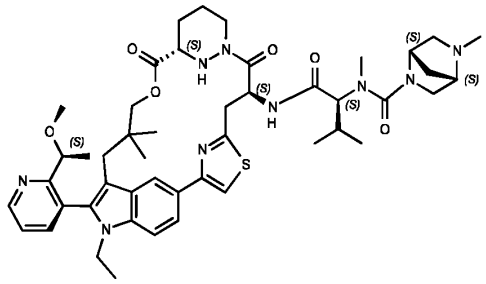
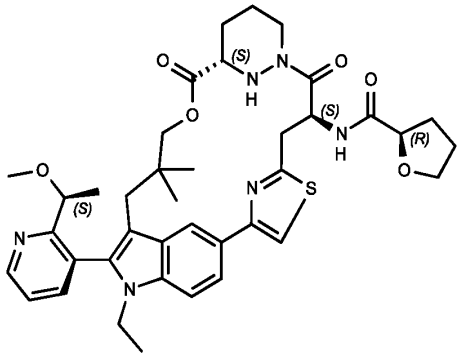
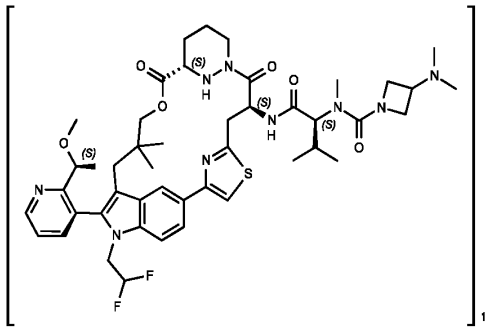
A293	
A294	
A295	
A296	

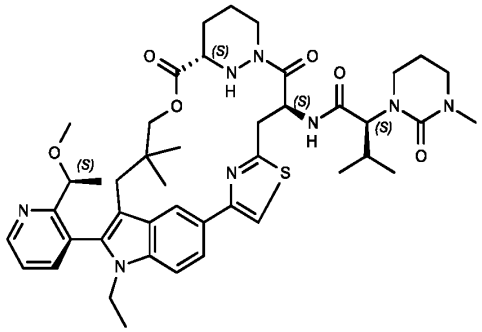
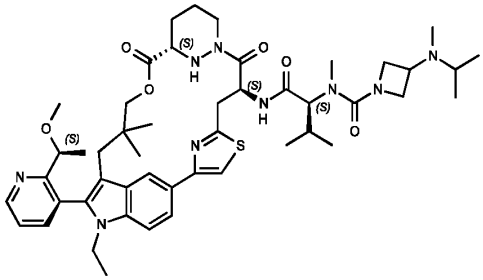
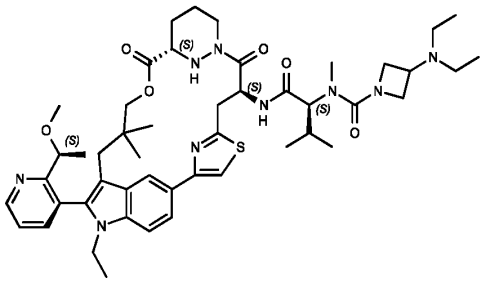
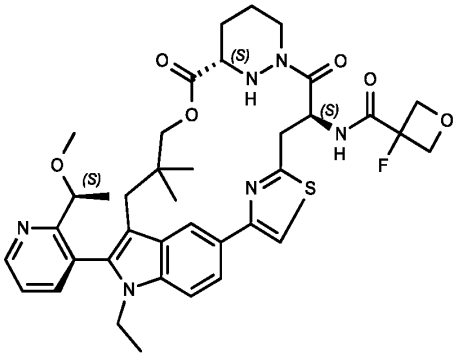
A297	
A298	
A299	
A300	

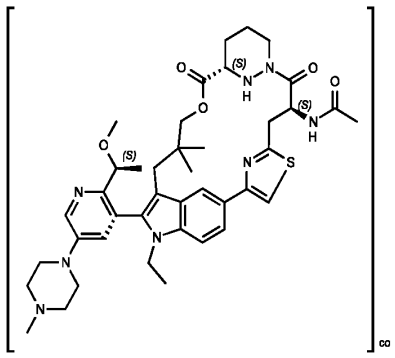
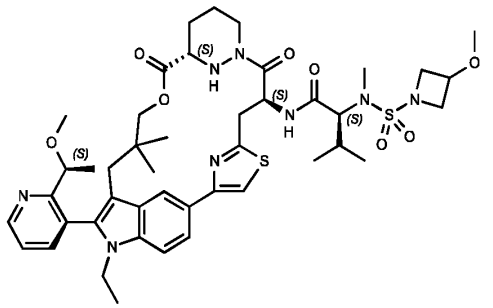
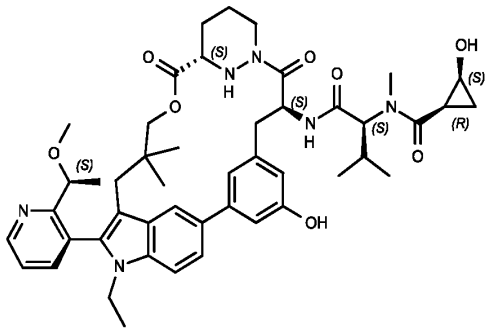
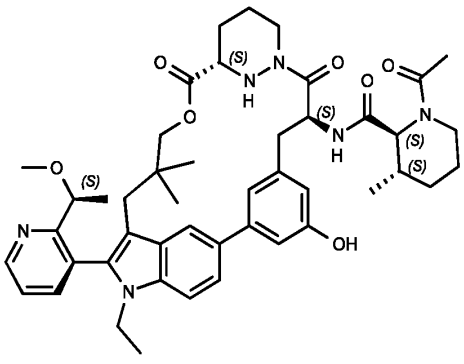
A301	
A302	
A303	
A304	

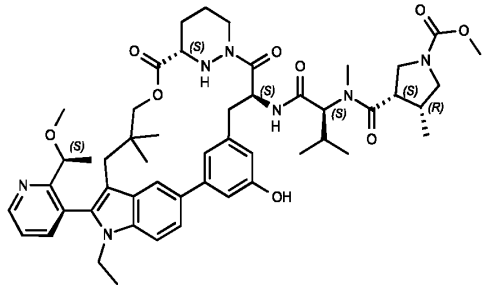
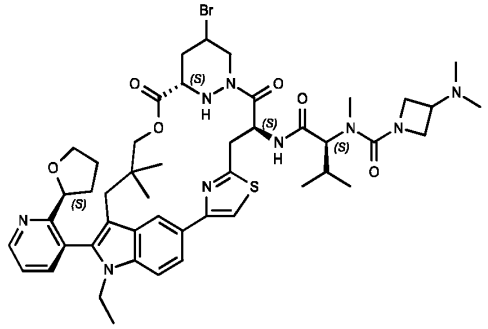
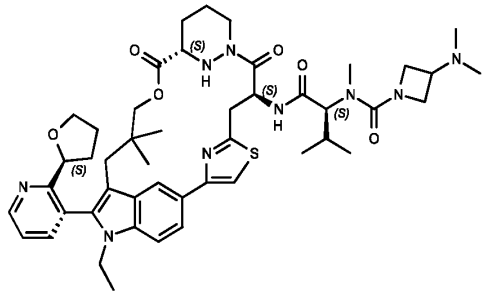
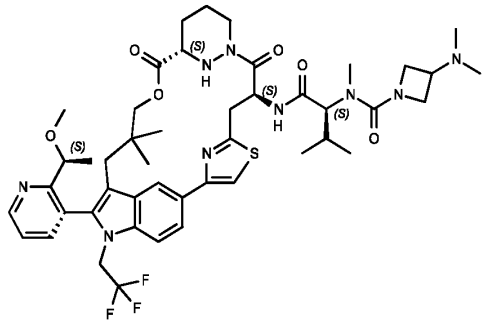
A305	
A306	
A307	
A308	

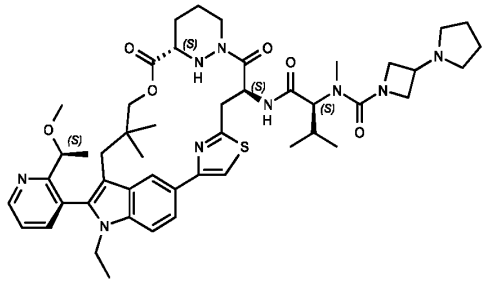
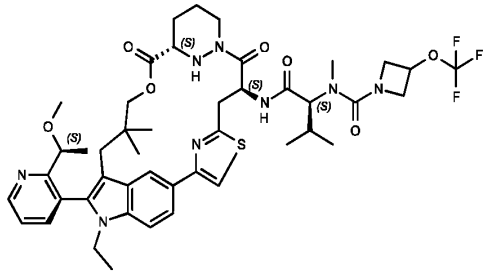
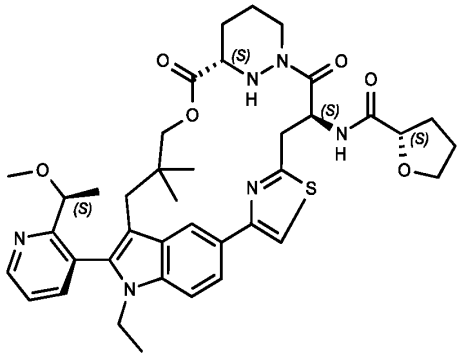
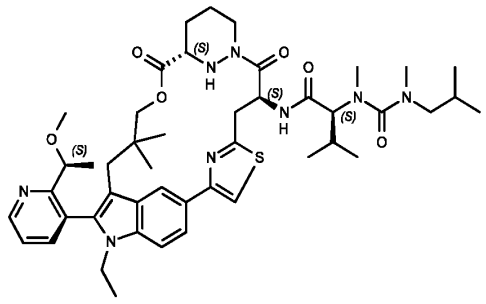
A309	
A310	
A311	
A312	

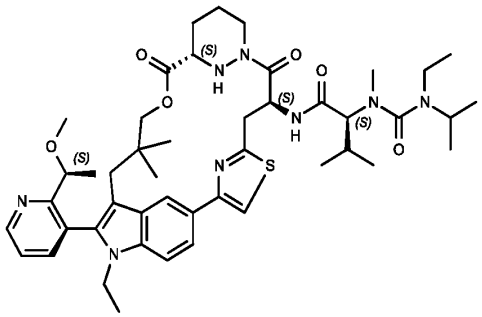
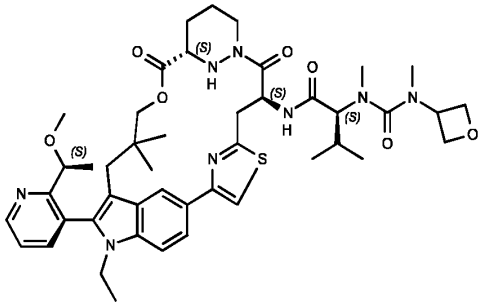
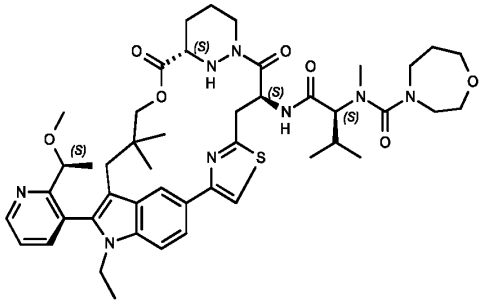
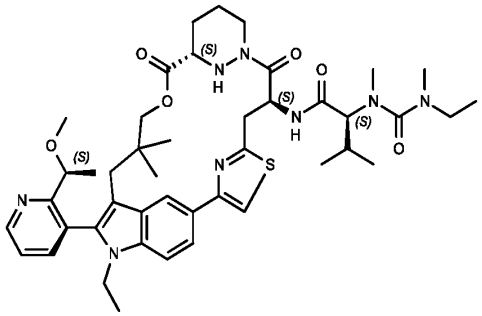
A313	
A314	
A315	
A316	

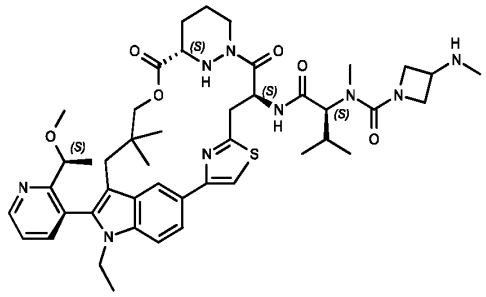
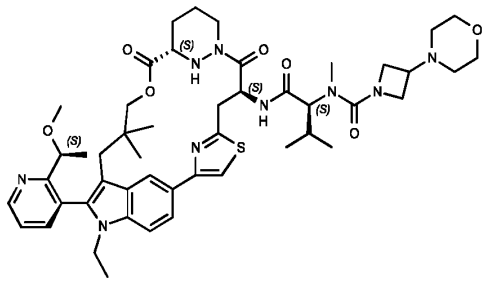
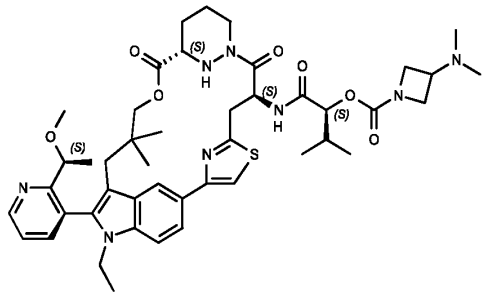
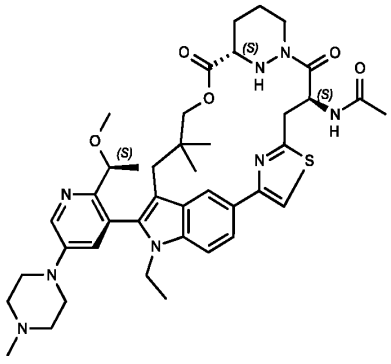
A317	
A318	
A319	
A320	

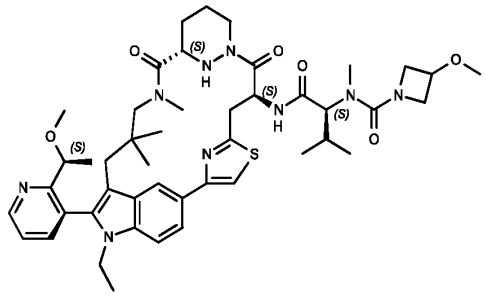
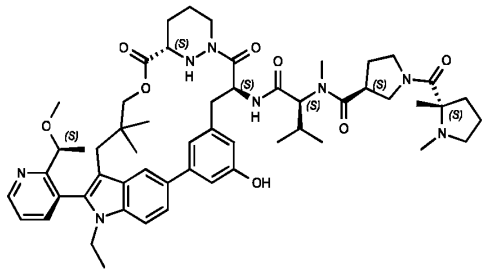
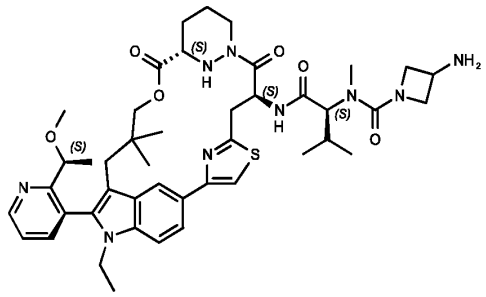
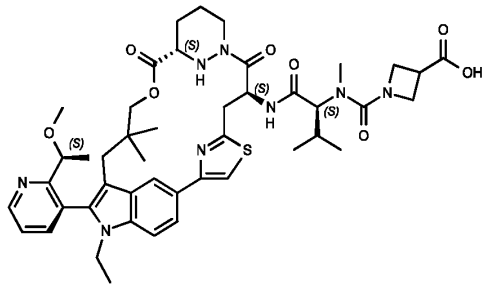
A321	
A322	
A323	
A324	

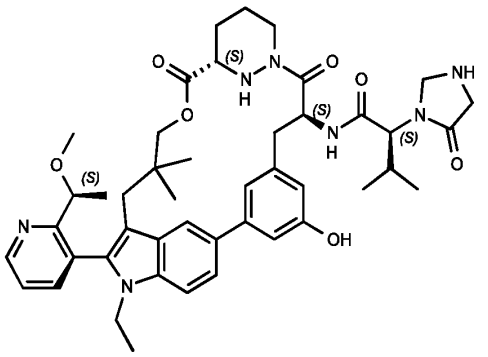
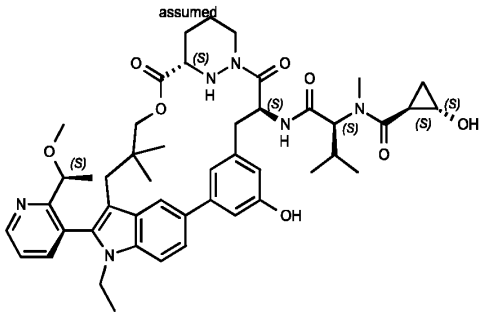
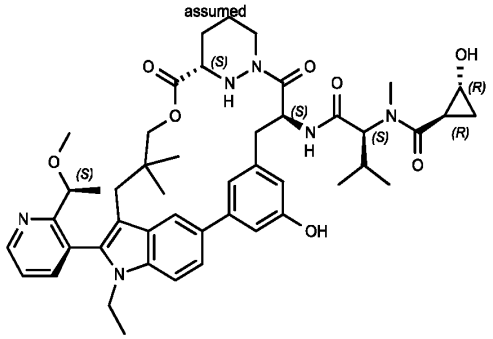
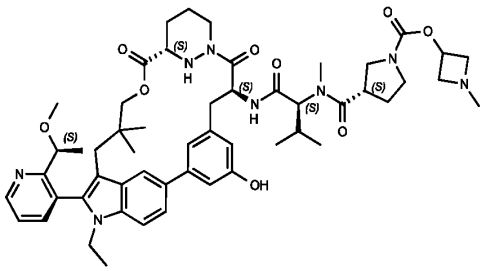
A325	
A326	
A327	
A328	

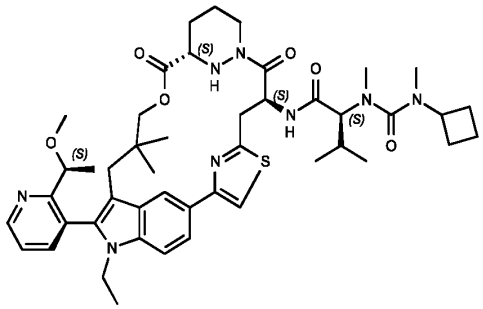
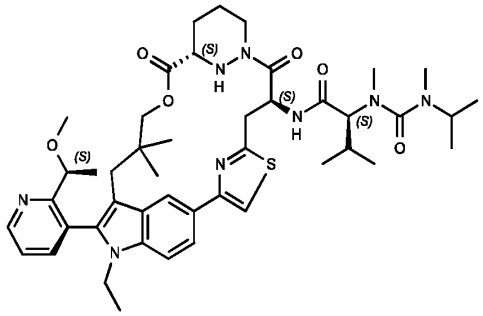
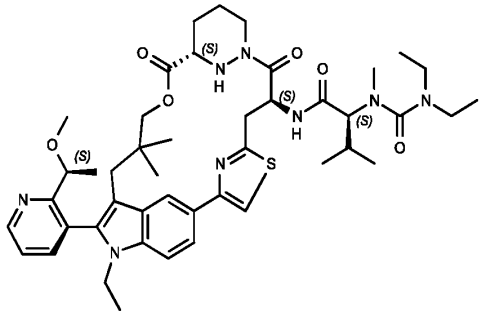
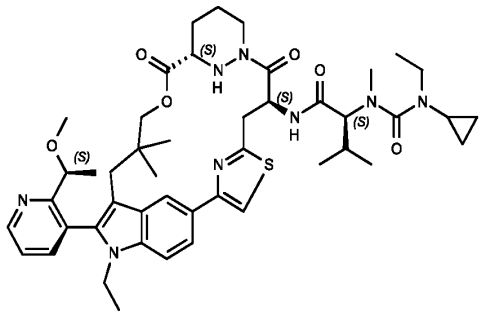
A329	
A330	
A331	
A332	

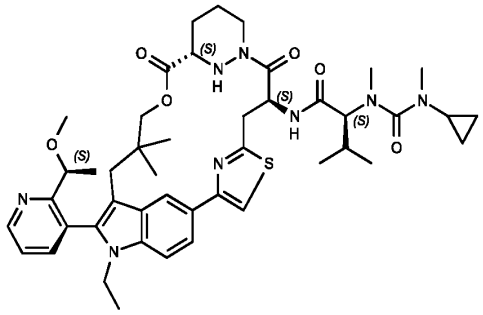
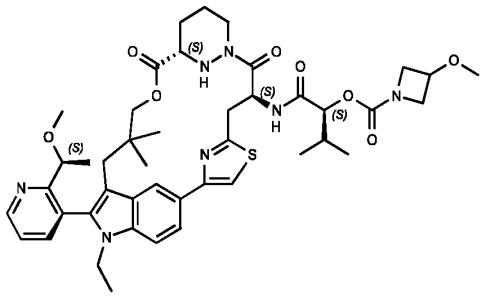
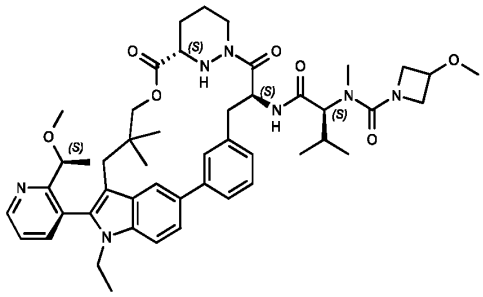
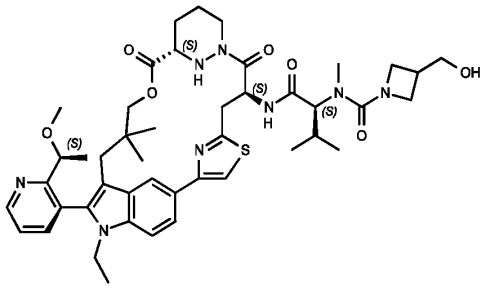
A333	
A334	
A335	
A336	

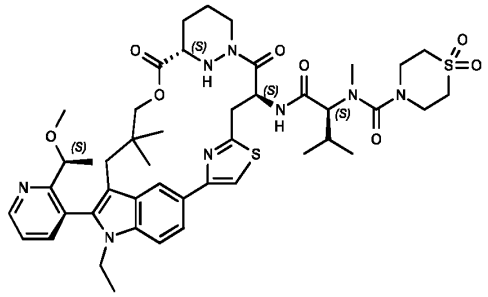
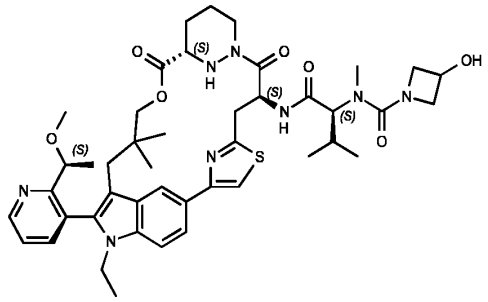
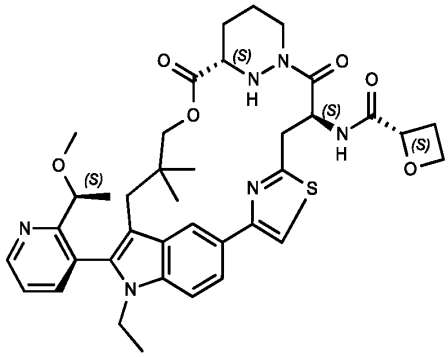
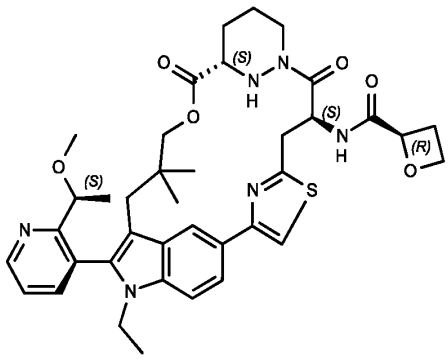
A337	
A338	
A339	
A340	

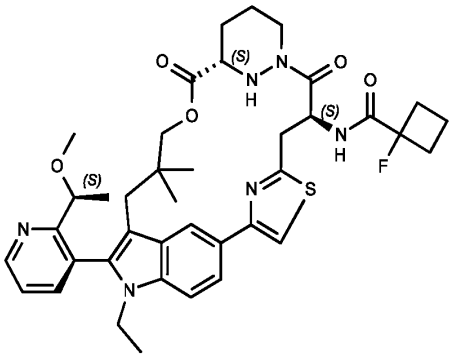
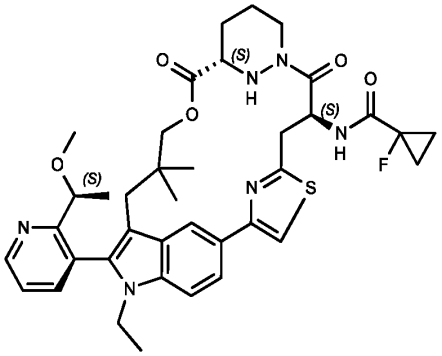
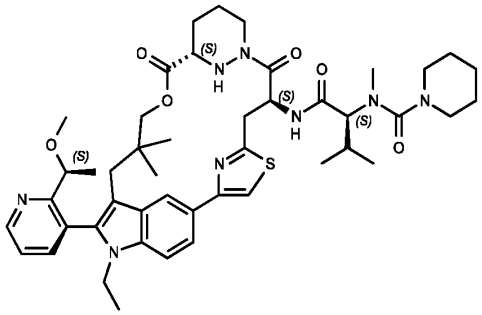
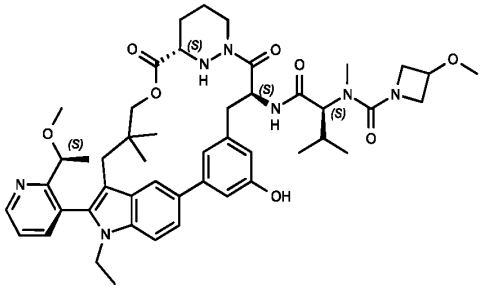
A341	
A342	
A343	
A344	

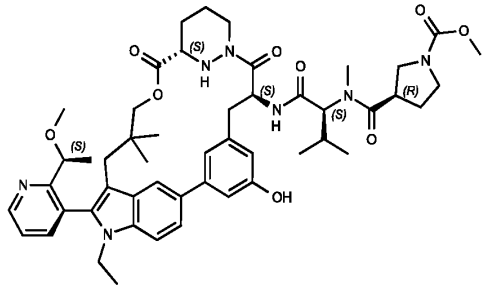
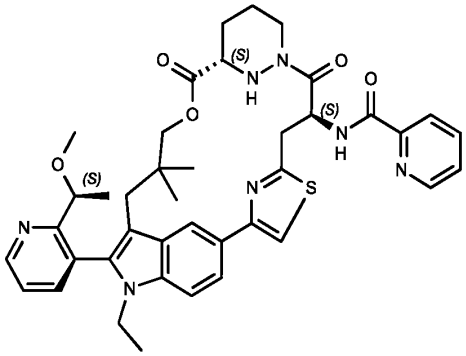
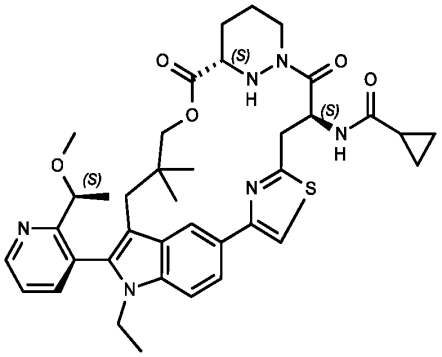
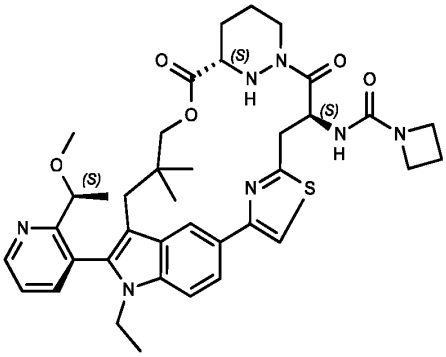
A345	
A346	
A347	
A348	

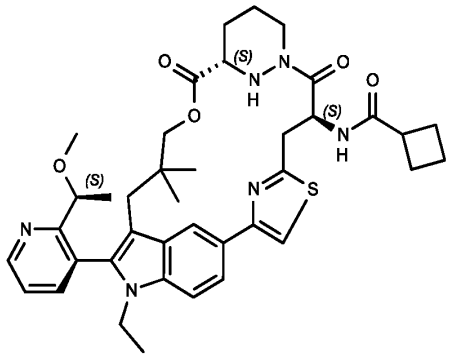
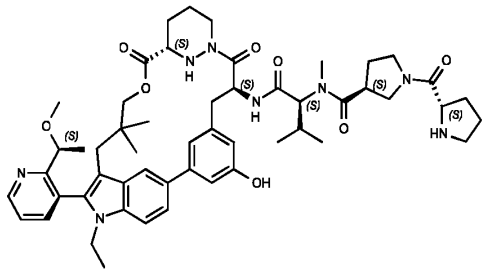
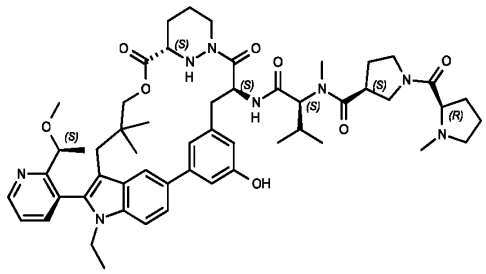
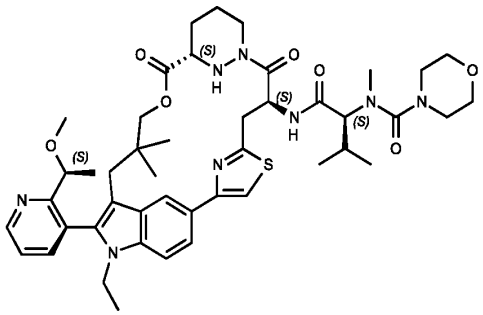
A349	
A350	
A351	
A352	

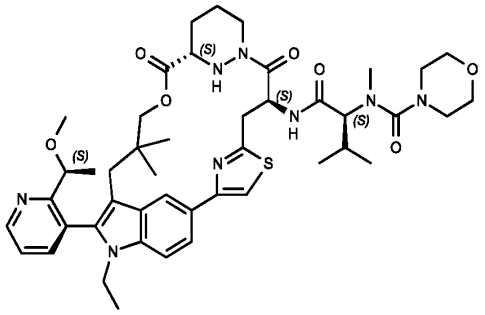
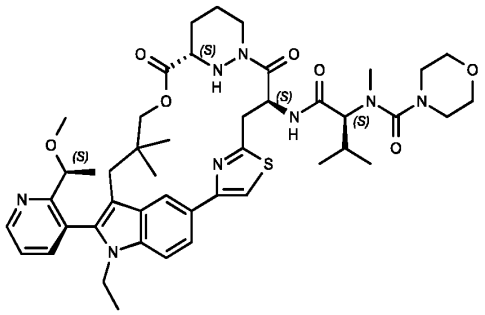
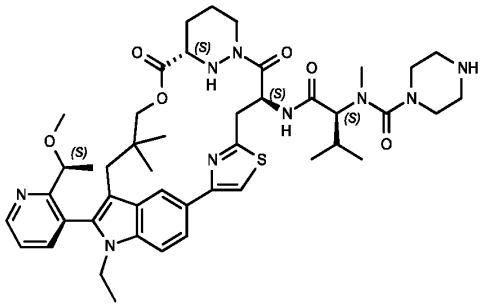
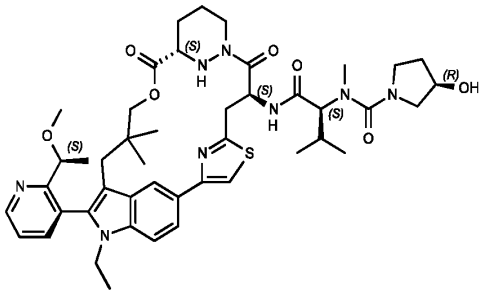
A353	
A354	
A355	
A356	

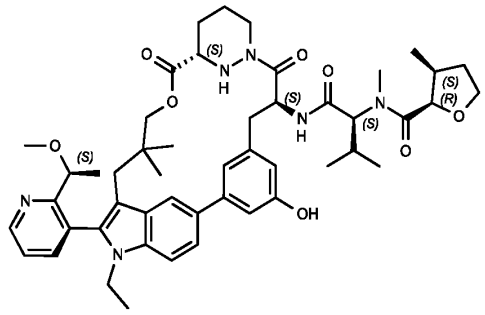
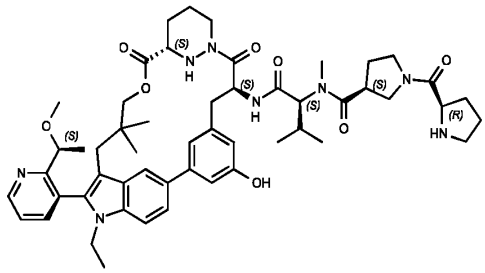
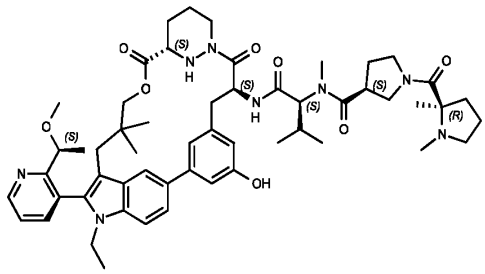
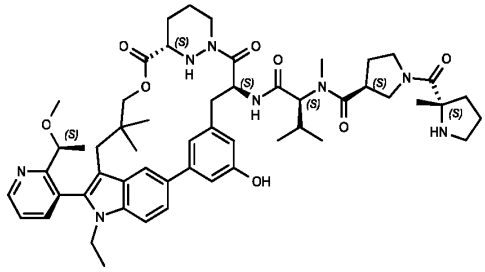
A357	
A358	
A359	
A360	

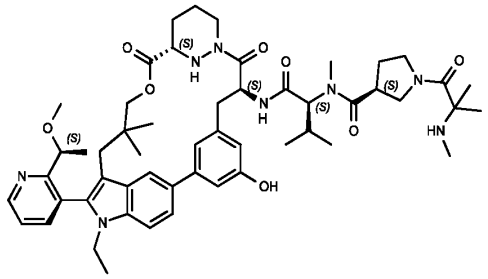
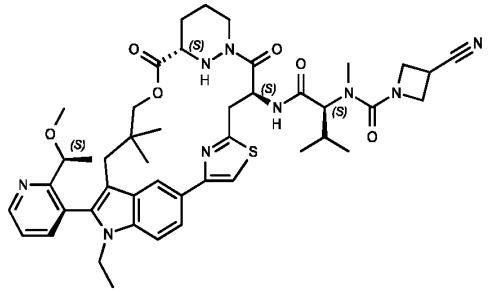
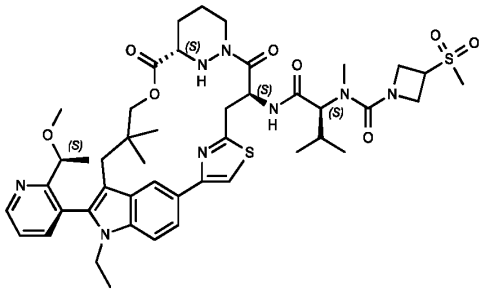
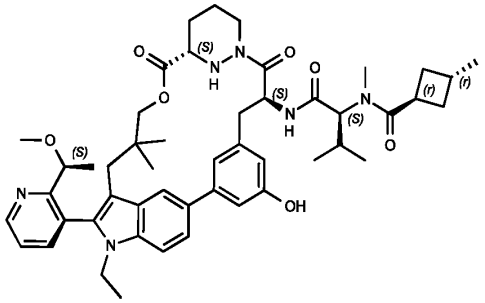
A361	
A362	
A363	
A364	

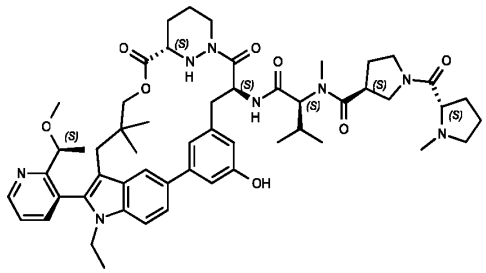
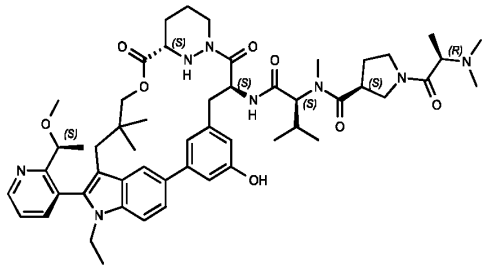
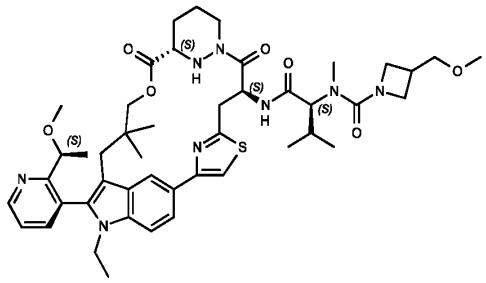
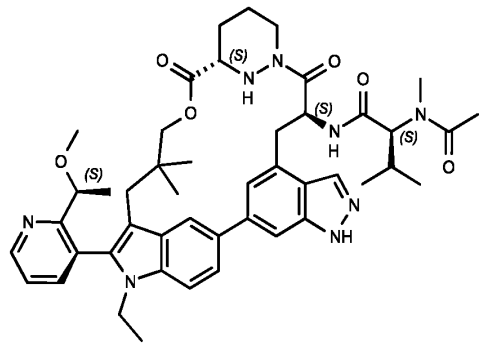
A365	
A366	
A367	
A368	

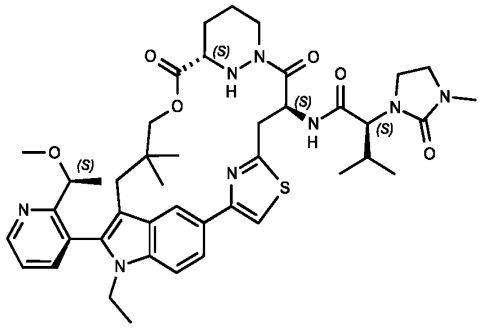
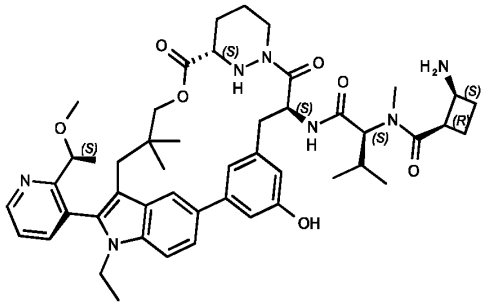
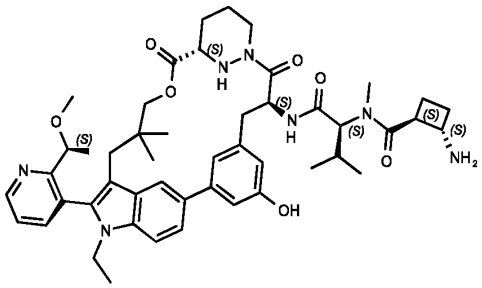
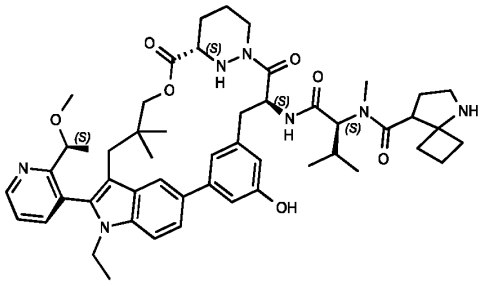
A369	
A370	
A371	
A372	

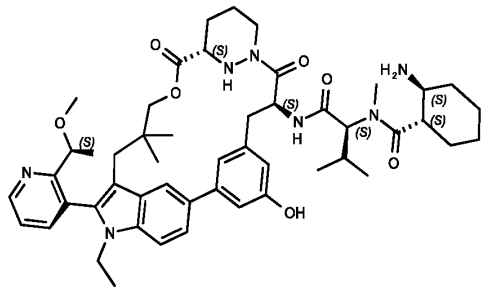
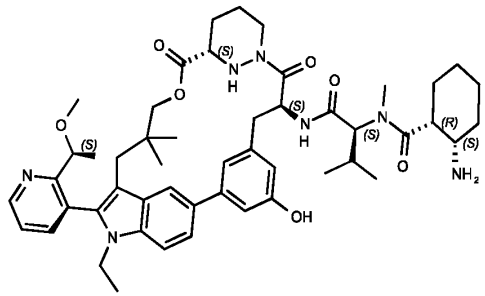
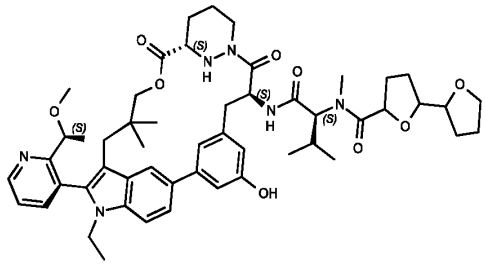
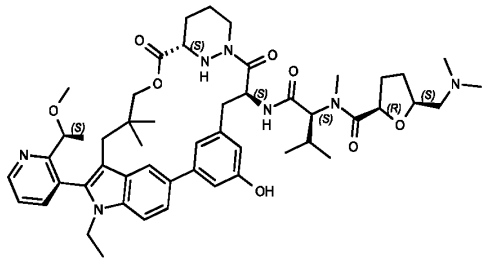
A373	
A374	
A375	
A376	

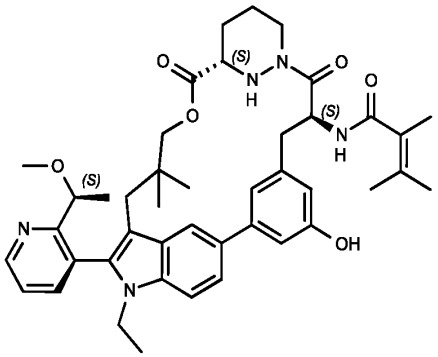
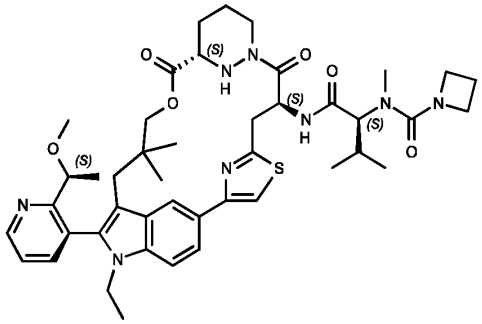
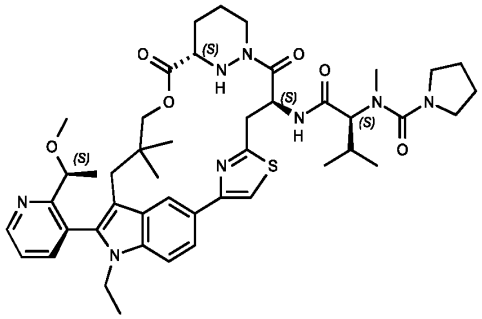
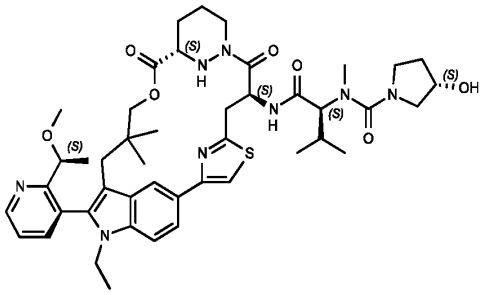
A377	
A378	
A379	
A380	

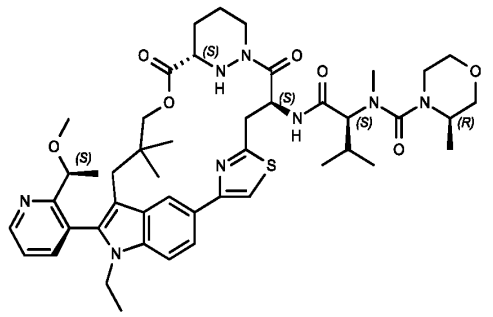
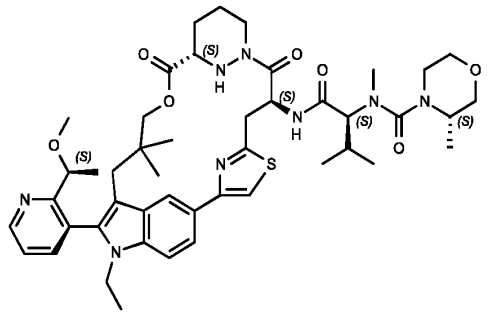
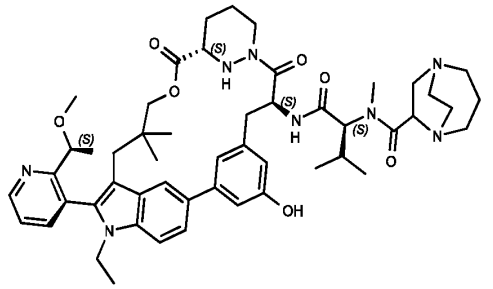
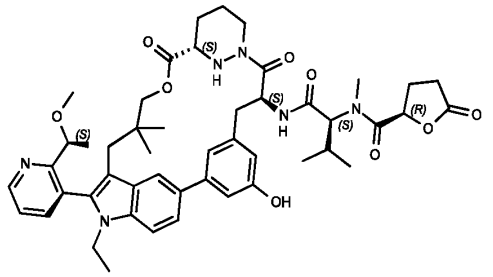
A381	
A382	
A383	
A384	

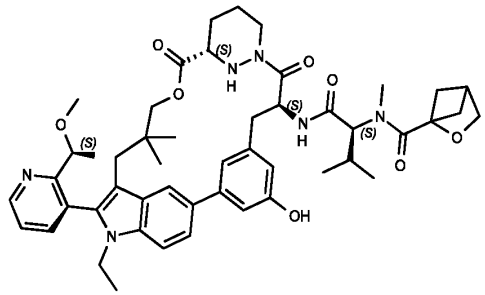
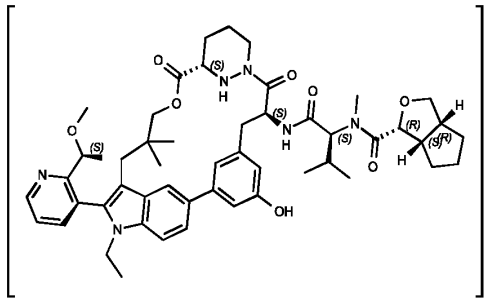
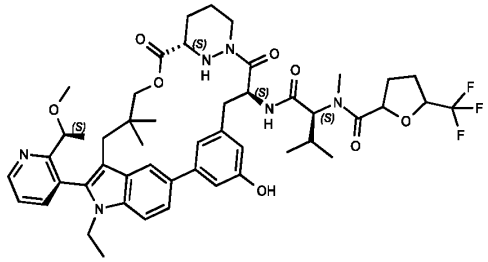
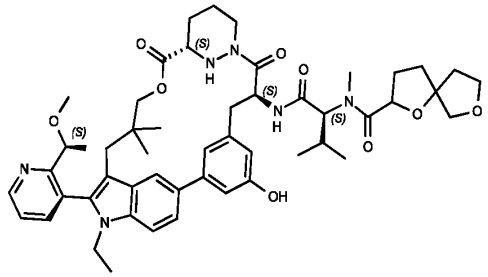
A385	
A386	
A387	
A388	

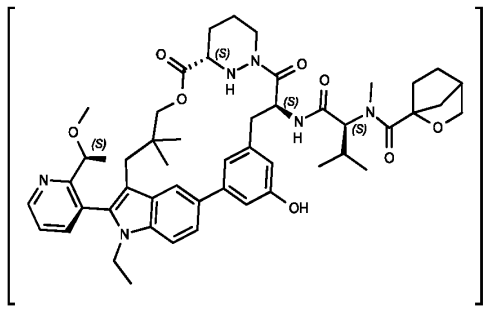
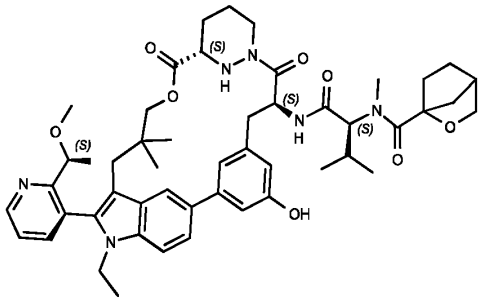
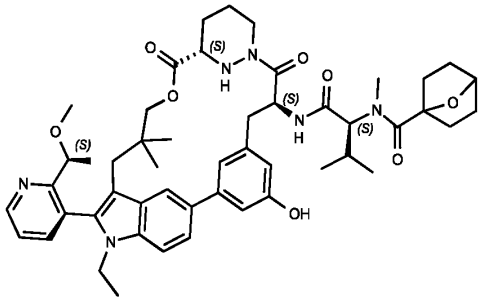
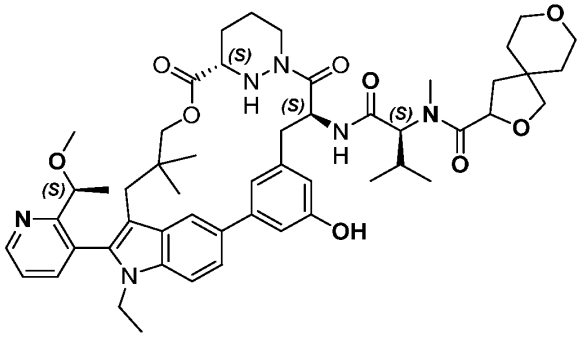
A389	
A391	
A392	
A393	

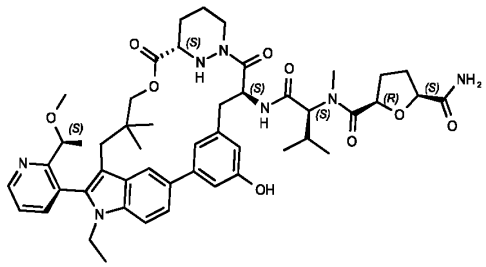
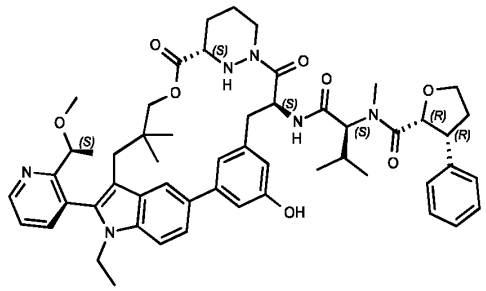
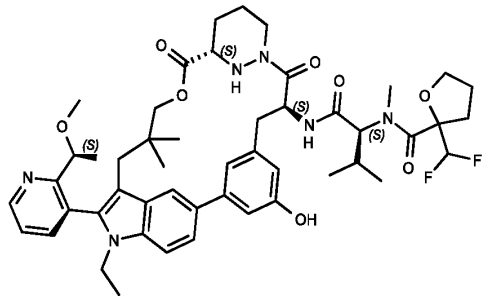
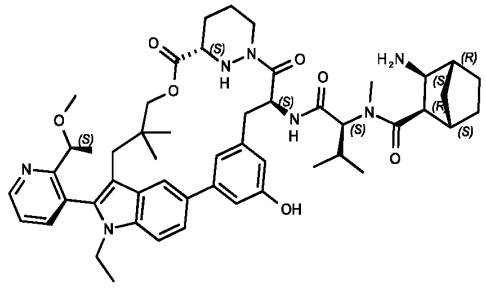
A394	
A395	
A396	
A397	

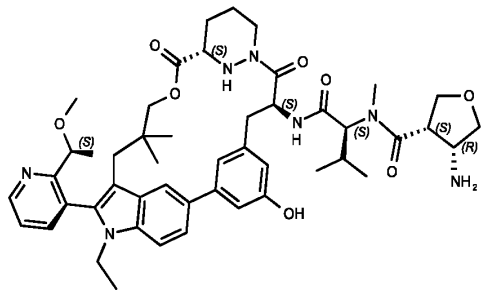
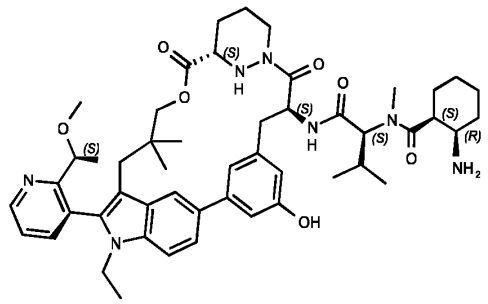
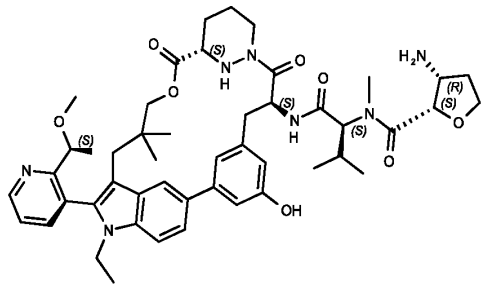
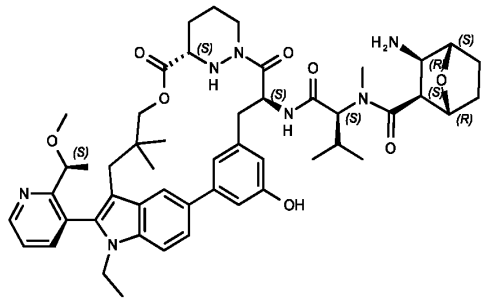
A398	
A399	
A400	
A401	

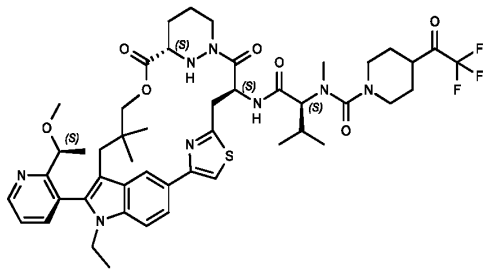
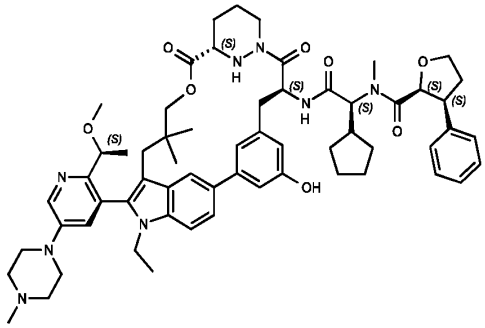
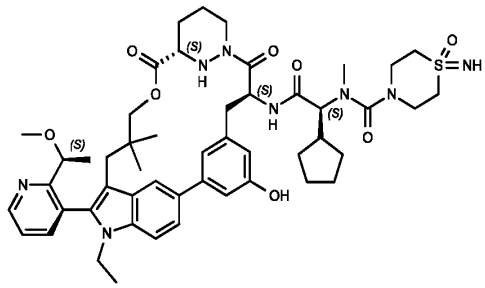
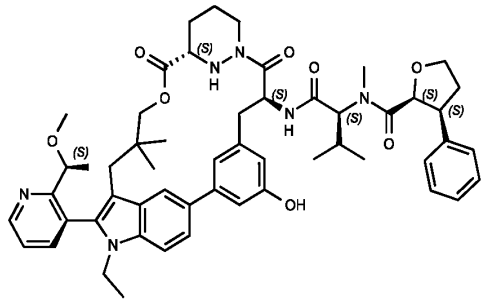
A402	
A403	
A404	
A405	

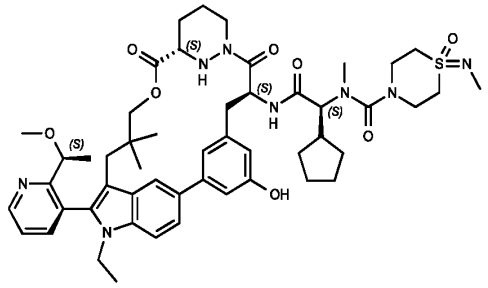
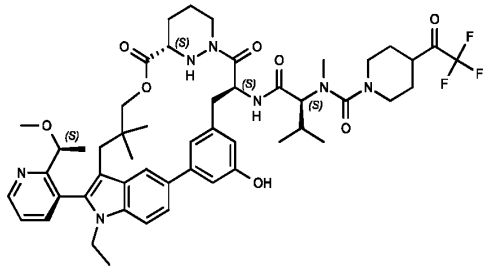
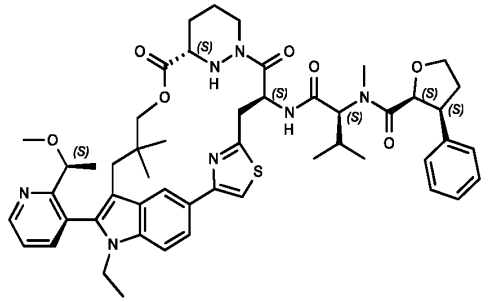
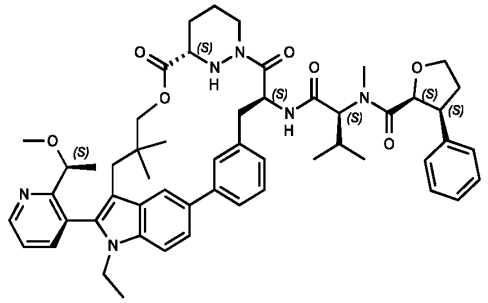
A406	
A407	
A408	
A409	

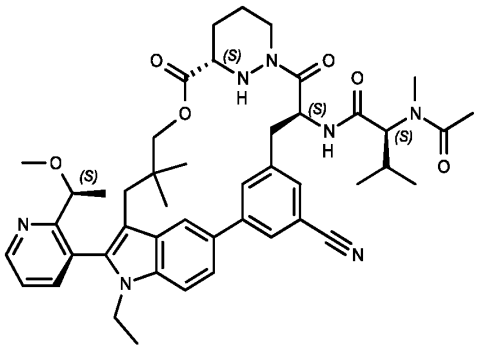
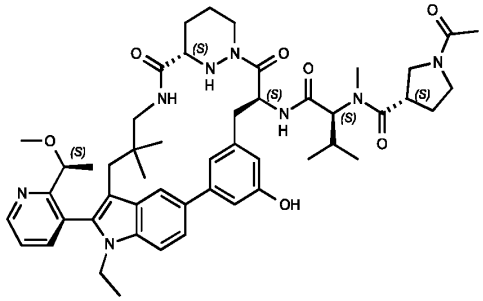
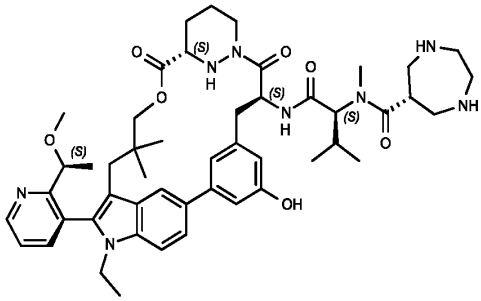
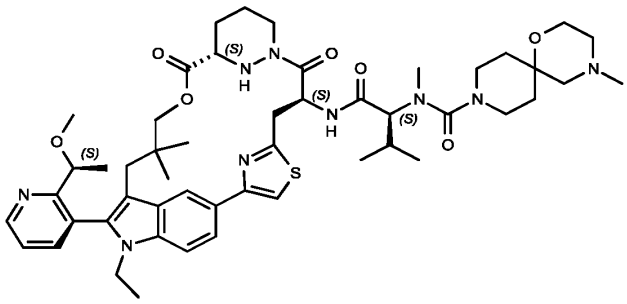
A410	
A411	
A412	
A413	

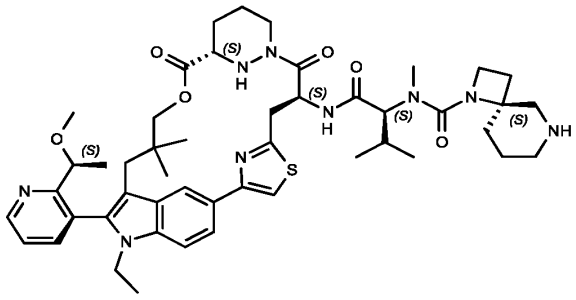
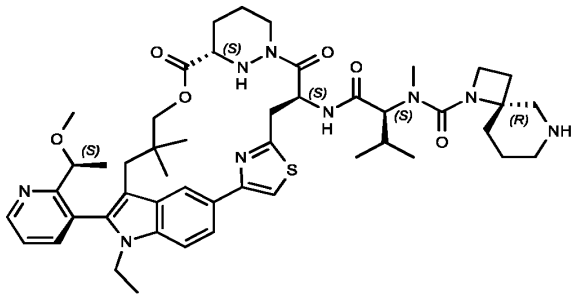
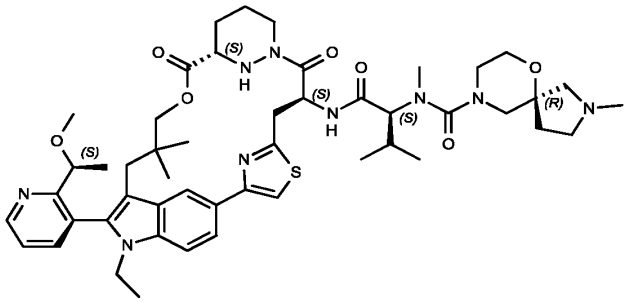
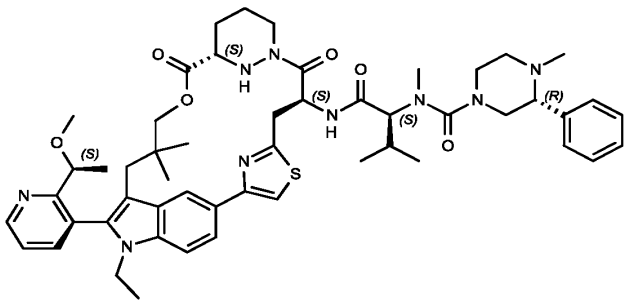
A414	
A415	
A416	
A417	

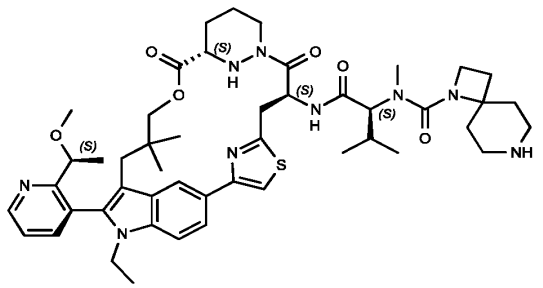
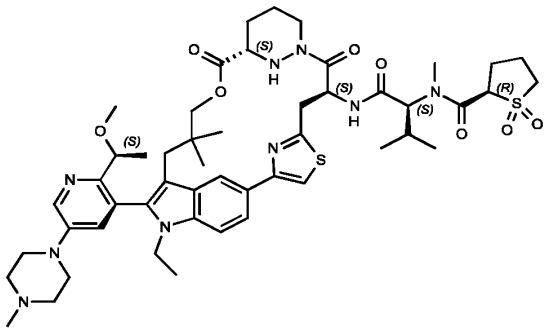
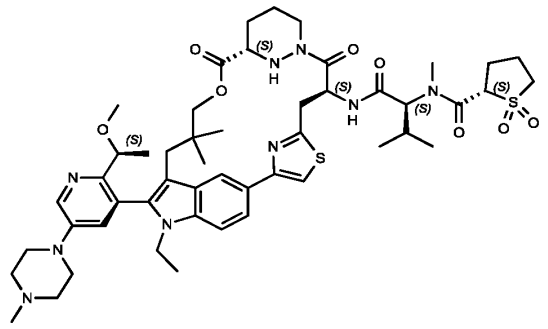
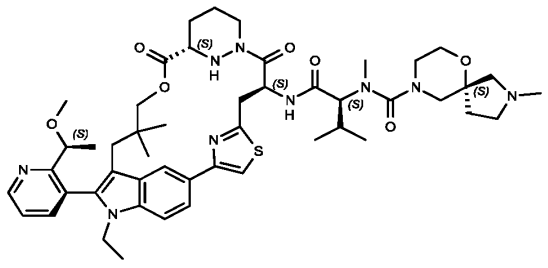
A418	
A419	
A420	
A421	

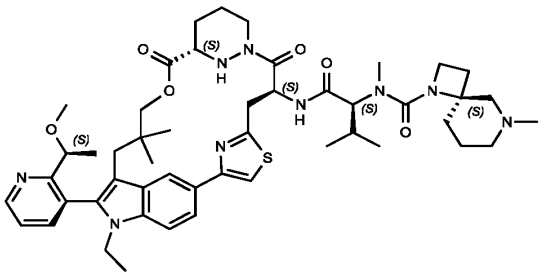
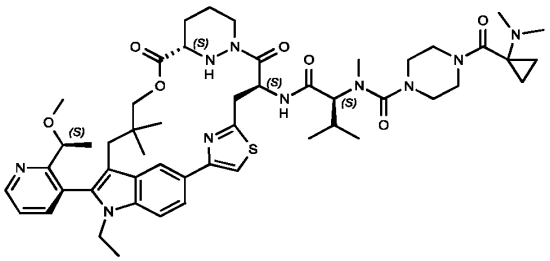
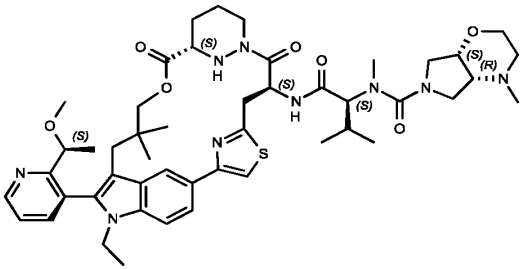
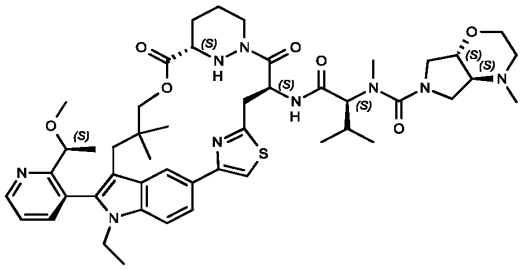
A422	
A423	
A424	
A425	

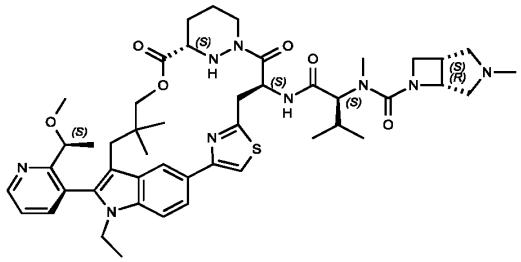
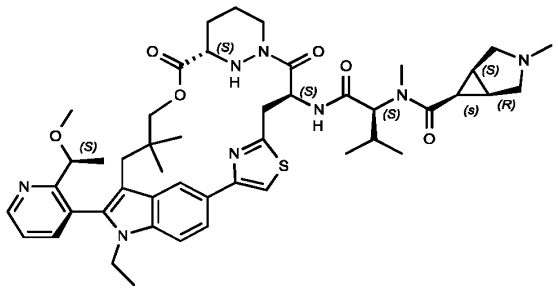
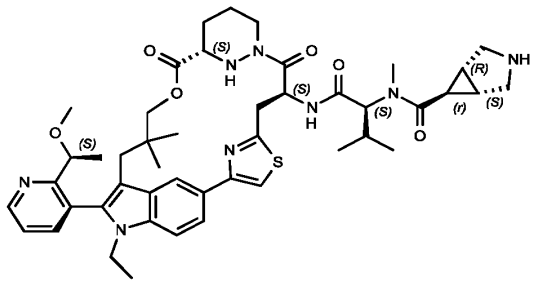
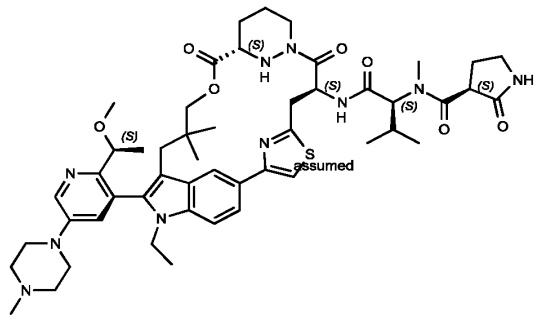
A426	
A427	
A428	
A429	

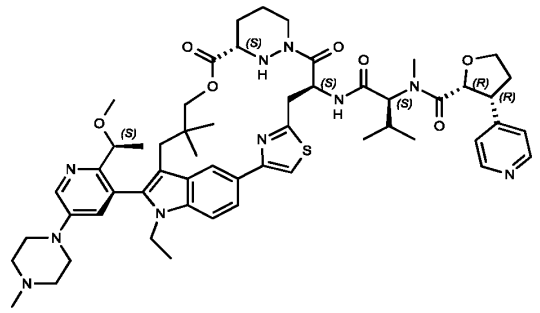
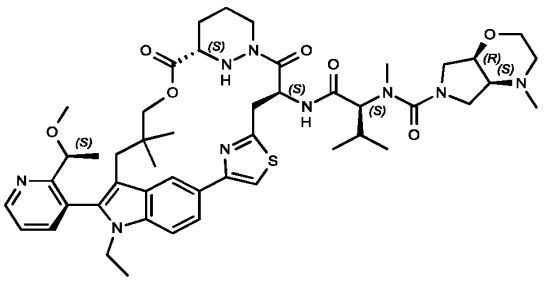
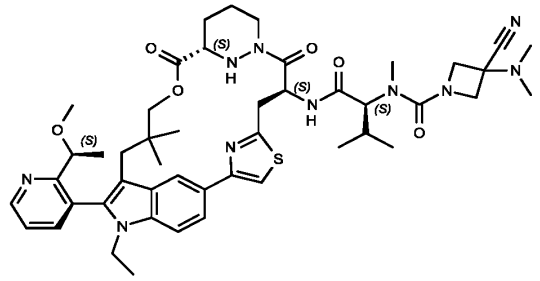
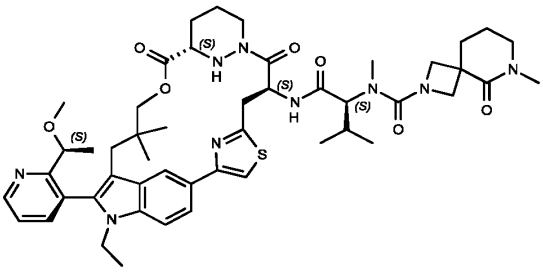
A430	
A431	
A432	
A433	

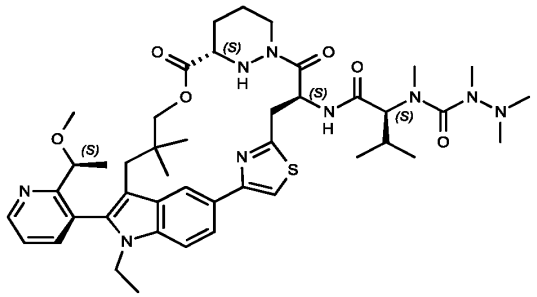
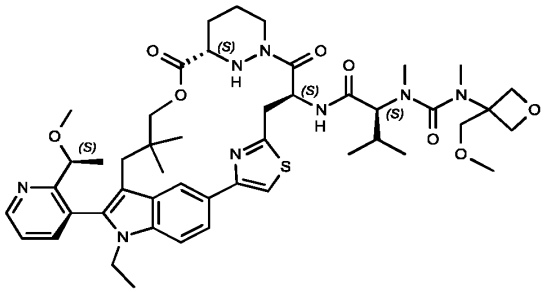
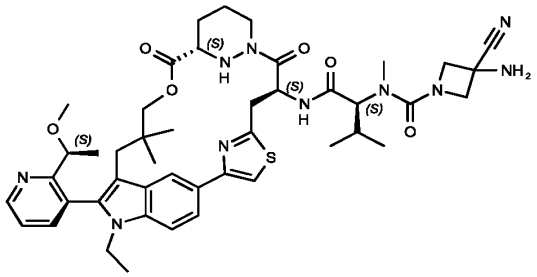
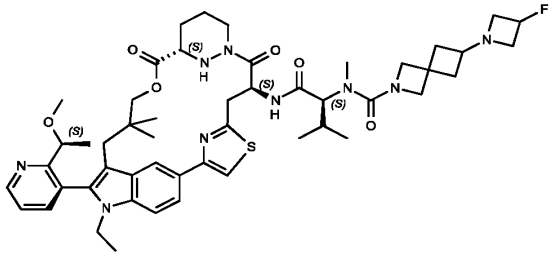
A434	
A435	
A436	
A437	

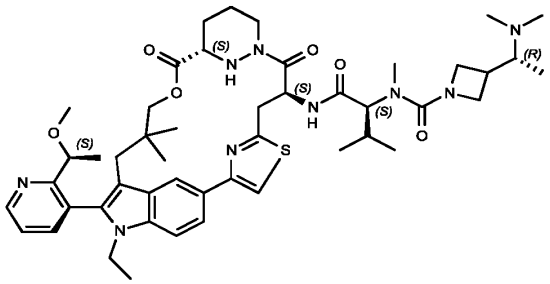
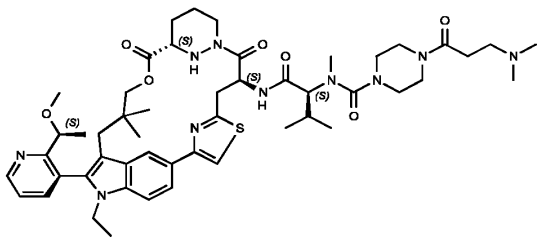
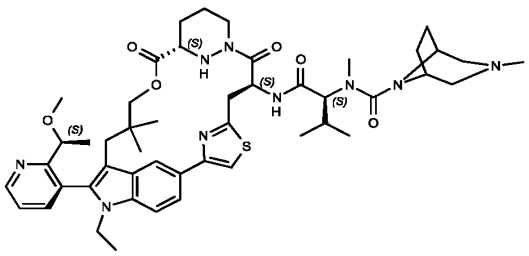
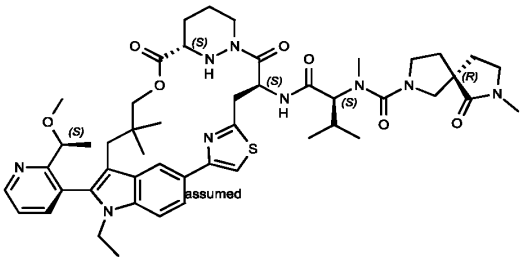
A438	
A439	
A440	
A441	

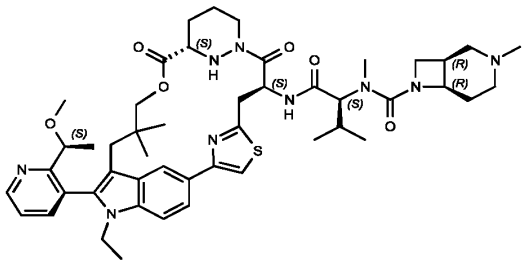
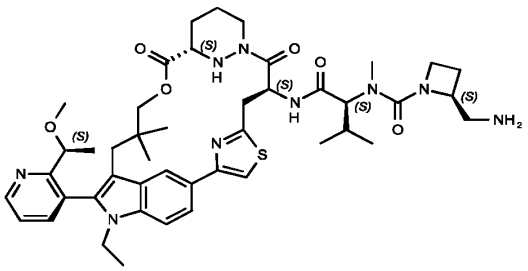
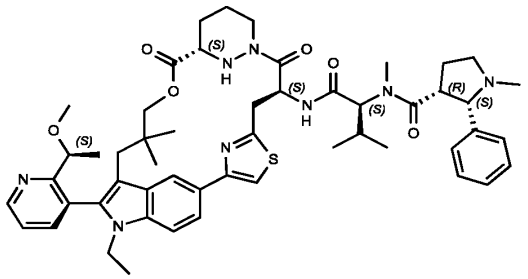
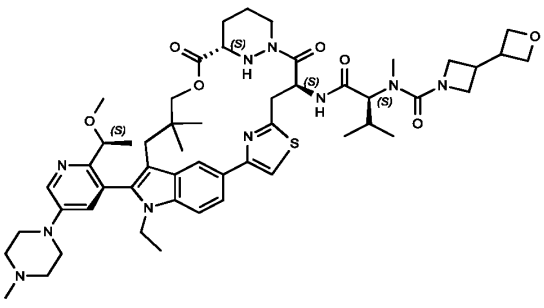
A442	
A443	
A444	
A445	

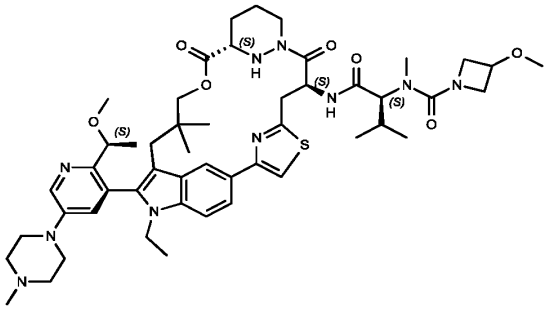
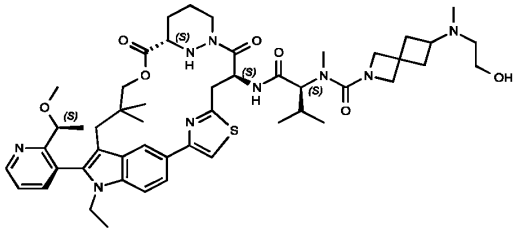
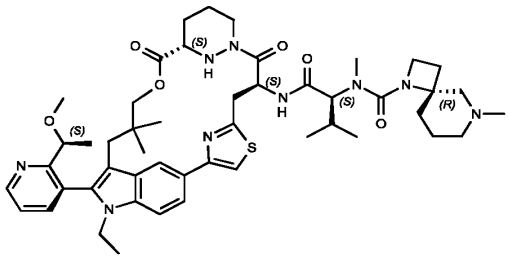
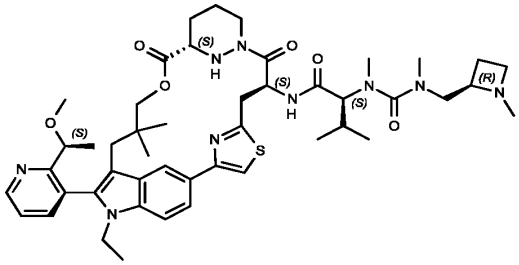
A446	
A447	
A448	
A449	

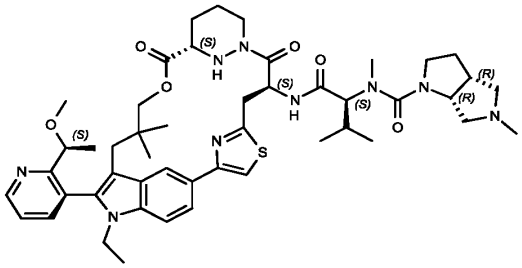
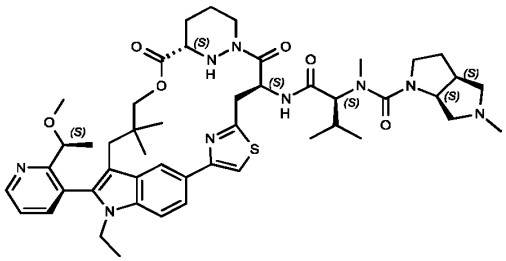
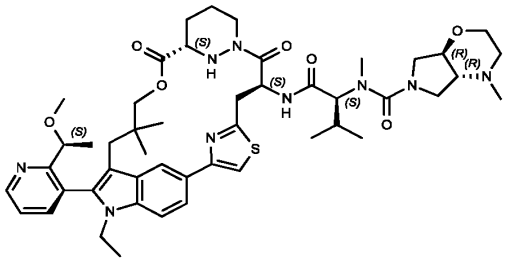
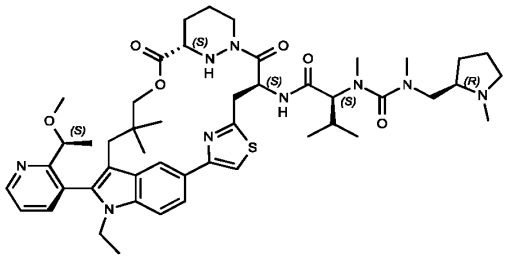
A450	
A451	
A452	
A453	

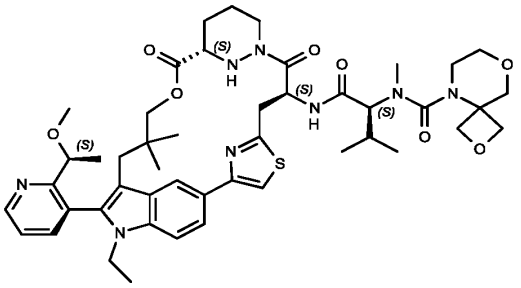
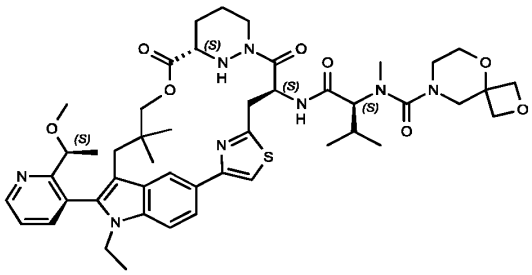
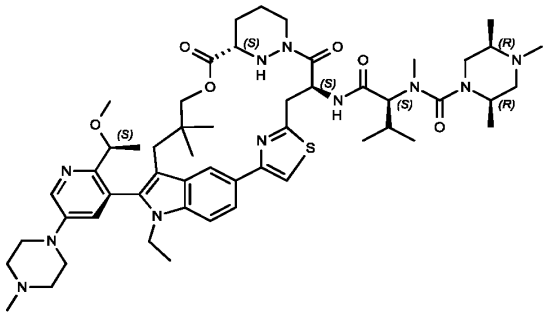
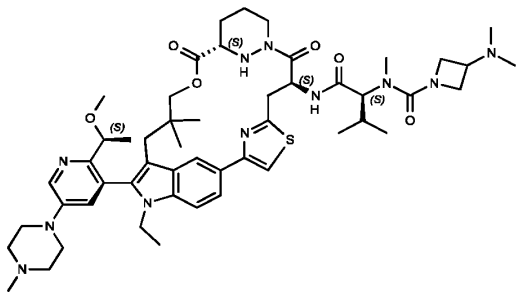
A454	
A455	
A456	
A457	

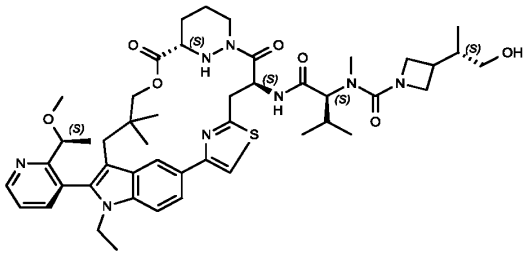
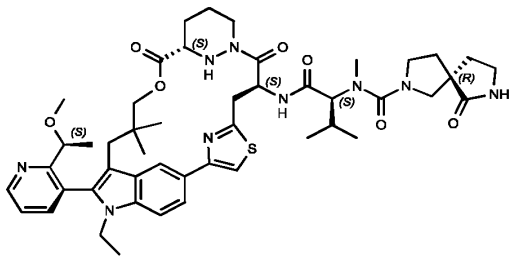
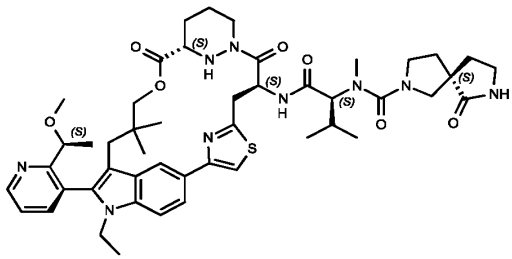
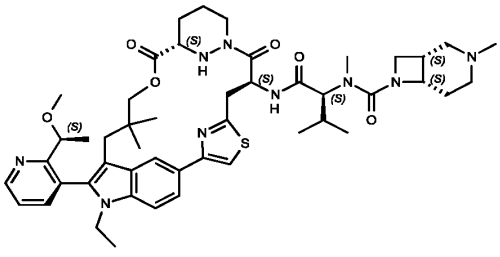
A458	
A459	
A460	
A461	

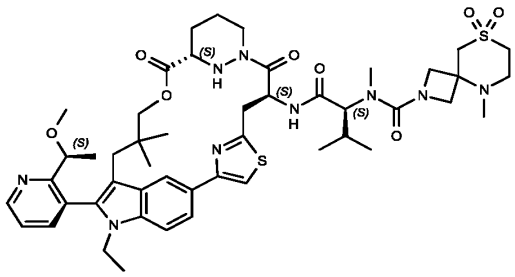
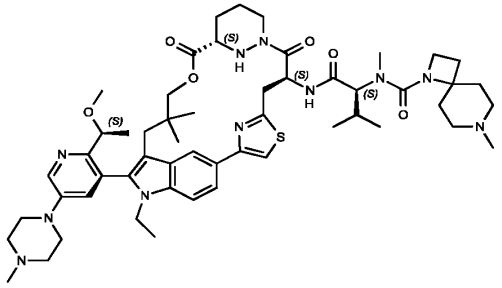
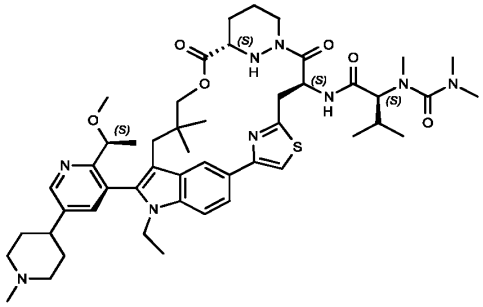
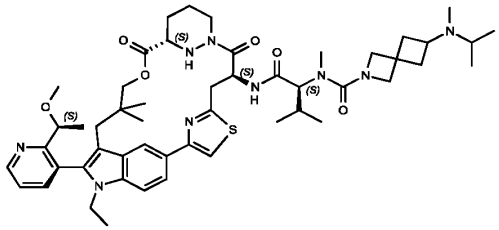
A462	
A463	
A464	
A465	

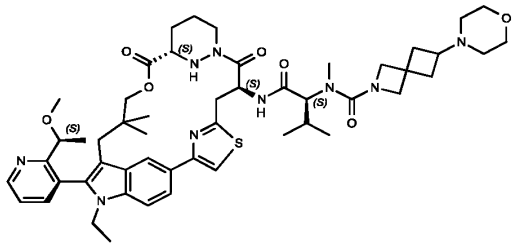
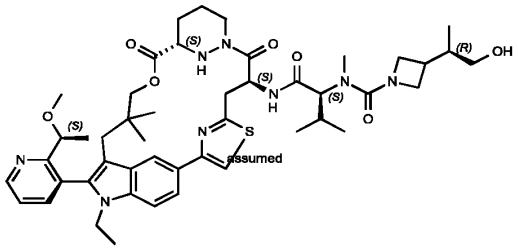
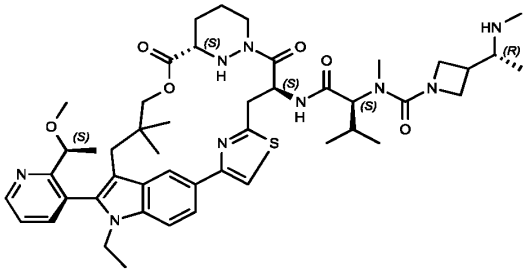
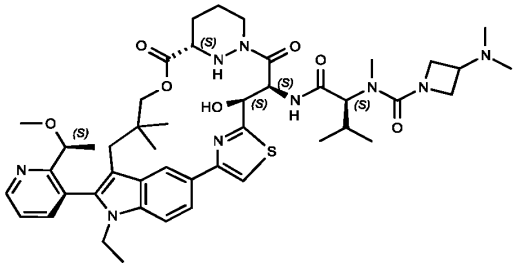
A466	
A467	
A468	
A469	

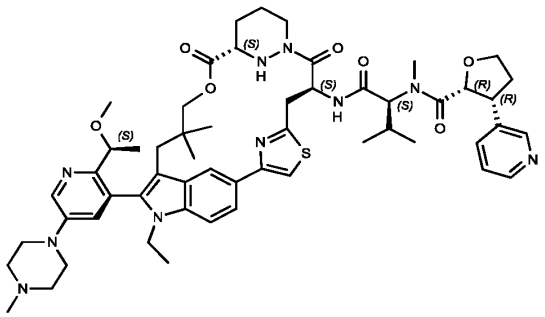
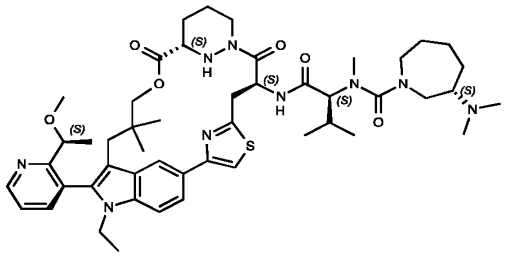
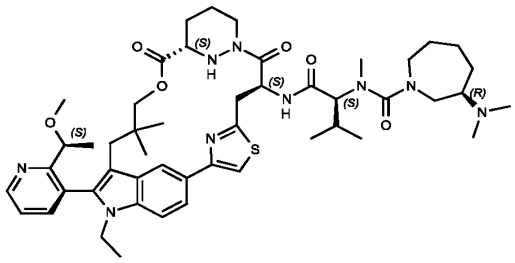
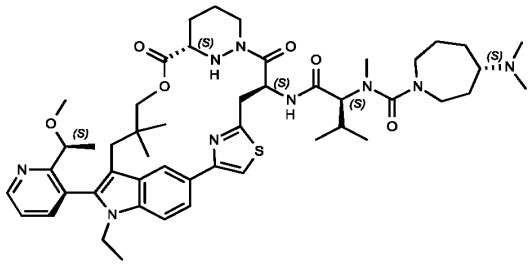
A470	
A471	
A472	
A473	

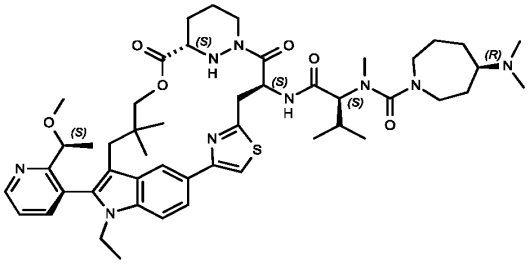
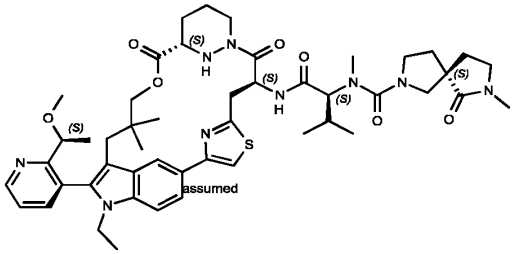
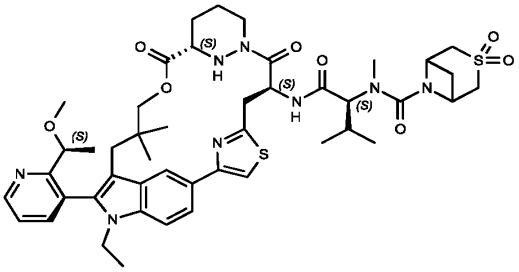
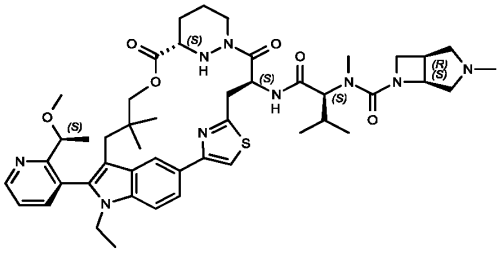
A474	
A475	
A476	
A477	

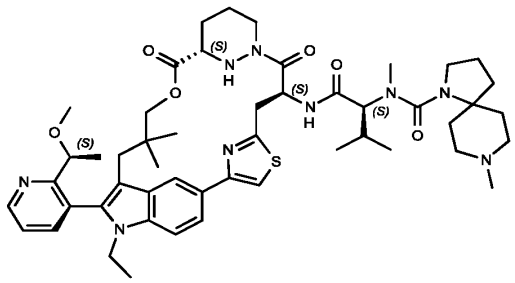
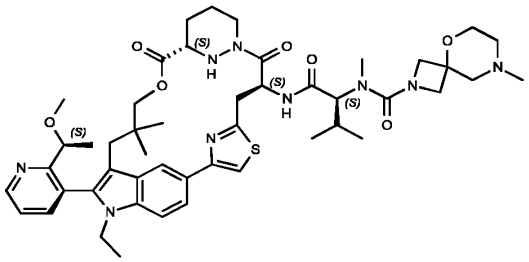
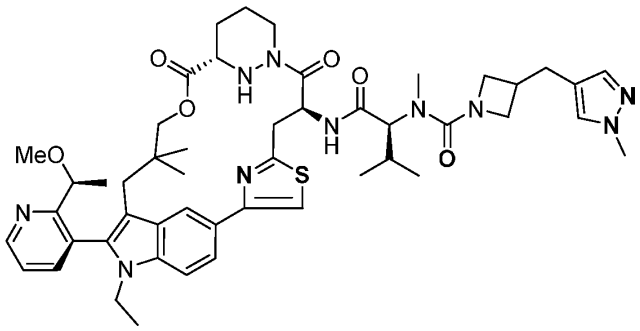
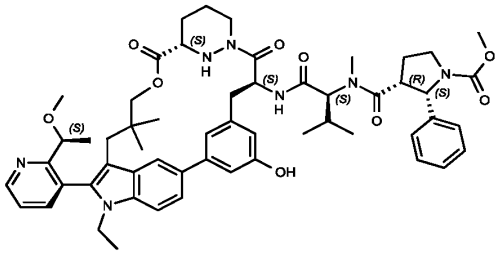
A478	
A479	
A480	
A481	

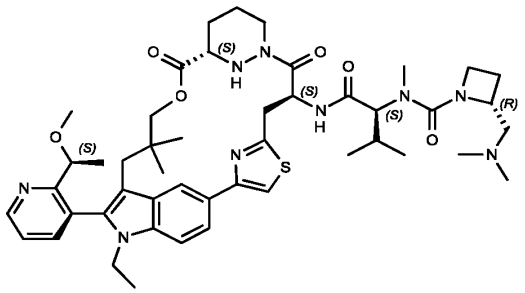
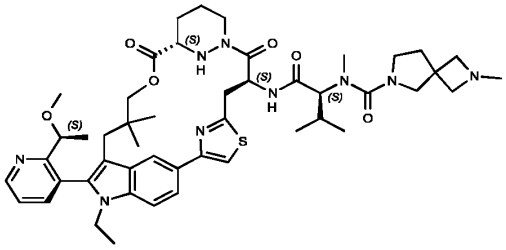
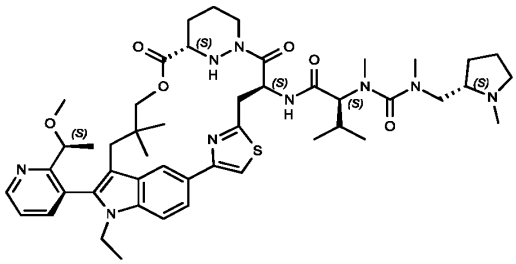
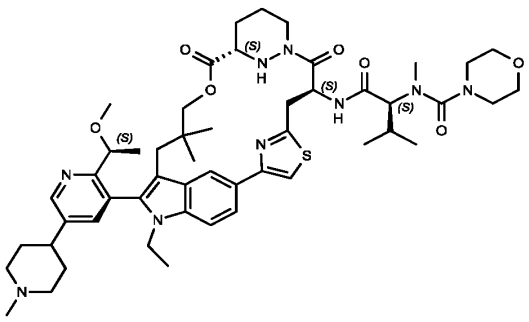
A482	
A483	
A484	
A485	

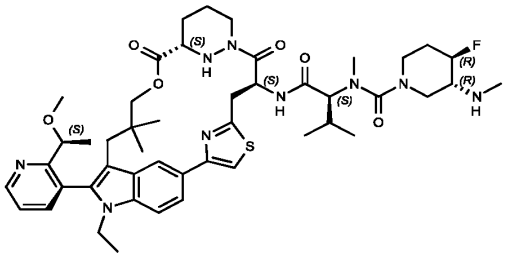
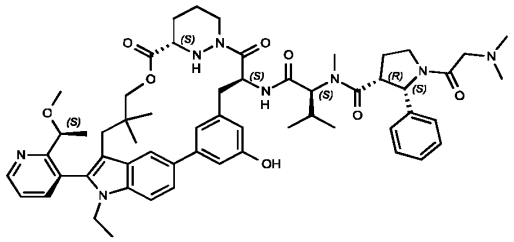
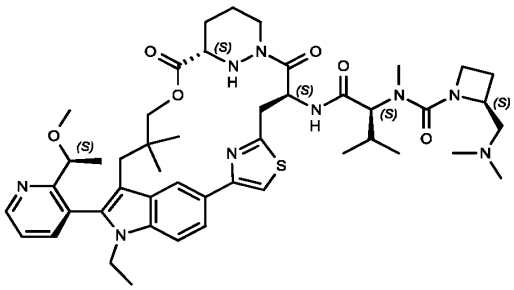
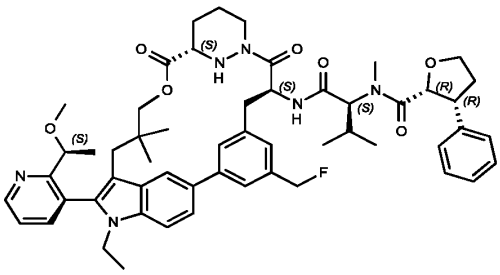
A486	
A487	
A488	
A489	

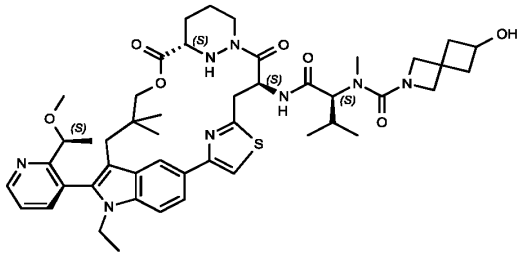
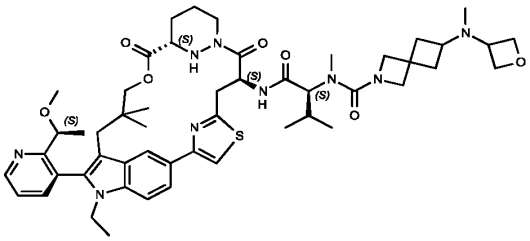
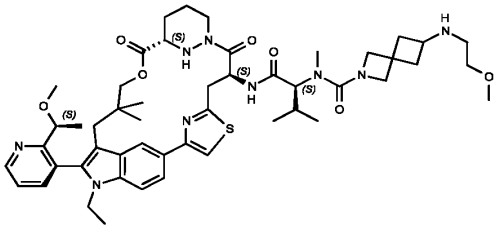
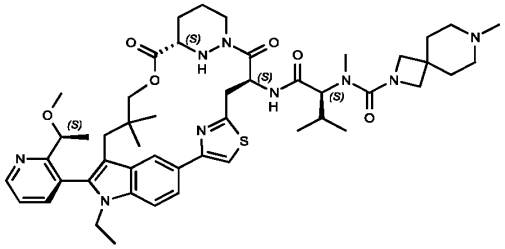
A490	
A491	
A492	
A493	

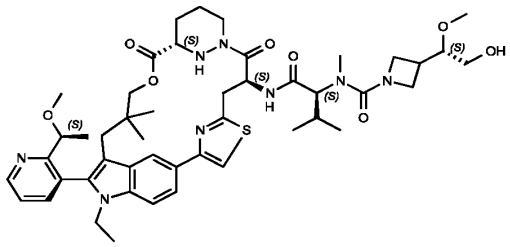
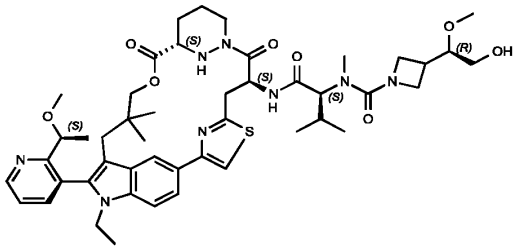
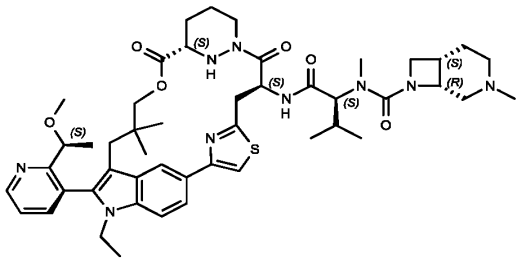
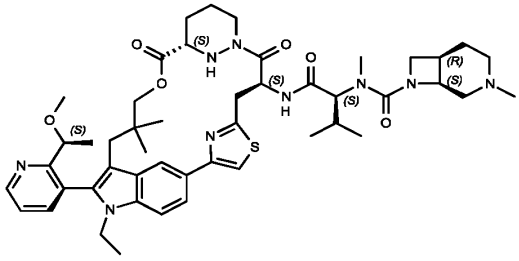
A494	
A495	
A496	
A497	

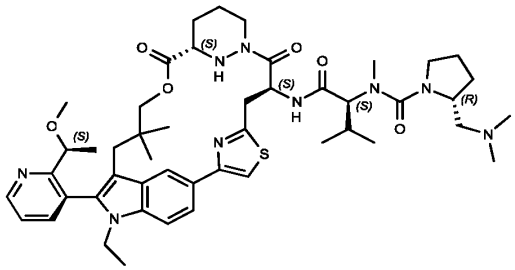
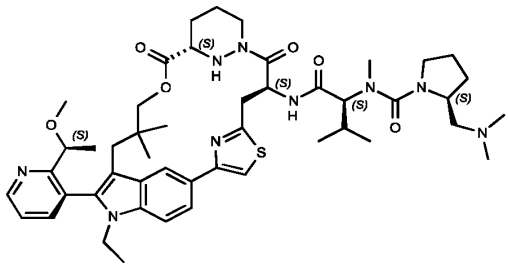
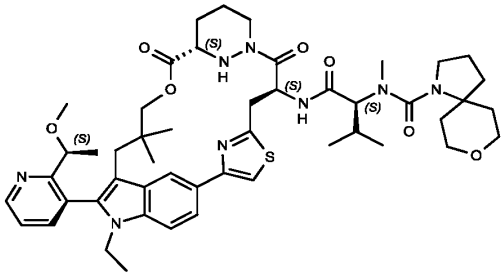
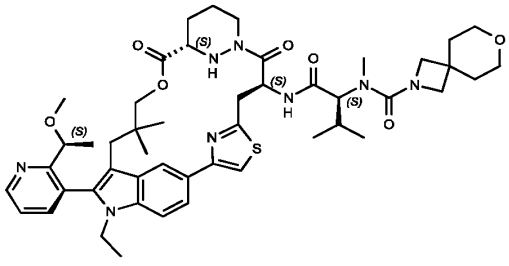
A498	
A499	
A500	
A501	

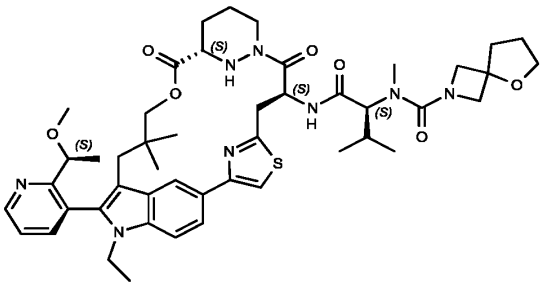
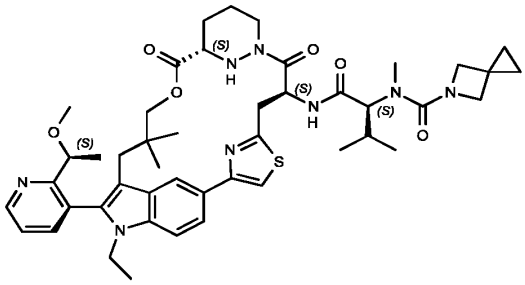
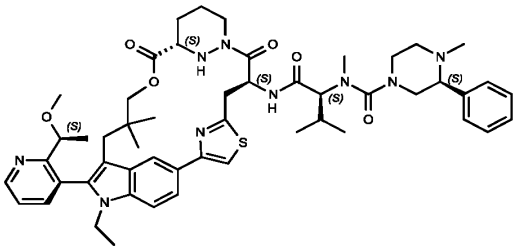
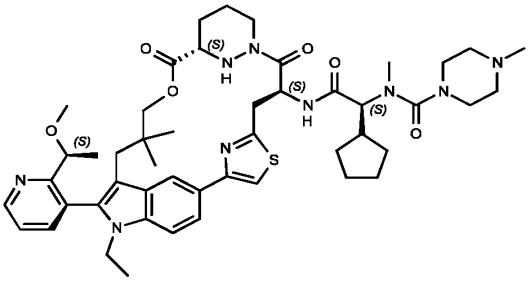
A502	
A503	
A504	
A505	

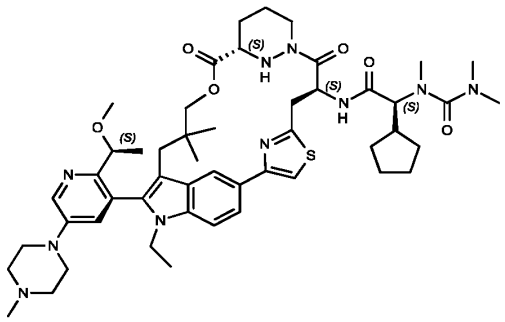
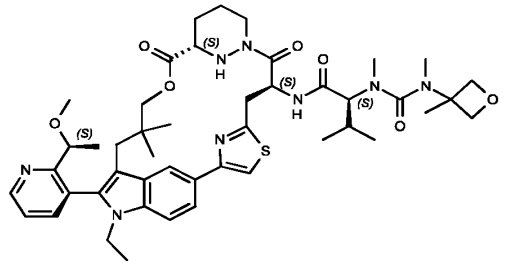
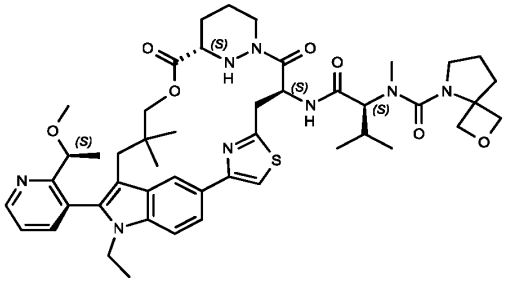
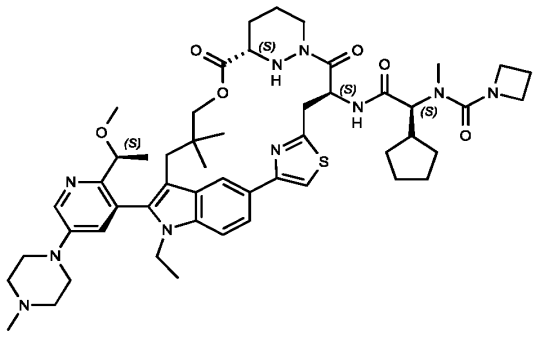
A506	
A507	
A508	
A509	

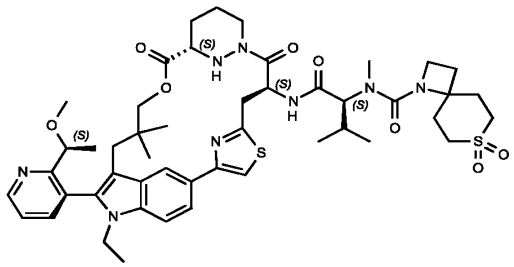
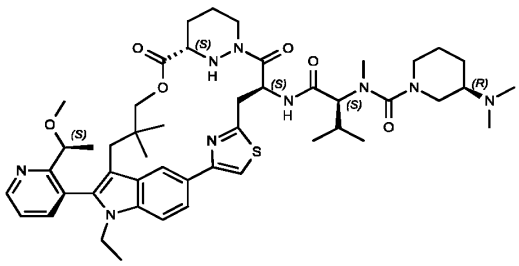
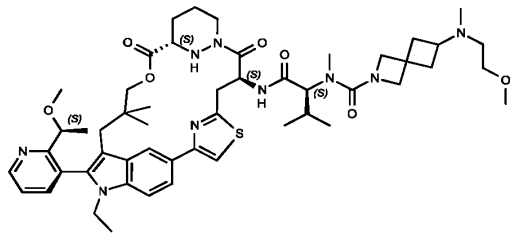
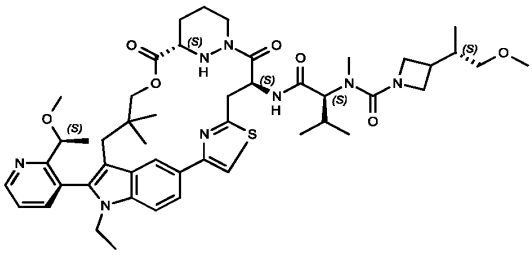
A510	
A511	
A512	
A513	

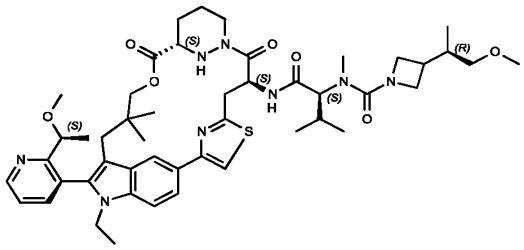
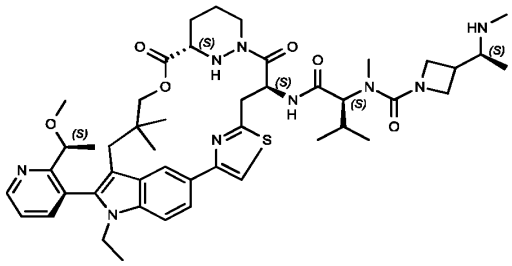
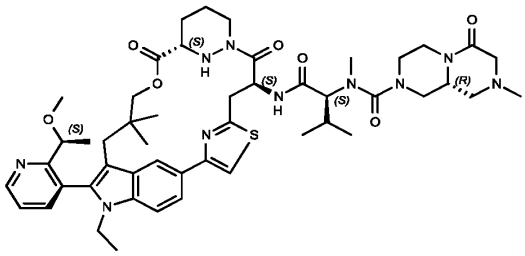
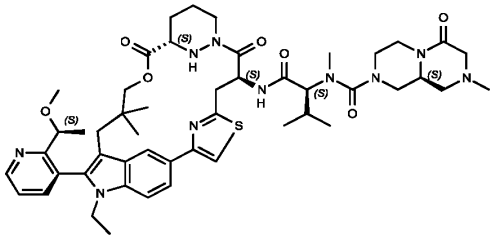
A514	
A515	
A516	
A517	

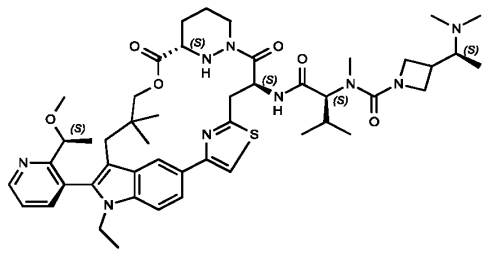
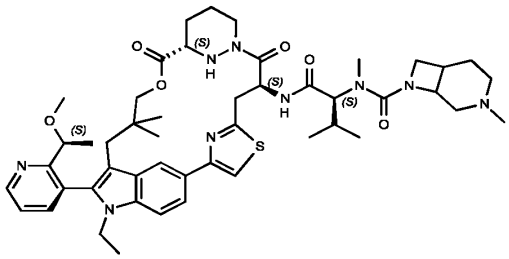
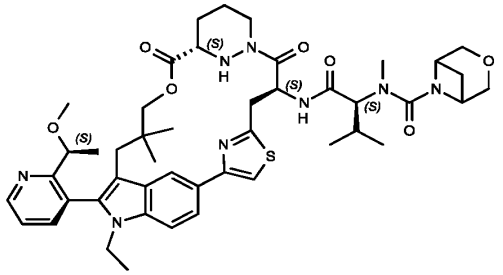
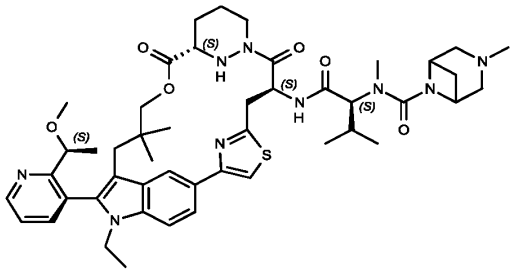
A518	
A519	
A520	
A521	

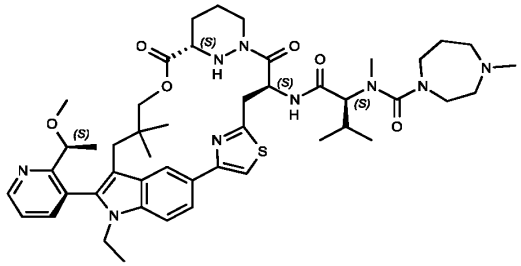
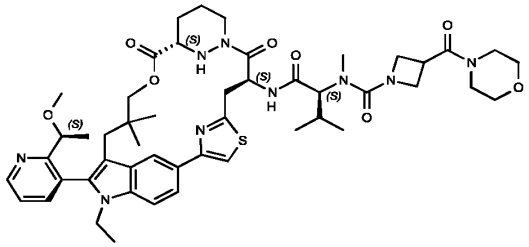
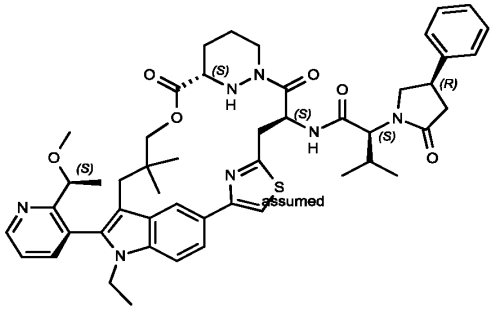
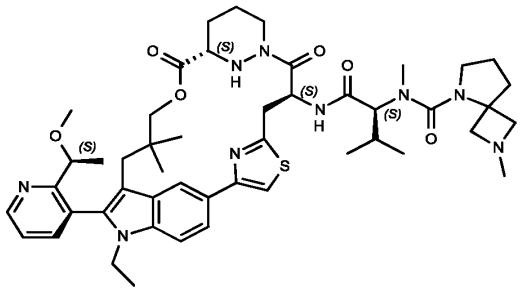
A522	
A523	
A524	
A525	

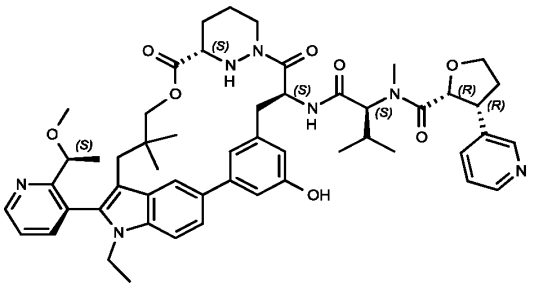
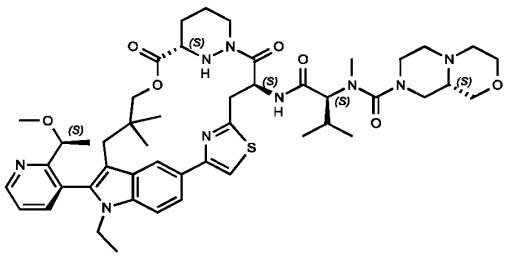
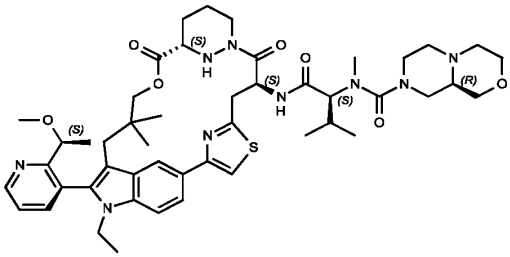
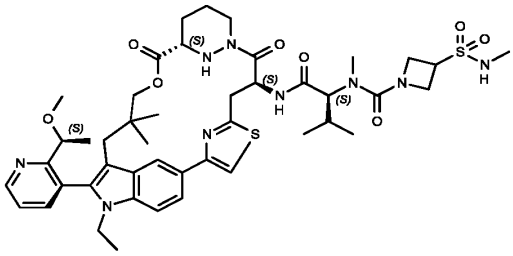
A526	
A527	
A528	
A529	

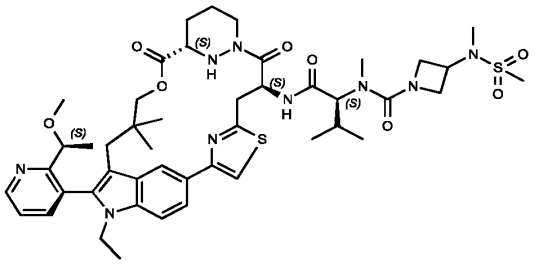
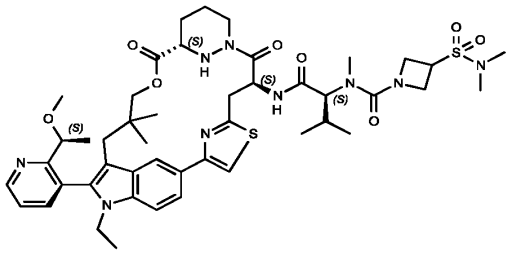
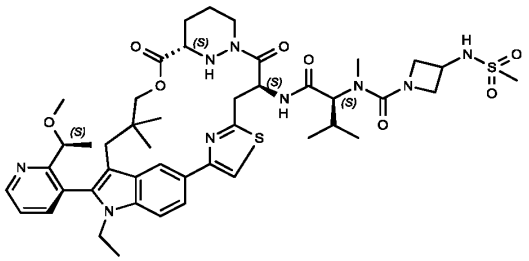
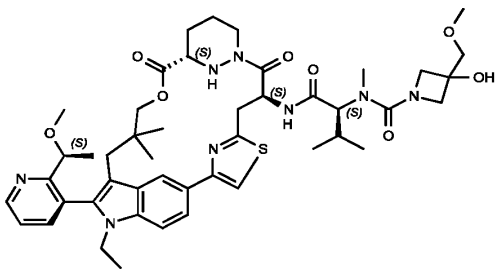
A530	
A531	
A532	
A533	

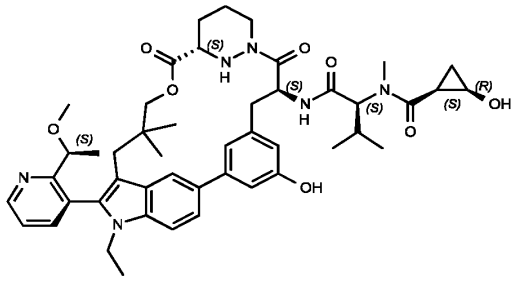
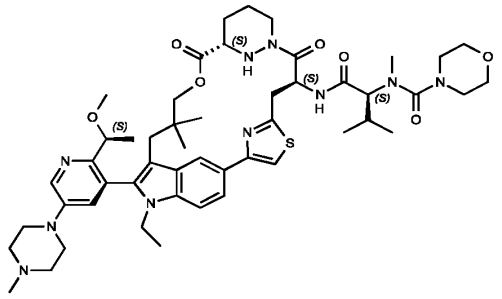
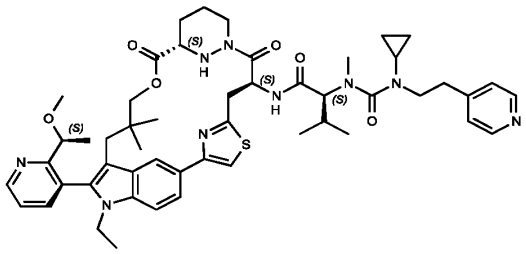
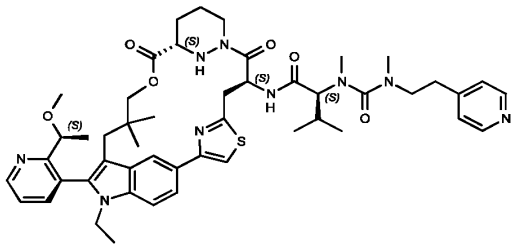
A534	
A535	
A536	
A537	

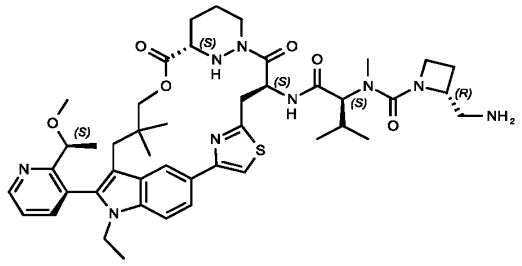
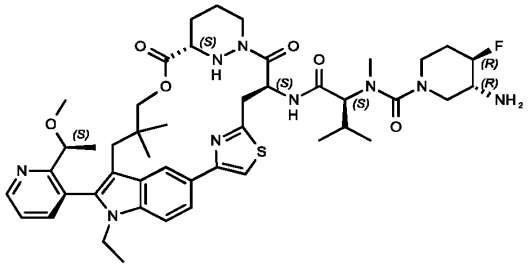
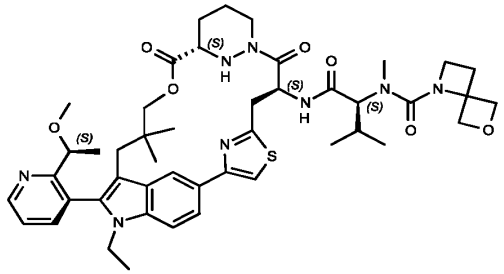
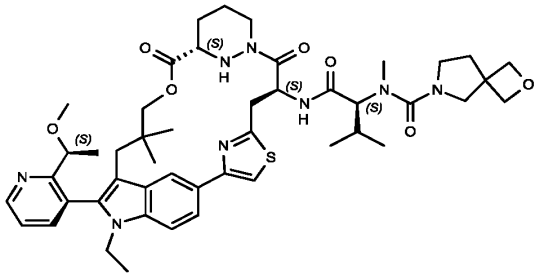
A538	
A539	
A540	
A541	

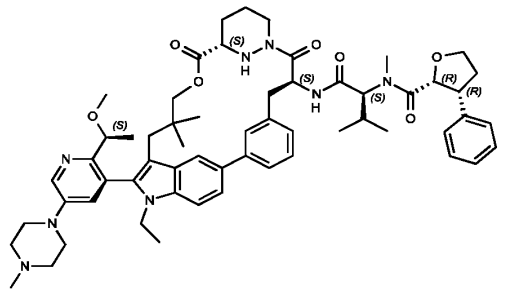
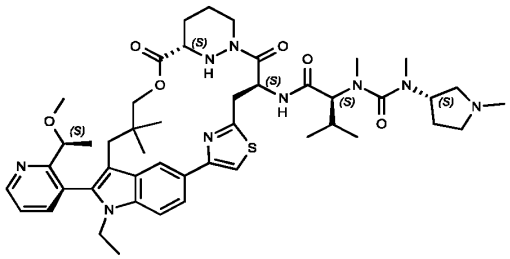
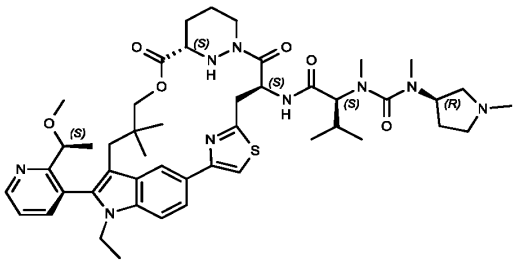
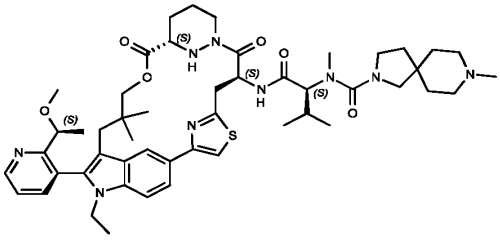
A542	
A543	
A544	
A545	

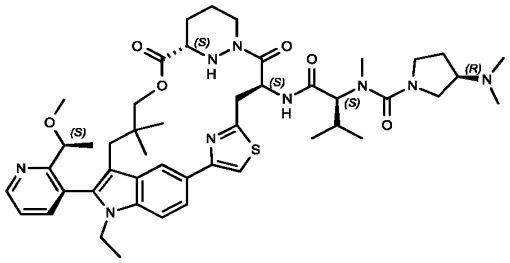
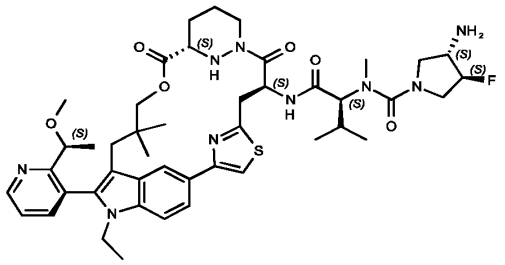
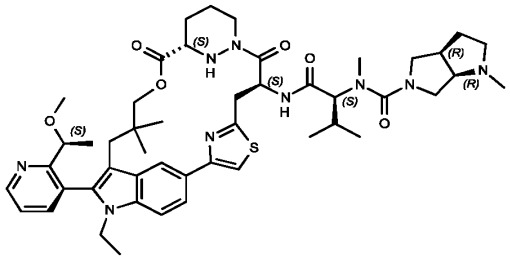
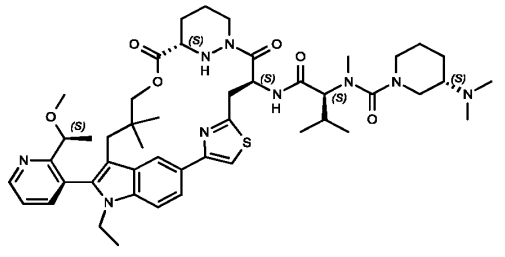
A546	
A547	
A548	
A549	

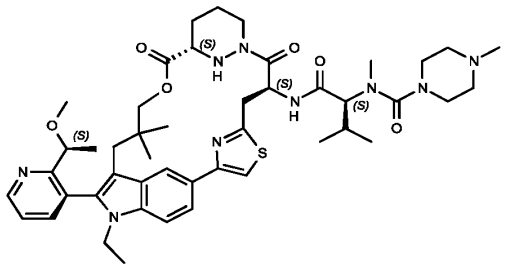
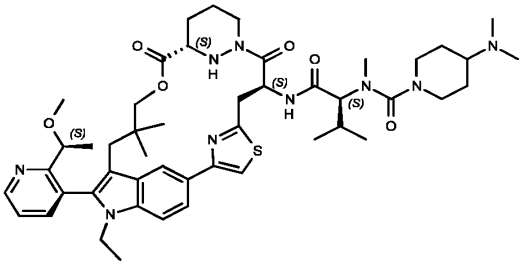
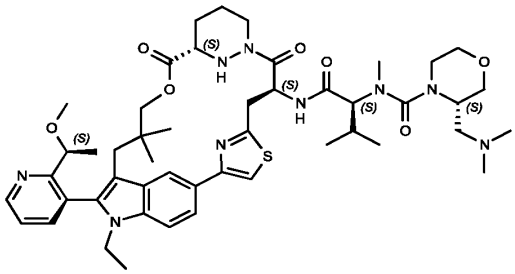
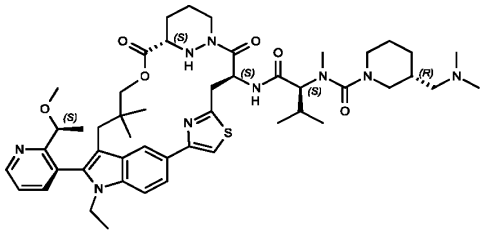
A550	
A551	
A552	
A553	

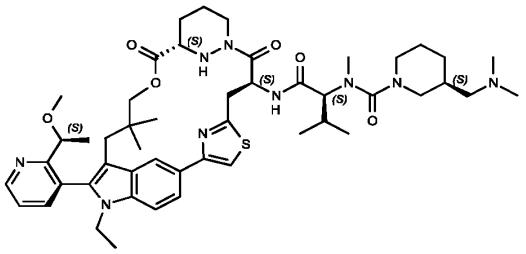
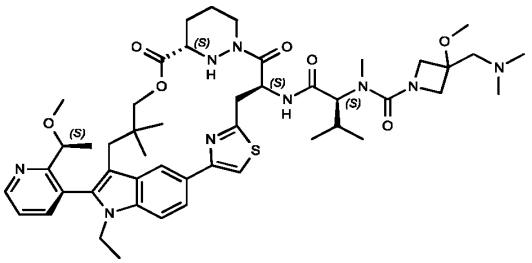
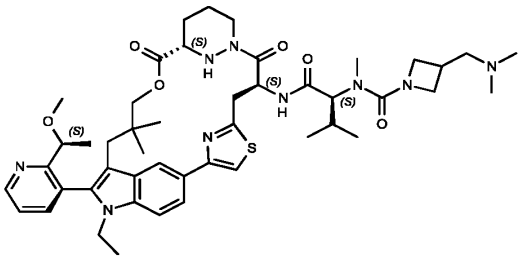
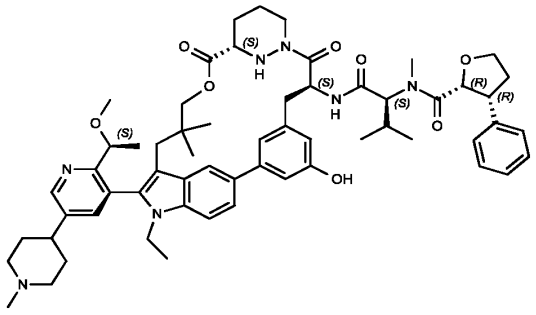
A554	
A555	
A556	
A557	

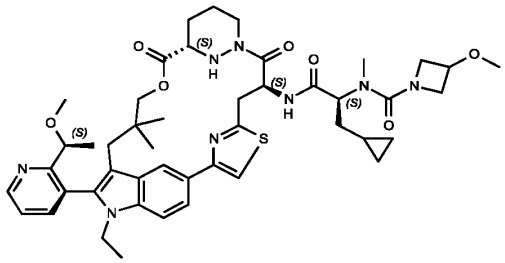
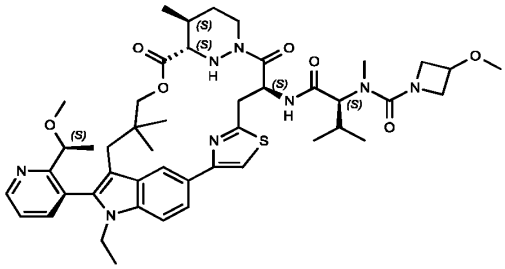
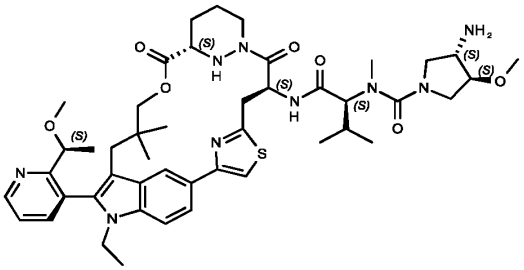
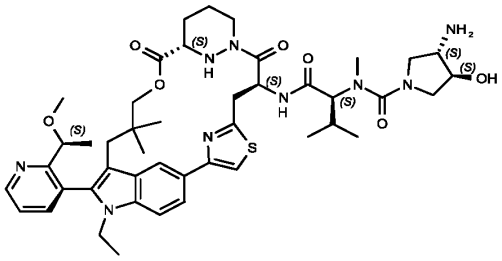
A558	
A559	
A560	
A561	

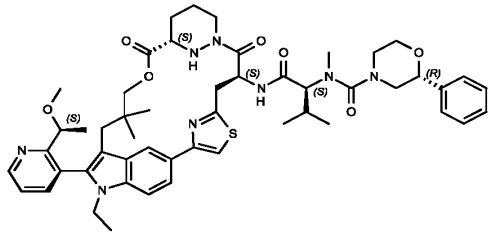
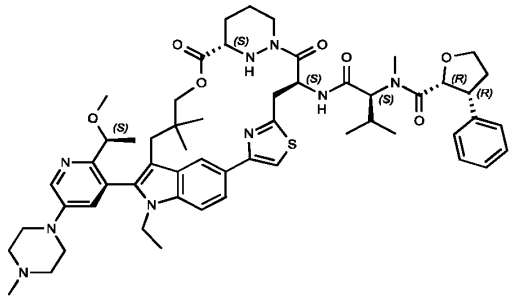
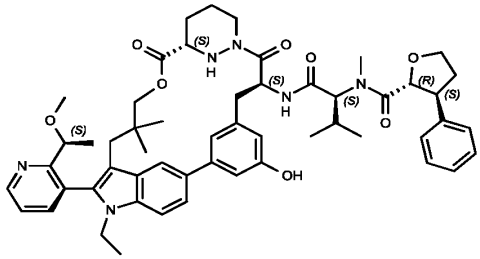
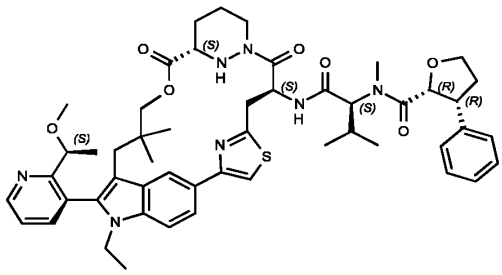
A562	
A563	
A564	
A565	

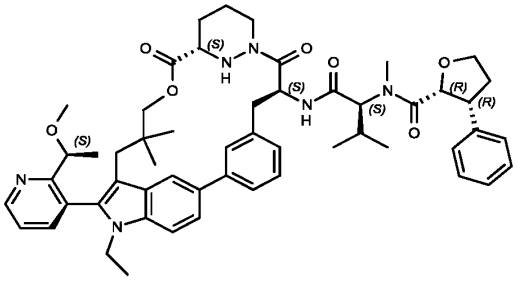
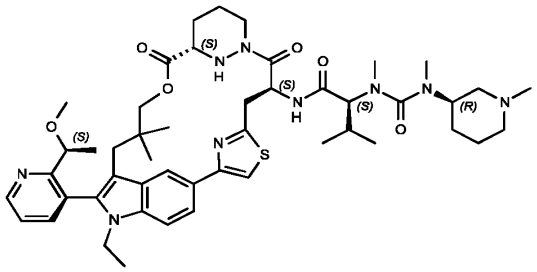
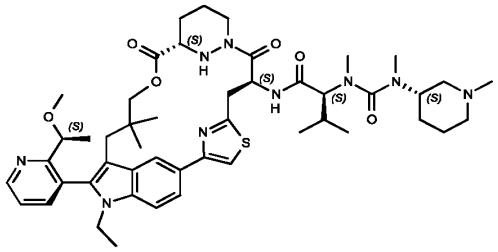
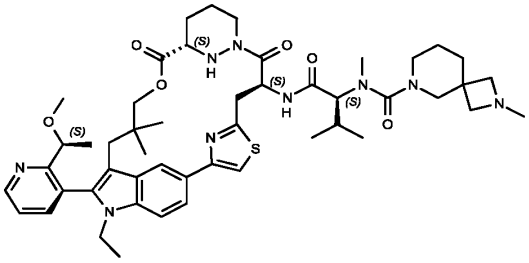
A566	
A567	
A568	
A569	

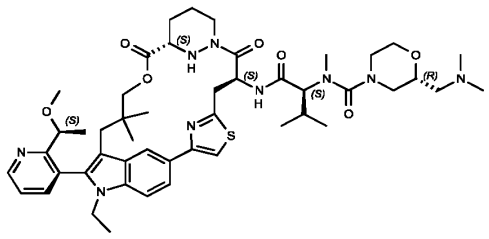
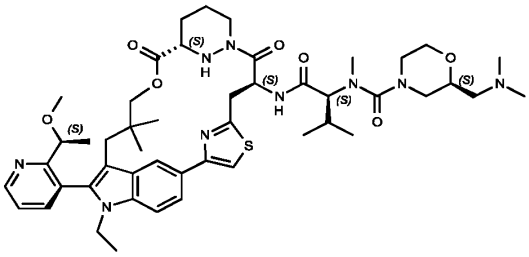
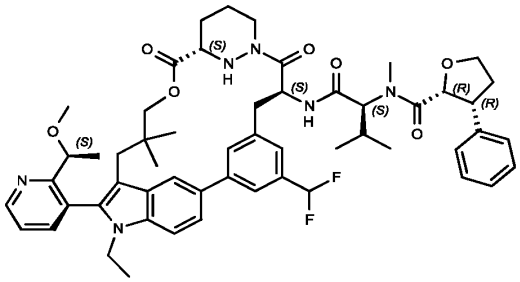
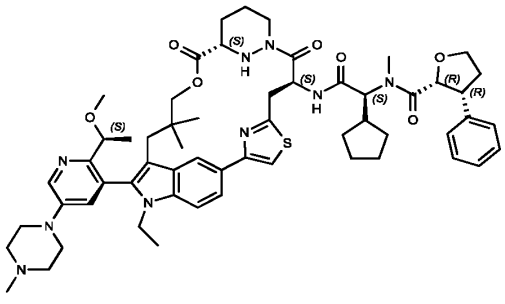
A570	
A571	
A572	
A573	

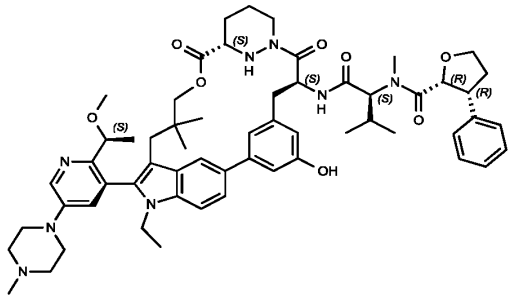
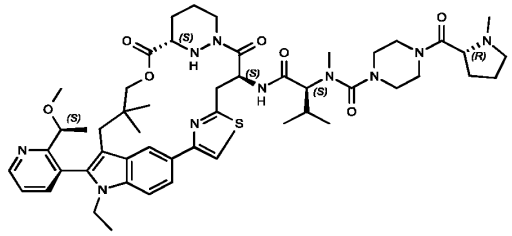
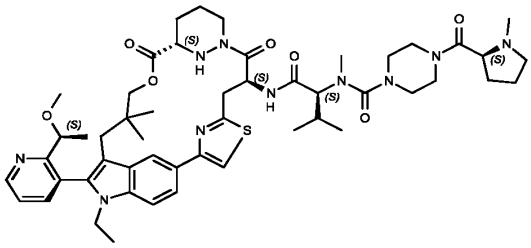
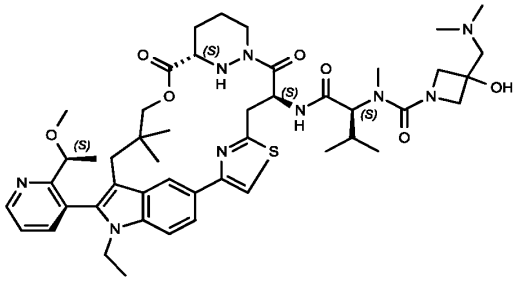
A574	
A575	
A576	
A577	

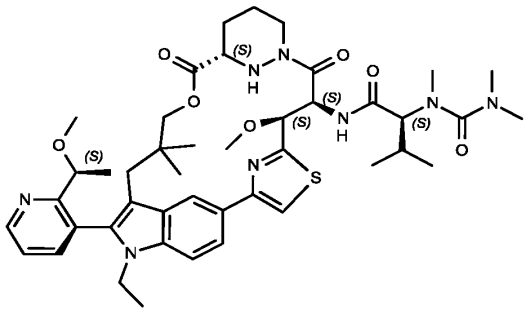
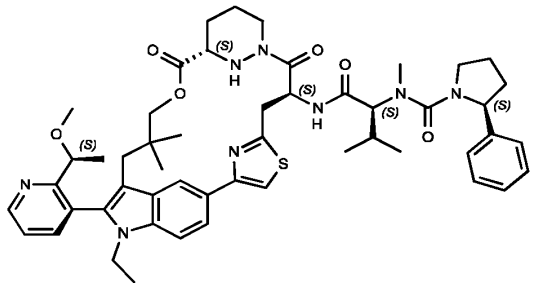
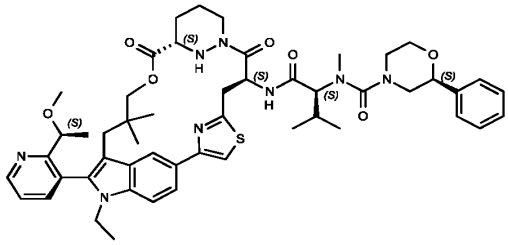
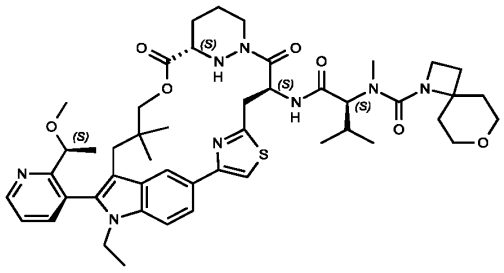
A578	
A579	
A580	
A581	

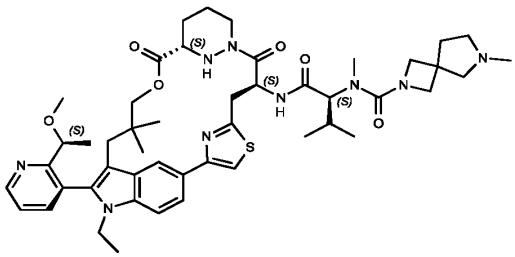
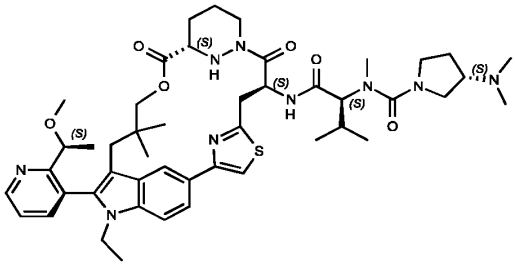
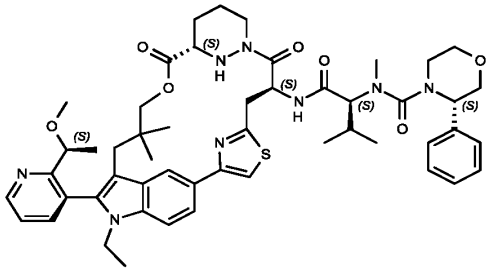
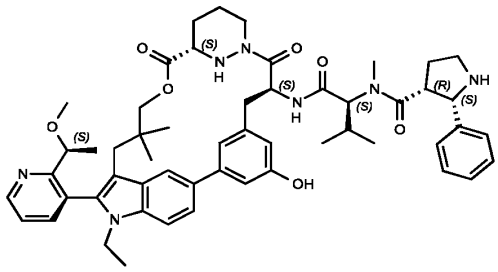
A582	
A583	
A584	
A585	

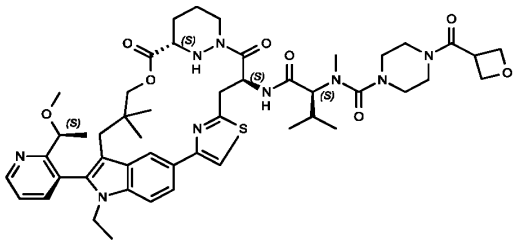
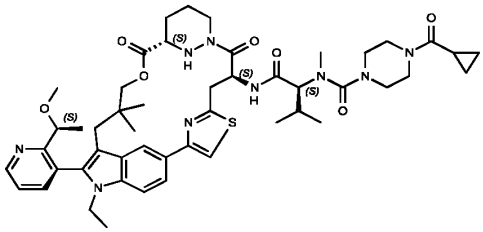
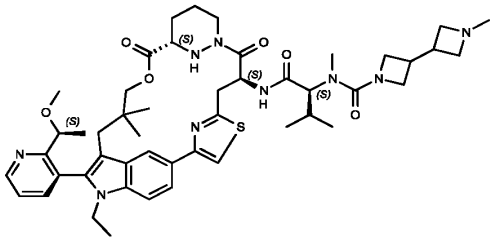
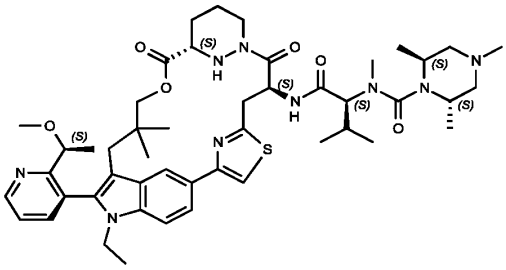
A586	
A587	
A588	
A589	

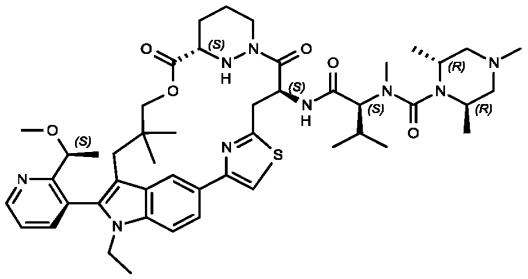
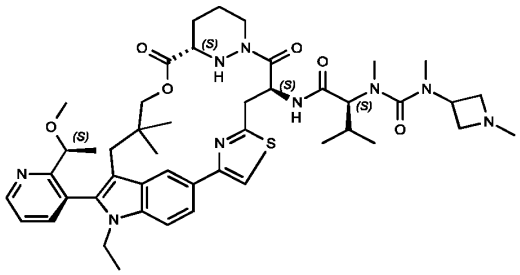
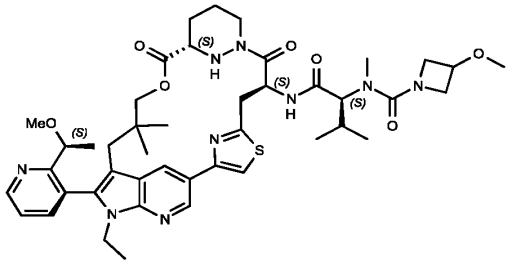
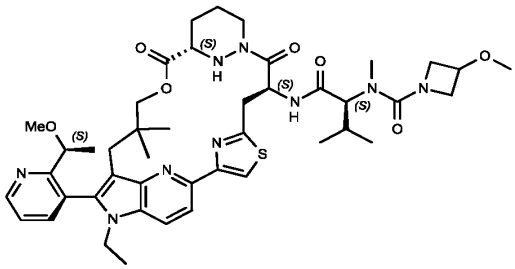
A590	
A591	
A592	
A593	

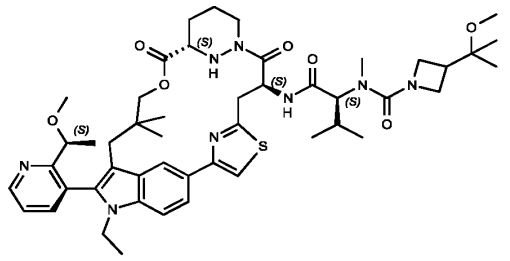
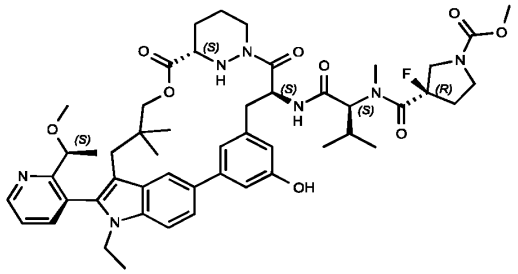
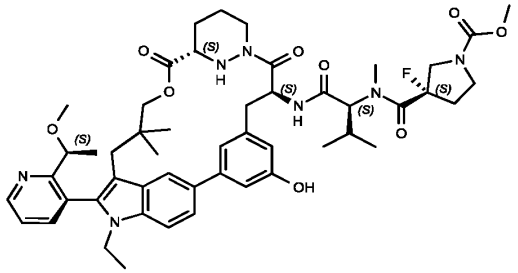
A594	
A595	
A596	
A597	

A598	
A599	
A600	
A601	

A602	
A603	
A604	
A605	

A606	
A607	
A608	
A609	

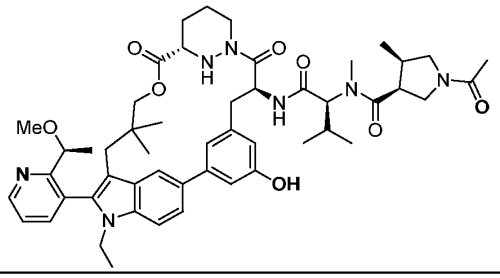
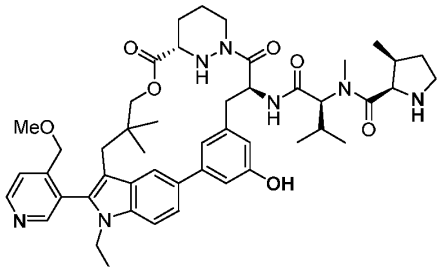
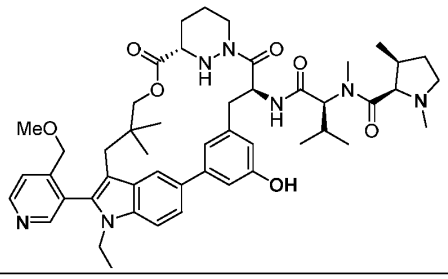
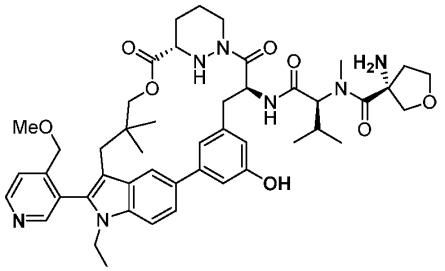
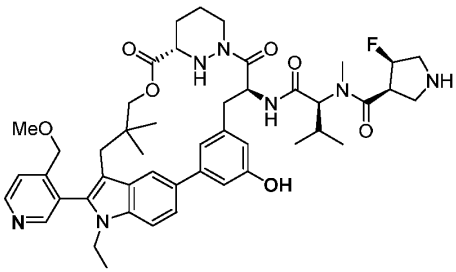
A610	
A611	
A612	
A613	

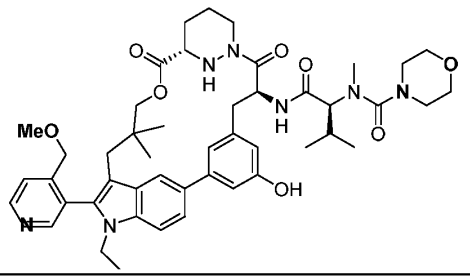
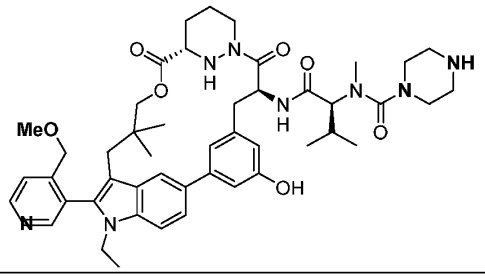
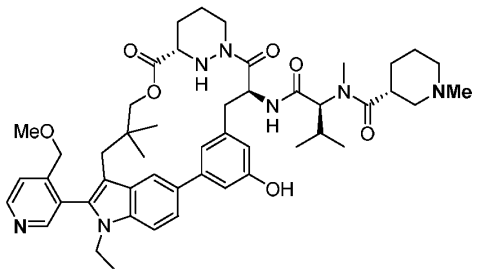
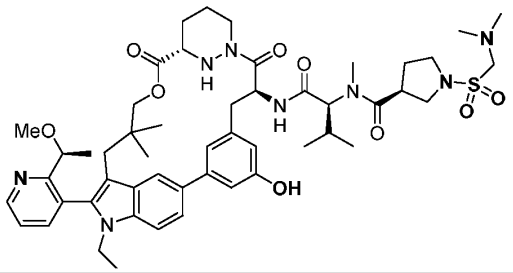
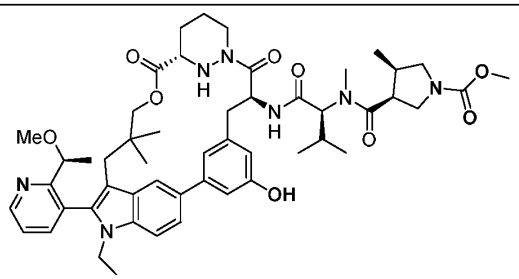
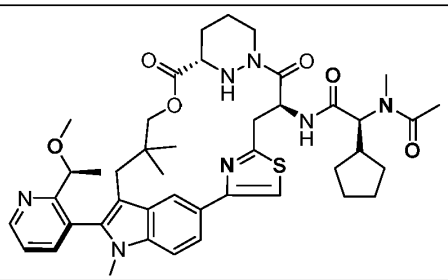
A614	
A615	
A616	

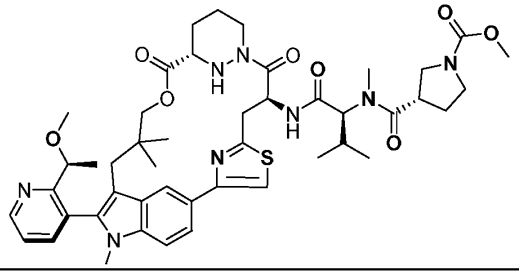
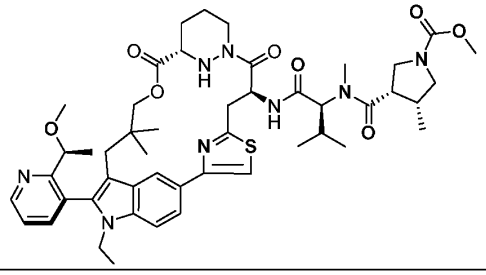
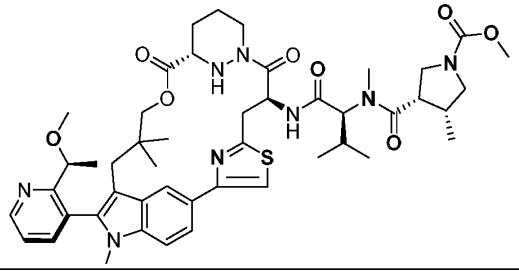
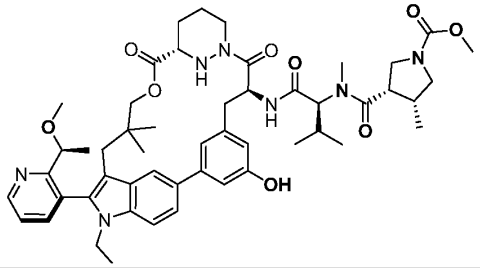
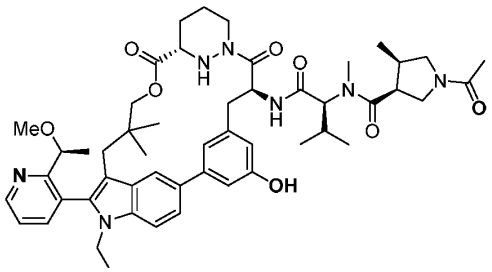
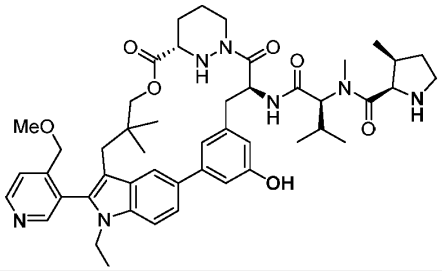
Note that some compounds are shown with bonds as flat or wedged. In some instances, the relative stereochemistry of stereoisomers has been determined; in some instances, the absolute stereochemistry has been determined. In some instances, a single Example number corresponds to a mixture of stereoisomers. All stereoisomers of the compounds of the foregoing table are contemplated by the present invention. In particular embodiments, an atropisomer of a compound of the foregoing table is contemplated. Any compound shown in brackets indicates that the compound is a disastereomer, and the absolute stereochemistry of such diastereomer may not be known.

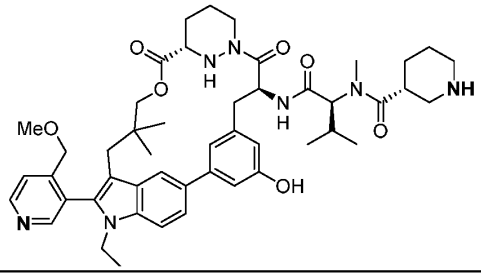
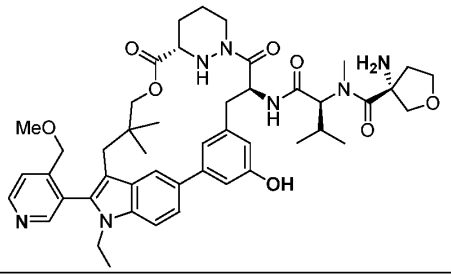
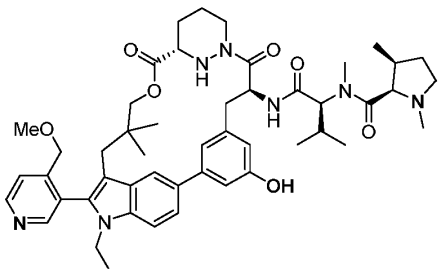
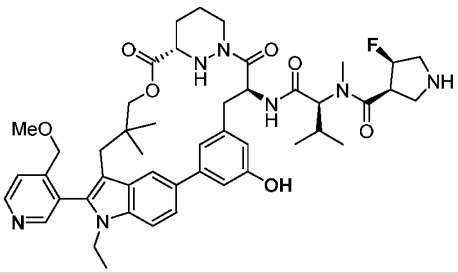
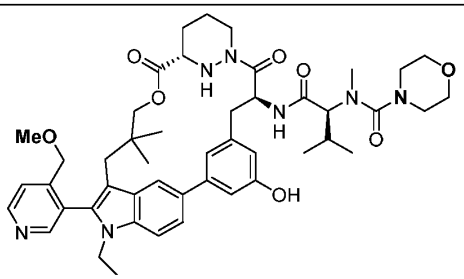
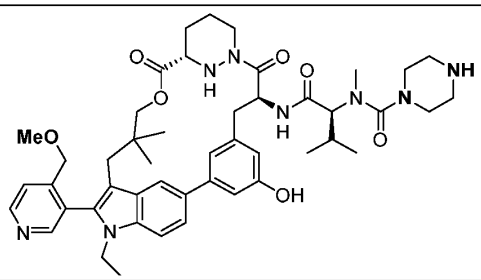
In some embodiments, a compound of Table 2 is provided, or a pharmaceutically acceptable salt thereof. In some embodiments, a compound of the present invention is selected from Table 2, or a pharmaceutically acceptable salt or atropisomer thereof.

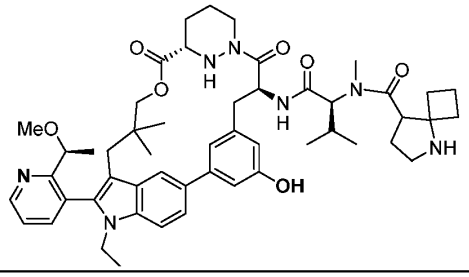
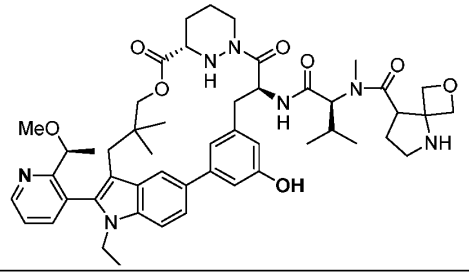
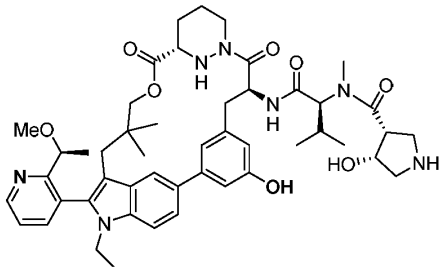
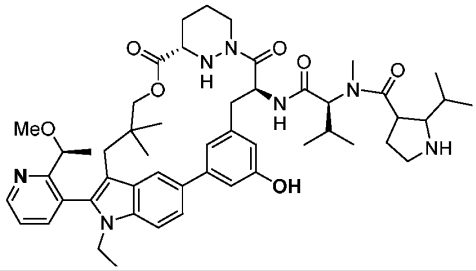
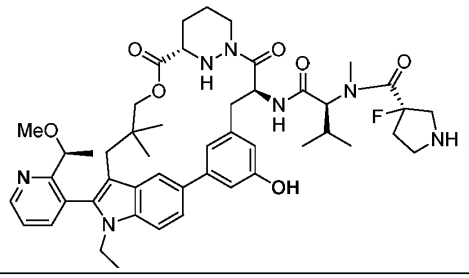
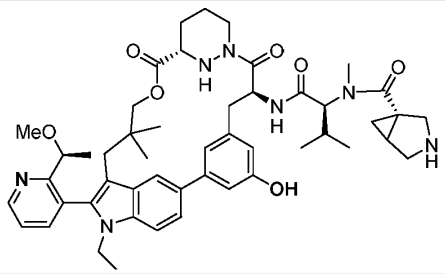
Table 2: Certain Compounds of the Present Invention

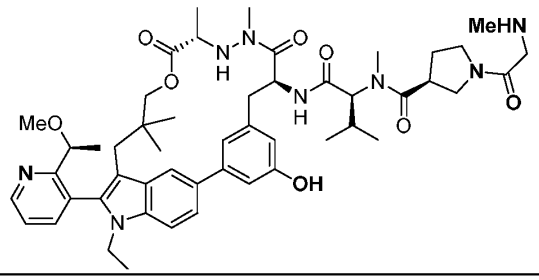
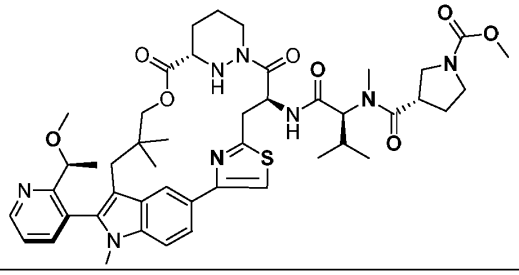
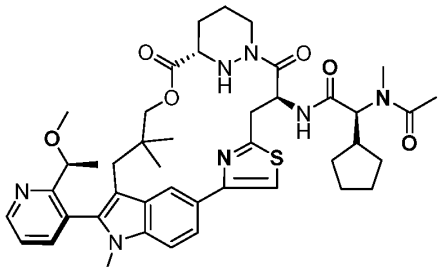
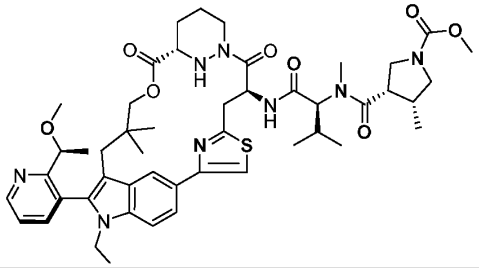
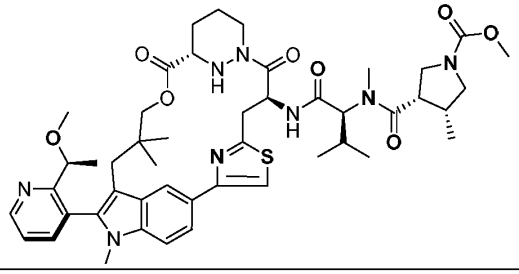
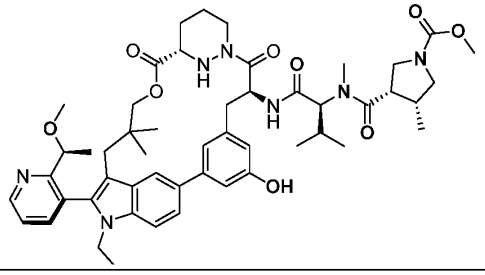
Ex#	Structure
B4	
B5	
B6	
B8	
B9	

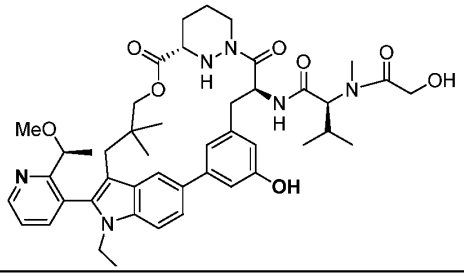
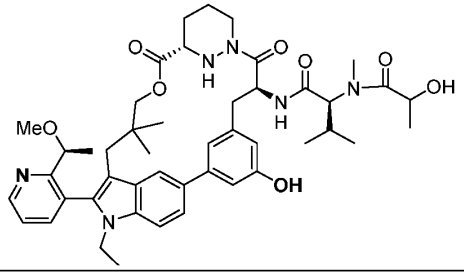
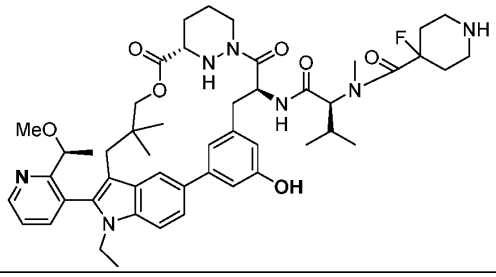
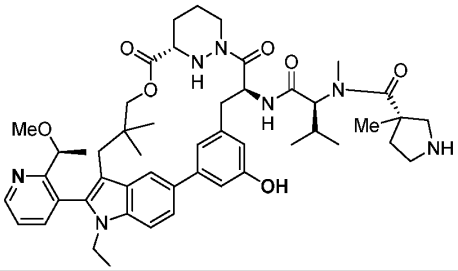
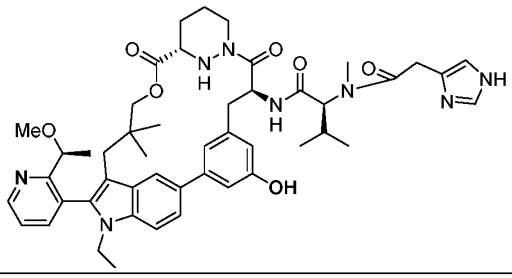
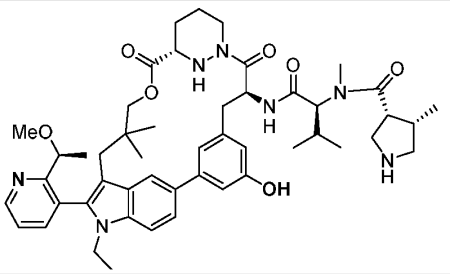
B12	
B13	
B19	
B44	
B47	
B57	

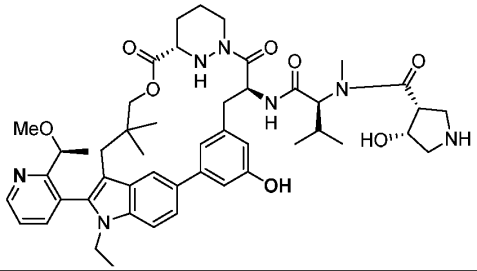
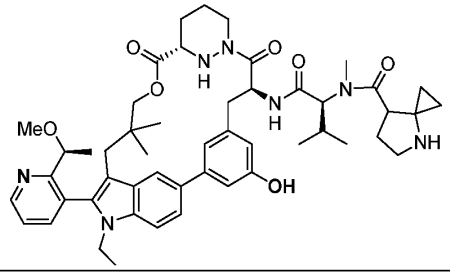
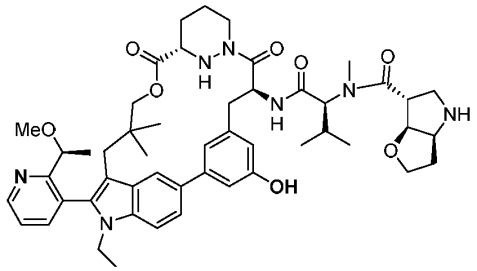
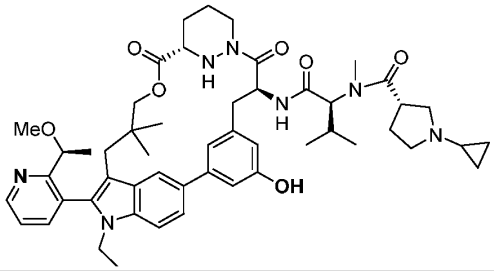
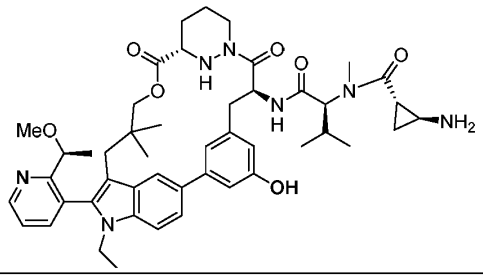
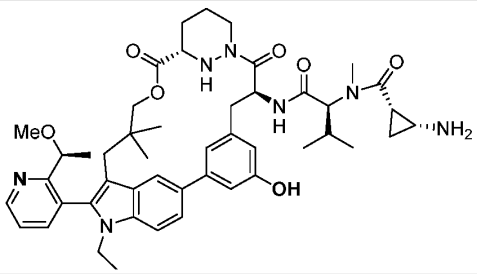
B58	
B59	
B60	
B61	
B66	
B67	

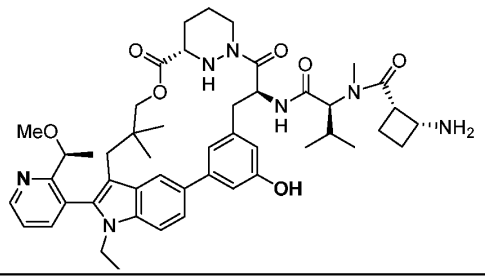
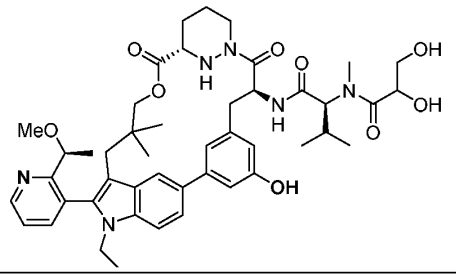
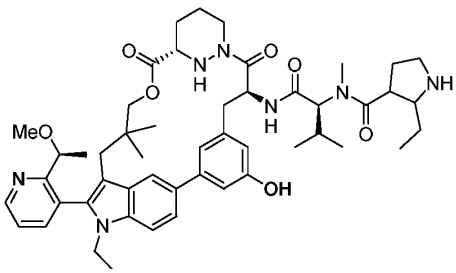
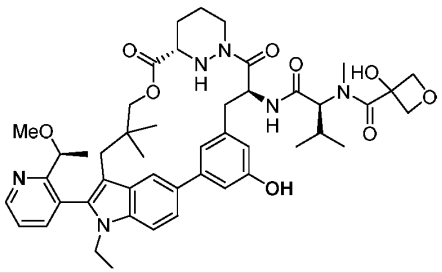
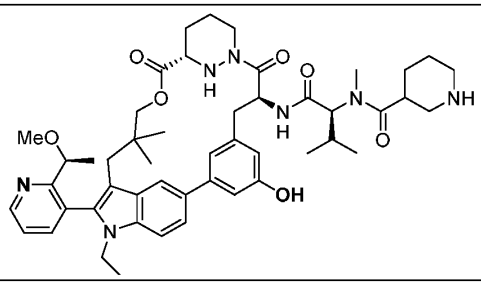
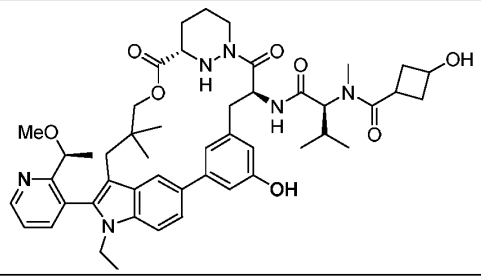
B69	
B71	
B73	
B74	
B80	
B81	

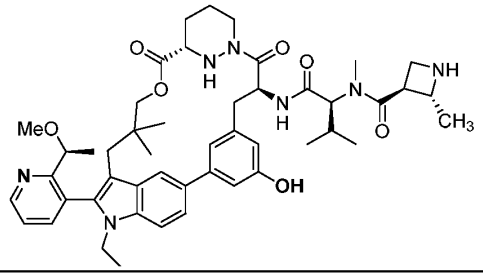
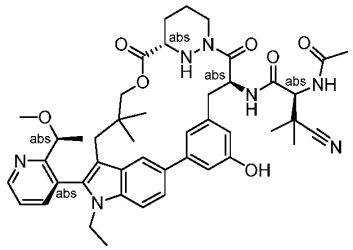
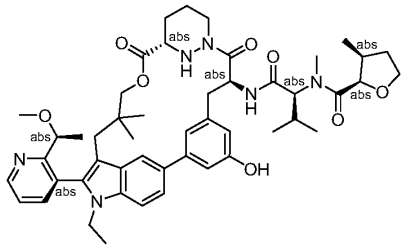
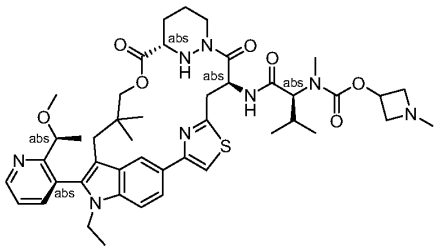
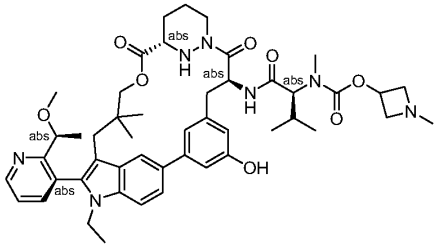
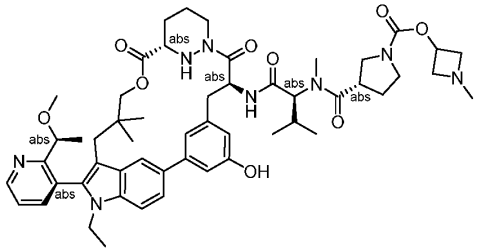
B94	
B95	
B96	
B97	
B99	
B100	

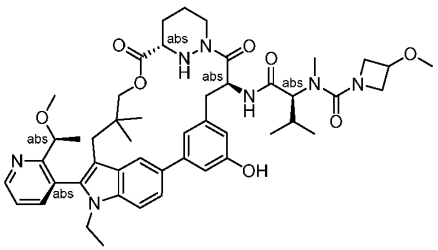
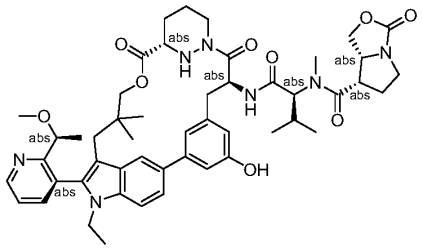
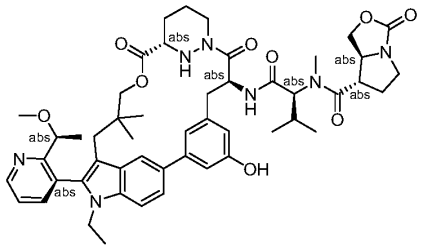
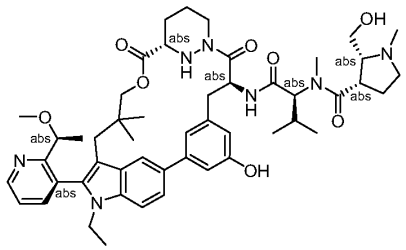
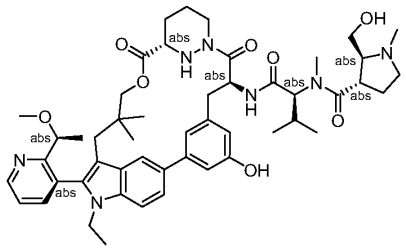
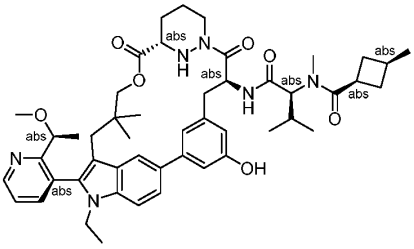
B104	
B106	
B107	
B109	
B110	
B111	

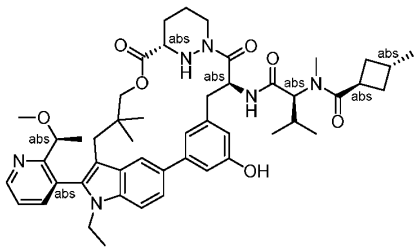
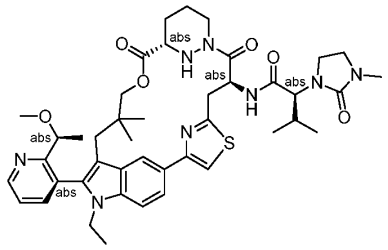
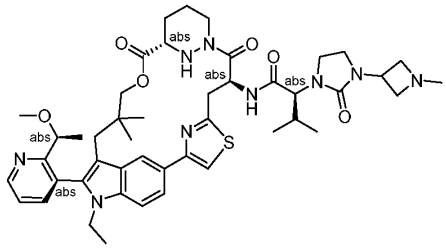
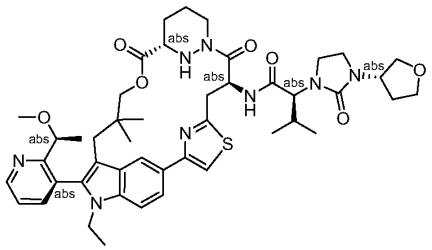
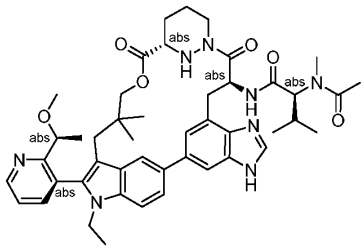
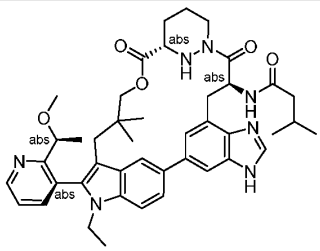
B112	
B113	
B114	
B117	
B119	
B122	

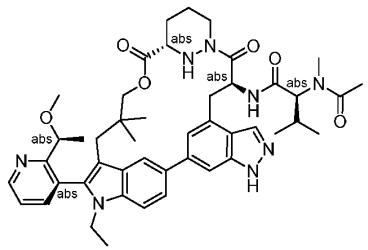
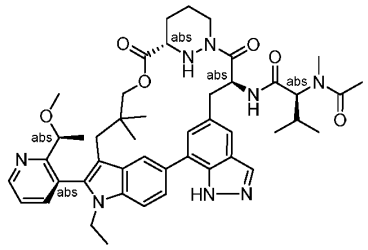
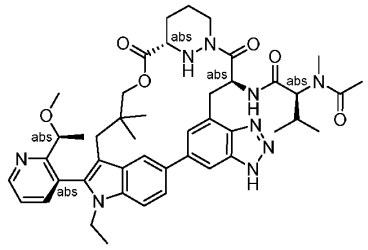
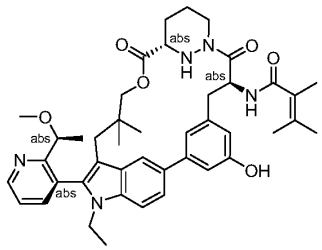
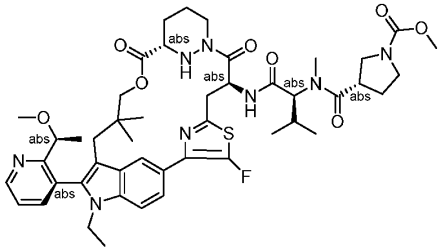
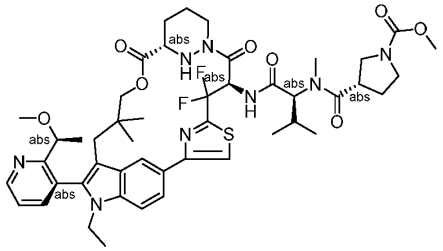
B123	
B124	
B126	
B128	
B129	
B130	

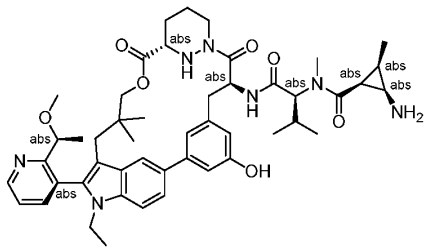
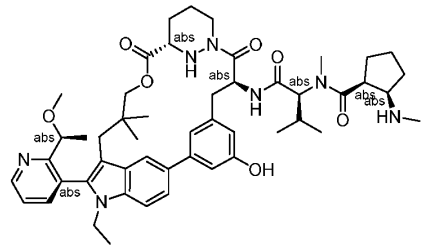
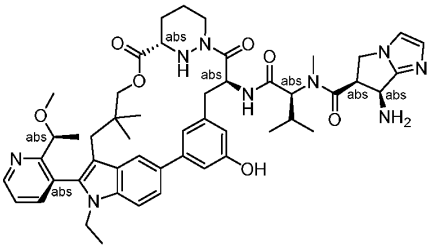
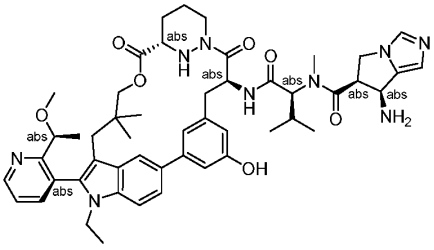
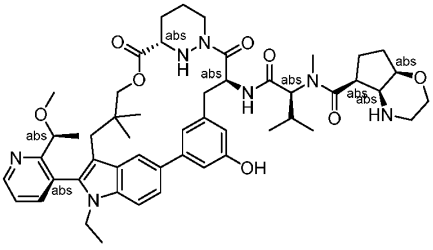
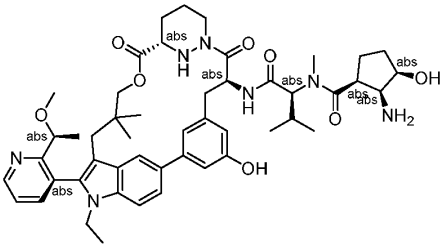
B133	
B134	
B135	
B137	
B138	
B139	

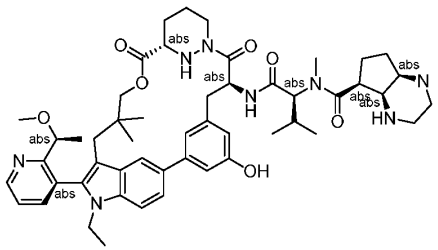
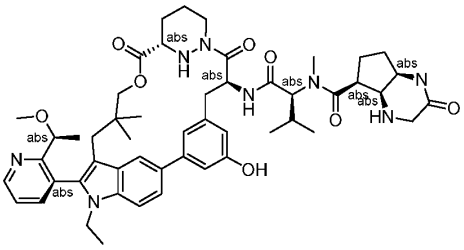
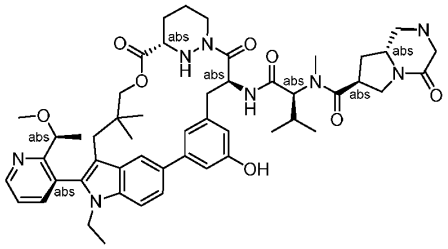
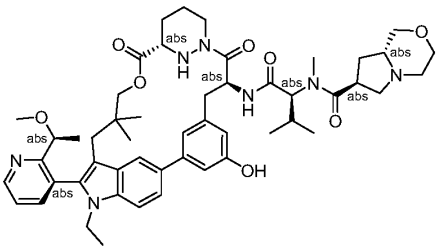
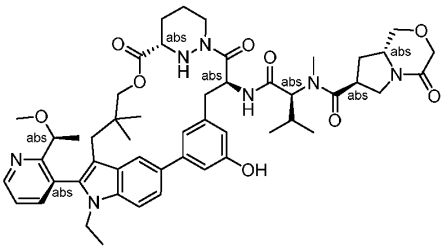
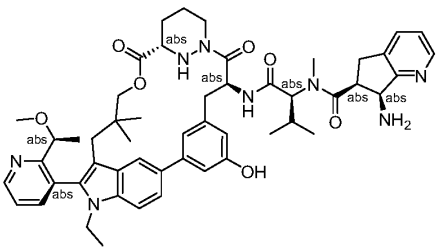
B141	
B143	
B144	
B145	
B146	
B147	

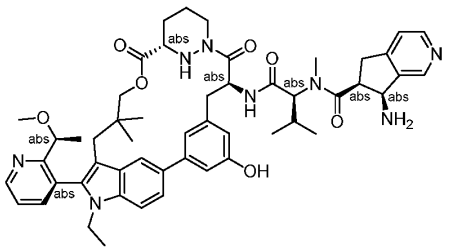
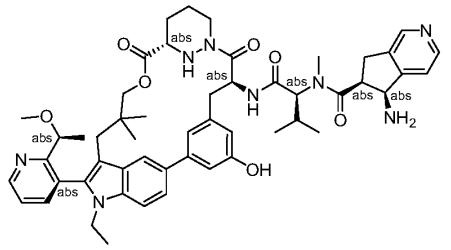
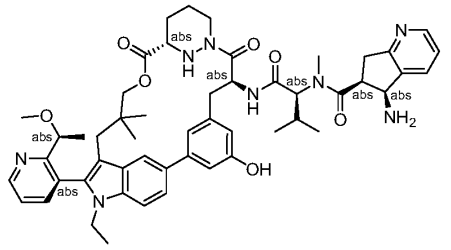
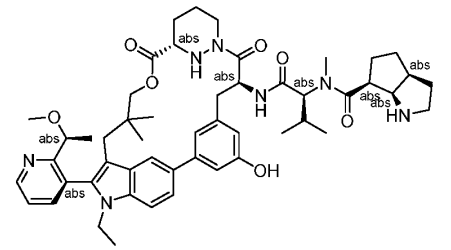
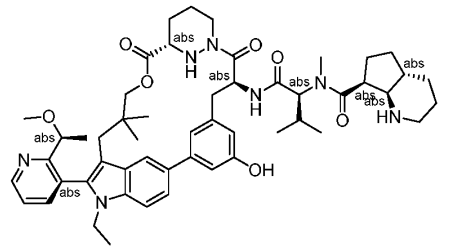
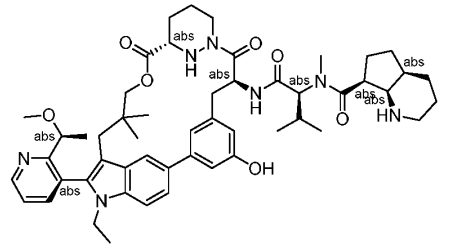
B148	
B149	
B150	
B151	
B152	
B153	

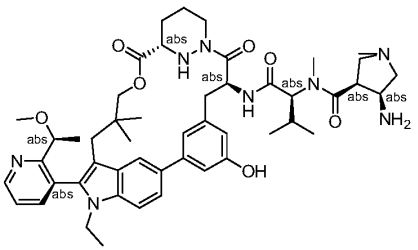
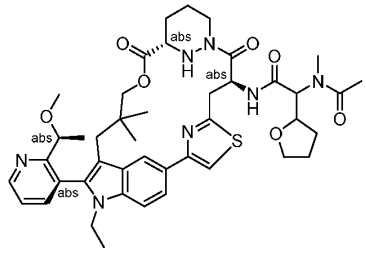
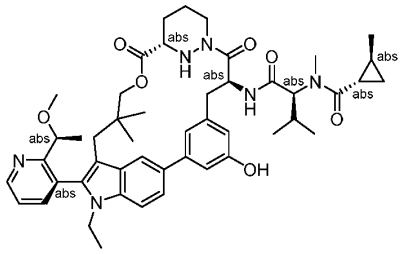
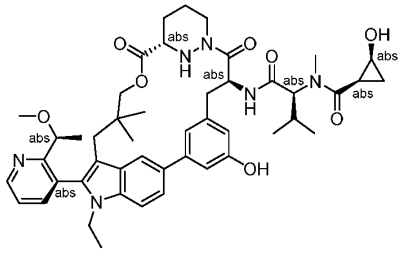
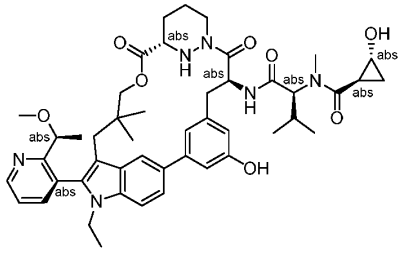
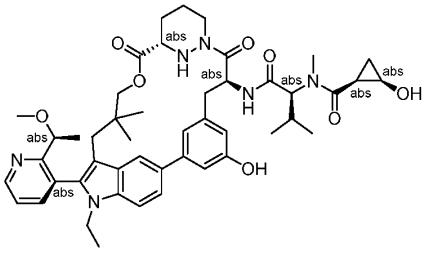
B154	
B155	
B156	
B157	
B158	
B159	

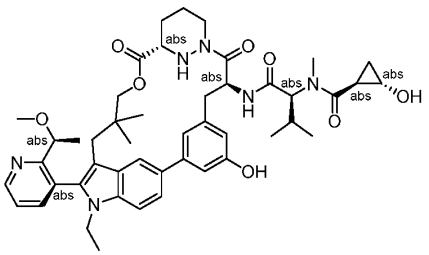
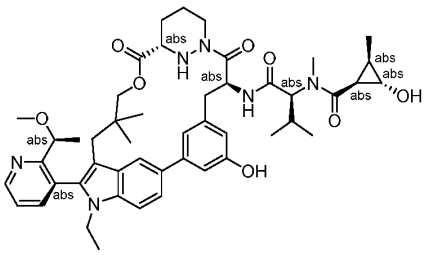
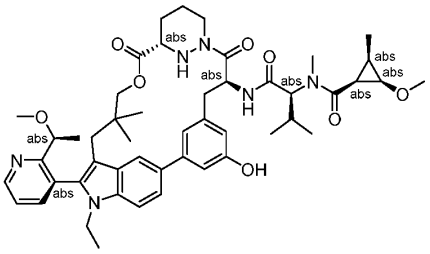
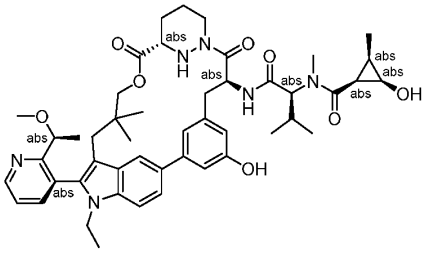
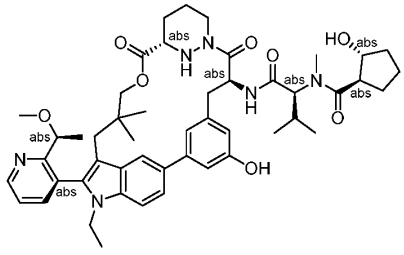
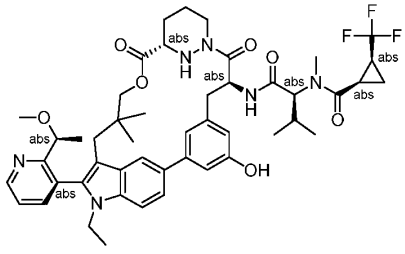
B160	
B161	
B162	
B163	
B164	
B165	

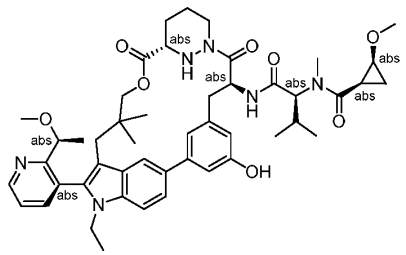
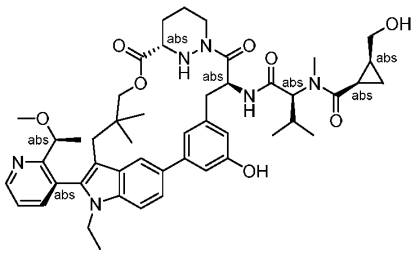
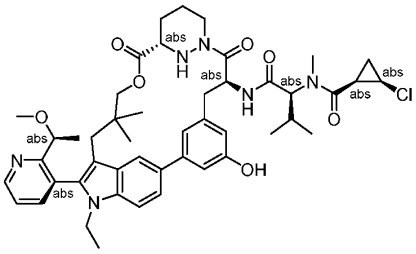
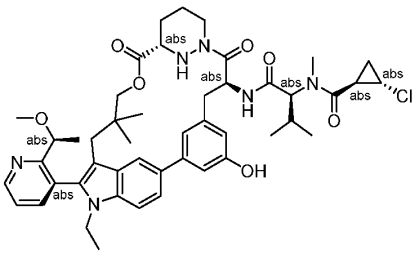
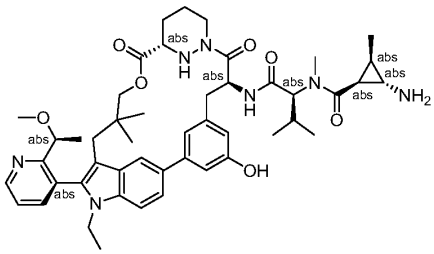
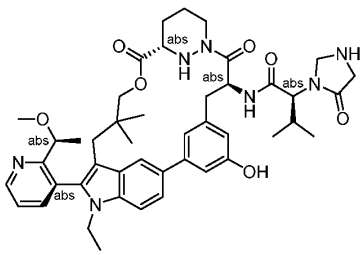
B166	
B167	
B168	
B169	
B170	
B171	

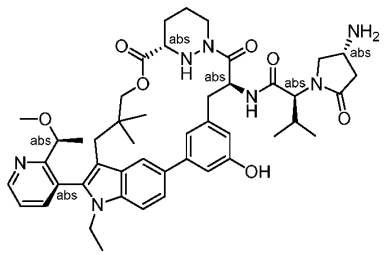
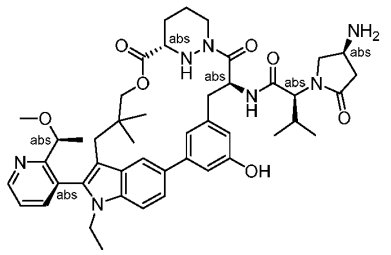
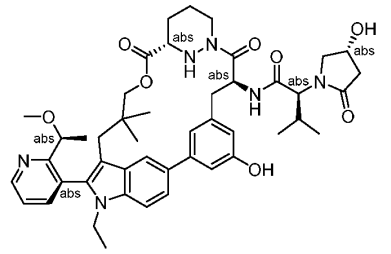
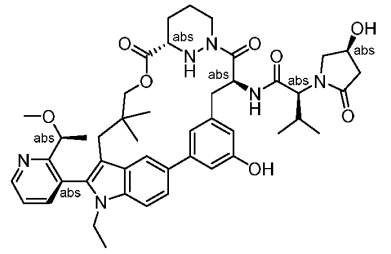
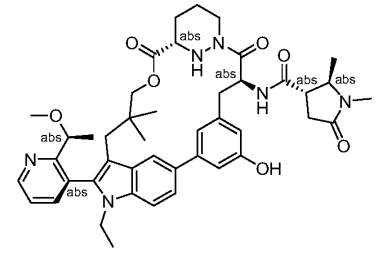
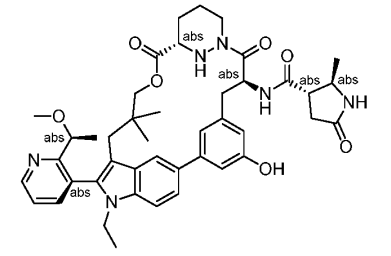
B172	
B173	
B174	
B175	
B176	
B177	

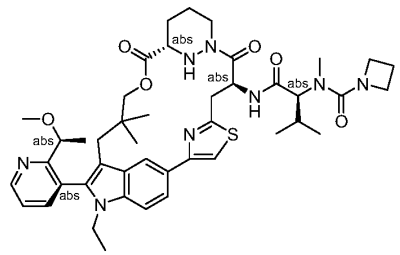
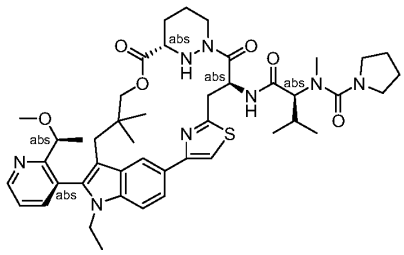
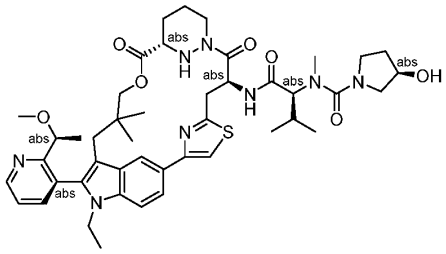
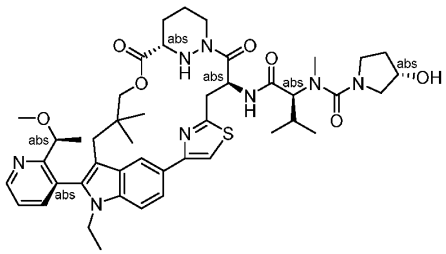
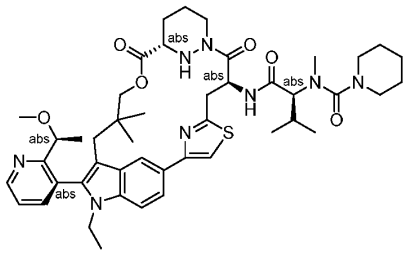
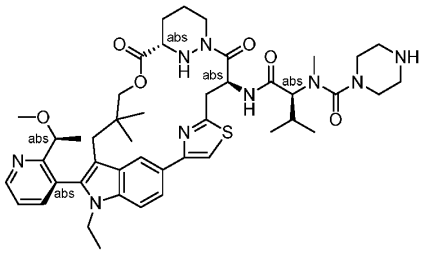
B178	
B179	
B180	
B181	
B182	
B183	

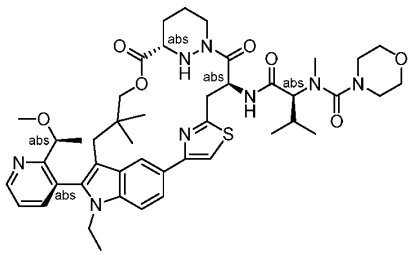
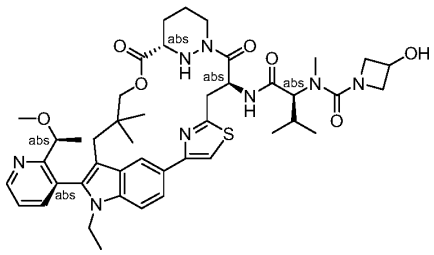
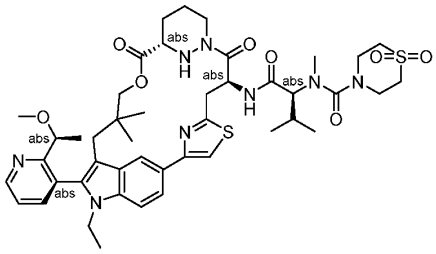
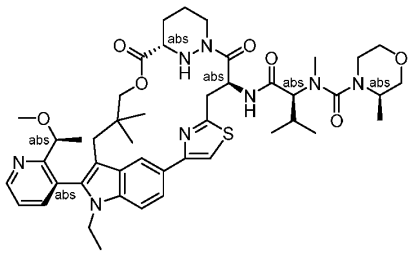
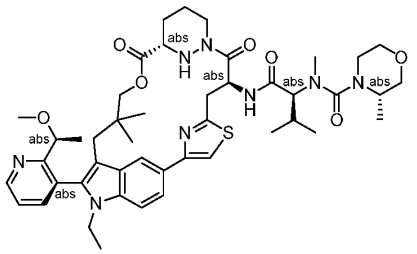
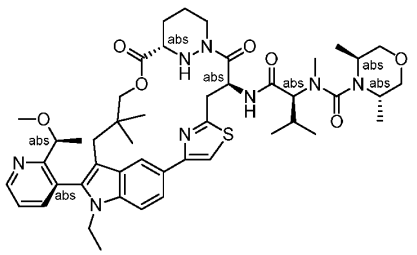
B184	
B185	
B186	
B187	
B188	
B189	

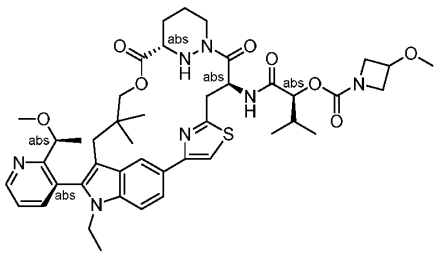
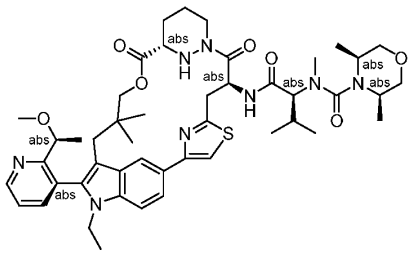
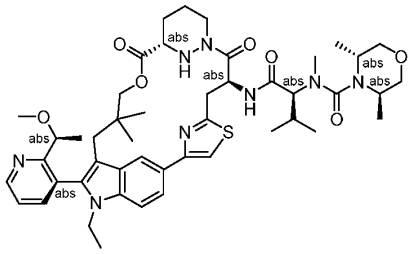
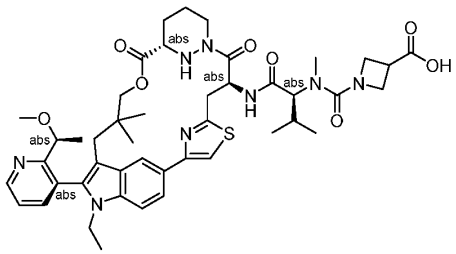
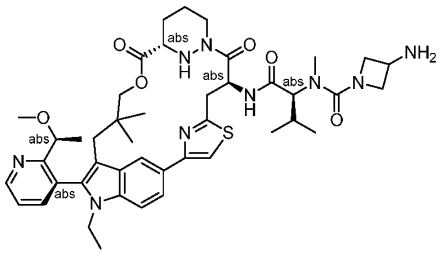
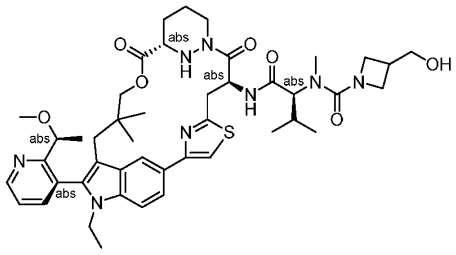
B190	
B191	
B192	
B193	
B194	
B195	

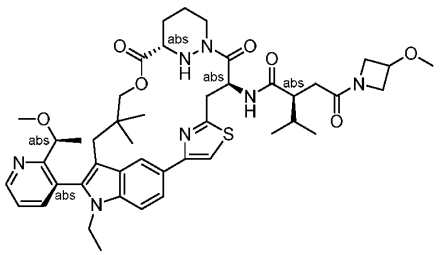
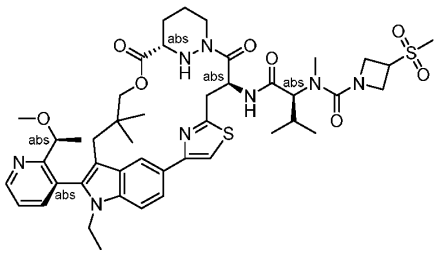
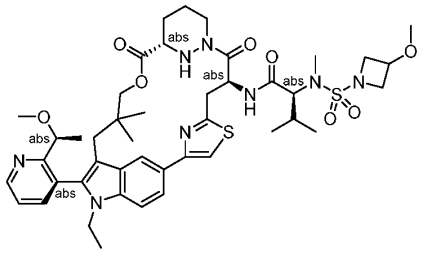
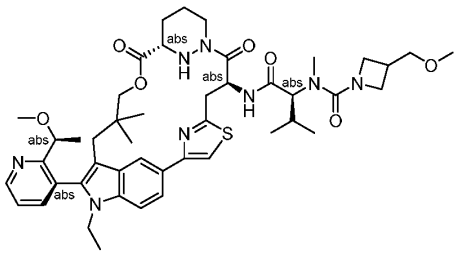
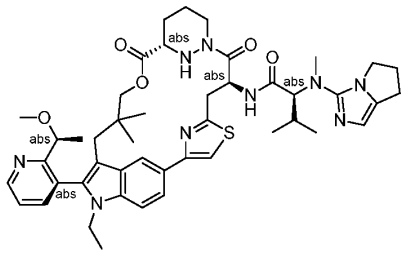
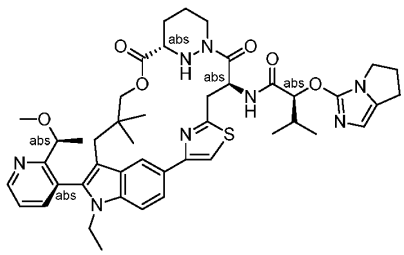
B196	
B197	
B198	
B199	
B200	
B201	

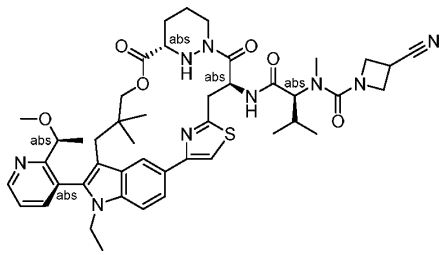
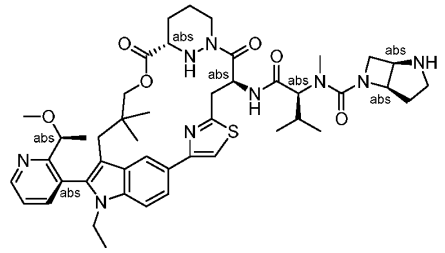
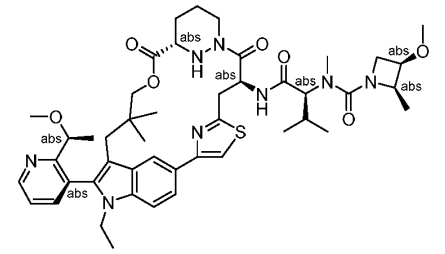
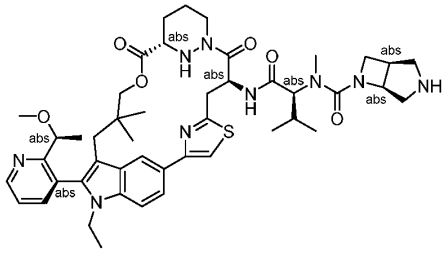
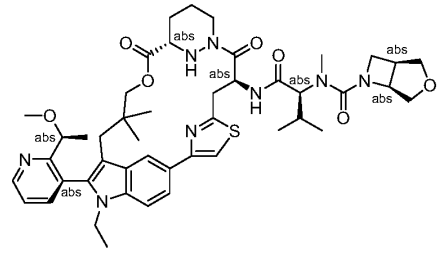
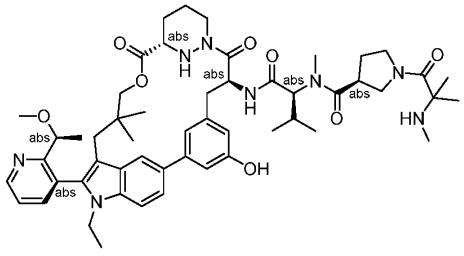
B202	
B203	
B204	
B205	
B206	
B207	

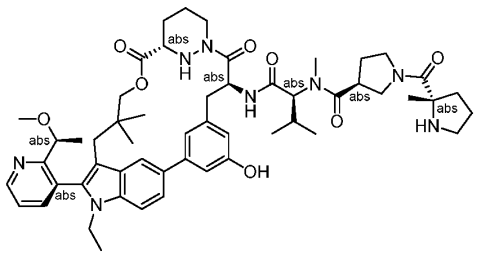
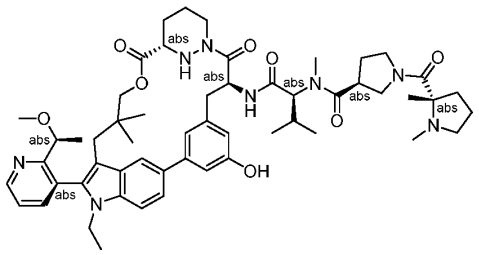
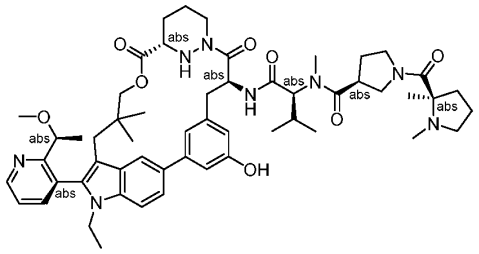
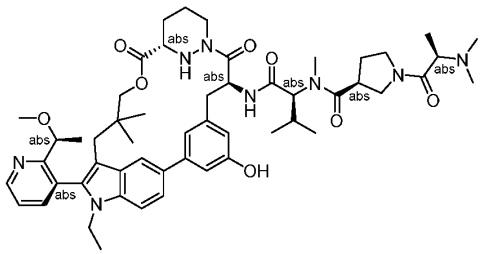
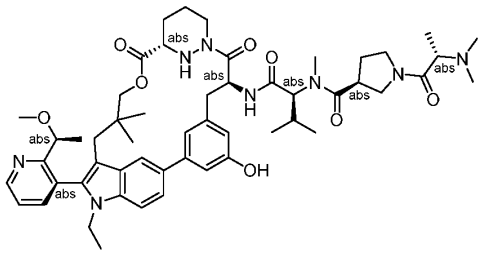
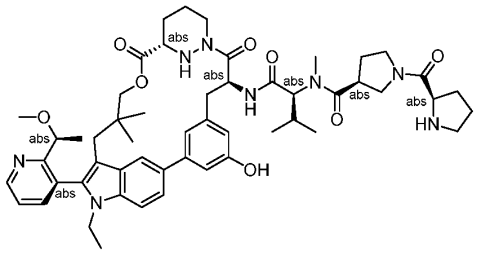
B208	
B209	
B210	
B211	
B212	
B213	

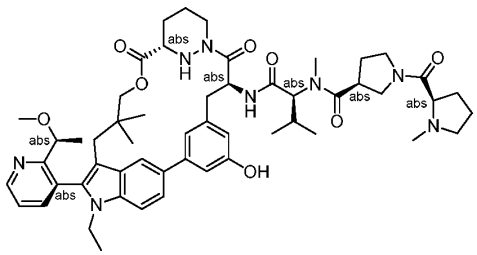
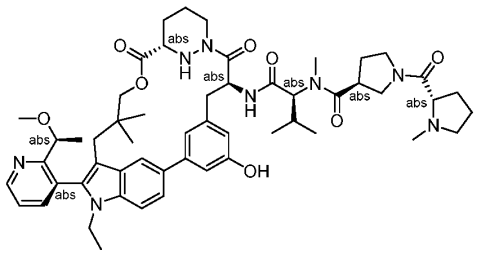
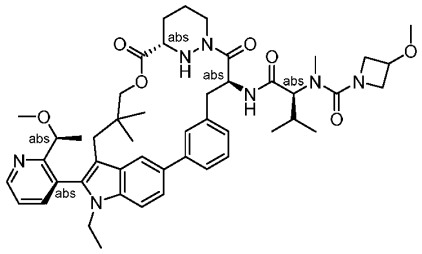
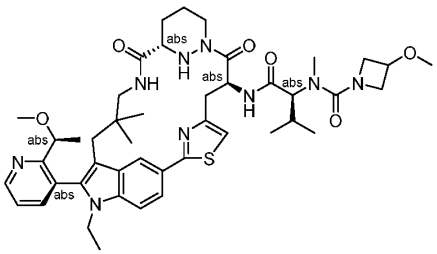
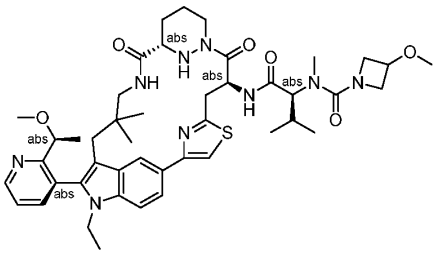
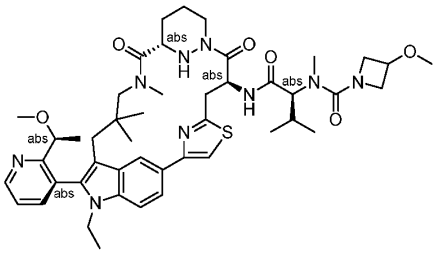
B214	
B215	
B216	
B217	
B218	
B219	

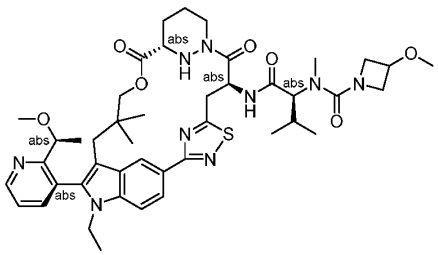
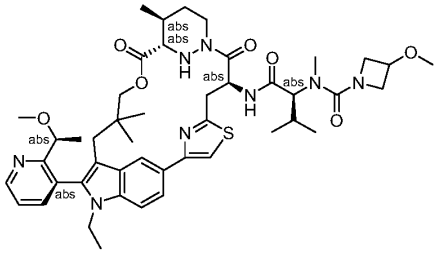
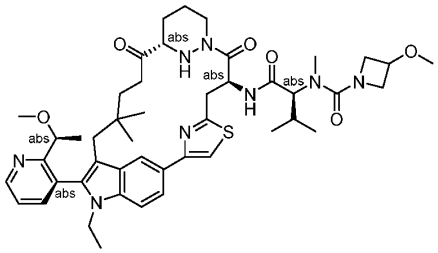
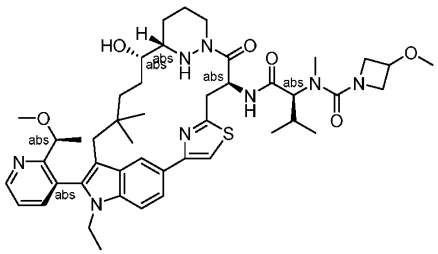
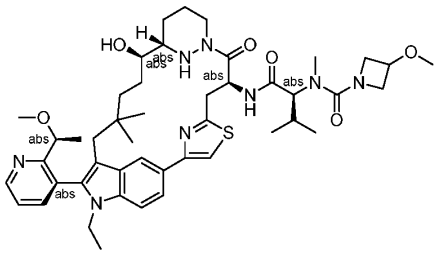
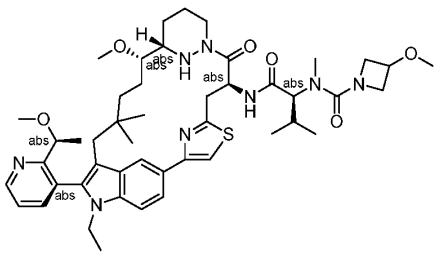
B220	
B221	
B222	
B223	
B224	
B225	

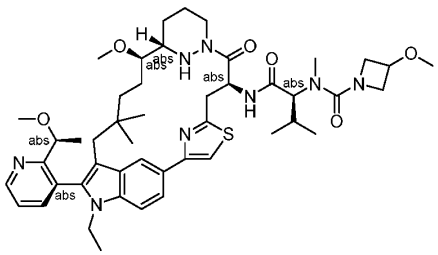
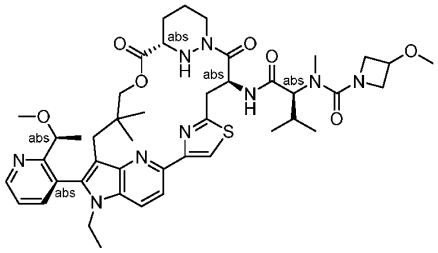
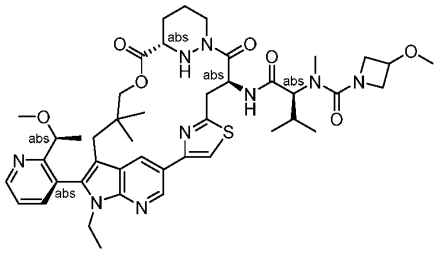
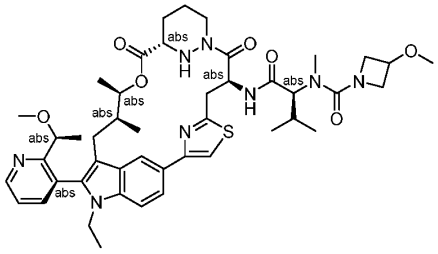
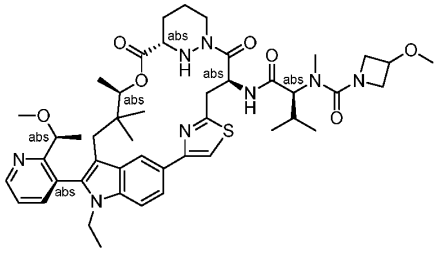
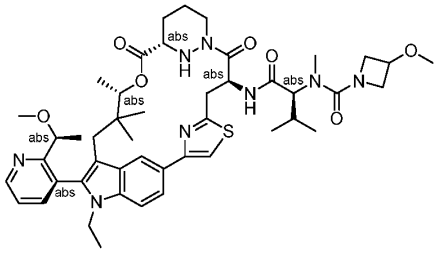
B226	
B227	
B228	
B229	
B230	
B231	

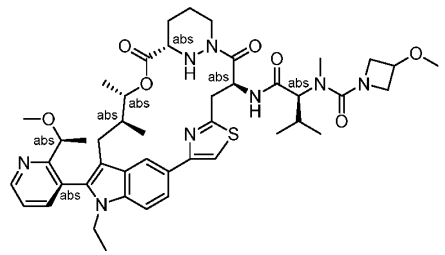
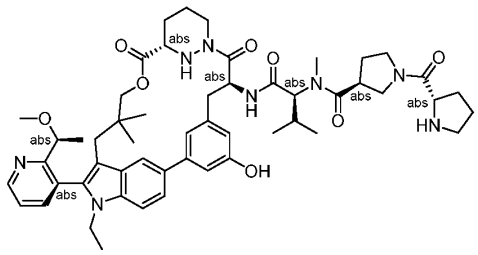
B232	
B233	
B234	
B235	
B236	
B237	

B238	
B239	
B240	
B241	
B242	
B243	

B244	
B245	
B246	
B247	
B248	
B249	

B250	
B251	
B252	
B253	
B254	
B255	

B256	
B257	
B258	
B259	
B260	
B261	

B262	
B263	

Note that some compounds are shown with bonds as flat or wedged. In some instances, the relative stereochemistry of stereoisomers has been determined; in some instances, the absolute stereochemistry has been determined. All stereoisomers of the compounds of the foregoing table are contemplated by the present invention. In particular embodiments, an atropisomer of a compound of the foregoing table is contemplated.

In some embodiments, a compound of the present invention is or acts as a prodrug, such as with respect to administration to a cell or to a subject in need thereof.

Also provided are pharmaceutical compositions comprising a compound of the present invention, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

With respect to compounds of the present invention, one stereoisomer may exhibit better inhibition than another stereoisomer. For example, one atropisomer may exhibit inhibition, whereas the other atropisomer may exhibit little or no inhibition.

Methods of Synthesis

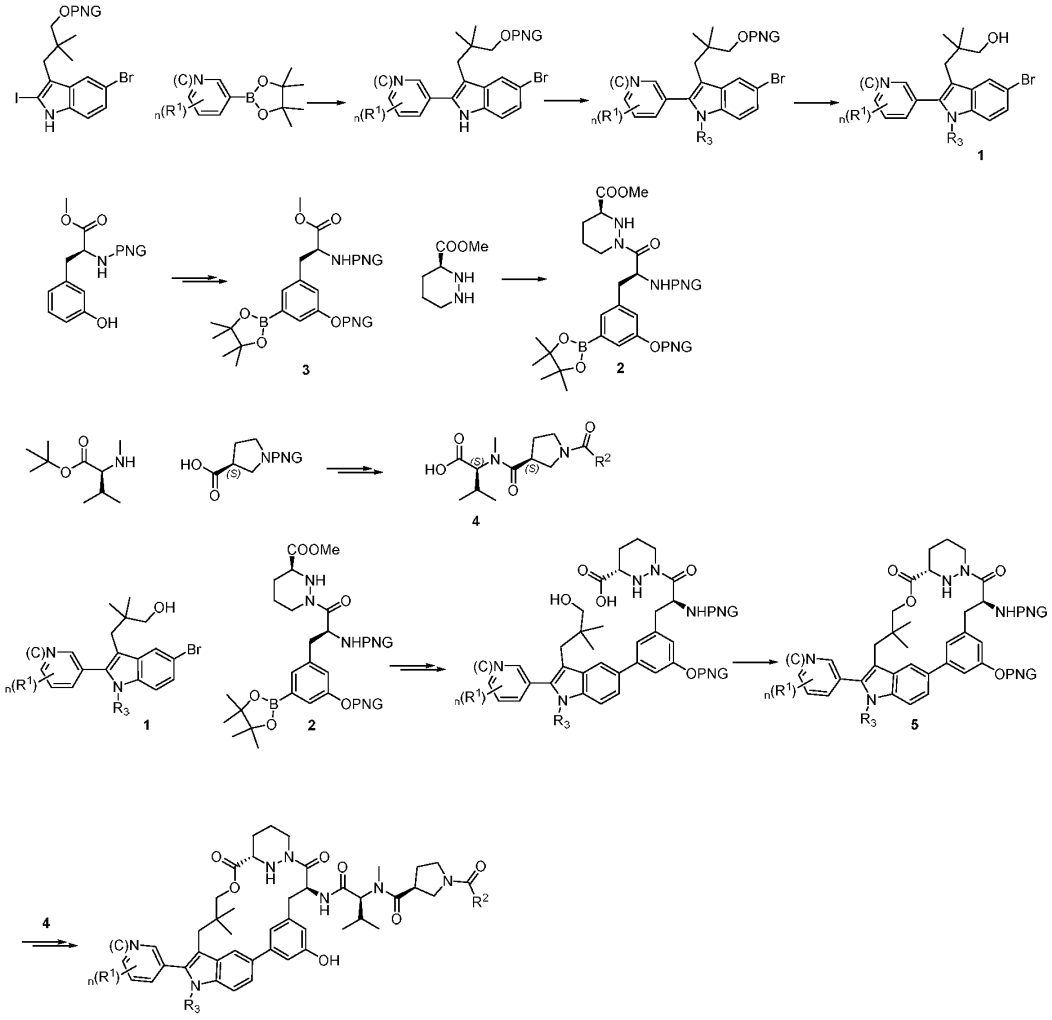
The compounds described herein may be made from commercially available starting materials or synthesized using known organic, inorganic, or enzymatic processes.

The compounds of the present invention can be prepared in a number of ways well known to those skilled in the art of organic synthesis. By way of example, compounds of the present invention can be synthesized using the methods described in the Schemes below, together with synthetic methods known in the art of synthetic organic chemistry, or variations thereon as appreciated by those skilled in the art. These methods include but are not limited to those methods described in the Schemes below.

Compounds of Table 1 herein were prepared using methods disclosed herein or were prepared using methods disclosed herein combined with the knowledge of one of skill in the art.

Compounds of Table 2 may be prepared using methods disclosed herein or may be prepared using methods disclosed herein combined with the knowledge of one of skill in the art.

Scheme 1. General synthesis of macrocyclic esters

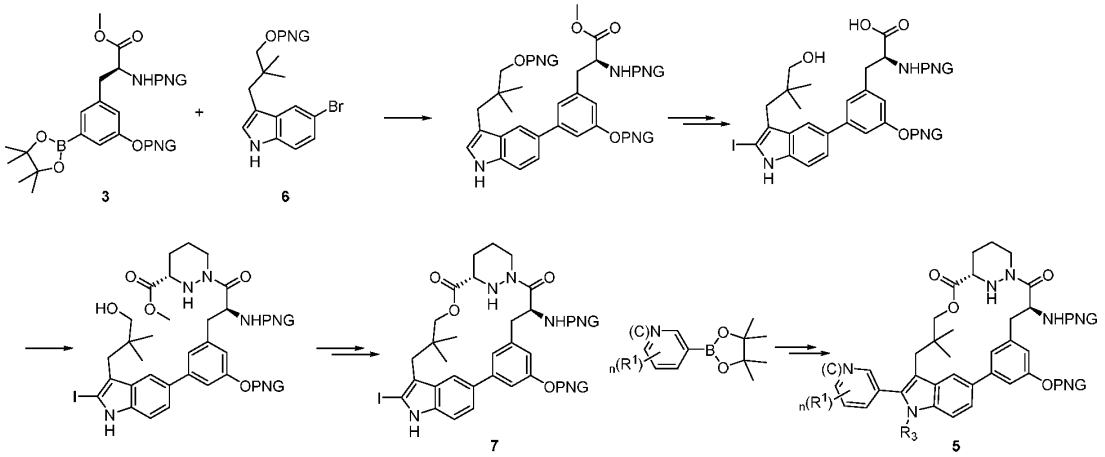


A general synthesis of macrocyclic esters is outlined in Scheme 1. An appropriately substituted Aryl Indole intermediate (**1**) can be prepared in three steps starting from protected 3-(5-bromo-2-iodo-1H-indol-3-yl)-2,2-dimethylpropan-1-ol and appropriately substituted boronic acid, including Palladium mediated coupling, alkylation, and de-protection reactions. Methyl-amino-hexahydropyridazine-3-carboxylate-boronic ester (**2**) can be prepared in three steps, including protection, Iridium catalyst mediated borylation, and coupling with methyl (*S*)-hexahydropyridazine-3-carboxylate.

An appropriately substituted acetylpyrrolidine-3-carbonyl-N-methyl-L-valine (**4**) can be made by coupling of methyl-L-valinate and protected (*S*)-pyrrolidine-3-carboxylic acid, followed by deprotection, coupling with an appropriately substituted carboxylic acid, and a hydrolysis step.

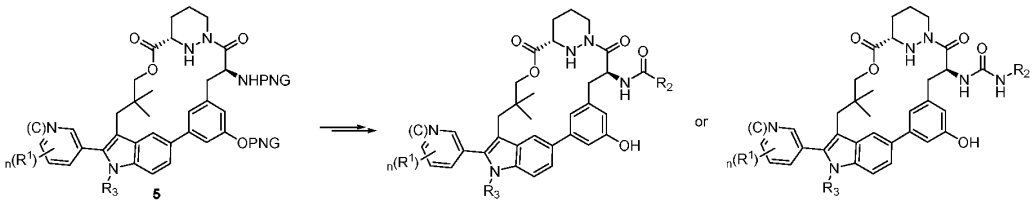
The final macrocyclic esters can be made by coupling of methyl-amino-hexahydropyridazine-3-carboxylate-boronic ester (**2**) and intermediate (**1**) in the presence of Pd catalyst followed by hydrolysis and macrolactonization steps to result in an appropriately protected macrocyclic intermediate (**5**). Deprotection and coupling with an appropriately substituted acetylpyrrolidine-3-carbonyl-N-methyl-L-valine (**4**) results in a macrocyclic product. Additional deprotection or functionalization steps are be required to produce a final compound. For example, a person of skill in the art would be able to install into a macrocyclic ester a desired -B-L-W group of a compound of Formula (I), where B, L and W are defined herein, including by using methods exemplified in the Example section herein.

Scheme 2. Alternative general synthesis of macrocyclic esters



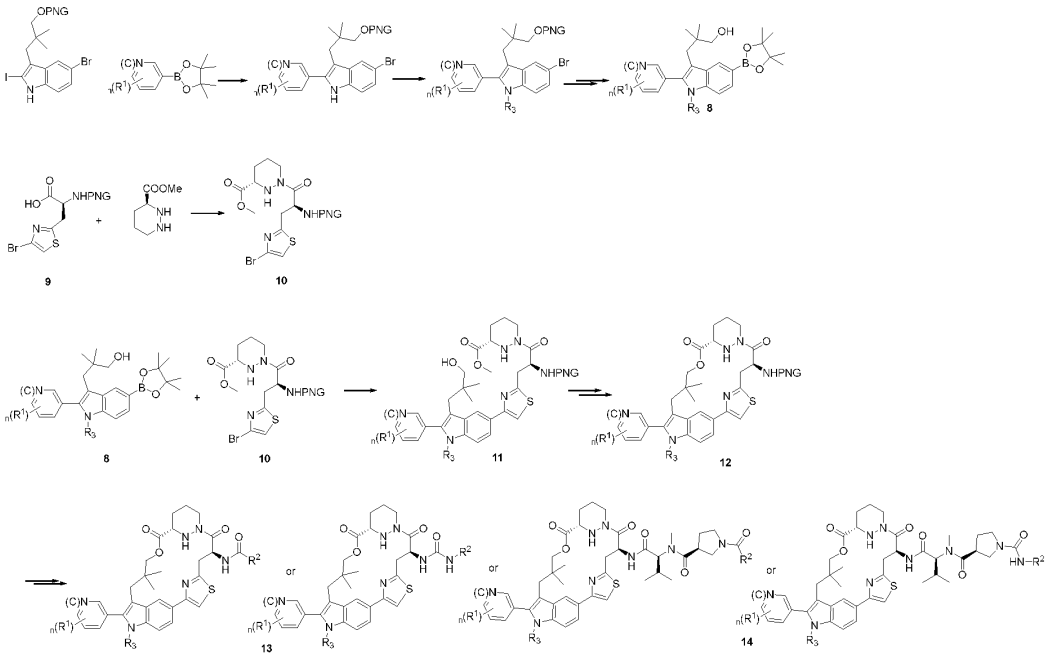
Alternatively, macrocyclic esters can be prepared as described in Scheme 2. An appropriately protected bromo-indolyl (**6**) can be coupled in the presence of Pd catalyst with boronic ester (**3**), followed by iodination, deprotection, and ester hydrolysis. Subsequent coupling with methyl (*S*)-hexahydropyridazine-3-carboxylate, followed by hydrolysis and macrolactonization can result in iodo intermediate (**7**). Coupling in the presence of Pd catalyst with an appropriately substituted boronic ester and alkylation can yield fully a protected macrocycle (**5**). Additional deprotection or functionalization steps are required to produce a final compound. For example, a person of skill in the art would be able to install into a macrocyclic ester a desired -B-L-W group of a compound of Formula (I), where B, L and W are defined herein, including by using methods exemplified in the Example section herein.

Scheme 3. General synthesis of macrocyclic esters



Alternatively, fully a protected macrocycle (**5**) can be deprotected and coupled with an appropriately substitututed coupling partners, and deprotected to results in a macrocyclic product. Additional deprotection or functionalization steps are be required to produce a final compound. For example, a person of skill in the art would be able to install into a macrocyclic ester a desired -B-L-W group of a compound of Formula (I), where B, L and W are defined herein, including by using methods exemplified in the Example section herein.

Scheme 4. General synthesis of macrocyclic esters



An alternative general synthesis of macrocyclic esters is outlined in Scheme 4. An appropriately substituted indolyl boronic ester (**8**) can be prepared in four steps starting from protected 3-(5-bromo-2-iodo-1H-indol-3-yl)-2,2-dimethylpropan-1-ol and appropriately substituted

boronic acid, including Palladium mediated coupling, alkylation, de-protection, and Palladium mediated borylation reactions.

Methyl-amino-3-(4-bromothiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (**10**) can be prepared via coupling of (S)-2-amino-3-(4-bromothiazol-2-yl)propanoic acid (**9**) with methyl (*S*)-hexahydropyridazine-3-carboxylate.

The final macrocyclic esters can be made by coupling of Methyl-amino-3-(4-bromothiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (**10**) and an appropriately substituted indolyl boronic ester (**8**) in the presence of Pd catalyst followed by hydrolysis and macrolactonization steps to result in an appropriately protected macrocyclic intermediate (**11**). Deprotection and coupling with an appropriately substituted carboxylic acid (or other coupling partner) or intermediate **4** can result in a macrocyclic product. Additional deprotection or functionalization steps could be required to produce a final compound **13** or **14**.

In addition, compounds of the disclosure can be synthesized using the methods described in the Examples below, together with synthetic methods known in the art of synthetic organic chemistry, or variations thereon as appreciated by those skilled in the art. These methods include but are not limited to those methods described in the Examples below. For example, a person of skill in the art would be able to install into a macrocyclic ester a desired -B-L-W group of a compound of Formula (I), where B, L and W are defined herein, including by using methods exemplified in the Example section herein.

Pharmaceutical Compositions and Methods of Use

Pharmaceutical Compositions and Methods of Administration

The compounds with which the invention is concerned are Ras inhibitors, and are useful in the treatment of cancer. Accordingly, one embodiment of the present invention provides pharmaceutical compositions containing a compound of the invention or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient, as well as methods of using the compounds of the invention to prepare such compositions.

As used herein, the term “pharmaceutical composition” refers to a compound, such as a compound of the present invention, or a pharmaceutically acceptable salt thereof, formulated together with a pharmaceutically acceptable excipient.

In some embodiments, a compound is present in a pharmaceutical composition in unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant population. In some embodiments, pharmaceutical compositions may be specially formulated for administration in solid or liquid form, including those adapted for the following: oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, e.g., those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin, lungs, or oral cavity; intravaginally or

intrarectally, for example, as a pessary, cream, or foam; sublingually; ocularly; transdermally; or nasally, pulmonary, and to other mucosal surfaces.

A "pharmaceutically acceptable excipient," as used herein, refers any inactive ingredient (for example, a vehicle capable of suspending or dissolving the active compound) having the properties of being nontoxic and non-inflammatory in a subject. Typical excipients include, for example: antiadherents, antioxidants, binders, coatings, compression aids, disintegrants, dyes (colors), emollients, emulsifiers, fillers (diluents), film formers or coatings, flavors, fragrances, glidants (flow enhancers), lubricants, preservatives, printing inks, sorbents, suspensing or dispersing agents, sweeteners, or waters of hydration. Excipients include, but are not limited to: butylated optionally substituted hydroxyltoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, crosslinked polyvinyl pyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, optionally substituted hydroxylpropyl cellulose, optionally substituted hydroxylpropyl methylcellulose, lactose, magnesium stearate, maltitol, mannitol, methionine, methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol, polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac, silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E, vitamin C, and xylitol. Those of ordinary skill in the art are familiar with a variety of agents and materials useful as excipients. See, e.g., e.g., Ansel, et al., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems. Philadelphia: Lippincott, Williams & Wilkins, 2004; Gennaro, et al., Remington: The Science and Practice of Pharmacy. Philadelphia: Lippincott, Williams & Wilkins, 2000; and Rowe, Handbook of Pharmaceutical Excipients. Chicago, Pharmaceutical Press, 2005. In some embodiments, a composition includes at least two different pharmaceutically acceptable excipients.

Compounds described herein, whether expressly stated or not, may be provided or utilized in salt form, e.g., a pharmaceutically acceptable salt form, unless expressly stated to the contrary. The term "pharmaceutically acceptable salt," as use herein, refers to those salts of the compounds described herein that are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and other animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, pharmaceutically acceptable salts are described in: Berge et al., *J. Pharmaceutical Sciences* 66:1-19, 1977 and in *Pharmaceutical Salts: Properties, Selection, and Use*, (Eds. P.H. Stahl and C.G. Wermuth), Wiley-VCH, 2008. The salts can be prepared in situ during the final isolation and purification of the compounds described herein or separately by reacting the free base group with a suitable organic acid.

The compounds of the invention may have ionizable groups so as to be capable of preparation as pharmaceutically acceptable salts. These salts may be acid addition salts involving inorganic or organic acids or the salts may, in the case of acidic forms of the compounds of the invention, be prepared from inorganic or organic bases. In some embodiments, the compounds are prepared or used as pharmaceutically acceptable salts prepared as addition products of pharmaceutically acceptable acids or bases. Suitable pharmaceutically acceptable acids and

bases are well-known in the art, such as hydrochloric, sulfuric, hydrobromic, acetic, lactic, citric, or tartaric acids for forming acid addition salts, and potassium hydroxide, sodium hydroxide, ammonium hydroxide, caffeine, various amines, and the like for forming basic salts. Methods for preparation of the appropriate salts are well-established in the art.

Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-optionally substituted hydroxyl-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine and the like.

As used herein, the term “subject” refers to any member of the animal kingdom. In some embodiments, “subject” refers to humans, at any stage of development. In some embodiments, “subject” refers to a human patient. In some embodiments, “subject” refers to non-human animals. In some embodiments, the non-human animal is a mammal (e.g., a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, or a pig). In some embodiments, subjects include, but are not limited to, mammals, birds, reptiles, amphibians, fish, or worms. In some embodiments, a subject may be a transgenic animal, genetically-engineered animal, or a clone.

As used herein, the term “dosage form” refers to a physically discrete unit of a compound (e.g., a compound of the present invention) for administration to a subject. Each unit contains a predetermined quantity of compound. In some embodiments, such quantity is a unit dosage amount (or a whole fraction thereof) appropriate for administration in accordance with a dosing regimen that has been determined to correlate with a desired or beneficial outcome when administered to a relevant population (i.e., with a therapeutic dosing regimen). Those of ordinary skill in the art appreciate that the total amount of a therapeutic composition or compound administered to a particular subject is determined by one or more attending physicians and may involve administration of multiple dosage forms.

As used herein, the term “dosing regimen” refers to a set of unit doses (typically more than one) that are administered individually to a subject, typically separated by periods of time. In some embodiments, a given therapeutic compound (e.g., a compound of the present invention) has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which are separated from one another by a time period of the same length; in some embodiments, a dosing regimen comprises a plurality of doses and at least two different time periods separating individual doses. In some embodiments, all doses within a dosing regimen are of the same unit dose amount. In some embodiments,

different doses within a dosing regimen are of different amounts. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount different from the first dose amount. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount same as the first dose amount. In some embodiments, a dosing regimen is correlated with a desired or beneficial outcome when administered across a relevant population (i.e., is a therapeutic dosing regimen).

A "therapeutic regimen" refers to a dosing regimen whose administration across a relevant population is correlated with a desired or beneficial therapeutic outcome.

The term "treatment" (also "treat" or "treating"), in its broadest sense, refers to any administration of a substance (e.g., a compound of the present invention) that partially or completely alleviates, ameliorates, relieves, inhibits, delays onset of, reduces severity of, or reduces incidence of one or more symptoms, features, or causes of a particular disease, disorder, or condition. In some embodiments, such treatment may be administered to a subject who does not exhibit signs of the relevant disease, disorder or condition or of a subject who exhibits only early signs of the disease, disorder, or condition. Alternatively, or additionally, in some embodiments, treatment may be administered to a subject who exhibits one or more established signs of the relevant disease, disorder or condition. In some embodiments, treatment may be of a subject who has been diagnosed as suffering from the relevant disease, disorder, or condition. In some embodiments, treatment may be of a subject known to have one or more susceptibility factors that are statistically correlated with increased risk of development of the relevant disease, disorder, or condition.

The term "therapeutically effective amount" means an amount that is sufficient, when administered to a population suffering from or susceptible to a disease, disorder, or condition in accordance with a therapeutic dosing regimen, to treat the disease, disorder, or condition. In some embodiments, a therapeutically effective amount is one that reduces the incidence or severity of, or delays onset of, one or more symptoms of the disease, disorder, or condition. Those of ordinary skill in the art will appreciate that the term "therapeutically effective amount" does not in fact require successful treatment be achieved in a particular individual. Rather, a therapeutically effective amount may be that amount that provides a particular desired pharmacological response in a significant number of subjects when administered to patients in need of such treatment. It is specifically understood that particular subjects may, in fact, be "refractory" to a "therapeutically effective amount." In some embodiments, reference to a therapeutically effective amount may be a reference to an amount as measured in one or more specific tissues (e.g., a tissue affected by the disease, disorder or condition) or fluids (e.g., blood, saliva, serum, sweat, tears, urine). Those of ordinary skill in the art will appreciate that, in some embodiments, a therapeutically effective amount may be formulated or administered in a single dose. In some embodiments, a therapeutically effective amount may be formulated or administered in a plurality of doses, for example, as part of a dosing regimen.

For use as treatment of subjects, the compounds of the invention, or a pharmaceutically acceptable salt thereof, can be formulated as pharmaceutical or veterinary compositions.

Depending on the subject to be treated, the mode of administration, and the type of treatment desired, e.g., prevention, prophylaxis, or therapy, the compounds, or a pharmaceutically acceptable salt thereof, are formulated in ways consonant with these parameters. A summary of such techniques may be found in *Remington: The Science and Practice of Pharmacy*, *21st Edition*, Lippincott Williams & Wilkins, (2005); and *Encyclopedia of Pharmaceutical Technology*, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York, each of which is incorporated herein by reference.

Compositions can be prepared according to conventional mixing, granulating or coating methods, respectively, and the present pharmaceutical compositions can contain from about 0.1% to about 99%, from about 5% to about 90%, or from about 1% to about 20% of a compound of the present invention, or pharmaceutically acceptable salt thereof, by weight or volume. In some embodiments, compounds, or a pharmaceutically acceptable salt thereof, described herein may be present in amounts totaling 1-95% by weight of the total weight of a composition, such as a pharmaceutical composition.

The composition may be provided in a dosage form that is suitable for intraarticular, oral, parenteral (e.g., intravenous, intramuscular), rectal, cutaneous, subcutaneous, topical, transdermal, sublingual, nasal, vaginal, intravesicular, intraurethral, intrathecal, epidural, aural, or ocular administration, or by injection, inhalation, or direct contact with the nasal, genitourinary, reproductive or oral mucosa. Thus, the pharmaceutical composition may be in the form of, e.g., tablets, capsules, pills, powders, granulates, suspensions, emulsions, solutions, gels including hydrogels, pastes, ointments, creams, plasters, drenches, osmotic delivery devices, suppositories, enemas, injectables, implants, sprays, preparations suitable for iontophoretic delivery, or aerosols. The compositions may be formulated according to conventional pharmaceutical practice.

As used herein, the term “administration” refers to the administration of a composition (e.g., a compound, or a preparation that includes a compound as described herein) to a subject or system. Administration to an animal subject (e.g., to a human) may be by any appropriate route. For example, in some embodiments, administration may be bronchial (including by bronchial instillation), buccal, enteral, interdermal, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intravenous, intraventricular, mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (including by intratracheal instillation), transdermal, vaginal or vitreal.

Formulations may be prepared in a manner suitable for systemic administration or topical or local administration. Systemic formulations include those designed for injection (e.g., intramuscular, intravenous or subcutaneous injection) or may be prepared for transdermal, transmucosal, or oral administration. A formulation will generally include a diluent as well as, in some cases, adjuvants, buffers, preservatives and the like. Compounds, or a pharmaceutically acceptable salt thereof, can be administered also in liposomal compositions or as microemulsions.

For injection, formulations can be prepared in conventional forms as liquid solutions or suspensions or as solid forms suitable for solution or suspension in liquid prior to injection or as emulsions. Suitable excipients include, for example, water, saline, dextrose, glycerol and the like. Such compositions may also contain amounts of nontoxic auxiliary substances such as wetting or

emulsifying agents, pH buffering agents and the like, such as, for example, sodium acetate, sorbitan monolaurate, and so forth.

Various sustained release systems for drugs have also been devised. See, for example, U.S. Patent No. 5,624,677.

Systemic administration may also include relatively noninvasive methods such as the use of suppositories, transdermal patches, transmucosal delivery and intranasal administration. Oral administration is also suitable for compounds of the invention, or a pharmaceutically acceptable salt thereof. Suitable forms include syrups, capsules, and tablets, as is understood in the art.

Each compound, or a pharmaceutically acceptable salt thereof, as described herein, may be formulated in a variety of ways that are known in the art. For example, the first and second agents of the combination therapy may be formulated together or separately. Other modalities of combination therapy are described herein.

The individually or separately formulated agents can be packaged together as a kit. Non-limiting examples include, but are not limited to, kits that contain, e.g., two pills, a pill and a powder, a suppository and a liquid in a vial, two topical creams, etc. The kit can include optional components that aid in the administration of the unit dose to subjects, such as vials for reconstituting powder forms, syringes for injection, customized IV delivery systems, inhalers, etc. Additionally, the unit dose kit can contain instructions for preparation and administration of the compositions. The kit may be manufactured as a single use unit dose for one subject, multiple uses for a particular subject (at a constant dose or in which the individual compounds, or a pharmaceutically acceptable salt thereof, may vary in potency as therapy progresses); or the kit may contain multiple doses suitable for administration to multiple subjects ("bulk packaging"). The kit components may be assembled in cartons, blister packs, bottles, tubes, and the like.

Formulations for oral use include tablets containing the active ingredient(s) in a mixture with non-toxic pharmaceutically acceptable excipients. These excipients may be, for example, inert diluents or fillers (e.g., sucrose, sorbitol, sugar, mannitol, microcrystalline cellulose, starches including potato starch, calcium carbonate, sodium chloride, lactose, calcium phosphate, calcium sulfate, or sodium phosphate); granulating and disintegrating agents (e.g., cellulose derivatives including microcrystalline cellulose, starches including potato starch, croscarmellose sodium, alginates, or alginic acid); binding agents (e.g., sucrose, glucose, sorbitol, acacia, alginic acid, sodium alginate, gelatin, starch, pregelatinized starch, microcrystalline cellulose, magnesium aluminum silicate, carboxymethylcellulose sodium, methylcellulose, optionally substituted hydroxylpropyl methylcellulose, ethylcellulose, polyvinylpyrrolidone, or polyethylene glycol); and lubricating agents, glidants, and antiadhesives (e.g., magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc). Other pharmaceutically acceptable excipients can be colorants, flavoring agents, plasticizers, humectants, buffering agents, and the like.

Two or more compounds may be mixed together in a tablet, capsule, or other vehicle, or may be partitioned. In one example, the first compound is contained on the inside of the tablet, and the second compound is on the outside, such that a substantial portion of the second compound is released prior to the release of the first compound.

Formulations for oral use may also be provided as chewable tablets, or as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent (e.g., potato starch, lactose, microcrystalline cellulose, calcium carbonate, calcium phosphate or kaolin), or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil. Powders, granulates, and pellets may be prepared using the ingredients mentioned above under tablets and capsules in a conventional manner using, e.g., a mixer, a fluid bed apparatus or a spray drying equipment.

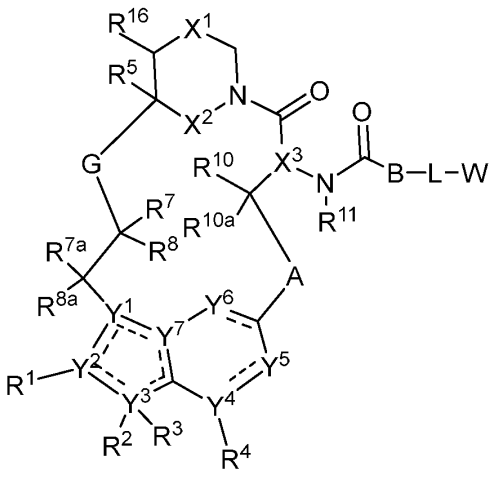
Dissolution or diffusion-controlled release can be achieved by appropriate coating of a tablet, capsule, pellet, or granulate formulation of compounds, or by incorporating the compound, or a pharmaceutically acceptable salt thereof, into an appropriate matrix. A controlled release coating may include one or more of the coating substances mentioned above or, e.g., shellac, beeswax, glycowax, castor wax, carnauba wax, stearyl alcohol, glyceryl monostearate, glyceryl distearate, glycerol palmitostearate, ethylcellulose, acrylic resins, dl-polylactic acid, cellulose acetate butyrate, polyvinyl chloride, polyvinyl acetate, vinyl pyrrolidone, polyethylene, polymethacrylate, methylmethacrylate, 2-optionally substituted hydroxylmethacrylate, methacrylate hydrogels, 1,3 butylene glycol, ethylene glycol methacrylate, or polyethylene glycols. In a controlled release matrix formulation, the matrix material may also include, e.g., hydrated methylcellulose, carnauba wax and stearyl alcohol, carbopol 934, silicone, glyceryl tristearate, methyl acrylate-methyl methacrylate, polyvinyl chloride, polyethylene, or halogenated fluorocarbon.

The liquid forms in which the compounds, or a pharmaceutically acceptable salt thereof, and compositions of the present invention can be incorporated for administration orally include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Generally, when administered to a human, the oral dosage of any of the compounds of the invention, or a pharmaceutically acceptable salt thereof, will depend on the nature of the compound, and can readily be determined by one skilled in the art. A dosage may be, for example, about 0.001 mg to about 2000 mg per day, about 1 mg to about 1000 mg per day, about 5 mg to about 500 mg per day, about 100 mg to about 1500 mg per day, about 500 mg to about 1500 mg per day, about 500 mg to about 2000 mg per day, or any range derivable therein.

Numbered Embodiments

[1] A compound, or pharmaceutically acceptable salt thereof, having the structure of Formula I:



Formula I

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- or >C=C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is hydrogen, cyano, optionally substituted amino, optionally substituted C_1-C_4 alkoxy, optionally substituted C_1-C_4 hydroxyalkyl, optionally substituted C_1-C_4 aminoalkyl, optionally substituted C_1-C_4 haloalkyl, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 guanidinoalkyl, C_0-C_4 alkyl optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 3 to 8-membered heteroaryl;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or S(O)$_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent or R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form C=C$R^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-

membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is hydrogen, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

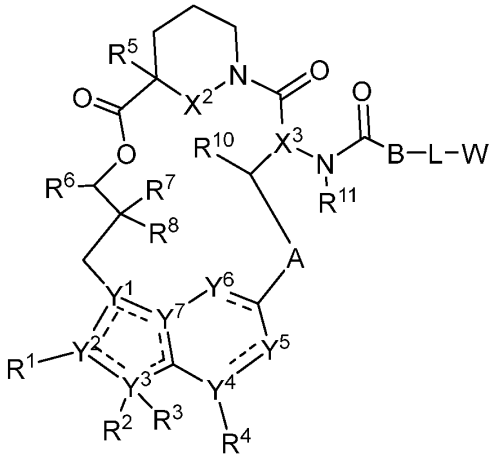
R^{10a} is hydrogen or halo;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{16} is hydrogen or C_1-C_3 alkyl.

[2] The compound, or pharmaceutically acceptable salt thereof, of paragraph [1], wherein G is optionally substituted C_1-C_4 heteroalkylene.

[3] The compound, or pharmaceutically acceptable salt thereof, of paragraph [1] or [2], wherein the compound has the structure of Formula Ic:



Formula Ic

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is hydrogen, optionally substituted amino, optionally substituted C_1-C_4 alkoxy, optionally substituted C_1-C_4 hydroxyalkyl, optionally substituted C_1-C_4 aminoalkyl, optionally substituted C_1-C_4 haloalkyl, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 guanidinoalkyl, C_0-C_4 alkyl optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 3 to 8-membered cycloalkyl, or optionally susbtituted 3 to 8-membered heteroaryl;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', $C(O)N(R')_2$, S(O)R', $S(O)_2R'$, or $S(O)_2N(R')_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent or R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), $C=N(O\text{-}C_1\text{-}C_3$ alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl; and

R^{11} is hydrogen or C_1-C_3 alkyl.

[4] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [3], wherein X^2 is NH.

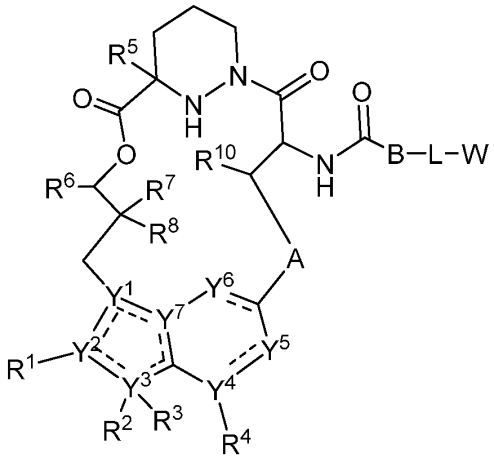
[5] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [4], wherein X^3 is CH.

[6] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [5], wherein R^{11} is hydrogen.

[7] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [5], wherein R^{11} is C_1-C_3 alkyl.

[8] The compound, or pharmaceutically acceptable salt thereof, of paragraph [7], wherein R^{11} is methyl.

[9] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [6], wherein the compound has the structure of Formula Id:



Formula Id

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-

membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is hydrogen, optionally substituted amino, optionally substituted C_1-C_4 alkoxy, optionally substituted C_1-C_4 hydroxyalkyl, optionally substituted C_1-C_4 aminoalkyl, optionally substituted C_1-C_4 haloalkyl, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 guanidinoalkyl, C_0-C_4 alkyl optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 3 to 8-membered cycloalkyl, or optionally susbtituted 3 to 8-membered heteroaryl;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent or R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered

heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; and

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl.

[10] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [9] wherein X^1 is optionally substituted C_1-C_2 alkylene.

[11] The compound, or pharmaceutically acceptable salt thereof, of paragraph [10], wherein X^1 is methylene.

[12] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [11], wherein R^5 is hydrogen.

[13] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [11], wherein R^5 is C_1-C_4 alkyl optionally substituted with halogen.

[14] The compound, or pharmaceutically acceptable salt thereof, of paragraph [13], wherein R^5 is methyl.

[15] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [14], wherein Y^4 is C.

[16] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [15], wherein R^4 is hydrogen.

[17] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [16], wherein Y^5 is CH.

[18] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [17], wherein Y^6 is CH.

[19] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [18], wherein Y^1 is C.

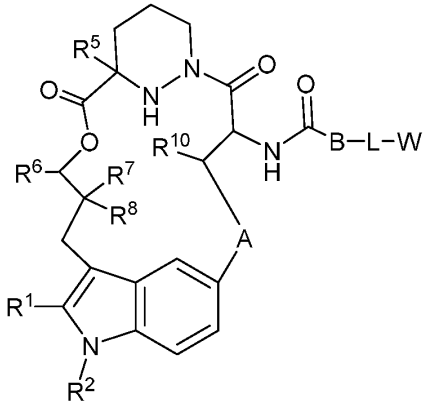
[20] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [19], wherein Y^2 is C.

[21] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [20], wherein Y^3 is N.

[22] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [21], wherein R^3 is absent.

[23] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [22], wherein Y^7 is C.

[24] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [6] or [9] to [23], wherein the compound has the structure of Formula Ie:



Formula Ie

wherein A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is hydrogen, optionally substituted amino, optionally substituted C_1-C_4 alkoxy, optionally substituted C_1-C_4 hydroxyalkyl, optionally substituted C_1-C_4 aminoalkyl, optionally substituted C_1-C_4 haloalkyl, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 guanidinoalkyl, C_0-C_4 alkyl optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 3 to 8-membered cycloalkyl, or optionally susbtituted 3 to 8-membered heteroaryl;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent or R^2 and R^3 combine with the atom to which they are attached to form an

optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; and

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl.

[25] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [3] to [24], wherein R^6 is hydrogen.

[26] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [25], wherein R^2 is hydrogen, cyano, optionally substituted C_1-C_6 alkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 6-membered heterocycloalkyl.

[27] The compound, or pharmaceutically acceptable salt thereof, of paragraph [26], wherein R^2 is optionally substituted C_1-C_6 alkyl.

[28] The compound, or pharmaceutically acceptable salt thereof, of paragraph [27], wherein R^2 is ethyl.

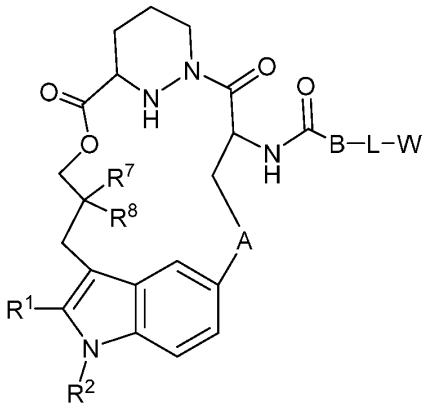
[29] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [28], wherein R^7 is optionally substituted C_1-C_3 alkyl.

[30] The compound, or pharmaceutically acceptable salt thereof, of paragraph [29], wherein R^7 is C_1-C_3 alkyl.

[31] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [30], wherein R^8 is optionally substituted C_1-C_3 alkyl.

[32] The compound, or pharmaceutically acceptable salt thereof, of paragraph [31], wherein R^8 is C_1-C_3 alkyl.

[33] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [32], wherein the compound has the structure of Formula If:



Formula If

wherein A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is hydrogen, optionally substituted amino, optionally substituted C_1-C_4 alkoxy, optionally substituted C_1-C_4 hydroxyalkyl, optionally substituted C_1-C_4 aminoalkyl, optionally substituted C_1-C_4 haloalkyl, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 guanidinoalkyl, C_0-C_4 alkyl optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 3 to 8-membered cycloalkyl, or optionally susbtituted 3 to 8-membered heteroaryl;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is C_1-C_6 alkyl or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

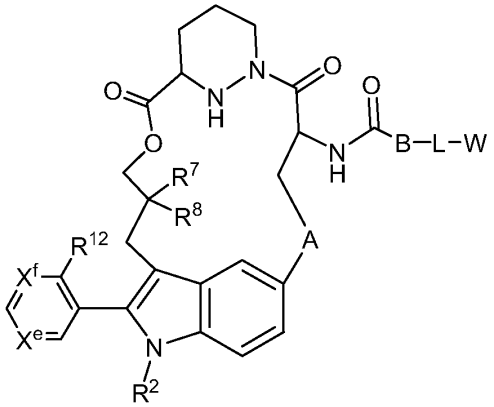
R^8 is C_1-C_3 alkyl; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

[34] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [33], wherein R^1 is 5 to 10-membered heteroaryl.

[35] The compound, or pharmaceutically acceptable salt thereof, of paragraph [34], wherein R^1 is optionally substituted 6-membered aryl or optionally substituted 6-membered heteroaryl.

[36] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [35], wherein the compound has the structure of Formula Ig:



Formula Ig

wherein A is, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is hydrogen, optionally substituted amino, optionally substituted C_1-C_4 alkoxy, optionally substituted C_1-C_4 hydroxyalkyl, optionally substituted C_1-C_4 aminoalkyl, optionally substituted C_1-C_4 haloalkyl, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 guanidinoalkyl, C_0-C_4 alkyl optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 3 to 8-membered cycloalkyl, or optionally susbtituted 3 to 8-membered heteroaryl;

R^2 is C_1-C_6 alkyl or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

R^8 is C_1-C_3 alkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

X^e is N, CH, or CR^{17};

X^f is N or CH;

R^{12} is optionally substituted C_1-C_6 alkyl or optionally substituted C_1-C_6 heteroalkyl; and

R^{17} is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl,

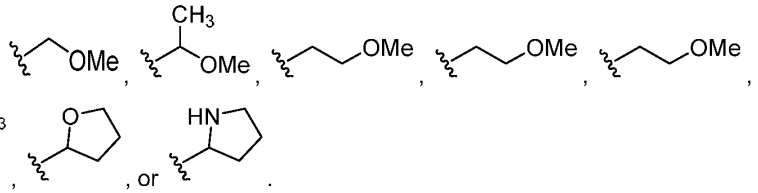
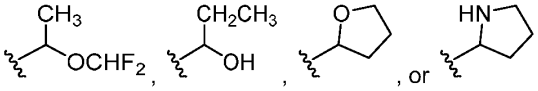
optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

[37] The compound, or pharmaceutically acceptable salt thereof, of paragraph [36], wherein X^e is N and X^f is CH.

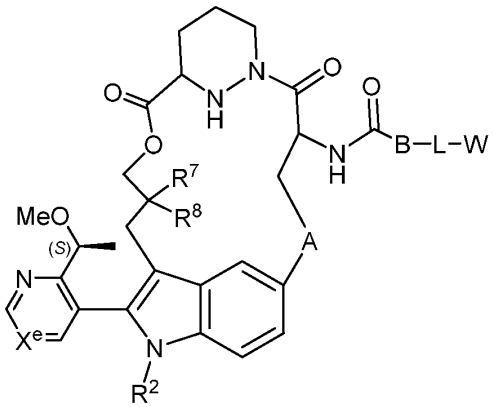
[38] The compound, or pharmaceutically acceptable salt thereof, of paragraph [36], wherein X^e is CH and X^f is N.

[39] The compound, or pharmaceutically acceptable salt thereof, of paragraph [36], wherein X^e is CR^{17} and X^f is N.

[40] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [36] to [39], wherein R^{12} is optionally substituted C_1-C_6 heteroalkyl.

[41] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [36] to [40], wherein R^{12} is  , or  .

[42] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [41], wherein the compound has the structure of Formula Ih:



Formula Ih

wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is hydrogen, optionally substituted amino, optionally substituted C_1-C_4 alkoxy, optionally substituted C_1-C_4 hydroxyalkyl, optionally substituted C_1-C_4 aminoalkyl, optionally substituted C_1-C_4 haloalkyl, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 guanidinoalkyl, C_0-C_4 alkyl

optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 3 to 8-membered cycloalkyl, or optionally substituted 3 to 8-membered heteroaryl;

R^2 is C_1-C_6 alkyl or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

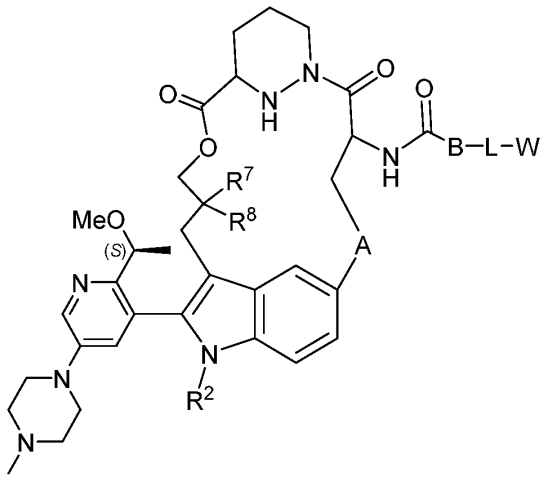
R^8 is C_1-C_3 alkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

X^e is CH, or CR^{17}; and

R^{17} is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

[43] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [42], wherein the compound has the structure of Formula Ii:



Formula Ii

wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is hydrogen, optionally substituted amino, optionally substituted C_1-C_4 alkoxy, optionally substituted C_1-C_4 hydroxyalkyl, optionally substituted C_1-C_4 aminoalkyl, optionally substituted C_1-C_4 haloalkyl, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_4 guanidinoalkyl, C_0-C_4 alkyl optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 3 to 8-membered cycloalkyl, or optionally substituted 3 to 8-membered heteroaryl;

R^2 is C_1-C_6 alkyl or 3 to 6-membered cycloalkyl;

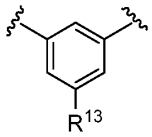
R^7 is C_1-C_3 alkyl;

R^8 is C_1-C_3 alkyl; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

[44] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [43], wherein A is optionally substituted 6-membered arylene.

[45] The compound, or pharmaceutically acceptable salt thereof, of paragraph [44], wherein A has the structure:

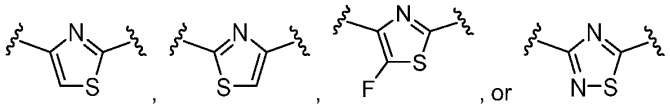


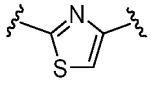
wherein R^{13} is hydrogen, hydroxy, amino, cyano, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl.

[46] The compound, or pharmaceutically acceptable salt thereof, of paragraph [45], wherein R^{13} is hydrogen.

[47] The compound, or pharmaceutically acceptable salt thereof, of paragraph [45], wherein R^{13} is hydroxy.

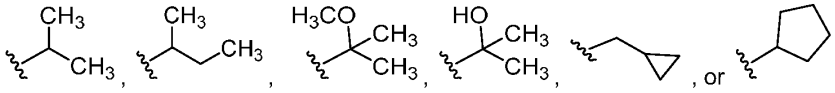
[48] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [43], wherein A is optionally substituted 5 to 6-membered heteroarylene.

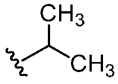
[49] The compound, or pharmaceutically acceptable salt thereof, of paragraph [48], wherein A is:  .

[50] The compound, or pharmaceutically acceptable salt thereof, of paragraph [49], wherein A is  .

[51] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [50], wherein B is -CHR^9-.

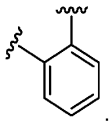
[52] The compound, or pharmaceutically acceptable salt thereof, of paragraph [51], wherein R^9 is optionally substituted C_1-C_6 alkyl or optionally substituted 3 to 6-membered cycloalkyl.

[53] The compound, or pharmaceutically acceptable salt thereof, of paragraph [52], wherein R^9 is:  .

[54] The compound, or pharmaceutically acceptable salt thereof, of paragraph [53], wherein R^9 is:  .

[55] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [50], wherein B is optionally substituted 6-membered arylene.

[56] The compound, or pharmaceutically acceptable salt thereof, of paragraph [55], wherein B is 6-membered arylene.

[57] The compound, or pharmaceutically acceptable salt thereof, of paragraph [56], wherein B is:  .

[58] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [50], wherein B is absent.

[59] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [58], wherein R^7 is methyl.

[60] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [59], wherein R^8 is methyl.

[61] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [60], wherein the linker is the structure of Formula II:

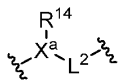
$$A^1\text{-}(B^1)_f\text{-}(C^1)_g\text{-}(B^2)_h\text{-}(D^1)\text{-}(B^3)_i\text{-}(C^2)_j\text{-}(B^4)_k\text{–}A^2$$

Formula II

where A^1 is a bond between the linker and B; A^2 is a bond between W and the linker; B^1, B^2, B^3, and B^4 each, independently, is selected from optionally substituted C_1-C_2 alkylene, optionally substituted C_1-C_3 heteroalkylene, O, S, and NR^N; R^N is hydrogen, optionally substituted C_1-C_4 alkyl, optionally substituted C_1-C_3 cycloalkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted C_1-C_7 heteroalkyl; C^1 and C^2 are each, independently, selected from carbonyl, thiocarbonyl, sulphonyl, or phosphoryl; f, g, h, i, j, and k are each, independently, 0 or 1; and D^1 is optionally substituted C_1-C_{10} alkylene, optionally substituted C_2-C_{10} alkenylene, optionally substituted C_2-C_{10} alkynylene, optionally substituted 3 to 14-membered heterocycloalkylene, optionally substituted 5 to 10-membered heteroarylene, optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 6 to 10-membered arylene, optionally substituted C_2-C_{10} polyethylene glycolene, or optionally substituted C_1-C_{10} heteroalkylene, or a chemical bond linking A^1-$(B^1)_f$-$(C^1)_g$-$(B^2)_h$- to -$(B^3)_i$-$(C^2)_j$-$(B^4)_k$–A^2.

[62] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [61], wherein the linker is acyclic.

[63] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [62], wherein the linker has the structure of Formula IIa:



Formula IIa

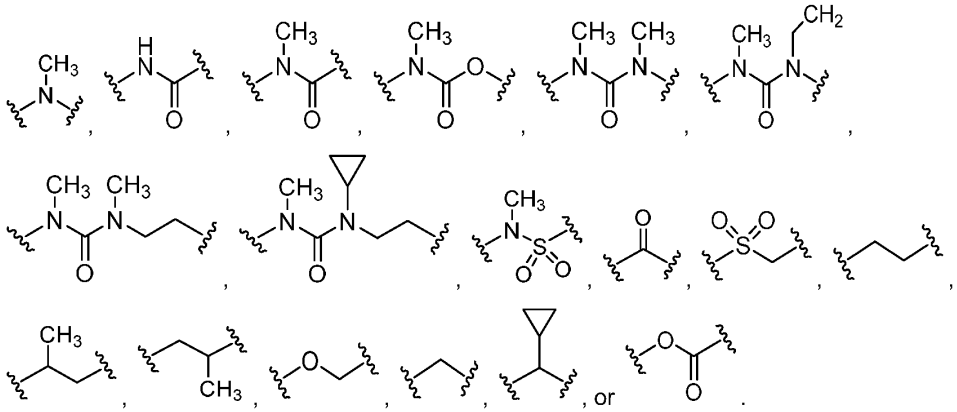
wherein X^a is absent or N;

R^{14} is absent, hydrogen, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_3 cycloalkyl; and

L^2 is absent, -C(O)-, -SO_2-, optionally substituted C_1-C_4 alkylene or optionally substituted C_1-C_4 heteroalkylene,

wherein at least one of X^a, R^{14}, or L^2 is present.

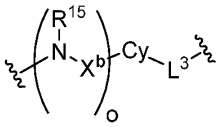
[64] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [63], wherein the linker has the structure:



[65] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [64], wherein the linker has the structure .

[66] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [61], wherein the linker is or comprises a cyclic group.

[67] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [61] or [66], wherein the linker has the structure of Formula IIb:



Formula IIb

wherein o is 0 or 1;

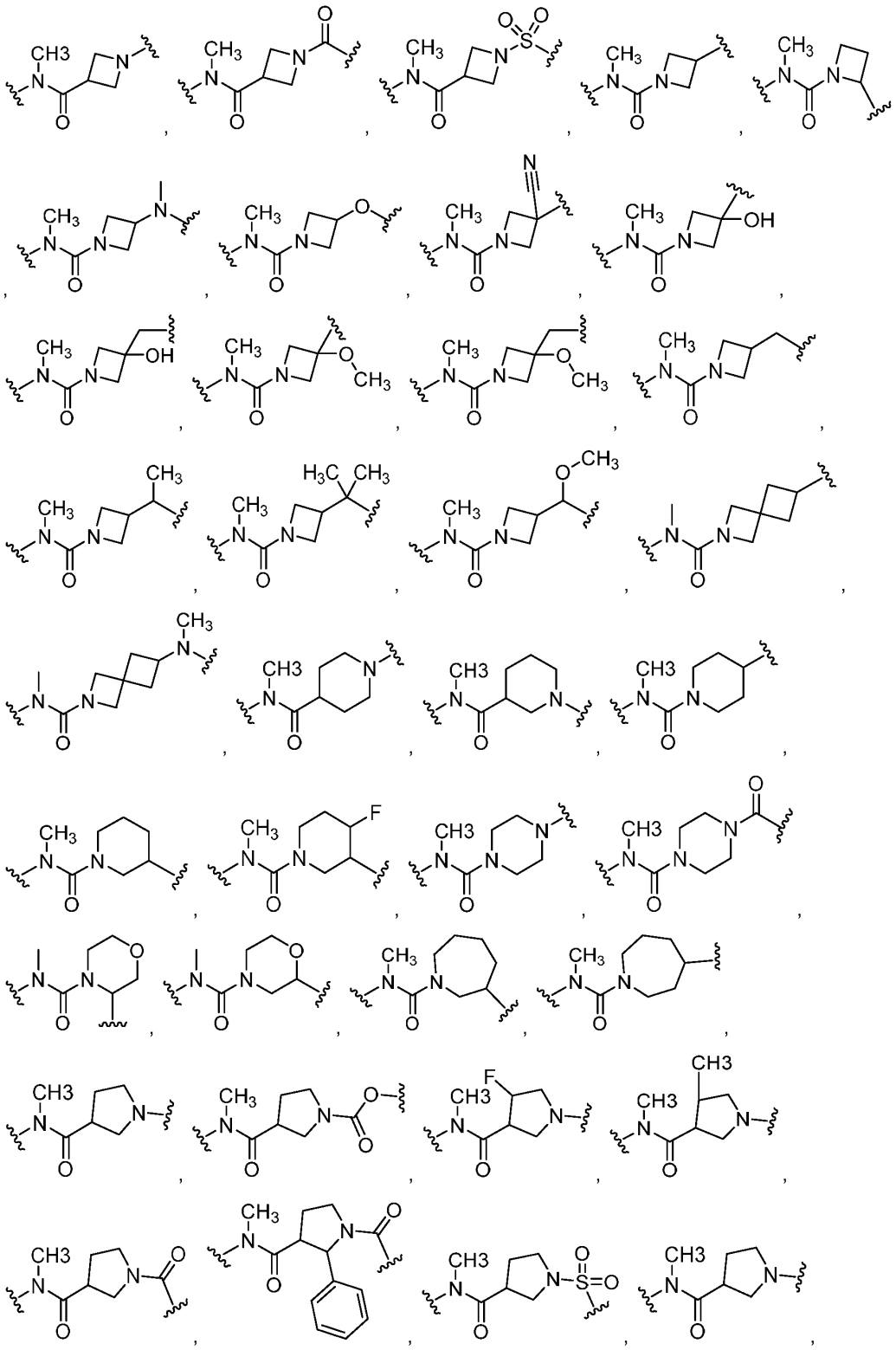
X^b is C(O) or SO_2;

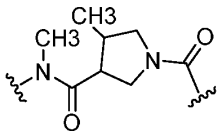
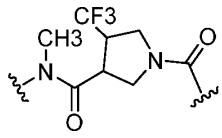
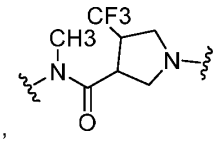
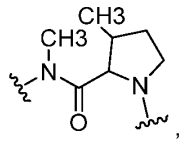
R^{15} is hydrogen or optionally substituted C_1-C_6 alkyl;

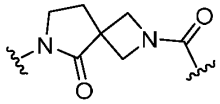
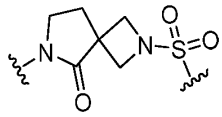
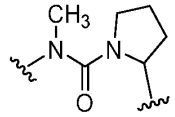
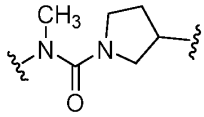
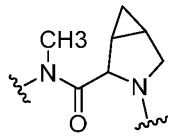
Cy is optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 3 to 8-membered heterocycloalkylene, optionally substituted 6-10 membered arylene, or optionally substituted 5 to 10-membered heteroarylene; and

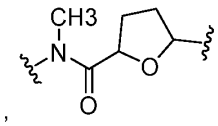
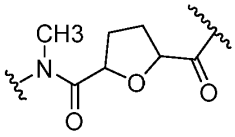
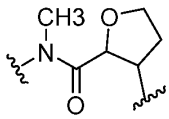
L^3 is absent, -C(O)-, -SO_2-, optionally substituted C_1-C_4 alkylene or optionally substituted C_1-C_4 heteroalkylene.

[68] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [67], wherein the linker has the structure:



 ,  ,  ,  ,

 ,  ,  ,  ,  ,

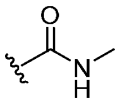
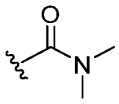
 ,  , or  .

[69] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [68], wherein W is hydrogen.

[70] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [68], wherein W is optionally substituted amino.

[71] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [70], wherein W is $-NHCH_3$ or $-N(CH_3)_2$.

[72] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [68], wherein W is optionally substituted amido.

[73] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [72], wherein W is  or  .

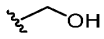
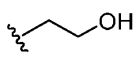
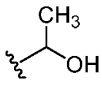
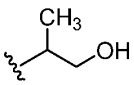
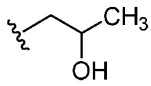
[74] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [68], wherein W is optionally substituted C_1-C_4 alkoxy.

[75] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [74], wherein W is methoxy or iso-propoxy.

[76] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [68], wherein W is optionally substituted C_1-C_4 alkyl.

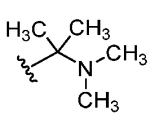
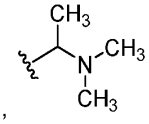
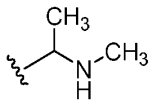
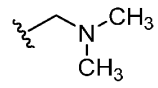
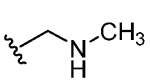
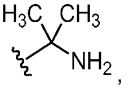
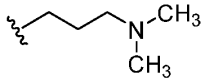
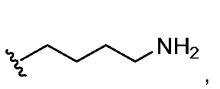
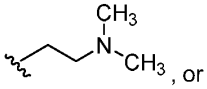
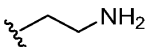
[77] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [76], wherein W is methyl, ethyl, iso-propyl, tert-butyl, or benzyl.

[78] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [68], wherein W is optionally substituted C_1-C_4 hydroxyalkyl.

[79] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [78], wherein W is  ,  ,  ,  , or  .

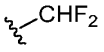
[80] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [68], wherein W is optionally substituted C_1-C_4 aminoalkyl.

[81] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [80],

wherein W is ,  ,  ,  ,  ,  ,  ,  ,  ,  , or  .

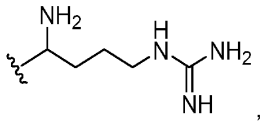
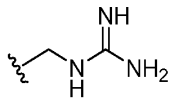
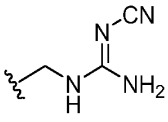
[82] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [68], wherein W is optionally substituted C_1-C_4 haloalkyl.

[83] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [82],

wherein W is ,  , , or .

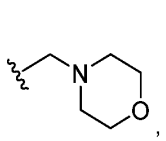
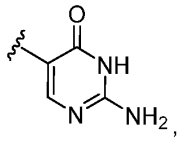
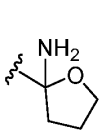
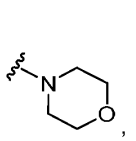
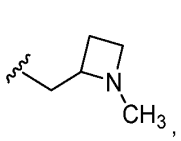
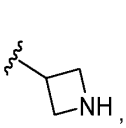
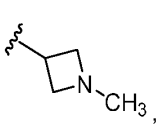
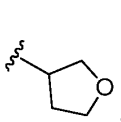
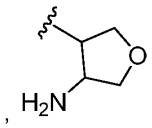
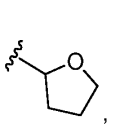
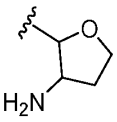
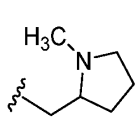
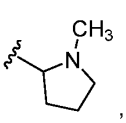
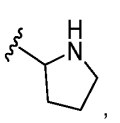
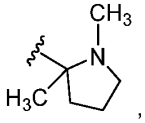
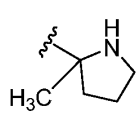
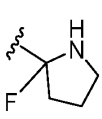
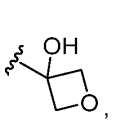
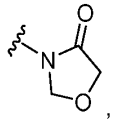
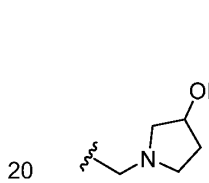
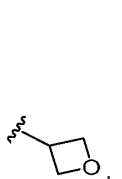
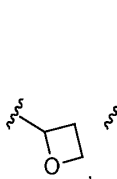
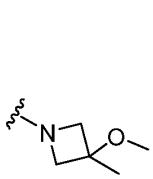
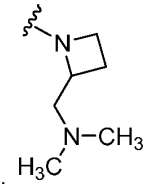
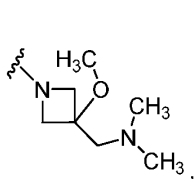
[84] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [68], wherein W is optionally substituted C_1-C_4 guanidinoalkyl.

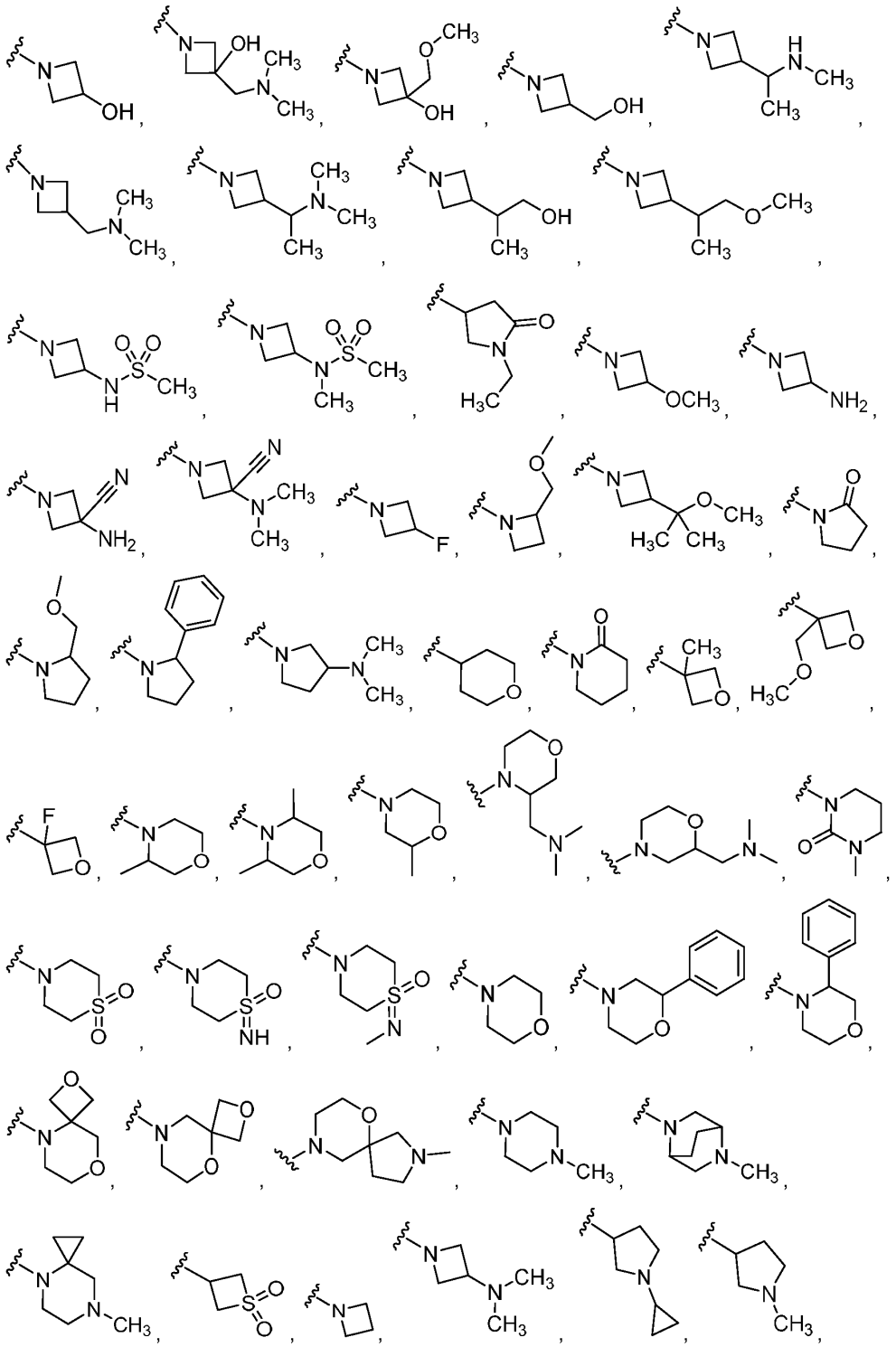
[85] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [84],

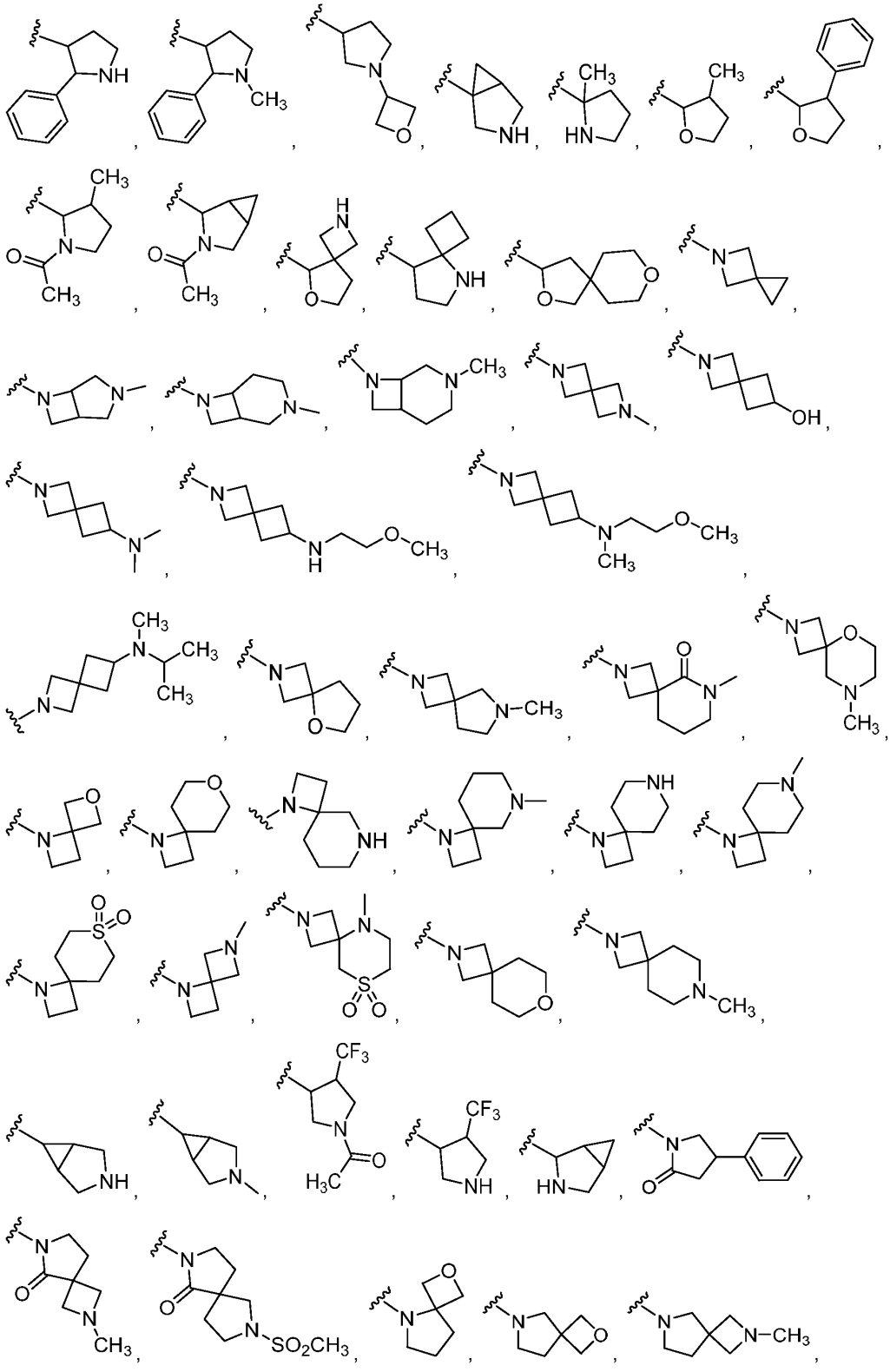
wherein W is  ,  , or  .

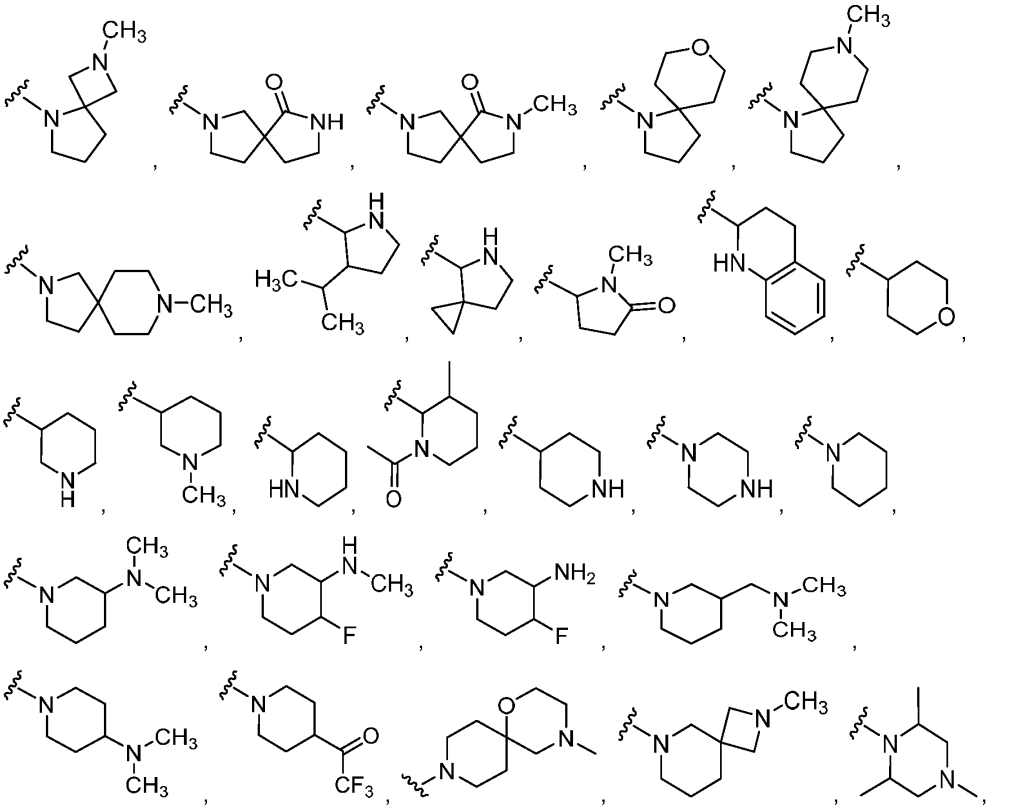
[86] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [68], wherein W is C_0-C_4 alkyl optionally substituted 3 to 11-membered heterocycloalkyl.

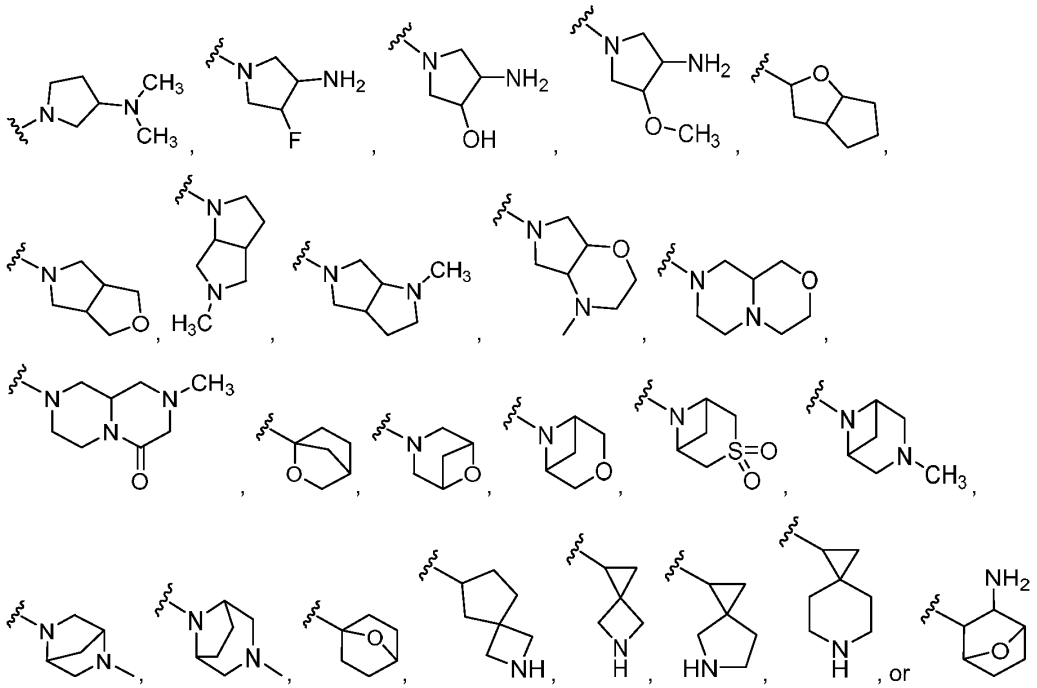
[87] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [86],

wherein W is  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  , ,  , ,  .









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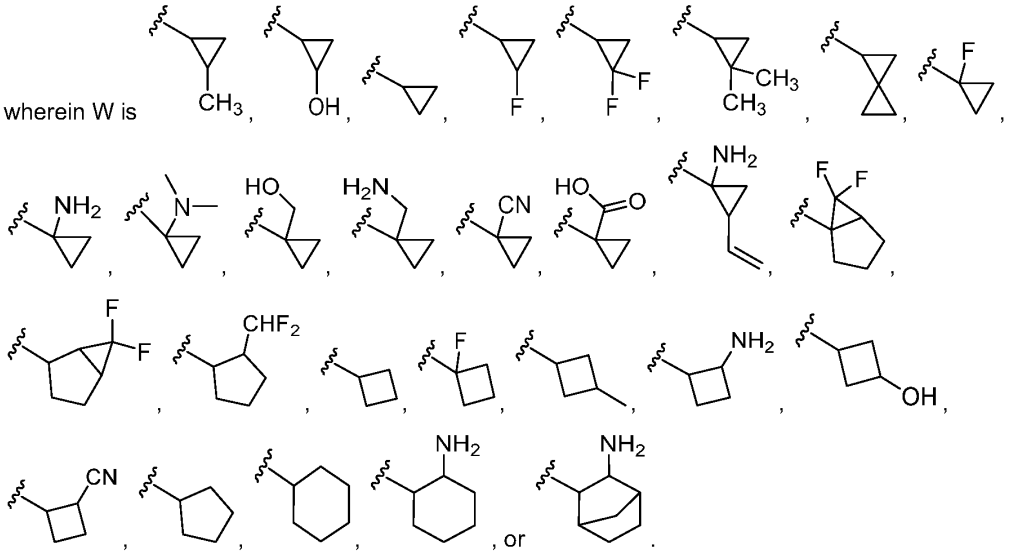
[88] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [68], wherein W is optionally substituted 3 to 8-membered cycloalkyl.

[89] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [88], wherein W is



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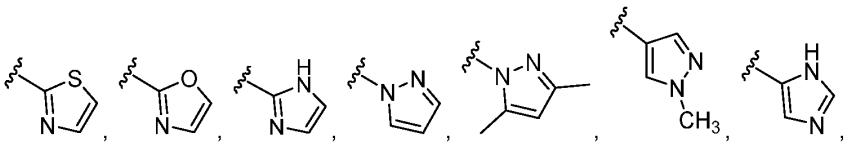
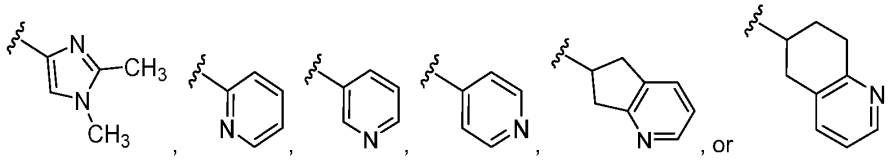
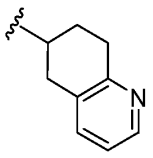
[90] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [68], wherein W is optionally substituted 3 to 8-membered heteroaryl.

[91] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [90], wherein W is  ,  , or  .

[92] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [68], wherein W is optionally substituted 6- to 10-membered aryl.

[93] The compound, or a pharmaceutically acceptable salt thereof, or paragraph [92], wherein W is phenyl, 4-hydroxy-phenyl, or 2,4-methoxy-phenyl.

[94] A compound, or a pharmaceutically acceptable salt thereof, of Table 1 or 2.

[95] A pharmaceutical composition comprising a compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [94] and a pharmaceutically acceptable excipient.

Examples

The disclosure is further illustrated by the following examples and synthesis examples, which are not to be construed as limiting this disclosure in scope or spirit to the specific procedures herein described. It is to be understood that the examples are provided to illustrate certain embodiments and that no limitation to the scope of the disclosure is intended thereby. It is to be further understood that resort may be had to various other embodiments, modifications, and equivalents thereof which may suggest themselves to those skilled in the art without departing from the spirit of the present disclosure or scope of the appended claims.

Chemical Syntheses

Definitions used in the following examples and elsewhere herein are:

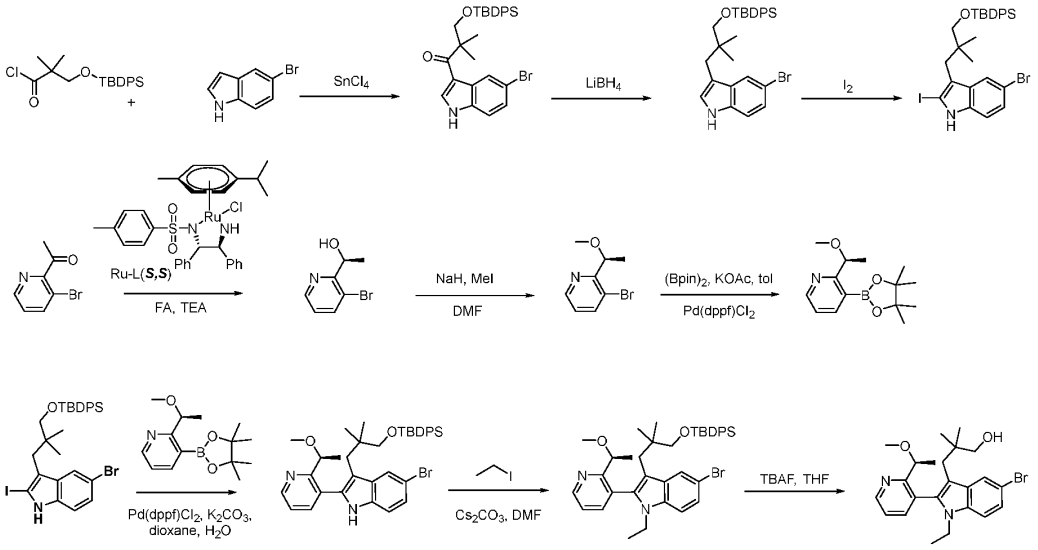
CH_2Cl_2, DCM	Methylene chloride, Dichloromethane
CH_3CN, MeCN	Acetonitrile
CuI	Copper (I) iodide
DIPEA	Diisopropylethyl amine
DMF	N,N-Dimethylformamide
EtOAc	Ethyl acetate
h	hour
H_2O	Water
HCl	Hydrochloric acid
K_3PO_4	Potassium phosphate (tribasic)
MeOH	Methanol
Na_2SO_4	Sodium sulfate
NMP	N-methyl pyrrolidone
$Pd(dppf)Cl_2$	[1,1′-Bis(diphenylphosphino)ferrocene]dichloropalladium(II)

Instrumentation

Mass spectrometry data collection took place with a Shimadzu LCMS-2020, an Agilent 1260LC-6120/6125MSD, a Shimadzu LCMS-2010EV, or a Waters Acquity UPLC, with either a QDa detector or SQ Detector 2. Samples were injected in their liquid phase onto a C-18 reverse phase. The compounds were eluted from the column using an acetonitrile gradient and fed into the mass analyzer. Initial data analysis took place with either Agilent ChemStation, Shimadzu LabSolutions, or Waters MassLynx. NMR data was collected with either a Bruker AVANCE III HD 400MHz, a Bruker Ascend 500MHz instrument, or a Varian 400MHz, and the raw data was analyzed with either TopSpin or Mestrelab Mnova.

Synthesis of Intermediates

Intermediate 1. Synthesis of 3-(5-bromo-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropan-1-ol



Intermediate 1.

Step 1. To a mixture of 3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropanoyl chloride (65 g, 137 mmol, crude) in DCM (120 mL) at 0 °C under an atmosphere of N_2 was added 1M $SnCl_4$ in DCM (137 mL, 137 mmol) slowly. The mixture was stirred at 0 °C for 30 min, then a solution of 5-bromo-1*H*-indole (26.8 g, 137 mmol) in DCM (40 mL) was added dropwise. The mixture was stirred at 0 °C for 45 min, then diluted with EtOAc (300 mL), washed with brine (100 mL x 4), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 1-(5-bromo-1*H*-indol-3-yl)-3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one (55 g, 75% yield). LCMS (ESI): m/z [M+Na] calc'd for $C_{29}H_{32}BrNO_2SiNa$ 556.1; found 556.3.

Step 2. To a mixture of 1-(5-bromo-1*H*-indol-3-yl)-3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one (50 g, 93.6 mmol) in THF (100 mL) at 0 °C under an atmosphere of N_2 was added $LiBH_4$ (6.1 g, 281 mmol). The mixture was heated to 60 °C and stirred for 20 h, then MeOH (10 mL) and EtOAc (100 mL) were added and the mixture washed with brine (50 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The residue was diluted with DCM (50 mL), cooled to 10 °C and diludine (9.5 g, 37.4 mmol) and $TsOH.H_2O$ (890 mg, 4.7 mmol) added. The mixture was stirred at 10 °C for 2 h, filtered, the filtrate concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 1-(5-bromo-1*H*-indol-3-yl)-3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one (41 g, 84% yield). LCMS (ESI): m/z [M+H] calc'd for $C_{29}H_{34}BrNOSi$ 519.2; found 520.1; ^1H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H),

7.75 - 7.68 (m, 5H), 7.46 - 7.35 (m, 6H), 7.23 - 7.19 (m, 2H), 6.87 (d, *J* = 2.1 Hz, 1H), 3.40 (s, 2H), 2.72 (s, 2H), 1.14 (s, 9H), 0.89 (s, 6H).

Step 3. To a mixture of 1-(5-bromo-1*H*-indol-3-yl)-3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one (1.5 g, 2.9 mmol) and I_2 (731 mg, 2.9 mmol) in THF (15 mL) at rt was added AgOTf (888 mg, 3.5 mmol). The mixture was stirred at rt for 2 h, then diluted with EtOAc (200 mL) and washed with saturated $Na_2S_2O_3$ (100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-iodo-1*H*-indole (900 mg, 72% yield) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (s, 1H), 7.68 (d, *J* = 1.3 Hz, 1H), 7.64 - 7.62 (m, 4H), 7.46 - 7.43 (m, 6H), 7.24 - 7.22 (d, 1H), 7.14 - 7.12 (dd, *J* = 8.6, 1.6 Hz, 1H), 3.48 (s, 2H), 2.63 (s, 2H), 1.08 (s, 9H), 0.88 (s, 6H).

Step 4. To a stirred mixture of HCOOH (66.3 g, 1.44 mol) in TEA (728 g, 7.2 mol) at 0 °C under an atmosphere of Ar was added (4*S*,5*S*)-2-chloro-2-methyl-1-(4-methylbenzenesulfonyl)-4,5-diphenyl-1,3-diaza-2-ruthenacyclopentane cymene (3.9 g, 6.0 mmol) portion-wise. The mixture was heated to 40 °C and stirred for 15 min, then cooled to rt and 1-(3-bromopyridin-2-yl)ethanone (120 g, 600 mmol) added in portions. The mixture was heated to 40 °C and stirred for an additional 2 h, then the solvent was concentrated under reduced pressure. Brine (2 L) was added to the residue, the mixture was extracted with EtOAc (4 x 700 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (1*S*)-1-(3-bromopyridin-2-yl)ethanol (100 g, 74% yield) a an oil. LCMS (ESI): m/z [M+H] calc'd for C_7H_8BrNO 201.1; found 201.9.

Step 5. To a stirred mixture of (1*S*)-1-(3-bromopyridin-2-yl)ethanol (100 g, 495 mmol) in DMF (1 L) at 0 °C was added NaH, 60% dispersion in oil (14.25 g, 594 mmol) in portions. The mixture was stirred at 0 °C for 1 h. MeI (140.5 g, 990 mmol) was added dropwise at 0 °C and the mixture was allowed to warm to rt and stirred for 2 h. The mixture was cooled to 0 °C and saturated NH_4Cl (5 L) was added. The mixture was extracted with EtOAc (3 x 1.5 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-bromo-2-[(1*S*)-1-methoxyethyl]pyridine (90 g, 75% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_8H_{10}BrNO$ 215.0; found 215.9.

Step 6. To a stirred mixture of 3-bromo-2-[(1*S*)-1-methoxyethyl]pyridine (90 g, 417 mmol) and Pd(dppf)Cl_2 (30.5 g, 41.7 mmol) in toluene (900 mL) at rt under an atmosphere of Ar was added bis(pinacolato)diboron (127 g, 500 mmol) and KOAc (81.8 g, 833 mmol) in portions. The mixture was heated to 100 °C and stirred for 3 h. The filtrate was concentrated under reduced pressure and the residue was purified by Al_2O_3 column chromatography to give 2-[(1*S*)-1-methoxyethyl]-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (100 g, 63% yield) as a semi-solid. LCMS (ESI): m/z [M+H] calc'd for $C_{14}H_{22}BNO_3$ 263.2; found 264.1.

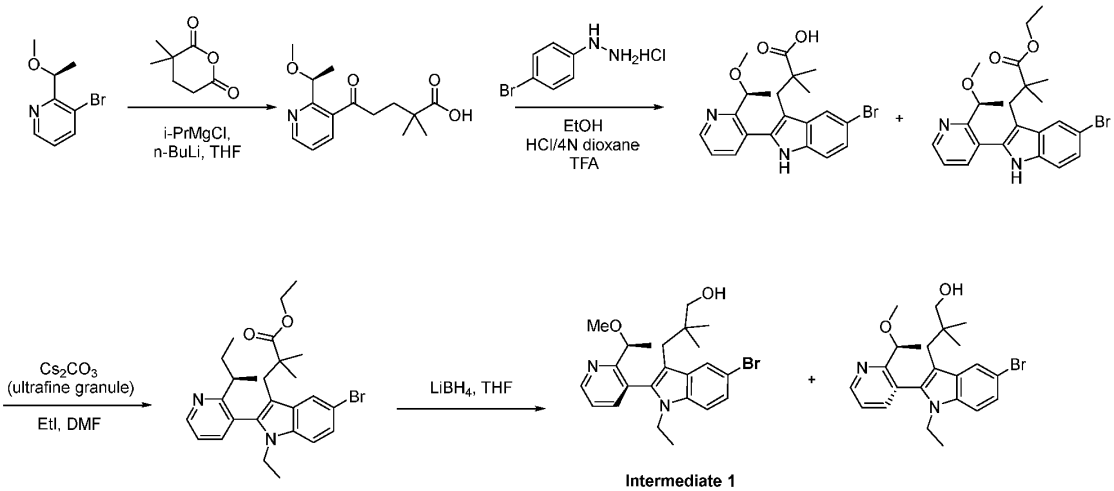
Step 7. To a stirred mixture of 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-2-iodo-1*H*-indole (140 g, 217 mmol) and 2-[(1*S*)-1-methoxyethyl]-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (100 g, 380 mmol) in 1,4-dioxane (1.4 L) at rt under an atmosphere of Ar was added K_2CO_3 (74.8 g, 541 mmol), Pd(dppf)Cl_2 (15.9 g, 21.7 mmol) and H_2O (280 mL) in portions. The mixture was heated to 85 °C and stirred for 4 h, then cooled, H_2O (5 L)

added and the mixture extracted with EtOAc (3 x 2 L). The combined organic layers were washed with brine (2 x 1 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-1*H*-indole (71 g, 45% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{37}H_{43}BrN_2O_2Si$ 654.2; found 655.1.

Step 8. To a stirred mixture of 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-1*H*-indole (71 g, 108 mmol) in DMF (0.8 L) at 0 °C under an atmosphere of N_2 was added Cs_2CO_3 (70.6 g, 217 mmol) and EtI (33.8 g, 217 mmol) in portions. The mixture was warmed to rt and stirred for 16 h then H_2O (4 L) added and the mixture extracted with EtOAc (3 x 1.5 L). The combined organic layers were washed with brine (2 x 1 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indole (66 g, 80% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{47}BrN_2O_2Si$ 682.3; found 683.3.

Step 9. To a stirred mixture of TBAF (172.6 g, 660 mmol) in THF (660 mL) at rt under an atmosphere of N_2 was added 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indole (66 g, 97 mmol) in portions. The mixture was heated to 50 °C and stirred for 16 h, cooled, diluted with H_2O (5 L) and extracted with EtOAc (3 x 1.5 L). The combined organic layers were washed with brine (2 x 1 L), dried over anhydrous Na_2SO_4 and filtered. After filtration, the filtrate was concentrated under reduced pressure. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-(5-bromo-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropan-1-ol (30 g, 62% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{29}BrN_2O_2$ 444.1; found 445.1.

Intermediate 1. Alternative Synthesis through Fisher Indole Route.



Intermediate 1

Step 1. To a mixture of *i*-PrMgCl (2M in in THF, 0.5 L) at -10 °C under an atmosphere of N_2 was added *n*-BuLi, 2.5 M in hexane (333 mL, 833 mmol) dropwise over 15 min. The mixture was stirred for 30 min at -10 °C then 3-bromo-2-[(1*S*)-1-methoxyethyl]pyridine (180 g, 833 mmol) in THF (0.5 L) added dropwise over 30 min at -10 °C. The resulting mixture was warmed to -5 °C and stirred for 1 h, then 3,3-dimethyloxane-2,6-dione (118 g, 833 mmol) in THF (1.2 L) was added dropwise over 30 min at -5 °C. The mixture was warmed to 0 °C and stirred for 1.5 h, then quenched with the addition of pre-cooled 4M HCl in 1,4-dioxane (0.6 L) at 0 °C to adjust pH ~5. The mixture was diluted with ice-water (3 L) and extracted with EtOAc (3 x 2.5 L). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 5-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-2,2-dimethyl-5-oxopentanoic acid (87 g, 34% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{15}H_{21}NO_4$ 279.2; found 280.1.

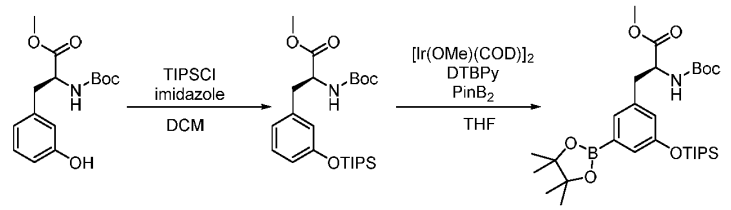
Step 2. To a mixture of 5-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-2,2-dimethyl-5-oxopentanoic acid (78 g, 279 mmol) in EtOH (0.78 L) at rt under an atmosphere of N_2 was added (4-bromophenyl)hydrazine HCl salt (68.7 g, 307 mmol) in portions. The mixture was heated to 85 °C and stirred for 2 h, cooled to rt, then 4M HCl in 1,4-dioxane (69.8 mL, 279 mmol) added dropwise. The mixture was heated to 85 °C and stirred for an additional 3 h, then concentrated under reduced pressure and the residue was dissolved in TFA (0.78 L). The mixture was heated to 60 °C and stirred for 1.5, concentrated under reduced pressure and the residue adjusted to pH ~5 with saturated $NaHCO_3$, then extracted with EtOAc (3 x 1.5 L). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-(5-bromo-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-1*H*-indol-3-yl)-2,2-dimethylpropanoic acid and ethyl (*S*)-3-(5-bromo-2-(2-(1-methoxyethyl)pyridin-3-yl)-1*H*-indol-3-yl)-2,2-dimethylpropanoate (78 g, crude). LCMS (ESI): m/z [M+H] calc'd for $C_{21}H_{23}BrN_2O_3$ 430.1 and $C_{23}H_{27}BrN_2O_3$ 458.1; found 431.1 and 459.1.

Step 3. To a mixture of 3-(5-bromo-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-1*H*-indol-3-yl)-2,2-dimethylpropanoic acid and ethyl (*S*)-3-(5-bromo-2-(2-(1-methoxyethyl)pyridin-3-yl)-1*H*-indol-3-

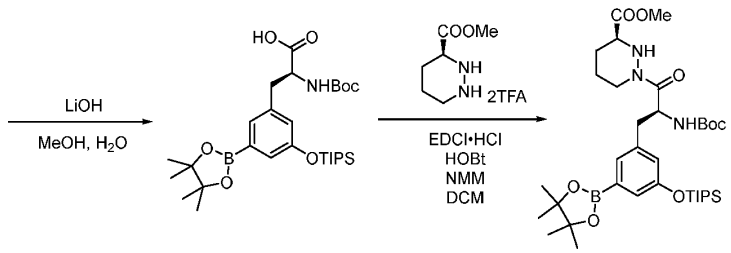
yl)-2,2-dimethylpropanoate (198 g, 459 mmol) in DMF (1.8 L) at 0 °C under an atmosphere of N_2 was added Cs_2CO_3 (449 g, 1.38 mol) in portions. EtI (215 g, 1.38 mmol) in DMF (200 mL) was then added dropwise at 0 °C. The mixture was warmed to rt and stirred for 4 h then diluted with brine (5 L) and extracted with EtOAc (3 x 2.5 L). The combined organic layers were washed with brine (2 x 1.5 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give ethyl 3-(5-bromo-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropanoate (160 g, 57% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{25}H_{31}BrN_2O_3$ 486.2; found 487.2.

Step 4. To a mixture of ethyl 3-(5-bromo-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropanoate (160 g, 328 mmol) in THF (1.6 L) at 0 °C under an atmosphere of N_2 was added $LiBH_4$ (28.6 g, 1.3 mol). The mixture was heated to 60 °C for 16 h, cooled, and quenched with pre-cooled (0 °C) aqueous NH_4Cl (5 L). The mixture was extracted with EtOAc (3 x 2 L) and the combined organic layers were washed with brine (2 x 1 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give to two atropisomers of 3-(5-bromo-1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (as single atropisomers) (60 g, 38% yield) and (40 g, 26% yield) both as solids. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{29}BrN_2O_2$ 444.1; found 445.2.

Intermediate 2 and Intermediate 4. Synthesis of (*S*)-1-((*S*)-2-((tert-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)propanoyl)hexahydropyridazine-3-carboxylate



Intermediate 4.



Intermediate 2.

Step 1. To a mixture of (*S*)-methyl 2-(*tert*-butoxycarbonylamino)-3-(3-hydroxyphenyl)propanoate (10.0 g, 33.9 mmol) in DCM (100 mL) was added imidazole (4.6 g, 67.8 mmol) and TIPSCl (7.8 g, 40.7 mmol). The mixture was stirred at rt overnight then diluted with DCM (200 mL) and washed with H_2O (150 mL x 3). The organic layer was dried over anhydrous Na_2SO_4,

filtered, concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (*S*)-methyl 2-(*tert*-butoxycarbonylamino)-3-(3-(triisopropylsilyloxy)phenyl)-propanoate (15.0 g, 98% yield) as an oil. LCMS (ESI): m/z [M+Na] calc'd for $C_{24}H_{41}NO_5SiNa$ 474.3; found 474.2.

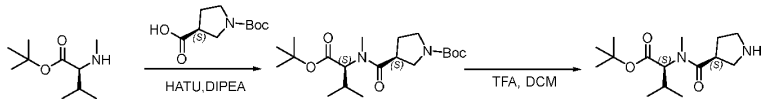
Step 2. A mixture of (*S*)-methyl 2-(*tert*-butoxycarbonylamino)-3-(3-(triisopropylsilyloxy)phenyl)-propanoate (7.5 g, 16.6 mmol), $PinB_2$ (6.3 g, 24.9 mmol), $[Ir(OMe)(COD)]_2$ (1.1 g, 1.7 mmol) and 4-*tert*-butyl-2-(4-*tert*-butyl-2-pyridyl)pyridine (1.3 g, 5.0 mmol) was purged with Ar (x3), then THF (75 mL) was added and the mixture placed under an atmosphere of Ar and sealed. The mixture was heated to 80 °C and stirred for 16 h, concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (*S*)-methyl 2-(*tert*-butoxycarbonylamino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-(triisopropylsilyloxy)phenyl)-propanoate (7.5 g, 78% yield) as a solid. LCMS (ESI): m/z [M+Na] calc'd for $C_{30}H_{52}BNO_7SiNa$ 600.4; found 600.4; ^1H NMR (300 MHz, CD_3OD) δ 7.18 (s, 1H), 7.11 (s, 1H), 6.85 (s, 1H), 4.34 (m, 1H), 3.68 (s, 3H), 3.08 (m, 1H), 2.86 (m, 1H), 1.41 - 1.20 (m, 26H), 1.20 - 1.01 (m, 22H), 0.98 - 0.79 (m, 4H).

Step 3. To a mixture of triisopropylsilyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)propanoate (4.95 g, 6.9 mmol) in MeOH (53 mL) at 0 °C was added LiOH (840 mg, 34.4 mmol) in H_2O (35 mL). The mixture was stirred at 0 °C for 2 h, then acidified to pH ~5 with 1M HCl and extracted with EtOAc (250 mL x 2). The combined organic layers were washed with brine (100 mL x 3), dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to give (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)propanoic acid (3.7 g, 95% yield), which was used directly in the next step without further purification. LCMS (ESI): m/z [M+NH_4] calc'd for $C_{29}H_{50}BNO_7SiNH_4$ 581.4; found 581.4.

Step 4. To a mixture of methyl (*S*)-hexahydropyridazine-3-carboxylate (6.48 g, 45.0 mmol) in DCM (200 mL) at 0 °C was added NMM (41.0 g, 405 mmol), (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)propanoic acid (24 g, 42.6 mmol) in DCM (50 mL) then HOBt (1.21 g, 9.0 mmol) and EDCI HCl salt (12.9 g, 67.6 mmol). The mixture was warmed to rt and stirred for 16 h, then diluted with DCM (200 mL) and washed with H_2O (3 x 150 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, the filtrate concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)propanoyl)hexahydropyridazine-3-

carboxylate (22 g, 71% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{35}H_{60}BN_3O_8Si$ 689.4; found 690.5.

Intermediate 3. Synthesis of (*S*)-*tert*-butyl 3-methyl-2-((*S*)-N-methylpyrrolidine-3-carboxamido)butanoate

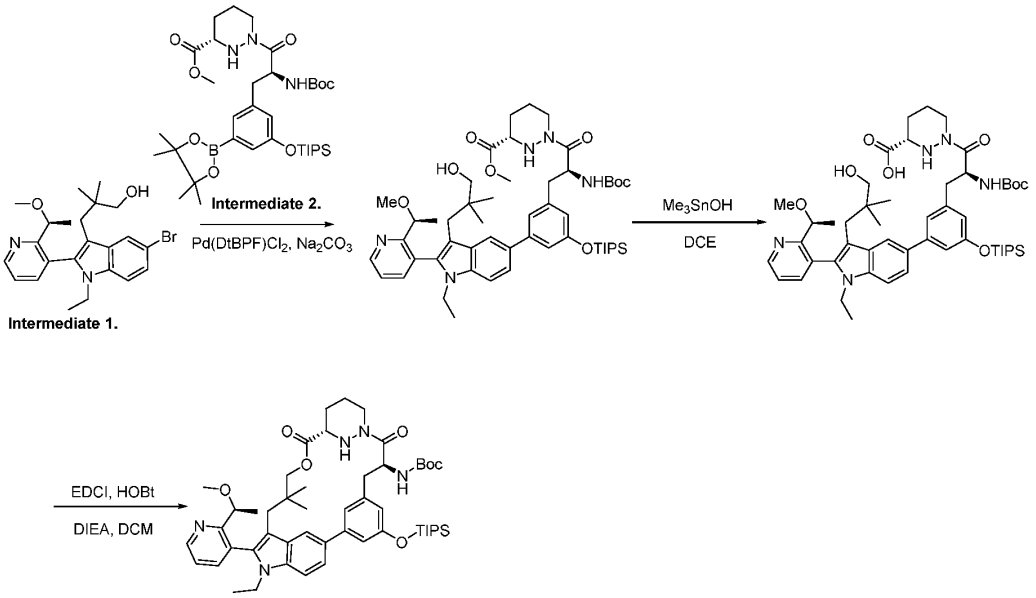


Intermediate 3.

Step 1. To a mixture of (*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-3-carboxylic acid (2.2 g, 10.2 mmol) in DMF (10 mL) at rt was added HATU (7.8 g, 20.4 mmol) and DIPEA (5 mL). After stirring at rt for 10 min, *tert*-butyl methyl-L-valinate (3.8g, 20.4 mmol) in DMF (10 mL) was added. The mixture was stirred at rt for 3 h, then diluted with DCM (40 mL) and H_2O (30 mL). The aqueous and organic layers were separated, and the organic layer was washed with H_2O (3 × 30 mL), brine (30 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (*S*)-*tert*-butyl 3-(((*S*)-1-(*tert*-butoxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)pyrrolidine-1-carboxylate (3.2 g, 82% yield) as an oil. LCMS (ESI): m/z [M+Na] calc'd for $C_{20}H_{36}N_2O_5Na$ 407.3; found 407.2.

Step 2. A mixture of (*S*)-*tert*-butyl 3-(((*S*)-1-(*tert*-butoxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)pyrrolidine-1-carboxylate (3.2 g, 8.4 mmol) in DCM (13 mL) and TFA (1.05 g, 9.2 mmol) was stirred at rt for 5 h. The mixture was concentrated under reduced pressure to give (*S*)-*tert*-butyl 3-methyl-2-((*S*)-*N*-methylpyrrolidine-3-carboxamido)butanoate (2.0 g, 84% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{15}H_{28}N_2O_3$ 284.2; found 285.2.

Intermediate 5. Synthesis of tert-butyl ((6^{3}S,4S)-11-ethyl-12-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-25-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate.



Intermediate 5.

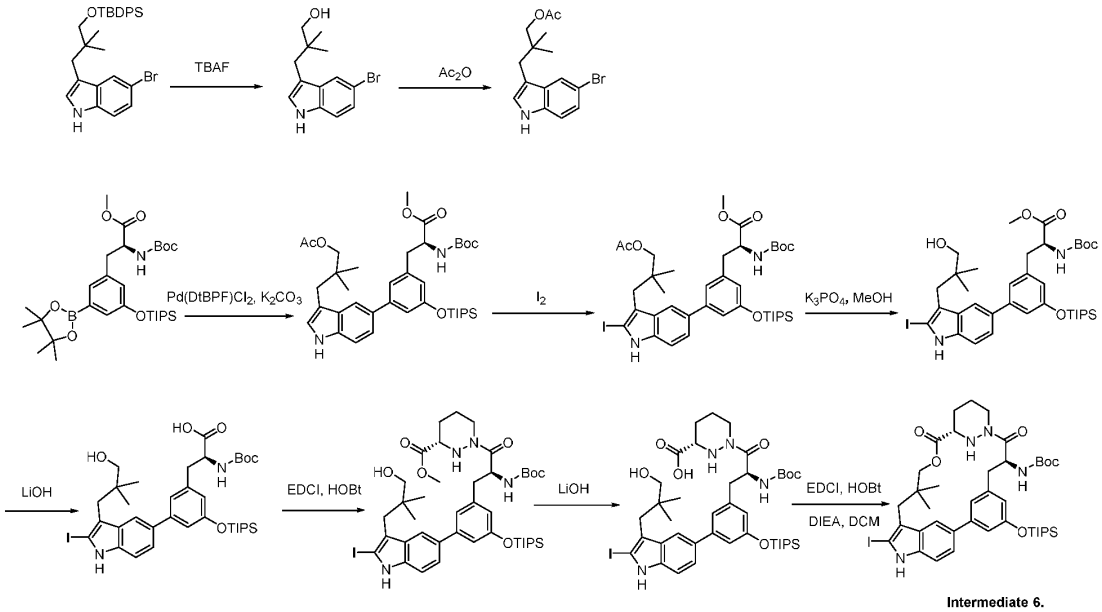
Step 1. To a stirred mixture of 3-(5-bromo-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropan-1-ol (30 g, 67 mmol) and methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate (55.8 g, 80.8 mmol) in 1,4-dioxane (750 mL) at rt under an atmosphere of Ar was added Na_2CO_3 (17.9 g, 168.4 mmol), $Pd(DtBPF)Cl_2$ (4.39 g, 6.7 mmol) and H_2O (150.00 mL) in portions. The mixture was heated to 85 °C and stirred for 3 h, cooled, diluted with H_2O (2 L) and extracted with EtOAc (3 x 1 L). The combined organic layers were washed with brine (2 x 500 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate (50 g, 72% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{52}H_{77}N_5O_8Si$ 927.6; found 928.8.

Step 2. To a stirred mixture of methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate (50 g, 54 mmol) in DCE (500 mL) at rt was added trimethyltin hydroxide (48.7 g, 269 mmol) in portion. The mixture was heated to 65 °C and stirred for 16 h, then filtered and the filter cake washed with DCM (3 x 150 mL). The filtrate was concentrated under reduced pressure to give (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-

carboxylic acid (70 g, crude), which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{75}N_5O_8Si$ 913.5; found 914.6.

Step 3. To a stirred mixture of (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid (70 g) in DCM (5 L) at 0 °C under an atmosphere of N_2 was added DIPEA (297 g, 2.3 mol), HOBT (51.7 g, 383 mmol) and EDCI (411 g, 2.1 mol) in portions. The mixture was warmed to rt and stirred for 16 h, then diluted with DCM (1 L), washed with brine (3 x 1 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (36 g, 42% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{73}N_5O_7Si$ 895.5; found 896.5.

Intermediate 6. Synthesis of *tert*-butyl N-[(8*S*,14*S*)-21-iodo-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate.



Intermediate 6.

Step 1. This reaction was undertaken on 5-batches in parallel on the scale illustrated below.

Into a 2L round-bottom flasks each were added 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1*H*-indole (100 g, 192 mmol) and TBAF (301.4 g, 1.15 mol) in THF (1.15 L) at rt. The resulting mixture was heated to 50 °C and stirred for 16 h, then the mixture was concentrated under reduced pressure. The combined residues were diluted with H_2O (5 L) and extracted with EtOAc (3 x 2 L). The combined organic layers were washed with brine (2 x 1.5 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-(5-bromo-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (310 g, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{16}BrNO$ 281.0 and 283.0; found 282.1 and 284.1.

Step 2. This reaction was undertaken on 2-batches in parallel on the scale illustrated below.

To a stirred mixture of 3-(5-bromo-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (135 g, 478 mmol) and TEA (145.2 g, 1.44 mol) in DCM (1.3 L) at 0 °C under an atmosphere of N_2 was added Ac_2O (73.3 g, 718 mmol) and DMAP (4.68 g, 38.3 mmol) in portions. The resulting mixture was stirred for 10 min at 0 °C, then washed with H_2O (3 x 2 L). The organic layers from each experiment were combined and washed with brine (2 x 1 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography to give 3-(5-bromo-1*H*-indol-3-yl)-2,2-dimethylpropyl acetate (304 g, 88% yield) as

a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.16 - 11.11 (m, 1H), 7.69 (d, J = 2.0 Hz, 1H), 7.32 (d, J = 8.6 Hz, 1H), 7.19 - 7.12 (m, 2H), 3.69 (s, 2H), 2.64 (s, 2H), 2.09 (s, 3H), 0.90 (s, 6H).

Step 3. This reaction was undertaken on 4-batches in parallel on the scale illustrated below.

Into a 2L round-bottom flasks were added methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-[(triisopropylsilyl)oxy]phenyl]propanoate (125 g, 216 mmol), 1,4-dioxane (1 L), H_2O (200 mL), 3-(5-bromo-1*H*-indol-3-yl)-2,2-dimethylpropyl acetate (73.7 g, 227 mmol), K_2CO_3 (59.8 g, 433 mmol) and Pd(DtBPF)Cl_2 (7.05 g, 10.8 mmol) at rt under an atmosphere of Ar. The resulting mixture was heated to 65 °C and stirred for 2 h, then diluted with H_2O (10 L) and extracted with EtOAc (3 x 3 L). The combined organic layers were washed with brine (2 x 2 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography to give methyl (2*S*)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (500 g, 74% yield) as an oil. LCMS (ESI): m/z [M+Na] calc'd for $C_{39}H_{58}N_2O_7SiNa$ 717.4; found 717.3.

Step 4. This reaction was undertaken on 3-batchs' in parallel on the scale illustrated below.

To a stirred mixture of methyl (2*S*)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (150 g, 216 mmol) and $NaHCO_3$ (21.76 g, 259 mmol) in THF (1.5 L) was added AgOTf (66.5 g, 259 mmol) in THF dropwise at 0 °C under an atmosphere of nitrogen. I_2 (49.3 g, 194 mmol) in THF was added dropwise over 1 h at 0 °C and the resulting mixture was stirred for an additional 10 min at 0 °C. The combined experiments were diluted with aqueous $Na_2S_2O_3$ (5 L) and extracted with EtOAc (3 x 3 L). The combined organic layers were washed with brine (2 x 1.5 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography to give methyl (2*S*)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (420 g, 71% yield) as an oil. LCMS (ESI): m/z [M+Na] calc'd for $C_{39}H_{57}IN_2O_7SiNa$, 843.3; found 842.9.

Step 5. This reaction was undertaken on 3-batches in parallel on the scale illustrated below.

To a 2L round-bottom flask were added methyl (2*S*)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (140 g, 171 mmol), MeOH (1.4 L) and K_3PO_4 (108.6 g, 512 mmol) at 0 °C. The mixture was warmed to rt and stirred for 1 h, then the combined experiments were diluted with H_2O (9 L) and extracted with EtOAc (3 x 3 L). The combined organic layers were washed with brine (2 x 2 L), dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to give methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-

dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoate (438g, crude) as a solid. LCMS (ESI): m/z [M+Na] calc'd for $C_{37}H_{55}IN_2O_6SiNa$ 801.3; found 801.6.

Step 6. This reaction was undertaken on 3-batches in parallel on the scale illustrated below.

To a stirred mixture of methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoate (146 g, 188 mmol) in THF (1.46 L) was added LiOH (22.45 g, 937 mmol) in H_2O (937 mL) dropwise at 0 °C. The resulting mixture was warmed to rt and stirred for 1.5 h [note: LCMS showed 15% de-TIPS product]. The mixture was acidified to pH 5 with 1M HCl (1M) and the combined experiments were extracted with EtOAc (3 x 3 L). The combined organic layers were washed with brine (2 x 2 L), dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to give (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoic acid (402 g, crude) as a solid. LCMS (ESI): m/z [M+Na] calc'd for $C_{36}H_{53}IN_2O_6SiNa$ 787.3; found 787.6.

Step 7. To a stirred mixture of (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoic acid (340 g, 445 mmol) and methyl (3*S*)-1,2-diazinane-3-carboxylate (96.1 g, 667 mmol) in DCM (3.5 L) was added NMM (225 g, 2.2 mol), EDCI (170 g, 889 mmol), HOBT (12.0 g, 88.9 mmol) portionwise at 0 °C. The mixture was warmed to rt and stirred for 16 h, then washed with H_2O (3 x 2.5 L), brine (2 x 1 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography to give methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate (310 g, 62% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{63}IN_4O_7Si$ 890.4; found 890.8.

Step 8. This reaction was undertaken on 3-batches in parallel on the scale illustrated below.

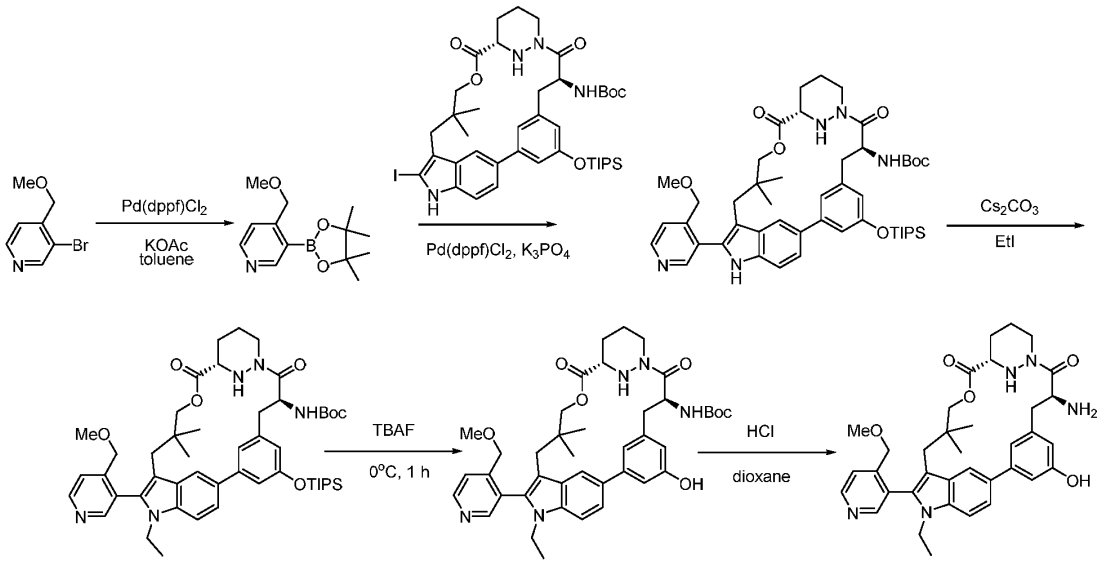
To a stirred mixture of methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate (85.0 g, 95.4 mmol) in THF (850 mL) each added LiOH (6.85 g, 286 mmol) in H_2O (410 mL) dropwise at 0 °C under an atmosphere of N_2. The mixture was stirred at 0 °C for 1.5 h [note: LCMS showed 15% de-TIPS product], then acidified to pH 5 with 1M HCl and the combined experiments extracted with EtOAc (3 x 2 L). The combined organic layers were washed with brine (2 x 1.5 L), dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to give (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid (240 g, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{61}IN_4O_7Si$ 876.3; found 877.6.

Step 9. This reaction was undertaken on 2-batches in parallel on the scale illustrated below.

To a stirred mixture of (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-

carboxylic acid (120 g, 137 mmol) in DCM (6 L) was added DIPEA (265 g, 2.05 mol), EDCI (394 g, 2.05 mol), HOBT (37 g, 274 mmol) in portions at 0 °C under an atmosphere of N_2. The mixture was warmed to rt and stirred overnight, then the combined experiments were washed with H_2O (3 x 6 L), brine (2 x 6 L), dried over anhydrous Na_2SO_4 and filtered. After filtration, the filtrate was concentrated under reduced pressure. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography to give *tert*-butyl *N*-[(8*S*,14*S*)-21-iodo-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate (140 g, 50% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{59}IN_4O_6Si$ 858.9; found 858.3.

Intermediate 7. Synthesis of (6^3S,4^S)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione



Step 1. To a mixture of 3-bromo-4-(methoxymethyl)pyridine (1.00 g, 5.0 mmol), 4,4,5,5-tetramethyl-2-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (1.51 g, 5.9 mmol) and KOAc (1.21 g, 12.3 mmol) in toluene (10 mL) at rt under an atmosphere of Ar was added Pd(dppf)Cl_2 (362 mg, 0.5 mmol). The mixture was heated to 110 °C and stirred overnight, then concentrated under reduced pressure to give 4-(methoxymethyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2- yl)pyridine, which was used directly in the next step directly without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{20}BNO_3$ 249.2; found 250.3.

Step 2. To a mixture of 4-(methoxymethyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (290 mg, 1.16 mmol), K_3PO_4 (371 mg, 1.75 mmol) and *tert*-butyl *N*-[(8*S*,14*S*)-21-iodo-18,18- dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate (500 mg, 0.58 mmol) in 1,4-dioxane (5 mL) and H_2O (1 mL) at rt under an atmosphere of Ar was added Pd(dppf)Cl_2 (43 mg, 0.06 mmol). The mixture was heated to 70 °C

and stirred for 2 h, then H_2O added and the mixture extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl *N*-[(8*S*,14*S*)-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate (370 mg,74% yield) as a foam. LCMS (ESI): m/z [M+H] calc'd for $C_{48}H_{67}N_5O_7Si$ 853.6; found 854.6.

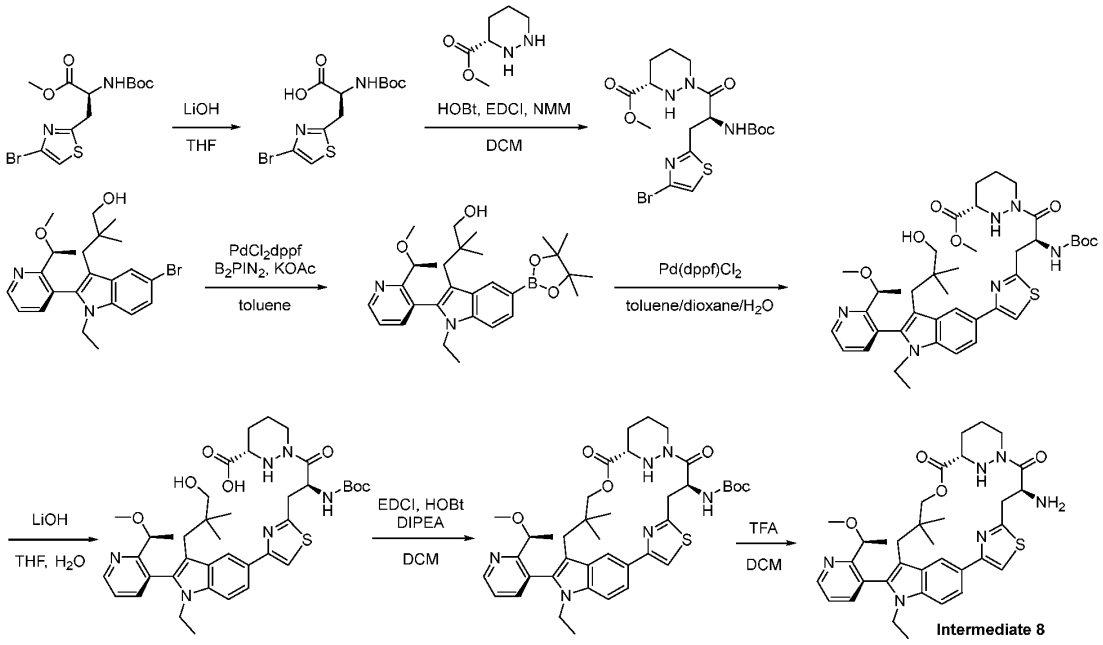
Step 3. A mixture of *tert*-butyl *N*-[(8*S*,14*S*)-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate (350 mg, 0.41 mmol), Cs_2CO_3 (267 mg, 0.82 mmol) and EtI (128 mg, 0.82 mmol) in DMF (4 mL) was stirred at 35 °C overnight. H_2O was added and the mixture was extracted with EtOAc (2 x 15 mL). The combined organic layers were washed with brine (15 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl *N*-[(8*S*,14*S*)-22-ethyl-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl] carbamate (350 mg, 97% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{71}N_5O_7Si$ 881.5; found 882.6.

Step 4. A mixture of *tert*-butyl *N*-[(8*S*,14*S*)-22-ethyl-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl] carbamate (350 mg, 0.4 mmol) and 1M TBAF in THF (0.48 mL, 0.480 mmol) in THF (3 mL) at 0 °C under an atmosphere of Ar was stirred for 1 h. The mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl *N*-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4- (methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate (230 mg, 80% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{51}N_5O_7$ 725.4; found 726.6.

Step 5. To a mixture of *tert*-butyl *N*-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate (200 mg, 0.28 mmol) in 1,4-dioxane (2 mL) at 0 °C under an atmosphere of Ar was added 4M HCl in 1,4-dioxane (2 mL, 8 mmol). The mixture was allowed to warm to rt and was stirred overnight, then concentrated under reduced pressure to give (6^3S,4^S)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-

1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (200 mg). LCMS (ESI): m/z [M+H] calc'd for $C_{36}H_{43}N_5O_5$ 625.3; found 626.5.

Intermediate 8. Synthesis of *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate



Step 1. To a solution of methyl (2*S*)-3-(4-bromo-1,3-thiazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (110 g, 301.2 mmol) in THF (500 mL) and H_2O (200 mL) at room temperature was added LiOH (21.64 g, 903.6 mmol). The solution was stirred for 1 h and was then concentrated under reduced pressure. The residue was adjusted to pH 6 with 1 M HCl and then extracted with DCM (3 x 500 mL). The combined organic layers were, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give (*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (108 g, crude). LCMS (ESI): m/z [M+H] calc'd for $C_{11}H_{16}BrN_2O_4S$ 351.0; found 351.0.

Step 2. To a solution of (*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (70 g, 199.3 mmol) in DCM (500 mL) at 0 °C was added methyl (3*S*)-1,2-diazinane-3-carboxylate bis(trifluoroacetic acid) salt (111.28 g, 298.96 mmol), NMM (219.12 mL. 1993.0 mmol), EDCI (76.41 g, 398.6 mmol) and HOBt (5.39 g, 39.89 mmol). The solution was warmed to room temperature and stirred for 1 h. The reaction was then quenched with H_2O (500 mL) and was extracted with EtOAc (3 x 500 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressured. The residue was purified by silica gel column chromatography to give methyl (*S*)-1-((*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (88.1 g, 93% yield). LCMS (ESI): m/z [M+H] calc'd for $C_{17}H_{26}BrN_4O_5S$ 477.1; found 477.1.

Step 3. To a solution of 3-(5-bromo-1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (60 g, 134.7 mmol) in toluene (500 mL) at room temperature was added bis(pinacolato)diboron (51.31 g, 202.1 mmol), $Pd(dppf)Cl_2$ (9.86 g, 13.4 mmol), and KOAc (26.44 g, 269 mmol). The reaction mixture was then heated to 90 °C and stirred for 2 h. The reaction solution was then cooled to room temperature and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give (*S*)-3-(1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (60.6 g, 94% yield). LCMS (ESI): m/z [M+H] calc'd for $C_{29}H_{42}BN_2O_4$ 493.32; found 493.3.

Step 4. To a solution of (*S*)-3-(1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (30 g, 60.9 mmol) in toluene (600 mL), dioxane (200 mL), and H_2O (200 mL) at room temperature was added methyl (*S*)-1-((*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (43.62 g, 91.4mmol), K_3PO_4 (32.23 g, 152.3 mmol) and $Pd(dppf)Cl_2$ (8.91 g, 12.18 mmol). The resulting solution was heated to 70 °C and stirred overnight. The reaction mixture was then cooled to room temperature and was quenched with H_2O (200 mL). The mixture was extracted with EtOAc and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (39.7 g, 85% yield). LCMS (ESI): m/z [M+H] calc'd for $C_{40}H_{55}N_6O_7S$ 763.4; found 763.3.

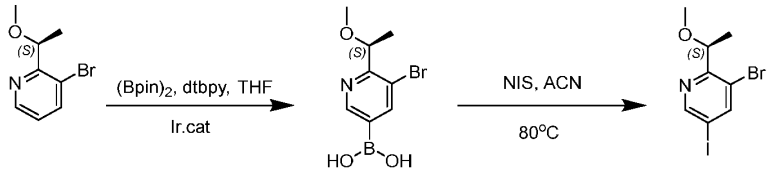
Step 5. To a solution of methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (39.7 g, 52.0 mmol) in THF (400 mL) and H_2O (100 mL) at room temperature was added $LiOH\bullet H_2O$ (3.74 g, 156.2 mmol). The mixture was stirred for 1.5 h and was then concentrated under reduced pressure. The residue was acidified to pH 6 with 1 M HCl and extracted with DCM (3 x 1000 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (37.9 g, crude). LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{53}N_6O_7S$ 749.4; found 749.4.

Step 6. To a solution of (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (37.9 g, 50.6 mmol), HOBt (34.19 g, 253.0 mmol) and DIPEA (264.4 mL, 1518 mmol) in DCM (4 L) at 0 °C was added EDCI (271.63 g, 1416.9 mmol). The resulting mixture was warmed to room temperature and stirred overnight. The reaction mixture was then quenched with H_2O and washed with 1 M HCl (4 x 1 L). The organic layer was separated and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-

pyridazinacycloundecaphane-4-yl)carbamate (30 g, 81% yield). LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{51}N_6O_6S$ 731.4; found 731.3.

Step 7. To a solution of *tert*-butyl ((6^3*S*,4*S*,*Z*)-11-ethyl-12-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-61,62,63,64,65,66-hexahydro-11*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (6 g, 8.21 mmol) in DCM (60 mL) at 0 °C was added TFA (30 mL). The mixture was stirred for 1 h and was then concentrated under reduced pressure to give (6^3*S*,4*S*,*Z*)-4-amino-11-ethyl-12-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-61,62,63,64,65,66-hexahydro-11*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (7.0 g, crude). LCMS (ESI): m/z [M+H] calc'd for $C_{34}H_{42}N_6O_4S$ 631.3; found: 630.3.

Intermediate 9. Synthesis of (*S*)-3-bromo-5-iodo-2- (1-methoxyethyl) pyridine.



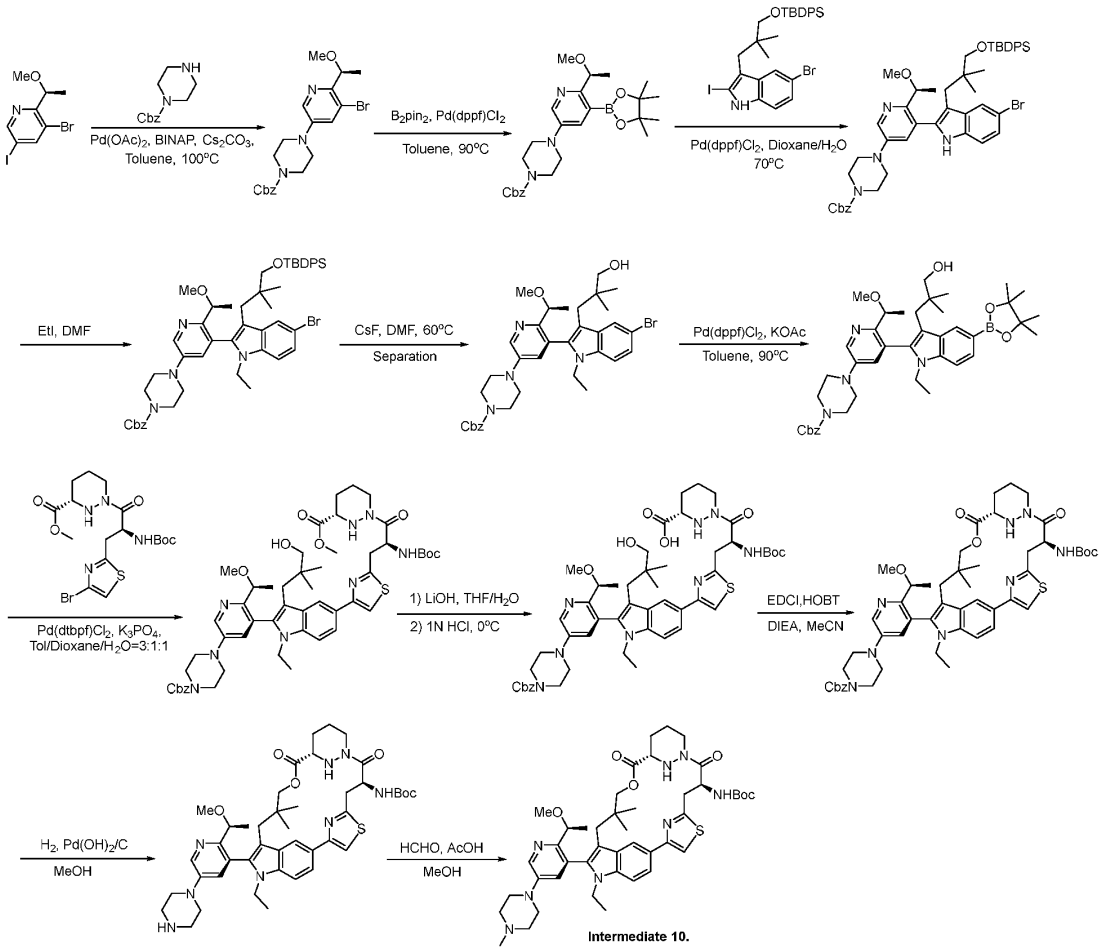
Intermediate 9.

Step 1. To a stirred solution of 3-bromo-2-[(1*S*)-1-methoxyethyl]pyridine (80.00 g, 370.24 mmol, 1.00 equiv) and bis(pinacolato)diboron (141.03 g, 555.3 mmol, 1.50 equiv) in THF (320 mL) was added dtbpy (14.91 g, 55.5 mmol) and chloro(1,5-cyclooctadiene)iridium(I) dimer (7.46 g, 11.1 mmol) under argon atmosphere. The resulting mixture was stirred for 16 h at 75 °C under argon atmosphere. The mixture was concentrated under reduced pressure. The resulting mixture was dissolved in EtOAc (200 mL) and the mixture was adjusted to pH 10 with Na_2CO_3 (40 g) and NaOH (10 g) (mass 4:1) in water (600 mL). The aqueous layer was extracted with EtOAc (800mL). The aqueous phase was acidified to pH = 6 with HCl (6 *N*) to precipitate the desired solid to afford 5-bromo-6-[(1*S*)-1-methoxyethyl]pyridin-3-ylboronic acid (50g, 52.0% yield) as a light-yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_8H_{11}BBrNO_3$ 259.0; found 260.0.

Step 2. To a stirred solution of 5-bromo-6-[(1*S*)-1-methoxyethyl]pyridin-3-ylboronic acid (23.00 g, 88.5 mmol) in ACN (230 mL) were added NIS (49.78 g, 221.2 mmol) at room temperature under argon atmosphere . The resulting mixture was stirred for overnight at 80 °C under argon atmosphere. The resulting mixture was concentrated under reduced pressure. The resulting mixture was dissolved in DCM (2.1 L) and washed with $Na_2S_2O_3$ (3 x 500 mL). The organic layer was dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford (*S*)-3-bromo-5-iodo-2-(1-methoxyethyl)pyridine (20 g, 66.0% yield). LCMS (ESI): m/z [M+H] calc'd for C_8H_9BrINO 340.9; found 341.7.

Intermediate 10. Synthesis of *tert*-butyl ((6^3*S*,4*S*,*Z*)-11-ethyl-12-(2-((*S*)-1-

methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate



Intermediate 10.

Step 1. Into a 3L 3-necked round-bottom flask purged and maintained with an inert atmosphere of argon, was placed 3-bromo-5-iodo-2-[(1*S*)-1-methoxyethyl]pyridine (147 g, 429.8 mmol) benzyl piperazine-1-carboxylate (94.69 g, 429.8 mmol), $Pd(OAc)_2$ (4.83 g, 21.4 mmol), BINAP (5.35 g, 8.6 mmol), Cs_2CO_3 (350.14 g, 1074.6 mmol), toluene (1 L). The resulting solution was stirred for overnight at 100 °C in an oil bath. The reaction mixture was cooled to 25 °C after reaction completed. The resulting mixture was concentrated under reduced pressure. The residue was applied onto a silica gel column with ethyl acetate/hexane (1:1). Removal of solvent under reduced pressure gave benzyl (*S*)-4-(5-bromo-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (135 g, 65.1% yield) as a dark yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{20}H_{24}BrN_3O_3$ 433.1; found 434.1.

Step 2. Into a 3-L 3-necked round-bottom flask purged and maintained with an inert atmosphere of argon, was placed benzyl 4-[5-bromo-6-[(1*S*)-1-methoxyethyl]pyridin-3-yl]piperazine-1-carboxylate (135 g, 310.8 mmol), bis(pinacolato)diboron (86.82 g, 341.9 mmol), $Pd(dppf)Cl_2$

(22.74 g, 31.0 mmol), KOAc (76.26 g, 777.5 mmol), Toluene (1 L). The resulting solution was stirred for 2 days at 90 °C in an oil bath. The reaction mixture was cooled to 25 °C. The resulting mixture was concentrated under vacuum. The residue was applied onto a neutral alumina column with ethyl acetate/hexane (1:3). Removal of solvent under reduced pressure gave benzyl (*S*)-4-(6-(1-methoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl)piperazine-1-carboxylate (167 g, crude) as a dark yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{26}H_{36}BN_3O_5$ 481.3; found 482.1.

Step 3. Into a 3-L 3-necked round-bottom flask purged and maintained with an inert atmosphere of argon, was placed (*S*)-4-(6-(1-methoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl)piperazine-1-carboxylate (167 g, 346.9 mmol), 5-bromo-3-[3-[(tert-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-2-iodo-1H-indole (224.27 g, 346.9 mmol), $Pd(dppf)Cl_2$ (25.38 g, 34.6 mmol), dioxane (600 mL), H_2O (200 mL), K_3PO_4 (184.09 g, 867.2 mmol), Toluene (200 mL). The resulting solution was stirred for overnight at 70 °C in an oil bath. The reaction mixture was cooled to 25 °C after reaction completed. The resulting mixture was concentrated under vacuum. The residue was applied onto a silica gel column with ethyl acetate/hexane (1:1). Removal of solvent under reduced pressure gave benzyl (*S*)-4-(5-(5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (146 g, 48.1% yield) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{49}H_{57}BrN_4O_4Si$ 872.3; found 873.3.

Step 4. To a stirred mixture of benzyl (*S*)-4-(5-(5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (146 g, 167.0 mmol) and Cs_2CO_3 (163.28 g, 501.1 mmol) in DMF (1200 mL) was added C_2H_5I (52.11 g, 334.0 mmol) in portions at 0 °C under N_2 atmosphere. The final reaction mixture was stirred at 25 °C for 12 h. Desired product could be detected by LCMS. The resulting mixture was diluted with EA (1 L) and washed with brine (3 x 1.5L). The organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to give benzyl (*S*)-4-(5-(5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1-ethyl-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (143 g, crude) as a yellow solid that was used directly for next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{61}BrN_4O_4Si$ 900.4; found 901.4.

Step 5. To a stirred mixture of benzyl benzyl (*S*)-4-(5-(5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1-ethyl-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (143 g, 158.5 mmol) in DMF (1250 mL) was added CsF (72.24 g, 475.5 mmol). Then the reaction mixture was stirred at 60 °C for 2 days under N_2 atmosphere. Desired product could be detected by LCMS. The resulting mixture was diluted with EA (1 L) and washed with brine (3 x 1L). Then the organic phase was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with PE/EA (1/3) to afford two atropisomers of benzyl (*S*)-4-(5-(5-bromo-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate **A** (38 g, 36% yield, RT = 1.677 min in 3 min LCMS(0.1% FA)) and **B** (34 g, 34% yield, RT = 1.578 min in 3 min LCMS(0.1% FA)) both as yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{35}H_{43}BrN_4O_4$ 663.2; found 662.2.

Step 6. Into a 500-mL 3-necked round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed benzyl (*S*)-4-(5-(5-bromo-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate **A** (14 g, 21.1 mmol), bis(pinacolato)diboron (5.89 g, 23.21 mmol), Pd(dppf)Cl_2 (1.54 g, 2.1 mmol), KOAc (5.18 g, 52.7 mmol), Toluene (150 mL). The resulting solution was stirred for 5 h at 90 °C in an oil bath. The reaction mixture was cooled to 25 °C. The resulting mixture was concentrated under vacuum. The residue was purified by silica gel column chromatography, eluted with PE/EA (1/3) to give benzyl (*S*)-4-(5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (12 g, 76.0% yield) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{55}BN_4O_6$ 710.4; found 711.3.

Step 7. Into a 250-mL round-bottom flask purged and maintained with an inert atmosphere of argon, was placed benzyl (*S*)-4-(5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (10.8 g, 15.2 mmol), methyl (3*S*)-1-[(2*S*)-3-(4-bromo-1,3-thiazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoyl]-1,2-diazinane-3-carboxylate (7.98 g, 16.7 mmol), Pd(dtbpf)Cl_2 (0.99 g, 1.52 mmol), K_3PO_4 (8.06 g, 37.9 mmol), Toluene (60 mL), dioxane (20 mL), H_2O (20 mL). The resulting solution was stirred for 3 h at 70 °C in an oil bath. The reaction mixture was cooled to 25 °C. The resulting solution was extracted with EtOAc (2 x 50 mL) and concentrated under reduced pressure. The residue was applied onto a silica gel column with ethyl acetate/hexane (10:1). Removal of solvent to give methyl (*S*)-1-((*S*)-3-(4-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-5-yl)thiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (8 g, 50.9% yield) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{52}H_{68}N_8O_9S$ 980.5; found 980.9.

Step 8. To a stirred mixture of methyl (*S*)-1-((*S*)-3-(4-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((S)-1-methoxyethyl)pyridin-3-yl)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-5-yl)thiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (12 g, 12.23 mmol) in THF (100 mL)/H_2O (100 mL) was added LiOH (2.45 g, 61.1 mmol) under N_2 atmosphere and the resulting mixture was stirred for 2 h at 25 °C. Desired product could be detected by LCMS. THF was concentrated under reduced pressure. The pH of aqueous phase was acidified to 5 with HCL (1N) at 0 °C. The aqueous layer was extracted with DCM (3 x 100ml). The organic phase was concentrated under reduced pressure to give (*S*)-1-((*S*)-3-(4-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-5-yl)thiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylic acid (10 g, 84.5% yield) as a light yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{66}N_8O_9S$ 966.5; found 967.0.

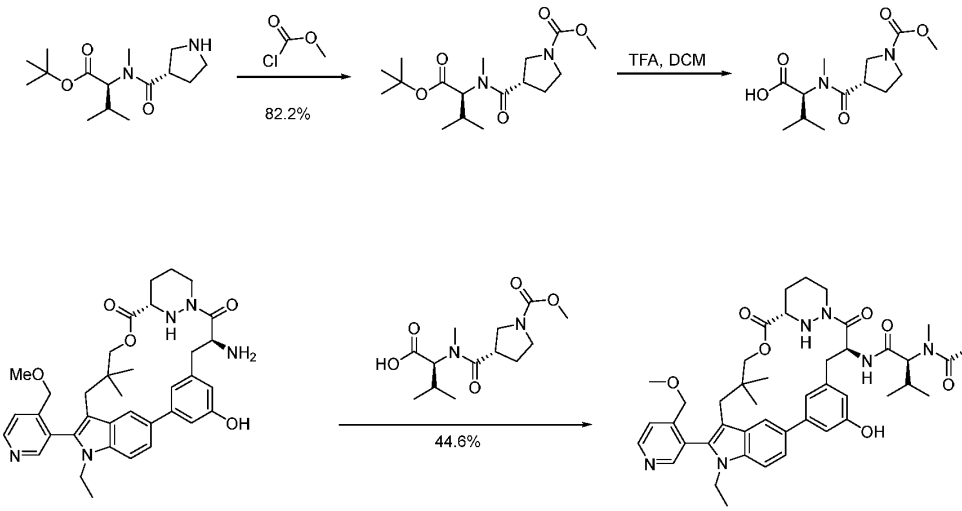
Step 9. Into a 3-L round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed (*S*)-1-((*S*)-3-(4-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-5-yl)thiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylic acid (18 g, 18.61 mmol), ACN (1.8 L), DIEA (96.21 g, 744.4 mmol), EDCI (107.03 g, 558.3 mmol), HOBT (25.15 g, 186.1

mmol). The resulting solution was stirred for overnight at 25 °C. The resulting mixture was concentrated under reduced pressure after reaction completed. The resulting solution was diluted with DCM (1 L). The resulting mixture was washed with HCl (3 x 1 L, 1N aqueous). The resulting mixture was washed with water (3 x 1 L). Then the organic layer was concentrated, the residue was applied onto a silica gel column with ethyl acetate/hexane (1:1). Removal of solvent under reduced pressure gave benzyl 4-(5-((6^3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-11-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-12-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (10.4 g, 54.8% yield) as a light yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{64}N_8O_8S$ 948.5; found 949.3.

Step 10. Into a 250-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed benzyl 4-(5-((6^3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-11-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-12-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (10.40 g, 10.9 mmol), Pd(OH)$_2$/C (5 g, 46.9 mmol), MeOH (100 mL). The resulting solution was stirred for 3 h at 25 °C under 2 atm H_2 atmosphere. The solids were filtered out and the filter cake was washed with MeOH (3 x 100 mL). Then combined organic phase was concentrated under reduced pressure to give *tert*-butyl ((6^3*S*,4*S*,*Z*)-11-ethyl-12-(2-((*S*)-1-methoxyethyl)-5-(piperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (8.5 g, 90.4% yield) as a light yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{58}N_8O_6S$ 814.4; found 815.3.

Step 11. Into a 1000-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed *tert*-butyl ((6^3*S*,4*S*,*Z*)-11-ethyl-12-(2-((*S*)-1-methoxyethyl)-5-(piperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (8.5 g, 10.4 mmol), MeOH (100 mL), AcOH (1.88 g, 31.2 mmol) and stirred for 15 mins. Then HCHO (1.88 g, 23.15 mmol, 37% aqueous solution) and $NaBH_3CN$ (788 mg, 12.5 mmol) was added at 25 °C. The resulting solution was stirred for 3 h at 25 °C. The resulting mixture was quenched with 100 mL water and concentrated under reduced pressure to remove MeOH. The resulting solution was diluted with 300 mL of DCM. The resulting mixture was washed with water (3 x 100 mL). Removal of solvent gave *tert*-butyl ((6^3*S*,4*S*,*Z*)-11-ethyl-12-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (8.2 g, 90.1% yield) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{60}N_8O_6S$ 828.4; found 829.3.

Example A11. Synthesis of methyl (3*S*)-3-{[(1*S*)-1-{[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamoyl}-2-methylpropyl](methyl)carbamoyl}pyrrolidine-1-carboxylate



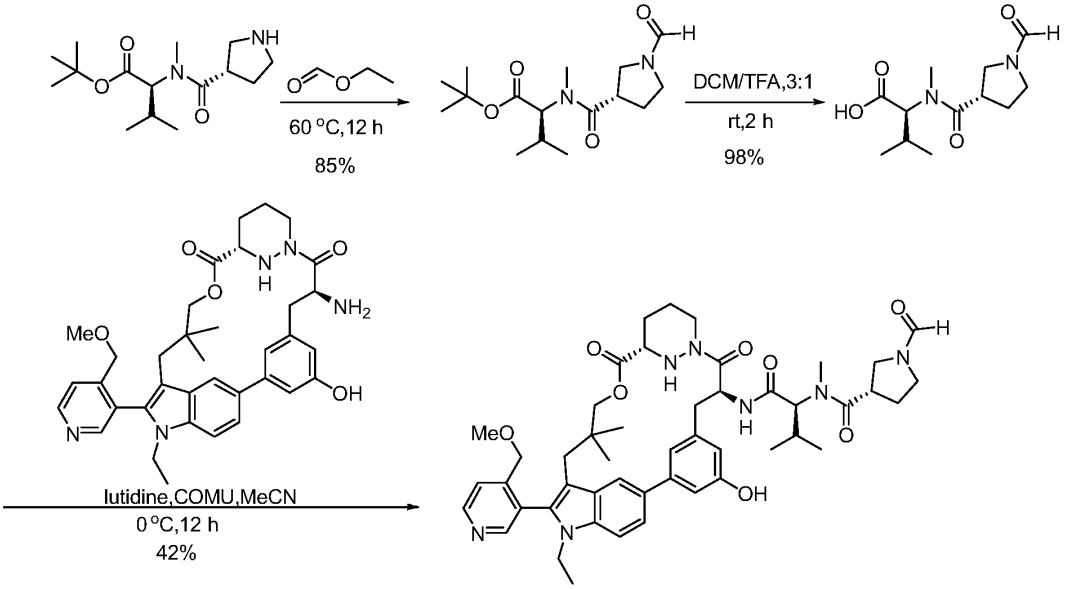
Step 1. To a mixture of *tert*-butyl *N*-methyl-*N*-((*S*)-pyrrolidine-3-carbonyl)-L-valinate (500 mg, 1.8 mmol) and TEA (356 mg, 3.5 mmol) in DCM (10 mL) at 0 °C was added methyl carbonochloridate (199 mg, 2.1 mmol) dropwise. The mixture was allowed to warm to rt and was stirred for 12 then concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (*S*)-3-(((*S*)-1-(*tert*-butoxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)pyrrolidine-1-carboxylate (550 mg, 82%) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{17}H_{30}N_2O_5$ 342.2; found 343.2.

Step 2. A mixture of methyl (*S*)-3-(((*S*)-1-(*tert*-butoxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)pyrrolidine-1-carboxylate (500 mg, 1.46 mmol), DCM (8 mL) and TFA (2 mL) was stirred at rt for 3 h. The mixture was concentrated under reduced pressure with azeotropic removal of H_2O using toluene (5 mL) to give *N*-((*S*)-1-(methoxycarbonyl)pyrrolidine-3-carbonyl)-*N*-methyl-L-valine (400 mg) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{22}N_2O_5$ 286.2; found 287.2.

Step 3. To a mixture of (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (80 mg, 0.13 mmol), *N*-((*S*)-1-(methoxycarbonyl)pyrrolidine-3-carbonyl)-*N*-methyl-L-valine (55 mg, 0.19 mmol) and DIPEA (165 mg, 1.3 mmol) in DMF (2 mL) at 0 °C was added COMU (77 mg, 0.18 mmol). The mixture was stirred at 0 °C for 2 h, then concentrated under reduced pressure and the residue was purified by prep-HPLC to give methyl (3*S*)-3-{[(1*S*)-1-{[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamoyl}-2-methylpropyl](methyl)carbamoyl}pyrrolidine-1-carboxylate (51 mg, 45% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{49}H_{63}N_7O_9$ 893.5; found 894.7; ^{1}H NMR (400 MHz,

DMSO-d_6) δ 9.33 (s, 1H), 8.88 - 8.66 (m, 2H), 8.62 (s, 1H), 8.17 - 8.06 (m, 1H), 7.92 (d, *J* = 8.7 Hz, 1H), 7.79 - 7.68 (m, 1H), 7.65 - 7.49 (m, 2H), 7.21 - 7.11 (m, 1H), 7.01 (d, *J* = 11.8 Hz, 1H), 6.71 - 6.40 (m, 1H), 5.54 - 5.30 (m, 1H), 5.28 - 4.99 (m, 1H), 4.87 - 4.56 (m, 1H), 4.46 - 4.21 (m, 3H), 4.11 - 3.89 (m, 3H), 3.70 (s, 1H), 3.65 - 3.59 (m, 4H), 3.35 (s, 2H), 3.24 (s, 2H), 3.18 - 3.07 (s, 1H), 3.00 - 2.58 (m, 8H), 2.22 - 2.01 (m, 4H), 1.81 (d, *J* = 11.4 Hz, 2H), 1.72 - 1.42 (m, 2H), 1.15 - 0.64 (m, 13H), 0.43 (d, *J* = 16.4 Hz, 3H).

Example A17. Synthesis of (2*S*)-*N*-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-2-{1-[(3*S*)-1-formylpyrrolidin-3-yl]-N-methylformamido}-3-methylbutanamide



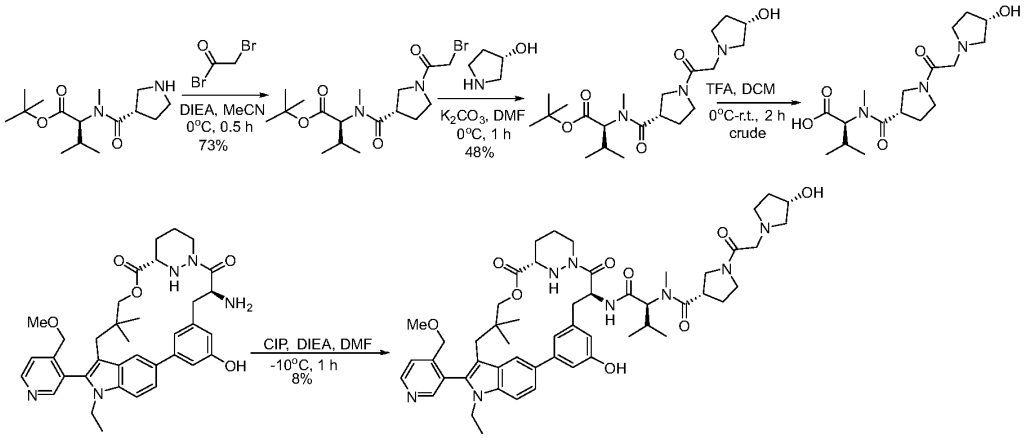
Step 1. A mixture of *tert*-butyl (2*S*)-3-methyl-2-[*N*-methyl-1-(3*S*)-pyrrolidin-3-ylformamido]butanoate (290 mg, 1.0 mmol) and ethyl formate (755 mg, 10.2 mmol) was heated to 60 °C and stirred for 12 h. The mixture was concentrated under reduced pressure to give *tert*-butyl (2*S*)-2-[1-[(3*S*)-1-formylpyrrolidin-3-yl]-*N*-methylformamido]-3-methylbutanoate (300 mg, 85% yield) as a solid. LCMS (ESI): m/z [M+H-tBu] calc'd for $C_{12}H_{20}N_2O_4$ 256.1; found 257.2.

Step 2. To a mixture of *tert*-butyl (2*S*)-2-[1-[(3*S*)-1-formylpyrrolidin-3-yl]-*N*-methylformamido]-3-methylbutanoate (290 mg, 0.93 mmol) in DCM (3 mL) at rt was added TFA (1 mL). The mixture was stirred at rt for 2 h, then concentrated under reduced pressure to give (2*S*)-2-[1-[(3*S*)-1-formylpyrrolidin-3-yl]-*N*-methylformamido]-3-methylbutanoic acid (260 mg, 98%) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{12}H_{20}N_2O_4$ 256.1; found 257.2.

Step 3. To a mixture of (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (60 mg, 0.1 mmol), 2,6-dimethylpyridine (15.4 mg, 0.14 mmol) and (2*S*)-2-[1-[(3*S*)-1-formylpyrrolidin-3-yl]-*N*-methylformamido]-3-methylbutanoic acid (37 mg, 0.14 mmol) in MeCN (2 mL) at 0 °C under an

atmosphere of N_2 was added COMU (62 mg, 0.14 mmol). The mixture was stirred at 0 °C for 12 h, then concentrated under reduced pressure and the residue was purified by prep-HPLC to give (2*S*)-*N*-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-2-{1-[(3*S*)-1-formylpyrrolidin-3-yl]-N-methylformamido}-3-methylbutanamide (35 mg, 42%) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{48}H_{61}N_7O_8$ 863.5; found 864.5; ^{1}H NMR (400 MHz, DMSO-*d*6) δ 8.79 - 8.61 (m, 2H), 8.51 (d, *J* = 7.8 Hz, 3H), 8.31 - 8.09 (m, 1H), 7.93 (s, 1H), 7.68 - 7.48 (m, 3H), 7.25 - 6.97 (m, 2H), 6.71 - 6.43 (m, 1H), 5.40 (d, *J* = 24.8 Hz, 1H), 5.22 (s, 1H), 4.86 - 4.34 (m, 1H), 4.23 (t, *J* = 13.8 Hz, 3H), 4.12 - 3.84 (m, 3H), 3.83 - 3.54 (m, 4H), 3.22 (d, *J* = 1.7 Hz, 2H), 3.09 (d, *J* = 14.3 Hz, 1H), 3.01 - 2.92 (m, 1H), 2.99 - 2.93 (m, 2H), 2.92 - 2.65 (m, 5H), 2.07 (d, *J* = 12.2 Hz, 4H), 1.80 (s, 1H), 1.74 - 1.48 (m, 2H), 1.08 (t, *J* = 7.1 Hz, 2H), 1.03 - 0.54 (m, 12H), 0.43 (d, *J* = 16.2 Hz, 3H).

Example A6. Synthesis of (2*S*)-N-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-2-{1-[(3*S*)-1-{2-[(3*R*)-3-hydroxypyrrolidin-1-yl]acetyl}pyrrolidin-3-yl]-*N*-methylformamido}-3-methylbutanamide



Step 1. A mixture of *tert*-butyl (2*S*)-3-methyl-2-[*N*-methyl-1-(3*S*)-pyrrolidin-3-ylformamido]butanoate (300 mg, 1.1 mmol) and DIPEA (409 mg, 3.2 mmol) in MeCN (4 mL) at 0 °C was added bromoacetyl bromide (256 mg, 1.3 mmol) dropwise. The mixture was stirred at 0 °C for 30 min, then concentrated under reduced pressure and the residue was purified by C18-silica gel column chromatography to give *tert*-butyl (2*S*)-2-[1-[(3*S*)-1-(2-bromoacetyl)pyrrolidin-3-yl]-*N*-methylformamido]-3-methylbutanoate (350mg, 73% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{17}H_{29}BrN_2O_4$ 404.1; found 405.2 and 407.2.

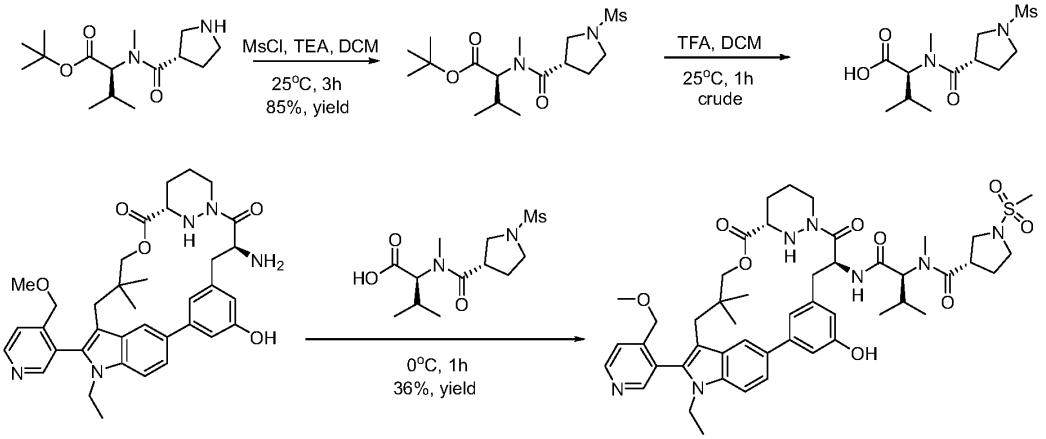
Step 2. To a mixture of *tert*-butyl (2*S*)-2-[1-[(3*S*)-1-(2-bromoacetyl)pyrrolidin-3-yl]-*N*-methylformamido]-3-methylbutanoate (110 mg, 0.27 mmol) and K_2CO_3 (75 mg, 0.54 mmol) in DMF (2 mL) at 0 °C was added (3*S*)-pyrrolidin-3-ol (36 mg, 0.41 mmol) dropwise. The mixture was stirred at 0 °C for 1 h, then concentrated under reduced pressure and the residue was purified by prep-HPLC to give *tert*-butyl (2*S*)-2-[1-[(3*S*)-1-[2-[(3*S*)-3-hydroxypyrrolidin-1-yl]acetyl]pyrrolidin-3-yl]-*N*-methylformamido]-3-methylbutanoate (60 mg, 48% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{21}H_{37}N_3O_5$ 411.3; found 412.5.

Step 3. To a mixture of *tert*-butyl (2*S*)-2-[1-[(3*S*)-1-[2-[(3*S*)-3-hydroxypyrrolidin-1-yl]acetyl]pyrrolidin-3-yl]-*N*-methylformamido]-3-methylbutanoate (60 mg, 0.15 mmol) in DCM (0.50 mL) at 0 °C was added TFA (0.50 mL, 6.7 mmol) dropwise. The mixture was warmed to rt and stirred for 2 h, then concentrated under reduced pressure with toluene (x 3) to give (2*S*)-2-[1-[(3*S*)-1-[2-[(3*S*)-3-hydroxypyrrolidin-1-yl]acetyl]pyrrolidin-3-yl]-*N*-methylformamido]-3-methylbutanoic acid (70 mg, crude) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{17}H_{29}N_3O_5$ 355.2; found 356.4.

Step 4. To a mixture of (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (60 mg, 0.1 mmol) and DIPEA (124 mg, 1.0 mmol) in DMF (1 mL) at -10 °C was added (2*S*)-2-[1-[(3*S*)-1-[2-[(3*S*)-3-hydroxypyrrolidin-1-yl]acetyl]pyrrolidin-3-yl]-*N*-methylformamido]-3-methylbutanoic acid (51 mg, 0.14 mmol) and CIP

(40 mg, 0.14 mmol) in portions. The mixture was stirred at -10 °C for 1 h, then diluted with H_2O (30 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (1 x 10 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by prep-HPLC to give (2*S*)-N-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-2-{1-[(3*S*)-1-{2-[(3*R*)-3-hydroxypyrrolidin-1-yl]acetyl}pyrrolidin-3-yl]-*N*-methylformamido}-3-methylbutanamide (8.6 mg, 8% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{53}H_{70}N_8O_9$ 962.5; found 963.5; ^{1}H NMR (400 MHz, CD_3OD) δ 8.70 (td, *J* = 5.1, 1.6 Hz, 1H), 8.66 - 8.48 (m, 1H), 8.07 - 7.90 (m, 1H), 7.76 (dd, *J* = 9.9, 5.2 Hz, 1H), 7.61 (tt, *J* = 9.9, 2.0 Hz, 1H), 7.52 (dt, *J* = 8.7, 3.5 Hz, 1H), 7.11 - 6.97 (m, 1H), 6.62 - 6.47 (m, 1H), 5.68 - 5.48 (m, 1H), 4.79 (dt, *J* = 11.2, 9.1 Hz, 1H), 4.53 - 4.18 (m, 4H), 4.16 - 3.86 (m, 3H), 3.85 - 3.56 (m, 7H), 3.55 - 3.46 (m, 1H), 3.42 (d, *J* = 4.6 Hz, 4H), 3.26 - 3.01 (m, 3H), 3.01 - 2.60 (m, 9H), 2.42 - 2.01 (m, 6H), 1.92 (s, 1H), 1.75 (s, 2H), 1.62 (q, *J* = 12.7 Hz, 1H), 1.26 - 0.80 (m, 13H), 0.61 - 0.40 (m, 3H).

Example A24. Synthesis of (2*S*)-*N*-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-2-{1-[(3*S*)-1-methanesulfonylpyrrolidin-3-yl]-*N*-methylformamido}-3-methylbutanamide



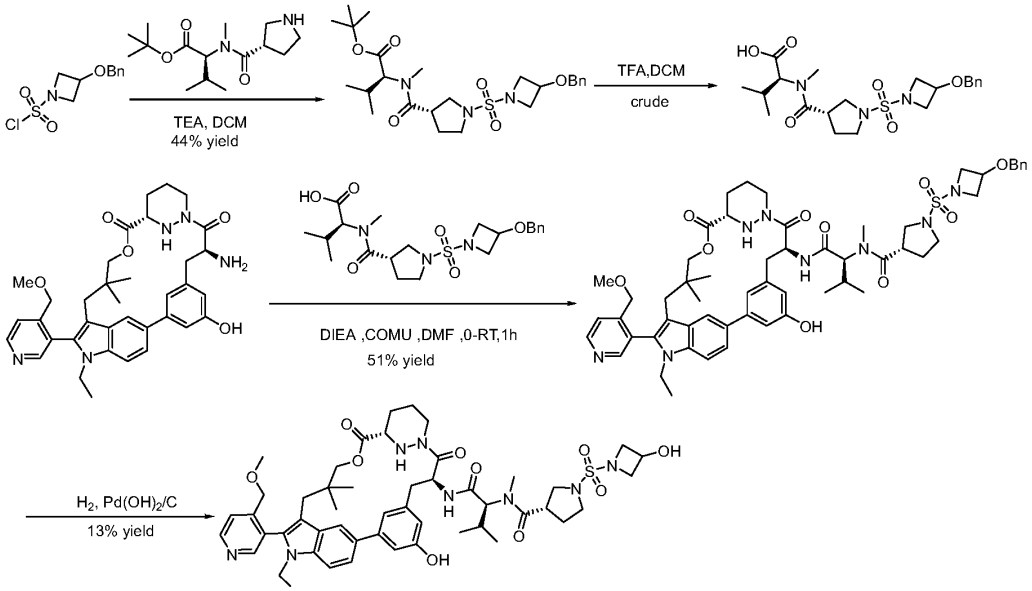
Step 1. To a mixture of *tert*-butyl *N*-methyl-*N*-((*S*)-pyrrolidine-3-carbonyl)-L-valinate (500 mg, 1.8 mmol) in DCM (8 mL)at 0 °C under an atmosphere of N_2 was added TEA (356 mg, 3.5 mmol), followed by MsCl (242 mg, 2.1 mmol). The mixture was warmed to rt and was stirred for 3 h, then washed with brine (2 x 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure and the residue was by purified by silica gel column chromatography to give *tert*-butyl *N*-methyl-*N*-((*S*)-1-(methylsulfonyl)pyrrolidine-3-carbonyl)-L-valinate (540 mg, 85%) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{16}H_{30}N_2O_5S$ 362.2; found 363.1.

Step 2. A mixture of *tert*-butyl *N*-methyl-*N*-((*S*)-1-(methylsulfonyl)pyrrolidine-3-carbonyl)-L-valinate (570 mg, 1.6 mmol), DCM (8 mL) and TFA (2 mL) at rt under an atmosphere of N_2 was stirred for 1 h. The mixture was concentrated under reduced pressure with toluene (5 mL) to give

N-methyl-*N*-((*S*)-1-(methylsulfonyl)pyrrolidine-3-carbonyl)-L-valine (500 mg) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{12}H_{22}N_2O_5S$ 305.1; found 306.2.

Step 3. To a mixture of (6^3S,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (80 mg, 0.13 mmol) in DMF (2 mL) at 0 °C under an atmosphere of N_2 was added DIPEA (165 mg, 1.3 mmol), *N*-methyl-*N*-((*S*)-1-(methylsulfonyl)pyrrolidine-3-carbonyl)-L-valine (59 mg, 0.19 mmol) and COMU (71 mg, 0.17 mmol). The mixture was stirred at 0 °C for 1 h, then concentrated under reduced pressure and the residue was purified by prep-HPLC to give (2*S*)-*N*-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.$1^{2,6}$.$1^{10,14}$.$0^{23,27}$]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-2-{1-[(3*S*)-1-methanesulfonylpyrrolidin-3-yl]-*N*-methylformamido}-3-methylbutanamide (42 mg, 36% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{48}H_{63}N_7O_9S$ 913.4; found 914.6; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.35 - 9.33 (m, 1H), 8.74 - 8.62 (m, 2H), 8.52 (s, 1H), 8.19 - 8.11 (m, 1H), 7.92 (s, 1H), 7.64 - 7.60 (m, 2H), 7.53 (t, *J* = 9.0 Hz, 1H), 7.22 - 7.10 (m, 1H), 7.02 (s, 1H), 6.58 - 6.48 (m, 1H), 5.37 - 5.24 (m, 1H), 5.19 - 5.04 (m, 1H), 4.30 - 4.18 (m, 3H), 4.07 - 3.91 (m, 3H), 3.75 - 3.49 (m, 6H), 3.22 (d, *J* = 1.5 Hz, 2H), 2.97 - 2.91 (m, 4H), 2.92 - 2.65 (m, 7H), 2.27 (s, 1H), 2.06 (d, *J* = 14.4 Hz, 3H), 1.85 (d, *J* = 35.3 Hz, 2H), 1.70 - 1.50 (m, 2H), 1.09 - 0.88 (m, 8H), 0.85 - 0.72 (m, 5H), 0.43 (d, *J* = 17.8 Hz, 3H).

Example A37. Synthesis of (2*S*)-N-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2},^{6}.1^{10},^{14}.0^{23},^{27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-2-{1-[(3*S*)-1-[(3-hydroxyazetidin-1-yl)sulfonyl]pyrrolidin-3-yl]-*N*-methylformamido}-3-methylbutanamide



Step 1. To a mixture of *tert*-butyl *N*-methyl-*N*-((*S*)-pyrrolidine-3-carbonyl)-L-valinate (500 mg, 1.8 mmol) in DCM (20 mL) ar rt was added TEA (356 mg, 3.5 mmol) and 3-(benzyloxy)azetidine-1-sulfonyl chloride (460 mg, 1.8 mmol). The mixture was stirred at rt overnight, then concentrated under reduced pressure and the residue was purified by prep-HPLC to give *tert*-butyl *N*-((*S*)-1-((3-(benzyloxy)azetidin-1-yl)sulfonyl)pyrrolidine-3-carbonyl)-*N*-methyl-L-valinate (390 mg, 44% yield) of as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{25}H_{39}N_3O_6S$ 509.3; found 510.5.

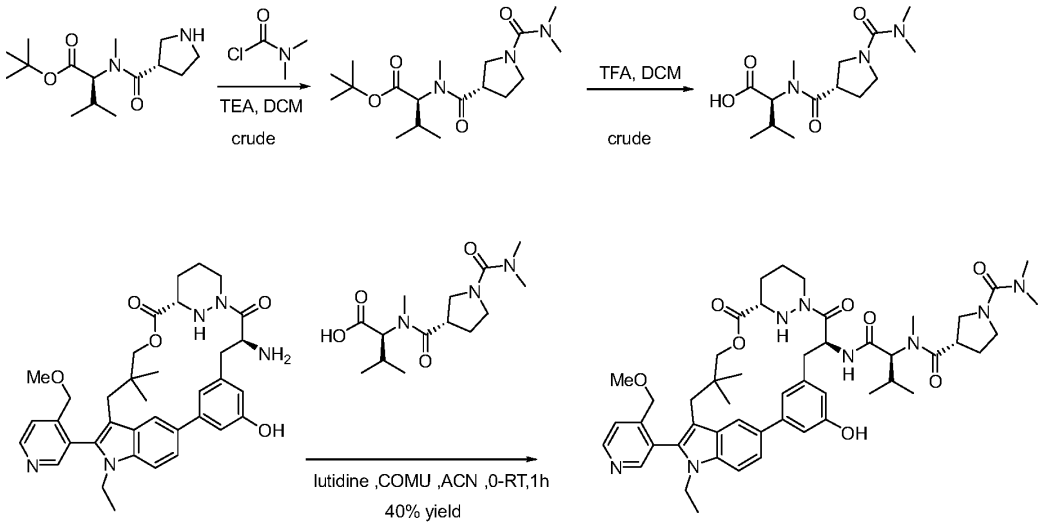
Step 2. A mixture of *tert*-butyl *N*-((*S*)-1-((3-(benzyloxy)azetidin-1-yl)sulfonyl)pyrrolidine-3-carbonyl)-*N*-methyl-L-valinate (390 mg, 0.77 mmol), DCM (4 mL) and TFA (1 mL) at rt under an atmosphere of N_2 was stirred at rt for 2 h. The mixture was concentrated under reduced pressure with toluene (10 mL x 2) to give *N*-((*S*)-1-((3-(benzyloxy)azetidin-1-yl)sulfonyl)pyrrolidine-3-carbonyl)-*N*-methyl-L-valine (370 mg, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{21}H_{31}N_3O_6S$ 453.2; found 454.5.

Step 3. To a mixture of (6^{3}*S*,4*S*)-4-amino-1^{1}-ethyl-2^{5}-hydroxy-1^{2}-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^{1},6^{2},6^{3},6^{4},6^{5},6^{6}-hexahydro-1^{1}*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (60 mg, 0.1 mmol) in DMF (8 mL) at 0 °C under an atmosphere of N_2 was added DIPEA (124 mg, 0.96 mmol), *N*-((*S*)-1-((3-(benzyloxy)azetidin-1-yl)sulfonyl)pyrrolidine-3-carbonyl)-*N*-methyl-L-valine (65 mg, 0.14 mmol) and COMU (58 mg, 0.13 mmol). The mixture was stirred at 0 °C for 1 h, then concentrated under reduced pressure and the residue was purified by prep-HPLC to give (3*S*)-1-((3-(benzyloxy)azetidin-1-yl)sulfonyl)-*N*-((2*S*)-1-(((6^{3}*S*,4*S*)-1^{1}-ethyl-2^{5}-hydroxy-1^{2}-(4-

(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methylpyrrolidine-3-carboxamide (52 mg, 51% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{57}H_{72}N_8O_{10}S$ 1060.5; found 1061.3.

Step 4. A mixture of (3*S*)-1-((3-(benzyloxy)azetidin-1-yl)sulfonyl)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methylpyrrolidine-3-carboxamide (55 mg, 0.05 mmol), MeOH (3 mL) and $Pd(OH)_2$/C (11 mg, 20% by weight) was stirred under a H_2 atmosphere for 12 h. The mixture was filtered, the filtrate was concentrated under reduced pressure and the residue was purified by prep-HPLC to give (2*S*)-N-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-2-{1-[(3*S*)-1-[(3-hydroxyazetidin-1-yl)sulfonyl]pyrrolidin-3-yl]-*N*-methylformamido}-3-methylbutanamide (6.5 mg, 13% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{66}N_8O_{10}S$ 970.5; found 971.2; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.33 - 9.29 (m, 1H), 8.75 - 8.65 (m, 2H), 8.52 (s, 0.5H), 8.15 - 8.06 (m, 0.5H), 7.92 (s, 1H), 7.65 - 7.50 (m, 3H), 7.22 - 7.14 (m, 1H), 7.02 (s, 1H), 6.58 - 6.46 (m, 1H), 5.84 - 5.80 (m, 1H), 5.28 - 5.22 (m, 0.6H), 4.75 - 4.69 (m, 0.4H), 4.45 - 4.12 (m, 4H), 4.05 - 3.88 (m, 5H), 3.72 - 3.50 (m, 7H), 3.22 (s, 2H), 3.12 - 3.04 (m, 1H), 2.94 - 2.70 (m, 7H), 2.29 - 2.03 (m, 5H), 1.90 - 1.77 (m, 2H), 1.76 - 1.45 (m, 2H), 1.24 (s, 1H), 1.08 - 1.02 (m, 2H), 1.01 - 0.72 (m, 12H), 0.5 - 0.43 (m, 3H).

Example A42. Synthesis of (3*S*)-N3-[(1*S*)-1-{[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamoyl}-2-methylpropyl]-*N*1,*N*1,*N*3-trimethylpyrrolidine-1,3-dicarboxamide



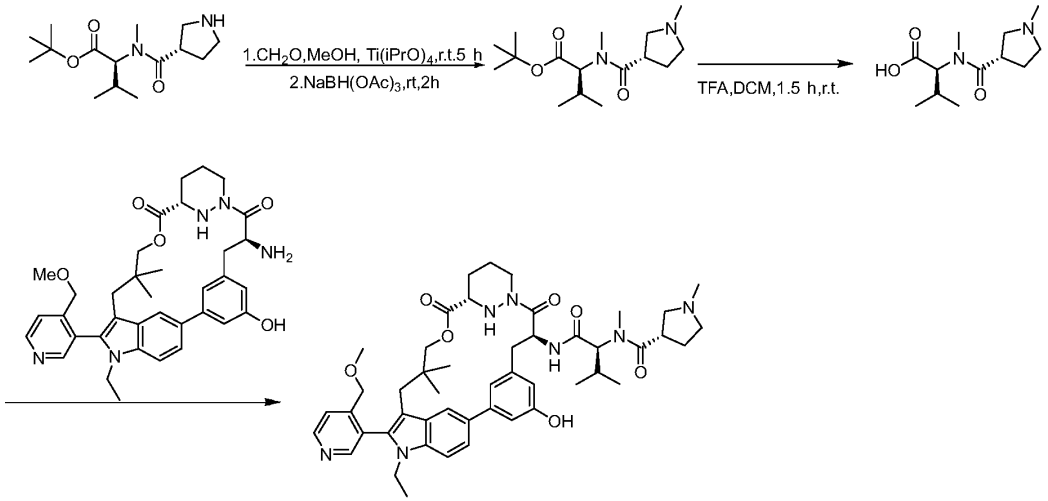
Step 1. To a mixture of *tert*-butyl (2*S*)-3-methyl-2-[*N*-methyl-1-(3*S*)-pyrrolidin-3-ylformamido]butanoate (200 mg, 0.7 mmol) and TEA (142 mg, 1.4 mmol) in DCM (10 mL) at 0 °C

under an atmosphere of N_2 was added dimethylcarbamyl chloride (91 mg, 0.84 mmol) in portions. The mixture was warmed to rt and stirred for 1 h, then H_2O added and the mixture extracted with DCM (3 x 50 mL). The combined organic layers were washed with brine (1 x 5 mL), dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to give *tert*-butyl (2*S*)-2-[1-[(3*S*)-1-(dimethylcarbamoyl)pyrrolidin-3-yl]-*N*-methylformamido]-3-methylbutanoate, which was used in the next step without further purification.

Step 2. A mixture of *tert*-butyl (2*S*)-2-[1-[(3*S*)-1-(dimethylcarbamoyl)pyrrolidin-3-yl]-*N*-methylformamido]-3-methylbutanoate (335 mg, 0.94 mmol) in DCM (10 mL) and TFA (2 mL, 26.9 mmol) was stirred at rt for 2 h. The mixture was concentrated under reduced pressure to give (2*S*)-2-[1-[(3*S*)-1-(dimethylcarbamoyl)pyrrolidin-3-yl]-*N*-methylformamido]-3-methylbutanoic acid, which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{14}H_{25}N_3O_4$ 299.2; found 300.2.

Step 3. To a mixture of (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (80 mg, 0.13 mmol) and (2*S*)-2-[1-[(3*S*)-1-(dimethylcarbamoyl)pyrrolidin-3-yl]-*N*-methylformamido]-3-methylbutanoic acid (57 mg, 0.19 mmol) in MeCN (3 mL) at 0 °C under an atmosphere of N_2 was added lutidine (137 mg, 1.3 mmol) and COMU (77 mg, 0.18 mmol) in portions. The mixture was stirred at 0 °C for 1 h, then concentrated under reduced pressure and the residue was purified by prep-HPLC to give (3*S*)-N3-[(1*S*)-1-{[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.$1^{2,6}$.$1^{10,14}$.$0^{23,27}$]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamoyl}-2-methylpropyl]-*N*1,*N*1,*N*3-trimethylpyrrolidine-1,3-dicarboxamide (45.6 mg, 39% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{66}N_8O_8$ 906.5; found 907.4; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.31 - 9.30 (m, 1H), 8.72 - 8.71 (m, 1H), 8.59 (d, *J* = 50.4 Hz, 1H), 7.92 - 7.90 (m,1H), 7.74 - 7.42 (m, 3H), 7.23 - 7.08 (m, 1H), 7.00 (d, *J* = 13.4 Hz, 1H), 6.56 - 6.49 (m, 1H), 5.45 - 5.32 (m, 1H), 5.26 - 5.04 (m, 1H), 4.87 - 4.64 (m, 1H), 4.53 - 4.35 (m, 1H), 4.32 - 4.09 (m, 3H), 4.12 - 3.81 (m, 3H), 3.81 - 3.37 (m, 6H), 3.23 (t, *J* = 1.6 Hz, 2H), 3.12 - 3.10 (m, 1H), 3.01 - 2.52 (m, 13H), 2.23 - 1.95 (m, 4H), 1.81 (s, 1H), 1.67 (s, 1H), 1.60 - 1.47 (m, 1H), 1.28 - 1.22 (m, 1H), 1.21 - 1.14 (m, 1H), 1.11 - 1.02 (m, 2H), 1.02 - 0.66 (m, 12H), 0.43 (d, *J* = 16.8 Hz, 3H).

Example A27. Synthesis of (2*S*)-*N*-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{*N*-methyl-1-[(3*S*)-1-methylpyrrolidin-3-yl]formamido}butanamide



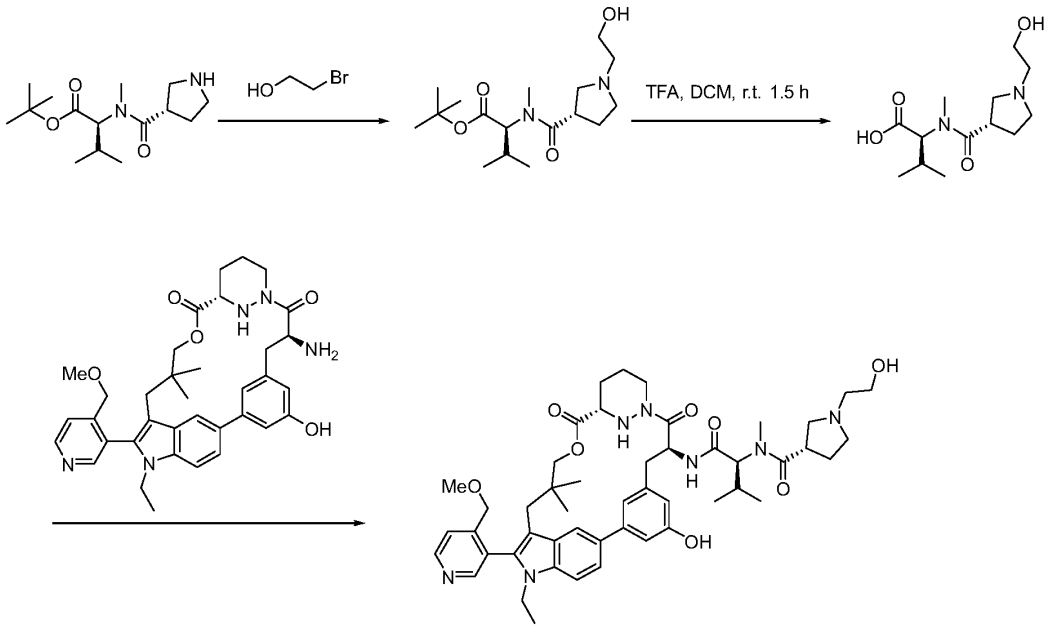
Step 1. A mixture of *tert*-butyl (2*S*)-3-methyl-2-[*N*-methyl-1-(3*S*)-pyrrolidin-3-ylformamido]butanoate (80 mg 0.28 mmol), Ti(Oi-Pr)$_4$ (88 mg, 0.31 mmol) and paraformaldehyde (26mg 0.29 mmol) in MeOH (2 mL) was stirred at rt under an atmosphere of air overnight. The mixture was cooled to 0 °C and NaBH(OAc)$_3$ (107 mg, 0.51 mmol) was added. The mixture was warmed to rt and stirred for 2 h, then cooled to 0 °C and H_2O (0.2 mL) added. The mixture was concentrated under reduced pressure and the residue was purified by C18-silica gel column chromatography to give *tert*-butyl (2*S*)-3-methyl-2-[*N*-methyl-1-[(3*S*)-1-methylpyrrolidin-3-yl]formamido]butanoate (97 mg, crude) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{16}H_{30}N_2O_3$ 298.2; found 299.3.

Step 2. A mixture of *tert*-butyl (2*S*)-3-methyl-2-[*N*-methyl-1-[(3*S*)-1-methylpyrrolidin-3-yl]formamido]butanoate (97 mg, 0.32 mmol) in DCM (2 mL) and TFA (1 mL, 13.5 mmol) was stirred at rt for 1 h, then the mixture was concentrated under reduced pressure to give (2*S*)-3-methyl-2-[*N*-methyl-1-[(3*S*)-1-methylpyrrolidin-3-yl]formamido]butanoic acid (100 mg, crude) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{12}H_{22}N_2O_3$ 242.2; found 243.2.

Step 3. To a mixture of (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (80 mg, 0.13 mmol) and (2*S*)-3-methyl-2-[*N*-methyl-1-[(3*S*)-1-methylpyrrolidin-3-yl]formamido]butanoic acid (47 mg, 0.19 mmol) in MeCN (2 mL) at 0 °C was added 2,6-dimethylpyridine (137 mg, 1.3 mmol) and COMU (77 mg, 0.18 mmol). The mixture was warmed to rt and stirred for 1 h, then concentrated under reduced pressure and the residue was purified by prep-HPLC to give (2*S*)-*N*-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{*N*-methyl-1-[(3*S*)-1-methylpyrrolidin-3-yl]formamido}butanamide (28 mg, 26% yield) as a

solid. LCMS (ESI): m/z [M+H] calc'd for $C_{48}H_{63}N_7O_7$ 849.5; found 850.5; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.31 (s, 1H), 8.72 (t, *J* = 5.1 Hz, 1H), 8.67 - 8.50 (m, 1H), 7.98 - 7.87 (m, 1H), 7.67 - 7.47 (m, 3H), 7.22 - 7.07 (m, 1H), 7.01 (s, 1H), 6.53 (d, *J* = 40.1 Hz, 1H), 5.44 - 5.00 (m, 2H), 4.46 - 4.12 (m, 3H), 4.08 - 3.79 (m, 3H), 3.79 - 3.45 (m, 3H), 3.22 (d, *J* = 1.2 Hz, 2H),3.14 - 2.94 (m, 2H), 2.92 - 2.55 (m, 10H), 2.43 - 2.20 (m, 4H), 2.19 - 1.92 (m, 4H), 1.81 (d, *J* = 11.9 Hz, 2H), 1.67 (s, 1H), 1.53 (s, 1H), 1.09 (t, *J* = 7.1 Hz, 1H), 1.02 - 0.91 (m, 3H), 0.91 - 0.80 (m, 5H), 0.80 - 0.67 (m, 3H), 0.42 (d, *J* = 21.7 Hz, 3H).

Example A23. Synthesis of (2*S*)-N-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2},^{6}.1^{10},^{14}.0^{23},^{27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-2-{1-[(3*S*)-1-(2-hydroxyethyl)pyrrolidin-3-yl]-*N*-methylformamido}-3-methylbutanamide



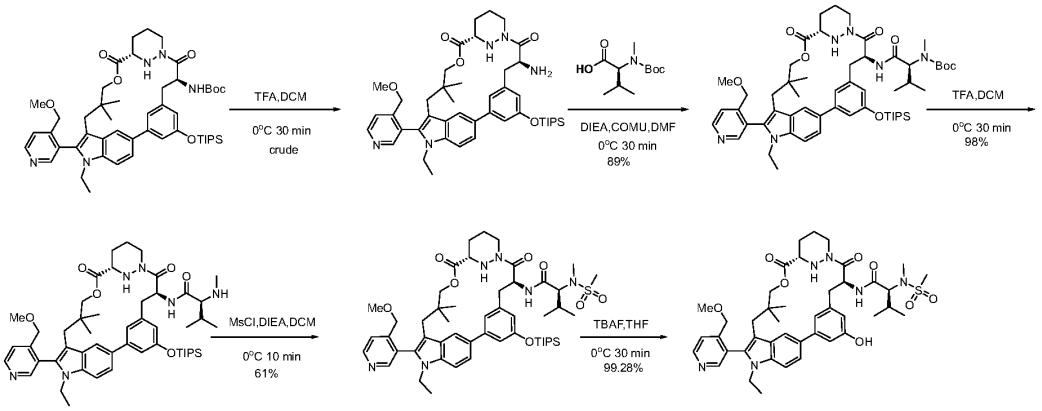
Step 1. To a mixture of *tert*-butyl (2*S*)-3-methyl-2-[*N*-methyl-1-(3*S*)-pyrrolidin-3-ylformamido]butanoate vanadium (200 mg, 0.6 mmol) and 2-bromoethanol (224 mg, 1.8 mmol) in DMF (5 mL) at rt was added Cs_2CO_3 (777 mg, 2.4 mmol) and KI (50 mg, 0.3 mmol). The mixture was stirred at rt for 16 h then diluted with H_2O and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine (2 x 100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by C18-silica gel column chromatography to give *tert*-butyl (2*S*)-2-[1-[(3*S*)-1-(2-hydroxyethyl)pyrrolidin-3-yl]-*N*-methylformamido]-3-methylbutanoate (201 mg, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{17}H_{32}N_2O_4$ 328.2; found 329.4.

Step 2. A mixture of *tert*-butyl (2*S*)-2-[1-[(3*S*)-1-(2-hydroxyethyl)pyrrolidin-3-yl]-*N*-methylformamido]-3-methylbutanoate (100 mg, 0.3 mmol) in DCM (1 mL) and TFA (0.50 mL) at rt was stirred for 1 h, then concentrated under reduced pressure to give (2*S*)-2-[1-[(3*S*)-1-(2-hydroxyethyl)pyrrolidin-3-yl]-*N*-methylformamido]-3-methylbutanoic acid (110 mg, crude) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{24}N_2O_4$ 272.2; found 273.2.

Step 3. To a mixture of (6^{3}*S*,4*S*)-4-amino-1^{1}-ethyl-2^{5}-hydroxy-1^{2}-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^{1},6^{2},6^{3},6^{4},6^{5},6^{6}-hexahydro-1^{1}*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (60 mg, 0.1 mmol) and (2*S*)-2-[1-[(3*S*)-1-(2-hydroxyethyl)pyrrolidin-3-yl]-*N*-methylformamido]-3-methylbutanoic acid (31 mg, 0.11 mmol) in MeCN (2 mL) at 0 °C under an atmosphere of N_2 was added 2,6-dimethylpyridine (103 mg, 1.0 mmol) and COMU (58 mg, 0.13 mmol). The mixture was warmed to rt and stirred for 1 h, then concentrated under reduced pressure and the residue was purified by prep-HPLC to give (2*S*)-

N-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-2-{1-[(3*S*)-1-(2-hydroxyethyl)pyrrolidin-3-yl]-*N*-methylformamido}-3-methylbutanamide (13 mg, 16% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{49}H_{65}N_7O_8$ 879.5; found 880.3; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.72 (t, *J* = 5.3 Hz, 1H), 8.68 - 8.58 (m, 1H), 8.52 (s, 1H), 7.93 (d, *J* = 10.6 Hz, 1H), 7.68 - 7.58 (m, 2H), 7.53 (d, *J* = 7.1 Hz, 1H), 7.21 - 7.07 (m, 1H), 7.01 (s, 1H), 6.52 (d, *J* = 42.8 Hz, 1H), 5.35 (d, *J* = 25.5 Hz, 1H), 5.22 - 4.97 (m, 1H), 4.59 - 4.35 (m, 1H), 4.23 (t, *J* = 13.8 Hz, 3H), 4.11 - 3.81 (m, 3H), 3.81 - 3.56 (m, 2H), 3.56 - 3.47 (m,3H), 3.22 (d, *J* = 1.2 Hz, 2H), 3.09 (d, *J* = 12.6 Hz, 1H), 2.99 - 2.65 (m, 10H), 2.57 - 2.53(m,1H), 2.47 - 2.19 (m, 2H), 2.14 - 2.08(m, 1H), 2.08 (s, 1H), 2.06 - 1.98 (m, 2H), 1.81 (s, 2H), 1.59 (d, *J* = 55.9 Hz, 2H), 1.14 - 0.67 (m, 13H), 0.42 (d, *J* = 22.1 Hz, 3H).

Example A57. Synthesis of (2*S*)-*N*-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-(*N*-methylmethanesulfonamido)butanamide



Step 1. A mixture of *tert*-butyl *N*-[(8*S*,14*S*)-22-ethyl-21-[2-(2-methoxyethyl)phenyl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate (880 mg, 1.2 mmol), DCM (10 mL) and TFA (5 mL) was stirred at 0 °C for 30 min. The mixture was concentrated under reduced pressure to give (8*S*,14*S*)-8-amino-22-ethyl-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaene-9,15-dione, that was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{45}H_{63}N_5O_5Si$ 781.5; found 782.7.

Step 2. To a mixture of (8*S*,14*S*)-8-amino-22-ethyl-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaene-9,15-dione (880 mg, 1.13 mmol) and (2*S*)-2-[(*tert*-butoxycarbonyl)(methyl)amino]-3-methylbutanoic acid (521 mg, 2.3 mmol) in DMF (8.8 mL) at 0 °C was added DIPEA (1.45 g, 11.3

mmol) and COMU (88 mg, 0.21 mmol). The mixture was stirred at 0 °C for 30 min, then diluted with H_2O (100 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine (3 x 100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by prep-TLC to give *tert*-butyl *N*-[(1*S*)-1-[[(8*S*,14*S*)-22-ethyl-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamoyl]-2-methylpropyl]-*N*-methylcarbamate (1 g, 89% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{56}H_{82}N_6O_8Si$ 994.6; found 995.5.

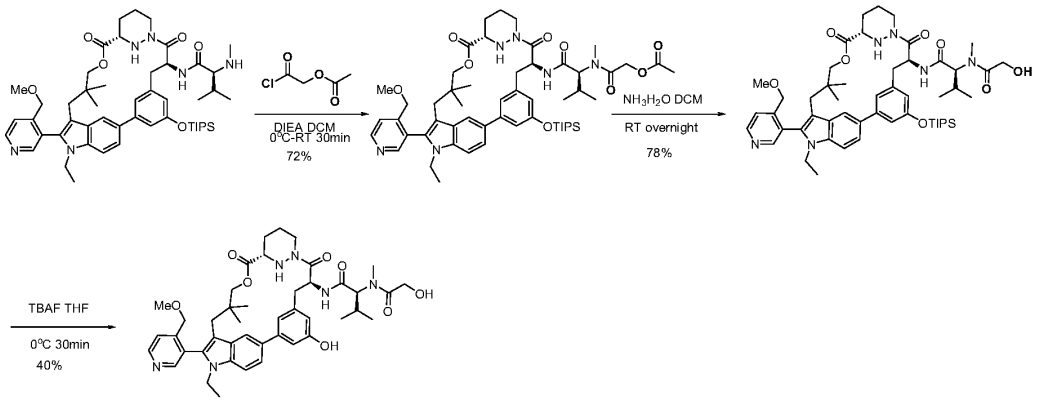
Step 3. A mixture of *tert*-butyl *N*-[(1*S*)-1-[[(8*S*,14*S*)-22-ethyl-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamoyl]-2-methylpropyl]-*N*-methylcarbamate (1.0 g, 1.0 mmol), DCM (10 mL) and TFA (5 mL) was stirred for 30 min. The mixture was concentrated under reduced pressure and the residue was basified to pH ~8 with saturated $NaHCO_3$, then extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (3 x 10 mL), dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to give (2*S*)-*N*-[(8*S*,14*S*)-22-ethyl-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-(methylamino)butanamide (880 mg, 98% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{74}N_6O_6Si$ 894.5; found 895.5.

Step 4. To a mixture of (2*S*)-*N*-[(8*S*,14*S*)-22-ethyl-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-(methylamino)butanamide (90 mg, 0.1 mmol) in DCM (2 mL) at 0 °C was added DIPEA (65 mg, 0.5 mmol) and MsCl (14 mg, 0.12 mmol). The mixture was stirred at 0 C for 30 min, then concentrated under reduced pressure and the residue diluted with H_2O (5mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were washed with brine (3 x 5 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by prep-TLC to give (2*S*)-*N*-[(8*S*,14*S*)-22-ethyl-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-(*N*-methylmethanesulfonamido)butanamide (60 mg, 61% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{52}H_{76}N_6O_8SSi$ 972.5; found 973.7.

Step 5. To a mixture of (2*S*)-*N*-[(8*S*,14*S*)-22-ethyl-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-(*N*-methylmethanesulfonamido)butanamide (60 mg, 0.06 mmol) in THF (2 mL) at 0 °C was added 1M TBAF in THF (6 □L, 0.006 mmol). The mixture was stirred at 0 °C for 30 min, then diluted with H_2O (5 mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were washed with brine (3 x 5 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was

concentrated under reduced pressure and the residue was purified by prep-TLC to give (2*S*)-*N*-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-(*N*-methylmethanesulfonamido)butanamide (50 mg, 99% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{56}N_6O_8S$ 816.4; found 817.5; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34 (d, *J* = 1.8 Hz, 1H), 8.72 (t, *J* = 5.2 Hz, 1H), 8.65 (d, *J* = 5.8 Hz, 1H), 7.99 - 7.86 (m, 1H), 7.71 - 7.45 (m, 3H), 7.19 (d, *J* = 41.5 Hz, 1H), 7.03 (t, *J* = 1.9 Hz, 1H), 6.66 (d, *J* = 10.4 Hz, 1H), 5.34 (q, *J* = 8.1 Hz, 1H), 5.14 (dd, *J* = 62.7, 12.2 Hz, 1H), 4.55 - 4.15 (m, 3H), 4.14 - 3.80 (m, 4H), 3.80 - 3.46 (m, 3H), 3.23 (s, 1H), 3.02 - 2.72 (m, 8H), 2.68 (s, 2H), 2.15 - 1.89 (m, 3H), 1.82 (d, *J* = 12.4 Hz, 1H), 1.76 - 1.62 (m, 1H), 1.54 (q, *J* = 12.7 Hz, 1H), 1.24 (s, 1H), 1.08 (t, *J* = 7.1 Hz, 2H), 1.03 - 0.86 (m, 9H), 0.81 (s, 2H), 0.46 (s, 3H).

Example A43. Synthesis of (2*S*)-*N*-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-2-(2-hydroxy-*N*-methylacetamido)-3-methylbutanamide



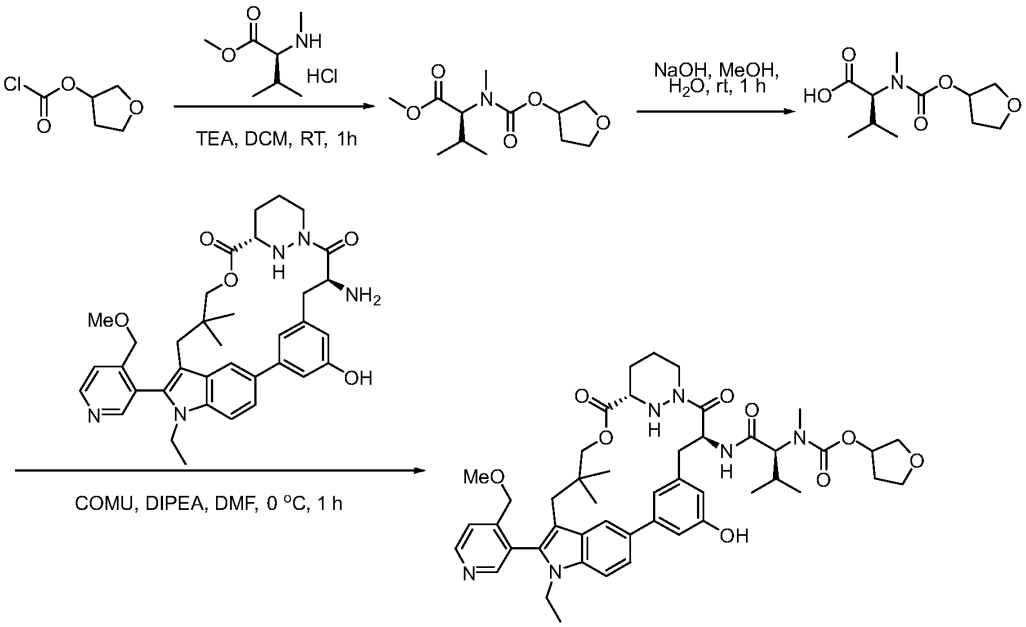
Step 1. To a mixture of (2*S*)-*N*-[(8*S*,14*S*)-22-ethyl-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-(methylamino)butanamide (100 mg, 0.11 mmol) in DCM (1 mL) at 0 °C was added DIPEA (72 mg, 0.56 mmol) and 2-chloro-2-oxoethyl acetate (11.53 mg, 0.11 mmol). The mixture was warmed to rt and stirred for 30 min, then concentrated under reduced pressure, diluted with water (3 mL) and extracted with EtOAc (3 x 3 mL). The combined organic layers were washed with brine (3 x 3 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by prep-TLC to give [[(1*S*)-1-[[(8*S*,14*S*)-22-ethyl-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamoyl]-2-methylpropyl](methyl)carbamoyl]methyl

acetate (80 mg, 72% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{55}H_{78}N_6O_9Si$ 994.6; found 995.7.

Step 2. A mixture of [[(1*S*)-1-[[(8*S*,14*S*)-22-ethyl-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamoyl]-2-methylpropyl](methyl)carbamoyl]methyl acetate (80 mg, 0.080 mmol), DCM (1 mL) and aqueous NH_4OH (0.8 mL) was stirred at rt overnight. H2O (5 mL) was added and the mixture was extracted with EtOAc (3 x 5 mL). The combined organic layers were washed with brine (3 x 5 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by prep-TLC to give (2*S*)-*N*-[(8*S*,14*S*)-22-ethyl-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-2-(2-hydroxy-*N*-methylacetamido)-3-methylbutanamide (60 mg, 78% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{53}H_{76}N_6O_8Si$ 952.6; found 953.7.

Step 3. A mixture of (2*S*)-*N*-[(8*S*,14*S*)-22-ethyl-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-2-(2-hydroxy-*N*-methylacetamido)-3-methylbutanamide (60 mg, 0.06 mmol), THF (2 mL) and 1M TBAF in THF (6 □L, 0.006 mmol) at 0 °C was stirred for 30 min. H_2O (3 mL) was added and the mixture was extracted with EtOAc (3 x 3 mL). The combined organic layers were washed with brine (3 x 3 mL), dried over anhydrous Na_2SO_4. The filtrate was concentrated under reduced pressure and the residue was purified by prep-TLC to give (2*S*)-*N*-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2},^{6}.1^{10},^{14}.0^{23},^{27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-2-(2-hydroxy-*N*-methylacetamido)-3-methylbutanamide (20 mg, 40% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{56}N_6O_8$ 796.4; found 797.6; ^{1}H NMR (400 MHz, CD_3OD) δ 8.70 (dd, *J* = 5.7, 4.4 Hz, 1H), 8.66 - 8.49 (m, 1H), 8.00 (dd, *J* = 4.6, 1.7 Hz, 1H), 7.76 (dd, *J* = 9.9, 5.2 Hz, 1H), 7.60 (dt, *J* = 8.7, 1.6 Hz, 1H), 7.56 - 7.47 (m, 1H), 7.29 - 7.18 (m, 1H), 7.10 - 6.98 (m, 1H), 6.54 (dt, *J* = 3.6, 1.7 Hz, 1H), 5.67 - 5.55 (m, 1H), 4.77 (dd, *J* = 11.2, 8.4 Hz, 1H), 4.57 - 4.39 (m, 3H), 4.39 - 4.20 (m, 3H), 4.19 - 3.91 (m, 2H), 3.90 - 3.65 (m, 3H), 3.60 (dd, *J* = 11.0, 1.8 Hz, 1H), 3.42 (s, 1H), 3.32 (s, 1H), 3.29 - 3.15 (m, 1H), 3.10 - 2.97 (m, 1H), 2.97 - 2.82 (m, 5H), 2.82 - 2.63 (m, 2H), 2.35 - 2.11 (m, 3H), 1.94 (d, *J* = 13.2 Hz, 1H), 1.82 - 1.49 (m, 3H), 1.31 (s, 1H), 1.19 (t, *J* = 7.2 Hz, 2H), 1.09 - 0.95 (m, 7H), 0.95 - 0.83 (m, 5H), 0.50 (d, *J* = 32.4 Hz, 3H).

Example A50. Synthesis of oxolan-3-yl-*N*-[(1*S*)-1-{[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2},^{6}.1^{10},^{14}.0^{23},^{27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamoyl}-2-methylpropyl]-*N*-methylcarbamate



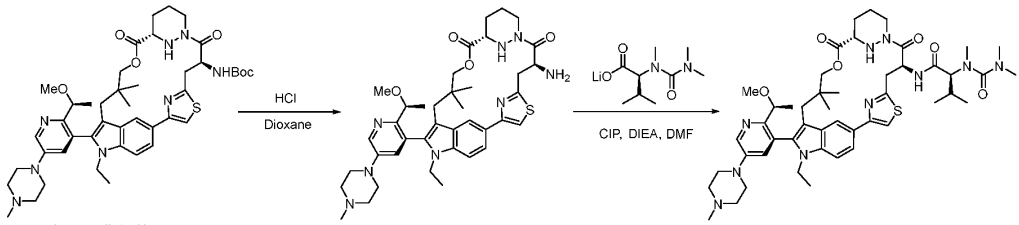
Step 1. To a mixture of methyl (2*S*)-3-methyl-2-(methylamino)butanoate (500 mg, 3.4 mmol) and TEA (1.44 mL, 14.2 mmol) in DCM (20 mL) at rt was added oxolan-3-yl carbonochloridate (1.04 g, 6.9 mmol). The mixture was stirred at rt for 1 h, then sat. NH_4Cl added and the mixture extracted with DCM (3 x 10 mL). The combined organic layers were washed with brine (1 x 10 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (2*S*)-3-methyl-2 [methyl (oxolan-3-yloxy)carbonyl]amino]butanoate (800 mg, 89% yield) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.57 - 4.05 (m, 1H), 3.99 - 3.78 (m, 4H), 3.70 (s, 3H), 3.26 (s, 1H), 2.99 - 2.68 (m, 3H), 2.26 - 1.83 (m, 3H), 1.06 - 0.76 (m, 6H).

Step 2. A mixture of methyl (2*S*)-3-methyl-2 [methyl (oxolan-3-yloxy)carbonyl]amino]butanoate (1 g, 3.9 mmol) and 2M NaOH (19.3 mL, 38.6 mmol) in MeOH (20 mL) was stirred at rt for 1 h. The mixture was concentrated under reduced pressure and the residue was extracted with MTBE (3 x 10 mL). The aqueous layer was acidified to pH ~2 with 2 M HCl then extracted with DCM (3 x 20 mL). The combined organic layers were washed with brine (2 x 10 mL), dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to give (2*S*)-3-methyl-2-[methyl[(oxolan-3-yloxy)carbonyl]amino]butanoic acid (630 mg, 67% yield) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.32 (br. s, 1H), 4.45 - 4.08 (m, 1H), 4.04 - 3.81 (m, 4H), 2.93 (d, *J* = 6.9 Hz, 3H), 2.38 - 1.93 (m, 3H), 1.06 (t, *J* = 5.6 Hz, 3H), 0.94 (d, *J* = 6.7 Hz, 3H).

Step 3. To a mixture of (6^3*S*,4*S*)- 4-amino- 1^1-ethyl-2^5-hydroxyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (80 mg, 0.13 mmol), (2*S*)-3-methyl-

2-[methyl[(oxolan-3-yloxy)carbonyl]amino]butanoic acid (63 mg, 0.26 mmol) and DIPEA (165 mg, 1.3 mmol) in DMF (2 mL) at 0 °C was added COMU (38 mg, 0.19 mmol). The mixture was stirred at 0 °C for 30 min, then the mixture was concentrated under reduced pressure and the residue was purified by prep-HPLC to give oxolan-3-yl-*N*-[(1*S*)-1-{[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamoyl}-2-methylpropyl]-*N*-methylcarbamate (50 mg, 45% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{47}H_{60}N_6O_9$ 852.4; found 853.5; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34 - 9.18 (m, 1H), 8.72 (t, *J* = 5.1 Hz, 1H), 8.58 (d, *J* = 47.8 Hz, 1H), 8.48 - 8.15 (m, 1H), 7.91 (s, 1H), 7.70 - 7.57 (m, 2H), 7.55 - 7.46 (m, 1H), 7.13 (d, *J* = 24.7 Hz, 1H), 7.01 (s, 1H), 6.56 (d, *J* = 9.2 Hz, 1H), 5.34 (s, 1H), 5.28 - 5.00 (m, 2H), 4.40 (d, *J* = 13.3 Hz, 1H), 4.33 - 4.14 (m, 4H), 4.12 - 3.45 (m, 10H), 3.23 (s, 1H), 3.10 (d, *J* = 14.5 Hz, 1H), 2.99 - 2.62 (m, 6H), 2.20 - 1.99 (m, 4H), 1.80 (s, 1H), 1.66 (s, 1H), 1.52 (d, *J* = 12.2 Hz, 1H), 1.09 (t, *J* = 7.1 Hz, 2H), 0.99 - 0.89 (m, 6H), 0.87 - 0.76 (m, 5H), 0.42 (d, *J* = 24.2 Hz, 3H).

Example A277. The synthesis of (2*S*)-N-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methyl-2-(1,3,3-trimethylureido)butanamide



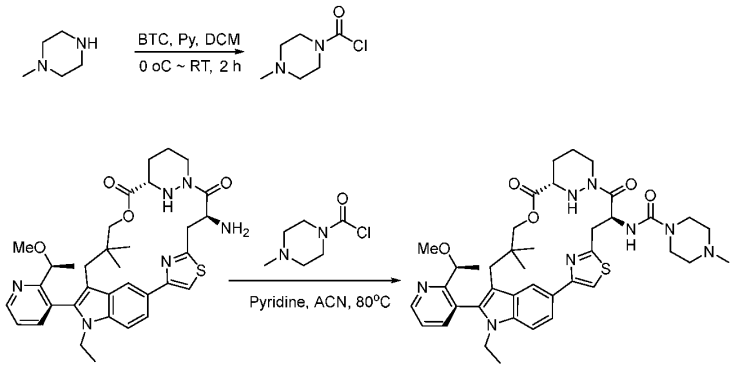
Intermediate 10

Step 1. A solution of Intermediate 10 (8.2 g, 9.89 mmol) in dioxane (40 mL) at 0 °C under nitrogen atmosphere, was added HCl (40 mL, 4M in dioxane). The reaction solution was stirred at 0 °C for 1 h, then concentrated under reduced pressure. The resulting mixture was diluted with DCM (600 mL) and saturated sodium bicarbonate aqueous solution (400 mL). The organic phase was separated and washed with brine (500 mL x 2), then concentrated under reduced pressure to afford (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (7.2 g, 94.8% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{52}N_8O_4S$ 728.4; found 729.3.

Step 2. A mixture of (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (6 g, 8.23 mmol) and lithium N-(dimethylcarbamoyl)-N-methyl-L-valinate (4.28 g, 20.58 mmol) in DMF (80 mL),was added DIEA (53.19 g, 411.55 mmol).The reaction mixture was stirred for 5 minutes, then added CIP (3.43 g, 12.35 mmol) in one portion. The resulting solution was stirred at 25 °C for 1 h, then quenched with

water (100 mL), extracted with EtOAc (300 mL). The organic layer was separated and washed with saturated ammonium chloride aqueous solution (100 mL x 3) and water (100 mL x 2). The combined organic layers were concentrated under reduced pressure. The residue was purified by reverse phase chromatography to afford (2*S*)-N-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methyl-2-(1,3,3-trimethylureido)butanamide (2.5 g, 33.2% yield) as a solid. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.52 – 8.34 (m, 3H), 7.82 (s, 1H), 7.79 – 7.69 (m, 1H), 7.60 – 7.50 (m, 1H), 7.26 – 7.16 (m, 1H), 5.64 – 5.50 (m, 1H), 5.20 – 5.09 (m, 1H), 4.40 – 4.08 (m, 5H), 3.92 – 3.82 (m, 1H), 3.66 – 3.50 (m, 2H), 3.37 – 3.35 (m. 1H), 3.30 – 3.28 (m, 1H), 3.28 – 3.20 (m, 4H), 3.19 – 3.15 (m, 3H), 3.12 – 3. 04 (m, 1H), 2.99 – 2.89 (m, 1H), 2.81 (s, 6H), 2.77 (s, 4H), 2.48 – 2.38 (m, 5H), 2.22 (s, 3H), 2.16 – 2.04 (m, 2H), 1.88 – 1.78 (m, 2H), 1.60 – 1.45 (m, 2H), 1.39 – 1.29 (m, 3H), 0.97 – 0.80 (m, 12H), 0.34 (s, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{48}H_{68}N_{10}O_6S$ 912.5; found 913.6.

Example A265. The synthesis of N-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-4-methylpiperazine-1-carboxamide



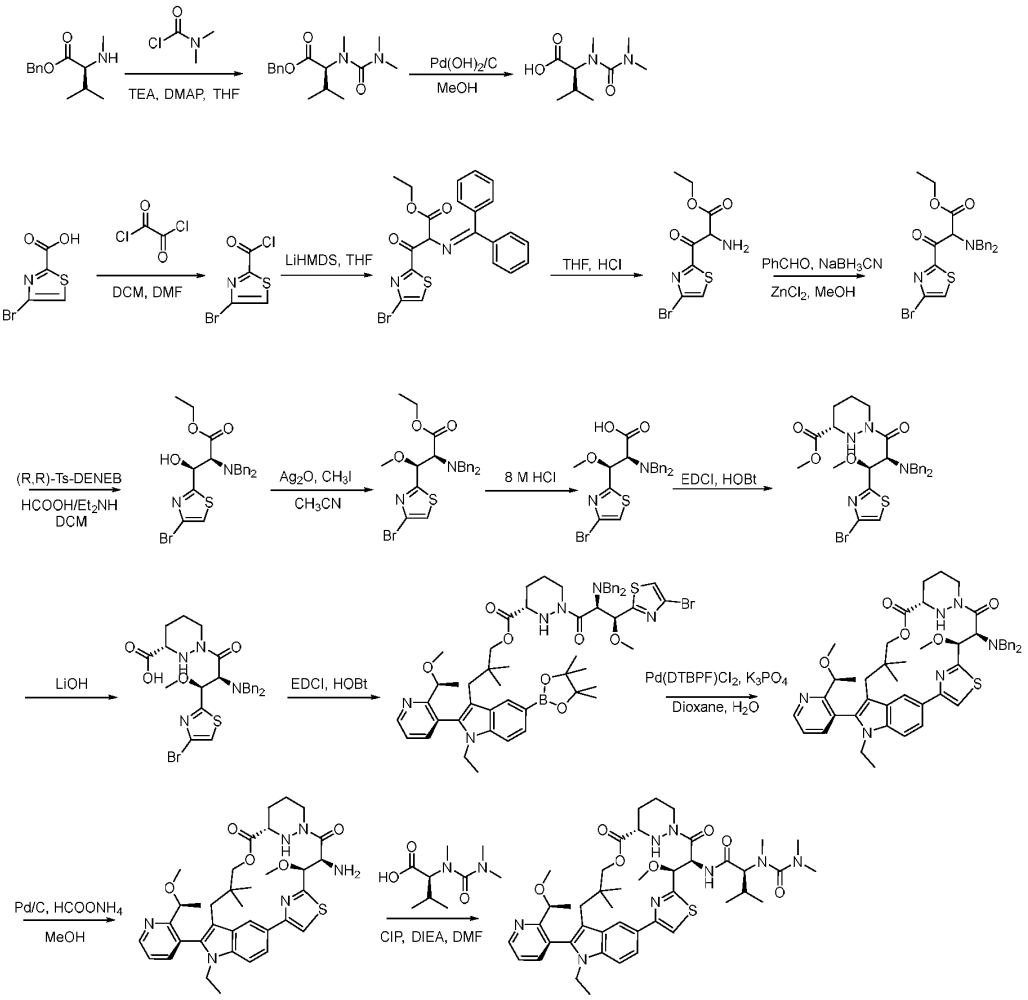
Intermediate 8

Step 1. To a stirred solution of 1-methylpiperazine (100 mg, 1.148 mmol) and Pyridine (275.78 mg, 3.44 mmol) in DCM (3 mL) were added BTC (112.5 mg, 0.38 mmol) in DCM (1 mL) dropwise at 0 °C under nitrogen atmosphere. The reaction was stirred for 2 hh 0 °C under nitrogen atmosphere. The resulting mixture was concentrated under reduced pressure to afford 4-methylpiperazine-1-carbonyl chloride (250 mg, crude) as an oil.

Step 2. To a stirred solution of Intermediate 8 (100 mg, 0.16 mmol) and pyridine (100 mg, 1.272 mmol) in ACN (2 mL) was added 4-methylpiperazine-1-carbonyl chloride (38.67 mg, 0.24 mmol) dropwise at 0 °C under nitrogen atmosphere. The reaction mixture was stirred for 2 hh at 0 °C under nitrogen atmosphere. The resulting mixture was diluted with water (100 mL) and extracted with EtOAc (100 mL x 3). The combined organic layers were washed with brine (50 mL x 3), dried over anhydrous Na_2SO_4, then filtered and concentrated under reduced pressure. The residue was

purified by reverse flash chromatography to give N-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-4-methylpiperazine-1-carboxamide (20 mg, 16.7% yield) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (dd, *J* = 4.8, 1.7 Hz, 1H), 8.50 (s, 1H), 8.14 (d, *J* = 2.5 Hz, 1H), 7.79 (d, *J* = 9.1 Hz, 2H), 7.77 – 7.72 (m, 1H), 7.58 (d, *J* = 8.6 Hz, 1H), 7.52 (dd, *J* = 7.7, 4.7 Hz, 1H), 6.82 (d, *J* = 9.0 Hz, 1H), 5.32 (t, *J* = 9.0 Hz, 1H), 4.99 (d, *J* = 12.1 Hz, 1H), 4.43 – 4.02 (m, 5H), 3.57 (d, *J* = 3.1 Hz, 2H), 3.26 (d, *J* = 8.4 Hz, 6H), 2.97 (d, *J* = 14.3 Hz, 1H), 2.80 – 2.66 (m, 1H), 2.55 (s, 1H), 2.40 (d, *J* = 14.4 Hz, 1H), 2.32 (d, *J* = 5.9 Hz, 4H), 2.21 (s, 3H), 2.09 (d, *J* = 12.1 Hz, 1H), 1.77 (d, *J* = 18.8 Hz, 2H), 1.52 (dd, *J* = 11.8, 5.4 Hz, 1H), 1.37 (d, *J* = 6.0 Hz, 3H), 1.24 (s, 1H), 0.90 (s, 3H), 0.85 (t, *J* = 7.0 Hz, 3H), 0.32 (s, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{40}H_{52}N_8O_5S$ 756.38; found 757.3.

Example A598. The synthesis of (2*S*)-N-((6^3*S*,3*S*,4*S*,*Z*)-1^1-ethyl-3-methoxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methyl-2-(1,3,3-trimethylureido)butanamide



Step 1. A mixture of benzyl (2*S*)-3-methyl-2-(methylamino)butanoate (500 mg, 2.26 mmol) and dimethylcarbamyl chloride (1.215 g, 11.3 mmol) in THF (5 mL), was added TEA (2.286 g, 22.59 mmol) and DMAP (276.02 mg, 2.26 mmol) in portions under nitrogen atmosphere. The reaction mixture was stirred at 65 °C for 12 hh under nitrogen atmosphere, then quenched with water (100 mL) and was extracted with EtOAc (50 mL x 3). The combined organic phase was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by reverse phase chromatography to afford benzyl N-(dimethylcarbamoyl)-N-methyl-L-valinate (400 mg, 58.3% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{16}H_{24}N_2O_3$ 292.2; found 293.1.

Step 2. A mixture of benzyl N-(dimethylcarbamoyl)-N-methyl-L-valinate (400 mg, 1.37 mmol) and palladium hydroxide on carbon (400 mg, 2.85 mmol) in MeOH (10 mL) was stirred for 4 hh under hydrogen atmosphere. The reaction mixture was filtered and the filter cake was washed

with MeOH (100 mL x 3). The filtrate was concentrated under reduced pressure to afford N-(dimethylcarbamoyl)-N-methyl-L-valine (200 mg, crude) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_9H_{18}N_2O_3$ 202.1; found 203.1.

Step 3. A solution of 4-bromo-1,3-thiazole-2-carboxylic acid (10 g, 48.07 mmol) in DCM (100 mL), was added oxalyl chloride (16.27 mL, 192.28 mmol) and DMF (0.11 mL, 1.53 mmol) at 0 °C. The reaction was stirred for at room temperature for 2 hh, then concentrated under reduced pressure to afford 4-bromo-1,3-thiazole-2-carbonyl chloride (10.8 g, crude).

Step 4. A solution of ethyl 2-[(diphenylmethylidene)amino]acetate (12.75 g, 47.69 mmol) in THF (100 mL) at -78 °C, was added LiHMDS (47.69 mL, 47.69 mmol), and stirred at -40 °C for 30 minutes. Then the reaction mixture was added a solution of 4-bromo-1,3-thiazole-2-carbonyl chloride (10.8 g, 47.69 mmol) in THF (100 mL) at -78 °C and stirred at room temperature for 12 hh. The resulting mixture was quenched with water (100 mL), extracted with EtOAc (100 mL x 3). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford ethyl 3-(4-bromothiazol-2-yl)-2-((diphenylmethylene)amino)-3-oxopropanoate (27 g, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{21}H_{17}BrN_2O_3S$ 456.0; found 457.0.

Step 5. A solution of ethyl 3-(4-bromothiazol-2-yl)-2-((diphenylmethylene)amino)-3-oxopropanoate (20 g, 43.73 mmol) in THF (150 mL) at 0 °C, was added 1 M HCl (100 mL) and stirred at room temperature for 2 hh. The resulting solution was concentrated and washed with ethyl ether(200 mL x 2). The water phase was adjusted pH to 8 with sodium bicarbonate solution, then extracted with EtOAc (100 mL x 3). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford ethyl 2-amino-3-(4-bromothiazol-2-yl)-3-oxopropanoate as an oil (9 g, crude). LCMS (ESI): m/z [M+H] calc'd for $C_8H_9BrN_2O_3S$ 292.0; found 292.9.

Step 6. A solution of ethyl 2-amino-3-(4-bromothiazol-2-yl)-3-oxopropanoate (10 g, 34.11 mmol) in MeOH (200 mL) at 0 °C, was added benzaldehyde (7.24 g, 68.23 mmol), zinc chloride (9.3 g, 68.23 mmol) and $NaBH_3CN$ (4.29 g, 68.23 mmol). The reaction was stirred at room temperature for 2 hh, then quenched with water (100 mL) and concentrated. The resulting mixture was extracted with EtOAc (100 mL x 3). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography to afford ethyl 3-(4-bromothiazol-2-yl)-2-(dibenzylamino)-3-oxopropanoate as a solid (8.4 g, 52 % yield). LCMS (ESI): m/z [M+H] calc'd for $C_{22}H_{21}BrN_2O_3S$ 472.1; found 473.0.

Step 7. A mixture of ethyl 3-(4-bromothiazol-2-yl)-2-(dibenzylamino)-3-oxopropanoate (5 g, 10.56 mmol) and (R,R)-TS-DENEB (1.375 g, 2.11 mmol) in DCM (100 mL), was added HCOOH (1.99 mL, 43.29 mmol) and diethylamine (2.2 mL, 2.11 mmol) dropwise at room temperature under nitrogen atmosphere. The reaction mixture was stirred at 50 °C for 12 hh under nitrogen atmosphere. The resulting mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford ethyl (2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-(dibenzylamino)-3-hydroxypropanoate (3.148 g, 60% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{22}H_{23}BrN_2O_3S$ 474.1; found 475.0.

Step 8. A mixture of ethyl (2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-(dibenzylamino)-3-hydroxypropanoate (1 g, 2.1 mmol) and Ag_2O (4.88 g, 21.06 mmol) in acetonitrile (10 mL), was added iodomethane (3.58 g, 25.22 mmol) in portions. The reaction mixture was stirred at 50 °C for 12 hh, then filtered. The filter cake was washed with MeOH (50 mL x 2). The filtrate was concentrated under reduced pressure to afford ethyl (2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-(dibenzylamino)-3-methoxypropanoate (1.06 g, crude) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{25}BrN_2O_3S$ 488.1; found 489.3.

Step 9. A mixture of ethyl (2S,3S)-3-(4-bromothiazol-2-yl)-2-(dibenzylamino)-3-hydroxypropanoate (1.06 g, 2.3 mmol) in HCl (10 ml, 8 M) was stirred at 80 °C for 12 hh and concentrated by reduced pressure. The residue was purified by reverse phase chromatography to afford (2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-(dibenzylamino)-3-methoxypropanoic acid (321mg, 31.7% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{21}H_{21}BrN_2O_3S$ 460.1; found 461.1.

Step 10. A solution of (2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-(dibenzylamino)-3-methoxypropanoic acid (4.61 g, 10 mmol) in DCM (100 mL) at 0 °C was added methyl (3*S*)-1,2-diazinane-3-carboxylate bis(trifluoroacetic acid) salt (3.72 g, 15 mmol), NMM (10.1 mL. 100 mmol), EDCI (3.8 g, 20 mmol) and HOBt (5.39 g, 39.89 mmol). The solution was warmed to room temperature and stirred for 1 h. The reaction was then quenched with H_2O (100 mL) and was extracted with EtOAc (100 mL x 3). The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressured. The residue was purified by silica gel column chromatography to give methyl (*S*)-1-((2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-(dibenzylamino)-3-methoxypropanoyl)hexahydropyridazine-3-carboxylate (5.11 g, 90% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{27}H_{31}BrN_4O_4S$ 587.1; found 586.1.

Step 11. A solution of methyl (*S*)-1-((2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-(dibenzylamino)-3-methoxypropanoyl)hexahydropyridazine-3-carboxylate (5.11 g, 9 mmol) in THF (100 mL)/H_2O (100 mL) was added LiOH (1.81 g, 45 mmol) under N_2 atmosphere and the resulting mixture was stirred for 2 hh at 25 °C. The resulting mixture was concentrated under reduced pressure, the residue was acidified to pH 5 with HCL (1N). The aqueous layer was extracted with DCM (50 mL x 3). The combined organic phase was concentrated under reduced pressure to give (*S*)-1-((2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-(dibenzylamino)-3-methoxypropanoyl)hexahydropyridazine-3-carboxylic acid (4.38 g , 85% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{26}H_{29}BrN_4O_4S$ 572.1; found 573.1.

Step 12. A mixture of (*S*)-1-((2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-(dibenzylamino)-3-methoxypropanoyl)hexahydropyridazine-3-carboxylic acid (1.15 g, 2 mmol) and (S)-3-(1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indol-3-yl)-2,2-dimethylpropan-1-ol (985 mg, 2 mmol) in DCM (50 mL), was added DIEA (1.034 g, 8 mmol), EDCI (1.15 g, 558.3 mmol), HOBT (270.2 mg, 2 mmol). The reaction solution was stirred at 25 °C for 16 hh. The resulting mixture was diluted with DCM (200 mL), washed with water (50 mL x 2) and brine (50 mL x 3) and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl (*S*)-1-((2S,3S)-3-(4-bromothiazol-2-yl)-2-(dibenzylamino)-3-methoxypropanoyl)hexahydropyridazine-3-carboxylate (1.13 g, 54% yield) as a solid. LCMS (ESI):

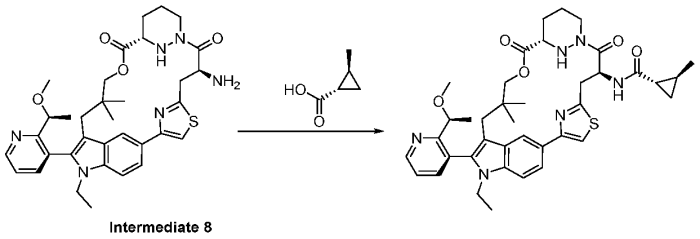
m/z [M+H] calc'd for $C_{55}H_{68}BBrN_6O_7S$ 1046.4; found 1047.4.

Step 13. A mixture of 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl (S)-1-((2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-(dibenzylamino)-3-methoxypropanoyl)hexahydropyridazine-3-carboxylate (250 mg, 0.24 mmol) and Pd(DtBPF)Cl_2 (15.55 mg, 0.024 mmol) in dioxane (5 mL) and water (1 mL), was added K_3PO_4 (126.59 mg, 0.6 mmol) in portions under nitrogen atmosphere. The reaction mixture was stirred at 80 °C for 2 hh under nitrogen atmosphere. The resulting mixture was diluted with water (20 mL) and extracted with EtOAc (10mL x 3), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford (6^3*S*,3*S*,4*S*,*Z*)-4-(dibenzylamino)-1^1-ethyl-3-methoxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (137 mg,44.38 %) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{49}H_{56}N_6O_5S$ 840.4; found 841.5.

Step 14. A mixture of ((6^3*S*,3*S*,4*S*,*Z*)-4-(dibenzylamino)-1^1-ethyl-3-methoxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (100 mg, 0.12 mmol) and Pd/C (253.06 mg, 2.38 mmol) in MeOH (10 mL), was added $HCOONH_4$ (149.94 mg, 2.38 mmol) in portions. The reaction mixture was stirred at 60 °C for 6 hh under hydrogen atmosphere. The resulting mixture was filtered, the filter cake was washed with MeOH (100 mL x 10). The filtrate was concentrated under reduced pressure to afford (6^3*S*,3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-3-methoxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (56 mg, crude) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{35}H_{44}N_6O_5S$ 660.3; found 661.2.

Step 15. A mixture of (6^3*S*,3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-3-methoxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (56 mg, 0.085 mmol) and N-(dimethylcarbamoyl)-N-methyl-L-valine (51.42 mg, 0.25 mmol) in DMF (2 mL), was added 2-Chloro-1,3-dimethylimidazolidinium hexafluorophosphate (47.55 mg, 0.17 mmol) and DIEA (547.62 mg, 4.24 mmol) in portions. The reaction mixture was stirred for 12 hh. The resulting mixture was purified by reverse phase chromatography to afford (2*S*)-N-((6^3*S*,3*S*,4*S*,*Z*)-1^1-ethyl-3-methoxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methyl-2-(1,3,3-trimethylureido)butanamide (1.5 mg, 2.06 % yield) as a solid. ^{1}H NMR (400 MHz, Methanol-d4) δ 8.74-8.77 (m, 1H), 8.61 (d, J = 1.6 Hz, 1H), 7.99 – 7.87 (m, 1H), 7.73 – 7.66 (m, 1H), 7.68 (s, 1H), 7.60 – 7.55 (m, 1H), 7.49 (d, J = 8.7 Hz, 1H), 7.31 (d, J = 51.0 Hz, 0H), 5.89 (s, 1H), 4.95 (s, 1H), 4.43 (d, J = 13.0 Hz, 1H), 4.36 (q, J = 6.2 Hz, 1H), 4.33 – 4.19 (m, 2H), 4.10 – 4.03 (m, 1H), 4.03 (d, J = 11.2 Hz, 1H), 3.78 – 3.67 (m, 2H), 3.65 (s, 0H), 3.46 (s, 3H), 3.34 (s, 4H), 3.01 (d, J = 10.3 Hz, 1H), 2.93 (s, 6H), 2.88 – 2.81 (m, 1H), 2.78 (s, 3H), 2.70 – 2.60 (m, 1H), 2.23 – 2.01 (m, 2H), 2.03 (s, 0H), 1.99 (d, J = 13.3 Hz, 1H), 1.91 – 1.74 (m, 1H), 1.69 – 1.54 (m, 1H), 1.45 (d, J = 6.2 Hz, 3H), 1.37 – 1.32 (m, 1H), 1.28 (s, 1H), 0.94 (p, J = 6.7 Hz, 12H), 0.51 (s, 3H), 0.10 (s, 1H). LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{60}N_8O_7$ 844.4; found 845.4.

Example A286. The synthesis of (1*S*,2*S*)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-6^4,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methylcyclopropane-1-carboxamide



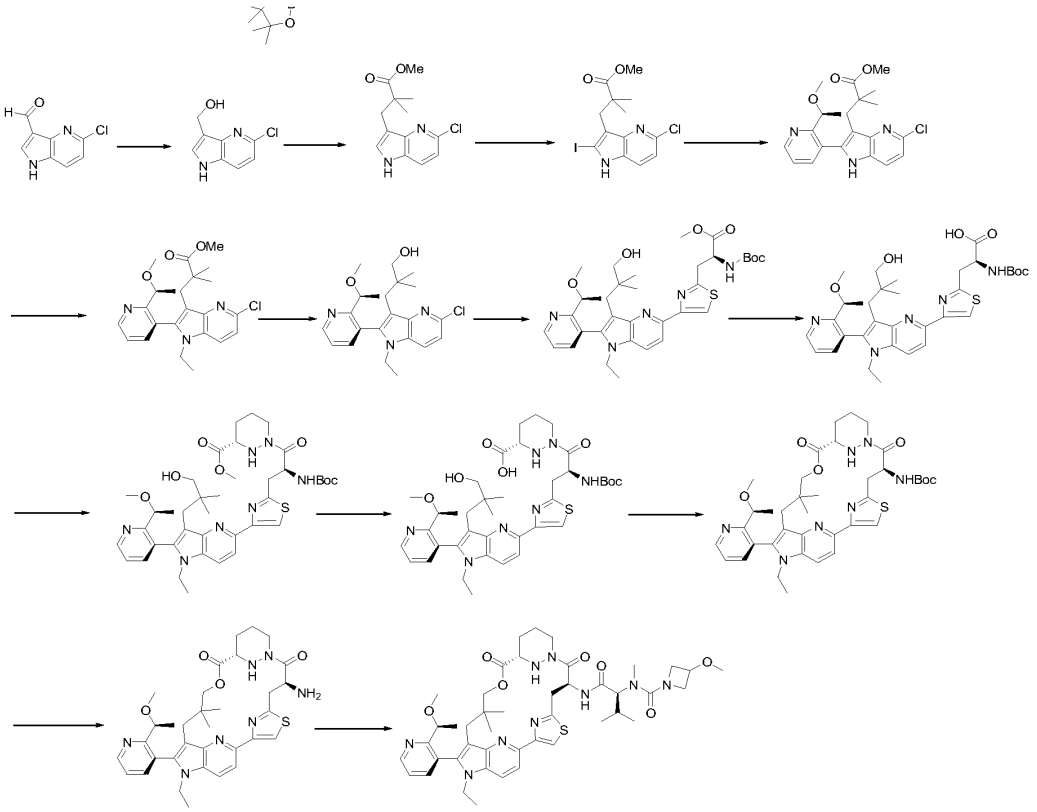
Intermediate 8

Step 1. A solution of Intermediate 8 (8 g, 10.95 mmol) in HCl (200 mL, 4M in 1,4-dioxane) was stirred at 0 °C for 2 hh, then concentrated under reduced pressure. The resulting mixture was diluted with DCM (60 mL) and saturated $NaHCO_3$ aqueous solution (40 mL). The organic phase was separated and washed with brine (50 mL x 2) and concentrated under reduced pressure to give (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (10.3 g, crude) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{34}H_{42}N_6O_4S$ 630.3; found 631.2.

Step 2. A stirred solution of (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (8 g, 12.68 mmol) in DMF (50 mL) at 0 °C, was added DIEA (9.83 g, 76.09 mmol), (1*S*,2*S*)-2-methylcyclopropane-1-carboxylic acid (1.52 g, 15.22 mmol) and HATU (14.47 g, 38.05 mmol). The reaction mixture was stirred at 0 °C for 2 hh and concentrated under reduced pressure. The residue was purified by reverse phase chromatography to afford (1*S*,2*S*)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methylcyclopropane-1-carboxamide (6.84 g, 56.37% yield) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.79 (dd, *J* = 4.7, 1.9 Hz, 1H), 8.59 – 8.40 (m, 2H), 7.95 – 7.86 (m, 1H), 7.82 – 7.71 (m, 2H), 7.66 – 7.53 (m, 2H), 5.57 (t, *J* = 9.0 Hz, 1H), 5.07 (s, 1H), 4.41 – 4.28 (m, 2H), 4.25 (d, *J* = 12.4 Hz, 1H), 4.17 (d, *J* = 10.8 Hz, 1H), 4.09 (d, *J* = 7.2 Hz, 1H), 3.58 (s, 2H), 3.32 (d, *J* = 14.6 Hz, 1H), 3.28 (s, 3H), 3.16 (dd, *J* = 14.7, 9.1 Hz, 1H), 2.95 (d, *J* = 14.4 Hz, 1H), 2.75 (m, *J* = 12.1, 7.1 Hz, 1H), 2.43 (d, *J* = 14.4 Hz, 1H), 2.13 – 2.00 (m, 1H), 1.76 (d, *J* = 22.0 Hz, 2H), 1.60 – 1.44 (m, 2H), 1.38 (d, *J* = 6.1 Hz, 3H), 1.07 (d, *J* = 1.9 Hz, 4H), 0.86 (dd, *J* = 14.1, 7.1 Hz, 7H), 0.59 – 0.49 (m, 1H), 0.34 (s, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{48}N_6O_5S$ 712.3; found 713.2.

Example A613. The synthesis of *N*-((2*S*)-1-(((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-pyrrolo[3,2-b]pyridina-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-

3-methyl-1-oxobutan-2-yl)-3-methoxy-N-methylazetidine-1-carboxamide



Step 1. A mixture of methyl (*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoate (920 mg, 2.5mmol), 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (1.6 g, 6.3 mmol), x-Phos (180 mg, 0.5 mmol), $Pd_2(dba)_3$-chloroform (130 mg, 0.13 mmol) and potassium acetate (740 mg, 7.5 mmol) in dioxane (25 mL) in a sealed tube under N_2 atmosphere, was stirred at 110 °C for 8 hh to afford crude methyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiazol-2-yl)propanoate as a solution. LCMS (ESI): m/z [M+H] calc'd for $C_{18}H_{29}BN_2O_6S$ 412.2; found 331.1.

Step 2. A mixture of 5-chloro-1*H*-pyrrolo[3,2-*b*]pyridine-3-carbaldehyde (7 g, 39 mmol) in MeOH (140 mL) under N_2 atmosphere, was added $NaBH_4$ (2.9 g, 78 mmol) at 0 °C. The reaction mixture was stirred at 10 °C for 2 hh and concentrated under reduced pressure. The residue was diluted with EtOAc (200 mL), washed with brine (25 mL), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford (5-chloro-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)methanol (3.5 g, 55% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_8H_7ClN_2O$ 182.0; found 183.0.

Step 3. A mixture of (5-chloro-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)methanol (3.5 g, 19 mmol) and ((1-methoxy-2-methylprop-1-en-1-yl)oxy)trimethylsilane (6.7 g, 38 mmol) in THF (50 mL), was dropwise added TMSOTf (3.8 g, 17.1 mmol) at 0 °C. The reaction mixture was stirred at 5 °C for 2

hh, then diluted with EtOAc (100 mL), washed with saturated $NaHCO_3$ aqueous (50 mL), and brine (50 mL x 2). The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford methyl 3-(5-chloro-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2,2-dimethylpropanoate (3 g, 59% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{15}ClN_2O_2$ 266.1; found 267.1.

Step 4. A mixture of methyl 3-(5-chloro-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2,2-dimethylpropanoate (3 g, 11 mmol) in anhydrous THF (50 mL) at 0 °C, was added AgOTf (4.3g, 17 mmol) and I_2 (2.9 g, 11 mmol). The reaction mixture was stirred at 0 °C for 2 hh, then quench with conc. Na_2SO_3 (20 mL), diluted with EtOAc (50 mL) and filtered. The filtrate was washed with brine (50 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified with silica gel column chromatography to afford methyl 3-(5-chloro-2-iodo-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2,2-dimethylpropanoate (2.3 g, 52% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{14}ClIN_2O_2$ 393.0; found 392.0

Step 5. A mixture of methyl 3-(5-chloro-2-iodo-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2,2-dimethylpropanoate (2.3 g, 5.9 mmol), 2-(2-(2-methoxyethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (1.6 g, 7.1 mmol) and K_2CO_3 (2.4 g, 18 mol) in dioxane (25 mL) and water (5 mL) under N_2 atmosphere, was added Pd(dppf)Cl2·DCM (480 mg, 0.59 mmol). The reaction mixture was stirred at 70 °C for 4 hh, then diluted with EtOAc (200 mL) and washed with brine (25 mL). The separated organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford methyl (*S*)-3-(5-chloro-2-(2-(1-methoxyethyl)pyridin-3-yl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2,2-dimethylpropanoate (2 g, yield 84%) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{21}H_{24}ClN_3O_3$ 401.2; found 402.2.

Step 6. A mixture of methyl (*S*)-3-(5-chloro-2-(2-(1-methoxyethyl)pyridin-3-yl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2,2-dimethylpropanoate (2 g, 5 mmol), cesium carbonate (3.3 g, 10 mmol) and EtI (1.6 g, 10 mmol) in DMF (30 mL) was stirred at 25 °C for 10 hh. The resulting mixture was diluted with EtOAc (100 mL), washed with brine (20 mL x 4). The separated organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford methyl (*S*)-3-(5-chloro-1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2,2-dimethylpropanoate as two diastereomers (P1: 0.7 g, 32% yield; P2: 0.6 g, 28% yield) both as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{28}ClN_3O_3$ 429.2; found 430.2.

Step 7. A mixture of methyl (*S*)-3-(5-chloro-1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2,2-dimethylpropanoate (P2, 1.2 g, 2.8 mmol) in anhydrous THF (20 mL) at 5 °C, was added $LiBH_4$ (120 mg, 5.6 mmol). The reaction mixture was stirred at 60 °C for 4 hh, then quenched with conc. NH_4Cl (20 mL), diluted with EtOAc (50 mL) and washed with brine (30 mL). The organic layer was separated, dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified with silica gel column chromatography to afford (*S*)-3-(5-chloro-1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2,2-dimethylpropan-1-ol (1 g, 89% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{22}H_{28}ClN_3O_2$ 401.2; found 402.2.

Step 8. A mixture of solution from Step 1 (360 mg, crude, 1 mmol) in dioxane (10 mL) and water (2 mL), was added (*S*)-3-(5-chloro-1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-1*H*-pyrrolo[3,2-

b]pyridin-3-yl)-2,2-dimethylpropan-1-ol (200 mg, 0.5 mmol), potassium carbonate (200 mg, 1.5 mmol) and Pd-118 (30 mg, 0.05 mmol). This reaction mixture was stirred at 70 °C for 3 hh, then diluted with EtOAc (40 mL), filtered. The filtrate was washed with brine , dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified with silica gel column chromatography to afford methyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-pyrrolo[3,2-b]pyridin-5-yl)thiazol-2-yl)propanoate (300 mg, 65% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{34}H_{45}N_5O_6S$ 651.3; found 652.3.

Step 9. A solution of methyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-pyrrolo[3,2-b]pyridin-5-yl)thiazol-2-yl)propanoate (280 mg, 0.43 mmol) in MeOH (4 mL), was added a solution of lithium hydroxide (51 mg, 2.15 mmol) in water (2 mL) at 20 °C. The reaction was stirred at 20 °C for 5 hh, then adjusted to pH = 3~4 with HCl (1 N). The resulting mixture was diluted with water (30 mL) and extracted with EtOAc (15 mL × 3). The combined organic phase was washed with brine (10 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-pyrrolo[3,2-b]pyridin-5-yl)thiazol-2-yl)propanoic acid (280 mg, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{33}H_{43}N_5O_6S$ 637.3; found 638.3.

Step 10. A solution of (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-pyrrolo[3,2-b]pyridin-5-yl)thiazol-2-yl)propanoic acid (274 mg, 0.43mmol) and methyl (*S*)-hexahydropyridazine-3-carboxylate (280 mg, 0.64 mmol) in DMF (3 mL) at 5 °C, was added a solution of HATU (245 mg, 0.64 mmol) and DIEA (555 mg, 4.3mmol) in DMF (2 mL). The reaction was stirred for 1 h, then diluted with EtOAc (20 mL) and water (20 mL). The organic layer was separated and washed with water (20 mL x 3) and brine (20 mL), dried over anhydrous sodium sulfate, filtered concentrated under reduced pressure. The residue was purified by silica gel chromatography to give methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-pyrrolo[3,2-b]pyridin-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (230 mg, 70% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{53}N_7O_7S$ 763.4; found 764.3.

Step 11. A solution of methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-pyrrolo[3,2-b]pyridin-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (230 mg, 0.3 mmol) in DCE (3 mL), was added trimethyltin hydroxide (300 mg, 1.4 mmol) under N_2 atmosphere. The reaction was stirred at 65 °C for 16 hh, then concentrated under reduced pressure. The residue was diluted with EtOAc (20 mL), washed with water (20 mL) and brine (10 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1H-pyrrolo[3,2-b]pyridin-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (200 mg, crude) as foam. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{51}N_7O_7S$ 749.4; found 750.3.

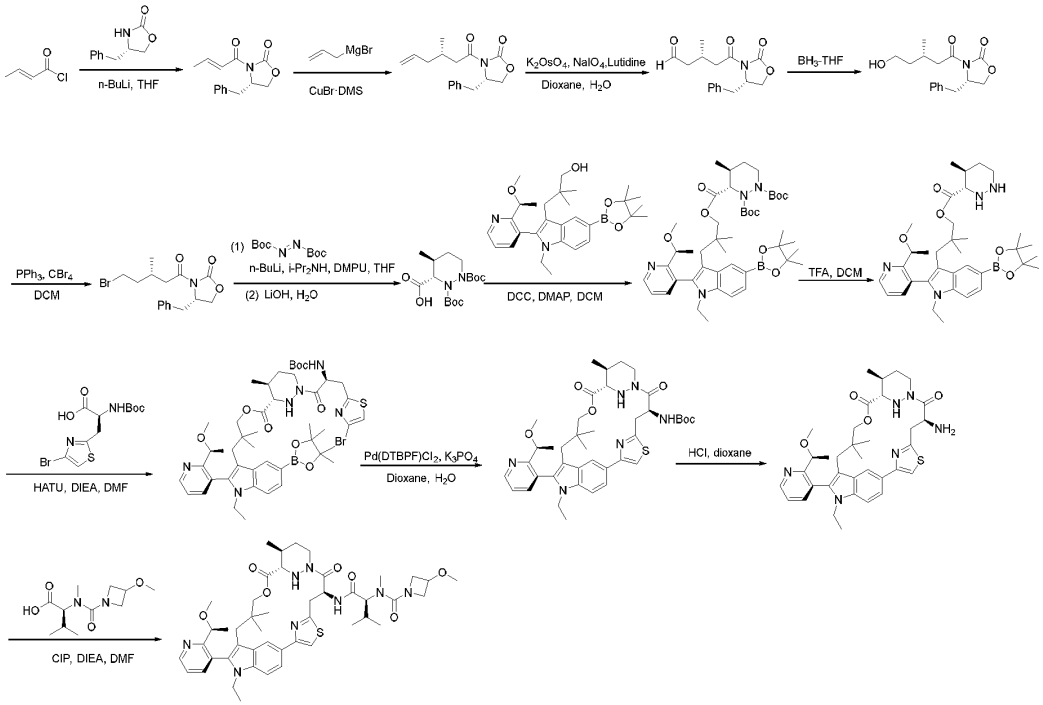
Step 12. A solution of (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-

2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1H-pyrrolo[3,2-b]pyridin-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (245 mg, 0.32mmol) in DCM (50 mL) at 5 °C, were added HOBt (432 mg, 3.2mmol), EDCI(1.8 g, 9.6mmol) and DIEA (1.65 g, 12.8mmol). The reaction mixture was stirred at 20 °C for 16 hh, then concentrated under reduced pressure. The residue was diluted with EtOAc (20 mL) and water (20 mL). The organic layer was separated and washed with water (30 mL x 3) and brine (30 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography to give *tert*-butyl ((6^3*S*,4*S*,*Z*)-11-ethyl-12-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11*H*-8-oxa-2(4,2)-thiazola-1(5,3)-pyrrolo[3,2-b]pyridina-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (100 mg, 43% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{49}N_7O_6S$ 731.4; found 732.3.

Step 13. A solution of *tert*-butyl ((6^3*S*,4*S*,*Z*)-11-ethyl-12-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11H-8-oxa-2(4,2)-thiazola-1(5,3)-pyrrolo[3,2-b]pyridina-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (80 mg, 0.11 mmol) in TFA (0.2 mL) and DCM (0.6 mL) was stirred at 20 °C for 1 h. The reaction was concentrated to afford (6^3*S*,4*S*,*Z*)-4-amino-11-ethyl-12-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11*H*-8-oxa-2(4,2)-thiazola-1(5,3)-pyrrolo[3,2-b]pyridina-6(1,3)-pyridazinacycloundecaphane-5,7-dione (72 mg, 95% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{33}H_{41}N_7O_4S$ 631.3; found 632.3.

Step 14. A solution of (6^{3}S,4S,Z)-4-amino-11-ethyl-12-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11H-8-oxa-2(4,2)-thiazola-1(5,3)-pyrrolo[3,2-b]pyridina-6(1,3)-pyridazinacycloundecaphane-5,7-dione (100 mg, 0.16 mmol) and (2S)-2-[(3-methoxyazetidin-1-yl)carbonyl(methyl)amino]-3-methylbutanoic acid (78 mg, 0.32 mmol) in DMF (5 mL) at 0 °C, was dropwise added a solution of DIEA (620 mg, 4.8 mmol) and HATU (91 mg, 0.24 mmol) in DMF (5 mL). The reaction mixture was stirred at 0°C for 2 hh, then diluted with EtOAc (50 mL), washed with water (25 mL x 3), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford N-((2S)-1-(((6^{3}S,4S,Z)-11-ethyl-12-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11H-8-oxa-2(4,2)-thiazola-1(5,3)-pyrrolo[3,2-b]pyridina-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-3-methoxy-N-methylazetidine-1-carboxamide (112.9 mg, 82% yield) as a solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.77-8.75 (dd, J = 4.8, 1.7 Hz, 1H), 7.96-7.94 (d, J = 8.6 Hz, 1H), 7.89-7.87 (dd, J = 8.4, 2.3 Hz, 2H), 7.77-7.74 (d, J = 8.6 Hz, 1H), 7.58-7.55 (dd, J = 7.8, 4.8 Hz, 1H), 5.73-5.70 (dd, J = 8.0, 2.7 Hz, 1H), 4.41-4.38 (dt, J = 8.5, 4.3 Hz, 2H), 4.33 – 4.26 (m, 3H), 4.24 – 4.17 (m, 3H), 4.04-4.01 (dd, J = 11.9, 3.0 Hz, 1H), 3.99-3.96 (m, 1H), 3.89 – 3.83 (m, 2H), 3.53-3.49 (dd, J = 9.7, 7.3 Hz, 2H), 3.46-3.45 (d, J = 3.0 Hz, 1H), 3.35 (s, 3H), 3.34-3.33 (d, J = 4.5 Hz, 3H), 3.28 (s, 1H), 2.89 (s, 3H), 2.78-2.71 (td, J = 13.2, 3.4 Hz, 1H), 2.52-2.48 (d, J = 14.1 Hz, 1H), 2.23 – 2.20 (m, 1H), 2.19-2.11 (d, J = 10.2 Hz, 1H), 1.91-1.88 (d, J = 13.5 Hz, 1H), 1.73-1.70 (dd, J = 9.0, 3.9 Hz, 1H), 1.56 – 1.50 (m, 1H), 1.47-1.46 (d, J = 6.1 Hz, 3H), 0.98 – 0.91 (m, 9H), 0.88 (s, 3H), 0.45 (s, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{59}N_9O_7S$ 857.4; found 858.3.

Example A579. The synthesis of N-((2*S*)-1-(((6^3*S*,6^4*S*,4*S*,*Z*)-11^1-ethyl-12^1-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-6^4,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-3-methoxy-N-methylazetidine-1-carboxamide



Step 1. A solution of (*S*)-4-benzyloxazolidin-2-one (10 g, 56.43 mmol) in THF (100 mL) was purged with nitrogen, was added of n-butyllithium (24.83 mL, 62.08 mmol) at -78 °C under nitrogen atmosphere, then stirred for at -78 °C for 15 minutes. The reaction mixture was added 2-butenoyl chloride (6.49 g, 62.08 mmol). The resulting solution was stirred at -78 °C for 30 minutes, then slowly warmed up to 0 °C and stirred for 15 minutes, quenched with saturated ammonium chloride solution (100 mL). The resulting solution was extracted with EtOAc (100 mL x 3) and the combined organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford (4*S*)-4-benzyl-3-[(2*E*)-but-2-enoyl]-1,3-oxazolidin-2-one (12.26 g, 88.57 % yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{14}H_{15}NO_3$ 245.1; found 246.1.

Step 2. A solution of CuBr·DMS (12.07 g, 58.71 mmol) in THF (120 mL) was purged and maintained nitrogen atmosphere, added of allylmagnesium bromide (58.71 mL, 58.71 mmol) at -78 °C. The reaction was stirred at -60 °C for 30 minutes under nitrogen atmosphere followed by addition of (4*S*)-4-benzyl-3-[(2*E*)-but-2-enoyl]-1,3-oxazolidin-2-one (12 g, 48.92 mmol) at -78 °C. The resulting solution was stirred at -50 °C for 3 more hh, then quenched with saturated ammonium chloride solution (100 mL) and extracted with EtOAc (60 mL x 3). The combined organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford (*S*)-4-benzyl-3-((*S*)-3-methylhex-5-

enoyl)oxazolidin-2-one (13.2 g, 93.89 % yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{17}H_{21}NO_3$ 287.2; found 288.2.

Step 3. A solution of (*S*)-4-benzyl-3-((*S*)-3-methylhex-5-enoyl)oxazolidin-2-one (13.2 g, 45.94 mmol) in dioxane (200 mL) and water (200 mL), was added 2,4-Lutidine (9.84 g, 91.87 mmol) followed with $K_2OsO_4{\cdot}2H_2O$ (1.69 g, 4.59 mmol) at 0 °C. The reaction solution was stirred at 0 °C for 15 minutes, then was added $NaIO_4$ (39.3 g, 183.74 mmol). The resulting mixture was stirred at 0 °C for 1 h, then extracted with EtOAc (150 mL x 3). The combined organic phase was hydrochloric acid (100 mL x 3), dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford (*S*)-5-((*S*)-4-benzyl-2-oxooxazolidin-3-yl)-3-methyl-5-oxopentanal (12.3 g, crude) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{16}H_{19}NO_4$ 289.1; found 290.1.

Step 4. A solution of (*S*)-5-((*S*)-4-benzyl-2-oxooxazolidin-3-yl)-3-methyl-5-oxopentanal (12.3 g, 42.51 mmol) in THF (200 mL) was purged and maintained with nitrogen atmosphere, then added borane-tetrahydrofuran complex (55.27 mL, 55.27 mmol) at 0 °C. The reaction was stirred at 0 °C for 30 minutes, then quenched with methanol (40 mL). The resulting mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford (*S*)-4-benzyl-3-((*S*)-5-hydroxy-3-methylpentanoyl)oxazolidin-2-one (9.6 g, 77.51 % yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{16}H_{21}NO_4$ 291.1; found 292.1.

Step 5. A solution of (*S*)-4-benzyl-3-((*S*)-5-hydroxy-3-methylpentanoyl)oxazolidin-2-one (9.6 g, 32.95 mmol) and CBr_4 (16.39 g, 49.43 mmol) in DCM (120 mL) at 0 °C, was added triphenylphosphine (12.96 g, 49.41 mmol). The reaction was stirred at 0 °C for 1 h, then quenched with ice water (100 mL) and extracted with DCM (100 mL x 3). The combined organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford (*S*)-4-benzyl-3-((*R*)-5-bromo-3-methylpentanoyl)oxazolidin-2-one (10 g, 85.67 % yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{16}H_{20}BrNO_4$ 353.1; found 354.1

Step 6. A mixture of n-BuLi (2.26 mL, 5.65 mmol) and diisopropylamine (571.3 mg, 5.65 mmol) in THF (10 mL) under nitrogen at -78 °C, was added a cooled (-78 °C) solution of (*S*)-4-benzyl-3-((R)-5-bromo-3-methylpentanoyl)oxazolidin-2-one (2 g, 5.65 mmol) in THF (9 mL). The reaction mixture was stirred at -78 °C for 30 minutes, then was added a solution of (*E*)-N-[(*tert*-butoxycarbonyl)imino](*tert*-butoxy)formamide (1.3 g, 5.65 mmol) in THF (10 mL), stirred for another 30 minutes at -78 °C. The resulting mixture was added DMPU (16 mL, 132.82 mmol) and warmed up to 0 °C and stirred for 90 minutes, followed by addition of a solution of $LiOH{\cdot}H_2O$ (1.18 g, 28.12 mmol) in water (20 mL). Then THF was removed under reduced pressure. The residue was washed with DCM (80 mL x 3). The aqueous phase was acidified to pH 5~6 with HCl (aq.), extracted with mixture of DCM/methanol (80 mL x 3, 10:1). The combined organic layers were dried over anhydrous sodium sulfate, filtered, concentrated under reduced pressure. The residue was purified by reverse phase chromatography to afford (3*S*,4*S*)-1,2-bis(*tert*-butoxycarbonyl)-4-methylhexahydropyridazine-3-carboxylic acid (296 mg, 15.22 % yield) as a solid. LCMS (ESI): m/z [M-H] calc'd for $C_{16}H_{28}N_2O_6$ 344.2; found 343.1.

Step 7. A mixture of (3*S*,4*S*)-1,2-bis(*tert*-butoxycarbonyl)-4-methylhexahydropyridazine-3-carboxylic acid (289 mg, 0.84 mmol) and (*S*)-3-(1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-5-

(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indol-3-yl)-2,2-dimethylpropan-1-ol (413.24 mg, 0.84 mmol) in DMF (10 mL) at 0 °C, was added DMAP (51.26 mg, 0.42 mmol) and DCC (692.53 mg, 3.36 mmol). The reaction solution was stirred at room temperature for 1 h, then quenched with water/ice (10 mL), extracted with EtOAc (15 mL x 3). The combined organic layers were washed with brine (50 mL x 3), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford 1,2-di-*tert*-butyl 3-(3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl) (3*S*,4*S*)-4-methyltetrahydropyridazine-1,2,3-tricarboxylate (538 mg, 78.3 % yield) as a solid. LCMS (ESI): m/z [M-H] calc'd for $C_{45}H_{67}BN_4O_9$ 818.5; found 819.4.

Step 8. A solution of 1,2-di-*tert*-butyl 3-(3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl) (3*S*,4*S*)-4-methyltetrahydropyridazine-1,2,3-tricarboxylate (508 mg, 0.62 mmol) in DCM (25 mL), was added TFA (25 mL) at 0 °C. The reaction solution was stirred at room temperature for 1 h. The resulting mixture was concentrated to afford 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl (3*S*,4*S*)-4-methylhexahydropyridazine-3-carboxylate (508 mg, crude) as an oil. LCMS (ESI): m/z [M-H] calc'd for $C_{35}H_{51}BN_4O_5$ 618.4; found 619.3.

Step 9. A solution of 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indol-3-yl)-2,2-dimethylpropyl (3*S*,4*S*)-4-methylhexahydropyridazine-3-carboxylate (508 mg, 0.82 mmol) and (*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (288.41 mg, 0.82 mmol) in DMF (50 mL) at 0 °C, was added DIEA (1061.31 mg, 8.21 mmol), HATU (468.35 mg, 1.23 mmol). The reaction solution was stirred at room temperature for 1 h, then quenched with ice water (30 mL) and extracted with EtOAc (30 mL x 3). The combined organic phase was washed with brine (50 mL x 3), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl (3*S*,4*S*)-1-((S)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)-4-methylhexahydropyridazine-3-carboxylate (431 mg, 55.14 % yield) as a solid. LCMS (ESI): m/z [M-H] calc'd for $C_{46}H_{64}BBrN_6O_8S$ 950.4; found 951.3.

Step 10. A mixture of Pd(DTBpf)Cl_2 (27.39 mg, 0.042 mmol) and K_3PO_4 (89.2 mg, 0.42 mmol) in dioxane (5 mL) and water (1 mL) was purged nitrogen, stirred at 60 °C for 5 minutes under nitrogen atmosphere, then added a solution of 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl (3*S*,4*S*)-1-((*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)-4-methylhexahydropyridazine-3-carboxylate (200 mg, 0.21 mmol) in dioxane (5 mL) and water (1 mL) at 60 °C. The reaction mixture was stirred at 60 °C for 1 h, then quenched with ice water (5 mL), extracted with EtOAc (15 mL x 3). The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford *tert*-butyl ((6^3*S*,6^4*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-6^4,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-

pyridazinacycloundecaphane-4-yl)carbamate (70 mg, 44.72 % yield) as a solid. LCMS (ESI): m/z [M-H] calc'd for $C_{40}H_{52}N_6O_6S$ 744.4; found 745.4.

Step 11. A solution of *tert*-butyl ((6^{3}S,6^{4}S,4S,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-6^4,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (70 mg, 0.094 mmol) in dioxane (5 mL), was added HCl in dioxane (5 mL, 4M). The reaction was stirred at room temperature for 1 h, then concentrated under reduced pressure to afford (6^3*S*,6^4*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-6^4,10,10-trimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (124 mg, crude) as an oil. LCMS (ESI): m/z [M-H] calc'd for $C_{36}H_{45}N_5O_4S$ 644.3; found 645.3.

Step 12. A mixture of (6^3*S*,6^4*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-6^4,10,10-trimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (112 mg, 0.17 mmol) and N-(3-methoxyazetidine-1-carbonyl)-N-methyl-L-valine (50.92 mg, 0.21 mmol) in DMF (3 mL) at 0 °C, was added DIEA (1.795 g, 13.9 mmol), 2-chloro-1,3-dimethylimidazolidinium hexafluorophosphate (72.57 mg, 0.26 mmol). The reaction was stirred at room temperature for 1 h and then filtered. The filtrate was purified by reverse phase chromatography to afford N-((2*S*)-1-(((6^3*S*,6^4*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-6^4,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-3-methoxy-N-methylazetidine-1-carboxamide (25.6 mg, 16.92 % yield) as a solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.76 (dd, *J* = 4.7, 1.8 Hz, 1H), 8.60 (s, 1H), 8.30 – 8.20 (m, 1H), 7.86 – 7.70 (m, 3H), 7.61 – 7.50 (m, 2H), 5.57 – 5.43 (m, 1H), 5.07 (d, *J* = 12.1 Hz, 1H), 4.39 –4.21 (m, 3H), 4.20 – 4.01 (m, 5H), 3.96 (d, *J* = 11.1 Hz, 1H), 3.82 (dd, *J* = 8.9, 3.6 Hz, 1H), 3.77 – 3.71 (m, 1H), 3.63 – 3.55 (m, 2H), 3.35 – 3.27 (m, 2H), 3.24 (s, 3H), 3.23 – 3.14 (m, 4H), 2.93 – 2.79 (m, 2H), 2.70 (s, 3H), 2.15 – 2.01 (m, 1H), 1.83 – 1.61 (m, 2H), 1.38 (d, *J* = 6.1 Hz, 4H), 0.98 (d, *J* = 6.4 Hz, 3H), 0.94 – 0.85 (m, 6H), 0.85 – 0.72 (m, 6H), 0.43 (s, 3H). LCMS (ESI): m/z [M-H] calc'd for $C_{46}H_{62}N_8O_7S$ 870.4; found 871.4.

The following table of compounds (Table 3) were prepared using the aforementioned methods or variations thereof, as is known to those of skill in the art.

Table 3: Exemplary Compounds Prepared by Methods of the Present Invention

Ex#	LCMS (ESI): m/z [M+H] Found	Ex#	LCMS (ESI): m/z [M+H] Found
A1	907.5	**A38**	835.0
A2	963.5	**A39**	839.7
A3	908.3	**A40**	793.7
A4	850.4	**A41**	878.4
A5	892.6	**A42**	907.4
A6	963.5	**A43**	797.6
A7	895.8	**A44**	807.7

A8	949.6	**A45**	920.5
A9	920.5	**A46**	865.5
A10	836.6	**A47**	894.4
A11	894.7	**A48**	895.8
A12	893.5	**A49**	837.4
A13	842.5	**A50**	853.5
A14	949.7	**A51**	892.5
A15	921.5	**A52**	806.3
A16	878.7	**A53**	798.0
A17	864.5	**A54**	786.5
A18	837.6	**A55**	781.6
A19	821.6	**A56**	821.0
A20	894.5	**A57**	817.5
A21	795.4	**A58**	767.4
A22	878.5	**A59**	823.5
A23	880.3	**A60**	876.6
A24	914.6	**A61**	779.6
A25	795.4	**A62**	863.7
A26	837.5	**A63**	848.6
A27	850.5	**A64**	833.7
A28	823.6	**A65**	866.7
A29	906.5	**A66**	838.4
A30	852.6	**A67**	810.5
A31	894.8	**A68**	838.7
A32	836.5	**A69**	851.7
A33		**A70**	823.5
A34	906.0	**A71**	786.5
A35	970.7	**A72**	842.5
A36	964.5	**A73**	864.5
A37	971.2	**A74**	852.5
A75	797.6	**A170**	870.5
A76	796.4	**A171**	879.5
A77	822	**A172**	811.5
A78	848.5	**A173**	871.2
A79	904.8	**A174**	837.4
A80	946.5	**A175**	874.5
A81	838.5	**A176**	807.5
A82	853.5	**A177**	773
A83	850.45	**A178**	787
A84	864.5	**A179**	787
A85	864.5	**A180**	784
A86	822.6	**A181**	784

A87	822.3	**A182**	722.9
A88	836.3	**A183**	722
A89	839.6	**A184**	762
A90	837.6	**A185**	872.18
A91	837.5	**A186**	745.7
A92	811.5	**A187**	829.9
A93	811.5	**A188**	829.9
A94	837.5	**A189**	759.6
A95	935.6	**A190**	775.9
A96	919.6	**A191**	808.7
A97	926.5	**A192**	770.8
A98	905.5	**A193**	802.7
A99	912.3	**A194**	789.8
A100	864.5	**A195**	796.7
A101	852.5	**A196**	744.7
A102	795.4	**A197**	798.9
A103	772.3	**A198**	840.9
A104	781.4	**A199**	753.9
A105	891.5	**A200**	758.9
A106	898.5	**A201**	984.4
A107	848.5	**A202**	934.4
A108	855.5	**A203**	941.5
A109	878.8	**A204**	950.4
A110	885.6	**A205**	857.3
A111	894.6	**A206**	890.4
A112	947.7	**A207**	791.7
A113	954.7	**A208**	793.6
A114	963.6	**A209**	867.5
A115	892.4	**A210**	858.5
A116	889.5	**A211**	922.6
A117	936.5	**A212**	798.4
A118	841.4	**A213**	867.7
A119	834.8	**A214**	797.5
A120	921.5	**A215**	946.5
A121	852.8	**A216**	904.8
A122	865.8	**A217**	862.6
A123	907.8	**A218**	835.5
A124	851.8	**A219**	849.6
A125	838	**A220**	931.4
A126	862.5	**A221**	911.3
A127	864.8	**A222**	853.2
A128	864.8	**A223**	835.5
A129	850.8	**A224**	821.6

A130	906	**A225**	748.8
A131	865.8	**A226**	913.8
A132	838.9	**A227**	894.0
A133	877.9	**A228**	877.9
A134	879.8	**A229**	897.8
A135	961.6	**A230**	879.9
A136	815.5	**A231**	893.9
A137	801.5	**A232**	852.9
A138	802.4	**A233**	950.6
A139	850.5	**A234**	917.3
A140	862.6	**A235**	897.3
A141	811.4	**A236**	780.8
A142	793.3	**A237**	919.4
A143	856.2	**A238**	842.4
A144	793.5	**A239**	826.4
A145	836.2	**A240**	851.8
A146	835.4	**A241**	851.7
A147	835.3	**A242**	878.9
A148	876.6	**A243**	864.8
A149	862.6	**A244**	883.5
A150	865.5	**A245**	828.4
A151	890.3	**A246**	821.4
A152	786.2	**A247**	912.8
A153	819.5	**A248**	893.6
A154	857.2	**A249**	888.8
A155	862.6	**A250**	899.8
A156	847.5	**A251**	864.7
A157	849.5	**A252**	905.8
A158	849.5	**A253**	750.7
A159	846.6	**A254**	787.8
A160	839.6	**A255**	851.6
A161	839.5	**A256**	795.4
A162	839.5	**A257**	852.6
A163	862.6	**A258**	766.8
A164	862.7	**A259**	864.5
A165	839.5	**A260**	853.4
A166	857.5	**A261**	773.8
A167	857.5	**A262**	878.7
A168	836.5	**A263**	780.8
A169	880.3		
A264	758.4	**A293**	898.7
A265	757.3	**A294**	912.7

A266	772.4	**A295**	882.3
A267	728.4	**A296**	912.3
A268	882.4	**A297**	921.3
A270	744.3	**A298**	883.2
A271	871.2	**A299**	871.3
A272	898.6	**A300**	898.5
A273	910.5	**A301**	869.3
A274	882.3	**A302**	893.5
A275	885.5	**A303**	924.4
A276	885.5	**A304**	841.2
A277	913.6	**A305**	841.5
A278	885.6	**A306**	914.5
A279	885.4	**A307**	896.5
A280	910.6	**A308**	896.4
A281	884.3	**A309**	871.3
A282	882.2	**A310**	871.4
A283	898.5	**A311**	896.5
A284	882.5	**A312**	883.5
A285	896.2	**A313**	896.6
A286	713.1	**A314**	882.6
A287	835.3	**A315**	729.3
A288	925.4	**A316**	906.5
A289	885.0	**A317**	827.4
A290	941.3	**A318**	898.5
A291	898.3	**A319**	898.3
A292	898.7	**A320**	733.2
A321	771.4	**A354**	844.6
A322	893.2	**A355**	850.5
A323	837.5	**A356**	855.5
A324	807.3	**A357**	905.4
A325	922.5	**A358**	843.5
A326	882.5	**A359**	715.2
A327	882.5	**A360**	715.2
A328	924.4	**A361**	731.3
A329	896.3	**A362**	717.3
A330	911.1	**A363**	855.5
A331	729.4	**A364**	866.5
A332	857.4	**A365**	908.6
A333	857.5	**A366**	736.1
A334	857.2	**A367**	699.1
A335	871.5	**A368**	714.1
A336	829.5	**A369**	713.3
A337	856.5	**A370**	947.6

A338	912.2	**A371**	961.4
A339	857.5	**A372**	857.5
A340	771.4	**A373**	857.5
A341	870.5	**A374**	857.5
A342	975.3	**A375**	856.5
A343	842.5	**A376**	857.5
A344	871.7	**A377**	865.6
A345	808.5	**A378**	947.3
A346	837.5	**A379**	975.6
A347	837.5	**A380**	961.3
A348	963.5	**A381**	850.6
A349	855.5	**A382**	852.2
A350	843.5	**A383**	905.1
A351	843.5	**A384**	849.5
A352	855.5	**A385**	961.6
A353	841.5	**A386**	949.3
A387	871.5	**A421**	892.9
A388	819.3	**A422**	951.3
A389	813.2	**A423**	1051.6
A391	851.7	**A424**	939.4
A392	851.7	**A425**	927.4
A393	891.8	**A426**	953.40
A394	879.8	**A427**	978.3
A395	879.8	**A428**	918.2
A396	921.8	**A429**	911.3
A397	909.8	**A430**	804.5
A398	736.4	**A431**	891.5
A399	827.5	**A432**	879.5
A400	841.5	**A433**	940.7
A401	857.5	**A434**	896.5
A402	871.4	**A435**	896.3
A403	871.5	**A436**	926.2
A404	906.9	**A437**	946.6
A405	865.9	**A438**	896.1
A406	863.8	**A439**	988.1
A407	891.9	**A440**	988.1
A408	919.9	**A441**	926.2
A409	908	**A442**	910.9
A410	878.0	**A443**	967.1
A411	878.0	**A444**	912.1
A412	878.0	**A445**	912.1
A413	922.0	**A446**	882.2
A414	894.9	**A447**	867.1

A415	928.0	**A448**	953.2
A416	901.9	**A449**	953.5
A417	890.9	**A450**	1017.2
A418	867.9	**A451**	912.2
A419	879.9	**A452**	895.2
A420	866.9	**A453**	924.2
A454	844.2	**A482**	960.1
A455	901.9	**A483**	1008.1
A456	867.2	**A484**	912.2
A457	940.6	**A485**	938.6
A458	898.5	**A486**	952.3
A459	954.8	**A487**	885.3
A460	896.2	**A488**	884.3
A461	924.2	**A489**	886.2
A462	896.2	**A490**	1017.9
A463	856.2	**A491**	912.6
A464	931.2	**A492**	912.6
A465	981.7	**A493**	912.6
A466	955.3	**A494**	912.6
A467	940.6	**A495**	924.2
A468	910.6	**A496**	917.0
A469	884.5	**A497**	882.1
A470	896.2	**A498**	924.6
A471	896.1	**A499**	912.1
A472	912.1	**A500**	921.2
A473	898.6	**A501**	984.1
A474	899.2	**A502**	884.6
A475	899.1	**A503**	896.1
A476	996.3	**A504**	898.1
A477	968.6	**A505**	954.7
A478	885.5	**A506**	902.1
A479	910.9	**A507**	1011.2
A480	910.1	**A508**	884.6
A481	896.9	**A509**	943.2
A510	883.5	**A538**	898.2
A511	952.3	**A539**	896.5
A512	940.6	**A540**	870.0
A513	910.3	**A541**	882.1
A514	901.2	**A542**	884.2
A515	901.2	**A543**	940.9

A516	896.3	**A544**	874.2
A517	896.2	**A545**	897.9
A518	898.6	**A546**	928.2
A519	898.6	**A547**	912.5
A520	911.2	**A548**	912.5
A521	897.2	**A549**	920.5
A522	883.2	**A550**	934.5
A523	853.2	**A551**	934.4
A524	946.2	**A552**	920.5
A525	896.2	**A553**	887.1
A526	940.3	**A554**	837.4
A527	871.2	**A555**	955.2
A528	883.2	**A556**	932.2
A529	951.8	**A557**	906.1
A530	945.1	**A558**	856.2
A531	898.3	**A559**	888.2
A532	954.5	**A560**	869.1
A533	899.5	**A561**	883.2
A534	899.5	**A562**	1009.3
A535	884.2	**A563**	884.6
A536	939.5	**A564**	884.6
A537	939.5	**A565**	924.2
A566	884.9	**A592**	961.2
A567	874.2	**A593**	1043.0
A568	896.2	**A594**	1025.8
A569	898.2	**A595**	967.5
A570	870.2	**A596**	967.5
A571	899.0	**A597**	900.1
A572	914.2	**A598**	845.4
A573	912.2	**A599**	917.2
A574	913.9	**A600**	933.1
A575	914.2	**A601**	898.0
A576	885.0	**A602**	897.0
A577	1024.7	**A603**	884.9
A578	869.5	**A604**	933.1
A579	871.4	**A605**	926.1
A580	886.9	**A606**	940.5
A581	872.1	**A607**	924.3
A582	933.2	**A608**	896.3

A583	1016.2	**A609**	898.4
A584	927.2	**A610**	898.5
A585	918.2	**A611**	870.5
A586	911.3	**A612**	858.4
A587	899.4	**A613**	858.3
A588	898.6	**A614**	899.4
A589	910.2	**A615**	926.4
A590	914.6	**A616**	926.4
A591	915.4		

Blank = not determined

Biological Assays

Potency assay: pERK

The purpose of this assay is to measure the ability of test compounds to inhibit K-Ras in cells. Activated K-Ras induces increased phosphorylation of ERK at Threonine 202 and Tyrosine 204 (pERK). This procedure measures a decrease in cellular pERK in response to test compounds. The procedure described below in NCI-H358 cells is applicable to K-Ras G12C.

Note: this protocol may be executed substituting other cell lines to characterize inhibitors of other RAS variants, including, for example, AsPC-1 (K-Ras G12D), Capan-1 (K-Ras G12V), or NCI-H1355 (K-Ras G13C).

NCI-H358 cells were grown and maintained using media and procedures recommended by the ATCC. On the day prior to compound addition, cells were plated in 384-well cell culture plates (40 µl/well) and grown overnight in a 37°C, 5% CO2 incubator. Test compounds were prepared in 10, 3-fold dilutions in DMSO, with a high concentration of 10 mM. On day of assay, 40 nl of test compound was added to each well of cell culture plate using an Echo550 liquid handler (LabCyte®). Concentrations of test compound were tested in duplicate. After compound addition, cells were incubated 4 hours at 37°C, 5% CO2. Following incubation, culture medium was removed and cells were washed once with phosphate buffered saline.

In some experiments, cellular pERK level was determined using the AlphaLISA SureFire Ultra p-ERK1/2 Assay Kit (PerkinElmer). Cells were lysed in 25 µl lysis buffer, with shaking at 600 RPM at room temperature. Lysate (10 µl) was transferred to a 384-well Opti-plate (PerkinElmer) and 5 µl acceptor mix was added. After a 2-hour incubation in the dark, 5 µl donor mix was added, plate was sealed and incubated 2 hours at room temperature. Signal was read on an Envision plate reader (PerkinElmer) using standard AlphaLISA settings. Analysis of raw data was carried out in Excel (Microsoft) and Prism (GraphPad). Signal was plotted vs. the decadal logarithm of compound concentration, and IC_{50} was determined by fitting a 4-parameter sigmoidal concentration response model.

In other experiments, cellular pERK was determined by In-Cell Western. Following compound treatment, cells were washed twice with 200 µl tris buffered saline (TBS) and fixed for 15 minutes with 150 µl 4% paraformaldehyde in TBS. Fixed cells were washed 4 times for 5 minutes

with TBS containing 0.1% Triton X-100 (TBST) and then blocked with 100 µl Odyssey blocking buffer (LI-COR) for 60 minutes at room temperature. Primary antibody (pERK, CST-4370, Cell Signaling Technology) was diluted 1:200 in blocking buffer, and 50 µl was added to each well and incubated overnight at 4°C. Cells were washed 4 times for 5 minutes with TBST. Secondary antibody (IR-800CW rabbit, LI-COR, diluted 1:800) and DNA stain DRAQ5 (LI-COR, diluted 1:2000) were added and incubated 1-2 hours at room temperature. Cells were washed 4 times for 5 minutes with TBST. Plates were scanned on a Li-COR Odyssey CLx Imager. Analysis of raw data was carried out in Excel (Microsoft) and Prism (GraphPad). Signal was plotted vs. the decadal logarithm of compound concentration, and IC_{50} was determined by fitting a 4-parameter sigmoidal concentration response model.

Determination of Cell Viability in RAS Mutant Cancer Cell Lines

Protocol: CellTiter-Glo® Cell Viability Assay

Note – The following protocol describes a procedure for monitoring cell viability of K-Ras mutant cancer cell lines in response to a compound of the invention. Other RAS isoforms may be employed, though the number of cells to be seeded will vary based on cell line used.

The purpose of this cellular assay was to determine the effects of test compounds on the proliferation of three human cancer cell lines (NCI-H358 (K-Ras G12C), AsPC-1 (K-Ras G12D), Capan-1 (K-Ras G12V)) over a 5-day treatment period by quantifying the amount of ATP present at endpoint using the CellTiter-Glo® 2.0 Reagent (Promega).

Cells were seeded at 250 cells/well in 40 µl of growth medium in 384-well assay plates and incubated overnight in a humidified atmosphere of 5% CO_2 at 37°C. On the day of the assay, 10 mM stock solutions of test compounds were first diluted into 3 mM solutions with 100% DMSO. Well-mixed compound solutions (15 µl) were transferred to the next wells containing 30 µl of 100% DMSO, and repeated until a 9-concentration 3-fold serial dilution was made (starting assay concentration of 10 µM). Test compounds (132.5 nl) were directly dispensed into the assay plates containing cells. The plates were shaken for 15 seconds at 300 rpm, centrifuged, and incubated in a humidified atmosphere of 5% CO_2 at 37°C for 5 days. On day 5, assay plates and their contents were equilibrated to room temperature for approximately 30 minutes. CellTiter-Glo® 2.0 Reagent (25 µl) was added, and plate contents were mixed for 2 minutes on an orbital shaker before incubation at room temperature for 10 minutes. Luminescence was measured using the PerkinElmer Enspire. Data were normalized by the following: (Sample signal/Avg. DMSO)*100. The data were fit using a four-parameter logistic fit.

Disruption of B-Raf Ras-binding Domain ($BRAF^{RBD}$) Interaction with K-Ras by Compounds of the Invention (also called a FRET assay or an MOA assay)

Note – The following protocol describes a procedure for monitoring disruption of K-Ras G12C (GMP-PNP) binding to $BRAF^{RBD}$ by a compound of the invention. This protocol may also be executed substituting other Ras proteins or nucleotides.

The purpose of this biochemical assay was to measure the ability of test compounds to facilitate ternary complex formation between a nucleotide-loaded K-Ras isoform and Cyclophilin A;

the resulting ternary complex disrupts binding to a $BRAF^{RBD}$ construct, inhibiting K-Ras signaling through a RAF effector. Data is reported as IC50 values.

In assay buffer containing 25 mM HEPES pH 7.3, 0.002% Tween20, 0.1% BSA, 100 mM NaCl and 5 mM $MgCl_2$, tagless Cyclophilin A, His6-K-Ras-GMPPNP, and GST-$BRAF^{RBD}$ were combined in a 384-well assay plate at final concentrations of 25 µM, 12.5 nM and 50 nM, respectively. Compound was present in plate wells as a 10-point 3-fold dilution series starting at a final concentration of 30 µM. After incubation at 25°C for 3 hours, a mixture of Anti-His Eu-W1024 and anti-GST allophycocyanin was then added to assay sample wells at final concentrations of 10 nM and 50 nM, respectively, and the reaction incubated for an additional 1.5 hours. TR-FRET signal was read on a microplate reader (Ex 320 nm, Em 665/615 nm). Compounds that facilitate disruption of a K-Ras:RAF complex were identified as those eliciting a decrease in the TR-FRET ratio relative to DMSO control wells.

Table 4: Biological Assay Data for Representative Compounds of the Present Invention

Ex#	FRET G12C IC50, uM	FRET G12V IC50, uM	FRET G12D IC50, uM	FRET G13C IC50, uM	FRET G13D IC50, uM	H358 pERK EC50, uM	Capan-1 pERK EC50, uM	ASPC-1 pERK EC50, uM	H358 Cell Viability IC50, uM
A74	0.45	3.67	0.228	0.091	0.212	0.334	0.413	0.468	
A73	0.082	0.905	0.061	0.013	0.033	0.036	0.197	0.079	
A3	0.029	0.043	0.545	0.099	0.16	0.018	0.008	0.144	0.057
A25	0.128	0.197	1.29	0.097	0.235	0.064	0.052	0.706	0.226
A12	0.068	0.329	0.154	0.148	0.219				4.29

Blank = not determined

Additional H358 Cell Viability assay data

*Key:

+++++: IC50 ≥ 10 uM

++++: 10 uM > IC50 ≥ 1 uM

+++: 1 uM > IC50 ≥ 0.1 uM

++: 0.1 uM > IC50 ≥ 0.01 uM

+: IC50 < 0.01 uM

Table 5. H358 Cell Viability assay data (K-Ras G12C, IC50, uM):

IC50*	Examples
+	A136,A159,A205,A277,A278,A289,A291,A296,A298,A302,A303,A304,A306,A309,A310,A325,A335,A338,A356,A358,A365,A372,A373,A374,A382,A399,A439,A443,A450,A457,A465,A466,A476,A477,A478,A483,A484,A487,A490,A500,A501,A505,A514,A515,A526,A529,A536,A543,A546,A551,A555,A561,A562,A573,A577,A583,A590,A593,A594,A606,A607
++	A114,A117,A13,A131,A138,A141,A147,A156,A160,A162,A164,A165,A170,A202,A204,A211,A217,A218,A223,A224,A233,A240,A242,A247,A248,A249,A250,A252,A273,A279,A280,A285,A286,A288,A290,A293,A294,A295,A297,A299,A3,A301,A305,A307,A3

	11,A312,A313,A316,A318,A319,A326,A329,A330,A333,A334,A336,A337,A342,A348,A349,A350,A351,A352,A353,A357,A363,A364,A375,A376,A377,A379,A383,A387,A389,A391,A392,A400,A401,A402,A403,A406,A415,A422,A433,A436,A440,A441,A444,A445,A451,A452,A454,A458,A459,A467,A481,A485,A486,A488,A489,A491,A492,A493,A494,A495,A498,A499,A502,A503,A506,A507,A509,A510,A511,A512,A513,A518,A520,A521,A522,A523,A525,A527,A528,A531,A532,A533,A534,A537,A538,A540,A541,A542,A547,A548,A549,A550,A552,A553,A557,A559,A560,A563,A564,A565,A566,A567,A568,A569,A570,A571,A574,A576,A578,A582,A584,A585,A587,A588,A589,A591,A595,A596,A597,A601,A603,A605,A614,A615
+++	A100,A11,A116,A121,A123,A124,A126,A130,A132,A137,A139,A143,A146,A152,A155,A157,A161,A166,A167,A168,A169,A171,A173,A174,A18,A184,A19,A201,A203,A209,A21,A210,A219,A221,A226,A228,A231,A232,A238,A239,A241,A243,A245,A25,A252,A26,A260,A264,A266,A267,A268,A270,A274,A276,A28,A281,A282,A283,A284,A287,A29,A292,A30,A308,A314,A315,A317,A321,A322,A323,A332,A339,A343,A346,A35,A354,A355,A360,A361,A362,A367,A368,A369,A378,A381,A384,A385,A386,A39,A393,A395,A396,A397,A407,A408,A409,A410,A411,A412,A413,A414,A416,A417,A418,A420,A421,A423,A437,A438,A442,A447,A449,A455,A461,A462,A471,A480,A482,A496,A497,A5,A504,A508,A524,A535,A539,A54,A544,A545,A554,A556,A572,A575,A579,A580,A581,A586,A592,A598,A600,A602,A604,A608,A610,A611,A612,A613,A616,A64,A7,A78,A8,A90,A91,A94,A95
++++	A1,A10,A101,A102,A104,A111,A117,A12,A120,A125,A127,A128,A129,A134,A148,A15,A16,A163,A17,A2,A20,A216,A22,A227,A23,A24,A244,A254,A254,A256,A258,A259,A261,A262,A27,A3,A320,A359,A36,A36,A37,A38,A4,A40,A405,A41,A42,A43,A44,A45,A453,A46,A464,A50,A51,A517,A519,A52,A53,A54,A55,A57,A58,A59,A599,A6,A60,A609,A61,A65,A66,A67,A70,A82,A83,A85,A9,A92,A97
+++++	A12,A133,A14,A31,A32,A4,A47,A48,A56,A62,A63,A68,A69,A84,A99

*Key:

+++++: IC50 ≥ 10 uM

++++: 10 uM > IC50 ≥ 1 uM

+++: 1 uM > IC50 ≥ 0.1 uM

++: 0.1 uM > IC50 ≥ 0.01 uM

+: IC50 < 0.01 uM

Table 6. Capan-1 Cell Viability assay data (K-Ras G12V, IC50, uM):

IC50*	Examples
+	A277,A450,A465,A466,A476,A477,A484,A500,A505,A526,A529,A555,A562,A577,A583,A593,A594
++	A114,A117,A132,A136,A138,A141,A156,A159,A162,A165,A170,A202,A204,A205,A210,A211,A218,A224,A233,A240,A247,A250,A278,A279,A280,A285,A288,A289,A290,A

	291,A293,A295,A296,A298,A3,A302,A303,A304,A306,A309,A310,A312,A313,A316,A318,A319,A325,A329,A330,A334,A335,A336,A338,A353,A356,A357,A358,A363,A364,A365,A372,A373,A374,A376,A377,A382,A383,A387,A389,A399,A400,A401,A402,A403,A415,A433,A436,A439,A440,A443,A444,A445,A451,A452,A454,A457,A458,A467,A472,A474,A475,A478,A481,A483,A485,A486,A487,A490,A491,A494,A499,A503,A506,A509,A510,A511,A512,A513,A514,A515,A518,A520,A521,A523,A525,A527,A528,A531,A532,A533,A534,A536,A537,A538,A540,A543,A546,A547,A548,A549,A550,A551,A561,A563,A565,A566,A569,A570,A571,A573,A574,A576,A587,A590,A591,A601,A603,A606,A607,A608,A614,A615
+++	A102,A11,A116,A121,A123,A126,A13,A131,A137,A139,A143,A146,A147,A152,A157,A160,A161,A164,A166,A167,A168,A169,A171,A173,A174,A18,A19,A201,A203,A209,A21,A217,A219,A221,A223,A226,A228,A232,A238,A239,A241,A242,A244,A245,A248,A249,A25,A252,A252,A254,A26,A264,A266,A267,A268,A273,A274,A275,A276,A281,A282,A283,A284,A286,A287,A292,A294,A297,A299,A30,A301,A305,A307,A308,A311,A314,A315,A320,A321,A322,A323,A326,A332,A333,A337,A342,A343,A346,A347,A348,A349,A350,A351,A352,A354,A355,A360,A361,A362,A367,A368,A369,A375,A379,A384,A385,A386,A39,A395,A396,A397,A406,A407,A409,A410,A411,A412,A413,A416,A419,A420,A422,A423,A437,A441,A447,A449,A455,A459,A461,A462,A468,A469,A470,A471,A473,A480,A482,A488,A489,A492,A493,A495,A496,A497,A498,A5,A501,A502,A504,A507,A508,A516,A517,A522,A524,A530,A535,A539,A54,A541,A542,A544,A545,A552,A553,A556,A557,A558,A559,A560,A564,A567,A568,A575,A578,A579,A580,A582,A584,A585,A586,A588,A589,A595,A596,A597,A598,A600,A602,A605,A609,A610,A611,A612,A613,A616,A90,A91,A94,A95
++++	A1,A100,A101,A106,A107,A111,A112,A113,A115,A124,A125,A129,A130,A135,A144,A148,A149,A155,A158,A16,A17,A175,A176,A179,A180,A182,A183,A184,A185,A200,A216,A220,A225,A225,A229,A230,A231,A235,A236,A237,A24,A246,A253,A254,A259,A260,A261,A262,A265,A270,A272,A28,A29,A300,A317,A324,A327,A331,A339,A345,A359,A366,A370,A371,A378,A380,A381,A388,A391,A392,A393,A394,A398,A40,A405,A408,A414,A417,A418,A421,A43,A434,A435,A438,A442,A453,A456,A460,A463,A464,A479,A519,A54,A554,A572,A581,A592,A599,A604,A61,A7,A76,A78,A8,A80,A82,A83,A85,A89,A93,A96,A97
+++++	A104,A104,A105,A108,A109,A110,A116,A117,A118,A119,A120,A122,A127,A128,A133,A134,A140,A142,A145,A150,A151,A153,A154,A163,A172,A177,A178,A178,A179,A181,A186,A187,A188,A189,A190,A191,A192,A193,A194,A195,A196,A197,A198,A199,A206,A207,A208,A212,A213,A214,A215,A222,A227,A227,A234,A236,A243,A251,A253,A255,A258,A271,A328,A340,A341,A344,A4,A404,A446,A448,A75,A79,A84,A86,A87,A87,A88,A92,A98,A99

Additional Ras-Raf disruption/FRET/MOA assay data (IC50, uM):

*Key:

+++++: IC50 ≥ 10 uM

++++: 10 uM > IC50 ≥ 1 uM

+++: 1 uM > IC50 ≥ 0.1 uM

++: 0.1 uM > IC50 ≥ 0.01 uM

+: IC50 < 0.01 uM

Table 7. KRAS G12D FRET data

IC50*	Examples
+	None
++	A1,A100,A111,A120,A124,A125,A127,A128,A129,A131,A133,A134,A135,A139,A140,A148,A159,A164,A223,A227,A228,A231,A242,A243,A247,A249,A325,A342,A348,A365,A370,A371,A378,A379,A380,A381,A385,A386,A391,A392,A393,A395,A397,A4,A415,A419,A427,A483,A494,A501,A507,A546,A573,A577,A584,A594,A605,A95
+++	A10,A101,A102,A106,A114,A12,A121,A122,A123,A126,A130,A132,A136,A14,A146,A147,A149,A15,A151,A155,A156,A157,A158,A160,A161,A162,A163,A165,A166,A167,A168,A169,A171,A174,A2,A201,A202,A204,A205,A209,A211,A216,A217,A218,A219,A224,A227,A229,A23,A230,A232,A233,A240,A241,A248,A248,A250,A251,A252,A252,A255,A259,A264,A265,A266,A267,A268,A27,A270,A273,A274,A275,A277,A278,A279,A280,A285,A286,A287,A288,A289,A290,A291,A294,A298,A3,A302,A303,A304,A306,A309,A31,A310,A311,A312,A313,A314,A32,A321,A323,A332,A333,A334,A335,A336,A343,A346,A347,A349,A350,A351,A353,A356,A358,A363,A364,A372,A373,A374,A376,A377,A382,A383,A384,A394,A396,A399,A400,A401,A402,A404,A405,A406,A407,A408,A409,A41,A410,A411,A412,A413,A414,A416,A417,A418,A420,A421,A422,A423,A424,A426,A432,A434,A435,A436,A438,A441,A443,A444,A447,A45,A450,A454,A457,A458,A459,A463,A465,A466,A467,A468,A469,A471,A475,A476,A477,A478,A48,A484,A485,A487,A488,A491,A492,A493,A498,A5,A500,A502,A503,A505,A506,A509,A514,A515,A518,A520,A523,A526,A528,A529,A531,A533,A534,A536,A537,A538,A542,A543,A545,A549,A551,A552,A554,A555,A557,A558,A559,A560,A561,A562,A563,A564,A565,A566,A567,A568,A569,A571,A574,A576,A578,A580,A581,A582,A583,A586,A587,A588,A589,A590,A591,A593,A595,A596,A6,A600,A601,A603,A606,A607,A608,A610,A611,A614,A615,A616,A62,A66,A67,A68,A7,A78,A79,A8,A80,A81,A83,A85,A87,A87,A88,A89,A99
++++	A105,A107,A108,A109,A11,A110,A112,A113,A117,A118,A119,A12,A13,A137,A138,A144,A152,A16,A17,A170,A173,A175,A176,A177,A178,A179,A179,A18,A184,A19,A20,A208,A21,A210,A213,A215,A22,A222,A225,A226,A236,A239,A24,A25,A253,A254,A254,A257,A26,A260,A262,A272,A276,A28,A282,A283,A284,A292,A293,A295,A296,A297,A299,A30,A300,A301,A305,A307,A308,A315,A316,A318,A319,A320,A322,A324,A326,A329,A33,A330,A331,A337,A338,A339,A344,A345,A35,A352,A354,A355,A357,A

	359,A36,A36,A360,A361,A362,A366,A367,A368,A369,A375,A38,A387,A389,A39,A4,A40,A403,A425,A428,A43,A431,A433,A437,A439,A44,A440,A442,A445,A448,A449,A451,A452,A455,A456,A46,A460,A461,A462,A464,A47,A470,A472,A473,A474,A479,A480,A481,A486,A489,A49,A490,A495,A496,A497,A499,A50,A504,A508,A51,A510,A511,A512,A513,A516,A517,A519,A521,A522,A524,A525,A527,A530,A532,A535,A539,A540,A541,A544,A547,A548,A550,A553,A556,A570,A572,A575,A579,A585,A592,A597,A598,A599,A602,A604,A609,A612,A64,A65,A69,A70,A76,A82,A84,A9,A90,A91,A92,A93,A94,A96,A97,A98,A613
+++++	A103,A103,A104,A104,A115,A116,A116,A117,A141,A142,A143,A145,A150,A153,A154,A172,A178,A180,A181,A182,A183,A185,A186,A187,A188,A189,A190,A191,A192,A193,A194,A195,A196,A197,A198,A199,A200,A203,A206,A207,A212,A214,A220,A221,A225,A234,A235,A236,A237,A238,A244,A245,A246,A253,A256,A258,A261,A271,A281,A29,A3,A317,A327,A328,A340,A341,A37,A388,A398,A42,A429,A430,A446,A453,A482,A52,A53,A54,A54,A55,A56,A57,A58,A59,A60,A61,A63,A75,A77,A86

Table 8. KRAS G12C FRET data

IC50*	Examples
+	A323,A325,A347,A501,A546,A577,A594
++	A1,A10,A100,A11,A111,A114,A117,A12,A120,A121,A125,A126,A127,A128,A129,A13,A131,A132,A135,A136,A139,A14,A140,A146,A147,A148,A149,A15,A151,A155,A156,A157,A159,A16,A160,A162,A164,A165,A166,A168,A17,A18,A19,A2,A20,A201,A202,A204,A205,A211,A216,A217,A218,A219,A223,A224,A226,A227,A228,A229,A230,A231,A233,A240,A241,A242,A243,A247,A248,A248,A249,A250,A252,A252,A255,A262,A264,A265,A266,A273,A274,A275,A277,A278,A279,A280,A285,A288,A289,A290,A291,A298,A3,A302,A303,A304,A306,A309,A310,A312,A316,A321,A330,A333,A334,A335,A336,A338,A342,A343,A346,A348,A349,A350,A351,A353,A356,A358,A363,A364,A365,A370,A371,A372,A373,A374,A376,A377,A378,A379,A380,A381,A382,A383,A384,A385,A386,A387,A391,A392,A393,A395,A396,A397,A399,A4,A400,A401,A402,A405,A406,A407,A408,A409,A410,A411,A412,A413,A414,A415,A418,A419,A420,A422,A424,A426,A427,A432,A438,A443,A444,A450,A452,A454,A457,A458,A459,A465,A466,A467,A471,A475,A477,A478,A483,A484,A487,A488,A489,A491,A493,A494,A498,A5,A500,A503,A505,A507,A509,A510,A514,A515,A523,A526,A528,A529,A533,A534,A536,A537,A538,A540,A543,A549,A550,A551,A552,A554,A555,A557,A558,A560,A561,A562,A565,A567,A569,A571,A573,A574,A576,A578,A581,A582,A583,A584,A586,A590,A591,A593,A595,A596,A598,A6,A600,A601,A605,A606,A607,A608,A614,A615,A616,A62,A67,A68,A7,A8,A87,A9,A95
+++	A101,A102,A105,A106,A12,A122,A123,A124,A130,A133,A134,A137,A138,A144,A158,A161,A163,A167,A169,A170,A171,A173,A174,A176,A178,A179,A179,A182,A183,A184,A207,A208,A209,A21,A210,A215,A22,A225,A227,A23,A232,A236,A24,A25,A251,A253,A254,A254,A257,A259,A26,A260,A267,A268,A27,A270,A276,A28,A282,A283,A2

	84,A286,A287,A29,A292,A293,A294,A295,A296,A297,A299,A30,A301,A305,A308,A31,A311,A313,A314,A315,A318,A319,A32,A320,A322,A324,A326,A329,A33,A331,A332,A337,A339,A34,A344,A345,A35,A352,A354,A355,A357,A359,A36,A36,A360,A361,A362,A366,A367,A368,A369,A37,A375,A38,A389,A39,A394,A4,A40,A403,A404,A41,A416,A417,A42,A421,A423,A425,A43,A431,A433,A434,A435,A436,A437,A439,A44,A440,A441,A445,A447,A449,A45,A451,A453,A455,A456,A46,A460,A461,A462,A463,A468,A469,A47,A472,A473,A474,A476,A479,A48,A480,A481,A485,A486,A49,A490,A492,A495,A496,A497,A499,A50,A502,A504,A506,A508,A51,A511,A512,A513,A518,A519,A52,A520,A521,A522,A524,A525,A527,A53,A530,A531,A532,A535,A541,A542,A544,A545,A547,A548,A553,A556,A559,A563,A564,A566,A568,A570,A579,A580,A585,A587,A588,A589,A592,A597,A602,A603,A610,A611,A612,A64,A65,A66,A69,A76,A78,A79,A80,A81,A82,A83,A84,A85,A86,A87,A88,A89,A90,A91,A93,A94,A96,A97,A98,A99
++++	A104,A107,A108,A109,A110,A112,A113,A115,A116,A117,A118,A119,A141,A142,A143,A150,A152,A175,A177,A178,A180,A181,A185,A199,A203,A206,A212,A213,A214,A220,A221,A222,A225,A236,A237,A238,A239,A244,A245,A246,A253,A256,A258,A261,A271,A272,A281,A3,A300,A307,A317,A388,A398,A428,A429,A442,A446,A448,A464,A470,A482,A516,A517,A539,A54,A54,A55,A56,A57,A572,A575,A58,A59,A599,A60,A604,A609,A61,A63,A70,A75,A77,A92,A613
+++++	A103,A103,A104,A116,A145,A153,A154,A172,A186,A187,A188,A189,A190,A191,A192,A193,A194,A195,A196,A197,A198,A200,A234,A235,A327,A328,A340,A341,A430

Table 9. KRAS G12S FRET data

IC50*	Examples
+	A501,A577,A594
++	A1,A10,A100,A111,A114,A120,A121,A124,A125,A127,A128,A129,A131,A135,A139,A140,A147,A148,A156,A159,A162,A164,A165,A2,A202,A204,A211,A217,A218,A219,A223,A224,A227,A228,A230,A242,A243,A247,A248,A248,A249,A250,A252,A252,A273,A275,A277,A291,A3,A312,A323,A325,A335,A342,A347,A348,A349,A351,A363,A365,A370,A371,A377,A378,A379,A380,A381,A385,A386,A391,A392,A393,A395,A396,A397,A4,A400,A405,A406,A407,A408,A409,A411,A414,A415,A418,A419,A422,A424,A427,A459,A465,A483,A491,A494,A498,A5,A500,A503,A507,A526,A529,A537,A546,A554,A555,A558,A561,A565,A573,A578,A584,A590,A6,A605,A606,A607,A615,A68,A7,A87,A95
+++	A101,A102,A11,A117,A119,A12,A122,A123,A126,A13,A130,A132,A133,A134,A136,A14,A146,A149,A15,A151,A155,A157,A158,A16,A160,A161,A163,A166,A167,A168,A169,A17,A170,A171,A173,A174,A18,A184,A19,A20,A201,A205,A209,A21,A215,A216,A22,A226,A227,A229,A23,A231,A232,A233,A236,A24,A240,A241,A25,A251,A254,A254,A255,A257,A259,A26,A260,A262,A264,A265,A266,A267,A268,A27,A270,A274,A276,A278,A279,A28,A280,A284,A285,A286,A287,A288,A289,A29,A290,A293,A294,A295,A296,A297,A298,A30,A301,A302,A303,A304,A306,A309,A31,A310,A311,A313,A31

	4,A315,A316,A318,A319,A32,A320,A321,A324,A326,A329,A33,A330,A331,A332,A333,A334,A336,A337,A338,A343,A344,A346,A35,A350,A352,A353,A354,A356,A357,A358,A36,A360,A361,A362,A364,A367,A368,A369,A37,A372,A373,A374,A375,A376,A38,A382,A383,A384,A387,A39,A394,A399,A40,A401,A402,A403,A404,A41,A410,A412,A413,A416,A417,A420,A421,A423,A426,A43,A431,A432,A433,A434,A435,A436,A437,A438,A441,A443,A444,A445,A447,A45,A450,A451,A452,A454,A455,A456,A457,A458,A46,A463,A466,A467,A468,A469,A47,A471,A472,A473,A474,A475,A476,A477,A478,A48,A480,A484,A485,A486,A487,A488,A489,A490,A492,A493,A495,A502,A505,A506,A508,A509,A51,A510,A511,A512,A513,A514,A515,A518,A520,A521,A522,A523,A525,A527,A528,A530,A531,A532,A533,A534,A535,A536,A538,A540,A541,A542,A543,A545,A547,A549,A550,A551,A552,A556,A557,A559,A560,A562,A563,A564,A566,A567,A568,A569,A570,A571,A574,A576,A580,A581,A582,A583,A585,A586,A587,A588,A589,A591,A593,A595,A596,A597,A598,A600,A601,A602,A603,A608,A610,A611,A614,A616,A62,A64,A65,A66,A67,A69,A76,A78,A79,A8,A80,A81,A83,A84,A85,A87,A88,A89,A9,A90,A91,A93,A94
++++	A105,A106,A107,A108,A109,A110,A112,A113,A115,A116,A118,A12,A137,A138,A141,A142,A143,A144,A152,A175,A176,A177,A178,A178,A179,A179,A180,A181,A182,A183,A185,A203,A207,A208,A210,A213,A214,A220,A221,A222,A225,A225,A236,A237,A238,A239,A244,A245,A246,A253,A256,A258,A261,A271,A272,A281,A282,A283,A292,A299,A3,A300,A305,A307,A308,A317,A322,A339,A34,A345,A355,A359,A36,A366,A388,A389,A398,A4,A42,A425,A428,A439,A44,A440,A442,A446,A448,A449,A453,A460,A461,A462,A464,A470,A479,A481,A482,A49,A496,A497,A499,A50,A504,A516,A517,A519,A52,A524,A53,A539,A54,A54,A544,A548,A55,A553,A56,A57,A572,A575,A579,A58,A59,A592,A599,A60,A604,A609,A61,A612,A63,A70,A75,A82,A86,A92,A96,A97,A98,A99,A613
+++++	A103,A103,A104,A104,A116,A117,A145,A150,A153,A154,A172,A186,A187,A188,A189,A190,A191,A192,A193,A194,A195,A196,A197,A198,A199,A200,A206,A212,A234,A235,A253,A327,A328,A340,A341,A429,A430,A77

Table 10. KRAS G13C FRET data

IC50*	Examples
+	A381,A325,A501,A594
++	A1,A10,A100,A101,A102,A111,A114,A121,A123,A124,A125,A126,A127,A128,A129,A130,A131,A132,A133,A134,A135,A139,A140,A146,A147,A148,A149,A151,A155,A156,A159,A160,A162,A164,A165,A166,A168,A169,A171,A184,A201,A202,A204,A21,A211,A215,A216,A217,A218,A219,A223,A224,A226,A227,A227,A228,A229,A23,A230,A231,A233,A240,A241,A242,A243,A247,A248,A248,A249,A25,A250,A251,A252,A252,A255,A266,A27,A275,A277,A3,A31,A323,A324,A342,A346,A347,A348,A349,A351,A364,A365,A370,A371,A377,A378,A379,A380,A384,A385,A386,A391,A392,A393,A394,A395,A396,A397,A4,A405,A406,A407,A408,A409,A41,A410,A413,A414,A415,A418,A4

	19,A420,A421,A422,A424,A426,A427,A432,A459,A465,A500,A507,A509,A526,A529,A546,A554,A555,A562,A573,A577,A578,A584,A605,A607,A615,A616,A67,A68,A78,A87,A88,A89,A95
+++	A105,A106,A107,A109,A11,A117,A118,A119,A12,A120,A122,A13,A136,A138,A14,A142,A144,A15,A157,A158,A16,A161,A163,A167,A17,A170,A174,A175,A176,A177,A178,A178,A179,A179,A18,A180,A181,A182,A183,A185,A19,A2,A20,A205,A207,A208,A209,A214,A22,A225,A225,A232,A236,A236,A24,A253,A254,A254,A256,A257,A258,A259,A26,A262,A264,A265,A267,A268,A270,A273,A274,A276,A278,A279,A28,A280,A284,A285,A286,A287,A288,A289,A290,A291,A293,A294,A295,A296,A297,A298,A30,A302,A303,A304,A306,A309,A310,A311,A312,A313,A314,A315,A316,A318,A319,A32,A320,A321,A322,A329,A33,A330,A331,A332,A333,A334,A335,A336,A337,A338,A343,A344,A345,A35,A350,A352,A353,A354,A355,A356,A357,A358,A359,A360,A361,A362,A363,A366,A367,A368,A369,A37,A372,A373,A374,A375,A376,A382,A383,A387,A388,A39,A398,A399,A4,A40,A400,A401,A402,A403,A404,A411,A412,A416,A417,A423,A425,A43,A431,A433,A434,A435,A436,A437,A438,A439,A441,A443,A444,A445,A447,A449,A45,A450,A451,A452,A454,A456,A457,A458,A46,A462,A463,A466,A467,A468,A469,A47,A470,A471,A472,A473,A474,A475,A476,A477,A478,A48,A480,A483,A484,A485,A486,A487,A488,A489,A490,A491,A492,A493,A494,A495,A497,A498,A5,A502,A503,A504,A505,A506,A508,A51,A510,A511,A512,A513,A514,A515,A518,A520,A521,A522,A523,A524,A525,A528,A530,A531,A532,A533,A534,A535,A536,A537,A538,A540,A541,A542,A543,A544,A545,A547,A548,A549,A55,A550,A551,A552,A553,A556,A557,A558,A559,A560,A561,A563,A564,A565,A566,A567,A568,A569,A570,A571,A574,A576,A579,A580,A581,A582,A583,A585,A586,A587,A588,A589,A590,A591,A592,A593,A595,A596,A597,A598,A6,A600,A601,A602,A603,A606,A608,A610,A611,A614,A62,A65,A66,A69,A7,A75,A76,A79,A8,A80,A81,A82,A83,A84,A85,A86,A87,A9,A90,A91,A92,A93,A94,A96,A98,A99
++++	A104,A104,A108,A110,A112,A113,A115,A116,A12,A137,A141,A143,A150,A152,A172,A173,A188,A199,A203,A206,A210,A212,A213,A220,A221,A222,A234,A235,A237,A238,A239,A244,A245,A246,A253,A260,A261,A271,A272,A281,A282,A283,A29,A292,A299,A3,A300,A301,A305,A307,A308,A317,A326,A339,A34,A36,A36,A38,A389,A42,A428,A44,A440,A442,A446,A448,A453,A455,A460,A461,A464,A479,A481,A482,A49,A496,A499,A50,A516,A517,A519,A52,A527,A53,A539,A54,A54,A56,A57,A572,A575,A58,A599,A604,A609,A61,A612,A63,A64,A70,A77,A97,A613
+++++	A103,A103,A116,A117,A145,A153,A154,A186,A187,A189,A190,A191,A192,A193,A194,A195,A196,A197,A198,A200,A327,A328,A340,A341,A429,A430,A59,A60

Table 11. KRAS G12V FRET data

IC50*	Examples
+	A325
++	A1,A11,A114,A117,A121,A135,A139,A140,A146,A147,A156,A159,A160,A162,A164,A165,A2,A201,A202,A204,A211,A218,A219,A223,A224,A230,A233,A247,A248,A249,A250,A252,A264,A265,A266,A275,A277,A278,A279,A3,A323,A342,A347,A348,A349,A365,A370,A371,A377,A378,A379,A380,A385,A391,A396,A399,A405,A407,A415,A422,A423,A424,A427,A454,A465,A477,A487,A5,A500,A501,A507,A526,A529,A546,A554,A555,A562,A577,A578,A584,A594,A605,A607,A615,A95
+++	A10,A100,A102,A12,A120,A123,A124,A125,A126,A127,A128,A129,A13,A131,A132,A136,A137,A138,A14,A148,A149,A15,A151,A155,A157,A158,A16,A161,A166,A167,A168,A17,A170,A171,A174,A176,A18,A184,A19,A20,A205,A209,A21,A215,A217,A22,A226,A227,A228,A229,A231,A232,A236,A24,A240,A241,A242,A243,A248,A25,A251,A252,A254,A254,A255,A257,A26,A262,A267,A268,A270,A273,A274,A28,A280,A285,A286,A287,A288,A289,A29,A290,A291,A293,A294,A295,A296,A297,A298,A30,A301,A302,A303,A304,A306,A309,A310,A311,A312,A313,A314,A315,A316,A318,A319,A320,A321,A322,A324,A326,A329,A33,A330,A331,A332,A333,A334,A335,A336,A337,A338,A343,A344,A345,A346,A35,A350,A351,A352,A353,A354,A355,A356,A357,A358,A359,A36,A36,A360,A361,A362,A363,A364,A366,A367,A368,A369,A37,A372,A373,A374,A375,A376,A38,A381,A382,A383,A384,A386,A387,A39,A392,A393,A394,A395,A397,A4,A40,A400,A401,A402,A403,A406,A408,A409,A410,A411,A412,A413,A414,A416,A418,A419,A420,A425,A426,A43,A431,A433,A436,A437,A438,A44,A441,A443,A444,A445,A450,A451,A452,A456,A457,A458,A459,A463,A466,A467,A471,A472,A474,A475,A476,A478,A480,A483,A484,A485,A486,A488,A489,A49,A490,A491,A492,A493,A494,A495,A496,A498,A503,A505,A506,A509,A510,A511,A512,A513,A514,A515,A520,A521,A522,A523,A525,A528,A530,A531,A532,A533,A534,A535,A536,A537,A538,A540,A541,A543,A545,A547,A548,A549,A550,A551,A552,A553,A557,A558,A559,A560,A561,A564,A565,A566,A567,A568,A569,A570,A571,A573,A574,A576,A579,A580,A581,A582,A583,A585,A586,A589,A590,A591,A593,A595,A596,A597,A598,A6,A600,A601,A602,A603,A606,A608,A610,A614,A616,A62,A64,A67,A68,A7,A76,A78,A8,A83,A87,A89,A9,A90,A91,A93,A94
++++	A101,A105,A106,A111,A112,A113,A115,A116,A117,A12,A122,A130,A133,A134,A141,A142,A143,A144,A152,A163,A169,A173,A177,A178,A178,A179,A179,A180,A181,A182,A183,A185,A199,A203,A207,A208,A210,A212,A214,A216,A220,A221,A225,A225,A227,A23,A236,A237,A238,A239,A244,A245,A246,A253,A253,A256,A258,A259,A260,A261,A27,A271,A276,A281,A282,A283,A284,A292,A299,A3,A300,A305,A307,A308,A31,A317,A32,A339,A34,A388,A389,A398,A404,A41,A417,A42,A421,A428,A432,A434,A435,A439,A440,A442,A447,A449,A453,A455,A46,A460,A461,A462,A464,A468,A469,A470,A473,A479,A48,A481,A482,A497,A499,A50,A502,A504,A508,A51,A516,A517,A518,A519,A52,A524,A527,A53,A539,A54,A54,A542,A544,A55,A556,A56,A563,A57,A5

	72,A575,A58,A587,A588,A59,A592,A599,A60,A604,A609,A61,A611,A612,A63,A65,A66,A69,A75,A79,A80,A81,A82,A84,A85,A86,A87,A88,A92,A96,A97,A98,A99,A613
+++++	A103,A103,A104,A104,A107,A108,A109,A110,A116,A118,A119,A145,A150,A153,A154,A172,A175,A186,A187,A188,A189,A190,A191,A192,A193,A194,A195,A196,A197,A198,A200,A206,A213,A222,A234,A235,A272,A327,A328,A340,A341,A4,A429,A430,A446,A448,A45,A47,A70,A77

Table 12. KRAS WT FRET data

IC50*	Examples
+	A594
++	A1,A10,A100,A111,A114,A121,A124,A125,A126,A127,A128,A129,A130,A131,A135,A139,A140,A146,A147,A148,A149,A151,A155,A156,A157,A159,A160,A162,A164,A165,A166,A168,A2,A202,A204,A211,A216,A217,A218,A219,A223,A224,A227,A228,A23,A230,A231,A241,A242,A243,A247,A248,A249,A252,A252,A274,A275,A277,A278,A287,A290,A291,A298,A3,A306,A312,A323,A325,A333,A335,A336,A342,A346,A347,A348,A349,A351,A363,A365,A370,A371,A372,A373,A374,A377,A378,A379,A380,A381,A385,A386,A391,A392,A393,A395,A396,A397,A4,A400,A402,A405,A406,A407,A409,A410,A411,A412,A413,A415,A418,A419,A422,A424,A426,A427,A443,A454,A459,A465,A475,A483,A487,A491,A493,A494,A498,A500,A501,A503,A505,A507,A526,A528,A529,A536,A537,A545,A546,A554,A555,A558,A560,A561,A562,A565,A571,A573,A574,A577,A578,A584,A590,A596,A600,A605,A606,A607,A615,A616,A87,A95
+++	A101,A102,A106,A11,A117,A12,A120,A122,A123,A13,A132,A133,A134,A136,A137,A138,A14,A144,A15,A158,A16,A161,A163,A167,A169,A17,A170,A171,A173,A174,A176,A178,A179,A179,A18,A184,A19,A20,A201,A205,A208,A209,A21,A210,A215,A22,A226,A227,A229,A232,A233,A236,A24,A240,A248,A25,A250,A251,A254,A254,A255,A257,A259,A26,A260,A262,A264,A265,A266,A267,A268,A27,A270,A273,A276,A279,A28,A280,A283,A284,A285,A286,A288,A289,A292,A293,A294,A295,A296,A297,A299,A30,A301,A302,A303,A304,A308,A309,A31,A310,A311,A313,A314,A315,A316,A318,A319,A32,A320,A321,A322,A324,A326,A329,A330,A331,A332,A334,A337,A338,A339,A343,A344,A345,A35,A350,A352,A353,A354,A355,A356,A357,A358,A360,A361,A362,A364,A367,A368,A369,A37,A375,A376,A38,A382,A383,A384,A387,A39,A394,A399,A40,A401,A403,A404,A408,A41,A414,A416,A417,A420,A421,A423,A43,A432,A433,A434,A435,A436,A437,A438,A439,A44,A440,A441,A444,A445,A447,A449,A45,A450,A451,A452,A455,A456,A457,A458,A46,A461,A463,A466,A467,A468,A469,A47,A471,A472,A473,A474,A476,A477,A478,A48,A480,A484,A485,A486,A488,A489,A49,A490,A492,A495,A496,A5,A50,A502,A504,A506,A508,A509,A51,A510,A511,A512,A513,A514,A515,A518,A519,A520,A521,A522,A523,A524,A525,A527,A530,A531,A532,A533,A534,A535,A538,A540,A541,A542,A543,A547,A548,A549,A550,A551,A552,A553,A556,A557,A559,A563,A564,A566,A567,A568,A569,A570,A576,A579,A580,A581,A582,A583,A585,A586,A587,A588,A589,A591,A593,A595,A597,A598,A6,A601,A602,A603,A608,

	A610,A611,A614,A62,A64,A65,A66,A67,A68,A7,A76,A78,A79,A8,A80,A81,A82,A83,A84,A85,A87,A88,A89,A9,A90,A91,A93,A94,A99
++++	A105,A107,A108,A109,A110,A112,A113,A115,A116,A118,A119,A12,A141,A142,A143,A150,A152,A175,A177,A178,A180,A181,A182,A183,A185,A199,A203,A206,A207,A213,A214,A220,A221,A222,A225,A225,A234,A235,A236,A237,A238,A239,A244,A245,A246,A253,A253,A256,A258,A261,A271,A272,A281,A282,A29,A3,A300,A305,A307,A317,A33,A34,A359,A36,A36,A366,A388,A389,A398,A4,A42,A425,A428,A429,A431,A442,A446,A448,A453,A460,A462,A464,A470,A479,A481,A482,A497,A499,A516,A517,A52,A53,A539,A54,A54,A544,A55,A56,A57,A572,A575,A58,A59,A592,A599,A60,A604,A609,A61,A612,A63,A69,A70,A75,A86,A92,A96,A97,A98,A613
+++++	A103,A103,A104,A104,A116,A117,A145,A153,A154,A172,A186,A187,A188,A189,A190,A191,A192,A193,A194,A195,A196,A197,A198,A200,A212,A327,A328,A340,A341,A430,A77

Table 13. KRAS G13D FRET data

IC50*	Examples (Example A55 not tested)
+	None
++	A1,A10,A100,A111,A114,A121,A124,A125,A127,A128,A129,A130,A131,A133,A134,A135,A139,A140,A148,A151,A155,A159,A162,A163,A164,A165,A169,A2,A202,A204,A211,A216,A217,A223,A224,A227,A227,A228,A229,A23,A231,A242,A243,A247,A248,A249,A251,A252,A255,A27,A275,A277,A3,A323,A325,A342,A347,A348,A349,A365,A370,A371,A378,A379,A380,A381,A385,A386,A391,A392,A393,A395,A396,A397,A4,A405,A407,A409,A41,A415,A419,A424,A426,A427,A432,A45,A459,A501,A507,A529,A546,A558,A573,A577,A578,A584,A594,A605,A607,A615,A67,A87,A88,A95
+++	A101,A102,A11,A117,A119,A12,A120,A122,A123,A126,A13,A132,A136,A14,A146,A147,A149,A15,A156,A157,A158,A16,A160,A161,A166,A167,A168,A17,A170,A171,A174,A176,A179,A18,A184,A19,A20,A201,A205,A209,A21,A215,A218,A219,A226,A230,A232,A233,A236,A24,A240,A241,A248,A25,A250,A252,A254,A254,A257,A259,A26,A262,A264,A265,A266,A267,A268,A270,A273,A274,A278,A279,A28,A280,A284,A285,A286,A287,A288,A289,A290,A291,A293,A294,A295,A298,A302,A303,A304,A306,A309,A31,A310,A311,A312,A313,A314,A315,A316,A318,A319,A32,A321,A324,A329,A330,A332,A333,A334,A335,A336,A337,A338,A343,A345,A346,A35,A350,A351,A352,A353,A356,A357,A358,A361,A363,A364,A369,A372,A373,A374,A375,A376,A377,A38,A382,A383,A384,A387,A39,A394,A399,A4,A40,A400,A401,A402,A403,A404,A406,A408,A410,A411,A412,A413,A414,A416,A417,A418,A420,A421,A422,A423,A43,A433,A434,A435,A436,A437,A438,A441,A442,A443,A444,A445,A447,A450,A452,A454,A457,A458,A46,A463,A465,A466,A467,A468,A469,A47,A471,A473,A475,A476,A477,A478,A48,A480,A483,A484,A485,A486,A487,A488,A489,A491,A492,A493,A494,A498,A5,A500,A502,A503,A504,A505,A506,A509,A51,A510,A512,A513,A514,A515,A518,A520,A523,A525,A526,A528,A531,A532,A533,A534,A535,A536,A537,A538,A540,A542,A543,A5

	45,A549,A550,A551,A552,A554,A555,A557,A559,A560,A561,A562,A563,A564,A565,A566,A567,A568,A569,A570,A571,A574,A576,A580,A581,A582,A583,A586,A587,A588,A589,A590,A591,A593,A595,A596,A597,A598,A6,A600,A601,A602,A603,A606,A608,A610,A611,A614,A616,A62,A64,A65,A66,A68,A7,A76,A78,A79,A8,A80,A81,A83,A84,A85,A87,A89,A9,A90,A93,A94
++++	A105,A106,A107,A108,A109,A110,A112,A113,A115,A116,A118,A12,A137,A138,A141,A142,A143,A144,A152,A173,A175,A177,A178,A178,A179,A180,A181,A182,A183,A185,A199,A203,A206,A207,A208,A210,A213,A214,A22,A222,A225,A225,A236,A238,A239,A246,A253,A256,A258,A260,A261,A272,A276,A281,A282,A283,A29,A292,A296,A297,A299,A30,A300,A301,A305,A307,A308,A317,A320,A322,A326,A33,A331,A339,A34,A344,A354,A355,A359,A36,A36,A360,A362,A366,A367,A368,A37,A388,A389,A398,A42,A425,A431,A439,A44,A440,A446,A448,A449,A451,A455,A456,A460,A461,A462,A464,A470,A472,A474,A479,A481,A482,A49,A490,A495,A496,A497,A499,A50,A508,A511,A516,A517,A519,A52,A521,A522,A524,A527,A53,A530,A539,A54,A541,A544,A547,A548,A553,A556,A56,A57,A572,A575,A579,A58,A585,A59,A592,A599,A604,A609,A61,A612,A63,A69,A70,A75,A77,A82,A86,A91,A92,A96,A97,A98,A99,A613
+++++	A103,A103,A104,A104,A116,A117,A145,A150,A153,A154,A172,A186,A187,A188,A189,A190,A191,A192,A193,A194,A195,A196,A197,A198,A200,A212,A220,A221,A234,A235,A237,A244,A245,A253,A271,A3,A327,A328,A340,A341,A428,A429,A430,A453,A60

Table 14. KRAS Q61H FRET data

IC50*	Examples
+	A159,A275,A415,A501,A546,A577,A594,A605
++	A102,A124,A136,A174,A204,A205,A226,A230,A236,A24,A25,A250,A266,A268,A273,A274,A277,A278,A279,A280,A285,A287,A289,A290,A291,A3,A302,A304,A306,A309,A310,A312,A316,A324,A334,A335,A346,A349,A356,A358,A37,A372,A373,A374,A376,A38,A382,A383,A387,A396,A399,A400,A401,A405,A406,A409,A422,A424,A426,A434,A438,A443,A444,A450,A454,A457,A459,A465,A466,A467,A468,A471,A474,A475,A477,A478,A480,A483,A484,A485,A487,A488,A491,A492,A493,A494,A498,A500,A502,A503,A505,A507,A509,A510,A514,A515,A520,A523,A526,A528,A529,A530,A531,A533,A534,A536,A537,A538,A540,A543,A545,A549,A550,A551,A552,A554,A555,A557,A558,A559,A560,A561,A562,A563,A564,A565,A566,A567,A569,A571,A573,A574,A578,A581,A582,A583,A584,A589,A590,A591,A593,A595,A596,A600,A601,A606,A607,A608,A610,A614,A615,A616,A89
+++	A138,A144,A152,A163,A170,A176,A177,A178,A178,A179,A179,A180,A181,A207,A208,A210,A225,A225,A253,A264,A265,A267,A270,A271,A272,A276,A281,A282,A283,A284,A286,A293,A297,A299,A305,A315,A320,A321,A331,A337,A344,A354,A357,A359,A360,A361,A362,A366,A367,A368,A369,A375,A388,A389,A423,A433,A435,A436,A437,A439,A440,A441,A442,A445,A447,A449,A451,A452,A453,A455,A456,A458,A460,

	A461,A462,A463,A464,A469,A470,A472,A473,A476,A479,A481,A482,A486,A489,A490,A495,A496,A497,A499,A504,A506,A508,A511,A512,A513,A518,A519,A521,A522,A524,A525,A527,A532,A535,A54,A541,A542,A544,A547,A548,A553,A556,A568,A57,A570,A572,A576,A579,A580,A585,A586,A587,A588,A592,A597,A598,A599,A602,A603,A604,A609,A61,A611,A612,A99
++++	A104,A145,A150,A220,A234,A237,A446,A448,A516,A517,A539,A575,A613
+++++	A154,A186,A189,A191,A328,A340

Table 15. NRAS G12C FRET data

IC50*	Examples
+	A323,A325,A501,A577,A578,A594
++	A1,A10,A100,A11,A114,A120,A121,A125,A127,A128,A129,A131,A135,A136,A139,A140,A146,A147,A148,A151,A156,A157,A159,A160,A162,A164,A165,A166,A168,A2,A201,A202,A204,A205,A211,A217,A218,A219,A223,A224,A228,A229,A230,A231,A233,A240,A242,A243,A247,A248,A248,A249,A250,A252,A252,A264,A265,A266,A267,A268,A273,A274,A275,A277,A278,A279,A280,A285,A288,A289,A290,A291,A298,A3,A302,A303,A304,A306,A309,A310,A312,A313,A316,A319,A321,A330,A333,A334,A335,A336,A338,A342,A343,A346,A347,A348,A349,A350,A351,A353,A356,A357,A358,A363,A364,A365,A370,A371,A372,A373,A374,A376,A377,A378,A379,A380,A381,A382,A383,A384,A385,A386,A387,A391,A392,A393,A395,A396,A397,A399,A4,A400,A401,A402,A405,A406,A407,A408,A409,A411,A413,A414,A415,A418,A419,A422,A424,A426,A427,A432,A436,A438,A443,A444,A450,A452,A454,A457,A458,A459,A465,A466,A467,A471,A475,A476,A477,A478,A483,A484,A487,A488,A489,A491,A493,A494,A498,A5,A500,A503,A505,A507,A509,A510,A514,A515,A523,A526,A528,A529,A531,A533,A534,A536,A537,A538,A540,A543,A545,A546,A549,A550,A551,A552,A554,A555,A557,A558,A559,A560,A561,A562,A565,A567,A569,A571,A573,A574,A576,A581,A582,A583,A584,A590,A591,A593,A595,A596,A598,A6,A600,A601,A605,A606,A607,A608,A614,A615,A616,A8,A87,A95
+++	A101,A102,A106,A111,A117,A122,A123,A124,A126,A13,A130,A132,A133,A134,A137,A138,A14,A149,A15,A155,A158,A16,A161,A163,A167,A169,A17,A170,A171,A173,A174,A176,A18,A184,A19,A20,A209,A21,A210,A215,A216,A226,A227,A227,A23,A232,A236,A24,A241,A25,A251,A254,A254,A255,A257,A259,A26,A260,A262,A27,A270,A276,A28,A282,A283,A284,A286,A287,A29,A292,A293,A294,A295,A296,A297,A299,A30,A301,A305,A308,A31,A311,A314,A315,A318,A32,A320,A322,A324,A326,A329,A331,A332,A337,A339,A344,A345,A35,A352,A354,A355,A359,A36,A36,A360,A361,A362,A366,A367,A368,A369,A37,A375,A38,A389,A39,A394,A40,A403,A404,A41,A410,A412,A416,A417,A420,A421,A423,A43,A431,A433,A434,A435,A437,A439,A44,A440,A441,A445,A447,A449,A451,A453,A455,A456,A46,A460,A461,A462,A463,A468,A469,A47,A472,A473,A474,A479,A480,A481,A482,A485,A486,A490,A492,A495,A496,A497,A499,A502,A504,A506,A508,A511,A512,A513,A518,A519,A520,A521,A522,A524,A525,A

	527,A530,A532,A535,A539,A541,A542,A544,A547,A548,A553,A556,A563,A564,A566,A568,A570,A579,A580,A585,A586,A587,A588,A589,A592,A597,A602,A603,A609,A610,A611,A612,A62,A64,A65,A66,A67,A68,A76,A78,A79,A80,A81,A83,A85,A87,A88,A89,A9,A90,A91,A93,A94,A96,A97,A98,A99
++++	A105,A107,A108,A109,A110,A112,A113,A115,A116,A117,A118,A119,A12,A141,A142,A143,A144,A150,A152,A175,A177,A178,A178,A179,A179,A180,A181,A182,A183,A185,A199,A203,A206,A207,A208,A212,A213,A214,A220,A221,A225,A225,A235,A236,A237,A238,A239,A244,A245,A246,A253,A253,A256,A258,A261,A271,A272,A281,A3,A300,A307,A317,A388,A398,A4,A42,A425,A428,A429,A442,A446,A448,A45,A464,A470,A49,A50,A51,A516,A517,A52,A53,A54,A54,A56,A57,A572,A575,A58,A59,A599,A60,A604,A61,A63,A75,A82,A84,A86,A92,A613
+++++	A103,A103,A104,A104,A116,A145,A153,A154,A172,A186,A187,A188,A189,A190,A191,A192,A193,A194,A195,A196,A197,A198,A200,A222,A234,A327,A328,A340,A341,A430,A77

Table 16. NRAS WT FRET data

IC50*	Examples
+	A501,A577,A594
++	A124,A136,A159,A204,A230,A25,A250,A273,A274,A275,A277,A278,A279,A287,A290,A291,A3,A304,A306,A309,A310,A312,A335,A346,A349,A356,A372,A373,A374,A387,A396,A399,A400,A405,A406,A409,A415,A422,A424,A426,A434,A438,A443,A450,A454,A457,A459,A465,A466,A471,A475,A477,A478,A483,A484,A487,A491,A493,A494,A498,A500,A503,A505,A507,A526,A528,A529,A531,A533,A536,A537,A545,A546,A554,A555,A558,A560,A561,A562,A565,A569,A571,A573,A574,A578,A584,A590,A591,A596,A600,A605,A606,A607,A615
+++	A102,A138,A144,A163,A170,A174,A176,A178,A179,A179,A205,A208,A210,A225,A226,A236,A24,A253,A264,A265,A266,A267,A268,A270,A276,A280,A283,A284,A285,A286,A289,A293,A297,A299,A302,A305,A315,A316,A320,A321,A324,A331,A334,A337,A344,A354,A357,A358,A359,A360,A361,A362,A366,A367,A368,A369,A37,A375,A376,A38,A382,A383,A401,A423,A433,A435,A436,A437,A439,A440,A441,A444,A445,A447,A449,A451,A452,A455,A456,A458,A463,A467,A468,A469,A472,A473,A474,A476,A479,A480,A481,A485,A486,A488,A489,A490,A492,A495,A496,A497,A502,A504,A506,A508,A509,A510,A511,A512,A513,A514,A515,A518,A519,A520,A521,A522,A523,A524,A525,A527,A530,A532,A534,A535,A538,A540,A541,A542,A543,A547,A548,A549,A550,A551,A552,A553,A556,A557,A559,A563,A564,A566,A567,A568,A570,A576,A579,A580,A581,A582,A583,A585,A586,A587,A588,A589,A593,A595,A597,A598,A601,A602,A603,A608,A610,A611,A612,A614,A616,A89,A99
++++	A104,A150,A152,A177,A178,A180,A181,A207,A220,A225,A234,A237,A271,A272,A281,A282,A388,A389,A442,A446,A448,A453,A460,A461,A462,A464,A470,A482,A499,A516,A517,A539,A54,A544,A57,A572,A575,A592,A599,A604,A609,A61,A613

+++++	A145,A154,A186,A189,A191,A328,A340

Table 17. NRAS Q61K FRET data

IC50*	Examples
+	A275
++	A136,A159,A170,A205,A266,A268,A277,A278,A279,A280,A285,A289,A290,A291,A302,A304,A306,A309,A310,A312,A316,A334,A335,A337,A344,A349,A356,A358,A372,A373,A374,A376,A382,A383,A387,A396,A399,A400,A401,A405,A409,A415,A422,A443,A444,A445,A450,A451,A452,A454,A457,A458,A459,A465,A466,A467,A471,A475,A477,A478,A483,A484,A485,A486,A487,A488,A489,A491,A493,A494,A500,A501,A503,A505,A510,A511,A512,A514,A515,A520,A522,A523,A526,A528,A529,A530,A531,A532,A533,A534,A536,A537,A538,A540,A543,A546,A549,A550,A551,A552,A555,A557,A560,A561,A565,A566,A567,A569,A571,A573,A574,A576,A577,A578,A580,A581,A582,A584,A590,A591,A594,A595,A596,A598,A600,A601,A606,A607,A608,A614
+++	A102,A124,A138,A144,A152,A174,A176,A204,A208,A210,A226,A230,A236,A24,A25,A250,A264,A265,A267,A270,A271,A273,A274,A276,A281,A283,A284,A286,A287,A293,A297,A299,A3,A305,A315,A320,A321,A324,A331,A346,A354,A357,A359,A360,A361,A362,A366,A367,A368,A369,A375,A38,A389,A406,A424,A426,A433,A434,A436,A437,A438,A439,A440,A441,A447,A449,A453,A455,A456,A460,A461,A462,A468,A469,A472,A474,A476,A479,A480,A481,A482,A490,A492,A495,A496,A497,A498,A499,A502,A506,A507,A509,A513,A521,A524,A525,A527,A535,A541,A542,A544,A545,A547,A548,A553,A554,A556,A558,A559,A562,A563,A564,A568,A570,A579,A583,A585,A586,A587,A588,A589,A592,A593,A597,A602,A603,A604,A605,A609,A610,A611,A612,A613,A615,A616,A89
++++	A104,A150,A163,A177,A178,A178,A179,A179,A180,A181,A207,A220,A225,A225,A234,A237,A253,A272,A282,A37,A388,A423,A435,A442,A446,A448,A463,A464,A470,A473,A504,A508,A516,A517,A518,A519,A539,A54,A57,A572,A575,A599,A61,A99
+++++	A145,A154,A186,A189,A191,A328,A340

Table 18. NRAS Q61R FRET data

IC50*	Examples
+	A577,A594
++	A136,A159,A275,A277,A278,A279,A291,A304,A306,A309,A310,A312,A335,A349,A356,A372,A373,A374,A396,A399,A400,A415,A422,A450,A454,A459,A465,A466,A475,A477,A483,A487,A494,A500,A501,A503,A505,A520,A526,A528,A529,A536,A546,A555,A560,A561,A562,A565,A578,A583,A584,A593,A601,A606,A607
+++	A102,A138,A170,A174,A204,A205,A210,A226,A230,A24,A25,A250,A264,A265,A266,A267,A268,A270,A273,A274,A276,A280,A283,A285,A286,A287,A289,A290,A293,A297,A299,A3,A302,A305,A316,A321,A334,A337,A344,A346,A354,A357,A358,A361,A369,A375,A376,A38,A382,A383,A387,A401,A405,A406,A409,A423,A424,A426,A43

	3,A434,A436,A437,A438,A439,A440,A441,A443,A444,A445,A449,A451,A452,A453,A455,A456,A457,A458,A461,A467,A468,A471,A472,A474,A476,A478,A480,A484,A485,A486,A488,A489,A490,A491,A492,A493,A495,A498,A499,A502,A506,A507,A509,A510,A511,A512,A513,A514,A515,A521,A522,A523,A525,A527,A530,A531,A532,A533,A534,A535,A537,A538,A540,A541,A542,A543,A545,A547,A548,A549,A550,A551,A552,A553,A554,A556,A557,A558,A559,A563,A564,A566,A567,A568,A569,A570,A571,A573,A574,A576,A579,A580,A581,A582,A585,A586,A587,A589,A590,A591,A595,A596,A597,A598,A600,A602,A603,A605,A608,A609,A610,A611,A614,A615,A616,A89
++++	A124,A144,A152,A163,A176,A177,A178,A179,A179,A180,A207,A208,A220,A225,A225,A236,A237,A253,A271,A272,A281,A282,A284,A315,A320,A324,A331,A359,A360,A362,A366,A367,A368,A37,A388,A389,A435,A442,A447,A448,A460,A462,A463,A464,A469,A470,A473,A479,A481,A482,A496,A497,A504,A508,A516,A517,A518,A519,A524,A539,A54,A544,A57,A572,A575,A588,A592,A599,A604,A61,A612,A99,A613
+++++	A104,A145,A150,A154,A178,A181,A186,A189,A191,A234,A328,A340,A446

In vitro Cell Proliferation Panels

Potency for inhibition of cell growth was assessed at CrownBio using standard methods. Briefly, cell lines were cultured in appropriate medium, and then plated in 3D methylcellulose. Inhibition of cell growth was determined by CellTiter-Glo® after 5 days of culture with increasing concentrations of compounds. Compound potency was reported as the 50% inhibition concentration (absolute IC50).

The assay took place over 7 days. On day 1, cells in 2D culture were harvested during logarithmic growth and suspended in culture medium at 1x105 cells/ml. Higher or lower cell densities were used for some cell lines based on prior optimization. 3.5 ml of cell suspension was mixed with 6.5% growth medium with 1% methylcellulose, resulting in a cell suspension in 0.65% methylcellulose. 90 µl of this suspension was distributed in the wells of 2 96-well plates. One plate was used for day 0 reading and 1 plate was used for the end-point experiment. Plates were incubated overnight at 37 C with 5% CO2. On day 2, one plate (for t0 reading) was removed and 10 µl growth medium plus 100 µl CellTiter-Glo® Reagent was added to each well. After mixing and a 10 minute incubation, luminescence was recorded on an EnVision Multi-Label Reader (Perkin Elmer). Compounds in DMSO were diluted in growth medium such that the final, maximum concentration of compound was 10 µM, and serial 4-fold dilutions were performed to generate a 9-point concentration series. 10 µl of compound solution at 10 times final concentration was added to wells of the second plate. Plate was then incubated for 120 hours at 37C and 5% CO2. On day 7 the plates were removed, 100 µl CellTiter-Glo® Reagent was added to each well, and after mixing and a 10 minute incubation, luminescence was recorded on an EnVision Multi-Label Reader (Perkin Elmer). Data was exported to GeneData Screener and modeled with a sigmoidal concentration response model in order to determine the IC50 for compound response.

Not all cell lines with a given RAS mutation may be equally sensitive to a RAS inhibitor targeting that mutation, due to differential expression of efflux transporters, varying dependencies

on RAS pathway activation for growth, or other reasons. This has been exemplified by the cell line KYSE-410 which, despite having a KRAS G12C mutation, is insensitive to the KRAS G12C (OFF) inhibitor MRTX-849 (Hallin et al., Cancer Discovery 10:54-71 (2020)), and the cell line SW1573, which is insensitive to the KRAS G12C (OFF) inhibitor AMG510 (Canon et al., Nature 575:217-223 (2019)).

Table 19: IC50 values for various cancer cell lines with Compound B, Compound C, and Compound D

*Key:

low sensitivity: IC50 ≥ 1 uM

moderately sensitive: 1 uM > IC50 ≥ 0.1 uM

very sensitive: IC50 < 0.1 uM

Cell Line	Histotype	Mutant	Cmpd B IC50*	Cmpd C IC50*	Cmpd D IC50*
A-375	Skin	BRAF V600E	low sensitivity	low sensitivity	low sensitivity
KYSE-410	HN/Esophagus	KRAS G12C	moderately sensitive		very sensitive
MIA PaCa-2	Pancreas	KRAS G12C	moderately sensitive	very sensitive	very sensitive
NCI-H358	Lung	KRAS G12C	moderately sensitive	very sensitive	very sensitive
SW1573	Lung	KRAS G12C	low sensitivity	low sensitivity	low sensitivity
SW837	Intestine/Large/Colorectum	KRAS G12C	moderately sensitive		moderately sensitive
LS513	Intestine/Large/Colorectum	KRAS G12D	moderately sensitive		moderately sensitive
HuCCT1	Liver/Bile duct	KRAS G12D	moderately sensitive		very sensitive
HCC1588	Lung	KRAS G12D	low sensitivity	low sensitivity	moderately sensitive
HPAC	Pancreas	KRAS G12D	moderately sensitive		very sensitive
AsPC-1	Pancreas	KRAS G12D	moderately sensitive	moderately sensitive	moderately sensitive
AGS	Stomach	KRAS G12D	moderately sensitive	very sensitive	moderately sensitive
HEC-1-A	Uterus	KRAS G12D	moderately sensitive		moderately sensitive
SW403	Intestine/Large/Colorectum	KRAS G12V	moderately sensitive		very sensitive
NOZ	Liver/Bile duct	KRAS G12V	moderately sensitive		moderately sensitive
NCI-H441	Lung	KRAS G12V	moderately sensitive	moderately sensitive	moderately sensitive
NCI-H727	Lung	KRAS G12V	moderately sensitive	very sensitive	very sensitive
OVCAR-5	Ovary	KRAS G12V	moderately sensitive		very sensitive
Capan-2	Pancreas	KRAS G12V	moderately sensitive		very sensitive
SW48	Intestine/Large/Colorectum	not MAPK (PIK3CA G914R, EGFR	low sensitivity	low sensitivity	low sensitivity

		G719S)			
NCI-H2009	Lung	other KRAS (G12A)	moderately sensitive		moderately sensitive
CAL-62	HN/Thyroid	other KRAS (G12R)	moderately sensitive		
A549	Lung	other KRAS (G12S)	moderately sensitive	moderately sensitive	moderately sensitive
TOV-21G	Ovary	other KRAS (G13C)	low sensitivity		moderately sensitive
DV-90	Lung	other KRAS (G13D)	low sensitivity		moderately sensitive
HCT116	Intestine/Large/Colorectum	other KRAS (G13D)	moderately sensitive		very sensitive
NCI-H747	Intestine/Large/Colorectum	other KRAS (G13D)	moderately sensitive		very sensitive
NCI-H460	Lung	other KRAS (Q61H)	moderately sensitive	moderately sensitive	moderately sensitive
Calu-6	Lung	other KRAS (Q61K)	moderately sensitive	very sensitive	moderately sensitive
SNU-668	Stomach	other KRAS (Q61K)	moderately sensitive		very sensitive
OZ	Liver/Bile duct	other KRAS (Q61L)	moderately sensitive		moderately sensitive
SW948	Intestine/Large/Colorectum	other KRAS (Q61L)	low sensitivity	moderately sensitive	moderately sensitive
BxPC-3	Pancreas	other MAPK (BRAF V487_P492delinsA)	low sensitivity	low sensitivity	low sensitivity
NCI-H1975	Lung	other MAPK (EGFR T790M, L858R)	moderately sensitive	moderately sensitive	very sensitive
NCI-H3122	Lung	other MAPK (EML4-ALK(E13,A20))	moderately sensitive		moderately sensitive
YCC-1	Stomach	other MAPK (KRAS Amp)			moderately sensitive
MeWo	Skin	other MAPK (NF1 mut)	low sensitivity	moderately sensitive	moderately sensitive

NCI-H1838	Lung	other MAPK (NF1 mut)	moderately sensitive	moderately sensitive	moderately sensitive
RL95-2	Uterus	other RAS (HRAS Q61H)			very sensitive
NCI-H1915	Lung	other RAS (HRAS Q61L)		moderately sensitive	moderately sensitive
L-363	Blood/Leukemia	other RAS (NRAS Q61H)			low sensitivity
CHP-212	Brain&Nerves	other RAS (NRAS Q61K)			moderately sensitive
HT-1080	Soft tissue	other RAS (NRAS Q61K)			moderately sensitive
NCI-H2087	Lung	other RAS (NRAS Q61K)			very sensitive
OCI-LY-19	Blood/Lymphoma	other RAS (NRAS Q61K)			moderately sensitive
SNU-387	Liver/bile duct	other RAS			moderately

		(NRAS Q61K)			sensitive
Hep G2	Liver/bile duct	other RAS (NRAS Q61L)		moderately sensitive	very sensitive
HL-60	Blood/Leukemia	other RAS (NRAS Q61L)			very sensitive
MOLP8	Blood/Myeloma	other RAS (NRAS Q61L)			moderately sensitive
SNU-719	Stomach	other RAS (NRAS Q61L)			moderately sensitive
TF-1	Blood/Leukemia	other RAS (NRAS Q61P)			moderately sensitive
ASH-3	HN/Thyroid	other RAS (NRAS Q61R)			moderately sensitive
SK-MEL-2	Skin	other RAS (NRAS Q61R)			moderately sensitive
SW1271	Lung	other RAS (NRAS Q61R)			moderately sensitive

In vivo PD and Efficacy Data with Compound A, a compound of the present invention

FIG. 1A:

Methods: The human pancreatic adenocarcinoma Capan-2 KRASG12V/wt xenograft model was used for a single-day treatment PK/PD study (FIG. 1A). Compound A (Capan-2 pERK K-Ras G12D EC50: 0.0037 uM) was administered at 100 mg/kg as a single dose or bid (second dose administered 8 hours after first dose) orally administered (po). The treatment groups with sample collections at various time points were summarized in Table 20 below. Tumor samples were collected to assess RAS/ERK signaling pathway modulation by measuring the mRNA level of human DUSP6 in qPCR assay, while accompanying blood plasma samples were collected to measure circulating Compound A levels.

Table 20. Summary of treatment groups, doses, and time points for single-dose PK/PD study using Capan-2 tumors.

Compound/group	**Dose/Regimen**	**PK, n = 3/time point**	**PD, n = 3/time point**
Vehicle control	10 ml/kg ip	1h, 24h	1h, 24h
Compound A	100 mg/kg po	1h, 2h, 8h, 12h, 24h	1h, 2h, 8h, 10h, 24h
Compound A	100 mg/kg po bid	1h, 2h, 8h, 12h, 24h	1h, 2h, 8h, 10h, 24h

Results: In FIG. 1A, Compound A delivered at 100 mg/kg as a single dose inhibited DUSP6 mRNA levels in tumors >95% through 10 hours. A second dose of Compound A 8 hours following first administration maintained pathway modulation of 93% through 24 hours. These data indicate Compound A provides strong MAPK pathway modulation with continued target coverage.

FIG. 1B:

Methods: Effects of Compound A on tumor cell growth in vivo were evaluated in the human pancreatic adenocarcinoma Capan-2 KRASG12V/wt xenograft model using female BALB/c nude mice (6-8 weeks old). Mice were implanted with Capan-2 tumor cells in 50% Matrigel (4 x 106 cells/mouse) subcutaneously in the flank. Once tumors reached an average size of ~180 mm3,

mice were randomized to treatment groups to start the administration of test articles or vehicle. Compound A was orally administered (po) twice daily at 100 mg/kg. A SHP2 inhibitor, RMC-4550 (commercially available), was administered orally every other day at 20 mg/kg. Body weight and tumor volume (using calipers) was measured twice weekly until study endpoints. Tumor regressions calculated as >10% decrease in starting tumor volume. All dosing arms were well tolerated.

Results: In FIG. 1B, single agent SHP2i RMC-4550 dosed every other day at 20 mg/kg po resulted in 39% TGI. Single-agent Compound A administered at 100 mg/kg po bid daily led to a TGI of 98%, with 4/10 (40%) individual animals achieving tumor regressions. Combination of Compound A and RMC-4550 resulted in total tumor regression of 35%, with individual tumor regressions observed in 7/9 (77.8%) individual animals at the end of treatment (Day 40 after treatment started) in Capan-2 CDX model with heterozygous KRASG12V. The anti-tumor activity of Compound A, and Combination arms was statistically significant compared with control group (***$p<0.001$, ordinary One-way ANOVA with multiple comparisons via a post-hoc Tukey's test), while RMC-4550 was not significant at these doses.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure come within known or customary practice within the art to which the invention pertains and may be applied to the essential features set forth herein.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

FIG. 1A

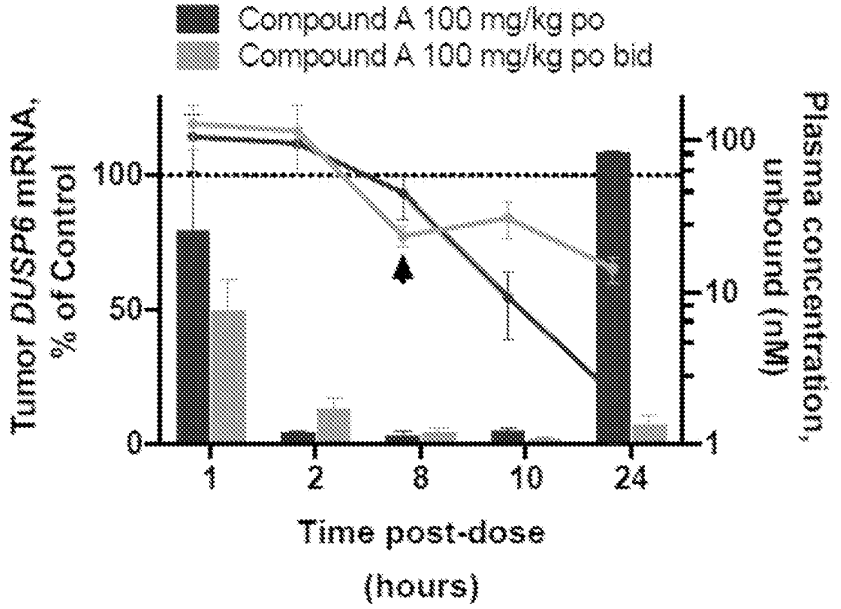
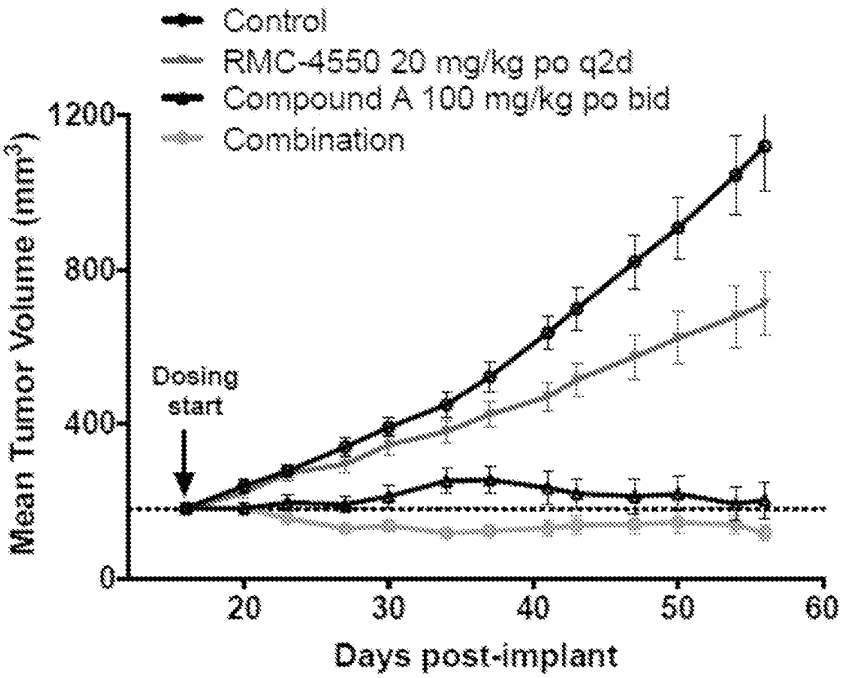


FIG. 1B



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APPENDIX B

RAS INHIBITORS

Background

The vast majority of small molecule drugs act by binding a functionally important pocket on a target protein, thereby modulating the activity of that protein. For example, cholesterol-lowering drugs known as statins bind the enzyme active site of HMG-CoA reductase, thus preventing the enzyme from engaging with its substrates. The fact that many such drug/target interacting pairs are known may have misled some into believing that a small molecule modulator could be discovered for most, if not all, proteins provided a reasonable amount of time, effort, and resources. This is far from the case. Current estimates are that only about 10% of all human proteins are targetable by small molecules. Bojadzic and Buchwald, Curr Top Med Chem 18: 674-699 (2019). The other 90% are currently considered refractory or intractable toward above-mentioned small molecule drug discovery. Such targets are commonly referred to as "undruggable." These undruggable targets include a vast and largely untapped reservoir of medically important human proteins. Thus, there exists a great deal of interest in discovering new molecular modalities capable of modulating the function of such undruggable targets.

It has been well established in literature that Ras proteins (K-Ras, H-Ras and N-Ras) play an essential role in various human cancers and are therefore appropriate targets for anticancer therapy. Indeed, mutations in Ras proteins account for approximately 30% of all human cancers in the United States, many of which are fatal. Dysregulation of Ras proteins by activating mutations, overexpression or upstream activation is common in human tumors, and activating mutations in Ras are frequently found in human cancer. For example, activating mutations at codon 12 in Ras proteins function by inhibiting both GTPase-activating protein (GAP)-dependent and intrinsic hydrolysis rates of GTP, significantly skewing the population of Ras mutant proteins to the "on" (GTP-bound) state (Ras(ON)), leading to oncogenic MAPK signaling. Notably, Ras exhibits a picomolar affinity for GTP, enabling Ras to be activated even in the presence of low concentrations of this nucleotide. Mutations at codons 13 (e.g., G13D) and 61 (e.g., Q61K) of Ras are also responsible for oncogenic activity in some cancers.

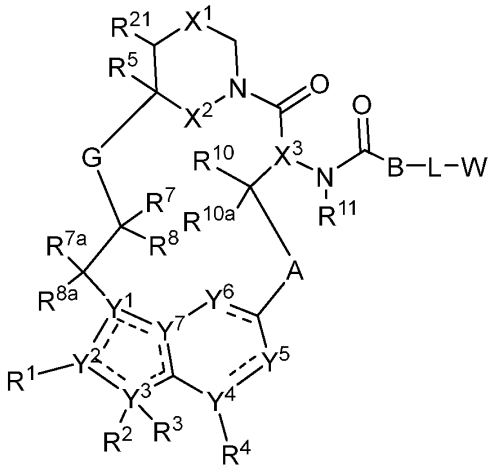
Despite extensive drug discovery efforts against Ras during the last several decades, a drug directly targeting Ras is still not approved. Additional efforts are needed to uncover additional medicines for cancers driven by the various Ras mutations.

Summary

Provided herein are Ras inhibitors. The approach described herein entails formation of a high affinity three-component complex, or conjugate, between a synthetic ligand and two intracellular proteins which do not interact under normal physiological conditions: the target protein of interest (e.g., Ras), and a widely expressed cytosolic chaperone (presenter protein) in the cell (e.g., cyclophilin A). More specifically, in some embodiments, the inhibitors of Ras described herein induce a new binding pocket in Ras by driving formation of a high affinity tri-complex, or

conjugate, between the Ras protein and the widely expressed cytosolic chaperone, cyclophilin A (CYPA). Without being bound by theory, the inventors believe that one way the inhibitory effect on Ras is effected by compounds of the invention and the complexes, or conjugates, they form is by steric occlusion of the interaction site between Ras and downstream effector molecules, such as RAF and PI3K, which are required for propagating the oncogenic signal.

As such, in some embodiments, the disclosure features a compound, or pharmaceutically acceptable salt thereof, of structural Formula I:



Formula I

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 10-membered heteroarylene;

B is absent, -CH(R^9)-, >C=C$R^9R^{9'}$, or >C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, a haloacetal, or an alkynyl sulfone;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', $C(O)N(R')_2$, S(O)R', $S(O)_2R'$, or $S(O)_2N(R')_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), $C=N(O\text{-}C_1\text{-}C_3$ alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally

substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is H, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl; or

R^9 and $R^{9'}$, combined with the atoms to which they are attached, form a 3 to 6-membered cycloalkyl or a 3 to 6-membered heterocycloalkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{21} is hydrogen or C_1-C_3 alkyl (e.g., methyl).

Also provided are pharmaceutical compositions comprising a compound of Formula I, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

Further provided is a conjugate, or salt thereof, comprising the structure of Formula IV:

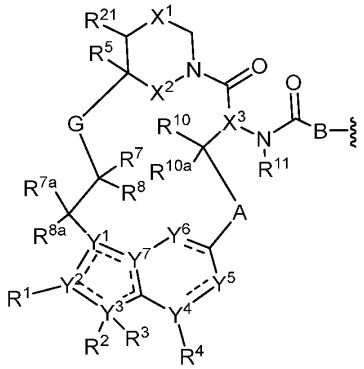
M-L-P

Formula IV

wherein L is a linker;

P is a monovalent organic moiety; and

M has the structure of Formula V:



Formula V

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-

membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is absent, -CH(R^9)-, >C=C$R^9R^{9'}$, or >C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or S(O)$_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is H, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl; or

R^9 and $R^{9'}$, combined with the atoms to which they are attached, form a 3 to 6-membered cycloalkyl or a 3 to 6-membered heterocycloalkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{21} is H or C_1-C_3 alkyl.

It is specifically contemplated that any limitation discussed with respect to one embodiment of the invention may apply to any other embodiment of the invention. Furthermore, any compound or composition of the invention may be used in any method of the invention, and any method of the invention may be used to produce or to utilize any compound or composition of the invention.

Brief Description of the Drawings

FIG. 1A and FIG. 1B: These figures illustrate a matched pair analysis of potencies of certain compounds of the present invention (Formula BB) (points on the right) and corresponding compounds of Formula AA (points on the left) wherein a H is replaced with (*S*)Me in the context of

two different cell-based assays. The y axes represent pERK EC50 (FIG. 1A) or CTG IC50 (FIG. 1B) as measured in an H358 cell line.

FIG. 2A and FIG. 2B: A compound of the present invention, Compound A, drove deep regressions in vivo in a NSCLC (KRAS G12C) xenograft model. Some animals exhibited complete responses (CR) = 3 consecutive tumor measurements ≤ 30 mm3. FIG. 2A shows Compound A dosed at 100 mg/kg by daily oral gavage led to tumor regression in NCI-H358 KRASG12C xenograft model, which is a sensitive model to KRASG12C inhibition alone. The spaghetti titer plot (FIG. 2B) displaying individual tumor growth is shown next to the tumor volume plot (FIG. 2A).

FIG. 3A and FIG. 3B: A compound of the present invention, Compound B, drove tumor xenograft regressions in combination with a MEK inhibitor, cobimetinib, in a NSCLC (KRAS G12C) model. FIG. 3A shows the combination of intermittent intravenous administration of Compound B at 50 mg/kg plus daily oral administration of cobimetinib at 2.5 mg/kg drove tumor regression, whereas each single agent led to tumor growth inhibition. End of study responses were shown as waterfall plots (FIG. 3B), which indicate 6 out 10 mice had tumor regression in the combination group, whereas no tumor regressions recorded in each single agent group.

FIG. 4A and FIG. 4B: A compound of the present invention, Compound C, dosed weekly with daily SHP2 inhibitor, RMC-4550, drove xenograft regressions in a NSCLC (KRAS G12C) model. In FIG. 4A, the combinatorial activity of once weekly intravenous administration of Compound C at 60 mg/kg plus daily oral administration of SHP2 inhibitor at 30 mg/kg is shown. End of study responses in individual tumors were plotted as a waterfall plot (FIG. 4B).

FIG. 5: A compound of the present invention, Compound D, combined with a MEK inhibitor, trametinib, suppressed in vitro growth durably in a long-term cell growth NSCLC (KRAS G12C) model.

Definitions and Chemical Terms

In this application, unless otherwise clear from context, (i) the term “a” means “one or more”; (ii) the term "or" is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternative are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or"; (iii) the terms “comprising” and “including” are understood to encompass itemized components or steps whether presented by themselves or together with one or more additional components or steps; and (iv) where ranges are provided, endpoints are included.

As used herein, the term “about” is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value. In certain embodiments, the term “about” refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of a stated value, unless otherwise stated or otherwise evident from the context (e.g., where such number would exceed 100% of a possible value).

As used herein, the term “adjacent” in the context of describing adjacent atoms refers to bivalent atoms that are directly connected by a covalent bond.

A "compound of the present invention" and similar terms as used herein, whether explicitly noted or not, refers to Ras inhibitors described herein, including compounds of Formula I and subformula thereof, and compounds of Table 1 and Table 2, as well as salts (e.g., pharmaceutically acceptable salts), solvates, hydrates, stereoisomers (including atropisomers), and tautomers thereof.

The term "wild-type" refers to an entity having a structure or activity as found in nature in a "normal" (as contrasted with mutant, diseased, altered, etc) state or context. Those of ordinary skill in the art will appreciate that wild-type genes and polypeptides often exist in multiple different forms (e.g., alleles).

Those skilled in the art will appreciate that certain compounds described herein can exist in one or more different isomeric (e.g., stereoisomers, geometric isomers, atropisomers, tautomers) or isotopic (e.g., in which one or more atoms has been substituted with a different isotope of the atom, such as hydrogen substituted for deuterium) forms. Unless otherwise indicated or clear from context, a depicted structure can be understood to represent any such isomeric or isotopic form, individually or in combination.

Compounds described herein can be asymmetric (e.g., having one or more stereocenters). All stereoisomers, such as enantiomers and diastereomers, are intended unless otherwise indicated. Compounds of the present disclosure that contain asymmetrically substituted carbon atoms can be isolated in optically active or racemic forms. Methods on how to prepare optically active forms from optically active starting materials are known in the art, such as by resolution of racemic mixtures or by stereoselective synthesis. Many geometric isomers of olefins, C=N double bonds, and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present disclosure. Cis and trans geometric isomers of the compounds of the present disclosure are described and may be isolated as a mixture of isomers or as separated isomeric forms.

In some embodiments, one or more compounds depicted herein may exist in different tautomeric forms. As will be clear from context, unless explicitly excluded, references to such compounds encompass all such tautomeric forms. In some embodiments, tautomeric forms result from the swapping of a single bond with an adjacent double bond and the concomitant migration of a proton. In certain embodiments, a tautomeric form may be a prototropic tautomer, which is an isomeric protonation states having the same empirical formula and total charge as a reference form. Examples of moieties with prototropic tautomeric forms are ketone - enol pairs, amide - imidic acid pairs, lactam - lactim pairs, amide - imidic acid pairs, enamine - imine pairs, and annular forms where a proton can occupy two or more positions of a heterocyclic system, such as, 1H- and 3H-imidazole, 1H-, 2H- and 4H-1,2,4-triazole, 1H- and 2H- isoindole, and 1H- and 2H-pyrazole. In some embodiments, tautomeric forms can be in equilibrium or sterically locked into one form by appropriate substitution. In certain embodiments, tautomeric forms result from acetal interconversion.

Unless otherwise stated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. Exemplary isotopes that can be incorporated into compounds of the present invention include isotopes of hydrogen, carbon,

nitrogen, oxygen, phosphorus, sulfur, fluorine, chlorine, and iodine, such as ^{2}H, ^{3}H, ^{11}C, ^{13}C, ^{14}C, ^{13}N, ^{15}N, ^{15}O, ^{17}O, ^{18}O, ^{32}P, ^{33}P, ^{35}S, ^{18}F, ^{36}Cl, ^{123}I and ^{125}I. Isotopically-labeled compounds (e.g., those labeled with ^{3}H and ^{14}C) can be useful in compound or substrate tissue distribution assays. Tritiated (i.e., ^{3}H) and carbon-14 (i.e., ^{14}C) isotopes can be useful for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium (i.e., ^{2}H) may afford certain therapeutic advantages resulting from greater metabolic stability (e.g., increased in vivo half-life or reduced dosage requirements). In some embodiments, one or more hydrogen atoms are replaced by ^{2}H or ^{3}H, or one or more carbon atoms are replaced by ^{13}C- or ^{14}C-enriched carbon. Positron emitting isotopes such as ^{15}O, ^{13}N, ^{11}C, and ^{18}F are useful for positron emission tomography (PET) studies to examine substrate receptor occupancy. Preparations of isotopically labelled compounds are known to those of skill in the art. For example, isotopically labeled compounds can generally be prepared by following procedures analogous to those disclosed for compounds of the present invention described herein, by substituting an isotopically labeled reagent for a non-isotopically labeled reagent.

As is known in the art, many chemical entities can adopt a variety of different solid forms such as, for example, amorphous forms or crystalline forms (e.g., polymorphs, hydrates, solvate). In some embodiments, compounds of the present invention may be utilized in any such form, including in any solid form. In some embodiments, compounds described or depicted herein may be provided or utilized in hydrate or solvate form.

At various places in the present specification, substituents of compounds of the present disclosure are disclosed in groups or in ranges. It is specifically intended that the present disclosure include each and every individual subcombination of the members of such groups and ranges. For example, the term "C_1-C_6 alkyl" is specifically intended to individually disclose methyl, ethyl, C_3 alkyl, C_4 alkyl, C_5 alkyl, and C_6 alkyl. Furthermore, where a compound includes a plurality of positions at which substituents are disclosed in groups or in ranges, unless otherwise indicated, the present disclosure is intended to cover individual compounds and groups of compounds (e.g., genera and subgenera) containing each and every individual subcombination of members at each position.

The term "optionally substituted X" (e.g., "optionally substituted alkyl") is intended to be equivalent to "X, wherein X is optionally substituted" (e.g., "alkyl, wherein said alkyl is optionally substituted"). It is not intended to mean that the feature "X" (e.g., alkyl) *per se* is optional. As described herein, certain compounds of interest may contain one or more "optionally substituted" moieties. In general, the term "substituted", whether preceded by the term "optionally" or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent, e.g., any of the substituents or groups described herein. Unless otherwise indicated, an "optionally substituted" group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. For example, in the term "optionally substituted C_1-C_6 alkyl-C_2-C_9 heteroaryl," the alkyl portion, the heteroaryl portion, or both, may be optionally substituted. Combinations of substituents envisioned by the present disclosure are preferably those that result

in the formation of stable or chemically feasible compounds. The term "stable", as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain embodiments, their recovery, purification, and use for one or more of the purposes disclosed herein.

Suitable monovalent substituents on a substitutable carbon atom of an "optionally substituted" group may be, independently, deuterium; halogen; $-(CH_2)_{0-4}R^\circ$; $-(CH_2)_{0-4}OR^\circ$; $-O(CH_2)_{0-4}R^\circ$; $-O-(CH_2)_{0-4}C(O)OR^\circ$; $-(CH_2)_{0-4}CH(OR^\circ)_2$; $-(CH_2)_{0-4}SR^\circ$; $-(CH_2)_{0-4}Ph$, which may be substituted with R°; $-(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ which may be substituted with R°; $-CH=CHPh$, which may be substituted with R°; $-(CH_2)_{0-4}O(CH_2)_{0-1}$-pyridyl which may be substituted with R°; 4-8 membered saturated or unsaturated heterocycloalkyl (e.g., pyridyl); 3-8 membered saturated or unsaturated cycloalkyl (e.g., cyclopropyl, cyclobutyl, or cyclopentyl); $-NO_2$; $-CN$; $-N_3$; $-(CH_2)_{0-4}N(R^\circ)_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)R^\circ$; $-N(R^\circ)C(S)R^\circ$; $-(CH_2)_{0-4}N(R^\circ)C(O)NR^\circ_2$; $-N(R^\circ)C(S)NR^\circ_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)OR^\circ$; $-N(R^\circ)N(R^\circ)C(O)R^\circ$; $-N(R^\circ)N(R^\circ)C(O)NR^\circ_2$; $-N(R^\circ)N(R^\circ)C(O)OR^\circ$; $-(CH_2)_{0-4}C(O)R^\circ$; $-C(S)R^\circ$; $-(CH_2)_{0-4}C(O)OR^\circ$; $-(CH_2)_{0-4}$-$C(O)$-$N(R^o)_2$; $-(CH_2)_{0-4}$-$C(O)$-$N(R^o)$-$S(O)_2$-R^o; $-C(NCN)NR^\circ_2$; $-(CH_2)_{0-4}C(O)SR^\circ$; $-(CH_2)_{0-4}C(O)OSiR^\circ_3$; $-(CH_2)_{0-4}OC(O)R^\circ$; $-OC(O)(CH_2)_{0-4}SR^\circ$; $-SC(S)SR^\circ$; $-(CH_2)_{0-4}SC(O)R^\circ$; $-(CH_2)_{0-4}C(O)NR^\circ_2$; $-C(S)NR^\circ_2$; $-C(S)SR^\circ$; $-(CH_2)_{0-4}OC(O)NR^\circ_2$; $-C(O)N(OR^\circ)R^\circ$; $-C(O)C(O)R^\circ$; $-C(O)CH_2C(O)R^\circ$; $-C(NOR^\circ)R^\circ$; $-(CH_2)_{0-4}SSR^\circ$; $-(CH_2)_{0-4}S(O)_2R^\circ$; $-(CH_2)_{0-4}S(O)_2OR^\circ$; $-(CH_2)_{0-4}OS(O)_2R^\circ$; $-S(O)_2NR^\circ_2$; $-(CH_2)_{0-4}S(O)R^\circ$; $-N(R^\circ)S(O)_2NR^\circ_2$; $-N(R^\circ)S(O)_2R^\circ$; $-N(OR^\circ)R^\circ$; $-C(NOR^\circ)NR^\circ_2$; $-C(NH)NR^\circ_2$; $-P(O)_2R^\circ$; $-P(O)R^\circ_2$; $-P(O)(OR^\circ)_2$; $-OP(O)R^\circ_2$; $-OP(O)(OR^\circ)_2$; $-OP(O)(OR^\circ)R^\circ$, $-SiR^\circ_3$; $-(C_{1-4}$ straight or branched alkylene$)O-N(R^\circ)_2$; or $-(C_{1-4}$ straight or branched alkylene$)C(O)O-N(R^\circ)_2$, wherein each R° may be substituted as defined below and is independently hydrogen, $-C_{1-6}$ aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, $-CH_2$-(5-6 membered heteroaryl ring), or a 3-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of R°, taken together with their intervening atom(s), form a 3-12-membered saturated, partially unsaturated, or aryl mono- or bicyclic ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, which may be substituted as defined below.

Suitable monovalent substituents on R° (or the ring formed by taking two independent occurrences of R° together with their intervening atoms), may be, independently, halogen, $-(CH_2)_{0-2}R^\bullet$, $-(haloR^\bullet)$, $-(CH_2)_{0-2}OH$, $-(CH_2)_{0-2}OR^\bullet$, $-(CH_2)_{0-2}CH(OR^\bullet)_2$; $-O(haloR^\bullet)$, $-CN$, $-N_3$, $-(CH_2)_{0-2}C(O)R^\bullet$, $-(CH_2)_{0-2}C(O)OH$, $-(CH_2)_{0-2}C(O)OR^\bullet$, $-(CH_2)_{0-2}SR^\bullet$, $-(CH_2)_{0-2}SH$, $-(CH_2)_{0-2}NH_2$, $-(CH_2)_{0-2}NHR^\bullet$, $-(CH_2)_{0-2}NR^\bullet_2$, $-NO_2$, $-SiR^\bullet_3$, $-OSiR^\bullet_3$, $-C(O)SR^\bullet$, $-(C_{1-4}$ straight or branched alkylene$)C(O)OR^\bullet$, or $-SSR^\bullet$ wherein each $R^\bullet$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently selected from C_{1-4} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents on a saturated carbon atom of R° include =O and =S.

Suitable divalent substituents on a saturated carbon atom of an "optionally substituted" group include the following: =O, =S, =NNR*$_2$, =NNHC(O)R*, =NNHC(O)OR*, =NNHS(O)$_2$R*, =NR*, =NOR*, -O(C(R*$_2$))$_{2-3}$O-, or -S(C(R*$_2$))$_{2-3}$S-, wherein each independent occurrence of R* is selected from hydrogen, C$_{1-6}$ aliphatic which may be substituted as defined below, or an unsubstituted 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents that are bound to vicinal substitutable carbons of an "optionally substituted" group include: -O(CR*$_2$)$_{2-3}$O-, wherein each independent occurrence of R* is selected from hydrogen, C$_{1-6}$ aliphatic which may be substituted as defined below, or an unsubstituted 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

Suitable substituents on the aliphatic group of R* include halogen, -R•, -(haloR•), -OH, -OR•, -O(haloR•), -CN, -C(O)OH, -C(O)OR•, -NH$_2$, -NHR•, -NR•$_2$, or -NO$_2$, wherein each R• is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C$_{1-4}$ aliphatic, -CH$_2$Ph, -O(CH$_2$)$_{0-1}$Ph, or a 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

Suitable substituents on a substitutable nitrogen of an "optionally substituted" group include -R†, -NR†$_2$, -C(O)R†, -C(O)OR†, -C(O)C(O)R†, -C(O)CH$_2$C(O)R†, -S(O)$_2$R†, -S(O)$_2$NR†$_2$, -C(S)NR†$_2$, -C(NH)NR†$_2$, or -N(R†)S(O)$_2$R†; wherein each R† is independently hydrogen, C$_{1-6}$ aliphatic which may be substituted as defined below, unsubstituted -OPh, or an unsubstituted 3-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of R†, taken together with their intervening atom(s) form an unsubstituted 3-12-membered saturated, partially unsaturated, or aryl mono- or bicyclic ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

Suitable substituents on an aliphatic group of R† are independently halogen, -R•, -(haloR•), -OH, -OR•, -O(haloR•), -CN, -C(O)OH, -C(O)OR•, -NH$_2$, -NHR•, -NR•$_2$, or -NO$_2$, wherein each R• is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C$_{1-4}$ aliphatic, -CH$_2$Ph, -O(CH$_2$)$_{0-1}$Ph, or a 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents on a saturated carbon atom of R† include =O and =S.

The term "acetyl," as used herein, refers to the group -C(O)CH$_3$.

The term "alkoxy," as used herein, refers to a -O-C$_1$-C$_{20}$ alkyl group, wherein the alkoxy group is attached to the remainder of the compound through an oxygen atom.

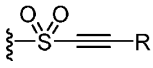
The term "alkyl," as used herein, refers to a saturated, straight or branched monovalent hydrocarbon group containing from 1 to 20 (e.g., from 1 to 10 or from 1 to 6) carbons. In some embodiments, an alkyl group is unbranched (i.e., is linear); in some embodiments, an alkyl group is

branched. Alkyl groups are exemplified by, but not limited to, methyl, ethyl, *n*- and *iso*-propyl, *n*-, *sec*-, *iso*- and *tert*-butyl, and neopentyl.

The term "alkylene," as used herein, represents a saturated divalent hydrocarbon group derived from a straight or branched chain saturated hydrocarbon by the removal of two hydrogen atoms, and is exemplified by methylene, ethylene, isopropylene, and the like. The term "C_x-C_y alkylene" represents alkylene groups having between x and y carbons. Exemplary values for x are 1, 2, 3, 4, 5, and 6, and exemplary values for y are 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, or 20 (e.g., C_1-C_6, C_1-C_{10}, C_2-C_{20}, C_2-C_6, C_2-C_{10}, or C_2-C_{20} alkylene). In some embodiments, the alkylene can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein.

The term "alkenyl," as used herein, represents monovalent straight or branched chain groups of, unless otherwise specified, from 2 to 20 carbons (e.g., from 2 to 6 or from 2 to 10 carbons) containing one or more carbon-carbon double bonds and is exemplified by ethenyl, 1-propenyl, 2-propenyl, 2-methyl-1-propenyl, 1-butenyl, and 2-butenyl. Alkenyls include both cis and trans isomers. The term "alkenylene," as used herein, represents a divalent straight or branched chain groups of, unless otherwise specified, from 2 to 20 carbons (e.g., from 2 to 6 or from 2 to 10 carbons) containing one or more carbon-carbon double bonds.

The term "alkynyl," as used herein, represents monovalent straight or branched chain groups from 2 to 20 carbon atoms (e.g., from 2 to 4, from 2 to 6, or from 2 to 10 carbons) containing a carbon-carbon triple bond and is exemplified by ethynyl, and 1-propynyl.

The term "alkynyl sulfone," as used herein, represents a group comprising the structure  , wherein R is any chemically feasible substituent described herein.

The term "amino," as used herein, represents -N(R^t)$_2$, e.g., -NH_2 and -N(CH_3)$_2$.

The term "aminoalkyl," as used herein, represents an alkyl moiety substituted on one or more carbon atoms with one or more amino moieties.

The term "amino acid," as described herein, refers to a molecule having a side chain, an amino group, and an acid group (e.g., -CO_2H or -SO_3H), wherein the amino acid is attached to the parent molecular group by the side chain, amino group, or acid group (e.g., the side chain). As used herein, the term "amino acid" in its broadest sense, refers to any compound or substance that can be incorporated into a polypeptide chain, e.g., through formation of one or more peptide bonds. In some embodiments, an amino acid has the general structure H_2N-C(H)(R)-COOH. In some embodiments, an amino acid is a naturally-occurring amino acid. In some embodiments, an amino acid is a synthetic amino acid; in some embodiments, an amino acid is a D-amino acid; in some embodiments, an amino acid is an L-amino acid. "Standard amino acid" refers to any of the twenty standard L-amino acids commonly found in naturally occurring peptides. Exemplary amino acids include alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, optionally substituted hydroxylnorvaline, isoleucine, leucine, lysine, methionine, norvaline, ornithine, phenylalanine, proline, pyrrolysine, selenocysteine, serine, taurine, threonine, tryptophan, tyrosine, and valine.

The term "aryl," as used herein, represents a monovalent monocyclic, bicyclic, or multicyclic ring system formed by carbon atoms, wherein the ring attached to the pendant group is

aromatic. Examples of aryl groups are phenyl, naphthyl, phenanthrenyl, and anthracenyl. An aryl ring can be attached to its pendant group at any heteroatom or carbon ring atom that results in a stable structure and any of the ring atoms can be optionally substituted unless otherwise specified.

The term "C_0," as used herein, represents a bond. For example, part of the term -N(C(O)-(C_0-C_5 alkylene-H)- includes -N(C(O)-(C_0 alkylene-H)-, which is also represented by -N(C(O)-H)-.

The terms "carbocyclic" and "carbocyclyl," as used herein, refer to a monovalent, optionally substituted C_3-C_{12} monocyclic, bicyclic, or tricyclic ring structure, which may be bridged, fused or spirocyclic, in which all the rings are formed by carbon atoms and at least one ring is non-aromatic. Carbocyclic structures include cycloalkyl, cycloalkenyl, and cycloalkynyl groups. Examples of carbocyclyl groups are cyclohexyl, cyclohexenyl, cyclooctynyl, 1,2-dihydronaphthyl, 1,2,3,4-tetrahydronaphthyl, fluorenyl, indenyl, indanyl, decalinyl, and the like. A carbocyclic ring can be attached to its pendant group at any ring atom that results in a stable structure and any of the ring atoms can be optionally substituted unless otherwise specified.

The term "carbonyl," as used herein, represents a C(O) group, which can also be represented as C=O.

The term "carboxyl," as used herein, means -CO_2H, (C=O)(OH), COOH, or C(O)OH or the unprotonated counterparts.

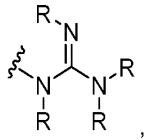
The term "cyano," as used herein, represents a -CN group.

The term "cycloalkyl," as used herein, represents a monovalent saturated cyclic hydrocarbon group, which may be bridged, fused or spirocyclic having from three to eight ring carbons, unless otherwise specified, and is exemplified by cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cycloheptyl.

The term "cycloalkenyl," as used herein, represents a monovalent, non-aromatic, saturated cyclic hydrocarbon group, which may be bridged, fused or spirocyclic having from three to eight ring carbons, unless otherwise specified, and containing one or more carbon-carbon double bonds.

The term "diastereomer," as used herein, means stereoisomers that are not mirror images of one another and are non-superimposable on one another.

The term "enantiomer," as used herein, means each individual optically active form of a compound of the invention, having an optical purity or enantiomeric excess (as determined by methods standard in the art) of at least 80% (i.e., at least 90% of one enantiomer and at most 10% of the other enantiomer), preferably at least 90% and more preferably at least 98%.

The term "guanidinyl," refers to a group having the structure:  , wherein each R is, independently, any any chemically feasible substituent described herein.

The term "guanidinoalkyl alkyl," as used herein, represents an alkyl moiety substituted on one or more carbon atoms with one or more guanidinyl moieties.

The term "haloacetyl," as used herein, refers to an acetyl group wherein at least one of the hydrogens has been replaced by a halogen.

The term "haloalkyl," as used herein, represents an alkyl moiety substituted on one or more carbon atoms with one or more of the same of different halogen moieties.

The term "halogen," as used herein, represents a halogen selected from bromine, chlorine, iodine, or fluorine.

The term "heteroalkyl," as used herein, refers to an "alkyl" group, as defined herein, in which at least one carbon atom has been replaced with a heteroatom (e.g., an O, N, or S atom). The heteroatom may appear in the middle or at the end of the radical.

The term "heteroaryl," as used herein, represents a monovalent, monocyclic or polycyclic ring structure that contains at least one fully aromatic ring: i.e., they contain $4n+2$ pi electrons within the monocyclic or polycyclic ring system and contains at least one ring heteroatom selected from N, O, or S in that aromatic ring. Exemplary unsubstituted heteroaryl groups are of 1 to 12 (e.g., 1 to 11, 1 to 10, 1 to 9, 2 to 12, 2 to 11, 2 to 10, or 2 to 9) carbons. The term "heteroaryl" includes bicyclic, tricyclic, and tetracyclic groups in which any of the above heteroaromatic rings is fused to one or more, aryl or carbocyclic rings, e.g., a phenyl ring, or a cyclohexane ring. Examples of heteroaryl groups include, but are not limited to, pyridyl, pyrazolyl, benzooxazolyl, benzoimidazolyl, benzothiazolyl, imidazolyl, thiazolyl, quinolinyl, tetrahydroquinolinyl, and 4-azaindolyl. A heteroaryl ring can be attached to its pendant group at any ring atom that results in a stable structure and any of the ring atoms can be optionally substituted unless otherwise specified. In some embodiment, the heteroaryl is substituted with 1, 2, 3, or 4 substituents groups.

The term "heterocycloalkyl," as used herein, represents a monovalent monocyclic, bicyclic or polycyclic ring system, which may be bridged, fused or spirocyclic, wherein at least one ring is non-aromatic and wherein the non-aromatic ring contains one, two, three, or four heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur. The 5-membered ring has zero to two double bonds, and the 6- and 7-membered rings have zero to three double bonds. Exemplary unsubstituted heterocycloalkyl groups are of 1 to 12 (e.g., 1 to 11, 1 to 10, 1 to 9, 2 to 12, 2 to 11, 2 to 10, or 2 to 9) carbons. The term "heterocycloalkyl" also represents a heterocyclic compound having a bridged multicyclic structure in which one or more carbons or heteroatoms bridges two non-adjacent members of a monocyclic ring, e.g., a quinuclidinyl group. The term "heterocycloalkyl" includes bicyclic, tricyclic, and tetracyclic groups in which any of the above heterocyclic rings is fused to one or more aromatic, carbocyclic, heteroaromatic, or heterocyclic rings, e.g., an aryl ring, a cyclohexane ring, a cyclohexene ring, a cyclopentane ring, a cyclopentene ring, a pyridine ring, or a pyrrolidine ring. Examples of heterocycloalkyl groups are pyrrolidinyl, piperidinyl, 1,2,3,4-tetrahydroquinolinyl, decahydroquinolinyl, dihydropyrrolopyridine, and decahydronapthyridinyl. A heterocycloalkyl ring can be attached to its pendant group at any ring atom that results in a stable structure and any of the ring atoms can be optionally substituted unless otherwise specified.

The term "hydroxy," as used herein, represents a -OH group.

The term "hydroxyalkyl," as used herein, represents an alkyl moiety substituted on one or more carbon atoms with one or more -OH moieties.

The term "isomer," as used herein, means any tautomer, stereoisomer, atropiosmer, enantiomer, or diastereomer of any compound of the invention. It is recognized that the

compounds of the invention can have one or more chiral centers or double bonds and, therefore, exist as stereoisomers, such as double-bond isomers (i.e., geometric E/Z isomers) or diastereomers (e.g., enantiomers (i.e., (+) or (-)) or cis/trans isomers). According to the invention, the chemical structures depicted herein, and therefore the compounds of the invention, encompass all the corresponding stereoisomers, that is, both the stereomerically pure form (e.g., geometrically pure, enantiomerically pure, or diastereomerically pure) and enantiomeric and stereoisomeric mixtures, e.g., racemates. Enantiomeric and stereoisomeric mixtures of compounds of the invention can typically be resolved into their component enantiomers or stereoisomers by well-known methods, such as chiral-phase gas chromatography, chiral-phase high performance liquid chromatography, crystallizing the compound as a chiral salt complex, or crystallizing the compound in a chiral solvent. Enantiomers and stereoisomers can also be obtained from stereomerically or enantiomerically pure intermediates, reagents, and catalysts by well-known asymmetric synthetic methods.

As used herein, the term “linker” refers to a divalent organic moiety connecting moiety B to moiety W in a compound of Formula I, such that the resulting compound is capable of achieving an IC50 of 2 uM or less in the Ras-RAF disruption assay protocol provided in the Examples below, and provided here:

The purpose of this biochemical assay is to measure the ability of test compounds to facilitate ternary complex formation between a nucleotide-loaded Ras isoform and cyclophilin A; the resulting ternary complex disrupts binding to a $BRAF^{RBD}$ construct, inhibiting Ras signaling through a RAF effector.

In assay buffer containing 25 mM HEPES pH 7.3, 0.002% Tween20, 0.1% BSA, 100 mM NaCl and 5 mM $MgCl_2$, tagless Cyclophilin A, His6-K-Ras-GMPPNP (or other Ras variant), and GST-$BRAF^{RBD}$ are combined in a 384-well assay plate at final concentrations of 25 µM, 12.5 nM and 50 nM, respectively. Compound is present in plate wells as a 10-point 3-fold dilution series starting at a final concentration of 30 µM. After incubation at 25°C for 3 hours, a mixture of Anti-His Eu-W1024 and anti-GST allophycocyanin is then added to assay sample wells at final concentrations of 10 nM and 50 nM, respectively, and the reaction incubated for an additional 1.5 hours. TR-FRET signal is read on a microplate reader (Ex 320 nm, Em 665/615 nm). Compounds that facilitate disruption of a Ras:RAF complex are identified as those eliciting a decrease in the TR-FRET ratio relative to DMSO control wells.

In some embodiments, the linker comprises 20 or fewer linear atoms. In some embodiments, the linker comprises 15 or fewer linear atoms. In some embodiments, the linker comprises 10 or fewer linear atoms. In some embodiments, the linker has a molecular weight of under 500 g/mol. In some embodiments, the linker has a molecular weight of under 400 g/mol. In some embodiments, the linker has a molecular weight of under 300 g/mol. In some embodiments, the linker has a molecular weight of under 200 g/mol. In some embodiments, the linker has a molecular weight of under 100 g/mol. In some embodiments, the linker has a molecular weight of under 50 g/mol.

As used herein, a “monovalent organic moiety” is less than 500 kDa. In some embodiments, a “monovalent organic moiety” is less than 400 kDa. In some embodiments, a

"monovalent organic moiety" is less than 300 kDa. In some embodiments, a "monovalent organic moiety" is less than 200 kDa. In some embodiments, a "monovalent organic moiety" is less than 100 kDa. In some embodiments, a "monovalent organic moiety" is less than 50 kDa. In some embodiments, a "monovalent organic moiety" is less than 25 kDa. In some embodiments, a "monovalent organic moiety" is less than 20 kDa. In some embodiments, a "monovalent organic moiety" is less than 15 kDa. In some embodiments, a "monovalent organic moiety" is less than 10 kDa. In some embodiments, a "monovalent organic moiety" is less than 1 kDa. In some embodiments, a "monovalent organic moiety" is less than 500 g/mol. In some embodiments, a "monovalent organic moiety" ranges between 500 g/mol and 500 kDa.

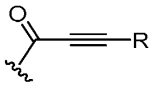
The term "stereoisomer," as used herein, refers to all possible different isomeric as well as conformational forms which a compound may possess (e.g., a compound of any formula described herein), in particular all possible stereochemically and conformationally isomeric forms, all diastereomers, enantiomers or conformers of the basic molecular structure, including atropisomers. Some compounds of the present invention may exist in different tautomeric forms, all of the latter being included within the scope of the present invention.

The term "sulfonyl," as used herein, represents an $-S(O)_2-$ group.

The term "thiocarbonyl," as used herein, refers to a -C(S)- group.

The term "vinyl ketone," as used herein, refers to a group comprising a carbonyl group directly connected to a carbon-carbon double bond.

The term "vinyl sulfone," as used herein, refers to a group comprising a sulfonyl group directed connected to a carbon-carbon double bond.

The term "ynone," as used herein, refers to a group comprising the structure 

, wherein R is any any chemically feasible substituent described herein.

Those of ordinary skill in the art, reading the present disclosure, will appreciate that certain compounds described herein may be provided or utilized in any of a variety of forms such as, for example, salt forms, protected forms, pro-drug forms, ester forms, isomeric forms (e.g., optical or structural isomers), isotopic forms, etc. In some embodiments, reference to a particular compound may relate to a specific form of that compound. In some embodiments, reference to a particular compound may relate to that compound in any form. In some embodiments, for example, a preparation of a single stereoisomer of a compound may be considered to be a different form of the compound than a racemic mixture of the compound; a particular salt of a compound may be considered to be a different form from another salt form of the compound; a preparation containing one conformational isomer ((Z) or (E)) of a double bond may be considered to be a different form from one containing the other conformational isomer ((E) or (Z)) of the double bond; a preparation in which one or more atoms is a different isotope than is present in a reference preparation may be considered to be a different form.

Detailed Description

Compounds

Provided herein are Ras inhibitors. The approach described herein entails formation of a high affinity three-component complex, or conjugate, between a synthetic ligand and two intracellular proteins which do not interact under normal physiological conditions: the target protein of interest (e.g., Ras), and a widely expressed cytosolic chaperone (presenter protein) in the cell (e.g., cyclophilin A). More specifically, in some embodiments, the inhibitors of Ras described herein induce a new binding pocket in Ras by driving formation of a high affinity tri-complex, or conjugate, between the Ras protein and the widely expressed cytosolic chaperone, cyclophilin A (CYPA). Without being bound by theory, the inventors believe that one way the inhibitory effect on Ras is effected by compounds of the invention and the complexes, or conjugates, they form is by steric occlusion of the interaction site between Ras and downstream effector molecules, such as RAF, which are required for propagating the oncogenic signal.

Without being bound by theory, the inventors postulate that both covalent and non-covalent interactions of a compound of the present invention with Ras and the chaperone protein (e.g., cyclophilin A) may contribute to the inhibition of Ras activity. In some embodiments, a compound of the present invention forms a covalent adduct with a side chain of a Ras protein (e.g., a sulfhydryl side chain of the cysteine at position 12 or 13 of a mutant Ras protein). Covalent adducts may also be formed with other side chains of Ras. In addition, or alternatively, non-covalent interactions may be at play: for example, van der Waals, hydrophobic, hydrophilic and hydrogen bond interactions, and combinations thereof, may contribute to the ability of the compounds of the present invention to form complexes and act as Ras inhibitors. Accordingly, a variety of Ras proteins may be inhibited by compounds of the present invention (e.g., K-Ras, N-Ras, H-Ras, and mutants thereof at positions 12, 13 and 61, such as G12C, G12D, G12V, G12S, G13C, G13D, and Q61L, and others described herein).

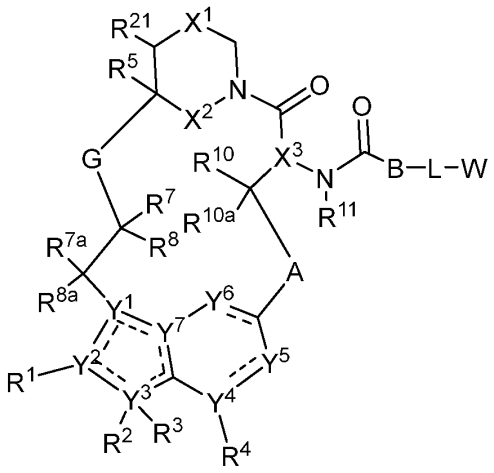
Methods of determining covalent adduct formation are known in the art. One method of determining covalent adduct formation is to perform a “cross-linking” assay, such as under these conditions (*Note – the following protocol describes a procedure for monitoring cross-linking of K-Ras G12C (GMP-PNP) to a compound of the invention. This protocol may also be executed substituting other Ras proteins or nucleotides).*

The purpose of this biochemical assay is to measure the ability of test compounds to covalently label *nucleotide*-loaded K-Ras isoforms. In assay buffer containing 12.5 mM HEPES pH 7.4, 75 mM NaCl, 1 mM $MgCl_2$, 1 mM BME, 5 µM Cyclophilin A and 2 µM test compound, a 5 µM stock of GMP-PNP-loaded K-Ras (1-169) G12C is diluted 10-fold to yield a final concentration of 0.5 µM; with final sample volume being 100 µL.

The sample is incubated at 25°C for a time period of up to 24 hours prior to quenching by the addition of 10 µL of 5% Formic Acid. Quenched samples are centrifuged at 15000 rpm for 15 minutes in a benchtop centrifuge before injecting a 10 µL aliquot onto a reverse phase C4 column and eluting into the mass spectrometer with an increasing acetonitrile gradient in the mobile phase.

Analysis of raw data may be carried out using Waters MassLynx MS software, with % bound calculated from the deconvoluted protein peaks for labeled and unlabeled K-Ras.

Accordingly, provided herein is a compound, or pharmaceutically acceptable salt thereof, having the structure of Formula I:



Formula I

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 10-membered heteroarylene;

B is absent, -CH(R^9)-, >C=C$R^9R^{9'}$, or >C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, a haloacetal, or an alkynyl sulfone;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or S(O)$_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', $C(O)N(R')_2$, S(O)R', $S(O)_2R'$, or $S(O)_2N(R')_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-

membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is H, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl; or

R^9 and $R^{9'}$, combined with the atoms to which they are attached, form a 3 to 6-membered cycloalkyl or a 3 to 6-membered heterocycloalkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo;

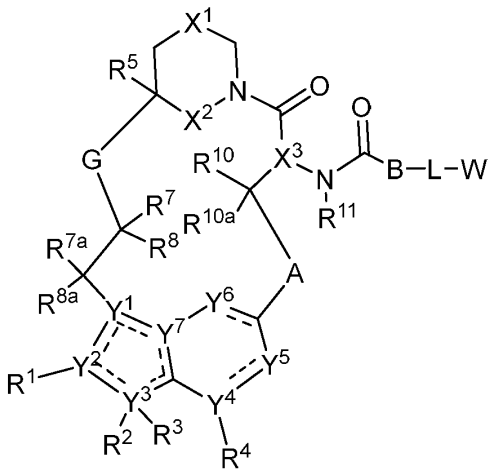
R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{21} is hydrogen or C_1-C_3 alkyl (e.g., methyl).

In some embodiments, R^9 is H, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

In some embodiments, R^{21} is hydrogen.

In some embodiments, provided herein is a compound, or pharmaceutically acceptable salt thereof, having the structure of Formula Ia:



Formula Ia

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-

membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 10-membered heteroarylene;

B is -CH(R^9)- or >C=C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, a haloacetal, or an alkynyl sulfone;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or S(O)$_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH$_2$, or N;

Y^6 is C(O), CH, CH$_2$, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

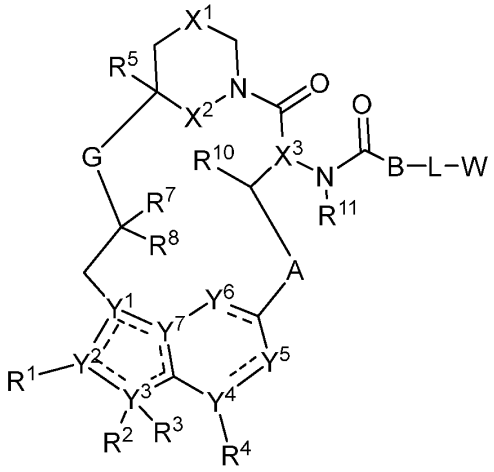
$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo; and

R^{11} is hydrogen or C_1-C_3 alkyl.

In some embodiments, the disclosure features a compound, or pharmaceutically acceptable salt thereof, of structural Formula Ib:



Formula Ib

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, a haloacetal, or an alkynyl sulfone;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

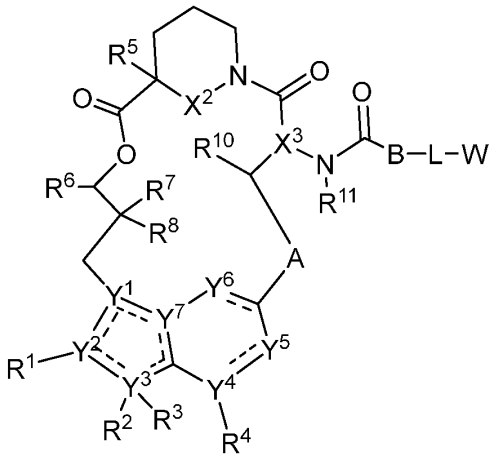
R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl; and

R^{11} is hydrogen or C_1-C_3 alkyl.

In some embodiments of compounds of the present invention, G is optionally substituted C_1-C_4 heteroalkylene.

In some embodiments, a compound having the structure of Formula Ic is provided, or a pharmaceutically acceptable salt thereof:



Formula Ic

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered

cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

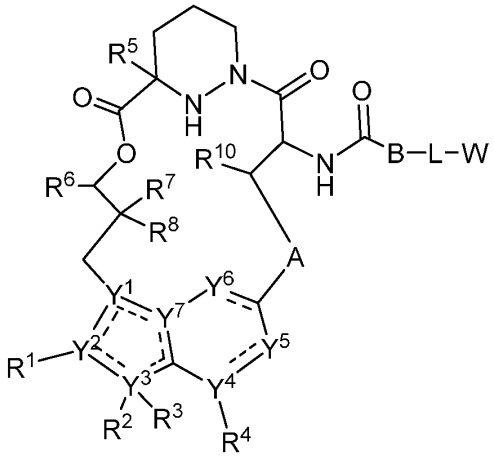
R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl; and

R^{11} is hydrogen or C_1-C_3 alkyl.

In some embodiments of compounds of the present invention, X^2 is NH. In some embodiments, X^3 is CH. In some embodiments, R^{11} is hydrogen. In some embodiments, R^{11} is C_1-C_3 alkyl. In some embodiments, R^{11} is methyl.

In some embodiments, a compound of the present invention has the structure of Formula Id, or a pharmaceutically acceptable salt thereof:



Formula Id

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

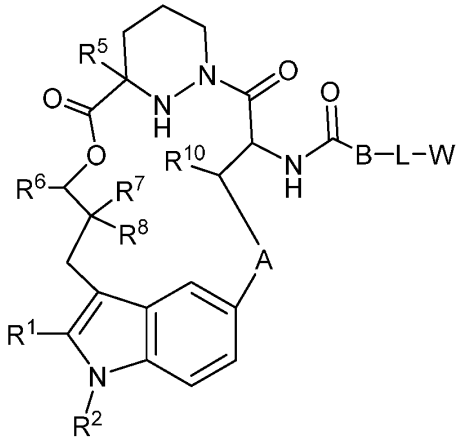
R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; and

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl.

In some embodiments of a compound of the present invention, X^1 is optionally substituted C_1-C_2 alkylene. In some embodiments, X^1 is methylene. In some embodiments, X^1 is methylene substituted with a C_1-C_6 alkyl group or a halogen. In some embodiments, X^1 is -CH(Br)-. In some embodiments, X^1 is -CH(CH_3)-. In some embodiments, R^5 is hydrogen. In some embodiments, R^5 is C_1-C_4 alkyl optionally substituted with halogen. In some embodiments, R^5 is methyl. In some embodiments, Y^4 is C. In some embodiments, R^4 is hydrogen. In some embodiments, Y^5 is CH.

In some embodiments, Y^6 is CH. In some embodiments, Y^1 is C. In some embodiments, Y^2 is C. In some embodiments, Y^3 is N. In some embodiments, R^3 is absent. In some embodiments, Y^7 is C.

In some embodiments, a compound of the present invention has the structure of Formula Ie, or a pharmaceutically acceptable salt thereof:



Formula Ie

wherein A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

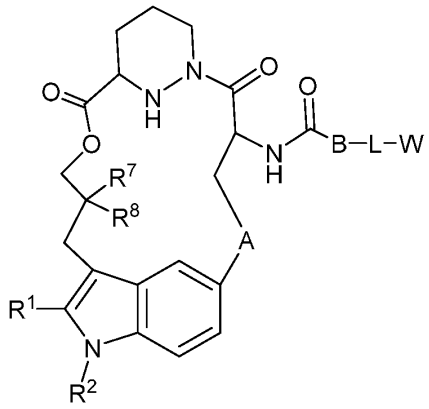
$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; and

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl.

In some embodiments of a compound of the present invention, R^6 is hydrogen. In some embodiments, R^2 is hydrogen, cyano, optionally substituted C_1-C_6 alkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 6-membered heterocycloalkyl. In some embodiments, R^2 is optionally substituted C_1-C_6 alkyl. In some embodiments, R_2 is fluoroalkyl. In some embodiments, R^2 is ethyl. In some embodiments, R_2 is $-CH_2CF_3$. In some embodiments, R_2 is C_2-C_6 alkynyl. In some embodiments, R_2 is -CHC≡CH. In some embodiments, R_2 is $-CH_2C{\equiv}CCH_3$. In some embodiments, R^7 is optionally substituted C_1-C_3 alkyl. In some embodiments, R^7 is C_1-C_3 alkyl. In some embodiments, R^8 is optionally substituted C_1-C_3 alkyl. In some embodiments, R^8 is C_1-C_3 alkyl.

In some embodiments, a compound of the present invention has the structure of Formula If, or a pharmaceutically acceptable salt thereof:



Formula If

wherein A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

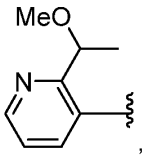
R^2 is C_1-C_6 alkyl or 3 to 6-membered cycloalkyl;

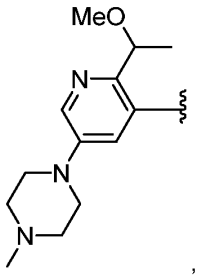
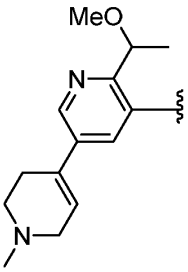
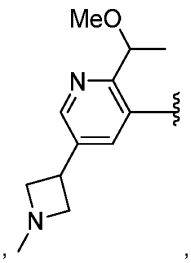
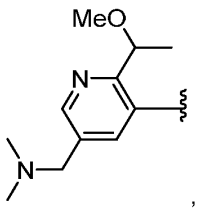
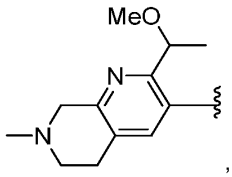
R^7 is C_1-C_3 alkyl;

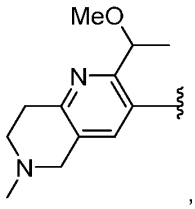
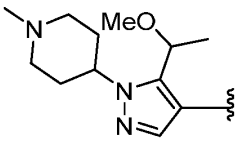
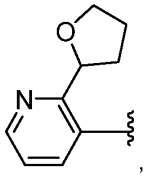
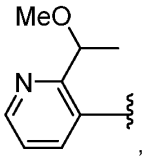
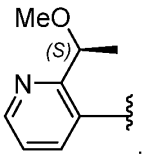
R^8 is C_1-C_3 alkyl; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

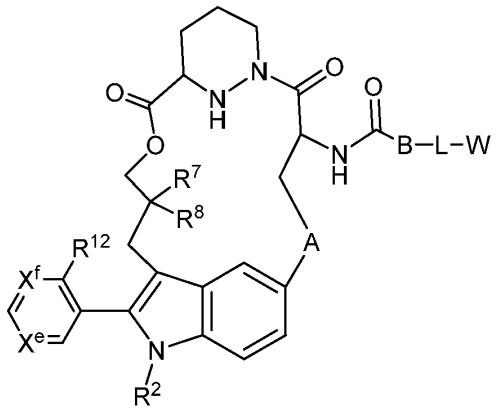
In some embodiments of a compound of the present invention, R^1 is optionally substituted 6 to 10-membered aryl, optionally substituted 3 to 6-membered cycloalkenyl, or optionally substituted 5 to 10-membered heteroaryl. In some embodiments, R^1 is optionally substituted 6-membered aryl, optionally substituted 6-membered cycloalkenyl, or optionally substituted 6-membered heteroaryl.

In some embodiments of a compound of the present invention, R_1 is  ,

 ,  ,  ,  ,  ,

 ,  , or  , or a stereoisomer (e.g., atropisomer) thereof. In some embodiments of a compound of the present invention, R_1 is  , or a stereoisomer (e.g., atropisomer) thereof. In some embodiments of a compound of the present invention, R_1 is  .

In some embodiments, a compound of the present invention has the structure of Formula Ig, or a pharmaceutically acceptable salt thereof:



Formula Ig

wherein A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

R^2 is C_1-C_6 alkyl, C_1-C_6 fluoroalkyl, or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

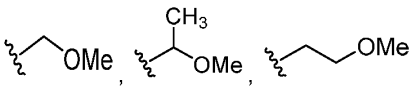
R^8 is C_1-C_3 alkyl; and

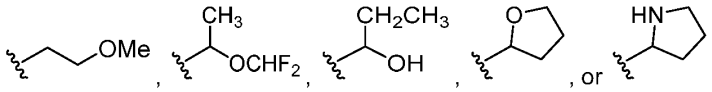
R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl

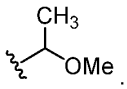
X^e and X^f are, independently, N or CH; and

R^{12} is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, or optionally substituted 3 to 6-membered heterocycloalkylene.

In some embodiments of a compound of the present invention, X^e is N and X^f is CH. In some embodiments, X^e is CH and X^f is N.

In some embodiments of a compound of the present invention, R^{12} is optionally substituted C_1-C_6 heteroalkyl. In some embodiments, R^{12} is 

, 

. In some embodiments, R^{12} is 

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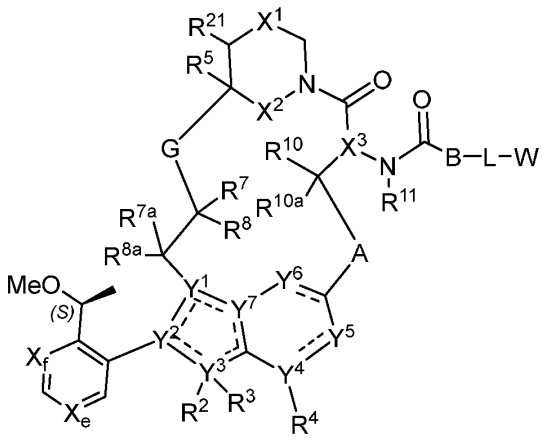
In some embodiments, a compound of the present invention has the structure of Formula VI, or a pharmaceutically acceptable salt thereof:



Formula VI

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene (e.g., phenyl or phenol), or optionally substituted 5 to 10-membered heteroarylene;

B is absent, -CH(R^9)-, >C=C$R^9R^{9'}$, or >C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, a haloacetal, or an alkynyl sulfone;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or S(O)$_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is H, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl; or

R^9 and $R^{9'}$, combined with the atoms to which they are attached, form a 3 to 6-membered cycloalkyl or a 3 to 6-membered heterocycloalkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

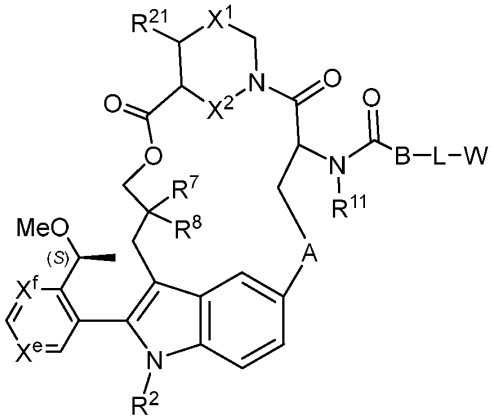
R^{10a} is hydrogen or halo;

R^{11} is hydrogen or C_1-C_3 alkyl;

R^{21} is hydrogen or C_1-C_3 alkyl (e.g., methyl); and

X^e and X^f are, independently, N or CH.

In some embodiments, a compound of the present invention has the structure of Formula VIa, or a pharmaceutically acceptable salt thereof:



Formula VIa

wherein A optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene (e.g., phenyl or phenol), or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

R^2 is C_1-C_6 alkyl, C_1-C_6 fluoroalkyl, or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

R^8 is C_1-C_3 alkyl; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

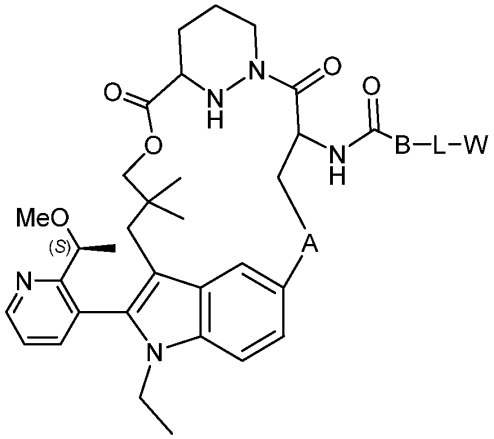
X^e and X^f are, independently, N or CH;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{21} is hydrogen or C_1-C_3 alkyl.

In some embodiments of a compound of the present invention, X^e is N and X^f is CH. In some embodiments, X^e is CH and X^f is N.

In some embodiments, a compound of the present invention has the structure of Formula VIb, or a pharmaceutically acceptable salt thereof:



Formula VIb

wherein A optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene (e.g., phenyl or phenol), or optionally substituted 5 to 6-membered heteroarylene;

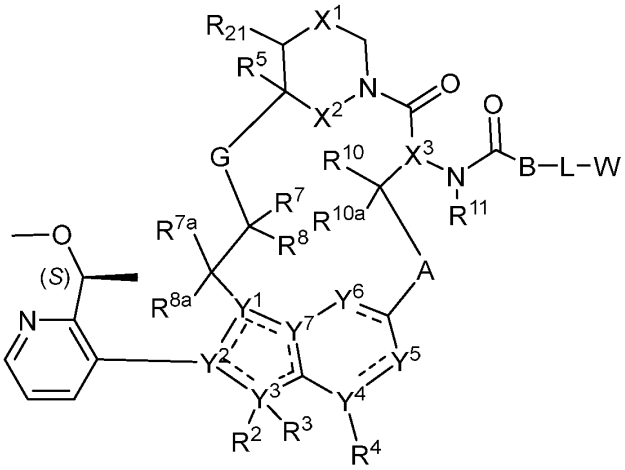
B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

L is absent or a linker; and

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone.In some embodiments of a compound of the present invention, A is optionally substituted 6-membered arylene.

In some embodiments, a compound of the present invention has the structure of Formula VIc (corresponding for Formula BB of FIG. 1A and FIG. 1B), or a pharmaceutically acceptable salt thereof:



Formula VIc

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene (e.g., phenyl or phenol), or optionally substituted 5 to 10-membered heteroarylene;

B is absent, -CH(R^9)-, >C=C$R^9R^{9'}$, or >C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, a haloacetal, or an alkynyl sulfone;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or S(O)$_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is H, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl; or

R^9 and $R^{9'}$, combined with the atoms to which they are attached, form a 3 to 6-membered cycloalkyl or a 3 to 6-membered heterocycloalkyl;

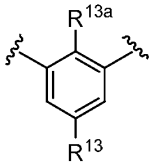
R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo;

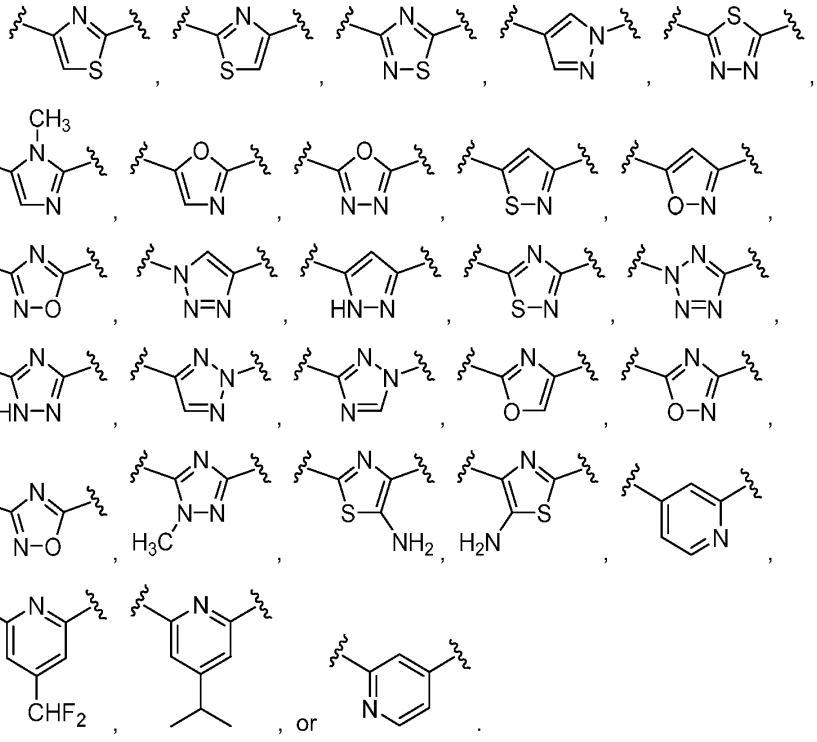
R^{11} is hydrogen or C_1-C_3 alkyl; and

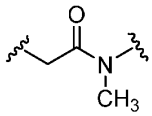
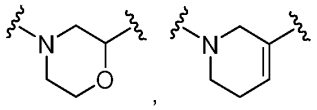
R^{21} is hydrogen or C_1-C_3 alkyl (e.g., methyl).

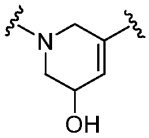
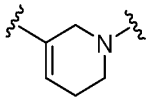
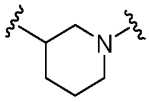
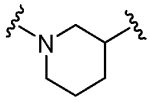
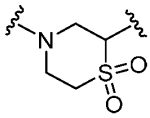
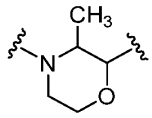
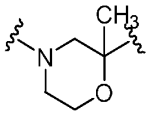
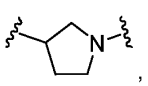
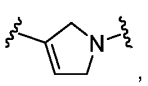
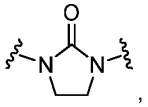
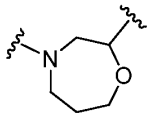
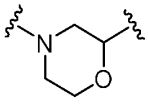
In some embodiments, A has the structure:

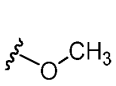
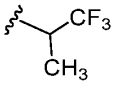
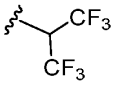
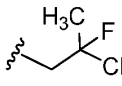


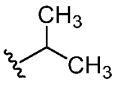
wherein R^{13} is hydrogen, halo, hydroxy, amino, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl; and R^{13a} is hydrogen or halo. In some embodiments, R^{13} is hydrogen. In some embodiments, R^{13} and R^{13a} are each hydrogen. In some embodiments, R^{13} is hydroxy, methyl, fluoro, or difluoromethyl.

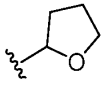
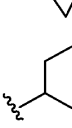
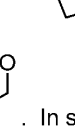
In some embodiments, A is optionally substituted 5 to 6-membered heteroarylene. In some embodiments, A is: 

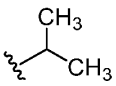
In some embodiments, A is optionally substituted C_1-C_4 heteroalkylene. In some embodiments, A is:  . In some embodiments, A is optionally substituted 3 to 6-membered heterocycloalkylene. In some embodiments, A is: 

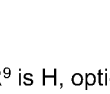
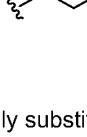













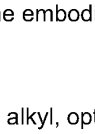
In some embodiments of a compound of the present invention, B is -CHR^9-. In some embodiments, R^9 is H, F, optionally substituted C$_1$-C$_6$ alkyl, optionally substituted C$_1$-C$_6$ heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl. In some embodiments, R^9 is:











In some embodiments of a compound of the present invention, B is optionally substituted 6-membered arylene. In some embodiments, B is 6-membered arylene. In some embodiments, B is:

In some embodiments of a compound of the present invention, R^7 is methyl.

In some embodiments of a compound of the present invention, R^8 is methyl.

In some embodiments, R^{21} is hydrogen.

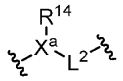
In some embodiments of a compound of the present invention, the linker is the structure of Formula II:

$$A^1\text{-}(B^1)_f\text{-}(C^1)_g\text{-}(B^2)_h\text{-}(D^1)\text{-}(B^3)_i\text{-}(C^2)_j\text{-}(B^4)_k\text{–}A^2$$

Formula II

where A^1 is a bond between the linker and B; A^2 is a bond between W and the linker; B^1, B^2, B^3, and B^4 each, independently, is selected from optionally substituted C$_1$-C$_2$ alkylene, optionally substituted C$_1$-C$_3$ heteroalkylene, O, S, and NR^N; R^N is hydrogen, optionally substituted C$_{1-4}$ alkyl,

optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted C_1-C_7 heteroalkyl; C^1 and C^2 are each, independently, selected from carbonyl, thiocarbonyl, sulphonyl, or phosphoryl; f, g, h, i, j, and k are each, independently, 0 or 1; and D^1 is optionally substituted C_1-C_{10} alkylene, optionally substituted C_2-C_{10} alkenylene, optionally substituted C_2-C_{10} alkynylene, optionally substituted 3 to 14-membered heterocycloalkylene, optionally substituted 5 to 10-membered heteroarylene, optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 6 to 10-membered arylene, optionally substituted C_2-C_{10} polyethylene glycolene, or optionally substituted C_1-C_{10} heteroalkylene, or a chemical bond linking A^1-$(B^1)_f$-$(C^1)_g$-$(B^2)_h$- to -$(B^3)_i$-$(C^2)_j$-$(B^4)_k$–A^2. In some embodiments, the linker is acyclic. In some embodiments, linker has the structure of Formula IIa:

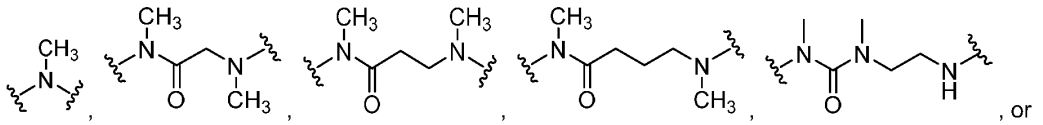


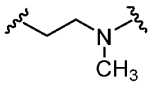
Formula IIa

wherein X^a is absent or N;

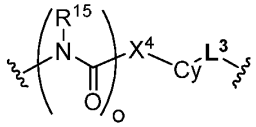
R^{14} is absent, hydrogen or optionally substituted C_1-C_6 alkyl; and

L^2 is absent, -SO_2-, optionally substituted C_1-C_4 alkylene or optionally substituted C_1-C_4 heteroalkylene, wherein at least one of X^a, R^{14}, or L^2 is present. In some embodiments, the linker has the structure:


, or


.

In some embodiments, the linker is or comprises a cyclic moiety. In some embodiments, the linker has the structure of Formula IIb:



Formula IIb

wherein o is 0 or 1;

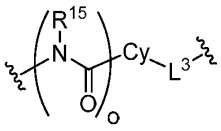
R^{15} is hydrogen or optionally substituted C_1-C_6 alkyl, optionally substituted 3 to 8-membered cycloalkylene, or optionally substituted 3 to 8-membered heterocycloalkylene;

X^4 is absent, optionally substituted C_1-C_4 alkylene, O, NCH_3, or optionally substituted C_1-C_4 heteroalkylene;

Cy is optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 3 to 8-membered heterocycloalkylene, optionally substituted 6-10 membered arylene, or optionally substituted 5 to 10-membered heteroarylene; and

L^3 is absent, $-SO_2-$, optionally substituted C_1-C_4 alkylene or optionally substituted C_1-C_4 heteroalkylene.

. In some embodiments, the linker has the structure of Formula IIb-1:



Formula IIb-1

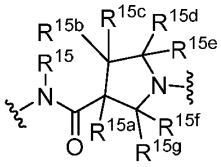
wherein o is 0 or 1;

R^{15} is hydrogen or optionally substituted C_1-C_6 alkyl, optionally substituted 3 to 8-membered cycloalkylene, or optionally substituted 3 to 8-membered heterocycloalkylene;

Cy is optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 3 to 8-membered heterocycloalkylene, optionally substituted 6-10 membered arylene, or optionally substituted 5 to 10-membered heteroarylene; and

L^3 is absent, $-SO_2-$, optionally substituted C_1-C_4 alkylene or optionally substituted C_1-C_4 heteroalkylene.

In some embodiments, the linker has the structure of Formula IIc:

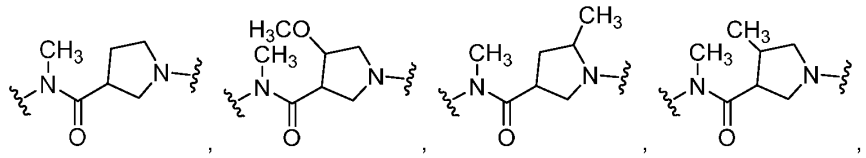


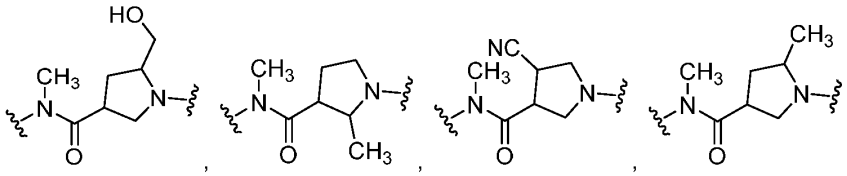
Formula IIc

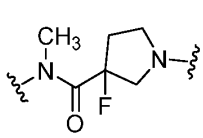
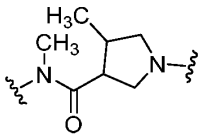
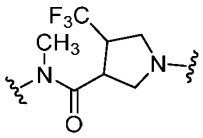
wherein R^{15} is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted 3 to 8-membered cycloalkylene, or optionally substituted 3 to 8-membered heterocycloalkylene; and

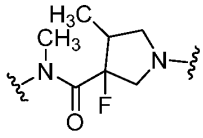
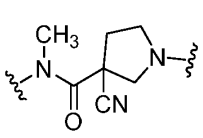
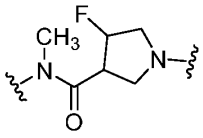
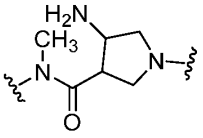
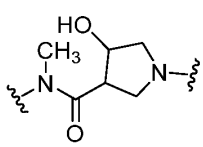
R^{15a}, R^{15b}, R^{15c}, R^{15d}, R^{15e}, R^{15f}, and R^{15g} are, independently, hydrogen, halo, hydroxy, cyano, amino, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 alkoxy, or , or R^{15b} and R^{15d} combine with the carbons to which they are attached to form an optionally substituted 3 to 8-membered cycloalkylene, or optionally substituted 3 to 8-membered heterocycloalkylene.

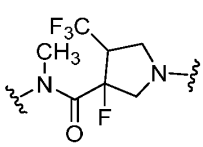
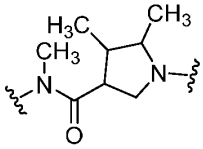
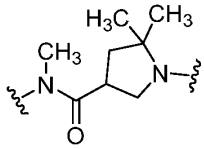
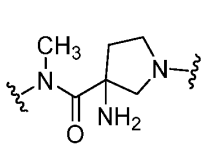
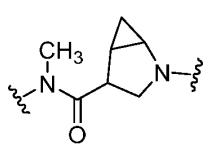
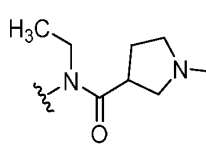
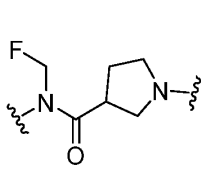
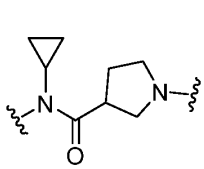
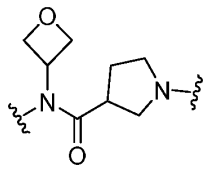
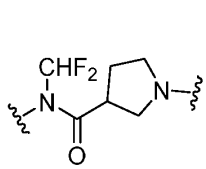
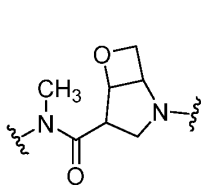
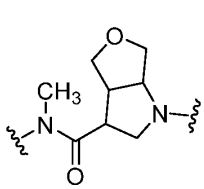
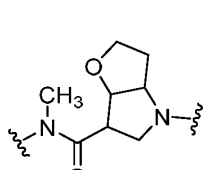
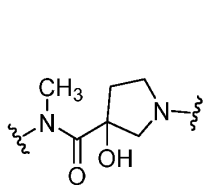
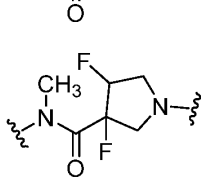
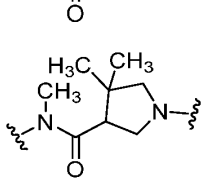
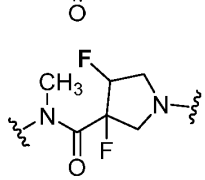
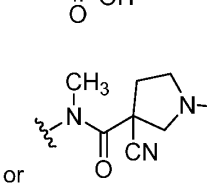
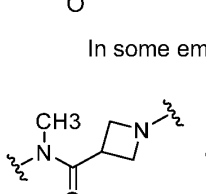
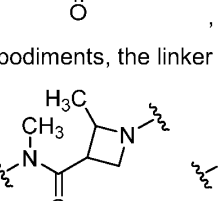
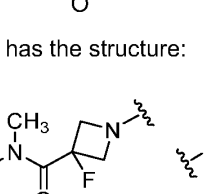
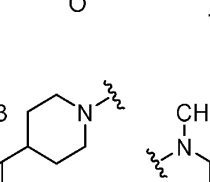
In some embodiments, the linker has the structure:



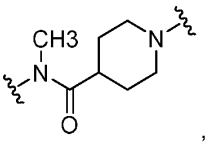
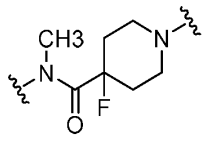
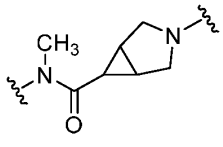
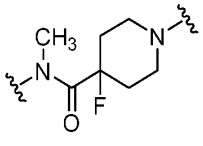
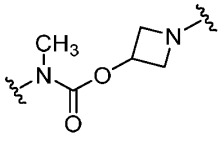
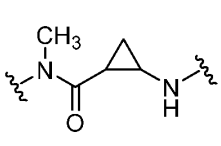


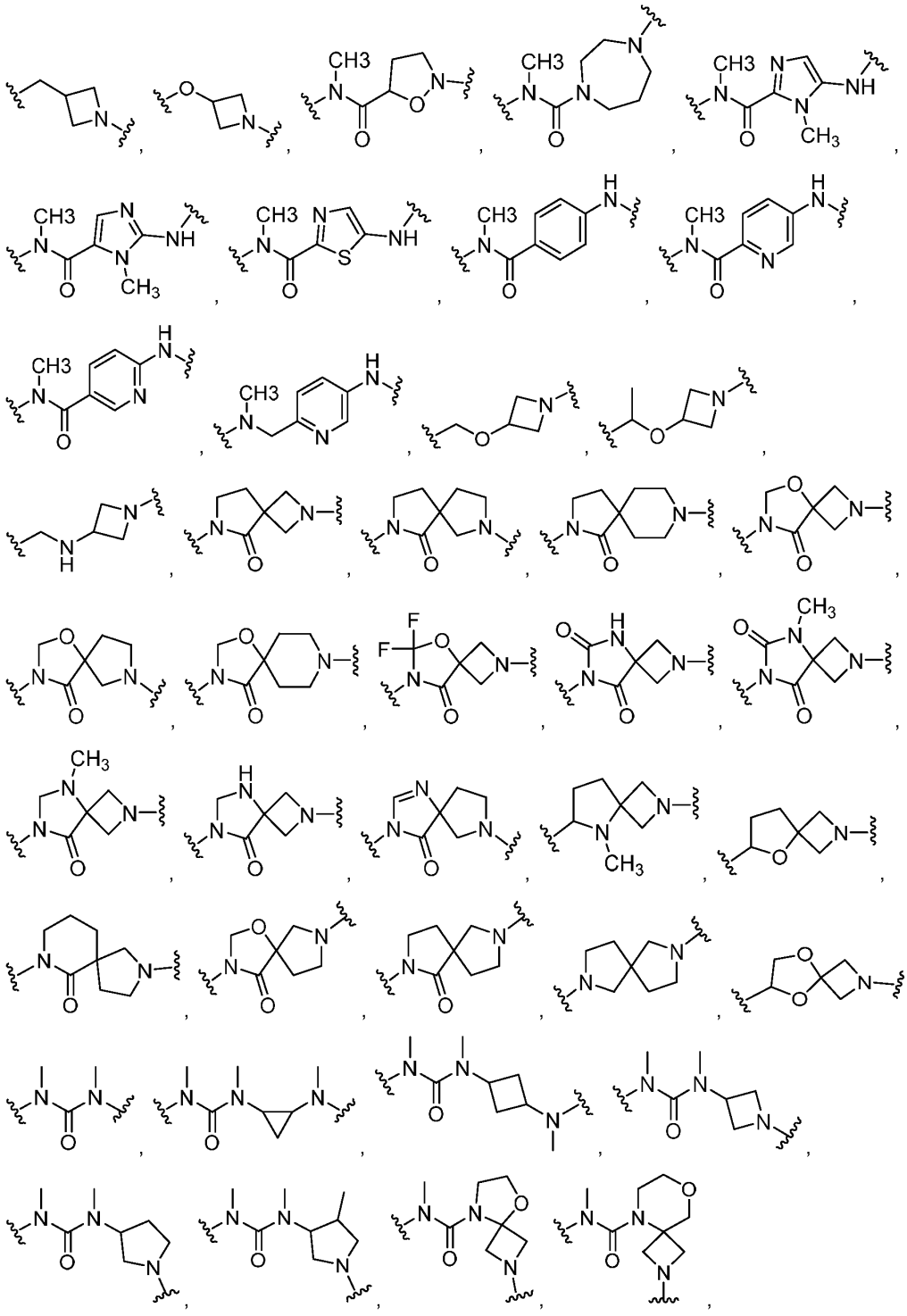




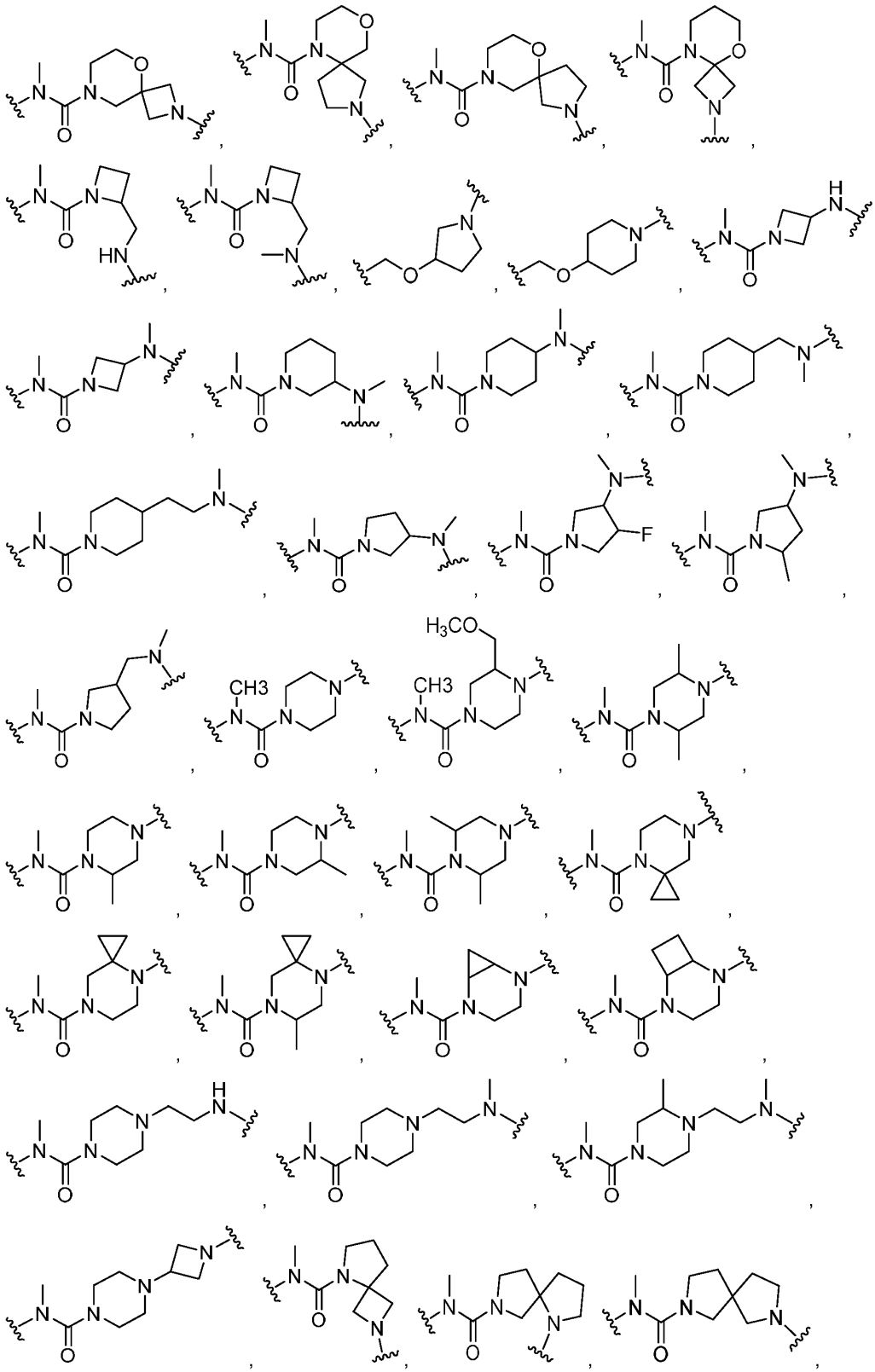






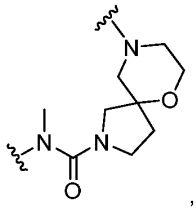
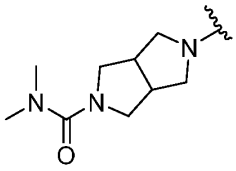
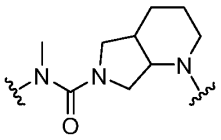
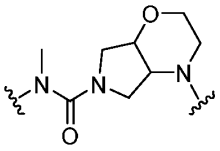
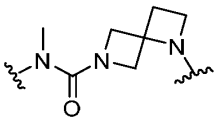
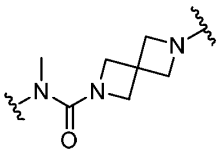
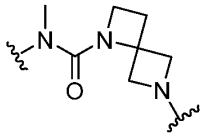
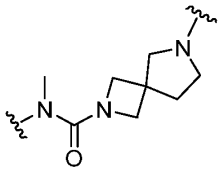
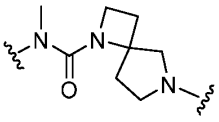
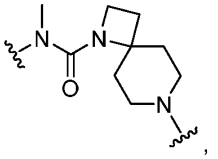
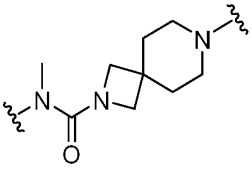
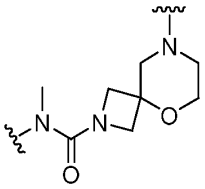
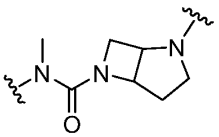
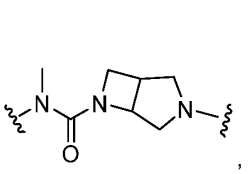
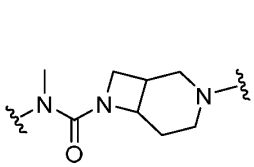
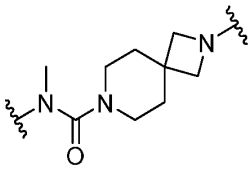
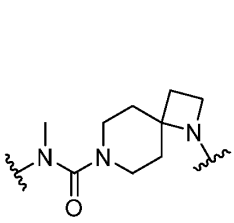
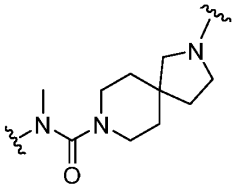
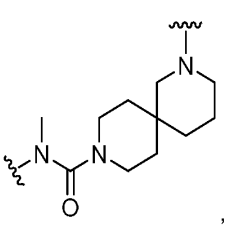
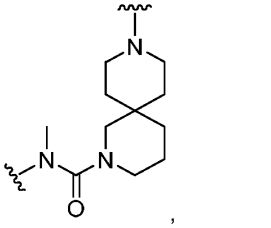























In some embodiments, the linker has the structure:





 ,  ,  ,  ,
 ,  ,  ,  ,
 ,  ,  ,  ,
 ,  ,  ,  ,
 ,  ,  ,  ,
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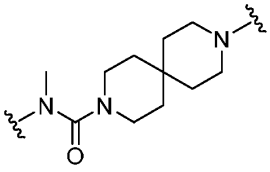
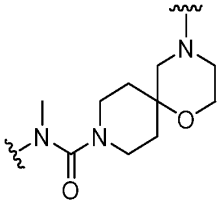
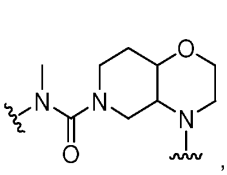
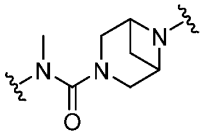
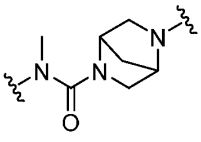
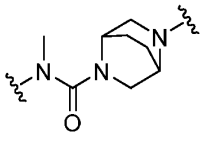
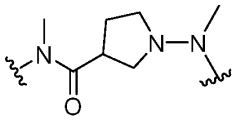
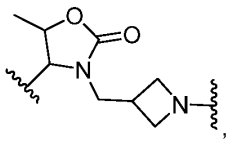
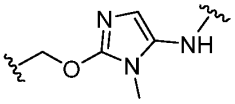
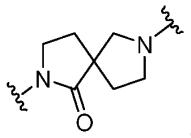
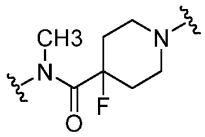
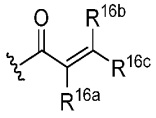
 ,  ,  ,  ,
 ,  ,  ,  , or
 .

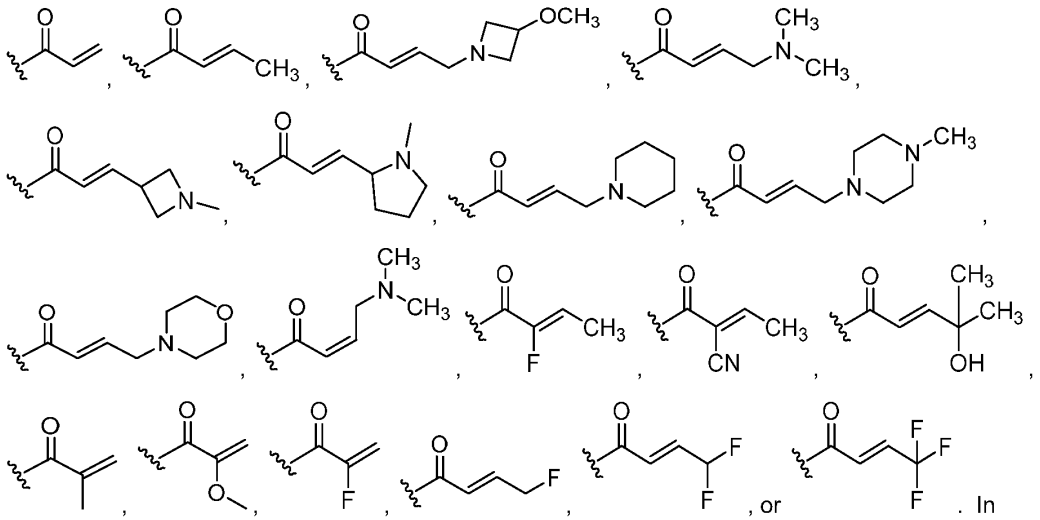
In some embodiments, the linker has the structure  .

In some embodiments, the linker has the structure  .In some embodiments of a compound of the present invention, W is a cross-linking group comprising a vinyl ketone. In some embodiments, W has the structure of Formula IIIa:

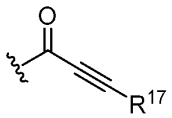


Formula IIIa

wherein R^{16a}, R^{16b}, and R^{16c} are, independently, hydrogen, -CN, halogen, or -C_1-C_3 alkyl optionally substituted with one or more substituents independently selected from -OH, -O-C_1-C_3 alkyl, -NH_2, -NH(C_1-C_3 alkyl), -N(C_1-C_3 alkyl)$_2$, or a 4 to 7-membered saturated heterocycloalkyl. In some embodiments, W is:



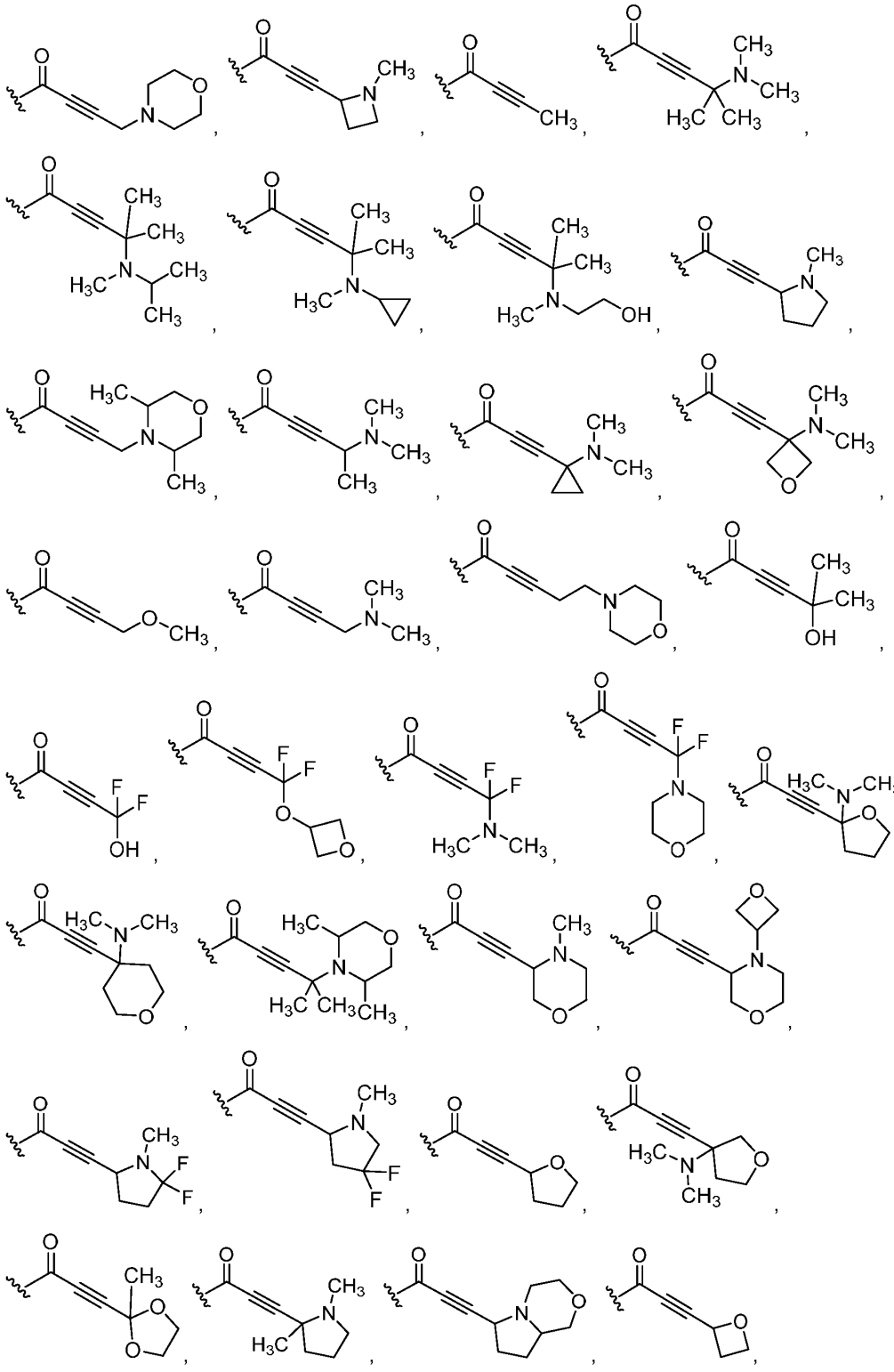
In some embodiments, W is a cross-linking group comprising an ynone. In some embodiments, W has the structure of Formula IIIb:

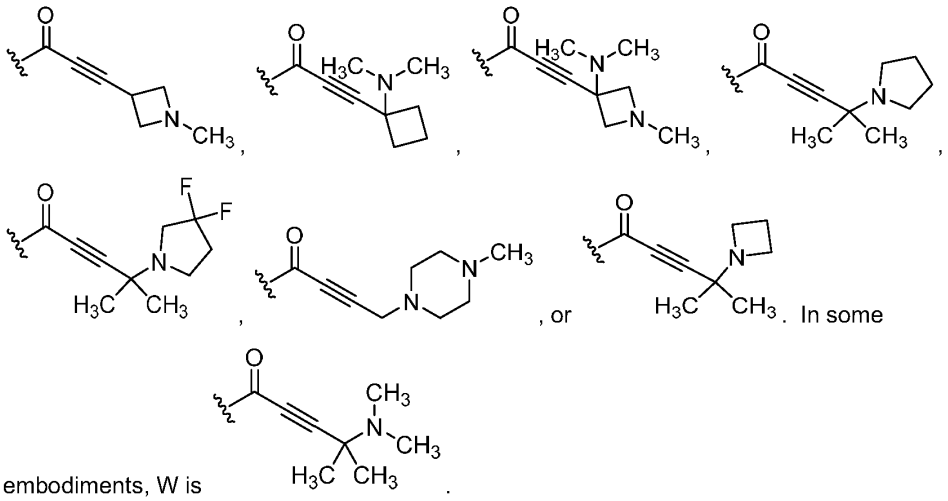


Formula IIIb

wherein R^{17} is hydrogen, -C_1-C_3 alkyl optionally substituted with one or more substituents independently selected from -OH, -O-C_1-C_3 alkyl, -NH_2, -NH(C_1-C_3 alkyl), -N(C_1-C_3 alkyl)$_2$, or a 4 to

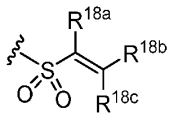
7-membered saturated heterocycloalkyl, or a 4 to 7-membered saturated heterocycloalkyl. In some embodiments, W is:





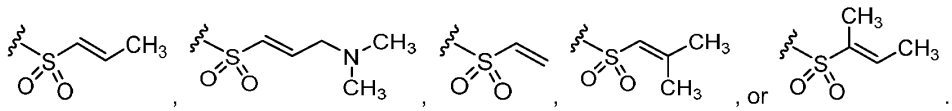
embodiments, W is .

In some embodiments, W is a cross-linking group comprising a vinyl sulfone. In some embodiments, W has the structure of Formula IIIc:

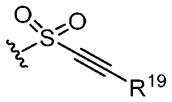


Formula IIIc

wherein R^{18a}, R^{18b}, and R^{18c} are, independently, hydrogen, -CN, or -C_1-C_3 alkyl optionally substituted with one or more substituents independently selected from -OH, -O-C_1-C_3 alkyl, -NH_2, -NH(C_1-C_3 alkyl), -N(C_1-C_3 alkyl)$_2$, or a 4 to 7-membered saturated heterocycloalkyl. In some embodiments, W is:

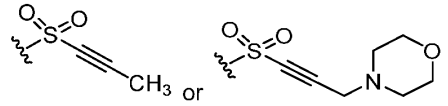


some embodiments, W is a cross-linking group comprising an alkynyl sulfone. In some embodiments, W has the structure of Formula IIId:

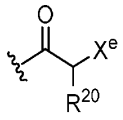


Formula IIId

wherein R^{19} is hydrogen, -C_1-C_3 alkyl optionally substituted with one or more substituents independently selected from -OH, -O-C_1-C_3 alkyl, -NH_2, -NH(C_1-C_3 alkyl), -N(C_1-C_3 alkyl)$_2$, or a 4 to 7-membered saturated heterocycloalkyl, or a 4 to 7-membered saturated heterocycloalkyl. In some embodiments, W is:



. In some embodiments, W has the structure of Formula IIIe:



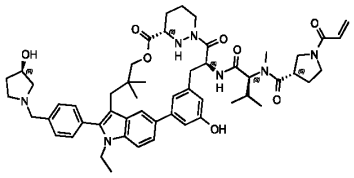
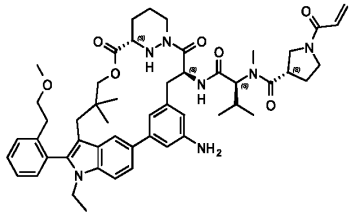
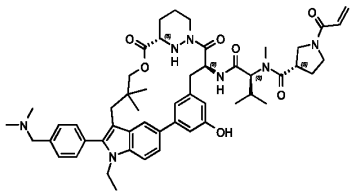
Formula IIIe

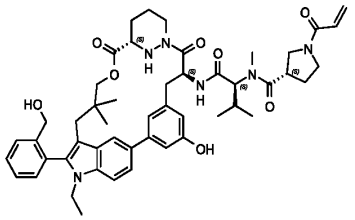
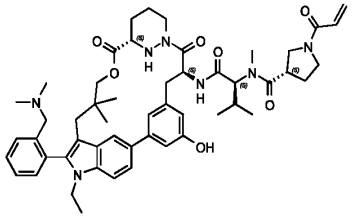
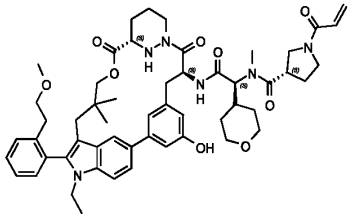
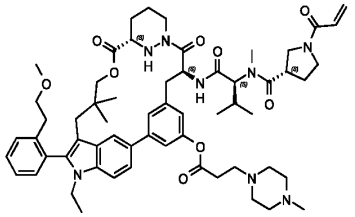
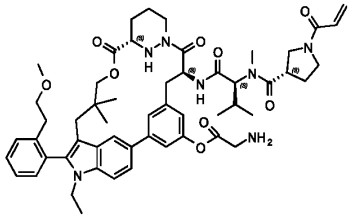
wherein X^e is a halogen; and

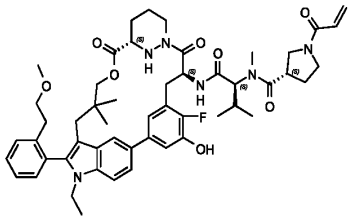
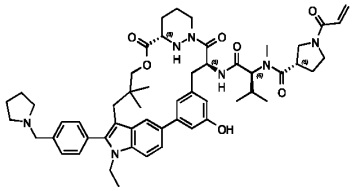
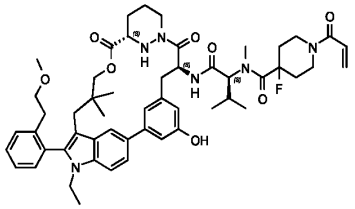
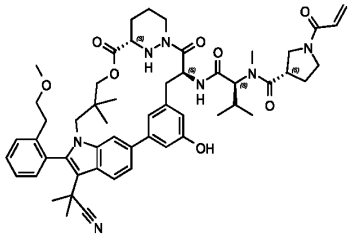
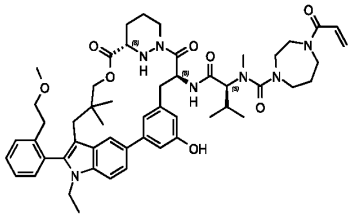
R^{20} is hydrogen, -C_1-C_3 alkyl optionally substituted with one or more substituents independently selected from -OH, -O-C_1-C_3 alkyl, -NH_2, -NH(C_1-C_3 alkyl), -N(C_1-C_3 alkyl)$_2$, or a 4 to 7-membered saturated heterocycloalkyl. In some embodiments, W is haloacetal. In some embodiments, W is not haloacetal.

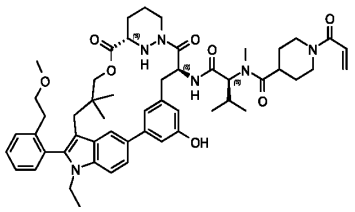
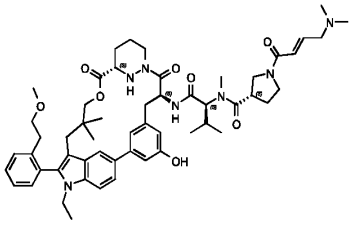
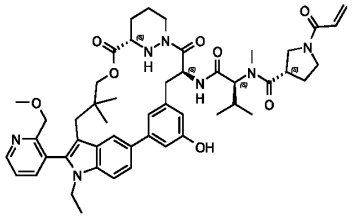
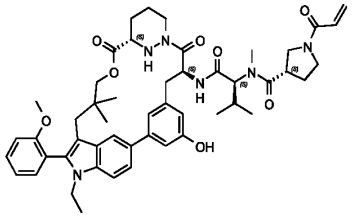
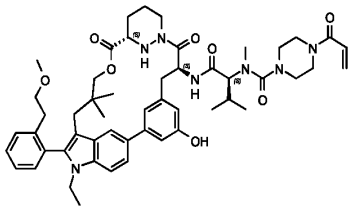
In some embodiments, a compound of the present invention is selected from Table 1, or a pharmaceutically acceptable salt or stereoisomer thereof. In some embodiments, a compound of the present invention is selected from Table 1, or a pharmaceutically acceptable salt or atropisomer thereof.

Table 1: Certain Compounds of the Present Invention

Ex#	Structure
A1	
A2	
A3	

Ex#	Structure
A4	
A5	
A6	
A7	
A8	

Ex#	Structure
A9	
A10	
A11	
A12	
A13	

Ex#	Structure
A14	
A15	
A16	
A17	
A18	

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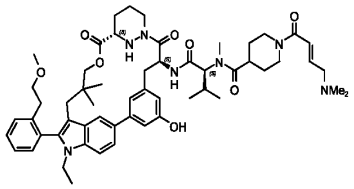
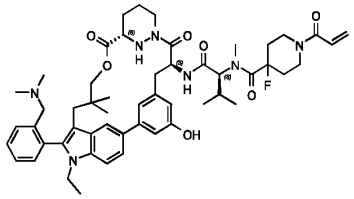
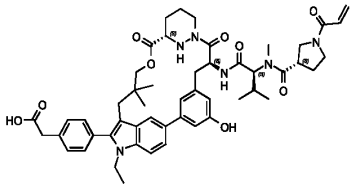
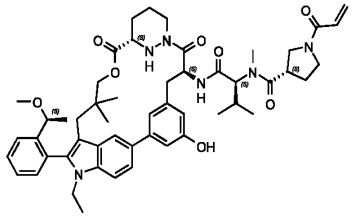
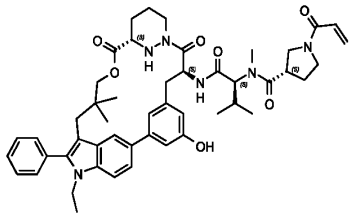
A19	
A20	
A21	
A22	
A23	

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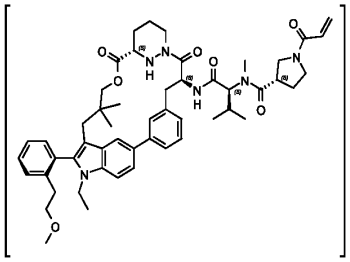
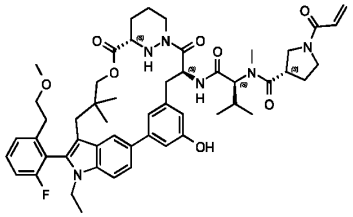
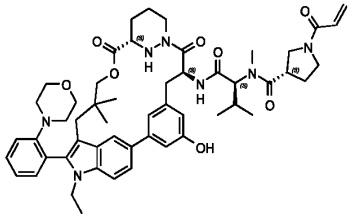
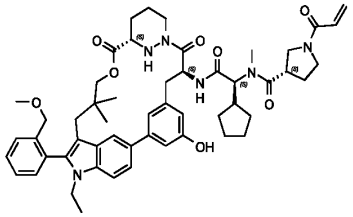
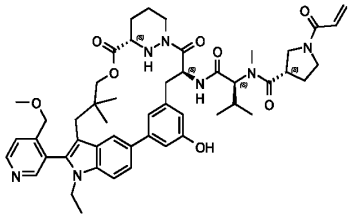
A24	
A25	
A26	
A27	
A28	

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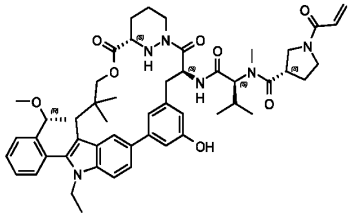
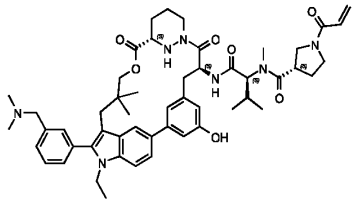
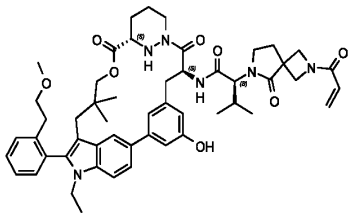
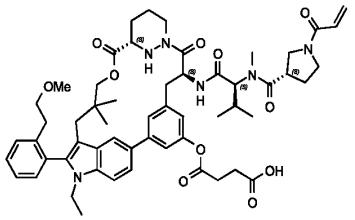
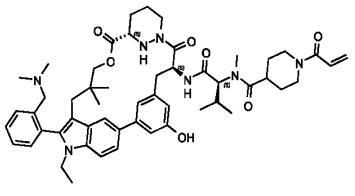
A29	
A30	
A31	
A32	
A33	

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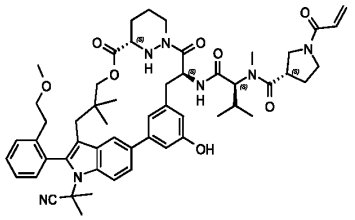
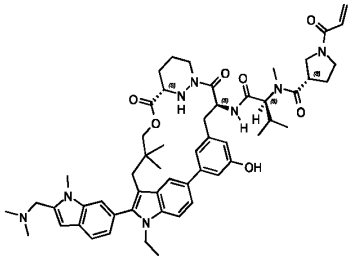
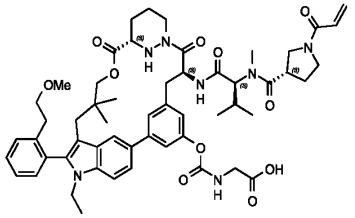
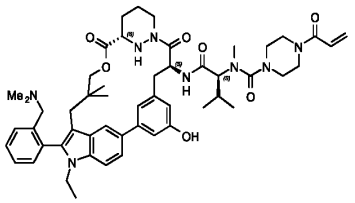
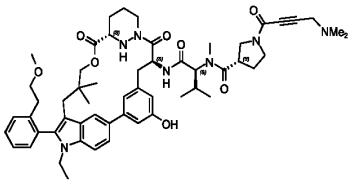
A34	
A35	
A36	
A37	
A38	

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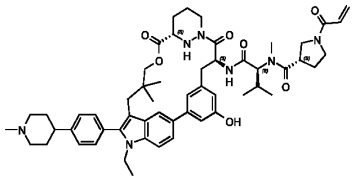
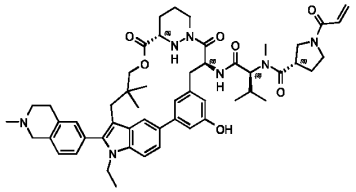
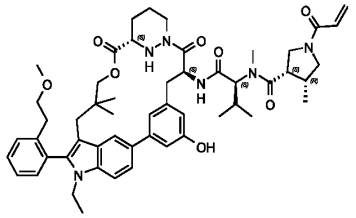
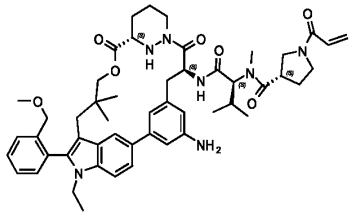
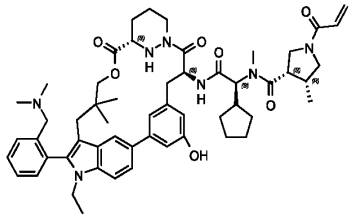
A39	
A40	
A41	
A42	
A43	

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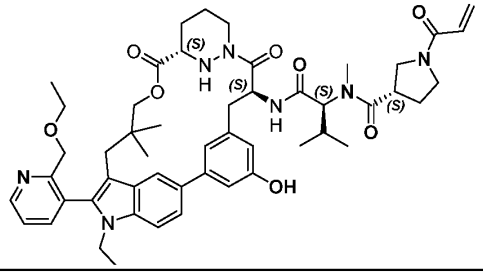
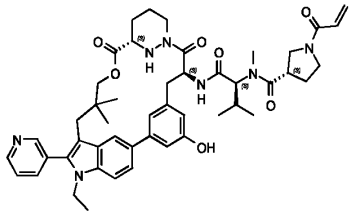
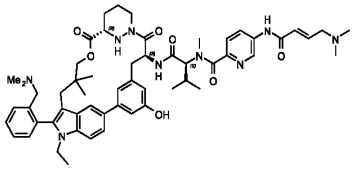
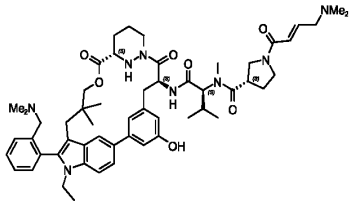
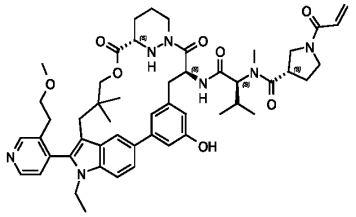
A44	
A45	
A46	
A47	
A48	

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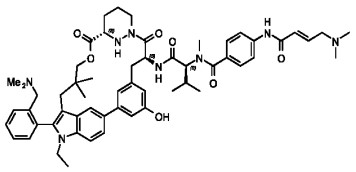
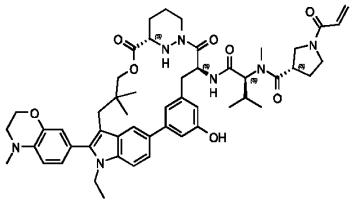
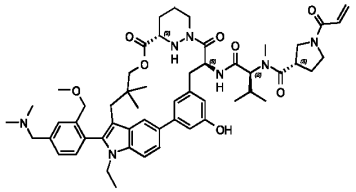
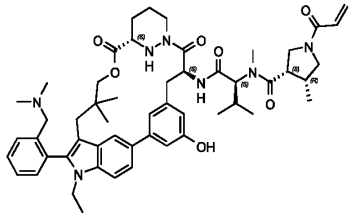
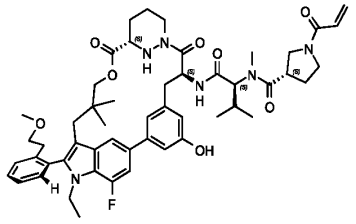
A49	
A50	
A51	
A52	
A53	

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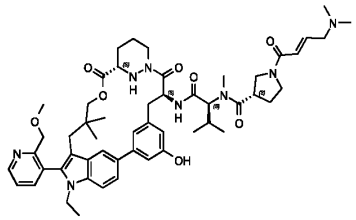
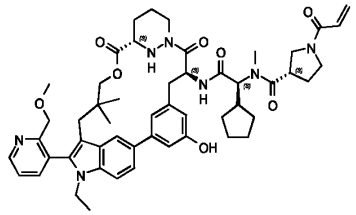
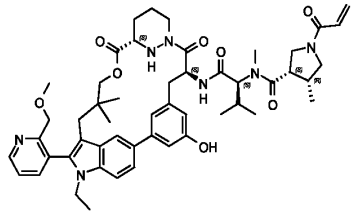
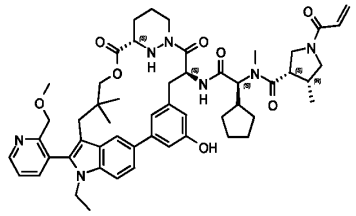
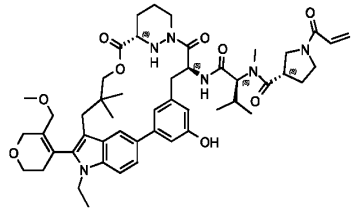
A54	
A55	
A56	
A57	
A58	

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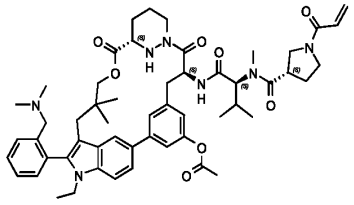
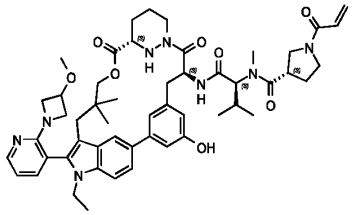
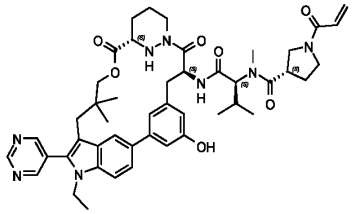
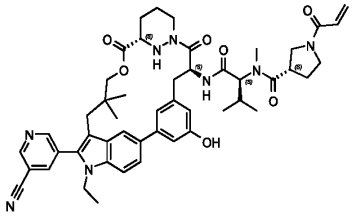
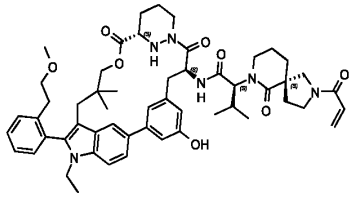
A59	
A60	
A61	
A62	
A63	

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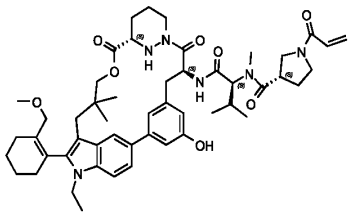
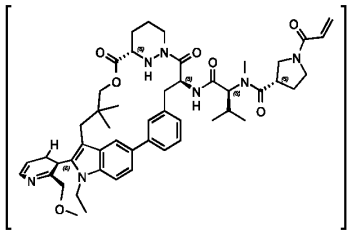
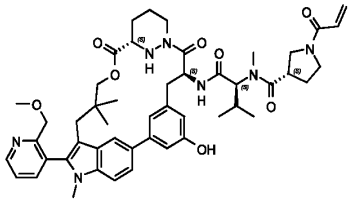
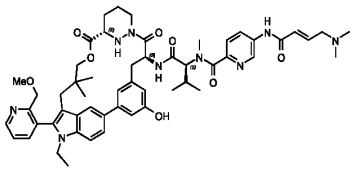
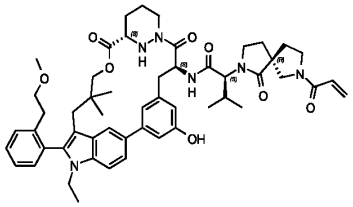
A64	
A65	
A66	
A67	
A68	

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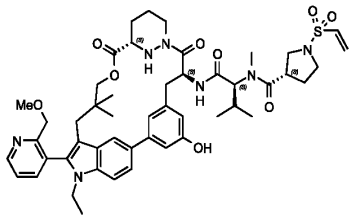
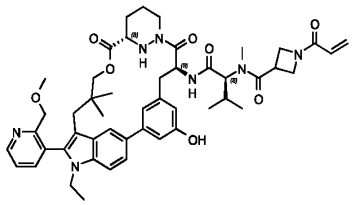
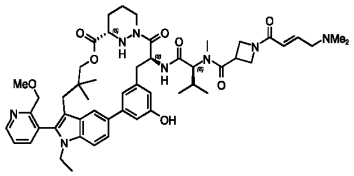
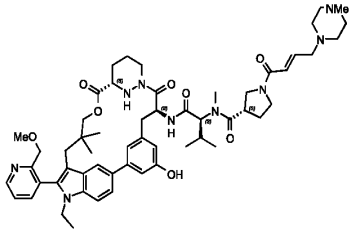
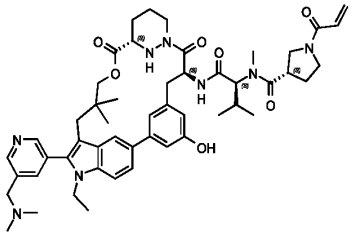
A69	
A70	
A71	
A72	
A73	

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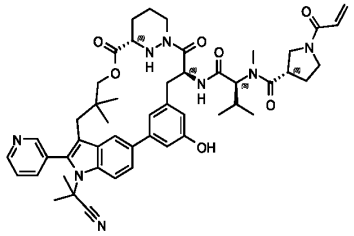
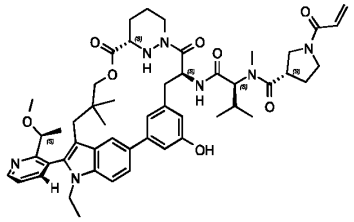
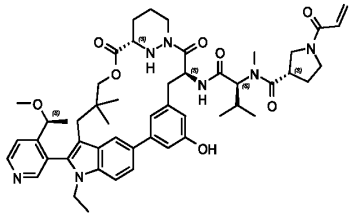
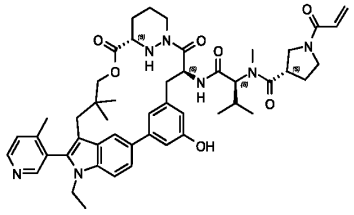
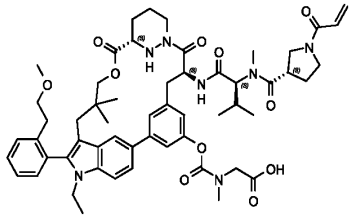
A74	
A75	
A76	
A77	
A78	

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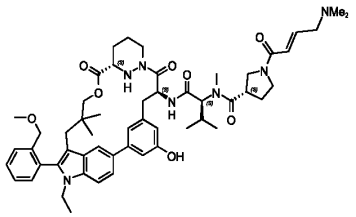
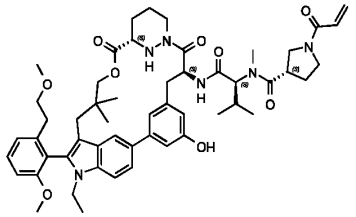
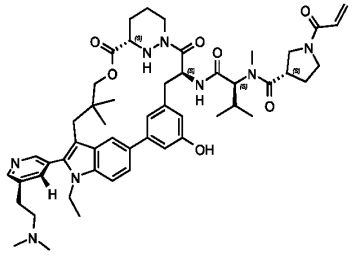
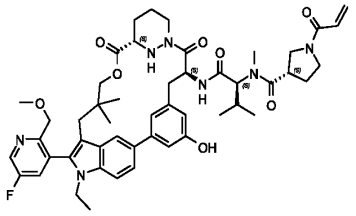
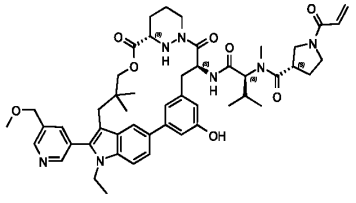
A79	
A80	
A81	
A82	
A83	

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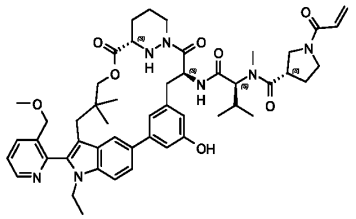
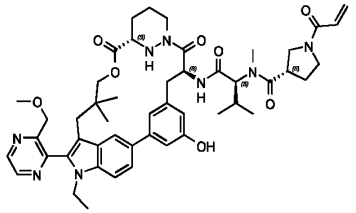
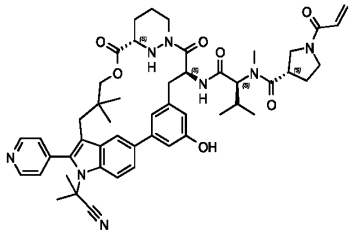
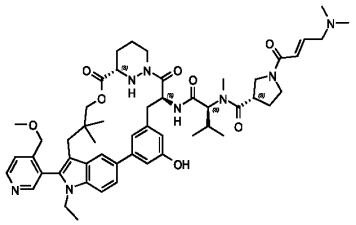
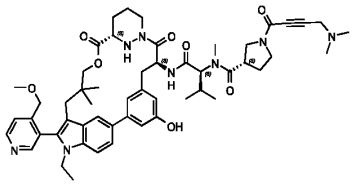
A84	
A85	
A86	
A87	
A88	

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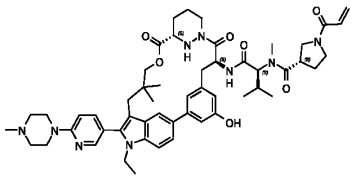
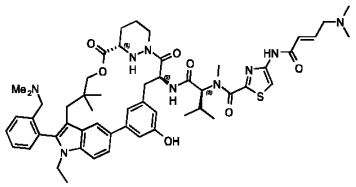
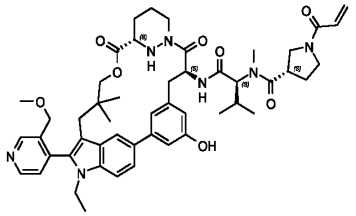
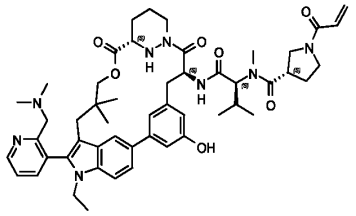
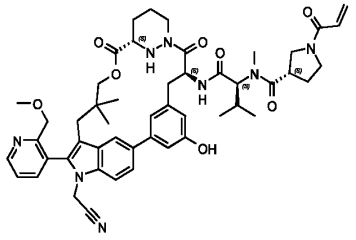
A89	
A90	
A91	
A92	
A93	

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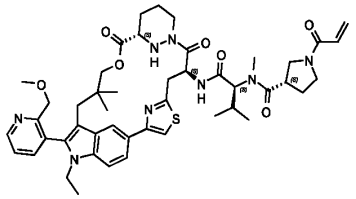
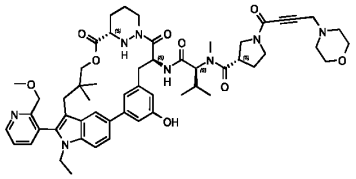
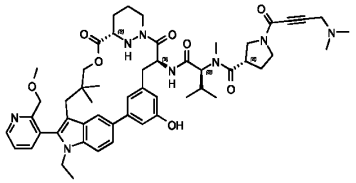
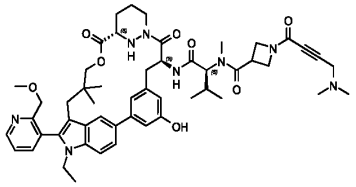
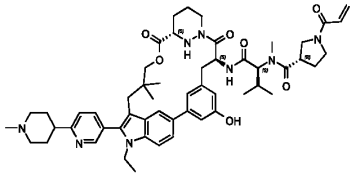
A94	
A95	
A96	
A97	
A98	

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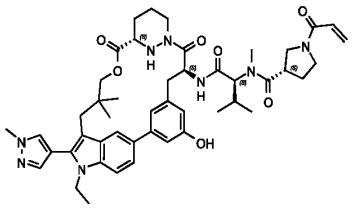
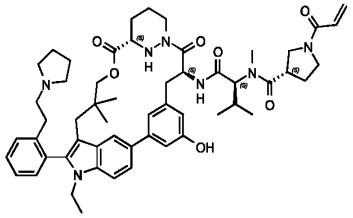
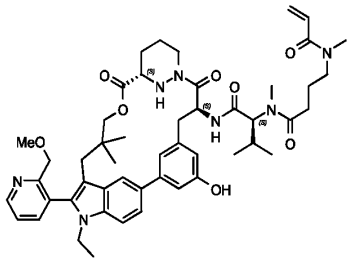
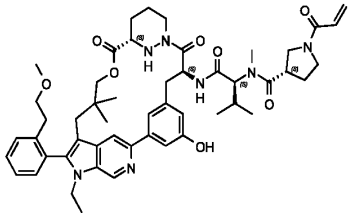
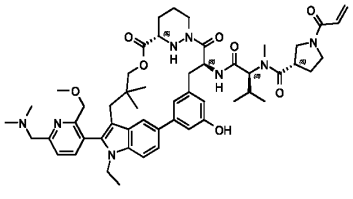
A99	
A100	
A101	
A102	
A103	

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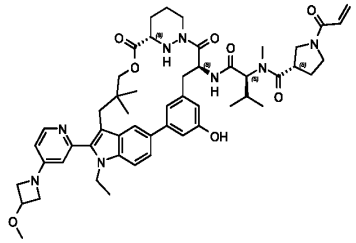
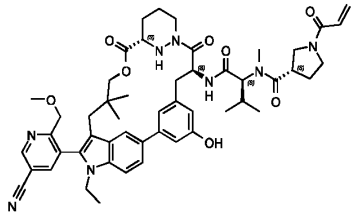
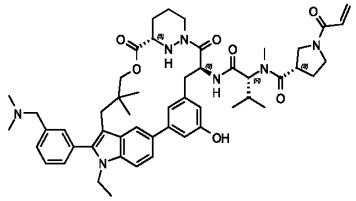
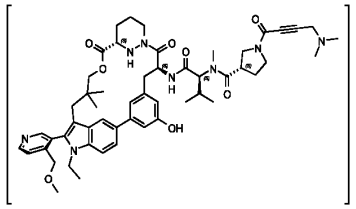
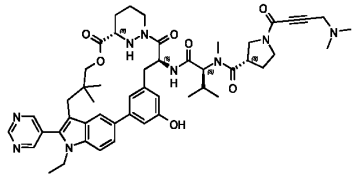
A104	
A105	
A106	
A107	
A108	

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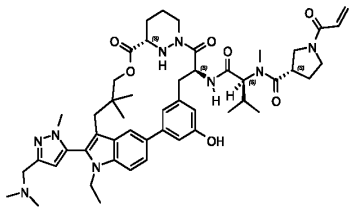
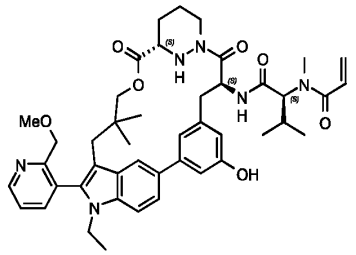
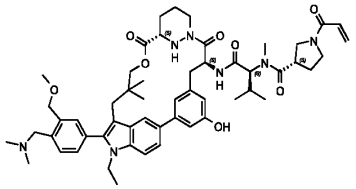
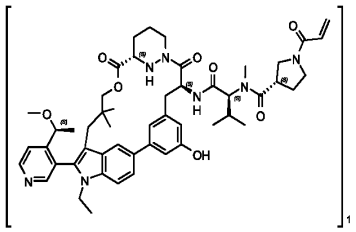
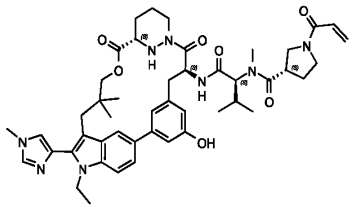
A109	
A110	
A111	
A112	
A113	

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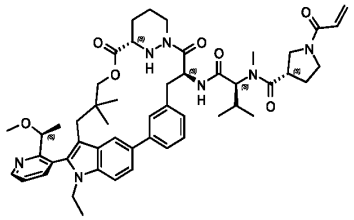
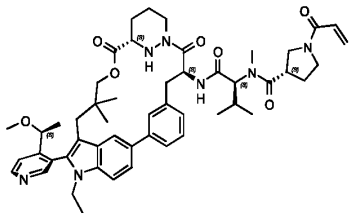
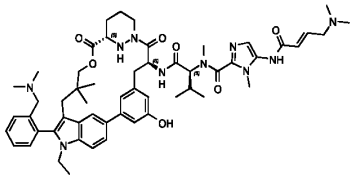
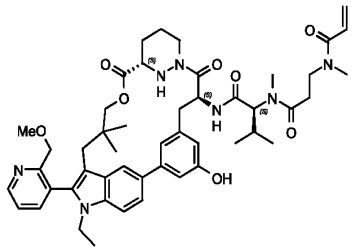
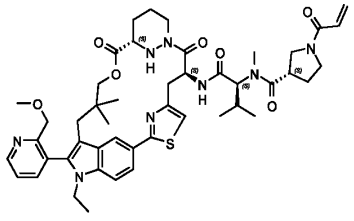
A114	
A115	
A116	
A117	
A118	

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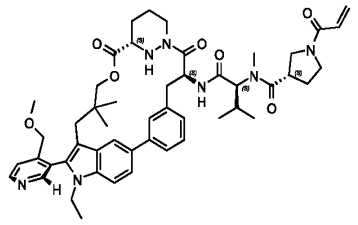
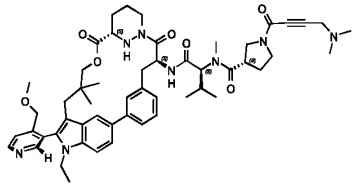
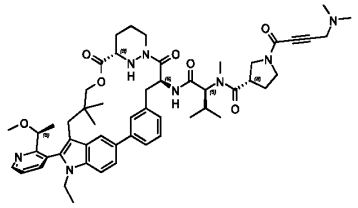
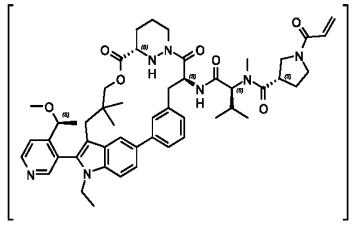
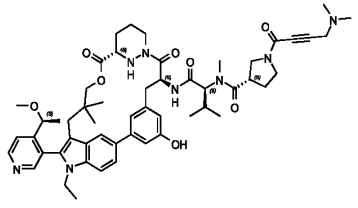
A119	
A120	
A121	
A122	
A123	

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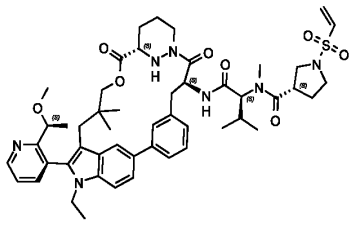
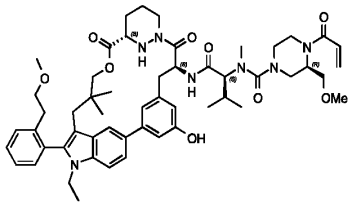
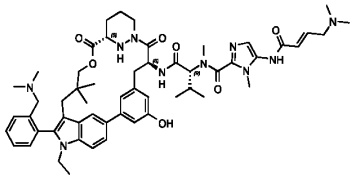
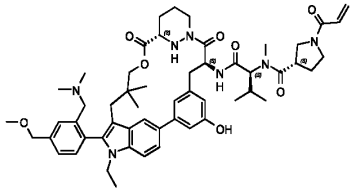
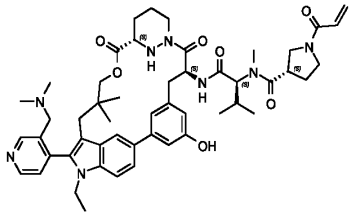
A124	
A125	
A126	
A127	
A128	

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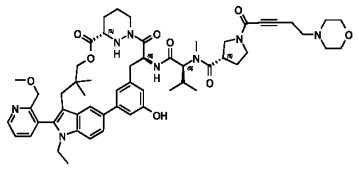
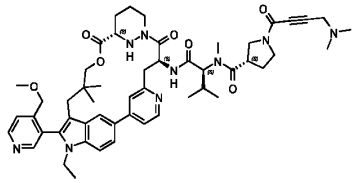
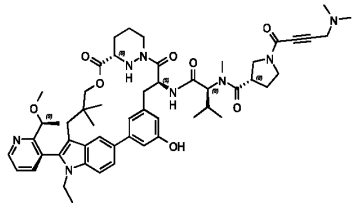
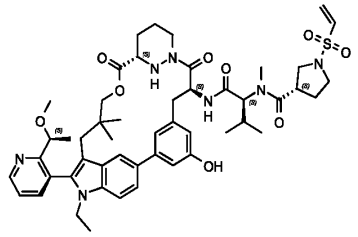
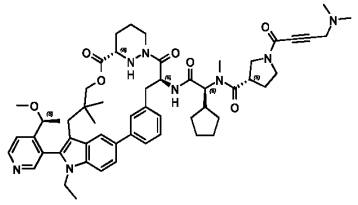
A129	
A130	
A131	
A132	
A133	

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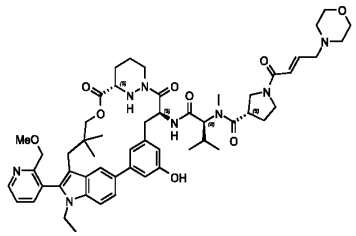
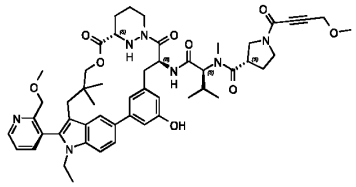
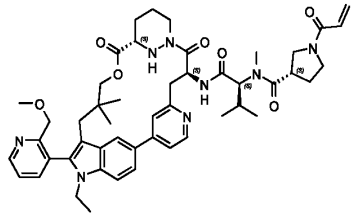
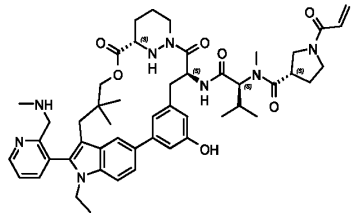
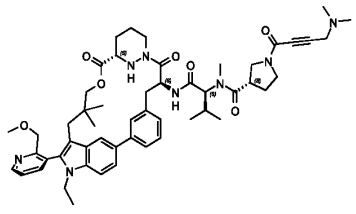
A134	
A135	
A136	
A137	
A138	

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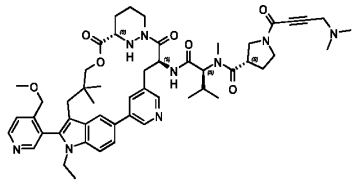
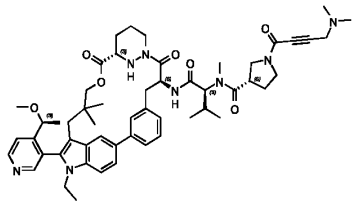
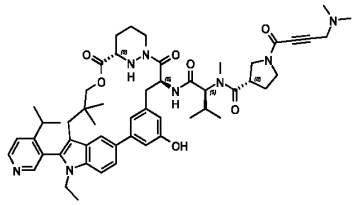
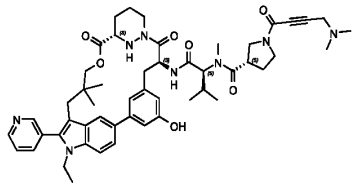
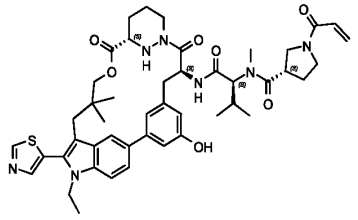
A139	
A140	
A141	
A142	
A143	

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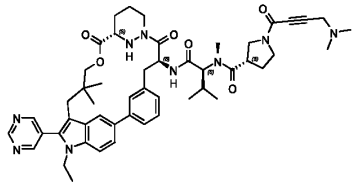
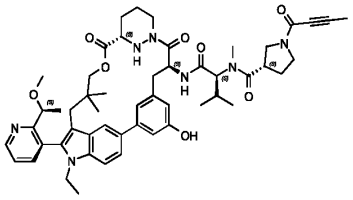
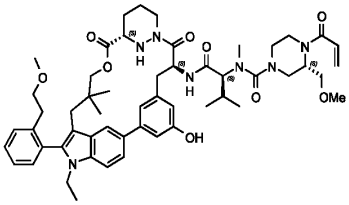
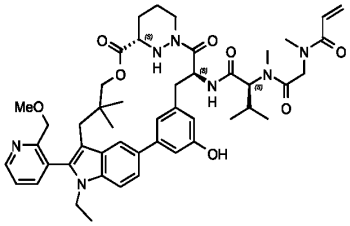
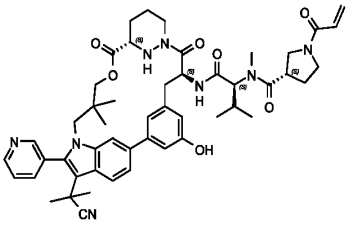
A144	
A145	
A146	
A147	
A148	

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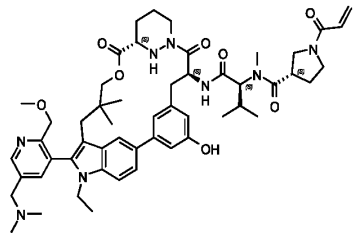
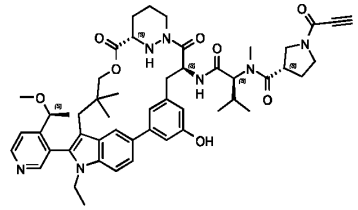
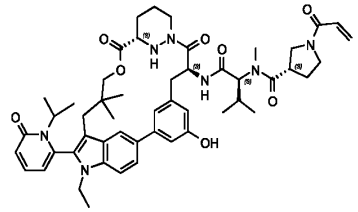
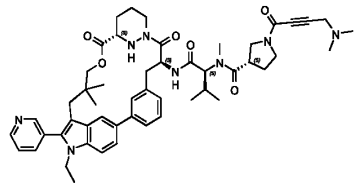
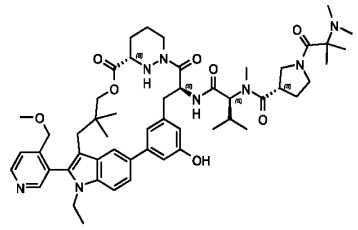
A149	
A150	
A151	
A152	
A153	

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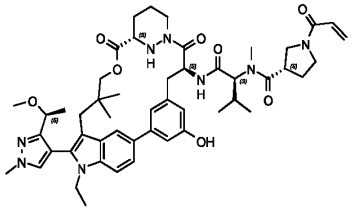
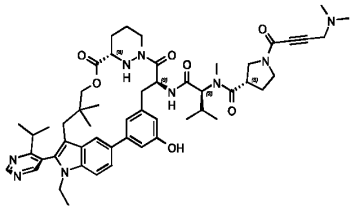
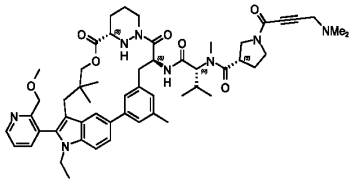
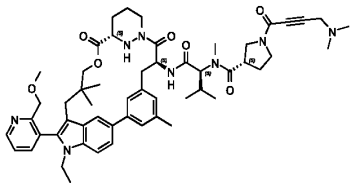
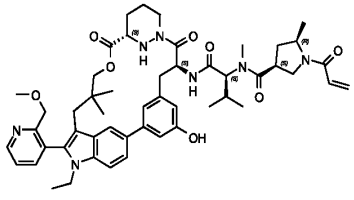
A154	
A155	
A156	
A157	
A158	

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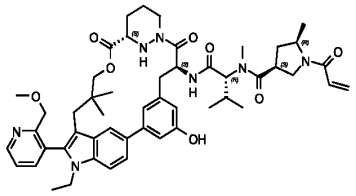
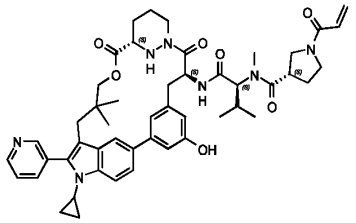
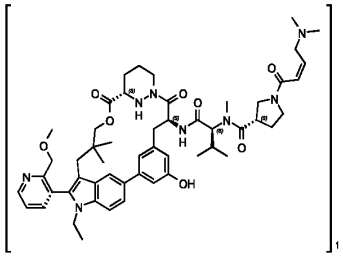
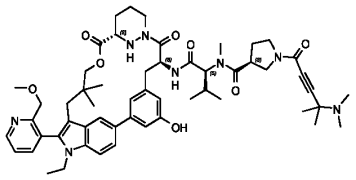
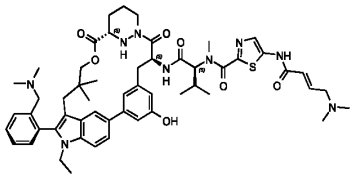
A159	
A160	
A161	
A162	
A163	

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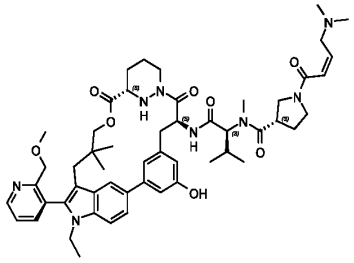
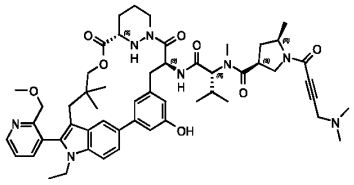
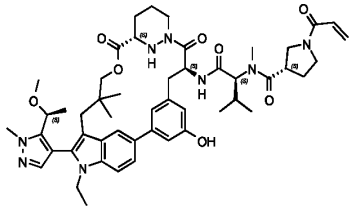
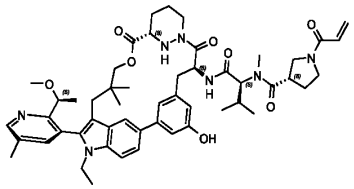
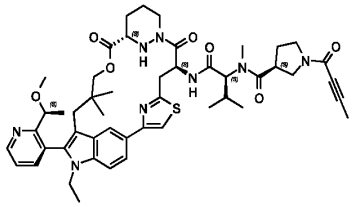
A164	
A165	
A166	
A167	
A168	

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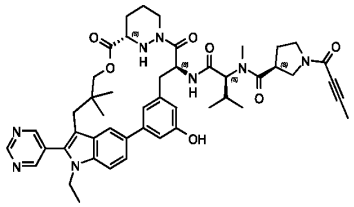
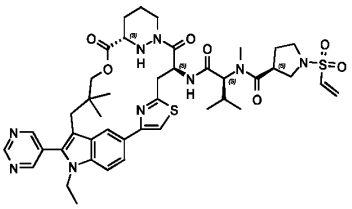
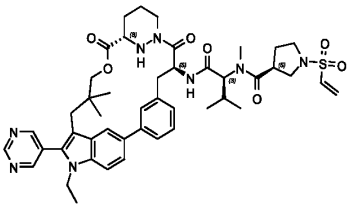
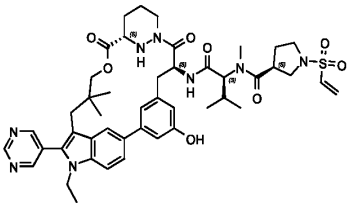
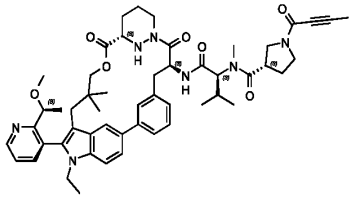
A169	
A170	
A171	
A172	
A173	

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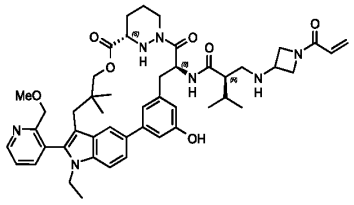
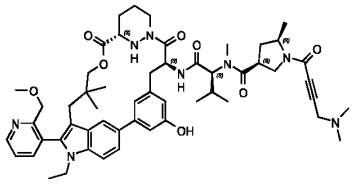
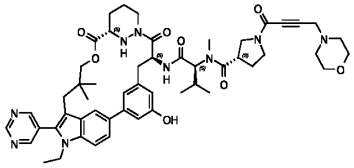
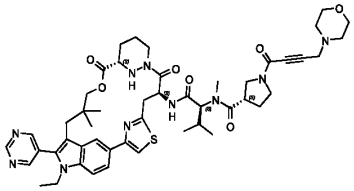
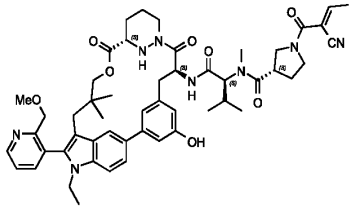
A174	
A175	
A176	
A177	
A178	

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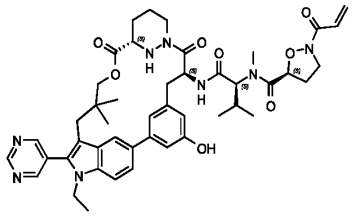
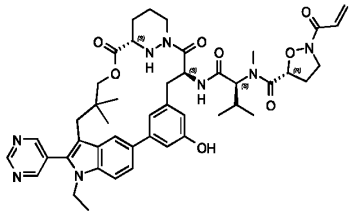
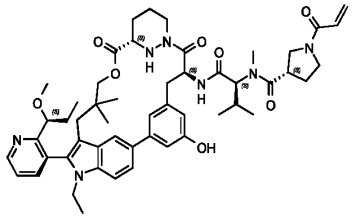
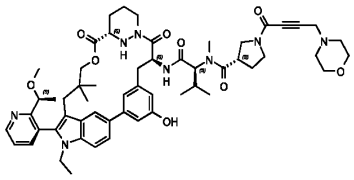
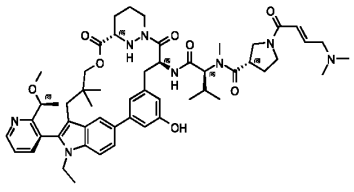
A179	
A180	
A181	
A182	
A183	

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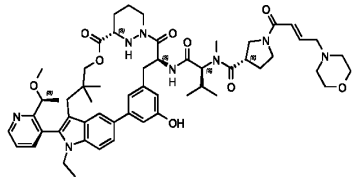
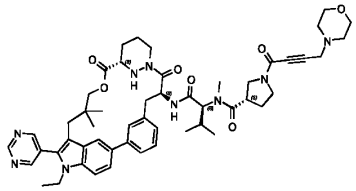
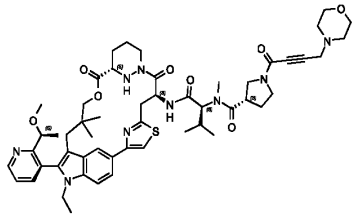
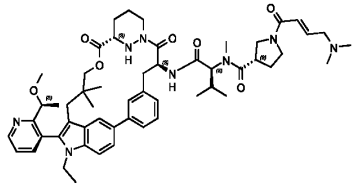
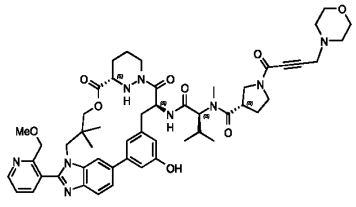
A184	
A185	
A186	
A187	
A188	

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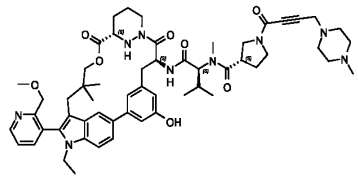
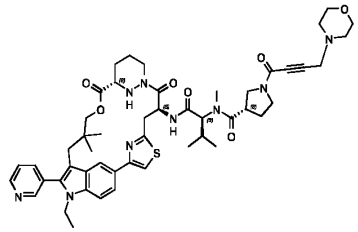
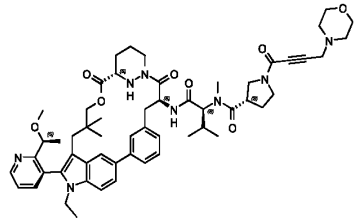
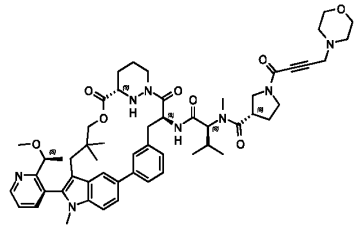
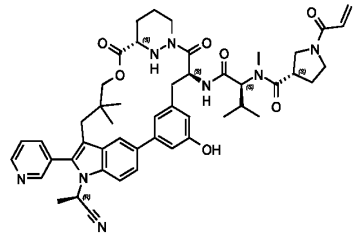
A189	
A190	
A191	
A192	
A193	

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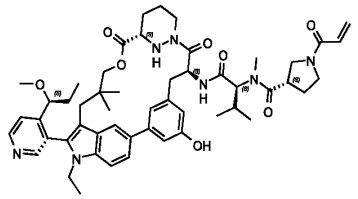
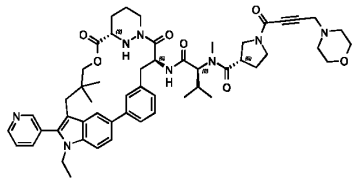
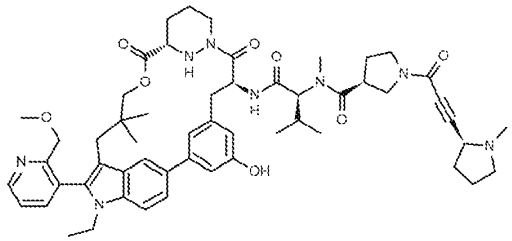
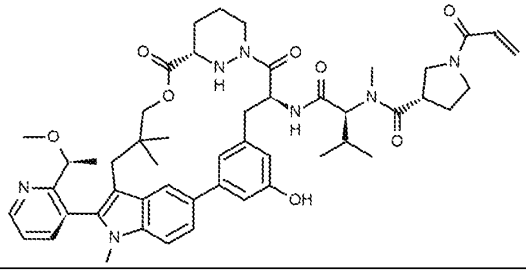
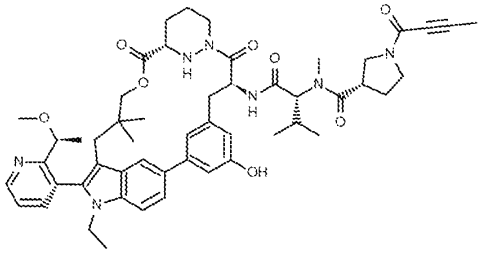
A194	
A195	
A196	
A197	
A198	

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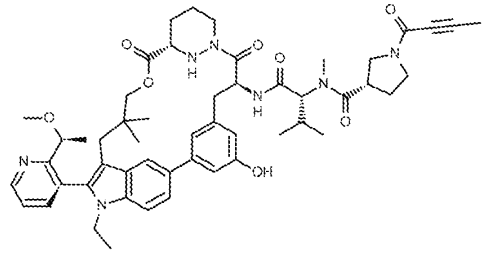
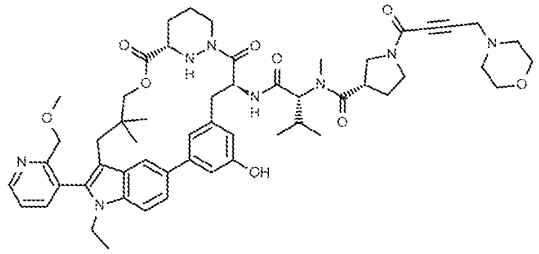
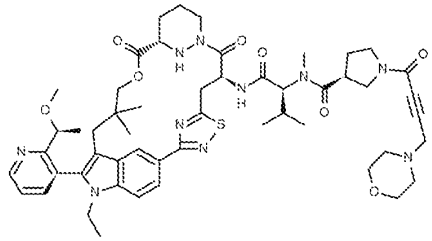
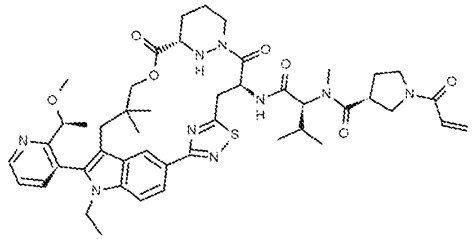
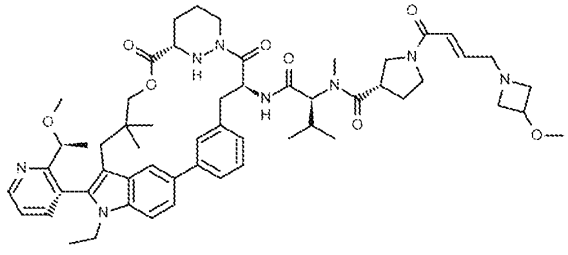
A199	
A200	
A201	
A202	
A203	

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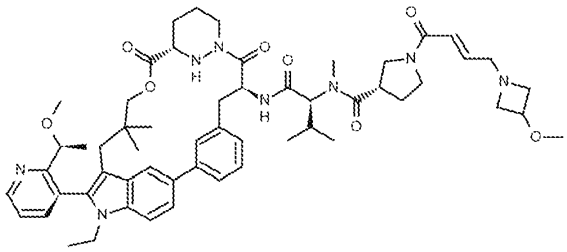
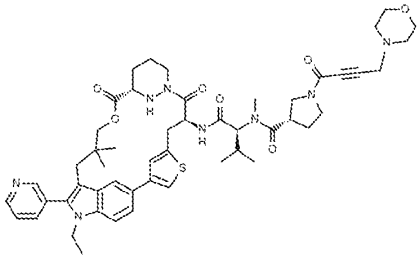
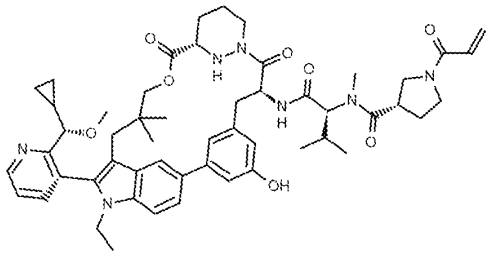
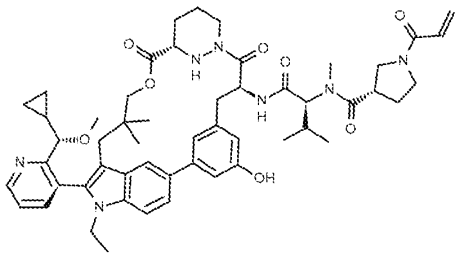
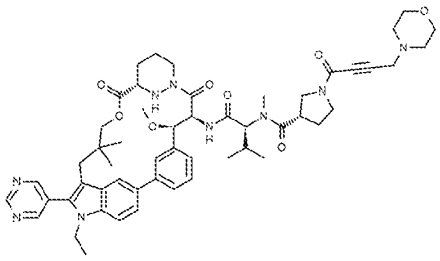
A204	
A205	
A206	
A207	
A208	

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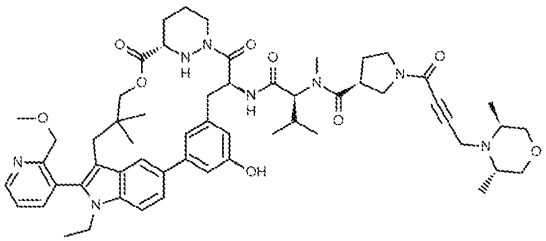
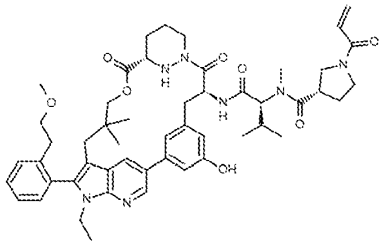
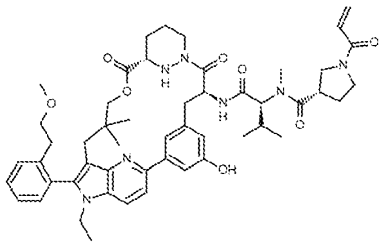
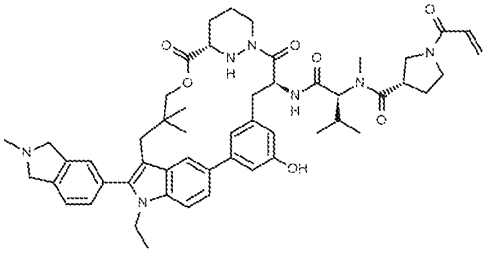
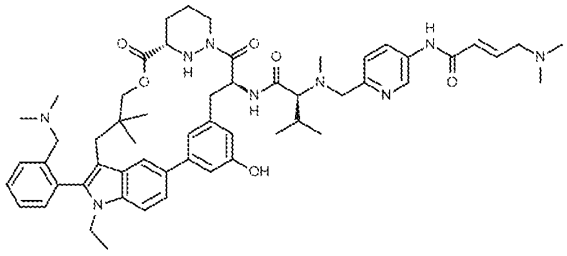
A209	
A210	
A211	
A212	
A213	

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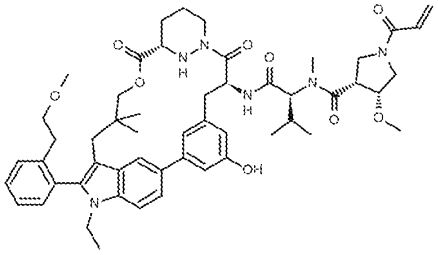
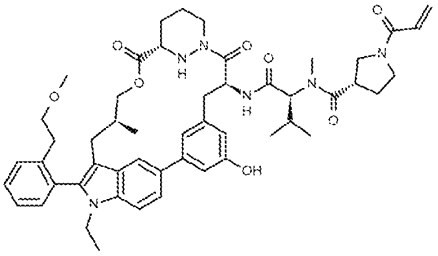
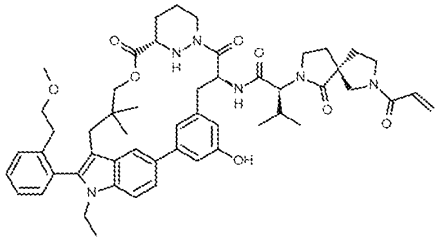
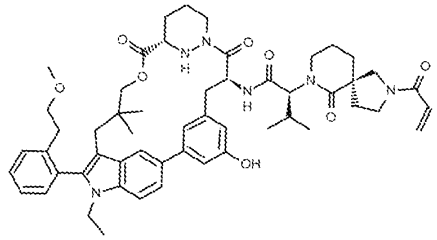
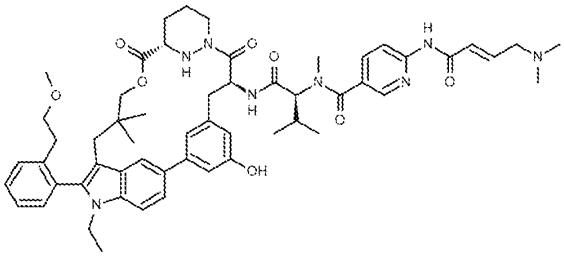
A214	
A215	
A216	
A217	
A218	

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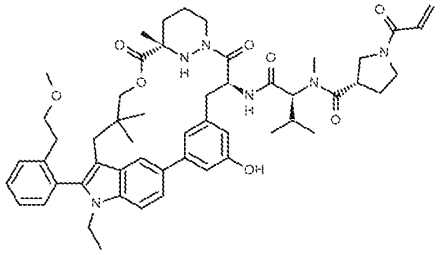
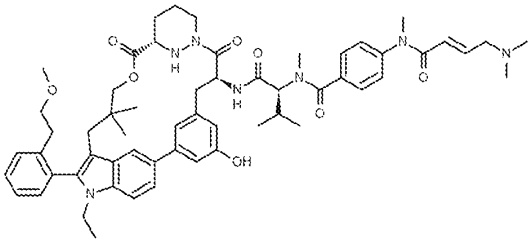
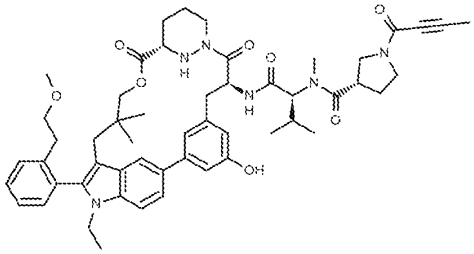
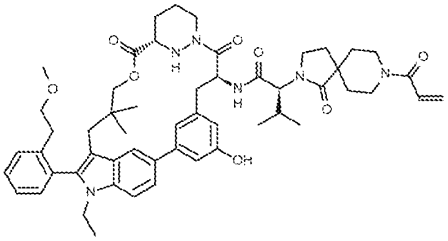
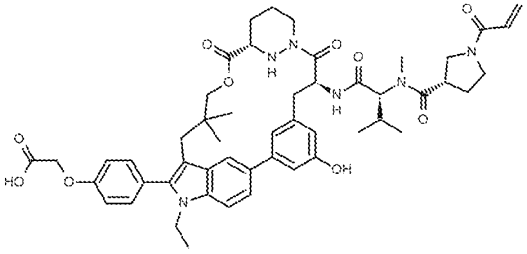
A219	
A220	
A221	
A222	
A223	

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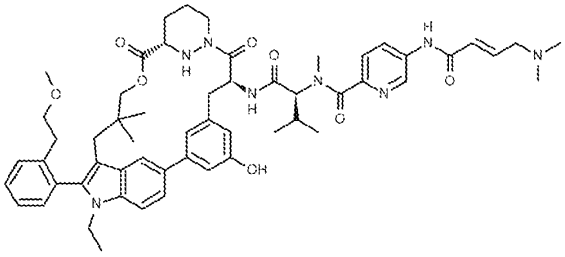
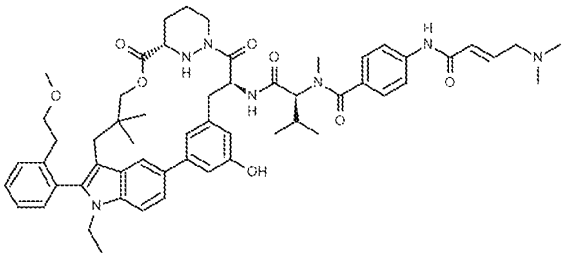
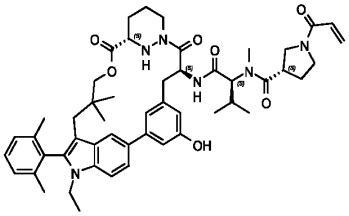
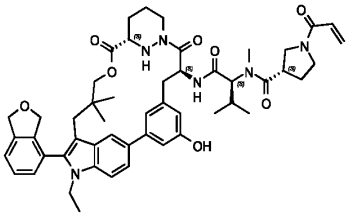
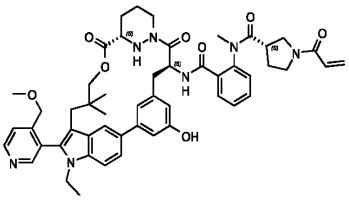
A224	
A225	
A226	
A227	
A228	

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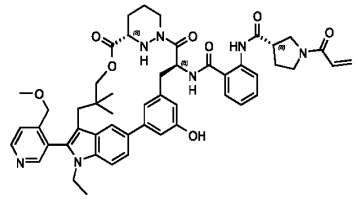
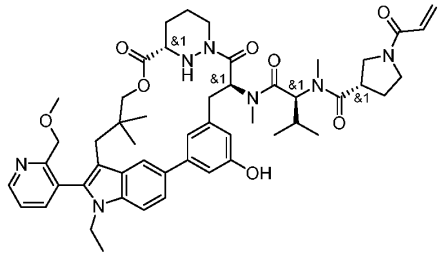
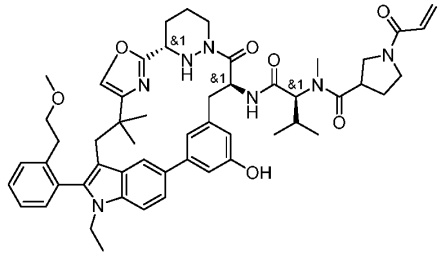
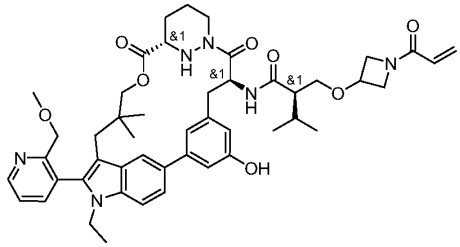
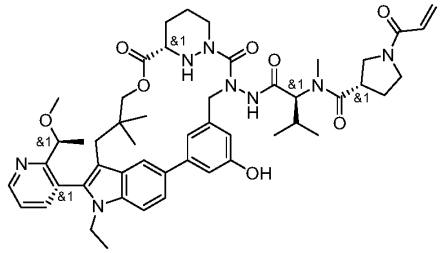
A229	
A230	
A231	
A232	
A233	

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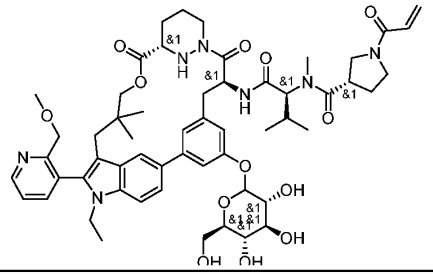
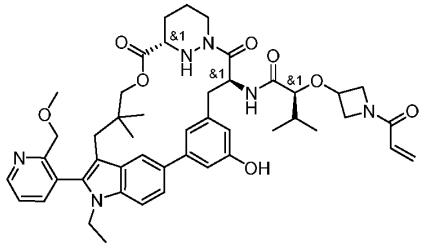
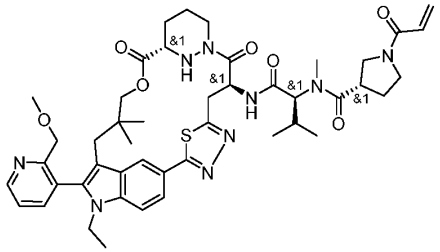
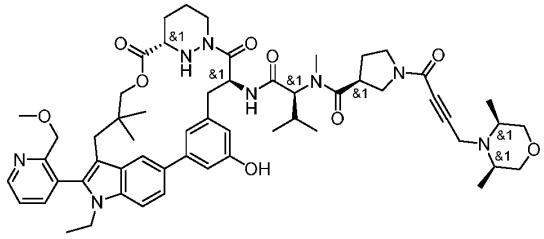
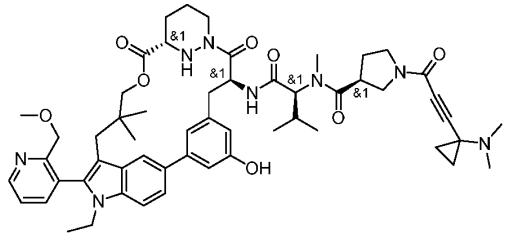
A234	
A235	
A236	
A237	
A238	

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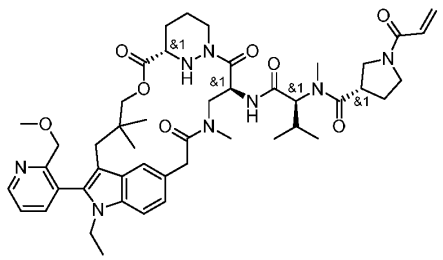
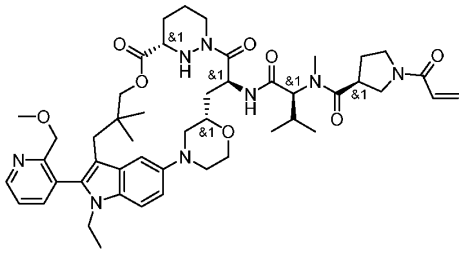
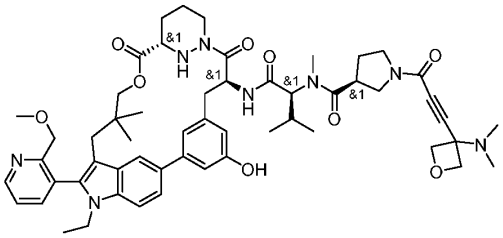
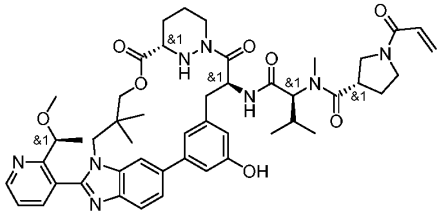
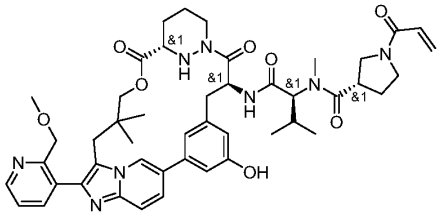
A239	
A240	
A241	
A242	
A243	

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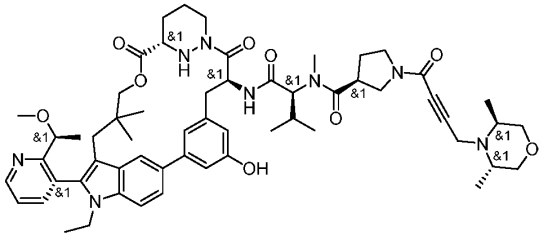
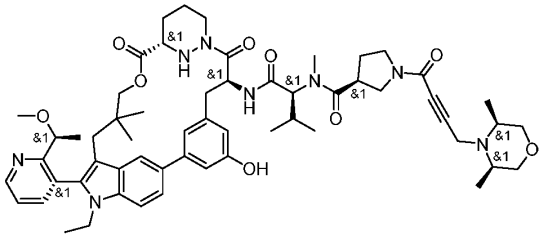
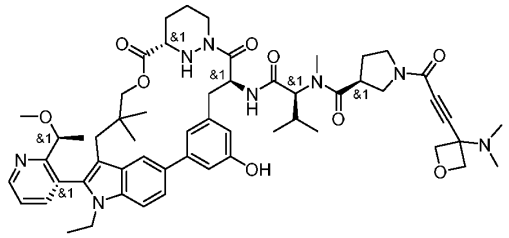
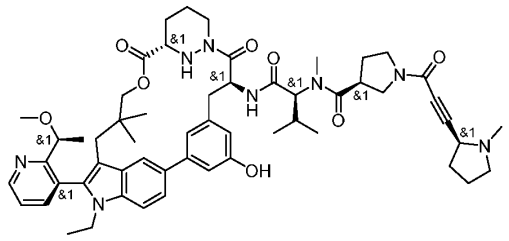
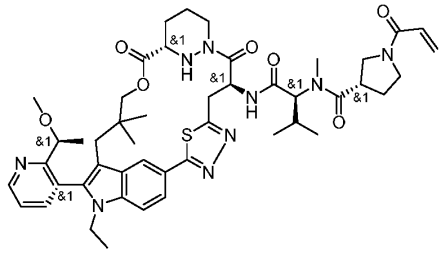
A244	
A245	
A246	
A247	
A248	

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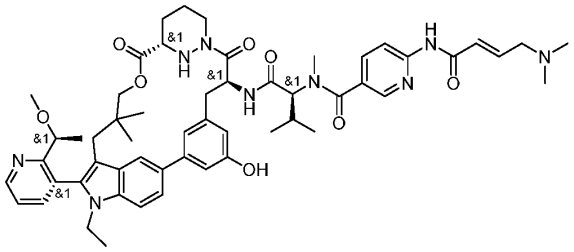
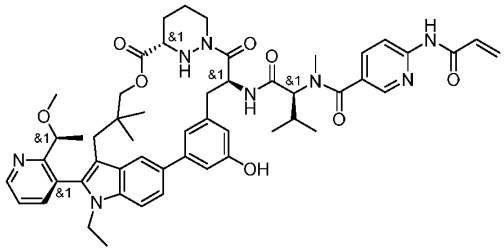
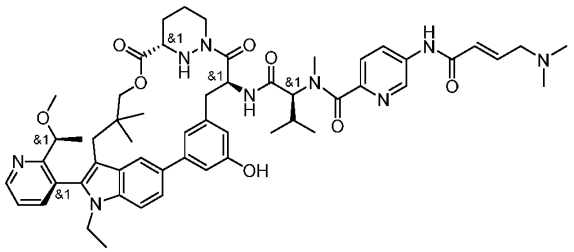
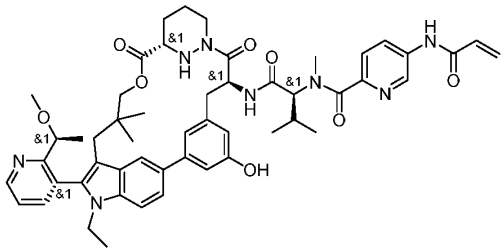
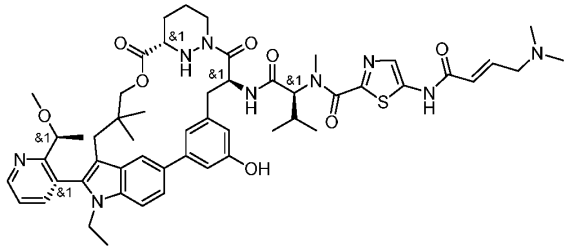
A249	
A250	
A251	
A252	
A253	

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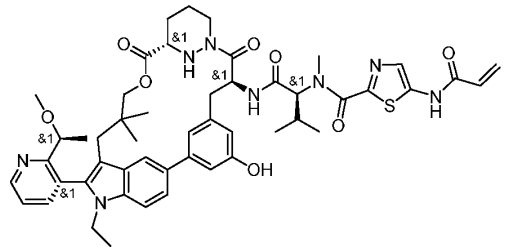
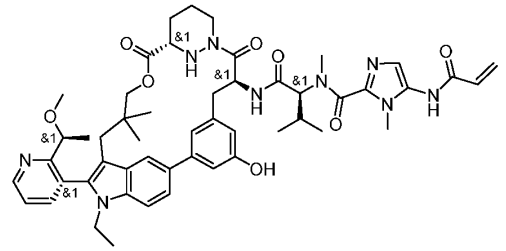
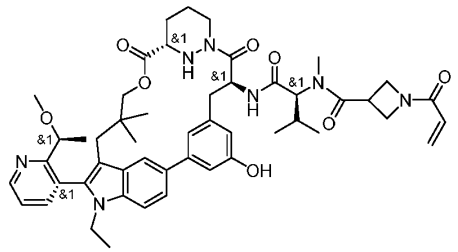
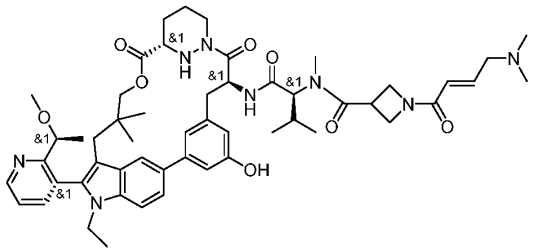
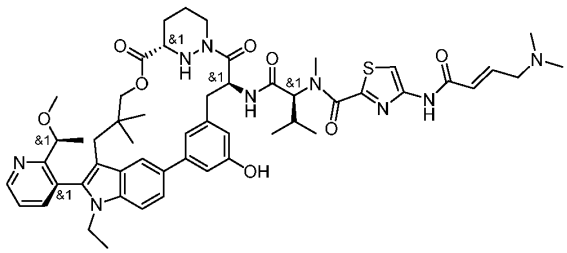
A254	
A255	
A256	
A257	
A258	

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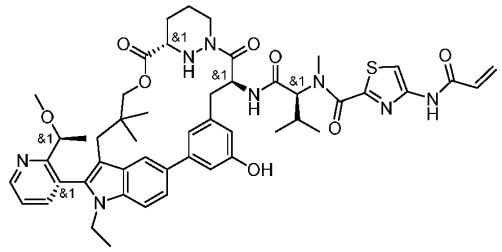
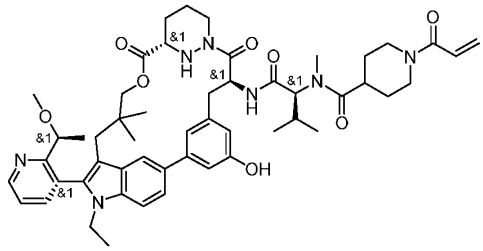
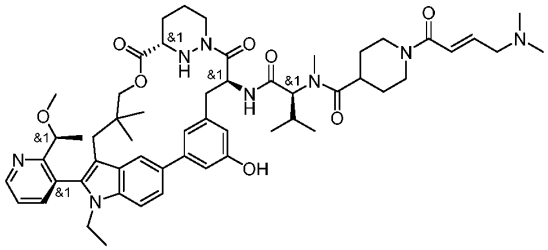
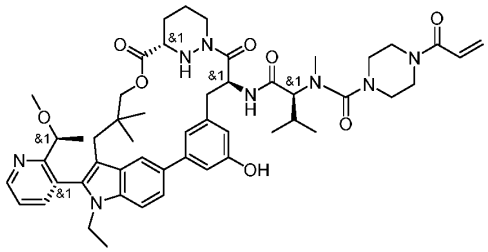
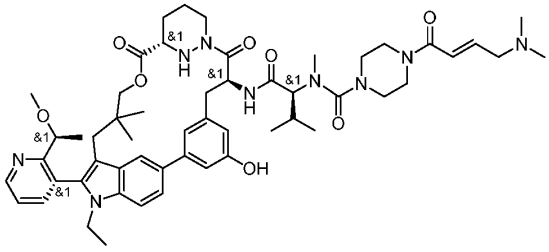
A259	
A260	
A261	
A262	
A263	

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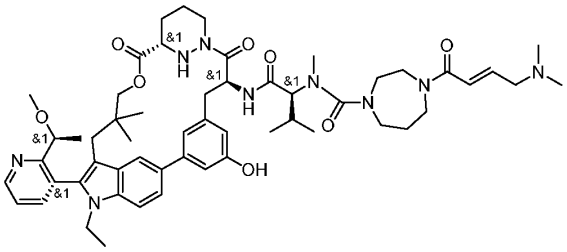
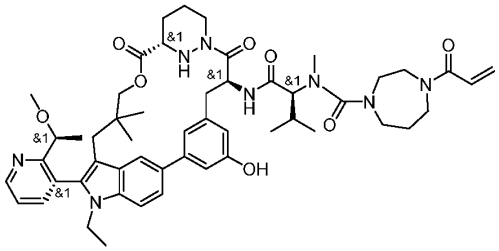
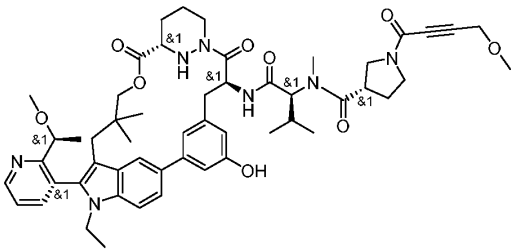
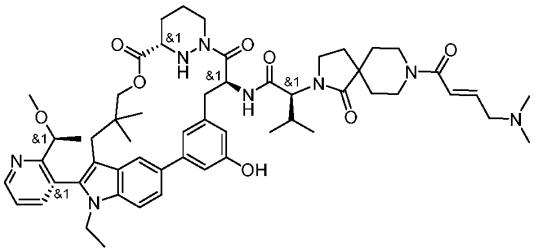
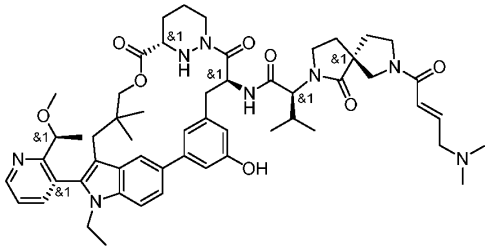
A264	
A265	
A266	
A267	
A268	

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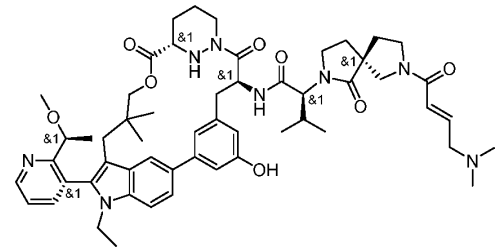
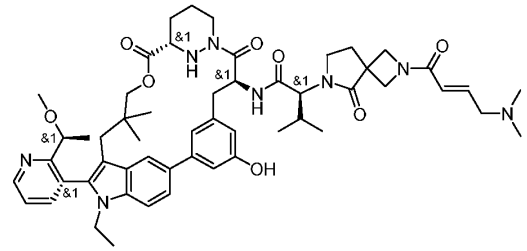
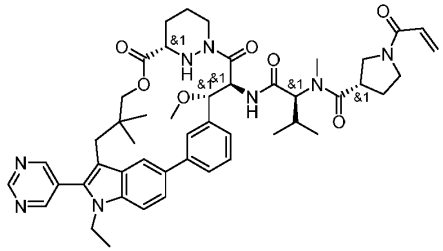
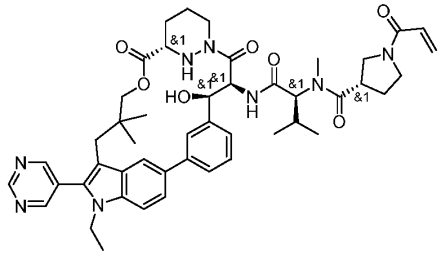
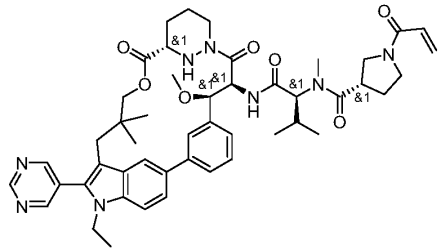
A269	
A270	
A271	
A272	
A273	

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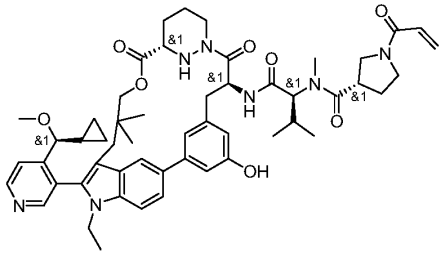
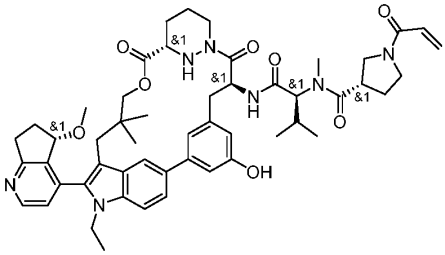
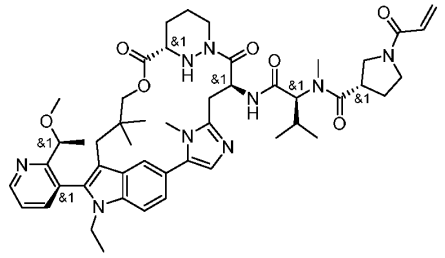
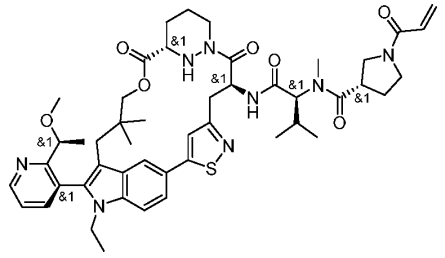
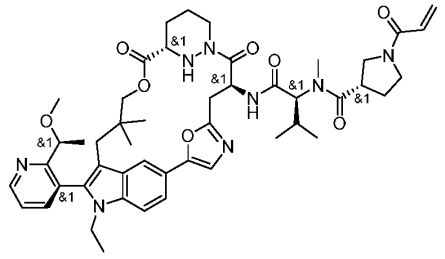
A274	
A275	
A276	
A277	
A278	

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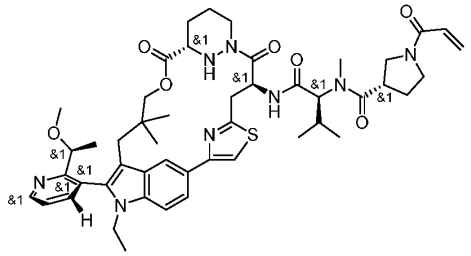
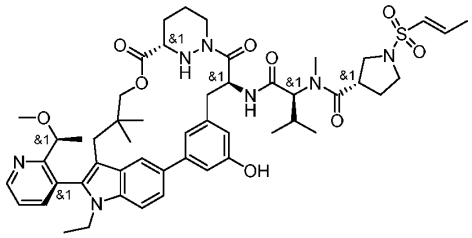
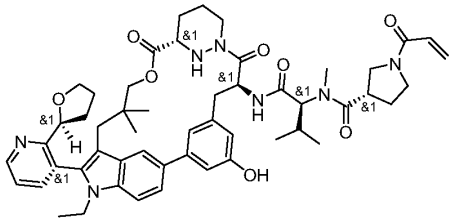
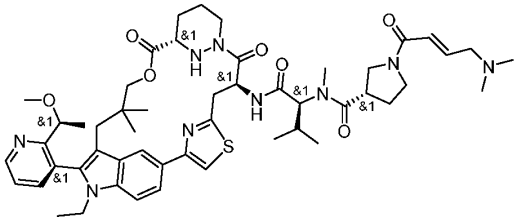
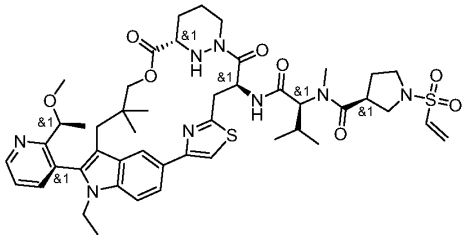
A279	
A280	
A281	
A282	
A283	

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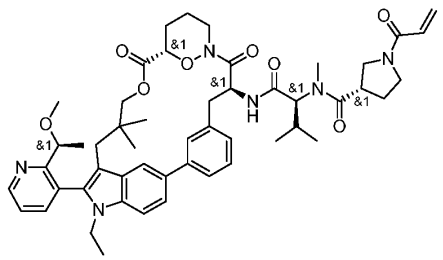
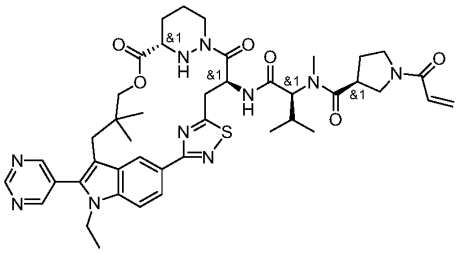
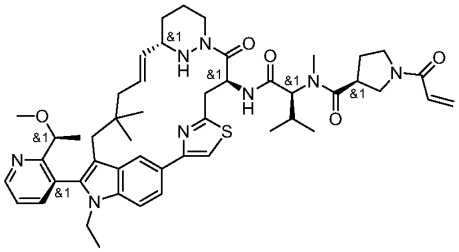
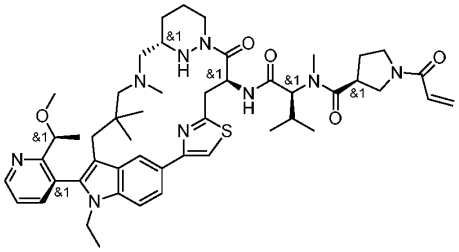
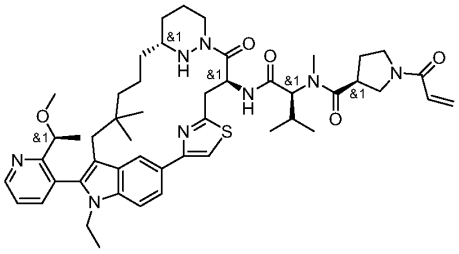
A284	
A285	
A286	
A287	
A288	

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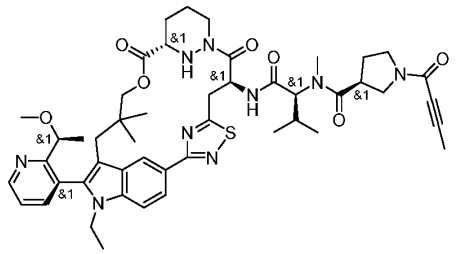
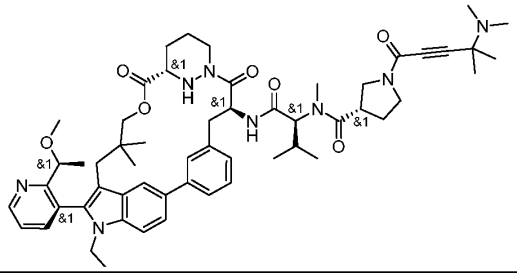
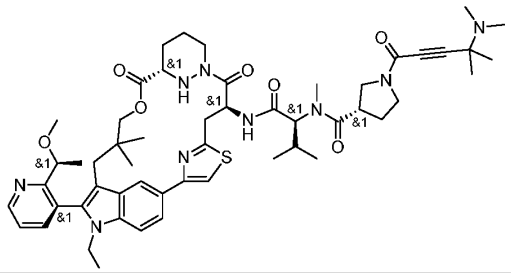
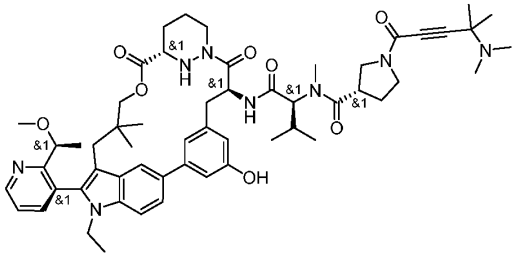
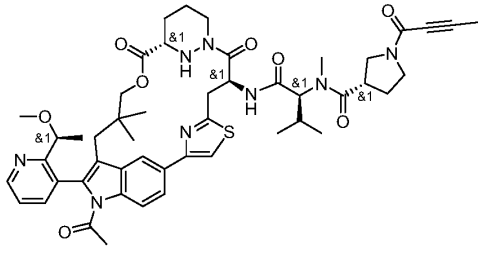
A289	
A290	
A291	
A292	
A293	

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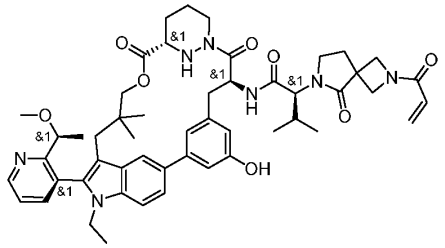
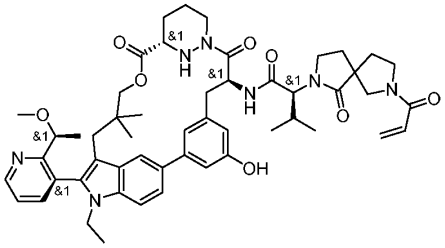
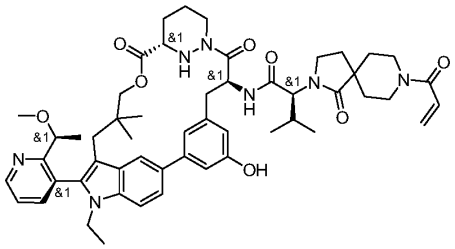
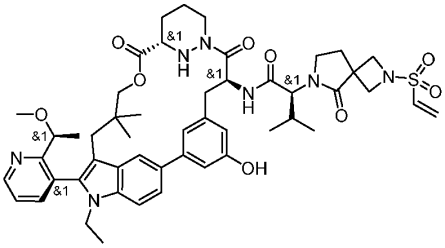
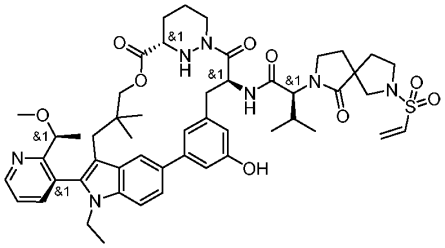
A294	
A295	
A296	
A297	
A298	

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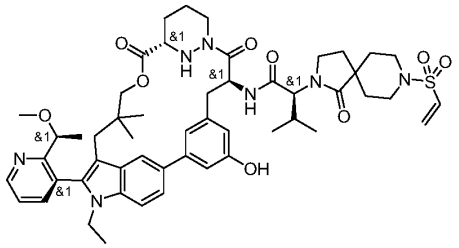
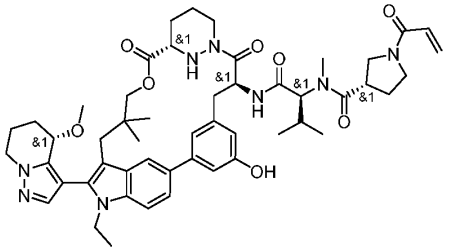
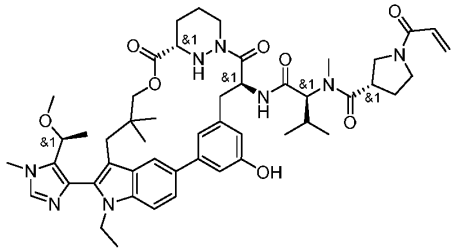
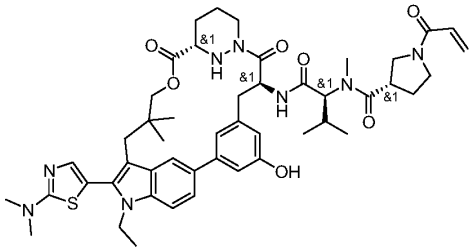
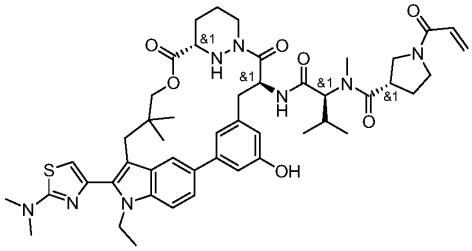
A299	
A300	
A301	
A302	
A303	

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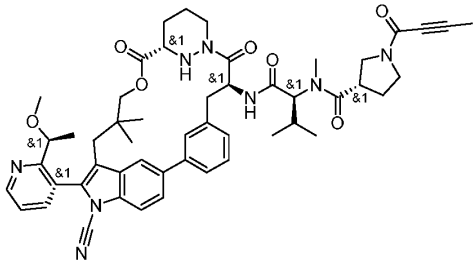
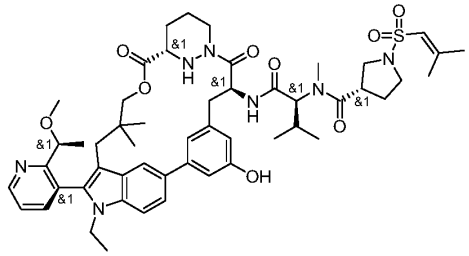
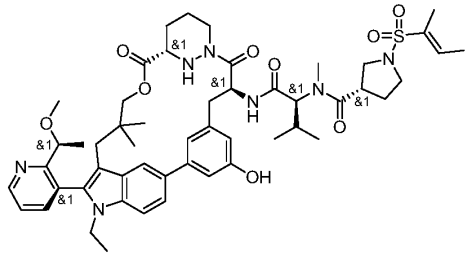
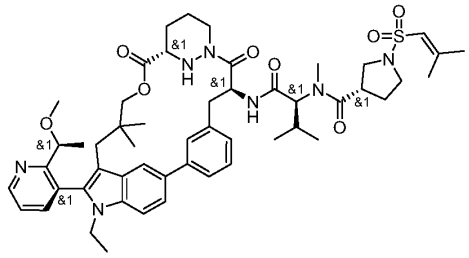
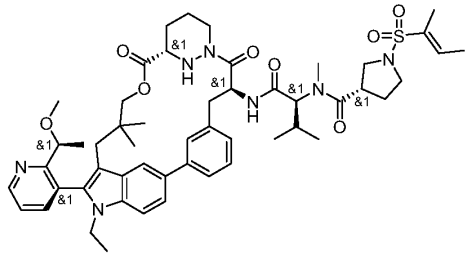
A304	
A305	
A306	
A307	
A308	

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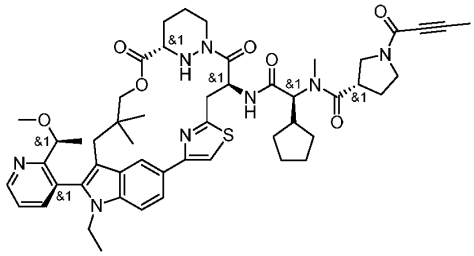
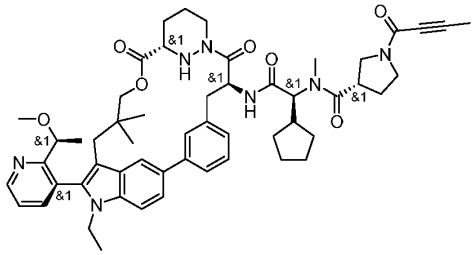
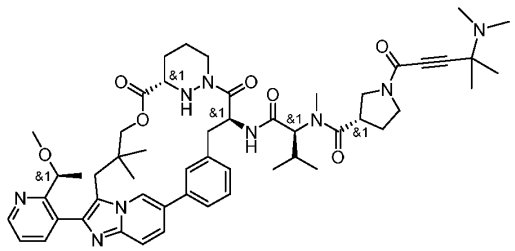
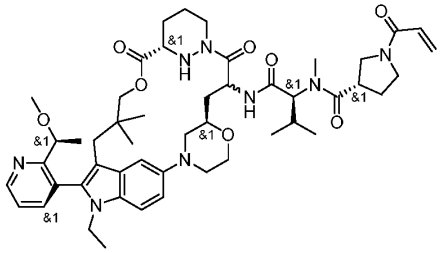
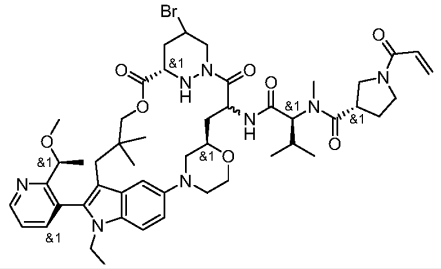
A309	
A310	
A311	
A312	
A313	

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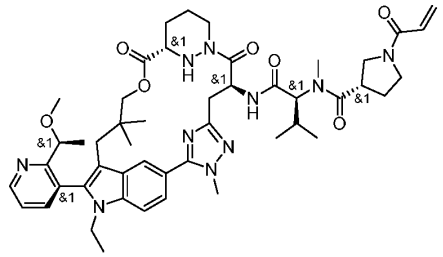
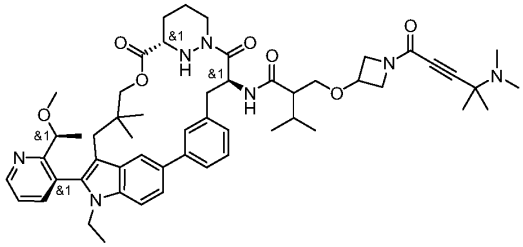
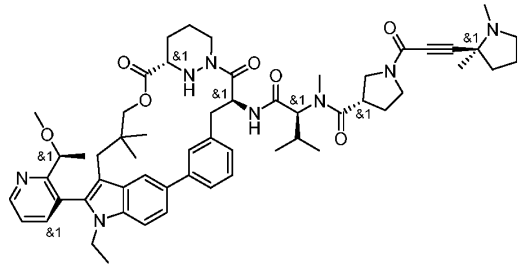
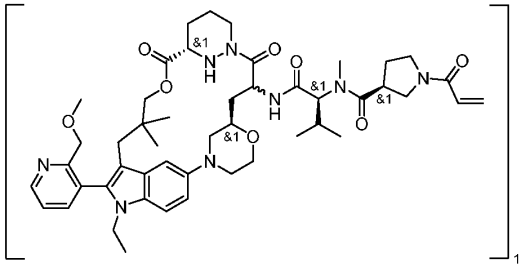
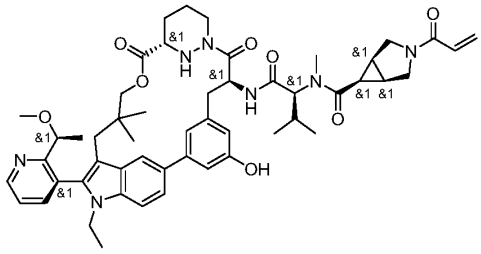
A314	
A316	
A317	
A318	
A319	

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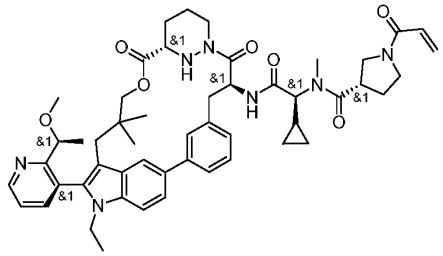
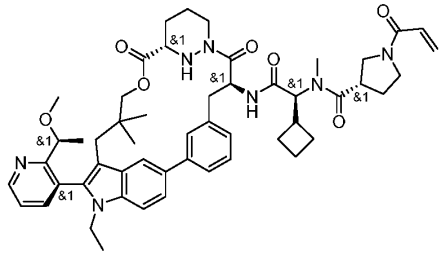
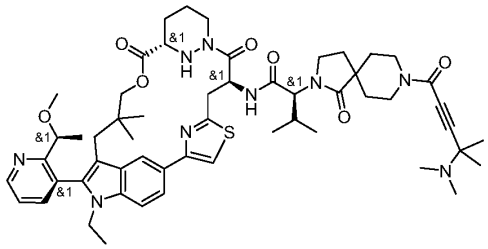
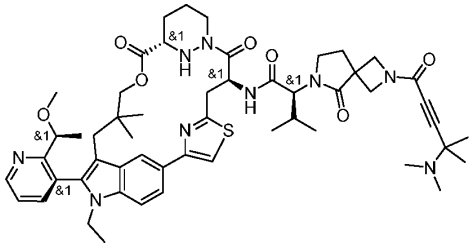
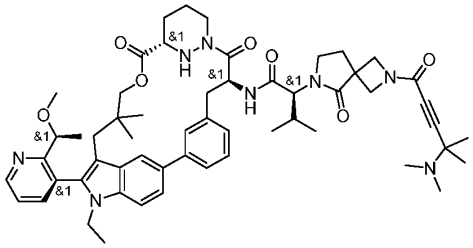
A320	
A321	
A322	
A323	
A324	

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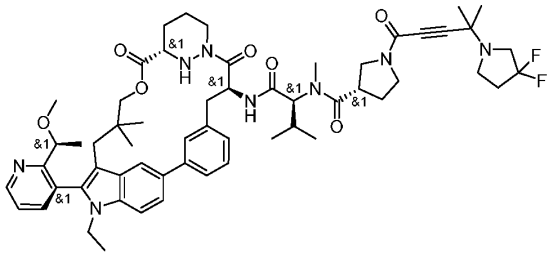
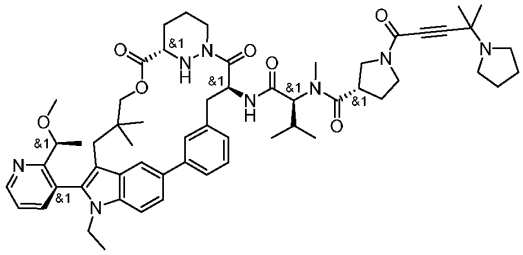
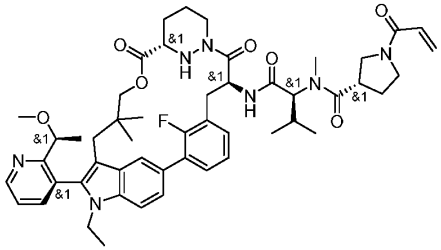
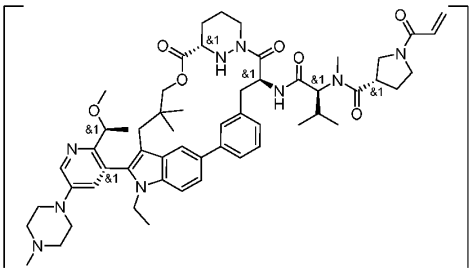
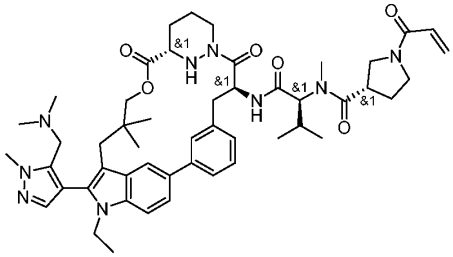
A325	
A326	
A327	
A328	
A329	

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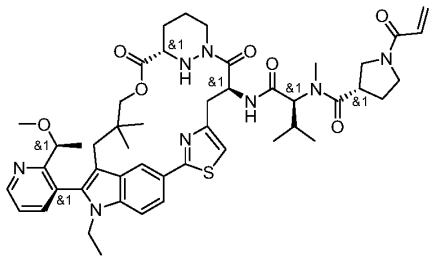
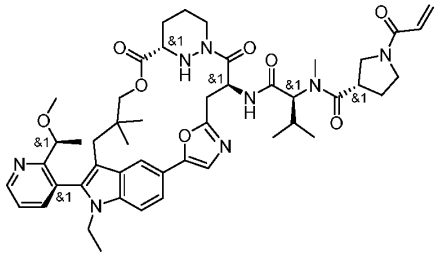
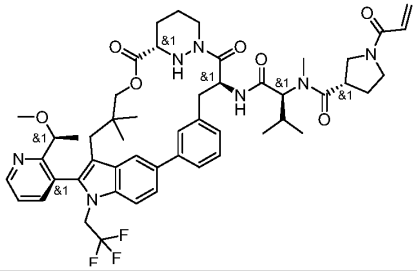
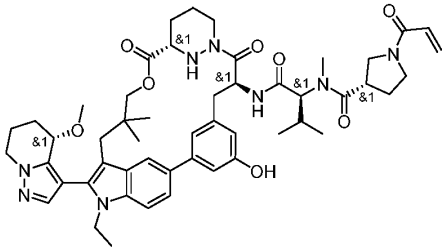
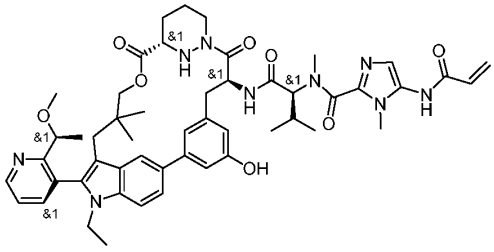
A330	
A331	
A332	
A333	
A334	

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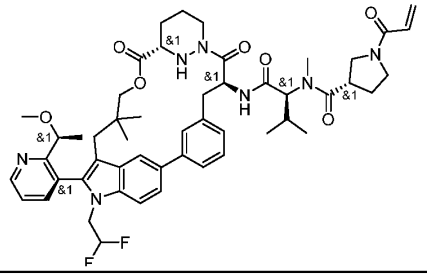
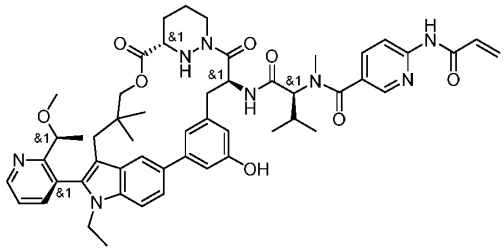
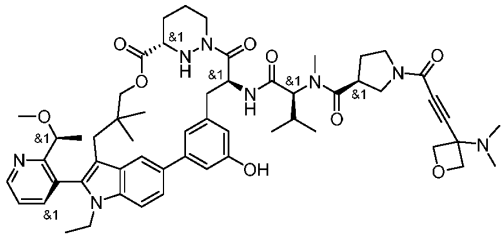
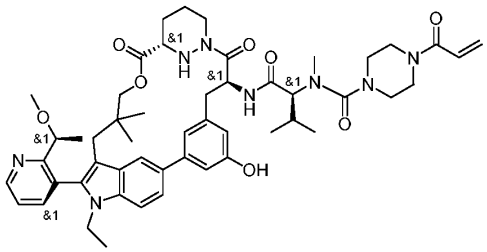
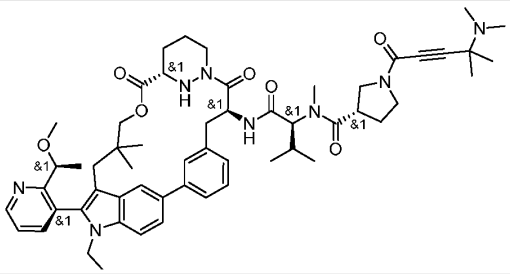
A335	
A336	
A337	
A338	
A339	

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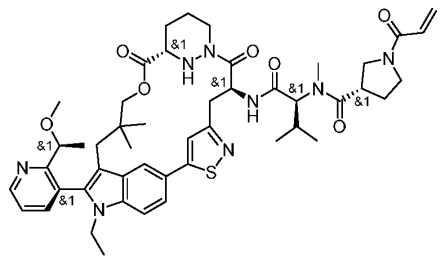
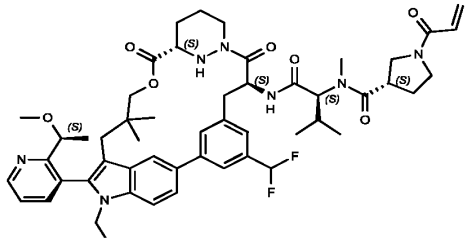
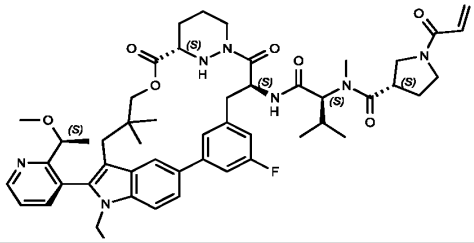
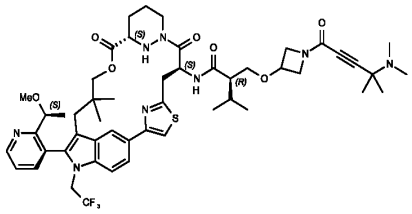
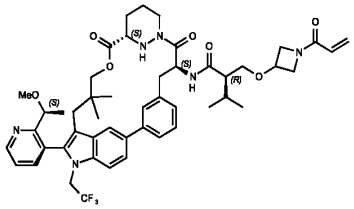
A340	
A341	
A342	
A343	
A344	

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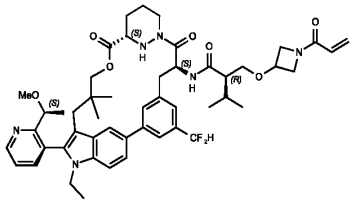
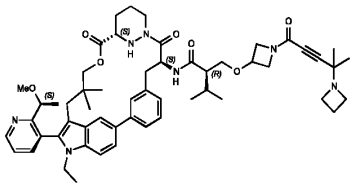
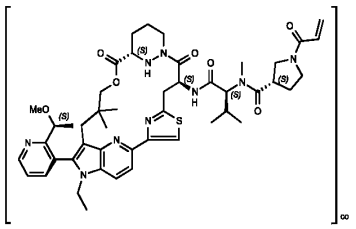
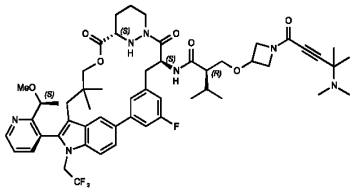
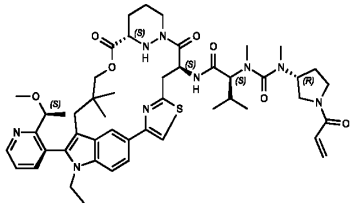
A345	
A346	
A347	
A348	
A349	

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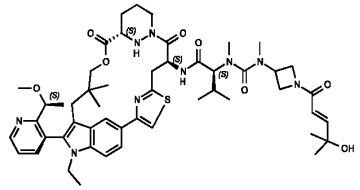
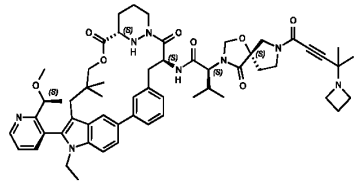
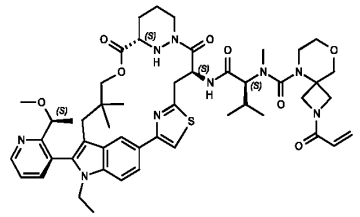
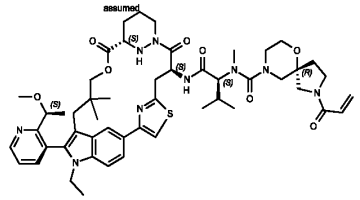
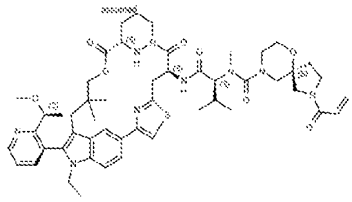
A350	
A351	
A352	
A353	
A354	

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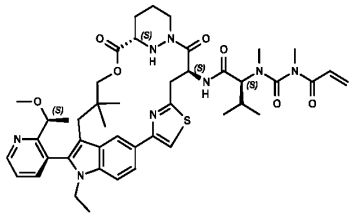
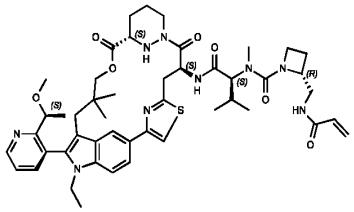
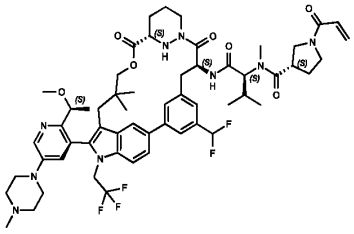
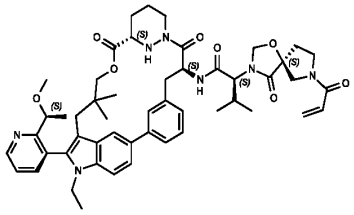
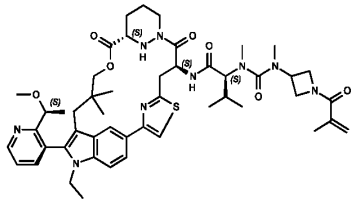
A355	
A356	
A357	
A358	
A359	

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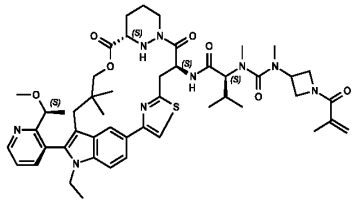
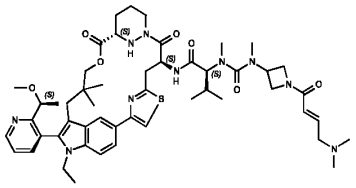
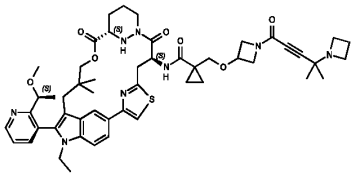
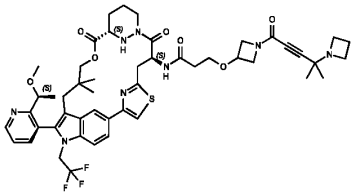
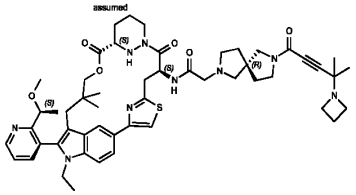
A360	
A361	
A362	
A363	
A364	

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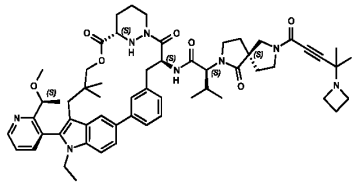
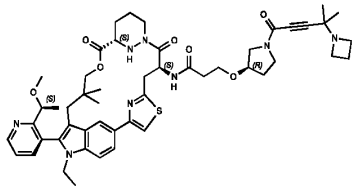
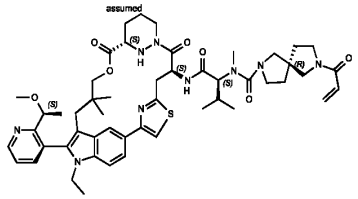
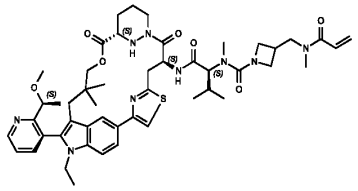
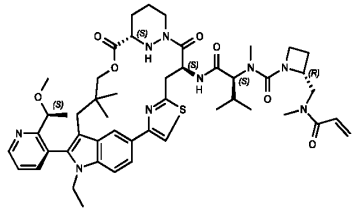
A365	
A366	
A367	
A368	
A369	

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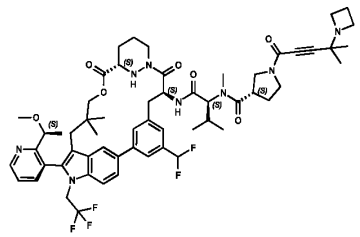
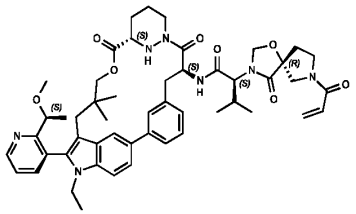
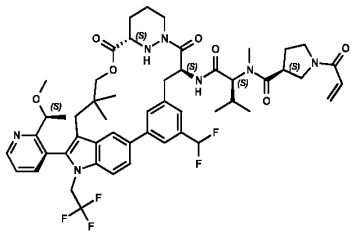
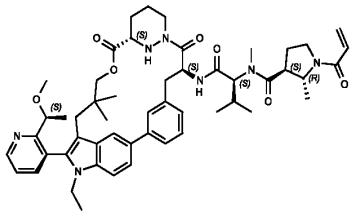
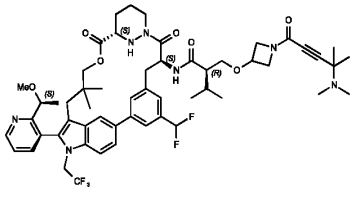
A370	
A371	
A372	
A373	
A374	

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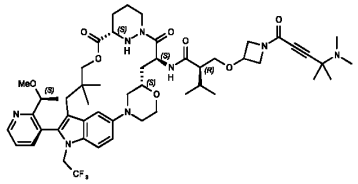
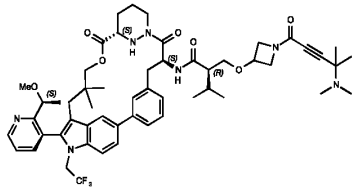
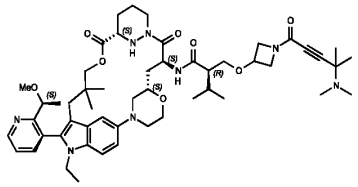
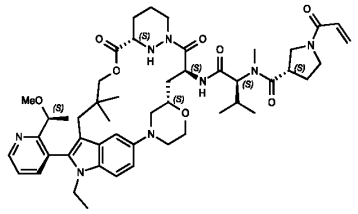
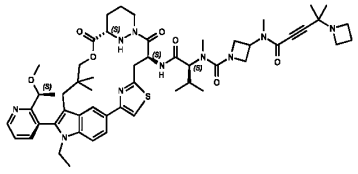
A375	
A376	
A377	
A378	
A379	

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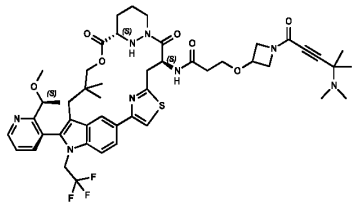
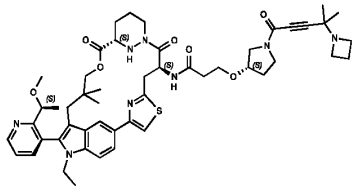
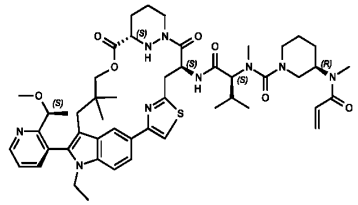
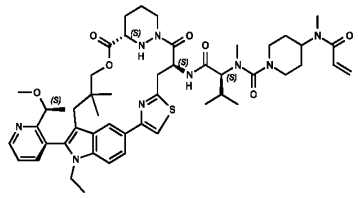
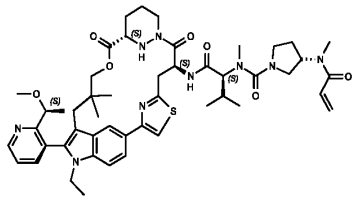
A380	
A381	
A382	
A383	
A384	

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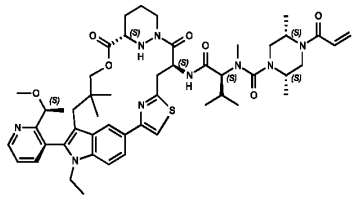
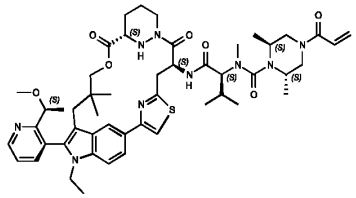
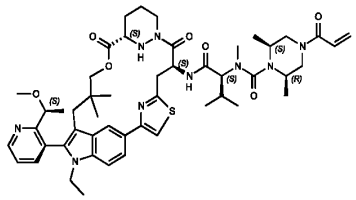
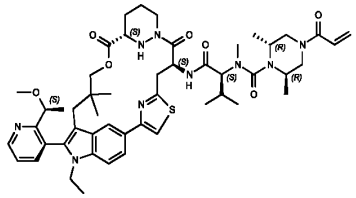
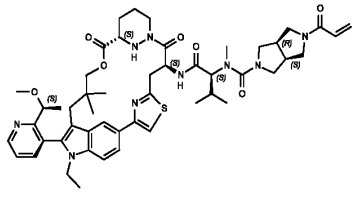
A385	
A386	
A387	
A388	
A389	

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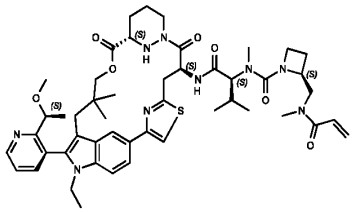
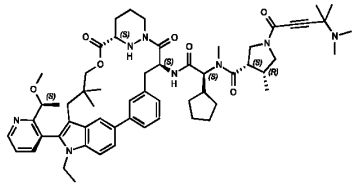
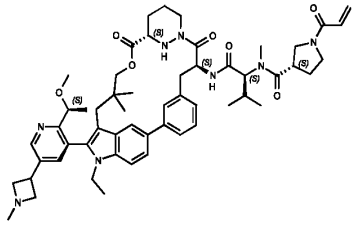
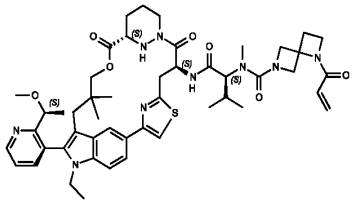
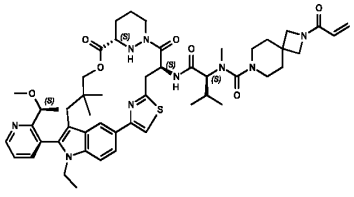
A390	
A391	
A392	
A393	
A394	

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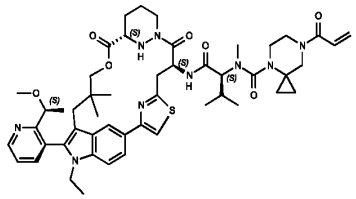
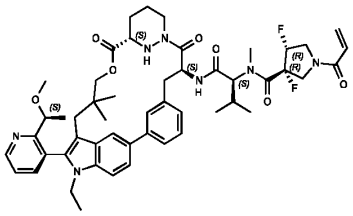
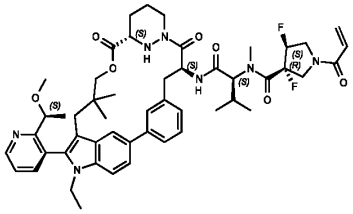
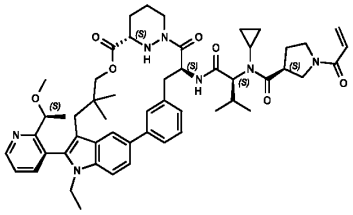
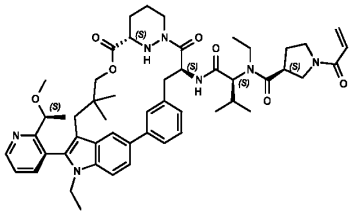
A395	
A396	
A397	
A398	
A399	

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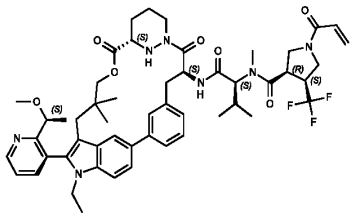
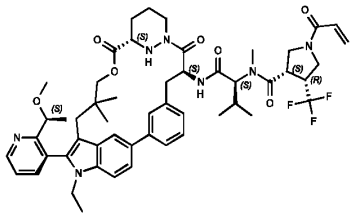
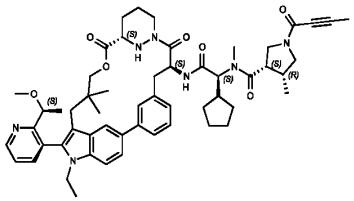
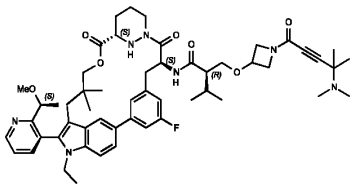
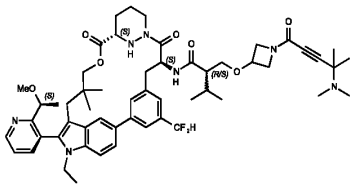
A400	
A401	
A402	
A403	
A404	

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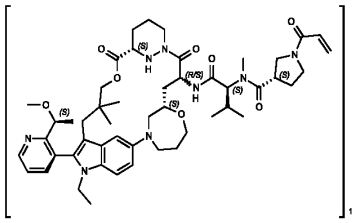
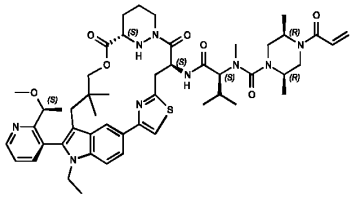
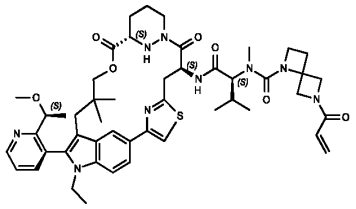
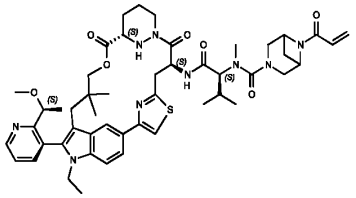
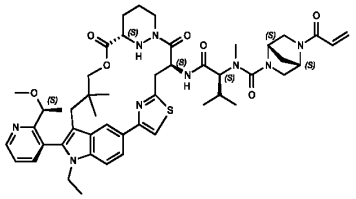
A405	
A406	
A407	
A408	
A409	

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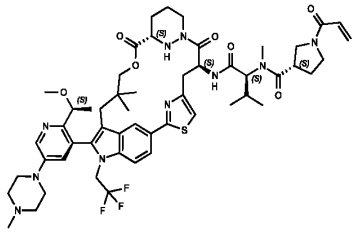
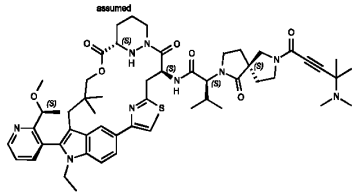
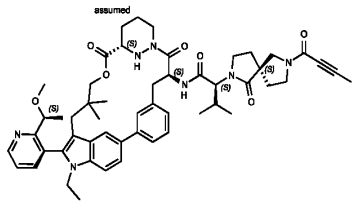
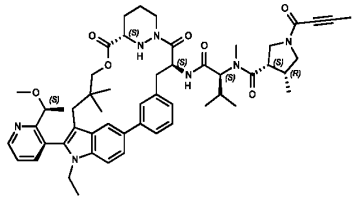
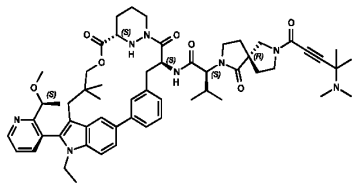
A410	
A411	
A412	
A413	
A414	

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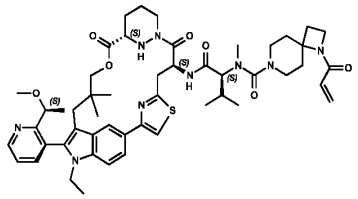
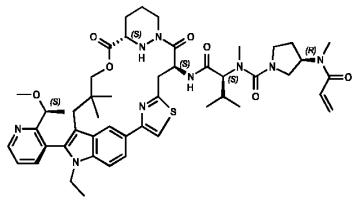
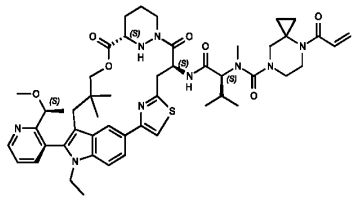
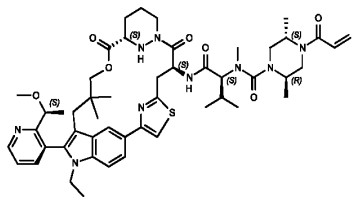
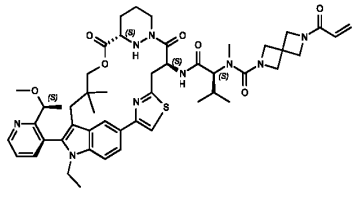
A415	
A416	
A417	
A418	
A419	

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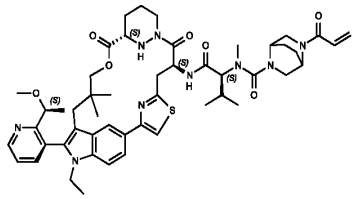
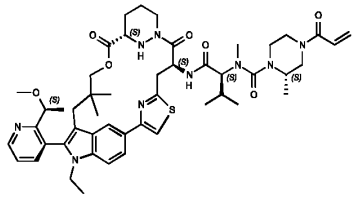
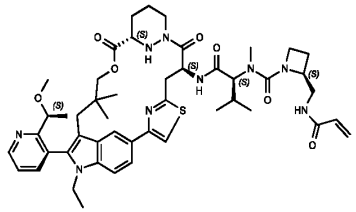
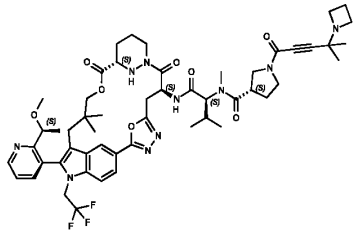
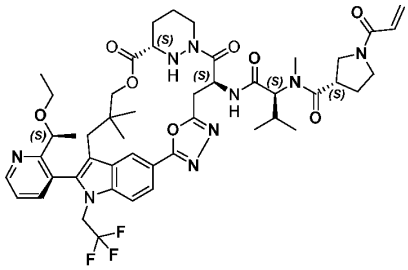
A420	
A421	
A422	
A423	
A424	

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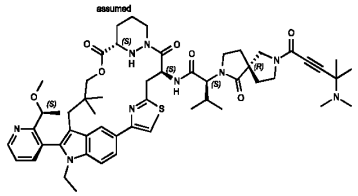
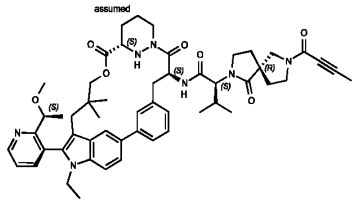
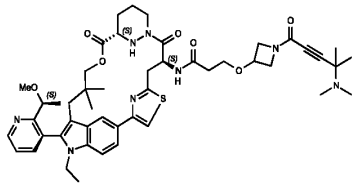
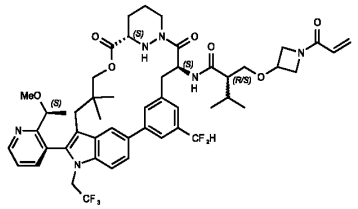
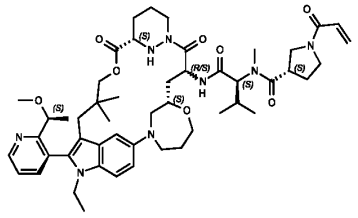
A425	
A426	
A427	
A428	
A429	

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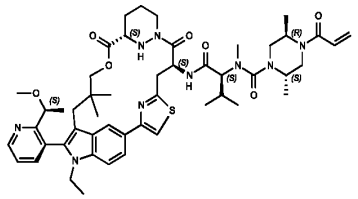
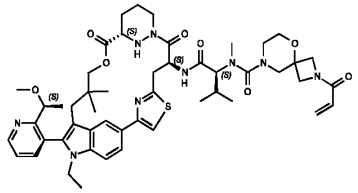
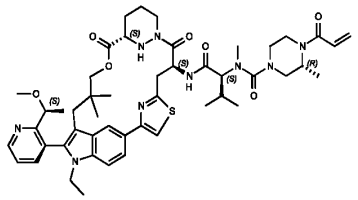
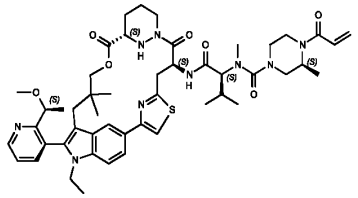
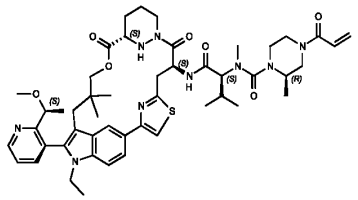
A430	
A431	
A432	
A433	
A334	

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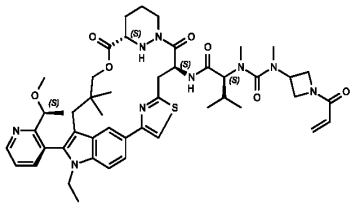
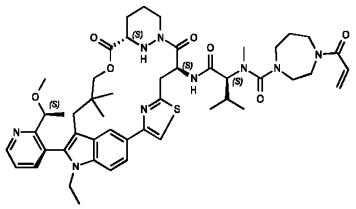
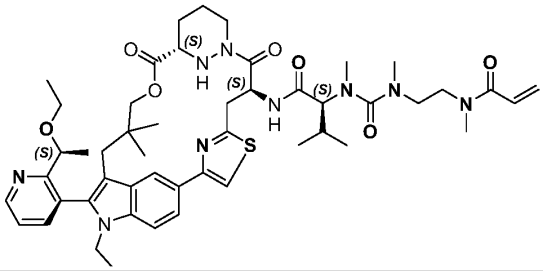
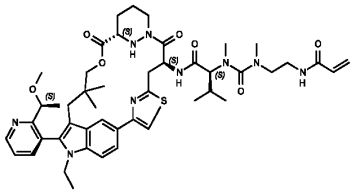
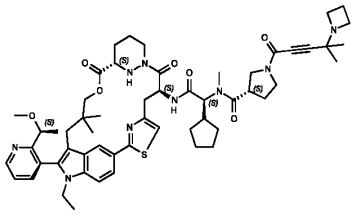
A435	
A436	
A437	
A438	
A439	

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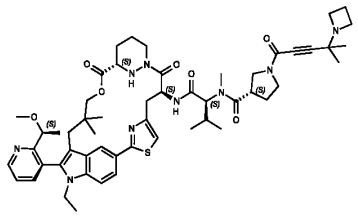
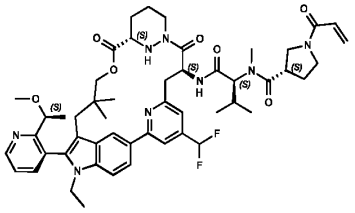
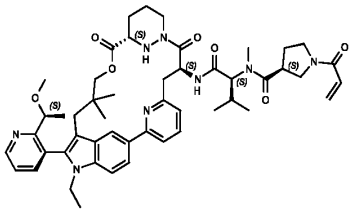
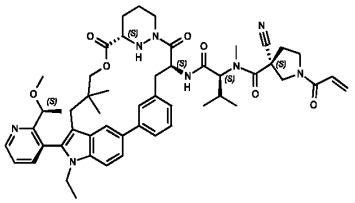
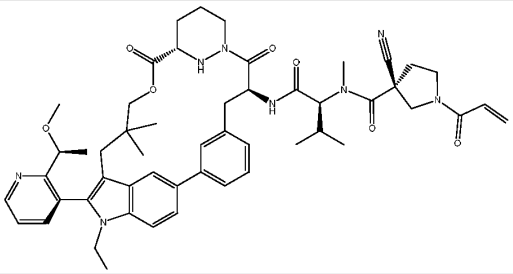
A440	
A441	
A442	
A443	
A444	

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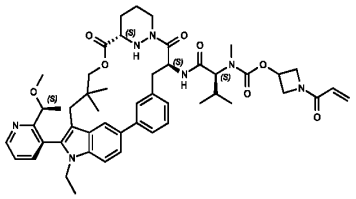
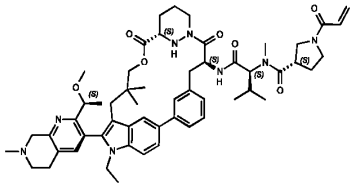
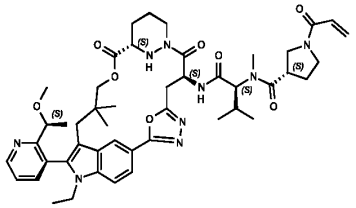
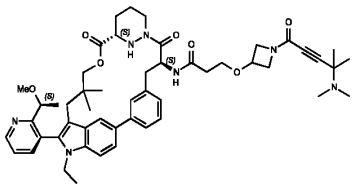
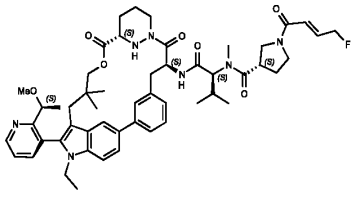
A445	
A446	
A447	
A448	
A449	

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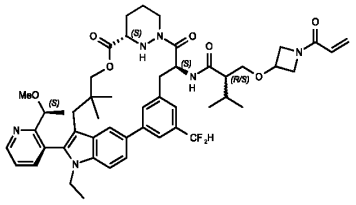
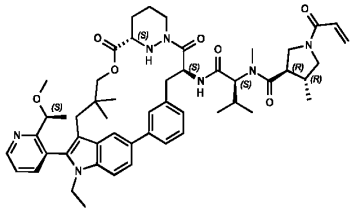
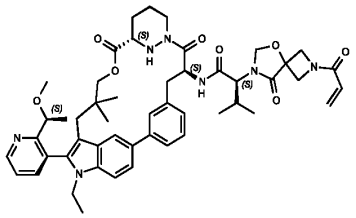
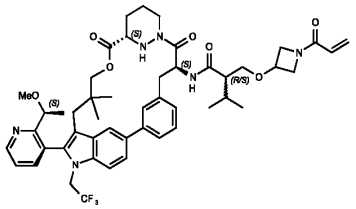
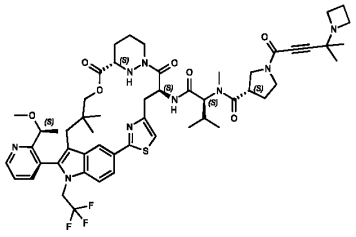
A450	
A451	
A452	
A453	
A454	

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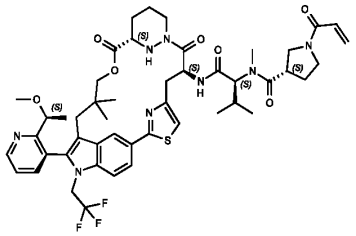
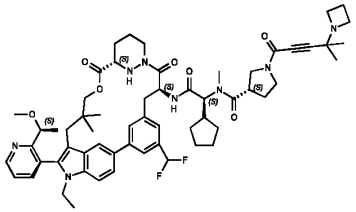
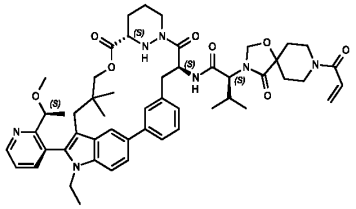
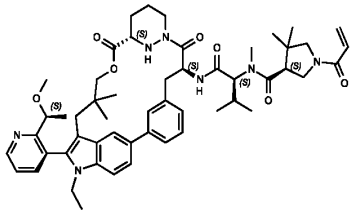
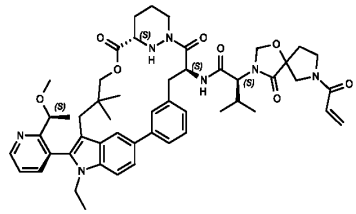
A455	
A456	
A457	
A458	
A459	

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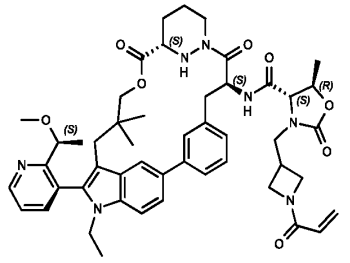
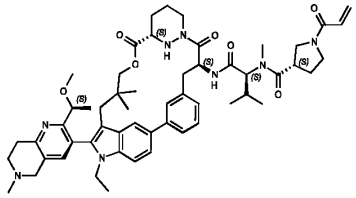
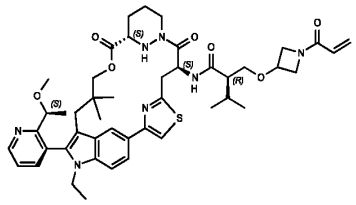
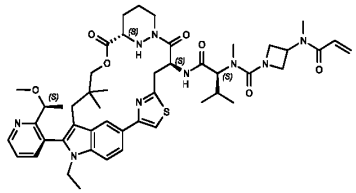
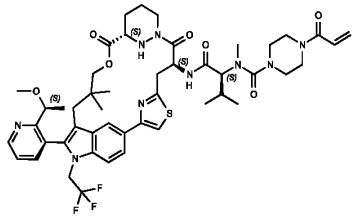
A460	
A461	
A462	
A463	
A464	

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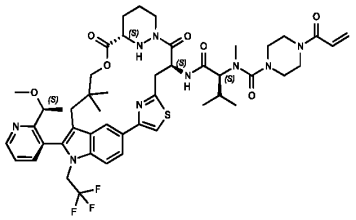
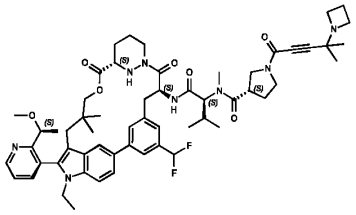
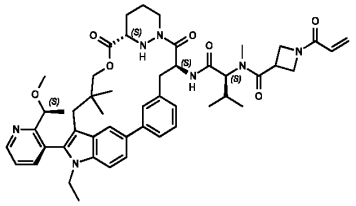
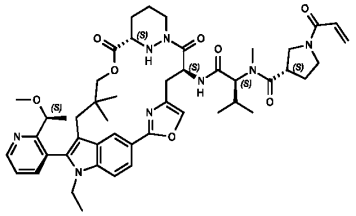
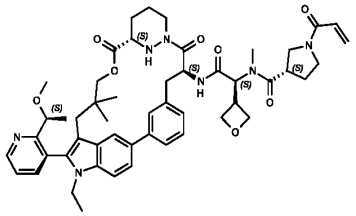
A465	
A466	
A467	
A468	
A469	

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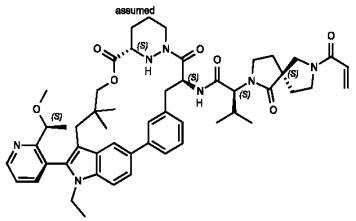
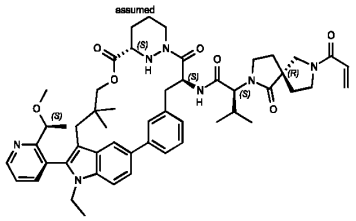
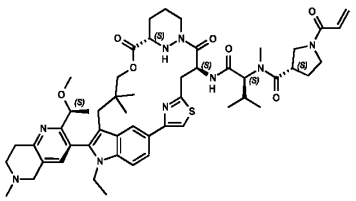
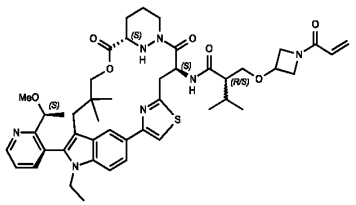
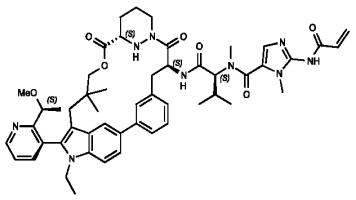
A470	
A471	
A472	
A473	
A474	

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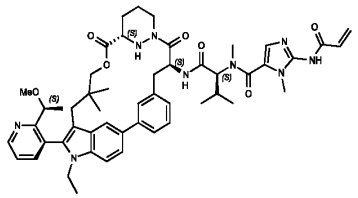
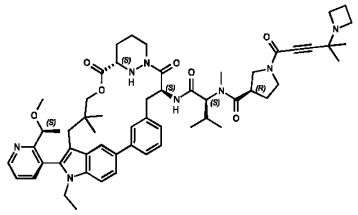
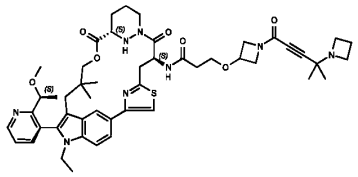
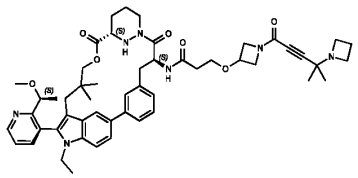
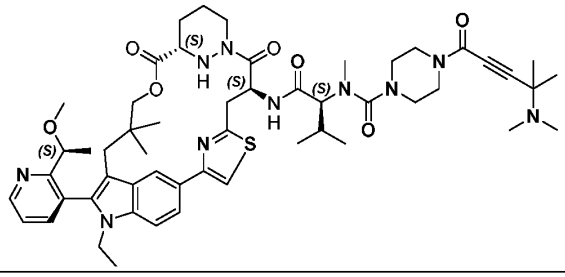
A475	
A476	
A477	
A478	
A479	

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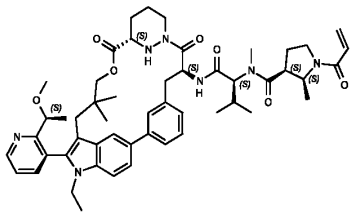
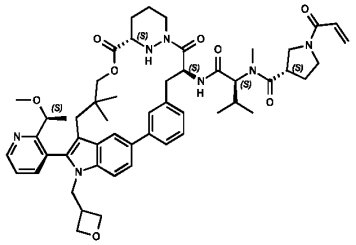
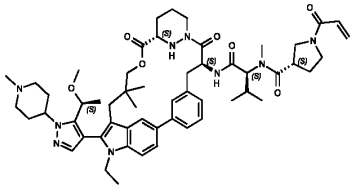
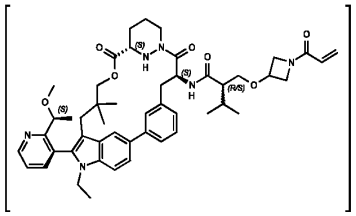
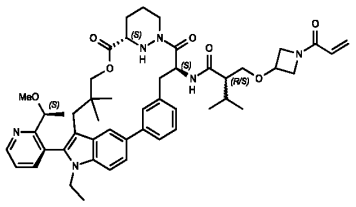
A480	
A481	
A482	
A483	
A484	

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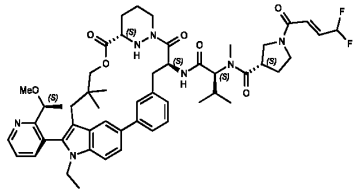
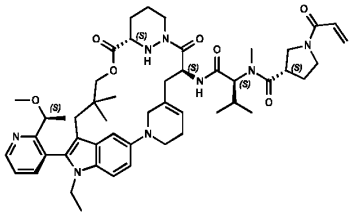
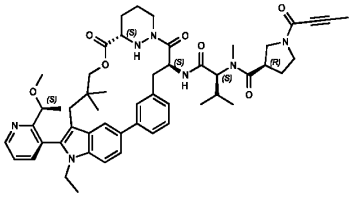
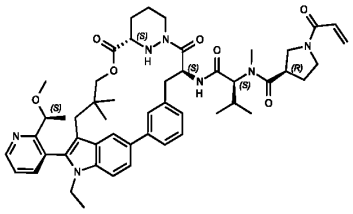
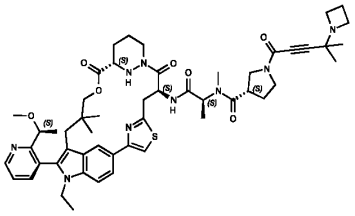
A485	
A486	
A487	
A488	
A489	

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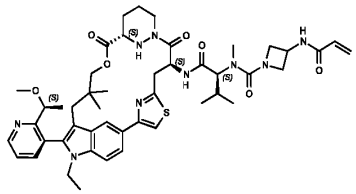
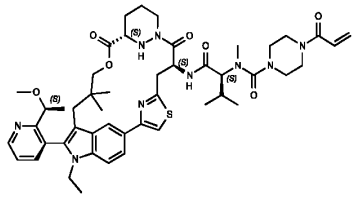
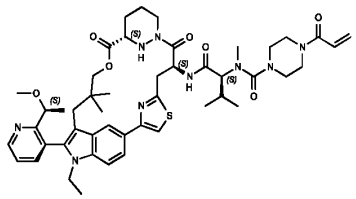
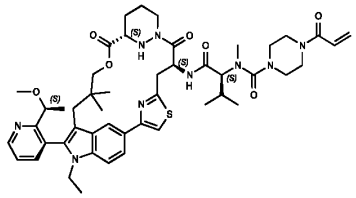
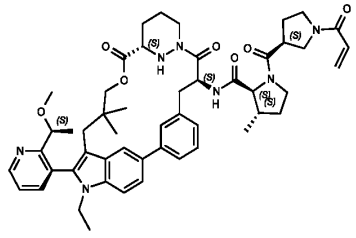
A490	
A491	
A492	
A493	
A494	

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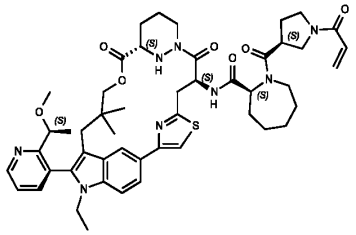
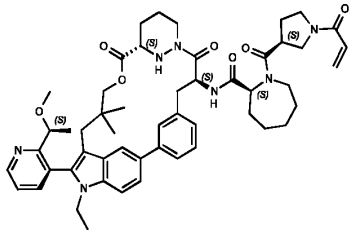
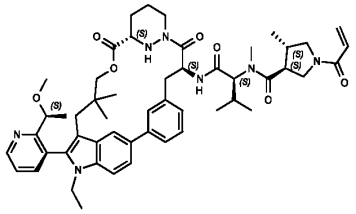
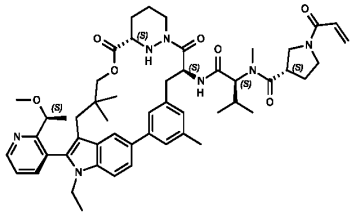
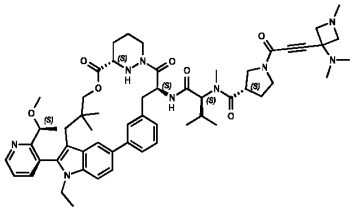
A495	
A496	
A497	
A498	
A499	

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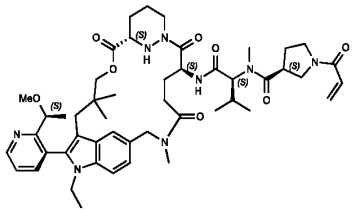
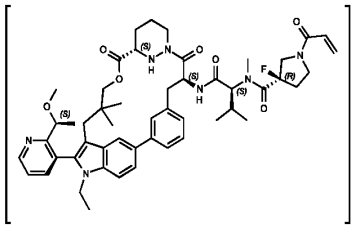
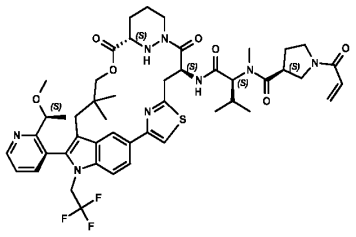
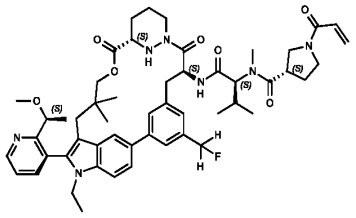
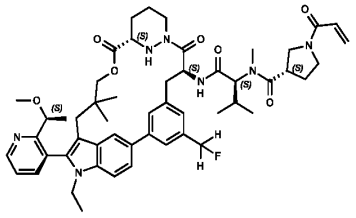
A500	
A501	
A502	
A503	
A504	

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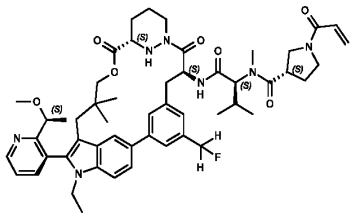
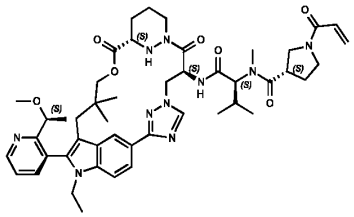
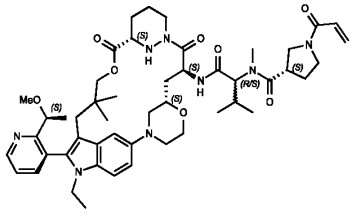
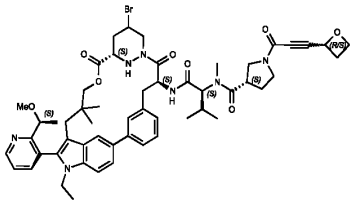
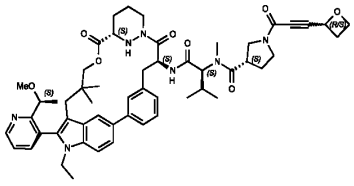
A505	
A506	
A507	
A508	
A509	

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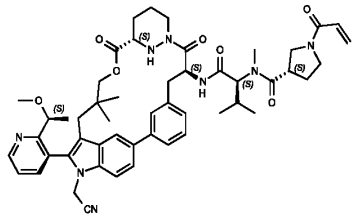
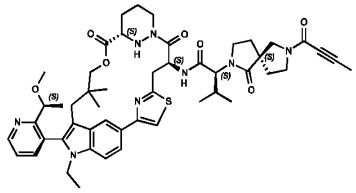
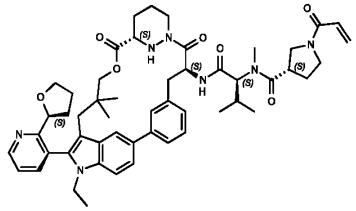
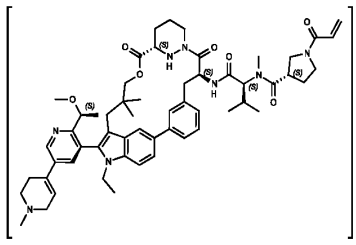
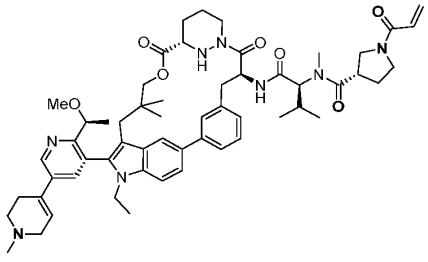
A510	
A511	
A512	
A513	
A514	

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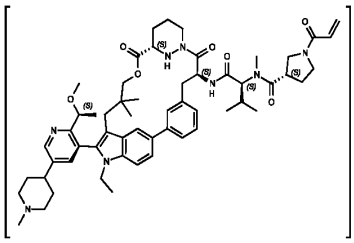
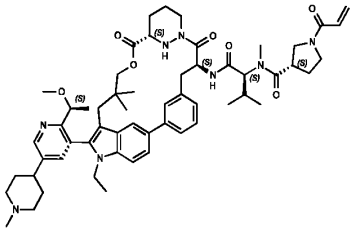
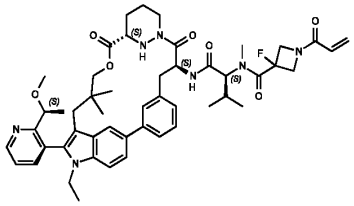
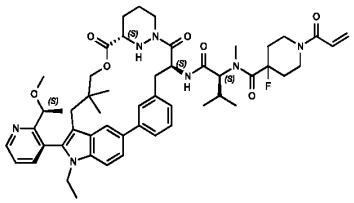
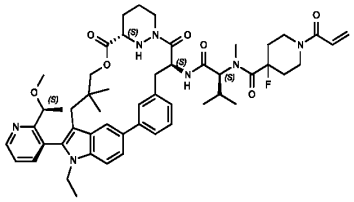
A515	
A516	
A517	
A518	
A519	

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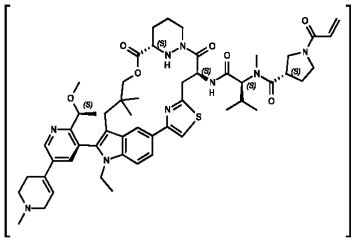
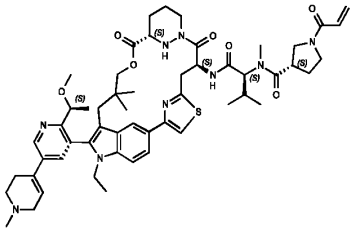
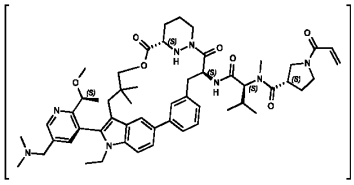
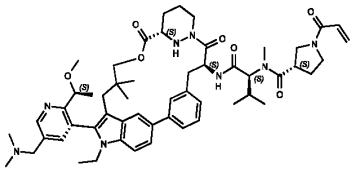
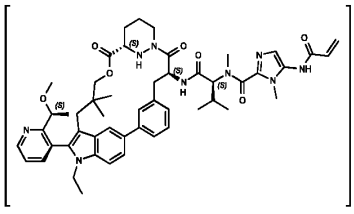
A520	
A521	
A522	
A523	
A524	

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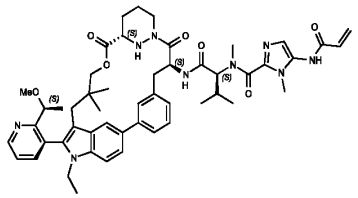
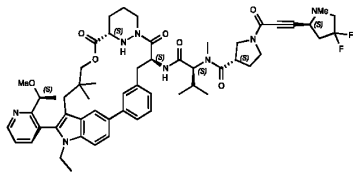
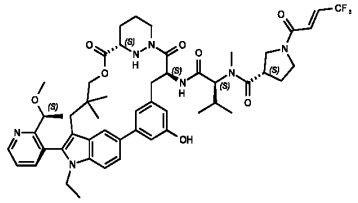
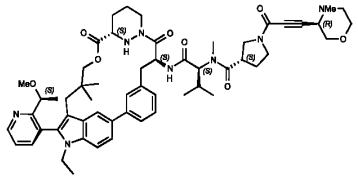
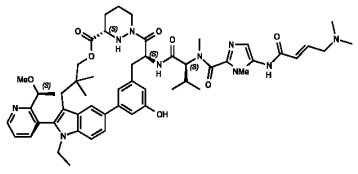
A525	
A526	
A527	
A528	
A529	

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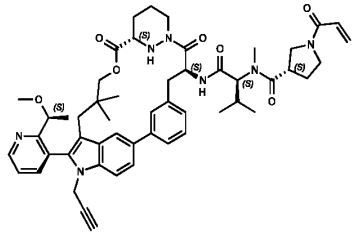
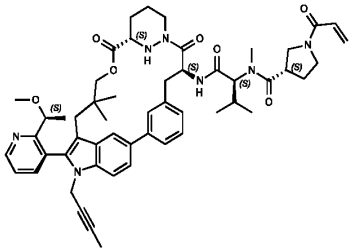
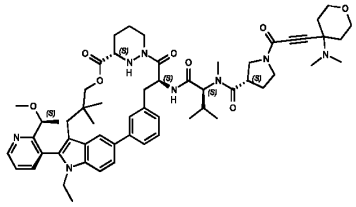
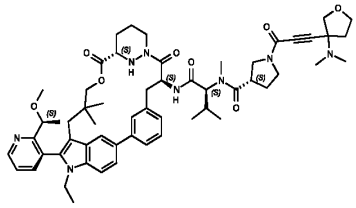
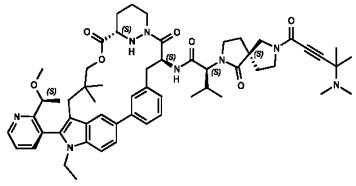
A530	
A531	
A532	
A533	
A534	

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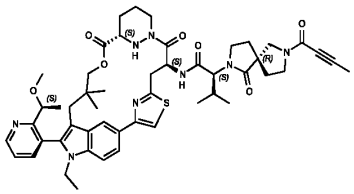
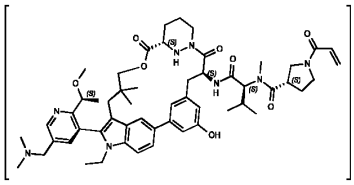
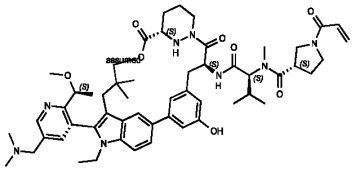
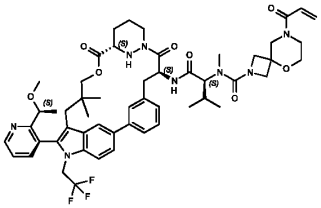
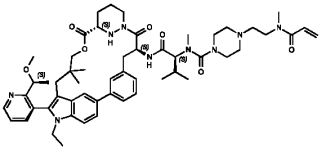
A535	
A536	
A537	
A538	
A539	

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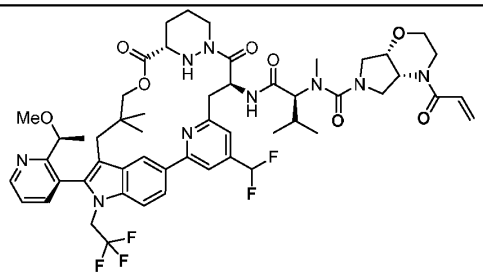
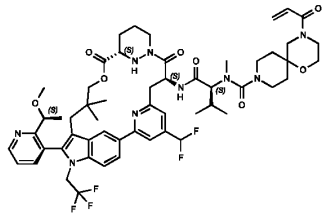
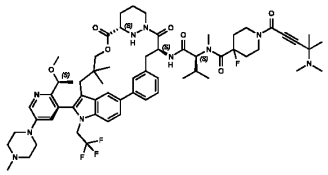
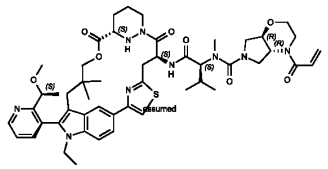
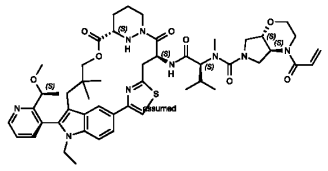
A540	
A541	
A542	
A543	
A544	

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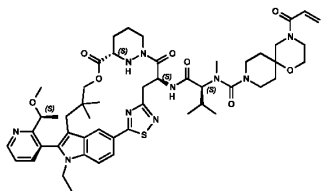
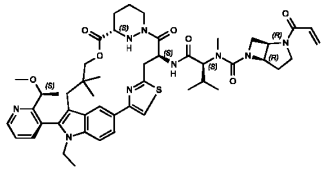
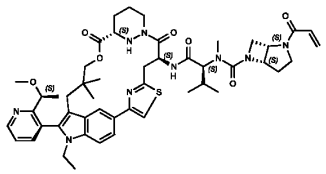
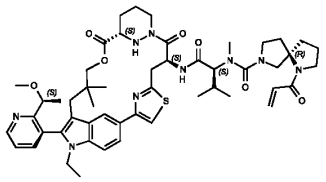
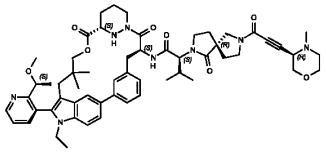
A545	
A546	
A547	
A548	
A549	

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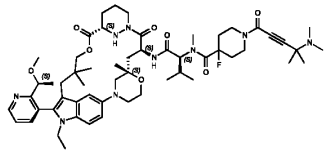
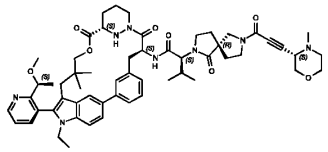
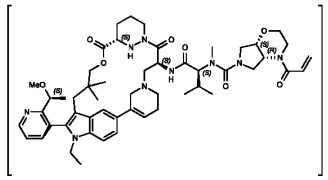
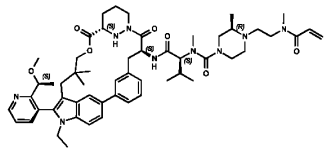
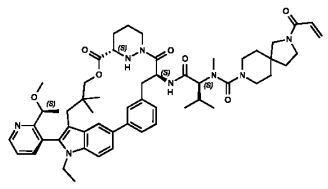
A550	
A551	
A552	
A553	
A554	

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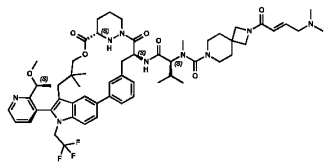
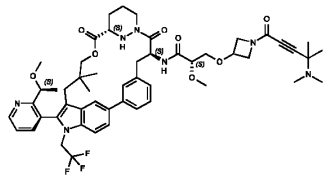
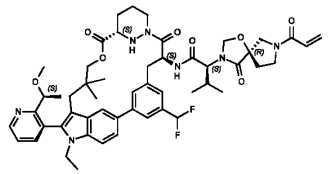
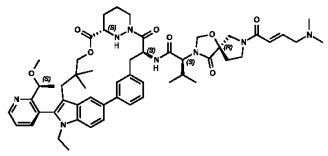
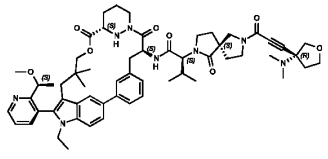
A555	
A556	
A557	
A558	
A559	

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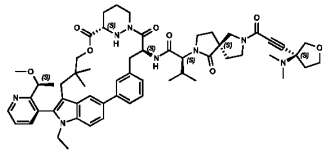
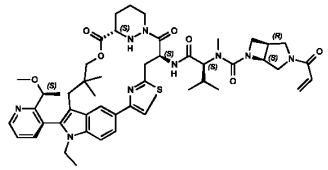
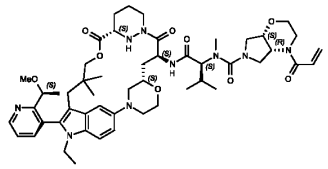
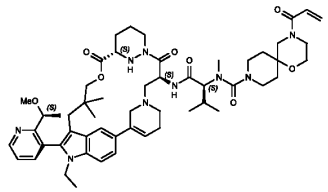
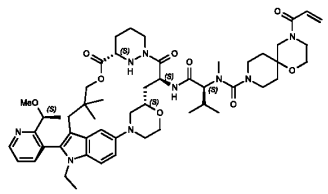
A560	
A561	
A562	
A563	
A564	

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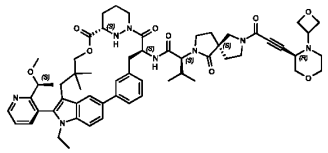
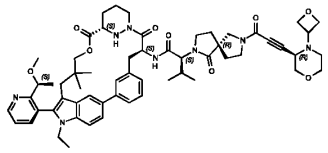
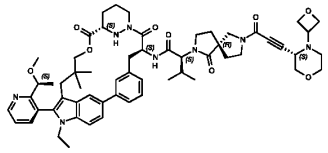
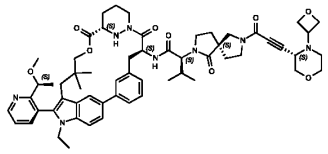
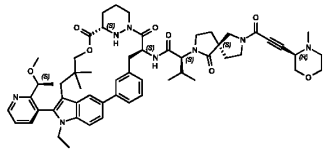
A565	
A566	
A567	
A568	
A569	

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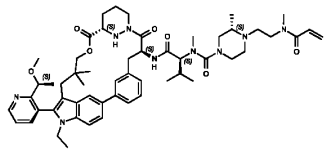
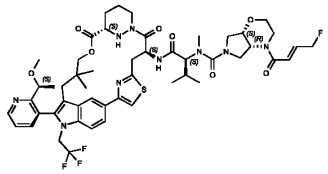
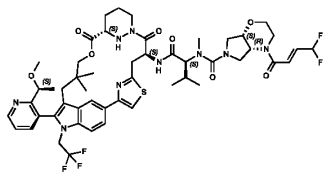
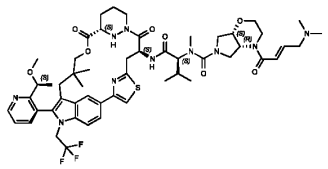
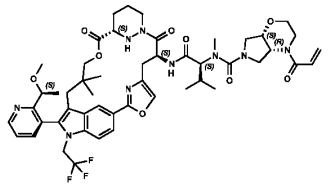
A570	
A571	
A572	
A573	
A574	

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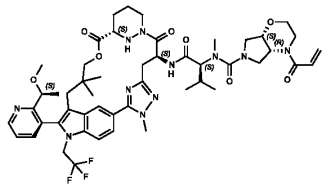
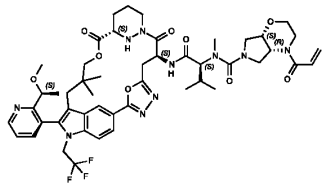
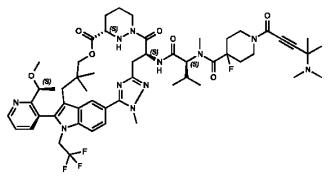
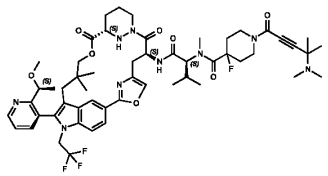
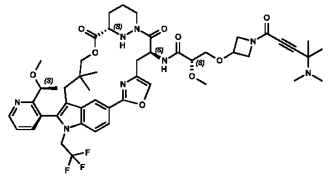
A575	
A576	
A577	
A578	
A579	

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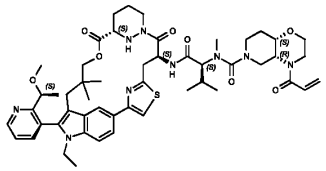
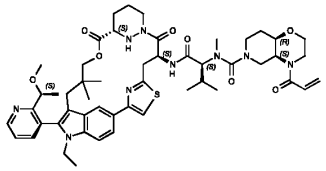
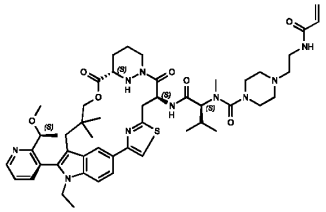
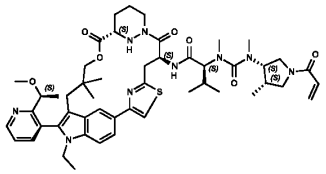
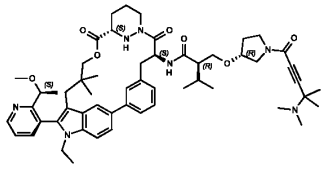
A580	
A581	
A582	
A583	
A584	

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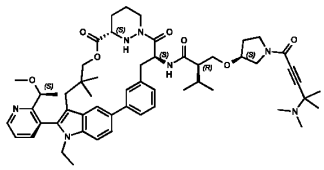
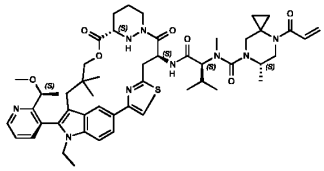
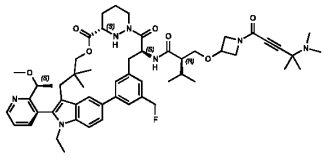
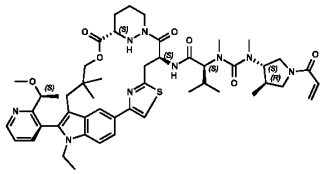
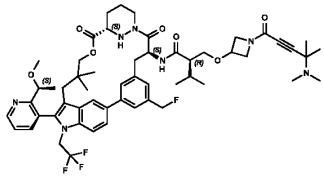
A585	
A586	
A587	
A588	
A589	

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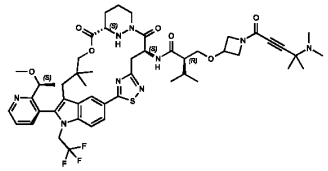
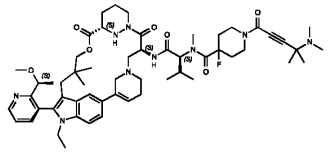
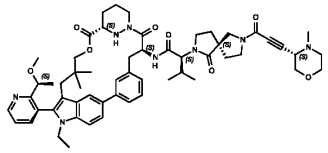
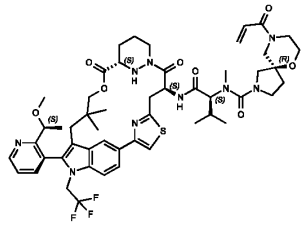
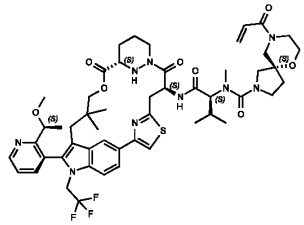
A590	
A591	
A592	
A593	
A594	

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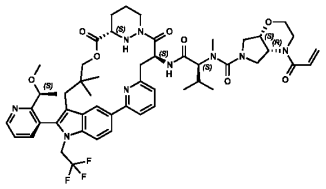
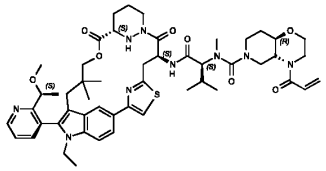
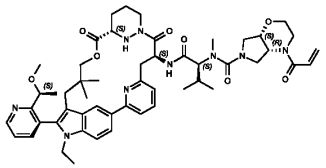
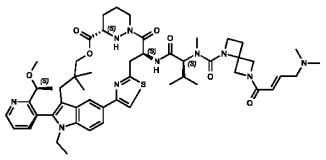
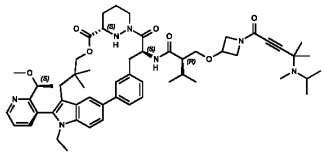
A595	
A596	
A597	
A598	
A599	

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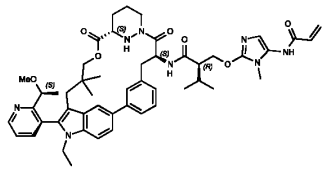
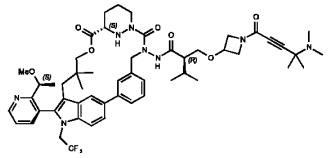
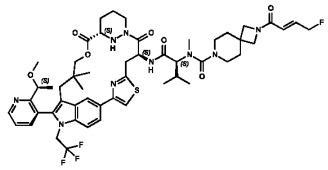
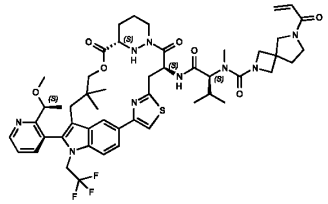
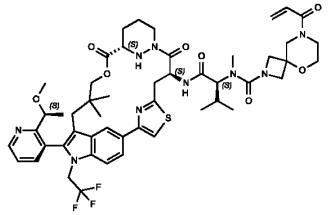
A600	
A601	
A602	
A603	
A604	

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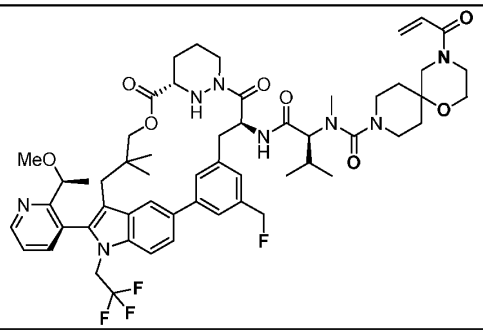
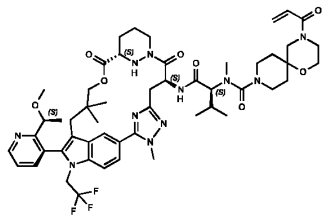
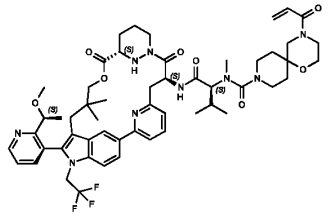
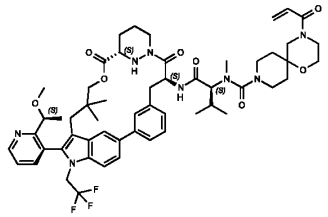
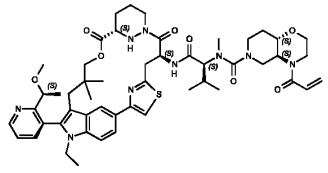
A605	
A606	
A607	
A608	
A609	

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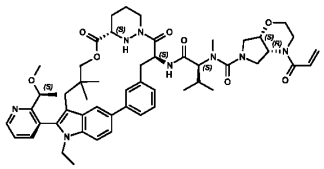
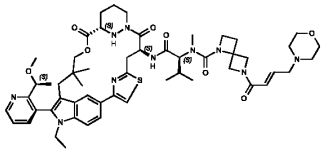
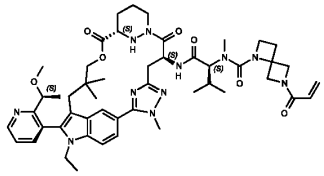
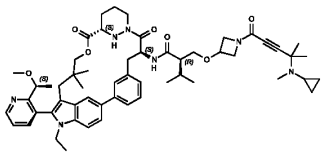
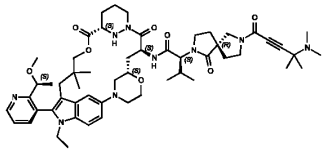
A610	
A611	
A612	
A613	
A614	

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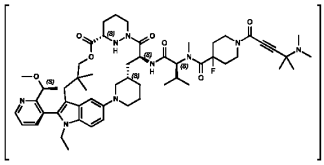
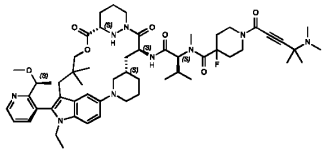
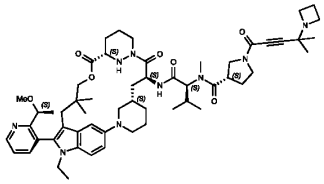
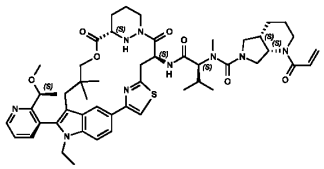
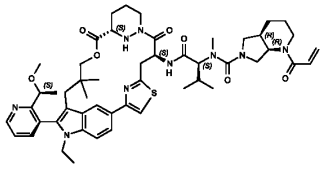
A615	
A616	
A617	
A618	
A619	

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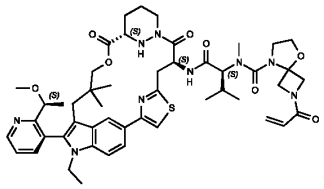
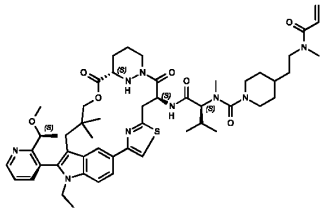
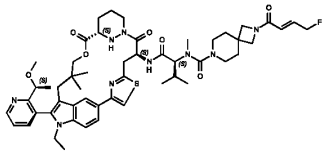
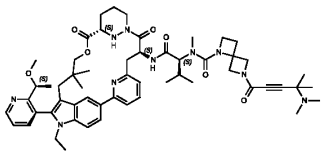
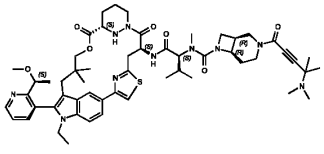
A620	
A621	
A622	
A623	
A624	

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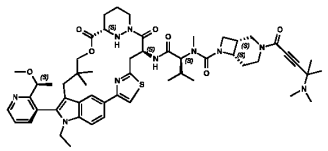
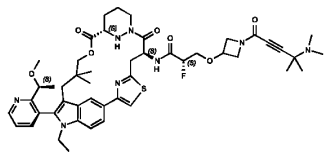
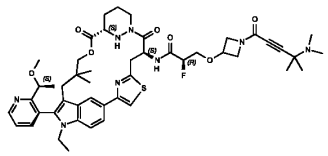
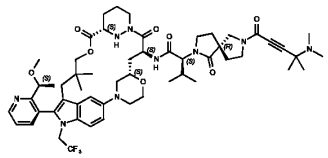
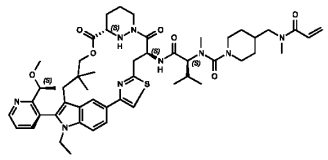
A625	
A626	
A627	
A628	
A629	

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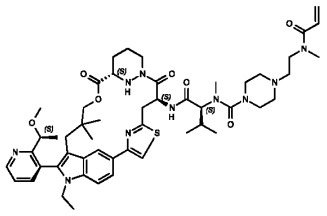
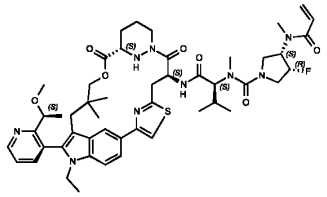
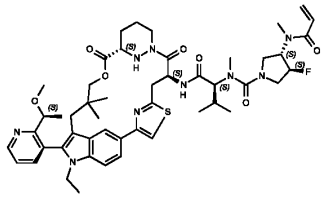
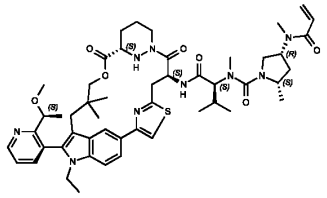
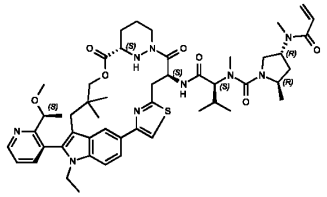
A630	
A631	
A632	
A633	
A634	

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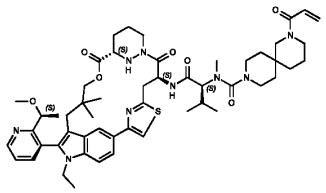
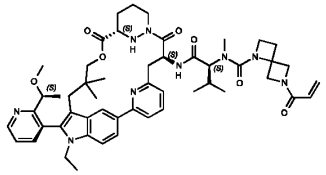
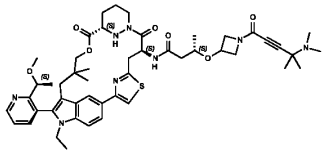
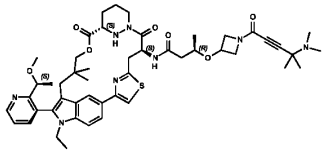
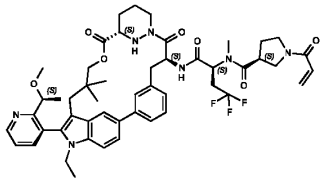
A635	
A636	
A637	
A638	
A639	

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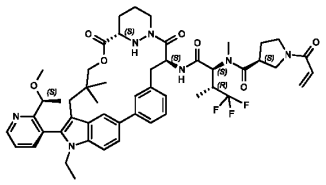
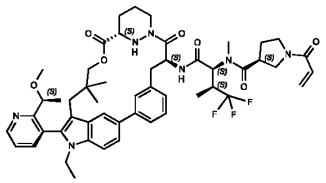
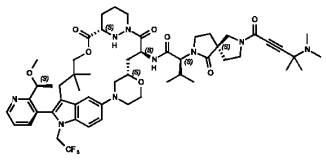
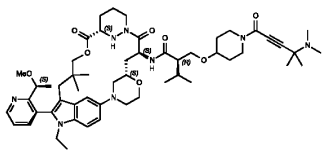
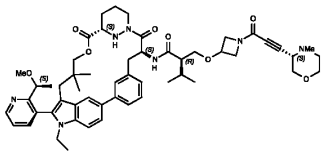
A640	
A641	
A642	
A643	
A644	

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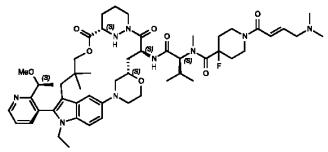
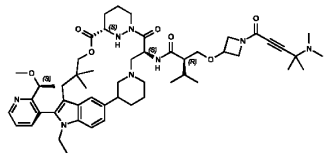
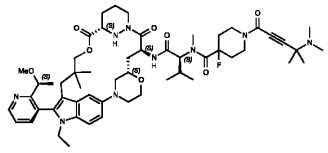
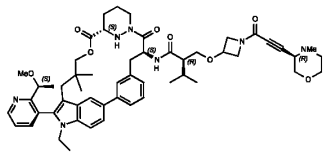
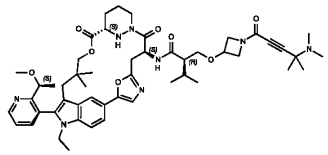
A645	
A646	
A647	
A648	
A649	

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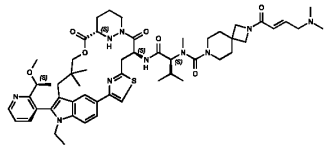
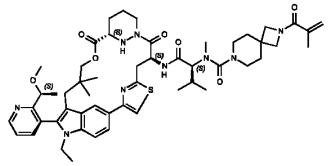
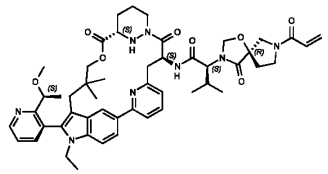
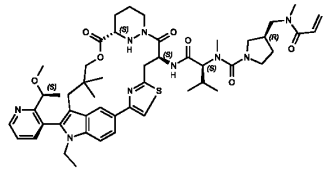
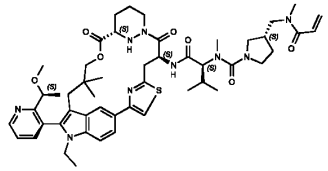
A650	
A651	
A652	
A653	
A654	

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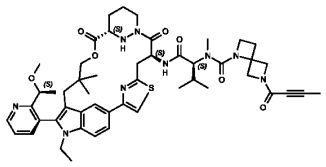
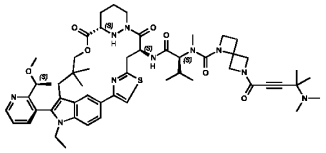
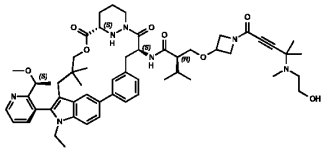
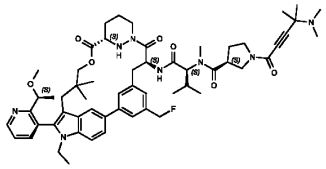
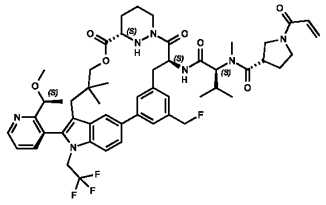
A655	
A656	
A657	
A658	
A659	

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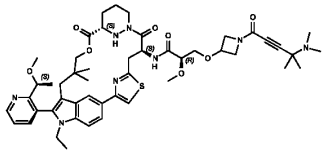
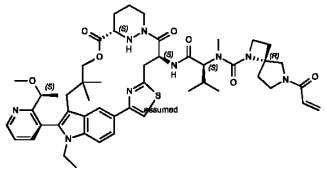
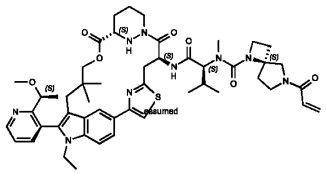
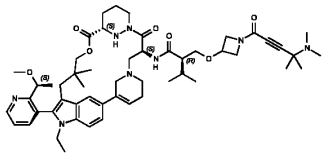
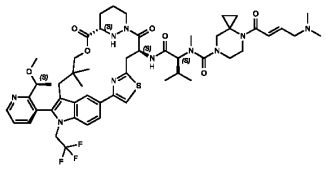
A660	
A661	
A662	
A663	
A664	

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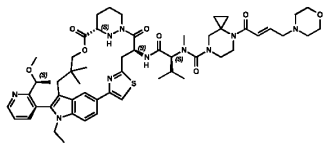
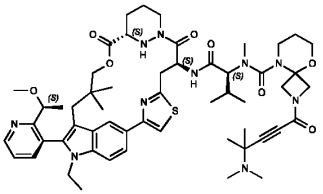
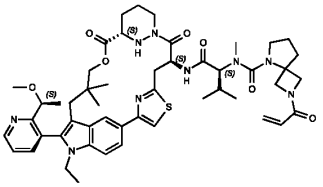
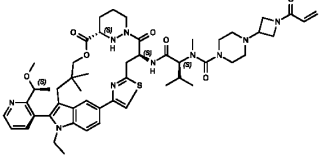
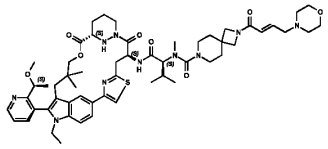
A665	
A666	
A667	
A668	
A669	

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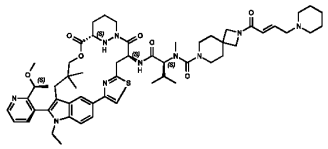
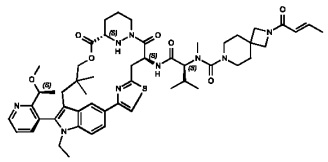
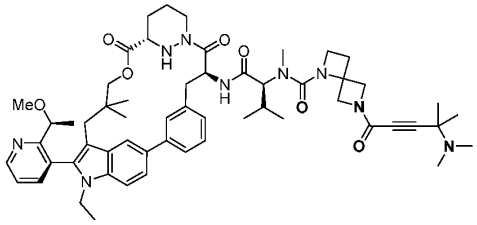
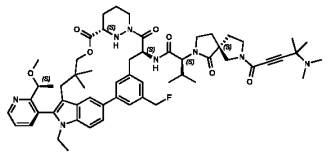
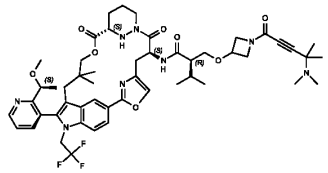
A670	
A671	
A672	
A673	
A674	

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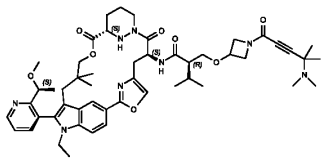
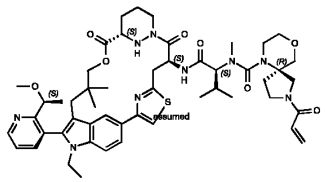
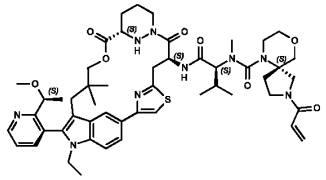
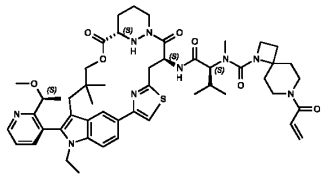
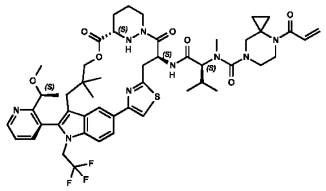
A675	
A676	
A677	
A678	
A679	

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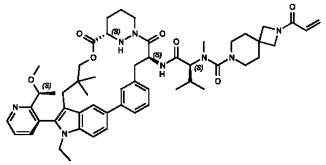
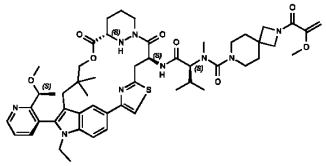
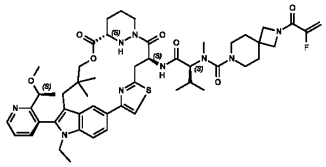
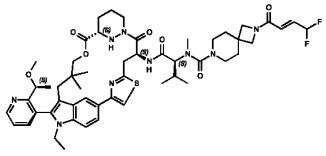
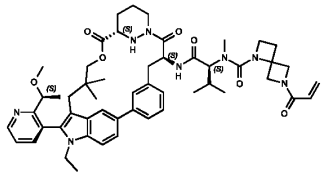
A680	
A681	
A682	
A683	
A684	

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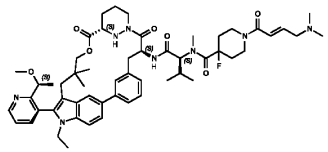
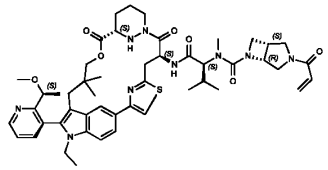
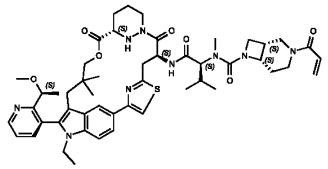
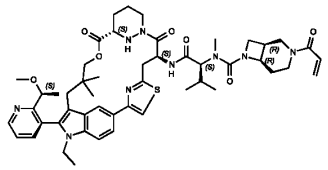
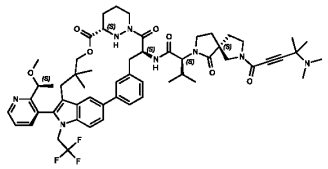
A685	
A686	
A687	
A688	
A689	

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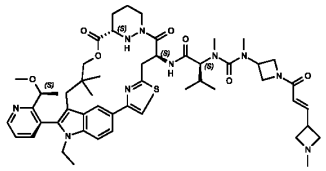
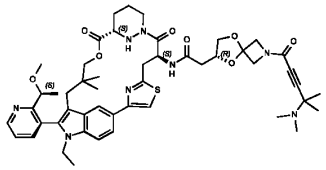
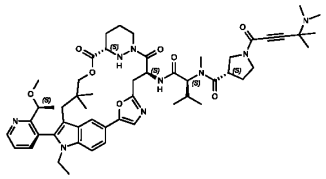
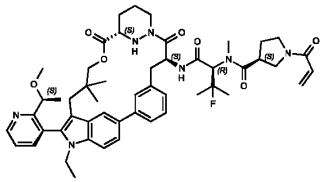
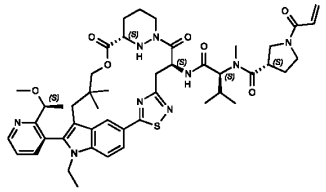
A690	
A691	
A692	
A693	
A694	

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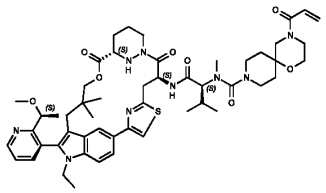
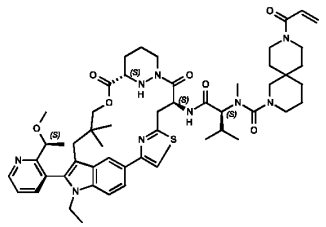
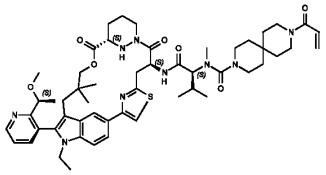
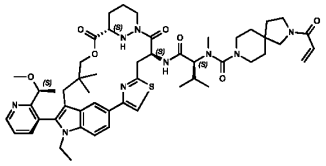
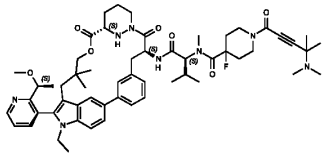
A695	
A696	
A697	
A698	
A699	

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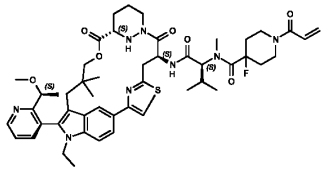
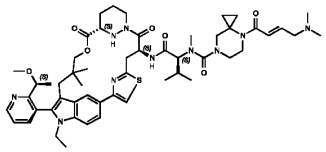
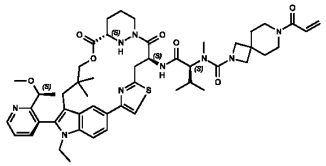
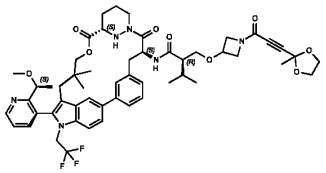
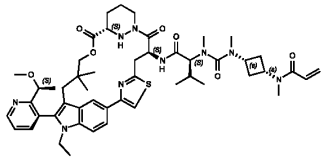
A700	
A701	
A702	
A703	
A704	

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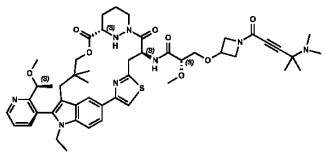
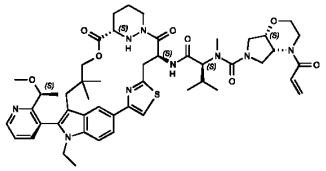
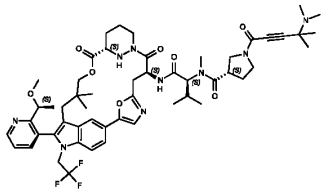
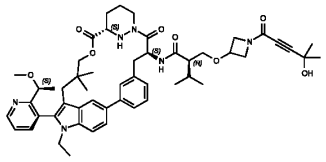
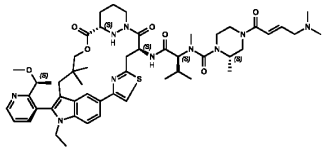
A705	
A706	
A707	
A708	
A709	

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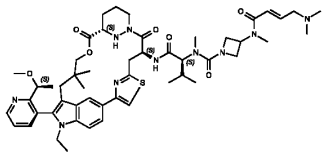
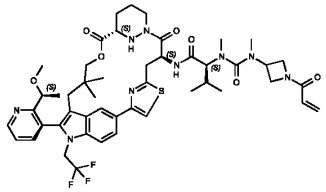
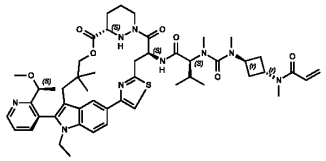
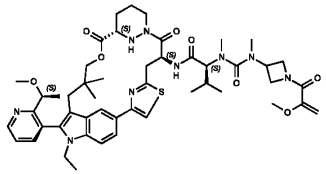
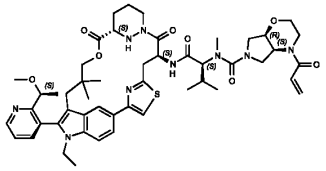
A710	
A711	
A712	
A713	
A714	

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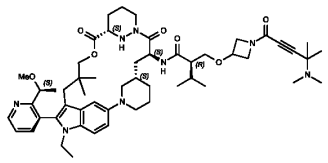
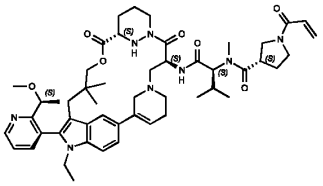
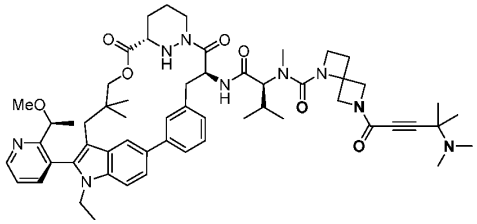
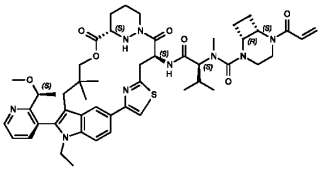
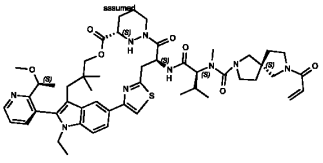
A715	
A716	
A717	
A718	
A719	

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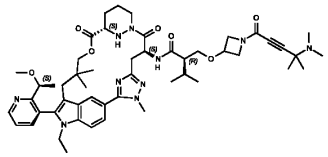
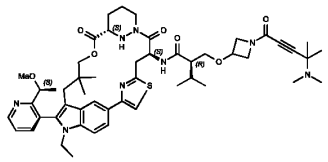
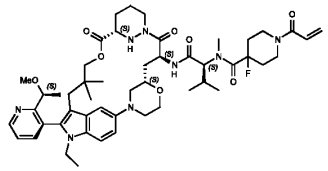
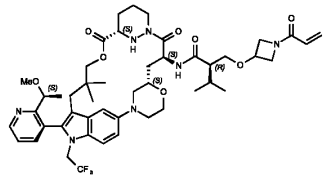
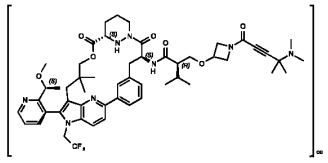
A720	
A721	
A722	
A723	
A724	

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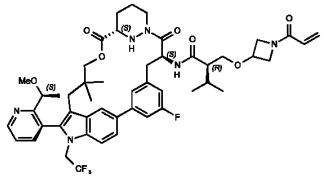
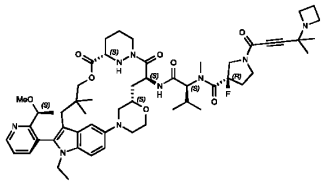
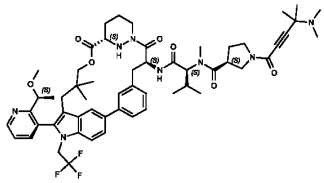
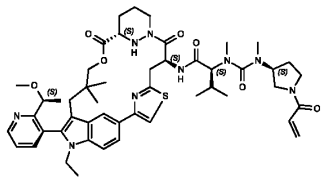
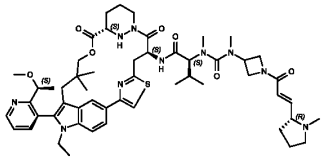
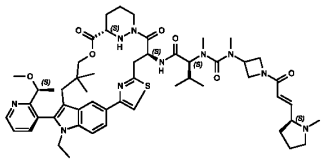
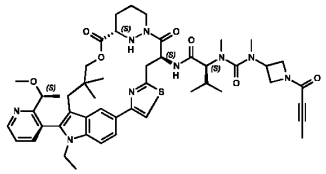
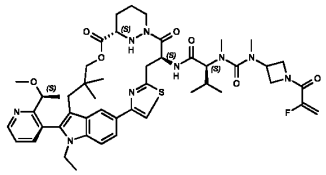
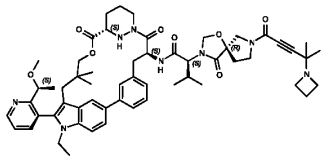
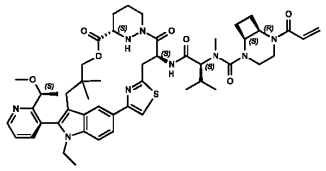
A725	
A726	
A727	
A728	
A729	

Ex#	Structure
A730	
A731	
A732	
A733	
A734	

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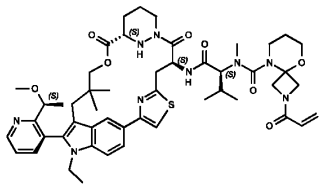
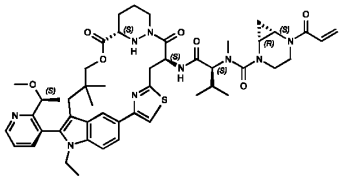
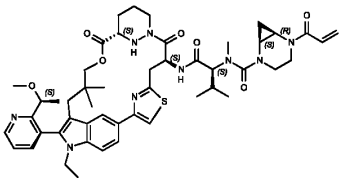
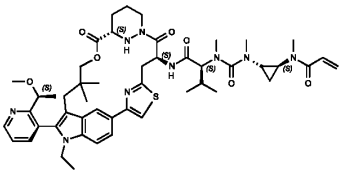
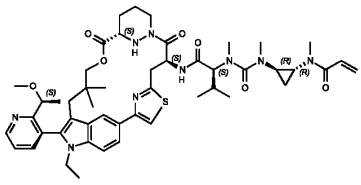
A735	
A736	
A737	
A738	
A739	

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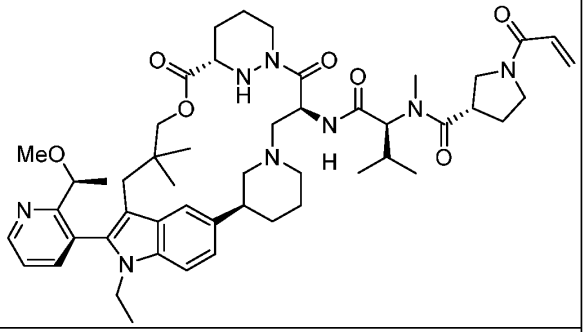
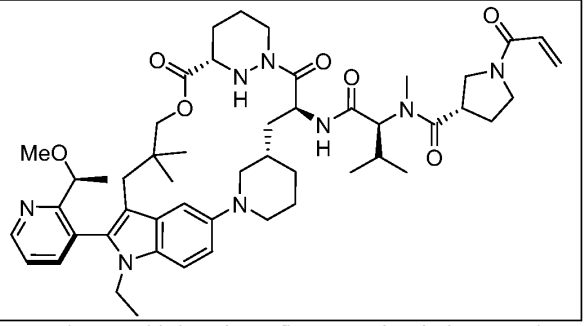
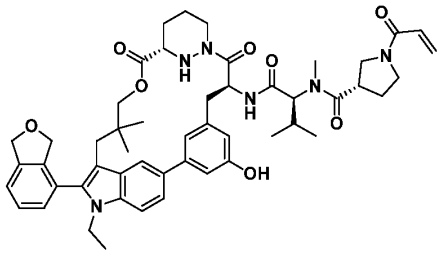
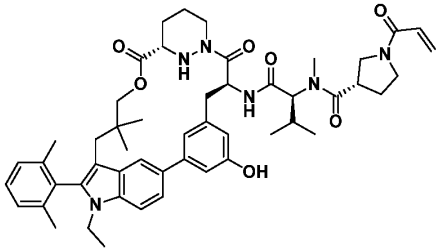
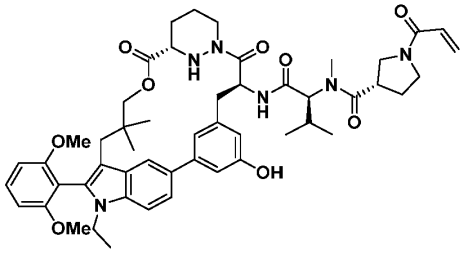
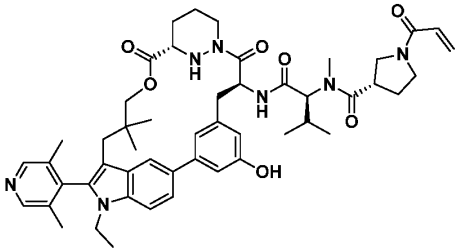
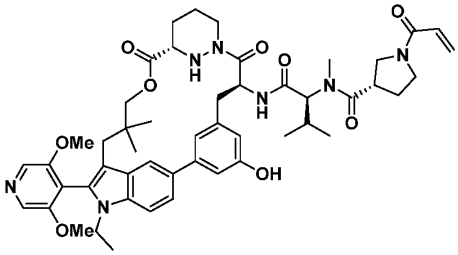
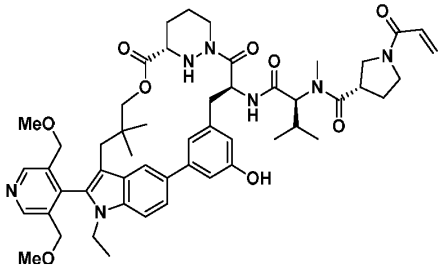
A740	
A741	

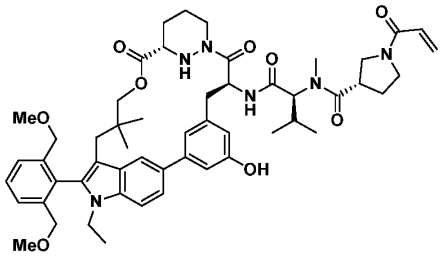
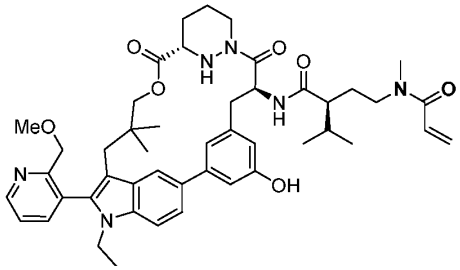
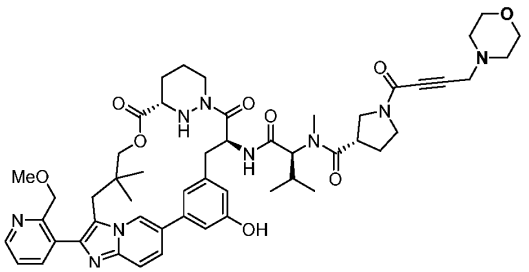
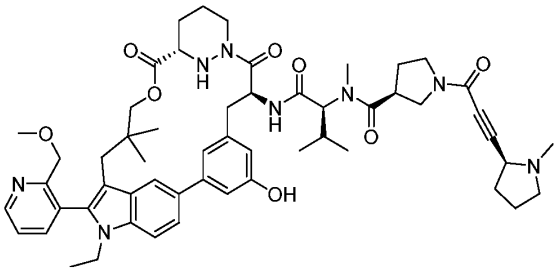
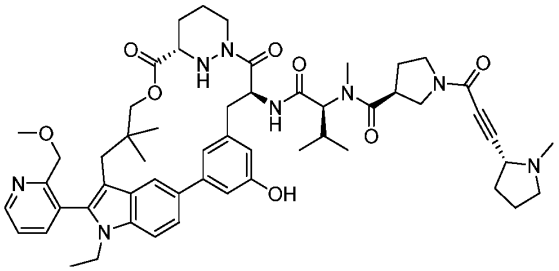
Note that some compounds are shown with bonds as flat or wedged. In some instances, the relative stereochemistry of stereoisomers has been determined; in some instances, the absolute stereochemistry has been determined. In some instances, a single Example number corresponds to a mixture of stereoisomers. All stereoisomers of the compounds of the foregoing table are contemplated by the present invention. In particular embodiments, an atropisomer of a compound of the foregoing table is contemplated.
Brackets are to be ignored.
*The activity of this stereoisomer may, in fact, be attributable to the presence of a small amount of the stereoisomer with the (S) configuration at the -NC(O)-CH$(CH_3)_2$-N(CH_3)- position.

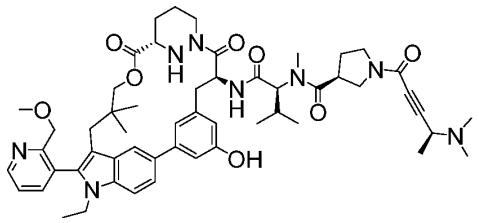
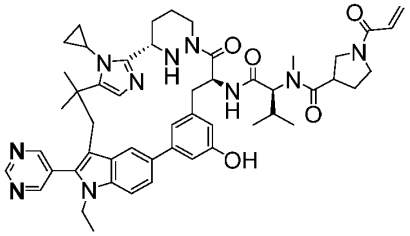
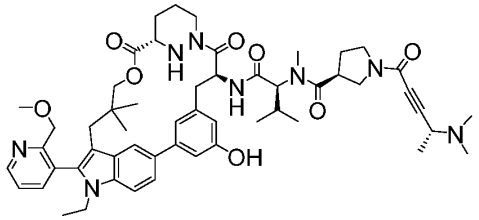
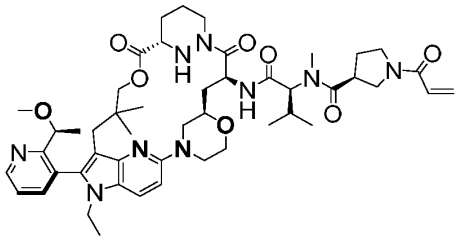
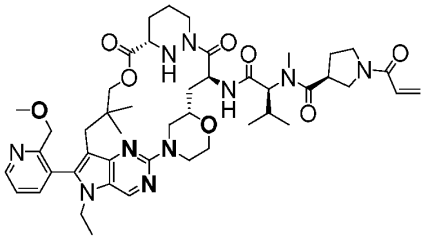
In some embodiments, a compound of Table 2 is provided, or a pharmaceutically acceptable salt thereof. In some embodiments, a compound of the present invention is selected from Table 2, or a pharmaceutically acceptable salt or atropisomer thereof.

Table 2: Certain Compounds of the Present Invention

Ex#	Structure
B1	

Ex#	Structure
B2	
B3	
B4	
B5	
B6	

Ex#	Structure
B7	
B11	
B12	
B13	
B18	

Ex#	Structure
B21	
B22	
B25	
B27	
B28	

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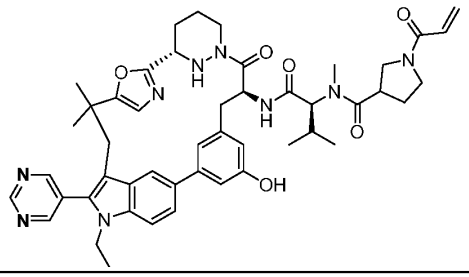
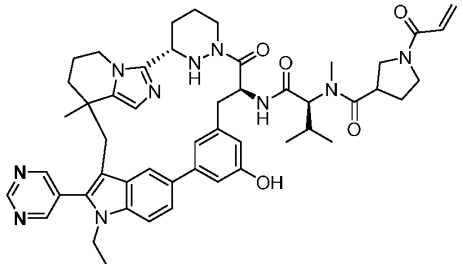
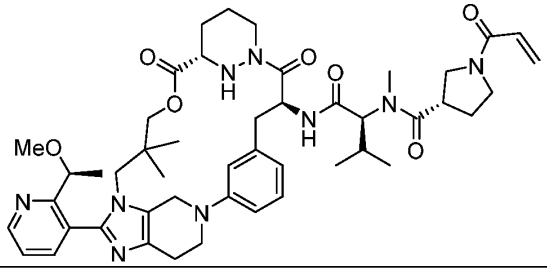
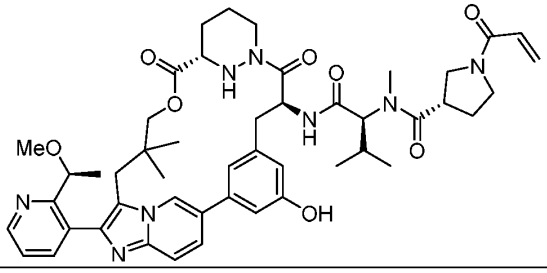
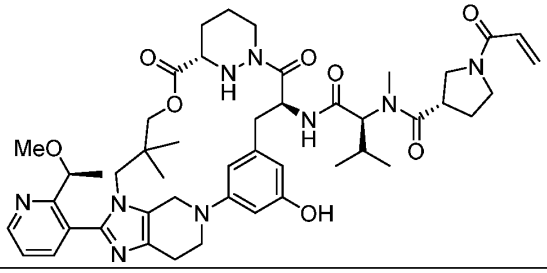
B29	
B30	
B32	
B34	
B38	

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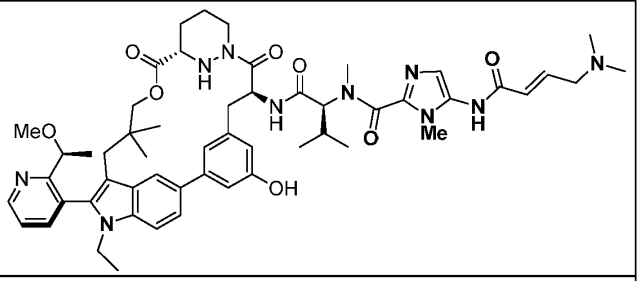
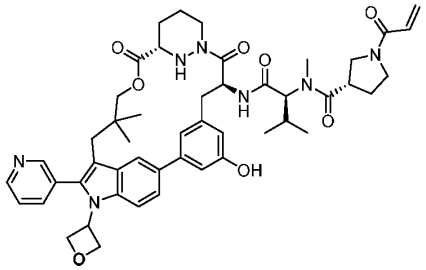
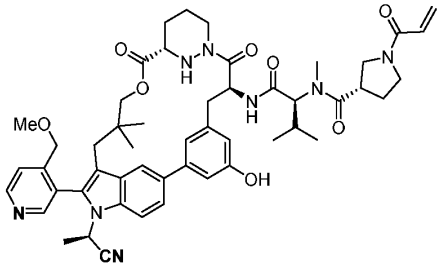
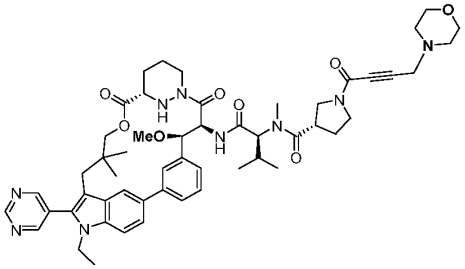
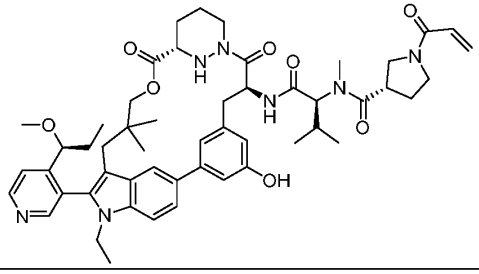
B47	
B64	
B65	
B66	
B70	

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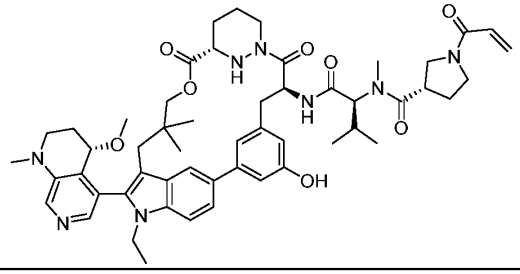
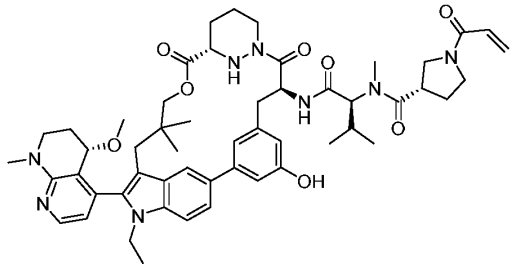
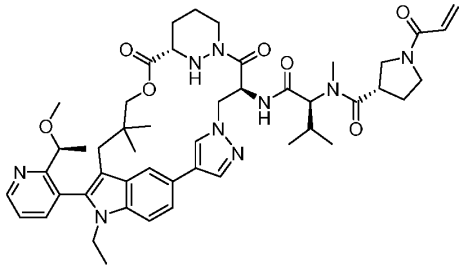
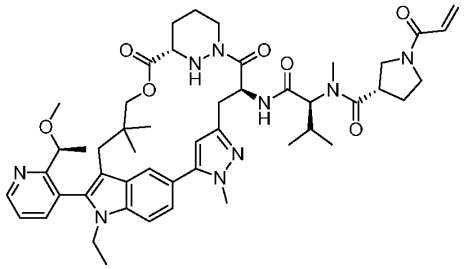
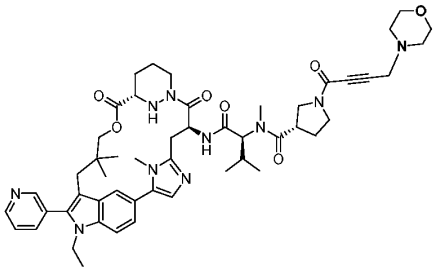
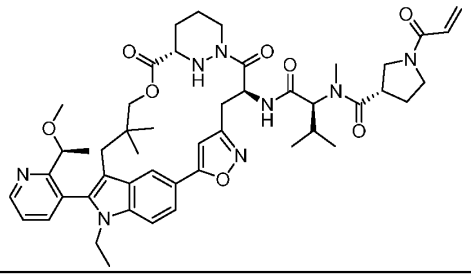
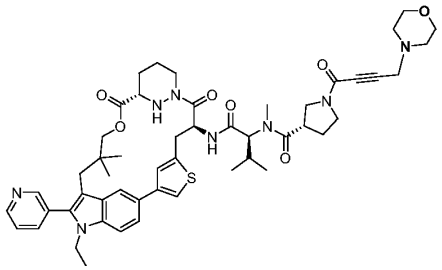
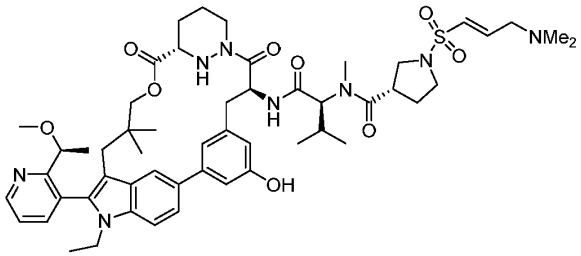
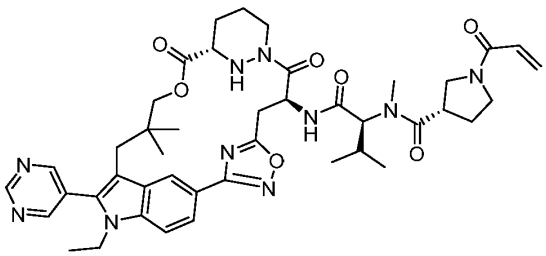
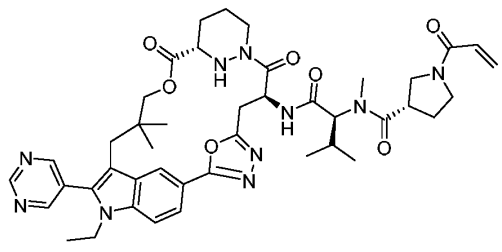
B73	
B74	
B75	
B76	
B77	

Ex#	Structure
B81	
B83	
B85	
B86	
B87	

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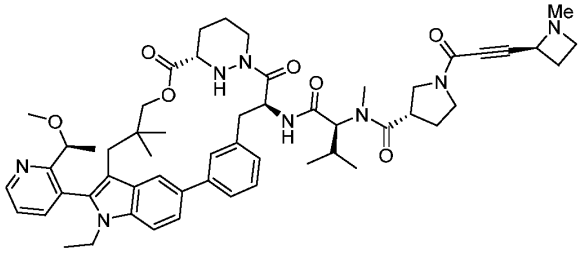
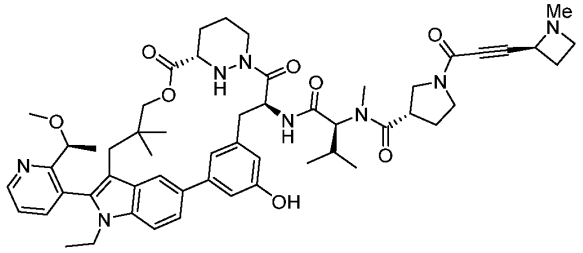
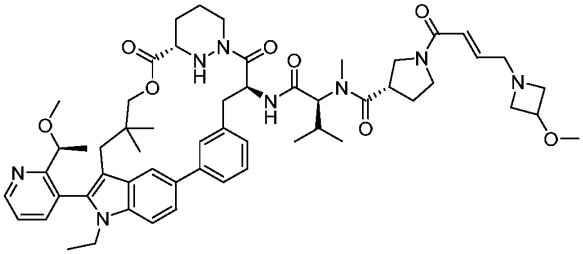
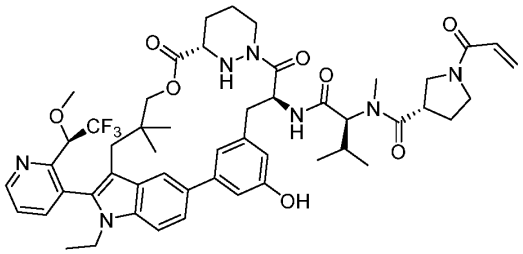
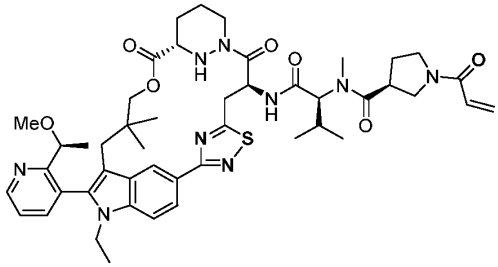
B88	
B89	
B90	
B91	
B96	

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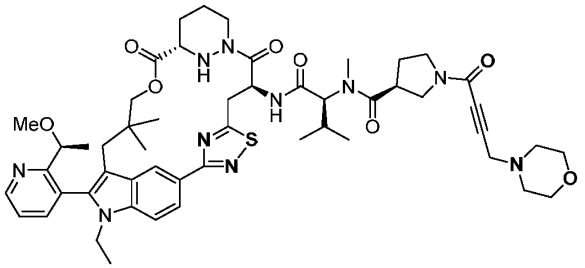
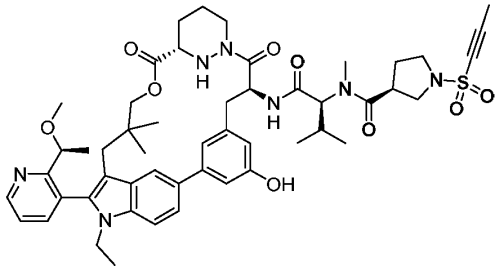
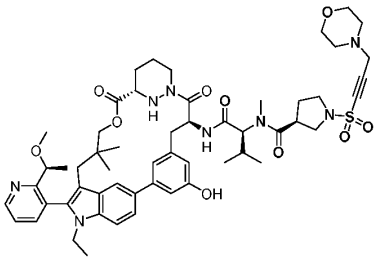
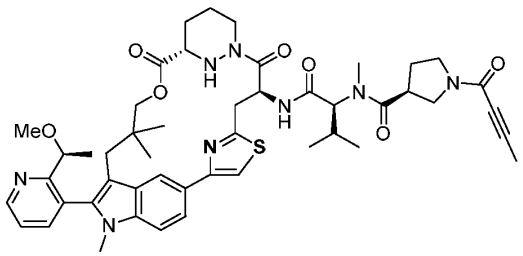
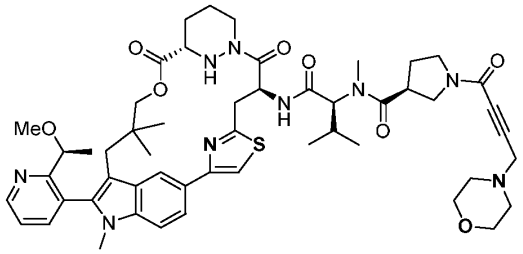
B97	
B102	
B103	
B104	
B106	

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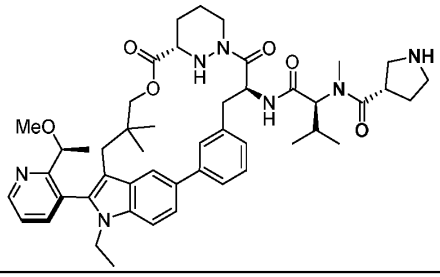
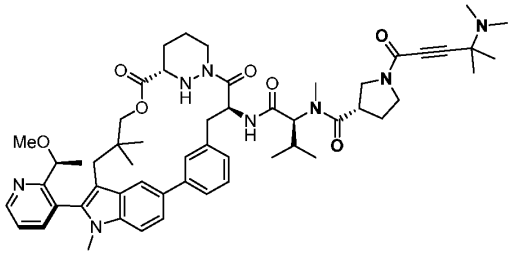
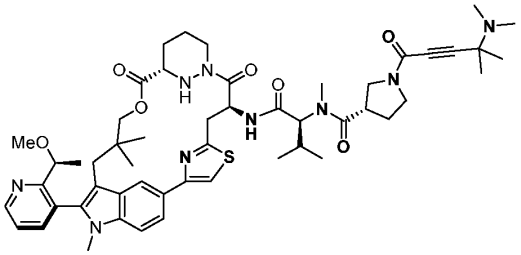
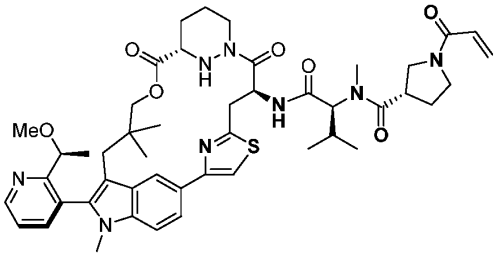
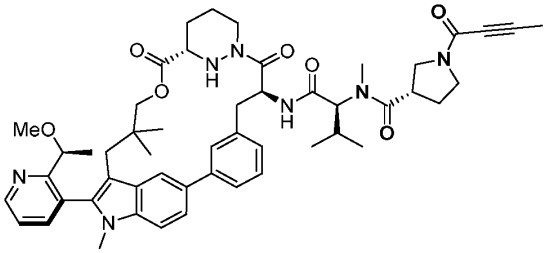
B107	
B109	
B111	
B112	
B113	

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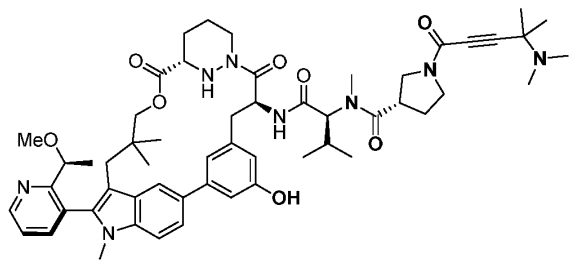
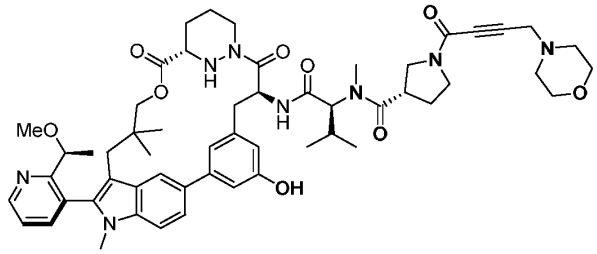
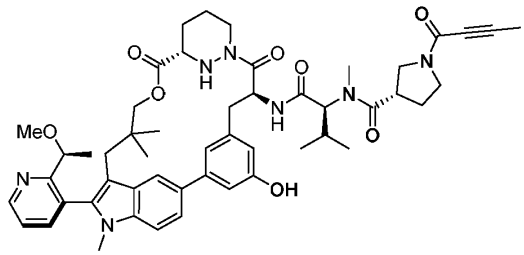
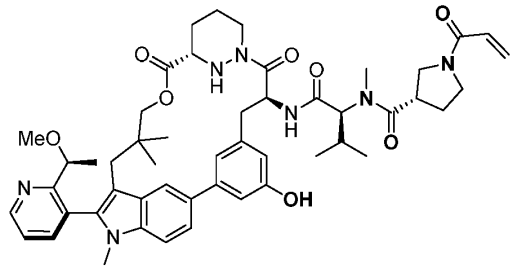
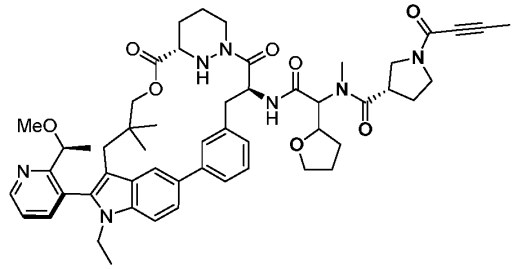
B115	
B116	
B117	
B118	
B119	

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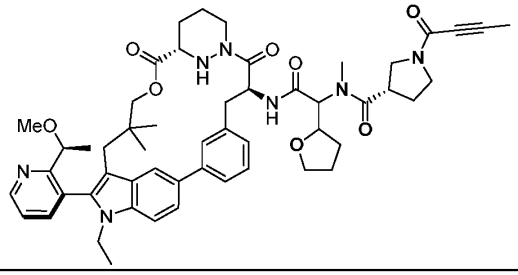
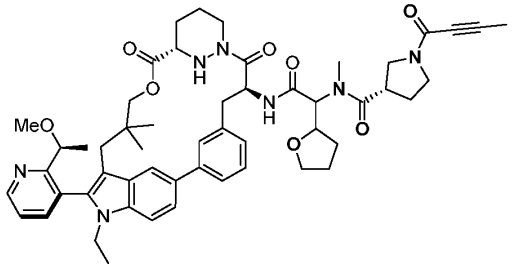
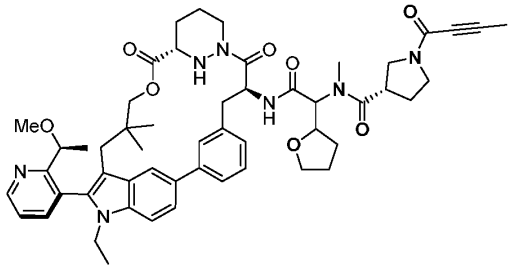
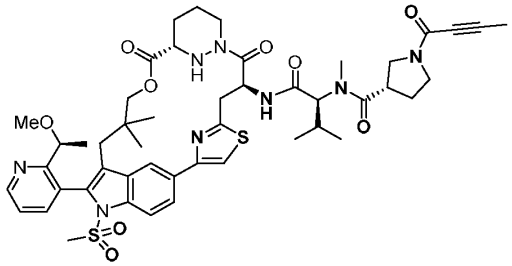
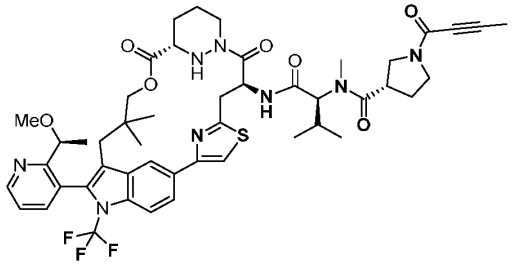
B120	
B121	
B122	
B123	
B124	

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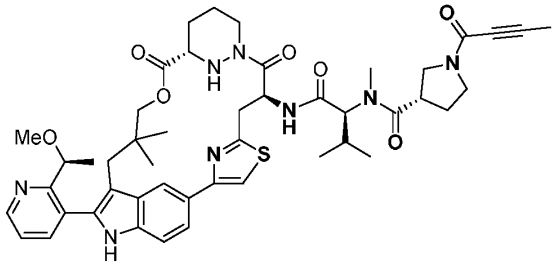
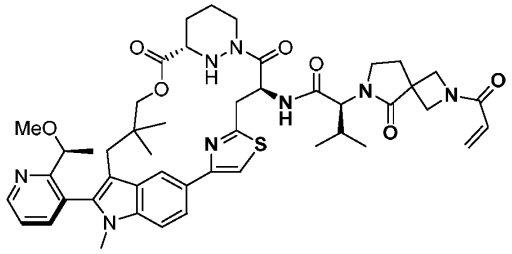
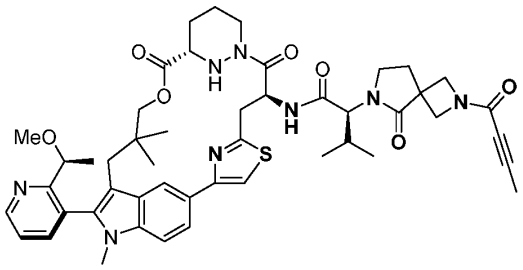
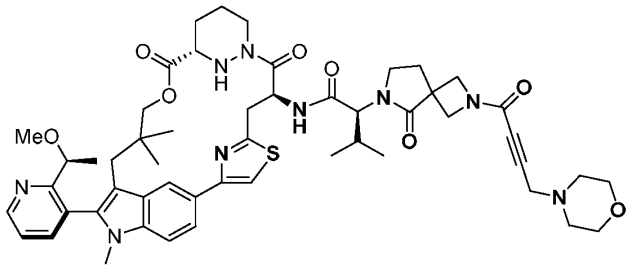
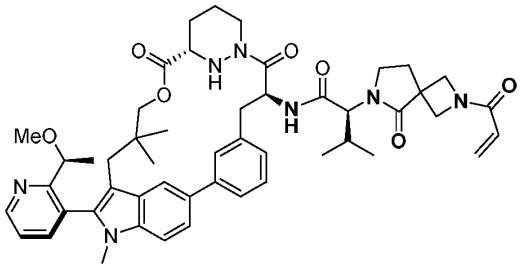
B126	
B127	
B128	
B129	
B130	

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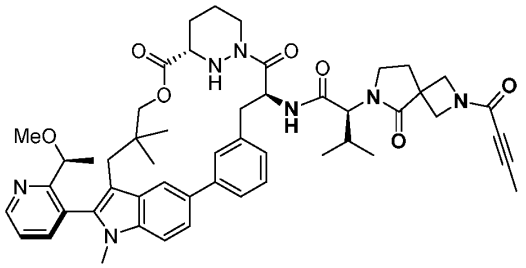
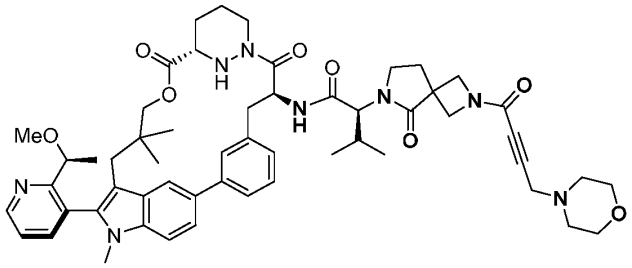
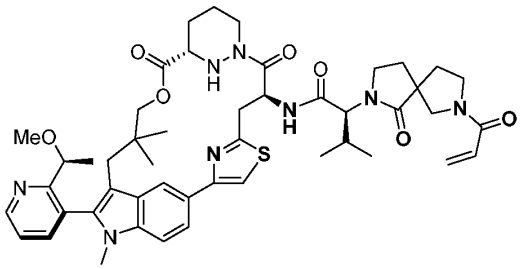
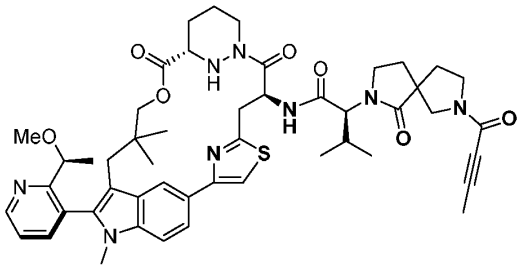
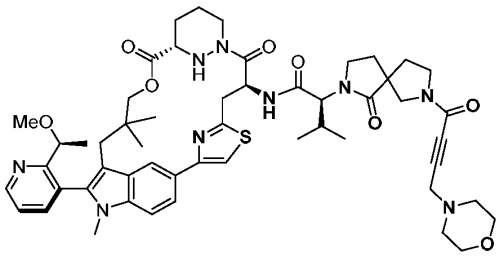
B131	
B132	
B139	
B140	
B141	

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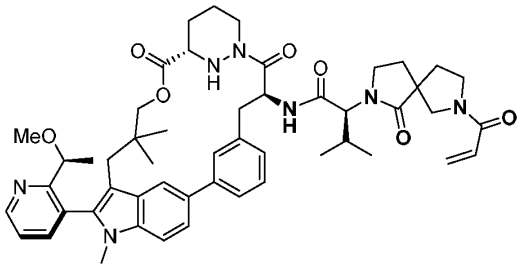
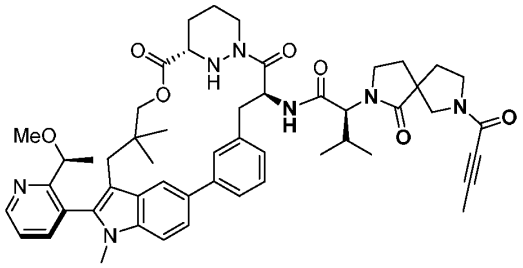
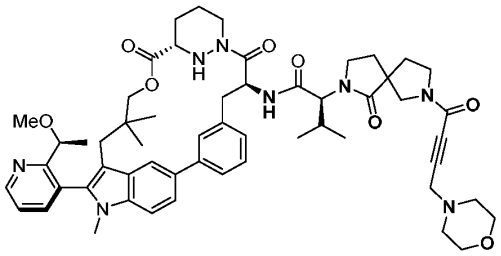
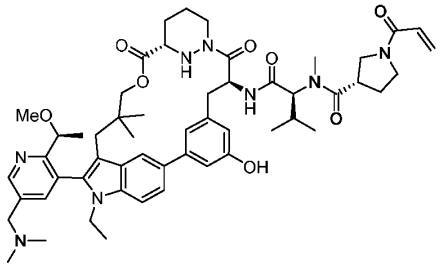
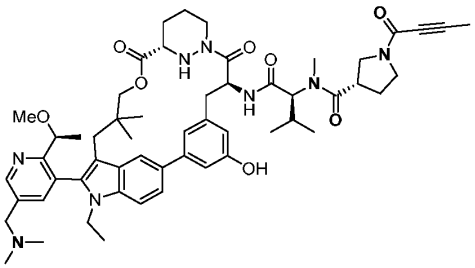
B142	
B143	
B144	
B145	
B146	

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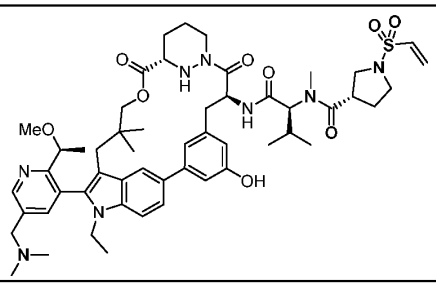
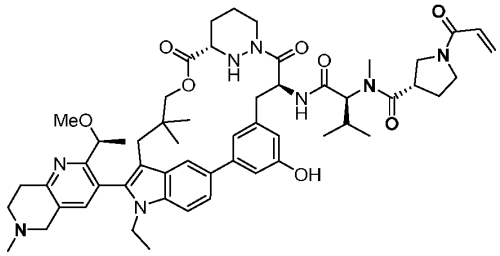
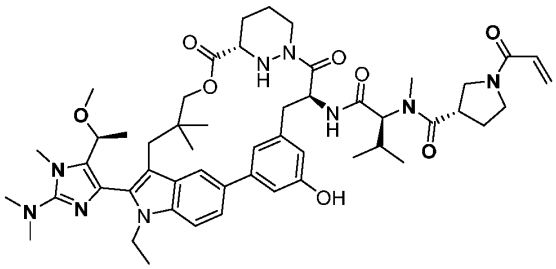
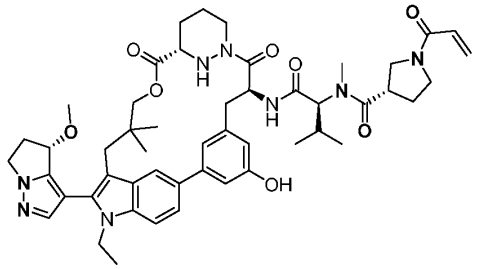
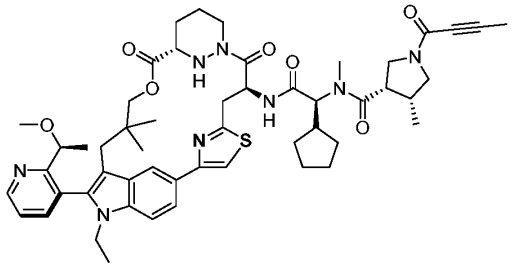
B147	
B148	
B149	
B150	
B161	

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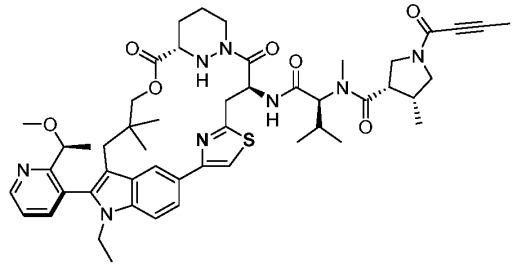
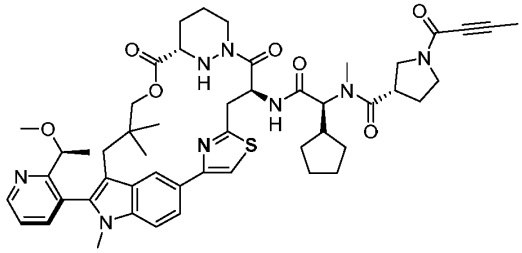
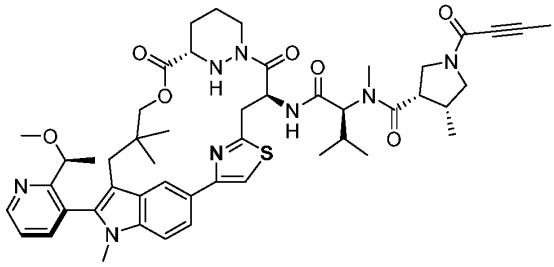
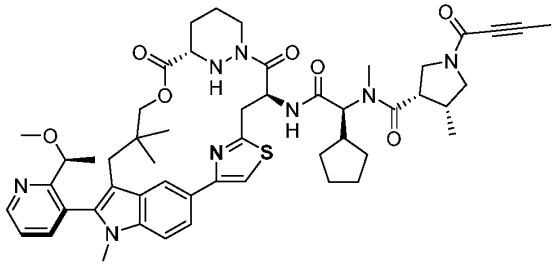
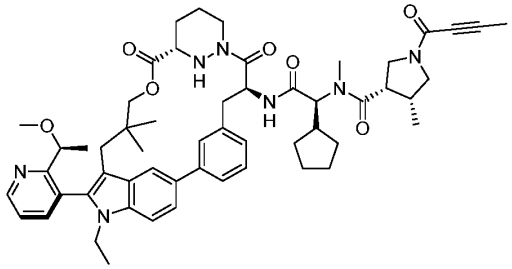
B162	
B163	
B164	
B165	
B167	

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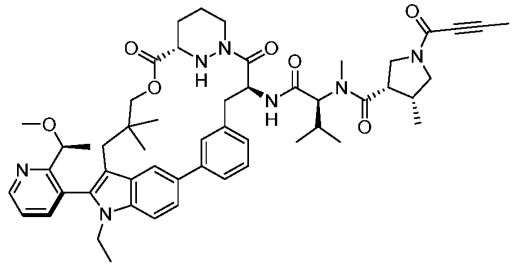
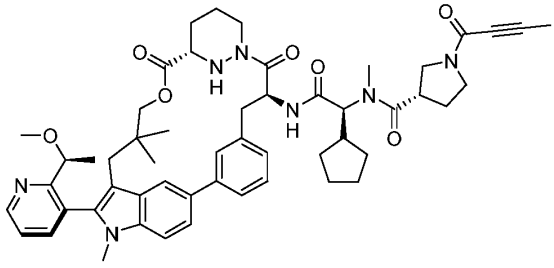
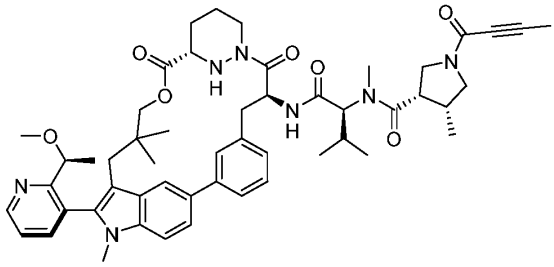
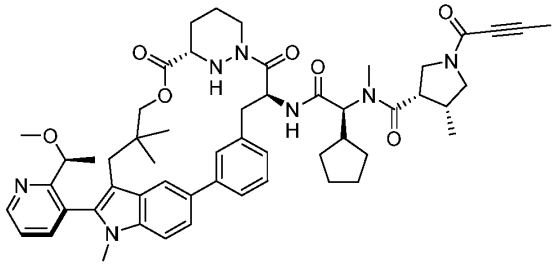
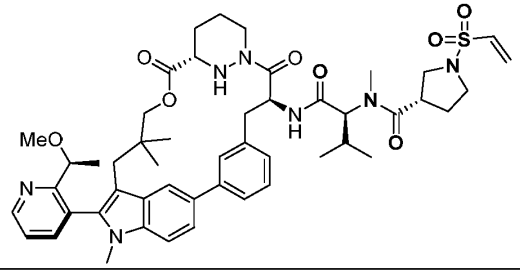
B168	
B169	
B170	
B171	
B172	

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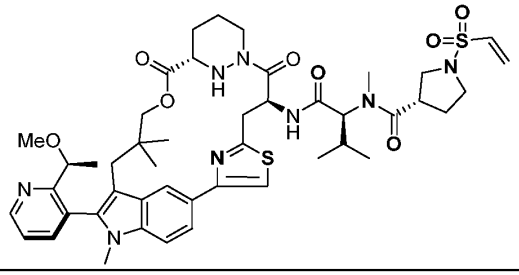
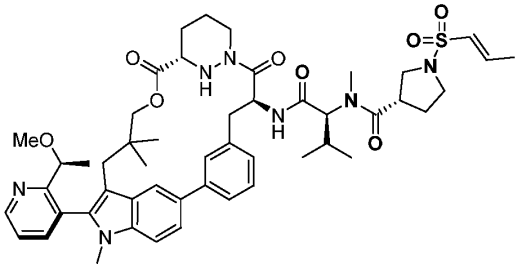
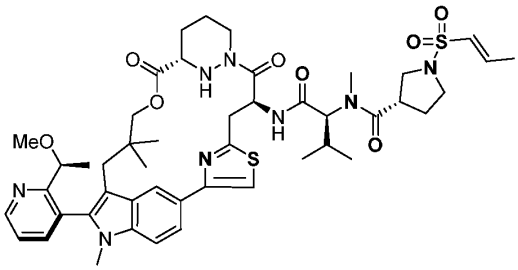
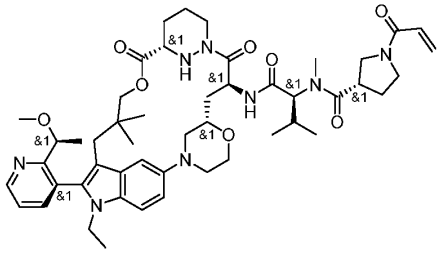
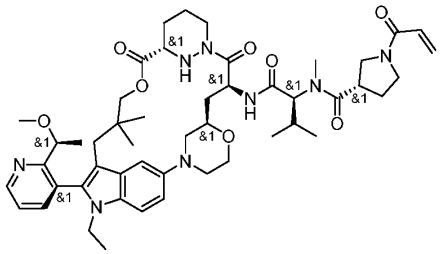
B173	
B174	
B175	
B176	
B177	

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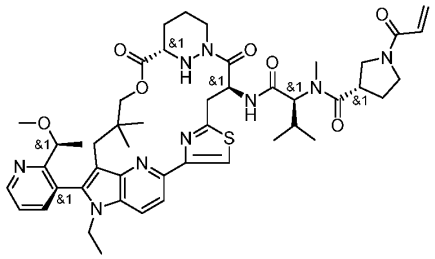
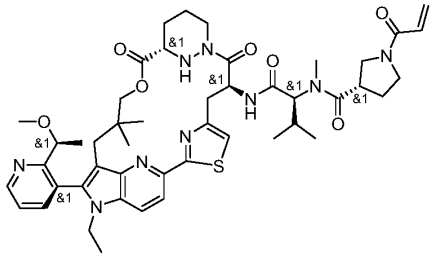
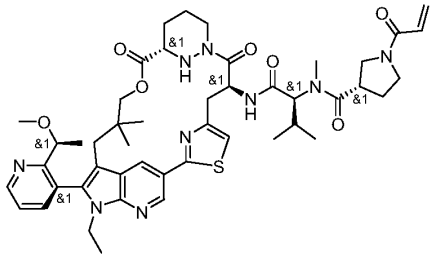
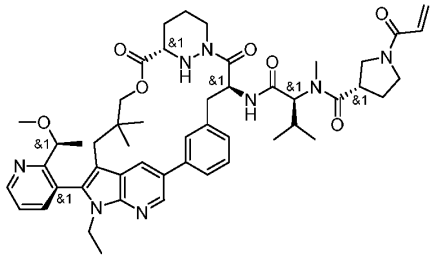
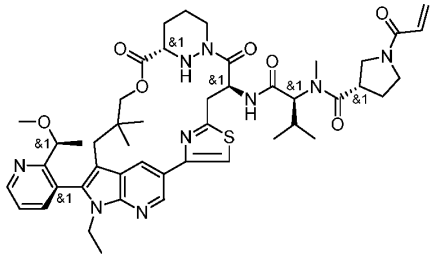
B178	
B179	
B180	
B181	
B182	

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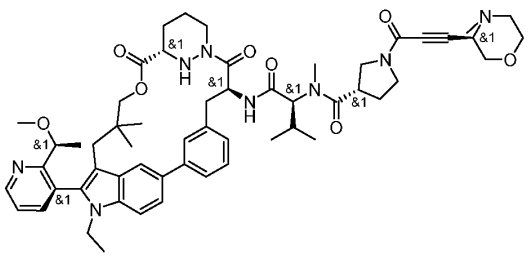
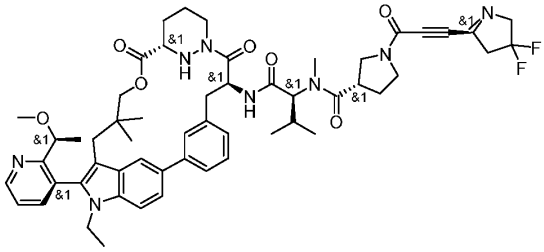
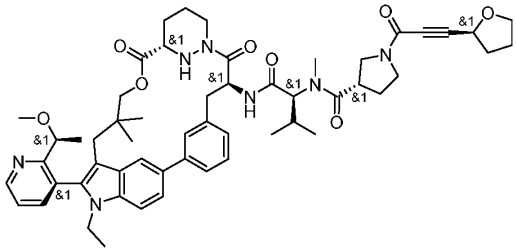
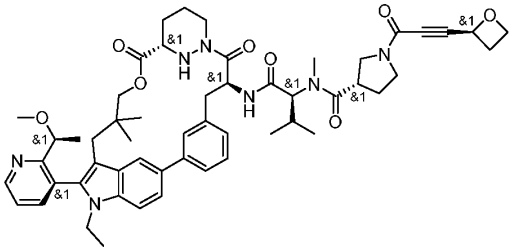
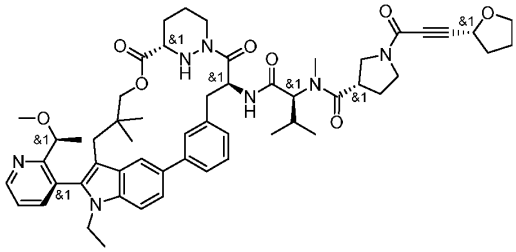
B183	
B184	
B185	
B186	
B187	

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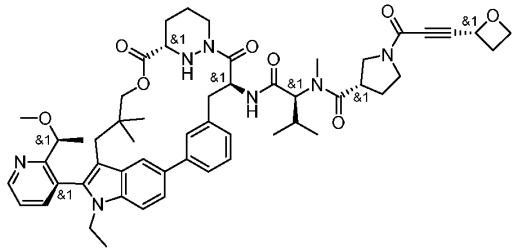
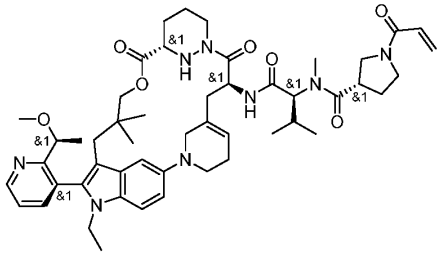
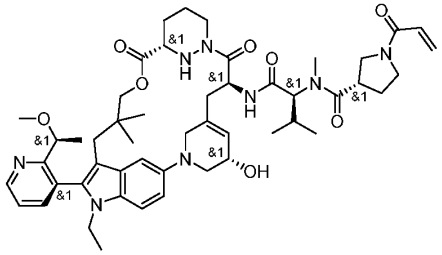
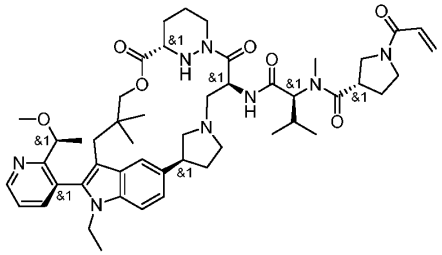
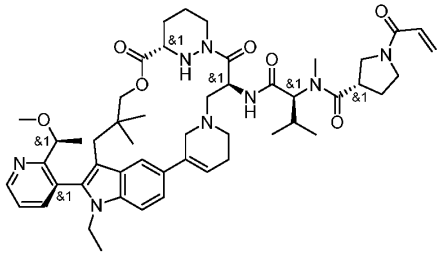
B188	
B189	
B190	
B191	
B192	

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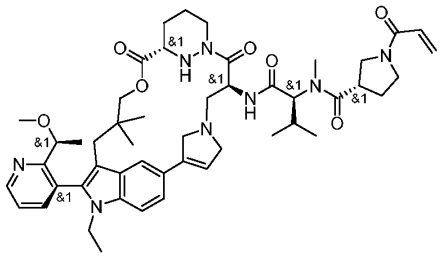
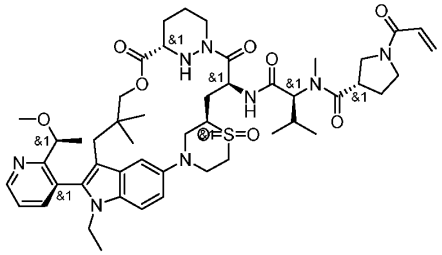
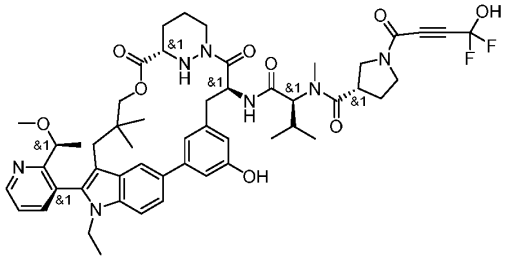
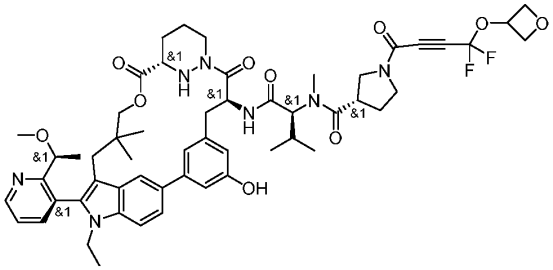
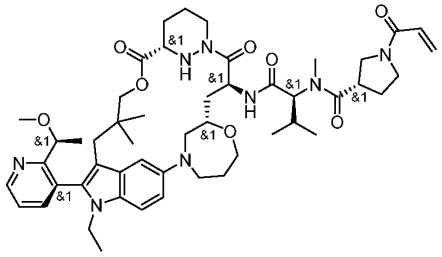
B194	
B195	
B196	
B197	
B198	

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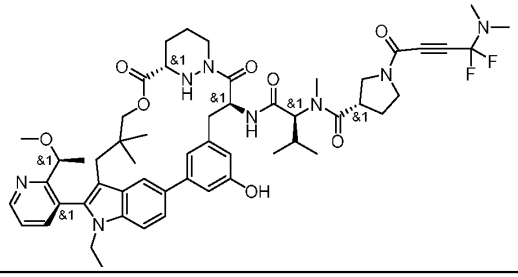
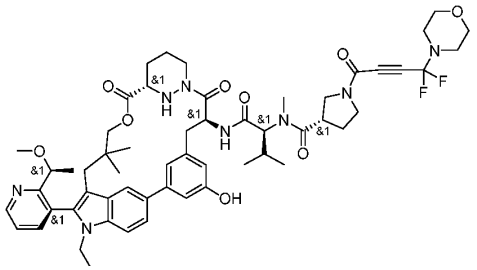
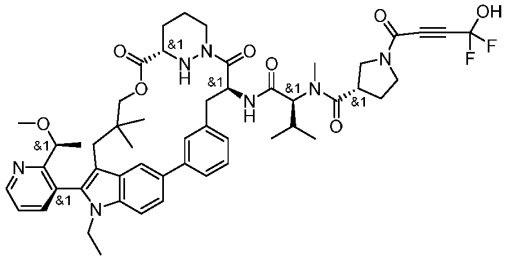
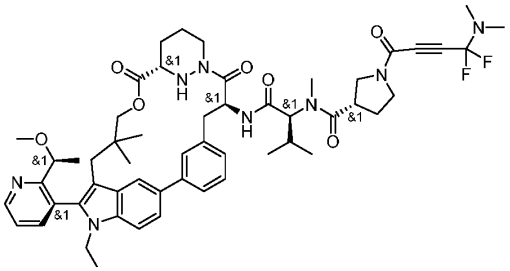
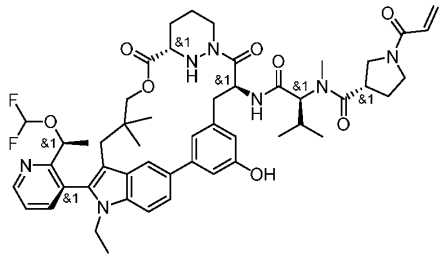
B199	
B200	
B201	
B202	
B203	

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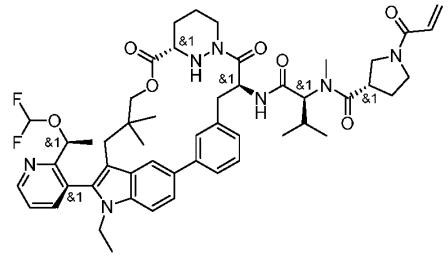
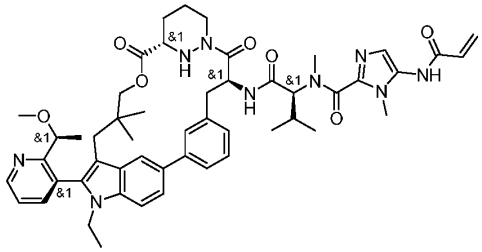
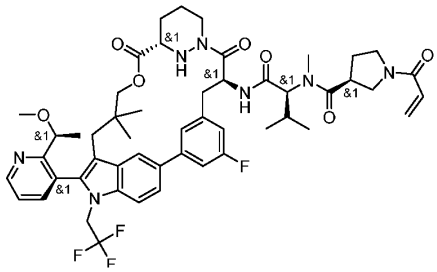
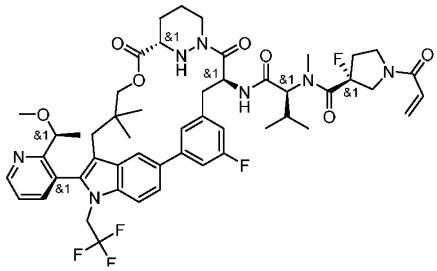
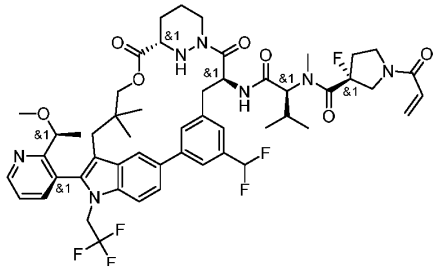
B204	
B205	
B206	
B207	
B208	

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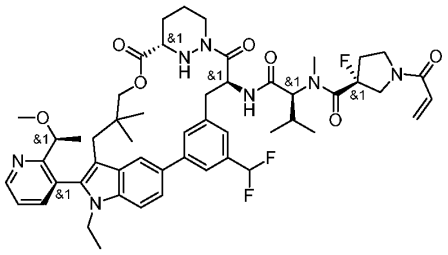
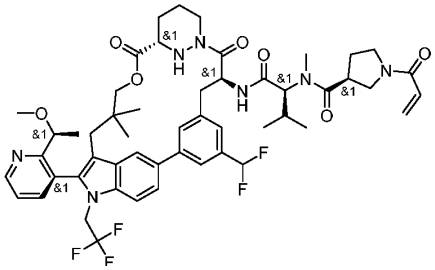
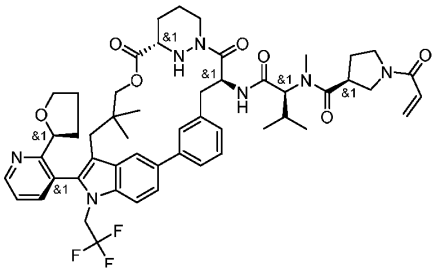
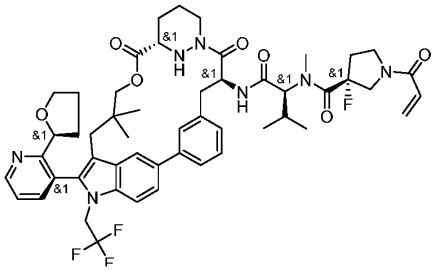
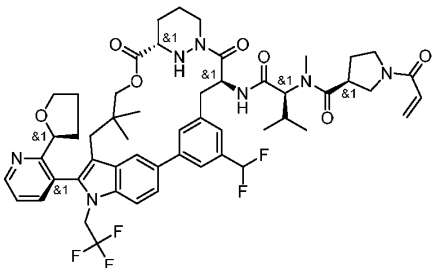
B209	
B210	
B211	
B212	
B213	

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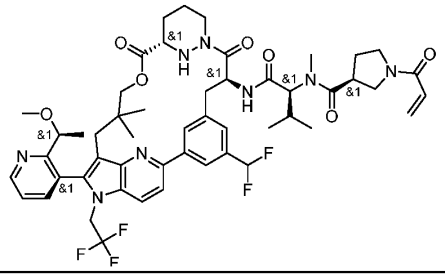
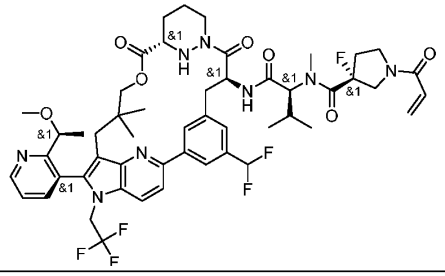
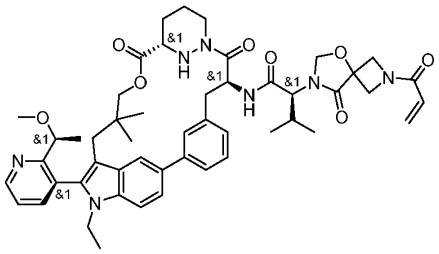
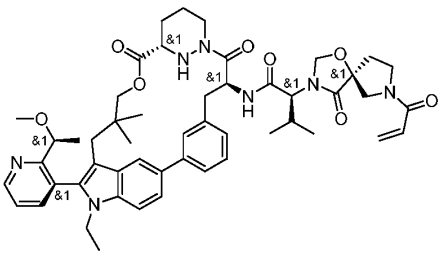
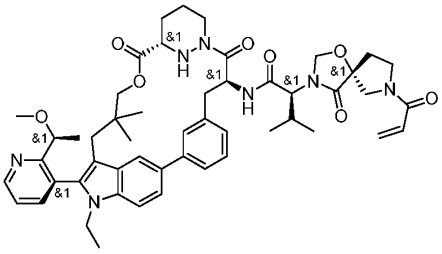
B214	
B215	
B216	
B217	
B218	

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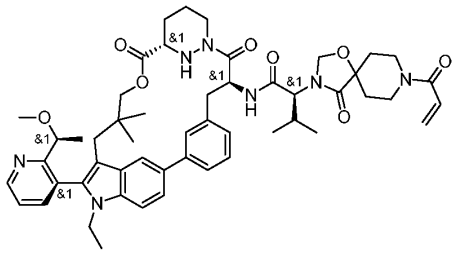
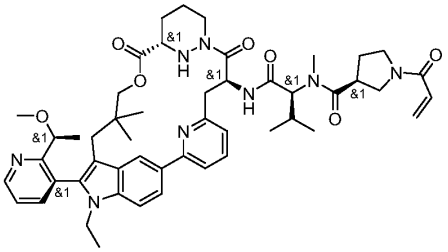
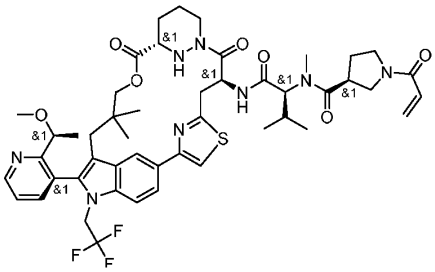
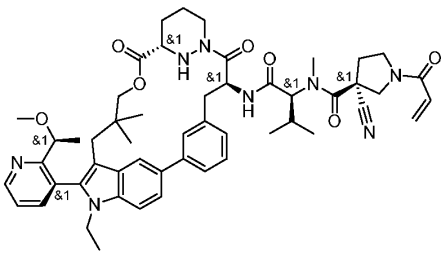
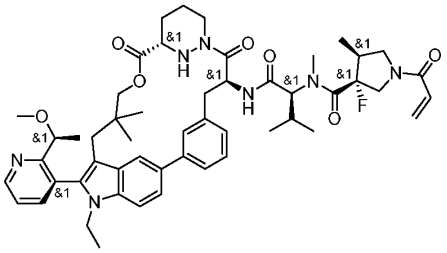
B219	
B220	
B221	
B222	
B223	

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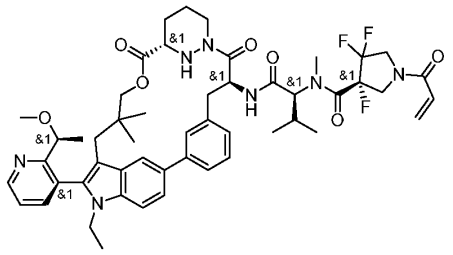
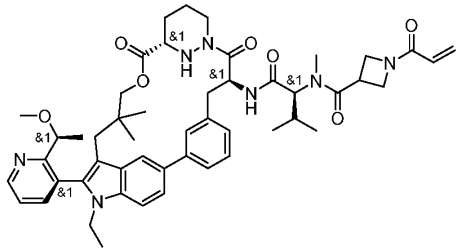
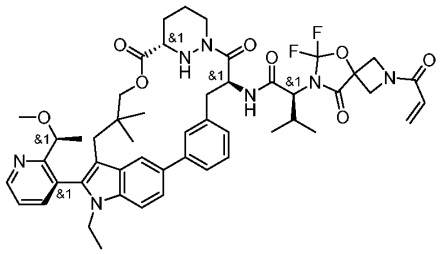
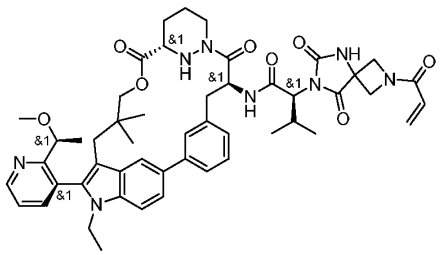
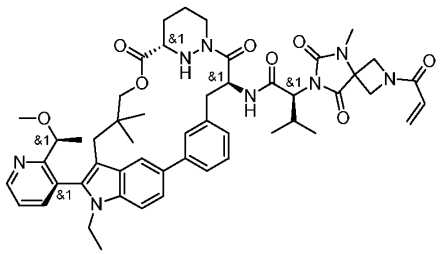
B224	
B225	
B226	
B227	
B228	

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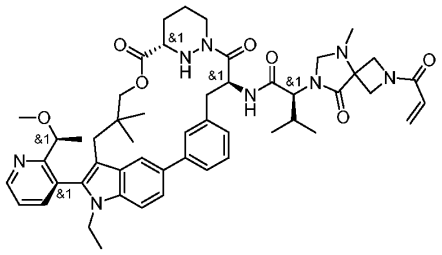
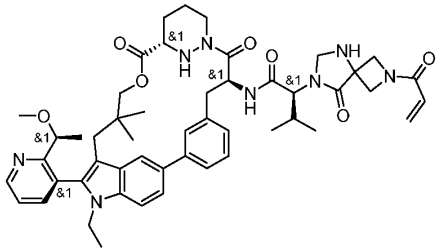
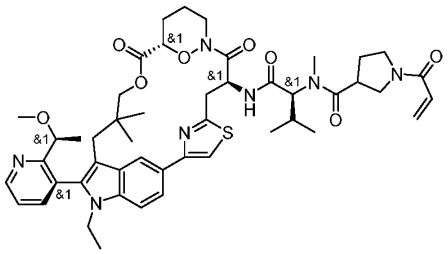
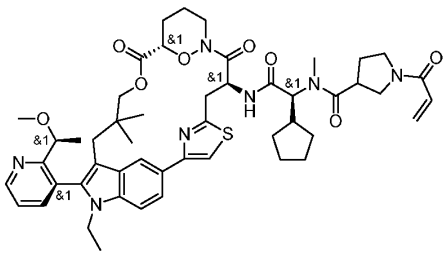
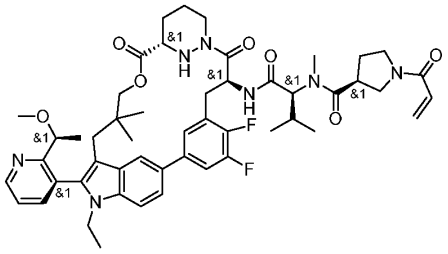
B229	
B230	
B231	
B232	
B233	

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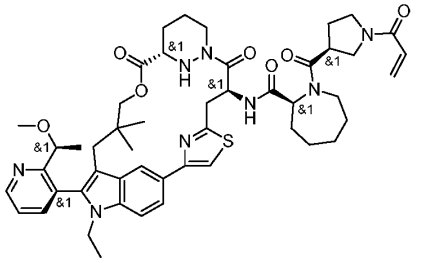
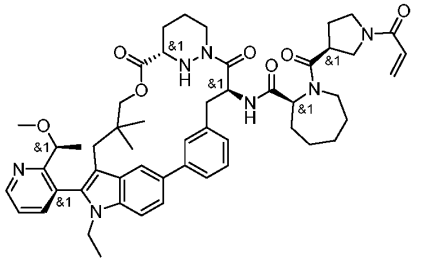
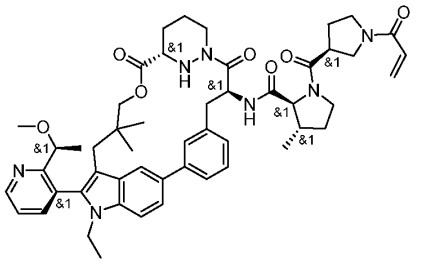
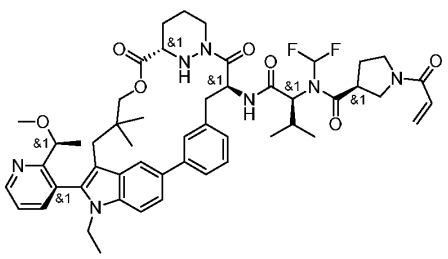
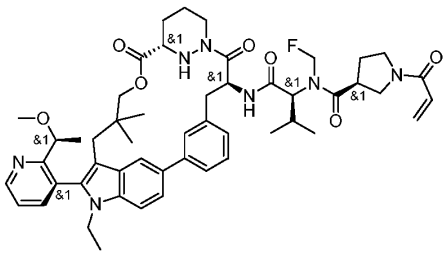
B234	
B235	
B236	
B237	
B238	

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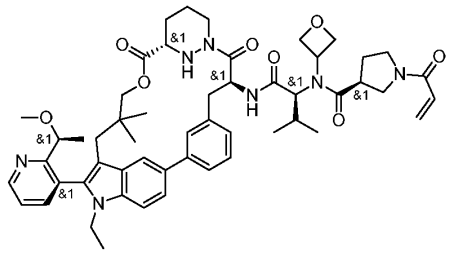
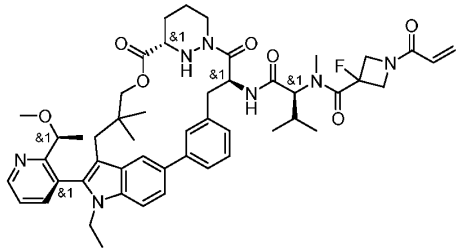
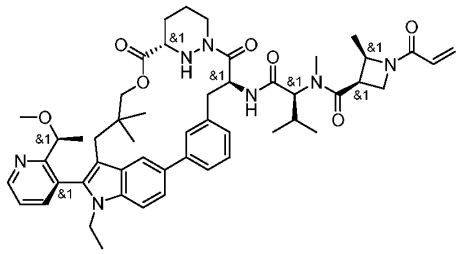
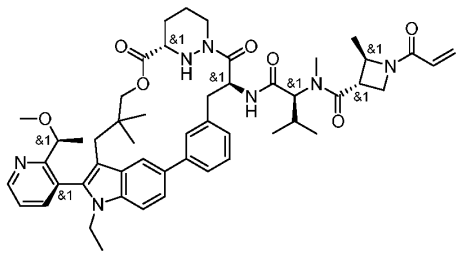
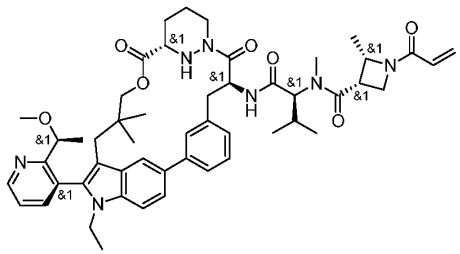
B239	
B240	
B241	
B242	
B243	

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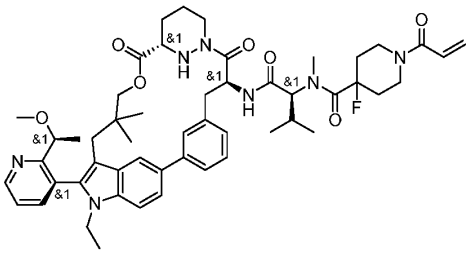
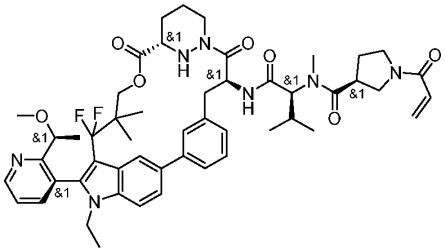
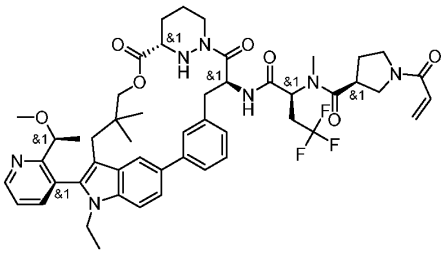
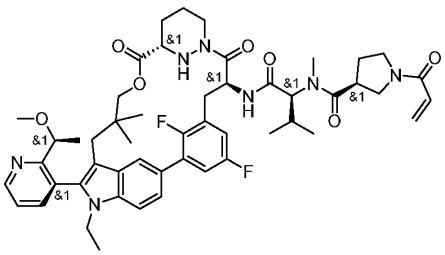
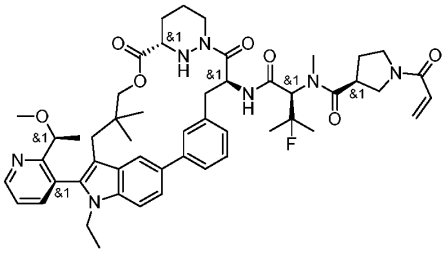
B244	
B245	
B246	
B247	
B248	

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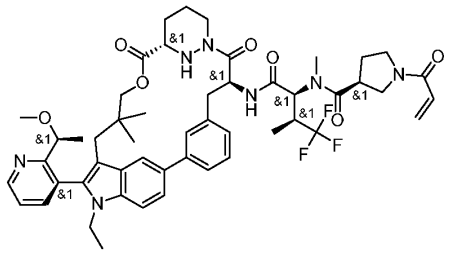
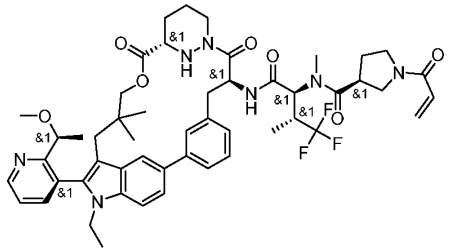
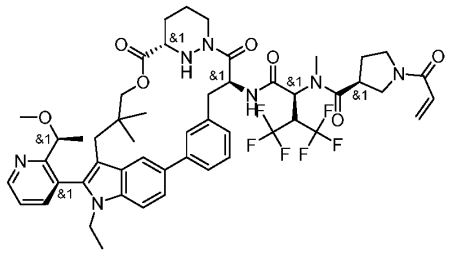
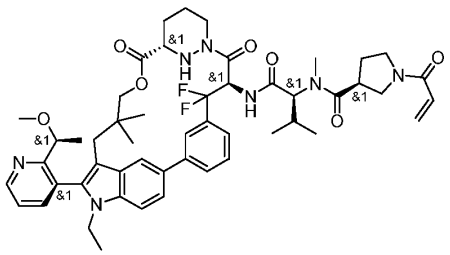
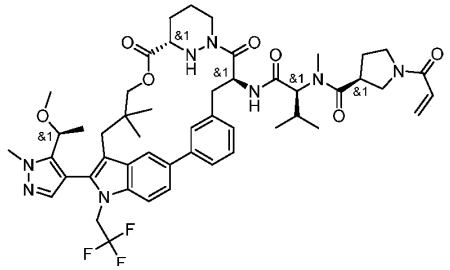
B249	
B250	
B251	
B252	
B253	

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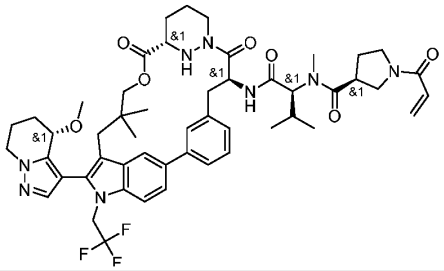
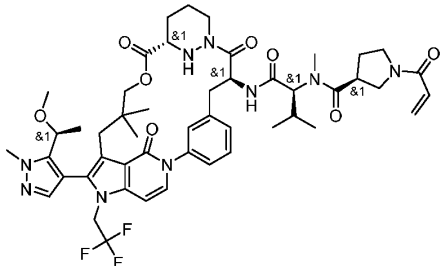
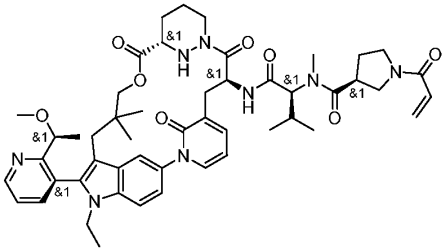
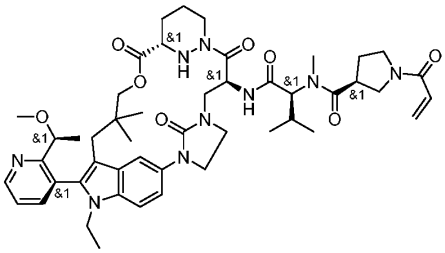
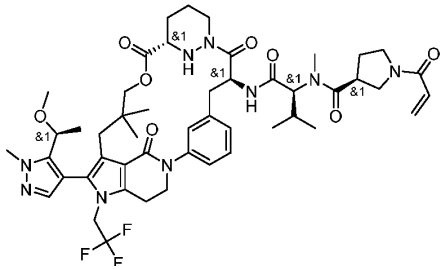
B254	
B255	
B256	
B257	
B258	

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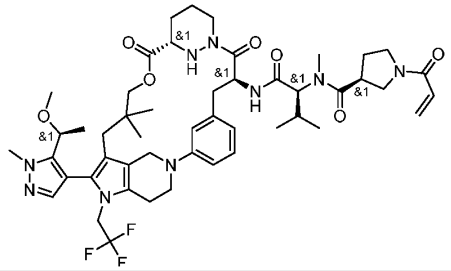
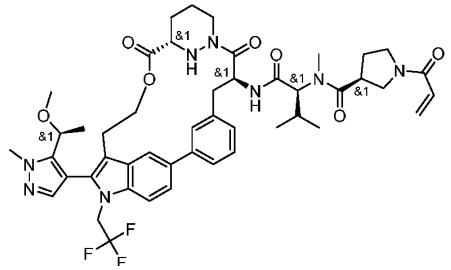
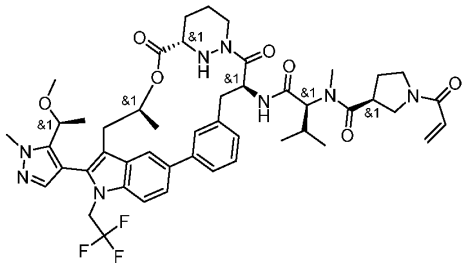
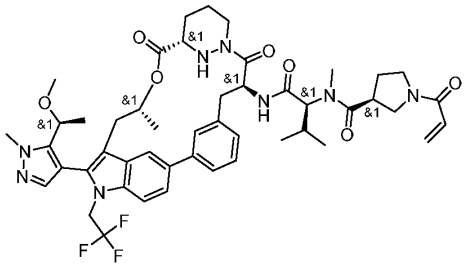
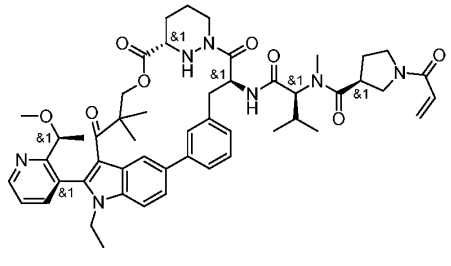
B259	
B260	
B261	
B262	
B263	

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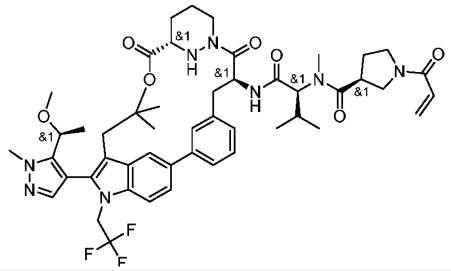
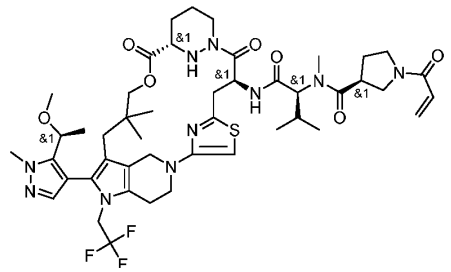
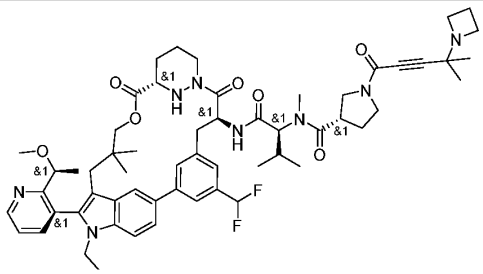
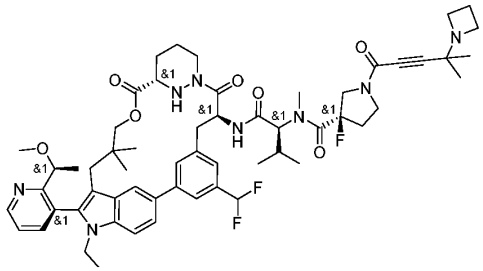
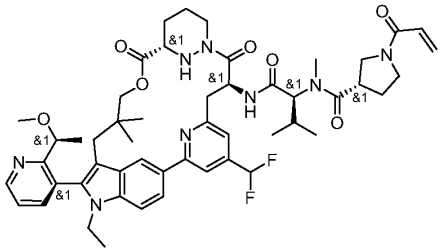
B264	
B265	
B266	
B267	
B268	

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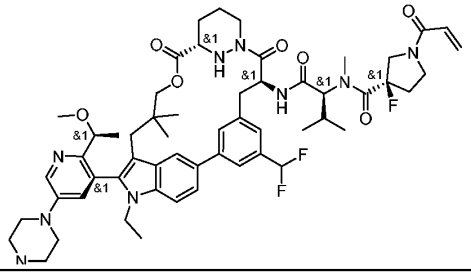
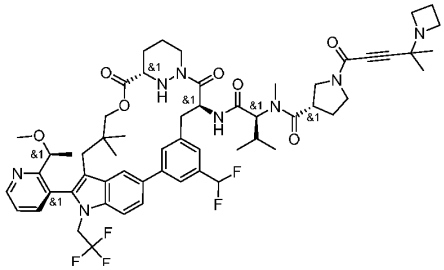
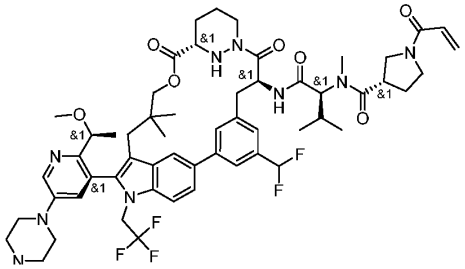
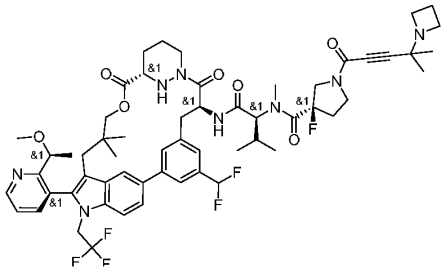
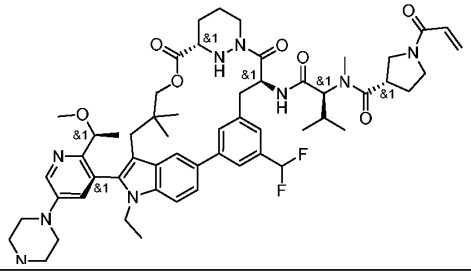
B269	
B270	
B271	
B272	
B273	

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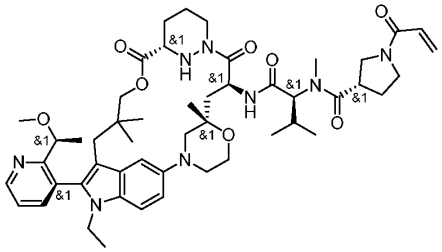
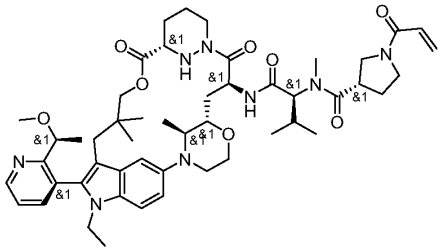
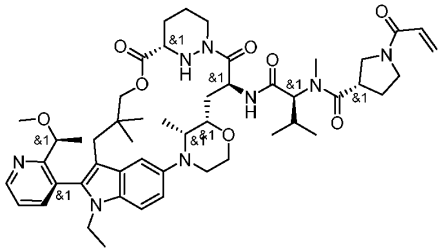
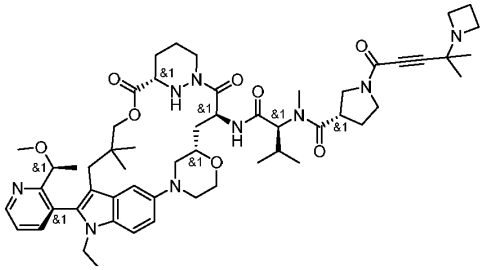
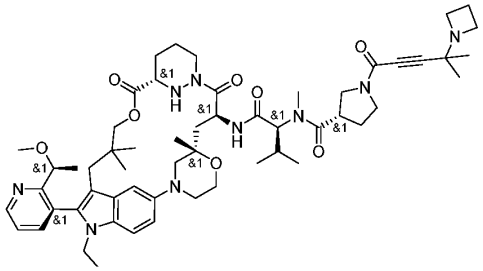
B274	
B275	
B276	
B277	
B278	

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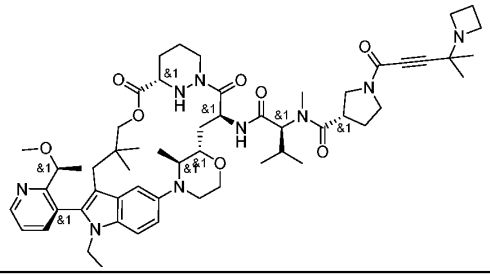
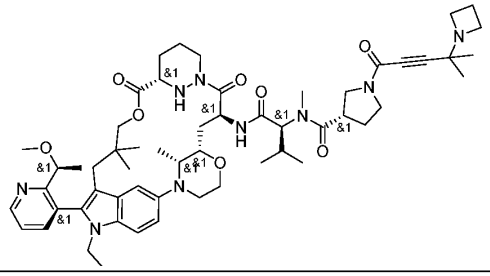
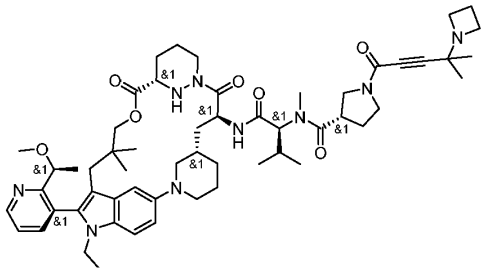
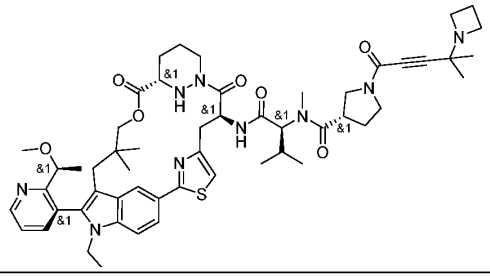
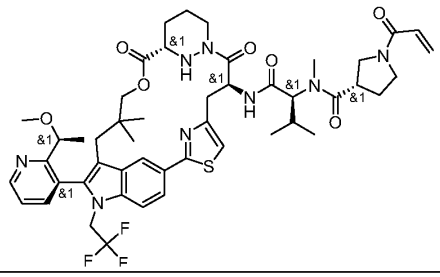
B279	
B280	
B282	
B283	
B284	

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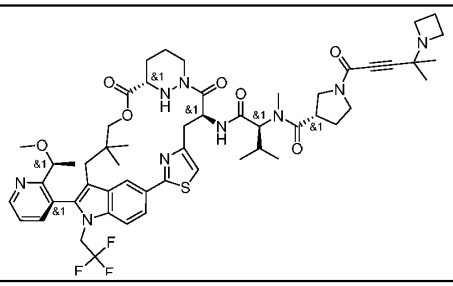
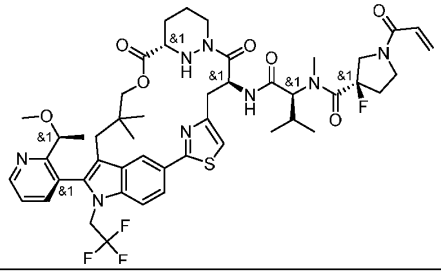
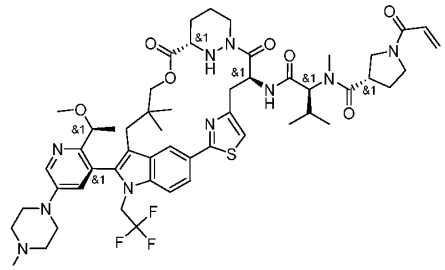
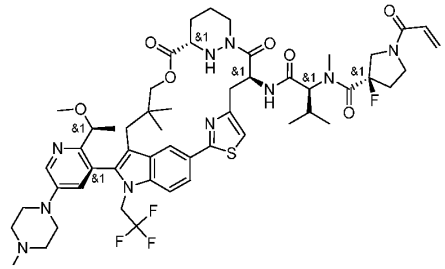
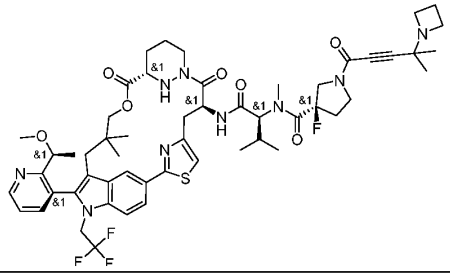
B285	
B286	
B287	
B288	
B289	

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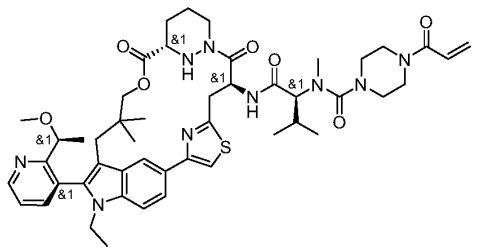
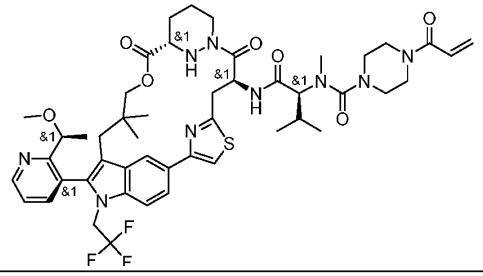
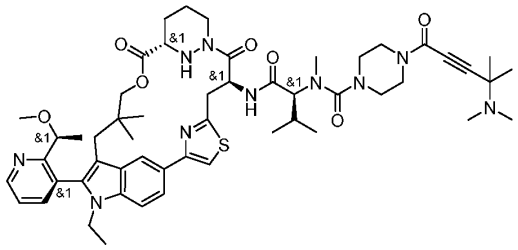
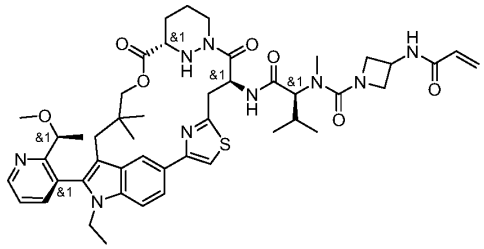
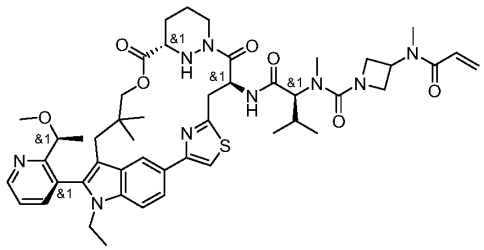
B290	
B291	
B292	
B293	
B294	

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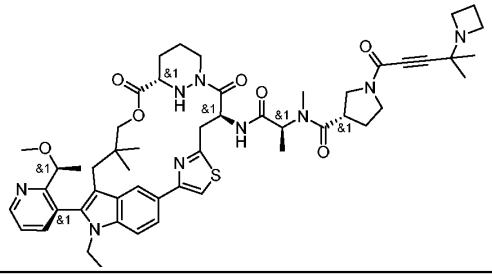
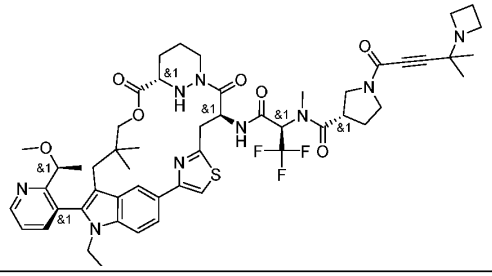
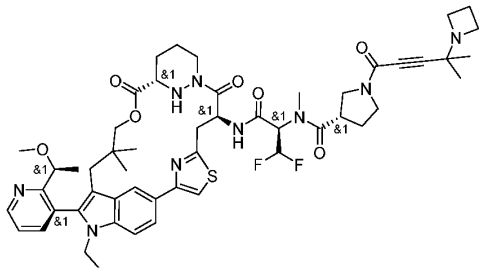
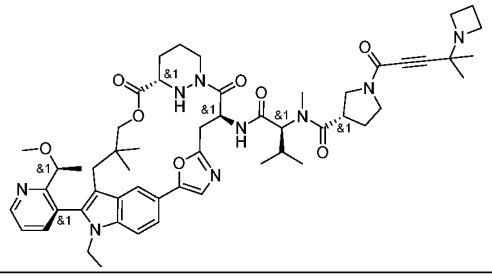
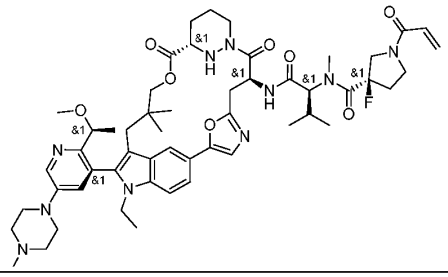
B295	
B296	
B297	
B298	
B299	

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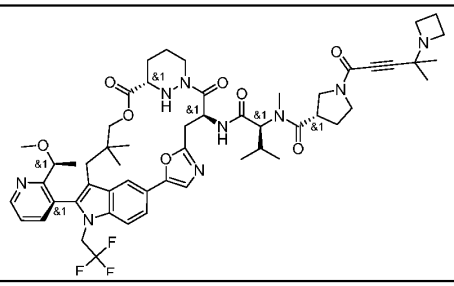
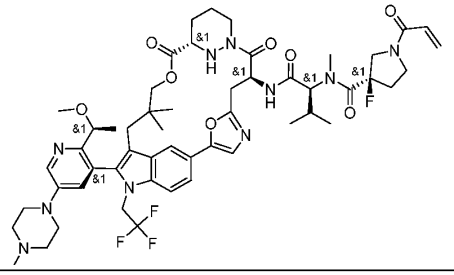
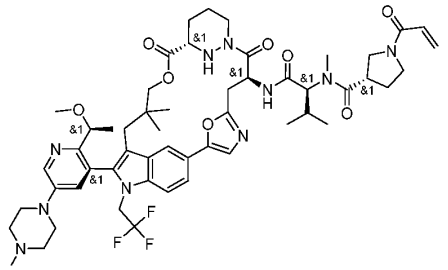
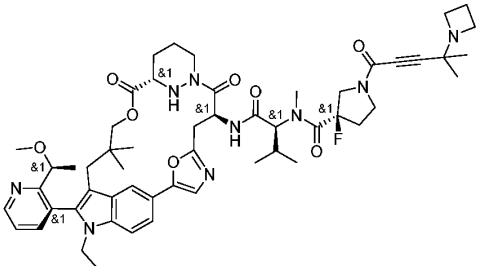
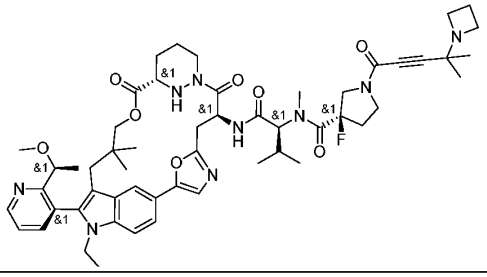
B300	
B301	
B302	
B303	
B304	

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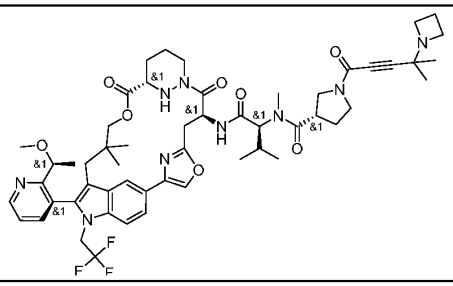
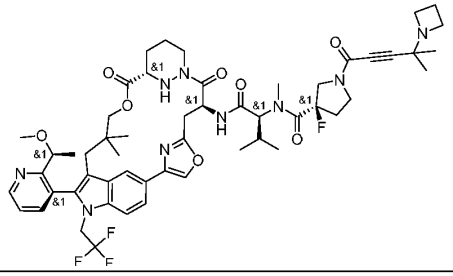
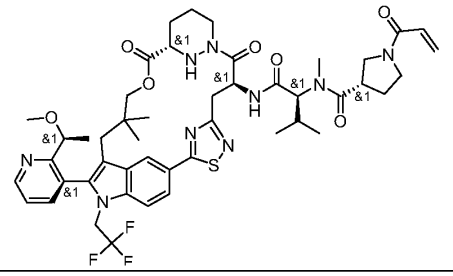
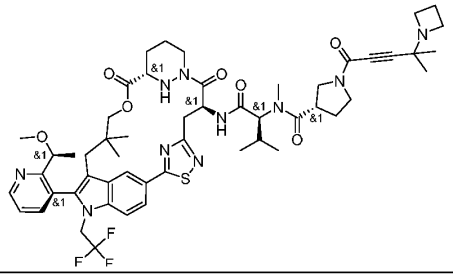
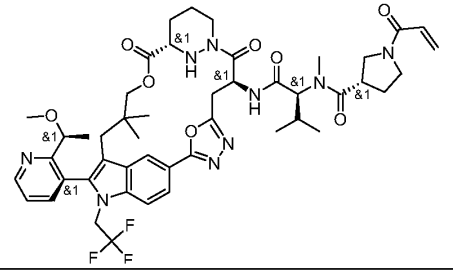
B305	
B306	
B307	
B308	
B309	

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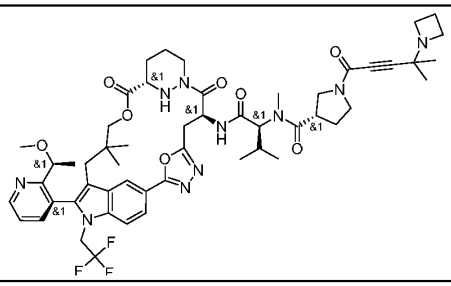
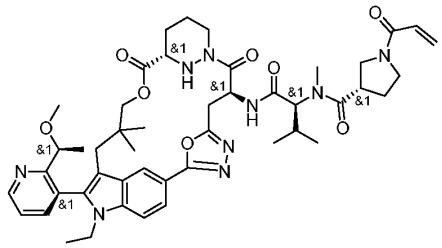
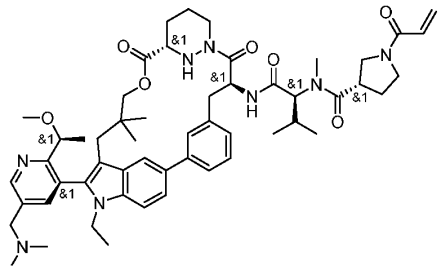
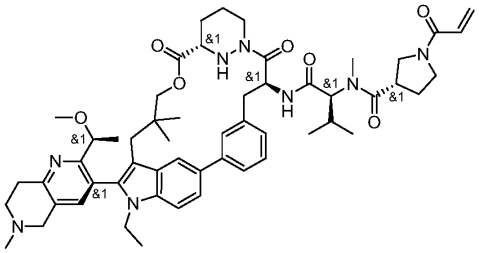
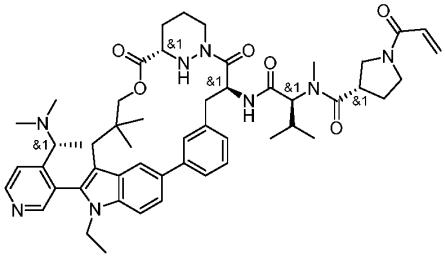
B310	
B311	
B312	
B313	
B314	

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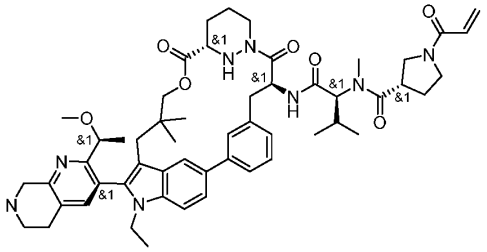
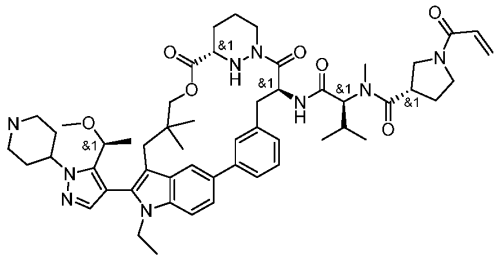
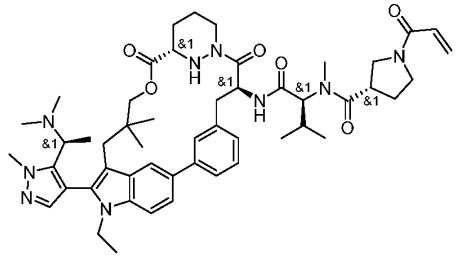
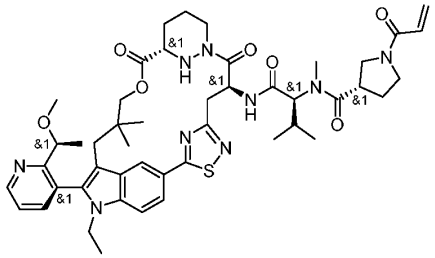
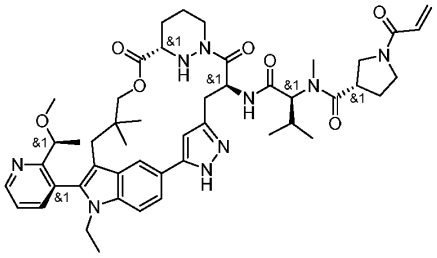
B315	
B316	
B317	
B318	
B319	

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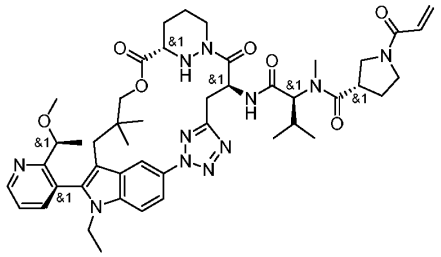
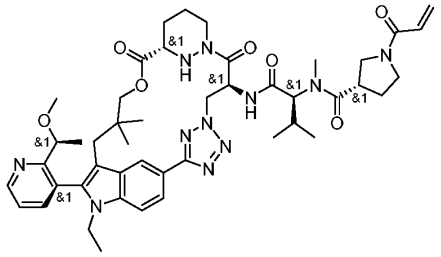
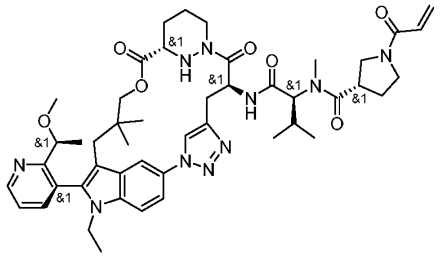
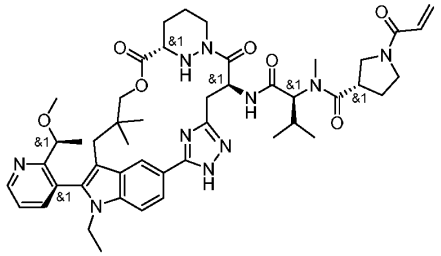
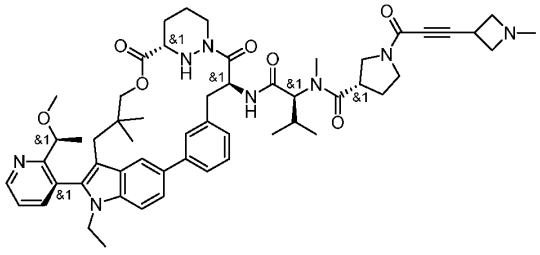
B320	
B321	
B322	
B323	
B324	

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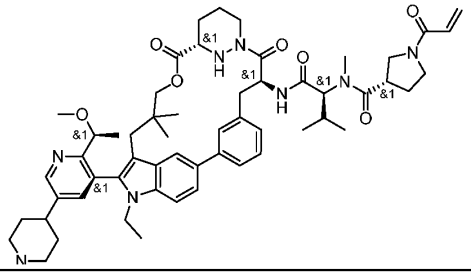
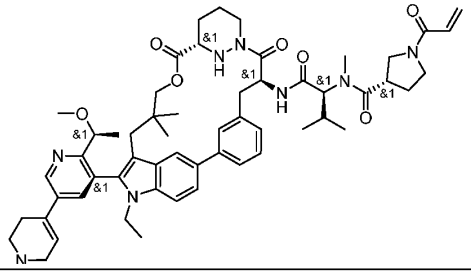
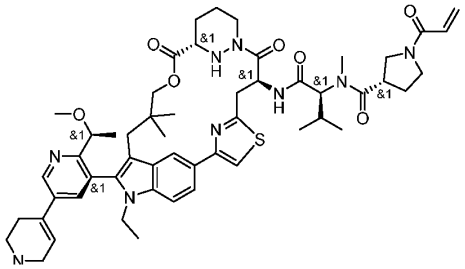
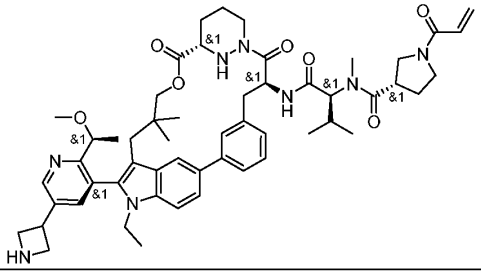
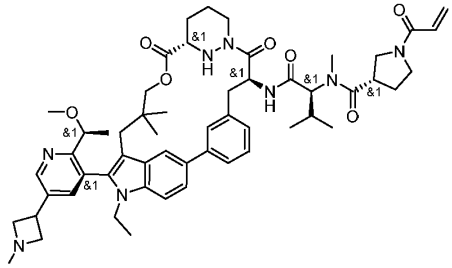
B325	
B326	
B327	
B328	
B329	

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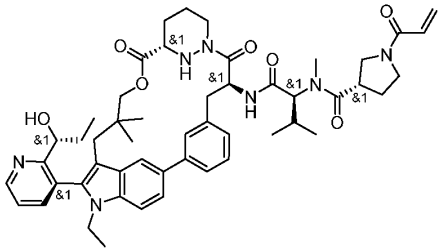
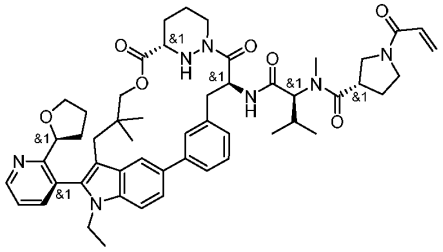
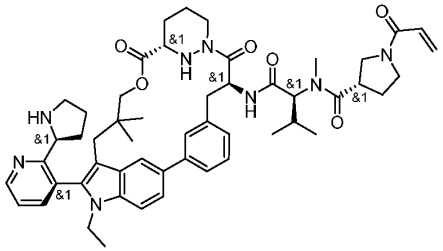
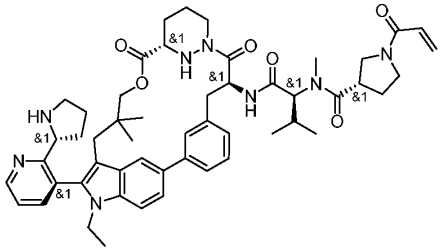
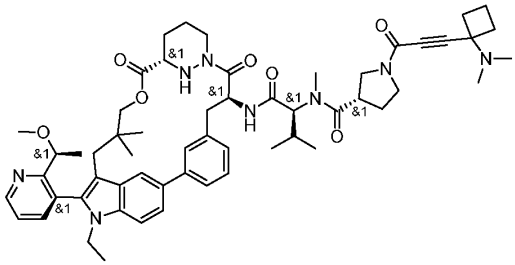
B330	
B331	
B332	
B333	
B334	

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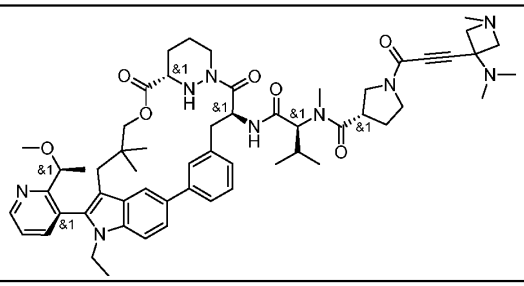
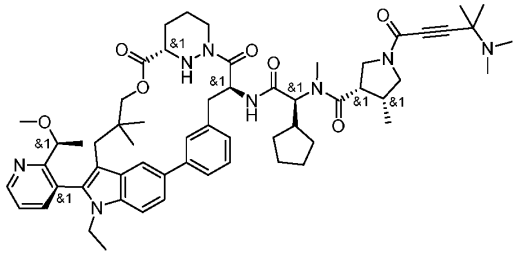
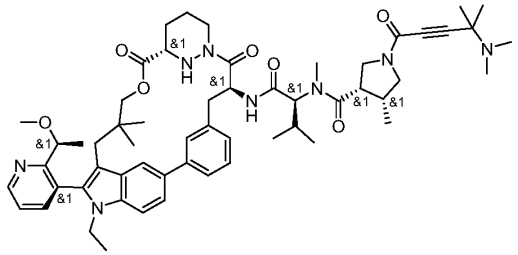
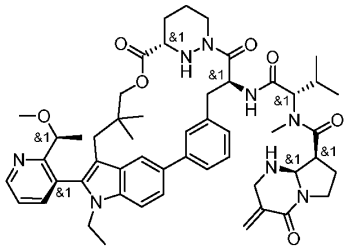
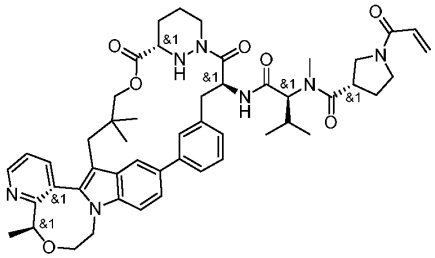
B335	
B336	
B337	
B338	
B339	

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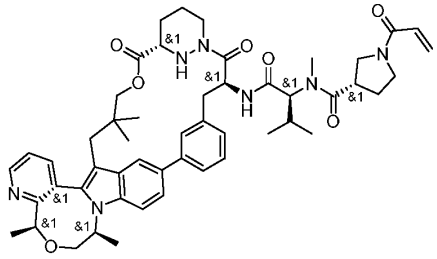
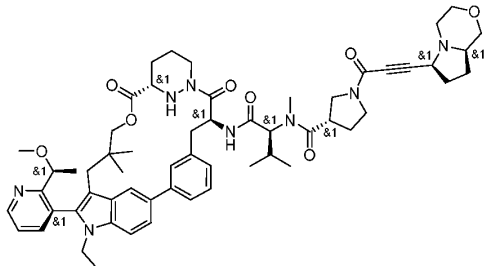
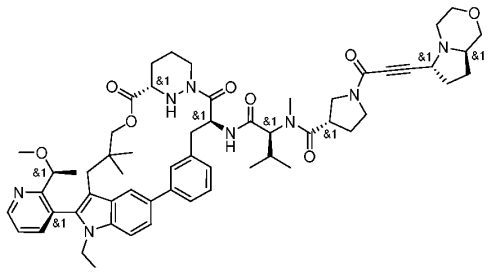
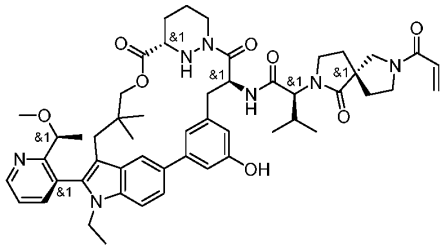
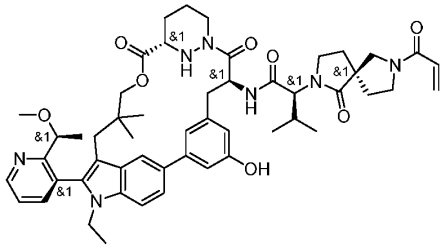
B340	
B341	
B342	
B343	
B344	

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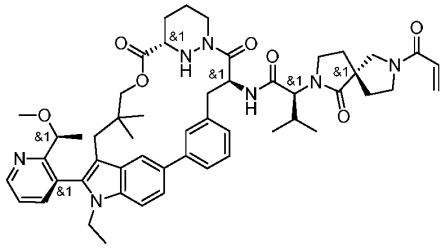
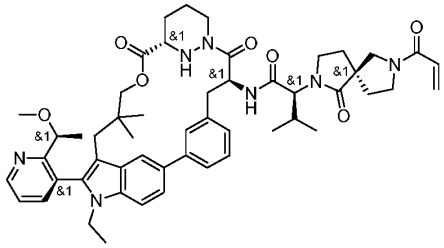
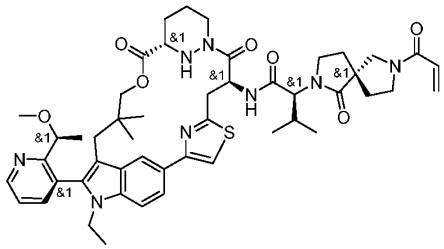
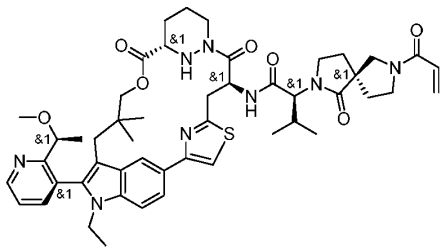
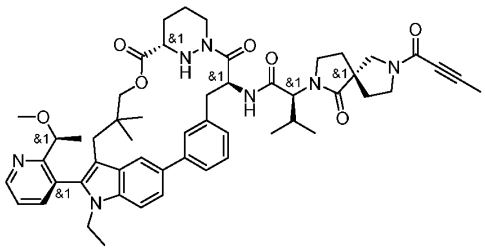
B345	
B346	
B347	
B348	
B349	

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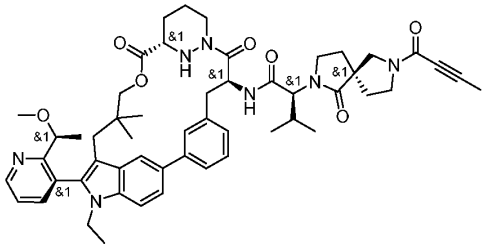
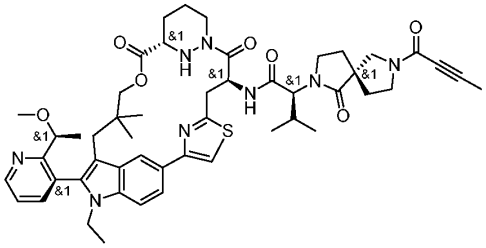
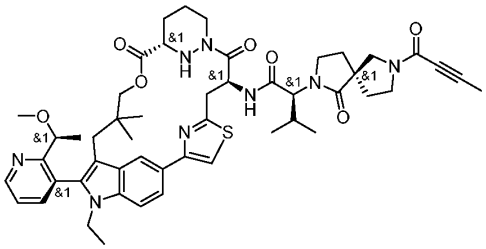
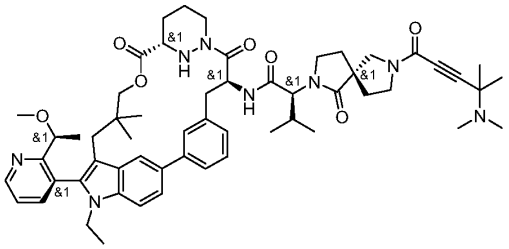
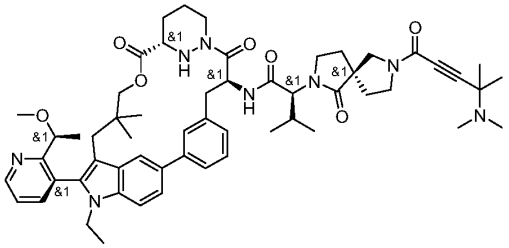
B350	
B351	
B352	
B353	
B354	

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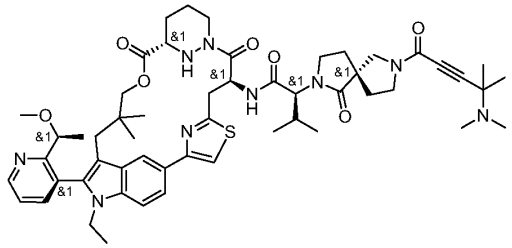
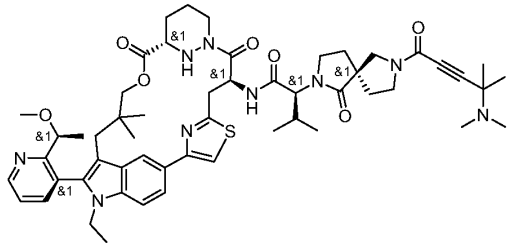
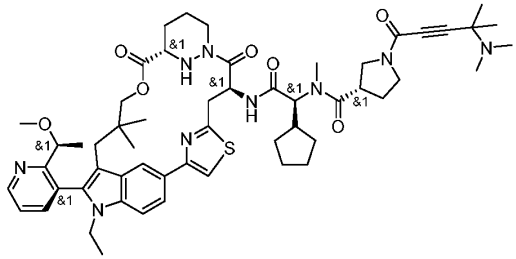
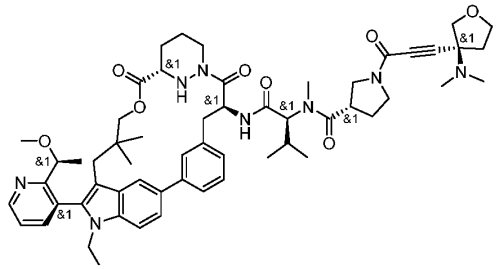
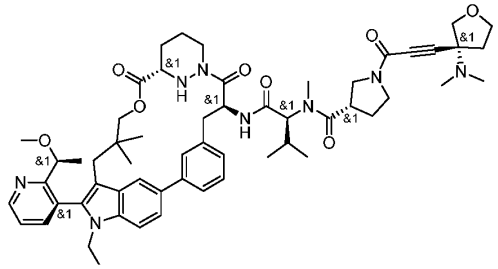
B355	
B356	
B357	
B358	
B359	

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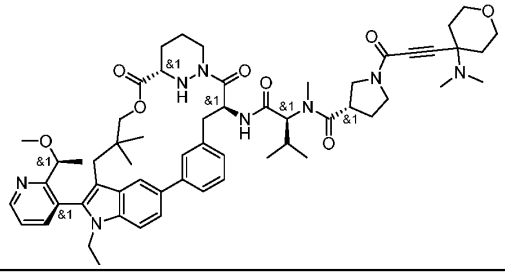
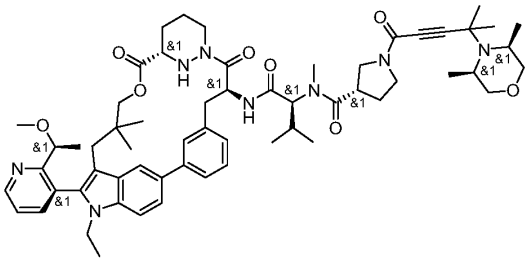
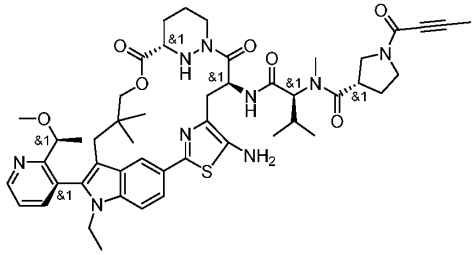
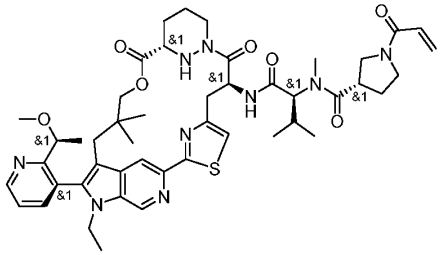
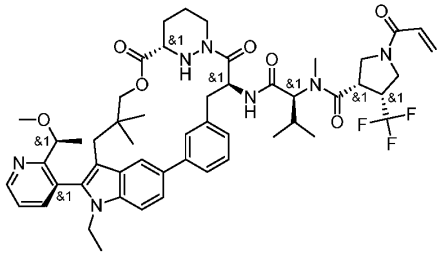
B360	
B361	
B362	
B363	
B364	

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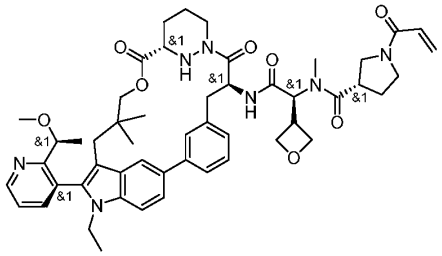
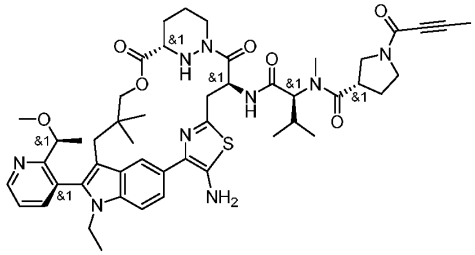
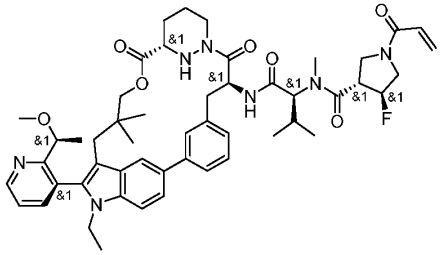
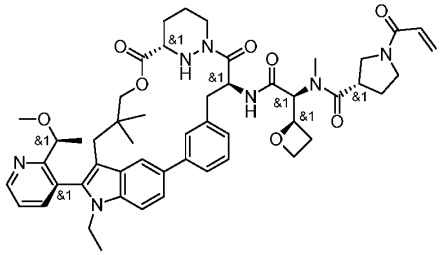
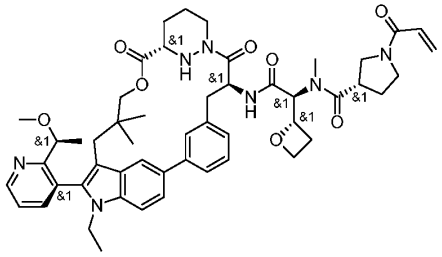
B365	
B366	
B367	
B368	
B369	

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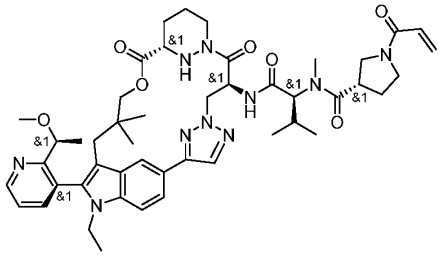
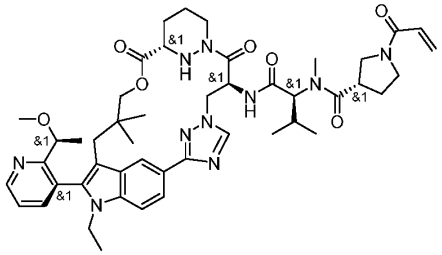
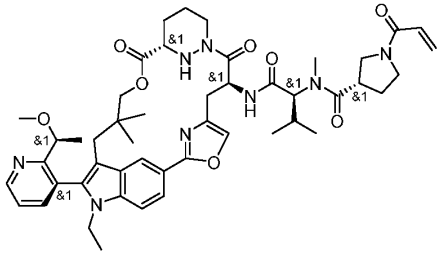
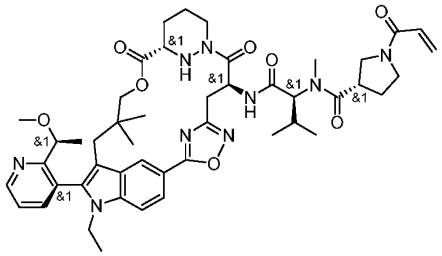
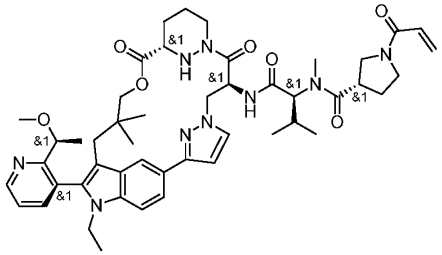
B370	
B371	
B372	
B373	
B374	

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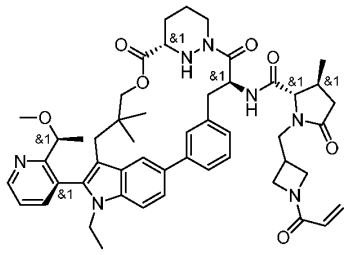
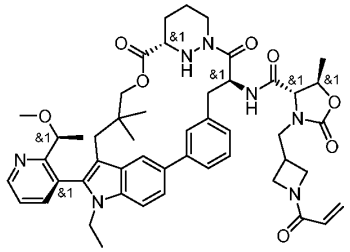
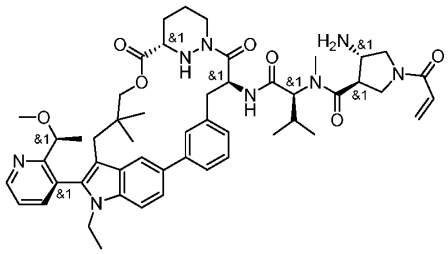
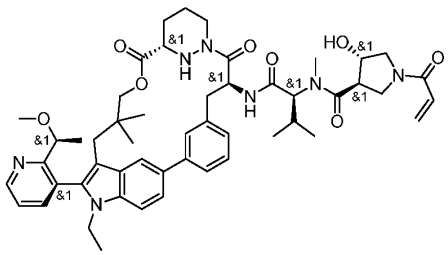
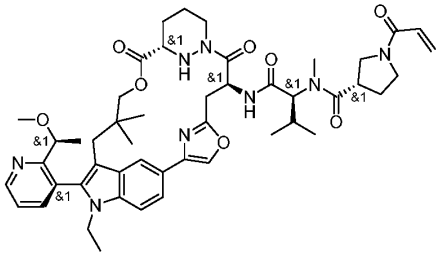
B375	
B376	
B377	
B378	
B379	

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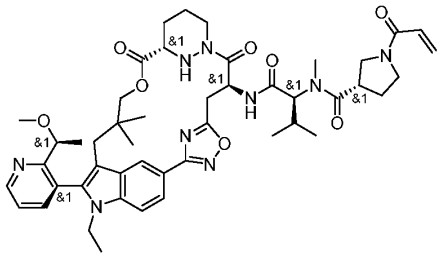
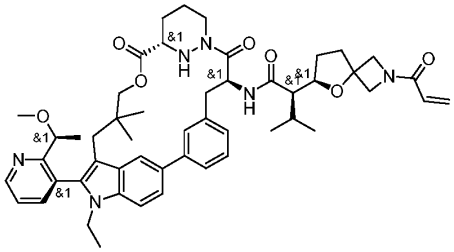
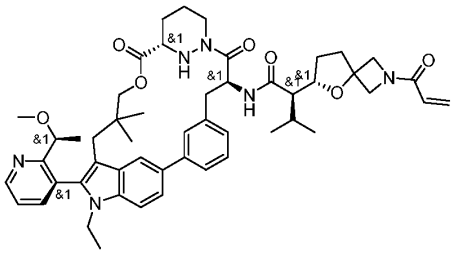
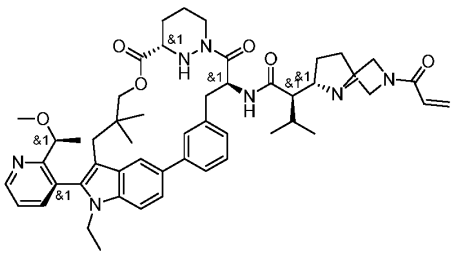
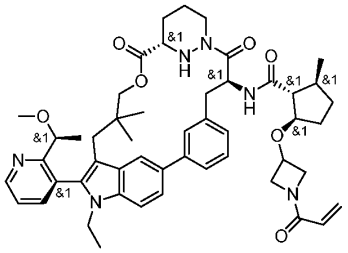
B380	
B381	
B382	
B383	
B384	

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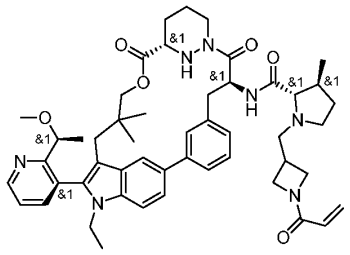
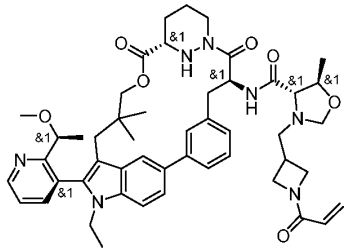
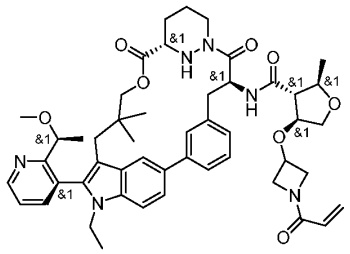
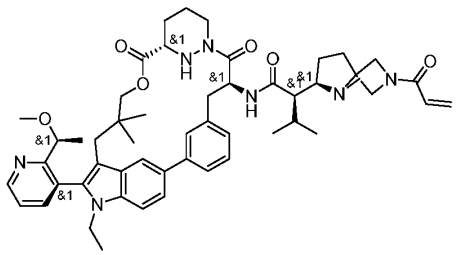
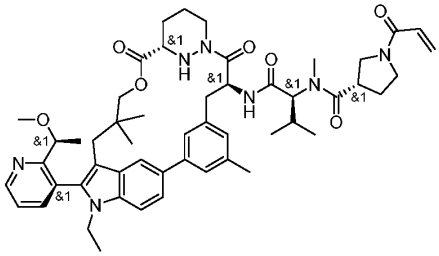
B385	
B386	
B387	
B388	
B389	

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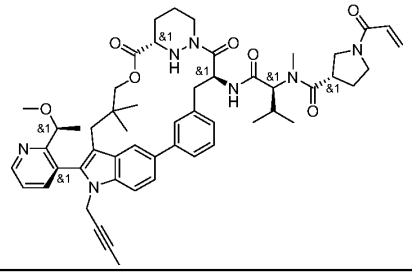
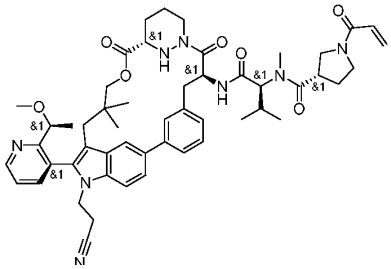
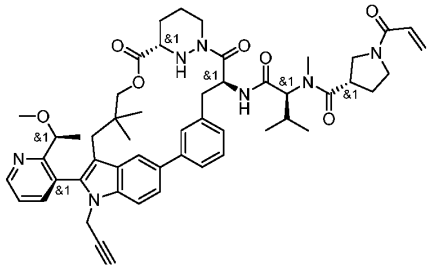
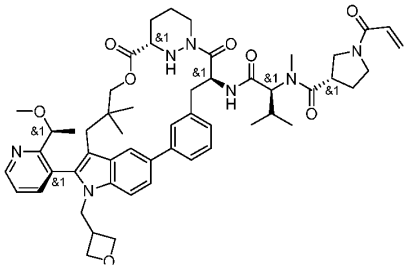
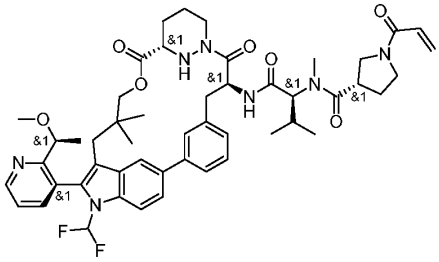
B390	
B391	
B392	
B393	
B394	

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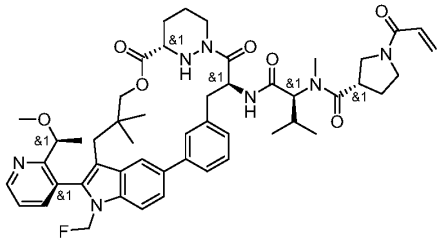
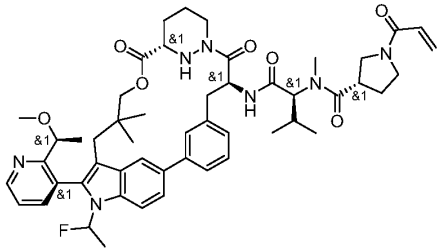
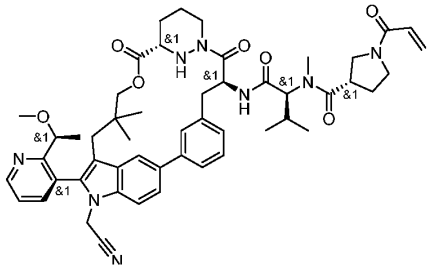
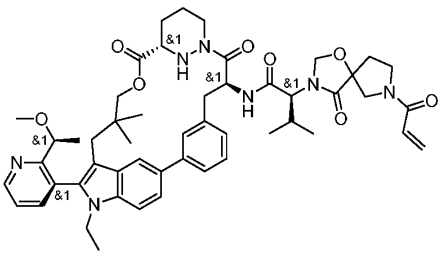
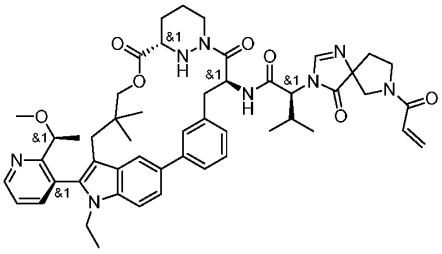
B395	
B396	
B397	
B398	
B399	

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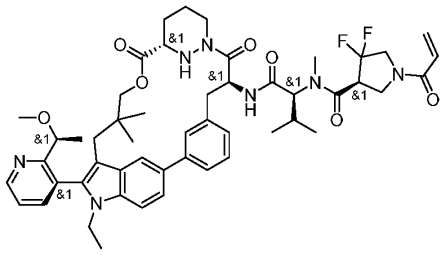
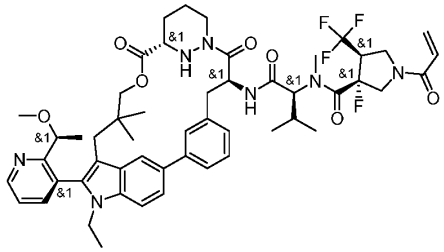
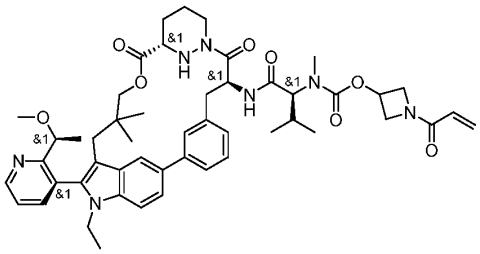
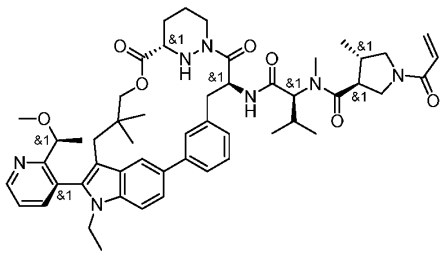
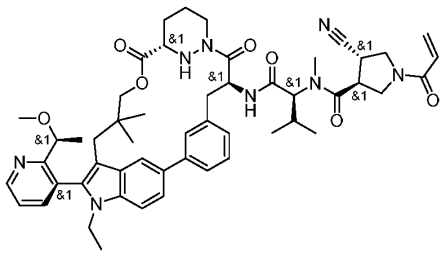
B400	
B401	
B402	
B403	
B404	

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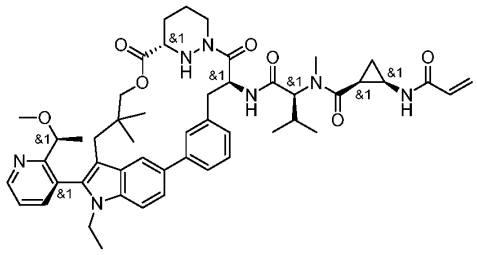
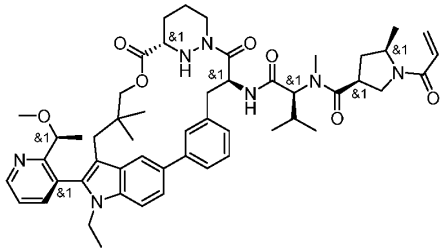
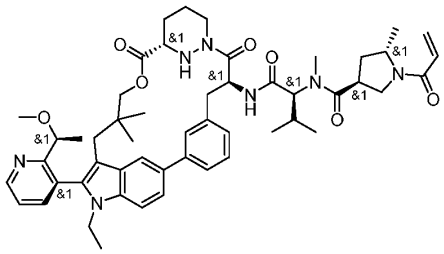
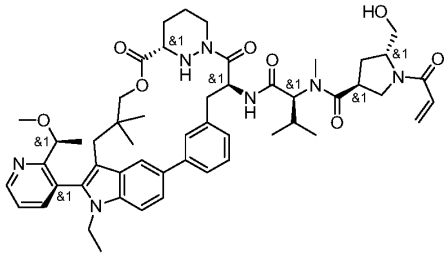
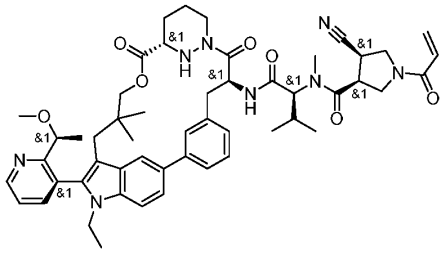
B405	
B406	
B407	
B408	
B409	

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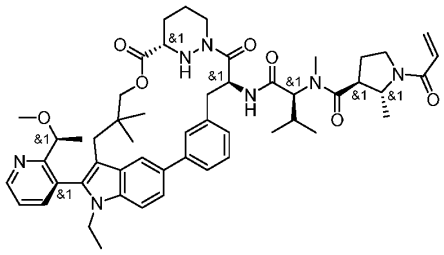
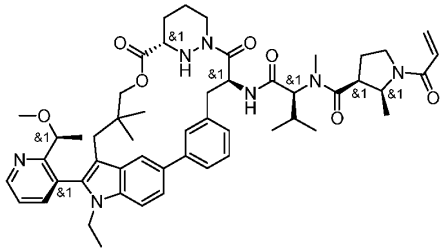
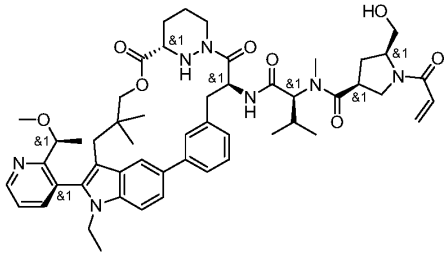
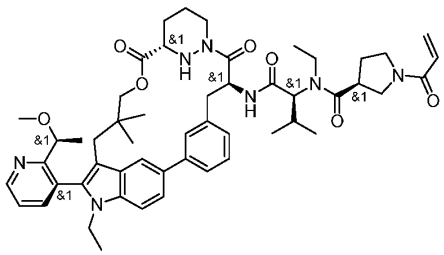
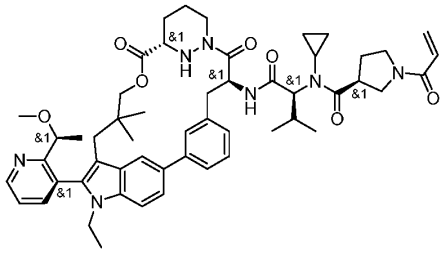
B410	
B411	
B412	
B413	
B414	

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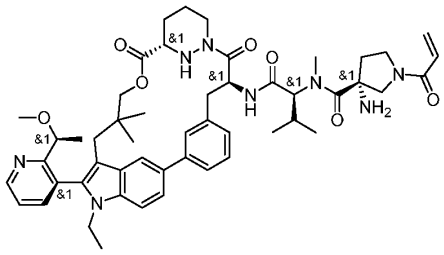
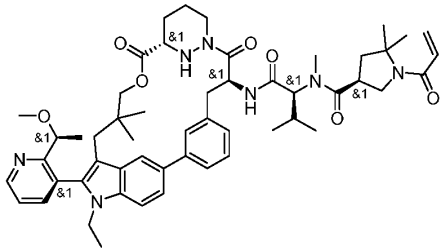
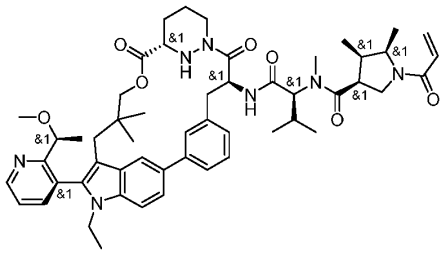
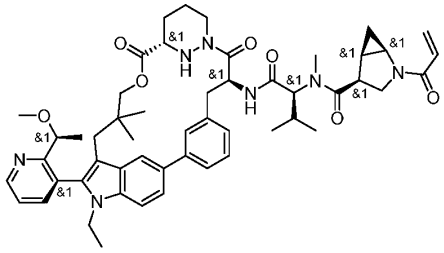
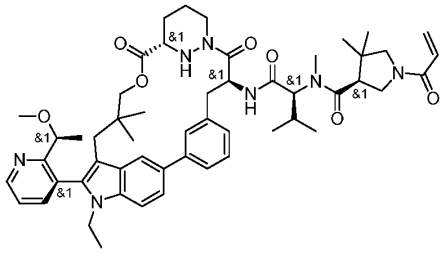
B415	
B416	
B417	
B418	
B419	

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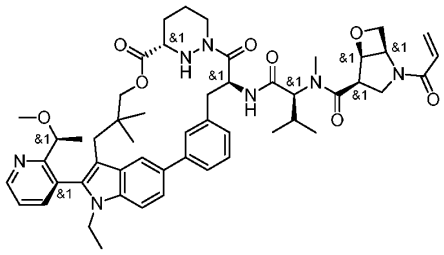
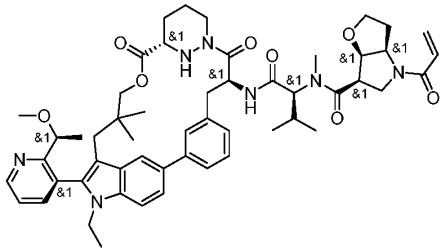
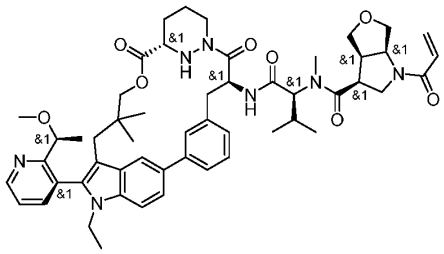
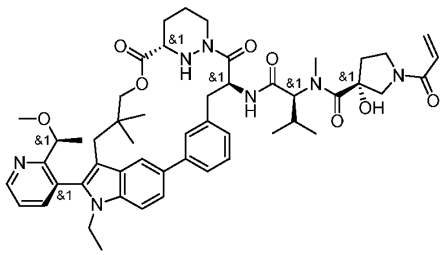
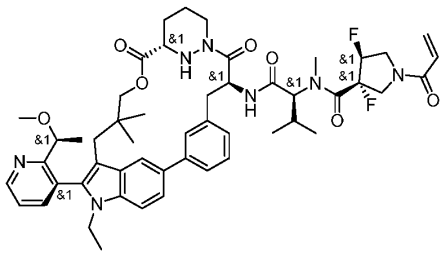
B420	
B421	
B422	
B423	
B424	

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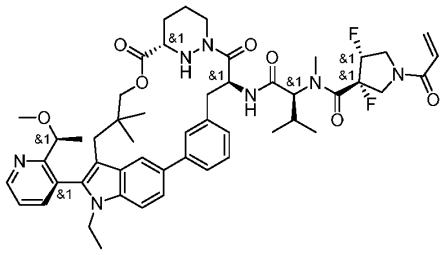
B425	

Note that some compounds are shown with bonds as flat or wedged. In some instances, the relative stereochemistry of stereoisomers has been determined; in some instances, the absolute stereochemistry has been determined. All stereoisomers of the compounds of the foregoing table are contemplated by the present invention. In particular embodiments, an atropisomer of a compound of the foregoing table is contemplated.

In some embodiments, a compound of the present invention is or acts as a prodrug, such as with respect to administration to a cell or to a subject in need thereof.

Also provided are pharmaceutical compositions comprising a compound of the present invention, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

Further provided is a conjugate, or salt thereof, comprising the structure of Formula IV:

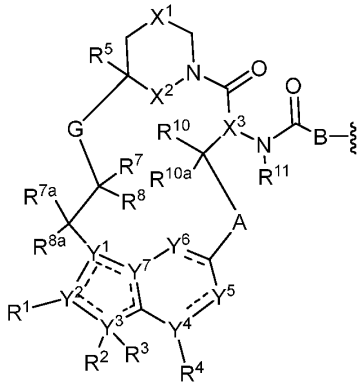
M-L-P

Formula IV

wherein L is a linker;

P is a monovalent organic moiety; and

M has the structure of Formula Va:



Formula Va

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is absent, -CH(R^9)-, >C=C$R^9R^{9'}$, or >C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', $S(O)_2$R', or $S(O)_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is H, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl; or

R^9 and $R^{9'}$, combined with the atoms to which they are attached, form a 3 to 6-membered cycloalkyl or a 3 to 6-membered heterocycloalkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo; and

R^{11} is hydrogen or C_1-C_3 alkyl.

In some embodiments the conjugate, or salt thereof, comprises the structure of Formula IV:

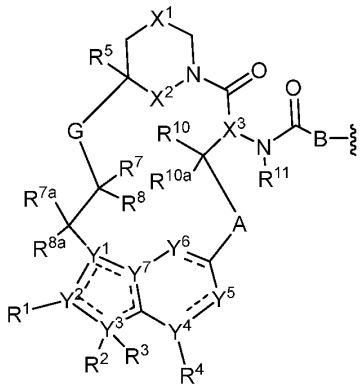
M-L-P

Formula IV

wherein L is a linker;

P is a monovalent organic moiety; and

M has the structure of Formula Vb:



Formula Vb

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- or >C=C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', $S(O)_2$R', or $S(O)_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered

cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo; and

R^{11} is hydrogen or C_1-C_3 alkyl.

In some embodiments, the conjugate has the structure of Formula IV:

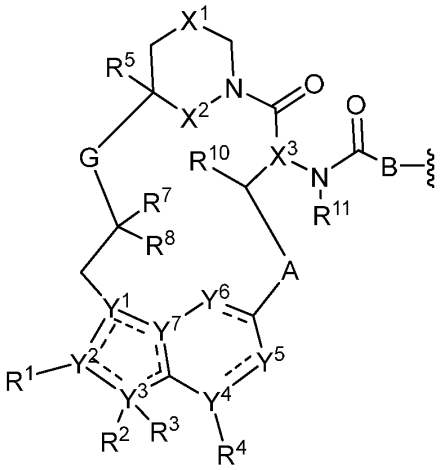
M-L-P

Formula IV

wherein L is a linker;

P is a monovalent organic moiety; and

M has the structure of Formula Vc:



Formula Vc

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or S(O)$_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form C=C$R^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl; and

R^{11} is hydrogen or C_1-C_3 alkyl.

In some embodiments, a compound of the present invention has the structure of of Formula IV:

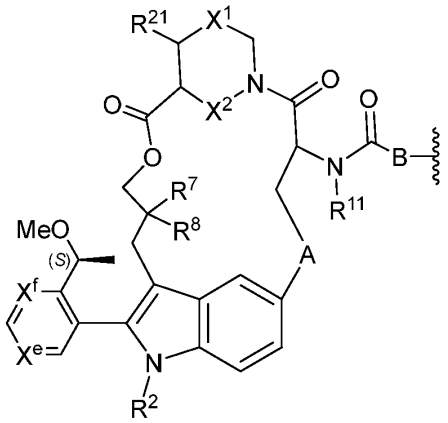
M-L-P

Formula IV

wherein L is a linker;

P is a monovalent organic moiety; and

M has the structure of Formula Vd:



Formula Vd

wherein A optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene (e.g., phenyl or phenol), or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

R^2 is C_1-C_6 alkyl, C_1-C_6 fluoroalkyl, or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

R^8 is C_1-C_3 alkyl; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

X^e and X^f are, independently, N or CH;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{21} is hydrogen or C_1-C_3 alkyl.

In some embodiments of a compound of the present invention, X^e is N and X^f is CH. In some embodiments, X^e is CH and X^f is N.

In some embodiments, a compound of the present invention has the structure of of Formula IV:

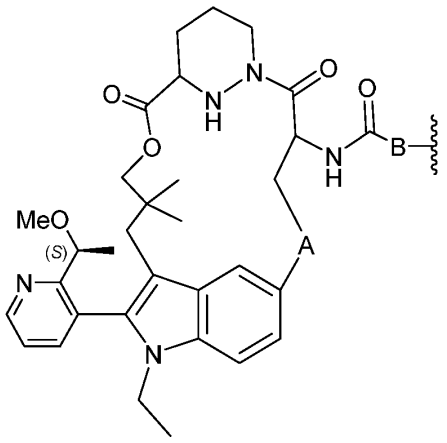
M-L-P

Formula IV

wherein L is a linker;

P is a monovalent organic moiety; and

M has the structure of Formula Ve:



Formula Ve

wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene (e.g., phenyl or phenol), or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

In some embodiments of a conjugate of the present invention, the linker has the structure of Formula II:

A^1-$(B^1)_f$-$(C^1)_g$-$(B^2)_h$-(D^1)-$(B^3)_i$-$(C^2)_j$-$(B^4)_k$–A^2

Formula II

where A^1 is a bond between the linker and B; A^2 is a bond between P and the linker; B^1, B^2, B^3, and B^4 each, independently, is selected from optionally substituted C_1-C_2 alkylene, optionally substituted C_1-C_3 heteroalkylene, O, S, and NR^N; R^N is hydrogen, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted C_1-C_7 heteroalkyl; C^1 and C^2 are each, independently, selected from carbonyl, thiocarbonyl, sulphonyl, or phosphoryl; f, g, h, i, j, and k are each, independently, 0 or 1; and D^1 is optionally substituted C_1–C_{10} alkylene, optionally substituted C_2-C_{10} alkenylene, optionally substituted C_2-C_{10} alkynylene, optionally substituted 3 to 14-membered heterocycloalkylene, optionally substituted 5 to 10-membered heteroarylene, optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 6 to 10-membered arylene, optionally substituted C_2-C_{10} polyethylene glycolene, or optionally substituted C_1-C_{10} heteroalkylene, or a chemical bond linking A^1-$(B^1)_f$-$(C^1)_g$-$(B^2)_h$- to -$(B^3)_i$-$(C^2)_j$-$(B^4)_k$–A^2.

In some embodiments of a conjugate of the present invention, the monovalent organic moiety is a protein, such as a Ras protein. In some embodiments, the Ras protein is K-Ras G12C, K-Ras G13C, H-Ras G12C, H-Ras G13C, N-Ras G12C, or N-Ras G13C. Other Ras proteins are described herein. In some embodiments, the linker is bound to the monovalent organic moiety through a bond to a sulfhydryl group of an amino acid residue of the monovalent organic moiety. In some embodiments, the linker is bound to the monovalent organic moiety through a bond to a carboxyl group of an amino acid residue of the monovalent organic moiety.

With respect to compounds of the present invention, one stereoisomer may exhibit better inhibition than another stereoisomer. For example, one atropisomer may exhibit inhibition, whereas the other atropisomer may exhibit little or no inhibition.

In some embodiments, a method or use described herein further comprises administering an additional anti-cancer therapy. In some embodiments, the additional anti-cancer therapy is a HER2 inhibitor, an EGFR inhibitor, a second Ras inhibitor, a SHP2 inhibitor, an SOS1 inhibitor, a Raf inhibitor, a MEK inhibitor, an ERK inhibitor, a PI3K inhibitor, a PTEN inhibitor, an AKT inhibitor, an mTORC1 inhibitor, a BRAF inhibitor, a PD-L1 inhibitor, a PD-1 inhibitor, a CDK4/6 inhibitor, or a combination thereof. In some embodiments, the additional anticancer therapy is a SHP2 inhibitor. Other additional anti-cancer therapies are described herein.

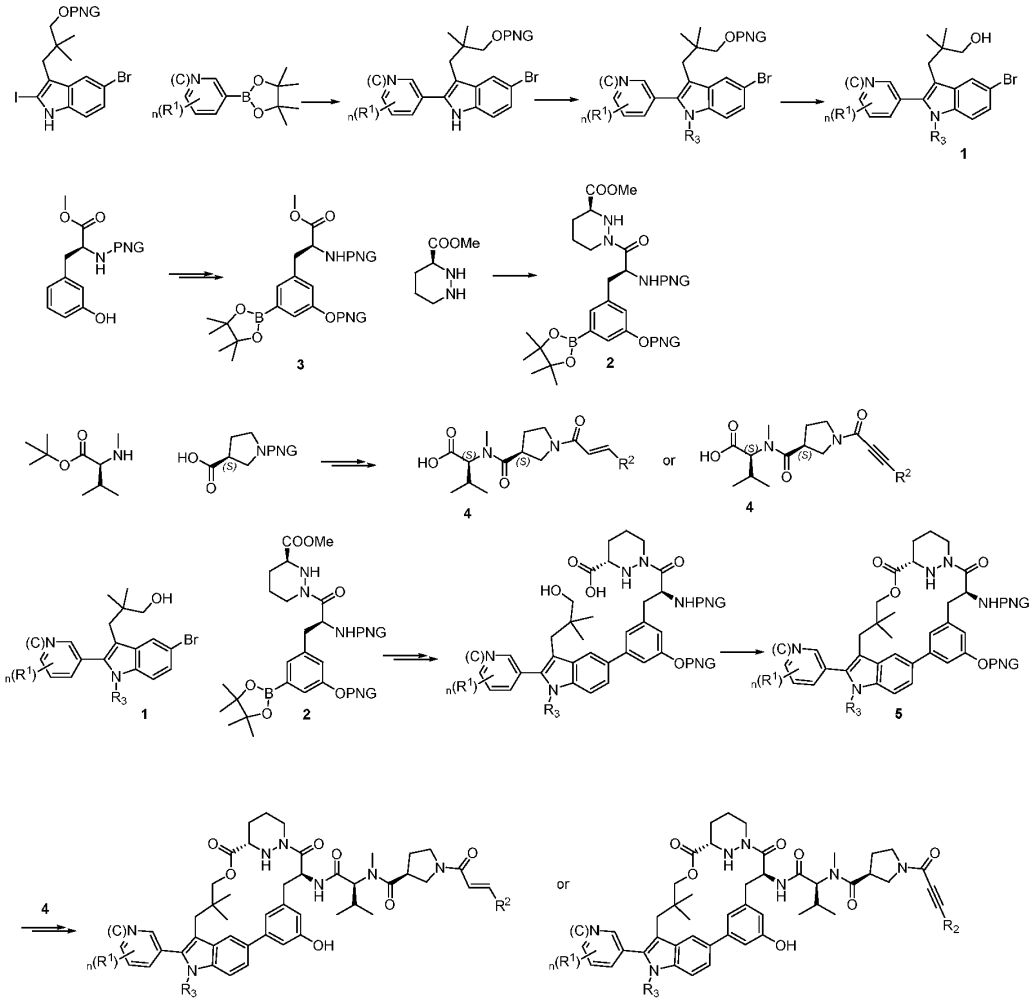
Methods of Synthesis

The compounds described herein may be made from commercially available starting materials or synthesized using known organic, inorganic, or enzymatic processes.

The compounds of the present invention can be prepared in a number of ways well known to those skilled in the art of organic synthesis. By way of example, compounds of the present invention can be synthesized using the methods described in the Schemes below, together with synthetic methods known in the art of synthetic organic chemistry, or variations thereon as

appreciated by those skilled in the art. These methods include but are not limited to those methods described in the Schemes below.

Scheme 1. General synthesis of macrocyclic esters



A general synthesis of macrocyclic esters is outlined in Scheme 1. An appropriately substituted aryl-3-(5-bromo-1-ethyl-1H-indol-3-yl)-2,2-dimethylpropan-1-ol (**1**) can be prepared in three steps starting from protected 3-(5-bromo-2-iodo-1H-indol-3-yl)-2,2-dimethylpropan-1-ol and appropriately substituted boronic acid, including palladium mediated coupling, alkylation, and de-protection reactions.

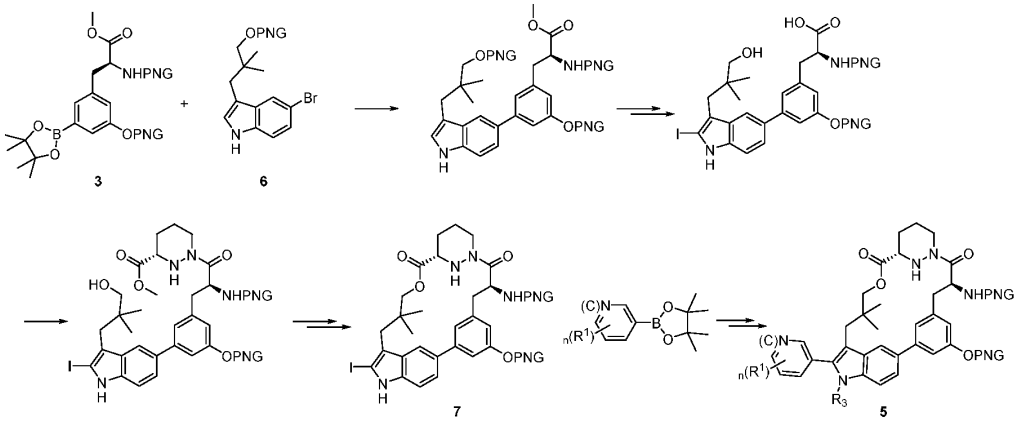
Methyl-amino-hexahydropyridazine-3-carboxylate-boronic ester (**2**) can be prepared in three steps, including protection, iridium catalyst mediated borylation, and coupling with methyl methyl (*S*)-hexahydropyridazine-3-carboxylate.

An appropriately substituted acetylpyrrolidine-3-carbonyl-N-methyl-L-valine (or an alternative aminoacid derivative (**4**) can be made by coupling of methyl-L-valinate and protected

(*S*)-pyrrolidine-3-carboxylic acid, followed by deprotection, coupling with a carboxylic acid containing an appropriately substituted Michael acceptor, and a hydrolysis step.

The final macrocyclic esters can be made by coupling of methyl-amino-hexahydropyridazine-3-carboxylate-boronic ester (**2**) and aryl-3-(5-bromo-1-ethyl-1H-indol-3-yl)-2,2-dimethylpropan-1-ol (**1**) in the presence of a Pd catalyst followed by hydrolysis and macrolactonization steps to result in an appropriately protected macrocyclic intermediate (**5**). Deprotection and coupling with an appropriately substituted intermediate **4** results in a macrocyclic product. Additional deprotection and/or functionalization steps can be required to produce the final compound.

Scheme 2. Alternative general synthesis of macrocyclic esters



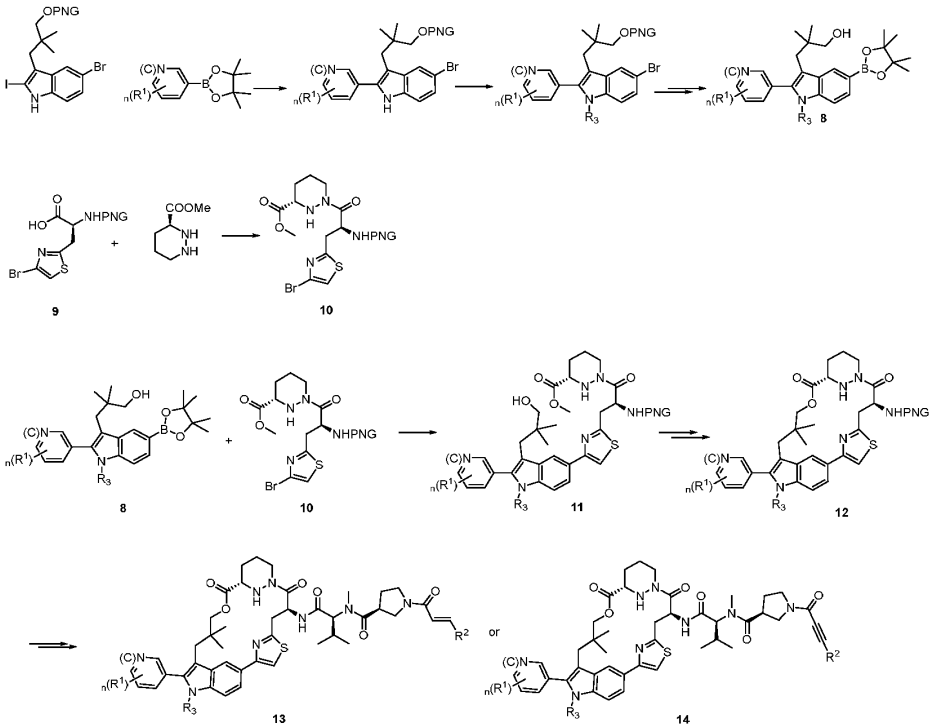
Alternatively, macrocyclic ester can be prepared as described in Scheme 2. An appropriately protected bromo-indolyl (**6**) coupled in the presence of a Pd catalyst with boronic ester (**3**), followed by iodination, deprotection, and ester hydrolysis. Subsequent coupling with methyl (*S*)-hexahydropyridazine-3-carboxylate, followed by hydrolysis and macrolactonization can result in iodo intermediate (**7**). Coupling in the presence of a Pd catalyst with an appropriately substituted boronic ester and alkyllation can yield fully protected macrocycle (**5**). Additional deprotection or functionalization steps are required to produce the final compound.

In addition, compounds of the disclosure can be synthesized using the methods described in the Examples below, together with synthetic methods known in the art of synthetic organic chemistry, or variations thereon as appreciated by those skilled in the art. These methods include but are not limited to those methods described in the Examples below. For example, a person of skill in the art would be able to install into a macrocyclic ester a desired -B-L-W group of a compound of Formula (I), where B, L and W are defined herein, including by using methods exemplified in the Example section herein.

Compounds of Table 1 herein were prepared using methods disclosed herein or were prepared using methods disclosed herein combined with the knowledge of one of skill in the art.

Compounds of Table 2 may be prepared using methods disclosed herein or may be prepared using methods disclosed herein combined with the knowledge of one of skill in the art.

Scheme 3. General synthesis of macrocyclic esters



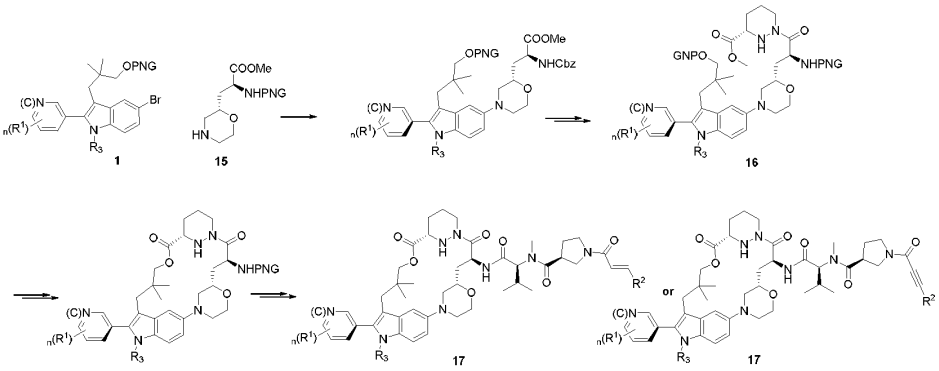
An alternative general synthesis of macrocyclic esters is outlined in Scheme 3. An appropriately substituted indolyl boronic ester (**8**) can be prepared in four steps starting from protected 3-(5-bromo-2-iodo-1H-indol-3-yl)-2,2-dimethylpropan-1-ol and appropriately substituted boronic acid, including Palladium mediated coupling, alkylation, de-protection, and Palladium mediated borylation reactions.

Methyl-amino-3-(4-bromothiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (**10**) can be prepared via coupling of (S)-2-amino-3-(4-bromothiazol-2-yl)propanoic acid (**9**) with methyl (*S*)-hexahydropyridazine-3-carboxylate.

The final macrocyclic esters can be made by coupling of Methyl-amino-3-(4-bromothiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (**10**) and an appropriately substituted indolyl boronic ester (**8**) in the presence of Pd catalyst followed by hydrolysis and macrolactonization steps to result in an appropriately protected macrocyclic intermediate (**11**). Deprotection and coupling

with an appropriately substituted intermediate **4** can result in a macrocyclic product. Additional deprotection or functionalization steps could be required to produce a final compound **13 or 14**.

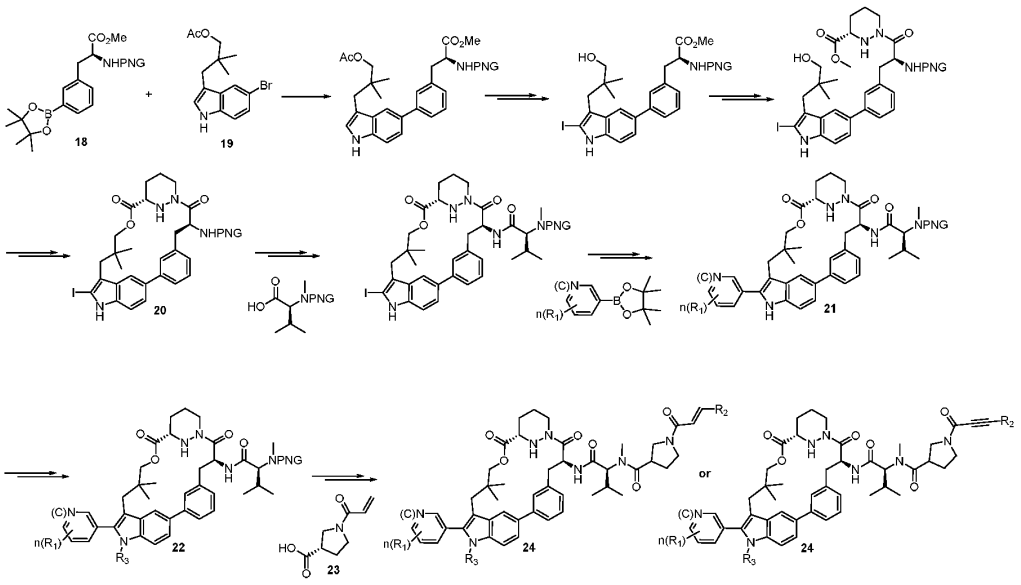
Scheme 4. General synthesis of macrocyclic esters



An alternative general synthesis of macrocyclic esters is outlined in Scheme 4. An appropriately substituted morpholine or an alternative herecyclic intermediate (**15**) can be coupled with appropriately protected Intermediate **1** via Palladium mediated coupling. Subsequent ester hydrolysis, and coupling with piperazoic ester results in intermediate **16**.

The macrocyclic esters can be made by hydrolysis, deprotection and macrocyclization sequence. Subsequent deprotection and coupling with Intermediate **4** (or analogs) result in an appropriately substituted final macrocyclic products. Additional deprotection or functionalization steps could be required to produce a final compound **17**.

Scheme 5. General synthesis of macrocyclic esters



An alternative general synthesis of macrocyclic esters is outlined in Scheme 5. An appropriately substituted macrocycle (**20**) can be prepared starting from an appropriately protected boronic ester **18** and bromo indolyl intermediate (**19**), including Palladium mediated coupling, hydrolysis, coupling with piperazoic ester, hydrolysis, de-protection, and macrocyclizarion steps. Subsequent coupling with an appropriately substituted protected aminoacid followed by palladium mediated coupling yiels intermediate **21**. Additional deprotection and derivatization steps, including alkyllation may be required at this point.

The final macrocyclic esters can be made by coupling of intermediate (**22**) and an appropriately substituted carboxylic acid intermediate (**23**). Additional deprotection or functionalization steps could be required to produce a final compound (**24**).

In addition, compounds of the disclosure can be synthesized using the methods described in the Examples below, together with synthetic methods known in the art of synthetic organic chemistry, or variations thereon as appreciated by those skilled in the art. These methods include but are not limited to those methods described in the Examples below. For example, a person of skill in the art would be able to install into a macrocyclic ester a desired -B-L-W group of a compound of Formula (I), where B, L and W are defined herein, including by using methods exemplified in the Example section herein.

Pharmaceutical Compositions and Methods of Use

Pharmaceutical Compositions and Methods of Administration

The compounds with which the invention is concerned are Ras inhibitors, and are useful in the treatment of cancer. Accordingly, one embodiment of the present invention provides pharmaceutical compositions containing a compound of the invention or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient, as well as methods of using the compounds of the invention to prepare such compositions.

As used herein, the term “pharmaceutical composition” refers to a compound, such as a compound of the present invention, or a pharmaceutically acceptable salt thereof, formulated together with a pharmaceutically acceptable excipient.

In some embodiments, a compound is present in a pharmaceutical composition in unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant population. In some embodiments, pharmaceutical compositions may be specially formulated for administration in solid or liquid form, including those adapted for the following: oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, e.g., those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin, lungs, or oral cavity; intravaginally or

intrarectally, for example, as a pessary, cream, or foam; sublingually; ocularly; transdermally; or nasally, pulmonary, and to other mucosal surfaces.

A "pharmaceutically acceptable excipient," as used herein, refers any inactive ingredient (for example, a vehicle capable of suspending or dissolving the active compound) having the properties of being nontoxic and non-inflammatory in a subject. Typical excipients include, for example: antiadherents, antioxidants, binders, coatings, compression aids, disintegrants, dyes (colors), emollients, emulsifiers, fillers (diluents), film formers or coatings, flavors, fragrances, glidants (flow enhancers), lubricants, preservatives, printing inks, sorbents, suspensing or dispersing agents, sweeteners, or waters of hydration. Excipients include, but are not limited to: butylated optionally substituted hydroxyltoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, crosslinked polyvinyl pyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, optionally substituted hydroxylpropyl cellulose, optionally substituted hydroxylpropyl methylcellulose, lactose, magnesium stearate, maltitol, mannitol, methionine, methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol, polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac, silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E, vitamin C, and xylitol. Those of ordinary skill in the art are familiar with a variety of agents and materials useful as excipients. See, e.g., e.g., Ansel, et al., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems. Philadelphia: Lippincott, Williams & Wilkins, 2004; Gennaro, et al., Remington: The Science and Practice of Pharmacy. Philadelphia: Lippincott, Williams & Wilkins, 2000; and Rowe, Handbook of Pharmaceutical Excipients. Chicago, Pharmaceutical Press, 2005. In some embodiments, a composition includes at least two different pharmaceutically acceptable excipients.

Compounds described herein, whether expressly stated or not, may be provided or utilized in salt form, e.g., a pharmaceutically acceptable salt form, unless expressly stated to the contrary. The term "pharmaceutically acceptable salt," as use herein, refers to those salts of the compounds described herein that are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and other animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, pharmaceutically acceptable salts are described in: Berge et al., *J. Pharmaceutical Sciences* 66:1-19, 1977 and in *Pharmaceutical Salts: Properties, Selection, and Use*, (Eds. P.H. Stahl and C.G. Wermuth), Wiley-VCH, 2008. The salts can be prepared in situ during the final isolation and purification of the compounds described herein or separately by reacting the free base group with a suitable organic acid.

The compounds of the invention may have ionizable groups so as to be capable of preparation as pharmaceutically acceptable salts. These salts may be acid addition salts involving inorganic or organic acids or the salts may, in the case of acidic forms of the compounds of the invention, be prepared from inorganic or organic bases. In some embodiments, the compounds are prepared or used as pharmaceutically acceptable salts prepared as addition products of pharmaceutically acceptable acids or bases. Suitable pharmaceutically acceptable acids and

bases are well-known in the art, such as hydrochloric, sulfuric, hydrobromic, acetic, lactic, citric, or tartaric acids for forming acid addition salts, and potassium hydroxide, sodium hydroxide, ammonium hydroxide, caffeine, various amines, and the like for forming basic salts. Methods for preparation of the appropriate salts are well-established in the art.

Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-optionally substituted hydroxyl-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like.

As used herein, the term “subject” refers to any member of the animal kingdom. In some embodiments, “subject” refers to humans, at any stage of development. In some embodiments, “subject” refers to a human patient. In some embodiments, “subject” refers to non-human animals. In some embodiments, the non-human animal is a mammal (e.g., a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, or a pig). In some embodiments, subjects include, but are not limited to, mammals, birds, reptiles, amphibians, fish, or worms. In some embodiments, a subject may be a transgenic animal, genetically-engineered animal, or a clone.

As used herein, the term “dosage form” refers to a physically discrete unit of a compound (e.g., a compound of the present invention) for administration to a subject. Each unit contains a predetermined quantity of compound. In some embodiments, such quantity is a unit dosage amount (or a whole fraction thereof) appropriate for administration in accordance with a dosing regimen that has been determined to correlate with a desired or beneficial outcome when administered to a relevant population (i.e., with a therapeutic dosing regimen). Those of ordinary skill in the art appreciate that the total amount of a therapeutic composition or compound administered to a particular subject is determined by one or more attending physicians and may involve administration of multiple dosage forms.

As used herein, the term “dosing regimen” refers to a set of unit doses (typically more than one) that are administered individually to a subject, typically separated by periods of time. In some embodiments, a given therapeutic compound (e.g., a compound of the present invention) has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which are separated from one another by a time period of the same length; in some embodiments, a dosing regimen comprises a plurality of doses and at least two different time periods separating individual doses. In some embodiments, all doses within a dosing regimen are of the same unit dose amount. In some embodiments,

different doses within a dosing regimen are of different amounts. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount different from the first dose amount. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount same as the first dose amount. In some embodiments, a dosing regimen is correlated with a desired or beneficial outcome when administered across a relevant population (i.e., is a therapeutic dosing regimen).

A "therapeutic regimen" refers to a dosing regimen whose administration across a relevant population is correlated with a desired or beneficial therapeutic outcome.

The term "treatment" (also "treat" or "treating"), in its broadest sense, refers to any administration of a substance (e.g., a compound of the present invention) that partially or completely alleviates, ameliorates, relieves, inhibits, delays onset of, reduces severity of, or reduces incidence of one or more symptoms, features, or causes of a particular disease, disorder, or condition. In some embodiments, such treatment may be administered to a subject who does not exhibit signs of the relevant disease, disorder or condition or of a subject who exhibits only early signs of the disease, disorder, or condition. Alternatively, or additionally, in some embodiments, treatment may be administered to a subject who exhibits one or more established signs of the relevant disease, disorder, or condition. In some embodiments, treatment may be of a subject who has been diagnosed as suffering from the relevant disease, disorder, or condition. In some embodiments, treatment may be of a subject known to have one or more susceptibility factors that are statistically correlated with increased risk of development of the relevant disease, disorder, or condition.

The term "therapeutically effective amount" means an amount that is sufficient, when administered to a population suffering from or susceptible to a disease, disorder, or condition in accordance with a therapeutic dosing regimen, to treat the disease, disorder, or condition. In some embodiments, a therapeutically effective amount is one that reduces the incidence or severity of, or delays onset of, one or more symptoms of the disease, disorder, or condition. Those of ordinary skill in the art will appreciate that the term "therapeutically effective amount" does not in fact require successful treatment be achieved in a particular individual. Rather, a therapeutically effective amount may be that amount that provides a particular desired pharmacological response in a significant number of subjects when administered to patients in need of such treatment. It is specifically understood that particular subjects may, in fact, be "refractory" to a "therapeutically effective amount." In some embodiments, reference to a therapeutically effective amount may be a reference to an amount as measured in one or more specific tissues (e.g., a tissue affected by the disease, disorder or condition) or fluids (e.g., blood, saliva, serum, sweat, tears, urine). Those of ordinary skill in the art will appreciate that, in some embodiments, a therapeutically effective amount may be formulated or administered in a single dose. In some embodiments, a therapeutically effective amount may be formulated or administered in a plurality of doses, for example, as part of a dosing regimen.

For use as treatment of subjects, the compounds of the invention, or a pharmaceutically acceptable salt thereof, can be formulated as pharmaceutical or veterinary compositions.

Depending on the subject to be treated, the mode of administration, and the type of treatment desired, e.g., prevention, prophylaxis, or therapy, the compounds, or a pharmaceutically acceptable salt thereof, are formulated in ways consonant with these parameters. A summary of such techniques may be found in *Remington: The Science and Practice of Pharmacy*, *21st Edition*, Lippincott Williams & Wilkins, (2005); and *Encyclopedia of Pharmaceutical Technology*, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York, each of which is incorporated herein by reference.

Compositions can be prepared according to conventional mixing, granulating or coating methods, respectively, and the present pharmaceutical compositions can contain from about 0.1% to about 99%, from about 5% to about 90%, or from about 1% to about 20% of a compound of the present invention, or pharmaceutically acceptable salt thereof, by weight or volume. In some embodiments, compounds, or a pharmaceutically acceptable salt thereof, described herein may be present in amounts totaling 1-95% by weight of the total weight of a composition, such as a pharmaceutical composition.

The composition may be provided in a dosage form that is suitable for intraarticular, oral, parenteral (e.g., intravenous, intramuscular), rectal, cutaneous, subcutaneous, topical, transdermal, sublingual, nasal, vaginal, intravesicular, intraurethral, intrathecal, epidural, aural, or ocular administration, or by injection, inhalation, or direct contact with the nasal, genitourinary, reproductive or oral mucosa. Thus, the pharmaceutical composition may be in the form of, e.g., tablets, capsules, pills, powders, granulates, suspensions, emulsions, solutions, gels including hydrogels, pastes, ointments, creams, plasters, drenches, osmotic delivery devices, suppositories, enemas, injectables, implants, sprays, preparations suitable for iontophoretic delivery, or aerosols. The compositions may be formulated according to conventional pharmaceutical practice.

As used herein, the term “administration” refers to the administration of a composition (e.g., a compound, or a preparation that includes a compound as described herein) to a subject or system. Administration to an animal subject (e.g., to a human) may be by any appropriate route. For example, in some embodiments, administration may be bronchial (including by bronchial instillation), buccal, enteral, interdermal, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intravenous, intraventricular, mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (including by intratracheal instillation), transdermal, vaginal, or vitreal.

Formulations may be prepared in a manner suitable for systemic administration or topical or local administration. Systemic formulations include those designed for injection (e.g., intramuscular, intravenous or subcutaneous injection) or may be prepared for transdermal, transmucosal, or oral administration. A formulation will generally include a diluent as well as, in some cases, adjuvants, buffers, preservatives and the like. Compounds, or a pharmaceutically acceptable salt thereof, can be administered also in liposomal compositions or as microemulsions.

For injection, formulations can be prepared in conventional forms as liquid solutions or suspensions or as solid forms suitable for solution or suspension in liquid prior to injection or as emulsions. Suitable excipients include, for example, water, saline, dextrose, glycerol and the like. Such compositions may also contain amounts of nontoxic auxiliary substances such as wetting or

emulsifying agents, pH buffering agents and the like, such as, for example, sodium acetate, sorbitan monolaurate, and so forth.

Various sustained release systems for drugs have also been devised. See, for example, U.S. Patent No. 5,624,677.

Systemic administration may also include relatively noninvasive methods such as the use of suppositories, transdermal patches, transmucosal delivery and intranasal administration. Oral administration is also suitable for compounds of the invention, or a pharmaceutically acceptable salt thereof. Suitable forms include syrups, capsules, and tablets, as is understood in the art.

Each compound, or a pharmaceutically acceptable salt thereof, as described herein, may be formulated in a variety of ways that are known in the art. For example, the first and second agents of the combination therapy may be formulated together or separately. Other modalities of combination therapy are described herein.

The individually or separately formulated agents can be packaged together as a kit. Non-limiting examples include, but are not limited to, kits that contain, e.g., two pills, a pill and a powder, a suppository and a liquid in a vial, two topical creams, etc. The kit can include optional components that aid in the administration of the unit dose to subjects, such as vials for reconstituting powder forms, syringes for injection, customized IV delivery systems, inhalers, etc. Additionally, the unit dose kit can contain instructions for preparation and administration of the compositions. The kit may be manufactured as a single use unit dose for one subject, multiple uses for a particular subject (at a constant dose or in which the individual compounds, or a pharmaceutically acceptable salt thereof, may vary in potency as therapy progresses); or the kit may contain multiple doses suitable for administration to multiple subjects ("bulk packaging"). The kit components may be assembled in cartons, blister packs, bottles, tubes, and the like.

Formulations for oral use include tablets containing the active ingredient(s) in a mixture with non-toxic pharmaceutically acceptable excipients. These excipients may be, for example, inert diluents or fillers (e.g., sucrose, sorbitol, sugar, mannitol, microcrystalline cellulose, starches including potato starch, calcium carbonate, sodium chloride, lactose, calcium phosphate, calcium sulfate, or sodium phosphate); granulating and disintegrating agents (e.g., cellulose derivatives including microcrystalline cellulose, starches including potato starch, croscarmellose sodium, alginates, or alginic acid); binding agents (e.g., sucrose, glucose, sorbitol, acacia, alginic acid, sodium alginate, gelatin, starch, pregelatinized starch, microcrystalline cellulose, magnesium aluminum silicate, carboxymethylcellulose sodium, methylcellulose, optionally substituted hydroxylpropyl methylcellulose, ethylcellulose, polyvinylpyrrolidone, or polyethylene glycol); and lubricating agents, glidants, and antiadhesives (e.g., magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc). Other pharmaceutically acceptable excipients can be colorants, flavoring agents, plasticizers, humectants, buffering agents, and the like.

Two or more compounds may be mixed together in a tablet, capsule, or other vehicle, or may be partitioned. In one example, the first compound is contained on the inside of the tablet, and the second compound is on the outside, such that a substantial portion of the second compound is released prior to the release of the first compound.

Formulations for oral use may also be provided as chewable tablets, or as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent (e.g., potato starch, lactose, microcrystalline cellulose, calcium carbonate, calcium phosphate or kaolin), or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil. Powders, granulates, and pellets may be prepared using the ingredients mentioned above under tablets and capsules in a conventional manner using, e.g., a mixer, a fluid bed apparatus or a spray drying equipment.

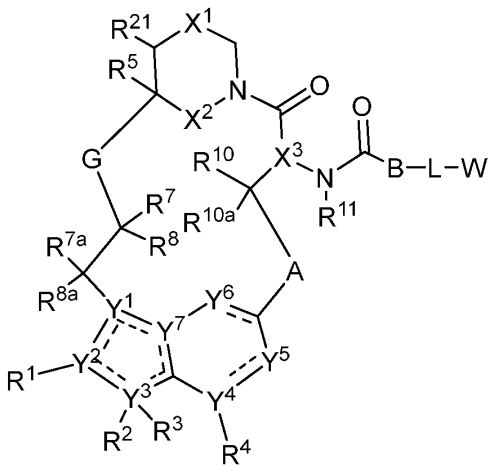
Dissolution or diffusion-controlled release can be achieved by appropriate coating of a tablet, capsule, pellet, or granulate formulation of compounds, or by incorporating the compound, or a pharmaceutically acceptable salt thereof, into an appropriate matrix. A controlled release coating may include one or more of the coating substances mentioned above or, e.g., shellac, beeswax, glycowax, castor wax, carnauba wax, stearyl alcohol, glyceryl monostearate, glyceryl distearate, glycerol palmitostearate, ethylcellulose, acrylic resins, dl-polylactic acid, cellulose acetate butyrate, polyvinyl chloride, polyvinyl acetate, vinyl pyrrolidone, polyethylene, polymethacrylate, methylmethacrylate, 2-optionally substituted hydroxylmethacrylate, methacrylate hydrogels, 1,3 butylene glycol, ethylene glycol methacrylate, or polyethylene glycols. In a controlled release matrix formulation, the matrix material may also include, e.g., hydrated methylcellulose, carnauba wax and stearyl alcohol, carbopol 934, silicone, glyceryl tristearate, methyl acrylate-methyl methacrylate, polyvinyl chloride, polyethylene, or halogenated fluorocarbon.

The liquid forms in which the compounds, or a pharmaceutically acceptable salt thereof, and compositions of the present invention can be incorporated for administration orally include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Generally, when administered to a human, the oral dosage of any of the compounds of the invention, or a pharmaceutically acceptable salt thereof, will depend on the nature of the compound, and can readily be determined by one skilled in the art. A dosage may be, for example, about 0.001 mg to about 2000 mg per day, about 1 mg to about 1000 mg per day, about 5 mg to about 500 mg per day, about 100 mg to about 1500 mg per day, about 500 mg to about 1500 mg per day, about 500 mg to about 2000 mg per day, or any range derivable therein.

Numbered Embodiments

[1] A compound, or pharmaceutically acceptable salt thereof, having the structure of Formula I:



Formula I

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 10-membered heteroarylene;

B is absent, -CH(R^9)-, >C=C$R^9R^{9'}$, or >C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', $S(O)_2$R', or $S(O)_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is H, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl; or

R^9 and $R^{9'}$, combined with the atoms to which they are attached, form a 3 to 6-membered cycloalkyl or a 3 to 6-membered heterocycloalkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

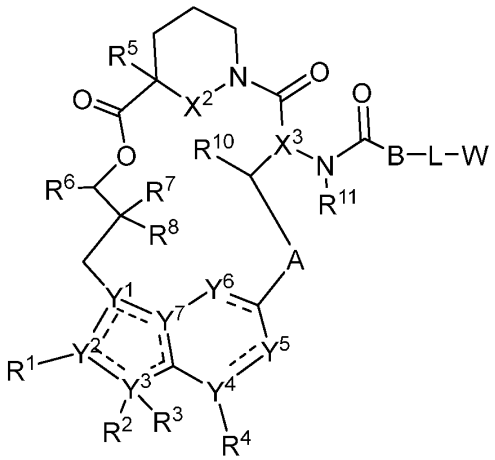
R^{10a} is hydrogen or halo;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{21} is H or C_1-C_3 alkyl.

[2] The compound, or pharmaceutically acceptable salt thereof, of paragraph [1], wherein G is optionally substituted C_1-C_4 heteroalkylene.

[3] The compound, or pharmaceutically acceptable salt thereof, of paragraph [1] or [2], wherein the compound has the structure of Formula Ic:



Formula Ic

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', $C(O)N(R')_2$, S(O)R', $S(O)_2R'$, or $S(O)_2N(R')_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), $C=N(O\text{-}C_1\text{-}C_3$ alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl; and

R^{11} is hydrogen or C_1-C_3 alkyl.

[4] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [3], wherein X^2 is NH.

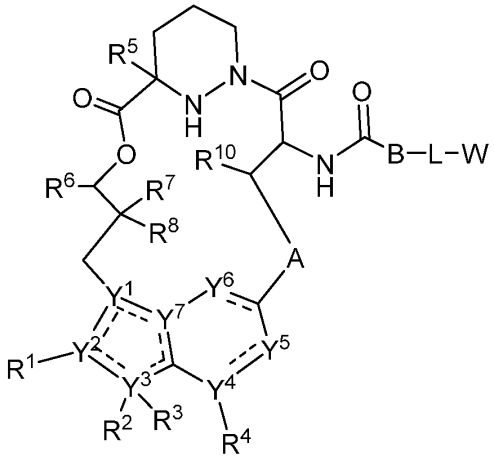
[5] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [4], wherein X^3 is CH.

[6] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [5], wherein R^{11} is hydrogen.

[7] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [5], wherein R^{11} is C_1-C_3 alkyl.

[8] The compound, or pharmaceutically acceptable salt thereof, of paragraph [7], wherein R^{11} is methyl.

[9] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [6], wherein the compound has the structure of Formula Id:



Formula Id

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form C=C$R^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; and

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl.

[10] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [9] wherein X^1 is optionally substituted C_1-C_2 alkylene.

[11] The compound, or pharmaceutically acceptable salt thereof, of paragraph [10], wherein X^1 is methylene.

[12] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [11], wherein R^5 is hydrogen.

[13] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [11], wherein R^5 is C_1-C_4 alkyl optionally substituted with halogen.

[14] The compound, or pharmaceutically acceptable salt thereof, of paragraph [13], wherein R^5 is methyl.

[15] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [14], wherein Y^4 is C.

[16] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [15], wherein R^4 is hydrogen.

[17] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [16], wherein Y^5 is CH.

[18] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [17], wherein Y^6 is CH.

[19] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [18], wherein Y^1 is C.

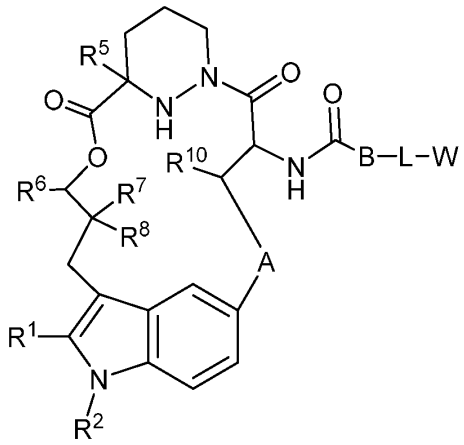
[20] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [19] wherein Y^2 is C.

[21] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [20] wherein Y^3 is N.

[22] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [21], wherein R^3 is absent.

[23] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [22], wherein Y^7 is C.

[24] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [6] or [9] to [23], wherein the compound has the structure of Formula Ie:



Formula Ie

wherein A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; and

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl.

[25] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [3] to [24], wherein R^6 is hydrogen.

[26] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [25], wherein R^2 is hydrogen, cyano, optionally substituted C_1-C_6 alkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 6-membered heterocycloalkyl.

[27] The compound, or pharmaceutically acceptable salt thereof, of paragraph [26], wherein R^2 is optionally substituted C_1-C_6 alkyl.

[28] The compound, or pharmaceutically acceptable salt thereof, of paragraph [27], wherein R^2 is ethyl.

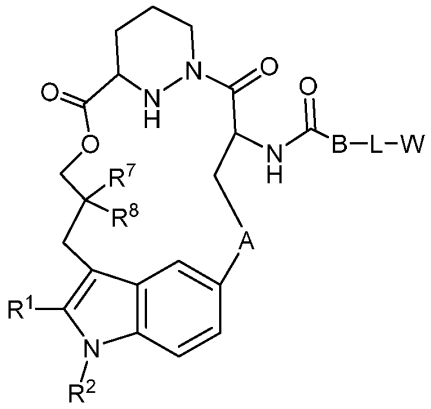
[29] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [28], wherein R^7 is optionally substituted C_1-C_3 alkyl.

[30] The compound, or pharmaceutically acceptable salt thereof, of paragraph 29, wherein R^7 is C_1-C_3 alkyl.

[31] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to 30, wherein R^8 is optionally substituted C_1-C_3 alkyl.

[32] The compound, or pharmaceutically acceptable salt thereof, of paragraph [31], wherein R^8 is C_1-C_3 alkyl.

[33] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [32], wherein the compound has the structure of Formula If:



Formula If

wherein A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is C_1-C_6 alkyl or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

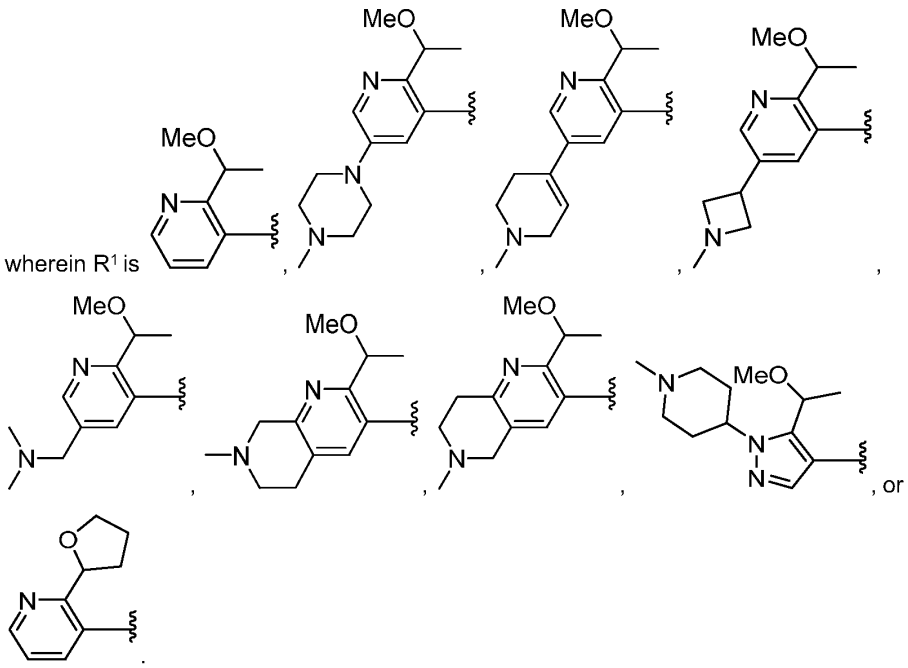
R^8 is C_1-C_3 alkyl; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

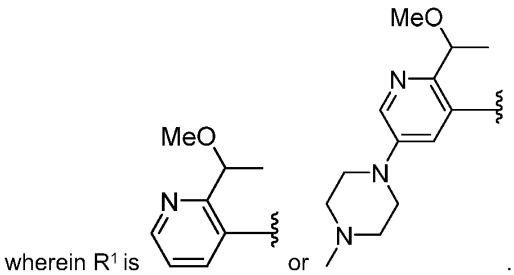
[34] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [33], wherein R^1 is optionally substituted 6 to 10-membered aryl, optionally substituted 3 to 6-membered cycloalkenyl, or optionally substituted 5 to 10-membered heteroaryl.

[35] The compound, or pharmaceutically acceptable salt thereof, of paragraph [34], wherein R^1 is optionally substituted 6-membered aryl, optionally substituted 6-membered cycloalkenyl, or optionally substituted 6-membered heteroaryl.

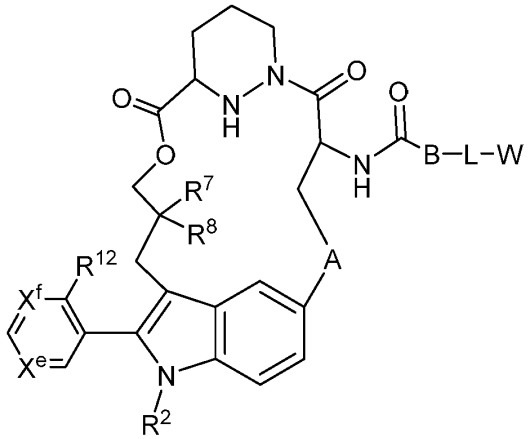
[36] The compound, or pharmaceutically acceptable salt thereof, of paragraph [35],

wherein R^1 is 

[37] The compound, or pharmaceutically acceptable salt thereof, of paragraph [36],

wherein R^1 is  or .

[38] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [37], wherein the compound has the structure of Formula Ig:



Formula Ig

wherein A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

R^2 is C_1-C_6 alkyl, C_1-C_6 fluoroalkyl, or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

R^8 is C_1-C_3 alkyl; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl

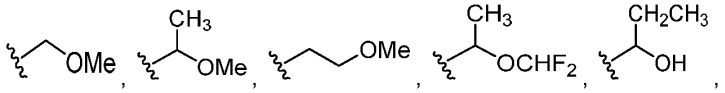
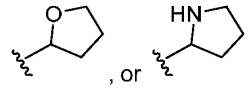
X^e and X^f are, independently, N or CH; and

R^{12} is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

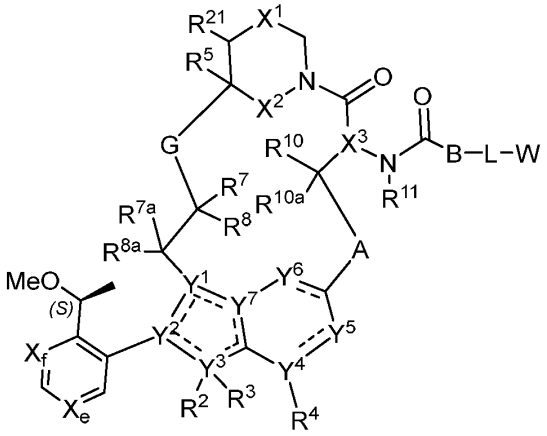
[39] The compound, or pharmaceutically acceptable salt thereof, of paragraph [38], wherein X^e is N and X^f is CH.

[40] The compound, or pharmaceutically acceptable salt thereof, of paragraph [38], wherein X^e is CH and X^f is N.

[41] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [38] to [40], wherein R^{12} is optionally substituted C_1-C_6 heteroalkyl.

[42] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [38] to [41], wherein R^{12} is  ,  , or .

[43] The compound, or pharmaceutically acceptable salt thereof, of paragraph [1] or [2], wherein the compound has the structure of Formula VI:



Formula VI

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 10-membered heteroarylene;

B is absent, -CH(R^9)-, >C=C$R^9R^{9'}$, or >C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, a haloacetal, or an alkynyl sulfone;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or S(O)$_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is H, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl; or

R^9 and $R^{9'}$, combined with the atoms to which they are attached, form a 3 to 6-membered cycloalkyl or a 3 to 6-membered heterocycloalkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

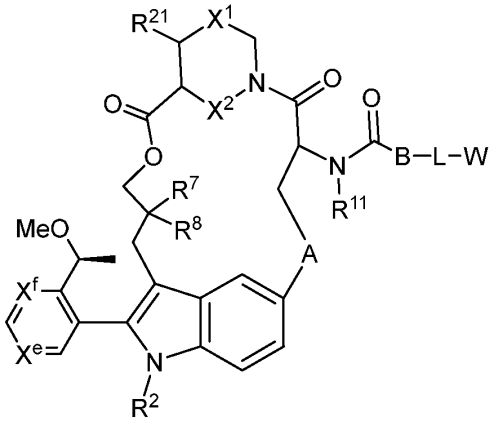
R^{10a} is hydrogen or halo;

R^{11} is hydrogen or C_1-C_3 alkyl;

R^{21} is hydrogen or C_1-C_3 alkyl (e.g., methyl); and

X^e and X^f are, independently, N or CH.

[44] The compound, or pharmaceutically acceptable salt thereof, of paragraph [43], wherein the compound has the structure of Formula VIa:



Formula VIa

wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', $C(O)N(R')_2$, S(O)R', $S(O)_2R'$, or $S(O)_2N(R')_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

R^2 is C_1-C_6 alkyl, C_1-C_6 fluoroalkyl, or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

R^8 is C_1-C_3 alkyl; and

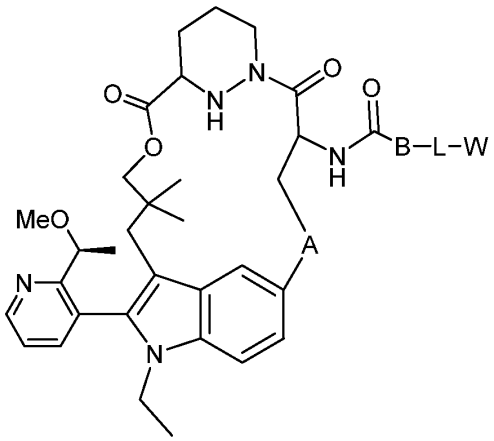
R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

X^e and X^f are, independently, N or CH;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{21} is hydrogen or C_1-C_3 alkyl.

[45] The compound, or pharmaceutically acceptable salt thereof, of paragraph [43] or [44], wherein the compound has the structure of Formula VIb:



Formula VIb

wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

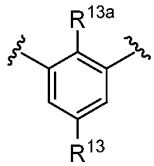
R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

L is absent or a linker; and

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone.

[46] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [45], wherein A is optionally substituted 6-membered arylene.

[47] The compound, or pharmaceutically acceptable salt thereof, of paragraph [46], wherein A has the structure:



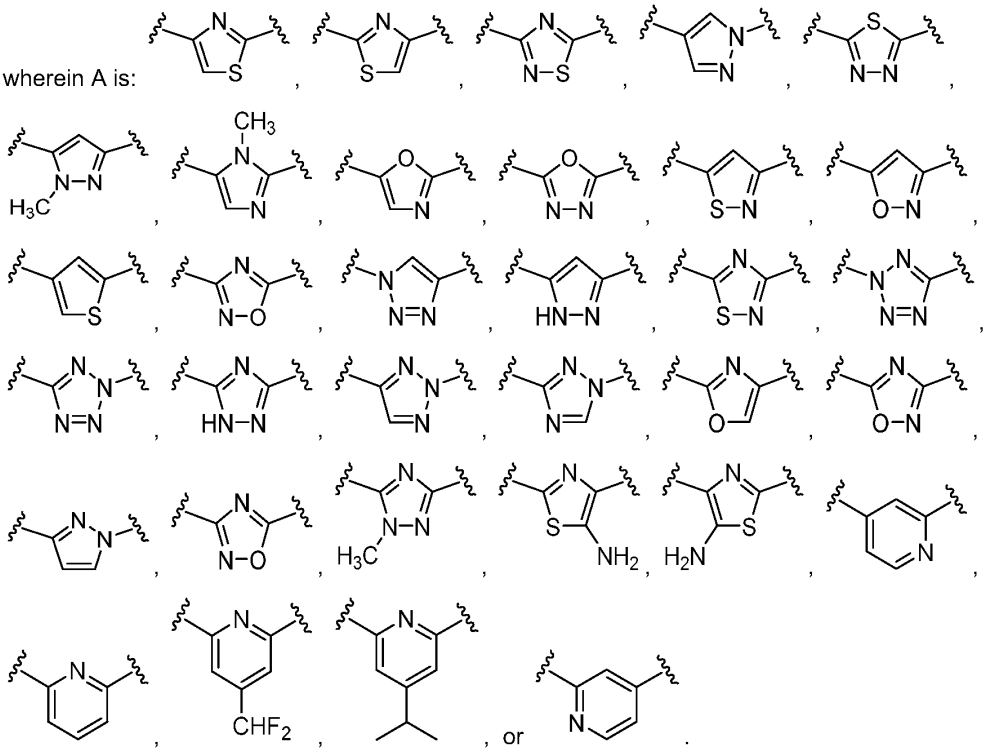
wherein R^{13} is hydrogen, halo, hydroxy, amino, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl; and

R^{13a} is hydrogen or halo.

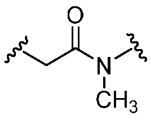
[48] The compound, or pharmaceutically acceptable salt thereof, of paragraph [47], wherein R^{13} and R^{13a} are each hydrogen.

[49] The compound, or pharmaceutically acceptable salt thereof, of paragraph [47], wherein R^{13} is hydroxy, methyl, fluoro, or difluoromethyl.

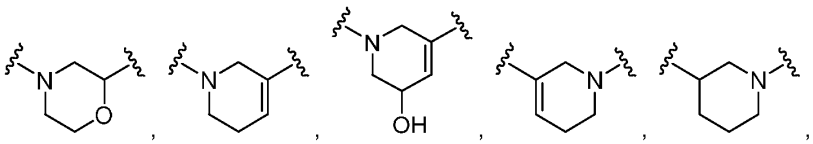
[50] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [45], wherein A is optionally substituted 5 to 6-membered heteroarylene.

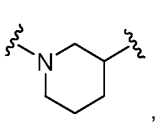
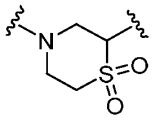
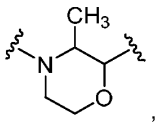
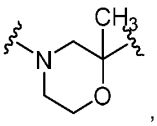
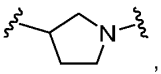
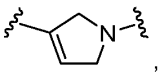
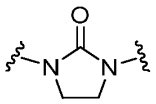
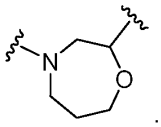
[51] The compound, or pharmaceutically acceptable salt thereof, of paragraph [50], wherein A is: 

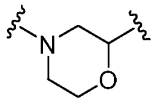
[52] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [45], wherein A is optionally substituted C_1-C_4 heteroalkylene.

[53] The compound, or pharmaceutically acceptable salt thereof, of paragraph [52], wherein A is:  .

[54] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [45], wherein A is optionally substituted 3 to 6-membered heterocycloalkylene.

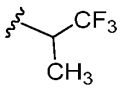
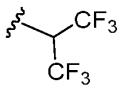
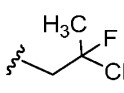
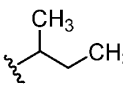
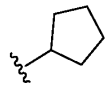
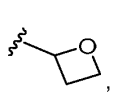
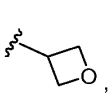
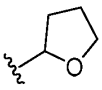
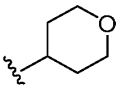
[55] The compound, or pharmaceutically acceptable salt thereof, of paragraph [54], wherein A is: 

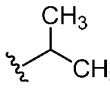







, or  .

[56] The compound, or pharmaceutically acceptable salt thereof, of paragraph [55], wherein A is  .

[57] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [56], wherein B is -CHR^9-.

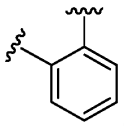
[58] The compound, or pharmaceutically acceptable salt thereof, of paragraph [57], wherein R^9 is F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

[59] The compound, or pharmaceutically acceptable salt thereof, of paragraph [58], wherein R^9 is: , , , , , , ,
 ,  ,  ,  , , ,  ,  ,
 ,  , or  .

[60] The compound, or pharmaceutically acceptable salt thereof, of paragraph [59], wherein R^9 is:  .

[61] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [56], wherein B is optionally substituted 6-membered arylene.

[62] The compound, or pharmaceutically acceptable salt thereof, of paragraph [61], wherein B is 6-membered arylene.

[63] The compound, or pharmaceutically acceptable salt thereof, of paragraph [61], wherein B is:  .

[64] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [63], wherein R^7 is methyl.

[65] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [64], wherein R^8 is methyl.

[66] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [65], wherein the linker is the structure of Formula II:

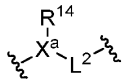
$$A^1\text{-}(B^1)_f\text{-}(C^1)_g\text{-}(B^2)_h\text{-}(D^1)\text{-}(B^3)_i\text{-}(C^2)_j\text{-}(B^4)_k\text{–}A^2$$

Formula II

where A^1 is a bond between the linker and B; A^2 is a bond between W and the linker; B^1, B^2, B^3, and B^4 each, independently, is selected from optionally substituted C_1-C_2 alkylene, optionally substituted C_1-C_3 heteroalkylene, O, S, and NR^N; R^N is hydrogen, optionally substituted C_1–C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted C_1-C_7 heteroalkyl; C^1 and C^2 are each, independently, selected from carbonyl, thiocarbonyl, sulphonyl, or phosphoryl; f, g, h, i, j, and k are each, independently, 0 or 1; and D^1 is optionally substituted C_1-C_{10} alkylene, optionally substituted C_2-C_{10} alkenylene, optionally substituted C_2-C_{10} alkynylene, optionally substituted 3 to 14-membered heterocycloalkylene, optionally substituted 5 to 10-membered heteroarylene, optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 6 to 10-membered arylene, optionally substituted C_2-C_{10} polyethylene glycolene, or optionally substituted C_1-C_{10} heteroalkylene, or a chemical bond linking $A^1\text{-}(B^1)_f\text{-}(C^1)_g\text{-}(B^2)_h$- to -$(B^3)_i\text{-}(C^2)_j\text{-}(B^4)_k\text{–}A^2$.

[67] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [66], wherein the linker is acyclic.

[68] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [67], wherein the linker has the structure of Formula IIa:



Formula IIa

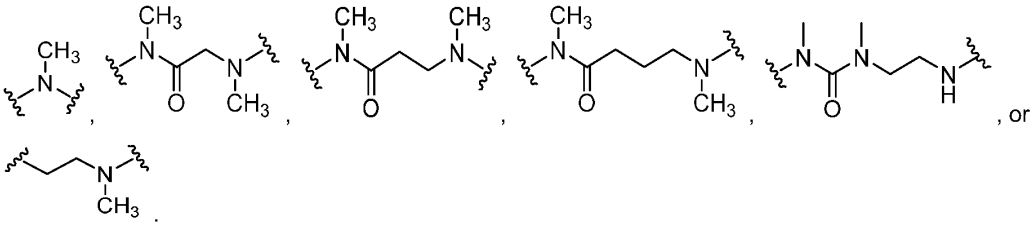
wherein X^a is absent or N;

R^{14} is absent, hydrogen or optionally substituted C_1-C_6 alkyl; and

L^2 is absent, -SO_2-, optionally substituted C_1-C_4 alkylene or optionally substituted C_1-C_4 heteroalkylene,

wherein at least one of X^a, R^{14}, or L^2 is present.

[69] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [68], wherein the linker has the structure:

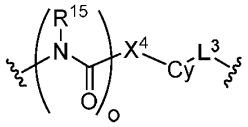


, or

.

[70] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [66], wherein the linker is or comprises a cyclic moiety.

[71] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [70], wherein the linker has the structure of Formula IIb:



Formula IIb

wherein o is 0 or 1;

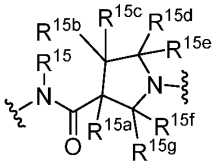
R^{15} is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted 3 to 8-membered cycloalkylene, or optionally substituted 3 to 8-membered heterocycloalkylene;

X^4 is absent, optionally substituted C_1-C_4 alkylene, O, NCH_3, or optionally substituted C_1-C_4 heteroalkylene;

Cy is optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 3 to 8-membered heterocycloalkylene, optionally substituted 6-10 membered arylene, or optionally substituted 5 to 10-membered heteroarylene; and

L^3 is absent, -SO_2-, optionally substituted C_1-C_4 alkylene or optionally substituted C_1-C_4 heteroalkylene.

[72] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [71], wherein the linker has the structure:

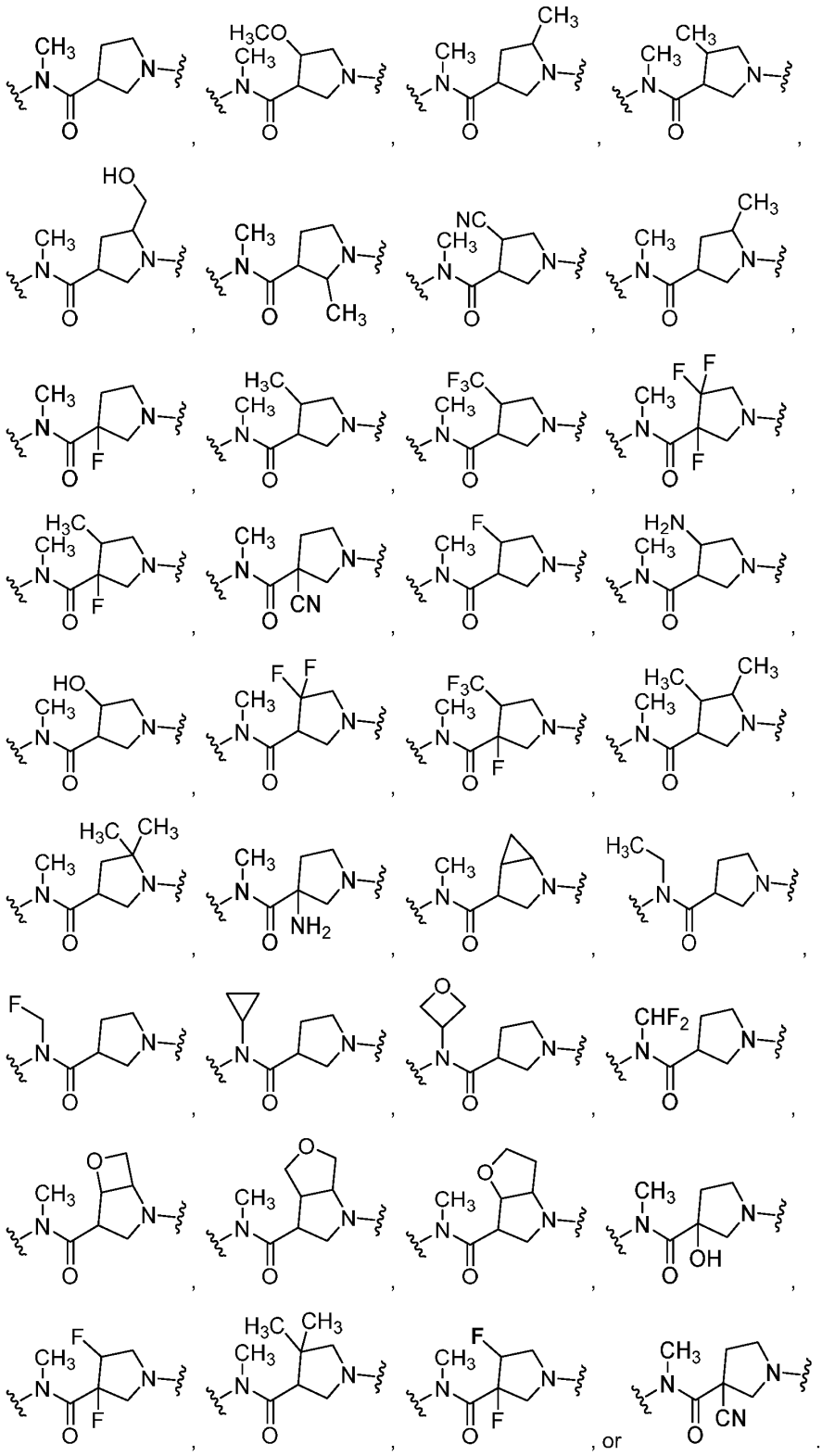


Formula IIc

wherein R^{15} is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted 3 to 8-membered cycloalkylene, or optionally substituted 3 to 8-membered heterocycloalkylene; and

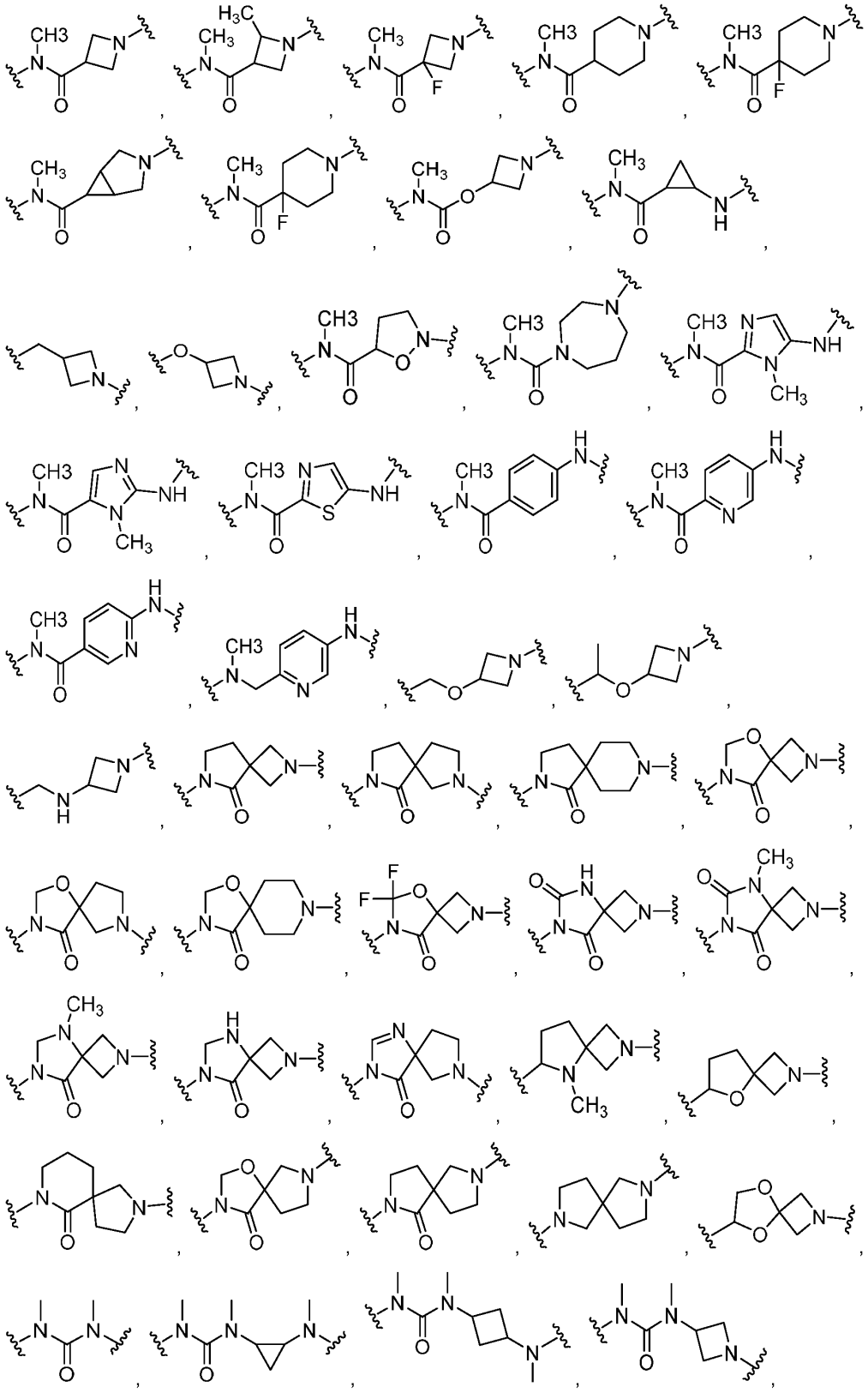
R^{15a}, R^{15b}, R^{15c}, R^{15d}, R^{15e}, R^{15f}, and R^{15g} are, independently, hydrogen, halo, hydroxy, cyano, amino, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 alkoxy, or , or R^{15b} and R^{15d} combine with the carbons to which they are attached to form an optionally substituted 3 to 8-membered cycloalkylene, or optionally substituted 3 to 8-membered heterocycloalkylene.

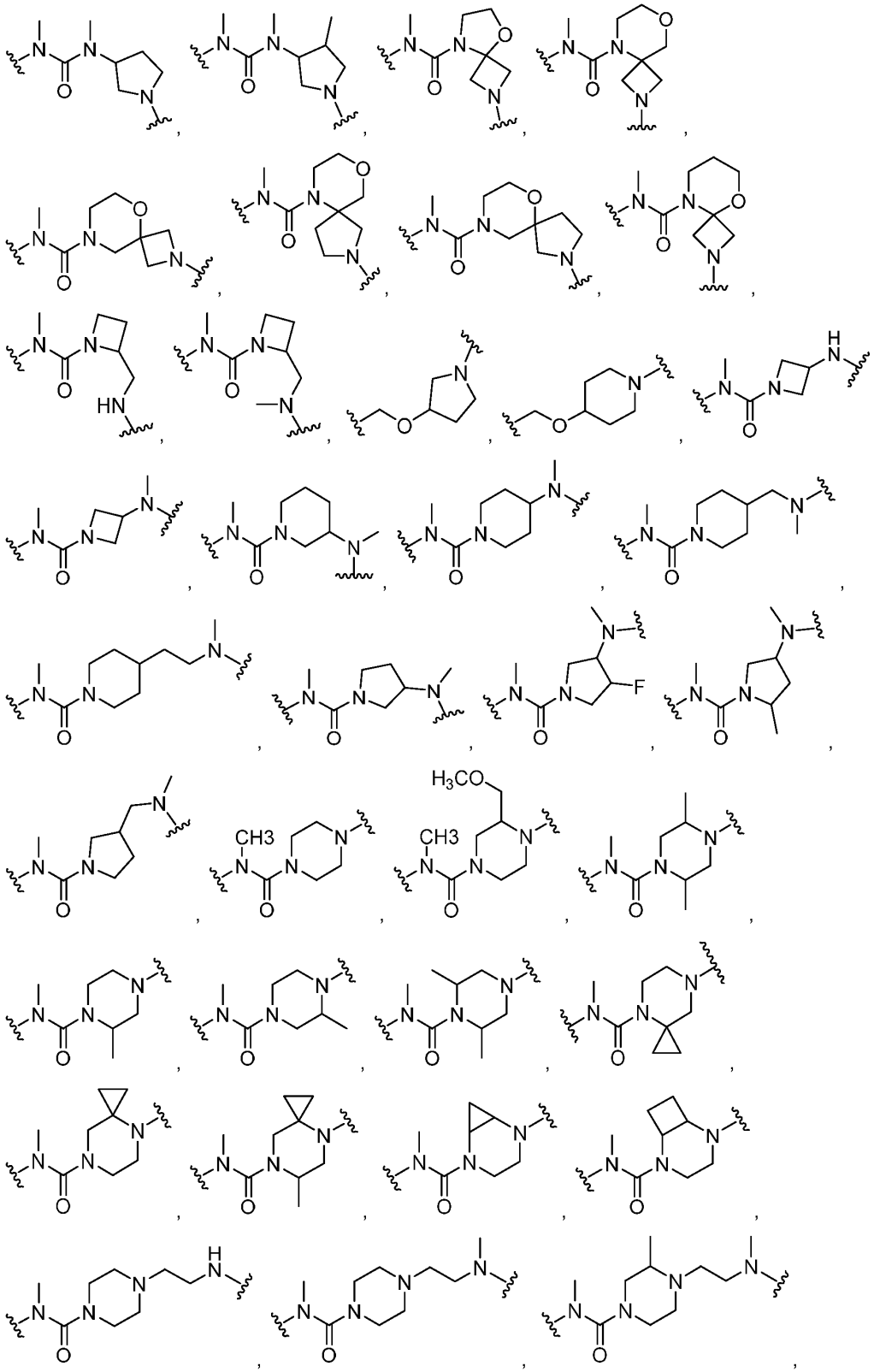
[73] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [72], wherein the linker has the structure:

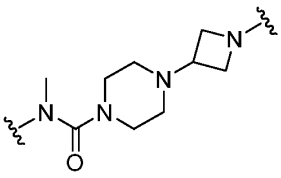
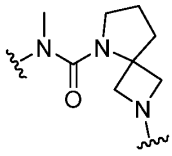
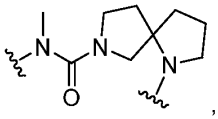
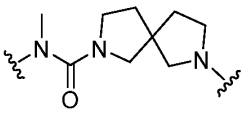
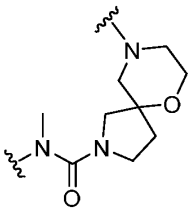
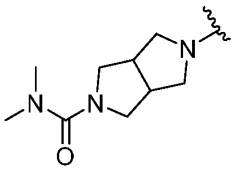
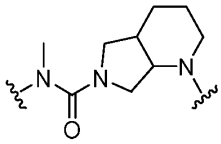
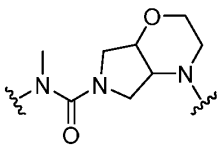
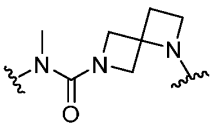
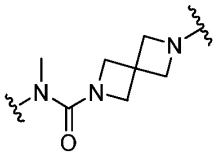
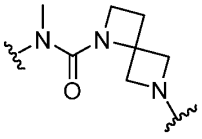
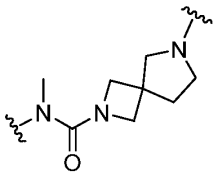
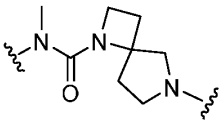
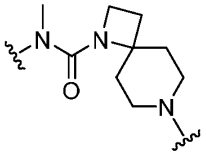
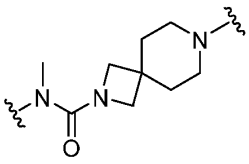
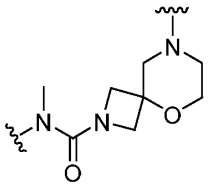
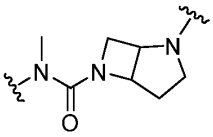
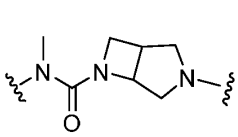
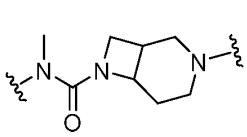
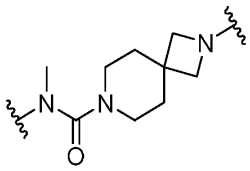
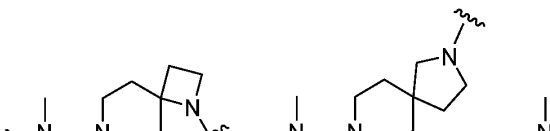
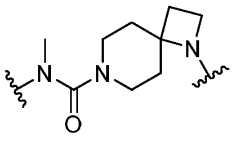
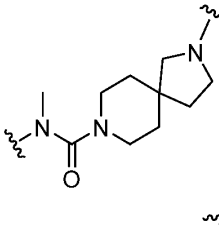
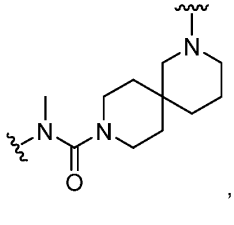
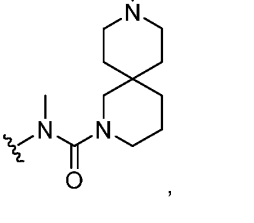
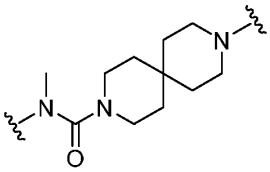
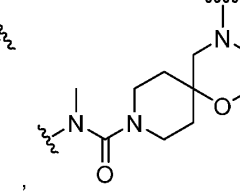
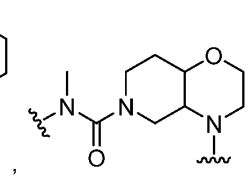
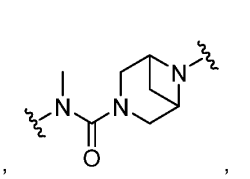


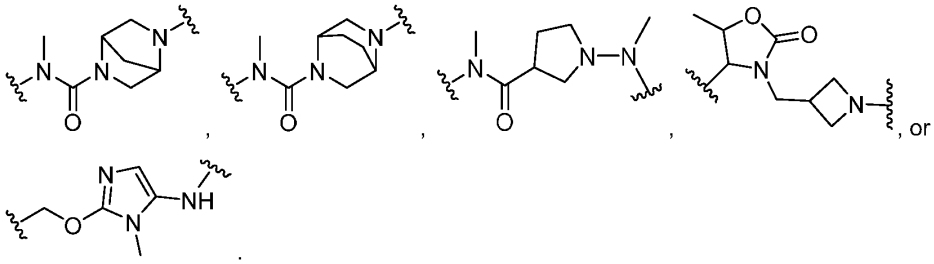
, or .

[74] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [71], wherein the linker has the structure:



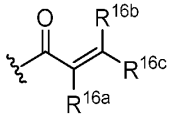




[75] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [74], wherein W is a cross-linking group comprising a vinyl ketone.

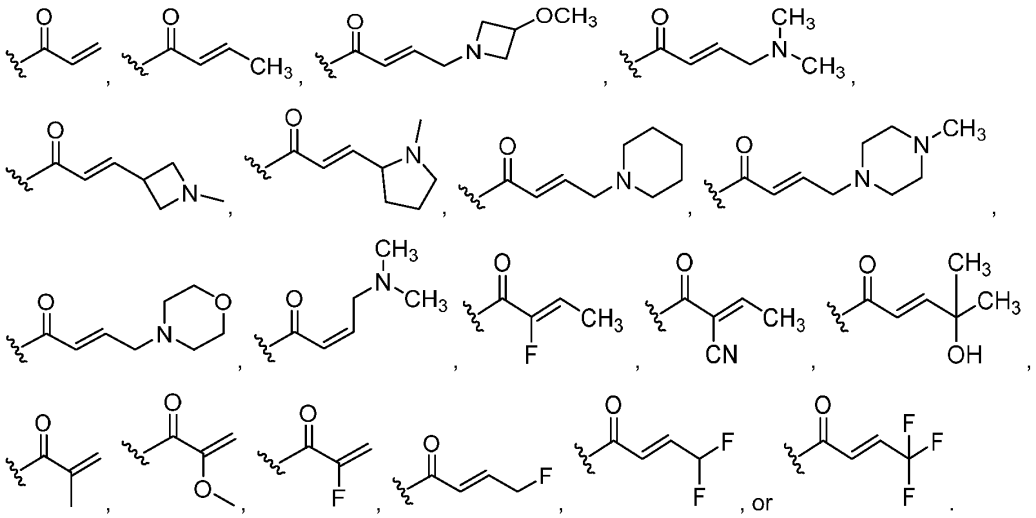
[76]. The compound, or a pharmaceutically acceptable salt thereof, of paragraph [75], wherein W has the structure of Formula IIIa:



Formula IIIa

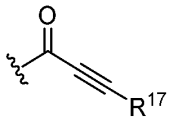
wherein R^{16a}, R^{16b}, and R^{16c} are, independently, hydrogen, -CN, halogen, or -C_1-C_3 alkyl optionally substituted with one or more substituents independently selected from -OH, -O-C_1-C_3 alkyl,
-NH_2, -NH(C_1-C_3 alkyl), -N(C_1-C_3 alkyl)$_2$, or a 4 to 7-membered saturated heterocycloalkyl.

[77] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [76], wherein W is:



[78] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [74], wherein W is a cross-linking group comprising an ynone.

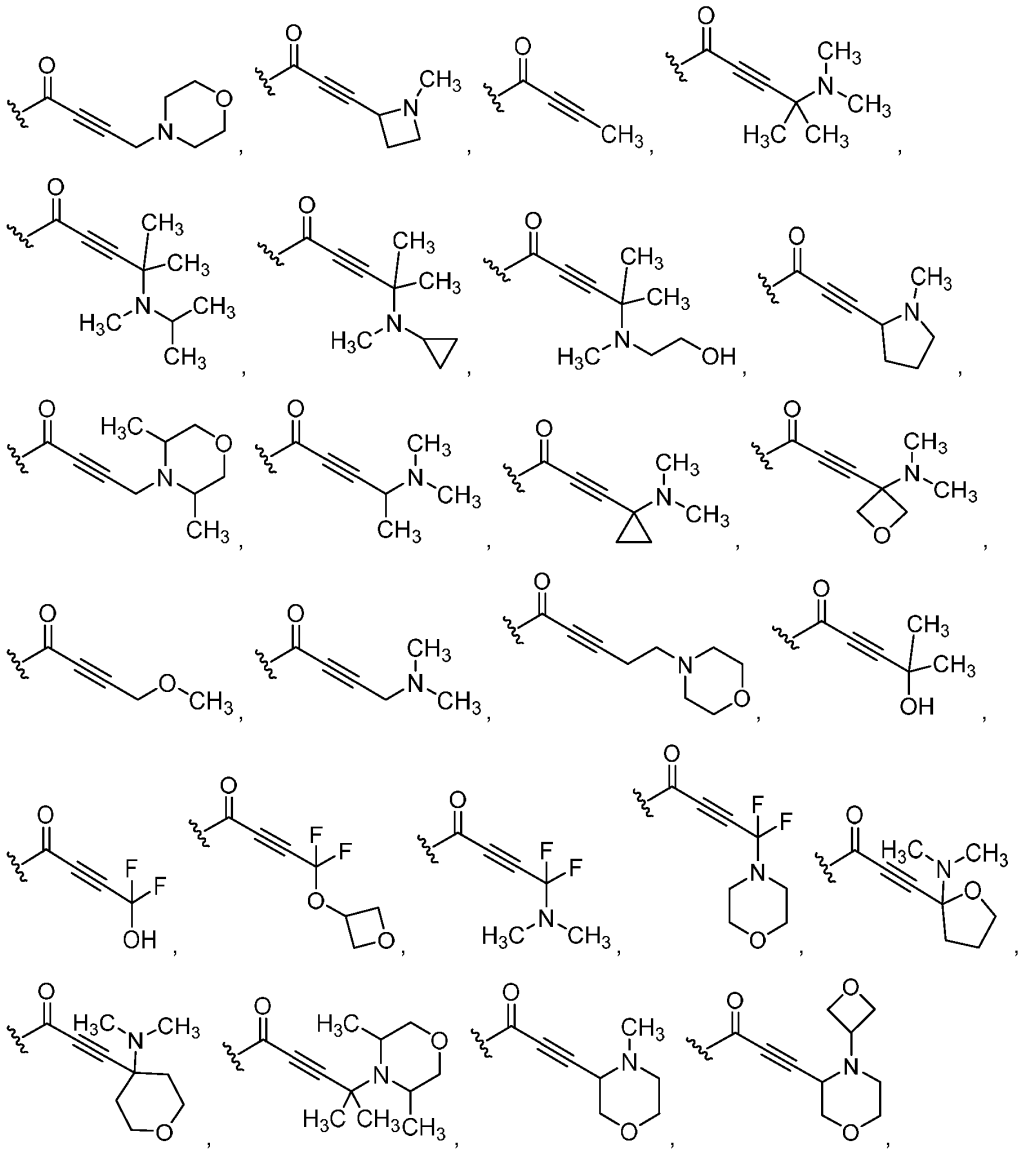
[79] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [78], wherein W has the structure of Formula IIIb:

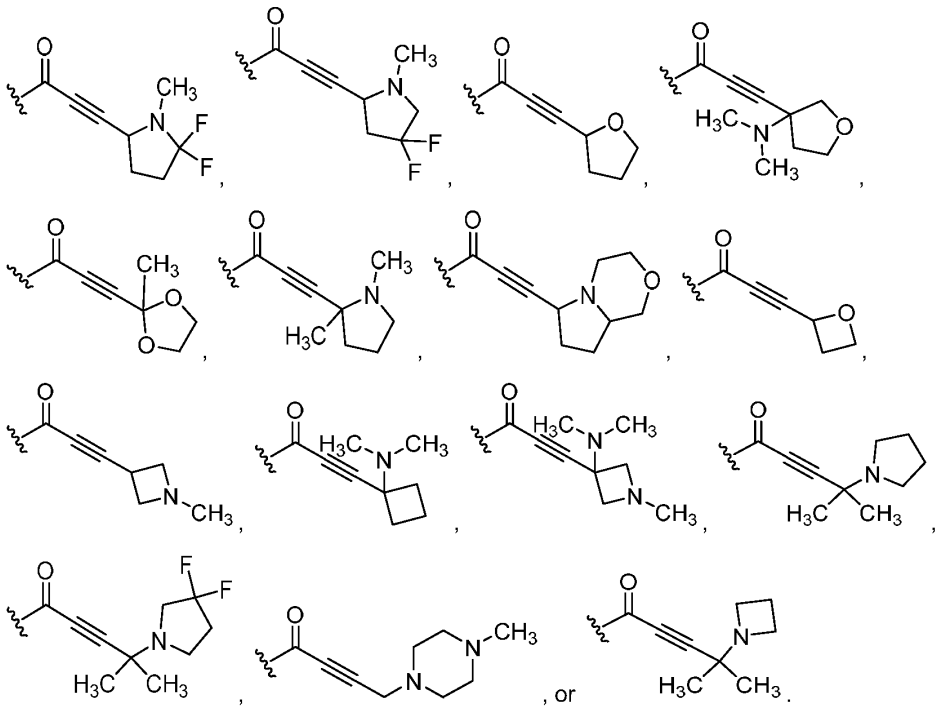


Formula IIIb

wherein R^{17} is hydrogen, -C_1-C_3 alkyl optionally substituted with one or more substituents independently selected from -OH, -O-C_1-C_3 alkyl, -NH_2, -NH(C_1-C_3 alkyl), -N(C_1-C_3 alkyl)$_2$, or a 4 to 7-membered saturated cycloalkyl, or a 4 to 7-membered saturated heterocycloalkyl.

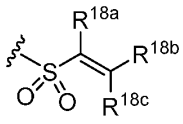
[80] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [79], wherein W is:





[81] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [74], wherein W is a cross-linking group comprising a vinyl sulfone.

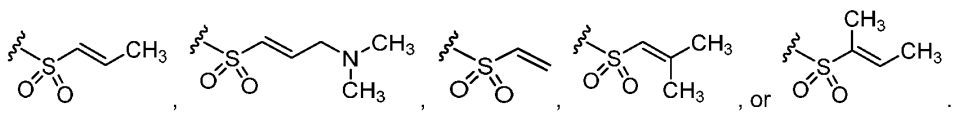
[82] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [81], wherein W has the structure of Formula IIIc:



Formula IIIc

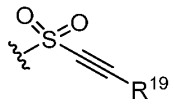
wherein R^{18a}, R^{18b}, and R^{18c} are, independently, hydrogen, -CN, or -C_1-C_3 alkyl optionally substituted with one or more substituents independently selected from -OH, -O-C_1-C_3 alkyl, -NH_2, -NH(C_1-C_3 alkyl), -N(C_1-C_3 alkyl)$_2$, or a 4 to 7-membered saturated heterocycloalkyl.

[83] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [82], wherein W is:



[84] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [74], wherein W is a cross-linking group comprising an alkynyl sulfone.

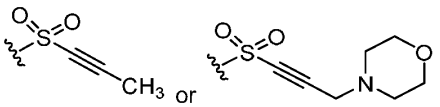
[85] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [84], wherein W has the structure of Formula IIId:



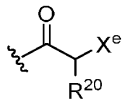
Formula IIId

wherein R^{19} is hydrogen, -C_1-C_3 alkyl optionally substituted with one or more substituents independently selected from -OH, -O-C_1-C_3 alkyl, -NH_2, -NH(C_1-C_3 alkyl), -N(C_1-C_3 alkyl)$_2$, or a 4 to 7-membered saturated heterocycloalkyl, or a 4 to 7-membered saturated heterocycloalkyl.

[86] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [85], wherein W is:



[87] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [74], wherein W has the structure of Formula IIIe:



Formula IIIe

wherein X^e is a halogen; and

R^{20} is hydrogen, -C_1-C_3 alkyl optionally substituted with one or more substituents independently selected from -OH, -O-C_1-C_3 alkyl, -NH_2, -NH(C_1-C_3 alkyl), -N(C_1-C_3 alkyl)$_2$, or a 4 to 7-membered saturated heterocycloalkyl.

[88] A compound, or a pharmaceutically acceptable salt thereof, selected from Table 1 or Table 2.

[89] A pharmaceutical composition comprising a compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [88], and a pharmaceutically acceptable excipient.

[90] A conjugate, or salt thereof, comprising the structure of Formula IV:

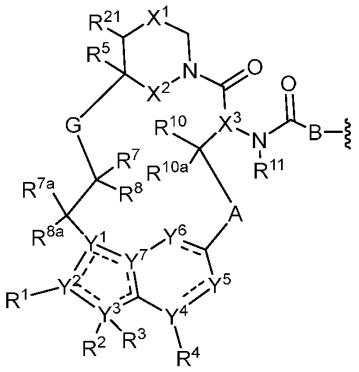
M-L-P

Formula IV

wherein L is a linker;

P is a monovalent organic moiety; and

M has the structure of Formula V:



Formula V

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is absent, -CH(R^9)-, >C=C$R^9R^{9'}$, or >C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or S(O)$_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered

cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is H, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl; or

R^9 and $R^{9'}$, combined with the atoms to which they are attached, form a 3 to 6-membered cycloalkyl or a 3 to 6-membered heterocycloalkyl;

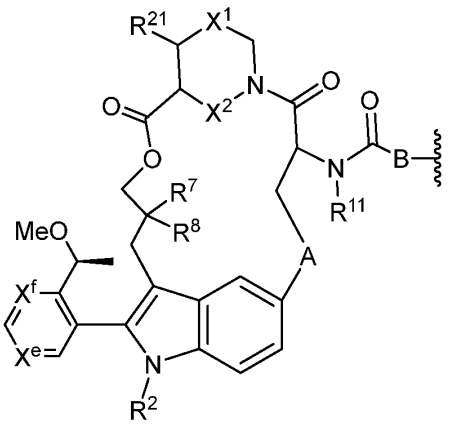
R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{21} is H or C_1-C_3 alkyl.

[91] The conjugate of paragraph [90], or salt thereof, wherein M has the structure of Formula Vd:



Formula Vd

wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', $C(O)N(R')_2$, S(O)R', $S(O)_2R'$, or $S(O)_2N(R')_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

R^2 is C_1-C_6 alkyl, C_1-C_6 fluoroalkyl, or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

R^8 is C_1-C_3 alkyl; and

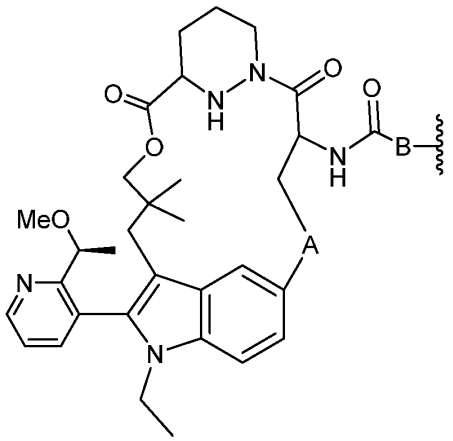
R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

X^e and X^f are, independently, N or CH;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{21} is hydrogen or C_1-C_3 alkyl.

[92] The conjugate of paragraph [91], or salt thereof, wherein M has the structure of Formula Ve:



Formula Ve

wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

[93] The conjugate, or salt thereof, of any one of paragraphs [90] to [92], wherein the linker has the structure of Formula II:

$$A^1\text{-}(B^1)_f\text{-}(C^1)_g\text{-}(B^2)_h\text{-}(D^1)\text{-}(B^3)_i\text{-}(C^2)_j\text{-}(B^4)_k\text{–}A^2$$

Formula II

where A^1 is a bond between the linker and B; A^2 is a bond between W and the linker; B^1, B^2, B^3, and B^4 each, independently, is selected from optionally substituted C_1-C_2 alkylene, optionally substituted C_1-C_3 heteroalkylene, O, S, and NR^N; R^N is hydrogen, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted C_1-C_7 heteroalkyl; C^1 and C^2 are each, independently, selected from carbonyl, thiocarbonyl, sulphonyl, or phosphoryl; f, g, h, i, j, and k are each, independently, 0 or 1; and D^1 is optionally substituted C_1-C_{10} alkylene, optionally substituted C_2-C_{10} alkenylene, optionally substituted C_2-C_{10} alkynylene, optionally substituted 3 to 14-membered heterocycloalkylene, optionally substituted 5 to 10-membered heteroarylene, optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 6 to 10-membered arylene, optionally substituted C_2-C_{10}

polyethylene glycolene, or optionally substituted C_1-C_{10} heteroalkylene, or a chemical bond linking A^1-$(B^1)_f$-$(C^1)_g$-$(B^2)_h$- to -$(B^3)_i$-$(C^2)_j$-$(B^4)_k$–A^2.

[94] The conjugate, or salt thereof, of any one of paragraphs [90] to [93], wherein the monovalent organic moiety is a protein.

[95] The conjugate, or salt thereof, of paragraph [94], wherein the protein is a Ras protein.

[96] The conjugate, or salt thereof, of paragraph [95], wherein the Ras protein is K-Ras G12C, K-Ras G13C, H-Ras G12C, H-Ras G13C, N-Ras G12C, or N-Ras G13C.

[97] The conjugate, or salt thereof, of any one of paragraphs [93] to [96], wherein the linker is bound to the monovalent organic moiety through a bond to a sulfhydryl group of an amino acid residue of the monovalent organic moiety.

Examples

The disclosure is further illustrated by the following examples and synthesis examples, which are not to be construed as limiting this disclosure in scope or spirit to the specific procedures herein described. It is to be understood that the examples are provided to illustrate certain embodiments and that no limitation to the scope of the disclosure is intended thereby. It is to be further understood that resort may be had to various other embodiments, modifications, and equivalents thereof which may suggest themselves to those skilled in the art without departing from the spirit of the present disclosure or scope of the appended claims.

Chemical Syntheses

Definitions used in the following examples and elsewhere herein are:

CH_2Cl_2, DCM	Methylene chloride, Dichloromethane
CH_3CN, MeCN	Acetonitrile
CuI	Copper (I) iodide
DIPEA	Diisopropylethyl amine
DMF	N,N-Dimethylformamide
EtOAc	Ethyl acetate
h	hour
H_2O	Water
HCl	Hydrochloric acid
K_3PO_4	Potassium phosphate (tribasic)
MeOH	Methanol
Na_2SO_4	Sodium sulfate
NMP	N-methyl pyrrolidone
$Pd(dppf)Cl_2$	[1,1′-Bis(diphenylphosphino)ferrocene]dichloropalladium(II)

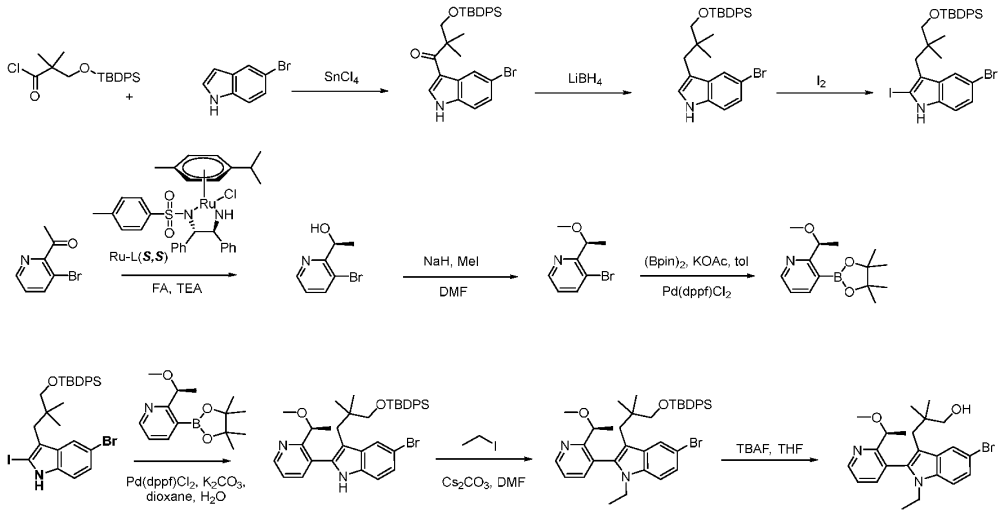
Instrumentation

Mass spectrometry data collection took place with a Shimadzu LCMS-2020, an Agilent 1260LC-6120/6125MSD, a Shimadzu LCMS-2010EV, or a Waters Acquity UPLC, with either a QDa detector or SQ Detector 2. Samples were injected in their liquid phase onto a C-18 reverse

phase. The compounds were eluted from the column using an acetonitrile gradient and fed into the mass analyzer. Initial data analysis took place with either Agilent ChemStation, Shimadzu LabSolutions, or Waters MassLynx. NMR data was collected with either a Bruker AVANCE III HD 400MHz, a Bruker Ascend 500MHz instrument, or a Varian 400MHz, and the raw data was analyzed with either TopSpin or Mestrelab Mnova.

Synthesis of Intermediates

Intermediate 1. Synthesis of 3-(5-bromo-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropan-1-ol



Intermediate 1.

Step 1. To a mixture of 3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropanoyl chloride (65 g, 137 mmol, crude) in DCM (120 mL) at 0 °C under an atmosphere of N_2 was added 1M $SnCl_4$ in DCM (137 mL, 137 mmol) slowly. The mixture was stirred at 0 °C for 30 min, then a solution of 5-bromo-1*H*-indole (26.8 g, 137 mmol) in DCM (40 mL) was added dropwise. The mixture was stirred at 0 °C for 45 min, then diluted with EtOAc (300 mL), washed with brine (100 mL x 4), dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 1-(5-bromo-1*H*-indol-3-yl)-3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one (55 g, 75% yield). LCMS (ESI): m/z [M+Na] calc'd for $C_{29}H_{32}BrNO_2SiNa$ 556.1; found 556.3.

Step 2. To a mixture of 1-(5-bromo-1*H*-indol-3-yl)-3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one (50 g, 93.6 mmol) in THF (100 mL) at 0 °C under an atmosphere of N_2 was added $LiBH_4$ (6.1 g, 281 mmol). The mixture was heated to 60 °C and stirred for 20 h, then MeOH (10 mL) and EtOAc (100 mL) were added and the mixture washed with brine (50 mL), dried over Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure. The residue was diluted with DCM (50 mL), cooled to 10 °C and diludine (9.5 g, 37.4 mmol) and $TsOH.H_2O$ (890 mg, 4.7 mmol) added. The mixture was stirred at 10 °C for 2 h, filtered, the filtrate concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 1-(5-bromo-1*H*-

indol-3-yl)-3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one (41 g, 84% yield). LCMS (ESI): m/z [M+H] calc'd for $C_{29}H_{34}BrNOSi$ 519.2; found 520.1; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.75 - 7.68 (m, 5H), 7.46 - 7.35 (m, 6H), 7.23 - 7.19 (m, 2H), 6.87 (d, *J* = 2.1 Hz, 1H), 3.40 (s, 2H), 2.72 (s, 2H), 1.14 (s, 9H), 0.89 (s, 6H).

Step 3. To a mixture of 1-(5-bromo-1*H*-indol-3-yl)-3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one (1.5 g, 2.9 mmol) and I_2 (731 mg, 2.9 mmol) in THF (15 mL) at rt was added AgOTf (888 mg, 3.5 mmol). The mixture was stirred at rt for 2 h, then diluted with EtOAc (200 mL) and washed with saturated $Na_2S_2O_3$ (100 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-iodo-1*H*-indole (900 mg, 72% yield) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (s, 1H), 7.68 (d, *J* = 1.3 Hz, 1H), 7.64 - 7.62 (m, 4H), 7.46 - 7.43 (m, 6H), 7.24 - 7.22 (d, 1H), 7.14 - 7.12 (dd, *J* = 8.6, 1.6 Hz, 1H), 3.48 (s, 2H), 2.63 (s, 2H), 1.08 (s, 9H), 0.88 (s, 6H).

Step 4. To a stirred mixture of HCOOH (66.3 g, 1.44 mol) in TEA (728 g, 7.2 mol) at 0 °C under an atmosphere of Ar was added (4*S*,5*S*)-2-chloro-2-methyl-1-(4-methylbenzenesulfonyl)-4,5-diphenyl-1,3-diaza-2-ruthenacyclopentane cymene (3.9 g, 6.0 mmol) portion-wise. The mixture was heated to 40 °C and stirred for 15 min, then cooled to rt and 1-(3-bromopyridin-2-yl)ethanone (120 g, 600 mmol) added in portions. The mixture was heated to 40 °C and stirred for an additional 2 h, then the solvent was concentrated under reduced pressure. Brine (2 L) was added to the residue, the mixture was extracted with EtOAc (4 x 700 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (1*S*)-1-(3-bromopyridin-2-yl)ethanol (100 g, 74% yield) a an oil. LCMS (ESI): m/z [M+H] calc'd for C_7H_8BrNO 201.1; found 201.9.

Step 5. To a stirred mixture of (1*S*)-1-(3-bromopyridin-2-yl)ethanol (100 g, 495 mmol) in DMF (1 L) at 0 °C was added NaH, 60% dispersion in oil (14.25 g, 594 mmol) in portions. The mixture was stirred at 0 °C for 1 h. MeI (140.5 g, 990 mmol) was added dropwise at 0 °C and the mixture was allowed to warm to rt and stirred for 2 h. The mixture was cooled to 0 °C and saturated NH_4Cl (5 L) was added. The mixture was extracted with EtOAc (3 x 1.5 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-bromo-2-[(1*S*)-1-methoxyethyl]pyridine (90 g, 75% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_8H_{10}BrNO$ 215.0; found 215.9.

Step 6. To a stirred mixture of 3-bromo-2-[(1*S*)-1-methoxyethyl]pyridine (90 g, 417 mmol) and Pd(dppf)Cl_2 (30.5 g, 41.7 mmol) in toluene (900 mL) at rt under an atmosphere of Ar was added bis(pinacolato)diboron (127 g, 500 mmol) and KOAc (81.8 g, 833 mmol) in portions. The mixture was heated to 100 °C and stirred for 3 h. The filtrate was concentrated under reduced pressure and the residue was purified by Al_2O_3 column chromatography to give 2-[(1*S*)-1-methoxyethyl]-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (100 g, 63% yield) as a semi-solid. LCMS (ESI): m/z [M+H] calc'd for $C_{14}H_{22}BNO_3$ 263.2; found 264.1.

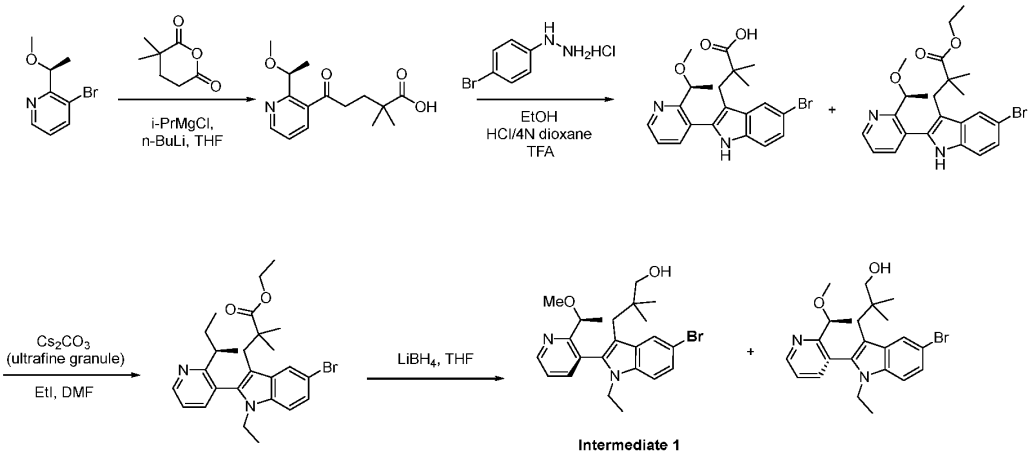
Step 7. To a stirred mixture of 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-2-iodo-1*H*-indole (140 g, 217 mmol) and 2-[(1*S*)-1-methoxyethyl]-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (100 g, 380 mmol) in 1,4-dioxane (1.4 L) at rt under an

atmosphere of Ar was added K_2CO_3 (74.8 g, 541 mmol), $Pd(dppf)Cl_2$ (15.9 g, 21.7 mmol), and H_2O (280 mL) in portions. The mixture was heated to 85 °C and stirred for 4 h, then cooled, H_2O (5 L) added, and the mixture extracted with EtOAc (3 x 2 L). The combined organic layers were washed with brine (2 x 1 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-1*H*-indole (71 g, 45% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{37}H_{43}BrN_2O_2Si$ 654.2; found 655.1.

Step 8. To a stirred mixture of 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-1*H*-indole (71 g, 108 mmol) in DMF (0.8 L) at 0 °C under an atmosphere of N_2 was added Cs_2CO_3 (70.6 g, 217 mmol) and EtI (33.8 g, 217 mmol) in portions. The mixture was warmed to rt and stirred for 16 h then H_2O (4 L) added and the mixture extracted with EtOAc (3 x 1.5 L). The combined organic layers were washed with brine (2 x 1 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indole (66 g, 80% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{47}BrN_2O_2Si$ 682.3; found 683.3.

Step 9. To a stirred mixture of TBAF (172.6 g, 660 mmol) in THF (660 mL) at rt under an atmosphere of N_2 was added 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indole (66 g, 97 mmol) in portions. The mixture was heated to 50 °C and stirred for 16 h, cooled, diluted with H_2O (5 L), and extracted with EtOAc (3 x 1.5 L). The combined organic layers were washed with brine (2 x 1 L), dried over anhydrous Na_2SO_4, and filtered. After filtration, the filtrate was concentrated under reduced pressure. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-(5-bromo-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropan-1-ol (30 g, 62% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{29}BrN_2O_2$ 444.1; found 445.1.

Intermediate 1. Alternative Synthesis through Fisher Indole Route.



Intermediate 1

Step 1. To a mixture of *i*-PrMgCl (2M in in THF, 0.5 L) at -10 °C under an atmosphere of N_2 was added *n*-BuLi, 2.5 M in hexane (333 mL, 833 mmol) dropwise over 15 min. The mixture was stirred for 30 min at -10 °C then 3-bromo-2-[(1*S*)-1-methoxyethyl]pyridine (180 g, 833 mmol) in THF (0.5 L) added dropwise over 30 min at -10 °C. The resulting mixture was warmed to -5 °C and stirred for 1 h, then 3,3-dimethyloxane-2,6-dione (118 g, 833 mmol) in THF (1.2 L) was added dropwise over 30 min at -5 °C. The mixture was warmed to 0 °C and stirred for 1.5 h, then quenched with the addition of pre-cooled 4M HCl in 1,4-dioxane (0.6 L) at 0 °C to adjust pH ~5. The mixture was diluted with ice-water (3 L) and extracted with EtOAc (3 x 2.5 L). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography to give 5-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-2,2-dimethyl-5-oxopentanoic acid (87 g, 34% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{15}H_{21}NO_4$ 279.2; found 280.1.

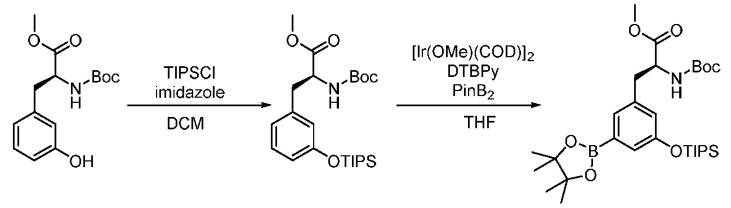
Step 2. To a mixture of 5-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-2,2-dimethyl-5-oxopentanoic acid (78 g, 279 mmol) in EtOH (0.78 L) at rt under an atmosphere of N_2 was added (4-bromophenyl)hydrazine HCl salt (68.7 g, 307 mmol) in portions. The mixture was heated to 85 °C and stirred for 2 h, cooled to rt, then 4M HCl in 1,4-dioxane (69.8 mL, 279 mmol) added dropwise. The mixture was heated to 85 °C and stirred for an additional 3 h, then concentrated under reduced pressure, and the residue was dissolved in TFA (0.78 L). The mixture was heated to 60 °C and stirred for 1.5 h, concentrated under reduced pressure, and the residue adjusted to pH ~5 with saturated $NaHCO_3$, then extracted with EtOAc (3 x 1.5 L). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate concentrated under reduced pressure, and the residue was purified by silica gel column chromatography to give 3-(5-bromo-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-1*H*-indol-3-yl)-2,2-dimethylpropanoic acid and ethyl (*S*)-3-(5-bromo-2-(2-(1-methoxyethyl)pyridin-3-yl)-1*H*-indol-3-yl)-2,2-dimethylpropanoate (78 g, crude). LCMS (ESI): m/z [M+H] calc'd for $C_{21}H_{23}BrN_2O_3$ 430.1 and $C_{23}H_{27}BrN_2O_3$ 458.1; found 431.1 and 459.1.

Step 3. To a mixture of 3-(5-bromo-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-1*H*-indol-3-yl)-2,2-dimethylpropanoic acid and ethyl (*S*)-3-(5-bromo-2-(2-(1-methoxyethyl)pyridin-3-yl)-1*H*-indol-3-yl)-2,2-dimethylpropanoate (198 g, 459 mmol) in DMF (1.8 L) at 0 °C under an atmosphere of N_2

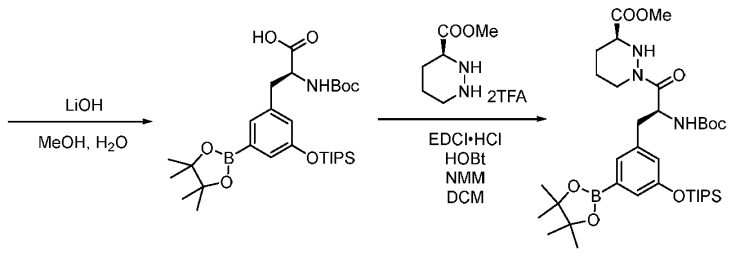
was added Cs_2CO_3 (449 g, 1.38 mol) in portions. EtI (215 g, 1.38 mmol) in DMF (200 mL) was then added dropwise at 0 °C. The mixture was warmed to rt and stirred for 4 h then diluted with brine (5 L) and extracted with EtOAc (3 x 2.5 L). The combined organic layers were washed with brine (2 x 1.5 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give ethyl 3-(5-bromo-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropanoate (160 g, 57% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{25}H_{31}BrN_2O_3$ 486.2; found 487.2.

Step 4. To a mixture of ethyl 3-(5-bromo-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropanoate (160 g, 328 mmol) in THF (1.6 L) at 0 °C under an atmosphere of N_2 was added $LiBH_4$ (28.6 g, 1.3 mol). The mixture was heated to 60 °C for 16 h, cooled, and quenched with pre-cooled (0 °C) aqueous NH_4Cl (5 L). The mixture was extracted with EtOAc (3 x 2 L) and the combined organic layers were washed with brine (2 x 1 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give to two atropisomers (as single atropisomers) of 3-(5-bromo-1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (60 g, 38% yield) and (40 g, 26% yield) both as solids. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{29}BrN_2O_2$ 444.1; found 445.2.

Intermediate 2 and Intermediate 4. Synthesis of (S)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)propanoyl)hexahydropyridazine-3-carboxylate



Intermediate 4.



Intermediate 2.

Step 1. To a mixture of (*S*)-methyl 2-(*tert*-butoxycarbonylamino)-3-(3-hydroxyphenyl)propanoate (10.0 g, 33.9 mmol) in DCM (100 mL) was added imidazole (4.6 g, 67.8 mmol) and TIPSCl (7.8 g, 40.7 mmol). The mixture was stirred at rt overnight then diluted with DCM (200 mL) and washed with H_2O (150 mL x 3). The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, and the residue was purified by silica gel column

chromatography to give (*S*)-methyl 2-(*tert*-butoxycarbonylamino)-3-(3-(triisopropylsilyloxy)phenyl)-propanoate (15 g, 98% yield) as an oil. LCMS (ESI): m/z [M+Na] calc'd for $C_{24}H_{41}NO_5SiNa$ 474.3; found 474.2.

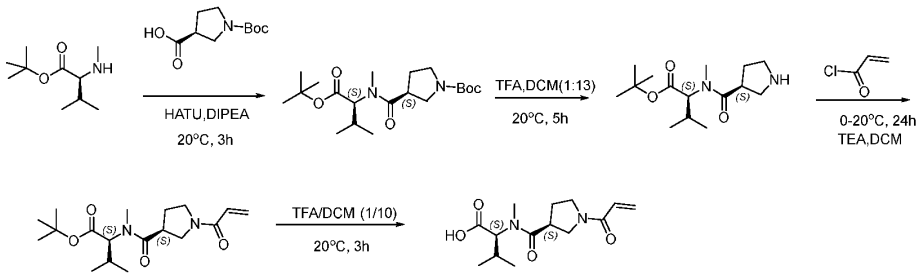
Step 2. A mixture of (*S*)-methyl 2-(*tert*-butoxycarbonylamino)-3-(3-(triisopropylsilyloxy)phenyl)-propanoate (7.5 g, 16.6 mmol), $PinB_2$ (6.3 g, 24.9 mmol), $[Ir(OMe)(COD)]_2$ (1.1 g, 1.7 mmol), and 4-*tert*-butyl-2-(4-*tert*-butyl-2-pyridyl)pyridine (1.3 g, 5.0 mmol) was purged with Ar (x3), then THF (75 mL) was added and the mixture placed under an atmosphere of Ar and sealed. The mixture was heated to 80 °C and stirred for 16 h, concentrated under reduced pressure, and the residue was purified by silica gel column chromatography to give (*S*)-methyl 2-(*tert*-butoxycarbonylamino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-(triisopropylsilyloxy)phenyl)-propanoate (7.5 g, 78% yield) as a solid. LCMS (ESI): m/z [M+Na] calc'd for $C_{30}H_{52}BNO_7SiNa$ 600.4; found 600.4; ^1H NMR (300 MHz, CD_3OD) δ 7.18 (s, 1H), 7.11 (s, 1H), 6.85 (s, 1H), 4.34 (m, 1H), 3.68 (s, 3H), 3.08 (m, 1H), 2.86 (m, 1H), 1.41 - 1.20 (m, 26H), 1.20 - 1.01 (m, 22H), 0.98 - 0.79 (m, 4H).

Step 3. To a mixture of triisopropylsilyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)propanoate (4.95 g, 6.9 mmol) in MeOH (53 mL) at 0 °C was added LiOH (840 mg, 34.4 mmol) in H_2O (35 mL). The mixture was stirred at 0 °C for 2 h, then acidified to pH ~5 with 1M HCl and extracted with EtOAc (250 mL x 2). The combined organic layers were washed with brine (100 mL x 3), dried over anhydrous Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure to give (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)propanoic acid (3.7 g, 95% yield), which was used directly in the next step without further purification. LCMS (ESI): m/z [M+NH_4] calc'd for $C_{29}H_{50}BNO_7SiNH_4$ 581.4; found 581.4.

Step 4. To a mixture of methyl (*S*)-hexahydropyridazine-3-carboxylate (6.48 g, 45.0 mmol) in DCM (200 mL) at 0 °C was added NMM (41.0 g, 405 mmol), (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)propanoic acid (24 g, 42.6 mmol) in DCM (50 mL) then HOBt (1.21 g, 9.0 mmol) and EDCI HCl salt (12.9 g, 67.6 mmol). The mixture was warmed to rt and stirred for 16 h, then diluted with DCM (200 mL) and washed with H_2O (3 x 150 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, the filtrate concentrated under reduced pressure, and the residue was purified by silica gel column chromatography to give methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)propanoyl)hexahydropyridazine-3-

carboxylate (22 g, 71% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{35}H_{60}BN_3O_8Si$ 689.4; found 690.5.

Intermediate 3. Synthesis of *N*-((*S*)-1-acryloylpyrrolidine-3-carbonyl)-*N*-methyl-L-valine



Intermediate 3

Step 1. To a mixture of (*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-3-carboxylic acid (2.2 g, 10.2 mmol) in DMF (10 mL) at rt was added HATU (7.8 g, 20.4 mmol) and DIPEA (5 mL). After stirring at rt for 10 min, *tert*-butyl methyl-L-valinate (3.8g, 20.4 mmol) in DMF (10 mL) was added. The mixture was stirred at rt for 3 h, then diluted with DCM (40 mL) and H_2O (30 mL). The aqueous and organic layers were separated and the organic layer was washed with H_2O (3 × 30 mL), brine (30 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (*S*)-*tert*-butyl 3-(((*S*)-1-(*tert*-butoxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)pyrrolidine-1-carboxylate (3.2 g, 82% yield) as an oil. LCMS (ESI): m/z [M+Na] calc'd for $C_{20}H_{36}N_2O_5Na$ 407.3; found 407.2.

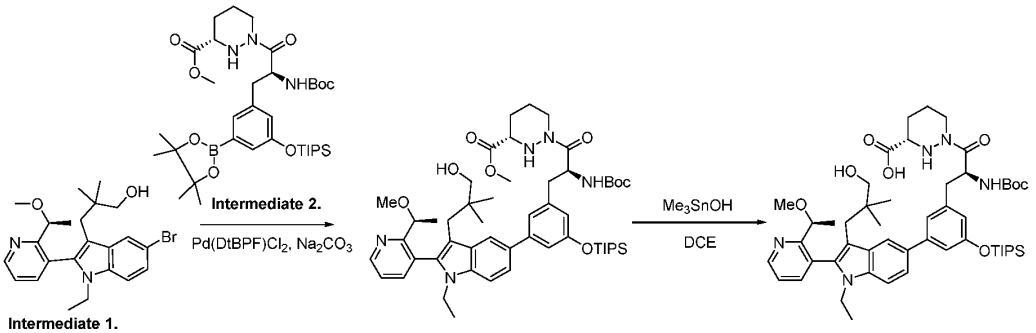
Step 2. A mixture of (*S*)-*tert*-butyl 3-(((*S*)-1-(*tert*-butoxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)pyrrolidine-1-carboxylate (3.2 g, 8.4 mmol) in DCM (13 mL) and TFA (1.05 g, 9.2 mmol) was stirred at rt for 5 h. The mixture was concentrated under reduced pressure to give (*S*)-*tert*-butyl 3-methyl-2-((*S*)-*N*-methylpyrrolidine-3-carboxamido)butanoate (2.0 g, 84% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{15}H_{28}N_2O_3$ 284.2; found 285.2.

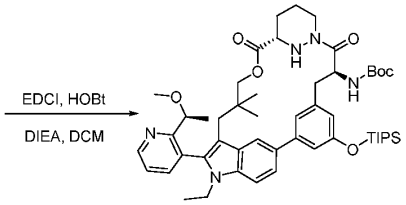
Step 3. To a mixture of (*S*)-*tert*-butyl 3-methyl-2-((*S*)-*N*-methylpyrrolidine-3-carboxamido)butanoate (600 mg, 2.1 mmol) in DCM (6 mL) at 0 °C was added TEA (342 mg, 3.36 mmol). After stirring at 0 °C for 10 mins, acryloyl chloride (284 mg, 3.2 mmol) in DCM (10 mL) was added. The mixture was warmed to rt and stirred for 24 h, then diluted with DCM (30 mL) and H_2O (30 mL). The aqueous and organic layers were separated and the organic layer was washed with H_2O (3 × 30 mL), brine (30 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl *N*-((*S*)-1-acryloylpyrrolidine-3-carbonyl)-*N*-methyl-L-valinate (500 mg, 70% yield) as an oil.

Step 4. To a mixture of *tert*-butyl *N*-((*S*)-1-acryloylpyrrolidine-3-carbonyl)-*N*-methyl-L-valinate (100 mg, 0.29 mmol) in DCM (3.0 mL) at 15 °C was added TFA (0.3 mL). The mixture was warmed to rt and stirred for 5 h, then the mixture was concentrated under reduced pressure to give *N*-((*S*)-1-acryloylpyrrolidine-3-carbonyl)-*N*-methyl-L-valine (150 mg) as a solid. The crude product

was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{14}H_{22}N_2O_4$ 282.2; found 283.2.

Intermediate 5. Synthesis of tert-butyl ((63S,4S)-11-ethyl-12-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-25-((triisopropylsilyl)oxy)-61,62,63,64,65,66-hexahydro-11H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate.





Intermediate 5.

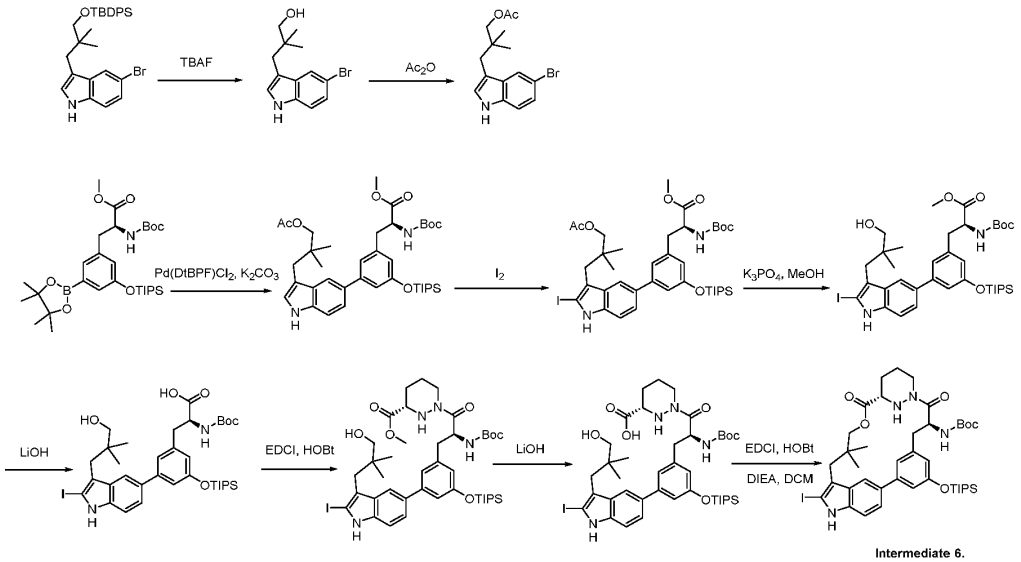
Step 1. To a stirred mixture of 3-(5-bromo-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropan-1-ol (30 g, 67 mmol) and methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate (55.8 g, 80.8 mmol) in 1,4-dioxane (750 mL) at rt under an atmosphere of Ar was added Na_2CO_3 (17.9 g, 168.4 mmol), $Pd(DtBPF)Cl_2$ (4.39 g, 6.7 mmol), and H_2O (150.00 mL) in portions. The mixture was heated to 85 °C and stirred for 3 h, cooled, diluted with H_2O (2 L), and extracted with EtOAc (3 x 1 L). The combined organic layers were washed with brine (2 x 500 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate (50 g, 72% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{52}H_{77}N_5O_8Si$ 927.6; found 928.8.

Step 2. To a stirred mixture of methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate (50 g, 54 mmol) in DCE (500 mL) at rt was added trimethyltin hydroxide (48.7 g, 269 mmol) in portion. The mixture was heated to 65 °C and stirred for 16 h, then filtered and the filter cake washed with DCM (3 x 150 mL). The

filtrate was concentrated under reduced pressure to give (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid (70 g, crude), which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{75}N_5O_8Si$ 913.5; found 914.6.

Step 3. To a stirred mixture of (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid (70 g) in DCM (5 L) at 0 °C under an atmosphere of N_2 was added DIPEA (297 g, 2.3 mol), HOBT (51.7 g, 383 mmol) and EDCI (411 g, 2.1 mol) in portions. The mixture was warmed to rt and stirred for 16 h, then diluted with DCM (1 L), washed with brine (3 x 1 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (36 g, 42% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{73}N_5O_7Si$ 895.5; found 896.5.

Intermediate 6. Synthesis of tert-butyl N-[(8S,14S)-21-iodo-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate.



Step 1. This reaction was undertaken on 5-batches in parallel on the scale illustrated below.

Into a 2L round-bottom flasks each were added 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1*H*-indole (100 g, 192 mmol) and TBAF (301.4 g, 1.15 mol) in THF (1.15 L) at rt. The resulting mixture was heated to 50 °C and stirred for 16 h, then the mixture was concentrated under reduced pressure. The combined residues were diluted with H_2O (5 L) and

extracted with EtOAc (3 x 2 L). The combined organic layers were washed with brine (2 x 1.5 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-(5-bromo-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (310 g, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{16}BrNO$ 281.0 and 283.0; found 282.1 and 284.1.

Step 2. This reaction was undertaken on two batches in parallel on the scale illustrated below.

To a stirred mixture of 3-(5-bromo-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (135 g, 478 mmol) and TEA (145.2 g, 1.44 mol) in DCM (1.3 L) at 0 °C under an atmosphere of N_2 was added Ac_2O (73.3 g, 718 mmol) and DMAP (4.68 g, 38.3 mmol) in portions. The resulting mixture was stirred for 10 min at 0 °C, then washed with H_2O (3 x 2 L). The organic layers from each experiment were combined and washed with brine (2 x 1 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography to give 3-(5-bromo-1*H*-indol-3-yl)-2,2-dimethylpropyl acetate (304 g, 88% yield) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.16 - 11.11 (m, 1H), 7.69 (d, *J* = 2.0 Hz, 1H), 7.32 (d, *J* = 8.6 Hz, 1H), 7.19 - 7.12 (m, 2H), 3.69 (s, 2H), 2.64 (s, 2H), 2.09 (s, 3H), 0.90 (s, 6H).

Step 3. This reaction was undertaken on four batches in parallel on the scale illustrated below.

Into a 2L round-bottom flasks were added methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-[(triisopropylsilyl)oxy]phenyl]propanoate (125 g, 216 mmol), 1,4-dioxane (1 L), H_2O (200 mL), 3-(5-bromo-1*H*-indol-3-yl)-2,2-dimethylpropyl acetate (73.7 g, 227 mmol), K_2CO_3 (59.8 g, 433 mmol), and Pd(DtBPF)Cl_2 (7.05 g, 10.8 mmol) at rt under an atmosphere of Ar. The resulting mixture was heated to 65 °C and stirred for 2 h, then diluted with H_2O (10 L) and extracted with EtOAc (3 x 3 L). The combined organic layers were washed with brine (2 x 2 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography to give methyl (2*S*)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (500 g, 74% yield) as an oil. LCMS (ESI): m/z [M+Na] calc'd for $C_{39}H_{58}N_2O_7SiNa$ 717.4; found 717.3.

Step 4. This reaction was undertaken on three batches in parallel on the scale illustrated below.

To a stirred mixture of methyl (2*S*)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (150 g, 216 mmol) and $NaHCO_3$ (21.76 g, 259 mmol) in THF (1.5 L) was added AgOTf (66.5 g, 259 mmol) in THF dropwise at 0 °C under an atmosphere of nitrogen. I_2 (49.3 g, 194 mmol) in THF was added dropwise over 1 h at 0 °C and the resulting mixture was stirred for an additional 10 min at 0 °C. The combined experiments were diluted with aqueous $Na_2S_2O_3$ (5 L) and extracted with EtOAc (3 x 3 L). The combined organic layers were washed with brine (2 x 1.5 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography to give methyl (2*S*)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-2-iodo-1*H*-

indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (420 g, 71% yield) as an oil. LCMS (ESI): m/z [M+Na] calc'd for $C_{39}H_{57}IN_2O_7SiNa$, 843.3; found 842.9.

Step 5. This reaction was undertaken on three batches in parallel on the scale illustrated below.

To a 2L round-bottom flask were added methyl (2*S*)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (140 g, 171 mmol), MeOH (1.4 L) and K_3PO_4 (108.6 g, 512 mmol) at 0 °C. The mixture was warmed to rt and stirred for 1 h, then the combined experiments were diluted with H_2O (9 L) and extracted with EtOAc (3 x 3 L). The combined organic layers were washed with brine (2 x 2 L), dried over anhydrous Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure to give methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoate (438g, crude) as a solid. LCMS (ESI): m/z [M+Na] calc'd for $C_{37}H_{55}IN_2O_6SiNa$ 801.3; found 801.6.

Step 6. This reaction was undertaken on three batches in parallel on the scale illustrated below.

To a stirred mixture of methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoate (146 g, 188 mmol) in THF (1.46 L) was added LiOH (22.45 g, 937 mmol) in H_2O (937 mL) dropwise at 0 °C. The resulting mixture was warmed to rt and stirred for 1.5 h [note: LCMS showed 15% de-TIPS product]. The mixture was acidified to pH 5 with 1M HCl (1M) and the combined experiments were extracted with EtOAc (3 x 3 L). The combined organic layers were washed with brine (2 x 2 L), dried over anhydrous Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure to give (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoic acid (402 g, crude) as a solid. LCMS (ESI): m/z [M+Na] calc'd for $C_{36}H_{53}IN_2O_6SiNa$ 787.3; found 787.6.

Step 7. To a stirred mixture of (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoic acid (340 g, 445 mmol) and methyl (3*S*)-1,2-diazinane-3-carboxylate (96.1 g, 667 mmol) in DCM (3.5 L) was added NMM (225 g, 2.2 mol), EDCI (170 g, 889 mmol), and HOBT (12.0 g, 88.9 mmol) portionwise at 0 °C. The mixture was warmed to rt and stirred for 16 h, then washed with H_2O (3 x 2.5 L), brine (2 x 1 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography to give methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate (310 g, 62% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{63}IN_4O_7Si$ 890.4; found 890.8.

Step 8. This reaction was undertaken on three batches in parallel on the scale illustrated below.

To a stirred mixture of methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate (85.0 g, 95.4 mmol) in THF (850 mL) each added LiOH (6.85 g, 286 mmol) in H_2O (410 mL) dropwise at 0 °C under an atmosphere of N_2. The mixture was stirred at 0 °C for

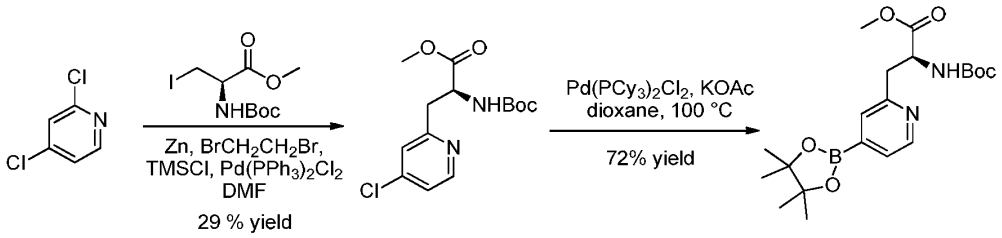
1.5 h [note: LCMS showed 15% de-TIPS product], then acidified to pH 5 with 1M HCl and the combined experiments extracted with EtOAc (3 x 2 L). The combined organic layers were washed with brine (2 x 1.5 L), dried over anhydrous Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure to give (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid (240 g, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{61}IN_4O_7Si$ 876.3; found 877.6.

Step 9.

This reaction was undertaken on two batches in parallel on the scale illustrated below.

To a stirred mixture of (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid (120 g, 137 mmol) in DCM (6 L) was added DIPEA (265 g, 2.05 mol), EDCI (394 g, 2.05 mol), and HOBT (37 g, 274 mmol) in portions at 0 °C under an atmosphere of N_2. The mixture was warmed to rt and stirred overnight, then the combined experiments were washed with H_2O (3 x 6 L), brine (2 x 6 L), dried over anhydrous Na_2SO_4, and filtered. After filtration, the filtrate was concentrated under reduced pressure. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography to give *tert*-butyl *N*-[(8*S*,14*S*)-21-iodo-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate (140 g, 50% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{59}IN_4O_6Si$ 858.9; found 858.3.

Intermediate 7. Synthesis of (*S*)-methyl 2-((*tert*-butoxycarbonyl)amino)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)propanoate

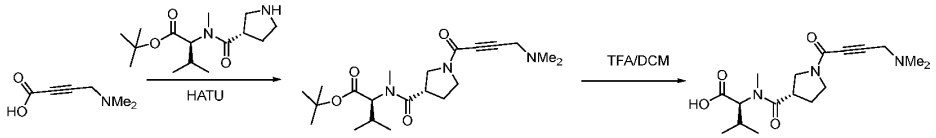


Step 1. Zn dust (28 g, 428 mmol) was added to a 1L, three necked, round bottomed flask, purged with N_2, and heated with a heat gun for 10 min under vacuum. The mixture was cooled to rt, and a solution of 1,2-dibromoethane (1.85 mL, 21.5 mmol) in DMF (90 mL) was added dropwise over 10 min. The mixture was heated at 90 °C for 30 min and re-cooled to rt. TMSCl (0.55 mL, 4.3 mmol) was added, and the mixture was stirred for 30 min at rt, then a mixture of (*R*)-methyl 2-((*tert*-butoxycarbonyl)amino)-3-iodopropanoate (22.5g, 71.4 mmol) in DMF (200 mL) was added dropwise over a period of 10 min. The mixture was heated at 35 °C and stirred for 2 h, then cooled to rt, and 2,4-dichloropyridine (16 g, 109 mmol) and $Pd(PPh_3)_2Cl_2$ (4 g, 5.7 mmol) added. The mixture was heated at 45 °C and stirred for 2 h, cooled, and filtered, then H_2O (1 L) and EtOAc (0.5 L) were added to the filtrate. The organic and aqueous layers were separated, and the aqueous layer was extracted with EtOAc (2 x 500 mL). The organic layers were dried over anhydrous

Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography to give (*S*)-methyl 2-((*tert*-butoxycarbonyl)amino)-3-(4-chloropyridin-2-yl)propanoate (6.5 g, 29% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{14}H_{19}ClN_2O_4$ 314.1; found 315.1.

Step 2. To a mixture of (*S*)-methyl 2-((*tert*-butoxycarbonyl)amino)-3-(4-chloropyridin-2-yl)propanoate (6.5 g, 20.6 mmol) in 1,4-dioxane (80 mL) at rt under an atmosphere of N_2 was added bis(pinacolato)diboron (6.3 g, 24.7 mmol), KOAc (8.1 g, 82.4 mmol), and $Pd(PCy_3)_2Cl_2$ (1.9 g, 2.5 mmol). The mixture was heated to 100 °C and stirred for 3 h, then H_2O (100 mL) added and the mixture extracted with EtOAc (3 x 200 mL). The organic layers were combined, washed with brine (2 x 100 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (*S*)-methyl 2-((*tert*-butoxycarbonyl)amino)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)propanoate (6 g, 72% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{20}H_{31}BN_2O_6$ 406.2; found 407.3.

Synthesis of Intermediate 8.

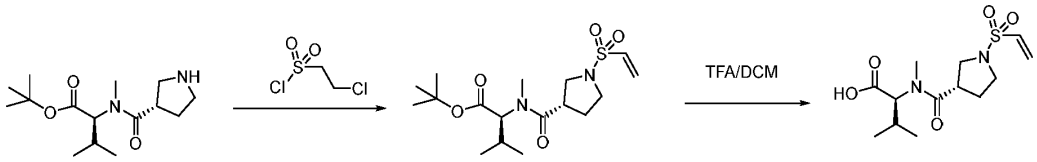


Step 1. To a mixture of 4-(dimethylamino)but-2-ynoic acid (900 mg, 7.0 mmol) in DMF (20 mL) at -5 °C was added *tert*-butyl *N*-methyl-*N*-((*S*)-pyrrolidine-3-carbonyl)-L-valinate (1.0 g, 3.5 mmol), DIPEA (2.2 g, 17.6 mmol) and HATU (2.7 g, 7.0 mmol) in portions. The mixture was stirred between -5 to 5 °C for 1 h, then diluted with EtOAc (100 mL) and ice-H_2O (100 mL). The aqueous and organic layers were separated and the organic layer was washed with H_2O (3 × 100 mL), brine (100 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl *N*-((*S*)-1-(4-(dimethylamino)but-2-ynoyl)pyrrolidine-3-carbonyl)-*N*-methyl-L-valinate (900 mg, 55% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{21}H_{35}N_3O_4$ 393.5; found 394.3.

Step 2. To a mixture of *tert*-butyl *N*-((*S*)-1-(4-(dimethylamino)but-2-ynoyl)pyrrolidine-3-carbonyl)-*N*-methyl-L-valinate (260 mg, 0.66 mmol) in DCM (6 mL) was added TFA (3 mL) at rt. The mixture was stirred at rt for 2 h, then the solvent was concentrated under reduced pressure to give (2*S*)-2-{1-[(3*S*)-1-[4-(dimethylamino)but-2-ynoyl]pyrrolidin-3-yl]-*N*-methylformamido}-3-

methylbutanoic acid (280 mg) as an impure oil. The crude product was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{17}H_{27}N_3O_4$ 337.2; found 338.3.

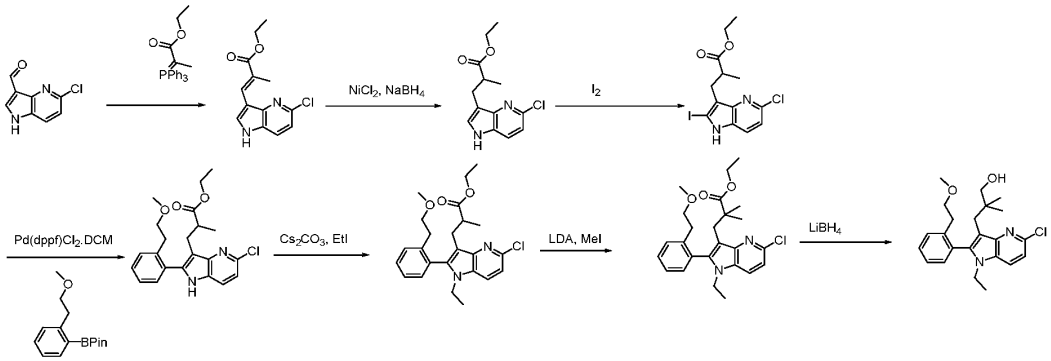
Synthesis of Intermediate 9.



Step 1. To a mixture of *tert*-butyl *N*-methyl-*N*-((*S*)-pyrrolidine-3-carbonyl)-L-valinate (500 mg, 1.8 mmol) in DCM (8 mL) at 5 °C was added TEA (533 mg, 5.3 mmol) followed by dropwise addition of 2-chloroethane-1-sulfonyl chloride (574 mg, 3.5 mmol) in DCM (2 mL) The mixture was stirred at 5 °C for 1 h, then diluted with H_2O (20 mL) and extracted with EtOAC (3 x 10 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl *N*-methyl-*N*-((*S*)-1-(vinylsulfonyl)pyrrolidine-3-carbonyl)-L-valinate (300 mg, 45% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{17}H_{30}N_2O_5S$ 374.2; found 375.2.

Step 2. To a mixture of *tert*-butyl *N*-methyl-*N*-((*S*)-1-(vinylsulfonyl)pyrrolidine-3-carbonyl)-L-valinate (123 mg, 0.33 mmol) in DCM (3 mL) at rt was added TFA (1 mL). The mixture was stirred at rt for 1 h, then concentrated under reduced pressure to give *N*-methyl-*N*-((*S*)-1-(vinylsulfonyl)pyrrolidine-3-carbonyl)-L-valine (130 mg, crude) as a solid, which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{22}N_2O_5S$ 318.1; found 319.1.

Synthesis of Intermediate 10.



Step 1. A mixture of 5-chloro-1*H*-pyrrolo[3,2-b]pyridine-3-carbaldehyde (8.5 g, 47.1 mmol) and ethyl 2-(triphenylphosphoranylidene)propionate (2.56 g, 70.7 mmol) in 1,4-dioxane (120 mL) was stirred at reflux for 4 h, then concentrated under reduced pressure. EtOAc (200 mL) was added and the mixture was washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column

chromatography to give ethyl (*E*)-3-(5-chloro-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2-methylacrylate (7.5 g, 60% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{13}ClN_2O_2$ 264.1; found 265.1.

Step 2. To a mixture of ethyl (*E*)-3-(5-chloro-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2-methylacrylate (7.5 g, 28.3 mmol) and $NiCl_2$ (4.8 g, 28.3 mmol) in 1:1 THF/MeOH (300 mL) was added $NaBH_4$ (21.5 g, 566 mmol) in 20 portions every 25 minutes. After complete addition, the mixture was stirred at rt for 30 min, then diluted with EtOAc (500 mL) and washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give ethyl 3-(5-chloro-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2-methylpropanoate (3.4 g, 45% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{15}ClN_2O_2$ 266.1; found 267.1.

Step 3. To a mixture of ethyl 3-(5-chloro-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2-methylpropanoate (7.0 g, 26.2 mmol) and AgOTf (6.7 g, 26.2 mmol) in THF (50 mL) at 0 °C was added I_2 (6.65 g, 26.2 mol). The mixture was stirred at 0 °C for 30 min then diluted with EtOAc (100 mL), washed with Na_2SO_3 (50 mL), brine (50 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give ethyl 3-(5-chloro-2-iodo-1*H*-pyrrolo[3,2-b]pyridin-3-yl)-2-methylpropanoate (6 g, 58% yield) as white solid. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{14}ClIN_2O_2$ 392.0; found 393.0.

Step 4. To a mixture of ethyl 3-(5-chloro-2-iodo-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2-methylpropanoate (6.0 g, 15.3 mmol) and 2-(2-(2-methoxyethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (5.6 g, 21.4 mmol) and K_2CO_3 (6.3 g, 45.9 mmol) in 1,4-dioxane (150 mL) and H_2O (30 mL) under an atmosphere of N_2 was added Pd(dppf)Cl_2.DCM (1.3 g, 3.1 mmol). The mixture was heated to 80 °C and stirred for 4 h, then diluted with EtOAc (500 mL), washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-(5-chloro-2-(2-(2-methoxyethyl)phenyl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2-methylpropanoate (5.5 g, 50% yield) as a viscous oil. LCMS (ESI): m/z [M+H] calc'd for $C_{22}H_{25}ClN_2O_3$ 400.2; found 401.2.

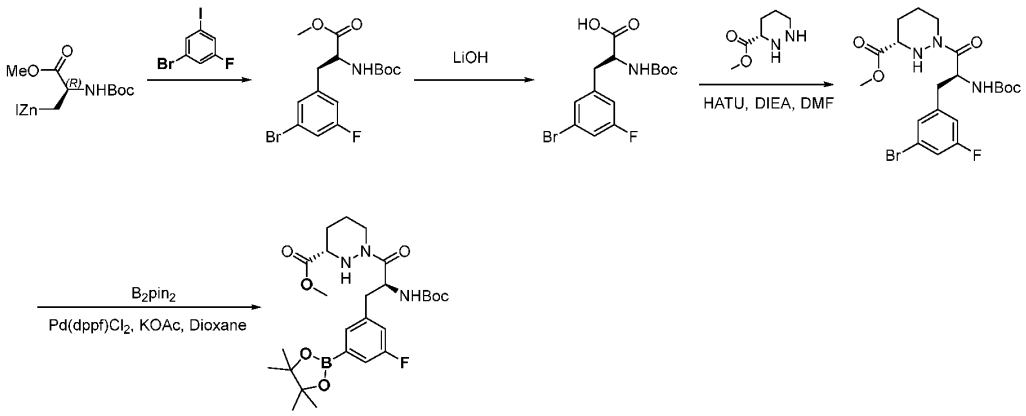
Step 5. A mixture of ethyl 3-(5-chloro-2-(2-(2-methoxyethyl)phenyl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2-methylpropanoate (5.5 g, 13.8 mmol), Cs_2CO_3 (8.9 g, 27.5 mmol), and EtI (3.5 g, 27.5 mmol) in DMF (30 mL) at rt was stirred for 10 h. The mixture was diluted with EtOAc (100 mL), washed with brine (20 mL x 4), dried over Na_2SO_4, filtered, and concentrated in vacuo. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give ethyl 3-(5-chloro-1-ethyl-2-(2-(2-methoxyethyl)phenyl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2-methylpropanoate (5.6 g, 95% yield) as a viscous oil. LCMS (ESI): m/z [M+H] calc'd for $C_{25}H_{31}ClN_2O_3$ 428.2; found 429.2.

Step 6. To a mixture of ethyl 3-(5-chloro-1-ethyl-2-(2-(2-methoxyethyl)phenyl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2-methylpropanoate (5.4 g, 12.6 mmol) in THF (50 mL) at -65 °C was added 2M LDA (25 mL, 50 mmol) and stirred at -65 °C for 1 h. MeI (3.6 g, 25 mmol) was added and the mixture was stirred at -65 °C for 2.5 h, then aqueous NH_4Cl and EtOAc (50 mL) were added. The aqueous and organic layers were separated and the organic layer was washed with brine (30 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give ethyl 3-(5-chloro-1-ethyl-2-(2-(2-

methoxyethyl)phenyl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2,2-dimethylpropanoate (3.2 g, 57% yield) as a viscous oil. LCMS (ESI): m/z [M+H] calc'd for $C_{25}H_{31}ClN_2O_3$ 442.2; found 443.2.

Step 7. To a mixture of ethyl 3-(5-chloro-1-ethyl-2-(2-(2-methoxyethyl)phenyl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2,2-dimethylpropanoate (1.0 g, 2.3 mmol) in THF (10 mL) at 5 °C was added $LiBH_4$ (196 mg, 9.0 mmol). The mixture was heated to 65 °C and stirred for 2 h then aqueous NH_4Cl and EtOAc (50 mL) added. The aqueous and organic layers were separated and the organic layer was washed with brine (30 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-(5-chloro-1-ethyl-2-(2-(2-methoxyethyl)phenyl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2,2-dimethylpropan-1-ol (0.75 g, 82% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{29}ClN_2O_2$ 400.2; found 401.2.

Intermediate 11: Methyl (3S)-1-{(2S)-2-(tert-butoxycarbonyl)amino-3-[3-fluoro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]propanoyl}-1,2-diazinane-3-carboxylate



Step 1. To a stirred solution of methyl (2R)-2-{[(tert-butoxy)carbonyl]amino}-3-(iodozincio)propanoate (12 g, 30 mmol, 1.2 eq) in DMF (100 mL) was added 1-bromo-3-fluoro-5-iodobenzene (7.5 g, 25 mmol, 1 eq) and $Pd(PPh_3)_2Cl_2$ (1.7 g, 2.5 mmol, 0.1 equiv) at 20°C under N_2 atmosphere. The resulting mixture was stirred for 2 hrs at 65°C under N_2 atmosphere. The reaction mixture was quenched with water and extracted with EA (200 mL x 2). The organic phase was washed with water (200 mL x 1) and brine (100 mL x 1) and concentrated to dryness to give a residue. The residue was purified by prep-TLC (PE/EA=10/1) to afford methyl 3-(3-bromo-5-fluorophenyl)-2-{[(tert-butoxy)carbonyl]amino}propanoate (6 g, 58% yield) as a colorless oil. LCMS (ESI) m/z = 398.1 $[M+Na]^+$, calculated for $C_{15}H_{19}BrFNO_4$: 375.0

Step 2. To a solution of methyl 3-(3-bromo-5-fluorophenyl)-2-{[(tert-butoxy)carbonyl]amino}propanoate(3.2 g, 8.5 mmol, 1 eq) in THF (50 mL) was added Lithium hydroxide(610.7 mg, 25.5 mmol, 3 eq) in H_2O (10 mL). Then the reaction mixture was stirred at 20 °C for 1 h. The mixture was adjusted to pH = 5.0 with 1 M HCl aqueous solution. The mixture was quenched with H_2O (150 mL) and extracted with EA (200 mL x 3). The combined organic layers was washed bine (50 mL), dried over Na_2SO_4 and concentrated to afford 3-(3-bromo-5-

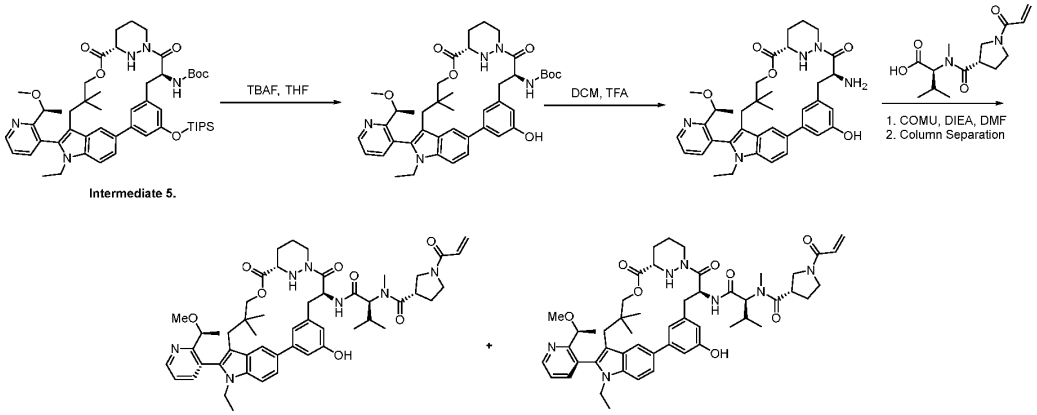
fluorophenyl)-2-{[(tert-butoxy)carbonyl]amino}propanoic acid (2.65 g, 68% yield) as a white solid. LCMS (ESI) m/z = 384.1 $[M+Na]^+$, calculated for $C_{14}H_{15}BrFNO_4$ MW: 361.0

Step 3. To a mixture of 3-(3-bromo-5-fluorophenyl)-2-{[(tert-butoxy)carbonyl]amino}propanoic acid(2.3 g, 6.4 mmol, 1 eq) and methyl (3S)-1,2-diazinane-3-carboxylate(1.66 g, 11.5 mmol, 1.8 eq) in DMF(150 mL) was added HATU(4.9 g, 12.8 mmol, 2 eq) and DIEA(16.5 g, 128 mmol, 20 eq) in DMF(50 mL) at 0 °C. Then the reaction mixture was stirred at 0 °C for 1 h. The mixture was quenched with H_2O (100 mL) and extracted with EA (300 mL x 3). The combined organic layers was washed bine (50 mL), dried over Na_2SO_4 and concentrated to give the residue, which was purified by Pre-HPLC eluting with acetonitrile in water (0.1%FA) from 60% to 70% in 10 minutes to give methyl (3S)-1-[(2S)-3-(3-bromo-5-fluorophenyl)-2-{[(tert-butoxy)carbonyl]amino}propanoyl]-1,2-diazinane-3-carboxylate(2.7 g, 78% yield) as a pale yellow solid. LCMS (ESI) m/z = 510.1 $[M+Na]^+$,calculated for $C_{20}H_{27}BrFNO_5$: 487.1.

Step 4. A mixture of methyl (S)-1-((S)-3-(3-bromo-5-fluorophenyl)-2-((tert-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (3 g, 6.16 mmol, 1 eq), 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (1.9 g, 7.4 mmol, 1.2 eq), KOAc (900 mg, 9.24 mmol, 1.5 eq) and Pd(dppf)Cl_2DCM (0.3 g, 0.37 mmol, 0.05 eq) in dioxane (50 mL) was heated at 100 °C for 17 h under N_2 atmosphere. The mixture was concentrated and purified by column chromatography (DCM/MeOH=100/1 to 40/1) to give methyl (3S)-1-(2S)-2-{(tert-butoxycarbonyl)amino-3-[3-fluoro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]propanoyl}-1,2-diazinane-3-carboxylate (2.6 g, 79% yield) as a yellow oil. LCMS (ESI) m/z = 536.2 $[M+H]^+$, calculated for $C_{26}H_{39}BFNO_7$: 535.3.

Compounds A341 and A342 may be prepared using methods disclosed herein via Intermediate 11.

Example A75. Synthesis of two atropisomers of (2*S*)-*N*-[(8*S*,14*S*,20*M*)-22-ethyl-4-hydroxy-21-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{*N*-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide.



Step 1. To a stirred mixture of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-

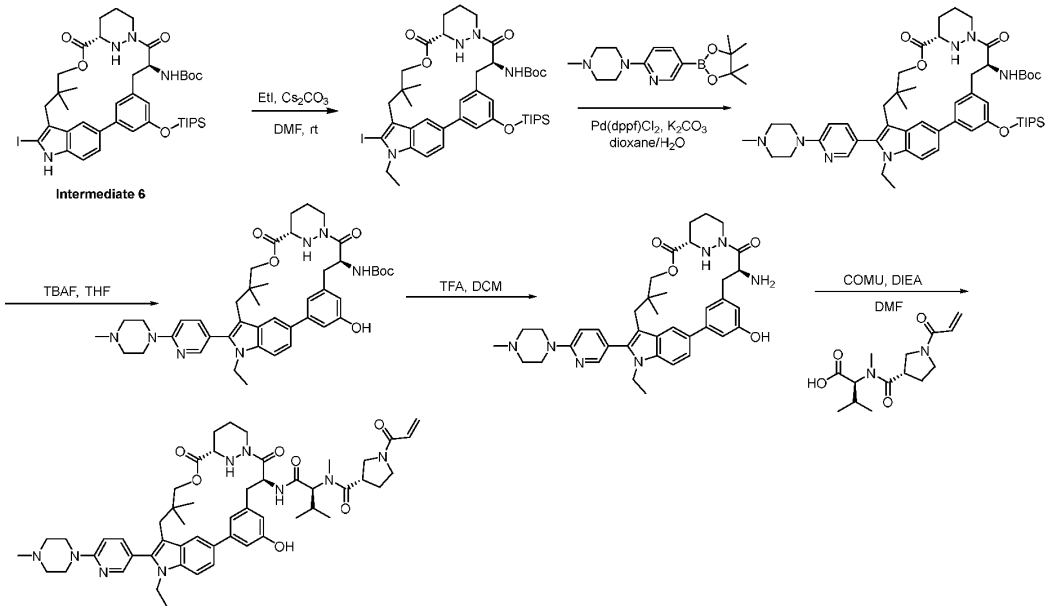
hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (18.0 g, 20.1 mmol) in THF (180 mL) at 0 °C was added a 1M solution of TBAF in THF (24.1 mL, 24.1 mmol). The mixture was stirred at 0 °C for 1 h, then diluted with brine (1.5 L) and extracted with EtOAc (3 x 1 L). The combined organic layers were washed with brine (2 x 500 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (11.5 g, 69% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{53}N_5O_7$ 739.4; found 740.4.

Step 2. To a stirred mixture of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (11.5 g, 15.5 mmol) in DCM (120 mL) at 0 °C was added TFA (60 mL, 808 mmol). The mixture was stirred at 0 °C for 1 h, then concentrated under reduced pressure and the residue again concentrated under reduced pressure with toluene (20 mL; repeated x3) to give (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (12 g, crude), which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{37}H_{45}N_5O_5$ 639.3; found 640.6.

Step 3. To a stirred mixture of (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (11.9 g, 18.6 mmol) in DMF (240 mL) at 0 °C under an atmosphere of N_2 was added DIPEA (48.1 g, 372 mmol), (2*S*)-3-methyl-2-[*N*-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido]butanoic acid (9.45 g, 33.5 mmol) and COMU (11.95 g, 27.9 mmol) in portions. The mixture was stirred ay 0 °C for 90 min, then diluted with brine (1.5 L) and extracted with EtOAc (3 x 1 L). The combined organic layers were washed with brine (2 x 500 mL), dried over anhydrous Na_2SO_4, and filtered. After filtration, the filtrate was concentrated under reduced pressure. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (x 2) to give two atropisomers of (2*S*)-*N*-[(8*S*,14*S*,20*M*)-22-ethyl-4-hydroxy-21-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{*N*-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide (2.7 g, 15.5%, yield) and (4.2 g, 24.7% yield) both as solids. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{65}N_7O_8$ 903.5; found 904.7; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.35 - 9.27 (m, 1H), 8.77 (dd, *J* = 4.7, 1.7 Hz, 1H), 7.95 (dq, *J* = 6.2, 2.0 Hz, 2H), 7.55 (ddd, *J* = 28.0, 8.2, 4.3 Hz, 3H), 7.08 (dd, *J* = 37.9, 6.2 Hz, 2H), 6.69 - 6.48 (m, 2H), 6.17 (ddt, *J* = 16.7, 7.2, 2.3 Hz, 1H), 5.74 - 5.62 (m, 1H), 5.43 - 5.34 (m, 1H), 5.12 - 5.00 (m, 1H), 4.25 (d, *J* = 12.3 Hz, 1H), 4.17 - 3.99 (m, 3H), 3.89 - 3.65 (m, 4H), 3.66 - 3.45 (m, 3H), 3.12 (s, 4H), 2.95 - 2.70 (m, 6H), 2.41 - 2.06 (m, 5H), 1.99 - 1.88 (m, 1H), 1.82 (d, *J* = 12.1 Hz, 2H), 1.54 (t, *J* = 12.0 Hz, 1H), 1.21 (dd, *J* = 6.3, 2.5 Hz, 3H), 1.11 (t, *J* = 7.1 Hz, 3H), 0.99 - 0.88 (m, 6H), 0.79 (ddd, *J* = 27.8, 6.7, 2.1 Hz, 3H), 0.48

(d, *J* = 3.7 Hz, 3H) and LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{65}N_7O_8$ 903.5; found 904.7; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34 - 9.27 (m, 1H), 8.77 (dd, *J* = 4.7, 1.7 Hz, 1H), 8.17 - 7.77 (m, 3H), 7.64 - 7.43 (m, 3H), 7.33 (d, *J* = 13.7 Hz, 1H), 7.05 - 6.94 (m, 1H), 6.69 - 6.41 (m, 2H), 6.26 - 5.94 (m, 1H), 5.73 - 5.63 (m, 1H), 5.50 - 5.20 (m, 2H), 4.40 - 4.15 (m, 3H), 4.00 - 3.40 (m, 9H), 3.11 (d, *J* = 4.4 Hz, 3H), 2.93 - 2.60 (m, 8H), 2.29 - 2.01 (m, 3H), 1.99 (s, 1H), 1.87 - 1.75 (m, 2H), 1.73 - 1.47 (m, 2H), 1.40 (d, *J* = 6.0 Hz, 3H), 1.01 - 0.88 (m, 6H), 0.85 - 0.65 (m, 7H), 0.56 (s, 3H).

Example A89. Synthesis of (2*S*)-N-[(8*S*,14*S*)-22-ethyl-4-hydroxy-18,18-dimethyl-21-[6-(4-methylpiperazin-1-yl)pyridin-3-yl]-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{*N*-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide



Step 1. To a mixture of tert-butyl ((6^{3}S,4S)-1^2-iodo-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (240.00 mg, 0.279 mmol, 1.00 equiv) and Cs_2CO_3 (182 mg, 0.558 mmol, 2 equiv) in DMF (5.00 mL) was added ethyl iodide (113.45 mg, 0.727 mmol, 2.60 equiv) dropwise at 0 °C. The reaction was stirred for 16 h at 25 °C. The resulting mixture was diluted with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (3 x 10 mL), and dried over anhydrous Na_2SO_4. The filtrate was concentrated under reduced pressure and the remaining residue was purified by silica gel column chromatography to afford tert-butyl ((6^{3}S,4S)-1^1-ethyl-1^2-iodo-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (190 mg, 77%yield) as a yellow solid.

Step 2. A mixture of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-iodo-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (500 mg, 0.54 mmol), 1-methyl-4-[5-(4,4,5,5-

tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl]piperazine (257 mg, 0.8 mmol), Pd(dppf)Cl_2 (83 mg, 0.11 mmol) and K_2CO_3 (156 mg, 1.1 mmol) in 1,4-dioxane (25 mL) and H_2O (5 mL) under an atmosphere of Ar was stirred at 80 °C for 2 h. The mixture was concentrated under reduced pressure and the residue was purified by prep-TLC to afford *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-10,10-dimethyl-1^2-(6-(4-methylpiperazin-1-yl)pyridin-3-yl)-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (400 mg, 76% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{53}H_{77}N_7O_6Si$ 935.6; found 936.6.

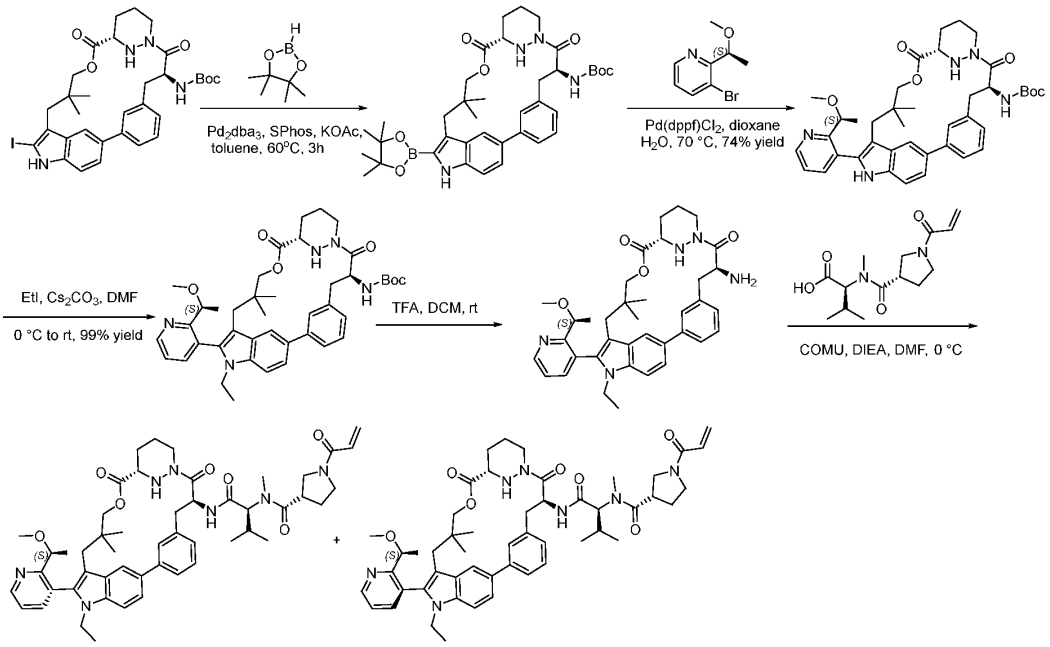
Step 3. A mixture of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-10,10-dimethyl-1^2-(6-(4-methylpiperazin-1-yl)pyridin-3-yl)-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (350 mg, 0.36 mmol) and 1M TBAF in THF (0.4 mL, 0.4 mmol) in THF (5 mL) was stirred at 0 °C for 1 h. The mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-10,10-dimethyl-1^2-(6-(4-methylpiperazin-1-yl)pyridin-3-yl)-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (290 mg, 100% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{57}N_7O_6$ 779.4; found 780.4.

Step 4. A mixture of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-10,10-dimethyl-1^2-(6-(4-methylpiperazin-1-yl)pyridin-3-yl)-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (300 mg, 0.37 mmol) in TFA (5 mL) and DCM (5 mL) was stirred at rt for 1 h. The mixture was concentrated under reduced pressure to give (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-10,10-dimethyl-1^2-(6-(4-methylpiperazin-1-yl)pyridin-3-yl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (300 mg, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{49}N_7O_4$ 679.4; found 680.3.

Step 5. To a mixture of (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-10,10-dimethyl-1^2-(6-(4-methylpiperazin-1-yl)pyridin-3-yl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (300 mg, 0.36 mmol) in DMF (3 mL) at 0 °C under an atmosphere of N_2 was added DIPEA (0.96 mL, 5.4 mmol) and (2*S*)-3-methyl-2-[*N*-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido]butanoic acid (213 mg, 0.72 mmol), followed by dropwise addition of COMU (243 mg, 0.56 mmol). H_2O was added at 0 °C and the mixture was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (3 x 10 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the crude residue was purified by Prep-HPLC to give (2*S*)-N-[(8*S*,14*S*)-22-ethyl-4-hydroxy-18,18-dimethyl-21-[6-(4-methylpiperazin-1-yl)pyridin-3-yl]-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{*N*-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide (45 mg, 13.2% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{53}H_{69}N_9O_7$ 943.5; found 944.8; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.39 - 9.23 (m, 1H), 8.64 - 8.60 (m, 1H), 8.19 - 8.16 (m, 1H), 8.15 (d, *J* = 6.2 Hz, 1H), 7.86 (s, 1H), 7.66 - 7.62 (m, 1H), 7.56-7.54 (m, 1H), 7.50 - 7.43 (m, 1H), 7.13 - 7.11 (m, 1H), 7.03 - 6.95 (m, 1H), 6.70 - 6.47 (m, 2H), 6.17 (ddt, *J* = 16.8, 6.4, 2.8 Hz, 1H), 5.76 - 5.63 (m, 1H),

5.45 - 5.33 (m, 1H), 5.11 (m, 1H), 4.75 - 4.72 (m, 1H), 4.28 - 4.24 (m, 1H), 4.11 - 3.98 (m, 4H), 3.91 - 3.76 (m, 1H), 3.73 - 3.71 (m, 1H), 3.59 - 3.56 (m, 7H), 3.51 - 3.40 (m, 2H), 3.08 - 2.94 (m, 1H), 2.94 - 2.92 (m, 2H), 2.92 - 2.87 (m, 2H), 2.86 - 2.83 (m, 2H), 2.80 - 2.65 (m, 2H), 2.83 - 2.82 (m, 3H), 2.28 - 2.25 (m, 3H), 2.08 - 2.05 (m, 2H), 2.02 - 1.96 (m, 1H), 1.87 - 1.78 (m, 1H), 1.74 - 1.66 (m, 1H), 1.56 - 1.48 (m, 1H), 1.11 - 1.08 (m, 4H), 0.99 - 0.92 (m, 2H), 0.89 - 0.87 (m, 5H), 0.82 - 0.73 (m, 2H).

Example A115. Synthesis of two atropisomers of (2*S*)-*N*-[(8*S*,14*S*,20*P*)-22-ethyl-21-{4-[(1*S*)-1-methoxyethyl]pyridin-3-yl}-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{*N*-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide



Step 1. A 1 L round-bottom flask was charged with tert-butyl ((6^{3}S,4S)-1^2-iodo-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (22.00 g, 32.042 mmol, 1.00 equiv), toluene (300.00 mL), $Pd_2(dba)_3$ (3.52 g, 3.845 mmol, 0.12 equiv), S-Phos (3.95 g, 9.613 mmol, 0.30 equiv), and KOAc (9.43 g, 96.127 mmol, 3.00 equiv) at room temperature. To the mixture was added 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (26.66 g, 208.275 mmol, 6.50 equiv) dropwise with stirring at room temperature. The resulting solution was stirred for 3 h at 60 °C. The resulting mixture was filtered, and the filter cake was washed with EtOAc. The filtrate was concentrated under reduced pressure and the remaining residue was purified by silica gel column chromatography to afford tert-butyl ((6^{3}S,4S)-10,10-dimethyl-5,7-dioxo-12-(4,4,5,5-tetramethyl- 1,3,2-dioxaborolan-2-yl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-

benzenacycloundecaphane-4-yl)carbamate (22 g, 90 %) as a light yellow solid. ESI-MS m/z = 687.3 $[M+H]^+$; Calculated MW: 686.4

Step 2. A mixture of *tert*-butyl ((6^3*S*,4*S*)-10,10-dimethyl-5,7-dioxo-1^2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (2.0 g, 2.8 mmol), 3-bromo-2-[(1*S*)-1-methoxyethyl]pyridine (0.60 g, 2.8 mmol), $Pd(dppf)Cl_2$ (0.39 g, 0.5 mmol), and K_3PO_4 (1.2 g, 6.0 mmol) in 1,4-dioxane (50 mL) and H_2O (10 mL) under an atmosphere of N_2 was heated to 70 °C and stirred for 2 h. The mixture was diluted with H_2O (50 mL) and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (3 x 50 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl ((6^3*S*,4*S*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (1.5 g, 74% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{40}H_{49}N_5O_6$ 695.4; found 696.5.

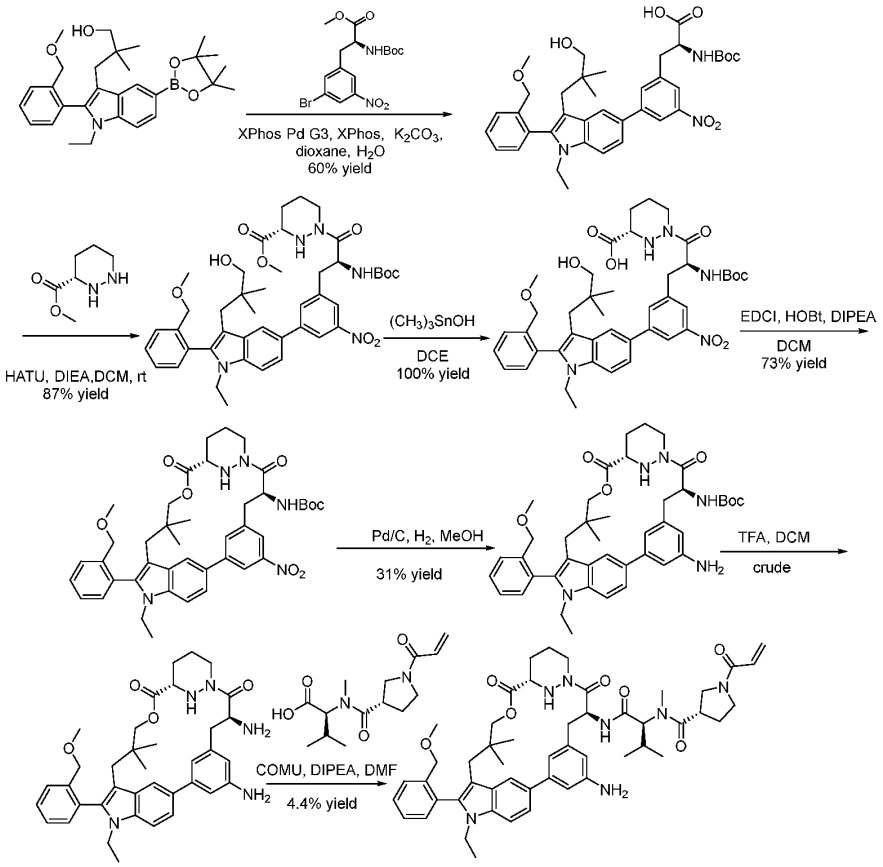
Step 3. A mixture of *tert*-butyl ((6^3*S*,4*S*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (1.5 g, 2.1 mmol), Cs_2CO_3 (2.1 g, 6.3 mmol), and ethyl iodide (0.43 mL, 5.1 mmol) in DMF (50 mL) was stirred at 0 °C for 16 h. The mixture was quenched at 0 °C with H_2O and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (3 x 50 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (1.5 g, 99% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{53}N_5O_6$ 723.4; found 724.6.

Step 4. A mixture of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (1.3 g, 1.7 mmol) in TFA (10 mL) and DCM (20 mL) was stirred at 0 °C for 2 h. The mixture was concentrated under reduced pressure to afford (6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (1.30 g, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{37}H_{45}N_5O_4$ 623.3; found 624.4.

Step 5. Into a 40-mL vial purged and maintained with an inert atmosphere of Ar, was placed (6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (250 mg, 0.4 mmol), (2*S*)-3-methyl-2-[*N*-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido]butanoic acid (226 mg, 0.8 mmol), DIPEA (774 mg, 6.0 mmol), and DMF (3 mL). A solution of COMU (257 mg, 0.6 mmol) in DMF (2 mL) was added at 0 °C and the resulting mixture was stirred at 0 °C for 1 h. The mixture was filtered, the filtrate was concentrated under reduced pressure, and the residue was purified by prep-HPLC to give two atropisomers of (2*S*)-*N*-[(8*S*,14*S*,20*P*)-22-ethyl-21-{4-[(1*S*)-1-methoxyethyl]pyridin-3-yl}-18,18-

dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{*N*-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide (56 mg, 15% yield) and (46 mg, 13% yield) both as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{65}N_7O_7$ 887.5; found 888.4; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.81 (s, 1H), 8.07 (s,1H), 8.05 - 7.96(m, 1H), 7.78 - 7.45 (m, 5H), 7.41 - 7.08(m, 2H), 6.66 -6.58 (m, 1H), 6.18 (d, *J* = 17.0 Hz, 1H), 5.75 - 5.67 (m, 1H), 5.46 - 5.31(m, 1H), 5.16 - 5.04 (m, 1H), 4.75 (dd, *J* = 10.9, 4.5 Hz, 1H), 4.31 - 4.21 (m, 2H), 4.11 - 3.95 (m, 3H), 3.87 - 3.71 (m, 5H), 3.74 - 3.54 (m, 3H), 3.11 (s, 4H), 2.95 (d, *J* = 9.7 Hz, 2H), 2.85 - 2.72 (m, 3H), 2.31 - 2.04 (m, 3H), 1.88 - 1.47(m, 2H), 1.24 - 1.21 (m, 3H), 1.16 - 1.08 (m, 3H), 1.03 - 0.91 (m, 6H), 0.85 -0.74 (m, 3H), 0.51 - 0.46 (m, 3H) and LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{65}N_7O_7$ 887.5; found 888.4; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (s, 1H), 8.71 - 8.63 (m, 0.5H), 8.23 - 8.17 (m, 0.5H), 8.00 (s, 1H), 7.85 (t, *J* = 9.9 Hz, 2H), 7.77 - 7.62 (m, 3H), 7.57 - 7.50 (m, 1H), 7.33 - 7.22 (m, 1H), 7.15 - 7.06 (m, 1H), 6.73 - 6.56 (m, 1H), 6.17 (ddd, *J* = 16.7, 6.1, 2.7 Hz, 1H), 5.76 - 5.64 (m, 1H), 5.49 - 5.29 (m, 2H), 4.70 (dd, *J* = 10.8, 3.5 Hz, 1H), 4.33 - 4.22 (m, 3H), 4.14 - 3.95 (m, 2H), 3.86 - 3.77 (m, 1H), 3.72 - 3.65 (m, 2H), 3.61 (t, J = 10.6 Hz, 3H), 3.46 - 3.42 (m, 1H), 3.13 (d, *J* = 4.8 Hz, 3H), 2.99 (d, *J* = 14.4 Hz, 1H), 2.95 - 2.70 (m, 6H), 2.24 - 1.99 (m, 4H), 1.95 - 1.44 (m, 4H), 1.40 (d, *J* = 6.1 Hz, 3H), 0.98 - 0.87 (m, 6H), 0.86 - 0.64 (m, 6H), 0.64 - 0.54 (m, 3H).

Example A2. Synthesis of (2*S*)-*N*-[(8*S*,14*S*)-4-amino-22-ethyl-21-[2-(2-methoxyethyl)phenyl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{N-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide



Step 1. Into a 25 mL sealed tube were added 3-[1-ethyl-2-[2-(methoxymethyl)phenyl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-3-yl]-2,2-dimethylpropan-1-ol (590 mg, 1.2 mmol), methyl (2*S*)-3-(3-bromo-5-nitrophenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (747 mg, 1.9 mmol), XPhos Pd G3 (105 mg, 0.12 mmol), XPhos (71 mg, 0.15 mmol), K_2CO_3 (427 mg, 3.1 mmol), and 1,4-dioxane (2 mL) under an atmosphere of N_2 at rt. The mixture was heated to 60 °C and stirred overnight, then cooled and H_2O added. The mixture was extracted with EtOAc (3 x 20 mL) and the combined organic layers were washed with brine (1 x 20 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)phenyl]indol-5-yl]-5-nitrophenyl]propanoic acid (500 mg, 61% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{37}H_{45}N_3O_8$ 659.3; found 660.4.

Step 2. A mixture of (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)phenyl]indol-5-yl]-5-nitrophenyl]propanoic acid (500 mg, 0.79 mmol), methyl (3*S*)-1,2-diazinane-3-carboxylate (164 mg, 1.1 mmol), DCM (6 mL), DIPEA (294 mg,

2.3 mmol) and HATU (432 mg, 1.1 mmol) was stirred at 0 °C for 1 h under an atmosphere of air. H_2O was added and the mixture was extracted with DCM (3 x 20 mL), then the combined organic layers were dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)phenyl]indol-5-yl]-5-nitrophenyl]propanoyl]-1,2-diazinane-3-carboxylate (520 mg, 87% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{55}N_5O_9$ 785.4; found 786.8

Step 3. Into a 40 mL sealed tube were added methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)phenyl]indol-5-yl]-5-nitrophenyl]propanoyl]-1,2-diazinane-3-carboxylate (510 mg, 0.65 mmol), DCE (5 mL) and trimethyltin hydroxide (587 mg, 3.3 mmol) at rt under an atmosphere of air. The mixture was heated to 60 °C and stirred overnight, cooled, and diluted with DCM (20 mL). The mixture was washed with 0.1 N $KHSO_4$ (3 x 20 mL), dried over anhydrous Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure to give (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)phenyl]indol-5-yl]-5-nitrophenyl]propanoyl]-1,2-diazinane-3-carboxylic acid (500 mg, 100%) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{53}N_5O_9$ 771.4; found 772.7.

Step 4. A mixture of (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)phenyl]indol-5-yl]-5-nitrophenyl]propanoyl]-1,2-diazinane-3-carboxylic acid (490 mg, 0.64 mmol), DCM (100 mL), DIPEA (2.5 g, 19.0 mmol), HOBT (429 mg, 3.2 mmol), and EDCI (3.65 g, 19.0 mmol) at room temperature was stirred at rt overnight under an atmosphere of air. H_2O was added and the mixture was extracted with DCM (3 x 60 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-(methoxymethyl)phenyl)-10,10-dimethyl-2^5-nitro-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (350 mg, 73% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{51}N_5O_8$ 753.4; found 754.2.

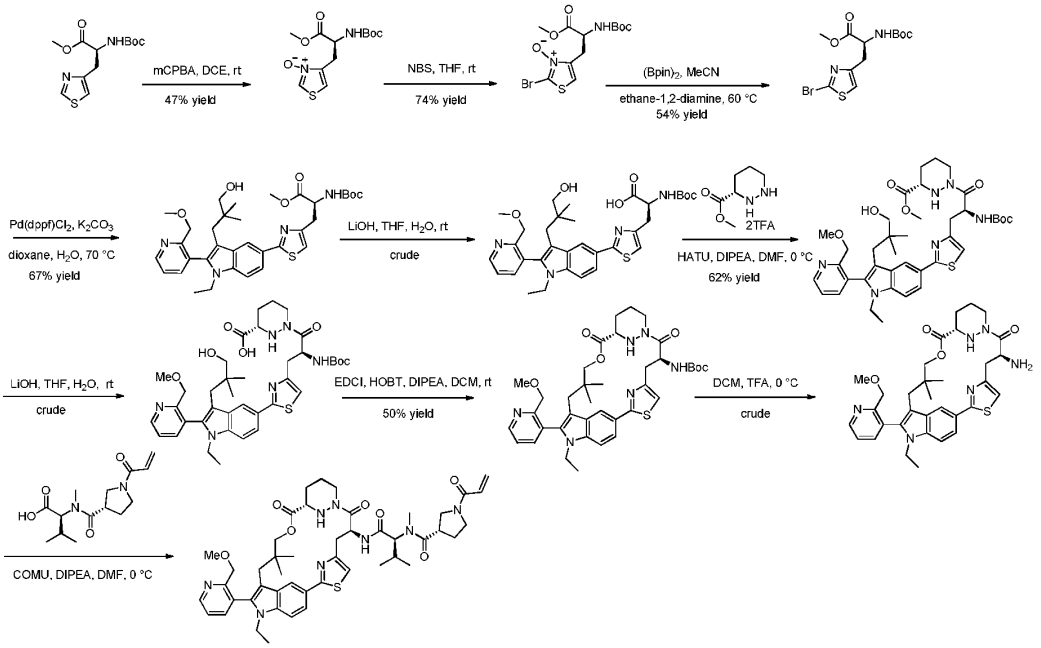
Step 5. A mixture of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-(methoxymethyl)phenyl)-10,10-dimethyl-2^5-nitro-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (200 mg, 0.27 mmol), MeOH (4 mL), and Pd on carbon (20 mg) was stirred at rt for 2 h under an atmosphere of H_2. The mixture was filtered, the filter cake was washed with MeOH (3 x 5 mL), and the filtrate was concentrated under reduced pressure to give *tert*-butyl ((6^3*S*,4*S*)-2^5-amino-1^1-ethyl-1^2-(2-(methoxymethyl)phenyl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (60 mg, 31% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{53}N_5O_6$ 723.4; found 724.4.

Step 6. Into an 8 mL vial were added *tert*-butyl ((6^3*S*,4*S*)-2^5-amino-1^1-ethyl-1^2-(2-(methoxymethyl)phenyl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (50 mg, 0.07 mmol), DCM (1 mL), and TFA (158 mg, 1.4 mmol) at 0 °C under an atmosphere of air. The mixture was stirred for at 0 °C for 2 h then concentrated under reduced pressure to give (6^3*S*,4*S*)-2^5,4-diamino-

1^1-ethyl-1^2-(2-(methoxymethyl)phenyl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (45 mg) as a solid, which was used directly in the next step directly without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{37}H_{45}N_5O_4$ 623.3; found 624.4.

Step 7. Into an 8 mL vial were added (6^3*S*,4*S*)-2^5,4-diamino-1^1-ethyl-1^2-(2-(methoxymethyl)phenyl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (40 mg, 0.06 mmol), DMF (1 mL), DIPEA (75 mg, 0.58 mmol), and COMU (41 mg, 0.1 mmol) at 0 °C under an atmosphere of air. The mixture was stirred at 0 °C for 1 h, then H_2O added. The mixture was extracted with EtOAc (3 x 30 mL), the combined organic layers were concentrated under reduced pressure, and purified by prep-HPLC to give (2*S*)-*N*-[(8*S*,14*S*)-4-amino-22-ethyl-21-[2-(2-methoxyethyl)phenyl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.$1^{2,6}$.$1^{10,14}$.$0^{23,27}$]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{N-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide (2.5 mg, 4.4% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{65}N_7O_7$ 887.5; found 888.6; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.74 - 8.55 (m, 1H), 7.89 (d, *J* = 9.6 Hz, 1H), 7.66 - 7.53 (m, 1H), 7.57 - 7.47 (m, 6H), 7.32 (t, *J* = 6.4 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 2H), 6.70 - 6.55 (m, 1H), 6.24 - 6.12 (m, 1H), 5.69 (ddd, *J* = 14.8, 8.0, 3.9 Hz, 1H), 5.41 (s, 1H), 5.09 - 4.80 (m, 2H), 4.26 (d, *J* = 10.1 Hz, 2H), 4.19 (s, 2H), 4.17 - 4.06 (m, 1H), 4.02 (dd, *J* = 12.0, 3.9 Hz, 1H), 3.92 (d, *J* = 8.0 Hz, 3H), 3.78 (d, *J* = 8.7 Hz, 5H), 3.29 (s, 2H), 3.14 (d, *J* = 1.9 Hz, 1H), 2.98 - 2.92 (m, 1H), 2.87 - 2.68 (m, 3H), 2.62 (d, *J* = 12.5 Hz, 3H), 2.15 - 1.99 (m, 4H), 1.80 (s, 1H), 1.68 - 1.53 (m, 2H), 1.08 (t, *J* = 7.1 Hz, 1H), 0.98 - 0.88 (m, 6H), 0.82 (dd, *J* = 23.3, 16.4 Hz, 3H), 0.74 (t, *J* = 7.2 Hz, 3H), 0.44 (s, 2H), 0.43 (s, 3H).

Example A118. Synthesis of (2*S*)-N-[(7*S*,13*S*)-21-ethyl-20-[2-(methoxymethyl)pyridin-3-yl]-17,17-dimethyl-8,14-dioxo-15-oxa-3-thia-9,21,27,28-tetraazapentacyclo[17.5.2.1^{2},^{5}.1^{9},^{13}.0^{22},^{26}]octacosa-1(25),2(28),4,19,22(26),23-hexaen-7-yl]-3-methyl-2-{*N*-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide



Step 1. A mixture of methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(1,3-thiazol-4-yl)propanoate (2.08 g, 7.26 mmol) and mCPBA (1.88 g, 10.9 mmol) in DCE (15 mL) at 0 °C under an atmosphere of N_2 was diluted with DCM (100 mL). The mixture was allowed to warm to rt and stirred for 16 h, then diluted with DCM, washed with H_2O (1 x 30 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 4-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methoxy-3-oxopropyl]-1,3-thiazol-3-ium-3-olate (1.15 g, 47% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{12}H_{18}N_2O_5S$ 302.1; found 303.2.

Step 2. To a mixture of 4-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methoxy-3-oxopropyl]-1,3-thiazol-3-ium-3-olate (1.15 g, 3.8 mmol) in THF at 0 °C under an atmosphere of N_2 was added NBS (0.74 g, 4.2 mmol) dropwise. The mixture was allowed to warm to rt and stirred for 2 h, then diluted with H_2O (500mL) and extracted with EtOAc (3 x 500 mL). The combined organic layers were washed with water (2 x 30 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 2-bromo-4-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methoxy-3-oxopropyl]-1,3-thiazol-3-ium-3-olate (1.2 g, 74% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{12}H_{17}BrN_2O_5S$ 380.0; found 381.0.

Step 3. To a stirred mixture of 2-bromo-4-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-methoxy-3-oxopropyl]-1,3-thiazol-3-ium-3-olate (1.2 g, 3.2 mmol) and 4,4,5,5-tetramethyl-2-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (1.04 g, 4.1 mmol) in MeCN at 70 °C under an

atmosphere of N_2 was added ethane-1,2-diamine (1.89 g, 31.5 mmol) in portions. The mixture was cooled to 60 °C and the mixture was stirred overnight, then diluted with water (500 mL) and extracted with EtOAc (3 x 400 mL). The combined organic layers were washed with brine (1 x 50 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (2*S*)-3-(2-bromo-1,3-thiazol-4-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (653 mg, 54% yield) as a solid.

Step 4. A 50 mL sealed tube was charged with 3-[1-ethyl-2-[2-(methoxymethyl)pyridin-3-yl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-3-yl]-2,2-dimethylpropan-1-ol (1.00 g, 2.1 mmol), K_2CO_3 (727 mg, 5.2 mmol), Pd(dppf)Cl_2 (153 mg, 0.21 mmol), and 2,4-dibromo-1,3-thiazole (1.0 g, 4.2 mmol) at rt under an atmosphere of N_2, then 1,4-dioxane (1.0 mL) and H_2O (0.20 mL) were added. The mixture was heated to 70 °C and stirred for 4 h, then cooled, diluted with H_2O (100 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine (3 x 100 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-[5-(4-bromo-1,3-thiazol-2-yl)-1-ethyl-2-[2-(methoxymethyl)pyridin-3-yl]indol-3-yl]-2,2-dimethylpropan-1-ol (727 mg, 67% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{34}H_{44}N_4O_6S$ 636.3; found 637.3.

Step 5. To a stirred mixture of methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[2-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-4-yl]propanoate (636 mg, 1.0 mmol) and LiOH.H_2O (126 mg, 3.0 mmol) in THF at 0 °C under an atmosphere of N_2 was added H_2O (1.24 mL) portionwise. The mixture was allowed to warm to rt and stirred for 1 h, then diluted with water (300 mL) and extracted with EtOAc (3 x 300 mL). The combined organic layers were washed with brine (1 x 100 mL), dried over anhydrous Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure to give (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[2-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-4-yl]propanoic acid (622 mg, crude), which was used in the next step directly without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{33}H_{42}N_4O_6S$ 622.3; found 623.2.

Step 6. To a stirred mixture of (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[2-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-4-yl]propanoic acid (622 mg, 1.0 mmol) and methyl (3*S*)-1,2-diazinane-3-carboxylate (288 mg, 2.0 mmol) in DMF at 0 °C under an atmosphere of N_2 was added HATU (570 mg, 1.5 mmol). The mixture was stirred at 0 °C for 1 h, then diluted with EtOAc and washed with H_2O (1 x 10 mL), dried over anhydrous Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure to give methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[2-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-4-yl]propanoyl]-1,2-diazinane-3-carboxylate (550 mg, 62% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{52}N_6O_7S$ 748.4; found 749.6.

Step 7. (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[2-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-4-yl]propanoyl]-1,2-diazinane-3-carboxylic acid was synthesized in a manner similar to (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid except methyl (3*S*)-1-[(2*S*)-

2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate was substituted with methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[2-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-4-yl]propanoyl]-1,2-diazinane-3-carboxylate. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{50}N_6O_7S$ 734.3; found 735.3.

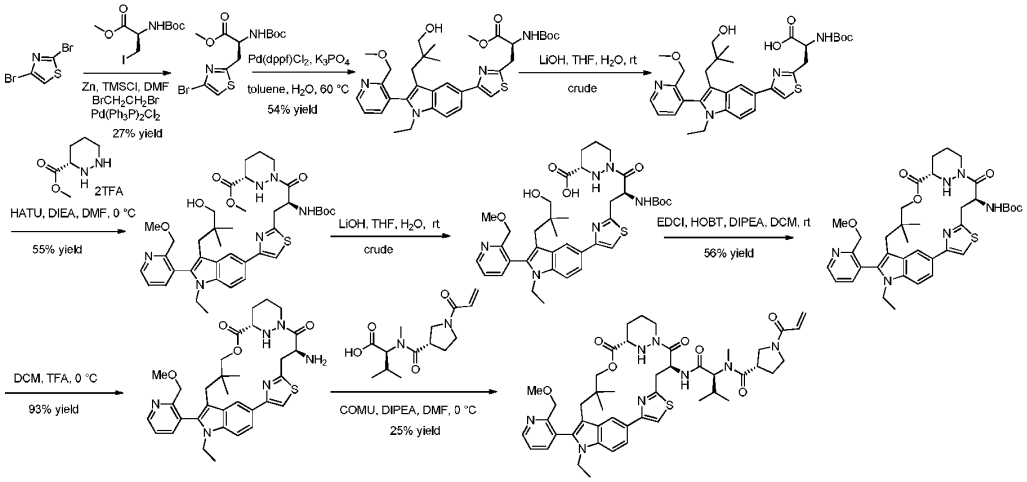
Step 8. *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(2,4)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate was synthesized in a manner similar to *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate except (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid was substituted with (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[2-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-4-yl]propanoyl]-1,2-diazinane-3-carboxylic acid. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{48}N_6O_6S$ 716.3; found 717.4.

Step 9. To a stirred mixture of *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(2,4)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (253 mg) in DCM at 0 °C under an atmosphere of N_2 was added TFA (1.0 mL) dropwise. The mixture was stirred at 0 °C for 1 h, then concentrated under reduced pressure and then repeated using toluene (20 mL x 3) to give (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(2,4)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (253 mg, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{33}H_{40}N_6O_4S$ 616.3; found 617.3.

Step 10. (2*S*)-N-[(7*S*,13*S*)-21-ethyl-20-[2-(methoxymethyl)pyridin-3-yl]-17,17-dimethyl-8,14-dioxo-15-oxa-3-thia-9,21,27,28-tetraazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacosa-1(25),2(28),4,19,22(26),23-hexaen-7-yl]-3-methyl-2-{*N*-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide was synthesized in a manner similar to (2*S*)-*N*-[(8*S*,14*S*)-4-amino-22-ethyl-21-[2-(2-methoxyethyl)phenyl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{N-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide except (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione was substituted with (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(2,4)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione. LCMS (ESI): m/z [M+H] calc'd for $C_{47}H_{60}N_8O_7S$ 880.4; found 881.6; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.75 (m, 1H), 8.55 (d, *J* = 6.7 Hz, 1H), 8.32 (d, *J* = 8.3 Hz, 1H), 7.99 (d, *J* = 7.7 Hz, 1H), 7.65 - 7.51 (m, 3H), 7.11 - 6.92 (m, 1H), 6.72 - 6.56 (m, 1H), 6.18 (dd, *J* = 16.8, 2.9 Hz, 1H), 5.82 - 5.65 (m, 1H), 5.61 - 5.46 (m, 1H), 5.02 (dd, *J* = 24.2, 12.2 Hz, 1H), 4.69 (d, *J* = 10.9 Hz, 1H), 4.37 - 4.11 (m, 5H), 4.05 - 3.79 (m, 4H), 3.76 - 3.50 (m, 6H), 3.47 (s, 2H), 3.08 (s, 3H), 3.04 (s, 1H), 2.98 (d, *J* = 1.9 Hz, 1H), 2.95 (d, *J* = 3.6 Hz, 2H), 2.83 (d, *J* = 2.0 Hz,

2H), 2.24 - 2.03 (m, 4H), 1.81 (s, 2H), 1.56 (s, 1H), 1.11 (t, *J* = 7.0 Hz, 2H), 1.02 - 0.87 (m, 8H), 0.80 (dd, *J* = 24.6, 6.6 Hz, 3H), 0.41 (s, 2H), 0.31 (s, 1H).

Example A194. Synthesis of (2*S*)-N-[(7S,13*S*)-21-ethyl-20-[2-(methoxymethyl)pyridin-3-yl]-17,17-dimethyl-8,14-dioxo-15-oxa-4-thia-9,21,27,28-tetraazapentacyclo[17.5.2.1^{2},^{5}.1^{9},^{13}.0^{22},^{26}]octacosa-1(25),2,5(28),19,22(26),23-hexaen-7-yl]-3-methyl-2-{*N*-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide



Step 1. A mixture of Zn (1.2 g, 182 mmol) and 1,2-dibromoethane (1.71 g, 9.1 mmol) and DMF (50 mL) was stirred for 30 min at 90 °C under an atmosphere of Ar. The mixture was allowed to rt, then TMSCl (198 mg, 1.8 mmol) was added dropwise over 30 min at rt. Methyl (2*R*)-2-[(*tert*-butoxycarbonyl) amino]-3-iodopropanoate (10.0 g, 30.4 mmol) in DMF (100 mL) was added dropwise over 10 min at rt. The mixture was heated to 35 °C and stirred for 2 h, then a mixture of 2,5-dibromo-1,3-thiazole (1.48 g, 60.8 mmol) and $Pd(PPh_3)2Cl_2$ (2.1 g, 3.0 mmol) in DMF (100 mL) was added dropwise .The mixture was heated to 70 °C and stirred for 2h, then filtered and the filtrate diluted with EtOAc (1 L) and washed with H_2O (3 x 1 L), dried with anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (2*S*)-3-(5-bromo-1,3-thiazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (3 g, 27 % yield) as a semi-solid. LCMS (ESI): m/z [M+H] calc'd for $C_{12}H_{17}BrN_2O_4S$ 364.0; found 365.1.

Step 2. Into a 20mL sealed tube were added 3-[1-ethyl-2-[2-(methoxymethyl)pyridin-3-yl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-3-yl]-2,2-dimethylpropan-1-ol (100 mg, 0.21 mmol), K_3PO_4 (111 mg, 0.52 mmol), $Pd(dppf)Cl_2$ (15 mg, 0.02 mmol), methyl (2*S*)-3-(4-bromo-1,3-thiazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (153 mg, 0.42 mmol), toluene (1 mL), and H_2O (0.2 mL) at rt under an atmosphere of N_2. The mixture was heated to 60 °C and stirred for 3 h, cooled, diluted with H_2O (10 mL) and extracted with EtOAc (10 mL x 3). The combined organic layers were washed with brine (3 x 10 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[4-[1-ethyl-3-(3-hydroxy-2,2-

dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-2-yl]propanoate (72 mg, 54% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{34}H_{44}N_4O_6S$ 636.3; found 637.2.

Step 3. A mixture of methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[4-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-2-yl]propanoate (40 mg, 0.06 mmol) and LiOH.H_2O (unspecified) in THF (1 mL) and H_2O (0.2 mL) was stirred at rt under an atmosphere of N_2 for 2 h. The mixture was acidified to pH 5 with aqueous $NaHSO_4$ and extracted with EtOAc (3 x 10mL). The combined organic layers were washed with brine (3 x 10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to give (2*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-(methoxymethyl)pyridin-3-yl)-1*H*-indol-5-yl)thiazol-2-yl)propanoic acid. The crude product was used in the next step directly without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{33}H_{42}N_4O_6S$ 622.3; found 623.3.

Step 4. Methyl (3*S*)-1-((2*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-(methoxymethyl)pyridin-3-yl)-1*H*-indol-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylate was synthesized in a manner similar to methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[2-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-4-yl]propanoyl]-1,2-diazinane-3-carboxylate except (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[2-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-4-yl]propanoic acid was substituted with (2*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-(methoxymethyl)pyridin-3-yl)-1*H*-indol-5-yl)thiazol-2-yl)propanoic acid. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{52}N_6O_7S$ 748.4; found 749.4.

Step 5. (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[4-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-2-yl]propanoyl]-1,2-diazinane-3-carboxylic acid was synthesized in a manner similar to (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid except methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate was substituted with methyl (3*S*)-1-((2*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-(methoxymethyl)pyridin-3-yl)-1*H*-indol-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylate. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{50}N_6O_7S$ 734.3; found 735.4.

Step 6. *Tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-12-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate was synthesized in a manner similar to *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate except (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid was substituted with (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[4-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-

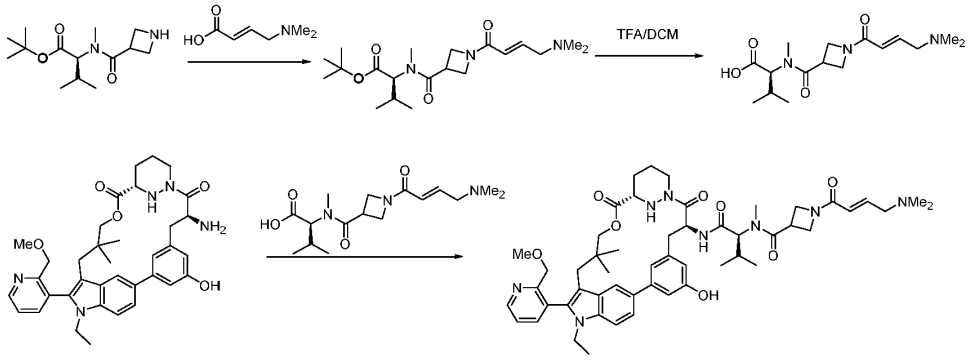
(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-2-yl]propanoyl]-1,2-diazinane-3-carboxylic acid. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{48}N_6O_6S$ 716.3; found 717.3.

Step 7. (6^3S,4*S*,*Z*)-4-amino-11^1-ethyl-12^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11^1H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione was synthesized in a manner similar to (6^3S,4*S*,*Z*)-4-amino-11^1-ethyl-12^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11^1H-8-oxa-2(2,4)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione except *tert*-butyl ((6^3S,4*S*,*Z*)-11^1-ethyl-12^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11^1H-8-oxa-2(2,4)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate was substituted with *tert*-butyl ((6^3S,4*S*,*Z*)-11^1-ethyl-12-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11^1H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate. LCMS (ESI): m/z [M+Na] calc'd for $C_{33}H_{40}N_6O_4SNa$ 639.3; found 640.3.

Step 8. (2*S*)-N-[(7S,13*S*)-21-ethyl-20-[2-(methoxymethyl)pyridin-3-yl]-17,17-dimethyl-8,14-dioxo-15-oxa-4-thia-9,21,27,28-tetraazapentacyclo[17.5.2.$1^{2,5}$.$1^{9,13}$.$0^{22,26}$]octacosa-1(25),2,5(28),19,22(26),23-hexaen-7-yl]-3-methyl-2-{*N*-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide was synthesized in a manner similar to (2*S*)-N-[(7*S*,13*S*)-21-ethyl-20-[2-(methoxymethyl)pyridin-3-yl]-17,17-dimethyl-8,14-dioxo-15-oxa-3-thia-9,21,27,28-tetraazapentacyclo[17.5.2.$1^{2,5}$.$1^{9,13}$.$0^{22,26}$]octacosa-1(25),2(28),4,19,22(26),23-hexaen-7-yl]-3-methyl-2-{*N*-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide except (6^3S,4*S*)-4-amino-11^1-ethyl-2^5-hydroxy-12^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione was substituted with (6^3S,4*S*,*Z*)-4-amino-11^1-ethyl-12^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11^1H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione. LCMS (ESI): m/z [M+H] calc'd for $C_{47}H_{60}N_8O_7S$ 880.4; found 881.5; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.70 (dt, *J* = 16.2, 8.1 Hz, 1H), 8.54 (ddd, *J* = 6.6, 4.7, 1.7 Hz, 1H), 8.50 (m, 1H), 7.96 (d, *J* = 7.8 Hz, 1H), 7.88 (t, *J* = 2.1 Hz, 2H), 6.70 - 6.57 (m, 2H), 6.24 - 6.13 (m, 2H), 5.75 (m, 1H), 5.55 (t, *J* = 7.3 Hz, 1H), 5.46 (d, *J* = 8.5 Hz, 1H), 5.14 (d, *J* = 13.0 Hz, 1H), 4.84 - 4.75 (m, 1H), 4.35 (d, *J* = 10.7 Hz, 1H), 4.28 - 4.19 (m, 4H), 3.91 (s, 3H), 3.87 (dd, *J* = 10.4, 8.1 Hz, 1H), 3.78 - 3.70 (m, 2H), 3.63 (t, *J* = 8.8 Hz, 2H), 3.61 - 3.49 (m, 2H), 2.87 (d, *J* = 1.1 Hz, 2H), 2.79 (s, 1H), 2.38 (s, 1H), 2.18 (s, 1H), 2.13 (d, *J* = 10.7 Hz, 4H), 1.96 (s, 2H), 1.81 (s, 1H), 1.53 (s,2H), 1.11 (t, *J* = 7.1 Hz, 2H), 0.99 - 0.89 (m, 7H), 0.93 - 0.81 (m, 2H), 0.78 (d, *J* = 6.6 Hz, 2H), 0.28 (s, 3H).

Example A71. Synthesis of (2*S*)-2-(1-{1-[(2*E*)-4-(dimethylamino)but-2-enoyl]azetidin-3-yl}-*N*-methylformamido)-*N*-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[2-(methoxymethyl)pyridin-3-yl]-

18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methylbutanamide



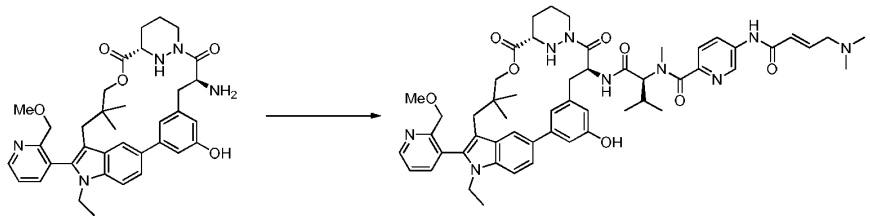
Step 1. To a mixture of *tert*-butyl *N*-(azetidine-3-carbonyl)-*N*-methyl-L-valinate (350 mg, 1.3 mmol) and (2*E*)-4-(dimethylamino)but-2-enoic acid (201 mg, 1.56 mmol) in DCM (8 mL) at 5 °C was added a solution of T3P, 50% in EtOAc (827 mg, 2.6 mmol) and DIPEA (1.7 g, 13 mmol) in DCM (2 mL). The mixture was stirred for 1 h, then diluted with EtOAc (20 mL) and H_2O (20 mL). The aqueous and organic layers were separated and the organic layer was washed with H_2O (3 × 10 mL), brine (10 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by prep-HPLC to give *tert*-butyl (*E*)-*N*-(1-(4-(dimethylamino)but-2-enoyl)azetidine-3-carbonyl)-*N*-methyl-L-valinate (200 mg, 39% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{20}H_{35}N_3O_4$ 381.3; found 382.3.

Step 2. To a mixture of *tert*-butyl (*E*)-*N*-(1-(4-(dimethylamino)but-2-enoyl)azetidine-3-carbonyl)-*N*-methyl-L-valinate (190 mg, 0.32 mmol) in DCM (3 mL) at rt was added TFA (1 mL). The mixture was stirred at rt for 1 h, then concentrated under reduced pressure to give (*E*)-*N*-(1-(4-(dimethylamino)but-2-enoyl)azetidine-3-carbonyl)-*N*-methyl-L-valine (190 mg, 90%) as a solid, which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{16}H_{27}N_3O_4$ 325.2; found 326.2.

Step 3. To a mixture of (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (172 mg, 0.27 mmol) and (*E*)-*N*-(1-(4-(dimethylamino)but-2-enoyl)azetidine-3-carbonyl)-*N*-methyl-L-valine (105 mg, 0.32 mmol) in DMF (2 mL) at 5 °C was added a mixture of HATU (133 mg, 0.297 mmol) and DIPEA (348 mg, 2.7 mmol) in DMF (1 mL). The mixture was stirred for 1h, then diluted with EtOAc (20 mL) and H_2O (20 mL). The aqueous and organic layers were separated, and the organic layer was washed with H_2O (3 × 10 mL), brine (10 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by prep-TLC to give (2*S*)-2-(1-{1-[(2*E*)-4-(dimethylamino)but-2-enoyl]azetidin-3-yl}-*N*-methylformamido)-*N*-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[2-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methylbutanamide (4.8 mg, 2% yield over 2 steps) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{52}H_{68}N_8O_8$ 932.5; found 933.5; ^{1}H NMR (400 MHz, CD_3OD) δ 8.71 (d, *J* = 3.2 Hz, 1H), 8.50 (s, 1.5H), 8.08 - 7.85 (m, 2H), 7.65 - 7.44 (m, 3H), 7.32 - 7.14 (m, 1H), 7.07 - 6.95 (m, 1H), 6.80 (dt, *J*

= 22.1, 6.8 Hz, 1H), 6.55 (d, *J* = 35.8 Hz, 1H), 6.30 (d, *J* = 15.4 Hz, 1H), 5.56 (dd, *J* = 13.8, 6.7 Hz, 1H), 4.76 (dd, *J* = 19.8, 10.5 Hz, 1H), 4.54 (dd, *J* = 15.9, 7.5 Hz, 2H), 4.48 - 4.38 (m, 2H), 4.36 - 4.23 (m, 3H), 4.22 - 4.14 (m, 1H), 3.96 (qd, *J* = 15.6, 7.9 Hz, 3H), 3.77 (ddd, *J* = 25.8, 23.4, 11.9 Hz, 2H), 3.58 (dd, *J* = 17.2, 8.3 Hz, 2H), 3.38 (s, 2H), 3.25 - 3.11 (m, 3H), 3.05 - 2.94 (m, 1H), 2.94 - 2.81 (m, 4H), 2.73 (dd, *J* = 20.9, 11.0 Hz, 1H), 2.45 (d, *J* = 6.9 Hz, 5H), 2.32 - 2.07 (m, 3H), 1.92 (d, *J* = 13.2 Hz, 1H), 1.72 (s, 1H), 1.64 - 1.51 (m, 1H), 1.18 (t, *J* = 7.0 Hz, 2H), 1.00 (ddd, *J* = 14.6, 11.8, 8.5 Hz, 6H), 0.92 - 0.81 (m, 4H), 0.55 - 0.41 (m, 3H).

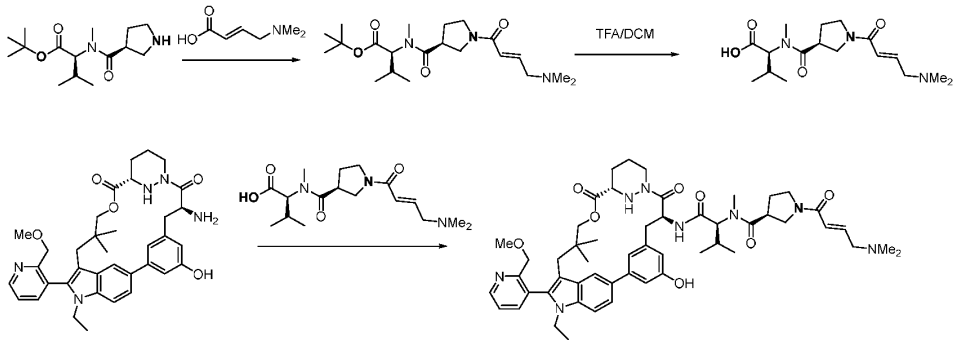
Example A67. Synthesis of (2*E*)-4-(dimethylamino)-N-(6-{[(1*S*)-1-{[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[2-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamoyl}-2-methylpropyl](methyl)carbamoyl}pyridin-3-yl)but-2-enamide



Step 1. To a mixture of (6^{3}*S*,4*S*)-4-amino-1^{1}-ethyl-2^{5}-hydroxy-1^{2}-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^{1},6^{2},6^{3},6^{4},6^{5},6^{6}-hexahydro-1^{1}*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione TFA salt (225 mg, 0.28 mmol) and (*E*)-*N*-(5-(4-(dimethylamino)but-2-enamido)picolinoyl)-*N*-methyl-L-valine TFA salt (260 mg crude, 0.56 mmol) in DMF (5 mL) at 0 °C were added DIPEA (0.46 mL, 2.8 mmol) followed by HATU (140 mg, 0.36 mmol). The mixture was stirred at 0-10 °C for 1 h, then concentrated under reduced pressure and the residue was purified by prep-HPLC to give (2*E*)-4-(dimethylamino)-N-(6-{[(1*S*)-1-{[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[2-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamoyl}-2-methylpropyl](methyl)carbamoyl}pyridin-3-yl)but-2-enamide TFA salt (23.3 mg, 8% yield over 2 steps) as a solid. LCMS (ESI): m/z [M+Na] calc'd for $C_{54}H_{67}N_9O_8Na$ 992.5; found 992.4; ^{1}H NMR (400 MHz, CD_3OD) δ 9.05 (d, *J* = 2.5 Hz, 1H), 8.85 - 8.71 (m, 1H), 8.43 (ddd, *J* = 33.3, 18.0, 2.6 Hz, 2H), 8.01 - 7.87 (m, 2H), 7.83 - 7.70 (m, 1H), 7.60 - 7.47 (m, 2H), 7.31 - 7.19 (m, 1H), 7.07 - 6.90 (m, 2H), 6.70 - 6.36 (m, 3H), 5.81 - 5.61 (m, 1H), 4.50 - 4.20 (m, 4H), 4.01 - 3.68 (m, 3H), 3.64 - 3.35 (m, 5H), 3.27 - 3.08 (m, 3H), 3.04 - 2.44 (m, 11H), 2.36 - 2.10 (m, 3H), 1.93 (d, *J* = 13.0 Hz, 1H), 1.61 (dd, *J* = 34.3, 21.6 Hz, 3H), 1.39 - 1.16 (m, 3H), 1.12 - 0.81 (m, 6H), 0.78 - 0.45 (m, 6H).

Example A54. Synthesis of (2*S*)-2-{1-[(3*S*)-1-[(2*E*)-4-(dimethylamino)but-2-enoyl]pyrrolidin-3-yl]-N-methylformamido}-N-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[2-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-

triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methylbutanamide



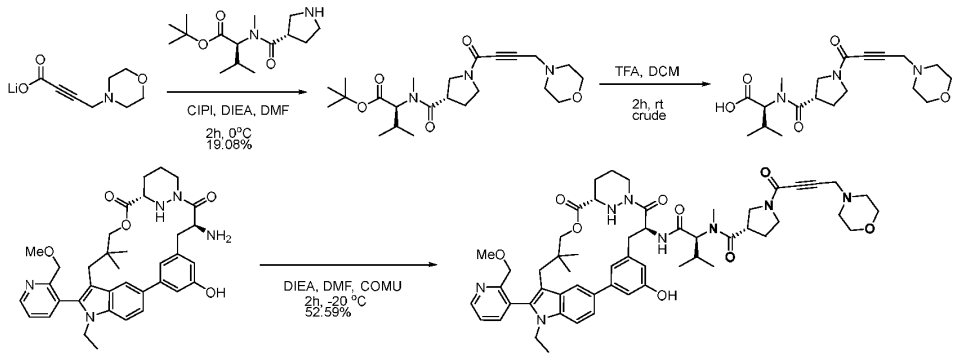
Step 1. To a mixture of *tert*-butyl *N*-methyl-*N*-((*S*)-pyrrolidine-3-carbonyl)-L-valinate (210 mg, 0.73 mmol) in DMF (4 mL) at rt were added 4-(dimethylamino)-4-methylpent-2-ynoic acid (450 mg, 2.9 mmol), DIPEA (1.2 mL, 7.3 mmol), and HATU (332 mg, 0.88 mmol). The mixture was stirred at rt for 1 h then diluted with EtOAc, and the mixture washed with H_2O, brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl *N*-((*S*)-1-(4-(dimethylamino)-4-methylpent-2-ynoyl)pyrrolidine-3-carbonyl)-*N*-methyl-L-valinate (140 mg, 45% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{39}N_3O_4$ 421.3; found 422.3.

Step 2. A mixture of *tert*-butyl *N*-((*S*)-1-(4-(dimethylamino)-4-methylpent-2-ynoyl)pyrrolidine-3-carbonyl)-*N*-methyl-L-valinate (130 mg, 0.31 mmol) in DCM (2 mL) and TFA (1 mL) was stirred at rt for 90 min. The mixture was concentrated under reduced pressure to give *N*-((*S*)-1-(4-(dimethylamino)-4-methylpent-2-ynoyl)pyrrolidine-3-carbonyl)-*N*-methyl-L-valine TFA salt (150 mg) as an oil, which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{19}H_{31}N_3O_4$ 365.2; found 366.2.

Step 3. (3*S*)-1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylpyrrolidine-3-carboxamide TFA salt was synthesized in a manner similar to 1-acryloyl-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylazetidine-3-carboxamide except (2*S*)-2-{1-[(3*S*)-1-[(2*E*)-4-(dimethylamino)but-2-enoyl]pyrrolidin-3-yl]-N-methylformamido}-N-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[2-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methylbutanamide TFA salt. (120 mg, 54% yield over 2 steps) as a solid. ^{1}H-NMR (400 MHz, CD_3OD) δ 8.76 - 8.68 (m, 1H), 8.44 (s, 1H), 8.02 - 7.94 (m, 1H), 7.94 - 7.84 (m, 1H), 7.65 - 7.43 (m, 3H), 7.27 - 7.14 (m, 1H), 7.06 - 6.96 (m, 1H), 6.65 - 6.48 (m, 1H), 5.62 - 5.46 (m, 1H), 4.81 - 4.57 (m, 1H), 4.46 - 4.22 (m, 3H), 4.10 - 3.35 (m, 11H), 3.26 - 2.93 (m, 6H), 2.91 - 2.51 (m, 4H), 2.42 - 2.09 (m, 9H), 1.95 - 1.87 (m, 1H), 1.85 - 1.40 (m, 6H), 1.38

- 1.10 (m, 6H), 1.07 - 0.81 (m, 9H), 0.56 - 0.38 (m, 3H). LCMS (ESI): m/z [M+H] $C_{52}H_{68}N_8O_8$ found 947.7.

Example A95. Synthesis of (2*S*)-*N*-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[2-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{N-methyl-1-[(3*S*)-1-[4-(morpholin-4-yl)but-2-ynoyl]pyrrolidin-3-yl]formamido}butanamide



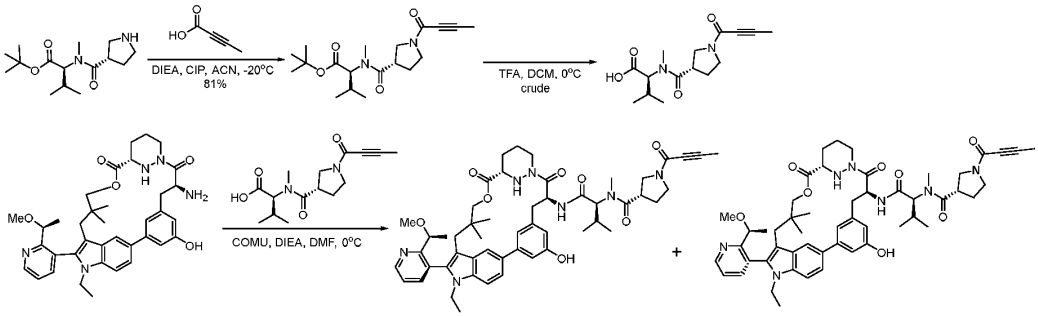
Step 1. A mixture of *tert*-butyl (2*S*)-3-methyl-2-[*N*-methyl-1-(3*S*)-pyrrolidin-3-ylformamido]butanoate (500 mg, 1.8 mmol), 4-(morpholin-4-yl)but-2-ynoic acid (1.49 g, 8.8 mmol), DIPEA (682 mg, 5.3 mmol) and CIP (635 mg, 2.3 mmol) in DMF (5 mL) was stirred at 0 °C for 2 h.. The mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl *N*-methyl-*N*-((*S*)-1-(4-morpholinobut-2-ynoyl)pyrrolidine-3-carbonyl)-*L*-valinate (150 mg, 19% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{37}N_3O_5$ 435.3; found 436.5.

Step 2. A mixture of *tert*-butyl *N*-methyl-*N*-((*S*)-1-(4-morpholinobut-2-ynoyl)pyrrolidine-3-carbonyl)-*L*-valinate (250 mg, 0.57 mmol) in DCM (5 mL) and TFA (2.5 mL) was stirred at rt for 2h. The mixture was concentrated under reduced pressure to give (2*S*)-3-methyl-2-[*N*-methyl-1-[(3*S*)-1-[4-(morpholin-4-yl)but-2-ynoyl]pyrrolidin-3-yl]formamido]butanoic acid (310mg, crude) as an oil, which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{19}H_{29}N_3O_5$ 379.2; found 380.2.

Step 3. A mixture of (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (250 mg, 0.4 mmol), DIPEA (516 mg, 4.0 mmol), (2*S*)-3-methyl-2-[*N*-methyl-1-[(3*S*)-1-[4-(morpholin-4-yl)but-2-ynoyl]pyrrolidin-3-yl]formamido]butanoic acid (182 mg, 0.48 mmol), and COMU (205 mg, 0.48 mmol) in DMF (3 mL) was stirred at -20 °C for 2 h. The mixture was diluted with H_2O (10 mL), then extracted with EtOAc (3 x 10 mL) and the combined organic layers were washed with brine (3 x 10 mL), dried over anhydrous Na_2SO_4, and filtered. The mixture was concentrated under reduced pressure and the residue was purified by reverse-phase silica gel column chromatography to give (2*S*)-*N*-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[2-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-

methyl-2-{N-methyl-1-[(3*S*)-1-[4-(morpholin-4-yl)but-2-ynoyl]pyrrolidin-3-yl]formamido}butanamide (207 mg, 53% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{55}H_{70}N_8O_9$ 986.5; found 987.8; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.39 - 9.28 (m, 1H), 8.74 (t, *J* = 4.8, 1H), 8.70 - 8.04 (m, 1H), 7.98 - 7.90 (m, 1.5H), 7.82 (d, *J* = 7.7 Hz, 0.5H), 7.63 - 7.46 (m, 3H), 7.26 - 7.10 (m, 1H), 7.03 (s, 1H), 6.58 - 6.43 (m, 1H), 5.44 - 5.30 (m, 1H), 5.06 (q, 0.5H), 4.72 (t, *J* = 11.0 , 0.5H), 4.39 - 4.20 (m, 3H), 4.15 (d, *J* = 11.1 Hz, 1H), 4.09 - 3.85 (m, 4H), 3.66 (s, 2H), 3.65 - 3.58 (m, 4H), 3.58 - 3.55 (m, 2H), 3.55 - 3.48 (m, 3H), 3.47 - 3.41 (m, 3H), 3.31 (s, 2H), 3.10 (s, 2H), 2.92 (s, 1H), 2.89 - 2.65 (m, 5H), 2.68 (s, 1H), 2.45 - 2.38 (m , 1H), 2.29 - 2.24 (m, 1H), 2.23 - 1.99 (m, 3H), 1.82 (d, *J* = 12.1 Hz, 1H), 1.76 - 1.62 (m, 1H), 1.61 - 1.45 (m, 1H), 1.14 - 1.04 (m, 2H), 1.02 - 0.92 (m, 3H), 0.91 - 0.86 (m, 3H), 0.83 - 0.77 (m, 3H), 0.77 - 0.70 (m, 2H), 0.50 - 0.35 (m, 3H).

Example A145. Synthesis of two atropisomers of (2*S*)-2-{1-[(3*S*)-1-(but-2-ynoyl)pyrrolidin-3-yl]-*N*-methylformamido}-*N*-[(8*S*,14*S*,20*M*)-22-ethyl-4-hydroxy-21-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methylbutanamide



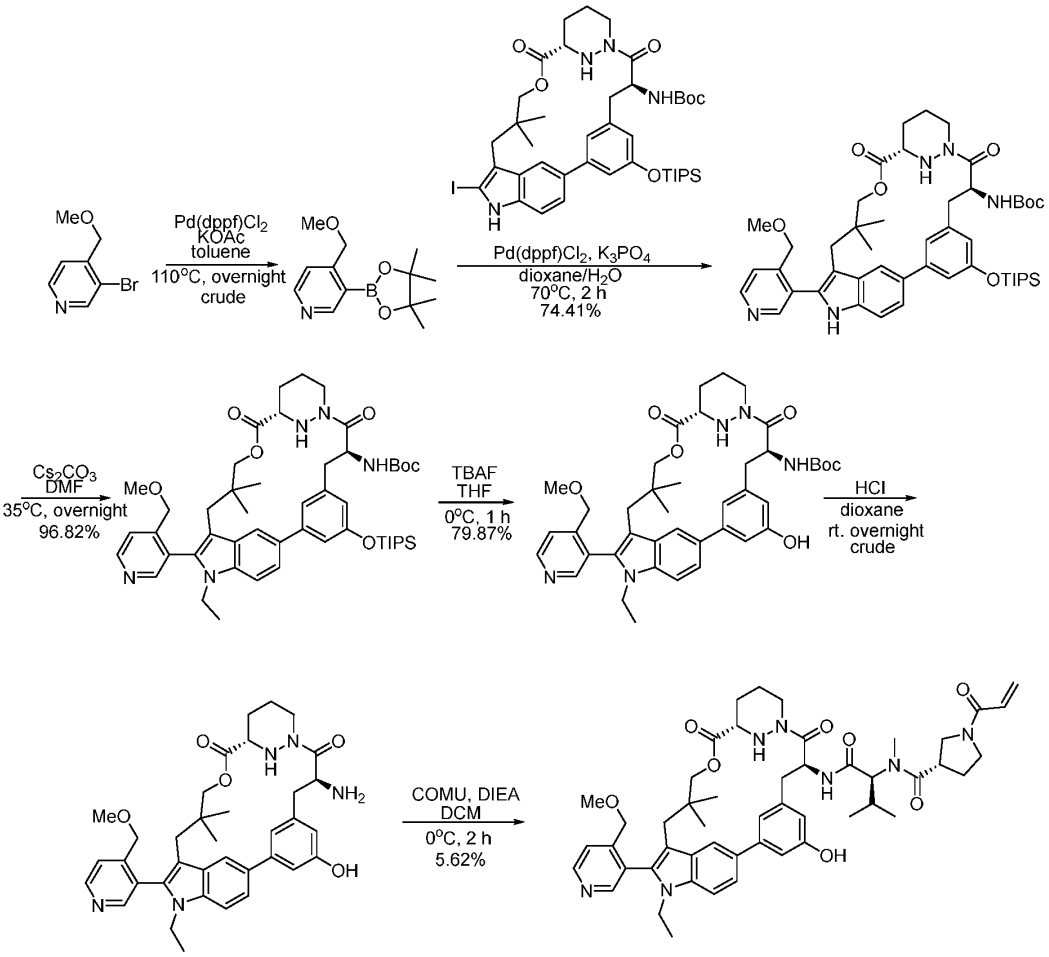
Step 1. To a mixture of but-2-ynoic acid (222 mg) and CIP (588 mg) in ACN (8 mL) at 0 °C under an atmosphere of Ar was added DIPEA (681 mg). The mixture was stirred at 0 °C then *tert*-butyl *N*-methyl-*N*-((*S*)-pyrrolidine-3-carbonyl)-L-valinate (500 mg) in ACN (3 mL) was added dropwise and the mixture stirred at 0 °C for 2 h. EtOAc was added and the mixture was washed with brine (3 x 20 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl *N*-((*S*)-1-(but-2-ynoyl)pyrrolidine-3-carbonyl)-*N*-methyl-L-valinate as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{19}H_{30}N_2O_4$ 350.2; found 352.1.

Step 2. A mixture of *tert*-butyl *N*-((*S*)-1-(but-2-ynoyl)pyrrolidine-3-carbonyl)-*N*-methyl-L-valinate (200 mg) in DCM (4 mL) and TFA (2 mL) was stirred at 0 °C for 2 h. The mixture was concentrated under reduced pressure with azeotropic removal of H_2O using toluene (4 mL x 2) to give *N*-((*S*)-1-(but-2-ynoyl)pyrrolidine-3-carbonyl)-*N*-methyl-L-valinate as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{15}H_{22}N_2O_4$ 294.2; found 295.2.

Step 3. Two atropisomers of (2*S*)-2-{1-[(3*S*)-1-(but-2-ynoyl)pyrrolidin-3-yl]-N-methylformamido}-N-[(8S,14S,20M)-22-ethyl-4-hydroxy-21-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methylbutanamide was synthesized in a manner

similar to (2*S*)-*N*-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[2-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{N-methyl-1-[(3*S*)-1-[4-(morpholin-4-yl)but-2-ynoyl]pyrrolidin-3-yl]formamido}butanamide except (2*S*)-3-methyl-2-[*N*-methyl-1-[(3*S*)-1-[4-(morpholin-4-yl)but-2-ynoyl]pyrrolidin-3-yl]formamido]butanoic acid was substituted with *N*-((*S*)-1-(but-2-ynoyl)pyrrolidine-3-carbonyl)-*N*-methyl-L-valinate. (43.3 mg, 12% yield) and (33 mg, 9% yield) both as solids. LCMS (ESI): m/z [M+H] calc'd for $C_{52}H_{65}N_7O_8$ 915.5; found 916.7; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34 - 9.27 (m, 1H), 8.78 (t, *J* = 2.5 Hz, 1H), 8.68 (t, *J* = 8.5 Hz, 0.5H), 8.20 - 8.11 (m, 0.6H), 7.95 (ddt, *J* = 5.4, 3.5, 1.7 Hz, 2H), 7.63 - 7.60(m, 1H), 7.61 - 7.49 (m, 2H), 7.13 (s, 1H), 7.03 (d, *J* = 6.2 Hz, 1H), 6.60 - 6.49 (d, *J* = 35.5 Hz, 1H), 5.43 - 5.39 (m, 1H), 5.12 - 5.00 (m, 0.7H), 4.74 (d, *J* = 10.6 Hz, 0.4H), 4.32 - 4.25 (m, 1H), 4.18 - 3.85 (m, 5H), 3.81 - 3.45 (m, 8H), 3.18 - 3.02 (m, 5H), 2.93 - 2.80 (m, 4H), 2.80 - 2.70 (m, 2H), 2.42 - 2.36 (m, 1H), 2.31 - 2.20 (m, 1H), 2.18 - 1.96 (m, 6H), 1.85 - 1.74 (m, 1H), 1.74 - 1.63 (m, 1H), 1.6 2- 1.42 (m, 1H), 1.32 - 1.16 (m, 4H), 1.15-1.05 (t, *J* = 6.3 Hz, 4H), 1.04 - 0.95 (m, 2H), 0.95 - 0.85 (m, 5H), 0.68 - 0.52(m, 4H), 0.52 - 0.37 (m, 4H). and LCMS (ESI): m/z [M+H] calc'd for $C_{52}H_{65}N_7O_8$ 915.5; found 916.7; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.36 - 9.28 (m, 1H), 8.77 (dd, *J* = 4.7, 1.8 Hz, 1H), 8.62 - 8.57 (m, 0.5H), 8.15 - 8.07 (m, 0.5H), 7.95 (s, 1H), 7.87 - 7.81 (m, 1H), 7.65 - 7.51 (m, 3H), 7.37 - 7.25 (m, 1H), 7.10 - 7.03 (m, 1H), 6.54 (d, *J* = 35.5Hz, 1H), 5.52 - 5.21 (m, 2H), 4.78 - 4.66 (m, 0.5H), 4.34 - 4.20 (m, 3H), 4.15 - 3.85(m, 4H), 3.85 - 3.42 (m, 7H), 3.22 - 3.11(m, 3H), 2.97- 2.72 (m, 7H), 2.62 - 2.54 (m, 1H), 2.28 - 1.96 (m, 7H), 1.95 - 1.74 (m, 2H), 1.73 - 1.44 (m, 2H), 1.42 - 1.37 (m, 3H), 1.28 - 1.14 (m, 1H), 1.03 - 0.85 (m, 6H), 0.83 - 0.72 (m, 7H), 0.71 - 0.55 (m, 3H).

Example A28. Synthesis of (2*S*)-*N*-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-[*N*-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido]butanamide



Step 1. To a mixture of 3-bromo-4-(methoxymethyl)pyridine (1.00 g, 5.0 mmol), 4,4,5,5-tetramethyl-2-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (1.51 g, 5.9 mmol) and KOAc (1.21 g, 12.3 mmol) in toluene (10 mL) at rt under an atmosphere of Ar was added $Pd(dppf)Cl_2$ (362 mg, 0.5 mmol). The mixture was heated to 110 °C and stirred overnight, then concentrated under reduced pressure to give 4-(methoxymethyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2- yl)pyridine, which was used directly in the next step directly without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{20}BNO_3$ 249.2; found 250.3.

Step 2. To a mixture of 4-(methoxymethyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (290 mg, 1.16 mmol), K_3PO_4 (371 mg, 1.75 mmol) and *tert*-butyl *N*-[(8*S*,14*S*)-21-iodo-18,18- dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate (500 mg, 0.58 mmol) in 1,4-dioxane (5 mL) and H_2O (1 mL) at rt under an

atmosphere of Ar was added Pd(dppf)Cl_2 (43 mg, 0.06 mmol). The mixture was heated to 70 °C and stirred for 2 h, then H_2O added and the mixture extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl *N*-[(8*S*,14*S*)-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate (370 mg,74% yield) as a foam. LCMS (ESI): m/z [M+H] calc'd for $C_{48}H_{67}N_5O_7Si$ 853.6; found 854.6.

Step 3. A mixture of *tert*-butyl *N*-[(8*S*,14*S*)-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate (350 mg, 0.41 mmol), Cs_2CO_3 (267 mg, 0.82 mmol) and EtI (128 mg, 0.82 mmol) in DMF (4 mL) was stirred at 35 °C overnight. H_2O was added and the mixture was extracted with EtOAc (2 x 15 mL). The combined organic layers were washed with brine (15 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl *N*-[(8*S*,14*S*)-22-ethyl-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl] carbamate (350 mg, 97% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{71}N_5O_7Si$ 881.5; found 882.6.

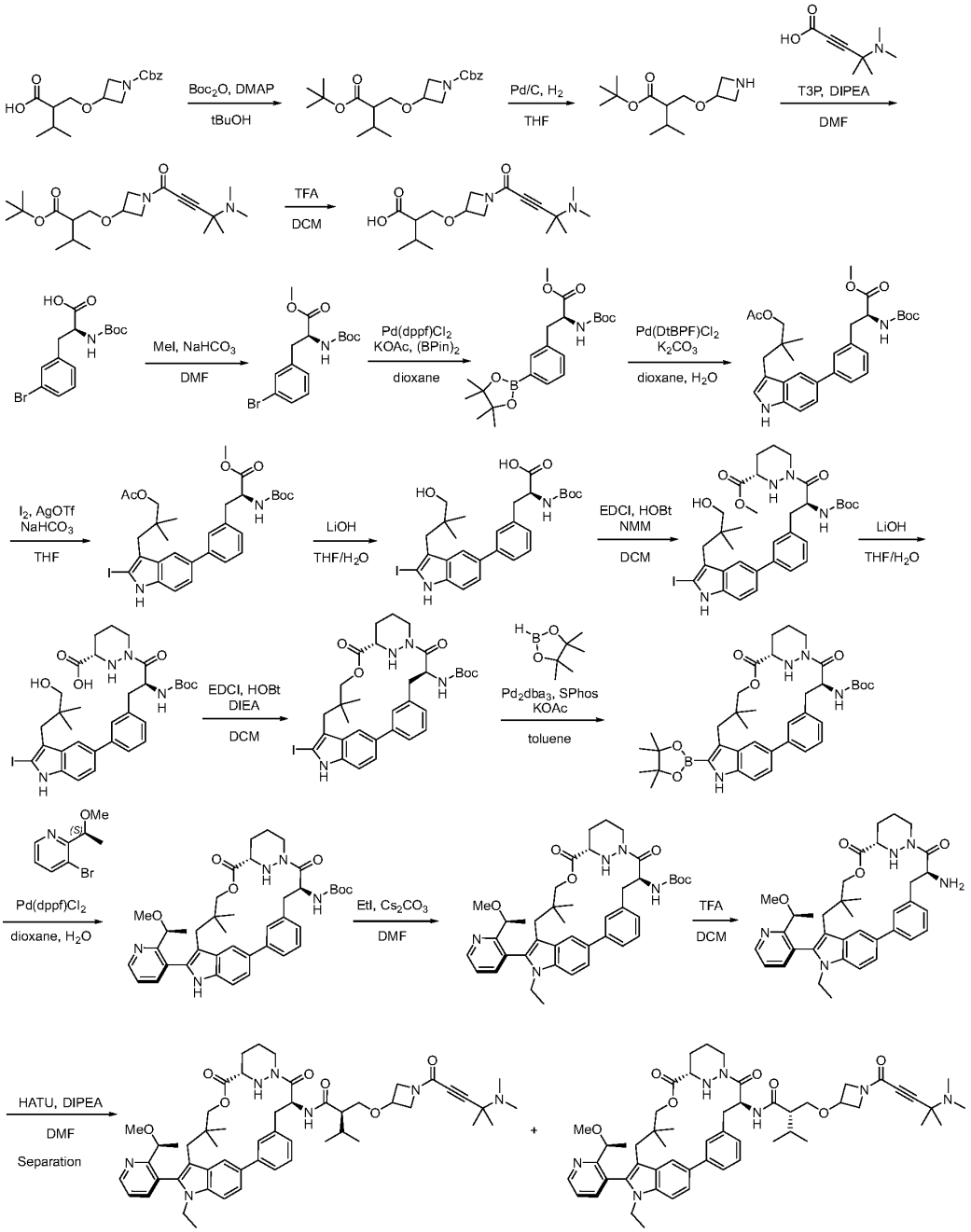
Step 4. A mixture of *tert*-butyl *N*-[(8*S*,14*S*)-22-ethyl-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl] carbamate (350 mg, 0.4 mmol) and 1M TBAF in THF (0.48 mL, 0.480 mmol) in THF (3 mL) at 0 °C under an atmosphere of Ar was stirred for 1 h. The mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl *N*-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4- (methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate (230 mg, 80% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{51}N_5O_7$ 725.4; found 726.6.

Step 5. To a mixture of *tert*-butyl *N*-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate (200 mg, 0.28 mmol) in 1,4-dioxane (2 mL) at 0 °C under an atmosphere of Ar was added 4M HCl in 1,4-dioxane (2 mL, 8 mmol). The mixture was allowed to warm to rt and was stirred overnight, then concentrated under reduced pressure to give (8*S*,14*S*)-8-amino-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-16-oxa-10,22,28-

triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaene-9,15-dione (200 mg). LCMS (ESI): m/z [M+H] calc'd for $C_{36}H_{43}N_5O_5$ 625.3; found 626.5.

Step 6. To a mixture of (2*S*)-3-methyl-2-[*N*-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido]butanoic acid (108 mg, 0.38 mmol) and (8*S*,14*S*)-8-amino-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaene-9,15-dione (200 mg, 0.32 mmol) in DCM (3 mL) at 0 °C was added DIPEA (165 mg, 1.3 mmol) and COMU (274 mg, 0.64 mmol) in portions. The mixture was stirred at 0 °C for 2 h, H_2O added and extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography then prep-HPLC to give (2*S*)-*N*-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-[*N*-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido]butanamide (16 mg, 5.6% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{63}N_7O_8$ 889.5; found 890.6; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.33 (dd, *J* = 9.1, 6.9 Hz, 1H), 8.79 - 8.46 (m, 2H), 7.93 (s, 1H), 7.68 - 7.58 (m, 2H), 7.53 (t, *J* = 8.5 Hz, 1H), 7.26 - 6.98 (m, 2H), 6.71 - 6.47 (m, 2H), 6.24 - 6.07 (m, 1H), 5.80 - 5.60 (m, 1H), 5.49 - 5.18 (m, 1H), 4.45 - 4.07 (m, 4H), 4.08 - 3.87 (m, 3H), 3.87 - 3.64 (m, 4H), 3.64 - 3.40 (m, 5H), 3.34 (s, 2H), 3.30 (s, 2H), 3.23 (d, *J* = 1.8 Hz, 1H), 2.94 - 2.74 (m, 6H), 2.16 - 2.01 (m, 3H), 1.82 - 1.47 (m, 3H), 1.08 (q, *J* = 8.9, 8.0 Hz, 1H), 1.00 - 0.88 (m, 6H), 0.82 (d, *J* = 10.8 Hz, 4H), 0.76 - 0.66 (m, 2H), 0.44 (d, *J* = 14.2 Hz, 3H).

Example A316. Synthesis of (2*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide



Step 1. To a mixture of 2-(((1-((benzyloxy)carbonyl)azetidin-3-yl)oxy)methyl)-3-methylbutanoic acid (650 mg, 2 mmol) and di-*tert*-butyl dicarbonate (883 mg, 4 mmol) in ^{t}BuOH (10 mL) was added 4-dimethylaminopyridine (124 mg, 1 mmol). The mixture was heated to 30 °C and

stirred for 1 h, then diluted with H_2O (50 mL) and extracted with EtOAc (50 mL x 3). The combined organic layers were concentrated under reduced pressure and the residue was purified by silica gel column chromatography to afford benzyl 3-(2-(*tert*-butoxycarbonyl)-3-methylbutoxy)azetidine-1-carboxylate (450 mg, 56% yield) as an oil. LCMS (ESI): m/z [M+Na] calc'd for $C_{21}H_{31}NO_5Na$ 400.2; found 400.2.

Step 2. A mixture of benzyl 3-(2-(*tert*-butoxycarbonyl)-3-methylbutoxy)azetidine-1-carboxylate (450 mg, 1.19 mmol) and Pd/C (50 mg) in THF (30 mL) was stirred for 2 h under an atmosphere of H_2 (15 psi). The mixture was filtered and the filtrate was concentrated under reduced pressure to give *tert*-butyl 2-((azetidin-3-yloxy)methyl)-3-methylbutanoate (300 mg, 100% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{26}NO_3$ 243.2; found 244.2; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.35 - 4.25 (m, 1H), 3.71 - 3.63 (m, 2H), 3.63 - 3.56 (m, 2H), 3.50 (t, *J* = 8.0 Hz, 1H), 3.43 (dd, *J* = 9.0, 4.0 Hz, 1H), 2.37 - 2.26 (m, 1H), 2.21 (br. s, 1H), 1.92 - 1.81 (m, 1H), 1.47 (s, 9H), 0.93 (d, *J* = 6.8 Hz, 6H).

Step 3. To a mixture of *tert*-butyl 2-((azetidin-3-yloxy)methyl)-3-methylbutanoate (270 mg, 1.11 mmol), 4-(dimethylamino)-4-methylpent-2-ynoic acid (860 mg, 5.55 mmol) and DIPEA (1.56 g, 11.1 mmol) in DMF (20 mL) at 0 °C was added T_3P (2.12 g, 6.7 mmol). The mixture was stirred at 0 °C for 1 h, diluted with EtOAc (200 mL), then washed with H_2O (30 mL x 5), brine (30 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl 2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-3-methylbutanoate (200 mg, 47% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{21}H_{36}N_2O_4$ 380.3; found 381.3.

Step 4. To a mixture of *tert*-butyl 2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-3-methylbutanoate (190 mg, 0.5 mmol) in DCM (4 mL) was added TFA (2 mL). The mixture was stirred for 1 h, then concentrated under reduced pressure to give 2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-3-methylbutanoic acid (162 mg, 100% yield) as an oil, which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{17}H_{28}N_2O_4$ 324.2; found 325.3.

Step 5. To a solution of (2*S*)-3-(3-bromophenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoic acid (100 g, 290 mmol) in DMF (1 L) at room temperature was added $NaHCO_3$ (48.8 g, 581.1 mmol) and MeI (61.9 g, 435.8 mmol). The reaction mixture was stirred for 16 h and was then quenched with H_2O (1 L) and extracted with EtOAc (3 x 1 L). The combined organic layers were washed with brine (3 x 500 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (13% EtOAc/pet. ether) to give methyl (*S*)-3-(3-bromophenyl)-2-((*tert*-butoxycarbonyl)amino)propanoate (109 g, crude). LCMS (ESI): m/z [M+Na] calc'd for $C_{15}H_{20}BrNO_4$ 380.05; found 380.0.

Step 6. To a stirred solution of methyl (2*S*)-3-(3-bromophenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (108 g, 301.5 mmol) and bis(pinacolato)diboron (99.53 g, 391.93 mmol) in 1,4-dioxane (3.2 L) was added KOAc (73.97 g, 753.70 mmol) and $Pd(dppf)Cl_2$ (22.06 g, 30.15 mmol). The reaction mixture was heated to 90 °C for 3 h and was then cooled to room temperature and extracted with EtOAc (2 x 3 L). The combined organic layers were washed with brine (3 x 800 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The

residue was purified by silica gel chromatography (5% EtOAc/pet. ether) to give methyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoate (96 g, 78.6% yield). LCMS (ESI): m/z [M+Na] calc'd for $C_{21}H_{32}BNO_6$ 428.22; found 428.1.

Step 7. To a mixture of methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]propanoate (94 g, 231.9 mmol) and 3-(5-bromo-1H-indol-3-yl)-2,2-dimethylpropyl acetate (75.19 g, 231.93 mmol) in 1,4-dioxane (1.5 L) and H_2O (300 mL) was added K_2CO_3 (64.11 g, 463.85 mmol) and Pd(DtBPF)Cl_2 (15.12 g, 23.19 mmol). The reaction mixture was heated to 70 °C and stirred for 4 h. The reaction mixture was extracted with EtOAc (2 x 2 L) and the combined organic layers were washed with brine (3 x 600 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (20% EtOAc/pet. ether) to give methyl (*S*)-3-(3-(3-(3-acetoxy-2,2-dimethylpropyl)-1H-indol-5-yl)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanoate (130 g, crude). LCMS (ESI): m/z [M+H] calc'd for $C_{30}H_{38}N_2O_6$ 523.28; found 523.1.

Step 8. To a solution of methyl (2*S*)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-1H-indol-5-yl]phenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (95.0 g, 181.8 mmol) and iodine (36.91 g, 145.41 mmol) in THF (1 L) at -10 °C was added AgOTf (70.0 g, 272.7 mmol) and $NaHCO_3$ (22.9 g, 272.65 mmol). The reaction mixture was stirred for 30 min and was then quenched by the addition of sat. $Na_2S_2O_3$ (100 mL) at 0 °C. The resulting mixture was extracted with EtOAc (3 x 1 L) and the combined organic layers were washed with brine (3 x 500 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (50% EtOAc/pet. ether) to give methyl (*S*)-3-(3-(3-(3-acetoxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanoate (49.3 g, 41.8% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{30}H_{37}IN_2O_6$: 649.18; found 649.1.

Step 9. To a solution of methyl (2*S*)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-2-iodo-1H-indol-5-yl]phenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (60 g, 92.5 mmol) in THF (600 mL) was added a solution of LiOH H_2O (19.41 g, 462.5 mmol) in H_2O (460 mL). The resulting solution was stirred overnight and then the pH was adjusted to 6 with HCl (1 M). The resulting solution was extracted with EtOAc (2 x 500 mL) and the combined organic layers was washed with sat. brine (2 x 500 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl)phenyl)propanoic acid (45 g, 82.1% yield). LCMS (ESI): m/z [M+Na] calc'd for $C_{27}H_{33}IN_2O_6$ 615.13; found 615.1.

Step 10. To a solution of (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl]phenyl]propanoic acid (30 g, 50.6 mmol) and methyl (3*S*)-1,2-diazinane-3-carboxylate (10.9 g, 75.9 mmol) in DCM (400 mL) was added NMM (40.97 g, 405.08 mmol), HOBT (2.05 g, 15.19 mmol), and EDCI (19.41 g, 101.27 mmol). The reaction mixture was stirred overnight and then the mixture was washed with sat. NH_4Cl (2 x 200 mL) and sat. brine (2 x 200 mL), and the mixture was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylate (14 g, 38.5% yield). LCMS (ESI): m/z [M+H] calc'd for $C_{33}H_{43}IN_4O_6$ 718.23; found 719.4.

Step 11. To a solution of methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylate (92 g, 128.0 mmol) in THF (920 mL) at 0 °C was added a solution of LiOH H_2O (26.86 g, 640.10 mmol) in H_2O (640 mL). The reaction mixture was stirred for 2 h and was then concentrated under reduced pressure to give (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylic acid (90 g, crude). LCMS (ESI): m/z [M+H] calc'd for $C_{32}H_{41}IN_4O_6$ 705.22; found 705.1.

Step 12. To a solution of of (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid (90 g, 127.73 mmol) in DCM (10 L) at 0 °C was added HOBt (34.52 g, 255.46 mmol), DIPEA (330.17 g, 2554.62 mmol) and EDCI (367.29 g, 1915.96 mmol). The reaction mixture was stirred for 16 h and was then concentrated under reduced pressure. The mixture was extracted with DCM (2 x 2 L) and the combined organic layers were washed with brine (3 x 1 L), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (50% EtOAc/pet. ether) to give *tert*-butyl ((6^3*S*,4*S*)-1^2-iodo-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (70 g, 79.8% yield). LCMS (ESI): m/z [M+H] calc'd for $C_{32}H_{39}IN_4O_5$ 687.21; found 687.1.

Step 13. A 1 L round-bottom flask was charged with *tert*-butyl ((6^3*S*,4*S*)-1^2-iodo-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (22.0 g, 32.042 mmol), toluene (300.0 mL), $Pd_2(dba)_3$ (3.52 g, 3.845 mmol), S-Phos (3.95 g, 9.613 mmol), and KOAc (9.43 g, 96.127 mmol) at room temperature. To the mixture was added 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (26.66 g, 208.275 mmol) dropwise with stirring at room temperature. The resulting solution was stirred for 3 h at 60 °C. The resulting mixture was filtered, and the filter cake was washed with EtOAc. The filtrate was concentrated under reduced pressure and the remaining residue was purified by silica gel column chromatography to afford *tert*-butyl ((6^3*S*,4*S*)-10,10-dimethyl-5,7-dioxo-12-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (22 g, 90 % yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{51}BN_4O_7$ 687.3; found 687.4.

Step 14. A mixture of *tert*-butyl ((6^3*S*,4*S*)-10,10-dimethyl-5,7-dioxo-1^2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (2.0 g, 2.8 mmol), 3-bromo-2-[(1*S*)-1-methoxyethyl]pyridine (0.60 g, 2.8 mmol), Pd(dppf)Cl_2 (0.39 g, 0.5 mmol), and K_3PO_4 (1.2 g, 6.0 mmol) in 1,4-dioxane (50 mL) and H_2O (10 mL) under an atmosphere of N_2 was heated to 70 °C and stirred for 2 h. The mixture was diluted with H_2O (50 mL) and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (3 x 50 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl ((6^3*S*,4*S*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-

2(1,3)-benzenacycloundecaphane-4-yl)carbamate (1.5 g, 74% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{40}H_{49}N_5O_6$ 695.4; found 696.5.

Step 15. To a solution of *tert*-butyl ((6^3*S*,4*S*)-1^2-(2-((*S*)-1-methoxyethyl) pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl) carbamate (20 g, 28.7 mmol) and Cs_2CO_3 (18.7 g, 57.5 mmol) in DMF (150 mL) at 0 °C was added a solution of ethyl iodide (13.45 g, 86.22 mmol) in DMF (50 mL). The resulting mixture was stirred overnight at 35 °C and was then diluted with H_2O (500 mL). The mixture was extracted with EtOAc (2 x 300 mL) and the combined organic layers were washed with brine (3 x 100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography to give *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (4.23 g, 18.8% yield) and the atropisomer (5.78 g, 25.7% yield) as solids. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{53}N_5O_6$ 724.4; found 724.6.

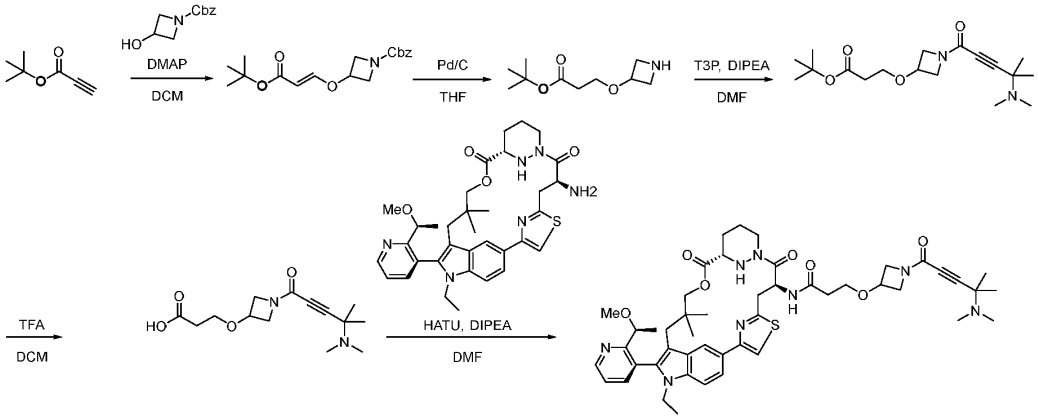
Step 16. A mixture of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (1.3 g, 1.7 mmol) in TFA (10 mL) and DCM (20 mL) was stirred at 0 °C for 2 h. The mixture was concentrated under reduced pressure to afford (6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (1.30 g, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{37}H_{45}N_5O_4$ 623.3; found 624.4.

Step 17. To a mixture of (6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (258 mg, 0.41 mmol) and 2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-3-methylbutanoic acid (162 mg, 0.5 mmol) in DMF (4 mL) at 0 °C was added a mixture of HATU (188 mg, 0.5 mmol) and DIPEA (534 mg, 4.14 mmol) in DMF (2 mL). The mixture was stirred at 0 °C for 1 h, then diluted with H_2O (30 mL) and extracted with EtOAc (30 mL x 3). The combined organic layers were concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide (250 mg, 64% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{54}H_{71}N_7O_7$ 929.5; found 930.5; ^{1}H NMR (400 MHz, CD_3OD) δ 8.90 - 8.79 (m, 1H), 8.54 - 8.21 (m, 1H), 8.15 - 7.91 (m, 2H), 7.88 - 7.67 (m, 2H), 7.65 - 7.52 (m, 2H), 7.47 - 7.15 (m, 2H), 5.80 - 5.52 (m, 1H), 4.53 - 4.23 (m, 5H), 4.23 - 3.93 (m, 3H), 3.90 - 3.76 (m, 2H), 3.75 - 3.58 (m, 3H), 3.57 - 3.44 (m, 1H), 3.38 (s, 1H), 3.29 - 3.26 (m, 2H), 3.21 - 2.85 (m, 8H), 2.82 - 2.65 (m, 3H), 2.51 - 2.30 (m, 1H), 2.24 - 2.03 (m, 1H), 1.99 - 1.87 (m, 1H), 1.86 - 1.69 (m, 6H), 1.67 - 1.57 (m, 2H), 1.57 - 1.39 (m, 4H), 1.45 - 1.05 (m, 2H), 1.04 - 0.96 (m, 3H), 0.96 - 0.88 (m, 3H), 0.88 - 0.79 (m, 3H), 0.79 - 0.63 (m, 3H), 0.56 (s, 1H).

Step 18. 2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-

hexahydro-1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide (180 mg, 0.194 mmol) was purified by prep-HPLC to afford (2*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide (41.8 mg, 23.2% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{54}H_{71}N_7O_7$ 930.5; found 930.5; ^{1}H NMR (400 MHz, MeOD) δ 8.74 (d, J = 4.0 Hz, 1H), 8.53 – 8.30 (m, 1H), 8.10 – 7.95 (m, 1H), 7.94 – 7.80 (m, 2H), 7.68 (t, *J* = 8.0 Hz, 1H), 7.65 – 7.58 (m, 1H), 7.58 – 7.46 (m, 2H), 7.38 – 7.17 (m, 2H), 5.73 – 5.60 (m, 1H), 4.52 – 4.40 (m, 1H), 4.35 – 4.15 (m, 4H), 4.14 – 3.95 (m, 2H), 3.90 – 3.72 (m, 3H), 3.71 – 3.45 (m, 4H), 3.30 – 3.20 (m, 3H), 3.06 – 2.72 (m, 5H), 2.49 – 2.28 (m, 4H), 2.28 – 2.20 (m, 3H), 2.18 – 2.06 (m, 1H), 2.00 – 1.90 (m, 1H), 1.90 – 1.52 (m, 4H), 1.52 – 1.40 (m, 5H), 1.40 – 1.22 (m, 4H), 1.09 – 0.92 (m, 8H), 0.90 – 0.75 (m, 3H), 0.71 – 0.52 (m, 3H) and (2*S*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide (51.2 mg, 28.4% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{54}H_{71}N_7O_7$ 930.5; found 930.3; ^{1}H NMR (400 MHz, MeOD) δ 8.74 (d, J = 4.0 Hz, 1H), 8.28 – 8.20 (m, 0.6H), 8.11 – 7.98 (m, 1H), 7.97 – 7.80 (m, 2H), 7.73 – 7.48 (m, 4H), 7.46 – 7.36 (m, 0.4H), 7.33 – 7.26 (m, 1H), 7.25 – 7.13 (m, 1H), 5.79 – 5.66 (m, 1H), 4.54 – 4.43 (m, 1H), 4.42 – 4.01 (m, 7H), 3.90 – 3.75 (m, 2H), 3.73 – 3.48 (m, 4H), 3.27 – 3.12 (m, 3H), 3.08 – 2.99 (m, 1H), 2.96 – 2.85 (m, 2H), 2.84 – 2.69 (m, 2H), 2.69 – 2.49 (m, 6H), 2.41 – 2.29 (m, 1H), 2.15 – 2.05 (m, 1H), 1.95 – 1.85 (m, 1H), 1.84 – 1.71 (m, 1H), 1.71 – 1.38 (m, 11H), 1.14 – 1.00 (m, 3H), 1.00 – 0.71 (m, 9H), 0.70 – 0.56 (m, 3H).

Example A427. Synthesis of 3-((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)propanamide



Step 1. To a mixture of *tert*-butyl prop-2-ynoate (5 g, 40 mmol) and [3-(3-hydroxyazetidin-1-yl)phenyl]methyl formate (4.1 g, 20 mmol) in DCM (150 mL) was added DMAP (9.8 g, 80 mmol). The mixture was stirred for 2 h, then diluted with H_2O and washed with H_2O (60 mL x 3). The

organic layer was dried over Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue purified by silica gel column chromatography to give benzyl (*E*)-3-((3-(*tert*-butoxy)-3-oxoprop-1-en-1-yl)oxy)azetidine-1-carboxylate (6.6 g, 90% yield) as an oil. LCMS (ESI): m/z [M+Na] calc'd for $C_{18}H_{23}NO_5Na$ 356.2; found 356.2.

Step 2. A mixture of benzyl (*E*)-3-((3-(*tert*-butoxy)-3-oxoprop-1-en-1-yl)oxy)azetidine-1-carboxylate (1.4 g, 4 mmol) and Pd/C (200 mg) in THF (10 mL) was stirred under an atmosphere of H_2 (1 atmosphere) for 16 h. The mixture was filtered and the filtrate and was concentrated under reduced pressure to give *tert*-butyl 3-(azetidin-3-yloxy)propanoate, which was used directly in the next step. LCMS (ESI): m/z [M+H] calc'd for $C_{10}H_{19}NO_3$ 201.1; found 202.2.

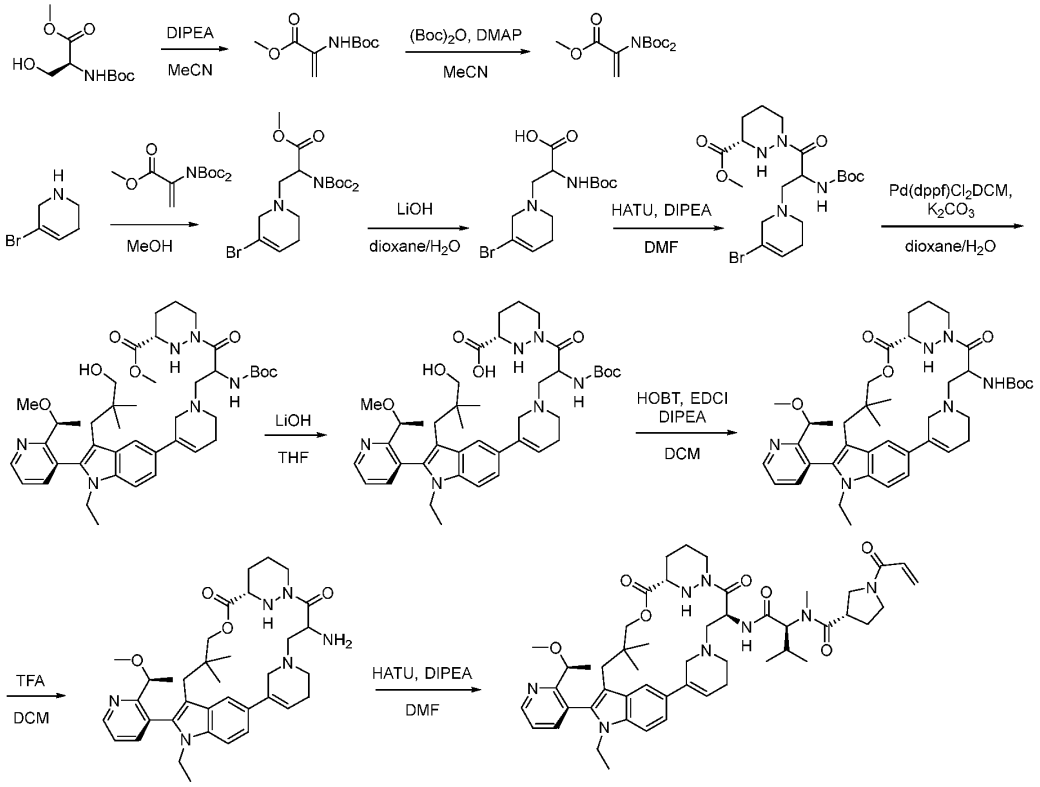
Step 3. To a mixture of *tert*-butyl 3-(azetidin-3-yloxy)propanoate (300 mg, 1.5 mmol) and 4-(dimethylamino)-4-methylpent-2-ynoic acid (2.3 g, 15 mmol) in DMF (15 mL) at 5 °C was added DIPEA (1.9 g, 15 mmol) and T3P (4.77 g, 7.5 mmol) dropwise. The mixture was stirred at 5 °C for 2 h, then H_2O and EtOAc (80 mL) were added. The organic and aqueous layers were separated and the organic layer was washed with H_2O (20 mL x 3), brine (30 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to afford *tert*-butyl 3-((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)propanoate (60 mg, 12% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{18}H_{30}N_2O_4$ 338.2; found 339.2.

Step 4. A mixture *tert*-butyl 3-((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)propanoate (70 mg, 0.21 mmol) in TFA / DCM (1:3, 2 mL) was stirred at 0 - 5 °C for 1 h, then concentrated under reduced pressure to give 3-({1-[4-(dimethylamino)-4-methylpent-2-ynoyl]azetidin-3-yl}oxy)propanoic acid (56 mg, 95% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{14}H_{22}N_2O_4$ 282.2; found 283.3.

Step 5. To a mixture of 3-((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)propanoic acid (56 mg, 0.19 mmol), (6^3S,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (90 mg, 0.14 mmol) and DIPEA (200 mg, 1.9 mmol) in DMF (1 mL) at 0 °C was added HATU (110 mg, 0.38 mmol) portion-wise. The mixture was stirred at 0 °C for 1 h, then H_2O added and the mixture extracted with EtOAx (150 mL x 2). The combined organic layers were washed with H_2O (150 mL) and brine (150 mL), then dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give 3-((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)-*N*-((6^3S,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)propanamide (12.6 mg, 7.5 % yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{48}H_{62}N_8O_7S$ 894.5; found 895.3; ^{1}H NMR (400 MHz, CD_3OD) δ 8.73 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.57 (s, 1H), 8.28 (s, 0.3H), 7.84 (m, 1H), 7.71 (m, 1H), 7.52 (m, 3H), 5.76 (dd, *J* = 30.2, 7.8 Hz, 1H), 4.40 (m, 4H), 4.32 - 4.12 (m, 4H), 4.06 (dd, *J* = 12.4, 6.0 Hz, 1H), 3.97 - 3.86 (m, 1H), 3.79 - 3.66 (m, 4H), 3.46 (dd, *J* = 14.8, 4.8 Hz, 1H), 3.41 - 3.33 (m, 3H), 3.29 - 3.19 (m, 1H), 3.17 - 3.05 (m, 1H), 2.79 (m, 1H), 2.73 - 2.50 (m, 3H), 2.49 - 2.43 (m, 3H), 2.38 (s, 3H), 2.21 (dd, *J*

= 12.6, 9.6 Hz, 1H), 1.95 (d, *J* = 12.8 Hz, 1H), 1.86 - 1.73 (m, 1H), 1.61 (dd, *J* = 12.6, 3.6 Hz, 1H), 1.51 (s, 2H), 1.46 - 1.43 (m, 4H), 1.38 - 1.27 (m, 3H), 1.01 - 0.86 (m, 6H), 0.44 (d, *J* = 11.6 Hz, 3H).

Example A716. Synthesis of (3*S*)-1-acryloyl-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylpyrrolidine-3-carboxamide



Step 1. To a solution of methyl (*tert*-butoxycarbonyl)-L-serinate (10 g, 45 mmol) in anhydrous MeCN (150 mL), was added DIPEA (17 g, 137 mmol). The reaction mixture was stirred at 45 °C for 2 h to give methyl 2-((*tert*-butoxycarbonyl)amino)acrylate in solution. LCMS (ESI): m/z [M+Na] calc'd for $C_9H_{15}NO_4$ 201.1; found 224.1.

Step 2. To a solution of methyl 2-((*tert*-butoxycarbonyl)amino)acrylate (12 g, 60 mmol) in anhydrous MeCN (150 mL) at 0 °C, was added 4-DMAP (13 g, 90 mmol) and $(Boc)_2O$ (26 g, 120 mmol). The reaction was stirred for 6 h, then quenched with H_2O (100 mL) and extracted with DCM (200 mL x 3). The combined organic layers were washed with brine (150 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give methyl 2-(bis(*tert*-butoxycarbonyl)amino)acrylate (12.5 g, 65% yield) as solid. LCMS (ESI): m/z [M+Na] calc'd for $C_{14}H_{23}NO_6$ 301.2; found 324.1.

Step 3. To a mixture of 5-bromo-1,2,3,6-tetrahydropyridine (8.0 g, 49 mmol) in MeOH (120 mL) under an atmosphere of Ar was added methyl 2-{*bis*[(*tert*-butoxy)carbonyl]amino}prop-2-enoate (22 g, 74 mmol). The mixture was stirred for 16 h, then concentrated under reduced pressure and

the residue was purified by silica gel column chromatography to give methyl 2-(bis(*tert*-butoxycarbonyl)amino)-3-(5-bromo-3,6-dihydropyridin-1(2*H*)-yl)propanoate (12 g, 47% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{19}H_{31}BrN_2O_6$ 462.1; found 463.1.

Step 4. To a mixture of methyl 2-(bis(*tert*-butoxycarbonyl)amino)-3-(5-bromo-3,6-dihydropyridin-1(2*H*)-yl)propanoate (14 g, 30 mmol) in 1,4-dioxane (30 mL) and H_2O (12 mL) was added LiOH (3.6 g, 151 mmol). The mixture was heated to 35 °C and stirred for 12 h, then 1M HCl was added and the pH adjusted to ~3-4. The mixture was extracted with DCM (300 mL x 2) and the combined organic layers were dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give 3-(5-bromo-3,6-dihydropyridin-1(2*H*)-yl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (10 g, 85% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{21}BrN_2O_4$ 348.1; found 349.0.

Step 5. To a mixture of 3-(5-bromo-3,6-dihydropyridin-1(2*H*)-yl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (10 g, 30 mmol), DIPEA (12 g, 93 mmol) and methyl (3*S*)-1,2-diazinane-3-carboxylate (5.4 g, 37 mmol) in DMF (100 mL) at 0 °C under an atmosphere of Ar was added HATU (13 g, 34 mmol). The mixture was stirred at 0 °C for 2 h, then H_2O added and the mixture extracted with EtOAc (300 mL x 2). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give methyl (3*S*)-1-(3-(5-bromo-3,6-dihydropyridin-1(2*H*)-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (9.0 g, 55% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{19}H_{31}BrN_4O_5$ 474.1; found 475.1.

Step 6. A mixture of methyl (3*S*)-1-(3-(5-bromo-3,6-dihydropyridin-1(2*H*)-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (9.0 g, 18 mmol), K_2CO_3 (4.5 g, 32 mmol), Pd(dppf)Cl_2.DCM (1.4 g, 2 mmol), 3-(1-ethyl-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-3-yl)-2,2-dimethylpropan-1-ol (9.8 g, 20 mmol) in 1,4-dioxane (90 mL) and H_2O (10 mL) under an atmosphere of Ar was heated to 75 °C and stirred for 2 h. H_2O was added and the mixture was extracted with EtOAc (200 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (3*S*)-1-(2-((*tert*-butoxycarbonyl)amino)-3-(5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)-3,6-dihydropyridin-1(2H)-yl)propanoyl)hexahydropyridazine-3-carboxylate (4.0 g, 25% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{60}N_6O_7$ 760.5; found 761.4.

Step 7. To a mixture of methyl (3*S*)-1-(2-((*tert*-butoxycarbonyl)amino)-3-(5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)-3,6-dihydropyridin-1(2H)-yl)propanoyl)hexahydropyridazine-3-carboxylate (4.1 g, 5.0 mmol) in THF (35 mL) at 0 °C was added LiOH (0.60 g, 27 mmol). The mixture was stirred at 0 °C for 1.5 h, then 1M HCl added to adjust pH to ~6-7 and the mixture extracted with EtOAc (200 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to give (3*S*)-1-(2-((*tert*-butoxycarbonyl)amino)-3-(5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)-3,6-dihydropyridin-1(2*H*)-

yl)propanoyl)hexahydropyridazine-3-carboxylic acid (3.6 g, 80% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{58}N_6O_7$ 746.4; found 747.4.

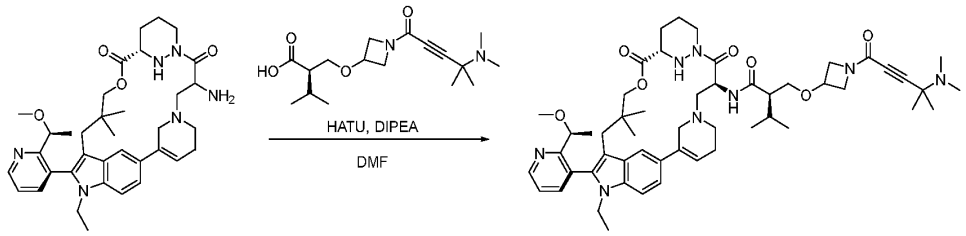
Step 8. To a mixture of (3*S*)-1-(2-((*tert*-butoxycarbonyl)amino)-3-(5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)-3,6-dihydropyridin-1(2*H*)-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (3.6 g , 5.0 mmol) and DIPEA (24 g ,190 mmol) in DCM (700 mL) under an atmosphere of Ar was added EDCI.HCl (28 g, 140 mmol) and HOBT (6.5 g, 50 mmol). The mixture was heated to 30 °C and stirred for 16 h at 30 ℃, then concentrated under reduced pressure. The residue was diluted with EtOAc (200 mL) and washed with H_2O (200 mL x 2), brine (200 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl ((6^3*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)carbamate (1.45 g, 40% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{56}N_6O_6$ 728.4; found 729.4.

Step 9. To a mixture of *tert*-butyl ((6^3*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)carbamate (130 mg, 0.20 mmol) in DCM (1.0 mL) at 0 °C was added TFA (0.3 mL). The mixture was warmed to room temperature and stirred for 2 h, then concentrated under reduced pressure to give (6^3*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-5,7-dione, which was used directly in the next step directly without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{36}H_{48}N_6O_4$ 628.4; found 629.4.

Step 10. To a mixture of ((6^3*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-5,7-dione (130 mg, 0.2 mmol), DIPEA (270 mg, 2.0 mmol) and (2*S*)-3-methyl-2-{*N*-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanoic acid (118 mg, 0.40 mmol) in DMF (3.0 mL) at 0 °C under an atmosphere of Ar was added HATU (87 mg, 0.30 mmol) in portions. The mixture was stirred at 0 °C for 1 h, then diluted with H_2O extracted with EtOAc (30 mL x 2). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (3*S*)-1-acryloyl-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylpyrrolidine-3-carboxamide (17.2 mg, 10% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{68}N_6O_4$ 892.5; found 893.5; ^{1}H NMR (400 MHz, CD_3OD) δ 8.74 (d, *J* = 4.4 Hz, 1H), 7.93 - 7.90 (m, 1H), 7.56 - 7.51 (m, 3H), 7.43 (d, *J* = 4.4 Hz, 1H), 6.63 - 6.53 (m, 2H), 6.33 - 6.23 (m, 2H),5.83 - 5.70 (m, 1H), 4.73 - 4.70 (d, *J* = 11.0 Hz, 1H), 4.48 - 4.45 (d, J = 13.0 Hz, 1H), 4.12 - 4.10 (m, 3H), 3.86 - 3.81(m, 4H), 3.79 - 3.75 (m, 1H), 3.72 - 3.69 (m, 3H), 3.57 - 3.47 (m, 2H), 3.21 - 3.09 (m, 1H), 3.07 - 3.04 (q, 4H), 3.02 - 2.95 (m, 3H), 2.86 - 2.82(m, 3H), 2.66 - 2.48 (m, 2H), 2.29 - 2.17 (m,

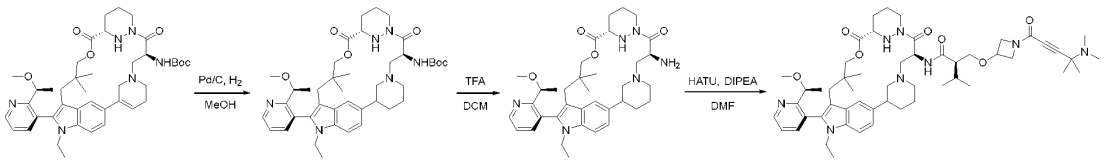
4H), 2.11 - 1.98 (m, 2H), 1.95 - 1.91 (m,1H), 1.45 (d, *J* = 6.2 Hz, 3H), 1.23 - 1.16 (m, 2H), 1.09 - 1.04 (m, 1H), 0.97 - 0.93 (m, 3H), 0.92 - 0.81 (m, 5H), 0.67 - 0.63 (m, 3H).

Example A663. The synthesis of (2*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)373yridine373-3-yl)oxy)methyl)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)373yridine-3-yl)-10,10-dimethyl-5,7-dioxo-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)-3-methylbutanamide



To a mixture of (6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)373yridine-3-yl)-10,10-dimethyl-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-5,7-dione (100 mg, 0.16 mmol), ®-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)373yridine373-3-yl)oxy)methyl)-3-methylbutanoic acid (80 mg, 0.24 mmol) and DIPEA (825 mg, 6.4 mmol) in DMF (2 mL) at 0 °C, was added HATU (95 mg, 0.24 mmol). The reaction mixture was stirred at 0 °C for 1 h, then poured into H_2O (60 mL), extracted with EtOAc (80 mL x 2). The combined organic layers were washed with H_2O (80 mL) and brine (80 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography to afford (2*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)373yridine373-3-yl)oxy)methyl)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)373yridine-3-yl)-10,10-dimethyl-5,7-dioxo-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)-3-methylbutanamide (55 mg, 36% yield) as solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.76 – 8.70 (m, 1H), 8.49 (dd, J = 4.3, 1.4 Hz, 0.1H), 7.93 – 7.87 (m, 1H), 7.58 – 7.50 (m, 3H), 7.41 (dd, J = 8.8, 3.2 Hz, 1H), 6.26 (d, J = 16.8 Hz, 1H), 5.96 (t, J = 9.6 Hz, 1H), 4.47 (d, J = 12.8 Hz, 1H), 4.39 – 4.28 (m, 2H), 4.21 – 3.97 (m, 5H), 3.96 – 3.70 (m, 5H), 3.68 – 3.54 (m, 3H), 3.51 – 3.35 (m, 1H), 3.11 (d, J = 22.7 Hz, 3H), 3.00 – 2.67 (m, 5H), 2.46 – 2.30 (m, 7H), 2.24 (s, 3H), 2.11 (d, J = 12.4 Hz, 1H), 1.92 (d, J = 13.2 Hz, 1H), 1.85 – 1.60 (m, 3H), 1.45 (d, J = 7.8 Hz, 6H), 1.32 (d, J = 16.0 Hz, 3H), 1.12 (dt, J = 24.5, 6.8 Hz, 3H), 0.95 (m, 6H), 0.76 (m, 6H). LCMS (ESI): m/z [M+H] calc'd for $C_{53}H_{74}N_8O_7$ 934.6; found 935.5.

Example A646. The synthesis of (2*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(3,1)-piperidinacycloundecaphane-4-yl)-3-methylbutanamide



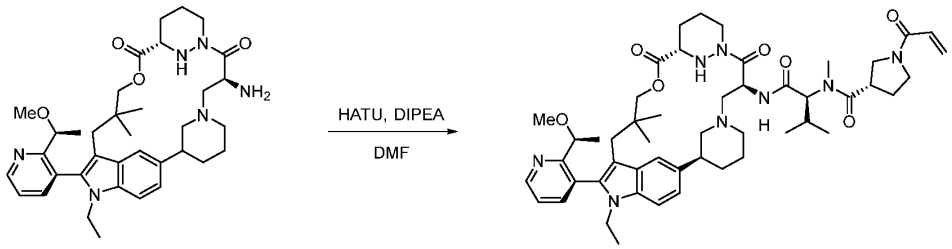
Step 1. A mixture of *tert*-butyl ((6^3*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)carbamate (0.2 g, 0.28 mmol) and Pd/C (0.2 g, 2 mmol) in MeOH (10 mL) was stirred at 25 °C for 16 h under an H_2 atmosphere. The reaction mixture was filtered through Celite, concentrated under reduced pressure to afford *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(3,1)-piperidinacycloundecaphane-4-yl)carbamate as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{58}N_6O_6$ 730.4; found 731.4.

Step 2. To a solution of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(3,1)-piperidinacycloundecaphane-4-yl)carbamate (150 mg, 0.2 mmol) in DCM (1.5 mL) at 0 °C was added TFA (0.5 mL). The reaction mixture was stirred at 20 °C for 1 h, then concentrated under reduced pressure to afford (6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(3,1)-piperidinacycloundecaphane-5,7-dione as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{36}H_{50}N_6O_4$ 630.4; found 631.4.

Step 3. To a mixture of (6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(3,1)-piperidinacycloundecaphane-5,7-dione (240 mg, 0.4 mmol), DIPEA (982 mg, 2 mmol) and (*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-3-methylbutanoic acid (148 mg, 0.45 mmol) in DMF (4 mL) at 0 °C under argon atmosphere, was added HATU (173 mg, 0.46 mmol) in portions. The reaction mixture was stirred at 0 °C under an argon atmosphere for 1 h, then quenched with H_2O at 0 °C. The resulting mixture was extracted with EtOAc (30 mL x 2). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by reverse phase chromatography to afford (2*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(3,1)-piperidinacycloundecaphane-4-yl)-3-methylbutanamide (150 mg, 38% yield) as solid. ^{1}H NMR (400 MHz, CD3OD) δ 8.72 (d, J = 4.8 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.53-7.49(m, 2H), 7.42 (d, J = 8.4 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 5.95-5.91 (m, 1H), 4.52-4.49 (m, 1H), 4.37-4.25 (m, 3H), 4.18-4.15 (m, 2H), 3.99-3.98 (m, 2H), 3.90-3.86 (m, 1H), 3.76-3.68 (m, 2H), 3.55-3.50(m, 2H), 3.39-3.36 (m, 2H), 3.20 (s, 3H), 3.02 (s, 3H), 2.89-2.79 (m, 3H), 2.62-2.50 (m, 2H),

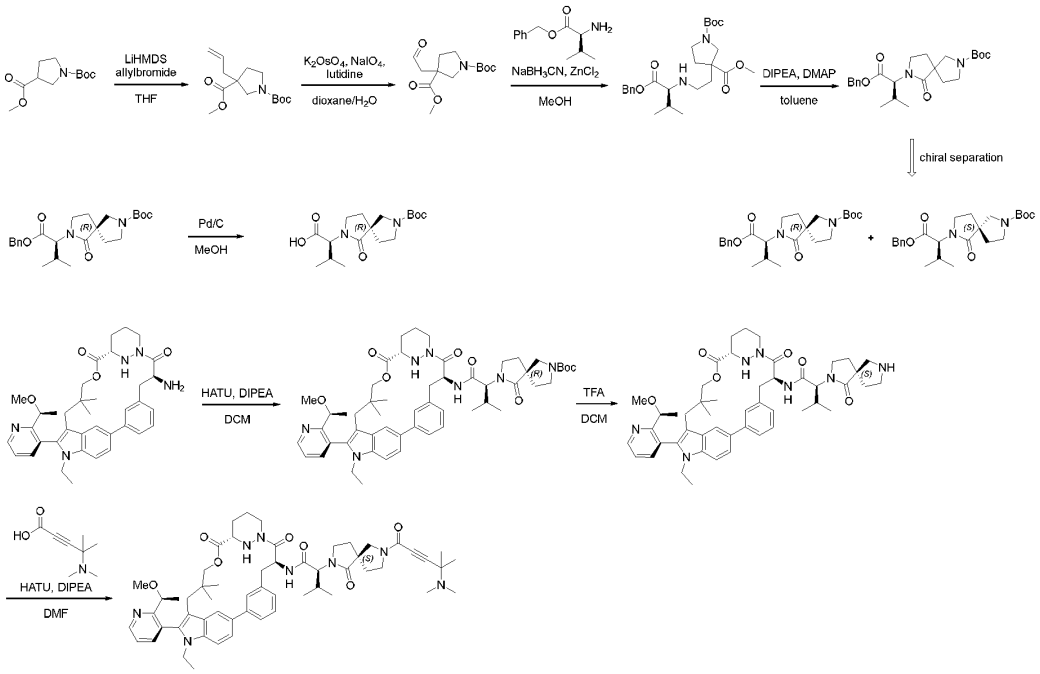
2.36 (s, 3H), 2.35-2.30 (m, 1H), 2.26(s, 3H), 2.20-1.15 (m, 1H), 1.97-1.93 (m, 3H), 1.81-1.76 (m, 4H), 1.64-1.61 (m, 2H), 1.46-1.43 (m, 6H), 1.36 (d, J = 14.8 Hz, 3H), 1.02 (s, 3H), 0.94 (m, 6H), 0.81 (s, 3H), 0.65 (s, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{53}H_{76}N_8O_7$ 936.6; found 937.5.

Example A740. Synthesis of (3*S*)-1-acryloyl-*N*-((2*S*)-1-(((2^3*S*,6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(3,1)-piperidinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylpyrrolidine-3-carboxamide



To a mixture of (6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(3,1)-piperidinacycloundecaphane-5,7-dione (140 mg, 0.20 mmol), DIPEA (570 mg, 4.4 mmol) and (2*S*)-3-methyl-2-{*N*-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanoic acid (124 mg, 0.40 mmol) in DMF (3.0 mL) at 0 °C under an atmosphere of Ar was added HATU (100 mg, 0.30 mmol) in portions. The mixture was stirred at 0 °C for 1 h, then H_2O was added and the mixture extracted with EtOAc (2 x 30 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (3*S*)-1-acryloyl-*N*-((2*S*)-1-(((2^3*S*,6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(3,1)-piperidinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methylpyrrolidine-3-carboxamide (41 mg, 20% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{70}N_8O_7$ 894.5; found 895.5; ^{1}H NMR (400 MHz, CD_3OD) δ 8.72 (d, *J* = 4.8 Hz, 1H), 7.87 (d, *J* = 7.6 Hz, 1H), 7.51 - 7.49 (m, 2H), 7.42 - 7.37 (m, 1H), 7.18 - 7.14 (m, 1H), 6.64 - 6.54 (m, 1H), 6.30 - 6.23 (m, 1H), 5.77 - 5.70 (m, 2H), 4.65 - 4.60 (m, 1H), 4.50 - 4.40 (m, 1H), 4.27 - 4.16 (m, 2H), 4.00 - 3.95 (m, 2H), 3.83 - 3.78(m, 2H), 3.73 - 3.60 (m, 4H), 3.51 - 3.36 (m, 3H), 3.22 - 3.19 (m, 4H), 3.07 (d, *J* = 6.8 Hz, 2H), 2.99 (d, *J* = 12.0 Hz, 3H), 2.90 - 2.78 (m, 2H), 2.75 - 2.64 (m, 3H), 2.20 - 2.10 (m, 4H), 2.02 - 1.93 (m, 3H), 1.87 - 1.64 (m, 4H), 1.45 (d, *J* = 4.8 Hz, 3H), 1.06 - 1.00 (m, 4H), 0.97 - 0.89 (m, 3H), 0.83 - 0.79 (m, 3H), 0.66 (s, 3H).

Example A534. (2*S*)-2-((*S*)-7-(4-(dimethylamino)-4-methylpent-2-ynoyl)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide



Step 1. To a mixture of 1-*tert*-butyl 3-methyl pyrrolidine-1,3-dicarboxylate (20.0 g, 87.2 mmol) in THF (150 mL) at -78 °C under an atmosphere of nitrogen was added 1M LiHMDS in THF (113.4 mL, 113.4 mmol). After stirring at -78 °C for 40 min, allyl bromide (13.72 g, 113.4 mmol) was added and the mixture was allowed to warm to room temperature and stirred for 4 h. The mixture was cooled to 0 °C, saturated NaCl (30 mL) was added and the mixture extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 1-(*tert*-butyl) 3-methyl 3-allylpyrrolidine-1,3-dicarboxylate (17 g, 72% yield) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.80 - 5.60 (m, 1H), 5.16 - 5.02 (m, 2H), 3.71 (s, 4H), 3.42 (d, *J* = 9.3 Hz, 2H), 3.27 (t, *J* = 11.2 Hz, 1H), 2.42 (d, *J* = 7.6 Hz, 2H), 2.38 - 2.24 (m, 1H), 2.05 (s, 1H), 1.85 (dt, *J* = 14.3, 7.5 Hz, 1H), 1.46 (s, 10H), 1.27 (t, *J* = 7.1 Hz, 1H).

Step 2. To a mixture of 1-(*tert*-butyl) 3-methyl 3-allylpyrrolidine-1,3-dicarboxylate (4.0 g, 14.9 mmol) and 2,6-dimethylpyridine (3.18 g, 29.7 mmol) in 1,4-dioxane (200 mL) and H_2O (100 mL) at 0 °C was added K_2OsO_4 $2H_2O$ (0.11 g, 0.3 mmol) in portions. The mixture was stirred for 15 min at 0 °C, then $NaIO_4$ (6.35 g, 29.7 mmol) was added in portions. The mixture was stirred at room temperature for 3 h at room temperature, then cooled to 0 °C and saturated aqueous $Na_2S_2O_3$ (50 mL) added. The mixture was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with 2 M HCl, then dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give 1-(*tert*-butyl) 3-methyl 3-(2-oxoethyl)pyrrolidine-1,3-dicarboxylate (4 g, 52% yield) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.80 - 5.60 (m, 1H), 5.16 -

5.04 (m, 2H), 3.72 (s, 3H), 3.41 (s, 3H), 3.28 (d, *J* = 11.0 Hz, 1H), 2.44 (s, 2H), 2.31 (d, *J* = 9.1 Hz, 1H), 1.85 (dt, *J* = 12.7, 7.5 Hz, 1H), 1.69 (s, 1H), 1.47 (s, 10H).

Step 3. To a mixture of 1-(*tert*-butyl) 3-methyl 3-(2-oxoethyl)pyrrolidine-1,3-dicarboxylate (6.30 g, 23.2 mmol), in MeOH (70 mL) at 0 °C was added benzyl (2*S*)-2-amino-3-methylbutanoate (7.22 g, 34.8 mmol) and $ZnCl_2$ (4.75 g, 34.8 mmol). The mixture was warmed to room temperature and stirred for 30 min, then cooled to 0 °C and $NaCNBH_3$ (2.92 g, 46.4 mmol) was added in portions. The mixture was warmed to room temperature and stirred for 2 h, then cooled to 0 °C and saturated aqueous NH_4Cl added. The mixture was extracted with EtOAc (3 x 200 mL) and the combined organic layers were washed with brine (150 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 1-(*tert*-butyl) 3-methyl 3-(2-(((*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)amino)ethyl)pyrrolidine-1,3-dicarboxylate (6.4 g, 54% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{25}H_{38}N_2O_6$ 462.3; found 463.4.

Step 4. To a mixture of 1-(*tert*-butyl) 3-methyl 3-(2-(((*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)amino)ethyl)pyrrolidine-1,3-dicarboxylate (4.50 g, 9.7 mmol) in toluene (50 mL) was added DIPEA (12.57 g, 97.3 mmol) and DMAP (1.19 g, 9.7 mmol). The resulting mixture was heated to 80 °C and stirred for 24 h, then concentrated under reduced pressure and the residue was purified by preparative-HPLC, then by chiral-HPLC to give *tert*-butyl (*R*)-7-((*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate (1.0 g, 32% yield) and *tert*-butyl (*S*)-7-((*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate (1.0 g, 32% yield) and as a an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{24}H_{34}N_2O_5$ 430.5; found 431.2 and LCMS (ESI): m/z [M+H] calc'd for $C_{24}H_{34}N_2O_5$ 430.3; found 431.2.

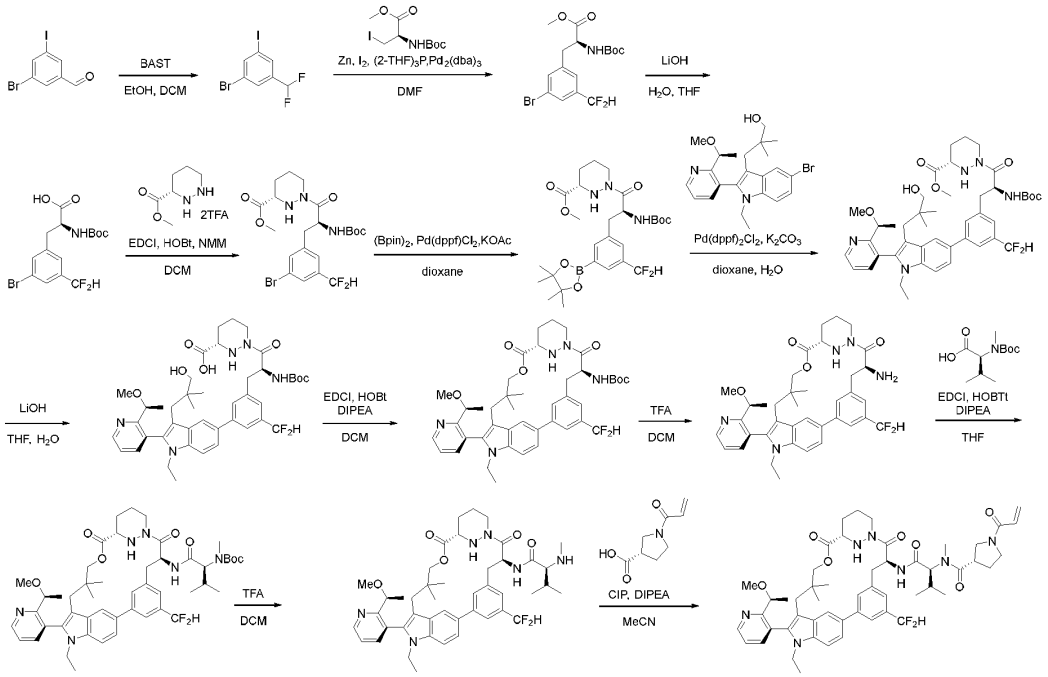
Step 5. A mixture of *tert*-butyl (*R*)-7-((*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate (4.0 g) and 10% Pd/C (1 g) in MeOH (40 mL) was stirred at room temperature under an atmosphere of H_2. The mixture was filtered through a pad of Celite pad and the filtrae was concentrated under reduced pressure to give (*S*)-2-((*R*)-7-(*tert*-butoxycarbonyl)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)-3-methylbutanoic acid (4.9 g) as a solid. LCMS (ESI): m/z [M-H] calc'd for $C_{17}H_{28}N_2O_5$ 340.2; found 339.3.

Step 6. To a mixture of (6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (500 mg, 0.8 mmol) in DCM at 0 °C were added DIPEA (829 mg, 6.4 mmol), ((*S*)-2-((*R*)-7-(*tert*-butoxycarbonyl)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)-3-methylbutanoic acid (273 mg, 0.8 mmol) and HATU (396 mg, 1.0 mmol) in portions over 1 min. The mixture was allowed to warm to room temperature and stirred 2 h, then concentrated under reduced pressure and the residue was purified by preparative-TLC to give *tert*-butyl (5*R*)-7-((2*S*)-1-((((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate (500 mg, 64% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{54}H_{71}N_7O_8$ 945.5; found 946.5.

Step 7. To a mixture of *tert*-butyl (5*R*)-7-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate (1.0 g, 1.06 mmol) in DCM (10 mL) at 0 °C was added TFA (3 mL) dropwise. The mixture was warmed to room temperature and stirred for 1 h, then concentrated under reduced pressure to give (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-((*S*)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)butanamide (1.3 g). LCMS (ESI): m/z [M-H] calc'd for $C_{49}H_{63}N_7O_6$ 846.1; found 845.5.

Step 8. To a mixture of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-((*S*)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)butanamide (500 mg, 0.59 mmol) and DIPEA (764 mg, 5.9 mmol) in DMF (5 mL) at 0 °C were added 4-(dimethylamino)-4-methylpent-2-ynoic acid (110 mg, 0.71 mmol) and HATU (292 mg, 0.77 mmol) in portions. The mixture was warmed to room temperature and stirred for 1 h, then H_2O (10 mL) was added and the mixture extracted with EtOAc (10 mL x 3). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (2*S*)-2-((*S*)-7-(4-(dimethylamino)-4-methylpent-2-ynoyl)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide (177 mg, 28.94% yield) as a white solid. LCMS (ESI): m/z [M+H] calc'd for $C_{57}H_{74}N_8O_7$ 982.6; found 983.8; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (dd, *J* = 4.7, 1.7 Hz, 1H), 8.52 (d, *J* = 7.9 Hz, 1H), 7.99 (d, *J* = 1.7 Hz, 1H), 7.83 (d, *J* = 10.2 Hz, 2H), 7.74 - 7.58 (m, 3H), 7.53 (dd, *J* = 7.7, 4.8 Hz, 1H), 7.24 (t, *J* = 7.6 Hz, 1H), 7.12 (d, *J* = 7.6 Hz, 1H), 5.32 (d, *J* = 9.7 Hz, 2H), 4.33 - 4.20 (m, 4H), 4.03 (dd, *J* = 15.0, 8.6 Hz, 2H), 3.88 - 3.82 (m, 1H), 3.63 (dq, *J* = 20.5, 10.3 Hz, 4H), 3.42 - 3.34 (m, 2H), 3.21 (s, 1H), 3.13 (d, *J* = 2.8 Hz, 3H), 2.87 (s, 2H), 2.83 - 2.72 (m, 2H), 2.69 - 2.62 (m, 1H), 2.21 (d, *J* = 22.6 Hz, 6H), 2.12 - 1.76 (m, 7H), 1.75 - 1.47 (m, 2H), 1.46 - 1.28 (m, 9H), 0.99 - 0.89 (m, 6H), 0.79 - 0.71 (m, 6H), 0.52 (s, 3H).

Example A341. Synthesis of (3*S*)-1-acryloyl-*N*-((2*S*)-1-(((6^3*S*,4*S*)-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methylpyrrolidine-3-carboxamide



Step 1. To a mixture of 3-bromo-5-iodobenzaldehyde (4.34 g, 14.0 mmol) in DCM at 0 °C under an atmosphere of N_2 was added BAST (6.8 g, 30.7 mmol) and EtOH (129 mg, 2.8 mmol) dropwise. The mixture was heated with microwave heating at 27 °C for 14 h. H_2O (500 mL) was added and the mixture was extracted with DCM (200 mL x 3), the combined organic layers were concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 1-bromo-3-(difluoromethyl)-5-iodobenzene (3.2 g, 65% yield) as a solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.16 (p, *J* = 1.2 Hz, 1H), 7.94 (p, *J* = 1.3 Hz, 1H), 7.81 (p, *J* = 1.3 Hz, 1H), 7.00 (t, *J* = 55.3 Hz, 1H).

Step 2. A mixture of Zn (2.28 g, 34.8 mmol) and I_2 (442 mg, 1.74 mmol) in DMF (20 mL) under an atmosphere of Ar was stirred at 50 °C for 0.5 h. To this mixture was added a solution of methyl (methyl (*R*)-2-((*tert*-butoxycarbonyl)amino)-3-iodopropanoate (2.39 g, 7.25 mmol) in DMF (20 mL) and the mixture was stirred at 50 °C for 2 h. After cooling, the mixture was added to 1-bromo-3-(difluoromethyl)-5-iodobenzene (2.90 g, 8.7 mmol), $Pd_2(dba)_3$ (239 mg, 0.26 mmol) and tri-2-furylphosphine (162 mg, 0.7 mmol) in DMF (20 mL). The mixture was heated to 70 °C and stirred for 2 h, then H_2O (200mL) was added and the mixture extracted with EtOAc (200 mL x 3). The combined organic layers were concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (*S*)-3-(3-bromo-5-(difluoromethyl)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanoate (560 mg, 19% yield) as a solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.65 (d, *J* = 10.0 Hz, 2H), 7.47 (s, 1H), 7.36 (d, *J* = 8.4 Hz, 1H), 7.00 (t, *J* = 55.6 Hz, 1H), 4.25

(td, *J* = 9.6, 4.7 Hz, 1H), 3.64 (s, 3H), 3.11 (dd, *J* = 13.6, 4.9 Hz, 1H), 3.00 - 2.80 (m, 1H), 1.32 (s, 9H).

Step 3. To a mixture of methyl (*S*)-3-(3-bromo-5-(difluoromethyl)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanoate (650 mg, 1.6 mmol) in THF (1.5 mL) at 0 °C under an atmosphere of N_2 was added LiOH (114 mg, 4.8 mmol) in H_2O (1.50 mL). The mixture was stirred at 0 °C for 1 h, then acidified to pH 5 with 1M HCl. The mixture was extracted with DCM / MeOH (10/1) (100 mL x 3) and the combined organic layers were dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give (*S*)-3-(3-bromo-5-(difluoromethyl)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (500 mg), which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{15}H_{18}BrF_2NO_4$ 393.0; found 392.1.

Step 4. To a mixture of methyl (3*S*)-1,2-diazinane-3-carboxylate (475 mg, 3.3 mmol) in DCM (10 mL) at 0 °C under an atmosphere of N_2 were added *N*-methylmorpholine (3.34 g, 33.0 mmol) and (*S*)-3-(3-bromo-5-(difluoromethyl)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (650 mg, 1.7 mmol) and HOBt (45 mg, 0.33 mmol) and EDCI (632 mg, 3.3 mmol). The mixture was warmed to room temperature and stirred for 16 h, then diluted with DCM (100 mL) and H_2O. The organic and aqueous layer was separated and the aqueous layer was extracted with DCM (100 mL x 3). The combined organic layers were dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (*S*)-1-((*S*)-3-(3-bromo-5-(difluoromethyl)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (510 mg, 56% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{21}H_{28}BrF_2N_3O_5$ 519.1; found 520.3.

Step 5. To a mixture of 4,4,5,5-tetramethyl-2-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (488 mg, 1.92 mmol) and methyl (*S*)-1-((*S*)-3-(3-bromo-5-(difluoromethyl)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (500 mg, 0.96 mmol) in 1,4-dioxane (5 mL) was added Pd(dppf)Cl_2 (70 mg, 0.07 mmol) and KOAc (236 mg, 2.4 mmol) in portions. The mixture was heated to 90 °C and stirred for 4 h then diluted with H_2O (100 mL). The mixture was extracted with DCM (100 mL x 3) and the combined organic layers were dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(difluoromethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylate (423 mg, 73% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{27}H_{40}BF_2N_3O_7$ 567.3; found 568.2.

Step 6. To a mixture of methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(difluoromethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylate (260 mg, 0.47 mmol), (*S*)-3-(5-bromo-1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol and Pd(dppf)Cl_2 (34 mg, 0.05 mmol) in 1,4-dioxane (3 mL) and H_2O (0.6 mL) was added K_2CO_3 (163 mg, 1.12 mmol). The mixture was heated to 60 °C and stirred for 16 h, then diluted with H_2O (100 mL) and extracted with DCM (100 mL x 3). The combined organic layers were dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica

gel column chromatography to give methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(difluoromethyl)-5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylate (350 mg, 78% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{57}F_2N_5O_7$ 805.4; found 806.6.

Step 7. To a mixture of methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(difluoromethyl)-5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylate (350 mg, 0.43 mmol) in THF (2.8 mL) at 0 °C was added LiOH H_2O (54 mg, 1.3 mmol) in H_2O (0.7 mL). The mixture was warmed to room temperature and stirred for 2 h, then acidified to pH 5 with 1M HCl and extracted with EtOAc (50 mL x 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to give (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(difluoromethyl)-5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylic acid (356 mg) was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{55}F_2N_5O_7$ 791.4; found 792.6.

Step 8. To a mixture of *S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(difluoromethyl)-5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylic acid (356 mg, 0.45 mmol) and DIPEA (1.74 g, 13.5 mmol) in DCM were added EDCI (2.41 g, 12.6 mmol) and HOBt (304 mg, 2.3 mmol). The mixture was stirred for 16 h then H_2O was added and the mixture extracted with EtOAc (200 mL x 3). The combined organic layers were washed with brine (50 mL x 4), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl ((6^3*S*,4*S*)-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (202 mg, 51% yield). LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{53}F_2N_5O_6$ 773.4; found 774.6.

Step 9. To a mixture of *tert*-butyl ((6^3*S*,4*S*)-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (202 mg, 0.26 mmol) in DCM (2 mL) at 0 °C was added TFA (1.0 mL) dropwise. The mixture was stirred at 0 °C for 1.5 h, then concentrated under reduced pressure and dried azeotropically with toluene (3 mL x 3) to give (6^3*S*,4*S*)-4-amino-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione, which was used directly in the next without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{45}F_2N_5O_4$ 673.3; found 674.5.

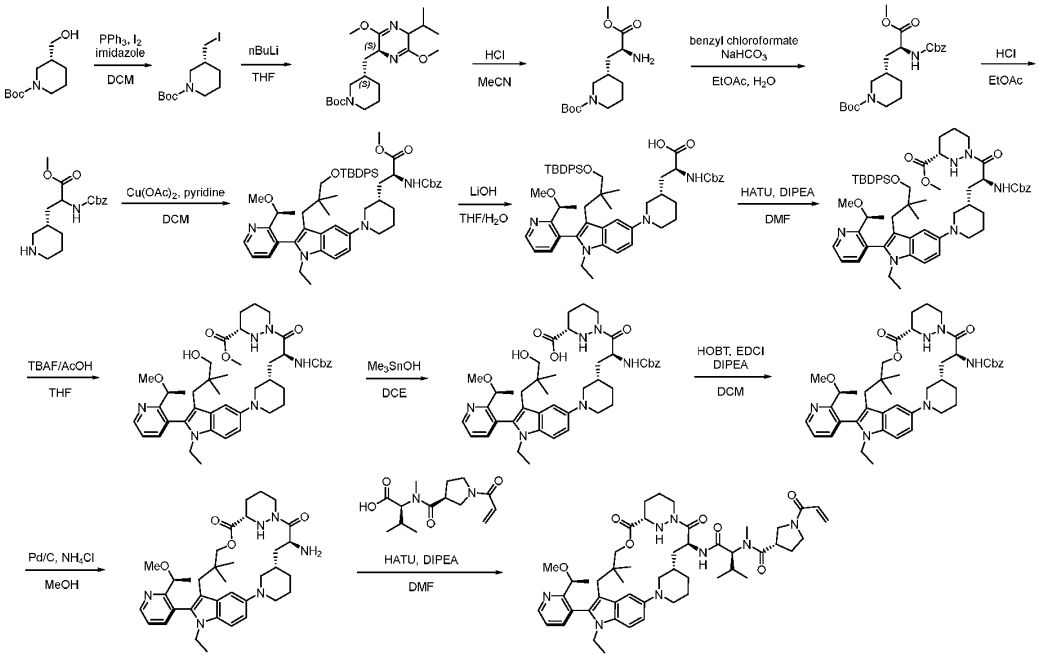
Step 10. To a mixture of (6^3*S*,4*S*)-4-amino-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (202 mg, 0.3 mmol) and (2*S*)-2-[(*tert*-butoxycarbonyl)(methyl)amino]-3-methylbutanoic acid (139 mg, 0.6 mmol) in THF under an atmosphere of Ar were added DIPEA (581 mg, 4.5 mmol), EDCI (86 mg, 0.45 mmol) and HOBt (61 mg, 0.45 mmol). The mixture was stirred for 16 h, then H_2O (100 mL) added and the mixture

extracted with EtOAc (200 mL x 3). The combined organic layers were washed with brine (50 mL x 3), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl ((2*S*)-1-(((6^3*S*,4*S*)-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (135 mg, 46% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{49}H_{64}F_2N_6O_7$ 886.5; found 887.6.

Step 11. To a mixture of *tert*-butyl ((2*S*)-1-(((6^3*S*,4*S*)-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (130 mg, 0.15 mmol) in DCM at 0 °C under an atmosphere of N_2 was added TFA (1.0 mL) dropwise. The mixture was stirred at 0 °C for 1.5 h, then concentrated under reduced pressure and dried azeotropically with toluene (3 mL x 3) to give (2*S*)-*N*-((6^3*S*,4*S*)-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide (130 mg), which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{56}F_2N_6O_5$ 786.4; found 787.6.

Step 12. To a mixture of (2*S*)-*N*-((6^3*S*,4*S*)-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide (130 mg, 0.17 mmol) and (3*S*)-1-(prop-2-enoyl)pyrrolidine-3-carboxylic acid (56 mg, 0.33 mmol) in MeCN (1.5 mL) at 0 °C under an atmosphere of N_2 were added DIPEA (427 mg, 3.3 mmol) and CIP (69 mg, 0.25 mmol). The mixture was stirred at 0 °C for 1 h, then H_2O (100 mL) was added and the mixture extracted with EtOAc (200 mL x 3). The combined organic layers were washed with brine (50 mL x 3), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (3*S*)-1-acryloyl-*N*-((2*S*)-1-(((6^3*S*,4*S*)-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methylpyrrolidine-3-carboxamide (58 mg, 36% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{52}H_{65}F_2N_7O_7$ 937.4; found 938.1; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.78 (dd, *J* = 4.8, 1.7 Hz, 1H), 8.43 - 8.21 (m, 1H), 8.02 (s, 2H), 7.93 - 7.81 (m, 2H), 7.76 (dd, *J* = 9.3, 3.9 Hz, 1H), 7.66 (d, *J* = 8.7 Hz, 1H), 7.56 (dd, *J* = 7.7, 4.8 Hz, 1H), 7.34 (d, *J* = 5.4 Hz, 1H), 7.20 - 6.86 (m, 1H), 6.80 - 6.40 (m, 1H), 6.15 (ddt, *J* = 16.8, 4.9, 2.4 Hz, 1H), 5.90 - 5.60 (m, 1H), 5.59 - 5.19 (m, 2H), 4.71 (dd, *J* = 10.7, 3.1 Hz, 1H), 4.40 - 4.17 (m, 3H), 4.12 - 3.90 (m, 3H), 3.85 - 3.71 (m, 1H), 3.61 (tdd, *J* = 23.4, 9.9, 4.3 Hz, 6H), 3.40 - 3.30 (m, 2H), 3.11 (d, *J* = 6.8 Hz, 3H), 3.08 - 2.90 (m, 2H), 2.87 (s, 2H), 2.84 (s, 3H), 2.69 - 2.30 (d, *J* = 16.5 Hz, 1H), 2.30 - 1.79 (m, 5H), 1.75 - 1.45 (m, 2H), 1.40 (d, *J* = 6.1 Hz, 3H), 1.05 - 0.85 (m, 6H), 0.85 - 0.66 (m, 6H), 0.57 (d, *J* = 11.8 Hz, 3H).

Example A741. Synthesis of (3*S*)-1-acryloyl-*N*-((2*S*)-1-(((2^3*S*,6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-piperidinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methylpyrrolidine-3-carboxamide



Step 1. A solution of *tert*-butyl (3*R*)-3-(hydroxymethyl) piperidine-1-carboxylate (10 g, 46.45 mmol) in DCM (200 mL) at 0 °C, was added PPh_3 (15.8 g, 60.4 mmol), Imidazole (4.7 g, 69.7 mmol) and I_2 (14.1 g, 55.74 mmol). The reaction suspension was stirred at 20 °C for 17 h, then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford *tert*-butyl (3*R*)-3-(iodomethyl) piperidine-1-carboxylate (10 g, 66% yield) as oil. LCMS (ESI): m/z [M+H] calc'd for $C_{11}H_{20}INO_2$ 325.1; found no mass.

Step 2. To a mixture of 3-isopropyl-2,5-dimethoxy-3,6-dihydropyrazine (10.8 g, 58.9 mmol) in THF (150 mL) at -60 ˚C under an atmosphere of N_2 was added n-BuLi (47 mL, 2.5 M in hexane, 117.7 mmol) dropwise. The mixture was warmed to 0 °C and was stirred for 2 h, then re-cooled to -60 °C, and a solution of *tert*-butyl (3*R*)-3-(iodomethyl)piperidin-1-yl formate (9.60 g, 29.4 mmol) in THF (50 mL) was slowly added dropwise. The mixture was stirred at -60 °C for 2 h then warmed to room temperature and stirred for 2 h. Saturated NH_4Cl (150 mL) was slowly added and the mixture extracted with EtOAc (150 mL x 2). The combined organic layers were washed with brine (200 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was reduced under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl (3*S*)-3-{[(2*S*)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl]methyl}piperidin-1-yl formate (5.3 g, 46% yield) as a gum. LCMS (ESI): m/z [M+H] calc'd for $C_{20}H_{35}N_3O_4$ 381.5; found 382.3.

Step 3. A mixture of *tert*-butyl (3*S*)-3-{[(2*S*)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl]methyl}piperidin-1-yl formate (5.30 g, 13.9 mol) in MeCN (4 mL) was added 1M HCl (27.7 mL, 27.7 mmol) dropwise. The mixture was stirred for 2 h, then saturated $NaHCO_3$ until ~pH 7-8, then

extracted with DCM (30 mL x 2). The combined organic layers was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give methyl (*S*)-*tert*-butyl 3-((*S*)-2-amino-3-methoxy-3-oxopropyl)piperidine-1-carboxylate (4.3 g, 95% yield) as an oil, which was used in next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{14}H_{26}N_2O_4$ 286.2; found 287.3.

Step 4. To a mixture of methyl (*S*)-*tert*-butyl 3-((*S*)-2-amino-3-methoxy-3-oxopropyl)piperidine-1-carboxylate (4.30 g, 15.0 mmol) in EtOAc (30 mL) and H_2O (20 mL) at -10 °C was added $NaHCO_3$ (3.77 g, 44.88 mmol). The mixture was stirred at -10 °C for 10 min, then a solution of benzyl chloroformate (3.83 g, 22.44 mmol) was added dropwise. The mixture was warmed to 0 °C and stirred for 1 h, then H_2O (50 mL) was added and the mixture extracted with EtOAc (50 mL x 2). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure to give *tert*-butyl (3*S*)-3-[(2*S*)-2-{[(benzyloxy)carbonyl] amino}-3-methoxy-3-oxopropyl]piperidine-1-carboxylate (4.0 g, 60% yield) as a gum. LCMS (ESI): m/z [M-Boc+H] calc'd for $C_{17}H_{24}N_2O_4$ 320.2; found 321.3.

Step 5. To a mixture of *tert*-butyl (3*S*)-3-[(2*S*)-2-{[(benzyloxy)carbonyl] amino}-3-methoxy-3-oxopropyl]piperidine-1-carboxylate (1.0 g, 2.38 mmol) in EtOAc (8 mL) was added 2M HCl in EtOAc (11.9 mL, 23.8 mmol). The mixture was stirred for 2 h, then saturated $NaHCO_3$ added until ~pH 8-9, and the mixture extracted with DCM (30 mL x 3). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give (2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-[(3*S*)-piperidin-3-yl]propanoate (740 mg, 91% yield) as a gum. LCMS (ESI): m/z [M+H] calc'd for $C_{17}H_{24}N_2O_4$ 320.2; found 321.2.

Step 6. To a mixture of (3-{3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl}-1-ethyl-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}indol-5-yl)boranediol (5.47 g, 8.43 mmol) and methyl (2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-[(3*S*)-piperidin-3-yl]propanoate (2.70 g, 8.43 mmol) in DCM (70mL) was added $Cu(OAc)_2$ (6.06 g, 16.86 mmol) and pyridine (2.0 g , 25.3 mmol). The mixture was stirred under an atmosphere of O_2 for 48 h, then diluted with DCM (200 mL) and washed with H_2O (150 mL x 2). The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl 2-{[(benzyloxy)carbonyl]amino}-3-[(3*S*)-1-(3-{3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl}-1-ethyl-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}indol-5-yl)piperidin-3-yl]propanoate (3.6 g, 42% yield) as a solid. LCMS (ESI): m/z [M/2+H] calc'd for $C_{56}H_{70}N_4O_6Si$ 462.3; found 462.3.

Step 7. To a mixture of methyl 2-{[(benzyloxy)carbonyl]amino}-3-[(3*S*)-1-(3-{3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl}-1-ethyl-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}indol-5-yl)piperidin-3-yl]propanoate (3.60 g, 3.57 mmol) in THF (60 mL) and H_2O (30 mL) was added LiOH (342 mg, 14.28 mmol). The mixture was stirred for 2 h, then diluted with H_2O (150 mL), then 1M HCl was added slowly until ~pH 3-4 and the mixture extracted with EtOAc (200 mL x 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to give 2-{[(benzyloxy)carbonyl]amino}-3-[(3*S*)-1-(3-{3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl}-1-ethyl-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}indol-5-yl)piperidin-3-yl]propanoic acid (3.3 g, 85% yield) as a solid, which was used directly in the next step without further purification. LCMS (ESI): m/z [M/2+H] calc'd for $C_{55}H_{68}N_4O_6Si$ 455.3; found 455.3.

Step 8. To a mixture of methyl 2-{[(benzyloxy)carbonyl]amino}-3-[(3*S*)-1-(3-{3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl}-1-ethyl-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}indol-5-yl)piperidin-3-yl]propanoate (3.30 g, 2.91 mmol) in DMF (40 mL) was added methyl (3*S*)-1,2-diazinane-3-carboxylate (0.42 g, 2.91 mmol), HATU (2.21 g, 5.82 mmol) and DIPEA (2.26 g, 17.46 mmol). The mixture was stirred for 3 h, then poured into ice-H_2O and extracted with EtOAc (120 mL x 2). The combined organic layers were washed with saturated $NaHCO_3$ (150 mL), brine (150 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (3*S*)-1-[(2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-[(3*S*)-1-(3-{3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl}-1-ethyl-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}indol-5-yl)piperidin-3-yl]propanoyl]-1,2-diazinane-3-carboxylate (2.9 g, 95% yield) as a gum. LCMS (ESI): m/z [M/2+H] calc'd for $C_{61}H_{78}N_6O_7Si$ 518.3; found 518.3.

Step 9. To a mixture of methyl (3*S*)-1-[(2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-[(3*S*)-1-(3-{3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl}-1-ethyl-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}indol-5-yl)piperidin-3-yl]propanoyl]-1,2-diazinane-3-carboxylate (1.70 g, 1.64 mmol) was added a mixture of 1M TBAF in THF (19.68 mL, 19.68 mmol) and AcOH (1.18 g, 19.68 mmol). The reaction was heated to 60 °C and stirred for 22 h, then diluted with EtOAc (80 mL) and washed with saturated $NaHCO_3$ (80 mL), H_2O (60 mL x 2) and brine (60 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give methyl (3*S*)-1-[(2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-[(3*S*)-1-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}indol-5-yl]piperidin-3-yl]propanoyl]-1,2-diazinane-3-carboxylate (1.0 g, 73% yield) as a solid. LCMS (ESI): m/z [M/2+H] calc'd for $C_{45}H_{60}N_6O_7$ 399.2; found 399.4.

Step 10. To a mixture m methyl (3*S*)-1-[(2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-[(3*S*)-1-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}indol-5-yl]piperidin-3-yl]propanoyl]-1,2-diazinane-3-carboxylate (1.0 g, 1.1 mmol) in 1,2-dichloroethane (10 mL) was added Me_3SnOH (1.42 g 7.84 mmol). The mixture was heated to 65 °C and stirred for 10 h, then filtered and the filtrate was concentrated under reduced pressure to give (3*S*)-1-[(2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-[(3*S*)-1-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}indol-5-yl]piperidin-3-yl]propanoyl]-1,2-diazinane-3-carboxylic acid (1.0 g, 99% yield) as a gum. The product was used in the next step without further purification. LCMS (ESI): m/z [M/2+H] calc'd for $C_{44}H_{58}N_6O_7$ 392.2; found 392.3.

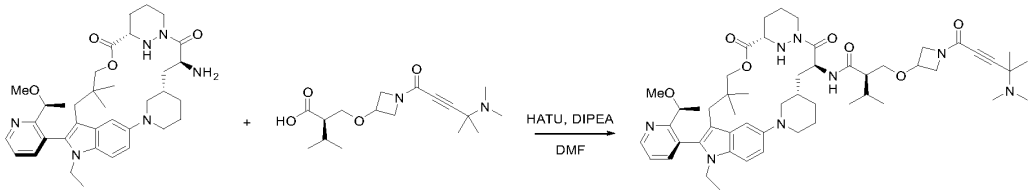
Step 11. To a mixture of (3*S*)-1-[(2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-[(3*S*)-1-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}indol-5-yl]piperidin-3-yl]propanoyl]-1,2-diazinane-3-carboxylic acid (1.0 g, 1.1 mmol) in DCM (30 mL) at 0 °C was added HOBT (1.51 g, 11.2 mmol), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide HCl (6.44 g, 33.6 mmol) and DIPEA (5.79 g, 44.8 mmol). The mixture was warmed to room temperature and stirred for 6 h, then diluted with H_2O and extracted with EtOAc (100 mL x 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give benzyl *N*-[(6*S*,8*S*,14*S*)-22-ethyl-21-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}-18,18-dimethyl-9,15-dioxo-16-oxa-2,10,22,28-

tetraazapentacyclo [18.5.2.1^{2,6}.1^{10, 14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]carbamate (340 mg, 36% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{56}N_6O_6$ 383.2; found 383.3.

Step 12. A mixture of benzyl *N*-[(6*S*,8*S*,14*S*)-22-ethyl-21-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}-18,18-dimethyl-9,15-dioxo-16-oxa-2,10,22,28-tetraazapentacyclo [18.5.2.1^{2,6}.1^{10, 14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]carbamate (250 mg, 0.33 mmol), Pd/C (100 mg) and NH_4Cl (353 mg, 6.6 mmol) in MeOH (5 mL) was stirred under an atmosphere of H_2 for 4 h. The mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was dissolved in DCM (30 mL) and washed with saturated $NaHCO_3$ (20 mL), H_2O (20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure to give (6*S*,8*S*,14*S*)-8-amino-22-ethyl-21-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}-18,18-dimethyl-16-oxa-2,10,22,28-tetraazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraene-9,15-dione, which was used in next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{36}H_{50}N_6O_4$ 631.4; found 631.4.

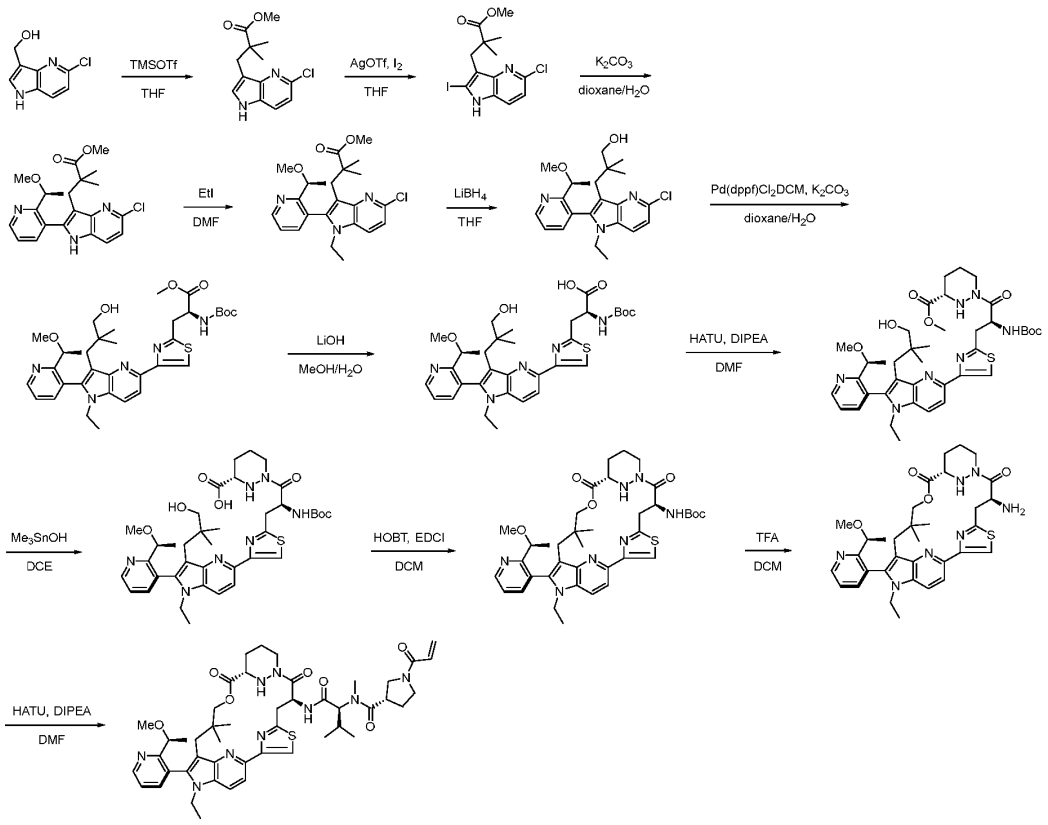
Step 13. To a mixture of (6*S*,8*S*,14*S*)-8-amino-22-ethyl-21-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}-18,18-dimethyl-16-oxa-2,10,22,28-tetraazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraene-9,15-dione (300 mg, 0.48 mmol), (2*S*)-3-methyl-2-{*N*-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanoic acid (136 mg, 0.48 mmol) and DIPEA (620 mg, 4.8 mmol) in DMF (5 mL) at 0 °C was added HATU (183 mg, 0.48 mmol). The mixture was stirred at 0-5 °C for 1 h, then diluted with EtOAc (50 mL), washed with H_2O (50 mL x 2), brine (50 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (2*S*)-*N*-[(6*S*,8*S*,14*S*)-22-ethyl-21-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}-18,18-dimethyl-9,15-dioxo-16-oxa-2,10,22,28-tetraazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]-3-methyl-2-{*N*-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide (90 mg, 20% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{70}N_8O_7$ 895.5; found 895.4; ^{1}H NMR (400 MHz, CD_3OD) δ 8.71 (dd, *J* = 4.8, 1.5 Hz, 1H), 7.86 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.51 (dd, *J* = 7.7, 4.8 Hz, 1H), 7.36 (dd, *J* = 8.9, 1.9 Hz, 1H), 7.22 (d, *J* = 12.1 Hz, 1H), 7.09 (dd, *J* = 8.9, 1.9 Hz, 1H), 6.59 (dt, *J* = 16.9, 9.9 Hz, 1H), 6.26 (ddd, *J* = 16.8, 5.0, 1.9 Hz, 1H), 5.80 - 5.67 (m, 1H), 5.59 - 5.46 (m, 1H), 4.93 (d, *J* = 12.4 Hz, 1H), 4.66 (dd, *J* = 11.1, 6.4 Hz, 1H), 4.45 (d, *J* = 12.6 Hz, 1H), 4.28 - 4.19 (m, 1H), 4.13 (dd, *J* = 14.5, 7.2 Hz, 1H), 4.02 - 3.87 (m, 1H), 3.87 - 3.36 (m, 11H), 3.16 (s, 2H), 3.10 (d, *J* = 3.4 Hz, 2H), 2.76 (dd, *J* = 26.9, 13.5 Hz, 3H), 2.61 (s, 1H), 2.35 - 1.97 (m, 5H), 1.78 (dd, *J* = 25.4, 22.1 Hz, 10H), 1.45 (d, *J* = 6.2 Hz, 3H), 1.04 (d, *J* = 6.2 Hz, 3H), 0.95 (dd, *J* = 6.5, 1.8 Hz, 3H), 0.83 (d, *J* = 6.6 Hz, 3H), 0.72 (d, *J* = 31.8 Hz, 6H).

Example A715. Synthesis of benzyl ((2^3*S*,6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-piperidinacycloundecaphane-4-yl)carbamate



To a solution of ((2^3*S*,6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-piperidinacycloundecaphane-5,7-dione (50 mg, 0.08 mmol), (*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-3-methylbutanoic acid (26 mg, 0.08 mmol) and DIPEA (31 mg, 0.24mmol) in DMF (1 mL) at 0 °C, was added HATU (30 mg, 0.08 mmol). The reaction mixture was stirred at 0-5 °C for 1 h, then diluted with EtOAc (20 mL), washed with H_2O (20 mL x 2) and brine (20 mL). The organic phase was separated and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give (2*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-*N*-((2^3*S*,6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-piperidinacycloundecaphane-4-yl)-3-methylbutanamide as solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.71 (d, J = 4.7 Hz, 1H), 8.24 (s, 1H), 8.10 (dd, J = 26.7, 7.8 Hz, 1H), 7.86 (d, J = 7.7 Hz, 1H), 7.51 (dd, J = 7.7, 4.8 Hz, 1H), 7.39 (dd, J = 8.9, 3.1 Hz, 1H), 7.26 (d, J = 16.6 Hz, 1H), 7.11 (d, J = 8.8 Hz, 1H), 5.61 (s, 1H), 4.50 – 4.28 (m, 3H), 4.27 – 4.07 (m, 3H), 3.98 (ddd, J = 25.6, 13.4, 5.1 Hz, 2H), 3.84 – 3.72 (m, 2H), 3.62 (dd, J = 10.7, 4.8 Hz, 2H), 3.55 (d, J = 7.1 Hz, 2H), 3.47 (d, J = 6.5 Hz, 2H), 3.16 (s, 3H), 3.03 – 2.91 (m, 1H), 2.76 (dd, J = 28.7, 15.2 Hz, 3H), 2.62 (s, 1H), 2.40 (t, J = 7.0 Hz, 3H), 2.33 (dd, J = 14.3, 5.0 Hz, 4H), 2.05 (d, J = 11.6 Hz, 1H), 1.99 – 1.64 (m, 10H), 1.64 – 1.55 (m, 1H), 1.51 – 1.42 (m, 6H), 1.37 (d, J = 12.3 Hz, 3H), 1.05 (s, 3H), 0.94 (ddd, J = 9.3, 6.7, 2.0 Hz, 6H), 0.76 (d, J = 3.8 Hz, 3H), 0.69 (s, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{53}H_{76}N_8O_7$ 936.6; found 937.4.

Example A347. Synthesis of (2*S*)-*N*-[(7*S*,13*S*)-21-ethyl-20-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}-17,17-dimethyl-8,14-dioxo-15-oxa-4-thia-9,21,25,27,28-pentaazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacosa-1(25),2,5(28),19,22(26),23-hexaen-7-yl]-3-methyl-2-{*N*-methyl-1-[(3*S*)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide



Step 1. To a mixture of (5-chloro-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)methanol (3.5 g, 19 mmol), and ((1-methoxy-2-methylprop-1-en-1-yl)oxy)trimethylsilane (6.7 g, 38 mmol) in THF (50 ml) at 0 °C was added TMSOTf (3.8 g, 17 mmol) dropwise. The mixture was stirred at 0-5 °C for 2 h, then diluted with EtOAc (100 mL) and washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL x 2). The organic layer was dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give methyl 3-(5-chloro-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2,2-dimethylpropanoate (3.0 g, 59% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{15}ClN_2O_2$ 266.1; found 267.1.

Step 2. To a mixture of methyl 3-(5-chloro-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2,2-dimethylpropanoate (3.0 g, 11 mmol) in anhydrous THF (50 mL) at 0 °C was added AgOTf (4.3g, 17 mmol) and I_2 (2.9 g, 11 mmol). The mixture was stirred at 0 °C for 2 h, then saturated Na_2SO_3 (20 mL) and EtOAc (50 mL) added. The mixture was filtered and the filtrate was washed with brine (50 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl 3-(5-chloro-2-iodo-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2,2-dimethylpropanoate (2.3 g, 52% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{15}ClIN_2O_2$ 392.0; found 393.0.

Step 3. To a mixture of methyl 3-(5-chloro-2-iodo-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2,2-dimethylpropanoate (2.3 g, 5.9 mmol) and 2-(2-(2-methoxyethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (1.6 g, 7.1 mmol) and K_2CO_3 (2.4 g, 18 mol) in 1,4-dioxane (25 mL) and H_2O (5 mL) under an atmosphere of N_2 was added Pd(dppf)Cl_2.DCM (480 mg, 0.59 mmol). The mixture was

heated to 70 °C and for 4 h, then diluted with EtOAc (200 mL) and washed with brine (25 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (*S*)-3-(5-chloro-2-(2-(1-methoxyethyl)pyridin-3-yl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2,2-dimethylpropanoate (2.0 g, 84% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{21}H_{24}ClN_3O_3$ 401.2; found 402.2.

Step 4. A mixture of ethyl 3-(5-chloro-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2-methylpropanoate (2.0 g, 5.0 mmol), Cs_2CO_3 (3.3 g, 10 mmol) and EtI (1.6 g, 10 mmol) in DMF (30 mL) was stirred for 10 h. The mixture was diluted with EtOAc (100 mL) and washed with brine (20 mL x 4), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give two diastereomers of methyl (*S*)-3-(5-chloro-1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2,2-dimethylpropanoate (0.7 g, 32% yield; 0.6 g, 28% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{28}ClN_3O_3$ 429.2; found 430.2.

Step 5. To a mixture of methyl (*S*)-3-(5-chloro-1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2,2-dimethylpropanoate (1.9 g, 4.4 mmol) in anhydrous THF (20 mL) at 0 °C was added $LiBH_4$ (200 mg, 8.8 mmol). The mixture was heated to 60 °C and stirred for 4 h, then saturated NH_4Cl (20 mL) and EtOAc (50 mL) added. The aqueous and organic layers were separated and the organic layer was washed with brine (30 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (*S*)-3-(5-chloro-1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2,2-dimethylpropan-1-ol (1.5 g, 85% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{22}H_{28}ClN_3O_2$ 401.2; found 402.2.

Step 6. To a mixture of (*S*)-3-(5-chloro-1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-1*H*-pyrrolo[3,2-*b*]pyridin-3-yl)-2,2-dimethylpropan-1-ol (550 mg, 1.37 mmol), (*S*)-(2-(2-((*tert*-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)thiazol-4-yl)boronic acid (907.4 mg, 2.74 mmol, 2 eq) and K_2CO_3 (568 mg, 4.11 mmol) in 1,4-dioxane (25 mL) and H_2O (5 mL) under an atmosphere of N_2 was added Pd(dppf)Cl_2.DCM (89 mg, 0.14 mmol). The mixture was heated to 70 °C and stirred for 4 h, then H_2O (50 mL) added and the mixture extracted with EtOAc (100 mL x 3). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-pyrrolo[3,2-b]pyridin-5-yl)thiazol-2-yl)propanoate (440 mg, 22% yield) as a solid, which was used directly in the next step. LCMS (ESI): m/z [M+H] calc'd for $C_{34}H_{45}N_5O_6S$ 651.3; found 652.3.

Step 7. To a mixture of (2*S*)-methyl 2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-pyrrolo[3,2-*b*]pyridin-5-yl)thiazol-2-yl)propanoate (280 mg, 0.43 mmol) in MeOH (4 mL) was added a solution of LiOH (51 mg, 2.2 mmol) in H_2O (2 mL). The mixture was stirred for 5 h, then pH adjusted to ~3-4 by addition of 1M HCl. The mixture was diluted with H_2O (30 mL) and extracted with EtOAc (15 mL × 3). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to give (2*S*)-2-((*tert*-

butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-pyrrolo[3,2-*b*]pyridin-5-yl)thiazol-2-yl)propanoic acid (280 mg) as solid, which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{33}H_{43}N_5O_6S$ 637.3; found 638.3.

Step 8. To a mixture of (2*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-pyrrolo[3,2-*b*]pyridin-5-yl)thiazol-2-yl)propanoic acid (274 mg, 0.43 mmol) and methyl (3*S*)-1,2-diazinane-3-carboxylate (280 mg, 0.64 mmol) in DMF (3 mL) at 0-5 °C was added a solution of HATU (245 mg, 0.64 mmol) and DIPEA (555 mg, 4.3 mmol) in DMF (2 mL). The mixture was stirred for 1 h, then diluted with EtOAc (20 mL) and H_2O (20 mL). The aqueous and organic layers were partitioned and the organic layer was washed with H_2O (20 mL x 3), brine (20 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (3*S*)-1-[(2*S*)-2-{[(*tert*-butoxy)carbonyl]amino}-3-{4-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}pyrrolo[3,2-*b*]pyridin-5-yl]-1,3-thiazol-2-yl}propanoyl]-1,2-diazinane-3-carboxylate (230 mg, 70% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{53}N_7O_7S$ 763.4; found 764.3.

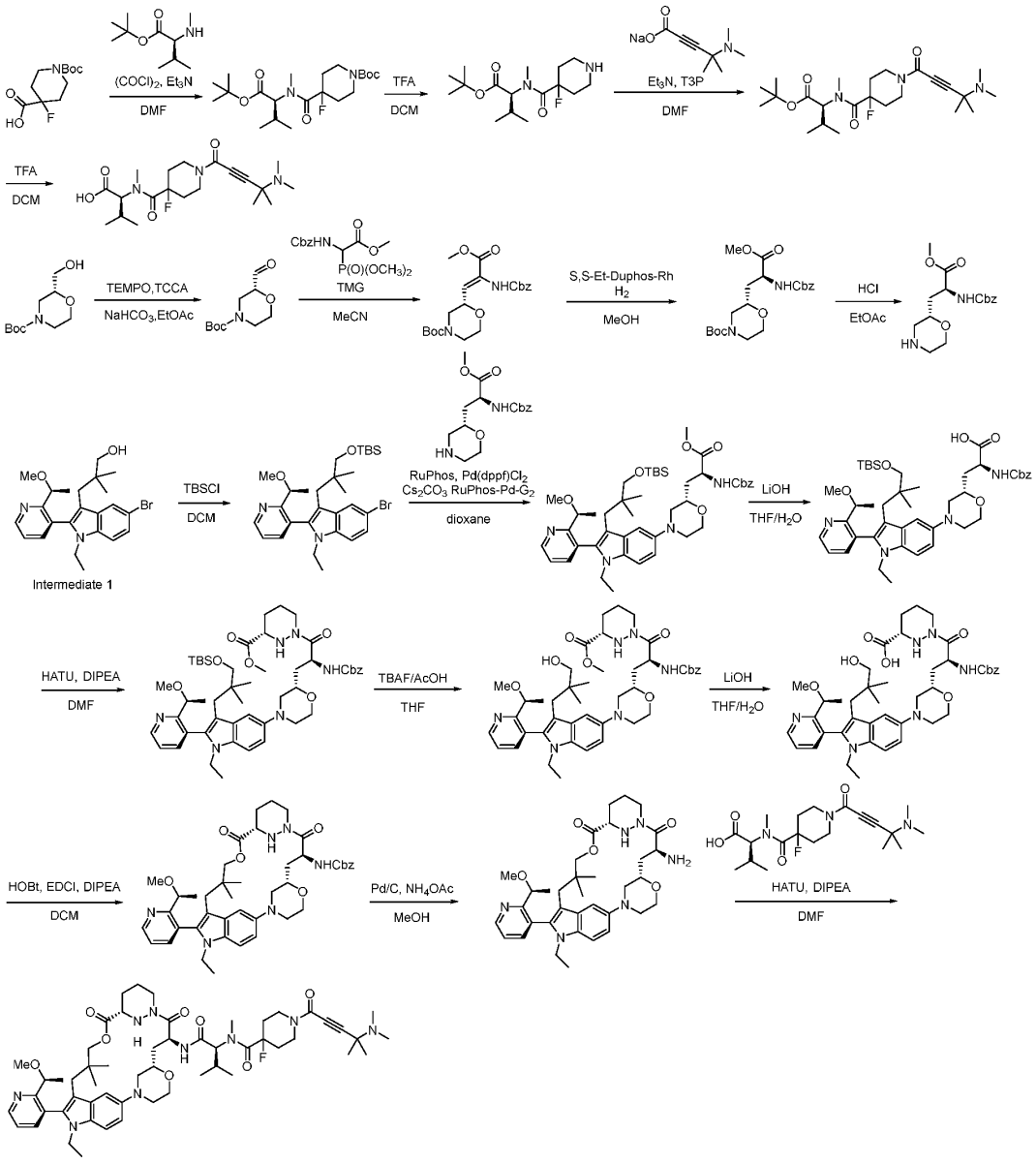
Step 9. To a mixture of methyl (3*S*)-1-[(2*S*)-2-{[(*tert*-butoxy)carbonyl]amino}-3-{4-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}pyrrolo[3,2-*b*]pyridin-5-yl]-1,3-thiazol-2-yl}propanoyl]-1,2-diazinane-3-carboxylate (230 mg, 0.3 mmol) in DCE (3 mL) under an atmosphere of N_2 was added Me_3SnOH (300 mg). The mixture was heated to 65 °C and stirred for 16 h, then concentrated under reduced pressure. The residue was diluted with EtOAc (20 mL), washed with H_2O (20 mL) and brine (10 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give (3*S*)-1-[(2*S*)-2-{[(*tert*-butoxy)carbonyl]amino}-3-{4-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}pyrrolo[3,2-*b*]pyridin-5-yl]-1,3-thiazol-2-yl}propanoyl]-1,2-diazinane-3-carboxylic acid (200 mg) as a foam, which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{51}N_7O_7S$ 749.4; found 750.3.

Step 10. To a mixture of (3*S*)-1-[(2*S*)-2-{[(*tert*-butoxy)carbonyl]amino}-3-{4-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}pyrrolo[3,2-*b*]pyridin-5-yl]-1,3-thiazol-2-yl}propanoyl]-1,2-diazinane-3-carboxylic acid (245 mg, 0.32 mmol) in DCM (50 mL) at 0-5 °C were added HOBT (432 mg, 3.2 mmol), EDCI HCl (1.8 g, 9.6 mmol) and DIPEA (1.65 g, 12.8 mmol). The mixture was warmed to room temperature and stirred for 16 h, then concentrated under reduced pressure. The residue was diluted with EtOAc (20 mL) and H_2O (20 mL) and the aqueous and organic layers were partitioned. The organic layer was washed with H_2O (30 mL x 3), brine (30 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-pyrrolo[3,2-b]pyridina-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (100 mg, 43% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{49}N_7O_6S$ 731.4; found 732.3.

Step 11. A mixture of *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-pyrrolo[3,2-b]pyridina-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate(80 mg, 0.11 mmol) in DCM (0.6 mL) and TFA (0.2 mL) was stirred for 1 h. The mixture was concentrated under reduced pressure to give (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-pyrrolo[3,2-b]pyridina-6(1,3)-pyridazinacycloundecaphane-5,7-dione (72 mg, 95% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{33}H_{41}N_7O_4S$ 631.3; found 632.3.

Step 12. To a mixture of (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-pyrrolo[3,2-b]pyridina-6(1,3)-pyridazinacycloundecaphane-5,7-dione (120 mg, 0.39 mmol) and DIPEA (335 mg, 2.6 mmol) in DMF (1 mL) at 0 °C was added HATU (60 mg, 0.16 mmol). The mixture was stirred at 0 °C for 1 h, then diluted with H_2O (110 mL) and extracted with EtOAc (80 mL x 2). The combined organic layers were washed with H_2O (100 mL), brine (100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give (3*S*)-1-acryloyl-*N*-((2*S*)-1-(((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-pyrrolo[3,2-b]pyridina-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylpyrrolidine-3-carboxamide (1.8 mg, 2% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{47}H_{61}N_9O_7S$ 895.4; found 896.3; ^{1}H NMR (400 MHz, CD_3OD) δ 8.72 (d, *J* = 4.5 Hz, 1H), 7.98 - 7.77 (m, 3H), 7.72 (dd, *J* = 12.0, 8.6 Hz, 1H), 7.54 (dd, *J* = 7.6, 4.8 Hz, 1H), 6.67 - 6.54 (m, 1H), 6.26 (m, 1H), 5.79 - 5.58 (m, 2H), 4.83 - 4.75 (m, 1H), 4.39 - 4.16 (m, 4H), 4.02 (dd, *J* = 28.0, 10.6 Hz, 2H), 3.89 - 3.65 (m, 6H), 3.50 (m, 4H), 3.34 (d, *J* = 6.2 Hz, 3H), 3.12 (d, *J* = 4.0 Hz, 2H), 3.00 (s, 1H), 2.73 (m, 1H), 2.48 - 2.37 (m, 1H), 2.31 - 2.07 (m, 4H), 1.88 (d, *J* = 11.2 Hz, 1H), 1.71 (d, *J* = 12.8 Hz, 1H), 1.44 (m, 7H), 0.97 (dd, *J* = 6.2, 4.4 Hz, 3H), 0.92 - 0.84 (m, 8H), 0.41 (d, *J* = 6.2 Hz, 3H).

Example 647. Synthesis of 1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-*N*-((2*S*)-1-(((2^2*S*,6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-4-fluoro-*N*-methylpiperidine-4-carboxamide



Step 1. A mixture of 1-[(*tert*-butoxy)carbonyl]-4-fluoropiperidine-4-carboxylic acid (2.0 g, 8.1 mmol) in DCM (20 mL) was added oxalic dichloride (1.34 g, 10.5 mmol) and DMF (30 mg, 0.4 mmol). The resulting solution was stirred at room temperature for 1 h. Et_3N (3.2 g, 3.2 mmol) and (2*S*)-3-methyl-2-(methylamino)butanoic acid (1.25 g, 9.5 mmol) were added and the mixture was stirred at room temperature for 1 h. H_2O (100 mL) was added and the mixture was extracted with EtOAc (50 mL x 3). The combined organic layers were concentrated under reduced pressure and

the residue was purified by silica gel column chromatography to give *tert*-butyl (*S*)-4-((1-(*tert*-butoxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)-4-fluoropiperidine-1-carboxylate (1.34 g, 45% yield) as a solid. LCMS (ESI): m/z [M+Na] calc'd for $C_{21}H_{37}FN_2O_5Na$ 439.3; found 439.3.

Step 2. A mixture of *tert*-butyl (*S*)-4-((1-(*tert*-butoxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)-4-fluoropiperidine-1-carboxylate (290 mg, 0.70 mmol) in DCM (4 mL) and TFA (2 mL) was stirred at room temperature for 2 h, then concentrated under reduced pressure to give *N*-(4-fluoropiperidine-4-carbonyl)-*N*-methyl-*L*-valine, which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{12}H_{21}FN_2O_3$ 260.2; found 261.2.

Step 3. To a solution of the *tert*-butyl *N*-(4-fluoropiperidine-4-carbonyl)-*N*-methyl-L-valinate (1.7 g, 5.3 mmol), sodium 4-(dimethylamino)-4-methylpent-2-ynoate (1.67 g, 9.4 mmol) and Et_3N (2.73 g, 36.9 mmol) in DMF (20 mL) stirred at 5 °C was added T3P (4.11 g, 10.7 mmol, 50wt% in EtOAc). The reaction mixture was stirred at 5 °C for 1 h. The resulting mixture was quenched with H_2O (100 mL) and extracted with EtOAc (50 mL x 3). The combined organic layers were concentrated and purified by silica gel column chromatography to give *tert*-butyl *N*-(1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-4-fluoropiperidine-4-carbonyl)-*N*-methyl-L-valinate (1.6 g, 74.0% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{24}H_{40}FN_3O_4$ 453.3; found 454.2.

Step 4. To a solution of *tert*-butyl *N*-(1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-4-fluoropiperidine-4-carbonyl)-*N*-methyl-L-valinate (50 mg, 0.11 mmol) in DCM (2 mL) was added TFA (1 mL). The reaction mixture was stirred at 20 °C for 2 h, then concentrated under reduced pressure to afford crude *N*-(1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-4-fluoropiperidine-4-carbonyl)-*N*-methyl-L-valine. It was used for the next step directly without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{20}H_{32}FN_3O_4$ 397.2; found 398.3.

Step 5. To a solution of *tert*-butyl (2*R*)-2-(hydroxymethyl)morpholin-4-yl formate (50 g, 230 mmol) in EtOAc (1 L) was added TEMPO (715 mg, 4.6 mmol) and $NaHCO_3$ (58 g, 690 mmol) at 20 °C. The mixture was cooled to -50 °C, then TCCA (56 g, 241mmol) in EtOAc (100 mL) was added dropwise over 30 min. The reaction mixture was warmed to 5 °C for 2 h, then quenched with 10% $Na_2S_2O_3$ (200 mL) and stirred for 20 min. The resulting mixture was filtered and the organic phase was separated from filtrate. The aqueous phase was extracted with EtOAc (100 mL x 2). The combined organic layers were washed with H_2O (100 mL) and brine (100 mL), and dried over anhydrous Na_2SO_4. The organic layer was concentrated under reduced pressure to afford *tert*-butyl (2*R*)-2-formylmorpholin-4-yl formate (50 g, crude) as an oil.

Step 6. To a solution of *tert*-butyl (2*R*)-2-formylmorpholin-4-yl formate (49 g, 153 mmol) and methyl 2-{[(benzyloxy)carbonyl]amino}-2-(dimethoxyphosphoryl)acetate (60 g, 183 mmol) in CAN (300 mL) was added tetramethylguanidine (35 g, 306 mmol) at 0~10 °C. The reaction mixture was stirred at 10 °C for 30 min then warmed to 20 °C for 2 h. The reaction mixture was diluted with DCM (200 mL) and washed with Citric acid (10%, 200 mL) and 10% $NaHCO_3$ aqueous solution (200 mL). The organic phase was concentrated under reduced pressure, and purified by silica gel column chromatography to afford *tert*-butyl (*S*,*Z*)-2-(2-(((benzyloxy)carbonyl)amino)-3-methoxy-3-oxoprop-1-en-1-yl)morpholine-4-carboxylate (36 g, 90% yield) as solid. LCMS (ESI): m/z [M+Na] calc'd for $C_{21}H_{28}N_2O_4$ 420.2; found: 443.1

Step 7. To a solution of *tert*-butyl (*S*,*Z*)-2-(2-(((benzyloxy)carbonyl)amino)-3-methoxy-3-oxoprop-1-en-1-yl)morpholine-4-carboxylate (49 g, 0.12 mol) in MeOH (500 mL) was added (*S*,*S*)-Et-DUPHOS-Rh (500 mg, 0.7 mmol). The mixture was stirred at 25 °C under an H_2 (60 psi) atmosphere for 48 h. The reaction was concentrated and purified by chromatography to give *tert*-butyl (*S*)-2-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-methoxy-3-oxopropyl)morpholine-4-carboxylate (44 g, 89.8% yield) as solid. LCMS (ESI): m/z [M+Na] calc'd for $C_{21}H_{30}N_2O_7$ 422.2; found: 445.2.

Step 8. To a stirred solution of *tert*-butyl (*S*)-2-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-methoxy-3-oxopropyl)morpholine-4-carboxylate (2.2 g, 5.2 mmol) in EtOAc (2 mL) was added HCl/EtOAc (25 mL) at 15 °C. The reaction was stirred at 15 °C for 2 h, then concentrated under reduced pressure to afford methyl (*S*)-2-(((benzyloxy)carbonyl)amino)-3-((*S*)-morpholin-2-yl)propanoate (1.51 g, 90.4% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{16}H_{22}N_2O_5$ 322.1; found 323.2.

Step 9. To a solution of 3-(5-bromo-1-ethyl-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}indol-3-yl)-2,2-dimethylpropan-1-ol(100 g, 0.22 mol) and 1H-imidazole(30.6 g, 0.45 mol) in DCM (800 mL) was added TBSCl (50.7 g, 0.34 mol) in DCM (200 mL) at 0 °C. The reaction was stirred at 25 °C for 2 h. The resulting solution was washed with H_2O (300 mL x 3) and brine (200 mL x 2), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified with silica gel column chromatography to give (*S*)-5-bromo-3-(3-((*tert*-butyldimethylsilyl)oxy)-2,2-dimethylpropyl)-1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-1*H*-indole (138 g, 90% yield) as an solid. LCMS (ESI): m/z [M+H] calc'd for $C_{29}H_{43}BrN_2O_2Si$ 558.2; found 559.2.

Step 10. To a stirred solution of Intermediate 1 (50 g, 89.3 mmol) in dioxane (500 mL) was added methyl (2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-[(2*S*)-morpholin-2-yl]propanoate from step 1 (31.7 g, 98.2 mmol), RuPhos (16.7 g, 35.7 mmol), Di-mu-chlorobis(2-amino-1,1-biphenyl-2-yl-C,N)dipalladium(II) (2.8 g, 4.4 mmol) and cesium carbonate (96 g, 295 mmol) followed by RuPhos-Pd-G2 (3.5 g, 4.4 mmol) at 105 °C under an N_2 atmosphere. The reaction mixture was stirred for 6 h at 105 °C under an N_2 atmosphere. The resulting mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by prep-TLC chromatography to afford methyl (2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-[(2*S*)-4-(3-{3-[(*tert*-butyldimethylsilyl)oxy]-2,2-dimethylpropyl}-1-ethyl-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}indol-5-yl)morpholin-2-yl]propanoate (55 g, 73% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{45}H_{64}N_4O_7Si$ 800.5; found 801.5.

Step 11. To a solution of methyl (2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-[(2*S*)-4-(3-{3-[(*tert*-butyldimethylsilyl)oxy]-2,2-dimethylpropyl}-1-ethyl-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}indol-5-yl)morpholin-2-yl]propanoate (10 g, 12 mmol) in THF (270 mL) was added LiOH (1.3 g, 31 mmol) in H_2O (45 mL) at 20 °C. The reaction was stirred at 20 °C for 2 h, then treated with 1N HCl to adjust pH to 4~5 at 0~5 °C. The resulting mixture was extracted with EtOAc (50 mL x 2). The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. The organic phase was then concentrated under reduced pressure to afford (2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-[(2*S*)-4-(3-{3-[(*tert*-butyldimethylsilyl)oxy]-2,2-dimethylpropyl}-1-ethyl-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}indol-5-yl)morpholin-2-yl]propanoic acid (9.5 g, 97% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{62}N_4O_7Si$ 786.4; found 787.4.

Step 12. To a stirred solution of (2*S*)-2-{[(benzyloxy)carbonyl]amino}-3-[(2*S*)-4-(3-{3-[(*tert*-butyldimethylsilyl)oxy]-2,2-dimethylpropyl}-1-ethyl-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}indol-5-yl)morpholin-2-yl]propanoic acid (10 g, 12.7 mmol) in DMF (150 mL), was added methyl (*S*)-hexahydropyridazine-3-carboxylate (2 g, 14 mmol), then cooled to 0 °C, DIPEA (32.8 g, 254 mmol) was added followed by HATU (9.7 g, 25.4 mmol) at 0~5 °C. The reaction mixture was stirred at 0~5 °C for 1 h. The resulting mixture was diluted with EtOAc (500 mL) and H_2O (200 mL). The organic layer was separated and washed with H_2O (100 mL x 2) and brine (100 mL), dried over anhydrous sodium sulfate. The solution was filtered and concentrated under reduced pressure, and the residue was purified by silica gel column chromatography to afford methyl (*S*)-1-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-((*S*)-4-(3-(3-((*tert*-butyldimethylsilyl)oxy)-2,2-dimethylpropyl)-1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (8 g, 70% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{72}N_6O_8Si$ 912.5; found 913.4.

Step 13. A solution of methyl (*S*)-1-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-((*S*)-4-(3-(3-((*tert*-butyldimethylsilyl)oxy)-2,2-dimethylpropyl)-1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (8.5 g, 9 mmol) in THF (8 mL) was added a mixture of tetrabutylammonium fluoride (1M in THF, 180 mL, 180 mmol) and AcOH (11 g, 200 mmol) at 20 °C. The reaction mixture was stirred at 75 °C for 3 h. The resulting mixture was diluted with EtOAc (150 mL) and washed with H_2O (20 mL x 6). The organic phase was concentrated under reduced pressure to give methyl (*S*)-1-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-((*S*)-4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (7.4 g, 100% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{58}N_6O_8$ 799.4; found 798.4.

Step 14. To a solution of methyl (*S*)-1-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-((*S*)-4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (8 g, 10 mmol) in THF (200 mL) was added lithium hydroxide (600 mg, 25 mmol) in H_2O (30 mL). The reaction mixture was stirred at 20 °C for 1 h, then treated with 1N HCl to adjust pH to 4~5 at 0~5 °C, and extracted with EtOAc (500 mL x 2). The organic phase was washed with brine, and concentrated under reduced pressure to afford (*S*)-1-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-((*S*)-4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (8 g, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{56}N_6O_8$ 784.4; found 785.4.

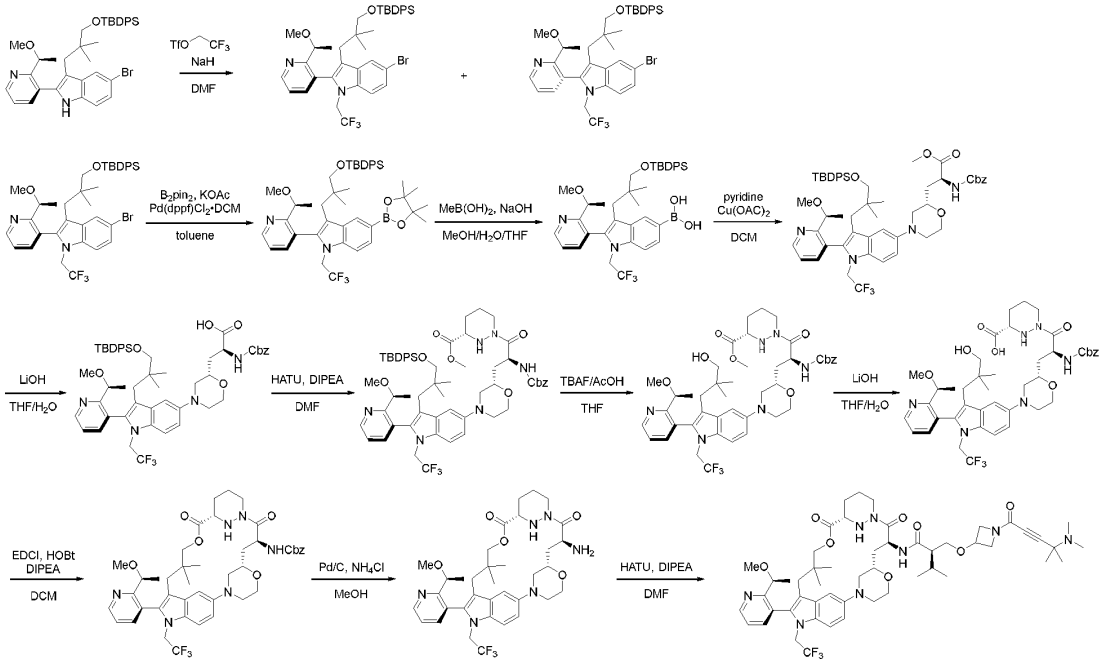
Step 15. To a stirred solution of (*S*)-1-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-((*S*)-4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (8 g, 10.2 mmol) and DIPEA (59 g, 459 mmol) in DCM (800 mL) was added EDCI (88 g, 458 mmol) and HOBT (27.6 g, 204 mmol) at 25 °C under an argon atmosphere. The reaction mixture was stirred at 25 °C for 16 h. The resulting mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography to afford benzyl ((2^2*S*,6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-

6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (5 g, 66% yield) as a solid; LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{54}N_6O_7$ 766.4; found 767.4.

Step 16. To a solution of benzyl ((2^2*S*,6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (400 mg, 0.5 mmol) in MeOH (20 mL) was added Pd/C (200 mg) and ammonium acetate (834 mg, 16 mmol) at 20 °C under an H_2 atmosphere and the mixture was stirred for 2 h. Then resulting mixture was filtered and concentrated under reduced pressure. The residue was redissolved in DCM (20 mL) and washed with H_2O (5 mL x 2), then concentrated under reduced pressure to afford (2^2*S*,6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (320 mg, 97% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{35}H_{48}N_6O_5$ 632.4; found 633.3.

Step 17. To a solution of the (2^2*S*,6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (50 mg, 0.079 mmol), *N*-(1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-4-fluoropiperidine-4-carbonyl)-*N*-methyl-L-valine (47 mg, 0.12 mmol) in DMF (2 mL) stirred at 0 °C was added HATU (36 mg, 0.09 mmol) and DIPEA (153 mg, 1.2 mmol) dropwise. The reaction was stirred at 0 °C for 1 h. The resulting mixture was purified by reverse phase to afford 1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-*N*-((2*S*)-1-(((2^2*S*,6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-4-fluoro-*N*-methylpiperidine-4-carboxamide (11.9 mg, 13.9% yield) as a solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.71 (dd, *J* = 4.8, 1.7 Hz, 1H), 7.86 (d, *J* = 7.8 Hz, 1H), 7.51 (dd, *J* = 7.8, 4.8 Hz, 1H), 7.39 (d, *J* = 8.9 Hz, 1H), 7.15 – 7.04 (m, 2H), 5.67 (d, *J* = 8.8 Hz, 1H), 4.62 (d, *J* = 11.2 Hz, 1H), 4.46 (d, *J* = 12.4 Hz, 1H), 4.39 – 4.27 (m, 2H), 4.23 (d, *J* = 6.1 Hz, 1H), 4.17 – 4.08 (m, 1H), 3.93 (s, 2H), 3.86 (s, 1H), 3.84 – 3.76 (m, 2H), 3.74 – 3.65 (m, 2H), 3.63 – 3.51 (m, 2H), 3.27 – 3.23 (m, 1H), 3.22 – 3.11 (m, 6H), 3.0 – 2.89 (m, 2H), 2.85 – 2.75 (m, 2H), 2.74 – 2.55 (m, 2H), 2.36 (d, *J* = 8.2 Hz, 6H), 2.32 – 2.21 (m, 2H), 2.20 – 2.02 (m, 5H), 1.92 (d, *J* = 12.5 Hz, 2H), 1.69 (dd, *J* = 43.8, 12.6 Hz, 2H), 1.46 (dt, *J* = 8.0, 4.9 Hz, 9H), 1.03 (d, *J* = 3.5 Hz, 3H), 0.90 (dd, *J* = 48.3, 6.5 Hz, 6H), 0.77 (d, *J* = 3.0 Hz, 3H), 0.69 (s, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{55}H_{78}FN_9O_8$ 1011.6; found 1012.5.

Example A375. Synthesis of (2*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-*N*-((2^2*S*,6^3*S*,4*S*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide



Step 1. To a mixture of 5-bromo-3-{3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl}-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}-1*H*-indole (10.0 g, 15.2 mmol) in anhydrous DMF (120 mL) at 0 °C under an atmosphere of N_2 was added NaH, 60% dispersion in oil (1.2 g, 30.4 mmol) and 2,2,2-trifluoroethyl trifluoromethanesulfonate (35.4 g, 152 mmol). The mixture was stirred at 0 °C for 1 h, then saturated NH_4Cl (30 mL) added and the mixture extracted with EtOAc (100 mL x 3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (*S*)-5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indole (8 g) as an oil and the other atropisomer (6 g, 48% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{44}BrF_3N_2O_2Si$ 736.2; found 737.1.

Step 2. To a mixture of (*S*)-5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indole (7.2 g, 9.7 mmol) in toulene (80 mL) under an atmosphere of N_2 was added 4,4,4′,4′,5,5,5′,5′-octamethyl-2,2′-bi(1,3,2-dioxaborolane) (2.7 g, 10.6 mmol), KOAc (1.9 g, 19.4 mmol) and Pd(dppf)Cl_2 DCM (0.8 g, 0.1 mmol). The mixture was heated to 90 °C and stirred for 8 h, then saturated NH_4Cl (30 mL) added and the mixture extracted with EtOAc (40 mL x 3). The combined organic layers were dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (*S*)-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-

1,3,2-dioxaborolan-2-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indole (6.1 g, 64% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{45}H_{56}BF_3N_2O_4Si$ 784.4; found 785.3.

Step 3. To a mixture of (*S*)-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indole (33 g, 42 mmol) in THF (120 mL) and MeOH (330 mL) at 0 °C under an atmosphere of N_2 was added $MeB(OH)_2$ (50.4 g, 841 mmol), then a mixture of NaOH (33.6 g, 841 mmol) in H_2O (120 mL). The mixture was warmed to room temperature and stirred for 16 h, then concentrated under reduced pressure. H_2O (500 mL) was added to the residue and the mixture extracted with EtOAc (300 mL x 3). The combined organic layers were washed with brine (300 mL), H_2O (300 mL), then concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (*S*)-(3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)boronic acid (20 g, 68% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{46}BF_3N_2O_4Si$ 702.3; found 703.3.

Step 4. Note: Three reactions were run in parallel – the yield reflects the sum of the products.

A mixture of (*S*)-(3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)boronic acid (1.85 g, 5.6 mmol) and methyl (*S*)-2-(((benzyloxy)carbonyl)amino)-3-((*S*)-morpholin-2-yl)propanoate in DCM (150 mL) under air was added pyridine (1.35 g, 16.9 mmol) and $Cu(OAc)_2$ (2.0 g, 11.3 mmol). The mixture was stirred for 48 h, then concentrated under reduced pressure. H_2O (300 mL) was added to the residue and the mixture was extracted with EtOAc (300 mL x 2). The combined organic layers were washed with brine (100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was silica gel column chromatography to give methyl (*S*)-2-(((benzyloxy)carbonyl)amino)-3-((*S*)-4-(3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)morpholin-2-yl)propanoate (9.2 g, 55% yield) as a solid. LCMS (ESI): m/z [M/2+H] calc'd for $C_{55}H_{65}F_3N_4O_7Si$ 490.2; found 490.3.

Step 5. To a mixture of methyl (*S*)-2-(((benzyloxy)carbonyl)amino)-3-((*S*)-4-(3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)morpholin-2-yl)propanoate (10.8 g, 11.0 mmol) in THF (50 mL) was added LiOH (528 mg, 22 mmol) in H_2O (10 mL). The mixture was stirred for 1 h, then cooled to 0-5 °C and acidified to pH~7 using 2N HCl (10 mL). The mixture was extracted with DCM (100 mL x 2) and the combined organic layers were dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give (*S*)-2-(((benzyloxy)carbonyl)amino)-3-((*S*)-4-(3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)morpholin-2-yl)propanoic acid (10.6 g, 100% yield) as a solid. LCMS (ESI): m/z [M/2+H] calc'd for $C_{54}H_{63}F_3N_4O_7Si$ 483.2; found 483.3.

Step 6. To a mixture of (*S*)-2-(((benzyloxy)carbonyl)amino)-3-((*S*)-4-(3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)morpholin-2-yl)propanoic acid (10.6 g, 11.0 mmol) and methyl (3*S*)-1,2-diazinane-3-carboxylate (15.8 g, 22.0 mmol) in DMF (150 mL) at 0 °C was added DIPEA (28.4 g, 220 mmol) and HATU (8.4 g, 22.0 mmol). The mixture was stirred at 0~5 °C for 1 h, then EtOAc

(500 mL) was added and the mixture was washed with H_2O (200 mL x 2), brine (100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (*S*)-1-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-((*S*)-4-(3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (11 g, 90% yield) as a solid. LCMS (ESI): m/z [M/2+H] calc'd for $C_{60}H_{73}F_3N_6O_8Si$ 546.3; found 546.3.

Step 7. To a mixture of methyl (*S*)-1-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-((*S*)-4-(3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (11.0 g, 10.1 mmol) in THF (10 mL) was added a mixture of AcOH (21.2 g, 353 mmol) and 1M TBAF in THF (300 mL, 300 mmol). The mixture was heated to 80 °C and stirred for 16 h, then concentrated under reduced pressure. EtOAc (800 mL) was added to the residue and the mixture was washed with H_2O (80 mL x 6), concentrated under reduced pressure and the residue was purified by preparative-HPLC to give methyl (*S*)-1-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-((*S*)-4-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (7.9 g, 91% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{55}F_3N_6O_8$ 852.4; found 853.3.

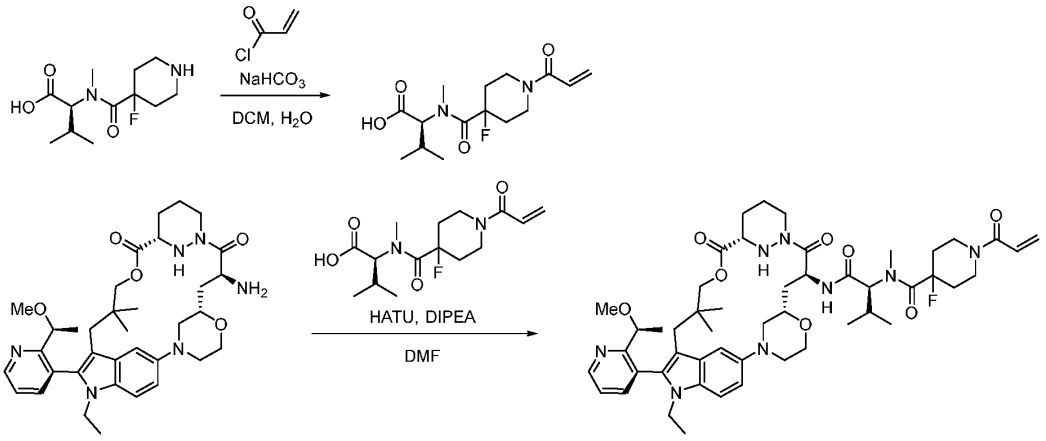
Step 8. To a mixture of methyl (*S*)-1-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-((*S*)-4-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (7.9 g, 9.3 mmol) in THF (50 mL) was added LiOH (443 mg, 18.5 mmol) in H_2O (10 mL). The mixture was stirred for 1 h, then cooled to 0-5 °C and acidified to ~pH 7 with 2N HCl (9 mL). The mixture was extracted with DCM (100 mL x 2) and the combined organic layers were dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give (*S*)-1-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-((*S*)-4-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (7.6 g, 98% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{53}F_3N_6O_8$ 838.4; found 839.3.

Step 9. To a mixture of (*S*)-1-((*S*)-2-(((benzyloxy)carbonyl)amino)-3-((*S*)-4-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (7.6 g, 9.0 mmol) and DIPEA (52.3 g, 405 mmol) in DCM (800 mL) under an atmosphere of Ar was added EDCI (77.6 g, 405 mmol) and HOBT (12 g, 90 mmol). The mixture was stirred for 16 h, then concentrated under reduced pressure. The residue was diluted with EtOAc (500 mL), washed with H_2O (100 mL x 2) and filtered. The organic layer was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give benzyl ((2^2*S*,6^3*S*,4*S*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (6.1 g, 74% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{51}F_3N_6O_7$ 820.4; found 821.3.

Step 10. To a mixture of benzyl ((2^2*S*,6^3*S*,4*S*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (700 mg, 0.85 mmol) in MeOH (30 mL) was added 10% Pd on C (317 mg) and NH_4Cl (909 mg). The mixture was stirred under an atmosphere of H_2 (1 atm) for 16 h, then filtered through Celite and the filter cake was washed with MeOH (150 mL). The filtrate was concentrated under reduced pressure, DCM (20 mL) was added to the residue and the mixture was washed with saturated $NaHCO_3$ (20 mL x 3). The organic layer was concentrated under reduced pressure to give (2^2*S*,6^3*S*,4*S*)-4-amino-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (660 mg, 95% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{35}H_{45}F_3N_6O_5$ 686.3; found 687.3; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (dd, *J* = 4.7, 1.7 Hz, 1H), 7.66 (d, *J* = 7.4 Hz, 1H), 7.43 - 7.30 (m, 2H), 7.12 - 7.01 (m, 2H), 4.90 - 4.83 (m, 1H), 4.68 (d, *J* = 12.5 Hz, 1H), 4.57 (dd, *J* = 16.2, 8.1 Hz, 1H), 4.24 (q, *J* = 6.1 Hz, 1H), 4.08 (d, *J* = 10.6 Hz, 1H), 3.97 - 3.82 (m, 4H), 3.80 - 3.68 (m, 2H), 3.55 (d, *J* = 11.6 Hz, 1H), 3.21 (d, *J* = 9.4 Hz, 1H), 2.93 (dd, *J* = 19.9, 9.3 Hz, 3H), 2.66 (t, *J* = 11.6 Hz, 1H), 2.47 (d, *J* = 14.5 Hz, 1H), 2.19 - 2.04 (m, 4H), 1.96 (d, *J* = 13.6 Hz, 2H), 1.80 - 1.71 (m, 2H), 1.66 - 1.59 (m, 1H), 1.47 (d, *J* = 6.1 Hz, 3H), 0.88 (s, 3H), 0.42 (s, 3H).

Step 11. To a mixture of (2^2*S*,6^3*S*,4*S*)-4-amino-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (300 mg, 0.4 mmol), (2*R*)-2-[({1-[4-(dimethylamino)-4-methylpent-2-ynoyl]azetidin-3-yl}oxy)methyl]-3-methylbutanoic acid (157 mg, 0.48 mmol) and DIPEA (569.0 mg, 0.4 mmol) in DMF (5 mL) at 0 °C was added HATU (217 mg, 0.57 mmol). The mixture was stirred at 0 °C for 0.5 h, then diluted with H_2O and extracted with EtOAc (20 mL x 3). The combined organic layers were washed with brine (20 mL x 3), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give (2*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-*N*-((2^2*S*,6^3*S*,4*S*)-12-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide (200 mg, 46% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{52}H_{71}F_3N_8O_8$ 992.5; found 993.4; ^{1}H NMR (400 MHz, CD_3OD) δ 8.74 (m, 1H), 8.00 (m, 1H), 7.85 (d, *J* = 7.7 Hz, 1H), 7.60 - 7.43 (m, 2H), 7.14 (t, *J* = 9.5 Hz, 2H), 5.63 (s, 1H), 5.06 (m, 1H), 4.64 (s, 1H), 4.52 - 4.31 (m, 3H), 4.27 - 4.05 (m, 3H), 3.97 (m, 1H), 3.92 - 3.66 (m, 6H), 3.59 (m, 2H), 3.46 (m, 2H), 3.25 (d, *J* = 5.3 Hz, 3H), 3.07 - 2.89 (m, 2H), 2.86 - 2.59 (m, 3H), 2.38 - 2.32 (m, 3H), 2.28 (s, 3H), 2.13 (m, 2H), 2.03 - 1.51 (m, 6H), 1.50 - 1.41 (m, 6H), 1.38 (d, *J* = 7.5 Hz, 3H), 0.98 (t, *J* = 8.7 Hz, 6H), 0.89 (t, *J* = 6.4 Hz, 3H), 0.54 (d, *J* = 8.4 Hz, 3H).

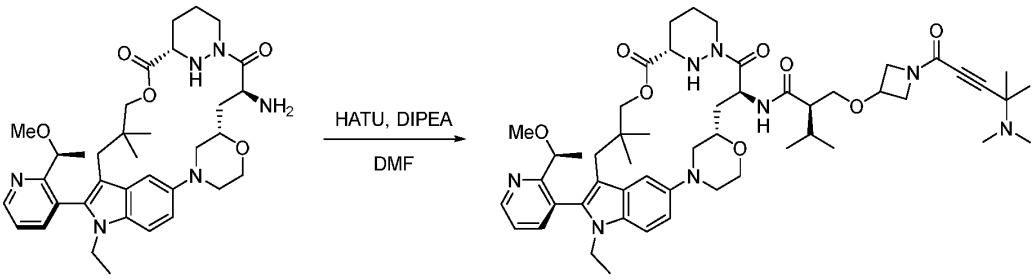
Example A722. Synthesis of 1-acryloyl-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-4-fluoro-*N*-methylpiperidine-4-carboxamide



Step 1. To a mixture of *N*-(4-fluoropiperidine-4-carbonyl)-*N*-methyl-*L*-valine (190 mg, 0.73 mmol) and $NaHCO_3$ (306 mg, 3.6 mmol) in DCM (2 mL) and H_2O (1 mL) at -10 °C was added prop-2-enoyl chloride (132 mg, 1.45 mmol). The mixture was stirred at 0-5 °C for 1 h, then diluted with DCM (20 mL) and washed with H_2O (20 mL x 2), brine (20 mL) and the organic layer dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *N*-(1-acryloyl-4-fluoropiperidine-4-carbonyl)-*N*-methyl-*L*-valine (120 mg, 52% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{15}H_{23}FN_2O_4$ 314.2; found 315.2.

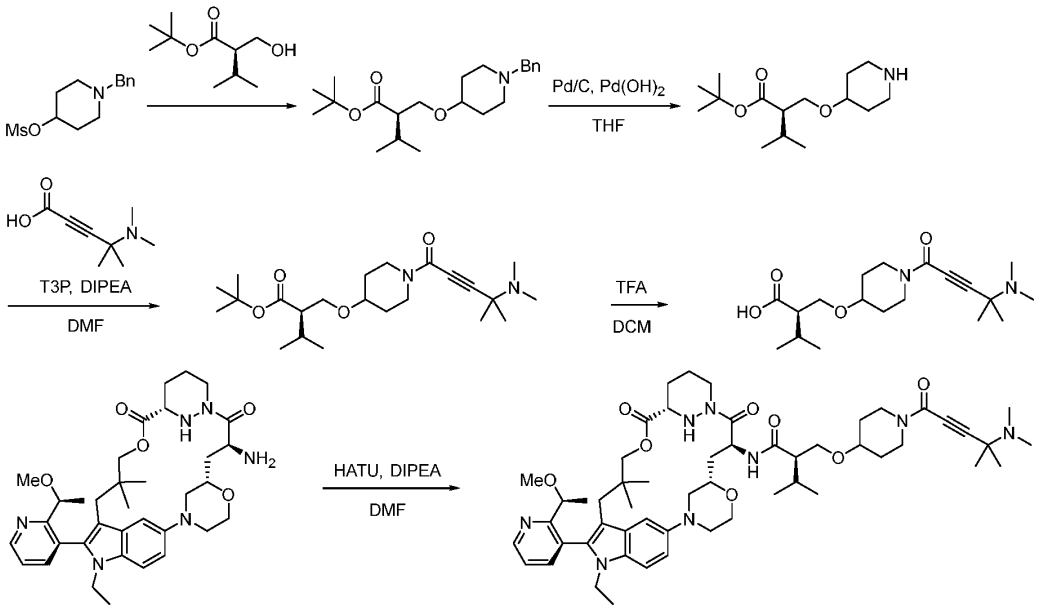
Step 2. To a mixture of (6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (153 mg, 0.24 mmol), *N*-(1-acryloyl-4-fluoropiperidine-4-carbonyl)-*N*-methyl-*L*-valine (106 mg, 0.34 mmol) in DMF(2 mL) at 5 °C was added HATU (110 mg, 0.29 mmol) and DIPEA (468 mg, 3.6 mmol) dropwise. The mixture was stirred at 5 °C for 1 h, then purified by preparative-HPLC to give 1-acryloyl-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-4-fluoro-*N*-methylpiperidine-4-carboxamide (69.5 mg, 29% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{69}FN_8O_8$ 928.5; found 929.4; ^{1}H NMR (400 MHz, CD_3OD) δ 8.71 (d, *J* = 3.2 Hz, 1H), 7.86 (d, *J* = 7.7 Hz, 1H), 7.51 (dd, *J* = 7.7, 4.8 Hz, 1H), 7.39 (d, *J* = 8.9 Hz, 1H), 7.18 - 7.02 (m, 2H), 6.80 (dd, *J* = 16.8, 10.7 Hz, 1H), 6.23 (d, *J* = 16.8 Hz, 1H), 5.77 (d, *J* = 10.6 Hz, 1H), 5.67 (d, *J* = 6.5 Hz, 1H), 4.61 (d, *J* = 11.1 Hz, 1H), 4.44 (t, *J* = 15.1 Hz, 2H), 4.23 (q, *J* = 6.1 Hz, 1H), 4.18 - 4.10 (m, 1H), 4.09 - 4.01 (m, 1H), 3.99 - 3.83 (m, 3H), 3.83 - 3.65 (m, 4H), 3.58 - 3.46 (m, 2H), 3.27 (s, 1H), 3.21 - 3.11 (m, 6H), 3.00 - 2.91 (m, 2H), 2.85 - 2.75 (m, 2H), 2.73 - 2.64 (m, 1H), 2.62 - 2.54 (m, 1H), 2.36 - 2.21 (m, 2H), 2.19 - 2.01 (m, 5H), 1.92 (d, *J* = 12.7 Hz, 2H), 1.79 - 1.57 (m, 2H), 1.44 (d, *J* = 6.2 Hz, 3H), 1.04 (t, *J* = 6.3 Hz, 3H), 0.98 - 0.81 (m, 6H), 0.76 (s, 3H), 0.68 (s, 3H).

Example A377. Synthesis of (2*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-*N*-((2^2*S*,6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide



Step 1. (2*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-*N*-((2^2*S*,6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide was synthesized in a manner similar to (2*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-*N*-((2^2*S*,6^3*S*,4*S*)-12-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide except (2^2*S*,6^3*S*,4*S*)-4-amino-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione was substituted with (2^2*S*,6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione and 3-({1-[4-(dimethylamino)-4-methylpent-2-ynoyl]azetidin-3-yl}oxy)propanoic acid was substituted with (2*R*)-2-[({1-[4-(dimethylamino)-4-methylpent-2-ynoyl]azetidin-3-yl}oxy)methyl]-3-methylbutanoic acid to give the desired product (25.6 mg, 26% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{52}H_{74}N_8O_8$ 938.6; found 939.5; ^{1}H NMR (400 MHz, CD_3OD) δ 8.72 (dd, *J* = 4.8, 1.5 Hz, 1H), 7.97 (dd, *J* = 20.0, 6.8 Hz, 1H), 7.87 (dd, *J* = 5.8, 2.6 Hz, 1H), 7.54 - 7.51 (m, 1H), 7.41 (d, *J* = 8.9 Hz, 1H), 7.14 (dd, *J* = 36.0, 10.4 Hz, 2H), 5.65 (s, 1H), 4.49 - 4.33 (m, 3H), 4.27 - 4.08 (m, 4H), 3.96 (d, *J* = 8.6 Hz, 2H), 3.87 (dd, *J* = 10.8, 3.6 Hz, 2H), 3.79 (dd, *J* = 10.8, 7.7 Hz, 3H), 3.69 - 3.58 (m, 3H), 3.43 (dd, *J* = 23.9, 11.7 Hz, 2H), 3.17 (d, *J* = 21.9 Hz, 3H), 3.00 - 2.95 (m, 1H), 2.70 (t, *J* = 14.0 Hz, 8H), 2.34 - 2.24 (m, 1H), 2.05 (d, *J* = 34.4 Hz, 3H), 1.92 - 1.82 (m, 2H), 1.69 - 1.62 (m, 5H), 1.57 (d, *J* = 11.5 Hz, 3H), 1.45 (d, *J* = 6.2 Hz, 3H), 1.33 (d, *J* = 12.6 Hz, 1H), 1.05 - 0.93 (m, 10H), 0.80 (d, *J* = 9.8 Hz, 3H), 0.64 (d, *J* = 12.2 Hz, 2H).

Example A643. Synthesis of (2*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)piperidin-4-yl)oxy)methyl)-*N*-((2^2*S*,6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide



Step 1. A mixture of 1-(1-methylphenyl)piperidin-4-yl methanesulfonate (2 g, 7.4 mmol) and *tert*-butyl (2*R*)-2-(hydroxymethyl)-3-methylbutanoate (1.39 g, 7.4 mmol) was stirred at 120 °C for 1 h, then purified by silica gel column chromatography to give *tert*-butyl (*R*)-2-(((1-benzylpiperidin-4-yl)oxy)methyl)-3-methylbutanoate (800 mg, 28% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{22}H_{35}NO_3$ 361.3; found 362.3.

Step 2. A mixture of *tert*-butyl (*R*)-2-(((1-benzylpiperidin-4-yl)oxy)methyl)-3-methylbutanoate (700 mg, 1.9 mmol) , 10% wet Pd/C (411 mg, 3.9 mmol) and 20% wet $Pd(OH)_2$/C (542mg, 3.9 mmol) in THF (30 mL) was stirred under an atmosphere of H_2 (15 psi) for 16 h. The mixture was filtered and the filtrate was concentrated under reduced pressure to give *tert*-butyl (*R*)-3-methyl-2-((piperidin-4-yloxy)methyl)butanoate (440 mg, 80% yield) as a an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{15}H_{29}NO_3$ 271.2; found 272.2.

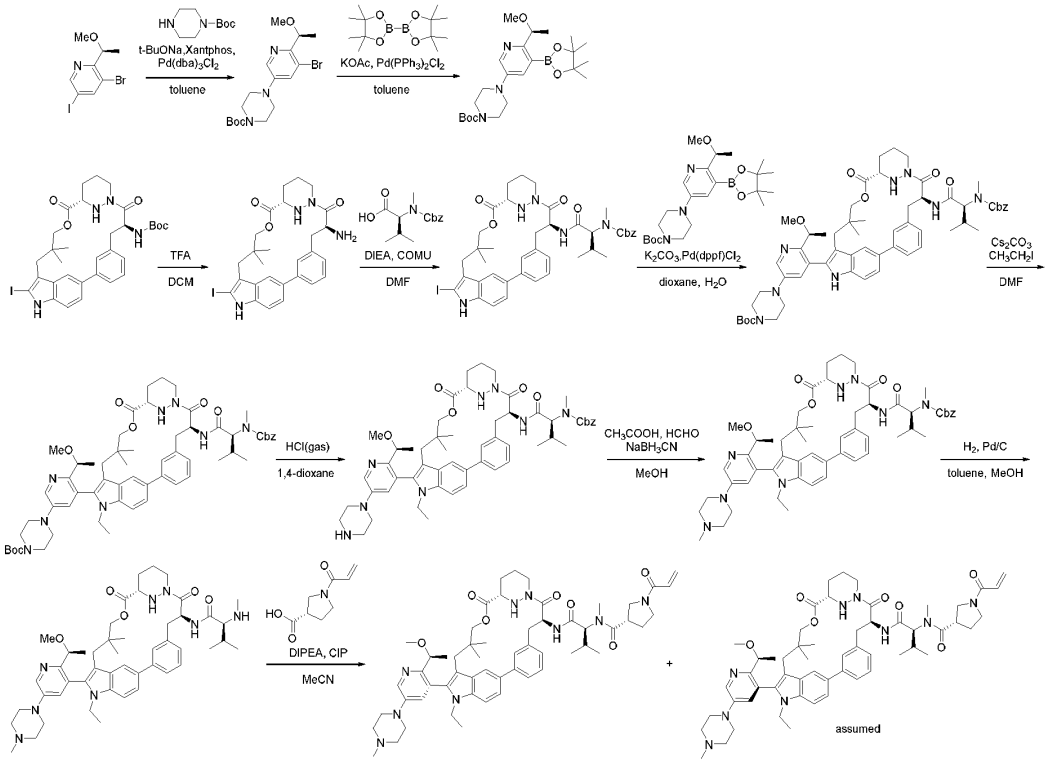
Step 3. To a mixture of *tert*-butyl (*R*)-3-methyl-2-((piperidin-4-yloxy)methyl)butanoate (440 mg, 1.6 mmol) 4-(dimethylamino)-4-methylpent-2-ynoic acid (3.77 g, 24.3 mmol) and DIPEA (2.09 g, 16.2 mmol) in DMF (50 mL) at 0 °C was added T3P (2.57 g, 8.1 mmol). The mixture was stirred at 0 °C for 1 h, then poured into H_2O (50 mL) and extracted with EtOAc (50mL x 3). The combined organic layers were washed with brine, concentrated under reduced pressure and the residue purified by silica gel column chromatography to give *tert*-butyl (*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)piperidin-4-yl)oxy)methyl)-3-methylbutanoate (190 mg, 27% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{40}N_2O_4$ 408.3; found 409.4.

Step 4. To a mixture of *tert*-butyl (*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)piperidin-4-yl)oxy)methyl)-3-methylbutanoate (180 mg, 0.47 mmol) in DCM (2 mL) was added

TFA (1 mL). The mixture was stirred at room temperature for 1 h, then concentrated under reduced pressure to give (*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)piperidin-4-yl)oxy)methyl)-3-methylbutanoic acid (170 mg, 98% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{19}H_{32}N_2O_4$ 352.2; found 353.2.

Step 5. (2*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)piperidin-4-yl)oxy)methyl)-*N*-((2^2*S*,6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide was synthesized in a manner similar to (2*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-*N*-((2^2*S*,6^3*S*,4*S*)-12-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide except (2^2*S*,6^3*S*,4*S*)-4-amino-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione was substituted with (2^2*S*,6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione and 3-({1-[4-(dimethylamino)-4-methylpent-2-ynoyl]azetidin-3-yl}oxy)propanoic acid was substituted with (2*R*)-2-[({1-[4-(dimethylamino)-4-methylpent-2-ynoyl]piperidin-4-yl}oxy)methyl]-3-methylbutanoic acid. (101 mg, 42% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{54}H_{78}N_8O_8$ 966.6; found 969.5; ^{1}H NMR (400 MHz, CD_3OD) δ 8.70 (d, *J* = 4.8 Hz, 1H), 7.81 - 7.76 (m, 1H), 7.56 - 7.46 (m, 1H), 7.43 - 7.34 (m, 1H), 7.24 - 7.01 (m, 2H), 5.66 - 5.54 (m, 1H), 4.50 - 4.40 (m, 1H), 4.31 - 4.22 (m, 1H), 4.19 - 4.08 (m, 1H), 4.02 - 3.82(m, 4H), 3.80 - 3.53 (m, 10H), 3.47 - 3.34 (m, 2H), 3.26 - 3.15 (m, 3H), 2.98 - 2.57 (m, 5H), 2.37 - 2.30 (m, 3H), 2.27 - 2.18 (m, 4H), 2.15 - 2.02 (m, 2H), 2.00 - 1.80 (m, 4H), 1.78 - 1.71 (m, 2H), 1.68 - 1.55 (m, 3H), 1.49 - 1.37 (m, 6H), 1.35 - 1.28 (m, 3H), 1.05 - 0.92 (m, 9H), 0.85 - 0.72 (m, 3H), 0.68 - 0.51 (m, 3H).

Example A328. Synthesis of two atropisomers of (3*S*)-1-acryloyl-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylpyrrolidine-3-carboxamide



Step 1. To a mixture of 3-bromo-5-iodo-2-[(1*S*)-1-methoxyethyl]pyridine (2.20 g, 6.4 mmol) and *tert*-butyl piperazine-1-carboxylate (1.20 g, 6.4 mmol) in toluene (50 mL) under an atmosphere of Ar were added *t*BuONa (0.74 g, 7.7 mmol) and portion-wise addition of $Pd_2(dba)_3$ (0.59 g, 0.64 mmol), followed by portion-wise addition of Xantphos (0.74 g, 1.3 mmol). The mixture was heated to 100 °C and stirred for 16 h then H_2O added and the mixture extracted with EtOAc (400 mL x 3). The combined organic layers were washed with brine (150 mL x 3), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give *tert*-butyl 4-[5-bromo-6-[(1*S*)-1-methoxyethyl]pyridin-3-yl]piperazine-1-carboxylate (1.7g, 61% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{17}H_{26}BrN_3O_3$ 399.1; found 400.1.

Step 2. A mixture of *tert*-butyl 4-[5-bromo-6-[(1*S*)-1-methoxyethyl]pyridin-3-yl]piperazine-1-carboxylate (1.76 g, 4.4 mmol) and 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (1.67 g, 6.6 mmol) in toluene (18 mL) under an atmosphere of Ar were added KOAc (0.95 g, 9.7 mmol) and $Pd(PPh_3)_2Cl_2$ (0.31 g, 0.44 mmol) in portions. The mixture was heated to 80 °C and stirred for 16 h, then diluted with H_2O and the mixture extracted with EtOAc (500 mL x 3). The combined organic layers were washed with brine (100 mL x 3), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column

chromatography to give *tert*-butyl 4-[6-[(1*S*)-1-methoxyethyl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl]piperazine-1-carboxylate (1.4 g, 68% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{38}BN_3O_5$ 447.4; found 448.2.

Step 3. To a mixture of *tert*-butyl ((6^3*S*,4*S*)-1^2-iodo-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (1.0 g, 1.5 mmol) in DCM (10 mL) at 0 'C under an atmosphere of N_2 was added TFA (5.0 mL, 67.3 mmol) in portions. The mixture was stirred at 0 °C for 1 h then concentrated under reduced pressure and dried azeotropically with toluene (3 mL x 3) to give (6^3*S*,4*S*)-4-amino-1^2-iodo-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (1.0 g), which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{27}H_{31}IN_4O_3$ 586.1; found 587.3.

Step 4. To a mixture of (6^3*S*,4*S*)-4-amino-1^2-iodo-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (1.0 g, 1.7 mmol) in DMF (15 mL) at 0 °C under an atmosphere of N_2 were added DIPEA (2.20 g, 17.0 mmol) and (2*S*)-2-[[(benzyloxy)carbonyl](methyl)amino]-3-methylbutanoic acid (0.90 g, 3.4 mmol) in portions, followed by COMU (1.10 g, 2.6 mmol) in portions over 10 min. The mixture was stirred at 0 °C for 1.5 h, then diluted with H_2O and the mixture was extracted with EtOAc (300 mL x 3). The combined organic layers were washed with brine (100 mL x 3), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give benzyl ((2*S*)-1-(((6^3*S*,4*S*)-1^2-iodo-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (790 mg, 53% yield) as a solid.

Step 5. To a mixture of benzyl ((2*S*)-1-(((6^3*S*,4*S*)-1^2-iodo-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (480 mg, 0.58 mmol) and *tert*-butyl 4-[6-[(1*S*)-1-methoxyethyl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl]piperazine-1-carboxylate (309 mg, 0.69 mmol) in 1,4-dioxane (8.0 mL) and H_2O (1.6 mL) under an atmosphere of Ar were added K_2CO_3 (199 mg, 1.4 mmol) and Pd(dppf)Cl_2 (42 mg, 0.06 mmol) in portions. The mixture was heated to 70 °C and stirred for 16 h, then diluted with H_2O and extracted with EtOAc (200 mL x 3). The combined organic layers were washed with brine (150 mL x 3), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl 4-(5-((6^3*S*,4*S*)-4-((*S*)-2-(((benzyloxy)carbonyl)(methyl)amino)-3-methylbutanamido)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (335 mg, 51% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{58}H_{74}N_8O_9$ 1026.6; found 1027.4.

Step 6. To a mixture of *tert*-butyl 4-(5-((6^3*S*,4*S*)-4-((*S*)-2-(((benzyloxy)carbonyl)(methyl)amino)-3-methylbutanamido)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-

benzenacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (335 mg, 0.33 mmol) in DMF (5 mL) at 0 °C under an atmosphere of N_2 were added Cs_2CO_3 (234 mg, 0.72 mmol) and iodoethane (102 mg, 0.65 mmol) in portions. The mixture was warmed to room temperature and stirred for 16 h, then diluted with H_2O and the mixture extracted with EtOAc (100 mL x 3). The combined organic layers were washed with brine (50 mL x 3), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give *tert*-butyl 4-(5-((6^3*S*,4*S*)-4-((*S*)-2-(((benzyloxy)carbonyl)(methyl)amino)-3-methylbutanamido)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (320 mg, 84% yield) as a light yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{60}H_{78}N_8O_9$ 1054.6; found 1055.8.

Step 7. A mixture of *tert*-butyl 4-(5-((6^3*S*,4*S*)-4-((*S*)-2-(((benzyloxy)carbonyl)(methyl)amino)-3-methylbutanamido)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (320 mg) in xx M HCl in 1,4-dioxane (3.0 mL) at 0 °C under an atmosphere of N_2 was stirred at room temperature for 2 h, then concentrated under reduced pressure to give benzyl ((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(piperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate, which was used directly in the next step further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{55}H_{70}N_8O_7$ 954.5; found 955.3.

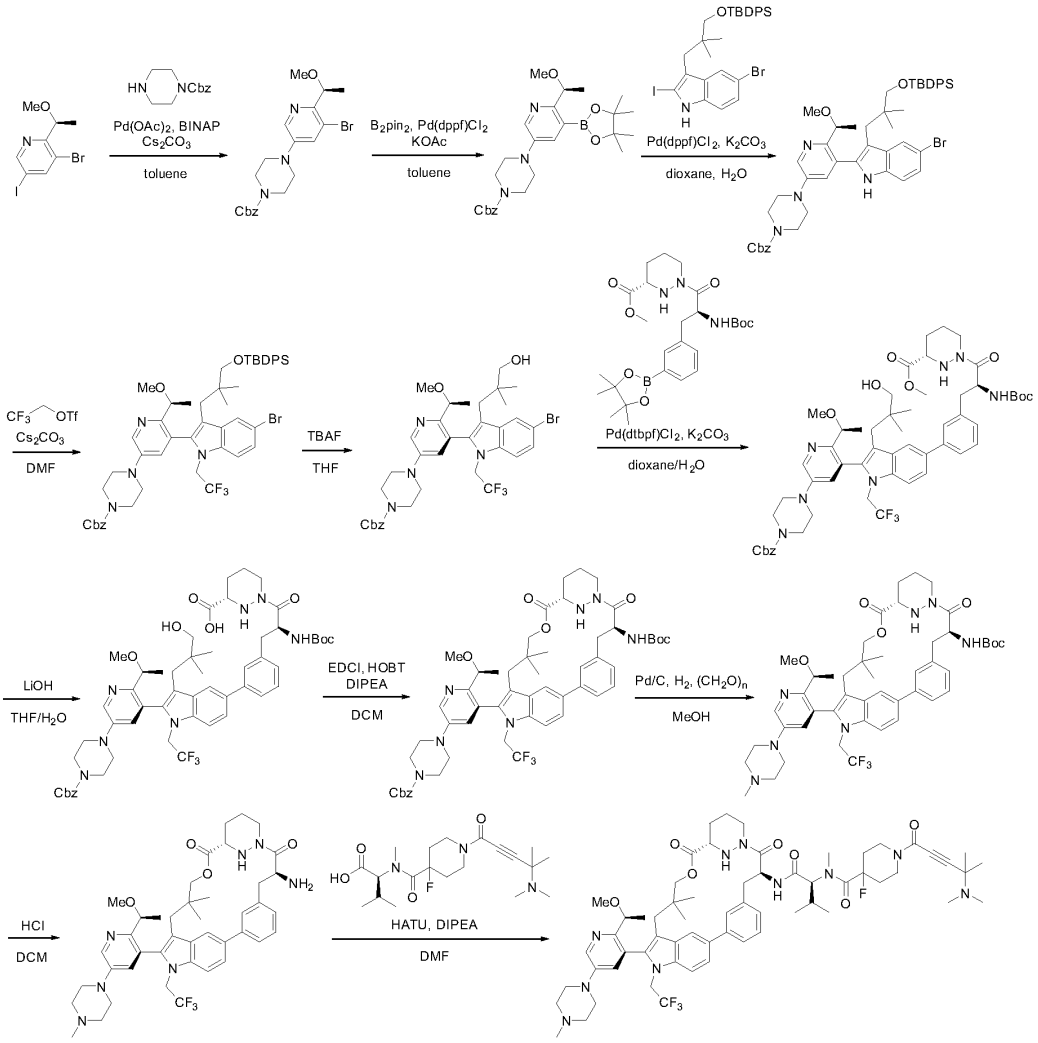
Step 8. To a mixture of benzyl ((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(piperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (320 mg, 0.34 mmol) and HCHO (60 mg, 2.0 mmol) in MeOH (3.0 mL) at 0 °C under an atmosphere of N_2 were added $NaCNBH_3$ (42 mg, 0.67 mmol) and AcOH (60 mg, 1.0 mmol) in portions. The mixture was warmed to room temperature and stirred for 2 h, then diluted with H_2O and the mixture extracted with DCM / MeOH (5:1) (200 mL x 3). The combined organic layers were washed with brine (100 mL x 3), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give benzyl ((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (160 mg, 59% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{56}H_{72}N_8O_7$ 968.6; found 969.6.

Step 9. To a mixture of benzyl ((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (160 mg, 0.17 mmol) in toluene (10 mL) and MeOH (1.0 mL) was

added Pd/C (130 mg, 1.2 mmol) in portions. The mixture was evacuated and re-filled with H_2 (x 3), then stirred under an atmosphere of H_2 for 16 h. The mixture was filtered and the filtrate was concentrated under reduced pressure to give (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide (140 mg), which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{48}H_{66}N_8O_5$ 834.5; found 835.5.

Step 10. To a mixture of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide (140 mg, 0.17 mmol) in ACN (2.0 mL) at 0 °C under an atmosphere of N_2 were added DIPEA (433 mg, 3.35 mmol), (3*S*)-1-(prop-2-enoyl)pyrrolidine-3-carboxylic acid (57 mg, 0.34 mmol) in portions and CIP (70 mg, 0.25 mmol) in portions over 10 min. The mixture was stirred at 0 °C for 1.5 h, then H_2O added and the mixture extracted with EtOAc (150 mL x 3). The combined organic layers were washed with brine (100 mL x 3), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give two atropisomers of (3*S*)-1-acryloyl-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylpyrrolidine-3-carboxamide (40 mg, 24% yield) as a solid and (20 mg, 12% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{56}H_{75}N_9O_7$ 985.6; found 986.7; ^{1}H NMR (400 MHz, DMSO-d_6) 8.47 (t, *J* = 2.1 Hz, 1H), 8.00 (d, *J* = 4.7 Hz, 1H), 7.78 - 7.59 (m, 3H), 7.58 - 7.48 (m, 1H), 7.42 - 7.30 (m, 1H), 7.23 (dq, *J* = 8.0, 4.0, 3.5 Hz, 1H), 7.15 - 7.03 (m, 1H), 6.75 - 6.50 (m, 1H), 6.18 (dt, *J* = 16.8, 2.7 Hz, 1H), 5.70 (tt, *J* = 9.3, 2.7 Hz, 1H), 5.48 - 5.23 (m, 1H), 5.06 (dd, *J* = 31.1, 12.3 Hz, 1H), 4.74 (dd, *J* = 11.0, 4.3 Hz, 1H), 4.33 - 4.15 (m, 2H), 4.01 (ddd, *J* = 36.1, 12.6, 7.6 Hz, 2H), 3.91 - 3.56 (m, 6H), 3.52 - 3.39 (m, 2H), 3.31 - 3.28 (m, 2H), 3.24 (d, *J* = 5.7 Hz, 4H), 3.06 (s, 4H), 2.93 (d, *J* = 9.8 Hz, 2H), 2.81 (d, *J* = 5.4 Hz, 3H), 2.47 - 2.43 (m, 4H), 2.22 (s, 4H), 2.09 (tq, *J* = 12.0, 7.4, 6.6 Hz, 3H), 1.81 (s, 1H), 1.74 (d, *J* = 11.7 Hz, 1H), 1.56 (d, *J* = 11.7 Hz, 1H), 1.20 (dd, *J* = 6.3, 1.5 Hz, 3H), 1.10 (td, *J* = 7.2, 2.4 Hz, 3H), 1.00 - 0.86 (m, 6H), 0.86 - 0.72 (m, 3H), 0.54 (d, *J* = 3.5 Hz, 3H) and LCMS (ESI): m/z [M-H] calc'd for $C_{56}H_{75}N_9O_7$ 985.6; found 984.4; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.46 (d, *J* = 3.0 Hz, 2H), 7.98 (s, 1H), 7.89 - 7.83 (m, 1H), 7.76 - 7.57 (m, 3H), 7.24 (s, 2H), 7.07 (s, 1H), 6.70 - 6.58 (m, 1H), 6.17 (d, *J* = 16.5 Hz, 1H), 5.73 - 5.67(m, 1H), 5.36 - 5.30 (m, 1H), 4.31 - 3.97 (m, 6H), 3.83 - 3.77 (m, 2H), 3.74 - 3.49 (m, 6H), 3.48 - 3.41 (m, 1H), 3.40 - 3.37 (m, 2H) 3.28 - 3.24 (m, 4H), 3.07 (s, 3H), 2.88 - 2.82 (m, 1H), 2.80 - 2.64 (m, 7H), 2.49 - 2.44 (m, 4H), 2.22 (s, 3H), 2.04 (d, *J* = 26.1 Hz, 3H), 1.85 - 1.79 (m, 1H), 1.67 - 1.55 (m, 2H), 1.35 (d, *J* = 6.1 Hz, 3H), 1.27 - 1.22 (m, 1H), 1.05 - 0.93 (m, 4H), 0.89 (d, *J* = 6.9 Hz, 2H), 0.79 (d, *J* = 12.4 Hz, 5H), 0.73 (d, *J* = 6.5 Hz, 1H), 0.56 (s, 3H).

Example A542. The synthesis of 1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-4-fluoro-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylpiperidine-4-carboxamide



Step 1. To a solution of (*S*)-3-bromo-5-iodo-2-(1-methoxyethyl)pyridine (15 g, 43.86 mmol), and benzyl piperazine-1-carboxylate (8.7 g, 39.48 mmol) in toluene (150 mL) at 0 °C, were added cesium carbonate (71.46 g, 219.32 mmol), BINAP (0.55 g, 0.88 mmol) and palladium acetate (0.49 g, 2.19 mmol) in portions. The reaction mixture was stirred at 90 °C for 12 h under an argon atmosphere. The resulting mixture was cooled down to room temperature, filtered and the filter cake was washed with EtOAc (150 mL x 3). The filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford benzyl (*S*)-4-(5-bromo-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (16 g, 84% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{58}N_6O_7$ 433.1; found 434.0.

Step 2. To a stirred solution of benzyl (*S*)-4-(5-bromo-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (22.7 g, 52.26 mmol), bis(pinacolato)diboron (19.91 g, 78.4 mmol) in toluene (230 mL) at 0 °C, were added potassium acetate (12.82 g, 130.66 mmol) and $Pd(dppf)Cl_2$·DCM (4.26 g, 5.23 mmol) in portions. The reaction mixture was stirred at 90 °C for 6 h under an argon atmosphere. The resulting mixture was filtered and the filter cake was washed with EtOAc (200 mL x 3). The filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford benzyl (*S*)-4-(6-(1-methoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl)piperazine-1-carboxylate (14.7 g, 58% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{26}H_{36}BN_3O_5$ 481.3; found 482.3.

Step 3. To a stirred solution of 5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-iodo-1*H*-indole (17.46 g, 27 mmol) in 1,4-dioxane (150 mL) and H_2O (30 mL) at 0 °C, were added potassium carbonate (9.33 g, 67.51 mmol) and $Pd(dppf)Cl_2$ DCM (2.2 g, 2.7 mmol) in portions, followed by benzyl (*S*)-4-(6-(1-methoxyethyl)-5-(4, 4, 5, 5-tetramethyl-1, 3, 2-dioxaborolan-2-yl) pyridin-3-yl) piperazine-1-carboxylate (13 g, 27 mmol). The reaction mixture was stirred at 70 °C for 12 h under an argon atmosphere. The resulting mixture was cooled to room temperature and quenched with H_2O, then extracted with EtOAc (200 mL x 3). The organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford benzyl (*S*)-4-(5-(5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (20 g, 84.7% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{49}H_{57}BN_4O_4Si$ 873.2; found 873.3.

Step 4. To a mixture of benzyl (*S*)-4-(5-(5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (19 g, 21.74 mmol) and Cs_2CO_3 (49.58 g, 152.17 mmol) in DMF (190 mL) at 0 °C under argon atmosphere, was dropwise added 2,2,2-trifluoroethyl trifluoromethanesulfonate (50.46 g, 217.39 mmol). The reaction mixture was stirred at room temperature for 12 h under an argon atmosphere, then quenched with H_2O, extracted with EtOAc (200 mL x 3). The combined organic layers were washed with brine (200 mL x 3), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford benzyl (*S*)-4-(5-(5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (17.6 g, 84.7% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{58}BF_3N_4O_4Si$ 954.2; found 955.3.

Step 5. To a solution of benzyl (*S*)-4-(5-(5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (18 g, 18.83 mmol), was added TBAF in THF (180.0 mL) at 0 °C. The reaction mixture was stirred at 40 °C for 12 h under an argon atmosphere, then quenched with cold H_2O. The resulting mixture was extracted with EtOAc (200 mL x 3). The combined organic layers were washed with brine (200 mL x 3), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford benzyl (*S*)-4-(5-(5-bromo-3-(3-hydroxy-2,2-dimethylpropyl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-2-yl)-6-(1-

methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (7.8 g, 57.7% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{35}H_{40}BrF_3N_4O_4$ 716.2; found 717.1.

Step 6. A solution of benzyl (*S*)-4-(5-(5-bromo-3-(3-hydroxy-2,2-dimethylpropyl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (1 g, 1.39 mmol) in 1,4-dioxane (10 mL) and H_2O (2 mL), was added methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylate (1.08 g, 2.09 mmol), potassium carbonate (481.47 mg, 3.48 mmol) and Pd(dtbpf)Cl_2 (181.64 mg, 0.28 mmol) in portions at 0 °C. The reaction mixture was stirred at 70 °C for 3 h under an argon atmosphere. The resulting mixture was cooled to room temperature, then quenched with H_2O and extracted with EtOAc (50 mL x 3). The combined organic layers were washed with brine (20 mL x 3), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel chromatography to afford methyl (*S*)-1-((*S*)-3-(3-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-(3-hydroxy-2,2-dimethylpropyl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (1.1 g, 77% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{55}H_{68}F_3N_7O_9$ 1027.5; found 1028.3.

Step 7. To a solution of methyl (*S*)-1-((*S*)-3-(3-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-(3-hydroxy-2,2-dimethylpropyl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (1.1 g, 1.07 mmol) in THF (8 mL) and H_2O (2 mL) at 0 °C, was dropwise added LiOH (2.2 mL, 1M aqueous) under an argon atmosphere. The reaction mixture was stirred for 2 h then concentrated under reduced pressure. The residue was acidified to pH 5 with citric acid (1M) and extracted with EtOAc (20 mL x 3). The combined organic layers were concentrated under reduced pressure. The residue was purified by reverse phase chromatography to afford (*S*)-1-((*S*)-3-(3-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-(3-hydroxy-2,2-dimethylpropyl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylic acid (750 mg, 69% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{54}H_{66}F_3N_7O_9$ 1013.5; found 1014.3.

Step 8. To a solution of (*S*)-1-((*S*)-3-(3-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-(3-hydroxy-2,2-dimethylpropyl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylic acid (0.75 g, 0.74 mmol) in DCM (75 mL) at 0 °C, were added in portions HOBT (0.5 g, 3.7 mmol), DIPEA (3.82 g, 29.58 mmol), and EDCI (4.25 g, 22.19 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 12 h under an argon atmosphere. The resulting mixture was concentrated under reduced pressure and extracted with EtOAc (100 mL x 3). The combined organic layers were washed with brine (50 mL x 3), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel chromatography to afford benzyl 4-(5-((6^3*S*,4*S*)-4-((*tert*-butoxycarbonyl)amino)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (0.5 g, 67.9% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{54}H_{64}F_3N_7O_8$ 995.5; found 996.3.

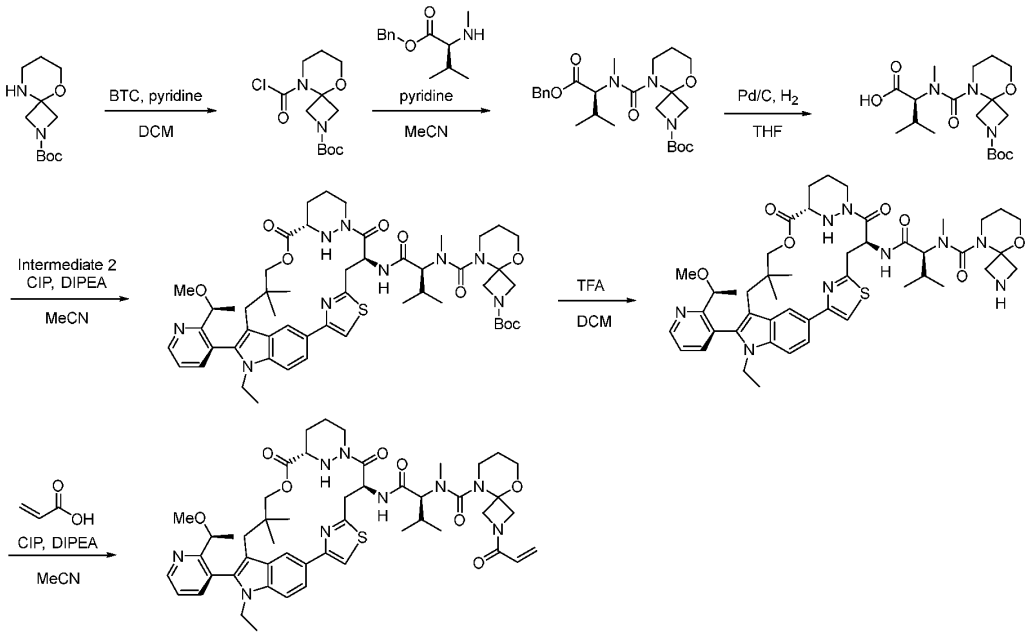
Step 9. To a mixture of benzyl 4-(5-((6^3*S*,4*S*)-4-((*tert*-butoxycarbonyl)amino)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (500 mg, 0.5 mmol) in MeOH (15 mL) at 0 °C, was added paraformaldehyde (135.64 mg, 1.5 mmol), and Pd/C (750 mg) in portions. The reaction mixture was stirred at room temperature for 12 h under a hydrogen atmosphere. The resulting mixture was filtered and the filter cake was washed with EtOAc (50 mL x 5). The filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography to afford *tert*-butyl ((6^3*S*,4*S*)-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (350 mg, 79.6% yield) as an solid. LCMS (ESI): m/z [M+H] calc'd for $C_{47}H_{60}F_3N_7O_8$ 875.5; found 876.5.

Step 10. To a solution of *tert*-butyl ((6^3*S*,4*S*)-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (300 mg, 0.34 mmol) in DCM (2 mL) at 0 °C, was dropwise added HCl in 1,4-dioxane (1 mL, 4M, 4 mmol). The reaction mixture was stirred at room temperature for 2 h, then concentrated under reduced pressure to give (6^3*S*,4*S*)-4-amino-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione hydrochloride (350 mg, crude) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{52}F_3N_7O_4$ 775.4; found 766.4.

Step 11. To a solution of (6^3*S*,4*S*)-4-amino-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione hydrochloride (150 mg, 0.19 mmol) and *N*-(1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-4-fluoropiperidine-4-carbonyl)-*N*-methyl-L-valine (154 mg, 0.39 mmol) in DMF (2 mL) at 0 °C, was dropwise added a mixture of DIPEA (1 g, 7.72 mmol) and HATU (110 mg, 0.29 mmol) in DMF (0.2 mL). The reaction mixture was stirred at 0 °C for 2 h under an argon atmosphere, then quenched with H_2O. The resulting mixture was extracted with EtOAc (20 mL x 3). The combined organic phase was washed with brine (10 mL x 3), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by reverse phase chromatography to afford 1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-4-fluoro-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylpiperidine-4-carboxamide (39.5 mg, 17% yield) as solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (d, *J* = 2.9 Hz, 1H), 8.32 (t, *J* = 7.3 Hz, 1H), 7.97 (s, 1H), 7.82-7.69 (m, 3H), 7.66 (t, *J* = 7.8 Hz, 1H), 7.33-7.07 (m, 3H), 5.50 (dd, *J* = 16.7, 8.6 Hz, 1H), 5.33 (t, *J* = 9.2 Hz, 1H), 5.16 (d, *J* = 12.2 Hz, 1H), 4.94-4.80 (m, 1H), 4.64 (d, *J*= 10.8 Hz, 1H), 4.33-4.16(m, 3H), 4.12-4.02 (m, 2H), 3.71-3.50 (m, 3H), 3.25 (s, 3H), 3.21-3.16 (m, 3H), 3.14-3.05 (m, 1H), 2.96 (t, *J* = 4.7 Hz, 4H), 2.84 (s, 1H), 2.82-2.72 (m, 2H),

2.59-2.53 (m, 1H), 2.47-2.40 (m, 4H), 2.22 (d, *J* = 2.9 Hz, 9H), 2.18-2.12 (m, 2H), 2.11-1.99 (m, 3H), 1.87-1.78 (m, 1H), 1.74-1.62(m, 1H), 1.59-1.48 (m, 1H), 1.40-1.32 (m, 9H), 1.01 (t, *J* = 7.7 Hz, 1H), 0.89 (s, 5H), 0.83 (d, *J* = 6.3 Hz, 1H), 0.77 (d, *J* = 6.6 Hz, 2H), 0.38 (s, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{58}N_6O_7$ 1154.6; found 1155.7.

Example A735. Synthesis of 2-acryloyl-*N*-((2*S*)-1-(((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methyl-5-oxa-2,9-diazaspiro[3.5]nonane-9-carboxamide



Step 1. To a stirred mixture of BTC (425.2 mg, 1.448 mmol) in DCM (10 mL) was added dropwise pyridine (1.04 g,13.16 mmol) and *tert*-butyl 5-oxa-2,9-diazaspiro[3.5]nonane-2-carboxylate (1 g, 4.39 mmol), the reaction mixture was stirred at room temperature for 2 h. The resulting mixture was concentrated under reduced pressure to give crude *tert*-butyl 9-(chlorocarbonyl)-5-oxa-2,9-diazaspiro[3.5]nonane-2-carboxylate.

Step 2. To a stirred solution of *tert*-butyl 9-(chlorocarbonyl)-5-oxa-2,9-diazaspiro[3.5]nonane-2-carboxylate (2.5 g, crude) in MeCN (20 mL) were added dropwise pyridine (1.04 g, 13.16 mmol) and benzyl (2*S*)-3-methyl-2-(methylamino)butanoate (970.66 mg, 4.38 mmol) at room temperature. The reaction mixture was stirred at 80 °C for 12 h and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford *tert*-butyl (*S*)-9-((1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)-5-oxa-2,9-diazaspiro[3.5]nonane-2-carboxylate (783 mg, 37.6% yield, two steps) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{25}H_{37}N_3O_6$ 475.3; found 476.3.

Step 3. A solution of *tert*-butyl (*S*)-9-((1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)-5-oxa-2,9-diazaspiro[3.5]nonane-2-carboxylate (783 mg, 1.65 mmol) and 10 wt% palladium on carbon (226.29 mg) in THF (10 mL) was stirred for 2 h at 50 °C under a hydrogen atmosphere. The resulting mixture was cooled to room temperature, filtered and the filter cake was washed with MeCN (10 mL x 3). The filtrate was concentrated under reduced pressure to give *N*-(2-(*tert*-butoxycarbonyl)-5-oxa-2,9-diazaspiro[3.5]nonane-9-carbonyl)-*N*-methyl-L-valine (591 mg, 98.8% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{18}H_{31}N_3O_6$ 385.2; found 386.3.

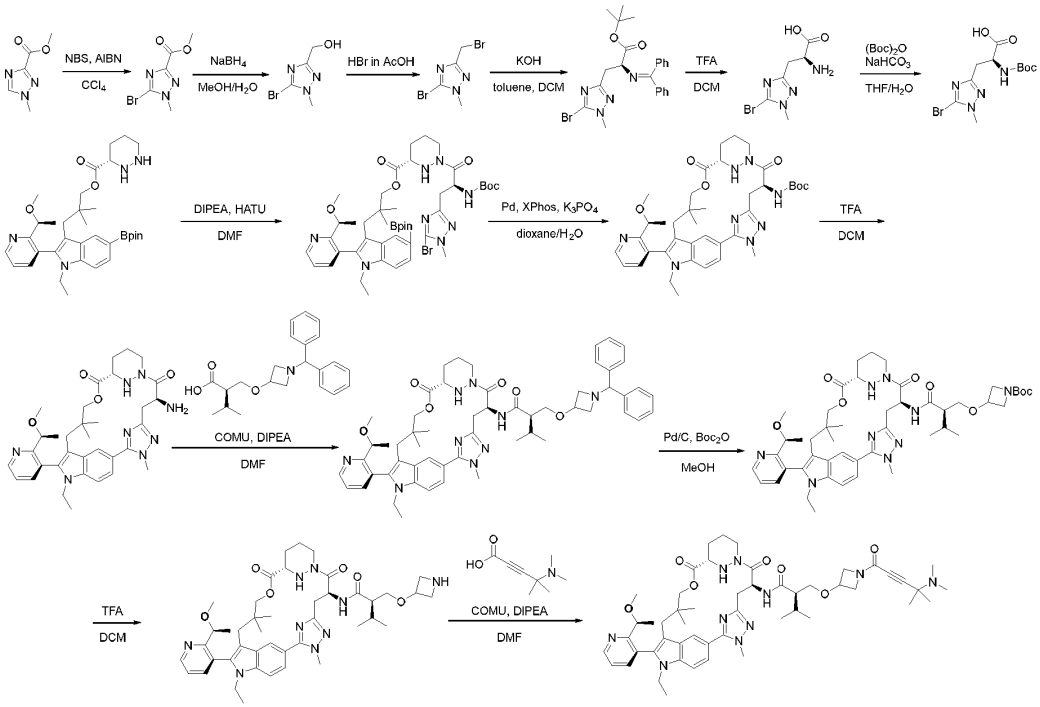
Step 4. To a stirred solution of intermediate 2 (731 mg, 1.16 mmol) and DIPEA (2.25 g, 17.38 mmol) in MeCN (50 mL) was added CIP (644.31 mg, 2.32 mmol) and *N*-(2-(*tert*-butoxycarbonyl)-5-oxa-2,9-diazaspiro[3.5]nonane-9-carbonyl)-*N*-methyl-*L*-valine (446.68 mg, 1.16 mmol) at room temperature. The reaction mixture was stirred for 2 h then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford *tert*-butyl 9-(((2*S*)-1-(((6^3*S*,4*S*,*Z*)-11-ethyl-12-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-61,62,63,64,65,66-hexahydro-11*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)-5-oxa-2,9-diazaspiro[3.5]nonane-2-carboxylate (752 mg, 65% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{52}H_{71}N_9O_9S$ 997.5; found 996.6.

Step 5. To a stirred solution of *tert*-butyl 9-(((2*S*)-1-(((6^3*S*,4*S*,*Z*)-11-ethyl-12-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-61,62,63,64,65,66-hexahydro-11*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)-5-oxa-2,9-diazaspiro[3.5]nonane-2-carboxylate (752 mg, 0.75 mmol) in DCM (40 mL) was added TFA (10 mL) in portions at room temperature. The reaction mixture was stirred for 2 h, then concentrated under reduced pressure. To the residue was added saturated aqueous sodium bicarbonate (100 mL) and DCM (100 mL). The aqueous layer was separated and extracted with DCM (100 mL x 2). The combined organic phase was dried over anhydrous sodium sulfate, filtrated and concentrated under reduced pressure to afford *N*-((2*S*)-1-(((6^3*S*,4*S*,*Z*)-11-ethyl-12-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-61,62,63,64,65,66-hexahydro-11*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methyl-5-oxa-2,9-diazaspiro[3.5]nonane-9-carboxamide (587 mg, 87% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{47}H_{63}N_9O_7S$ 897.5; found 898.4.

Step 6. A stirred solution of *N*-((2*S*)-1-(((6^3*S*,4*S*,*Z*)-11-ethyl-12-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-61,62,63,64,65,66-hexahydro-11*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methyl-5-oxa-2,9-diazaspiro[3.5]nonane-9-carboxamide (586 mg, 0.65 mmol) in MeCN (10 mL) was added acrylic acid (47 mg, 0.65 mmol), DIPEA (421 mg, 3.26 mmol), CIP (362 mg, 1.3 mmol). The reaction mixture was stirred for 12 h and concentrated under reduced pressure. The residue was purified by reverse phase chromatography to afford 2-acryloyl-*N*-((2*S*)-1-(((6^3*S*,4*S*,*Z*)-11-ethyl-12-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-61,62,63,64,65,66-hexahydro-11*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methyl-5-oxa-2,9-diazaspiro[3.5]nonane-9-carboxamide (194 mg, 27% yield) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.78 (dd, *J* = 4.8, 1.7 Hz, 1H), 8.48 (d, *J* = 1.6 Hz, 2H), 7.85-

7.65 (m, 3H), 7.65-7.42(m,2H), 6.30 (mm,1H), 6.10 (m, *J* = 17.0, 1H), 5.78-5.50 (m, *J* = 10.3, 2H), 5.10 (dd, 1H),4.40-3.80 (m, 14H), 3.60 – 3.10 (m, 10H), 2.94 (d, *J* = 14.5 Hz, 1H), 2.85 (s, 4H), 2.42 (dd, 1H), 2.07 (dd, 2H), 1.80 (s, 2H), 1.55 (s, 3H), 1.32 (d, 3H), 0.95 – 0.75 (m, 12H), 0.33 (s, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{65}N_9O_8S$ 951.5; found 952.6.

Example A720. Synthesis of (2*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*,2^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-4-yl)-3-methylbutanamide



Step 1. To a stirred solution of methyl 1-methyl-1,2,4-triazole-3-carboxylate (7.0 g, 49.60 mmol) in CCl_4 (70. mL) was added NBS (13.24 g, 74.40 mmol) and AIBN (11.40 g, 69.44 mmol) in portions at 25 °C under an argon atmosphere. The resulting mixture was stirred for 24 h at 80 °C. The resulting mixture was filtered, the filtrate was cooled to 20 °C and kept at 20 °C for 30 min. The resulting mixture was filtered. The filter cake was washed with H_2O (3 x 50 mL) and pet. ether (3 x 100 mL). The filter cake was dried under reduced pressure. This resulted in methyl 5-bromo-1-methyl-1,2,4-triazole-3-carboxylate (10 g, crude) as a light yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_5H_6BrN_3O_2$ 219.0; found 219.9.

Step 2. To a stirred solution of methyl 5-bromo-1-methyl-1,2,4-triazole-3-carboxylate (10.0 g, 45.50 mmol) in MeOH (150.0 mL) and H_2O (30.0 mL) was added $NaBH_4$ (6.88 g, 181.80 mmol) in portions at -5 °C under a nitrogen atmosphere. The resulting mixture was stirred for 2 h at 0~10 °C. Desired product could be detected by LCMS. The reaction was quenched with brine (100 mL) at 0 °C. The resulting mixture was extracted with pet. ether (100 mL). The aqueous layer was

separated and filtered. The filter cake was washed with MeOH (2 x 50 mL). The filtrate was concentrated under reduced pressure to afford (5-bromo-1-methyl-1,2,4-triazol-3-yl)methanol (6 g, crude) as a light yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_4H_6BrN_3O$ 191.98; found 192.0.

Step 3. A solution of (5-bromo-1-methyl-1,2,4-triazol-3-yl)methanol (6.0 g) and HBr in AcOH (144.0 mL) was stirred for overnight at 80 °C .The mixture was neutralized to pH 9 with saturated $NaHCO_3$ (aq.). The resulting mixture was extracted with EtOAc (3 x 60 mL). The combined organic layers were washed with brine (3x100 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. This resulted in 5-bromo-3-(bromomethyl)-1-methyl-1,2,4-triazole (6 g, crude) as a white solid. LCMS (ESI): m/z [M+H] calc'd for $C_4H_5Br_2N_3$ 253.89; found 253.8.

Step 4. To a stirred mixture of 5-bromo-3-(bromomethyl)-1-methyl-1,2,4-triazole (6.0 g, 23.54 mmol) and *tert*-butyl 2-[(diphenylmethylidene)amino]acetate (6.95 g, 23.54 mmol) in toluene (42 mL) and DCM (18.0 mL) was added (2*R*,4*R*,5*S*)-1-(anthracen-9-ylmethyl)-5-ethenyl-2-[(*S*)-(prop-2-en-1-yloxy)(quinolin-4-yl)methyl]-1-azabicyclo[2.2.2]octan-1-ium bromide (1.43 g, 2.35 mmol) in portions at 0 °C under argon atmosphere. The resulting mixture was stirred and KOH (60 mL) in H_2O was added. The resulting mixture was stirred for 24 h at -10 °C under an argon atmosphere. Desired product could be detected by LCMS. The reaction was quenched with sat. NH_4Cl (aq.) at 0 °C. The resulting mixture was extracted with EtOAc (3 x 100mL). The combined organic layers were washed with brine (1 x 200 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by Prep-TLC to afford *tert*-butyl (2*S*)-3-(5-bromo-1-methyl-1,2,4-triazol-3-yl)-2-[(diphenylmethylidene)amino]propanoate (5 g, 38.6% yield) as a yellow oil. LCMS (ESI): m/z [M+H] calc'd for $C_{21}H_{21}BrN_4O_2$ 469.12; found 469.1.

Step 5. To a stirred solution of *tert*-butyl (2*S*)-3-(5-bromo-1-methyl-1,2,4-triazol-3-yl)-2-[(diphenylmethylidene)amino]propanoate (5.0 g, 10.65 mmol) in DCM (50.0 mL) was added TFA (25.0 mL) dropwise at 0 °C under argon atmosphere. The resulting mixture was stirred for 16 h at room temperature under an argon atmosphere. The resulting mixture was concentrated under reduced pressure to afford (2*S*)-2-amino-3-(5-bromo-1-methyl-1,2,4-triazol-3-yl)propanoic acid (6 g, crude) as a brown oil. LCMS (ESI): m/z [M+H] calc'd for $C_6H_9BrN_4O_2$ 249.00; found 249.0.

Step 6. To a stirred solution of (2*S*)-2-amino-3-(5-bromo-1-methyl-1,2,4-triazol-3-yl)propanoic acid (6.0 g, 24.09 mmol) in THF (36.0 mL) was added $NaHCO_3$ (10.14 g, 120.69 mmol), Boc_2O (7.89 g, 36.14 mmol) in portions at 0 °C under an argon atmosphere. The resulting mixture was stirred for 16 h at room temperature. Desired product could be detected by LCMS. The resulting mixture was concentrated under reduced pressure. The mixture was purified by reverse phase chromatography to afford (2*S*)-3-(5-bromo-1-methyl-1,2,4-triazol-3-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoic acid (3 g, 33.9% yield) as a white solid. LCMS (ESI): m/z [M+H] calc'd for $C_{11}H_{17}BrN_4O_4$ 349.05; found 349.0.

Step 7. To a stirred solution of 2-[[(2*M*)-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-3-yl]methyl]-2-methylpropyl (3*S*)-1,2-diazinane-3-carboxylate (1.0 g, 1.65 mmol) in DMF (10.0 mL) was added DIPEA (4.28 g, 33.08 mmol), (2*S*)-3-(5-bromo-1-methyl-1,2,4-triazol-3-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoic acid (0.69 g, 1.98

mmol) and HATU (0.75 g, 1.99 mmol) in portions at 0 °C. The resulting mixture was stirred for 2 h at 20 °C under an argon atmosphere. Desired product could be detected by LCMS. The resulting mixture was quenched with H_2O (100 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (3x100 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by reverse phase chromatography to afford 2-[[(2*M*)-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-3-yl]methyl]-2-methylpropyl (3*S*)-1-[(2*S*)-3-(5-bromo-1-methyl-1,2,4-triazol-3-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoyl]-1,2-diazinane-3-carboxylate (800 mg, 46.5% yield) as a light yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{45}H_{64}BBrN_8O_8$ 935.42; found 935.2.

Step 8. To a stirred solution of 2-[[(2*M*)-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-3-yl]methyl]-2-methylpropyl (3*S*)-1-[(2*S*)-3-(5-bromo-1-methyl-1,2,4-triazol-3-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoyl]-1,2-diazinane-3-carboxylate (800.0 mg, 0.86 mmol) in dioxane (10.0 mL) were added K_3PO_4 (0.45 g, 2.12 mmol), XPhos (122.26 mg, 0.27 mmol), XPhos Pd G3 (0.22 g, 0.27 mmol) and H_2O (2.0 mL) at room temperature. The resulting mixture was stirred for 3 h at 75 °C under an argon atmosphere. Desired product could be detected by LCMS. The resulting mixture was extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine (3 x 60 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by reverse phase chromatography to afford *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*,2^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-4-yl)carbamate (400 mg, 56.8% yield) as a light yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{52}N_8O_6$ 729.41; found 729.3.

Step 9. To a solution of *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*,2^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-4-yl)carbamate (400.0 mg, 0.56 mmol) in DCM (1 mL) was added TFA (0.5 mL). The reaction was stirred for 1 h at room temperature under an argon atmosphere. After concentration, the mixture was neutralized to pH 8 with saturated $NaHCO_3$ (aq., 20 mL). The mixture was extracted with DCM (3 x 20 mL). The organic layers were dried over Na_2SO_4 and concentrated to afford (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*,2^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-5,7-dione (500 mg, crude) as a light yellow solid. ESI-MS m/z = 629.3 $[M+H]^+$; Calculated MW:628.3. LCMS (ESI): m/z [M+H] calc'd for $C_{34}H_{44}N_8O_4$ 629.36; found 629.3.

Step 10. To a stirred solution of (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*,2^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-5,7-dione (170.0 mg, 0.27 mmol) and (*R*)-2-(((1-benzhydrylazetidin-3-yl)oxy)methyl)-3-methylbutanoic acid (114.68 mg, 0.32 mmol) in DMF (5 mL) were added DIPEA (698.86 mg, 5.41 mmol) and HATU (123.36 mg, 0.32 mmol) dropwise at 0 °C under an air atmosphere. The resulting mixture was stirred for 2 h at 0 °C. The resulting mixture was diluted with 25 mL H_2O. The resulting mixture was extracted with EtOAc (3 x

25 mL). The combined organic layers were washed with brine (3 x 25 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford (2*R*)-2-(((1-benzhydrylazetidin-3-yl)oxy)methyl)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6- hexahydro-1^1*H*,2^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-4-yl)-3-methylbutanamide (180 mg, crude) as an off-white oil. LCMS (ESI): m/z [M+H] calc'd for $C_{56}H_{69}N_9O_6$ 964.54; found 964.4.

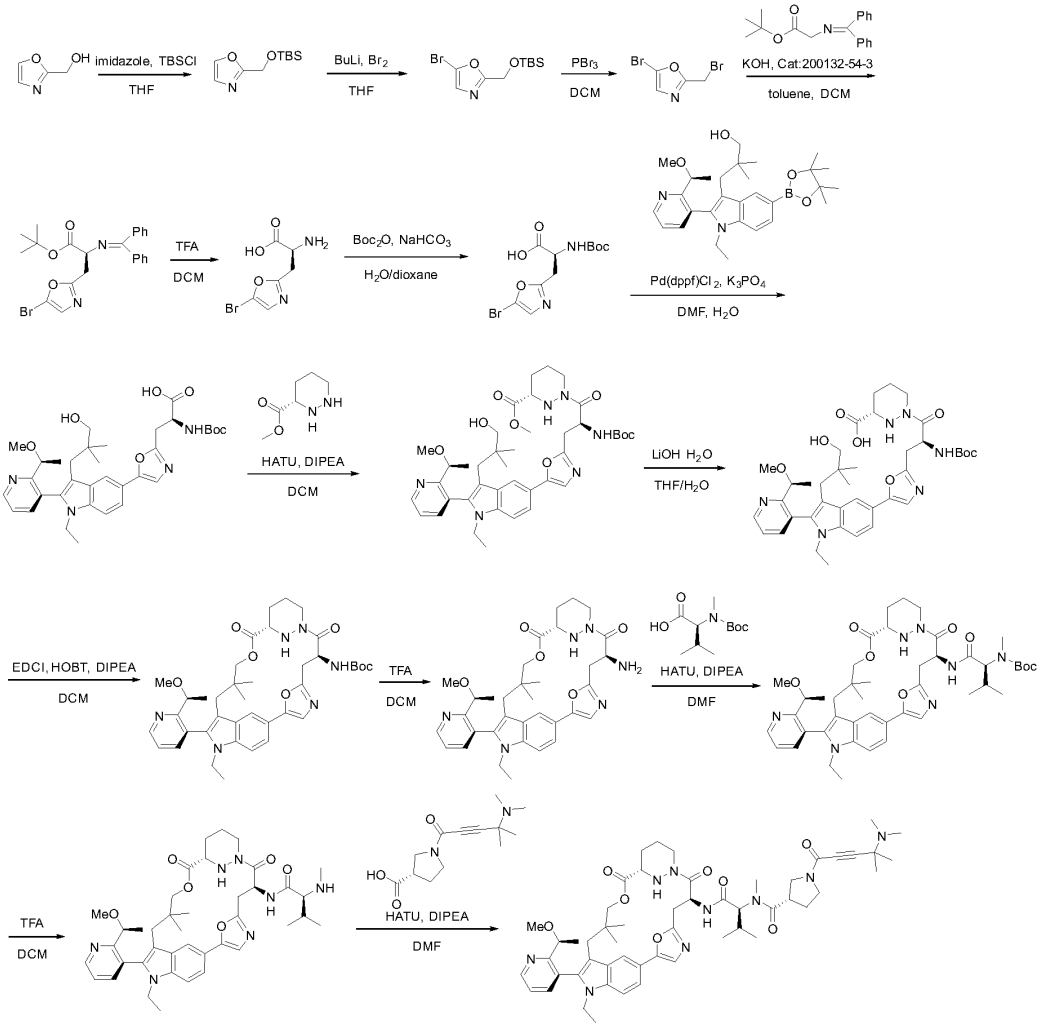
Step 11. To a stirred solution of (2*R*)-2-(((1-benzhydrylazetidin-3-yl)oxy)methyl)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6- hexahydro-1^1*H*,2^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-4-yl)-3-methylbutanamide (180.0 mg, 0.19 mmol) and Pd/C (90.0 mg, 0.85 mmol) in MeOH(10 mL) was added Boc_2O (81.48 mg, 0.37 mmol) at room temperature under a hydrogen atmosphere. The resulting mixture was stirred overnight at room temperature. The resulting mixture was filtered, the filter cake was washed with MeOH (3x10 mL). The filtrate was concentrated under reduced pressure to afford *tert*-butyl 3-((2*R*)-2-(((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6- hexahydro-1^1*H*,2^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-4-yl)carbamoyl)-3-methylbutoxy)azetidine-1-carboxylate (80 mg, 47.7% yield) as an off-white solid. LCMS (ESI): m/z [M+H] calc'd for $C_{48}H_{67}N_9O_8$ 898.52; found 898.4.

Step 12. To a stirred solution of *tert*-butyl 3-((2*R*)-2-(((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6- hexahydro-1^1*H*,2^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-4-yl)carbamoyl)-3-methylbutoxy)azetidine-1-carboxylate in DCM (2 mL) was added TFA (1.0 mL) dropwise at 0 °C under an air atmosphere. The resulting mixture was stirred for 1 h at 0 °C. The resulting mixture was concentrated under reduced pressure to afford (2*R*)-2-((azetidin-3-yloxy)methyl)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*,2^1*H* -8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-4-yl)-3-methylbutanamide (85 mg, crude) as a yellow green oil.

Step 13. To a stirred solution of (2*R*)-2-((azetidin-3-yloxy)methyl)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6- hexahydro-1^1*H*,2^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-4-yl)-3-methylbutanamide (80.0 mg, 0.10 mmol) and 4-(dimethylamino)-4-methylpent-2-ynoic acid (38.90 mg, 0.25 mmol) in DMF (2 mL) were added DIPEA (518.27 mg, 4.01 mmol) and COMU (51.52 mg, 0.12 mmol) in portions at 0 °C The reaction mixture was stirred under an air atmosphere for 2 h. The crude product (150 mg) was purified by reverse phase chromatography to afford (2*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*,2^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-4-yl)-3-methylbutanamide (15.3 mg, 16.3% yield) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (dd, J = 4.8, 1.7 Hz, 1H), 8.15 (d, J = 1.7 Hz, 1H), 8.07 (d, J = 8.1 Hz, 1H), 7.85 – 7.78 (m, 1H), 7.70 (d, J = 8.6 Hz, 1H), 7.58 – 7.48 (m, 2H), 5.82 (s, 1H), 4.95 (d, J = 11.7 Hz, 1H), 4.41 – 4.30 (m, 5H), 4.30 (d, J = 8.2 Hz, 2H), 4.25 (d, J = 5.6 Hz, 4H), 4.10 (td, J = 17.1, 16.1, 9.1 Hz, 2H), 3.99 – 3.82 (m, 3H), 3.71 –

3.60 (m, 1H), 3.54 – 3.43 (m, 3H), 3.39 (s, 2H), 3.22 (d, *J* = 1.6 Hz, 1H), 2.92 (d, *J* = 13.6 Hz, 1H), 2.86 – 2.77 (m, 2H), 2.45 (s, 6H), 2.37 (q, *J* = 7.7 Hz, 1H), 2.17 (d, *J* = 6.6 Hz, 2H), 2.03 (d, *J* = 10.2 Hz, 2H), 1.78 – 1.66 (m, 3H), 1.47 (t, *J* = 10.9 Hz, 6H), 1.35 – 1.28 (m, 12H), 0.32 (s, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{70}N_{10}O_7$ 935.55; found 935.3.

Example A692. Synthesis of (3*S*)-1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(5,2)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylpyrrolidine-3-carboxamide



Step 1. To a solution of 1,3-oxazol-2-ylmethanol (5.0 g, 50.46 mmol) in THF (75 mL), were added imidazole (8.59 mg, 0.13 mmol), and TBSCl (11.41 mg, 0.08 mmol) at 0 °C. The resulting solution was stirred for 5 h then concentrated under reduced pressure. The crude material was purified by silica gel column chromatography to afford 2-[[(*tert*-butyldimethylsilyl)oxy]methyl]-1,3-

oxazole (10 g, 92.8% yield) as colorless oil. LCMS (ESI): m/z [M+H] calc'd for $C_{10}H_{19}NO_2Si$ 214.13; found 214.3.

Step 2. To a solution of 2-[[(*tert*-butyldimethylsilyl)oxy]methyl]-1,3-oxazole (10.0 g, 46.87 mmol) in THF (150.0 mL, 1851.45 mmol) at -78 °C was added *n*-BuLi (22.4 mL, 56.25 mmol) over 10 min and stirred for 30 min at -78 °C under an argon atmosphere. Then the solution of Br_2 (3.6 mL, 70.31 mmol) in THF (10 mL) was added over 10 min to the solution at -78 °C. The resulting solution was slowly warmed to room temperature and stirred for 2 h. The resulting mixture was diluted with NH_4Cl/H_2O (100 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure and purified by silica gel column chromatography to afford 5-bromo-2-[[(*tert*-butyldimethylsilyl)oxy]methyl]-1,3-oxazole (5.3 g, 38.6% yield) as yellow oil. LCMS (ESI): m/z [M+H] calc'd for $C_{10}H_{18}BrNO_2Si$ 292.04; found 292.0.

Step 3. To a solution of 5-bromo-2-[[(*tert*-butyldimethylsilyl)oxy]methyl]-1,3-oxazole (4.0 g, 13.69 mmol) in DCM (60.0 mL) was added PBr_3 (7.41 g, 27.37 mmol) at 0 °C under an argon atmosphere. The resulting solution was stirred for 4 h then diluted with $NaHCO_3/H_2O$ (30mL). The mixture was extracted with EtOAc (3 x 40 mL). The organic layers were concentrated under reduced pressure and purified by silica gel column chromatography to afford 5-bromo-2-(bromomethyl)-1,3-oxazole (2.5 g, 75.7% yield) as yellow oil. LCMS (ESI): m/z [M+H] calc'd for $C_4H_3Br_2NO$ 239.87; found 241.9.

Step 4. A mixture of 5-bromo-2-(bromomethyl)-1,3-oxazole (9.0 g, 37.36 mmol), Cat:200132-54-3 (2.26 g, 3.74 mmol), DCM (45.0 mL), toluene (90.0 mL), KOH (20.96 g, 373.63 mmol), H_2O (42 mL),and *tert*-butyl 2-[(diphenylmethylidene)amino]acetate (13.24 g, 44.82 mmol) at 0 °C was stirred for 4 h then diluted with H_2O (30 mL). The mixture was extracted with DCM (3 x 40 mL). The organic layers were concentrated under reduced pressure and purified by reverse phase column chromatography to afford *tert*-butyl (2*S*)-3-(5-bromo-1,3-oxazol-2-yl)-2-[(diphenylmethylidene)amino]propanoate (4.8 g, 28.2% yield) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{23}BrN_2O_3$ 455.10; found 457.1.

Step 5. A mixture of *tert*-butyl (2*S*)-3-(5-bromo-1,3-oxazol-2-yl)-2-[(diphenylmethylidene)amino]propanoate (1.20 g, 2.64 mmol), DCM (10.0 mL, 157.30 mmol), and TFA (5.0 mL, 67.32 mmol) at 0 °C was stirred for 2 h then concentrated under reduced pressure to afford (*S*)-3-(5-bromooxazol-2-yl)-2-((2,2,2-trifluoroacetyl)-l4-azaneyl)propanoic acid (0.5 g, 81.3% yield) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_6H_7BrN_2O_3$ 234.97; found 237.0.

Step 6. A mixture of (*S*)-3-(5-bromooxazol-2-yl)-2-((2,2,2-trifluoroacetyl)-l4-azaneyl)propanoic acid (500.0 mg, 2.13 mmol), Boc_2O (928.56 mg, 4.26 mmol), dioxane (2.50 mL), H_2O (2.50 mL), and $NaHCO_3$ (714.84 mg, 8.51 mmol) at 0 °C was stirred for 3 h. The resulting solution was purified by reverse phase column chromatography to afford (2*S*)-3-(5-bromo-1,3-oxazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoic acid (0.65 g, 91.1% yield) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{11}H_{15}BrN_2O_5$ 335.02; found 334.8.

Step 7. To a solution of (2*S*)-3-(5-bromo-1,3-oxazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoic acid (500.0 mg, 1.49 mmol) and 3-[(2*M*)-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-3-yl]-2,2-

dimethylpropan-1-ol (808.16 mg, 1.64 mmol) in DMF(5.0 mL) and H_2O(1.0 mL) were added K_3PO_4 (791.67 mg, 3.73 mmol) and Pd(dppf)Cl_2 (109.16 mg, 0.15 mmol). The resulting mixture was stirred for 2 h at 70 °C under an argon atmosphere. The mixture was purified by reverse phase column chromatography to afford (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[5-[(2*M*)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-1,3-oxazol-2-yl]propanoic acid (600 mg, 64.79% yield) as a light brown solid. LCMS (ESI): m/z [M+H] calc'd for $C_{34}H_{44}N_4O_7$ 621.33; found 621.3.

Step 8. To a stirred mixture of methyl (3*S*)-1,2-diazinane-3-carboxylate(627.10 mg, 4.350 mmol) and (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[5-[(2*M*)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-1,3-oxazol-2-yl]propanoic acid (900.0 mg, 1.45 mmol) in DCM (10.0 mL) were added HATU (661.54 mg, 1.74 mmol) and DIPEA (3747.71 mg, 29.00 mmol) at 0 °C. The resulting mixture was stirred for 2 h. Desired product could be detected by LCMS. The resulting mixture was concentrated under reduced pressure and the crude material was purified by silica gel column chromatography to afford methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[5-[(2*M*)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-1,3-oxazol-2-yl]propanoyl]-1,2-diazinane-3-carboxylate (900mg,83.11%) as a brown yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{40}H_{54}N_6O_8$ 747.41; found 747.2.

Step 9. To a stirred mixture of methyl (*3S*)-1-[(*2S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[5-[(2M)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(*1S*)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-1,3-oxazol-2-yl]propanoyl]-1,2-diazinane-3-carboxylate (2000.0 mg, 2.68 mmol) in THF (18. mL) and H_2O (6.0 mL) was added LiOH.H_2O (337.10 mg, 8.03 mmol) at 0 °C. The resulting mixture was stirred for 2 h. Desired product could be detected by LCMS. The reaction was quenched with H_2O at 0 °C and adjusted to pH 6 with 1N HCl solution. The resulting mixture was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (1x10 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[5-[(2M)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-1,3-oxazol-2-yl]propanoyl]-1,2-diazinane-3-carboxylic acid (1300 mg, 66.2% yield) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{52}N_6O_8$ 733.39; found 733.3.

Step 10. To a stirred mixture of (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[5-[(2M)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-1,3-oxazol-2-yl]propanoyl]-1,2-diazinane-3-carboxylic acid (1.2 g, 1.64 mmol) and DIPEA (8.5 g, 65.50 mmol) in DCM (120.0 mL) were added HOBT (1.8 g, 13.10 mmol) and EDCI (7.8 g, 40.93 mmol) at 0 °C. The resulting mixture was stirred for 2 h. Desired product could be detected by LCMS. The mixture was concentrated under reduced pressure and purified by silica gel column chromatography to afford *tert*-butyl ((6^3*S*,4*S*)-11-ethyl-12-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11*H*-8-oxa-2(5,2)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (660 mg, 56.4% yield) as a brown yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{50}N_6O_7$ 715.38; found 715.3.

Step 11. A mixture of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(5,2)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (20.0 mg, 0.028 mmol) and TFA (3.0 mL) in DCM (6.0 mL) at 0 °C was stirred for 2 h. Desired product could be detected by LCMS. The resulting mixture was concentrated under reduced pressure to afford (6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(5,2)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (160mg, crude) as a yellow green solid. LCMS (ESI): m/z [M+H] calc'd for $C_{34}H_{42}N_6O_5$ 615.33; found 615.2.

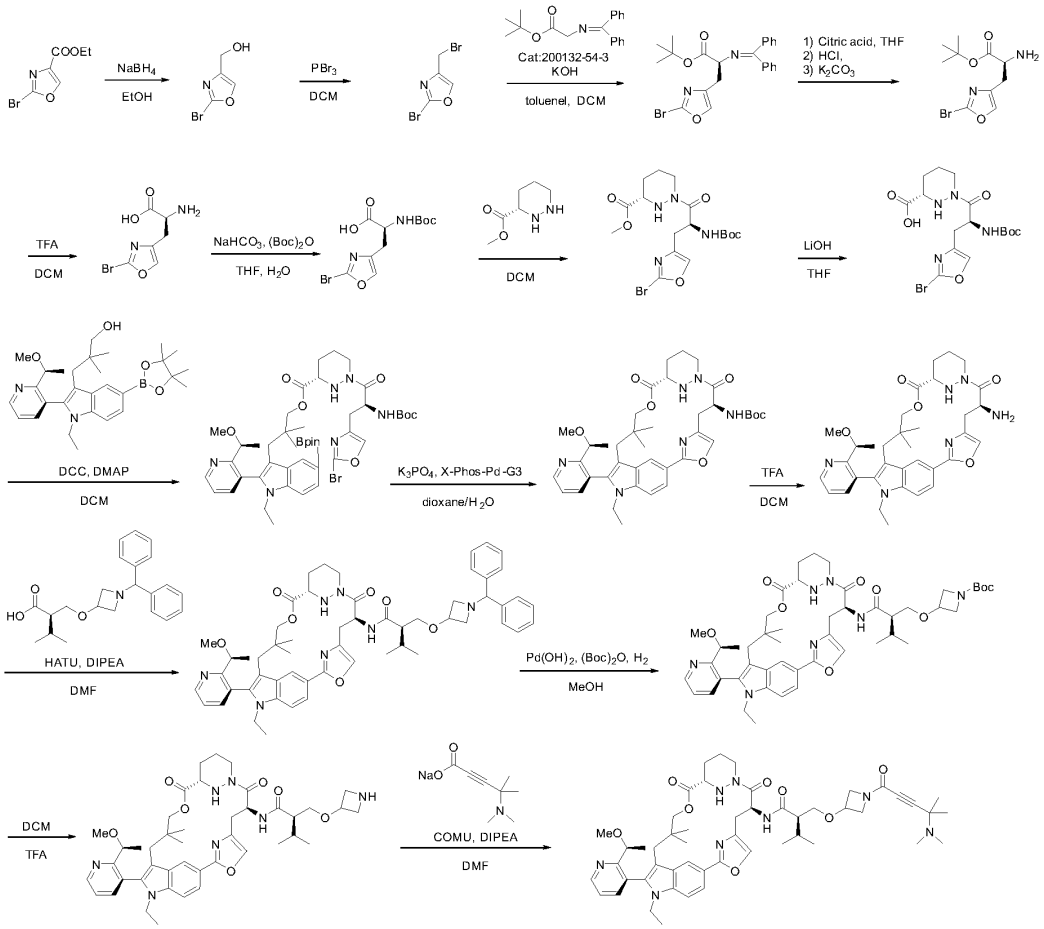
Step 12. To a stirred mixture of (6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(5,2)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (180.0 mg, 0.293 mmol) and (2*S*)-2-[(*tert*-butoxycarbonyl)(methyl)amino]-3-methylbutanoic acid (135.45 mg, 0.59 mmol) in DMF (2.0 mL) were added HATU (133.60 mg, 0.35 mmol) and DIPEA (756.86 mg, 5.86 mmol) at 0 °C. The resulting mixture was stirred for 2 h. Desired product could be detected by LCMS. The mixture was purified by reverse phase column chromatography to afford *tert*-butyl ((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(5,2)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (160 mg, 66% yield) as a brown yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{45}H_{61}N_7O_8$ 828.47; found 828.4.

Step 13. To a stirred mixture of *tert*-butyl ((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(5,2)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (160.0 mg, 0.19 mmol) in DCM (2.0 mL) was added TFA (1.0 mL) at 0 °C. The resulting mixture was stirred for 2 h. Desired product could be detected by LCMS. The resulting mixture was concentrated under reduced pressure to afford (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(5,2)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide (130 mg, crude) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{40}H_{53}N_7O_6$ 728.41; found 728.5.

Step 14. To a stirred mixture of ((2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(5,2)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide (130.0 mg, 0.18 mmol) and (3*S*)-1-[4-(dimethylamino)-4-methylpent-2-ynoyl]pyrrolidine-3-carboxylic acid (180.25 mg, 0.72 mmol) in DMF (2.0 mL) were added DIPEA (461.64 mg, 3.57 mmol) and HATU (135.81 mg, 0.36 mmol) at 0 °C. The resulting mixture was stirred for 2 h. Desired product could be detected by LCMS. The mixture was purified by reverse phase column chromatography to afford (3*S*)-1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(5,2)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylpyrrolidine-3-carboxamide (55.3 mg, 31.3% yield) as a solid. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.77 (dd, *J* = 4.8,

1.7 Hz, 1H), 8.09 – 8.00 (m, 1H), 7.92 (s, 1H), 7.88 – 7.80 (m, 1H), 7.62 (d, *J* = 8.7 Hz, 1H), 7.59 – 7.49 (m, 2H), 7.39 – 7.30 (m, 1H), 5.69 (p, *J* = 8.8 Hz, 1H), 5.44 (d, *J* = 12.1 Hz, 1H), 4.67 (d, *J* = 10.7 Hz, 1H), 4.30 – 4.15 (m, 3H), 3.99 (dt, *J* = 13.2, 6.4 Hz, 3H), 3.89 – 3.79 (m, 1H), 3.62 (ddd, *J* = 30.5, 18.6, 11.4 Hz, 5H), 3.39 (dd, *J* = 9.4, 3.8 Hz, 2H), 3.21 – 3.13 (m, 1H), 3.08 (d, *J* = 15.0 Hz, 3H), 2.99 – 2.74 (m, 6H), 2.26 – 2.18 (m, 5H), 2.16 (s, 2H), 2.14 – 1.94 (m, 3H), 1.86 – 1.68 (m, 2H), 1.57 (q, *J* = 9.2, 5.8 Hz, 1H), 1.44 – 1.27 (m, 9H), 0.94 (d, *J* = 6.6 Hz, 4H), 0.89 (dd, *J* = 6.5, 2.5 Hz, 2H), 0.80 (d, *J* = 6.3 Hz, 2H), 0.77 – 0.69 (m, 4H), 0.58 (d, *J* = 20.4 Hz, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{53}H_{71}N_9O_8$ 962.55; found 962.5.

Example A675. Synthesis of (2*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide



Step 1. A mixture of 2-bromo-4-(ethoxycarbonyl)-1,3-oxazol-5-ylium (6.83 g, 31.19 mmol), EtOH (100.0 mL) and $NaBH_4$ (4.72 g, 124.76 mmol) at 0 °C was stirred for 6 h at 0 °C under an air atmosphere. The reaction was quenched with H_2O at 0 °C. The resulting mixture was extracted with EtOAc (3 x 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After

filtration, the filtrate was concentrated under reduced pressure to afford (2-bromo-1,3-oxazol-4-yl)methanol (4.122 g, 74.3% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_4H_4BrNO_2$ 177.95; found 178.0.

Step 2. A mixture of (2-bromo-1,3-oxazol-4-yl)methanol (4.30 g, 24.16 mmol), DCM (50 mL) and phosphorus tribromide (9809.39 mg, 36.24 mmol) at 0 °C was stirred overnight at 0 °C under an air atmosphere. The reaction was quenched by the addition of $NaHCO_3$ (aq.) at 0 °C. The resulting mixture was extracted with EtOAc (3 x 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford 2-bromo-4-(bromomethyl)-1,3-oxazole (3.28 g, 56.4% yield) as a liquid. LCMS (ESI): m/z [M+H] calc'd for $C_4H_3Br_2NO$ 239.87; found 239.9.

Step 3. A mixture of 2-bromo-4-(bromomethyl)-1,3-oxazole(3280.0 mg, 13.62 mmol), KOH (9M, 10 mL), 30 mL mixture of toluene/DCM (7/3) and *tert*-butyl 2-[(diphenylmethylidene)amino]acetate (5228.74 mg, 17.70 mmol) at -16 °C was stirred overnight under an air atmosphere. The resulting mixture was extracted with EtOAc (3 x 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford *tert*-butyl (2*S*)-3-(2-bromo-1,3-oxazol-4-yl)-2-[(diphenylmethylidene)amino]propanoate (8.33 g, 80.6% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{23}BrN_2O_3$ 455.10; found 455.1.

Step 4. A mixture of *tert*-butyl (2*S*)-3-(2-bromo-1,3-oxazol-4-yl)-2-[(diphenylmethylidene)amino] propanoate (4100.0 mg, 9.0 mmol) and citric acid (1 N) (40.0 mL, 0.21 mmol), in THF (40 mL) at room temperature was stirred overnight under an air atmosphere. The reaction was quenched by the addition of HCl (aq.) (100 mL) at 0 °C. The aqueous layer was extracted with EtOAc (3 x 100 mL). K_2CO_3 (aq.) (200 mL) was added to the resulting mixture and extracted with EtOAc (3 x 100 mL). The organic layer was concentrated under reduced pressure to afford *tert*-butyl (2*S*)-2-amino-3-(2-bromo-1,3-oxazol-4-yl)propanoate (1730 mg, 66% yield) as a dark yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{10}H_{15}BrN_2O_3$ 291.03; found 291.0.

Step 5. A mixture of *tert*-butyl (2*S*)-2-amino-3-(2-bromo-1,3-oxazol-4-yl)propanoate (1780.0 mg, 6.11 mmol), TFA (10.0 mL) and DCM (10.0 mL) at 0 °C was stirred for overnight under an air atmosphere. The resulting mixture was concentrated under reduced pressure to afford (2*S*)-2-amino-3-(2-bromo-1,3-oxazol-4-yl)propanoic acid (1250 mg, 87% yield) as a dark yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_6H_7BrN_2O_3$ 234.97; found 234.9.

Step 6. A mixture of di-*tert*-butyl dicarbonate(4178.58 mg, 19.15 mmol), THF (10 mL), H_2O (10 mL),(2*S*)-2-amino-3-(2-bromo-1,3-oxazol-4-yl)propanoic acid (1500.0 mg, 6.38 mmol) and $NaHCO_3$ (3216.74 mg, 38.29 mmol) at room temperature was stirred overnight under an air atmosphere. The reaction was quenched with H_2O at room temperature. The resulting mixture was concentrated under reduced pressure. The resulting mixture was extracted with EtOAc (3 x 100 mL). The aqueous layer was acidified to pH 6 with 1 M HCl (aq.). The resulting mixture was extracted with EtOAc (3 x 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford (2*S*)-3-(2-bromo-1,3-oxazol-4-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoic acid (920 mg, 43.0% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{11}H_{15}BrN_2O_5$ 335.02; found 335.0.

Step 7. A mixture of 3-(2-bromo-1,3-oxazol-4-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoic acid (850.0 mg, 2.54 mmol), methyl 1,2-diazinane-3-carboxylate (1.88 g, 13.04 mmol), DIPEA (1966.68 mg, 15.22 mmol), DCM (30.0 mL) and HATU (1446.48 mg, 3.80 mmol) at 0 °C was stirred for 3 h under an air atmosphere. The resulting mixture was extracted with DCM (3 x 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The resulting mixture was purified by reverse flash chromatography to afford methyl 1-[3-(2-bromo-1,3-oxazol-4-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoyl]-1,2-diazinane-3-carboxylate (610 mg, 52.1% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{17}H_{25}BrN_4O_6$ 461.10; found 461.0.

Step 8. A mixture of methyl 1-[3-(2-bromo-1,3-oxazol-4-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoyl]-1,2-diazinane-3-carboxylate (570.0 mg, 1.24 mmol), LiOH (2.0 mL, 1 M aq.) and THF (2.0 mL) at 0 °C was stirred for 3 h under an air atmosphere. The resulting mixture was concentrated under reduced pressure. The resulting mixture was extracted with EtOAc (3 x 50 mL). The combined aqueous layers were acidified to pH 5 with 1N HCl (aq.). The aqueous phase was extracted with EtOAc (3 x 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford 1-[3-(2-bromo-1,3-oxazol-4-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoyl]-1,2-diazinane-3-carboxylic acid (500 mg, 90.5% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{16}H_{23}BrN_4O_6$ 447.09; found 446.8.

Step 9. A mixture of 1-[3-(2-bromo-1,3-oxazol-4-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoyl]-1,2-diazinane-3-carboxylic acid (450.0 mg, 1.01 mmol), 3-[(2*M*)-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-3-yl]-2,2-dimethylpropan-1-ol (743.19 mg, 1.51 mmol), DMAP (24.58 mg, 0.20 mmol), DCM (15.0 mL) and DCC (311.37 mg, 1.51 mmol) at 0 °C was stirred for 3 h under an air atmosphere. The resulting mixture was extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by Prep-TLC to afford 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl(*S*)-1-((*S*)-3-(2-bromooxazol-4-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (330 mg, 35.6% yield) as a white solid. LCMS (ESI): m/z [M+H] calc'd for $C_{45}H_{62}BBrN_6O_9$ 921.39; found 921.4.

Step 10. A mixture of 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl(*S*)-1-((*S*)-3-(2-bromooxazol-4-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (290.0 mg, 0.33 mmol), K_3PO_4 (206.64 mg, 0.97 mmol), X-Phos (30.94 mg, 0.07 mmol), XPhos Pd G3 (54.93 mg, 0.07 mmol), dioxane (5. mL) and H_2O (1.0 mL) at 70 °C was stirred for 4 h under an argon atmosphere. The resulting mixture was extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by Prep-TLC to afford *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-

oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (130 mg, 56.0% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{50}N_6O_7$ 715.38; found 715.3.

Step 11. A mixture of *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (120.0 mg), DCM (2.0 mL) and TFA (0.2 mL) at room temperature was stirred for 6 h under an air atmosphere. The resulting mixture was concentrated under reduced pressure. to afford (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (90 mg, 87.2% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{34}H_{42}N_6O_5$ 615.33; found 615.3.

Step 12. A mixture of (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (200.0 mg, 0.33 mmol), (2*R*)-2-([[1-(diphenylmethyl)azetidin-3-yl]oxy]methyl)-3-methylbutanoic acid (172.49 mg, 0.49 mmol), DIPEA (420.48 mg, 3.25 mmol), DMF (3.0 mL) and HATU (148.44 mg, 0.39 mmol) at 0 °C was stirred for 3 h under an air atmosphere. The resulting mixture was extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by Prep-TLC to afford (2*R*)-2-(((1-benzhydrylazetidin-3-yl)oxy)methyl)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide (154 mg, 77.0% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{56}H_{67}N_7O_7$ 950.52; found 950.6.

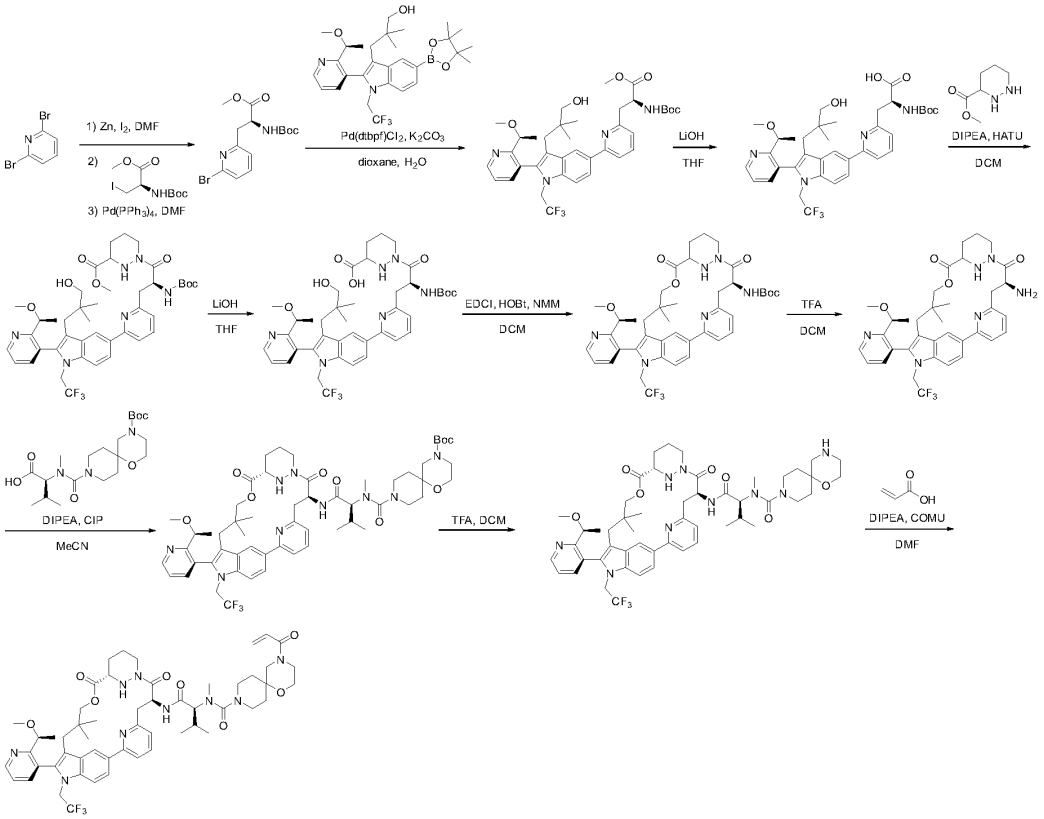
Step 13. A mixture of (2*R*)-2-(((1-benzhydrylazetidin-3-yl)oxy)methyl)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide (240.0 mg, 0.25 mmol), $(Boc)_2O$ (165.37 mg, 0.76 mmol), MeOH (5.0 mL) and $Pd(OH)_2$ (72.0 mg, 0.51 mmol) at room temperature was stirred overnight under an H_2 atmosphere. The resulting mixture was filtered, the filter cake was washed with MeOH (3 x 5 mL). The filtrate was concentrated under reduced pressure. The residue was purified by Prep-TLC to afford *tert*-butyl 3-((2*R*)-2-(((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamoyl)-3-methylbutoxy)azetidine-1-carboxylate (150 mg, 67.2% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{48}H_{65}N_7O_9$ 884.49; found 884.2.

Step 14. A mixture of *tert*-butyl 3-((2*R*)-2-(((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamoyl)-3-methylbutoxy)azetidine-1-carboxylate (150.0 mg), DCM (2.0 mL) and TFA (0.40 mL) at 0 °C was stirred for 3 h under an air atmosphere. The resulting mixture was concentrated under reduced pressure to afford (2*R*)-2-((azetidin-3-yloxy)methyl)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(2,4)-

oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide (120 mg, 90.2% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{57}N_7O_7$ 784.44; found 784.2.

Step 15. A mixture of (2*R*)-2-((azetidin-3-yloxy)methyl)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide (130.0 mg, 0.17 mmol), sodium 4-(dimethylamino)-4-methylpent-2-ynoate (44.07 mg, 0.25 mmol), DMF (3.0 mL), DIPEA (64.29 mg, 0.50 mmol) and COMU (106.46 mg, 0.25 mmol) at 0 °C was stirred for 3 h under an air atmosphere. The resulting mixture was extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The crude product was purified by reverse phase chromatography to afford (2*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide (25 mg, 16.4% yield) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (dd, *J* = 4.8, 1.8 Hz, 1H), 8.55 (s, 1H), 8.14 (dd, *J* = 8.3, 2.9 Hz, 1H), 7.82 (d, *J* = 7.6 Hz, 1H), 7.76 – 7.65 (m, 3H), 7.54 (dd, *J* = 7.7, 4.7 Hz, 1H), 7.54 – 7.02 (m, 1H), 5.72 (td, *J* = 7.4, 3.4 Hz, 1H), 4.97 (d, *J* = 11.9 Hz, 1H), 4.46 – 4.24 (m, 6H), 4.18 – 4.04 (m, 2H), 3.94 (dd, *J* = 32.9, 7.8 Hz, 1H), 3.77 – 3.63 (m, 2H), 3.57 (s, 1H), 3.49 (s, 2H), 3.21 (s, 3H), 2.90 (d, *J* = 14.6 Hz, 1H), 2.87 – 2.79 (m, 1H), 2.72 (td, *J* = 15.5, 14.6, 3.1 Hz, 2H), 2.46 (s, 1H), 2.43 – 2.26 (m, 6H), 2.11 – 1.99 (m, 1H), 1.82 – 1.66 (m, 2H), 1.56 – 1.37 (m, 11H), 0.89 (dt, *J* = 12.3, 7.7 Hz, 12H), 0.35 (s, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{68}N_8O_8$ 921.52; found 921.5.

Example A607. The synthesis of (2*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-*N*-((2^3*S*,6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-piperidinacycloundecaphane-4-yl)-3-methylbutanamide



Step 1. A mixture of Zn (44.18 g, 675.41 mmol) and I_2 (8.58 g, 33.77 mmol) in DMF (120 mL) was stirred for 30 min at 50 °C under an argon atmosphere, followed by the addition of methyl (2*R*)-2-[(*tert*-butoxycarbonyl) amino]-3-iodopropanoate (72.24 g, 219.51 mmol) in DMF (200 mL). The reaction mixture was stirred at 50 °C for 2 h under an argon atmosphere. Then a mixture of 2,6-dibromo-pyridine (40 g, 168.85 mmol) and $Pd(PPh_3)_4$ (39.02 g, 33.77 mmol) in DMF (200 mL) was added. The resulting mixture was stirred at 75 °C for 2 h, then cooled down to room temperature and extracted with EtOAc (1 L x 3). The combined organic layers were washed with H_2O (1 L x 3), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford methyl (2*S*)-3-(6-bromopyridin-2-yl)-2-[(*tert*-butoxycarbonyl) amino] propanoate (41 g, 67% yield) as oil. LCMS (ESI): m/z [M+H] calc'd for $C_{14}H_{19}BrN_2O_4$ 358.1; found 359.1.

Step 2. To a solution of 3-[(2M)-2-[2-[(1*S*)-1-methoxyethyl] pyridin-3-yl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-(2,2,2-trifluoroethyl) indol-3-yl]-2,2-dimethylpropan-1-ol (45.0 g, 82.35 mmol) in dioxane (400 mL) and H_2O (80 mL), were added potassium carbonate (28.45 g, 205.88 mmol), methyl (2*S*)-3-(6-bromopyridin-2-yl)-2-[(*tert*-butoxycarbonyl) amino] propanoate

(35.5 g, 98.8 mmol), Pd(dtbpf)Cl_2 (5.37 g, 8.24 mmol) at room temperature. The reaction mixture was stirred at 70 °C for 2 h under a nitrogen atmosphere. The resulting mixture was extracted with EtOAc (500 mL x 3). The combined organic layers were washed with H_2O (300 mL x 3), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography to afford methyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(6-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)pyridin-2-yl)propanoate (48 g, 83% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{37}H_{45}F_3N_4O_6$ 698.3; found 699.4.

Step 3. A solution of methyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(6-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)pyridin-2-yl)propanoate (52 g, 74.42 mmol) in THF (520 mL), was added LiOH (74.41 mL, 223.23 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 3 h. The resulting mixture was acidified to pH 5 with HCl (aq.) and extracted with EtOAc (1 L x 3). The combined organic layers were washed with H_2O (1 L x 3), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(6-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)pyridin-2-yl)propanoic acid (50 g, 98% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{36}H_{43}F_3N_4O_6$ 684.3; found 685.1.

Step 4. To a solution of (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(6-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)pyridin-2-yl)propanoic acid (55 g, 80.32 mmol) in DCM (600 mL), were added DIPEA (415.23 g, 3212.82 mmol), and HATU (45.81 g, 120.48 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 12 h and then quenched with H_2O. The resulting mixture was extracted with EtOAc (1 L x 3). The combined organic layers were washed with H_2O (1 L), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford methyl 1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(6-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)pyridin-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (63 g, 96% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{53}F_3N_6O_7$ 810.4; found 811.3.

Step 5. A solution of methyl 1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(6-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)pyridin-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (50 g, 61.66 mmol) in THF (500 mL) and 3M LiOH (61.66 mL, 184.980 mmol) at 0 °C was stirred at room temperature for 3 h, then acidified to pH 5 with HCl (aq.). The resulting mixture was extracted with EtOAc (800 mL x 3). The combined organic layers were washed with H_2O (800 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford 1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(6-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)pyridin-2-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (48 g, 97% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{51}F_3N_6O_7$ 796.3; found 797.1.

Step 6. To a solution of 1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(6-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)pyridin-2-

yl)propanoyl)hexahydropyridazine-3-carboxylic acid (50 g, 62.74 mmol) in DCM (10 L) at 0 °C, were added DIPEA (243.28 g, 1882.32 mmol), EDCI (360.84 g, 1882.32 mmol) and HOBT (84.78 g, 627.44 mmol). The reaction mixture was stirred at room temperature for 3 h, quenched with H_2O and concentrated under reduced pressure. The residue was extracted with EtOAc (2 L x 3). The combined organic layers were washed with H_2O (2 L x 3), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford *tert*-butyl ((4*S*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(2,6)-pyridinacycloundecaphane-4-yl)carbamate (43.6 g, 89% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{49}F_3N_6O_6$ 778.3; found 779.3.

Step 7. To a solution of *tert*-butyl ((4*S*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(2,6)-pyridinacycloundecaphane-4-yl)carbamate (300 mg) in DCM (10 mL), was added TFA (3 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. The resulting mixture was diluted with toluene (10 mL) and concentrated under reduced pressure three times to afford (4*S*)-4-amino-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(2,6)-pyridinacycloundecaphane-5,7-dione (280 mg, crude) as oil. LCMS (ESI): m/z [M+H] calc'd for $C_{36}H_{41}F_3N_6O_4$ 679.2; found 678.3.

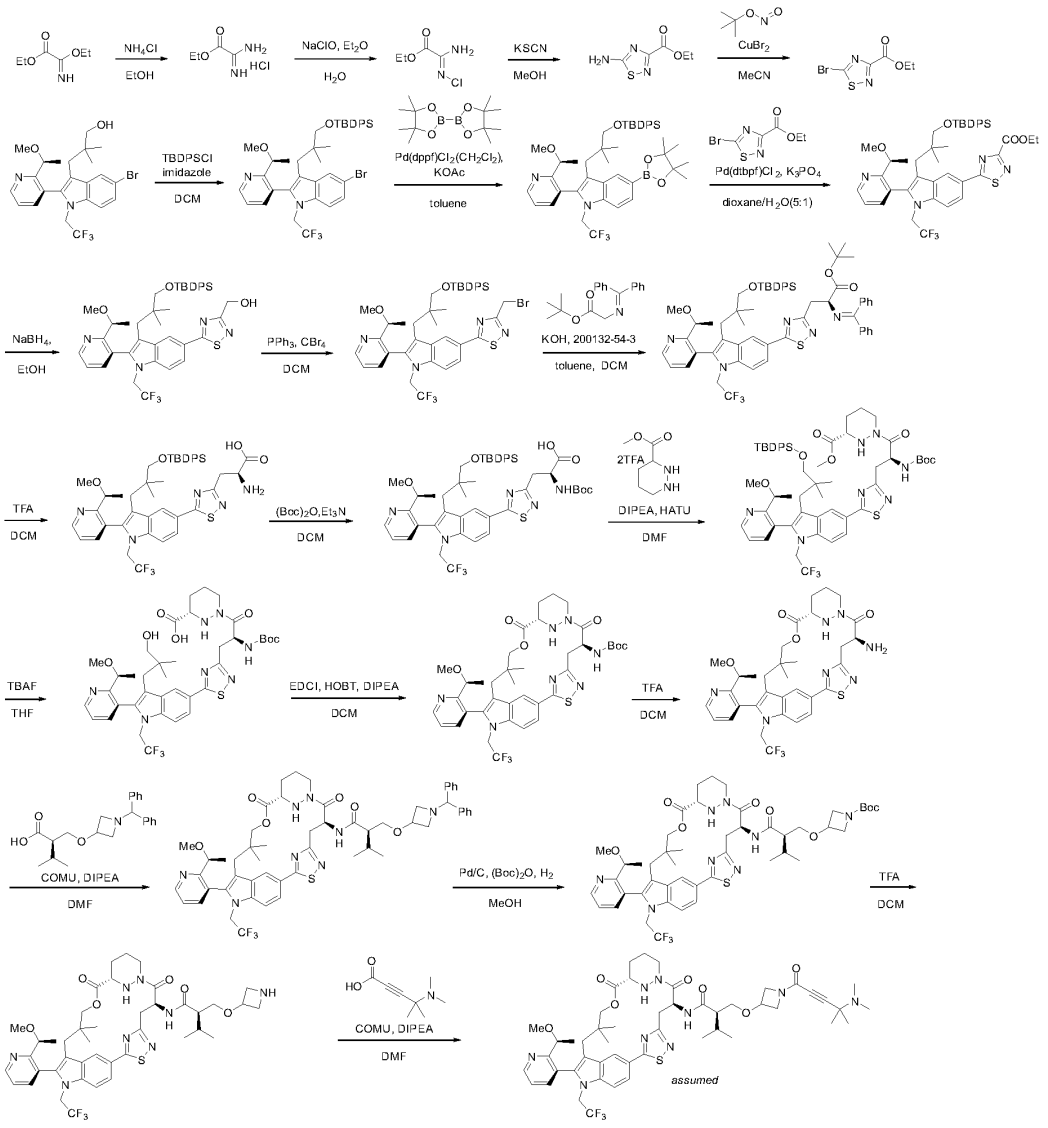
Step 8. To a solution of (4*S*)-4-amino-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(2,6)-pyridinacycloundecaphane-5,7-dione (140 mg, 0.21 mmol) in MeCN (2 mL), were added DIPEA (266.58 mg, 2.06 mmol), *N*-(4-(*tert*-butoxycarbonyl)-1-oxa-4,9-diazaspiro[5.5]undecane-9-carbonyl)-*N*-methyl-L-valine (127.94 mg, 0.31 mmol) and CIP (114.68 mg, 0.41 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. The resulting mixture was concentrated under reduced pressure. The residue was extracted with EtOAc (10 mL x 3). The combined organic layers were washed with H_2O (10 mL x 3), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography to afford *tert*-butyl 9-(((2*S*)-1-(((6^3*S*,4*S*)-1^2-(2-((*S*)-1-methoxyethyl) pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(2,6)-pyridinacycloundecaphane-4-yl) amino)-3-methyl-1-oxobutan-2-yl) (methyl)carbamoyl)-1-oxa-4,9-diazaspiro [5.5] undecane-4-carboxylate (170 mg, 76% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{56}H_{74}F_3N_9O_9$ 1073.5; found 1074.6.

Step 9. To a solution of *tert*-butyl 9-(((2*S*)-1-(((6^3*S*,4*S*)-1^2-(2-((*S*)-1-methoxyethyl) pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(2,6)-pyridinacycloundecaphane-4-yl) amino)-3-methyl-1-oxobutan-2-yl) (methyl)carbamoyl)-1-oxa-4,9-diazaspiro [5.5] undecane-4-carboxylate (160 mg, 0.15 mmol) in DCM (5 mL) at 0 °C, was dropwise added TFA (1.5 mL). The reaction mixture was stirred at 0 °C for 1 h. The resulting mixture was diluted with toluene (10 mL) and concentrated under reduced pressure three times to afford *N*-((2*S*)-1-(((6^3*S*,4*S*)-1^2-(2-((*S*)-1-methoxyethyl) pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-

6(1,3)-pyridazina-2(2,6)-pyridinacycloundecaphane-4-yl) amino)-3-methyl-1-oxobutan-2-yl)-*N*-methyl-1-oxa-4,9-diazaspiro [5.5] undecane-9-carboxamide (150 mg, crude) as oil. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{65}F_3N_9O_7$ 973.5; found 974.4.

Step 10. To a solution of *N*-((2*S*)-1-(((6^3*S*,4*S*)-1^2-(2-((*S*)-1-methoxyethyl) pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(2,6)-pyridinacycloundecaphane-4-yl) amino)-3-methyl-1-oxobutan-2-yl)-*N*-methyl-1-oxa-4,9-diazaspiro [5.5] undecane-9-carboxamide (150 mg, 0.15 mmol) in DMF (3 mL), were added DIPEA (199.01 mg, 1.54 mmol), acrylic acid (16.64 mg, 0.23 mmol) and COMU (98.39 mg, 0.23 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 1 h and concentrated under reduced pressure. The residue was extracted with EtOAc (10 mL x 3). The combined organic layers were washed with H_2O (10 mL x 3), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography and reverse phase chromatography to afford 4-acryloyl-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(2,6)-pyridinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methyl-1-oxa-4,9-diazaspiro[5.5]undecane-9-carboxamide (53mg, 33% yield) as solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.79 (dd, J = 4.8, 1.9 Hz, 2H), 8.09 (d, J = 31.4 Hz, 1H), 7.96 (d, J = 8.9 Hz, 1H), 7.90 – 7.72 (m, 3H), 7.64 (t, J = 7.8 Hz, 1H), 7.56 (dd, J = 7.8, 4.7 Hz, 1H), 7.03 (s, 1H), 6.86 (dd, J = 16.6, 10.4 Hz, 1H), 6.20 (d, J = 14.0 Hz, 1H), 5.73 (d, J = 12.2 Hz, 2H), 5.47 (s, 1H), 5.35 (d, J = 11.8 Hz, 1H), 4.68 (s, 1H), 4.28 (d, J = 12.5 Hz, 1H), 4.13 (d, J = 6.4 Hz, 1H), 3.92 (s, 1H), 3.84 – 3.64 (m, 6H), 3.59 (d, J = 14.0 Hz, 3H), 3.50 (s, 3H), 3.10 (s, 5H), 3.02 (d, J = 13.3 Hz, 2H), 2.85 (d, J = 12.1 Hz, 3H), 2.08 – 1.89 (m, 2H), 1.81 (s, 1H), 1.75 – 1.51 (m, 5H), 1.39 (d, J = 6.1 Hz, 4H), 1.24 (s, 0H), 0.91 – 0.66 (m, 10H), 0.53 (s, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{54}H_{68}F_3N_9O_8$ 1027.5; found 1028.1.

Example A590. The synthesis of (2*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-*N*-((6^3*S*,4*S*,*Z*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide



Step 1. To a mixture of ethyl 2-ethoxy-2-iminoacetate (25.0 g, 172.23 mmol) and EtOH (250.0 mL) at 0 °C was added ammonium chloride (9.21 g, 172.23 mmol) in portions then stirred for 4 h at room temperature under an argon atmosphere. The resulting mixture was concentrated under reduced pressure and washed with Et_2O (3 x 200 mL). The organic layers were combined and concentrated under reduced pressure. This resulted in ethyl 2-amino-2-iminoacetate hydrochloride (20g, crude) as a light yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_4H_8N_2O_2$ 117.07; found 116.9.

Step 2. To a mixture of ethyl 2-amino-2-iminoacetate hydrochloride (13.30 g, 87.17 mmol), H_2O (50.0 mL) and Et_2O (100.0 mL) at 0 °C was added sodium hypochlorite pentahydrate (7.79 g, 104.60 mmol) dropwise. The resulting mixture was stirred for 3 h under an argon atmosphere. The mixture was extracted with Et_2O (3x 200 mL). The resulting solution was washed with brine (3 x 100 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford ethyl (*Z*)-2-amino-2-(chloroimino) acetate (7 g, crude) as a light yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_4H_7ClN_2O_2$ 151.03; found 150.8.

Step 3. To a solution of ethyl (*Z*)-2-amino-2-(chloroimino) acetate (8.40 g, 55.792 mmol) and MeOH (130.0 mL) at 0 °C was added potassium thiocyanate (5.42 g, 55.79 mmol) in portions. The resulting mixture was stirred for 4 h at room temperature under an argon atmosphere. The reaction was quenched with H_2O/Ice. The mixture was extracted with EtOAc (5 x 100 mL). The resulting organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford ethyl 5-amino-1, 2, 4-thiadiazole-3-carboxylate (2.3 g, 23.8% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_5H_7N_3O_2S$ 174.03; found 173.8.

Step 4. To a solution of ethyl 5-amino-1, 2, 4-thiadiazole-3-carboxylate (5.80 g, 33.49 mmol), MeCN (90.0 mL) and $CuBr_2$ (11.22 g, 50.23 mmol) at 0 °C was added 2-methyl-2-propylnitrit (6.91 g, 66.98 mmol) dropwise under an argon atmosphere. The mixture was stirred for 30 min. The mixture was then stirred for 4 h at 50 °C. The mixture was cooled to 0 °C and quenched with H_2O/Ice. The mixture was extracted with EtOAc (3x100 mL). The resulting organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography to afford ethyl 5-bromo-1, 2, 4-thiadiazole-3-carboxylate (6.2 g, 78.1% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_5H_5BrN_2O_2S$ 236.93; found 237.1.

Step 5. To a solution of (*S*)-3-(5-bromo-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (16.60 g, 33.24 mmol), DCM (170.0 mL) and imidazole (5.66 g, 83.10 mmol) at 0 °C was added *tert*-butyl-chlorodiphenylsilane (11.88 g, 43.21 mmol) dropwise. The resulting mixture was stirred for 3 h at room temperature under an argon atmosphere. The reaction was quenched with H_2O/Ice. The mixture was extracted with EtOAc (3x 200 mL). The organic layer was washed with brine (3x100 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford (*S*)-5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indole (22 g, 89.7% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{44}BrF_3N_2O_2Si$ 737.24; found 737.0.

Step 6. To a solution of (*S*)-5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indole (28.0 g, 37.95 mmol), toluene (270.0 mL), KOAc (9.31 g, 94.88 mmol) and bis(pinacolato)diboron (19.27 g, 75.90 mmol) at 0 °C was added $Pd(dppf)Cl_2.CH_2Cl_2$ (6.18 g, 7.59 mmol) in portions. The resulting mixture was stirred for 3 h at 90 °C under an argon atmosphere. The mixture was cooled to room temperature and quenched with H_2O/Ice. The mixture was extracted with EtOAc (3 x 200 mL). The resulting organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford (*S*)-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-

dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indole (28.2 g, 94.7% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{45}H_{56}BF_3N_2O_4Si$ 785.41; found 785.4.

Step 7. To a solution of (*S*)-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indole (19.60 g, 24.97 mmol), 1,4-dioxane (200 mL), H_2O (40 mL), ethyl 5-bromo-1, 2, 4-thiadiazole-3-carboxylate (5.92 g, 24.97 mmol) and K_3PO_4 (13.25 g, 62.43 mmol) at 0 °C was added Pd(dtbpf)Cl_2 (1.63 g, 2.50 mmol) in portions. The resulting mixture was stirred for 1.5 h at 75 °C under an argon atmosphere. The mixture was cooled to 0 °C and quenched with H_2O/Ice and extracted with EtOAc (3x200 mL). The resulting organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford ethyl (*S*)-5-(3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)-1,2,4-thiadiazole-3-carboxylate (14 g, 68.8% yield) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{49}F_3N_4O_4SSi$ 815.33; found 815.2.

Step 8. To a solution of ethyl (*S*)-5-(3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)-1,2,4-thiadiazole-3-carboxylate (13.60 g, 16.69 mmol) and EtOH (140.0 mL) at 0 °C was added $NaBH_4$ (3.16 g, 83.43 mmol) in portions. The resulting mixture was stirred for 3 h then quenched with H_2O/Ice. The resulting mixture was washed with brine (3 x 100 mL) and extracted with EtOAc (3 x 200 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford (*S*)-5-(3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)-1,2,4-thiadiazol-3-ol (9.7 g, 75.2% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{47}F_3N_4O_3SSi$ 773.32; found 773.3.

Step 9. To a solution of (*S*)-5-(3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)-1,2,4-thiadiazol-3-ol (9.70 g, 12.55 mmol), DCM (100.0 mL) and CBr_4 (8.32 g, 25.10 mmol) at 0 °C was added PPh_3 (6.58 g, 25.10 mmol) in DCM (20.0 mL) dropwise. The resulting mixture was stirred for 2 h under an argon atmosphere then quenched with H_2O/Ice. The mixture was extracted with DCM (3 x 200 mL). The resulting organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by reverse flash chromatography to afford (*S*)-3-(bromomethyl)-5-(3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)-1,2,4-thiadiazole (9.5 g, 90.6% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{46}BrF_3N_4O_2SSi$ 835.23; found 834.9.

Step 10. To a stirred solution of (*S*)-3-(bromomethyl)-5-(3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)-1,2,4-thiadiazole (9.40 g, 11.25 mmol), toluene (84.0 mL), DCM (36.0 mL), *tert*-butyl 2-[(diphenylmethylidene)amino]acetate (3.32 g, 11.25 mmol) and O-Allyl-*N*-(9-anthracenylmethyl)cinchonidinium bromide (0.68 g, 1.13 mmol) at 0 °C was added 9M KOH aqueous (94.0 mL) dropwise. The resulting mixture was stirred overnight under an argon

atmosphere. The mixture was extracted with EtOAc (3 x 200 mL) and the organic phase was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford *tert*-butyl (*S*)-3-(5-(3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)-1,2,4-thiadiazol-3-yl)-2-((diphenylmethylene)amino)propanoate (9 g, 76.2% yield)as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{61}H_{66}F_3N_5O_4SSi$ 1050.46; found 1050.8.

Step 11. To a solution of *tert*-butyl (*S*)-3-(5-(3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)-1,2,4-thiadiazol-3-yl)-2-((diphenylmethylene)amino)propanoate (8.0 g, 7.62 mmol) and DCM (40.0 mL) at 0 °C solution was added TFA (40.0 mL) dropwise. The resulting mixture was stirred overnight at room temperature then concentrated under reduced pressure. The residue was basified to pH 8 with $NaHCO_3$. The mixture was extracted with EtOAc (3 x 200 mL). The organic phase was concentrated under reduced pressure. The residue was purified by reverse flash chromatography to afford (*S*)-2-amino-3-(5-(3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)-1,2,4-thiadiazol-3-yl)propanoic acid (5 g, 79.1% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{50}F_3N_5O_4SSi$ 830.34; found 830.2.

Step 12. To a solution of (*S*)-2-amino-3-(5-(3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)-1,2,4-thiadiazol-3-yl)propanoic acid (4.70 g, 5.66 mmol), DCM (50.0 mL) and Et_3N (2.86 g, 28.31 mmol) at 0 °C was added $(Boc)_2O$ (1.36 g, 6.23 mmol) dropwise. The resulting mixture was stirred for 3 h at room temperature under an argon atmosphere then concentrated under reduced pressure and purified by reverse flash chromatography to afford (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(5-(3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)-1,2,4-thiadiazol-3-yl)propanoic acid (5 g, 94.9% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{49}H_{58}F_3N_5O_6SSi$ 930.39; found 930.3.

Step 13. To a mixture of (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(5-(3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)-1,2,4-thiadiazol-3-yl)propanoic acid (5.30 g, 5.70 mmol), DMF (60.0 mL), methyl 1,2-diazinane-3-carboxylate (1.64 g, 11.40 mmol) and DIPEA (22.09 g, 170.94 mmol) at 0 °C was added HATU (2.82 g, 7.41 mmol) in DMF (5 mL) dropwise. The resulting mixture was stirred for 3 h at room temperature under an argon atmosphere. The reaction was then quenched with H_2O/Ice. The mixture was extracted with EtOAc (3 x 100 mL) and the organic phase was washed with brine (3 x 100 mL). The resulting mixture was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(5-(3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)-1,2,4-thiadiazol-3-yl)propanoyl)hexahydropyridazine-3-carboxylate (5.6 g) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{55}H_{68}F_3N_7O_7SSi$ 1056.47; found 1056.2.

Step 14. A mixture of methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(5-(3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-

trifluoroethyl)-1*H*-indol-5-yl)-1,2,4-thiadiazol-3-yl)propanoyl)hexahydropyridazine-3-carboxylate (5.60 g, 5.30 mmol) and TBAF in THF (56.0 mL) was stirred overnight at 40 °C under an argon atmosphere. The reaction was quenched with sat. NH_4Cl (aq.). The mixture was extracted with EtOAc (3 x 100 mL) and the organic phase was concentrated under reduced pressure. The residue was purified by reverse flash chromatography to afford (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(5-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)-1,2,4-thiadiazol-3-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (4.1 g, 96.2% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{48}F_3N_7O_7S$ 804.34; found 804.3.

Step 15. To a solution of (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(5-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1*H*-indol-5-yl)-1,2,4-thiadiazol-3-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (4.0 g, 5.0 mmol) and DCM (450.0 mL) at 0 °C were added DIPEA (51.45 g, 398.08 mmol), HOBt (6.72 g, 49.76 mmol) and EDCI (57.23 g, 298.55 mmol) in portions. The resulting mixture was stirred for 16 h at room temperature under an argon atmosphere. The reaction was quenched with H_2O/Ice and extracted with EtOAc (3 x 30 mL). The organic phase was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (1.7 g, 43.5% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{46}F_3N_7O_6S$ 786.33; found 786.3.

Step 16. To a solution of ((6^3*S*,4*S*,*Z*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (300.0 mg, 0.38 mmol) and DCM (2.0 mL) at 0 °C was added TFA (1.0 mL) dropwise. The resulting mixture was stirred for 2 h at room temperature then concentrated under reduced pressure. The residue was basified to pH 8 with saturated $NaHCO_3$ (aq.). The mixture was extracted with EtOAc (3 x 20 mL). The organic phase was concentrated under reduced pressure to afford (6^3*S*,4*S*,*Z*)-4-amino-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (270 mg, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{33}H_{38}F_3N_7O_4S$ 686.27; found 686.1.

Step 17. To a solution of (6^3*S*,4*S*,*Z*)-4-amino-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (160.0 mg, 0.23 mmol), (*R*)-2-(((1-benzhydrylazetidin-3-yl)oxy)methyl)-3-methylbutanoic acid (123.70 mg, 0.35 mmol) and DMF (2.0 mL) at 0 °C were added DIPEA (603.09 mg, 4.660 mmol) and COMU (119.91 mg, 0.28 mmol) in DMF (0.5 mL). The resulting mixture was stirred for 2 h at room temperature under an argon atmosphere. The reaction was quenched with H_2O/Ice and extracted with EtOAc (3 x 20 mL). The organic phase was washed with brine (3 x 10 mL) and concentrated under reduced pressure. The residue was purified by Prep-TLC to afford (2*R*)-2-(((1-benzhydrylazetidin-3-yl)oxy)methyl)-*N*-((6^3*S*,4*S*,*Z*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-

pyridazinacycloundecaphane-4-yl)-3-methylbutanamide (160 mg, 67.2% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{55}H_{63}F_3N_8O_6S$ 1021.46; found 1021.4.

Step 18. To a solution of (2*R*)-2-(((1-benzhydrylazetidin-3-yl)oxy)methyl)-*N*-((6^3*S*,4*S*,*Z*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide (160.0 mg, 0.16 mmol) and MeOH (5.0 mL) at 0 °C was added $(Boc)_2O$ (85.49 mg, 0.39 mmol) dropwise followed by Pd/C (320.0 mg) in portions. The resulting mixture was stirred overnight at room temperature under a hydrogen atmosphere. The resulting mixture was filtered and the filter cake was washed with EtOAc (3 x 20 mL). The filtrate was concentrated under reduced pressure. The residue was purified by Prep-TLC to afford *tert*-butyl 3-((2*R*)-2-(((6^3*S*,4*S*,*Z*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamoyl)-3-methylbutoxy)azetidine-1-carboxylate (80 mg, 53.5% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{47}H_{61}F_3N_8O_8S$ 955.44; found 955.2.

Step 19. To a solution of *tert*-butyl 3-((2*R*)-2-(((6^3*S*,4*S*,*Z*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamoyl)-3-methylbutoxy)azetidine-1-carboxylate (120.0 mg, 0.13 mmol) and DCM (0.80 mL) at 0 °C was added TFA (0.4 mL) dropwise and the resulting mixture was stirred for 2 h at room temperature. The mixture was basified to pH 8 with saturated $NaHCO_3$ (aq.). The mixture was extracted with EtOAc (3 x 10 mL) and concentrated under reduced pressure. The residue was purified by reverse flash chromatography to afford (2*R*)-2-((azetidin-3-yloxy)methyl)-*N*-((6^3*S*,4*S*,*Z*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide (40 mg, 37.2% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{53}F_3N_8O_6S$ 855.38; found 855.3.

Step 20. To a solution of (2*R*)-2-((azetidin-3-yloxy)methyl)-*N*-((6^3*S*,4*S*,*Z*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide (32.0 mg, 0.037 mmol),4-(dimethylamino)-4-methylpent-2-ynoic acid (11.62 mg, 0.074 mmol) and DMF (0.50 mL) at 0 °C were added DIPEA (193.49 mg, 1.48 mmol) and COMU (19.23 mg, 0.044 mmol) in DMF (0.1 mL) dropwise. The resulting mixture was stirred for 2 h at room temperature under an argon atmosphere. The reaction was quenched with H_2O/Ice and extracted with EtOAc (3 x 20 mL). The organic phase was washed with brine (3 x 10 mL) and concentrated under reduced pressure. The crude product (60 mg) was purified by reverse phase chromatography to afford (2*R*)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-*N*-((6^3*S*,4*S*,*Z*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide (11.7 mg, 30.9% yield) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.80 (dd, *J* = 4.7, 1.8 Hz, 1H), 8.58 (s, 1H), 8.30 (d, *J* = 8.9 Hz, 1H), 7.92 (d, *J* = 8.6 Hz, 1H), 7.84-7.69 (m, 2H), 7.56 (dd, *J* = 7.8, 4.8 Hz, 1H), 5.78 (t, *J* = 8.6 Hz, 2H), 5.10

(d, *J* = 12.1 Hz, 1H), 4.91 (dd, *J* = 16.9, 8.8 Hz, 1H), 4.31 (d, *J* = 6.6 Hz, 6H), 4.05 (dd, *J* = 16.3, 6.6 Hz, 2H), 3.87 (d, *J* = 6.3 Hz, 1H), 3.75 (d, *J* = 11.3 Hz, 1H), 3.61 (d, *J* = 11.0 Hz, 1H), 3.53 (d, *J* = 9.8 Hz, 1H), 3.45 (s, 3H), 3.25 (s, 1H), 3.09 (d, *J* = 10.4 Hz, 1H), 3.01 (d, *J* = 14.5 Hz, 1H), 2.79 (s, 1H), 2.45-2.35 (m, 2H), 2.20 (d, *J* = 5.4 Hz, 6H), 2.15 (d, *J* = 12.0 Hz, 1H), 1.81 (d, 2H), 1.74-1.65 (m, 1H), 1.54 (s, 1H), 1.41-1.30 (m, 9H), 1.24 (s, 1H), 0.94 (s, 3H), 0.90-0.81 (m, 5H), 0.29 (s, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{64}F_3N_9O_7S$ 992.47; found 992.5.

The following table of compounds (Table 3) were prepared using the aforementioned methods or variations thereof, as is known to those of skill in the art.

Table 3: Exemplary Compounds Prepared by Methods of the Present Invention

Ex#	LCMS (ESI): m/z [M+H] Found	Ex#	LCMS (ESI): m/z [M+H] Found
A1	944.5	**A116**	986.5
A2	888.6	**A117**	879.5
A3	903.2	**A118**	881.6
A4	876.3	**A119**	874.5
A5	903.4	**A120**	929.7
A6	945.8	**A121**	943.6
A7	1058.1	**A122**	888.8
A8	960.8	**A123**	959.3
A9	921.7	**A124**	924.6
A10	928.8	**A125**	962.5
A11	936.4	**A126**	986.5
A12	942.5	**A127**	946.6
A13	932.5	**A128**	903.4
A14	917.5	**A129**	1001.2
A15	961.5	**A130**	930.5
A16	890.6	**A131**	959.5
A17	875.4	**A132**	940.5
A18	918.5	**A133**	969.7
A19	975.6	**A134**	990.5
A20	934.5	**A135**	933.5
A21	903.3	**A136**	875.4
A22	903.6	**A137**	889.5
A23	844.6	**A138**	929.4
A24	887.6	**A139**	930.4
A25	921.4	**A140**	943.7
A26	930.4	**A141**	943.8
A27	915.6	**A142**	901.7
A28	890.6	**A143**	852.3
A29	903.5	**A144**	886.7
A30	902.7	**A145**	916.7
A31	901.4	**A146**	963.4
A32	1003.4	**A147**	864.4

Ex#	LCMS (ESI): m/z [M+H] Found	Ex#	LCMS (ESI): m/z [M+H] Found
A33	917.4	**A148**	885.5
A34	942.7	**A149**	947.5
A35	956.1	**A150**	902.7
A36	1005.5	**A151**	904.5
A37	917.5	**A152**	885.5
A38	959.5	**A153**	949.6
A39	942.9	**A154**	907.5
A40	915.7	**A155**	944.5
A41	917.5	**A156**	943.7
A42	888.6	**A157**	943.5
A43	942.5	**A158**	904.5
A44	904.35	**A159**	904.5
A45	846.35	**A160**	858.5
A46	982.5	**A161**	947.4
A47	960.1	**A162**	973.5
A48	905.3	**A163**	989.4
A49	981.8	**A164**	947.5
A50	916.9	**A165**	960.3
A51	946.7	**A166**	907.7
A52	916.5	**A167**	918.5
A53	921.6	**A168**	907.4
A54	947.7	**A169**	859.4
A55	916.5	**A170**	874.3
A56	904.5	**A171**	867.5
A57	930.5	**A172**	883.6
A58	895.5	**A173**	900.5
A59	944.7	**A174**	848.4
A60	931.5	**A175**	959.5
A61	847.7	**A176**	944.5
A62	871.5	**A177**	935.4
A63	930.4	**A178**	930.4
A64	893.7	**A179**	849.5
A65	874.7	**A180**	849.5
A66	876.5	**A181**	918.8
A67	970.5	**A182**	1002.2
A68	915.4	**A183**	961.5
A69	927.3	**A184**	1003.5
A70	876.4	**A185**	928.5
A71	933.5	**A186**	992.5
A72	1003.5	**A187**	945.5
A73	903.6	**A188**	960.4
A74	885.5	**A189**	1000.6
A75	904.7	**A190**	934.3
A76	904.5	**A191**	985.5

Ex#	LCMS (ESI): m/z [M+H] Found	Ex#	LCMS (ESI): m/z [M+H] Found
A77	860.6	**A192**	971.5
A78	1018.5	**A193**	871.6
A79	947.5	**A194**	918.5
A80	933.3	**A195**	927.4
A81	917.8	**A196**	972.3
A82	908.7	**A197**	896.4
A83	890.6	**A198**	916.6
A84	890.6	**A199**	916.6
A85	891.5	**A200**	987.4
A86	885.5	**A201**	993.7
A87	947.7	**A202**	896.4
A88	945.5	**A203**	987.6
A89	944.8	**A204**	987.6
A90	898	**A205**	933.5
A91	890.5	**A206**	930.5
A92	903.4	**A207**	930.5
A93	901.4	**A208**	958.8
A94	881.5	**A209**	1015.7
A95	987.8	**A210**	906.5
A96	945.7	**A211**	905.5
A97	931.8	**A212**	901.3
A98	943.8	**A213**	969.5
A99	849.7	**A214**	933.6
A100	942.8	**A215**	890.3
A101	893.4	**A216**	916.4
A102	904.4	**A217**	930.5
A103	947.5	**A218**	983.5
A104	931.7	**A219**	917.4
A105	915.6	**A220**	996.5
A106	902.5	**A221**	915.4
A107	945.7	**A222**	930.05
A108	902.3	**A223**	920.01
A109	906.5	**A224**	983.5
A110	793.4	**A225**	982.7
A111	946.7	**A226**	873.7
A112	905.14	**A227**	887.7
A113	849.6	**A228**	911
A114	888.4	**A229**	896.7
A115	888.4		
A230	905.5	**A286**	877.4
A231	912.4	**A287**	894.7
A232	849.4	**A288**	879.3
A233	905.4	**A289**	908.7

Ex#	LCMS (ESI): m/z [M+H] Found	Ex#	LCMS (ESI): m/z [M+H] Found
A234	1053.3	**A290**	971.6
A235	835.5	**A291**	978.5
A236	882.3	**A292**	987.5
A237	1015.4	**A293**	921.4
A238	971.5	**A294**	902.8
A239	869.4	**A295**	916.4
A240	884.4	**A296**	930.8
A241	987.4	**A297**	938.4
A242	877.4	**A298**	952.6
A243	863.5	**A299**	966.7
A244	1029.6	**A300**	919.6
A245	1029.5	**A301**	907.7
A246	501.4	**A302**	895.8
A247	985.5	**A303**	895.4
A248	896.4	**A304**	897.5
A249	984.5	**A305**	968.6
A250	927.4	**A306**	968.7
A251	985.4	**A307**	952.6
A252	927.3	**A308**	952.7
A253	990.4	**A309**	933.6
A254	933.3	**A310**	926.8
A255	930.7	**A311**	944.7
A256	890.4	**A312**	897.5
A257	947.4	**A313**	976.5
A258	990.3	**A314**	893.3
A259	933.3	**A316**	930.5
A260	918.4	**A317**	982.6
A261	975.5	**A318**	883.5
A262	919.4	**A319**	916.5
A263	976.5	**A320**	886.6
A264	990.5	**A321**	900.6
A265	933.5	**A322**	1004.4
A266	945.5	**A323**	976.5
A267	987.5	**A324**	969.5
A268	973.5	**A325**	1033.7
A269	487.3	**A326**	997.5
A270	959.4	**A327**	906.6
A271	861.3	**A328**	986.7
A272	847.6	**A329**	890.5
A273	861.6	**A330**	895.6
A274	930.5	**A331**	879.5
A275	916.3	**A332**	942.5
A276	892.8	**A333**	919.6
A277	895.7	**A334**	930.7

Ex#	LCMS (ESI): m/z [M+H] Found	Ex#	LCMS (ESI): m/z [M+H] Found
A278	879.5	**A335**	924.5
A279	895.4	**A336**	927.4
A280	954.6	**A337**	501.4
A281	916.6	**A338**	919.4
A282	952.5	**A339**	971.6
A283	931.4	**A340**	895.7
A284	889.5	**A341**	938.5
A285	839.4	**A342**	906.8

Ex#	LCMS (ESI): m/z [M+H] Found	Ex#	LCMS (ESI): m/z [M+H] Found
A343	992.4	**A370**	1087.3
A344	901.40	**A371**	902.5
A345	897.40	**A372**	992.1
A346	942.50	**A373**	902.6
A347	896.30	**A374**	1,034.40
A348	1,002.30	**A375**	993.50
A349	924.6	**A376**	984.40
A350	968.2	**A377**	939.50
A351	997.6	**A378**	897.50
A352	952.7	**A379**	1005.6
A353	966.3	**A380**	949.4
A354	966.2	**A381**	921.5
A355	855.6	**A382**	938.5
A356	910.4	**A383**	938.5
A357	1091.0	**A384**	924.5
A358	902.5	**A385**	938.3
A359	904.7	**A386**	938.4
A360	924.5	**A387**	938.5
A361	967.5	**A388**	938.5
A362	936.6	**A389**	936.5
A363	961.4	**A390**	924.5
A364	946.5	**A391**	1011.6
A365	995.6	**A392**	958.0
A366	921.5	**A393**	922.4
A367	950.6	**A394**	950.3
A368	924.5	**A395**	936.5
A369	924.5	**A396**	924.4
A397	924.7	**A424**	934.6
A398	914.6	**A425**	1012.5
A399	902.5	**A426**	912.5
A400	956.6	**A427**	895.30
A401	955.9	**A428**	951.30
A402	940.8	**A429**	911.50
A403	948.40	**A430**	938.5
A404	980.40	**A431**	952.3
A405	911.50	**A432**	924.2
A406	938.7	**A433**	924.2
A407	922.5	**A434**	924.7
A408	922.5	**A435**	910.5
A409	922.6	**A436**	924.6
A410	1046.9	**A437**	912.5
A411	990.7	**A438**	898.5
A412	912.7	**A439**	1016.6
A413	914.7	**A440**	990.5

Ex#	LCMS (ESI): m/z [M+H] Found	Ex#	LCMS (ESI): m/z [M+H] Found
A414	983.5	**A441**	939.6
A415	950.5	**A442**	889.2
A416	924.6	**A443**	913.5
A417	936.7	**A444**	913.5
A418	938.5	**A445**	890.1
A419	922.3	**A446**	957.6
A420	936.7	**A447**	880.5
A421	924.5	**A448**	888.4
A422	910.5	**A449**	920.5
A423	1028.9	**A450**	897.2
A451	902.6	**A478**	900.5
A452	888.4	**A479**	993.6
A453	901.3	**A480**	902.2
A454	1044.6	**A481**	930.6
A455	949.2	**A482**	974.6
A456	1059.5	**A483**	847.5
A457	916.7	**A484**	847.4
A458	916.2	**A485**	938.4
A459	902.3	**A486**	893.5
A460	874.5	**A487**	900.6
A461	957.9	**A488**	888.5
A462	854.3	**A489**	962.6
A463	910.7	**A490**	896.5
A464	964.5	**A491**	910.2
A465	964.5	**A492**	910.5
A466	1033.7	**A493**	910.5
A467	874.5	**A494**	886.3
A468	879.6	**A495**	907.5
A469	902.6	**A496**	900.4
A470	900.6	**A497**	902.7
A471	900.6	**A498**	902.6
A472	964.6	**A499**	998.7
A473	854.3	**A500**	897.5
A474	914.4	**A501**	906.3
A475	914.40	**A502**	949.2
A476	983.6	**A503**	920.6
A477	907.5	**A504**	920.8
A505	920.0	**A532**	1013.6
A506	879.3	**A533**	999.3
A507	897.4	**A534**	983.4
A508	942.4	**A535**	919.5
A509	942.4	**A536**	961.7
A510	899.3	**A537**	961.6
A511	919.5	**A532**	1013.6

Ex#	LCMS (ESI): m/z [M+H] Found	Ex#	LCMS (ESI): m/z [M+H] Found
A512	900.5	**A533**	999.3
A513	983.7	**A534**	983.4
A514	983.7	**A535**	919.5
A515	985.7	**A536**	961.7
A516	985.7	**A537**	961.6
A517	892.6	**A538**	999.7
A518	920.6	**A539**	960.4
A519	920.4	**A540**	1050.2
A520	990.6	**A541**	1078.7
A521	990.6	**A542**	1155.7
A522	945.3	**A543**	952.6
A523	945.4	**A544**	952.6
A524	914.5	**A545**	981.2
A525	914.4	**A546**	922.3
A526	1005.4	**A547**	922.5
A527	972.4	**A548**	950.5
A528	985.4	**A549**	997.4
A529	987.4	**A550**	1025.6
A530	898.5	**A551**	997.5
A531	912.3	**A552**	950.4
A553	974.6	**A580**	966.6
A554	957.5	**A581**	966.6
A555	1054.4	**A582**	953.5
A556	972.5	**A583**	938.5
A557	952.4	**A584**	944.7
A558	959.6	**A585**	944.6
A559	1011.7	**A586**	950.6
A560	1011.6	**A587**	962.6
A561	922.5	**A588**	938.5
A562	954.3	**A589**	1016.6
A563	978.5	**A590**	992.5
A564	982.5	**A591**	1008.7
A565	1039.3	**A592**	997.5
A566	1039.4	**A593**	1020.5
A567	1039.4	**A594**	1020.6
A568	1039.3	**A595**	1000.6
A569	997.4	**A596**	966.7
A570	974.7	**A597**	946.6
A571	1038.5	**A598**	979.6
A572	1056.5	**A599**	958.7
A573	1063.5	**A600**	887.4
A574	990.6	**A601**	985.5
A575	1004.5	**A602**	1036.5
A576	991.5	**A603**	989.9

Ex#	LCMS (ESI): m/z [M+H] Found	Ex#	LCMS (ESI): m/z [M+H] Found
A577	1062.3	**A604**	1006.1
A578	1048.3	**A605**	1059.7
A579	963.3	**A606**	1032.6
A607	1028.1	**A634**	938.6
A608	1027.1	**A635**	978.6
A609	966.6	**A636**	916.6
A610	945.6	**A637**	909.5
A611	1021.5	**A638**	909.5
A612	920.6	**A639**	928.3
A613	956.1	**A640**	942.0
A614	992.5	**A641**	942.6
A615	1010.5	**A642**	1047.5
A616	1010.5	**A643**	966.6
A617	990.5	**A644**	944.4
A618	950.7	**A645**	986.5
A619	950.4	**A646**	937.5
A620	938.1	**A647**	1012.5
A621	966.1	**A648**	944.5
A622	982.6	**A649**	921.6
A623	999.6	**A650**	1007.6
A624	1019.5	**A651**	964.2
A625	1019.5	**A652**	1903.1
A626	913.5	**A653**	938.6
A627	913.4	**A654**	938.6
A628	1046.5	**A655**	934.4
A629	952.2	**A656**	1005.5
A630	967.6	**A657**	960.7
A631	942.5	**A658**	1003.3
A632	942.6	**A659**	974.6
A633	938.6	**A660**	925.5
A661	936.6	**A688**	936.5
A662	936.6	**A689**	1036.9
A663	935.5	**A690**	979.6
A664	1047.5	**A691**	923.5
A665	1035.5	**A692**	962.5
A666	1035.2	**A693**	906.5
A667	936.3	**A694**	896.1
A668	965.6	**A695**	980.7
A669	1049.7	**A696**	978.4
A670	1047.7	**A697**	978.3
A671	964.6	**A698**	964.3
A672	997.9	**A699**	1003.3
A673	1015.3	**A700**	927.2
A674	975.5	**A701**	993.7

Ex#	LCMS (ESI): m/z [M+H] Found	Ex#	LCMS (ESI): m/z [M+H] Found
A675	921.5	**A702**	950.6
A676	966.5	**A703**	985.5
A677	966.4	**A704**	938.6
A678	950.5	**A705**	925.5
A679	990.2	**A706**	952.5
A680	943.6	**A707**	1016.5
A681	980.6	**A708**	903.2
A682	968.6	**A709**	981.5
A683	1000.6	**A710**	967.5
A684	915.0	**A711**	964.5
A685	977.6	**A712**	937.9
A686	922.5	**A713**	939.9
A687	936.4	**A714**	952.5
A715	937.4	**A733**	997.6
A716	893.5	**A734**	936.5
A717	924.6	**A735**	952.5
A718	936.6	**A736**	922.2
A719	950.5	**A737**	922.1
A720	935.3	**A738**	924.5
A721	937.3	**A739**	924.5
A722	929.4	**A730**	993.2
A723	910.4	**A731**	922.7
A724	985.4	**A732**	928.6
A725	919.3	**A733**	997.6
A726	1010.4	**A734**	936.5
A727	1025.5	**A735**	952.5
A728	924.6	**A736**	922.2
A729	993.5	**A737**	922.1
A730	993.2	**A738**	924.5
A731	922.7	**A739**	924.5
A732	928.6	**A740**	895.5
A741	895.4		

Blank = not determined

Matched Pair Analysis

FIGs. 1A-1B compare the potency in two different cell-based assays of compounds of Formula BB of the present invention (points on the right) and corresponding compounds of Formula AA (points on the left) wherein a H is replaced with (*S*)Me. The y axes represent pERK EC50 (FIG. 1A) or CTG IC50 (FIG. 1B) as measured in an H358 cell line. Assay protocols are below. The linked points represent a matched pair that differs only between H and (*S*)Me substitution. Each

compound of Formula BB demonstrated reduced potency in cell assays compared to the corresponding compound of Formula AA.

Biological Assays

Potency assay: pERK

The purpose of this assay is to measure the ability of test compounds to inhibit K-Ras in cells. Activated K-Ras induces increased phosphorylation of ERK at Threonine 202 and Tyrosine 204 (pERK). This procedure measures a decrease in cellular pERK in response to test compounds. The procedure described below in NCI-H358 cells is applicable to K-Ras G12C.

Note: This protocol may be executed substituting other cell lines to characterize inhibitors of other RAS variants, including, for example, AsPC-1 (K-Ras G12D), Capan-1 (K-Ras G12V), or NCI-H1355 (K-Ras G13C).

NCI-H358 cells were grown and maintained using media and procedures recommended by the ATCC. On the day prior to compound addition, cells were plated in 384-well cell culture plates (40 µl/well) and grown overnight in a 37°C, 5% CO2 incubator. Test compounds were prepared in 10, 3-fold dilutions in DMSO, with a high concentration of 10 mM. On the day of assay, 40 nL of test compound was added to each well of cell culture plate using an Echo550 liquid handler (LabCyte®). Concentrations of test compound were tested in duplicate. After compound addition, cells were incubated 4 hours at 37°C, 5% CO2. Following incubation, culture medium was removed and cells were washed once with phosphate buffered saline.

In some experiments, cellular pERK level was determined using the AlphaLISA SureFire Ultra p-ERK1/2 Assay Kit (PerkinElmer). Cells were lysed in 25 µL lysis buffer, with shaking at 600 RPM at room temperature. Lysate (10 µL) was transferred to a 384-well Opti-plate (PerkinElmer) and 5 µL acceptor mix was added. After a 2-hour incubation in the dark, 5 µL donor mix was added, plate was sealed, and incubated 2 hours at room temperature. Signal was read on an Envision plate reader (PerkinElmer) using standard AlphaLISA settings. Analysis of raw data was carried out in Excel (Microsoft) and Prism (GraphPad). Signal was plotted vs. the decadal logarithm of compound concentration, and IC_{50} was determined by fitting a 4-parameter sigmoidal concentration response model.

In other experiments, cellular pERK was determined by In-Cell Western. Following compound treatment, cells were washed twice with 200 µL tris buffered saline (TBS) and fixed for 15 minutes with 150 µL 4% paraformaldehyde in TBS. Fixed cells were washed 4 times for 5 minutes with TBS containing 0.1% Triton X-100 (TBST) and then blocked with 100 µL Odyssey blocking buffer (LI-COR) for 60 minutes at room temperature. Primary antibody (pERK, CST-4370, Cell Signaling Technology) was diluted 1:200 in blocking buffer, and 50 µL was added to each well and incubated overnight at 4°C. Cells were washed 4 times for 5 minutes with TBST. Secondary antibody (IR-800CW rabbit, LI-COR, diluted 1:800) and DNA stain DRAQ5 (LI-COR, diluted 1:2000) were added and incubated 1-2 hours at room temperature. Cells were washed 4 times for 5 minutes with TBST. Plates were scanned on a Li-COR Odyssey CLx Imager. Analysis of raw data was carried out in Excel (Microsoft) and Prism (GraphPad). Signal was plotted vs. the decadal

logarithm of compound concentration, and IC_{50} was determined by fitting a 4-parameter sigmoidal concentration response model.

The following compounds exhibited a pERK EC50 of under 5 uM (H358 KRAS G12C):

A48,A15,A272,A174,A163,A453,A447,A279,A240,A214,A225,A136,A226,A219,A228,A21,A12,A78
,A424,A219,A378,A224,A4,A53,A187,A218,A213,A314,A220,A208,A24,A9,A126,A345,A46,A203,
A210,A184,A469,A366,A113,A328,A693,A639,A364,A100,A249,A486,A307,A347,A33,A210,A192,
A285,A468,A185,A612,A109,A284,A200,A2,A6,A606,A325,A139,A496,A393,A561,A125,A494,A54
7,A215,A258,A195,A259,A212,A637,A53,A63,A68,A178,A189,A205,A78,A254,A690,A563,A14,A1
9,A92,A576,A278,A331,A42,A67,A209,A350,A562,A652,A703,A623,A191,A241,A199,A193,A478,
A251,A177,A222,A23,A59,A26,A211,A106,A279,A120,A7,A134,A521,A116,A467,A694,A729,A151
,A110,A277,A340,A221,A723,A13,A442,A611,A50,A190,A553,A696,A211,A303,A613,A37,A146,A
666,A688,A216,A390,A548,A238,A160,A183,A164,A451,A481,A524,A1,A186,A37,A635,A71,A269
,A289,A489,A400,A731,A497,A568,A274,A253,A471,A720,A241,A179,A180,A426,A117,A363,A71
6,A423,A217,A708,A227,A3,A12,A8,A381,A84,A408,A85,A171,A263,A473,A258,A564,A118,A103,
A565,A641,A655,A47,A11,A392,A169,A487,A640,A206,A449,A358,A192,A148,A4,A41,A5,A18,A3
01,A10,A65,A554,A159,A264,A99,A79,A142,A143,A25,A98,A80,A101,A730,A212,A359,A61,A441,
A283,A413,A717,A145,A182,A62,A181,A233,A232,A634,A495,A34,A251,A539,A632,A54,A327,A3
7,A196,A607,A645,A35,A214,A225,A638,A40,A52,A268,A448,A575,A176,A593,A15,A17,A94,A17
0,A713,A93,A402,A64,A261,A399,A422,A214,A225,A625,A31,A119,A135,A281,A676,A709,A81,A
32,A633,A39,A646,A662,A124,A732,A320,A81,A187,A354,A45,A570,A165,A66,A20,A455,A431,A
270,A250,A457,A153,A404,A710,A541,A127,A373,A369,A557,A349,A598,A618,A60,A636,A499,A
87,A156,A680,A477,A406,A330,A202,A535,A617,A737,A201,A302,A722,A209,A374,A631,A29,A5
55,A420,A380,A111,A306,A173,A628,A672,A51,A167,A588,A512,A194,A282,A412,A701,A583,A3
96,A678,A649,A27,A204,A626,A257,A614,A409,A172,A372,A353,A58,A728,A74,A619,A144,A183
,A538,A445,A531,A360,A361,A459,A536,A344,A267,A574,A677,A530,A415,A30,A73,A152,A490,
A702,A714,A483,A567,A43,A310,A319,A86,A321,A656,A739,A115,A130,A155,A608,A648,A168,A
485,A738,A129,A650,A715,A488,A147,A121,A470,A115,A133,A510,A421,A309,A335,A387,A386,
A734,A95,A430,A604,A458,A592,A384,A664,A197,A725,A89,A83,A586,A622,A305,A498,A668,A4
27,A630,A158,A644,A735,A70,A683,A352,A341,A719,A674,A70,A44,A501,A438,A698,A377,A417
,A154,A433,A104,A184,A603,A280,A712,A237,A105,A394,A605,A517,A704,A566,A77,A356,A454
,A600,A643,A112,A569,A529,A247,A463,A437,A718,A472,A461,A558,A48,A671,A395,A670,A681
,A687,A382,A82,A686,A342,A436,A296,A16,A545,A533,A416,A149,A207,A371,A596,A675,A132,
A419,A56,A579,A733,A573,A707,A597,A697,A75,A653,A362,A615,A332,A69,A162,A128,A432,A6
54,A22,A397,A526,A582,A418,A91,A260,A97,A191,A55,A581,A375,A522,A108,A367,A610,A552,
A571,A57,A543,A661,A138,A196,A246,A337,A446,A265,A96,A509,A123,A627,A651,A682,A157,A
572,A624,A691,A532,A462,A580,A695,A186,A316,A540,A590,A665,A244,A166,A587,A629,A595,
A518,A519,A131,A502,A726,A452,A141,A181,A262,A338,A155,A389,A124,A275,A414,A546,A67
9,A425,A669,A28,A520,A88,A131,A589,A621,A182,A297,A594,A283,A194,A250,A336,A706,A252
,A440,A107,A724,A525,A388,A175,A300,A333,A659,A346,A150,A476,A368,A528,A503,A504,A50
5,A684,A76,A736,A551,A383,A491,A492,A493,A410,A316,A295,A559,A511,A38,A140,A663,A334
,A700,A692,A348,A584,A513,A657,A328,A515,A317,A135,A660,A351,A544,A281,A685,A602,A55

6,A385,A326,A464,A465,A403,A133,A299,A667,A255,A334,A256,A585,A642,A133,A443,A435,A560,A444,A439,A324,A120,A407,A527,A245,A370,A537,A247,A474,A475,A705,A323,A112,A298,A609,A673,A292,A599,A132,A145,A266,A601,A466,A549,A379,A727,A167,A711,A75,A76,A121,A357,A620,A316,A479,A290,A339,A322,A376,A456,A391,A291,A550,A343,A721,A689,A411,A578,A616,A534,A365,A658,A699,A577,A647,A591,A542,A279,A294.

Determination of Cell Viability in RAS Mutant Cancer Cell Lines

Protocol: CellTiter-Glo® Cell Viability Assay

Note – The following protocol describes a procedure for monitoring cell viability of K-Ras mutant cancer cell lines in response to a compound of the invention. Other RAS isoforms may be employed, though the number of cells to be seeded will vary based on cell line used.

The purpose of this cellular assay was to determine the effects of test compounds on the proliferation of three human cancer cell lines (NCI-H358 (K-Ras G12C), AsPC-1 (K-Ras G12D), and Capan-1 (K-Ras G12V)) over a 5-day treatment period by quantifying the amount of ATP present at endpoint using the CellTiter-Glo® 2.0 Reagent (Promega).

Cells were seeded at 250 cells/well in 40 µL of growth medium in 384-well assay plates and incubated overnight in a humidified atmosphere of 5% CO_2 at 37°C. On the day of the assay, 10 mM stock solutions of test compounds were first diluted into 3 mM solutions with 100% DMSO. Well-mixed compound solutions (15 µL) were transferred to the next wells containing 30 µL of 100% DMSO, and repeated until a 9-concentration 3-fold serial dilution was made (starting assay concentration of 10 µM). Test compounds (132.5 nL) were directly dispensed into the assay plates containing cells. The plates were shaken for 15 seconds at 300 rpm, centrifuged, and incubated in a humidified atmosphere of 5% CO_2 at 37 °C for 5 days. On day 5, assay plates and their contents were equilibrated to room temperature for approximately 30 minutes. CellTiter-Glo® 2.0 Reagent (25 µL) was added, and plate contents were mixed for 2 minutes on an orbital shaker before incubation at room temperature for 10 minutes. Luminescence was measured using the PerkinElmer Enspire. Data were normalized by the following: (Sample signal/Avg. DMSO)*100. The data were fit using a four-parameter logistic fit.

Disruption of B-Raf Ras-binding Domain ($BRAF^{RBD}$) Interaction with K-Ras by Compounds of the Invention (also called a FRET assay or an MOA assay)

Note – The following protocol describes a procedure for monitoring disruption of K-Ras G12C (GMP-PNP) binding to $BRAF^{RBD}$ by a compound of the invention. This protocol may also be executed substituting other Ras proteins or nucleotides.

The purpose of this biochemical assay was to measure the ability of test compounds to facilitate ternary complex formation between a nucleotide-loaded K-Ras isoform and Cyclophilin A; the resulting ternary complex disrupts binding to a $BRAF^{RBD}$ construct, inhibiting K-Ras signaling through a RAF effector. Data is reported as IC50 values.

In assay buffer containing 25 mM HEPES pH 7.3, 0.002% Tween20, 0.1% BSA, 100 mM NaCl and 5 mM $MgCl_2$, tagless Cyclophilin A, His6-K-Ras-GMPPNP, and GST-$BRAF^{RBD}$ were combined in a 384-well assay plate at final concentrations of 25 µM, 12.5 nM and 50 nM,

respectively. Compound was present in plate wells as a 10-point 3-fold dilution series starting at a final concentration of 30 μM. After incubation at 25°C for 3 hours, a mixture of Anti-His Eu-W1024 and anti-GST allophycocyanin was then added to assay sample wells at final concentrations of 10 nM and 50 nM, respectively, and the reaction incubated for an additional 1.5 hours. TR-FRET signal was read on a microplate reader (Ex 320 nm, Em 665/615 nm). Compounds that facilitate disruption of a K-Ras:RAF complex were identified as those eliciting a decrease in the TR-FRET ratio relative to DMSO control wells.

Table 4: Biological Assay Data for Representative Compounds of the Present Invention

Ex#	H358 pERK (K-Ras G12C) EC50, uM	H358 Cell Viability (K-Ras G12C) IC50, uM	FRET (K-Ras G12C) IC50, uM	FRET (K-Ras G13C) IC50, uM
A208	0.013	0.004	0.023	7.48
A193	0.067	0.054	0.295	10.2
A186	0.013	0.005	0.038	0.94
A89a	0.003	0.0008	0.009	0.82
A89b	0.028	0.011	0.034	0.58

Table 5. Additional H358 Cell Viability assay data (K-Ras G12C, IC50, uM):

*Key:

++++: IC50 ≥ 1 uM

+++: 1 uM > IC50 ≥ 0.1 uM

++: 0.1 uM > IC50 ≥ 0.01 uM

+: IC50 < 0.01 uM

IC50*	Examples
+	A104,A107,A108,A112,A120,A121,A123,A124,A128,A131,A131,A132,A133,A133,A135,A138,A140,A141,A145,A150,A155,A157,A162,A166,A167,A175,A181,A182,A183,A186,A191,A194,A196,A237,A244,A245,A246,A247,A250,A255,A256,A260,A266,A275,A28,A281,A283,A290,A291,A292,A295,A296,A298,A299,A300,A309,A316,A316,A316,A317,A322,A323,A324,A326,A328,A332,A333,A334,A334,A336,A337,A339,A343,A344,A346,A348,A351,A352,A356,A357,A365,A368,A370,A371,A375,A376,A377,A379,A38,A380,A382,A383,A384,A385,A388,A389,A391,A394,A395,A396,A397,A403,A407,A410,A411,A414,A416,A419,A425,A427,A435,A439,A440,A443,A444,A445,A446,A452,A454,A456,A461,A462,A463,A464,A465,A466,A472,A474,A475,A476,A479,A483,A48

	5,A488,A491,A492,A493,A502,A503,A504,A505,A509,A511,A513,A515,A517,A518,A519,A520,A522,A525,A526,A527,A528,A532,A533,A534,A537,A540,A542,A543,A544,A546,A549,A55,A550,A551,A552,A556,A559,A56,A560,A566,A567,A57,A571,A572,A577,A578,A579,A581,A584,A585,A587,A589,A590,A591,A592,A594,A595,A596,A597,A599,A600,A601,A602,A604,A609,A610,A615,A616,A620,A621,A624,A627,A629,A631,A636,A642,A643,A644,A647,A657,A658,A659,A660,A661,A663,A665,A667,A673,A674,A675,A677,A684,A685,A686,A689,A691,A692,A699,A700,A704,A705,A706,A707,A711,A712,A714,A718,A721,A724,A726,A727,A733,A735,A736,A738,A75,A76,A76,A88,A91,A96
++	A10,A105,A111,A112,A115,A115,A119,A12,A121,A124,A129,A130,A132,A133,A135,A143,A144,A145,A147,A149,A15,A152,A154,A155,A156,A158,A16,A165,A167,A168,A172,A173,A176,A181,A182,A184,A187,A194,A197,A20,A201,A202,A204,A207,A209,A212,A214,A214,A22,A225,A225,A232,A233,A238,A241,A247,A250,A251,A252,A257,A261,A262,A264,A265,A267,A268,A269,A27,A270,A280,A281,A282,A283,A297,A30,A302,A305,A306,A31,A310,A319,A320,A321,A327,A330,A335,A338,A34,A341,A342,A349,A35,A353,A354,A358,A359,A360,A361,A362,A363,A367,A369,A37,A372,A373,A374,A381,A386,A387,A39,A392,A393,A399,A40,A400,A402,A404,A406,A408,A409,A41,A412,A413,A415,A417,A418,A420,A421,A422,A423,A426,A43,A430,A431,A432,A433,A436,A437,A438,A44,A441,A442,A448,A449,A45,A451,A455,A457,A458,A459,A467,A468,A47,A470,A471,A473,A477,A478,A48,A481,A487,A489,A490,A495,A497,A498,A499,A5,A501,A51,A510,A512,A52,A524,A529,A530,A531,A535,A536,A538,A54,A541,A545,A555,A558,A564,A565,A568,A569,A573,A574,A575,A58,A580,A582,A583,A586,A588,A598,A60,A603,A605,A607,A608,A61,A611,A613,A614,A617,A618,A619,A62,A622,A623,A625,A626,A628,A630,A633,A634,A637,A638,A64,A640,A641,A645,A646,A648,A649,A650,A651,A653,A654,A655,A656,A66,A662,A664,A666,A668,A669,A670,A671,A672,A676,A678,A679,A680,A681,A682,A683,A687,A69,A694,A695,A697,A698,A70,A70,A701,A702,A703,A708,A709,A710,A713,A715,A716,A717,A719,A720,A722,A723,A725,A728,A73,A730,A732,A734,A737,A739,A74,A75,A77,A79,A81,A82,A83,A86,A87,A89,A93,A94,A95,A97,A98,A99

+++	A1,A100,A101,A103,A106,A109,A11,A110,A113,A114,A116,A117,A118,A120,A127,A13,A134,A139,A14,A142,A144,A146,A148,A151,A153,A159,A160,A164,A169,A17,A170,A171,A177,A18,A183,A185,A186,A19,A190,A191,A192,A192,A193,A195,A196,A199,A200,A205,A206,A209,A210,A211,A211,A212,A215,A216,A217,A219,A219,A221,A222,A226,A227,A23,A241,A249,A25,A251,A253,A254,A258,A258,A259,A26,A263,A274,A277,A278,A279,A284,A289,A29,A3,A301,A303,A304,A304,A304,A304,A308,A314,A32,A325,A328,A329,A33,A331,A340,A345,A347,A350,A355,A364,A366,A37,A37,A378,A390,A398,A4,A401,A42,A424,A447,A453,A469,A482,A484,A486,A494,A496,A50,A506,A507,A508,A514,A521,A523,A53,A53,A539,A547,A548,A553,A554,A557,A561,A562,A563,A570,A576,A59,A593,A6,A606,A612,A63,A632,A635,A639,A65,A652,A67,A68,A688,A690,A693,A696,A7,A71,A729,A731,A78,A8,A80,A81,A84,A85,A9,A92
++++	A102,A12,A122,A125,A126,A136,A137,A148,A15,A161,A163,A174,A178,A179,A180,A184,A187,A188,A189,A198,A2,A203,A208,A21,A210,A210,A210,A211,A211,A213,A213,A214,A214,A215,A216,A217,A218,A220,A220,A221,A222,A223,A223,A224,A224,A225,A225,A228,A229,A230,A231,A231,A231,A234,A235,A236,A239,A239,A24,A240,A242,A243,A248,A261,A271,A272,A273,A276,A279,A279,A283,A285,A286,A287,A288,A293,A307,A311,A312,A313,A318,A318,A32,A4,A405,A428,A429,A450,A46,A460,A48,A480,A49,A500,A516,A72,A78,A90

Additional Ras-Raf disruption/FRET/MOA assay data (IC50, uM):

*Key:

+++++: IC50 ≥ 10 uM

++++: 10 uM > IC50 ≥ 1 uM

+++: 1 uM > IC50 ≥ 0.1 uM

++: 0.1 uM > IC50 ≥ 0.01 uM

+: IC50 < 0.01 uM

Table 6. KRAS G12S FRET data

IC50*	Examples
+	none

++	A028,A075,A076,A076,A087,A112,A145,A155,A167,A181,A183,A184,A194,A233,A255,A256,A260,A262,A264,A265,A266,A268,A270,A275,A292,A294,A295,A298,A299,A300,A319,A333,A334,A334,A338,A367,A382,A383,A385,A388,A389,A418,A433,A464,A465,A479,A491,A492,A493,A527,A537,A542,A577,A596,A598,A602,A609,A621,A629,A664,A665,A679,A712,A734,A736
+++	A004,A005,A012,A015,A016,A022,A027,A029,A032,A034,A037,A037,A041,A043,A044,A047,A048,A051,A052,A054,A055,A056,A057,A058,A064,A070,A070,A072,A074,A077,A079,A082,A083,A086,A088,A091,A093,A095,A096,A097,A101,A103,A105,A107,A123,A127,A128,A129,A131,A132,A135,A141,A147,A148,A149,A150,A153,A154,A158,A164,A166,A178,A182,A189,A193,A194,A196,A206,A207,A237,A241,A244,A245,A246,A247,A250,A251,A252,A257,A261,A261,A263,A267,A269,A280,A281,A281,A296,A297,A305,A306,A328,A336,A337,A349,A350,A351,A353,A354,A357,A360,A361,A368,A369,A379,A384,A386,A387,A393,A394,A395,A397,A406,A407,A408,A409,A410,A415,A416,A417,A419,A420,A421,A430,A431,A432,A435,A436,A437,A438,A443,A455,A463,A470,A490,A502,A515,A517,A518,A519,A529,A536,A538,A539,A541,A543,A544,A545,A548,A554,A555,A557,A558,A560,A564,A570,A571,A572,A573,A575,A578,A580,A581,A582,A583,A586,A591,A594,A603,A604,A605,A606,A608,A611,A612,A618,A620,A622,A624,A625,A630,A631,A632,A633,A634,A635,A636,A650,A651,A653,A654,A655,A656,A659,A661,A662,A667,A668,A669,A670,A671,A672,A677,A678,A680,A681,A682,A683,A684,A685,A686,A687,A689,A690,A695,A696,A697,A698,A699,A700,A701,A702,A704,A709,A710,A711,A713,A714,A717,A718,A719,A728,A729,A730,A731,A732,A737
++++	A001,A003,A006,A007,A008,A010,A013,A014,A017,A018,A019,A020,A025,A030,A031,A033,A035,A036,A038,A039,A040,A045,A050,A053,A060,A061,A062,A063,A066,A067,A068,A069,A071,A073,A075,A081,A081,A089,A092,A098,A099,A104,A108,A109,A110,A111,A112,A113,A115,A115,A117,A119,A120,A121,A124,A125,A131,A132,A133,A133,A134,A135,A137,A140,A143,A145,A146,A151,A155,A159,A161,A162,A167,A168,A169,A172,A173,A175,A176,A179,A180,A181,A182,A183,A184,A186,A187,A197,A199,A202,A204,A209,A210,A211,A212,A213,A214,A214,A215,A216,A217,A221,A223,A225,A225,A226,A227,A238,

	A241,A247,A249,A250,A251,A253,A254,A258,A258,A259,A277,A278,A282,A283,A290,A291,A301,A302,A309,A310,A314,A317,A320,A321,A324,A325,A326,A327,A330,A331,A332,A335,A339,A340,A341,A342,A343,A344,A347,A348,A356,A358,A359,A363,A364,A365,A366,A370,A371,A372,A373,A376,A378,A380,A381,A390,A391,A392,A396,A399,A401,A402,A411,A412,A413,A414,A422,A423,A424,A425,A426,A427,A439,A440,A442,A444,A445,A446,A447,A449,A451,A452,A454,A456,A457,A458,A459,A461,A462,A466,A468,A469,A471,A472,A473,A474,A475,A476,A481,A485,A487,A488,A489,A495,A498,A499,A501,A503,A504,A505,A506,A507,A509,A510,A511,A512,A513,A520,A521,A522,A525,A526,A528,A530,A531,A532,A533,A534,A535,A540,A546,A547,A549,A550,A551,A552,A553,A559,A561,A562,A563,A565,A566,A567,A568,A569,A574,A579,A585,A588,A590,A592,A593,A595,A597,A600,A601,A607,A610,A616,A619,A623,A626,A628,A637,A638,A639,A640,A641,A642,A645,A647,A652,A658,A660,A673,A676,A688,A691,A692,A693,A694,A705,A706,A707,A716,A720,A722,A723,A725,A726,A727,A733,A738,A739
+++++	A002,A004,A009,A011,A012,A015,A021,A023,A024,A026,A032,A036,A037,A038,A042,A046,A048,A049,A053,A059,A065,A078,A078,A080,A084,A085,A090,A094,A100,A102,A106,A114,A116,A118,A120,A121,A122,A124,A126,A130,A133,A136,A138,A139,A142,A144,A144,A148,A152,A156,A157,A160,A163,A165,A170,A171,A174,A177,A185,A186,A187,A188,A190,A191,A191,A192,A192,A195,A196,A198,A200,A201,A203,A205,A208,A209,A210,A210,A210,A211,A211,A211,A212,A213,A214,A214,A215,A216,A217,A218,A218,A219,A219,A219,A219,A220,A220,A221,A222,A222,A223,A224,A224,A225,A225,A228,A229,A230,A231,A231,A231,A232,A234,A235,A236,A239,A239,A240,A242,A243,A248,A271,A272,A273,A274,A276,A279,A279,A279,A283,A283,A284,A285,A286,A287,A288,A289,A293,A303,A304,A304,A304,A304,A307,A308,A311,A312,A313,A316,A316,A316,A318,A318,A322,A323,A328,A329,A345,A346,A352,A355,A362,A374,A375,A377,A398,A400,A403,A404,A405,A428,A429,A441,A448,A450,A453,A460,A467,A477,A478,A480,A482,A483,A484,A486,A494,A496,A497,A500,A508,A514,A516,A523,A524,A556,A576,A584,A587,A589,A599,A613,A614,A615,A617,A627,A643,A644,A646,A648,A649,A657,A663,A666,A674,A675,A703,A708,A715,A721,A724,A735

Table 7. KRAS G12D FRET data

IC50*	Examples
+	none
++	A183,A264,A265,A268,A319,A388,A464,A465,A664
+++	A028,A054,A075,A076,A076,A087,A107,A112,A123,A131,A145,A153,A155,A167,A184,A189,A194,A233,A247,A255,A256,A257,A260,A261,A262,A263,A266,A269,A270,A275,A292,A294,A295,A298,A300,A333,A334,A334,A338,A349,A353,A354,A367,A368,A382,A383,A384,A385,A386,A387,A389,A394,A407,A409,A417,A418,A432,A433,A436,A479,A491,A492,A493,A527,A529,A537,A542,A555,A577,A578,A594,A596,A598,A602,A603,A605,A608,A609,A612,A621,A622,A629,A633,A650,A651,A653,A654,A662,A665,A667,A669,A671,A678,A679,A681,A682,A683,A685,A696,A697,A698,A700,A701,A704,A709,A710,A711,A712,A719,A734,A736,A737
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	,A588,A591,A593,A595,A600,A604,A606,A607,A610,A611,A618,A619,A620,A623,A624,A625,A626,A630,A631,A632,A634,A635,A636,A640,A645,A647,A655,A656,A658,A659,A660,A661,A668,A670,A672,A673,A676,A677,A680,A684,A686,A687,A688,A689,A690,A691,A694,A695,A699,A702,A707,A713,A714,A717,A718,A722,A727,A728,A729,A730,A731,A732,A738,A739
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Table 8. KRAS G13C FRET data

IC50*	Examples
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++	A031,A037,A038,A046,A067,A069,A071,A075,A076,A076,A090,A108,A112,A116,A121,A123,A124,A131,A132,A133,A133,A135,A140,A141,A145,A150,A155,A163,A167,A172,A178,A182,A183,A194,A196,A218,A224,A233,A244,A245,A246,A247,A249,A250,A251,A254,A256,A257,A258,A259,A260,A261,A263,A264,A265,A266,A267,A268,A275,A280,A281,A283,A295,A296,A300,A305,A306,A319,A333,A334,A337,A379,A383,A385,A388,A389,A394,A396,A397,A417,A418,A421,A432,A433,A438,A452,A464,A465,A479,A491,A492,A493,A517,A525,A527,A537,A538,A539,A540,A541,A545,A546,A552,A553,A554,A555,A564,A570,A571,A572,A573,A574,A575,A577,A578,A591,A594,A595,A597,A600,A602,A603,A604,A605,A606,A607,A608,A609,A610,A621,A622,A629,A630,A632,A650,A664,A668,A679,A680,A695,A698,A699,A706,A711,A714,A718,A719,A734,A736,A737
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	A423,A425,A430,A431,A435,A436,A437,A439,A440,A443,A444,A445,A449,A451,A454,A455,A457,A458,A459,A463,A466,A470,A471,A476,A485,A487,A488,A490,A497,A501,A502,A503,A504,A505,A509,A510,A513,A515,A518,A519,A524,A534,A536,A543,A544,A547,A549,A550,A551,A557,A558,A559,A560,A561,A562,A563,A566,A567,A576,A580,A581,A582,A583,A586,A593,A596,A598,A601,A611,A612,A618,A619,A620,A624,A625,A631,A633,A634,A635,A636,A640,A642,A645,A647,A651,A652,A653,A654,A655,A658,A659,A660,A661,A662,A665,A667,A669,A670,A671,A672,A673,A678,A681,A682,A684,A685,A686,A687,A688,A689,A690,A692,A694,A696,A697,A700,A701,A702,A704,A707,A709,A710,A712,A717,A722,A725,A726,A727,A728,A729,A730,A731,A732,A733,A738,A739
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Table 9. KRAS G12V FRET data

IC50*	Examples
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	A163,A165,A170,A171,A174,A177,A185,A186,A187,A188,A190,A191,A192,A192,A195,A196,A198,A200,A201,A202,A203,A205,A208,A209,A210,A210,A210,A211,A211,A211,A212,A213,A214,A214,A214,A214,A215,A216,A217,A218,A219,A219,A219,A219,A220,A220,A221,A222,A222,A223,A224,A225,A225,A225,A225,A228,A229,A230,A231,A231,A231,A234,A235,A236,A239,A239,A240,A243,A248,A250,A271,A272,A273,A274,A276,A279,A279,A279,A283,A283,A284,A285,A286,A287,A288,A293,A303,A304,A304,A304,A304,A308,A311,A312,A313,A316,A316,A318,A318,A322,A323,A327,A328,A329,A345,A347,A352,A355,A371,A374,A377,A400,A404,A405,A425,A428,A429,A441,A442,A448,A450,A453,A460,A467,A471,A478,A480,A482,A484,A486,A494,A496,A497,A500,A508,A514,A516,A523,A524,A535,A568,A569,A576,A587,A589,A613,A614,A615,A617,A627,A643,A644,A646,A648,A649,A657,A663,A666,A674,A675,A715,A724,A735

Table 10. KRAS WT FRET data

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Table 11. KRAS G12C FRET data

IC50*	Examples
+	A038,A075,A076,A088,A095,A096,A097,A107,A108,A112,A116,A120,A121,A129,A130,A131,A131,A132,A133,A133,A135,A140,A141,A145,A157,A162,A167,A175,A176,A182,A183,A184,A194,A196,A209,A244,A245,A246,A247,A247,A255,A256,A257,A266,A268,A270,A290,A291,A292,A294,A298,A299,A300,A316,A316,A316,A317,A322,A323,A324,A326,A333,A334,A337,A339,A343,A346,A348,A351,A365,A375,A376,A377,A379,A380,A381,A388,A391,A403,A407,A411,A414,A425,A427,A474,A475,A477,A479,A509,A527,A529,A534,A542,A543,A544,A549,A550,A551,A556,A577,A578,A579,A591,A599,A600,A601,A609,A620,A626,A627,A628,A638,A642,A647,A658,A660,A663,A667,A673,A684,A689,A691,A692,A699,A705,A707,A711,A721,A726,A727,A735
++	A004,A005,A012,A015,A016,A020,A022,A027,A028,A029,A031,A032,A037,A0

	37,A038,A043,A044,A047,A048,A051,A052,A054,A055,A056,A057,A058,A064,A067,A069,A070,A070,A071,A072,A074,A076,A077,A079,A082,A083,A086,A087,A091,A093,A103,A104,A105,A115,A121,A123,A124,A126,A127,A128,A132,A133,A134,A135,A138,A139,A142,A144,A148,A149,A150,A152,A153,A154,A155,A155,A156,A158,A164,A165,A166,A172,A178,A179,A181,A182,A183,A186,A187,A189,A191,A193,A196,A200,A201,A204,A207,A209,A214,A214,A225,A225,A233,A237,A238,A241,A241,A250,A250,A251,A251,A252,A253,A260,A261,A262,A263,A264,A265,A267,A269,A275,A280,A281,A283,A295,A296,A297,A305,A319,A328,A332,A334,A336,A338,A342,A344,A349,A350,A352,A353,A359,A361,A362,A363,A366,A367,A368,A370,A371,A382,A383,A384,A385,A386,A387,A389,A394,A396,A397,A400,A410,A416,A417,A418,A421,A423,A432,A433,A435,A436,A437,A438,A439,A440,A443,A444,A445,A448,A452,A454,A456,A458,A459,A463,A464,A465,A466,A476,A478,A485,A489,A490,A491,A492,A493,A499,A501,A503,A504,A505,A510,A513,A515,A517,A518,A519,A525,A526,A528,A532,A533,A536,A537,A538,A545,A552,A558,A559,A560,A566,A567,A568,A569,A572,A573,A575,A580,A581,A584,A585,A587,A589,A590,A592,A594,A595,A596,A597,A598,A602,A603,A610,A612,A614,A615,A616,A617,A621,A622,A624,A629,A632,A634,A636,A637,A643,A644,A645,A646,A649,A650,A651,A653,A657,A659,A661,A662,A664,A665,A668,A669,A671,A672,A674,A675,A678,A679,A681,A682,A683,A685,A686,A697,A698,A700,A701,A703,A704,A706,A709,A710,A712,A715,A719,A720,A722,A724,A730,A733,A734,A736,A737
+++	A001,A003,A006,A007,A008,A010,A011,A013,A014,A017,A018,A019,A023,A025,A026,A030,A033,A034,A035,A039,A040,A041,A042,A045,A046,A050,A053,A053,A060,A061,A062,A063,A065,A066,A068,A073,A075,A078,A078,A080,A081,A081,A089,A092,A094,A098,A099,A101,A109,A110,A111,A112,A113,A115,A117,A118,A119,A120,A125,A137,A143,A144,A145,A146,A147,A151,A159,A160,A161,A167,A168,A169,A173,A174,A177,A180,A181,A184,A185,A188,A190,A191,A192,A194,A195,A197,A199,A205,A206,A208,A210,A211,A212,A213,A215,A216,A217,A218,A220,A221,A222,A223,A224,A226,A227,A229,A232,A249,A254,A258,A258,A259,A261,A277,A278,A281,A282,A284,A301,A302,A306,A310,A314,A320,A321,A327,A330,A331,A335,A340,A341,A347,A354,A356,A357,A358,A360,A364,A369,A372,A373,A374,A378,A390,A392,A393,A395,A399,A4

	02,A404,A406,A408,A409,A412,A413,A415,A419,A420,A422,A424,A426,A430,A431,A442,A446,A447,A449,A451,A455,A457,A461,A462,A468,A469,A470,A471,A472,A473,A481,A483,A484,A486,A487,A488,A494,A495,A497,A498,A502,A506,A507,A508,A511,A512,A520,A522,A524,A530,A531,A539,A540,A541,A546,A547,A548,A553,A554,A555,A557,A561,A562,A563,A564,A565,A570,A571,A574,A576,A582,A583,A586,A588,A593,A604,A605,A606,A607,A608,A611,A613,A618,A619,A623,A625,A630,A631,A633,A635,A639,A640,A641,A648,A652,A654,A655,A656,A670,A676,A677,A680,A687,A688,A690,A693,A694,A695,A696,A702,A708,A713,A714,A716,A717,A718,A723,A725,A728,A729,A731,A732,A738,A739
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Table 12. KRAS G13D FRET data

IC50*	Examples
+	None
++	A075,A076,A112,A155,A183,A260,A261,A262,A263,A264,A265,A267,A268,A270,A294,A296,A298,A300,A319,A333,A338,A388,A464,A465,A527,A537,A542,A664
+++	A028,A054,A096,A105,A107,A123,A128,A131,A132,A141,A145,A149,A150,A153,A158,A164,A167,A181,A182,A184,A189,A194,A196,A207,A233,A244,A245,A246,A247,A250,A251,A252,A253,A255,A256,A257,A258,A261,A266,A269,A275,A280,A281,A281,A292,A295,A297,A299,A305,A306,A334,A334,A336,A337,A349,A350,A353,A354,A357,A361,A367,A368,A379,A382,A383,A384,A385,A3

	86,A387,A389,A394,A395,A397,A406,A407,A410,A415,A416,A417,A418,A421,A432,A433,A436,A438,A463,A479,A490,A491,A492,A493,A518,A519,A529,A536,A538,A543,A544,A545,A555,A558,A573,A577,A578,A580,A581,A582,A591,A594,A596,A598,A602,A603,A605,A606,A608,A609,A611,A612,A620,A621,A622,A624,A629,A631,A633,A650,A651,A653,A654,A655,A659,A661,A662,A665,A667,A669,A670,A671,A672,A678,A679,A681,A682,A683,A684,A685,A686,A687,A690,A695,A697,A698,A699,A700,A701,A704,A709,A710,A711,A712,A718,A719,A729,A730,A734,A736,A737
++++	A016,A038,A069,A075,A088,A095,A103,A104,A108,A110,A111,A112,A116,A117,A120,A120,A121,A121,A125,A127,A129,A130,A131,A132,A133,A133,A133,A134,A135,A138,A140,A142,A144,A144,A145,A146,A147,A148,A152,A154,A155,A156,A157,A159,A161,A162,A163,A166,A175,A178,A179,A180,A181,A182,A183,A187,A193,A194,A197,A206,A209,A212,A213,A232,A237,A238,A241,A241,A247,A249,A251,A254,A258,A259,A277,A278,A282,A290,A302,A314,A321,A326,A328,A330,A331,A332,A335,A339,A340,A341,A342,A344,A351,A356,A358,A359,A360,A363,A364,A365,A366,A369,A370,A372,A373,A380,A381,A390,A391,A393,A396,A399,A401,A408,A409,A411,A412,A413,A419,A420,A422,A424,A427,A430,A431,A435,A437,A439,A440,A443,A444,A445,A446,A449,A451,A452,A454,A455,A456,A457,A458,A459,A466,A468,A469,A470,A472,A473,A474,A475,A476,A485,A487,A488,A489,A499,A501,A502,A503,A504,A505,A509,A510,A511,A513,A515,A517,A520,A521,A522,A525,A534,A539,A540,A541,A546,A547,A548,A549,A550,A551,A552,A553,A554,A557,A559,A560,A561,A562,A563,A564,A566,A567,A570,A571,A572,A574,A575,A583,A586,A588,A593,A595,A597,A600,A601,A604,A607,A610,A616,A618,A619,A623,A625,A626,A630,A632,A634,A635,A636,A637,A638,A640,A641,A642,A645,A647,A652,A656,A658,A660,A668,A673,A676,A677,A680,A688,A689,A691,A693,A694,A696,A702,A706,A707,A713,A714,A717,A720,A722,A725,A726,A727,A728,A731,A732,A738,A739
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Table 13. KRAS Q61H FRET data

IC50*	Examples
+	A297,A299,A388,A598,A621
++	A012,A022,A075,A076,A077,A082,A087,A105,A148,A167,A181,A207,A262,A275,A281,A300,A333,A338,A349,A350,A353,A354,A367,A368,A379,A382,A383,A384,A385,A386,A387,A389,A394,A407,A416,A417,A463,A491,A492,A493,A537,A542,A543,A544,A545,A557,A577,A578,A580,A582,A586,A594,A596,A602,A603,A605,A606,A608,A609,A611,A612,A620,A622,A629,A631,A633,A650,A651,A653,A654,A655,A656,A661,A662,A664,A665,A667,A668,A669,A671,A672,A676,A678,A679,A681,A682,A683,A684,A686,A687,A696,A697,A698,A701,A702,A704,A709,A710,A711,A712,A718,A719,A734,A736,A737,A739
+++	A016,A045,A064,A074,A083,A086,A089,A104,A115,A166,A172,A193,A252,A2

	77,A314,A328,A330,A332,A340,A341,A342,A344,A351,A356,A357,A358,A360,A361,A365,A366,A369,A372,A373,A390,A393,A468,A474,A475,A494,A502,A503,A504,A505,A522,A534,A538,A539,A540,A541,A546,A547,A548,A549,A551,A552,A553,A554,A555,A558,A559,A560,A561,A564,A566,A567,A570,A571,A572,A573,A574,A575,A581,A583,A588,A591,A593,A595,A597,A600,A604,A607,A610,A618,A619,A623,A624,A625,A626,A630,A632,A634,A635,A636,A640,A659,A660,A670,A673,A677,A680,A685,A688,A689,A690,A694,A695,A699,A700,A706,A713,A714,A717,A725,A728,A729,A730,A731,A732,A738
++++	A023,A025,A026,A050,A053,A060,A061,A062,A080,A084,A085,A102,A113,A136,A143,A151,A160,A168,A202,A210,A211,A226,A227,A242,A278,A290,A301,A302,A303,A331,A335,A339,A343,A345,A346,A347,A348,A352,A359,A362,A363,A364,A370,A371,A376,A380,A381,A391,A392,A403,A427,A442,A447,A461,A472,A481,A495,A496,A498,A531,A550,A562,A563,A565,A568,A569,A576,A579,A584,A585,A589,A590,A592,A601,A616,A627,A637,A638,A639,A641,A642,A645,A647,A652,A658,A666,A674,A691,A692,A693,A703,A705,A707,A708,A716,A720,A721,A722,A723,A726,A727,A733,A735
+++++	A170,A171,A205,A240,A243,A248,A285,A304,A304,A313,A316,A329,A355,A374,A375,A377,A378,A482,A486,A507,A587,A599,A613,A614,A615,A617,A628,A643,A644,A646,A648,A649,A657,A663,A675,A715,A724

Table 14. NRAS G12C FRET data

IC50*	Examples
+	A038,A075,A076,A088,A095,A096,A107,A108,A112,A116,A120,A121,A123,A129,A130,A131,A131,A132,A133,A133,A135,A140,A141,A145,A155,A157,A162,A167,A175,A176,A182,A183,A184,A194,A196,A209,A244,A245,A246,A247,A247,A250,A255,A257,A266,A268,A270,A290,A292,A294,A297,A298,A299,A300,A316,A316,A316,A323,A324,A326,A333,A334,A336,A337,A339,A343,A348,A351,A365,A375,A376,A377,A380,A388,A391,A407,A411,A414,A427,A435,A474,A475,A479,A509,A527,A529,A534,A537,A542,A543,A544,A550,A551,A556,A577,A578,A579,A591,A600,A601,A609,A612,A616,A620,A626,A627,A628,A638,A642,A647,A658,A660,A663,A667,A673,A684,A689,A691,A692,A699,A705,A707,A711,A721,A727,A735

++	A016,A054,A069,A103,A104,A105,A115,A121,A124,A126,A127,A128,A132,A133,A134,A135,A138,A142,A144,A148,A149,A150,A152,A153,A154,A155,A158,A164,A165,A166,A172,A178,A179,A181,A182,A183,A186,A187,A189,A191,A196,A200,A204,A207,A233,A237,A241,A241,A250,A251,A251,A252,A256,A260,A261,A262,A263,A264,A265,A267,A269,A275,A281,A291,A295,A296,A305,A317,A319,A322,A328,A332,A334,A338,A344,A346,A349,A350,A352,A353,A354,A361,A362,A363,A367,A368,A370,A371,A379,A381,A382,A383,A384,A385,A386,A387,A389,A394,A396,A397,A400,A403,A410,A417,A418,A419,A421,A423,A425,A432,A433,A436,A437,A438,A439,A440,A443,A444,A445,A448,A452,A454,A456,A458,A459,A463,A464,A465,A466,A476,A477,A478,A485,A489,A490,A491,A492,A493,A499,A501,A503,A504,A505,A510,A513,A515,A517,A518,A519,A525,A526,A528,A532,A533,A536,A538,A545,A549,A552,A558,A559,A560,A566,A567,A569,A572,A573,A574,A575,A580,A581,A582,A584,A585,A587,A589,A590,A592,A594,A595,A596,A597,A598,A599,A602,A603,A610,A614,A615,A617,A621,A622,A624,A629,A631,A632,A633,A634,A636,A637,A643,A644,A645,A646,A649,A650,A651,A653,A654,A657,A659,A661,A662,A664,A665,A668,A669,A671,A672,A674,A675,A676,A678,A679,A681,A682,A683,A685,A686,A695,A697,A698,A700,A701,A703,A704,A706,A709,A710,A712,A715,A719,A720,A722,A724,A726,A730,A733,A734,A736,A737
+++	A065,A075,A094,A109,A111,A112,A113,A115,A117,A118,A119,A120,A137,A139,A143,A144,A145,A146,A147,A151,A156,A159,A161,A167,A169,A173,A177,A180,A181,A184,A185,A188,A190,A192,A193,A194,A195,A197,A199,A201,A206,A209,A210,A211,A212,A214,A225,A226,A227,A232,A238,A249,A253,A254,A258,A258,A259,A261,A277,A278,A280,A281,A282,A283,A301,A302,A306,A310,A314,A320,A321,A327,A330,A331,A335,A340,A341,A342,A347,A356,A357,A358,A359,A360,A364,A366,A369,A372,A373,A374,A378,A390,A392,A393,A395,A399,A402,A404,A406,A408,A409,A412,A413,A415,A416,A420,A422,A424,A430,A431,A442,A446,A447,A449,A451,A455,A457,A461,A462,A467,A468,A469,A470,A471,A472,A473,A481,A483,A486,A487,A488,A494,A495,A497,A498,A502,A506,A507,A508,A511,A512,A520,A522,A524,A530,A531,A539,A540,A541,A546,A547,A548,A553,A554,A555,A557,A561,A562,A563,A564,A565,A568,A570,A571,A576,A583,A586,A588,A593,A604,A605,A606,A607,A608,A611,A6

	18,A619,A623,A625,A630,A635,A639,A640,A641,A648,A652,A655,A656,A670,A677,A680,A687,A688,A690,A693,A694,A696,A702,A708,A713,A714,A716,A717,A718,A723,A725,A728,A729,A731,A732,A738,A739
++++	A024,A102,A106,A110,A124,A125,A136,A160,A163,A168,A170,A171,A174,A186,A187,A191,A192,A202,A203,A205,A208,A210,A211,A212,A213,A228,A229,A235,A240,A242,A248,A274,A276,A279,A283,A284,A289,A303,A307,A309,A313,A325,A328,A345,A398,A401,A426,A441,A453,A460,A480,A484,A496,A514,A516,A521,A523,A535,A613,A666
+++++	A114,A122,A148,A198,A210,A210,A211,A211,A213,A214,A214,A225,A225,A230,A231,A231,A231,A234,A236,A239,A239,A243,A271,A272,A273,A279,A279,A283,A285,A286,A287,A288,A293,A304,A304,A304,A304,A308,A311,A312,A318,A318,A329,A355,A405,A428,A429,A450,A482,A500

Table 15. NRAS Q61R FRET data

IC50*	Examples
+	A388
++	A367,A368,A382,A383,A385,A386,A387,A389,A407,A417,A491,A492,A493,A542,A577,A594,A596,A598,A602,A603,A609,A611,A612,A621,A629,A633,A655,A664,A665,A667,A669,A678,A679,A697,A704,A712,A719,A734,A736,A737
+++	A012,A022,A075,A076,A082,A087,A105,A148,A167,A181,A207,A262,A275,A281,A297,A299,A300,A314,A333,A338,A349,A350,A353,A354,A356,A357,A360,A361,A369,A379,A384,A393,A394,A416,A463,A502,A537,A538,A539,A541,A543,A544,A545,A546,A548,A554,A555,A570,A571,A572,A573,A575,A578,A580,A581,A582,A583,A586,A591,A593,A595,A597,A604,A605,A606,A607,A608,A610,A618,A619,A620,A622,A624,A625,A630,A631,A632,A634,A635,A636,A650,A651,A653,A654,A656,A659,A661,A662,A668,A670,A671,A672,A676,A677,A680,A681,A682,A683,A684,A685,A686,A687,A688,A690,A695,A696,A698,A699,A700,A701,A702,A706,A709,A710,A711,A713,A714,A717,A718,A728,A732,A738
++++	A016,A064,A074,A077,A083,A086,A089,A104,A115,A166,A168,A193,A252,A277,A290,A328,A330,A332,A335,A339,A340,A341,A342,A343,A344,A347,A351,A352,A358,A359,A363,A364,A365,A366,A370,A372,A373,A380,A381,A390,A3

	91,A392,A442,A461,A472,A474,A475,A481,A494,A495,A496,A498,A503,A504,A505,A522,A531,A534,A540,A547,A549,A550,A551,A552,A553,A557,A558,A559,A560,A561,A564,A566,A567,A574,A588,A600,A601,A623,A626,A638,A639,A640,A641,A647,A652,A658,A660,A673,A689,A691,A693,A694,A720,A722,A725,A727,A729,A730,A731,A739
+++++	A023,A025,A026,A045,A050,A053,A060,A061,A062,A080,A084,A085,A102,A113,A136,A143,A151,A160,A170,A171,A172,A202,A205,A210,A211,A226,A227,A240,A242,A243,A248,A278,A285,A301,A302,A303,A304,A304,A313,A316,A329,A331,A345,A346,A348,A355,A362,A371,A374,A375,A376,A377,A378,A403,A427,A447,A468,A482,A486,A507,A556,A562,A563,A565,A568,A569,A576,A579,A584,A585,A587,A589,A590,A592,A599,A613,A614,A615,A616,A617,A627,A628,A637,A642,A643,A644,A645,A646,A648,A649,A657,A663,A666,A674,A675,A692,A703,A705,A707,A708,A715,A716,A721,A723,A724,A726,A733,A735

Table 16. NRAS Q61K FRET data

IC50*	Examples
+	A388
++	A262,A297,A299,A338,A349,A350,A353,A354,A367,A368,A369,A382,A383,A384,A385,A386,A387,A389,A394,A407,A416,A417,A463,A491,A492,A493,A542,A543,A544,A545,A577,A580,A582,A594,A596,A598,A602,A603,A609,A611,A612,A620,A621,A622,A629,A631,A633,A650,A651,A653,A654,A655,A661,A662,A664,A665,A667,A668,A669,A671,A678,A679,A681,A682,A683,A686,A687,A696,A697,A698,A701,A702,A704,A709,A710,A711,A712,A718,A719,A734,A736,A737,A739
+++	A012,A022,A074,A075,A076,A077,A082,A086,A087,A105,A148,A167,A181,A207,A275,A277,A281,A300,A314,A330,A333,A340,A356,A360,A361,A379,A390,A393,A502,A537,A538,A539,A541,A546,A547,A548,A554,A555,A561,A570,A571,A572,A573,A574,A575,A578,A581,A583,A586,A588,A593,A595,A597,A604,A605,A606,A607,A608,A610,A618,A619,A623,A624,A625,A630,A632,A634,A635,A636,A656,A660,A670,A672,A676,A677,A680,A684,A685,A688,A690,A694,

	A695,A699,A700,A706,A713,A714,A717,A728,A729,A730,A731,A732,A738
++++	A016,A025,A045,A050,A060,A061,A064,A080,A083,A089,A104,A115,A143,A151,A166,A168,A172,A193,A202,A210,A211,A227,A252,A278,A290,A328,A331,A332,A335,A339,A341,A342,A343,A344,A347,A351,A352,A357,A358,A359,A363,A364,A365,A366,A370,A372,A373,A380,A381,A391,A427,A442,A461,A468,A472,A474,A475,A481,A494,A495,A496,A498,A503,A504,A505,A522,A531,A534,A540,A549,A550,A551,A552,A553,A557,A558,A559,A560,A562,A563,A564,A566,A567,A576,A590,A591,A600,A601,A626,A637,A638,A639,A640,A641,A647,A652,A659,A673,A689,A691,A693,A705,A720,A722,A725,A727,A735
+++++	A023,A026,A053,A062,A084,A085,A102,A113,A136,A160,A170,A171,A205,A226,A240,A242,A243,A248,A285,A301,A302,A303,A304,A304,A313,A316,A329,A345,A346,A348,A355,A362,A371,A374,A375,A376,A377,A378,A392,A403,A447,A482,A486,A507,A556,A565,A568,A569,A579,A584,A585,A587,A589,A592,A599,A613,A614,A615,A616,A617,A627,A628,A642,A643,A644,A645,A646,A648,A649,A657,A658,A663,A666,A674,A675,A692,A703,A707,A708,A715,A716,A721,A723,A724,A726,A733

Table 17. NRAS WT FRET data

IC50*	Examples
+	A388
++	A075,A076,A087,A167,A262,A275,A297,A299,A300,A333,A338,A349,A367,A368,A382,A383,A385,A386,A387,A389,A407,A417,A491,A492,A493,A537,A542,A577,A594,A596,A598,A602,A603,A605,A609,A612,A621,A629,A633,A664,A665,A667,A669,A679,A697,A704,A709,A711,A712,A719,A734,A736,A737
+++	A012,A016,A022,A074,A077,A082,A086,A105,A148,A166,A181,A207,A252,A277,A281,A340,A350,A351,A353,A354,A356,A357,A358,A360,A361,A369,A379,A384,A393,A394,A416,A463,A502,A538,A539,A541,A543,A544,A545,A546,A547,A548,A550,A552,A554,A555,A557,A558,A564,A570,A571,A572,A573,A575,A578,A580,A581,A582,A583,A586,A591,A593,A595,A600,A604,A606,A607,A608,A610,A611,A618,A620,A622,A624,A625,A630,A631,A632,A634,A635,A636,A647,A650,A651,A653,A654,A655,A656,A659,A660,A661,A662,A668,A670,A671,A672,A676,A677,A678,A680,A681,A682,A683,A684,A685,A686,A687,A688,

	A690,A695,A696,A698,A699,A700,A701,A702,A710,A713,A714,A717,A718,A722,A728,A729,A730,A731,A732,A738,A739
++++	A025,A045,A060,A061,A062,A064,A083,A089,A104,A115,A151,A168,A172,A193,A202,A210,A211,A227,A278,A290,A314,A328,A330,A331,A332,A335,A339,A341,A342,A343,A344,A347,A348,A352,A359,A362,A363,A364,A365,A366,A370,A371,A372,A373,A375,A376,A378,A380,A381,A390,A391,A392,A427,A447,A461,A468,A472,A474,A475,A481,A494,A495,A496,A498,A503,A504,A505,A507,A522,A531,A534,A540,A549,A551,A553,A556,A559,A560,A561,A562,A563,A566,A567,A574,A576,A579,A584,A585,A588,A589,A590,A597,A601,A616,A619,A623,A626,A637,A638,A639,A640,A641,A642,A645,A648,A652,A658,A673,A689,A691,A693,A694,A703,A705,A706,A707,A708,A716,A720,A721,A723,A725,A726,A727,A735
+++++	A023,A026,A050,A053,A080,A084,A085,A102,A113,A136,A143,A160,A170,A171,A205,A226,A240,A242,A243,A248,A285,A302,A303,A304,A304,A313,A316,A329,A345,A346,A355,A374,A377,A403,A442,A482,A486,A565,A568,A569,A587,A592,A599,A613,A614,A615,A617,A627,A628,A643,A644,A646,A649,A657,A663,A666,A674,A675,A692,A715,A724,A733

In vitro Cell Proliferation Panels

Potency for inhibition of cell growth was assessed at CrownBio using standard methods. Briefly, cell lines were cultured in appropriate medium, and then plated in 3D methylcellulose. Inhibition of cell growth was determined by CellTiter-Glo® after 5 days of culture with increasing concentrations of compounds. Compound potency was reported as the 50% inhibition concentration (absolute IC50).

The assay took place over 7 days. On day 1, cells in 2D culture were harvested during logarithmic growth and suspended in culture medium at 1x105 cells/ml. Higher or lower cell densities were used for some cell lines based on prior optimization. 3.5 ml of cell suspension was mixed with 6.5% growth medium with 1% methylcellulose, resulting in a cell suspension in 0.65% methylcellulose. 90 µl of this suspension was distributed in the wells of 2 96-well plates. One plate was used for day 0 reading and 1 plate was used for the end-point experiment. Plates were incubated overnight at 37 C with 5% CO2. On day 2, one plate (for t0 reading) was removed and 10 µl growth medium plus 100 µl CellTiter-Glo® Reagent was added to each well. After mixing and a 10 minute incubation, luminescence was recorded on an EnVision Multi-Label Reader (Perkin Elmer). Compounds in DMSO were diluted in growth medium such that the final, maximum concentration of compound was 10 µM, and serial 4-fold dilutions were performed to generate a 9-point concentration series. 10 µl of compound solution at 10 times final concentration was added to

wells of the second plate. Plate was then incubated for 120 hours at 37C and 5% CO2. On day 7 the plates were removed, 100 µl CellTiter-Glo® Reagent was added to each well, and after mixing and a 10 minute incubation, luminescence was recorded on an EnVision Multi-Label Reader (Perkin Elmer). Data was exported to GeneData Screener and modeled with a sigmoidal concentration response model in order to determine the IC50 for compound response.

Not all cell lines with a given RAS mutation may be equally sensitive to a RAS inhibitor targeting that mutation, due to differential expression of efflux transporters, varying dependencies on RAS pathway activation for growth, or other reasons. This has been exemplified by the cell line KYSE-410 which, despite8 having a KRAS G12C mutation, is insensitive to the KRAS G12C (OFF) inhibitor MRTX-849 (Hallin et al., Cancer Discovery 10:54-71 (2020)), and the cell line SW1573, which is insensitive to the KRAS G12C (OFF) inhibitor AMG510 (Canon et al., Nature 575:217-223 (2019)).

Table 18: IC50 values for various cancer cell lines with Compound B

*Key:

low sensitivity: IC50 ≥ 1 uM

moderately sensitive: 1 uM > IC50 ≥ 0.1 uM

very sensitive: IC50 < 0.1 uM

Cell Line	Histotype	Mutant	IC50*
NCI-H358	Lung	KRAS G12C	very sensitive
MIA PaCa-2	Pancreas	KRAS G12C	very sensitive
SW837	Intestine/Large/Colorectum	KRAS G12C	very sensitive
KYSE-410	HN/Esophagus	KRAS G12C	moderately sensitive
NCI-H727	Lung	KRAS G12V	moderately sensitive
OVCAR-5	Ovary	KRAS G12V	moderately sensitive
Capan-2	Pancreas	KRAS G12V	moderately sensitive
NCI-H747	Intestine/Large/Colorectum	other KRAS (G13D)	moderately sensitive
NCI-H441	Lung	KRAS G12V	moderately sensitive
HEC-1-A	Uterus	KRAS G12D	moderately sensitive
NOZ	Liver/Bile duct	KRAS G12V	moderately sensitive
HCT116	Intestine/Large/Colorectum	other KRAS (G13D)	moderately sensitive
Calu-6	Lung	other KRAS (Q61K)	moderately sensitive
HuCCT1	Liver/Bile duct	KRAS G12D	moderately sensitive

NCI-H2009	Lung	other KRAS (G12A)	low sensitivity
NCI-H1975	Lung	other MAPK (EGFR T790M, L858R)	low sensitivity
SW1573	Lung	KRAS G12C	not sensitive
AGS	Stomach	KRAS G12D	low sensitivity
AsPC-1	Pancreas	KRAS G12D	low sensitivity
SNU-668	Stomach	other KRAS (Q61K)	low sensitivity
HPAC	Pancreas	KRAS G12D	low sensitivity
NCI-H1838	Lung	other MAPK (NF1 mut)	low sensitivity
NCI-H3122	Lung	other MAPK (EML4-ALK(E13,A20))	low sensitivity
NCI-H460	Lung	other KRAS (Q61H)	low sensitivity
SW403	Intestine/Large/Colorectum	KRAS G12V	low sensitivity
A549	Lung	other KRAS (G12S)	low sensitivity
CAL-62	HN/Thyroid	other KRAS (G12R)	low sensitivity
DV-90	Lung	other KRAS (G13D)	low sensitivity
OZ	Liver/Bile duct	other KRAS (Q61L)	low sensitivity
A-375	Skin	BRAF V600E	low sensitivity
BxPC-3	Pancreas	other MAPK (BRAF V487_P492delinsA)	low sensitivity
SW48	Intestine/Large/Colorectum	not MAPK (PIK3CA G914R, EGFR G719S)	low sensitivity
HCC1588	Lung	KRAS G12D	low sensitivity
TOV-21G	Ovary	other KRAS (G13C)	low sensitivity
SW948	Intestine/Large/Colorectum	other KRAS (Q61L)	low sensitivity
LS513	Intestine/Large/Colorectum	KRAS G12D	low sensitivity
MeWo	Skin	other MAPK (NF1 mut)	low sensitivity

Table 19: Summary of IC50 results for various cancer cell lines with several compounds of the present invention (Compounds B and E-M)

*Key:

(L) low sensitivity: IC50 ≥ 1 uM

(M) moderately sensitive: 1 uM > IC50 ≥ 0.1 uM

(V) very sensitive: IC50 < 0.1 uM

Cell Line	Histotype	Mutant	E	F	G	H	I	J	K	L	B	M
SW837	Intestine/Large/ Colorectum	KRAS G12C	V	V	V	V	V	V	V	V	V	V
MIA PaCa-2	Pancreas	KRAS G12C	V	V	V	V	V	V	V	V	V	V
KYSE-410	HN/Esophagus	KRAS G12C	L	L	L	L	L	L	L	L	L	L
H358	Lung	KRAS G12C	V	V	V	V	V	V	V	V	V	V
H2122	Lung	KRAS G12C	V	V	V	V	V	V	V	V	V	V
H1373	Lung	KRAS G12C	V	V	V	V	V	V	V	V	V	V
H23	Lung	KRAS G12C	V	V	V	V	V	V	V	V	V	V
H1792	Lung	KRAS G12C	V	V	V	V	V	V	V	V	V	V
AsPC-1	Pancreas	KRAS G12D	L	L	M	L	L	M	L	L	M	L
A375	Skin	BRAF V600E	L	L	L	L	L	L	L	L	L	L
H1975	Lung	other MAPK (EGFR T790M, L858R)	M	L	M	L	L	M	L	M	M	L
HCC1588	Lung	KRAS G12D	L	L	L	L	L	L	L	L	L	L
H441	Lung	KRAS G12V	M	M	M	L	M	V	L	M	V	L

In vivo NSCLC K-Ras G12C Xenograft Models

Compound A:

Methods:

The effects of a compound of the present invention, Compound A (H358 pERK K-Ras G12C EC50: 0.001 uM), on tumor cell growth in vivo were evaluated in the human non-small cell lung cancer NCI-H358 KRASG12C xenograft model using female BALB/c nude mice (6-8 weeks old). Mice were implanted with NCI-H358 tumor cells in 50% Matrigel (5 x 106 cells/mouse) subcutaneously in the flank. At the indicated tumor volume (dotted line, FIG. 2A), mice were randomized to treatment groups to start the administration of test articles or vehicle. Compound A was administered by oral gavage daily at the dose of 100 mg/kg. Body weight and tumor volume (using calipers) was measured twice weekly until study endpoints. Spaghetti plot (FIG. 2B) shows the tumor volume change in individual tumors during the course of treatment.

Results:

FIG. 2A shows Compound A dosed at 100 mg/kg by daily oral gavage led to tumor regression in NCI-H358 KRASG12C xenograft model, which is a sensitive model to KRASG12C inhibition alone. The spaghetti titer plot (FIG. 2B) displaying individual tumor growth is shown next to the tumor volume plot (FIG. 2A). Over the treatment course of 28 days, Compound A drove tumor regression in all 10 animals bearing NCI-H358 KRASG12C tumors.

Compound B:

Methods:

The combinatorial effect of a compound of the present invention, Compound B (H358 pERK K-Ras G12C EC50: 0.003 uM), with cobimetinib on tumor cell growth in vivo were evaluated in the human non-small cell lung cancer NCI-H358 KRASG12C xenograft model using female BALB/c nude mice (6-8 weeks old). Mice were implanted with NCI-H358 tumor cells in 50% Matrigel (5 x 106 cells/mouse) subcutaneously in the flank. At indicated tumor volume (dotted line, FIG. 3A), mice were randomized to treatment groups to start the administration of test articles or vehicle. Compound B was administered by intermittent (twice weekly) intravenous injection at the dose of 50 mg/kg. Cobimetinib was administered by daily oral gavage at 2.5 mg/kg. The combination of Compound B and cobimetinib at their respective single-agent dose and regimen was also tested. Body weight and tumor volume (using calipers) was measured twice weekly until study endpoints. End of study responses in individual tumors were plotted as a waterfall plot (FIG. 3B), and the numbers indicate number of tumor regression in each group. Tumor regression is defined as greater than 10% reduction of tumor volume at the end of study relative to initial volume.

Results:

FIG. 3A shows the combination of intermittent intravenous administration of Compound B at 50 mg/kg plus daily oral administration of cobimetinib at 2.5 mg/kg drove tumor regression, whereas each single agent led to tumor growth inhibition. End of study responses were shown as

waterfall plots (FIG. 3B), which indicate 6 out 10 mice had tumor regression in the combination group, whereas no tumor regressions recorded in each single agent group.

Compound C:

Methods:

The combinatorial effect of a compound of the present invention, Compound C (H358 pERK K-Ras G12C EC50: 0.007 uM), with a SHP2 inhibitor, RMC-4550, on tumor cell growth in vivo were evaluated in the human non-small cell lung cancer NCI-H358 KRASG12C xenograft model using female BALB/c nude mice (6-8 weeks old). Mice were implanted with NCI-H358 tumor cells in 50% Matrigel (5 x 106 cells/mouse) subcutaneously in the flank. At indicated tumor volume (dotted line, FIG. 4A), mice were randomized to treatment groups to start the administration of test articles or vehicle. Compound C was administered by once weekly intravenous injection at the dose of 60 mg/kg. SHP2 inhibitor was administered by daily oral gavage at 30 mg/kg. The combination of Compound C and SHP2 inhibitor at their respective single-agent dose and regimen was also tested. Body weight and tumor volume (using calipers) was measured twice weekly until study endpoints. End of study responses in individual tumors were plotted as a waterfall plot (FIG. 4B), and the numbers indicate number of tumor regression in each group. Tumor regression is defined as greater than 10% reduction of tumor volume at the end of study relative to initial volume.

Results:

In FIG. 4A, the combinatorial activity of once weekly intravenous administration of Compound C at 60 mg/kg plus daily oral administration of SHP2 inhibitor at 30 mg/kg is shown. The combination treatment had similar anti-tumor activity as the single agent SHP2 inhibitor, but the combination treatment led to 8 out of 10 mice with tumor regression, whereas single agent SHP2 inhibitor led to 5 out of 10 mice with tumor regressions. Single agent Compound C administered once weekly via intravenous injection led to tumor growth inhibition with one tumor regression.

Cell Proliferation Assay

Methods:

NCI-H358 cells were plated in 12-well tissue culture plates at a density of 100,000 cells/well in RPMI 1640 (10% FBS, 1% PenStrep) and cultured overnight at 37°C, 5% CO2. The following day, cells were treated with either trametinib (10 nM) or a compound of the present invention, Compound D (H358 pERK K-Ras G12C EC50: 0.024 uM), (17 nM). These concentrations represent the EC50 values from a 72-hour proliferation assay using the CellTiter-Glo® reagent (Promega). Additionally, cells were treated with the combination of trametinib and Compound D at the above indicated concentrations. The plate was placed in the Incucyte S3 live cell analysis system (37°C, 5% CO2) and confluence was measured by recording images at 6-hour intervals for a maximum of 40 days, or until wells reached maximal confluence. Media and drug were replaced

at 3-4 day intervals. Data are plotted as % confluence over the time course of the experiment for each single agent and respective combination (FIG. 5).

Results:

As shown in FIG. 5, treatment of NCI-H358 cells with submaximal (EC50) concentrations of Compound D or MEK inhibitor results in a short period of growth inhibition, followed by proliferation. Cells reach maximal confluence in ~10 days after the addition of drug. The combination of the MEK inhibitor, trametinib, with Compound D resulted in complete and sustained inhibition of cell growth throughout the duration of the assay.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure come within known or customary practice within the art to which the invention pertains and may be applied to the essential features set forth herein.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

FIG. 1A

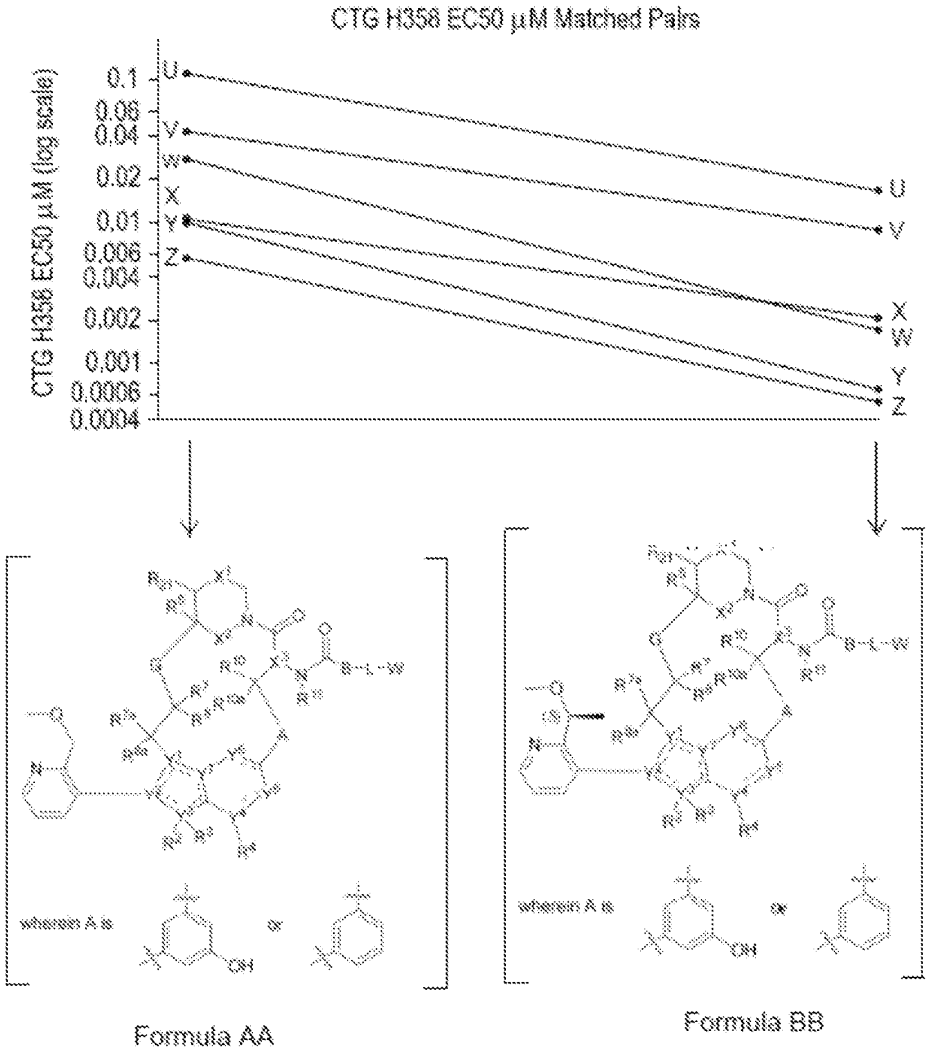

CTG H358 EC50 µM Matched Pairs
CTG H358 EC50 µM (log scale)
0.1
0.08
0.04
0.02
0.01
0.006
0.004
0.002
0.001
0.0006
0.0004
U
V
W
X
Y
Z
wherein A is
or
Formula AA
Formula BB

FIG. 1B

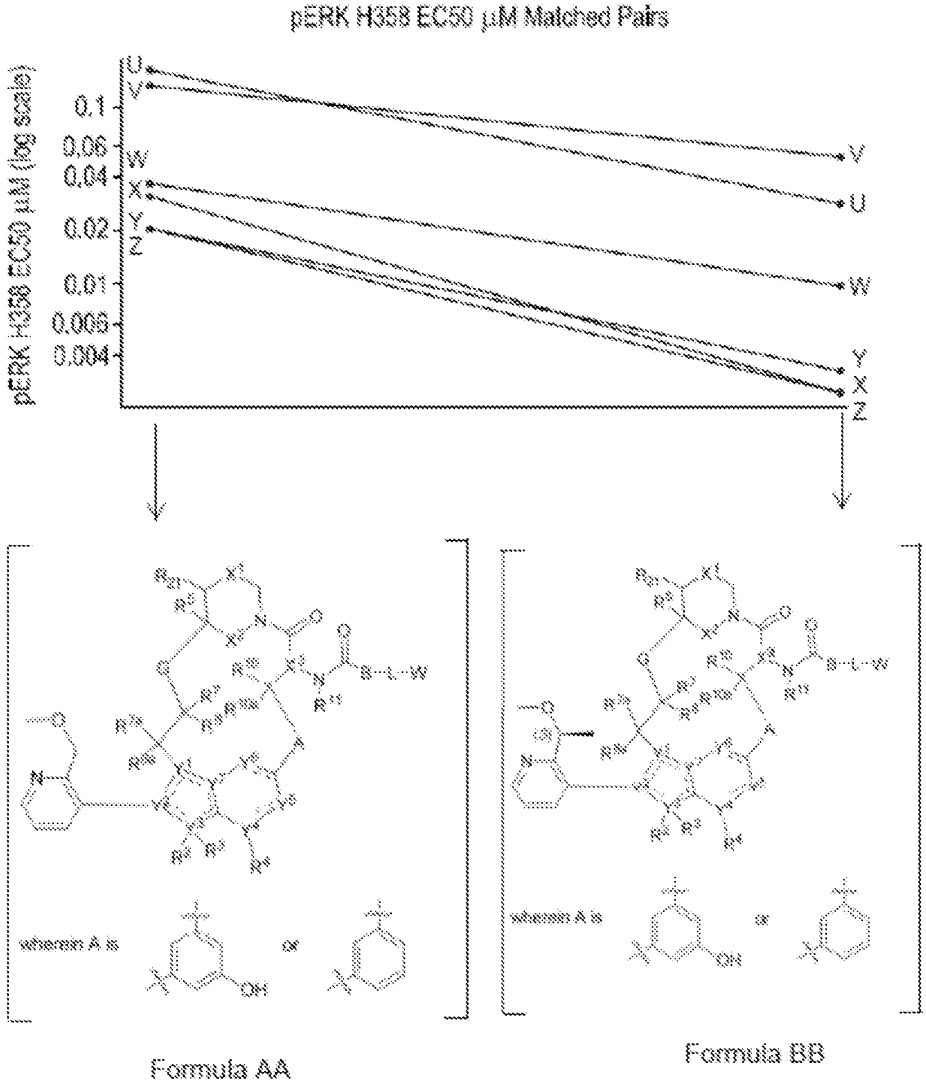

pERK H358 EC50 µM Matched Pairs
pERK H358 EC50 µM (log scale)
0.1
0.06
0.04
0.02
0.01
0.006
0.004
U
V
W
X
Y
Z
wherein A is
or
Formula AA
Formula BB

FIG. 2A

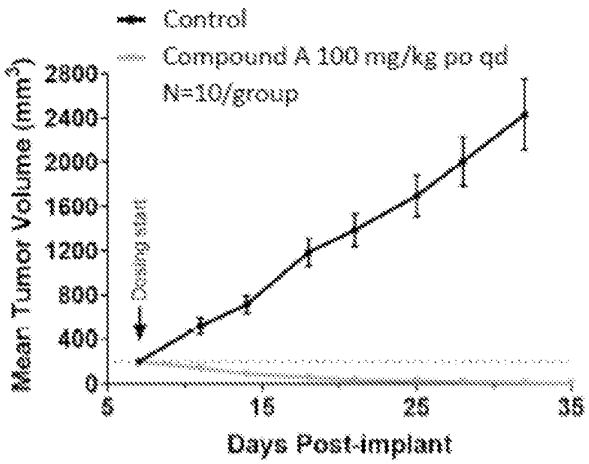

Control
Compound A 100 mg/kg po qd
N=10/group
Mean Tumor Volume (mm3)
2800
2400
2000
1600
1200
800
400
0
Dosing start
5
15
25
35
Days Post-implant

FIG. 2B

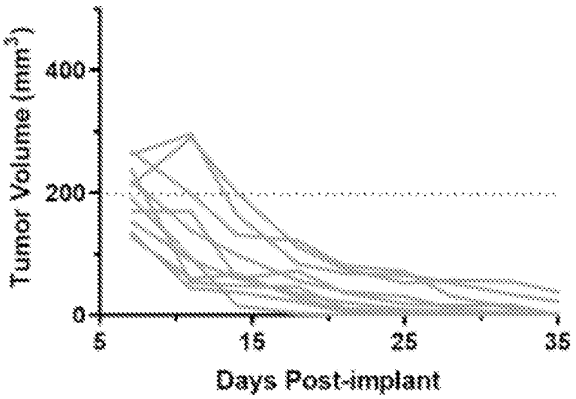

Tumor Volume (mm3)
400
200
0
5
15
25
35
Days Post-implant

FIG. 3A

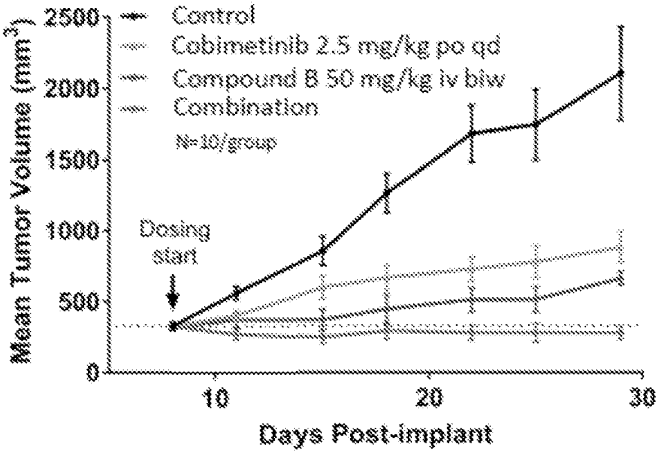

Mean Tumor Volume (mm3)
2500
2000
1500
1000
500
0
Control
Cobimetinib 2.5 mg/kg po qd
Compound B 50 mg/kg iv biw
Combination
N=10/group
Dosing start
10
20
30
Days Post-implant

FIG. 3B

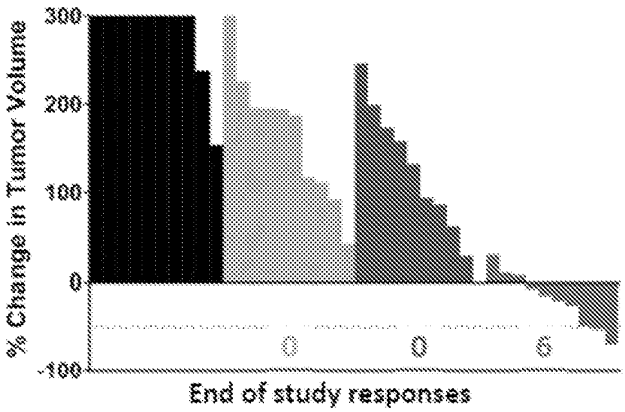

% Change in Tumor Volume
300
200
100
0
-100
0
0
6
End of study responses

FIG. 4A

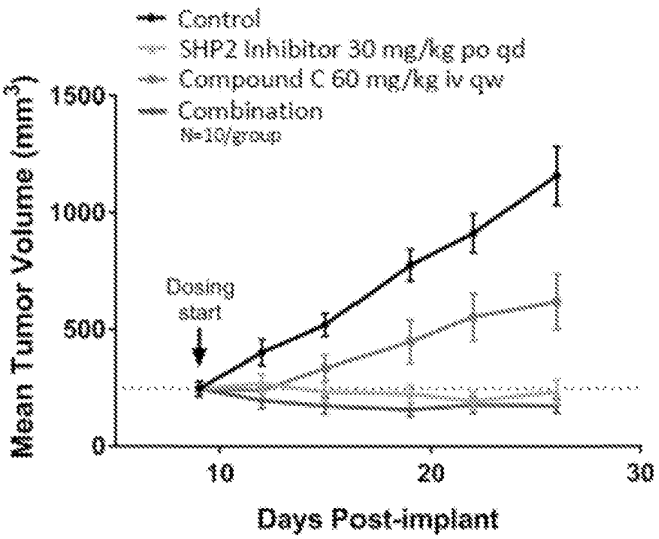

Control
SHP2 Inhibitor 30 mg/kg po qd
Compound C 60 mg/kg iv qw
Combination
N=10/group
Mean Tumor Volume (mm3)
1500
1000
500
0
Dosing start
10
20
30
Days Post-implant

FIG. 4B

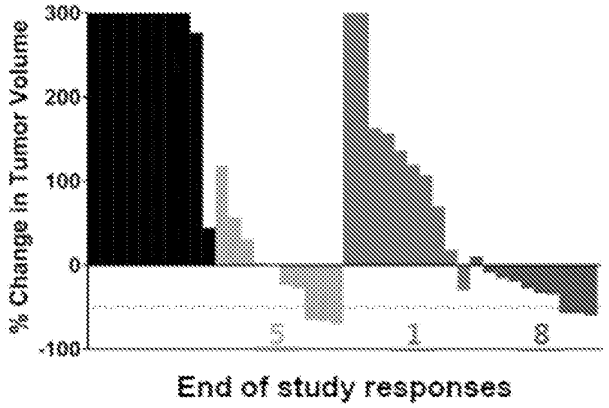
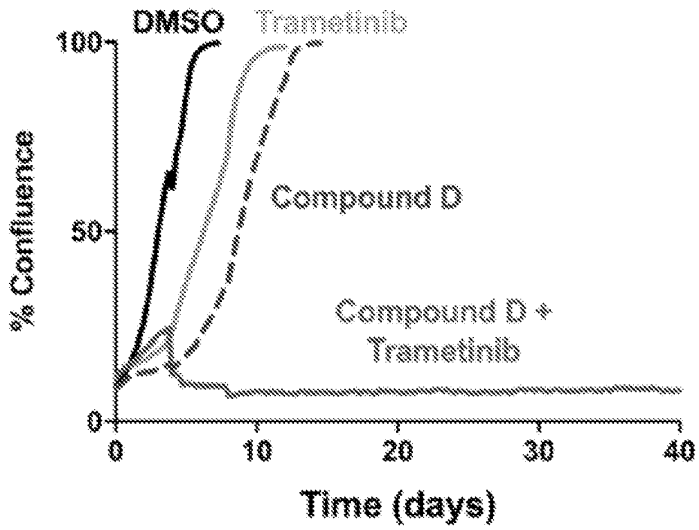

% Change in Tumor Volume
300
200
100
0
-100
5
1
8
End of study responses

FIG. 5


DMSO
Trametinib
Compound D
Compound D +
Trametinib
% Confluence
100
50
0
0
10
20
30
40
Time (days)

APPENDIX C

RAS INHIBITORS

Background

The vast majority of small molecule drugs act by binding a functionally important pocket on a target protein, thereby modulating the activity of that protein. For example, cholesterol-lowering drugs known as statins bind the enzyme active site of HMG-CoA reductase, thus preventing the enzyme from engaging with its substrates. The fact that many such drug/target interacting pairs are known may have misled some into believing that a small molecule modulator could be discovered for most, if not all, proteins provided a reasonable amount of time, effort, and resources. This is far from the case. Current estimates are that only about 10% of all human proteins are targetable by small molecules. Bojadzic and Buchwald, Curr Top Med Chem 18: 674-699 (2019). The other 90% are currently considered refractory or intractable toward above-mentioned small molecule drug discovery. Such targets are commonly referred to as “undruggable.” These undruggable targets include a vast and largely untapped reservoir of medically important human proteins. Thus, there exists a great deal of interest in discovering new molecular modalities capable of modulating the function of such undruggable targets.

It has been well established in literature that Ras proteins (K-Ras, H-Ras and N-Ras) play an essential role in various human cancers and are therefore appropriate targets for anticancer therapy. Indeed, mutations in Ras proteins account for approximately 30% of all human cancers in the United States, many of which are fatal. Dysregulation of Ras proteins by activating mutations, overexpression or upstream activation is common in human tumors, and activating mutations in Ras are frequently found in human cancer. For example, activating mutations at codon 12 in Ras proteins function by inhibiting both GTPase-activating protein (GAP)-dependent and intrinsic hydrolysis rates of GTP, significantly skewing the population of Ras mutant proteins to the “on” (GTP-bound) state (Ras(ON)), leading to oncogenic MAPK signaling. Notably, Ras exhibits a picomolar affinity for GTP, enabling Ras to be activated even in the presence of low concentrations of this nucleotide. Mutations at codons 13 (e.g., G13D) and 61 (e.g., Q61K) of Ras are also responsible for oncogenic activity in some cancers.

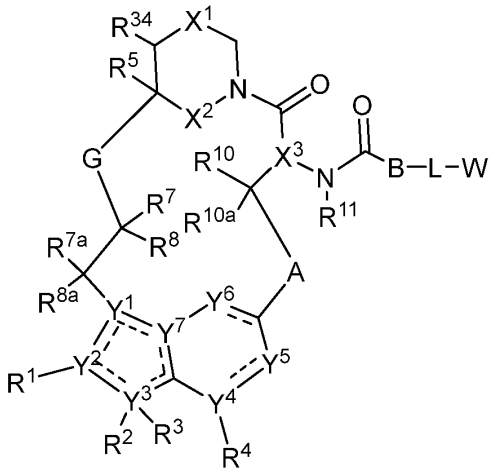
Despite extensive drug discovery efforts against Ras during the last several decades, a drug directly targeting Ras is still not approved. Additional efforts are needed to uncover additional medicines for cancers driven by the various Ras mutations.

Summary

Provided herein are Ras inhibitors. The approach described herein entails formation of a high affinity three-component complex, or conjugate, between a synthetic ligand and two intracellular proteins which do not interact under normal physiological conditions: the target protein of interest (e.g., Ras), and a widely expressed cytosolic chaperone (presenter protein) in the cell (e.g., cyclophilin A). More specifically, in some embodiments, the inhibitors of Ras described

herein induce a new binding pocket in Ras by driving formation of a high affinity tri-complex, or conjugate, between the Ras protein and the widely expressed cytosolic chaperone, cyclophilin A (CYPA). Without being bound by theory, the inventors believe that one way the inhibitory effect on Ras is effected by compounds of the invention and the complexes, or conjugates, they form is by steric occlusion of the interaction site between Ras and downstream effector molecules, such as RAF and PI3K, which are required for propagating the oncogenic signal.

As such, in some embodiments, the disclosure features a compound, or pharmaceutically acceptable salt thereof, of structural Formula I:



Formula I

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 10-membered heteroarylene;

B is -CH(R^9)- or >C=C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-

1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', $C(O)N(R')_2$, S(O)R', $S(O)_2R'$, or $S(O)_2N(R')_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is hydrogen, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{34} is hydrogen or C_1-C_3 alkyl (e.g., methyl).

Also provided are pharmaceutical compositions comprising a compound of Formula I, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

Further provided is a conjugate, or salt thereof, comprising the structure of Formula IV:

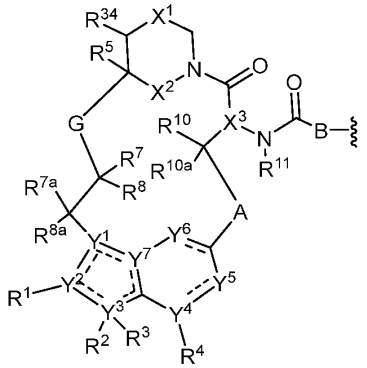
M-L-P

Formula IV

wherein L is a linker;

P is a monovalent organic moiety; and

M has the structure of Formula V:



Formula V

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- or >C=C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', $S(O)_2$R', or $S(O)_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is hydrogen, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{34} is hydrogen or C_1-C_3 alkyl (e.g., methyl).

It is specifically contemplated that any limitation discussed with respect to one embodiment of the invention may apply to any other embodiment of the invention. Furthermore, any compound or composition of the invention may be used in any method of the invention, and any method of the invention may be used to produce or to utilize any compound or composition of the invention.

Brief Description of the Figures

FIG. 1A: A compound of the present invention, Compound A, deeply and durably inhibits oncogenic signals in a pancreatic CDX model (HPAC CDX model, PDAC, KRAS G12D/WT). Single dose experiment, n = 3/time point, all dose levels well tolerated.

FIG. 1B: Treatment of KRAS G12D tumors in vivo with a compound of the present invention, Compound A, drives tumor regressions in a pancreatic CDX model (HPAC CDX model, PDAC, KRAS G12D/WT). n = 10/group, ***p<0.001. All dose levels well tolerated.

Definitions and Chemical Terms

In this application, unless otherwise clear from context, (i) the term “a” means “one or more”; (ii) the term "or" is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternative are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or"; (iii) the terms “comprising” and “including” are understood to encompass itemized components or steps whether presented by themselves or together with one or more additional components or steps; and (iv) where ranges are provided, endpoints are included.

As used herein, the term “about” is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value. In certain embodiments, the term “about” refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of a stated value, unless otherwise stated or otherwise evident from the context (e.g., where such number would exceed 100% of a possible value).

As used herein, the term “adjacent” in the context of describing adjacent atoms refers to bivalent atoms that are directly connected by a covalent bond.

A “compound of the present invention” and similar terms as used herein, whether explicitly noted or not, refers to Ras inhibitors described herein, including compounds of Formula I and subformula thereof, and compounds of Table 1 and Table 2, as well as salts (e.g., pharmaceutically acceptable salts), solvates, hydrates, stereoisomers (including atropisomers), and tautomers thereof.

The term “wild-type” refers to an entity having a structure or activity as found in nature in a “normal” (as contrasted with mutant, diseased, altered, etc) state or context. Those of ordinary skill in the art will appreciate that wild-type genes and polypeptides often exist in multiple different forms (e.g., alleles).

Those skilled in the art will appreciate that certain compounds described herein can exist in one or more different isomeric (e.g., stereoisomers, geometric isomers, atropisomers, tautomers) or isotopic (e.g., in which one or more atoms has been substituted with a different isotope of the atom, such as hydrogen substituted for deuterium) forms. Unless otherwise indicated or clear from context, a depicted structure can be understood to represent any such isomeric or isotopic form, individually or in combination.

Compounds described herein can be asymmetric (e.g., having one or more stereocenters). All stereoisomers, such as enantiomers and diastereomers, are intended unless otherwise

indicated. Compounds of the present disclosure that contain asymmetrically substituted carbon atoms can be isolated in optically active or racemic forms. Methods on how to prepare optically active forms from optically active starting materials are known in the art, such as by resolution of racemic mixtures or by stereoselective synthesis. Many geometric isomers of olefins, C=N double bonds, and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present disclosure. Cis and trans geometric isomers of the compounds of the present disclosure are described and may be isolated as a mixture of isomers or as separated isomeric forms.

In some embodiments, one or more compounds depicted herein may exist in different tautomeric forms. As will be clear from context, unless explicitly excluded, references to such compounds encompass all such tautomeric forms. In some embodiments, tautomeric forms result from the swapping of a single bond with an adjacent double bond and the concomitant migration of a proton. In certain embodiments, a tautomeric form may be a prototropic tautomer, which is an isomeric protonation states having the same empirical formula and total charge as a reference form. Examples of moieties with prototropic tautomeric forms are ketone - enol pairs, amide - imidic acid pairs, lactam - lactim pairs, amide - imidic acid pairs, enamine - imine pairs, and annular forms where a proton can occupy two or more positions of a heterocyclic system, such as, 1H- and 3H-imidazole, 1H-, 2H- and 4H-1,2,4-triazole, 1H- and 2H- isoindole, and 1H- and 2H-pyrazole. In some embodiments, tautomeric forms can be in equilibrium or sterically locked into one form by appropriate substitution. In certain embodiments, tautomeric forms result from acetal interconversion.

Unless otherwise stated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. Exemplary isotopes that can be incorporated into compounds of the present invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, sulfur, fluorine, chlorine, and iodine, such as ^{2}H, ^{3}H, ^{11}C, ^{13}C, ^{14}C, ^{13}N, ^{15}N, ^{15}O, ^{17}O, ^{18}O, ^{32}P, ^{33}P, ^{35}S, ^{18}F, ^{36}Cl, ^{123}I and ^{125}I. Isotopically-labeled compounds (e.g., those labeled with ^{3}H and ^{14}C) can be useful in compound or substrate tissue distribution assays. Tritiated (i.e., ^{3}H) and carbon-14 (i.e., ^{14}C) isotopes can be useful for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium (i.e., ^{2}H) may afford certain therapeutic advantages resulting from greater metabolic stability (e.g., increased in vivo half-life or reduced dosage requirements). In some embodiments, one or more hydrogen atoms are replaced by ^{2}H or ^{3}H, or one or more carbon atoms are replaced by ^{13}C- or ^{14}C-enriched carbon. Positron emitting isotopes such as ^{15}O, ^{13}N, ^{11}C, and ^{18}F are useful for positron emission tomography (PET) studies to examine substrate receptor occupancy. Preparations of isotopically labelled compounds are known to those of skill in the art. For example, isotopically labeled compounds can generally be prepared by following procedures analogous to those disclosed for compounds of the present invention described herein, by substituting an isotopically labeled reagent for a non-isotopically labeled reagent.

As is known in the art, many chemical entities can adopt a variety of different solid forms such as, for example, amorphous forms or crystalline forms (e.g., polymorphs, hydrates, solvate).

In some embodiments, compounds of the present invention may be utilized in any such form, including in any solid form. In some embodiments, compounds described or depicted herein may be provided or utilized in hydrate or solvate form.

At various places in the present specification, substituents of compounds of the present disclosure are disclosed in groups or in ranges. It is specifically intended that the present disclosure include each and every individual subcombination of the members of such groups and ranges. For example, the term "C_1-C_6 alkyl" is specifically intended to individually disclose methyl, ethyl, C_3 alkyl, C_4 alkyl, C_5 alkyl, and C_6 alkyl. Furthermore, where a compound includes a plurality of positions at which substituents are disclosed in groups or in ranges, unless otherwise indicated, the present disclosure is intended to cover individual compounds and groups of compounds (e.g., genera and subgenera) containing each and every individual subcombination of members at each position.

The term "optionally substituted X" (e.g., "optionally substituted alkyl") is intended to be equivalent to "X, wherein X is optionally substituted" (e.g., "alkyl, wherein said alkyl is optionally substituted"). It is not intended to mean that the feature "X" (e.g., alkyl) *per se* is optional. As described herein, certain compounds of interest may contain one or more "optionally substituted" moieties. In general, the term "substituted", whether preceded by the term "optionally" or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent, e.g., any of the substituents or groups described herein. Unless otherwise indicated, an "optionally substituted" group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. For example, in the term "optionally substituted C_1-C_6 alkyl-C_2-C_9 heteroaryl," the alkyl portion, the heteroaryl portion, or both, may be optionally substituted. Combinations of substituents envisioned by the present disclosure are preferably those that result in the formation of stable or chemically feasible compounds. The term "stable", as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain embodiments, their recovery, purification, and use for one or more of the purposes disclosed herein.

Suitable monovalent substituents on a substitutable carbon atom of an "optionally substituted" group may be, independently, deuterium;
halogen; $-(CH_2)_{0-4}R^\circ$; $-(CH_2)_{0-4}OR^\circ$; $-O(CH_2)_{0-4}R^\circ$;
$-O-(CH_2)_{0-4}C(O)OR^\circ$; $-(CH_2)_{0-4}CH(OR^\circ)_2$; $-(CH_2)_{0-4}SR^\circ$; $-(CH_2)_{0-4}Ph$, which may be substituted with R°; $-(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ which may be substituted with R°; -CH=CHPh, which may be substituted with R°; $-(CH_2)_{0-4}O(CH_2)_{0-1}$-pyridyl which may be substituted with R°; 4 to 8-membered saturated or unsaturated heterocycloalkyl (e.g., pyridyl); 3 to 8-membered saturated or unsaturated cycloalkyl (e.g., cyclopropyl, cyclobutyl, or
cyclopentyl); $-NO_2$; -CN; $-N_3$; $-(CH_2)_{0-4}N(R^\circ)_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)R^\circ$;
$-N(R^\circ)C(S)R^\circ$; -
$(CH_2)_{0-4}N(R^\circ)C(O)NR^\circ_2$; $-N(R^\circ)C(S)NR^\circ_2$; $-(CH_2)_{0-4}N(R^\circ)C(O)OR^\circ$; $-N(R^\circ)N(R^\circ)C(O)R^\circ$;

-N(R°)N(R°)C(O)NR°$_2$; -N(R°)N(R°)C(O)OR°; -(CH$_2$)$_{0-4}$C(O)R°; -C(S)R°; -(CH$_2$)$_{0-4}$C(O)OR°; -(CH$_2$)$_{0-4}$-C(O)-N(R°)$_2$; -(CH$_2$)$_{0-4}$-C(O)-N(R°)-S(O)$_2$-R°; -C(NCN)NR°$_2$; -(CH$_2$)$_{0-4}$C(O)SR°; -(CH$_2$)$_{0-4}$C(O)OSiR°$_3$; -(CH$_2$)$_{0-4}$OC(O)R°; -OC(O)(CH$_2$)$_{0-4}$SR°; -SC(S)SR°; -(CH$_2$)$_{0-4}$SC(O)R°; -(CH$_2$)$_{0-4}$C(O)NR°$_2$;

-C(S)NR°$_2$; -C(S)SR°; -(CH$_2$)$_{0-4}$OC(O)NR°$_2$; -C(O)N(OR°)R°; -C(O)C(O)R°; -C(O)CH$_2$C(O)R°; -C(NOR°)R°; -(CH$_2$)$_{0-4}$SSR°; -(CH$_2$)$_{0-4}$S(O)$_2$R°; -(CH$_2$)$_{0-4}$S(O)$_2$OR°; -(CH$_2$)$_{0-4}$OS(O)$_2$R°; -S(O)$_2$NR°$_2$; -(CH$_2$)$_{0-4}$S(O)R°; -N(R°)S(O)$_2$NR°$_2$; -N(R°)S(O)$_2$R°; -N(OR°)R°; -C(NOR°)NR°$_2$; -C(NH)NR°$_2$; -P(O)$_2$R°;

-P(O)R°$_2$; -P(O)(OR°)$_2$; -OP(O)R°$_2$; -OP(O)(OR°)$_2$; -OP(O)(OR°)R°, -SiR°$_3$; -(C$_1$-C$_4$ straight or branched alkylene)O-N(R°)$_2$; or -(C$_1$-C$_4$ straight or branched alkylene)C(O)O-N(R°)$_2$, wherein each R° may be substituted as defined below and is independently hydrogen, -C$_1$-C$_6$ aliphatic, -CH$_2$Ph, -O(CH$_2$)$_{0-1}$Ph, -CH$_2$-(5 to 6 membered heteroaryl ring), or a 3 to 6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of R°, taken together with their intervening atom(s), form a 3 to 12-membered saturated, partially unsaturated, or aryl mono- or bicyclic ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, which may be substituted as defined below.

Suitable monovalent substituents on R° (or the ring formed by taking two independent occurrences of R° together with their intervening atoms), may be, independently,

halogen, -(CH$_2$)$_{0-2}$R•,

-(haloR•), -(CH$_2$)$_{0-2}$OH, -(CH$_2$)$_{0-2}$OR•, -(CH$_2$)$_{0-2}$CH(OR•)$_2$; -O(haloR•), -CN, -N$_3$, -(CH$_2$)$_{0-2}$C(O)R•, -(CH$_2$)$_{0-2}$C(O)OH, -(CH$_2$)$_{0-2}$C(O)OR•, -(CH$_2$)$_{0-2}$SR•, -(CH$_2$)$_{0-2}$SH, -(CH$_2$)$_{0-2}$NH$_2$, -(CH$_2$)$_{0-2}$NHR•, -(CH$_2$)$_{0-2}$NR•$_2$, -NO$_2$, -SiR•$_3$, -OSiR•$_3$, -C(O)SR•, -(C$_1$-$_4$ straight or branched alkylene)C(O)OR•, or -SSR• wherein each R• is unsubstituted or where preceded by “halo” is substituted only with one or more halogens, and is independently selected from C$_1$-C$_4$ aliphatic, -CH$_2$Ph, -O(CH$_2$)$_{0-1}$Ph, or a 5 to 6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents on a saturated carbon atom of R° include =O and =S.

Suitable divalent substituents on a saturated carbon atom of an “optionally substituted” group include the following: =O, =S, =NNR*$_2$, =NNHC(O)R*, =NNHC(O)OR*, =NNHS(O)$_2$R*, =NR*, =NOR*,

-O(C(R*$_2$))$_{2-3}$O-, or -S(C(R*$_2$))$_{2-3}$S-, wherein each independent occurrence of R* is selected from hydrogen, C$_1$-C$_6$ aliphatic which may be substituted as defined below, or an unsubstituted 5 to 6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents that are bound to vicinal substitutable carbons of an “optionally substituted” group include: -O(CR*$_2$)$_{2-3}$O-, wherein each independent occurrence of R* is selected from hydrogen, C$_1$-C$_6$ aliphatic which may be substituted as defined below, or an unsubstituted 5 to 6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

Suitable substituents on the aliphatic group of R^* include halogen, $-R^\bullet$, $-(haloR^\bullet)$, $-OH$, $-OR^\bullet$, $-O(haloR^\bullet)$, $-CN$, $-C(O)OH$, $-C(O)OR^\bullet$, $-NH_2$, $-NHR^\bullet$, $-NR^\bullet_2$, or $-NO_2$, wherein each $R^\bullet$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_1-C_4 aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5 to 6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

Suitable substituents on a substitutable nitrogen of an "optionally substituted" group include $-R^\dagger$, $-NR^\dagger_2$, $-C(O)R^\dagger$, $-C(O)OR^\dagger$, $-C(O)C(O)R^\dagger$, $-C(O)CH_2C(O)R^\dagger$, $-S(O)_2R^\dagger$, $-S(O)_2NR^\dagger_2$, $-C(S)NR^\dagger_2$, $-C(NH)NR^\dagger_2$, or $-N(R^\dagger)S(O)_2R^\dagger$; wherein each $R^\dagger$ is independently hydrogen, C_1-C_6 aliphatic which may be substituted as defined below, unsubstituted $-OPh$, or an unsubstituted 3 to 6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of $R^\dagger$, taken together with their intervening atom(s) form an unsubstituted 3 to 12-membered saturated, partially unsaturated, or aryl mono- or bicyclic ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

Suitable substituents on an aliphatic group of $R^\dagger$ are independently halogen, $-R^\bullet$, $-(haloR^\bullet)$, $-OH$, $-OR^\bullet$, $-O(haloR^\bullet)$, $-CN$, $-C(O)OH$, $-C(O)OR^\bullet$, $-NH_2$, $-NHR^\bullet$, $-NR^\bullet_2$, or $-NO_2$, wherein each $R^\bullet$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_1-C_4 aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 5 to 6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents on a saturated carbon atom of $R^\dagger$ include =O and =S.

The term "acetyl," as used herein, refers to the group $-C(O)CH_3$.

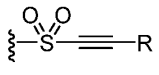
The term "alkoxy," as used herein, refers to a $-O$-C_1-C_{20} alkyl group, wherein the alkoxy group is attached to the remainder of the compound through an oxygen atom.

The term "alkyl," as used herein, refers to a saturated, straight or branched monovalent hydrocarbon group containing from 1 to 20 (e.g., from 1 to 10 or from 1 to 6) carbons. In some embodiments, an alkyl group is unbranched (i.e., is linear); in some embodiments, an alkyl group is branched. Alkyl groups are exemplified by, but not limited to, methyl, ethyl, *n*- and *iso*-propyl, *n*-, *sec*-, *iso*- and *tert*-butyl, and neopentyl.

The term "alkylene," as used herein, represents a saturated divalent hydrocarbon group derived from a straight or branched chain saturated hydrocarbon by the removal of two hydrogen atoms, and is exemplified by methylene, ethylene, isopropylene, and the like. The term "C_x-C_y alkylene" represents alkylene groups having between x and y carbons. Exemplary values for x are 1, 2, 3, 4, 5, and 6, and exemplary values for y are 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, or 20 (e.g., C_1-C_6, C_1-C_{10}, C_2-C_{20}, C_2-C_6, C_2-C_{10}, or C_2-C_{20} alkylene). In some embodiments, the alkylene can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein.

The term “alkenyl,” as used herein, represents monovalent straight or branched chain groups of, unless otherwise specified, from 2 to 20 carbons (e.g., from 2 to 6 or from 2 to 10 carbons) containing one or more carbon-carbon double bonds and is exemplified by ethenyl, 1-propenyl, 2-propenyl, 2-methyl-1-propenyl, 1-butenyl, and 2-butenyl. Alkenyls include both cis and trans isomers. The term “alkenylene,” as used herein, represents a divalent straight or branched chain groups of, unless otherwise specified, from 2 to 20 carbons (e.g., from 2 to 6 or from 2 to 10 carbons) containing one or more carbon-carbon double bonds.

The term “alkynyl,” as used herein, represents monovalent straight or branched chain groups from 2 to 20 carbon atoms (e.g., from 2 to 4, from 2 to 6, or from 2 to 10 carbons) containing a carbon-carbon triple bond and is exemplified by ethynyl, and 1-propynyl.

The term “alkynyl sulfone,” as used herein, represents a group comprising the structure  , wherein R is any chemically feasible substituent described herein.

The term “amino,” as used herein, represents $-N(R^{\dagger})_2$, e.g., $-NH_2$ and $-N(CH_3)_2$.

The term “aminoalkyl,” as used herein, represents an alkyl moiety substituted on one or more carbon atoms with one or more amino moieties.

The term “amino acid,” as described herein, refers to a molecule having a side chain, an amino group, and an acid group (e.g., $-CO_2H$ or $-SO_3H$), wherein the amino acid is attached to the parent molecular group by the side chain, amino group, or acid group (e.g., the side chain). As used herein, the term “amino acid” in its broadest sense, refers to any compound or substance that can be incorporated into a polypeptide chain, e.g., through formation of one or more peptide bonds. In some embodiments, an amino acid has the general structure H_2N-C(H)(R)-COOH. In some embodiments, an amino acid is a naturally-occurring amino acid. In some embodiments, an amino acid is a synthetic amino acid; in some embodiments, an amino acid is a D-amino acid; in some embodiments, an amino acid is an L-amino acid. “Standard amino acid” refers to any of the twenty standard L-amino acids commonly found in naturally occurring peptides. Exemplary amino acids include alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, optionally substituted hydroxylnorvaline, isoleucine, leucine, lysine, methionine, norvaline, ornithine, phenylalanine, proline, pyrrolysine, selenocysteine, serine, taurine, threonine, tryptophan, tyrosine, and valine.

The term “aryl,” as used herein, represents a monovalent monocyclic, bicyclic, or multicyclic ring system formed by carbon atoms, wherein the ring attached to the pendant group is aromatic. Examples of aryl groups are phenyl, naphthyl, phenanthrenyl, and anthracenyl. An aryl ring can be attached to its pendant group at any heteroatom or carbon ring atom that results in a stable structure and any of the ring atoms can be optionally substituted unless otherwise specified.

The term “C_0,” as used herein, represents a bond. For example, part of the term -N(C(O)-(C_0-C_5 alkylene-H)- includes -N(C(O)-(C_0 alkylene-H)-, which is also represented by -N(C(O)-H)-.

The terms “carbocyclic” and “carbocyclyl,” as used herein, refer to a monovalent, optionally substituted 3 to 12-membered monocyclic, bicyclic, or tricyclic ring structure, which may be bridged,

fused or spirocyclic, in which all the rings are formed by carbon atoms and at least one ring is non-aromatic. Carbocyclic structures include cycloalkyl, cycloalkenyl, and cycloalkynyl groups. Examples of carbocyclyl groups are cyclohexyl, cyclohexenyl, cyclooctynyl, 1,2-dihydronaphthyl, 1,2,3,4-tetrahydronaphthyl, fluorenyl, indenyl, indanyl, decalinyl, and the like. A carbocyclic ring can be attached to its pendant group at any ring atom that results in a stable structure and any of the ring atoms can be optionally substituted unless otherwise specified.

The term "carbonyl," as used herein, represents a C(O) group, which can also be represented as C=O.

The term "carboxyl," as used herein, means $-CO_2H$, (C=O)(OH), COOH, or C(O)OH or the unprotonated counterparts.

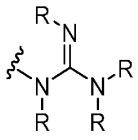
The term "cyano," as used herein, represents a -CN group.

The term "cycloalkyl," as used herein, represents a monovalent saturated cyclic hydrocarbon group, which may be bridged, fused, or spirocyclic having from three to eight ring carbons, unless otherwise specified, and is exemplified by cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cycloheptyl.

The term "cycloalkenyl," as used herein, represents a monovalent, non-aromatic, saturated cyclic hydrocarbon group, which may be bridged, fused, or spirocyclic having from three to eight ring carbons, unless otherwise specified, and containing one or more carbon-carbon double bonds.

The term "diastereomer," as used herein, means stereoisomers that are not mirror images of one another and are non-superimposable on one another.

The term "enantiomer," as used herein, means each individual optically active form of a compound of the invention, having an optical purity or enantiomeric excess (as determined by methods standard in the art) of at least 80% (i.e., at least 90% of one enantiomer and at most 10% of the other enantiomer), preferably at least 90% and more preferably at least 98%.

The term "guanidinyl," refers to a group having the structure:  , wherein each R is, independently, any any chemically feasible substituent described herein.

The term "guanidinoalkyl alkyl," as used herein, represents an alkyl moiety substituted on one or more carbon atoms with one or more guanidinyl moieties.

The term "haloacetyl," as used herein, refers to an acetyl group wherein at least one of the hydrogens has been replaced by a halogen.

The term "haloalkyl," as used herein, represents an alkyl moiety substituted on one or more carbon atoms with one or more of the same of different halogen moieties.

The term "halogen," as used herein, represents a halogen selected from bromine, chlorine, iodine, or fluorine.

The term "heteroalkyl," as used herein, refers to an "alkyl" group, as defined herein, in which at least one carbon atom has been replaced with a heteroatom (e.g., an O, N, or S atom). The heteroatom may appear in the middle or at the end of the radical.

The term “heteroaryl,” as used herein, represents a monovalent, monocyclic or polycyclic ring structure that contains at least one fully aromatic ring: i.e., they contain 4*n*+2 pi electrons within the monocyclic or polycyclic ring system and contains at least one ring heteroatom selected from N, O, or S in that aromatic ring. Exemplary unsubstituted heteroaryl groups are of 1 to 12 (e.g., 1 to 11, 1 to 10, 1 to 9, 2 to 12, 2 to 11, 2 to 10, or 2 to 9) carbons. The term “heteroaryl” includes bicyclic, tricyclic, and tetracyclic groups in which any of the above heteroaromatic rings is fused to one or more, aryl or carbocyclic rings, e.g., a phenyl ring, or a cyclohexane ring. Examples of heteroaryl groups include, but are not limited to, pyridyl, pyrazolyl, benzooxazolyl, benzoimidazolyl, benzothiazolyl, imidazolyl, thiazolyl, quinolinyl, tetrahydroquinolinyl, and 4-azaindolyl. A heteroaryl ring can be attached to its pendant group at any ring atom that results in a stable structure and any of the ring atoms can be optionally substituted unless otherwise specified. In some embodiment, the heteroaryl is substituted with 1, 2, 3, or 4 substituents groups.

The term “heterocycloalkyl,” as used herein, represents a monovalent, monocyclic, bicyclic or polycyclic ring system, which may be bridged, fused, or spirocyclic, wherein at least one ring is non-aromatic and wherein the non-aromatic ring contains one, two, three, or four heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur. The 5-membered ring has zero to two double bonds, and the 6- and 7-membered rings have zero to three double bonds. Exemplary unsubstituted heterocycloalkyl groups are of 1 to 12 (e.g., 1 to 11, 1 to 10, 1 to 9, 2 to 12, 2 to 11, 2 to 10, or 2 to 9) carbons. The term “heterocycloalkyl” also represents a heterocyclic compound having a bridged multicyclic structure in which one or more carbons or heteroatoms bridges two non-adjacent members of a monocyclic ring, e.g., a quinuclidinyl group. The term “heterocycloalkyl” includes bicyclic, tricyclic, and tetracyclic groups in which any of the above heterocyclic rings is fused to one or more aromatic, carbocyclic, heteroaromatic, or heterocyclic rings, e.g., an aryl ring, a cyclohexane ring, a cyclohexene ring, a cyclopentane ring, a cyclopentene ring, a pyridine ring, or a pyrrolidine ring. Examples of heterocycloalkyl groups are pyrrolidinyl, piperidinyl, 1,2,3,4-tetrahydroquinolinyl, decahydroquinolinyl, dihydropyrrolopyridine, and decahydronapthyridinyl. A heterocycloalkyl ring can be attached to its pendant group at any ring atom that results in a stable structure and any of the ring atoms can be optionally substituted unless otherwise specified.

The term “hydroxy,” as used herein, represents a -OH group.

The term “hydroxyalkyl,” as used herein, represents an alkyl moiety substituted on one or more carbon atoms with one or more -OH moieties.

The term “isomer,” as used herein, means any tautomer, stereoisomer, atropiosmer, enantiomer, or diastereomer of any compound of the invention. It is recognized that the compounds of the invention can have one or more chiral centers or double bonds and, therefore, exist as stereoisomers, such as double-bond isomers (i.e., geometric E/Z isomers) or diastereomers (e.g., enantiomers (i.e., (+) or (-)) or cis/trans isomers). According to the invention, the chemical structures depicted herein, and therefore the compounds of the invention, encompass all the corresponding stereoisomers, that is, both the stereomerically pure form (e.g., geometrically pure, enantiomerically pure, or diastereomerically pure) and enantiomeric and stereoisomeric

mixtures, e.g., racemates. Enantiomeric and stereoisomeric mixtures of compounds of the invention can typically be resolved into their component enantiomers or stereoisomers by well-known methods, such as chiral-phase gas chromatography, chiral-phase high performance liquid chromatography, crystallizing the compound as a chiral salt complex, or crystallizing the compound in a chiral solvent. Enantiomers and stereoisomers can also be obtained from stereomerically or enantiomerically pure intermediates, reagents, and catalysts by well-known asymmetric synthetic methods.

As used herein, the term "linker" refers to a divalent organic moiety connecting moiety B to moiety W in a compound of Formula I, such that the resulting compound is capable of achieving an IC50 of 2 uM or less in the Ras-RAF disruption assay protocol provided in the Examples below, and provided here:

The purpose of this biochemical assay is to measure the ability of test compounds to facilitate ternary complex formation between a nucleotide-loaded Ras isoform and cyclophilin A; the resulting ternary complex disrupts binding to a $BRAF^{RBD}$ construct, inhibiting Ras signaling through a RAF effector.

In assay buffer containing 25 mM HEPES pH 7.3, 0.002% Tween20, 0.1% BSA, 100 mM NaCl and 5 mM $MgCl_2$, tagless Cyclophilin A, His6-K-Ras-GMPPNP (or other Ras variant), and GST-$BRAF^{RBD}$ are combined in a 384-well assay plate at final concentrations of 25 µM, 12.5 nM and 50 nM, respectively. Compound is present in plate wells as a 10-point 3-fold dilution series starting at a final concentration of 30 µM. After incubation at 25°C for 3 hours, a mixture of Anti-His Eu-W1024 and anti-GST allophycocyanin is then added to assay sample wells at final concentrations of 10 nM and 50 nM, respectively, and the reaction incubated for an additional 1.5 hours. TR-FRET signal is read on a microplate reader (Ex 320 nm, Em 665/615 nm). Compounds that facilitate disruption of a Ras:RAF complex are identified as those eliciting a decrease in the TR-FRET ratio relative to DMSO control wells.

In some embodiments, the linker comprises 20 or fewer linear atoms. In some embodiments, the linker comprises 15 or fewer linear atoms. In some embodiments, the linker comprises 10 or fewer linear atoms. In some embodiments, the linker has a molecular weight of under 500 g/mol. In some embodiments, the linker has a molecular weight of under 400 g/mol. In some embodiments, the linker has a molecular weight of under 300 g/mol. In some embodiments, the linker has a molecular weight of under 200 g/mol. In some embodiments, the linker has a molecular weight of under 100 g/mol. In some embodiments, the linker has a molecular weight of under 50 g/mol.

As used herein, a "monovalent organic moiety" is less than 500 kDa. In some embodiments, a "monovalent organic moiety" is less than 400 kDa. In some embodiments, a "monovalent organic moiety" is less than 300 kDa. In some embodiments, a "monovalent organic moiety" is less than 200 kDa. In some embodiments, a "monovalent organic moiety" is less than 100 kDa. In some embodiments, a "monovalent organic moiety" is less than 50 kDa. In some embodiments, a "monovalent organic moiety" is less than 25 kDa. In some embodiments, a

"monovalent organic moiety" is less than 20 kDa. In some embodiments, a "monovalent organic moiety" is less than 15 kDa. In some embodiments, a "monovalent organic moiety" is less than 10 kDa. In some embodiments, a "monovalent organic moiety" is less than 1 kDa. In some embodiments, a "monovalent organic moiety" is less than 500 g/mol. In some embodiments, a "monovalent organic moiety" ranges between 500 g/mol and 500 kDa.

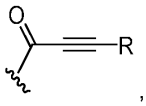
The term "stereoisomer," as used herein, refers to all possible different isomeric as well as conformational forms which a compound may possess (e.g., a compound of any formula described herein), in particular all possible stereochemically and conformationally isomeric forms, all diastereomers, enantiomers or conformers of the basic molecular structure, including atropisomers. Some compounds of the present invention may exist in different tautomeric forms, all of the latter being included within the scope of the present invention.

The term "sulfonyl," as used herein, represents an -$S(O)_2$- group.

The term "thiocarbonyl," as used herein, refers to a -C(S)- group.

The term "vinyl ketone," as used herein, refers to a group comprising a carbonyl group directly connected to a carbon-carbon double bond.

The term "vinyl sulfone," as used herein, refers to a group comprising a sulfonyl group directed connected to a carbon-carbon double bond.

The term "ynone," as used herein, refers to a group comprising the structure 

, wherein R is any any chemically feasible substituent described herein.

Those of ordinary skill in the art, reading the present disclosure, will appreciate that certain compounds described herein may be provided or utilized in any of a variety of forms such as, for example, salt forms, protected forms, pro-drug forms, ester forms, isomeric forms (e.g., optical or structural isomers), isotopic forms, etc. In some embodiments, reference to a particular compound may relate to a specific form of that compound. In some embodiments, reference to a particular compound may relate to that compound in any form. In some embodiments, for example, a preparation of a single stereoisomer of a compound may be considered to be a different form of the compound than a racemic mixture of the compound; a particular salt of a compound may be considered to be a different form from another salt form of the compound; a preparation containing one conformational isomer ((Z) or (E)) of a double bond may be considered to be a different form from one containing the other conformational isomer ((E) or (Z)) of the double bond; a preparation in which one or more atoms is a different isotope than is present in a reference preparation may be considered to be a different form.

Detailed Description

Compounds

Provided herein are Ras inhibitors. The approach described herein entails formation of a high affinity three-component complex, or conjugate, between a synthetic ligand and two intracellular proteins which do not interact under normal physiological conditions: the target protein

of interest (e.g., Ras), and a widely expressed cytosolic chaperone (presenter protein) in the cell (e.g., cyclophilin A). More specifically, in some embodiments, the inhibitors of Ras described herein induce a new binding pocket in Ras by driving formation of a high affinity tri-complex, or conjugate, between the Ras protein and the widely expressed cytosolic chaperone, cyclophilin A (CYPA). Without being bound by theory, the inventors believe that one way the inhibitory effect on Ras is effected by compounds of the invention and the complexes, or conjugates, they form is by steric occlusion of the interaction site between Ras and downstream effector molecules, such as RAF, which are required for propagating the oncogenic signal.

Without being bound by theory, the inventors postulate that both covalent and non-covalent interactions of a compound of the present invention with Ras and the chaperone protein (e.g., cyclophilin A) may contribute to the inhibition of Ras activity. In some embodiments, a compound of the present invention forms a covalent adduct with a side chain of a Ras protein (e.g., the $-CH_2-COOH$ or $-CH_2-COO-$ side chain of the aspartic acid at position 12 or 13 of a mutant Ras protein). Covalent adducts may also be formed with other side chains of Ras. In addition or alternatively, non-covalent interactions may be at play: for example, van der Waals, hydrophobic, hydrophilic, and hydrogen bond interactions, and combinations thereof, may contribute to the ability of the compounds of the present invention to form complexes and act as Ras inhibitors. Accordingly, a variety of Ras proteins may be inhibited by compounds of the present invention (e.g., K-Ras, N-Ras, H-Ras, and mutants thereof at positions 12, 13 and 61, such as G12C, G12D, G12V, G12S, G13C, G13D, and Q61L, and others described herein).

Methods of determining covalent adduct formation are known in the art. One method of determining covalent adduct formation is to perform a “cross-linking” assay, such as described in the Examples, and below:

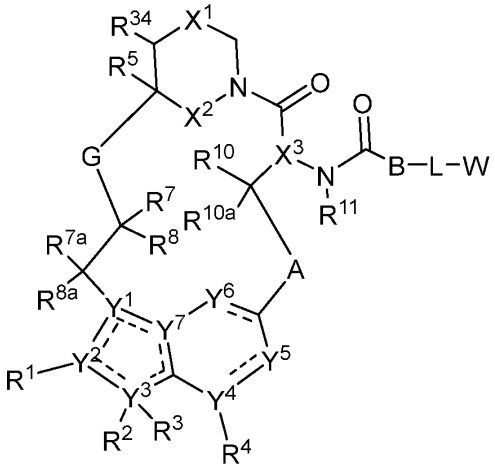
Note – the following protocol describes a procedure for monitoring cross-linking of K-Ras G12C (GMP-PNP) to a compound of the invention. This protocol may also be executed substituting other Ras proteins or nucleotides, such as K-Ras G12D.

The purpose of this biochemical assay is to measure the ability of test compounds to covalently label *nucleotide*-loaded K-Ras isoforms. In assay buffer containing 12.5 mM HEPES pH 7.4, 75 mM NaCl, 1 mM $MgCl_2$, 1 mM BME, 5 µM Cyclophilin A and 2 µM test compound, a 5 µM stock of GMP-PNP-loaded K-Ras (1-169) G12C is diluted 10-fold to yield a final concentration of 0.5 µM; with final sample volume being 100 µL.

The sample is incubated at 25°C for a time period of up to 24 hours prior to quenching by the addition of 10 µL of 5% Formic Acid. Quenched samples are centrifuged at 15000 rpm for 15 minutes in a benchtop centrifuge before injecting a 10 µL aliquot onto a reverse phase C4 column and eluting into the mass spectrometer with an increasing acetonitrile gradient in the mobile phase. Analysis of raw data may be carried out using Waters

MassLynx MS software, with % bound calculated from the deconvoluted protein peaks for labeled and unlabeled K-Ras.

Accordingly, provided herein is a compound, or pharmaceutically acceptable salt thereof, having the structure of Formula I:



Formula I

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 10-membered heteroarylene;

B is -CH(R^9)- or >C=C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', $C(O)N(R')_2$, S(O)R', $S(O)_2R'$, or $S(O)_2N(R')_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), $C=N(O\text{-}C_1\text{-}C_3$ alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is hydrogen, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo; and

R^{11} is hydrogen or C_1-C_3 alkyl; and

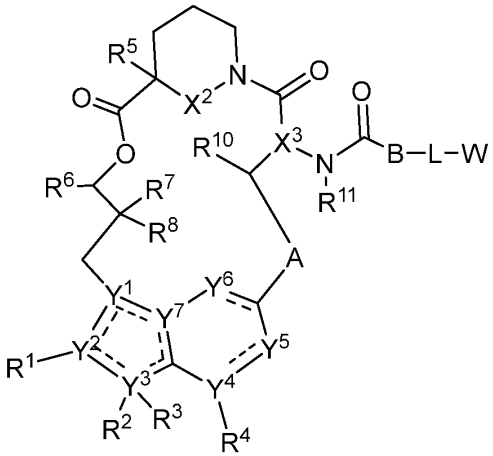
R^{34} is hydrogen or C_1-C_3 alkyl (e.g., methyl).

In some embodiments, R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

In some embodiments, R^{34} is hydrogen.

In some embodiments of compounds of the present invention, G is optionally substituted C_1-C_4 heteroalkylene.

In some embodiments, a compound of the present invention has the structure of Formula Ia, or a pharmaceutically acceptable salt thereof:



Formula Ia

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

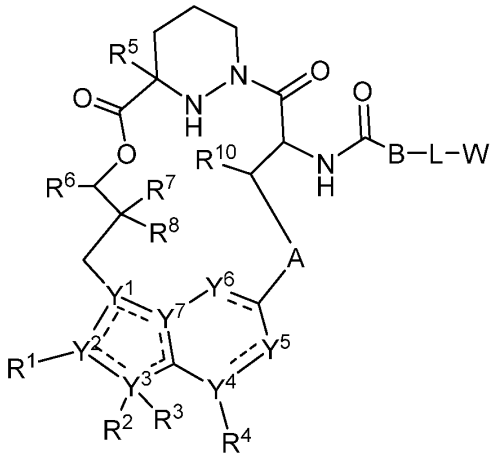
R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl; and

R^{11} is hydrogen or C_1-C_3 alkyl.

In some embodiments of compounds of the present invention, X^2 is NH. In some embodiments, X^3 is CH.

In some embodiments of compounds of the present invention, R^{11} is hydrogen. In some embodiments, R^{11} is C_1-C_3 alkyl, such as methyl.

In some embodiments, a compound of the present invention has the structure of Formula Ib, or a pharmaceutically acceptable salt thereof:



Formula Ib

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; and

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl.

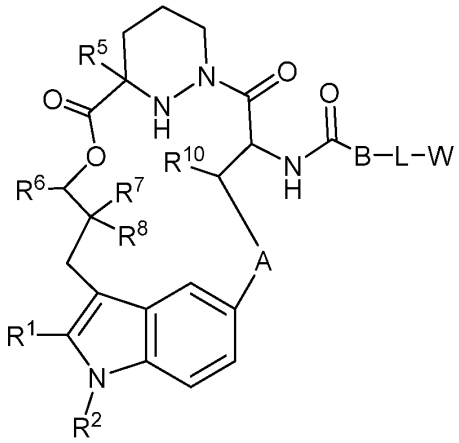
In some embodiments of compounds of the present invention, X^1 is optionally substituted C_1-C_2 alkylene. In some embodiments, X^1 is methylene.

In some embodiments of compounds of the present invention, R^4 is hydrogen.

In some embodiments of compounds of the present invention, R^5 is hydrogen. In some embodiments, R^5 is C_1-C_4 alkyl optionally substituted with halogen. In some embodiments, R^5 is methyl.

In some embodiments of compounds of the present invention, Y^4 is C. In some embodiments, R^4 is hydrogen. In some embodiments, Y^5 is CH. In some embodiments, Y^6 is CH. In some embodiments, Y^1 is C. In some embodiments, Y^2 is C. In some embodiments, Y^3 is N. In some embodiments, R^3 is absent. In some embodiments, Y^7 is C.

In some embodiments, a compound of the present invention has the structure of Formula Ic, or a pharmaceutically acceptable salt thereof:



Formula Ic

wherein A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; and

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl.

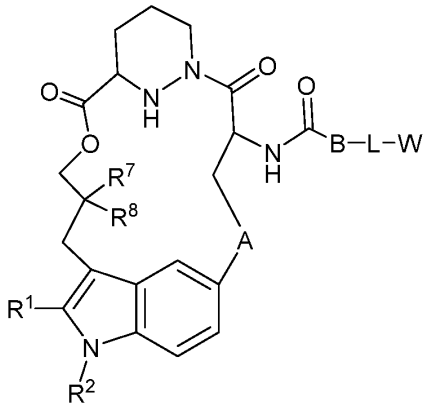
In some embodiments of compounds of the present invention, R^6 is hydrogen.

In some embodiments of compounds of the present invention, R^2 is hydrogen, cyano, optionally substituted C_1-C_6 alkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 6-membered heterocycloalkyl. In some embodiments, R^2 is optionally substituted C_1-C_6 alkyl, such as ethyl.

In some embodiments of compounds of the present invention, R^7 is optionally substituted C_1-C_3 alkyl. In some embodiments, R^7 is C_1-C_3 alkyl.

In some embodiments of compounds of the present invention, R^8 is optionally substituted C_1-C_3 alkyl. In some embodiments, R^8 is C_1-C_3 alkyl.

In some embodiments, a compound of the present invention has the structure of Formula Id, or a pharmaceutically acceptable salt thereof:



Formula Id

wherein A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is C_1-C_6 alkyl or 3 to 6-membered cycloalkyl;

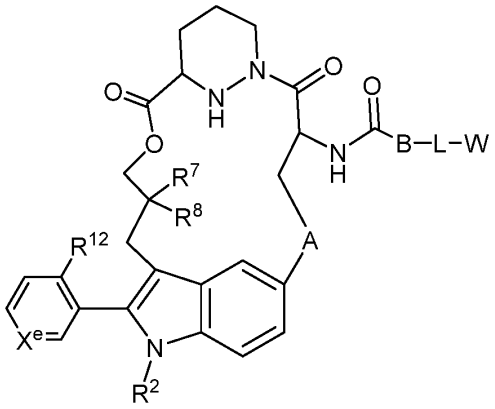
R^7 is C_1-C_3 alkyl;

R^8 is C_1-C_3 alkyl; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

In some embodiments of compounds of the present invention, R^1 is 5 to 10-membered heteroaryl. In some embodiments, R^1 is optionally substituted 6-membered aryl or optionally substituted 6-membered heteroaryl.

In some embodiments, a compound of the present invention has the structure of Formula Ie, or a pharmaceutically acceptable salt thereof:



Formula Ie

wherein A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

R^2 is C_1-C_6 alkyl or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

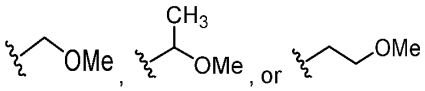
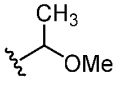
R^8 is C_1-C_3 alkyl; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl

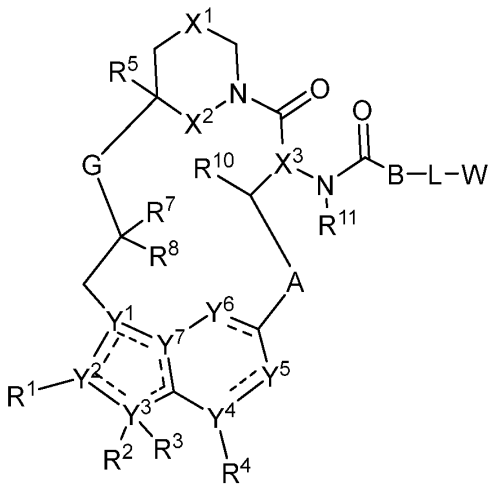
X^e is N or CH; and

R^{12} is optionally substituted C_1-C_6 alkyl or optionally substituted C_1-C_6 heteroalkyl.

In some embodiments of compounds of the present invention, X^e is N. In some embodiments, X^e is CH.

In some embodiments of compounds of the present invention, R^{12} is optionally substituted C_1-C_6 heteroalkyl. In some embodiments, R^{12} is  . In some embodiments, R^{12} is  .

In some embodiments, a compound of the present invention has the structure of Formula If, or a pharmaceutically acceptable salt thereof:



Formula If

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-

1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', $C(O)N(R')_2$, S(O)R', $S(O)_2R'$, or $S(O)_2N(R')_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), $C=N(O\text{-}C_1\text{-}C_3$ alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

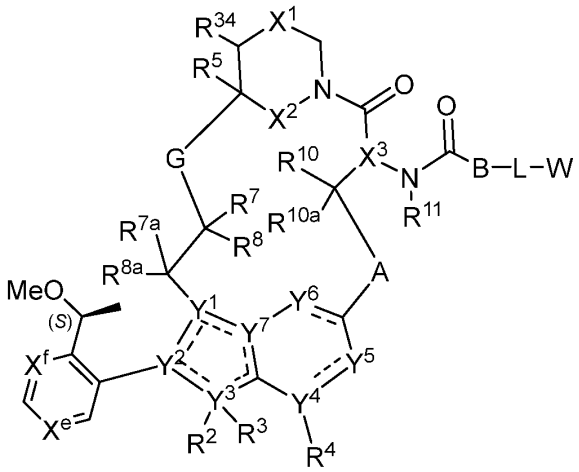
$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl; and

R^{11} is hydrogen or C_1-C_3 alkyl.

In some embodiments, a compound of the present invention has the structure of Formula VI, or a pharmaceutically acceptable salt thereof:



Formula VI

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene (e.g., phenyl or phenol), or optionally substituted 5 to 10-membered heteroarylene;

B is -CH(R^9)- or >C=C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', $C(O)N(R')_2$, S(O)R', $S(O)_2R'$, or $S(O)_2N(R')_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), $C=N(O\text{-}C_1\text{-}C_3$ alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is hydrogen, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

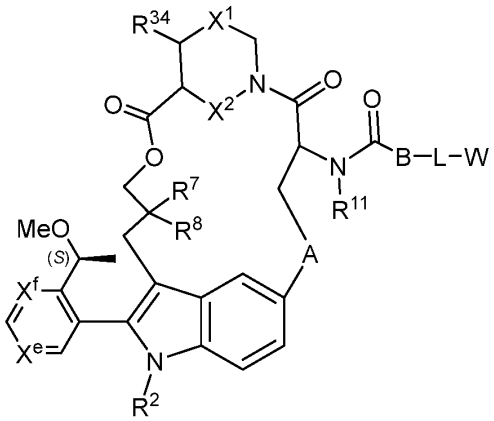
R^{10a} is hydrogen or halo;

R^{11} is hydrogen or C_1-C_3 alkyl;

R^{34} is hydrogen or C_1-C_3 alkyl; and

X^e and X^f are, independently, N or CH.

In some embodiments, a compound of the present invention has the structure of Formula VIa, or a pharmaceutically acceptable salt thereof:



Formula VIa

wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene (e.g., phenyl or phenol), or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered

heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', $C(O)N(R')_2$, S(O)R', $S(O)_2R'$, or $S(O)_2N(R')_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

R^2 is C_1-C_6 alkyl or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

R^8 is C_1-C_3 alkyl; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

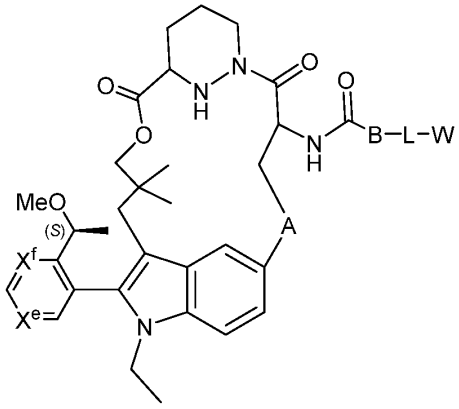
X^e and X^f are, independently, N or CH;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{21} is hydrogen or C_1-C_3 alkyl.

In some embodiments of a compound of the present invention, X^e is N and X^f is CH. In some embodiments, X^e is CH and X^f is N.

In some embodiments, a compound of the present invention has the structure of Formula VIb, or a pharmaceutically acceptable salt thereof:



Formula VIb

wherein A optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene (e.g., phenyl or phenol), or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

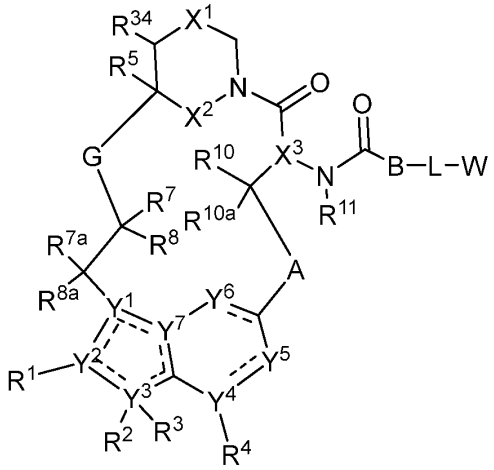
W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; and

X^e and X^f are, independently, N or CH.

In some embodiments of a compound of the present invention, X^e is N and X^f is CH. In some embodiments, X^e is CH and X^f is N.

In some embodiments, a compound of the present invention has the structure of Formula VII, or a pharmaceutically acceptable salt thereof:



Formula VII

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 10-membered heteroarylene;

B is -CH(R^9)- or >C=C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or S(O)$_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

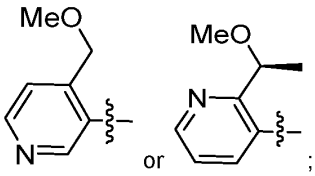
Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is



;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is hydrogen, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl;

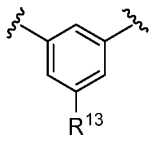
R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo;

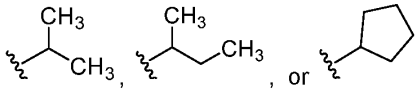
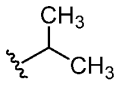
R^{11} is hydrogen or C_1-C_3 alkyl; and

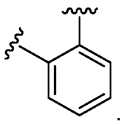
R^{34} is hydrogen or C_1-C_3 alkyl (e.g., methyl).

In some embodiments of compounds of the present invention, A is optionally substituted 6-membered arylene. In some embodiments, A has the structure:



wherein R^{13} is hydrogen, hydroxy, amino, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl. In some embodiments, R^{13} is hydrogen. In some embodiments, R^{13} is hydroxy.

In some embodiments of compounds of the present invention, B is -CHR^9-. In some embodiments, R^9 is optionally substituted C_1-C_6 alkyl or optionally substituted 3 to 6-membered cycloalkyl. In some embodiments, R^9 is:  . In some embodiments, R^9 is:  . In some embodiments, R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

In some embodiments, B is optionally substituted 6-membered arylene. In some embodiments, B is 6-membered arylene. In some embodiments, B is:  .

In some embodiments of compounds of the present invention, R^7 is methyl.

In some embodiments of compounds of the present invention, R^8 is methyl.

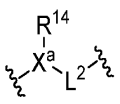
In some embodiments, R^{34} is hydrogen.

In some embodiments of compounds of the present invention, the linker is the structure of Formula II:

$$A^1\text{-}(B^1)_f\text{-}(C^1)_g\text{-}(B^2)_h\text{-}(D^1)\text{-}(B^3)_i\text{-}(C^2)_j\text{-}(B^4)_k\text{–}A^2$$

Formula II

where A^1 is a bond between the linker and B; A^2 is a bond between W and the linker; B^1, B^2, B^3, and B^4 each, independently, is selected from optionally substituted C_1-C_2 alkylene, optionally substituted C_1-C_3 heteroalkylene, O, S, and NR^N; R^N is hydrogen, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted C_1-C_7 heteroalkyl; C^1 and C^2 are each, independently, selected from carbonyl, thiocarbonyl, sulphonyl, or phosphoryl; f, g, h, i, j, and k are each, independently, 0 or 1; and D^1 is optionally substituted C_1-C_{10} alkylene, optionally substituted C_2-C_{10} alkenylene, optionally substituted C_2-C_{10} alkynylene, optionally substituted 3 to 14-membered heterocycloalkylene, optionally substituted 5 to 10-membered heteroarylene, optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 6 to 10-membered arylene, optionally substituted C_2-C_{10} polyethylene glycolene, or optionally substituted C_1-C_{10} heteroalkylene, or a chemical bond linking A^1-$(B^1)_f$-$(C^1)_g$-$(B^2)_h$- to -$(B^3)_i$-$(C^2)_j$-$(B^4)_k$–A^2. In some embodiments, the linker is acyclic. In some embodiments, the linker has the structure of Formula IIa:

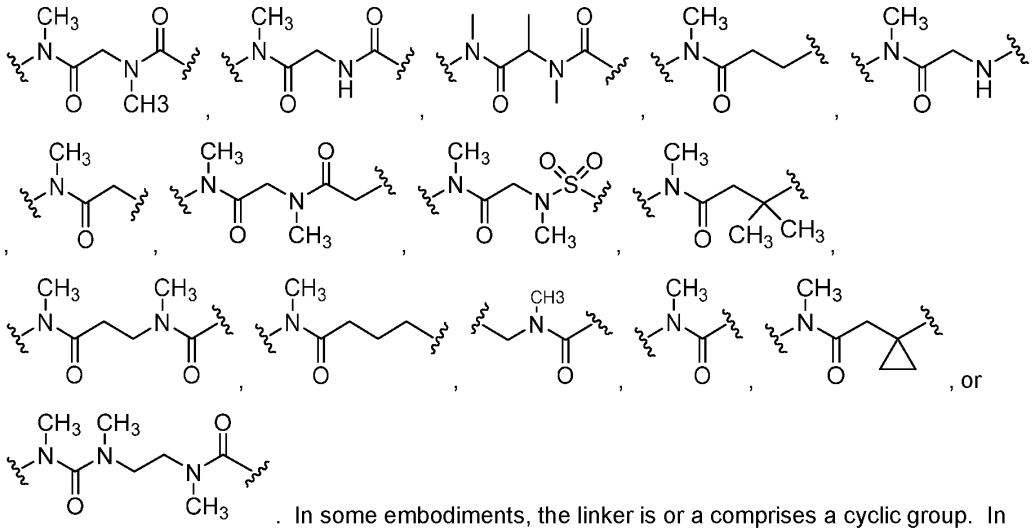


Formula IIa

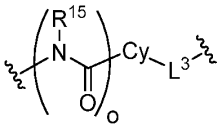
wherein X^a is absent or N;

R^{14} is absent, hydrogen or optionally substituted C_1-C_6 alkyl; and

L^2 is absent, -SO_2-, optionally substituted C_1-C_4 alkylene or optionally substituted C_1-C_4 heteroalkylene, wherein at least one of X^a, R^{14}, or L^2 is present. In some embodiments, the linker has the structure:



. In some embodiments, the linker is or a comprises a cyclic group. In some embodiments, the linker has the structure of Formula IIb:



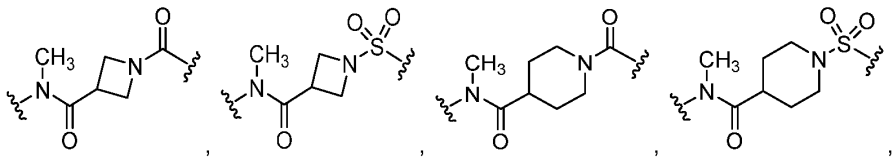
Formula IIb

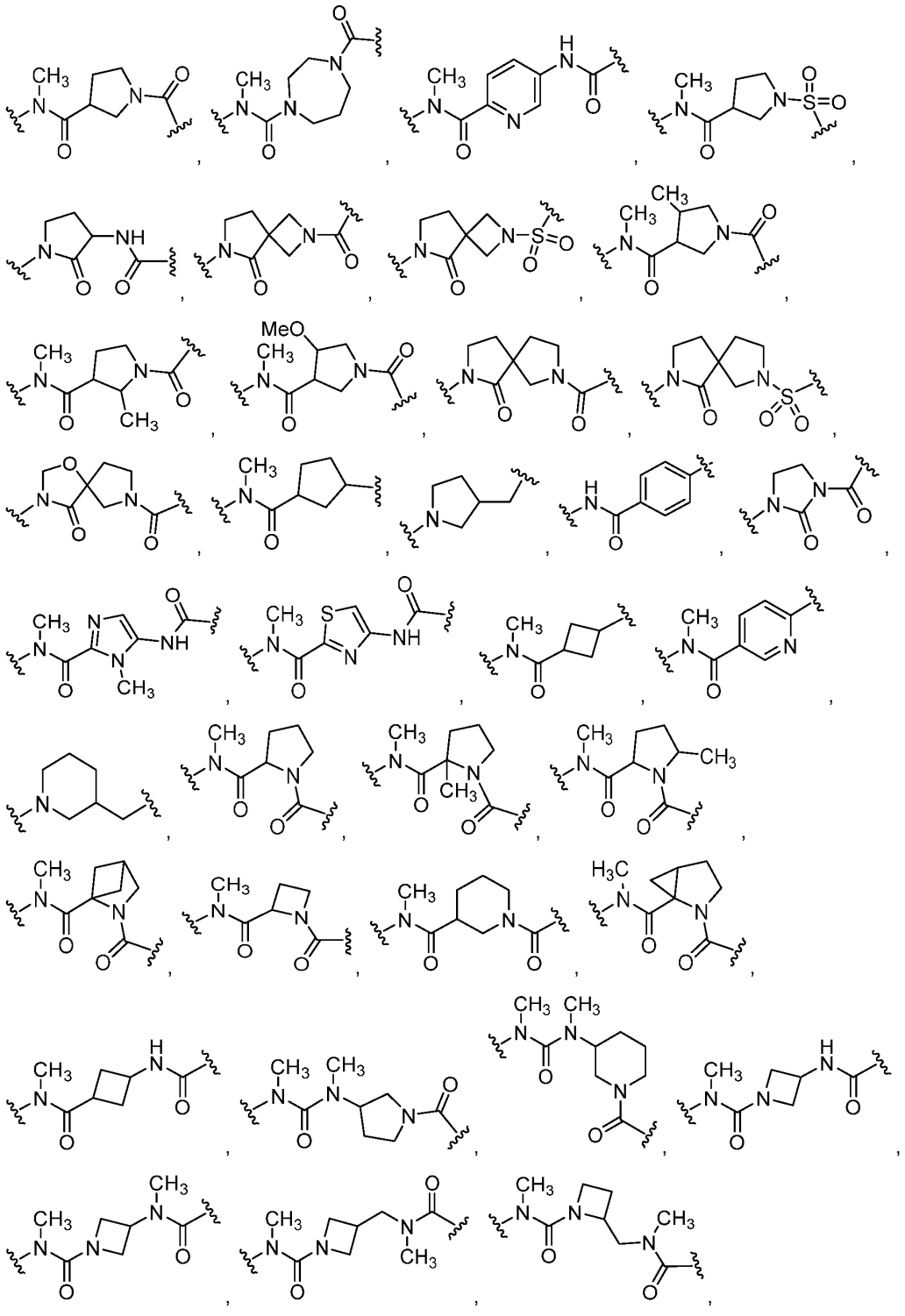
wherein o is 0 or 1;

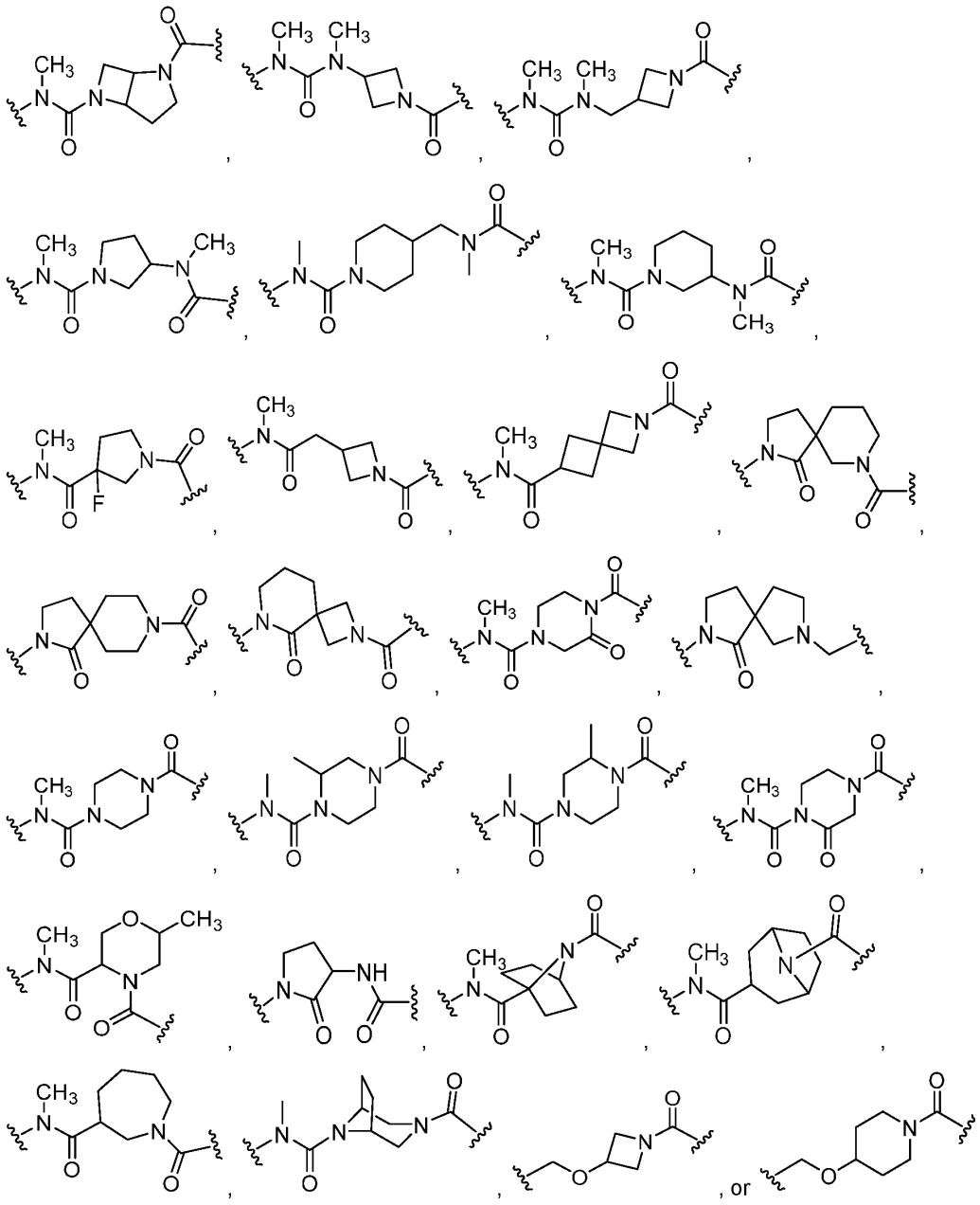
R^{15} is hydrogen or optionally substituted C_1-C_6 alkyl;

Cy is optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 3 to 8-membered heterocycloalkylene, optionally substituted 6-10 membered arylene, or optionally substituted 5 to 10-membered heteroarylene; and

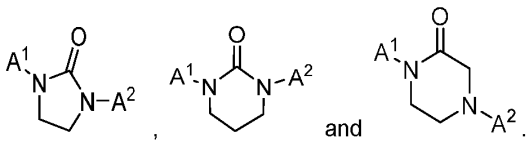
L^3 is absent, -SO_2-, optionally substituted C_1-C_4 alkylene or optionally substituted C_1-C_4 heteroalkylene. In some embodiments, the linker has the structure:





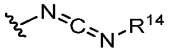


. In some embodiments, a linker of Formula II is selected from the group consisting of



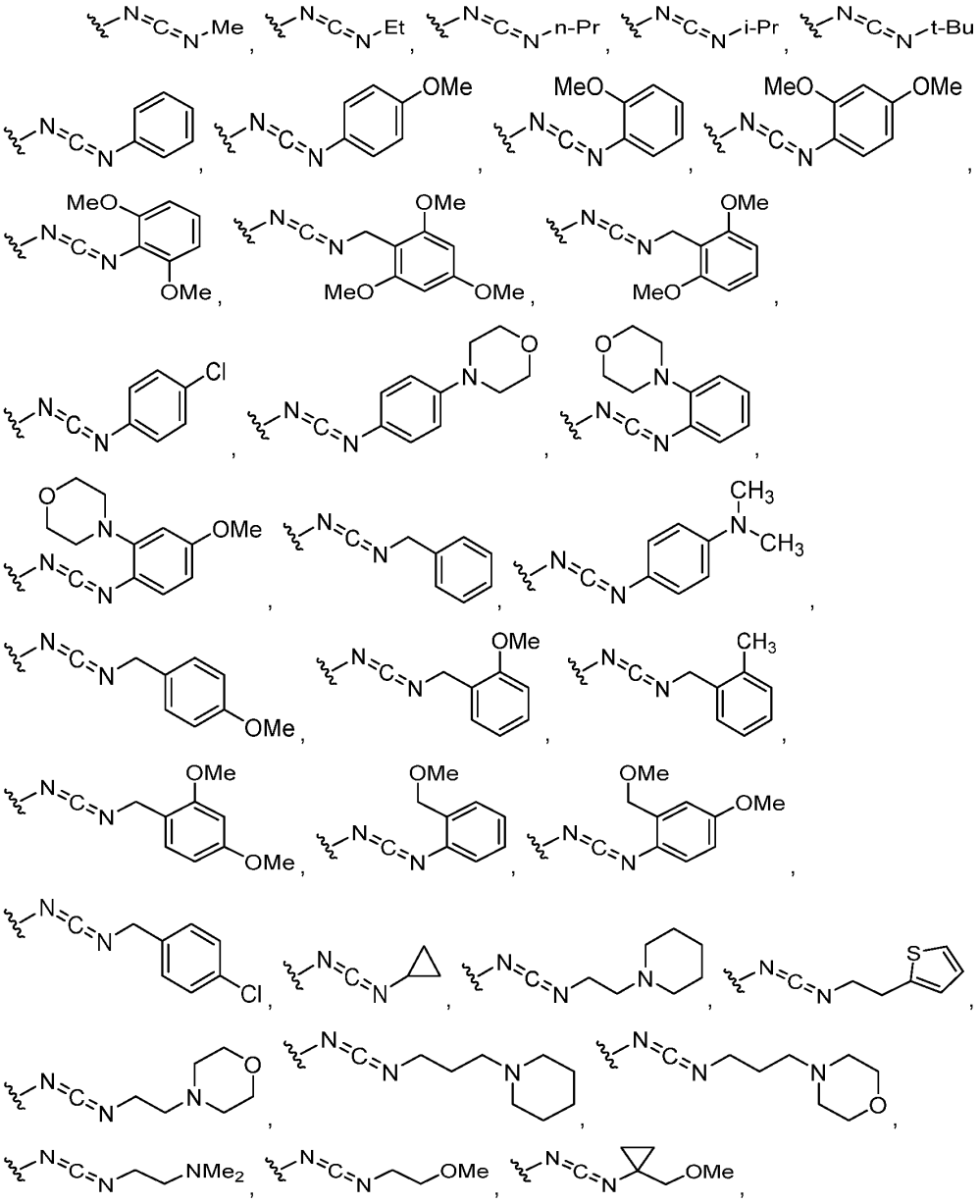
and

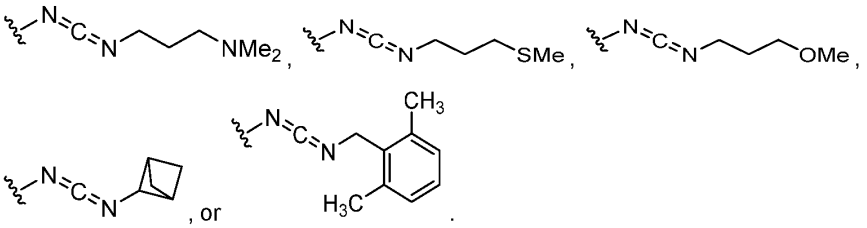
In some embodiments of compounds of the present invention, W comprises a carbodiimide. In some embodiments, W has the structure of Formula IIIa:



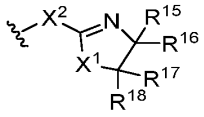
Formula IIIa

wherein R^{14} is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 6 to 10-membered aryl, optionally substituted 3 to 14-membered heterocycloalkyl, or optionally substituted 5 to 10-membered heteroaryl. In some embodiments, W has the structure:





In some embodiments, W comprises an oxazoline or thiazoline. In some embodiments, W has the structure of Formula IIIb:

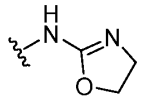


Formula IIb

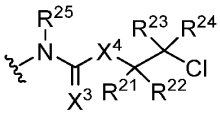
wherein X^1 is O or S;

X^2 is absent or NR^{19};

R^{15}, R^{16}, R^{17}, and R^{18} are, independently, hydrogen or optionally substituted C_1-C_6 alkyl; and

R^{19} is hydrogen, C(O)(optionally substituted C_1-C_6 alkyl), optionally substituted C_1-C_6 alkyl, optionally substituted 6 to 10-membered aryl, optionally substituted 3 to 14-membered heterocycloalkyl, or optionally substituted 5 to 10-membered heteroaryl. In some embodiments, W is  .

In some embodiments, W comprises a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, or a chloroethyl thiocarbamate. In some embodiments, W has the structure of Formula IIIc:

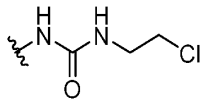


Formula IIIc

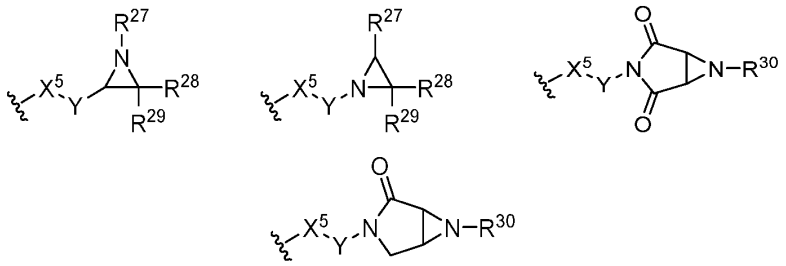
wherein X^3 is O or S;

X^4 is O, S, NR^{26};

R^{21}, R^{22}, R^{23}, R^{24}, and R^{26} are, independently, hydrogen or optionally substituted C_1-C_6 alkyl; and

R^{25} is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted 6 to 10-membered aryl, optionally substituted 3 to 14-membered heterocycloalkyl, or optionally substituted 5 to 10-membered heteroaryl. In some embodiments, W is  .

In some embodiments, W comprises an aziridine. In some embodiments, W has the structure of Formula IIId1, Formula IIId2, Formula IIId3, or Formula IIId4:



Formula IIId1 Formula IIId2 Formula IIId3 Formula IIId4

wherein X^5 is absent or NR^{30};

Y is absent or C(O), C(S), S(O), SO_2, or optionally substituted C_1-C_3 alkylene;

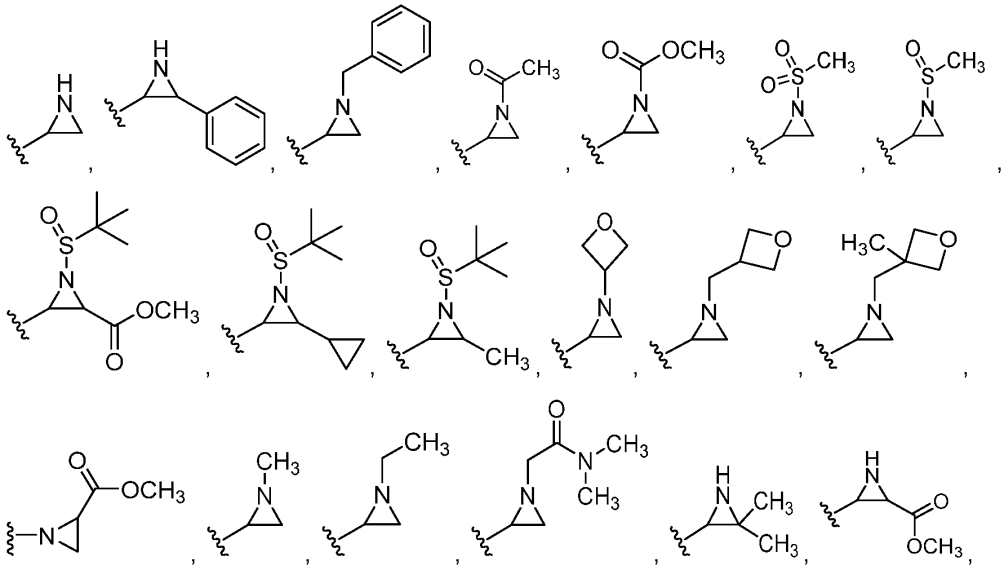
R^{27} is hydrogen, $-C(O)R^{32}$, $-C(O)OR^{32}$, $-SOR^{33}$, $-SO_2R^{33}$, optionally substituted C_1-C_6 alkyl, optionally substituted 6 to 10-membered aryl, optionally substituted 3 to 14-membered heterocycloalkyl, or optionally substituted 5 to 10-membered heteroaryl;

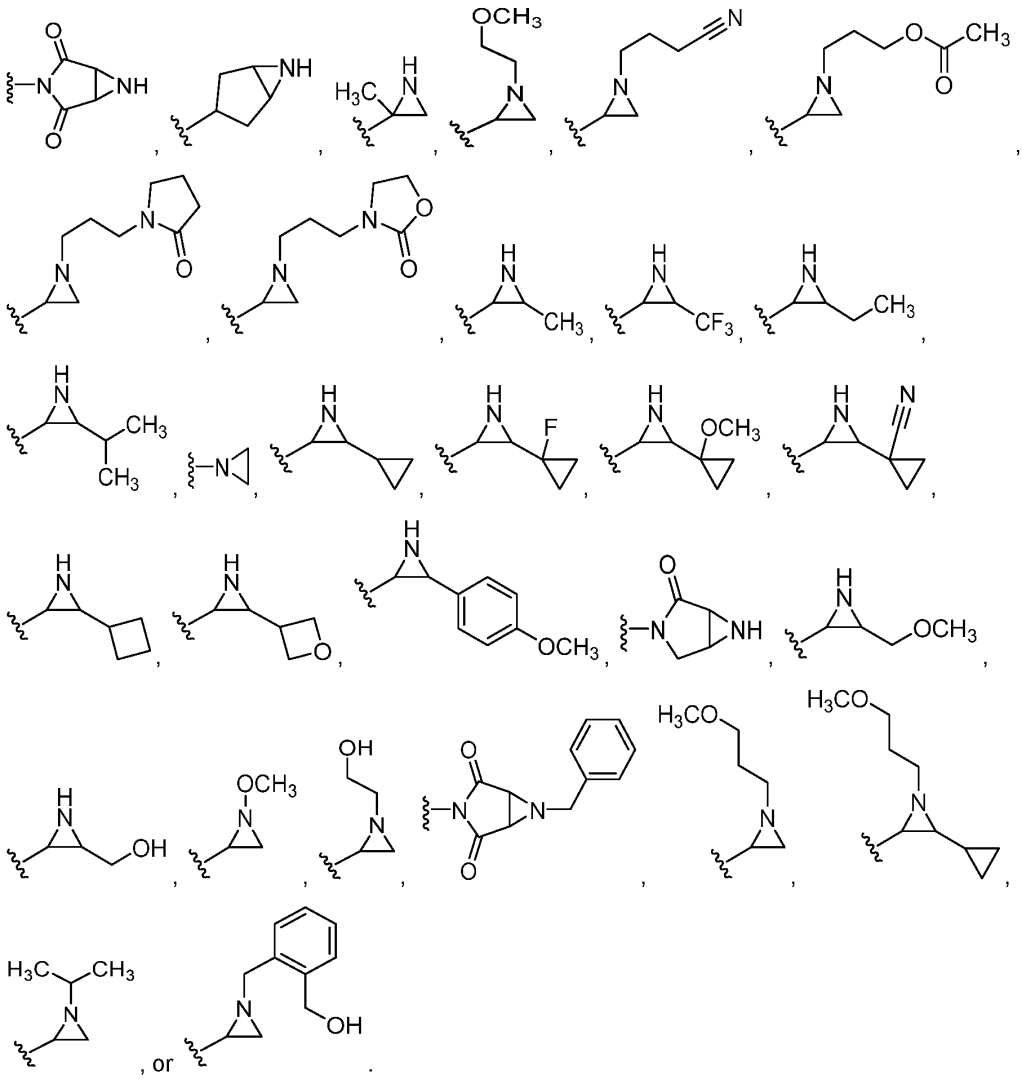
R^{28} and R^{29} are, independently, hydrogen, CN, $C(O)R^{31}$, CO_2R^{31}, $C(O)R^{31}R^{31}$ optionally substituted C_1-C_6 alkyl, optionally substituted 3 to 10-membered cycloalkyl, optionally substituted 6 to 10-membered aryl, optionally substituted 3 to 14-membered heterocycloalkyl, or optionally substituted 5 to 10-membered heteroaryl;

each R^{31} is, independently, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted 6 to 10-membered aryl, optionally substituted 3 to 14-membered heterocycloalkyl, or optionally substituted 5 to 10-membered heteroaryl;

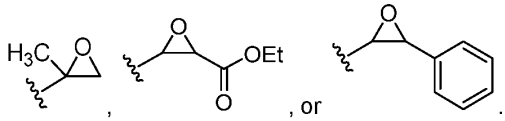
R^{30} is hydrogen or optionally substituted C_1-C_6 alkyl; and

R^{32} and R^{33} are, independently, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted 6 to 10-membered aryl, optionally substituted 3 to 14-membered heterocycloalkyl, or optionally substituted 5 to 10-membered heteroaryl. In some embodiments, W is:





In some embodiments, W comprises an epoxide. In some embodiments, W is ,

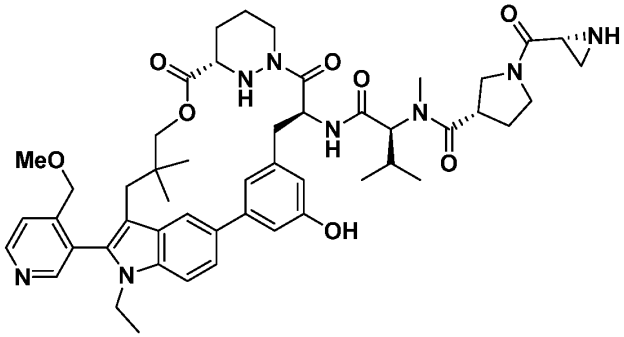
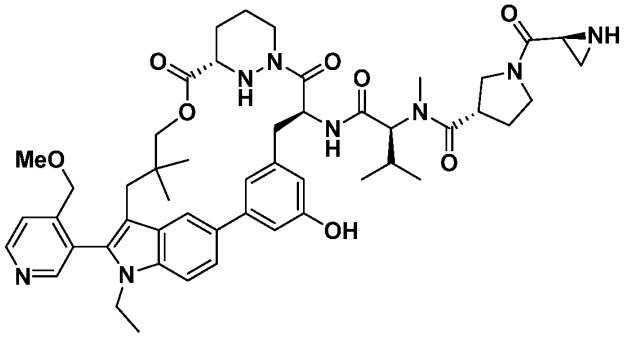
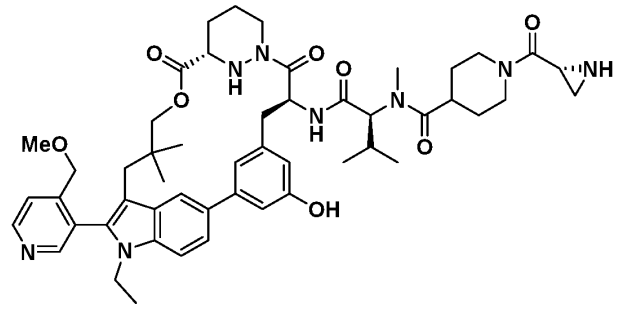


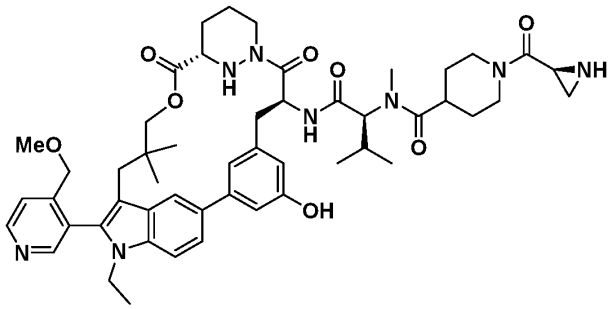
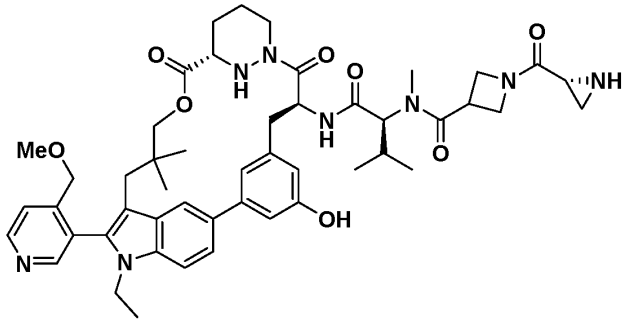
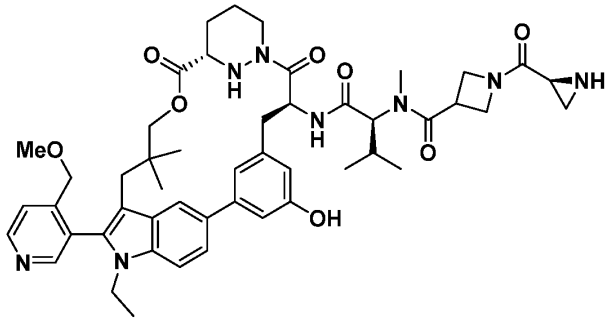
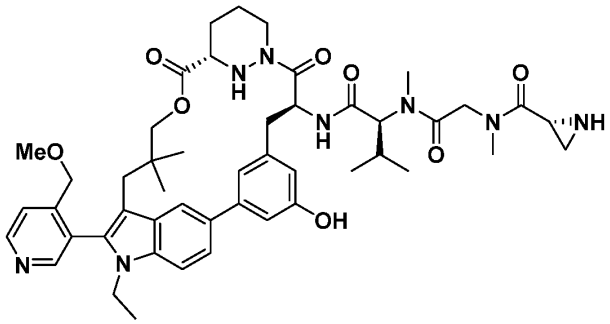
In some embodiments, W is a cross-linking group bound to an organic moiety that is a Ras binding moiety, i.e., RBM-W, wherein upon contact of an RBM-W compound with a Ras protein, the RBM-W binds to the Ras protein to form a conjugate. For example, the W moiety of an RBM-W compound may bind, e.g., cross-link, with an amino acid of the Ras protein to form the conjugate. In some embodiments, the Ras binding moiety is a K-Ras binding moiety. In some embodiments, the K-Ras binding moiety binds to a residue of a K-Ras Switch-II binding pocket of the K-Ras protein. In some embodiments, the Ras binding moiety is an H-Ras binding moiety that binds to a

residue of an H-Ras Switch-II binding pocket of an H-Ras protein. In some embodiments, the Ras binding moiety is an N-Ras binding moiety that binds to a residue of an N-Ras Switch-II binding pocket of an N-Ras protein. The W of an RBM-W compound may comprise any W described herein. The Ras binding moiety typically has a molecular weight of under 1200 Da. See, e.g., see, e.g., Johnson et al., 292:12981-12993 (2017) for a description of Ras protein domains, incorporated herein by reference.

In some embodiments, a compound of the present invention is selected from Table 1, or a pharmaceutically acceptable salt or stereoisomer thereof. In some embodiments, a compound of the present invention is selected from Table 1, or a pharmaceutically acceptable salt or atropisomer thereof.

Table 1: Certain Compounds of the Present Invention

Ex#	Structure
1	
2	
3	

Ex#	Structure
4	
5	
6	
7	

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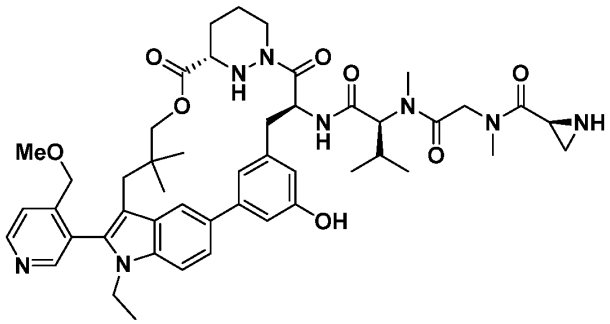
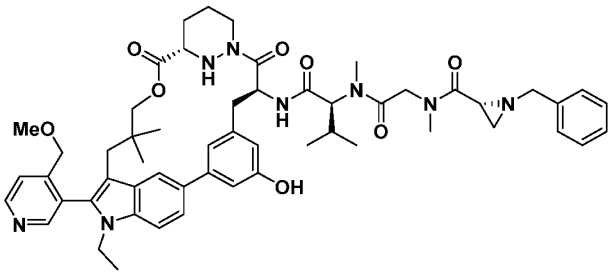
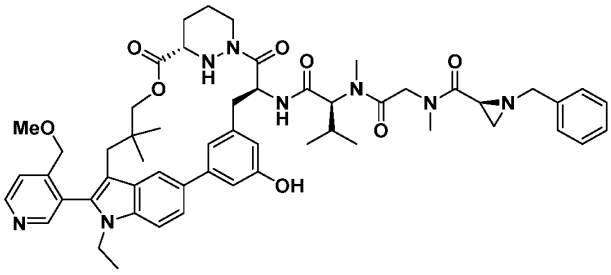
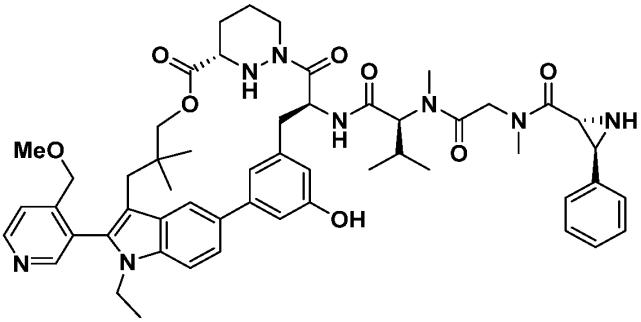
8	
9	
10	
11	

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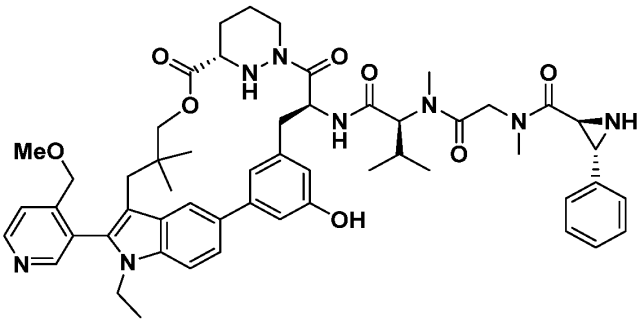
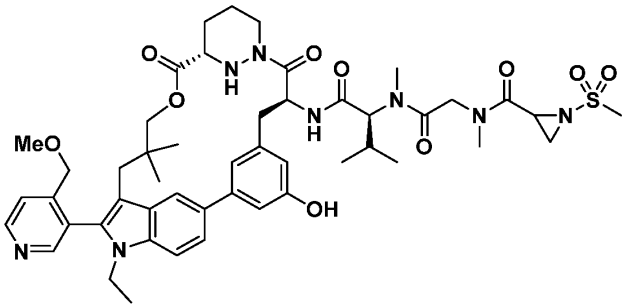
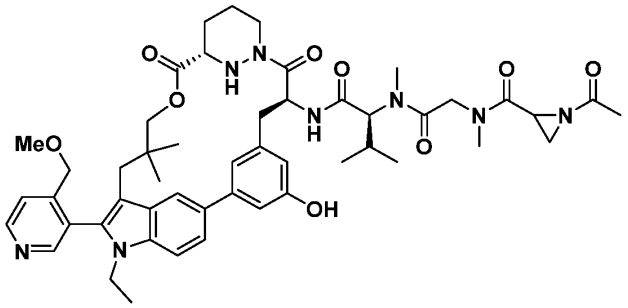
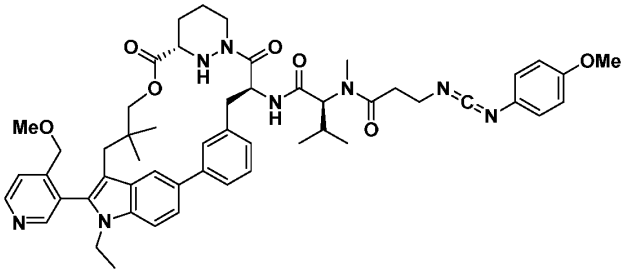
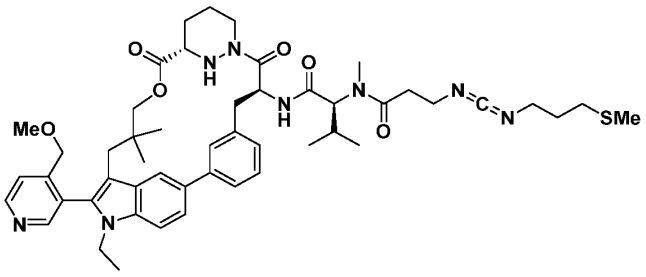
12	
13	
14	
15 A and B	
16 A and B	

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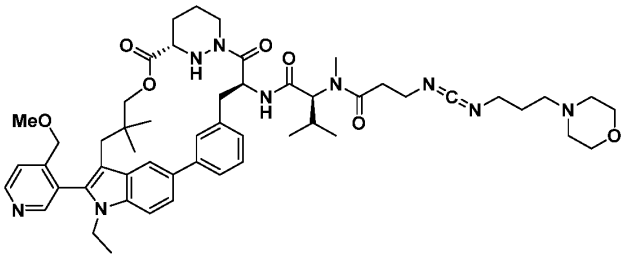
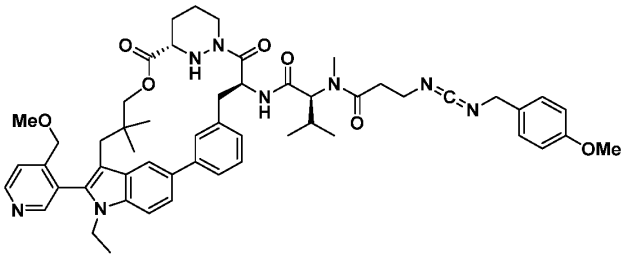
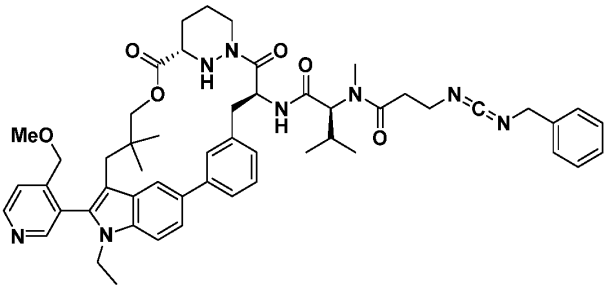
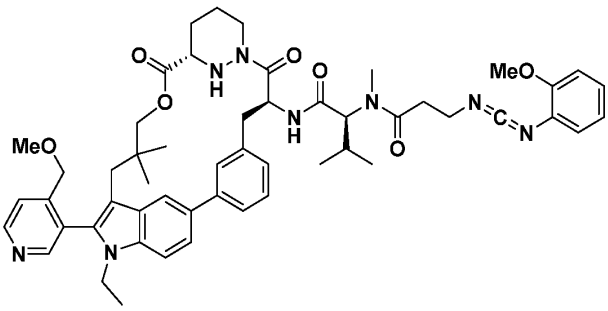
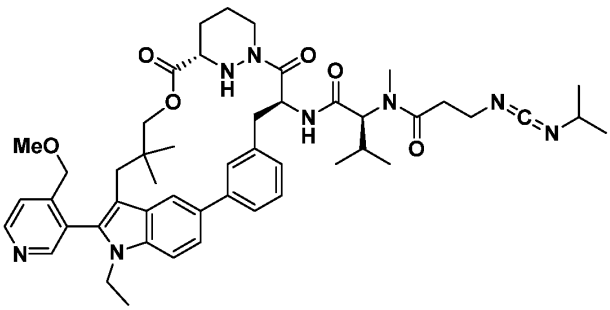
17 A and B	
18 A and B	
19 A and B	
20 A and B	
21 A and B	

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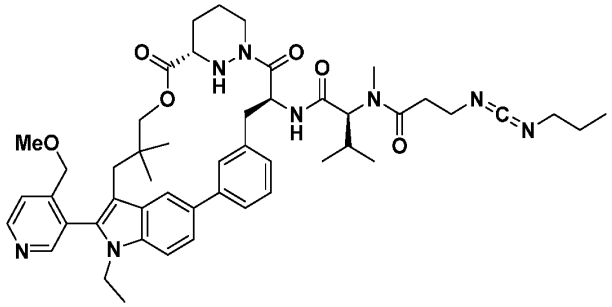
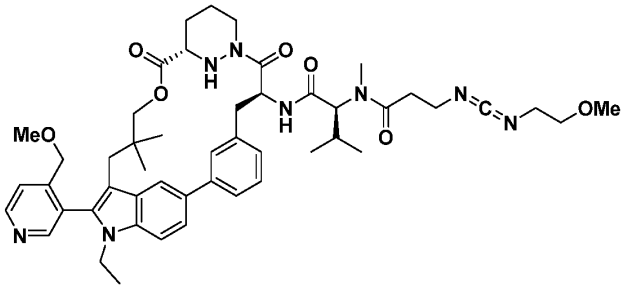
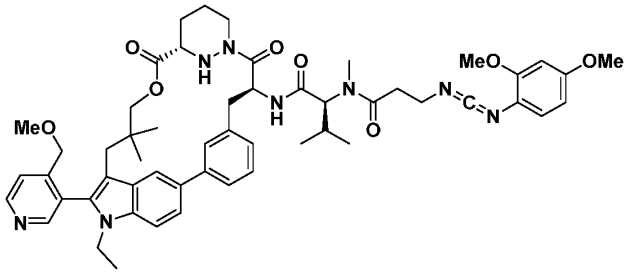
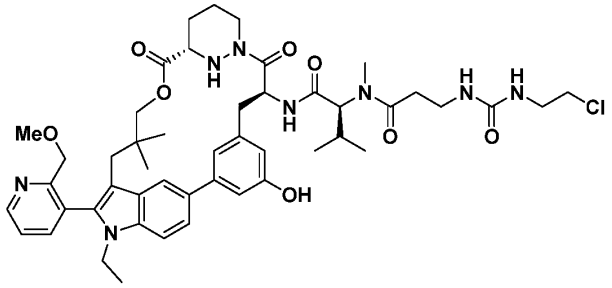
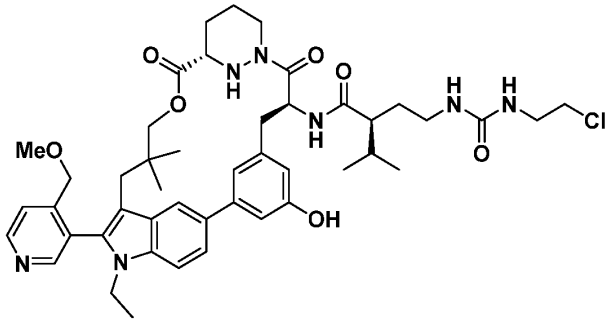
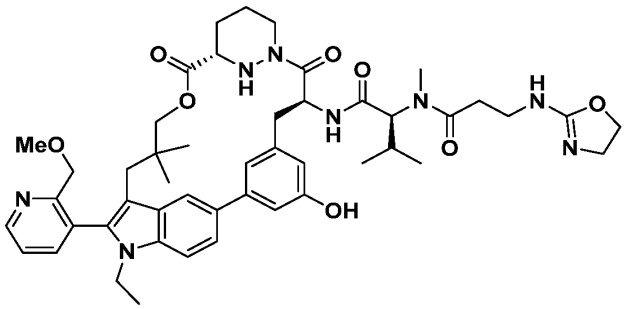
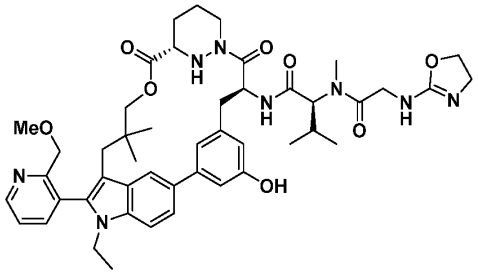
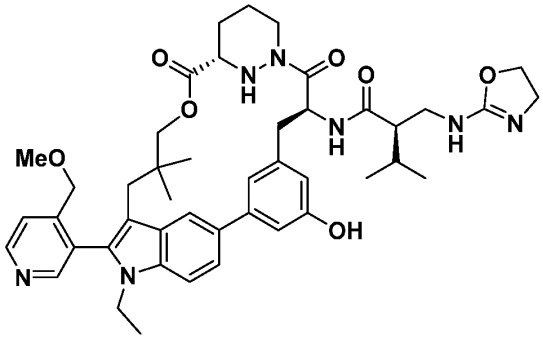
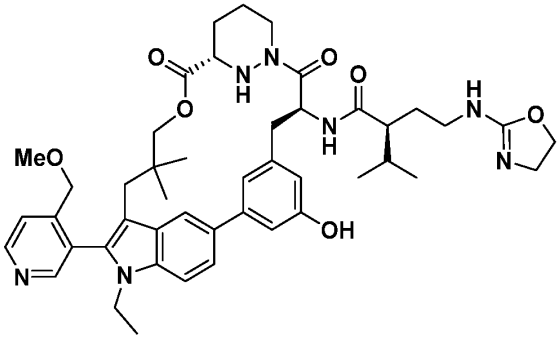
22 A and B	
23 A and B	
24 A and B	
25	
26	

Ex#	Structure
27	
28	
29	
30	

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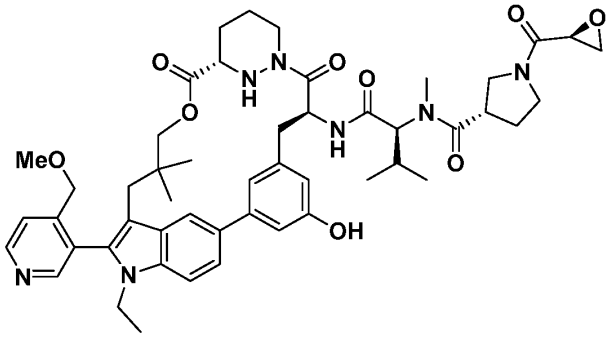
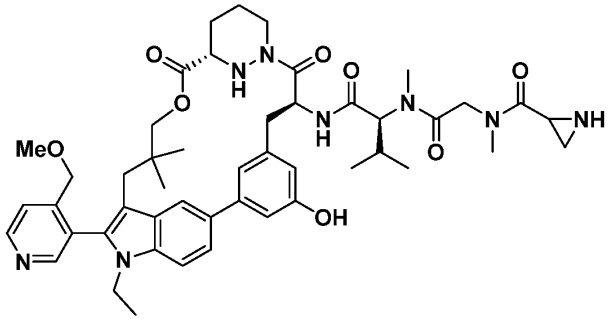
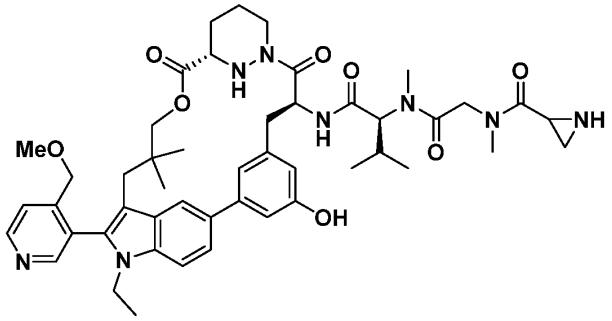
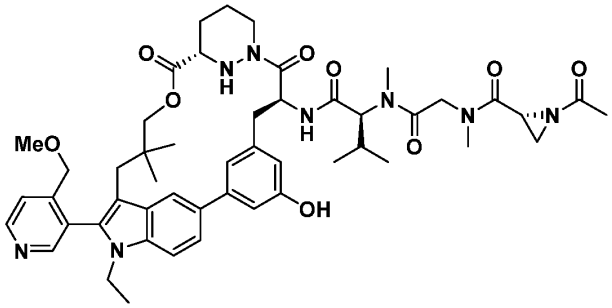
31	
32 A and B	
33	
34	

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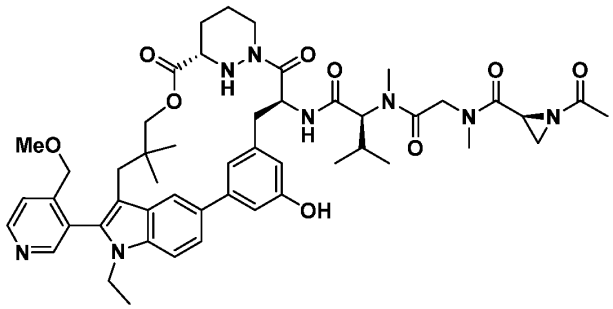
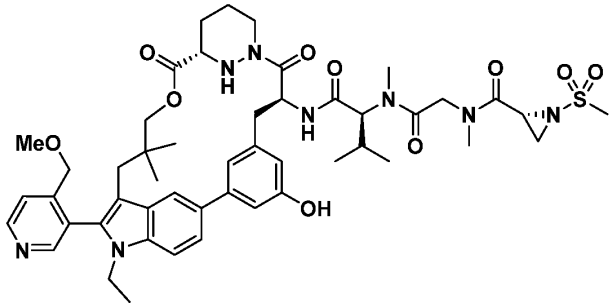
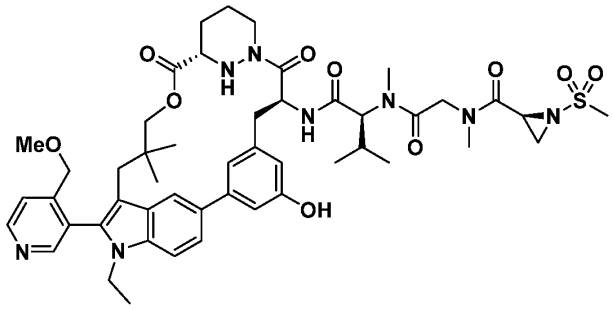
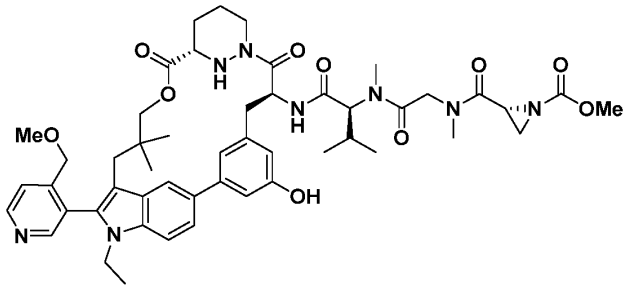
35	
36	
37	
38	

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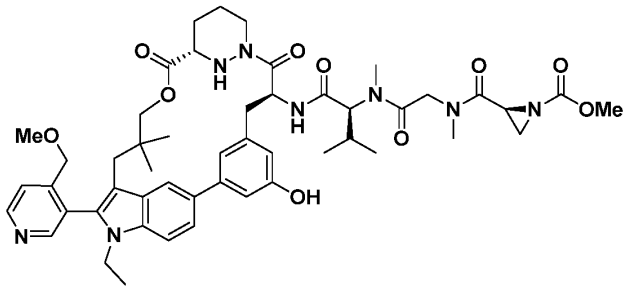
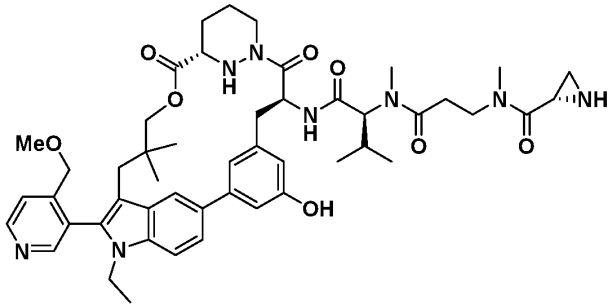
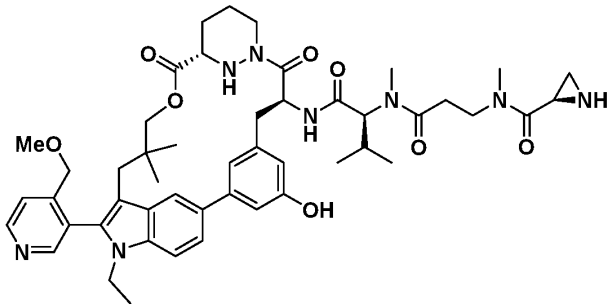
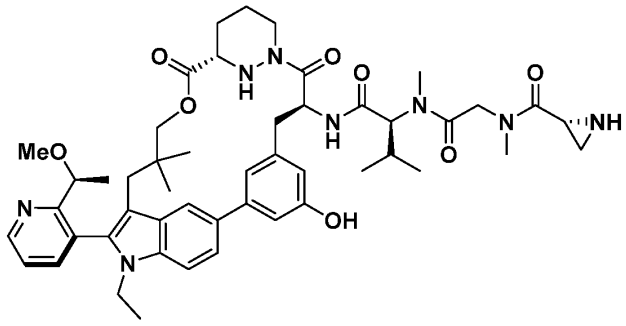
39	
40	
41	
42A	

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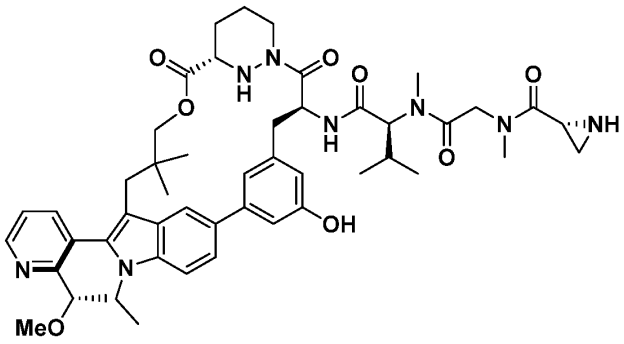
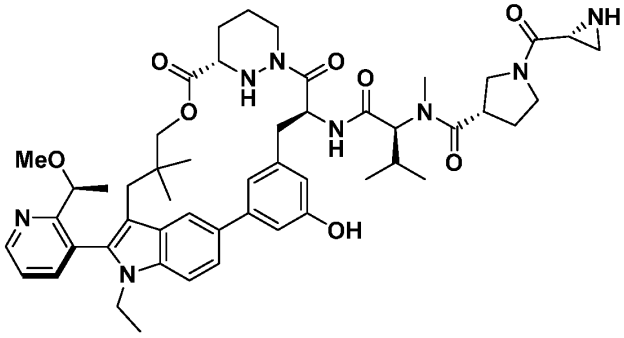
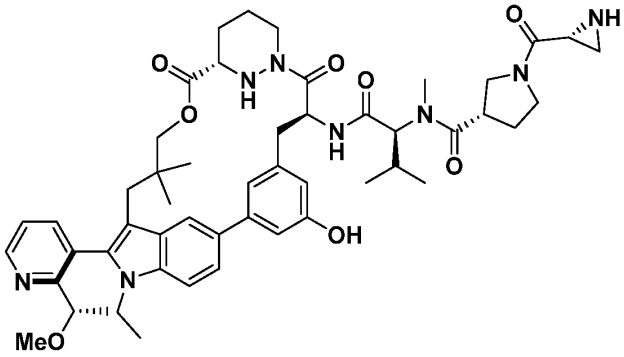
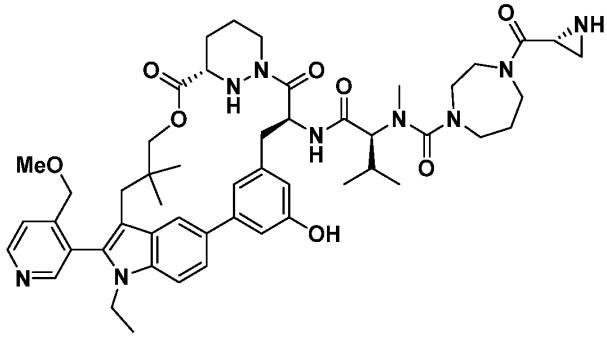
42B	
43A	
43B	
44	

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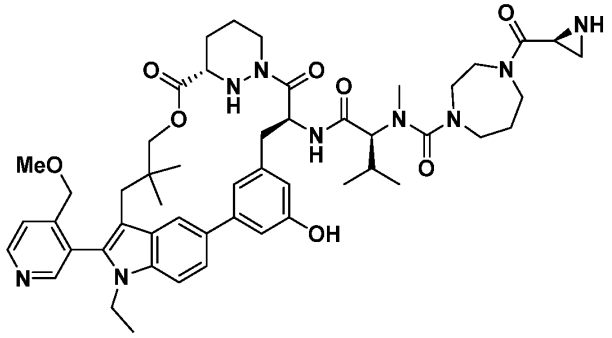
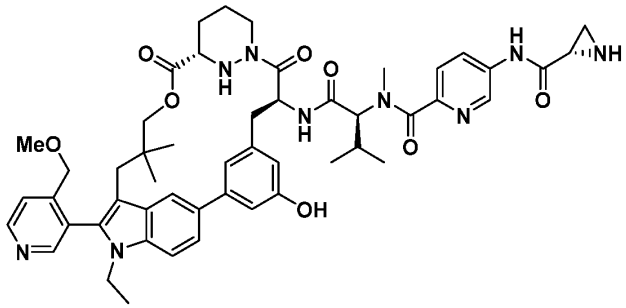
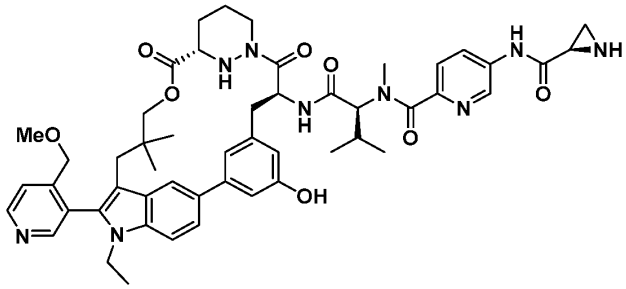
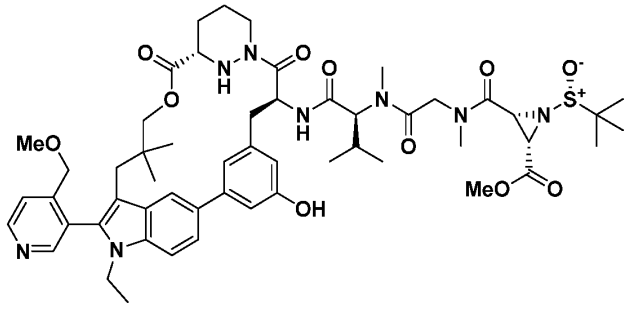
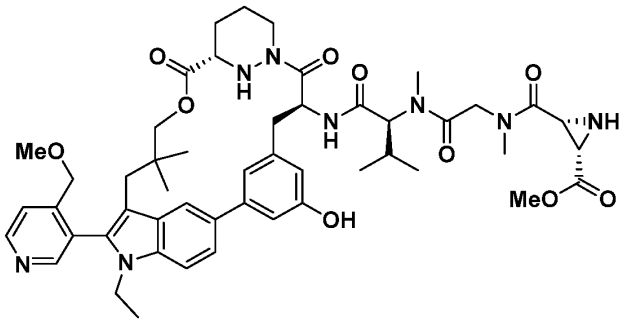
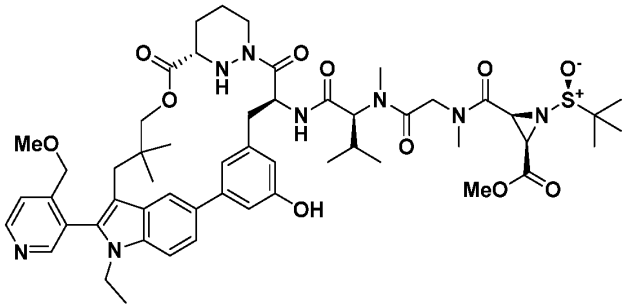
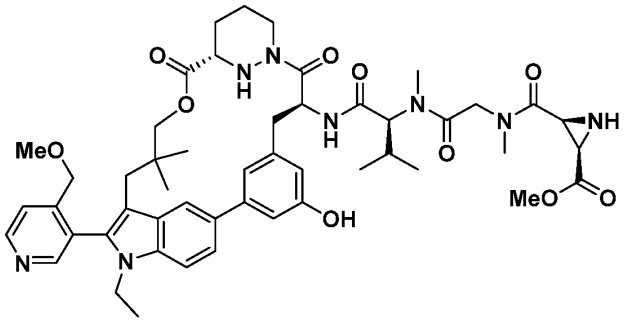
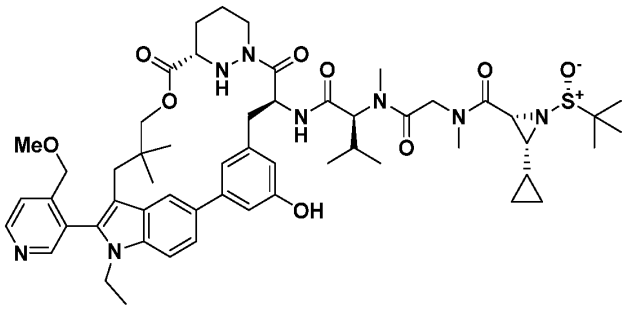
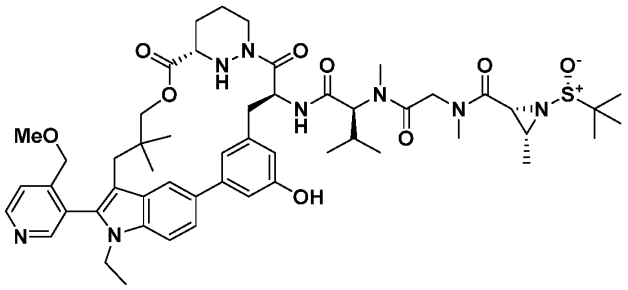
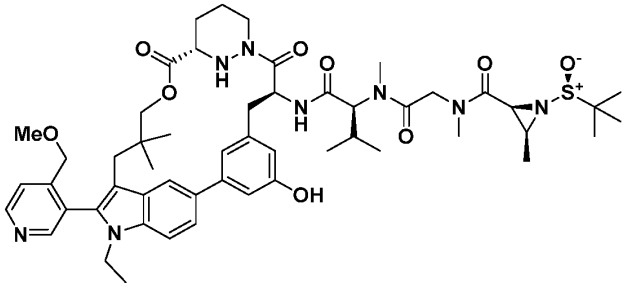
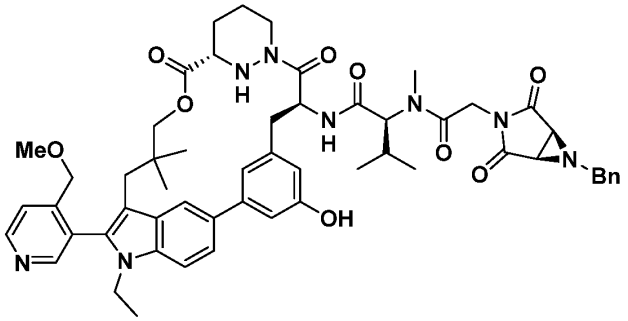
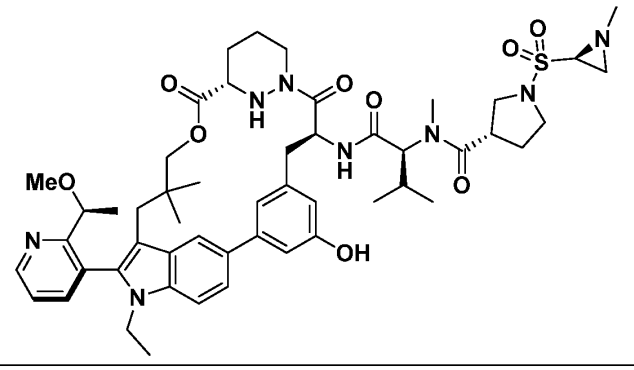
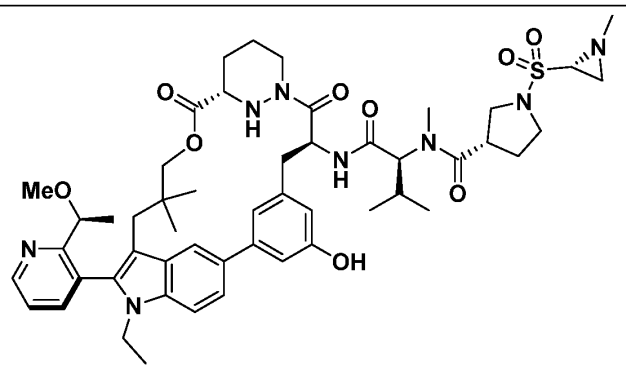
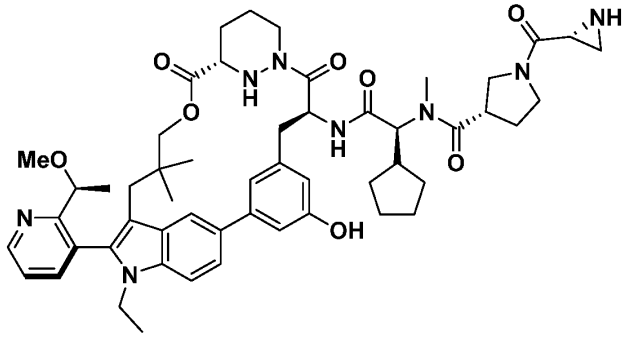
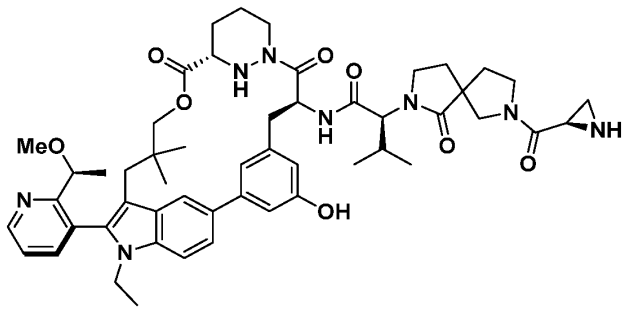
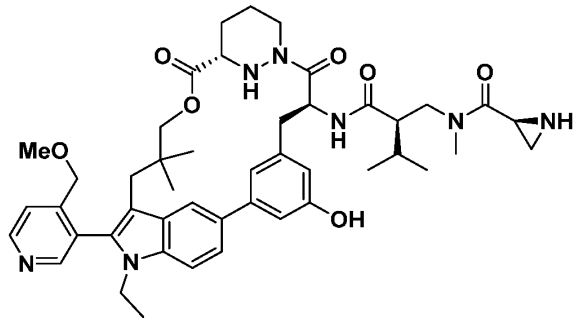
45	
46	
47 A and B	
48	

Ex#	Structure
49	
50	
51	
52	

Ex#	Structure
53	
54	
55	
56*	

Ex#	Structure
57*	
58	
59	
60	

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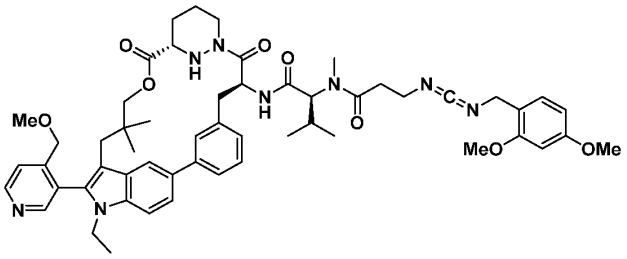
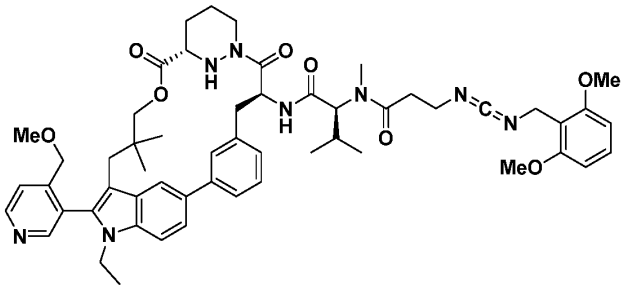
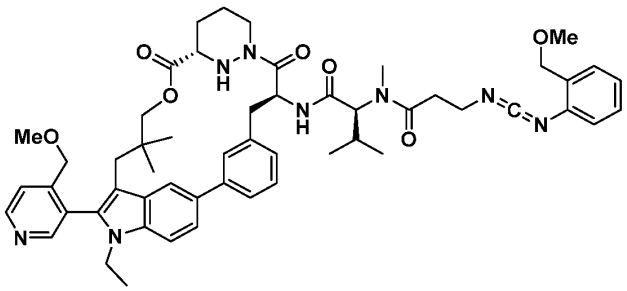
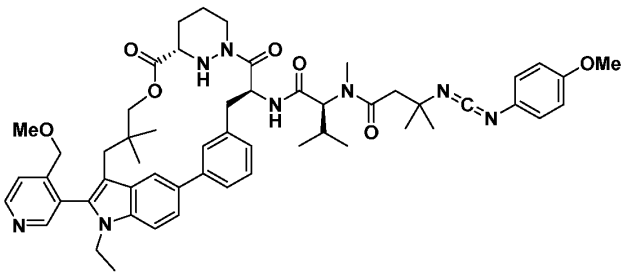
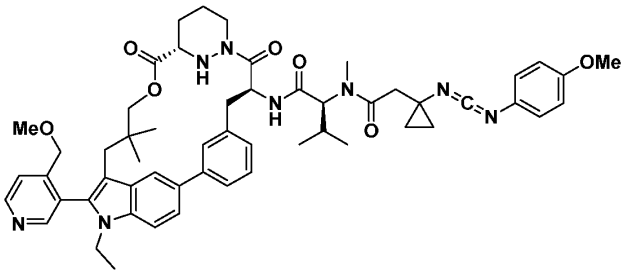
61 A and B	
62 A and B	
63	
64 A and B	
65 A and B	

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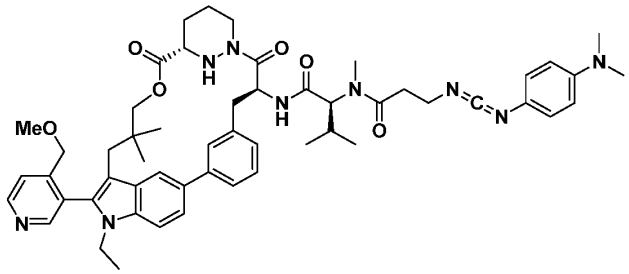
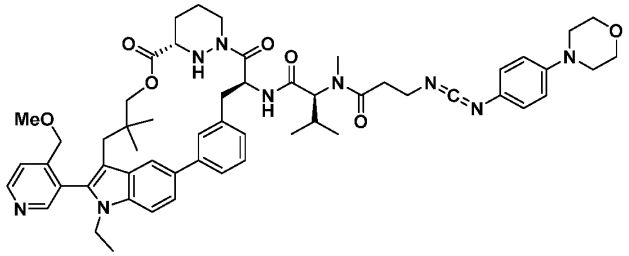
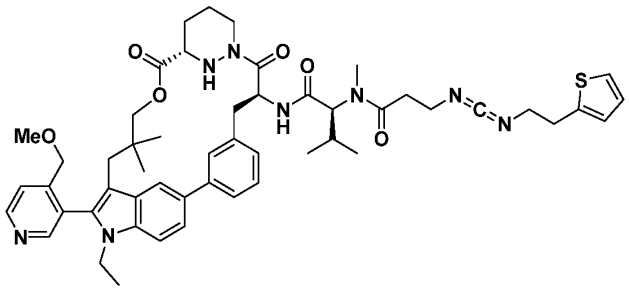
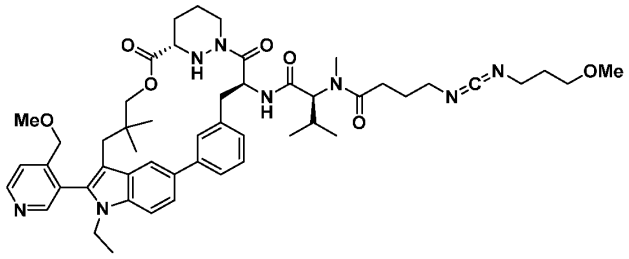
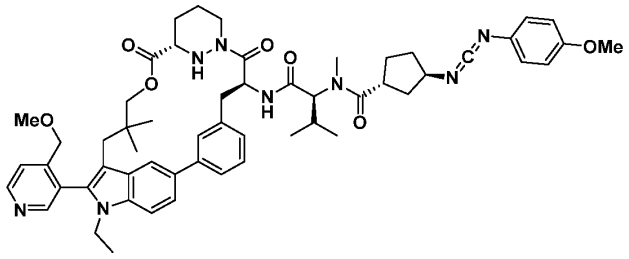
66	
67	
68 A and B	
69 A and B	
70 A and B	

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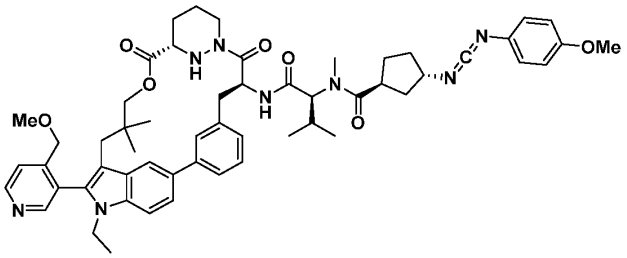
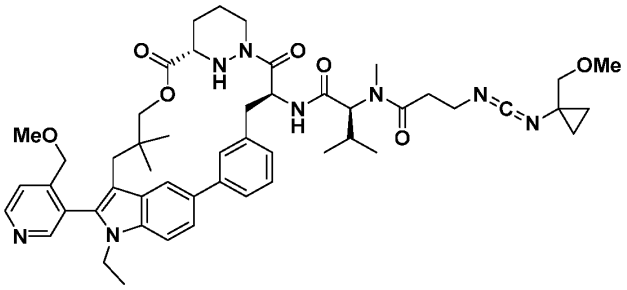
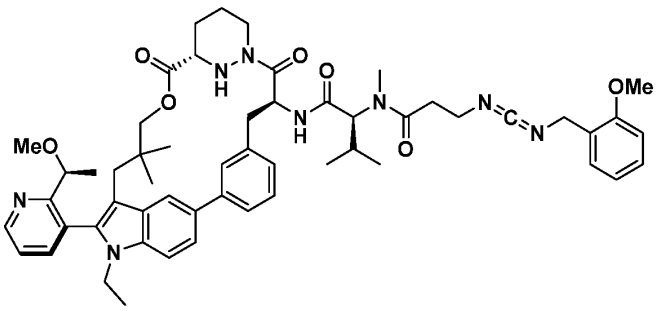
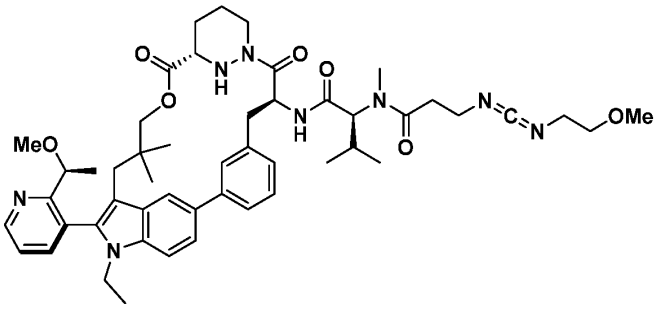
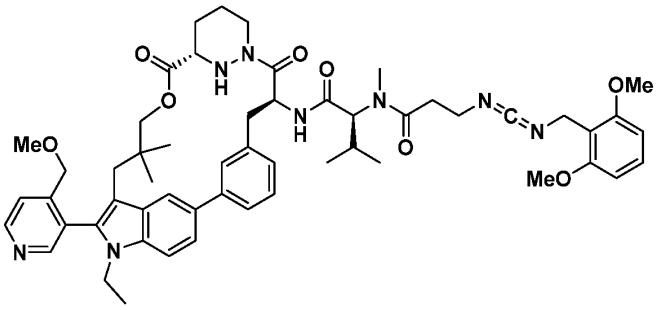
71 A and B	
72 A and B	
73	
74	
75	

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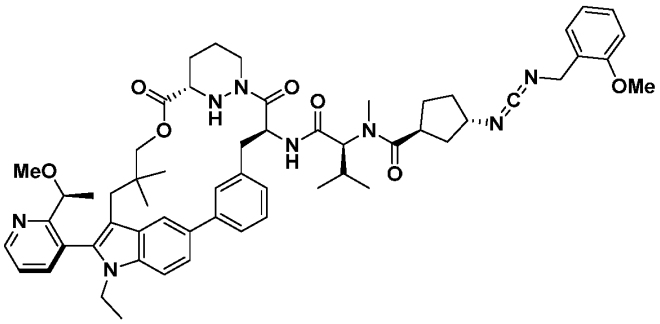
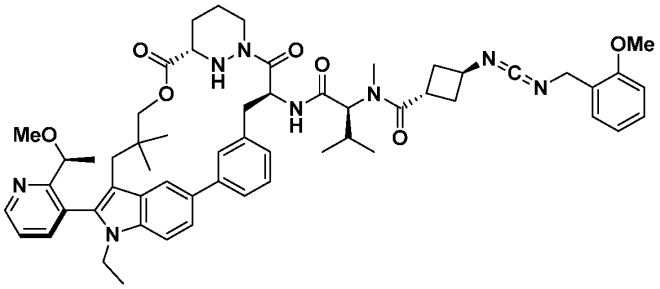
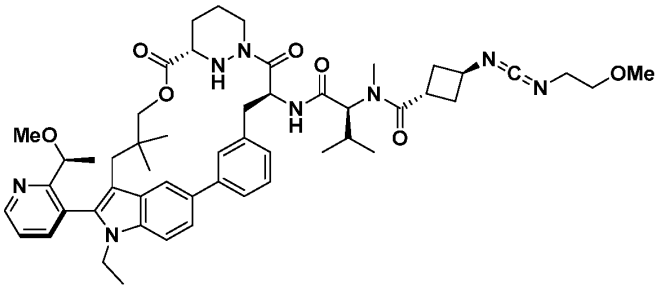
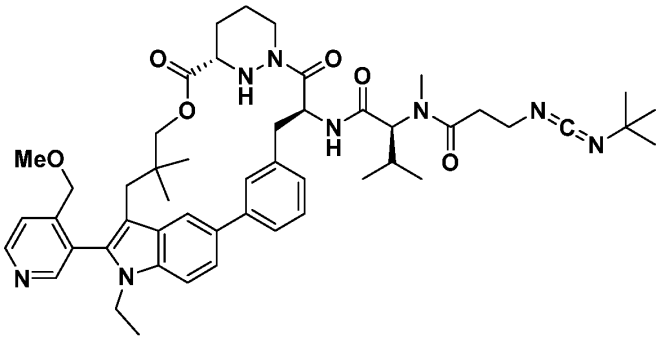
76	
77	
78	
79 A and B	

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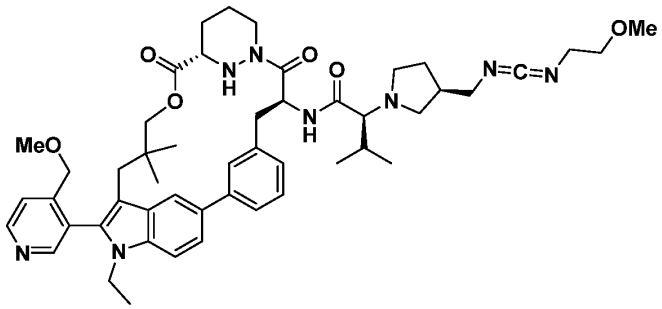
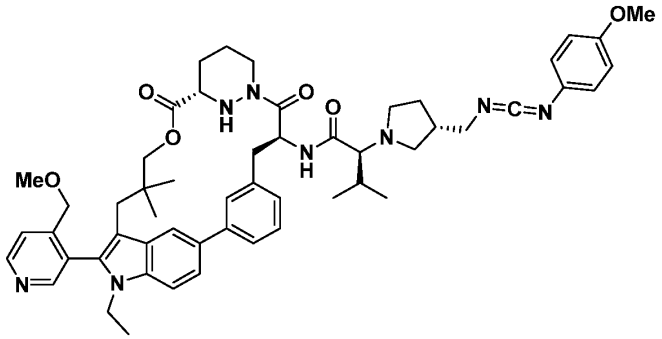
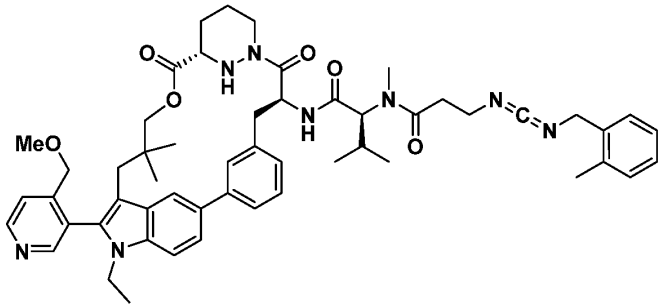
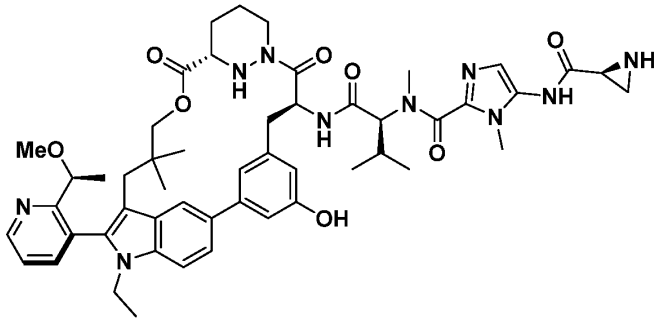
80	
81	
82 A and B	
83	

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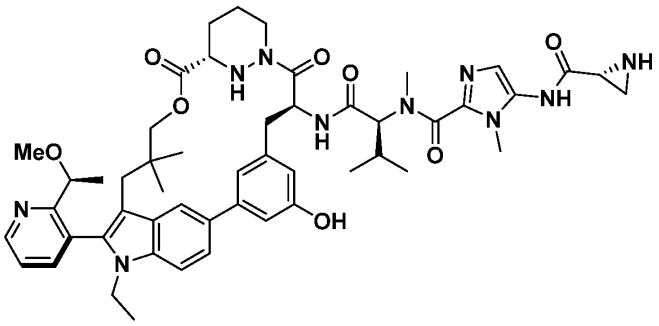
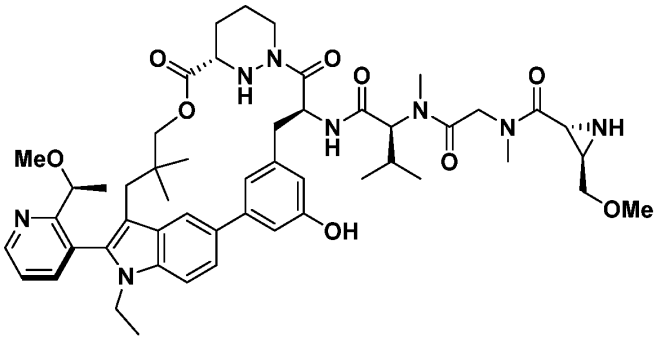
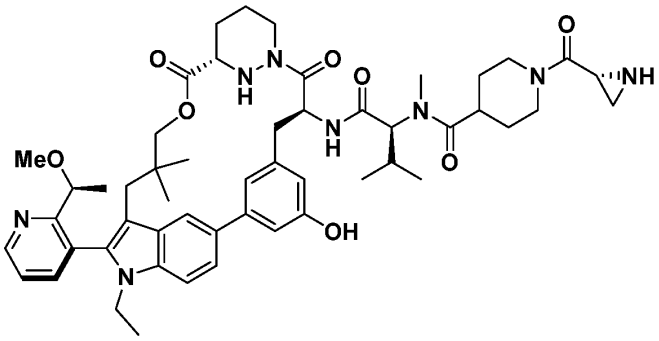
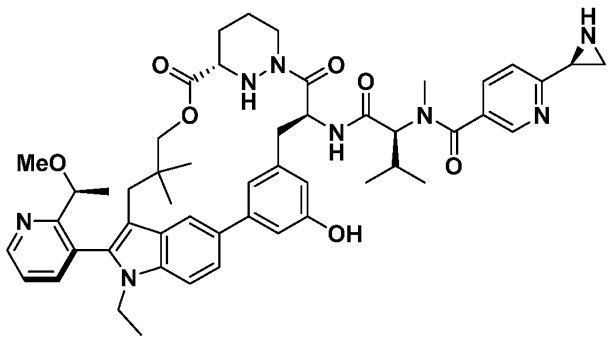
84	
85	
86	
87	

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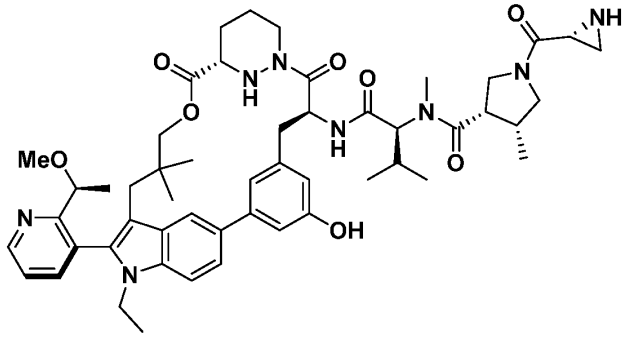
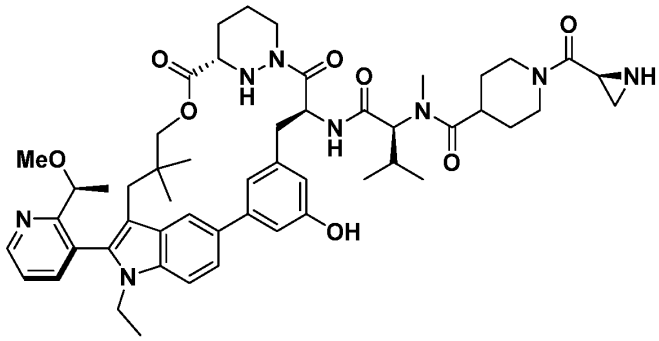
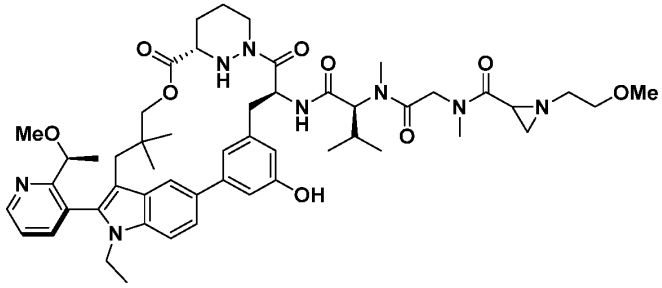
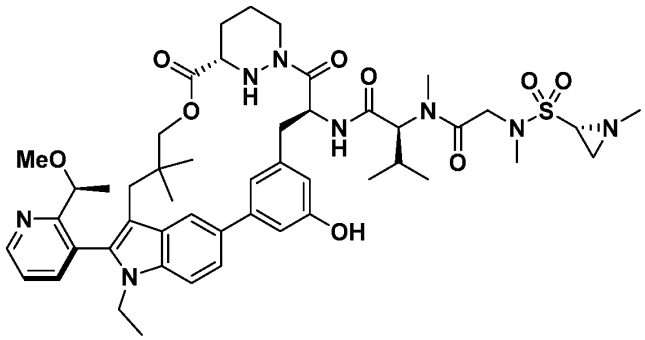
88	
89	
90	
91*	

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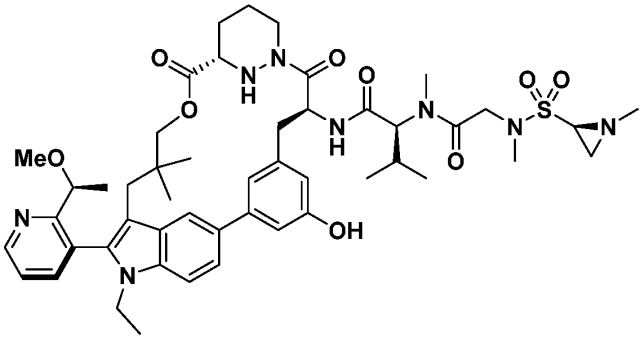
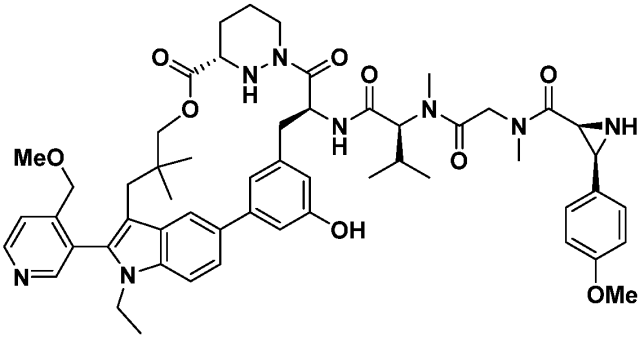
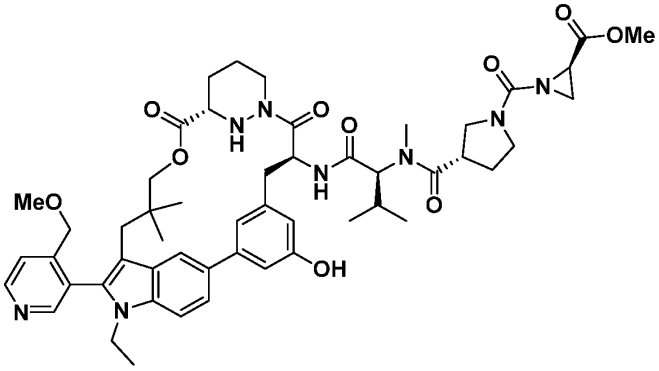
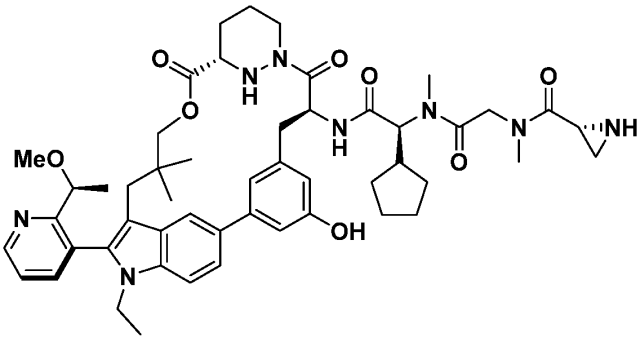
92*	
93	
94	
95	

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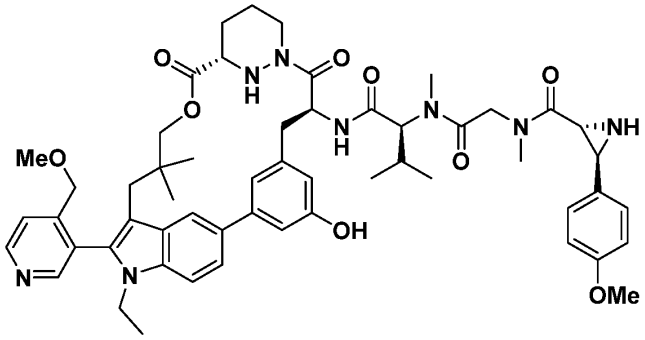
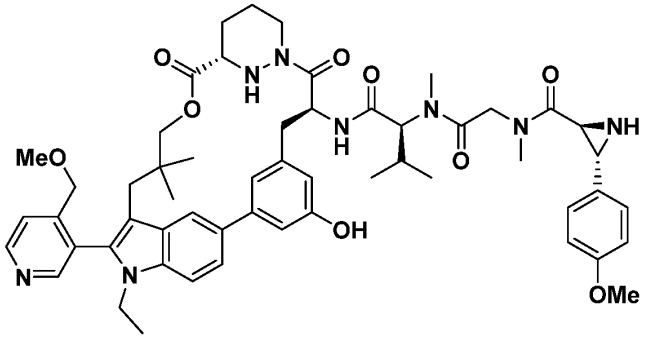
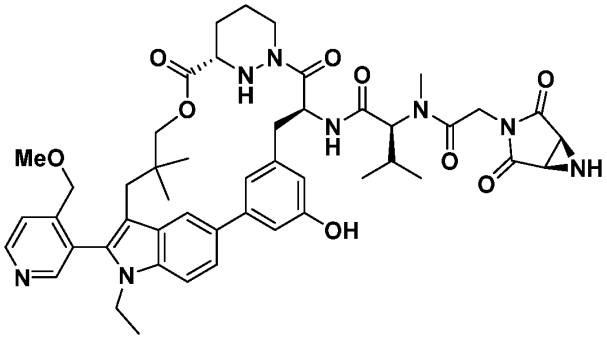
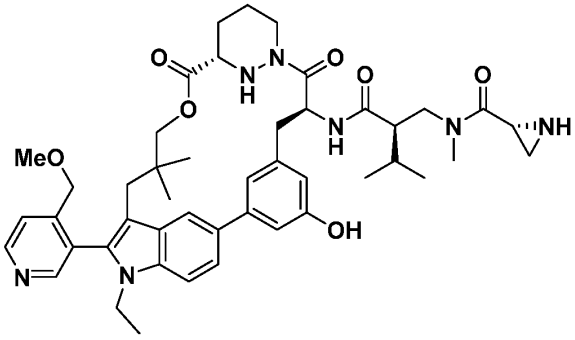
96	
97	
98	
99	

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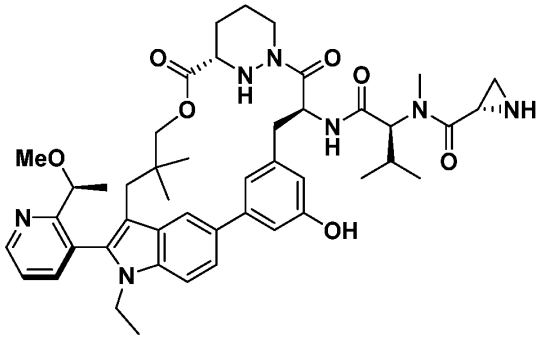
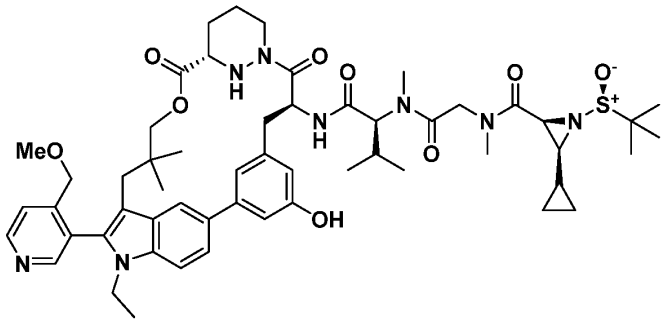
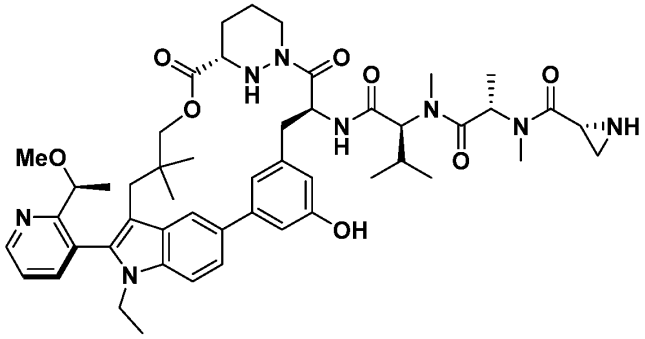
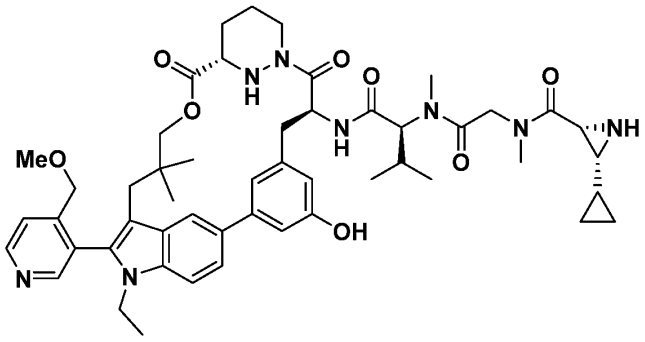
100	
101	
102	
103	

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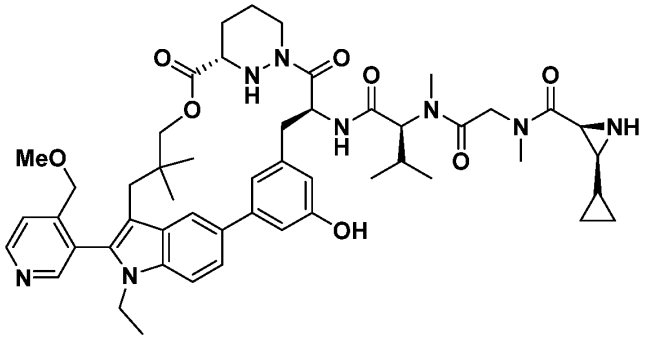
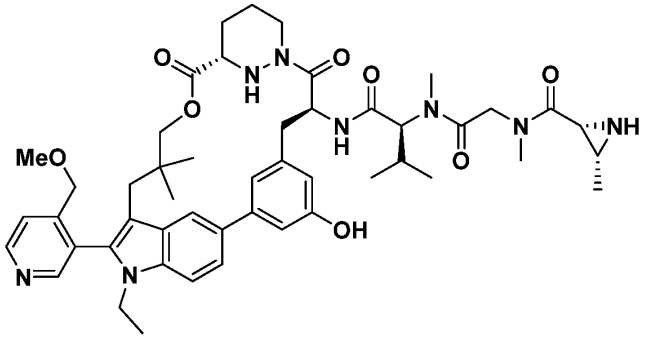
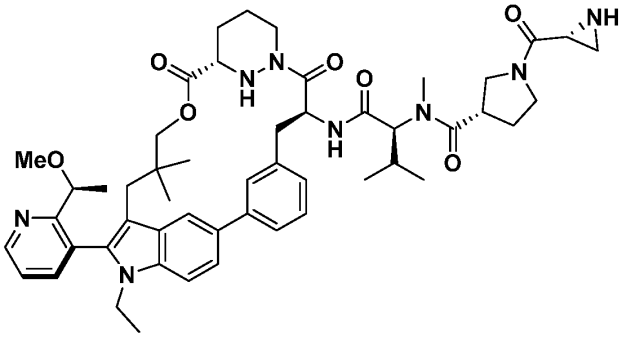
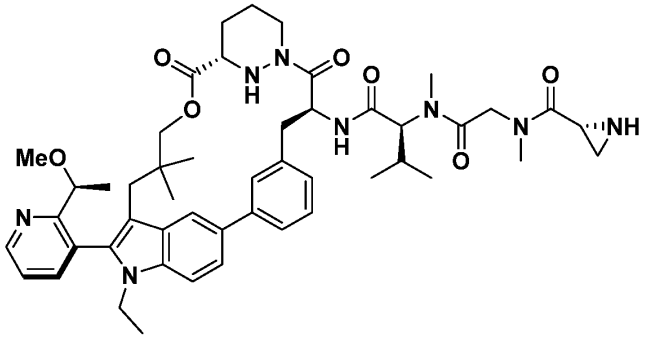
104	
105	
106	
107	

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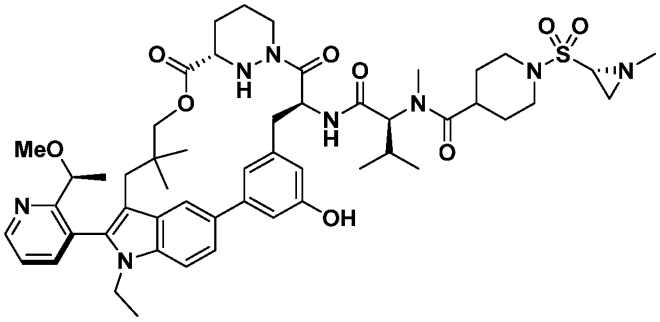
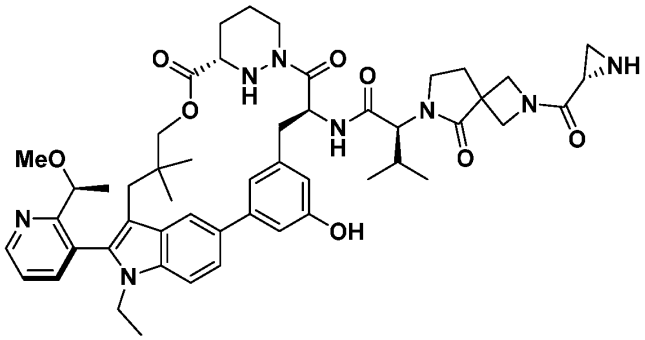
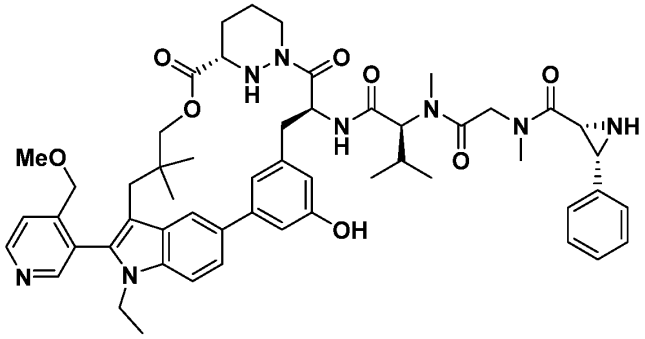
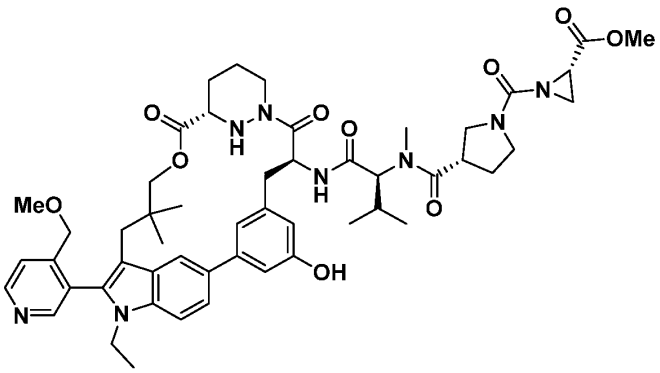
108*	
109	
110	
111	

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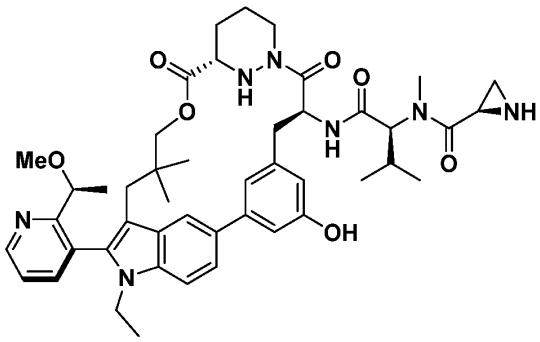
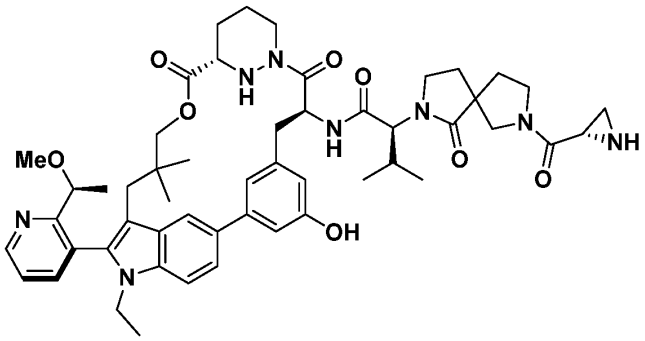
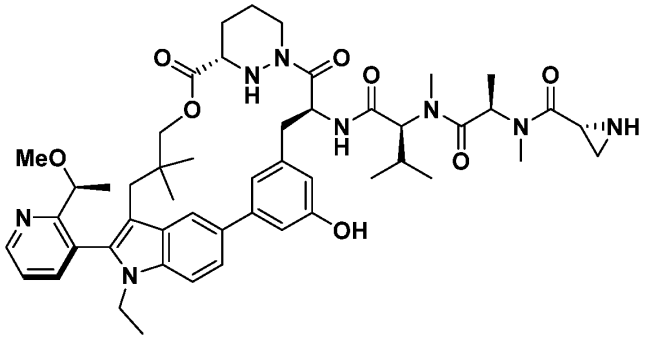
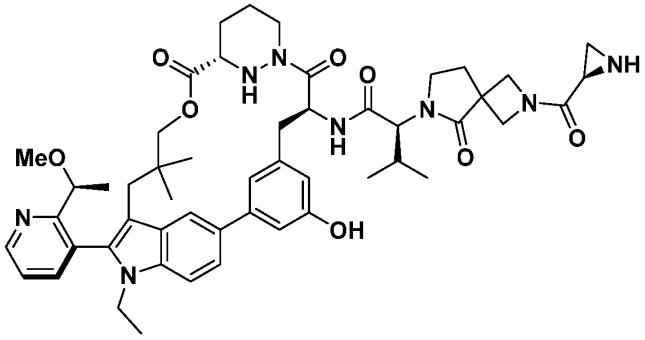
112	
113	
114	
115	

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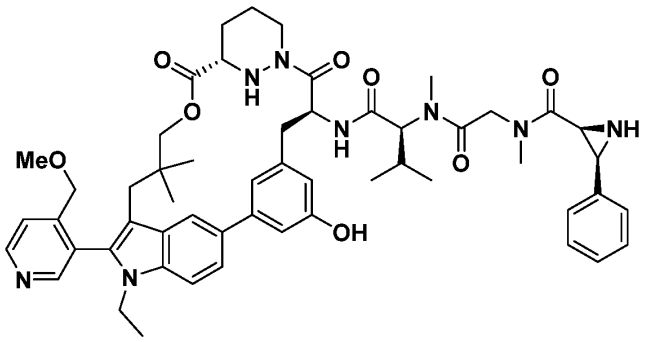
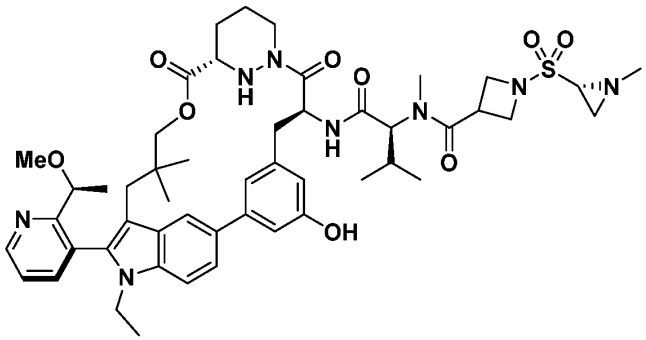
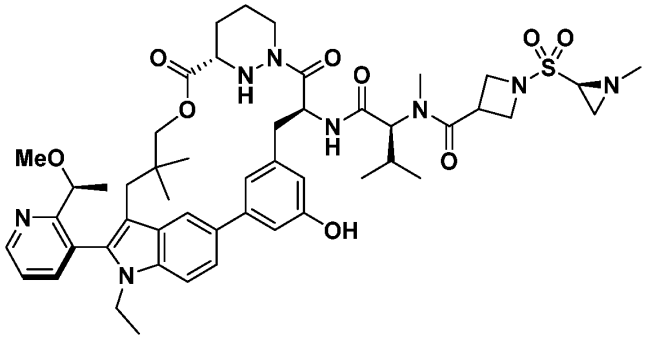
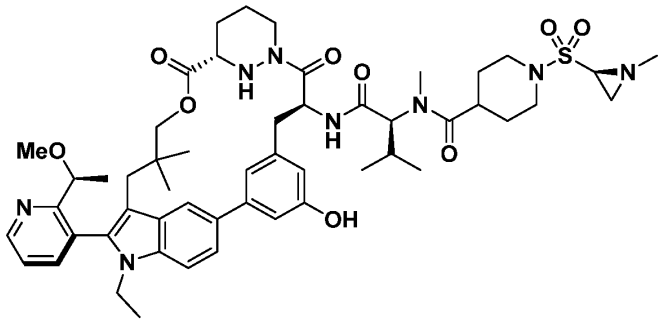
116	
117*	
118*	
119*	

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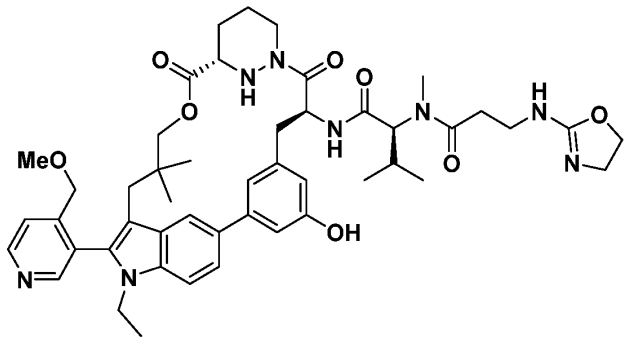
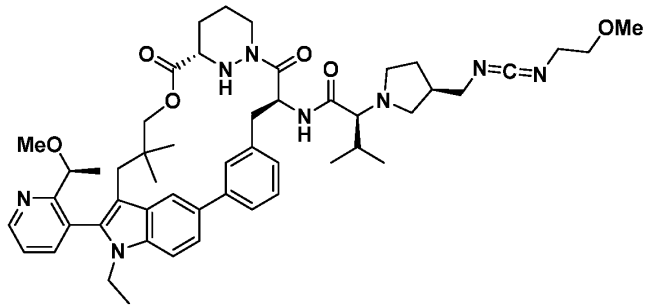
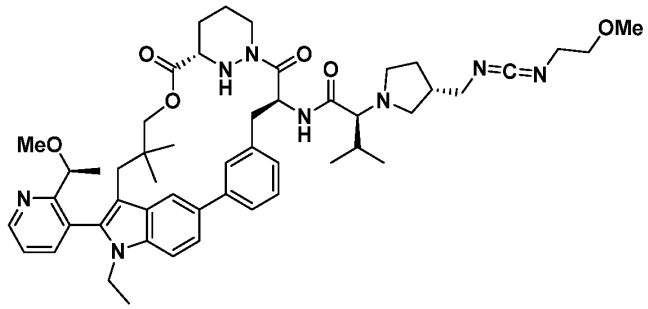
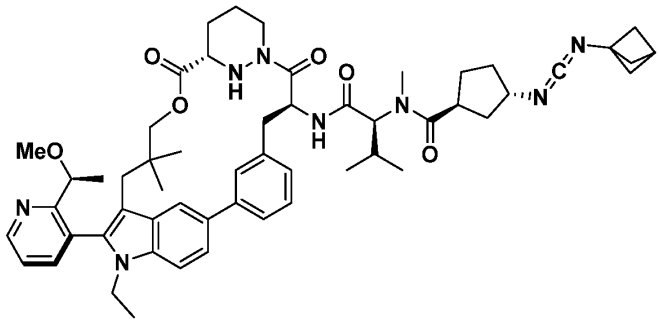
120	
121	
122	
123	

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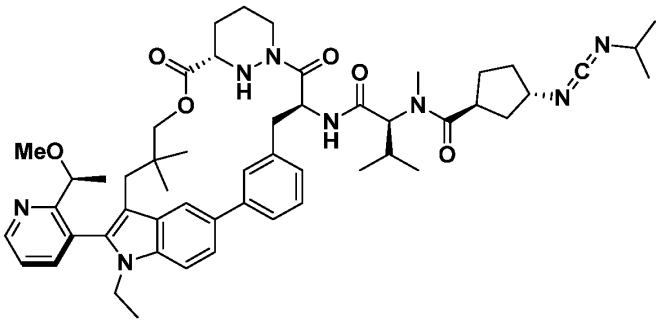
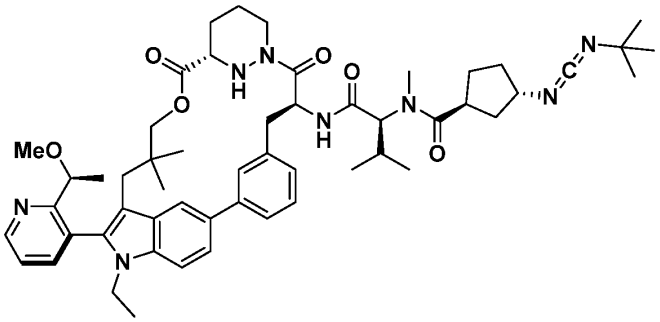
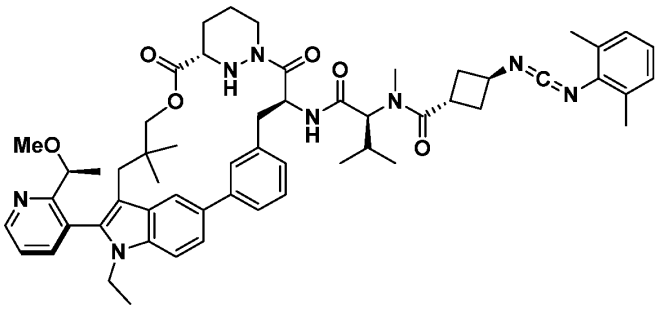
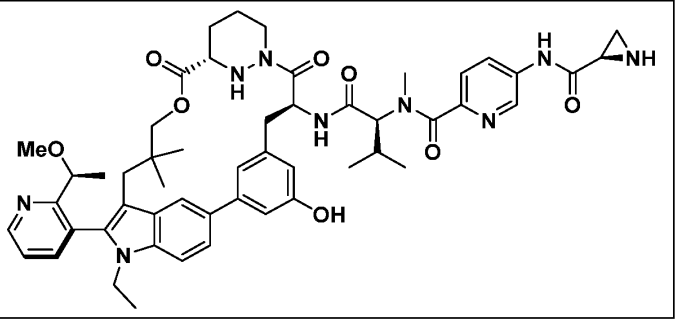
124	
125	
126	
127	

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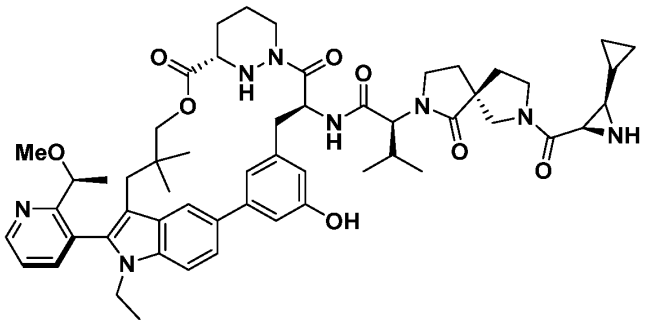
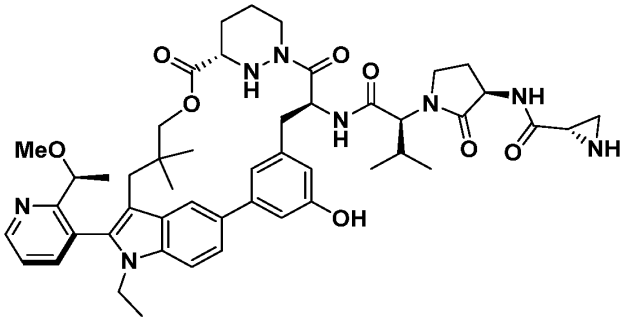
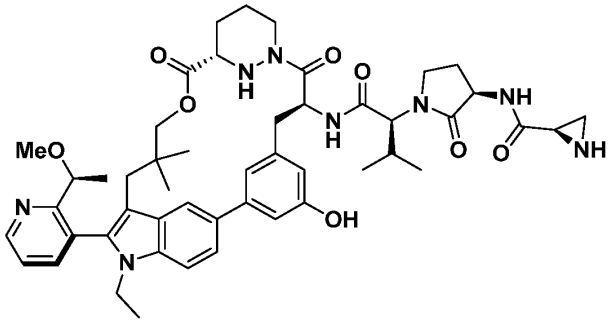
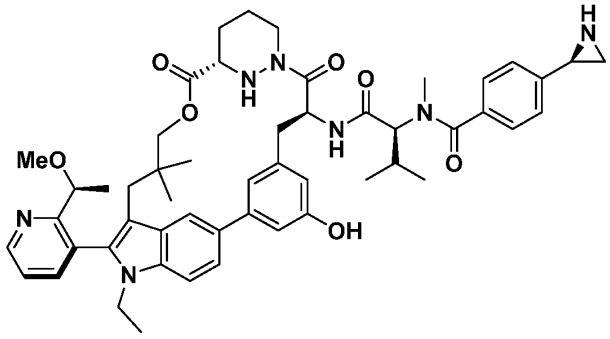
128	
129	
130	
131	

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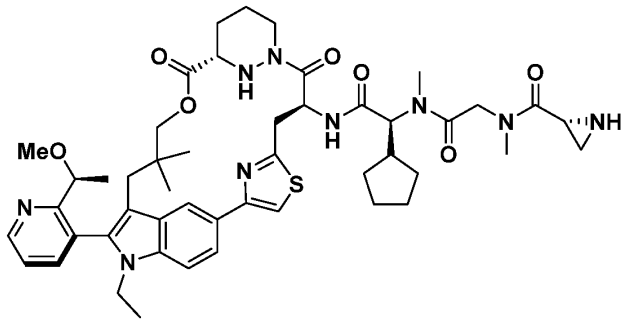
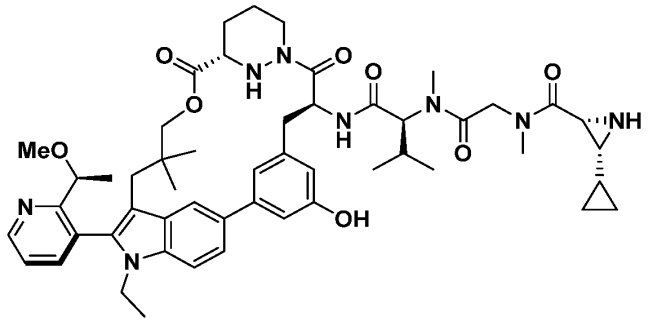
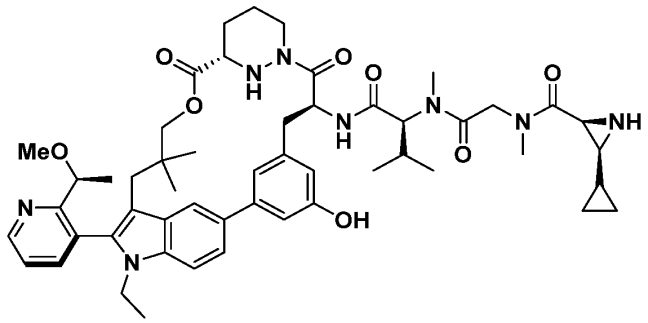
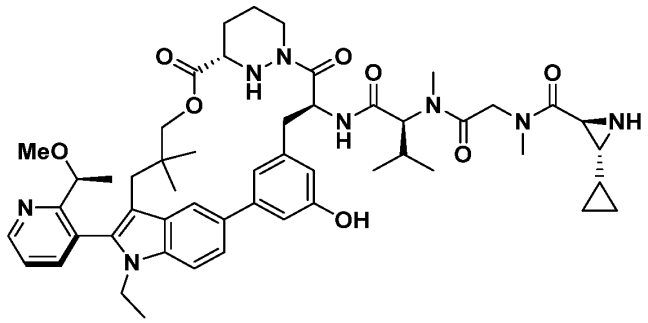
132	
133	
134	
135	

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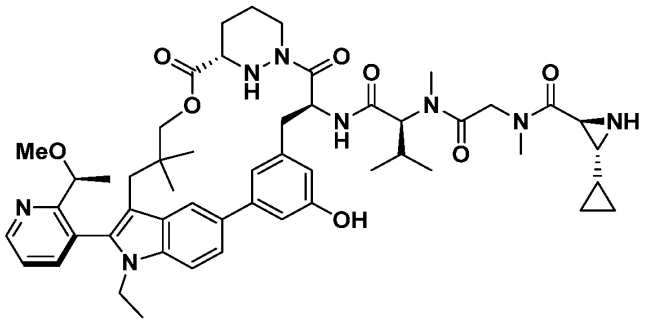
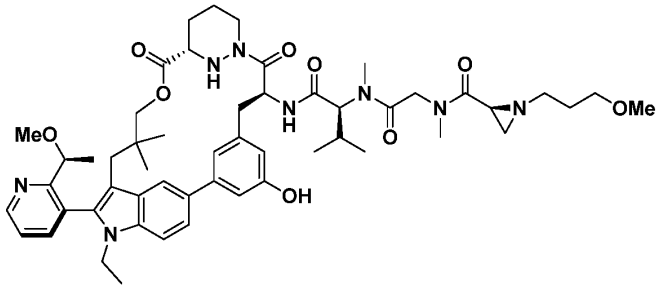
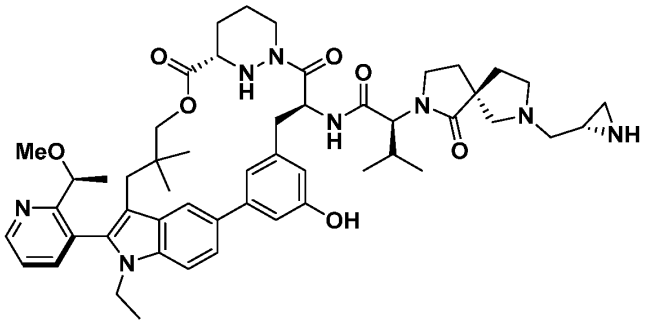
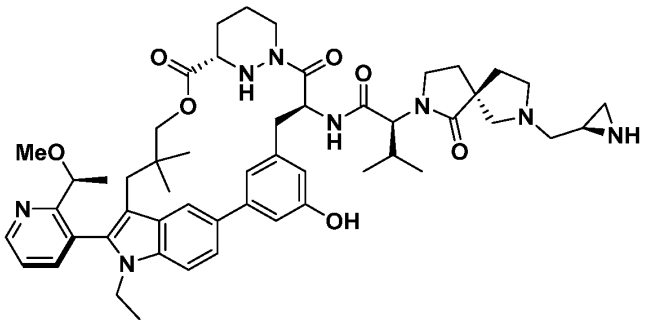
136	
137	
138	
139	

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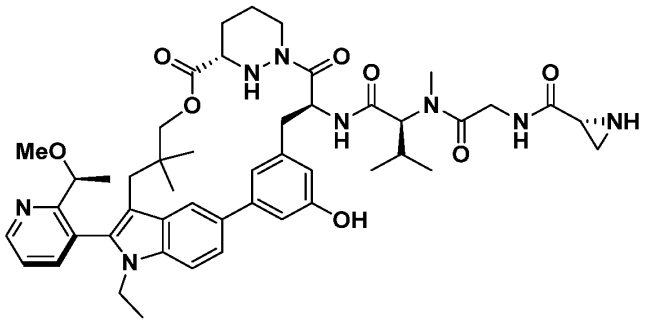
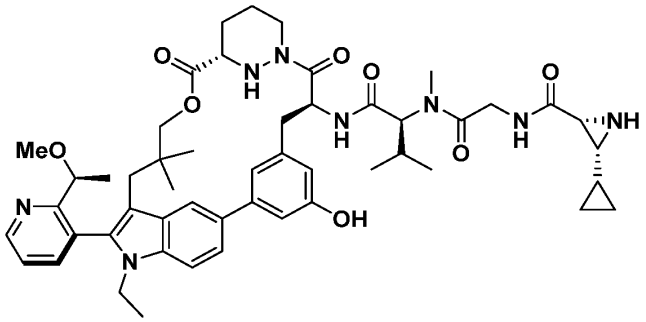
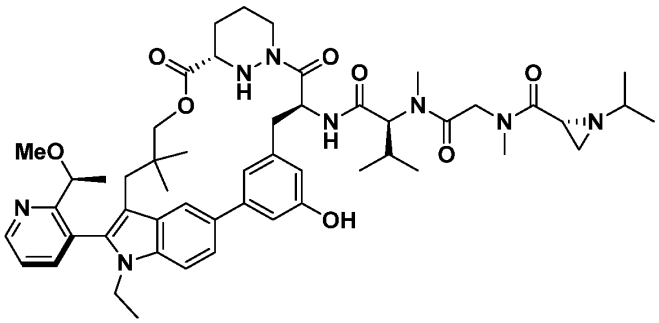
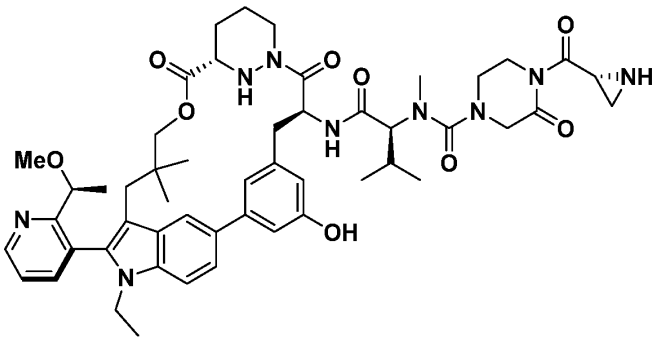
140	
141	
142	
143	

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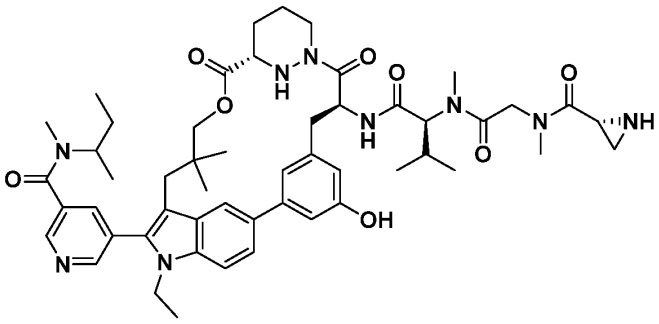
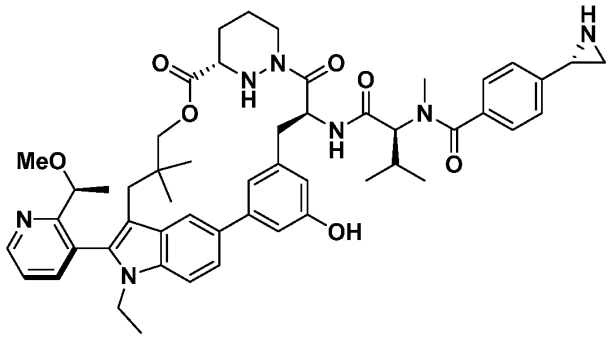
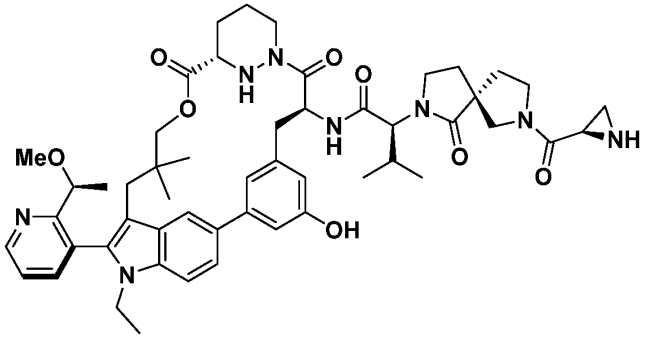
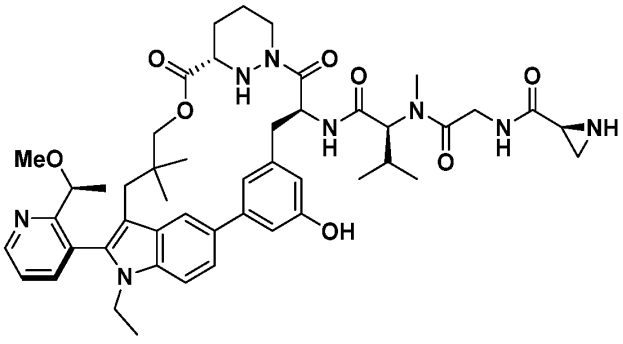
144	
145	
146	
147	

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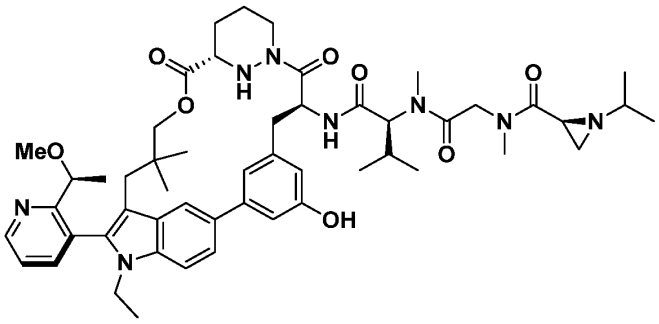
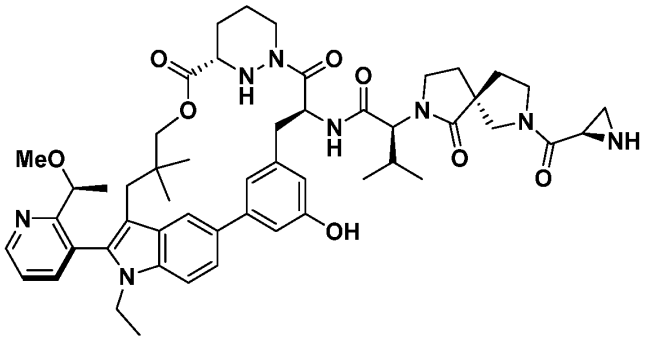
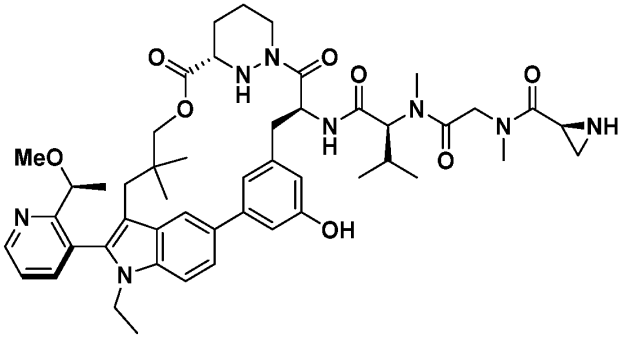
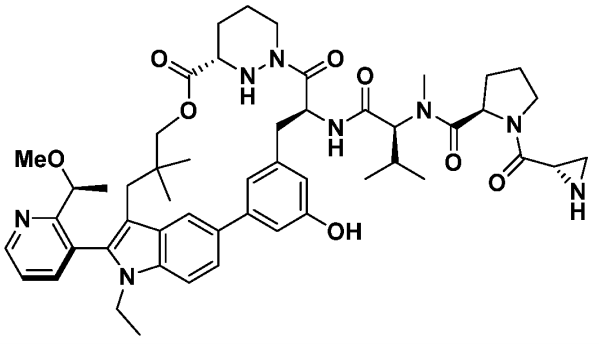
148	
149	
150	
151	

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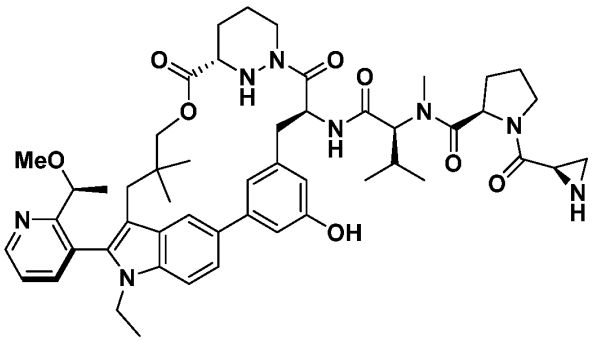
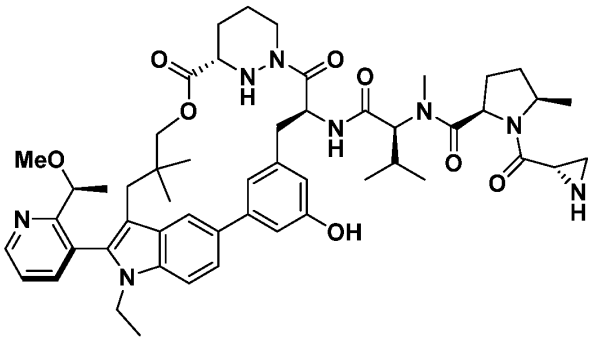
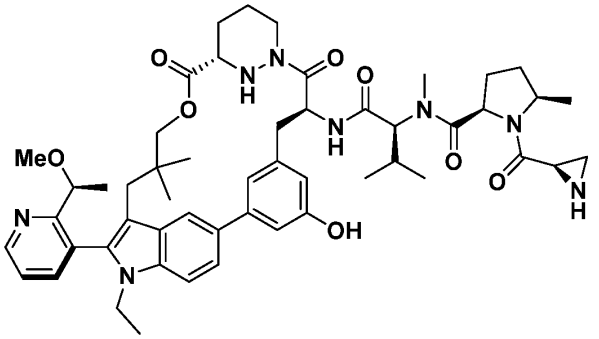
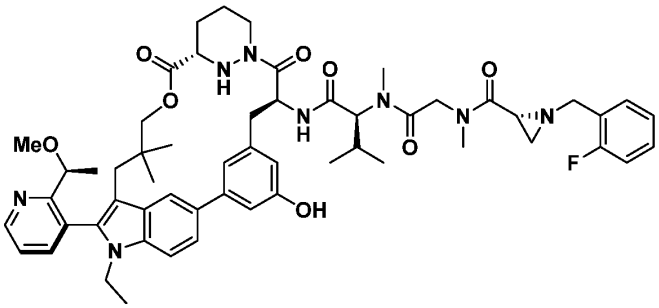
152	
153	
154	
155	

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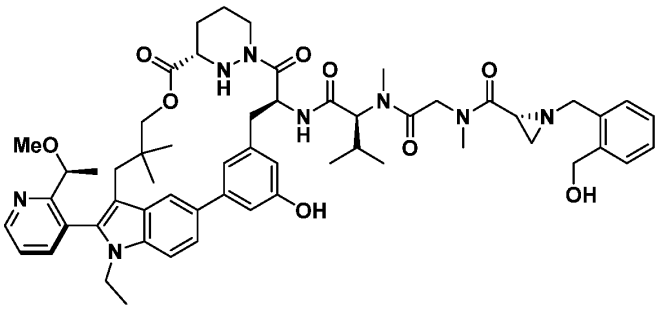
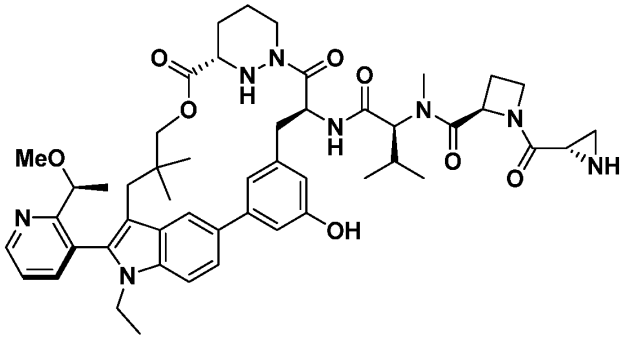
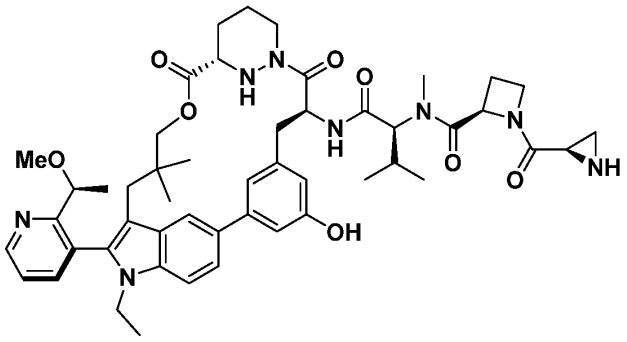
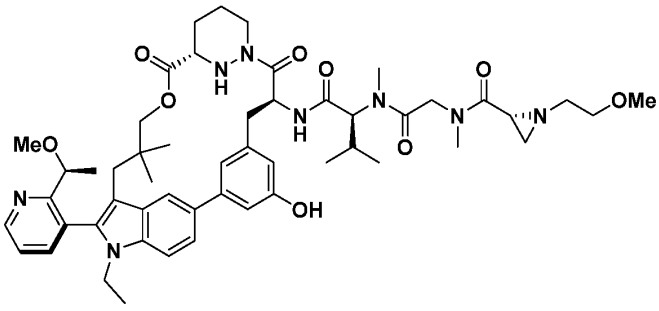
156	
157	
158	
159	

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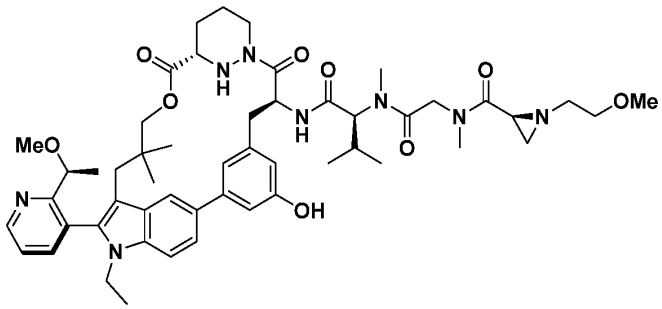
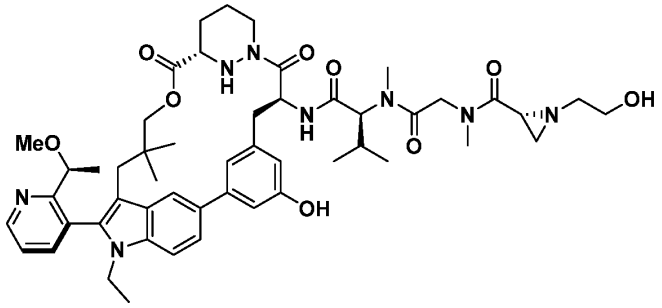
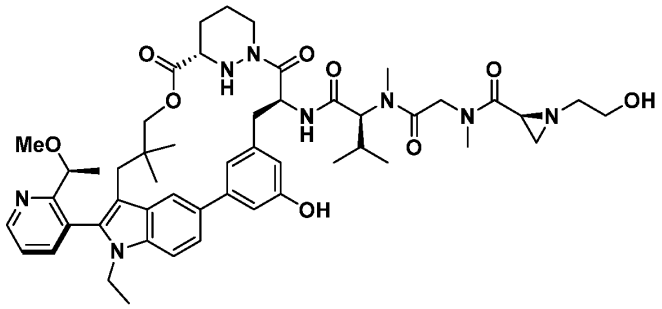
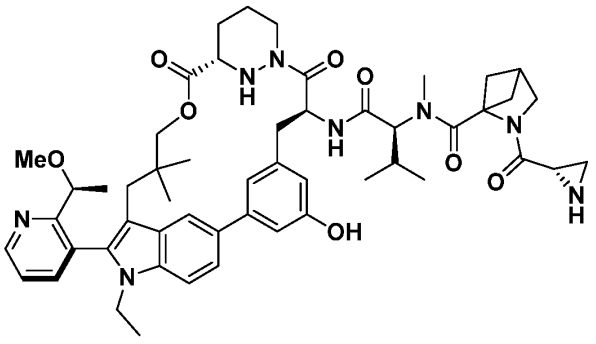
160	
161	
162	
163	

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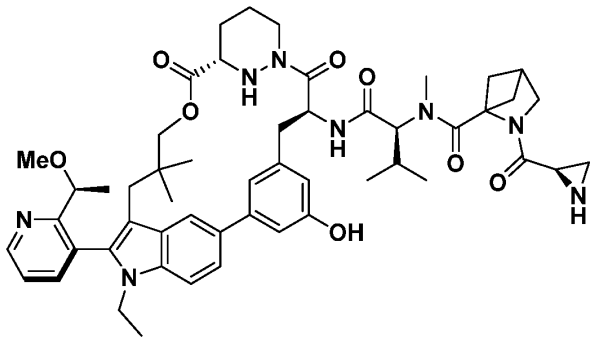
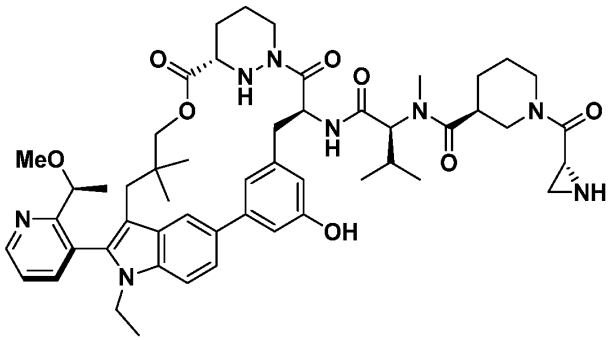
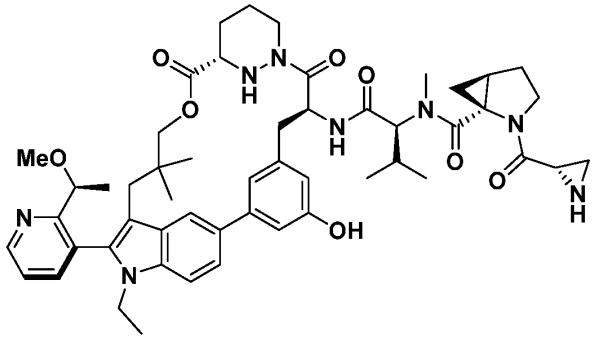
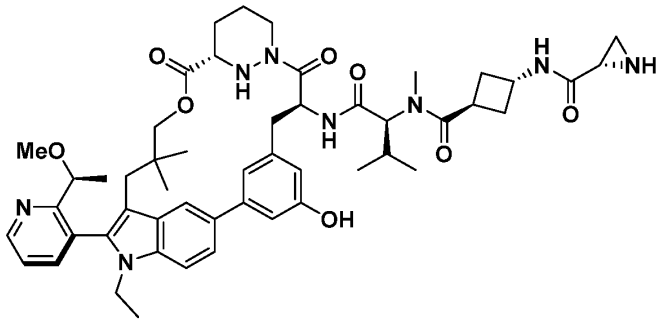
164	
165	
166	
167	

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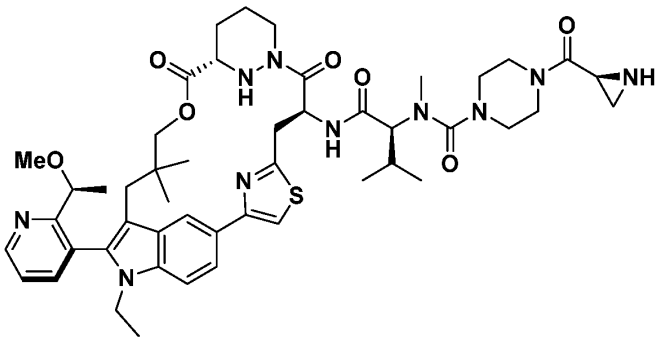
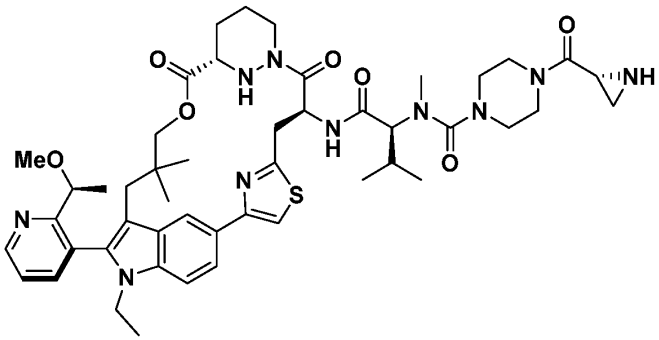
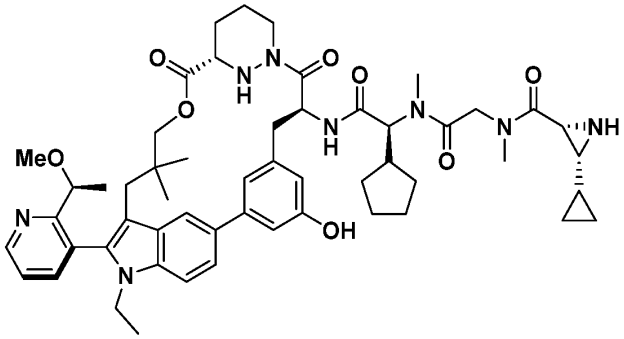
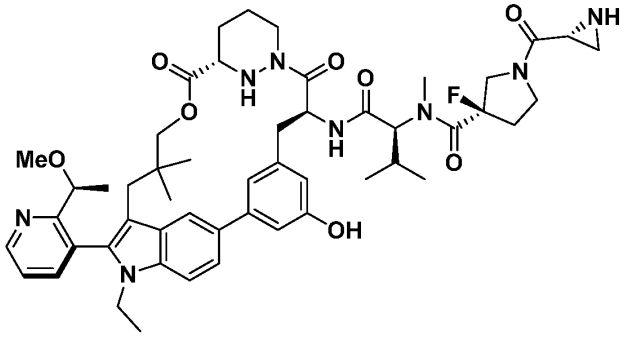
168	
169	
170	
171	

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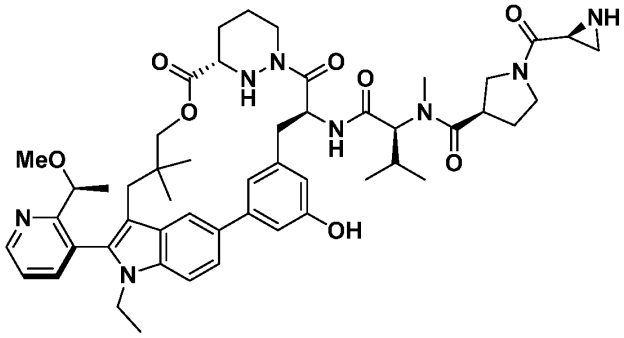
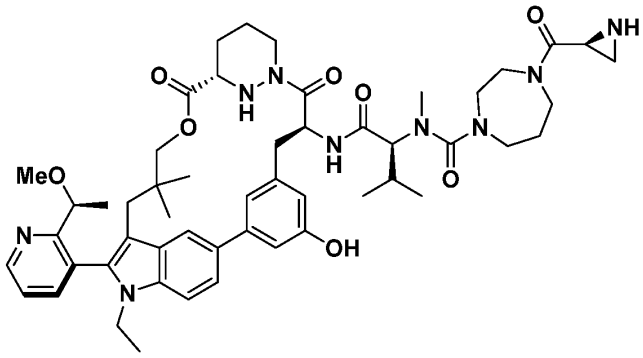
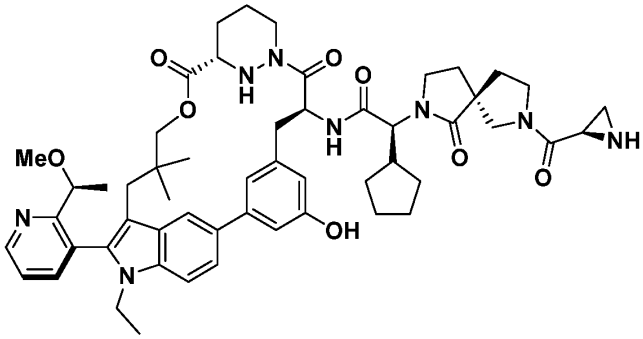
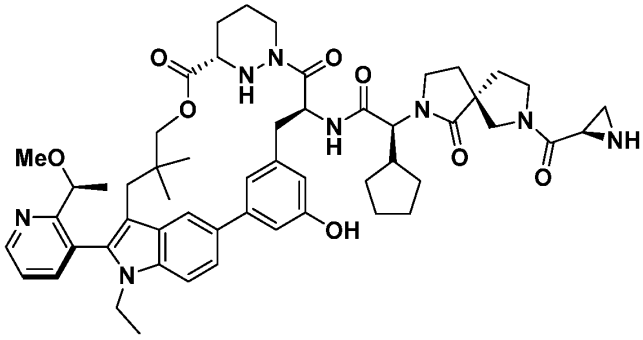
172	
173	
174	
175	

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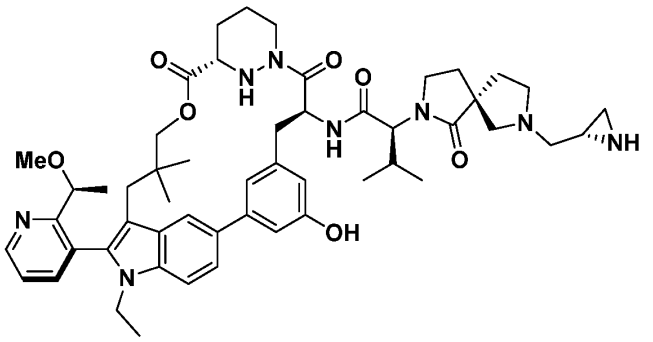
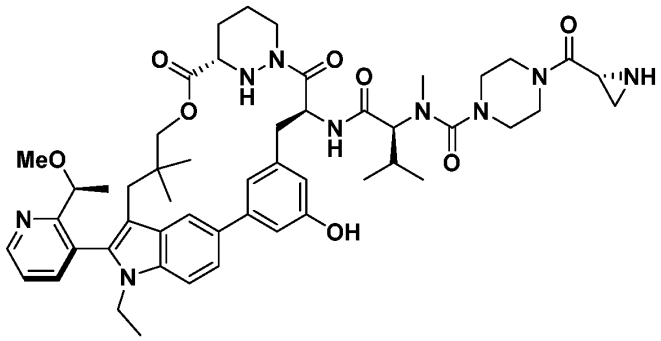
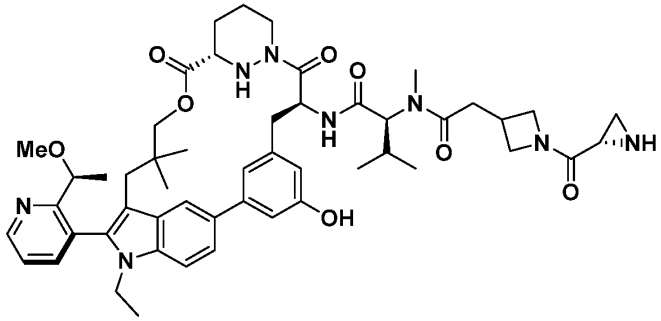
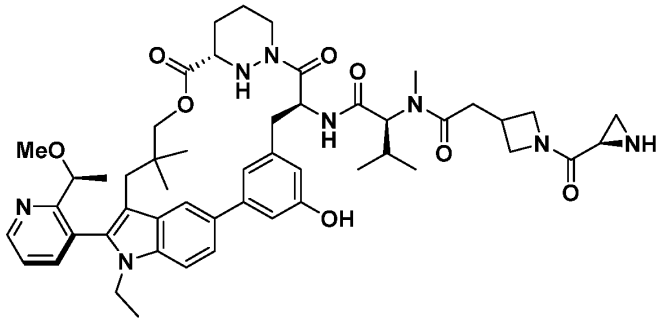
176	
177	
178	
179	

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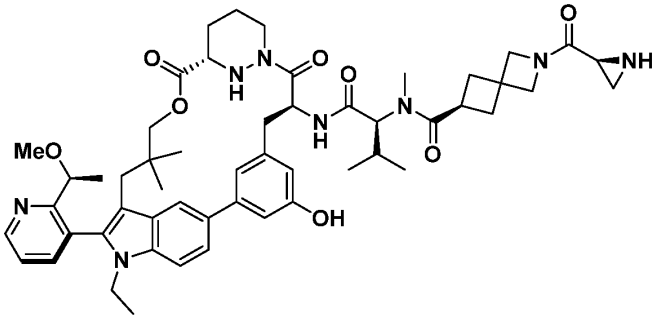
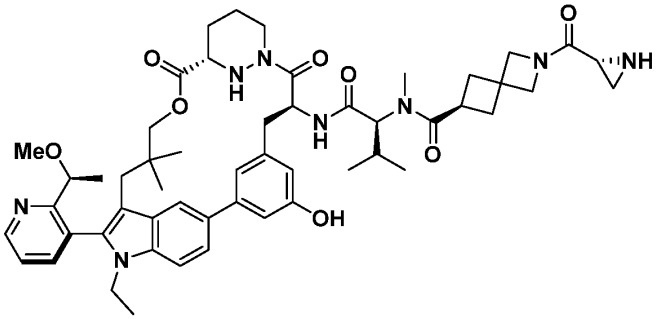
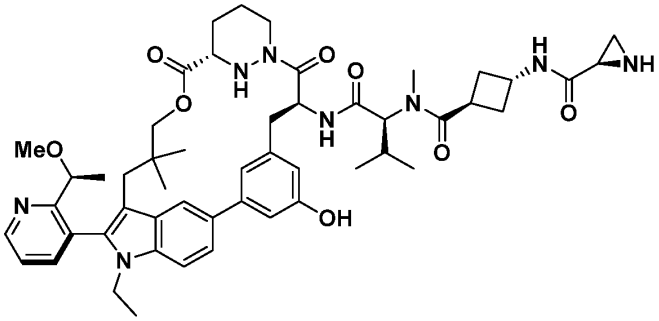
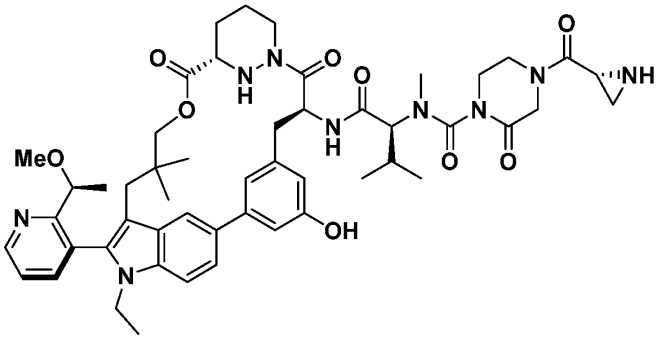
180	
181	
182	
183	

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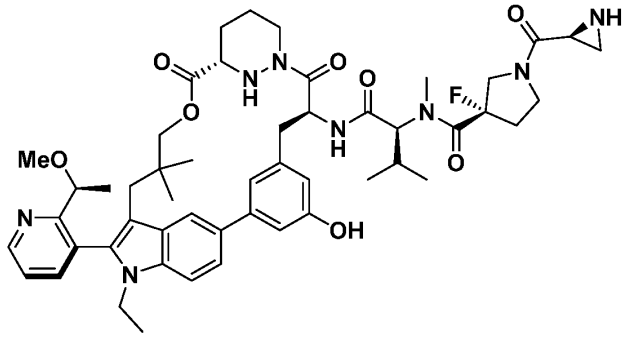
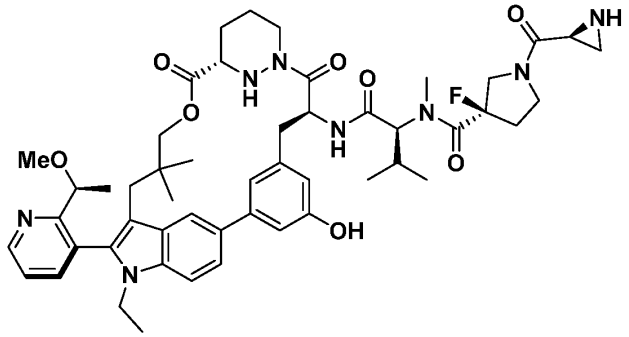
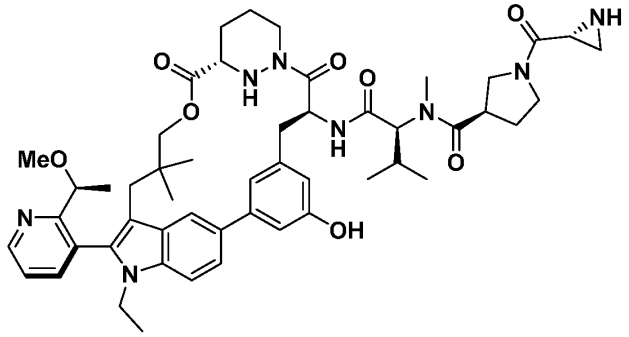
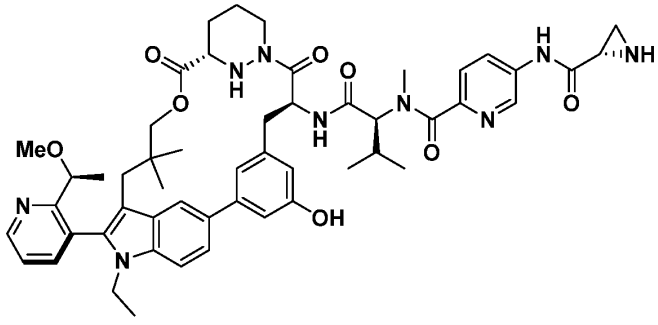
184	
185	
186	
187	

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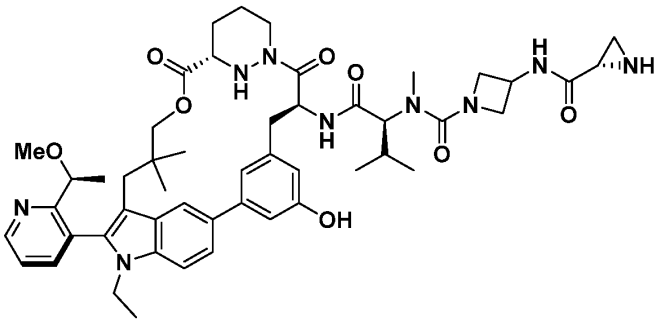
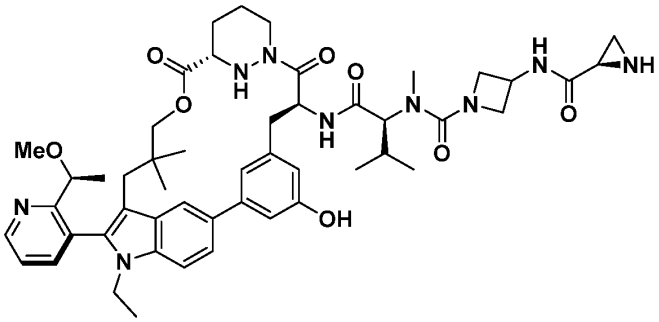
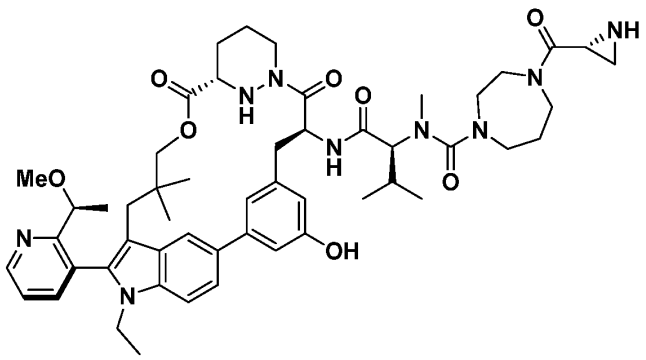
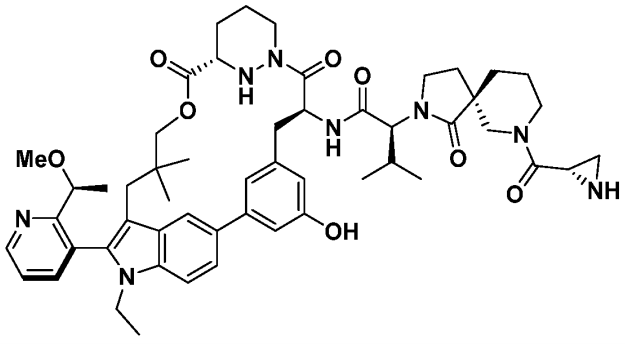
188	
189	
190	
191	

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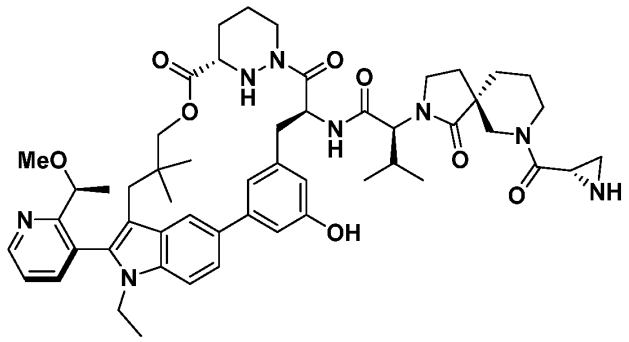
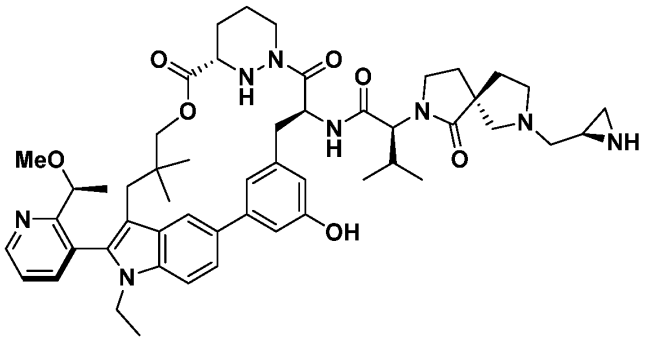
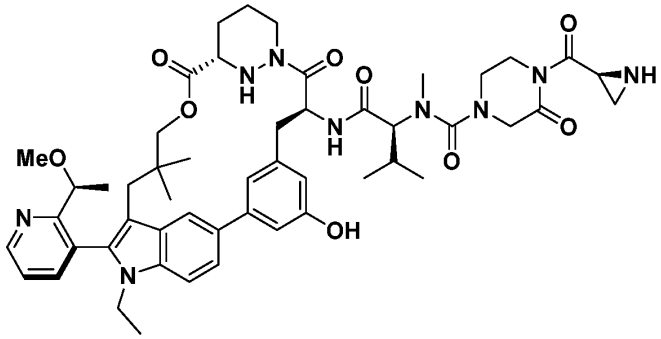
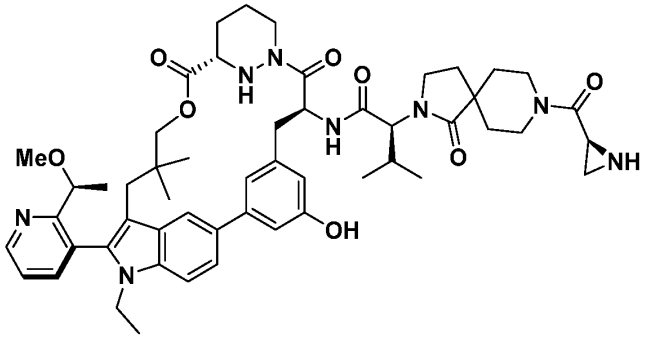
192	
193	
194	
195	

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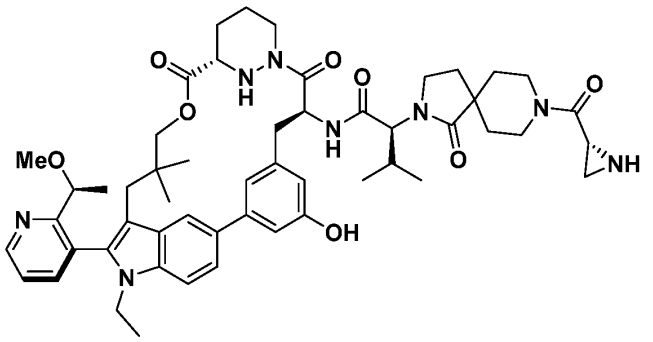
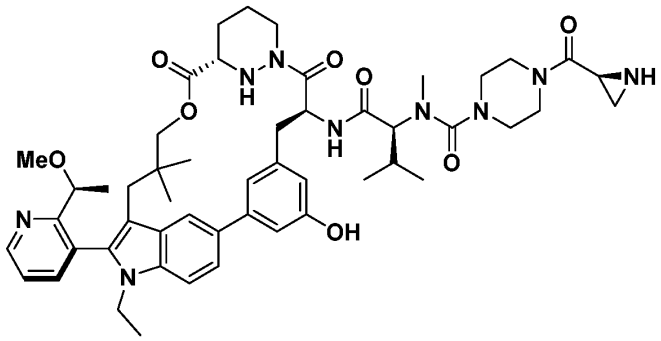
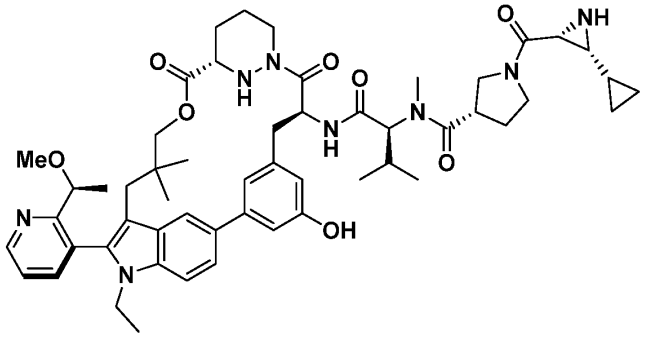
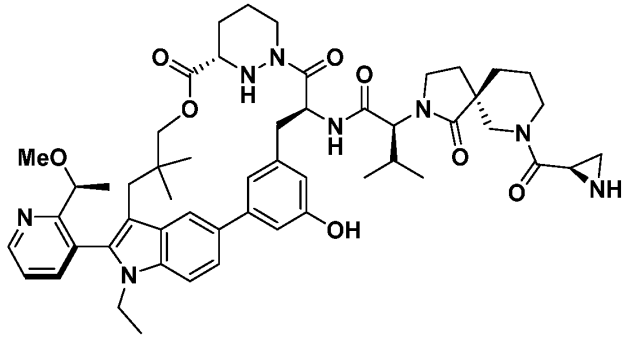
196	
197	
198	
199	

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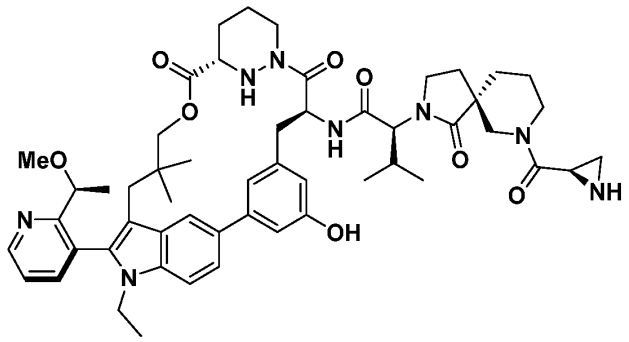
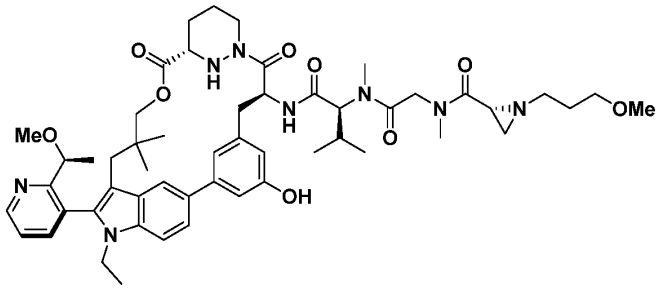
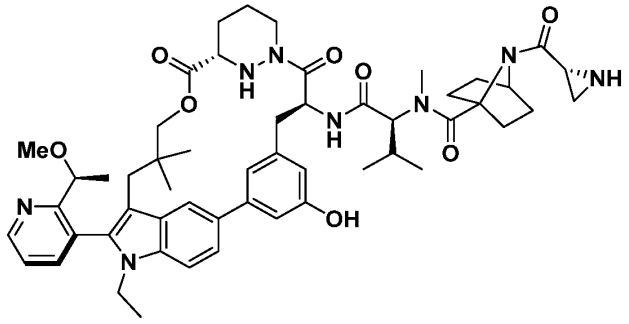
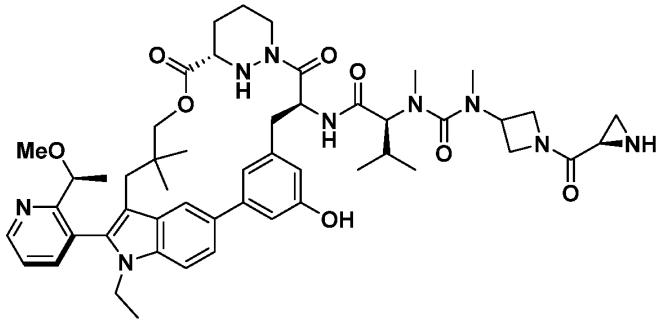
200	
201	
202	
203	

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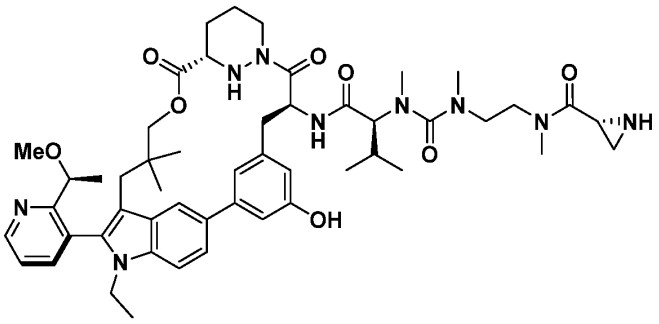
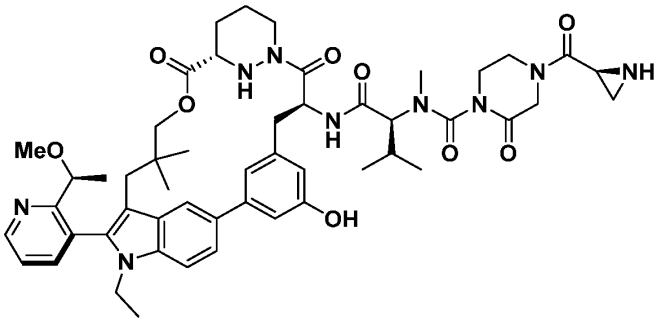
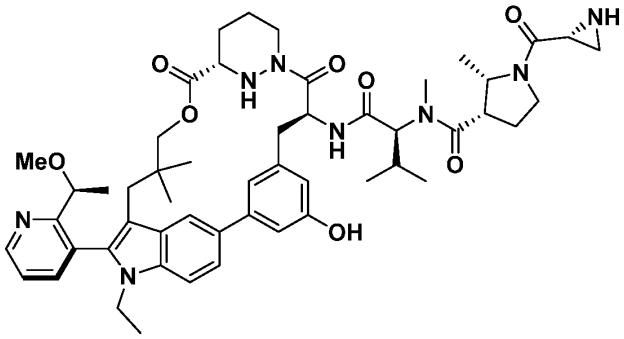
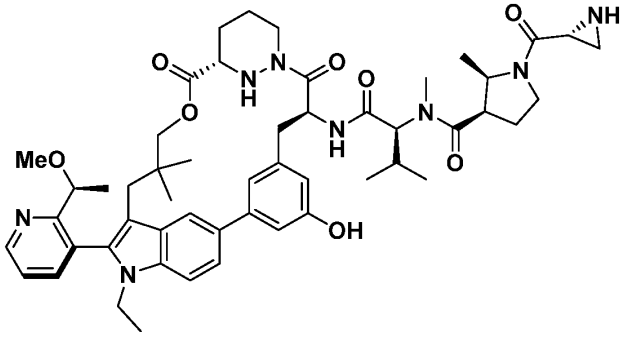
204	
205	
206	
207	

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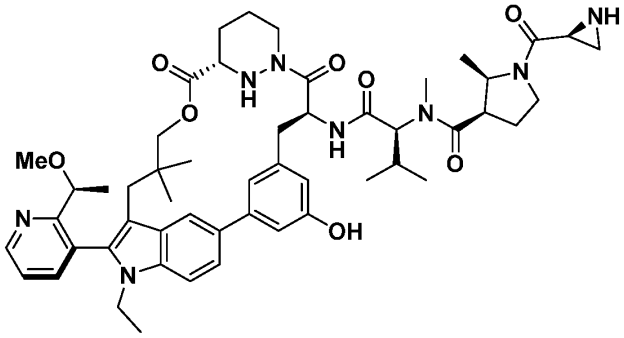
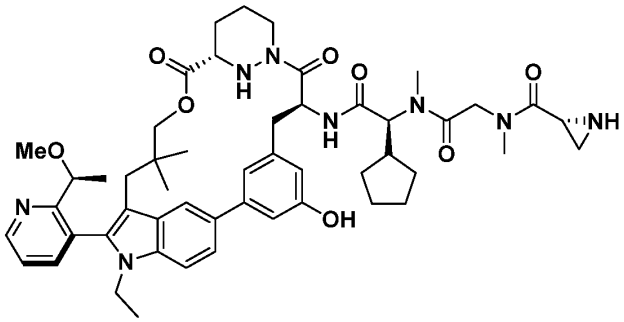
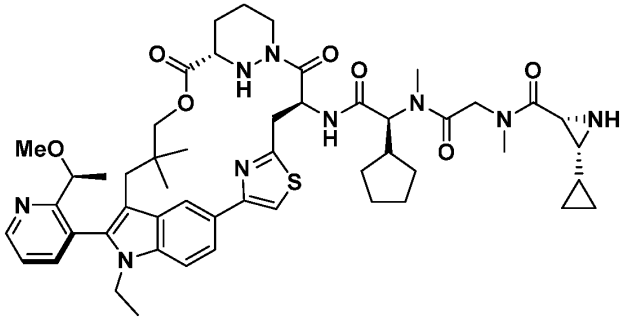
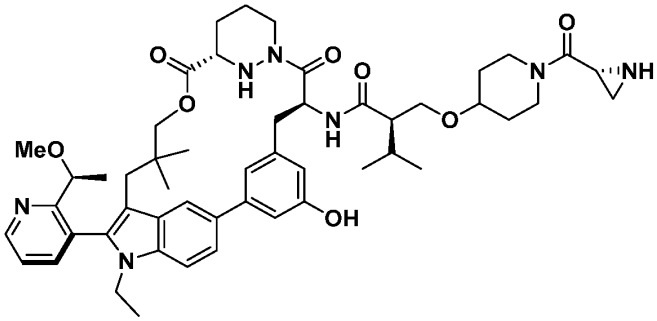
208	
209	
210	
211	

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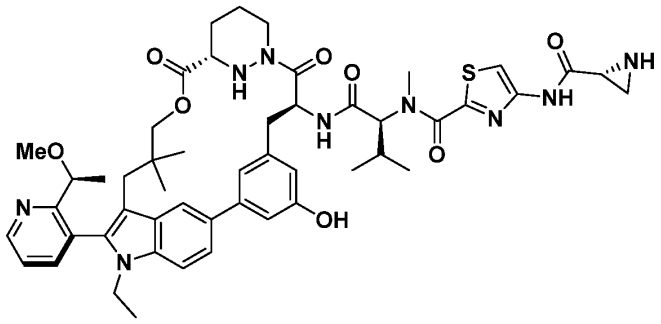
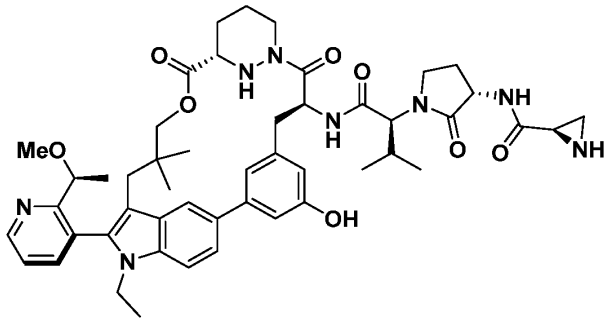
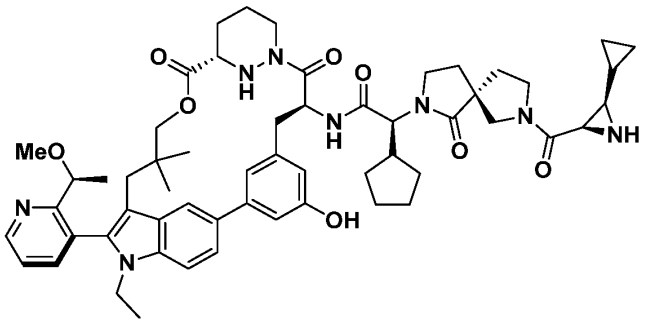
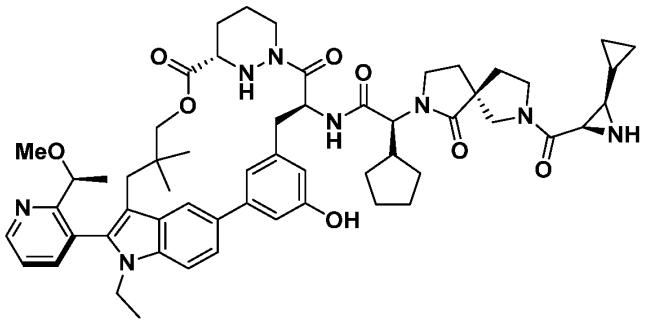
212	
213	
214	
215	

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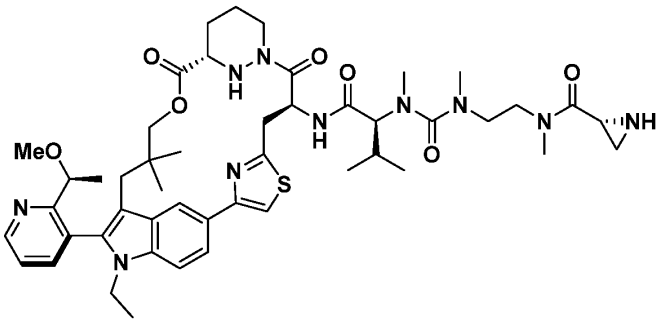
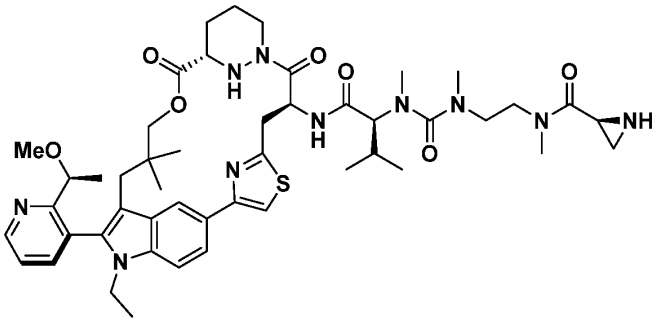
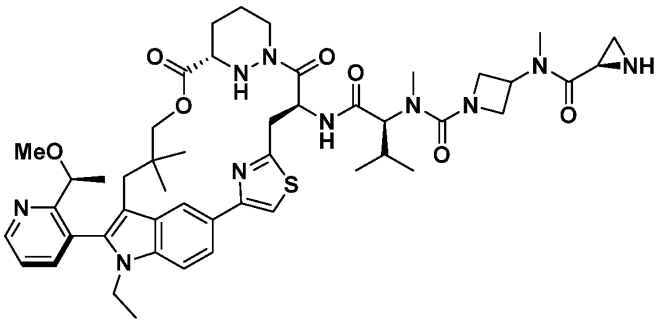
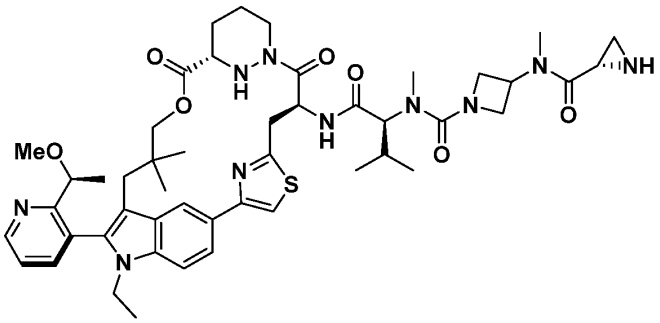
216	
217	
218	
219	

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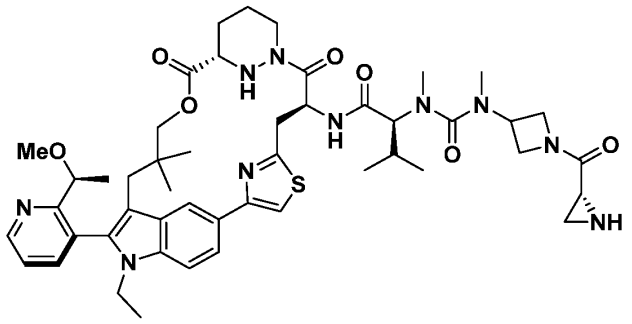
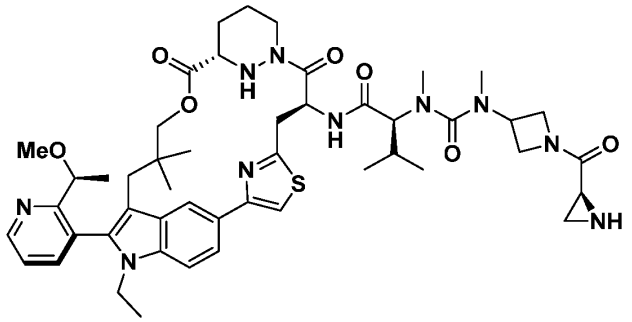
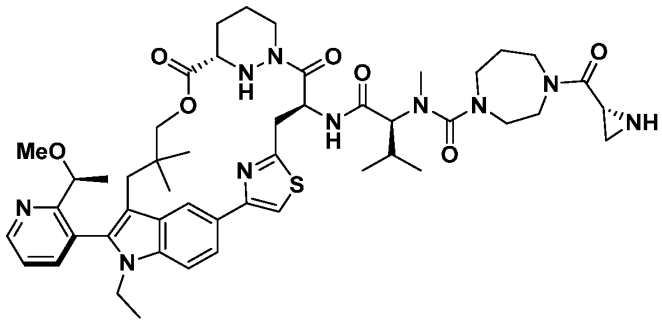
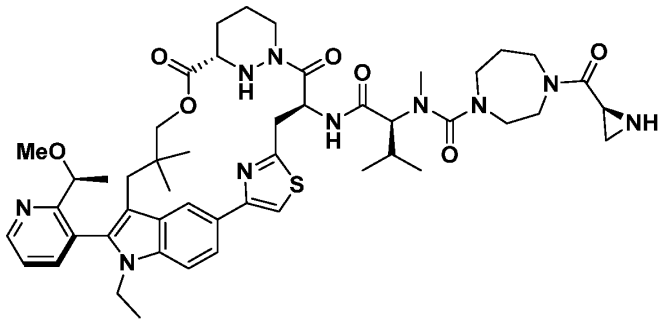
220	
221	
222	
223	

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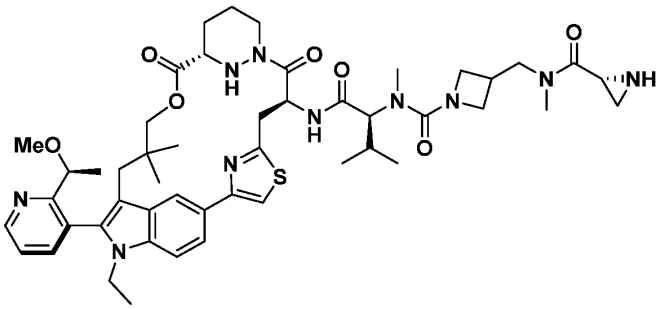
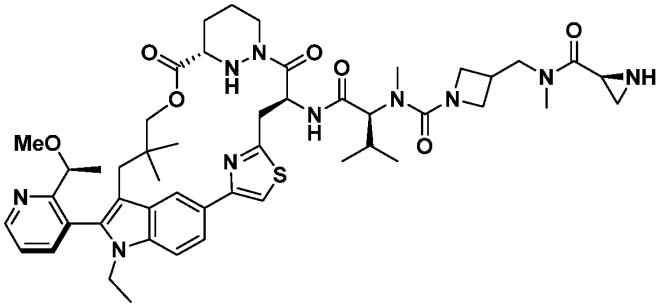
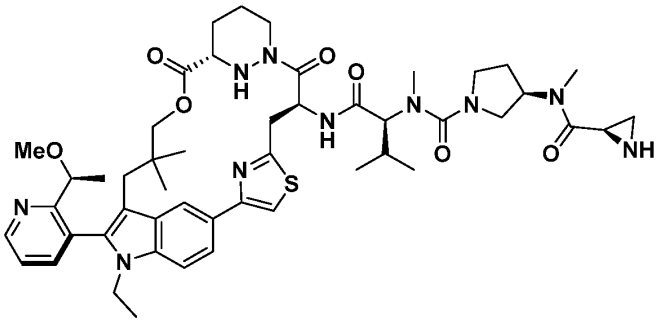
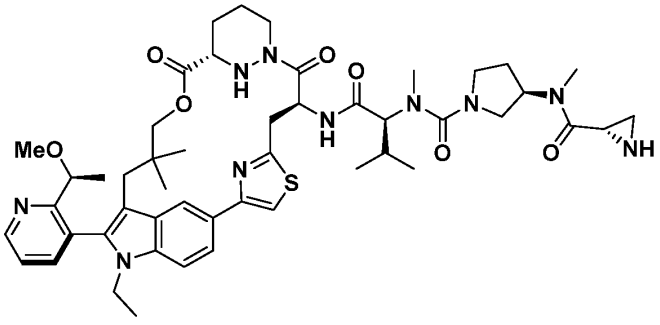
224	
225	
226	
227	

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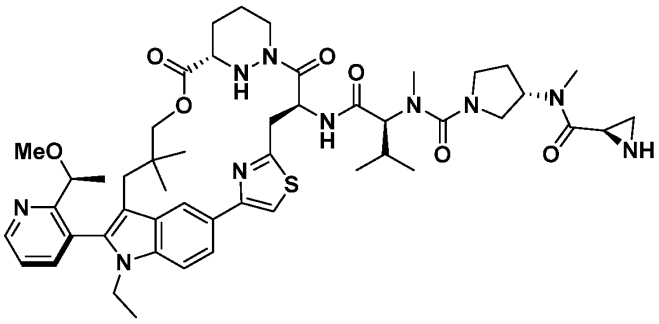
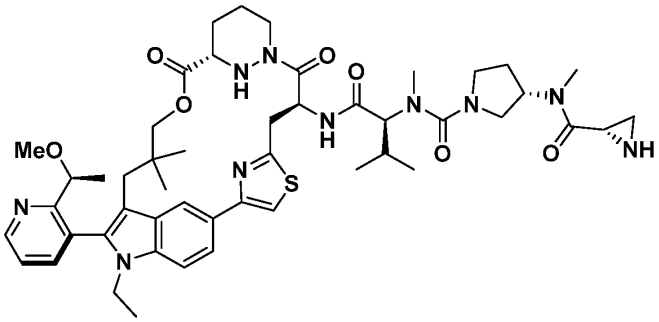
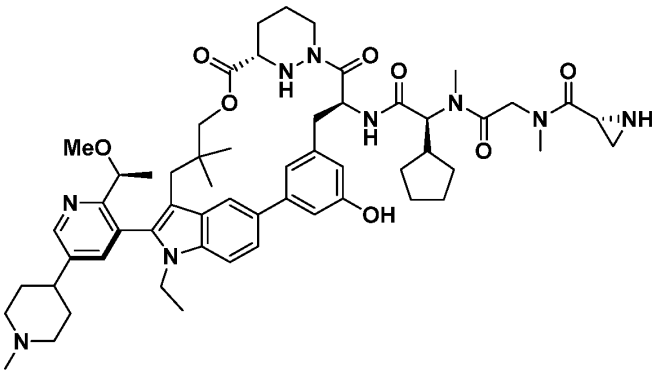
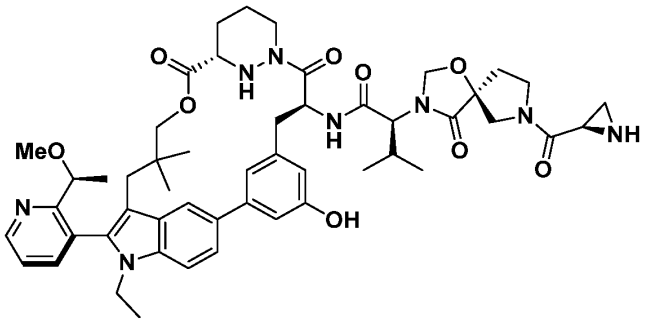
228	
229	
230	
231	

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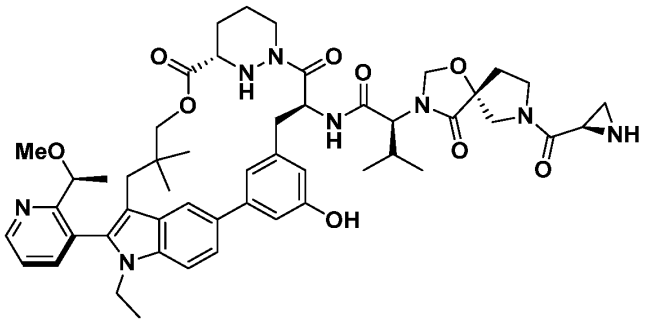
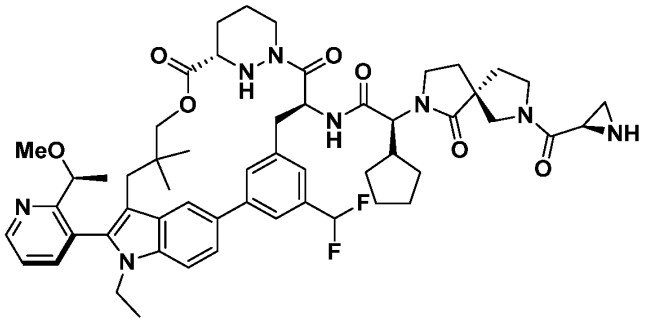
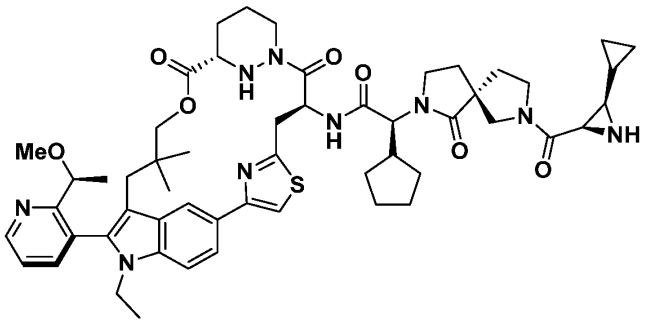
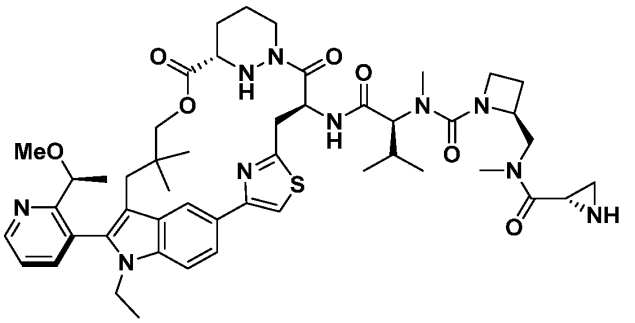
232	
233	
234	
235	

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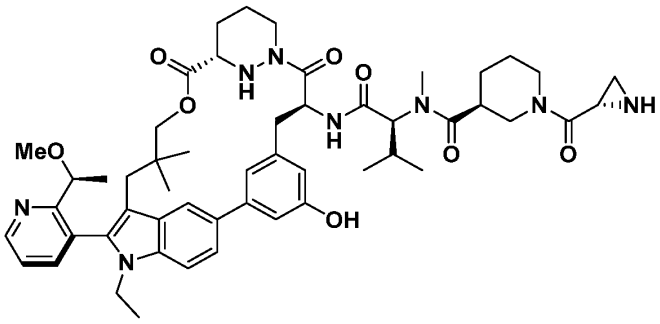
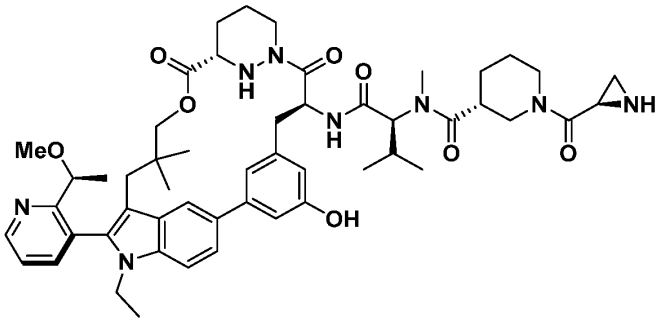
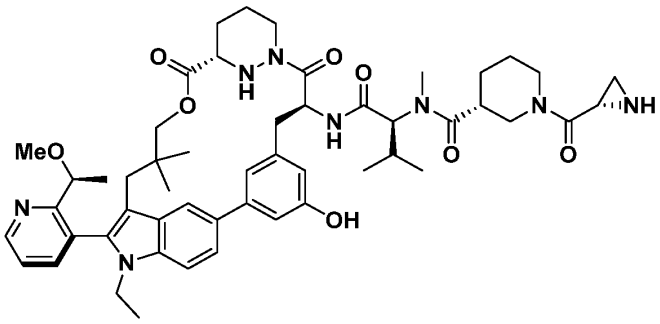
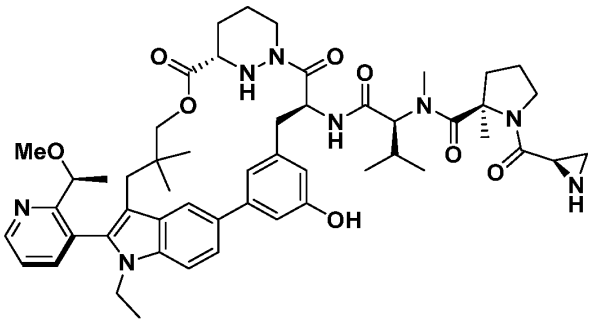
236	
237	
238	
239	

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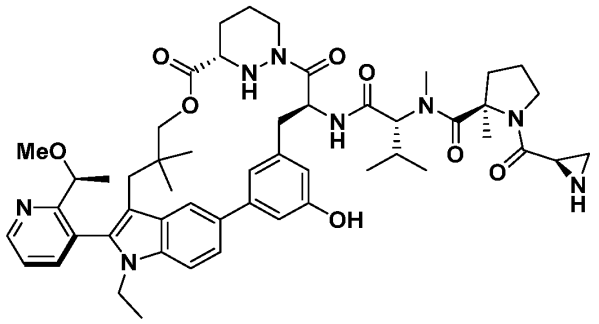
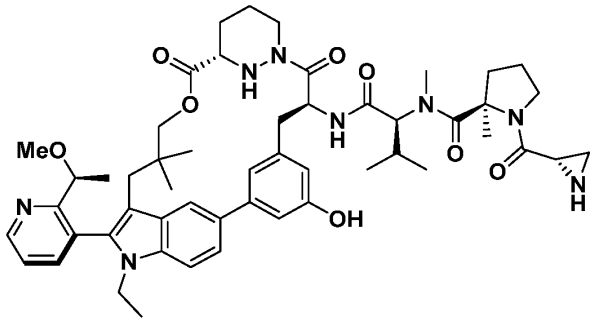
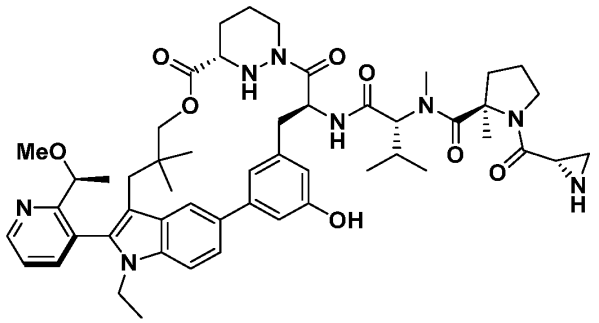
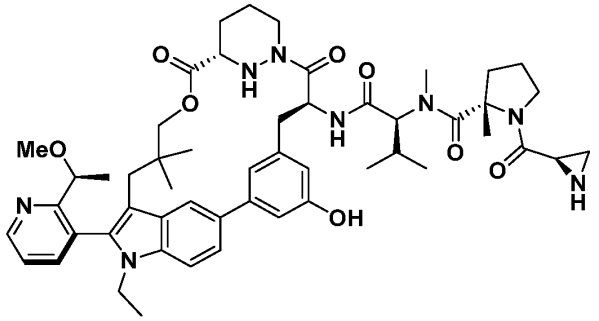
240	
241	
242	
243	

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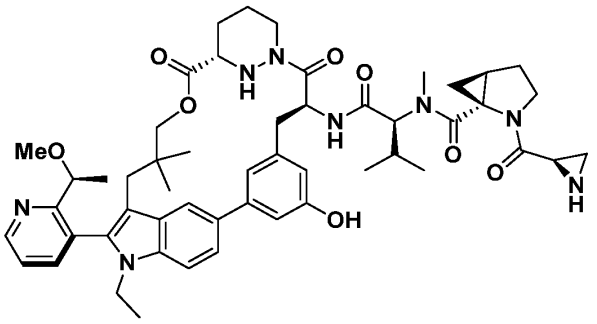
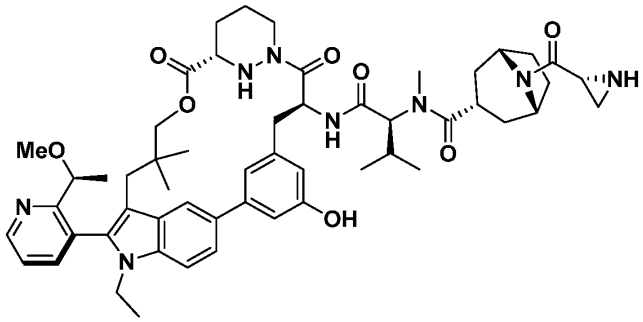
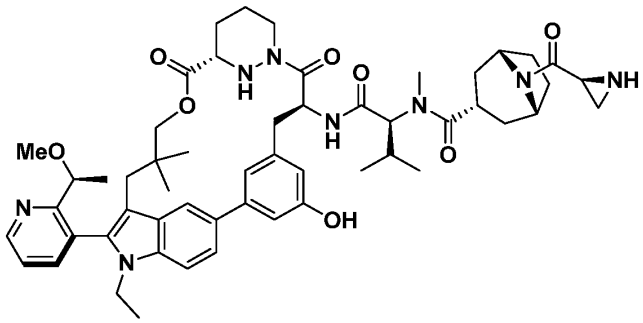
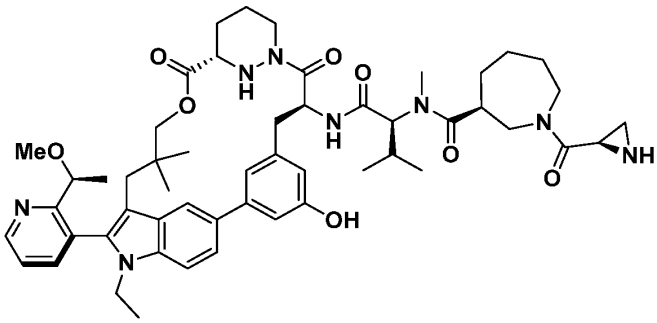
244	
245	
246	
247	

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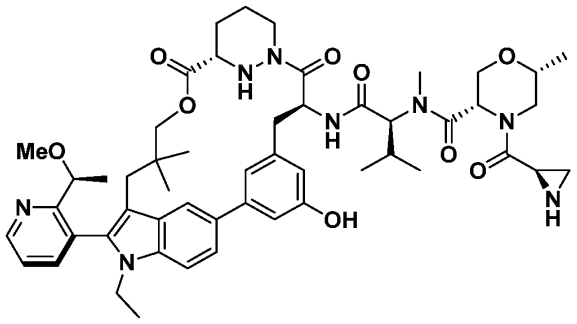
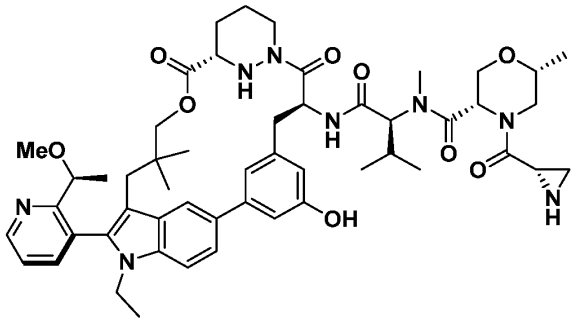
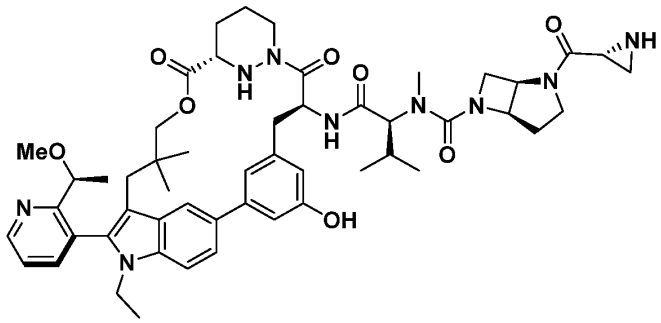
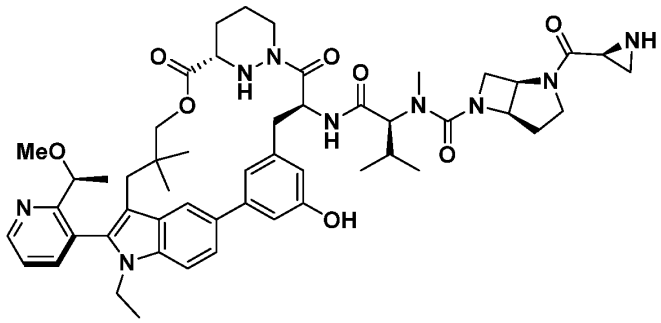
248	
249	
250	
251	

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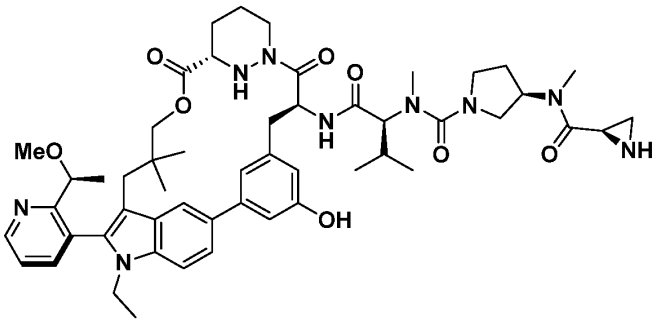
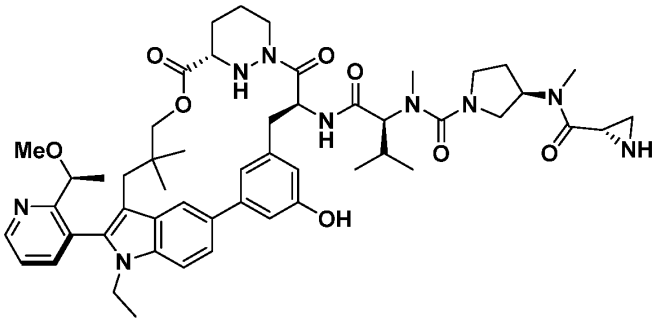
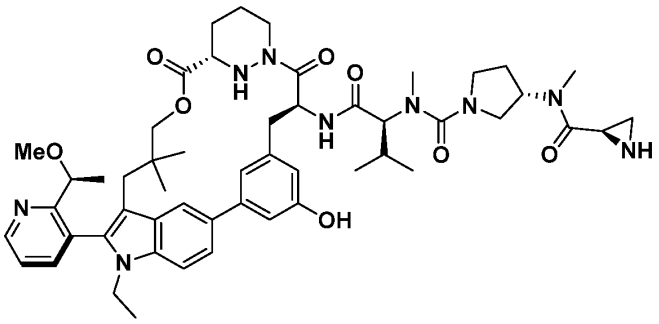
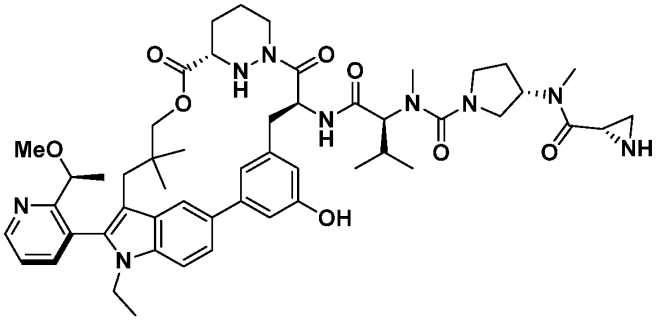
252	
253	
254	
255	

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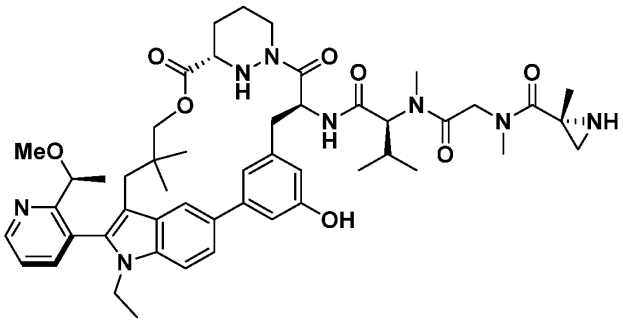
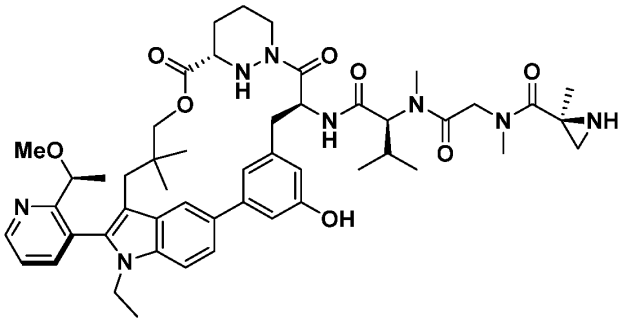
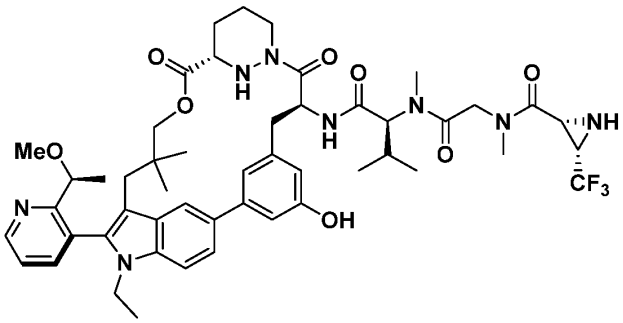
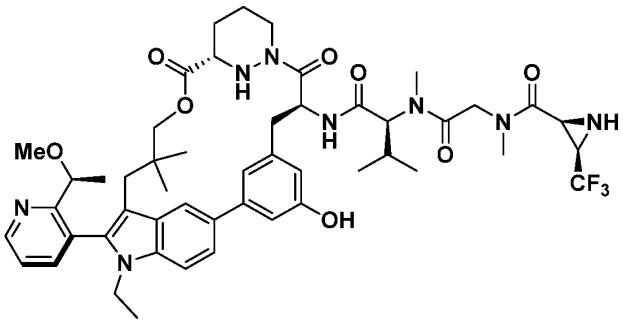
256	
257	
258*	
259*	

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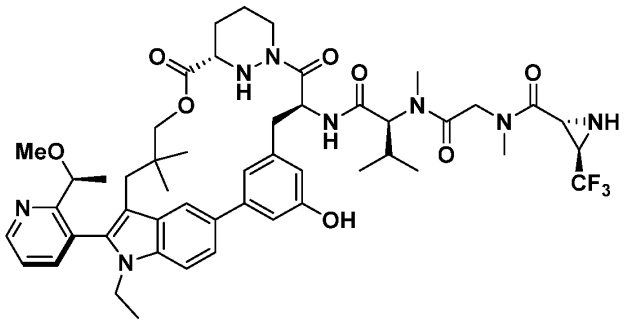
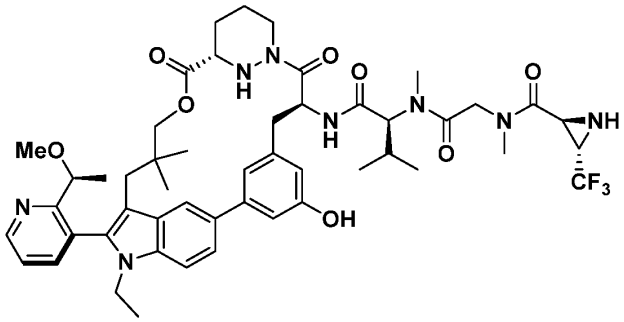
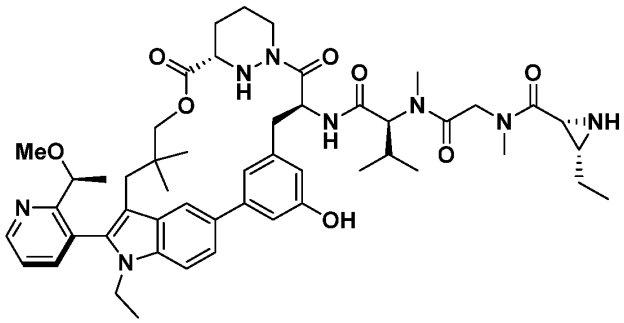
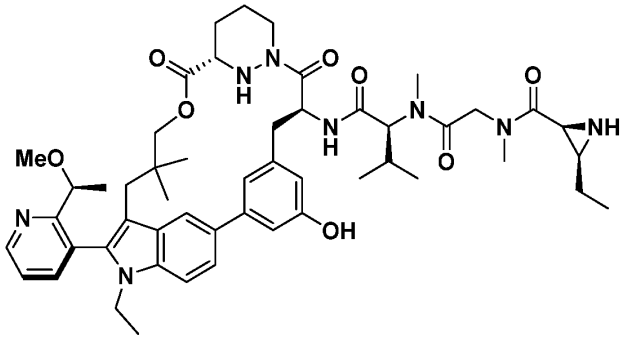
260*	
261*	
262	
263	

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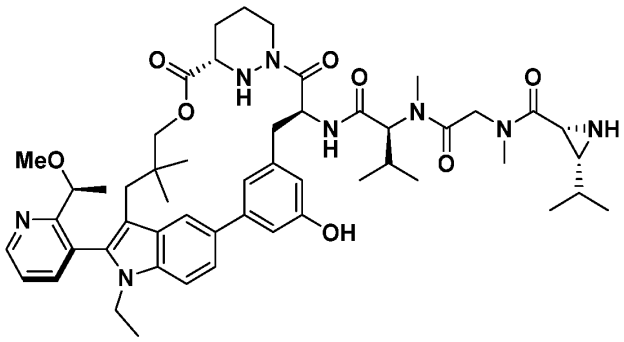
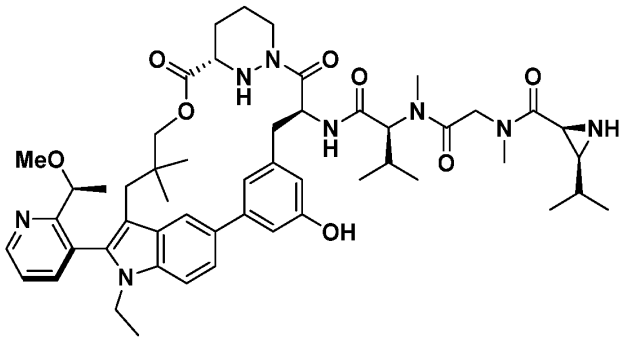
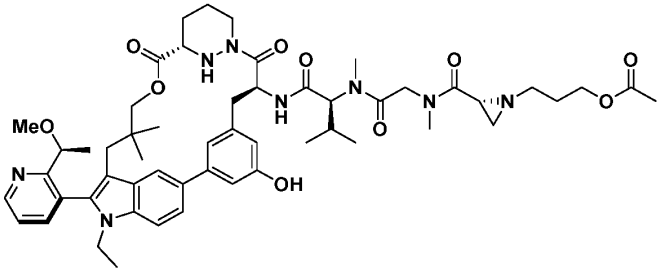
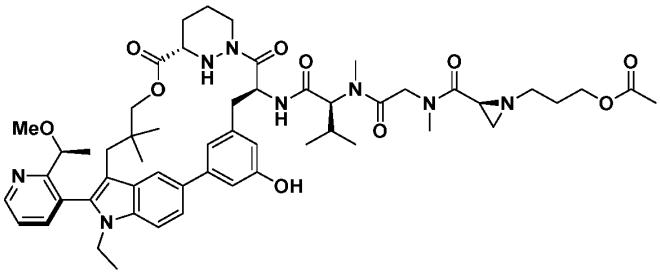
264	
265	
266	
267	

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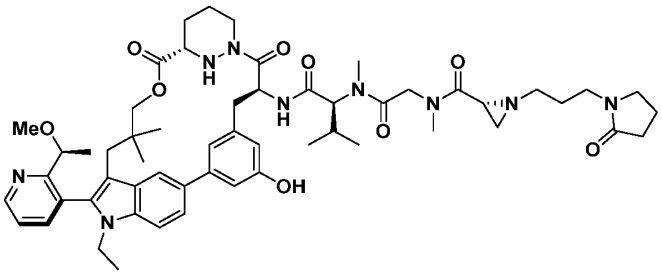
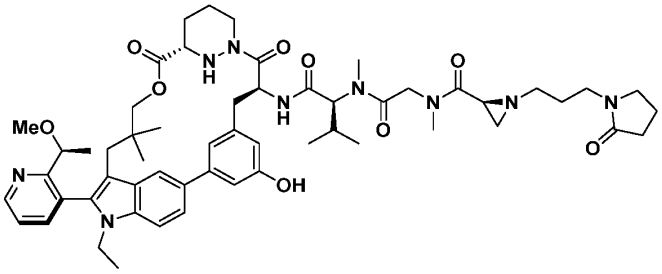
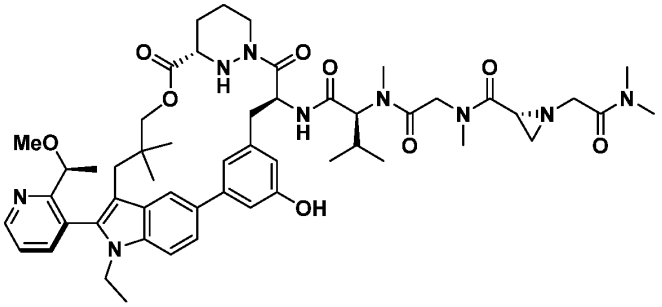
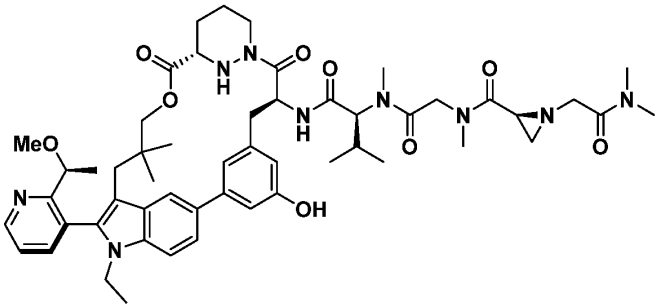
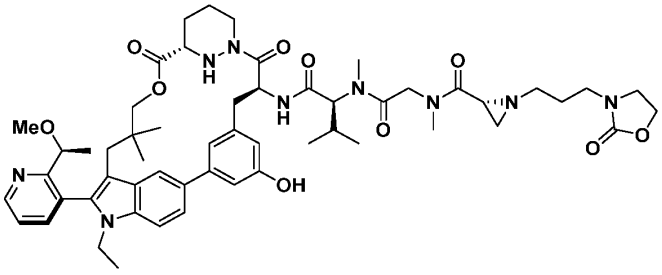
268	
269	
270	
271	
272	

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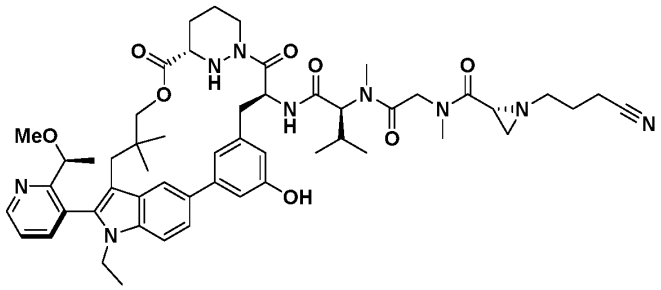
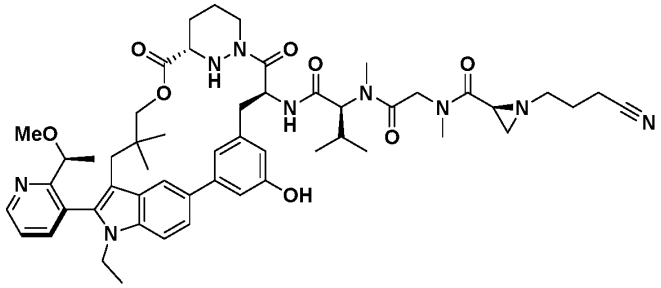
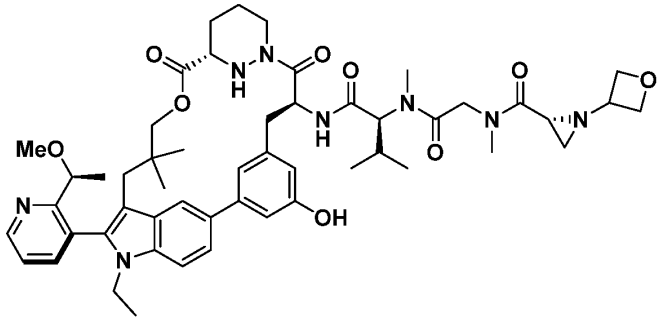
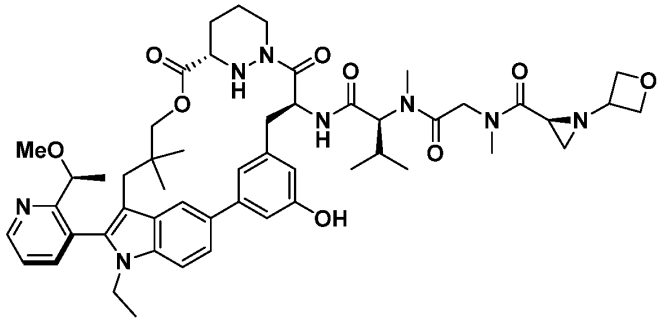
273	
274	
275	
276	

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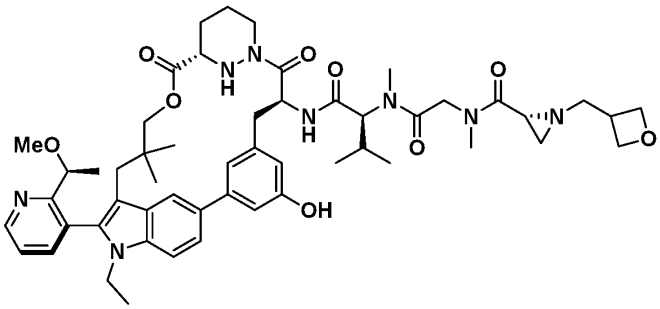
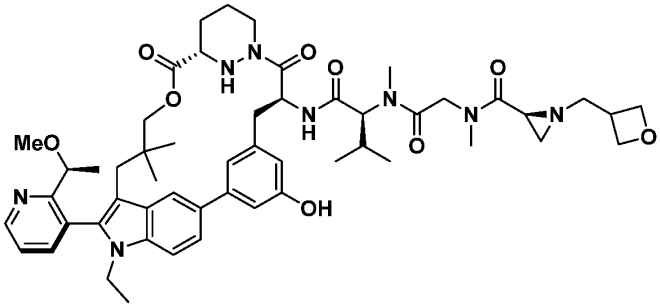
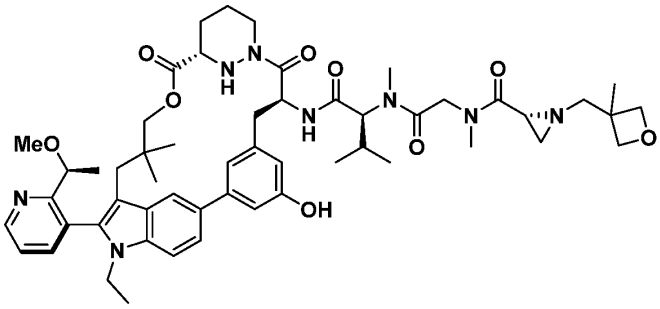
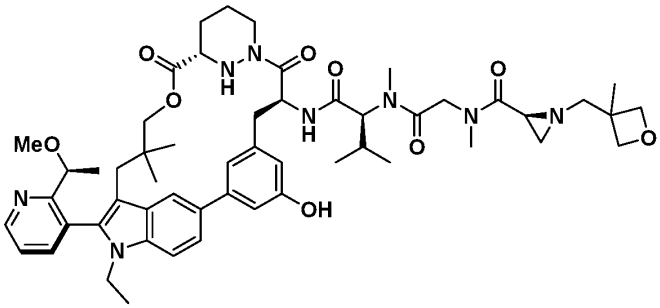
277	
278	
279	
280	

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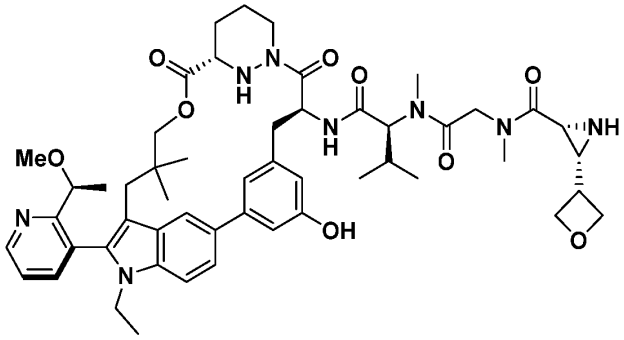
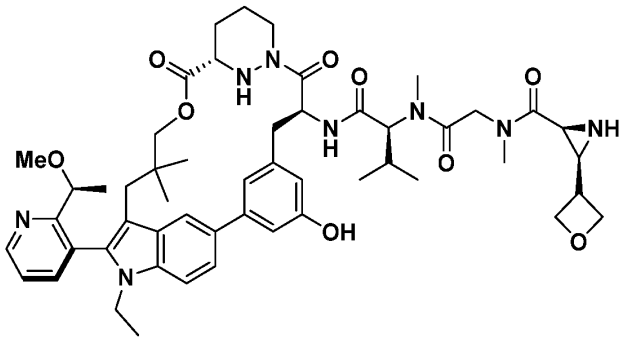
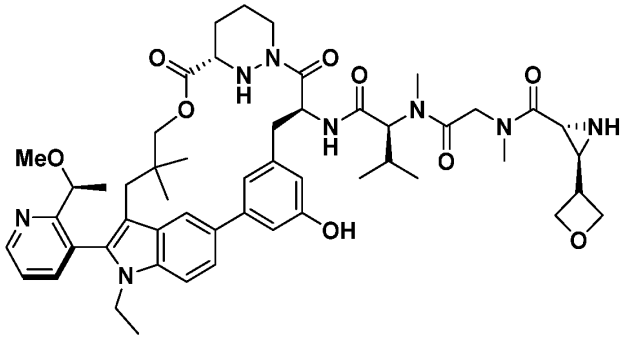
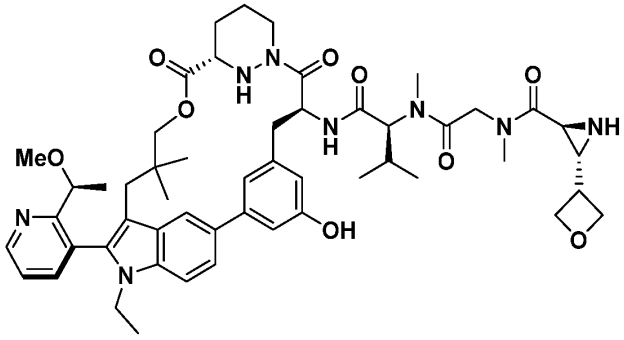
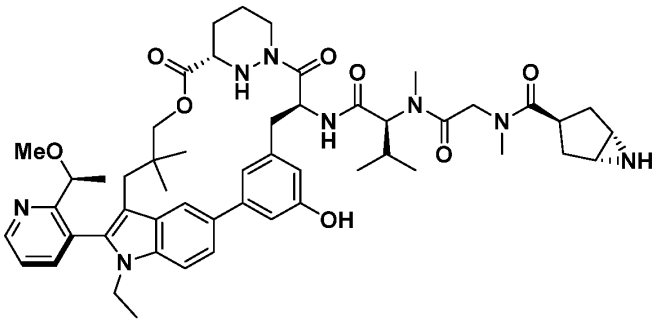
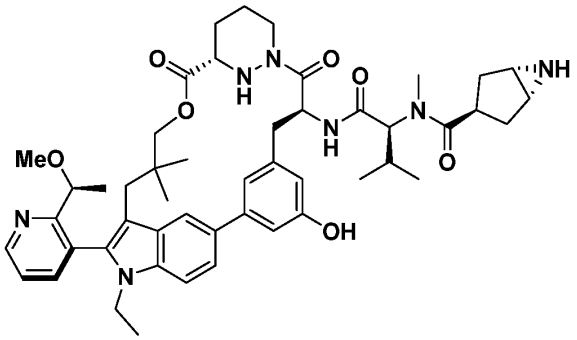
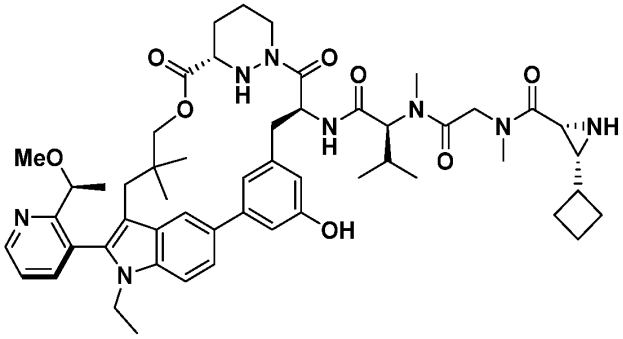
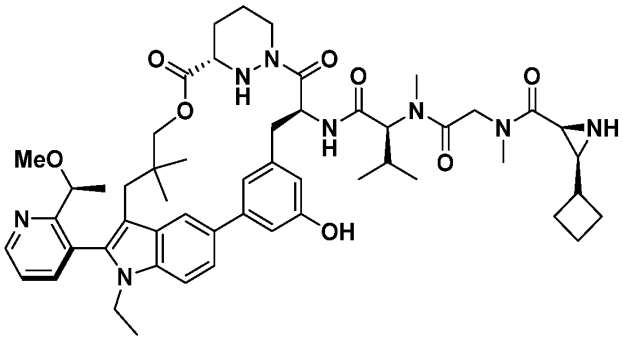
281*	
282*	
283*	
284*	

Ex#	Structure
285	
286	
287	
288	

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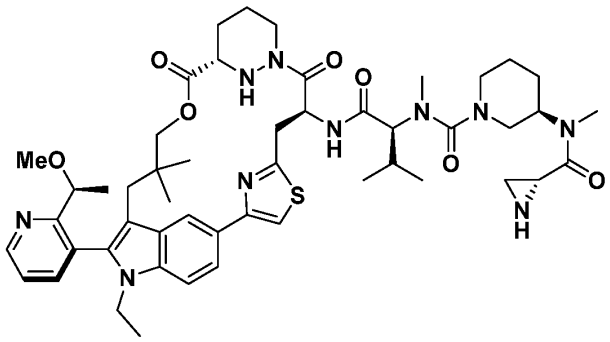
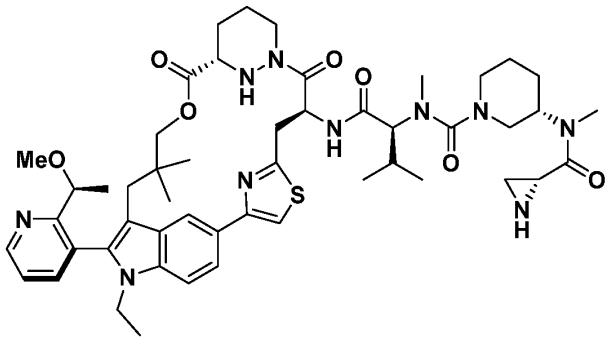
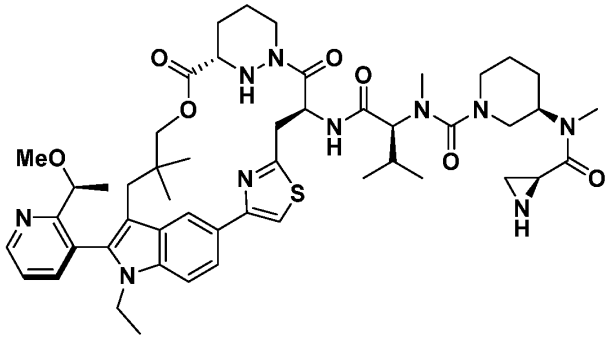
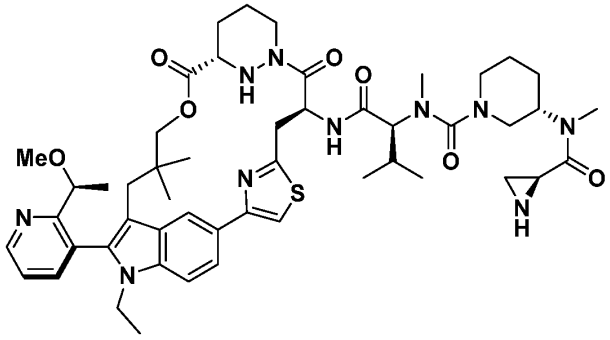
289	
290	
291	
292	

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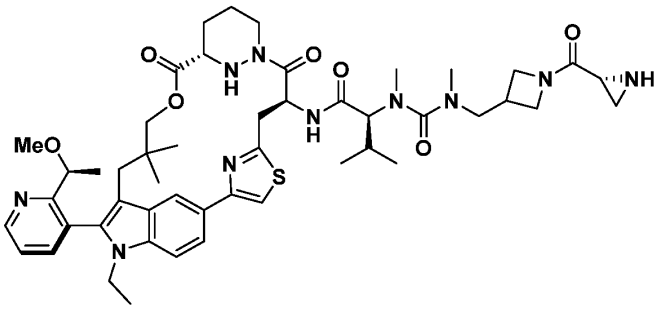
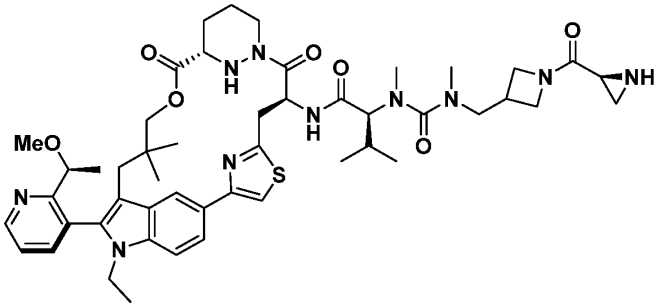
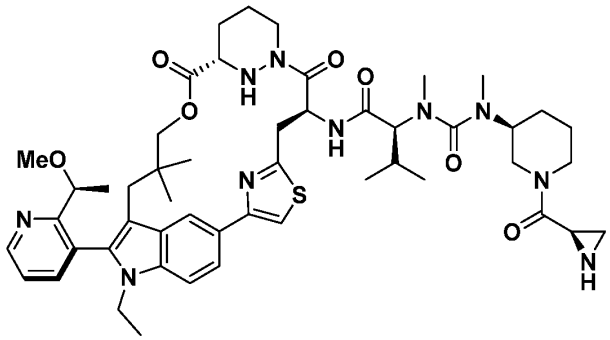
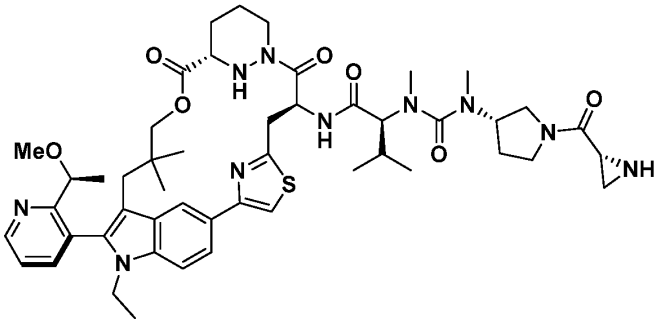
293	
294	
295	
296	

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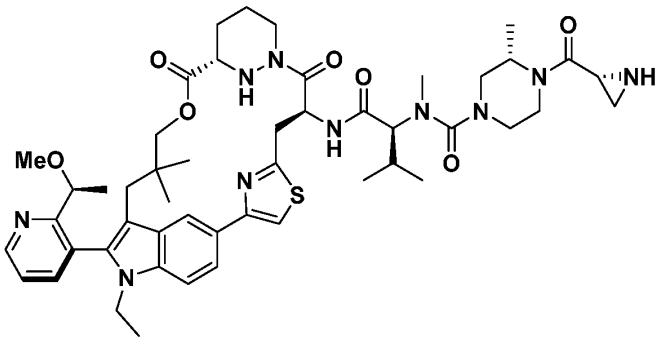
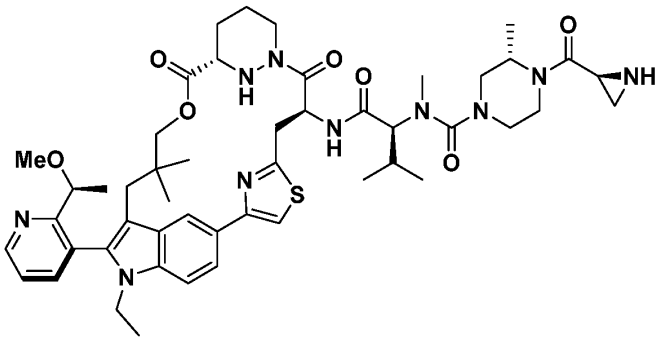
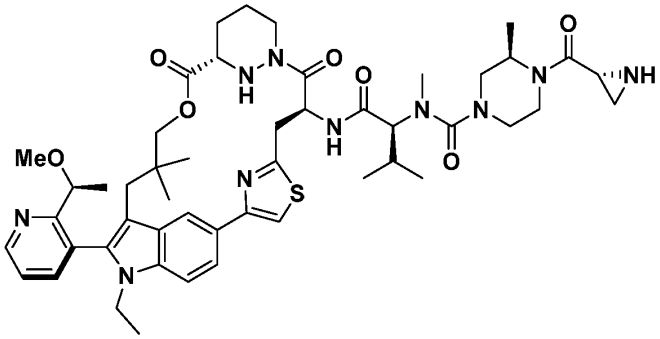
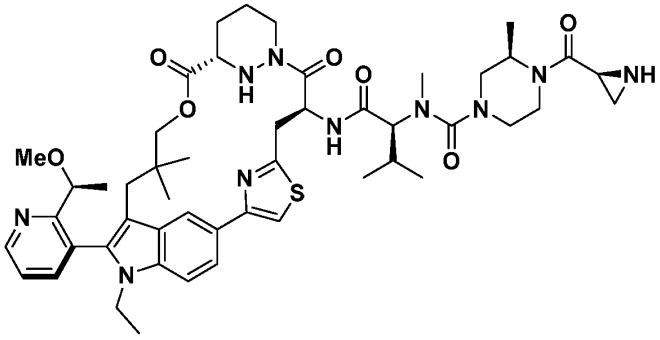
297	
298	
299	
300	

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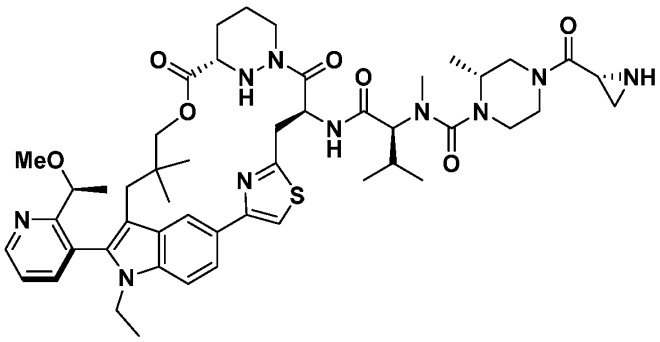
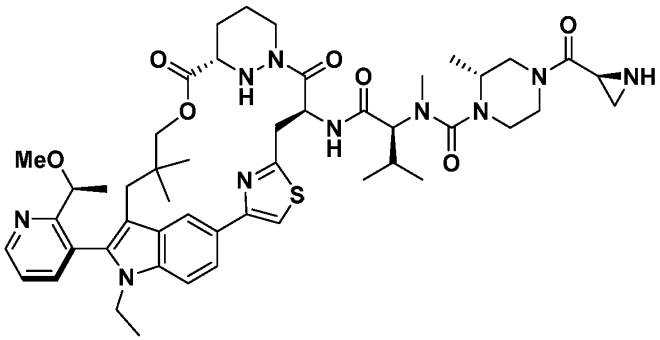
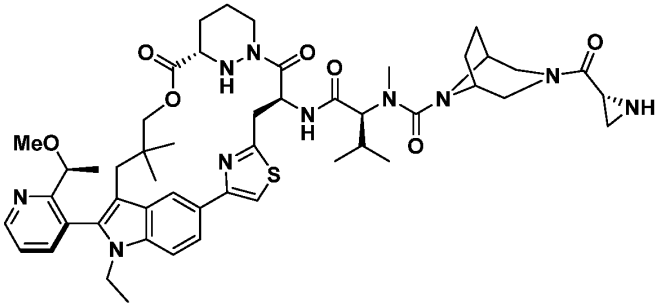
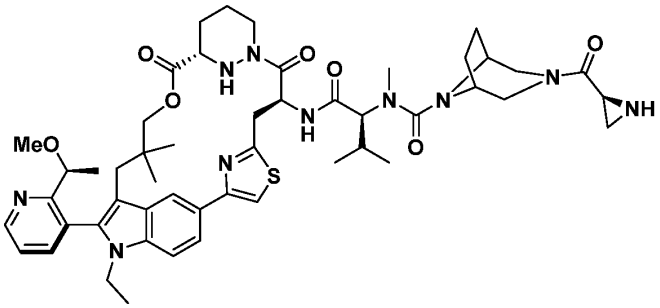
301	
302	
303	
304	

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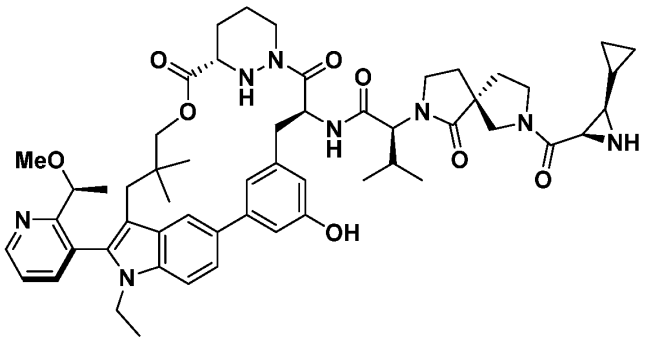
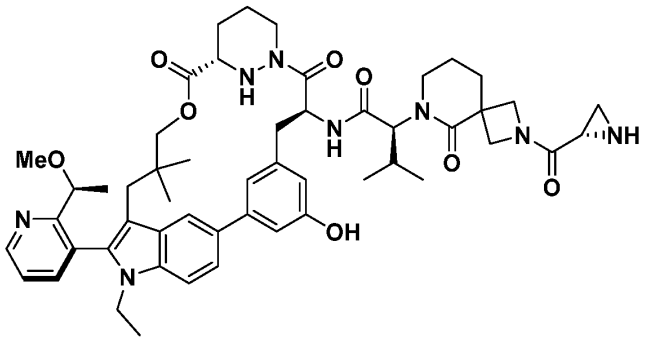
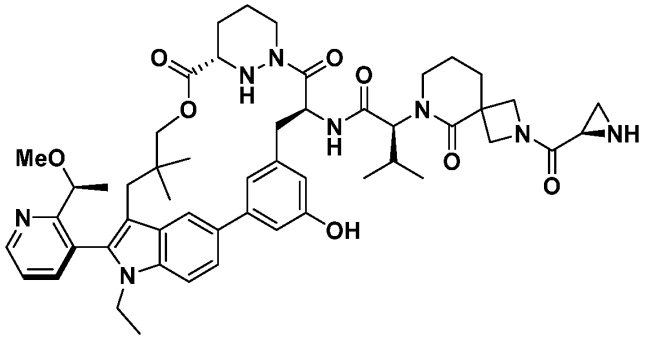
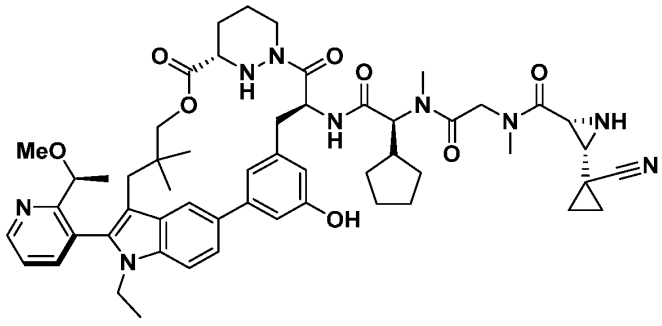
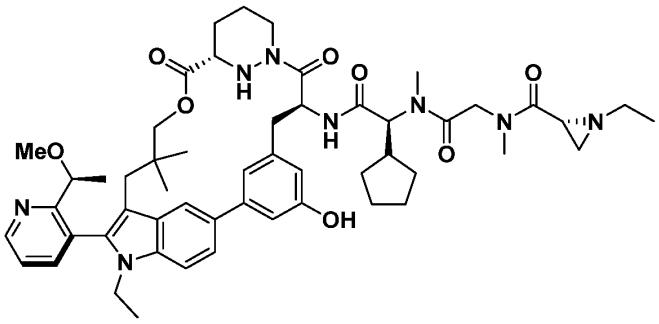
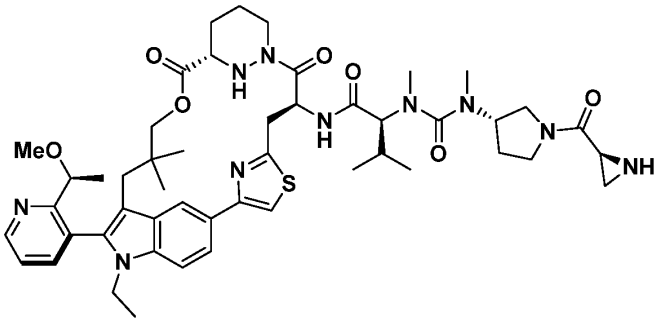
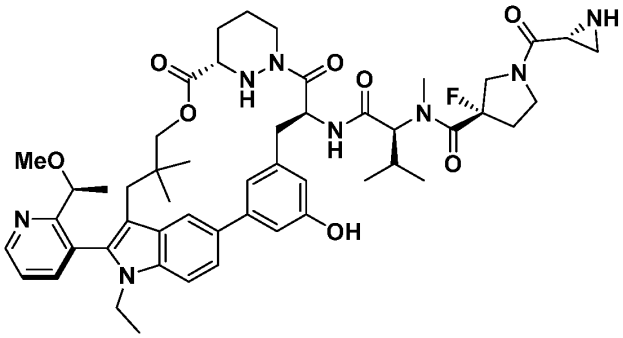
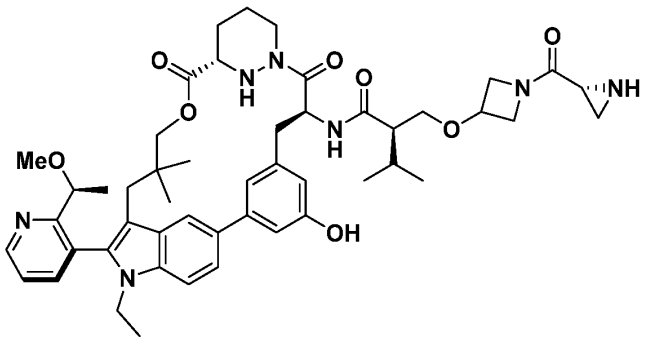
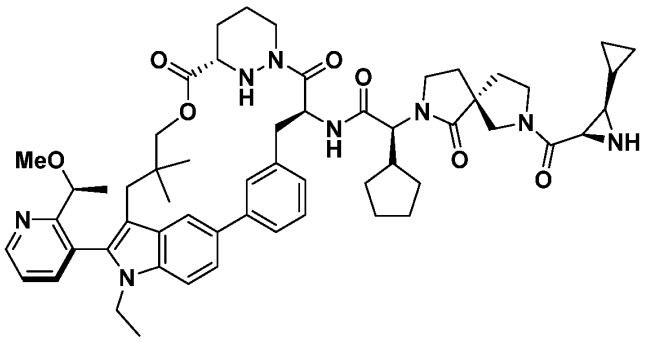
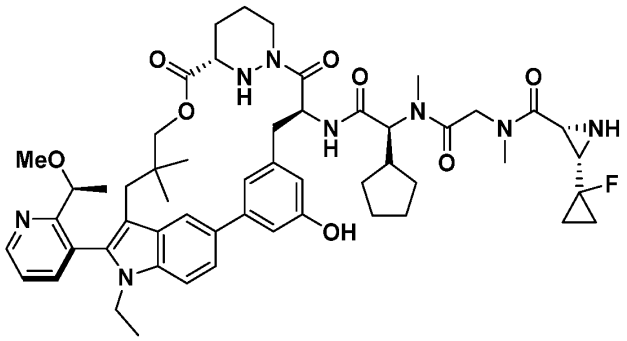
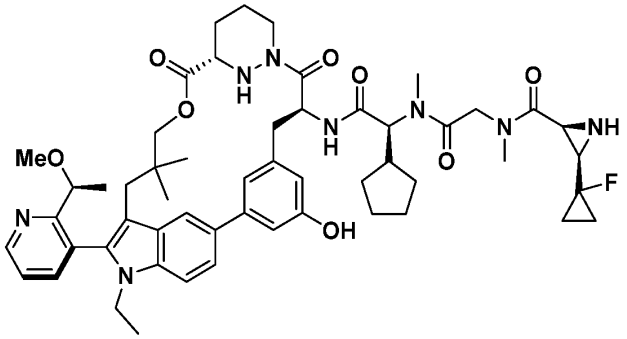
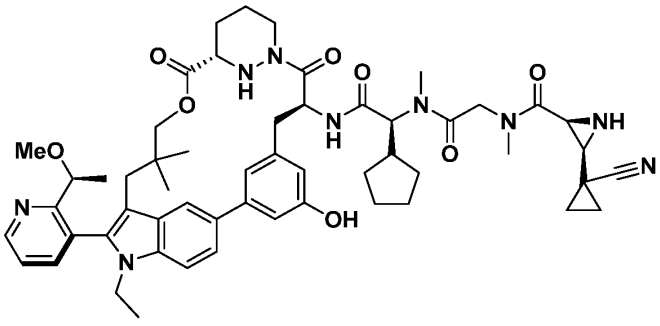
305	
306	
307	
308*	

Ex#	Structure
309	
310	
311	
312	

Ex#	Structure
313	
314*	
315*	
316*	

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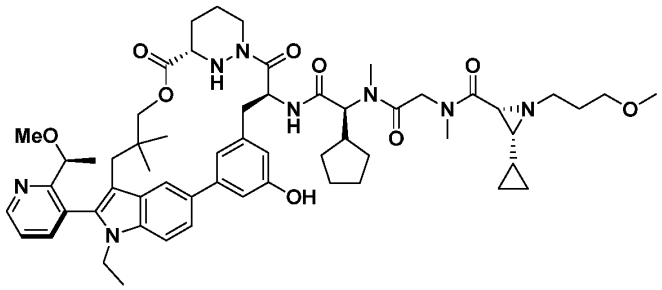
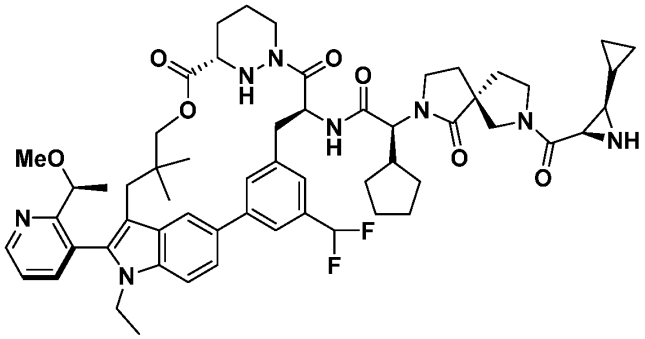
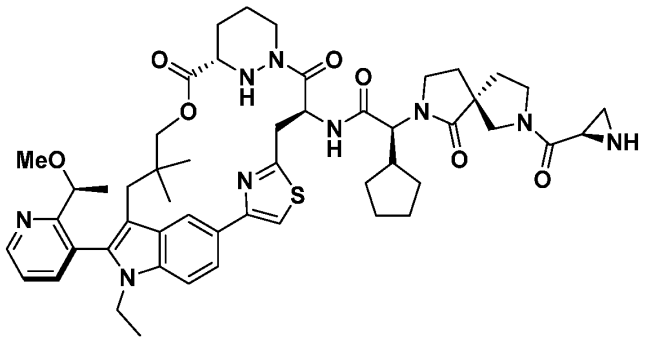
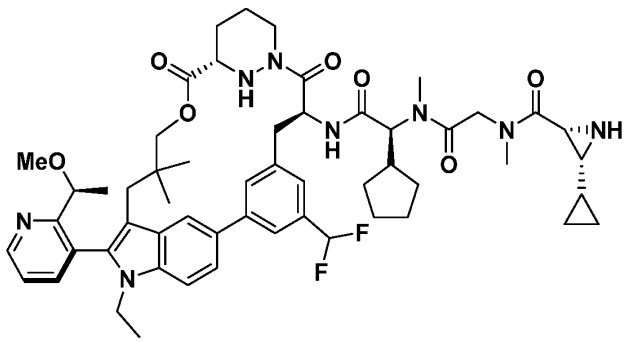
317	
318	
319	
320	

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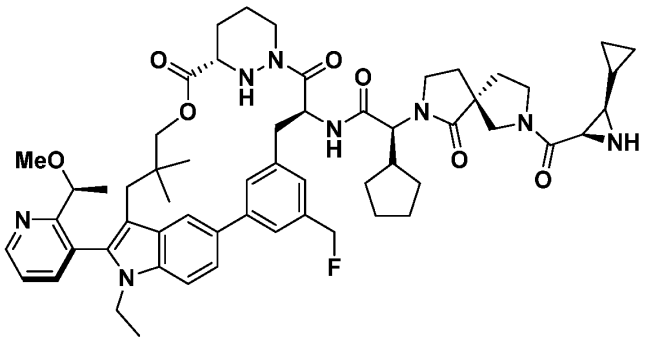
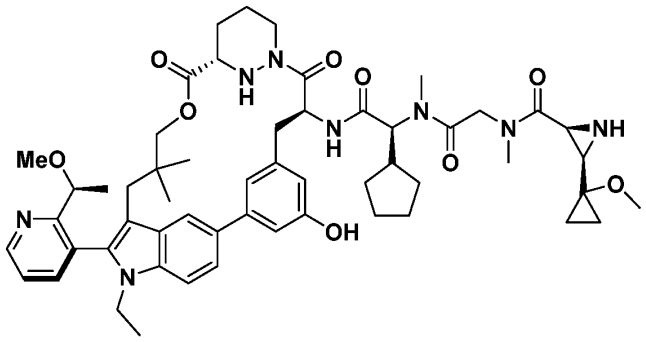
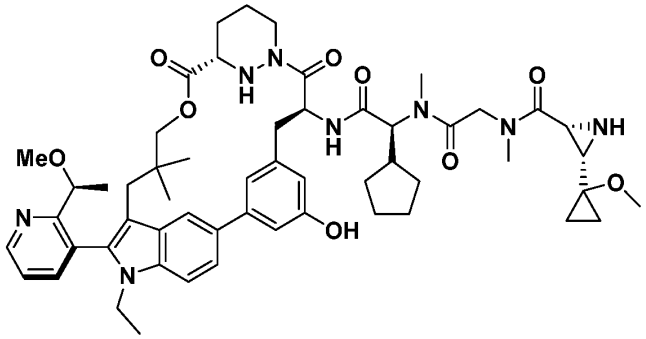
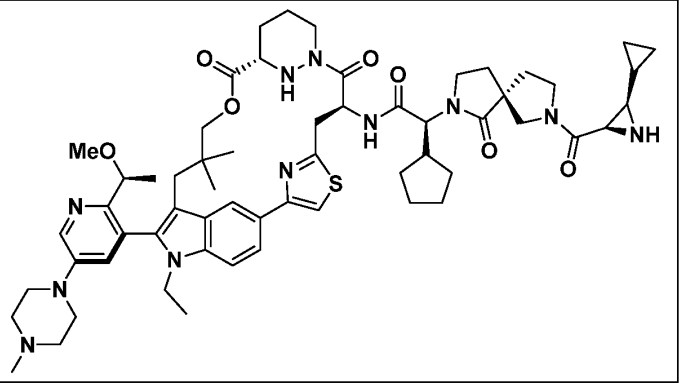
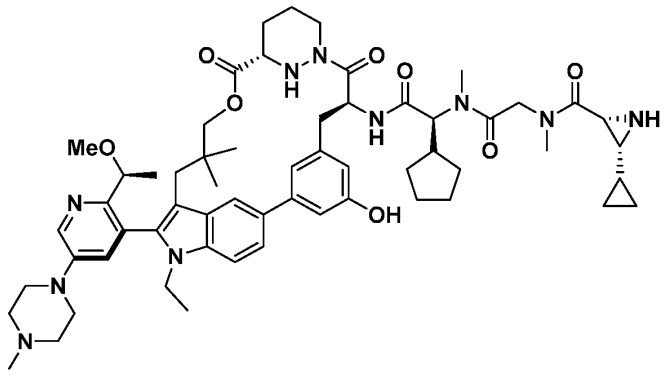
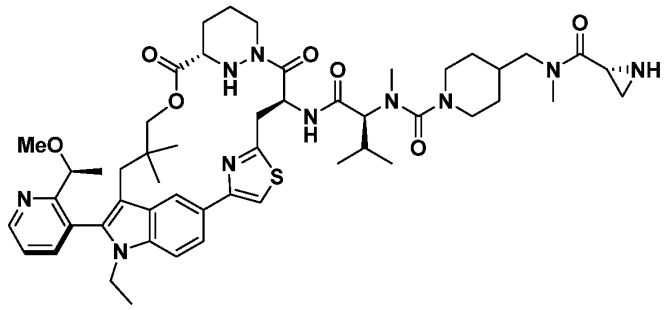
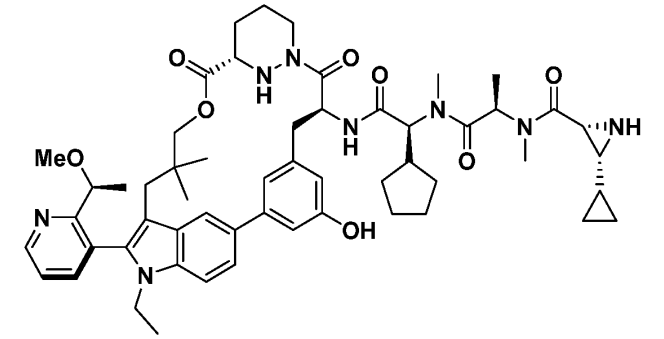
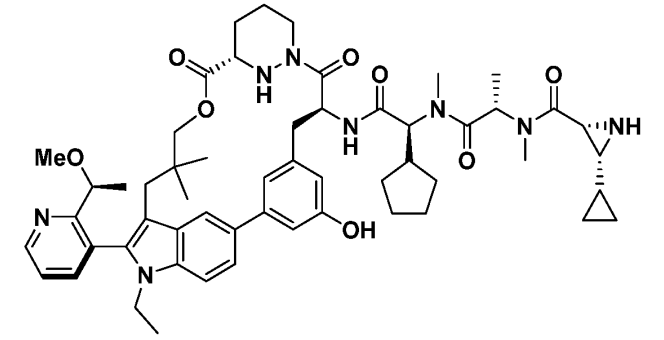
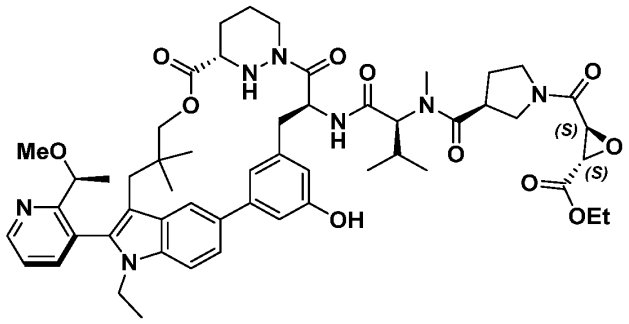
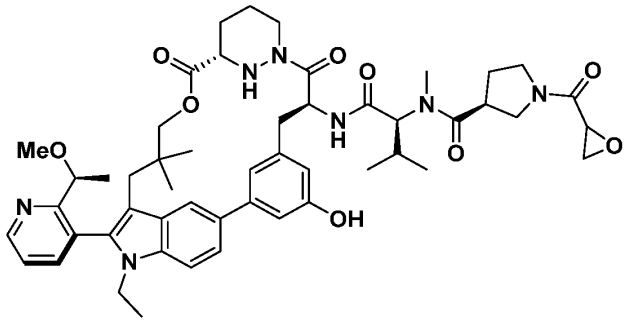
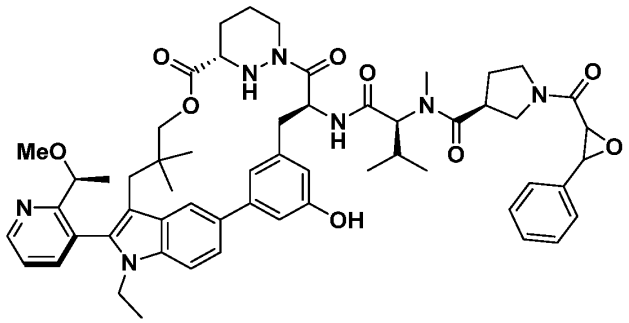
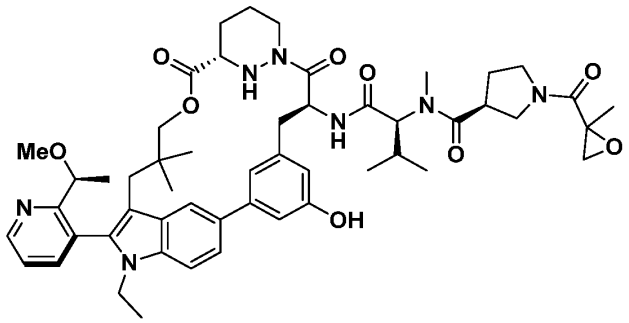
321	
322*	
323*	
324	

Ex#	Structure
325	
326	
327	
328	

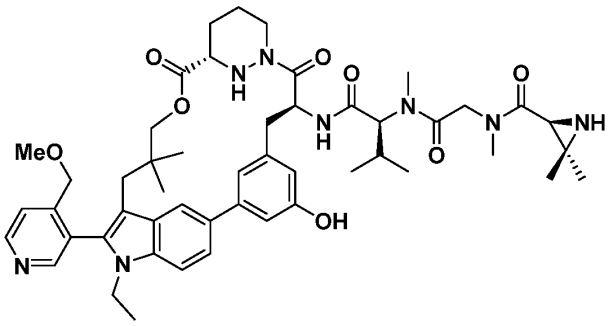
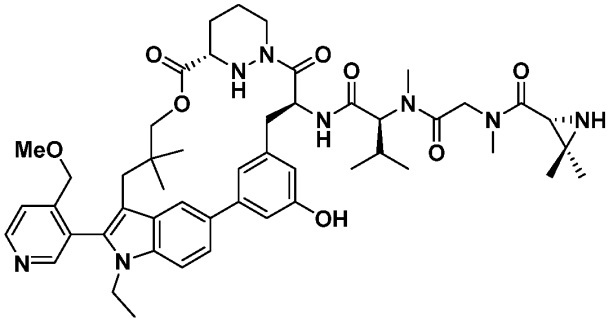
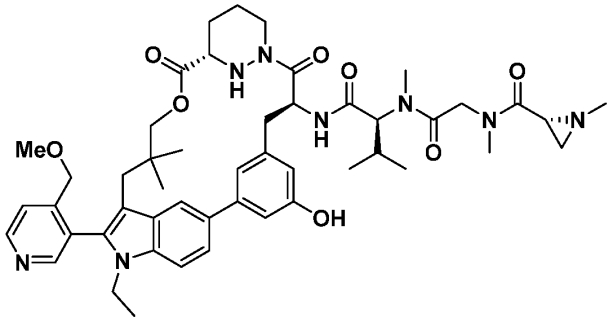
Ex#	Structure
329	
330	
331	
332	

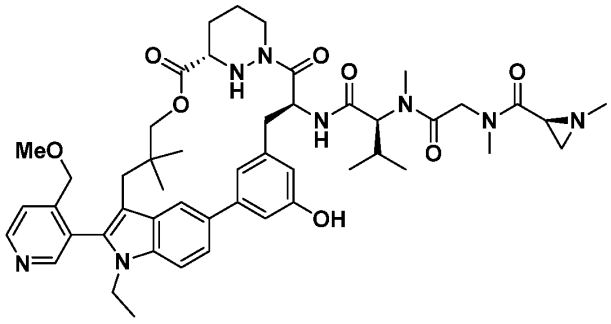
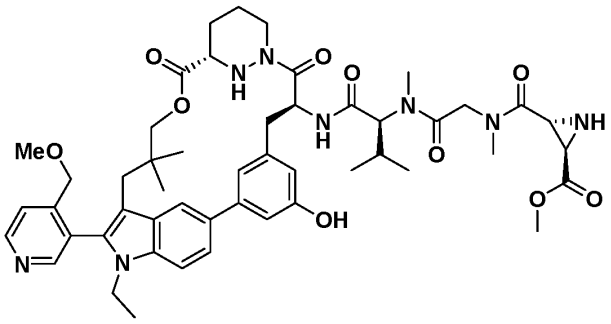
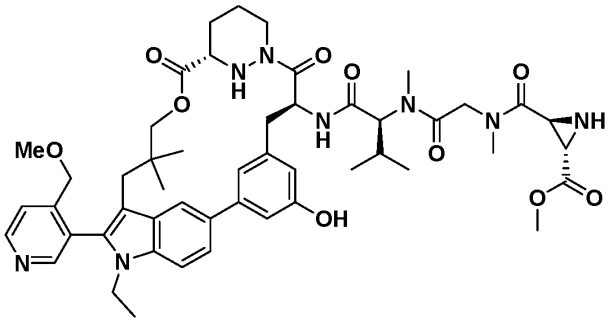
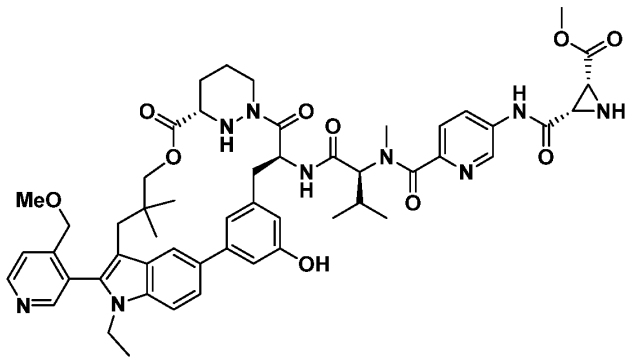
* Stereochemistry of the aziridine carbon is assumed.

Note that some compounds are shown with bonds as flat or wedged. In some instances, the relative stereochemistry of stereoisomers has been determined; in some instances, the absolute stereochemistry has been determined. In some instances, a single Example number corresponds to a mixture of stereoisomers. All stereoisomers of the compounds of the foregoing table are contemplated by the present invention. In particular embodiments, an atropisomer of a compound of the foregoing table is contemplated.

In some embodiments, a compound of Table 2 is provided, or a pharmaceutically acceptable salt thereof. In some embodiments, a compound of the present invention is selected from Table 2, or a pharmaceutically acceptable salt or atropisomer thereof.

Table 2: Certain Compounds of the Present Invention

Ex#	Structure
B5	
B6	
B8	

Ex#	Structure
B9	
B16	
B19	
B29	

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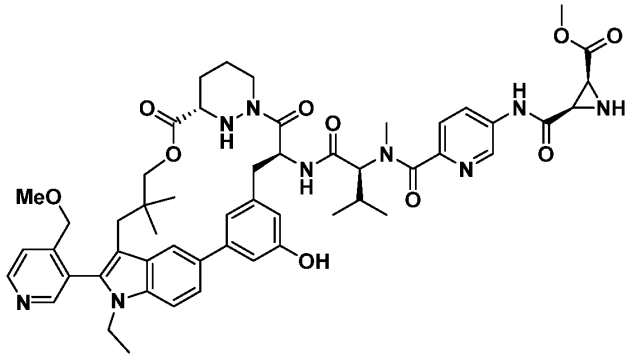
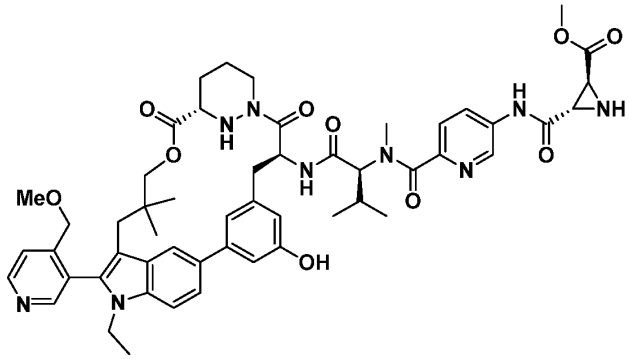
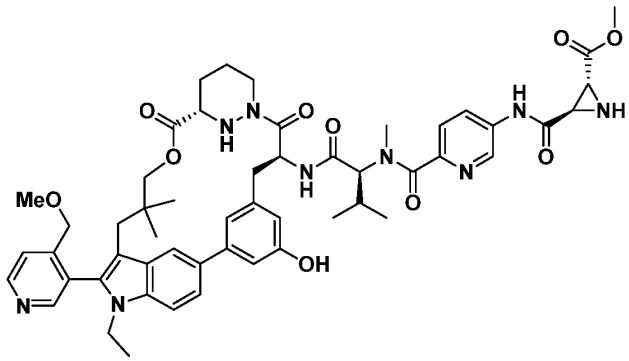
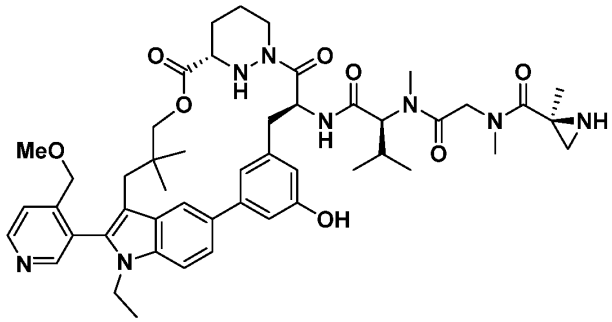
B30	
B31	
B32	
B35	

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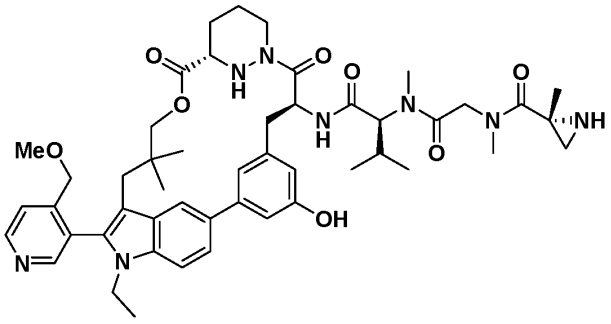
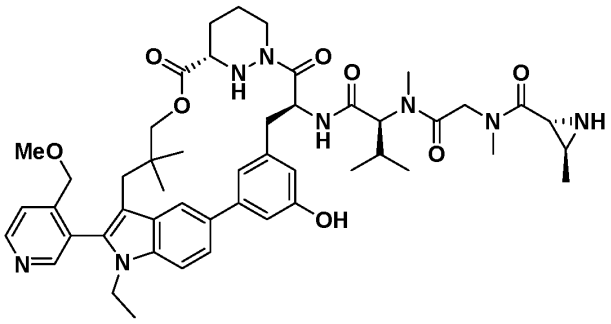
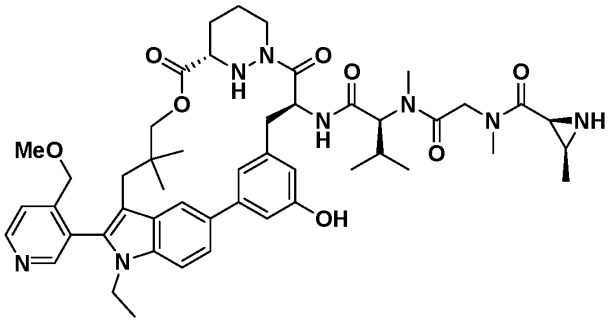
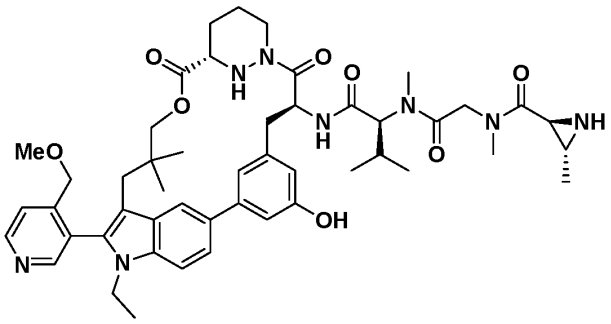
B36	
B37	
B38	
B40	

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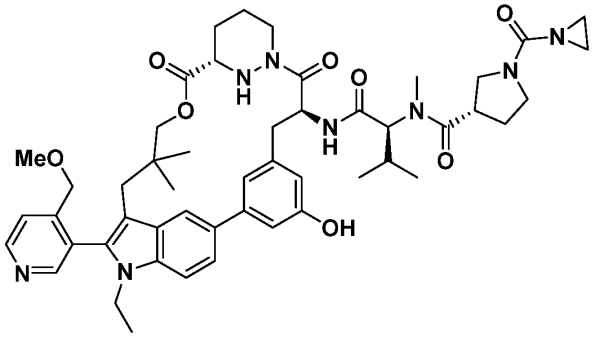
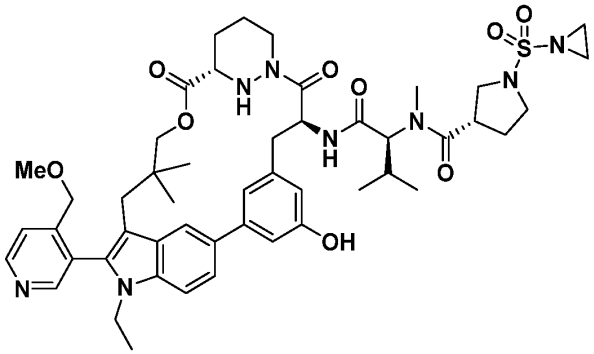
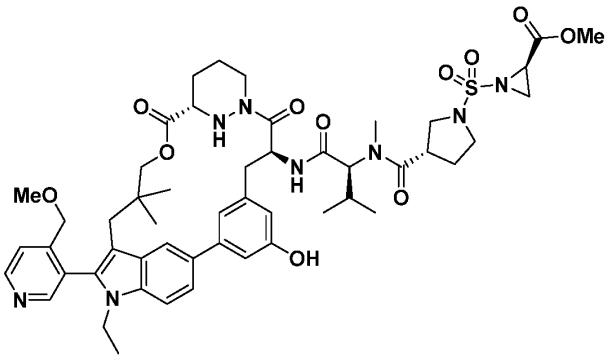
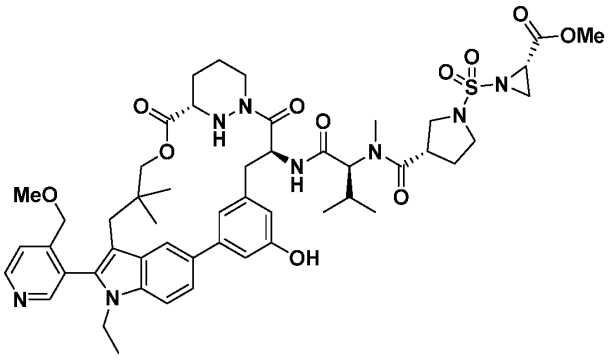
B41	
B42	
B43	
B44	

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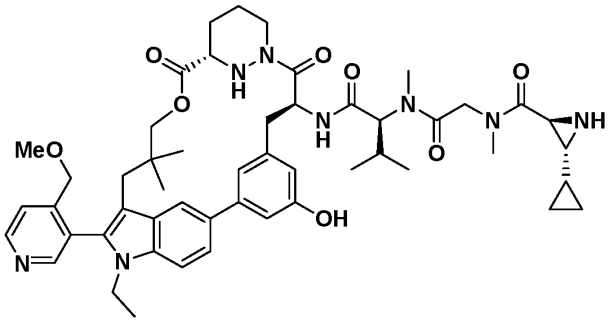
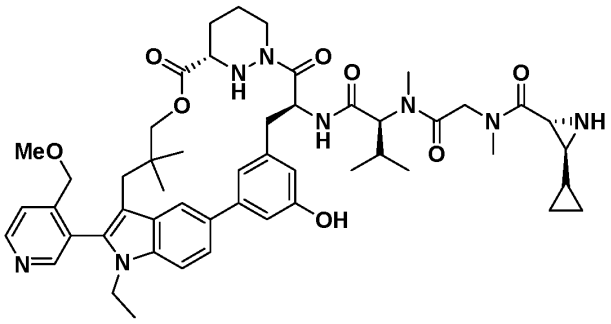
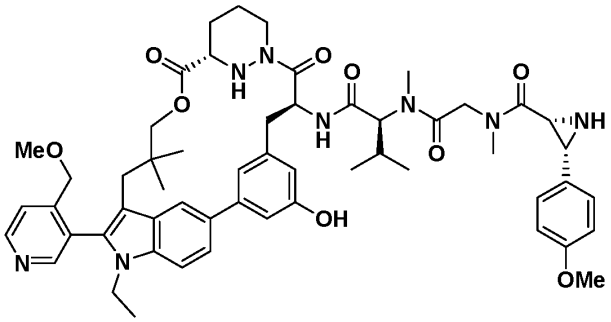
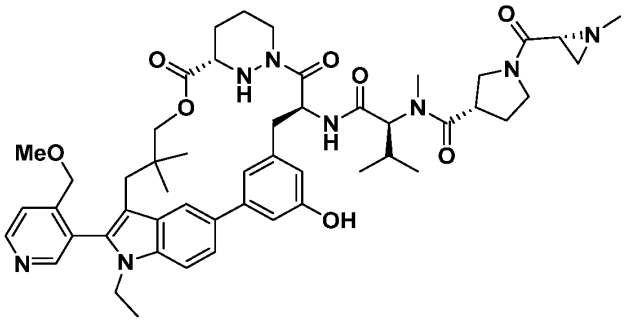
B46	
B48	
B51	
B53	

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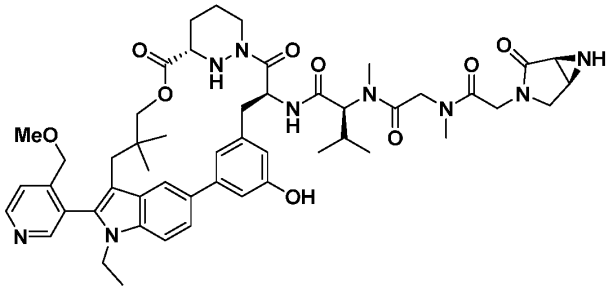
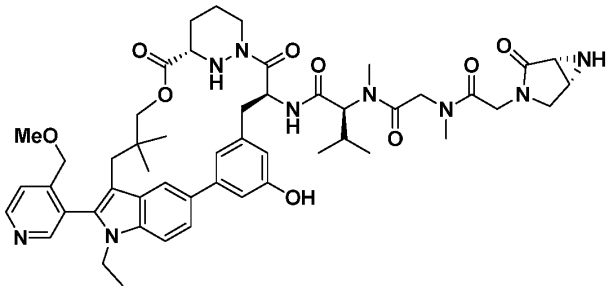
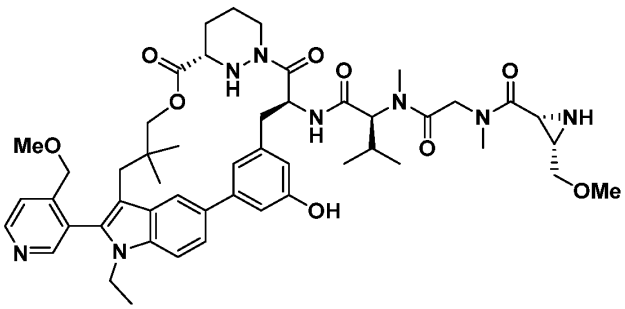
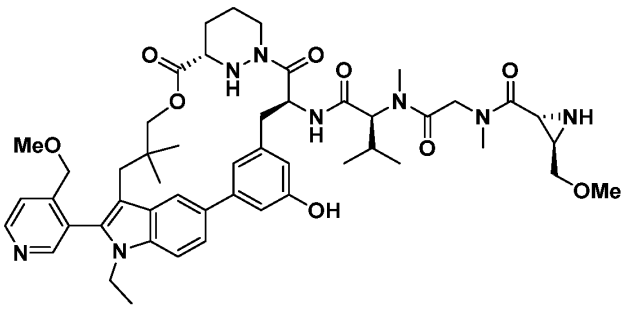
B54	
B55	
B56	
B57	

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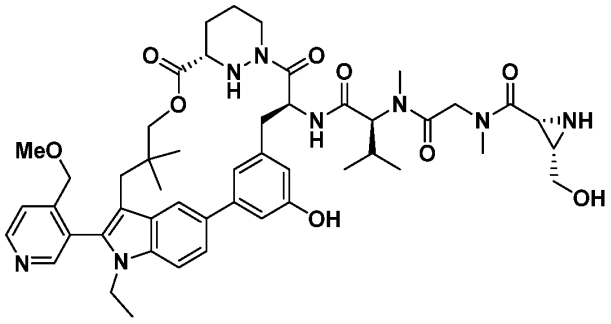
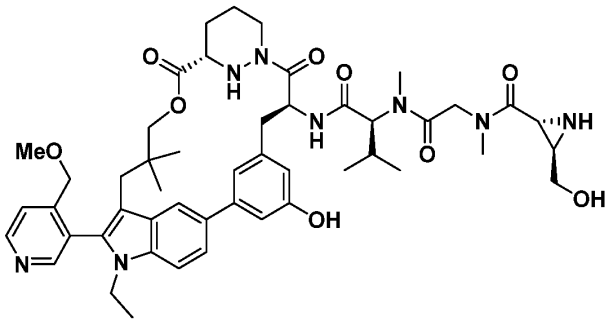
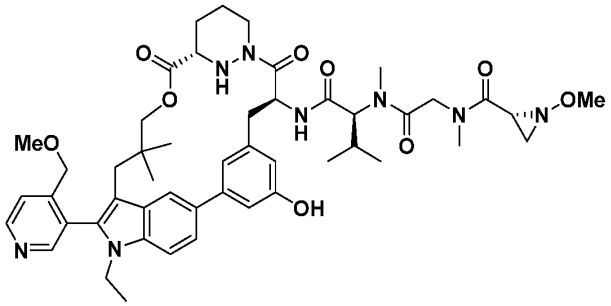
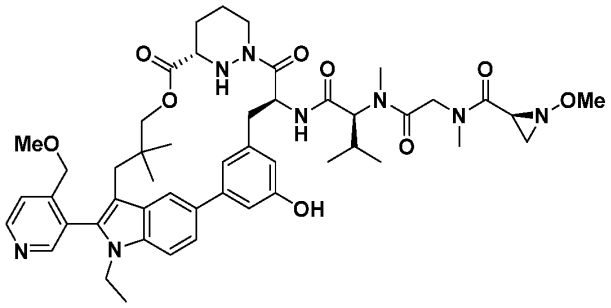
B58	
B59	
B60	
B61	

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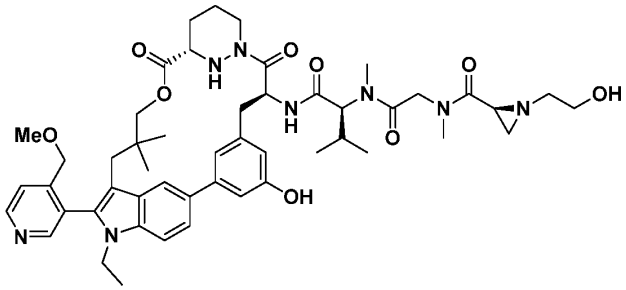
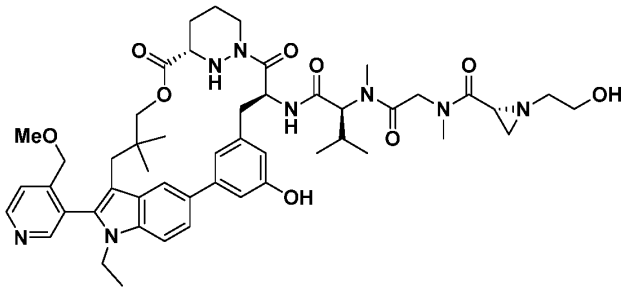
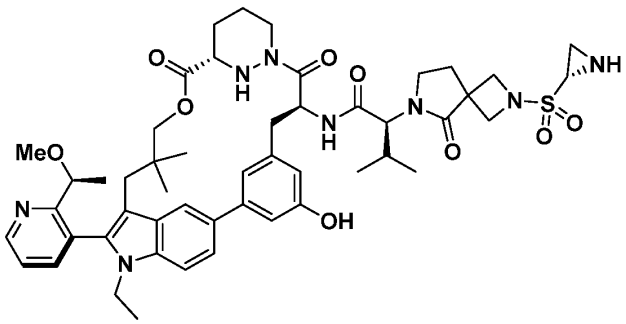
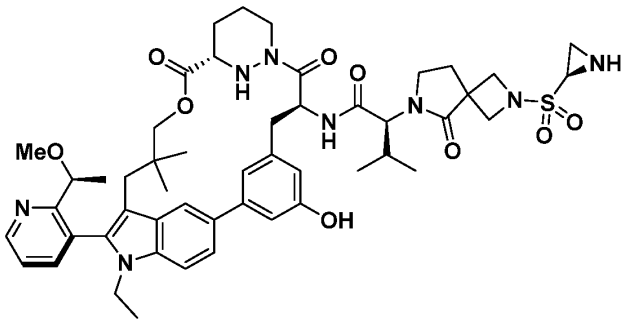
B62	
B63	
B73	
B74	

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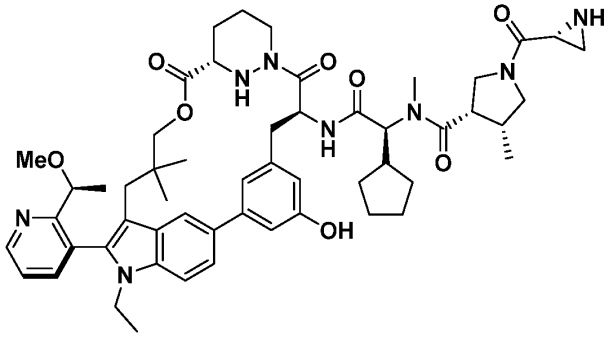
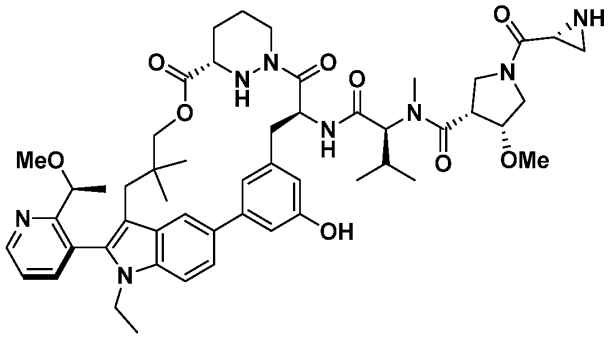
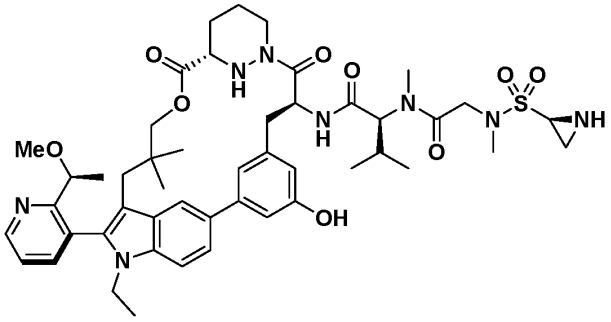
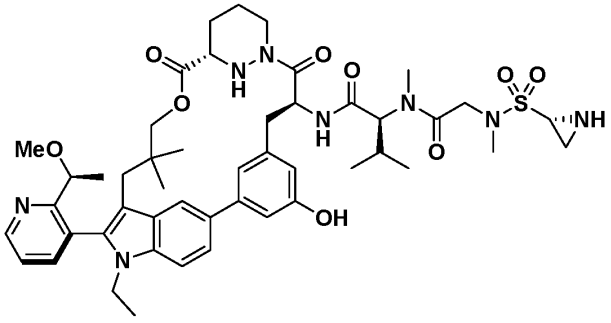
B76	
B78	
B79	
B80	

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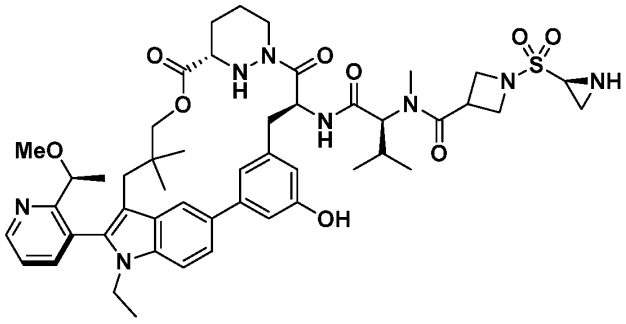
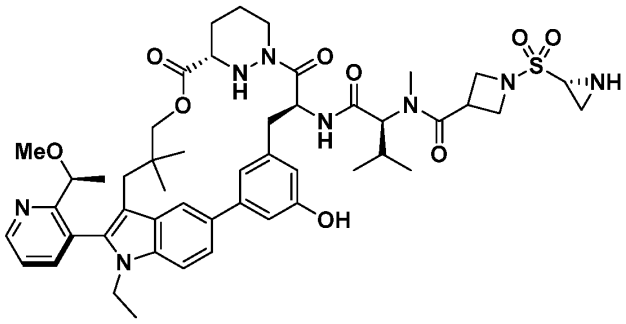
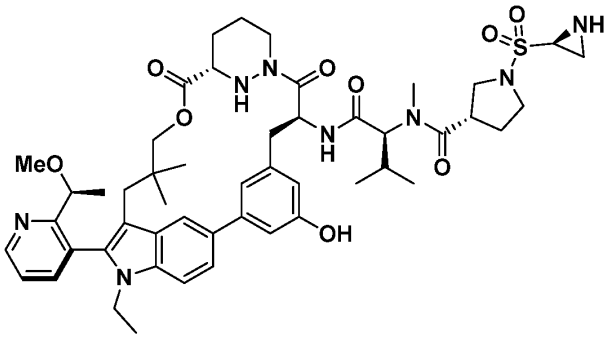
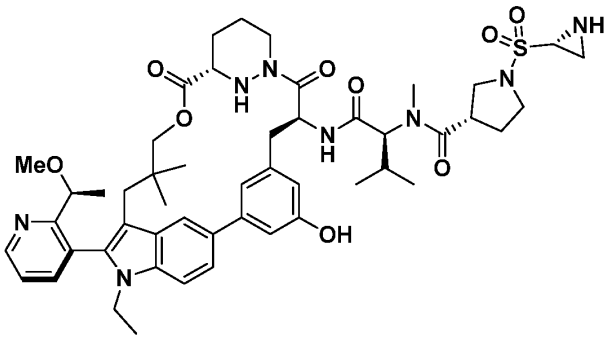
B83	
B84	
B87	
B88	

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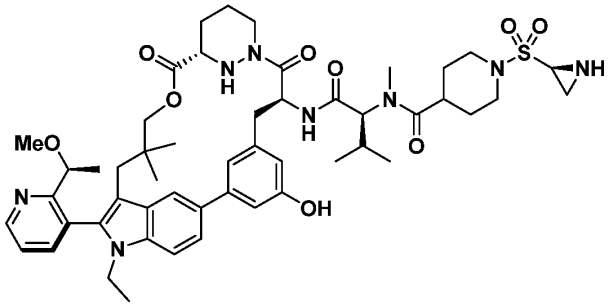
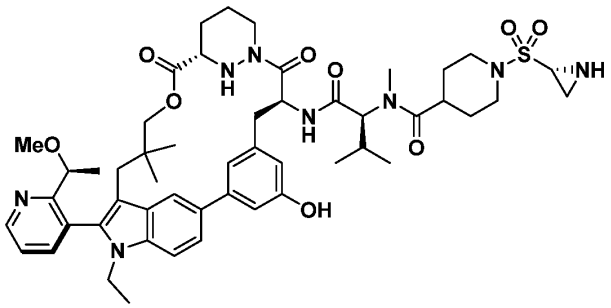
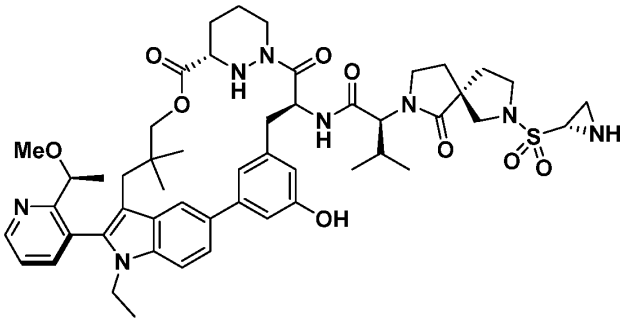
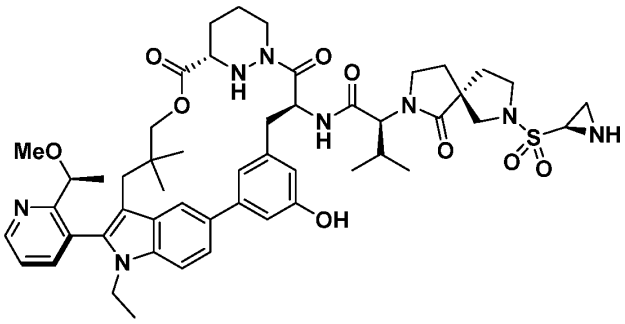
B91	
B92	
B97	
B98	

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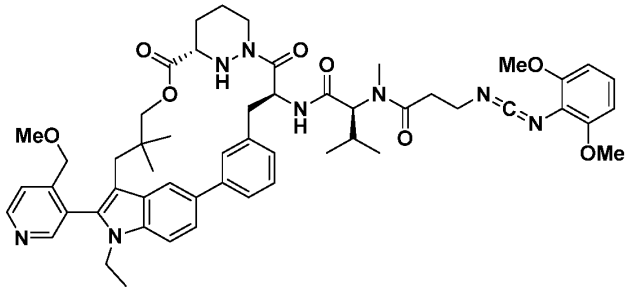
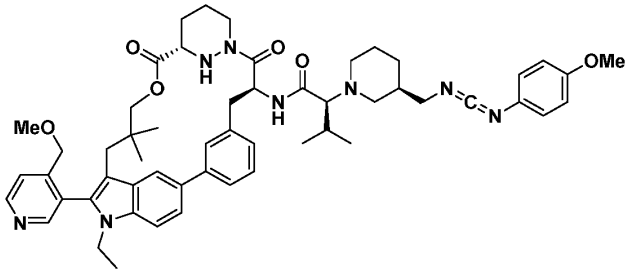
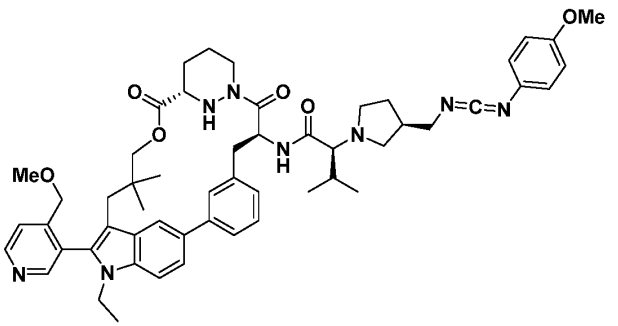
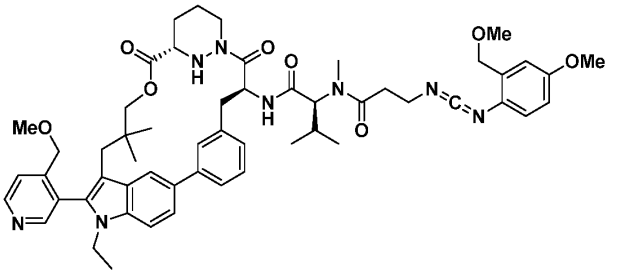
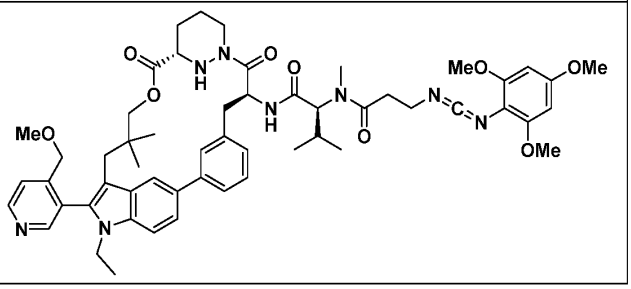
B101	
B108	
B109	
B113	
B116	

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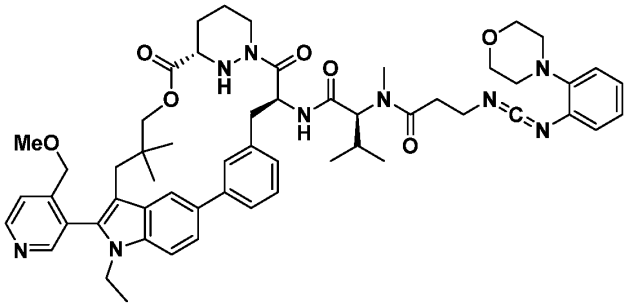
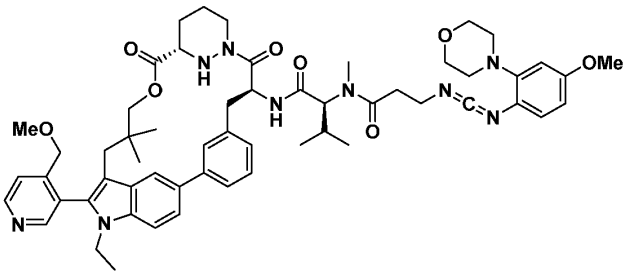
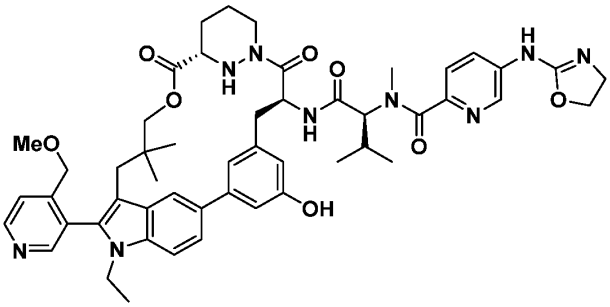
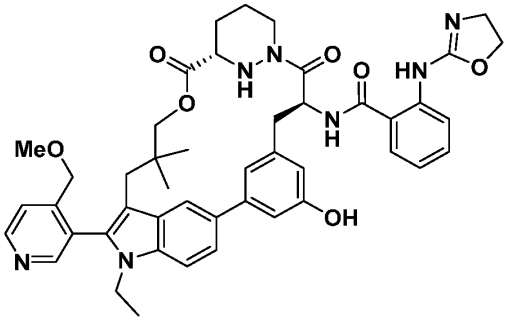
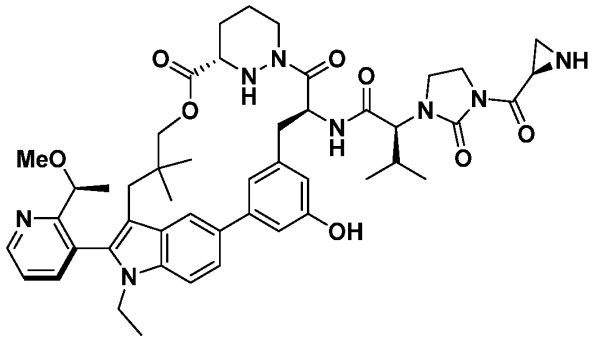
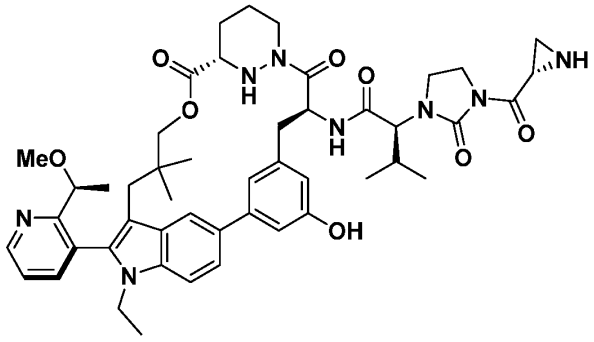
B117	
B118	
B119	
B120	

Ex#	Structure
B122	
B123	

Note that some compounds are shown with bonds as flat or wedged. In some instances, the relative stereochemistry of stereoisomers has been determined; in some instances, the absolute stereochemistry has been determined. All stereoisomers of the compounds of the foregoing table are contemplated by the present invention. In particular embodiments, an atropisomer of a compound of the foregoing table is contemplated.

In some embodiments, a compound of the present invention is or acts as a prodrug, such as with respect to administration to a cell or to a subject in need thereof.

Also provided are pharmaceutical compositions comprising a compound of the present invention, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

Further provided is a conjugate, or salt thereof, comprising the structure of Formula IV:

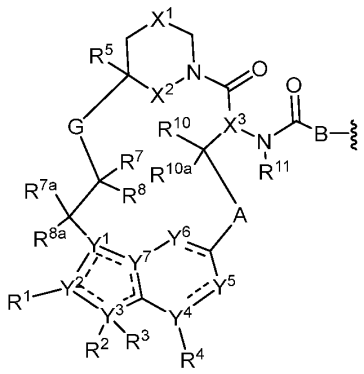
M-L-P

Formula IV

wherein L is a linker;

P is a monovalent organic moiety; and

M has the structure of Formula Va:



Formula Va

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- or >C=C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or S(O)$_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo; and

R^{11} is hydrogen or C_1-C_3 alkyl.

In some embodiments, the conjugate has the structure of Formula IV:

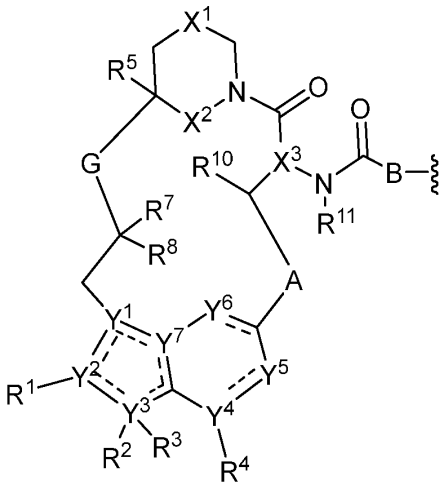
M-L-P

Formula IV

wherein L is a linker;

P is a monovalent organic moiety; and

M has the structure of Formula Vb:



Formula Vb

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or S(O)$_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', $C(O)N(R')_2$, S(O)R', $S(O)_2R'$, or $S(O)_2N(R')_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), $C=N(O\text{-}C_1\text{-}C_3$ alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl; and

R^{11} is hydrogen or C_1-C_3 alkyl.

In some embodiments, the conjugate has the structure of Formula IV:

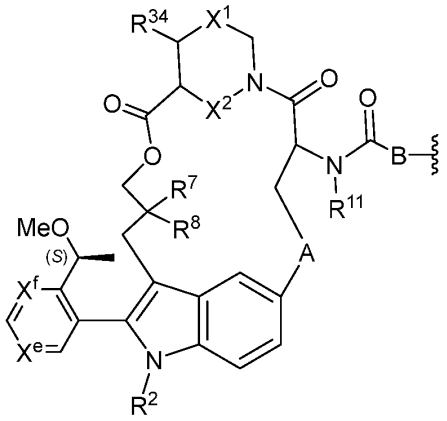
M-L-P

Formula IV

wherein L is a linker;

P is a monovalent organic moiety; and

M has the structure of Formula Vc:



Formula Vc

wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene (e.g., phenyl or phenol), or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', $C(O)N(R')_2$, S(O)R', $S(O)_2R'$, or $S(O)_2N(R')_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

X^e and X^f are, independently, N or CH;

R^2 is C_1-C_6 alkyl or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

R^8 is C_1-C_3 alkyl; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{34} is hydrogen or C_1-C_3 alkyl.

In some embodiments of a compound of the present invention, X^e is N and X^f is CH. In some embodiments, X^e is CH and X^f is N.

In some embodiments, the conjugate has the structure of Formula IV:

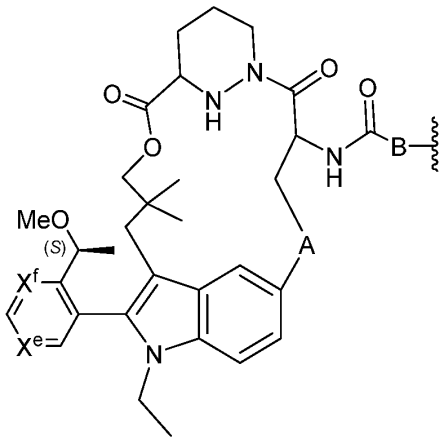
M-L-P

Formula IV

wherein L is a linker;

P is a monovalent organic moiety; and

M has the structure of Formula Vd:



Formula Vd

wherein A optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene (e.g., phenyl or phenol), or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; and

X^e and X^f are, independently, N or CH.

In some embodiments of a compound of the present invention, X^e is N and X^f is CH. In some embodiments, X^e is CH and X^f is N.

In some embodiments of conjugates of the present invention, the linker has the structure of Formula II:

$$A^1\text{-}(B^1)_f\text{-}(C^1)_g\text{-}(B^2)_h\text{-}(D^1)\text{-}(B^3)_i\text{-}(C^2)_j\text{-}(B^4)_k\text{–}A^2$$

Formula II

where A^1 is a bond between the linker and B; A^2 is a bond between P and the linker; B^1, B^2, B^3, and B^4 each, independently, is selected from optionally substituted C_1-C_2 alkylene, optionally substituted C_1-C_3 heteroalkylene, O, S, and NR^N; R^N is hydrogen, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted C_1-C_7 heteroalkyl; C^1 and C^2 are each, independently, selected from carbonyl, thiocarbonyl, sulphonyl, or phosphoryl; f, g, h, i, j, and k are each, independently, 0 or 1; and D^1 is optionally substituted C_1-C_{10} alkylene, optionally substituted C_2-C_{10} alkenylene, optionally substituted C_2-C_{10} alkynylene, optionally substituted 3 to 14-membered heterocycloalkylene, optionally substituted 5 to 10-membered heteroarylene, optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 6 to 10-membered arylene, optionally substituted C_2-C_{10} polyethylene glycolene, or optionally substituted C_1-C_{10} heteroalkylene, or a chemical bond linking A^1-$(B^1)_f$-$(C^1)_g$-$(B^2)_h$- to -$(B^3)_i$-$(C^2)_j$-$(B^4)_k$–A^2. In some embodiments of conjugates of the present invention, the linker is bound to the monovalent organic moiety through a bond to a carboxyl group of an amino acid residue of the monovalent organic moiety.

In some embodiments of conjugates of the present invention, the monovalent organic moiety is a protein. In some embodiments, the protein is a Ras protein. In some embodiments, the Ras protein is K-Ras G12D or K-Ras G13D.

With respect to compounds of the present invention, one stereoisomer may exhibit better inhibition than another stereoisomer. For example, one atropisomer may exhibit inhibition, whereas the other atropisomer may exhibit little or no inhibition.

Methods of Synthesis

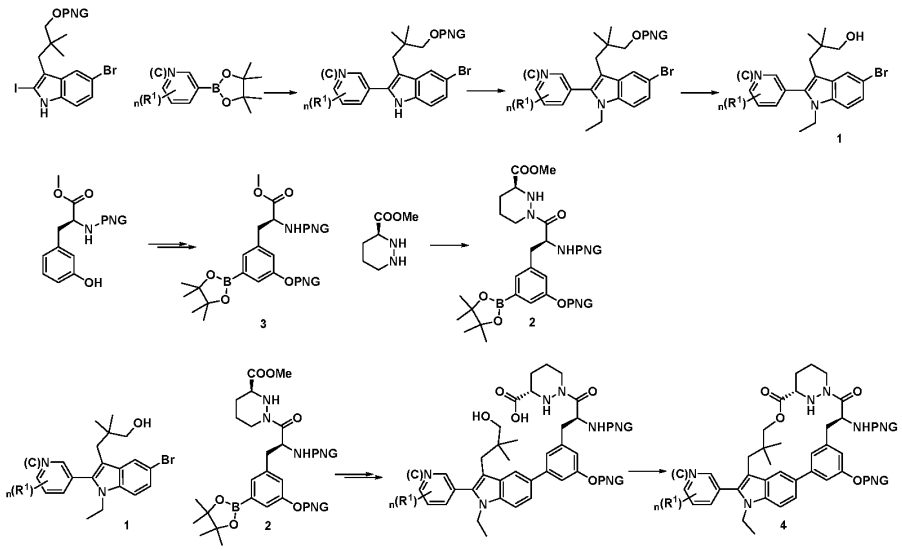
The compounds described herein may be made from commercially available starting materials or synthesized using known organic, inorganic, or enzymatic processes.

The compounds of the present invention can be prepared in a number of ways well known to those skilled in the art of organic synthesis. By way of example, compounds of the present invention can be synthesized using the methods described in the Schemes below, together with synthetic methods known in the art of synthetic organic chemistry, or variations thereon as appreciated by those skilled in the art. These methods include but are not limited to those methods described in the Schemes below.

Compounds of Table 1 herein were prepared using methods disclosed herein or were prepared using methods disclosed herein combined with the knowledge of one of skill in the art.

Compounds of Table 2 may be prepared using methods disclosed herein or may be prepared using methods disclosed herein combined with the knowledge of one of skill in the art.

Scheme 1. General synthesis of macrocyclic esters

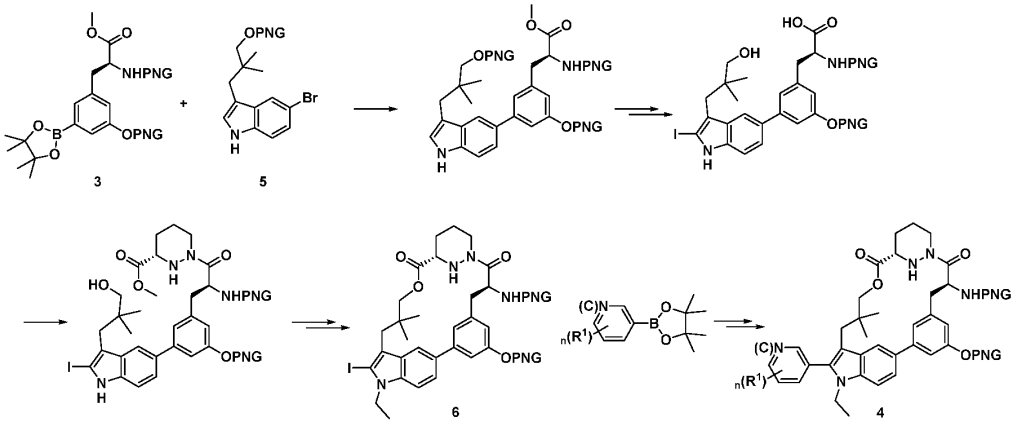


A general synthesis of macrocyclic esters is outlined in Scheme 1. An appropriately substituted aryl-3-(5-bromo-1-ethyl-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (**1**) can be prepared in three steps starting from protected 3-(5-bromo-2-iodo-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol and appropriately substituted boronic acid, including palladium mediated coupling, alkylation, and de-protection reactions.

Methyl-amino-hexahydropyridazine-3-carboxylate-boronic ester (**2**) can be prepared in three steps, including protection, iridium catalyst mediated borylation, and coupling with methyl (*S*)-hexahydropyridazine-3-carboxylate.

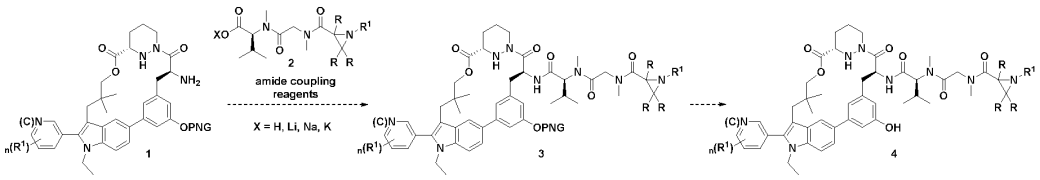
The final macrocyclic esters can be made by coupling of methyl-amino-hexahydropyridazine-3-carboxylate-boronic ester (**2**) and aryl-3-(5-bromo-1-ethyl-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (**1**) in the presence of Pd catalyst followed by hydrolysis and macrolactonization steps to result in an appropriately protected macrocyclic intermediate (**4**). Additional deprotection or functionalization steps are required to produce a final compound. For example, a person of skill in the art would be able to install into a macrocyclic ester a desired -B-L-W group of a compound of Formula (I), where B, L and W are defined herein, including by using methods exemplified in certain Schemes below and in the Example section herein.

Scheme 2. Alternative general synthesis of macrocyclic esters



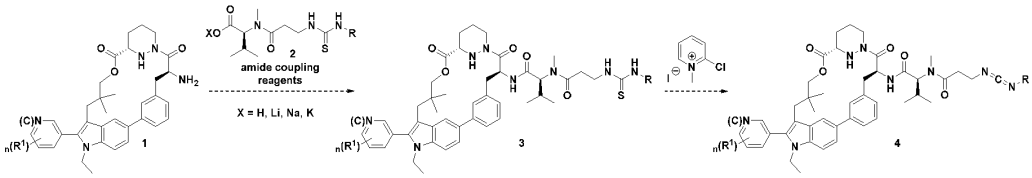
Alternatively, macrocyclic esters can be prepared as described in Scheme 2. An appropriately protected bromo-indolyl (**5**) can be coupled in the presence of Pd catalyst with boronic ester (**3**), followed by iodination, deprotection, and ester hydrolysis. Subsequent coupling with methyl (*S*)-hexahydropyridazine-3-carboxylate, followed by hydrolysis and macrolactonization can result in iodo intermediate (**6**). Coupling in the presence of Pd catalyst with an appropriately substituted boronic ester can yield fully a protected macrocycle (**4**). Additional deprotection or functionalization steps are required to produce a final compound. For example, a person of skill in the art would be able to install into a macrocyclic ester a desired -B-L-W group of a compound of Formula (I), where B, L and W are defined herein, including by using methods exemplified in certain Schemes below and in the Example section herein.

Scheme 3. General synthesis of aziridine containing macrocycles



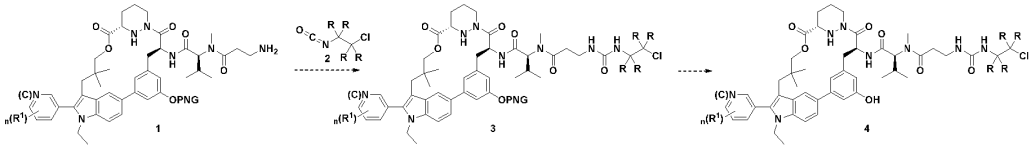
As shown in Scheme 3, compounds of this type may be prepared by the reaction of an appropriate amine (**1**) with an aziridine containing carboxylic acid (**2**) in the presence of standard amide coupling reagents, followed by deprotection of the aziridine, if R^1 is a protecting group, and deprotection of the phenol, if required, to produce the final compound (**4**).

Scheme 4. General synthesis of carbodiimide containing macrocycles



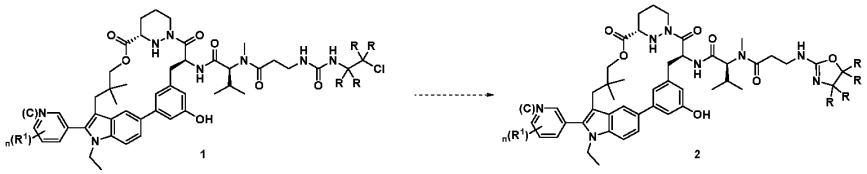
As shown in Scheme 4, compounds of this type may be prepared by the reaction of an appropriate amine (**1**) with a thiourea containing carboxylic acid (**2**) in the presence of standard amide coupling reagents, followed by conversion of the thiourea (**3**) to a carbodiimide (**4**) in the presence of 2-chloro-1-methylpyridin-1-ium iodide.

Scheme 5. General synthesis of chloroethyl urea containing macrocycles



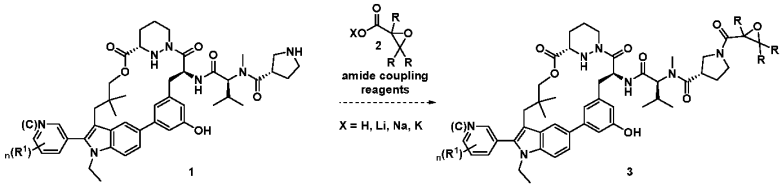
As shown in Scheme 5, compounds of this type may be prepared by the reaction of an appropriate amine (**1**) with an isocyanate (**2**) under basic conditions, followed by deprotection of the phenol, if required, to produce the final compound (**4**).

Scheme 6. General synthesis of amino oxazoline containing macrocycles



As shown in Scheme 6, compounds of this type may be prepared by cyclization of an appropriate chloroethyl urea (**1**) under elevated temperatures to produce the final compound (**2**).

Scheme 7. General synthesis of epoxide containing macrocycles



As shown in Scheme 7, compounds of this type may be prepared by the reaction of an appropriate amine (**1**) with an epoxide containing carboxylic acid (**2**) in the presence of standard amide coupling reagents to produce the final compound (**3**).

In addition, compounds of the disclosure can be synthesized using the methods described in the Examples below, together with synthetic methods known in the art of synthetic organic

chemistry, or variations thereon as appreciated by those skilled in the art. These methods include but are not limited to those methods described in the Examples below. For example, a person of skill in the art would be able to install into a macrocyclic ester a desired -B-L-W group of a compound of Formula (I), where B, L and W are defined herein, including by using methods exemplified in certain Schemes above and in the Example section herein.

Pharmaceutical Compositions and Methods of Use

Pharmaceutical Compositions and Methods of Administration

The compounds with which the invention is concerned are Ras inhibitors, and are useful in the treatment of cancer. Accordingly, one embodiment of the present invention provides pharmaceutical compositions containing a compound of the invention or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient, as well as methods of using the compounds of the invention to prepare such compositions.

As used herein, the term “pharmaceutical composition” refers to a compound, such as a compound of the present invention, or a pharmaceutically acceptable salt thereof, formulated together with a pharmaceutically acceptable excipient.

In some embodiments, a compound is present in a pharmaceutical composition in unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant population. In some embodiments, pharmaceutical compositions may be specially formulated for administration in solid or liquid form, including those adapted for the following: oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, e.g., those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin, lungs, or oral cavity; intravaginally or intrarectally, for example, as a pessary, cream, or foam; sublingually; ocularly; transdermally; or nasally, pulmonary, and to other mucosal surfaces.

A “pharmaceutically acceptable excipient,” as used herein, refers any inactive ingredient (for example, a vehicle capable of suspending or dissolving the active compound) having the properties of being nontoxic and non-inflammatory in a subject. Typical excipients include, for example: antiadherents, antioxidants, binders, coatings, compression aids, disintegrants, dyes (colors), emollients, emulsifiers, fillers (diluents), film formers or coatings, flavors, fragrances, glidants (flow enhancers), lubricants, preservatives, printing inks, sorbents, suspensing or dispersing agents, sweeteners, or waters of hydration. Excipients include, but are not limited to: butylated optionally substituted hydroxyltoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, crosslinked polyvinyl pyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, optionally substituted hydroxylpropyl cellulose, optionally substituted hydroxylpropyl methylcellulose, lactose, magnesium stearate, maltitol,

mannitol, methionine, methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol, polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac, silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E, vitamin C, and xylitol. Those of ordinary skill in the art are familiar with a variety of agents and materials useful as excipients. See, e.g., e.g., Ansel, et al., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems. Philadelphia: Lippincott, Williams & Wilkins, 2004; Gennaro, et al., Remington: The Science and Practice of Pharmacy. Philadelphia: Lippincott, Williams & Wilkins, 2000; and Rowe, Handbook of Pharmaceutical Excipients. Chicago, Pharmaceutical Press, 2005. In some embodiments, a composition includes at least two different pharmaceutically acceptable excipients.

Compounds described herein, whether expressly stated or not, may be provided or utilized in salt form, e.g., a pharmaceutically acceptable salt form, unless expressly stated to the contrary. The term “pharmaceutically acceptable salt,” as use herein, refers to those salts of the compounds described herein that are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and other animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, pharmaceutically acceptable salts are described in: Berge et al., *J. Pharmaceutical Sciences* 66:1-19, 1977 and in *Pharmaceutical Salts: Properties, Selection, and Use*, (Eds. P.H. Stahl and C.G. Wermuth), Wiley-VCH, 2008. The salts can be prepared in situ during the final isolation and purification of the compounds described herein or separately by reacting the free base group with a suitable organic acid.

The compounds of the invention may have ionizable groups so as to be capable of preparation as pharmaceutically acceptable salts. These salts may be acid addition salts involving inorganic or organic acids or the salts may, in the case of acidic forms of the compounds of the invention, be prepared from inorganic or organic bases. In some embodiments, the compounds are prepared or used as pharmaceutically acceptable salts prepared as addition products of pharmaceutically acceptable acids or bases. Suitable pharmaceutically acceptable acids and bases are well-known in the art, such as hydrochloric, sulfuric, hydrobromic, acetic, lactic, citric, or tartaric acids for forming acid addition salts, and potassium hydroxide, sodium hydroxide, ammonium hydroxide, caffeine, various amines, and the like for forming basic salts. Methods for preparation of the appropriate salts are well-established in the art.

Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-optionally substituted hydroxyl-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate,

valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like.

As used herein, the term "subject" refers to any member of the animal kingdom. In some embodiments, "subject" refers to humans, at any stage of development. In some embodiments, "subject" refers to a human patient. In some embodiments, "subject" refers to non-human animals. In some embodiments, the non-human animal is a mammal (e.g., a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, or a pig). In some embodiments, subjects include, but are not limited to, mammals, birds, reptiles, amphibians, fish, or worms. In some embodiments, a subject may be a transgenic animal, genetically-engineered animal, or a clone.

As used herein, the term "dosage form" refers to a physically discrete unit of a compound (e.g., a compound of the present invention) for administration to a subject. Each unit contains a predetermined quantity of compound. In some embodiments, such quantity is a unit dosage amount (or a whole fraction thereof) appropriate for administration in accordance with a dosing regimen that has been determined to correlate with a desired or beneficial outcome when administered to a relevant population (i.e., with a therapeutic dosing regimen). Those of ordinary skill in the art appreciate that the total amount of a therapeutic composition or compound administered to a particular subject is determined by one or more attending physicians and may involve administration of multiple dosage forms.

As used herein, the term "dosing regimen" refers to a set of unit doses (typically more than one) that are administered individually to a subject, typically separated by periods of time. In some embodiments, a given therapeutic compound (e.g., a compound of the present invention) has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which are separated from one another by a time period of the same length; in some embodiments, a dosing regimen comprises a plurality of doses and at least two different time periods separating individual doses. In some embodiments, all doses within a dosing regimen are of the same unit dose amount. In some embodiments, different doses within a dosing regimen are of different amounts. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount different from the first dose amount. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount same as the first dose amount. In some embodiments, a dosing regimen is correlated with a desired or beneficial outcome when administered across a relevant population (i.e., is a therapeutic dosing regimen).

A "therapeutic regimen" refers to a dosing regimen whose administration across a relevant population is correlated with a desired or beneficial therapeutic outcome.

The term "treatment" (also "treat" or "treating"), in its broadest sense, refers to any administration of a substance (e.g., a compound of the present invention) that partially or

completely alleviates, ameliorates, relieves, inhibits, delays onset of, reduces severity of, or reduces incidence of one or more symptoms, features, or causes of a particular disease, disorder, or condition. In some embodiments, such treatment may be administered to a subject who does not exhibit signs of the relevant disease, disorder or condition, or of a subject who exhibits only early signs of the disease, disorder, or condition. Alternatively, or additionally, in some embodiments, treatment may be administered to a subject who exhibits one or more established signs of the relevant disease, disorder, or condition. In some embodiments, treatment may be of a subject who has been diagnosed as suffering from the relevant disease, disorder, or condition. In some embodiments, treatment may be of a subject known to have one or more susceptibility factors that are statistically correlated with increased risk of development of the relevant disease, disorder, or condition.

The term “therapeutically effective amount” means an amount that is sufficient, when administered to a population suffering from or susceptible to a disease, disorder, or condition in accordance with a therapeutic dosing regimen, to treat the disease, disorder, or condition. In some embodiments, a therapeutically effective amount is one that reduces the incidence or severity of, or delays onset of, one or more symptoms of the disease, disorder, or condition. Those of ordinary skill in the art will appreciate that the term “therapeutically effective amount” does not in fact require successful treatment be achieved in a particular individual. Rather, a therapeutically effective amount may be that amount that provides a particular desired pharmacological response in a significant number of subjects when administered to patients in need of such treatment. It is specifically understood that particular subjects may, in fact, be “refractory” to a “therapeutically effective amount.” In some embodiments, reference to a therapeutically effective amount may be a reference to an amount as measured in one or more specific tissues (e.g., a tissue affected by the disease, disorder or condition) or fluids (e.g., blood, saliva, serum, sweat, tears, urine). Those of ordinary skill in the art will appreciate that, in some embodiments, a therapeutically effective amount may be formulated or administered in a single dose. In some embodiments, a therapeutically effective amount may be formulated or administered in a plurality of doses, for example, as part of a dosing regimen.

For use as treatment of subjects, the compounds of the invention, or a pharmaceutically acceptable salt thereof, can be formulated as pharmaceutical or veterinary compositions. Depending on the subject to be treated, the mode of administration, and the type of treatment desired, e.g., prevention, prophylaxis, or therapy, the compounds, or a pharmaceutically acceptable salt thereof, are formulated in ways consonant with these parameters. A summary of such techniques may be found in *Remington: The Science and Practice of Pharmacy*, *21st Edition*, Lippincott Williams & Wilkins, (2005); and *Encyclopedia of Pharmaceutical Technology*, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York, each of which is incorporated herein by reference.

Compositions can be prepared according to conventional mixing, granulating, or coating methods, respectively, and the present pharmaceutical compositions can contain from about 0.1% to about 99%, from about 5% to about 90%, or from about 1% to about 20% of a compound of the

present invention, or pharmaceutically acceptable salt thereof, by weight or volume. In some embodiments, compounds, or a pharmaceutically acceptable salt thereof, described herein may be present in amounts totaling 1-95% by weight of the total weight of a composition, such as a pharmaceutical composition.

The composition may be provided in a dosage form that is suitable for intraarticular, oral, parenteral (e.g., intravenous, intramuscular), rectal, cutaneous, subcutaneous, topical, transdermal, sublingual, nasal, vaginal, intravesicular, intraurethral, intrathecal, epidural, aural, or ocular administration, or by injection, inhalation, or direct contact with the nasal, genitourinary, reproductive, or oral mucosa. Thus, the pharmaceutical composition may be in the form of, e.g., tablets, capsules, pills, powders, granulates, suspensions, emulsions, solutions, gels including hydrogels, pastes, ointments, creams, plasters, drenches, osmotic delivery devices, suppositories, enemas, injectables, implants, sprays, preparations suitable for iontophoretic delivery, or aerosols. The compositions may be formulated according to conventional pharmaceutical practice.

As used herein, the term “administration” refers to the administration of a composition (e.g., a compound, or a preparation that includes a compound as described herein) to a subject or system. Administration to an animal subject (e.g., to a human) may be by any appropriate route. For example, in some embodiments, administration may be bronchial (including by bronchial instillation), buccal, enteral, interdermal, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intravenous, intraventricular, mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (including by intratracheal instillation), transdermal, vaginal, or vitreal.

Formulations may be prepared in a manner suitable for systemic administration or topical or local administration. Systemic formulations include those designed for injection (e.g., intramuscular, intravenous or subcutaneous injection) or may be prepared for transdermal, transmucosal, or oral administration. A formulation will generally include a diluent as well as, in some cases, adjuvants, buffers, preservatives and the like. Compounds, or a pharmaceutically acceptable salt thereof, can be administered also in liposomal compositions or as microemulsions.

For injection, formulations can be prepared in conventional forms as liquid solutions or suspensions or as solid forms suitable for solution or suspension in liquid prior to injection or as emulsions. Suitable excipients include, for example, water, saline, dextrose, glycerol, and the like. Such compositions may also contain amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and the like, such as, for example, sodium acetate, sorbitan monolaurate, and so forth.

Various sustained release systems for drugs have also been devised. See, for example, U.S. Patent No. 5,624,677.

Systemic administration may also include relatively noninvasive methods such as the use of suppositories, transdermal patches, transmucosal delivery, and intranasal administration. Oral administration is also suitable for compounds of the invention, or pharmaceutically acceptable salts thereof. Suitable forms include syrups, capsules, and tablets, as is understood in the art.

Each compound, or a pharmaceutically acceptable salt thereof, as described herein, may be formulated in a variety of ways that are known in the art. For example, the first and second agents of the combination therapy may be formulated together or separately. Other modalities of combination therapy are described herein.

The individually or separately formulated agents can be packaged together as a kit. Non-limiting examples include, but are not limited to, kits that contain, e.g., two pills, a pill and a powder, a suppository and a liquid in a vial, two topical creams, etc. The kit can include optional components that aid in the administration of the unit dose to subjects, such as vials for reconstituting powder forms, syringes for injection, customized IV delivery systems, inhalers, etc. Additionally, the unit dose kit can contain instructions for preparation and administration of the compositions. The kit may be manufactured as a single use unit dose for one subject, multiple uses for a particular subject (at a constant dose or in which the individual compounds, or a pharmaceutically acceptable salt thereof, may vary in potency as therapy progresses); or the kit may contain multiple doses suitable for administration to multiple subjects ("bulk packaging"). The kit components may be assembled in cartons, blister packs, bottles, tubes, and the like.

Formulations for oral use include tablets containing the active ingredient(s) in a mixture with non-toxic pharmaceutically acceptable excipients. These excipients may be, for example, inert diluents or fillers (e.g., sucrose, sorbitol, sugar, mannitol, microcrystalline cellulose, starches including potato starch, calcium carbonate, sodium chloride, lactose, calcium phosphate, calcium sulfate, or sodium phosphate); granulating and disintegrating agents (e.g., cellulose derivatives including microcrystalline cellulose, starches including potato starch, croscarmellose sodium, alginates, or alginic acid); binding agents (e.g., sucrose, glucose, sorbitol, acacia, alginic acid, sodium alginate, gelatin, starch, pregelatinized starch, microcrystalline cellulose, magnesium aluminum silicate, carboxymethylcellulose sodium, methylcellulose, optionally substituted hydroxylpropyl methylcellulose, ethylcellulose, polyvinylpyrrolidone, or polyethylene glycol); and lubricating agents, glidants, and antiadhesives (e.g., magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc). Other pharmaceutically acceptable excipients can be colorants, flavoring agents, plasticizers, humectants, buffering agents, and the like.

Two or more compounds may be mixed together in a tablet, capsule, or other vehicle, or may be partitioned. In one example, the first compound is contained on the inside of the tablet, and the second compound is on the outside, such that a substantial portion of the second compound is released prior to the release of the first compound.

Formulations for oral use may also be provided as chewable tablets, or as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent (e.g., potato starch, lactose, microcrystalline cellulose, calcium carbonate, calcium phosphate or kaolin), or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil. Powders, granulates, and pellets may be prepared using the ingredients mentioned above under tablets and capsules in a conventional manner using, e.g., a mixer, a fluid bed apparatus or a spray drying equipment.

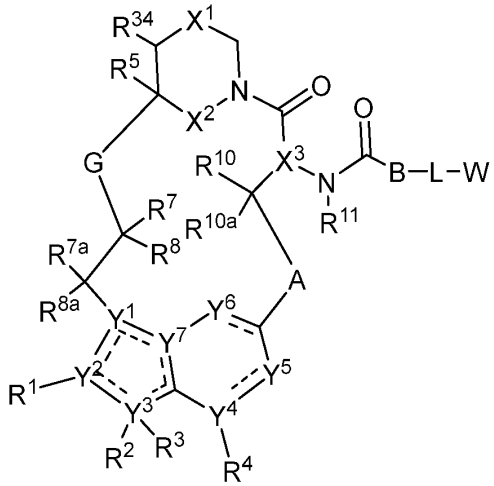
Dissolution or diffusion-controlled release can be achieved by appropriate coating of a tablet, capsule, pellet, or granulate formulation of compounds, or by incorporating the compound, or a pharmaceutically acceptable salt thereof, into an appropriate matrix. A controlled release coating may include one or more of the coating substances mentioned above or, e.g., shellac, beeswax, glycowax, castor wax, carnauba wax, stearyl alcohol, glyceryl monostearate, glyceryl distearate, glycerol palmitostearate, ethylcellulose, acrylic resins, dl-polylactic acid, cellulose acetate butyrate, polyvinyl chloride, polyvinyl acetate, vinyl pyrrolidone, polyethylene, polymethacrylate, methylmethacrylate, 2-optionally substituted hydroxylmethacrylate, methacrylate hydrogels, 1,3 butylene glycol, ethylene glycol methacrylate, or polyethylene glycols. In a controlled release matrix formulation, the matrix material may also include, e.g., hydrated methylcellulose, carnauba wax and stearyl alcohol, carbopol 934, silicone, glyceryl tristearate, methyl acrylate-methyl methacrylate, polyvinyl chloride, polyethylene, or halogenated fluorocarbon.

The liquid forms in which the compounds, or a pharmaceutically acceptable salt thereof, and compositions of the present invention can be incorporated for administration orally include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Generally, when administered to a human, the oral dosage of any of the compounds of the invention, or a pharmaceutically acceptable salt thereof, will depend on the nature of the compound, and can readily be determined by one skilled in the art. A dosage may be, for example, about 0.001 mg to about 2000 mg per day, about 1 mg to about 1000 mg per day, about 5 mg to about 500 mg per day, about 100 mg to about 1500 mg per day, about 500 mg to about 1500 mg per day, about 500 mg to about 2000 mg per day, or any range derivable therein.

Numbered Embodiments

[1] A compound, or pharmaceutically acceptable salt thereof, having the structure of Formula I:



Formula I

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 10-membered heteroarylene;

B is -CH(R^9)- or >C=C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', $S(O)_2$R', or $S(O)_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2 or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is hydrogen, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

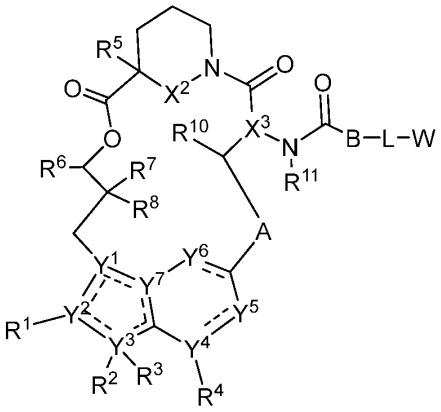
R^{10a} is hydrogen or halo;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{34} is hydrogen or C_1-C_3 alkyl.

[2] The compound, or pharmaceutically acceptable salt thereof, of paragraph [1], wherein G is optionally substituted C_1-C_4 heteroalkylene.

[3] The compound, or pharmaceutically acceptable salt thereof, of paragraph [1] or [2], wherein the compound has the structure of Formula Ia:



Formula Ia

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl; and

R^{11} is hydrogen or C_1-C_3 alkyl.

[4] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [3], wherein X^2 is NH.

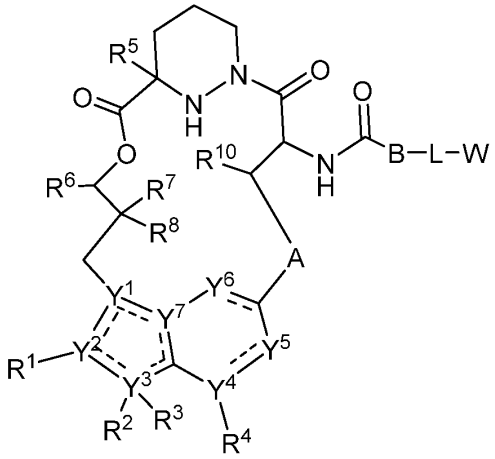
[5] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [4], wherein X^3 is CH.

[6] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [5], wherein R^{11} is hydrogen.

[7] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [5], wherein R^{11} is C_1-C_3 alkyl.

[8] The compound, or pharmaceutically acceptable salt thereof, of paragraph [7], wherein R^{11} is methyl.

[9] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [6], wherein the compound has the structure of Formula Ib:



Formula Ib

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form C=C$R^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; and

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl.

[10] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [9] wherein X^1 is optionally substituted C_1-C_2 alkylene.

[11] The compound, or pharmaceutically acceptable salt thereof, of paragraph [10], wherein X^1 is methylene.

[12] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [11], wherein R^5 is hydrogen.

[13] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [11], wherein R^5 is C_1-C_4 alkyl optionally substituted with halogen.

[14] The compound, or pharmaceutically acceptable salt thereof, of paragraph [13], wherein R^5 is methyl.

[15] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [14], wherein Y^4 is C.

[16] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [15], wherein R^4 is hydrogen.

[17] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [16], wherein Y^5 is CH.

[18] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [17], wherein Y^6 is CH.

[19] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [18], wherein Y^1 is C.

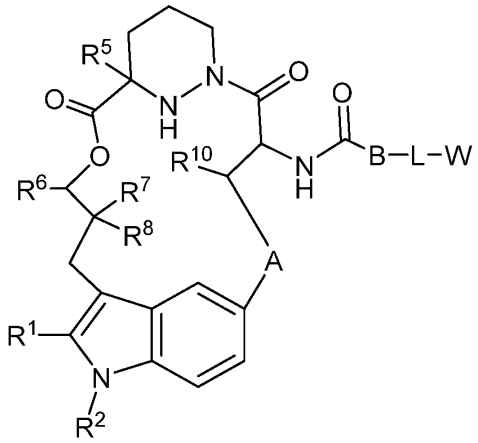
[20] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [19], wherein Y^2 is C.

[21] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [20], wherein Y^3 is N.

[22] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [21], wherein R^3 is absent.

[23] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [22], wherein Y^7 is C.

[24] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [6] or [9] to [23], wherein the compound has the structure of Formula Ic:



Formula Ic

wherein A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; and

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl.

[25] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [24], wherein R^6 is hydrogen.

[26] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [25], wherein R^2 is hydrogen, cyano, optionally substituted C_1-C_6 alkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 6-membered heterocycloalkyl.

[27] The compound, or pharmaceutically acceptable salt thereof, of paragraph [26], wherein R^2 is optionally substituted C_1-C_6 alkyl.

[28] The compound, or pharmaceutically acceptable salt thereof, of paragraph [27], wherein R^2 is ethyl.

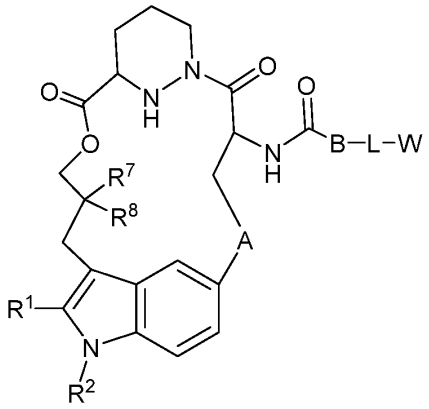
[29] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [28], wherein R^7 is optionally substituted C_1-C_3 alkyl.

[30] The compound, or pharmaceutically acceptable salt thereof, of paragraph [29], wherein R^7 is C_1-C_3 alkyl.

[31] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [30], wherein R^8 is optionally substituted C_1-C_3 alkyl.

[32] The compound, or pharmaceutically acceptable salt thereof, of paragraph [31], wherein R^8 is C_1-C_3 alkyl.

[33] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [32], wherein the compound has the structure of Formula Id:



Formula Id

wherein A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is C_1-C_6 alkyl or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

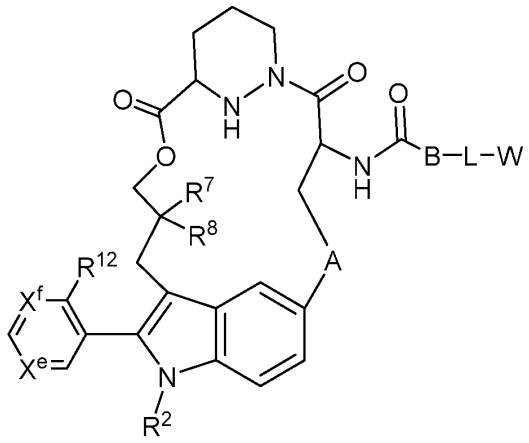
R^8 is C_1-C_3 alkyl; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

[34] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [33], wherein R^1 is 5 to 10-membered heteroaryl.

[35] The compound, or pharmaceutically acceptable salt thereof, of paragraph [34], wherein R^1 is optionally substituted 6-membered aryl or optionally substituted 6-membered heteroaryl.

[36] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [35], wherein the compound has the structure of Formula Ie:



Formula Ie

wherein A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

R^2 is C_1-C_6 alkyl or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

R^8 is C_1-C_3 alkyl; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl

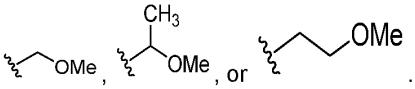
X^e and X^f are, independently, N or CH; and

R^{12} is optionally substituted C_1-C_6 alkyl or optionally substituted C_1-C_6 heteroalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

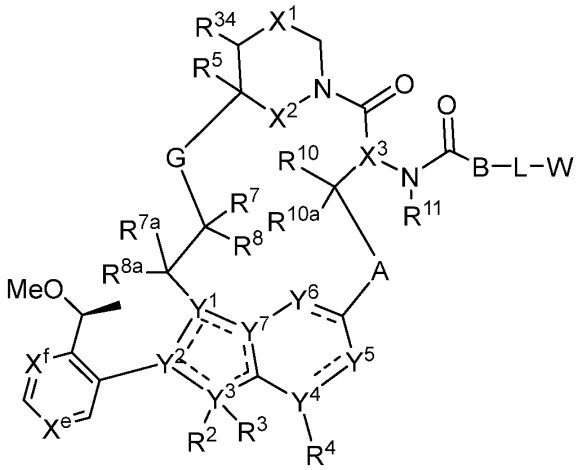
[37] The compound, or pharmaceutically acceptable salt thereof, of paragraph [36], wherein X^e is N and X^f is CH.

[38] The compound, or pharmaceutically acceptable salt thereof, of paragraph [36], wherein X^e is CH and X^f is N.

[39] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [36] to [38], wherein R^{12} is optionally substituted C_1-C_6 heteroalkyl.

[40] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [36] to [39], wherein R^{12} is  .

[41] The compound, or pharmaceutically acceptable salt thereof, of paragraph [1] or [2], wherein the compound has the structure of Formula VI:



Formula VI

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 10-membered heteroarylene;

B is -CH(R^9)- or >C=C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', $C(O)N(R')_2$, S(O)R', $S(O)_2R'$, or $S(O)_2N(R')_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), C=N(O-C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is hydrogen, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

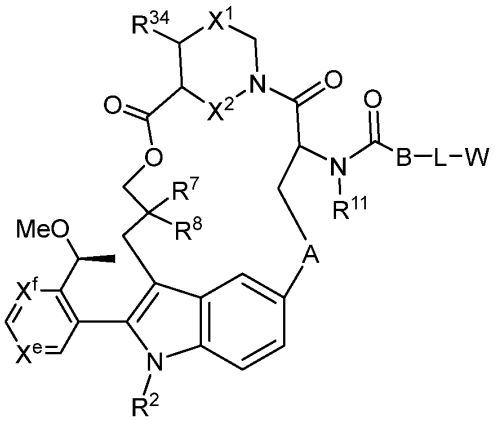
R^{10a} is hydrogen or halo;

R^{11} is hydrogen or C_1-C_3 alkyl;

R^{34} is hydrogen or C_1-C_3 alkyl; and

X^e and X^f are, independently, N or CH.

[42] The compound, or pharmaceutically acceptable salt thereof, of paragraph [41], wherein the compound has the structure of Formula VIa:



Formula VIa

wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered

heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', $C(O)N(R')_2$, S(O)R', $S(O)_2R'$, or $S(O)_2N(R')_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

R^2 is C_1-C_6 alkyl or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

R^8 is C_1-C_3 alkyl; and

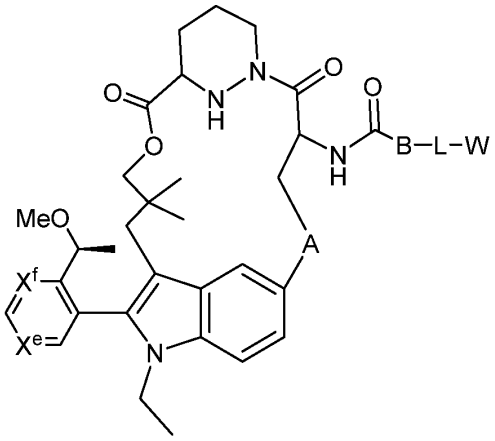
R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

X^e and X^f are, independently, N or CH;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{21} is hydrogen or C_1-C_3 alkyl.

[43] The compound, or pharmaceutically acceptable salt thereof, of paragraph [41] or [42], wherein the compound has the structure of Formula VIb:



Formula VIb

wherein A optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

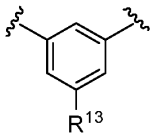
W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; and

X^e and X^f are, independently, N or CH.

[44] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [43], wherein A is optionally substituted 6-membered arylene.

[45] The compound, or pharmaceutically acceptable salt thereof, of paragraph [44], wherein A has the structure:



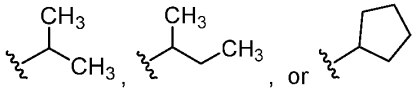
wherein R^{13} is hydrogen, hydroxy, amino, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl.

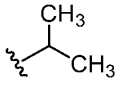
[46] The compound, or pharmaceutically acceptable salt thereof, of paragraph [45], wherein R^{13} is hydrogen.

[47] The compound, or pharmaceutically acceptable salt thereof, of paragraph [45], wherein R^{13} is hydroxy.

[48] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [47], wherein B is -CHR^9-.

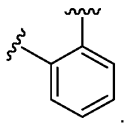
[49] The compound, or pharmaceutically acceptable salt thereof, of paragraph [48], wherein R^9 is optionally substituted C_1-C_6 alkyl or optionally substituted 3 to 6-membered cycloalkyl.

[50] The compound, or pharmaceutically acceptable salt thereof, of paragraph [49], wherein R^9 is:  .

[51] The compound, or pharmaceutically acceptable salt thereof, of paragraph [50], wherein R^9 is:  .

[52] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [47], wherein B is optionally substituted 6-membered arylene.

[53] The compound, or pharmaceutically acceptable salt thereof, of paragraph [52], wherein B is 6-membered arylene.

[54] The compound, or pharmaceutically acceptable salt thereof, of paragraph [53], wherein B is:  .

[55] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [54], wherein R^7 is methyl.

[56] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [55], wherein R^8 is methyl.

[57] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [56], wherein the linker is the structure of Formula II:

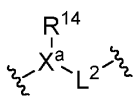
$$A^1\text{-}(B^1)_f\text{-}(C^1)_g\text{-}(B^2)_h\text{-}(D^1)\text{-}(B^3)_i\text{-}(C^2)_j\text{-}(B^4)_k\text{–}A^2$$

Formula II

where A^1 is a bond between the linker and B; A^2 is a bond between W and the linker; B^1, B^2, B^3, and B^4 each, independently, is selected from optionally substituted C_1-C_2 alkylene, optionally substituted C_1-C_3 heteroalkylene, O, S, and NR^N; R^N is hydrogen, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted C_1-C_7 heteroalkyl; C^1 and C^2 are each, independently, selected from carbonyl, thiocarbonyl, sulphonyl, or phosphoryl; f, g, h, i, j, and k are each, independently, 0 or 1; and D^1 is optionally substituted C_1-C_{10} alkylene, optionally substituted C_2-C_{10} alkenylene, optionally substituted C_2-C_{10} alkynylene, optionally substituted 3 to 14-membered heterocycloalkylene, optionally substituted 5 to 10-membered heteroarylene, optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 6 to 10-membered arylene, optionally substituted C_2-C_{10} polyethylene glycolene, or optionally substituted C_1-C_{10} heteroalkylene, or a chemical bond linking $A^1\text{-}(B^1)_f\text{-}(C^1)_g\text{-}(B^2)_h$- to -$(B^3)_i\text{-}(C^2)_j\text{-}(B^4)_k\text{–}A^2$.

[58] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [57], wherein the linker is acyclic.

[59] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [58], wherein the linker has the structure of Formula IIa:



Formula IIa

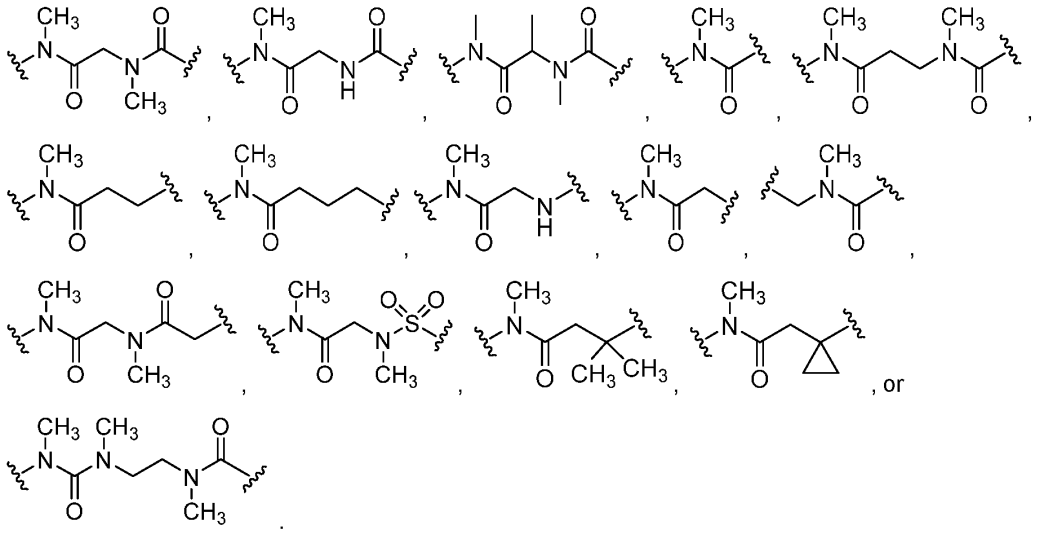
wherein X^a is absent or N;

R^{14} is absent, hydrogen or optionally substituted C_1-C_6 alkyl; and

L^2 is absent, $-SO_2-$, optionally substituted C_1-C_4 alkylene or optionally substituted C_1-C_4 heteroalkylene,

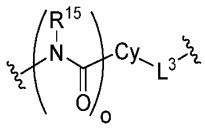
wherein at least one of X^a, R^{14}, or L^2 is present.

[60] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [59], wherein the linker has the structure:



[61] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [57], wherein the linker is or a comprises a cyclic group.

[62] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [57] or [61], wherein the linker has the structure of Formula IIb:



Formula IIb

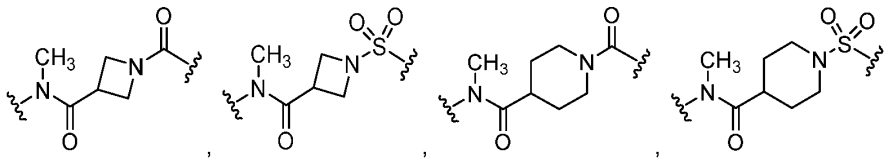
wherein o is 0 or 1;

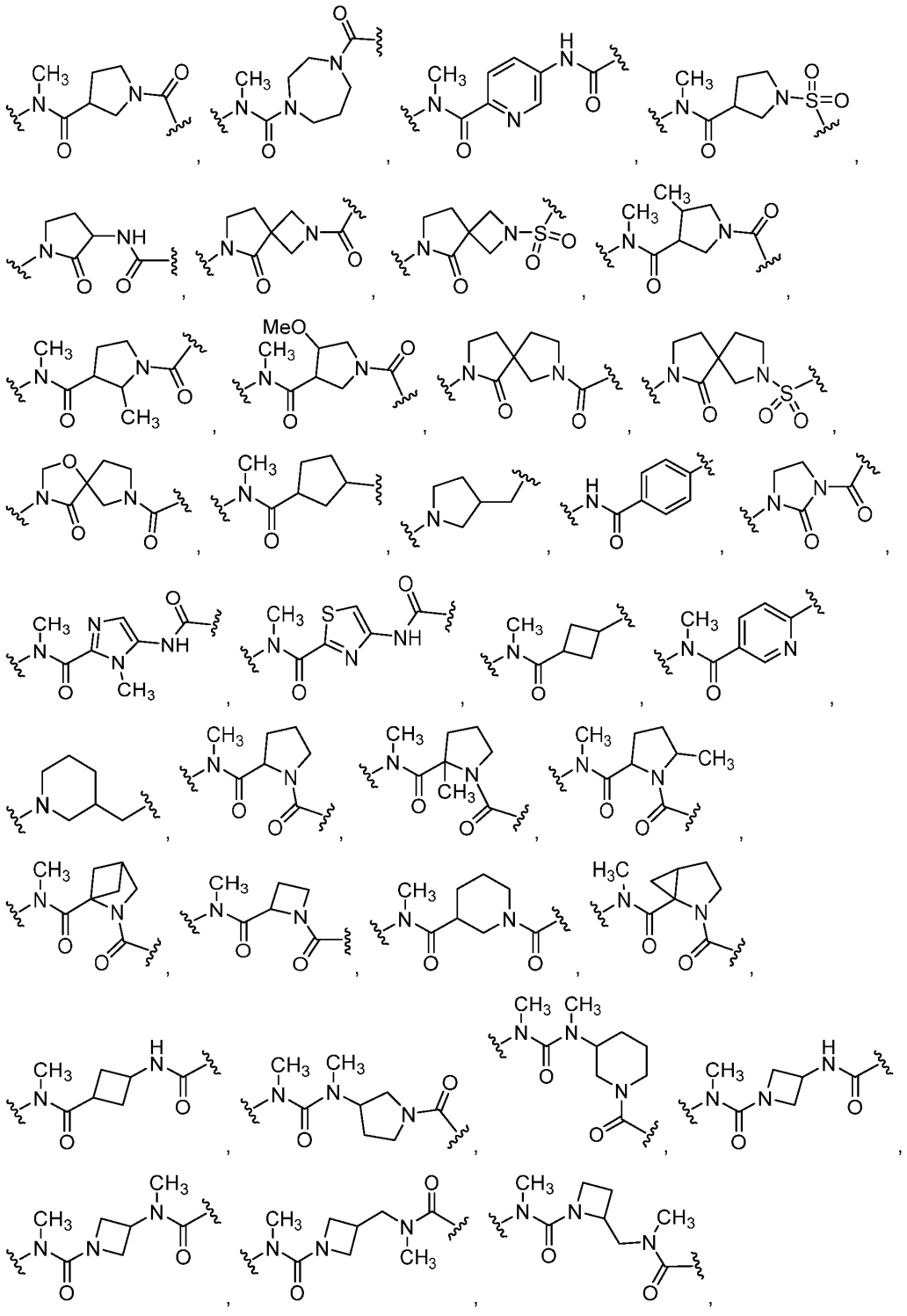
R^{15} is hydrogen or optionally substituted C_1-C_6 alkyl;

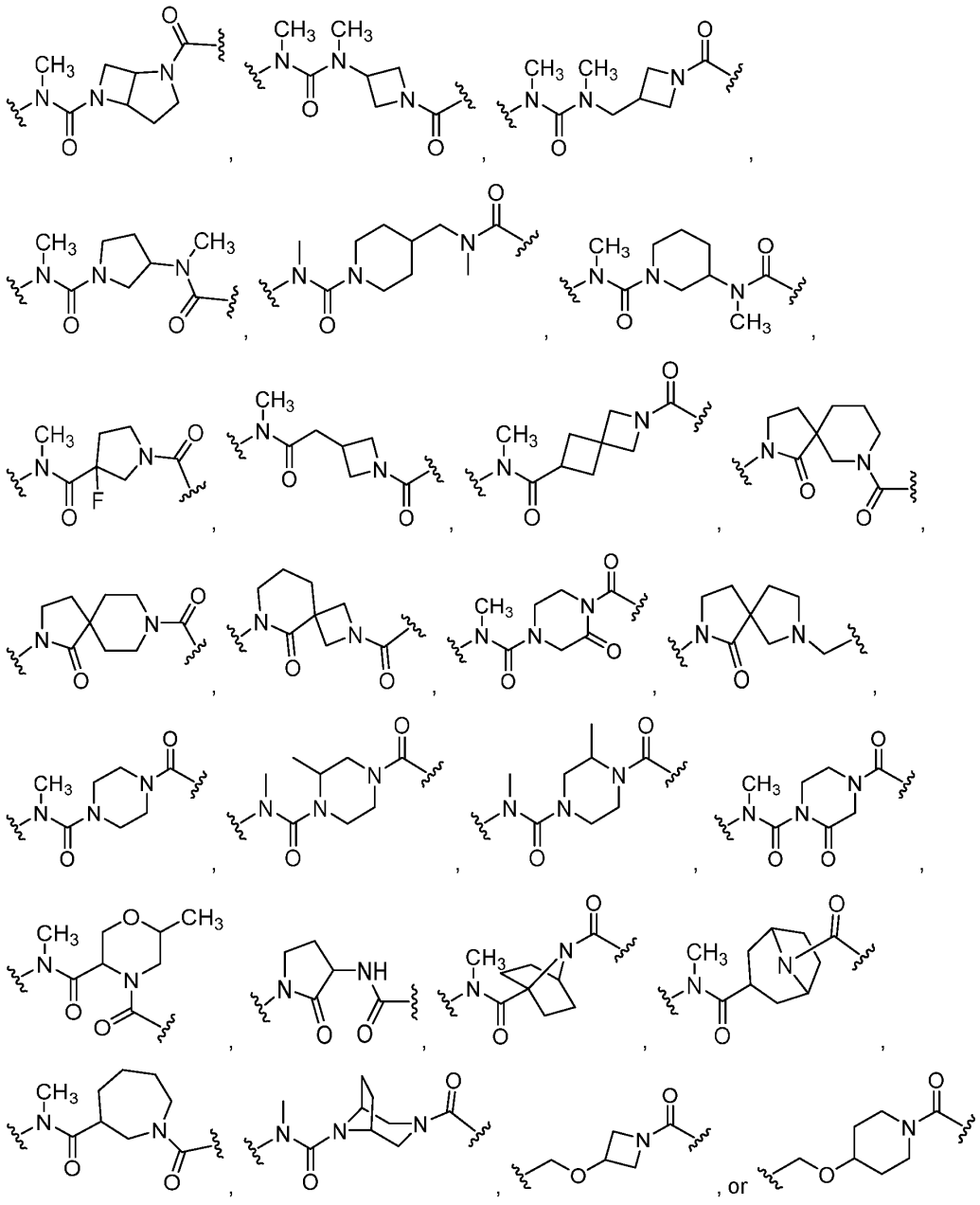
Cy is optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 3 to 8-membered heterocycloalkylene, optionally substituted 6-10 membered arylene, or optionally substituted 5 to 10-membered heteroarylene; and

L^3 is absent, $-SO_2-$, optionally substituted C_1-C_4 alkylene or optionally substituted C_1-C_4 heteroalkylene.

[63] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [62], wherein the linker has the structure:



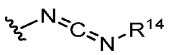




.

[64] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [63], wherein W comprises a carbodiimide.

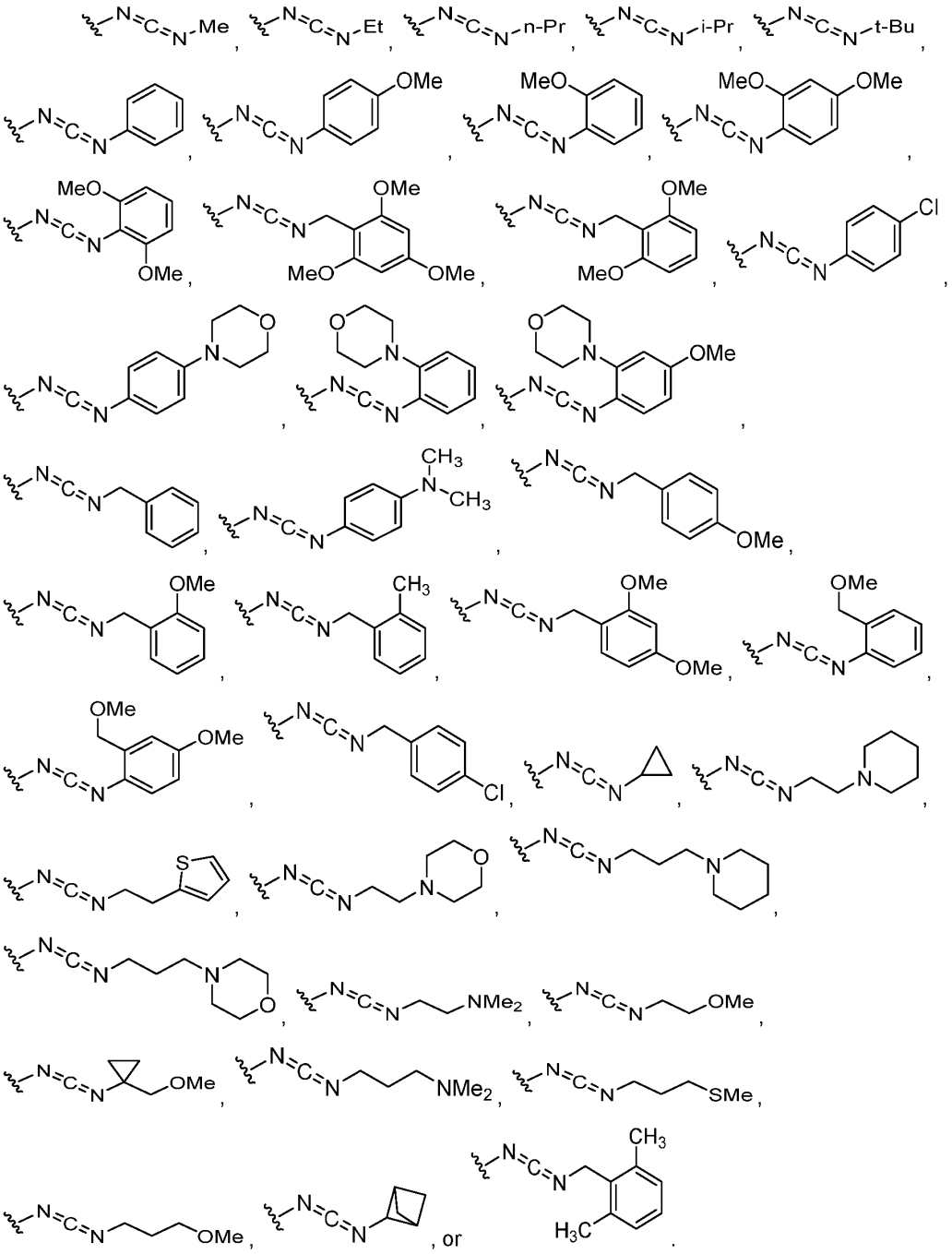
[65] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [64], wherein W has the structure of Formula IIIa:



Formula IIIa

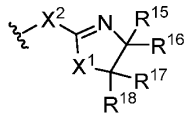
wherein R^{14} is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 6 to 10-membered aryl, optionally substituted 3 to 14-membered heterocycloalkyl, or optionally substituted 5 to 10-membered heteroaryl.

[66] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [65], wherein W has the structure:



[67] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [63], wherein W comprises an oxazoline or thiazoline.

[68] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [67], wherein W has the structure of Formula IIIb:



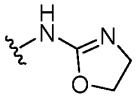
Formula IIb

wherein X^1 is O or S;

X^2 is absent or NR^{19};

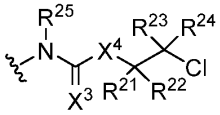
R^{15}, R^{16}, R^{17}, and R^{18} are, independently, hydrogen or optionally substituted C_1-C_6 alkyl; and

R^{19} is hydrogen, C(O)(optionally substituted C_1-C_6 alkyl), optionally substituted C_1-C_6 alkyl, optionally substituted 6 to 10-membered aryl, optionally substituted 3 to 14-membered heterocycloalkyl, or optionally substituted 5 to 10-membered heteroaryl.

[69] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [68], wherein W is  .

[70] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [63], wherein W comprises a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, or a chloroethyl thiocarbamate.

[71] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [70], wherein W has the structure of Formula IIIc:



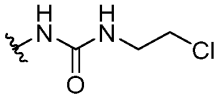
Formula IIIc

wherein X^3 is O or S;

X^4 is O, S, NR^{26};

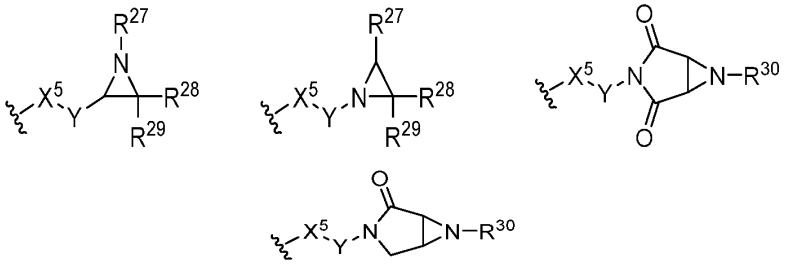
R^{21}, R^{22}, R^{23}, R^{24}, and R^{26} are, independently, hydrogen or optionally substituted C_1-C_6 alkyl; and

R^{25} is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted 6 to 10-membered aryl, optionally substituted 3 to 14-membered heterocycloalkyl, or optionally substituted 5 to 10-membered heteroaryl.

[72] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [71], wherein W is  .

[73] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [63], wherein W comprises an aziridine.

[74] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [73], wherein W has the structure of Formula IIId1, Formula IIId2, Formula IIId3, or Formula IIId4:



Formula IIId1 Formula IIId2 Formula IIId3 Formula IIId4

wherein X^5 is absent or NR^{30};

Y is absent or C(O), C(S), S(O), SO_2, or optionally substituted C_1-C_3 alkylene;

R^{27} is hydrogen, $-C(O)R^{32}$, $-C(O)OR^{32}$, $-SO_2R^{33}$, $-SOR^{33}$, optionally substituted C_1-C_6 alkyl, optionally substituted 6 to 10-membered aryl, optionally substituted 3 to 14-membered heterocycloalkyl, or optionally substituted 5 to 10-membered heteroaryl;

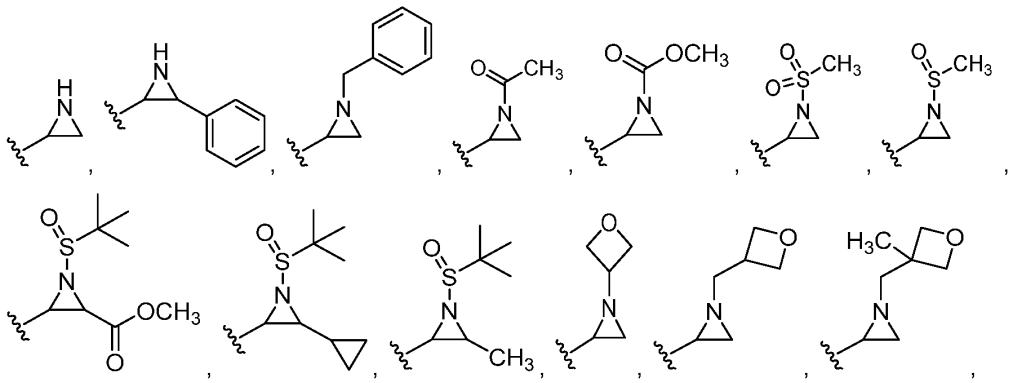
R^{28} and R^{29} are, independently, hydrogen, CN, $C(O)R^{31}$, CO_2R^{31}, $C(O)R^{31}R^{31}$ optionally substituted C_1-C_6 alkyl, optionally substituted 3 to 10-membered cycloalkyl, optionally substituted 6 to 10-membered aryl, optionally substituted 3 to 14-membered heterocycloalkyl, or optionally substituted 5 to 10-membered heteroaryl;

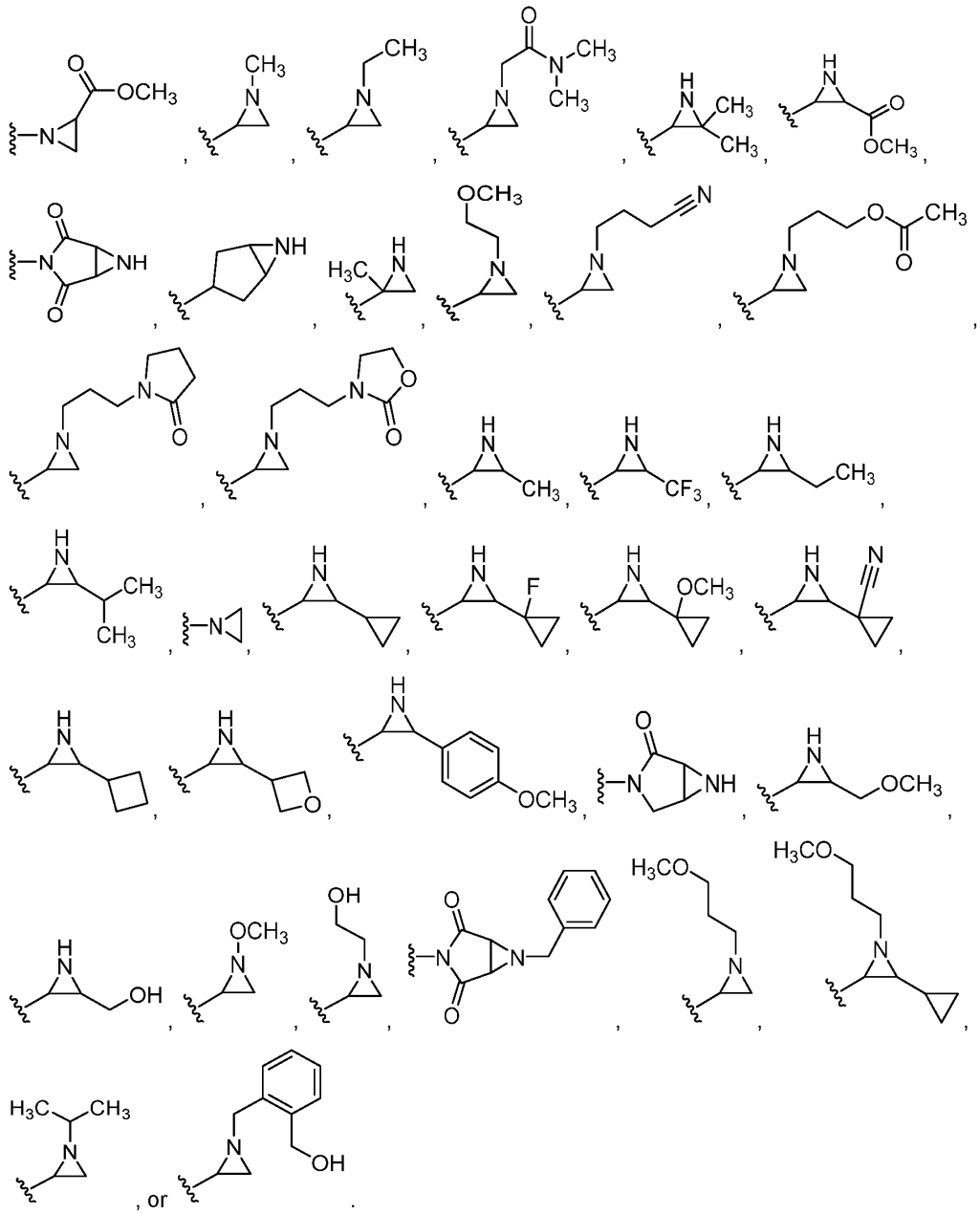
each R^{31} is, independently, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted 6 to 10-membered aryl, optionally substituted 3 to 14-membered heterocycloalkyl, or optionally substituted 5 to 10-membered heteroaryl;

R^{30} is hydrogen or optionally substituted C_1-C_6 alkyl; and

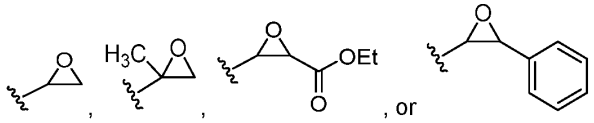
R^{32} and R^{33} are, independently, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted 6 to 10-membered aryl, optionally substituted 3 to 14-membered heterocycloalkyl, or optionally substituted 5 to 10-membered heteroaryl.

[75] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [73] or [74], wherein W is:





[76] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [63], wherein W comprises an epoxide.

[77] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [76], wherein W is  .

[78] A compound, or a pharmaceutically acceptable salt thereof, of Table 1 or Table 2.

[79] A pharmaceutical composition comprising a compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [78] and a pharmaceutically acceptable excipient.

[80] A conjugate, or salt thereof, comprising the structure of Formula IV:

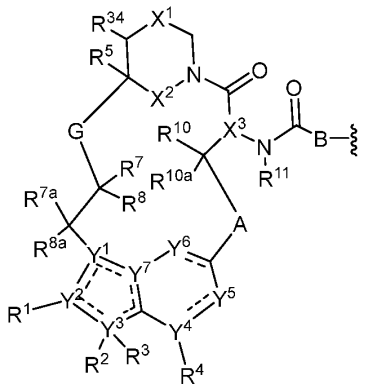
M-L-P

Formula IV

wherein L is a linker;

P is a monovalent organic moiety; and

M has the structure of Formula V:



Formula V

wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is -N(H or CH_3)C(O)-(CH_2)- where the amino nitrogen is bound to the carbon atom of -CH(R^{10})-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- or >C=C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of -N(R^{11})C(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, -C(O)O-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, -C(O)NH-**C**H(R^6)- where **C** is bound to -C(R^7R^8)-, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or S(O)$_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', $C(O)N(R')_2$, S(O)R', $S(O)_2R'$, or $S(O)_2N(R')_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C=CR^{7'}R^{8'}$; C=N(OH), $C=N(O\text{-}C_1\text{-}C_3$ alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxyl, optionally substituted C_1-C_3 alkyl, optionally

substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is hydrogen, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

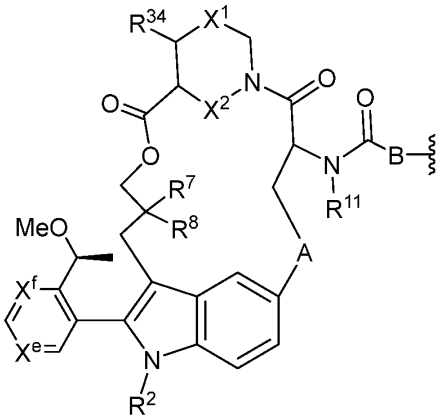
$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl;

R^{10a} is hydrogen or halo;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{34} is hydrogen or C_1-C_3 alkyl.

[81] A conjugate, or salt thereof, of paragraph [80], wherein M has the structure of Formula Vc:



Formula Vc

wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

X^e and X^f are, independently, N or CH;

R^2 is C_1-C_6 alkyl or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

R^8 is C_1-C_3 alkyl; and

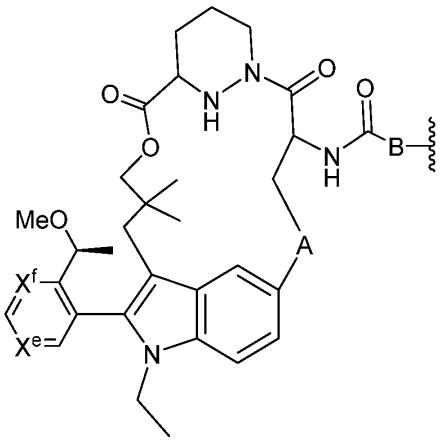
R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{34} is hydrogen or C_1-C_3 alkyl.

In some embodiments of a compound of the present invention, X^e is N and X^f is CH. In some embodiments, X^e is CH and X^f is N.

[82] The conjugate, or salt thereof, of paragraph [80] or [81], wherein M has the structure of Formula Vd:



Formula Vd

wherein A optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is -CH(R^9)- where the carbon is bound to the carbonyl carbon of -NHC(O)-, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a carbodiimide, an oxazoline, a thiazoline, a chloroethyl urea, a chloroethyl thiourea, a chloroethyl carbamate, a chloroethyl thiocarbamate, an aziridine, a trifluoromethyl ketone, a boronic acid, a boronic ester, an *N*-ethoxycarbonyl-2-ethoxy-

1,2-dihydroquinoline (EEDQ), an iso-EEDQ or other EEDQ derivative, an epoxide, an oxazolium, or a glycal;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; and

X^e and X^f are, independently, N or CH.

[83] The conjugate, or salt thereof, of any one of paragraphs [80] to [82], wherein the linker has the structure of Formula II:

$$A^1\text{-}(B^1)_f\text{-}(C^1)_g\text{-}(B^2)_h\text{-}(D^1)\text{-}(B^3)_i\text{-}(C^2)_j\text{-}(B^4)_k\text{–}A^2$$

Formula II

where A^1 is a bond between the linker and B; A^2 is a bond between P and the linker; B^1, B^2, B^3, and B^4 each, independently, is selected from optionally substituted C_1-C_2 alkylene, optionally substituted C_1-C_3 heteroalkylene, O, S, and NR^N; R^N is hydrogen, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted C_1-C_7 heteroalkyl; C^1 and C^2 are each, independently, selected from carbonyl, thiocarbonyl, sulphonyl, or phosphoryl; f, g, h, i, j, and k are each, independently, 0 or 1; and D^1 is optionally substituted C_1-C_{10} alkylene, optionally substituted C_2-C_{10} alkenylene, optionally substituted C_2-C_{10} alkynylene, optionally substituted 3 to 14-membered heterocycloalkylene, optionally substituted 5 to 10-membered heteroarylene, optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 6 to 10-membered arylene, optionally substituted C_2-C_{10} polyethylene glycolene, or optionally substituted C_1-C_{10} heteroalkylene, or a chemical bond linking $A^1\text{-}(B^1)_f\text{-}(C^1)_g\text{-}(B^2)_h$- to -$(B^3)_i\text{-}(C^2)_j\text{-}(B^4)_k\text{–}A^2$.

[84] The conjugate, or salt thereof, of any one of paragraphs [80] to [83], wherein the monovalent organic moiety is a protein.

[85] The conjugate, or salt thereof, of paragraph [84], wherein the protein is a Ras protein.

[86] The conjugate, or salt thereof, of paragraph [85], wherein the Ras protein is K-Ras G12D or K-Ras G13D.

[87] The conjugate, or salt thereof, of any one of paragraphs [80] to [86], wherein the linker is bound to the monovalent organic moiety through a bond to a carboxyl group of an amino acid residue of the monovalent organic moiety.

Examples

The disclosure is further illustrated by the following examples and synthesis examples, which are not to be construed as limiting this disclosure in scope or spirit to the specific procedures herein described. It is to be understood that the examples are provided to illustrate certain embodiments and that no limitation to the scope of the disclosure is intended thereby. It is to be further understood that resort may be had to various other embodiments, modifications, and equivalents thereof which may suggest themselves to those skilled in the art without departing from the spirit of the present disclosure or scope of the appended claims.

Chemical Syntheses

Definitions used in the following examples and elsewhere herein are:

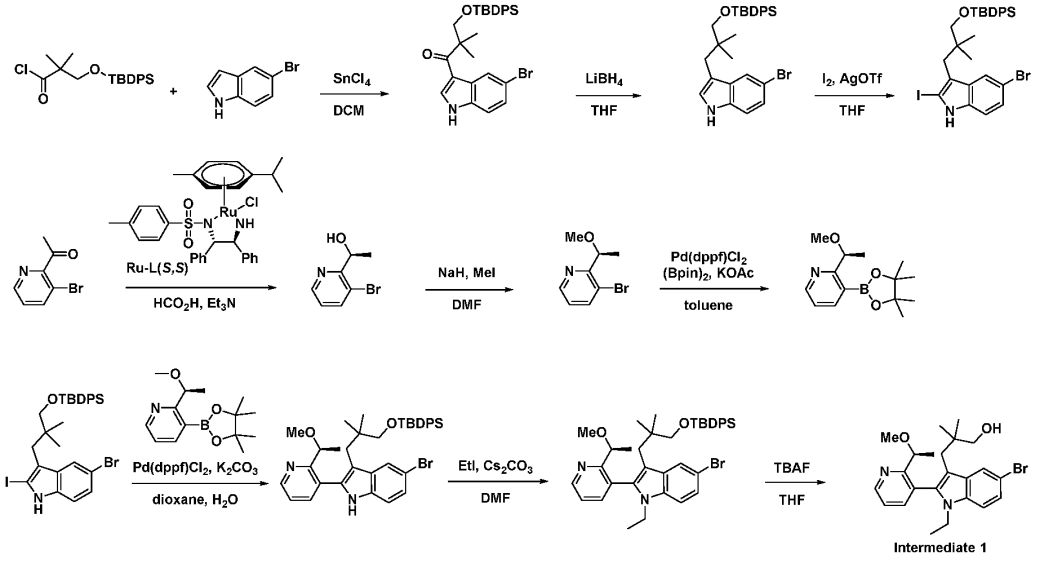
CH_2Cl_2, DCM	Methylene chloride, Dichloromethane
CH_3CN, MeCN	Acetonitrile
CuI	Copper (I) iodide
DIPEA	Diisopropylethyl amine
DMF	*N*,*N*-Dimethylformamide
EtOAc	Ethyl acetate
h	hour
H_2O	Water
HCl	Hydrochloric acid
K_3PO_4	Potassium phosphate (tribasic)
MeOH	Methanol
Na_2SO_4	Sodium sulfate
NMP	*N*-methyl pyrrolidone
$Pd(dppf)Cl_2$	[1,1′-Bis(diphenylphosphino)ferrocene]dichloropalladium(II)

Instrumentation

Mass spectrometry data collection took place with a Shimadzu LCMS-2020 or Waters Acquity UPLC with either a QDa detector or SQ Detector 2. Samples were injected in their liquid phase onto a C-18 reverse phase column to remove assay buffer and prepare the samples for the mass spectrometer. The compounds were eluted from the column using an acetonitrile gradient and fed into the mass analyzer. Initial data analysis took place with either Shimadzu LabSolutions or Waters MassLynx. NMR data was collected with either a Bruker AVANCE III HD 400MHz or a Bruker Ascend 500MHz instrument and the raw data was analyzed with either TopSpin or Mestrelab Mnova.

Synthesis of Intermediates

Intermediate 1. Synthesis of 3-(5-bromo-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropan-1-ol



Step 1: Synthesis of 1-(5-bromo-1*H*-indol-3-yl)-3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one

To a mixture of 3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropanoyl chloride (65 g, 137 mmol, crude) in DCM (120 mL) at 0 °C under an atmosphere of N_2 was added 1M $SnCl_4$ in DCM (137 mL,
137 mmol) slowly. The mixture was stirred at 0 °C for 30 min, then a solution of 5-bromo-1*H*-indole (26.8 g, 137 mmol) in DCM (40 mL) was added dropwise. The mixture was stirred at 0 °C for 45 min, then diluted with EtOAc (300 mL), washed with brine (4 x 100 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 1-(5-bromo-1*H*-indol-3-yl)-3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one (55 g, 75% yield). LCMS (ESI) *m/z*: [M + Na] calcd for $C_{29}H_{32}BrNO_2SiNa$ 556.1; found 556.3.

Step 2: Synthesis of 1-(5-bromo-1*H*-indol-3-yl)-3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one

To a mixture of 1-(5-bromo-1*H*-indol-3-yl)-3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one (50 g, 93.6 mmol) in THF (100 mL) at 0 °C under an atmosphere of N_2 was added $LiBH_4$ (6.1 g,
281 mmol). The mixture was heated to 60 °C and stirred for 20 h, then MeOH (10 mL) and EtOAc (100 mL) were added and the mixture washed with brine (50 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The residue was diluted with DCM (50 mL), cooled to 10 °C and diludine (9.5 g, 37.4 mmol) and $TsOH\bullet H_2O$ (890 mg, 4.7 mmol) were added. The mixture was stirred at 10 °C for 2 h, filtered, the filtrate concentrated under reduced pressure

and the residue was purified by silica gel column chromatography to give 1-(5-bromo-1*H*-indol-3-yl)-3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one (41 g, 84% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{29}H_{34}BrNOSi$: 519.2; found 520.1; ^1H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.75 - 7.68 (m, 5H), 7.46 - 7.35 (m, 6H), 7.23 - 7.19 (m, 2H), 6.87 (d, *J* = 2.1 Hz, 1H), 3.40 (s, 2H), 2.72 (s, 2H), 1.14 (s, 9H), 0.89 (s, 6H).

Step 3: Synthesis of 5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-iodo-1*H*-indole

To a mixture of 1-(5-bromo-1*H*-indol-3-yl)-3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one (1.5 g, 2.9 mmol) and I_2 (731 mg, 2.9 mmol) in THF (15 mL) at room temperature was added AgOTf (888 mg, 3.5 mmol). The mixture was stirred at room temperature for 2 h, then diluted with EtOAc (200 mL) and washed with saturated $Na_2S_2O_3$ (100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-iodo-1*H*-indole (900 mg, 72% yield) as a solid. ^1H NMR (400 MHz, DMSO-d_6) δ 11.70 (s, 1H), 7.68 (d, *J* = 1.3 Hz, 1H), 7.64 - 7.62 (m, 4H), 7.46 - 7.43 (m, 6H), 7.24 - 7.22 (d, 1H), 7.14 - 7.12 (dd, *J* = 8.6, 1.6 Hz, 1H), 3.48 (s, 2H), 2.63 (s, 2H), 1.08 (s, 9H), 0.88 (s, 6H).

Step 4: Synthesis of (1*S*)-1-(3-bromopyridin-2-yl)ethanol

To a stirred mixture of HCOOH (66.3 g, 1.44 mol) in Et_3N (1002 mL, 7.2 mol) at 0 °C under an atmosphere of Ar was added (4*S*,5*S*)-2-chloro-2-methyl-1-(4-methylbenzenesulfonyl)-4,5-diphenyl-1,3-diaza-2-ruthenacyclopentane cymene (3.9 g, 6.0 mmol) portion-wise. The mixture was heated to 40 °C and stirred for 15 min, then cooled to room temperature and 1-(3-bromopyridin-2-yl)ethanone (120 g,
600 mmol) added in portions. The mixture was heated to 40 °C and stirred for an additional 2 h, then the solvent was concentrated under reduced pressure. Brine (2 L) was added to the residue, the mixture was extracted with EtOAc (4 x 700 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (1*S*)-1-(3-bromopyridin-2-yl)ethanol (100 g, 74% yield) a an oil. LCMS (ESI) *m/z*: [M + H] calcd for C_7H_8BrNO: 201.98; found 201.9.

Step 5: Synthesis of 3-bromo-2-[(1*S*)-1-methoxyethyl]pyridine

To a stirred mixture of (1*S*)-1-(3-bromopyridin-2-yl)ethanol (100 g, 495 mmol) in DMF (1 L) at
0 °C was added NaH, 60% dispersion in oil (14.25 g, 594 mmol) in portions. The mixture was stirred at 0 °C for 1 h. MeI (140.5 g, 990 mmol) was added dropwise at 0 °C and the mixture was allowed to warm to room temperature and stirred for 2 h. The mixture was cooled to 0 °C and saturated NH_4Cl (5 L) was added. The mixture was extracted with EtOAc (3 x 1.5 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-bromo-2-[(1*S*)-1-methoxyethyl]pyridine (90 g, 75% yield) as an oil. LCMS (ESI) *m/z*: [M + H] calcd for $C_8H_{10}BrNO$: 215.99; found 215.9.

Step 6: Synthesis of 2-[(1*S*)-1-methoxyethyl]-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine

To a stirred mixture of 3-bromo-2-[(1*S*)-1-methoxyethyl]pyridine (90 g, 417 mmol) in toluene (900 mL) at room temperature under an atmosphere of Ar was added bis(pinacolato)diboron (127 g,
500 mmol) and KOAc (81.8 g, 833 mmol) and $Pd(dppf)Cl_2$ (30.5 g, 41.7 mmol). The mixture was heated to
100 °C and stirred for 3 h. The filtrate was concentrated under reduced pressure and the residue was purified by Al_2O_3 column chromatography to give 2-[(1*S*)-1-methoxyethyl]-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (100 g, 63% yield) as a semi-solid. LCMS (ESI) *m/z*: [M + H] calcd for $C_{14}H_{22}BNO_3$: 264.17; found 264.1.

Step 7: Synthesis of 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-1*H*-indole

To a stirred mixture of 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-2-iodo-1*H*-indole (140 g, 217 mmol) and 2-[(1*S*)-1-methoxyethyl]-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (100 g, 380 mmol) in 1,4-dioxane (1.4 L) at room temperature under an atmosphere of Ar was added K_2CO_3 (74.8 g, 541 mmol), $Pd(dppf)Cl_2$ (15.9 g, 21.7 mmol) and H_2O (280 mL) in portions. The mixture was heated to 85 °C and stirred for 4 h, then cooled, H_2O (5 L) added and the mixture extracted with EtOAc (3 x 2 L). The combined organic layers were washed with brine (2 x 1 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-1*H*-indole (71 g, 45% yield) as a solid. LCMS (ESI) *m/z*: [M + H] calcd for $C_{37}H_{43}BrN_2O_2Si$: 655.23; found 655.1.

Step 8: Synthesis of 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indole

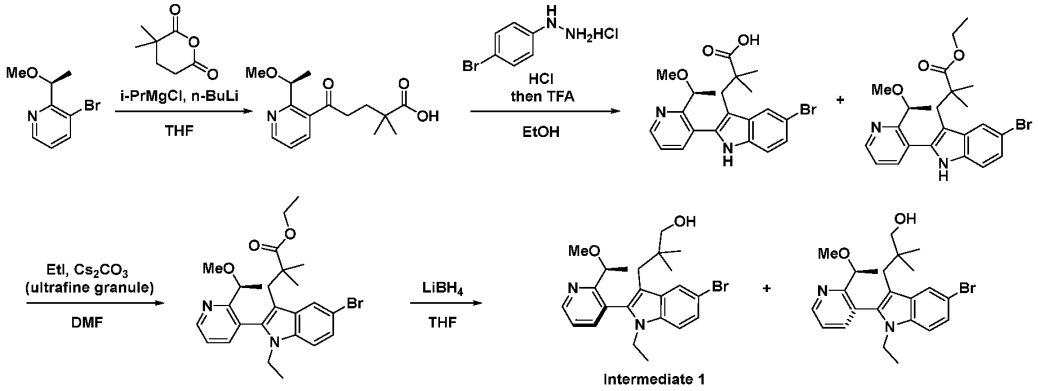
To a stirred mixture of 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-1*H*-indole (71 g, 108 mmol) in DMF (0.8 L) at 0 °C under an atmosphere of N_2 was added Cs_2CO_3 (70.6 g, 217 mmol) and EtI (33.8 g, 217 mmol) in portions. The mixture was warmed to room temperature and stirred for 16 h then H_2O (4 L) added and the mixture extracted with EtOAc (3 x 1.5 L). The combined organic layers were washed with brine (2 x 1 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indole (66 g, 80% yield) as an oil. LCMS (ESI) *m/z*: [M + H] calcd for $C_{39}H_{47}BrN_2O_2Si$: 683.26; found 683.3.

Step 9: Synthesis of 3-(5-bromo-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropan-1-ol

To a stirred mixture of TBAF (172.6 g, 660 mmol) in THF (660 mL) at room temperature under an atmosphere of N_2 was added 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-

dimethylpropyl]-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indole (66 g, 97 mmol) in portions. The mixture was heated to 50 °C and stirred for 16 h, cooled, diluted with H_2O (5 L) and extracted with EtOAc (3 x 1.5 L). The combined organic layers were washed with brine (2 x 1 L), dried over anhydrous Na_2SO_4 and filtered. After filtration, the filtrate was concentrated under reduced pressure. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-(5-bromo-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropan-1-ol (30 g, 62% yield) as a solid. LCMS (ESI) *m/z*: [M + H] calcd for $C_{23}H_{29}BrN_2O_2$: 445.14; found 445.1.

Intermediate 1. Alternative Synthesis through Fisher Indole Route.



Step 1: Synthesis of 5-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-2,2-dimethyl-5-oxopentanoic acid

To a mixture of *i*-PrMgCl (2M in in THF, 0.5 L) at -10 °C under an atmosphere of N_2 was added *n*-BuLi, 2.5 M in hexane (333 mL, 833 mmol) dropwise over 15 min. The mixture was stirred for 30 min at -10 °C then 3-bromo-2-[(1*S*)-1-methoxyethyl]pyridine (180 g, 833 mmol) in THF (0.5 L) added dropwise over 30 min at -10 °C. The resulting mixture was warmed to -5 °C and stirred for 1 h, then 3,3-dimethyloxane-2,6-dione (118 g, 833 mmol) in THF (1.2 L) was added dropwise over 30 min at -5 °C. The mixture was warmed to 0 °C and stirred for 1.5 h, then quenched with the addition of pre-cooled 4M HCl in 1,4-dioxane (0.6 L) at 0 °C to adjust pH ~5. The mixture was diluted with H_2O (3 L) at 0 °C and extracted with EtOAc (3 x 2.5 L). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 5-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-2,2-dimethyl-5-oxopentanoic acid (87 g, 34% yield) as a solid. LCMS (ESI) *m/z*: [M + H] calcd for $C_{15}H_{21}NO_4$: 280.15; found 280.1.

Step 2: Synthesis of 3-(5-bromo-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-1*H*-indol-3-yl)-2,2-dimethylpropanoic acid and ethyl (*S*)-3-(5-bromo-2-(2-(1-methoxyethyl)pyridin-3-yl)-1*H*-indol-3-yl)-2,2-dimethylpropanoate

To a mixture of 5-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-2,2-dimethyl-5-oxopentanoic acid (78 g, 279 mmol) in EtOH (0.78 L) at room temperature under an atmosphere of N_2 was added (4-bromophenyl)hydrazine HCl salt (68.7 g, 307 mmol) in portions. The mixture was heated to 85 °C

and stirred for 2 h, cooled to room temperature, then 4M HCl in 1,4-dioxane (69.8 mL, 279 mmol) added dropwise. The mixture was heated to 85 °C and stirred for an additional 3 h, then concentrated under reduced pressure and the residue was dissolved in TFA (0.78 L). The mixture was heated to 60 °C and stirred for 1.5 h, concentrated under reduced pressure and the residue adjusted to pH ~5 with saturated $NaHCO_3$, then extracted with EtOAc (3 x 1.5 L). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-(5-bromo-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-1*H*-indol-3-yl)-2,2-dimethylpropanoic acid and ethyl (*S*)-3-(5-bromo-2-(2-(1-methoxyethyl)pyridin-3-yl)-1*H*-indol-3-yl)-2,2-dimethylpropanoate (78 g, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{21}H_{23}BrN_2O_3$: 430.1 and $C_{23}H_{27}BrN_2O_3$: 459.12; found 431.1 (carboxylic acid) and 459.1.

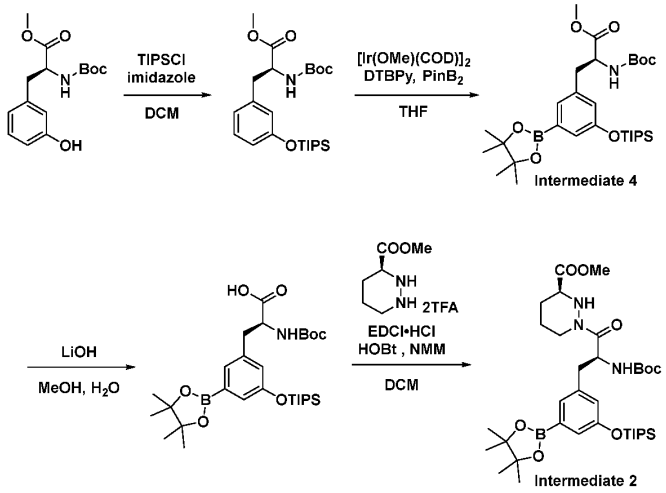
Step 3: Synthesis of ethyl 3-(5-bromo-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropanoate

To a mixture of 3-(5-bromo-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-1*H*-indol-3-yl)-2,2-dimethylpropanoic acid and ethyl (*S*)-3-(5-bromo-2-(2-(1-methoxyethyl)pyridin-3-yl)-1*H*-indol-3-yl)-2,2-dimethylpropanoate (198 g, 459 mmol) in DMF (1.8 L) at 0 °C under an atmosphere of N_2 was added Cs_2CO_3 (449 g, 1.38 mol) in portions. EtI (215 g, 1.38 mmol) in DMF (200 mL) was then added dropwise at 0 °C. The mixture was warmed to room temperature and stirred for 4 h then diluted with brine (5 L) and extracted with EtOAc (3 x 2.5 L). The combined organic layers were washed with brine (2 x 1.5 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give ethyl 3-(5-bromo-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropanoate (160 g, 57% yield) as a solid. LCMS (ESI) *m/z*: [M + H] calcd for $C_{25}H_{31}BrN_2O_3$: 487.17; found 487.2.

Step 4: Synthesis of 3-(5-bromo-1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol

To a mixture of ethyl 3-(5-bromo-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropanoate (160 g, 328 mmol) in THF (1.6 L) at 0 °C under an atmosphere of N_2 was added $LiBH_4$ (28.6 g, 1.3 mol). The mixture was heated to 60 °C for 16 h, cooled, and quenched with pre-cooled (0 °C) aqueous NH_4Cl (5 L). The mixture was extracted with EtOAc (3 x 2 L) and the combined organic layers were washed with brine (2 x 1 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give to two atropisomers of 3-(5-bromo-1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (as single atropisomers) (60 g, 38% yield) and (40 g, 26% yield) both as solids. LCMS (ESI) *m/z*: [M + H] calcd for $C_{23}H_{29}BrN_2O_2$: 445.14; found 445.2.

Intermediate 2 and Intermediate 4. Synthesis of (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)propanoyl)hexahydropyridazine-3-carboxylate



Step 1: Synthesis of (*S*)-methyl 2-(*tert*-butoxycarbonylamino)-3-(3-(triisopropylsilyloxy)phenyl)-propanoate

To a mixture of (*S*)-methyl 2-(*tert*-butoxycarbonylamino)-3-(3-hydroxyphenyl)propanoate (10.0 g, 33.9 mmol) in DCM (100 mL) was added imidazole (4.6 g, 67.8 mmol) and TIPSCl (7.8 g, 40.7 mmol). The mixture was stirred at room temperature overnight then diluted with DCM (200 mL) and washed with H_2O (3 x 150 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (*S*)-methyl 2-(*tert*-butoxycarbonylamino)-3-(3-(triisopropylsilyloxy)phenyl)-propanoate (15.0 g, 98% yield) as an oil. LCMS (ESI) *m/z*: [M + Na] calcd for $C_{24}H_{41}NO_5SiNa$: 474.22; found 474.2.

Step 2: Synthesis of (*S*)-methyl 2-(*tert*-butoxycarbonylamino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-(triisopropylsilyloxy)phenyl)-propanoate

A mixture of (*S*)-methyl 2-(*tert*-butoxycarbonylamino)-3-(3-(triisopropylsilyloxy)phenyl)-propanoate (7.5 g, 16.6 mmol), $PinB_2$ (6.3 g, 24.9 mmol), $[Ir(OMe)(COD)]_2$ (1.1 g, 1.7 mmol) and 4-*tert*-butyl-2-(4-*tert*-butyl-2-pyridyl)pyridine (1.3 g, 5.0 mmol) was purged with Ar, then THF (75 mL) was added and the mixture placed under an atmosphere of Ar and sealed. The mixture was heated to 80 °C and stirred for 16 h, concentrated under reduced pressure, and the residue was purified by silica gel column chromatography to give (*S*)-methyl 2-(*tert*-butoxycarbonylamino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-(triisopropylsilyloxy)phenyl)-propanoate (7.5 g, 78% yield) as a solid. LCMS (ESI) *m/z*: [M + Na] calcd for $C_{30}H_{52}BNO_7SiNa$: 600.35; found 600.4; ^1H NMR (300 MHz, CD_3OD) δ 7.18 (s, 1H), 7.11 (s, 1H), 6.85 (s, 1H), 4.34 (m, 1H), 3.68 (s, 3H), 3.08 (m, 1H), 2.86 (m, 1H), 1.41 - 1.20 (m, 26H), 1.20 - 1.01 (m, 22H), 0.98 - 0.79 (m, 4H).

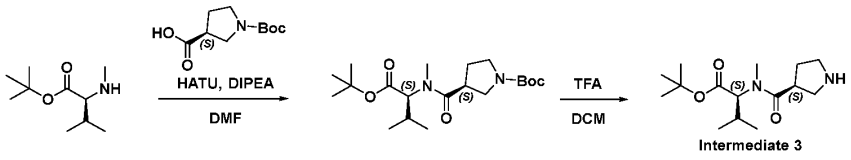
Step 3: Synthesis of (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)propanoic acid

To a mixture of triisopropylsilyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)propanoate (4.95 g, 6.9 mmol) in MeOH (53 mL) at 0 °C was added LiOH (840 mg, 34.4 mmol) in H_2O (35 mL). The mixture was stirred at 0 °C for 2 h, then acidified to pH ~5 with 1M HCl and extracted with EtOAc (2 x 250 mL). The combined organic layers were washed with brine (3 x 100 mL), dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to give (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)propanoic acid (3.7 g, 95% yield), which was used directly in the next step without further purification. LCMS (ESI) *m/z*: [M + NH_4] calcd for $C_{29}H_{50}BNO_7SiNH_4$: 581.38; found 581.4.

Step 4: Synthesis of methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)propanoyl)hexahydropyridazine-3-carboxylate

To a mixture of methyl (*S*)-hexahydropyridazine-3-carboxylate (6.48 g, 45.0 mmol) in DCM (200 mL) at 0 °C was added NMM (41.0 g, 405 mmol), (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)propanoic acid (24 g, 42.6 mmol) in DCM (50 mL) then HOBt (1.21 g, 9.0 mmol) and EDCI HCl salt (12.9 g, 67.6 mmol). The mixture was warmed to room temperature and stirred for 16 h, then diluted with DCM (200 mL) and washed with H_2O (3 x 150 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, the filtrate concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)propanoyl)hexahydropyridazine-3-carboxylate (22 g, 71% yield) as an oil. LCMS (ESI) *m/z*: [M + H] calcd for $C_{35}H_{60}BN_3O_8Si$: 690.42; found 690.5.

Intermediate 3. Synthesis of (*S*)-*tert*-butyl 3-methyl-2-((*S*)-N-methylpyrrolidine-3-carboxamido)butanoate



Step 1: Synthesis of (*S*)-*tert*-butyl 3-(((*S*)-1-(*tert*-butoxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)pyrrolidine-1-carboxylate

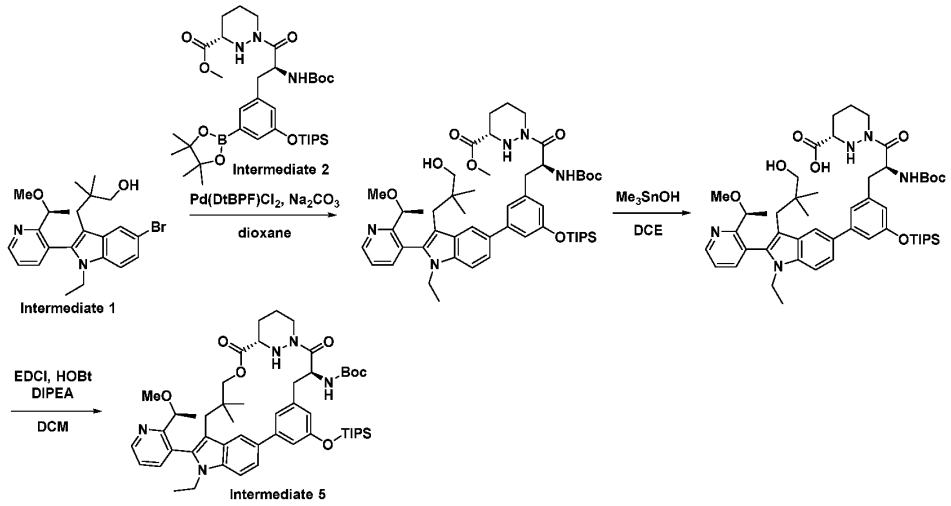
To a mixture of (*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-3-carboxylic acid (2.2 g, 10.2 mmol) in DMF (10 mL) at room temperature was added HATU (7.8 g, 20.4 mmol) and DIPEA (5 mL). After stirring at room temperature for 10 min, *tert*-butyl methyl-*L*-valinate (3.8g, 20.4 mmol) in DMF (10 mL) was added. The mixture was stirred at room temperature for 3 h, then diluted with DCM (40

mL) and H_2O (30 mL). The aqueous and organic layers were separated, and the organic layer was washed with H_2O (3 x 30 mL), brine (30 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (*S*)-*tert*-butyl 3-(((*S*)-1-(*tert*-butoxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)pyrrolidine-1-carboxylate (3.2 g, 82% yield) as an oil. LCMS (ESI) *m/z*: [M + Na] calcd for $C_{20}H_{36}N_2O_5Na$: 407.25; found 407.2.

Step 2: Synthesis of (*S*)-*tert*-butyl 3-methyl-2-((*S*)-*N*-methylpyrrolidine-3-carboxamido)butanoate

A mixture of (*S*)-*tert*-butyl 3-(((*S*)-1-(*tert*-butoxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)pyrrolidine-1-carboxylate (3.2 g, 8.4 mmol) in DCM (13 mL) and TFA (1.05 g, 9.2 mmol) was stirred at room temperature for 5 h. The mixture was concentrated under reduced pressure to give (*S*)-*tert*-butyl 3-methyl-2-((*S*)-*N*-methylpyrrolidine-3-carboxamido)butanoate (2.0 g, 84% yield) as an oil. LCMS (ESI) *m/z*: [M + H] calcd for $C_{15}H_{28}N_2O_3$: 285.21; found 285.2.

Intermediate 5. Synthesis of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate.



Step 1: Synthesis of methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate

To a stirred mixture of 3-(5-bromo-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropan-1-ol (30 g, 67 mmol) and methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate (55.8 g, 80.8 mmol) in 1,4-dioxane (750 mL) at room temperature under an atmosphere of Ar was added Na_2CO_3 (17.9 g, 168.4 mmol), Pd(DtBPF)Cl_2 (4.39 g, 6.7 mmol), and

H_2O (150 mL) in portions. The mixture was heated to 85 °C and stirred for 3 h, cooled, diluted with H_2O (2 L), and extracted with EtOAc (3 x 1 L). The combined organic layers were washed with brine (2 x 500 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate (50 g, 72% yield) as a solid. LCMS (ESI) *m/z*: [M + H] calcd for $C_{52}H_{77}N_5O_8Si$: 928.56; found 928.8.

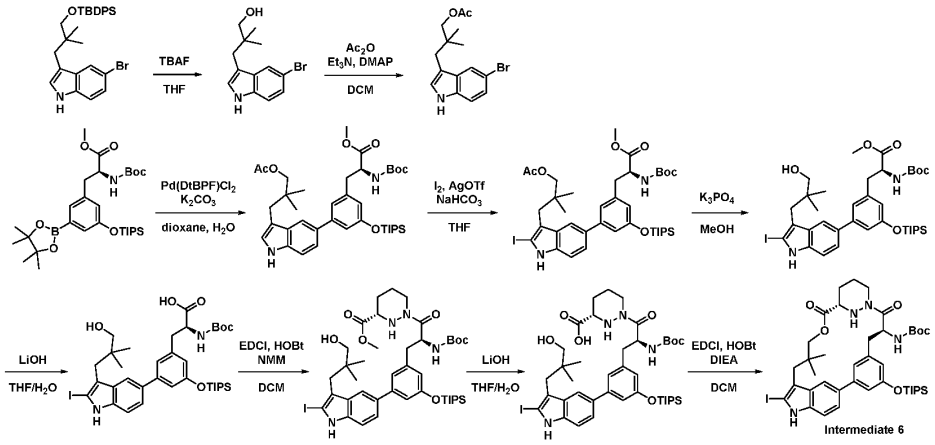
Step 2: Synthesis of (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid

To a stirred mixture of methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate (50 g, 54 mmol) in DCE (500 mL) at room temperature was added trimethyltin hydroxide (48.7 g, 269 mmol) in portions. The mixture was heated to 65 °C and stirred for 16 h, then filtered and the filter cake washed with DCM (3 x 150 mL). The filtrate was concentrated under reduced pressure to give (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid (70 g, crude), which was used directly in the next step without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{51}H_{75}N_5O_8Si$: 914.55; found 914.6.

Step 3: Synthesis of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate

To a stirred mixture of (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid (70 g) in DCM (5 L) at 0 °C under an atmosphere of N_2 was added DIPEA (400 mL, 2.3 mol), HOBT (51.7 g, 383 mmol) and EDCI (411 g, 2.1 mol) in portions. The mixture was warmed to room temperature and stirred for 16 h, then diluted with DCM (1 L), washed with brine (3 x 1 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (36 g, 42% yield) as a solid. LCMS (ESI) *m/z*: [M + H] calcd for $C_{51}H_{73}N_5O_7Si$: 896.54; found 896.5.

Intermediate 6. Synthesis of *tert*-butyl ((6^3*S*,4*S*)-1^2-iodo-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1-*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate.



Step 1: Synthesis of 3-(5-bromo-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol

This reaction was undertaken on five batches in parallel on the scale illustrated below. Into a 2L round-bottom flask were added 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1*H*-indole (100 g, 192 mmol) and TBAF (301.4 g, 1.15 mol) in THF (1.15 L) at room temperature. The resulting mixture was heated to 50 °C and stirred for 16 h, then the mixture was concentrated under reduced pressure.

At this stage the residues from all five batches were combined, diluted with H_2O (5 L), and extracted with EtOAc (3 x 2 L). The combined organic layers were washed with brine (2 x 1.5 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-(5-bromo-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (310 g, crude) as a solid. LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{16}BrNO$: 282.05 and 284.05; found 282.1 and 284.1.

Step 2: Synthesis of 3-(5-bromo-1*H*-indol-3-yl)-2,2-dimethylpropyl acetate

This reaction was undertaken on two batches in parallel in accordance with the procedure below. To a stirred mixture of 3-(5-bromo-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (135 g, 478 mmol) and Et_3N (200 mL, 1.44 mol) in DCM (1.3 L) at 0 °C under an atmosphere of N_2 was added Ac_2O (73.3 g, 718 mmol) and DMAP (4.68 g, 38.3 mmol) in portions. The resulting mixture was stirred for 10 min at 0 °C, then washed with H_2O (3 x 2 L).

At this stage, the organic layers from both batches were combined and washed with brine (2 x 1 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography to give 3-(5-bromo-1*H*-indol-3-yl)-2,2-dimethylpropyl acetate (304 g, 88% yield) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.16 - 11.11 (m, 1H), 7.69 (d, *J* = 2.0 Hz, 1H), 7.32 (d, *J* = 8.6 Hz, 1H), 7.19 - 7.12 (m, 2H), 3.69 (s, 2H), 2.64 (s, 2H), 2.09 (s, 3H), 0.90 (s, 6H).

Step 3: Synthesis of methyl (2*S*)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoate

This reaction was undertaken on four batches in parallel in accordance with the procedure below. Into a 2L round-bottom flasks were added methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-[(triisopropylsilyl)oxy]phenyl]propanoate (125 g, 216

mmol), 1,4-dioxane (1 L), H_2O (200 mL), 3-(5-bromo-1*H*-indol-3-yl)-2,2-dimethylpropyl acetate (73.7 g, 227 mmol), K_2CO_3 (59.8 g, 433 mmol), and Pd(DtBPF)Cl_2 (7.05 g, 10.8 mmol) at room temperature under an atmosphere of Ar. The resulting mixture was heated to 65 °C and stirred for 2 h, then diluted with H_2O (10 L) and extracted with EtOAc (3 x 3 L). The combined organic layers were washed with brine (2 x 2 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure.

At this point the residue from all four batches was combined and purified by column chromatography to give methyl (2*S*)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (500 g, 74% yield) as an oil. LCMS (ESI) *m/z*: [M + Na] calcd for $C_{39}H_{58}N_2O_7SiNa$: 717.39; found 717.3.

Step 4: Synthesis of methyl (2*S*)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoate

This reaction was undertaken on three batches in parallel in accordance with the procedure below. To a stirred mixture of methyl (2*S*)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (150 g, 216 mmol) and $NaHCO_3$ (21.76 g, 259 mmol) in THF (1.5 L) was added AgOTf (66.5 g, 259 mmol) in THF dropwise at 0 °C under an atmosphere of nitrogen. I_2 (49.3 g, 194 mmol) in THF was added dropwise over 1 h at 0 °C and the resulting mixture was stirred for an additional 10 min at 0 °C. The combined experiments were diluted with aqueous $Na_2S_2O_3$ (5 L) and extracted with EtOAc (3 x 3 L). The combined organic layers were washed with brine (2 x 1.5 L), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to a residue.

At this stage, the residue from all three batches was combined and purified by column chromatography to give methyl (2*S*)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (420 g, 71% yield) as an oil. LCMS (ESI) *m/z*: [M + Na] calcd for $C_{39}H_{57}IN_2O_7SiNa$: 843.29; found 842.9.

Step 5: Synthesis of methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoate

This reaction was undertaken on three batches in parallel in accordance with the procedure below. To a 2L round-bottom flask were added methyl (2*S*)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (140 g,
171 mmol), MeOH (1.4 L), and K_3PO_4 (108.6 g, 512 mmol) at 0 °C. The mixture was warmed to room temperature and stirred for 1 h, then the combined experiments were diluted with H_2O (9 L) and extracted with EtOAc (3 x 3 L). The combined organic layers were washed with brine (2 x 2 L), dried over anhydrous Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure.

At this stage the residue from all three batches was combined to give methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-

[(triisopropylsilyl)oxy]phenyl]propanoate (438 g, crude) as a solid. LCMS (ESI) *m/z*: [M + Na] calcd for $C_{37}H_{55}IN_2O_6SiNa$: 801.28; found 801.6.

Step 6: Synthesis of (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoic acid

This reaction was undertaken on three batches in parallel in accordance with the procedure below. To a stirred mixture of methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoate (146 g, 188 mmol) in THF (1.46 L) was added LiOH (22.45 g, 937 mmol) in H_2O (937 mL) dropwise at 0 °C. The resulting mixture was warmed to room temperature and stirred for 1.5 h [note: LCMS showed 15% de-TIPS product]. The mixture was acidified to pH 5 with 1M HCl (1M) and the combined experiments were extracted with EtOAc (3 x 3 L). The combined organic layers were washed with brine (2 x 2 L), dried over anhydrous Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure.

At this stage the residue from all three batches was combined to give (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoic acid (402 g, crude) as a solid. LCMS (ESI) *m/z*: [M + Na] calcd for $C_{36}H_{53}IN_2O_6SiNa$: 787.26; found 787.6.

Step 7: Synthesis of methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate

To a stirred mixture of (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoic acid (340 g, 445 mmol) and methyl (3*S*)-1,2-diazinane-3-carboxylate (96.1 g, 667 mmol) in DCM (3.5 L) was added NMM (225 g, 2.2 mol), EDCI (170 g, 889 mmol), and HOBt (12.0 g, 88.9 mmol) portionwise at 0 °C. The mixture was warmed to room temperature and stirred for 16 h, then washed with H_2O (3 x 2.5 L), brine (2 x 1 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography to give methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate (310 g, 62% yield) as an oil. LCMS (ESI) *m/z*: [M + H] calcd for $C_{42}H_{63}IN_4O_7Si$: 891.36; found 890.8.

Step 8: Synthesis of (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid

This reaction was undertaken on three batches in parallel in accordance with the procedure below. To a stirred mixture of methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-

2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate (85.0 g, 95.4 mmol) in THF (850 mL) was added LiOH (6.85 g, 286 mmol) in H_2O (410 mL) dropwise at 0 °C under an atmosphere of N_2. The mixture was stirred at 0 °C for 1.5 h [note: LCMS showed 15% de-TIPS product], then acidified to pH 5 with 1M HCl

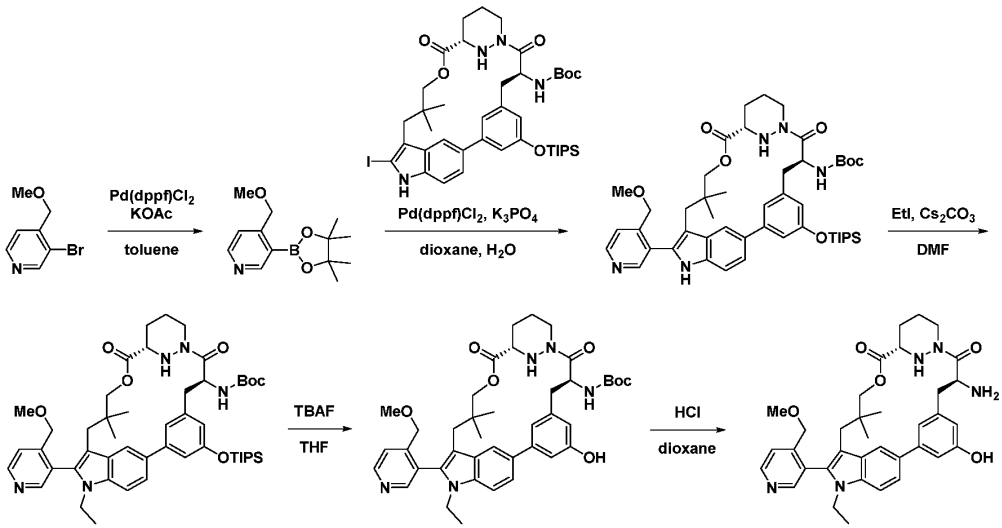
At this stage the mixtures from all three batches was combined and extracted with EtOAc (3 x 2 L). The combined organic layers were washed with brine (2 x 1.5 L), dried over anhydrous Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure to give (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid (240 g, crude) as a solid. LCMS (ESI) *m/z*: [M + H] calcd for $C_{41}H_{61}IN_4O_7Si$: 877.35; found 877.6.

Step 9: Synthesis of *tert*-butyl ((6^3*S*,4*S*)-1^2-iodo-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1-*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate

This reaction was undertaken on two batches in parallel in accordance with the procedure below. To a stirred mixture of (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid (120 g, 137 mmol) in DCM (6 L) was added DIPEA (357 mL, 2.05 mol), EDCI (394 g, 2.05 mol), and HOBT (37 g, 274 mmol) in portions at 0 °C under an atmosphere of N_2. The mixture was warmed to room temperature and stirred overnight.

At this stage the solutions from both batches were combined and washed with H_2O (3 x 6 L), brine (2 x 6 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography to give *tert*-butyl ((6^3*S*,4*S*)-1^2-iodo-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1-*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (140 g, 50% yield) as a solid. LCMS (ESI) *m/z*: [M + H] calcd for $C_{41}H_{59}IN_4O_6Si$: 859.33; found 858.3.

Intermediate 7. Synthesis of (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione



Step 1: Synthesis of 4-(methoxymethyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine

To a mixture of 3-bromo-4-(methoxymethyl)pyridine (1.0 g, 5.0 mmol), 4,4,5,5-tetramethyl-2-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (1.51 g, 5.9 mmol) and KOAc (1.21 g, 12.3 mmol) in toluene (10 mL) at room temperature under an atmosphere of Ar was added $Pd(dppf)Cl_2$
(362 mg, 0.5 mmol). The mixture was heated to 110 °C and stirred overnight, then concentrated under reduced pressure to give 4-(methoxymethyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine, which was used directly in the next step directly without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{20}BNO_3$: 250.16; found 250.3.

Step 2: Synthesis of give *tert*-butyl ((6^3*S*,4*S*)-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1-*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate

To a mixture of 4-(methoxymethyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (290 mg, 1.16 mmol), K_3PO_4 (371 mg, 1.75 mmol) and *tert*-butyl ((6^3*S*,4*S*)-1^2-iodo-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1-*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (500 mg, 0.58 mmol) in 1,4-dioxane (5 mL) and H_2O
(1 mL) at room temperature under an atmosphere of Ar was added $Pd(dppf)Cl_2$ (43 mg, 0.06 mmol). The mixture was heated to 70 °C and stirred for 2 h, then H_2O was added and the mixture extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl ((6^3*S*,4*S*)-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-

hexahydro-1^1-*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (370 mg, 74% yield) as a foam. LCMS (ESI) *m/z*: [M + H] calcd for $C_{48}H_{67}N_5O_7Si$: 854.49; found 854.6.

Step 3: Synthesis of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1-*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate

A mixture of *tert*-butyl ((6^3*S*,4*S*)-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1-*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (350 mg, 0.41 mmol), Cs_2CO_3 (267 mg, 0.82 mmol), and EtI (128 mg, 0.82 mmol) in DMF (4 mL) was stirred at 35 °C overnight. H_2O was added and the mixture was extracted with EtOAc (2 x 15 mL). The combined organic layers were washed with brine (15 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1-*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (350 mg, 97% yield) as an oil. LCMS (ESI) *m/z*: [M + H] calcd for $C_{50}H_{71}N_5O_7Si$: 882.52; found 882.6.

Step 4: Synthesis of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate

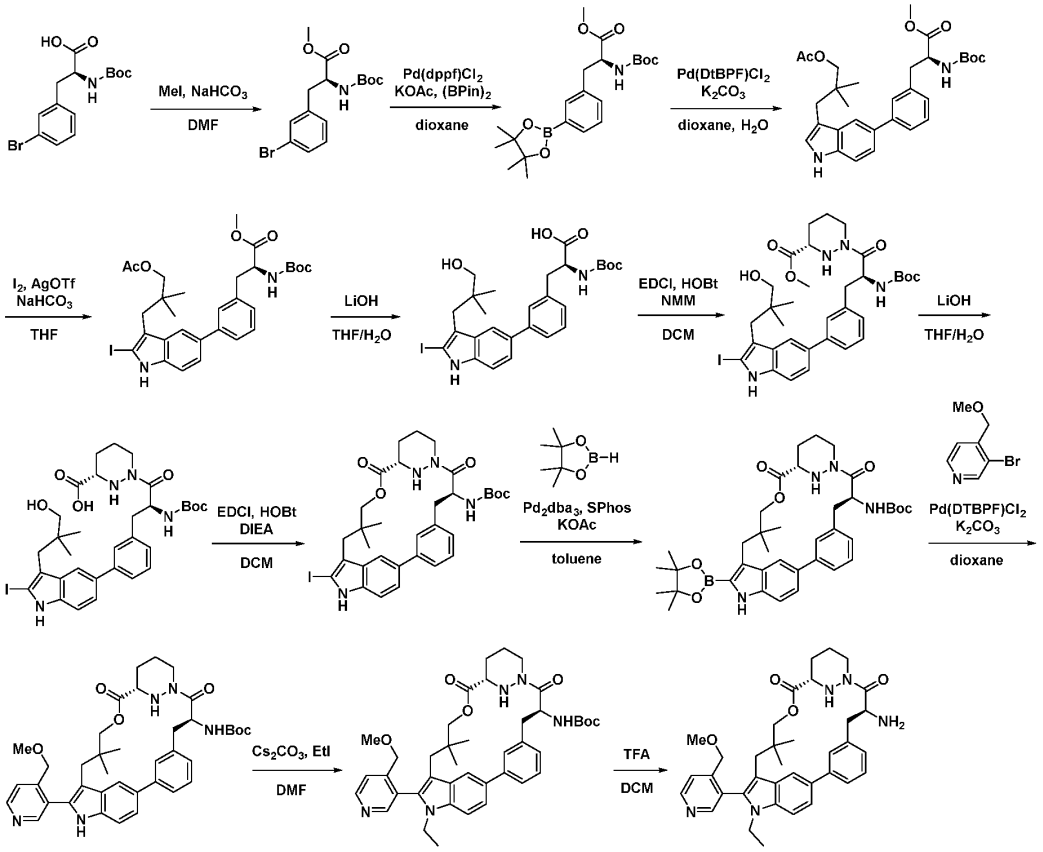
A mixture of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1-*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (350 mg, 0.4 mmol) and 1M TBAF in THF (0.48 mL, 0.480 mmol) in THF (3 mL) at 0 °C under an atmosphere of Ar was stirred for 1 h. The mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (230 mg, 80% yield) as an oil. LCMS (ESI) *m/z*: [M + H] calcd for $C_{41}H_{51}N_5O_7$: 726.39; found 726.6.

Step 5: Synthesis of (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione

To a mixture of *tert*-butyl *N*-[(8*S*,14*S*)-22-ethyl-4-hydroxy-21-[4- (methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate (200 mg, 0.28 mmol) in 1,4-dioxane (2 mL) at 0 °C under an atmosphere of Ar was added 4M HCl in 1,4-dioxane (2 mL, 8 mmol). The mixture was allowed to warm to room temperature and was stirred overnight, then concentrated under reduced pressure to give (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-

hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (200 mg). LCMS (ESI) *m/z*: [M + H] calcd for $C_{36}H_{43}N_5O_5$: 626.34; found 626.5.

Intermediate 8. Synthesis of (6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione



Step 1: Synthesis of methyl (*S*)-3-(3-bromophenyl)-2-((*tert*-butoxycarbonyl)amino)propanoate

To a solution of (2*S*)-3-(3-bromophenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoic acid (100 g, 290 mmol) in DMF (1 L) at room temperature was added $NaHCO_3$ (48.8 g, 581.1 mmol) and MeI (61.9 g, 435.8 mmol). The reaction mixture was stirred for 16 h and was then quenched with H_2O (1 L) and extracted with EtOAc (3 x 1 L). The combined organic layers were washed with brine (3 x 500 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (13% EtOAc/pet. ether) to afford the desired product (109 g, crude). LCMS (ESI) *m/z*: [M + Na] calcd for $C_{15}H_{20}BrNO_4$: 380.05; found 380.0.

Step 2: Synthesis of methyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoate

To a stirred solution of methyl (2*S*)-3-(3-bromophenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (108 g, 301.5 mmol) and bis(pinacolato)diboron (99.53 g, 391.93

mmol) in 1,4-dioxane (3.2 L) was added KOAc (73.97 g, 753.70 mmol) and Pd(dppf)Cl_2 (22.06 g, 30.15 mmol). The reaction mixture was heated to 90 °C for 3 h and was then cooled to room temperature and extracted with EtOAc (2 x 3 L). The combined organic layers were washed with brine (3 x 800 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (5% EtOAc/pet. ether) to afford the desired product (96 g, 78.6% yield). LCMS (ESI) *m/z*: [M + Na] calcd for $C_{21}H_{32}BNO_6$: 428.22; found 428.1.

Step 3: Synthesis of methyl (*S*)-3-(3-(3-(3-acetoxy-2,2-dimethylpropyl)-1H-indol-5-yl)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanoate

To a mixture of methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]propanoate (94 g, 231.9 mmol) and 3-(5-bromo-1H-indol-3-yl)-2,2-dimethylpropyl acetate (75.19 g, 231.93 mmol) in 1,4-dioxane (1.5 L) and H_2O (300 mL) was added K_2CO_3 (64.11 g, 463.85 mmol) and Pd(DtBPF)Cl_2 (15.12 g, 23.19 mmol). The reaction mixture was heated to 70 °C and stirred for 4 h. The reaction mixture was extracted with EtOAc (2 x 2 L) and the combined organic layers were washed with brine (3 x 600 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (20% EtOAc/pet. ether) to afford the desired product (130 g, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{30}H_{38}N_2O_6$: 523.28; found 523.1.

Step 4: Synthesis of methyl (*S*)-3-(3-(3-(3-acetoxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanoate

To a solution of methyl (2*S*)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-1H-indol-5-yl]phenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (95.0 g, 181.8 mmol) and iodine (36.91 g, 145.41 mmol) in THF (1 L) at -10 °C was added AgOTf (70.0 g, 272.7 mmol) and $NaHCO_3$ (22.9 g, 272.65 mmol). The reaction mixture was stirred for 30 min and was then quenched by the addition of sat. $Na_2S_2O_3$ (100 mL) at 0 °C. The resulting mixture was extracted with EtOAc (3 x 1 L) and the combined organic layers were washed with brine (3 x 500 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (50% EtOAc/pet. ether) to afford the desired product (49.3 g, 41.8% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{30}H_{37}IN_2O_6$: 649.18; found 649.1.

Step 5: Synthesis of (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl)phenyl)propanoic acid

To a solution of methyl (2*S*)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-2-iodo-1H-indol-5-yl]phenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (60 g, 92.5 mmol) in THF (600 mL) was added a solution of LiOH•H_2O (19.41 g, 462.5 mmol) in H_2O (460 mL). The resulting solution was stirred overnight and then the pH was adjusted to 6 with HCl (1 M). The resulting solution was extracted with EtOAc (2 x 500 mL) and the combined organic layers was washed with sat. brine (2 x 500 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (45 g, 82.1% yield). LCMS (ESI) *m/z*: [M + Na] calcd for $C_{27}H_{33}IN_2O_5$: 615.13; found 615.1.

Step 6: Synthesis of methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylate

To a solution of (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl]phenyl]propanoic acid (30 g, 50.6 mmol) and methyl (3*S*)-1,2-diazinane-3-carboxylate (10.9 g, 75.9 mmol) in DCM (400 mL) was added NMM (40.97 g, 405.08 mmol), HOBT (2.05 g, 15.19 mmol), and EDCI (19.41 g, 101.27 mmol). The reaction mixture was stirred overnight and then the mixture was washed with sat. NH_4Cl (2 x 200 mL) and sat. brine (2 x 200 mL), and the mixture was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (14 g, 38.5% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{33}H_{43}IN_4O_6$: 718.23; found 719.4.

Step 7: Synthesis of (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylic acid

To a solution of methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylate (92 g, 128.0 mmol) in THF (920 mL) at 0 °C was added a solution of $LiOH \bullet H_2O$ (26.86 g, 640.10 mmol) in H_2O (640 mL). The reaction mixture was stirred for 2 h and was then concentrated under reduced pressure to afford the desired product (90 g, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{32}H_{41}IN_4O_6$: 705.22; found 705.1).

Step 8: Synthesis of *tert*-butyl ((6^3*S*,4*S*)-1^2-iodo-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate

To a solution of of (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid (90 g, 127.73 mmol) in DCM (10 L) at 0 °C was added HOBt (34.52 g, 255.46 mmol), DIPEA (330.17 g, 2554.62 mmol) and EDCI (367.29 g, 1915.96 mmol). The reaction mixture was stirred for 16 h and was then concentrated under reduced pressure. The mixture was extracted with DCM (2 x 2 L) and the combined organic layers were washed with brine (3 x 1 L), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (50% EtOAc/pet. ether) to afford the desired product (70 g, 79.8% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{32}H_{39}IN_4O_5$: 687.21; found 687.1.

Step 9: Synthesis of *tert*-butyl ((6^3*S*,4*S*)-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate

To a solution of *tert*-butyl ((6^3*S*,4*S*)-1^2-iodo-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (22.0 g,
32.0 mmol) in toluene (300.0 mL) was added $Pd_2(dba)_3$ (3.52 g, 3.85 mmol), S-Phos (3.95 g, 9.61 mmol), and KOAc (9.43 g, 96.13 mmol) followed by 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (26.66 g, 208.3 mmol), dropwise. The resulting solution was heated to 60 °C and stirred for 3 h. The reaction mixture was then cooled to room temperature, filtered, the filter cake was washed with EtOAc, and the filtrate was concentrated under reduced pressure. The residue was purified by

normal phase chromatography to afford the desired product (22 g, 90% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{38}H_{51}BN_4O_7$: 687.39; found 687.3.

Step 10: Synthesis of *tert*-butyl ((6^3*S*,4*S*)-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate

To a mixture of *tert*-butyl ((6^3*S*,4*S*)-10,10-dimethyl-5,7-dioxo-12-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (3.0 g, 4.37 mmol) and 3-bromo-4-(methoxymethyl)pyridine (1.766 g, 8.74 mmol) in dioxane/H_2O (5/1) at 60 °C was added K_2CO_3 (2.415 g, 17.48 mmol) and Pd(DTBPF)Cl_2 (0.5695 g, 0.874 mmol). The reaction mixture was stirred for 4 h. The reaction mixture was cooled to room temperature and was extracted with EtOAc (300 mL). The solution was washed with brine (3 x 100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by normal phase chromatography (50% EtOAc/pet. ether) to afford the desired product (1.96 g, 65.8% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{39}H_{47}N_5O_6$: 682.36; found 682.7.

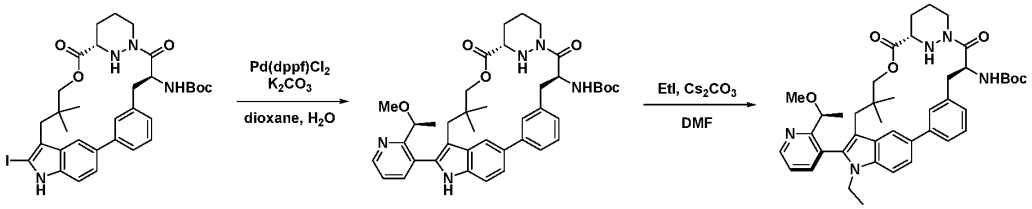
Step 11: Synthesis of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate

To a solution of *tert*-butyl ((6^3*S*,4*S*)-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (1.96 g, 2.88 mmol) and ethyl iodide (0.347 mL, 4.31 mmol) in DMF (20.0 mL) was added Cs_2CO_3 (2.342 g, 7.19 mmol). The resulting mixture was stirred at room temperature for 5 h and then diluted with EtOAc (200 mL). The mixture was washed with H_2O (3 x 100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by normal phase chromatography (50% EtOAc/pet. ether) to afford the desired product (1.24 g, 61% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{41}H_{51}N_5O_6$: 710.39; found 710.7.

Step 12: Synthesis of (6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione

To a solution of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (1.09 g, 1.54 mmol) in DCM (1.5 mL) at 0 °C was added TFA (1.50 mL). The reaction mixture was stirred for 1 h, concentrated under reduced pressure, and then azeotroped with toluene (3 x 20 mL) to afford the desired crude product (1.09 g) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{36}H_{43}N_5O_4$: 610.34; found 610.4.

Intermediate 9. Synthesis of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl) pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl) carbamate



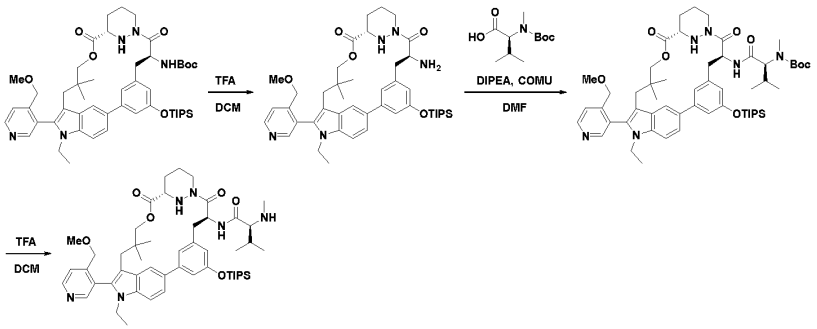
Step 1: Synthesis of *tert*-butyl ((6^3*S*,4*S*)-1^2-(2-((*S*)-1-methoxyethyl) pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl) carbamate

To a solution of *tert*-butyl ((6^3*S*,4*S*)-1^2-iodo-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (13 g, 18.93 mmol) and 2-[(1*S*)-1-methoxyethyl]-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (14.95 g, 56.8 mmol) in dioxane (130 mL) and H_2O (26 mL) was added K_2CO_3 (5.23 g, 37.9 mmol) and $Pd(dppf)Cl_2$ (1.39 g, 1.89 mmol). The reaction mixture was stirred for 4 h at 70 °C. The mixture was cooled to room temperature, filtered, and washed with EtOAc (3 x 100 mL). The filtrate was washed with brine (2 x 100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by prep-TLC (10% MeOH/DCM) to afford the desired product (21 g, 85.3% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{40}H_{49}N_5O_6$: 696.38; found 696.4.

Step 2: Synthesis of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl) pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl) carbamate

To a solution of *tert*-butyl ((6^3*S*,4*S*)-1^2-(2-((*S*)-1-methoxyethyl) pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl) carbamate (20 g, 28.7 mmol) and Cs_2CO_3 (18.7 g, 57.5 mmol) in DMF (150 mL) at 0 °C was added a solution of ethyl iodide (13.45 g, 86.22 mmol) in DMF (50 mL). The resulting mixture was stirred overnight at 35 °C and was then diluted with H_2O (500 mL). The mixture was extracted with EtOAc (2 x 300 mL) and the combined organic layers were washed with brine (3 x 100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (10%→50% EtOAc/pet. ether) to afford the desired product (4.23 g, 18.8% yield) and the atropisomer (5.78 g, 25.7% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{42}H_{53}N_5O_6$: 724.41; found 724.4.

Intermediate 10. Synthesis of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide



Step 1: Synthesis of (6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione

A mixture of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (880 mg, 1.2 mmol), DCM (10 mL), and TFA (5 mL) was stirred at 0 °C for 30 min. The mixture was concentrated under reduced pressure to afford the desired product, which was used directly in the next step without further purification. LCMS (ESI) *m/z:* [M + H] calcd for $C_{45}H_{63}N_5O_5Si$: 782.47; found 782.7.

Step 2: Synthesis of *tert*-butyl ((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-25-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate

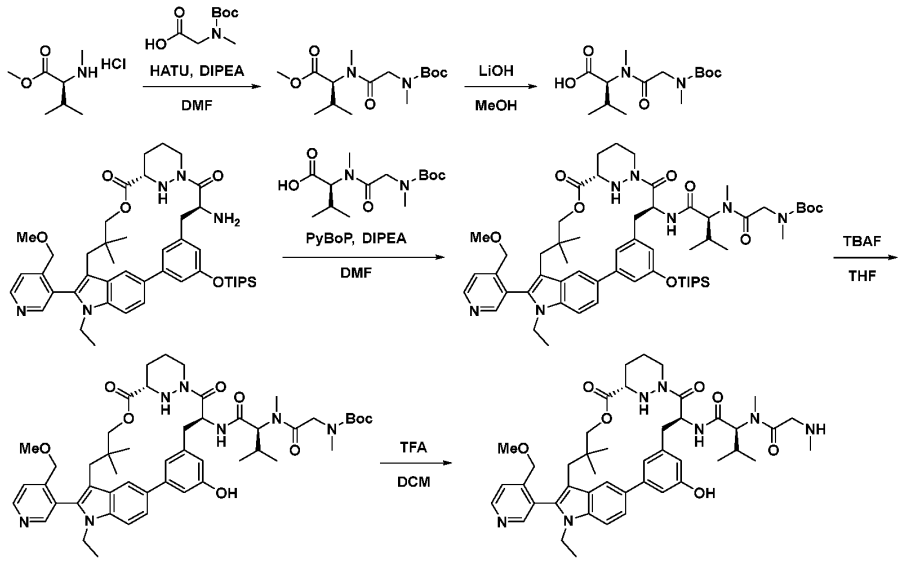
To a mixture of (6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (880 mg, 1.13 mmol) and *N*-(*tert*-butoxycarbonyl)-*N*-methyl-*L*-valine (521 mg, 2.3 mmol) in DMF (8.8 mL) at 0 °C was added DIPEA (1.95 mL, 11.3 mmol) and COMU
(88 mg, 0.21 mmol). The mixture was stirred at 0 °C for 30 min, then diluted with H_2O (100 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine (3 x 100 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by prep-TLC to afford the desired product (1 g, 89% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{56}H_{82}N_6O_8Si$: 995.61; found 995.5.

Step 3: Synthesis of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide

A mixture of *tert*-butyl ((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-25-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-

6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (1.0 g, 1.0 mmol), DCM (10 mL) and TFA (5 mL) was stirred for 30 min. The mixture was concentrated under reduced pressure and the residue was basified to pH ~8 with sat. $NaHCO_3$, then extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (3 x 10 mL), dried over Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure to afford the desired product (880 mg, 98% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{51}H_{74}N_6O_6Si$: 895.55; found 895.5.

Intermediate 11. Synthesis of (2*S*)-*N*-((63*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(*N*-methyl-2-(methylamino)acetamido)butanamide



Step 1: Synthesis of methyl *N*-(*N*-(*tert*-butoxycarbonyl)-*N*-methylglycyl)-*N*-methyl-*L*-valinate

To a solution of methyl methyl-*L*-valinate hydrochloride (2.0 g, 11.01 mmol) and *N*-(*tert*-butoxycarbonyl)-*N*-methylglycine (3.12 g, 16.51 mmol) in DMF (60.0 mL) at 0 °C was added DIPEA (9.58 mL, 55.01 mmol) and HATU (8.37 g, 22.02 mmol). The reaction mixture was stirred overnight and was then quenched with H_2O (100 mL). The mixture was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by reverse phase chromatography (40→60% MeCN/H_2O) to afford the desired product (2.9 g, 83% yield) as an oil. LCMS (ESI) *m/z*: [M + H] calcd for $C_{15}H_{28}N_2O_5$: 317.21; found 317.4.

Step 2: Synthesis of *N*-(*N*-(*tert*-butoxycarbonyl)-*N*-methylglycyl)-*N*-methyl-*L*-valine

To a solution of methyl *N*-(*N*-(*tert*-butoxycarbonyl)-*N*-methylglycyl)-*N*-methyl-*L*-valinate (3.70 g, 11.69 mmol) in THF (37.0 mL) was added a solution of LiOH•H_2O (1.96 g, 46.71 mmol) in H_2O (47.0 mL). The reaction mixture was stirred for 4 h, and then 1M HCl was added until the pH was adjusted to 5. The resulting solution was extracted with EtOAc (3 x 100 mL) and the combined

organic layers were washed with brine (3 x 50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by reverse phase chromatography (60→60% MeCN/H_2O) to afford the desired product (1.47 g, 41.6% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{14}H_{26}N_2O_5$: 303.19; found 303.4.

Step 3: Synthesis of *tert*-butyl (2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)(methyl)carbamate

To a solution of (6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (300.0 mg, 0.384 mmol) and *N*-(*N*-(*tert*-butoxycarbonyl)-*N*-methylglycyl)-*N*-methyl-*L*-valine (173.9 mg, 0.575 mmol) in DMF (3.0 mL) at 0 °C was added DIPEA (0.534 mL, 3.069 mmol) and PyBOP (399.2 mg, 0.767 mmol). The reaction mixture was stirred for 2 h and was then diluted with H_2O (30 mL). The resulting mixture was extracted with EtOAc (3 x 20 mL) and the combined organic layers were washed with brine (3 x 20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by normal phase chromatography (25% EtOAc/pet. ether) to afford the desired product (300 mg, 73% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{59}H_{87}N_7O_9Si$: 1066.64; found 1067.4.

Step 4: Synthesis of *tert*-butyl (2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)(methyl)carbamate

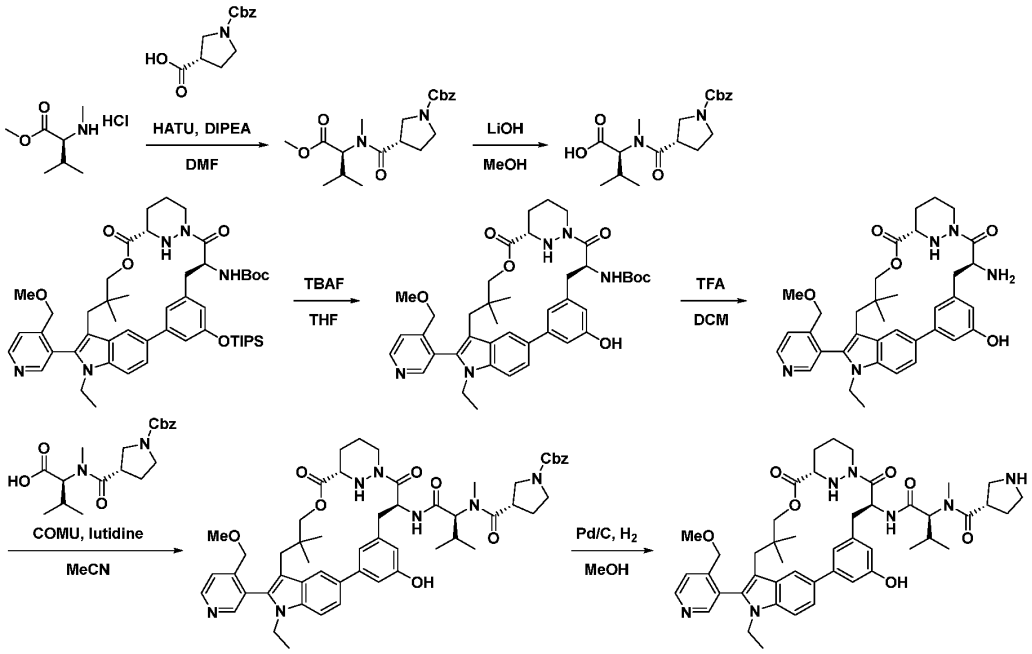
To a solution of *tert*-butyl (2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)(methyl)carbamate (355.0 mg) in THF (4.0 mL) at 0 °C was added TBAF (1.0 mL). The reaction mixture was stirred for 1 h and was then concentrated under reduced pressure. The residue was purified by normal phase chromatography (25% EtOAc/pet. ether) to afford the desired product (280 mg, 92% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{50}H_{67}N_7O_9$: 910.51; found 911.0.

Step 5: Synthesis of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(*N*-methyl-2-(methylamino)acetamido)butanamide

To a solution of *tert*-butyl (2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)(methyl)carbamate (150.0 mg, 0.165 mmol) in DCM (2.0 mL) at 0 °C was added TFA (0.70 mL). The reaction mixture was stirred for 1 h and was then

concentrated under reduced pressure to afford the desired crude product (150 mg) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{45}H_{59}N_7O_7$: 810.46; found 810.4.

Intermediate 12. Synthesis of (3*S*)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylpyrrolidine-3-carboxamide



Step 1: Synthesis of benzyl (*S*)-3-(((*S*)-1-methoxy-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)pyrrolidine-1-carboxylate

To a solution of methyl methyl-*L*-valinate hydrochloride (2.0 g, 13.8 mmol) and (*S*)-1-((benzyloxy)carbonyl)pyrrolidine-3-carboxylic acid (4.12 mg, 16.5 mmol) in DMF (20.0 mL) at 0 °C was added DIPEA (12 mL, 68.870 mmol). The reaction mixture was stirred for 0.5 h, and then HATU (7.856 mg, 20.66 mmol) was added. The resulting mixture was warmed to room temperature and stirred for 1 h. The reaction mixture was then diluted with EtOAc (800 mL) and was washed with sat. NH_4Cl (500 mL) and brine (3 x 350 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by normal phase chromatography (0→80% EtOAc/pet. ether) to afford the desired product (3.8 g, 73% yield) as an oil. LCMS (ESI) *m/z:*
[M + H] calcd for $C_{20}H_{28}N_2O_5$: 377.21; found 377.2.

Step 2: Synthesis of *N*-((*S*)-1-((benzyloxy)carbonyl)pyrrolidine-3-carbonyl)-*N*-methyl-*L*-valine

To a solution of benzyl (*S*)-3-(((*S*)-1-methoxy-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)pyrrolidine-1-carboxylate (1.125 g, 2.99 mmol) in MeOH (10.0 mL) was added

a solution of LiOH (180.0 mg, 7.52 mmol) in H_2O (2 mL). The reaction mixture was stirred for 4 h and was then quenched with sat. aq. NH_4Cl. The mixture with extracted with EtOAc (3 x 60 mL) and the combined organic layers were concentrated under reduced pressure to afford the desired product. LCMS (ESI) *m/z:* [M + H] calcd for $C_{19}H_{26}N_2O_5$: 363.19; found 363.2.

Step 3: Synthesis of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate

To a solution of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (1.70 g, 1.93 mmol) in THF (20 mL) at 0 °C was added TBAF (755.7 mg, 2.89 mmol). The reaction mixture was stirred for 2 h and was then quenched with H_2O (200 mL). The resulting mixture was extracted with EtOAc (3 x 200 mL) and the combined organic layers were washed with brine (3 x 200 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by normal phase chromatography (17% EtOAc/pet. ether) to afford the desired product (1.1 g, 70% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{41}H_{51}N_5O_7$: 726.39; found 726.7.

Step 4: Synthesis of (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione

To a solution of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (500.0 mg, 0.689 mmol) in DCM (10.0 mL) at 0 °C was added TFA (0.527 mL, 6.888 mmol). The resulting mixture was stirred for 1 h and then was concentrated under reduced pressure to afford the desired crude product (500 mg) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{36}H_{43}N_5O_5$: 626.34; found 626.4.

Step 5: Synthesis of benzyl (3*S*)-3-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)pyrrolidine-1-carboxylate

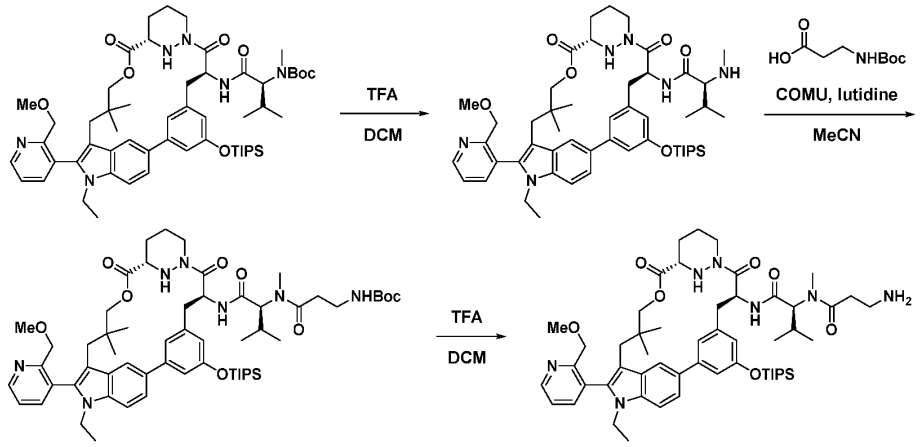
To a solution of *N*-((*S*)-1-((benzyloxy)carbonyl)pyrrolidine-3-carbonyl)-*N*-methyl-*L*-valine (676.4 mg, 6.31 mmol) in MeCN (10.0 mL) at 0 °C was added COMU (432.5 mg, 1.01 mmol). The reaction mixture was stirred for 5 min followed by the addition of (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (395.0 mg, 0.631 mmol). The reaction mixture was warmed to room temperature and stirred for 20 h. The mixture was then concentrated under reduced pressure, taken up in EtOAc (100 mL), and washed with brine (3 x 5 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by normal phase chromatography to afford a crude solid (0.81 g), which was then purified by reversed phase chromatography (MeCN/H_2O) to afford

the desired product (174 mg, 29% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{55}H_{67}N_7O_9$: 970.51; found 970.8.

Step 6: Synthesis of (3*S*)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylpyrrolidine-3-carboxamide

To a solution of benzyl (3*S*)-3-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)pyrrolidine-1-carboxylate (174.0 mg, 0.179 mmol) in MeOH (20.0 mL) was added Pd/C (87.0 mg, 0.08 mmol) followed by 2% aq. HCl (one drop). The reaction mixture was stirred at room temperature under a H_2 atmosphere (1 atm) for 14 h, at which point the reaction mixture was purged with N_2, filtered, and concentrated under reduced pressure to afford the crude product (130 mg, 86.7% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{47}H_{61}N_7O_7$: 836.47; found 836.5.

Intermediate 13. Synthesis of (2*S*)-2-(3-amino-*N*-methylpropanamido)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide



Step 1: Synthesis of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide

To a solution of *tert*-butyl ((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (212.4 mg, 212 µmol) in DCM (500 µL) at 0 °C was added TFA (500 µL, 6.52 mmol). After 2 h, the reaction was diluted with DCM (10 mL) and H_2O (10 mL), and then sat. aq. $NaHCO_3$ was added until the solution was pH 9. The aqueous layer was extracted with DCM (10

mL) and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the crude product (194 mg, 103% yield). LCMS (ESI) *m/z:* [M + H] calcd for $C_{51}H_{74}N_6O_6Si$: 895.55; found 895.7.

Step 2: Synthesis of *tert*-butyl (3-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-3-oxopropyl)carbamate

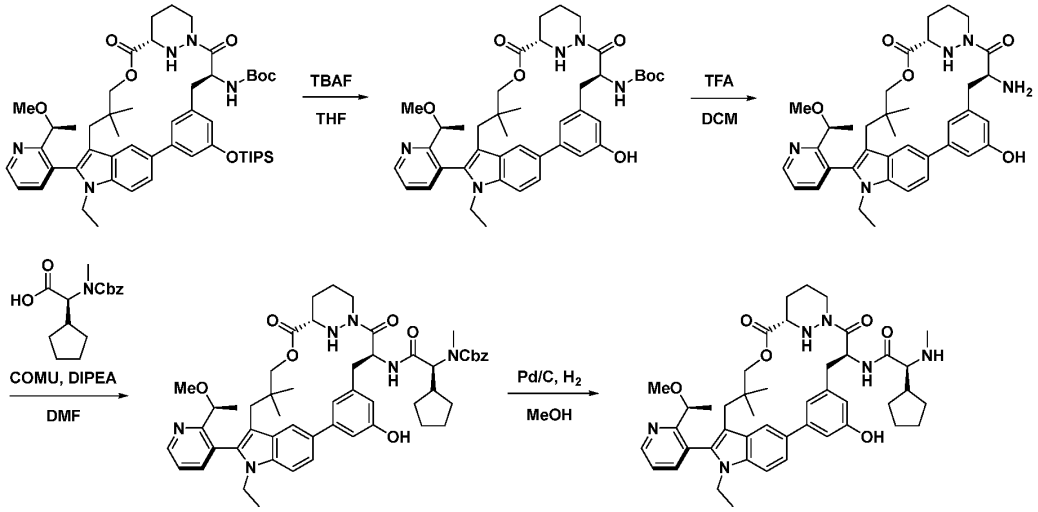
To a mixture of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide (150 mg, 167 µmol), COMU (88.5 mg, 206 µmol), and 3-((*tert*-butoxycarbonyl)amino)propanoic acid (39.6 mg, 209 µmol) in MeCN (1.66 mL) was added 2,6-lutidine (77.7 µL, 668 µmol). The reaction was stirred for 18 h at room temperature and then for 1 h at 55 °C. The reaction mixture was cooled to room temperature and was concentrated under reduced pressure. The crude residue was purified by reverse phase chromatography (20→60% MeCN/H_2O) to afford the product (132 mg, 67% yield). LCMS (ESI) *m/z:* [M + H] calcd for $C_{59}H_{87}N_7O_9Si$: 1066.64; found 1066.7.

Step 3: Synthesis of (2*S*)-2-(3-amino-*N*-methylpropanamido)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide

To a solution of *tert*-butyl (3-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-3-oxopropyl)carbamate (120 mg, 112 µmol) in DCM (560 µL) at 0 °C was added TFA (560 µL, 7.30 mmol). After 40 min, the reaction was diluted with DCM (10 mL) and then sat. aq. $NaHCO_3$ was added. The organic layer was dried over Na_2SO_4, filtered, and then concentrated under reduced pressure to afford the product (106 mg, 98% yield), which was used in the next step without purification. LCMS (ESI) *m/z:* [M + H] calcd for $C_{54}H_{79}N_7O_7Si$: 966.59; found 966.8.

Intermediate 14. Synthesis of (2*S*)-2-cyclopentyl-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-

oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-2-(methylamino)acetamide



Step 1: Synthesis of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate

To a stirred solution of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (18.0 g, 20.1 mmol) in THF (180 mL) at 0 °C was added a 1M solution of TBAF in THF (24.1 mL, 24.1 mmol). The mixture was stirred at 0 °C for 1 h, then diluted with brine (1.5 L), and extracted with EtOAc (3 x 1 L). The combined organic layers were washed with brine (2 x 500 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by normal phase chromatography afforded the desired product (11.5 g, 69% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{42}H_{53}N_5O_7$: 740.40; found 740.4.

Step 2: Synthesis of (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione

To a stirred solution of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (11.5 g, 15.5 mmol) in DCM (120 mL) at 0 °C was added TFA (60 mL, 808 mmol). The mixture was stirred at 0 °C for 1 h, then concentrated under reduced pressure and the residue again concentrated under reduced pressure with toluene (3 x 20 mL) to afford the desired crude product (12 g). LCMS (ESI) *m/z*: [M + H] calcd for $C_{37}H_{45}N_5O_5$: 640.35; found 640.6.

Step 3: Synthesis of benzyl ((1*S*)-1-cyclopentyl-2-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-

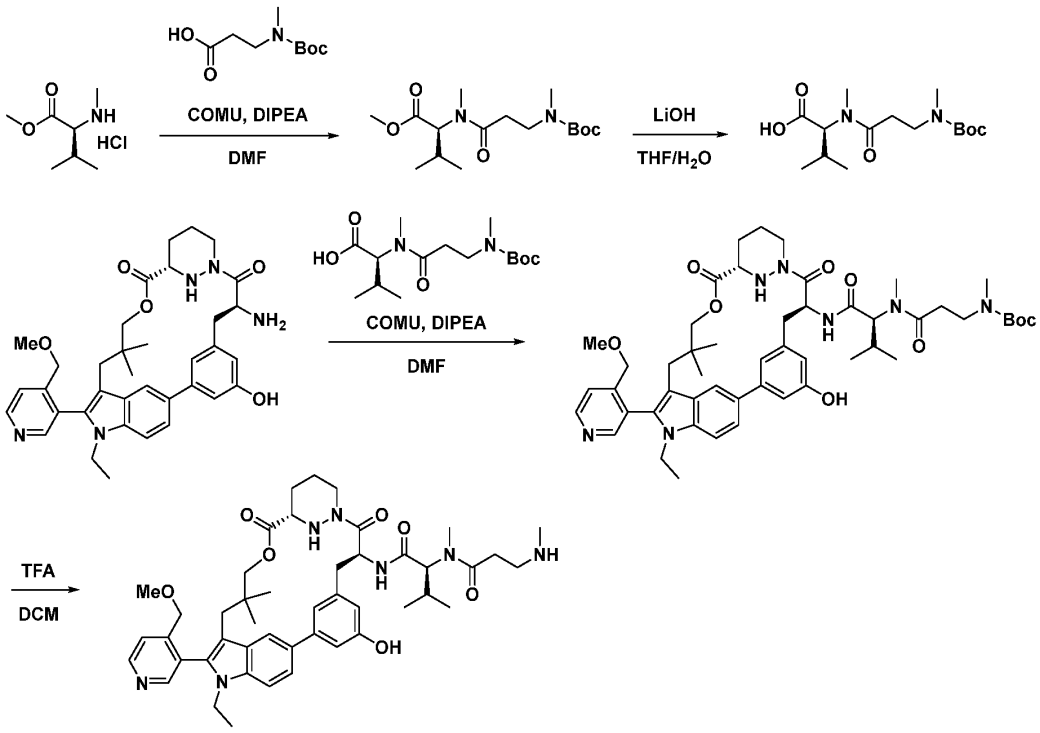
indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-2-oxoethyl)(methyl)carbamate

To a stirred solution of (6^3S,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (400.0 mg, 0.63 mmol) in DMF (4.0 mL) at 0 °C was added DIPEA (1.09 mL, 6.25 mmol) and (*S*)-2-(((benzyloxy)carbonyl)(methyl)amino)-2-cyclopentylacetic acid (255.0 mg, 0.88 mmol) followed by COMU (347.8 mg, 0.81 mmol). The resulting mixture was stirred at 0 °C for 1 h and was then diluted with H_2O (40 mL). The aqueous layer was extracted with EtOAc (3 x 15 mL) and the combined organic layers were washed with brine (2 x 10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by prep-TLC (25% EtOAc/pet. ether) to afford the desired product (510 mg, 80% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{53}H_{64}N_6O_8$: 913.49; found 913.6.

Step 4: Synthesis of (2*S*)-2-cyclopentyl-*N*-((6^3S,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-2-(methylamino)acetamide

To a stirred solution of benzyl ((1*S*)-1-cyclopentyl-2-(((6^3S,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-2-oxoethyl)(methyl)carbamate
(480.0 mg, 0.53 mmol), in MeOH (25 mL) was added Pd/C (200.0 mg, 1.88 mmol). The resulting mixture was placed under an atmosphere of H_2 (1 atm) and stirred for 2 h. The mixture was filtered, the filter cake was washed with MeOH (3 x 10 mL), and the filtrate was concentrated under reduced pressure to afford the desired crude product (440 mg). LCMS (ESI) *m/z*: [M + H] calcd for $C_{45}H_{58}N_6O_6$: 779.45; found 779.4.

Intermediate 15. Synthesis of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(*N*-methyl-3-(methylamino)propanamido)butanamide



Step 1: Synthesis of methyl *N*-(3-((*tert*-butoxycarbonyl)(methyl)amino)propanoyl)-*N*-methyl-*L*-valinate

To a solution of methyl methyl-*L*-valinate hydrochloride (1.0 g, 6.89 mmol) in DMF (20.0 mL) at 0 °C was added DIPEA (5.92 mL, 0.034 mmol), 3-((*tert*-butoxycarbonyl)(methyl)amino)propanoic acid (2.10 g, 0.010 mmol), and COMU (3.54 g, 8.27 mmol). The resulting mixture was stirred for 30 min and then quenched with H_2O (20 mL). The aqueous layer was extracted with EtOAc (3 x 20 mL) and the combined organic layers were washed with brine (3 x 20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by reverse phase chromatography (0→100% MeCN/H_2O) to afford the desired product (2 g, 87.9% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{16}H_{30}N_2O_5$: 331.22; found 331.2.

Step 2: Synthesis of *N*-(3-((*tert*-butoxycarbonyl)(methyl)amino)propanoyl)-*N*-methyl-*L*-valine

To a solution of *N*-(3-((*tert*-butoxycarbonyl)(methyl)amino)propanoyl)-*N*-methyl-*L*-valinate (1.0 g, 3.03 mmol) in THF (20.0 mL) and H_2O (4.0 mL) was added LiOH (0.14 g, 6.05 mmol). The resulting mixture was stirred for 3 h at room temperature. The mixture was acidified to pH 3 with

HCl (1N) and was then extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (3 x 20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired crude product (800 mg, 83.6% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{15}H_{28}N_2O_5$: 317.21; found 317.2.

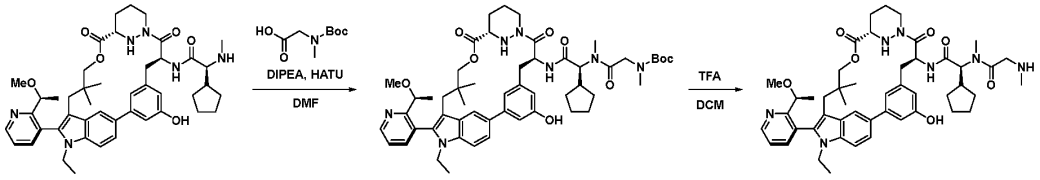
Step 3: Synthesis of *tert*-butyl (3-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-3-oxopropyl)(methyl)carbamate

To a solution of (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (600.0 mg, 0.96 mmol) in DMF (6.0 mL) at 0 °C was added DIPEA (1.67 mL, 9.59 mmol), *N*-(3-((*tert*-butoxycarbonyl)(methyl)amino)propanoyl)-*N*-methyl-*L*-valine (455.1 mg, 1.44 mmol), and COMU (492.5 mg, 1.15 mmol). The resulting mixture was stirred for 30 min and was then quenched with H_2O (60 mL). The aqueous layer was extracted with EtOAc (3 x 60 mL) and the combined organic layers were washed with brine (3 x 60 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by reverse phase chromatography (0→100% MeCN/H_2O) to afford the desired product (650 mg, 73.4% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{51}H_{69}N_7O_9$: 924.52; found 924.6.

Step 4: Synthesis of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(*N*-methyl-3-(methylamino)propanamido)butanamide

To a solution of *tert*-butyl (3-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-3-oxopropyl)(methyl)carbamate (650.0 mg) in DCM (7.0 mL) at 0 °C was added TFA (3.5 mL). The resulting mixture was stirred for 30 min and was then concentrated under reduced pressure. The resulting residue was diluted with toluene (3 x 10 mL) and concentrated under reduced pressure to afford the desired crude product. LCMS (ESI) *m/z*: [M + H] calcd for $C_{46}H_{61}N_7O_7$: 824.47; found 824.6.

Intermediate 16. Synthesis of (2*S*)-2-cyclopentyl-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-2-(*N*-methyl-2-(methylamino)acetamido)acetamide



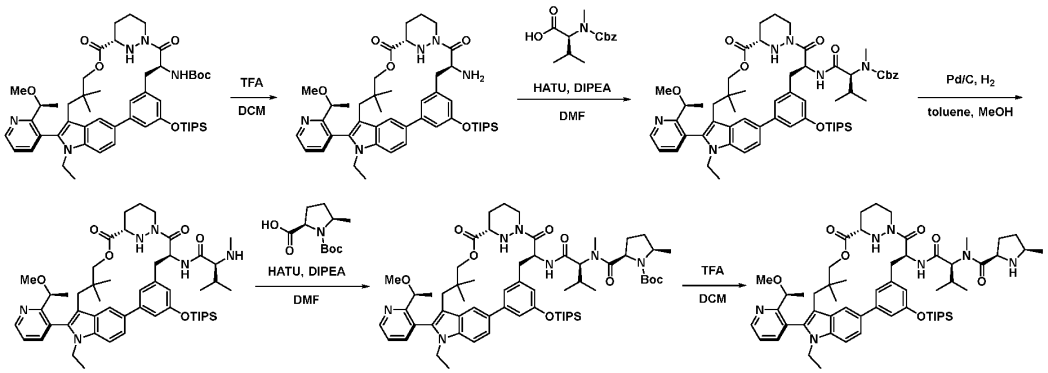
Step 1: Synthesis of *tert*-butyl (2-(((1*S*)-1-cyclopentyl-2-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-2-oxoethyl)(methyl)amino)-2-oxoethyl)(methyl)carbamate

To a mixture of (2*S*)-2-cyclopentyl-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-2-(methylamino)acetamide (300.0 mg, 0.385 mmol), DIPEA (0.657 mL, 3.851 mmol), and *N*-(*tert*-butoxycarbonyl)-*N*-methylglycine (109.30 mg, 0.578) in DMF (3.0 mL) at 0 °C was added HATU (175.72 mg, 0.462 mmol). The resulting mixture was stirred at 0 °C for 30 min and was then diluted with H_2O (30 mL). The resulting mixture was extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (3 x 30 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by prep-TLC (50% EtOAc/pet. ether) to afford the desired product (300 mg, 82.0% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{53}H_{71}N_7O_9$: 950.54; found 950.4.

Step 2: Synthesis of (2*S*)-2-cyclopentyl-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-2-(*N*-methyl-2-(methylamino)acetamido)acetamide

To a mixture of *tert*-butyl (2-(((1*S*)-1-cyclopentyl-2-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-2-oxoethyl)(methyl)amino)-2-oxoethyl)(methyl)carbamate (300.0 mg, 0.316 mmol) in DCM (3.0 mL) at 0 °C was added TFA (1.50 mL). The resulting mixture was stirred at 0 °C for 30 min and was then concentrated under reduced pressure to afford the desired crude product. LCMS (ESI) *m/z*: [M + H] calcd for $C_{48}H_{63}N_7O_7$: 850.49; found 850.5.

Intermediate 17. Synthesis of (2*R*,5*R*)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*,5-dimethylpyrrolidine-2-carboxamide



Step 1: Synthesis of (6^3S,4*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione

To a solution of *tert*-butyl ((6^3S,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (20.0 g, 22.315 mmol) in DCM (150.0 mL) at 0 °C was added TFA (50.0 mL). The resulting mixture was warmed to room temperature and stirred for 2 h and then concentrated under reduced pressure. The residue was dissolved in EtOAc (100 mL) and the solution was neutralized to pH 8 with sat. aq. $NaHCO_3$. The solution was extracted with EtOAc (3 x 150 mL) and the combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (17.86 g, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{46}H_{65}N_5O_5Si$: 796.49; found 795.5

Step 2: Synthesis of benzyl ((2*S*)-1-(((6^3S,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)- 6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate

To a solution of (6^3S,4*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (17.86 g, 22.433 mmol) and (2*S*)-2-[[(benzyloxy)carbonyl](methyl)amino]-3-methylbutanoic acid (8.93 g, 33.65 mmol) in DMF (150.0 mL) at 0 °C was added DIPEA (19.5 mL, 112.17 mmol) and HATU (17.06 g, 44.87 mmol). The resulting mixture was warmed to room temperature and stirred for 2 h. The reaction mixture was cooled to 0 °C and was quenched by the addition of H_2O (500 mL). The mixture was extracted with EtOAc (3 x 150 mL) and the combined organic layers were washed with brine (200 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by normal phase chromatography (25% EtOAc/pet. ether) to afford the desired product (19.0 g, 81.2% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{60}H_{82}N_6O_8Si$: 1043.61; found 1042.6

Step 3: Synthesis of (2*S*)-*N*-((6^3S,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide

To a solution of benzyl ((2*S*)-1-(((6^3S,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)- 6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (1.20 g, 1.150 mmol) in MeOH (1.2 mL) and toluene (1.2 mL) was added Pd/C (10%, 240 mg). The resulting mixture was placed under an atmosphere of H_2 (1 atm) and stirred overnight. The mixture was filtered and concentrated under reduced pressure to afford the desired product (1.05 g, 97.4% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{52}H_{76}N_6O_6Si$: 909.57; found 909.3.

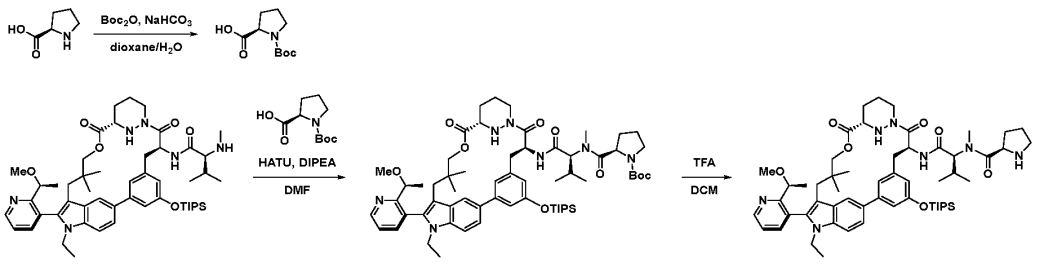
Step 4: Synthesis of *tert*-butyl (2*R*,5*R*)-2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)-5-methylpyrrolidine-1-carboxylate

To a solution of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide (500 mg, 0.550 mmol) in DMF (5 mL) at 0 °C was added DIPEA (0.94 mL, 5.499 mmol) and (2*R*,5*R*)-1-(*tert*-butoxycarbonyl)-5-methylpyrrolidine-2-carboxylic acid (504.29 mg, 2.199 mmol) followed by HATU (627.23 mg, 1.650 mmol) in portions. The resulting mixture was warmed to room temperature and stirred for 1 h. Purification by reverse phase chromatography (0→100% MeCN/H_2O) afforded the desired product (147 mg, 22.2% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{63}H_{93}N_7O_9Si$: 1120.69; found 1120.6.

Step 5: Synthesis of (2*R*,5*R*)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*,5-dimethylpyrrolidine-2-carboxamide

To a solution of *tert*-butyl (2*R*,5*R*)-2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)-5-methylpyrrolidine-1-carboxylate (150.0 mg, 0.134 mmol) in DCM at 0 °C was added TFA (1.50 mL, 13.155 mmol) dropwise. The resulting mixture was warmed to room temperature and stirred for 2 h and was then basified to pH 8 with sat. $NaHCO_3$. The resulting mixture was extracted with EtOAc (3 x 5 mL) and the combined organic layers were washed with brine (2 x 5 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (85 mg, 54.1% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{58}H_{85}N_7O_7Si$: 1020.64; found 1020.4.

Intermediate 18. Synthesis of (2*R*)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-i-methylpyrrolidine-2-carboxamide



Step 1: Synthesis of (*tert*-butoxycarbonyl)-*D*-proline

To a solution of *D*-proline (5.0 g, 43.43 mmol) in 1,4-dioxane (50 mL) and sat. $NaHCO_3$ (50 mL) at 0 °C was added Boc_2O (14.217 g, 65.143 mmol) in portions. The resulting mixture was stirred for 2 h at room temperature and was then extracted with EtOAc (100 mL). The aqueous layer was acidified to pH 6 with HCl and was then extracted into EtOAc (3 x 100 mL). The combined organic layers were washed with H_2O (2 x 100 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product which was used without further purification. LCMS (ESI) *m/z*: [M - H] calcd for $C_{10}H_{17}NO_4$: 214.11; found 214.0.

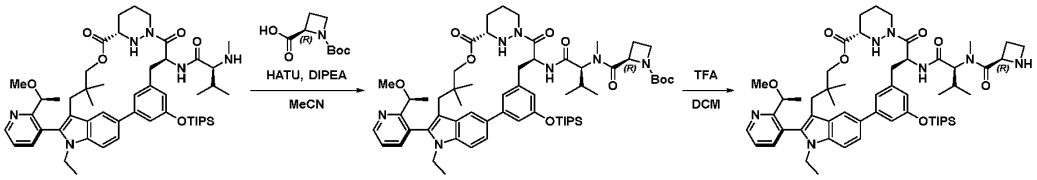
Step 2: Synthesis of *tert*-butyl (2*R*)-2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)pyrrolidine-1-carboxylate

To a solution of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide (142.03 mg, 0.660 mmol) in DMF was added DIPEA (0.710 mL, 5.499 mmol) followed by HATU (250.89 mg, 0.660 mmol) in portions. The resulting mixture was heated to 40 °C and stirred for 2 h. Purification by reverse phase chromatography (0→100% MeCN/H_2O) afforded the desired product (350 mg, 54.6% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{62}H_{91}N_7O_9Si$: 1106.67; found 1106.8.

Step 3: Synthesis of (2*R*)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-i-methylpyrrolidine-2-carboxamide

To a solution of *tert*-butyl (2*R*)-2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)pyrrolidine-1-carboxylate (350.0 mg, 0.325 mmol) in DCM (4 mL) at 0 °C was added TFA (2.0 mL). The resulting mixture was stirred for 30 min at 0 °C and then was concentrated under reduced pressure. The residue was dissolved in toluene (5 mL) then concentrated under reduced pressure three times to afford the desired product which was used without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{57}H_{83}N_7O_7Si$: 1006.62; found 1006.4.

Intermediate 19. Synthesis of (2*R*)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylazetidine-2-carboxamide



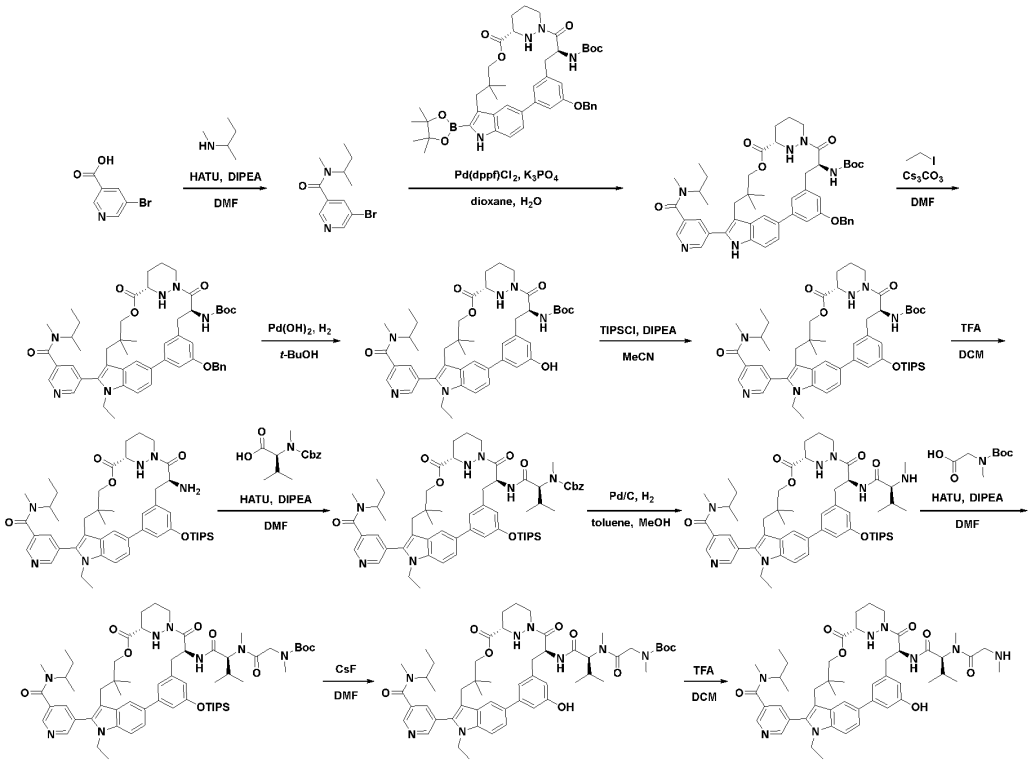
Step 1: Synthesis of *tert*-butyl (2*R*)-2-(((2*S*)-1-(((6^3*S*,4*S*)-11-ethyl-12-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-25-((triisopropylsilyl)oxy)-61,62,63,64,65,66-hexahydro-11*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)azetidine-1-carboxylate

To a mixture of (2*S*)-*N*-((6^3*S*,4*S*)-11-ethyl-12-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-25-((triisopropylsilyl)oxy)-61,62,63,64,65,66-hexahydro-11*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide (1.0 g, 1.10 mmol), (*R*)-1-(*tert*-butoxycarbonyl)azetidine-2-carboxylic acid (0.33 g, 1.650 mmol) and HATU (1.25 g, 3.299 mmol) in MeCN (20 mL) at 0 °C was added DIPEA (0.94 mL, 5.499 mmol). The resulting mixture was stirred at 0 °C for 3 h and then was concentrated under reduced pressure. Purification by prep-TLC (10% MeOH/DCM) afforded the desired product (800 mg, 59.9% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{61}H_{89}N_7O_9Si$: 1092.65; found 1092.6.

Step 2: Synthesis of (2*R*)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-11-ethyl-12-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-25-((triisopropylsilyl)oxy)-61,62,63,64,65,66-hexahydro-11*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylazetidine-2-carboxamide

To a mixture of *tert*-butyl (2*R*)-2-(((2*S*)-1-(((6^3*S*,4*S*)-11-ethyl-12-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-25-((triisopropylsilyl)oxy)-61,62,63,64,65,66-hexahydro-11*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)azetidine-1-carboxylate (400.0 mg, 0.366 mmol) in DCM (8.0 mL) at 0 °C was added TFA (4.0 mL). When the reaction was complete the mixture was concentrated under reduced pressure to afford the desired product (400 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{56}H_{81}N_7O_7Si$: 992.61; found 992.4.

Intermediate 20. Synthesis of *N*-(*sec*-butyl)-5-((6^3*S*,4*S*)-11-ethyl-25-hydroxy-10,10-dimethyl-4-((*S*)-3-methyl-2-(*N*-methyl-2-(methylamino)acetamido)butanamido)-5,7-dioxo-61,62,63,64,65,66-hexahydro-11*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-12-yl)-*N*-methylnicotinamide



Step 1: Synthesis of 5-bromo-*N*-(*sec*-butyl)-*N*-methylnicotinamide

To a solution of 5-bromonicotinic acid (2.0 g, 9.901 mmol) and HATU (5.65 g, 14.851 mmol) in DMF (40 mL) at 0 °C was added DIPEA (5.2 mL 9.9 mmol). The resulting mixture was stirred for 30 min at 0 °C and then *N*-methylbutan-2-amine (0.91 g, 10.396 mmol) was added. The resulting mixture was warmed to room temperature and stirred overnight, then diluted with H_2O (40 mL). The mixture was extracted with EtOAc (3 x 30 mL) and the combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (50% EtOAc/pet. ether) to afford the desired product (1.96 g, 73.2% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{11}H_{15}BrN_2O$: 271.04; found 271.1.

Step 2: Synthesis of *tert*-butyl ((6^3*S*,4*S*)-25-(benzyloxy)-1^2-(5-(*sec*-butyl(methyl)carbamoyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate

To a solution of 5-bromo-*N*-(*sec*-butyl)-*N*-methylnicotinamide (800.0 mg, 2.95 mmol) and K_3PO_3 (1.565 g, 7.376 mmol) in 1,4-dioxane (30.0 mL) and H_2O (6.0 mL) was added *tert*-butyl ((6^3*S*,4*S*)-2^5-(benzyloxy)-10,10-dimethyl-5,7-dioxo-1^2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (2.81 g, 3.540 mmol) and Pd(dppf)Cl_2 (215.87 mg, 0.295 mmol). The resulting mixture was heated to 85 °C and stirred for 3 h. The mixture was then cooled to room temperature, quenched with H_2O, and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with H_2O (100 mL), dried over Na_2SO_4, filtered, and

concentrated. The residue was purified by silica gel chromatography (10% MeOH/DCM) to afford the desired product (2.2 g, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{50}H_{60}N_6O_7$: 857.46; found 857.5.

Step 3: Synthesis of *tert*-butyl ((6^3*S*,4*S*)-2^5-(benzyloxy)-1^2-(5-(*sec*-butyl(methyl)carbamoyl)pyridin-3-yl)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate

To a solution of *tert*-butyl ((6^3*S*,4*S*)-25-(benzyloxy)-1^2-(5-(*sec*-butyl(methyl)carbamoyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (2.10 g, 2.450 mmol) and Cs_2CO_3 (2.39 g, 7.351 mmol) in DMF (20.0 mL) was added ethyl iodide (0.57 g, 3.675 mmol). The resulting mixture was stirred for 3 h at room temperature and was then quenched with H_2O (200 mL). The resulting mixture was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (10% MeOH/DCM) to afford the desired product (800 mg, 36.9% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{52}H_{64}N_6O_7$: 885.49; found 885.5.

Step 4: Synthesis of *tert*-butyl ((6^3*S*,4*S*)-1^2-(5-(*sec*-butyl(methyl)carbamoyl)pyridin-3-yl)-1^1-ethyl-2^5-hydroxy-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate

To a solution of *tert*-butyl ((6^3*S*,4*S*)-2^5-(benzyloxy)-1^2-(5-(*sec*-butyl(methyl)carbamoyl)pyridin-3-yl)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (770.0 mg, 0.870 mmol) in *tert*-BuOH (20.0 mL) was added $Pd(OH)_2$/C (24.42 mg, 0.174 mmol). The resulting suspension was stirred overnight at 50 °C under a hydrogen atmosphere (1 atm). The mixture was then cooled to room temperature, filtered and the filter cake was washed with MeOH (3 x 30 mL). The filtrate was concentrated under reduced pressure to afford the desired product (810 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{45}H_{58}N_6O_7$: 795.44; found 795.5.

Step 5: Synthesis of *tert*-butyl ((6^3*S*,4*S*)-1^2-(5-(*sec*-butyl(methyl)carbamoyl)pyridin-3-yl)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate

To a solution of *tert*-butyl ((6^3*S*,4*S*)-1^2-(5-(*sec*-butyl(methyl)carbamoyl)pyridin-3-yl)-1^1-ethyl-2^5-hydroxy-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (800.0 mg, 1.0 mmol) and DIPEA (0.876 mL, 5.031 mmol) in MeCN (10.0 mL) was added chlorotris(propan-2-yl)silane (291.02 mg, 1.509 mmol). The resulting mixture was stirred for 3 h and was then quenched with H_2O. The resulting mixture was extracted with EtOAc (3 x 50 mL) and the combined organic layers were washed with H_2O (3 x 30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (10% MeOH/DCM) to afford the desired product (800 mg, 83.6% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{54}H_{78}N_6O_7Si$: 951.58; found 950.8.

Step 6: Synthesis of 5-((6^3*S*,4*S*)-4-amino-1^1-ethyl-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-1^2-yl)-*N*-(*sec*-butyl)-*N*-methylnicotinamide

To a solution of *tert*-butyl ((6^3*S*,4*S*)-1^2-(5-(*sec*-butyl(methyl)carbamoyl)pyridin-3-yl)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (720.0 mg, 0.757 mmol) in DCM (10.0 mL) at 0 °C was added TFA (3.0 mL, 40.4 mmol). The resulting mixture was stirred for 2 h and was then concentrated under reduced pressure. The residue was cooled to at 0 °C and neutralized with sat. aq. $NaHCO_3$. The resulting mixture was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with H_2O (3 x 30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (540 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{49}H_{70}N_6O_5Si$: 851.53; found 851.8.

Step 7: Synthesis of benzyl ((2*S*)-1-(((6^3*S*,4*S*)-1^2-(5-(*sec*-butyl(methyl)carbamoyl)pyridin-3-yl)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate

To a solution of 5-((6^3*S*,4*S*)-4-amino-1^1-ethyl-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-1^2-yl)-*N*-(*sec*-butyl)-*N*-methylnicotinamide (530.0 mg, 0.623 mmol) and *N*-((benzyloxy)carbonyl)-*N*-methyl-*L*-valine (198.23 mg, 0.747 mmol) in DMF (10.0 mL) were added HATU (473.49 mg, 1.245 mmol) and DIPEA (0.542 mL, 3.113 mmol). The resulting mixture was stirred for 2 h and was then quenched with H_2O and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with H_2O (3 x 30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (10% MeOH/DCM) to afford the desired product (720 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{63}H_{87}N_7O_8Si$: 1098.65; found 1098.7.

Step 8: Synthesis of *N*-(*sec*-butyl)-5-((6^3*S*,4*S*)-1^1-ethyl-10,10-dimethyl-4-((*S*)-3-methyl-2-(methylamino)butanamido)-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-1^2-yl)-*N*-methylnicotinamide

To a solution of benzyl ((2*S*)-1-(((6^3*S*,4*S*)-1^2-(5-(*sec*-butyl(methyl)carbamoyl)pyridin-3-yl)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (670.0 mg, 0.610 mmol) in toluene (10.0 mL) and MeOH (1.0 mL) was added Pd/C (12.98 mg, 0.122 mmol). The suspension was stirred overnight under a hydrogen atmosphere (1 atm) and was then filtered, and the filter cake washed with MeOH (3 x 50 mL). The filtrate was concentrated under reduced pressure to afford the desired product (600 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{55}H_{81}N_7O_6Si$: 964.61; found 964.8.

Step 9: Synthesis of *tert*-butyl (2-(((2*S*)-1-(((6^3*S*,4*S*)-1^2-(5-(*sec*-butyl(methyl)carbamoyl)pyridin-3-yl)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-

benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)(methyl)carbamate

To a solution of *N*-(*sec*-butyl)-5-((6^3*S*,4*S*)-1^1-ethyl-10,10-dimethyl-4-((*S*)-3-methyl-2-(methylamino)butanamido)-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-1^2-yl)-*N*-methylnicotinamide (490.0 mg, 0.508 mmol) and *N*-(*tert*-butoxycarbonyl)-*N*-methylglycine (114.4 mg, 0.610 mmol) in DMF (10.0 mL) was added HATU (386.39 mg, 1.016 mmol) and DIPEA (0.443 mL, 2.540 mmol). The resulting mixture was stirred for 2 h and was then quenched with H_2O and extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with H_2O (3 x 30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (560 mg, 79.3% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{63}H_{94}N_8O_9Si$: 1135.70; found 1136.3.

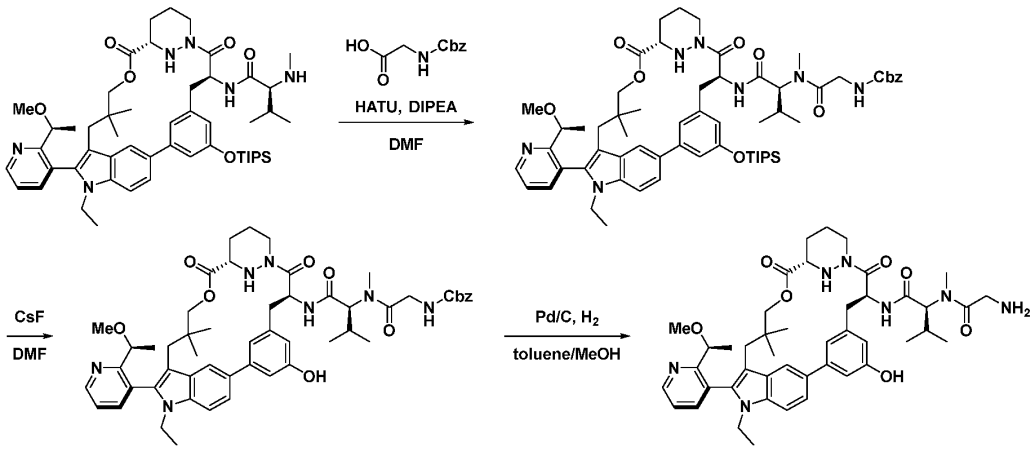
Step 10: Synthesis of *tert*-butyl (2-(((2*S*)-1-(((6^3*S*,4*S*)-1^2-(5-(*sec*-butyl(methyl)carbamoyl)pyridin-3-yl)-1^1-ethyl-2^5-hydroxy-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)(methyl)carbamate

To a solution of *tert*-butyl (2-(((2*S*)-1-(((6^3*S*,4*S*)-1^2-(5-(*sec*-butyl(methyl)carbamoyl)pyridin-3-yl)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)(methyl)carbamate (540.0 mg, 0.476 mmol) in DMF (10.0 mL) was added CsF (288.94 mg, 1.90 mmol). The resulting mixture was stirred for 2 h and was then quenched with H_2O and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with H_2O (3 x 30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (10% MeOH/DCM) to afford the desired product (430 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{54}H_{74}N_8O_9$: 979.57; found 980.0.

Step 11: Synthesis of *N*-(*sec*-butyl)-5-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-10,10-dimethyl-4-((*S*)-3-methyl-2-(*N*-methyl-2-(methylamino)acetamido)butanamido)-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-1^2-yl)-*N*-methylnicotinamide

To a solution of *tert*-butyl (2-(((2*S*)-1-(((6^3*S*,4*S*)-1^2-(5-(*sec*-butyl(methyl)carbamoyl)pyridin-3-yl)-1^1-ethyl-2^5-hydroxy-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)(methyl)carbamate (400.0 mg, 0.408 mmol) in DCM (10.0 mL) at 0 °C was added TFA (3.0 mL, 40.4 mmol). The reaction was stirred for 1 h and was thenquenched with sat. aq. $NaHCO_3$. The mixture was extracted with EtOAc (3 x 50 mL) and the combined organic layers were washed with H_2O (3 x 30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (380 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{49}H_{66}N_8O_7$: 879.51; found 879.5.

Intermediate 21. Synthesis of (2*S*)-2-(2-amino-*N*-methylacetamido)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide



Step 1: Synthesis of benzyl (2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)- 6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)carbamate

To a solution of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide (2.50 g, 2.75 mmol) and ((benzyloxy)carbonyl)glycine (690 mg, 3.30 mmol) in DMF (25 mL) at 0 °C was added HATU (2.10 g, 5.50 mmol) followed by DIPEA (1.5 mL, 8.25 mmol). The reaction mixture was stirred for 2 h and was then quenched with H_2O and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with H_2O (3 x 10 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (50% EtOAC/hexanes) afforded desired product (2.0 g, 72% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{62}H_{85}N_7O_9Si$: 1100.63; found 1100.7.

Step 2: Synthesis of benzyl (2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)carbamate

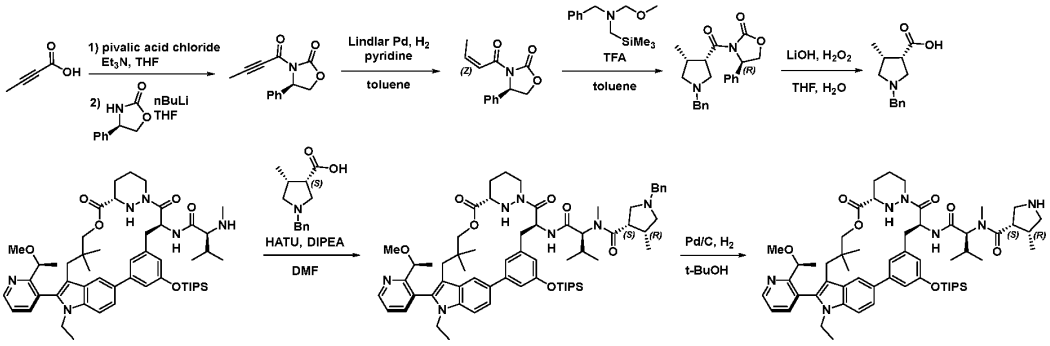
To a solution of benzyl (2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)- 6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)carbamate (400 mg, 0.36 mmol) in DMF at 0 °C was added CsF (220 mg, 1.5 mmol). The reaction mixture was stirred for 2 h and was then quenched with H_2O and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with H_2O (3 x 10 mL),

dried with Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (300 mg, 87% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{53}H_{65}N_7O_9$: 944.49; found 944.4.

Step 3: Synthesis of (2*S*)-2-(2-amino-*N*-methylacetamido)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide

To a solution of benzyl (2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)carbamate (300 mg, 0.32 mmol) in toluene (10 mL) and MeOH (1 mL) was added Pd/C (50 mg, 0.47 mmol). The suspension was stirred overnight under an atmosphere of hydrogen (1 atm). The reaction mixture was then was filtered and the filter cake was washed with EtOAc (3 x 10 mL). The filtrate was concentrated under reduced pressure to afford the desired product (180 mg, 43% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{45}H_{59}N_7O_7$: 810.46; found 810.5.

Intermediate 22. (3*S*,4*R*)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*,4-dimethylpyrrolidine-3-carboxamide



Step 1: Synthesis of (*R*)-3-(but-2-ynoyl)-4-phenyloxazolidin-2-one

To a solution of 2-butynoic acid (5.0 g, 59.47 mmol) in THF (100 mL) at -78 °C was added pivalic acid chloride (7.39 g, 61.26 mmol) and Et_3N (6.2 mL, 61.85 mmol) and then the mixture was stirred for 15 min and then warmed to 0 °C and stirred for 45 min. In a second flask, to a solution of (4*R*)-4-phenyl-1,3-oxazolidin-2-one (9.70 g, 59.47 mmol) in THF (100 mL) at -78 °C was added *n*-BuLi (2.5 M in hexane, 25 mL, 62.5 mmol). The mixture was stirred at -78 °C for 15 min and was then added to the initial mixture. The combined solutions were warmed to room temperature and stirred overnight. The reaction solution was quenched with sat. NH_4Cl (200 mL) and then the mixture was extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine (200 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by normal phase chromatography (20% EtOAc/pet. ether) afforded the desired product (6.0 g, 44.0% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{11}NO_3$: 230.08; found 229.9.

Step 2: Synthesis of (*R*,*Z*)-3-(but-2-enoyl)-4-phenyloxazolidin-2-one

To a solution of (*R*)-3-(but-2-ynoyl)-4-phenyloxazolidin-2-one (6.0 g, 26.17 mmol) in pyridine (6.0 mL) and toluene (60.0 mL) at 0 °C was added Lindlar Pd catalyst (594.57 mg, 2.88 mmol). The resulting mixture was stirred for 30 min at 0 °C under a hydrogen atmosphere (1 atm). The mixture was filtered, and the filter cake was washed with toluene (10.0 mL). The filtrate was concentrated under reduced pressure to afford the desired product (5.5 g, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{13}NO_3$: 232.10; found 231.9.

Step 3: (*R*)-3-((3*S*,4*R*)-1-benzyl-4-methylpyrrolidine-3-carbonyl)-4-phenyloxazolidin-2-one

To a solution of (*R*,*Z*)-3-(but-2-enoyl)-4-phenyloxazolidin-2-one (3.0 g, 12.97 mmol) and benzyl(methoxymethyl)[(trimethylsilyl)methyl]amine (3.70 g, 15.57 mmol) in toluene (20.0 mL) at 0 °C was added TFA (1.30 mL, 0.87 mmol). The resulting mixture was warmed to room temperature and stirred overnight. The mixture was then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (20% EtOAc/pet. ether) to afford the desired product (2 g, 42.3% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{22}H_{24}N_2O_3$: 365.19; found 365.2.

Step 4: Synthesis of (3*S*,4*R*)-1-benzyl-4-methylpyrrolidine-3-carboxylic acid

A solution of LiOH●H_2O (0.16 g, 6.860 mmol) and H_2O_2 (0.13 g, 3.76 mmol) in H_2O (5 mL) was added to a solution of (*R*)-3-((3*S*,4*R*)-1-benzyl-4-methylpyrrolidine-3-carbonyl)-4-phenyloxazolidin-2-one (1.0 g, 2.74 mmol) in THF (15.0 mL) at 0 °C. The resulting mixture was stirred for 2 h and was then quenched with H_2O (30 mL) and sodium sulfite (0.69 g, 5.48 mmol) and the solution was extracted with EtOAc (2 x 50 mL). The aqueous phase was adjusted to pH 4 with NaH_2PO_4●H_2O and 10% HCl, and the brine was added. The solution was extracted with *i*-PrOH/DCM (1:3, 5 x 50 mL) and the combined organic layers were washed with brine (40 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (400 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{17}NO_2$: 220.14; found 220.2.

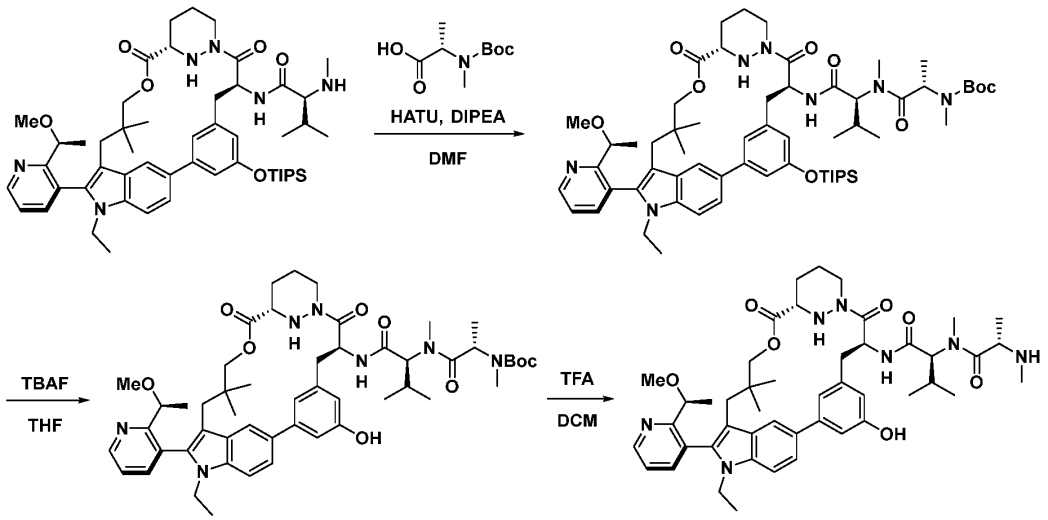
Step 5: Synthesis of (3*S*,4*R*)-1-benzyl-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*,4-dimethylpyrrolidine-3-carboxamide

To a mixture of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide (414.67 mg, 0.456 mmol) and (3*S*,4*R*)-1-benzyl-4-methylpyrrolidine-3-carboxylic acid (200.0 mg, 0.912 mmol) in DMF (5.0 mL) at 0 °C was added HATU (693.58 mg, 1.824 mmol) and DIPEA (0.794 mL, 4.560 mmol). The resulting mixture was warmed to room temperature and stirred for 2 h. The reaction was quenched with the addition of sat. aq. NH_4Cl (40 mL) and then extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (2 x 20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by prep-TLC (9% MeOH/DCM) to afford the desired product (350 mg, 34.6% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{65}H_{91}N_7O_7Si$: 1110.68; found 1110.9.

Step 6: (3*S*,4*R*)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*,4-dimethylpyrrolidine-3-carboxamide

To a solution of (3*S*,4*R*)-1-benzyl-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)- 6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*,4-dimethylpyrrolidine-3-carboxamide (300.0 mg, 0.270 mmol) in *t*-BuOH (10.0 mL) was added Pd/C (60.08 mg, 0.565 mmol). The resulting suspension was stirred overnight under a hydrogen atmosphere (1 atm). The mixture was then filtered, the filter cake was washed with MeOH (2 x 5 mL), and the filtrate was concentrated under reduced pressure to afford the desired product (280 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{58}H_{85}N_7O_7Si$: 1020.64; found 1020.8.

Intermediate 23. Synthesis of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-((*S*)-*N*-methyl-2-(methylamino)propanamido)butanamide



Step 1: Synthesis of *tert*-butyl ((2*S*)-1-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-1-oxopropan-2-yl)(methyl)carbamate

To a solution of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide (500.0 mg, 0.55 mmol), DIPEA (480 mL, 2.75 mmol) and (2*S*)-2-[(*tert*-butoxycarbonyl)(methyl)amino]propanoic acid (167.63 mg, 0.825 mmol) in DMF (5.0 mL) at 0 °C

was added HATU (271.80 mg, 0.715 mmol). The mixture was warmed to room temperature and stirred for 4 h. The reaction was then quenched with H_2O and extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with brine (5 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (50% EtOAc/pet. ether) to afford the desired product (550 mg, 91.4% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{61}H_{91}N_7O_9Si$: 1094.67; found 1094.5.

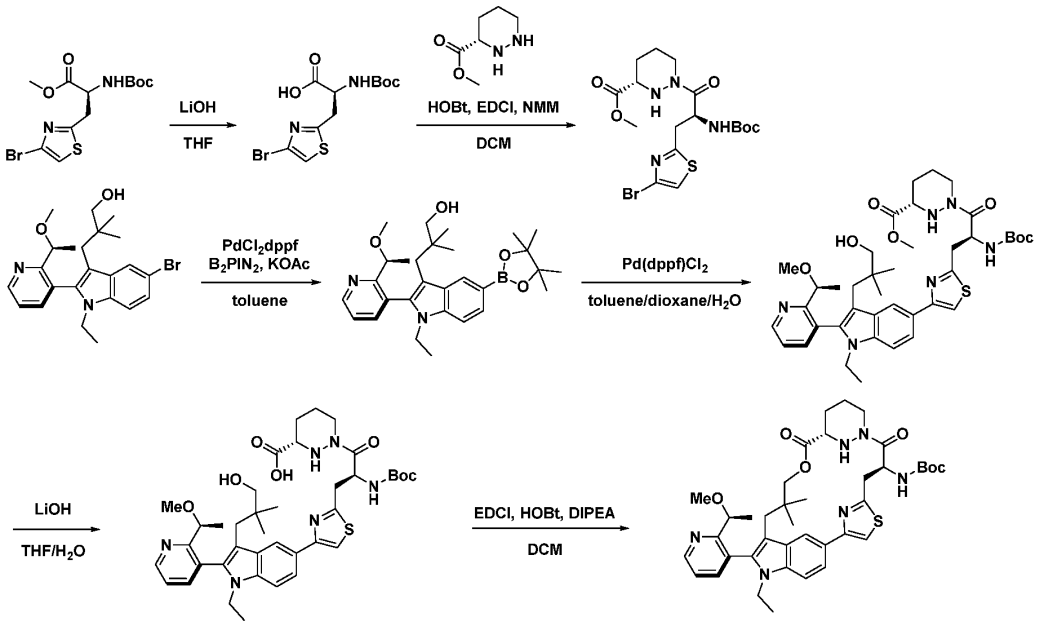
Step 2: Synthesis of *tert*-butyl ((2*S*)-1-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-1-oxopropan-2-yl)(methyl)carbamate

To a solution of *tert*-butyl ((2*S*)-1-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-1-oxopropan-2-yl)(methyl)carbamate (540 mg, 0.493 mmol) in THF (5.0 mL) at 0 °C was added TBAF (1M in THF, 0.59 mL, 0.592 mmol). The mixture was warmed to room temperature and stirred for 30 min. The reaction was quenched with H_2O and was then extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, filtered, After filtration, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (20% EtOAc/pet. ether) to afford the desired product (320 mg, 69.1% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{52}H_{71}N_7O_9$: 938.534; found 938.4.

Step 3: Synthesis of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-((*S*)-*N*-methyl-2-(methylamino)propanamido)butanamide

To a solution of *tert*-butyl ((2*S*)-1-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-1-oxopropan-2-yl)(methyl)carbamate (300.0 mg, 0.320 mmol) in DCM (3.0 mL) at 0 °C and was added TFA (1.0 mL). The mixture was warmed to room temperature and stirred for 2 h. The mixture was concentrated under reduced pressure to afford the desired product (300 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{47}H_{63}N_7O_7$: 838.49; found 838.4.

Intermediate 24. Synthesis of *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate



Step 1: Synthesis of (*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid

To a solution of methyl (2*S*)-3-(4-bromo-1,3-thiazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (110 g, 301.2 mmol) in THF (500 mL) and H_2O (200 mL) at room temperature was added LiOH (21.64 g, 903.6 mmol). The resulting solution was stirred for 1 h and was then concentrated under reduced pressure. The resulting residue was adjusted to pH 6 with 1 M HCl and then extracted with DCM (3 x 500 mL). The combined organic layers were, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (108 g, crude). LCMS (ESI) *m/z:* [M + H] calcd for $C_{11}H_{15}BrN_2O_4S$: 351.00; found 351.0.

Step 2: Synthesis of methyl (*S*)-1-((*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate

To a solution of (*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (70 g, 199.3 mmol) in DCM (500 mL) at 0 °C was added methyl (3*S*)-1,2-diazinane-3-carboxylate bis(trifluoroacetic acid) salt (111.28 g, 298.96 mmol), NMM (219.12 mL. 1993.0 mmol), EDCI (76.41 g, 398.6 mmol) and HOBt (5.39 g, 39.89 mmol). The resulting solution was warmed to room temperature and stirred for 1 h. The reaction was then quenched with H_2O (500 mL) and was extracted with EtOAc (3 x 500 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressured. The residue was purified by silica gel chromatography (0→50% EtOAc/pet. ether) to afford the desired product (88.1 g, 92.6% yield). LCMS (ESI) *m/z:* [M + H] calcd for $C_{17}H_{25}BrN_4O_5S$: 477.08; found 477.1.

Step 3: Synthesis of (*S*)-3-(1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol

To a solution of 3-(5-bromo-1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (60 g, 134.7 mmol) in toluene (500 mL) at room temperature was added

bis(pinacolato)diboron (51.31 g, 202.1 mmol), Pd(dppf)Cl_2 (9.86 g, 13.48 mmol) and KOAc (26.44 g, 269.4 mmol). Then reaction mixture was then heated to 90 °C and stirred for 2 h. The reaction solution was then cooled to room temperature and concentrated under reduced pressure. Purification by silica gel chromatography (0→50% EtOAc/pet. ether) afforded the desired product (60.6 g, 94.0% yield). LCMS (ESI) *m/z:* [M + H] calcd for $C_{29}H_{41}BN_2O_4$: 493.32; found 493.3.

Step 4: Synthesis of methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylate

To a solution of (*S*)-3-(1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (30 g, 60.9 mmol) in toluene (600 mL), dioxane (200 mL), and H_2O (200 mL) at room temperature was added methyl (*S*)-1-((*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (43.62 g, 91.4mmol), K_3PO_4 (32.23 g, 152.3 mmol) and Pd(dppf)Cl_2 (8.91 g, 12.18 mmol). The resulting solution was heated to 70 °C and stirred overnight. The reaction mixture was then cooled to room temperature and was quenched with H_2O (200 mL). The resulting mixture was extracted with EtOAc (3 x 1000 mL) and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (0→90% EtOAc/pet. ether) to afford the desired product (39.7 g, 85.4% yield). LCMS (ESI) *m/z:* [M + H] calcd for $C_{40}H_{54}N_6O_7S$: 763.39; found 763.3.

Step 5: Synthesis of (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylic acid

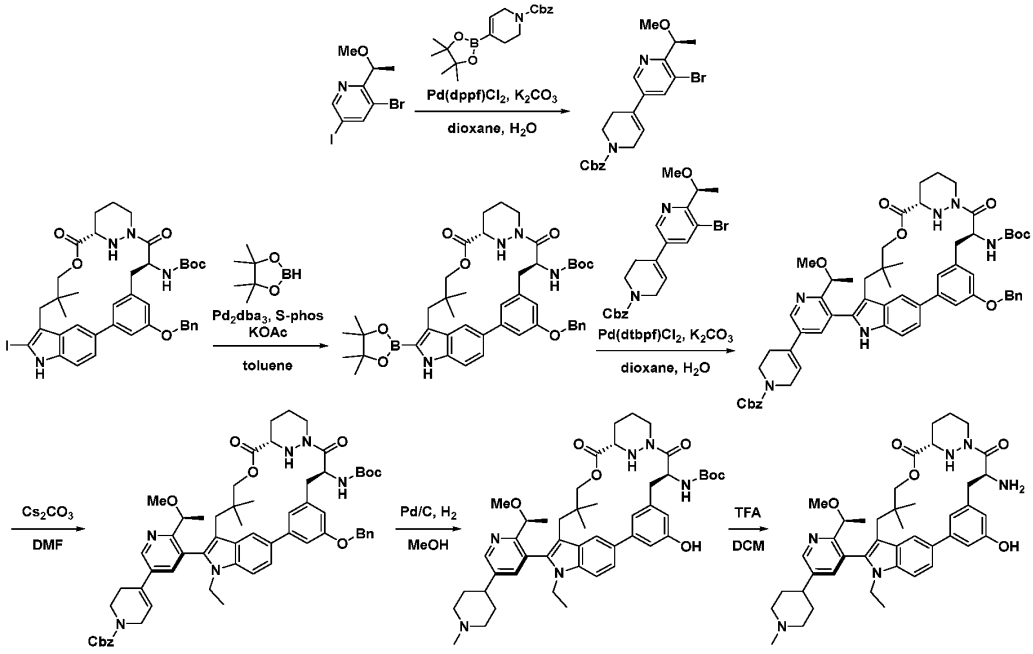
To a solution of methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (39.7 g, 52.0 mmol) in THF (400 mL) and H_2O (100 mL) at room temperature was added LiOH●H_2O (3.74 g, 156.2 mmol). The resulting mixture was stirred for 1.5 h and was then concentrated under reduced pressure. The residue was acidified to pH 6 with 1 M HCl and extracted with DCM (3 x 1000 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (37.9 g, crude). LCMS (ESI) *m/z:* [M + H] calcd for $C_{39}H_{52}N_6O_7S$: 749.37; found 749.4.

Step 6: Synthesis of *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate

To a solution of (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (37.9 g, 50.6 mmol), HOBt (34.19 g, 253.0 mmol) and DIPEA (264.4 mL, 1518 mmol) in DCM (4 L) at 0 °C was added EDCI (271.63 g, 1416.9 mmol). The resulting mixture was warmed to room temperature and stirred overnight. The reaction mixture was then quenched with H_2O and washed with 1 M HCl (4 x 1 L). The organic layer was separated and concentrated under reduced pressure. The residue was purified by silica gel

chromatography (0→70% EtOAc/pet. ether) to afford the desired product (30 g, 81.1% yield). LCMS (ESI) *m/z:* [M + H] calcd for $C_{39}H_{50}N_6O_6S$: 731.36; found 731.3.

Intermediate 25. Synthesis of (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)-5-(1-methylpiperidin-4-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione



Step 1: Synthesis of benzyl (*S*)-5-bromo-6-(1-methoxyethyl)-3',6'-dihydro-[3,4'-bipyridine]-1'(2'*H*)-carboxylate

To a solution of (*S*)-3-bromo-5-iodo-2-(1-methoxyethyl)pyridine (6.0 g, 17.55 mmol) and benzyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2*H*)-carboxylate (7.23 g, 21.05 mmol) in dioxane (70 mL) and H_2O (14 mL) was added K_2CO_3 (6.06 g, 43.86 mmol) and $Pd(dppf)Cl_2$ (1.28 g, 1.76 mmol). The reaction mixture was heated to 60 °C and stirred for 3 h. The mixture was diluted with H_2O (50 mL) then extracted into EtOAc (3 x 100 mL). The combined organic layers were washed with brine (3 x 50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (25% EtOAc/pet. ether) afforded the desired product (7.1 g, 94% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{21}H_{23}BrN_2O_3$: 431.10; found 431.1.

Step 2: Synthesis of *tert*-butyl ((6^3*S*,4*S*)-2^5-(benzyloxy)-10,10-dimethyl-5,7-dioxo-1^2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate

To a solution of *tert*-butyl ((6^3*S*,4*S*)-2^5-(benzyloxy)-1^2-iodo-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (5.0 g, 6.31 mmol), $Pd_2(dba)_3$ (690 mg, 757 µmol), S-

Phos (0.78 g, 1.89 mmol), and KOAc (2.17 g, 22.08 mmol) in toluene (75 mL) was added 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (5.65 g, 44.15 mmol). The reaction mixture was heated to 60 °C and stirred for 3 h. The reaction was quenched with H_2O at 0 °C then extracted into EtOAc (3 x 100 mL). The combined organic layers were washed with brine (3 x 30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (50% EtOAc/pet. ether) afforded the desired product (4.5 g, 90% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{75}H_{57}BN_4O_8$: 793.43; found 793.4.

Step 3: Synthesis of benzyl 5-((6^3*S*,4*S*)-2^5-(benzyloxy)-4-((*tert*-butoxycarbonyl)amino)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)-3',6'-dihydro-[3,4'-bipyridine]-1'(2'*H*)-carboxylate

To a solution of *tert*-butyl ((6^3*S*,4*S*)-2^5-(benzyloxy)-10,10-dimethyl-5,7-dioxo-1^2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (4.0 g, 5.05 mmol) and benzyl (*S*)-5-bromo-6-(1-methoxyethyl)-3',6'-dihydro-[3,4'-bipyridine]-1'(2'*H*)-carboxylate (2.61 g, 6.06 mmol) in dioxane (50 mL) and H_2O (10 mL) was added K_2CO_3 (1.74 g, 12.6 mmol) and Pd(dtbpf)Cl_2 (330 mg, 505 µmol). The reaction mixture was heated to 70 °C. After 3 h the reaction was diluted with H_2O (40 mL) and extracted into EtOAc (3 x 100 mL). The combined organic layers were washed with brine (2 x 50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (50% EtOAc/pet. ether) afforded the desired product (4.1 g, 80% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{60}H_{68}N_6O_9$: 1017.51; found 1017.4.

Step 4: Synthesis of benzyl 5-((6^3*S*,4*S*)-2^5-(benzyloxy)-4-((*tert*-butoxycarbonyl)amino)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)-3',6'-dihydro-[3,4'-bipyridine]-1'(2'*H*)-carboxylate

To a solution of benzyl 5-((6^3*S*,4*S*)-2^5-(benzyloxy)-4-((*tert*-butoxycarbonyl)amino)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)-3',6'-dihydro-[3,4'-bipyridine]-1'(2'*H*)-carboxylate (4.0 g, 3.93 mmol) and Cs_2CO_3 (3.84 g, 11.80 mmol) in DMF (30 mL) at 0 °C was added iodoethane (2.45 g, 15.73 mmol). The reaction mixture was warmed to room temperature. After 3 h the reaction mixture was diluted with H_2O (100 mL) and extracted into EtOAc (3 x 200 mL). The combined organic layers were washed with brine (3 x 100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (66% EtOAc/pet. ether) afforded the desired product (1.4 g, 34% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{62}H_{72}N_6O_9$: 1045.54; found 1045.5.

Step 5: Synthesis of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)-5-(1-methylpiperidin-4-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate

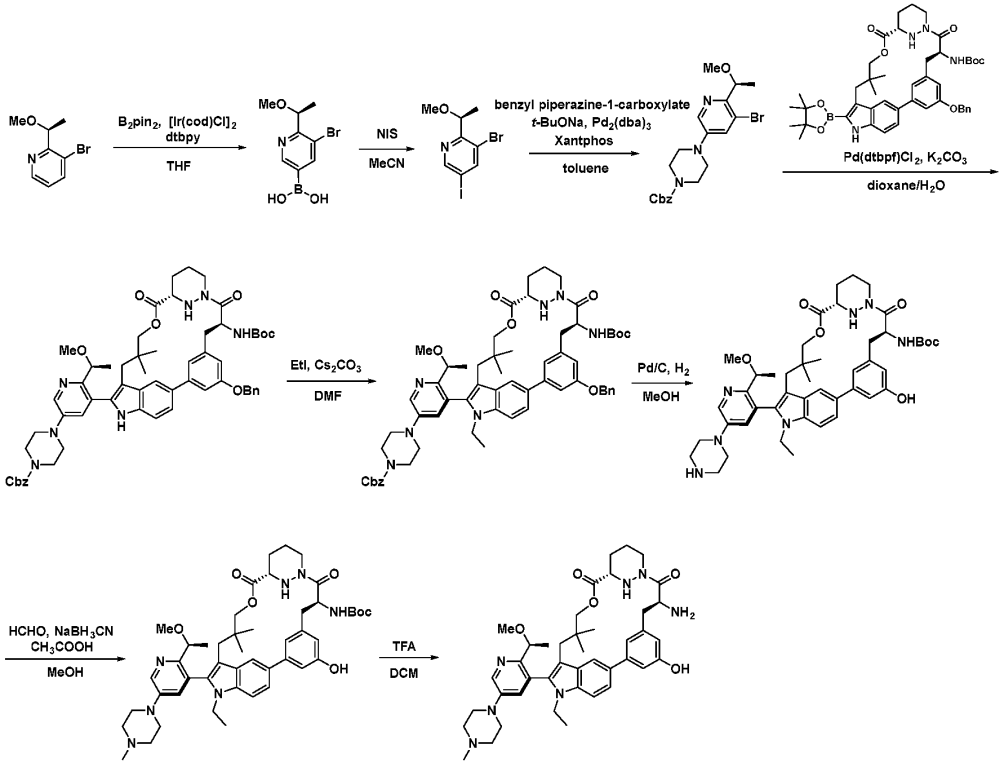
A solution of benzyl 5-((6^3*S*,4*S*)-2^5-(benzyloxy)-4-((*tert*-butoxycarbonyl)amino)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-

2(1,3)-benzenacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)-3',6'-dihydro-[3,4'-bipyridine]-1'(2'*H*)-carboxylate (1.29 g, 1.23 mmol) and Pd/C (700 mg) in MeOH (30 mL) was stirred for 72 h at room temperature under H_2 atmosphere. The reaction mixture was then filtered with MeOH (3 x 50 mL). The filtrate was concentrated under reduced pressure which afforded the desired product (850 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{48}H_{64}N_6O_7$: 837.49; found 837.7.

Step 6: Synthesis of (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)-5-(1-methylpiperidin-4-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione

To a solution of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)-5-(1-methylpiperidin-4-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (840 mg, 1.00 mmol) in DCM (10 mL) at 0 °C was added TFA (3.0 mL, 40.4 mmol). The reaction mixture was warmed to room temperature. After 2 h the reaction was cooled to 0 °C, quenched with sat. at. $NaHCO_3$, and extracted into EtOAc (3 x 50 mL). The combined organic layers were washed with brine (2 x 30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure which afforded product (670 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{43}H_{56}N_6O_5$: 737.44; found 737.3.

Intermediate 26. Synthesis of (6^3*S*, 4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridine-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione



Step 1: Synthesis of (*S*)-(5-bromo-6-(1-methoxyethyl)pyridin-3-yl)boronic acid

To a solution of (*S*)-3-bromo-2-(1-methoxyethyl)pyridine (40 g, 185 mmol) and bis(pinacolato)diboron (70.5 g, 278 mmol) in THF (1.6 L) at 75 °C was added 4,4'-di-*tert*-butyl-2,2'-bipyridine (7.45 g, 27.7 mmol) and $[Ir(cod)Cl]_2$ (1.24 mg, 1.85 mmol). After 16 h the mixture was concentrated under reduced pressure and the residue diluted with H_2O (1 L). The aqueous layer extracted with DCM/MeOH (2 L, 5:1), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Following purification by reverse phase chromatography (10→50% MeCN/H_2O, 0.1% HCO_2H) the combined product fractions were partially concentrated under reduced pressure. The aqueous layer was extracted with DCM/MeOH (3000 mL, 5:1), dried with Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (35.0 g, 65.5% yield). LCMS (ESI) *m/z*: [M + Na] calcd for $C_8H_{11}BBrNO_3$: 282.00; found 281.1.

Step 2: Synthesis of (*S*)-3-bromo-5-iodo-2-(1-methoxyethyl)pyridine

To a solution of (*S*)-(5-bromo-6-(1-methoxyethyl)pyridin-3-yl)boronic acid (35.0 g, 135 mmol) in MeCN (100 mL) was added *N*-iodosuccinimide (60.6 g, 269 mmol). The resulting reaction mixture was stirred overnight and then concentrated under reduced pressure. Purification by normal phase chromatography (10% EtOAc/pet. ether) afforded the desired product (40.0 g, 78.1% yield). LCMS (ESI) *m/z*: [M + H] calcd for C_8H_9BrINO: 341.90; found 341.8.

Step 3: Synthesis of benzyl (*S*)-4-(5-bromo-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate

To a solution of (*S*)-3-bromo-5-iodo-2-(1-methoxyethyl)pyridine (7.0 g, 20.5 mmol) and benzyl piperazine-1-carboxylate (9.0 g, 40.8 mmol) in toluene (70 mL) were added $Pd_2(dba)_3$ (375 mg, 0.409 mmol), Xantphos (1.18 g, 2.05 mmol) and sodium *tert*-butoxide (2.29 g, 24.6 mmol). The resulting mixture was heated to 120 °C and stirred for 16 h then cooled to room temperature and concentrated under reduced pressure. Purification by normal phase chromatography (25% EtOAc/pet. ether) afforded the desired product (5.0 g, 50.6% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{20}H_{24}BrN_3O_3$: 434.11; found 434.0.

Step 4: Synthesis of benzyl 4-(5-((6^3*S*,4*S*)-2^5-(benzyloxy)-4-((*tert*-butoxycarbonyl)amino)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate

To a solution of benzyl (*S*)-4-(5-bromo-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (3.29 g, 7.56 mmol) and *tert*-butyl ((6^3*S*,4*S*)-2^5-(benzyloxy)-10,10-dimethyl-5,7-dioxo-1^2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (50 g, 6.31 mmol) dioxane (40 mL) and H_2O (10 mL) were added K_2CO_3 (1.74 g, 12.614 mmol) and Pd(dtbpf)Cl_2 (822 mg, 1.26 mmol) and the resulting mixture was heated to 80 °C for 2 h. The reaction mixture was then concentrated under reduced pressure and diluted with H_2O (1 L). The aqueous layer was extracted with EtOAc (3 x 200 mL) and the combined organic layers were washed with H_2O, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by normal phase chromatography (50% EtOAc/pet. ether) afforded the desired product (5.0 g, 73.8% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{59}H_{69}N_7O_9$: 1020.54; found 1020.6.

Step 5: Synthesis of benzyl 4-(5-((6^3*S*, 4*S*)-2^5-(benzyloxy) -4-((*tert*-butoxycarbonyl)amino)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate

To a stirred solution of benzyl 4-(5-((6^3*S*,4*S*)-2^5-(benzyloxy)-4-((*tert*-butoxycarbonyl)amino)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (5.0 g, 5 mmol) in DMF (50 mL) at 0 °C was added Cs_2CO_3 (3.19 g, 9.80 mmol) and ethyl iodide (1.53 g, 10 mmol). The resulting mixture was stirred for 2 h at room temperature and then diluted with H_2O (200 mL). The aqueous layer was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with H_2O, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by normal phase chromatography (33% EtOAc/pet. ether) afforded the desired product (1.8 g, 35% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{61}H_{73}N_7O_9$: 1048.56; found 1048.4.

Step 6: Synthesis of *tert*-butyl ((6^3*S*, 4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)-5-(piperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-61,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate

To a stirred solution of benzyl 4-(5-((6^3*S*, 4*S*)-2^5-(benzyloxy) -4-((*tert*-butoxycarbonyl)amino)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (1.80 g, 1.72 mmol) in MeOH (20 mL) was added Pd/C (900 mg). The resulting mixture was stirred for 2 h at room temperature under a hydrogen atmosphere, filtered, and the filter cake washed with MeOH. The filtrate was concentrated under reduced pressure to afford the crude desired product which was used without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{46}H_{61}N_7O_7$: 824.47; found 824.3.

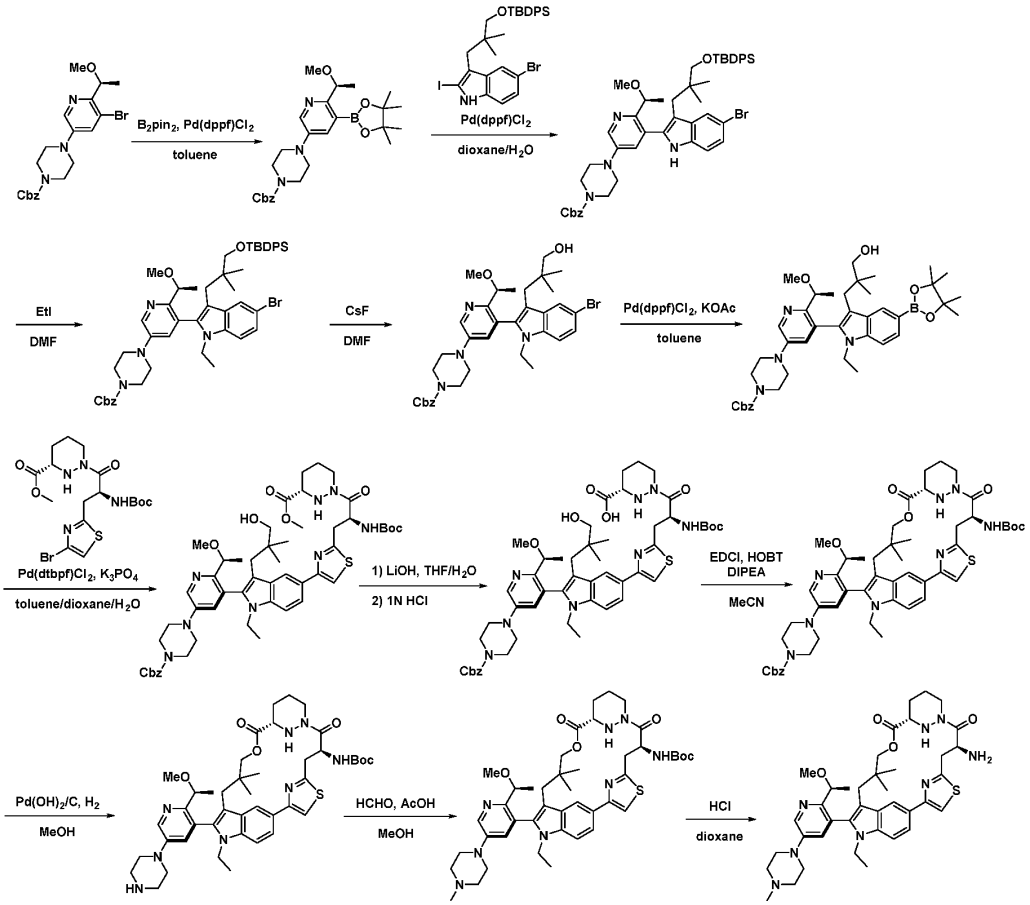
Step 7: Synthesis of *tert*-butyl((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate

To a stirred solution of *tert*-butyl ((6^3*S*, 4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)-5-(piperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate carbamate (590 mg, 0.716 mmol) and HCHO (129 mg, 1.43 mmol, 37 wt% in H_2O) in MeOH (6 ml) at 0 °C were added CH_3COOH (122 mg, 2.02 mmol) and $NaBH_3CN$ (85.3 mg, 1.35 mmol). The resulting mixture was warmed to room temperature and stirred for 2 h. The reaction mixture was then concentrated under reduced pressure and diluted with H_2O (100 mL). The aqueous layer was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with H_2O, dried with Na_2SO_4, filtered, concentrated under reduced pressure. pressure to afford the crude desired product which was used without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{47}H_{63}N_7O_9$: 838.49; found 838.4.

Step 8: Synthesis of (6^3*S*, 4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridine-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione

To a stirred solution of *tert*-butyl((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (590 mg, 0.704 mmol) in DCM (6 mL) at 0 °C was added TFA (3.0 mL). The resulting mixture was stirred for 30 min and then concentrated under reduced pressure to afford the crude desired product which was used without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{42}H_{55}N_7O_5$: 738.44; found 738.4.

Intermediate 27. Synthesis of (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione



Step 1: Synthesis of benzyl (*S*)-4-(5-bromo-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate

Into a 3-L 3-necked round-bottom flask purged and maintained with an inert atmosphere of argon, was placed benzyl 4-[5-bromo-6-[(1*S*)-1-methoxyethyl]pyridin-3-yl]piperazine-1-carboxylate (135 g, 310.821 mmol), bis(pinacolato)diboron (86.82 g, 341.903 mmol), $Pd(dppf)Cl_2$ (22.74 g, 31.082 mmol), KOAc (76.26 g, 777.052 mmol), and toluene (1 L). The resulting solution was stirred for 2 days at 90 °C in an oil bath. The reaction mixture was cooled to room temperature. The resulting mixture was concentrated under reduced pressure. The residue was purified by neutral alumina column chromatography (25% EtOAc/hexanes) to afford the desired product (167 g, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{26}H_{36}BN_3O_5$: 481.3; found 482.1.

Step 2: Synthesis of benzyl (*S*)-4-(5-(5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1H-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate

Into a 3-L 3-necked round-bottom flask purged and maintained with an inert atmosphere of argon, was placed (*S*)-4-(6-(1-methoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl)piperazine-1-carboxylate (167 g, 346.905 mmol), 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-

dimethylpropyl]-2-iodo-1*H*-indole (224.27 g, 346.905 mmol), Pd(dppf)Cl_2 (25.38 g, 34.69 mmol), dioxane (600 mL), H_2O (200 mL), K_3PO_4 (184.09 g, 867.262 mmol), and toluene (200 mL). The resulting solution was stirred for overnight at 70 °C in an oil bath. The reaction mixture was cooled to room temperature after reaction completed. The resulting mixture was concentrated under reduced pressure. The residue was purified by normal phase column chromatography (50% EtOAc/hexanes) to afford the desired product (146 g, 48.2% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{49}H_{57}BrN_4O_4Si$: 872.3; found 873.3.

Step 3: Synthesis of benzyl (*S*)-4-(5-(5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1-ethyl-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate

To a stirred mixture of benzyl (*S*)-4-(5-(5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (146 g, 167.047 mmol) and Cs_2CO_3 (163.28 g, 501.14 mmol) in DMF (1200 mL) was added C_2H_5I (52.11 g, 334.093 mmol) in portions at 0 °C under N_2 atmosphere. The final reaction mixture was stirred at room temperature for 12h. The resulting mixture was diluted with EtOAc (1 L) and washed with brine (3 x 1.5L). The organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford the desired product (143 g, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{51}H_{61}BrN_4O_4Si$: 900.4; found 901.4.

Step 4: Synthesis of benzyl (*S*)-4-(5-(5-bromo-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate

To a stirred mixture of benzyl (*S*)-4-(5-(5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1-ethyl-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (143 g, 158.526 mmol) in DMF (1250 mL) was added CsF (72.24 g, 475.578 mmol). The reaction mixture was stirred at 60 °C for 2 days under N_2 atmosphere. The resulting mixture was diluted with EtOAc (1 L) and washed with brine (3 x 1 L). The organic phase was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to afford two atropisomers of benzyl (*S*)-4-(5-(5-bromo-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate **A** (38 g, 36% yield, RT = 1.677 min in 3 min LCMS(0.1% FA)) and **B** (34 g, 34% yield, RT = 1.578 min in 3 min LCMS(0.1% FA)). LCMS (ESI) *m/z*: [M + H] calcd for $C_{35}H_{43}BrN_4O_4$: 663.2; found 662.2.

Step 5: Synthesis of benzyl (*S*)-4-(5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate

Into a 500-mL 3-necked round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed benzyl (*S*)-4-(5-(5-bromo-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate **A** (14 g, 21.095 mmol), bis(pinacolato)diboron (5.89 g, 23.205 mmol), Pd(dppf)Cl_2 (1.54 g, 2.11 mmol), KOAc (5.18 g, 52.738 mmol), and toluene (150 mL). The resulting solution was stirred for 5 h at 90 °C in an oil bath. The reaction mixture was then cooled to room temperature. The resulting mixture was concentrated under reduced pressure and the residue was purified by silica gel column

chromatography to afford the desired product (12 g, 76.0% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{41}H_{55}BN_4O_6$: 710.4; found 711.3.

Step 6: Synthesis of methyl (*S*)-1-((*S*)-3-(4-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-5-yl)thiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate

Into a 250-mL round-bottom flask purged and maintained with an inert atmosphere of argon, was placed benzyl (*S*)-4-(5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (10.8 g, 15.196 mmol), methyl (3*S*)-1-[(2*S*)-3-(4-bromo-1,3-thiazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoyl]-1,2-diazinane-3-carboxylate (7.98 g, 16.716 mmol), Pd(dtbpf)Cl_2 (0.99 g, 1.52 mmol), K_3PO_4 (8.06 g, 37.99 mmol), toluene (60 mL), dioxane (20 mL), and H_2O (20 mL). The resulting solution was stirred for 3 h at 70 °C in an oil bath. The reaction mixture was then cooled to room temperature. The resulting solution was extracted with EtOAc (2 x 50 mL) and concentrated under reduced pressure. The residue was purified by normal phase column chromatography to afford the desired product (8 g, 50.9% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{52}H_{68}N_8O_9S$: 980.5; found 980.9.

Step 7: Synthesis of (*S*)-1-((*S*)-3-(4-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-5-yl)thiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylic acid

To a stirred mixture of methyl (*S*)-1-((*S*)-3-(4-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-5-yl)thiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (12 g, 12.230 mmol) in THF (100 mL) and H_2O (100 mL) was added LiOH (2.45 g, 61.148 mmol) under N_2 atmosphere and the resulting mixture was stirred for 2 h at room temperature. The resulting mixture was concentrated under reduced pressure and the pH of aqueous phase was acidified to 5 with HCl (1N) at 0 °C. The aqueous layer was extracted with DCM (3 x 100 mL). The organic phase was concentrated under reduced pressure to afford the desired product (10 g, 84.5% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{51}H_{66}N_8O_9S$: 966.5; found 967.0.

Step 8: Synthesis of benzyl 4-(5-((6^3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate

Into a 3-L round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed (*S*)-1-((*S*)-3-(4-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-5-yl)thiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylic acid (18 g, 18.61 mmol), MeCN (1.8 L), DIPEA (96.21 g, 744.417 mmol), EDCI (107.03 g, 558.313 mmol), HOBT (25.15 g, 186.104 mmol). The resulting solution was stirred for overnight at room temperature. The resulting mixture was concentrated under reduced pressure after reaction completed. The resulting solution was diluted with DCM (1 L) and was washed with HCl (3 x 1 L, 1N aqueous). The resulting mixture was washed with H_2O (3 x 1 L) and then the organic layer was concentrated. The residue was

purified by normal phase column chromatography (50% EtOAc/hexanes) to afford the desired product (10.4 g, 54.9% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{51}H_{64}N_8O_8S$: 948.5; found 949.3.

Step 9: Synthesis of *tert*-butyl ((6^3*S*,4S,*Z*)-11-ethyl-12-(2-((*S*)-1-methoxyethyl)-5-(piperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate

Into a 250-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed benzyl 4-(5-((6^3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-11-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-12-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (10.40 g, 10.957 mmol), $Pd(OH)_2$/C (5 g, 46.984 mmol), and MeOH (100 mL). The resulting solution was stirred for 3 h at room temperature under a 2 atm H_2 atmosphere. The solids were filtered out and the filter cake was washed with MeOH (3 x 100 mL). The combined organic phase was concentrated under reduced pressure to afford the desired product (8.5 g, 90.4% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{43}H_{58}N_8O_6S$: 814.4; found 815.3.

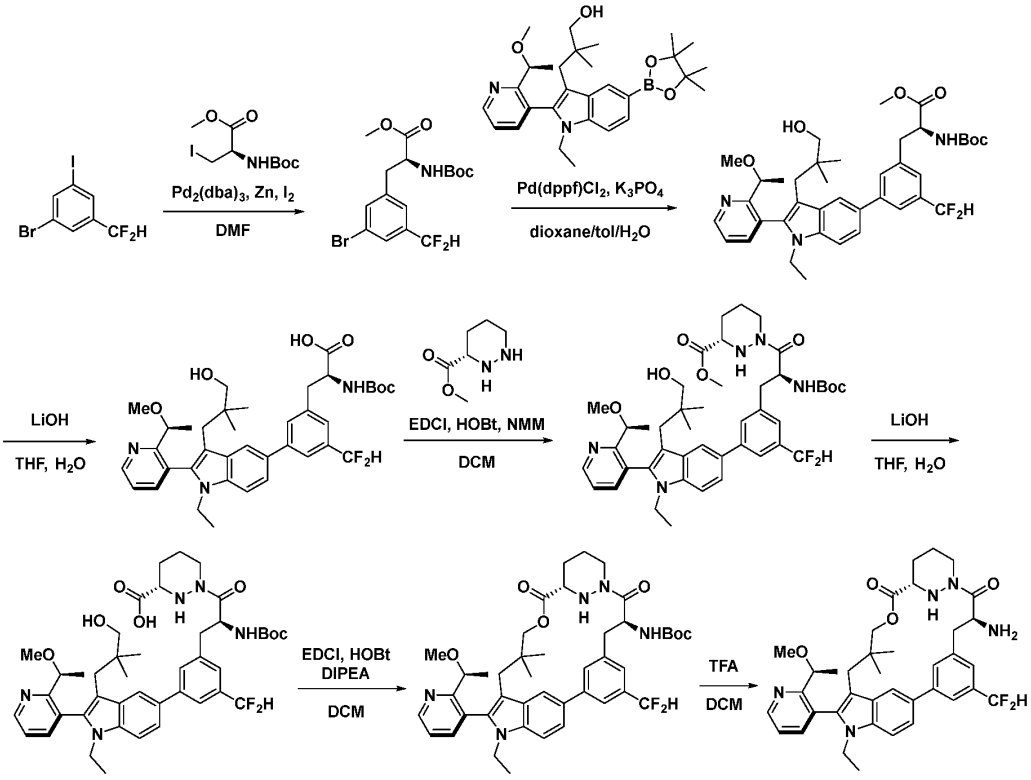
Step 10: Synthesis of *tert*-butyl ((6^3*S*,4*S*,*Z*)-11-ethyl-12-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate

Into a 1000-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed *tert*-butyl ((6^3*S*,4S,*Z*)-11-ethyl-12-(2-((*S*)-1-methoxyethyl)-5-(piperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (8.5 g, 10.429 mmol), MeOH (100 mL), AcOH (1.88 g, 31.286 mmol) and stirred for 15 min. Then HCHO (1.88 g, 23.15 mmol, 37% aqueous solution) and $NaBH_3CN$ (788 mg, 12.5 mmol) was added at room temperature. The resulting solution was stirred for 3 hr. The resulting mixture was quenched with H_2O (100 mL) and concentrated under reduced pressure to remove MeOH. The resulting solution was diluted with DCM (300 mL) and was washed with H_2O (3 x 100 mL). The organic phase was concentrated under reduced pressure to afford the desired product (8.2 g, 90.1% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{44}H_{60}N_8O_6S$: 828.4; found 829.3.

Step 10: Synthesis of (6^3*S*,4*S*,*Z*)-4-amino-11-ethyl-12-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione

Into a 250-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed *tert*-butyl ((6^3*S*,4*S*,*Z*)-11-ethyl-12-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (8.20 g, 9.891 mmol) and dioxane (40 mL), followed by the addition of HCl in 1,4-dioxane (4M, 40 mL) at 0 °C. The resulting solution was stirred for 1 h at 0 °C. The mixture was then concentrated under reduced pressure. The resulting solution was diluted with DCM (600 mL) and sat. aq. $NaHCO_3$ (400 mL). The organic phase was then washed twice with brine (500 mL). The organic phase was concentrated under reduced pressure to afford the desired product (7.2 g, 94.9% yield).

Intermediate 28. Synthesis of (6^3*S*,4*S*)-4-amino-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione



Step 1: Synthesis of methyl (*S*)-3-(3-bromo-5-(difluoromethyl)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanoate

Into a 1000 mL 3-necked round-bottom flask was added Zn powder (43.42 g, 663.835 mmol) and I_2 (1.30 g, 5.106 mmol) in DMF (400 mL) at room temperature. To the above mixture was added a solution of methyl (2*R*)-2-[(*tert*-butoxycarbonyl)amino]-3-iodopropanoate (36.42 g, 110.64 mmol) in DMF (10 mL). The mixture was heated to 30 °C for 10 min. To the mixture was then added a solution of methyl (2*R*)-2-[(*tert*-butoxycarbonyl)amino]-3-iodopropanoate (72.83 g, 221.28 mmol) in DMF (20 mL) dropwise at room temperature. The resulting mixture was stirred for 30 min. The resulting mixture was filtered and the solution was added to a mixture of 1-bromo-3-(difluoromethyl)-5-iodobenzene (85.0 g, 255.321 mmol), tris(furan-2-yl) phosphane (3.56 g, 15.319 mmol), and $Pd_2(dba)_3$ (4.68 g, 5.106 mmol) in DMF (400 mL) at room temperature under argon atmosphere. The reaction mixture was heated to 60 °C for 10 min and was then removed from the oil bath and was stirred for 1 h until the temperature of the resulting mixture cooled down to 50 °C. The reaction was quenched with aq. NH_4Cl (3000 mL) and the aqueous layer was extracted with EtOAc (3 x 1000 mL). The combined organic layers were washed with brine (2 x 1000 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was

purified by silica gel column chromatography (9% EtOAc/pet. ether) to afford the desired product (59 g, 56.6% yield).

Step 2: Synthesis of methyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(difluoromethyl)-5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)phenyl)propanoate

A mixture of methyl (2*S*)-3-[3-bromo-5-(difluoromethyl)phenyl]-2-[(*tert*-butoxycarbonyl)amino] propanoate (90.0 g, 220.459 mmol), (*S*)-3-(1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (1.50 g, 3.046 mmol), $Pd(dppf)Cl_2$ (16.13 g, 22.046 mmol) and K_3PO_4 (116.99 g, 551.148 mmol) in dioxane (600 mL), H_2O (200 mL), and toluene(200 mL) was stirred for 2 h at 70 °C. The resulting mixture was concentrated under reduced pressure and then diluted with H_2O (300 mL). The mixture was extracted with EtOAc (3 x 500 mL). The combined organic layers were washed with H_2O (3 x 500 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (50% EtOAc/pet. ether) to afford the desired product (128 g, 83.7% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{39}H_{49}F_2N_3O_6$: 694.37; found 694.2.

Step 3: Synthesis of (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(difluoromethyl)-5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)phenyl)propanoic acid

To a stirred solution of methyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(difluoromethyl)-5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)phenyl)propanoate (125.0 g, 180.159 mmol) in THF (800 mL) was added $LiOH \bullet H_2O$ (11.48 g, 479.403 mmol) in H_2O (200 mL) dropwise at 0 °C. The resulting mixture was stirred for 2 h at 0 °C. The mixture was acidified to pH 6 with 1 M HCl (aq.) and was then extracted with EtOAc (3 x 800 mL). The combined organic layers were washed with brine (2 x 200 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford the desired product (125 g, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{38}H_{47}F_2N_3O_6$: 680.37; found 680.2.

Step 4: Synthesis of methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(difluoromethyl)-5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylate

To a stirred solution of methyl (3*S*)-1,2-diazinane-3-carboxylate (39.77 g, 275.814 mmol) and NMM (185.98 g, 1838.760 mmol) in DCM (1500 mL) was (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(difluoromethyl)-5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)phenyl)propanoic acid (125.0 g, 183.876 mmol), HOBt (12.42 g, 91.938 mmol) and EDCI (70.50 g, 367.752 mmol) in portions at 0 °C. The resulting mixture was stirred at room temperature for 16 h. The reaction mixture was then washed with 0.5 M HCl (2 x 1000 mL) and brine (2 x 800 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (50% EtOAc/pet.ether) to afford the desired product (110 g, 74.2% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{44}H_{57}F_2N_5O_7$: 806.43; found 806.2.

Step 5: Synthesis of (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(difluoromethyl)-5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylic acid

To a stirred solution of methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(difluoromethyl)-5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylate (110.0 g, 136.482 mmol) in THF (800 mL) was added a solution of $LiOH \bullet H_2O$ (17.18 g, 409.446 mmol) in H_2O (200 mL) in portions at 0 °C. The resulting mixture was stirred for 2 h at 0 °C and was then neutralized to pH 6 with 0.5 M HCl. The resulting mixture was extracted with EtOAc (3 x 800 mL) and the combined organic layers were washed with brine (2 x 600 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (100 g, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{43}H_{55}F_2N_5O_7$: 792.42; found 792.4.

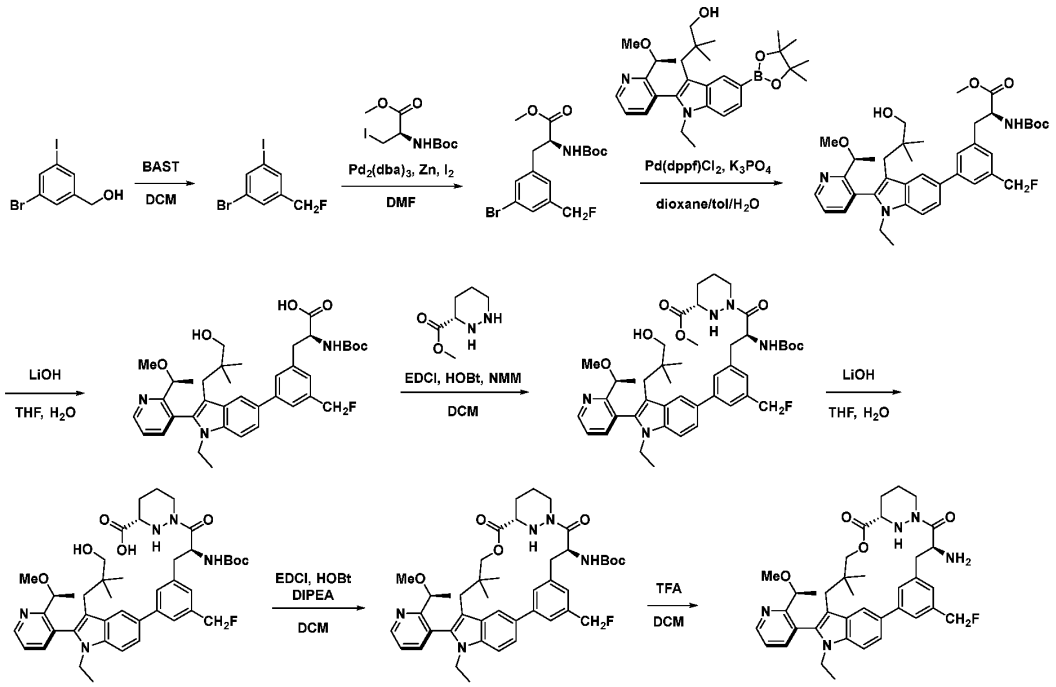
Step 6: Synthesis of *tert*-butyl ((6^3*S*,4*S*)-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate

To a stirred solution of (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(difluoromethyl)-5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylic acid (100.0 g, 126.273 mmol) in DCM (6000 mL) was added DIPEA (163.20 g, 1262.730 mmol), HOBt (85.31 g, 631.365 mmol), and EDCI (363.10 g, 1894.095 mmol) dropwise at 0 °C. The resulting mixture was stirred overnight at room temperature. The mixture was then washed with 0.5 M HCl (2 x2 000 mL) and brine (2 x 2000 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (50% EtOAc/pet. ether) to afford the desired product (70 g, 71.6% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{43}H_{53}F_2N_5O_6$: 774.41; found 774.0.

Step 7: Synthesis of (6^3*S*,4*S*)-4-amino-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione

To a stirred solution of *tert*-butyl ((6^3*S*,4*S*)-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (202.0 mg, 0.261 mmol) in DCM (2 mL) was added TFA (1.0 mL) dropwise at 0 °C. The resulting mixture was stirred for 1.5 h at 0 °C and was then concentrated under reduced pressure to afford the desired product. LCMS (ESI) *m/z*: [M + H] calcd for $C_{38}H_{45}F_2N_5O_4$: 674.35; found 674.5.

Intermediate 29. Synthesis of (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-(fluoromethyl)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione



Step 1: Synthesis of 1-bromo-3-(fluoromethyl)-5-iodobenzene

To a solution of (3-bromo-5-iodophenyl)methanol (175.0 g, 559.227 mmol) in DCM (2 L) was added BAST (247.45 g, 1118.454 mmol) dropwise at 0 °C. The resulting mixture was stirred for 16 h at room temperature. The reaction was quenched with sat. aq. $NaHCO_3$ at 0 °C. The organic layers were washed with H_2O (3 x 700 mL) and dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (3% EtOAc/pet. ether) to afford the desired product (120 g, 68% yield).

Step 2: Synthesis of methyl (2*S*)-3-[3-bromo-5-(fluoromethyl)phenyl]-2-[(*tert*-butoxycarbonyl)amino]propanoate

Into a 1000 mL 3-necked round-bottom flask was added Zn powder (32.40 g, 495.358 mmol) in DMF (350.0 mL) and I_2 (967.12 mg, 3.810 mmol). To the mixture was added a solution of methyl (2*R*)-2-[(*tert*-butoxycarbonyl)amino]-3-iodopropanoate (27.0 g, 82.03 mmol) in DMF (10 mL). The mixture was heated to 30 °C for 10 min. To the mixture was then added a solution of methyl (2*R*)-2-[(*tert*-butoxycarbonyl)amino]-3-iodopropanoate (54.0 g, 164.07 mmol) in DMF (20 mL). The resulting mixture was stirred for 30 min at room temperature and was filtered. The resulting solution was added to a mixture of 1-bromo-3-(fluoromethyl)-5-iodobenzene (60 g, 190.522 mmol), tris(furan-2-yl)phosphane (2.65 g, 11.431 mmol), and $Pd_2(dba)_3$ (3.49 g, 3.810 mmol) in DMF (400 mL) at room temperature under argon atmosphere and the reaction mixture was heated to 60 °C for 10 min then removed the oil bath. The resulting mixture was stirred for about 1 h until the temperature cooled down to 50 °C. The reaction was quenched with aq. NH_4Cl (3000 mL) and the

resulting mixture was extracted with EtOAc (3 x 1000 mL). The combined organic layers were washed with brine (2x 1000 mL) and dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (9% EtOAc/pet. ether) to afford the desired product (45 g, 60% yield).

Step 3: Synthesis of methyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin -3-yl)-1H-indol-5-yl)-5-(fluoromethyl)phenyl)propanoate

A mixture of methyl (2*S*)-3-[3-bromo-5-(fluoromethyl)phenyl]-2-[(*tert*-butoxycarbonyl)amino]propanoate (75.28 g, 192.905 mmol), (*S*)-3-(1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2- dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (95 g, 192.905 mmol), $Pd(dppf)Cl_2$ (14.11 g, 19.291 mmol) and K_2CO_3 (53.32 g, 385.810 mmol) in dioxane (900 mL) and H_2O (180 mL) was stirred for 2 h at 80 °C. The resulting mixture was concentrated under reduced pressure and was then diluted with H_2O. The resulting mixture was extracted with EtOAc (3 x 1200 mL) and the combined organic layers were washed with H_2O (3 x 500 mL) and dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (50% EtOAc/pet. ether) to afford the desired product (105 g, 80% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{39}H_{50}FN_3O_6$: 676.38; found 676.1.

Step 4: Synthesis of (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)-5-(fluoromethyl)phenyl)propanoic acid

To a stirred solution of methyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin -3-yl)-1H-indol-5-yl)-5-(fluoromethyl)phenyl)propanoate (108 g, 159.801 mmol) in THF (500 mL) was added a solution of $LiOH \bullet H_2O$ (11.48 g, 479.403 mmol) in H_2O (500 mL) at 0 °C. The resulting mixture was stirred for 2 h at 0 °C and was then acidified to pH 6 with 1 M HCl (aq.). The mixture was extracted with EtOAc (3 x 800 mL) and the combined organic layers were washed with brine (2x 200 mL) and dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford the desired product (101 g, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{38}H_{48}FN_3O_6$: 662.36; found 662.1.

Step 5: Synthesis of methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)-5-(fluoromethyl)phenyl)propanoyl)hexahydropyridazine-3-carboxylate

To a stirred solution of (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)-5-(fluoromethyl)phenyl)propanoic acid (103 g, 155.633 mmol) and NMM (157.42 g, 1556.330 mmol) in DCM (1200 mL) was added methyl (3*S*)-1,2- diazinane-3-carboxylate (33.66 g, 233.449 mmol), HOBt (10.51 g, 77.816 mmol) and EDCI (59.67 g, 311.265 mmol) in portions at 0 °C. The resulting mixture was stirred a t room temperature for 16 h. The organic layers were then washed with 0.5 M HCl (2 x 1000 mL) and brine (2 x 800 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated

under reduced pressure. The residue was purified by silica gel column chromatography (50% EtOAc/pet. ether) to afford the desired product (103 g, 83% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{44}H_{58}FN_5O_7$: 788.44; found 788.1.

Step 6: Synthesis of (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)-5-(fluoromethyl)phenyl)propanoyl)hexahydropyridazine-3-carboxylic acid

To a stirred solution of methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)-5-(fluoromethyl)phenyl)propanoyl)hexahydropyridazine-3-carboxylate (103 g, 130.715 mmol) in THF (700 mL) was added a solution of $LiOH{\bullet}H_2O$ (27.43 g, 653.575 mmol) in H_2O (700 mL) at 0 °C .The resulting mixture was stirred for 2 h at 0 °C and was then neutralized to pH 6 with 1 M HCl.The resulting mixture was extracted with EtOAc (3 x 800 mL) and the combined organic layers were washed with brine (2 x 600 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (101 g, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{43}H_{56}FN_5O_7$: 774.43; found 774.1.

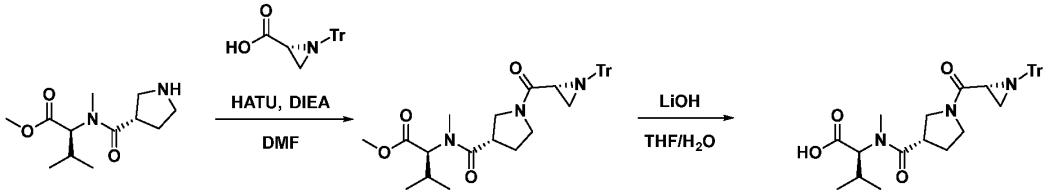
Step 7: Synthesis of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-2^5-(fluoromethyl)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl) -10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate

To a stirred solution of (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(3-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)-5-(fluoromethyl)phenyl)propanoyl)hexahydropyridazine-3-carboxylic acid (101 g, 130.50 mmol) in DCM (5500 mL) was added DIPEA (227.31 mL, 1305.0 mmol) and HOBt (88.17 g, 652.499 mmol), and EDCI (375.26 g, 1957.498 mmol) at 0 °C. The resulting mixture was stirred at room temperature overnight. The mixture was then washed with 0.5 M HCl (2 x 2000 mL), brine (2 x 2000 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (50% EtOAc/pet. ether) to afford the desired product (68 g, 65% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{43}H_{54}FN_5O_6$: 756.42; found 756.4.

Step 8: Synthesis of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-(fluoromethyl)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide

To a stirred solution of *tert*-butyl ((6^3*S*,4*S*)-1^1-ethyl-2^5-(fluoromethyl)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl) -10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (350 mg, 0.403 mmol) in DCM (4 mL) was added TFA (1.50 mL) at 0 °C. The resulting mixture was stirred at room temperature for 1.5 h and was then concentrated under reduced pressure to afford the desired product (600 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{38}H_{46}FN_5O_4$: 656.36; found 656.4.

Intermediate A-1. Synthesis of *N*-methyl-*N*-((*S*)-1-((*R*)-1-tritylaziridine-2-carbonyl)pyrrolidine-3-carbonyl)-*L*-valine



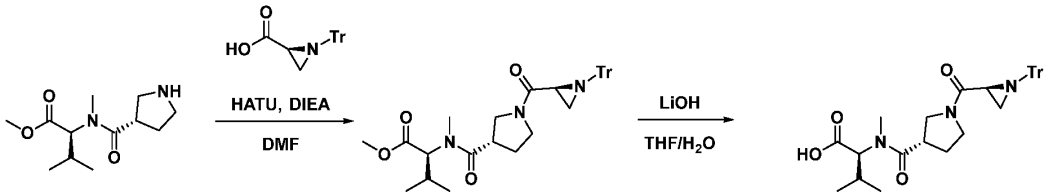
Step 1: Synthesis of methyl *N*-methyl-*N*-((*S*)-1-((*R*)-1-tritylaziridine-2-carbonyl)pyrrolidine-3-carbonyl)-*L*-valinate

To a mixture of methyl *N*-methyl-*N*-((*S*)-pyrrolidine-3-carbonyl)-*L*-valinate (0.840 g, 3.47 mmol) and (*R*)-1-tritylaziridine-2-carboxylic acid (1.713 g, 5.2 mmol) in DMF (20 mL) at 0 °C was added DIPEA (3.0 mL, 17.33 mmol) and HATU (2.636 g, 6.93 mmol). The reaction mixture was stirred for 3 h, at which point the mixture was extracted with EtOAc (200 mL). The EtOAc layer was washed with brine (3 x 50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by reverse phase chromatography (10→50% MeCN/H_2O) to afford the desired product (1.02 g, 53% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{34}H_{39}N_3O_4$: 554.30; found 554.3.

Step 2: Synthesis of *N*-methyl-*N*-((*S*)-1-((*R*)-1-tritylaziridine-2-carbonyl)pyrrolidine-3-carbonyl)-*L*-valine

To a solution of methyl *N*-methyl-*N*-((*S*)-1-((*R*)-1-tritylaziridine-2-carbonyl)pyrrolidine-3-carbonyl)-*L*-valinate (1.0 g, 1.81 mmol) in THF (10 mL) at 0 °C was added a solution of LiOH•H_2O (0.3789 g, 9.03 mmol) in H_2O (9.0 mL). After 3 h, the reaction solution was neutralized to pH 7 with sat. aq. NH_4Cl. The resulting mixture was extracted with EtOAc (3 x 50 mL) and the combined organic layers were washed with brine (3 x 20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the crude product (740 mg, 75.9% yield) as a solid. LCMS (ESI) *m/z:* [M - H] calcd for $C_{33}H_{37}N_3O_4$: 538.27; found 538.2.

Intermediate A-2. Synthesis of *N*-methyl-*N*-((*S*)-1-((*S*)-1-tritylaziridine-2-carbonyl)pyrrolidine-3-carbonyl)-*L*-valine



Step 1: Synthesis of methyl *N*-methyl-*N*-((*S*)-1-((*S*)-1-tritylaziridine-2-carbonyl)pyrrolidine-3-carbonyl)-*L*-valinate

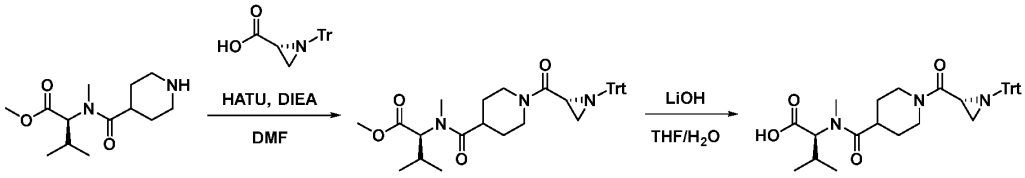
To a mixture of methyl *N*-methyl-*N*-((*S*)-pyrrolidine-3-carbonyl)-*L*-valinate (0.800 g, 3.30 mmol) and (*S*)-1-tritylaziridine-2-carboxylic acid (1.305 g, 3.96 mmol) in DMF (16 mL) at 0 °C was

added DIPEA (2.9 mL, 16.5 mmol) and HATU (1.88 g, 4.9 mmol). The reaction mixture was warmed to room temperature and stirred for 1 h, at which point the mixture was diluted with EtOAc. The mixture was washed with sat. NH_4Cl and the resulting aqueous layer extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by reverse phase chromatography (10→80% MeCN/H_2O) to afford the desired product (1.17 g, 64% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{34}H_{39}N_3O_4$: 554.30; found 554.3.

Step 2: Synthesis of *N*-methyl-*N*-((*S*)-1-((*S*)-1-tritylaziridine-2-carbonyl)pyrrolidine-3-carbonyl)-*L*-valine

To a stirred solution of methyl *N*-methyl-*N*-((*S*)-1-((*S*)-1-tritylaziridine-2-carbonyl)pyrrolidine-3-carbonyl)-*L*-valinate (1.10 g, 1.99 mmol) in THF (10.0 mL) at 0 °C was added a 1M solution of LiOH
(9.93 mL, 9.93 mmol). The reaction mixture was warmed to room temperature and stirred for 16 h. The reaction mixture was cooled to 0 °C and quenched with sat. aq. NH_4Cl to pH 6. The resulting mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired crude product (1.2 g). LCMS (ESI) *m/z:* [M + H] calcd for $C_{33}H_{37}N_3O_4$: 540.29; found 540.3.

Intermediate A-3. Synthesis of *N*-methyl-N-(1-((*R*)-1-tritylaziridine-2-carbonyl)piperidine-4-carbonyl)-*L*-valine



Step 1: Synthesis of methyl *N*-methyl-*N*-(1-((*R*)-1-tritylaziridine-2-carbonyl)piperidine-4-carbonyl)-*L*-valinate

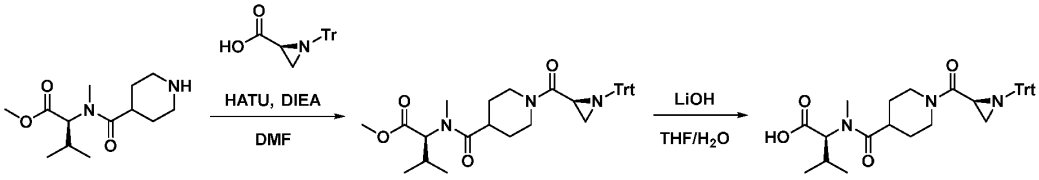
To a solution of (*R*)-1-tritylaziridine-2-carboxylic acid (1.157 g, 3.51 mmol) and methyl *N*-methyl-*N*-(piperidine-4-carbonyl)-*L*-valinate (0.600 g, 2.34 mmol) in DMF (20 mL) at 0 °C was added DIPEA (0.204 mL, 11.70 mmol) and HATU (1.780 g, 4.68 mmol. After 3 h, the reaction mixture was extracted with EtOAc (200 mL). The combined organic layers were washed with brine (3 x 50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by reverse phase chromatography (10→50% MeCN/H_2O) to afford the desired product

(740 mg, 55.7% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{35}H_{41}N_3O_4$: 568.32; found 568.3.

Step 2: Synthesis of *N*-methyl-*N*-(1-((*R*)-1-tritylaziridine-2-carbonyl)piperidine-4-carbonyl)-*L*-valine

To a solution of methyl *N*-methyl-*N*-(1-((*R*)-1-tritylaziridine-2-carbonyl)piperidine-4-carbonyl)-*L*-valinate (0.700 g, 1.23 mmol) in THF (7.0 mL) at 0 °C was added a solution of LiOH•H_2O (0.259 g, 6.17 mmol) in H_2O (6.0 mL). The resulting solution was warmed to room temperature and stirred for 3 h. The reaction mixture was diluted with EtOAc (100 mL) and was washed with sat. brine (5 x 50 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the crude product (700 mg) as a solid. LCMS (ESI) *m/z:* [M - H] calcd for $C_{34}H_{39}N_3O_4$: 552.29; found 552.2.

Intermediate A-4. Synthesis of *N*-methyl-N-(1-((*S*)-1-tritylaziridine-2-carbonyl)piperidine-4-carbonyl)-*L*-valine.



Step 1: Synthesis of methyl *N*-methyl-*N*-(1-((*S*)-1-tritylaziridine-2-carbonyl)piperidine-4-carbonyl)-*L*-valinate

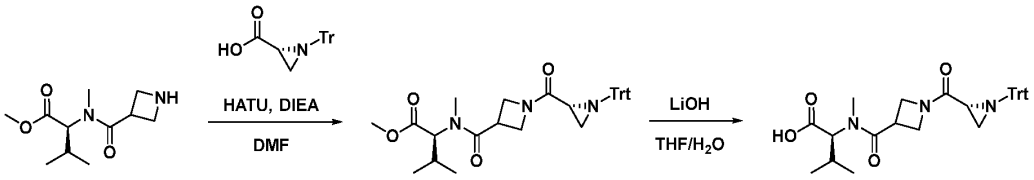
To a solution of methyl *N*-methyl-*N*-(piperidine-4-carbonyl)-*L*-valinate (0.550 g, 2.15 mmol) and (*S*)-1-tritylaziridine-2-carboxylic acid (0.848 g, 2.57 mmol) in DMF (10.0 mL) at 0 °C was added DIPEA (1.9 mL, 10.7 mmol) and HATU (1.2 g, 3.2 mmol). The reaction mixture was warmed to room temperature and stirred for 1 h. The reaction mixture was diluted with EtOAc (50 mL) and washed with sat. NH_4Cl (60 mL). The aqueous layer was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (200 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by reverse phase chromatography (10→80% MeCN/H_2O) to afford the desired product (1.2 g, 98.5% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{35}H_{41}N_3O_4$: 568.32; found 568.3.

Step 2: Synthesis of *N*-methyl-*N*-(1-((*S*)-1-tritylaziridine-2-carbonyl)piperidine-4-carbonyl)-*L*-valine

To a solution of methyl *N*-methyl-*N*-(1-((*S*)-1-tritylaziridine-2-carbonyl)piperidine-4-carbonyl)-*L*-valinate (1.20 g, 2.11 mmol) in THF (11.0 mL) at 0 °C was added 1M LiOH (10.57 mL, 10.57 mmol). The resulting solution was warmed to room temperature and stirred for 16 h. The reaction mixture was cooled to 0 °C and quenched with sat. NH_4Cl until pH 6. The resulting mixture was extracted with EtOAc

(3 x 50 mL) and the combined organic layers were washed with brine (3 x 50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the crude product (900 mg). LCMS (ESI) *m/z:* [M - H] calcd for $C_{34}H_{39}N_3O_4$: 554.29; found 554.3.

Intermediate A-5. Synthesis of *N*-methyl-*N*-(1-((*R*)-1-tritylaziridine-2-carbonyl)azetidine-3-carbonyl)-*L*-valine



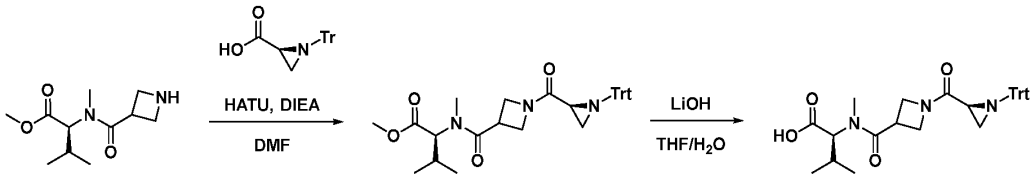
Step 1: Synthesis of methyl *N*-methyl-*N*-(1-((*R*)-1-tritylaziridine-2-carbonyl)azetidine-3-carbonyl)-*L*-valinate

To a solution of methyl *N*-(azetidine-3-carbonyl)-*N*-methyl-*L*-valinate (0.410 g, 1.79 mmol) and (*R*)-1-tritylaziridine-2-carboxylic acid (0.887 g, 2.69 mmol) in DMF (10 mL) at 0 °C was added DIPEA (1.56 mL, 8.98 mmol) and HATU (1.37 g, 3.59 mmol). The reaction mixture was stirred for 1 h. The resulting mixture was then extracted with EtOAc (3 x 50 mL) and the combined organic layers were washed with brine (3 x 20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by reverse phase chromatography (10→80% MeCN/H_2O) to afford the desired product (650 mg, 67% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{33}H_{37}N_3O_4$: 540.29; found 540.3.

Step 2: Synthesis of *N*-methyl-*N*-(1-((*R*)-1-tritylaziridine-2-carbonyl)azetidine-3-carbonyl)-*L*-valine

To a solution of methyl *N*-methyl-*N*-(1-((*R*)-1-tritylaziridine-2-carbonyl)azetidine-3-carbonyl)-*L*-valinate (0.650 mg, 1.20 mmol) in THF (10 mL) at 0 °C was added a 1M solution of LiOH•H_2O (6.03 mL). The reaction mixture was stirred for 3 h. The resulting mixture was then quenched with sat. NH_4Cl until pH 7. The resulting mixture was extracted with EtOAc (3 x 20 mL) and the combined organic layers were washed with brine (3 x 20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired crude product (588 mg) as a solid. LCMS (ESI) *m/z:* [M - H] calcd for $C_{32}H_{35}N_3O_4$: 526.27; found 526.3.

Intermediate A-6. Synthesis of *N*-methyl-*N*-(1-((*S*)-1-tritylaziridine-2-carbonyl)azetidine-3-carbonyl)-*L*-valine



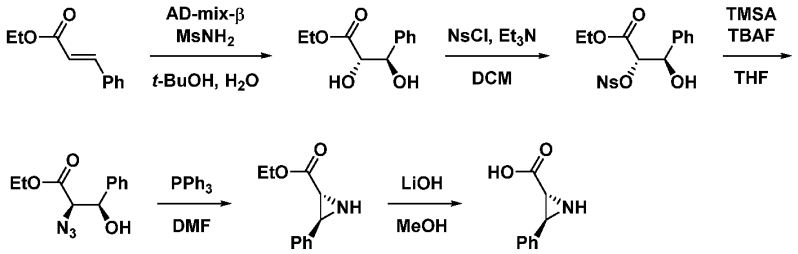
Step 1: Synthesis of methyl *N*-methyl-*N*-(1-((*S*)-1-tritylaziridine-2-carbonyl)azetidine-3-carbonyl)-*L*-valinate

To a solution of methyl *N*-(azetidine-3-carbonyl)-*N*-methyl-*L*-valinate (0.550 g, 2.41 mmol) and (*S*)-1-tritylaziridine-2-carboxylic acid (0.952 g, 2.89 mmol) in DMF (10 mL) at 0 °C was added DIPEA (2.1 mL, 12.05 mmol) and HATU (1.37 g, 3.61 mmol). The reaction mixture was warmed to room temperature and stirred for 1 h. The resulting mixture was diluted with EtOAc (50 mL) and washed with sat. NH_4Cl (60 mL). The aqueous layer was then extracted with EtOAc (3 x 50 mL) and the combined organic layers were washed with brine (200 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by reverse phase chromatography (10→80% MeCN/H_2O) to afford the desired product (820 mg, 63% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{33}H_{37}N_3O_4$: 540.29; found 540.3.

Step 2: Synthesis of *N*-methyl-*N*-(1-((*S*)-1-tritylaziridine-2-carbonyl)azetidine-3-carbonyl)-*L*-valine

To a solution of methyl *N*-methyl-*N*-(1-((*S*)-1-tritylaziridine-2-carbonyl)azetidine-3-carbonyl)-*L*-valinate (0.800 g, 1.48 mmol) in THF (8.0 mL) at 0 °C was added 1M LiOH (7.41 mL, 7.41 mmol). The reaction mixture was warmed to room temperature and stirred for 16 h and was then cooled to 0 °C and quenched with sat. NH_4Cl until pH 6. The resulting mixture was extracted with EtOAc (3 x 50 mL) and the combined organic layers were washed with brine (150 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by reverse phase chromatography (10→80% MeCN/H_2O+0.5% NH_4HCO_3) to afford the desired product (440 mg, 56% yield) as a solid. LCMS (ESI) *m/z:* [M - H] calcd for $C_{32}H_{35}N_3O_4$: 524.25; found 524.2.

Intermediate A-7. Synthesis of (2*R*,3*S*)-3-phenylaziridine-2-carboxylic acid



Step 1: Synthesis of ethyl (2*S*,3*R*)-2,3-dihydroxy-3-phenylpropanoate

To a solution of ethyl cinnamate (2.0 g, 11.4 mmol) in *t*-BuOH (35.0 mL) and H_2O (35.0 mL) at 0 °C was added AD-mix-β (15.83 g, 20.32 mmol), and methanesulfonamide (1.08 g, 11.3 mmol). The reaction mixture was stirred at room temperature for 16 h. The reaction was cooled to 0 °C and quenched with aq. $KHSO_4$. The resulting mixture was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with brine (2 x 90 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by normal phase chromatography (50% EtOAc/pet. ether) to afford the desired product (2.2 g, 82% yield) as a solid.

Step 2: Synthesis of ethyl (2*S*,3*R*)-3-hydroxy-2-(((4-nitrophenyl)sulfonyl)oxy)-3-phenylpropanoate

To a solution of ethyl (2*S*,3*R*)-2,3-dihydroxy-3-phenylpropanoate (2.0 g, 9.5 mmol) and Et_3N (3.97 mL, 28.5 mmol) in DCM (30.0 mL) at 0 °C was added 4-nitrobenzenesulfonyl chloride (2.11 g, 9.51 mmol). The resulting mixture was stirred for 1 h and was then diluted with H_2O (300 mL). The mixture was extracted with DCM (3 x 100 mL) and the combined organic layers were washed with brine (2 x 100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by prep-TLC (50% EtOAc/pet. ether) to afford the desired product (2.8 g, 67% yield) as a solid.

Step 3: Synthesis of ethyl (2*R*,3*R*)-2-azido-3-hydroxy-3-phenylpropanoate

To a solution of ethyl (2*S*,3*R*)-3-hydroxy-2-(((4-nitrophenyl)sulfonyl)oxy)-3-phenylpropanoate (2.80 g, 7.08 mmol) in THF (30 mL) at room temperature was added trimethylsilyl azide (1.63 g, 14.2 mmol) and TBAF (1M in THF, 14.16 mL, 14.16 mmol). The reaction mixture was heated to 60 °C and was stirred for 16 h. The reaction mixture was then cooled to room temperature, diluted with H_2O (150 mL), and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (2 x 30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by normal phase chromatography (50% EtOAc/pet. ether) to afford the desired product (1.2 g, 64% yield) as an oil.

Step 4: Synthesis of ethyl (2*R*,3*S*)-3-phenylaziridine-2-carboxylate

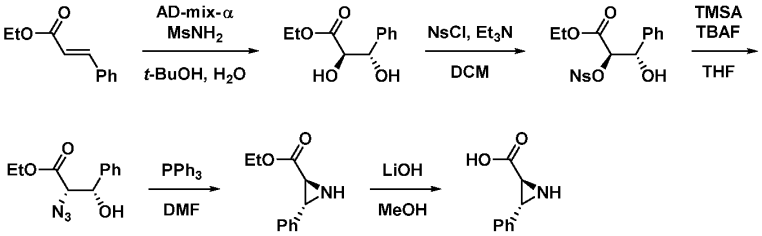
To a solution of ethyl (2*R*,3*R*)-2-azido-3-hydroxy-3-phenylpropanoate (1.20 g, 5.10 mmol) in DMF (15.0 mL) was added PPh_3 (1.61 g, 6.12 mmol). The reaction mixture was stirred at room

temperature for 30 min and then heated to 80 °C for an additional 16 h. The reaction mixture was then cooled to room temperature, diluted with H_2O (100 mL), and extracted with EtOAc (3 x 40 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO, filtered, and concentrated under reduced pressure. The residue was purified by normal phase chromatography (16% EtOAc/pet. ether) to afford the desired product (620 mg, 57% yield) as an oil. LCMS (ESI) *m/z:* [M + H] calcd for $C_{11}H_{13}NO_2$: 192.10; found 192.0.

Step 5: Synthesis of (2*R*,3*S*)-3-phenylaziridine-2-carboxylic acid

To a solution of ethyl (2*R*,3*S*)-3-phenylaziridine-2-carboxylate (0.100 g, 0.523 mmol) in MeOH (0.70 mL) at 0 °C was added a solution of LiOH (18.8 mg, 0.784 mmol) in H_2O (0.70 mL). The reaction mixture was stirred for 1 h. The mixture was then diluted with MeCN (10 mL), and the resulting precipitate was collected by filtration and washed with MeCN (2 x 10 mL) to afford the crude desired product (70 mg) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_9H_9NO_2$: 164.07; found 164.0.

Intermediate A-8. Synthesis of (2*S*,3*R*)-3-phenylaziridine-2-carboxylic acid



Step 1: Synthesis of ethyl (2*R*,3*S*)-2,3-dihydroxy-3-phenylpropanoate

To a solution of ethyl cinnamate (2.0 g, 11.4 mmol) in *t*-BuOH (35.0 mL) and H_2O (35.0 mL) at 0 °C was added AD-mix-α (15.83 g, 20.32 mmol), and methanesulfonamide (1.08 g, 11.3 mmol). The reaction mixture was stirred at room temperature for 16 h. The reaction was cooled to 0 °C and quenched with aq. $KHSO_4$. The resulting mixture was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with brine (2 x 80 mL), dried over Na_2SO_4, filtered, and

concentrated under reduced pressure. The residue was purified by normal phase chromatography (50% EtOAc/pet. ether) to afford the desired product (2.2 g, 82% yield) as a solid.

Step 2: Synthesis of ethyl (2*R*,3*S*)-3-hydroxy-2-(((4-nitrophenyl)sulfonyl)oxy)-3-phenylpropanoate

To a solution of ethyl (2*R*,3*S*)-2,3-dihydroxy-3-phenylpropanoate (2.10 g, 9.99 mmol) and Et_3N (4.18 mL, 29.9 mmol) in DCM (30.0 mL) at 0 °C was added 4-nitrobenzenesulfonyl chloride (2.21 g,
9.99 mmol). The resulting mixture was stirred for 1 h and was then diluted with H_2O (200 mL). The mixture was extracted with DCM (3 x 80 mL) and the combined organic layers were washed with brine
(2 x 80 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by prep-TLC (50% EtOAc/pet. ether) to afford the desired product (3.0 g, 68% yield) as a solid.

Step 3: Synthesis of ethyl (2*S*,3*S*)-2-azido-3-hydroxy-3-phenylpropanoate

To a solution of ethyl (2*R*,3*S*)-3-hydroxy-2-(((4-nitrophenyl)sulfonyl)oxy)-3-phenylpropanoate (3.0 g, 7.59 mmol) in THF (30 mL) at room temperature was added trimethylsilyl azide (1.75 g, 15.2 mmol) and TBAF (1M in THF, 15.18 mL, 15.18 mmol). The reaction mixture was heated to 60 °C and was stirred for 16 h. The reaction mixture was then cooled to room temperature, diluted with H_2O (150 mL), and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (2 x 30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by normal phase chromatography (50% EtOAc/pet. ether) to afford the desired product (1.4 g, 70% yield) as an oil.

Step 4: Synthesis of ethyl (2*S*,3*R*)-3-phenylaziridine-2-carboxylate

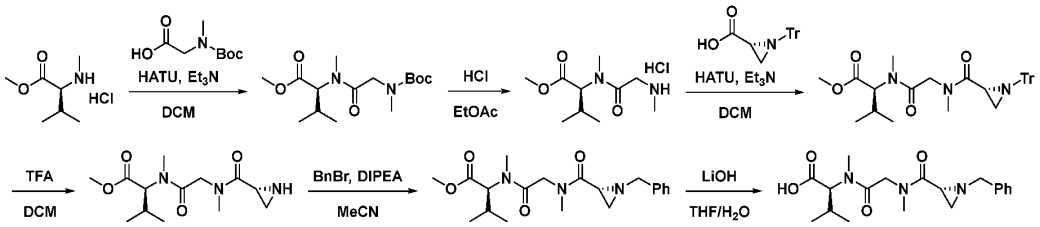
To a solution of ethyl (2*S*,3*S*)-2-azido-3-hydroxy-3-phenylpropanoate (1.40 g, 5.95 mmol) in DMF (20.0 mL) was added PPh_3 (1.87 g, 7.14 mmol). The reaction mixture was stirred at room temperature for 30 min and then heated to 80 °C for an additional 16 h. The reaction mixture was then cooled to room temperature, diluted with H_2O (150 mL), and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (40 mL), dried over Na_2SO, filtered, and concentrated under reduced pressure. The residue was purified by normal phase chromatography (16% EtOAc/pet. ether) to afford the desired product (720 mg, 56% yield) as an oil. LCMS (ESI) *m/z:* [M + H] calcd for $C_{11}H_{13}NO_2$: 192.10; found 192.0.

Step 5: Synthesis of (2*S*,3*R*)-3-phenylaziridine-2-carboxylic acid

To a solution of ethyl (2*S*,3*R*)-3-phenylaziridine-2-carboxylate (0.100 g, 0.523 mmol) in MeOH (0.70 mL) at 0 °C was added a solution of LiOH (18.8 mg, 0.784 mmol) in H_2O (0.70 mL). The reaction mixture was stirred for 1 h. The mixture was then diluted with MeCN (10 mL), and the resulting precipitate was collected by filtration and washed with MeCN (2 x 10 mL) to afford the

crude desired product (68 mg) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_9H_9NO_2$: 164.07; found 164.0.

Intermediate A-9. Synthesis of *N*-(*N*-((*R*)-1-benzylaziridine-2-carbonyl)-*N*-methylglycyl)-*N*-methyl-*L*-valine



Step 1: Synthesis of methyl *N*-(*N*-(*tert*-butoxycarbonyl)-*N*-methylglycyl)-*N*-methyl-*L*-valinate

To a solution of methyl methyl-*L*-valinate hydrochloride (4.0 g, 22.0 mmol) and *N*-(*tert*-butoxycarbonyl)-*N*-methylglycine (5.0 g, 26.4 mmol) in DCM (100.0 mL) was added Et_3N (9.2 mL, 66.1 mmol) and HATU (10.88 g, 28.63 mmol). The reaction mixture was stirred for 4 h. The reaction was then neutralized to pH 7 with sat. aq. $NaHCO_3$. The mixture was extracted with DCM and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by normal phase chromatography (50% EtOAc/pet. ether) to afford the desired product (6.2 g, 89% yield) as an oil. LCMS (ESI) *m/z:* [M + H] calcd for $C_{15}H_{28}N_2O_5$: 317.21; found 317.2.

Step 2: Synthesis of methyl *N*-methyl-*N*-(methylglycyl)-*L*-valinate hydrochloride

To a solution of methyl *N*-(*N*-(*tert*-butoxycarbonyl)-*N*-methylglycyl)-*N*-methyl-*L*-valinate (4.97 g, 15.7 mmol) in EtOAc (150.0 mL) at 0 °C was added HCl (4M in dioxane, 50.0 mL, 200 mmol). The reaction mixture was stirred for 3 h and then concentrated under reduced pressure to afford the desired crude product (4.26 g, 107% yield) as an oil. LCMS (ESI) *m/z:* [M + H] calcd for $C_{10}H_{20}N_2O_3$: 217.16; found 217.1.

Step 3: Synthesis of methyl *N*-methyl-*N*-(*N*-methyl-*N*-((*R*)-1-tritylaziridine-2-carbonyl)glycyl)-*L*-valinate

To a solution of methyl *N*-methyl-*N*-(methylglycyl)-*L*-valinate hydrochloride (1.0 g, 3.9 mmol) and (*R*)-1-tritylaziridine-2-carboxylic acid (1.30 g, 3.94 mmol) in DCM (25.0 mL) was added Et_3N (2.76 mL, 19.8 mmol) and HATU (1.81 g, 4.76 mmol). The reaction mixture was stirred for 1 h. The reaction was then neutralized to pH 7 with sat. aq. $NaHCO_3$. The mixture was extracted with DCM and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by normal phase chromatography (50% EtOAc/pet. ether) to afford the desired product (1.1 g, 52.6% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{32}H_{37}N_3O_4$: 528.29; found 528.2.

Step 4: Synthesis of methyl *N*-(*N*-((*R*)-aziridine-2-carbonyl)-*N*-methylglycyl)-*N*-methyl-*L*-valinate

To a solution methyl *N*-methyl-*N*-(*N*-methyl-*N*-((*R*)-1-tritylaziridine-2-carbonyl)glycyl)-*L*-valinate (1.0 g, 3.9 mmol) in DCM (6 mL) at 0 °C was added TFA (2 mL). The reaction mixture was

warmed to room temperature and stirred for 1 h, then concentrated under reduced pressure to afford the desired crude product (250 mg) as an oil. LCMS (ESI) *m/z:* [M + H] calcd for $C_{13}H_{23}N_3O_4$: 286.18; found 286.1.

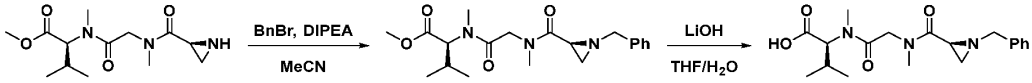
Step 5: Synthesis of methyl *N*-(*N*-((*R*)-1-benzylaziridine-2-carbonyl)-*N*-methylglycyl)-*N*-methyl-*L*-valinate

To a solution of methyl *N*-(*N*-((*R*)-aziridine-2-carbonyl)-*N*-methylglycyl)-*N*-methyl-*L*-valinate (220.0 mg, 0.771 mmol) in MeCN (2.0 mL) was added DIPEA (537 µL, 3.08 mmol) and benzyl bromide (101 µL, 0.848 mmol). The reaction mixture was stirred for 6 h. The reaction mixture was then concentrated under reduced pressure. The residue was purified by prep-TLC (9% MeOH/DCM) to afford the desired product (261 mg, 90% yield) as an oil. LCMS (ESI) *m/z:* [M + H] calcd for $C_{20}H_{29}N_3O_4$: 376.22; found 376.2.

Step 6: Synthesis of *N*-(*N*-((*R*)-1-benzylaziridine-2-carbonyl)-*N*-methylglycyl)-*N*-methyl-*L*-valine

To a solution of methyl *N*-(*N*-((*R*)-1-benzylaziridine-2-carbonyl)-*N*-methylglycyl)-*N*-methyl-*L*-valinate (261.0 mg, 0.695 mmol) in THF (3.38 mL) was added a solution of LiOH (83.2 mg, 3.48 mmol) in H_2O (3.50 mL). The reaction mixture was stirred for 1 h. The reaction was then quenched with sat. aq. NH_4Cl. The resulting mixture was extracted with EtOAc and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by reverse phase chromatography (10→50% MeCN/H_2O) to afford the desired product (230 mg, 91% yield) as an oil. LCMS (ESI) *m/z:* [M + H] calcd for $C_{19}H_{27}N_3O_4$: 362.21; found 362.2.

Intermediate A-10. Synthesis of *N*-(*N*-((*R*)-1-benzylaziridine-2-carbonyl)-*N*-methylglycyl)-*N*-methyl-*L*-valine



Step 1: Synthesis of methyl *N*-(*N*-((*S*)-1-benzylaziridine-2-carbonyl)-*N*-methylglycyl)-*N*-methyl-*L*-valinate

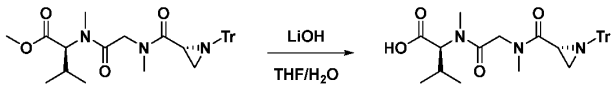
To a solution of methyl *N*-(*N*-((*S*)-aziridine-2-carbonyl)-*N*-methylglycyl)-*N*-methyl-*L*-valinate (362.0 mg, 1.269 mmol) in MeCN (6.0 mL) at 0 °C was added DIPEA (883 µL, 5.08 mmol) and benzyl bromide (165 µL, 1.39 mmol). The reaction mixture was then warmed to room temperature and stirred overnight. The reaction mixture was then concentrated under reduced pressure. The residue was purified by prep-TLC (7% MeOH/DCM) to afford the desired product (287 mg, 60% yield) as an oil. LCMS (ESI) *m/z:* [M + H] calcd for $C_{20}H_{29}N_3O_4$: 376.22; found 376.2.

Step 2: Synthesis of *N*-(*N*-((*S*)-1-benzylaziridine-2-carbonyl)-*N*-methylglycyl)-*N*-methyl-*L*-valine

To a solution of methyl *N*-(*N*-((*S*)-1-benzylaziridine-2-carbonyl)-*N*-methylglycyl)-*N*-methyl-*L*-valinate (270.0 mg, 0.719 mmol) in THF (3.6 mL) was added a solution of LiOH (86.1 mg, 3.59 mmol) in H_2O (3.60 mL). The reaction mixture was stirred for 30 min. The reaction was then quenched with sat. aq. NH_4Cl. The resulting mixture was extracted with EtOAc (3 x 15 mL) and the

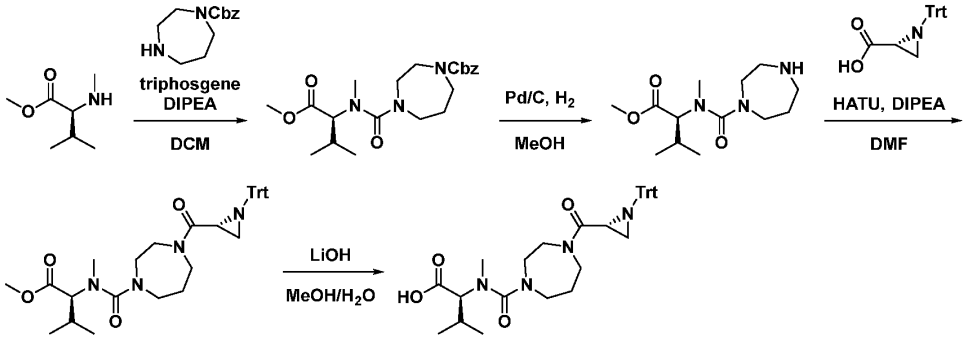
combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired crude product (240 mg, 92% yield) as an oil. LCMS (ESI) *m/z:* [M + H] calcd for $C_{19}H_{27}N_3O_4$: 362.21; found 362.2.

Intermediate A-11. Synthesis of *N*-methyl-*N*-(*N*-methyl-*N*-((*R*)-1-tritylaziridine-2-carbonyl)glycyl)-*L*-valine



To a solution of methyl *N*-methyl-*N*-(*N*-methyl-*N*-((*R*)-1-tritylaziridine-2-carbonyl)glycyl)-*L*-valinate (1.30 g, 2.46 mmol) in THF (10.0 mL) at 0 °C was added a solution of LiOH (177.0 mg, 7.39 mmol) in H_2O (7.40 mL). The resulting mixture was warmed to room temperature, stirred for 3 h, and was then acidified to pH 5 with HCl (aq). The resulting mixture was extracted with EtOAc (3 x 80 mL) and the combined organic layers were washed with brine (2 x 50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired crude product (1 g, 71% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{31}H_{35}N_3O_4$: 514.27; found 514.3.

Intermediate A-12. Synthesis of *N*-methyl-*N*-(4-((*R*)-1-tritylaziridine-2-carbonyl)-1,4-diazepane-1-carbonyl)-*L*-valine



Step 1: Synthesis of benzyl (*S*)-4-((1-methoxy-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)-1,4-diazepane-1-carboxylate

To a solution of methyl *N*-methyl-*L*-valinate (2.50 g, 17.22 mmol) in DCM at 0 °C was added DIPEA (1.8 mL, 10.33 mmol) followed by triphosgene (2.55 g, 8.61 mmol). The resulting mixture was stirred for 3 h at 0 °C. To the mixture was then added benzyl 1,4-diazepane-1-carboxylate (4.03 g, 17.20 mmol). The resulting mixture was warmed to room temperature and stirred overnight. The reaction was cooled to 0 °C and was quenched with $NaHCO_3$. The aqueous layer was extracted with EtOAc (2 x 30 mL) and the combined organic layers were washed with brine (2 x 30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by normal

phase chromatography (25% EtOAc/pet. ether) to afford the desired product (3.5 g, 50.1% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{21}H_{31}N_3O_5$: 406.23; found 406.5.

Step 2: Synthesis of methyl *N*-(1,4-diazepane-1-carbonyl)-*N*-methyl-*L*-valinate

To a solution of benzyl (*S*)-4-((1-methoxy-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)-1,4-diazepane-1-carboxylate (2.0 g, 4.93 mmol) in MeOH (20 mL) was added Pd/C (10%wt, 1 g). The mixture was placed under a hydrogen atmosphere (1 atm) and stirred for 2 h. The reaction mixture was filtered through a Celite and concentrated under reduced pressure to afford the desired crude product (1.3 g, 97.1% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{25}N_3O_3$: 272.20; found 272.3.

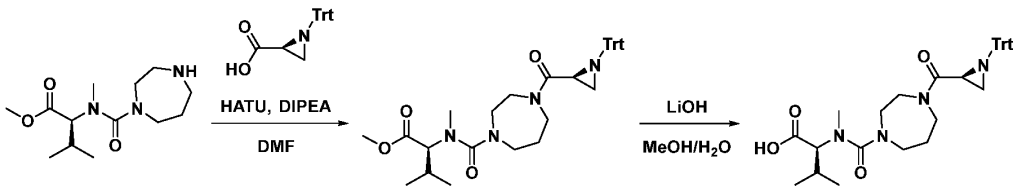
Step 3: Synthesis of methyl *N*-methyl-*N*-(4-((*R*)-1-tritylaziridine-2-carbonyl)-1,4-diazepane-1-carbonyl)-*L*-valinate

To a solution of methyl *N*-(1,4-diazepane-1-carbonyl)-*N*-methyl-*L*-valinate (1.0 g, 3.69 mmol) and (*R*)-1-tritylaziridine-2-carboxylic acid (1.46 g, 4.42 mmol) in DMF at 0 °C was added DIPEA (1.93 mL, 11.06 mmol) followed by HATU (2.10 g, 5.52 mmol). The resulting mixture was warmed to room temperature and stirred for 1 h. The reaction mixture was then diluted with H_2O (15 mL) and the aqueous layer was extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine
(3 x 30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by normal phase chromatography (25% EtOAc/pet. ether) to afford the desired product (1.6 g, 74.5% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{35}H_{42}N_4O_4$: 583.33; found 583.5.

Step 4: Synthesis of *N*-methyl-*N*-(4-((*R*)-1-tritylaziridine-2-carbonyl)-1,4-diazepane-1-carbonyl)-*L*-valine

To a solution of methyl *N*-methyl-*N*-(4-((*R*)-1-tritylaziridine-2-carbonyl)-1,4-diazepane-1-carbonyl)-*L*-valinate (1.60 g, 2.75 mmol) in MeOH (10.0 mL) and H_2O (5.0 mL) at 0 °C was added LiOH (0.66 g, 27.56 mmol). The resulting mixture was warmed to room temperature and stirred overnight. The reaction mixture was acidified to pH 5 with HCl (aq) and the aqueous layer was extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (3 x 30 mL), dried over Na_2SO_4, filtered, concentrated under reduced pressure to afford the desired crude product (1.4 g, 95.6% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{34}H_{40}N_4O_4$: 569.31; found 569.5.

Intermediate A-13. Synthesis of *N*-methyl-*N*-(4-((*S*)-1-tritylaziridine-2-carbonyl)-1,4-diazepane-1-carbonyl)-*L*-valine



Step 1: Synthesis of methyl *N*-methyl-*N*-(4-((*S*)-1-tritylaziridine-2-carbonyl)-1,4-diazepane-1-carbonyl)-*L*-valinate

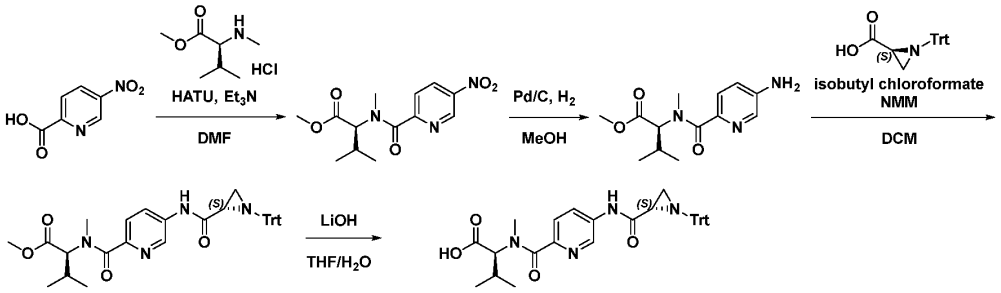
To a solution of methyl *N*-(1,4-diazepane-1-carbonyl)-*N*-methyl-*L*-valinate (1.16 g, 4.28 mmol) and (*S*)-1-tritylaziridine-2-carboxylic acid (1.69 g, 5.13 mmol) in DMF (10 mL) at 0 °C was

added DIPEA (2.23 mL, 12.82 mmol) followed by HATU (2.44 g, 6.41 mmol). The resulting mixture was stirred for 1 h at 0 °C. The reaction mixture was then diluted with H_2O (15 mL) and the aqueous layer was extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with brine (3 x 15 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by normal phase chromatography (17% EtOAc/pet. ether) to afford the desired product (2 g, 80.3% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{35}H_{42}N_4O_4$: 583.33; found 583.5.

Step 2: Synthesis of *N*-methyl-*N*-(4-((*S*)-1-tritylaziridine-2-carbonyl)-1,4-diazepane-1-carbonyl)-*L*-valine

To a solution of methyl *N*-methyl-*N*-(4-((*S*)-1-tritylaziridine-2-carbonyl)-1,4-diazepane-1-carbonyl)-*L*-valinate (1.0 g, 1.72 mmol) in MeOH (8.0 mL) and H_2O (4.0 mL) at 0 °C was added LiOH (411 mg, 17.16 mmol). The resulting mixture was warmed to room temperature and stirred overnight. The reaction mixture was acidified to pH 5 with HCl (aq) and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (3 x 10 mL), dried over Na_2SO_4, filtered, concentrated under reduced pressure to afford the desired crude product (0.6 g, 61.5% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{34}H_{40}N_4O_4$: 569.31; found 569.5.

Intermediate A-14. Synthesis of *N*-methyl-*N*-(5-((*S*)-1-tritylaziridine-2-carboxamido)picolinoyl)-*L*-valine



Step 1: Synthesis of methyl *N*-methyl-*N*-(5-nitropicolinoyl)-*L*-valinate

To a solution of methyl *N*-methyl-*L*-valinate hydrochloride (190.0 mg, 1.31 mmol) and 5-nitropicolinic acid (200.0 mg, 1.19 mmol) in DMF (2 mL) at 0 °C was added HATU (678.6 mg, 1.79 mmol) and Et_3N (0.332 mL, 2.38 mmol). The resulting mixture was warmed to room temperature and stirred for 2 h. The resulting mixture was then extracted with EtOAc (2 x 50 mL) and the combined organic layers were washed with H_2O (20 mL) and brine (20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by normal phase chromatography (33% EtOAc/pet. ether) to afford the desired product (210 mg, 59.8% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{17}N_3O_5$: 296.12; found 296.0.

Step 2: Synthesis of methyl *N*-(5-aminopicolinoyl)-*N*-methyl-*L*-valinate

To a solution of methyl *N*-methyl-*N*-(5-nitropicolinoyl)-*L*-valinate (5.0 g, 16.93 mmol) in MeOH (50.0 mL) was added Pd/C (2.50 g). The reaction mixture was placed under a hydrogen atmosphere (1 atm) and was stirred for 2 h. The mixture was filtered, the filter cake was washed with MeOH (2 x

20 mL), and the filtrate was concentrated under reduced pressure to afford the desired crude product (5.3 g). LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{19}N_3O_3$: 266.15; found 266.0.

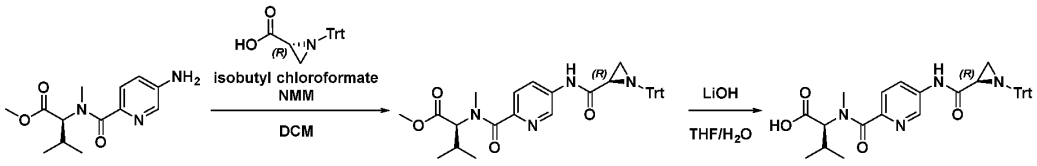
Step 3: Synthesis of methyl *N*-methyl-*N*-(5-((*S*)-1-tritylaziridine-2-carboxamido)picolinoyl)-*L*-valinate

To a solution (*S*)-1-tritylaziridine-2-carboxylic acid (55.9 mg, 0.17 mmol) in DCM at 0 °C was added isobutyl chloroformate (21.7 µL, 0.23 mmol) and *N*-methylmorpholine (66.8 µL, 0.61 mmol). The resulting mixture was stirred for 1 h and then methyl *N*-(5-aminopicolinoyl)-*N*-methyl-*L*-valinate (30.0 mg, 0.11 mmol) was added. The resulting mixture was warmed to room temperature and stirred for an additional 5 h. The mixture was extracted with DCM (3 x 50 mL) and the combined organic layers were washed with sat. $NaHCO_3$ (30 mL) and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by normal phase chromatography (33% EtOAc/pet. ether) to afford the desired product (1.09 g, 66.9% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{35}H_{36}N_4O_4$: 577.28; found 577.1.

Step 4: Synthesis of *N*-methyl-*N*-(5-((*S*)-1-tritylaziridine-2-carboxamido)picolinoyl)-*L*-valine

To a solution methyl *N*-methyl-*N*-(5-((*S*)-1-tritylaziridine-2-carboxamido)picolinoyl)-*L*-valinate (100.0 mg, 0.17 mmol) in THF (0.5 mL) at 0 °C was added a solution of LiOH (20.76 mg, 0.87 mmol) in H_2O (0.5 mL). The resulting mixture was warmed to room temperature and stirred for 6 h. The mixture was acidified to pH 5 with 1 M citric acid. The resulting mixture was extracted with EtOAc (3 x 20 mL) and the combined organic layers were washed with brine (5 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired crude product (76.8 mg, 78.7% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{34}H_{34}N_4O_4$: 563.27; found 563.3.

Intermediate A-15. Synthesis of *N*-methyl-*N*-(5-((*R*)-1-tritylaziridine-2-carboxamido)picolinoyl)-*L*-valine



Step 1: Synthesis of methyl *N*-methyl-*N*-(5-((*R*)-1-tritylaziridine-2-carboxamido)picolinoyl)-*L*-valinate

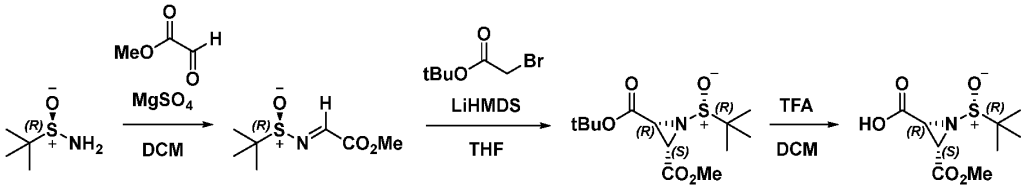
To a solution (*R*)-1-tritylaziridine-2-carboxylic acid (1396.7 mg, 4.24 mmol) in DCM (8 mL) at 0 °C was added isobutyl chloroformate (440 µL, 3.39 mmol) and *N*-methylmorpholine (466 µL, 4.24 mmol). The resulting mixture was stirred for 1 h and then methyl *N*-(5-aminopicolinoyl)-*N*-methyl-*L*-valinate (750.0 mg, 2.83 mmol) was added. The resulting mixture was warmed to room temperature and stirred for an additional 5 h. The mixture was quenched by the addition of $NaHCO_3$ and the aqueous layer was extracted with DCM (2 x 100 mL). The combined organic layers were washed with brine (120 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The

residue was purified by normal phase chromatography (50% EtOAc/pet. ether) to afford the desired product (580 mg, 35.6% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{35}H_{36}N_4O_4$: 577.28; found 577.2.

Step 2: Synthesis of *N*-methyl-*N*-(5-((*R*)-1-tritylaziridine-2-carboxamido)picolinoyl)-*L*-valine

To a solution methyl *N*-methyl-*N*-(5-((*R*)-1-tritylaziridine-2-carboxamido)picolinoyl)-*L*-valinate (558.0 mg, 0.97 mmol) in THF (14 mL) at 0 °C was added a solution of LiOH (115.9 mg, 4.84 mmol) in H_2O (14 mL). The resulting mixture was warmed to room temperature and stirred for 6 h. The mixture was acidified to pH 5 with 1 M citric acid. The resulting mixture was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired crude product (580 mg, 78.7% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{34}H_{34}N_4O_4$: 563.27; found 563.2.

Intermediate A-16. Synthesis of (2*R*,3*S*)-1-((*R*)-*tert*-butylsulfinyl)-3-(methoxycarbonyl)aziridine-2-carboxylic acid



Step 1: Synthesis of methyl (*R*,*E*)-2-((*tert*-butylsulfinyl)imino)acetate

To a solution of (*R*)-2-methylpropane-2-sulfinamide (13.21 g, 109.01 mmol) and methyl 2-oxoacetate (8.0 g, 90.85 mmol) in DCM (130 mL) at room temperature was added $MgSO_4$ (54.67 g, 454.23 mmol). The resulting mixture was heated to 35 °C and stirred for 16 h. The resulting mixture was filtered, the filter cake washed with EtOAc (3 x 50 mL), and the filtrate was concentrated under reduced pressure. The residue was purified by normal phase chromatography (25% EtOAc/pet. ether) to afford the desired (5.8 g, 33.4% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_7H_{13}NO_3S$: 192.07; found 191.9.

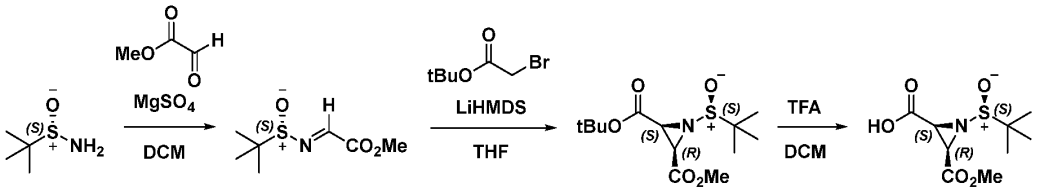
Step 2: Synthesis of 2-(*tert*-butyl) 3-methyl (2*R*,3*S*)-1-((*R*)-*tert*-butylsulfinyl)aziridine-2,3-dicarboxylate

To a solution of 1M LiHMDS (61.40 mL, 61.40 mmol) in THF (300.0 mL) at -78 °C was added *tert*-butyl 2-bromoacetate (11.83 g, 60.65 mmol). The resulting mixture was stirred for 30 min. To the reaction mixture was then added methyl methyl (*R*,*E*)-2-((*tert*-butylsulfinyl)imino)acetate (5.8 g, 30.33 mmol). The resulting mixture was warmed to -60 °C and stirred for 2.5 h. The reaction was warmed to 0 °C and quenched with sat. NH_4Cl (aq.). The resulting mixture was extracted with EtOAc (3 x 200 mL). The combined organic layers were washed with brine (500 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by reverse phase chromatography (10→50% MeCN/H_2O) to afford the desired product (1.34 g, 4.5% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{23}NO_5S$: 306.14; found 306.2.

Step 3: Synthesis of (2*R*,3*S*)-1-((*R*)-*tert*-butylsulfinyl)-3-(methoxycarbonyl)aziridine-2-carboxylic acid

To a solution of 2-(*tert*-butyl) 3-methyl (2*R*,3*S*)-1-((*R*)-*tert*-butylsulfinyl)aziridine-2,3-dicarboxylate (302.0 mg, 0.99 mmol) in DCM (3.0 mL) at 0 °C was added TFA (1.50 mL). The resulting mixture was stirred for 1 h and then concentrated under reduced pressure to afford the desired crude product
(300 mg). LCMS (ESI) *m/z*: [M + H] calcd for $C_9H_{15}NO_5S$: 250.07; found 250.1.

Intermediate A-17. Synthesis of (2*R*,3*S*)-1-((*S*)-*tert*-butylsulfinyl)-3-(methoxycarbonyl)aziridine-2-carboxylic acid



Step 1: Synthesis of methyl (*S*,*E*)-2-((*tert*-butylsulfinyl)imino)acetate

To a solution of (*S*)-2-methylpropane-2-sulfinamide (9.81 g, 80.94 mmol) and methyl 2-oxoacetate (5.94 g, 67.45 mmol) in DCM (100 mL) at room temperature was added $MgSO_4$ (40.60 g, 337.26 mmol). The resulting mixture was heated to 35 °C and stirred for 16 h. The resulting mixture was filtered, the filter cake washed with EtOAc (3 x 50 mL), and the filtrate was concentrated under reduced pressure. The residue was purified by normal phase chromatography (25% EtOAc/pet. ether) to afford the desired
(5.68 g, 44.0% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_7H_{13}NO_3S$: 192.07; found 191.1.

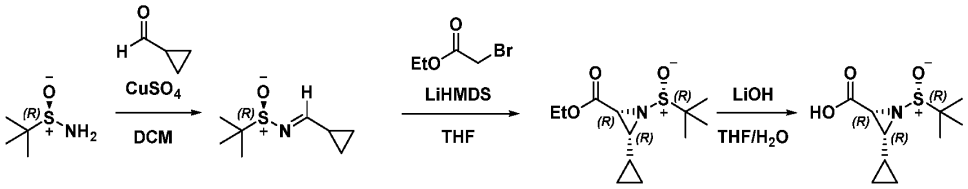
Step 2: Synthesis of 2-(*tert*-butyl) 3-methyl (2*R*,3*S*)-1-((*S*)-*tert*-butylsulfinyl)aziridine-2,3-dicarboxylate

To a solution of 1M LiHMDS (59.40 mL, 59.40 mmol) in THF (300.0 mL) at -78 °C was added *tert*-butyl 2-bromoacetate (11.59 g, 59.40 mmol). The resulting mixture was stirred for 30 min. To the reaction mixture was then added methyl methyl (*S*,*E*)-2-((*tert*-butylsulfinyl)imino)acetate (5.68 g, 29.70 mmol). The resulting mixture was warmed to -60 °C and stirred for 2.5 h. The reaction was warmed to 0 °C and quenched with sat. NH_4Cl (aq.). The resulting mixture was extracted with EtOAc (3 x 200 mL). The combined organic layers were washed with brine (500 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by reverse phase chromatography (10→50% MeCN/H_2O) to afford the desired product (1.26 g, 13.9% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{23}NO_5S$: 306.14; found 306.1.

Step 3: Synthesis of (2*R*,3*S*)-1-((*S*)-*tert*-butylsulfinyl)-3-(methoxycarbonyl)aziridine-2-carboxylic acid

To a solution of 2-(*tert*-butyl) 3-methyl (2*R*,3*S*)-1-((*S*)-*tert*-butylsulfinyl)aziridine-2,3-dicarboxylate (457.0 mg, 1.50 mmol) in DCM (6.0 mL) at 0 °C was added TFA (3.0 mL). The resulting mixture was stirred for 1 h and then concentrated under reduced pressure to afford the desired crude product
(450 mg). LCMS (ESI) *m/z*: [M + H] calcd for $C_9H_{15}NO_5S$: 250.07; found 250.1.

Intermediate A-18. Synthesis of (2*R*,3*R*)-1-((*R*)-*tert*-butylsulfinyl)-3-cyclopropylaziridine-2-carboxylic acid



Step 1: Synthesis of (*R*,*E*)-*N*-(cyclopropylmethylene)-2-methylpropane-2-sulfinamide

To a solution of (*R*)-2-methylpropane-2-sulfinamide (1.0 g, 8.25 mmol) and cyclopropanecarbaldehyde (1.16 g, 16.55 mmol) in DCM (50 mL) at room temperature was added $CuSO_4$ (3.95 g, 24.75 mmol). The resulting mixture was stirred overnight. The reaction mixture was then filtered, the filter cake washed with EtOAc, and the filtrate was concentrated under reduced pressure. The residue was purified by prep-TLC (17% EtOAc/pet. ether) to afford the desired product (1.4 g, 97.9% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_8H_{15}NOS$: 174.10; found 174.1.

Step 2: Synthesis of ethyl (2*R*,3*R*)-1-((*R*)-*tert*-butylsulfinyl)-3-cyclopropylaziridine-2-carboxylate

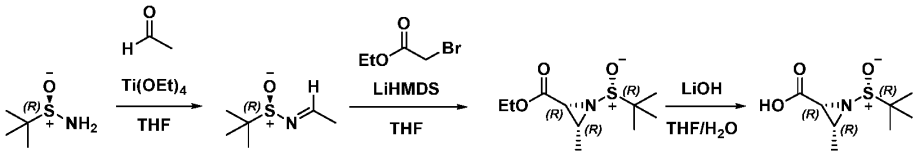
To a solution of 1M LiHMDS (23 mL, 23 mmol) in THF (50.0 mL) at -78 °C was added ethyl bromoacetate (3.83 g, 22.95 mmol). The resulting mixture was warmed to -70 °C and stirred for 1 h. To the reaction mixture was then added (*R*,*E*)-*N*-(cyclopropylmethylene)-2-methylpropane-2-sulfinamide

(2.0 g, 11.48 mmol). The resulting mixture was stirred for 1 h at -70 °C. The reaction mixture was warmed to 0 °C and quenched with H_2O. The aqueous layer was extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by prep-TLC (25% EtOAc/pet. ether) to afford the desired product (1.8 g, 60.5% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{12}H_{21}NO_3S$: 306.14; found 260.13.

Step 3: Synthesis of (2*R*,3*R*)-1-((*R*)-*tert*-butylsulfinyl)-3-cyclopropylaziridine-2-carboxylic acid

To a solution of ethyl (2*R*,3*R*)-1-((*R*)-*tert*-butylsulfinyl)-3-cyclopropylaziridine-2-carboxylate (900.0 mg, 3.47 mmol) in THF (3.0 mL) and H_2O (3.0 mL) at 0 °C was added $LiOH\bullet H_2O$ (218.4 mg, 5.21 mmol). The resulting mixture was stirred for 1 h and was then quenched by H_2O. The aqueous layer was extracted with EtOAc (3 x 50) and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired crude product (400 mg, 29.9% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{10}H_{17}NO_3S$: 232.10; found 232.1.

Intermediate A-19. Synthesis of (2*R*,3*R*)-1-((*R*)-*tert*-butylsulfinyl)-3-methylaziridine-2-carboxylic acid



Step 1: Synthesis of (*R*,*E*)-*N*-ethylidene-2-methylpropane-2-sulfinamide

To a solution of (*R*)-2-methylpropane-2-sulfinamide (3.0 g, 24.75 mmol) and tetraethoxytitanium (1.7 g, 7.43 mmol) in THF (30 mL) at 0 °C was added acetaldehyde (218.1 mg, 4.95 mmol). The resulting mixture was stirred for 20 min and was then quenched with H_2O (100 mL). The suspension was filtered, and the filter cake washed with EtOAc (3 x 100 mL). The aqueous layer was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with brine (3 x 100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by normal phase chromatography (9% EtOAc/pet. ether) afforded desired product (3 g, 82% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_6H_{13}NOS$: 148.08; found 148.0.

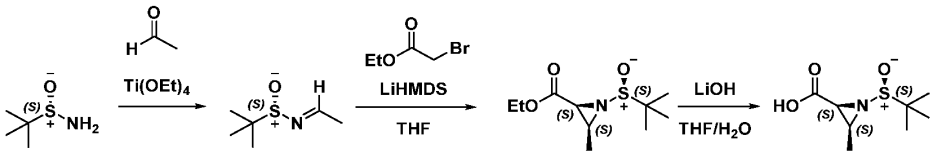
Step 2: Synthesis of ethyl (2*R*,3*R*)-1-((*R*)-*tert*-butylsulfinyl)-3-methylaziridine-2-carboxylate

To a solution of 1M LiHMDS (40.75 mL, 40.75 mmol) in THF (30.0 mL) at -78 °C was added ethyl bromoacetate (6.80 g, 40.75 mmol). The resulting mixture was stirred for 1 h. To the reaction mixture was then added (*R*,*E*)-*N*-ethylidene-2-methylpropane-2-sulfinamide (3.0 g, 20.38 mmol). The resulting mixture was stirred for 2 h at -78 °C and then quenched with H_2O (300 mL). The aqueous layer was extracted with EtOAc (3 x 300 mL) and the combined organic layers were washed with brine (3 x 100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by reverse phase chromatography (10→50% MeCN/H_2O) to afford the desired product (1.4 g, 29.5% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{10}H_{19}NO_3S$: 234.12; found 234.1.

Step 3: Synthesis of (2*R*,3*R*)-1-((*R*)-*tert*-butylsulfinyl)-3-methylaziridine-2-carboxylic acid

To a solution of ethyl (2*R*,3*R*)-1-((*R*)-*tert*-butylsulfinyl)-3-methylaziridine-2-carboxylate (1.0 g, 4.29 mmol) in THF (6.4 mL) and H_2O (6.4 mL) at 0 °C was added LiOH•H_2O (539.5 mg, 12.86 mmol). The resulting mixture was warmed to room temperature and stirred for 2 h and was then neutralized to pH 5 with HCl (aq.) and sat. NH_4Cl (aq.). The aqueous layer was extracted with EtOAc (3 x 10 mL) and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired crude product (489 mg, 55.6% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_8H_{15}NO_3S$: 206.09; found 206.0.

Intermediate A-20. Synthesis of (2*S*,3*S*)-1-(*S*)-*tert*-butylsulfinyl)-3-methylaziridine-2-carboxylic acid



Step 1: Synthesis of (*S*,*E*)-*N*-ethylidene-2-methylpropane-2-sulfinamide

To a mixture of (*S*)-2-methylpropane-2-sulfinamide (5.0 g, 41.25 mmol) and tetraethoxytitanium (18.82 g, 82.51 mmol) at 0 °C was added acetaldehyde (3.63 g, 82.51 mmol). The resulting mixture was warmed to room temperature and stirred for 30 min and was then quenched with H_2O (100 mL). The suspension was filtered, and the filter cake washed with EtOAc (3 x 100 mL). The aqueous layer was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with brine (3 x 100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford desired crude product (3.9 g, 64% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_6H_{13}NOS$: 148.08; found 148.2.

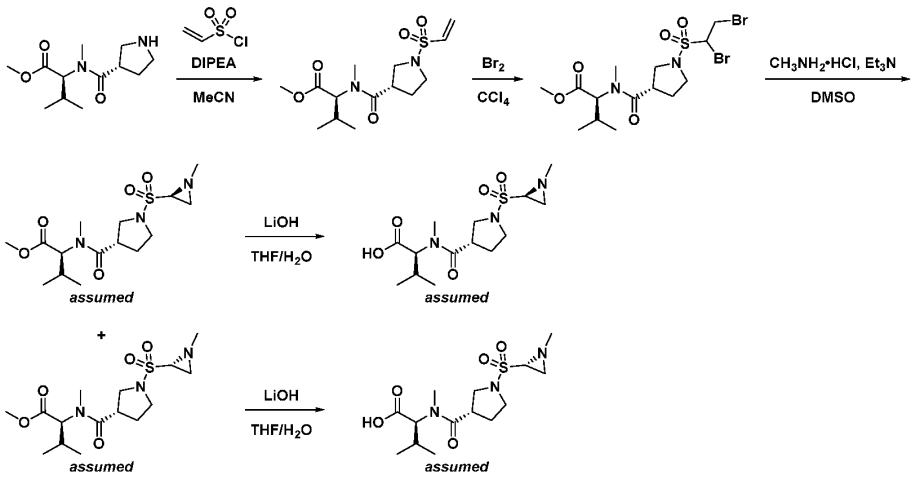
Step 2: Synthesis of ethyl (2*S*,3*S*)-1-((*S*)-*tert*-butylsulfinyl)-3-methylaziridine-2-carboxylate

To a solution of 1M LiHMDS (40.75 mL, 40.75 mmol) in THF (30.0 mL) at -78 °C was added ethyl bromoacetate (6.80 g, 40.75 mmol). The resulting mixture was stirred for 1 h. To the reaction mixture was then added (*S*,*E*)-*N*-ethylidene-2-methylpropane-2-sulfinamide (3.0 g, 20.38 mmol). The resulting mixture was stirred for 2 h at -78 °C and then quenched with H_2O. The aqueous layer was extracted with EtOAc (3 x 200 mL) and the combined organic layers were washed with brine (3 x 300 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by reverse phase chromatography (10→50% MeCN/H_2O) to afford the desired product (2 g, 42% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{10}H_{19}NO_3S$: 234.12; found 234.0.

Step 3: Synthesis of (2*S*,3*S*)-1-((*S*)-*tert*-butylsulfinyl)-3-methylaziridine-2-carboxylic acid

To a solution of ethyl (2*S*,3*S*)-1-((*S*)-*tert*-butylsulfinyl)-3-methylaziridine-2-carboxylate (80.0 mg, 0.34 mmol) in THF (1.0 mL) and H_2O (0.2 mL) at 0 °C was added LiOH•H_2O (32.9 mg, 1.37 mmol). The resulting mixture was warmed to room temperature and stirred for 4 h and was then acidified to pH 3 with HCl (aq.). The aqueous layer was extracted with EtOAc (3 x 10 mL) and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired crude product (70 mg, 99% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_8H_{15}NO_3S$: 206.09; found 206.0.

Intermediate A-21 and A-22. Synthesis of *N*-methyl-*N*-((*S*)-1-(((*R*)-1-methylaziridin-2-yl)sulfonyl)pyrrolidine-3-carbonyl)-*L*-valine and *N*-methyl-*N*-((*S*)-1-(((*S*)-1-methylaziridin-2-yl)sulfonyl)pyrrolidine-3-carbonyl)-*L*-valine



Step 1: Synthesis of methyl *N*-methyl-*N*-((*S*)-1-(vinylsulfonyl)pyrrolidine-3-carbonyl)-*L*-valinate

To a mixture of methyl *N*-methyl-*N*-((*S*)-pyrrolidine-3-carbonyl)-*L*-valinate (7.0 g, 28.89 mmol) in MeCN (200 mL) at -20 °C was added DIPEA (10.0 mL, 57.78 mmol) followed by ethenesulfonyl chloride (4.0 g, 31.78 mmol). The resulting solution was stirred for 2 h at -20 °C and was then diluted with EtOAc (800 mL). The resulting solution was washed with brine (3 x 100 mL) and concentrated under reduced pressure. Purification by normal phase chromatography (50% EtOAc/pet. ether) afforded the desired product (4.8 g, 49.9%, yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{14}H_{24}N_2O_5S$: 333.15; found 333.1.

Step 2: Synthesis of methyl *N*-((3*S*)-1-((1,2-dibromoethyl)sulfonyl)pyrrolidine-3-carbonyl)-*N*-methyl-*L*-valinate

To a solution of methyl *N*-methyl-*N*-((*S*)-1-(vinylsulfonyl)pyrrolidine-3-carbonyl)-*L*-valinate (4.5 g, 13.54 mmol) in CCl_4 (100 mL) at 0 °C was added Br_2 (2.77 mL, 54.15 mmol). The resulting solution was stirred for overnight and was then quenched by the addition of sat. $NaHCO_3$ (100 mL). The aqueous layer was extracted with EtOAc (3 x 200 mL) and the combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by normal phase chromatography (25% EtOAc/ pet. ether) afforded the desired product (2.6 g, 39.0% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{14}H_{24}Br_2N_2O_5S$: 492.99; found 493.0.

Step 3: Synthesis of methyl *N*-methyl-*N*-((*S*)-1-(((*R*)-1-methylaziridin-2-yl)sulfonyl)pyrrolidine-3-carbonyl)-*L*-valinate and methyl *N*-methyl-*N*-((*S*)-1-(((*S*)-1-methylaziridin-2-yl)sulfonyl)pyrrolidine-3-carbonyl)-*L*-valinate

To a solution of methyl *N*-((3*S*)-1-((1,2-dibromoethyl)sulfonyl)pyrrolidine-3-carbonyl)-*N*-methyl-*L*-valinate (2.6 g, 5.28 mmol) in DMSO (250 mL) was added methanamine hydrochloride (1.07 g, 15.85 mmol) and Et_3N (7.37 mL, 52.82 mmol). The reaction mixture was heated to 75 °C and stirred overnight. The mixture was then cooled to room temperature and diluted with EtOAc (1.5 L). The resulting mixture was washed with sat. NH_4Cl (2 x 200 mL) and brine (2 x 200 mL) and the organic layer was then concentrated under reduced pressure. Purification by reverse phase chromatography (40→60% MeCN/H_2O) afforded a mixture of the desired products. The

diastereomers were separated by prep-SFC (28% MeOH/CO_2) to afford methyl *N*-methyl-*N*-((*S*)-1-(((*R*)-1-methylaziridin-2-yl)sulfonyl)pyrrolidine-3-carbonyl)-*L*-valinate (0.46 g, 24% yield) and methyl *N*-methyl-*N*-((*S*)-1-(((*S*)-1-methylaziridin-2-yl)sulfonyl)pyrrolidine-3-carbonyl)-*L*-valinate (0.35 g, 18.3% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{15}H_{27}N_3O_5S$: 362.17; found 362.1.

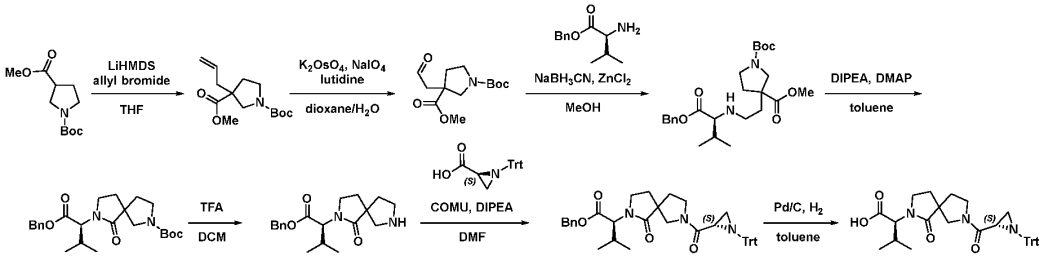
Step 4: Synthesis of *N*-methyl-*N*-((*S*)-1-(((*R*)-1-methylaziridin-2-yl)sulfonyl)pyrrolidine-3-carbonyl)-*L*-valine

To a solution of methyl *N*-methyl-*N*-((*S*)-1-(((*R*)-1-methylaziridin-2-yl)sulfonyl)pyrrolidine-3-carbonyl)-*L*-valinate (200.0 mg, 0.55 mmol) in THF (2.0 mL) and H_2O (2.0 mL) at 0 °C was added LiOH (53.0 mg, 2.21 mmol). The resulting solution was stirred for 2 h at 0 °C and then the reaction mixture was acidified to pH 6 with 1M HCl. The aqueous layer was extracted with EtOAc and the combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by reverse phase chromatography (5→55% MeCN/H_2O) afforded the desired product (110 mg, 57.2%, yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{14}H_{25}N_3O_5S$: 348.16; found 348.1.

Step 5: Synthesis of *N*-methyl-*N*-((*S*)-1-(((*S*)-1-methylaziridin-2-yl)sulfonyl)pyrrolidine-3-carbonyl)-*L*-valine

To a solution of methyl *N*-methyl-*N*-((*S*)-1-(((*S*)-1-methylaziridin-2-yl)sulfonyl)pyrrolidine-3-carbonyl)-*L*-valinate (200.0 mg, 0.55 mmol) in THF (2.0 mL) and H_2O (2.0 mL) at 0 °C was added LiOH (53.0 mg, 2.21 mmol). The resulting solution was stirred for 2 h at 0 °C and then the reaction mixture was acidified to pH 6 with 1M HCl. The aqueous layer was extracted with EtOAc and the combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by reverse phase chromatography (5→55 MeCN/H_2O) afforded the desired product (121 mg, 62.9%, yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{14}H_{25}N_3O_5S$: 348.16; found 348.1.

Intermediate A-23. Synthesis of (2*S*)-3-methyl-2-(1-oxo-7-((*S*)-1-tritylaziridine-2-carbonyl)-2,7-diazaspiro[4.4]nonan-2-yl)butanoic acid



Step 1: Synthesis of 1-(*tert*-butyl) 3-methyl 3-allylpyrrolidine-1,3-dicarboxylate

To a mixture of 1-(*tert*-butyl) 3-methyl pyrrolidine-1,3-dicarboxylate (10 g, 43.616 mmol) in THF (100 mL) at -78 °C was added 1M LiHMDS (65.42 mL, 65.424 mmol) dropwise. The resulting mixture was stirred at -78 °C for 1 h and then a solution of allyl bromide (7.91 g, 65.423 mmol) in THF was added dropwise over 10 min. The resulting mixture was stirred at -78 °C for an additional 2 h and was then quenched by the addition of sat. NH_4Cl at 0 °C. The resulting mixture was

extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with brine (2 x 80 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (20% EtOAc/pet. ether) afforded the desired product (10 g, 76% yield).

Step 2: Synthesis of 1-(*tert*-butyl) 3-methyl 3-(2-oxoethyl)pyrrolidine-1,3-dicarboxylate

To a mixture of 1-(*tert*-butyl) 3-methyl 3-allylpyrrolidine-1,3-dicarboxylate (11.0 g, 40.84 mmol) and 2,6-lutidine (8.75 g, 81.68 mmol) in dioxane (190 mL) and H_2O (19 mL) at 0 °C was added $K_2OsO_4 \bullet 2H_2O$ (0.75 g, 2.04 mmol). The resulting mixture was stirred at 0 °C for 15 min and then $NaIO_4$ (34.94 g, 163.36 mmol) was added in portions. The mixture was warmed to room temperature and stirred for an additional 3 h, then was quenched by the addition of sat. Na_2S2O_3 at 0 °C. The resulting mixture was extracted with EtOAc (3 x 300 mL) and the combined organic layers were washed with brine (200 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by reverse phase chromatography (0→40% MeCN/H_2O, 0.1% HCO_2H) afforded the desired product (6.4 g, 51% yield).

Step 3: Synthesis of 1-(*tert*-butyl) 3-methyl 3-(2-(((*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)amino)ethyl)pyrrolidine-1,3-dicarboxylate

To a mixture of 1-(*tert*-butyl) 3-methyl 3-(2-oxoethyl)pyrrolidine-1,3-dicarboxylate (6.30 g, 23.220 mmol) and benzyl *L*-valinate (7.22 g, 34.831 mmol) in MeOH (70 mL) at 0 °C was added $ZnCl_2$ (4.75 g, 34.831 mmol). The resulting mixture was warmed to room temperature and stirred for 30 min, then cooled to 0 °C. $NaBH_3CN$ (2.92 g, 46.441 mmol) was added in portions then the mixture was warmed to room temperature and stirred for 2 h. The reaction was quenched by the addition of sat. NH_4Cl at 0 °C and the resulting mixture was then extracted with EtOAc (3 x 200 mL). The combined organic layers were washed with brine (150 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (33% EtOAc/pet. ether) afforded the desired product (6.4 g, 53% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{25}H_{38}N_2O_6$: 463.28; found 463.3.

Step 4: Synthesis of *tert*-butyl 7-((*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate

To a mixture of 1-(*tert*-butyl) 3-methyl 3-(2-(((*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)amino)ethyl)pyrrolidine-1,3-dicarboxylate (4.50 g, 9.728 mmol) and DIPEA (16.6 mL, 97.28 mmol) in toluene (50 mL) was added DMAP (1.19 g, 9.728 mmol) and then mixture was heated to 80 °C. After 24 h the reaction was cooled to room temperature and concentrated under reduced pressure. Purification by reverse phase chromatography (15→60% MeCN/H_2O, 0.1% HCO_2H) afforded the desired product (3 g, 64% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{24}H_{34}N_2O_5$: 431.26; found 431.2.

Step 5: Synthesis of benzyl (2*S*)-3-methyl-2-(1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)butanoate

To a solution of *tert*-butyl 7-((*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate (400.0 mg, 0.929 mmol) in DCM (3.0 mL) at 0 °C was added TFA (1.50 mL, 20.195 mmol) dropwise. The resulting mixture was stirred at 0 °C for 1 h and was then concentrated under reduced pressure. The TFA residue was further removed by azeotropic

distillation with toluene three times to afford the desired product (400 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{19}H_{26}N_2O_3$: 331.20; found 331.1.

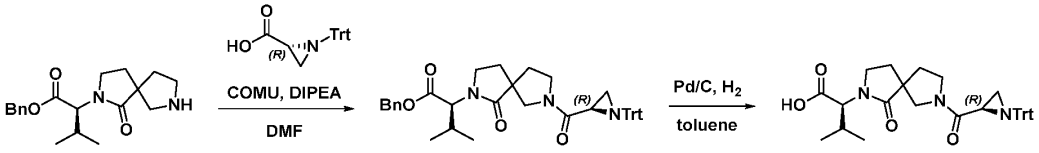
Step 6: Synthesis of benzyl (2*S*)-3-methyl-2-(1-oxo-7-((*S*)-1-tritylaziridine-2-carbonyl)-2,7-diazaspiro[4.4]nonan-2-yl)butanoate

To a solution of benzyl (2*S*)-3-methyl-2-(1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)butanoate (400.0 mg, 1.21 mmol) and DIPEA (2.06 mL, 12.11 mmol) in DMF (5 mL) at 0 °C was added (*S*)-1-tritylaziridine-2-carboxylic acid (558.26 mg, 1.695 mmol) followed by COMU (673.55 mg, 1.574 mmol) in portions. The resulting mixture was stirred at 0 °C for 1 h and was then diluted with H_2O (50 mL). The aqueous layer was extracted with EtOAc (3 x 20 mL) and the combined organic layers were washed with brine (20 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by prep-TLC (33% EtOAc/pet. ether) afforded the desired product (510 mg, 59% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{41}H_{43}N_3O_4$: 642.34; found 642.3.

Step 7: Synthesis of (2*S*)-3-methyl-2-(1-oxo-7-((*S*)-1-tritylaziridine-2-carbonyl)-2,7-diazaspiro[4.4]nonan-2-yl)butanoic acid

To a mixture of benzyl (2*S*)-3-methyl-2-(1-oxo-7-((*S*)-1-tritylaziridine-2-carbonyl)-2,7-diazaspiro[4.4]nonan-2-yl)butanoate (480.0 mg, 0.748 mmol) in toluene (35.0 mL) was added Pd/C 200.0 mg, 1.879 mmol). The resulting mixture was placed under an atmosphere of H_2 (1 atm), heated to 50 °C and stirred for 3 h. The mixture was cooled to room temperature, filtered, the filter cake was washed with MeOH (3 x 10 mL), and the filtrate was concentrated under reduced pressure to afford the desired product (310 mg, 67% yield). LCMS (ESI) *m/z*: [M - H] calcd for $C_{34}H_{37}N_3O_4$: 550.27; found 550.3.

Intermediate A-24. Synthesis of (2*S*)-3-methyl-2-(1-oxo-7-((*R*)-1-tritylaziridine-2-carbonyl)-2,7-diazaspiro[4.4]nonan-2-yl)butanoic acid



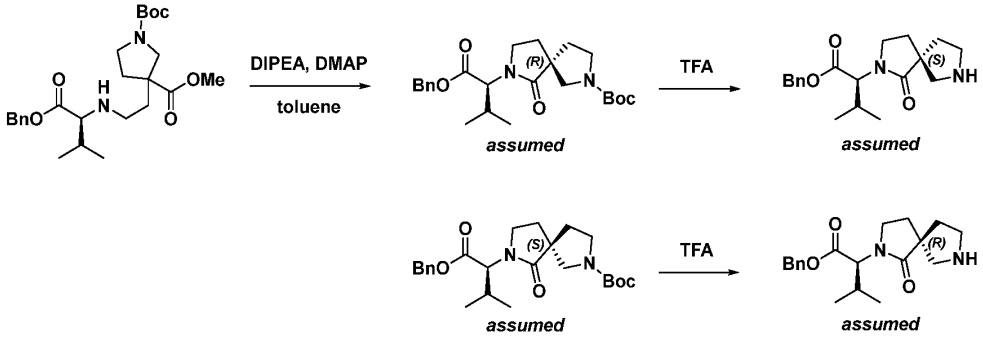
Step 1: Synthesis of benzyl (2*S*)-3-methyl-2-(1-oxo-7-((*R*)-1-tritylaziridine-2-carbonyl)-2,7-diazaspiro[4.4]nonan-2-yl)butanoate

To a solution of benzyl (2*S*)-3-methyl-2-(1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)butanoate (400.0 mg, 1.21 mmol) and (*R*)-1-tritylaziridine-2-carboxylic acid (518.4 mg, 1.57 mmol) in DMF (4.0 mL) at 0 °C was added DIPEA (1.0 mL, 6.05 mmol) followed by COMU (621.7 mg, 1.45 mmol). The resulting mixture was stirred for 1 h and was then diluted with H_2O (40 mL). The aqueous layer was extracted with EtOAc (3 x 15 mL) and the combined organic layers were washed with brine (2 x 10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by prep-TLC (33% EtOAc/pet. ether) to afford the desired product (540 mg, 62% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{41}H_{43}N_3O_4$: 642.33; found 642.4.

Step 2: Synthesis of (2*S*)-3-methyl-2-(1-oxo-7-((*R*)-1-tritylaziridine-2-carbonyl)-2,7-diazaspiro[4.4]nonan-2-yl)butanoic acid

To a solution of benzyl (2*S*)-3-methyl-2-(1-oxo-7-((*R*)-1-tritylaziridine-2-carbonyl)-2,7-diazaspiro[4.4]nonan-2-yl)butanoate (510.0 mg, 0.80 mmol) in toluene (30 mL) was added Pd/C (250.0 mg, 2.35 mmol). The resulting mixture was placed under a hydrogen atmosphere (1 atm), heated to 50 °C, and stirred for 3 h. The reaction was then cooled to room temperature, filtered, the filter cake was washed with MeOH (3 x 10 mL), and the filtrate was concentrated under reduced pressure to afford the desired crude product (330 mg). LCMS (ESI) *m/z*: [M + H] calcd for $C_{34}H_{37}N_3O_4$: 552.29; found 552.3.

Intermediate A-25 and A-26. Synthesis of benzyl (*S*)-3-methyl-2-((*S*)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)butanoate and benzyl (*S*)-3-methyl-2-((*R*)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)butanoate



Step 1: Synthesis of *tert*-butyl (*R*)-7-((*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate and *tert*-butyl (*S*)-7-((*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate

To a mixture of 1-(*tert*-butyl) 3-methyl 3-(2-(((*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)amino)ethyl)pyrrolidine-1,3-dicarboxylate (4.50 g, 9.728 mmol) and DIPEA (16.6 mL, 97.28 mmol) in toluene (50 mL) was added DMAP (1.19 g, 9.728 mmol) and then mixture was heated to 80 °C. After 24 h the reaction was cooled to room temperature and concentrated under reduced pressure. Purification by reverse phase chromatography (10→50% MeCN/H_2O, 0.1% HCO_2H). The diastereomers were then separated by chiral prep-SFC (30% EtOH/CO_2) to afford *tert*-butyl (*R*)-7-((*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate (1.0 g, 32% yield, LCMS (ESI) *m/z*: [M + H] calcd for $C_{24}H_{34}N_2O_5$: 431.26; found 431.2) and *tert*-butyl (*S*)-7-((*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate carboxylate (1.0 g, 32% yield, LCMS (ESI) *m/z*: [M + H] calcd for $C_{24}H_{34}N_2O_5$: 431.26; found 431.2).

Step 2: Synthesis of benzyl (*S*)-3-methyl-2-((*S*)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)butanoate

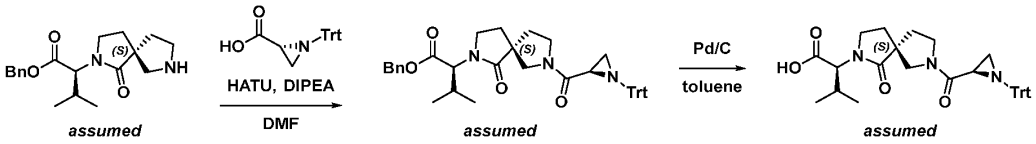
To a solution of *tert*-butyl (5*R*)-7-[(2*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl]-6-oxo-2,7-diazaspiro[4.4] nonane-2-carboxylate (1.40 g, 3.25 mmol) in DCM (14 mL) at 0 °C was added TFA (5.0 mL, 67.3 mmol). The resulting mixture was stirred at 0 °C for 1 h and was then concentrated under reduced pressure. The mixture was diluted with H_2O (20 mL) and was basified to pH 8 with sat. $NaHCO_3$ (aq.) at 0 °C. The resulting mixture was extracted with EtOAc (3 x 50 mL) and

combined organic layers were washed with brine (40 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (1.4 g, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{19}H_{26}N_2O_3$: 331.20; found 331.2).

Step 3: Synthesis of benzyl (*S*)-3-methyl-2-((*R*)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)butanoate

To a solution of *tert*-butyl (5*S*)-7-[(2*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl]-6-oxo-2,7-diazaspiro[4.4] nonane-2-carboxylate (1.0 g, 2.3 mmol) in DCM (10 mL) at 0 °C was added TFA (4.0 mL, 53.9 mmol). The resulting mixture was stirred at 0 °C for 1 h and was then concentrated under reduced pressure. The mixture was diluted with H_2O (10 mL) and was basified to pH 8 with sat. $NaHCO_3$ (aq.) at 0 °C. The resulting mixture was extracted with EtOAc (3 x 20 mL) and combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (1.0 g, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{19}H_{26}N_2O_3$: 331.20; found 331.1).

Intermediate A-27. Synthesis of (2*S*)-3-methyl-2-(1-oxo-7-((*R*)-1-tritylaziridine-2-carbonyl)-2,7-diazaspiro[4.4]nonan-2-yl)butanoic acid



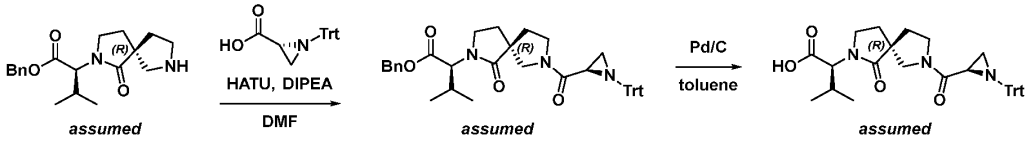
Step 1: Synthesis of benzyl (*S*)-3-methyl-2-((*S*)-1-oxo-7-((*R*)-1-tritylaziridine-2-carbonyl)-2,7-diazaspiro[4.4]nonan-2-yl)butanoate

To a solution of benzyl (*S*)-3-methyl-2-((*S*)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)butanoate (400 mg, 1.2 mmol) and DIPEA (1.1 mL, 6.1 mmol) in DMF (5.0 mL) at 0 °C was added (*R*)-1-tritylaziridine-2-carboxylic acid (480 mg, 1.5 mmol) and HATU (550 mg, 1.5 mmol). The resulting mixture was stirred for 1 h then purified by reverse phase chromatography (15→80% MeCN/H_2O, 0.5% NH_4HCO_3) to afford the desired product (500 mg, 57% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{41}H_{43}N_3O_4$: 642.34; found 642.3.

Step 2: Synthesis of (2*S*)-3-methyl-2-(1-oxo-7-((*R*)-1-tritylaziridine-2-carbonyl)-2,7-diazaspiro[4.4]nonan-2-yl)butanoic acid

A solution of benzyl (*S*)-3-methyl-2-((*S*)-1-oxo-7-((*R*)-1-tritylaziridine-2-carbonyl)-2,7-diazaspiro[4.4]nonan-2-yl)butanoate (450 mg, 0.70 mmol) and Pd/C (120 mg, 1.13 mmol) in toluene (30 mL) at 50 °C was stirred under a hydrogen atmosphere (1 atm). The mixture was stirred for 3 h and then was filtered, and the filter cake was washed with MeOH (3 x 30 mL). The filtrate was concentrated under reduced pressure to afford the desired product (430 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{34}H_{37}N_3O_4$: 552.29; found 552.3.

Intermediate 28. Synthesis of (*S*)-3-methyl-2-((*R*)-1-oxo-7-((*R*)-1-tritylaziridine-2-carbonyl)-2,7-diazaspiro[4.4]nonan-2-yl)butanoic acid



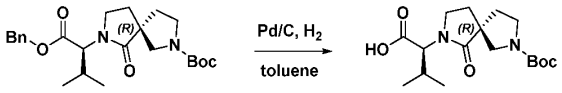
Step 1: Synthesis of benzyl (*S*)-3-methyl-2-((*R*)-1-oxo-7-((*R*)-1-tritylaziridine-2-carbonyl)-2,7-diazaspiro[4.4]nonan-2-yl)butanoate

To a solution of benzyl (*S*)-3-methyl-2-((*R*)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)butanoate (500 mg, 1.5 mmol) and DIPEA (1.3 mL, 7.6 mmol) in DMF (7.0 mL) at 0 °C was added (*R*)-1-tritylaziridine-2-carboxylic acid (550 mg, 1.7 mmol) and HATU (630 mg, 1.7 mmol). The resulting mixture was stirred for 1 h then purification by reverse phase chromatography (10→80% MeCN/H_2O, 0.5% NH_4HCO_3) afforded desired product (700 mg, 64% yield) as an off-white solid. LCMS (ESI) *m/z*: [M + H] calcd for $C_{41}H_{43}N_3O_4$: 642.34; found 642.3.

Step 2: Synthesis of (*S*)-3-methyl-2-((*R*)-1-oxo-7-((*R*)-1-tritylaziridine-2-carbonyl)-2,7-diazaspiro[4.4]nonan-2-yl)butanoic acid

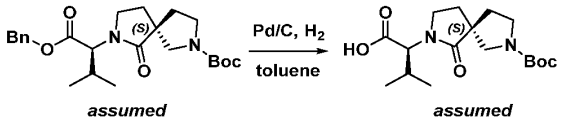
A solution of benzyl (*S*)-3-methyl-2-((*R*)-1-oxo-7-((*R*)-1-tritylaziridine-2-carbonyl)-2,7-diazaspiro[4.4]nonan-2-yl)butanoate (650 mg, 0.70 mmol) and Pd/C (140 mg, 1.3 mmol) in toluene (30 mL) at 50 °C was stirred under a hydrogen atmosphere (1 atm). The mixture was stirred for 3 h and then was filtered, and the filter cake was washed with MeOH (3 x 30 mL). The filtrate was concentrated under reduced pressure to afford the desired product (550 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{34}H_{37}N_3O_4$: 552.29; found 552.3.

Intermediate A-29. Synthesis of (*S*)-2-((*R*)-7-(*tert*-butoxycarbonyl)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)-3-methylbutanoic acid



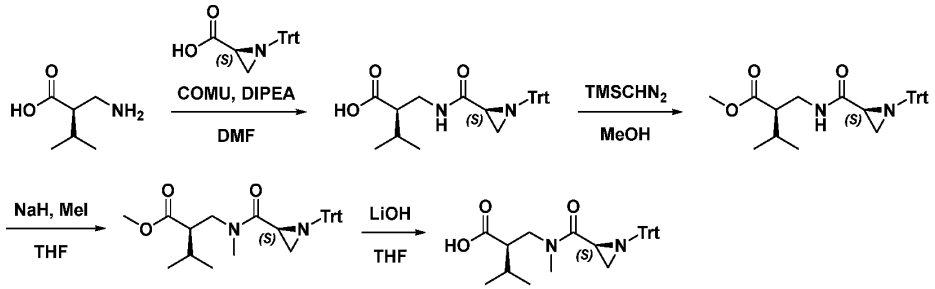
To a solution of *tert*-butyl (*R*)-7-((*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate (600 mg, 1.4 mmol) in toluene (20 mL) was added Pd/C (120 mg, 1.1 mmol). The reaction mixture was heated at 50 °C and stirred under a hydrogen atmosphere (1 atm) for 3 h. The mixture was filtered, and the filter cake was washed with MeOH (3 x 20 mL). The filtrate was concentrated under reduced pressure to afford the desired product (550 mg, crude). LCMS (ESI) *m/z*: [M - H] calcd for $C_{17}H_{28}N_2O_5$: 339.19; found 339.1.

Intermediate A-30. Synthesis of (*S*)-2-((*S*)-7-(*tert*-butoxycarbonyl)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)-3-methylbutanoic acid



To a solution of *tert*-butyl (*S*)-7-((*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate (550 mg, 1.3 mmol) in toluene (30 mL) was added Pd/C (120 mg, 1.1 mmol). The reaction mixture was heated at 50 °C and stirred under a hydrogen atmosphere (1 atm) for 3 h. The mixture was filtered, and the filter cake was washed with MeOH (3 x 20 mL). The filtrate was concentrated under reduced pressure to afford the desired product (550 mg, crude). LCMS (ESI) *m/z*: [M - H] calcd for $C_{17}H_{28}N_2O_5$: 339.19; found 339.2.

Intermediate A-31. Synthesis of (*R*)-3-methyl-2-(((*S*)-*N*-methyl-1-tritylaziridine-2-carboxamido)methyl)butanoic acid



Step 1: Synthesis of (*R*)-3-methyl-2-(((*S*)-1-tritylaziridine-2-carboxamido)methyl)butanoic acid

To a solution of (*S*)-1-tritylaziridine-2-carboxylic acid (1 g, 2.9 mmol) in DMF (10 mL) at 0 °C was added DIPEA (2.5 mL, 14.55 mmol) followed by COMU (1.12 g, 2.62 mmol). The resulting mixture was stirred for 20 min and (*R*)-2-(aminomethyl)-3-methylbutanoic acid (382.0 mg, 2.91 mmol) was added. The resulting mixture was warmed to room temperature and stirred for an additional 2 h. The reaction mixture was then quenched with H_2O and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by reverse phase chromatography (30→70% MeCN/H_2O + 0.1% NH_4HCO_3) to afford the desired product (850 mg, 63% yield). LCMS (ESI) *m/z*: [M - H] calcd for $C_{28}H_{30}N_2O_3$: 441.22; found 441.2.

Step 2: Synthesis of methyl (*R*)-3-methyl-2-(((*S*)-1-tritylaziridine-2-carboxamido)methyl)butanoate

To a solution of (*R*)-3-methyl-2-(((*S*)-*N*-methyl-1-tritylaziridine-2-carboxamido)methyl)butanoic acid (840.0 mg, 1.90 mmol) in MeOH (5.0 mL) at 0 °C was added $TMSCHN_2$ (10.0 mL, 0.45 mmol). The resulting mixture was warmed to room temperature and stirred for 2 h, at which point the reaction mixture was concentrated under reduced pressure. The residue was purified by reverse phase chromatography (30→80% MeCN/H_2O + 0.1% NH_4HCO_3) to

afford the desired product (450 mg, 52% yield). LCMS (ESI) *m/z*: [M - H] calcd for $C_{29}H_{32}N_2O_3$: 455.23; found 455.1.

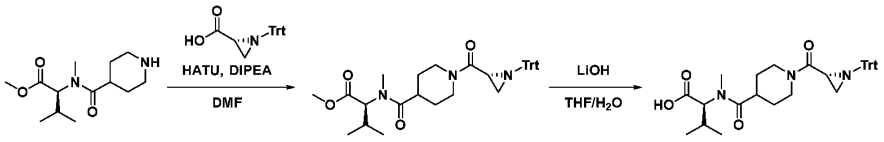
Step 3: Synthesis of methyl (*R*)-3-methyl-2-(((*S*)-*N*-methyl-1-tritylaziridine-2-carboxamido)methyl)butanoate

To a solution of methyl (*R*)-3-methyl-2-(((*S*)-1-tritylaziridine-2-carboxamido)methyl)butanoate (440.0 mg, 0.96 mmol) in THF (5.0 mL) at 0 °C was added NaH (46.25 mg, 1.93 mmol). The resulting mixture was stirred for 30 min and then MeI (1.37 g, 9.65 mmol) was added. The resulting mixture was warmed to room temperature and stirred for an additional 4 h. The reaction mixture was then quenched with H_2O and the aqueous layer was extracted with EtOAc (3 x 300 mL). The combined organic layers were washed with brine (3 x 200 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by reverse phase chromatography (10→90% MeCN/H_2O + 0.1% NH_4HCO_3) to afford the desired product (340 mg, 75% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{30}H_{34}N_2O_3$: 471.26; found 471.3.

Step 4: Synthesis of (*R*)-3-methyl-2-(((*S*)-*N*-methyl-1-tritylaziridine-2-carboxamido)methyl)butanoic acid

To a solution of methyl (*R*)-3-methyl-2-(((*S*)-*N*-methyl-1-tritylaziridine-2-carboxamido)methyl)butanoate (340.0 mg, 0.72 mmol) in MeOH (3.0 mL) and H_2O (3.0 mL) was added LiOH•H2O (242.5 mg, 5.78 mmol). The resulting mixture was stirred for 16 h at room temperature and was then acidified to pH 4 with $KHSO_4$ (1 N). The resulting mixture was extracted with EtOAc
(3 x 300 mL) and the combined organic layers were washed with brine (3 x 300 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by reverse phase chromatography (10→80% MeCN/H_2O + 0.1% NH_4HCO_3) to afford the desired product (260 mg). LCMS (ESI) *m/z*: [M + H] calcd for $C_{29}H_{32}N_2O_3$: 455.23; found 455.1.

Intermediate A-32. Synthesis of *N*-methyl-*N*-(1-((*R*)-1-tritylaziridine-2-carbonyl)piperidine-4-carbonyl)-*L*-valine



Step 1: Synthesis of methyl *N*-methyl-*N*-(1-((*R*)-1-tritylaziridine-2-carbonyl)piperidine-4-carbonyl)-*L*-valinate

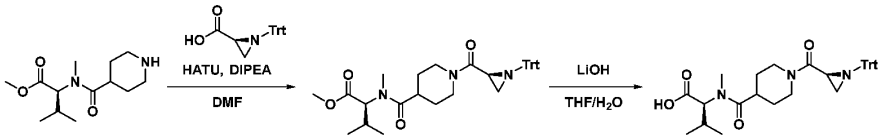
To a mixture of methyl *N*-methyl-*N*-(piperidine-4-carbonyl)-*L*-valinate (750 mg, 2.93 mmol) and (*R*)-1-tritylaziridine-2-carboxylic acid (1.13 g, 3.43 mmol) in DMF (7 mL) at 0 °C was added DIPEA (2.50 mL, 14.62 mmol) followed by HATU (2.20 g, 5.79 mmol) in portions. The resulting mixture was warmed to room temperature and stirred for 3 h. The reaction mixture was diluted with EtOAc (300 mL) and the mixture was washed with brine (2 x 150 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by normal phase chromatography (50%

EtOAc/hexanes) afforded the desired product (1.5 g, 90.3% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{35}H_{41}N_3O_4$: 568.32; found 568.3.

Step 2: Synthesis of *N*-methyl-*N*-(1-((*R*)-1-tritylaziridine-2-carbonyl)piperidine-4-carbonyl)-*L*-valine

To a solution of methyl *N*-methyl-*N*-(1-((*R*)-1-tritylaziridine-2-carbonyl)piperidine-4-carbonyl)-*L*-valinate (500 mg, 0.881 mmol) in THF (5 mL) at 0 °C was added a solution of LiOH (111 mg, 2.64 mmol) in H_2O (2.6 mL). The resulting mixture was warmed to room temperature and stirred for 4 h. The reaction mixture was diluted with H_2O (300 mL) and acidified to pH 5 with 1M HCl. The resulting mixture was extracted with DCM (3 x 100 mL) and the combined organic layers were washed with brine (2 x 150 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (600 mg, crude) which was used without further purification. LCMS (ESI) *m/z*: [M - H] calcd for $C_{34}H_{39}N_3O_4$: 552.29; found 552.3.

Intermediate A-33. Synthesis of *N*-methyl-*N*-(1-((*S*)-1-tritylaziridine-2-carbonyl)piperidine-4-carbonyl)-*L*-valine



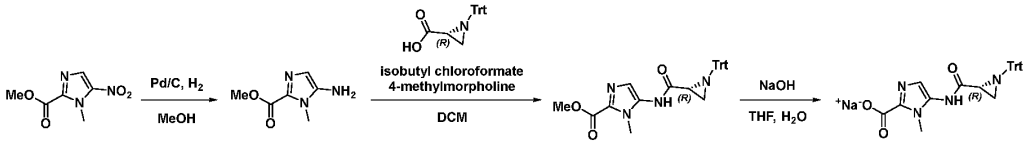
Step 1: Synthesis of methyl *N*-methyl-*N*-(1-((*S*)-1-tritylaziridine-2-carbonyl)piperidine-4-carbonyl)-*L*-valinate

To a mixture of methyl *N*-methyl-*N*-(piperidine-4-carbonyl)-*L*-valinate (0.90 g, 3.511 mmol) and (*S*)-1-tritylaziridine-2-carboxylic acid (2.31 g, 7.022 mmol) in DMF (10 mL) at 0 °C was added DIPEA (3.06 mL, 17.57 mmol) and HATU (2.67 g, 7.022 mmol). The resulting mixture was warmed to room temperature and stirred for 2 h. The reaction mixture was diluted with EtOAc (50 mL) and the mixture was washed with H_2O, brine (100 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by normal phase chromatography (100% EtOAc) afforded the desired product (1.47 g, 73.7% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{35}H_{41}N_3O_4$: 568.32; found 568.3.

Step 2: Synthesis of *N*-methyl-*N*-(1-((*S*)-1-tritylaziridine-2-carbonyl)piperidine-4-carbonyl)-*L*-valine

To a solution of methyl *N*-methyl-*N*-(1-((*S*)-1-tritylaziridine-2-carbonyl)piperidine-4-carbonyl)-*L*-valinate (1.0 g, 1.76 mmol) in THF (15 mL) at 0 °C was added a solution of LiOH (370 mg, 8.80 mmol) in H_2O (15 mL). The resulting mixture was warmed to room temperature and stirred for 3 h. The reaction mixture was acidified to pH 6 with 1M HCl. The aqueous layer was extracted with EtOAc (2 x 50 mL) and the combined organic layers were dried with Na_2SO_4, filtered, and concentrated under reduced pressure to adfford the desired product (1.33 g, crude) which was used without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{34}H_{39}N_3O_4$: 554.30; found 554.3.

Intermediate A-34. Synthesis of sodium (*R*)-1-methyl-5-(1-tritylaziridine-2-carboxamido)-1*H*-imidazole-2-carboxylate



Step 1: Synthesis of methyl 5-amino-1-methyl-1*H*-imidazole-2-carboxylate

To a mixture of methyl 1-methyl-5-nitro-1*H*-imidazole-2-carboxylate (1.0 g, 5.401 mmol) in MeOH (15 mL) was added Pd/C (500 mg). The resulting mixture was placed under an atmosphere of H_2 (1 atm) and stirred for 3 h. The mixture was filtered, the filter cake was washed with MeOH (3 x 20 mL), and the filtrate was concentrated under reduced pressure to afford the desired product (1.0 g, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_6H_9N_3O_2$: 156.08; found 156.1.

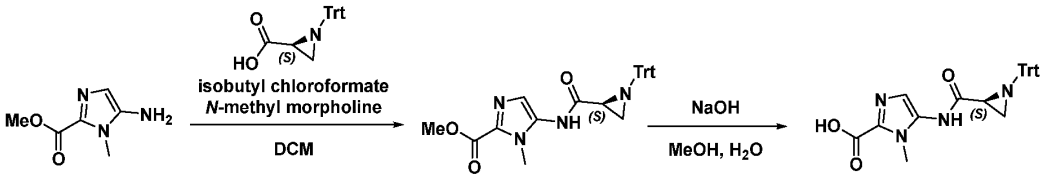
Step 2: Synthesis of methyl (*R*)-1-methyl-5-(1-tritylaziridine-2-carboxamido)-1*H*-imidazole-2-carboxylate

To a mixture of (*R*)-1-tritylaziridine-2-carboxylic acid (2.55 g, 7.741 mmol) in DCM (12.0 mL) at 0 °C was added a solution of isobutyl chloroformate (845.06 mg, 6.187 mmol) and *N*-methylmorpholine (1.04 g, 10.282 mmol) in DCM in portions over 30 min. To the resulting mixture was added methyl 5-amino-1-methyl-1*H*-imidazole-2-carboxylate (800.0 mg, 5.156 mmol). The mixture was stirred at room temperature overnight then diluted with DCM (300 mL) and washed with H_2O (3 x 100 mL). The organic layer was washed with brine (2 x 150 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (25% EtOAc/hexanes) afforded the desired product (1.2 g, 49.9% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{28}H_{26}N_4O_3$: 467.21; found 467.2.

Step 3: Synthesis of sodium (*R*)-1-methyl-5-(1-tritylaziridine-2-carboxamido)-1*H*-imidazole-2-carboxylate

To a mixture of methyl (*R*)-1-methyl-5-(1-tritylaziridine-2-carboxamido)-1*H*-imidazole-2-carboxylate (300 mg, 0.643 mmol) in THF (3 mL) was added a solution of NaOH (38.58 mg, 0.965 mmol) in H_2O. The resulting mixture was stirred for 2 h and then concentrated under reduced pressure to afford the desired product (400 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{27}H_{24}N_4O_3$: 453.19; found 453.2.

Intermediate A-35. Synthesis of (*S*)-1-methyl-5-(1-tritylaziridine-2-carboxamido)-1*H*-imidazole-2-carboxylic acid



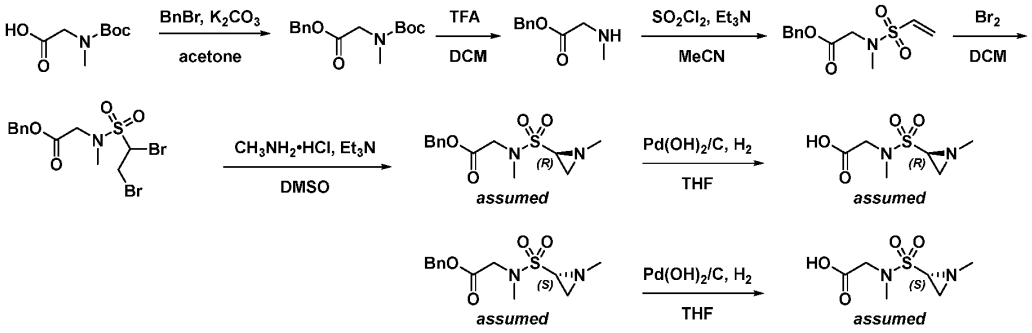
Step 1: Synthesis of methyl (*S*)-1-methyl-5-(1-tritylaziridine-2-carboxamido)-1*H*-imidazole-2-carboxylate

To a mixture of (*S*)-1-tritylaziridine-2-carboxylic acid (1.18 g, 3.577 mmol) in DCM (15 mL) at 0 °C was added isobutyl chloroformate (423.41 mg, 3.100 mmol) and *N*-methylmorpholine (0.39 mL, 3.862 mmol) dropwise. The resulting mixture was stirred at 0 °C for 1 h then methyl 5-amino-1-methyl-1*H*-imidazole-2-carboxylate (370.0 mg, 2.385 mmol) was added. The mixture was warmed to room temperature and stirred overnight. The reaction was quenched with sat. $NaHCO_3$ at 0 °C and the resulting mixture was extracted with DCM (2 x 100 mL). The combined organic layers were washed with brine (150 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (100% EtOAc) afforded the desired product (380 mg, 34.2% yield). LCMS (ESI) *m/z*: [M + H] calcd for C28H26N4O3: 467.21; found 467.3.

Step 2: Synthesis of (*S*)-1-methyl-5-(1-tritylaziridine-2-carboxamido)-1*H*-imidazole-2-carboxylic acid

To a mixture of methyl (*S*)-1-methyl-5-(1-tritylaziridine-2-carboxamido)-1*H*-imidazole-2-carboxylate (380.0 mg, 0.815 mmol) in MeOH (5 mL) at 0 °C was added NaOH (146.60 mg, 3.665 mmol) in H_2O (3.6 mL) dropwise. The resulting mixture was warmed to room temperature and stirred for 6 h then was acidified to pH 6 with 1M HCl. The resulting mixture was extracted with DCM (2 x 100 mL), and the combined organic layers were washed with brine (150 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (350 mg, crude). LCMS (ESI) *m/z*: [M - H] calcd for $C_{27}H_{24}N_4O_3$: 451.17; found 451.1.

Intermediate A-36 and A-37. Synthesis of (*R*)-*N*-methyl-*N*-((1-methylaziridin-2-yl)sulfonyl)glycine and (*S*)-*N*-methyl-*N*-((1-methylaziridin-2-yl)sulfonyl)glycine



Step 1: Synthesis of benzyl *N*-(*tert*-butoxycarbonyl)-*N*-methylglycinate

To a stirred mixture of [(*tert*-butoxycarbonyl)(methyl)amino]acetic acid (15. g, 79.28 mmol) in acetone (150 mL) was added BnBr (14.14 mL, 82.70 mmol) and K_2CO_3 (21.91 g, 158.55 mmol) in portions at 0 °C . The resulting mixture was stirred for 4 h at room temperature. The resulting mixture was filtered, the filter cake was washed with acetone (3 x 100 mL), and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (33% EtOAc/pet. ether) to afford the desired product (15.2 g, 68.6% yield). LCMS (ESI) *m/z*: [M + Na] calcd for $C_{15}H_{21}NO_4$: 302.14; found 302.0.

Step 2: Synthesis of benzyl methylglycinate

To a stirred solution of benzyl *N*-(*tert*-butoxycarbonyl)-*N*-methylglycinate (10.0 g, 35.80 mmol) in DCM (100 mL) was added TFA (50 mL) dropwise at 0 °C. The resulting mixture was stirred

for 1 h at 0 °C and then the resulting mixture was concentrated under reduced pressure to afford the desired product (7.80 g, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{10}H_{13}NO_2$: 180.10; found 179.1.

Step 3: Synthesis of benzyl *N*-methyl-*N*-(vinylsulfonyl)glycinate

To a solution of benzyl methylglycinate (15.60 g, 87.04 mmol) and Et_3N (36.4 mL, 261.1 mmol) in MeCN (300 mL) at -70 °C was added a solution of 2-chloroethanesulfonyl chloride (17.03 g, 104.47 mmol) in MeCN (150 mL). The resulting mixture was warmed to room temperature and stirred for 20 min. The reaction mixture was cooled -50 °C and additional Et_3N (36.4 mL, 261.1 mmol) was added to reaction mixture. The reaction mixture was warmed to room temperature and stirred for 1 h. The reaction was then quenched with H_2O at 0 °C. The mixture was acidified to pH 6 with 1 M HCl aq and was then extracted with DCM (800 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (33% EtOAc/pet. ether) to afford the desired product (7.53 g, 32.1% yield). LCMS (ESI) *m/z*: [M + H_2O] calcd for $C_{12}H_{15}NO_4S$: 287.08; found 287.2.

Step 4: Synthesis of benzyl *N*-((1,2-dibromoethyl)sulfonyl)-*N*-methylglycinate

To a solution of benzyl *N*-methyl-*N*-(vinylsulfonyl)glycinate (5.58 g, 20.7 mmol) in DCM (50 mL) at -20 °C was added a solution of Br_2 (1.06 mL, 6.64 mmol) in DCM (10 mL). The resulting mixture was warmed to room temperature and stirred overnight. The reaction mixture was then cooled to 0 °C and quenched with sat. aq. $Na_2S_2O_3$ (30 mL). The resulting mixture was washed with sat. aq. Na_2HCO_3 and then extracted with DCM (2 x 200 mL), the combined organic layers dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (33% EtOAc/pet. ether) to afford the desired product (5.1 g, 57.4% yield). LCMS (ESI) *m/z*: [M + H_2O] calcd for $C_{12}H_{15}Br_2NO_4S$: 444.92; found 444.9.

Step 5: Synthesis of benzyl (*R*)-*N*-methyl-*N*-((1-methylaziridin-2-yl)sulfonyl)glycinate and benzyl (*S*)-*N*-methyl-*N*-((1-methylaziridin-2-yl)sulfonyl)glycinate

To a stirred solution of benzyl *N*-((1,2-dibromoethyl)sulfonyl)-*N*-methylglycinate (7.20 g, 16.78 mmol) and methylamine hydrochloride (3.39 g, 50.2 mmol) in DMSO (750 mL) was added Et_3N (23.32 mL, 230.47 mmol). The resulting mixture was stirred for 2 h at room temperature then heated to 75 °C and stirred overnight. The reaction mixture was cooled to room temperature and extracted with EtOAc (2 x 1000 mL). The combined organic layers were washed with H_2O (1500 mL) and brine (1500 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc) to afford a mixture of diastereomers. The diastereomers were separated by prep-SFC (10% EtOH/Hex) to afford benzyl (*R*)-*N*-methyl-*N*-((1-methylaziridin-2-yl)sulfonyl)glycinate (500 mg, 31.3% yield) and benzyl (*S*)-*N*-methyl-*N*-((1-methylaziridin-2-yl)sulfonyl)glycinate (600 mg, 37.5% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{18}N_2O_4S$: 299.11; found 299.0.

Step 6: Synthesis of (*R*)-*N*-methyl-*N*-((1-methylaziridin-2-yl)sulfonyl)glycine

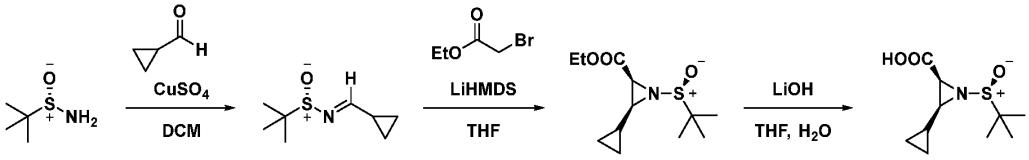
A suspension of benzyl (*R*)-*N*-methyl-*N*-((1-methylaziridin-2-yl)sulfonyl)glycinate (300.0 mg) and $Pd(OH)_2/C$ (150.0 mg) in THF at room temperature was stirred under an atmosphere of hydrogen (1 atm) for 3 h. The mixture was filtered, the filter cake was washed with MeOH (3 x 20

mL), and the filtrate was concentrated under reduced pressure to afford the desired product (206 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_6H_{12}N_2O_4S$: 209.06; found 209.0.

Step 7: Synthesis of (*S*)-*N*-methyl-*N*-((1-methylaziridin-2-yl)sulfonyl)glycine

A suspension of benzyl (*R*)-*N*-methyl-*N*-((1-methylaziridin-2-yl)sulfonyl)glycinate (300.0 mg, 1.01 mmol) and $Pd(OH)_2/C$ (150.0 mg) in THF at room temperature was stirred under an atmosphere of hydrogen (1 atm) for 3 h. The mixture was filtered, the filter cake was washed with MeOH (3 x 20 mL), and the filtrate was concentrated under reduced pressure to afford the desired product (216 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_6H_{12}N_2O_4S$: 209.06; found 209.1.

Intermediate A-38. Synthesis of (2*S*,3*S*)-1-(*tert*-butylsulfinyl)-3-cyclopropylaziridine-2-carboxylic acid



Step 1: Synthesis of (*E*)-*N*-(cyclopropylmethylene)-2-methylpropane-2-sulfinamide

To a suspension of (*S*)-2-methylpropane-2-sulfinamide (4.0 g, 33.0 mmol) and $CuSO_4$ (15.80 g, 99.01 mmol) in DCM (200.0 mL) was added cyclopropanecarbaldehyde (4.63 g, 66.0 mmol). The resulting mixture was stirred overnight and was then filtered, the filter cake was washed with DCM (3 x 100 mL), and the filtrate was concentrated under reduced pressure to afford the desired product (3.5 g, 61.2% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_8H_{15}NOS$: 174.10; found 174.1.

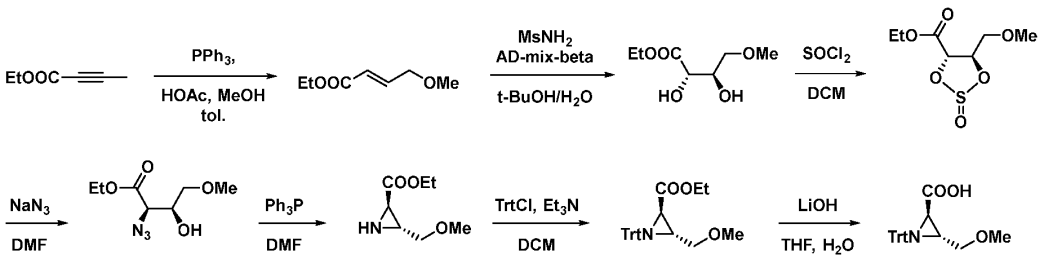
Step 2: Synthesis of ethyl (2*S*,3*S*)-1-(*tert*-butylsulfinyl)-3-cyclopropylaziridine-2-carboxylate

To a solution of ethyl bromoacetate (481.91 mg, 2.886 mmol) in THF (5.0 mL) at -78 °C was added LiHMDS (2.90 mL, 2.90 mmol). The resulting mixture was stirred for 2 h at -78 °C and then a solution of (*E*)-*N*-(cyclopropylmethylene)-2-methylpropane-2-sulfinamide (250.0 mg, 1.443 mmol) was added. The resulting mixture was stirred for 2 h at -78 °C and was then was then quenched with H_2O at 0 °C. The aqueous layer was extracted with EtOAc (3 x 50 mL), and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by prep-TLC (17% EtOAc/pet. ether) to afford the desired product (250 mg, 66.8% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{12}H_{21}NO_3S$: 260.13; found 260.1.

Step 3: Synthesis of (2*S*,3*S*)-1-(*tert*-butylsulfinyl)-3-cyclopropylaziridine-2-carboxylic acid

A solution of ethyl (2*S*,3*S*)-1-(*tert*-butylsulfinyl)-3-cyclopropylaziridine-2-carboxylate (500.0 mg, 1.928 mmol) in THF (2.0 mL) and H_2O (2.0 mL) at 0 °C was added $LiOH\bullet H_2O$ (121.34 mg, 2.89 mmol). The reaction mixture was stirred for 1 h and was then acidified to pH 6 with 1 M HCl (aq.). The resulting mixture was extracted with EtOAc (2 x 10 mL) and the combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure to afford the desired product (400 mg, 89.7% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{10}H_{17}NO_3S$: 232.10; found 232.0.

Intermediate A-39. Synthesis of (2*R*,3*R*)-3-(methoxymethyl)-1-tritylaziridine-2-carboxylic acid



Step 1: Synthesis of ethyl (*E*)-4-methoxybut-2-enoate

To a solution of ethyl but-2-ynoate (10.0 g, 89.18 mmol) in MeOH (8.80 mL, 118.594 mmol) and HOAc (1.05 mL, 18.3 mmol) was added a solution of PPh_3 (1.20 g, 4.58 mmol) in toluene (60.0 mL). The resulting solution heated to 110 °C and stirred overnight. The reaction mixture was cooled to room temperature and was then diluted with H_2O (60 mL). The resulting solution was extracted with EtOAc (2 x 60), and the combined organic layers were washed with brine (2 x 20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (9% EtOAc/pet. ether) to afford the desired product (4.9 g, 38.1% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_7H_{12}O_3$: 145.09; found 144.9.

Step 2: Synthesis of ethyl (2*S*,3*R*)-2,3-dihydroxy-4-methoxybutanoate

To a solution of ethyl (*E*)-4-methoxybut-2-enoate (5.0 g, 34.68mmol), and methanesulfonamide (3.30 g, 34.68 mmol) in *t*-BuOH (150.0 mL) and H_2O (100.0 mL) was added AD-mix-β (48.63 g, 62.43 mmol). The resulting solution was heated to 30 °C and stirred overnight. The solution was then cooled to room temperature and adjusted to pH 2 with $KHSO_4$. The resulting solution was extracted with EtOAc (2 x 100 mL) and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (1.28 g, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_7H_{14}O_5$: 179.09; found 179.0.

Step 3: Synthesis of ethyl (4*S*,5*R*)-5-(methoxymethyl)-1,3,2-dioxathiolane-4-carboxylate 2-oxide

To a solution of ethyl (2*S*,3*R*)-2,3-dihydroxy-4-methoxybutanoate (4.10 g, 23.01 mmol) in DCM (20.0 mL) at 0 °C was added $SOCl_2$ (5.47 g, 45.9 mmol). The resulting mixture was heated to 50 °C and stirred for 3 h. The reaction mixture was then cooled to room temperature and concentrated under reduced pressure to afford the desired product (4.0 g, crude).

Step 4: Synthesis of ethyl (2*R*,3*S*)-2-azido-3-hydroxy-4-methoxybutanoate

To a solution of ethyl (4*S*,5*R*)-5-(methoxymethyl)-1,3,2-dioxathiolane-4-carboxylate 2-oxide (4.0 g crude, 17.84 mmol) in DMF (20.0 mL) at 0 °C was added NaN_3 (5.80 g, 89.22 mmol). The resulting mixture was heated to 35 °C and stirred overnight. The reaction mixture was then diluted with H_2O (200 mL) and was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (3 x 50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (17% EtOAc/pet. ether) to

afford the desired product (1.0 g, 27.6% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_7H_{13}N_3O_4$: 204.10; found 204.0.

Step 5: Synthesis of ethyl (2*R*,3*R*)-3-(methoxymethyl)aziridine-2-carboxylate

To a solution of ethyl (2*R*,3*S*)-2-azido-3-hydroxy-4-methoxybutanoate(1.0 g, 4.92 mmol) in DMF (10 mL) at 0 °C was added PPh_3 (1.29 g, 4.92 mmol) in portions over 30 min. The reaction solution was then warmed to room temperature and stirred for 30 min. The reaction mixture was then heated to 85 °C and stirred until the reaction was complete. The reaction mixture was then concentrated under reduced pressure and purified by prep-TLC (33% EtOAc/pet. ether) to afford the desired product (480 mg, 61.3% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_7H_{13}NO_3$: 160.10; found 160.1.

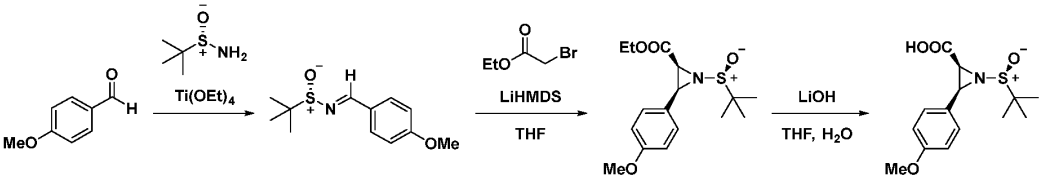
Step 6: Synthesis of ethyl (2*R*,3*R*)-3-(methoxymethyl)-1-tritylaziridine-2-carboxylate

To a solution of ethyl (2*R*,3*R*)-3-(methoxymethyl)aziridine-2-carboxylate (480.0 mg, 3.02 mmol) and Et_3N (2.1 mL, 15.0 mmol) in DCM (10 mL) at 0 °C was added Trt-Cl (1.681 g, 6.031 mmol). The resulting mixture was warmed to room temperature and stirred for 2 h. The mixture was concentrated then concentrated under reduced pressure and the residue was purified by prep-TLC (5% EtOAc/pet. ether) to afford the desired product (700 mg, crude).

Step 7: Synthesis of (2*R*,3*R*)-3-(methoxymethyl)-1-tritylaziridine-2-carboxylic acid

To a solution of ethyl (2*R*, 3*R*)-3-(methoxymethyl)-1-(triphenylmethyl)aziridine-2-carboxylate (200.0 mg, 0.498 mmol) in THF (5.0 mL) and H_2O (5 mL) was added $LiOH•H_2O$ (41.81 mg, 0.996 mmol). The resulting solution was stirred at room temperature for 24 h. The mixture was then diluted with H_2O (10 mL) and extracted with EtOAc (20 mL). The aqueous layer was then acidified to pH 7 with sat. aq. NH_4Cl and extracted with EtOAc (2 x 10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (60 mg, 32.3% yield). LCMS (ESI) *m/z*: [M - H] calcd for $C_{24}H_{23}NO_3$: 372.16; found 372.1.

Intermediate A-40. Synthesis of (2*S*,3*S*)-1-(*tert*-butylsulfinyl)-3-(4-methoxyphenyl)aziridine-2-carboxylic acid



Step 1: (*E*)-*N*-(4-methoxybenzylidene)-2-methylpropane-2-sulfinamide

A solution of (*S*)-2-methylpropane-2-sulfinamide (2.50 g) and anisaldehyde (2.81 g) in $Ti(OEt)_4$ (20.0 mL) was stirred at 70 °C for 1 h. The resulting mixture was cooled to room temperature, diluted with EtOAc (60 mL), and then poured into H_2O. The mixture was filtered, and the filter cake was washed with EtOAc (3 x 50 mL). The resulting mixture was extracted with EtOAc (3 x 50 mL) and the combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column

chromatography (25% EtOAc/pet. ether) to afford the desired product (4 g, 81.0% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{12}H_{17}NO_2S$: 240.11; found 240.1.

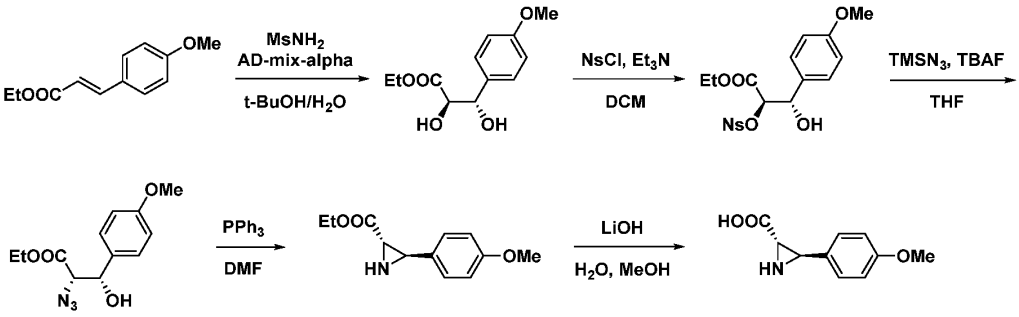
Step 2: Synthesis of ethyl (2*S*,3*S*)-1-(*tert*-butylsulfinyl)-3-(4-methoxyphenyl)aziridine-2-carboxylate

To a solution of ethyl 2-bromoacetate (5.60 g, 33.5 mmol) in THF (100 mL) at -78 °C was added LiHMDS (1M in THF, 34 mL, 33.473 mmol). After 30 min a solution of (*E*)-*N*-(4-methoxybenzylidene)-2-methylpropane-2-sulfinamide (4 g, 16.74 mmol) in THF (20 mL) was added. The resulting mixture was stirred at -78 °C for additional 3 h. The reaction was then quenched with sat. aq. NH_4Cl. The mixture was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (25% EtOAc/pet. ether) to afford the desired product (2.7 g, 49.6% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{16}H_{23}NO_4S$: 326.14; found 326.1.

Step 3: Synthesis of (2*S*,3*S*)-1-(*tert*-butylsulfinyl)-3-(4-methoxyphenyl)aziridine-2-carboxylic acid

To a solution of ethyl (2*S*,3*S*)-1-(*tert*-butylsulfinyl)-3-(4-methoxyphenyl)aziridine-2-carboxylate (800.0 mg, 2.68 mmol) in THF (2.0 mL) at 0 °C was added a solution of $LiOH\bullet H_2O$ (309.46 mg, 7.38 mmol) in H_2O (3.0 mL). The resulting mixture was warmed to room temperature and stirred for 4 h. The mixture was then acidified to pH 6 with sat. aq. NH_4Cl and then extracted with EtOAc (3 x 50 mL). The combined organic layers were dried over Na_2SO_4, filtered. and concentrated under reduced pressure to afford the desired product (690 mg, 94.4% yield). LCMS (ESI) *m/z*: [M - H] calcd for $C_{14}H_{19}NO_4S$: 296.10; found 296.2.

Intermediate A-41. Synthesis of (2*S*,3*R*)-3-(4-methoxyphenyl)aziridine-2-carboxylic acid



Step 1: Synthesis of ethyl (2*R*,3*S*)-2,3-dihydroxy-3-(4-methoxyphenyl)propanoate

To a solution of ethyl *p*-methoxycinnamate (5.0 g, 24.24 mmol) in *t*BuOH (70.0 mL) and H_2O (70.0 mL) at 0 °C was added AD-mix-α (33.80 g, 43.39 mmol) and methanesulfonamide (2.31 mg, 0.024 mmol). The resulting mixture was warmed to room temperature and stirred overnight. The reaction was then cooled to 0 °C and quenched with $KHSO_4$ (aq.). The mixture was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with brine (2 x 90 mL),

dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (50% EtOAc/pet. ether) to afford the desired product (5.7 g, 88.1% yield).

Step 2: Synthesis of ethyl (2*R*,3*S*)-3-hydroxy-3-(4-methoxyphenyl)-2-(((4-nitrophenyl)sulfonyl)oxy)propanoate

To a solution of ethyl (2*R*,3*S*)-2,3-dihydroxy-3-(4-methoxyphenyl)propanoate (3.0 g, 12.49 mmol) and Et_3N (0.174 mL, 1.249 mmol) in DCM (30.0 mL) at 0 °C was added 4-nitrobenzenesulfonyl chloride (2.76 g, 12.49 mmol). The resulting mixture was stirred for 1 h and was then diluted with H_2O. The mixture was extracted with DCM (3 x 100 mL) and the combined organic layers were washed with brine (2 x 100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by prep-TLC (50% EtOAc/pet. ether) to afford the desired product (3.8 g, 68.0% yield). LCMS (ESI) *m/z*: [M + Na] calcd for $C_{18}H_{19}NO_9S$: 448.07; found 448.2.

Step 3: Synthesis of ethyl (2*S*,3*S*)-2-azido-3-hydroxy-3-(4-methoxyphenyl)propanoate

To a solution of ethyl (2*R*,3*S*)-3-hydroxy-3-(4-methoxyphenyl)-2-(((4-nitrophenyl)sulfonyl)oxy)propanoate (1.20 g, 2.82 mmol) in THF at 0 °C was added TBAF (1M in THF, 5.64 mL, 5.64 mmol) and $TMSN_3$ (648.79 mg, 5.64 mmol). The resulting mixture was heated to at 60 °C and stirred for 16 h. The reaction was then cooled to at 0 °C and quenched with sat. aq. NH_4Cl. The mixture was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with H_2O (2 x 100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by prep-TLC (33% EtOAc/pet. ether) to afford the desired product (540 mg, 70.7% yield).

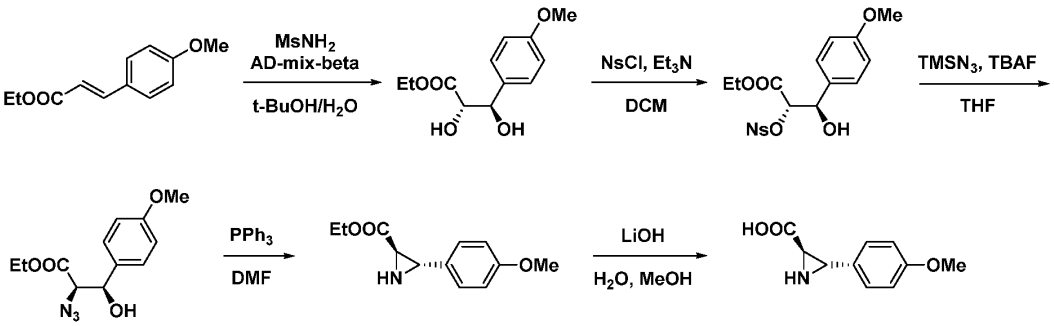
Step 4: Synthesis of ethyl (2*S*,3*R*)-3-(4-methoxyphenyl)aziridine-2-carboxylate

To a solution of ethyl (2*S*,3*S*)-2-azido-3-hydroxy-3-(4-methoxyphenyl)propanoate (440.0 mg, 1.659 mmol) in DMF was added PPh_3 (522.06 mg, 1.99 mmol). The resulting mixture was stirred at room temperature for 30 min and was then heated to 80 °C and stirred overnight. The mixture was then extracted with EtOAc (3 x 100 mL), and the combined organic layers were washed with H_2O (2 x 100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by prep-TLC (25% EtOAc/pet. ether) to afford the desired product (200 mg, 51.8% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{12}H_{15}NO_3$: 222.12; found 222.1.

Step 5: Synthesis of (2*S*,3*R*)-3-(4-methoxyphenyl)aziridine-2-carboxylic acid

To a solution of ethyl (2*S*,3*R*)-3-(4-methoxyphenyl)aziridine-2-carboxylate (200.0 mg, 0.904 mmol) in MeOH and H_2O at 0 °C was added $LiOH \bullet H_2O$ (86.6 mg, 3.62 mmol). The resulting mixture was stirred for 1 h and was then neutralized to pH 7 with HCl (aq.). The mixture was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with H_2O (2 x 100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (180 mg, 97.9% yield). LCMS (ESI) *m/z*: [M - H] calcd for $C_{10}H_{11}NO_3$: 192.07; found 192.0.

Intermediate A-42. Synthesis of (2*R*,3*S*)-3-(4-methoxyphenyl)aziridine-2-carboxylic acid



Step 1: Synthesis of ethyl (2*S*,3*R*)-2,3-dihydroxy-3-(4-methoxyphenyl)propanoate

To a solution of ethyl *p*-methoxycinnamate (5.0 g, 24.24 mmol) in *t*BuOH (70.0 mL) and H_2O (70.0 mL) at 0 °C was added AD-mix-ß (33.80 g, 43.39 mmol) and methanesulfonamide (2.31 mg, 0.024 mmol). The resulting mixture was warmed to room temperature and stirred overnight. The reaction was then cooled to 0 °C and quenched with $KHSO_4$ (aq.). The mixture was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with brine (2 x 90 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (50% EtOAc/pet. ether) to afford the desired product (5.7 g, 88.1% yield).

Step 2: Synthesis of ethyl (2*S*,3*R*)-3-hydroxy-3-(4-methoxyphenyl)-2-(((4-nitrophenyl)sulfonyl)oxy)propanoate

To a solution of ethyl (2*S*,3*R*)-2,3-dihydroxy-3-(4-methoxyphenyl)propanoate (5.80 g, 24.14 mmol) and Et_3N (10.1 mL, 72.42 mmol) in DCM (30.0 mL) at 0 °C was added 4-nitrobenzenesulfonyl chloride (5.34 g, 24.1 mmol). The resulting mixture was stirred for 1 h and was then diluted with H_2O. The mixture was extracted with DCM (3 x 100 mL) and the combined organic layers were washed with brine (2 x 100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by prep-TLC (50% EtOAc/pet. ether) to afford the desired product (7.2 g, 67.0% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{18}H_{19}NO_9S$: 426.09; found 426.2.

Step 3: Synthesis of ethyl (2*R*,3*R*)-2-azido-3-hydroxy-3-(4-methoxyphenyl)propanoate

To a solution of ethyl (2*S*,3*R*)-3-hydroxy-3-(4-methoxyphenyl)-2-(((4-nitrophenyl)sulfonyl)oxy)propanoate (5.0 g, 11.75 mmol) in THF at 0 °C was added TBAF (1M in THF, 23.5 mL, 23.51 mmol) and $TMSN_3$ (2.7 g, 23.5 mmol). The resulting mixture was heated to at 60 °C and stirred for 16 h. The reaction was then cooled to at 0 °C and quenched with sat. aq. NH_4Cl. The mixture was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with H_2O (2 x 100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by prep-TLC (33% EtOAc/pet. ether) to afford the desired product (2.3 g, 70.1% yield).

Step 4: Synthesis of ethyl (2*R*,3*S*)-3-(4-methoxyphenyl)aziridine-2-carboxylate

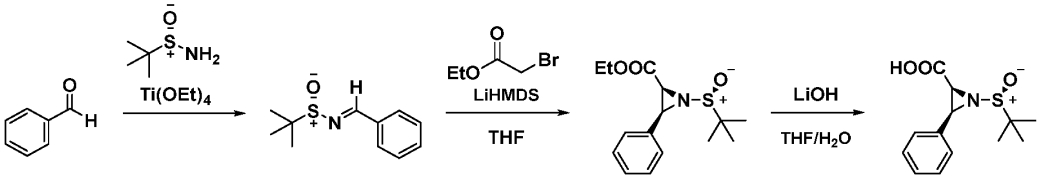
To a solution of ethyl (2*R*,3*R*)-2-azido-3-hydroxy-3-(4-methoxyphenyl)propanoate (2.30 g, 8.67 mmol) in DMF was added PPh_3 (2.73 g, 10.4 mmol). The resulting mixture was stirred at room

temperature for 30 min and was then heated to 80 °C and stirred overnight. The mixture was then extracted with EtOAc (3 x 100 mL), and the combined organic layers were washed with H_2O (2 x 100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by prep-TLC (25% EtOAc/pet. ether) to afford the desired product (1.6 g, 79.2% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{12}H_{15}NO_3$: 222.12; found 222.1.

Step 5: Synthesis of (2*R*,3*S*)-3-(4-methoxyphenyl)aziridine-2-carboxylic acid

To a solution of ethyl (2*S*,3*R*)-3-(4-methoxyphenyl)aziridine-2-carboxylate (200.0 mg, 0.904 mmol) in MeOH and H_2O at 0 °C was added LiOH●H_2O (86.6 mg, 3.62 mmol). The resulting mixture was stirred for 1 h and was then neutralized to pH 7 with HCl (aq.). The mixture was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with H_2O (2 x 100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (180 mg, 97.9% yield). LCMS (ESI) *m/z*: [M - H] calcd for $C_{10}H_{11}NO_3$: 192.07; found 192.0.

Intermediate A-43. Synthesis of (2*S*,3*S*)-1-((*S*)-*tert*-butylsulfinyl)-3-phenylaziridine-2-carboxylic acid



Step 1: Synthesis of (*S*,*E*)-*N*-benzylidene-2-methylpropane-2-sulfinamide

A solution of (*S*)-2-methylpropane-2-sulfinamide (2.50 g, 20.6 mmol), titanium ethoxide (9.41 g, 41.25 mmol) and benzaldehyde (2.19 g, 20.7 mmol) was heated at 70 °C for 1 h, cooled, and diluted with H_2O (250 mL). The aqueous layer was extracted with EtOAc (3 x 80 mL) and the combined organic layers were washed with brine (2 x 100 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure to afford the desired product (4.3 g, crude) which was used without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{11}H_{15}NOS$: 210.10; found 210.2.

Step 2: Synthesis of ethyl (2*S*,3*S*)-1-((*S*)-*tert*-butylsulfinyl)-3-phenylaziridine-2-carboxylate

To a solution of ethyl bromoacetate (798 mg, 4.78 mmol) in THF (15 mL) at -78 °C was added LiHMDS (1M in THF, 4.78 mL, 4.78 mmol). After 1 h, (*S*,*E*)-*N*-benzylidene-2-methylpropane-2-sulfinamide (500 mg, 2.39 mmol) in THF (5 mL) was added in portions over 20 min. The reaction mixture was stirred at -78 °C for 2 h and then quenched by the addition of sat. NH_4Cl. The aqueous layer was extracted with EtOAc (3 x 40 mL) and the combined organic layers were washed with brine (2 x 30 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by reverse phase chromatography (30→60% MeCN/H_2O, 0.1% HCO_2H) afforded the desired product (480 mg, 61% yield).

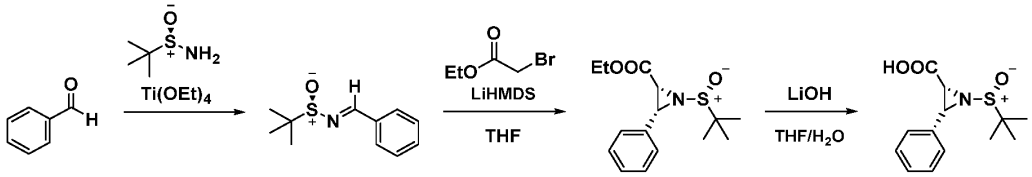
LCMS (ESI) *m/z*: [M + H] calcd for $C_{15}H_{21}NO_3S$: 296.13; found 296.2.

Step 3: Synthesis (2*S*,3*S*)-1-((*S*)-*tert*-butylsulfinyl)-3-phenylaziridine-2-carboxylic acid

To a solution of ethyl (2*S*,3*S*)-1-((*S*)-*tert*-butylsulfinyl)-3-phenylaziridine-2-carboxylate (600 mg, 2.03 mmol) in THF (4.0 mL) at 0 °C was added a solution of LiOH (97.2 mg, 4.06 mmol) in H_2O

(4.0 mL). The resulting mixture was stirred for 2 h at 0 °C and then acidified to pH 5 with 1 M HCl. The aqueous layer was extracted with EtOAc (3 x 40 mL) and the combined organic layers were washed with brine (2 x 20 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired compound (450mg, crude) which was used without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{17}NO_3S$: 268.10; found 268.1.

Intermediate A-44. Synthesis of (2*R*,3*R*)-1-((*R*)-*tert*-butylsulfinyl)-3-phenylaziridine-2-carboxylic acid



Step 1: Synthesis (*R*,*E*)-*N*-benzylidene-2-methylpropane-2-sulfinamide

A solution (*R*)-2-methylpropane-2-sulfinamide (2.50 g, 20.6 mmol), titanium tetraethoxide (9.41 g, 41.3 mmol) and benzaldehyde (2.19 g, 20.6 mmol) was heated 70 °C for 1 h, cooled, and diluted with H_2O (250 mL). The aqueous layer was extracted with EtOAc (3 x 90 mL) and the combined organic layers were washed with brine (2 x 100 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure to afford the desired product (4.2 g, crude) which was used without further purification. LCMS (ESI) *m/z:* [M + H] calcd for $C_{11}H_{15}NOS$: 210.10; found 210.1.

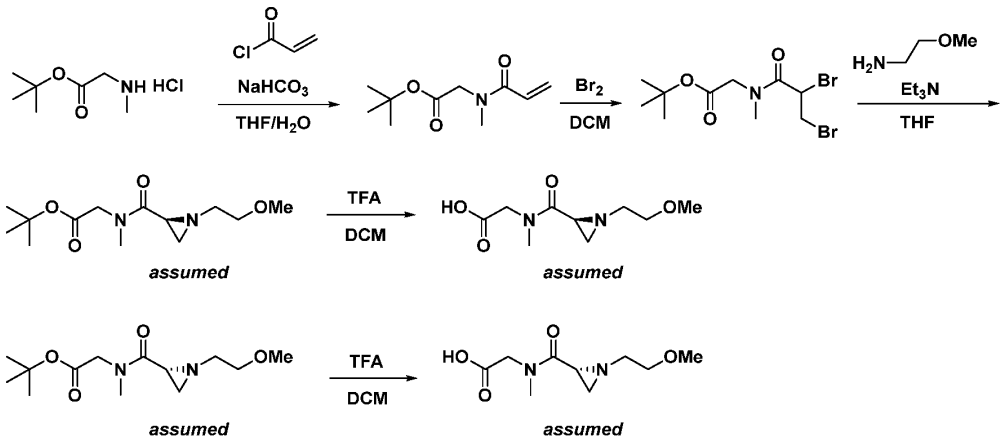
Step 2: Synthesis of ethyl (2*R*,3*R*)-1-((*R*)-*tert*-butylsulfinyl)-3-phenylaziridine-2-carboxylate

To a solution of ethyl bromoacetate (6.38 g, 38.2 mmol) in THF (150 mL) at -78 °C was added LiHMDS (1M in THF, 7.19 mL, 42.9 mmol). After 1 h, (*R*,*E*)-*N*-benzylidene-2-methylpropane-2-sulfinamide (4.0 g, 19.1 mmol) in THF (50 mL) was added in portions over 20 min. The reaction mixture was stirred at -78 °C for 2 h and then quenched by the addition of sat. NH_4Cl. The aqueous layer was extracted with EtOAc (3 x 80 mL) and the combined organic layers were washed with brine (2 x 60 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure. Purification by reverse phase chromatography (30→60% MeCN/H_2O, 0.1% HCO_2H) afforded the desired product (3.9 g, 62% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{15}H_{21}NO_3S$: 296.13; found 296.2.

Step 3: Synthesis (2*R*,3*R*)-1-((*R*)-*tert*-butylsulfinyl)-3-phenylaziridine-2-carboxylic acid

To a solution of ethyl (2*R*,3*R*)-1-((*R*)-*tert*-butylsulfinyl)-3-phenylaziridine-2-carboxylate (200 mg, 0.677 mmol) in THF (1.5 mL) at 0 °C was added a solution of LiOH (32.4 mg, 1.35 mmol) in H_2O (1.3 mL). The resulting mixture was stirred for 2 h at 0 °C and then acidified to pH 5 with 1M HCl. The aqueous layer was extracted with EtOAc (3 x 20 mL) and the combined organic layers were washed with brine (2 x 10 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired compound (220 mg, crude) which was used without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{17}NO_3S$: 268.10; found 268.4.

Intermediate A-45 and A-46. Synthesis of and (*S*)-*N*-(1-(2-methoxyethyl)aziridine-2-carbonyl)-*N*-methylglycine and (*R*)-*N*-(1-(2-methoxyethyl)aziridine-2-carbonyl)-*N*-methylglycine



Step 1: Synthesis of *tert*-butyl *N*-acryloyl-*N*-methylglycinate

To a mixture of *tert*-butyl methylglycinate hydrochloride (1.0 g, 5.5 mmol) and $NaHCO_3$ (1.39 g, 16.5 mmol) in THF (10 mL) and H_2O (5.0 mL) at 0 °C was added acryloyl chloride (750 mg, 8.26 mmol). The resulting solution was stirred for 2 h at room temperature and the reaction was then quenched by the addition H_2O (50 mL). The aqueous layer was extracted with EtOAc (2 x 50 mL) and the combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by normal phase chromatography (10→33% EtOAc/pet. ether) afforded the desired product (900 mg, 73.8% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{10}H_{17}NO_3$: 200.13; found 200.2.

Step 2: Synthesis of *tert*-butyl *N*-(2,3-dibromopropanoyl)-*N*-methylglycinate

To a solution of *tert*-butyl *N*-acryloyl-*N*-methylglycinate (2.0 g, 10.1 mmol) in DCM (40 mL) at -20 °C was added Br_2 (3.21 g, 20.1 mmol). The resulting mixture was stirred for 2 h at -20 °C and then quenched by the addition of $Na_2S_2O_3$ (100 mL). The aqueous layer was extracted with DCM (2 x 100 mL) and the combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated under reduced pressure to afford the desired product (2.4 g, crude) which was used without further purification. LCMS (ESI) *m/z*: [M + Na] calcd for $C_{10}H_{17}Br_2NO_3$: 381.96; found 381.8.

Step 3: Synthesis of *tert*-butyl (*S*)-*N*-(1-(2-methoxyethyl)aziridine-2-carbonyl)-*N*-methylglycinate and *tert*-butyl (*R*)-*N*-(1-(2-methoxyethyl)aziridine-2-carbonyl)-*N*-methylglycinate

To a solution of *tert*-butyl *N*-(2,3-dibromopropanoyl)-*N*-methylglycinate (4.0 g, 11.1 mmol) and 2-methoxyethan-1-amine (4.18 g, 55.7 mmol) in THF (40 mL) was added Et_3N (4.66 mL, 33.4 mmol). The resulting solution was stirred at 35 °C overnight was then quenched by the addition of H_2O. The aqueous layer was extracted with DCM (2 x 100 mL) and the combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by reverse phase chromatography (30→50% MeCN/H_2O) afforded a mixture of the desired products. The enantiomers were separated by chiral preparative normal phase chromatography (hexane, 10 mM NH_3-MeOH/EtOH) to afford *tert*-butyl (*S*)-*N*-(1-(2-

methoxyethyl)aziridine-2-carbonyl)-*N*-methylglycinate (400 mg, 33.3% yield) and *tert*-butyl (*R*)-*N*-(1-(2-methoxyethyl)aziridine-2-carbonyl)-*N*-methylglycinate (360 mg, 30% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{24}N_2O_4$: 273.18; found 273.0.

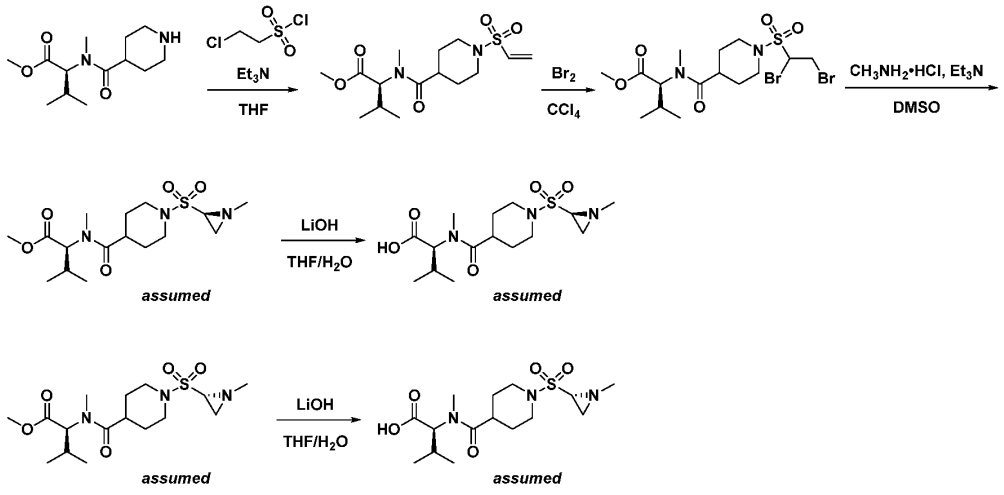
Step 4: Synthesis of (*S*)-*N*-(1-(2-methoxyethyl)aziridine-2-carbonyl)-*N*-methylglycine

To a solution of *tert*-butyl (*S*)-*N*-(1-(2-methoxyethyl)aziridine-2-carbonyl)-*N*-methylglycinate (250 mg, 0.918 mmol) in DCM (6.0 mL) at 0 °C was added TFA (3.0 mL). The resulting mixture was stirred at 2 h at 0 °C and then concentrated under reduced pressure to afford the desired product (250 mg, crude) which was used without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_9H_{16}N_2O_4$: 217.12; found 217.1.

Step 5: Synthesis of (*R*)-*N*-(1-(2-methoxyethyl)aziridine-2-carbonyl)-*N*-methylglycine

To a solution *tert*-butyl (*R*)-*N*-(1-(2-methoxyethyl)aziridine-2-carbonyl)-*N*-methylglycinate (180 mg, 0.661 mmol) in DCM (6.0 mL) at 0 °C was added TFA (3.0 mL). The resulting mixture was stirred at 2 h at 0 °C and then concentrated under reduced pressure to afford the desired product (150mg, crude) which was used without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_9H_{16}N_2O_4$: 217.12; found 217.1.

Intermediate A-47 and A-48. Synthesis of *N*-methyl-*N*-(1-(((*R*)-1-methylaziridin-2-yl)sulfonyl)piperidine-4-carbonyl)-*L*-valine and *N*-methyl-*N*-(1-(((*S*)-1-methylaziridin-2-yl)sulfonyl)piperidine-4-carbonyl)-*L*-valine



Step 1: Synthesis of methyl *N*-methyl-*N*-(1-(vinylsulfonyl)piperidine-4-carbonyl)-*L*-valinate

To a solution of 2-chloroethanesulfonyl chloride (1.91 g, 11.7 mmol) in THF (20 mL) at -70 °C was added methyl *N*-methyl-*N*-(piperidine-4-carbonyl)-*L*-valinate (2.0 g, 7.8 mmol) followed by Et_3N (790 □L, 780 □mol). After warming to -50 °C additional Et_3N (790 □L, 780 □mol) was added and the reaction mixture warmed to room temperature. After 1 h the reaction was quenched at 0 °C by the addition of H_2O (30 mL), acidified to pH 6 with 1M HCl, and extracted with $CHCl_3$ (3 x 30 mL). The combined organic layers were dried with $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by normal phase chromatography (50% EtOAc/pet. ether) afforded

the desired product (560 mg, 20.7% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{15}H_{26}N_2O_5S$: 347.17; found 347.2.

Step 2: Synthesis of methyl *N*-(1-((1,2-dibromoethyl)sulfonyl)piperidine-4-carbonyl)-*N*-methyl-*L*-valinate

To a solution of methyl *N*-methyl-*N*-(1-(vinylsulfonyl)piperidine-4-carbonyl)-*L*-valinate (580 mg, 1.67 mmol) in CCl_4 (28 mL) at room temperature was added Br_2 (580 mg, 1.67 mmol) The resulting mixture was stirred overnight at room temperature and then quenched by the addition of sat. $NaHCO_3$ (30 mL). The aqueous layer was extracted with DCM (3 x 30 mL) and the combined organic layers were dried with Na_2SO_4, filtered and concentrated under reduced pressure to afford the desired product which was used without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{15}H_{26}Br_2N_2O_5S$: 506.99; found 506.9.

Step 3: Synthesis of methyl *N*-methyl-*N*-(1-(((*R*)-1-methylaziridin-2-yl)sulfonyl)piperidine-4-carbonyl)-*L*-valinate and methyl *N*-methyl-*N*-(1-(((*S*)-1-methylaziridin-2-yl)sulfonyl)piperidine-4-carbonyl)-*L*-valinate

To a solution of methyl *N*-(1-((1,2-dibromoethyl)sulfonyl)piperidine-4-carbonyl)-*N*-methyl-*L*-valinate (4.80 g, 9.481 mmol) in DMSO (48 mL) was added methanamine hydrochloride (1.92 g, 28.436 mmol) and Et_3N (13.2 mL, 94.8 mmol). The reaction mixture was heated to 75 °C and stirred overnight. The mixture was then cooled to 0 °C, diluted NH_4Cl, and extracted with EtOAc (600 mL). The organic layer was washed with brine, dried with Na_2SO_4, filtered, concentrated under reduced pressure. Purification with normal phase chromatography (86% EtOAc/hexane) afforded a mixture of the desired products. The diastereomers were separated by prep-SFC chromatography (20% IPA/CO_2) to afford methyl *N*-methyl-*N*-(1-(((*R*)-1-methylaziridin-2-yl)sulfonyl)piperidine-4-carbonyl)-*L*-valinate (700 mg, 38.9% yield) and methyl *N*-methyl-*N*-(1-(((*S*)-1-methylaziridin-2-yl)sulfonyl)piperidine-4-carbonyl)-*L*-valinate (790 mg, 43.9% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{16}H_{29}N_3O_5S$: 376.19; found 376.1.

Step 4: Synthesis of *N*-methyl-*N*-(1-(((*R*)-1-methylaziridin-2-yl)sulfonyl)piperidine-4-carbonyl)-*L*-valine

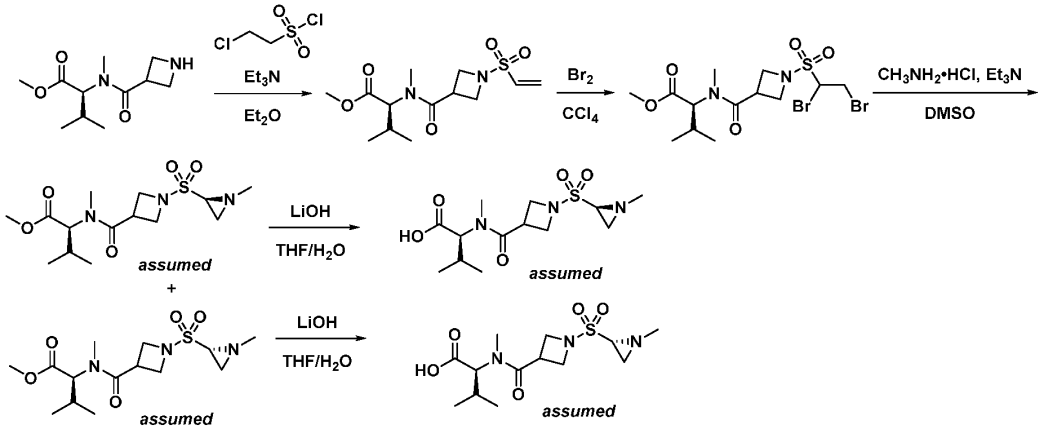
To a solution of methyl *N*-methyl-*N*-(1-(((*R*)-1-methylaziridin-2-yl)sulfonyl)piperidine-4-carbonyl)-*L*-valinate (200 mg, 0.533 mmol) in THF (2.0 mL) at 0 °C was added 1M LiOH (1 mL) The resulting mixture was stirred for 3 h at room temperature and then acidified to pH 6 with 1M HCl. The aqueous layer was extracted with EtOAc (3 x 10 mL) and the combined organic layers were dried with Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product which was used without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{15}H_{27}N_3O_5S$: 362.18; found 362.2.

Step 5: Synthesis of *N*-methyl-*N*-(1-(((*S*)-1-methylaziridin-2-yl)sulfonyl)piperidine-4-carbonyl)-*L*-valine

To a solution methyl *N*-methyl-*N*-(1-(((*S*)-1-methylaziridin-2-yl)sulfonyl)piperidine-4-carbonyl)-*L*-valinate (300 mg, 0.799 mmol) in THF (3.0 mL) at 0 °C was added 1M LiOH (3.0 mL). The resulting mixture was stirred for 3 h at room temperature and then acidified to pH 6 with 1M HCl. The aqueous layer was extracted with EtOAc (3 x 10 mL) and the combined organic layers

dried with Na_2SO_4, filtered and concentrated under reduced pressure to afford the desired product which was used without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{15}H_{27}N_3O_5S$: 362.18; found 362.2.

Intermediate A-49 and A-50. Synthesis of *N*-methyl-*N*-(1-(((*R*)-1-methylaziridin-2-yl)sulfonyl)azetidine-3-carbonyl)-*L*-valine and *N*-methyl-*N*-(1-(((*S*)-1-methylaziridin-2-yl)sulfonyl)azetidine-3-carbonyl)-*L*-valine



Step 1: Synthesis of methyl *N*-methyl-*N*-(1-(vinylsulfonyl)azetidine-3-carbonyl)-*L*-valinate

To a solution of 2-chloroethanesulfonyl chloride (357 mg, 2.19 mmol) in Et_2O (4.0 mL) at -70 °C was added an Et_2O (4.0 mL) solution of methyl *N*-(azetidine-3-carbonyl)-*N*-methyl-*L*-valinate (500 mg, 2.19 mmol) followed by Et_3N (0.304 mL, 2.19 mmol). The resulting mixture was stirred for 30 min at -50 °C at which time Et_3N (0.304 mL, 2.19 mmol) was added. The resulting mixture was stirred for 1 h at room temperature and then quenched with H_2O at 0 °C. The mixture was acidified to pH 6 with 1M HCl and extracted with $CHCl_3$ (3 x 10 mL). The combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by normal phase chromatography (50% EtOAc/pet. ether) afforded the desired product (180 mg, 25.8% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{22}N_2O_5S$: 319.13; found 319.1.

Step 2: Synthesis of methyl *N*-(1-((1,2-dibromoethyl)sulfonyl)azetidine-3-carbonyl)-*N*-methyl-*L*-valinate

To a solution of methyl *N*-methyl-*N*-(1-(vinylsulfonyl)azetidine-3-carbonyl)-*L*-valinate (460 mg, 1.45 mmol) in CCl_4 (6.0 mL) at room temperature was added a CCl_4 (2.0 mL) solution of Br_2 (346 mg, 2.17 mmol). The resulting mixture was stirred overnight and then quenched at 0 °C by the addition of sat. $NaHCO_3$ and $Na_2S_2O_3$. The aqueous layer was extracted with DCM (3 x 10 mL) and the combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated under reduced pressure to afford the desired product (500 mg) which was used without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{22}Br_2N_2O_5S$: 478.97; found 478.0.

Step 3: Synthesis of methyl *N*-methyl-*N*-(1-(((*R*)-1-methylaziridin-2-yl)sulfonyl)azetidine-3-carbonyl)-*L*-valinate and methyl *N*-methyl-*N*-(1-(((*S*)-1-methylaziridin-2-yl)sulfonyl)azetidine-3-carbonyl)-*L*-valinate

To a solution of methyl *N*-(1-((1,2-dibromoethyl)sulfonyl)azetidine-3-carbonyl)-*N*-methyl-*L*-valinate (260 mg, 0.54 mmol) in DMSO (4.0 mL) was added methanamine hydrochloride (110.0 mg, 1.63 mmol) and Et_3N (0.758 mL, 5.44 mmol). The resulting mixture was heated to 75 °C and stirred overnight. The mixture was then cooled to room temperature, diluted with H_2O (10 mL), and extracted with EtOAc (3 x 5 mL). The combined organic layers were washed with brine, dried with Na_2SO_4, filtered and concentrated under reduced pressure. Purification by normal phase chromatography (50% EtOAc/pet. ether) afforded a mixture of the desired products. The diastereomers were separated by chiral prep normal phase chromatography (hexane, 10 mM NH_3-MeOH /IPA) to afford methyl *N*-methyl-*N*-(1-(((*R*)-1-methylaziridin-2-yl)sulfonyl)azetidine-3-carbonyl)-*L*-valinate (0.59 g, 35% yield) and methyl *N*-methyl-*N*-(1-(((*S*)-1-methylaziridin-2-yl)sulfonyl)azetidine-3-carbonyl)-*L*-valinate (0.56 g, 33% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{14}H_{25}N_3O_5S$: 348.16; found 348.2.

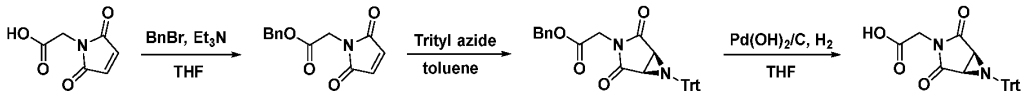
Step 4: Synthesis of *N*-methyl-*N*-(1-(((*R*)-1-methylaziridin-2-yl)sulfonyl)azetidine-3-carbonyl)-*L*-valine

To a solution of methyl *N*-methyl-*N*-(1-(((*R*)-1-methylaziridin-2-yl)sulfonyl)azetidine-3-carbonyl)-*L*-valinate (225.0 mg, 0.65 mmol) in THF (1.5 mL) at 0 °C was added LiOH (77.0mg, 3.23 mmol) dissolved in H_2O (1.5 mL). The resulting mixture was stirred for 2 h at room temperature and then acidified to pH 6 with 1M HCl. The aqueous layer was extracted with EtOAc and the combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (270 mg) which was used without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{23}N_3O_5S$: 334.15; found 334.0.

Step 5: Synthesis of *N*-methyl-*N*-(1-(((*S*)-1-methylaziridin-2-yl)sulfonyl)azetidine-3-carbonyl)-*L*-valine

To a solution of methyl *N*-methyl-*N*-(1-(((*S*)-1-methylaziridin-2-yl)sulfonyl)azetidine-3-carbonyl)-*L*-valinate (365.0 mg, 1.05 mmol) in THF (2.0 mL) and H_2O (2.0 mL) at 0 °C was added LiOH hydrate (132.0 mg, 3.15 mmol). The resulting mixture was stirred for 2 h at room temperature then acidified to pH 6 with 1M HCl and diluted with H_2O (20 mL). The aqueous layer was extracted with EtOAc and the combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (257 mg, crude) which was used without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{23}N_3O_5S$: 334.15; found 334.3.

Intermediate A-51. Synthesis of 2-((1*R*,5*S*)-2,4-dioxo-6-trityl-3,6-diazabicyclo[3.1.0]hexan-3-yl)acetic acid



Step 1: Synthesis of benzyl 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetate

To a solution of 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetic acid (5.0 g, 32.2 mmol) and Et_3N (13.5 mL, 96.7 mmol) in THF (80 mL) at 0 °C was added benzyl bromide (11.03 g, 64.5 mmol). The resulting mixture was stirred overnight at room temperature and then filtered. The filter cake was washed with THF (3 x 40 mL) and the filtrate was concentrated under reduced pressure. Purification by silica gel chromatography (16% EtOAc/hexanes) afforded the desired product (4.4 g, 55.7% yield) LCMS (ESI) *m/z*: [2M + Na] calcd for $C_{13}H_{11}NO_4$: 513.14; found 513.2.

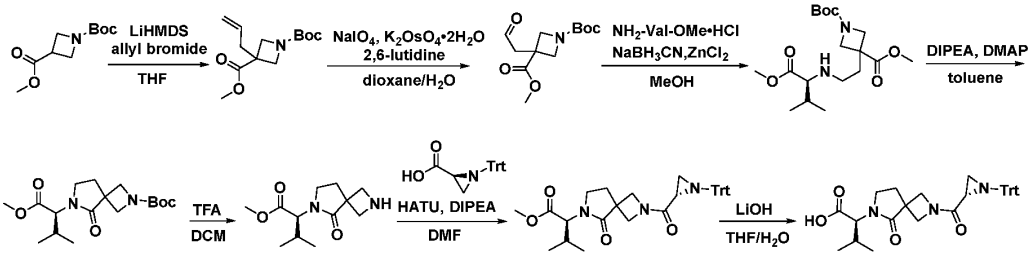
Step 2: Synthesis of benzyl 2-((1*R*,5*S*)-2,4-dioxo-6-trityl-3,6-diazabicyclo[3.1.0]hexan-3-yl)acetate

To a solution of benzyl 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetate of (1.0 g, 4.0 mmol) in toluene (10 mL) was added trityl azide (1.36 g, 4.89 mmol). The resulting mixture was stirred overnight at 120 °C and then concentrated under reduced pressure. Purification by reverse flash chromatography (50→80% MeCN/H_2O) afforded the desired product (400 mg, 19.5% yield). LCMS (ESI) *m/z*: [M + Na] calcd for $C_{32}H_{26}N_2O_4$: 525.19; found 525.2.

Step 3: Synthesis of 2-((1*R*,5*S*)-2,4-dioxo-6-trityl-3,6-diazabicyclo[3.1.0]hexan-3-yl)acetic acid

To a solution of benzyl 2-((1*R*,5*S*)-2,4-dioxo-6-trityl-3,6-diazabicyclo[3.1.0]hexan-3-yl)acetate (220 mg, 0.438 mmol) in THF (8.0 mL) was added $Pd(OH)_2/C$ (60 mg). The resulting solution was placed under a hydrogen atmosphere for 3 h using a H_2 balloon, filtered through Celite, and concentrated under reduced pressure to afford the desired product (160 mg, crude) which was used without further purification. LCMS (ESI) *m/z*: [M - H] calcd for $C_{25}H_{20}N_2O_4$: 411.13; found 411.2.

Intermediate A-52. Synthesis of (*S*)-3-methyl-2-(5-oxo-2-((*S*)-1-tritylaziridine-2-carbonyl)-2,6-diazaspiro[3.4]octan-6-yl)butanoic acid



Step 1: Synthesis of 1-(*tert*-butyl) 3-methyl 3-allylazetidine-1,3-dicarboxylate

To a solution of 1-(*tert*-butyl) 3-methyl azetidine-1,3-dicarboxylate (20.0 g, 92.9 mmol) and LiHMDS (140 mL, 1M in THF, 139 mmol) in THF (200 mL) at -78 °C was added allyl bromide (16.9 g, 139 mmol). The resulting solution was stirred overnight at room temperature and then quenched

with the addition of sat. NH_4Cl (100 mL) and diluted with EtOAc (800 mL). The organic layer was washed with brine (3 x 300 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure. Purification by silica gel column chromatography (17% EtOAc/pet. ether) afforded the desired product (15.0 g, 63.2% yield). LCMS (ESI) *m/z*: [M + H - *t*Bu] calcd for $C_{13}H_{21}NO_4$: 200.10; found 200.0

Step 2: Synthesis of 1-(*tert*-butyl) 3-methyl 3-(2-oxoethyl)azetidine-1,3-dicarboxylate

To a solution of 1-(*tert*-butyl) 3-methyl 3-allylazetidine-1,3-dicarboxylate (6.0 g, 23 mmol) and 2,6-lutidine (504 mg, 47.0 mmol) in dioxane (60 mL) and H_2O (60 mL) at 0 °C was added $K_2OsO_4 \bullet 2H_2O$ (433 mg, 1.18 mmol). The resulting mixture was stirred at room temperature for 15 min then $NaIO_4$ (20.1 g, 94.0 mmol) was added at 0 °C. The reaction was stirred for 3 h at room temperature and then quenched with sat. $Na_2S_2O_3$ at 0 °C. The aqueous layer was extracted with EtOAc (2 x 400 mL) and the combined organic layers were washed with 1 M HCl (2 x 80 mL), brine (2 x 100 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure to afford the desired product (2.84 g, crude) which was used without further purification. LCMS (ESI) *m/z*: [M - H] calcd for $C_{12}H_{19}NO_5$: 256.12; found 256.0

Step 3: Synthesis of 1-(*tert*-butyl) 3-methyl (*S*)-3-(2-((1-methoxy-3-methyl-1-oxobutan-2-yl)amino)ethyl)azetidine-1,3-dicarboxylate

To a solution of 1-(*tert*-butyl) 3-methyl 3-(2-oxoethyl)azetidine-1,3-dicarboxylate (13.0 g, 50.5 mmol) and methyl *L*-valinate hydrochloride (7.29 g, 55.6 mmol) in MeOH (130 mL) at 0 °C were added $ZnCl_2$ (7.57 g, 55.6 mmol) and $NaBH_3CN$ (6.35 g, 101 mmol).The resulting mixture was stirred at room temperature overnight, partially concentrated under reduced pressure and diluted with EtOAc (500 mL). The resulting solution was washed with brine (3 x 200 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by normal phase chromatography (10→66% EtOAc/pet. ether) afforded the desired product (7.72 g, 41.0% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{18}H_{32}N_2O_6$: 373.24; found 373.1.

Step 4: Synthesis of *tert*-butyl (*S*)-6-(1-methoxy-3-methyl-1-oxobutan-2-yl)-5-oxo-2,6-diazaspiro[3.4]octane-2-carboxylate

To a solution of 1-(*tert*-butyl) 3-methyl (*S*)-3-(2-((1-methoxy-3-methyl-1-oxobutan-2-yl)amino)ethyl)azetidine-1,3-dicarboxylate (6.0 g, 16 mmol) and DIPEA (28.0 mL, 161 mmol) in toluene (60 mL) at room temperature was added DMAP (197 mg, 1.61 mmol). The resulting mixture was stirred at 80 °C overnight, diluted with EtOAc (50 mL), washed with H_2O (50 mL), brine (3 x 50 mL) dried with Na_2SO_4, and filtered, and concentrated under reduced pressure. Purification by reverse phase chromatography 45→80% MeCN/H_2O) afforded the desired product (4.3 g, 78.4% yield). LCMS (ESI) *m/z*: [M + H - *t*Bu] calcd for $C_{17}H_{28}N_2O_5$: 285.15; found 285.0

Step 5: Synthesis of methyl (*S*)-3-methyl-2-(5-oxo-2,6-diazaspiro[3.4]octan-6-yl)butanoate

To a solution of *tert*-butyl (*S*)-6-(1-methoxy-3-methyl-1-oxobutan-2-yl)-5-oxo-2,6-diazaspiro[3.4]octane-2-carboxylate (2.7 g, 7.9 mmol) in DCM (27 mL) at room temperature was added TFA (8.10 mL, 71.0 mmol).The resulting mixture was stirred for 1 h and then concentrated under reduced pressure to afford the desired product, (1.70 g, crude) which was used without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{12}H_{20}N_2O_3$: 241.16; found 240.1.

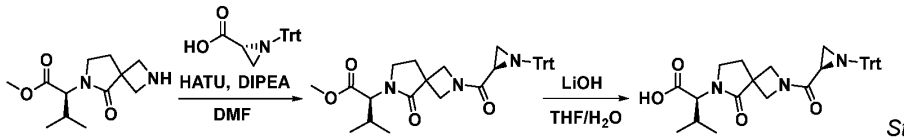
Step 6: Synthesis of methyl (*S*)-3-methyl-2-(5-oxo-2-((*S*)-1-tritylaziridine-2-carbonyl)-2,6-diazaspiro[3.4]octan-6-yl)butanoate

To a solution methyl (*S*)-3-methyl-2-(5-oxo-2,6-diazaspiro[3.4]octan-6-yl)butanoate (700 mg, 2.91 mmol) and (*S*)-1-tritylaziridine-2-carboxylic acid (1.15 g, 3.50 mmol) in DMF (7.0 mL) at 0 °C was added DIPEA (2.5 mL,14.6 mmol). After 30 min HATU (1.66 g, 4.37 mmol) was added and the resulting mixture was stirred at room temperature for 1 h. The reaction was then diluted with EtOAc (20 mL) and the organic layer was washed with sat. NH_4Cl (50 mL), brine (3 x 50 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by normal phase chromatography (0→80% EtOAc/pet. ether) afforded the desired product (300 mg, 18.7% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{34}H_{37}N_3O_4$: 552.29; found 552.2.

Step 7: Synthesis of (*S*)-3-methyl-2-(5-oxo-2-((*S*)-1-tritylaziridine-2-carbonyl)-2,6-diazaspiro[3.4]octan-6-yl)butanoic acid

To a solution of methyl (*S*)-3-methyl-2-(5-oxo-2-((*S*)-1-tritylaziridine-2-carbonyl)-2,6-diazaspiro[3.4]octan-6-yl)butanoate (700 mg, 1.27 mmol) in THF (10 mL) and H_2O (2.0 mL) at 0 °C was added LiOH (152 mg, 6.34 mmol). After 30 min the reaction mixture was warmed to room temperature for 1 h and then acidified to pH 6 with 1M HCl. The aqueous layer was extracted with EtOAc (3 x 50 mL) and the combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (300 mg, 18.7% yield) which was used without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{33}H_{35}N_3O_4$: 538.27; found 538.2.

Intermediate A-53. Synthesis of (*S*)-3-methyl-2-(5-oxo-2-((*R*)-1-tritylaziridine-2-carbonyl)-2,6-diazaspiro[3.4]octan-6-yl)butanoic acid



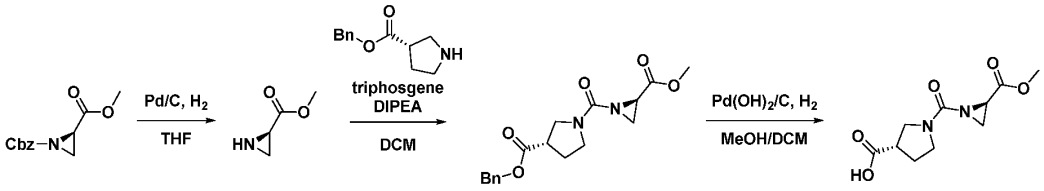
Step 1: Synthesis of methyl (*S*)-3-methyl-2-(5-oxo-2-((*R*)-1-tritylaziridine-2-carbonyl)-2,6-diazaspiro[3.4]octan-6-yl)butanoate

To a solution (*R*)-1-tritylaziridine-2-carboxylic acid (1.0 g, 3.0 mmol) and methyl (*S*)-3-methyl-2-(5-oxo-2,6-diazaspiro[3.4]octan-6-yl)butanoate (875 mg, 3.64 mmol) in DMF (10 mL) at 0 °C was added DIPEA (2.64 mL, 15.2 mmol). After 30 min HATU (1.73 g, 4.554 mmol) was added and the resulting mixture was stirred for 1 h at room temperature. The reaction was then diluted with EtOAc (20 mL) and the organic layer was washed with sat. NH_4Cl (50 mL), brine (3 x 50 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel chromatography (0→80% EtOAc/pet. ether) afforded the desired product (789 mg, 47% yield). LCMS (ESI) *m/z:* [M + H] calcd for $C_{34}H_{37}N_3O_4$: 552.29; found 552.3.

Step 2: Synthesis of (*S*)-3-methyl-2-(5-oxo-2-((*R*)-1-tritylaziridine-2-carbonyl)-2,6-diazaspiro[3.4]octan-6-yl)butanoic acid

To a stirred solution of methyl (*S*)-3-methyl-2-(5-oxo-2-((*R*)-1-tritylaziridine-2-carbonyl)-2,6-diazaspiro[3.4]octan-6-yl)butanoate (900 mg, 1.63 mmol) in THF (10 mL) and H_2O (2.5 mL) at 0 °C was added LiOH (156 mg, 6.53 mmol). After 30 min the reaction mixture was warmed to room temperature for 1 h and then acidified to pH 6 with 1M HCl. The aqueous layer was extracted with EtOAc (3 x 50 mL) and the combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (240 mg, 27.4% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{33}H_{35}N_3O_4$: 538.27; found 538.2.

Intermediate A-54. Synthesis of (*S*)-1-((*R*)-2-(methoxycarbonyl)aziridine-1-carbonyl)pyrrolidine-3-carboxylic acid



Step 1: Synthesis of methyl (*R*)-aziridine-2-carboxylate

A suspension of 1-benzyl 2-methyl (*R*)-aziridine-1,2-dicarboxylate (1.50 g, 6.4 mmol) and Pd/C (300 mg, 2.8 mmol) in THF (15 mL) under an atmosphere of hydrogen (1 atm) was stirred for 3 h before the solids were removed by filtration. The crude solution was concentrated under reduced pressure which afforded desired product (600 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_4H_7NO_2$: 102.06; found 102.3.

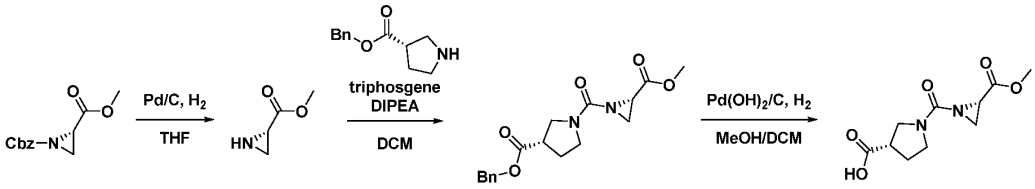
Step 2: Synthesis of benzyl (*S*)-1-((*R*)-2-(methoxycarbonyl)aziridine-1-carbonyl)pyrrolidine-3-carboxylate

To solution of methyl (*R*)-aziridine-2-carboxylate (1.0 g, 9.90 mmol) and benzyl (*S*)-pyrrolidine-3-carboxylate (2.63 g, 10.9 mmol, HCl salt) in DCM (30.0 mL) at -10 °C was added DIPEA (10.3 mL, 59.3 mmol) followed by triphosgene (880 mg, 2.97 mmol). The resulting solution was stirred for 30 min and was then quenched by the addition of H_2O (50 mL). The aqueous layer was extracted with DCM (2 x 100 mL), washed with brine (2 x 50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Purification by prep-TLC (50% EtOAc/pet. ether) afforded desired product (1.30 g, 28% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{17}H_{20}N_2O_5$: 333.15; found 333.2.

Step 3: Synthesis of (*S*)-1-((*R*)-2-(methoxycarbonyl)aziridine-1-carbonyl)pyrrolidine-3-carboxylic acid

To a solution of benzyl (*S*)-1-((*R*)-2-(methoxycarbonyl)aziridine-1-carbonyl)pyrrolidine-3-carboxylate (200 mg, 600 µmol) in MeOH (5 mL) and DCM (5 mL) under H_2 was added $Pd(OH)_2/C$ (130 mg, 90 µmol). The resulting mixture was stirred for 30 min at room temperature and then the mixture was filtered. The filter cake was washed with MeOH (2 x 10 mL) and the filtrate was concentrated under reduced pressure which afforded desired product (140 mg, 96% yield) as an off-white solid. LCMS (ESI) *m/z*: [M + H] calcd for $C_{10}H_{14}N_2O_5$: 243.10; found 243.3.

Intermediate A-55. Synthesis of (*S*)-1-((*S*)-2-(methoxycarbonyl)aziridine-1-carbonyl)pyrrolidine-3-carboxylic acid



Step 1: Synthesis of methyl (*S*)-aziridine-2-carboxylate

A suspension of 1-benzyl 2-methyl (*S*)-aziridine-1,2-dicarboxylate (200 mg, 850 µmol) and Pd/C (20 mg, 38 µmol) in THF (4.0 mL) under an atmosphere of hydrogen (1 atm) was stirred for 2 h before the solids were removed by filtration. The crude solution was concentrated under reduced pressure which afforded desired product (92 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_4H_7NO_2$: 102.06; found 102.3.

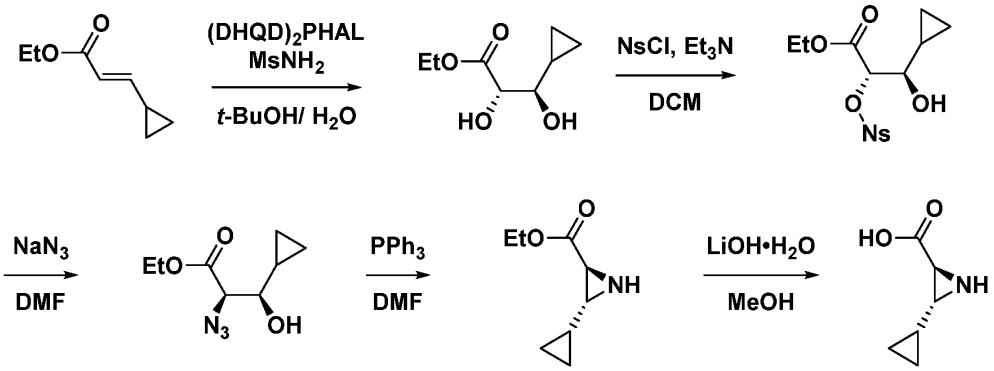
Step 2: Synthesis of benzyl (*S*)-1-((*S*)-2-(methoxycarbonyl)aziridine-1-carbonyl)pyrrolidine-3-carboxylate

To a solution of methyl (*S*)-aziridine-2-carboxylate (900 mg, 8.9 mmol) and benzyl (*S*)-pyrrolidine-3-carboxylate (2.37 g, 9.80 mmol, HCl salt) in DCM (30 mL) at -10 °C was added DIPEA (9.30 mL, 53.4 mmol) followed by triphosgene (790 mg, 2.67 mmol). The resulting solution was stirred for 30 min and was then quenched by the addition of H_2O (50 mL). The aqueous layer was extracted with DCM (2 x 100 mL), washed with brine (2 x 50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by prep-TLC (50% EtOAc/pet. ether) afforded desired product (360 mg, 8.5% yield) as an off-white oil. LCMS (ESI) *m/z*: [M + H] calcd for $C_{17}H_{20}N_2O_5$: 333.15; found 333.2.

Step 3: Synthesis of (*S*)-1-((*S*)-2-(methoxycarbonyl)aziridine-1-carbonyl)pyrrolidine-3-carboxylic acid

To a solution of benzyl (*S*)-1-((*S*)-2-(methoxycarbonyl)aziridine-1-carbonyl)pyrrolidine-3-carboxylate (130 mg, 390 µmol) in MeOH (3 mL) and DCM (3 mL) under H_2 was added $Pd(OH)_2/C$ (55 mg, 39 µmol). The resulting solution was stirred for 30 min at room temperature and then the reaction mixture was filtered. The filter cake was washed with MeOH (2 x 10 mL) and the filtrate was concentrated under reduced pressure which afforded desired product (90 mg, 95% yield) as an off-white solid. LCMS (ESI) *m/z*: [M + H] calcd for $C_{10}H_{14}N_2O_5$: 243.10; found 243.3.

Intermediate A-56. Synthesis of (2*R*,3*S*)-3-cyclopropylaziridine-2-carboxylic acid



Step 1: Synthesis of ethyl (2*S*,3*R*)-3-cyclopropyl-2,3-dihydroxypropanoate

A solution of ethyl (*E*)-3-cyclopropylacrylate (10.4 mL, 71 mmol) in *tert*-BuOH (270 mL) and H_2O (270 mL) was stirred at 0 °C. After 5 min $MsNH_2$ (6.8 g, 71 mmol) and $(DHQD)_2PHAL$ (100 g, 130 mmol) were added and the reaction mixture was warmed to room temperature. After stirring overnight, sat. Na_2SO_3 was added and the mixture was stirred for 30 min. The mixture was acidified to pH 6 with KH_2PO_4. Purification by silica gel column chromatography (33% EtOAc/pet. ether) afforded desired product (5.5 g, 44% yield).

Step 2: Synthesis of ethyl (2*S*,3*R*)-3-cyclopropyl-3-hydroxy-2-(((4-nitrophenyl)sulfonyl)oxy)propanoate

A solution of ethyl (2*S*,3*R*)-3-cyclopropyl-2,3-dihydroxypropanoate (5.40 g, 31.0 mmol) and Et_3N (13.0 mL, 93.0 mmol) in DCM (20 mL) was stirred at 0 °C and a solution of 4-nitrobenzenesulfonyl chloride (6.53 g, 29.5 mmol) in DCM (10 mL) was added. The reaction mixture was stirred for 1.5 h and was then extracted with DCM (3 x 200 mL). The combined organic layers were washed with brine (100 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (33% EtOAc/pet. ether) afforded desired product (6.9 g, 62% yield).

Step 3: Synthesis of ethyl (2*R*,3*R*)-2-azido-3-cyclopropyl-3-hydroxypropanoate

A mixture of ethyl (2*S*,3*R*)-3-cyclopropyl-3-hydroxy-2-(((4-nitrophenyl)sulfonyl)oxy)propanoate (6.90 g, 19.2 mmol) and NaN_3 (6.24 g, 96.0 mmol) in DMF (70.0 mL) was heated to 50 °C. The reaction mixture was stirred for 5 h and then extracted with EtOAc (3 x 200 mL). The combined organic layers were washed with brine (100 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (20% EtOAc/pet. ether) afforded desired product (2.8 g, 73% yield).

Step 4: Synthesis of ethyl (2*R*,3*S*)-3-cyclopropylaziridine-2-carboxylate

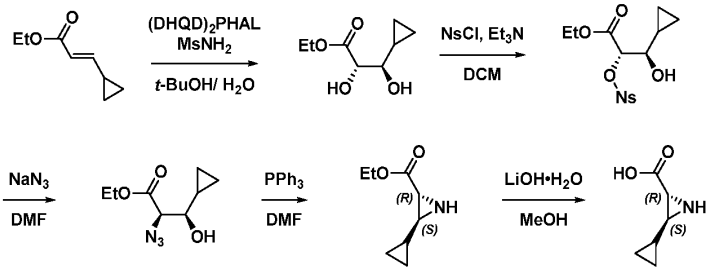
A mixture of triphenylphosphine (1.84 g, 7.02 mmol) in DMF (5 mL) was stirred at 0 °C. After 5 min ethyl (2*R*,3*R*)-2-azido-3-cyclopropyl-3-hydroxypropanoate (1.40 g, 7.03 mmol) was added and the reaction was warmed to room temperature. The reaction mixture was heated to 80 °C and stirred for 1 h. The mixture was then cooled to room temperature and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (50 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel column

chromatography (20% EtOAc/pet. ether) afforded the desired product (230 mg, 46% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_8H_{13}NO_2$: 156.10; found 156.2.

Step 5: Synthesis of lithium (2*R*,3*S*)-3-cyclopropylaziridine-2-carboxylate

To a mixture of ethyl (2*R*,3*S*)-3-cyclopropylaziridine-2-carboxylate (230 mg, 1.5 mmol) in MeOH (3.0 mL) was added LiOH•H_2O (125 mg, 3.0 mmol). The reaction was stirred for 3 h and then filtered. The filtrate was concentrated under reduced pressure which afforded the desired product (150 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_6H_9NO_2$: 128.07; found 128.2.

Intermediate A-57. Synthesis of (2*R*,3*S*)-3-cyclopropylaziridine-2-carboxylic acid



Step 1: Synthesis of ethyl (2*S*,3*R*)-3-cyclopropyl-2,3-dihydroxypropanoate

A solution of ethyl (*E*)-3-cyclopropylacrylate (10.4 mL, 71 mmol) in *tert*-BuOH (270 mL) and H_2O (270 mL) was stirred at 0 °C. After 5 min $MsNH_2$ (6.8 g, 71 mmol) and $(DHQD)_2PHAL$ (100 g, 130 mmol) were added and the reaction mixture was warmed to room temperature. After stirring overnight, sat. Na_2SO_3 was added and the mixture was stirred for 30 min. The mixture was acidified to pH 6 with KH_2PO_4. Purification by silica gel column chromatography (33% EtOAC/pet. ether) afforded desired product (5.5 g, 44% yield).

Step 2: Synthesis of ethyl (2*S*,3*R*)-3-cyclopropyl-3-hydroxy-2-(((4-nitrophenyl)sulfonyl)oxy)propanoate

A solution of ethyl (2*S*,3*R*)-3-cyclopropyl-2,3-dihydroxypropanoate (5.40 g, 31.0 mmol) and Et_3N (13.0 mL, 93.0 mmol) in DCM (20 mL) was stirred at 0 °C and a solution of 4-nitrobenzenesulfonyl chloride (6.53 g, 29.5 mmol) in DCM (10 mL) was added. The reaction mixture was stirred for 1.5 h and was then extracted with DCM (3 x 200 mL). The combined organic layers were washed with brine (100 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (33% EtOAc/pet. ether) afforded desired product (6.9 g, 62% yield).

Step 3: Synthesis of ethyl (2*R*,3*R*)-2-azido-3-cyclopropyl-3-hydroxypropanoate

A mixture of ethyl (2*S*,3*R*)-3-cyclopropyl-3-hydroxy-2-(((4-nitrophenyl)sulfonyl)oxy)propanoate (6.90 g, 19.2 mmol) and NaN_3 (6.24 g, 96.0 mmol) in DMF (70.0 mL) was heated to 50 °C. The reaction mixture was stirred for 5 h and then extracted with EtOAc (3 x 200 mL). The combined organic layers were washed with brine (100 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (20% EtOAc/pet. ether) afforded desired product (2.8 g, 73% yield).

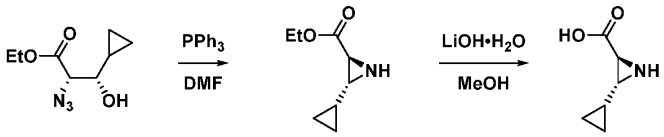
Step 4: Synthesis of ethyl (2*R*,3*S*)-3-cyclopropylaziridine-2-carboxylate

A mixture of triphenylphosphine (1.84 g, 7.02 mmol) in DMF (5 mL) was stirred at 0 °C. After 5 min ethyl (2*R*,3*R*)-2-azido-3-cyclopropyl-3-hydroxypropanoate (1.40 g, 7.03 mmol) was added and the reaction was warmed to room temperature. The reaction mixture was heated to 80 °C and stirred for 1 h. The mixture was then cooled to room temperature and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (50 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (20% EtOAc/pet. ether) afforded the desired product (230 mg, 46% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_8H_{13}NO_2$: 156.10; found 156.2.

Step 5: Synthesis of lithium (2*R*,3*S*)-3-cyclopropylaziridine-2-carboxylate

To a mixture of ethyl (2*R*,3*S*)-3-cyclopropylaziridine-2-carboxylate (230 mg, 1.5 mmol) in MeOH (3.0 mL) was added LiOH•H_2O (125 mg, 3.0 mmol). The reaction was stirred for 3 h and then filtered. The filtrate was concentrated under reduced pressure which afforded the desired product (150 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_6H_9NO_2$: 128.07; found 128.2.

Intermediate A-58. Synthesis of (2*S*,3*R*)-3-cyclopropylaziridine-2-carboxylic acid



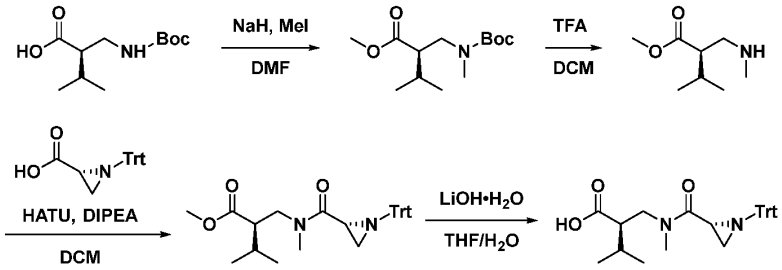
Step 1: Synthesis of ethyl (2*S*,3*R*)-3-cyclopropylaziridine-2-carboxylate

A mixture of PPh_3 (1.4 g, 5.4 mmol) in DMF (15.0 mL) was stirred at 0 °C. After 30 min, ethyl (2*S*,3*S*)-2-azido-3-cyclopropyl-3-hydroxypropanoate (980 mg, 4.92 mmol) was added. The reaction mixture was heated to 80 °C. After 2 h the reaction was quenched by the addition of H_2O (20 mL) and was extracted with EtOAc (3 x 30 mL). Purification by silica gel column chromatography (17% EtOAc/pet. ether) afforded desired product (500 mg, 65% yield).

Step 2: Synthesis of lithium (2*S*,3*R*)-3-cyclopropylaziridine-2-carboxylate

To a solution of ethyl (2*S*,3*R*)-3-cyclopropylaziridine-2-carboxylate (450 mg, 2.9 mmol) in THF (6.0 mL) and H_2O (2.0 mL) was added LiOH (90 mg, 3.8 mmol). The reaction was stirred for 2 h and then filtered. The filtrate was concentrated under reduced pressure which afforded the desired product (300 mg, crude).

Intermediate A-59. Synthesis of (*R*)-3-methyl-2-(((*R*)-*N*-methyl-1-tritylaziridine-2-carboxamido)methyl)butanoic acid



Step 1: Synthesis of methyl (*R*)-2-(((*tert*-butoxycarbonyl)(methyl)amino)methyl)-3-methylbutanoate

To a solution of (*R*)-2-(((*tert*-butoxycarbonyl)amino)methyl)-3-methylbutanoic acid (500 mg, 2.16 mmol) in DMF (10.0 mL) at 0 °C was added NaH (130 mg, 5.40 mmol). After 30 min, MeI (540 µL, 8.65 mmol) was added and the reaction was warmed to room temperature. After 2 h the reaction was cooled to 0 °C and quenched by the addition of sat. aq. NH_4Cl (10 mL). The resulting mixture was extracted with EtOAc (3 x 20 mL) and the combined organic layers were washed with brine (40 mL), dried with Na_2SO_4, and concentrated under reduced pressure. Purification by prep-TLC (9% EtOAc/pet. ether) afforded the desired product (500 mg, 89.2% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{25}NO_4$: 260.19; found 260.2.

Step 2: Synthesis of methyl (*R*)-3-methyl-2-((methylamino)methyl)butanoate

To a solution of methyl (*R*)-2-(((*tert*-butoxycarbonyl)(methyl)amino)methyl)-3-methylbutanoate (500 mg, 1.93 mmol) in DCM (5.0 mL) at 0 °C was added TFA (2.50 mL) dropwise. The resulting mixture was warmed to room temperature. After 2 h the reaction mixture was concentrated under reduced pressure to afford desired product (600 mg, crude) as a yellow solid.

Step 3: Synthesis of methyl (*R*)-3-methyl-2-(((*R*)-*N*-methyl-1-tritylaziridine-2-carboxamido)methyl)butanoate

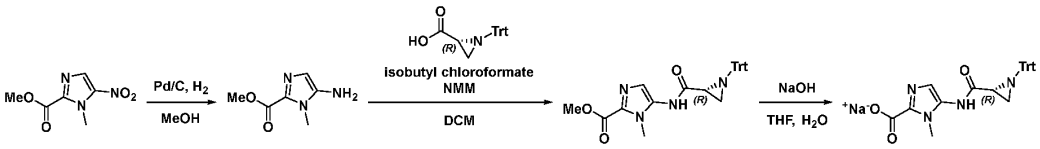
To a solution of methyl (*R*)-3-methyl-2-((methylamino)methyl)butanoate (550 mg, 3.45 mmol) and (*R*)-1-tritylaziridine-2-carboxylic acid (1.25 g, 3.80 mmol) in DCM (5.0 mL) at 0 °C was added DIPEA (1.81 mL, 10.4 mmol) followed by HATU (1.58 g, 4.15 mmol). The resulting mixture was warmed to room temperature. After 2 h the reaction was quenched with H_2O (30 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (50 mL), dried with Na_2SO_4, and concentrated under reduced pressure. Purification by silica gel column chromatography (9% EtOAc/pet. ether) afforded the desired product (300 mg, 19% yield) as a yellow oil. LCMS (ESI) *m/z*: [M + H] calcd for $C_{30}H_{34}N_2O_3$: 471.27; found 471.3.

Step 4: Synthesis of (*R*)-3-methyl-2-(((*R*)-*N*-methyl-1-tritylaziridine-2-carboxamido)methyl)butanoic acid

To a solution of methyl (*R*)-3-methyl-2-(((*R*)-*N*-methyl-1-tritylaziridine-2-carboxamido)methyl)butanoate (200 mg, 0.425 mmol) in THF (2.0 mL) at 0 °C was added $LiOH•H_2O$ (89 mg, 2.13 mmol) in H_2O (2.0 mL) dropwise. The resulting mixture was warmed to

room temperature. After 5 h the mixture was neutralized to pH 7 with 1M HCl. The reaction was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (30 mL), dried with Na_2SO_4, and concentrated under reduced pressure to afford product (200 mg, crude) as an off-white solid. The crude product was used in the next step directly without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{29}H_{32}N_2O_3$: 457.25; found 457.2.

Intermediate A-60. Synthesis of sodium (*R*)-1-methyl-5-(1-tritylaziridine-2-carboxamido)-1*H*-imidazole-2-carboxylate



Step 1: Synthesis of methyl 5-amino-1-methyl-1*H*-imidazole-2-carboxylate

A mixture of methyl 1-methyl-5-nitro-1H-imidazole-2-carboxylate (1.0 g, 5.401 mmol) and Pd/C (500.0 mg) in MeOH (15 mL) at room temperature was stirred under an atmosphere of hydrogen (1 atm) for 3 h. The mixture was filtered and the filter cake was washed with MeOH (3 x 20 mL). The filtrate was concentrated under reduced pressure to afford the desired product (1.0 g, crude). LCMS (ESI) *m/z*: [M + Na] calcd for $C_6H_9N_3O_2$: 156.08; found 156.1.

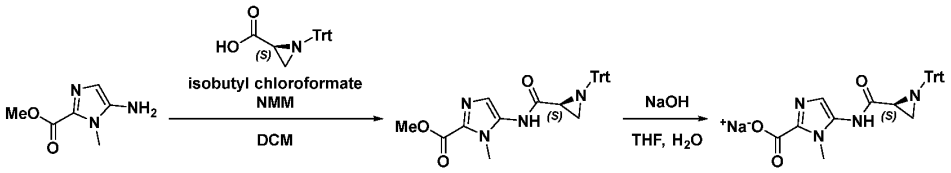
Step 2: Synthesis of methyl (*R*)-1-methyl-5-(1-tritylaziridine-2-carboxamido)-1 -imidazole-2-carboxylate

A solution of (*R*)-1-tritylaziridine-2-carboxylic acid (2.55 g, 7.741 mmol) in DCM (12.0 mL) at 0 °C was added in portions over 30 min to a solution of isobutyl chloroformate (845.1 mg, 6.187 mmol) and *N*-methylmorpholine (1.04 g, 10.282 mmol) in DCM. To the mixture was then added methyl 5-amino-1-methyl-1*H*-imidazole-2-carboxylate (800.0 mg, 5.156 mmol) at 0 °C. The resulting mixture was warmed to room temperature and stirred overnight. The mixture was diluted with DCM (300 mL) and washed with H_2O (3 x 100 mL), washed with brine (2 x 150 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (25% EtOAc/hexanes) to afford the final product (1.2 g, 49.9% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{28}H_{26}N_4O_3$: 467.21; found 467.2.

Step 3: Synthesis of sodium (*R*)-1-methyl-5-(1-tritylaziridine-2-carboxamido)-1*H*-imidazole-2-carboxylate

To a solution of methyl (*R*)-1-methyl-5-(1-tritylaziridine-2-carboxamido)-1 -imidazole-2-carboxylate (300.0 mg, 0.643 mmol) in THF (3 mL) at room temperature was added NaOH•H_2O (38.6 mg, 0.965 mmol). The resulting solution was warmed to room temperature and stirred for 2 h. The solution was concentrated under reduced pressure to afford the desired product (400 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{27}H_{24}N_4O_3$: 453.19; found 453.2.

Intermediate A-61. Synthesis of (*S*)-1-methyl-5-(1-tritylaziridine-2-carboxamido)-1*H*-imidazole-2-carboxylic acid



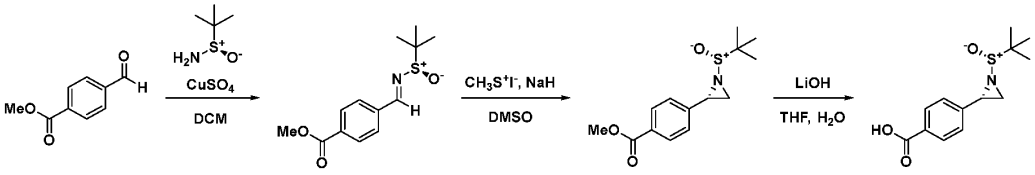
Step 1: Synthesis of methyl (*S*)-1-methyl-5-(1-tritylaziridine-2-carboxamido)-1*H*-imidazole-2-carboxylate

To a solution of (*S*)-1-tritylaziridine-2-carboxylic acid (1.18 g, 3.577 mmol) in DCM (15 mL) at 0 °C was added isobutyl chloroformate (423.4 mg, 3.100 mmol) and *N*-methylmorpholine (0.39 mL) 3.862 mmol). The resulting mixture was stirred for 1 h at 0 °C and then methyl 5-amino-1-methyl-1H-imidazole-2-carboxylate (370.0 mg, 2.385 mmol) was added and the resulting mixture was warmed to at room temperature and stirred overnight. The reaction mixture was quenched with sat. aq. $NaHCO_3$ at 0 °C before being extracted with DCM (2 x 100 mL). The combined organic layers were washed with brine (150 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (100% EtOAc) to afford the final product (380 mg, 34.2% yield) as a yellow solid. LCMS (ESI) *m/z*: [M + Na] calcd for $C_{28}H_{26}N_4O_3$: 467.21; found 467.3.

Step 2: Synthesis of (*S*)-1-methyl-5-(1-tritylaziridine-2-carboxamido)-1*H*-imidazole-2-carboxylic

To a solution of NaOH (146.6 mg, 3.665 mmol) in H_2O (3.6 mL) at 0 °C was added a solution of methyl (*S*)-1-methyl-5-(1-tritylaziridine-2-carboxamido)-1*H*-imidazole-2-carboxylate (380.0 mg, 0.815 mmol) in MeOH (5 mL). The resulting solution was warmed to room temperature and stirred for 6 h. The mixture was acidified to pH 6 with aq. 1 M HCl before being extracted with DCM (2 x 100 mL). The combined organic layers were washed with brine (150 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (350 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{27}H_{24}N_4O_3$: 451.18; found 451.1.

Intermediate A-62. Synthesis of 4-((2*S*)-1-(*tert*-butylsulfinyl)aziridin-2-yl)benzoic acid



Step 1: Synthesis of methyl (*E*)-4-(((*tert*-butylsulfinyl)imino)methyl)benzoate

To a solution of methyl 4-formylbenzoate (100.0 mg, 0.609 mmol) and 2-methylpropane-2-sulfinamide (76.0 mg, 0.627 mmol) in DCM (2.0 mL) was added $CuSO_4$ (291.7 mg, 1.827 mmol). The resulting solution was stirred overnight at room temperature and was then filtered. The filter cake was washed with EtOAc (3 x 200 mL) and the filtrate was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (67% EtOAc/pet.

ether) to afford the desired product (2 g, 61.4% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{17}NO_3S$: 268.10; found 268.0.

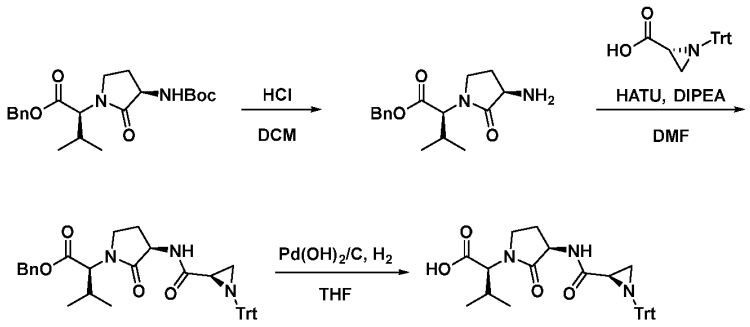
Step 2: Synthesis of methyl 4-((2*S*)-1-(*tert*-butylsulfinyl)aziridin-2-yl)benzoate

Methyl (*E*)-4-(((*tert*-butylsulfinyl)imino)methyl)benzoate (500.0 mg, 1.863 mmol), $Me_3S^+I^-$ (1.14 g, 5.590 mmol), and 60% NaH (134.15 mg, 5.590 mmol) were dissolved in DMSO (10.0 mL) at room temperature. The resulting mixture was stirred for 2 h and then the reaction was quenched by the addition of sat. aq. NH_4Cl (10 mL). The resulting mixture was extracted with EtOAc (3 x 50 mL) and the combined organic layers were washed with brine (2 x 100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (10% EtOAc/pet. ether) to afford the desired product (300 mg, 57.0% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{14}H_{19}NO_3S$: 282.12; found 282.1.

Step 3: Synthesis of 4-((2*S*)-1-(*tert*-butylsulfinyl)aziridin-2-yl)benzoic acid

To a solution of methyl 4-((2*S*)-1-(*tert*-butylsulfinyl)aziridin-2-yl)benzoate (400.0 mg, 1.422 mmol) in THF (5.0 mL) and H_2O (1.0 mL) was added LiOH (103.0 mg, 4.301 mmol). The resulting mixture was stirred overnight at room temperature and was then acidified to pH ~3 with 1 M HCl. The mixture was extracted with EtOAc (3 x 200 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by prep-TLC (10% MeOH/DCM) to afford the desired product (130 mg, 91.2% yield). LCMS (ESI) *m/z*: [M - H] calcd for $C_{13}H_{17}NO_3S$: 266.09; found 266.0.

Intermediate A-63. Synthesis of (*S*)-3-methyl-2-((*R*)-2-oxo-3-((*R*)-1-tritylaziridine-2-carboxamido)pyrrolidin-1-yl)butanoic acid



Step 1: Synthesis of benzyl (*S*)-2-((*R*)-3-amino-2-oxopyrrolidin-1-yl)-3-methylbutanoate

To a solution of benzyl (*S*)-2-((*R*)-3-((*tert*-butoxycarbonyl)amino)-2-oxopyrrolidin-1-yl)-3-methylbutanoate (1.0 g, 2.561 mmol) in DCM (10.0 mL) was added 4M HCl in 1,4-dioxane (5.0 mL) at 0 °C. The resulting mixture was stirred for 2 h at room temperature under an argon atmosphere. The resulting mixture was concentrated under reduced pressure to afford the desired crude product (890 mg, crude) as a yellow solid. LCMS (ESI) *m/z*: [M + H] calcd for $C_{16}H_{22}N_2O_3$: 291.17; found 291.1.

Step 2: Synthesis of benzyl (*S*)-3-methyl-2-((*R*)-2-oxo-3-((*R*)-1-tritylaziridine-2-carboxamido)pyrrolidin-1-yl)butanoate

To a solution of benzyl (*S*)-2-((*R*)-3-amino-2-oxopyrrolidin-1-yl)-3-methylbutanoate (450.0 mg, 1.550 mmol) and (*R*)-1-tritylaziridine-2-carboxylic acid (765.8 mg, 2.325 mmol) in DMF were added HATU (1.179 g, 3.100 mmol) and DIPEA (1.35 mL, 7.75 mmol) dropwise at 0 °C. The resulting mixture was stirred for 2 h at room temperature and was then extracted with EtOAc (2 x 100 mL). The combined organic layers were washed with H_2O, brine (20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (50% EtOAc/pet. ether) to afford the desired product (470 mg, 50.4% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{38}H_{39}N_3O_4$: 602.31; found 602.3.

Step 3: Synthesis of (*S*)-3-methyl-2-((*R*)-2-oxo-3-((*R*)-1-tritylaziridine-2-carboxamido)pyrrolidin-1-yl)butanoic acid

A suspension of benzyl (*S*)-3-methyl-2-((*R*)-2-oxo-3-((*R*)-1-tritylaziridine-2-carboxamido)pyrrolidin-1-yl)butanoate (430.0 mg, 0.715 mmol) and $Pd(OH)_2/C$ (230.0 mg, 1.638 mmol) were in THF (5 mL) was stirred for 3 h under and atmosphere of hydrogen (1 atm). The resulting mixture was filtered and the filter cake was washed with MeOH (2 x 50 mL). The filtrate was concentrated under reduced pressure to afford the crude final product (16 mg, crude) as a white solid. LCMS (ESI) *m/z*: [M + H] calcd for $C_{31}H_{33}N_3O_4$: 510.24; found 510.1.

Intermediate A-64. Synthesis of potassium (*S*)-1-isopropylaziridine-2-carboxylate

Step 1: Synthesis benzyl isopropyl-*L*-serinate

To a solution of benzyl *L*-serinate (3.65 g, 18.69 mmol), KOAc (1.83 g, 18.69 mmol), and acetone (2.5 mL, 33.66 mmol) in DCM (60.0 mL) was added $NaBH(AcO)_3$ (4.76 g, 22.436 mmol) in portions at 0 °C. The resulting mixture was stirred overnight at room temperature. The reaction was quenched by the addition of sat. aq. $NaHCO_3$ (50 mL) at room temperature. The resulting mixture was extracted with DCM (3 x 80 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (67% EtOAc/hexanes) to afford the desired product (2.7 g, 60.9% yield) as an off-white solid. LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{19}NO_3$: 238.14; found 238.2.

Step 2: Synthesis of benzyl (*S*)-1-isopropylaziridine-2-carboxylate

To a solution of benzyl isopropyl-*L*-serinate (2.70 g, 11.378 mmol), Et_3N (4.75 mL, 34.134 mmol) and DMAP (2.57 mg, 0.021 mmol) in DCM (50.0 mL) was added a solution of TsCl (2.60 g, 13.65 mmol) in DCM dropwise at 0 °C. The resulting mixture was stirred overnight at room temperature and was then stirred for 4 h at 40 °C. The reaction mixture was diluted with H_2O (80 mL) and was then extracted with DCM (2 x 50 mL). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue

was purified by silica gel chromatography (20% EtOAc/hexanes) to afford the desired product (2.3 g, 93.2% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{17}NO_2$: 220.13; found 220.1.

Step 3: Synthesis of potassium (*S*)-1-isopropylaziridine-2-carboxylate

To a solution of benzyl (*S*)-1-isopropylaziridine-2-carboxylate (800.0 mg, 3.65 mmol) and H_2O (6.0 mL) and THF (8.0 mL) was added a solution of KOH (245.62 mg, 4.378 mmol) in H_2O (2.0 mL) dropwise at 0 °C. The resulting mixture was stirred for 2 h at room temperature. The mixture was diluted with H_2O (10 mL) and the aqueous layer was washed with MTBE (3 x 8 mL). The aqueous layer was dried by lyophilization to afford the desired product (400 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_6H_{11}NO_2$: 130.09; found 130.0.

Intermediate A-65. Synthesis of potassium (*R*)-1-isopropylaziridine-2-carboxylate

Step 1: Synthesis benzyl isopropyl-*D*-serinate

To a solution of benzyl *D*-serinate (2.10 g, 10.757 mmol), KOAc (1.06 g, 10.757 mmol), and acetone (1.2 mL, 16.136 mmol) in DCM (40.0 mL) was added a solution of $NaBH(AcO)_3$ (2.96 g, 13.984 mmol) in portions at 0 °C. The resulting mixture was stirred overnight at room temperature. The reaction was quenched by the addition of sat. aq. $NaHCO_3$ (50 mL) and the mixture was extracted with DCM (3 x 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (67% EtOAc/hexanes) to afford the desired product (1.7 g, 66.6% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{19}NO_3$: 238.14; found 238.0.

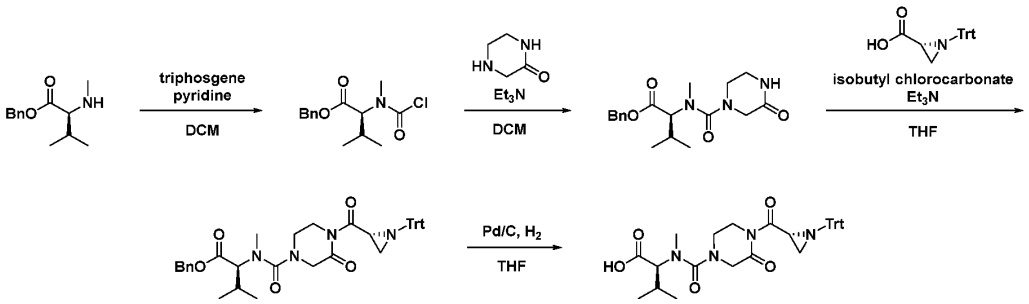
Step 2: Synthesis of benzyl (*R*)-1-isopropylaziridine-2-carboxylate

To a solution of benzyl isopropyl-*D*-serinate (1.75 g, 7.375 mmol), Et_3N (2.58 mL, 18.437 mmol) and DMAP (90.09 mg, 0.737 mmol) in DCM (30.0 mL) was added a solution of TsCl (1.69 g, 8.850 mmol) in DCM dropwise at 0 °C. The resulting mixture was stirred overnight at room temperature before being stirred for 4 h at 40 °C. The mixture was diluted with H_2O (80 mL) and then extracted with DCM (3 x 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (20% EtOAc/hexanes) to afford the desired product (1.4 g, 86.6% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{17}NO_2$: 220.13; found 219.9.

Step 3: Synthesis of potassium (*R*)-1-isopropylaziridine-2-carboxylate

To a solution of benzyl (*R*)-1-isopropylaziridine-2-carboxylate (600.0 mg, 2.736 mmol) in H_2O (3.0 mL) and THF (5.0 mL) was added a solution of KOH (184.22 mg, 3.283 mmol) in H_2O (2.0 mL) dropwise at 0 °C. The resulting mixture was stirred for 2 h at room temperature. The mixture was then diluted with H_2O (10 mL) and the aqueous layer was washed with MTBE (3 x 8 mL). The aqueous layer was then dried by lyophilization to afford the desired product (260 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_6H_{11}NO_2$: 130.09; found 130.1.

Intermediate A-66. Synthesis of *N*-methyl-*N*-(3-oxo-4-((*R*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valine



Step 1: Synthesis of benzyl *N*-(chlorocarbonyl)-*N*-methyl-*L*-valinate

To a solution of benzyl methyl-*L*-valinate (2.0 g, 9.038 mmol) in DCM (20.0 mL) was added a solution of triphosgene (800 mg, 2.711 mmol) and pyridine (2.14 g, 27.113 mmol) in DCM (20.0 mL) dropwise at 0 °C. The resulting mixture was stirred for 2 h at room temperature before being diluted with EtOAc. The solution was stirred for 30 min at room temperature and was then filtered. The filtrate was concentrated under reduced pressure to afford the crude product which was used in the next step directly without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{14}H_{18}ClNO_3$: 284.11; found 283.1.

Step 2: Synthesis of benzyl *N*-methyl-*N*-(3-oxopiperazine-1-carbonyl)-*L*-valinate

To a solution of piperazin-2-one (100.0 mg, 0.999 mmol) and Et_3N (0.487 mL, 3.496 mmol) in DCM (5.0 mL) was added a solution of benzyl *N*-(chlorocarbonyl)-*N*-methyl-*L*-valinate (311.75 mg, 1.099 mmol) in DCM (5 mL) dropwise at 0 °C. The resulting mixture was stirred for 4 h at room temperature. The mixture was then diluted with H_2O (5 mL), the aqueous layer was extracted with DCM (3 x 5 mL), and the combined organic layers were concentrated under reduced pressure. The residue was purified by prep-TLC (100% EtOAc) to afford the desired product (200 mg, 57.6% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{18}H_{25}N_3O_4$: 348.19; found 348.1.

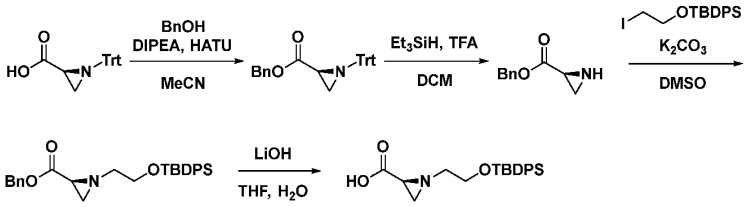
Step 3: Synthesis of benzyl *N*-methyl-*N*-(3-oxo-4-((*R*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valinate

To a solution of (*R*)-1-tritylaziridine-2-carboxylic acid (711.11 mg, 2.159 mmol) in THF was added Et_3N (0.40 mL, 2.878 mmol) and isobutyl chlorocarbonate (255.53 mg, 1.871 mmol) dropwise at 0 °C under a nitrogen atmosphere. The resulting mixture was stirred for 1 h at room temperature and then benzyl *N*-methyl-*N*-(3-oxopiperazine-1-carbonyl)-*L*-valinate (500.0 mg, 1.439 mmol) was added at room temperature. The resulting mixture was warmed to 70 °C and stirred overnight. The reaction was then cooled to room temperature and concentrated under reduced pressure. The residue was purified by prep-TLC (EtOAc/50% pet. ether) to afford the desired product (200 mg, 21.1% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{40}H_{42}N_4O_5$: 659.32; found 677.4.

Step 4: Synthesis of *N*-methyl-*N*-(3-oxo-4-((*R*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valine

A suspension of benzyl *N*-methyl-*N*-(3-oxo-4-((*R*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valinate (140.0 mg, 0.212 mmol) and Pd/C (50.0 mg) in THF (3 mL) was stirred for 2 h under a hydrogen atmosphere (1 atm). The mixture was then filtered and the filter cake was washed with MeOH (3 x 15 mL). The filtrate was concentrated under reduced pressure to afford the desired product (100 mg, crude). LCMS (ESI) *m/z*: [M - H] calcd for $C_{33}H_{36}N_4O_5$: 567.26; found 567.1.

Intermediate A-67. Synthesis of (*S*)-1-(2-((*tert*-butyldiphenylsilyl)oxy)ethyl)aziridine-2-carboxylic acid



Step 1: Synthesis of benzyl (*S*)-1-tritylaziridine-2-carboxylate

To a solution of (*S*)-1-tritylaziridine-2-carboxylic acid (500.0 mg, 1.518 mmol), benzyl alcohol (246.2 mg, 2.277 mmol) and DIPEA (0.793 mL, 4.554 mmol) in MeCN (10.0 mL) was added HATU (1.73 mg, 4.554 mmol). The resulting solution was stirred for 3 h at room temperature and was then concentrated under reduced pressure. The crude residue was purified by prep-TLC (50% EtOAc/pet. ether) to afford the desired product (300 mg, 47.1% yield) as an off-white slid. LCMS (ESI) *m/z*: [M + Na] calcd for $C_{29}H_{25}NO_2$: 442.18; found 442.3.

Step 2: Synthesis of benzyl (*S*)-aziridine-2-carboxylate

To a solution of benzyl (*S*)-1-tritylaziridine-2-carboxylate (300.0 mg, 0.715 mmol) in DCM (5.0 mL) at 0 °C was added TFA (326.2 mg, 2.860 mmol) and Et_3SiH (332.6 mg, 2.860 mmol). The resulting mixture was stirred at 0 °C for 3 h and was then concentrated under reduced pressure. The residue was purified by prep-TLC (10% MeOH/DCM) to afford the desired product (130 mg, 82.1% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{10}H_{11}NO_2$: 178.09; found 178.2.

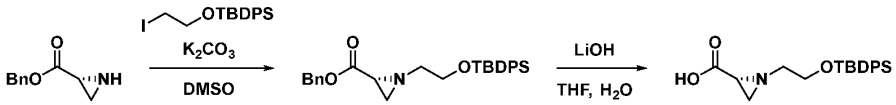
Step 3: Synthesis of benzyl (*S*)-1-(2-((*tert*-butyldiphenylsilyl)oxy)ethyl)aziridine-2-carboxylate

To a solution of benzyl (*S*)-aziridine-2-carboxylate (400.0 mg, 2.257 mmol) and *tert*-butyl(2-iodoethoxy)diphenylsilane (1.85 g, 4.52 mmol) in DMSO (10.0 mL) was added K_2CO_3 (935.9 mg, 6.772 mmol) at room temperature. The mixture was stirred at 60 °C for 5 h. The mixture was diluted with H_2O (30.0 mL) and was extracted with EtOAc (2 x 30 mL). The combined organic layers were washed with brine (2 x 50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by prep-TLC (20% EtOAc/pet. ether) to afford the desired product (200 mg, 15.4% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{28}H_{33}NO_3Si$: 460.23; found 460.0.

Step 4: Synthesis of lithium (*S*)-1-(2-((*tert*-butyldiphenylsilyl)oxy)ethyl)aziridine-2-carboxylate

To a solution of benzyl (*S*)-1-(2-((*tert*-butyldiphenylsilyl)oxy)ethyl)aziridine-2-carboxylate (200.0 mg, 0.435 mmol) in MeOH (2.0 mL) was added LiOH•H_2O (36.5 mg, 0.870 mmol). The resulting mixture was stirred overnight and was then concentrated under reduced pressure to afford the desired product (200 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{21}H_{27}NO_3Si$: 370.18; found 370.1.

Intermediate A-68. Synthesis of (*R*)-1-(2-((*tert*-butyldiphenylsilyl)oxy)ethyl)aziridine-2-carboxylic acid



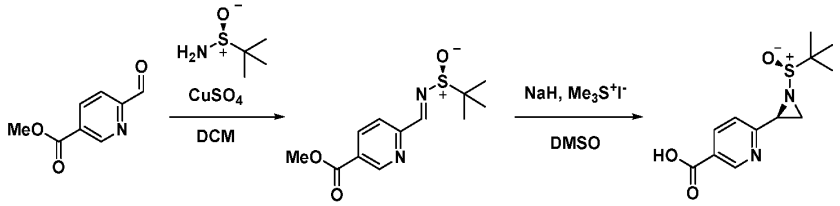
Step 1: Synthesis of methyl benzyl (*R*)-1-(2-((*tert*-butyldiphenylsilyl)oxy)ethyl)aziridine-2-carboxylate

To a solution of benzyl (*R*)-aziridine-2-carboxylate (600.0 mg, 3.386 mmol) and K_2CO_3 (1.87 g, 13.544 mmol) in DMSO (8.0 mL) was added *tert*-butyl(2-iodoethoxy)diphenylsilane (1.39 g, 3.386 mmol) in portions at room temperature. The resulting mixture was stirred at 80 °C for 16 h. The reaction mixture was then cooled to room temperature and concentrated under reduced pressure. The residue was purified by reverse phase chromatography (60→90% MeCN/H_2O) to afford the desired product (150 mg, 9.6% yield) as a colorless solid. LCMS (ESI) *m/z*: [M + Na] calcd for $C_{28}H_{33}NO_3Si$: 482.21; found 482.3.

Step 2: Synthesis of (*R*)-1-(2-((*tert*-butyldiphenylsilyl)oxy)ethyl)aziridine-2-carboxylic acid

To a solution of methyl benzyl (*R*)-1-(2-((*tert*-butyldiphenylsilyl)oxy)ethyl)aziridine-2-carboxylate (180.0 mg, 0.392 mmol) in H_2O (2.0 mL) and THF (3.0 mL) at 0 °C was added a solution of LiOH•H_2O (32.87 mg, 0.392 mmol) in H_2O (1.0 mL). The resulting mixture was diluted with H_2O (6.0 mL) and the aqueous layer was washed with MTBE (3 x 4 mL). The aqueous layer was dried by lyophilization which afforded the desired product (140 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{21}H_{27}NO_3Si$: 370.18; found 370.0.

Intermediate A-69. Synthesis of 6-((2*S*)-1-(*tert*-butylsulfinyl)aziridin-2-yl)nicotinic acid



Step 1: Synthesis of methyl (*E*)-6-(((*tert*-butylsulfinyl)imino)methyl)nicotinate

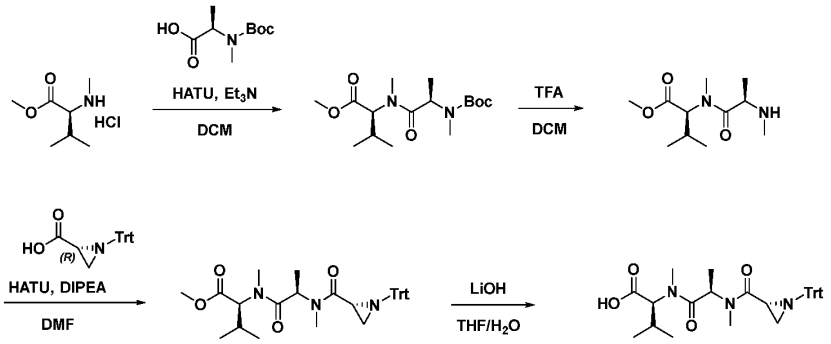
To a mixture of methyl 6-formylnicotinate (2.0 g, 12.11 mmol) and 2-methylpropane-2-sulfinamide (2.94 g, 24.26 mmol) in DCM (60 mL) was added $CuSO_4$ (5.80 g, 36.34 mmol). The resulting mixture was stirred at room temperature for 18 h. The reaction mixture was filtered, the

filter cake was washed with DCM (3 x 30 mL), and the filtrate was concentrated under reduced pressure. Purification by normal phase chromatography (66% EtOAc/pet. ether) afforded the desired product (2.581 g, 80% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{12}H_{16}N_2O_3S$: 269.10; found 269.1.

Step 2: Synthesis of 6-((2*S*)-1-(*tert*-butylsulfinyl)aziridin-2-yl)nicotinic acid

To a suspension of NaH (60%, 179.76 mg, 7.491 mmol) in DMSO (20 mL) at 0 °C was added $Me_3S^+I^-$ (1.53 g, 7.491 mmol) and the resulting mixture was warmed to room temperature and stirred for 1 h. To the reaction mixture was added a solution of methyl (*E*)-6-(((*tert*-butylsulfinyl)imino)methyl)nicotinate (670.0 mg, 2.497 mmol) in DMSO (20 mL) in portions. The mixture was stirred at room temperature for 3 h and was then diluted with EtOAc. The mixture was acidified to pH 4 with 1 M HCl and then the aqueous layer was extracted with EtOAc. The combined organic layers were concentrated under reduced pressure. Purification by reverse phase chromatography (10→15% MeCN/H_2O) afforded the desired product (313 mg, 45% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{12}H_{16}N_2O_3S$: 269.10; found 269.1.

Intermediate A-70. Synthesis of *N*-methyl-*N*-(*N*-methyl-*N*-((*R*)-1-tritylaziridine-2-carbonyl)-*D*-alanyl)-*L*-valine



Step 1: Synthesis of methyl *N*-(*N*-(*tert*-butoxycarbonyl)-*N*-methyl-*D*-alanyl)-*N*-methyl-*L*-valinate

To a solution of methyl-*L*-valinate hydrochloride (1.0 g, 5.51 mmol) and *N*-(*tert*-butoxycarbonyl)-*N*-methyl-*D*-alanine (1.34 g, 6.59 mmo) in DCM (20.0 mL) at 0 °C was added Et_3N (2.3 mL, 16.51 mmol) and HATU (2.72 g, 7.16 mmol). The mixture was warmed to room temperature and stirred for 4 h. The reaction mixture was then diluted with DCM (20 mL) and washed with sat. aq. NH_4Cl (2 x 40 mL) and brine (40 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (20% EtOAc/pet. ether) to afford the desired product (1.5 g, 82.5% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{16}H_{30}N_2O_5$: 331.23; found 331.1.

Step 2: Synthesis of methyl *N*-methyl-*N*-(methyl-*D*-alanyl)-*L*-valinate

To a solution of methyl *N*-(*N*-(*tert*-butoxycarbonyl)-*N*-methyl-*D*-alanyl)-*N*-methyl-*L*-valinate (1.50 g, 4.54 mmol) in DCM (9.0 mL) at 0 °C was added TFA (4.5 mL). The resulting mixture was warmed to room temperature and stirred for 1 h. The reaction mixture was concentrated under

reduced pressure to afford the desired product (1 g, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{11}H_{22}N_2O_3$: 231.17; found 231.2.

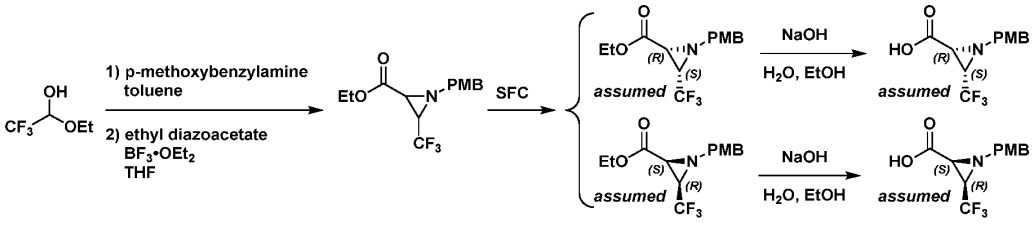
Step 3: Synthesis of methyl *N*-methyl-*N*-(*N*-methyl-*N*-((*R*)-1-tritylaziridine-2-carbonyl)-*D*-alanyl)-*L*-valinate

To a solution of methyl *N*-methyl-*N*-(methyl-*D*-alanyl)-*L*-valinate (900.0 mg, 3.91 mmol) and (*R*)-1-tritylaziridine-2-carboxylic acid (1.544 g, 4.689 mmol) in DMF (20.0 mL) at 0 °C was added DIPEA (3.4 mL, 19.54 mmol) and HATU (2.228 g, 5.86 mmol). The mixture was warmed to room temperature and stirred for 1 h. The reaction mixture was diluted with H_2O (50 mL) and extracted with EtOAc (3 x 50 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (33% EtOAc/pet. ether) to afford the desired product (1.2g, 56.7% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{33}H_{39}N_3O_4$: 542.30; found 542.3.

Step 4: Synthesis of *N*-methyl-*N*-(*N*-methyl-*N*-((*R*)-1-tritylaziridine-2-carbonyl)-*D*-alanyl)-*L*-valine

To a solution of methyl *N*-methyl-*N*-(*N*-methyl-*N*-((*R*)-1-tritylaziridine-2-carbonyl)-*D*-alanyl)-*L*-valinate (200.0 mg, 0.369 mmol) in THF (2.0 mL) at 0 °C was added a solution of LiOH•H_2O (77 mg, 1.84 mmol) in H_2O (1.85 mL). The resulting mixture was warmed to room temperature and stirred overnight. The mixture was adjusted to pH 9 with 1 M HCl and then adjusted to pH 7 with aq. NH_4Cl. The aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (200mg, crude. LCMS (ESI) *m/z*: [M + H] calcd for $C_{32}H_{37}N_3O_4$: 528.29; found 528.3.

Intermediate A-71 and A-72. Synthesis of (2*R*,3*S*)-1-(4-methoxybenzyl)-3-(trifluoromethyl)aziridine-2-carboxylic acid and (2*S*,3*R*)-1-(4-methoxybenzyl)-3-(trifluoromethyl)aziridine-2-carboxylic acid



Step 1: Synthesis of ethyl 1-(4-methoxybenzyl)-3-(trifluoromethyl)aziridine-2-carboxylate

A solution of 1-ethoxy-2,2,2-trifluoroethan-1-ol (2.17 mL, 18.37 mmol) and *p*-methoxybenzylamine (1.89 mL, 14.58 mmol) in toluene (46 mL) was refluxed for 16 h under Dean-Stark conditions. The reaction was concentrated under reduced pressure and the resulting residue was dissolved in THF (80 mL) and cooled to -78 °C. BF_3•Et_2O (0.360 mL, 2.92 mmol) was added to the solution, followed by dropwise addition of ethyl diazoacetate (1.83 mL, 17.50 mmol). The reaction was stirred for 4 h at room temperature. The reaction mixture was quenched by addition of aq. sat. $NaHCO_3$ (5 mL), and the resulting solution was extracted with DCM (3 x 50 mL). The combined organic layers were washed with H_2O (20 mL) and brine (10 mL). The organic phase was

dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (1→10% EtOAc/pet. ether) afford the desired product (2 g, 45.2 yield).

Step 2: Synthesis of ethyl (2*R*,3*S*)-1-(4-methoxybenzyl)-3-(trifluoromethyl)aziridine-2-carboxylate and ethyl (2*S*,3*R*)-1-(4-methoxybenzyl)-3-(trifluoromethyl)aziridine-2-carboxylate

Ethyl 1-(4-methoxybenzyl)-3-(trifluoromethyl)aziridine-2-carboxylate (1 g) was purified by SFC separation (column: REGIS(*S,S*)WHELK-O1(250 mm * 25 mm, 10 um); mobile phase: [Neu-IPA]; B%: 13% - 13%, min) to afford ethyl (2*R*,3*S*)-1-(4-methoxybenzyl)-3-(trifluoromethyl)aziridine-2-carboxylate (530 mg) and ethyl (2*S*,3*R*)-1-(4-methoxybenzyl)-3-(trifluoromethyl)aziridine-2-carboxylate (470 mg).

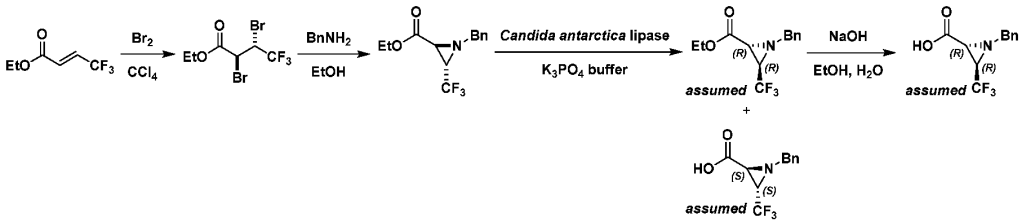
Step 3: Synthesis of (2*R*,3*S*)-1-(4-methoxybenzyl)-3-(trifluoromethyl)aziridine-2-carboxylic acid

To a solution of ethyl (2*R*,3*S*)-1-(4-methoxybenzyl)-3-(trifluoromethyl)aziridine-2-carboxylate (430 mg, 1.42 mmol) in EtOH (4 mL) and H_2O (6 mL) was added NaOH (113.42 mg, 2.84 mmol). The mixture was stirred at room temperature for 5 h. The mixture was acidified with aq. HCl (2M) to pH = 1 - 2. The reaction mixture was poured into H_2O (3 mL) and the aqueous phase was extracted with EtOAc (3 x 3 mL). The combined organic phase was washed with brine (5 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (350 mg, 89.1% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{12}H_{11}FNO_3$:274.08; found 274.1

Step 4: Synthesis of (2*S*,3*R*)-1-(4-methoxybenzyl)-3-(trifluoromethyl)aziridine-2-carboxylic acid

To a solution of ethyl (2*S*,3*R*)-1-(4-methoxybenzyl)-3-(trifluoromethyl)aziridine-2-carboxylate (370 mg, 1.22 mmol) in H_2O (2 mL) and EtOH (4 mL) was added NaOH (97.59 mg, 2.44 mmol). The mixture was stirred at room temperature for 5 h. The mixture was brought to pH = 1 – 2 with the addition of aq. HCl (2 M). The reaction mixture was poured into H_2O (3 mL) and the aqueous phase was extracted with EtOAc (3 x 3 mL). The combined organic phase was washed with brine (5 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (300 mg, 89.0% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{12}H_{11}FNO_3$:234.08; found 234.2

Intermediate A-73 and A-74. Synthesis of (2*S*,3*S*)-1-benzyl-3-(trifluoromethyl)aziridine-2-carboxylic acid and (2*R*,3*R*)-1-benzyl-3-(trifluoromethyl)aziridine-2-carboxylic acid



Step 1: Synthesis of ethyl (2*S*,3*R*)-2,3-dibromo-4,4,4-trifluorobutanoate

To a solution of ethyl (*E*)-4,4,4-trifluorobut-2-enoate (5 g, 29.74 mmol, 4.42 mL) in CCl_4 (90 mL) was added Br_2 (1.69 mL, 32.72 mmol) and the solution was stirred at 75 °C for 5 h. The reaction mixture was concentrated under reduced pressure to give the desired product (10.72 g, crude).

Step 2: Synthesis of ethyl (2*S*,3*S*)-1-benzyl-3-(trifluoromethyl)aziridine-2-carboxylate

To a solution of ethyl (2*S*,3*R*)-2,3-dibromo-4,4,4-trifluorobutanoate (10.72 g, 32.69 mmol) in EtOH (30 mL) was slowly added the solution of $BnNH_2$ (12.47 mL, 114.42 mmol) in EtOH (120 mL) at -5 °C under N_2. The mixture was warmed to room temperature and stirred for 15 h. The mixture was concentrated under reduced pressure and EtOAc (120 mL) was added to the residue. The precipitate was filtered off and the filtrate was washed with aqueous HCl (3%, 180 mL) and H_2O (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (20% EtOAc/pet. ether) to afford the desired product (6.02 g,67.4% yield).

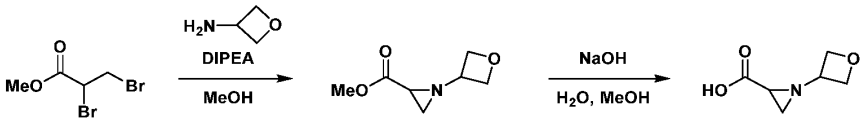
Step 3: Synthesis of ethyl (2*R*,3*R*)-1-benzyl-3-(trifluoromethyl)aziridine-2-carboxylate and (2*S*,3*S*)-1-benzyl-3-(trifluoromethyl)aziridine-2-carboxylic acid

Ethyl (2*R*,3*R*)-1-benzyl-3-(trifluoromethyl)aziridine-2-carboxylate and (2*S*,3*S*)-1-benzyl-3-(trifluoromethyl)aziridine-2-carboxylic acid were synthesized in Enzyme Screening Platform, based on the procedure in *Tetrahedron Asymmetry* **1999**, *10*, 2361.

Step 5: Synthesis of (2*R*,3*R*)-1-benzyl-3-(trifluoromethyl)aziridine-2-carboxylic acid

To a solution of ethyl (2*R*,3*R*)-1-benzyl-3-(trifluoromethyl)aziridine-2-carboxylate (200 mg, 731.93 µmol) in EtOH (5 mL) was added NaOH (2 M, 548.95 µL) and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure to remove EtOH. Then to the mixture was added HCl (1 M) to adjust pH to 1, and extracted with EtOAc (3 x 5 mL). The combined organic layers were washed with brine (2 x 10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford the desired product (138 mg, 76.9% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{11}H_{10}F_3NO_2$: 246.07; found 245.9.

Intermediate A-75. Synthesis of 1-(oxetan-3-yl)aziridine-2-carboxylic acid



Step 1: Synthesis of methyl 1-(oxetan-3-yl)aziridine-2-carboxylate

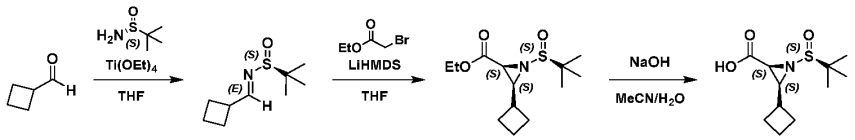
To a solution of methyl 2,3-dibromopropanoate (515.46 µL, 4.07 mmol) in MeOH (15 mL) was added DIPEA (3.54 mL, 20.33 mmol). After addition, the mixture was stirred for 15 min, and then oxetan-3-amine (297.25 mg, 4.07 mmol) was added dropwise. The resulting mixture was stirred at room temperature for 12 h. The reaction mixture was poured into H_2O (20 mL), the aqueous phase was extracted with DCM (2 x 25mL). The combined organic phase was washed with brine (20 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure. The

residue was purified by silica gel chromatography (10%→30% EtOAc/pet. ether) to afford the desired product (380 mg, 59.5% yield).

Step 2: Synthesis of 1-(oxetan-3-yl)aziridine-2-carboxylic acid

To a solution of methyl 1-(oxetan-3-yl)aziridine-2-carboxylate (280 mg, 1.78 mmol) in EtOH (3 mL) was added NaOH (2 M, 1.34 mL) at room temperature and the resulting mixture was stirred for 3 h. The reaction mixture was adjusted to pH 8 by the addition of HCl (1 M), and lyophilized to afford the desired product (200 mg, 78.4% yield).

Intermediate A-76. Synthesis of (2*S*,3*S*)-1-((*S*)-*tert*-butylsulfinyl)-3-cyclobutylaziridine-2-carboxylic acid



Step 1: Synthesis of (*S*,*E*)-*N*-(cyclobutylmethylene)-2-methylpropane-2-sulfinamide

To a solution of cyclobutanecarbaldehyde (0.5 g, 5.94 mmol) in THF (10 mL) was added (*S*)-2-methylpropane-2-sulfinamide (792.48 mg, 6.54 mmol) and $Ti(OEt)_4$ (2.47 mL, 11.89 mmol). The mixture was stirred at 75 °C for 3 h. The reaction mixture was cooled to room temperature and quenched by addition brine (30 mL), and filtered to remove solids. The mixture was extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (2 x 10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by silica gel chromatography (2%→10% EtOAc/pet. ether) to afford the desired product (907.3 mg, 39.9% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_9H_{17}NOS$: 188.1; found 188.3.

Step 2: Synthesis of ethyl (2*S*,3*S*)-1-((*S*)-*tert*-butylsulfinyl)-3-cyclobutylaziridine-2-carboxylate

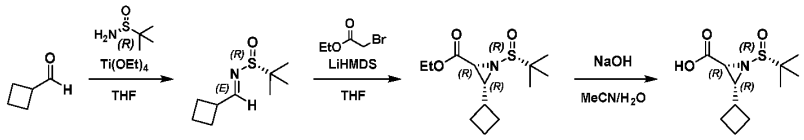
To a solution of ethyl 2-bromoacetate (1.60 g, 9.61 mmol, 1.06 mL) in THF (9 mL) was added LiHMDS (1 M, 9.61 mL) at -78 °C, after 2 min, (*S*,*E*)-*N*-(cyclobutylmethylene)-2-methylpropane-2-sulfinamide (0.9 g, 4.81 mmol) was added. The mixture was stirred at -78 °C for 2 h. The reaction mixture was quenched by addition H_2O (25 mL) at -78 °C and warmed to room temperature, then the mixture extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (2 x 5 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue, which was purified by silica gel chromatography (10%→20% EtOAc/pet. ether) to afford the desired product (426 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{23}NO_3S$: 274.14; found 274.3.

Step 3: Synthesis of (2*S*,3*S*)-1-((*S*)-*tert*-butylsulfinyl)-3-cyclobutylaziridine-2-carboxylic acid

To a solution of (2*S*,3*S*)-1-((*S*)-*tert*-butylsulfinyl)-3-cyclobutylaziridine-2-carboxylate (100 mg, 365.78 µmol) in MeCN (0.5 mL) and H_2O (0.5 mL) was added NaOH (21.95 mg, 548.67 µmol) at 0 °C, the mixture was warmed to room temperature and stirred for 2 h. The reaction mixture was adjusted to pH 5 by addition aq. 10% citric acid (~10 mL) and was then extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (2 x 5 mL), dried over Na_2SO_4,

filtered and concentrated under reduced pressure to afford the desired product (92.6 mg, crude). LCMS (ESI) m/z: [M + H] calcd for $C_{11}H_{19}NO_3S$: 246.11; found 246.3.

Intermediate A-77. Synthesis of (2*R*,3*R*)-1-((*R*)-*tert*-butylsulfinyl)-3-cyclobutylaziridine-2-carboxylic acid



Step 1: Synthesis of (*R*,*E*)-*N*-(cyclobutylmethylene)-2-methylpropane-2-sulfinamide

To a solution of cyclobutanecarbaldehyde (0.25 g, 2.97 mmol) in THF (5 mL) was added (*R*)-2-methylpropane-2-sulfinamide (396.24 mg, 3.27 mmol) and $Ti(OEt)_4$ (1.36 g, 5.94 mmol, 1.23 mL). The mixture was stirred at 75 °C for 3 h in two batches. The two batches were combined and the reaction mixture was quenched by the addition of brine (15 mL). The solution was extracted with EtOAc (3 x 20 mL) and the combined organic layers were washed with brine (2 x 5 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue, which was purified by silica gel chromatography (10%→20% EtOAc/pet. ether) to afford the desired product (786.7 mg, 70.7% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_9H_{17}NOS$: 188.1; found 188.3.

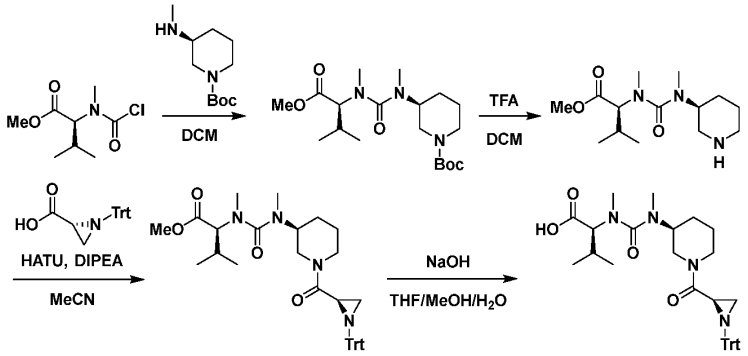
Step 2: Synthesis of ethyl (2*R*,3*R*)-1-((*R*)-*tert*-butylsulfinyl)-3-cyclobutylaziridine-2-carboxylate

To a solution of ethyl 2-bromoacetate (236.19 µL , 2.14 mmol) in THF (2 mL) was added LiHMDS (1 M, 2.14 mL) at -78 °C, after 30 min, (*R*,*E*)-*N*-(cyclobutylmethylene)-2-methylpropane-2-sulfinamide (0.2 g, 1.07 mmol) was added. The mixture was warmed to -40 °C and stirred for 4 h. The reaction mixture was quenched by addition H_2O (18 mL) at -40 °C and warmed to room temperature The mixture was extracted with EtOAc (3 x 15 mL) and the combined organic layers were washed with brine (2 x 5 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue, which was purified by prep-TLC (20% EtOAc/pet. ether) to afford the desired product (0.1 g, crude). LCMS (ESI) m/z: [M + H] calcd for $C_{13}H_{23}NO_3S$: 274.14; found 274.3.

Step 3: Synthesis of (2*R*,3*R*)-1-((*R*)-*tert*-butylsulfinyl)-3-cyclobutylaziridine-2-carboxylic acid

In two batches, to a solution ethyl (2*R*,3*R*)-1-((*R*)-*tert*-butylsulfinyl)-3-cyclobutylaziridine-2-carboxylate (25 mg, 91.44 µmol) in MeCN (0.25 mL) and H_2O (0.25 mL) was added NaOH (5.49 mg, 137.17 µmol) at 0 °C, the mixture was warmed to room temperature and stirred for 5 h. The reaction mixtures were combined, and adjust to pH to 5 with aq. 10% citric acid (10 mL), then extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (2 x 5 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford the desired product (53 mg, crude). LCMS (ESI) m/z: [M + H] calcd for $C_{11}H_{19}NO_3S$: 246.11; found 246.2.

Intermediate A-78. Synthesis of *N*-methyl-*N*-(methyl((*S*)-1-((*R*)-1-tritylaziridine-2-carbonyl)piperidin-3-yl)carbamoyl)-*L*-valine



Step 1: Synthesis of *tert*-butyl (*S*)-3-(3-((*S*)-1-methoxy-3-methyl-1-oxobutan-2-yl)-1,3-dimethylureido)piperidine-1-carboxylate

A mixture of methyl *N*-(chlorocarbonyl)-*N*-methyl-*L*-valinate (1.94 g, 9.34 mmol) in DCM was added to a solution of (*S*)-*tert*-butyl 3-(methylamino)piperidine-1-carboxylate (2.80 g, 13.08 mmol) in DCM (18 mL) at 0 °C. The mixture was stirred at 40 °C for 3 h. The mixture was added to saturated aq. NH_4Cl (80 mL), and the aqueous phase was extracted with DCM (3 x 40 mL). the combined organic phase was washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (30%→100% EtOAc/pet. ether) to afford the desired product (1.9 g, 55.3% yield). LCMS (ESI) m/z: [M + H] calculated for $C_{19}H_{35}N_3O_5$: 386.26; found 386.2.

Step 2: Synthesis of methyl *N*-methyl-*N*-(methyl((*S*)-piperidin-3-yl)carbamoyl)-*L*-valinate

To a solution of give *tert*-butyl (*S*)-3-(3-((*S*)-1-methoxy-3-methyl-1-oxobutan-2-yl)-1,3-dimethylureido)piperidine-1-carboxylate (1 g, 2.59 mmol) in DCM (10 mL) was added TFA (3.84 mL, 51.88 mmol) at 0 °C. The reaction was stirred at room temperature for 1 h. The mixture was added into saturated aq. Na_2CO_3 (100 mL) at 0 °C to adjust to pH 9. The aqueous phase was extracted with DCM (3 x 50 mL) and the combined organic phases were washed with brine (10 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure to afford the desired product (710 mg, crude). LCMS (ESI) m/z: [M + H] calculated for $C_{14}H_{27}N_3O_3$: 286.21; found 286.1.

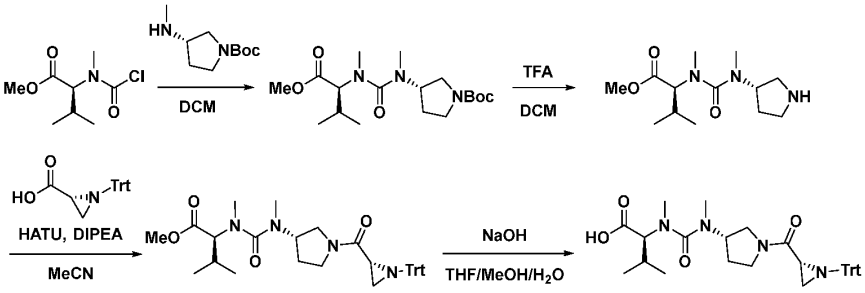
Step 3: Synthesis of methyl *N*-methyl-*N*-(methyl((*S*)-1-((*R*)-1-tritylaziridine-2-carbonyl)piperidin-3-yl)carbamoyl)-*L*-valinate

To a solution of (*R*)-1-tritylaziridine-2-carboxylic acid (1.24 g, 2.63 mmol, 70% purity) in MeCN (5 mL) at 0 °C was added DIPEA (1.22 mL, 7.01 mmol) and HATU (1.33 g, 3.50 mmol) followed by methyl *N*-methyl-*N*-(methyl((*S*)-piperidin-3-yl)carbamoyl)-*L*-valinate (500 mg, 1.75 mmol). The reaction mixture was warmed to room temperature and stirred 30 min. The mixture was added to saturated aq. NH_4Cl (100 mL) and the aqueous phase was extracted with DCM (3 x 50 mL). The combined organic phase was washed with brine (60 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (50%→100% EtOAc/pet. ether) to afford the desired product (650 mg, 49.7% yield). LCMS (ESI) m/z: [M + H] calculated for $C_{36}H_{44}N_4O_4$: 597.34; found 597.3

Step 4: Synthesis of *N*-methyl-*N*-(methyl((*S*)-1-((*R*)-1-tritylaziridine-2-carbonyl)piperidin-3-yl)carbamoyl)-*L*-valine

NaOH (58.34 mg, 1.46 mmol) was added to a solution of methyl *N*-methyl-*N*-(methyl((*S*)-1-((*R*)-1-tritylaziridine-2-carbonyl)piperidin-3-yl)carbamoyl)-*L*-valinate (640 mg, 857.97 μmol) in THF (4 mL), MeOH (1.3 mL), and H_2O (1.3 mL). The mixture was stirred at room temperature for 20 h. The reaction solution was directly lyophilized to afford the desired product (700 mg, crude). LCMS (ESI) m/z: [M + H] calculated for $C_{35}H_{42}N_4O_4$: 583.32; found 583.4.

Intermediate A-79. Synthesis of *N*-methyl-*N*-(methyl((*S*)-1-((*R*)-1-tritylaziridine-2-carbonyl)pyrrolidin-3-yl)carbamoyl)-*L*-valine



Step 1: Synthesis of *tert*-butyl (*S*)-3-(3-((*S*)-1-methoxy-3-methyl-1-oxobutan-2-yl)-1,3-dimethylureido)pyrrolidine-1-carboxylate

A solution of methyl *N*-(chlorocarbonyl)-*N*-methyl-*L*-valinate (1.14 g, 5.49 mmol) in DCM (10 mL) was added to a solution of *tert*-butyl (*S*)-3-(methylamino)pyrrolidine-1-carboxylate (1.54 g, 7.69 mmol) in DCM (10 mL) at 0 °C. The mixture was warmed to room temperature and stirred for 2 h. The mixture was then added to sat. NH_4Cl (50 mL), and the aqueous phase was extracted with DCM (3 x 30 mL). The combined organic phase was washed with brine (30 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (30%→100% EtOAc/pet. ether) to afford the desired product (1.07 g, 52.5% yield).

Step 2: Synthesis of methyl *N*-methyl-*N*-(methyl((*S*)-pyrrolidin-3-yl)carbamoyl)-*L*-valinate

To a solution of *tert*-butyl (*S*)-3-(3-((*S*)-1-methoxy-3-methyl-1-oxobutan-2-yl)-1,3-dimethylureido)pyrrolidine-1-carboxylate (1.05 g, 2.83 mmol) in DCM (11 mL) at 0 °C was added TFA (4.19 mL, 56.53 mmol). The reaction was then warmed to room temperature and stirred for 1 h. The mixture was added to sat. Na_2CO_3 (200 mL) at 0 °C dropwise to adjust to pH 9. The aqueous phase was extracted with DCM (3 x 100 mL), and the combined organic phase was washed with brine (100 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure to afford the desired product (800 mg, crude).

Step 3: Synthesis of methyl *N*-methyl-*N*-(methyl((*S*)-1-((*R*)-1-tritylaziridine-2-carbonyl)pyrrolidin-3-yl)carbamoyl)-*L*-valinate

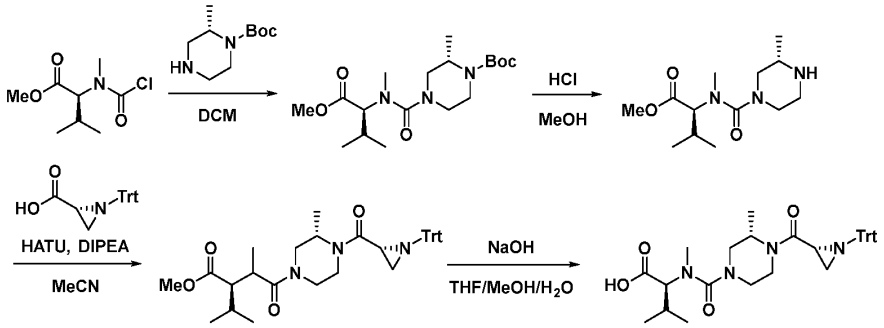
To a solution of (*R*)-1-tritylaziridine-2-carboxylic acid (1.04 g, 2.21 mmol) in MeCN (4 mL) at 0 °C was added HATU (1.12 g, 2.95 mmol), and DIPEA (1.03 mL, 5.90 mmol) followed by methyl *N*-methyl-*N*-(methyl((*S*)-pyrrolidin-3-yl)carbamoyl)-*L*-valinate (400 mg, 1.47 mmol). The mixture was

warmed to room temperature and stirred for 0.5 h. The mixture was poured into NH_4Cl aq. (50 mL) and extracted with DCM (3 x 20 mL). The combined organic phases were washed with brine (30 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (50%→100% EtOAc/pet. ether) to afford the desired product (580 mg, 67.5% yield).

Step 4: Synthesis of *N*-methyl-*N*-(methyl((*S*)-1-((*R*)-1-tritylaziridine-2-carbonyl)pyrrolidin-3-yl)carbamoyl)-*L*-valine

To a solution of methyl *N*-methyl-*N*-(methyl((*S*)-1-((*R*)-1-tritylaziridine-2-carbonyl)pyrrolidin-3-yl)carbamoyl)-*L*-valinate (650 mg, 1.12 mmol) in THF (3.9 mL) and MeOH (1.3 mL) was added a solution of NaOH (89.23 mg, 2.23 mmol) in H_2O (1.3 mL). The mixture was stirred at room temperature for 16 h. The reaction mixture was diluted with H_2O (10 mL) and then lyophilized directly to afford the desired product (700 mg, crude). LCMS (ESI) m/z: [M + H] calcd for $C_{34}H_{40}N_4O_4$: 569.30; found, 569.4.

Intermediate A-80. Synthesis of *N*-methyl-*N*-((*S*)-3-methyl-4-((*R*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valine



Step 1: Synthesis of *tert*-butyl (*S*)-4-(((*S*)-1-methoxy-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)-2-methylpiperazine-1-carboxylate

To a solution of methyl *tert*-butyl (*S*)-2-methylpiperazine-1-carboxylate (3.31 g, 16.52 mmol) in DCM (30 mL) at 0 °C was added a solution of methyl *N*-(chlorocarbonyl)-*N*-methyl-*L*-valinate in DCM (0.55 M, 30 mL). The mixture was stirred at room temperature for 30 min. The reaction mixture was diluted with H_2O (30 mL) and extracted with DCM (3 x 20 mL). The combined organic layers were washed with brine (2 x 15 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by silica gel chromatography (20%→50% EtOAc/pet. ether) to afford the desired product (5 g, 81.5% yield). LCMS (ESI) m/z: [M + H] calcd for $C_{18}H_{33}N_3O_5$:372.2; found 372.1.

Step 2: Synthesis of methyl *N*-methyl-*N*-((*S*)-3-methylpiperazine-1-carbonyl)-*L*-valinate

To *tert*-butyl (*S*)-4-(((*S*)-1-methoxy-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)-2-methylpiperazine-1-carboxylate (3 g, 8.08 mmol) was added a solution of 4M HCl in MeOH (30 mL). The mixture was stirred at room temperature for 1 h. The reaction mixture was adjusted to pH 8 with saturated aq. $NaHCO_3$, and was then diluted with H_2O (50 mL) and extracted with DCM (3 x

30 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford the desired product (1.8 g, 82.1% yield).

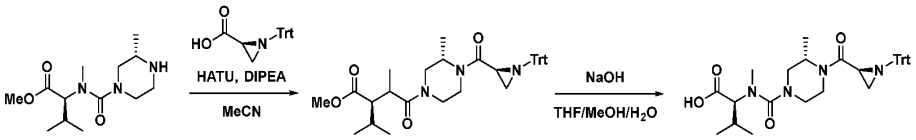
Step 3: Synthesis of methyl *N*-methyl-*N*-((*S*)-3-methyl-4-((*R*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valinate

To a solution of (2*R*)-1-tritylaziridine-2-carboxylic acid (971.10 mg, 2.95 mmol) in MeCN (10 mL) was added HATU (1.35 g, 3.54 mmol), DIPEA (1.54 mL, 8.84 mmol) and methyl *N*-methyl-*N*-((*S*)-3-methylpiperazine-1-carbonyl)-*L*-valinate (0.8 g, 2.95 mmol). The mixture was stirred at room temperature for 12 h. The reaction mixture was then diluted with H_2O (20 mL) and extracted with DCM (3 x 15 mL). The combined organic layers were washed with brine 20 mL (2 x 10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by silica gel chromatography (30%→50% EtOAc/pet. ether) to afford the desired product (0.35 g, 20.4% yield). LCMS (ESI) m/z: [M + H] calculated for $C_{35}H_{42}N_4O_4$: 583.3; found 583.2.

Step 4: Synthesis of *N*-methyl-*N*-((*S*)-3-methyl-4-((*R*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valine

To a solution of methyl *N*-methyl-*N*-((*S*)-3-methyl-4-((*R*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valinate (200 mg, 343.21 µmol) in H_2O (1 mL), THF (1 mL), and MeOH (1 mL) at 0 °C was added LiOH•H_2O (14.40 mg, 343.21 µmol). The mixture was stirred at room temperature for 8 h and was then lyophilized directly to afford the desired product (390 mg, 98.7% yield). LCMS (ESI) m/z: [M + Na] calculated for $C_{34}H_{40}N_4O_4$: 591.3; found 591.2.

Intermediate A-81. Synthesis of *N*-methyl-*N*-((*S*)-3-methyl-4-((*S*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valine



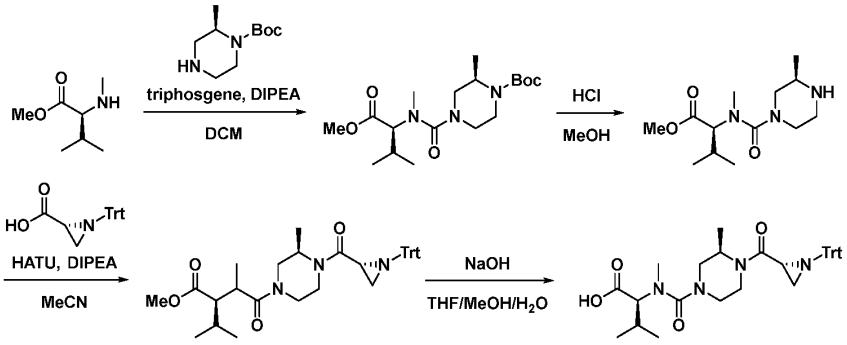
Step 1: Synthesis of methyl *N*-methyl-*N*-((*S*)-3-methyl-4-((*S*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valinate

To a solution of methyl *N*-methyl-*N*-((*S*)-3-methylpiperazine-1-carbonyl)-*L*-valinate (500 mg, 1.84 mmol) in MeCN (5 mL) at 0 °C was added (*S*)-1-tritylaziridine-2-carboxylic acid (1.30 g, 2.76 mmol, 70% purity), HATU (1.05 g, 2.76 mmol) and DIPEA (962.85 µL, 5.53 mmol). The mixture was stirred at toom temperature for 30 min. The reaction mixture was then diluted with H_2O (10 mL) and extracted with DCM (3 x 5 mL). The combined organic layers were washed with brine (2 x 5 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by silica gel chromatography (20%→33% EtOAc/pet. ether) to afford the desired product (0.5 g, 46.6% yield). LCMS (ESI) m/z: [M + Na] calculated for $C_{35}H_{42}O_4N_4$:605.2; found 605.2

Step 2: Synthesis of *N*-methyl-*N*-((*S*)-3-methyl-4-((*S*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valine

To a solution of methyl *N*-methyl-*N*-((*S*)-3-methyl-4-((*S*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valinate (400 mg, 686.42 μmol) in H_2O (2 mL), THF (2 mL), and MeOH (2 mL) at 0 °C was added LiOH•H_2O (28.80 mg, 686.42 μmol). The mixture was stirred at room temperature for 8 h. The mixture was lyophilized directly to afford then desired product (390 mg, 98.7% yield). LCMS (ESI) m/z: [M + Na] calculated for $C_{34}H_{40}N_4O_4$: 591.3; found 591.2.

Intermediate A-82. Synthesis of *N*-methyl-*N*-((*R*)-3-methyl-4-((*R*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valine



Step 1: Synthesis of *tert*-butyl (*R*)-4-(((*S*)-1-methoxy-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)-2-methylpiperazine-1-carboxylate

To a mixture of methyl methyl-*L*-valinate hydrochloride (3 g, 16.51 mmol) and DIPEA (17.26 mL, 99.09 mmol) in DCM (60 mL) at 0 °C was added bis(trichloromethyl) carbonate (2.45 g, 8.26 mmol) in one portion. The mixture was stirred at 0 °C for 30 min, then *tert*-butyl (*R*)-2-methylpiperazine-1-carboxylate (3.31 g, 16.51 mmol) was added to the mixture. The mixture was stirred at 0 °C for 1 h, then the pH of the solution was adjusted to 8 with sat. $NaHCO_3$. The residue was poured into H_2O (20 mL) and stirred for 5 min. The aqueous phase was extracted with EtOAc (2 x 20 mL), and the combined organic phase was washed with sat. $NaHCO_3$ (20 mL), dried with Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (1%→10% EtOAc/pet. ether) to afford the desired product (3.1 g, 50.5% yield). LCMS (ESI) m/z: [M + H] calculated for $C_{18}H_{33}N_3O_5$: 372.3; found 372.2.

Step 2: Synthesis of methyl *N*-methyl-*N*-((*R*)-3-methylpiperazine-1-carbonyl)-*L*-valinate

To a mixture of *tert*-butyl (*R*)-4-(((*S*)-1-methoxy-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)-2-methylpiperazine-1-carboxylate (2.5 g, 6.73 mmol) was added 4M HCl in MeOH (25 mL) at 0 °C. The mixture was stirred at room temperature for 1 h. The mixture was then concentrated under reduced pressure to afford the desired product (2 g, 96.5% yield).

Step 3: Synthesis of methyl *N*-methyl-*N*-((*R*)-3-methyl-4-((*R*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valinate

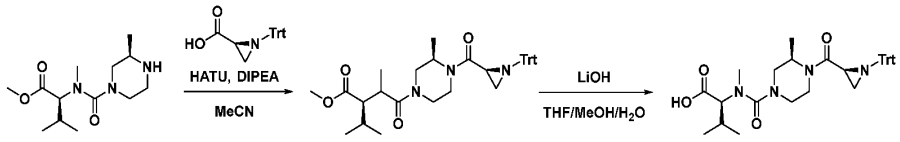
To a mixture of (*R*)-1-tritylaziridine-2-carboxylic acid (1.03 g, 3.12 mmol) and HATU (1.11 g, 2.92 mmol) in MeCN (1 mL) was added DIPEA (1.36 mL, 7.80 mmol) followed by methyl *N*-methyl-*N*-((*R*)-3-methylpiperazine-1-carbonyl)-*L*-valinate (600 mg, 1.95 mmol). The mixture was stirred at room temperature for 1 h. The mixture was then concentrated under reduced pressure. The residue

was purified by silica gel chromatography (1%→50% EtOAc/pet. ether) to afford the desired product (450 mg, 39.62% yield). LCMS (ESI) m/z: [M + H] calculated for $C_{35}H_{42}N_4O_4$: 583.3; found 583.2.

Step 4: Synthesis of *N*-methyl-*N*-((*R*)-3-methyl-4-((*R*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valine

To a mixture of methyl *N*-methyl-*N*-((*R*)-3-methyl-4-((*R*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valinate (450 mg, 772.23 μmol) in H_2O (1 mL), MeOH (1 mL), and THF (3 mL) was added LiOH•H_2O (48.60 mg, 1.16 mmol). The mixture was stirred at room temperature for 10 h and was then lyophilized to afford the desired product (410 mg, 92.4% yield). LCMS (ESI) m/z: [M + Na] calculated for $C_{34}H_{40}N_4O_4$: 591.3; found 591.3.

Intermediate A-83. Synthesis of *N*-methyl-*N*-((*R*)-3-methyl-4-((*S*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valine



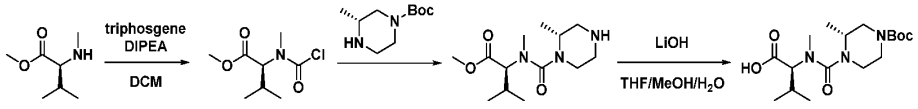
Step 1: Synthesis of methyl *N*-methyl-*N*-((*R*)-3-methyl-4-((*S*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valinate

To a mixture of (*S*)-1-tritylaziridine-2-carboxylic acid (1.03 g, 3.12 mmol) and HATU (1.11 g, 2.92 mmol) in MeCN (1 mL) was added DIPEA (1.36 mL, 7.80 mmol) followed by methyl *N*-methyl-*N*-((*R*)-3-methylpiperazine-1-carbonyl)-*L*-valinate (600 mg, 1.95 mmol). The mixture was stirred at room temperature for 1 h and was then concentrated under reduced pressure. The residue was purified by silica gel chromatography (0%→50% EtOAc/pet. ether) to afford the desired product (430 mg, 37.8% yield). LCMS (ESI) m/z: [M + H] calculated for $C_{35}H_{42}N_4O_4$: 583.3; found 583.2

Step 2: Synthesis of *N*-methyl-*N*-((*R*)-3-methyl-4-((*S*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valine

To a mixture of methyl *N*-methyl-*N*-((*R*)-3-methyl-4-((*S*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valinate (430 mg, 737.91 μmol) in H_2O (1 mL), MeOH (1 mL), and THF (3 mL) was added LiOH•H_2O (46.44 mg, 1.11 mmol). The mixture was stirred at room temperature for 10 h and was then lyophilized to afford the desired product (370 mg, 87.3% yield). LCMS (ESI) m/z: [M + Na] calculated for $C_{34}H_{40}N_4O_4$: 591.3; found 591.3.

Intermediate A-84. Synthesis of *N*-((*R*)-4-(*tert*-butoxycarbonyl)-2-methylpiperazine-1-carbonyl)-*N*-methyl-*L*-valine



Step 1: Synthesis of methyl *N*-(chlorocarbonyl)-*N*-methyl-*L*-valinate

To a solution of methyl methyl-*L*-valinate hydrochloride (1.8 g, 9.91 mmol) in DCM (20 mL) at 0 °C was added DIPEA (5.18 mL, 29.73 mmol) followed by bis(trichloromethyl) carbonate (1.47 g, 4.95 mmol). The mixture was stirred at 0 °C for 20 min. The reaction mixture used for the next step directly without workup.

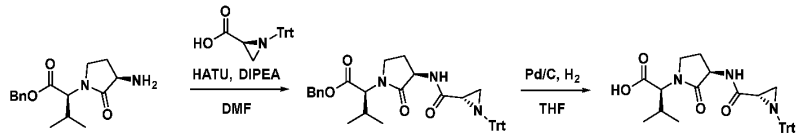
Step 2: Synthesis of *tert*-butyl (*R*)-4-(((*S*)-1-methoxy-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)-3-methylpiperazine-1-carboxylate

A solution of methyl *N*-(chlorocarbonyl)-*N*-methyl-*L*-valinate (1.03 g, 4.96 mmol) in DCM (10 mL) was added to a solution of *tert*-butyl (3*R*)-3-methylpiperazine-1-carboxylate (993.41 mg, 4.96 mmol) in DCM (1 mL) at 0 °C. The mixture was then stirred at 0 °C for 15 min. The mixture was added to aq. NH_4Cl (10 mL) and the solution was then extracted with DCM (3 x 10 mL). The combined organic phase was washed with brine (2 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography (0%→50% EtOAc/pet. ether) to afford the desired product (750 mg, 36.2% yield).

Step 3: Synthesis of *N*-((*R*)-4-(*tert*-butoxycarbonyl)-2-methylpiperazine-1-carbonyl)-*N*-methyl-*L*-valine

To a solution of *tert*-butyl (*R*)-4-(((*S*)-1-methoxy-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)-3-methylpiperazine-1-carboxylate (700 mg, 1.88 mmol) in THF (0.5 mL) and H_2O (0.5 mL) at 0 °C was added LiOH•H_2O (237.23 mg, 5.65 mmol). The mixture was stirred at room temperature for 3 h. The pH of the reaction mixture was adjusted to 6 - 7 with 1 N HCl. The mixture was extracted with EtOAc (3 x 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure to afford the desired product (600 mg, 85.5% yield).

Intermediate A-85. Synthesis of (*S*)-3-methyl-2-((*R*)-2-oxo-3-((*S*)-1-tritylaziridine-2-carboxamido)pyrrolidin-1-yl)butanoic acid



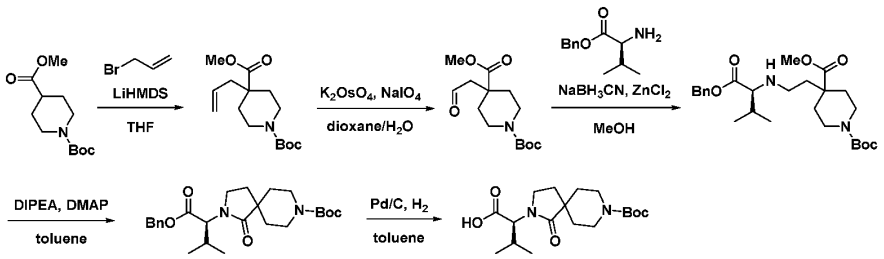
Step 1: Synthesis of benzyl (*S*)-3-methyl-2-((*R*)-2-oxo-3-((*S*)-1-tritylaziridine-2-carboxamido)pyrrolidin-1-yl)butanoate

To a mixture of benzyl (2*S*)-2-[(3*R*)-3-amino-2-oxopyrrolidin-1-yl]-3-methylbutanoate (420.0 mg, 1.446 mmol), DIPEA (934.73 mg, 7.232 mmol) and (2*S*)-1-(triphenylmethyl)aziridine-2-carboxylic acid (619.40 mg, 1.880 mmol) in DMF (5 mL) at 0 °C was added HATU (659.99 mg, 1.736 mmol). The resulting mixture was stirred at room temperature for 2 h. The reaction mixture was quenched with H_2O. The resulting mixture was extracted with EtOAc (2 x 10 mL), and the combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (30% EtOAc/pet. ether) to afford the desired product (480 mg, 55.2% yield). LCMS (ESI) *m/z*: $[M - H]^-$ calcd for $C_{38}H_{38}N_3O_4$: 600.29; found 600.3

Step 2: Synthesis of (*S*)-3-methyl-2-((*R*)-2-oxo-3-((*S*)-1-tritylaziridine-2-carboxamido)pyrrolidin-1-yl)butanoic acid

A suspension of benzyl (2*S*)-3-methyl-2-[(3*R*)-2-oxo-3-[(2*S*)-1-(triphenylmethyl)aziridine-2-amido]pyrrolidin-1-yl]butanoate (450.0 mg, 0.748 mmol) and Pd/C (200 mg) in THF (5 mL) at room temperature was stirred for 3 h under a hydrogen atmosphere. The mixture was then filtered and concentrated under reduced pressure to afford the desired product (400 mg, crude). LCMS (ESI) *m/z*: [M - H] calcd for $C_{31}H_{32}N_3O_4$: 510.24; found 510.2.

Intermediate A-86. Synthesis of (*S*)-2-(8-(*tert*-butoxycarbonyl)-1-oxo-2,8-diazaspiro[4.5]decan-2-yl)-3-methylbutanoic acid



Step 1: Synthesis of 1-(*tert*-butyl) 4-methyl 4-allylpiperidine-1,4-dicarboxylate

To a solution of 1-*tert*-butyl 4-methyl piperidine-1,4-dicarboxylate (5.0 g, 20.551 mmol) in THF (50 mL) at -78 °C was added LiHMDS (27 mL, 26.714 mmol, 1M in THF) followed by allyl bromide (3.23 g, 26.716 mmol). The resulting mixture was stirred overnight at room temperature. The reaction was quenched with sat. aq. NH_4Cl (aq.) and the combined organic layers were washed with brine (3 x 100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (50% EtOAc/pet. ether) to afford the desired product (4.5 g, 73.4% yield).

Step 2: Synthesis of 1-(*tert*-butyl) 4-methyl 4-(2-oxoethyl)piperidine-1,4-dicarboxylate

To a solution of 1-*tert*-butyl 4-methyl 4-(prop-2-en-1-yl)piperidine-1,4-dicarboxylate (1.0 g, 3.529 mmol) and $K_2OsO_4 \bullet 2H_2O$ (1.3 g, 3.529 mmol) in 1,4-dioxane (5 mL) and H_2O (5mL) at 0 °C was added $NaIO_4$ (1.51 g, 7.058 mmol). The resulting mixture was stirred at room temperature for 5 h. The mixture was extracted with EtOAc (3 x 50 mL) and the combined organic layers were washed with H_2O (3 x 100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was used in the next step directly without further purification to afford the desired product (800 mg, 75.% yield). LCMS (ESI) *m/z*: [M - H] calcd for $C_{14}H_{23}NO_5$: 284.16; found 284.0.

Step 3: Synthesis of 1-(*tert*-butyl) 4-methyl (*S*)-4-(2-((1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)amino)ethyl)piperidine-1,4-dicarboxylate

To a solution of 1-*tert*-butyl 4-methyl 4-(2-oxoethyl)piperidine-1,4-dicarboxylate (4.0 g, 14.018 mmol) and benzyl (2*S*)-2-amino-3-methylbutanoate (3.49 g, 16.822 mmol) in MeOH (40 mL) at 0 °C was added $ZnCl_2$ (2.10 g, 15.420 mmol) and $NaBH_3CN$ (1.76 g, 28.037 mmol). The resulting mixture was stirred at room temperature for 2 h. The reaction was quenched with sat. aq. NH_4Cl and the combined organic layers were washed with brine (3 x 100 mL), dried over Na_2SO_4,

filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (50% EtOAc/pet. ether) to afford the desired product. LCMS (ESI) *m/z*: [M + H] calcd for $C_{26}H_{40}N_2O_6$: 477.29; found 477.3.

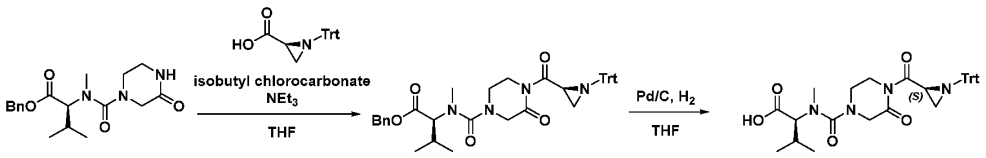
Step 4: Synthesis of *tert*-butyl (*S*)-2-(1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)-1-oxo-2,8-diazaspiro[4.5]decane-8-carboxylate

To a solution of 1-*tert*-butyl 4-methyl 4-(prop-2-en-1-yl)piperidine-1,4-dicarboxylate (2.20 g, 4.616 mmol) and DIPEA (5.97 g, 46.159 mmol) in toluene was added DMAP (0.56 g, 4.616 mmol) in portions at 120 °C. The resulting mixture was stirred overnight at 120 °C. The reaction was cooled to room temperature and quenched with sat. aq. NH_4Cl. The combined organic layers were washed with brine (3 x 100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by Prep-TLC (50% EtOAc/pet. ether) to afford the desired product (1.5 g, 50.2% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{25}H_{36}N_2O_5$: 445.26; found 445.3.

Step 5: Synthesis of (*S*)-2-(8-(*tert*-butoxycarbonyl)-1-oxo-2,8-diazaspiro[4.5]decan-2-yl)-3-methylbutanoic acid

To a solution of *tert*-butyl 2-[(2*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl]-1-oxo-2,8-diazaspiro[4.5]decane-8-carboxylate (2.40 g, 5.398 mmol) in toluene (25 mL) at room temperature was added Pd/C (2.40 g, 22.552 mmol). The resulting suspension was stirred overnight at room temperature under an H_2 atmosphere. The mixture was concentrated under reduced pressure, filtered, the filter cake washed with EtOAc (3 x 50 mL), and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (50% EtOAc/pet. ether) to afford the desired product (2. 2 g, 72.5% yield). LCMS (ESI) *m/z*: [M - H] calcd for $C_{18}H_{30}N_2O_5$: 353.22; found 353.2.

Intermediate A-87. Synthesis of *N*-methyl-*N*-(3-oxo-4-((*S*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valine



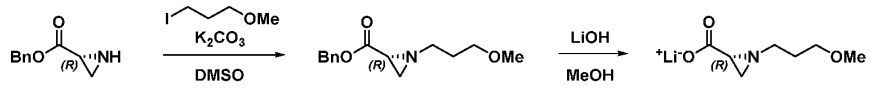
Step 1: Synthesis of benzyl *N*-methyl-*N*-(3-oxo-4-((*S*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valinate

To a solution of (2*S*)-1-(triphenylmethyl)aziridine-2-carboxylic acid (2.13 g, 6.466 mmol) in THF (10 mL) at 0 °C was added Et_3N (0.87 g, 8.598 mmol) and isobutyl chlorocarbonate (1.44 g, 10.54 mmol). The resulting mixture was stirred at room temperature for 1 h, and then benzyl (2*S*)-3-methyl-2-[methyl(3-oxopiperazine-1-carbonyl)amino]butanoate (1.50 g, 4.318 mmol) was added. The resulting mixture was stirred overnight at 70 °C. The reaction mixture was then concentrated under reduced pressure. The residue was purified by Prep-TLC (50% EtOAc/pet. ether) to afford the desired product (900 mg, 31.6% yield). LCMS (ESI) *m/z*: [M - H] calcd for $C_{40}H_{42}N_4O_5$: 657.32; found 657.1.

Step 2: Synthesis of *N*-methyl-*N*-(3-oxo-4-((*S*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valine

A solution of benzyl *N*-methyl-*N*-(3-oxo-4-((*S*)-1-tritylaziridine-2-carbonyl)piperazine-1-carbonyl)-*L*-valinate (500 mg) and Pd/C (50 mg) in THF (5 mL) was stirred for 2 h at room temperature under a hydrogen atmosphere. The resulting mixture was filtered, the filter cake was washed with MeOH (3 x 30 mL), and the filtrate was concentrated under reduced pressure to afford the desired product (460 mg, crude). LCMS (ESI) *m/z*: [M - H] calcd for $C_{33}H_{36}N_4O_5$: 567.27; found 567.1.

Intermediate A-88. Synthesis of lithium (*R*)-1-(3-methoxypropyl)aziridine-2-carboxylate



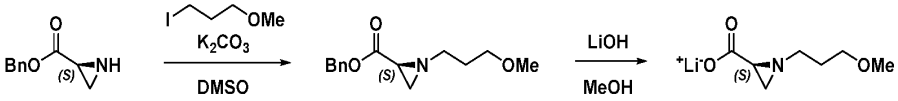
Step 1: Synthesis of benzyl (*R*)-1-(3-methoxypropyl)aziridine-2-carboxylate

To a mixture of benzyl (*R*)-aziridine-2-carboxylate (350.0 mg, 1.975 mmol) and K_2CO_3 (545.95 mg, 3.950 mmol) in DMSO (4 mL) at 60 °C was added 1-iodo-3-methoxypropane (790.13 mg, 3.950 mmol). The resulting mixture was stirred for 2 h and was then cooled to room temperature, diluted with brine (50 mL), and extracted with EtOAc (3 x 20 mL). The combined organic layers were concentrated under reduced pressure. The crude product was purified by reverse phase chromatography (30%→38% MeCN/H_2O) to afford the desired product (170 mg, 31.1% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{14}H_{19}NO_3$: 250.14; found 250.2.

Step 2: Synthesis of lithium (*R*)-1-(3-methoxypropyl)aziridine-2-carboxylate

A mixture of benzyl (*R*)-1-(3-methoxypropyl)aziridine-2-carboxylate (170 mg, 0.682 mmol) and LiOH (57.23 mg, 1.364 mmol) in MeOH (2 mL) was stirred at 0 °C for 1 h. The mixture was concentrated under reduced pressure to afford the desired product (200 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_7H_{13}NO_3$: 160.09; found 160.3.

Intermediate A-89. Synthesis of lithium (S)-1-(3-methoxypropyl)aziridine-2-carboxylate



Step 1: Synthesis of benzyl (*S*)-1-(3-methoxypropyl)aziridine-2-carboxylate

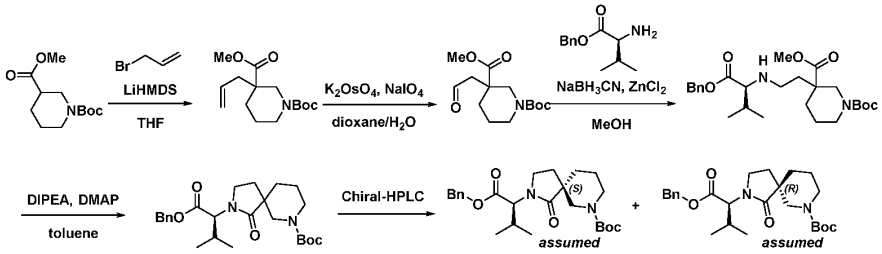
To a mixture of benzyl (*S*)-aziridine-2-carboxylate (250 mg, 1.411 mmol) and K_2CO_3 (389.96 mg, 2.822 mmol) in DMSO (4 mL) at 60 °C was added 1-iodo-3-methoxypropane (564.38 mg, 2.822 mmol). The resulting mixture was stirred for 2 h and was then cooled to room temperature, diluted with brine (50 mL), and extracted with EtOAc (3 x 20 mL). The combined organic layers were concentrated under reduced pressure. The crude product was purified by reverse phase

chromatography (25%→40% H_2O/MeCN) to afford the desired product (234 mg, 63.2% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{14}H_{19}NO_3$: 250.14; found 250.2.

Step 2: Synthesis of lithium (*S*)-1-(3-methoxypropyl)aziridine-2-carboxylate

A mixture of benzyl (*S*)-1-(3-methoxypropyl) aziridine-2-carboxylate (230 mg, 0.923 mmol) and LiOH•H_2O (77.43 mg, 1.845 mmol) in MeOH (3 mL) was stirred for 1 h at 0 °C. The resulting mixture was concentrated under reduced pressure to afford the desired product (320 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_7H_{13}NO_3$: 160.09; found 160.1.

Intermediate A-90. Synthesis of *tert*-butyl (*S*)-2-((*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)-1-oxo-2,7-diazaspiro[4.5]decane-7-carboxylate and *tert*-butyl (*R*)-2-((*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)-1-oxo-2,7-diazaspiro[4.5]decane-7-carboxylate



Step 1: Synthesis of 1-(*tert*-butyl) 3-methyl 3-allylpiperidine-1,3-dicarboxylate

To a solution of 1-*tert*-butyl 3-methyl piperidine-1,3-dicarboxylate (10.0 g, 41.101 mmol) and LiHMDS (82 mL, 82.202 mmol, 1M in THF) in THF (100 mL) at -78 °C was added allyl bromide (9.94 g, 82.202 mmol). The reaction was warmed to room temperature and stirred overnight. The solution was then quenched with sat. aq. NH_4Cl and diluted with EtOAc (500 mL). The organic layer was washed with brine (3 x 150 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford the desired product (9.9 g, 85% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{15}H_{25}NO_4$: 284.18; found 284.0.

Step 2: Synthesis of 1-(*tert*-butyl) 3-methyl 3-(2-oxoethyl)piperidine-1,3-dicarboxylate

To a solution of 1-(*tert*-butyl) 3-methyl 3-allylpiperidine-1,3-dicarboxylate1-(*tert*-butyl) 3-methyl 3-allylpiperidine-1,3-dicarboxylate (9.1 g, 32.114 mmol) and 2,6-lutidine (6.88 g, 64.227 mmol) in dioxane (180 mL) and H_2O (180 mL) at 0 °C was added K_2OsO_4•$2H_2O$ (591.61 mg, 1.606 mmol). The resulting mixture was stirred for 15 min at room temperature and was then cooled to at 0 °C and $NaIO_4$ (27.47 g, 128.455 mmol) was added in portions. The resulting mixture was stirred for 3 h at room temperature. And thee reaction was then quenched with sat. aq. $Na_2S_2O_3$ at 0 °C. The resulting mixture was extracted with EtOAc (2 x 500 mL), and thee combined organic layers were washed with 1M HCl (2 x 200 mL), brine (2 x 200 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (7.5 g, 81.9% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{14}H_{23}NO_5$: 286.16; found 286.1.

Step 3: Synthesis of 1-(*tert*-butyl) 3-methyl 3-(2-(((*R*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)amino)ethyl)piperidine-1,3-dicarboxylate

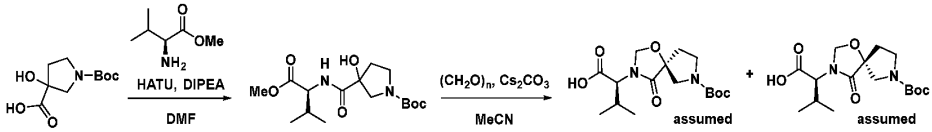
To a solution of 1-(*tert*-butyl) 3-methyl 3-(2-oxoethyl)piperidine-1,3-dicarboxylate (9.0 g, 31.541 mmol) and benzyl (2*S*)-2-amino-3-methylbutanoate (7.19 g, 34.695 mmol) in MeOH (90 mL)

at 0 °C was added $ZnCl_2$ (4.73g, 34.695 mmol) and $NaBH_3CN$ (3.96g, 63.083 mmol). The resulting mixture was stirred overnight at room temperature. Desired product could be detected by LCMS, and it was concentrated under reduced pressure and extracted with EtOAc (1200 mL). The organic layer was washed with brine (3 x 150 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by reverse phase chromatography afforded the desired product (9.9 g, 65.9% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{26}H_{40}N_2O_6$: 477.29; found 477.2.

Step 4: Synthesis of *tert*-butyl (*S*)-2-((*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)-1-oxo-2,7-diazaspiro[4.5]decane-7-carboxylate and *tert*-butyl (*R*)-2-((*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)-1-oxo-2,7-diazaspiro[4.5]decane-7-carboxylate

To a solution of 1-(*tert*-butyl) 3-methyl 3-(2-(((*R*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)amino)ethyl)piperidine-1,3-dicarboxylate (9.9 g, 20.772 mmol) and DIPEA (26.84 g, 207.715 mmol) in toluene (100 mL) was added DMAP (5.07 g, 41.543 mmol). The resulting mixture was stirred at 80 °C for 50 h. The resulting mixture was concentrated under reduced pressure and the residue was taken up in EtOAc (1000 mL). The organic layer was washed with brine (3 x 150 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by CHIRAL-HPLC (50% EtOH/Hex) to afford *tert*-butyl (*S*)-2-((*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)-1-oxo-2,7-diazaspiro[4.5]decane-7-carboxylate (1.75 g) and *tert*-butyl (*R*)-2-((*S*)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)-1-oxo-2,7-diazaspiro[4.5]decane-7-carboxylate (1.98 g). LCMS (ESI) *m/z*: [M + H] calcd for $C_{25}H_{36}N_2O_5$: 445.26; found 445.2.

Intermediates A-91 and A-92. Synthesis of (*S*)-2-((*S*)-7-(*tert*-butoxycarbonyl)-4-oxo-1-oxa-3,7-diazaspiro[4.4]nonan-3-yl)-3-methylbutanoic acid and (*S*)-2-((*R*)-7-(*tert*-butoxycarbonyl)-4-oxo-1-oxa-3,7-diazaspiro[4.4]nonan-3-yl)-3-methylbutanoic acid



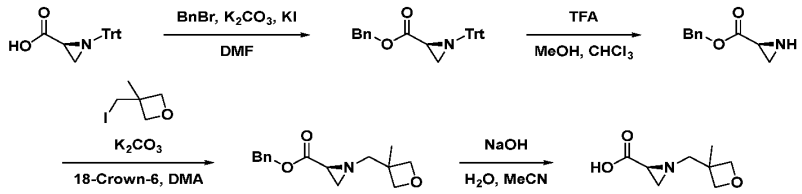
Step 1: Synthesis of *tert*-butyl 3-hydroxy-3-(((*S*)-1-methoxy-3-methyl-1-oxobutan-2-yl)carbamoyl)pyrrolidine-1-carboxylate

To a solution of 1-(*tert*-butoxycarbonyl)-3-hydroxypyrrolidine-3-carboxylic acid (800 mg, 3.46 mmol) and DIPEA (3.01 mL, 17.3 mmol) in DMF (10 mL) at 0 °C was added methyl *L*-valinate (681 mg, 5.19 mmol) and HATU (1.71 g, 4.497 mmol). The resulting mixture was warmed to room temperature and stirred for 2 h then diluted with H_2O (20 mL) and extracted into EtOAc (3 x 20 mL). The combined organic layers were washed with brine (2 x 20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by reverse phase chromatography (30→55% MeCN/H_2O, 0.1% NH_4HCO_3) afforded the desired product (1 g, 76% yield). LCMS (ESI) *m/z*: [M + Na] calcd for $C_{16}H_{28}N_2O_6$: 367.18; found 366.9.

Step 2: Synthesis of (*S*)-2-((*S*)-7-(*tert*-butoxycarbonyl)-4-oxo-1-oxa-3,7-diazaspiro[4.4]nonan-3-yl)-3-methylbutanoic acid and (*S*)-2-((*R*)-7-(*tert*-butoxycarbonyl)-4-oxo-1-oxa-3,7-diazaspiro[4.4]nonan-3-yl)-3-methylbutanoic acid

To a solution of *tert*-butyl 3-hydroxy-3-(((*S*)-1-methoxy-3-methyl-1-oxobutan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (1.0 g, 2.90 mmol) and Cs_2CO_3 (1.89 g, 5.81 mmol) in MeCN (15 mL) at 0 °C was added paraformaldehyde (436 mg, 14.5 mmol). The resulting mixture was heated to 80 °C and stirred overnight. Purification by reverse phase chromatography (10→40% MeCN/H_2O, 0.1% NH_4HCO_3) afforded a mixture of the desired products. The diastereomers were separated by prep-SFC (30% EtOH/hexanes, 0.3% TFA) to afford (*S*)-2-((*S*)-7-(*tert*-butoxycarbonyl)-4-oxo-1-oxa-3,7-diazaspiro[4.4]nonan-3-yl)-3-methylbutanoic acid (250 mg, 24% yield) and (*S*)-2-((*R*)-7-(*tert*-butoxycarbonyl)-4-oxo-1-oxa-3,7-diazaspiro[4.4]nonan-3-yl)-3-methylbutanoic acid (200 mg, 19% yield). LCMS (ESI) *m/z*: [M + Na] calcd for $C_{16}H_{26}N_2O_6$: 365.17; found 365.0.

Intermediate A-93. Synthesis of (*S*)-1-((3-methyloxetan-3-yl)methyl)aziridine-2-carboxylic acid



Step 1: Synthesis of benzyl (*S*)-1-tritylaziridine-2-carboxylate

To a mixture of (*S*)-1-tritylaziridine-2-carboxylic acid (3.0 g, 9.11 mmol) and benzyl bromide (2.16 mL, 18.22 mmol) in DMF (30 mL) was added K_2CO_3 (2.25 g, 18.22 mmol) and KI (76 mg, 455 µmol). The reaction mixture was heated to 50 °C and stirred for 30 min then was cooled to room temperature and diluted with H_2O (30 mL) and EtOAc (30 mL). The aqueous layer was extracted with EtOAc (3 x 40 mL), and the combined organic layers were washed with brine (5 x 70 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (4.7 g, crude).

Step 2: Synthesis of benzyl (*S*)-aziridine-2-carboxylate

To a mixture of benzyl (*S*)-1-tritylaziridine-2-carboxylate (3.4 g, 8.10 mmol) in MeOH (17.5 mL) and $CHCl_3$ (17.5 mL) at 0 °C was added TFA (9.0 mL, 122 mmol). The reaction mixture was stirred for 30 min then was poured into sat. aq. $NaHCO_3$ (50 mL), extracted into DCM (4 x 35 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (6→100% EtOAc/pet. ether) afforded the desired product (445 mg, 31% yield).

Step 3: Synthesis of benzyl (*S*)-1-((3-methyloxetan-3-yl)methyl)aziridine-2-carboxylate

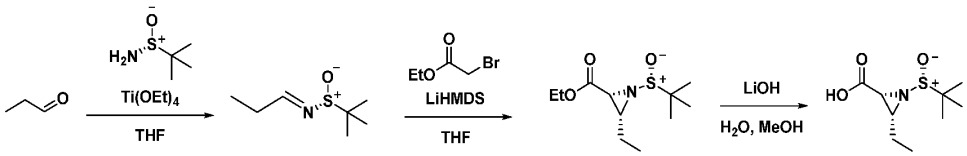
To a mixture of benzyl (*S*)-aziridine-2-carboxylate (440 mg, 2.48 mmol) and 3-(iodomethyl)-3-methyloxetane (2.11 g, 9.93 mmol) in DMA (5 mL) was added K_2CO_3 (1.72 g, 12.42 mmol) and 18-crown-6 (32.8 mg, 124 µmol). The reaction mixture was heated to 80 °C and stirred for 12 h, and was then was diluted with H_2O (25 mL) and EtOAc (25 mL). The aqueous layer was extracted with EtOAc (3 x 20 mL), and the combined organic layers were washed with brine (5 x 45 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by prep-TLC

(50% EtOAc/pet. ether) afforded the desired product (367 mg, 57% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{15}H_{19}NO_3$: 262.14; found 262.0.

Step 4: Synthesis of (*S*)-1-((3-methyloxetan-3-yl)methyl)aziridine-2-carboxylic acid

To a mixture of benzyl (*S*)-1-((3-methyloxetan-3-yl)methyl)aziridine-2-carboxylate (100 mg, 383 µmol) in MeCN (500 µL) and H_2O (500 µL) at 0 °C was added NaOH (23 mg, 574 µmol). The reaction mixture was stirred at 0 °C for 1 h then was concentrated under reduced pressure to afford the desired product (100 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_8H_{13}NO_3$: 172.10; found 172.0.

Intermediate A-94. Synthesis of (2*R*,3*R*)-1-(*tert*-butylsulfinyl)-3-ethylaziridine-2-carboxylic acid



Step 1: Synthesis of (*R,E*)-2-methyl-*N*-propylidenepropane-2-sulfinamide

To a solution of propionaldehyde (6.27 mL, 86.1 mmol) in THF (200 mL) was added (*R*)-2-methylpropane-2-sulfinamide (10.4 g, 86.1 mmol) and titanium ethoxide (51 mL, 170 mmol). The reaction mixture was heated to 70 °C for 3 h then cooled to room temperature and quenched with H_2O (50 mL), filtered, and extracted into EtOAc (3 x 30 mL). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (9→17% EtOAc/pet. ether) afforded the desired product (4.0 g, 29% yield).

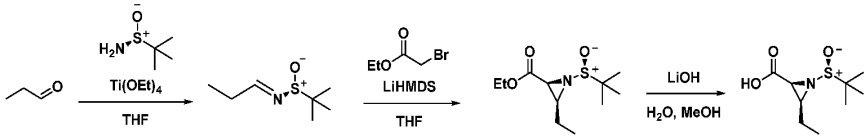
Step 2: Synthesis of ethyl (2*R*,3*R*)-1-(*tert*-butylsulfinyl)-3-ethylaziridine-2-carboxylate

To a solution of ethyl 2-bromoacetate (2.74 mL, 24.8 mmol) in THF (40 mL) at -78 °C was added LiHMDS (24.80 mL, 1 M in THF). After 30 min (*R,E*)-2-methyl-*N*-propylidenepropane-2-sulfinamide (2.0 g, 12.4 mmol) in THF (20 mL) was added to the reaction mixture. The mixture was stirred for 1 h then warmed to room temperature, quenched with H_2O (50 mL), and extracted into EtOAc (3 x 50 mL). The combined organic layers were washed with brine (2 x 50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (17→25% EtOAc/pet. ether) afforded product (1.34 g, 44% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{11}H_{21}NO_3S$: 248.13; found 248.1.

Step 3: Synthesis of (2*R*,3*R*)-1-(*tert*-butylsulfinyl)-3-ethylaziridine-2-carboxylic acid

To a solution of ethyl (2*R*,3*R*)-1-(*tert*-butylsulfinyl)-3-ethylaziridine-2-carboxylate (600 mg, 2.4 mmol) in MeOH (3 mL) and H_2O (3 mL) was added LiOH (70 mg, 2.9 mmol). The resulting mixture was stirred for 16 h then diluted with H_2O (20 mL) and washed with DCM (3 x 10 mL). Lyophilization of the aqueous layer afforded product (600 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_9H_{17}NO_3S$: 220.10; found 220.3.

Intermediate A-95. Synthesis of (2*S*,3*S*)-1-(*tert*-butylsulfinyl)-3-ethylaziridine-2-carboxylic acid



Step 1: Synthesis of (*S,E*)-2-methyl-*N*-propylidenepropane-2-sulfinamide

To a solution of propionaldehyde (6.27 mL, 86.1 mmol) in THF (50 mL) was added (*S*)-2-methylpropane-2-sulfinamide (10.4 g, 86.1 mmol) and titanium ethoxide (51 mL, 170 mmol). The reaction mixture was heated to 70 °C for 3 h then cooled to room temperature and quenched with H_2O (30 mL), filtered, and extracted into DCM (3 x 100 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (25% EtOAc/pet. ether) afforded product (4.6 g, 33% yield).

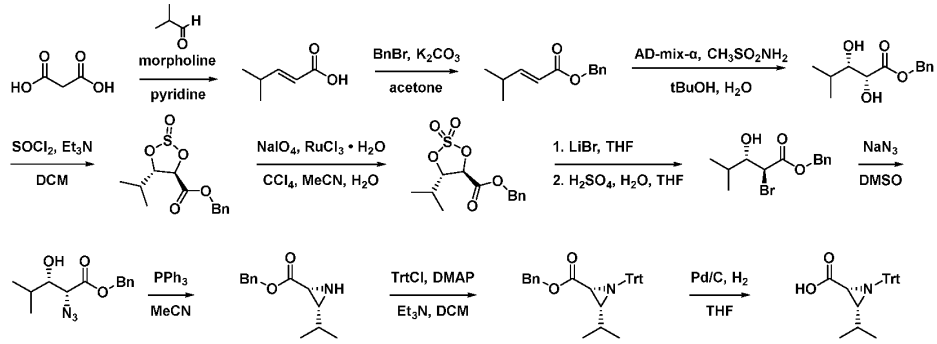
Step 2: Synthesis of ethyl (2*S*,3*S*)-1-(*tert*-butylsulfinyl)-3-ethylaziridine-2-carboxylate

To a solution of ethyl 2-bromoacetate (2.74 mL, 24.8 mmol) in THF (40 mL) at -78 °C was added LiHMDS (24.80 mL, 1M in THF). After 30 min (*S,E*)-2-methyl-*N*-propylidenepropane-2-sulfinamide (2.0 g, 12.4 mmol) in THF (20 mL) was added to the reaction mixture. The mixture was stirred for 1 h then warmed to room temperature, quenched with H_2O (20 mL), and extracted into EtOAc (3 x 20 mL). The combined organic layers were washed with brine (2 x 25 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by reverse phase chromatography (31→51% MeCN/H_2O, 10 mM NH_4HCO_3) afforded product (600 mg, 20% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{11}H_{21}NO_3S$: 248.13; found 248.1.

Step 3: Synthesis of (2*S*,3*S*)-1-(*tert*-butylsulfinyl)-3-ethylaziridine-2-carboxylic acid

To a solution of ethyl (2*S*,3*S*)-1-(*tert*-butylsulfinyl)-3-ethylaziridine-2-carboxylate (600 mg, 2.4 mmol) in MeOH (300 µL) and H_2O (300 µL) was added LiOH (87 mg, 3.6 mmol). The resulting mixture was stirred for 12 h then diluted with H_2O (20 mL) and washed with DCM (3 x 10 mL). Lyophilization of the aqueous layer afforded product (600 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_9H_{17}NO_3S$: 220.10; found 220.2.

Intermediate A-96. Synthesis of (2*R*,3*R*)-3-isopropyl-1-tritylaziridine-2-carboxylic acid



Step 1: Synthesis of (*E*)-4-methylpent-2-enoic acid

Two batches of a solution of malonic acid (25.0 mL, 240 mmol), isobutyraldehyde (34.7 mL, 380 mmol) and morpholine (380 µL, 4.32 mmol) in pyridine (75 mL) were stirred for 24 h then were heated to 115 °C and stirred for 12 h. The combined reaction mixtures were poured into H_2SO_4 (1M, 800 mL) and extracted into EtOAc (3 x 300 mL). The combined organic layers were washed with brine (300 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dissolved in NaOH (1 M, 500 mL), washed with EtOAc (2 x 200 mL), acidified to pH = 4 – 2 with HCl (4M), and extracted into EtOAc (3 x 300 mL). The combined organic layers were washed with brine (300 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure which afforded product (54 g, 98% yield).

Step 2: Synthesis of benzyl (*E*)-4-methylpent-2-enoate

To two batches of a solution of (*E*)-4-methylpent-2-enoic acid (6.25 mL, 52.6 mmol) in acetone (90 mL) was added K_2CO_3 (13.8 g, 100 mmol) and the mixtures were stirred for 30 min. Then a solution of benzyl bromide (6.31 mL, 53.1 mmol) in acetone (10 mL) was added and the mixtures were heated to 75 °C for 5 h. The reaction mixtures were cooled to room temperature and concentrated under reduced pressure. The residue was dissolved in EtOAc (200 mL) and H_2O (200 mL) then extracted into EtOAc (2 x 200 mL). The combined organic layers were washed with brine (300 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel chromatography (0→10% EtOAc/pet. ether) afforded product (9.0 g, 42% yield).

Step 3: Synthesis of benzyl (2*R*,3*S*)-2,3-dihydroxy-4-methylpentanoate

To a solution of AD-mix-α (61.7 g) and methanesulfonamide (4.19 g, 44.1 mmol) in *tert*-BuOH (225 mL) and H_2O (225 mL) was added benzyl (*E*)-4-methylpent-2-enoate (9 g, 44.1 mmol). The mixture was stirred at room temperature for 12 h then Na_2SO_3 (67.5 g) was added and stirred for 30 min. The reaction mixture was diluted with EtOAc (300 mL) and H_2O (300 mL) and extracted into EtOAc (3 x 300 mL), washed with brine (300 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel chromatography (0→25% EtOAc/pet. ether) afforded product (8.3 g, 79% yield). LCMS (ESI) *m/z*: [M + Na] calcd for $C_{13}H_{18}O_4$: 261.11; found 261.0.

Step 4: Synthesis of benzyl (4*R*,5*S*)-5-isopropyl-1,3,2-dioxathiolane-4-carboxylate 2-oxide

To a solution of benzyl (2*R*,3*S*)-2,3-dihydroxy-4-methylpentanoate (10 g, 42.0 mmol) in DCM (100 mL) at 0 °C was added Et_3N (17.5 mL, 126 mmol) and $SOCl_2$ (4.26 mL, 58.8 mmol). The reaction mixture was stirred 30 min then was diluted with DCM (30 mL) and H_2O (100 mL), extracted into DCM (3 x 50 mL), washed with brine (100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure which afforded product (11.0 g, 92% yield).

Step 5: Synthesis of benzyl (4*R*,5*S*)-5-isopropyl-1,3,2-dioxathiolane-4-carboxylate 2,2-dioxide

To a solution of benzyl (4*R*,5*S*)-5-isopropyl-1,3,2-dioxathiolane-4-carboxylate 2-oxide (11 g, 38.7 mmol) in H_2O (250 mL), MeCN (125 mL), and CCl_4 (125 mL) was added $NaIO_4$ (3.22 mL, 58.0 mmol) and $RuCl_3 \cdot H_2O$ (872 mg, 3.87 mmol). The mixture was stirred at room temperature for 1 h then was diluted with EtOAc (200 mL) and H_2O (50 mL), filtered, and the filtrate was extracted into EtOAc (3 x 200 mL). The combined organic layers were washed sequentially with brine (200

mL) and sat. aq. Na_2CO_3 (300 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel chromatography (0→17% EtOAc/pet. ether) afforded product (11 g, 95% yield).

Step 6: Synthesis of benzyl (2*S*,3*S*)-2-bromo-3-hydroxy-4-methylpentanoate

To a solution of benzyl (4*R*,5*S*)-5-isopropyl-1,3,2-dioxathiolane-4-carboxylate 2,2-dioxide (11 g, 36.6 mmol) in THF (520 mL) was added LiBr (3.49 mL, 139 mmol). The reaction mixture was stirred at room temperature for 5 h and then concentrated under reduced pressure. The residue was diluted in THF (130 mL) and H_2O (65 mL), cooled to 0 °C, then H_2SO_4 solution (20% aq., 1.3 L) was added and the mixture was warmed to room temperature and stirred for 24 h. The mixture was diluted with EtOAc (1.0 L), extracted into EtOAc (2 x 300 mL), washed sequentially with Na_2CO_3 (sat. aq., 300 mL) and brine (300 mL), then was concentrated under reduced pressure. Purification by silica gel chromatography (0→17% EtOAc/pet. ether) afforded product (10 g, 81% yield).

Step 7: Synthesis of benzyl (2*R*,3*S*)-2-azido-3-hydroxy-4-methylpentanoate

To a solution of benzyl (2*S*,3*S*)-2-bromo-3-hydroxy-4-methylpentanoate (10 g, 33.2 mmol) in DMSO (100 mL) was added NaN_3 (4.32 g, 66.4 mmol). The reaction mixture was stirred at room temperature for 12 h then was diluted with EtOAc (300 mL) and H_2O (200 mL). The aqueous phase was extracted into EtOAc (2 x 200 mL), washed with brine (200 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel chromatography (0→17% EtOAc/pet. ether) afforded product (7.5 g, 79% yield).

Step 8: Synthesis of benzyl (2*R*,3*R*)-3-isopropylaziridine-2-carboxylate

To a solution of benzyl (2*R*,3*S*)-2-azido-3-hydroxy-4-methylpentanoate (7.5 g, 28.5 mmol) in MeCN (150 mL) was added PPh_3 (7.70 g, 29.3 mmol). The reaction mixture was stirred at room temperature for 1 h and then heated to 70 °C and stirred for 4 h. The reaction mixture was concentrated under reduced pressure and purification by silica gel chromatography (0→17% EtOAc/pet. ether) afforded product (4.5 g, 66% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{17}NO_2$: 220.13; found 220.0.

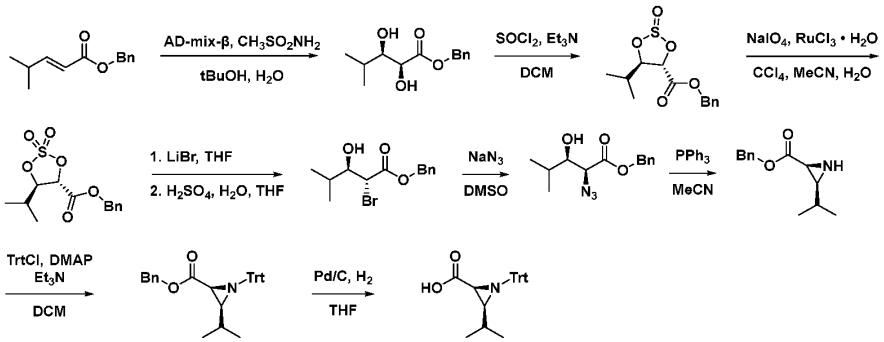
Step 9: Synthesis of benzyl (2*R*,3*R*)-3-isopropyl-1-tritylaziridine-2-carboxylate

To a solution of benzyl (2*R*,3*R*)-3-isopropylaziridine-2-carboxylate (2 g, 9.12 mmol) in DCM (30 mL) at 0 °C was added Et_3N (3.81 mL, 27.4 mmol) and trityl chloride (3.05 g, 10.9 mmol) followed by DMAP (111 mg, 912 µmol). The reaction mixture was stirred at 0 °C for 1 h and then was diluted with DCM (50 mL) and H_2O (50 mL) then extracted into DCM (2 x 30 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel chromatography (0→25% DCM/pet. ether) afforded product (3.1 g, 72% yield).

Step 10: Synthesis of (2*R*,3*R*)-3-isopropyl-1-tritylaziridine-2-carboxylic acid

Two solutions of benzyl (2*R*,3*R*)-3-isopropyl-1-tritylaziridine-2-carboxylate (200 mg, 430 µmol) and Pd/C (100 mg) in THF (4 mL) were stirred for 1 h at room temperature under H_2 atmosphere. The reaction mixtures were combined, filtered, and concentrated under reduced pressure. Purification by silica gel chromatography (0→50% EtOAc/pet. ether) afforded product (160 mg, 51% yield).

Intermediate A-97. Synthesis of (2*S*,3*S*)-1-benzyl-3-isopropylaziridine-2-carboxylic acid



Step 1: Synthesis of benzyl (2*S*,3*R*)-2,3-dihydroxy-4-methylpentanoate

To a solution of AD-mix-ß (61.7 g) and methanesulfonamide (4.19 g, 44.1 mmol) in *tert*-BuOH (225 mL) and H_2O (225 mL) was added benzyl (*E*)-4-methylpent-2-enoate (9 g, 44.1 mmol). The mixture was stirred at room temperature for 12 h then Na_2SO_3 (67.5 g) was added and stirred for 30 min. The reaction mixture was diluted with EtOAc (300 mL) and H_2O (300 mL) and extracted into EtOAc (3 x 300 mL), washed with brine (300 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel chromatography (0→25% EtOAc/pet. ether) afforded product (8.8 g, 84% yield). LCMS (ESI) *m/z*: [M + Na] calcd for $C_{13}H_{18}O_4$: 261.11; found 261.0.

Step 2: Synthesis of benzyl (4*S*,5*R*)-5-isopropyl-1,3,2-dioxathiolane-4-carboxylate 2-oxide

To a solution of benzyl (2*S*,3*R*)-2,3-dihydroxy-4-methylpentanoate (11.6 g, 48.7 mmol) in DCM (116 mL) at 0 °C was added Et_3N (20.3 mL, 146 mmol) and $SOCl_2$ (4.94 mL, 68.2 mmol). The reaction mixture was stirred 30 min then was diluted with DCM (100 mL) and H_2O (100 mL), extracted into DCM (3 x 100 mL), washed with brine (200 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure which afforded product (13.0 g, 94% yield).

Step 3: Synthesis of benzyl (4*S*,5*R*)-5-isopropyl-1,3,2-dioxathiolane-4-carboxylate 2,2-dioxide

To a solution of benzyl (4*S*,5*R*)-5-isopropyl-1,3,2-dioxathiolane-4-carboxylate 2-oxide (13 g, 45.7 mmol) in H_2O (290 mL), MeCN (145 mL), and CCl_4 (145 mL) was added $NaIO_4$ (3.80 mL, 68.6 mmol) and $RuCl_3$•H_2O (1.03 g, 4.57 mmol). The mixture was stirred at room temperature for 1 h then was diluted with DCM (500 mL) and H_2O (300 mL), filtered, and the filtrate was extracted into DCM (3 x 200 mL). The combined organic layers were washed sequentially with brine (500 mL) and sat. aq. Na_2CO_3 (300 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel chromatography (0→17% EtOAc/pet. ether) afforded product (11.5 g, 80% yield).

Step 4: Synthesis of benzyl (2*R*,3*R*)-2-bromo-3-hydroxy-4-methylpentanoate

To a solution of benzyl (4*S*,5*R*)-5-isopropyl-1,3,2-dioxathiolane-4-carboxylate 2,2-dioxide (11.5 g, 38.3 mmol) in THF (520 mL) was added LiBr (3.65 mL, 146 mmol). The reaction mixture

was stirred at room temperature for 5 h and then concentrated under reduced pressure. The residue was diluted in THF (130 mL) and H_2O (65 mL), cooled to 0 °C, then H_2SO_4 solution (20% aq., 1.3 L) was added and the mixture was warmed to room temperature and stirred for 24 h. The mixture was diluted with EtOAc (1.0 L), washed with Na_2CO_3 (sat. aq., 300 mL), then was concentrated under reduced pressure. Purification by silica gel chromatography (0→17% EtOAc/pet. ether) afforded product (10 g, 83% yield).

Step 5: Synthesis of benzyl (2*S*,3*R*)-2-azido-3-hydroxy-4-methylpentanoate

To a solution of benzyl (2*R*,3*R*)-2-bromo-3-hydroxy-4-methylpentanoate (10 g, 33.2 mmol) in DMSO (100 mL) was added NaN_3 (4.33 g, 66.6 mmol). The reaction mixture was stirred at room temperature for 12 h then was diluted with EtOAc (300 mL) and H_2O (200 mL). The mixture was extracted into EtOAc (2 x 200 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel chromatography (0→17% EtOAc/pet. ether) afforded product (7.5 g, 76% yield).

Step 6: Synthesis of benzyl (2*S*,3*S*)-3-isopropylaziridine-2-carboxylate

To a solution of benzyl (2*S*,3*R*)-2-azido-3-hydroxy-4-methylpentanoate (7.5 g, 28.5 mmol) in MeCN (150 mL) was added PPh_3 (7.70 g, 29.3 mmol). The reaction mixture was stirred at room temperature for 1 h and then heated to 70 °C and stirred for 3 h. The reaction mixture was concentrated under reduced pressure and purification by silica gel chromatography (0→17% EtOAc/pet. ether) afforded product (4.5 g, 64% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{17}NO_2$: 220.13; found 220.1.

Step 7: Synthesis of benzyl (2*S*,3*S*)-1-benzyl-3-isopropylaziridine-2-carboxylate

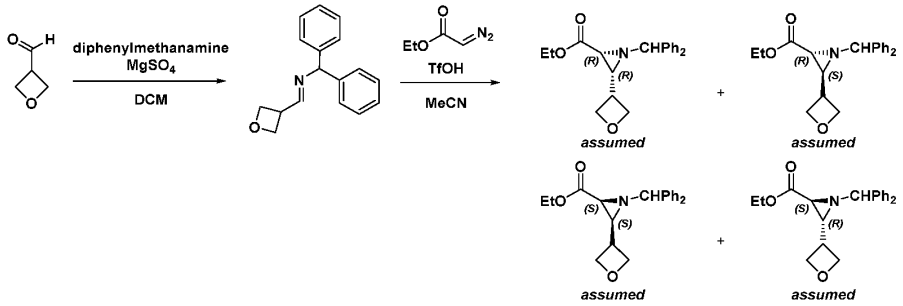
To a solution of benzyl (2*S*,3*S*)-3-isopropylaziridine-2-carboxylate (1 g, 4.56 mmol) in MeCN (10 mL) was added K_2CO_3 (3.15 g, 22.8 mmol) and benzyl bromide (812 µL, 6.84 mmol). The reaction mixture was stirred at room temperature for 6 h then was diluted with EtOAc (30 mL) and H_2O (30 mL), extracted into EtOAc (2 x 30 mL), washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by silica gel chromatography (0→17% EtOAc/pet. ether) afforded product (1.3 g, 89% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{20}H_{23}NO_2$: 310.18; found 310.1.

Step 8: Synthesis of (2*S*,3*S*)-1-benzyl-3-isopropylaziridine-2-carboxylic acid

To a solution of benzyl (2*S*,3*S*)-1-benzyl-3-isopropylaziridine-2-carboxylate (600 mg, 1.94 mmol) in THF (6 mL), MeCN (3 mL), and H_2O (6 mL) at 0°C was added $LiOH•H_2O$ (163 mg, 3.88 mmol). The reaction mixture was stirred at room temperature for 1 h and was adjusted to pH = 7-8 with HCl (0.5M). Lyophilization afforded product (750 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{13}H_{17}NO_2$: 220.13; found 220.1.

Intermediate A-98, A-99, A-100, and A-101. Synthesis of ethyl (2*R*,3*R*)-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate, ethyl (2*S*,3S)-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-

carboxylate, ethyl (2*R*,3*S*)-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate, and ethyl (2*S*,3R)-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate



Step 1: Synthesis of *N*-benzhydryl-1-(oxetan-3-yl)methanimine

To a solution oxetane-3-carbaldehyde (5.0 g, 58 mmol) and $MgSO_4$ (6.99 g, 58.1 mmol) in DCM (120 mL) at 0 °C was added diphenylmethanamine (12.1 mL, 69.7 mmol). The mixture was stirred for

12 h at room temperature then filtered and concentrated under reduced pressure to afford the desired compound (14 g, 95.9% yield) which was used without further purification.

Step 2: Synthesis of ethyl *cis*-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate and ethyl *trans*-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate

To a solution of *N*-benzhydryl-1-(oxetan-3-yl)methanimine (10 g, 39.79 mmol) in MeCN (150 mL) was added TfOH (878 mL, 9.95 mmol) and after 5 min ethyl diazoacetate (5.0 mL, 47.8 mmol) was added. The reaction mixture was stirred for 12 h at room temperature then cooled to 0 °C and quenched by the addition of saturated $NaHCO_3$ (300 mL). The aqueous layer was extracted with EtOAc (3 x 200 mL) and the combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by reverse phase chromatography (50→65% MeCN/H_2O, 10 mM NH_4HCO_3) afforded racemic ethyl *cis*-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate (1.1 g, 8.2% yield) and racemic ethyl *trans*-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate (780 mg, 5.8% yield)

Step 3: Separation of racemic ethyl *cis*-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate: ethyl (2*R*,3*R*)-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate and ethyl (2*S*,3*S*)-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate

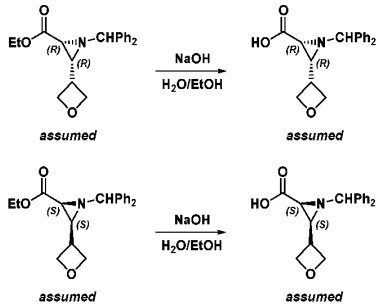
Racemic ethyl *cis*-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate (800 mg, 2.37 mmol) was separated by chiral prep-SFC (25% MeOH/CO_2) to afford ethyl (2*R*,3*R*)-1-benzhydryl-3-(oxetan-3-yl) aziridine-2-carboxylate (320 mg, 40% yield) and ethyl (2*S*,3*S*)-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate (320 mg,40% yield).

Step 4: Separation of racemic ethyl *trans*-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate: ethyl (2*R*,3*S*)-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate and ethyl (2*S*,3*R*)-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate

Racemic ethyl trans1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate (700 mg, 2.07 mmol) was separated by chiral prep-SFC (25% EtOH, 0.1% NH_4OH/CO_2) to afford ethyl (2*R*,3*S*)-1-

benzhydryl-3-(oxetan-3-yl) aziridine-2-carboxylate (300 mg, 42% yield) and ethyl (2*S*,3*R*)-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate (320 mg, 43% yield).

Intermediate A-102 and A-103. Synthesis of (2*R*,3*R*)-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylic acid and (2*S*,3*S*)-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylic acid



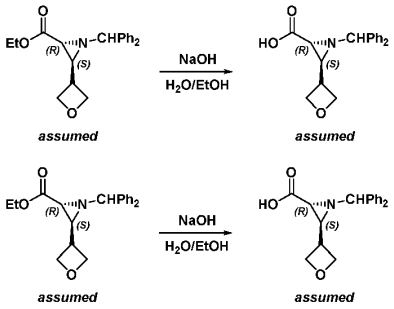
Step 1: Synthesis of (2*R*,3*R*)-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylic acid

To a solution of ethyl (2*R*,3*R*)-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate (156 mg, 463 mmol) in EtOH (3 mL) was added 2M NaOH (347 mL, 696 mmol). The reaction mixture was stirred for 3 h at room temperature and then concentrated under reduced pressure. The concentrate was acidified to pH 5 with 1M HCl and extracted with DCM (3 x 5 mL) and the combined organic layers were washed with brine, dried with Na_2SO_4, filtered and concentrated under reduced pressure to afford the desired compound (110 mg, 72.6% yield).

Step 2: Synthesis of (2*S*,3*S*)-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylic acid

To a solution of ethyl (2*S*,3*S*)-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate (150 mg, 444 mmol) in EtOH (5 mL) was added 2M NaOH (333 mL, 666 mmol). The reaction mixture was stirred for 3 h at room temperature and then acidified to pH 5 with 1M HCl. The aqueous layer extracted with DCM (3 x 10 mL) and the combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired compound (120 mg, 86.1% yield).

Intermediate A-104 and A-105. Synthesis of sodium (2*R*,3*S*)-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate and sodium (2*S*,3*R*)-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate



Step 1: Synthesis of sodium (2*R*,3*S*)-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate

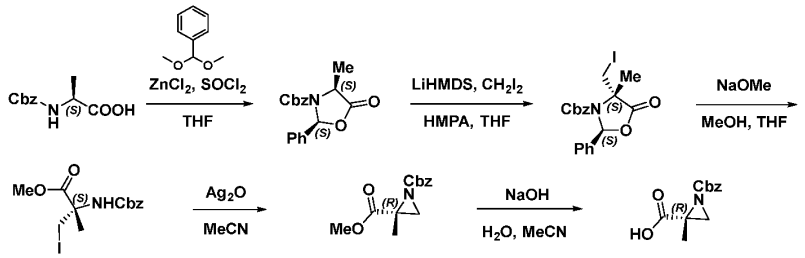
To a solution of ethyl (2*R*,3*S*)-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate (150 mg, 444 mmol) in EtOH (3 mL) was added 2M NaOH (333.42 mL, 666 mmol). The reaction mixture was stirred for

3 h at room temperature and then the pH was adjusted to pH 8 with 1M HCl. The resulting solution was lyophilized to afford the desired compound (165 mg, crude) which was used without further purification. LCMS (ESI) *m/z*: [M] calcd for $C_{19}H_{18}NO_3$: 308.13; found 308.0.

Step 2: Synthesis of sodium (2*S*,3*R*)-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate

To a solution of ethyl (2*S*,3*R*)-1-benzhydryl-3-(oxetan-3-yl)aziridine-2-carboxylate (170 mg, 503 mmol) in EtOH (3 mL) was added 2M NaOH (378 mL, 754 mmol). The reaction mixture was stirred for 3 h at room temperature and then the pH was adjusted to pH 8 with 1M HCl. The resulting solution was lyophilized to afford the desired compound (230 mg, crude) which was used without further purification. LCMS (ESI) *m/z*: [M] calcd for $C_{19}H_{18}NO_3$: 308.13; found 308.0.

Intermediate A-106. Synthesis of (*R*)-1-((benzyloxy)carbonyl)-2-methylaziridine-2-carboxylic acid



Step 1: Synthesis of benzyl (2*S*,4*S*)-4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate

To a mixture of ((benzyloxy)carbonyl)-*L*-alanine (25 g, 111.99 mmol) and (dimethoxymethyl)benzene (71.38 mL, 115.35 mmol) in THF (180 mL) was added $SOCl_2$ (8.94 g, 123.19 mmol) in one portion at 0 °C. The mixture was stirred for 10 min before $ZnCl_2$ (5.77 mL, 123.26 mmol) was added to the solution, then the mixture was stirred at 0 °C for 4 h. The reaction mixture was quenched by dropwise addition of cold H_2O and adjusted to pH = 5 with sat. $NaHCO_3$, then extracted with EtOAc (2 x 100 mL). The organic phase was washed with a aq. sat. $NaHCO_3$ (30 mL) and brine (30 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (1→10% EtOAc/pet. ether) to afford product (15 g, 43% yield).

Step 2: Synthesis of benzyl (2*S*,4*S*)-4-(iodomethyl)-4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate

HMPA (5.22 mL, 29.74 mmol) and LHMDS (1 M, 6.62 mL) were mixed in THF (45 mL) under N_2 atmosphere at 20 °C. This solution was cooled to −78 °C and a solution of benzyl (2*S*,4*S*)-4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate (2.0 g, 6.42 mmol) in THF (12 mL) was added dropwise with stirring. After stirring an additional 30 min, a solution of CH_2I_2 (1.55 mL, 19.27 mmol) in THF (6 mL) was added dropwise. The mixture was stirred at -78 °C for 90 min. The

mixture was warmed to 0 °C and quenched with sat. aq. NH_4Cl (70 mL). The mixture was extracted with EtOAc (2 x 30 mL), and the combined organic layers was washed with sat. aq. NH_4Cl (20 mL), H_2O (2 x 20 mL), and brine (30 mL) dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (1→20% EtOAc/pet. ether) to afford product (1.2 g, 41.4% yield).

Step 3: Synthesis of methyl (*S*)-2-(((benzyloxy)carbonyl)amino)-3-iodo-2-methylpropanoate

To a mixture of benzyl (2*S*,4*S*)-4-(iodomethyl)-4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate (1.2 g, 2.66 mmol) in THF (20 mL) was added a solution of NaOMe (957.69 mg, 5.32 mmol, 30% purity) in MeOH (9 mL) dropwise over 10 min at -40 °C under N_2. The mixture was stirred at -40 °C for 2 h, then warmed to -20 °C and stirred for 1 h. The reaction was quenched by addition of H_2O (20 mL), and the resulting mixture was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (1→20% EtOAc/pet. ether) to afford product (870 mg, 2.24 mmol, 84.4% yield).

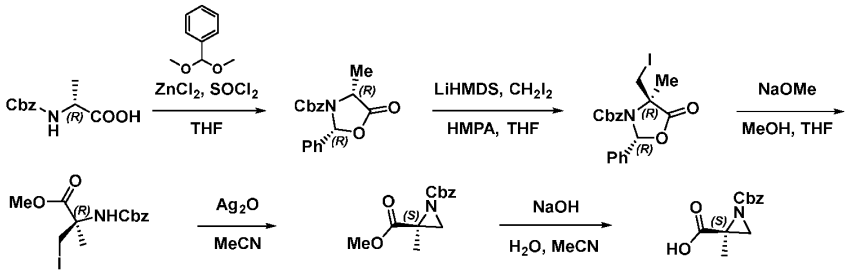
Step 4: Synthesis of 1-benzyl 2-methyl (*R*)-2-methylaziridine-1,2-dicarboxylate

To a mixture of methyl (*S*)-2-(((benzyloxy)carbonyl)amino)-3-iodo-2-methylpropanoate (0.87 g, 2.31 mmol) in MeCN (125 mL) was added Ag_2O (1.60 g, 6.92 mmol) in one portion at room temperature. The mixture was stirred at 90 °C for 30 min. The mixture was filtered and concentrated under reduced pressure to afford product (500 mg, 2.01 mmol, 86.9% yield).

Step 5: Synthesis of 1-benzyl 2-methyl (*R*)-2-methylaziridine-1,2-dicarboxylate

To a mixture of 1-benzyl 2-methyl (*R*)-2-methylaziridine-1,2-dicarboxylate (250 mg, 1.0 mmol) in MeCN (2.5 mL) and H_2O (2.5 mL) was added NaOH (40.12 mg, 1.0 mmol) in one portion at 0 °C under N_2. The mixture was stirred at 0 °C for 30 min. The mixture was concentrated under reduced pressure to afford crude product (256 mg, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{12}H_{12}NO_4$: 234.1; found 234.1.

Intermediate A-107. Synthesis of (*S*)-1-((benzyloxy)carbonyl)-2-methylaziridine-2-carboxylic acid



Step 1: Synthesis of benzyl (2*R*,4*R*)-4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate

Five batches were completed in parallel. To a mixture of ((benzyloxy)carbonyl)-*D*-alanine (5 g, 22.40 mmol) and (dimethoxymethyl)benzene (3.71 mL, 24.64 mmol) in THF (35 mL) was added $SOCl_2$ (1.79 mL, 24.64 mmol) in one portion at 0 °C. After the mixture was stirred for 10 min, $ZnCl_2$ (1.15 mL, 24.64 mmol) was added to the solution. Then the mixture was stirred at 0 °C for 4 h. The

give batches were combined and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (1→10% EtOAc/pet. ether) to afford product (20 g, 57.4% yield).

Step 2: Synthesis of benzyl (2*R*,4*R*)-4-(iodomethyl)-4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate

Four batches were completed in parallel. THF (300 mL), HMPA (13.06 mL, 74.34 mmol) and LHMDS (1 M, 16.54 mL) were mixed under N_2 atmosphere at 20 °C with stirring. The solution was cooled to −78 °C and a solution of benzyl (2*R*,4*R*)-4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate (5 g, 16.06 mmol) in THF (84 mL) was added dropwise. After stirring an additional 30 min, a solution of CH_2I_2 (3.89 mL, 48.18 mmol) in THF (33 mL) was added dropwise. The mixture was stirred at -78 °C for 90 min. The four batches were combined and warmed to 0 °C. Sat. aq. NH_4Cl (100 mL) was added to the combined solution and the resulting mixture was extracted with EtOAc (2 x 100 mL). The combined EtOAc layers was washed with sat. aq. NH_4Cl (50 mL), H_2O (2 x 20 mL), and brine (30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (1→17% EtOAc/pet. ether) to afford product (16 g, 55.2% yield).

Step 3: Synthesis of methyl (*R*)-2-(((benzyloxy)carbonyl)amino)-3-iodo-2-methylpropanoate

To a mixture of benzyl (2*R*,4*R*)-4-(iodomethyl)-4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate (16 g, 35.46 mmol) in THF (90 mL) was added NaOMe (12.77 g, 70.91 mmol, 30% purity) dropwise over 10 min at -40 °C under N_2. The mixture was stirred at -40 °C for 2 h, then warmed to -20 °C and stirred for 1 h. The reaction was quenched by addition of H_2O (100 mL), and the resulting mixture was extracted with diethyl ether (3×100 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (1→17% EtOAc/pet. ether) to afford product (10 g, 74.8% yield.

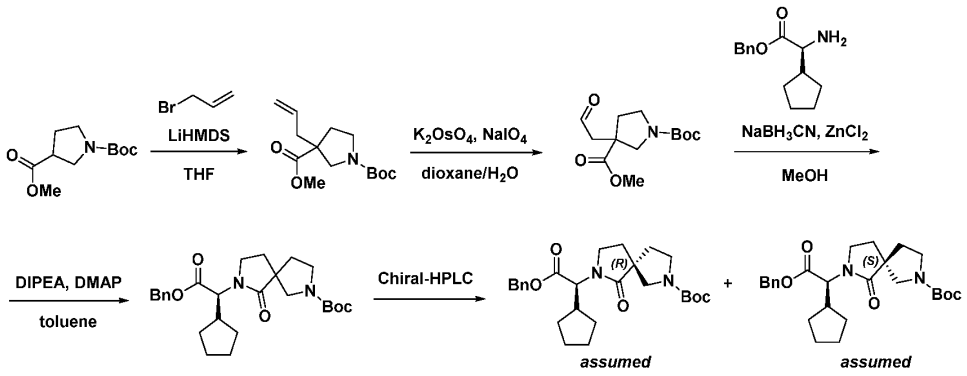
Step 4: Synthesis of 1-benzyl 2-methyl (*S*)-2-methylaziridine-1,2-dicarboxylate

Four batches were completed in parallel. To a mixture of methyl (*R*)-2-(((benzyloxy)carbonyl)amino)-3-iodo-2-methylpropanoate (8 g, 21.20 mmol) in MeCN (800 mL) was added Ag_2O (14.76 g, 63.64 mmol) in one portion at 20 °C. The mixture was stirred at 90 °C for 30 min. The four batches were combined, filtered, and concentrated under reduced pressure to afford product (5.1 g, 90.9% yield.

Step 5: Synthesis of (*S*)-1-((benzyloxy)carbonyl)-2-methylaziridine-2-carboxylic acid

To a solution of 1-benzyl 2-methyl (*S*)-2-methylaziridine-1,2-dicarboxylate (1 g, 4.01 mmol) in MeCN (5 mL) was added a solution of NaOH (240.69 mg, 6.02 mmol) in H_2O (5 mL) at 0 °C, then the mixture was stirred at 0 °C for 30 min. The mixture was lyophilized directly to afford crude product (1.05 g, crude). LCMS (ESI) *m/z*: [M + H] calcd for $C_{12}H_{12}NO_4$: 234.08; found 234.2.

Intermediates A-108 and A-109. Synthesis of *tert*-butyl (*R*)-7-((*S*)-2-(benzyloxy)-1-cyclopentyl-2-oxoethyl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate and *tert*-butyl (*S*)-7-((*S*)-2-(benzyloxy)-1-cyclopentyl-2-oxoethyl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate



Step 1: Synthesis of 1-(*tert*-butyl) 3-methyl 3-allylpyrrolidine-1,3-dicarboxylate

To a solution of 1-*(tert*-butyl) 3-methyl pyrrolidine-1,3-dicarboxylate (10.0 g, 43.6 mmol) in THF (100 mL) at -78 °C was added LiHMDS (65.0 mL, 65.4 mmol, 1 M in THF). After 1 h allyl bromide (5.63 mL, 65.4 mmol) was added and the resulting mixture was warmed to room temperature overnight. The reaction was quenched at 0 °C by the addition of NH_4Cl (200 mL). The aqueous layer was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by normal phase chromatography (5% EtOAc/pet. ether) afforded the desired product (10.0 g, 76.6% yield).

Step 3: Synthesis of 1-(*tert*-butyl) 3-methyl 3-(2-oxoethyl)pyrrolidine-1,3-dicarboxylate

To a solution of 1-(*tert*-butyl) 3-methyl 3-allylpyrrolidine-1,3-dicarboxylate (10.0 g, 37.1 mmol) and 2,6-dimethylpyridine (8.65 mL, 80.7 mmol) in dioxane (571 mL) and H_2O (142 mL) at 0 °C was added K_2OsO_4•$2H_2O$ (0.27 g, 0.73 mmol). After 15 min $NaIO_4$ (23.82 g, 111.4mmol) was added and the resulting mixture was stirred overnight at room temperature and then was diluted with H_2O (200 mL). The aqueous layer extracted with EtOAc (3 x 200 mL) and the combined organic layers were washed with 2 M HCl, dried with Na_2SO_4, filtered and concentrated under reduced pressure to afford the desired product (9.7 g, crude) which was used without further purification.

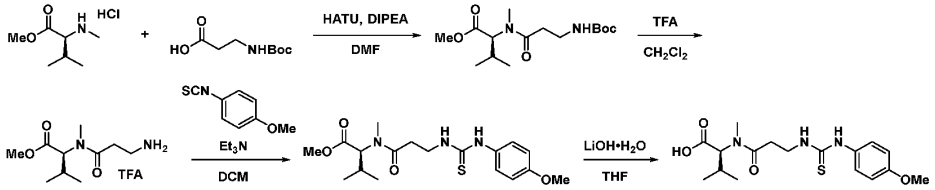
Step 4: Synthesis of 1-*(tert*-butyl) 3-methyl 3-(2-(((*S*)-2-(benzyloxy)-1-cyclopentyl-2-oxoethyl)amino)ethyl)pyrrolidine-1,3-dicarboxylate

To a solution of 1-(*tert*-butyl) 3-methyl 3-(2-oxoethyl)pyrrolidine-1,3-dicarboxylate (9.60 g, 35.4 mmol) in MeOH (100 mL) at 0 °C was added benzyl (*S*)-2-amino-2-cyclopentylacetate (12.38 g, 53.075 mmol) and zinc chloride (7.23 g, 53.1 mmol). After 30 min $NaBH_3CN$ (4.45 g, 70.8 mmol) was added and the resulting mixture stirred for 2 h at room temperature, concentrated under reduced pressure and the residue diluted with H_2O (150 mL). The aqueous layer was extracted with EtOAc (3 x 50 mL) and the combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and then concentrated under reduced pressure. Purification by normal phase chromatography (20% EtOAc/pet. ether) afforded the desired product (11.1 g, 64.2% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{27}H_{40}N_2O_6$: 489.30; found 489.3.

Step 5: Synthesis of *tert*-butyl (*R*)-7-((*S*)-2-(benzyloxy)-1-cyclopentyl-2-oxoethyl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate and *tert*-butyl (*S*)-7-((*S*)-2-(benzyloxy)-1-cyclopentyl-2-oxoethyl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate

To a solution of stirred solution of 1-*(tert*-butyl) 3-methyl 3-(2-(((*S*)-2-(benzyloxy)-1-cyclopentyl-2-oxoethyl)amino)ethyl)pyrrolidine-1,3-dicarboxylate (11.1 g, 22.7 mmol) in toluene (120 mL) was added DIPEA (39.6 mL, 227 mmol) and DMAP (2.78 g, 22.7 mmol). The resulting mixture was stirred for 2 days at 80 °C and then concentrated under reduced pressure. Purification by reverse phase chromatography (20→70% MeCN/H_2O, 0.1% HCO_2H) afforded a mixture of desired products. The diastereomers were separated by prep-SFC (30% EtOH/CO_2) to afford *tert*-butyl (*R*)-7-((*S*)-2-(benzyloxy)-1-cyclopentyl-2-oxoethyl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate (3.73 g, 44.4% yield) LCMS (ESI) *m/z*: [M + H] calcd for $C_{26}H_{36}N_2O_5$: 457.27; found 457.3 and *tert*-butyl (*S*)-7-((*S*)-2-(benzyloxy)-1-cyclopentyl-2-oxoethyl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate (3.87 g, 46.1% yield)) LCMS (ESI) *m/z*: [M + H] calcd for $C_{26}H_{36}N_2O_5$: 457.27; found 457.3.

Intermediate B-1. Synthesis of *N*-(3-(3-(4-methoxyphenyl)thioureido)propanoyl)-*N*-methyl-*L*-valine



Step 1: Synthesis of methyl *N*-(3-((*tert*-butoxycarbonyl)amino)propanoyl)-*N*-methyl-*L*-valinate

To a solution of 3-((*tert*-butoxycarbonyl)amino)propanoic acid (1.04 g, 5.50 mmol) in DMF (6 mL) was added DIPEA (2.38 mL, 13.7 mmol) followed by HATU (2.71 g, 7.15 mmol). The reaction mixture was stirred for 5 min and methyl methyl-*L*-valinate hydrochloride (1 g, 5.50 mmol) was added. The reaction was stirred at room temperature for 3 h and was then quenched with H_2O. The aqueous layer was extracted with EtOAc (3 x 10 mL) and the combined organic layers were washed with brine, and dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired crude product.

Step 2: Synthesis of methyl *N*-(3-aminopropanoyl)-*N*-methyl-*L*-valinate trifluoroacetic acid

To a solution of methyl *N*-(3-((*tert*-butoxycarbonyl)amino)propanoyl)-*N*-methyl-*L*-valinate (1.74 g, 5.50 mmol) in DCM (3 mL) was added TFA (2.09 mL, 27.4 mmol). The reaction was stirred at room temperature overnight and was then concentrated under reduced pressure to afford a solution of the desired crude product as a 33.5% solution in TFA.

Step 3: Synthesis of methyl *N*-(3-(3-(4-methoxyphenyl)thioureido)propanoyl)-*N*-methyl-*L*-valinate

To a 33.5 wt% solution of methyl *N*-(3-aminopropanoyl)-*N*-methyl-*L*-valinate trifluoroacetic acid (800 mg, 0.811 mmol) in TFA was added DCM (5 mL) followed by Et_3N (593 µL, 4.26 mmol)

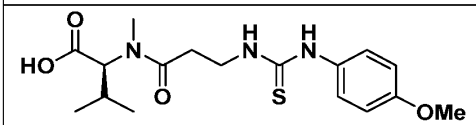
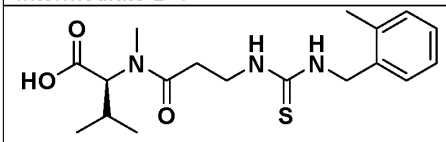
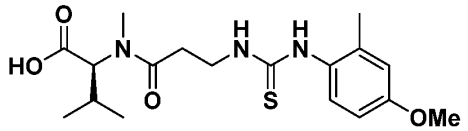
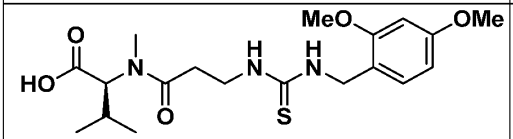
and 4-methoxyphenyl isothiocyanate (117.0 µL, 852 µmol). The reaction was stirred at room temperature for
3 h. The reaction mixture was then washed with H_2O (2 x 5 mL), aq. NH_4Cl (5 mL), and brine (5 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford the crude product (290.2 mg 89.2% yield) as an oil, which was taken on without purification. LCMS (ESI) *m/z:*
[M + H] calcd for $C_{18}H_{27}N_3O_4S$: 382.18; found 382.2.

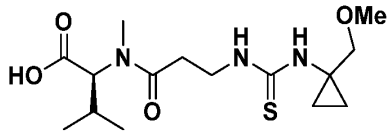
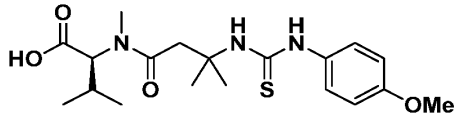
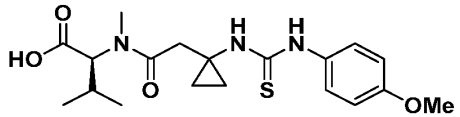
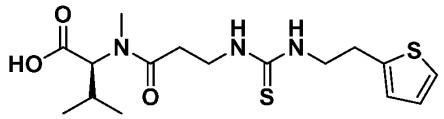
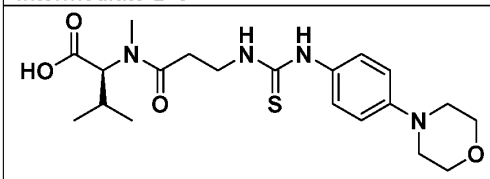
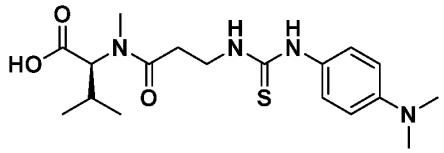
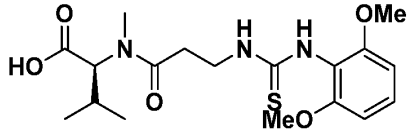
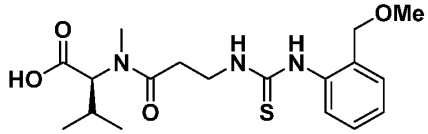
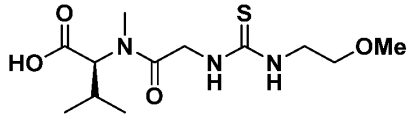
Step 4: Synthesis of *N*-(3-(3-(4-methoxyphenyl)thioureido)propanoyl)-*N*-methyl-*L*-valine

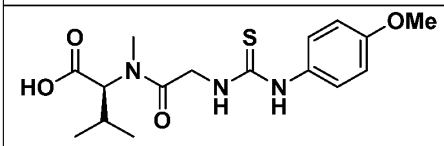
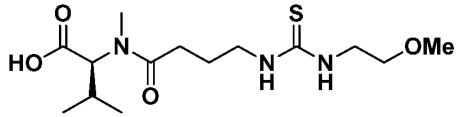
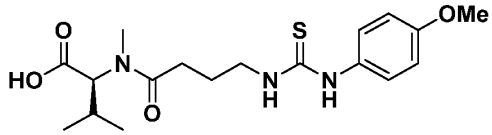
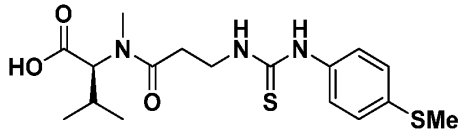
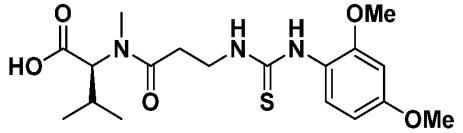
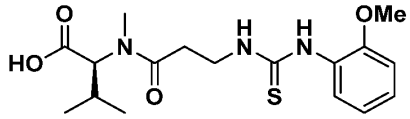
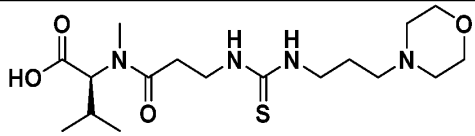
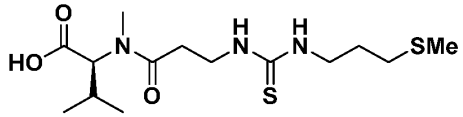
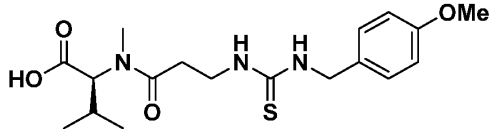
To a solution of methyl *N*-(3-(3-(4-methoxyphenyl)thioureido)propanoyl)-*N*-methyl-*L*-valinate (290.2 mg, 0.76 mmol) in THF (1 mL) was added a solution of $LiOH\bullet H_2O$ (41.4 mg, 0.99 mmol) in H_2O (300 µL). The reaction mixture was stirred overnight and was then acidified with HCl (4 M in dioxane,
120 µL, 0.48 mmol). The solution was then concentrated, the residue was dissolved in EtOAc, and the organic layer washed with H_2O (3 x 5 mL) and brine (5 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to afford the crude product (215.1 mg 77.0% yield), which was taken forward without further purification. LCMS (ESI) *m/z:* [M + H] calcd for $C_{17}H_{25}N_3O_4S$: 368.16; found 368.2.

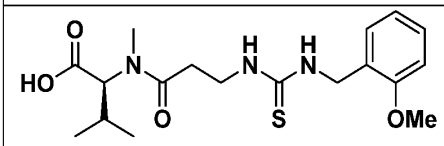
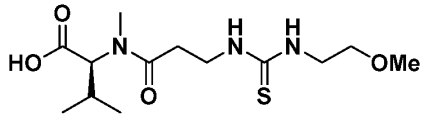
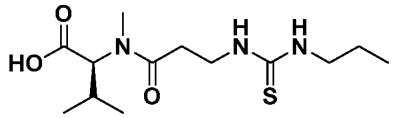
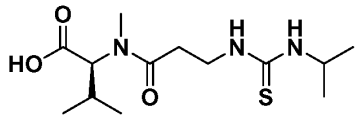
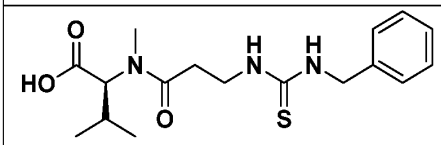
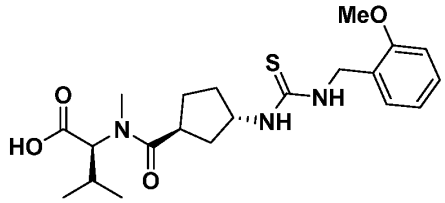
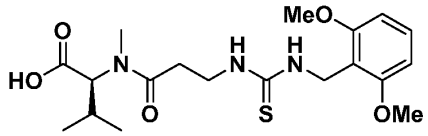
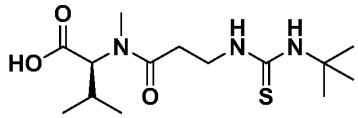
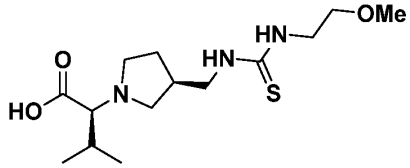
The following table of compounds were prepared using the methods or variations thereof used to synthesize Intermediate B-1.

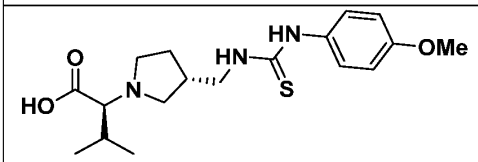
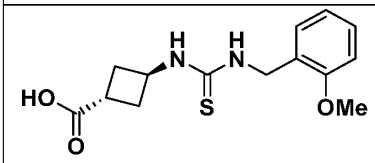
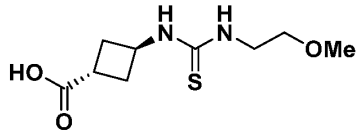
Table 3: Intermediate B

Structure	Molecular Formula	Calculated MW	Observed MW
 Intermediate B-1	$C_{17}H_{25}N_3O_4S$	[M + H] = 368.16	[M + H] = 368.5
 Intermediate B-2	$C_{18}H_{27}N_3O_3S$	[M + H] = 366.19	[M + H] = 366.6
 Intermediate B-3	$C_{18}H_{27}N_3O_4S$	[M + H] = 382.18	[M + H] = 382.5
 Intermediate B-4	$C_{19}H_{29}N_3O_5S$	[M + H] = 412.19	[M + H] = 412.6

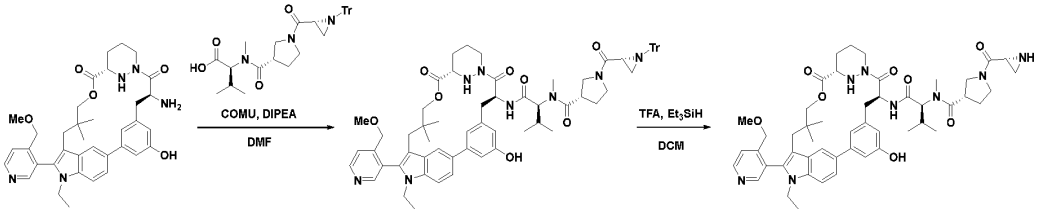
Structure	Molecular Formula	Calculated MW	Observed MW
 Intermediate B-5	$C_{15}H_{27}N_3O_4S$	[M + H] = 346.18	[M + H] = 346.6
 Intermediate B-6	$C_{19}H_{29}N_3O_4S$	[M + H] = 396.20	[M + H] = 396.6
 Intermediate B-7	$C_{19}H_{27}N_3O_4S$	[M + H] = 394.18	[M + H] = 394.7
 Intermediate B-8	$C_{16}H_{25}N_3O_3S_2$	[M + H] = 372.14	[M + H] = 327.6
 Intermediate B-9	$C_{20}H_{30}N_4O_4S$	[M + H] = 423.21	[M + H] = 423.6
 Intermediate B-10	$C_{18}H_{28}N_4O_3S$	[M + H] = 381.20	[M + H] = 381.6
 Intermediate B-11	$C_{18}H_{27}N_3O_5S$	[M + H] = 398.17	[M + H] = 398.6
 Intermediate B-12	$C_{18}H_{27}N_3O_4S$	[M + H] = 382.18	[M + H] = 382.6
 Intermediate B-13	$C_{12}H_{23}N_3O_4S$	[M + Na] = 328.13	[M + Na] = 328.5

Structure	Molecular Formula	Calculated MW	Observed MW
 Intermediate B-14	$C_{16}H_{23}N_3O_4S$	[M + H] = 354.15	[M + H] = 354.5
 Intermediate B-15	$C_{14}H_{27}N_3O_4S$	[M + H] = 334.18	[M + H] = 334.6
 Intermediate B-16	$C_{18}H_{27}N_3O_4S$	[M + H] = 382.18	[M + H] = 382.6
 Intermediate B-17	$C_{17}H_{25}N_3O_3S_2$	[M + H] = 384.14	[M + H] = 384.3
 Intermediate B-18	$C_{18}H_{27}N_3O_5S$	[M + H] = 398.17	[M + H] = 398.4
 Intermediate B-19	$C_{17}H_{25}N_3O_4S$	[M + H] = 368.16	[M + H] = 368.4
 Intermediate B-20	$C_{17}H_{32}N_4O_4S$	[M + H] = 389.22	[M + H] = 389.4
 Intermediate B-21	$C_{14}H_{27}N_3O_3S_2$	[M + H] = 350.16	[M + H] = 350.4
 Intermediate B-22	$C_{18}H_{27}N_3O_4S$	[M + H] = 382.18	[M + H] = 382.4

Structure	Molecular Formula	Calculated MW	Observed MW
 Intermediate B-23	$C_{18}H_{27}N_3O_4S$	[M + H] = 382.18	[M + H] = 382.4
 Intermediate B-24	$C_{13}H_{25}N_3O_4S$	[M + H] = 320.16	[M + H] = 320.3
 Intermediate B-25	$C_{13}H_{25}N_3O_3S$	[M + H] = 304.17	[M + H] = 304.5
 Intermediate B-26	$C_{13}H_{25}N_3O_3S$	[M + H] = 304.17	[M + H] = 304.3
 Intermediate B-27	$C_{17}H_{25}N_3O_3S$	[M + H] = 352.17	[M + H] = 352.4
 Intermediate B-28	$C_{21}H_{31}N_3O_4S$	[M + H] = 422.21	[M + H] = 422.7
 Intermediate B-29	$C_{19}H_{29}N_3O_5S$	[M + H] = 412.19	[M + H] = 412.2
 Intermediate B-30	$C_{14}H_{27}N_3O_3S$	[M + H] = 318.19	[M + H] = 318.6
 Intermediate B-31	$C_{14}H_{27}N_3O_3S$	[M + H] = 318.19	[M + H] = 318.7

Structure	Molecular Formula	Calculated MW	Observed MW
 Intermediate B-32	$C_{18}H_{27}N_3O_3S$	[M + H] = 366.19	[M + H] = 366.7
 Intermediate B-33	$C_{14}H_{18}N_2O_3S$	[M + H] = 295.11	[M + H] = 295.6
 Intermediate B-34	$C_9H_{16}N_2O_3S$	[M + H] = 233.10	[M + H] = 233.4

Example 1. Synthesis of (3*S*)-1-((*R*)-aziridine-2-carbonyl)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylpyrrolidine-3-carboxamide



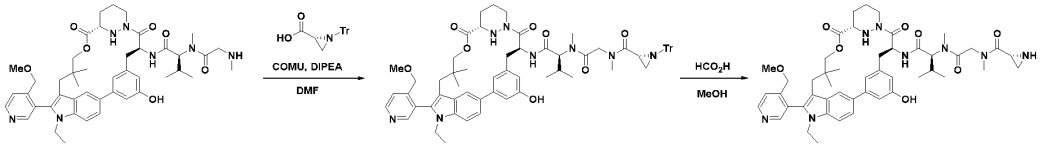
Step 1: Synthesis of (3*S*)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methyl-1-((*R*)-1-tritylaziridine-2-carbonyl)pyrrolidine-3-carboxamide

To a solution of (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (520.0 mg, 0.831 mmol) and *N*-methyl-*N*-((*S*)-1-((*R*)-1-tritylaziridine-2-carbonyl)pyrrolidine-3-carbonyl)-*L*-valine (0.6727 g, 1.25 mmol) in DMF (10 mL) at 0 °C was added COMU (0.5338 mg, 1.25 mmol) followed by DIPEA (1.16 mL, 6.65 mmol). After 2 h, the reaction mixture was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with brine (3 x 30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by reverse phase chromatography (10→50% MeCN/H_2O) to afford the desired product (500 mg, 52.4% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{69}H_{78}N_8O_8$: 1147.60; found 1147.8.

Step 2: Synthesis of (3*S*)-1-((*R*)-aziridine-2-carbonyl)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylpyrrolidine-3-carboxamide

To a stirred solution of (3*S*)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methyl-1-((*R*)-1-tritylaziridine-2-carbonyl)pyrrolidine-3-carboxamide (145.0 mg, 0.126 mmol) in DCM (3 mL) at 0 °C was added Et_3SiH (58.8 mg, 0.505 mmol) followed by TFA (57.6 mg, 0.505 mmol). After 1 h, DIPEA was added to the reaction mixture until pH 8. The resulting mixture was concentrated under reduced pressure, and the residue was purified by reverse phase chromatography (10→50% MeCN/H_2O) to afford the desired product (70 mg, 61.2% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{50}H_{64}N_8O_8$: 905.49; found 905.7.

Example 7. Synthesis of (2*R*)-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methylaziridine-2-carboxamide



Step 1: Synthesis of (2*R*)-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methyl-1-tritylaziridine-2-carboxamide

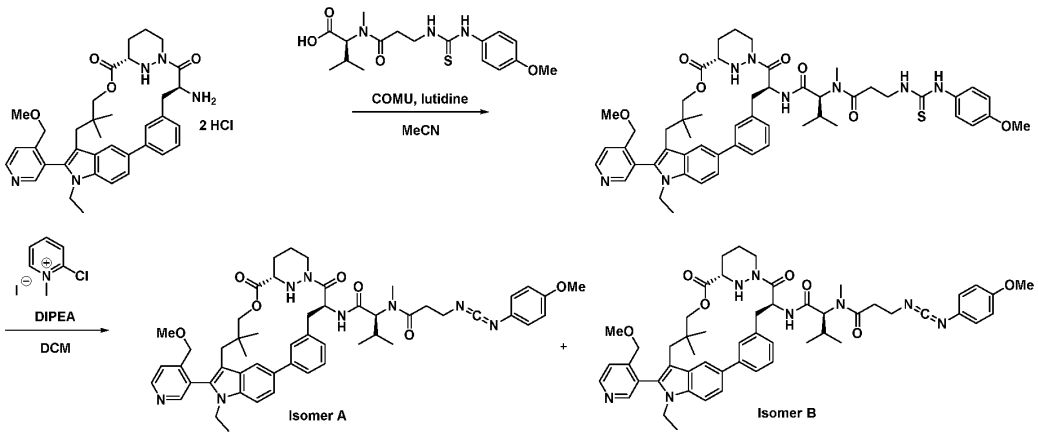
To a solution of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(*N*-methyl-2-(methylamino)acetamido)butanamide (285.7 mg, 0.353 mmol) in DMF (3.0 mL) at 0 °C was added (*R*)-1-tritylaziridine-2-carboxylic acid (232.4 mg, 0.705 mmol) followed by DIPEA (0.61 mL, 4.7 mmol) and COMU (211.4 mg, 0.494 mmol). The resulting mixture was warmed to room temperature and stirred for 1 h. The reaction mixture was diluted with H_2O (15 mL) and the mixture was extracted with EtOAc (3 x 4 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by prep-TLC (12% EtOAc/pet.

ether) to afford the desired product (301 mg, 68% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{67}H_{76}N_8O_8$: 1121.59; found 1121.8.

Step 2: Synthesis of (2*R*)-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methylaziridine-2-carboxamide

To a solution of (2*R*)-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methyl-1-tritylaziridine-2-carboxamide (301.0 mg, 0.268 mmol) in MeOH (3.0 mL) at 0 °C was added HCO_2H (1.50 mL). The reaction mixture was stirred for 1 h and then neutralized to pH 8 with DIPEA. The resulting mixture was diluted with H_2O (15 mL) and extracted with EtOAc (3 x 4 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by reverse phase chromatography (30→60% MeCN/H_2O) to afford the desired product (89.9 mg, 38% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{48}H_{62}N_8O_8$: 879.48; found 879.7.

Example 15. Synthesis of two isomers, 15A and 15B, of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-2-(3-((((4-methoxyphenyl)imino)methylene)amino)-*N*-methylpropanamido)-3-methylbutanamide



Step 1: Synthesis of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-2-(3-(3-(4-methoxyphenyl)thioureido)-*N*-methylpropanamido)-3-methylbutanamide

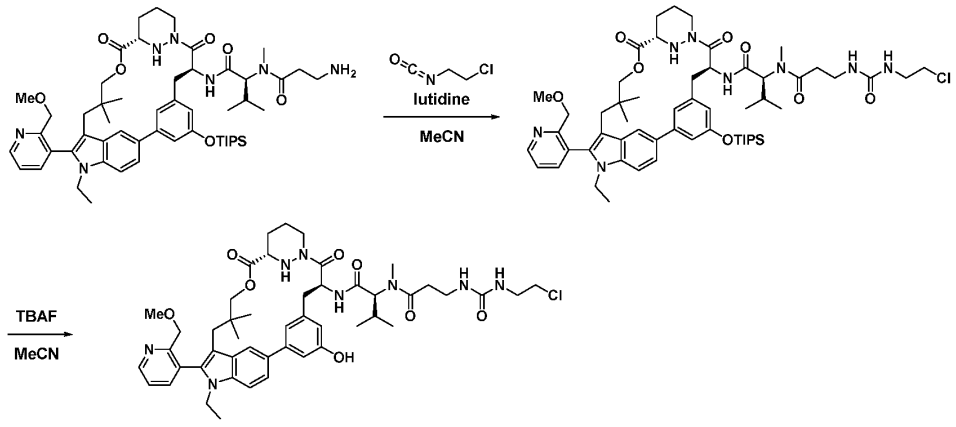
To a solution of (6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (108 mg, 168 µmol) and *N*-(3-(3-(4-

methoxyphenyl)thioureido)propanoyl)-*N*-methyl-*L*-valine (61.9 mg, 168 µmol) in MeCN (2 mL) at 0 °C was added 2,6-lutidine (97.8 µL, 840 µmol) followed by COMU (78.8 mg, 184 µmol). After 1 h at 0 °C the reaction was diluted with EtOAc and the organic portion washed with H_2O (15 mL) and brine (15 mL), dried over Na_2SO_4, and concentrated under reduced pressure. Purification by silica gel chromatography (20→100% EtOAc/Hex then 0→5% MeOH/EtOAc) afforded the desired product (117.0 mg 72.6% yield). LCMS (ESI) *m/z:* [M + H] calcd for $C_{53}H_{66}N_8O_7S$: 959.49; found 959.5.

Step 2: Synthesis of two isomers of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-2-(3-((((4-methoxyphenyl)imino)methylene)amino)-*N*-methylpropanamido)-3-methylbutanamide

To a solution of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-2-(3-(3-(4-methoxyphenyl)thioureido)-*N*-methylpropanamido)-3-methylbutanamide (117.0 mg, 121 µmol) in DCM (1 mL) was added DIPEA (63.2 µL, 363 µmol) followed by 2-chloro-1-methylpyridin-1-ium iodide (42.6 mg, 181 µmol). The reaction mixture was stirred overnight, at which point the solid was filtered and the crude solution was purified by reverse phase chromatography (40→100 MeCN/H_2O + 0.4% NH_4OH) to afford two separated isomers as the desired earlier eluting isomer 15A (6.9 mg, 6.2% yield) and later eluting isomer 15B (2.5 mg, 2.2% yield). LCMS (ESI) *m/z:*
[M + H] calcd for $C_{53}H_{64}N_8O_7$: 925.50; found 925.5 and LCMS (ESI) *m/z:* [M + H] calcd for $C_{53}H_{64}N_8O_7$: 925.50; found 925.6.

Example 25. Synthesis of (2*S*)-2-(3-(3-(2-chloroethyl)ureido)-*N*-methylpropanamido)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide



Step 1: Synthesis of (2*S*)-2-(3-(3-(2-chloroethyl)ureido)-*N*-methylpropanamido)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-

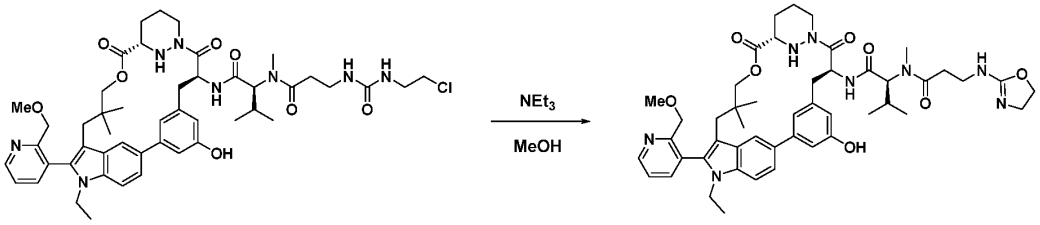
((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide

To a solution of (2*S*)-2-(3-amino-*N*-methylpropanamido)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide (106 mg, 109 µmol) in MeCN (544 µL) at 0 °C was added 1-chloro-2-isocyanatoethane (9.29 µL, 109 µmol) followed by Et_3N (15.1 µL, 109 µmol). After 12 min, the reaction was diluted with DCM (10 mL) and a solution of 1% formic acid in H_2O (10 mL). The aqueous layer was extracted with DCM (10 mL) and the combined organic layers were dried over Na_2SO_4, filtered, and then concentrated under reduced pressure to afford the desired product (117 mg, 100% yield), which was used in the next step without purification. LCMS (ESI) *m/z:* [M + H] calcd for $C_{57}H_{83}ClN_8O_8Si$: 1071.59; found 1071.5.

Step 2: Synthesis of (2*S*)-2-(3-(3-(2-chloroethyl)ureido)-*N*-methylpropanamido)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide

To a solution of (2*S*)-2-(3-(3-(2-chloroethyl)ureido)-*N*-methylpropanamido)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide (117 mg, 109 µmol) in MeCN (1.1 mL) at 0 °C was added TBAF (1M in dioxane,
109 µL, 109 µmol). After 5 min, the reaction was concentrated under reduced pressure and the crude residue was purified by normal phase chromatography (20→100% B/A, B=10% MeOH/EtOAc, A=hexanes) followed by reverse phase chromatography (20→60% MeCN/H_2O) to afford the final product (82.2 mg, 82% yield). LCMS (ESI) *m/z:* [M + H] calcd for $C_{48}H_{63}ClN_8O_8$: 915.45; found 915.7.

Example 30. Synthesis of (2*S*)-2-(3-((4,5-dihydrooxazol-2-yl)amino)-*N*-methylpropanamido)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide

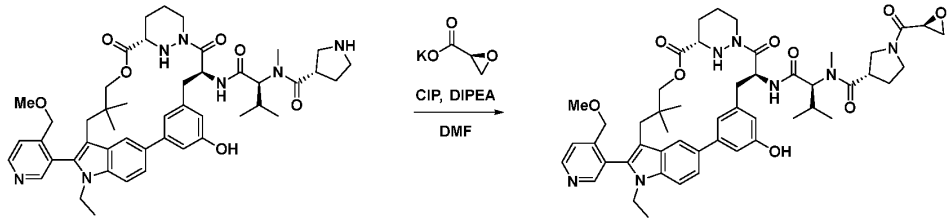


A solution of (2*S*)-2-(3-(3-(2-chloroethyl)ureido)-*N*-methylpropanamido)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-

hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide

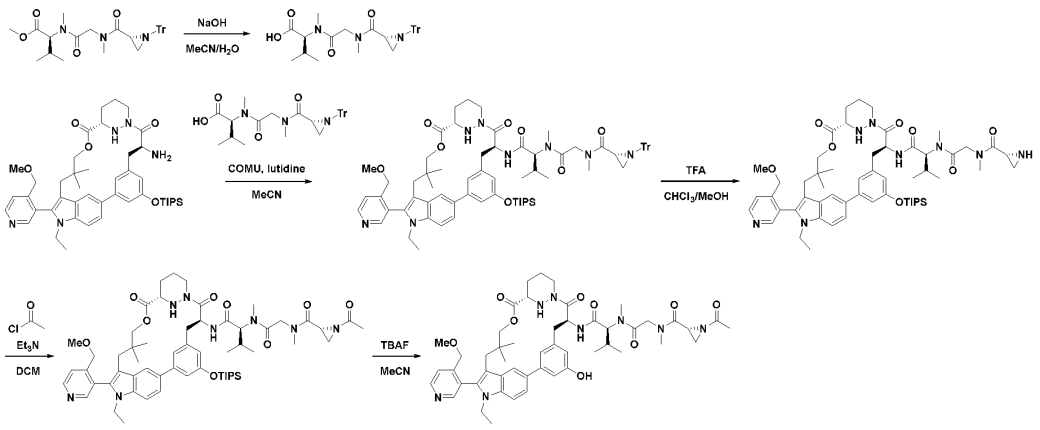
(55.0 mg, 60.0 µmol) and Et_3N (25.1 µL, 180 µmol) in MeOH (1.2 mL) was heated in the microwave at 150 °C for 1 min. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The crude residue was then purified by reverse phase chromatography (30→100% MeCN/H_2O + 0.4% NH_4OH) to afford the final product (21.1 mg, 40% yield). LCMS (ESI) *m/z:* [M + H] calcd for $C_{48}H_{62}N_8O_8$: 879.48; found 879.4.

Example 31. Synthesis of (3*S*)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methyl-1-((*S*)-oxirane-2-carbonyl)pyrrolidine-3-carboxamide



To a solution of potassium (*S*)-oxirane-2-carboxylate (16.98 mg, 0.135 mmol), 2-chloro-1,3-dimethylimidazolidinium hexafluorophosphate (87.46 mg, 0.314 mmol), and DIPEA (0.156 mL, 0.897 mmol) in DMF (1.5 mL) at 0 °C was added (3*S*)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylpyrrolidine-3-carboxamide (75.0 mg, 0.09 mmol). The resulting mixture was stirred overnight at room temperature, at which point it was diluted with EtOAc (100 mL). The organic layer was washed with brine (3 x 5 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by reverse phase chromatography (25→55% MeCN/H_2O) afforded the desired product (6.3 mg, 7.8% yield) as a solid. LCMS (ESI) *m/z:* [M + H] calcd for $C_{50}H_{63}N_7O_9$: 906.48; found 906.7.

Example 34. Synthesis of (2*R*)-1-acetyl-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-N-methylaziridine-2-carboxamide



Step 1: Synthesis of (2*R*)-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methyl-1-tritylaziridine-2-carboxamide

To a solution of (6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (260 mg, 0.332 mmol) and *N*-methyl-*N*-(*N*-methyl-*N*-((*R*)-1-tritylaziridine-2-carbonyl)glycyl)-*L*-valine (204 mg, 0.399 mmol) in MeCN (3.3 mL) at 0 °C was added lutidine (192 µL, 1.66 mmol) followed by COMU (156 mg, 0.366 mmol). The reaction stirred at 0 °C for 1 h and was then diluted with EtOAc. The mixture was washed with H_2O/brine (1:1), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by normal phase chromatography (0→100% EtOAc/hexanes) afforded the desired product (116 mg, 27% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{76}H_{96}N_8O_8Si$: 1277.72; found 1277.7.

Step *2*: Synthesis of (2*R*)-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methylaziridine-2-carboxamide

To a solution of (2*R*)-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methyl-1-tritylaziridine-2-carboxamide (400 mg, 0.313 mmol) in MeOH (1.56 mL) and chloroform (1.56 mL) at 0 °C was added TFA (191 µL, 2.50 mmol). The reaction stirred at 0 °C for 2 h and was then quenched with lutidine (364 µL, 3.13 mmol). The reaction mixture was diluted with DCM, washed with H_2O, and concentrated under reduced pressure. Purification by reverse phase chromatography (10→100% MeCN/H_2O) afforded the desired product (100 mg, 31% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{57}H_{82}N_8O_8Si$: 1035.61; found 1035.6.

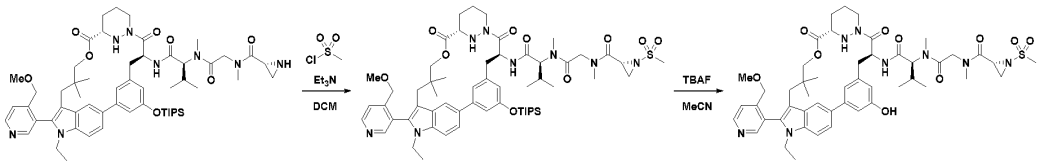
Step *3*: Synthesis of (2*R*)-1-acetyl-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methylaziridine-2-carboxamide

To a solution of (2*R*)-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methylaziridine-2-carboxamide (33 mg, 0.032 mmol) in DCM (637 µL) at 0 °C was added Et_3N (22.1 µL, 0.159 mmol) followed by acetyl chloride (4.54 µL, 0.064 mmol). The reaction stirred at 0 °C for 1 h. The reaction was then diluted with DCM, washed with $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired crude product (37 mg, 100% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{59}H_{84}N_8O_9Si$: 1077.62; found 1077.6.

Step *4*: Synthesis of (2*R*)-1-acetyl-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methylaziridine-2-carboxamide

To a solution of (2*R*)-1-acetyl-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methylaziridine-2-carboxamide (34 mg, 0.032 mmol) in MeCN (631 µL) at 0 °C was added TBAF (1M in THF, 31.5 µL, 0.032 mmol). The reaction stirred for 10 min and was then diluted with DCM, washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by reverse phase chromatography (10→100% MeCN/H_2O) afforded the desired product (8.5 mg, 29% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{50}H_{64}N_8O_9$: 921.49; found 921.5.

Example 36. Synthesis of (2*R*)-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methyl-1-(methylsulfonyl)aziridine-2-carboxamide



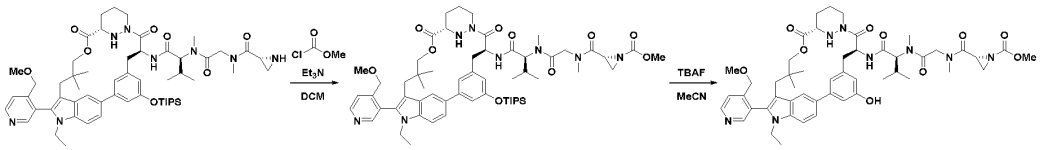
Step 1: Synthesis of (2*R*)-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methyl-1-(methylsulfonyl)aziridine-2-carboxamide

To a solution of (2*R*)-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methylaziridine-2-carboxamide (33 mg, 0.032 mmol) in DCM (637 µL) at 0 °C was added Et_3N (22.1 µL, 0.159 mmol) followed by methanesulfonyl chloride (4.93 µL, 0.064 mmol). The reaction was cooled to 0 °C for 1 h and was then diluted with DCM, washed with $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired crude product (35 mg, 100% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{58}H_{84}N_8O_{10}SSi$: 1113.59; found 1113.6.

Step 2: Synthesis of (2*R*)-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methyl-1-(methylsulfonyl)aziridine-2-carboxamide

To a solution of (2*R*)-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methyl-1-(methylsulfonyl)aziridine-2-carboxamide (35 mg, 0.032 mmol) in MeCN (646 µL) at 0 °C was added TBAF (1M in THF, 32.3 µL, 0.032 mmol). The reaction stirred for 10 min and was then diluted with DCM, washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by reverse phase chromatography (10→100% MeCN/H_2O) afforded the desired product (20 mg, 65% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{49}H_{64}N_8O_{10}S$: 957.45; found 957.5.

Example 38. Synthesis of methyl (2*R*)-2-((2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)(methyl)carbamoyl)aziridine-1-carboxylate



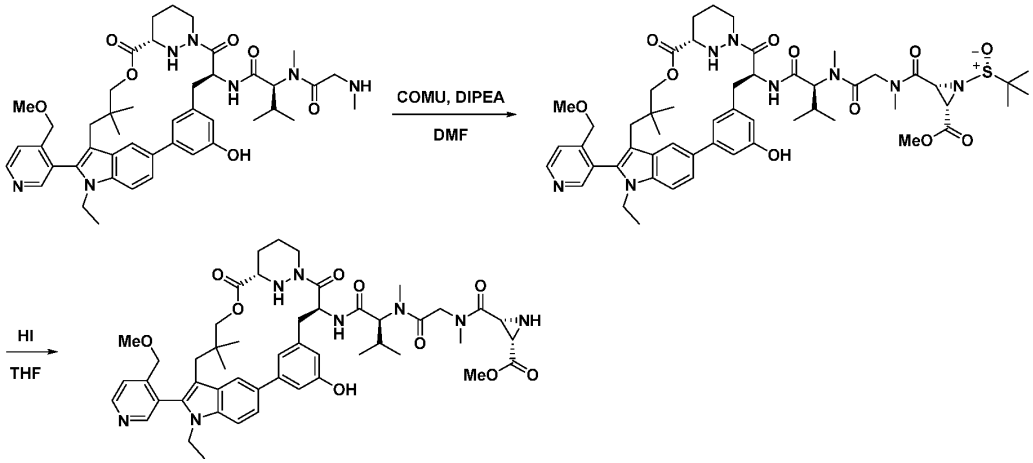
Step 1: Synthesis of methyl (2*R*)-2-((2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)(methyl)carbamoyl)aziridine-1-carboxylate

To a solution of (2*R*)-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methylaziridine-2-carboxamide (46 mg, 0.044 mmol) in DCM (888 µL) at 0 °C was added E_3N (30.8 µL, 0.22 mmol) followed by methyl chloroformate (4.46 µL, 0.058 mmol). The reaction stirred at 0 °C for 1 h and then the reaction was diluted with DCM, washed with $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired crude product (56 mg, 100% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{59}H_{84}N_8O_{10}Si$: 1093.62; found 1093.7.

Step 2: Synthesis of methyl (2*R*)-2-((2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)(methyl)carbamoyl)aziridine-1-carboxylate

To a solution of methyl (2*R*)-2-((2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)(methyl)carbamoyl)aziridine-1-carboxylate (56 mg, 0.051 mmol) in MeCN (1.0 mL) at 0 °C was added TBAF (1M in THF, 51.2 µL, 0.051 mmol). The reaction stirred for 15 min and was then diluted with DCM, washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by reverse phase chromatography (10→100% MeCN/H_2O) afforded the desired product (17 mg, 36% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{50}H_{64}N_8O_{10}$: 937.48; found 937.6.

Example 48 and 49. Synthesis of methyl (2*S*,3*R*)-1-((*R*)-*tert*-butylsulfinyl)-3-((2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)(methyl)carbamoyl)aziridine-2-carboxylate and methyl (2*S*,3*R*)-3-((2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)(methyl)carbamoyl)aziridine-2-carboxylate



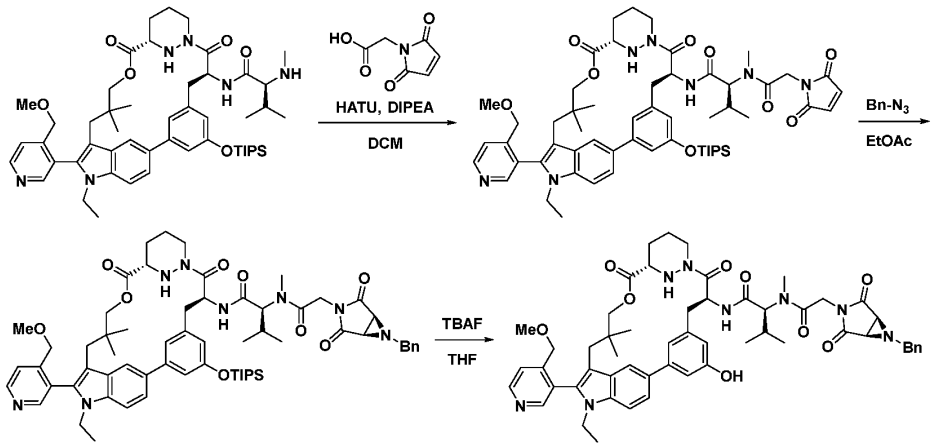
Step 1: Synthesis of methyl (2*S*,3*R*)-1-((*R*)-*tert*-butylsulfinyl)-3-((2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)(methyl)carbamoyl)aziridine-2-carboxylate

To a solution of (2*S*)-*N*-((63*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(*N*-methyl-2-(methylamino)acetamido)butanamide (267.0 mg, 0.33 mmol) and (2*R*,3*S*)-1-((*R*)-*tert*-butylsulfinyl)-3-(methoxycarbonyl)aziridine-2-carboxylic acid (246.5 mg, 0.99 mmol) in DMF (4.5 mL) at 0 °C was added DIPEA (0.574 mL, 3.3 mmol) followed by a solution of COMU (211.8 mg, 0.49 mmol) in DMF (0.5 mL). The resulting mixture was stirred for 1 h at 0 °C and was then quenched with sat. NH_4Cl. The aqueous layer was extracted with EtOAc (3 x 20 mL) and the combined organic layers were washed with brine (2 x 50 mL), dried over Na_2SO_4, filtered, concentrated under reduced pressure. The crude product was purified by reverse phase chromatography (35→65% MeCN/H_2O) to afford the desired product (253 mg,73.7% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{54}H_{72}N_8O_{11}S$: 1041.51; found 1041.8.

Step 2: Synthesis of methyl (2*S*,3*R*)-3-((2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)(methyl)carbamoyl)aziridine-2-carboxylate

To a solution of methyl (2*S*,3*R*)-1-((*R*)-*tert*-butylsulfinyl)-3-((2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)(methyl)carbamoyl)aziridine-2-carboxylate (200.0 mg, 0.19 mmol) in THF (4.0 mL) at 0 °C was added HI (1.0 mL), dropwise. The resulting mixture was stirred for 10 min at 0 °C and was then basified to pH 7 with DIPEA. The mixture was extracted with EtOAc (3 x 30 mL) and the combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by reverse phase chromatography (35→65% MeCN/H_2O) to afford the desired product (13.2 mg, 7.3% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{50}H_{64}N_8O_{10}$: 937.48; found 938.6.

Example 55. Synthesis of (2*S*)-2-(2-((1*R*,5*S*)-6-benzyl-2,4-dioxo-3,6-diazabicyclo[3.1.0]hexan-3-yl)-*N*-methylacetamido)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide



Step 1: Synthesis of (2*S*)-2-(2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N*-methylacetamido)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide

To a solution of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide (600.0 mg, 0.67 mmol) and 2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)acetic acid (124.7 mg, 0.80 mmol) in DCM (6.0 mL) at 0 °C was added DIPEA (0.934 mL, 5.36 mmol) followed by HATU (382.2 mg, 1.01 mmol). The reaction mixture was warmed to room temperature and stirred for 3 h. The reaction was then quenched by the addition of H_2O (20 mL). The aqueous layer was extracted with DCM (2 x 50 mL) and the combined organic layers were washed with brine (2 x 50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by normal phase chromatography (10→20% EtOAc/pet. ether) to afford the desired product (260 mg, 33.8% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{57}H_{77}N_7O_9Si$: 1032.56; found 1032.8.

Step 2: Synthesis of (2*S*)-2-(2-((1*R*,5*S*)-6-benzyl-2,4-dioxo-3,6-diazabicyclo[3.1.0]hexan-3-yl)-*N*-methylacetamido)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide

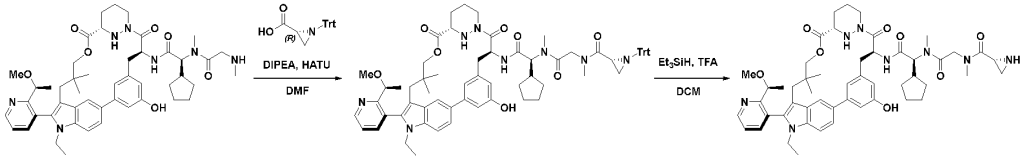
To a solution of (2*S*)-2-(2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-*N*-methylacetamido)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide (250.0 mg, 0.24 mmol) in EtOAc (2.0 mL) was added (azidomethyl)benzene (80.6 mg, 0.61 mmol). The reaction mixture was heated to 80 °C and stirred for 2 h. The reaction mixture was then heated to 120 °C and stirred for 2 days. The

reaction mixture was then cooled to room temperature and quenched with H_2O. The aqueous layer was extracted with EtOAc and the combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by normal phase chromatography to afford the desired product (50 mg, 18.1% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{64}H_{84}N_8O_9Si$: 1137.62; found 1138.3.

Step 3: Synthesis of (2*S*)-2-(2-((1*R*,5*S*)-6-benzyl-2,4-dioxo-3,6-diazabicyclo[3.1.0]hexan-3-yl)-*N*-methylacetamido)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide

To a solution of (2*S*)-2-(2-((1*R*,5*S*)-6-benzyl-2,4-dioxo-3,6-diazabicyclo[3.1.0]hexan-3-yl)-*N*-methylacetamido)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(4-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide (50.0 mg, 0.04 mmol) in THF (0.5 mL) at 0 °C was added 1M TBAF (0.07 mL, 0.07 mmol). The reaction mixture was stirred for 1 h. The reaction mixture was then diluted with H_2O and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by prep-TLC followed by reverse phase chromatography (45→72% MeCN/H_2O) to afford the desired product (20 mg, 46.4% yield). LCMS (ESI) *m/z*: [M + Na] calcd for $C_{55}H_{64}N_8O_9$: 1003.47; found 1003.8.

Example 95. Synthesis of (2*R*)-*N*-(2-(((1*S*)-1-cyclopentyl-2-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-2-oxoethyl)(methyl)amino)-2-oxoethyl)-*N*-methylaziridine-2-carboxamide



Step 1: Synthesis of (2*R*)-*N*-(2-(((1*S*)-1-cyclopentyl-2-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-2-oxoethyl)(methyl)amino)-2-oxoethyl)-*N*-methyl-1-tritylaziridine-2-carboxamide

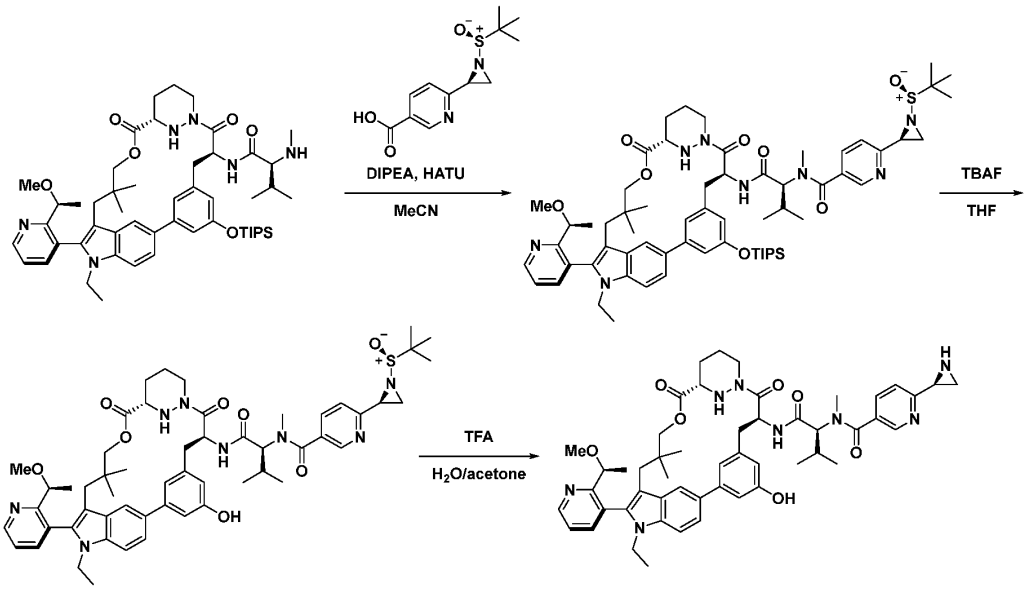
To a mixture of (2*S*)-2-cyclopentyl-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-2-(*N*-methyl-2-(methylamino)acetamido)acetamide (321.2 mg, 0.276 mmol), DIPEA (0.472 mL, 2.764 mmol), and (*R*)-1-tritylaziridine-2-carboxylic acid (136.59 mg, 0.415 mmol) in DMF (3.0 mL) at 0 °C was added HATU (126.14 mg, 0.332 mmol). The resulting mixture was stirred at 0 °C for 30 min, then diluted with H_2O (30 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (3 x 10 mL), dried with Na_2SO_4, filtered, and concentrated under reduced

pressure. Purification by prep-TLC (50% EtOAc/pet. ether) afforded the desired product (200 mg, 62.3% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{70}H_{80}N_8O_8$: 1161.62; found 1161.5.

Step 2: Synthesis of (2*R*)-*N*-(2-(((1*S*)-1-cyclopentyl-2-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-2-oxoethyl)(methyl)amino)-2-oxoethyl)-*N*-methylaziridine-2-carboxamide

To a mixture of (2*R*)-*N*-(2-(((1*S*)-1-cyclopentyl-2-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-2-oxoethyl)(methyl)amino)-2-oxoethyl)-*N*-methyl-1-tritylaziridine-2-carboxamide (195.0 mg, 0.168 mmol) in DCM (2.0 mL) at 0 °C was added Et_3SiH (78.09 mg, 0.672 mmol) and TFA (76.57 mg, 0.672 mmol). The resulting mixture was stirred at 0 °C for 30 min then basified to pH 8 with DIPEA and concentrated under reduced pressure. Purification by reverse phase chromatography (25→55% MeCN/H_2O) to afford the desired product (60 mg, 38.9% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{51}H_{66}N_8O_8$: 919.51; found 919.5.

Example 87. Synthesis of 6-((*S*)-aziridin-2-yl)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylnicotinamide



Step 1: Synthesis of 6-((2*S*)-1-(*tert*-butylsulfinyl)aziridin-2-yl)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-25-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylnicotinamide

To a mixture of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)- 6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide (198.24 mg, 0.218 mmol) and DIPEA (0.074 mL, 0.436 mmol) in MeCN (10 mL) at 0 °C was added HATU (200 mg, 0.526 mmol) and the resulting mixture was stirred for 3 min. To the mixture was then added a solution of 6-((2*S*)-1-(*tert*-butylsulfinyl)aziridin-2-yl)nicotinic acid (117.0 mg, 0.436 mmol) in MeCN (10 mL) in portions. The resulting mixture was stirred overnight at 0 °C and then was then quenched with H_2O extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (20 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product (430 mg, 85.0% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{64}H_{90}N_8O_8SSi$: 1159.65; found 1159.8.

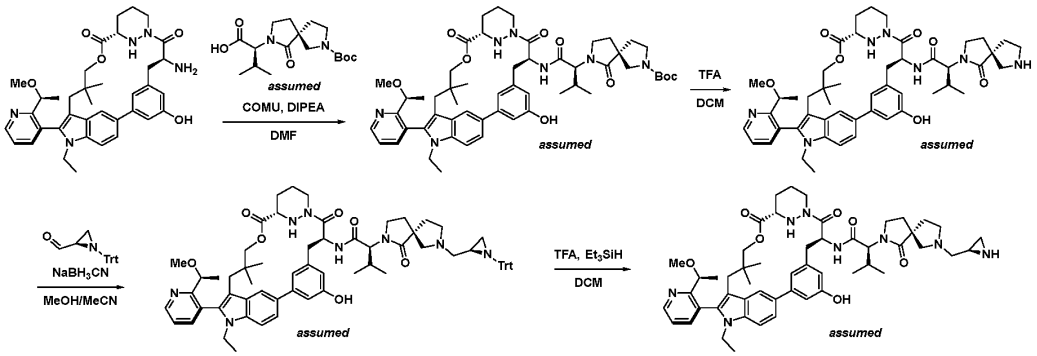
Step 2: Synthesis of 6-((2*S*)-1-(*tert*-butylsulfinyl)aziridin-2-yl)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylnicotinamide

To a solution of 6-((2*S*)-1-(*tert*-butylsulfinyl)aziridin-2-yl)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-25-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylnicotinamide (430.0 mg, 0.371 mmol) in THF (50.0 mL) at 0 °C was added TBAF (1 M in THF, 1.1 mL, 1.11 mmol) in portions. The resulting mixture was stirred at 0 °C for 2 h and was then concentrated under reduced pressure. The residue was purified by prep-TLC (5% MeOH/DCM) to afford the desired product (290 mg, 78% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{55}H_{70}N_8O_8S$: 1003.51; found 1003.8.

Step 3: Synthesis of 6-((*S*)-aziridin-2-yl)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylnicotinamide

To a solution of 6-((2*S*)-1-(*tert*-butylsulfinyl)aziridin-2-yl)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylnicotinamide (150.0 mg, 0.150 mmol) in H_2O (15.0 mL) and acetone (15.0 mL) at 0 °C was added TFA (7.50 mL, 100.97 mmol) in portions. The resulting mixture was warmed to room temperature and stirred for 48 h and then was neutralized to pH 8 with sat. $NaHCO_3$. The aqueous layer was extracted with EtOAc, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by reverse phase chromatography (38→58% MeCN/H_2O) afforded the desired product (10.0 mg, 7.4% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{51}H_{62}N_8O_7$: 899.48; found 899.5.

Example 139. Synthesis of (2*S*)-2-((*S*)-7-(((*R*)-aziridin-2-yl)methyl)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide



Step 1: Synthesis of *tert*-butyl (5*R*)-7-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate

To a solution of (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (600 mg, 0.94 mmol) and DIPEA (820 µL, 4.7 mmol) in DMF (8 mL) at 0 °C was added (*S*)-2-((*R*)-7-(*tert*-butoxycarbonyl)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)-3-methylbutanoic acid (380 mg, 1.13 mmol) and COMU (440 mg, 1.03 mmol). The reaction mixture was stirred for 1 h then was diluted with H_2O (100 mL) and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (30 mL), dried with Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure. Purification by Prep-TLC (EtOAc) afforded the desired product (600 mg, 66% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{54}H_{71}N_7O_9$: 962.54; found 962.5.

Step 2: Synthesis of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-((*S*)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)butanamide

To a solution of *tert*-butyl (5*R*)-7-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate (600 mg, 0.62 mmol) in DCM (6 mL) at 0 °C was added TFA (3.0 mL, 40 mmol). The reaction mixture was stirred for 2 h and then was concentrated under reduced pressure. The residue was diluted with H_2O (100 mL), basified to pH 8 with sat. aq. $NaHCO_3$, and extracted with EtOAc (3 x 60 mL). The combined organic layers were washed with brine (30 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure to afford the

desired product (430 mg, 79% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{49}H_{63}N_7O_7$: 862.49; found 862.5.

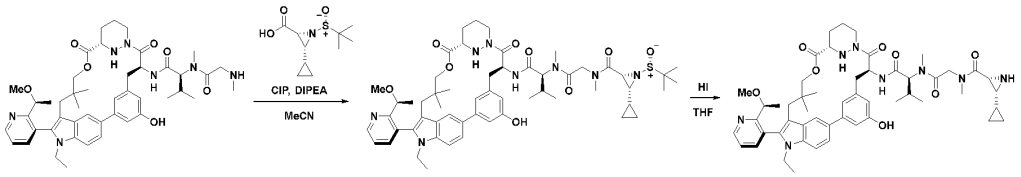
Step 3: Synthesis of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-((*S*)-1-oxo-7-(((*S*)-1-tritylaziridin-2-yl)methyl)-2,7-diazaspiro[4.4]nonan-2-yl)butanamide

To a solution of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-((*S*)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)butanamide (200 mg, 0.23 mmol) and (*R*)-1-tritylaziridine-2-carbaldehyde (110 mg, 0.35 mmol) in MeOH (0.50 mL) and MeCN (4.0 mL) was added $NaBH_3CN$ (29 mg, 0.46 mmol). The reaction mixture was stirred for 2 h then was quenched with sat. aq. NH_4Cl and was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (30 mL), dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by Prep-TLC (EtOAc) afforded desired product (145 mg, 53% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{71}H_{82}N_8O_7$: 1159.64; found 1159.6.

Step 4: Synthesis of (2*S*)-2-((*S*)-7-(((*R*)-aziridin-2-yl)methyl)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide

To a solution of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-((*S*)-1-oxo-7-(((*S*)-1-tritylaziridin-2-yl)methyl)-2,7-diazaspiro[4.4]nonan-2-yl)butanamide (140 mg, 0.12 mmol) in DCM (2.0 mL) 0 °C was added TFA (74 µL, 0.97 mmol) and Et_3SiH (150 µL, 0.97 mmol). The reaction mixture was stirred for 30 min then was basified to pH 8 with DIPEA. The resulting mixture was concentrated under reduced pressure. Purification by reverse phase chromatography (30→60% MeCN/H_2O) afforded the desired product (37.5 mg, 31% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{52}H_{68}N_8O_7$: 917.53; found 917.4.

Example 133. Synthesis of (2*R*,3*R*)-3-cyclopropyl-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methylaziridine-2-carboxamide



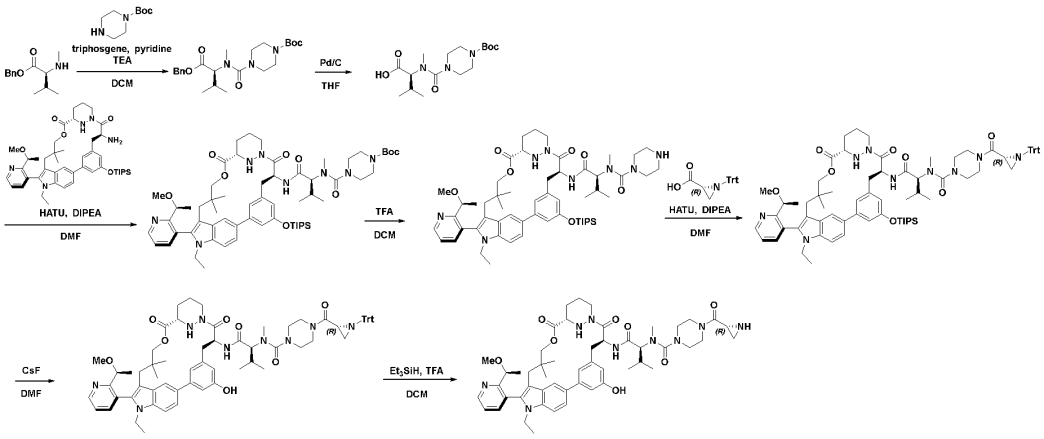
Step 1: Synthesis of (2*R*,3*R*)-1-(*tert*-butylsulfinyl)-3-cyclopropyl-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methylaziridine-2-carboxamide

To a solution of (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(*N*-methyl-2-(methylamino)acetamido)butanamide (50 mg, 61 μmol) and (2*R*,3*R*)-1-(*tert*-butylsulfinyl)-3-cyclopropylaziridine-2-carboxylic acid (21 mg, 91 μmol) in MeCN at 0 °C was added DIPEA (210 μL, 1.2 mmol) and CIP (25 mg, 91 μmol). The resulting mixture was stirred for 2 h and was then concentrated under reduced pressure. Purification by Prep-TLC (9% EtOAc/pet. ether) afforded the desired product (270 mg, 54% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{56}H_{76}N_8O_9S$: 1037.56; found 1037.4.

Step 2: Synthesis of (2*R*,3*R*)-3-cyclopropyl-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methylaziridine-2-carboxamide

To a mixture of (2*R*,3*R*)-1-(*tert*-butylsulfinyl)-3-cyclopropyl-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methylaziridine-2-carboxamide (230 mg, 0.22 mmol) in THF at 0 °C was added HI (0.50 mL, 3.8 mmol, 57% wt in H_2O). The reaction mixture was stirred for 10 min and then neutralized to pH 8 with DIPEA and concentrated under reduced pressure. Purification by reverse phase chromatography (40→60% MeCN/H_2O) afforded desired product (20 mg, 11% yield) as a white solid. LCMS (ESI) *m/z*: [M + H] calcd for $C_{52}H_{68}N_8O_8$: 933.53; found 933.6.

Example 177. Synthesis of 4-((*R*)-aziridine-2-carbonyl)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylpiperazine-1-carboxamide



Step 1: Synthesis of *tert*-butyl (*S*)-4-((1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)piperazine-1-carboxylate

Into a 100-mL vial were added benzyl methyl-*L*-valinate (2.0 g, 9.038 mmol) and triphosgene (0.89 g, 2.982 mmol) in DCM (30 mL) followed by pyridine (2.14 g, 27.113 mmol) in portions at 0 °C under an N_2 atmosphere. The mixture was stirred for 2 h at room temperature. The crude product was used in the next step directly without further purification. Then, the resulting mixture was added to *tert*-butyl piperazine-1-carboxylate (2.22 g, 11.912 mmol) in DCM (25 mL) and Et_3N (2.78 g, 27.489 mmol) in portions at room temperature under an N_2 atmosphere. The resulting mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, (30% EtOAc/pet. ether) to afford the desired product (3.6 g, 90.6% yield). LCMS (ESI) *m/z:* [M + H] calcd for $C_{23}H_{35}N_3O_5$: 434.26; found 434.2.

Step 2: Synthesis of *N*-(4-(*tert*-butoxycarbonyl)piperazine-1-carbonyl)-*N*-methyl-*L*-valine

Into a 100-mL vial were added *tert*-butyl (*S*)-4-((1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl) piperazine-1-carboxylate (2.95 g, 6.804 mmol) and Pd/C (1.48 g) in THF (25 mL). the reaction was stirred for overnight at room temperature under a hydrogen atmosphere. The resulting mixture was filtered, the filter cake was washed with EtOAc (3 x 50 mL), and the combined organic layers were concentrated under reduced pressure to afford the desired product (2.4 g, crude). LCMS (ESI) *m/z:* [M + H] calcd for $C_{16}H_{29}N_3O_5$: 344.21; found 344.4.

Step 3: Synthesis of *tert*-butyl 4-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)piperazine-1-carboxylate

Into a 50-mL vial was added (6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloun decaphane-5,7-dione (1.0 g, 1.256 mmol) and *N*-(4-(*tert*-

butoxycarbonyl) piperazine-1-carbonyl)-*N*-methyl-*L*-valine (647.04 mg, 1.884 mmol) in DMF (8 mL) followed by HATU (668.63 mg, 1.758 mmol) and DIPEA (811.69 mg, 6.280 mmol) in portions at room temperature. The resulting mixture was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (2 x 20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (50% EtOAc/pet. ether) to afford the desired product (1.08 g, 76.7% yield). LCMS (ESI) *m/z:* [M + H] calcd for $C_{62}H_{92}N_8O_9Si$: 1121.68; found 1122.0.

Step 4: Synthesis of *N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylpiperazine-1-carboxamide

Into a 100-mL vial was added *tert*-butyl 4-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)piperazine-1-carboxylate (1.08 g, 0.963 mmol) and TFA (3.0 mL, 40.39 mmol) in DCM (12 mL). The reaction was stirred for 2 h at room temperature under an N_2 atmosphere. The resulting mixture was concentrated under reduced pressure to afford the desired product (907 mg, crude). LCMS (ESI) *m/z:* [M + Na] calcd for $C_{57}H_{83}N_8O_7Si$: 1042.61; found 1043.9.

Step 5: Synthesis of *N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methyl-4-((*R*)-1-tritylaziridine-2-carbonyl)piperazine-1-carboxamide

Into a 40-mL vial was added *N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylpiperazine-1-carboxamide (400.0 mg, 0.392 mmol) and (*R*)-1-tritylaziridine-2-carboxylic acid (193.49 mg, 0.587 mmol) in DMF (3.5 mL) followed by HATU (208.46 mg, 0.548 mmol) and DIPEA (253.06 mg, 1.958 mmol) in portions at room temperature under an N_2 atmosphere. The resulting mixture was extracted with EtOAc (3 x 60 mL) and the combined organic layers were washed with brine (2 x 10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography, (50% EtOAc/pet. ether) to afford the desired product (367 mg, 70.3% yield). LCMS (ESI) *m/z:* [M + H -TIPS] calcd for $C_{79}H_{101}N_9O_8Si$: 1176.63; found 1176.2.

Step 6: Synthesis of *N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methyl-4-((*R*)-1-tritylaziridine-2-carbonyl)piperazine-1-carboxamide

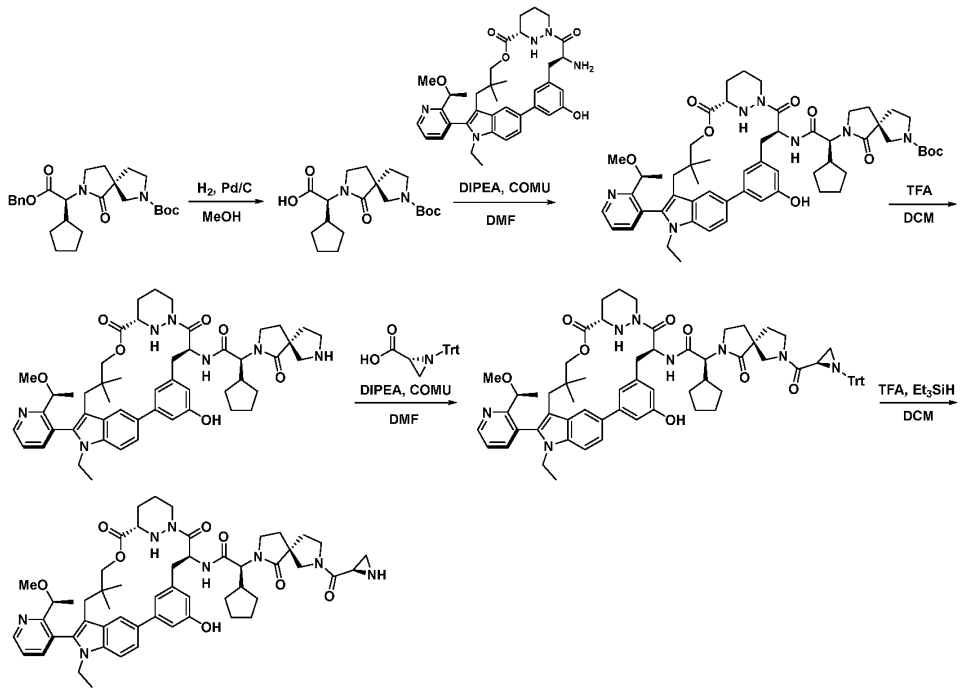
Into a 100-mL vial was added *N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-

hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methyl-4-((*R*)-1-tritylaziridine-2-carbonyl)piperazine-1-carboxamide (161.0 mg, 0.121 mmol) and CsF (91.75 mg, 0.604 mmol) in DMF (1.5 mL). The reaction was stirred for 2 h at room temperature and was then extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (2 x 10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by Prep-TLC (50% EtOAc/pet. ether) to afford the desired product (101 mg, 71.1% yield). LCMS (ESI) *m/z:* [M + H] calcd for $C_{70}H_{81}N_9O_8$: 1176.62; found 1176.9.

Step 7: Synthesis of 4-((*R*)-aziridine-2-carbonyl)-*N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methylpiperazine-1-carboxamide

Into a 40-mL vial was added *N*-((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacyclou ndecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-*N*-methyl-4-((*R*)-1-tritylaziridine-2-carbonyl) piperazine-1-carboxamide (101.0 mg, 0.086 mmol) and Et_3SiH (49.91 mg, 0.429 mmol) in DCM (2.0 mL) was added TFA (48.94 mg, 0.429 mmol) in portions at room temperature under an N_2 atmosphere. The mixture was basified to pH 8 with DIPEA. The crude product was purified by Prep-HPLC to afford the desired product (29.6 mg, 36.9% yield). LCMS (ESI) *m/z:* [M + H] calcd for $C_{51}H_{67}N_9O_8$: 934.51; found 934.3.

Example 175. Synthesis of (2*S*)-2-((*S*)-7-((*R*)-aziridine-2-carbonyl)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)-2-cyclopentyl-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)acetamide



Step 1: Synthesis of (*S*)-2-((*R*)-7-(*tert*-butoxycarbonyl)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)-2-cyclopentylacetic acid

To a solution stirred solution of *tert*-butyl (*R*)-7-((*S*)-2-(benzyloxy)-1-cyclopentyl-2-oxoethyl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate (1.0 g, 2.19 mmol) in MeOH (10 mL) at 0 °C was added Pd/C (200 mg). The resulting mixture was stirred for 1 h at room temperature under a hydrogen atmosphere, filtered, and the filter cake washed with MeOH (5 x10 mL). The filtrate was concentrated under reduced pressure to afford the desired product (895 mg, crude) which was used without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{19}H_{30}N_2O_5$: 376.23; found 367.1.

Step 2: Synthesis of *tert*-butyl (5*R*)-7-((1*S*)-1-cyclopentyl-2-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-2-oxoethyl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate

To a stirred solution of (6^3*S*,4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (702 mg, 1.10 mmol) and DIPEA (1.91 mL, 1.10 mmol) in DMF (500 mL) at 0 °C was added (*S*)-2-((*R*)-7-(*tert*-butoxycarbonyl)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)-2-cyclopentylacetic acid (523 mg, 1.43 mmol) and COMU (517 mg,

1.21 mmol). After 1 h at room temperature the reaction mixture was diluted with H_2O (150 mL). The aqueous layer was extracted with EtOAc (3 x 50 mL) and the combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by normal phase chromatography (40% EtOAc/pet. ether) afforded the desired product (978 mg, 90.2% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{56}H_{73}N_7O_9$: 988.56; found 988.7.

Step 3: Synthesis of (2*S*)-2-cyclopentyl-*N*- ((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-2-((*S*)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)acetamide

To a stirred solution of *tert*-butyl (5*R*)-7-((1*S*)-1-cyclopentyl-2-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-2-oxoethyl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate (300 mg, 0.304 mmol) in DCM (3.0 mL) at 0 °C was added TFA (1.5 mL). The resulting mixture was stirred for 30 min at room temperature. The reaction mixture was then diluted with toluene (2 mL) and concentrated under reduced pressure three times to afford the desired product (270 mg, crude) which was used without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{51}H_{65}N_7O_7$: 888.50; found 888.5.

Step 4: Synthesis of (2*S*)-2-cyclopentyl-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-2-((*S*)-1-oxo-7-((*R*)-1-tritylaziridine-2-carbonyl)-2,7-diazaspiro[4.4]nonan-2-yl)acetamide

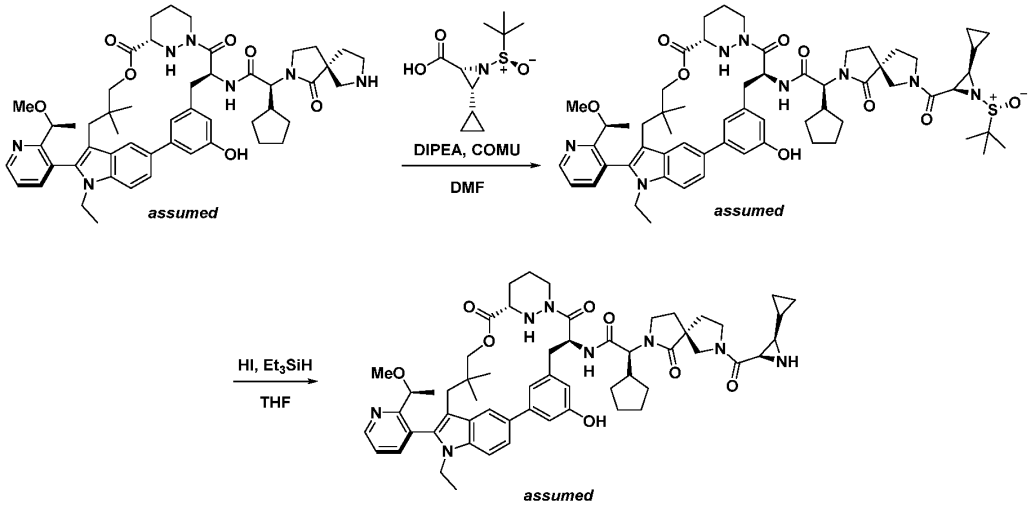
To a stirred solution of (2*S*)-2-cyclopentyl-*N*- ((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-2-((*S*)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)acetamide (270 mg, 0.304 mmol) and DIPEA (0.53 mL, 3.0 mmol) in DMF (3.0 mL) at 0 °C was added (*R*)-1-tritylaziridine-2-carboxylic acid (130 mg, 0.395 mmol) and COMU (143 mg, 0.334 mmol). After 1h at room temperature the reaction mixture was diluted with H_2O (30 mL). The aqueous layer was extracted with EtOAc (3 x 3 mL) and the combined organic layers were washed with brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by prep-TLC (5% MeOH/DCM) afforded the desired product (332 mg, 91.1% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{73}H_{82}N_8O_8$: 1199.64; found 1199.7.

Step 5: Synthesis of (2*S*)-2-((*S*)-7-((*R*)-aziridine-2-carbonyl)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)-2-cyclopentyl-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)acetamide

To a stirred solution of (2*S*)-2-cyclopentyl-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-2-((*S*)-1-oxo-7-((*R*)-1-tritylaziridine-2-carbonyl)-2,7-diazaspiro[4.4]nonan-2-yl)acetamide (309 mg, 0.258 mmol) in DCM (3.0 mL) at 0 °C was added Et_3SiH (164 mL, 1.03 mmol) and TFA (79 mL, 1.03 mmol). After 30 min the reaction

mixture was basified to pH 8 with DIPEA and concentrated under reduced pressure. Purification by reverse phase chromatography (30→60% MeCN/H_2O) afforded the desired product (36 mg, 14.2% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{54}H_{68}N_8O_8$: 957.53; found 957.3.

Example 214. Synthesis of (2*S*)-2-cyclopentyl-2-((*S*)-7-((2*R*,3*R*)-3-cyclopropylaziridine-2-carbonyl)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)acetamide



Step 1: Synthesis of (2*S*)-2-((5*S*)-7-((2*R*,3*R*)-1-(*tert*-butylsulfinyl)-3-cyclopropylaziridine-2-carbonyl)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)-2-cyclopentyl-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)acetamide

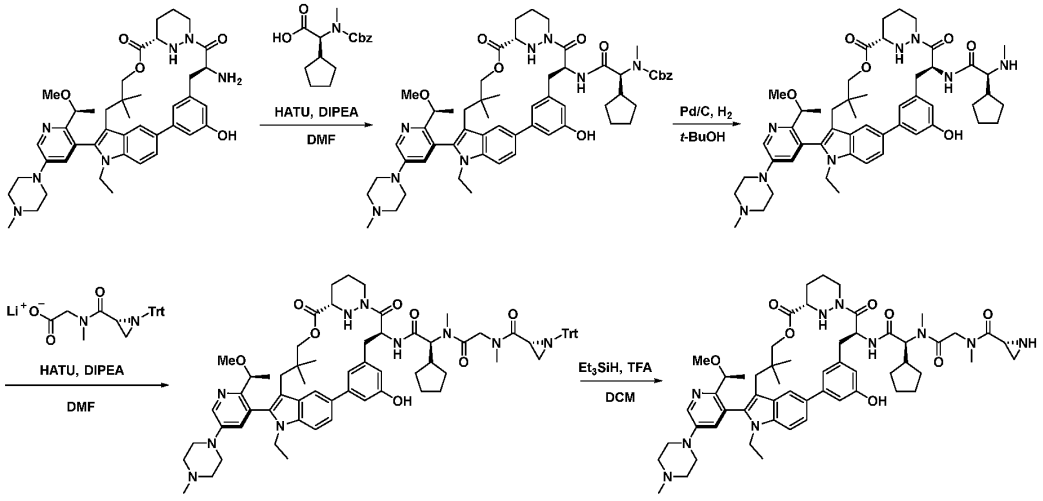
To a stirred solution of (2*S*)-2-cyclopentyl-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-2-((*S*)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)acetamide (270 mg, 0.30 mmol) in DMF (3.0 mL) at 0 °C was added DIPEA (530 µL, 3.0 mmol) and (2*R*,3*R*)-1-(*tert*-butylsulfinyl)-3-cyclopropylaziridine-2-carboxylic acid (105 mg, 0.46 mmol) followed by COMU (140 mg, 0.33 mmol). The resulting mixture was stirred for 1 h at room temperature and was then diluted with H_2O (30 mL). The reaction mixture was extracted into EtOAc (3 x 7 mL). The combined organic layers were washed with brine (3 x 10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by Prep-TLC (6% MeOH/DCM) afforded the desired product (237 mg, 71% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{61}H_{80}N_8O_9S$: 1101.58; found 1101.3.

Step 2: Synthesis of (2*S*)-2-cyclopentyl-2-((*S*)-7-((2*R*,3*R*)-3-cyclopropylaziridine-2-carbonyl)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-

methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)acetamide

To a stirred solution of (2*S*)-2-((5*S*)-7-((2*R*,3*R*)-1-(*tert*-butylsulfinyl)-3-cyclopropylaziridine-2-carbonyl)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)-2-cyclopentyl-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)acetamide (230 mg, 0.21 mmol) in THF (2.5 mL) at 0 °C was added Et_3SiH (130 µL, 0.83 mmol) and HI (125 µL, 0.41 mmol, 57% in H_2O). The resulting mixture was stirred for 30 min at room temperature then cooled to 0 °C and neutralized to pH 8. The mixture was concentrated under reduced pressure. Purification by Prep-TLC (8.3% MeOH/DCM) afforded the desired product (46 mg, 21% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{57}H_{72}N_8O_8$: 997.55; found 997.2.

Example 209. Synthesis of (2*R*)-*N*-(2-(((1*S*)-1-cyclopentyl-2-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-2-oxoethyl)(methyl)amino)-2-oxoethyl)-*N*-methylaziridine-2-carboxamide



Step 1: Synthesis of benzyl ((1*S*)-1-cyclopentyl-2-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-2-oxoethyl)(methyl)carbamate

To a stirred solution of (6^3*S*, 4*S*)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridine-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (490 mg, 0.664 mmol) and (*S*)-2-(((benzyloxy)carbonyl)(methyl)amino)-2-cyclopentylacetic acid (232 mg, 0.797 mmol) in DMF (5 mL) at

0 °C was added DIPEA (1.19 mL, 6.64 mmol) and HATU (303 mg, 0.797 mmol). The resulting mixture was stirred for 1 h at room temperature and then diluted with H_2O (20 mL). The aqueous

phase was extracted with EtOAc (3 x 20 mL) and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by reverse phase chromatography (0→100% MeCN/H_2O, 0.1% NH_4HCO_3) afforded the desired product (420 mg, 59.4% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{58}H_{74}N_8O_8$: 1011.57; found 1011.6.

Step 2: Synthesis of (2*S*)-2-cyclopentyl-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-2-(methylamino)acetamide

To a stirred solution of benzyl ((1*S*)-1-cyclopentyl-2-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-2-oxoethyl)(methyl)carbamate (450 mg, 0.445 mmol) in *t*-BuOH (10 mL) was added Pd/C (90 mg). The resulting mixture was warmed to 40 °C overnight under a hydrogen atmosphere, then filtered and the filter cake washed with MeOH. The filtrate was concentrated under reduced pressure to afford the desired product (420 mg, crude) which was used without further purification. LCMS (ESI) *m/z*: [M + H] calcd for $C_{50}H_{68}N_8O_6$: 877.54; found 877.5.

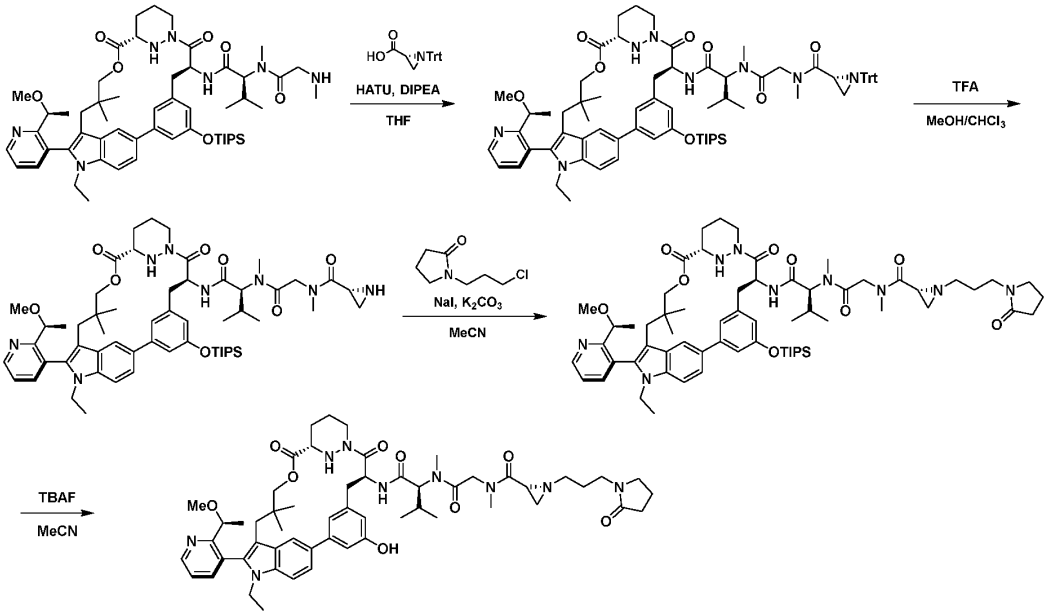
Step 3: Synthesis of (2*R*)-*N*-(2-(((1*S*)-1-cyclopentyl-2-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-2-oxoethyl)(methyl)amino)-2-oxoethyl)-*N*-methyl-1-tritylaziridine-2-carboxamide

To a stirred solution of (2*S*)-2-cyclopentyl-*N*-((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-2-(methylamino)acetamide (130 mg, 0.148 mmol) and lithium (*R*)-*N*-methyl-*N*-(1-tritylaziridine-2-carbonyl)glycinate (78.3 mg, 0.193 mmol) in DMF (2 mL) at 0 °C was added DIPEA (264 mL, 1.48 mmol) and HATU (68 mg, 0.178 mmol). The resulting mixture was stirred for 1 h at room temperature and then diluted with H_2O (20 mL). The aqueous phase was extracted with EtOAc (3 x 10 mL) and the combined organic layers were washed with H_2O, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by prep-TLC (10% MeOH/DCM) afforded the desired product (100 mg, 50.9% yield). LCMS (ESI) *m/z*: [M + Na] calcd for $C_{75}H_{90}N_{10}O_8$: 1281.69; found 1281.9.

Step 4: Synthesis of (2*R*)-*N*-(2-(((1*S*)-1-cyclopentyl-2-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-2-oxoethyl)(methyl)amino)-2-oxoethyl)-*N*-methylaziridine-2-carboxamide

To a stirred solution of (2*R*)-*N*-(2-(((1*S*)-1-cyclopentyl-2-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-2-oxoethyl)(methyl)amino)-2-oxoethyl)-*N*-methyl-1-tritylaziridine-2-carboxamide (100 mg, 0.079 mmol) in DCM (1.0 mL) at 0 °C was added Et_3SiH (51 mL, 0.318 mmol) and TFA (24 mL, 0.318 mmol). After 30 min the reaction mixture was basified to pH 8 with DIPEA and concentrated under reduced pressure. Purification by reverse phase chromatography (30→55% MeCN/H_2O) afforded the desired product (14mg, 16.5% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{56}H_{76}N_{10}O_8$: 1017.59; found 1017.6.

Example 268. Synthesis of (2*R*)-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methyl-1-(3-(2-oxopyrrolidin-1-yl)propyl)aziridine-2-carboxamide



Step 1: Synthesis of (2*R*)-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methyl-1-tritylaziridine-2-carboxamide

To a solution of (1*S*)-1-tritylaziridine-2-carboxylic acid (537.6 mg, 1.63 mmol), (2*S*)-*N*-((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(*N*-methyl-2-(methylamino)acetamido)butanamide

(800 mg, 0.816 mmol) in THF (8 mL) was added DIPEA (0.711 mL, 4.08 mmol), HATU (465.4 mg, 1.22 mmol) at 0 °C, the reaction was warmed to room temperature and stirred for 2 h. To the reaction was added H_2O (20 mL), the aqueous phase was extracted with DCM (3 x 30 mL) and the combined organic phases were washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0→100% EtOAc/pet. ether) to afford the desired product (1 g, 94.9% yield).

Step 2: Synthesis of (2*R*)-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methylaziridine-2-carboxamide

To a solution of (2*R*)-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridine-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methyl-1-tritylaziridine-2-carboxamide (1 g, 0.774 mmol) in MeOH (5 mL) and $CHCl_3$ (5 mL) was added TFA (1.15 mL, 15.48 mmol) at 0 °C. The reaction was warmed to room temperature and stirred for 2 h. The reaction mixture was added dropwise to aq. $NaHCO_3$ (30 mL) at 0 °C. Then the pH was adjusted to pH 7-8 with using aq. $NaHCO_3$ at 0 °C. The mixture was extracted with DCM (3 x 20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the desired product product (960 mg, crude), which was used directly in the next step without further purification.

Step 3: Synthesis of (2*R*)-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methyl-1-(3-(2-oxopyrrolidin-1-yl)propyl)aziridine-2-carboxamide

To a solution of (2*R*)-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridine-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methylaziridine-2-carboxamide (960 mg, 0.915 mmol) in MeCN (10 mL) was added 1-(3-chloropropyl)pyrrolidin-2-one (887.1 mg, 5.49 mmol), K_2CO_3 (1.14 g, 8.23 mmol), NaI (411.4 mg, 2.74 mmol), the reaction was stirred at 80 °C for 24 h. To the reaction was added H_2O (20 mL), the aqueous phase was extracted with EtOAc (3 x 20 mL). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by reverse phase chromatography (73→93% MeCN/H_2O, 10 mM NH_4HCO_3) to afford product (80 mg, 7.5% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{65}H_{96}N_9O_9Si$: 1174.7; found 1174.7.

Step 4: Synthesis of (2*R*)-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-2^5-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methyl-1-(3-(2-oxopyrrolidin-1-yl)propyl)aziridine-2-carboxamide

To a solution of (2*R*)-*N*-(2-(((2*S*)-1-(((6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridine-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)amino)-2-oxoethyl)-*N*-methyl-1-(3-(2-oxopyrrolidin-1-yl)propyl)aziridine-2-carboxamide (80 mg, 0.068 mmol) in THF (1 mL) was added TBAF (1 M, 0.082 mL). The reaction was stirred for 1 h and then was added to H_2O (10 mL), the aqueous phase was extracted with EtOAc (3 x 10 mL). The combined organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by reverse phase chromatography (25→65% MeCN/H_2O, 10 mM NH_4HCO_3 to afford the desired product (42 mg, 60.4% yield). LCMS (ESI) *m/z*: [M + H] calcd for $C_{56}H_{76}N_9O_9$: 1018.6; found 1018.5.

The following table of compounds (Table 4) were prepared using the aforementioned methods or variations thereof, as is known to those of skill in the art.

Table 4: Exemplary Compounds Prepared by Methods of the Present Invention

Ex#	Molecular Formula	Calculated MW	Observed MW LCMS (ESI) *m/z*
1	$C_{50}H_{64}N_8O_8$	[M + H] = 905.49	[M + H] = 905.7
2	$C_{50}H_{64}N_8O_8$	[M + H] = 905.49	[M + H] = 905.7
3	$C_{51}H_{66}N_8O_8$	[M + H] = 919.51	[M + H] = 919.7
4	$C_{51}H_{66}N_8O_8$	[M + H] = 919.51	[M + H] = 919.6
5	$C_{49}H_{62}N_8O_8$	[M + H] = 891.48	[M + H] = 891.7
6	$C_{49}H_{62}N_8O_8$	[M + H] = 891.48	[M + H] = 891.7
7	$C_{48}H_{62}N_8O_8$	[M + H] = 879.48	[M + H] = 879.7
8	$C_{48}H_{62}N_8O_8$	[M + H] = 879.48	[M + H] = 879.7
9	$C_{56}H_{68}N_8O_8$	[M + H] = 969.52	[M + H] = 969.5
10	$C_{56}H_{68}N_8O_8$	[M + H] = 969.52	[M + H] = 969.5
11	$C_{54}H_{66}N_8O_8$	[M + H] = 955.51	[M + H] = 955.7
12	$C_{54}H_{66}N_8O_8$	[M + H] = 955.51	[M + H] = 955.7
13	$C_{49}H_{64}N_8O_{10}S$	[M + H] = 957.45	[M + H] = 957.5
14	$C_{50}H_{64}N_8O_9$	[M + H] = 921.49	[M + H] = 921.5
15A	$C_{53}H_{64}N_8O_7$	[M + H] = 925.50	[M + H] = 925.5
15B	$C_{53}H_{64}N_8O_7$	[M + H] = 925.50	[M + H] = 925.5
16A	$C_{50}H_{66}N_8O_6S$	[M + H] = 907.49	[M + H] = 907.5
16B	$C_{50}H_{66}N_8O_6S$	[M + H] = 907.49	[M + H] = 907.5
17A	$C_{53}H_{71}N_9O_7$	[M + H] = 946.56	[M + H] = 946.6
17B	$C_{53}H_{71}N_9O_7$	[M + H] = 946.56	[M + H] = 946.6
18A	$C_{54}H_{66}N_8O_7$	[M + H] = 940.52	[M + H] = 939.6
18B	$C_{54}H_{66}N_8O_7$	[M + H] = 940.52	[M + H] = 939.6
19A	$C_{53}H_{64}N_8O_6$	[M + H] = 909.50	[M + H] = 909.6

Ex#	Molecular Formula	Calculated MW	Observed MW LCMS (ESI) *m/z*
19B	$C_{53}H_{64}N_8O_6$	[M + H] = 909.50	[M + H] = 909.5
20A	$C_{53}H_{64}N_8O_7$	[M + H] = 925.50	[M + H] = 925.5
20B	$C_{53}H_{64}N_8O_7$	[M + H] = 925.50	[M + H] = 925.5
21A	$C_{49}H_{64}N_8O_6$	[M + H] = 861.50	[M + H] = 861.5
21B	$C_{49}H_{64}N_8O_6$	[M + H] = 861.50	[M + H] = 861.5
22A	$C_{49}H_{64}N_8O_6$	[M + H] = 861.50	[M + H] = 861.6
22B	$C_{49}H_{64}N_8O_6$	[M + H] = 861.50	[M + H] = 861.5
23A	$C_{49}H_{64}N_8O_7$	[M + H] = 877.50	[M + H] = 877.6
23B	$C_{49}H_{64}N_8O_7$	[M + H] = 877.50	[M + H] = 877.6
24A	$C_{54}H_{66}N_8O_8$	[M + H] = 955.51	[M + H] = 955.6
24B	$C_{54}H_{66}N_8O_8$	[M + H] = 955.51	[M + H] = 955.6
25	$C_{48}H_{63}ClN_8O_8$	[M + H] = 915.45	[M + H] = 915.7
26	$C_{46}H_{60}ClN_7O_7$	[M + H] = 858.43	[M + H] = 858.7
27	$C_{48}H_{62}N_8O_8$	[M + H] = 879.48	[M + H] = 879.4
28	$C_{47}H_{60}N_8O_8$	[M + H] = 865.46	[M + H] = 865.7
29	$C_{45}H_{57}N_7O_7$	[M + H] = 808.44	[M + H] = 808.8
30	$C_{46}H_{59}N_7O_7$	[M + H] = 822.46	[M + H] = 822.4
31	$C_{50}H_{63}N_7O_9$	[M + H] = 906.48	[M + H] = 906.7
32A	$C_{54}H_{66}N_8O_7$	[M + H] = 940.18	[M + H] = 939.6
32B	$C_{54}H_{66}N_8O_7$	[M + H] = 940.18	[M + H] = 939.6
33	$C_{48}H_{62}N_8O_8$	[M + H] = 879.48	[M + H] = 878.5
34	$C_{50}H_{64}N_8O_9$	[M + H] = 921.49	[M + H] = 921.5
35	$C_{50}H_{64}N_8O_9$	[M + H] = 921.49	[M + H] = 921.6
36	$C_{49}H_{64}N_8O_{10}S$	[M + H] = 957.45	[M + H] = 957.5
37	$C_{49}H_{64}N_8O_{10}S$	[M + H] = 957.45	[M + H] = 957.5
38	$C_{50}H_{64}N_8O_{10}$	[M + H] = 937.48	[M + H] = 937.6
39	$C_{50}H_{64}N_8O_{10}$	[M + H] = 937.48	[M + H] = 937.5
40	$C_{49}H_{64}N_8O_8$	[M + H] = 893.49	[M + H] = 893.7
41	$C_{49}H_{64}N_8O_8$	[M + H] = 893.49	[M + H] = 893.8
42A	$C_{49}H_{64}N_8O_8$	[M + H] = 893.49	[M + H] = 893.7
42B	$C_{49}H_{64}N_8O_8$	[M + H] = 893.49	[M + H] = 893.7
43A	$C_{51}H_{66}N_8O_8$	[M + H] = 919.51	[M + H] = 919.8
43B	$C_{51}H_{66}N_8O_8$	[M + H] = 919.51	[M + H] = 919.7
44	$C_{51}H_{67}N_9O_8$	[M + H] = 934.52	[M + H] = 934.8
45	$C_{51}H_{67}N_9O_8$	[M + H] = 934.52	[M + H] = 934.7
46	$C_{51}H_{61}N_9O_8$	[M + H] = 928.47	[M + H] = 928.7
47A	$C_{51}H_{61}N_9O_8$	[M + H] = 928.47	[M + H] = 928.7

Ex#	Molecular Formula	Calculated MW	Observed MW LCMS (ESI) *m/z*
47B	$C_{51}H_{61}N_9O_8$	[M + H] = 928.47	[M + H] = 928.7
48	$C_{54}H_{72}N_8O_{11}S$	[M + H] = 1041.51	[M + H] = 1041.8
49	$C_{50}H_{64}N_8O_{10}$	[M + H] = 937.48	[M + H] = 938.6
50	$C_{54}H_{72}N_8O_{11}S$	[M + H] = 1041.51	[M + H] = 1041.6
51	$C_{50}H_{64}N_8O_{10}$	[M + H] = 937.48	[M + H] = 937.8
52	$C_{55}H_{74}N_8O_9S$	[M + H] = 1023.54	[M + H] = 1023.8
53	$C_{53}H_{72}N_8O_9S$	[M + Na] = 1019.50	[M + Na] = 1019.8
54	$C_{53}H_{72}N_8O_9S$	[M + H] = 997.52	[M + H] = 997.7
55	$C_{55}H_{64}N_8O_9$	[M + H] = 1003.47	[M + H] = 1003.8
56	$C_{51}H_{68}N_8O_9S$	[M + Na] = 991.47	[M + Na] = 991.7
57	$C_{51}H_{68}N_8O_9S$	[M + H] = 969.49	[M + H] = 969.8
58	$C_{53}H_{68}N_8O_8$	[M + H] = 945.52	[M + H] = 945.8
59	$C_{52}H_{66}N_8O_8$	[M + H] = 931.51	[M + H] = 931.8
60	$C_{46}H_{59}N_7O_7$	[M + H] = 822.46	[M + H] = 822.7
61A	$C_{55}H_{68}N_8O_8$	[M + H] = 969.52	[M + H] = 969.6
61B	$C_{55}H_{68}N_8O_8$	[M + H] = 969.52	[M + H] = 969.6
62A	$C_{55}H_{68}N_8O_8$	[M + H] = 955.51	[M + H] = 955.5
62B	$C_{55}H_{68}N_8O_8$	[M + H] = 955.51	[M + H] = 955.5
63	$C_{54}H_{66}N_8O_7$	[M + H] = 939.51	[M + H] = 939.6
64A	$C_{55}H_{68}N_8O_7$	[M + H] = 953.53	[M + H] = 953.6
64B	$C_{55}H_{68}N_8O_7$	[M + H] = 953.53	[M + H] = 953.6
65A	$C_{55}H_{66}N_8O_7$	[M + H] = 951.51	[M + H] = 951.7
65B	$C_{55}H_{66}N_8O_7$	[M + H] = 951.51	[M + H] = 951.6
66	$C_{54}H_{67}N_9O_6$	[M + H] = 938.53	[M + H] = 938.5
67	$C_{56}H_{69}N_9O_7$	[M + H] = 980.54	[M + H] = 980.1
68A	$C_{52}H_{64}N_8O_6S$	[M + H] = 929.47	[M + H] = 929.5
68B	$C_{52}H_{64}N_8O_6S$	[M + H] = 929.47	[M + H] = 929.5
69A	$C_{51}H_{68}N_8O_7$	[M + H] = 891.51	[M + H] = 891.5
69B	$C_{51}H_{68}N_8O_7$	[M + H] = 891.51	[M + H] = 891.5
70A	$C_{56}H_{68}N_8O_7$	[M + H] = 965.53	[M + H] = 965.6
70B	$C_{56}H_{68}N_8O_7$	[M + H] = 965.53	[M + H] = 965.6
71A	$C_{56}H_{68}N_8O_7$	[M + H] = 965.53	[M + H] = 965.5
71B	$C_{56}H_{68}N_8O_7$	[M + H] = 965.53	[M + H] = 965.5
72A	$C_{51}H_{66}N_8O_7$	[M + H] = 903.51	[M + H] = 903.5
72B	$C_{51}H_{66}N_8O_7$	[M + H] = 903.51	[M + H] = 903.6
73	$C_{55}H_{68}N_8O_7$	[M + H] = 953.53	[M + H] = 953.6
74	$C_{50}H_{66}N_8O_7$	[M + H] = 891.51	[M + H] = 891.6

Ex#	Molecular Formula	Calculated MW	Observed MW LCMS (ESI) *m/z*
75	$C_{55}H_{68}N_8O_8$	[M + H] = 969.52	[M + H] = 969.9
76	$C_{58}H_{72}N_8O_7$	[M + H] = 993.56	[M + H] = 993.5
77	$C_{57}H_{70}N_8O_7$	[M + H] = 979.54	[M + H] = 979.5
78	$C_{52}H_{68}N_8O_7$	[M + H] = 917.53	[M + H] = 917.5
79A	$C_{50}H_{66}N_8O_6$	[M + H] = 875.52	[M + H] = 875.6
79B	$C_{50}H_{66}N_8O_6$	[M + H] = 875.52	[M + H] = 875.6
80	$C_{50}H_{66}N_8O_6$	[M + H] = 875.52	[M + H] = 875.6
81	$C_{54}H_{66}N_8O_6$	[M + H] = 923.52	[M + H] = 923.5
82A	$C_{54}H_{66}N_8O_6$	[M + H] = 923.52	[M + H] = 923.6
82B	$C_{54}H_{66}N_8O_6$	[M + H] = 923.52	[M + H] = 923.6
83	$C_{51}H_{64}N_{10}O_8$	[M + H] = 945.50	[M + H] = 945.6
84	$C_{51}H_{64}N_{10}O_8$	[M + H] = 945.50	[M + H] = 945.6
85	$C_{51}H_{68}N_8O_9$	[M + H] = 937.52	[M + H] = 837.6
86	$C_{52}H_{68}N_8O_8$	[M + H] = 933.52	[M + H] = 933.7
87	$C_{51}H_{62}N_8O_7$	[M + H] = 899.48	[M + H] = 899.5
88	$C_{52}H_{68}N_8O_8$	[M + H] = 933.52	[M + H] = 933.6
89	$C_{52}H_{68}N_8O_8$	[M + H] = 933.52	[M + H] = 933.6
90	$C_{52}H_{70}N_8O_9$	[M + H] = 951.53	[M + H] = 951.6
91	$C_{49}H_{66}N_8O_9S$	[M + H] = 943.48	[M + H] = 943.6
92	$C_{49}H_{66}N_8O_9S$	[M + H] = 943.48	[M + H] = 943.5
93	$C_{55}H_{68}N_8O_9$	[M + H] = 985.52	[M + H] = 985.7
94	$C_{52}H_{66}N_8O_{10}$	[M + H] = 963.50	[M + H] = 963.6
95	$C_{51}H_{66}N_8O_8$	[M + H] = 919.51	[M + H] = 919.5
96	$C_{55}H_{68}N_8O_9$	[M + H] = 985.52	[M + H] = 985.7
97	$C_{55}H_{68}N_8O_9$	[M + H] = 985.52	[M + H] = 985.7
98	$C_{48}H_{58}N_8O_9$	[M + H] = 891.44	[M + H] = 891.6
99	$C_{46}H_{59}N_7O_7$	[M + H] = 822.46	[M + H] = 822.6
100	$C_{46}H_{59}N_7O_7$	[M + H] = 822.46	[M + H] = 822.6
101	$C_{55}H_{74}N_8O_9S$	[M + Na] = 1045.52	[M + Na] = 1045.7
102	$C_{50}H_{66}N_8O_8$	[M + H] = 907.51	[M + H] = 907.7
103	$C_{51}H_{66}N_8O_8$	[M + H] = 919.51	[M + H] = 919.5
104	$C_{51}H_{66}N_8O_8$	[M + H] = 919.51	[M + H] = 919.7
105	$C_{49}H_{64}N_8O_8$	[M + H] = 893.49	[M + H] = 893.7
106	$C_{51}H_{66}N_8O_7$	[M + H] = 903.51	[M + H] = 930.7
107	$C_{49}H_{64}N_8O_7$	[M + H] = 877.50	[M + H] = 877.7
108	$C_{52}H_{70}N_8O_9S$	[M + H] = 983.51	[M + H] = 983.8
109	$C_{51}H_{64}N_8O_8$	[M + H] = 917.49	[M + H] = 917.7

Ex#	Molecular Formula	Calculated MW	Observed MW LCMS (ESI) *m/z*
110	$C_{54}H_{66}N_8O_8$	[M + H] = 955.51	[M + H] = 955.7
111	$C_{52}H_{66}N_8O_{10}$	[M + H] = 963.50	[M + H] = 963.8
112	$C_{46}H_{59}N_7O_7$	[M + H] = 822.46	[M + H] = 822.6
113	$C_{52}H_{66}N_8O_8$	[M + Na] = 953.49	[M + Na] = 953.6
114	$C_{50}H_{66}N_8O_8$	[M + H] = 907.51	[M + H] = 907.7
115	$C_{51}H_{64}N_8O_8$	[M + H] = 917.49	[M + H] = 917.6
116	$C_{54}H_{66}N_8O_8$	[M + H] = 955.51	[M + H] = 955.7
117	$C_{50}H_{66}N_8O_9S$	[M + H] = 955.48	[M + H] = 955.6
118	$C_{50}H_{66}N_8O_9S$	[M + H] = 955.48	[M + H] = 955.8
119	$C_{52}H_{70}N_8O_9S$	[M + H] = 983.51	[M + H] = 983.7
120	$C_{48}H_{62}N_8O_8$	[M + H] = 879.48	[M + H] = 880.2
121	$C_{51}H_{68}N_8O_6$	[M + H] = 889.53	[M + H] = 889.6
122	$C_{51}H_{68}N_8O_6$	[M + H] = 889.53	[M + H] = 889.6
123	$C_{55}H_{70}N_8O_6$	[M + H] = 939.55	[M + H] = 939.6
124	$C_{53}H_{70}N_8O_6$	[M + H] = 915.55	[M + H] = 915.6
125	$C_{54}H_{72}N_8O_6$	[M + H] = 929.57	[M + H] = 929.6
126	$C_{57}H_{70}N_8O_6$	[M + H] = 963.55	[M + H] = 963.6
127	$C_{52}H_{63}N_9O_8$	[M + H] = 942.49	[M + H] = 942.4
128	$C_{55}H_{70}N_8O_8$	[M + H] = 971.54	[M + H] = 971.5
129	$C_{49}H_{62}N_8O_8$	[M + H] = 891.48	[M + H] = 891.4
130	$C_{49}H_{62}N_8O_8$	[M + H] = 891.48	[M + H] = 891.5
131	$C_{52}H_{63}N_7O_7$	[M + H] = 898.49	[M + H] = 898.4
132	$C_{52}H_{63}N_7O_7$	[M + H] = 910.46	[M + H] = 910.4
133	$C_{52}H_{68}N_8O_8$	[M + H] = 933.52	[M + H] = 933.6
134	$C_{52}H_{68}N_8O_8$	[M + H] = 919.51	[M + H] = 919.5
135	$C_{52}H_{68}N_8O_8$	[M + H] = 933.52	[M + H] = 933.5
136	$C_{52}H_{68}N_8O_8$	[M + H] = 933.52	[M + H] = 933.6
137	$C_{53}H_{72}N_8O_9$	[M + H] = 965.55	[M + H] = 966.2
138	$C_{52}H_{68}N_8O_7$	[M + H] = 917.53	[M + H] = 918.2
139	$C_{52}H_{68}N_8O_7$	[M + H] = 917.53	[M + H] = 917.4
140	$C_{48}H_{62}N_8O_8$	[M + H] = 879.48	[M + H] = 879.4
141	$C_{51}H_{66}N_8O_8$	[M + H] = 919.51	[M + H] = 919.1
142	$C_{52}H_{70}N_8O_8$	[M + H] = 935.54	[M + H] = 936.3
143	$C_{51}H_{65}N_9O_9$	[M + H] = 965.52	[M + H] = 966.5
144	$C_{52}H_{69}N_9O_8$	[M + H] = 948.53	[M + H] = 948.5
145	$C_{52}H_{63}N_7O_7$	[M + H] = 898.49	[M + H] = 898.5
146	$C_{52}H_{66}N_8O_8$	[M + H] = 931.51	[M + H] = 931.5

Ex#	Molecular Formula	Calculated MW	Observed MW LCMS (ESI) *m/z*
147	$C_{48}H_{62}N_8O_8$	[M + H] = 879.48	[M + H] = 879.5
148	$C_{52}H_{70}N_8O_8$	[M + H] = 934.53	[M + H] = 936.3
149	$C_{52}H_{66}N_8O_8$	[M + H] = 931.51	[M + H] = 931.5
150	$C_{49}H_{64}N_8O_8$	[M + H] = 893.49	[M + H] = 893.5
151	$C_{51}H_{66}N_8O_8$	[M + H] = 919.51	[M + H] = 919.5
152	$C_{51}H_{66}N_8O_8$	[M + H] = 919.51	[M + H] = 919.5
153	$C_{52}H_{68}N_8O_8$	[M + H] = 933.52	[M + H] = 934.5
154	$C_{52}H_{68}N_8O_8$	[M + H] = 933.52	[M + H] = 934.5
155	$C_{56}H_{69}FN_8O_8$	[M + H] = 1001.53	[M + H] = 1001.9
156	$C_{57}H_{72}N_8O_9$	[M + H] = 1013.55	[M + H] = 1013.9
157	$C_{50}H_{64}N_8O_8$	[M + H] = 905.49	[M + H] = 905.5
158	$C_{50}H_{64}N_8O_8$	[M + H] = 905.49	[M + H] = 905.6
159	$C_{52}H_{70}N_8O_9$	[M + H] = 951.53	[M + H] = 951.6
160	$C_{52}H_{70}N_8O_9$	[M + H] = 951.53	[M + H] = 951.6
161	$C_{51}H_{68}N_8O_9$	[M + H] = 937.52	[M + H] = 937.6
162	$C_{51}H_{68}N_8O_9$	[M + H] = 937.52	[M + H] = 937.6
163	$C_{52}H_{66}N_8O_8$	[M + H] = 931.51	[M + H] = 931.5
164	$C_{52}H_{66}N_8O_8$	[M + H] = 931.51	[M + H] = 931.5
165	$C_{52}H_{68}N_8O_8$	[M + H] = 933.53	[M + H] = 933.5
166	$C_{52}H_{66}N_8O_8$	[M + H] = 931.51	[M + H] = 931.5
167	$C_{51}H_{66}N_8O_8$	[M + H] = 919.51	[M + H] = 919.7
168	$C_{48}H_{64}N_{10}O_7S$	[M + H] = 925.48	[M + H] = 925.30
169	$C_{48}H_{64}N_{10}O_7S$	[M + H] = 925.48	[M + H] = 925.30
170	$C_{54}H_{70}N_8O_8$	[M + H] = 959.54	[M + H] = 959.40
171	$C_{51}H_{65}FN_8O_8$	[M + H] = 937.50	[M + H] = 937.3
172	$C_{51}H_{66}N_8O_8$	[M + H] = 919.51	[M + H] = 919.3
173	$C_{52}H_{69}N_9O_8$	[M + H] = 948.54	[M + H] = 948.5
174	$C_{54}H_{68}N_8O_8$	[M + H] = 957.53	[M + H] = 957.3
175	$C_{54}H_{68}N_8O_8$	[M + H] = 957.53	[M + H] = 957.3
176	$C_{52}H_{68}N_8O_7$	[M + H] = 917.53	[M + H] = 918.1
177	$C_{51}H_{67}N_9O_8$	[M + H] = 934.52	[M + H] = 934.3
178	$C_{51}H_{66}N_8O_8$	[M - H] = 917.49	[M - H] = 917.6
179	$C_{51}H_{66}N_8O_8$	[M - H] = 917.49	[M - H] = 917.7
180	$C_{53}H_{68}N_8O_8$	[M + H] = 945.53	[M + H] = 945.6
181	$C_{53}H_{68}N_8O_8$	[M + H] = 943.51	[M + H] = 943.6
182	$C_{51}H_{66}N_8O_8$	[M + H] = 919.51	[M + H] = 919.8
183	$C_{51}H_{65}N_9O_9$	[M + H] = 918.50	[M + H] = 948.6

Ex#	Molecular Formula	Calculated MW	Observed MW LCMS (ESI) *m/z*
184	$C_{51}H_{65}FN_8O_8$	[M + H] = 937.50	[M + H] = 937.3
185	$C_{51}H_{65}FN_8O_8$	[M + H] = 937.50	[M + H] = 937.2
186	$C_{51}H_{66}N_8O_8$	[M + H] = 919.51	[M + H] = 919.5
187	$C_{52}H_{63}N_9O_8$	[M + H] = 942.49	[M + H] = 942.3
188	$C_{50}H_{65}N_9O_8$	[M + H] = 920.51	[M + H] = 920.3
189	$C_{50}H_{65}N_9O_8$	[M + H] = 920.51	[M + H] = 920.3
190	$C_{52}H_{69}N_9O_8$	[M + H] = 948.54	[M + H] = 948.5
191	$C_{53}H_{68}N_8O_8$	[M + H] = 945.53	[M + H] = 945.5
192	$C_{53}H_{68}N_8O_8$	[M + H] = 945.53	[M + H] = 945.4
193	$C_{52}H_{68}N_8O_7$	[M + H] = 917.53	[M + H] = 918.0
194	$C_{51}H_{65}N_9O_9$	[M + H_2O] = 965.50	[M + H] = 966.4
195	$C_{53}H_{68}N_8O_8$	[M + H] = 945.53	[M + H] = 945.5
196	$C_{53}H_{68}N_8O_8$	[M + H] = 945.53	[M + H] = 945.4
197	$C_{51}H_{67}N_9O_8$	[M + H] = 934.52	[M + H] = 934.5
198	$C_{54}H_{70}N_8O_8$	[M + H] = 959.54	[M + H] = 959.3
199	$C_{53}H_{68}N_8O_8$	[M + H] = 945.53	[M + H] = 945.4
200	$C_{53}H_{68}N_8O_8$	[M + H] = 945.53	[M + H] = 945.5
201	$C_{53}H_{72}N_8O_9$	[M + H] = 965.55	[M + H] = 965.4
202	$C_{53}H_{68}N_8O_8$	[M + H] = 945.53	[M + H] = 945.8
203	$C_{51}H_{67}N_9O_8$	[M + H] = 934.52	[M + H] = 934.8
204	$C_{51}H_{69}N_9O_8$	[M + H] = 936.54	[M + H] = 936.8
205	$C_{51}H_{65}N_9O_9$	[M + H] = 948.50	[M + H] = 948.5
206	$C_{52}H_{68}N_8O_8$	[M + H] = 933.53	[M + H] = 933.7
207	$C_{52}H_{68}N_8O_8$	[M + H] = 933.53	[M + H] = 933.5
208	$C_{52}H_{68}N_8O_8$	[M + H] = 933.53	[M + H] = 933.7
209	$C_{56}H_{76}N_{10}O_8$	[M + H] = 1017.59	[M + H] = 1017.6
210	$C_{51}H_{67}N_9O_7S$	[M + H] = 950.50	[M + H] = 950.2
211	$C_{51}H_{67}N_7O_8$	[M + H] = 906.52	[M + H] = 906.4
212	$C_{50}H_{61}N_9O_8S$	[M + H] = 948.45	[M + H] = 948.1
213	$C_{49}H_{62}N_8O_8$	[M + H] = 891.48	[M + H] = 891.4
214	$C_{57}H_{72}N_8O_8$	[M + H] = 997.56	[M + H] = 997.2
215	$C_{57}H_{72}N_8O_8$	[M + H] = 997.56	[M + H] = 997.2
216	$C_{48}H_{66}N_{10}O_7S$	[M + H] = 927.49	[M + H] = 927.5
217	$C_{48}H_{66}N_{10}O_7S$	[M + H] = 927.49	[M + H] = 927.4
218	$C_{48}H_{64}N_{10}O_7S$	[M + H] = 925.48	[M + H] = 925.4
219	$C_{48}H_{64}N_{10}O_7S$	[M + H] = 925.48	[M + H] = 925.1
220	$C_{48}H_{64}N_{10}O_7S$	[M + H] = 925.48	[M + H] = 925.4

Ex#	Molecular Formula	Calculated MW	Observed MW LCMS (ESI) *m/z*
221	$C_{48}H_{64}N_{10}O_7S$	[M + H] = 925.48	[M + H] = 925.4
222	$C_{49}H_{66}N_{10}O_7S$	[M + H] = 939.49	[M + H] = 939.5
223	$C_{49}H_{66}N_{10}O_7S$	[M + H] = 939.49	[M + H] = 939.5
224	$C_{49}H_{66}N_{10}O_7S$	[M + H] = 939.49	[M + H] = 940.0
225	$C_{49}H_{66}N_{10}O_7S$	[M + H] = 939.49	[M + H] = 939.2
226	$C_{49}H_{66}N_{10}O_7S$	[M + H] = 939.49	[M + H] = 939.1
227	$C_{49}H_{66}N_{10}O_7S$	[M + H] = 939.49	[M + H] = 939.9
228	$C_{49}H_{66}N_{10}O_7S$	[M + H] = 939.49	[M + H] = 940.0
229	$C_{49}H_{66}N_{10}O_7S$	[M + H] = 939.49	[M + H] = 939.1
230	$C_{57}H_{77}N_9O_8$	[M + H] = 1016.60	[M + H] = 1016.6
231	$C_{51}H_{64}N_8O_9$	[M + H] = 933.49	[M + H] = 933.1
232	$C_{51}H_{64}N_8O_9$	[M + H] = 933.49	[M + H] = 933.2
233	$C_{55}H_{68}F_2N_8O_7$	[M + H] = 991.53	[M + H] = 992.0
234	$C_{54}H_{69}N_9O_7S$	[M + H] = 988.51	[M + H] = 988.1
235	$C_{49}H_{66}N_{10}O_7S$	[M + H] = 939.49	[M + H] = 939.5
236	$C_{52}H_{68}N_8O_8$	[M + H] = 933.53	[M + H] = 933.5
237	$C_{52}H_{68}N_8O_8$	[M + H] = 933.53	[M + H] = 933.5
238	$C_{52}H_{68}N_8O_8$	[M + H] = 933.53	[M + H] = 933.5
239	$C_{52}H_{68}N_8O_8$	[M + H] = 933.53	[M + H] = 933.5
240	$C_{52}H_{68}N_8O_8$	[M + H] = 933.53	[M + H] = 933.5
241	$C_{52}H_{68}N_8O_8$	[M + H] = 933.53	[M + H] = 933.5
242	$C_{52}H_{68}N_8O_8$	[M + H] = 933.53	[M + H] = 933.5
243	$C_{52}H_{68}N_8O_8$	[M + H] = 933.53	[M + H] = 933.5
244	$C_{52}H_{66}N_8O_8$	[M + H] = 931.51	[M + H] = 931.5
245	$C_{54}H_{70}N_8O_8$	[M + H] = 959.54	[M + H] = 959.5
246	$C_{54}H_{70}N_8O_8$	[M + H] = 959.54	[M + H] = 959.5
247	$C_{53}H_{70}N_8O_8$	[M + H] = 947.54	[M + H] = 947.4
248	$C_{52}H_{68}N_8O_9$	[M + H] = 949.52	[M + H] = 949.5
249	$C_{52}H_{68}N_8O_9$	[M + H] = 949.52	[M + H] = 949.5
250	$C_{52}H_{67}N_9O_8$	[M + H] = 946.52	[M + H] = 946.5
251	$C_{52}H_{67}N_9O_8$	[M + H] = 946.52	[M + H] = 946.5
252	$C_{52}H_{69}N_9O_8$	[M + H] = 948.54	[M + H] = 948.5
253	$C_{52}H_{69}N_9O_8$	[M + H] = 948.54	[M + H] = 948.5
254	$C_{52}H_{69}N_9O_8$	[M + H] = 948.54	[M + H] = 948.5
255	$C_{52}H_{69}N_9O_8$	[M + H] = 948.54	[M + H] = 948.5
256	$C_{50}H_{66}N_8O_8$	[M + H] = 907.51	[M + H] = 907.5
257	$C_{50}H_{66}N_8O_8$	[M + H] = 907.51	[M + H] = 907.5

Ex#	Molecular Formula	Calculated MW	Observed MW LCMS (ESI) *m/z*
258	$C_{50}H_{63}F_3N_8O_8$	[M + H] = 961.48	[M + H] = 961.5
259	$C_{50}H_{63}F_3N_8O_8$	[M + H] = 961.48	[M + H] = 961.5
260	$C_{50}H_{63}F_3N_8O_8$	[M + H] = 961.48	[M + H] = 961.5
261	$C_{50}H_{63}F_3N_8O_8$	[M + H] = 961.48	[M + H] = 961.5
262	$C_{51}H_{68}N_8O_8$	[M + H] = 921.53	[M + H] = 921.5
263	$C_{51}H_{68}N_8O_8$	[M + H] = 921.53	[M + H] = 921.5
264	$C_{52}H_{70}N_8O_8$	[M + H] = 935.54	[M + H] = 935.3
265	$C_{52}H_{70}N_8O_8$	[M + H] = 935.54	[M + H] = 935.3
266	$C_{54}H_{72}N_8O_{10}$	[M + H] = 993.55	[M + H] = 993.5
267	$C_{54}H_{72}N_8O_{10}$	[M + H] = 993.55	[M + H] = 993.5
268	$C_{56}H_{75}N_9O_9$	[M + H] = 1018.58	[M + H] = 1018.6
269	$C_{56}H_{75}N_9O_9$	[M + H] = 1018.58	[M + H] = 1018.5
270	$C_{53}H_{71}N_9O_9$	[M + H] = 978.55	[M + H] = 978.5
271	$C_{53}H_{71}N_9O_9$	[M + H] = 978.55	[M + H] = 978.5
272	$C_{55}H_{73}N_9O_{10}$	[M + H] = 1020.56	[M + H] = 1020.5
273	$C_{53}H_{69}N_9O_8$	[M + H] = 960.54	[M + H] = 960.5
274	$C_{53}H_{69}N_9O_8$	[M + H] = 960.54	[M + H] = 960.5
275	$C_{52}H_{68}N_8O_9$	[M + H] = 949.52	[M + H] = 949.3
276	$C_{52}H_{68}N_8O_9$	[M + H] = 949.52	[M + H] = 949.3
277	$C_{53}H_{70}N_8O_9$	[M + H] = 963.54	[M + H] = 963.5
278	$C_{53}H_{70}N_8O_9$	[M + H] = 963.54	[M + H] = 963.5
279	$C_{54}H_{72}N_8O_9$	[M + H] = 977.55	[M + H] = 977.5
280	$C_{54}H_{72}N_8O_9$	[M + H] = 977.55	[M + H] = 977.6
281	$C_{52}H_{68}N_8O_9$	[M + H] = 949.52	[M + H] = 949.3
282	$C_{52}H_{68}N_8O_9$	[M + H] = 949.52	[M + H] = 949.3
283	$C_{52}H_{68}N_8O_9$	[M + H] = 949.52	[M + H] = 949.5
284	$C_{52}H_{68}N_8O_9$	[M + H] = 949.52	[M + H] = 949.5
285	$C_{52}H_{68}N_8O_8$	[M + H] = 933.53	[M + H] = 933.5
286	$C_{49}H_{63}N_7O_7$	[M + H] = 862.49	[M + H] = 862.4
287	$C_{53}H_{70}N_8O_8$	[M + H] = 947.54	[M + H] = 947.5
288	$C_{53}H_{70}N_8O_8$	[M + H] = 947.54	[M + H] = 947.5
289	$C_{50}H_{68}N_{10}O_7S$	[M + H] = 953.51	[M + H] = 953.5
290	$C_{50}H_{68}N_{10}O_7S$	[M + H] = 953.51	[M + H] = 953.4
291	$C_{50}H_{68}N_{10}O_7S$	[M + H] = 953.51	[M + H] = 953.5
292	$C_{50}H_{68}N_{10}O_7S$	[M + H] = 953.51	[M + H] = 953.5
293	$C_{49}H_{66}N_{10}O_7S$	[M + H] = 939.49	[M + H] = 939.5
294	$C_{49}H_{66}N_{10}O_7S$	[M + H] = 939.49	[M + H] = 939.5

Ex#	Molecular Formula	Calculated MW	Observed MW LCMS (ESI) *m/z*
295	$C_{50}H_{68}N_{10}O_7S$	[M + H] = 953.51	[M + H] = 953.5
296	$C_{49}H_{66}N_{10}O_7S$	[M + H] = 939.49	[M + H] = 939.4
297	$C_{49}H_{66}N_{10}O_7S$	[M + H] = 939.49	[M + H] = 939.5
298	$C_{49}H_{66}N_{10}O_7S$	[M + H] = 939.49	[M + H] = 939.5
299	$C_{49}H_{66}N_{10}O_7S$	[M + H] = 939.49	[M + H] = 939.5
300	$C_{49}H_{66}N_{10}O_7S$	[M + H] = 939.49	[M + H] = 939.4
301	$C_{49}H_{66}N_{10}O_7S$	[M + H] = 939.49	[M + H] = 939.4
302	$C_{49}H_{66}N_{10}O_7S$	[M + H] = 939.49	[M + H] = 939.4
303	$C_{50}H_{66}N_{10}O_7S$	[M + H] = 951.49	[M + H] = 951.5
304	$C_{50}H_{66}N_{10}O_7S$	[M + H] = 951.49	[M + H] = 951.5
305	$C_{55}H_{70}N_8O_8$	[M + H] = 971.54	[M + H] = 971.1
306	$C_{52}H_{66}N_8O_8$	[M + H] = 931.51	[M + H] = 931.2
307	$C_{52}H_{66}N_8O_8$	[M + H] = 931.51	[M + H] = 931.2
308	$C_{55}H_{69}N_9O_8$	[M + H] = 984.54	[M + H] = 984.5
309	$C_{53}H_{70}N_8O_8$	[M + H] = 947.54	[M + H] = 947.5
310	$C_{49}H_{66}N_{10}O_7S$	[M + H] = 939.49	[M + H] = 939.5
311	$C_{51}H_{65}FN_8O_8$	[M + H] = 937.50	[M + H] = 937.2
312	$C_{49}H_{63}N_7O_8$	[M + H] = 878.48	[M + H] = 878.4
313	$C_{57}H_{72}N_8O_7$	[M + H] = 981.56	[M + H] = 981.5
314	$C_{54}H_{69}FN_8O_8$	[M + H] = 977.53	[M + H] = 977.5
315	$C_{54}H_{69}FN_8O_8$	[M + H] = 977.53	[M + H] = 977.5
316	$C_{55}H_{69}N_9O_8$	[M + H] = 984.54	[M + H] = 984.5
317	$C_{58}H_{78}N_8O_9$	[M + H] = 1031.60	[M + H] = 1031.5
318	$C_{58}H_{72}F_2N_8O_7$	[M + H] = 1031.56	[M + H] = 1031.5
319	$C_{58}H_{72}F_2N_8O_7$	[M + H] = 948.48	[M + H] = 948.4
320	$C_{51}H_{65}N_9O_7S$	[M + H] = 993.54	[M + H] = 993.5
321	$C_{55}H_{70}F_2N_8O_7$	[M + H] = 1013.57	[M + H] = 1013.5
322	$C_{55}H_{70}F_2N_8O_7$	[M + H] = 989.55	[M + H] = 989.5
323	$C_{58}H_{73}FN_8O_7$	[M + H] = 989.55	[M + H] = 989.5
324	$C_{58}H_{73}FN_8O_7$	[M + H] = 1086.60	[M + H] = 1086.6
325	$C_{55}H_{72}N_8O_9$	[M + H] = 1057.63	[M + H] = 1057.4
326	$C_{55}H_{72}N_8O_9$	[M + H] = 967.52	[M + H] = 967.5
327	$C_{59}H_{79}N_{11}O_7S$	[M + H] = 973.56	[M + H] = 973.4
328	$C_{59}H_{79}N_{11}O_7S$	[M + H] = 973.56	[M + H] = 973.5
329	$C_{54}H_{69}N_7O_{11}$	[M + H] = 992.52	[M + H] = 992.4
330	$C_{51}H_{65}N_7O_9$	[M + H] = 920.49	[M + H] = 920.6
331	$C_{57}H_{69}N_7O_9$	[M + H] = 996.53	[M + H] = 996.6

Ex#	Molecular Formula	Calculated MW	Observed MW LCMS (ESI) *m/z*
332	$C_{52}H_{67}N_7O_9$	[M + H] = 934.51	[M + H] = 934.6

Biological Assays

Compounds 1-2,4-18A, 19A-19B, 21A-24A, 27-32A, 33-43A, 44-45, 47B-54, 56-59, 68A, 69A, 71B, 72A, 73-78, 79B-82A, 83-97, 100-110, 112-117, 119-234, 236-294, and 297-332 exhibited: a) a % cross-linking to $KRAS^{G12D}$ of greater than zero within a 24-hour incubation timeframe in the assay described below; and/or b) an IC50 of 2 μM or less in the $KRAS^{G12D}$-B-Raf (AsPC-1) disruption assay described below.

Potency assay: pERK

The purpose of this assay is to measure the ability of test compounds to inhibit K-Ras in cells. Activated K-Ras induces increased phosphorylation of ERK at Threonine 202 and Tyrosine 204 (pERK). This procedure measures a decrease in cellular pERK in response to test compounds. The procedure described below in NCI-H358 cells is applicable to K-Ras G12C.

Note: This protocol may be executed substituting other cell lines to characterize inhibitors of other RAS variants, including, for example, AsPC-1 (K-Ras G12D), Capan-1 (K-Ras G12V), or NCI-H1355 (K-Ras G13C).

NCI-H358 cells were grown and maintained using media and procedures recommended by the ATCC. On the day prior to compound addition, cells were plated in 384-well cell culture plates (40 µl/well) and grown overnight in a 37°C, 5% CO2 incubator. Test compounds were prepared in 10, 3-fold dilutions in DMSO, with a high concentration of 10 mM. On the day of assay, 40 nL of test compound was added to each well of cell culture plate using an Echo550 liquid handler (LabCyte®). Concentrations of test compound were tested in duplicate. After compound addition, cells were incubated 4 hours at 37°C, 5% CO2. Following incubation, culture medium was removed and cells were washed once with phosphate buffered saline.

In some experiments, cellular pERK level was determined using the AlphaLISA SureFire Ultra p-ERK1/2 Assay Kit (PerkinElmer). Cells were lysed in 25 µL lysis buffer, with shaking at 600 RPM at room temperature. Lysate (10 µL) was transferred to a 384-well Opti-plate (PerkinElmer) and 5 µL acceptor mix was added. After a 2-hour incubation in the dark, 5 µL donor mix was added, plate was sealed, and incubated 2 hours at room temperature. Signal was read on an Envision plate reader (PerkinElmer) using standard AlphaLISA settings. Analysis of raw data was carried out in Excel (Microsoft) and Prism (GraphPad). Signal was plotted vs. the decadal logarithm of compound concentration, and IC_{50} was determined by fitting a 4-parameter sigmoidal concentration response model.

In other experiments, cellular pERK was determined by In-Cell Western. Following compound treatment, cells were washed twice with 200 µL tris buffered saline (TBS) and fixed for 15 minutes with 150 µL 4% paraformaldehyde in TBS. Fixed cells were washed 4 times for 5 minutes with TBS containing 0.1% Triton X-100 (TBST) and then blocked with 100 µL Odyssey

blocking buffer (LI-COR) for 60 minutes at room temperature. Primary antibody (pERK, CST-4370, Cell Signaling Technology) was diluted 1:200 in blocking buffer, and 50 µL was added to each well and incubated overnight at 4°C. Cells were washed 4 times for 5 minutes with TBST. Secondary antibody (IR-800CW rabbit, LI-COR, diluted 1:800) and DNA stain DRAQ5 (LI-COR, diluted 1:2000) were added and incubated 1-2 hours at room temperature. Cells were washed 4 times for 5 minutes with TBST. Plates were scanned on a Li-COR Odyssey CLx Imager. Analysis of raw data was carried out in Excel (Microsoft) and Prism (GraphPad). Signal was plotted vs. the decadal logarithm of compound concentration, and IC_{50} was determined by fitting a 4-parameter sigmoidal concentration response model.

The following compounds exhibited a pERK EC50 of under 5 uM (AsPC-1 KRAS G12D): 179,157,178,327,205,106,242,121,183,36,158,196,84,17AandB,87,187,114,182,255,254,185,236,124,197,1,107,192,34,118,296,78,89,104,74,306,310,105,152,269,229,221,294,117,119,240,151,193,86,245,128,163,272,270,79AandB,232,140,138,293,38,94,110,172,271,246,72AandB,108,35,14,127,7,153,39,190,96,227,13,77,286,215,244,184,284,275,147,295,204,50,161,129,176,51,290,226,218,164,282,167,162,131,228,292,233,308,304,48,9,113,298,277,54,57,219,173,220,268,49,149,247,120,154,307,56,166,11,53,101,10,8,238,97,303,132,186,52,297,93,85,83,280,103,200,276,278,144,165,199,33,139,112,224,177,241,273,237,274,191,243,319,320,225,59,311,207,239,279,160,289,171,156,92,202,43A,266,208,281,159,300,210,223,217,283,216,231,299,90,91,267,155,259,291,258,257,262,222,137,100,256,88,316,142,318,146,198,288,302,174,265,322,12,168,42A,201,301,263,248,287,58,305,260,134,169,313,314,323,234,136,148,102,315,141,150,309,326,261,321,175,230,249,264,95,285,135,133,170,317,328,214,209,324,325.

Determination of Cell Viability in RAS Mutant Cancer Cell Lines

Protocol: CellTiter-Glo® Cell Viability Assay

Note – The following protocol describes a procedure for monitoring cell viability of K-Ras mutant cancer cell lines in response to a compound of the invention. Other RAS isoforms may be employed, though the number of cells to be seeded will vary based on cell line used.

The purpose of this cellular assay was to determine the effects of test compounds on the proliferation of three human cancer cell lines (NCI-H358 (K-Ras G12C), AsPC-1 (K-Ras G12D), and Capan-1 (K-Ras G12V)) over a 5-day treatment period by quantifying the amount of ATP present at endpoint using the CellTiter-Glo® 2.0 Reagent (Promega).

Cells were seeded at 250 cells/well in 40 µL of growth medium in 384-well assay plates and incubated overnight in a humidified atmosphere of 5% CO_2 at 37°C. On the day of the assay, 10 mM stock solutions of test compounds were first diluted into 3 mM solutions with 100% DMSO. Well-mixed compound solutions (15 µL) were transferred to the next wells containing 30 µL of 100% DMSO, and repeated until a 9-concentration 3-fold serial dilution was made (starting assay concentration of 10 µM). Test compounds (132.5 nL) were directly dispensed into the assay plates containing cells. The plates were shaken for 15 seconds at 300 rpm, centrifuged, and incubated in a humidified atmosphere of 5% CO_2 at 37 °C for 5 days. On day 5, assay plates and their contents were equilibrated to room temperature for approximately 30 minutes. CellTiter-Glo® 2.0 Reagent

(25 µL) was added, and plate contents were mixed for 2 minutes on an orbital shaker before incubation at room temperature for 10 minutes. Luminescence was measured using the PerkinElmer Enspire. Data were normalized by the following: (Sample signal/Avg. DMSO)*100. The data were fit using a four-parameter logistic fit.

*Key:

+++++: IC50 ≥ 10 uM

++++: 10 uM > IC50 ≥ 1 uM

+++: 1 uM > IC50 ≥ 0.1 uM

++: 0.1 uM > IC50 ≥ 0.01 uM

+: IC50 < 0.01 uM

Table 5. H358 Cell Viability assay data (K-Ras G12C, IC50, uM):

IC50*	Examples of A compounds
+	1,44,58,59,90,95,106,136,137,142,146,148,150,155,156,159,160,165,171,174,175,186,201,207,209,222,231,236,241,261,266,267,273,274,279,280,299,301,307,319,324,325,328,42A,43A
++	2,5,7,8,9,10,13,33,41,83,85,100,102,132,133,135,144,168,169,170,191,208,214,216,217,223,224,225,226,227,228,230,234,239,248,249,256,257,258,259,260,262,263,264,265,270,276,277,283,287,288,289,290,291,292,293,296,297,298,300,302,303,316,317,322,323,326,42B,43B
+++	3,4,6,11,12,14,40,45,46,48,50,52,56,63,77,103,107,141,202,210,243,285,294,304,313,318,320,321
++++	26,29,30,31,49,60,66,96,15 A and B,16 A and B,16 A and B,17 A and B,17 A and B,18 A and B,18 A and B,19 A and B,19 A and B,20 A and B,20 A and B,22 A and B,23 A and B,24 A and B,24 A and B,32 A and B,32 A and B,47 A and B,62 A and B,62 A and B,68 A and B,68 A and B,69 A and B,69 A and B
+++++	15 A and B,25,27,28,47 A and B,67

Table 6. AsPC-1 Cell Viability assay data (K-Ras G12D, IC50, uM):

IC50*	Examples of A compounds
+	209,325
++	95,133,170,175,214,230,249,285,309,313,317,321,324

+++	7,12,58,59,77,88,90,100,102,103,135,136,137,141,142,144,146,148,150,155,156,159,160,168,169,171,174,186,198,201,207,210,216,217,222,223,224,225,228,231,234,239,241,243,248,256,257,258,259,260,261,262,263,264,265,266,267,270,273,274,277,279,281,283,287,288,289,291,297,298,299,300,301,302,303,305,307,311,314,315,318,319,320,323,326,328,19 A and B,42A,43A
++++	1,8,9,10,11,13,14,29,34,35,38,39,44,48,49,52,53,54,56,57,60,63,73,74,75,76,78,80,81,83,84,85,86,87,91,92,96,97,101,104,105,106,107,108,110,112,113,114,117,119,120,121,122,128,129,132,134,139,140,147,149,151,152,153,154,157,158,161,162,163,164,165,166,167,172,173,176,177,184,185,187,190,191,192,193,196,197,199,200,202,204,208,215,218,219,220,221,226,227,229,232,233,236,237,238,240,242,244,245,246,247,255,268,269,272,275,276,278,280,282,284,286,290,292,293,294,295,296,304,306,308,310,316,322,327,16 A and B,16 A and B,17 A and B,17 A and B,18 A and B,18 A and B,22 A and B,23 A and B,32 A and B,42B,61 A and B,61 A and B,62 A and B,62 A and B,64 A and B,65 A and B,65 A and B,68 A and B,69 A and B,69 A and B,70 A and B,71 A and B,72 A and B,72 A and B,79 A and B,79 A and B,82 A and B,82 A and B
+++++	2,3,4,5,6,28,31,36,37,40,41,45,46,50,51,55,66,67,89,93,94,98,99,109,111,115,116,118,126,127,130,131,138,143,145,178,179,180,181,182,183,188,189,194,195,203,205,206,211,212,213,235,250,251,252,253,254,271,312,15 A and B,15 A and B,20 A and B,20 A and B,24 A and B,24 A and B,32 A and B,43B,47 A and B,47 A and B,64 A and B,70 A and B,71 A and B

Disruption of B-Raf Ras-binding Domain ($BRAF^{RBD}$) Interaction with K-Ras by Compounds of the Invention (also called a FRET assay or an MOA assay)

Note – The following protocol describes a procedure for monitoring disruption of K-Ras G12C (GMP-PNP) binding to $BRAF^{RBD}$ by a compound of the invention. This protocol may also be executed substituting other Ras proteins or nucleotides, such as K-Ras G12D and K-Ras G13D.

The purpose of this biochemical assay was to measure the ability of test compounds to facilitate ternary complex formation between a nucleotide-loaded K-Ras isoform and Cyclophilin A; the resulting ternary complex disrupts binding to a $BRAF^{RBD}$ construct, inhibiting K-Ras signaling through a RAF effector. Data is reported as IC50 values.

In assay buffer containing 25 mM HEPES pH 7.3, 0.002% Tween20, 0.1% BSA, 100 mM NaCl and 5 mM $MgCl_2$, tagless Cyclophilin A, His6-K-Ras-GMPPNP, and GST-$BRAF^{RBD}$ were combined in a 384-well assay plate at final concentrations of 25 µM, 12.5 nM, and 50 nM, respectively. Compound was present in plate wells as a 10-point 3-fold dilution series starting at a final concentration of 30 µM. After incubation at 25 °C for 3 hours, a mixture of anti-His Eu-W1024 and anti-GST allophycocyanin was then added to assay sample wells at final concentrations of 10 nM and 50 nM, respectively, and the reaction incubated for an additional 1.5 hours. TR-FRET signal was read on a microplate reader (Ex 320 nm, Em 665/615 nm). Compounds that facilitate

disruption of a K-Ras:RAF complex were identified as those eliciting a decrease in the TR-FRET ratio relative to DMSO control wells.

Ras-Raf disruption/FRET/MOA assay data (IC50, uM):

*Key:

+++++: IC50 ≥ 10 uM

++++: 10 uM > IC50 ≥ 1 uM

+++: 1 uM > IC50 ≥ 0.1 uM

++: 0.1 uM > IC50 ≥ 0.01 uM

+: IC50 < 0.01 uM

Table 7. KRAS G13D FRET data

IC50*	Examples of A compounds
+	None
++	12,58,83,85,86,88,89,95,100,109,113,115,120,129,130,133,135,136,137,138,139,140,143,148,149,150,157,158,162,167,170,171,172,173,174,175,176,177,182,186,193,194,195,196,197,198,204,209,230,231,239,241,248,249,256,257,262,263,264,265,274,276,285,286,288,306,307,309,325,329,330,332,258*,259*,260*,261*,281*,282*,283*,284*,314*,315*,316*,42A,43A
+++	1,2,3,4,5,7,8,9,11,13,14,33,34,35,36,37,38,39,40,41,44,45,48,49,50,51,52,53,54,56,57,59,84,87,90,91,92,93,94,96,97,101,102,103,104,105,108,110,111,112,116,117,118,119,127,128,131,134,141,142,144,145,146,147,151,152,153,155,156,159,160,161,163,164,165,166,168,169,178,179,180,181,183,184,185,187,188,189,190,191,199,200,201,202,203,205,206,207,208,212,213,214,215,216,217,218,219,220,221,222,223,224,225,226,227,228,229,232,236,237,238,242,243,244,245,246,247,252,253,254,255,266,267,268,269,270,271,272,273,275,277,278,279,280,287,289,291,293,294,297,298,299,300,301,302,303,305,308,311,317,322,323,326,328,331,47 A and B,69 A and B
++++	6,10,25,26,27,28,29,30,31,46,55,66,67,73,74,98,106,107,114,126,132,154,192,210,211,233,235,240,250,251,290,292,295,296,304,310,312,313,318,319,320,321,324,15 A and B,17 A and B,18 A and B,19 A and B,24 A and B,32 A and B,32 A and B,42B,43B,47 A and B,62 A and B,72 A and B
+++++	60,63,75,76,77,78,80,81,99,121,122,123,124,125,234,327,15 A and B,16 A and B,16 A and B,17 A and B,18 A and B,19 A and B,20 A and B,20 A and B,22 A and B,23 A and B,24 A and B,61 A and B,61 A and B,62 A and B,64 A and B,64 A and B,65 A and B,65 A and B,68 A and B,68 A and B,69 A and B,70 A and B,70 A and B,71 A and B,71 A and B,72 A and B,79 A and B,79 A and B,82 A and B,82 A and B

Table 8. KRAS G12S FRET data

IC50*	Examples of A compounds

+	325,264,281,135
++	263,258,230,249,287,282,209,139,315,170,283,267,150,260,274,284,42A,167,276,261,133,137,12,256,136,257,285,288,265,88,85,231,316,330,269,262,280,148,186,278,182,248,259,309,162,317,314,332,160,140,175,305,95,308,158,113,273,323,268,173,322,306,43A,214,177,171,161,146,272,207,329,157,197,201,147,103,208,200,328,241,172,90,89,105,149,279,100,159,238,93,271,91,48,174,7,35,155,307,53,36,52,92,190,156,237,302,326,59,8,142,37,33,11,141,97,127,10,13,277,58,104,275,222,129,266,204,39,191,110,176,301,96,331,54,198,239,130,255,134,83,50,1,34,289,86,38,47 A and B,168,143,169,270,298
+++	14,115,152,109,49,102,223,189,44,291,144,297,120,202,153,187,185,188,56,138,195,224,254,51,178,244,286,194,45,225,181,246,9,205,166,101,196,252,2,116,243,193,216,253,179,293,217,192,112,245,311,199,203,94,165,3,163,232,151,5,294,108,183,46,215,299,119,117,4,300,164,303,184,213,227,145,57,128,304,6,220,229,218,226,228,87,251,131,84,219,111,221,242,154,310,247,180,40,47 A and B,41,290,118,236,292,31,324,296,321,250,212,206,55,126
++++	210,240,77,121,211,319,42B,313,132,114,98,320,295,318,43B,235,122,107,62 A and B,312,24 A and B,106,74,234,78,66,15 A and B,233,73,69 A and B,63,29,67,22 A and B,80,23 A and B
+++++	25,124,32 A and B,327,27,28,26,62 A and B,72 A and B,20 A and B,79 A and B,19 A and B,16 A and B,123,24 A and B,30,17 A and B,15 A and B,19 A and B,32 A and B,18 A and B,18 A and B,17 A and B,16 A and B,20 A and B,69 A and B,60,68 A and B,68 A and B,65 A and B,65 A and B,64 A and B,64 A and B,70 A and B,71 A and B,70 A and B,71 A and B,72 A and B,61 A and B,61 A and B,82 A and B,82 A and B,81,79 A and B,76,75,99,125

Table 9. KRAS G13C FRET data

IC50*	Examples of A compounds
+	84,85,89,129,135,136,139,140,182,195,205,209,230,231,256,257,258,260,282,283,284,285,325,329,330,42A
++	3,4,7,8,11,12,13,14,34,35,36,37,38,39,41,44,45,48,49,50,51,53,54,56,57,58,59,77,78,83,86,87,88,90,91,92,93,94,95,96,97,100,102,103,104,105,108,109,112,113,115,117,118,119,120,124,127,128,130,131,133,134,137,138,141,142,143,144,145,146,147,148,149,150,152,155,156,157,158,159,160,161,162,167,169,170,171,172,173,174,175,176,177,178,179,180,181,183,184,185,186,187,188,189,190,191,193,194,196,197,198,200,201,203,204,207,208,212,213,214,215,232,237,238,239,241,245,246,248,249,251,252,253,254,255,259,261,262,263,264,265,266,267,268,269,270,271,272,273,274,275,276,277,278,279,280,281,286,287,288,297,301,305,306,307,308,309,311,314,315,316,317,322,323,326,328,331,332,43A,47 A and B,47 A and B,69 A and B
+++	1,2,5,6,9,10,31,33,40,46,52,55,66,74,76,98,101,106,107,110,111,114,116,121,122,12

	3,125,126,151,153,154,163,164,165,166,168,192,199,202,206,211,216,217,218,219,220,221,222,223,224,225,226,227,228,229,233,236,243,244,247,250,289,290,291,292,293,294,296,298,299,300,302,303,310,312,313,318,319,320,321,324,22 A and B,42B,70 A and B
++++	25,27,28,29,63,67,73,80,132,210,234,235,240,242,295,304,327,15 A and B,16 A and B,17 A and B,17 A and B,18 A and B,19 A and B,20 A and B,23 A and B,24 A and B,32 A and B,43B,62 A and B,65 A and B,68 A and B,69 A and B,72 A and B,79 A and B
+++++	26,30,60,75,81,99,15 A and B,16 A and B,18 A and B,19 A and B,20 A and B,24 A and B,32 A and B,61 A and B,61 A and B,62 A and B,64 A and B,64 A and B,65 A and B,68 A and B,70 A and B,71 A and B,71 A and B,72 A and B,79 A and B,82 A and B,82 A and B

Table 10. KRAS G12V FRET data

IC50*	Examples of A compounds
+	325
++	1,11,12,13,36,44,48,50,58,59,83,85,88,90,93,95,96,97,100,103,113,133,135,136,137,139,140,141,146,147,148,149,150,151,155,156,157,158,159,160,161,162,165,167,170,171,172,173,174,175,177,182,186,189,190,192,197,200,201,204,207,208,209,214,230,231,239,241,248,249,256,257,258,259,260,261,262,263,264,265,267,268,269,272,273,274,276,278,279,280,281,282,283,284,285,287,288,302,305,306,307,309,314,315,316,317,322,323,326,328,329,330,331,332,42A,43A
+++	2,3,4,5,6,7,8,9,10,14,31,33,34,35,37,38,39,40,41,45,49,51,52,53,54,55,56,57,84,86,87,89,91,92,94,101,102,104,105,108,109,110,111,112,115,116,117,118,119,120,127,128,129,130,131,134,142,143,144,145,152,153,154,163,164,166,168,169,176,178,179,180,181,183,184,185,187,188,191,194,195,196,198,199,202,203,205,206,212,213,215,216,217,218,219,220,221,222,223,224,225,226,227,228,229,232,236,237,238,243,244,245,246,247,250,251,252,253,254,255,266,270,271,275,277,286,289,290,291,292,293,294,297,298,299,300,301,303,304,308,310,311,321,324,47 A and B
++++	29,46,66,67,74,77,78,80,98,106,107,114,121,122,123,124,126,132,138,193,210,211,233,234,235,240,242,295,296,312,313,318,319,320,15 A and B,16 A and B,22 A and B,24 A and B,42B,43B,47 A and B,62 A and B,69 A and B,72 A and B,79 A and B
+++++	73,25,26,27,28,30,60,63,75,76,81,99,125,327,15 A and B,16 A and B,17 A and B,17 A and B,18 A and B,18 A and B,19 A and B,19 A and B,20 A and B,20 A and B,23 A and B,24 A and B,32 A and B,32 A and B,61 A and B,61 A and B,62 A and B,64 A and B,64 A and B,65 A and B,65 A and B,68 A and B,68 A and B,69 A and B,70 A and B,70 A and B,71 A and B,71 A and B,72 A and B,79 A and B,82 A and B,82 A and B

Table 11. KRAS G12D FRET data

IC50*	Examples of A compounds
+	214,305,325
++	59,88,95,103,113,133,135,136,137,139,140,141,146,148,150,167,170,171,173,175,186,198,209,230,231,241,248,249,256,258,260,261,263,264,265,267,269,274,276,278,280,281,282,283,284,285,287,309,315,316,330,42A,43A
+++	1,2,7,8,12,13,14,33,34,35,36,37,38,39,40,41,44,45,48,49,50,51,52,53,54,56,58,73,74,83,85,86,89,90,91,92,93,96,97,100,101,102,104,105,110,112,115,120,127,128,129,130,131,134,138,142,143,144,147,149,151,152,153,155,156,157,158,159,160,161,162,163,164,165,166,168,169,172,174,176,177,178,179,181,182,184,185,187,188,189,190,191,192,193,194,196,197,199,200,201,202,203,204,207,208,215,216,217,222,223,224,225,227,236,237,238,239,242,243,244,245,252,253,254,255,257,259,262,266,268,270,271,272,273,275,277,279,286,288,289,291,293,294,297,298,299,300,301,302,303,304,306,307,308,311,313,314,317,321,322,323,324,326,328,329,331,332,70 A and B
++++	16 A and B,3,4,5,6,9,10,11,29,31,46,57,63,66,67,76,77,78,80,84,87,94,98,106,107,108,109,111,114,116,117,118,119,121,122,123,124,125,126,132,145,154,180,183,195,205,206,210,211,212,213,218,219,220,221,226,228,229,232,233,234,235,240,246,247,250,251,290,292,295,296,310,312,318,319,320,327,15 A and B,17 A and B,17 A and B,18 A and B,19 A and B,22 A and B,23 A and B,24 A and B,32 A and B,42B,43B,47 A and B,47 A and B,62 A and B,68 A and B,69 A and B,72 A and B,82 A and B
+++++	25,26,27,28,30,55,60,75,81,99,15 A and B,16 A and B,18 A and B,19 A and B,20 A and B,20 A and B,24 A and B,32 A and B,61 A and B,61 A and B,62 A and B,64 A and B,64 A and B,65 A and B,65 A and B,68 A and B,69 A and B,70 A and B,71 A and B,71 A and B,72 A and B,79 A and B,79 A and B,82 A and B

Table 12. KRAS WT FRET data

IC50*	Examples of A compounds
+	264,325
++	12,13,35,36,37,44,45,52,53,54,58,59,83,85,86,88,89,90,91,92,93,95,96,97,100,102,103,105,109,110,112,113,115,116,120,129,130,133,134,135,136,137,138,139,140,141,143,146,147,148,149,150,155,156,157,158,159,160,161,162,167,168,169,170,171,172,173,174,175,176,177,182,183,186,189,190,191,193,195,196,197,198,201,204,205,207,208,209,213,214,222,223,230,231,237,238,239,241,245,246,248,249,252,253,256,257,258,259,260,261,262,263,265,267,268,269,271,272,273,274,276,278,279,280,281,282,283,284,285,287,288,289,291,297,298,301,302,305,306,307,308,309,314,315,316,317,322,323,326,328,329,330,331,332,42A,43A
+++	1,2,3,4,5,6,7,8,9,10,11,14,33,34,38,39,40,41,48,49,50,51,56,57,84,87,94,98,101,104,108,111,117,118,119,121,127,128,131,142,144,145,151,152,153,154,163,164,165,166,178,179,180,181,184,185,187,188,192,194,199,200,202,203,206,211,212,215,216,21

	7,218,219,220,221,224,225,226,227,228,229,232,236,240,242,243,244,247,250,251,254,255,266,270,275,277,286,290,292,293,294,295,296,299,300,303,304,310,311,312,318,321,324,47 A and B
++++	25,26,27,29,30,31,46,55,63,66,67,73,74,76,77,78,80,106,107,114,122,123,124,126,132,210,233,234,235,313,319,320,327,15 A and B,16 A and B,22 A and B,23 A and B,24 A and B,42B,43B,47 A and B,62 A and B,69 A and B,79 A and B
+++++	28,60,75,81,99,125,15 A and B,16 A and B,17 A and B,17 A and B,18 A and B,18 A and B,19 A and B,19 A and B,20 A and B,20 A and B,24 A and B,32 A and B,32 A and B,61 A and B,61 A and B,62 A and B,64 A and B,64 A and B,65 A and B,65 A and B,68 A and B,68 A and B,69 A and B,70 A and B,70 A and B,71 A and B,71 A and B,72 A and B,72 A and B,79 A and B,82 A and B,82 A and B

Table 13. KRAS G12C FRET data

IC50*	Examples of A compounds
+	1,7,41,58,88,90,95,109,113,115,135,137,139,140,142,146,148,149,150,151,156,161,162,165,167,171,174,175,183,184,186,190,192,196,198,200,201,203,204,209,230,231,241,249,252,263,264,266,267,268,269,270,271,272,273,274,280,281,306,307,309,311,312,325,329,330,331,42A,43A
++	2,3,4,5,6,8,9,10,11,12,13,14,31,33,34,35,36,37,38,39,44,45,48,49,50,51,52,53,54,56,59,73,74,77,78,83,84,85,86,87,89,91,92,93,94,96,97,100,101,102,103,104,105,106,110,114,117,119,124,125,128,129,130,131,133,134,136,138,141,143,144,147,152,153,154,155,157,158,159,160,168,169,170,172,173,176,177,178,179,181,182,185,188,189,191,193,195,197,202,205,206,207,208,211,213,214,215,216,217,220,222,223,224,225,232,236,237,238,239,243,244,245,246,247,248,250,251,253,254,255,256,257,258,259,260,261,262,265,275,276,277,278,279,282,283,284,285,287,288,289,291,293,294,297,298,299,301,302,305,308,314,315,316,317,319,322,323,326,328,332,16 A and B,17 A and B,18 A and B,22 A and B,23 A and B,32 A and B,43B,68 A and B,69 A and B,82 A and B
+++	40,46,55,57,66,67,75,76,80,81,98,107,108,111,112,116,118,120,121,122,123,126,127,132,145,163,164,166,180,187,194,199,210,212,218,219,221,226,227,228,229,233,234,235,240,242,286,290,292,295,296,300,303,304,310,313,318,320,321,324,15 A and B,16 A and B,17 A and B,19 A and B,20 A and B,24 A and B,42B,47 A and B,61 A and B,62 A and B,70 A and B,72 A and B,79 A and B
++++	25,26,27,28,29,63,327,18 A and B,32 A and B,47 A and B,62 A and B,65 A and B,68 A and B,69 A and B,71 A and B,72 A and B,82 A and B
+++++	30,60,99,15 A and B,19 A and B,20 A and B,24 A and B,61 A and B,64 A and B,64 A and B,65 A and B,70 A and B,71 A and B,79 A and B

Table 14. KRAS Q61H FRET data

IC50*	Examples of A compounds
+	209,230,231,246,249,258,264,281,325
++	36,37,91,92,108,117,119,141,144,163,164,166,167,170,186,202,203,204,205,207,208,212,213,214,215,216,217,218,220,222,223,224,225,227,228,229,232,237,238,239,241,244,245,248,252,253,254,255,256,257,259,260,261,262,263,265,266,267,268,269,271,272,273,274,275,276,277,278,279,280,282,283,284,285,286,287,288,289,291,293,294,297,298,299,300,301,302,303,304,305,306,307,308,309,311,314,315,316,317,322,323,326,328,329,330,331,332
+++	165,206,210,211,219,221,226,233,235,236,240,242,243,247,250,251,270,290,292,295,296,310,312,313,318,319,320,321,324
++++	234,327
+++++	None

Table 15. NRAS G12C FRET data

IC50*	Examples of A compounds
+	1,5,7,41,58,59,86,88,90,95,109,113,115,135,136,137,138,139,140,142,146,148,149,150,151,155,156,159,160,161,162,165,167,171,174,175,184,186,192,196,198,200,201,203,204,209,230,231,241,249,252,263,264,266,267,268,269,270,271,272,273,274,276,278,280,281,284,306,307,309,311,312,325,329,330,42A,43A
++	2,3,6,8,9,10,11,12,13,14,33,34,35,36,37,38,39,44,45,48,49,50,52,53,54,73,74,77,78,83,84,85,87,89,91,92,93,96,97,100,101,102,103,104,105,106,110,114,124,125,128,129,130,131,133,134,141,143,144,147,152,153,154,157,158,168,169,170,172,173,176,177,178,179,181,182,183,185,188,189,190,191,193,195,197,202,205,206,207,208,211,213,214,215,216,217,218,222,223,224,225,227,228,232,236,237,238,239,243,244,245,246,247,248,251,253,254,255,256,257,258,259,260,261,262,265,275,277,279,282,283,285,287,288,289,291,293,294,297,298,299,300,301,302,305,308,314,315,316,317,319,322,323,326,328,331,332,16 A and B,17 A and B,18 A and B,22 A and B,23 A and B,43B,68 A and B,69 A and B
+++	219,4,31,40,46,51,55,56,57,66,67,75,76,80,94,107,108,111,112,116,117,118,119,120,121,122,123,126,127,132,145,163,164,166,180,187,194,199,210,212,220,221,226,229,233,235,240,242,250,286,290,292,295,296,303,304,310,313,320,321,324,15 A and B,16 A and B,17 A and B,19 A and B,20 A and B,32 A and B,42B,47 A and B,61 A and B,62 A and B,70 A and B,71 A and B,72 A and B,72 A and B,79 A and B,82 A and B
++++	318,26,28,29,63,81,98,234,327,18 A and B,24 A and B,47 A and B,62 A and B,68 A and B,69 A and B,82 A and B
+++++	25,27,30,60,99,15 A and B,19 A and B,20 A and B,24 A and B,32 A and B,61 A and B,64 A and B,64 A and B,65 A and B,65 A and B,70 A and B,71 A and B,79 A and B

Table 16. NRAS WT FRET data

IC50*	Examples of A compounds
+	258,264,315,325
++	37,91,92,117,141,167,170,186,204,205,207,208,209,213,214,217,222,223,224,225,230,231,237,238,239,241,245,246,248,249,252,253,255,256,257,259,260,261,262,263,265,267,268,269,272,273,274,276,278,279,280,281,282,283,284,285,287,288,289,291,297,298,301,302,305,306,307,308,309,311,314,316,317,322,323,326,328,329,330,331,332
+++	36,108,119,144,163,164,165,166,202,203,206,211,212,215,216,218,219,220,221,226,227,228,229,232,236,242,243,244,247,250,251,254,266,270,271,275,277,286,290,292,293,294,295,296,299,300,303,304,310,312,321,324
++++	210,233,234,235,240,313,318,319,320
+++++	327

Table 17. NRAS Q61K FRET data

IC50*	Examples of A compounds
+	None
++	167,170,216,217,218,219,220,222,223,224,225,227,228,229,231,246,249,258,260,264,281,289,291,293,294,297,298,299,300,301,302,315,325,326,329
+++	36,37,91,92,108,117,119,141,163,186,202,203,204,205,207,208,209,212,213,214,221,226,230,232,235,237,238,239,241,245,248,252,253,254,255,256,257,259,261,262,263,265,266,267,268,269,271,272,273,274,275,276,277,278,279,280,282,283,284,285,286,287,288,290,292,295,296,303,304,305,306,307,308,309,310,311,314,316,317,319,322,323,324,328,330,331,332
++++	144,164,165,166,206,210,211,215,233,234,236,242,243,244,247,250,251,270,312,313,318,320,321
+++++	240,327

Table 18. NRAS Q61R FRET data

IC50*	Examples of A compounds
+	None
++	170,209,222,223,224,230,249,258,264,289,297,298,301,302,325,326,329
+++	36,37,91,92,108,117,141,163,167,186,202,203,204,205,207,208,212,213,214,216,217,218,219,220,221,225,226,227,228,229,231,232,237,238,239,241,244,245,246,248,252,253,254,255,256,257,259,260,261,262,263,265,266,267,268,269,271,272,273,274,275,276,278,279,280,281,282,283,284,285,286,287,288,290,291,292,293,294,296,299,300,303,304,305,306,307,308,309,310,311,314,315,316,317,322,323,324,328,330,331,332
++++	119,144,164,165,166,206,210,211,215,234,235,236,240,242,243,247,250,251,270,277,295,312,313,318,319,320,321

+++++	233,327

Cross-linking of Ras Proteins with Compounds of the Invention to Form Conjugates

Note – The following protocol describes a procedure for monitoring cross-linking of K-Ras G12C (GMP-PNP) to a compound of the invention. This protocol may also be executed substituting other Ras proteins or nucleotides, such as such as K-Ras G12D and K-Ras G13D.

The purpose of this biochemical assay was to measure the ability of test compounds to covalently label nucleotide-loaded K-Ras isoforms. In assay buffer containing 12.5 mM HEPES pH 7.4, 75 mM NaCl, 1 mM $MgCl_2$, 1 mM BME, 5 µM Cyclophilin A, and 2 µM test compound, a 5 µM stock of GMP-PNP-loaded K-Ras (1-169) G12C was diluted 10-fold to yield a final concentration of 0.5 µM; with final sample volume being 100 µL.

The sample was incubated at 25 °C for a time period of up to 24 hours prior to quenching by the addition of 10 µL of 5% Formic Acid. Quenched samples were centrifuged at 15000 rpm for 15 minutes in a benchtop centrifuge before injecting a 10 µL aliquot onto a reverse phase C4 column and eluting into the mass spectrometer with an increasing acetonitrile gradient in the mobile phase. Analysis of raw data was carried out using Waters MassLynx MS software, with % bound calculated from the deconvoluted protein peaks for labeled and unlabeled K-Ras.

In vitro Cell Proliferation Panels

Potency for inhibition of cell growth was assessed at CrownBio using standard methods. Briefly, cell lines were cultured in appropriate medium, and then plated in 3D methylcellulose. Inhibition of cell growth was determined by CellTiter-Glo® after 5 days of culture with increasing concentrations of compounds. Compound potency was reported as the 50% inhibition concentration (absolute IC50).

The assay took place over 7 days. On day 1, cells in 2D culture were harvested during logarithmic growth and suspended in culture medium at 1x105 cells/ml. Higher or lower cell densities were used for some cell lines based on prior optimization. 3.5 ml of cell suspension was mixed with 6.5% growth medium with 1% methylcellulose, resulting in a cell suspension in 0.65% methylcellulose. 90 µl of this suspension was distributed in the wells of 2 96-well plates. One plate was used for day 0 reading and 1 plate was used for the end-point experiment. Plates were incubated overnight at 37 C with 5% CO2. On day 2, one plate (for t0 reading) was removed and 10 µl growth medium plus 100 µl CellTiter-Glo® Reagent was added to each well. After mixing and a 10 minute incubation, luminescence was recorded on an EnVision Multi-Label Reader (Perkin Elmer). Compounds in DMSO were diluted in growth medium such that the final, maximum concentration of compound was 10 µM, and serial 4-fold dilutions were performed to generate a 9-point concentration series. 10 µl of compound solution at 10 times final concentration was added to wells of the second plate. Plate was then incubated for 120 hours at 37C and 5% CO2. On day 7 the plates were removed, 100 µl CellTiter-Glo® Reagent was added to each well, and after mixing and a 10 minute incubation, luminescence was recorded on an EnVision Multi-Label Reader

(Perkin Elmer). Data was exported to GeneData Screener and modeled with a sigmoidal concentration response model in order to determine the IC50 for compound response.

Not all cell lines with a given RAS mutation may be equally sensitive to a RAS inhibitor targeting that mutation, due to differential expression of efflux transporters, varying dependencies on RAS pathway activation for growth, or other reasons. This has been exemplified by the cell line KYSE-410 which, despite having a KRAS G12C mutation, is insensitive to the KRAS G12C (OFF) inhibitor MRTX-849 (Hallin et al., Cancer Discovery 10:54-71 (2020)), and the cell line SW1573, which is insensitive to the KRAS G12C (OFF) inhibitor AMG510 (Canon et al., Nature 575:217-223 (2019)).

Table 19: IC50 values for various cancer cell lines with Compound B

*Key:

low sensitivity: IC50 ≥ 1 uM

moderately sensitive: 1 uM > IC50 ≥ 0.1 uM

very sensitive: IC50 < 0.1 uM

blank = not measured

Cell Line	Histotype	Cancer Driver/Mutant	IC50*
A-375	Skin	BRAF V600E	low sensitivity
KYSE-410	HN/Esophagus	KRAS G12C	moderately sensitive
MIA PaCa-2	Pancreas	KRAS G12C	very sensitive
NCI-H358	Lung	KRAS G12C	very sensitive
SW1573	Lung	KRAS G12C	low sensitivity
SW837	Intestine/Large/Colorectum	KRAS G12C	very sensitive
LS513	Intestine/Large/Colorectum	KRAS G12D	moderately sensitive
HuCCT1	Liver/Bile duct	KRAS G12D	very sensitive
HCC1588	Lung	KRAS G12D	low sensitivity
HPAC	Pancreas	KRAS G12D	very sensitive
AsPC-1	Pancreas	KRAS G12D	moderately sensitive
AGS	Stomach	KRAS G12D	very sensitive
HEC-1-A	Uterus	KRAS G12D	very sensitive
SW403	Intestine/Large/Colorectum	KRAS G12V	moderately sensitive
NOZ	Liver/Bile duct	KRAS G12V	low sensitivity
NCI-H441	Lung	KRAS G12V	moderately sensitive
NCI-H727	Lung	KRAS G12V	moderately sensitive
OVCAR-5	Ovary	KRAS G12V	moderately sensitive
Capan-2	Pancreas	KRAS G12V	moderately sensitive
SW48	Intestine/Large/Colorectum	not MAPK (PIK3CA G914R, EGFR G719S)	
NCI-H2009	Lung	other KRAS (G12A)	moderately sensitive
CAL-62	HN/Thyroid	other KRAS (G12R)	low sensitivity

A549	Lung	other KRAS (G12S)	low sensitivity
TOV-21G	Ovary	other KRAS (G13C)	low sensitivity
DV-90	Lung	other KRAS (G13D)	low sensitivity
HCT116	Intestine/Large/Colorectum	other KRAS (G13D)	moderately sensitive
NCI-H747	Intestine/Large/Colorectum	other KRAS (G13D)	moderately sensitive
NCI-H460	Lung	other KRAS (Q61H)	moderately sensitive
Calu-6	Lung	other KRAS (Q61K)	moderately sensitive
SNU-668	Stomach	other KRAS (Q61K)	moderately sensitive
OZ	Liver/Bile duct	other KRAS (Q61L)	moderately sensitive
SW948	Intestine/Large/Colorectum	other KRAS (Q61L)	low sensitivity
BxPC-3	Pancreas	other MAPK (BRAF V487_P492delinsA)	low sensitivity
NCI-H1975	Lung	other MAPK (EGFR T790M, L858R)	moderately sensitive
NCI-H3122	Lung	other MAPK (EML4-ALK(E13,A20))	moderately sensitive
YCC-1	Stomach	other MAPK (KRAS Amp)	
MeWo	Skin	other MAPK (NF1 mut)	low sensitivity
NCI-H1838	Lung	other MAPK (NF1 mut)	moderately sensitive

In vivo PD and Efficacy Data with Compound A, a compound of the present invention

FIG. 1A:

Methods: The human pancreatic adenocarcinoma HPAC KRAS G12D/wt xenograft model was used for a single-dose PD study. Compound A (AsPC-1 pERK K-Ras G12D EC50: 0.036 uM) was administered at 30 and 60 mg/kg by intraperitoneal injection (ip injection). The treatment groups with sample collections at various time points were summarized in Table 20 below. Tumor samples were collected to assess RAS/ERK signaling pathway modulation by measuring the mRNA level of human DUSP6 in qPCR assay.

Table 20. Summary of treatment groups, doses, and time points for single-dose PD study using HPAC tumors.

Compound/group	Dose/Regimen	PD, n = 3/time point
Vehicle control	10 ml/kg ip	1h, 24h
Compound A	30 mg/kg ip	1h, 4h, 8h, 24h
Compound A	60 mg/kg ip	1h, 4h, 6h, 24h

Results: In FIG. 1A, Compound A at either 30 mg/kg or 60 mg/kg led to inhibition of DUSP6 mRNA levels in tumors at all time points tested, indicating strong MAPK pathway

modulation. The inhibitory effects of Compound A on DUSP6 mRNA levels are durable even 24 hours after drug administration.

FIG. 1B:

Methods: Effects of Compound A on tumor cell growth in vivo were evaluated in the human pancreatic adenocarcinoma HPAC KRAS G12D/wt xenograft model using female BALB/c nude mice (6-8 weeks old). Mice were implanted with HPAC tumor cells in PBS (3 x 106 cells/mouse) subcutaneously in the flank. Once tumors reached an average size of ~150 mm3, mice were randomized to treatment groups to start the administration of test articles or vehicle. Compound A was administered by intraperitoneal injection once daily. Body weight and tumor volume (using calipers) was measured twice weekly until study endpoints.

Results: Single-agent Compound A administered at 10 mg/kg ip daily led to a TGI of 89.9% at Day 28, while both 30 mg/kg and 60 mg/kg Compound A dosed ip daily resulted in complete regression of all tumors in the group (complete regression defined as >85% tumor regression from baseline) at the end of treatment (Day 35 after treatment started) in HPAC CDX model with heterozygous KRAS G12D. The anti-tumor activity of all 3 tested doses of Compound A was statistically significant compared with control group (***$p<0.001$, ordinary One-way ANOVA with multiple comparisons via a post-hoc Tukey's test).

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure come within known or customary practice within the art to which the invention pertains and may be applied to the essential features set forth herein.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

FIG. 1A

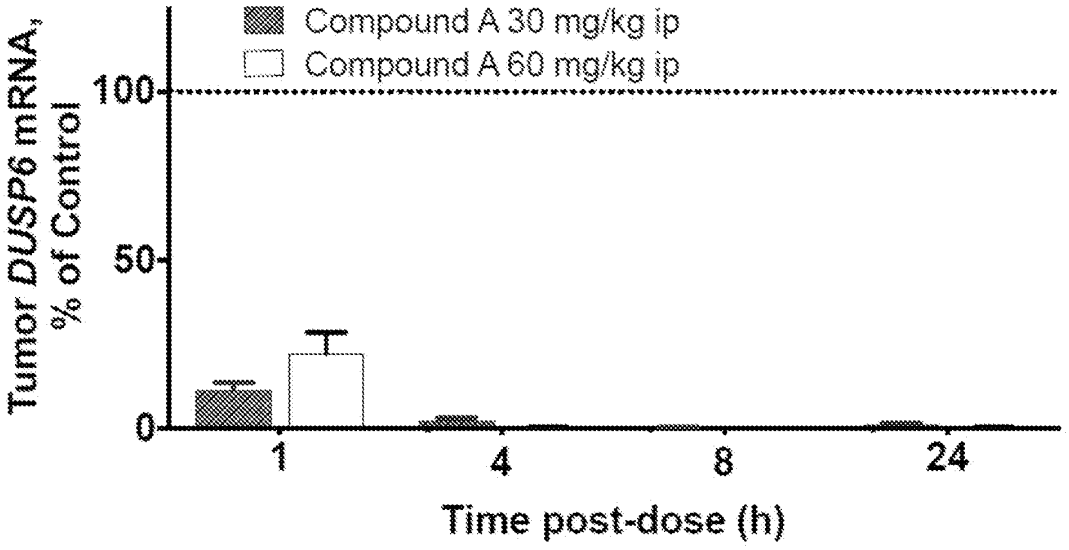
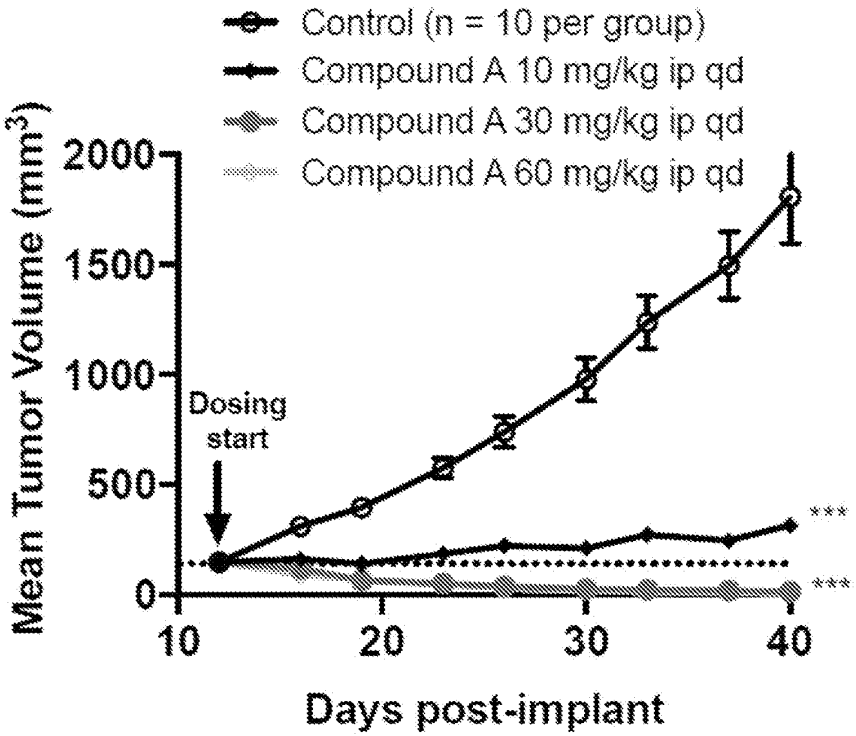

Compound A 30 mg/kg ip
Compound A 60 mg/kg ip
Tumor DUSP6 mRNA, % of Control
100
50
0
1
4
8
24
Time post-dose (h)

FIG. 1B


Control (n = 10 per group)
Compound A 10 mg/kg ip qd
Compound A 30 mg/kg ip qd
Compound A 60 mg/kg ip qd
Mean Tumor Volume (mm3)
2000
1500
1000
500
0
Dosing start
***
***
10
20
30
40
Days post-implant

APPENDIX D

RAS INHIBITORS

Background

The vast majority of small molecule drugs act by binding a functionally important pocket on a target protein, thereby modulating the activity of that protein. For example, cholesterol-lowering drugs known as statins bind the enzyme active site of HMG-CoA reductase, thus preventing the enzyme from engaging with its substrates. The fact that many such drug/target interacting pairs are known may have misled some into believing that a small molecule modulator could be discovered for most, if not all, proteins provided a reasonable amount of time, effort, and resources. This is far from the case. Current estimates are that only about 10% of all human proteins are targetable by small molecules. Bojadzic and Buchwald, Curr Top Med Chem 18: 674-699 (2019). The other 90% are currently considered refractory or intractable toward above-mentioned small molecule drug discovery. Such targets are commonly referred to as "undruggable." These undruggable targets include a vast and largely untapped reservoir of medically important human proteins. Thus, there exists a great deal of interest in discovering new molecular modalities capable of modulating the function of such undruggable targets.

It has been well established in literature that Ras proteins (K-Ras, H-Ras and N-Ras) play an essential role in various human cancers and are therefore appropriate targets for anticancer therapy. Indeed, mutations in Ras proteins account for approximately 30% of all human cancers in the United States, many of which are fatal. Dysregulation of Ras proteins by activating mutations, overexpression or upstream activation is common in human tumors, and activating mutations in Ras are frequently found in human cancer. For example, activating mutations at codon 12 in Ras proteins function by inhibiting both GTPase-activating protein (GAP)-dependent and intrinsic hydrolysis rates of GTP, significantly skewing the population of Ras mutant proteins to the "on" (GTP-bound) state (Ras(ON)), leading to oncogenic MAPK signaling. Notably, Ras exhibits a picomolar affinity for GTP, enabling Ras to be activated even in the presence of low concentrations of this nucleotide. Mutations at codons 13 (e.g., G13D) and 61 (e.g., Q61K) of Ras are also responsible for oncogenic activity in some cancers.

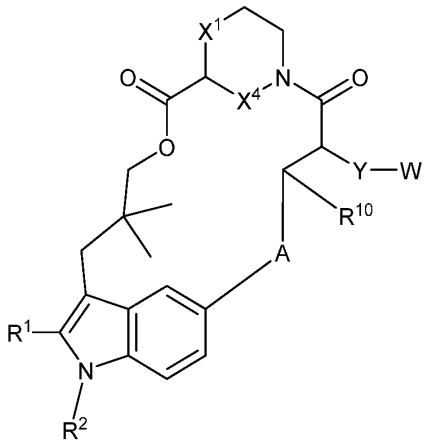
Despite extensive drug discovery efforts against Ras during the last several decades, a drug directly targeting Ras is still not approved. Additional efforts are needed to uncover additional medicines for cancers driven by the various Ras mutations.

Summary

Provided herein are Ras inhibitors. The approach described herein entails formation of a high affinity three-component complex between a synthetic ligand and two intracellular proteins which do not interact under normal physiological conditions: the target protein of interest (e.g., Ras), and a widely expressed cytosolic chaperone (presenter protein) in the cell (e.g., cyclophilin A). More specifically, in some embodiments, the inhibitors of Ras described herein induce a new

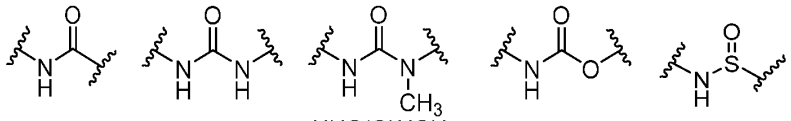
binding pocket in Ras by driving formation of a high affinity tri-complex between the Ras protein and the widely expressed cytosolic chaperone, cyclophilin A (CYPA). Without being bound by theory, the inventors believe that one way the inhibitory effect on Ras is effected by compounds of the invention and the complexes they form is by steric occlusion of the interaction site between Ras and downstream effector molecules, such as RAF and PI3K, which are required for propagating the oncogenic signal.

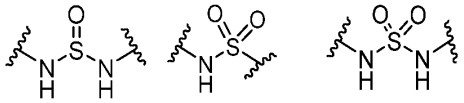
As such, in some embodiments, the disclosure features a compound, or pharmaceutically acceptable salt thereof, of structural Formula I:



Formula I

wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, optionally substituted 5 to 6-membered heteroarylene, optionally substituted C_2-C_4 alkylene, or optionally substituted C_2-C_4 alkenylene;

Y is  -NHC(O)-, -NHC(O)NH-, -NHC(O)NCH$_3$-, -NHC(O)O-, -NHC(S)-,

 -NHC(S)NH-, -NHS(O)$_2$-, or -NHS(O)$_2$NH-;

W is hydrogen, C_1-C_4 alkyl, optionally substituted C_1-C_3 heteroalkyl, optionally substituted 3 to 10-membered heterocycloalkyl, optionally substituted 3 to 10-membered cycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

X^1 and X^4 are each, independently, CH_2 or NH;

R^1 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 15-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl,

optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; and

R^{10} is hydrogen, hydroxy, optionally substituted C_1-C_3 alkyl, or optionally substituted C_1-C_6 heteroalkyl. In some embodiments, R^{10} is hydrogen.

Also provided are pharmaceutical compositions comprising a compound of Formula I, or a pharmaceutically acceptable salt thereof, and a pharamceuticall acceptable excipient.

It is specifically contemplated that any limitation discussed with respect to one embodiment of the invention may apply to any other embodiment of the invention. Furthermore, any compound or composition of the invention may be used in any method of the invention, and any method of the invention may be used to produce or to utilize any compound or composition of the invention.

Definitions and Chemical Terms

In this application, unless otherwise clear from context, (i) the term “a” means “one or more”; (ii) the term "or" is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternative are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or"; (iii) the terms “comprising” and “including” are understood to encompass itemized components or steps whether presented by themselves or together with one or more additional components or steps; and (iv) where ranges are provided, endpoints are included.

As used herein, the term “about” is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value. In certain embodiments, the term “about” refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of a stated value, unless otherwise stated or otherwise evident from the context (e.g., where such number would exceed 100% of a possible value).

As used herein, the term “adjacent” in the context of describing adjacent atoms refers to bivalent atoms that are directly connected by a covalent bond.

A “compound of the present invention” and similar terms as used herein, whether explicitly noted or not, refers to Ras inhibitors described herein, including compounds of Formula I and subformula thereof, and compounds of Table 1 and Table 1-1, as well as salts (e.g., pharmaceutically acceptable salts), solvates, hydrates, stereoisomers (including atropisomers), and tautomers thereof.

The term “wild-type” refers to an entity having a structure or activity as found in nature in a “normal” (as contrasted with mutant, diseased, altered, etc) state or context. Those of ordinary skill in the art will appreciate that wild-type genes and polypeptides often exist in multiple different forms (e.g., alleles).

Those skilled in the art will appreciate that certain compounds described herein can exist in one or more different isomeric (e.g., stereoisomers, geometric isomers, atropisomers, tautomers) or isotopic (e.g., in which one or more atoms has been substituted with a different isotope of the atom, such as hydrogen substituted for deuterium) forms. Unless otherwise indicated or clear from context, a depicted structure can be understood to represent any such isomeric or isotopic form, individually or in combination.

Compounds described herein can be asymmetric (e.g., having one or more stereocenters). All stereoisomers, such as enantiomers and diastereomers, are intended unless otherwise indicated. Compounds of the present disclosure that contain asymmetrically substituted carbon atoms can be isolated in optically active or racemic forms. Methods on how to prepare optically active forms from optically active starting materials are known in the art, such as by resolution of racemic mixtures or by stereoselective synthesis. Many geometric isomers of olefins, C=N double bonds, and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present disclosure. Cis and trans geometric isomers of the compounds of the present disclosure are described and may be isolated as a mixture of isomers or as separated isomeric forms.

In some embodiments, one or more compounds depicted herein may exist in different tautomeric forms. As will be clear from context, unless explicitly excluded, references to such compounds encompass all such tautomeric forms. In some embodiments, tautomeric forms result from the swapping of a single bond with an adjacent double bond and the concomitant migration of a proton. In certain embodiments, a tautomeric form may be a prototropic tautomer, which is an isomeric protonation states having the same empirical formula and total charge as a reference form. Examples of moieties with prototropic tautomeric forms are ketone - enol pairs, amide - imidic acid pairs, lactam - lactim pairs, amide - imidic acid pairs, enamine - imine pairs, and annular forms where a proton can occupy two or more positions of a heterocyclic system, such as, 1H- and 3H-imidazole, 1H-, 2H- and 4H-1,2,4-triazole, 1H- and 2H- isoindole, and 1H- and 2H-pyrazole. In some embodiments, tautomeric forms can be in equilibrium or sterically locked into one form by appropriate substitution. In certain embodiments, tautomeric forms result from acetal interconversion.

Unless otherwise stated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. Exemplary isotopes that can be incorporated into compounds of the present invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, sulfur, fluorine, chlorine, and iodine, such as ^{2}H, ^{3}H, ^{11}C, ^{13}C, ^{14}C, ^{13}N, ^{15}N, ^{15}O, ^{17}O, ^{18}O, ^{32}P, ^{33}P, ^{35}S, ^{18}F, ^{36}Cl, ^{123}I and ^{125}I. Isotopically-labeled compounds (e.g., those labeled with ^{3}H and ^{14}C) can be useful in compound or substrate tissue distribution assays. Tritiated (i.e., ^{3}H) and carbon-14 (i.e., ^{14}C) isotopes can be useful for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium (i.e., ^{2}H) may afford certain therapeutic advantages resulting from greater metabolic stability (e.g., increased in vivo half-life or reduced dosage requirements). In some embodiments, one or more hydrogen atoms are replaced by ^{2}H or ^{3}H, or one or more carbon atoms are replaced by ^{13}C- or ^{14}C-enriched

carbon. Positron emitting isotopes such as ^{15}O, ^{13}N, ^{11}C, and ^{18}F are useful for positron emission tomography (PET) studies to examine substrate receptor occupancy. Preparations of isotopically labelled compounds are known to those of skill in the art. For example, isotopically labeled compounds can generally be prepared by following procedures analogous to those disclosed for compounds of the present invention described herein, by substituting an isotopically labeled reagent for a non-isotopically labeled reagent.

As is known in the art, many chemical entities can adopt a variety of different solid forms such as, for example, amorphous forms or crystalline forms (e.g., polymorphs, hydrates, solvate). In some embodiments, compounds of the present invention may be utilized in any such form, including in any solid form. In some embodiments, compounds described or depicted herein may be provided or utilized in hydrate or solvate form.

At various places in the present specification, substituents of compounds of the present disclosure are disclosed in groups or in ranges. It is specifically intended that the present disclosure include each and every individual subcombination of the members of such groups and ranges. For example, the term "C_1-C_6 alkyl" is specifically intended to individually disclose methyl, ethyl, C_3 alkyl, C_4 alkyl, C_5 alkyl, and C_6 alkyl. Furthermore, where a compound includes a plurality of positions at which substituents are disclosed in groups or in ranges, unless otherwise indicated, the present disclosure is intended to cover individual compounds and groups of compounds (e.g., genera and subgenera) containing each and every individual subcombination of members at each position.

The term "optionally substituted X" (e.g., "optionally substituted alkyl") is intended to be equivalent to "X, wherein X is optionally substituted" (e.g., "alkyl, wherein said alkyl is optionally substituted"). It is not intended to mean that the feature "X" (e.g., alkyl) *per se* is optional. As described herein, certain compounds of interest may contain one or more "optionally substituted" moieties. In general, the term "substituted", whether preceded by the term "optionally" or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent, e.g., any of the substituents or groups described herein. Unless otherwise indicated, an "optionally substituted" group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. For example, in the term "optionally substituted C_1-C_6 alkyl-C_2-C_9 heteroaryl," the alkyl portion, the heteroaryl portion, or both, may be optionally substituted. Combinations of substituents envisioned by the present disclosure are preferably those that result in the formation of stable or chemically feasible compounds. The term "stable", as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain embodiments, their recovery, purification, and use for one or more of the purposes disclosed herein.

Suitable monovalent substituents on a substitutable carbon atom of an "optionally substituted" group may be, independently, deuterium;
halogen; $-(CH_2)_{0-4}R^{\circ}$; $-(CH_2)_{0-4}OR^{\circ}$; $-O(CH_2)_{0-4}R^{\circ}$;

-O-$(CH_2)_{0-4}$C(O)OR°; -$(CH_2)_{0-4}$CH(OR°)$_2$; -$(CH_2)_{0-4}$SR°; -$(CH_2)_{0-4}$Ph, which may be substituted with R°; -$(CH_2)_{0-4}$O$(CH_2)_{0-1}$Ph which may be substituted with R°; -CH=CHPh, which may be substituted with R°; -$(CH_2)_{0-4}$O$(CH_2)_{0-1}$-pyridyl which may be substituted with R°; 4-11 membered saturated or unsaturated heterocycloalkyl (e.g., 4-8 membered saturated or unsaturated heterocycloalkyl (e.g., pyridyl)) which may be further optionally substituted (e.g., with a methyl); 3-8 membered saturated or unsaturated cycloalkyl (e.g., cyclopropyl, cyclobutyl, or cyclopentyl); -NO_2; -CN; -N_3; -$(CH_2)_{0-4}$N(R°)$_2$; -$(CH_2)_{0-4}$N(R°)C(O)R°; -N(R°)C(S)R°; -$(CH_2)_{0-4}$N(R°)C(O)NR°$_2$; -N(R°)C(S)NR°$_2$; -$(CH_2)_{0-4}$N(R°)C(O)OR°; -N(R°)N(R°)C(O)R°; -N(R°)N(R°)C(O)NR°$_2$; -N(R°)N(R°)C(O)OR°; -$(CH_2)_{0-4}$C(O)R°; -C(S)R°; -$(CH_2)_{0-4}$C(O)OR°; -$(CH_2)_{0-4}$-C(O)-N(R°)$_2$; -$(CH_2)_{0-4}$-C(O)-N(R°)-S(O)$_2$-R°; -C(NCN)NR°$_2$; -$(CH_2)_{0-4}$C(O)SR°; -$(CH_2)_{0-4}$C(O)OSiR°$_3$; -$(CH_2)_{0-4}$OC(O)R°; -OC(O)$(CH_2)_{0-4}$SR°; -SC(S)SR°; -$(CH_2)_{0-4}$SC(O)R°; -$(CH_2)_{0-4}$C(O)NR°$_2$; -C(S)NR°$_2$; -C(S)SR°; -$(CH_2)_{0-4}$OC(O)NR°$_2$; -C(O)N(OR°)R°; -C(O)C(O)R°; -C(O)CH_2C(O)R°; -C(NOR°)R°; -$(CH_2)_{0-4}$SSR°; -$(CH_2)_{0-4}$S(O)$_2$R°; -$(CH_2)_{0-4}$S(O)$_2$OR°; -$(CH_2)_{0-4}$OS(O)$_2$R°; -S(O)$_2$NR°$_2$; -$(CH_2)_{0-4}$S(O)R°; -N(R°)S(O)$_2$NR°$_2$; -N(R°)S(O)$_2$R°;

-N(OR°)R°; -C(NOR°)NR°$_2$; -C(NH)NR°$_2$; -P(O)$_2$R°; -P(O)R°$_2$; -P(O)(OR°)$_2$; -OP(O)R°$_2$; -OP(O)(OR°)$_2$;

-OP(O)(OR°)R°, -SiR°$_3$; -(C_{1-4} straight or branched alkylene)O-N(R°)$_2$; or -(C_{1-4} straight or branched alkylene)C(O)O-N(R°)$_2$, wherein each R° may be substituted as defined below and is independently hydrogen, -C_{1-6} aliphatic, -CH_2Ph, -O$(CH_2)_{0-1}$Ph, -CH_2-(5-6 membered heteroaryl ring), or a 3-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of R°, taken together with their intervening atom(s), form a 3-12-membered saturated, partially unsaturated, or aryl mono- or bicyclic ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, which may be substituted as defined below.

Suitable monovalent substituents on R° (or the ring formed by taking two independent occurrences of R° together with their intervening atoms), may be, independently, halogen, -$(CH_2)_{0-2}$R•,

-(haloR•), -$(CH_2)_{0-2}$OH, -$(CH_2)_{0-2}$OR•, -$(CH_2)_{0-2}$CH(OR•)$_2$; -O(haloR•), -CN, -N_3, -$(CH_2)_{0-2}$C(O)R•, -$(CH_2)_{0-2}$C(O)OH, -$(CH_2)_{0-2}$C(O)OR•, -$(CH_2)_{0-2}$SR•, -$(CH_2)_{0-2}$SH, -$(CH_2)_{0-2}$NH$_2$, -$(CH_2)_{0-2}$NHR•, -$(CH_2)_{0-2}$NR•$_2$, -NO_2, -SiR•$_3$, -OSiR•$_3$, -C(O)SR•, -(C_{1-4} straight or branched alkylene)C(O)OR•, or -SSR• wherein each R• is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently selected from C_{1-4} aliphatic, -CH_2Ph, -O$(CH_2)_{0-1}$Ph, or a 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents on a saturated carbon atom of R° include =O and =S.

Suitable divalent substituents on a saturated carbon atom of an "optionally substituted" group include the following: =O, =S, =NNR*$_2$, =NNHC(O)R*, =NNHC(O)OR*, =NNHS(O)$_2$R*, =NR*, =NOR*,

-O(C(R*2))2-3O-, or -S(C(R*2))2-3S-, wherein each independent occurrence of R* is selected from hydrogen, C1-6 aliphatic which may be substituted as defined below, or an unsubstituted 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents that are bound to vicinal substitutable carbons of an "optionally substituted" group include: -O(CR*2)2-3O-, wherein each independent occurrence of R* is selected from hydrogen, C1-6 aliphatic which may be substituted as defined below, or an unsubstituted 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

Suitable substituents on the aliphatic group of R* include halogen, -R•, -(haloR•), -OH, -OR•, -O(haloR•), -CN, -C(O)OH, -C(O)OR•, -NH2, -NHR•, -NR•2, or -NO2, wherein each R• is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C1-4 aliphatic, -CH2Ph, -O(CH2)0-1Ph, or a 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

Suitable substituents on a substitutable nitrogen of an "optionally substituted" group include -R†, -NR†2, -C(O)R†, -C(O)OR†, -C(O)C(O)R†, -C(O)CH2C(O)R†, -S(O)2R†, -S(O)2NR†2, -C(S)NR†2, -C(NH)NR†2, or -N(R†)S(O)2R†; wherein each R† is independently hydrogen, C1-6 aliphatic which may be substituted as defined below, unsubstituted -OPh, or an unsubstituted 3-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of R†, taken together with their intervening atom(s) form an unsubstituted 3-12-membered saturated, partially unsaturated, or aryl mono- or bicyclic ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

Suitable substituents on an aliphatic group of R† are independently halogen, -R•, -(haloR•), -OH, -OR•, -O(haloR•), -CN, -C(O)OH, -C(O)OR•, -NH2, -NHR•, -NR•2, or -NO2, wherein each R• is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C1-4 aliphatic, -CH2Ph, -O(CH2)0-1Ph, or a 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents on a saturated carbon atom of R† include =O and =S.

The term "acetyl," as used herein, refers to the group -C(O)CH3.

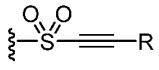
The term "alkoxy," as used herein, refers to a -O-C1-C20 alkyl group, wherein the alkoxy group is attached to the remainder of the compound through an oxygen atom.

The term "alkyl," as used herein, refers to a saturated, straight or branched monovalent hydrocarbon group containing from 1 to 20 (e.g., from 1 to 10 or from 1 to 6) carbons. In some embodiments, an alkyl group is unbranched (i.e., is linear); in some embodiments, an alkyl group is branched. Alkyl groups are exemplified by, but not limited to, methyl, ethyl, *n*- and *iso*-propyl, *n*-, *sec*-, *iso*- and *tert*-butyl, and neopentyl.

The term “alkylene,” as used herein, represents a saturated divalent hydrocarbon group derived from a straight or branched chain saturated hydrocarbon by the removal of two hydrogen atoms, and is exemplified by methylene, ethylene, isopropylene, and the like. The term “C_x-C_y alkylene” represents alkylene groups having between x and y carbons. Exemplary values for x are 1, 2, 3, 4, 5, and 6, and exemplary values for y are 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, or 20 (e.g., C_1-C_6, C_1-C_{10}, C_2-C_{20}, C_2-C_6, C_2-C_{10}, or C_2-C_{20} alkylene). In some embodiments, the alkylene can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein.

The term “alkenyl,” as used herein, represents monovalent straight or branched chain groups of, unless otherwise specified, from 2 to 20 carbons (e.g., from 2 to 6 or from 2 to 10 carbons) containing one or more carbon-carbon double bonds and is exemplified by ethenyl, 1-propenyl, 2-propenyl, 2-methyl-1-propenyl, 1-butenyl, and 2-butenyl. Alkenyls include both cis and trans isomers. The term “alkenylene,” as used herein, represents a divalent straight or branched chain groups of, unless otherwise specified, from 2 to 20 carbons (e.g., from 2 to 6 or from 2 to 10 carbons) containing one or more carbon-carbon double bonds.

The term “alkynyl,” as used herein, represents monovalent straight or branched chain groups from 2 to 20 carbon atoms (e.g., from 2 to 4, from 2 to 6, or from 2 to 10 carbons) containing a carbon-carbon triple bond and is exemplified by ethynyl, and 1-propynyl.

The term “alkynyl sulfone,” as used herein, represents a group comprising the structure  , wherein R is any chemically feasible substituent described herein.

The term “amino,” as used herein, represents $-N(R^{\dagger})_2$, e.g., $-NH_2$ and $-N(CH_3)_2$.

The term “aminoalkyl,” as used herein, represents an alkyl moiety substituted on one or more carbon atoms with one or more amino moieties.

The term “amino acid,” as described herein, refers to a molecule having a side chain, an amino group, and an acid group (e.g., $-CO_2H$ or $-SO_3H$), wherein the amino acid is attached to the parent molecular group by the side chain, amino group, or acid group (e.g., the side chain). As used herein, the term “amino acid” in its broadest sense, refers to any compound or substance that can be incorporated into a polypeptide chain, e.g., through formation of one or more peptide bonds. In some embodiments, an amino acid has the general structure H_2N-C(H)(R)-COOH. In some embodiments, an amino acid is a naturally-occurring amino acid. In some embodiments, an amino acid is a synthetic amino acid; in some embodiments, an amino acid is a D-amino acid; in some embodiments, an amino acid is an L-amino acid. “Standard amino acid” refers to any of the twenty standard L-amino acids commonly found in naturally occurring peptides. Exemplary amino acids include alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, optionally substituted hydroxylnorvaline, isoleucine, leucine, lysine, methionine, norvaline, ornithine, phenylalanine, proline, pyrrolysine, selenocysteine, serine, taurine, threonine, tryptophan, tyrosine, and valine.

The term “aryl,” as used herein, represents a monovalent monocyclic, bicyclic, or multicyclic ring system formed by carbon atoms, wherein the ring attached to the pendant group is aromatic. Examples of aryl groups are phenyl, naphthyl, phenanthrenyl, and anthracenyl. An aryl

ring can be attached to its pendant group at any heteroatom or carbon ring atom that results in a stable structure and any of the ring atoms can be optionally substituted unless otherwise specified.

The term "C_0," as used herein, represents a bond. For example, part of the term -N(C(O)-(C_0-C_5 alkylene-H)- includes -N(C(O)-(C_0 alkylene-H)-, which is also represented by -N(C(O)-H)-.

The terms "carbocyclic" and "carbocyclyl," as used herein, refer to a monovalent, optionally substituted C_3-C_{12} monocyclic, bicyclic, or tricyclic ring structure, which may be bridged, fused or spirocyclic, in which all the rings are formed by carbon atoms and at least one ring is non-aromatic. Carbocyclic structures include cycloalkyl, cycloalkenyl, and cycloalkynyl groups. Examples of carbocyclyl groups are cyclohexyl, cyclohexenyl, cyclooctynyl, 1,2-dihydronaphthyl, 1,2,3,4-tetrahydronaphthyl, fluorenyl, indenyl, indanyl, decalinyl, and the like. A carbocyclic ring can be attached to its pendant group at any ring atom that results in a stable structure and any of the ring atoms can be optionally substituted unless otherwise specified.

The term "carbonyl," as used herein, represents a C(O) group, which can also be represented as C=O.

The term "carboxyl," as used herein, means -CO_2H, (C=O)(OH), COOH, or C(O)OH or the unprotonated counterparts.

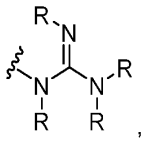
The term "cyano," as used herein, represents a -CN group.

The term "cycloalkyl," as used herein, represents a monovalent saturated cyclic hydrocarbon group, which may be bridged, fused or spirocyclic having from three to eight ring carbons, unless otherwise specified, and is exemplified by cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cycloheptyl.

The term "cycloalkenyl," as used herein, represents a monovalent, non-aromatic, saturated cyclic hydrocarbon group, which may be bridged, fused or spirocyclic having from three to eight ring carbons, unless otherwise specified, and containing one or more carbon-carbon double bonds.

The term "diastereomer," as used herein, means stereoisomers that are not mirror images of one another and are non-superimposable on one another.

The term "enantiomer," as used herein, means each individual optically active form of a compound of the invention, having an optical purity or enantiomeric excess (as determined by methods standard in the art) of at least 80% (i.e., at least 90% of one enantiomer and at most 10% of the other enantiomer), preferably at least 90% and more preferably at least 98%.

The term "guanidinyl," refers to a group having the structure:  , wherein each R is, independently, any any chemically feasible substituent described herein.

The term "guanidinoalkyl alkyl," as used herein, represents an alkyl moiety substituted on one or more carbon atoms with one or more guanidinyl moieties.

The term "haloacetyl," as used herein, refers to an acetyl group wherein at least one of the hydrogens has been replaced by a halogen.

The term “haloalkyl,” as used herein, represents an alkyl moiety substituted on one or more carbon atoms with one or more of the same of different halogen moieties.

The term “halogen,” as used herein, represents a halogen selected from bromine, chlorine, iodine, or fluorine.

The term "heteroalkyl," as used herein, refers to an "alkyl" group, as defined herein, in which at least one carbon atom has been replaced with a heteroatom (e.g., an O, N, or S atom). The heteroatom may appear in the middle or at the end of the radical.

The term “heteroaryl,” as used herein, represents a monovalent, monocyclic or polycyclic ring structure that contains at least one fully aromatic ring: i.e., they contain $4n+2$ pi electrons within the monocyclic or polycyclic ring system and contains at least one ring heteroatom selected from N, O, or S in that aromatic ring. Exemplary unsubstituted heteroaryl groups are of 1 to 12 (e.g., 1 to 11, 1 to 10, 1 to 9, 2 to 12, 2 to 11, 2 to 10, or 2 to 9) carbons. The term “heteroaryl” includes bicyclic, tricyclic, and tetracyclic groups in which any of the above heteroaromatic rings is fused to one or more, aryl or carbocyclic rings, e.g., a phenyl ring, or a cyclohexane ring. Examples of heteroaryl groups include, but are not limited to, pyridyl, pyrazolyl, benzooxazolyl, benzoimidazolyl, benzothiazolyl, imidazolyl, thiazolyl, quinolinyl, tetrahydroquinolinyl, and 4-azaindolyl. A heteroaryl ring can be attached to its pendant group at any ring atom that results in a stable structure and any of the ring atoms can be optionally substituted unless otherwise specified. In some embodiment, the heteroaryl is substituted with 1, 2, 3, or 4 substituents groups.

The term “heterocycloalkyl,” as used herein, represents a monovalent monocyclic, bicyclic or polycyclic ring system, which may be bridged, fused or spirocyclic, wherein at least one ring is non-aromatic and wherein the non-aromatic ring contains one, two, three, or four heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur. The 5-membered ring has zero to two double bonds, and the 6- and 7-membered rings have zero to three double bonds. Exemplary unsubstituted heterocycloalkyl groups are of 1 to 12 (e.g., 1 to 11, 1 to 10, 1 to 9, 2 to 12, 2 to 11, 2 to 10, or 2 to 9) carbons. The term “heterocycloalkyl” also represents a heterocyclic compound having a bridged multicyclic structure in which one or more carbons or heteroatoms bridges two non-adjacent members of a monocyclic ring, e.g., a quinuclidinyl group. The term “heterocycloalkyl” includes bicyclic, tricyclic, and tetracyclic groups in which any of the above heterocyclic rings is fused to one or more aromatic, carbocyclic, heteroaromatic, or heterocyclic rings, e.g., an aryl ring, a cyclohexane ring, a cyclohexene ring, a cyclopentane ring, a cyclopentene ring, a pyridine ring, or a pyrrolidine ring. Examples of heterocycloalkyl groups are pyrrolidinyl, piperidinyl, 1,2,3,4-tetrahydroquinolinyl, decahydroquinolinyl, dihydropyrrolopyridine, and decahydronapthyridinyl. A heterocycloalkyl ring can be attached to its pendant group at any ring atom that results in a stable structure and any of the ring atoms can be optionally substituted unless otherwise specified.

The term “hydroxy,” as used herein, represents a -OH group.

The term “hydroxyalkyl,” as used herein, represents an alkyl moiety substituted on one or more carbon atoms with one or more -OH moieties.

The term “isomer,” as used herein, means any tautomer, stereoisomer, atropiosmer, enantiomer, or diastereomer of any compound of the invention. It is recognized that the compounds of the invention can have one or more chiral centers or double bonds and, therefore, exist as stereoisomers, such as double-bond isomers (i.e., geometric E/Z isomers) or diastereomers (e.g., enantiomers (i.e., (+) or (-)) or cis/trans isomers). According to the invention, the chemical structures depicted herein, and therefore the compounds of the invention, encompass all the corresponding stereoisomers, that is, both the stereomerically pure form (e.g., geometrically pure, enantiomerically pure, or diastereomerically pure) and enantiomeric and stereoisomeric mixtures, e.g., racemates. Enantiomeric and stereoisomeric mixtures of compounds of the invention can typically be resolved into their component enantiomers or stereoisomers by well-known methods, such as chiral-phase gas chromatography, chiral-phase high performance liquid chromatography, crystallizing the compound as a chiral salt complex, or crystallizing the compound in a chiral solvent. Enantiomers and stereoisomers can also be obtained from stereomerically or enantiomerically pure intermediates, reagents, and catalysts by well-known asymmetric synthetic methods.

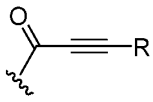
The term “stereoisomer,” as used herein, refers to all possible different isomeric as well as conformational forms which a compound may possess (e.g., a compound of any formula described herein), in particular all possible stereochemically and conformationally isomeric forms, all diastereomers, enantiomers or conformers of the basic molecular structure, including atropisomers. Some compounds of the present invention may exist in different tautomeric forms, all of the latter being included within the scope of the present invention.

The term “sulfonyl,” as used herein, represents an -$S(O)_2$- group.

The term “thiocarbonyl,” as used herein, refers to a -C(S)- group.

The term “vinyl ketone,” as used herein, refers to a group comprising a carbonyl group directly connected to a carbon-carbon double bond.

The term “vinyl sulfone,” as used herein, refers to a group comprising a sulfonyl group directed connected to a carbon-carbon double bond.

The term “ynone,” as used herein, refers to a group comprising the structure 

, wherein R is any any chemically feasible substituent described herein.

Those of ordinary skill in the art, reading the present disclosure, will appreciate that certain compounds described herein may be provided or utilized in any of a variety of forms such as, for example, salt forms, protected forms, pro-drug forms, ester forms, isomeric forms (e.g., optical or structural isomers), isotopic forms, etc. In some embodiments, reference to a particular compound may relate to a specific form of that compound. In some embodiments, reference to a particular compound may relate to that compound in any form. In some embodiments, for example, a preparation of a single stereoisomer of a compound may be considered to be a different form of the compound than a racemic mixture of the compound; a particular salt of a compound may be considered to be a different form from another salt form of the compound; a preparation containing

one conformational isomer ((Z) or (E)) of a double bond may be considered to be a different form from one containing the other conformational isomer ((E) or (Z)) of the double bond; a preparation in which one or more atoms is a different isotope than is present in a reference preparation may be considered to be a different form.

Brief Description of the Drawings

FIG. 1A is a graph demonstrating the in vivo efficacy of Compound A in a human pancreatic adenocarcinoma HPAC $KRAS^{G12D/wt}$ xenograft model using female BALB/c nude mice. The graph shows tumor volume (mm^3) vs. days post-implant of the mouse xenograft model. Mice were randomized to treatment groups prior to administration of test articles or vehicle. Compound A was administered by oral gavage once every other day.

FIG. 1B is a graph demonstrating dose-dependent exposure of Compound A in blood and tumor samples from BALB/c nude mice (6-8 weeks old, human non-small cell lung cancer (NSCLC) NCI-H441 $KRAS^{G12V/wt}$ xenograft model), monitored through 72 hours following dose. Pharmacokinetics were analyzed based on total concentration (nM) of Compound A in tumors or blood, following a single oral gavage dose of Compound A at 10, 25 or 50 mg/kg to 72 hours. At each time point, tumor or blood was sampled from n=3 animals.

FIG. 1C is a graph showing PK (10 mg/kg, po) and PD (% tumor DUSP relative to control, 10 and 25 mg/kg, po) in naïve animals treated with a single dose of Compound A.

FIG. 1D is a graph demonstrating the in vivo efficacy of Compound A in the NCI-H441 CDX model with heterozygous $KRAS^{G12V}$. NCI-H441 cells were implanted in 50% Matrigel. Animals were randomized and treatment was initiated at average tumor volume of ~155 mm^3. Animals were dosed with Compound A 10 or 25 mg/kg po qd or Control for 32 days. All dose levels were tolerated. n = 10/group. ***p<0.0001 by one-way ANOVA.

FIG. 1E shows end of study responses for Compound A in the NCI-H441 CDX model with heterozygous $KRAS^{G12V}$. NCI-H441 end of study tumors were graphed as % change in tumor volume compared to volume at treatment initiation. R (regressions) = number of regressions >10% from initial. CR (complete response) = number of regressions >80% from initial. Each animal represented as a separate bar.

FIG. 1F shows shows % body weight change in animals treated with Compound A (in the NCI-H441 CDX model with heterozygous $KRAS^{G12V}$). NCI-H441 cell-derived xenografts were measured twice weekly by caliper measurements. Body weight change graphed as percentage of animals starting body weight.

FIG. 2A is a graph demonstrating in vivo efficacy of Compound A in the human pancreatic Capan-2 CDX xenograft model with heterozygous $KRAS^{G12V}$ using female BALB/c nude mice. The graph shows tumor volume (mm^3) vs. days post-implant of the mouse xenograft model. Capan-2 cells were implanted in 50% Matrigel. Animals were randomized and treatment was initiated at average tumor volume of ~166 mm^3. Animals were dosed with Compound A 10 mg/kg po qd or 25 mg/kg po q2d or Control for 28 days. All dose levels were tolerated. n = 8/group. **p=0.01, ***p<0.0001 by one-way ANOVA.

FIG. 2B shows end of study responses for Compound A in the human pancreatic Capan-2 CDX xenograft model with heterozygous $KRAS^{G12V}$. Capan-2 end of study tumors were graphed as % change in tumor volume compared to volume at treatment initiation. R (regressions) = number of regressions >10% from initial. Each animal represented as a separate bar.

FIG. 2C shows % body weight change in animals treated with Compound A in the human pancreatic Capan-2 CDX xenograft model with heterozygous $KRAS^{G12V}$. Capan-2 cell-derived xenografts were measured twice weekly by caliper measurements. Body weight change graphed as percentage of animals starting body weight.

FIG. 2D is a graph demonstrating in vivo efficacy of Compound A in the human colorectal SW403 $KRAS^{G12V/wt}$ xenograft model using female BALB/c nude mice. The graph shows tumor volume (mm^3) vs. days post-implant of the mouse xenograft model. SW403 cells were implanted in 50% Matrigel. Animals were randomized and treatment was initiated at average tumor volume of ~171mm^3. Animals were dosed with Compound A 25 mg/kg po qd or 50 mg/kg po q2d or Control for 28 days. All dose levels were tolerated. n = 8/group. ***$p<0.0001$ by one-way ANOVA.

FIG. 2E shows end of study responses for Compound A in the human colorectal SW403 $KRAS^{G12V/wt}$ xenograft model. SW403 end of study tumors were graphed as % change in tumor volume compared to volume at treatment initiation. R (regressions) = number of regressions >10% from initial. CR (complete response) = number of regressions >80% from initial. Each animal represented as a separate bar.

FIG. 2F shows % body weight change in animals treated with Compound A in the human colorectal SW403 $KRAS^{G12V/wt}$ xenograft model. SW403 cell-derived xenografts were measured twice weekly by caliper measurements. Body weight change graphed as percentage of animals starting body weight.

FIG. 3 demonstrates in vitro efficacy of Compound A in multiple RAS-driven cancer cell lines. Each graph shows cell proliferation (% relative to control) vs. log M [Compound A]. Potency of in vitro cell proliferation inhibition of Capan-1 ($KRAS^{G12V}$), AsPC-1 ($KRAS^{G12D}$), HCT116 ($KRAS^{G13D}$), SK-MEL-30 ($NRAS^{Q61K}$), NCI-H1975 ($EGFR^{T790M/L858R}$), and A375 ($BRAF^{V600E}$) cells exposed to Compound A for 120 hours. Data represent the mean of multiple experiments.

FIG. 4A demonstrates in vivo efficacy of Compound A (25 mg/kg po qd) in the human pancreatic adenocarcinoma HPAC $KRAS^{G12D/wt}$ xenograft model using female BALB/c nude mice. The graph shows tumor volume (mm^3) vs. days post-implant of the mouse xenograft model. HPAC cells were implanted in 50% Matrigel. Animals were randomized and treatment was initiated at average tumor volume of ~142 mm^3. Animals were dosed with Compound A 25 mg/kg po qd or Control for 28 days. Dose level was tolerated. n = 9-10/group. ***$p<0.0001$ by one-way ANOVA.

FIG. 4B shows end of study responses for Compound A in the human pancreatic adenocarcinoma HPAC $KRAS^{G12D/wt}$ xenograft model. HPAC end of study tumors were graphed as % change in tumor volume compared to volume at treatment initiation. CR (complete response) = number of regressions >80% from initial. Each animal represented as a separate bar.

FIG. 4C shows % body weight change in animals treated with Compound A in the human pancreatic adenocarcinoma HPAC $KRAS^{G12D/wt}$ xenograft model. HPAC cell-derived xenografts

were measured twice weekly by caliper measurements. Body weight change graphed as percentage of animals starting body weight.

FIG. 4D demonstrates in vivo efficacy of Compound A in the human colorectal GP2d $KRAS^{G12D/wt}$ xenograft model using female BALB/c nude mice. The graph shows tumor volume (mm^3) vs. days post-implant of the mouse xenograft model. GP2d cells were implanted in 50% Matrigel. Animals were randomized and treatment was initiated at average tumor volume of ~154 mm^3. Animals were dosed with Compound A 25 mg/kg po qd or Control for 28 days. Dose level was tolerated. n = 10/group. ***p<0.0001 by one-way ANOVA.

FIG. 4E shows end of study responses for Compound A in the human colorectal GP2d $KRAS^{G12D/wt}$ xenograft model. GP2d end of study tumors were graphed as % change in tumor volume compared to volume at treatment initiation. Each animal represented as a separate bar.

FIG. 4F shows % body weight change in animals treated with Compound A in the human colorectal GP2d $KRAS^{G12D/wt}$ xenograft model. GP2d cell-derived xenografts were measured twice weekly by caliper measurements. Body weight change graphed as percentage of animals starting body weight.

FIG. 5A demonstrates in vitro efficacy of Compound A in down-regulating immune checkpoint proteins in NCI-H358 $KRAS^{G12C}$ Cells in Vitro. FIG. 5A shows cell surface expression of PD-L1, PVR and CD73 on H358 cells following 48-hour treatment with Compound A in the presence of Interferon gamma (IFNγ), as measured by flow cytometry. Each graph shows mean fluorescence intensity ((MFI), for each respective immune checkpoint protein) vs. log M [Compound A].

FIG. 5B demonstrates in vitro efficacy of Compound A in down-regulating immune checkpoint proteins in SW900 $KRAS^{G12C}$ Cells in Vitro. FIG. 5B shows cell surface expression of PD-L1, PVR and CD73 on SW900 cells following 48-hour treatment with Compound A in the presence of Interferon gamma (IFNγ), as measured by flow cytometry. Each graph shows mean fluorescence intensity ((MFI), for each respective immune checkpoint protein) vs. log M [Compound A].

FIG. 5C demonstrates in vitro efficacy of Compound A in down-regulating immune checkpoint proteins in Capan-2 $KRAS^{G12C}$ Cells in Vitro. FIG. 5C shows cell surface expression of PD-L1, PVR and CD73 on Capan-2 cells following 48-hour treatment with Compound A in the presence of Interferon gamma (IFNγ), as measured by flow cytometry. Each graph shows mean fluorescence intensity ((MFI), for each respective immune checkpoint protein) vs. log M [Compound A].

FIGs. 6A-6B demonstrate that Compound A, a $KRAS^{MULTI}$(ON) inhibitor disclosed herein, is active against RAS oncogene switching mutations observed in $KRAS^{G12C}$(OFF) resistance. FIG. 6A is a heatmap representing cellular RAS/RAF disruption assay results regarding various KRAS mutations in the presence of different RAS inhibitors. FIG. 6B shows the IC50 value associated with each colored bar of the heatmap.

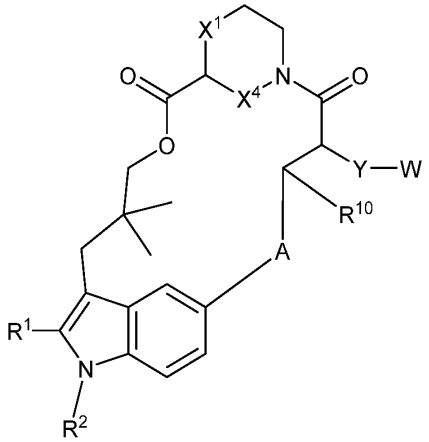
Detailed Description

Compounds

Provided herein are Ras inhibitors. The approach described herein entails formation of a high affinity three-component complex between a synthetic ligand and two intracellular proteins which do not interact under normal physiological conditions: the target protein of interest (e.g., Ras), and a widely expressed cytosolic chaperone (presenter protein) in the cell (e.g., cyclophilin A). More specifically, in some embodiments, the inhibitors of Ras described herein induce a new binding pocket in Ras by driving formation of a high affinity tri-complex between the Ras protein and the widely expressed cytosolic chaperone, cyclophilin A (CYPA). Without being bound by theory, the inventors believe that one way the inhibitory effect on Ras is effected by compounds of the invention and the complexes they form is by steric occlusion of the interaction site between Ras and downstream effector molecules, such as RAF, which are required for propagating the oncogenic signal.

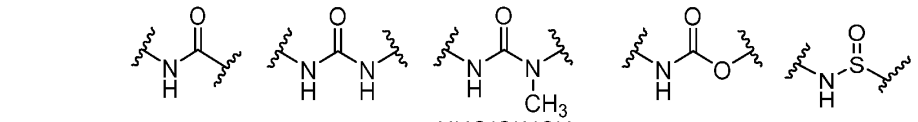
Without being bound by theory, the inventors postulate that non-covalent interactions of a compound of the present invention with Ras and the chaperone protein (e.g., cyclophilin A) may contribute to the inhibition of Ras activity. For example, van der Waals, hydrophobic, hydrophilic and hydrogen bond interactions, and combinations thereof, may contribute to the ability of the compounds of the present invention to form complexes and act as Ras inhibitors. Accordingly, a variety of Ras proteins may be inhibited by compounds of the present invention (e.g., a wild-type Ras or Ras^{amp}, or K-Ras, N-Ras, H-Ras, and mutants thereof at positions 12, 13 and 61, such as G12C, G12D, G12V, G12S, G13C, G13D, and Q61L, and others described herein, as well as combinations of Ras proteins).

Accordingly, provided herein are compounds, or pharmaceutically acceptable salts thereof, having the structure of Formula I:

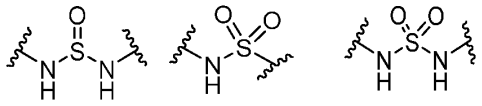


Formula I

wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, optionally substituted 5 to 6-membered heteroarylene, optionally substituted C_2-C_4 alkylene, or optionally substituted C_2-C_4 alkenylene;



Y is -NHC(O)-, -NHC(O)NH-, -NHC(O)NCH$_3$-, -NHC(O)O-, -NHC(S)-,



-NHC(S)NH-, -NHS(O)$_2$-, or -NHS(O)$_2$NH-;

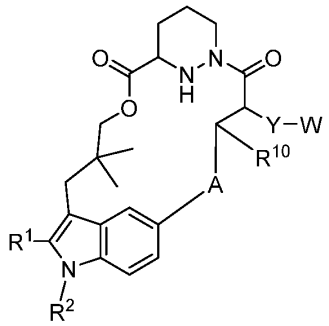
W is hydrogen, C_1-C_4 alkyl, optionally substituted C_1-C_3 heteroalkyl, optionally substituted 3 to 10-membered heterocycloalkyl, optionally substituted 3 to 10-membered cycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

X^1 and X^4 are each, independently, CH_2 or NH;

R^1 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 15-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;and

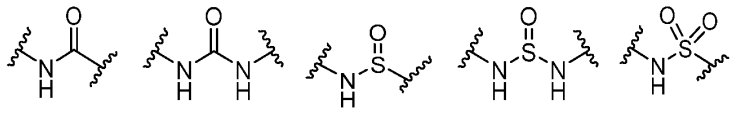
R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; and R^{10} is hydrogen, hydroxy, optionally substituted C_1-C_3 alkyl, or optionally substituted C_1-C_6 heteroalkyl.

In some embodiments, the compound, or pharmaceutically acceptable salt thereof, has the structure of Formula Ia:

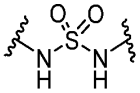


Formula Ia

wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;



Y is -NHC(O)-, -NHC(O)NH-, -NHC(S)-, -NHC(S)NH-, $-NHS(O)_2-$, or



$-NHS(O)_2NH-$;

W is hydrogen, C_1-C_4 alkyl, optionally substituted 3 to 10-membered heterocycloalkyl, optionally substituted 3 to 10-membered cycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^1 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; and

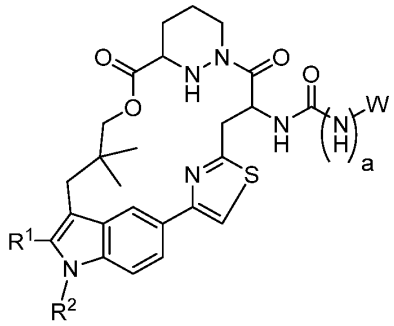
R^{10} is hydrogen or optionally substituted C_1-C_6 heteroalkyl. In some embodiments, R^{10} is hydrogen.

In some embodiments, R^1 is optionally substituted 6 to 10-membered aryl or optionally substituted 5 to 10-membered heteroaryl. In some embodiments, R^1 is optionally substituted phenyl or optionally substituted pyridine.

In some embodiments, A is optionally substituted thiazole, optionally substituted triazole, optionally substituted morpholino, optionally substituted piperidinyl, optionally substituted pyridine, or optionally substituted phenyl. In some embodiments, A is optionally substituted thiazole, optionally substituted triazole, optionally substituted morpholino, or phenyl. In some embodiments, A is not an optionally substituted phenyl or benzimidazole. In some embodiments, A is not hydroxyphenyl.

In some embodiments, Y is -NHC(O)- or -NHC(O)NH-.

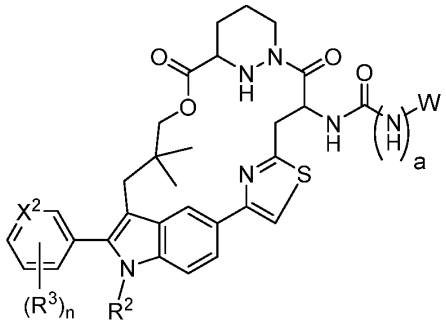
In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula II:



Formula II,

wherein a is 0 or 1.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula II-1:



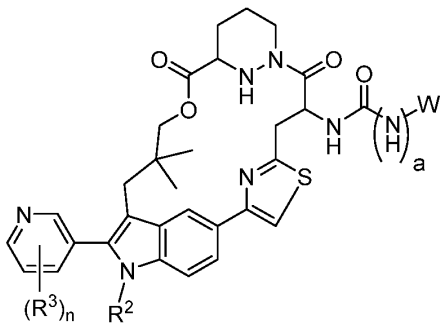
Formula II-1,

wherein X^2 is N or CH;

each R^3 is independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl (e.g., optionally substituted 3 to 6-membered heterocycloalkyl), optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; and

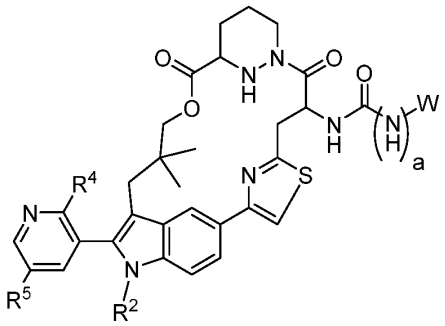
n is an integer from 1 to 4.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula II-2:



Formula II-2.

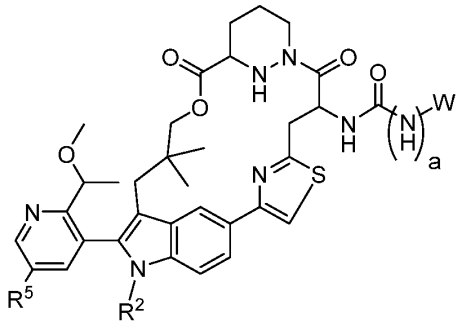
In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula II-3:



Formula II-3,

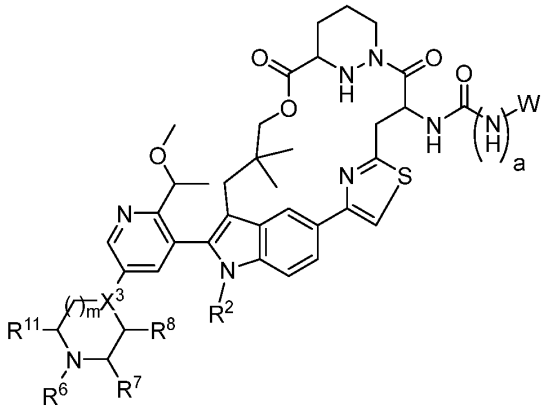
wherein R^4 and R^5 are each independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl (e.g., optionally substituted 3 to 6-membered heterocycloalkyl), optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula II-4:



Formula II-4.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula II-5:



Formula II-5,

wherein X^3 is N or CH;

m is 1 or 2;

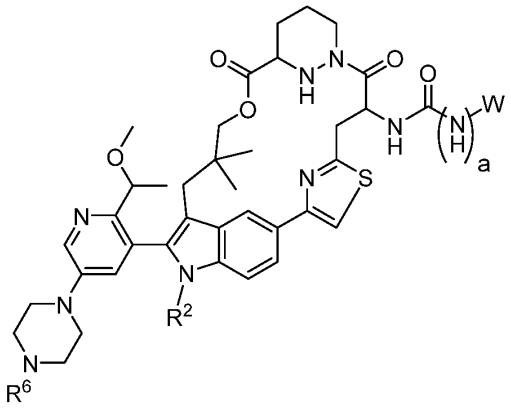
R^6, R^7, R^8, and R^{11} are each independently selected from hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; or

R^6 and R^7 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^8 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered heterocycloalkyl; or

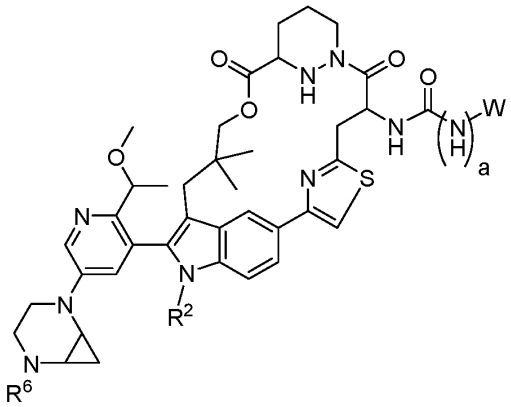
R^7 and R^{11} combine with the atoms to which they are attached to form an optionally substituted 4 to 8-membered heterocycloalkyl. In some embodiments, X^3 is N. In some embodiments, m is 1. In some embodiments, R^{11} is H. In some embodiments, X^3 is N, m is 1, and R^{11} is H.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula II-6:



Formula II-6.

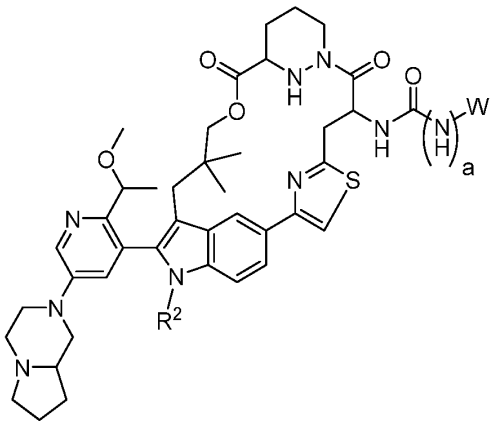
In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula II-7:

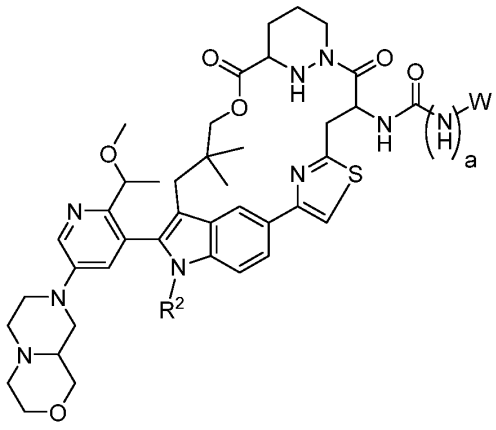


Formula II-7.

In some embodiments (e.g., of any one of Formulae II-6 or II-7), R^6 is methyl.

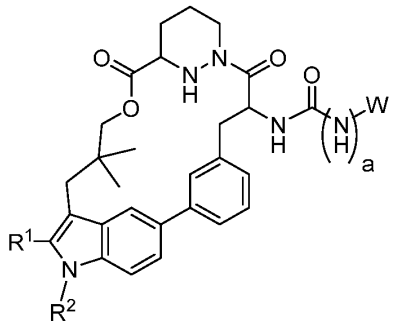
In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula II-8 or Formula II-9:





Formula II-8, Formula II-9.

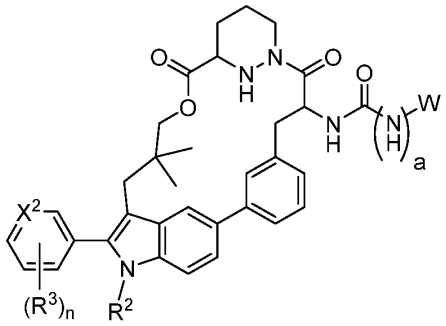
In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula III:



Formula III,

wherein a is 0 or 1.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula III-1:



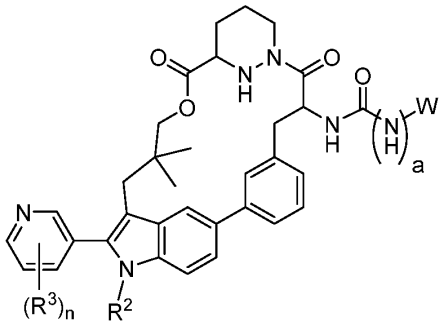
Formula III-1,

wherein X^2 is N or CH;

each R^3 is independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl (e.g., optionally substituted 3 to 6-membered heterocycloalkyl), optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; and

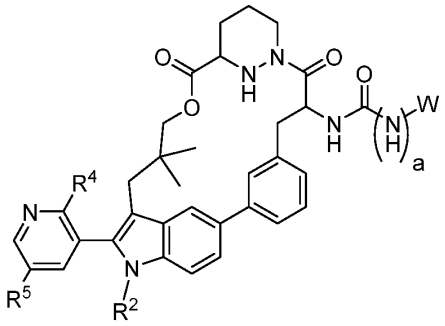
n is an integer from 1 to 4.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula III-2:



Formula III-2.

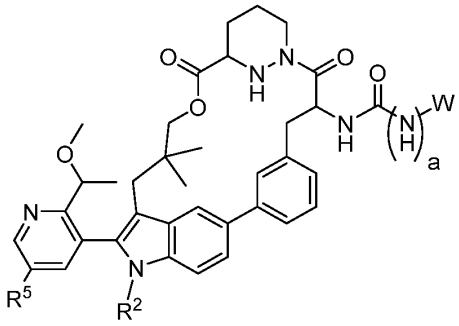
In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula III-3:



Formula III-3,

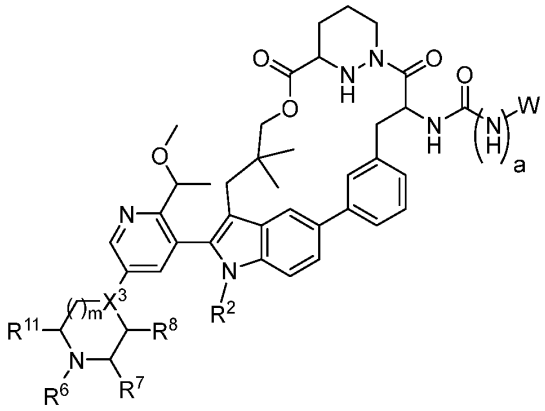
wherein R^4 and R^5 are each independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl (e.g., optionally substituted 3 to 6-membered heterocycloalkyl), optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula III-4:



Formula III-4.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula III-5:



Formula III-5,

wherein X^3 is N or CH;

m is 1 or 2;

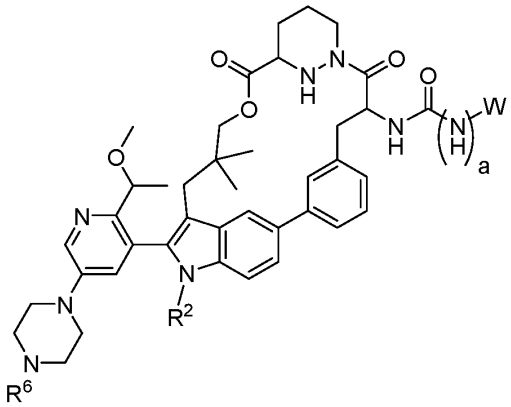
R^6, R^7, R^8, and R^{11} are each independently selected from hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; or

R^6 and R^7 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^8 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered heterocycloalkyl; or

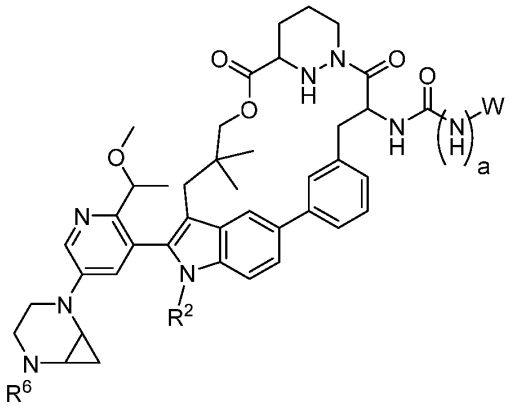
R^7 and R^{11} combine with the atoms to which they are attached to form an optionally substituted 4 to 8-membered heterocycloalkyl. In some embodiments, X^3 is N. In some embodiments, m is 1. In some embodiments, R^{11} is hydrogen. In some embodiments, X^3 is N, m is 1, and R^{11} is H.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula III-6:



Formula III-6.

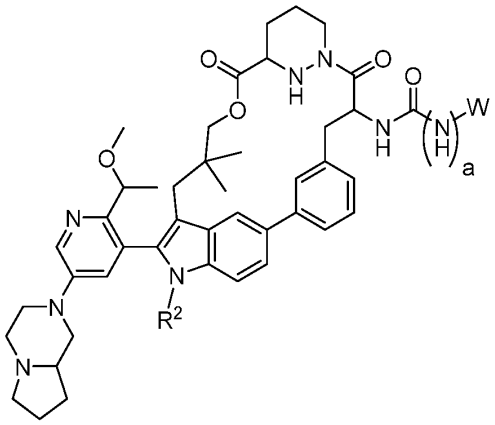
In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula III-7:



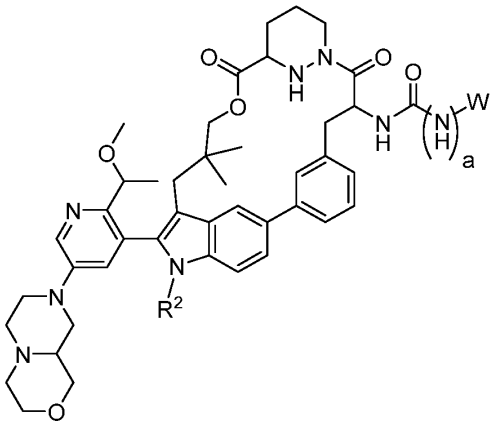
Formula III-7.

In some embodiments (e.g., of any one of Formulae III-6 or III-7), R^6 is methyl.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula III-8 or Formula III-9:

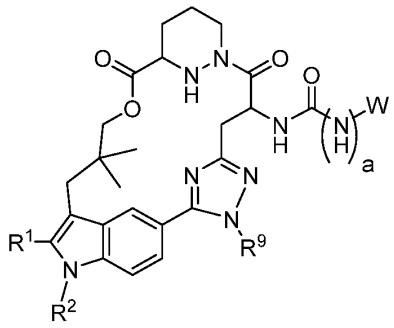


Formula III-8,



Formula III-9.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula IV:

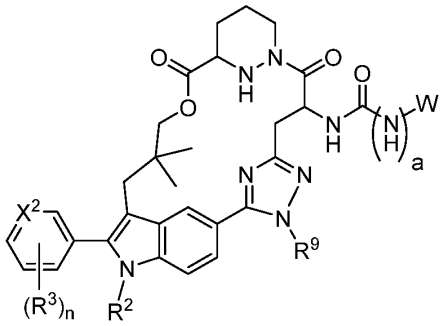


Formula IV,

wherein R^9 is H or C_1-C_6 alkyl; and

a is 0 or 1.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula IV-1:



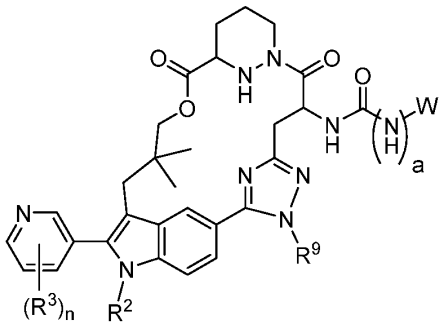
Formula IV-1,

wherein X^2 is N or CH;

each R^3 is independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl (e.g., optionally substituted 3 to 6-membered heterocycloalkyl), optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; and

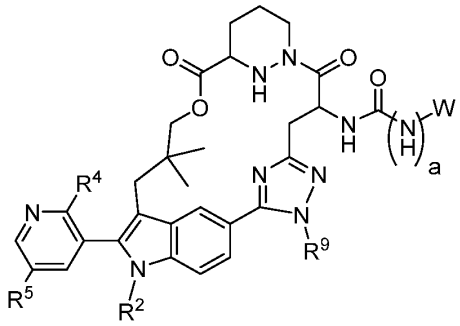
n is an integer from 1 to 4.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula IV-2:



Formula IV-2.

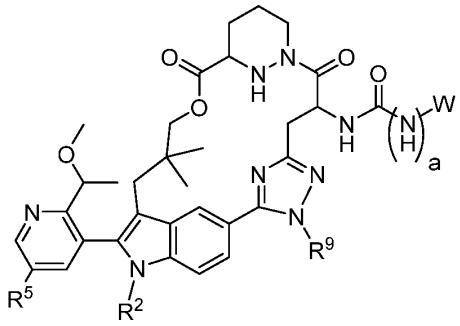
In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula IV-3:



Formula IV-3,

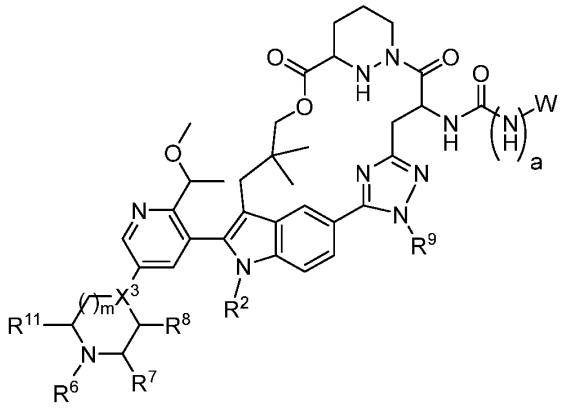
wherein R^4 and R^5 are each independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl (e.g., optionally substituted 3 to 6-membered heterocycloalkyl), optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula IV-4:



Formula IV-4.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula IV-5:



Formula IV-5,

wherein X^3 is N or CH;

m is 1 or 2;

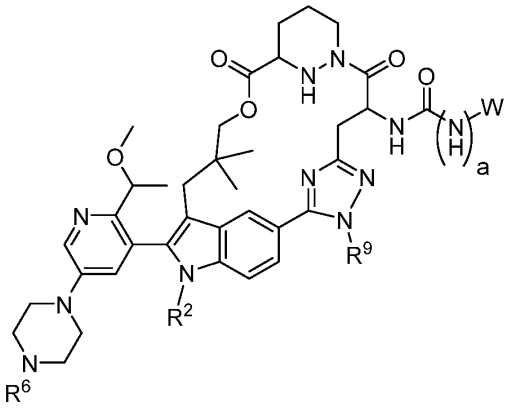
R^6, R^7, R^8, and R^{11} are each independently selected from hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; or

R^6 and R^7 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^8 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered heterocycloalkyl; or

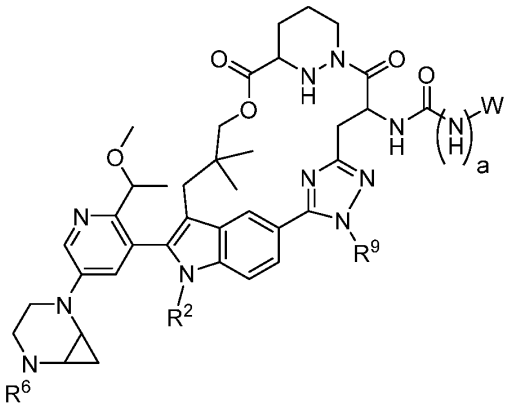
R^7 and R^{11} combine with the atoms to which they are attached to form an optionally substituted 4 to 8-membered heterocycloalkyl. In some embodiments, X^3 is N. In some embodiments, m is 1. In some embodiments, R^{11} is hydrogen. In some embodiments, X^3 is N, m is 1, and R^{11} is H.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula IV-6:



Formula IV-6.

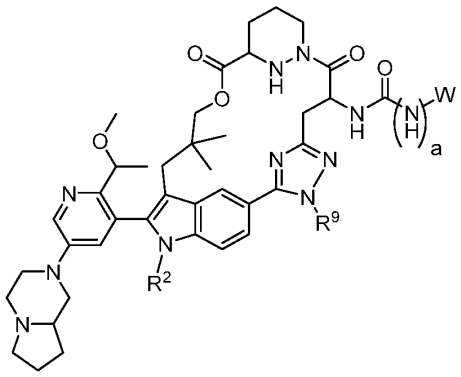
In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula IV-7:



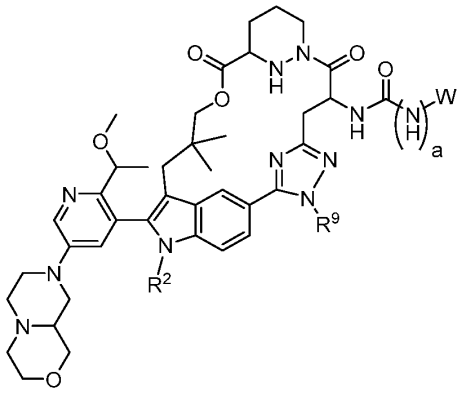
Formula IV-7.

In some embodiments (e.g., of any one fo Formulae IV-6 or IV-7), R^6 is methyl.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula IV-8 or Formula IV-9:



Formula IV-8,

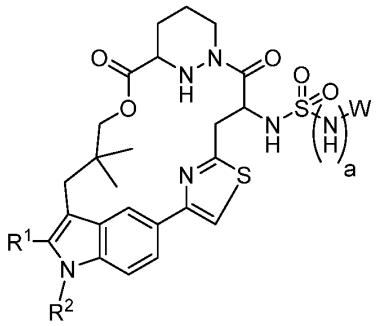


Formula IV-9.

In some embodiments (e.g., of any one of Formulae IV, IV-1, IV-2, IV-3, IV-4, IV-5, IV-6, IV-7, IV-8, or IV-9), R^9 is methyl.

In some embodiments, Y is $-NHS(O)_2-$ or $-NHS(O)_2NH-$.

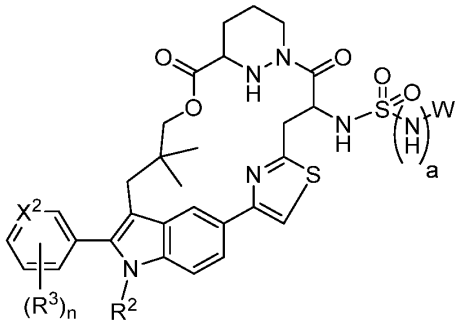
In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula V:



Formula V,

wherein a is 0 or 1.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula V-1:



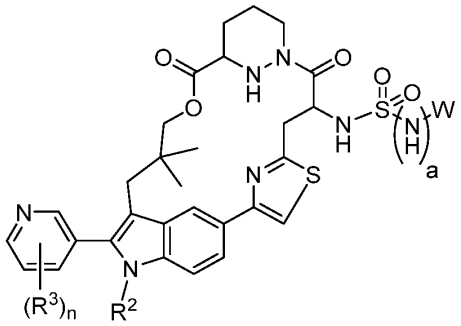
Formula V-1,

wherein X^2 is N or CH;

each R^3 is independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl (e.g., optionally substituted 3 to 6-membered heterocycloalkyl), optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; and

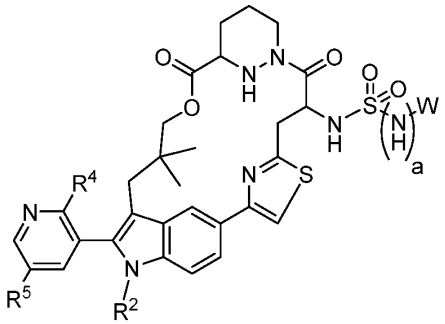
n is an integer from 1 to 4.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula V-2:



Formula V-2.

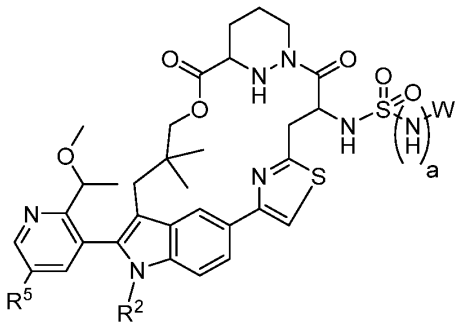
In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula V-3:



Formula V-3,

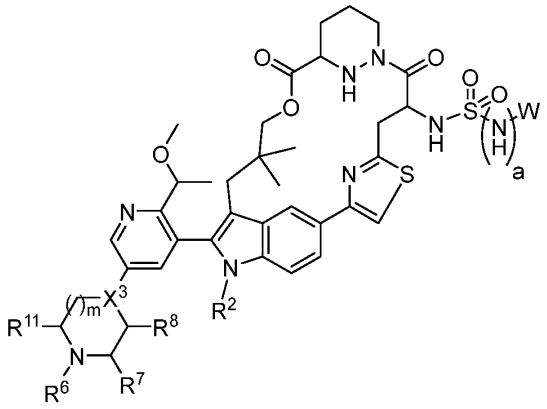
wherein R^4 and R^5 are each independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl (e.g., optionally substituted 3 to 6-membered heterocycloalkyl), optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula V-4:



Formula V-4.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula V-5:



Formula V-5,

wherein X^3 is N or CH;

m is 1 or 2;

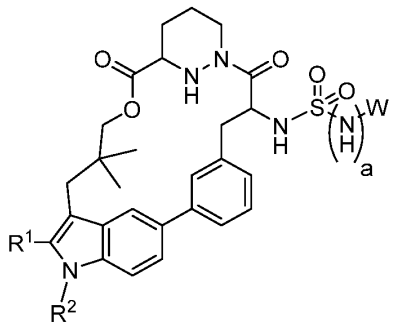
R^6, R^7, R^8, and R^{11} are each independently selected from hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; or

R^6 and R^7 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^8 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^{11} combine with the atoms to which they are attached to form an optionally substituted 4 to 8-membered heterocycloalkyl. In some embodiments, X^3 is N. In some embodiments, m is 1. In some embodiments, R^{11} is hydrogen. In some embodiments, X^3 is N, m is 1, and R^{11} is H.

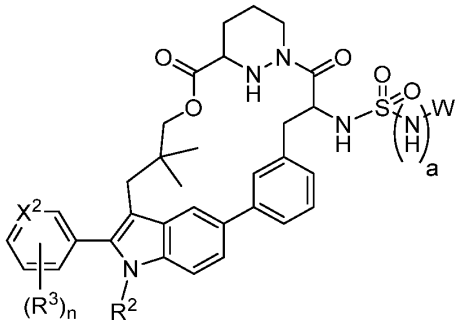
In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula VI:



Formula VI,

wherein a is 0 or 1.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula VI-1:



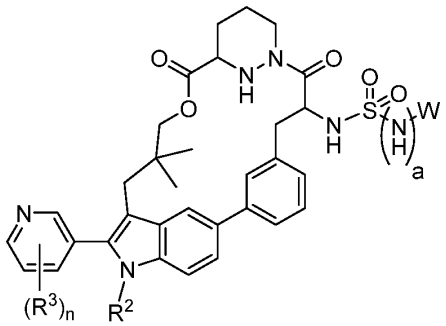
Formula VI-1,

wherein X^2 is N or CH;

each R^3 is independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl (e.g., optionally substituted 3 to 6-membered heterocycloalkyl), optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; and

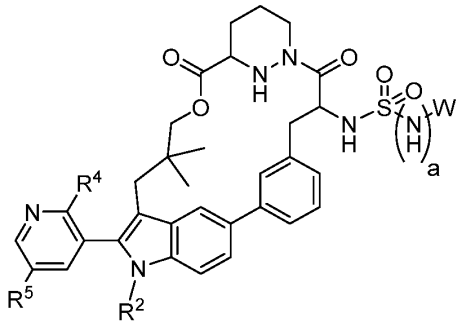
n is an integer from 1 to 4.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula VI-2:



Formula VI-2.

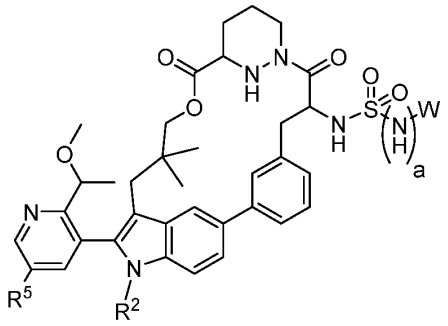
In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula VI-3:



Formula VI-3,

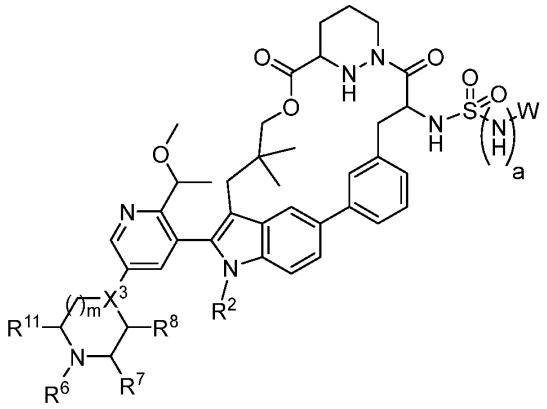
wherein R^4 and R^5 are each independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl (e.g., optionally substituted 3 to 6-membered heterocycloalkyl), optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula VI-4:



Formula VI-4.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula VI-5:



Formula VI-5,

wherein X^3 is N or CH;

m is 1 or 2;

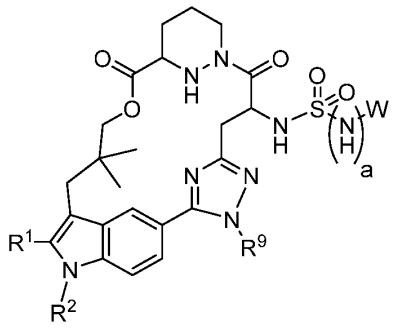
R^6, R^7, R^8, and R^{11} are each independently selected from hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; or

R^6 and R^7 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^8 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^{11} combine with the atoms to which they are attached to form an optionally substituted 4 to 8-membered heterocycloalkyl. In some embodiments, X^3 is N. In some embodiments, m is 1. In some embodiments, R^{11} is hydrogen. In some embodiments, X^3 is N, m is 1, and R^{11} is H.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula VII:

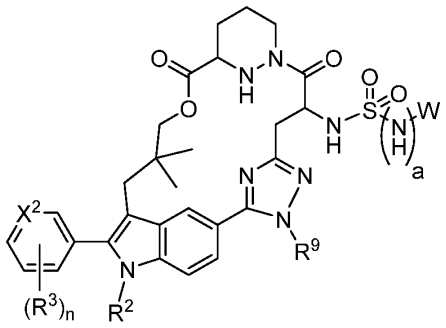


Formula VII,

wherein R^9 is H or C_1-C_6 alkyl; and

a is 0 or 1.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula VII-1:



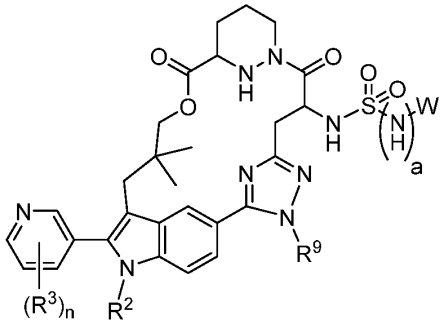
Formula VII-1,

wherein X^2 is N or CH;

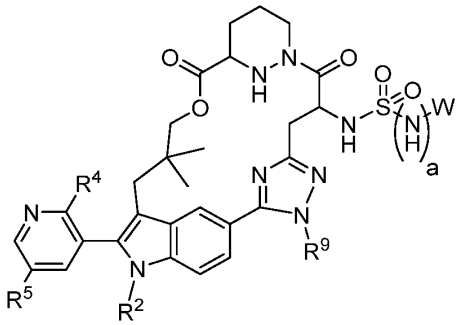
each R^3 is independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl (e.g., optionally substituted 3 to 6-membered heterocycloalkyl), optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; and

n is an integer from 1 to 4.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula VII-2:



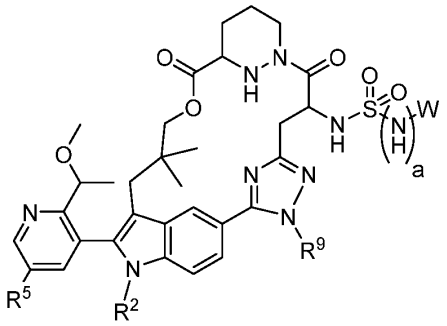
In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula VII-3:



Formula VII-3,

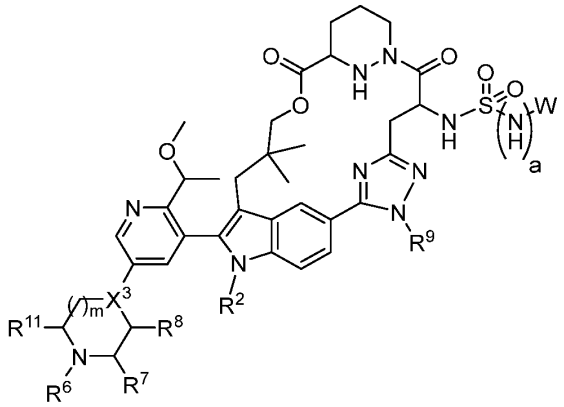
wherein R^4 and R^5 are each independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl (e.g., optionally substituted 3 to 6-membered heterocycloalkyl), optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula VII-4:



Formula VII-4.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula VII-5:



Formula VII-5,

wherein X^3 is N or CH;

m is 1 or 2;

R^6, R^7, R^8, and R^{11} are each independently selected from hydrogren, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; or

R^6 and R^7 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^8 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered heterocycloalkyl; or

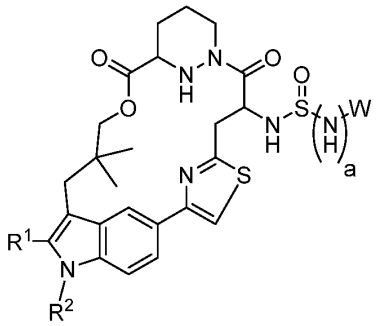
R^7 and R^{11} combine with the atoms to which they are attached to form an optionally substituted 4 to 8-membered heterocycloalkyl. In some embodiments, X^3 is N. In some

embodiments, m is 1. In some embodiments, R^{11} is hydrogen. In some embodiments, X^3 is N, m is 1, and R^{11} is H.

In some embodiments (e.g., of any one of Formulae VII, VII-1, VII-2, VII-3, VII-4, or VII-5), R^9 is methyl.

In some embodiments, Y is -NHS(O)- or -NHS(O)NH-.

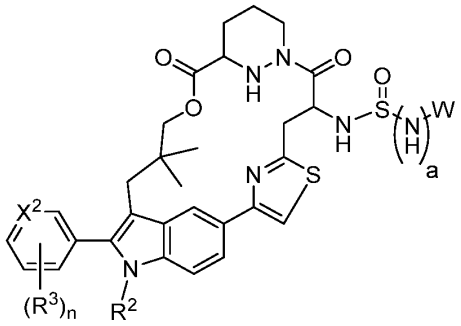
In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula VIII:



Formula VIII,

wherein a is 0 or 1.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula VIII-1:



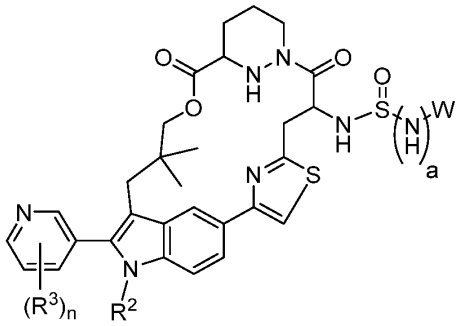
Formula VIII-1,

wherein X^2 is N or CH;

each R^3 is independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl (e.g., optionally substituted 3 to 6-membered heterocycloalkyl), optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; and

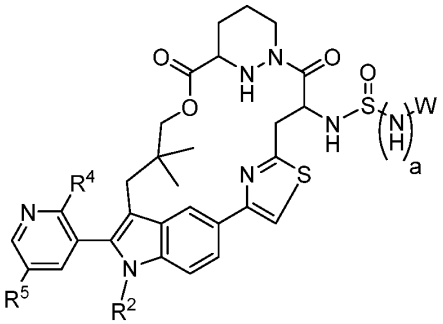
n is an integer from 1 to 4.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula VIII-2:



Formula VIII-2.

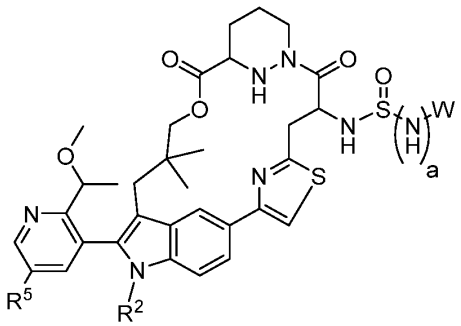
In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula VIII-3:



Formula VIII-3,

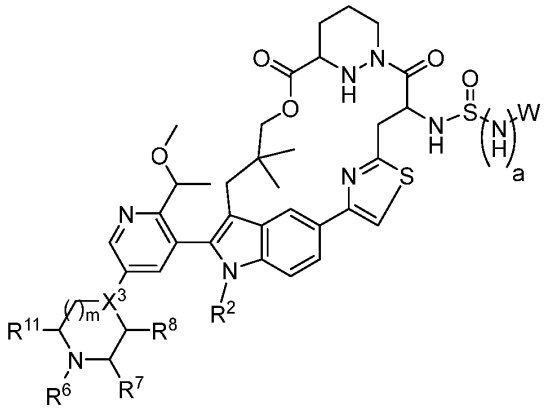
wherein R^4 and R^5 are each independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl (e.g., optionally substituted 3 to 6-membered heterocycloalkyl), optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula VIII-4:



Formula VIII-4.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula VIII-5:



Formula VIII-5,

wherein X^3 is N or CH;

m is 1 or 2;

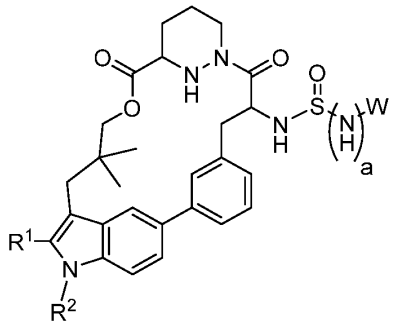
R^6, R^7, R^8, and R^{11} are each independently selected from hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; or

R^6 and R^7 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^8 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^{11} combine with the atoms to which they are attached to form an optionally substituted 4 to 8-membered heterocycloalkyl. In some embodiments, X^3 is N. In some embodiments, m is 1. In some embodiments, R^{11} is hydrogen. In some embodiments, X^3 is N, m is 1, and R^{11} is H.

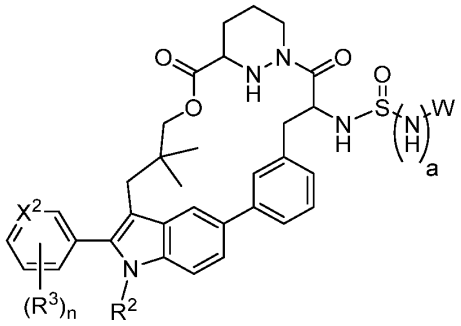
In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula IX:



Formula IX,

wherein a is 0 or 1.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula IX-1:



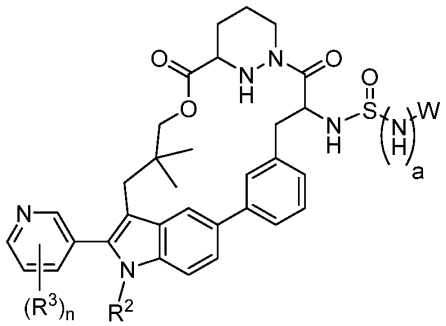
Formula IX-1,

wherein X^2 is N or CH;

each R^3 is independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl (e.g., optionally substituted 3 to 6-membered heterocycloalkyl), optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; and

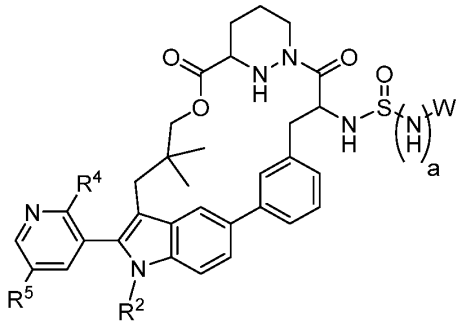
n is an integer from 1 to 4.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula IX-2:



Formula IX-2.

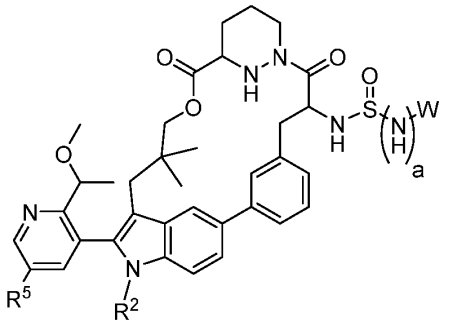
In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula IX-3:



Formula IX-3,

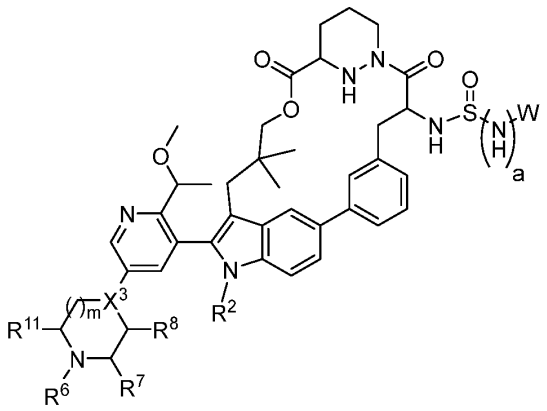
wherein R^4 and R^5 are each independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl (e.g., optionally substituted 3 to 6-membered heterocycloalkyl), optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula IX-4:



Formula IX-4.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula IX-5:



Formula IX-5,

wherein X^3 is N or CH;

m is 1 or 2;

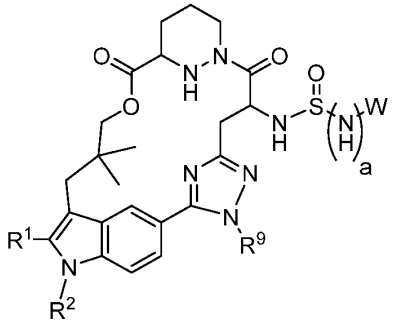
R^6, R^7, R^8, and R^{11} are each independently selected from hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; or

R^6 and R^7 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^8 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^{11} combine with the atoms to which they are attached to form an optionally substituted 4 to 8-membered heterocycloalkyl. In some embodiments, X^3 is N. In some embodiments, m is 1. In some embodiments, R^{11} is hydrogen. In some embodiments, X^3 is N, m is 1, and R^{11} is H.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula X:

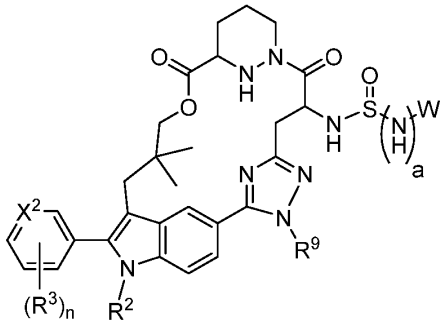


Formula X,

wherein R^9 is H or C_1-C_6 alkyl; and

a is 0 or 1.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula X-1:



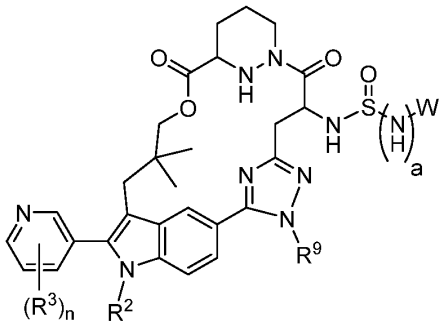
Formula X-1,

wherein X^2 is N or CH;

each R^3 is independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl (e.g., optionally substituted 3 to 6-membered heterocycloalkyl), optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; and

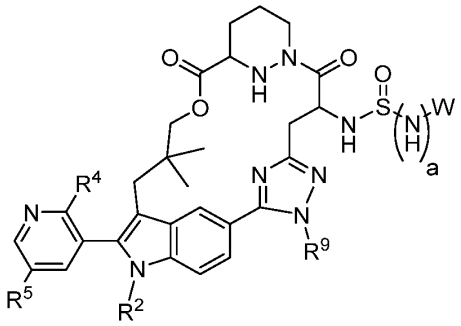
n is an integer from 1 to 4.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula X-2:



Formula X-2.

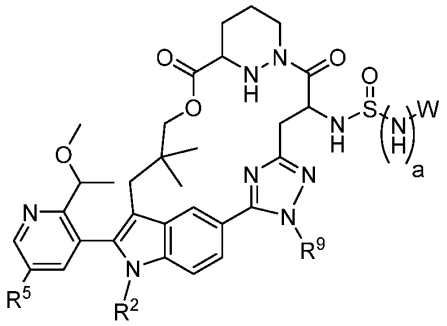
In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula X-3:



Formula X-3,

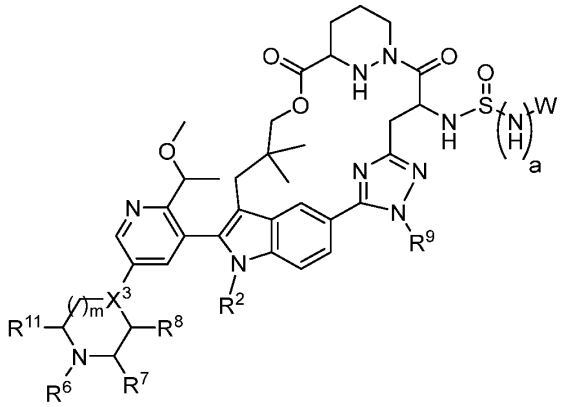
wherein R^4 and R^5 are each independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl (e.g., optionally substituted 3 to 6-membered heterocycloalkyl), optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula X-4:



Formula X-4.

In some embodiments, the compound, or a pharmaceutically acceptable salt thereof, has the structure of Formula X-5:



Formula X-5,

wherein X^3 is N or CH;

m is 1 or 2;

R^6, R^7, R^8, and R^{11} are each independently selected from hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; or

R^6 and R^7 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^8 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^{11} combine with the atoms to which they are attached to form an optionally substituted 4 to 8-membered heterocycloalkyl. In some embodiments, X^3 is N. In some

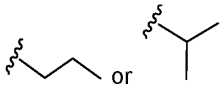
embodiments, m is 1. In some embodiments, R^{11} is hydrogen. In some embodiments, X^3 is N, m is 1, and R^{11} is H.

In some embodiments (e.g., of any one of Formulae X, X-1, X-2, X-3, X-4, or X-5), R^9 is methyl.

In some embodiments of any aspect described herein, a is 0. In some embodiments of any of the above, a is 0.

In some embodiments of any aspect described herein, R^2 is optionally substituted C_1-C_6 alkyl. In some embodiments, R^2 is selected from $-CH_2CH_3$ or $-CH_2CF_3$.

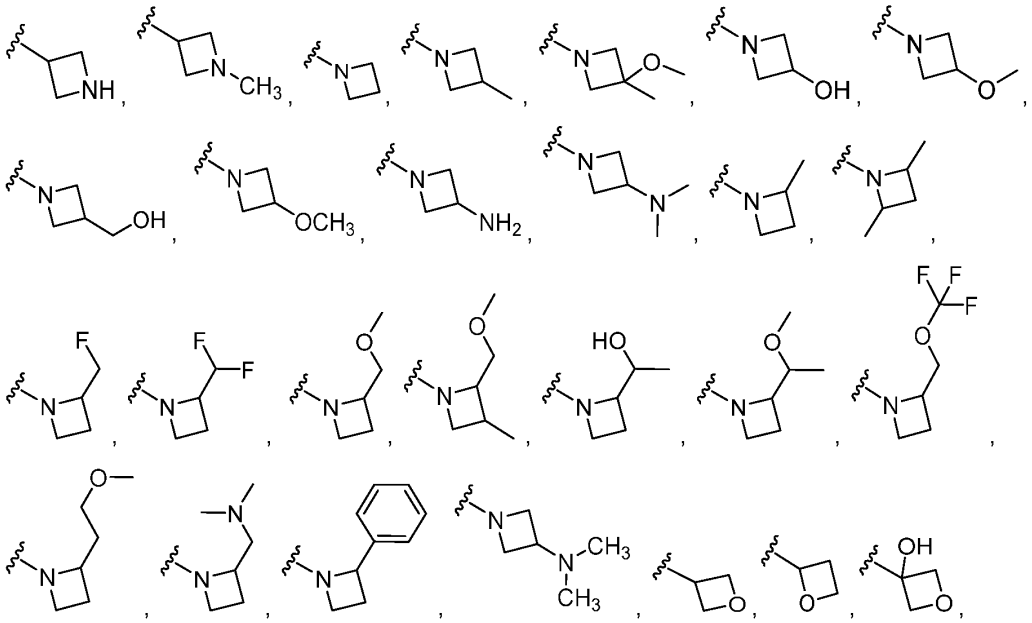
In some embodiments of any aspect described herein, W is C_1-C_4 alkyl. In some embodiments, W is:

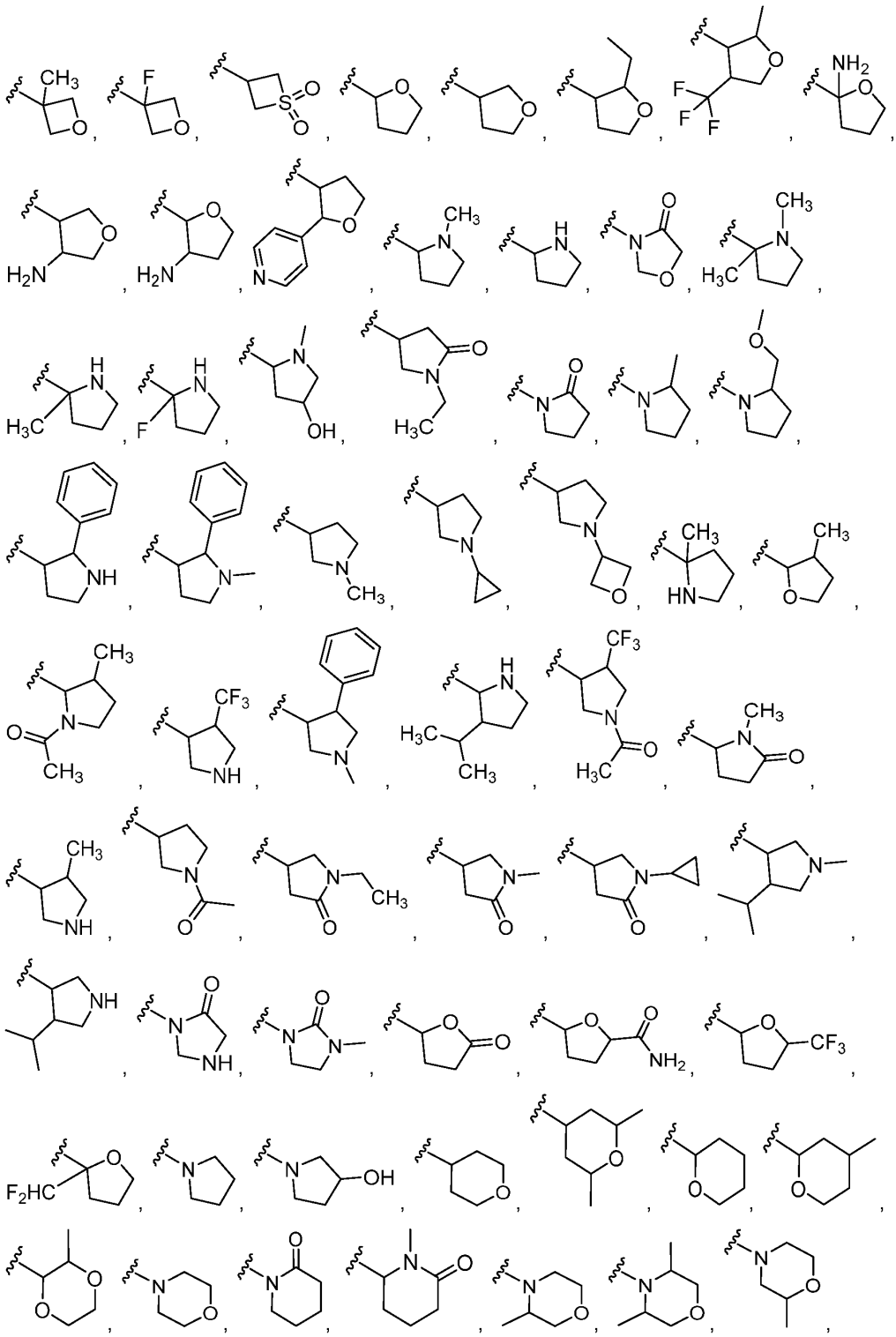


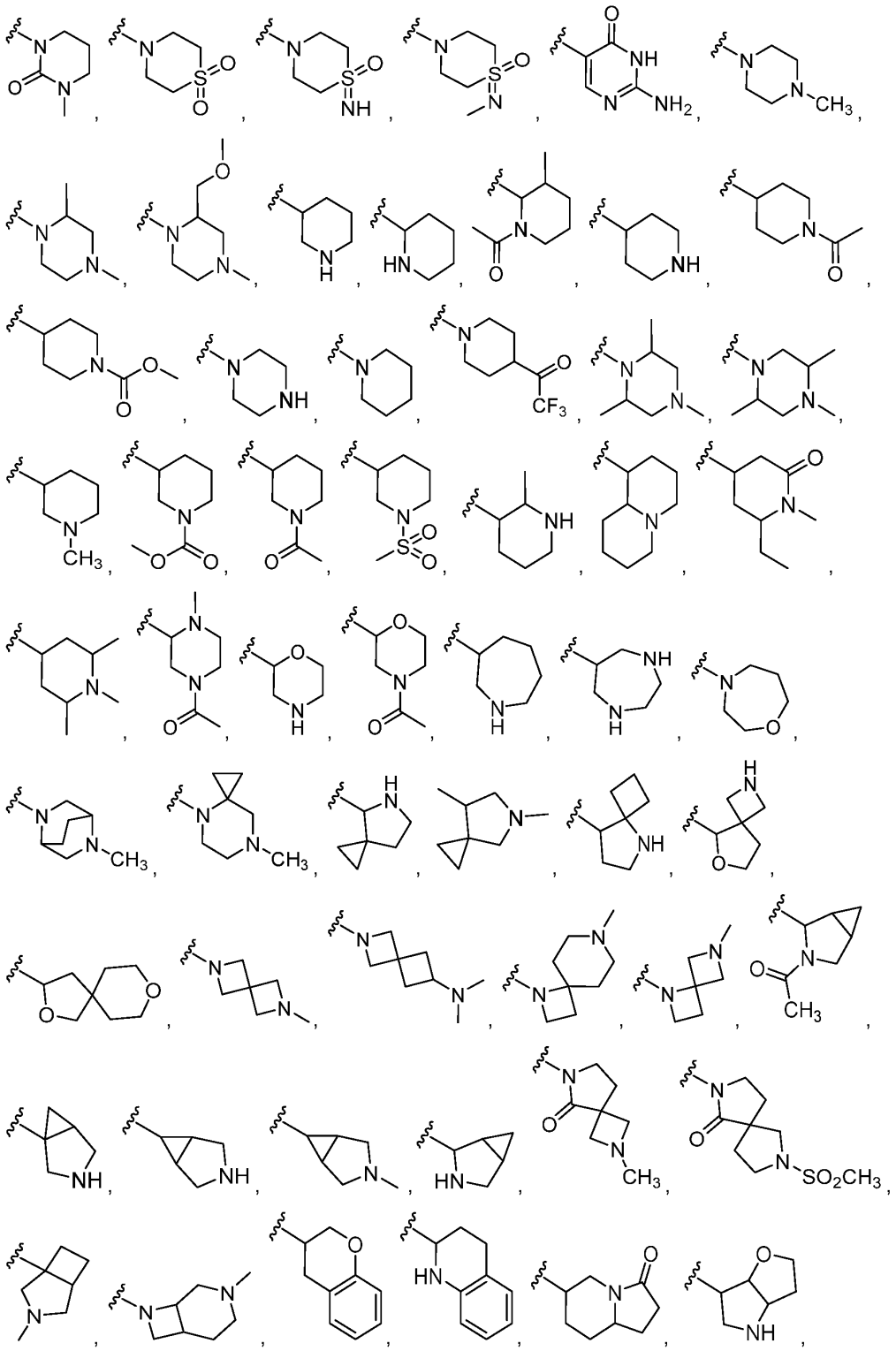
In some embodiments of any aspect described herein, W is optionally substituted cyclopropyl, optionally substituted cyclobutyl, optionally substituted cyclopentyl, or optionally substituted cyclohexyl, optionally substituted piperidine, optionally substituted piperazine, optionally substituted pyridine, or optionally substituted phenyl.

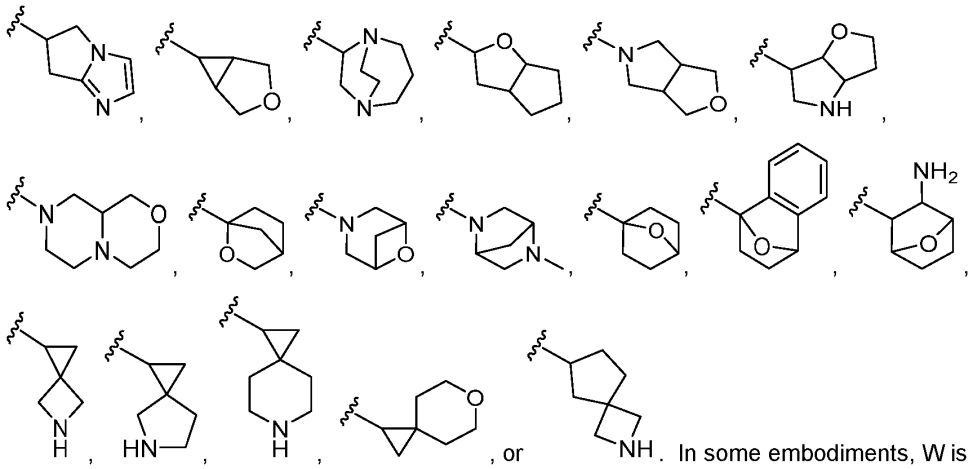
In some embodiments of any aspect described herein, W is optionally substituted 3 to 10-membered heterocycloalkyl, optionally substituted 3 to 10-membered cycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

In some embodiments of any aspect described herein, W is optionally substituted 3 to 10-membered heterocycloalkyl. In some embodiments, W is selected from the following, or a stereoisomer thereof:

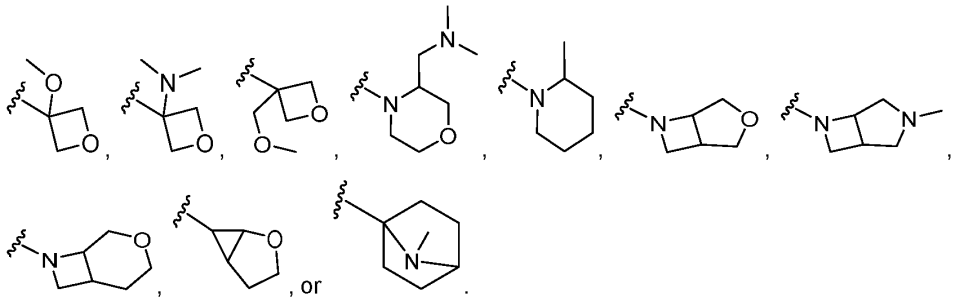




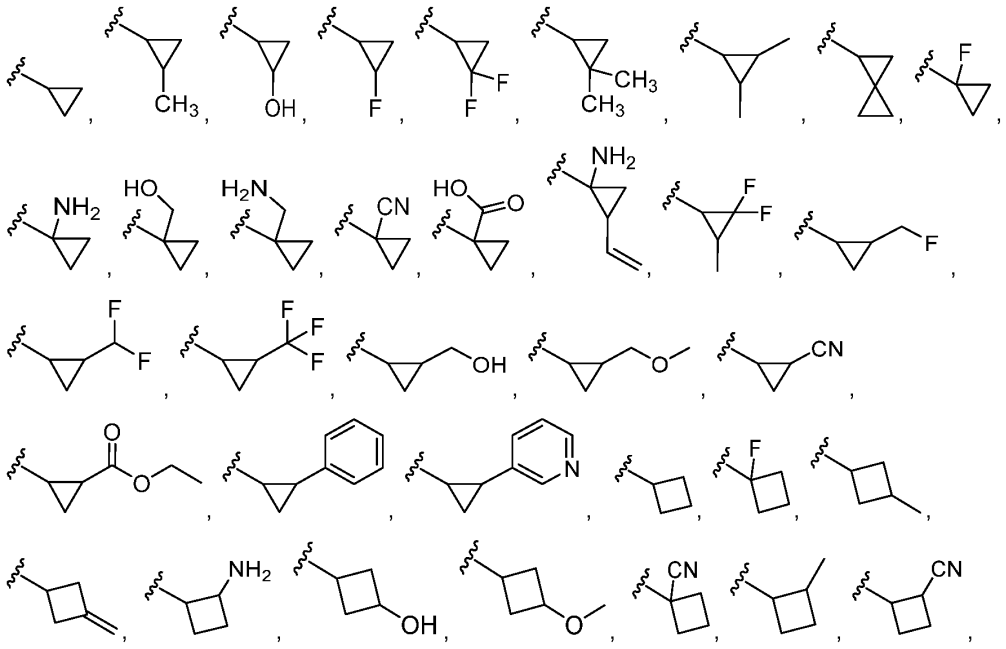


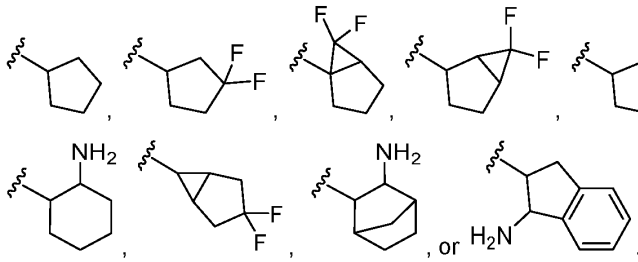


selected from the following, or a stereoisomer thereof:

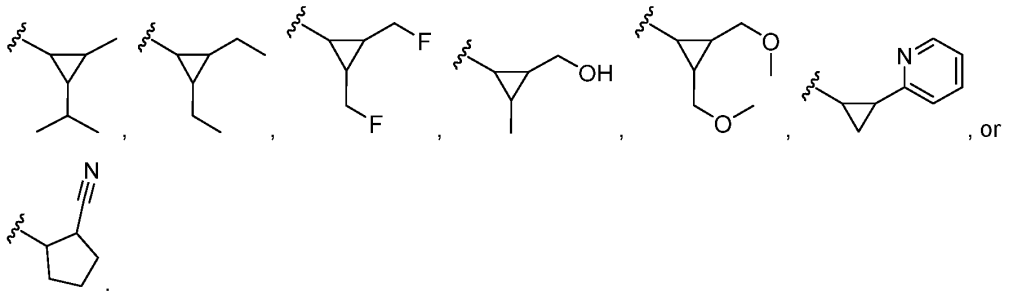


In some embodiments of any aspect described herein, W is optionally substituted 3 to 10-membered cycloalkyl. In some embodiments, W is selected from the following, or a stereoisomer thereof:





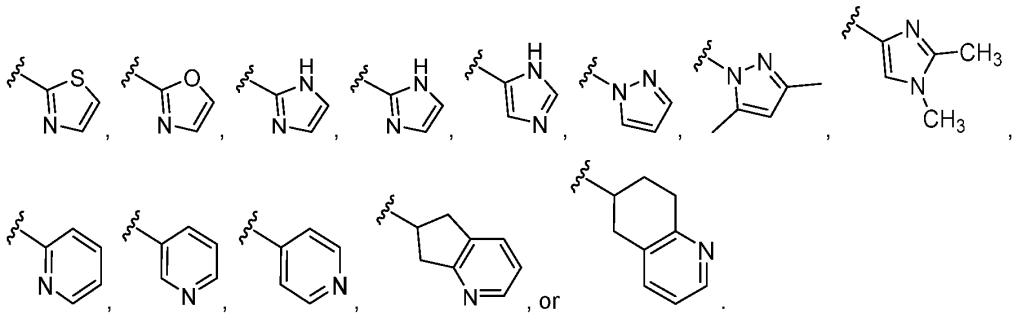
. In some embodiments, W is selected from the following, or a stereoisomer thereof:



, or

.

In some embodiments of any aspect described herein, W is optionally substituted 5 to 10-membered heteroaryl. In some embodiments, W is selected from the following, or a stereoisomer thereof:

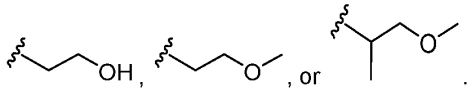


, or

.

In some embodiments of any aspect described herein, W is optionally substituted 6 to 10-membered aryl. In some embodiments, W is optionally substituted phenyl.

In some embodiments of any aspect described herein, W is optionally substituted C_1-C_3 heteroalkyl. In some embodiments, W is selected from the following, or a stereoisomer thereof:

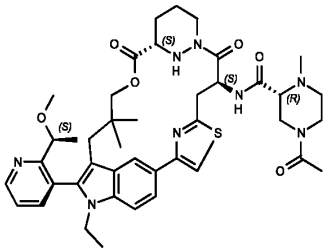
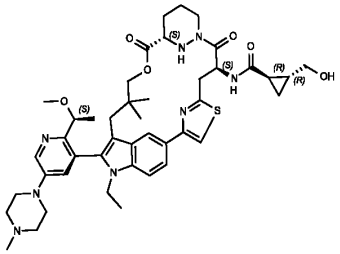
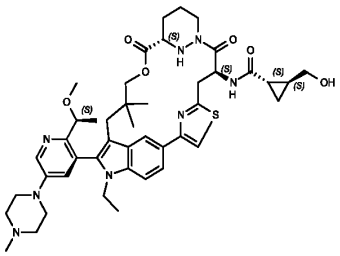
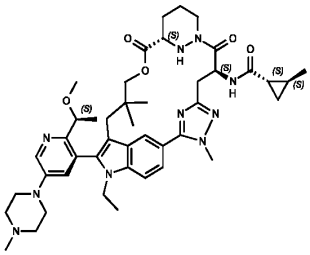
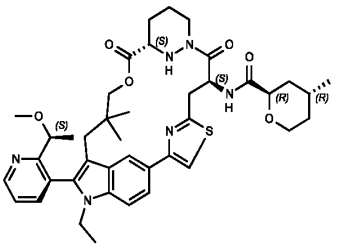


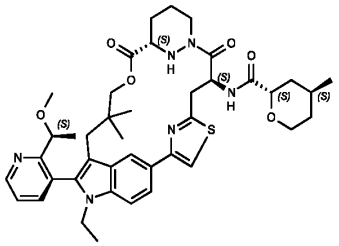
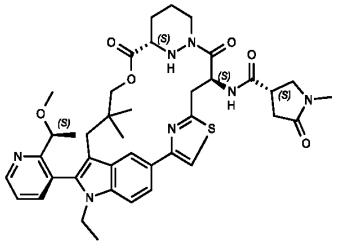
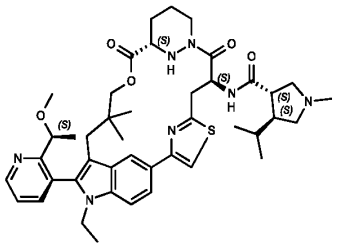
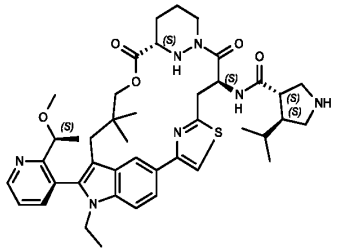
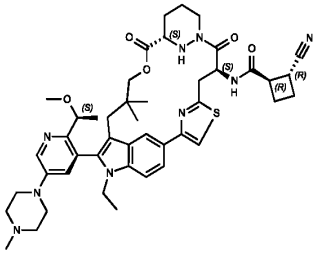
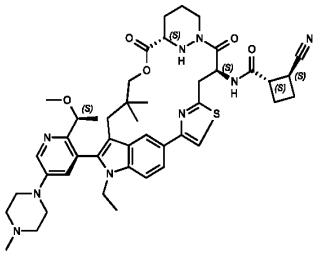
, or

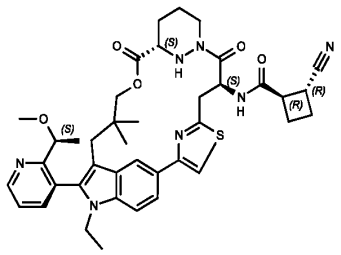
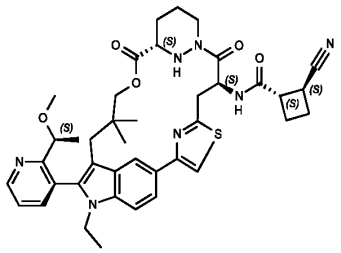
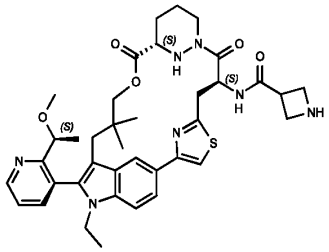
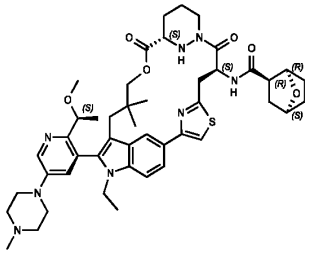
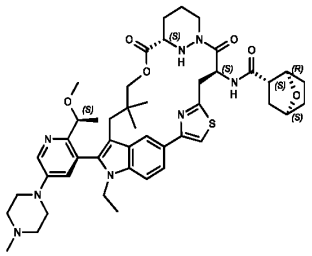
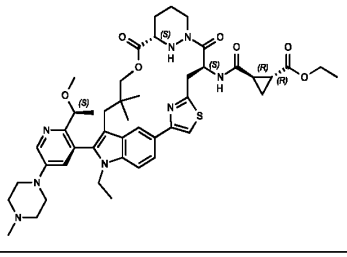
.

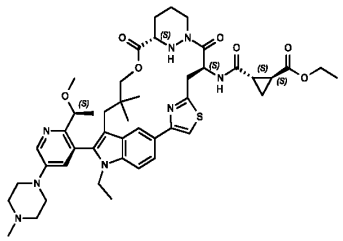
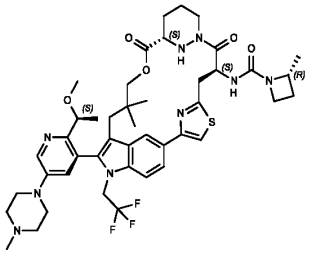
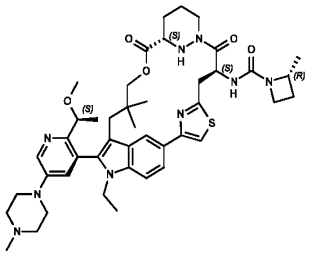
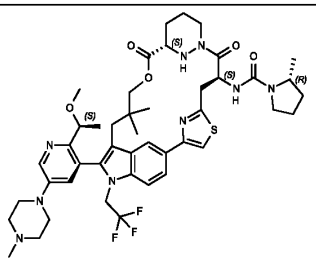
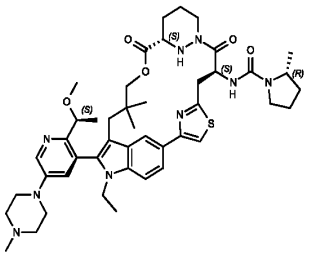
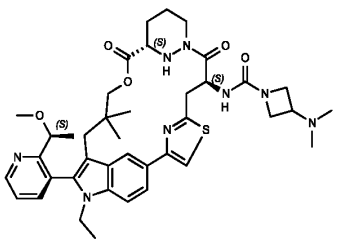
In some embodiments, a compound of the present invention is selected from Table 1, or a pharmaceutically acceptable salt or stereoisomer thereof. In some embodiments, a compound of the present invention is selected from Table 1, or a pharmaceutically acceptable salt or atropisomer thereof.

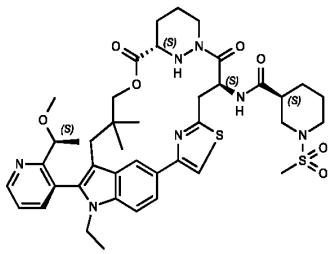
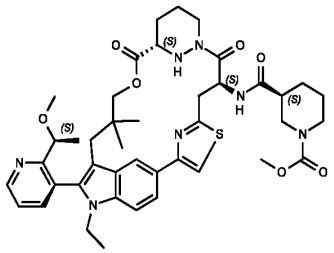
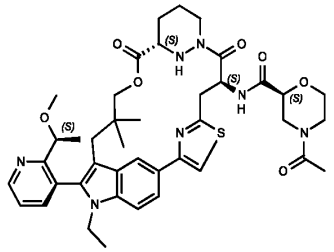
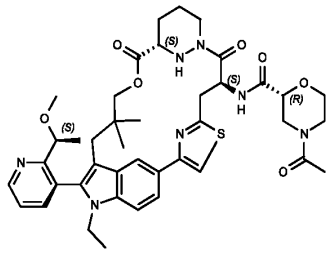
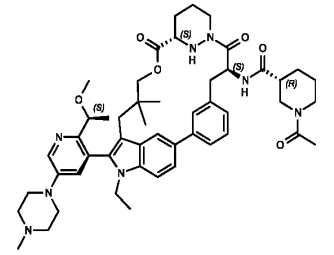
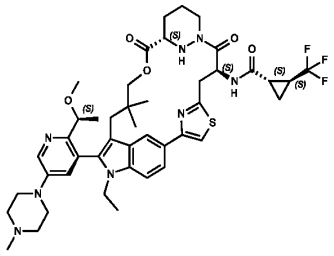
Table 1: Certain Compounds of the Present Invention

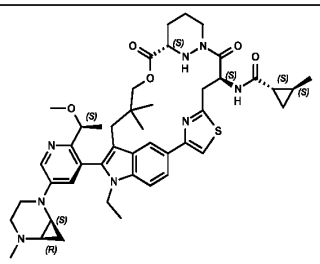
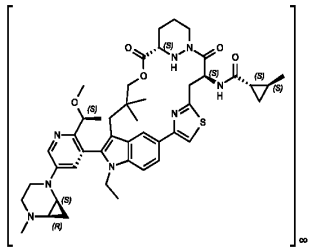
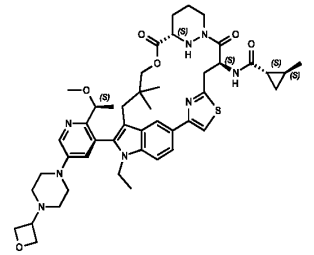
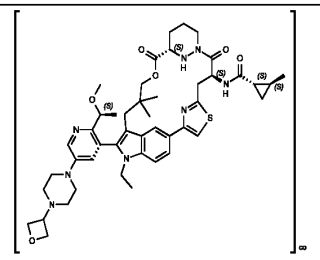
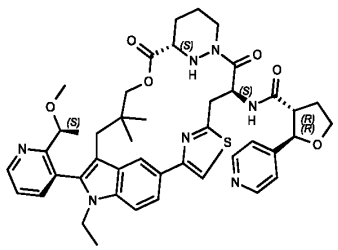
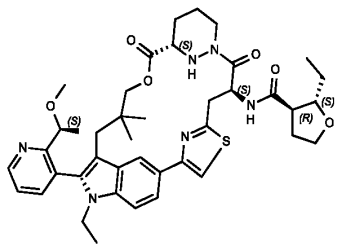
Ex. #	Structure
A1	
A2	
A3	
A4	
A5	

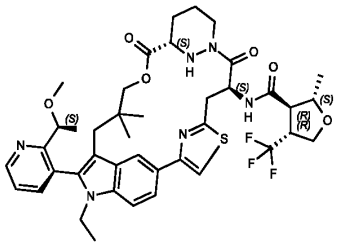
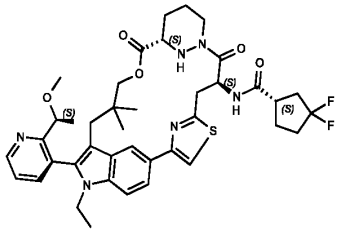
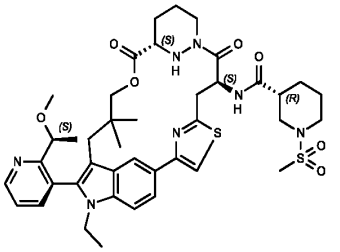
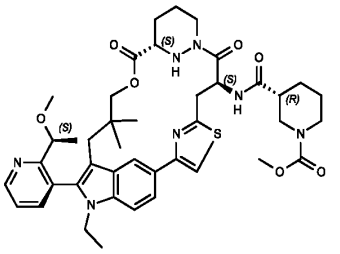
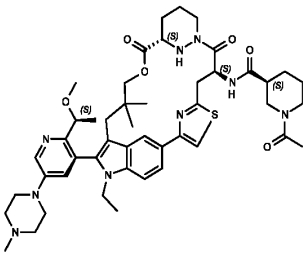
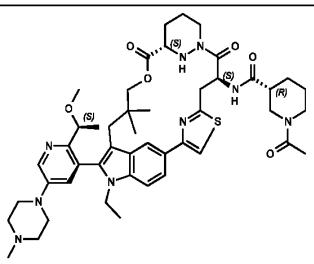
A6	
A7	
A8	
A9	
A10	
A11	

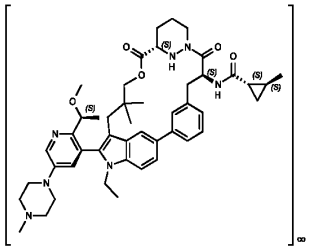
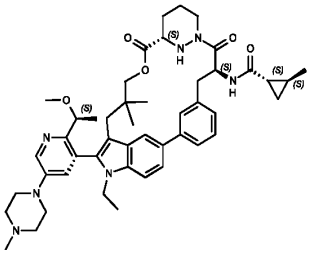
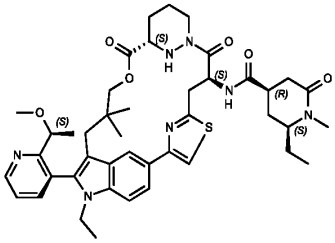
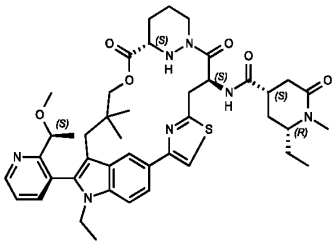
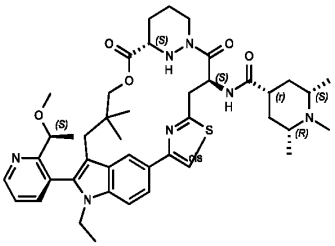
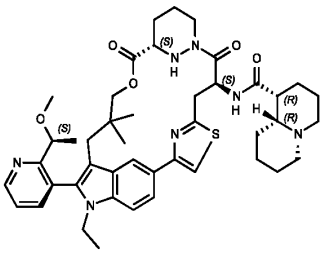
A12	
A13	
A14	
A15	
A16	
A17	

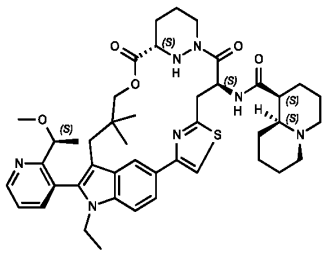
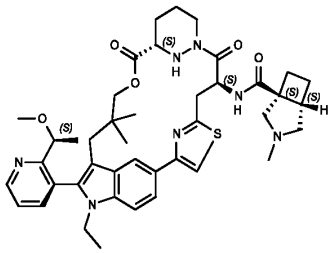
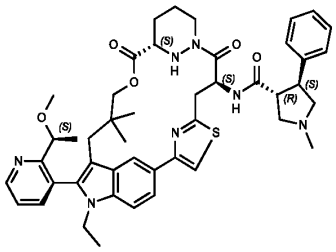
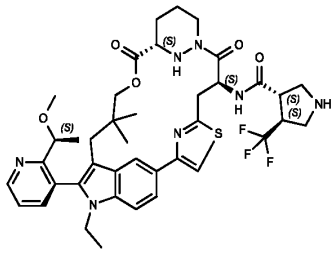
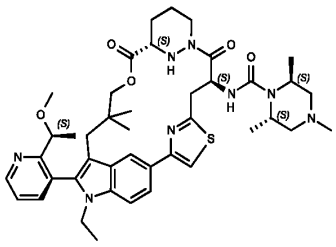
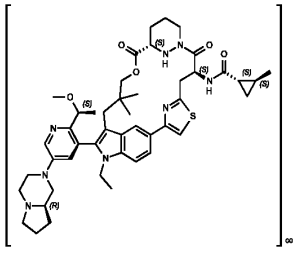
A18	
A19	
A20	
A21	
A22	
A23	

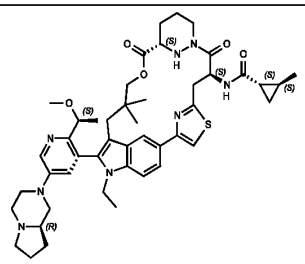
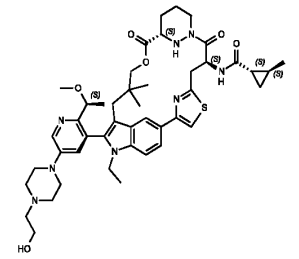
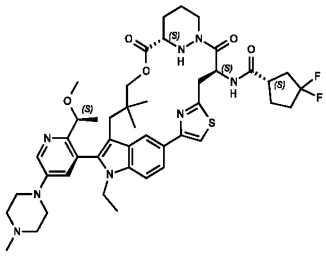
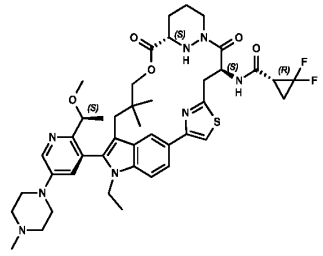
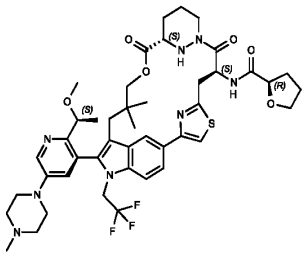
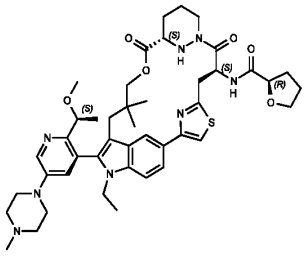
A24	
A25	
A26	
A27	
A28	
A29	

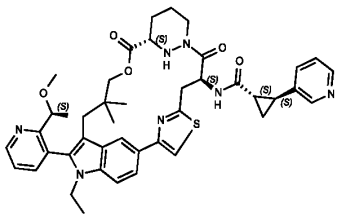
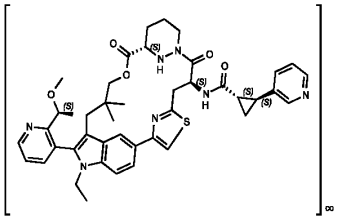
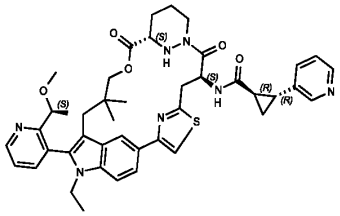
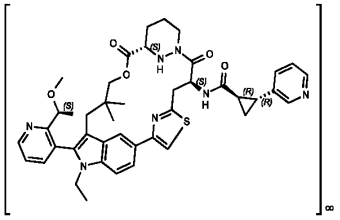
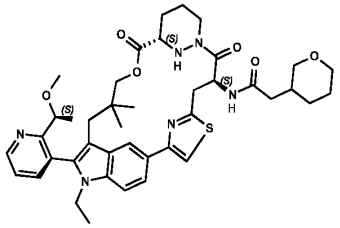
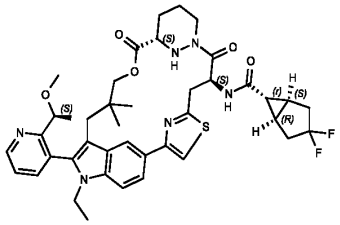
A30	
A31	
A32	
A33	
A34	
A35	

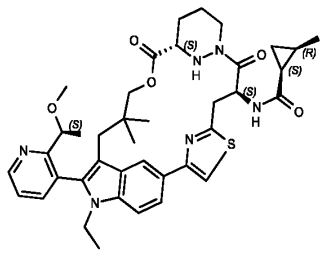
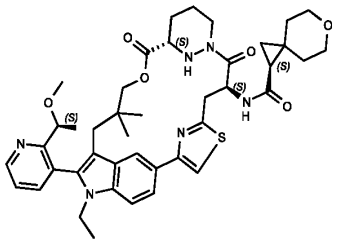
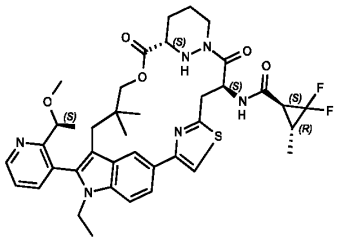
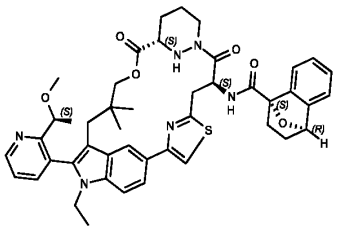
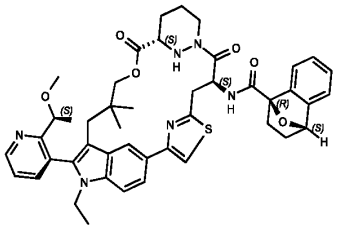
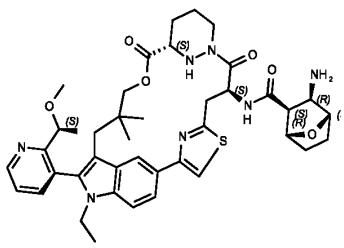
A36	
A37	
A38	
A39	
A40	
A41	

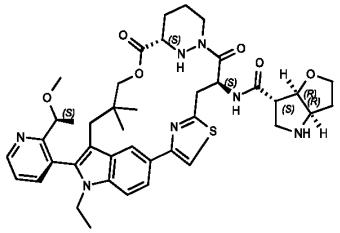
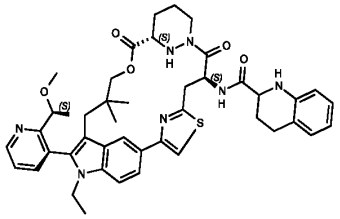
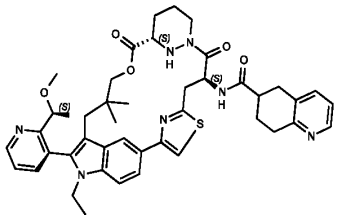
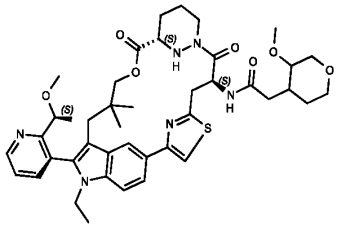
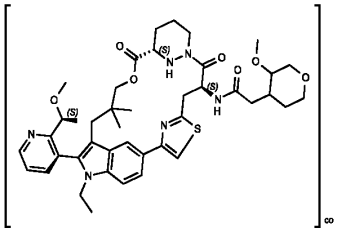
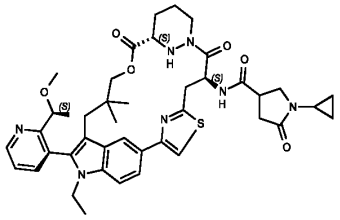
A42	
A43	
A44	
A45	
A46	
A47	

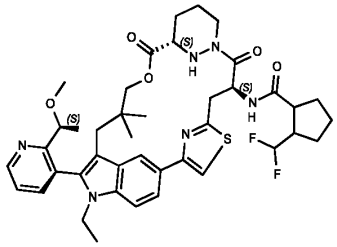
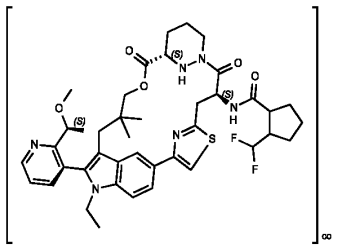
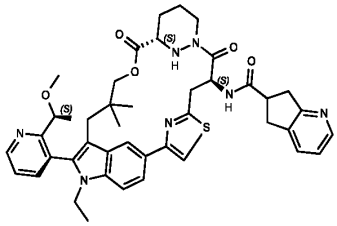
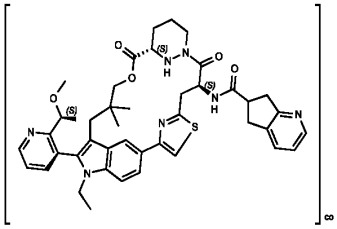
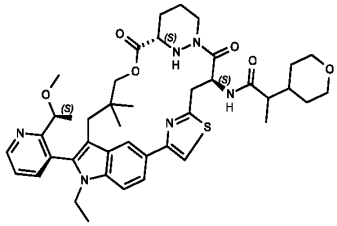
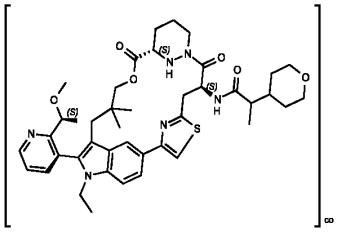
A48	
A49	
A50	
A51	
A52	
A53	

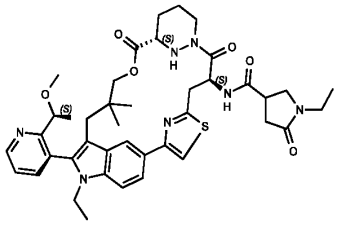
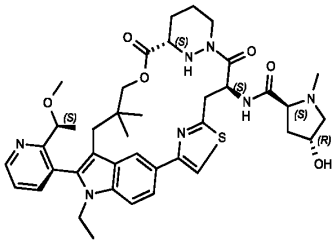
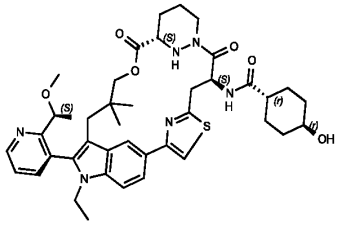
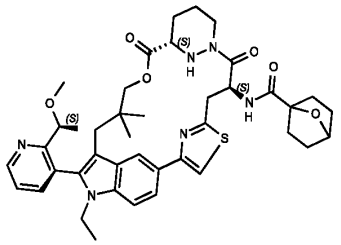
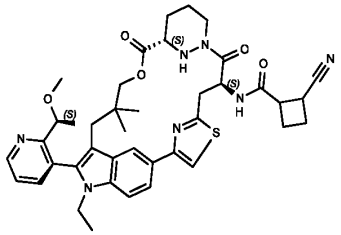
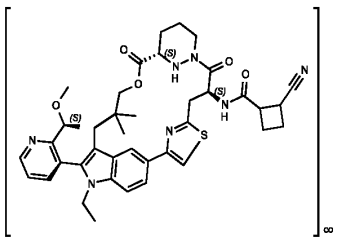
A54	
A55	
A56	
A57	
A58	
A59	

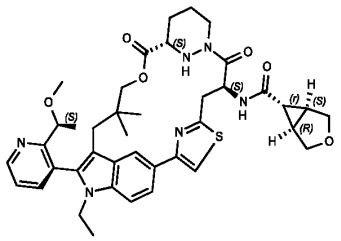
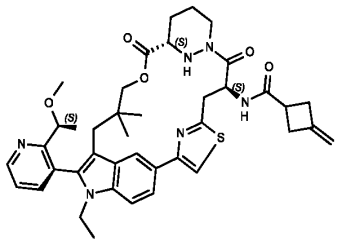
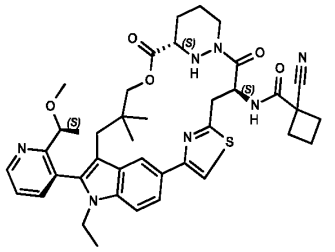
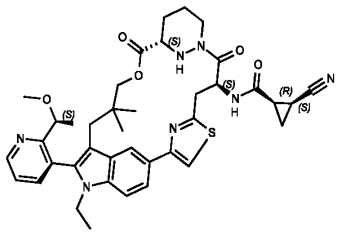
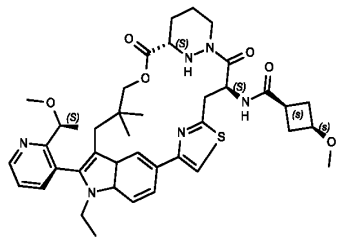
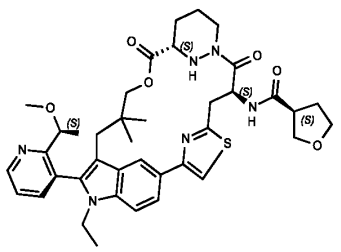
A60	
A61	
A62	
A63	
A64	
A65	

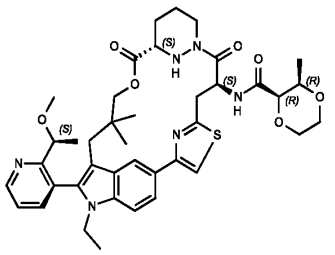
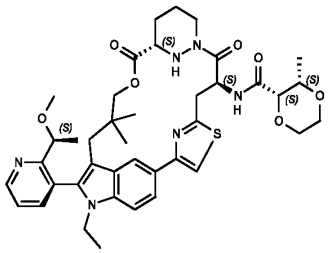
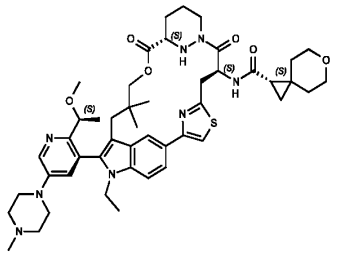
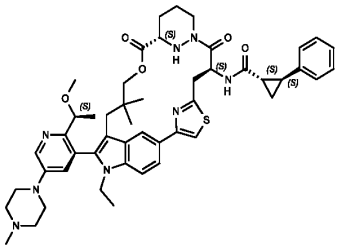
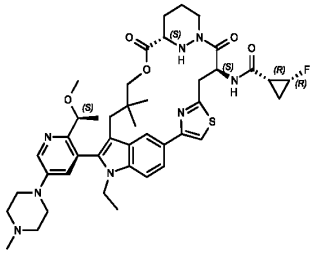
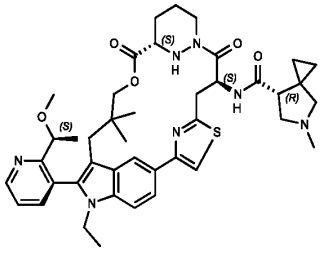
A66	
A67	
A68	
A69	
A70	
A71	

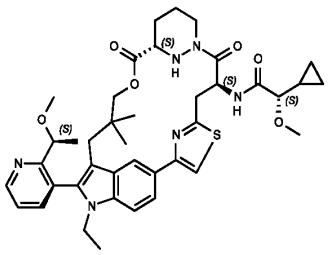
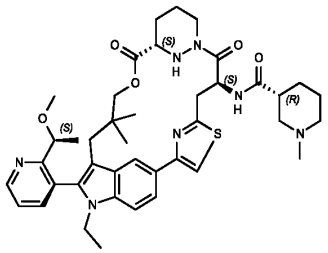
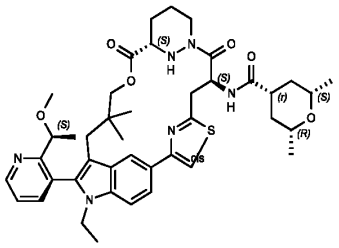
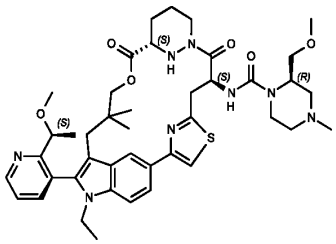
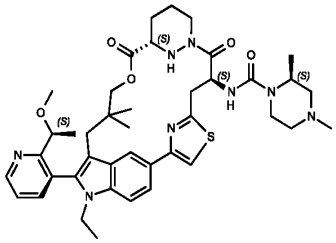
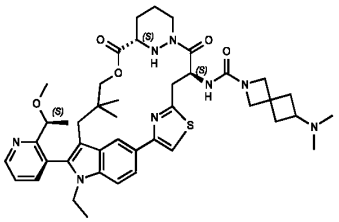
A72	
A73	
A74	
A75	
A76	
A77	

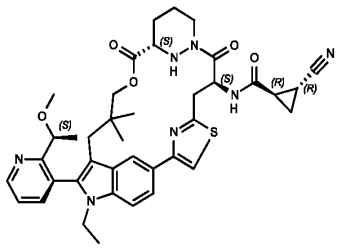
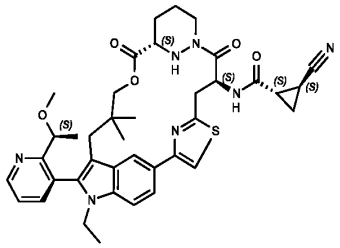
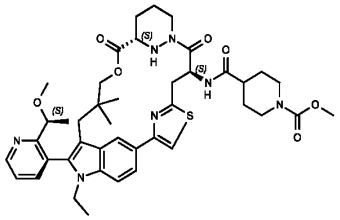
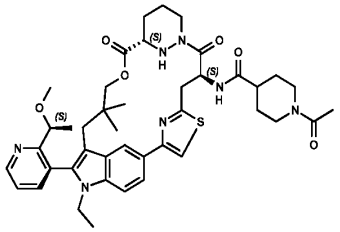
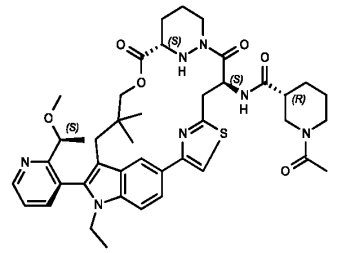
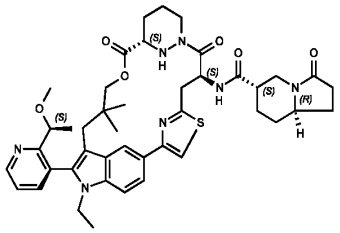
A78	
A79	
A80	
A81	
A82	
A83	

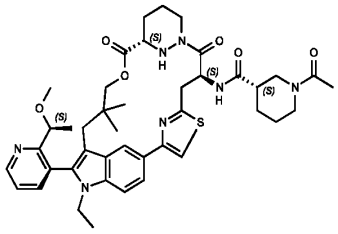
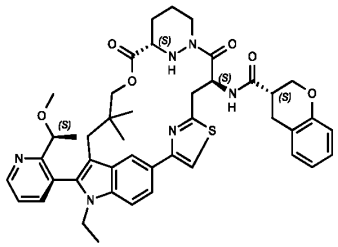
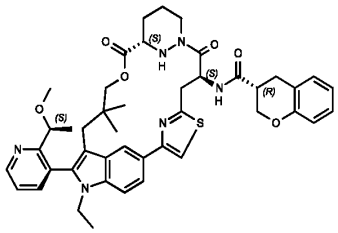
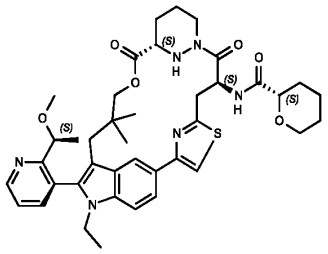
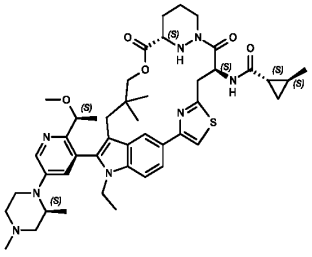
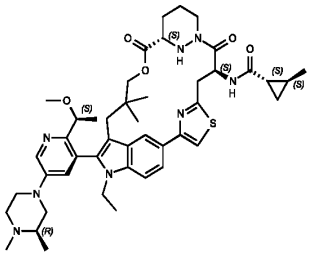
A84	
A85	
A86	
A87	
A88	
A89	

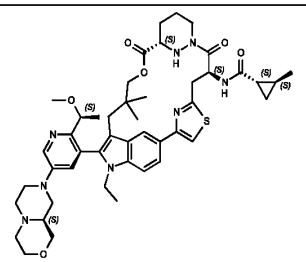
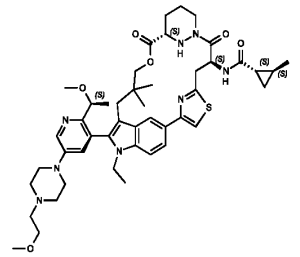
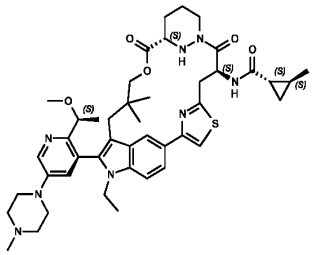
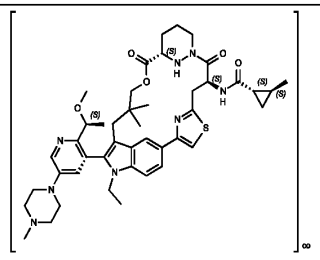
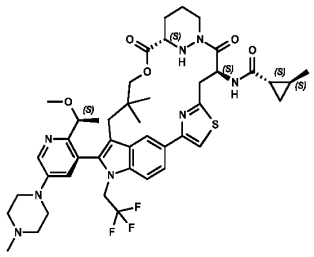
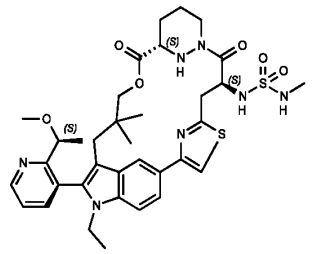
A90	
A91	
A92	
A93	
A94	
A95	

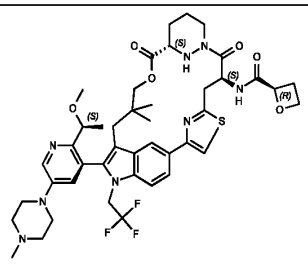
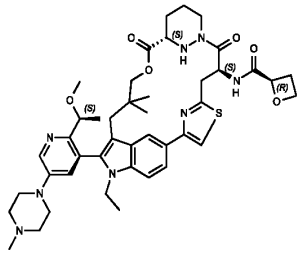
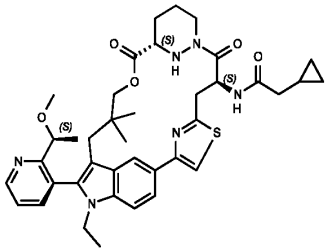
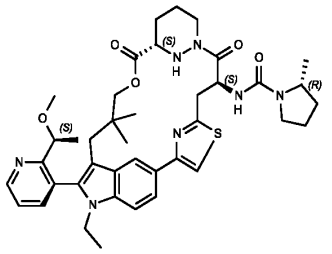
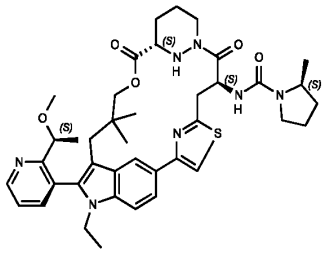
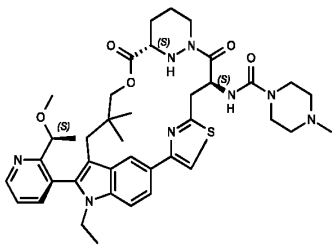
A96	
A97	
A98	
A99	
A100	
A101	

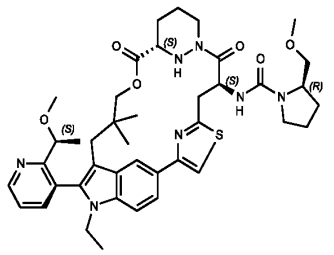
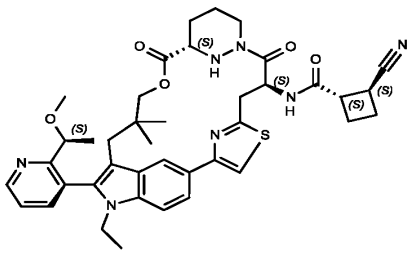
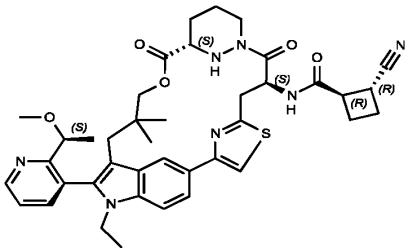
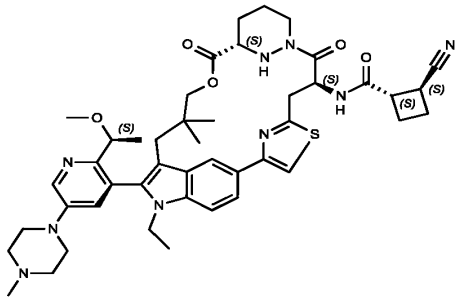
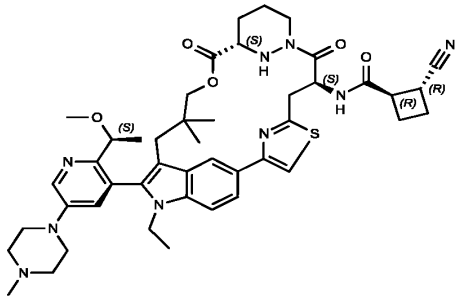
A102	
A103	
A104	
A105	
A106	
A107	

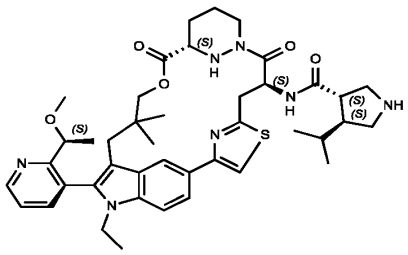
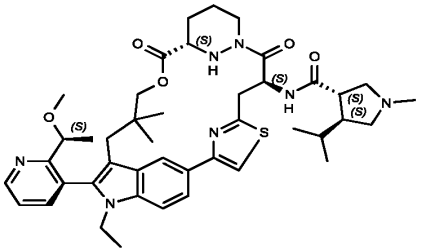
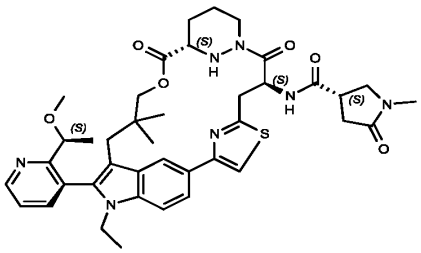
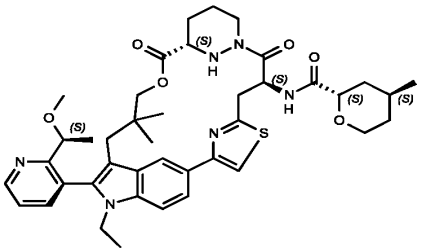
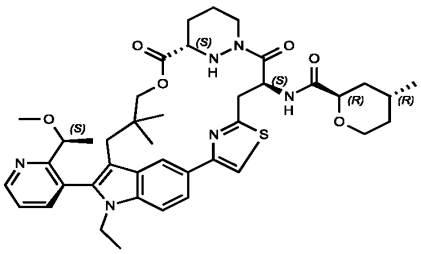
A108	
A109	
A110	
A111	
A112	
A113	

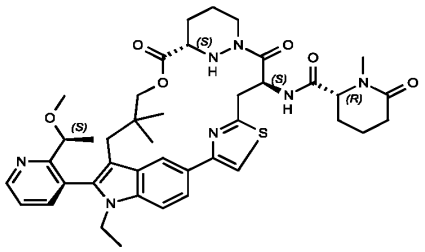
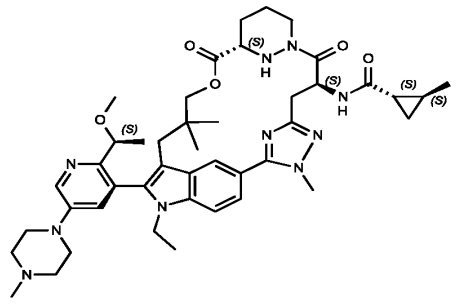
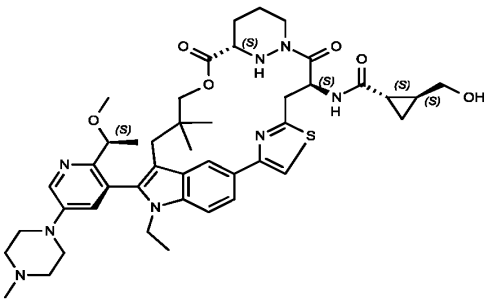
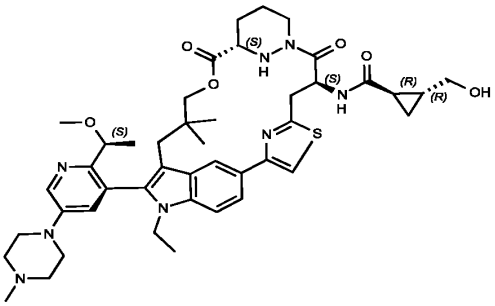
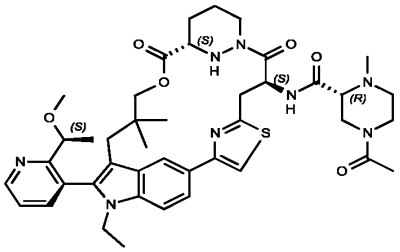
A114	
A115	
A116	
A117	
A118	
A119	

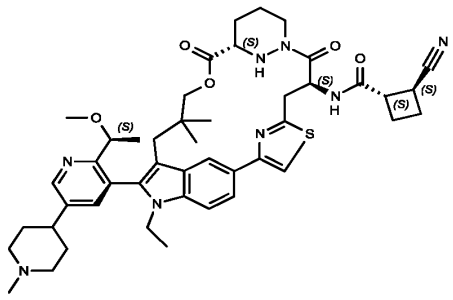
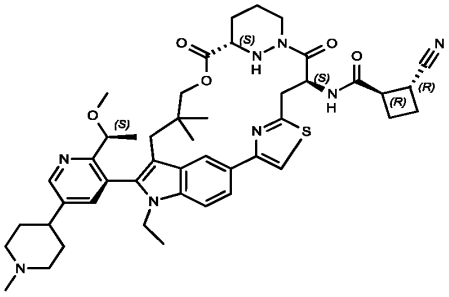
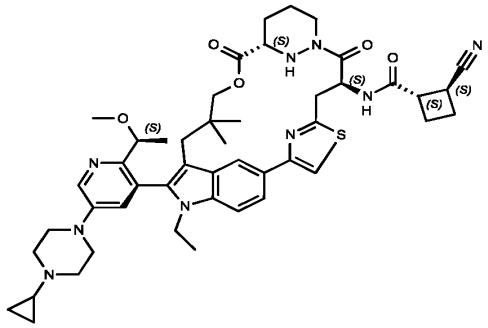
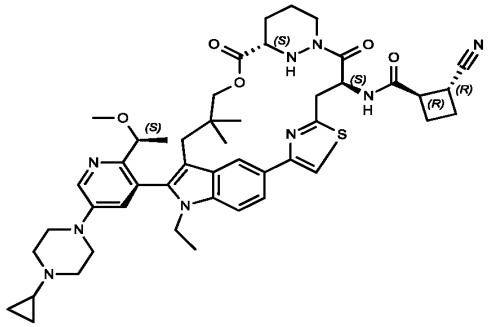
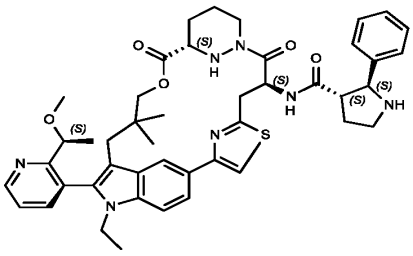
A120	
A121	
A122	
A123	
A124	
A125	

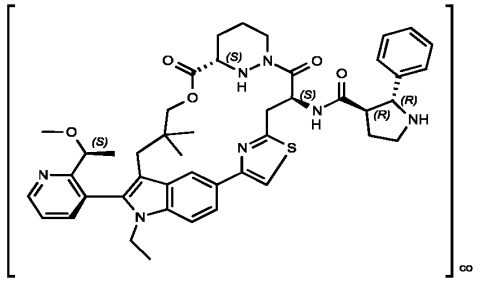
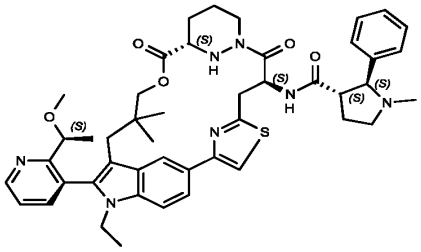
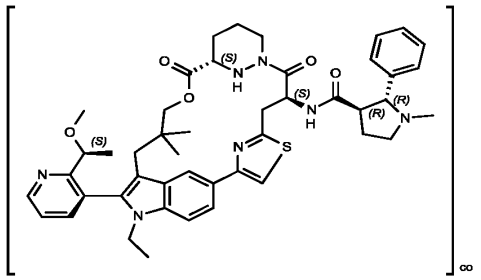
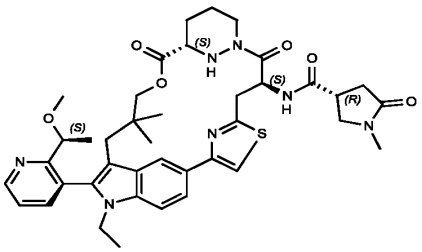
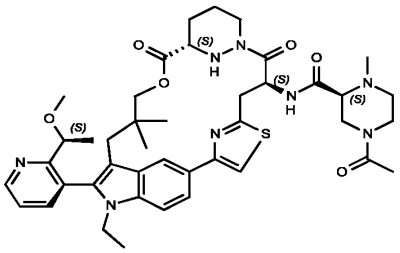
A126	
A127	
A128	
A129	
A130	
A131	

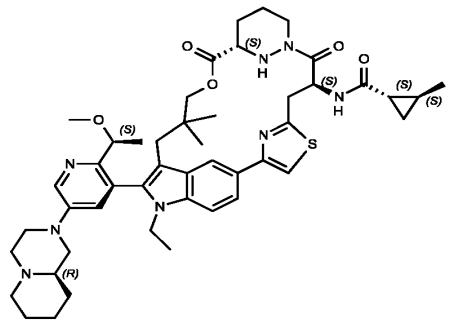
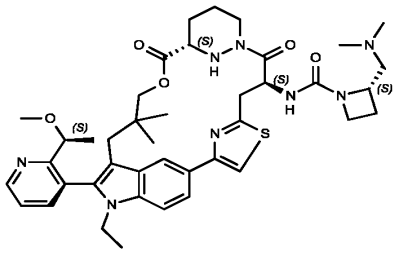
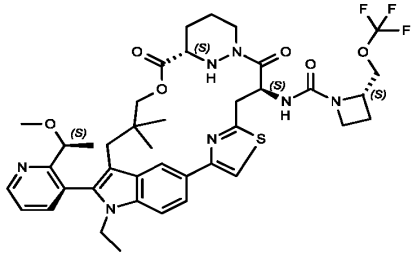
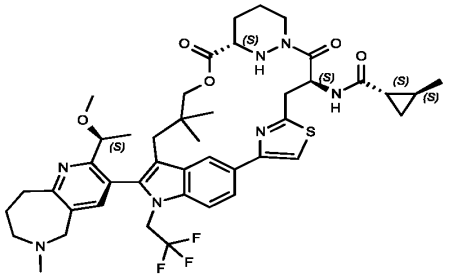
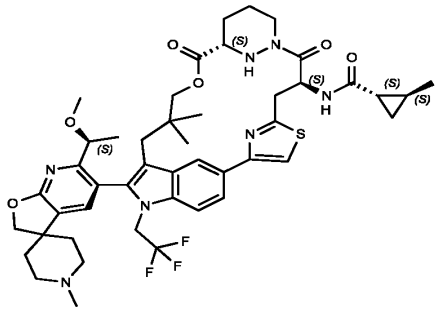
A132	
A133	
A134	
A135	
A136	

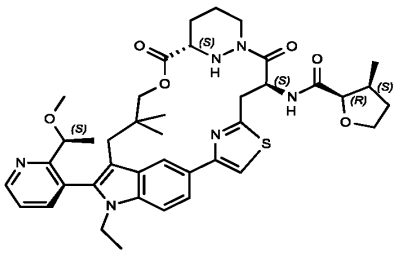
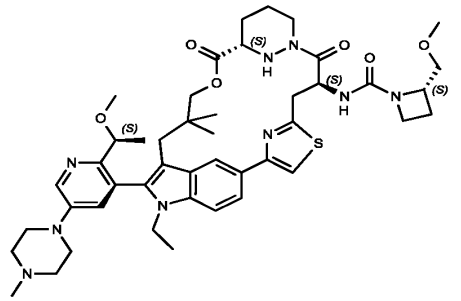
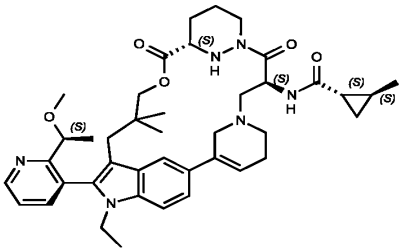
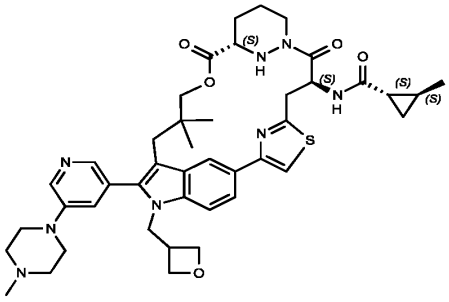
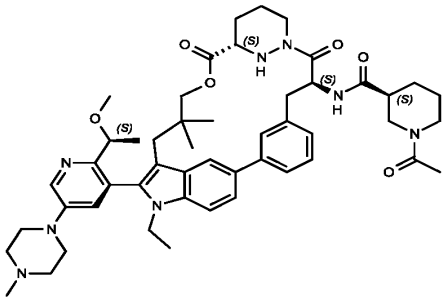
A137	
A138	
A139	
A140	
A141	

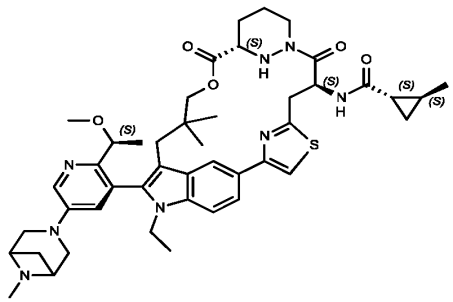
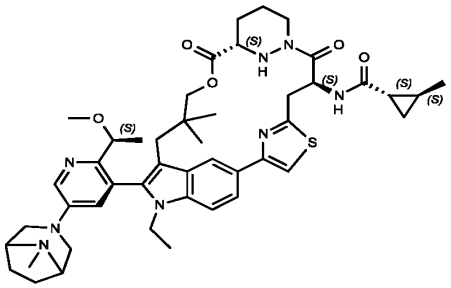
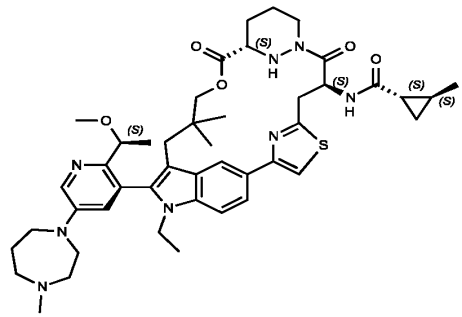
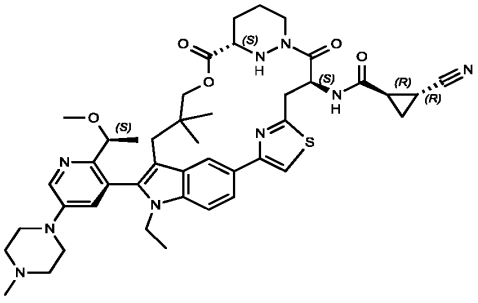
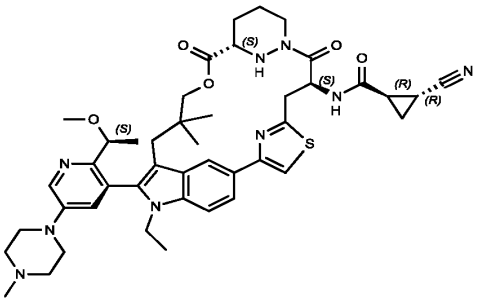
A142	
A143	
A144	
A145	
A146	

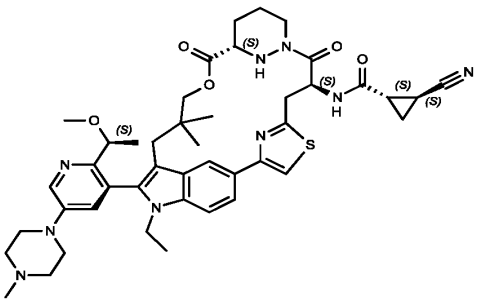
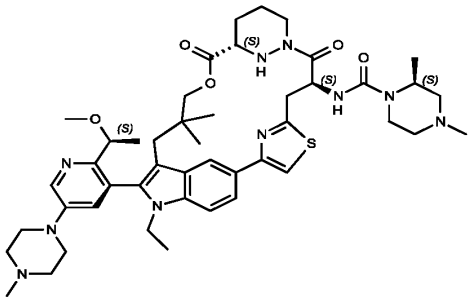
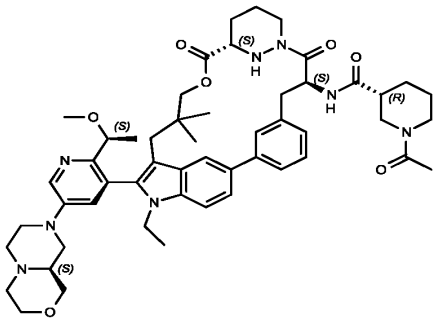
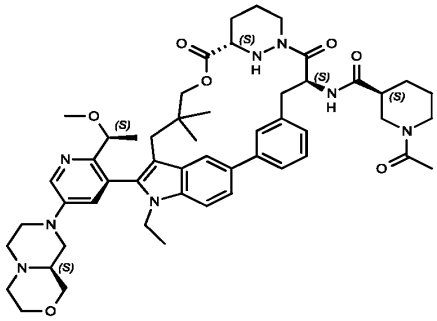
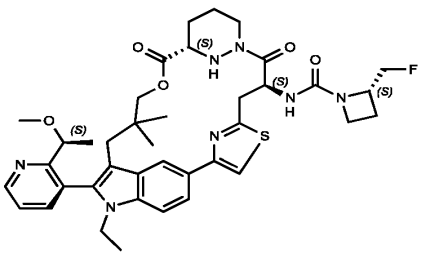
A147	
A148	
A149	
A150	
A151	

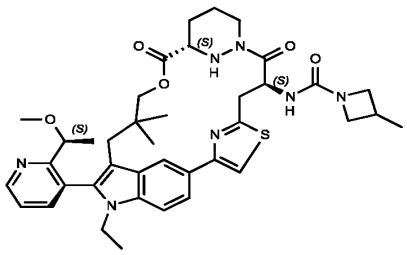
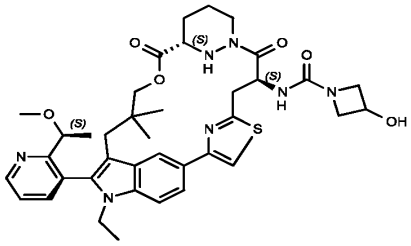
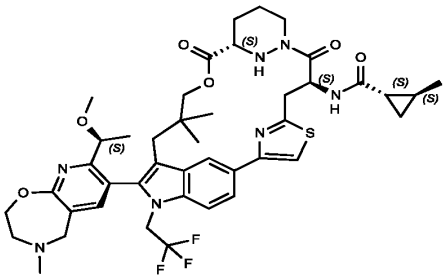
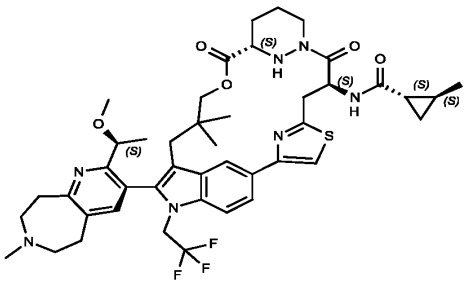
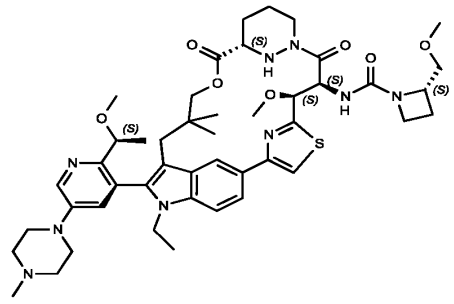
A152	
A153	
A154	
A155	
A156	

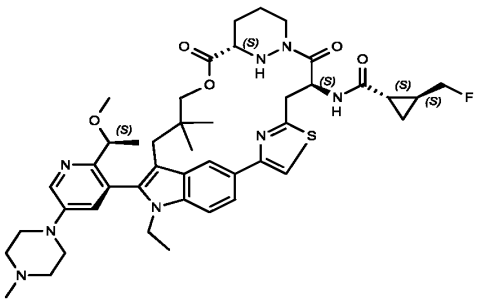
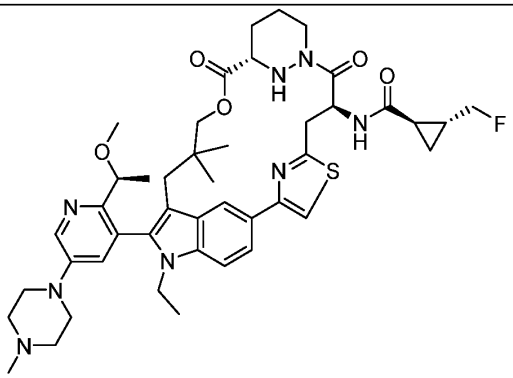
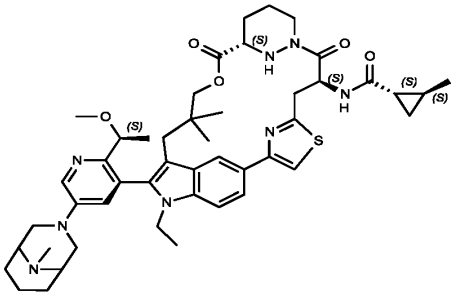
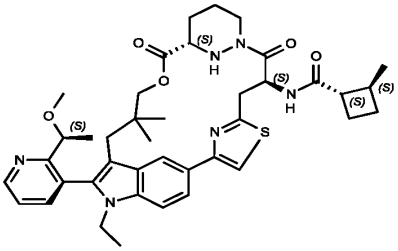
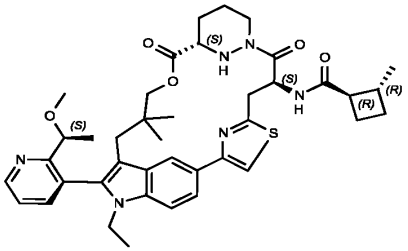
A157	
A158	
A159	
A160	
A161	

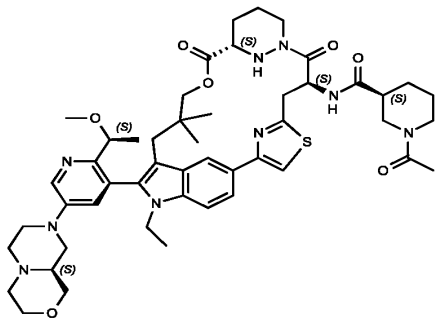
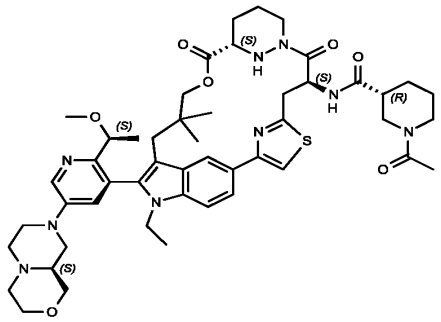
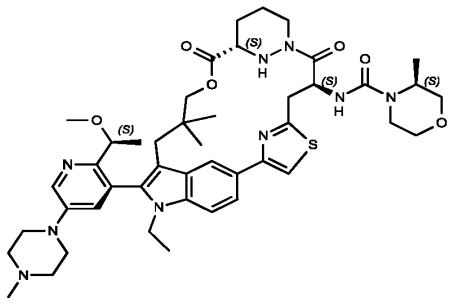
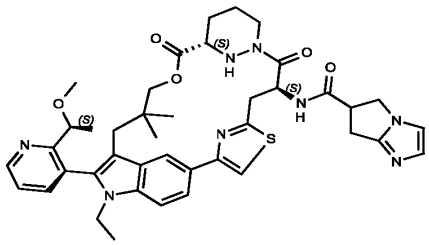
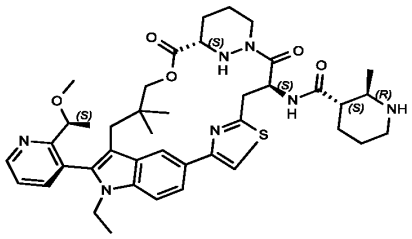
A162	
A163	
A164	
A165	
A166	

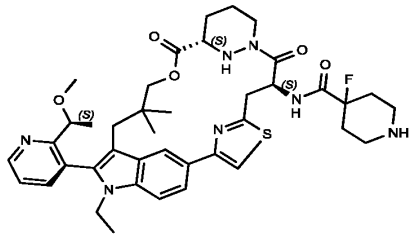
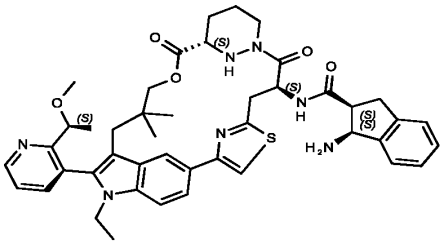
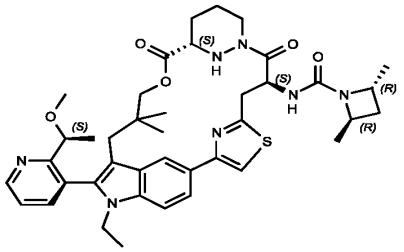
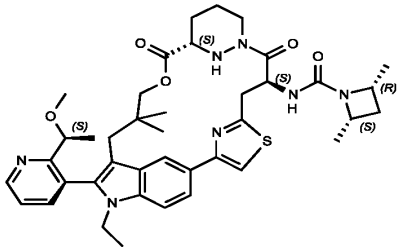
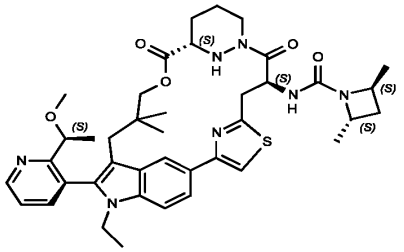
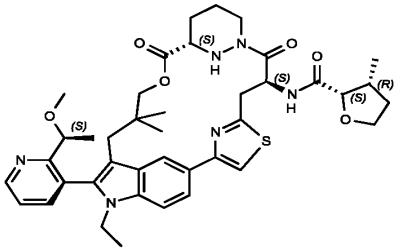
A167	
A168	
A169	
A170	
A171	

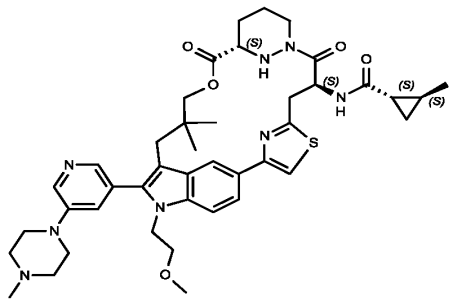
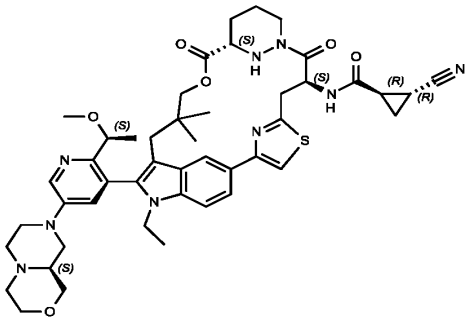
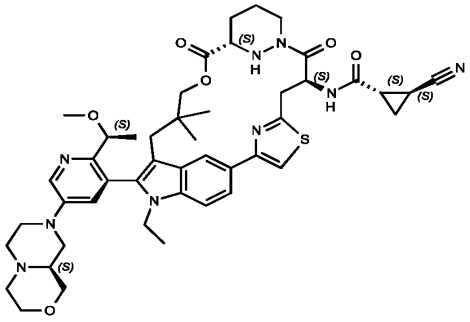
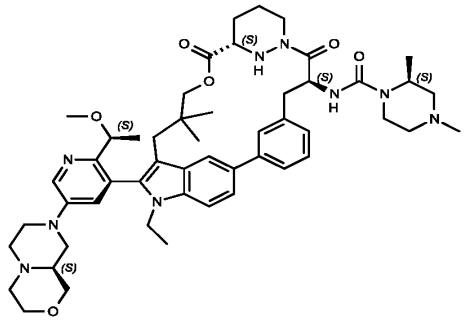
A172	
A173	
A174	
A175	
A176	

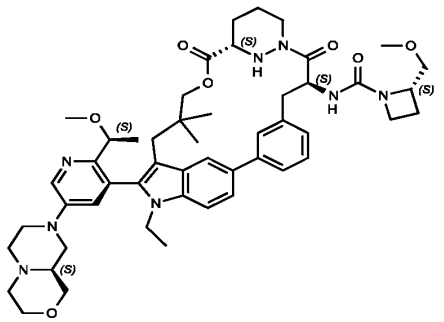
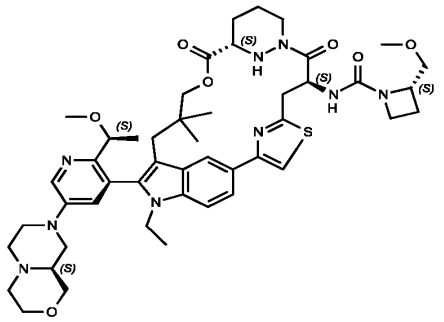
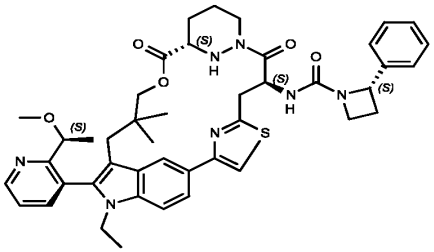
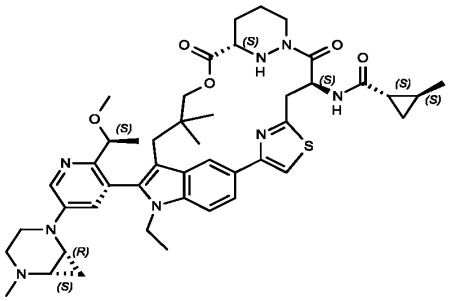
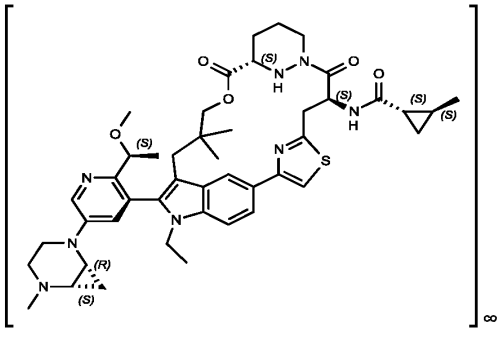
A177	
A178	
A179	
A180	
A181	

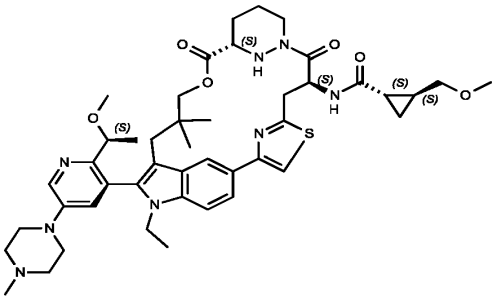
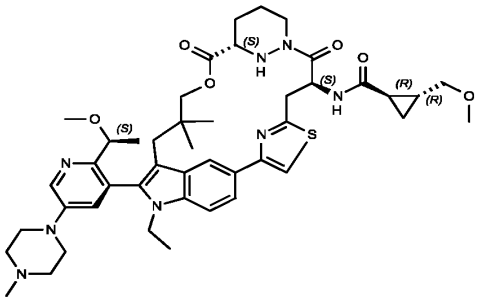
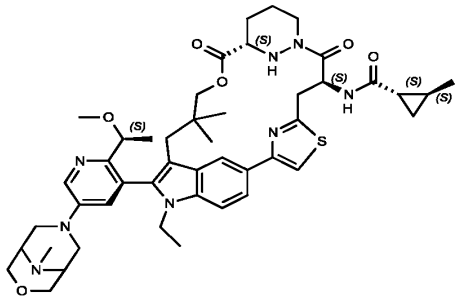
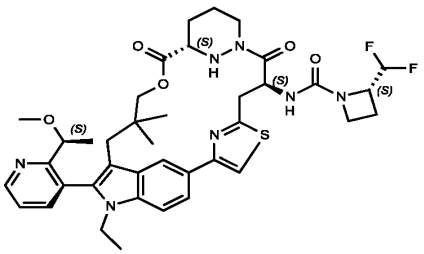
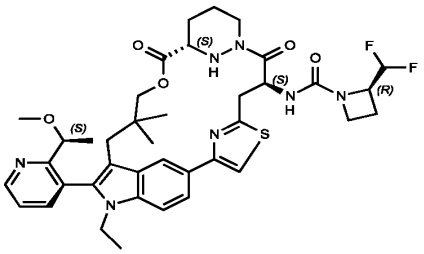
A182	
A183	
A184	
A185	
A186	

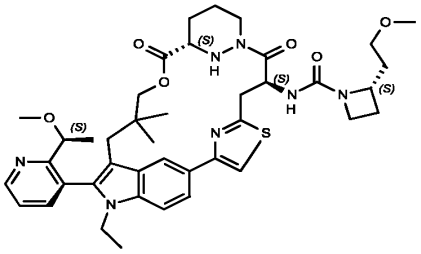
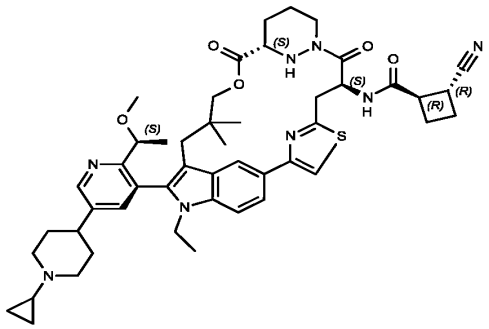
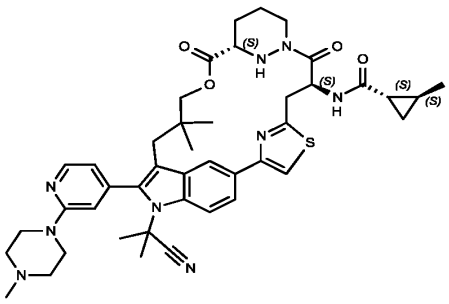
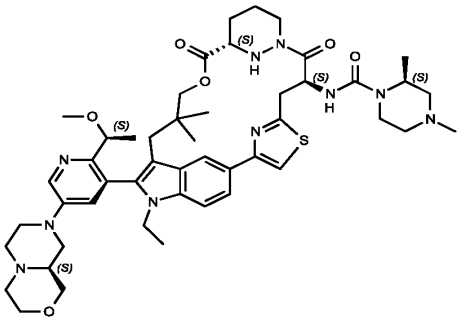
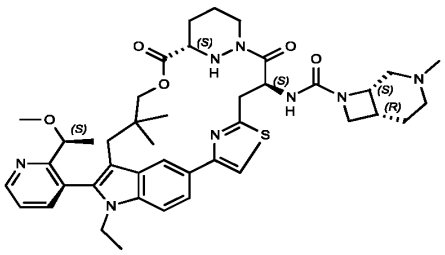
A187	
A188	
A189	
A190	
A191	

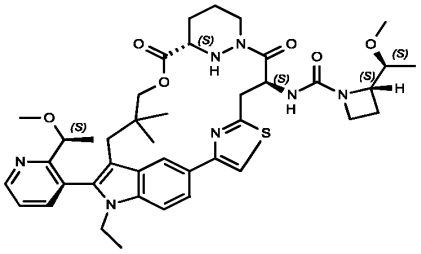
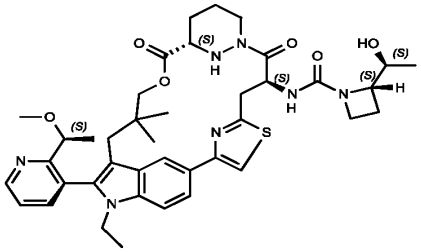
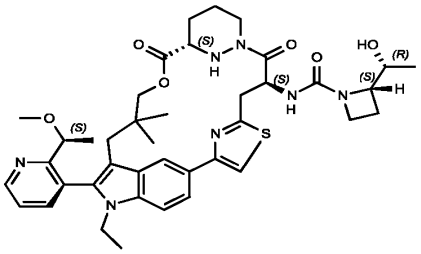
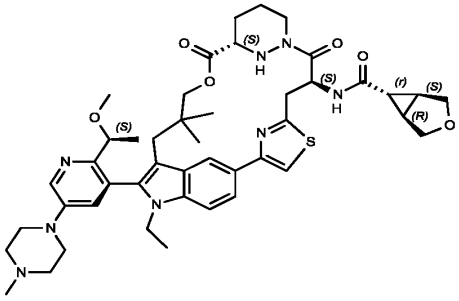
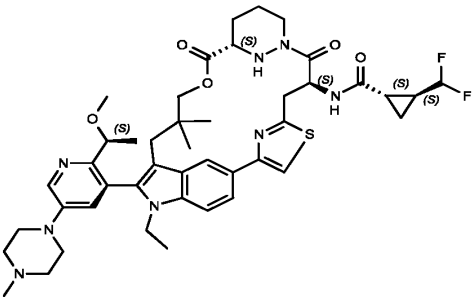
A192	
A193	
A194	
A195	
A196	
A197	

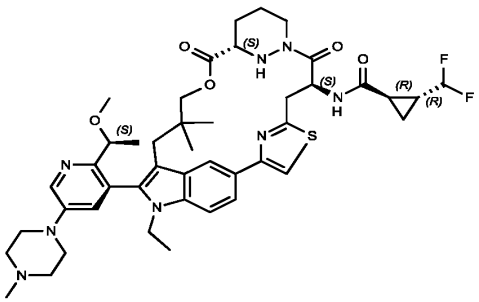
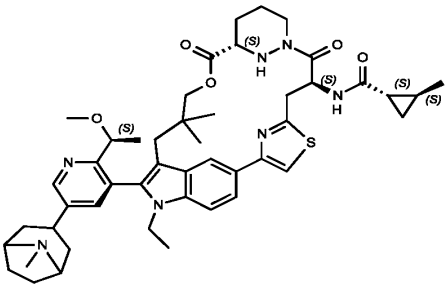
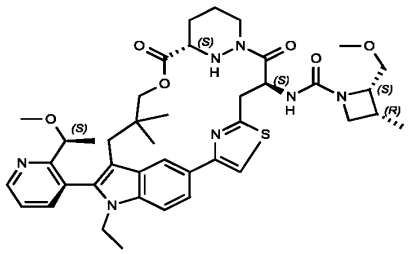
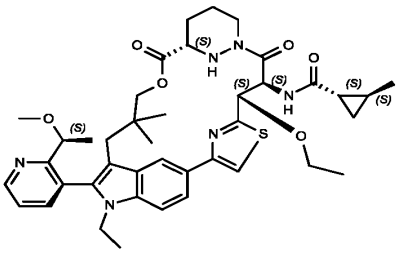
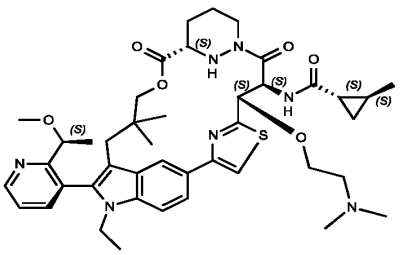
A198	
A199	
A200	
A201	

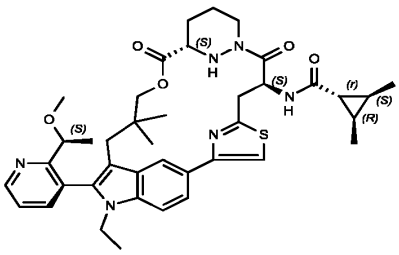
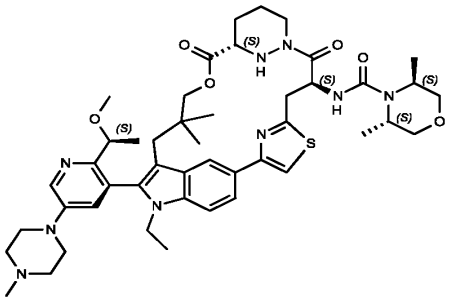
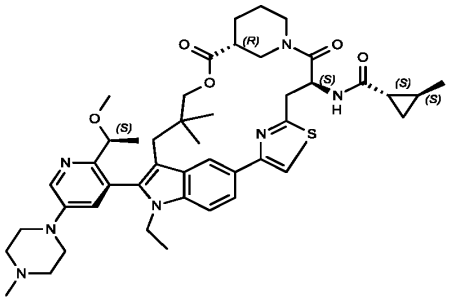
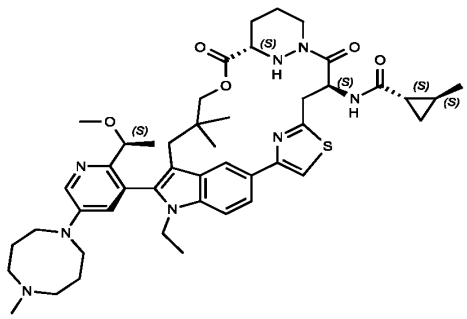
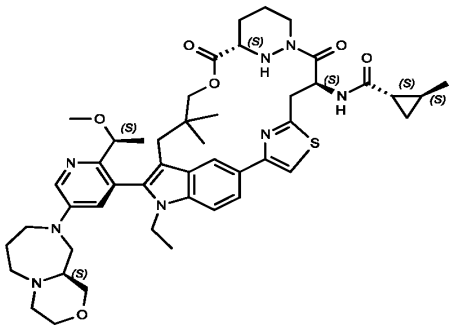
A202	
A203	
A204	
A205	
A206	

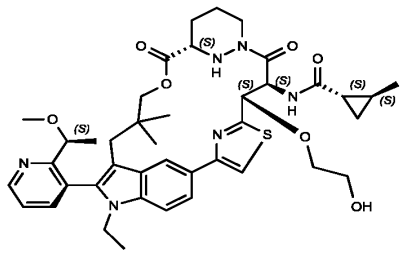
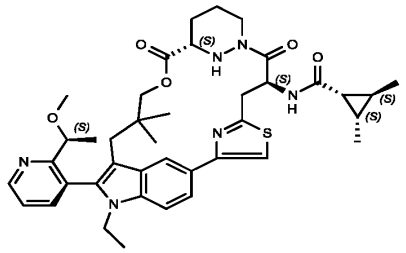
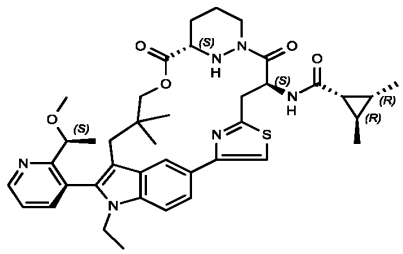
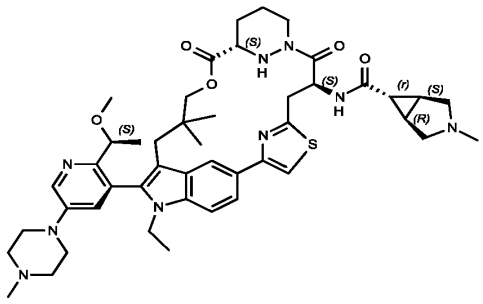
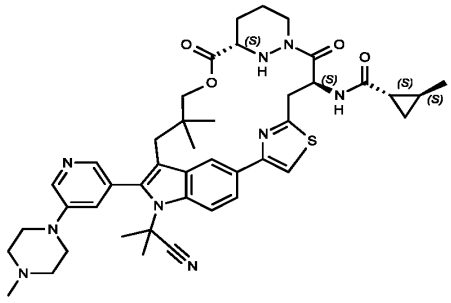
A207	
A208	
A209	
A210	
A211	

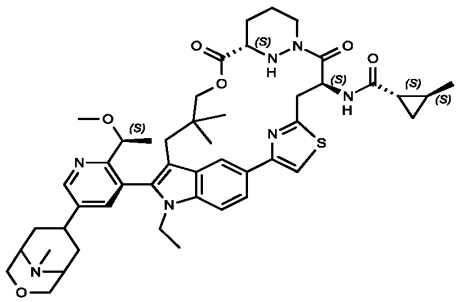
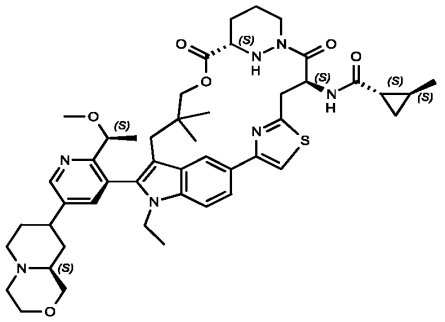
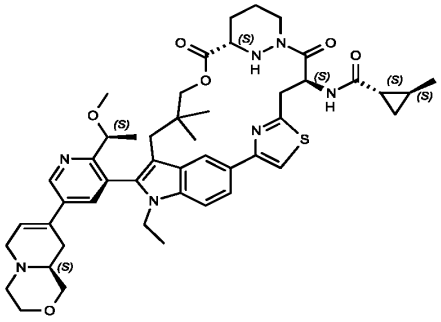
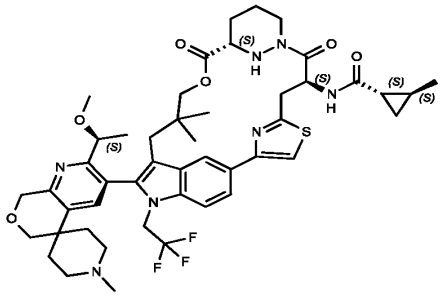
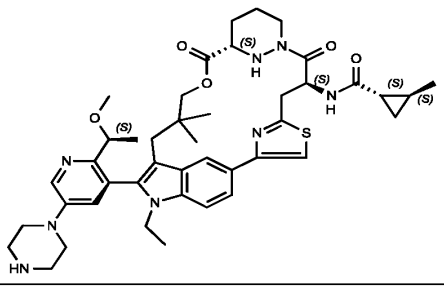
A212	
A213	
A214	
A215	
A216	

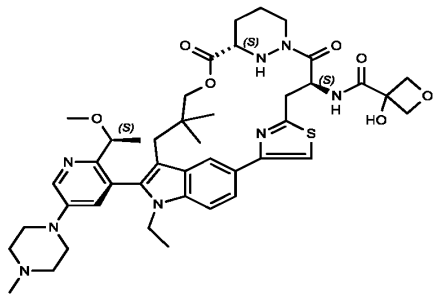
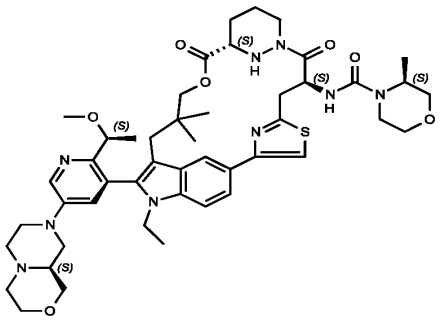
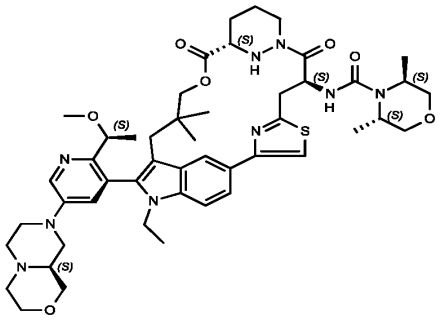
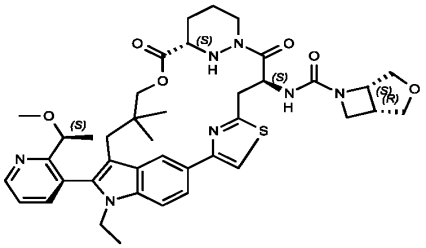
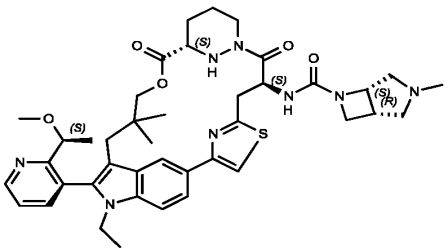
A217	
A218	
A219	
A220	
A221	

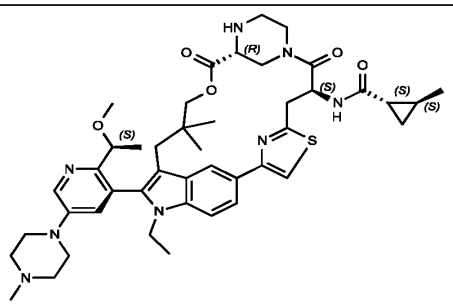
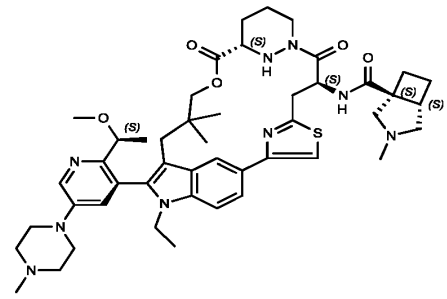
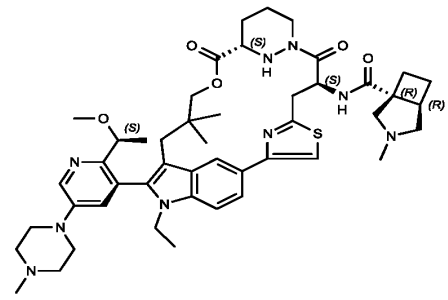
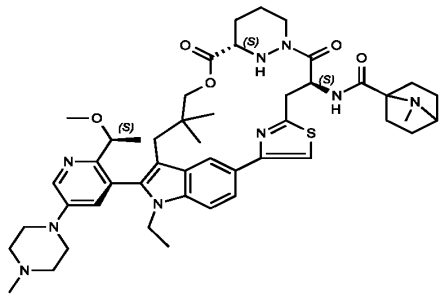
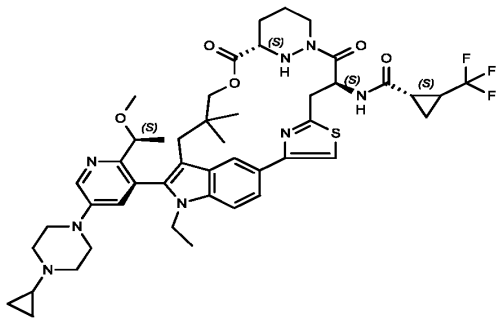
A222	
A223	
A224	
A225	
A226	

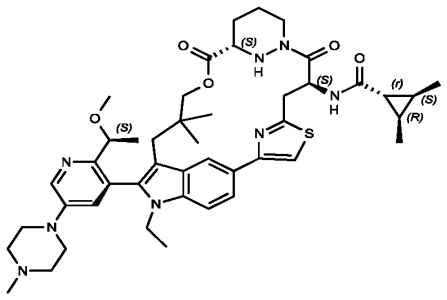
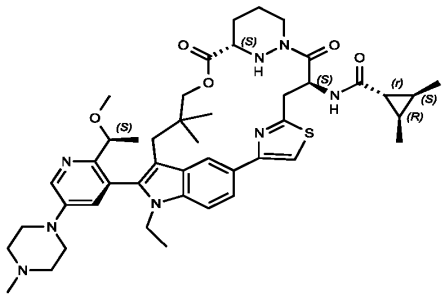
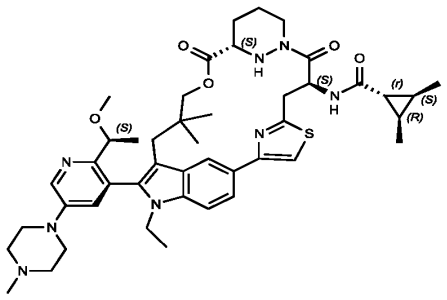
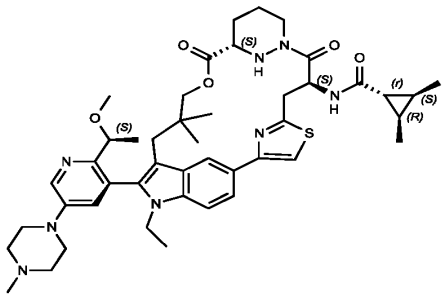
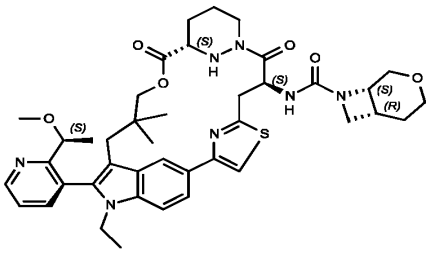
A227	
A228	
A229	
A230	
A231	

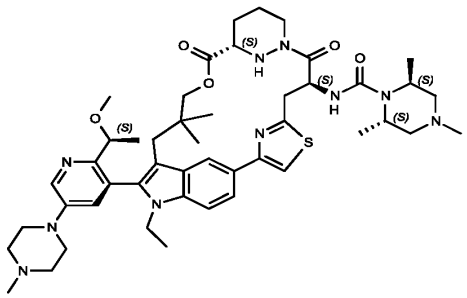
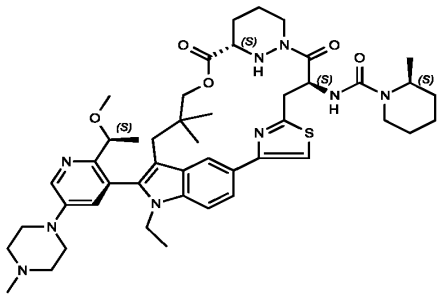
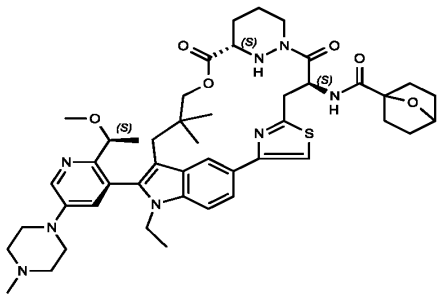
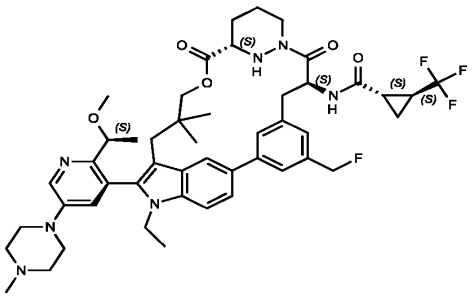
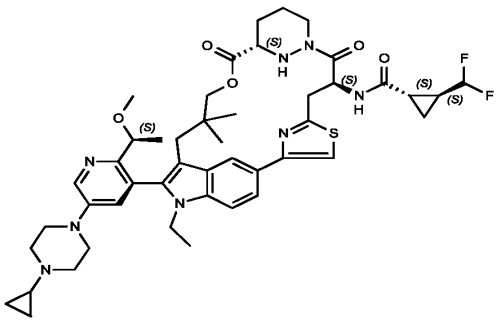
A232	
A233	
A234	
A235	
A236	

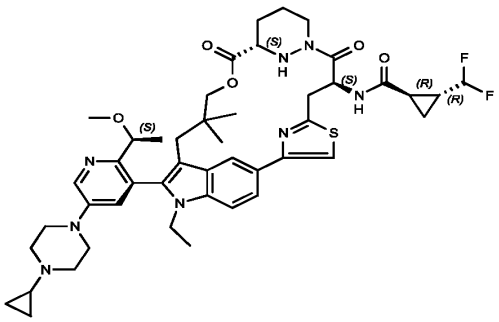
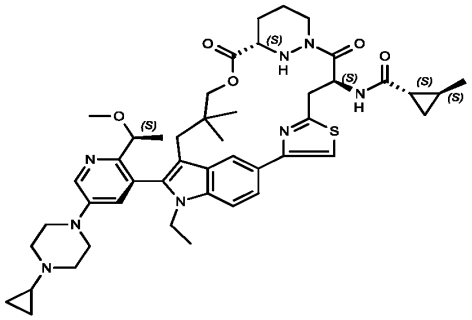
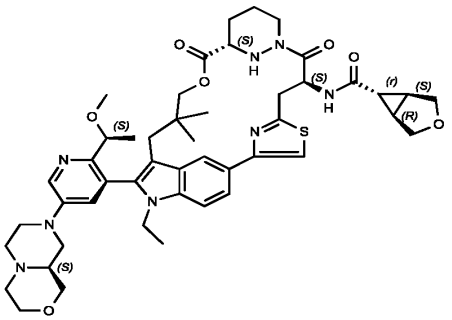
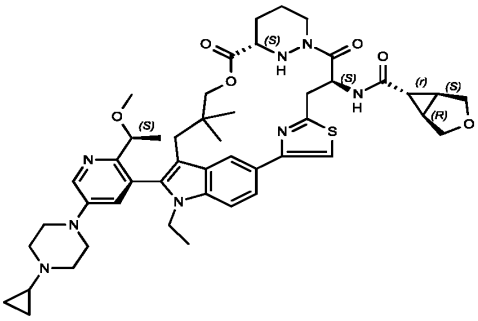
A237	
A238	
A239	
A240	
A241	

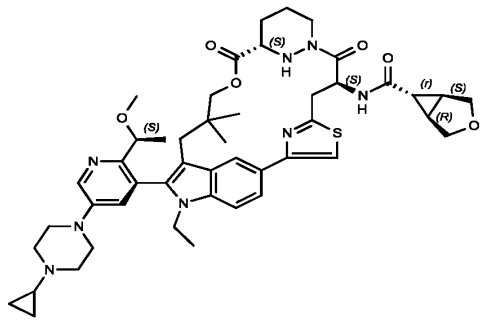
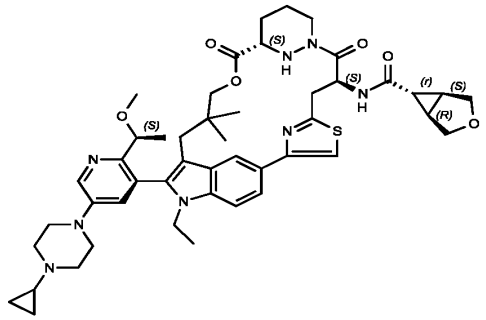
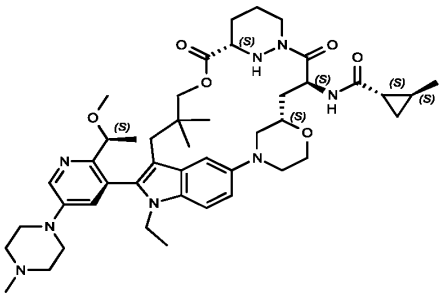
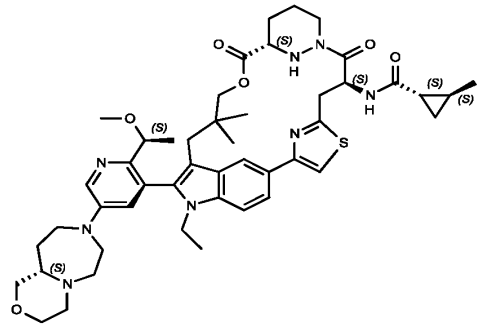
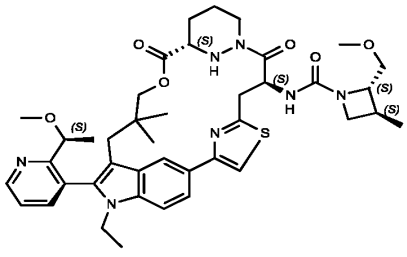
A242	
A243	
A244	
A245	
A246	

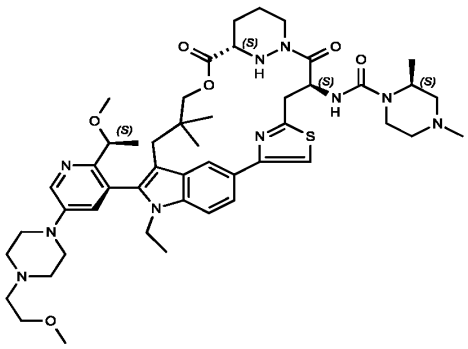
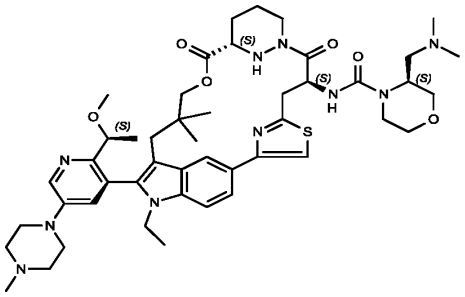
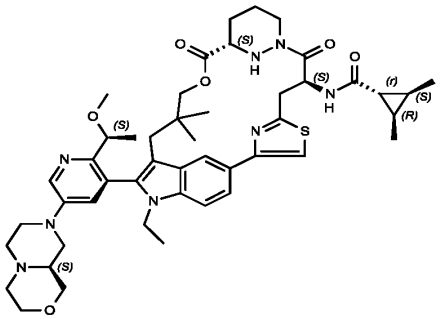
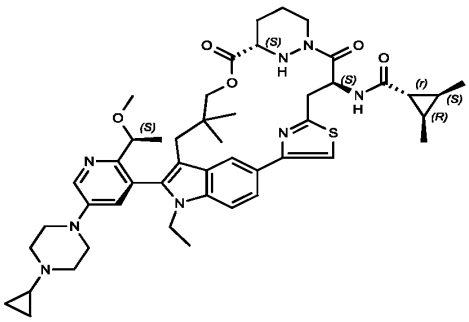
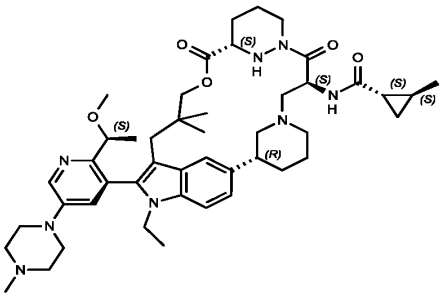
A247	
A248	
A249	
A250	
A251	

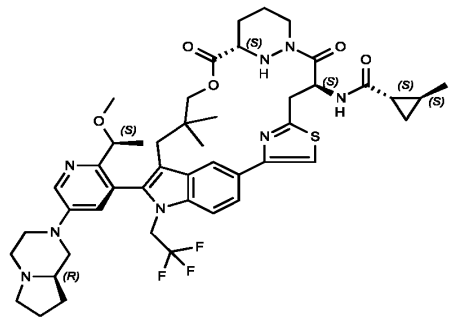
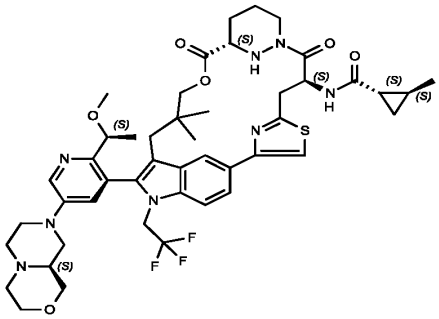
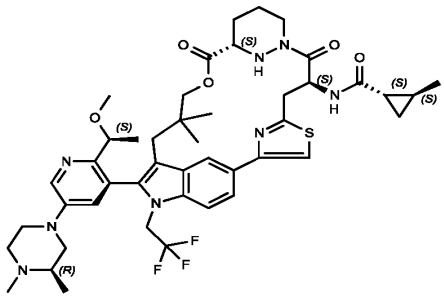
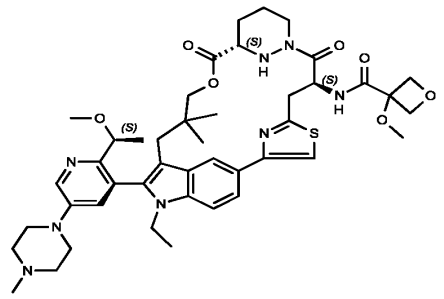
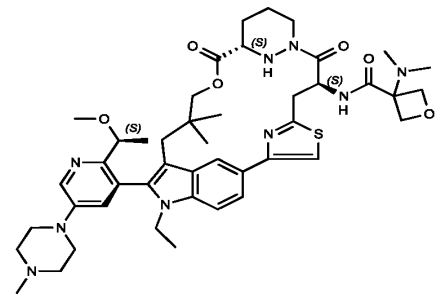
A252	
A253	
A254	
A255	
A256	

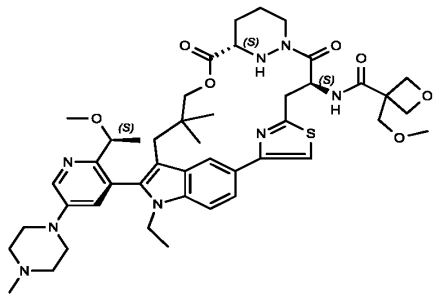
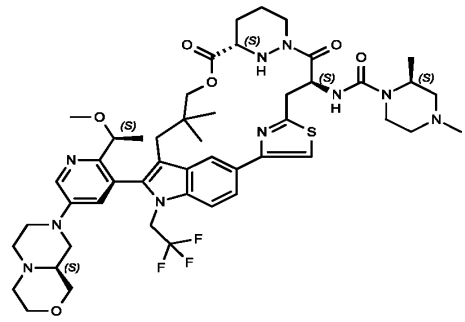
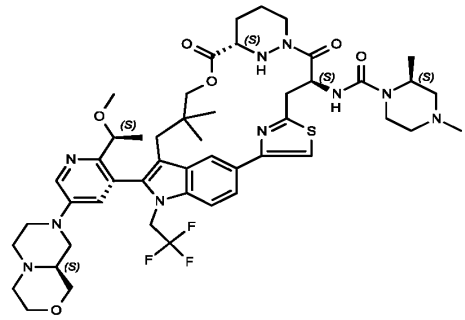
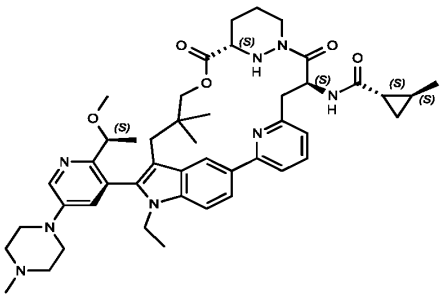
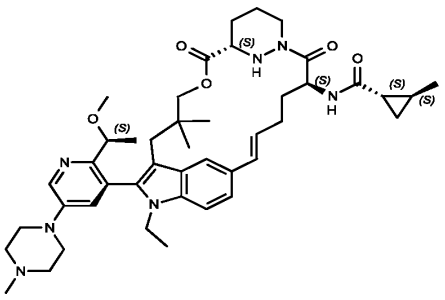
A257	
A258	
A259	
A260	
A261	

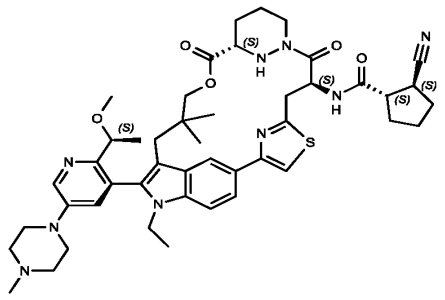
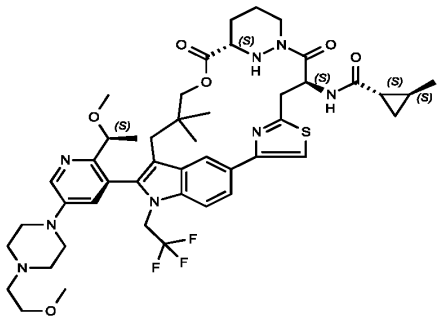
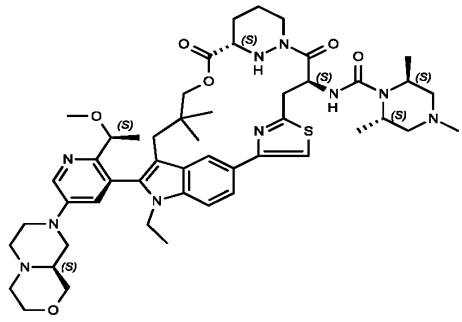
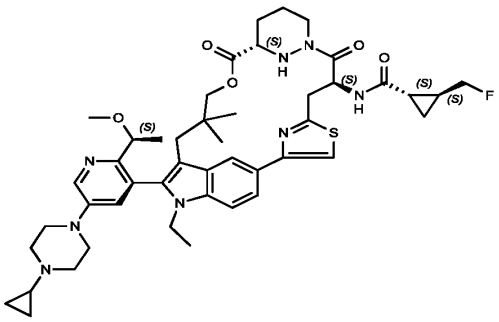
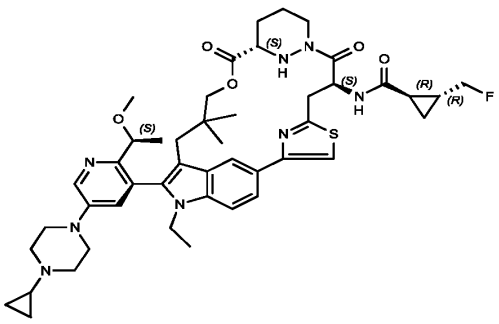
A262	
A263	
A264	
A265	

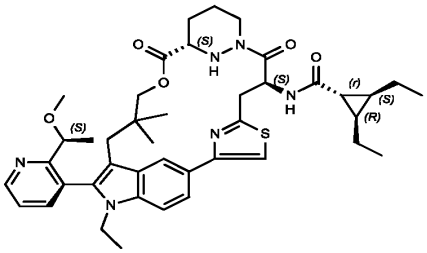
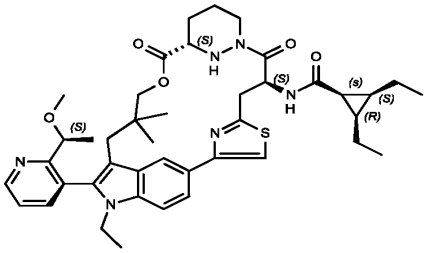
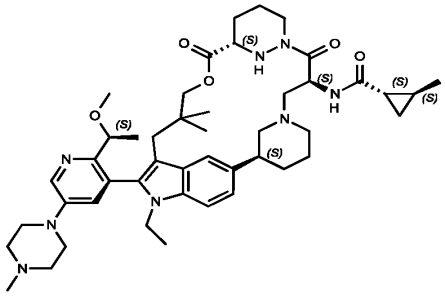
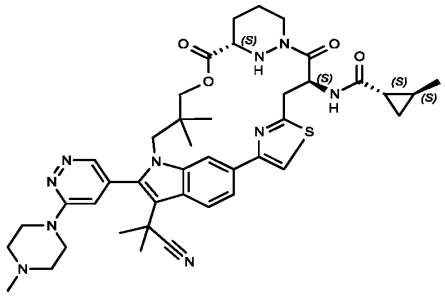
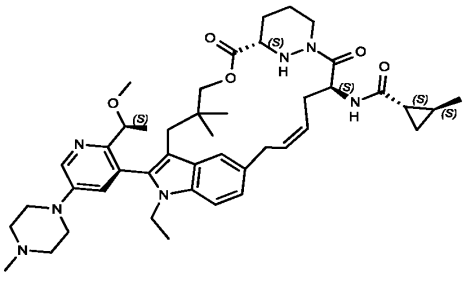
A266	
A267	
A268	
A269	
A270	

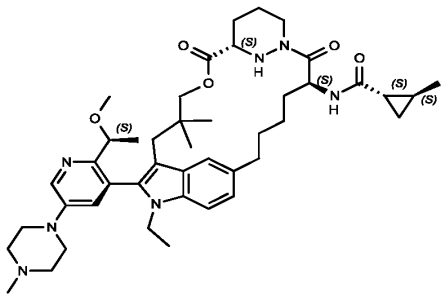
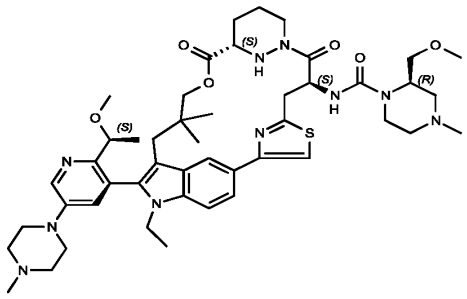
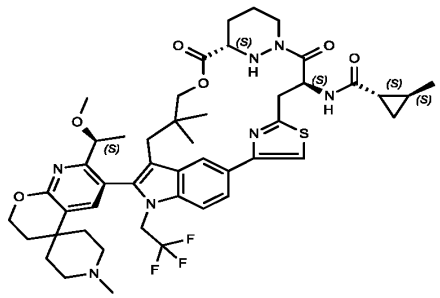
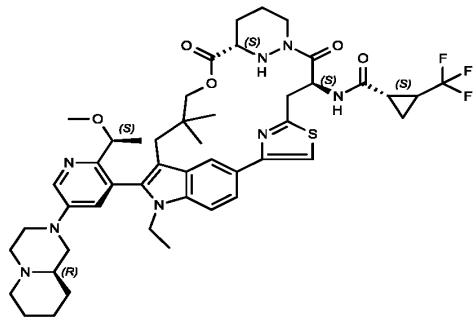
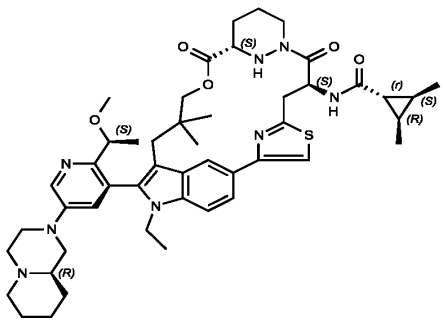
A271	
A272	
A273	
A274	
A275	

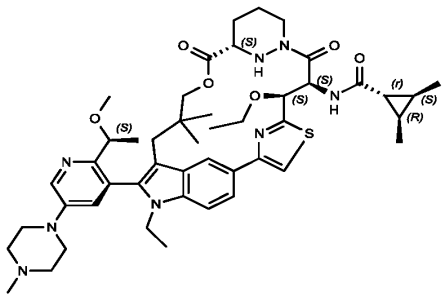
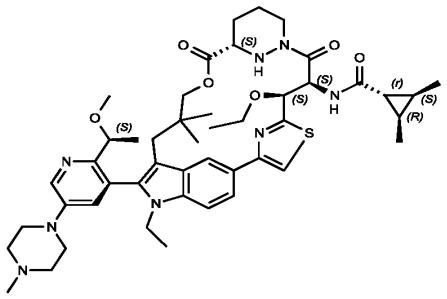
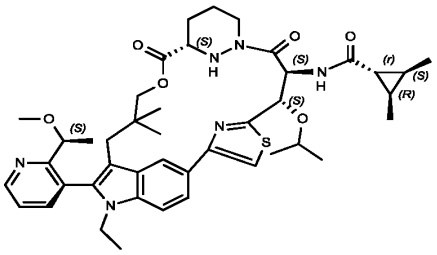
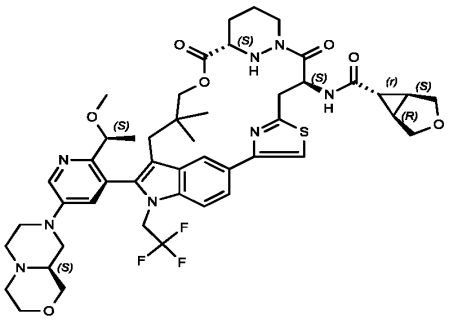
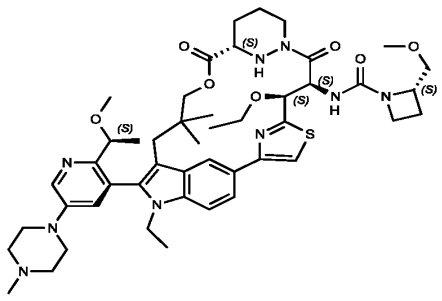
A276	
A277	
A278	
A279	
A280	

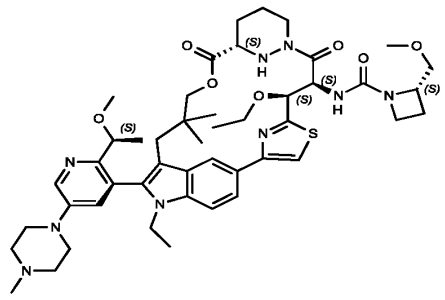
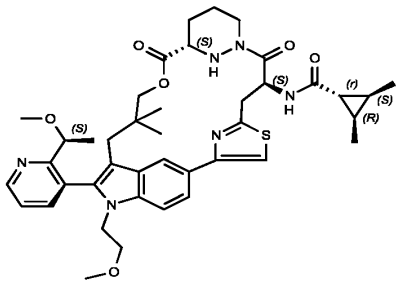
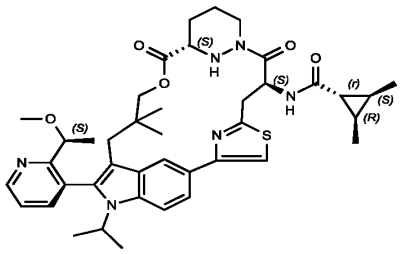
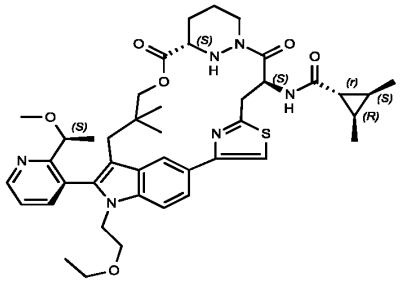
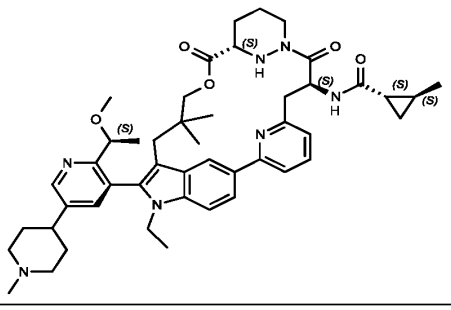
A281	
A282	
A283	
A284	
A285	

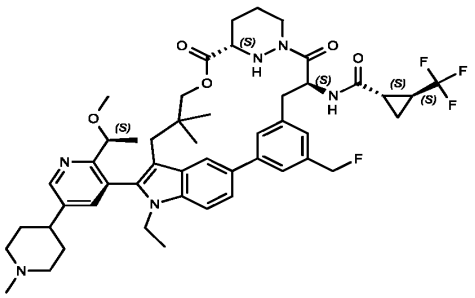
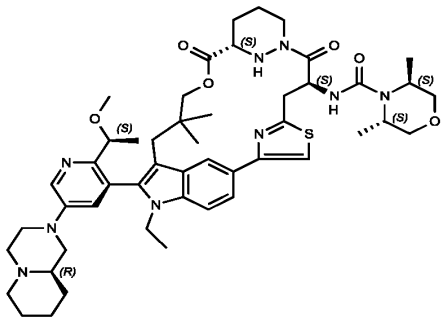
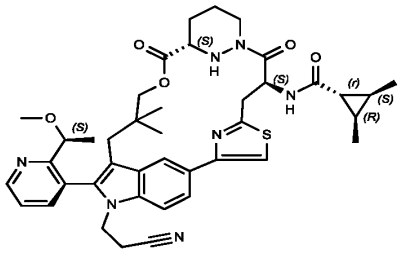
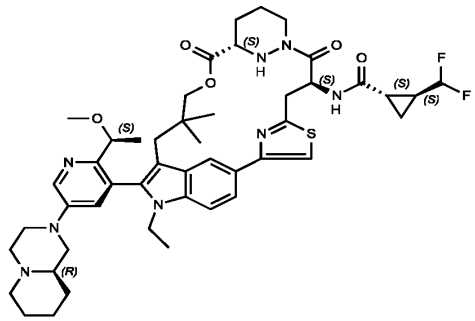
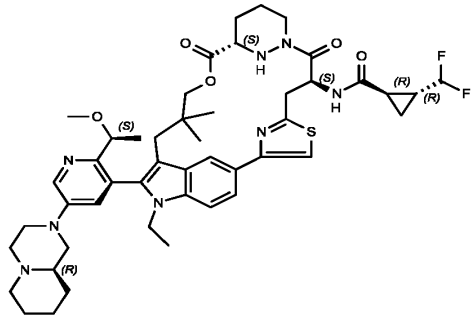
A286	
A287	
A288	
A289	
A290	

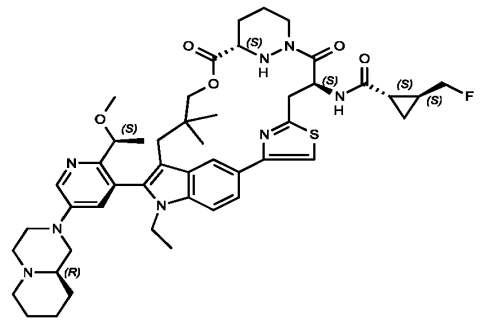
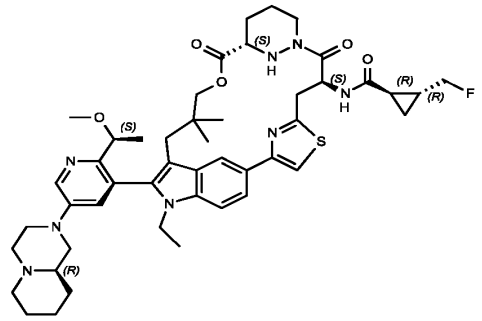
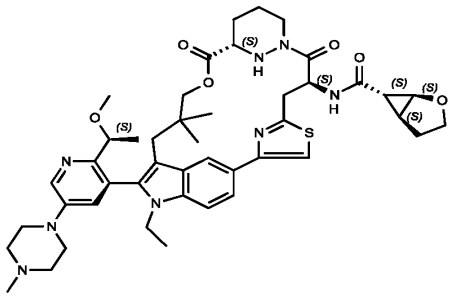
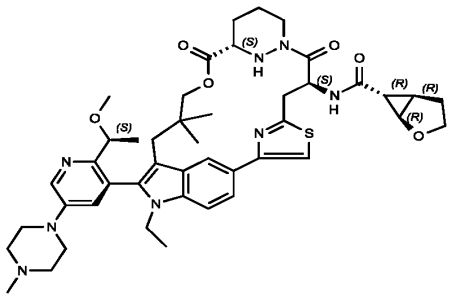
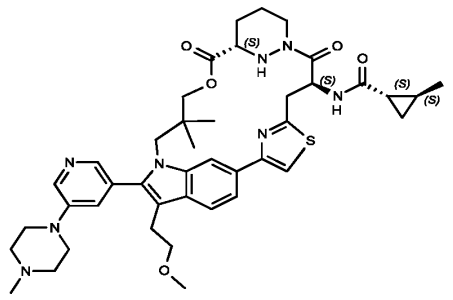
A291	
A292	
A293	
A294	
A295	

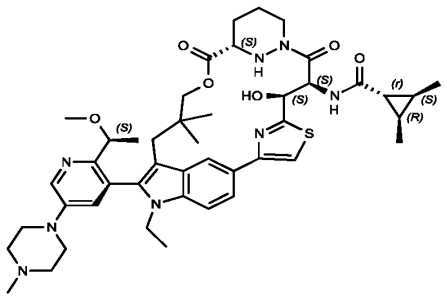
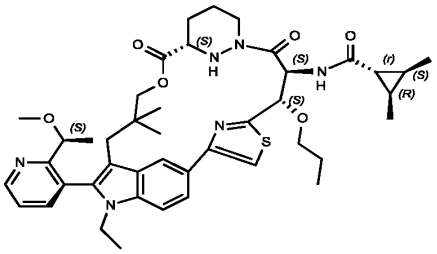
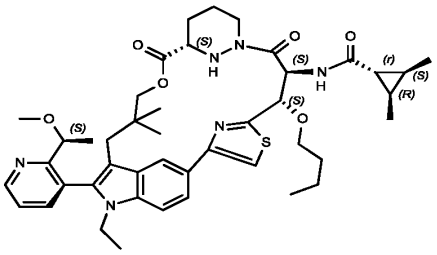
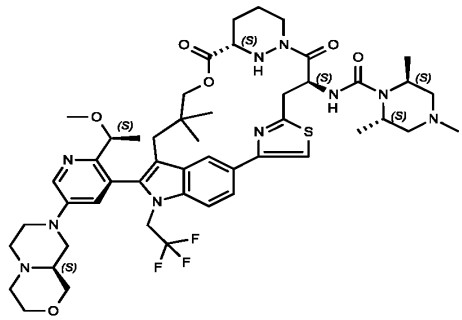
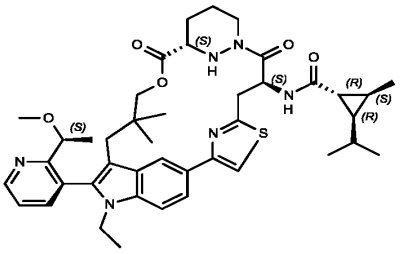
A296	
A297	
A298	
A299	
A300	

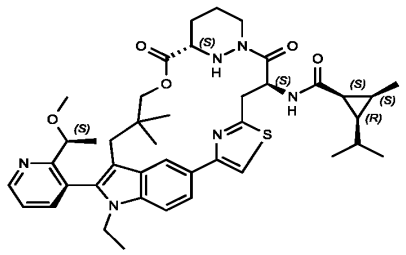
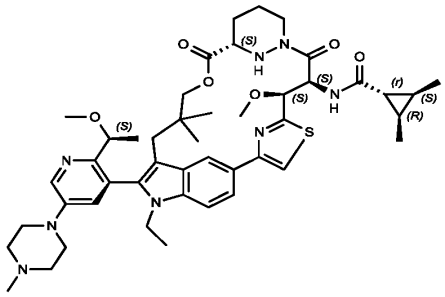
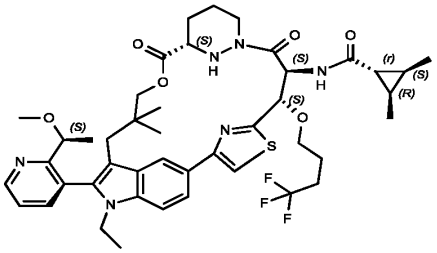
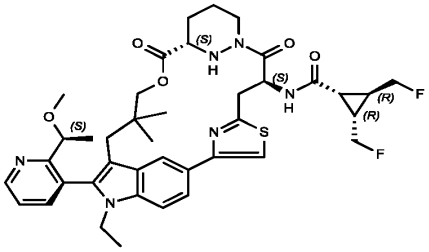
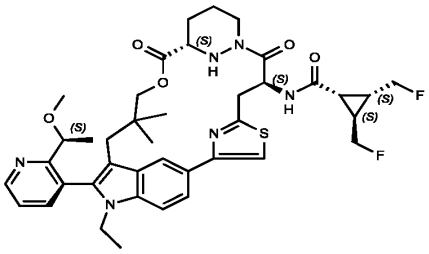
A301	
A302	
A303	
A304	
A305	

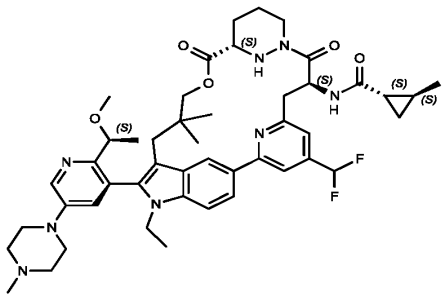
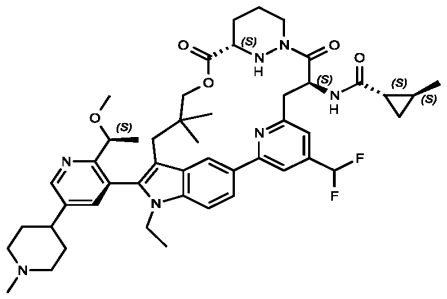
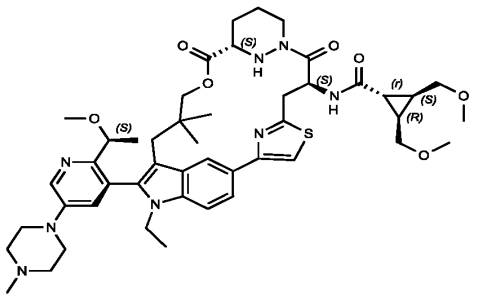
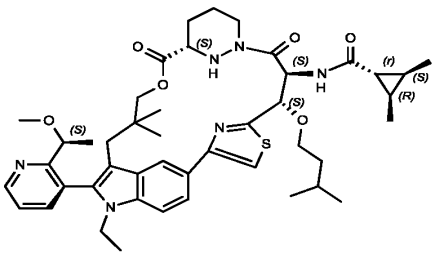
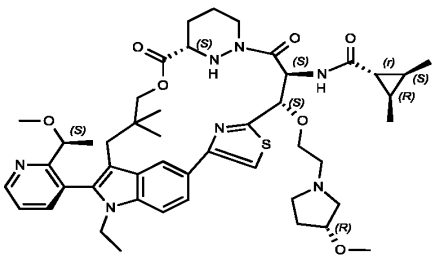
A306	
A307	
A308	
A309	
A310	

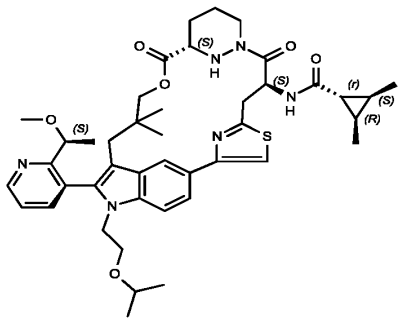
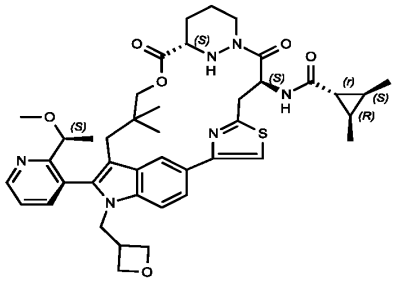
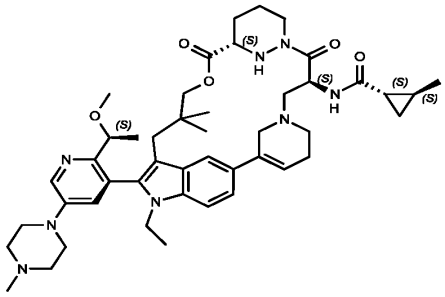
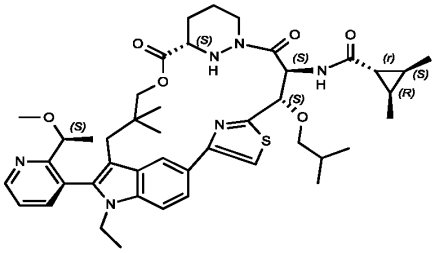
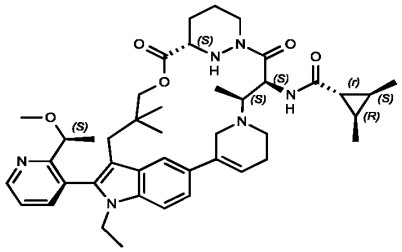
A311	
A312	
A313	
A314	
A315	

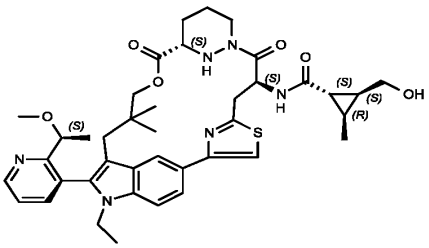
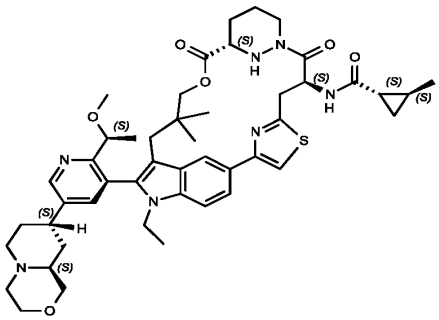
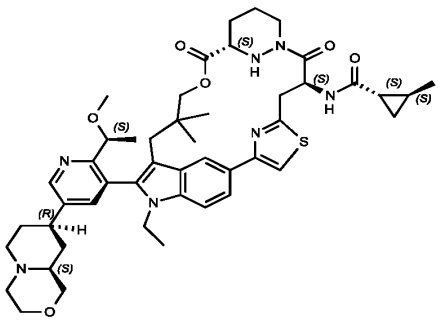
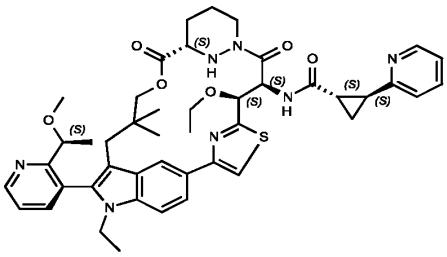
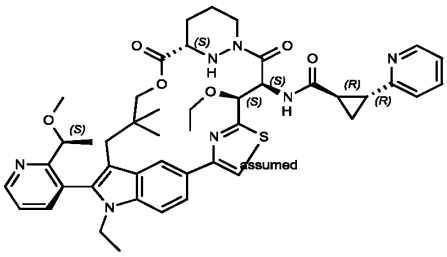
A316	
A317	
A318	
A319	
A320	

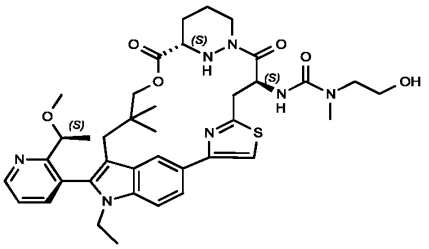
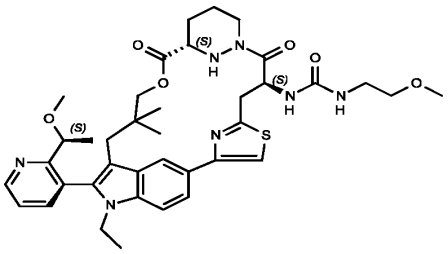
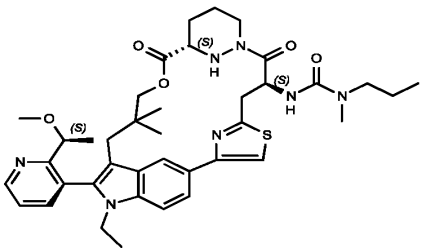
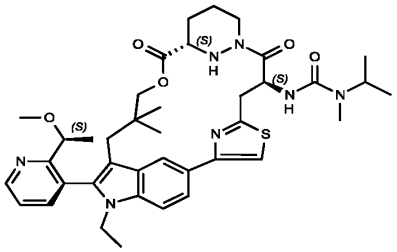
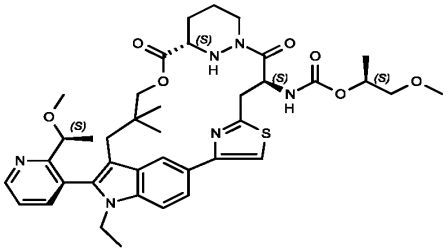
A321	
A322	
A323	
A324	
A325	

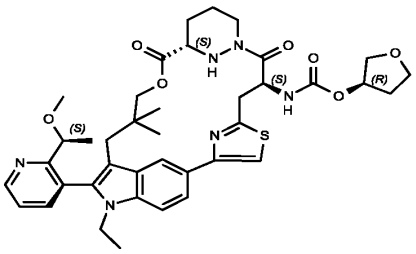
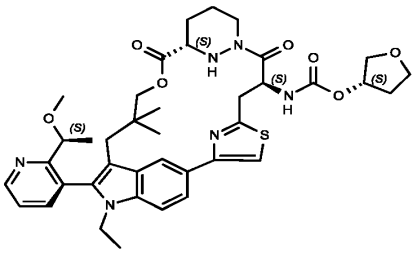
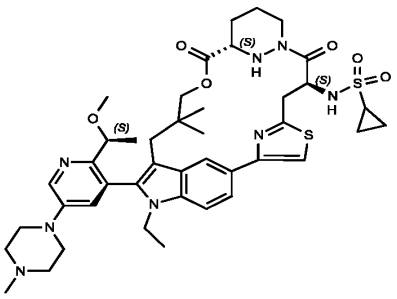
A326	
A327	
A328	
A329	
A330	

A331	
A332	
A333	
A334	
A335	

A336	
A337	
A338	
A339	
A340	

A341	
A342	
A343	
A344	
A345	

A346	
A347	
A348	
A349	
A350	

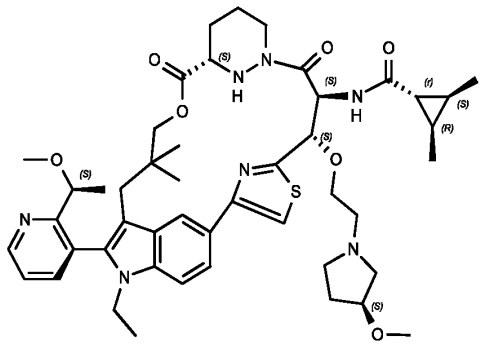
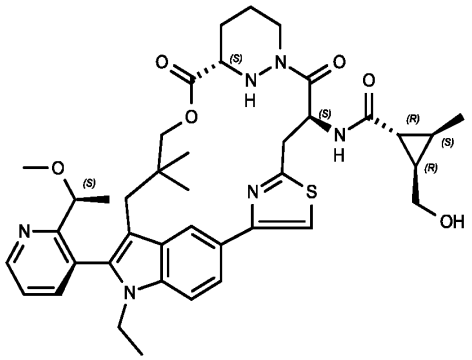
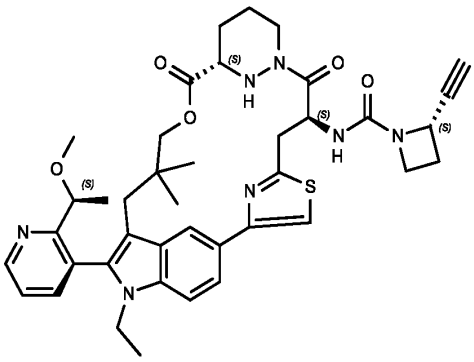
A351	
A352	
A353	

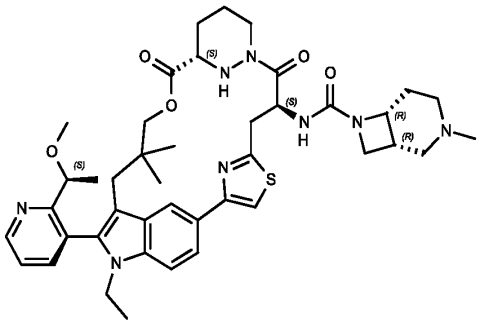
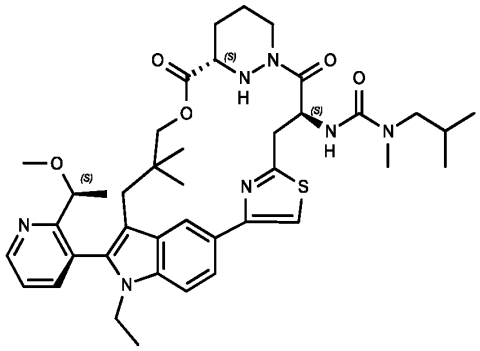
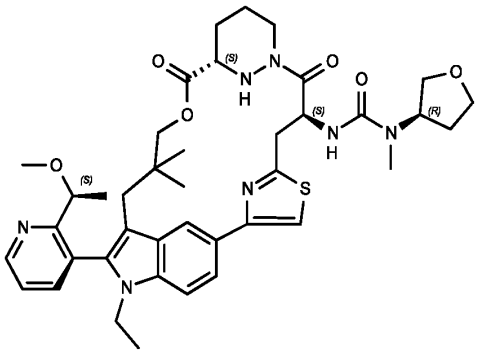
Note that some compounds are shown with bonds as flat or wedged. In some instances, the relative stereochemistry of stereoisomers has been determined; in some instances, the absolute stereochemistry has been determined. All stereoisomers of the compounds of the foregoing table are contemplated by the present invention. In particular embodiments, an atropisomer of a compound of the foregoing table is contemplated. Any compound shown in brackets indicates that the compound is a diastereomer, and the absolute stereochemistry of such diastereomer may not be known.

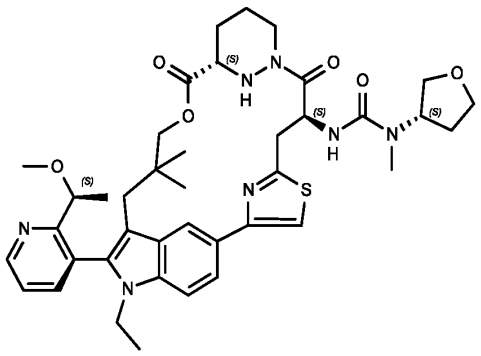
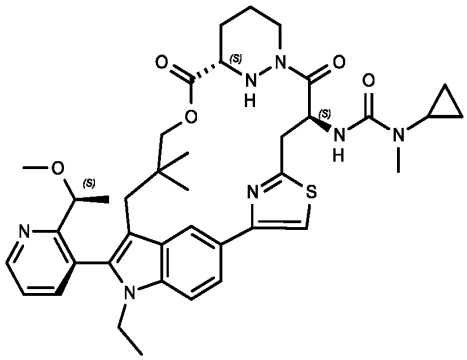
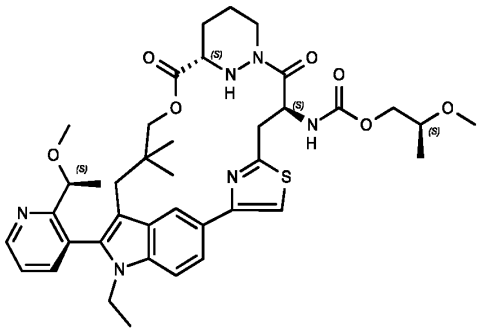
In some embodiments, a compound of the present invention is selected from Table 1-1, or a pharmaceutically acceptable salt or stereoisomer thereof. In some embodiments, a compound of the present invention is selected from Table 1-1, or a pharmaceutically acceptable salt or atropisomer thereof.

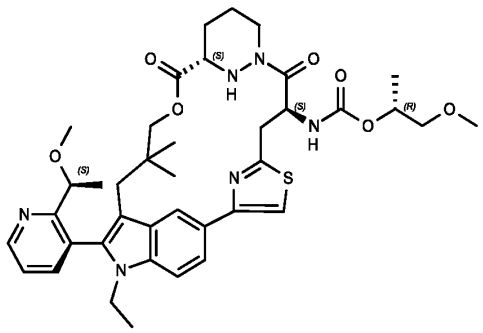
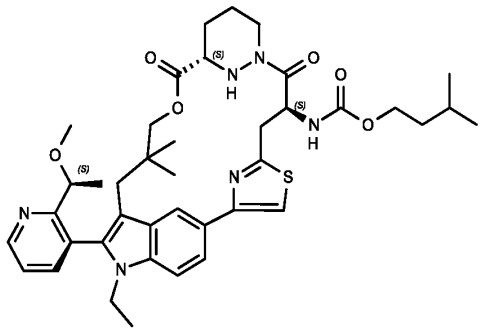
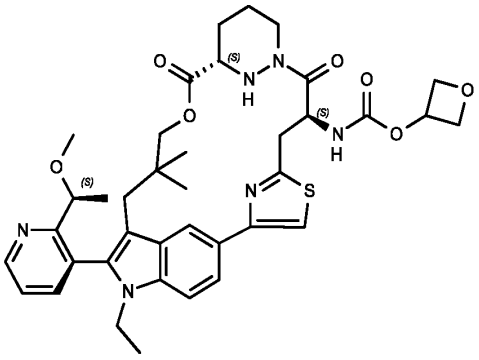
Table 1-1: Certain Compounds of the Present Invention

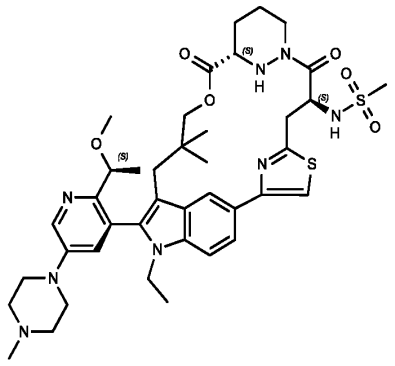
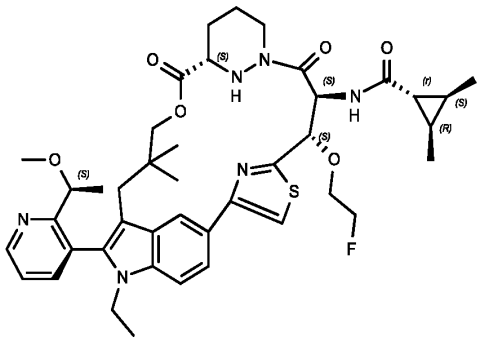
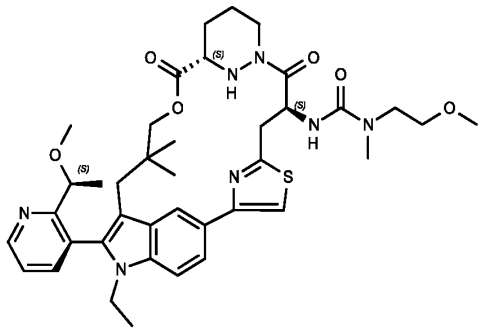
Ex#	Structure

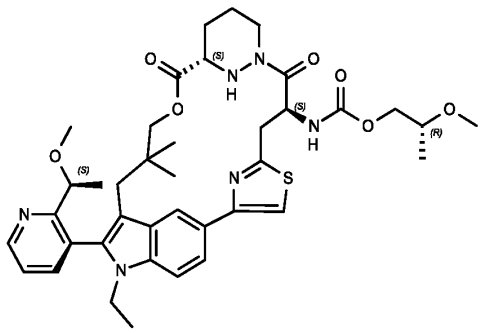
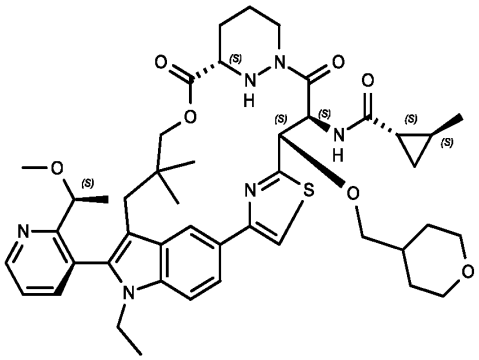
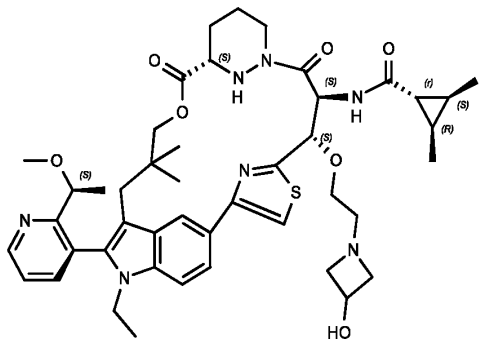
A354	
A355	
A356	

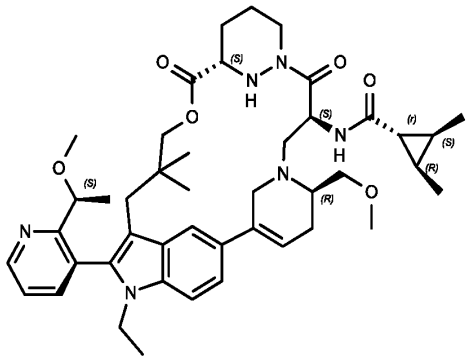
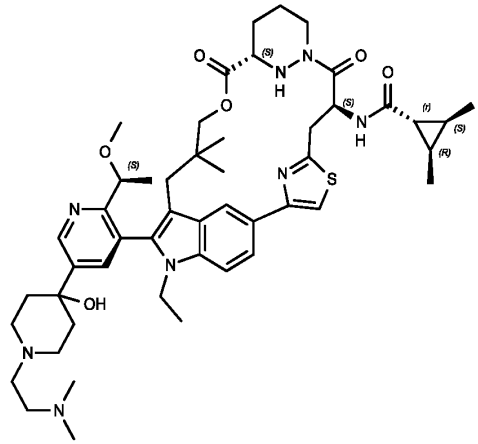
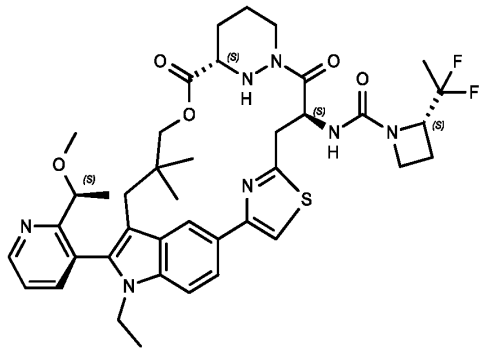
A357	
A358	
A359	

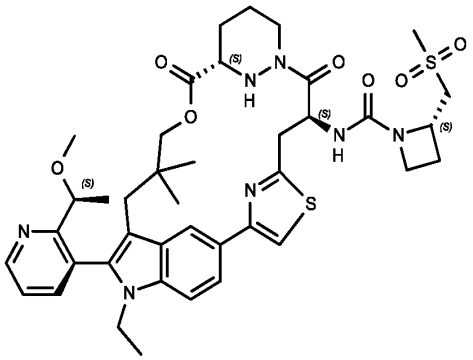
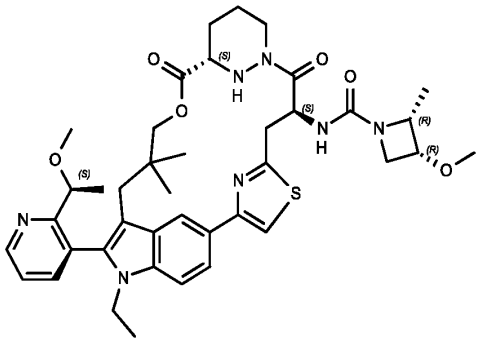
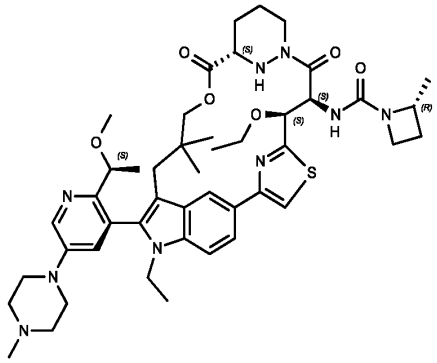
A360	
A361	
A362	

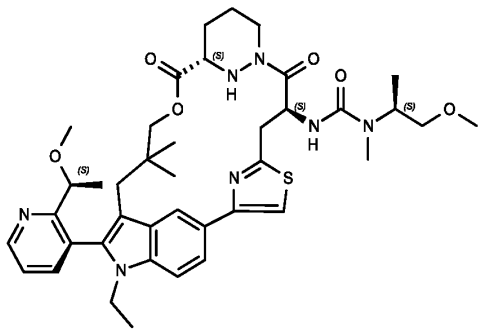
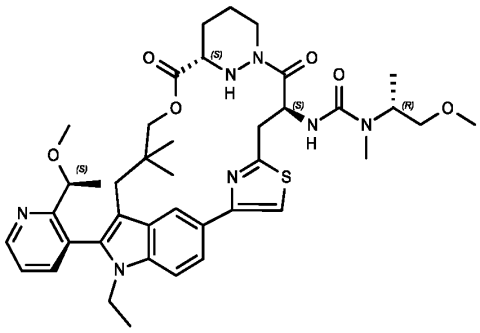
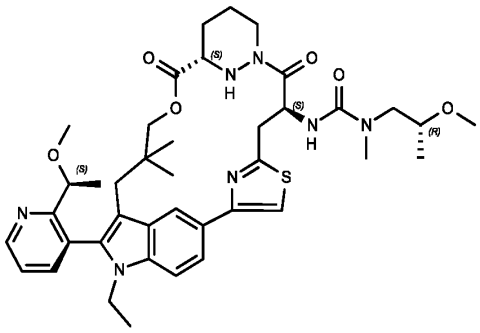
A363	
A364	
A365	

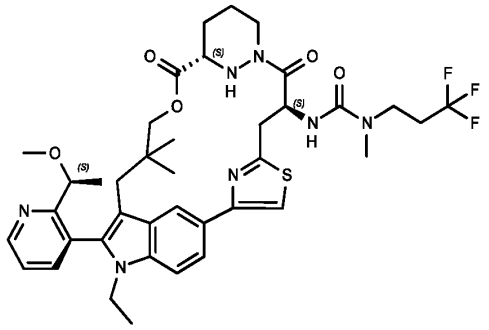
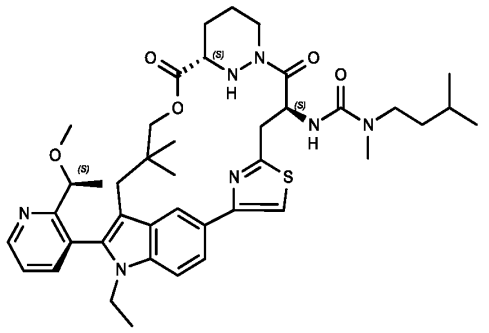
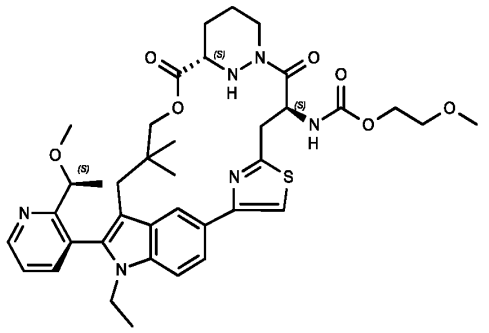
A366	
A367	
A368	

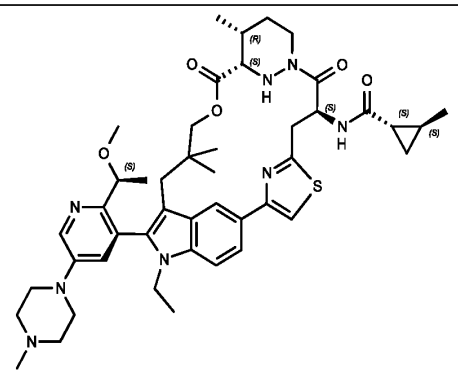
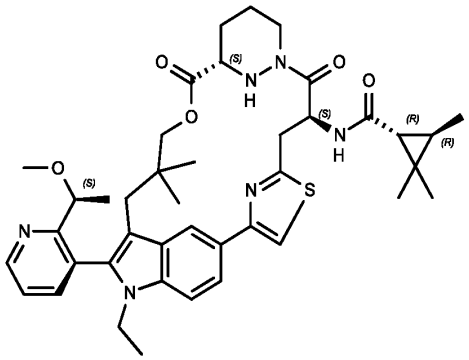
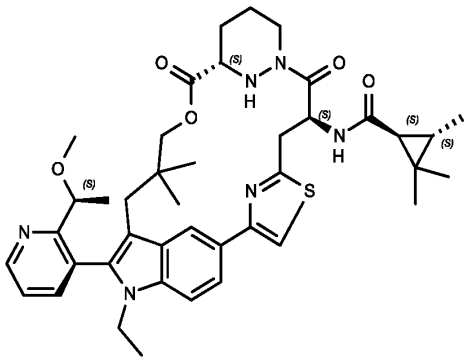
A369	
A370	
A371	

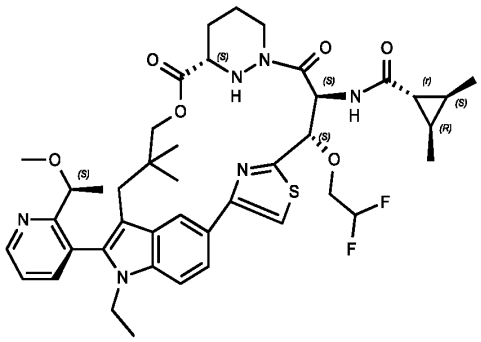
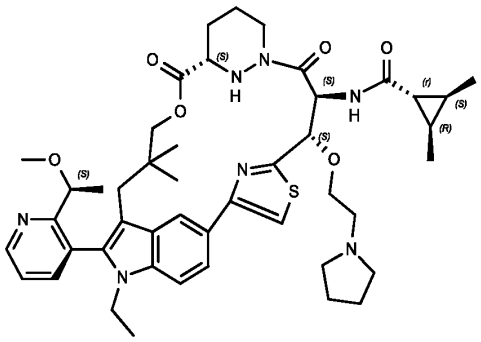
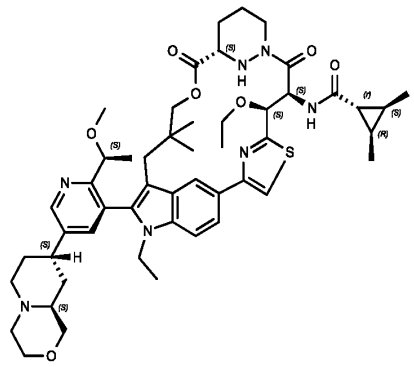
A372	
A373	
A374	

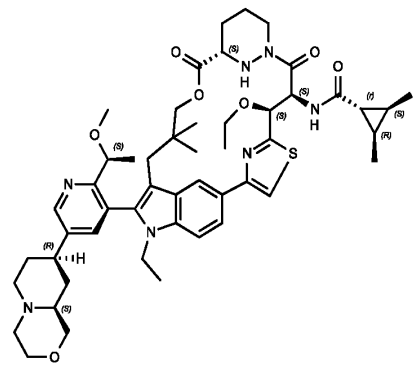
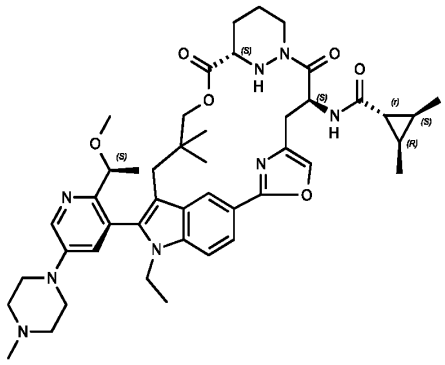
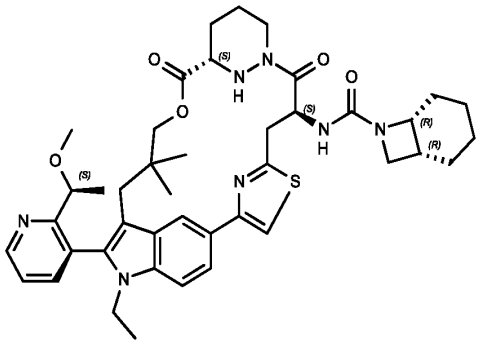
A375	
A376	
A377	

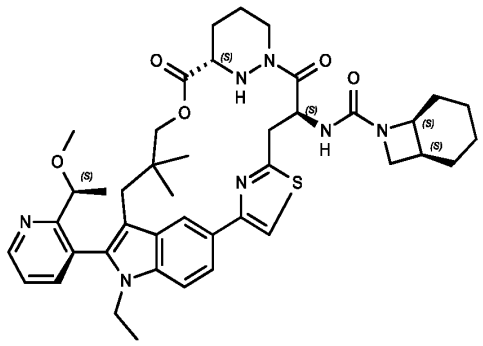
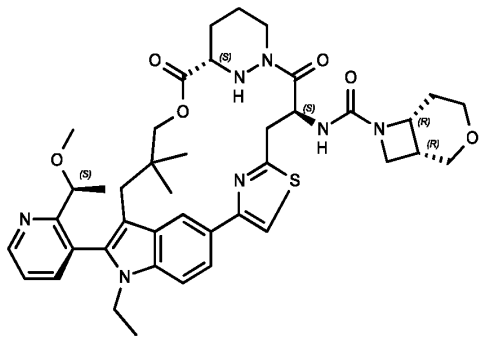
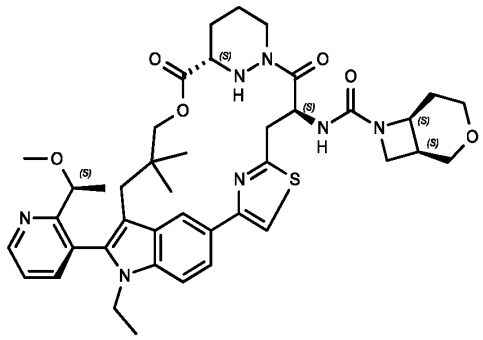
A378	
A379	
A380	

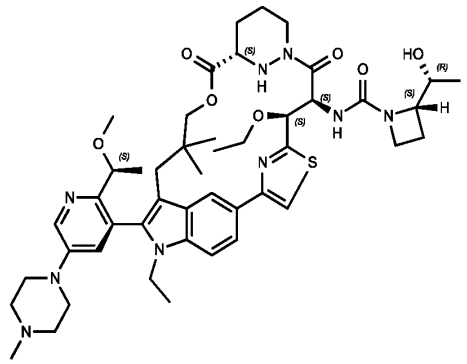
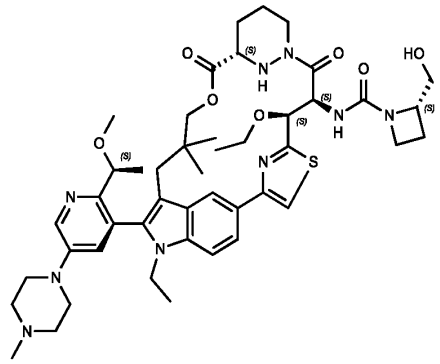
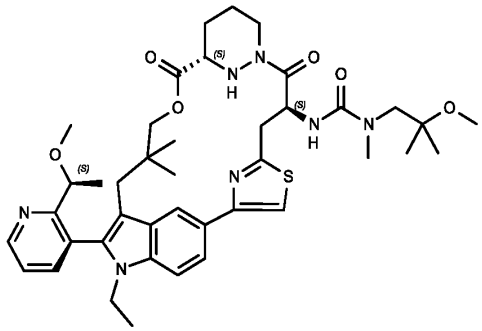
A381	
A382	
A383	

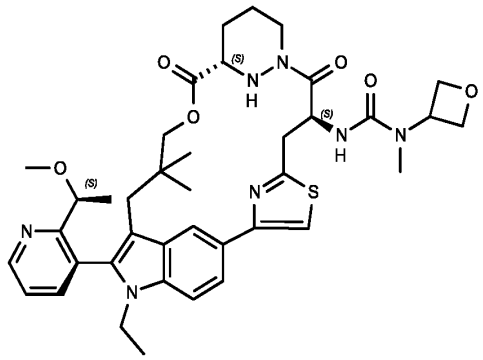
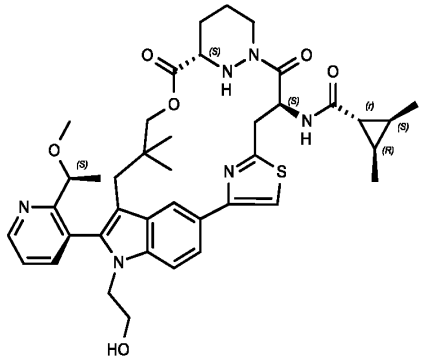
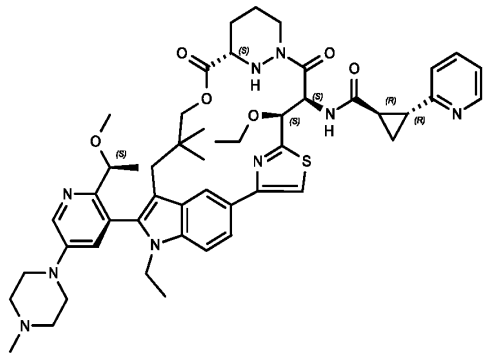
A384	
A385	
A386	

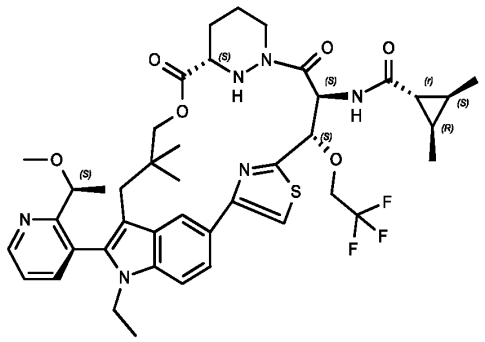
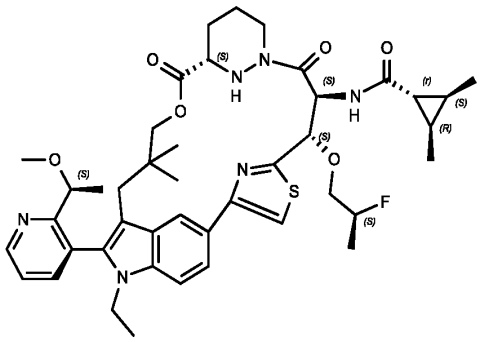
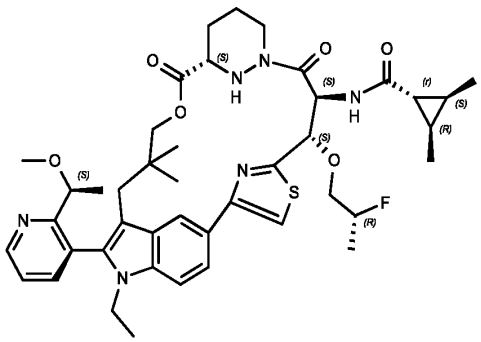
A387	
A388	
A389	

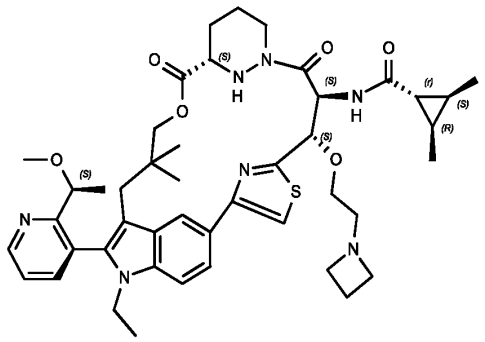
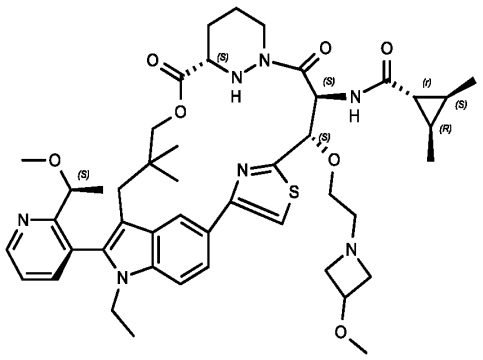
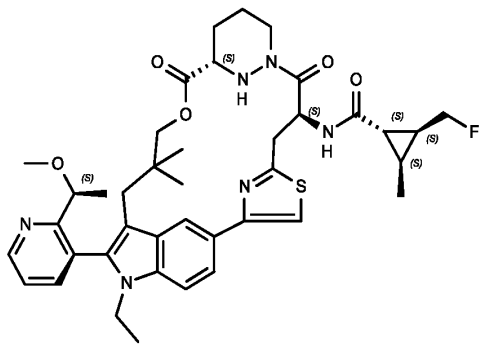
A390	
A391	
A392	

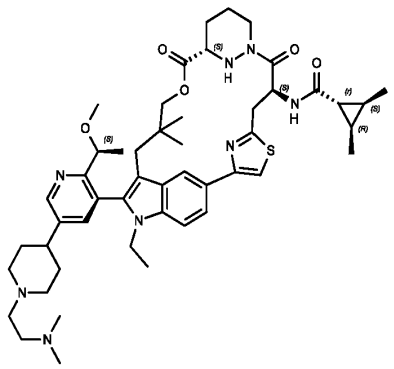
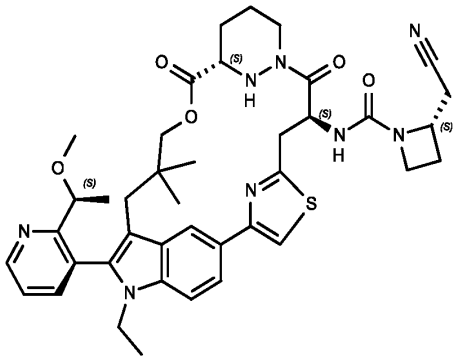
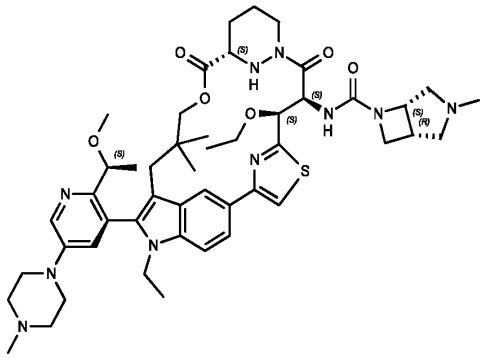
A393	
A394	
A395	

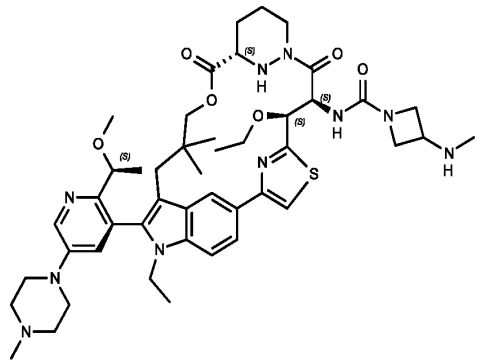
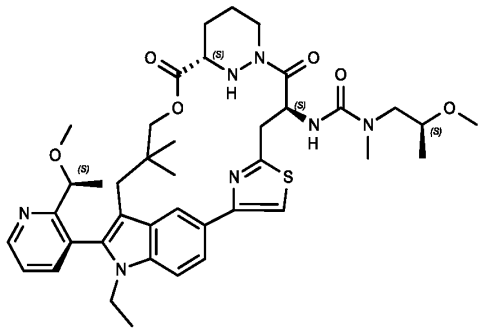
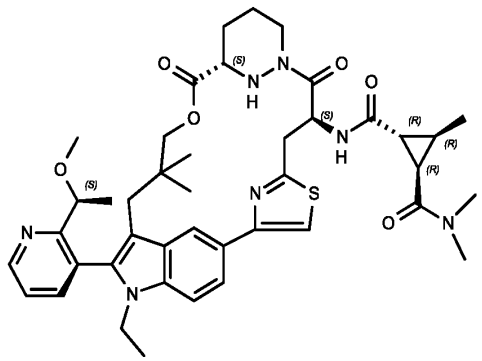
A396	
A397	
A398	

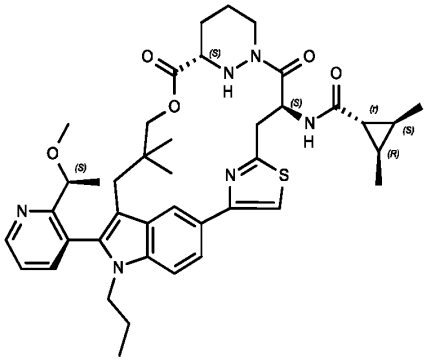
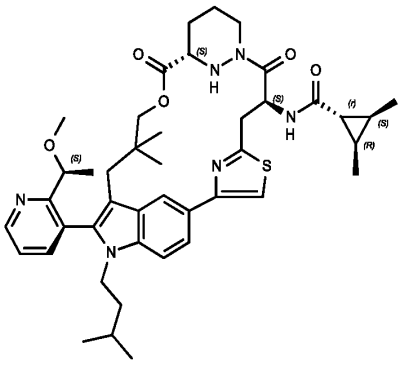
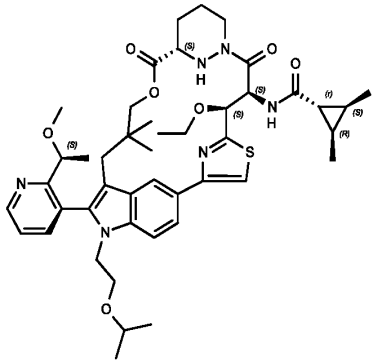
A399	
A400	
A401	

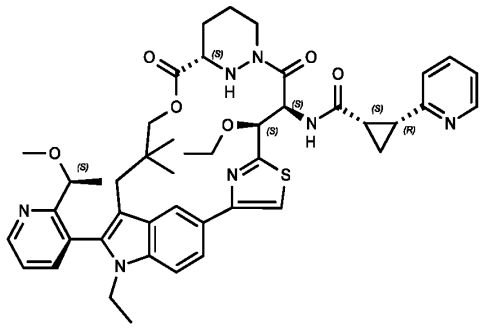
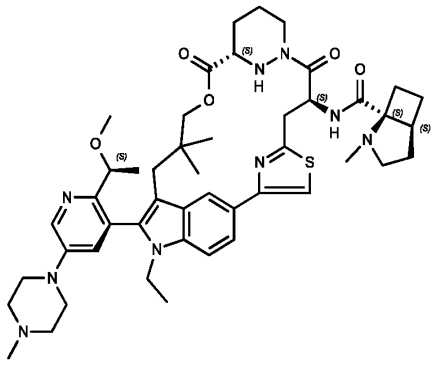
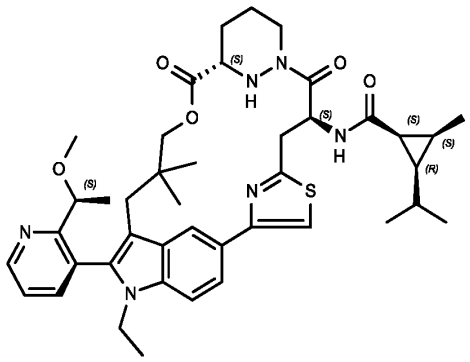
A402	
A403	
A404	

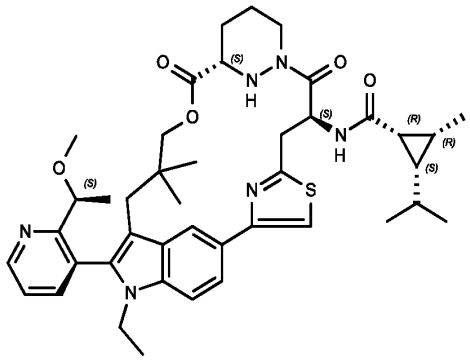
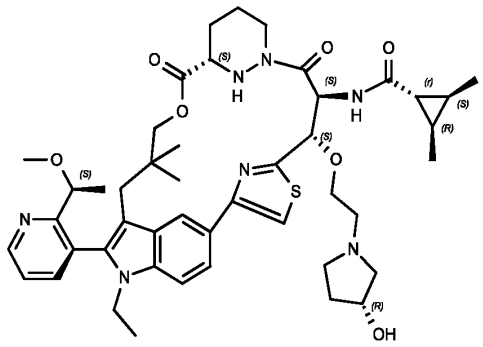
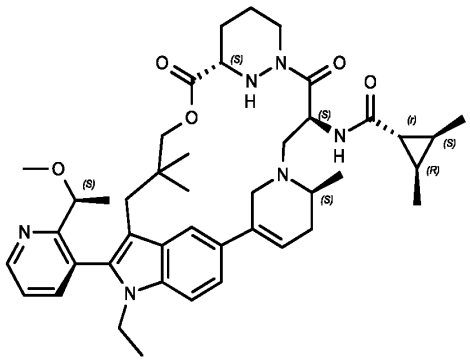
A405	
A406	
A407	

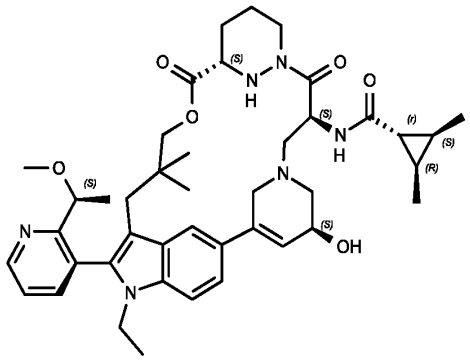
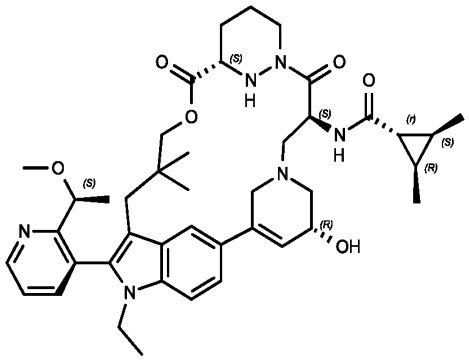
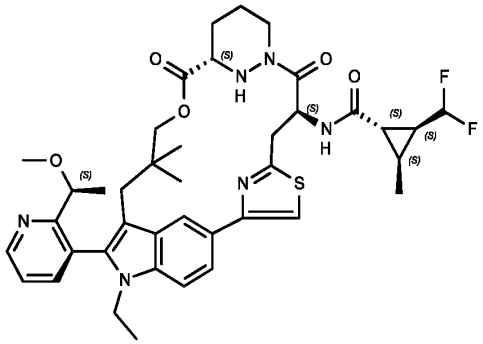
A408	
A409	
A410	

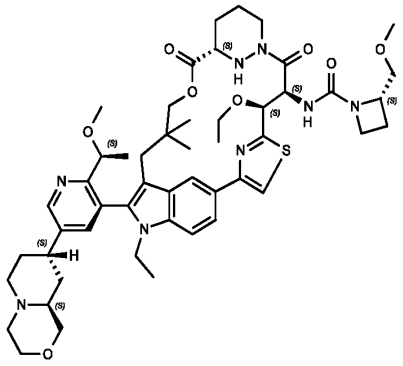
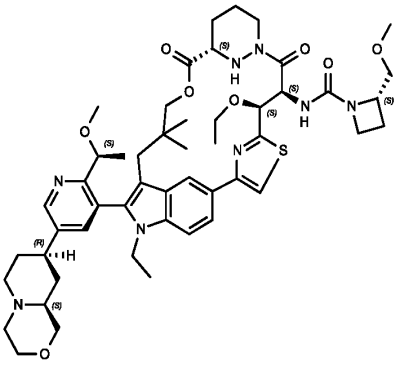
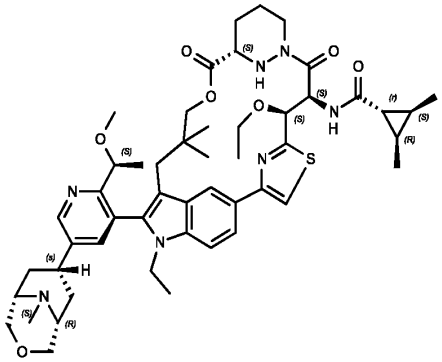
A411	
A412	
A413	

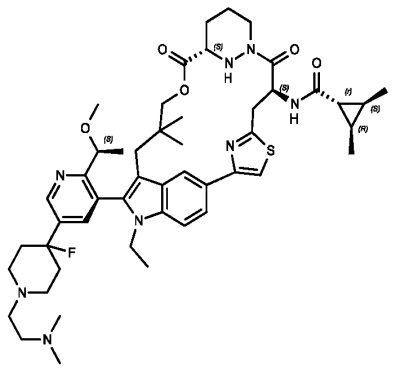
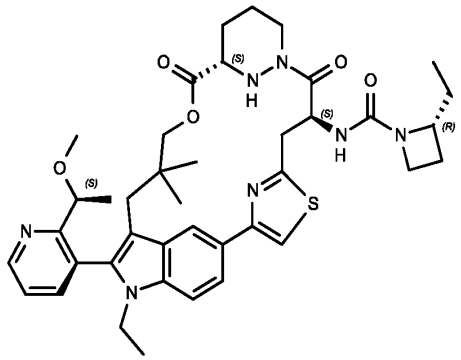
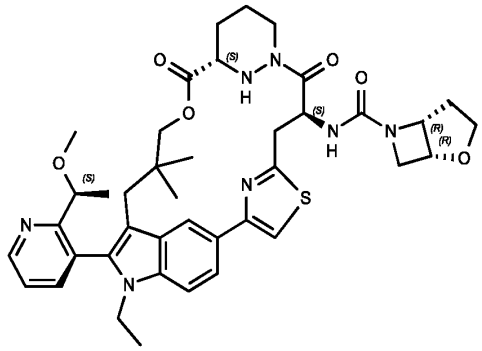
A414	
A415	
A416	

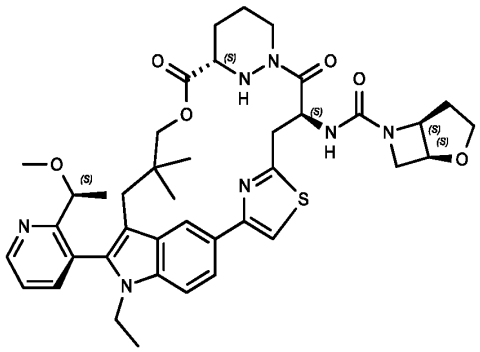
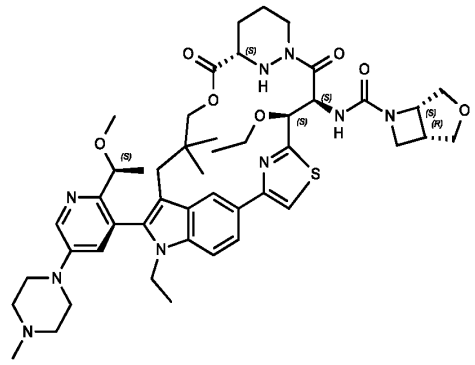
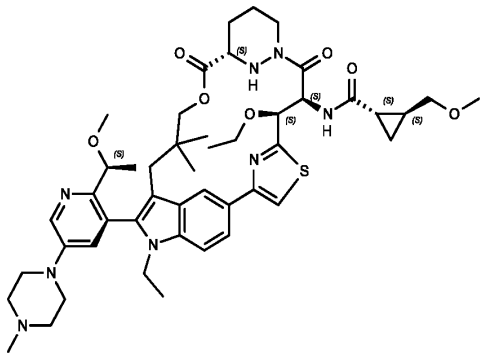
A417	
A418	
A419	

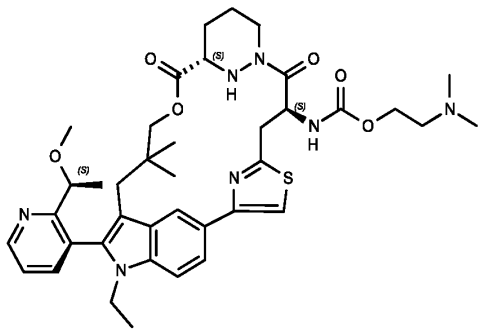
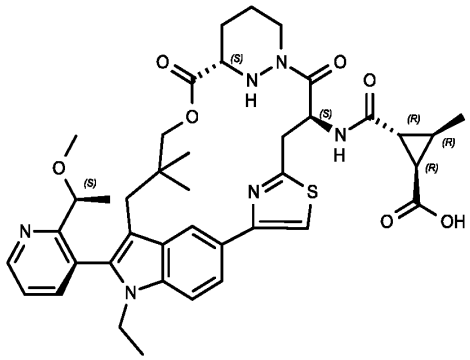
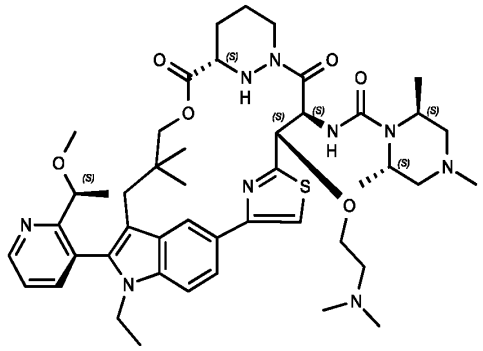
A420	
A421	
A422	

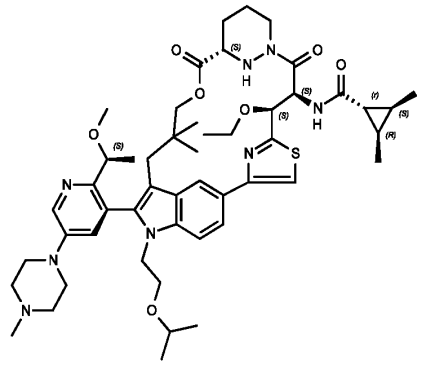
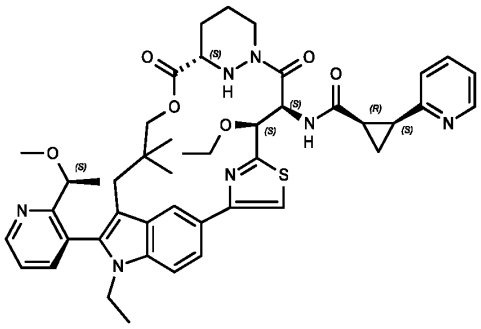
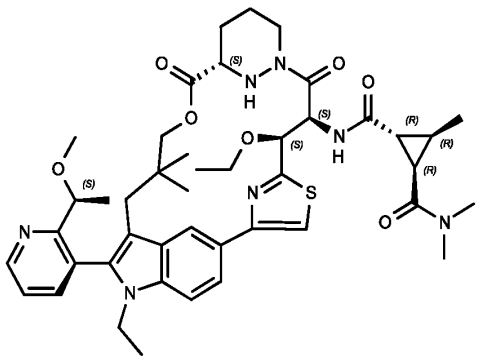
A423	
A424	
A425	

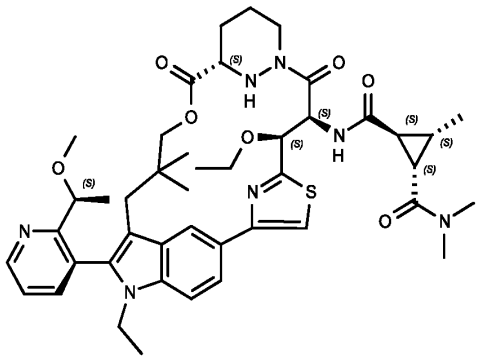
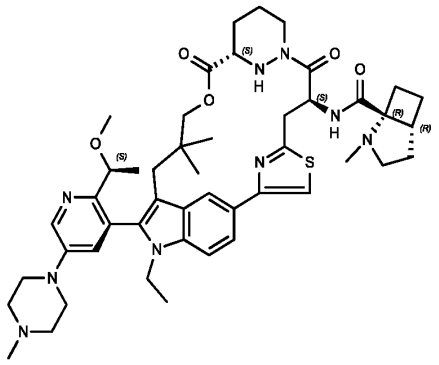
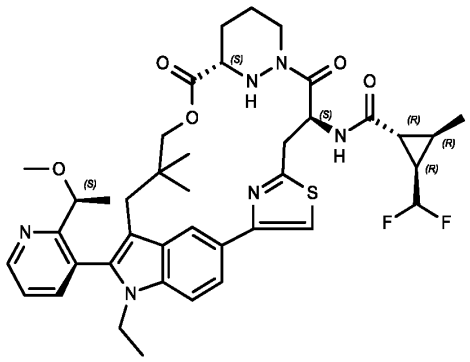
A426	
A427	
A428	

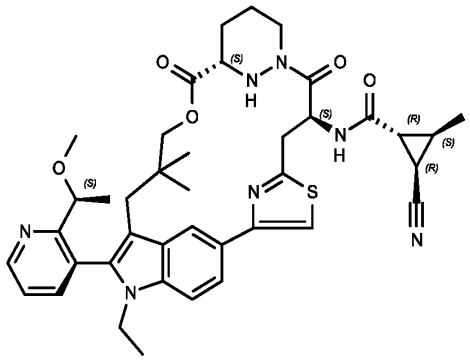
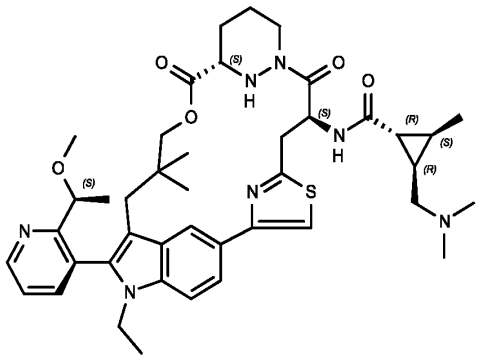
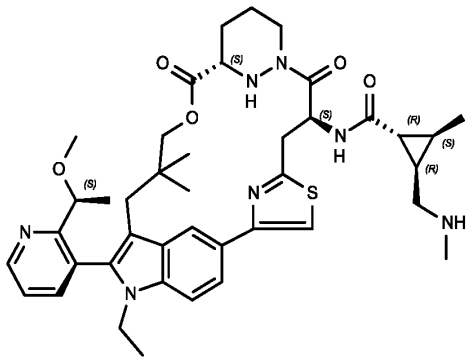
A429	
A430	
A431	

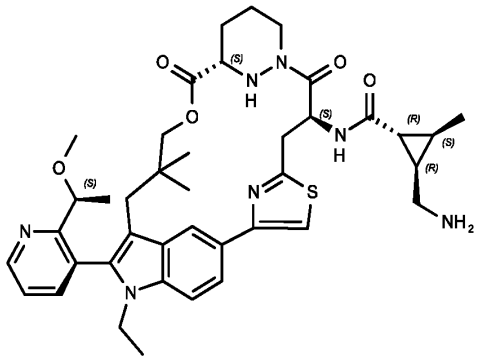
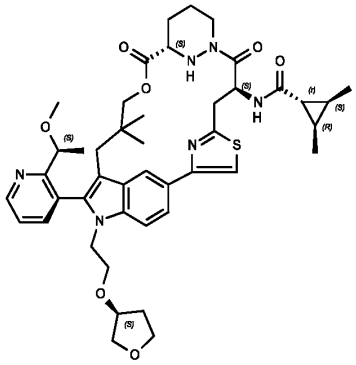
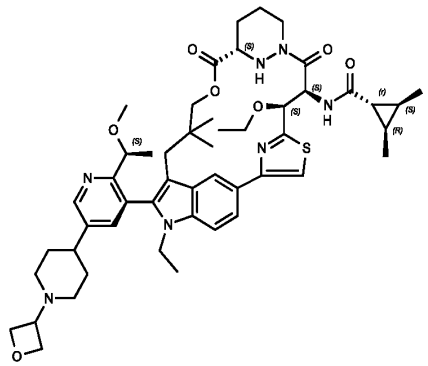
A432	
A433	
A434	

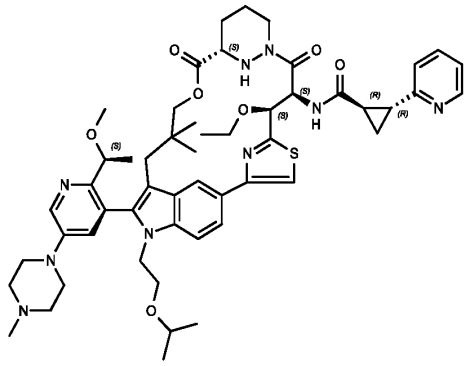
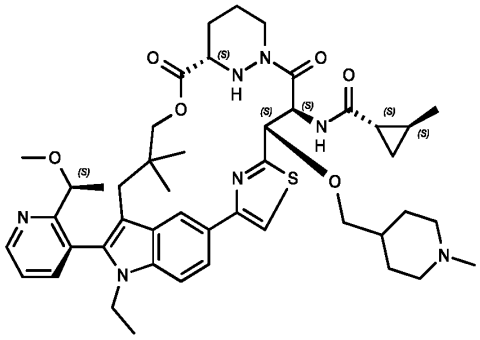
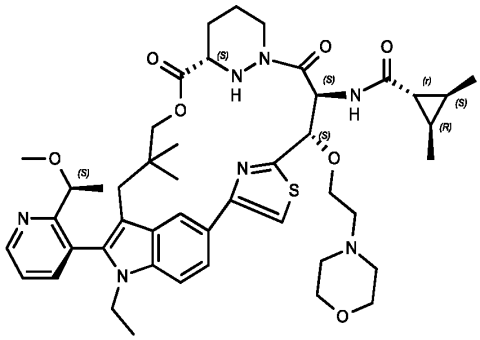
A435	
A436	
A437	

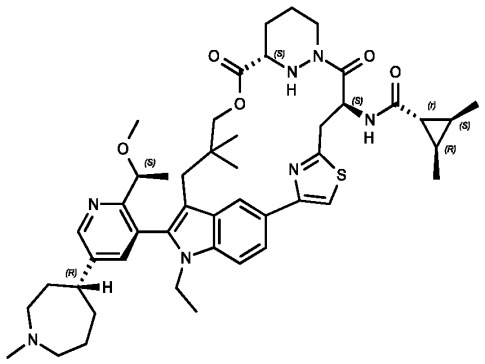
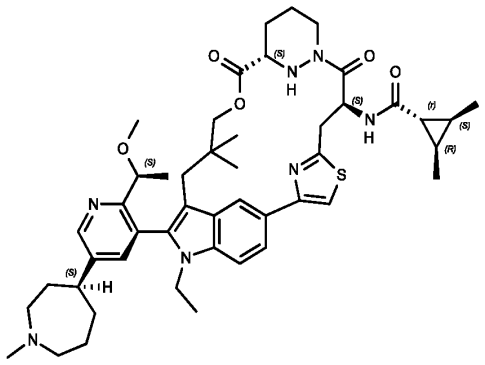
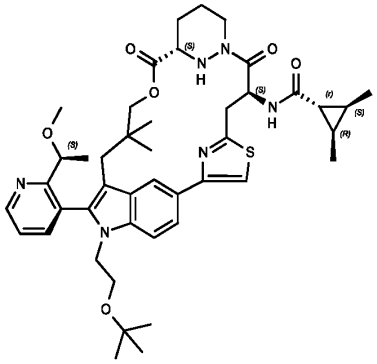
A438	
A439	
A440	

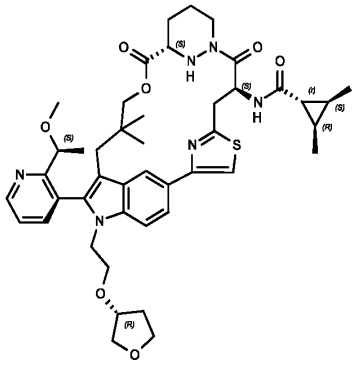
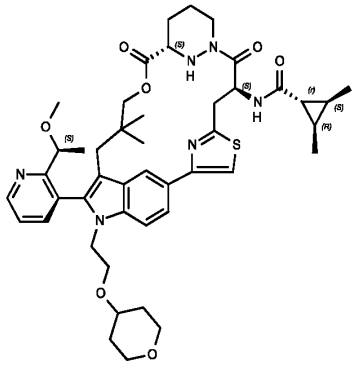
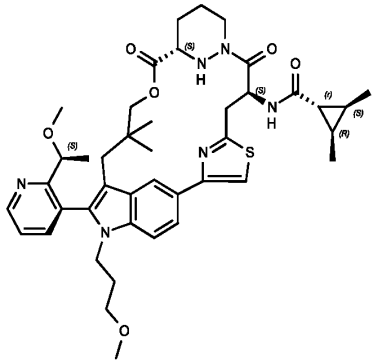
A441	
A442	
A443	

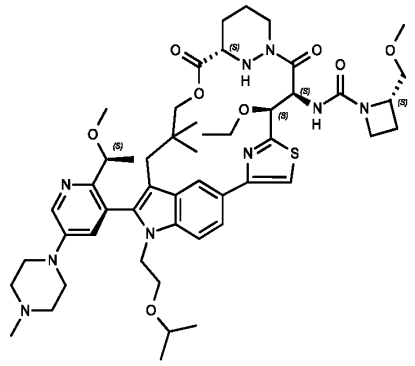
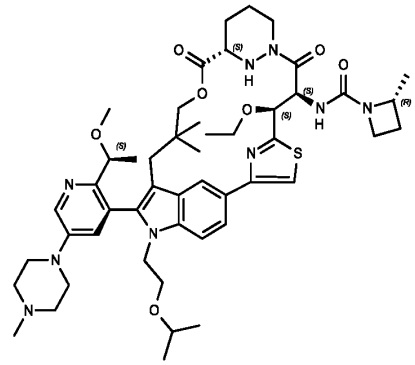
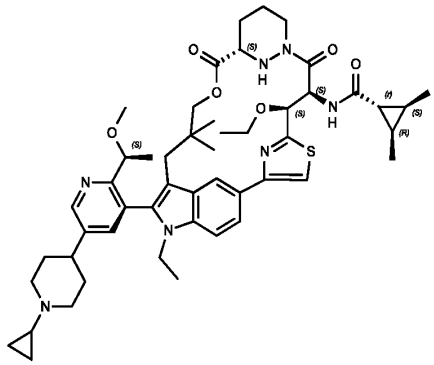
A444	
A445	
A446	

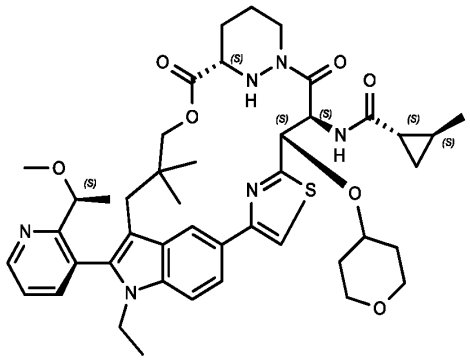
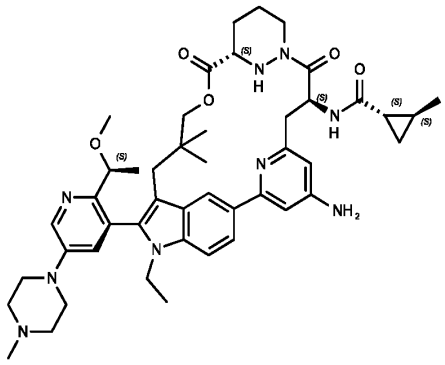
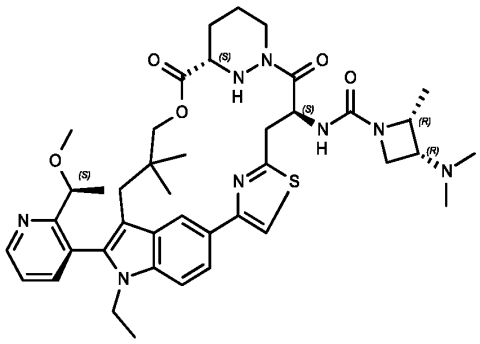
A447	
A448	
A449	

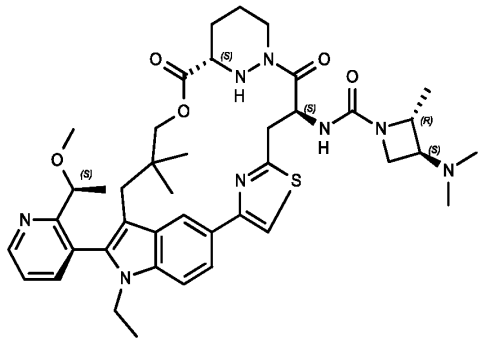
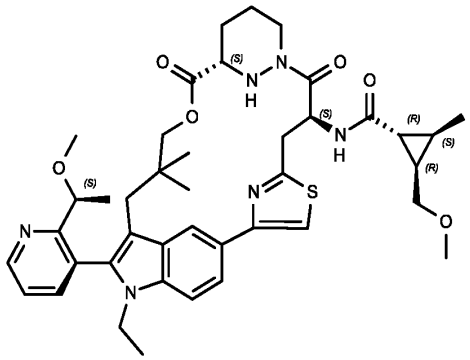
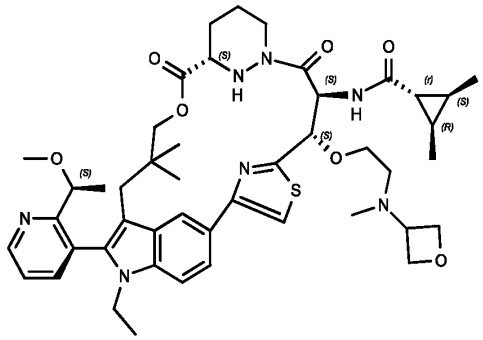
A450	
A451	
A452	

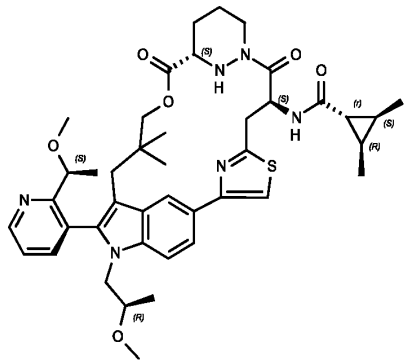
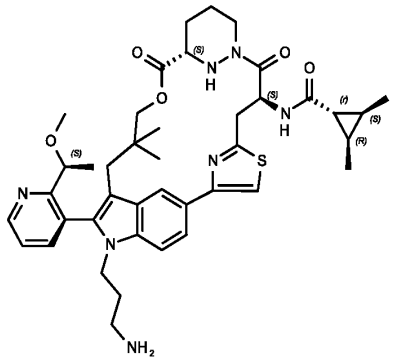
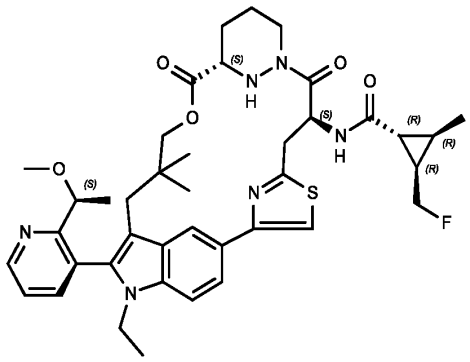
A453	
A454	
A455	

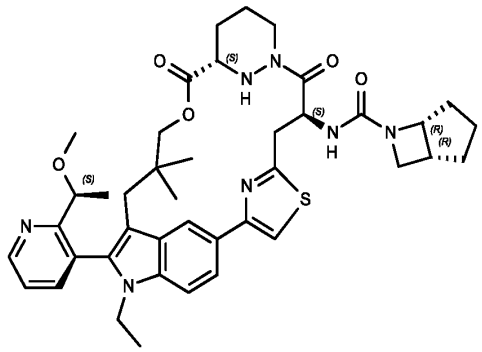
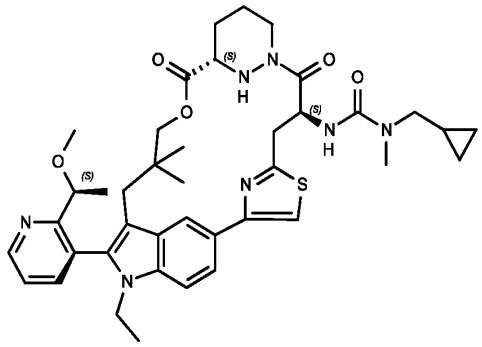
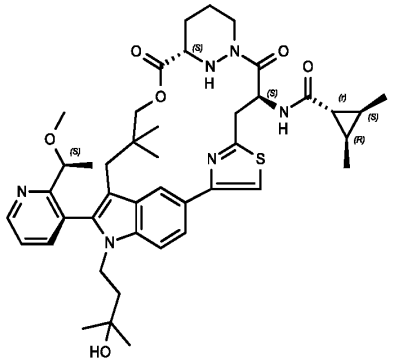
A456	
A457	
A458	

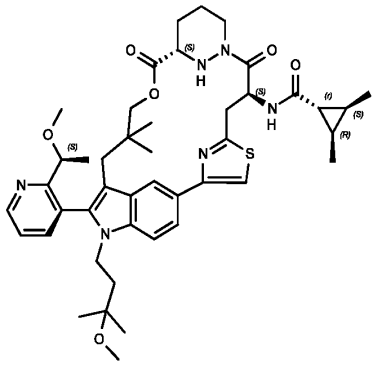
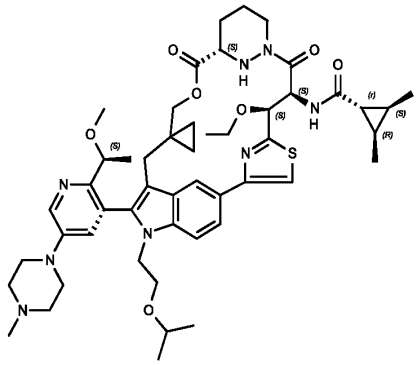
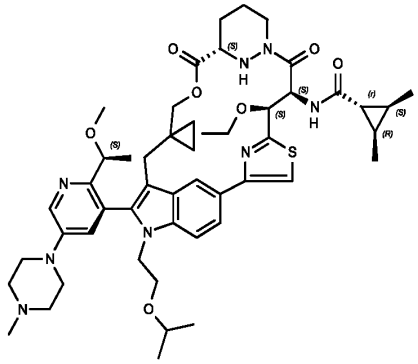
A459	
A460	
A461	

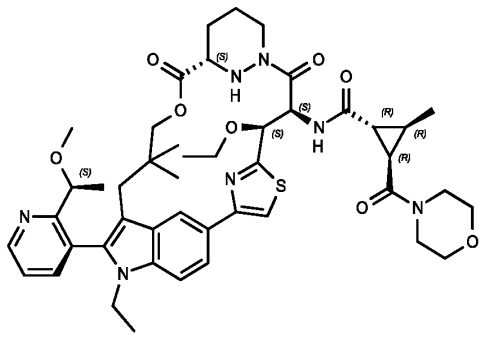
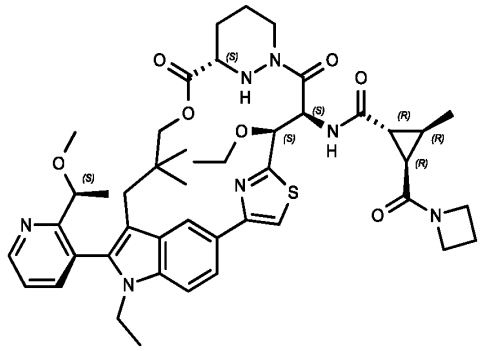
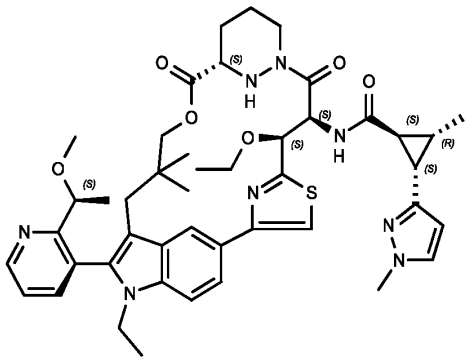
A462	
A463	
A464	

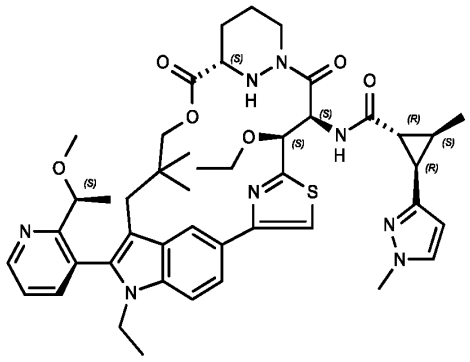
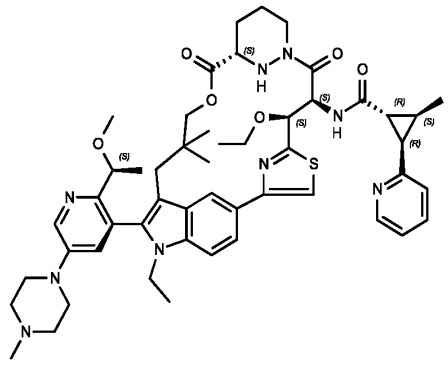
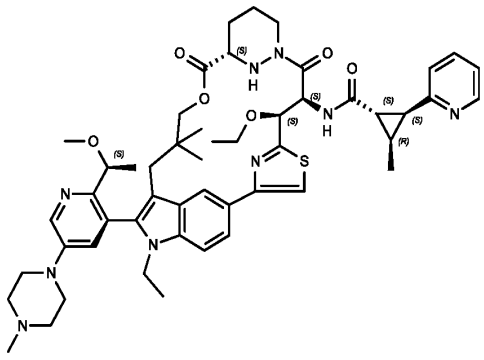
A465	
A466	
A467	

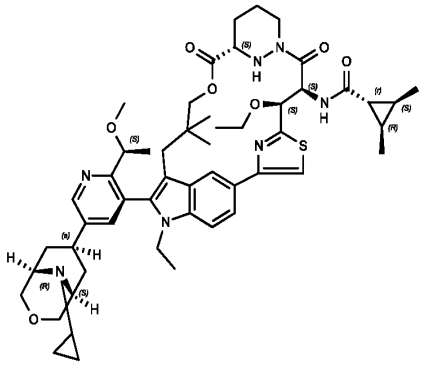
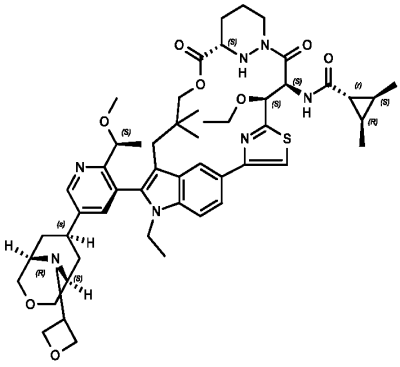
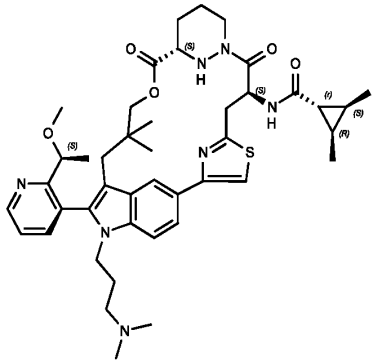
A468	
A469	
A470	

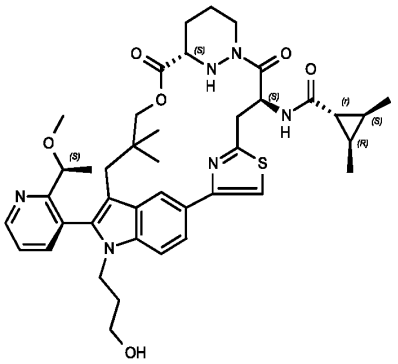
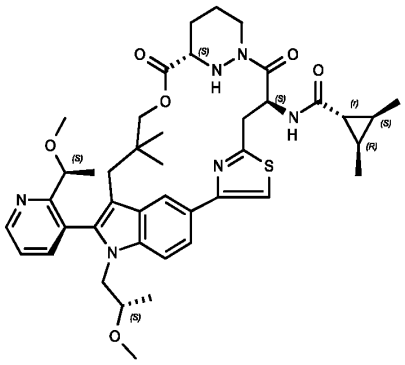
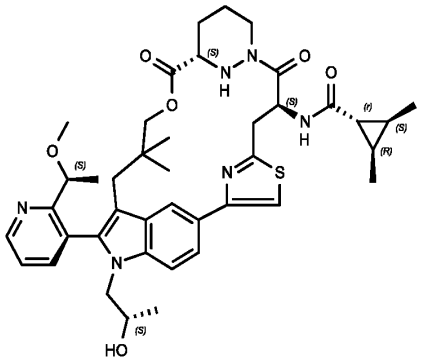
A471	
A472	
A473	

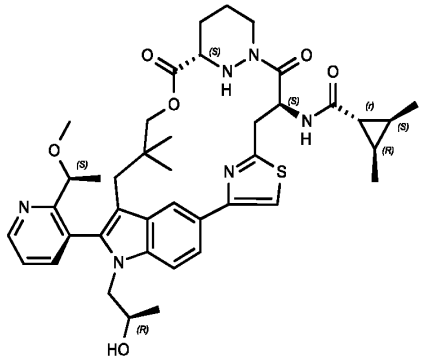
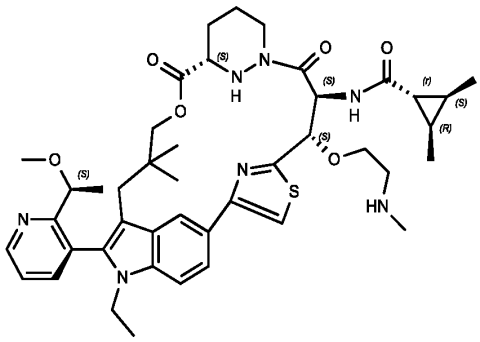
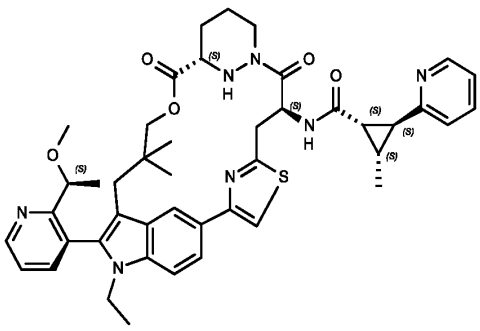
A474	
A475	
A476	

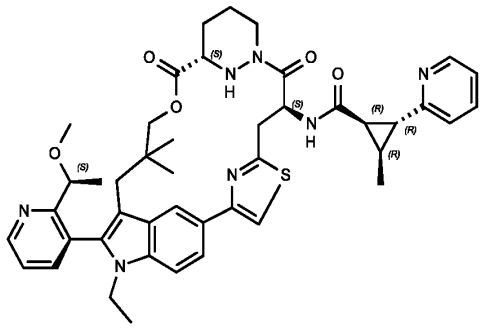
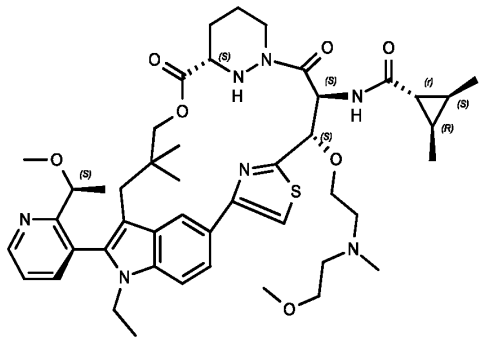
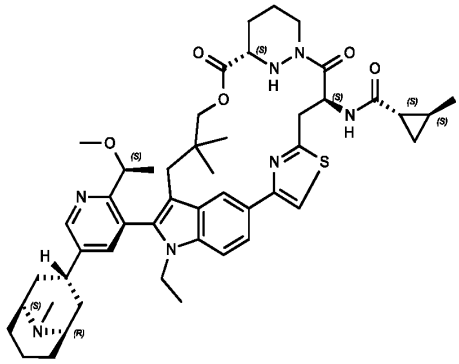
A477	
A478	
A479	

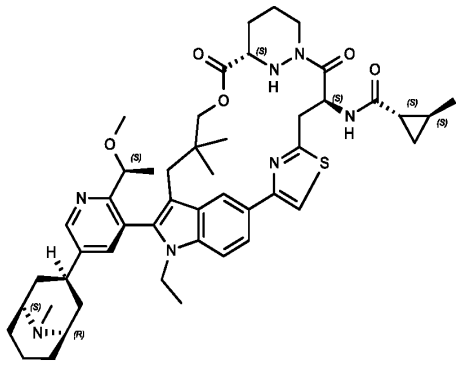
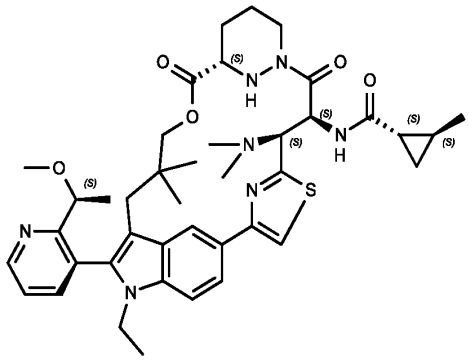
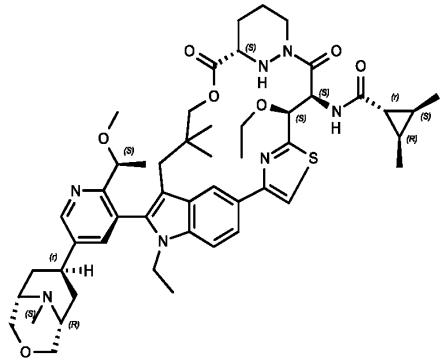
A480	
A481	
A482	

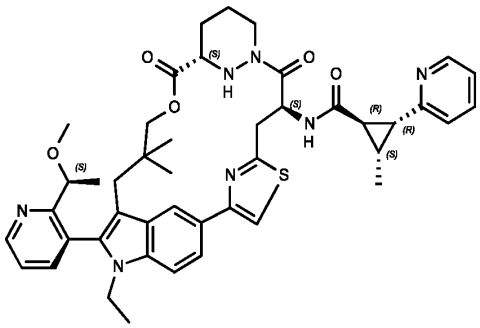
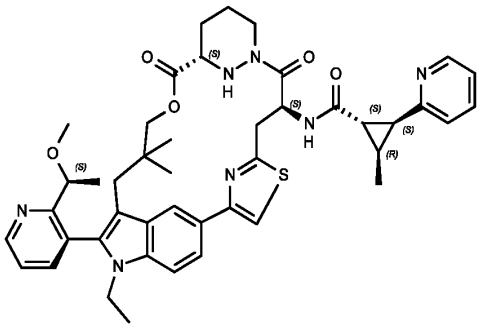
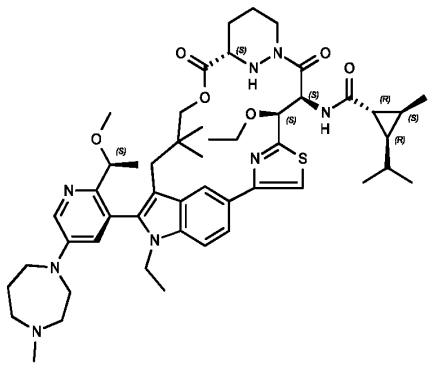
A483	
A484	
A485	

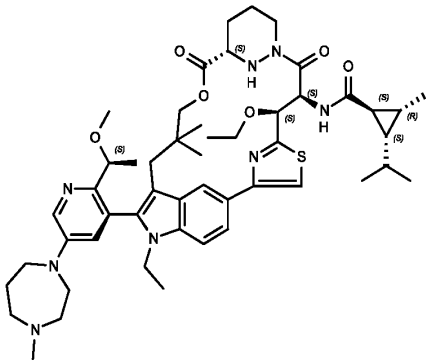
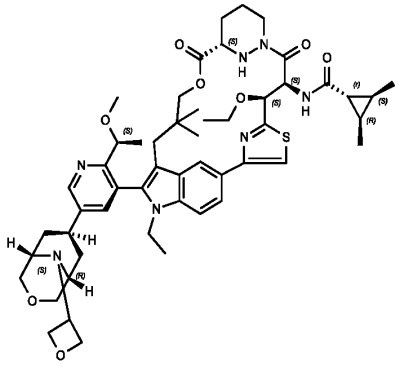
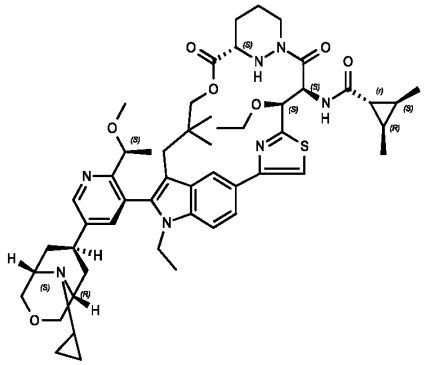
A486	
A487	
A488	

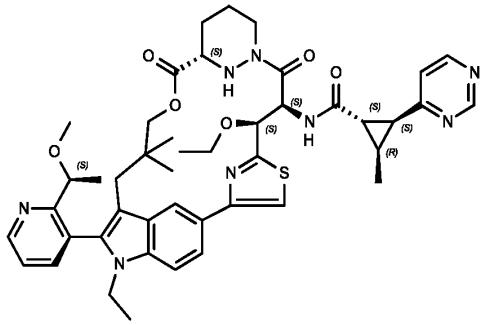
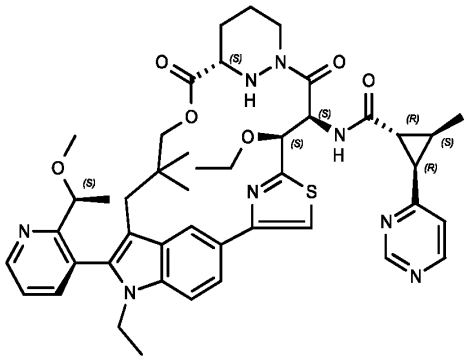
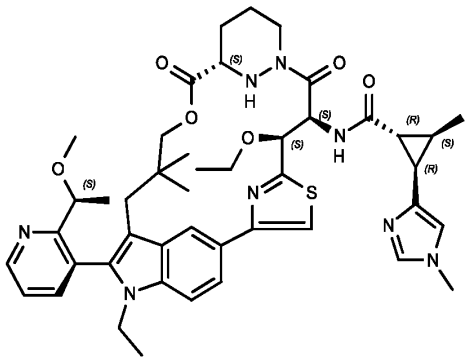
A489	
A490	
A491	

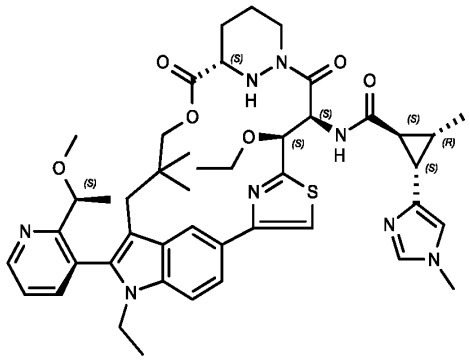
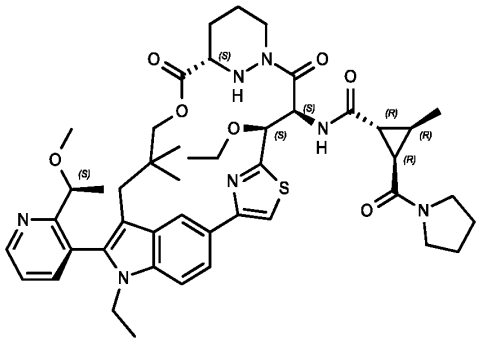
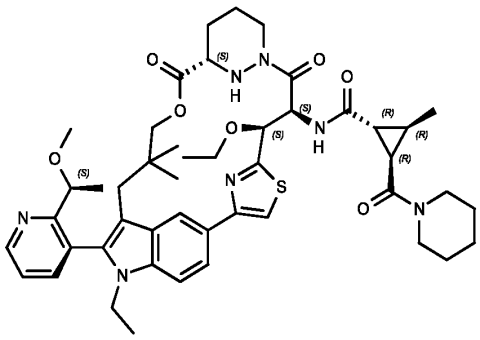
A492	
A493	
A494	

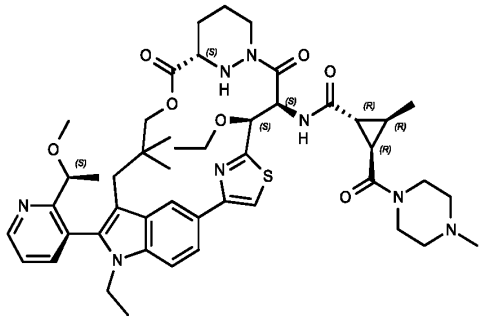
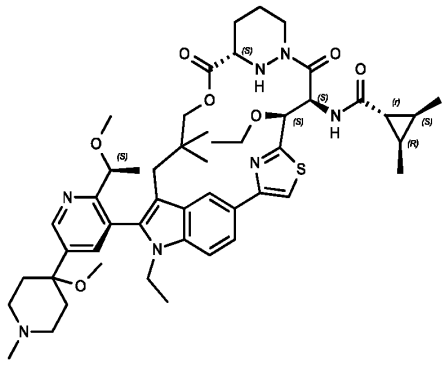
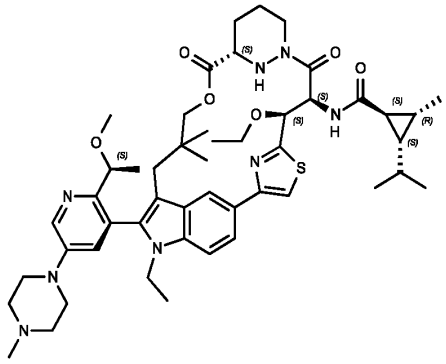
A495	
A496	
A497	

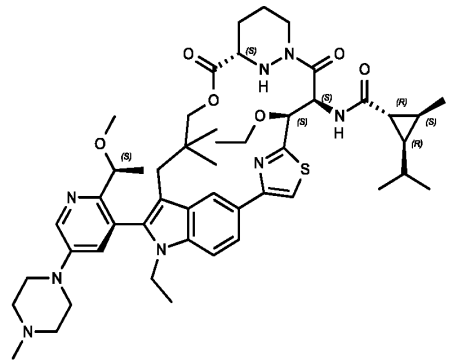
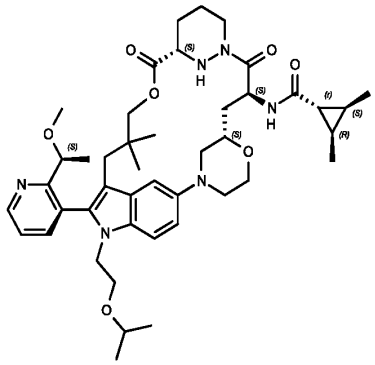
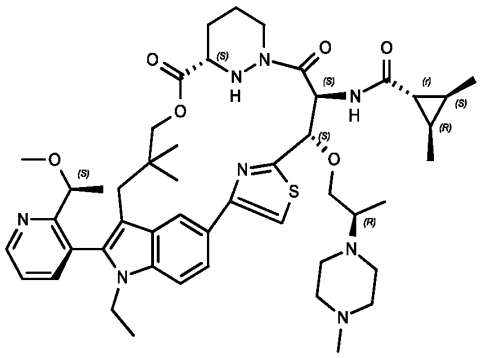
A498	
A499	
A500	

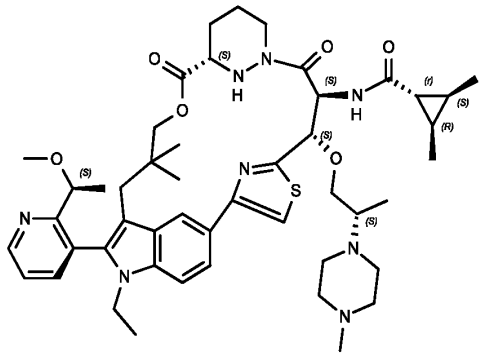
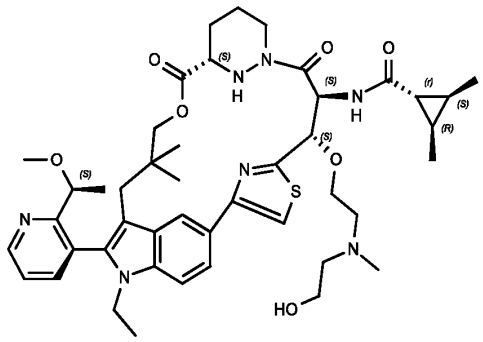
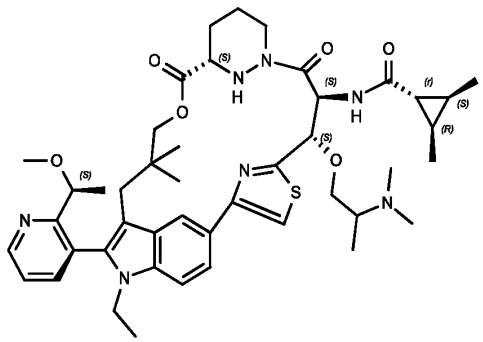
A501	
A502	
A503	

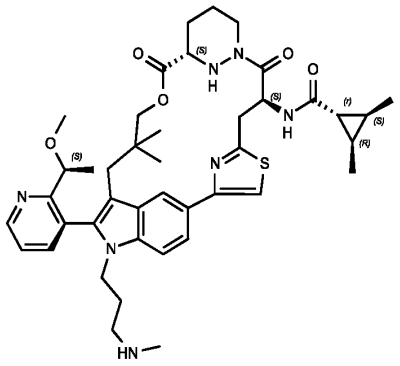
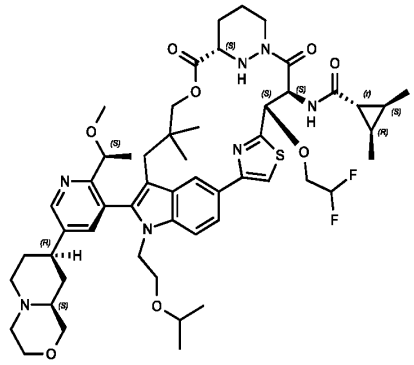
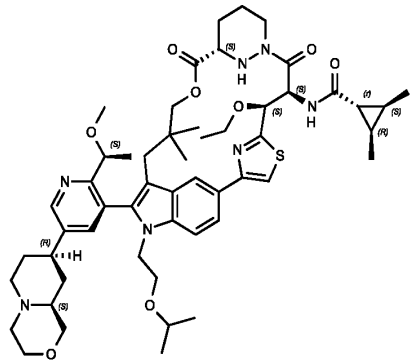
A504	
A505	
A506	

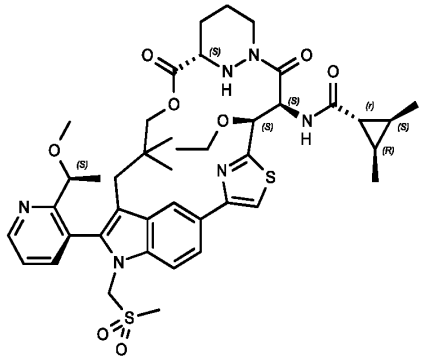
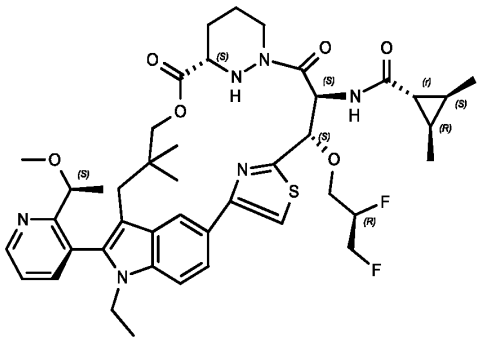
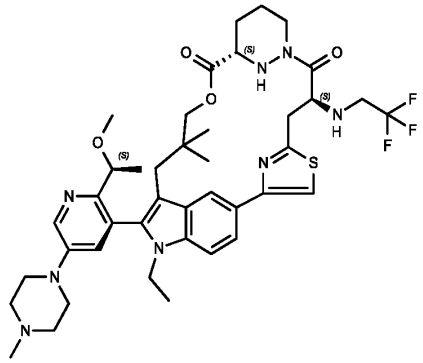
A507	
A508	
A509	

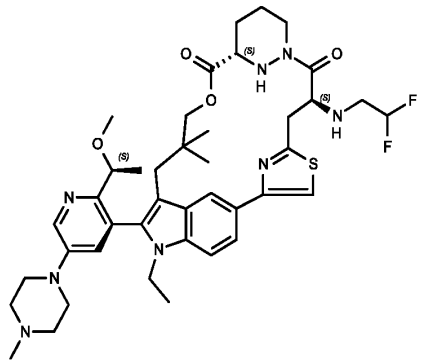
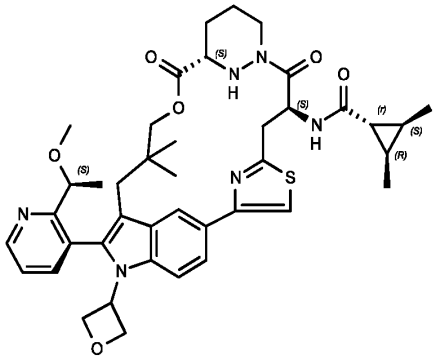
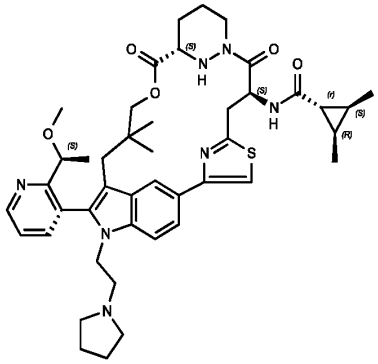
A510	
A511	
A512	

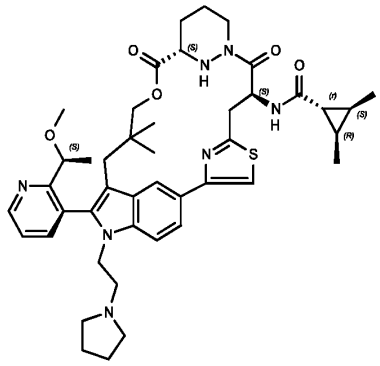
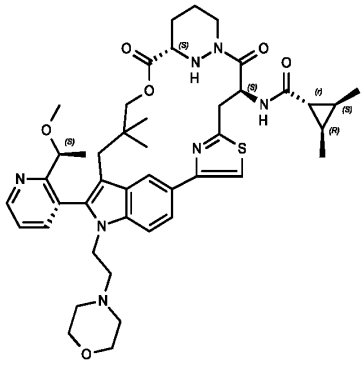
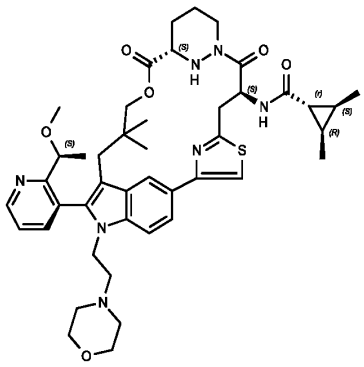
A513	
A514	
A515	

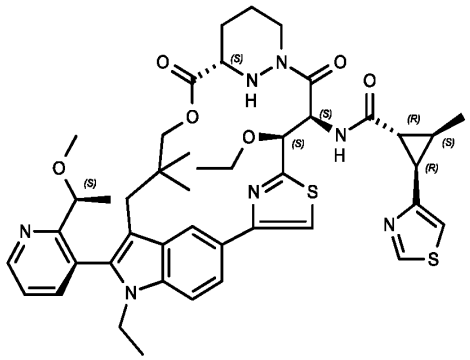
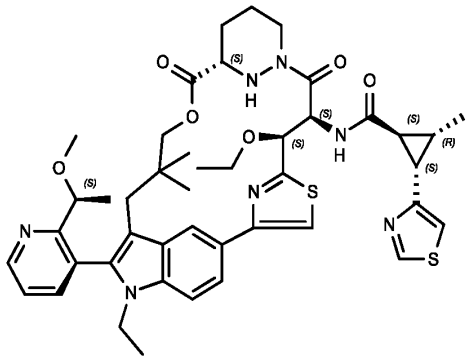
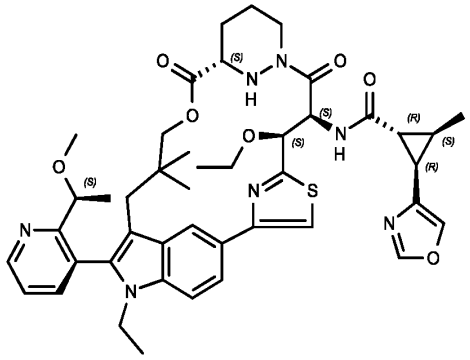
A516	
A517	
A518	

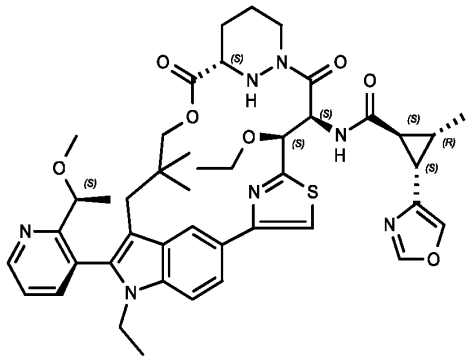
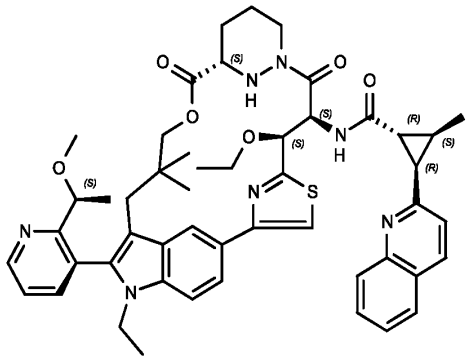
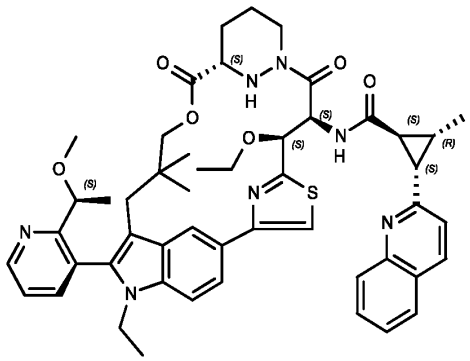
A519	
A520	
A521	

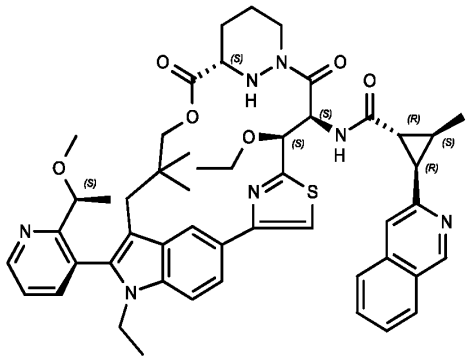
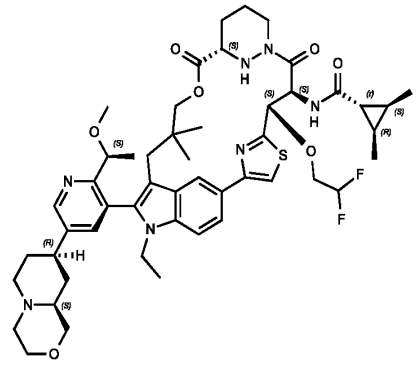
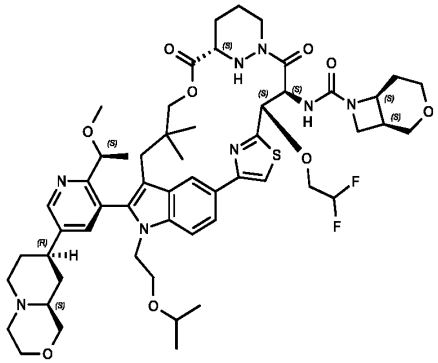
A522	
A523	
A524	

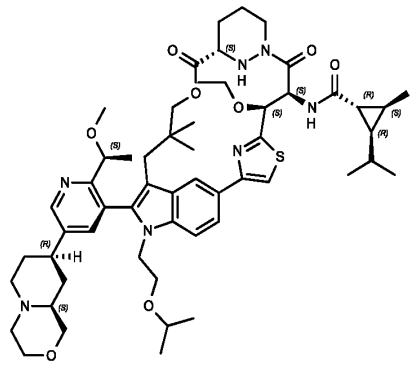
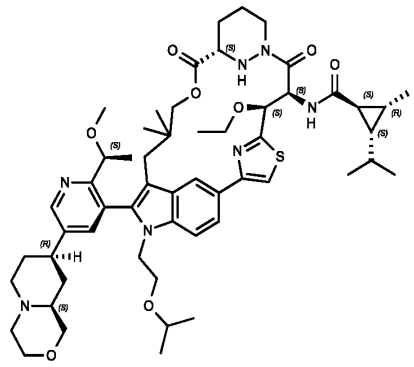
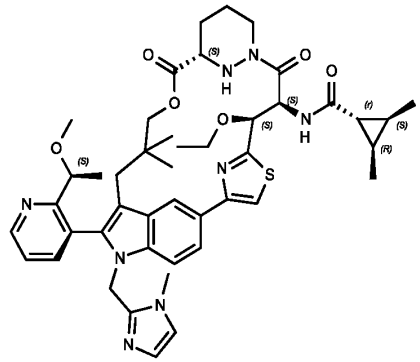
A525	
A526	
A527	

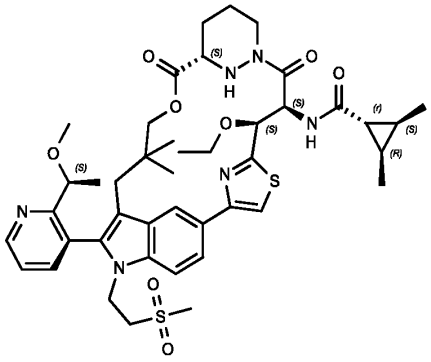
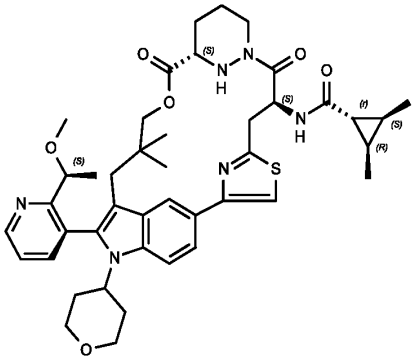
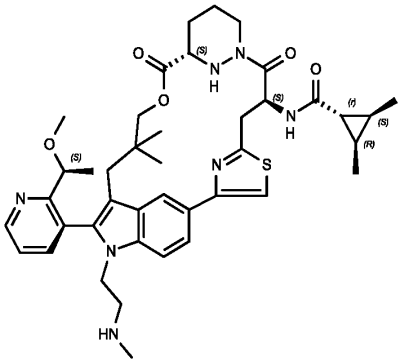
A528	
A529	
A530	

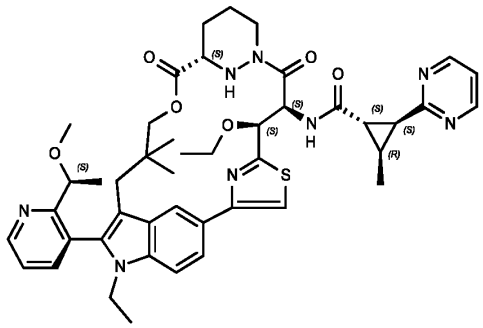
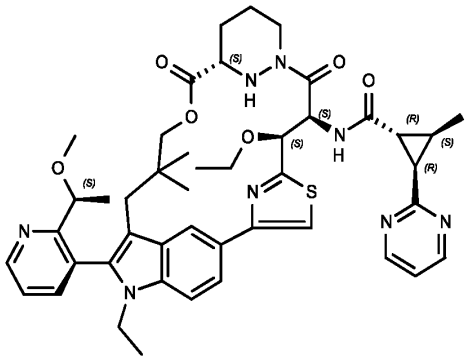
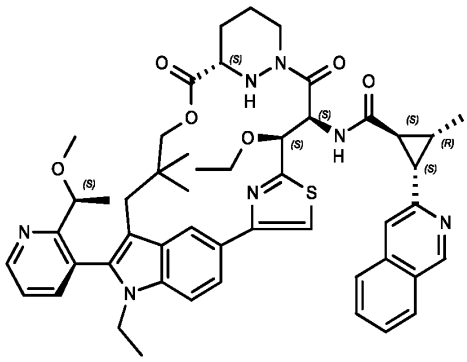
A531	
A532	
A533	

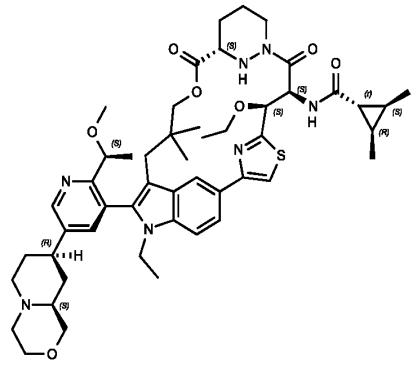
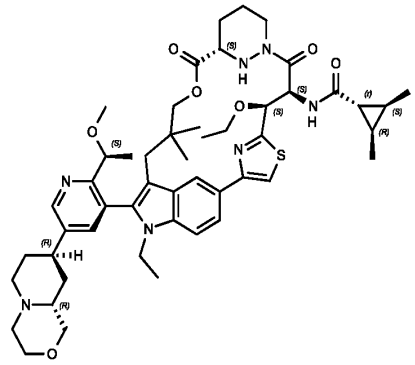
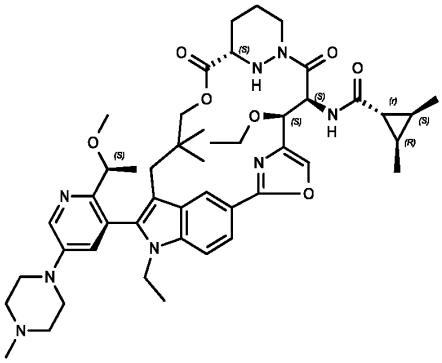
A534	
A535	
A536	

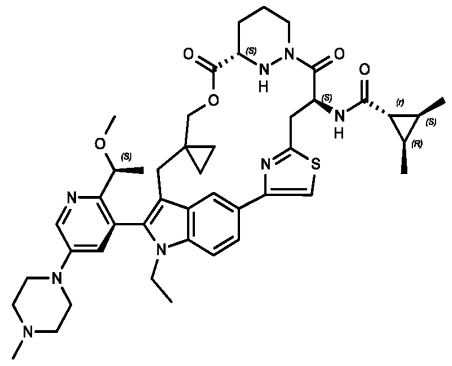
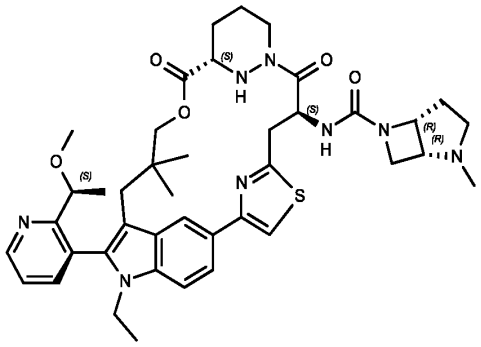
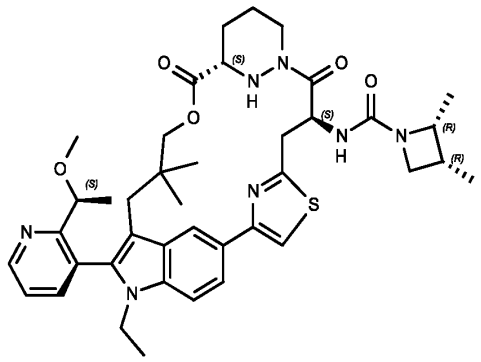
A537	
A538	
A539	

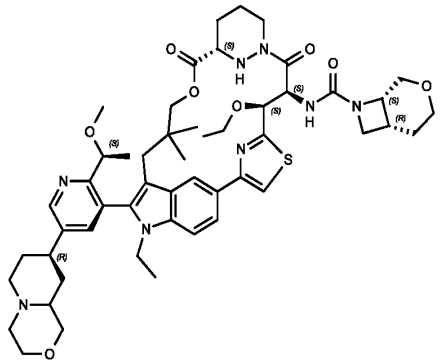
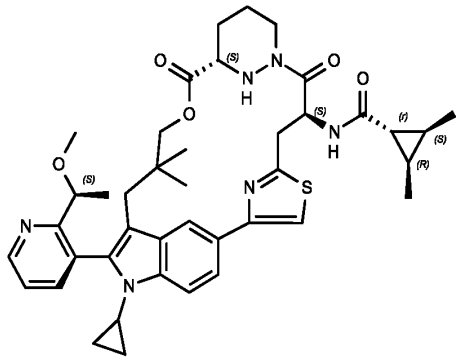
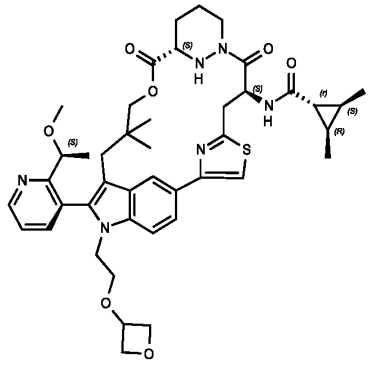
A540	
A541	
A542	

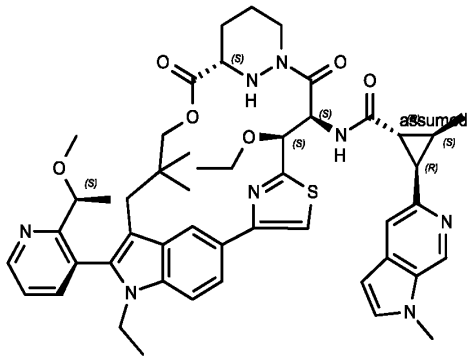
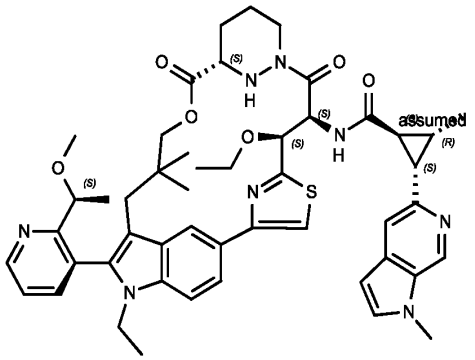
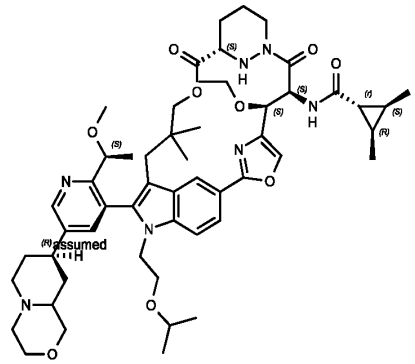
A543	
A544	
A545	

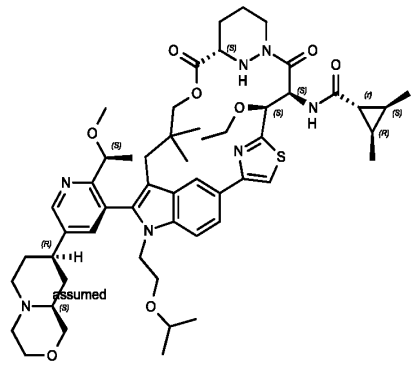
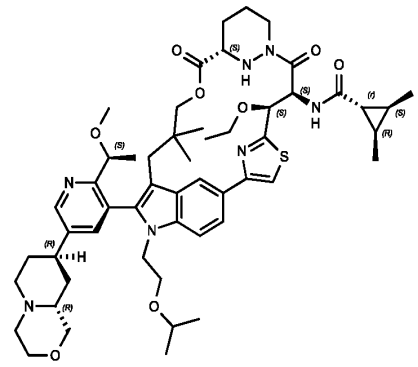
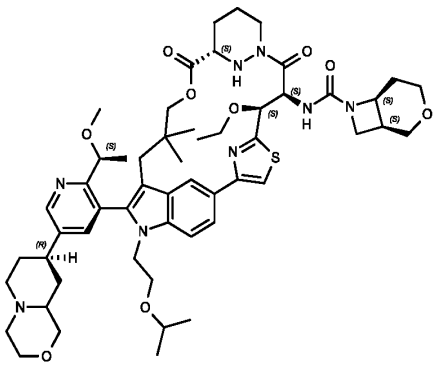
A546	
A547	
A548	

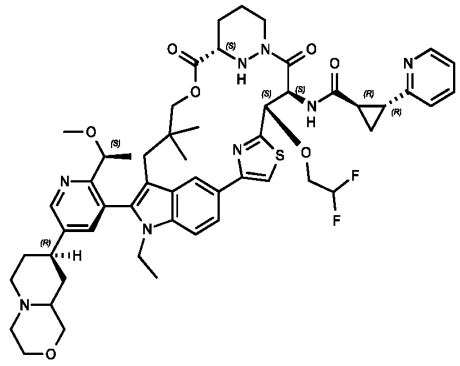
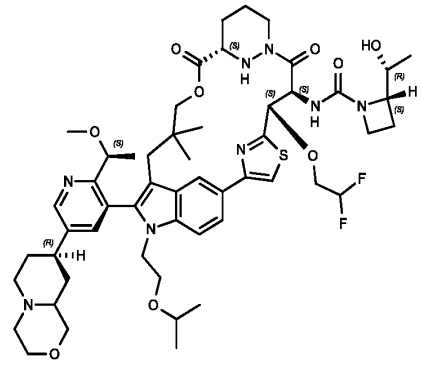
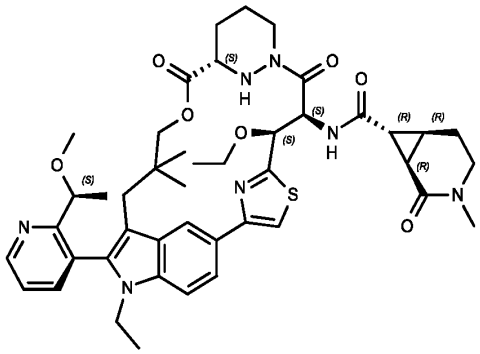
A549	
A550	
A551	

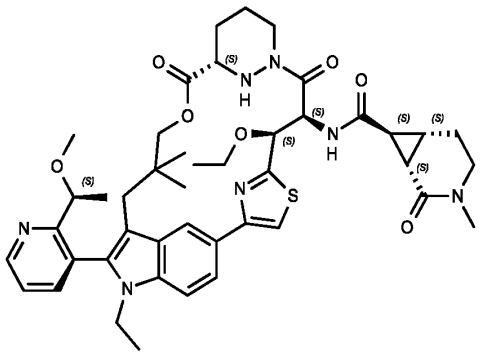
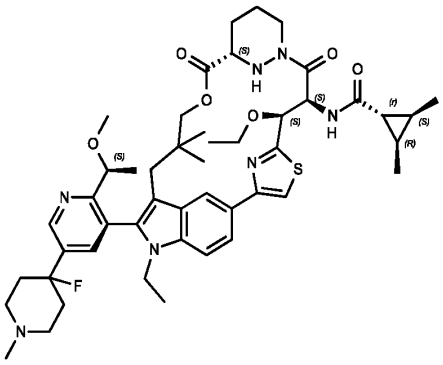
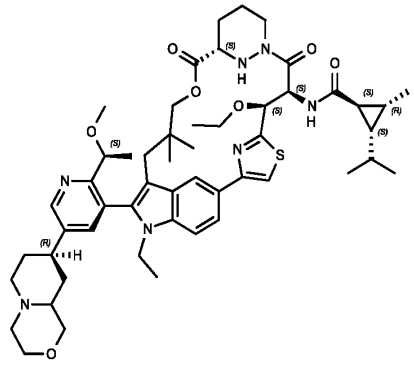
A552	
A553	
A554	

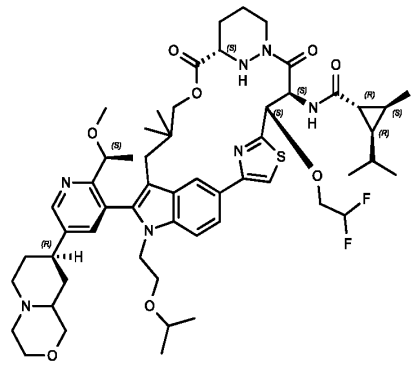
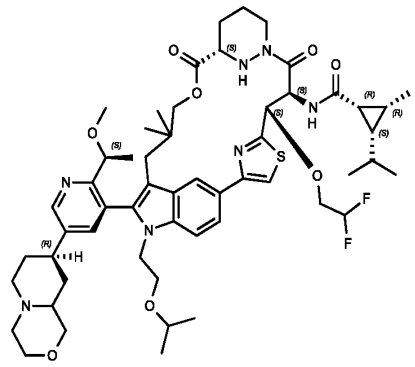
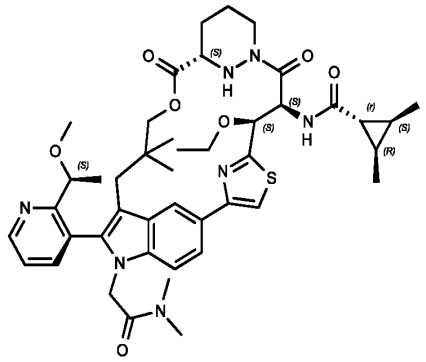
A555	
A556	
A557	

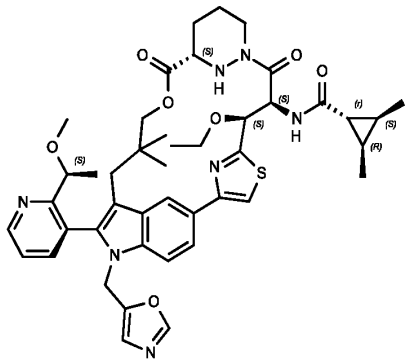
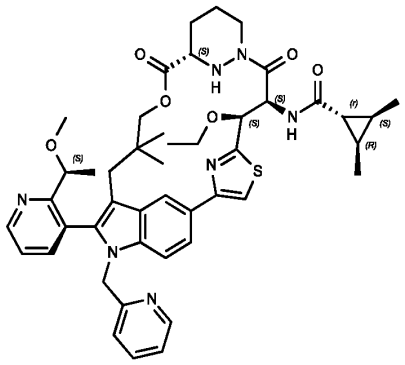
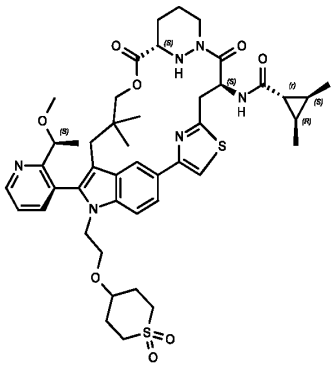
A558	
A559	
A560	

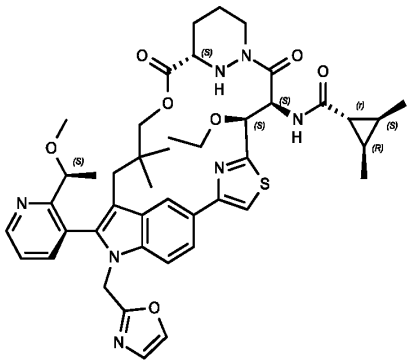
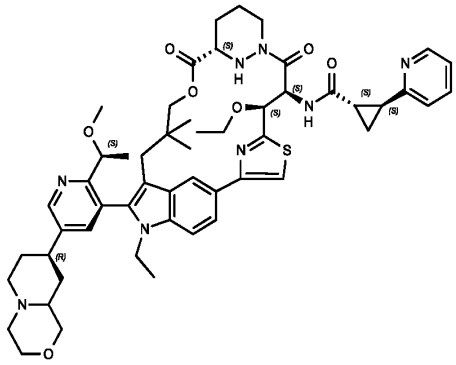
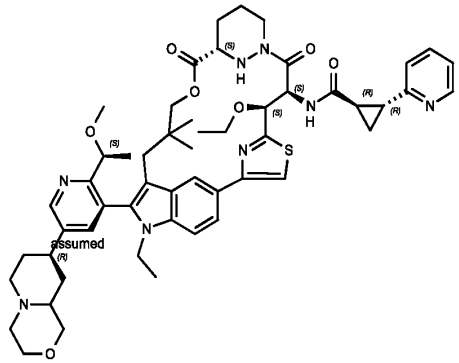
A561	
A562	
A563	

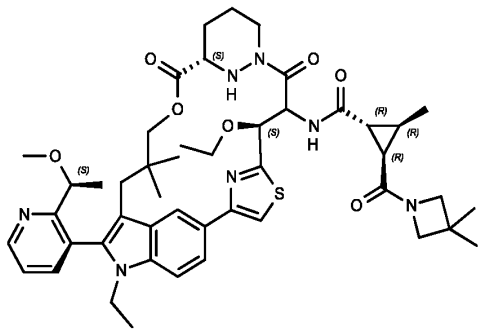
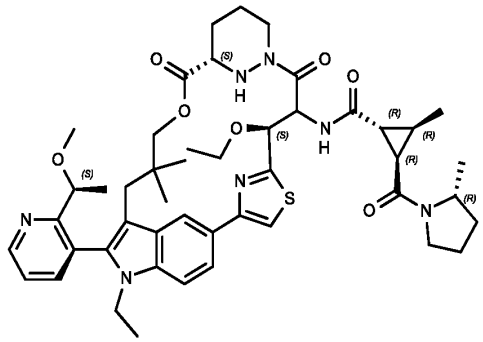
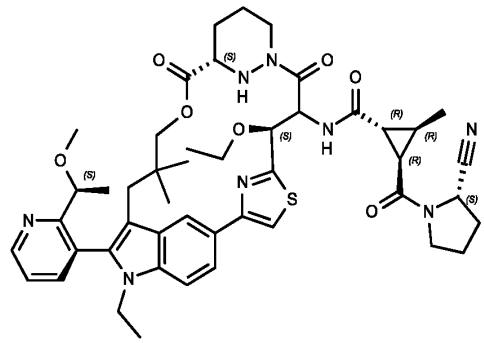
A564	
A565	
A566	

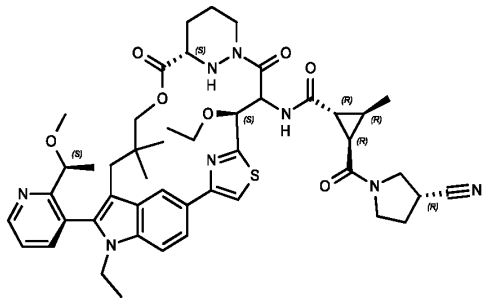
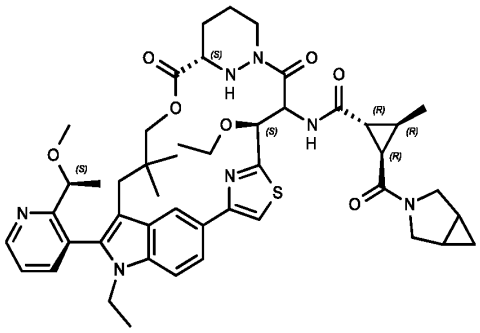
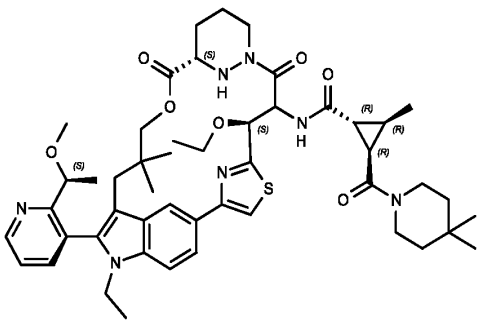
A567	
A568	
A569	

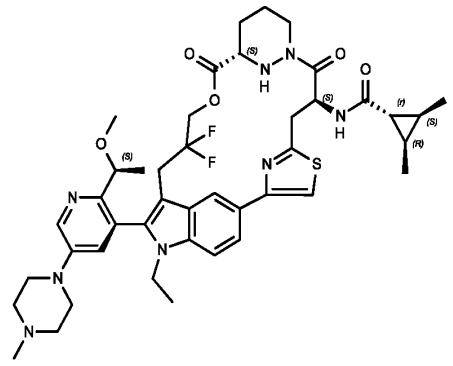
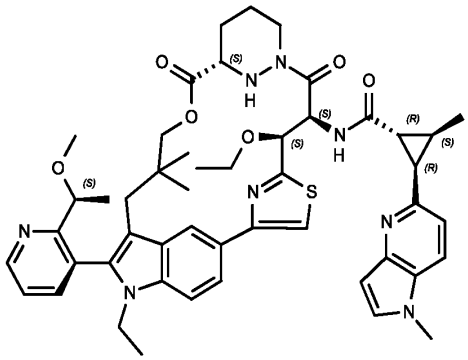
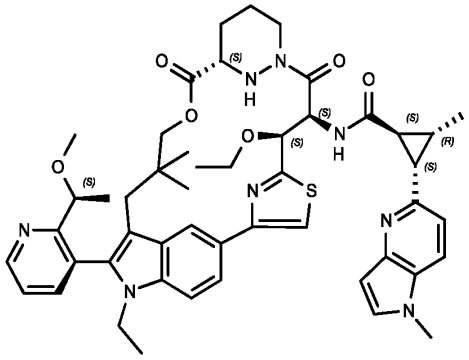
A570	
A571	
A572	

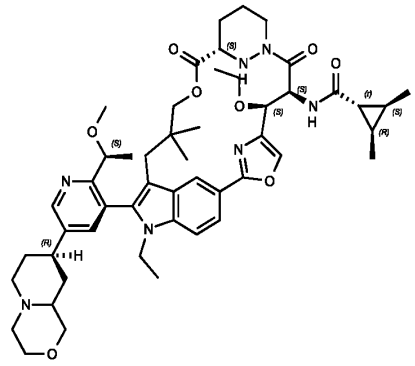
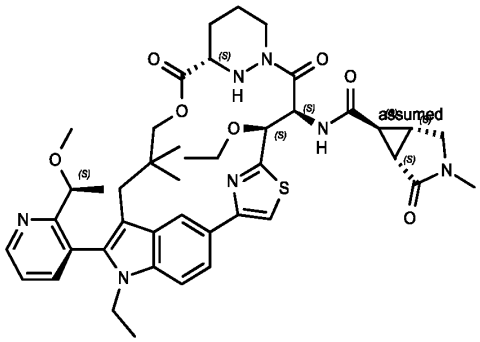
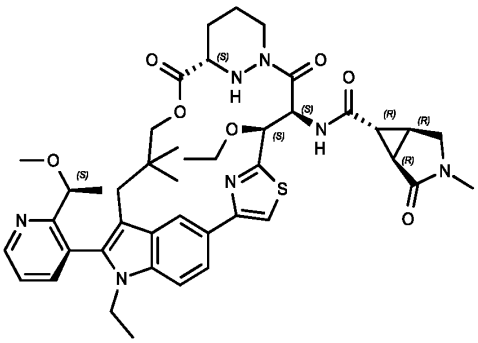
A573	
A574	
A575	

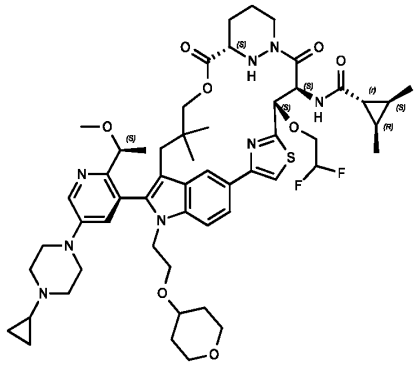
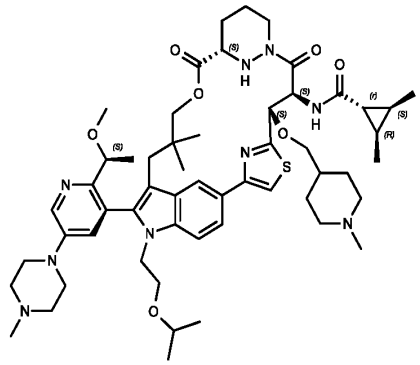
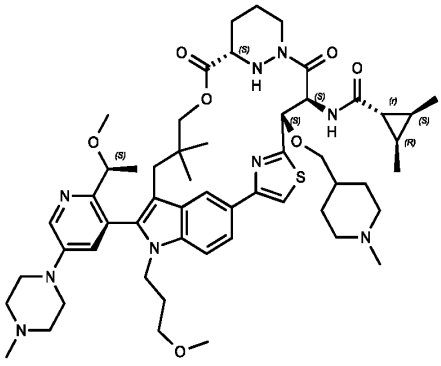
A576	
A577	
A578	

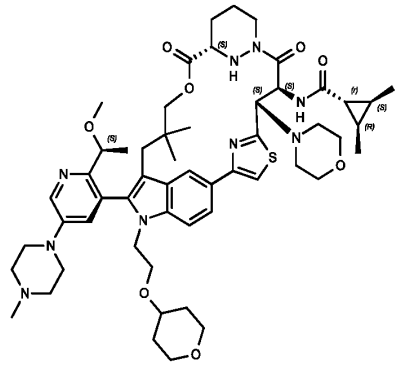
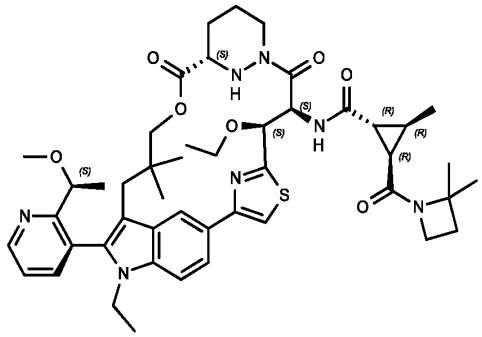
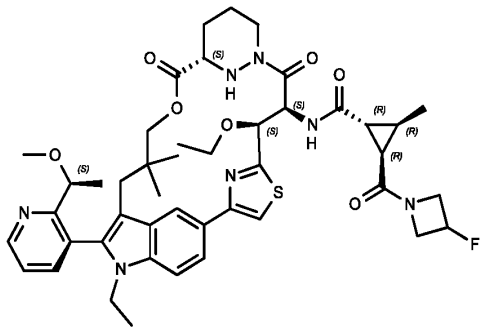
A579	
A580	
A581	

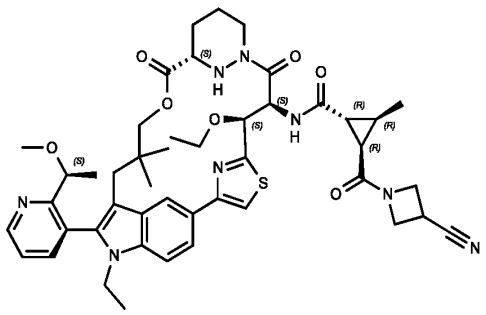
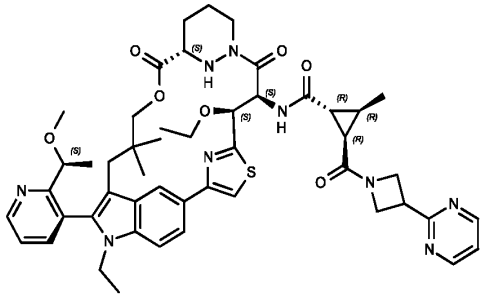
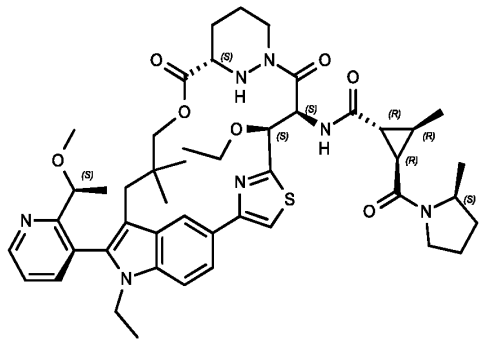
A582	
A583	
A584	

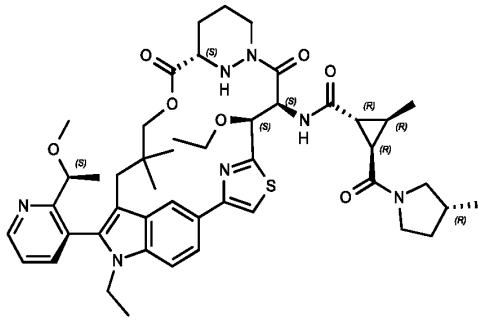
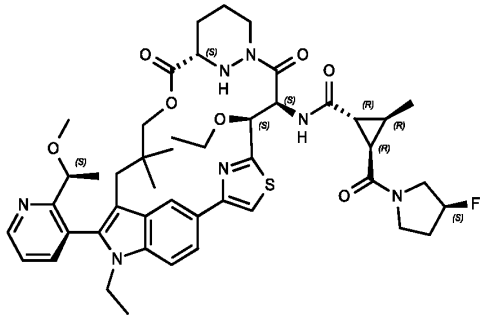
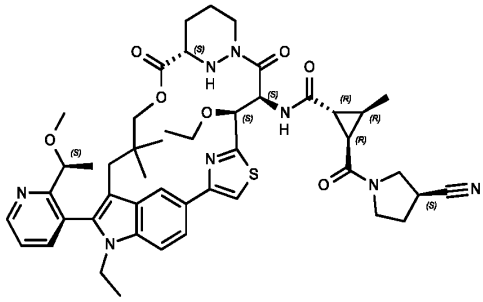
A585	
A586	
A587	

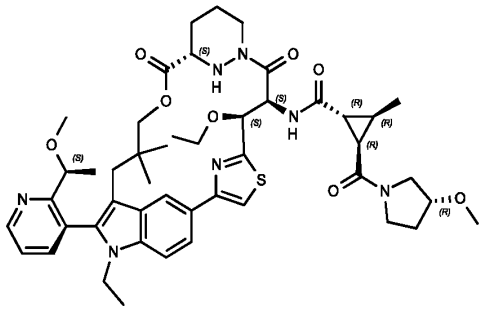
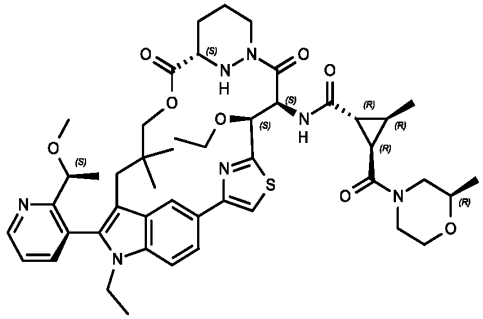
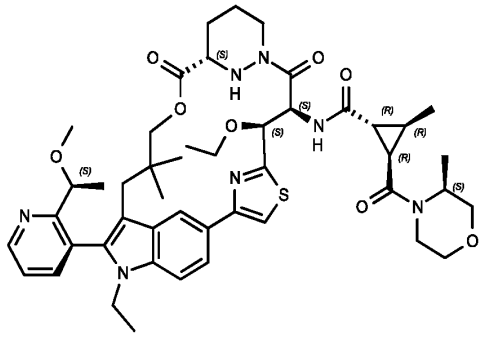
A588	
A589	
A590	

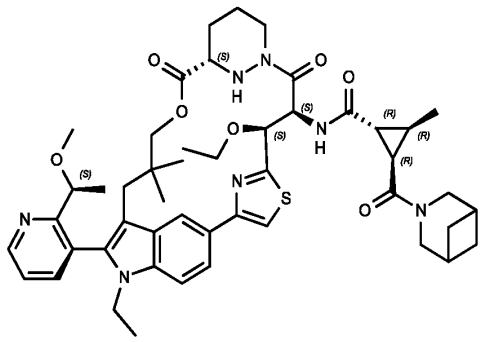
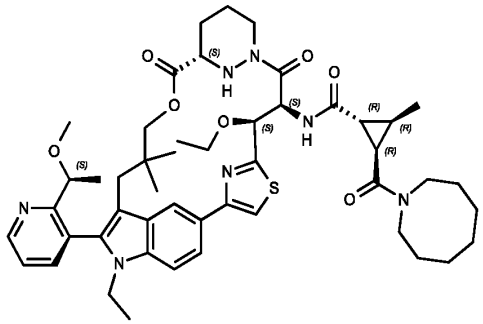
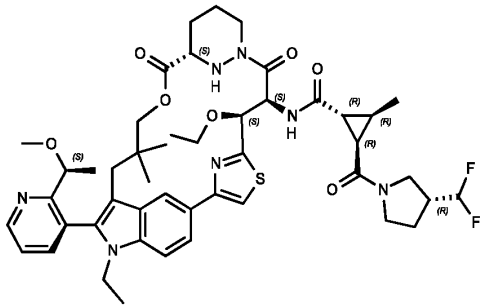
A591	
A592	
A593	

A594	
A595	
A596	

A597	
A598	
A599	

A600	
A601	
A602	

A603	
A604	
A605	

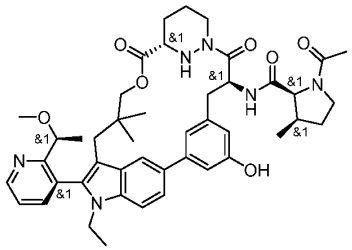
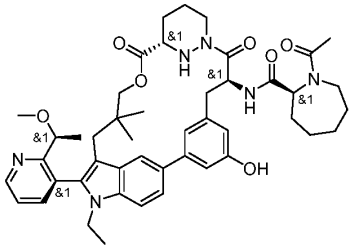
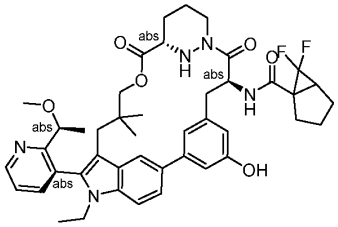
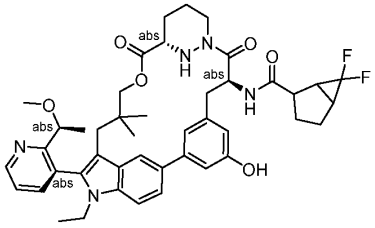
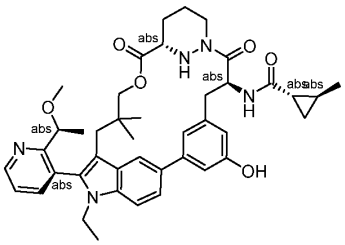
A606	
A607	
A608	

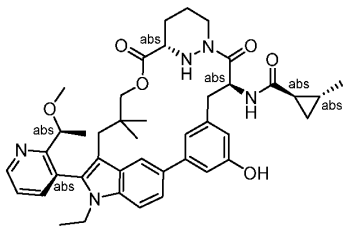
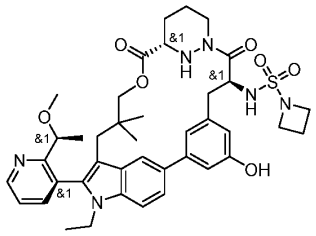
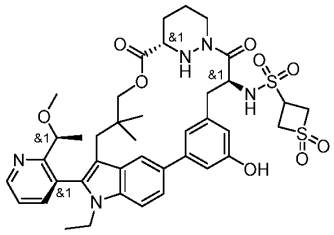
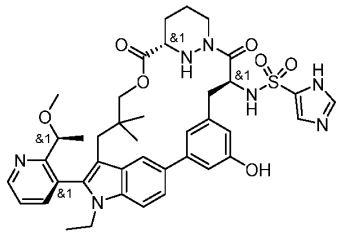
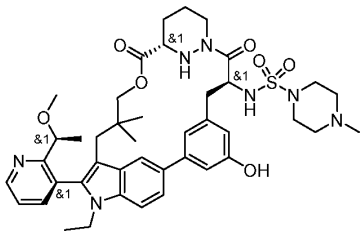
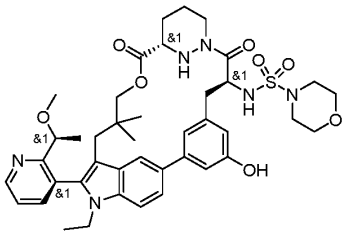
In some embodiments, a compound of the present invention is a compound selected from Table 2, or a pharmaceutically acceptable salt or stereoisomer thereof. In some embodiments, a compound of the present invention is a compound selected from Table 2, or a pharmaceutically acceptable salt or atropisomer thereof

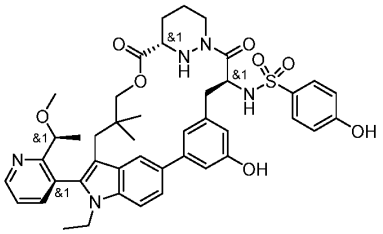
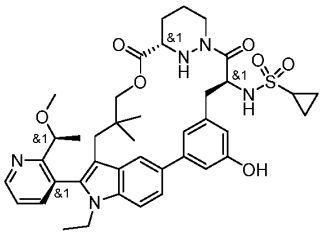
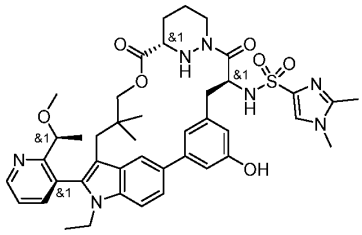
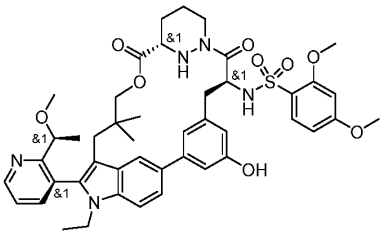
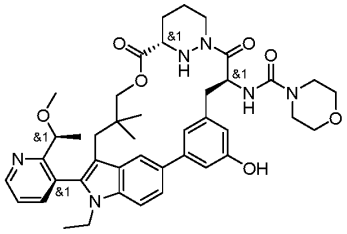
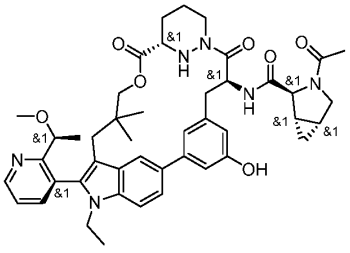
In some embodiments, a compound of the present invention is not a compound selected from Table 2. In some embodiments, a compound of the present invention is not a compound selected from Table 2, or a pharmaceutically acceptable salt or stereoisomer thereof. In some

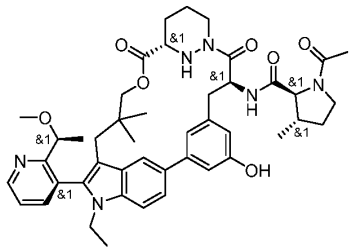
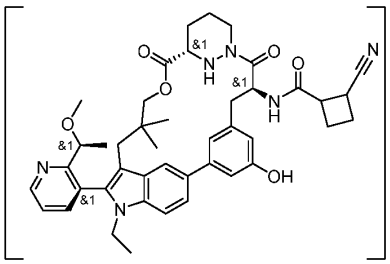
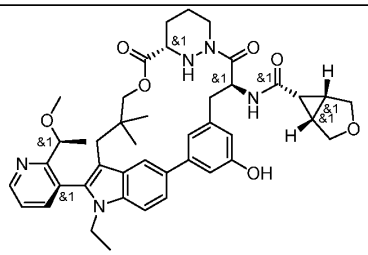
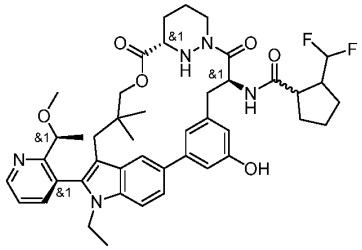
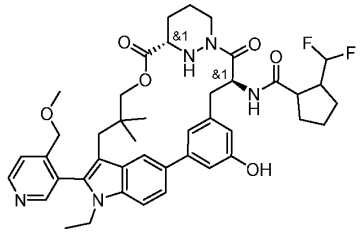
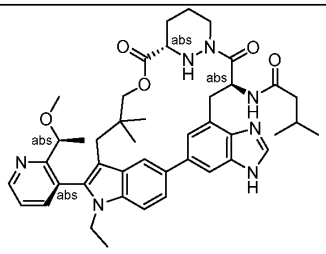
embodiments, a compound of the present invention is not a compound selected from Table 2, or a pharmaceutically acceptable salt or atropisomer thereof.

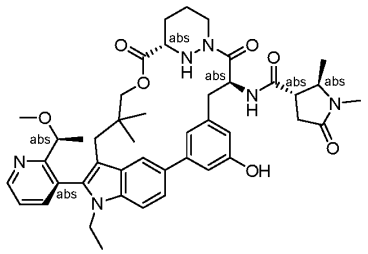
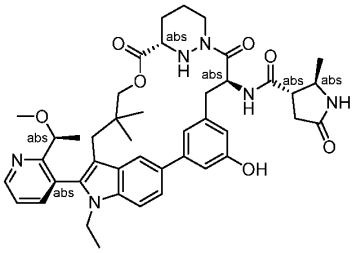
Table 2: Certain Compounds

Ex. #	Structure
B1	
B2	
B3	
B4	
B5	

B6	
B7	
B8	
B9	
B10	
B11	

B12	
B13	
B14	
B15	
B16	
B17	

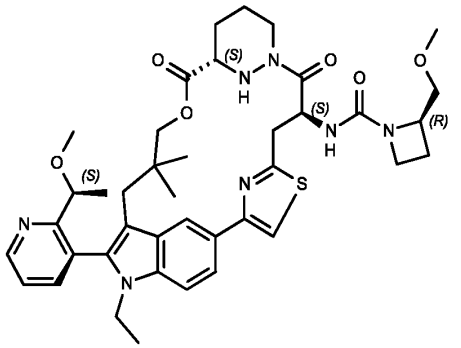
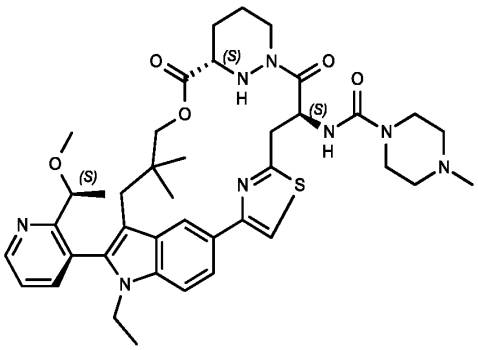
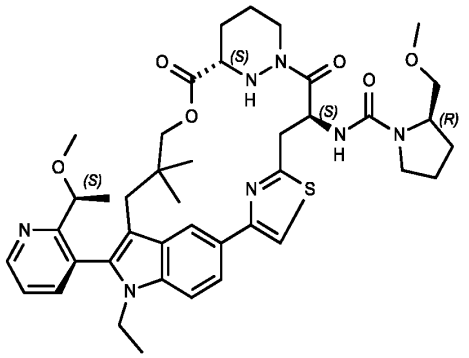
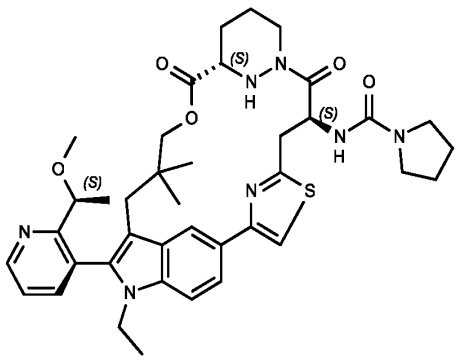
B18	
B19	
B20	
B21	
B22	
B23	

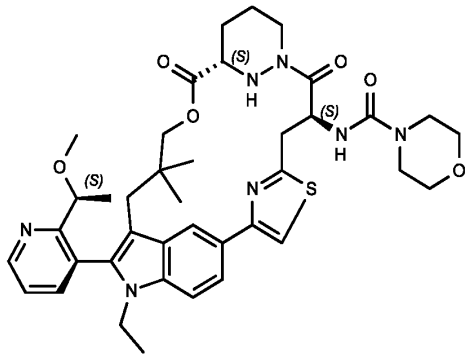
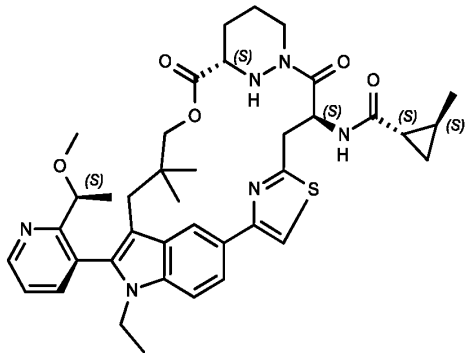
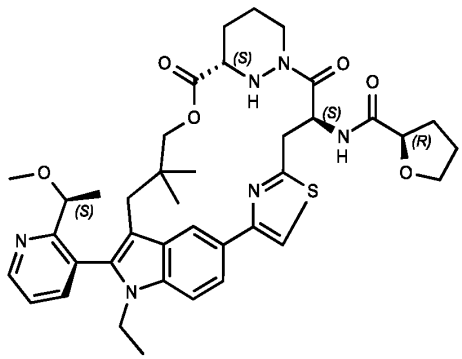
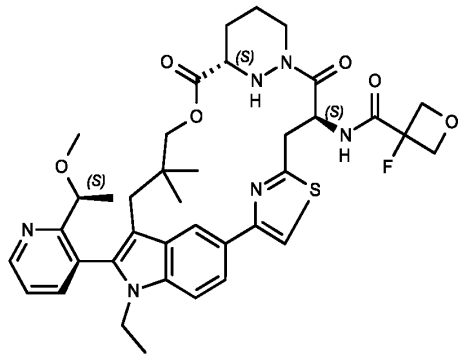
B24	
B25	

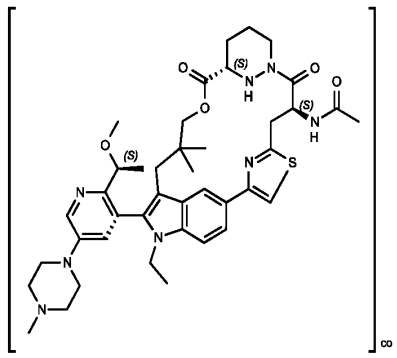
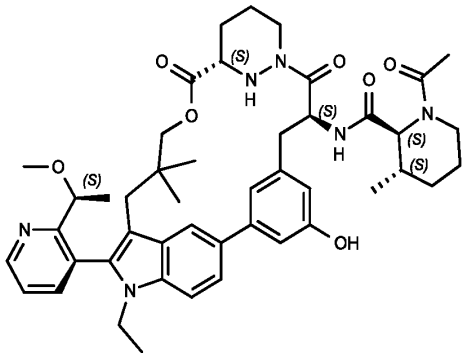
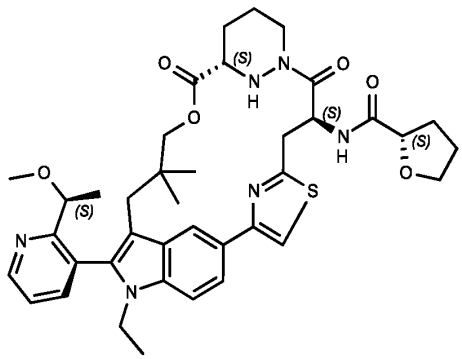
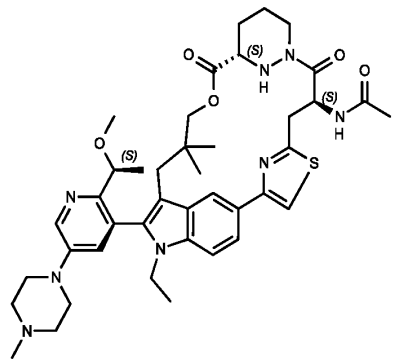
In some embodiments, a compound of the present invention is a compound selected from Table 3 (e.g., C1-C20 or C1-C21), or a pharmaceutically acceptable salt or stereoisomer thereof. In some embodiments, a compound of the present invention is a compound selected from Table 3 (e.g., C1-C20 or C1-C21), or a pharmaceutically acceptable salt or atropisomer thereof.

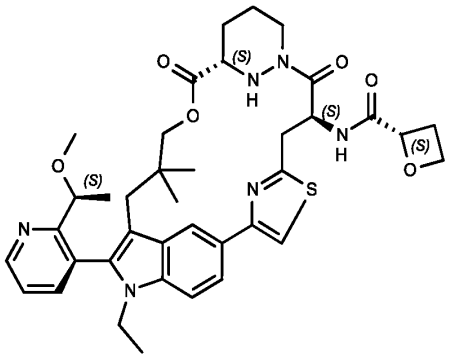
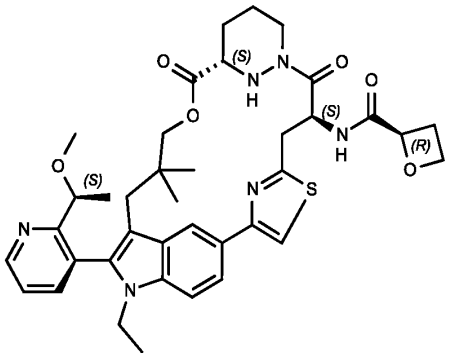
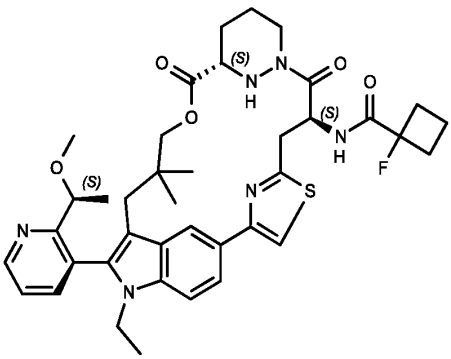
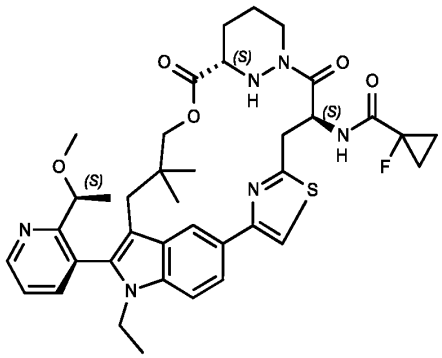
In some embodiments, a compound of the present invention is not a compound selected from Table 3 (e.g., C1-C20 or C1-C21). In some embodiments, a compound of the present invention is not a compound selected from Table 3 (e.g., C1-C20 or C1-C21), or a pharmaceutically acceptable salt or stereoisomer thereof. In some embodiments, a compound of the present invention is not a compound selected from Table 3 (e.g., C1-C20 or C1-C21), or a pharmaceutically acceptable salt or atropisomer thereof.

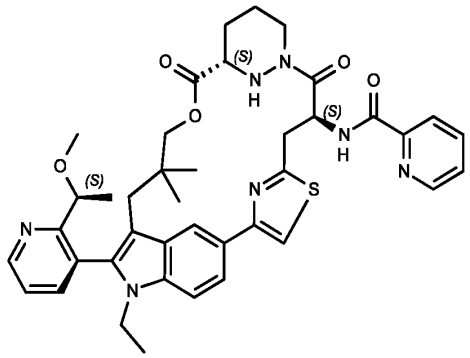
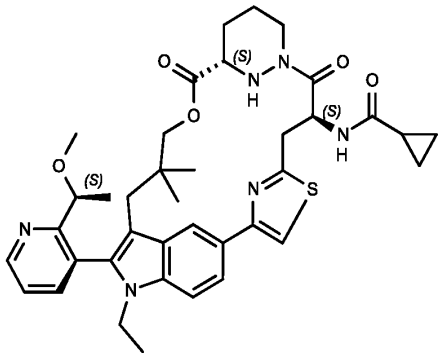
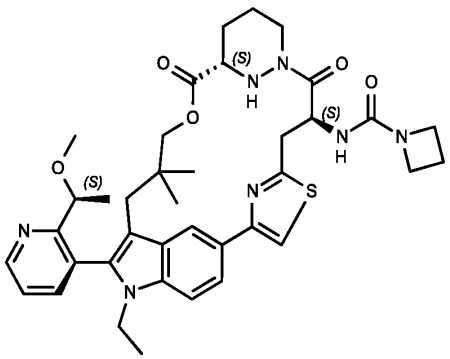
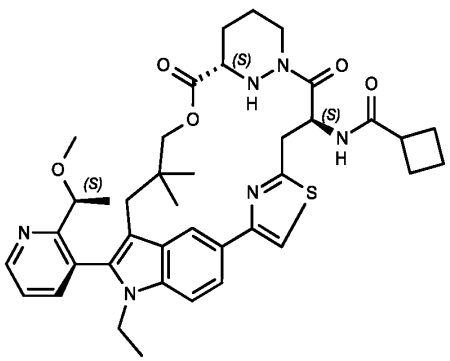
Table 3: Certain Compounds

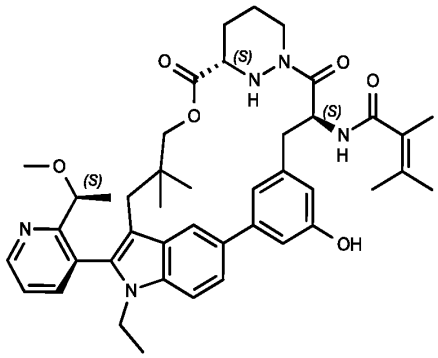
Ex. #	Structure
C1	
C2	
C3	
C4	

C5	
C6	
C7	
C8	

C9	
C10	
C11	
C12	

C13	
C14	
C15	
C16	

C17	
C18	
C19	
C20	

C21	

In some embodiments, a compound of the present invention has improved oral bioavailability compared to what is known in the art. Methods of measuring oral bioavailability are known in the art, and one such method is provided in the Examples below.

In some embodiments, a compound of the present invention is or acts as a prodrug, such as with respect to administration to a cell or to a subject in need thereof.

Also provided are pharmaceutical compositions comprising a compound of the present invention, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

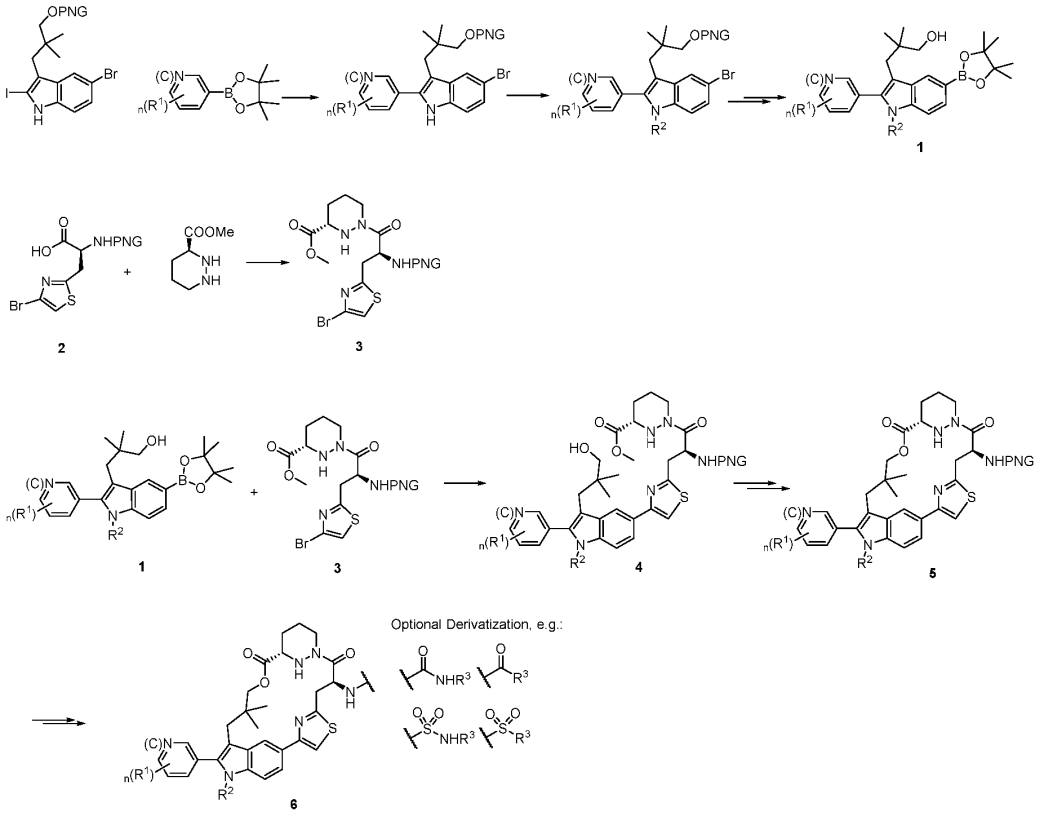
With respect to compounds of the present invention, one stereoisomer may exhibit better inhibition than another stereoisomer. For example, one atropisomer may exhibit inhibition, whereas the other atropisomer may exhibit little or no inhibition.

Methods of Synthesis

The compounds described herein may be made from commercially available starting materials or synthesized using known organic, inorganic, or enzymatic processes.

The compounds of the present invention can be prepared in a number of ways well known to those skilled in the art of organic synthesis. By way of example, compounds of the present invention can be synthesized using the methods described in the Schemes below, together with synthetic methods known in the art of synthetic organic chemistry, or variations thereon as appreciated by those skilled in the art. These methods include but are not limited to those methods described in the Schemes below.

Scheme 1. General synthesis of macrocyclic esters



A general synthesis of macrocyclic esters is outlined in Scheme 1. An appropriately substituted indolyl boronic ester (**1**) can be prepared in four steps starting from protected 3-(5-bromo-2-iodo-1H-indol-3-yl)-2,2-dimethylpropan-1-ol and appropriately substituted boronic acid, including palladium mediated coupling, alkylation, de-protection, and palladium mediated borylation reactions.

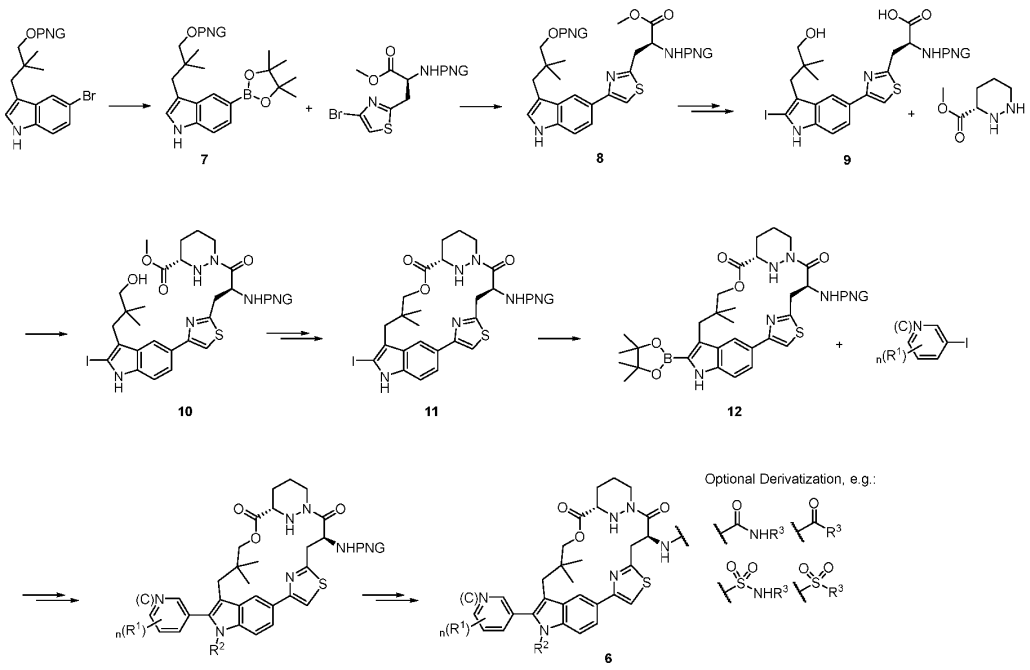
Methyl-amino-3-(4-bromothiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (**3**) can be prepared via coupling of (S)-2-amino-3-(4-bromothiazol-2-yl)propanoic acid (**2**) with methyl (*S*)-hexahydropyridazine-3-carboxylate.

The final macrocyclic esters can be made by coupling of methyl-amino-3-(4-bromothiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (**3**) and an appropriately substituted indolyl boronic ester (**1**) in the presence of Pd catalyst followed by hydrolysis and macrolactonization steps to result in an appropriately protected macrocyclic intermediate (**5**). Deprotection and coupling with an appropriately substituted carboxylic acid (or other coupling partner) can result in a macrocyclic product. Additional deprotection or functionalization steps could be required to produce a final compound **6**.

Further, with respect to Scheme 1, the thiazole may be replaced with an alternative optionally substituted 5 to 6-membered heteroarylene, or an optionally substituted 3 to 6-membered

cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene (e.g., morpholino), or optionally substituted 6-membered arylene (e.g., phenyl).

Scheme 2. Alternative general synthesis of macrocyclic esters



Alternatively, macrocyclic esters can be prepared as described in Scheme 2. An appropriately substituted and protected indolyl boronic ester (**7**) can be coupled in the presence of Pd catalyst with (S)-2-amino-3-(4-bromothiazol-2-yl)propanoic acid, followed by iodination, deprotection, and ester hydrolysis. Subsequent coupling with methyl (*S*)-hexahydropyridazine-3-carboxylate, followed by hydrolysis and macrolactonization can result in iodo intermediate (**11**). Subsequent palladium mediated borylation and coupling in the presence of Pd catalyst with an appropriately substituted iodo aryl or iodo heteroaryl intermediate can yield an appropriately protected macrocyclic intermediate. Alkylation, deprotection and coupling with an appropriately substituted carboxylic acid carboxylic acid (or other coupling partner) results in a macrocyclic product. Additional deprotection or functionalization steps could be required to produce a final compound **6**.

Further, with respect to Scheme 2, the thiazole may be replaced with an alternative optionally substituted 5 to 6-membered heteroarylene, or an optionally substituted 3 to 6-membered

cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene (e.g., morpholino), or optionally substituted 6-membered arylene (e.g., phenyl).

Compounds of Table 1 and Table 1-1 herein were prepared using methods disclosed herein or were prepared using methods described herein combined with the knowledge of one of skill in the art.

Pharmaceutical Compositions and Methods of Use

Pharmaceutical Compositions and Methods of Administration

The compounds with which the invention is concerned are Ras inhibitors, and are useful in the treatment of cancer. Accordingly, one embodiment of the present invention provides pharmaceutical compositions containing a compound of the invention or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient, as well as methods of using the compounds of the invention to prepare such compositions.

As used herein, the term “pharmaceutical composition” refers to a compound, such as a compound of the present invention, or a pharmaceutically acceptable salt thereof, formulated together with a pharmaceutically acceptable excipient.

In some embodiments, a compound is present in a pharmaceutical composition in unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant population. In some embodiments, pharmaceutical compositions may be specially formulated for administration in solid or liquid form, including those adapted for the following: oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, e.g., those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin, lungs, or oral cavity; intravaginally or intrarectally, for example, as a pessary, cream, or foam; sublingually; ocularly; transdermally; or nasally, pulmonary, and to other mucosal surfaces.

A “pharmaceutically acceptable excipient,” as used herein, refers any inactive ingredient (for example, a vehicle capable of suspending or dissolving the active compound) having the properties of being nontoxic and non-inflammatory in a subject. Typical excipients include, for example: antiadherents, antioxidants, binders, coatings, compression aids, disintegrants, dyes (colors), emollients, emulsifiers, fillers (diluents), film formers or coatings, flavors, fragrances, glidants (flow enhancers), lubricants, preservatives, printing inks, sorbents, suspensing or dispersing agents, sweeteners, or waters of hydration. Excipients include, but are not limited to: butylated optionally substituted hydroxyltoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, crosslinked polyvinyl pyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, optionally substituted hydroxylpropyl cellulose, optionally substituted hydroxylpropyl methylcellulose, lactose, magnesium stearate, maltitol,

mannitol, methionine, methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol, polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac, silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E, vitamin C, and xylitol. Those of ordinary skill in the art are familiar with a variety of agents and materials useful as excipients. See, e.g., e.g., Ansel, et al., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems. Philadelphia: Lippincott, Williams & Wilkins, 2004; Gennaro, et al., Remington: The Science and Practice of Pharmacy. Philadelphia: Lippincott, Williams & Wilkins, 2000; and Rowe, Handbook of Pharmaceutical Excipients. Chicago, Pharmaceutical Press, 2005. In some embodiments, a composition includes at least two different pharmaceutically acceptable excipients.

Compounds described herein, whether expressly stated or not, may be provided or utilized in salt form, e.g., a pharmaceutically acceptable salt form, unless expressly stated to the contrary. The term “pharmaceutically acceptable salt,” as use herein, refers to those salts of the compounds described herein that are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and other animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, pharmaceutically acceptable salts are described in: Berge et al., *J. Pharmaceutical Sciences* 66:1-19, 1977 and in *Pharmaceutical Salts: Properties, Selection, and Use*, (Eds. P.H. Stahl and C.G. Wermuth), Wiley-VCH, 2008. The salts can be prepared in situ during the final isolation and purification of the compounds described herein or separately by reacting the free base group with a suitable organic acid.

The compounds of the invention may have ionizable groups so as to be capable of preparation as pharmaceutically acceptable salts. These salts may be acid addition salts involving inorganic or organic acids or the salts may, in the case of acidic forms of the compounds of the invention, be prepared from inorganic or organic bases. In some embodiments, the compounds are prepared or used as pharmaceutically acceptable salts prepared as addition products of pharmaceutically acceptable acids or bases. Suitable pharmaceutically acceptable acids and bases are well-known in the art, such as hydrochloric, sulfuric, hydrobromic, acetic, lactic, citric, or tartaric acids for forming acid addition salts, and potassium hydroxide, sodium hydroxide, ammonium hydroxide, caffeine, various amines, and the like for forming basic salts. Methods for preparation of the appropriate salts are well-established in the art.

Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-optionally substituted hydroxyl-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate,

valerate salts and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine and the like.

As used herein, the term "subject" refers to any member of the animal kingdom. In some embodiments, "subject" refers to humans, at any stage of development. In some embodiments, "subject" refers to a human patient. In some embodiments, "subject" refers to non-human animals. In some embodiments, the non-human animal is a mammal (e.g., a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, or a pig). In some embodiments, subjects include, but are not limited to, mammals, birds, reptiles, amphibians, fish, or worms. In some embodiments, a subject may be a transgenic animal, genetically-engineered animal, or a clone.

As used herein, the term "dosage form" refers to a physically discrete unit of a compound (e.g., a compound of the present invention) for administration to a subject. Each unit contains a predetermined quantity of compound. In some embodiments, such quantity is a unit dosage amount (or a whole fraction thereof) appropriate for administration in accordance with a dosing regimen that has been determined to correlate with a desired or beneficial outcome when administered to a relevant population (i.e., with a therapeutic dosing regimen). Those of ordinary skill in the art appreciate that the total amount of a therapeutic composition or compound administered to a particular subject is determined by one or more attending physicians and may involve administration of multiple dosage forms.

As used herein, the term "dosing regimen" refers to a set of unit doses (typically more than one) that are administered individually to a subject, typically separated by periods of time. In some embodiments, a given therapeutic compound (e.g., a compound of the present invention) has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which are separated from one another by a time period of the same length; in some embodiments, a dosing regimen comprises a plurality of doses and at least two different time periods separating individual doses. In some embodiments, all doses within a dosing regimen are of the same unit dose amount. In some embodiments, different doses within a dosing regimen are of different amounts. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount different from the first dose amount. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount same as the first dose amount. In some embodiments, a dosing regimen is correlated with a desired or beneficial outcome when administered across a relevant population (i.e., is a therapeutic dosing regimen).

A "therapeutic regimen" refers to a dosing regimen whose administration across a relevant population is correlated with a desired or beneficial therapeutic outcome.

The term "treatment" (also "treat" or "treating"), in its broadest sense, refers to any administration of a substance (e.g., a compound of the present invention) that partially or

completely alleviates, ameliorates, relieves, inhibits, delays onset of, reduces severity of, or reduces incidence of one or more symptoms, features, or causes of a particular disease, disorder, or condition. In some embodiments, such treatment may be administered to a subject who does not exhibit signs of the relevant disease, disorder or condition or of a subject who exhibits only early signs of the disease, disorder, or condition. Alternatively, or additionally, in some embodiments, treatment may be administered to a subject who exhibits one or more established signs of the relevant disease, disorder or condition. In some embodiments, treatment may be of a subject who has been diagnosed as suffering from the relevant disease, disorder, or condition. In some embodiments, treatment may be of a subject known to have one or more susceptibility factors that are statistically correlated with increased risk of development of the relevant disease, disorder, or condition.

The term “therapeutically effective amount” means an amount that is sufficient, when administered to a population suffering from or susceptible to a disease, disorder, or condition in accordance with a therapeutic dosing regimen, to treat the disease, disorder, or condition. In some embodiments, a therapeutically effective amount is one that reduces the incidence or severity of, or delays onset of, one or more symptoms of the disease, disorder, or condition. Those of ordinary skill in the art will appreciate that the term “therapeutically effective amount” does not in fact require successful treatment be achieved in a particular individual. Rather, a therapeutically effective amount may be that amount that provides a particular desired pharmacological response in a significant number of subjects when administered to patients in need of such treatment. It is specifically understood that particular subjects may, in fact, be “refractory” to a “therapeutically effective amount.” In some embodiments, reference to a therapeutically effective amount may be a reference to an amount as measured in one or more specific tissues (e.g., a tissue affected by the disease, disorder or condition) or fluids (e.g., blood, saliva, serum, sweat, tears, urine). Those of ordinary skill in the art will appreciate that, in some embodiments, a therapeutically effective amount may be formulated or administered in a single dose. In some embodiments, a therapeutically effective amount may be formulated or administered in a plurality of doses, for example, as part of a dosing regimen.

For use as treatment of subjects, the compounds of the invention, or a pharmaceutically acceptable salt thereof, can be formulated as pharmaceutical or veterinary compositions. Depending on the subject to be treated, the mode of administration, and the type of treatment desired, e.g., prevention, prophylaxis, or therapy, the compounds, or a pharmaceutically acceptable salt thereof, are formulated in ways consonant with these parameters. A summary of such techniques may be found in *Remington: The Science and Practice of Pharmacy*, *21st Edition*, Lippincott Williams & Wilkins, (2005); and *Encyclopedia of Pharmaceutical Technology*, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York, each of which is incorporated herein by reference.

Compositions can be prepared according to conventional mixing, granulating or coating methods, respectively, and the present pharmaceutical compositions can contain from about 0.1% to about 99%, from about 5% to about 90%, or from about 1% to about 20% of a compound of the

present invention, or pharmaceutically acceptable salt thereof, by weight or volume. In some embodiments, compounds, or a pharmaceutically acceptable salt thereof, described herein may be present in amounts totaling 1-95% by weight of the total weight of a composition, such as a pharmaceutical composition.

The composition may be provided in a dosage form that is suitable for intraarticular, oral, parenteral (e.g., intravenous, intramuscular), rectal, cutaneous, subcutaneous, topical, transdermal, sublingual, nasal, vaginal, intravesicular, intraurethral, intrathecal, epidural, aural, or ocular administration, or by injection, inhalation, or direct contact with the nasal, genitourinary, reproductive or oral mucosa. Thus, the pharmaceutical composition may be in the form of, e.g., tablets, capsules, pills, powders, granulates, suspensions, emulsions, solutions, gels including hydrogels, pastes, ointments, creams, plasters, drenches, osmotic delivery devices, suppositories, enemas, injectables, implants, sprays, preparations suitable for iontophoretic delivery, or aerosols. The compositions may be formulated according to conventional pharmaceutical practice.

As used herein, the term “administration” refers to the administration of a composition (e.g., a compound, or a preparation that includes a compound as described herein) to a subject or system. Administration to an animal subject (e.g., to a human) may be by any appropriate route. For example, in some embodiments, administration may be bronchial (including by bronchial instillation), buccal, enteral, interdermal, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intravenous, intraventricular, mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (including by intratracheal instillation), transdermal, vaginal or vitreal.

Formulations may be prepared in a manner suitable for systemic administration or topical or local administration. Systemic formulations include those designed for injection (e.g., intramuscular, intravenous or subcutaneous injection) or may be prepared for transdermal, transmucosal, or oral administration. A formulation will generally include a diluent as well as, in some cases, adjuvants, buffers, preservatives and the like. Compounds, or a pharmaceutically acceptable salt thereof, can be administered also in liposomal compositions or as microemulsions.

For injection, formulations can be prepared in conventional forms as liquid solutions or suspensions or as solid forms suitable for solution or suspension in liquid prior to injection or as emulsions. Suitable excipients include, for example, water, saline, dextrose, glycerol and the like. Such compositions may also contain amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, such as, for example, sodium acetate, sorbitan monolaurate, and so forth.

Various sustained release systems for drugs have also been devised. See, for example, U.S. Patent No. 5,624,677.

Systemic administration may also include relatively noninvasive methods such as the use of suppositories, transdermal patches, transmucosal delivery and intranasal administration. Oral administration is also suitable for compounds of the invention, or a pharmaceutically acceptable salt thereof. Suitable forms include syrups, capsules, and tablets, as is understood in the art.

Each compound, or a pharmaceutically acceptable salt thereof, as described herein, may be formulated in a variety of ways that are known in the art. For example, the first and second agents of the combination therapy may be formulated together or separately. Other modalities of combination therapy are described herein.

The individually or separately formulated agents can be packaged together as a kit. Non-limiting examples include, but are not limited to, kits that contain, e.g., two pills, a pill and a powder, a suppository and a liquid in a vial, two topical creams, etc. The kit can include optional components that aid in the administration of the unit dose to subjects, such as vials for reconstituting powder forms, syringes for injection, customized IV delivery systems, inhalers, etc. Additionally, the unit dose kit can contain instructions for preparation and administration of the compositions. The kit may be manufactured as a single use unit dose for one subject, multiple uses for a particular subject (at a constant dose or in which the individual compounds, or a pharmaceutically acceptable salt thereof, may vary in potency as therapy progresses); or the kit may contain multiple doses suitable for administration to multiple subjects ("bulk packaging"). The kit components may be assembled in cartons, blister packs, bottles, tubes, and the like.

Formulations for oral use include tablets containing the active ingredient(s) in a mixture with non-toxic pharmaceutically acceptable excipients. These excipients may be, for example, inert diluents or fillers (e.g., sucrose, sorbitol, sugar, mannitol, microcrystalline cellulose, starches including potato starch, calcium carbonate, sodium chloride, lactose, calcium phosphate, calcium sulfate, or sodium phosphate); granulating and disintegrating agents (e.g., cellulose derivatives including microcrystalline cellulose, starches including potato starch, croscarmellose sodium, alginates, or alginic acid); binding agents (e.g., sucrose, glucose, sorbitol, acacia, alginic acid, sodium alginate, gelatin, starch, pregelatinized starch, microcrystalline cellulose, magnesium aluminum silicate, carboxymethylcellulose sodium, methylcellulose, optionally substituted hydroxylpropyl methylcellulose, ethylcellulose, polyvinylpyrrolidone, or polyethylene glycol); and lubricating agents, glidants, and antiadhesives (e.g., magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc). Other pharmaceutically acceptable excipients can be colorants, flavoring agents, plasticizers, humectants, buffering agents, and the like.

Two or more compounds may be mixed together in a tablet, capsule, or other vehicle, or may be partitioned. In one example, the first compound is contained on the inside of the tablet, and the second compound is on the outside, such that a substantial portion of the second compound is released prior to the release of the first compound.

Formulations for oral use may also be provided as chewable tablets, or as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent (e.g., potato starch, lactose, microcrystalline cellulose, calcium carbonate, calcium phosphate or kaolin), or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil. Powders, granulates, and pellets may be prepared using the ingredients mentioned above under tablets and capsules in a conventional manner using, e.g., a mixer, a fluid bed apparatus or a spray drying equipment.

Dissolution or diffusion-controlled release can be achieved by appropriate coating of a tablet, capsule, pellet, or granulate formulation of compounds, or by incorporating the compound, or a pharmaceutically acceptable salt thereof, into an appropriate matrix. A controlled release coating may include one or more of the coating substances mentioned above or, e.g., shellac, beeswax, glycowax, castor wax, carnauba wax, stearyl alcohol, glyceryl monostearate, glyceryl distearate, glycerol palmitostearate, ethylcellulose, acrylic resins, dl-polylactic acid, cellulose acetate butyrate, polyvinyl chloride, polyvinyl acetate, vinyl pyrrolidone, polyethylene, polymethacrylate, methylmethacrylate, 2-optionally substituted hydroxylmethacrylate, methacrylate hydrogels, 1,3 butylene glycol, ethylene glycol methacrylate, or polyethylene glycols. In a controlled release matrix formulation, the matrix material may also include, e.g., hydrated methylcellulose, carnauba wax and stearyl alcohol, carbopol 934, silicone, glyceryl tristearate, methyl acrylate-methyl methacrylate, polyvinyl chloride, polyethylene, or halogenated fluorocarbon.

The liquid forms in which the compounds, or a pharmaceutically acceptable salt thereof, and compositions of the present invention can be incorporated for administration orally include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Generally, when administered to a human, the oral dosage of any of the compounds of the invention, or a pharmaceutically acceptable salt thereof, will depend on the nature of the compound, and can readily be determined by one skilled in the art. A dosage may be, for example, about 0.001 mg to about 2000 mg per day, about 1 mg to about 1000 mg per day, about 5 mg to about 500 mg per day, about 100 mg to about 1500 mg per day, about 500 mg to about 1500 mg per day, about 500 mg to about 2000 mg per day, or any range derivable therein.

Examples

The disclosure is further illustrated by the following examples and synthesis examples, which are not to be construed as limiting this disclosure in scope or spirit to the specific procedures herein described. It is to be understood that the examples are provided to illustrate certain embodiments and that no limitation to the scope of the disclosure is intended thereby. It is to be further understood that resort may be had to various other embodiments, modifications, and equivalents thereof which may suggest themselves to those skilled in the art without departing from the spirit of the present disclosure or scope of the appended claims.

Chemical Syntheses

Definitions used in the following examples and elsewhere herein are:

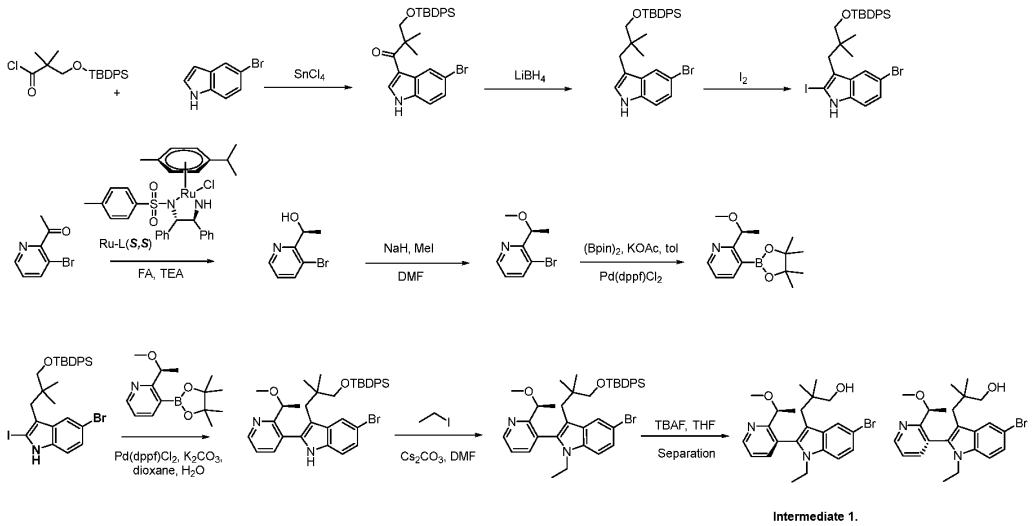
B_2pin_2	Bis(pinacolato)diboron
BINAP	2,2′-Bis(diphenylphosphino)-1,1′-binaphthyl
CH_2Cl_2, DCM	Methylene chloride, Dichloromethane
CH_3CN, MeCN	Acetonitrile
CuI	Copper (I) iodide
DIPEA, DIEA	Diisopropylethyl amine
DMF	N,N-Dimethylformamide
EA	Ethyl acetate
EDCI	N-Ethyl-N'-carbodiimide hydrochloride
EtOAc	Ethyl acetate
h	hour
H_2O	Water
HCl	Hydrochloric acid
HOBt	Hydroxybenzotriazole
K_3PO_4	Potassium phosphate (tribasic)
MeOH	Methanol
Na_2SO_4	Sodium sulfate
NMM	N-methylmorpholine
NMP	N-methyl pyrrolidone
$Pd(dppf)Cl_2$	[1,1′-Bis(diphenylphosphino)ferrocene]dichloropalladium(II)
PE	Petroleum ether
rt	Room temperature
TFA	Trifluoroacetic acid

Instrumentation

Mass spectrometry data collection took place with a Shimadzu LCMS-2020, an Agilent 1260LC-6120/6125MSD, a Shimadzu LCMS-2010EV, or a Waters Acquity UPLC, with either a QDa detector or SQ Detector 2. Samples were injected in their liquid phase onto a C-18 reverse phase. The compounds were eluted from the column using an acetonitrile gradient and fed into the mass analyzer. Initial data analysis took place with either Agilent ChemStation, Shimadzu LabSolutions, or Waters MassLynx. NMR data was collected with either a Bruker AVANCE III HD 400MHz, a Bruker Ascend 500MHz instrument, or a Varian 400MHz, and the raw data was analyzed with either TopSpin or Mestrelab Mnova.

Synthesis of Intermediates

Intermediate 1. Synthesis of 3-(5-bromo-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropan-1-ol



Intermediate 1.

Step 1

To a mixture of 3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropanoyl chloride (65 g, 137 mmol, crude) in DCM (120 mL) at 0 °C under an atmosphere of N_2 was added 1M $SnCl_4$ in DCM (137 mL, 137 mmol) slowly. The mixture was stirred at 0 °C for 30 min, then a solution of 5-bromo-1*H*-indole (26.8 g, 137 mmol) in DCM (40 mL) was added dropwise. The mixture was stirred at 0 °C for 45 min, then diluted with EtOAc (300 mL), washed with brine (400 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 1-(5-bromo-1*H*-indol-3-yl)-3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one (55 g, 75% yield). LCMS (ESI): m/z [M+Na] calc'd for $C_{29}H_{32}BrNO_2SiNa$ 556.1; found 556.3.

Step 2

To a mixture of 1-(5-bromo-1*H*-indol-3-yl)-3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one (50 g, 93.6 mmol) in THF (100 mL) at 0 °C under an atmosphere of N_2 was added $LiBH_4$ (6.1 g, 281 mmol). The mixture was heated to 60 °C and stirred for 20 h, then MeOH (10 mL) and EtOAc (100 mL) were added and the mixture washed with brine (50 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure. The residue was diluted with DCM (50 mL), cooled to 10 °C and diludine (9.5 g, 37.4 mmol) and TsOH. H_2O (890 mg, 4.7 mmol) added. The mixture was stirred at 10 °C for 2 h, filtered, the filtrate concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 1-(5-bromo-1*H*-indol-3-yl)-3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one (41 g, 84% yield). LCMS (ESI): m/z [M+H] calc'd for $C_{29}H_{34}BrNOSi$ 519.2; found 520.1; ^1H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H),

7.75 - 7.68 (m, 5H), 7.46 - 7.35 (m, 6H), 7.23 - 7.19 (m, 2H), 6.87 (d, *J* = 2.1 Hz, 1H), 3.40 (s, 2H), 2.72 (s, 2H), 1.14 (s, 9H), 0.89 (s, 6H).

Step 3

To a mixture of 1-(5-bromo-1*H*-indol-3-yl)-3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one (1.5 g, 2.9 mmol) and I_2 (731 mg, 2.9 mmol) in THF (15 mL) at rt was added AgOTf (888 mg, 3.5 mmol). The mixture was stirred at rt for 2 h, then diluted with EtOAc (200 mL) and washed with saturated $Na_2S_2O_3$ (100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-iodo-1*H*-indole (900 mg, 72% yield) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (s, 1H), 7.68 (d, *J* = 1.3 Hz, 1H), 7.64 - 7.62 (m, 4H), 7.46 - 7.43 (m, 6H), 7.24 - 7.22 (d, 1H), 7.14 - 7.12 (dd, *J* = 8.6, 1.6 Hz, 1H), 3.48 (s, 2H), 2.63 (s, 2H), 1.08 (s, 9H), 0.88 (s, 6H).

Step 4

To a stirred mixture of HCOOH (66.3 g, 1.44 mol) in TEA (728 g, 7.2 mol) at 0 °C under an atmosphere of Ar was added (4*S*,5*S*)-2-chloro-2-methyl-1-(4-methylbenzenesulfonyl)-4,5-diphenyl-1,3-diaza-2-ruthenacyclopentane cymene (3.9 g, 6.0 mmol) portion-wise. The mixture was heated to 40 °C and stirred for 15 min, then cooled to rt and 1-(3-bromopyridin-2-yl)ethanone (120 g, 600 mmol) added in portions. The mixture was heated to 40 °C and stirred for an additional 2 h, then the solvent was concentrated under reduced pressure. Brine (2 L) was added to the residue, the mixture was extracted with EtOAc (4 x 700 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (1*S*)-1-(3-bromopyridin-2-yl)ethanol (100 g, 74% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for C_7H_8BrNO 201.1; found 201.9.

Step 5

To a stirred mixture of (1*S*)-1-(3-bromopyridin-2-yl)ethanol (100 g, 495 mmol) in DMF (1 L) at 0 °C was added NaH, 60% dispersion in oil (14.25 g, 594 mmol) in portions. The mixture was stirred at 0 °C for 1 h. MeI (140.5 g, 990 mmol) was added dropwise at 0 °C and the mixture was allowed to warm to rt and stirred for 2 h. The mixture was cooled to 0 °C and saturated NH_4Cl (5 L) was added. The mixture was extracted with EtOAc (3 x 1.5 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-bromo-2-[(1*S*)-1-methoxyethyl]pyridine (90 g, 75% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_8H_{10}BrNO$ 215.0; found 215.9.

Step 6

To a stirred mixture of 3-bromo-2-[(1*S*)-1-methoxyethyl]pyridine (90 g, 417 mmol) and Pd(dppf)Cl_2 (30.5 g, 41.7 mmol) in toluene (900 mL) at rt under an atmosphere of Ar was added bis(pinacolato)diboron (127 g, 500 mmol) and KOAc (81.8 g, 833 mmol) in portions. The mixture was heated to 100 °C and stirred for 3 h. The filtrate was concentrated under reduced pressure and the residue was purified by Al_2O_3 column chromatography to give 2-[(1*S*)-1-methoxyethyl]-3-

(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (100 g, 63% yield) as a semi-solid. LCMS (ESI): m/z [M+H] calc'd for $C_{14}H_{22}BNO_3$ 263.2; found 264.1.

Step 7

To a stirred mixture of 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-2-iodo-1*H*-indole (140 g, 217 mmol) and 2-[(1*S*)-1-methoxyethyl]-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (100 g, 380 mmol) in 1,4-dioxane (1.4 L) at rt under an atmosphere of Ar was added K_2CO_3 (74.8 g, 541 mmol), $Pd(dppf)Cl_2$ (15.9 g, 21.7 mmol) and H_2O (280 mL) in portions. The mixture was heated to 85 °C and stirred for 4 h, then cooled, H_2O (5 L) added and the mixture extracted with EtOAc (3 x 2 L). The combined organic layers were washed with brine (2 x 1 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-1*H*-indole (71 g, 45% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{37}H_{43}BrN_2O_2Si$ 654.2; found 655.1.

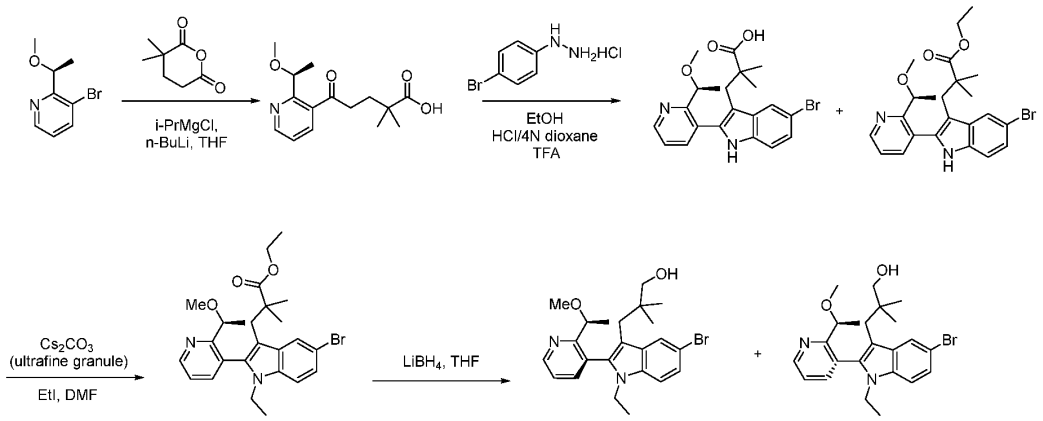
Step 8

To a stirred mixture of 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-1*H*-indole (71 g, 108 mmol) in DMF (0.8 L) at 0 °C under an atmosphere of N_2 was added Cs_2CO_3 (70.6 g, 217 mmol) and EtI (33.8 g, 217 mmol) in portions. The mixture was warmed to rt and stirred for 16 h then H_2O (4 L) added and the mixture extracted with EtOAc (3 x 1.5 L). The combined organic layers were washed with brine (2 x 1 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indole (66 g, 80% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{47}BrN_2O_2Si$ 682.3; found 683.3.

Step 9

To a stirred mixture of TBAF (172.6 g, 660 mmol) in THF (660 mL) at rt under an atmosphere of N_2 was added 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indole (66 g, 97 mmol) in portions. The mixture was heated to 50 °C and stirred for 16 h, cooled, diluted with H_2O (5 L) and extracted with EtOAc (3 x 1.5 L). The combined organic layers were washed with brine (2 x 1 L), dried over anhydrous Na_2SO_4 and filtered. After filtration, the filtrate was concentrated under reduced pressure. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give two atropisomers of 3-(5-bromo-1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (as single atropisomers) both as solids. (Combined 30 g, 62% yield) both as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{29}BrN_2O_2$ 444.1; found 445.1.

Intermediate 1. Alternative Synthesis through Fisher Indole Route



Intermediate 1

Step 1

To a mixture of *i*-PrMgCl (2M in in THF, 0.5 L) at -10 °C under an atmosphere of N_2 was added *n*-BuLi, 2.5 M in hexane (333 mL, 833 mmol) dropwise over 15 min. The mixture was stirred for 30 min at -10 °C then 3-bromo-2-[(1*S*)-1-methoxyethyl]pyridine (180 g, 833 mmol) in THF (0.5 L) added dropwise over 30 min at -10 °C. The resulting mixture was warmed to -5 °C and stirred for 1 h, then 3,3-dimethyloxane-2,6-dione (118 g, 833 mmol) in THF (1.2 L) was added dropwise over 30 min at -5 °C. The mixture was warmed to 0 °C and stirred for 1.5 h, then quenched with the addition of pre-cooled 4M HCl in 1,4-dioxane (0.6 L) at 0 °C to adjust pH ~5. The mixture was diluted with ice-water (3 L) and extracted with EtOAc (3 x 2.5 L). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 5-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-2,2-dimethyl-5-oxopentanoic acid (87 g, 34% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{15}H_{21}NO_4$ 279.2; found 280.1.

Step 2

To a mixture of 5-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-2,2-dimethyl-5-oxopentanoic acid (78 g, 279 mmol) in EtOH (0.78 L) at rt under an atmosphere of N_2 was added (4-bromophenyl)hydrazine HCl salt (68.7 g, 307 mmol) in portions. The mixture was heated to 85 °C and stirred for 2 h, cooled to rt, then 4M HCl in 1,4-dioxane (69.8 mL, 279 mmol) added dropwise. The mixture was heated to 85 °C and stirred for an additional 3 h, then concentrated under reduced pressure and the residue was dissolved in TFA (0.78 L). The mixture was heated to 60 °C and stirred for 1.5, concentrated under reduced pressure and the residue adjusted to pH ~5 with saturated $NaHCO_3$, then extracted with EtOAc (3 x 1.5 L). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-(5-bromo-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-1*H*-indol-3-yl)-2,2-dimethylpropanoic acid and ethyl (*S*)-3-(5-bromo-2-(2-

(1-methoxyethyl)pyridin-3-yl)-1*H*-indol-3-yl)-2,2-dimethylpropanoate (78 g, crude). LCMS (ESI): m/z [M+H] calc'd for $C_{21}H_{23}BrN_2O_3$ 430.1 and $C_{23}H_{27}BrN_2O_3$ 458.1; found 431.1 and 459.1.

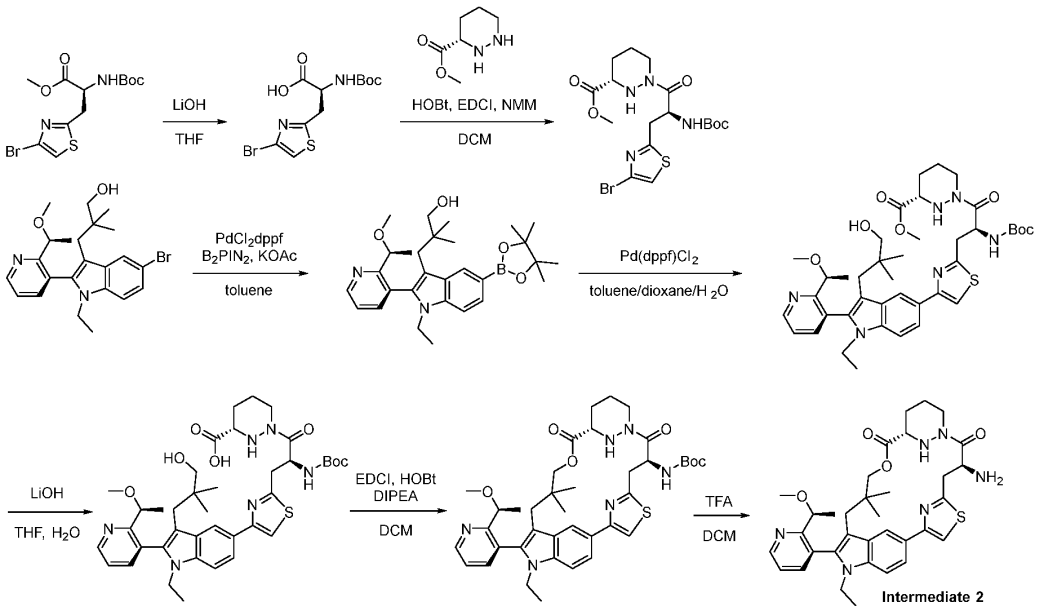
Step 3

To a mixture of 3-(5-bromo-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]-1*H*-indol-3-yl)-2,2-dimethylpropanoic acid and ethyl (*S*)-3-(5-bromo-2-(2-(1-methoxyethyl)pyridin-3-yl)-1*H*-indol-3-yl)-2,2-dimethylpropanoate (198 g, 459 mmol) in DMF (1.8 L) at 0 °C under an atmosphere of N_2 was added Cs_2CO_3 (449 g, 1.38 mol) in portions. EtI (215 g, 1.38 mmol) in DMF (200 mL) was then added dropwise at 0 °C. The mixture was warmed to rt and stirred for 4 h then diluted with brine (5 L) and extracted with EtOAc (3 x 2.5 L). The combined organic layers were washed with brine (2 x 1.5 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give ethyl 3-(5-bromo-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropanoate (160 g, 57% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{25}H_{31}BrN_2O_3$ 486.2; found 487.2.

Step 4

To a mixture of ethyl 3-(5-bromo-1-ethyl-2-[2-[(1*S*)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropanoate (160 g, 328 mmol) in THF (1.6 L) at 0 °C under an atmosphere of N_2 was added $LiBH_4$ (28.6 g, 1.3 mol). The mixture was heated to 60 °C for 16 h, cooled, and quenched with pre-cooled (0 °C) aqueous NH_4Cl (5 L). The mixture was extracted with EtOAc (3 x 2 L) and the combined organic layers were washed with brine (2 x 1 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give to two atropisomers of 3-(5-bromo-1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (as single atropisomers) (60 g, 38% yield) and (40 g, 26% yield) both as solids. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{29}BrN_2O_2$ 444.1; found 445.2.

Intermediate 2. Synthesis of *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate



Step 1

To a solution of methyl (2*S*)-3-(4-bromo-1,3-thiazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (110 g, 301.2 mmol) in THF (500 mL) and H_2O (200 mL) at room temperature was added LiOH (21.64 g, 903.6 mmol). The solution was stirred for 1 h and was then concentrated under reduced pressure. The residue was adjusted to pH 6 with 1 M HCl and then extracted with DCM (3 x 500 mL). The combined organic layers were, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give (*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (108 g, crude). LCMS (ESI): m/z [M+H] calc'd for $C_{11}H_{16}BrN_2O_4S$ 351.0; found 351.0.

Step 2

To a solution of (*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (70 g, 199.3 mmol) in DCM (500 mL) at 0 °C was added methyl (3*S*)-1,2-diazinane-3-carboxylate bis(trifluoroacetic acid) salt (111.28 g, 298.96 mmol), NMM (219.12 mL. 1993.0 mmol), EDCI (76.41 g, 398.6 mmol) and HOBt (5.39 g, 39.89 mmol). The solution was warmed to room temperature and stirred for 1 h. The reaction was then quenched with H_2O (500 mL) and was extracted with EtOAc (3 x 500 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressured. The residue was purified by silica gel column chromatography to give methyl (*S*)-1-((*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (88.1 g, 93% yield). LCMS (ESI): m/z [M+H] calc'd for $C_{17}H_{26}BrN_4O_5S$ 477.1; found 477.1.

Step 3

To a solution of 3-(5-bromo-1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (60 g, 134.7 mmol) in toluene (500 mL) at room temperature was added bis(pinacolato)diboron (51.31 g, 202.1 mmol), Pd(dppf)Cl_2 (9.86 g, 13.4 mmol), and KOAc (26.44 g, 269 mmol). The reaction mixture was then heated to 90 °C and stirred for 2 h. The reaction solution was then cooled to room temperature and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give (*S*)-3-(1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (60.6 g, 94% yield). LCMS (ESI): m/z [M+H] calc'd for $C_{29}H_{42}BN_2O_4$ 493.32; found 493.3.

Step 4

To a solution of (*S*)-3-(1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (30 g, 60.9 mmol) in toluene (600 mL), dioxane (200 mL), and H_2O (200 mL) at room temperature was added methyl (*S*)-1-((*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (43.62 g, 91.4mmol), K_3PO_4 (32.23 g, 152.3 mmol) and Pd(dppf)Cl_2 (8.91 g, 12.18 mmol). The resulting solution was heated to 70 °C and stirred overnight. The reaction mixture was then cooled to room temperature and was quenched with H_2O (200 mL). The mixture was extracted with EtOAc and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (39.7 g, 85% yield). LCMS (ESI): m/z [M+H] calc'd for $C_{40}H_{55}N_6O_7S$ 763.4; found 763.3.

Step 5

To a solution of methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (39.7 g, 52.0 mmol) in THF (400 mL) and H_2O (100 mL) at room temperature was added LiOH•H_2O (3.74 g, 156.2 mmol). The mixture was stirred for 1.5 h and was then concentrated under reduced pressure. The residue was acidified to pH 6 with 1 M HCl and extracted with DCM (3 x 1000 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (37.9 g, crude). LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{53}N_6O_7S$ 749.4; found 749.4.

Step 6

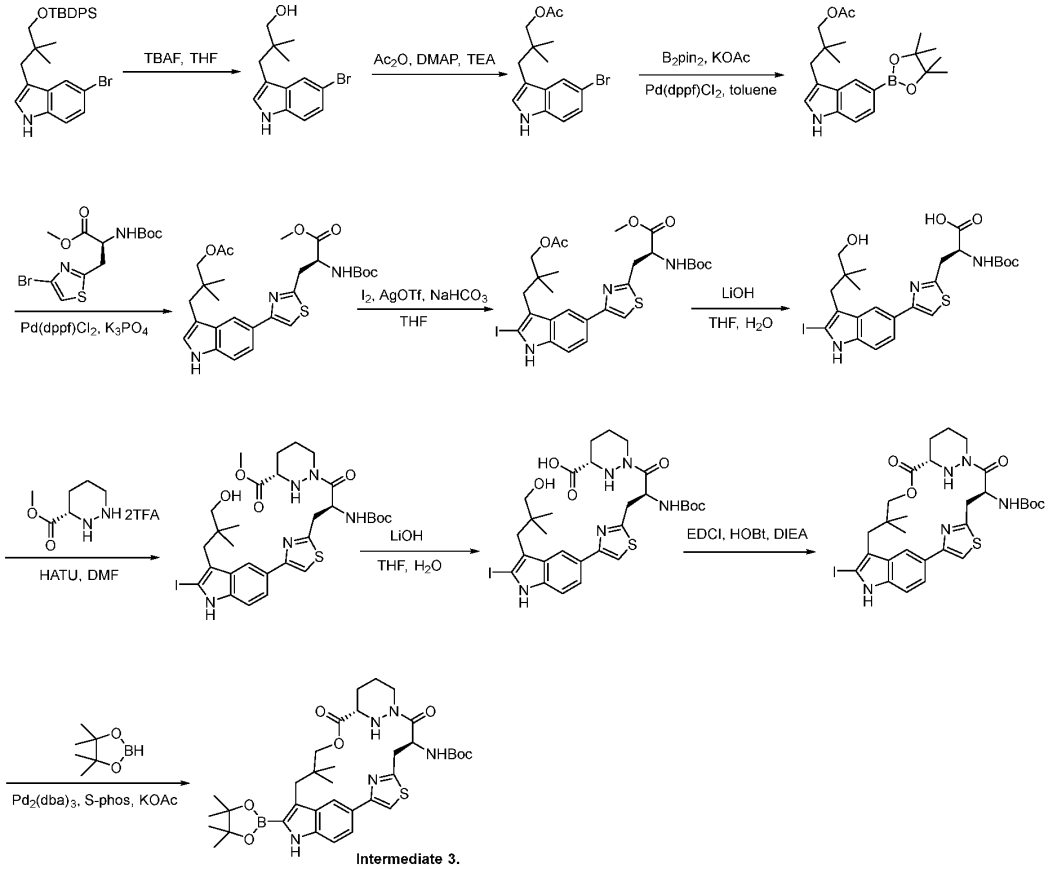
To a solution of (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1*H*-indol-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (37.9 g, 50.6 mmol), HOBt (34.19 g, 253.0 mmol) and DIPEA (264.4 mL, 1518 mmol) in DCM (4 L) at 0 °C was added EDCI (271.63 g, 1416.9 mmol). The resulting mixture was warmed to room temperature and stirred overnight. The reaction mixture was then quenched with H_2O and washed with 1 M HCl (4 x 1 L). The organic layer was separated and concentrated under reduced pressure. The residue was purified by silica gel column

chromatography to give *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (30 g, 81% yield). LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{51}N_6O_6S$ 731.4; found 731.3.

Step 7

To a solution of *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (6 g, 8.21 mmol) in DCM (60 mL) at 0 °C was added TFA (30 mL). The mixture was stirred for 1 h and was then concentrated under reduced pressure to give (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (7.0 g, crude). LCMS (ESI): m/z [M+H] calc'd for $C_{34}H_{42}N_6O_4S$ 631.3; found: 630.3.

Intermediate 3. Synthesis of *tert*-butyl ((6^3*S*,4*S*,*Z*)-10,10-dimethyl-5,7-dioxo-1^2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate



Intermediate 3.

Step 1

To a stirred solution of 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1H-indole (100 g, 192.0 mmol) in THF (1000 mL) were added TBAF(261.17 g, 998.8 mmol) in portion at room temperature. The resulting mixture was stirred for 16 h at 50 °C. The resulting mixture was concentrated under reduced pressure. The resulting mixture was extracted with EtOAc (2 L). The combined organic layers were washed with water (6 L), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with PE/EtOAc (3:1) to afford 3-(5-bromo-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (54 g, 96.63%). LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{16}BrNO$ 281.0; found 282.0.

Step 2

To a stirred solution of 3-(5-bromo-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (54 g, 191.3 mmol) in DCM (300 mL) were added TEA (58.09 g, 574.1 mmol) and Ac_2O (18.95 g, 185.6 mmol) and DMAP (1.17 g, 9.5 mmol) dropwise at 0 °C. The resulting mixture was washed with water (3 x 500 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. This resulted in 3-(5-bromo-1*H*-indol-3-yl)-2,2-dimethylpropyl acetate (54 g, 80.6%) as a yellow solid. The crude product was used in the next step directly without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{15}H_{18}BrNO_2$ 323.0; found 324.0.

Step 3

To a stirred solution of 3-(5-bromo-1H-indol-3-yl)-2,2-dimethylpropyl acetate(54 g, 166.5 mmol) in toluene (600 mL) were added KOAc (40.87 g, 416.3 mmol) and B_2pin_2 (105.76 g, 416.3 mmol) and $Pd(dppf)Cl_2$ (12.19 g, 16.6 mmol) in portions at room temperature under argon atmosphere. The resulting mixture was stirred for 3 h at 90 °C under argon atmosphere. The resulting mixture was concentrated under reduced pressure. The resulting mixture was extracted with EtOAc (1 L). The combined organic layers were washed with water (3x1 L), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with PE/EtOAc (3:1) to afford 2,2-dimethyl-3-[5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl]propyl acetate borane (55 g, 76.57%) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{21}H_{30}BNO_4$ 371.2; found 372.2.

Step 4

To a stirred solution of 2,2-dimethyl-3-[5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl]propyl acetate (54 g, 145.443 mmol) and methyl (2*S*)-3-(4-bromo-1,3-thiazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (79.68 g, 218.1 mmol) and K_3PO_4 (77.18 g, 363.6 mmol) in toluene (330 mL) and dioxane (110 mL) and H_2O (110 mL) were added $Pd(dppf)Cl_2$ (10.64 g, 14.5 mmol) in portions at room temperature under argon atmosphere. The resulting mixture was stirred for 36 h at 70 °C under argon atmosphere. The resulting mixture was concentrated under vacuum. The resulting mixture was extracted with EtOAc (3 L). The combined organic layers were washed with water (3x2 L), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with PE/EtOAc (1:1) to afford methyl (2*S*)-3-(4-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-1H-indol-5-yl]-1,3-

thiazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (54 g, 60.78%) as a yellow oil. LCMS (ESI): m/z [M+H] calc'd for $C_{27}H_{35}N_3O_6S$ 529.2; found 530.2.

Step 5

To a stirred solution of methyl (2*S*)-3-(4-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-1*H*-indol-5-yl]-1,3-thiazol-2-yl)-2-[(tert-butoxycarbonyl)amino]propanoate (54 g, 101.954 mmol) in THF (450 mL) were added $NaHCO_3$ (10.28 g, 122.3 mmol) and AgOTf (31.44 g, 122.3 mmol) dropwise at 0 °C. To the stirred solution was added I_2 (23.29 g, 91.6 mmol) in THF (100 mL) dropwise at 0 °C. The resulting mixture was stirred for 15 min at 0 °C. The reaction was quenched with sat. $Na_2S_2O_3$ (aq.) at 0 °C. The resulting mixture was extracted with EtOAc (1 L). The combined organic layers were washed with water (3 L), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with PE/EtOAc (5:1) to afford methyl (2*S*)-3-(4-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-2-iodo-1*H*-indol-5-yl]-1,3-thiazol-2-yl)-2-[(*tert*-butoxycarbonyl)mino] propanoate (40 g, 53.80%) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{27}H_{34}IN_3O_6S$ 655.1; found 656.1.

Step 6

To a stirred solution of methyl (2*S*)-3-(4-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-2-iodo-1*H*-indol-5-yl]-1,3-thiazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoate (40 g, 61.01 mmol) in THF (300 mL) and H_2O (100 mL) were added LiOH (4.38 g, 183.05 mmol) dropwise at 0 °C. The resulting mixture was stirred for overnight at room temperature. The residue was acidified to pH 6 with conc. HCl. The resulting mixture was extracted with EtOAc (500 mL). The combined organic layers were washed with water (3 x 500 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. This resulted in (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[4-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-1,3-thiazol-2-yl]propanoic acid (40 g, crude) as a yellow oil. The crude product was used in the next step directly without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{24}H_{30}IN_3O_5S$ 599.1.1; found 600.1.

Step 7

To a stirred solution of (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[4-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-1,3-thiazol-2-yl]propanoic acid (40 g, 66.72 mmol) and methyl (3*S*)-1,2-diazinane-3-carboxylate (28.86 g, 200.17 mmol) and HOBT (1.8 g, 13.35 mmol) and DIEA (172.47 g, 1334.5 mmol) in DCM (350 mL) were added EDCI (31.98 g, 166.8 mmol) dropwise at 0 °C. The resulting mixture was stirred for overnight at room temperature under nitrogen atmosphere. The resulting mixture was washed with water (1.5 L), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with PE/EtOAc (1:1) to afford methyl (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[4-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-1,3-thiazol-2-yl]propanoyl]-1,2-diazinane-3-carboxylate (28 g, 43.9%) as a yellow oil. LCMS (ESI): m/z [M+H] calc'd for $C_{30}H_{40}IN_5O_6S$ 725.1.1; found 726.1

Step 8

To a stirred solution of methyl (3S)-1-[(2S)-2-[(*tert*-butoxycarbonyl)amino]-3-[4-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-1,3-thiazol-2-yl]propanoyl]-1,2-diazinane-3-

carboxylate (28 g, 38.5 mmol) in THF (240 mL) were added LiOH (2.77 g, 115.7 mmol) in H_2O (80 mL) dropwise at 0 °C. The resulting mixture was stirred for 2 h at room temperature. The mixture was acidified to pH 6 with conc. HCl. The resulting mixture was extracted with EtOAc (300 mL). The combined organic layers were washed with water (3x300 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. This resulted in (3S)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[4-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-1,3-thiazol-2-yl]propanoyl]-1,2-diazinane-3-carboxylic acid (25 g, crude) as a yellow oil. The crude product was used in the next step directly without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{29}H_{38}IN_5O_6S$ 711.1; found 712.2.

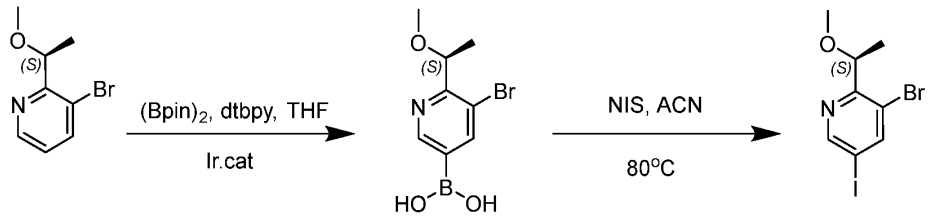
Step 9

To a stirred solution of (3*S*)-1-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[4-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1*H*-indol-5-yl]-1,3-thiazol-2-yl]propanoyl]-1,2-diazinane-3-carboxylic acid (25 g, 35.13 mmol) and HOBT (23.74 g, 175.6 mmol) and DIPEA (136.21 g, 1053.9 mmol) in DCM (2 L) were added EDCI (188.5 g, 983.6 mmol) in portions at 0 °C. The resulting mixture was stirred for overnight at room temperature under nitrogen atmosphere. The resulting mixture was washed with water (6 L), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with PE/EtOAc (2:1) to afford *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^2-iodo-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (13 g, 45.88%) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{29}H_{36}IN_5O_5S$ 693.1; found 694.0.

Step 10

To a stirred mixture of *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^2-iodo-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (13 g, 18.7 mmol) and KOAc (6.44 g, 65.6 mmol) and s-Phos (2.31 g, 5.62 mmol) in toluene (120 mL) were added $Pd_2(dba)_3$ (2.06 g, 2.25 mmol) in portions at room temperature under argon atmosphere. To the stirred solution were added 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (17.99 g, 140.5 mmol) dropwise at 0 °C under argon atmosphere. The resulting mixture was stirred for 3 h at 60°C under argon atmosphere. The reaction was quenched with sat. NH_4Cl (aq.) at 0 °C. The resulting mixture was concentrated under vacuum. The resulting mixture was extracted with EtOAc (200 mL). The combined organic layers were washed with water (3 x 300 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with PE/EtOAc (3:1) to afford *tert*-butyl ((6^3*S*,4*S*,*Z*)-10,10-dimethyl-5,7-dioxo-1^2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (10 g, 68.6% yield) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{35}H_{48}BN_5O_7S$ 693.3; found 694.4.

Intermediate 4. Synthesis of (*S*)-3-bromo-5-iodo-2- (1-methoxyethyl) pyridine



Intermediate 4.

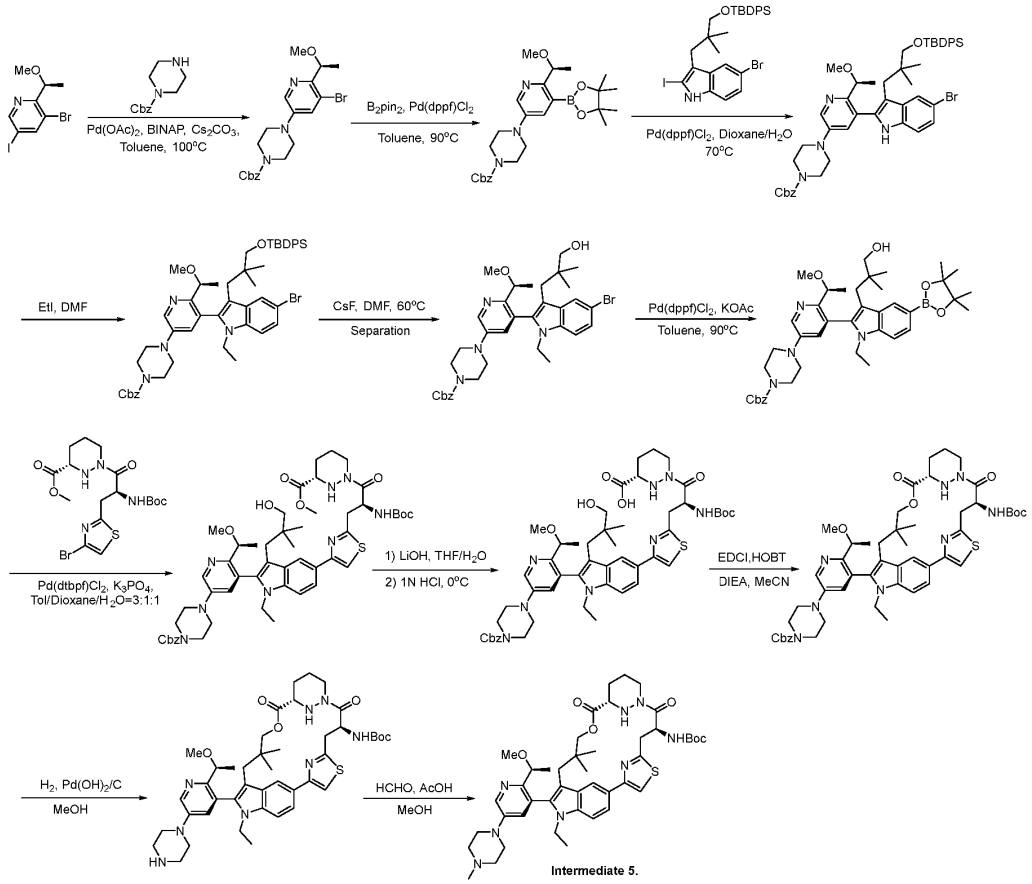
Step 1

To a stirred solution of 3-bromo-2-[(1*S*)-1-methoxyethyl]pyridine (80.00 g, 370.24 mmol, 1.00 equiv) and bis(pinacolato)diboron (141.03 g, 555.3 mmol, 1.50 equiv) in THF (320 mL) was added dtbpy (14.91 g, 55.5 mmol) and Chloro(1,5-cyclooctadiene)iridium(I) dimer (7.46 g, 11.1 mmol) under argon atmosphere. The resulting mixture was stirred for 16 h at 75 °C under argon atmosphere. The mixture was concentrated under reduced pressure. The resulting mixture was dissolved in EtOAc (200 mL) and the mixture was adjusted to pH 10 with Na_2CO_3 (40 g) and NaOH (10 g) (mass 4:1) in water (600 mL). The aqueous layer was extracted with EtOAc (800mL). The aqueous phase was acidified to pH = 6 with HCl (6 *N*) to precipitate the desired solid to afford 5-bromo-6-[(1S)-1-methoxyethyl]pyridin-3-ylboronic acid (50g, 52.0%yield) as a light-yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_8H_{11}BBrNO_3$ 259.0; found 260.0.

Step 2

To a stirred solution of 5-bromo-6-[(1*S*)-1-methoxyethyl]pyridin-3-ylboronic acid (23.00 g, 88.5 mmol) in ACN (230 mL) were added NIS (49.78 g, 221.2 mmol) at room temperature under argon atmosphere. The resulting mixture was stirred for overnight at 80 °C under argon atmosphere. The resulting mixture was concentrated under reduced pressure. The resulting mixture was dissolved in DCM (2.1 L) and washed with $Na_2S_2O_3$ (3 x 500 mL). The organic layer was dried over anhydrous Na2SO4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford (*S*)-3-bromo-5-iodo-2-(1-methoxyethyl)pyridine (20 g, 66.0%yield). LCMS (ESI): m/z [M+H] calc'd for C_8H_9BrINO 340.9; found 341.7.

Intermediate 5. Synthesis of *tert*-butyl ((6^3*S*,4*S*,*Z*)-11-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate



Step 1

Into a 3L 3-necked round-bottom flask purged and maintained with an inert atmosphere of argon, was placed 3-bromo-5-iodo-2-[(1*S*)-1-methoxyethyl]pyridine (147 g, 429.8 mmol) benzyl piperazine-1-carboxylate (94.69 g, 429.8 mmol), $Pd(OAc)_2$ (4.83 g, 21.4 mmol), BINAP (5.35 g, 8.6 mmol), Cs_2CO_3 (350.14 g, 1074.6 mmol), toluene (1 L). The resulting solution was stirred for overnight at 100 °C in an oil bath. The reaction mixture was cooled to 25 °C after reaction completed. The resulting mixture was concentrated under reduced pressure. The residue was applied onto a silica gel column with ethyl acetate/hexane (1:1). Removal of solvent under reduced pressure gave benzyl (*S*)-4-(5-bromo-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (135 g, 65.1% yield) as a dark yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{20}H_{24}BrN_3O_3$ 433.1; found 434.1.

Step 2

Into a 3-L 3-necked round-bottom flask purged and maintained with an inert atmosphere of argon, was placed benzyl 4-[5-bromo-6-[(1*S*)-1-methoxyethyl]pyridin-3-yl]piperazine-1-carboxylate (135 g, 310.8 mmol), bis(pinacolato)diboron (86.82 g, 341.9 mmol), $Pd(dppf)Cl_2$ (22.74 g, 31.0

mmol), KOAc (76.26 g, 777.5 mmol), Toluene (1 L). The resulting solution was stirred for 2 days at 90 °C in an oil bath. The reaction mixture was cooled to 25 °C. The resulting mixture was concentrated under vacuum. The residue was applied onto a neutral alumina column with ethyl acetate/hexane (1:3). Removal of solvent under reduced pressure gave benzyl (*S*)-4-(6-(1-methoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl)piperazine-1-carboxylate (167 g, crude) as a dark yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{26}H_{36}BN_3O_5$ 481.3; found 482.1.

Step 3

Into a 3-L 3-necked round-bottom flask purged and maintained with an inert atmosphere of argon, was placed (*S*)-4-(6-(1-methoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl)piperazine-1-carboxylate (167 g, 346.9 mmol), 5-bromo-3-[3-[(tert-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-2-iodo-1H-indole (224.27 g, 346.9 mmol), $Pd(dppf)Cl_2$ (25.38 g, 34.6 mmol), dioxane (600 mL), H_2O (200 mL), K_3PO_4 (184.09 g, 867.2 mmol), Toluene (200 mL). The resulting solution was stirred for overnight at 70 °C in an oil bath. The reaction mixture was cooled to 25 °C after reaction completed. The resulting mixture was concentrated under vacuum. The residue was applied onto a silica gel column with ethyl acetate/hexane (1:1). Removal of solvent under reduced pressure gave benzyl (*S*)-4-(5-(5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (146 g, 48.1% yield) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{49}H_{57}BrN_4O_4Si$ 872.3; found 873.3.

Step 4

To a stirred mixture of benzyl (*S*)-4-(5-(5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (146 g, 167.0 mmol) and Cs_2CO_3 (163.28 g, 501.1 mmol) in DMF (1200 mL) was added C_2H_5I (52.11 g, 334.0 mmol) in portions at 0 °C under N_2 atmosphere. The final reaction mixture was stirred at 25 °C for 12 h. Desired product could be detected by LCMS. The resulting mixture was diluted with EA (1 L) and washed with brine (3 x 1.5L). The organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to give benzyl (*S*)-4-(5-(5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1-ethyl-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (143 g, crude) as a yellow solid that was used directly for next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{61}BrN_4O_4Si$ 900.4; found 901.4.

Step 5

To a stirred mixture of benzyl benzyl (*S*)-4-(5-(5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1-ethyl-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (143 g, 158.5 mmol) in DMF (1250 mL) was added CsF (72.24 g, 475.5 mmol). Then the reaction mixture was stirred at 60 °C for 2 days under N_2 atmosphere. Desired product could be detected by LCMS. The resulting mixture was diluted with EA (1 L) and washed with brine (3 x 1L). Then the organic phase was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with PE/EA (1/3) to afford two atropisomers of benzyl (*S*)-4-(5-(5-bromo-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-

yl)piperazine-1-carboxylate **A** (38 g, 36% yield, RT = 1.677 min in 3 min LCMS(0.1% FA)) and **B** (34 g, 34% yield, RT = 1.578 min in 3 min LCMS(0.1% FA)) both as yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{35}H_{43}BrN_4O_4$ 663.2; found 662.2.

Step 6

Into a 500-mL 3-necked round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed benzyl (*S*)-4-(5-(5-bromo-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate **A** (14 g, 21.1 mmol), bis(pinacolato)diboron (5.89 g, 23.21 mmol), Pd(dppf)Cl_2 (1.54 g, 2.1 mmol), KOAc (5.18 g, 52.7 mmol), Toluene (150 mL). The resulting solution was stirred for 5 h at 90 °C in an oil bath. The reaction mixture was cooled to 25 °C. The resulting mixture was concentrated under vacuum. The residue was purified by silica gel column chromatography, eluted with PE/EA (1/3) to give benzyl (*S*)-4-(5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (12 g, 76.0% yield) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{55}BN_4O_6$ 710.4; found 711.3.

Step 7

Into a 250-mL round-bottom flask purged and maintained with an inert atmosphere of argon, was placed benzyl (*S*)-4-(5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (10.8 g, 15.2 mmol), methyl (3*S*)-1-[(2*S*)-3-(4-bromo-1,3-thiazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoyl]-1,2-diazinane-3-carboxylate (7.98 g, 16.7 mmol), Pd(dtbpf)Cl_2 (0.99 g, 1.52 mmol), K_3PO_4 (8.06 g, 37.9 mmol), Toluene (60 mL), dioxane (20 mL), H_2O (20 mL). The resulting solution was stirred for 3 h at 70 °C in an oil bath. The reaction mixture was cooled to 25 °C. The resulting solution was extracted with EtOAc (2 x 50 mL) and concentrated under reduced pressure. The residue was applied onto a silica gel column with ethyl acetate/hexane (10:1). Removal of solvent to give methyl (*S*)-1-((*S*)-3-(4-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-5-yl)thiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (8 g, 50.9% yield) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{52}H_{68}N_8O_9S$ 980.5; found 980.9.

Step 8

To a stirred mixture of methyl (*S*)-1-((*S*)-3-(4-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((S)-1-methoxyethyl)pyridin-3-yl)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-5-yl)thiazol-2-yl)-2-((tert-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (12 g, 12.23 mmol) in THF (100 mL)/H_2O (100 mL) was added LiOH (2.45 g, 61.1 mmol) under N_2 atmosphere and the resulting mixture was stirred for 2 h at 25 °C. Desired product could be detected by LCMS. THF was concentrated under reduced pressure. The pH of aqueous phase was acidified to 5 with HCL (1N) at 0 °C. The aqueous layer was extracted with DCM (3 x 100ml). The organic phase was concentrated under reduced pressure to give (*S*)-1-((*S*)-3-(4-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-5-yl)thiazol-2-yl)-2-((*tert*-

butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylic acid (10 g, 84.5% yield) as a light yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{66}N_8O_9S$ 966.5; found 967.0.

Step 9

Into a 3-L round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed (*S*)-1-((*S*)-3-(4-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-5-yl)thiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylic acid (18 g, 18.61 mmol), ACN (1.8 L), DIEA (96.21 g, 744.4 mmol), EDCI (107.03 g, 558.3 mmol), HOBT (25.15 g, 186.1 mmol). The resulting solution was stirred for overnight at 25 °C. The resulting mixture was concentrated under vacuum after reaction completed. The resulting solution was diluted with DCM (1 L). The resulting mixture was washed with HCl (3 x 1 L, 1N aqueous). The resulting mixture was washed with water (3 x 1 L). Then the organic layer was concentrated, the residue was applied onto a silica gel column with ethyl acetate/hexane (1:1). Removal of solvent under reduced pressure gave benzyl 4-(5-((6^3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (10.4 g, 54.8% yiels) as a light yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{64}N_8O_8S$ 948.5; found 949.3.

Step 10

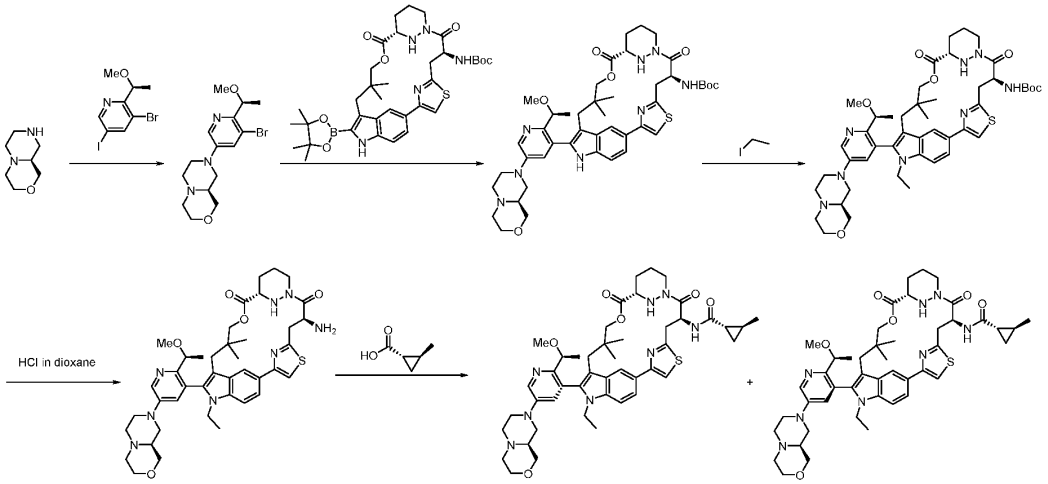
Into a 250-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed benzyl 4-(5-((6^3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (10.40 g, 10.9 mmol), $Pd(OH)_2$/C (5 g, 46.9 mmol), MeOH (100 mL). The resulting solution was stirred for 3 h at 25 °C under 2 atm H_2 atmosphere. The solids were filtered out and the filter cake was washed with MeOH (3 x 100 mL). Then combined organic phase was concentrated under reduced pressure to give *tert*-butyl ((6^3*S*,4S,*Z*)-1*1*-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(piperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (8.5 g, 90.4% yield) as a light yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{58}N_8O_6S$ 814.4; found 815.3.

Step 11

Into a 1000-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed *tert*-butyl ((6^3*S*,4S,*Z*)-1*1*-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(piperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (8.5 g, 10.4 mmol), MeOH (100 mL), AcOH (1.88 g, 31.2 mmol) and stirred for 15 mins. Then HCHO (1.88 g, 23.15 mmol, 37% aqueous solution) and $NaBH_3CN$ (788 mg, 12.5 mmol) was added at 25 °C. The resulting solution was stirred for 3 h at 25 °C. The resulting mixture was quenched with 100 mL water and concentrated under reduced pressure to remove MeOH. The resulting solution was diluted with 300 mL of DCM. The resulting mixture was washed with water (3 x 100 mL). Removal of solvent gave

tert-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (8.2 g, 90.1% yield) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{60}N_8O_6S$ 828.4; found 829.3.

Example A120. (1*S*,2*S*)-N-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(5-((*S*)-hexahydropyrazino[2,1-c][1,4]oxazin-8(1H)-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methylcyclopropane-1-carboxamide



Step 1

To a stirred solution of 3-bromo-5-iodo-2-[(1*S*)-1-methoxyethyl]pyridine (1 g, 2.92 mmol) (*S*)-octahydropyrazino[2,1-*c*][1,4]oxazine (498.9 mg, 3.1 mmol) and Potassium tert-butoxide (656.25 mg, 5.8 mmol) in Toluene (15 mL) were added $Pd_2(dba)_3$ (53.55 mg, 0.06 mmol) and XantPhos (169.2 mg, 0.29 mmol) at room temperature under nitrogen atmosphere. The resulting mixture was stirred for 3 h at 100 °C under nitrogen atmosphere. After completion of reaction, the solution was concentrated under reduced pressure. The residue was purified by reverse flash chromatography (conditions: column, C18 silica gel; mobile phase, ACN in water (0.05% TFA), 0% to 100% gradient in 40 min; detector, UV 254 nm) to afford (*S*)-8-(5-bromo-6-((*S*)-1-methoxyethyl)pyridin-3-yl)octahydropyrazino[2,1-*c*][1,4]oxazine (670 mg, 57.2% yield) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{15}H_{22}BrN_3O_2$ 355.1; found 356.1.

Step 2

To a mixture of (*S*)-8-(5-bromo-6-((*S*)-1-methoxyethyl)pyridin-3-yl)octahydropyrazino[2,1-*c*][1,4]oxazine (670 mg, 1.88 mmol), **Intermediate 3** (1.56 g, 2.26 mmol) and K_2CO_3 (779.74 mg, 5.6 mmol) in Toluene (9 mL), H_2O (3 mL) and 1,4-dioxane (3 mL) was added $Pd(dppf)Cl_2$ (137.61 mg, 0.19 mmol) at room temperature under nitrogen atmosphere. The resulting mixture was stirred for overnight at 65 °C under nitrogen atmosphere. After completion of reaction, the solution was concentrated under reduced pressure. The residue was purified by reverse flash chromatography (conditions: column, C18 silica gel; mobile phase, ACN in water (0.05% TFA), 0% to 100% gradient

in 30 min; detector, UV 254 nm) to afford *tert*-butyl ((6^3*S*,4*S*, *Z*)-1^2-(5-((*S*)-hexahydropyrazino[2,1-*c*][1,4]oxazin-8(1*H*)-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (1.4 g, 88.3% yield) as a white solid. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{58}N_8O_7S$ 842.4; found 843.2.

Step 3

To a stirred mixture of *tert*-butyl ((6^3*S*,4*S*, *Z*)-1^2-(5-((*S*)-hexahydropyrazino[2,1-*c*][1,4]oxazin-8(1*H*)-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (1.4 g, 1.66 mmol) and Cs_2CO_3 (1.62 g, 4.97 mmol) in DMF (10 mL) was added ethyl iodide (0.39 g, 2.5 mmol) dropwise at 0 °C. The resulting mixture was stirred for 2 h at room temperature. After completion of reaction, the solution was concentrated under reduced pressure. The residue was purified by reverse flash chromatography (conditions: column, C18 silica gel; mobile phase, ACN in water (0.05% TFA), 0% to 100% gradient in 30 min; detector, UV 254 nm) to afford *tert*-butyl ((6^3*S*,4*S*, *Z*)-1^1-ethyl-1^2-(5-((*S*)-hexahydropyrazino[2,1-*c*][1,4]oxazin-8(1*H*)-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (800 mg, 49.7% yield) as a brown yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{46}H_{62}N_8O_7S$ 870.4; found 871.2.

Step 4

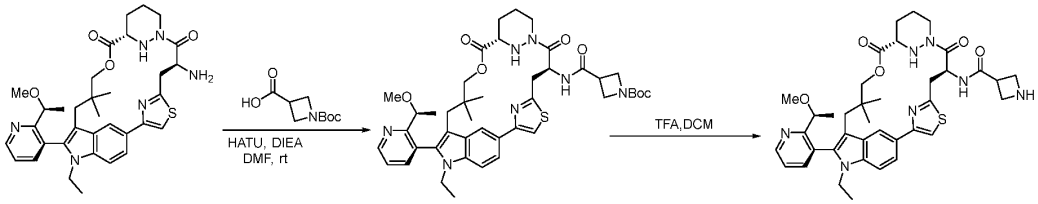
Into a 50 mL round-bottom flask were added *tert*-butyl ((6^3 *S*, 4*S*, *Z*)-1^1-ethyl-1^2-(5-((*S*)-hexahydropyrazino[2,1-*c*][1,4]oxazin-8(1*H*)-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (250 mg, 0.29 mmol) and HCl (4M in 1,4-dioxane, 10 mL) at 0 °C. The resulting mixture was stirred for 1 h at room temperature. The resulting mixture was concentrated. The resulting mixture was diluted with 30 mL of dichloromethane and 20 mL saturated $NaHCO_3$ aqueous solution. The organic phase was washed twice with 30 mL brine. Removal of solvent under reduced pressure resulted in (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(5-((*S*)-hexahydropyrazino[2,1-c][1,4]oxazin-8(1*H*)-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (170.00 mg, crude) as a brown solid. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{54}N_8O_5S$ 770.4; found 771.2.

Step 5

To a stirred solution of (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(5-((*S*)-hexahydropyrazino[2,1-c][1,4]oxazin-8(1*H*)-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (170 mg, 0.22 mmol) in DMF (8 mL) were added DIEA (2.8g, 22 mmol), (1*S*,2*S*)-2-methylcyclopropane-1-carboxylic acid (33 mg, 0.33 mmol) and HATU (125 mg, 0.33 mmol) at 0 °C. The resulting mixture was stirred for 2 h at room temperature. After completion of reaction, the solution was concentrated under reduced pressure. The crude product was purified by Prep-HPLC

to afford 50 mg racemated product. The racemate was purified by Prep-CHIRAL-HPLC with the following conditions (Column: CHIRAL ART Cellulose-SB, 2*25cm, 5um; Mobile Phase A: MtBE (10 mM NH_3-MeOH), Mobile Phase B: EtOH; Flow rate: 20 mL/min; Gradient: 50% B to 50% B in 7 min; 275/210 nm) to afford two atropisomers of (1*S*,2*S*)-N-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(5-((*S*)-hexahydropyrazino[2,1-c][1,4]oxazin-8(1*H*)-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methylcyclopropane-1-carboxamide (as single atropisomer) (10.7 mg, 5.1% yield) and (6 mg, 3.03%) both as white solid. LCMS (ESI): m/z [M+H] calc'd for $C_{46}H_{60}N_8O_6S$ 852.3; found 853.5. Isomer 1. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.53 (d, 2H), 8.46 (d, 1H), 7.79 (s, 1H), 7.71 (d, 1H), 7.51 (d, 1H), 7.38 (d, 1H), 5.55 (t, 1H), 5.05 (d, 1H), 4.22 (t, 2H), 3.99 – 3.80 (m, 4H), 3.82 – 3.59 (m, 5H), 3.54 (d, 2H), 3.39 (d, 1H), 3.14 (t, 2H), 3.07 (s, 3H), 2.99 (s, 1H), 2.81 (t, 3H), 2.67 (d, 1H), 2.44 – 2.34 (m, 2H), 2.30 (s, 1H), 2.21 – 2.07 (m, 2H), 1.80 (s, 2H), 1.51 (s, 2H), 1.21 (d, 4H), 1.15 – 0.93 (m, 7H), 0.87 (s, 3H), 0.65 (m, 2H), 0.52 (s, 4H). Isomer 2. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.54 – 8.39 (m, 3H), 7.79 (s, 1H), 7.75 – 7.67 (m, 1H), 7.55 (d, 1H), 7.22 (d, 1H), 5.56 (t, 1H), 5.07 (d, 1H), 4.34 – 4.09 (m, 5H), 3.83 – 3.62 (m, 4H), 3.55 (d, 3H), 3.21 (s, 3H), 3.14 (d, 2H), 2.94 – 2.64 (m, 5H), 2.46 – 2.36 (m, 2H), 2.32 – 2.15 (m, 3H), 2.08 (d, 1H), 1.79 (s, 2H), 1.49 (s, 2H), 1.33 (d, 3H), 1.25 (d, 1H), 1.06 (s, 4H), 0.90 (d, 7H), 0.54 (d, 1H), 0.34 (s, 3H).

Example A14. N-[(7*S*,13*S*,19*M*)-21-ethyl-20-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}-17,17-dimethyl-8,14-dioxo-15-oxa-4-thia-9,21,27,28-tetraazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacosa-1(25),2,5(28),19,22(26),23-hexaen-7-yl]azetidine-3-carboxamide



Step 1

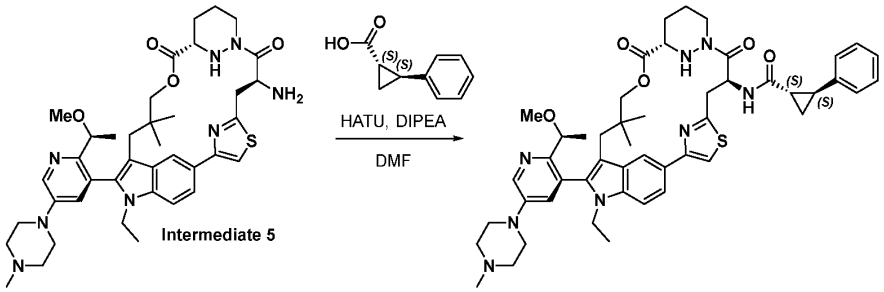
To a stirred solution of (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (450.00 mg, 0.71 mmol, 1.00 equiv) and 1-(*tert*-butoxycarbonyl)azetidine-3-carboxylic acid (215.3 mg, 1.07 mmol) in DMF (5.00 mL) were added DIEA (460.99 mg, 3.5 mmol) and HATU (379.7 mg, 1 mmol) in portions at room temperature under N_2 atmosphere until the reaction was complete by LCMS. The resulting mixture was extracted with EtOAc (3 x 20 mL) and the combined organic layers were washed with brine (2x20 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure and the resulting residue was purified by silica gel column chromatography to afford *tert*-butyl 3-(((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-

pyridazinacycloundecaphane-4-yl)carbamoyl)azetidine-1-carboxylate (520 mg, 90%) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{55}N_7O_7S$ ESI-MS 813.4; found: 814.4

Step 2

To a solution of *tert*-butyl 3-(((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamoyl)azetidine-1-carboxylate (170.00 mg, 0.21 mmol) in DCM (1.6 mL) was added TFA (0.4 mL, 5.3 mmol) dropwised at 0 °C. It was stirred for 2 h at room temperature under N_2 atmosphere and then concentrated under reduced pressure. The crude product was purified by Prep-HPLC to afford N-[(7*S*,13*S*,19*M*)-21-ethyl-20-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}-17,17-dimethyl-8,14-dioxo-15-oxa-4-thia-9,21,27,28-tetraazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacosa-1(25),2,5(28),19,22(26),23-hexaen-7-yl]azetidine-3-carboxamide (44.7 mg, 30% yield) as a white solid. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{47}N_7O_5S$ 713.3; found 714.1.

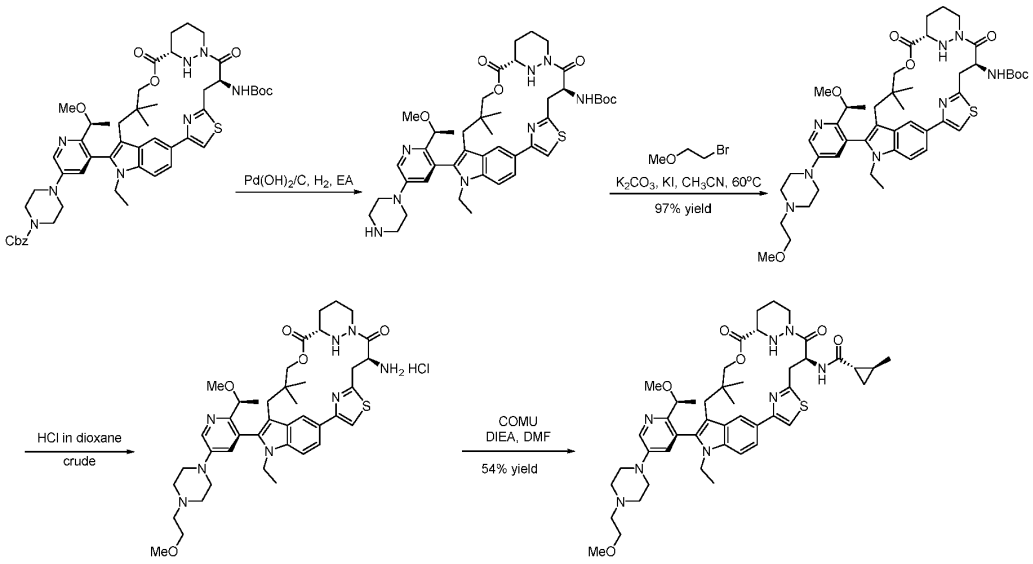
Example A99. (1*S*,2*S*)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-phenylcyclopropane-1-carboxamide



To a solution of *tert*-butyl ((6^3*S*,4*S*,*Z*)-11-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (50.0 mg, 0.069 mmol), (1*S*,2*S*)-2-phenylcyclopropane-1-carboxylic acid (16.69 mg, 0.103 mmol), and DIPEA (44.32 mg, 0.343 mmol) in DMF (0.50 mL) at 0 °C was added HATU (78.24 mg, 0.206 mmol). The resulting mixture was warmed to room temperature and stirred for 3 h. The crude product was purified by prep-HPLC to give (1*S*,2*S*)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-phenylcyclopropane-1-carboxamide (34 mg, 51% yield) as an off-white solid. LCMS (ESI): m/z [M+H] calc'd for $C_{49}H_{60}N_8O_5S$ 873.5; found 874.1.

Example A121. Synthesis of (1*S*,2*S*)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-(2-methoxyethyl)piperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-

hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methylcyclopropane-1-carboxamide



Step 1

A mixture of benzyl 4-(5-((6^3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-12-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (380 mg, 0.4 mmol) in EtOAc (10 mL) was added $Pd(OH)_2/C$ (600 mg, 20 mol%) was hydrogenated at rt overnight. The mixture was filtered through a pad of Celite® and the filtrate was concentrated under reduced pressure to give *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(piperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (310 mg, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{58}N_8O_6S$ 814.4; found 815.5.

Step 2

To a mixture of *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(piperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (300 mg, 0.37 mmol) and 1-bromo-2-methoxyethane (56 mg, 0.41 mmol) in MeCN (10 mL) at rt was added KI (61 mg, 0.37 mmol) and K_2CO_3 (51 mg, 0.37 mmol) in portions. The mixture was heated to 60 °C and stirred for 2 h, then diluted with H_2O (5 mL). The residue was purified by preparative-HPLC to give *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-(2-methoxyethyl)piperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (310 mg, 97% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{46}H_{64}N_8O_7S$ 872.5; found 873.6.

Step 3

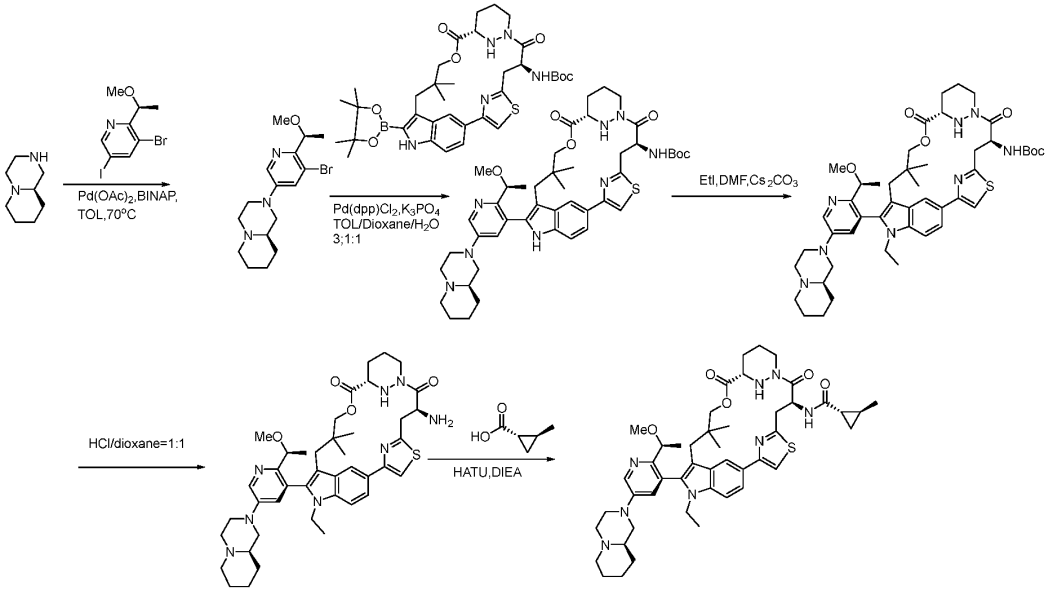
A mixture of *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-(2-methoxyethyl)piperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-

8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (300 mg, 0.34 mmol) in 4 M HCl in 1,4-dioxane, (10 mL) was stirred at rt for 1 h, then concentrated under reduced pressure to give (6^3S,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-(2-methoxyethyl)piperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione HCl salt (315 mg, crude) as a solid, which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{56}N_8O_5S$ 772.4; found 773.3.

Step 4

A mixture of (6^3S,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-(2-methoxyethyl)piperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione HCl salt (300 mg, 0.39 mmol) and (1*S*,2*S*)-2-methylcyclopropane-1-carboxylic acid (97 mg, 0.97 mmol) in DMF (5 mL) at 0 °C was added DIPEA (1.00 g, 7.77 mmol) dropwise, then COMU (249 mg, 0.58 mmol) in portions. The mixture was allowed to warm to rt and stirred for 2 h, then concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (1*S*,2*S*)-*N*-((6^3S,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-(2-methoxyethyl)piperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methylcyclopropane-1-carboxamide (178 mg, 54% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{46}H_{62}N_8O_6S$ 854.5; found 855.5; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 - 8.46 (m, 2H), 7.80 (d, *J* = 3.1 Hz, 1H), 7.77 - 7.70 (m, 1H), 7.57 (d, *J* = 8.6 Hz, 1H), 7.40 (d, *J* = 2.8 Hz, 1H), 5.55 (d, *J* = 9.2 Hz, 1H), 4.37 - 4.09 (m, 6H), 4.00 (s, 2H), 3.83 - 3.71 (m, 2H), 3.57 (s, 3H), 3.38 (t, *J* = 4.8 Hz, 2H), 3.32 (d, *J* = 2.5 Hz, 4H), 3.22 (s, 7H), 3.20 - 3.11 (m, 1H), 2.94 (d, *J* = 14.4 Hz, 1H), 2.76 (t, *J* = 11.4 Hz, 1H), 2.44 (d, *J* = 14.2 Hz, 1H), 2.07 (d, *J* = 12.0 Hz, 1H), 1.80 (s, 2H), 1.60 - 1.47 (m, 2H), 1.34 (d, *J* = 6.1 Hz, 3H), 1.07 (d, *J* = 1.8 Hz, 4H), 0.95 - 0.82 (m, 7H), 0.55 (d, *J* = 7.4 Hz, 1H), 0.35 (s, 3H).

Example A157. Synthesis of (1*S*,2*S*)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-((*R*)-octahydro-2*H*-pyrido[1,2-*a*]pyrazin-2-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methylcyclopropane-1-carboxamide



Step 1

5-[(9*aR*)-octahydropyrido[1,2-a]pyrazin-2-yl]-3-bromo-2-[(1*S*)-1-methoxyethyl]pyridine was synthesized in a manner similar to (*S*)-8-(5-bromo-6-((*S*)-1-methoxyethyl)pyridin-3-yl)octahydropyrazino[2,1-*c*][1,4]oxazine except (*S*)-octahydropyrazino[2,1-*c*][1,4]oxazine was substituted with (*S*)-octahydropyrazino[2,1-*c*][1,4]oxazine (1.5 g, 60% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{16}H_{24}BrN_3O$ 353.1; found 354.1.

Step 2

Tert-butyl ((6^3*S*,4*S*,*Z*)-1^2-(2-((*S*)-1-methoxyethyl)-5-((*R*)-octahydro-2*H*-pyrido[1,2-*a*]pyrazin-2-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate was synthesized in a manner similar to *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^2-(5-((*S*)-hexahydropyrazino[2,1-*c*][1,4]oxazin-8(1*H*)-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate except (*S*)-8-(5-bromo-6-((*S*)-1-methoxyethyl)pyridin-3-yl)octahydropyrazino[2,1-*c*][1,4]oxazine was substituted with 5-[(9*aR*)-octahydropyrido[1,2-a]pyrazin-2-yl]-3-bromo-2-[(1*S*)-1-methoxyethyl]pyridine and K_2CO_3 was substituted with K_3PO_4 to give (800 mg, 83% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{45}H_{60}N_8O_6S$ 840.4; found 841.4.

Step 3

Tert-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-((*R*)-octahydro-2*H*-pyrido[1,2-*a*]pyrazin-2-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate was synthesized in a

manner similar to *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(5-((*S*)-hexahydropyrazino[2,1-*c*][1,4]oxazin-8(1*H*)-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,66-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate except *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^2-(5-((*S*)-hexahydropyrazino[2,1-*c*][1,4]oxazin-8(1*H*)-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate was substituted with *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^2-(2-((*S*)-1-methoxyethyl)-5-((*R*)-octahydro-2*H*-pyrido[1,2-*a*]pyrazin-2-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate to give (220 mg, 27% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{47}H_{64}N_8O_6S$ 868.5; found 869.5.

Step 4

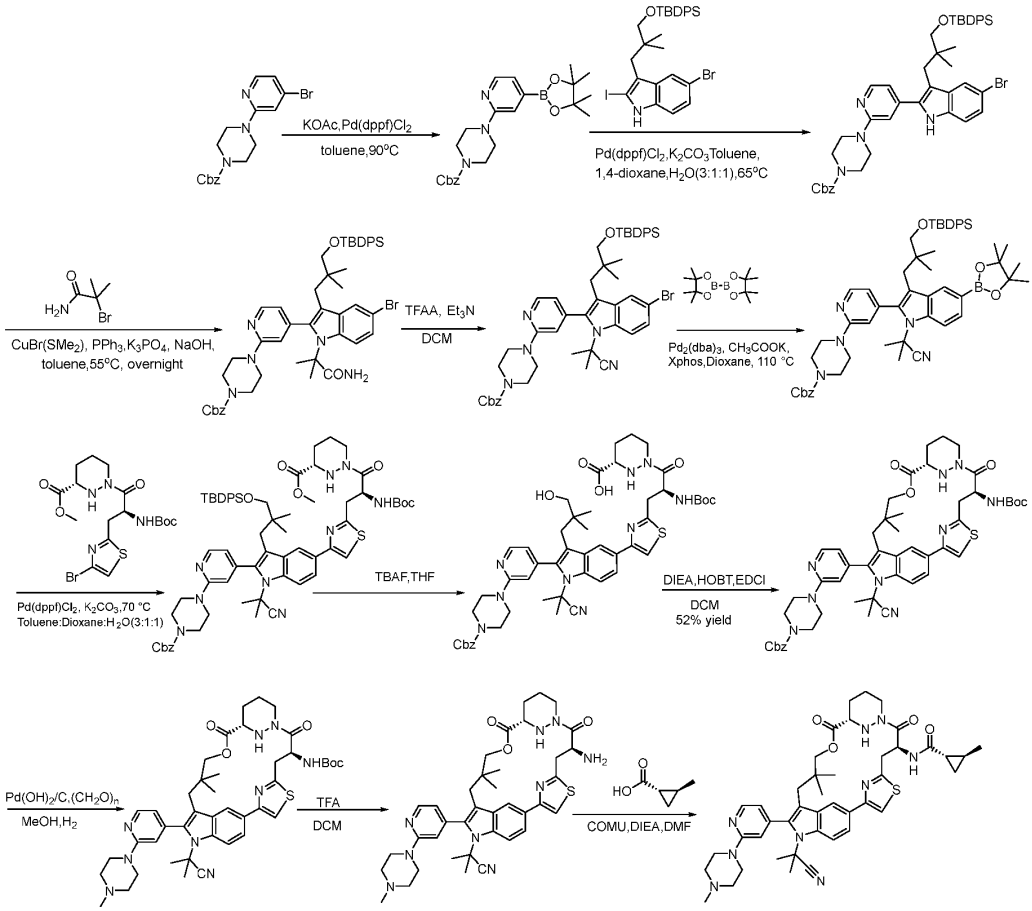
A mixture of *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-((*R*)-octahydro-2*H*-pyrido[1,2-*a*]pyrazin-2-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (220 mg, 0.25 mmol) in 1,4-dioxane (2 mL) at 0 °C was added 4M HCl in 1,4-dioxane (1 mL). The mixture was stirred at 0 °C for 1 h then concentrated under reduced pressure to give (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-((*R*)-octahydro-2*H*-pyrido[1,2-*a*]pyrazin-2-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (220 mg, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{56}N_8O_4S$ 768.4; found 769.4.

Step 5

(1*S*,2*S*)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-((*R*)-octahydro-2*H*-pyrido[1,2-*a*]pyrazin-2-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methylcyclopropane-1-carboxamide was synthesized in a manner similar to (1*S*,2*S*)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(5-((*S*)-hexahydropyrazino[2,1-*c*][1,4]oxazin-8(1*H*)-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methylcyclopropane-1-carboxamide except (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(5-((*S*)-hexahydropyrazino[2,1-*c*][1,4]oxazin-8(1*H*)-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione HCl salt was substituted with (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-((*R*)-octahydro-2*H*-pyrido[1,2-*a*]pyrazin-2-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione to give (13 mg, 8% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{47}H_{62}N_8O_5S$ 850.5; found 851.6; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 - 8.41 (m, 3H), 7.79 (s, 1H), 7.72 - 7.70 (m, 1H), 7.55 - 7.30 (m, 1H), 7.20 - 7.10 (m, 1H), 5.56 - 5.46 (m, 1H), 5.08 - 5.00 (m, 1H), 4.39 - 4.04 (m, 5H), 3.72 - 7.62 (m, 2H), 3.57 - 3.47 (m, 2H), 3.21 - 3.11 (m, 3H), 3.15 - 3.08 (m, 1H), 2.94 (m, 1H), 2.79 - 2.69 (m, 4H), 2.45 - 2.35 (m, 3H), 2.24 - 2.22 (m, 1H), 2.08 - 2.00 (m, 1H), 2.01 - 1.88 (m, 2H), 1.81 - 1.65 (m, 3H), 1.59 (d, *J* = 12.1 Hz, 2H), 1.54 -

1.38 (m, 2H), 1.33 - 1.30m, 3H), 1.28 - 1.12 (m, 3H), 1.06 - 0.86 (m, 4H), 0.96 - 0.79 (m, 6H), 0.55 - 0.50 (m, 1H), 0.34 (s, 3H).

Example A214. Synthesis of (1*S*,2*S*)-*N*-((6^3*S*,4*S*,*Z*)-1^1-(2-cyanopropan-2-yl)-10,10-dimethyl-1^2-(2-(4-methylpiperazin-1-yl)pyridin-4-yl)-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methylcyclopropane-1-carboxamide



Step 1

A mixture of benzyl 4-(4-bromopyridin-2-yl)piperazine-1-carboxylate (8.09 g, 21.5 mmol), bis(pinacolato)diboron (8.19 g, 32.3 mmol), KOAc (6.33 g, 64.5 mmol), Pd(dppf)Cl_2 (0.79 g, 1.1 mmol) in toluene (100 mL) under an atmosphere of Ar was heated to 90 °C and stirred for 2 h. The mixture was concentrated under vacuum, H2O (50 mL) was added to the residue and the mixture was extracted with EtOAc (2 x 100 mL). The combined organic layers were washed with brine (2 x 100 mL), dried, filtered and the filtrate concentrated under reduced pressure to give benzyl 4-[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl]piperazine-1-carboxylate (9.2 g, 100% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{30}BN_3O_4$ 423.2; found 424.2.

Step 2

A mixture of benzyl 4-[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl]piperazine-1-carboxylate (5.00 g, 11.8 mmol), 5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-2-iodo-1*H*-indole (7.64 g, 11.8 mmol), $Pd(dppf)Cl_2$ (0.86 g, 1.2 mmol), K_2CO_3 (6.27 g, 45.4 mmol) in toluene (45 mL), 1,4-dioxane (15 mL), H_2O (15 mL) under an atmosphere of N_2 was heated to 70 °C and stirred for 2 h. H_2O (50 mL) was added and the mixture was extracted with EtOAc (2 x 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give benzyl 4-[4-(5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1*H*-indol-2-yl)pyridin-2-yl]piperazine-1-carboxylate (4.9 g, 51% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{46}H_{51}BrN_4O_3Si$ 814.3; found 815.4.

Step 3

A mixture of benzyl 4-[4-(5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1*H*-indol-2-yl)pyridin-2-yl]piperazine-1-carboxylate (4.5 g, 5.5 mmol), 2-bromo-2-methylpropanamide (2.75 g, 16.6 mmol), K_3PO_4 (2.34 g, 11.0 mmol), NaOH (0.57 g, 14.3 mmol), Ph_3P (0.29 g, 1.1 mmol), copper bromide-dimethyl sulfide (0.23 g, 1.1 mmol) in toluene (50 mL) under an atmosphere of N_2 was heated to 45 °C and stirred for 2 days. H_2O (50 mL) was added and the mixture was extracted with EtOAc (2 x 50 mL). The combined organic layers were washed with brine (2 x 50 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give benzyl 4-[4-(5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1-(1-carbamoyl-1-methylethyl)indol-2-yl)pyridin-2-yl]piperazine-1-carboxylate (1.5 g, 30% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{58}BrN_5O_4Si$ 899.3; found 900.4.

Step 4

A mixture of benzyl 4-[4-(5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1-(1-carbamoyl-1-methylethyl)indol-2-yl)pyridin-2-yl]piperazine-1-carboxylate (1.40 g, 1.6 mmol), Et_3N (0.47 g, 4.7 mmol) and TFAA (0.65 g, 3.1 mmol) in DCM (20 mL) , 3 equiv) was stirred at rt for 2 h. H_2O (20 mL) was added and the mixture was extracted with DCM (2 x 20 mL). The combined organic layers were washed with brine (2 x 50 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give benzyl 4-[4-(5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1-(1-cyano-1-methylethyl)indol-2-yl)pyridin-2-yl]piperazine-1-carboxylate (1.3 g, 95% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{56}BrN_5O_3Si$ 881.3; found 882.4.

Step 5

A mixture of benzyl 4-[4-(5-bromo-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1-(1-cyano-1-methylethyl)indol-2-yl)pyridin-2-yl]piperazine-1-carboxylate (1.50 g, 1.7 mmol), bis(pinacolato)diboron (5.21 g, 20.5 mmol), $Pd_2(dba)_3$ (0.38 g, 0.4 mmol), KOAc (1.21 g, 12.3 mmol), X-Phos (0.20 g, 0.4 mmol) in 1,4-dioxane (25 mL) under an atmosphere of N_2 was heated to 110 °C and stirred for 2 h. H_2O (25 mL) was added and the mixture was extracted with EtOAc (2 x 25 mL). The combined organic layers were washed with brine (2 x 25 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica

gel column chromatography to give benzyl 4-[4-(3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1-(1-cyano-1-methylethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-2-yl)pyridin-2-yl]piperazine-1-carboxylate (1.6 g, 94% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{56}H_{68}BN_5O_5Si$ 929.5; found 930.4.

Step 6

A mixture of benzyl 4-[4-(3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1-(1-cyano-1-methylethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-2-yl)pyridin-2-yl]piperazine-1-carboxylate (1.60 g, 1.7 mmol), methyl (3*S*)-1-[(2*S*)-3-(4-bromo-1,3-thiazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]propanoyl]-1,2-diazinane-3-carboxylate (0.82 g, 1.7 mmol), K_2CO_3 (0.79 g, 5.8 mmol), $Pd(dppf)Cl_2$ (0.13 g, 0.17 mmol) in toluene (12 mL), 1,4-dioxane (4 mL) and H_2O (4 mL) under an atmosphere of N_2 was heated to 70 °C and stirred for 5 h. H_2O (30 mL) was added and the mixture was extracted with EtOAc (2 x 30 mL). The combined organic layers were washed with brine (2 x 30 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give benzyl 4-[4-(5-[2-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[(3*S*)-3-(methoxycarbonyl)-1,2-diazinan-1-yl]-3-oxopropyl]-1,3-thiazol-4-yl]-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1-(1-cyano-1-methylethyl)indol-2-yl)pyridin-2-yl]piperazine-1-carboxylate (650 mg, 31% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{67}H_{81}N_9O_8SSi$ 1199.6; found 1200.5.

Step 7

To a mixture of benzyl 4-[4-(5-[2-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-[(3*S*)-3-(methoxycarbonyl)-1,2-diazinan-1-yl]-3-oxopropyl]-1,3-thiazol-4-yl]-3-[3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1-(1-cyano-1-methylethyl)indol-2-yl)pyridin-2-yl]piperazine-1-carboxylate (650 mg, 0.54 mmol) in THF (15 mL) under an atmosphere of N2 was added TBAF (1.42 g, 5.4 mmol). The mixture was stirred at rt overnight then the mixture adjusted to pH ~6 with 1M HCl and extracted with EtOAc (2 x 50 mL). The combined organic layers were washed with brine (2 x 50 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (3*S*)-1-[(2*S*)-3-[4-[2-(2-[4-[(benzyloxy)carbonyl]piperazin-1-yl]pyridin-4-yl)-1-(1-cyano-1-methylethyl)-3-(3-hydroxy-2,2-dimethylpropyl)indol-5-yl]-1,3-thiazol-2-yl]-2-[(*tert*-butoxycarbonyl)amino]propanoyl]-1,2-diazinane-3-carboxylic acid (370 mg, 72% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{61}N_9O_8S$ 947.4; found 948.5.

Step 8

A mixture of (3*S*)-1-[(2*S*)-3-[4-[2-(2-[4-[(benzyloxy)carbonyl]piperazin-1-yl]pyridin-4-yl)-1-(1-cyano-1-methylethyl)-3-(3-hydroxy-2,2-dimethylpropyl)indol-5-yl]-1,3-thiazol-2-yl]-2-[(*tert*-butoxycarbonyl)amino]propanoyl]-1,2-diazinane-3-carboxylic acid (370 mg, 0.39 mmol), DIPEA (1.51 g, 11.7 mmol), HOBT (264 mg, 1.95 mmol), EDCI (2.09 g, 10.9 mmol) in DCM (370 mL) was stirred at rt overnight. H_2O (100 mL) was added and the mixture was extracted with EtOAc (2 x 100 mL). The combined organic layers were washed with brine (2 x 100 mL), dried over Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to give benzyl 4-(4-((6^3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-1^1-(2-cyanopropan-2-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-

hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-12-yl)pyridin-2-yl)piperazine-1-carboxylate (187 mg, 52% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{59}N_9O_7S$ 929.4; found 930.8.

Step 9

A mixture of benzyl 4-(4-((6^3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-1^1-(2-cyanopropan-2-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-12-yl)pyridin-2-yl)piperazine-1-carboxylate (170 mg, 0.18 mmol), paraformaldehyde (165 mg, 1.8 mmol), $Pd(OH)_2$/C (170 mg, 1.2 mmol) in MeOH (25 mL) was stirred under an atmosphere of H_2 overnight. The mixture was filtered and the filtrate was concentrated under reduced pressure to give *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-(2-cyanopropan-2-yl)-10,10-dimethyl-1^2-(2-(4-methylpiperazin-1-yl)pyridin-4-yl)-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (110 mg, 74% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{55}N_9O_5S$ 809.4; found 810.9.

Step 10

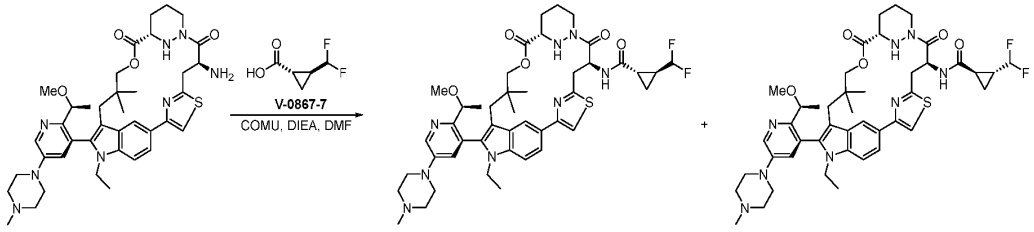
A mixture of *tert*-butyl ((6^3*S*,4*S*,*Z*)-1^1-(2-cyanopropan-2-yl)-10,10-dimethyl-1^2-(2-(4-methylpiperazin-1-yl)pyridin-4-yl)-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (110 mg, 0.14 mmol) and TFA (5.0 mL, 67.3 mmol) in DCM (5 mL) was stirred at rt for 1 h, then concentrated under reduced pressure to give 2-((6^3*S*,4*S*,*Z*)-4-amino-10,10-dimethyl-1^2-(2-(4-methylpiperazin-1-yl)pyridin-4-yl)-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-1^1-yl)-2-methylpropanenitrile (96 mg, 100% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{47}N_9O_3S$ 709.4; found 710.5.

Step 11

A mixture of 2-((6^3*S*,4*S*,*Z*)-4-amino-10,10-dimethyl-1^2-(2-(4-methylpiperazin-1-yl)pyridin-4-yl)-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-1^1-yl)-2-methylpropanenitrile (110 mg, 0.16 mmol), (1*S*,2*S*)-2-methylcyclopropane-1-carboxylic acid (47 mg, 0.47 mmol), COMU (66 mg, 0.16 mmol), DIPEA (1.00 g, 7.75 mmol) in DMF (5 mL) was stirred at rt for 2 h. H_2O (10 mL) was added and the mixture was extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with brine (2 x 10 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (1*S*,2*S*)-*N*-((6^3*S*,4*S*,*Z*)-1^1-(2-cyanopropan-2-yl)-10,10-dimethyl-1^2-(2-(4-methylpiperazin-1-yl)pyridin-4-yl)-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methylcyclopropane-1-carboxamide (21 mg ,17% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{53}N_9O_4S$ 791.4; found 792.4; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.51 (d, *J* = 7.1 Hz, 2H), 8.21 (d, *J* = 5.0 Hz, 1H), 8.16 (d, *J* = 5.0 Hz, 1H), 7.89 (d, *J* = 1.8 Hz, 1H), 7.83 (d, *J* = 1.5 Hz, 2H), 7.06 (s, 1H), 6.96 (s, 1H), 6.74 - 6.68 (m, 1H), 5.54 (q, *J* = 8.6 Hz, 1H), 5.04 (d, *J* = 12.2 Hz, 1H), 4.20 (q, *J* = 12.4 Hz, 2H), 3.66 (dd, *J* = 16.0, 10.9 Hz, 1H), 3.55 (s, 5H), 3.50 (d, *J* = 10.9 Hz, 1H), 3.35 (s, 1H), 3.15 (dd, *J* = 14.8, 9.2 Hz, 1H), 2.93 (dd, *J* = 14.4, 6.3 Hz, 1H), 2.77 (s, 1H), 2.38 (dd, *J* =

10.9, 5.5 Hz, 4H), 2.20 (d, *J* = 5.3 Hz, 3H), 2.09 (s, 1H), 2.06 (s, 1H), 2.09 - 1.99 (m, 3H), 1.82 - 1.51 (d, J = 4.0 Hz, 4H), 1.07 (s, 4H), 0.90 - 0.86(d, *J* = 3.0 Hz, 4H), 0.55 (d, *J* = 6.8 Hz, 1H), 0.47 - 0.38 (m, 3H).

Examples A221 and A222. Synthesis of (1*S*,2*S*)-2-(difluoromethyl)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)cyclopropane-1-carboxamide and (1*R*,2*R*)-2-(difluoromethyl)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)cyclopropane-1-carboxamide

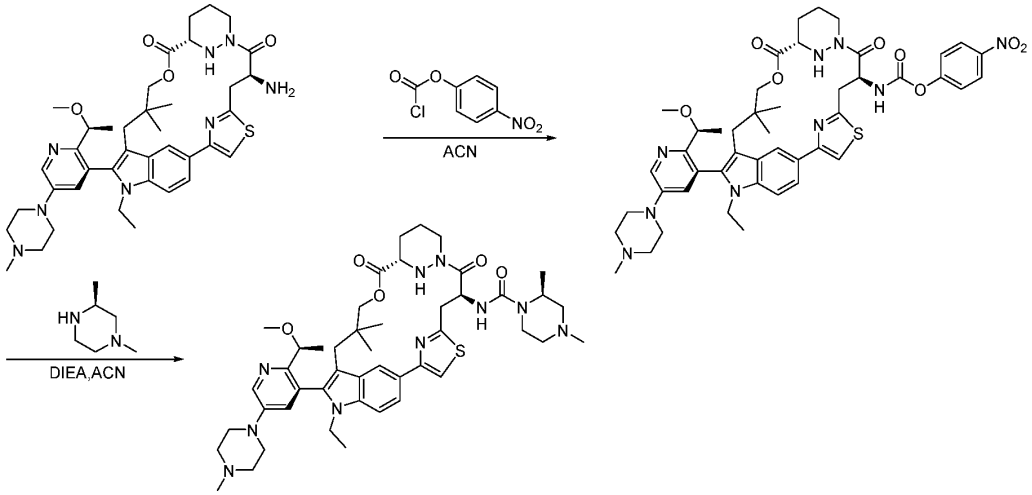


Step 1

To a mixture of (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (200 mg, 0.27 mmol) and *trans*-2-(difluoromethyl)cyclopropane-1-carboxylic acid (56 mg, 0.41 mmol) in DMF (8 mL) at 0 °C was added DIPEA (177 mg, 1.37 mmol) dropwise, followed by COMU (235 mg, 0.55 mmol). The mixture was allowed to warm to rt and stirred for 1 h, then diluted with EtOAc (10 mL) and H_2O (50 mL). The aqueous and organic layers were separated and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (2 x 10 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (1*S*,2*S*)-2-(difluoromethyl)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)cyclopropane-1-carboxamide (32mg, 27% yield) and (1*R*,2*R*)-2-(difluoromethyl)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)cyclopropane-1-carboxamide (31mg, 27% yield) both as solids. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{56}F_2N_8O_5S$ 846.4; found 847.3; ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.80 (s, 1H), 8.46 (dd, *J* = 13.4, 2.2 Hz, 2H), 7.83 (s, 2H), 7.73 (dd, *J* = 8.7, 1.6 Hz, 1H), 7.56 (d, *J* = 8.6 Hz, 1H), 7.23 (d, *J* = 2.8 Hz, 1H), 5.95 (d, *J* = 5.3 Hz, 1H), 5.57 (t, *J* = 9.1 Hz, 1H), 5.12 (d, *J* = 12.2 Hz, 1H), 4.14 (d, *J* = 6.4 Hz, 5H), 3.57 (s, 2H), 3.21 (s, 4H), 2.93 (d, *J* = 14.3 Hz, 2H), 2.78 - 2.68 (m, 1H), 2.67 (p, *J* = 1.9 Hz, 3H), 2.46 - 2.28 (m, 3H), 2.25 (s, 2H), 2.17 - 1.92 (m, 2H), 1.79 (s, 2H), 1.66 (dt, *J* = 9.7, 5.0 Hz, 1H), 1.51 (t, *J* = 9.0 Hz, 1H), 1.33 (d, *J* = 6.1 Hz,

4H), 1.24 (d, *J* = 5.6 Hz, 2H), 0.96 (s, 1H), 0.95 - 0.72 (m, 6H), 0.35 (s, 3H) and LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{56}F_2N_8O_5S$ 846.4; found 847.3; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.87 (d, *J* = 9.0 Hz, 1H), 8.46 (dd, *J* = 11.3, 2.2 Hz, 2H), 7.80 (s, 2H), 7.73 (dd, *J* = 8.7, 1.6 Hz, 1H), 7.56 (d, *J* = 8.7 Hz, 1H), 7.23 (d, *J* = 2.9 Hz, 1H), 6.09 (d, *J* = 5.1 Hz, 1H), 5.94 (d, *J* = 5.1 Hz, 1H), 5.80 (d, *J* = 5.2 Hz, 1H), 5.59 (t, *J* = 9.1 Hz, 1H), 5.13 (d, *J* = 12.2 Hz, 1H), 4.50 - 4.06 (m, 5H), 3.57 (s, 2H), 3.21 (s, 4H), 2.93 (d, *J* = 14.1 Hz, 2H), 2.67 (p, *J* = 1.9 Hz, 4H), 2.39 - 2.15 (m, 4H), 2.17 - 1.82 (m, 2H), 1.77 (d, *J* = 16.1 Hz, 2H), 1.44 (d, *J* = 43.3 Hz, 1H), 1.35 - 1.24 (m, 4H), 1.15 - 0.91 (m, 2H), 0.91 (s, 6H), 0.34 (s, 3H).

Example A173. Synthesis of (2*S*)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,4-dimethylpiperazine-1-carboxamide



Step 1

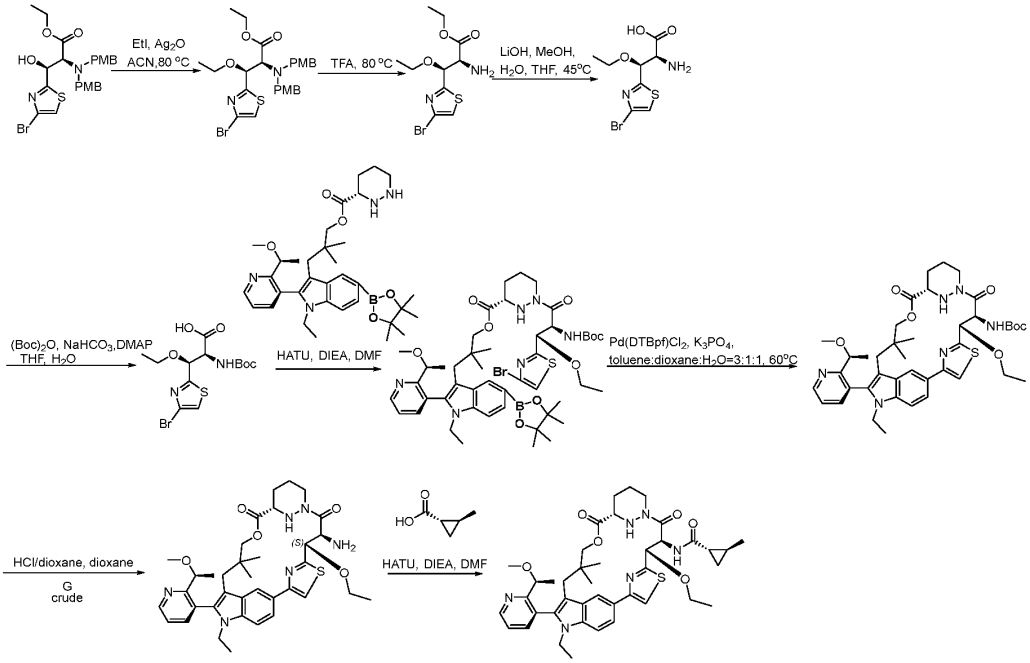
To a mixture of (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (80 mg, 0.11 mmol) in DCM (6 mL) at 0 °C was added pyridine (2 mL), then 4-nitrophenyl carbonochloridate (55 mg, 0.28 mmol) in portions. The mixture was allowed to warm to rt and stirred for 1 h at room temperature, then washed with 1M $NaHSO_4$ (10 mL) and H_2O (10 mL). The organic layer was concentrated under reduced pressure. to give 4-nitrophenyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (98 mg, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{46}H_{55}N_9O_8S$ 893.4; found 894.2.

Step 2

To a mixture of 4-nitrophenyl ((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (98 mg, 0.11

mmol) and (*S*)-1,3-dimethylpiperazine (63 mg, 0.55 mmol) in ACN (5 mL) at 0 °C was added DIPEA (43 mg, 0.33 mmol) in ACN (2 mL. The crude product was purified by preparative-HPLC to give (2*S*)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,4-dimethylpiperazine-1-carboxamide (13 mg, 13% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{46}H_{64}N_{10}O_5S$ 868.5; found 869.3; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.47 (s, 2H), 7.80 (s, 1H), 7.73 (s, 1H), 7.55 (s, 1H), 7.23 (s, 1H), 6.81 - 6.70 (m, 1H), 5.33 - 5.25 (m, 1H), 4.99 (s, 1H), 4.40 - 3.97 (m, 6H), 3.72 (s, 1H), 3.61 - 3.47 (m, 3H), 3.31 - 3.22 (m, 8H), 3.02 - 2.72 (m, 5H), 2.66 (s, 1H), 2.60 - 2.51 (m, 3H), 2.49 - 2.37 (m, 2H), 2.35 - 2.13 (m, 6H), 2.12 - 1.97 (m, 2H), 1.95 - 1.66 (m, 3H), 1.55 (s, 1H), 1.33 (s, 3H), 1.27 - 1.19 (m, 4H), 0.99 - 0.82 (m, 6H), 0.33 (s, 3H).

Example A225. Synthesis of (1*S*,2*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methylcyclopropane-1-carboxamide



Step 1

To a mixture of ethyl (2*S*,3*S*)-2-[*bis*[(4-methoxyphenyl)methyl]amino]-3-(4-bromo-1,3-thiazol-2-yl)-3-hydroxypropanoate (1.00 g, 1.9 mmol) and Ag_2O (4.33 g, 18.7 mmol) in ACN (10 mL) at 0 °C under an atmosphere of N_2 was added ethyl iodide (2.91 g, 18.7 mmol) dropwise. The mixture was heated to 80 °C and stirred for 4 h, then filtered and the filter cake was washed with ACN (3 x 5 mL). The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give ethyl (2*S*,3*S*)-2-[*bis*[(4-methoxyphenyl)methyl]amino]-

3-(4-bromo-1,3-thiazol-2-yl)-3-ethoxypropanoate (557 mg, 53% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{26}H_{31}BrN_2O_5S$ 562.1; found 563.2.

Step 2

Into a 40 mL sealed tube were added ethyl (2*S*,3*S*)-2-[*bis*[(4-methoxyphenyl)methyl]amino]-3-(4-bromo-1,3-thiazol-2-yl)-3-ethoxypropanoate (530 mg) and TFA (10 mL) under an atmosphere of N_2. The mixture was heated to 80 °C and stirred overnight, then concentrated under reduced pressure to give ethyl (2*S*,3*S*)-2-amino-3-(4-bromo-1,3-thiazol-2-yl)-3-ethoxypropanoate that was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{10}H_{15}BrN_2O_3S$ 322.0; found 323.0.

Step 3

A mixture of ethyl (2*S*,3*S*)-2-amino-3-(4-bromo-1,3-thiazol-2-yl)-3-ethoxypropanoate (890 mg, 2.8 mmol), $LiOH.H_2O$ (1.16 g, 27.6 mmol), MeOH (6 mL), THF (2 mL) and H_2O (2 mL) was stirred at 45 °C for 2 h. The mixture was concentrated under reduced pressure to give (2*S*,3*S*)-2-amino-3-(4-bromo-1,3-thiazol-2-yl)-3-ethoxypropanoic acid (that was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_8H_{11}BrN_2O_3S$ 294.0; found 294.9.

Step 4

To a mixture of (2*S*,3*S*)-2-amino-3-(4-bromo-1,3-thiazol-2-yl)-3-ethoxypropanoic acid (890 mg, 3.0 mmol), $NaHCO_3$ (507 mg, 6.0 mmol) and DMAP (37 mg, 0.3 mmol) in THF / H_2O (1:1) at 0 °C was added $(Boc)_2O$ (1.97 g, 9.0 mmol). The mixture was warmed to rt and stirred overnight then concentrated under reduced pressure to remove THF and the residue was acidified to pH ~6 with HCl. The mixture was extracted with EtOAc (3 x 5mL) and the combined organic layers were dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (2*S*,3*S*)-3-(4-bromo-1,3-thiazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]-3-ethoxypropanoic acid (369 mg, 31 % yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{19}BrN_2O_5S$ 394.0; found 395.0.

Step 5

To a mixture of 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl-(*S*)-hexahydropyridazine-3-carboxylate (584 mg, 0.91 mmol) and (2*S*,3*S*)-3-(4-bromo-1,3-thiazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]-3-ethoxypropanoic acid (360 mg, 0.91 mmol) in DMF at 0 °C was added DIPEA (1.59 mL, 9.1 mmol) and HATU (693 mg, 1.8 mmol). The mixture was allowed to warm to rt and stirred for 1 h at room temperature, then cooled to 0 °C and H2O added. The mixture was extracted with EtOAc (2 x 5 mL) and the combined organic layers were washed with brine (3 x 10 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl (*S*)-1-((2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-ethoxypropanoyl)hexahydropyridazine-3-carboxylate (410 mg, 46 %) yield as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{47}H_{66}BBrN_6O_9S$ 980.4; found 981.3.

Step 6

Into a 50 mL Schlenk tube were added 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl (*S*)-1-((2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-ethoxypropanoyl)hexahydropyridazine-3-carboxylate (390 mg, 0.4 mmol) , Pd(DTBpf)Cl_2 (78 mg, 0.12 mmol), K_3PO_4 (211 mg, 1.0 mmol), toluene (9 mL), 1,4-dioxane (3 mL) and H_2O (3 mL) under an atmosphere of Ar. The mixture was heated to 60 °C and stirred for 1 h, then extracted with EtOAc (3 x 15 mL). The combined organic layers were dried over anhydrous Na2SO4, filtered, the filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (96 mg, 31% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{54}N_6O_7S$ 774.4; found 775.4.

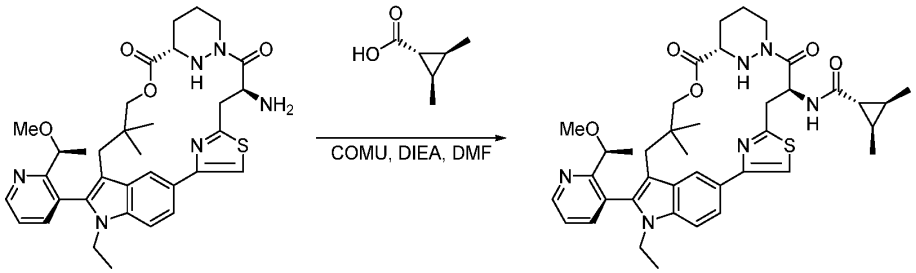
Step 7

A mixture of *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (92 mg, 0.12 mmol), HCl in 1,4-dioxane (2.5 mL) and 1,4-dioxane (2.5 mL) was stirred at rt for 1 h. The mixture was concentrated under reduced pressure to give *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (100 mg), which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{36}H_{46}N_6O_5S$ 674.3; found 675.3.

Step 8

To a mixture of *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (90 mg, 0.13 mmol) and (1*S*,2*S*)-2-methylcyclopropane-1-carboxylic acid (27 mg, 0.27 mmol) in DMF at 0 °C was added DIPEA (172 mg, 1.3 mmol) and HATU (101 mg, 0.27 mmol). The mixture was allowed to warm to rt and stirred for 1 h, then cooled to 0 °C, H_2O added and the mixture extracted with EtOAc (2 x 5 mL). The combined organic layers were washed with brine (3 x 20 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (1*S*,2*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methylcyclopropane-1-carboxamide (21 mg, 21% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{52}N_6O_6S$ 756.4; found 757.6; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (dd, *J* = 4.8, 1.7 Hz, 1H), 8.50 (s, 1H), 7.91 (s, 1H), 7.84 - 7.71 (m, 3H), 7.61 - 7.56 (m, 1H), 7.55 - 7.50 (m, 1H), 5.91 - 5.85 (m, 1H), 5.20 - 5.14 (m, 1H), 4.92 (s, 1H), 4.36 - 4.21 (m, 3H), 4.17 - 4.07 (m, 2H), 3.68 - 3.56 (m 3H), 3.54 - 3.46 (m, 1H), 3.22 (s, 3H), 2.90 - 2.73 (m, 2H), 2.09 - 2.03 (m, 1H), 1.88 - 1.73 (m,3H), 1.55 - 1.43 (m, 1H), 1.37 (d, *J* = 6.0 Hz, 3H), 1.20 (t, *J* = 6.9 Hz, 3H), 1.04 (s,4H), 0.93 - 0.71 (m, 7H), 0.51 (d, *J* = 6.3 Hz, 1H), 0.38 (s, 3H).

Example A227. Synthesis of (1*R*,2*R*,3*S*)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide



Step 1

To a mixture of (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (126 mg, 0.20 mmol) and (1*R*,2*R*,3*S*)-2,3-dimethylcyclopropane-1-carboxylic acid (34 mg, 0.30 mmol) in DMF (5 mL) at 0 °C was added DIPEA (129 mg, 1.0 mmol) and COMU (171 mg, 0.4 mmol). The mixture was warmed to rt and stirred for 1 h, then diluted with EtOAc (20 mL) and H_2O (20 mL). The aqueous layer was extracted with EtOAc (3 x 10 mL) and the combined organic layers were washed with brine (2 x 10 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (1*R*,2*R*,3*S*)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide (29mg, 25% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{40}H_{50}N_6O_5S$ 726.4; found 727.3; ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.75 (dd, *J* = 4.8, 1.7 Hz, 1H), 8.49 (d, *J* = 1.6 Hz, 1H), 8.40 (d, *J* = 9.0 Hz, 1H), 7.82 (s, 3H), 7.58 (d, *J* = 8.6 Hz, 1H), 7.52 (dd, *J* = 7.7, 4.7 Hz, 1H), 5.56 (t, *J* = 9.0 Hz, 1H), 5.07 (d, *J* = 12.2 Hz, 1H), 4.44 - 3.99 (m, 5H), 3.57 (s, 1H), 3.25 (s, 1H), 3.16 (d, *J* = 9.3 Hz, 3H), 2.94 (d, *J* = 14.3 Hz, 1H), 2.81 - 2.70 (m, 1H), 2.67 (p, *J* = 1.9 Hz, 1H), 2.44 - 2.27 (m, 1H), 2.16 - 2.01 (m, 1H), 1.78 (s, 2H), 1.53 (s, 1H), 1.37 (d, *J* = 6.0 Hz, 3H), 1.26 - 1.13 (m, 3H), 1.07 (dd, *J* = 9.4, 5.4 Hz, 6H), 0.93 - 0.77 (m, 6H), 0.32 (s, 3H).

The following table of compounds were prepared using the aforementioned methods or variations thereof, as would be known to those of skill in the art.

Table 4: Exemplary Compounds Prepared by Methods of the Present Invention

Ex#	LCMS (ESI): m/z [M+H] Found	Ex#	LCMS (ESI): m/z [M+H] Found
A1	799.2	**A178**	730.2
A2	827.5	**A179**	852.4
A3	827.5	**A180**	850.5
A4	809.5	**A181**	886.6
A5	757.4	**A182**	829
A6	757.4	**A183**	829
A7	756.4	**A184**	852.1
A8	784.2	**A185**	727.2
A9	770.2	**A186**	727.2
A10	836.2	**A187**	924.6
A11	836.3	**A188**	924.6
A12	738.3	**A189**	856.6
A13	738.3	**A190**	765.7
A14	714.1	**A191**	756.8
A15	853.4	**A192**	760.8
A16	853.2	**A193**	790.8
A17	869.2	**A194**	742.7
A18	869.2	**A195**	742.6
A19	880.3	**A196**	742.4
A20	826.2	**A197**	742.9
A21	894.1	**A198**	783
A22	840.2	**A199**	864.6
A23	757.4	**A200**	864.5
A24	820.2	**A201**	904.7
A25	800.3	**A202**	891.7
A26	786.4	**A203**	898.9
A27	786.4	**A204**	790.4
A28	875.6	**A205**	823.7
A29	865.5	**A206**	823.8
A30	823.5	**A207**	841.6
A31	823.5	**A208**	841.6
A32	853.5	**A209**	853.8
A33	853.5	**A210**	764.4
A34	806.8	**A211**	764.3

A35	757.7	**A212**	772.3
A36	811.7	**A213**	861.6
A37	763.7	**A214**	792.8
A38	820.4	**A215**	911.7
A39	800.5	**A216**	783.6
A40	882.2	**A217**	772.7
A41	881.9	**A218**	758.4
A42	804.6	**A219**	757.9
A43	804.6	**A220**	839.7
A44	798.2	**A221**	847.3
A45	798.2	**A222**	847.3
A46	784.3	**A223**	837
A47	796.4	**A224**	772.6
A48	796.4	**A225**	757.6
A49	768.4	**A226**	800.6
A50	818.2	**A227**	727.3
A51	796.2	**A228**	870.7
A52	785.2	**A229**	810.7
A53	837.5	**A230**	839.9
A54	837.6	**A231**	867.7
A55	841.5	**A232**	773.3
A56	861.2	**A233**	727.3
A57	833.1	**A234**	727.4
A58	881.4	**A235**	852.6
A59	827.2	**A236**	792.6
A60	776.7	**A237**	852.9
A61	776.7	**A238**	852.8
A62	776.7	**A239**	850.6
A63	776.7	**A240**	906.6
A64	757.7	**A241**	797.6
A65	775.7	**A242**	829.6
A66	713.6	**A243**	898.7
A67	769.7	**A244**	912.7
A68	749.7	**A245**	756.2
A69	803.7	**A246**	769.6
A70	803.7	**A247**	811.6
A71	770.7	**A248**	866.6
A72	770.8	**A249**	866.7
A73	790.8	**A250**	866.8
A74	790.8	**A251**	891.4
A75	787.7	**A252**	825.7
A76	787.8	**A253**	825.5

A77	782.8	**A254**	825.6
A78	777.8	**A255**	825.8
A79	777.8	**A256**	770.3
A80	776.7	**A257**	883.8
A81	776.37	**A258**	854.7
A82	771.8	**A259**	853.5
A83	771.8	**A260**	890.5
A84	770	**A261**	873.6
A85	758.7	**A262**	873.6
A86	757.7	**A263**	837.3
A87	755.7	**A264**	881.7
A88	738.7	**A265**	865.3
A89	738.7	**A266**	865.5
A90	741.7	**A267**	865.1
A91	725.7	**A268**	813.7
A92	738.7	**A269**	867.4
A93	724.7	**A270**	772.5
A94	743.7	**A271**	913.7
A95	729.8	**A272**	899.7
A96	759.5	**A273**	867.5
A97	759.4	**A274**	851.7
A98	867.9	**A275**	811.7
A99	874.1	**A276**	891.3
A100	815.9	**A277**	907.5
A101	768.5	**A278**	897.6
A102	743.4	**A279**	843.8
A103	756.5	**A280**	856.7
A104	771.5	**A281**	857.7
A105	801.6	**A282**	965.6
A106	771.2	**A283**	965.5
A107	797.6	**A284**	805.4
A108	724.4	**A285**	768.5
A109	724.4	**A286**	850.9
A110	800.4	**A287**	909.6
A111	784.4	**A288**	925.8
A112	784.4	**A289**	855.6
A113	796.4	**A290**	855.6
A114	784.4	**A291**	755.2
A115	791.4	**A292**	755.2
A116	791.4	**A293**	811.8
A117	743.4	**A294**	793.6
A118	825.5	**A295**	384.9

A119	825.5	**A296**	770.6
A120	853.5	**A297**	899.7
A121	855.5	**A298**	906.8
A122	811.5	**A299**	905.5
A123	811.6	**A300**	865.5
A124	865.5	**A301**	869.6
A125	724.3	**A302**	869.4
A126	867.4	**A303**	785.5
A127	813.5	**A304**	935.4
A128	713.2	**A305**	901.0
A129	742.3	**A306**	901.0
A130	742.3	**A307**	757.4
A131	757.3	**A308**	741.4
A132	772.4	**A309**	771.4
A133	738.3	**A310**	804.7
A134	738.3	**A311**	889.7
A135	836.3	**A312**	910.5
A136	836.2	**A313**	752.4
A137	770.2	**A314**	887.4
A138	784.2	**A315**	887.5
A139	756.4	**A316**	869.5
A140	757.4	**A317**	869.5
A141	757.4	**A318**	839.7
A142	770.4	**A319**	839.6
A143	809.5	**A320**	783.8
A144	827.5	**A321**	841.8
A145	827.5	**A322**	785.3
A146	400.4	**A323**	799.4
A147	835.5	**A324**	979.6
A148	835.5	**A325**	755.1
A149	862.6	**A326**	755.1
A150	862.5	**A327**	855.9
A151	804	**A328**	853.6
A152	804.5	**A329**	763.0
A153	818.5	**A330**	763.0
A154	818.5	**A331**	855.5
A155	756.4	**A332**	854.5
A156	799.4	**A333**	885.9
A157	851.6	**A334**	813.5
A158	771	**A335**	870.7
A159	812.5	**A336**	785.4
A160	850.6	**A337**	769.5

A161	892.6	**A338**	809.9
A162	743.4	**A339**	799.6
A163	856.5	**A340**	739.6
A164	711.4	**A341**	743.5
A165	795.8	**A342**	852.7
A166	875.6	**A343**	852.7
A167	823.5	**A344**	820.4
A168	837.7	**A345**	820.4
A169	825.6	**A346**	732.6
A170	822.5	**A347**	732.6
A171	822.7	**A348**	730.6
A172	822.5	**A349**	730.3
A173	869.3	**A350**	747.5
A174	917.7	**A351**	745.3
A175	917.7	**A352**	745.3
A176	746.4	**A353**	833.8
A177	728.2		

The following table of compounds were prepared using the aforementioned methods or variations thereof, as would be known to those of skill in the art, or the methods described below.

Table 5: Exemplary Compounds Prepared by Methods of the Present Invention

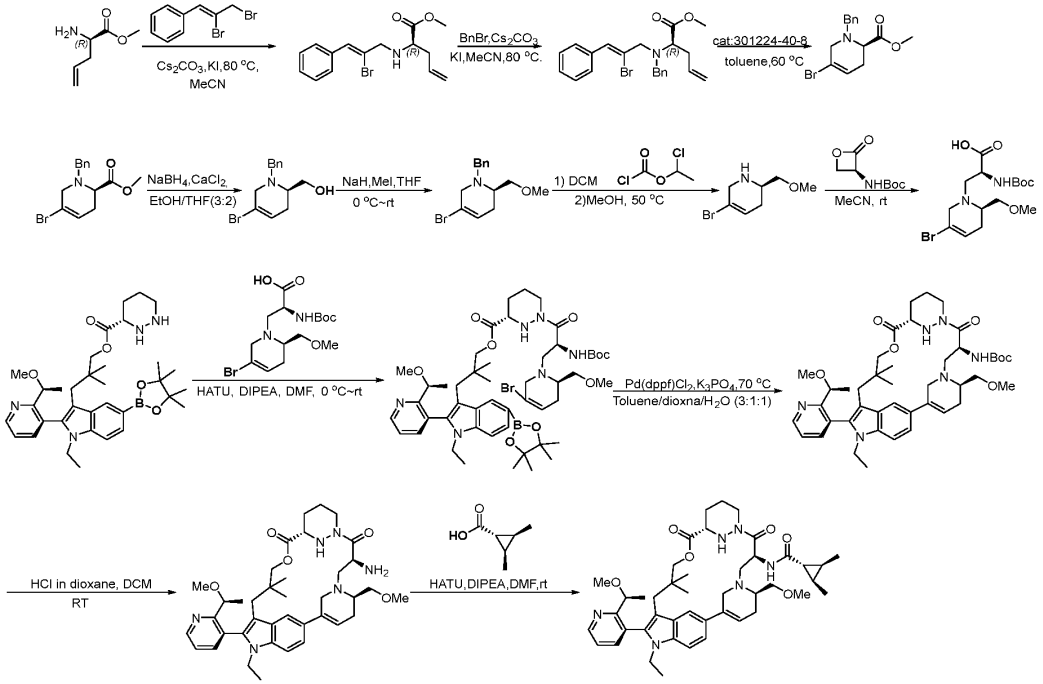
Ex#	LCMS (ESI): m/z [M+H] Found	Ex#	LCMS (ESI): m/z [M+H] Found
A354	870.8	**A482**	932.4
A355	743.5	**A483**	936.4
A356	738.5	**A484**	952.5
A357	783.4	**A485**	784.3
A358	744.5	**A486**	757.5
A359	758.6	**A487**	771.5
A360	758.6	**A488**	757.2
A361	728.4	**A489**	757.25
A362	747.6	**A490**	800.3
A363	747.5	**A491**	790.4
A364	745.6	**A492**	790.6
A365	731.6	**A493**	858.3
A366	807.4	**A494**	850.5
A367	789.4	**A495**	850.4
A368	746.5	**A496**	756.4
A369	747.4	**A497**	910.4
A370	827.4	**A498**	790.3
A371	842.0	**A499**	790.3
A372	769.2	**A500**	911.8
A373	897.4	**A501**	911.8
A374	778.3	**A502**	952.5

A375	806.4	**A503**	936.4
A376	758.4	**A504**	835.2
A377	871	**A505**	835.2
A378	760.5	**A506**	837.4
A379	760.35	**A507**	837.3
A380	760.5	**A508**	854.6
A381	784.4	**A509**	868.6
A382	758.5	**A510**	883.7
A383	733.4	**A511**	898.4
A384	825.3	**A512**	897.8
A385	741.2	**A513**	897.8
A386	741.2	**A514**	787.7
A387	807.5	**A515**	883.5
A388	840.3	**A516**	883.55
A389	910.7	**A517**	844.3
A390	910.4	**A518**	828.35
A391	809.8	**A519**	770.3
A392	768.6	**A520**	1004.3
A393	768.5	**A521**	968.9
A394	770.6	**A522**	835.5
A395	770.6	**A523**	821.3
A396	900.9	**A524**	811.5
A397	886.2	**A525**	793.4
A398	774.3	**A526**	755.2
A399	744.6	**A527**	796.6
A400	743.6	**A528**	796.3
A401	918.6	**A529**	812.5
A402	825.6	**A530**	812.3
A403	803.5	**A531**	840.2
A404	803.5	**A532**	840.2
A405	826.25	**A533**	824.2
A406	856.7	**A534**	824.2
A407	745.4	**A535**	884.3
A408	881.9	**A536**	884.3
A409	753.6	**A537**	884.25
A410	911.8	**A538**	946.3
A411	885.9	**A539**	1047.6
A412	760.6	**A540**	996.9
A413	784.3	**A541**	997.0
A414	741.4	**A542**	837.3
A415	769.5	**A543**	849.3
A416	829.7	**A544**	783.3
A417	820.4	**A545**	756.6
A418	866.8	**A546**	835.7
A419	755.3	**A547**	835.4
A420	755.3	**A548**	884.3

A421	856.7	**A549**	910.3
A422	739.5	**A550**	910.7
A423	740.4	**A551**	853.8
A424	741.4	**A552**	823.3
A425	763.2	**A553**	769.3
A426	941.8	**A554**	742.5
A427	941.3	**A555**	953.8
A428	910.7	**A556**	739.3
A429	899.2	**A557**	799.2
A430	742.3	**A558**	887.6
A431	756.6	**A559**	887.6
A432	756.6	**A560**	952.5
A433	898.9	**A561**	968.35
A434	885.4	**A562**	968.8
A435	746.6	**A563**	1011.7
A436	757.3	**A564**	995.3
A437	872.3	**A565**	1035.7
A438	927.9	**A566**	826.5
A439	820.2	**A567**	826.6
A440	828.3	**A568**	886.7
A441	828.2	**A569**	938.8
A442	866.5	**A570**	1032.4
A443	763.3	**A571**	1032.3
A444	738.1	**A572**	828.4
A445	770.2	**A573**	824.2
A446	756.7	**A574**	834.3
A447	742.7	**A575**	875.2
A448	813.6	**A576**	824.3
A449	910.8	**A577**	959.4
A450	976.9	**A578**	959.4
A451	840.7	**A579**	868.5
A452	856.4	**A580**	868.6
A453	838.7	**A581**	879.25
A454	838.4	**A582**	879.6
A455	799.4	**A583**	866.4
A456	813.6	**A584**	896.2
A457	827.6	**A585**	833.3
A458	771.2	**A586**	887.4
A459	958.4	**A587**	887.4
A460	928.4	**A588**	894.4
A461	894.8	**A589**	812.3
A462	813.5	**A590**	812.3
A463	820.7	**A591**	1031.7
A464	771.6	**A592**	1010.5
A465	771.6	**A593**	996.6
A466	757.7	**A594**	1010.4

A467	856.35	**A595**	868.6
A468	771.3	**A596**	858.5
A469	756.3	**A597**	865.2
A470	745.4	**A598**	918.6
A471	754.5	**A599**	868.6
A472	742.2	**A600**	868.25
A473	785.5	**A601**	872.3
A474	799.5	**A602**	879.6
A475	925.5	**A603**	884.6
A476	925.4	**A604**	884.4
A477	870.3	**A605**	884.7
A478	840.6	**A606**	880.2
A479	837.6	**A607**	896.7
A480	837.6	**A608**	904.3
A481	932.4		

Example A372. Synthesis of (1*r*,2*R*,3*S*)-*N*-((2^2*R*,6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-2^2-(methoxymethyl)-10,10-dimethyl-5,7-dioxo-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide



Step 1.

To a mixture of methyl (*R*)-2-aminopent-4-enoate (8.0 g, 36.5 mmol) and (*Z*)-(2,3-dibromoprop-1-en-1-yl)benzene (15.1 g, 54.8 mmol) in MECN (80 mL) under an atmosphere of N_2 was added Cs_2CO_3 (35.7 g, 109.6 mmol) and KI (12.13 g, 73.1 mmol) in portions. The mixture was

heated to 80 °C and stirred overnight, then filtered and the filter cake was washed with MeCN (3 x 20 mL). The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (*R*,*Z*)-2-((2-bromo-3-phenylallyl)amino)pent-4-enoate (12.0 g, 91% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{15}H_{18}BrNO_2$ 323.1; found 324.1.

Step 2.

To a mixture of methyl (*R*,*Z*)-2-((2-bromo-3-phenylallyl)amino)pent-4-enoate (12.0 g, 37.0 mmol) and BnBr (12.66 g, 74.0 mmol) in MeCN (120 mL) under an atmosphere of N_2 was added Cs_2CO_3 (24.12 g, 74.0 mmol) and KI (6.14 g, 37.0 mmol) in portions. The mixture was stirred at room temperature overnight, then filtered and the filter cake was washed with MeCN (3 x 20 mL). The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (*R*,*Z*)-2-(benzyl(2-bromo-3-phenylallyl)amino)pent-4-enoate (6.0 g, 35% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{22}H_{24}BrNO_2$ 415.1; found 416.1 [for ^{81}Br].

Step 3.

To a mixture of methyl (*R*,*Z*)-2-(benzyl(2-bromo-3-phenylallyl)amino)pent-4-enoate (5.8 g, 14.0 mmol) and [1,3-bis(2,4,6-trimethylphenyl)imidazolidin-2-ylidene]dichloro[[2-(propan-2-yloxy)phenyl]methylidene]ruthenium (2.63 g, 4.2 mmol) in toluene (580 mL) under an atmosphere of Ar was heated to 60 °C and stirred for 30 min. The mixture was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give methyl (*R*)-1-benzyl-5-bromo-1,2,3,6-tetrahydropyridine-2-carboxylate (3.7 g, 77% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{14}H_{16}BrNO_2$ 309.0; found 310.1.

Step 4.

To a mixture of methyl (*R*)-1-benzyl-5-bromo-1,2,3,6-tetrahydropyridine-2-carboxylate (3.7 g, 11.9 mmol) and $CaCl_2$ (2.65 g, 23.9 mmol) in EtOH (22 mL) and THF (15 mL) at 0 °C under an atmosphere of N_2 was added $NaBH_4$ (1.80 g, 47.7 mmol) in portions. The mixture was warmed to room temperature and stirred for 2 h, then cooled to 0 °C, and MeOH and H_2O were added. The mixture was extracted with DCM (2 x 50 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative HPLC to give (*R*)-(1-benzyl-5-bromo-1,2,3,6-tetrahydropyridin-2-yl)methanol (2.8 g, 75% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{13}H_{16}BrNO$ 281.0; found 282.3.

Step 5.

To a mixture of (*R*)-(1-benzyl-5-bromo-1,2,3,6-tetrahydropyridin-2-yl)methanol (1.0 g, 3.5 mmol) in THF (10 mL) at 0 °C was added NaH, 60% dispersion in oil (0.26 g, 10.6 mmol). The mixture was stirred for 15 min, then MeI (0.75 g, 5.3 mmol) was added and the mixture was allowed to warm to room temperature and stirred for 1 h. The mixture was cooled to 0 °C, saturated NH_4Cl was added and the mixture was extracted with EtOAc (2 x 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue

was purified by preparative-HPLC to give (*R*)-1-benzyl-5-bromo-2-(methoxymethyl)-1,2,3,6-tetrahydropyridine (1.0 g, 86% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{14}H_{18}BrNO$ 295.1; found 296.2.

Step 6.

A mixture of (*R*)-1-benzyl-5-bromo-2-(methoxymethyl)-1,2,3,6-tetrahydropyridine (1.0 g, 3.4 mmol) and 1-chloroethyl chloroformate (2 mL, 14.0 mmol) in DCM (10 mL) under an atmosphere of N_2 was stirred at room temperature overnight. The mixture was washed with brine (2 x 10 mL) and the combined aqueous layers were extracted with DCM (10mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. The residue was dissolved in MeOH (10 mL), the mixture was heated to 50 °C under an atmosphere of N_2 and stirred for 2 h, then concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (*R*)-5-bromo-2-(methoxymethyl)-1,2,3,6-tetrahydropyridine (500 mg, 72% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_7H_{12}BrNO$ 205.0; found 206.1.

Step 7.

To a mixture of (*R*)-5-bromo-2-(methoxymethyl)-1,2,3,6-tetrahydropyridine (600 mg, 2.9 mmol) and *tert*-butyl *N*-[(3*S*)-2-oxooxetan-3-yl]carbamate (382 mg, 2.0mmol) in MeCN (6 mL) under an atmosphere of N_2 was stirred overnight. The mixture was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (*S*)-3-((*R*)-5-bromo-2-(methoxymethyl)-3,6-dihydropyridin-1(2*H*)-yl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (500 mg, 39% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{15}H_{25}BrN_2O_5$ 392.1; found 393.1.

Step 8.

To a mixture of 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl (*S*)-hexahydropyridazine-3-carboxylate (1.25 g, 2.07 mmol) and (*S*)-3-((*R*)-5-bromo-2-(methoxymethyl)-3,6-dihydropyridin-1(2*H*)-yl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (0.49 g, 1.24 mmol) and DIPEA (2.67 g, 20.7 mmol) in DMF (15 mL) under an atmosphere of N_2 was added HATU (0.79 g, 2.07 mmol) in portions. The mixture was warmed to room temperature and stirred for 1 h, then H_2O added and the mixture was extracted with EtOAc (50mL). The organic layer was washed with brine (3 x 50 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl (*S*)-1-((*S*)-3-((*R*)-5-bromo-2-(methoxymethyl)-3,6-dihydropyridin-1(2*H*)-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (880 mg, 39% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{49}H_{72}BBrN_6O_9$ 980.5; found 981.4 [for ^{81}Br].

Step 9.

To a mixture of 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl (*S*)-1-((*S*)-3-((*R*)-5-bromo-2-(methoxymethyl)-3,6-dihydropyridin-1(2*H*)-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (850 mg, 0.87 mmol) and K_3PO_4 (460 mg, 2.17 mmol) in toluene (4.5 mL), 1,4-dioxane (1.5 mL) and H_2O (1.5 mL) under an atmosphere of N_2 was added Pd(dppf)Cl_2 (64 mg, 0.09 mmol) in portions. The mixture was heated to 70 °C and stirred for 3 h at 70°C, then concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl ((2^2*R*,6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-2^2-(methoxymethyl)-10,10-dimethyl-5,7-dioxo-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)carbamate (220 mg, 30% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{43}H_{60}N_6O_7$ 772.5; found 773.4.

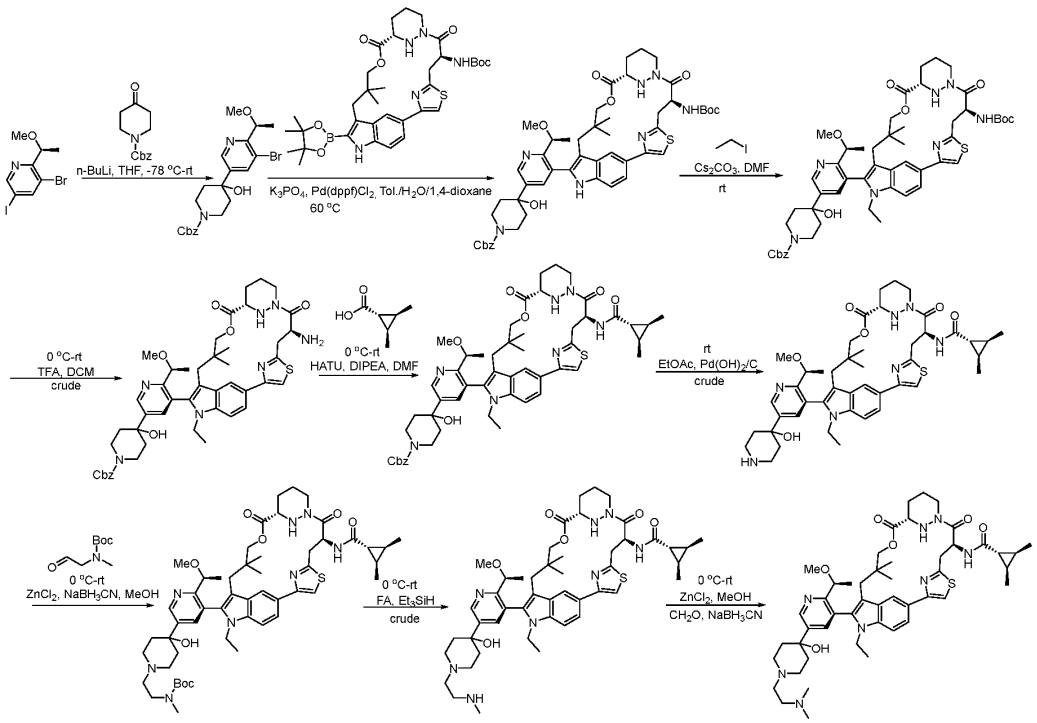
Step 10.

A mixture of *tert*-butyl ((2^2*R*,6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-2^2-(methoxymethyl)-10,10-dimethyl-5,7-dioxo-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)carbamate (100 mg, 0.13 mmol) and HCl in 1,4-dioxane (2 mL) in DCM (2 mL) was stirred at room temperature for 1 h. The mixture was concentrated under reduced pressure to give (2^2*R*,6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-2^2-(methoxymethyl)-10,10-dimethyl-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-5,7-dione (119 mg) as a solid, that was used directly in the next step without further purification.

Step 11.

To a mixture of (2^2*R*,6^3*S*,4*S*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-2^2-(methoxymethyl)-10,10-dimethyl-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-5,7-dione (20 mg, 0.18 mmol), (1*r*,2*R*,3*S*)-2,3-dimethylcyclopropanecarboxylic acid, and DIPEA (114 mg, 0.89 mmol) in DMF (2 mL) was added HATU (81 mg, 0.21 mmol) in portions. The mixture was stirred at room temperature for 1 h, then purified by preparative-HPLC to give (1*r*,2*R*,3*S*)-*N*-((2^2*R*,6^3*S*,4*S*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-2^2-(methoxymethyl)-10,10-dimethyl-5,7-dioxo-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide (24 mg, 18% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{44}H_{60}N_6O_6$ 768.5; found 769.2; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.90 - 8.65 (m, 1H), 8.10 - 7.95 (m, 1H), 7.90 - 7.75 (m, 1H), 7.57 - 7.45 (m, 3H), 7.40 (s, 1H), 6.26 (s, 1H), 5.65 - 5.50 (m, 1H), 5.45 - 5.30 (m, 1H), 4.39 - 4.25 (m, 1H), 4.24 - 4.20 (m, 1H), 4.19 - 4.10 (m, 1H), 4.09 - 4.00 (m, 1H), 3.88 - 3.76 (m, 1H), 3.73 - 3.62 (m, 2H), 3.62 - 3.55 (m, 2H), 3.26 (s, 5H), 3.14 - 3.00 (m, 2H), 2.96 - 2.87 (m, 1H), 2.79 - 2.70 (m, 5H), 2.43 (s, 1H), 2.15 - 2.06 (m, 2H), 1.95 - 1.86 (m, 1H), 1.86 - 1.72 (m, 1H), 1.58 - 1.49 (m, 2H), 1.40 - 1.30 (m, 3H), 1.10 - 1.00 (m, 6H), 0.99 - 0.90 (m, 6H), 0.82 (s, 3H), 0.52 (s, 3H).

Example A373. Synthesis of (1*r*,2*R*,3*S*)-*N*-((6^3*S*,4*S*,*Z*)-1^2-(5-(1-(2-(dimethylamino)ethyl)-4-hydroxypiperidin-4-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide



Step 1.

To a mixture of (*S*)-3-bromo-5-iodo-2-(1-methoxyethyl)pyridine (5.0 g, 14.6 mmol) in THF (40 mL) at -78 °C under an atmosphere of N_2 was added n-BuLi in hexanes (5.85 mL, 14.6 mmol) dropwise. The mixture was stirred at -78 °C for 1 h, then benzyl 4-oxopiperidine-1-carboxylate (6.82 g, 29.2 mmol) was added. The mixture was allowed to warm to 0 °C. Saturated NH_4Cl (3 mL) was added, the mixture was diluted with H_2O (20 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give benzyl (*S*)-4-(5-bromo-6-(1-methoxyethyl)pyridin-3-yl)-4-hydroxypiperidine-1-carboxylate (800 mg, 12% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{21}H_{25}BrN_2O_4$ 448.1; found 449.2.

Step 2.

To a mixture of *tert*-butyl ((6^3*S*,4*S*,*Z*)-10,10-dimethyl-5,7-dioxo-1^2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (500 mg, 0.72 mmol) and benzyl (*S*)-4-(5-bromo-6-(1-methoxyethyl)pyridin-3-yl)-4-hydroxypiperidine-1-carboxylate (648 mg, 1.4 mmol) in toluene (9 mL), H_2O (3 mL) and 1,4-dioxane (3 mL) under an atmosphere of N_2 was added K_3PO_4 (459 mg, 2.16

mmol) and $Pd(dppf)Cl_2.CH_2Cl_2$ (59 mg, 0.07 mmol) in portions. The mixture was heated to 60 °C and stirred for 4 h, then diluted with H_2O (10 mL) and extracted with EtOAc (3 x10 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give benzyl 4-(5-((6^3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-4-hydroxypiperidine-1-carboxylate (1.16 g, 75% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{50}H_{61}N_7O_9S$ 935.4; found 936.4.

Step 3.

To a mixture of benzyl 4-(5-((6^3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-4-hydroxypiperidine-1-carboxylate (1.1 g, 1.18 mmol) and Cs_2CO_3 (1.91 g, 5.88 mmol) in DMF (15 mL) at 0 °C was added iodoethane (0.64 g, 4.1 mmol) dropwise. The mixture was warmed to room temperature and stirred for 2 h, then diluted with H_2O (15 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give benzyl 4-(5-((6^3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-4-hydroxypiperidine-1-carboxylate (110 mg, 9% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{52}H_{65}N_7O_9S$ 963.5; found 964.4.

Step 4.

To a mixture of benzyl 4-(5-((6^3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-4-hydroxypiperidine-1-carboxylate (110 mg, 0.11 mmol) in DCM (3 mL) at 0 °C was added TFA (1 mL) dropwise. The mixture was warmed to room temperature and stirred for 1.5 h, then concentrated under reduced pressure to give benzyl 4-(5-((6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-4-hydroxypiperidine-1-carboxylate (120 mg) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{47}H_{57}N_7O_7S$ 863.4; found 864.5.

Step 5.

To a mixture of benzyl 4-(5-((6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-4-hydroxypiperidine-1-carboxylate (120 mg, 0.14 mmol) and (1*R*,2*R*,3*S*)-2,3-dimethylcyclopropane-1-carboxylic acid (32 mg, 0.28 mmol) in DMF (4 mL) at 0 °C was added DIPEA (180 mg, 1.39 mmol) and HATU (158 mg,

0.42 mmol) in portions. The mixture was warmed to room temperature and stirred for 2 h, then diluted with H_2O (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by reverse flash chromatography with the following conditions: column, C18 silica gel; mobile phase, MECN in water (0.05% TFA), 0% to 100% gradient in 300 min; detector, UV 254 nm. To afford benzyl 4-(5-((6^3*S*,4*S*,*Z*)-4-((1*r*,2*R*,3*S*)-2,3-dimethylcyclopropane-1-carboxamido)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-4-hydroxypiperidine-1-carboxylate (120 mg, 89% yield) as a brown solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{53}H_{65}N_7O_8S$ 959.4; found 960.4.

Step 6.

A mixture of benzyl 4-(5-((6^3*S*,4*S*,*Z*)-4-((1*r*,2*R*,3*S*)-2,3-dimethylcyclopropane-1-carboxamido)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-4-hydroxypiperidine-1-carboxylate (120 mg, 0.13 mmol) and $Pd(OH)_2$, 30% on carbon (80 mg, 0.25 mmol) in EtOAc (2 mL) was stirred under an atmosphere of H_2 overnight. The mixture was filtered, the filter cake was washed with MeOH (3 x 8 mL) and the filtrate was concentrated under reduced pressure to give (1*r*,2*R*,3*S*)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(5-(4-hydroxypiperidin-4-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide (45 mg) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{45}H_{59}N_7O_6S$ 825.4; found 826.4.

Step 7.

To a mixture of (1*r*,2*R*,3*S*)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(5-(4-hydroxypiperidin-4-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide (50 mg, 0.06 mmol) and *tert*-butyl *N*-methyl-*N*-(2-oxoethyl)carbamate (52 mg, 0.31 mmol) in MeOH (2 mL) at 0 °C was added $ZnCl_2$ (83 mg, 0.61 mmol) in portions. The mixture was warmed to room temperature and stirred for 30 min, then cooled to 0 °C and $NaBH_3CN$ (11 mg, 0.18 mmol) added in portions. The mixture was warmed to room temperature and stirred for 2 h, then the residue was purified by preparative-HPLC to give *tert*-butyl (2-(4-(5-((6^3*S*,4*S*,*Z*)-4-((1*r*,2*R*,3*S*)-2,3-dimethylcyclopropane-1-carboxamido)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-4-hydroxypiperidin-1-yl)ethyl)(methyl)carbamate (30 mg, 50% yield) as a yellow oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{53}H_{74}N_8O_8S$ 982.5; found 983.6.

Step 8.

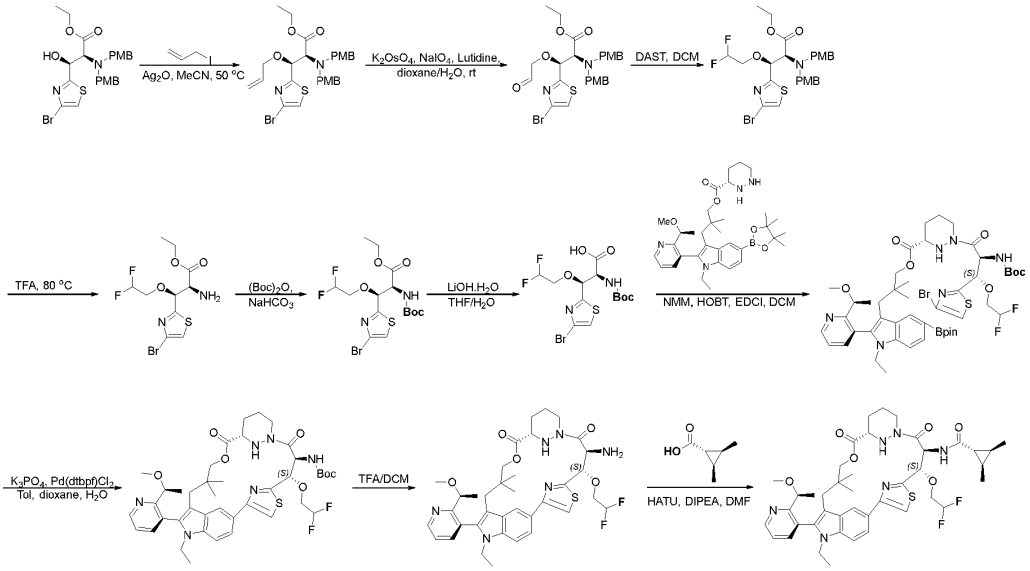
To a mixture of *tert*-butyl (2-(4-(5-((6^3*S*,4*S*,*Z*)-4-((1*r*,2*R*,3*S*)-2,3-dimethylcyclopropane-1-carboxamido)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-

thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-4-hydroxypiperidin-1-yl)ethyl)(methyl)carbamate (30 mg, 0.03 mmol) in FA (1.5 mL) at 0 °C was added Et_3SiH (18 mg, 0.16 mmol). The mixture was warmed to room temperature and stirred for 40 min, then concentrated under reduced pressure to give (1*r*,2*R*,3*S*)-*N*-((63*S*,4*S*,*Z*)-1^1-ethyl-1^2-(5-(4-hydroxy-1-(2-(methylamino)ethyl)piperidin-4-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide (25 mg) as a solid, which was used directly in the next step without further purification. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{48}H_{66}N_8O_6S$ 882.5; found 883.6.

Step 9.

To a mixture of (1*r*,2*R*,3*S*)-*N*-((63*S*,4*S*,*Z*)-11-ethyl-1^2-(5-(4-hydroxy-1-(2-(methylamino)ethyl)piperidin-4-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide (30 mg, 0.03 mmol) and formaldehyde (31 mg, 1.0 mmol) in MeOH (3 mL) at 0 °C was added $ZnCl_2$ (46 mg, 0.34 mmol) in portions. The mixture was warmed to room temperature and stirred for 30 min, then cooled to 0 °C and $NaBH_3CN$ (6.4 mg, 0.10 mmol) was added in portions. The mixture was warmed to room temperature and stirred for 2 h, then purified by preparative-HPLC to give (1*r*,2*R*,3*S*)-*N*-((63*S*,4*S*,*Z*)-1^2-(5-(1-(2-(dimethylamino)ethyl)-4-hydroxypiperidin-4-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide (0.7 mg, 2% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{49}H_{68}N_8O_6S$ 896.5; found 897.4; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.83 (d, J = 2.3 Hz, 1H), 8.49 (d, J = 1.6 Hz, 1H), 8.38 (d, J = 9.1 Hz, 2H), 7.81 (s, 1H), 7.78 - 7.68 (m, 2H), 7.57 (d, J = 8.7 Hz, 1H), 5.57 (t, J = 9.3 Hz, 1H), 5.06 (d, J = 12.1 Hz, 1H), 4.36 - 4.07 (m, 6H), 3.56 (s, 2H), 3.24 (s, 3H), 3.18 - 3.11 (m, 1H), 2.93 (d, J = 14.5 Hz, 1H), 2.75 - 2.60 (m, 2H), 2.43 (d, J = 11.4 Hz, 4H), 2.37 (s, 2H), 2.15 (s, 7H), 2.02 (d, J = 16.7 Hz, 4H), 1.78 (s, 2H), 1.66 (d, J = 12.6 Hz, 2H), 1.50 (s, 1H), 1.36 (d, J = 6.1 Hz, 3H), 1.24 (s, 1H), 1.16 (s, 2H), 1.15 - 0.93 (m, 6H), 0.92 - 0.63 (m, 6H), 0.31 (s, 3H).

Example A387. Synthesis of (1*r*,2*R*,3*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-(2,2-difluoroethoxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide



Step 1.

To a mixture of ethyl (2*S*,3*S*)-2-(bis(4-methoxybenzyl)amino)-3-(4-bromothiazol-2-yl)-3-hydroxypropanoate (1.0 g, 1.9 mmol) in MeCN (10 mL) at room temperature under an atmosphere of N_2 was added Ag_2O (2.17 g, 9.4 mmol) and allyl iodide (1.57 g, 9.36 mmol). The resulting mixture was heated to 60 °C and stirred for 16 h, then filtered, and the filter cake was washed with EtOAc (3 x 20 mL). The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give ethyl (2*S*,3*S*)-3-(allyloxy)-2-(bis(4-methoxybenzyl)amino)-3-(4-bromothiazol-2-yl)propanoate (1.0 g, 93% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{27}H_{31}BrN_2O_5S$ 574.1 & 576.1; found 575.1 & 577.1.

Step 2.

To a mixture of ethyl (2*S*,3*S*)-3-(allyloxy)-2-(bis(4-methoxybenzyl)amino)-3-(4-bromothiazol-2-yl)propanoate (1.0 g, 1.7 mmol) in 1,4-dioxane (10 mL) and H_2O (10 mL) at 0 °C was added 2,6-lutidine (0.37 g, 3.48 mmol) and $K_2OsO_4 \cdot 2H_2O$ (0.03 g, 0.09 mmol). The mixture was stirred at 0 °C for 15 min then $NaIO_4$ (1.49 g, 6.95 mmol) was added in portions. The mixture was warmed to room temperature and stirred for 2.5 h, then diluted with H_2O (50 mL) and extracted with EtOAc (3 x 50mL). The combined organic layers were washed with brine (2 x 50 mL), dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to give ethyl (2*S*,3*S*)-2-(bis(4-methoxybenzyl)amino)-3-(4-bromothiazol-2-yl)-3-(2-oxoethoxy)propanoate (1.2 g) as an oil, which was used directly in the next step without further purification. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{26}H_{29}BrN_2O_6S$ 576.1 & 578.1; found 577.4 & 579.4.

Step 3.

To a mixture of ethyl (2*S*,3*S*)-2-(bis(4-methoxybenzyl)amino)-3-(4-bromothiazol-2-yl)-3-(2-oxoethoxy)propanoate (1.2 g, 2.1 mmol) in DCM (20 mL) at -15 °C under an atmosphere of N_2 was

added DAST (0.37 g, 2.3 mmol) dropwise. The mixture was warmed to room temperature and stirred for 1.5 h, then re-cooled to 0 °C and further DAST (0.37 g, 2.3 mmol) added dropwise. The mixture was warmed to room temperature and stirred for 1 h, then cooled to 0 °C and saturated NH_4Cl (2 mL) added. The mixture was diluted with H_2O (20 mL) and extracted with DCM (3 x 20mL). The combined organic layers were washed with brine (2 x 50 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give ethyl (2*S*,3*S*)-2-(bis(4-methoxybenzyl)amino)-3-(4-bromothiazol-2-yl)-3-(2,2-difluoroethoxy)propanoate (270 mg, 22% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{26}H_{29}BrF_2N_2O_5S$ 598.1 & 600.1; found 599.1 & 601.1.

Step 4.

A mixture of ethyl (2*S*,3*S*)-2-(bis(4-methoxybenzyl)amino)-3-(4-bromothiazol-2-yl)-3-(2,2-difluoroethoxy)propanoate (240 mg, 0.40 mmol) in TFA (3 mL) was heated to 80 °C and stirred for 8 h, then concentrated under reduced pressure to give ethyl (2*S*,3*S*)-2-amino-3-(4-bromothiazol-2-yl)-3-(2,2-difluoroethoxy)propanoate (245 mg) as an oil, that was used directly in the next step without further purification. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{10}H_{13}BrF_2N_2O_3S$ 358.0 & 360.0; found 359.0 & 361.0.

Step 5.

To a mixture of ethyl (2*S*,3*S*)-2-amino-3-(4-bromothiazol-2-yl)-3-(2,2-difluoroethoxy)propanoate (230 mg, 0.64 mmol) and $NaHCO_3$ (108 mg, 1.29 mmol) in H_2O (0.9 mL) and THF (3 mL) was added $(Boc)_2O$ (147 mg, 0.67 mmol). The mixture was stirred at room temperature overnight, then diluted with H_2O (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (3 x 20 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give ethyl (2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-(2,2-difluoroethoxy)propanoate (145 mg, 49% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{15}H_{21}BrF_2N_2O_5S$ 458.0 & 460.0; found 459.0 & 461.0.

Step 6.

To a mixture of ethyl (2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-(2,2-difluoroethoxy)propanoate (140 mg, 0.31 mmol) in THF (1.5 mL) at 0 °C was added $LiOH.H_2O$ (64 mg, 1.53 mmol) in H_2O (1.5 mL) dropwise. The mixture was warmed to room temperature and stirred for 1 h, then acidified to pH ~5 with aqueous HCl, then extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (3 x 20 mL), dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to give (2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-(2,2-difluoroethoxy)propanoic acid (100 mg) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{13}H_{17}BrF_2N_2O_5S$ 430.0 & 432.0; found 431.0 & 433.0.

Step 7.

To a mixture of 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl-(*S*)-hexahydropyridazine-3-carboxylate (134 mg, 0.22 mmol) and NMM (335 mg, 3.32 mmol) in DCM (6 mL) at 0 °C under an atmosphere of N_2 was added (2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-(2,2-difluoroethoxy)propanoic acid (95 mg, 0.22 mmol) and HOBT (6 mg, 0.04 mmol) and EDCI (85 mg, 0.44 mmol). The mixture was warmed to room temperature and stirred overnight, then diluted with H_2O (10 mL) and extracted with DCM (3 x 10 mL). The combined organic layers were washed with brine (3 x 20 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl-(*S*)-1-((2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-(2,2-difluoroethoxy)propanoyl)hexahydropyridazine-3-carboxylate (70 mg, 36% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{47}H_{64}BBrF_2N_6O_9S$ 1016.4 & 1018.4; found 1017.3 & 1019.4.

Step 8.

To a mixture of mixture of 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl-(*S*)-1-((2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-(2,2-difluoroethoxy)propanoyl)hexahydropyridazine-3-carboxylate (50 mg, 0.05 mmol) and K_3PO_4 (31 mg, 0.15 mmol) in toluene (3 mL), 1,4-dioxane (1 mL) and H_2O (1 mL) under an atmosphere of N_2 was added Pd(DtBPF)Cl_2 (13 mg, 0.02 mmol) in portions. The mixture was heated to 60 °C and stirred for 1 h, then was extracted with EtOAc (3 x 10mL). The combined organic layers were washed with brine (3 x 20 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-3-(2,2-difluoroethoxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (27 mg, 59% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{41}H_{52}F_2N_6O_7S$ 810.4; found 811.4.

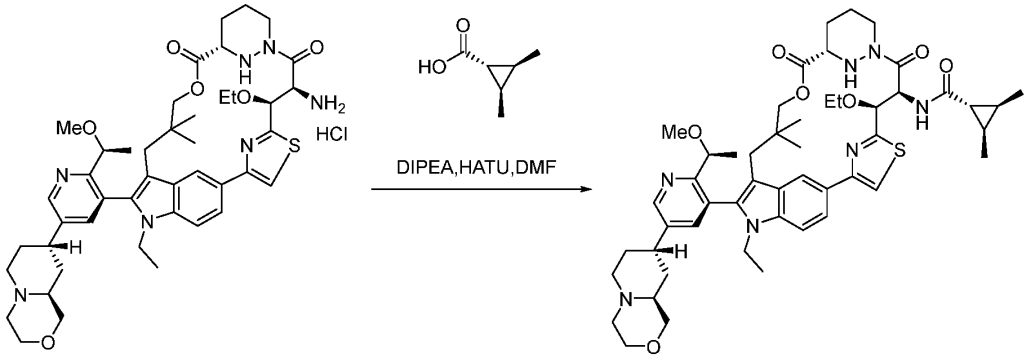
Step 9.

To a mixture *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-3-(2,2-difluoroethoxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (25 mg, 0.02 mmol) in DCM (1.5 mL) at 0 °C was added TFA (0.5 mL) dropwise. The mixture was warmed to room temperature and stirred for 1 h, then concentrated under reduced pressure to give (6^3*S*,3*S*,4*S*,*Z*)-4-amino-3-(2,2-difluoroethoxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (25 mg) as a solid, which was used directly in the next step without further purification. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{36}H_{44}F_2N_6O_5S$ 710.3; found 711.3.

Step 10.

To a mixture of (6^3S,3*S*,4*S*,*Z*)-4-amino-3-(2,2-difluoroethoxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (25 mg, 0.04 mmol) and (1*r*,2*R*,3*S*)-2,3-dimethylcyclopropane-1-carboxylic acid (6 mg, 0.05 mmol) in DMF (1 mL) at 0 °C under an atmosphere of N_2 was added DIPEA (45 mg, 0.35 mmol) and HATU (27 mg, 0.07 mmol) in portions. The mixture was warmed to room temperature and stirred for 2 h, then H_2O (10 mL) added and the mixture was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (3 x 20 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (1*r*,2*R*,3*S*)-*N*-((6^3S,3*S*,4*S*,*Z*)-3-(2,2-difluoroethoxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide (3.3 mg, 12% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{42}H_{52}F_2N_6O_6S$ 806.4; found 807.2; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.95 - 8.70 (m, 1H), 8.50 (s, 1H), 8.13 (s, 1H), 7.95 (s, 1H), 7.88 - 7.79 (m, 2H), 7.76 (d, *J* = 8.6 Hz, 1H), 7.59 (d, *J* = 8.7 Hz, 1H), 7.54 (dd, *J* = 7.7, 4.8 Hz, 1H), 7.27 - 6.88 (m, 1H), 6.36 - 6.04 (m, 1H), 5.97 (d, *J* = 9.9 Hz, 1H), 5.20 (d, *J* = 12.8 Hz, 2H), 4.25 (dd, *J* = 20.3, 10.5 Hz, 4H), 4.14 - 3.92 (m, 2H), 3.92 - 3.73 (m, 1H), 3.59 (q, *J* = 10.9 Hz, 2H), 3.20 (s, 3H), 2.81 (d, *J* = 13.7 Hz, 2H), 2.12 - 1.93 (m, 1H), 1.78 (d, *J* = 23.7 Hz, 2H), 1.59 - 1.45 (m, 2H), 1.38 (d, *J* = 6.1 Hz, 3H), 1.21 (d, *J* = 20.3 Hz, 1H), 1.16 - 1.01 (m, 7H), 0.95 - 0.73 (m, 6H), 0.43 (s, 3H).

Example A389. Synthesis of (1*r*,2*R*,3*S*)-*N*-((6^3S,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-((8*S*,9*aS*)-octahydropyrido[2,1-*c*][1,4]oxazin-8-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide

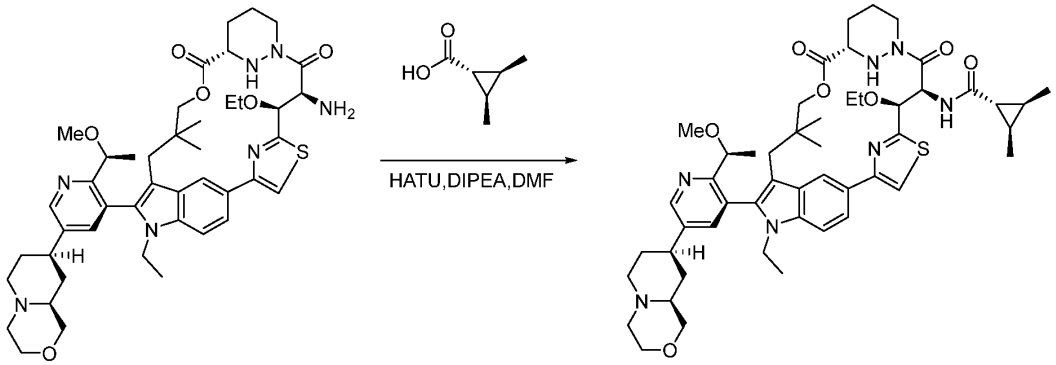


Step 1.

To a mixture of (6^3S,3*S*,4*S*,*Z*)-4-amino-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-((8*S*,9*aS*)-octahydropyrido[2,1-*c*][1,4]oxazin-8-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione HCl salt (90 mg, 0.11 mmol) and (1*R*,2*R*,3*S*)-2,3-dimethylcyclopropane-1-carboxylic acid (19 mg, 0.17 mmol) in DMF (3 mL) at 0 °C was added DIPEA (429 mg, 3.3 mmol) and HATU (63 mg, 0.17

mmol) in portions. The mixture was warmed to room temperature and stirred for 2 h, then diluted with H_2O (10 mL) and the mixture was extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with brine (3 x 10 mL), then concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (1*r*,2*R*,3*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-((8*S*,9*aS*)-octahydropyrido[2,1-*c*][1,4]oxazin-8-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide (13.8 mg, 13% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{50}H_{67}N_7O_7S$ 909.5; found 910.7; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (s, 1H), 8.50 (s, 1H), 7.93 (s, 1H), 7.83 - 7.51 (m, 4H), 5.96 - 5.77 (m, 1H), 5.17 (d, *J* = 11.5 Hz, 1H), 4.92 (s, 1H), 4.45 - 4.03 (m, 5H), 3.57 (ddd, *J* = 34.6, 24.2, 13.9 Hz, 8H), 3.23 (d, *J* = 8.3 Hz, 4H), 3.08 (d, *J* = 10.4 Hz, 2H), 2.84 (d, *J* = 45.9 Hz, 3H), 2.67 (s, 2H), 2.23 (s, 2H), 2.17 - 2.01 (m, 7H), 1.81 (s, 3H), 1.53 (s, 4H), 1.45 - 1.30 (m, 4H), 1.24 (s, 1H), 1.16 (td, *J* = 7.0, 1.9 Hz, 4H), 1.06 (dd, *J* = 12.1, 5.1 Hz, 6H), 0.97 - 0.76 (m, 8H), 0.38 (s, 3H).

Example A390. Synthesis of (1*r*,2*R*,3*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)-5-((8*R*,9*aS*)-octahydropyrido[2,1-*c*][1,4]oxazin-8-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide

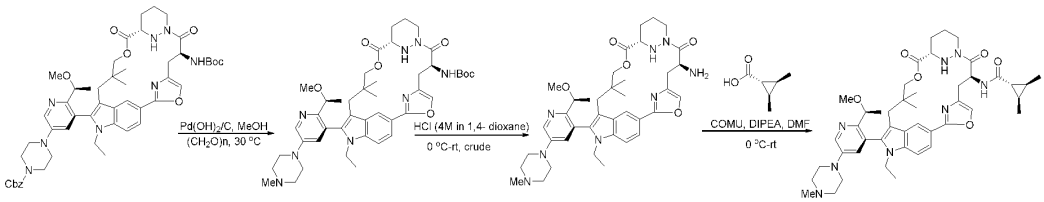


Step 1.

To a mixture of (6^3*S*,3*S*,4*S*,*Z*)-4-amino-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-((8*R*,9*aS*)-octahydropyrido[2,1-c][1,4]oxazin-8-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (100 mg, 0.12 mmol) and (1*R*,2*R*,3*S*)-2,3-dimethylcyclopropane-1-carboxylic acid (21 mg, 0.18 mmol) in DMF (3 mL) at -10 °C under an atmosphere of N_2 was added DIPEA (476 mg, 3.7 mmol) and HATU (56 mg, 0.15 mmol, 1.2 equiv) in portions. The mixture was stirred at -10 °C for 1.5 h, then diluted with brine (5 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (3 x 10 mL), dried over anhydrous Na_2SO_4 and the filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (1*r*,2*R*,3*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)-5-((8*R*,9*aS*)-octahydropyrido[2,1-*c*][1,4]oxazin-8-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-

hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide (22 mg, 20% yield) as a solid. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{50}H_{67}N_7O_7S$ 909.5; found 910.7; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.65 (d, *J* = 2.1 Hz, 1H), 8.49 (s, 1H), 7.92 (s, 1H), 7.74 (d, *J* = 8.5 Hz, 1H), 7.68 - 7.54 (m, 3H), 5.88 (d, *J* = 9.8 Hz, 1H), 5.17 (d, *J* = 12.1 Hz, 1H), 4.93 (s, 1H), 4.36 - 3.99 (m, 5H), 3.80 - 3.43 (m, 7H), 3.24 - 3.05 (m, 4H), 2.92 - 2.72 (m, 4H), 2.70 - 2.59 (m, 1H), 2.28 - 1.99 (m, 5H), 1.89 - 1.59 (m, 6H), 1.52 (q, *J* = 8.2, 6.3 Hz, 2H), 1.42 - 1.22 (m, 5H), 1.20 - 0.99 (m, 12H), 0.86 (d, *J* = 25.0 Hz, 7H), 0.39 (s, 3H).

Example A391. Synthesis of (1*r*,2*R*,3*S*)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide



Step 1.

A mixture of benzyl 4-(5-((6^3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (150 mg, 0.16 mmol), paraformaldehyde (36 mg, 0.81 mmol) and $Pd(OH)_2$, 30% weight on carbon (151 mg, 0.32 mmol) in MeOH (3 mL) was hydrogenated at 30 °C for 2 h. The mixture was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure to give *tert*-butyl ((6^3*S*,4*S*,*Z*)-11-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (134 mg) as a solid. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{44}H_{60}N_8O_7$ 812.5; found 813.4.

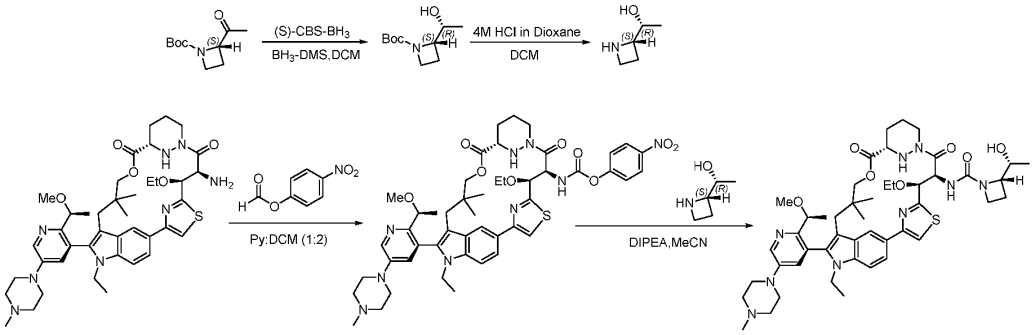
Step 2.

To a mixture of *tert*-butyl ((6^3*S*,4*S*,*Z*)-11-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (134 mg, 0.17 mmol) in DCM (1.5 mL) at 0 °C was added TFA (1.50 mL) in portions. The mixture was warmed to room temperature and stirred for 2 h, then concentrated under reduced pressure to give (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (318 mg) as an oil. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{39}H_{52}N_8O_5$ 712.4; found 713.4.

Step 3.

To a mixture of (6^3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (130 mg, 0.18 mmol), (1*R*,2*R*,3*S*)-2,3-dimethylcyclopropane-1-carboxylic acid (42 mg, 0.36 mmol) and DIPEA (236 mg, 1.8 mmol) in DMF (2 mL) at 0 °C was added COMU (117 mg, 0.27 mmol) in portions. The mixture was warmed to room temperature and stirred for 2h, then the residue was purified by preparative-HPLC to give (1*r*,2*R*,3*S*)-*N*-((6^3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide (28 mg, 19% yield) as a solid. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{45}H_{60}N_8O_6$ 808.5; found 809.8; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.66 - 8.45 (m, 2H), 8.29 (d, *J* = 8.0 Hz, 1H), 7.88 (s, 1H), 7.74 (d, *J* = 9.4 Hz, 2H), 7.30 (s, 1H), 5.81 (s, 1H), 5.00 (d, *J* = 11.7 Hz, 1H), 4.53 - 4.06 (m, 5H), 3.77 - 3.59 (m, 2H), 3.34 (s, 4H), 3.21 (s, 3H), 3.05 - 2.61 (m, 5H), 2.52 (s, 4H), 2.28 (s, 3H), 2.05 (s, 1H), 1.83 (s, 1H), 1.56 (s, 2H), 1.48 - 1.32 (m, 3H), 1.30 - 0.98 (m, 12H), 0.93 (s, 3H), 0.46 (s, 3H).

Example A396. Synthesis of (2*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-((*R*)-1-hydroxyethyl)azetidine-1-carboxamide



Step 1.

To a mixture of *tert*-butyl (*S*)-2-acetylazetidine-1-carboxylate (543 mg, 1.96 mmol) in DCM (330 mL) at room temperature under an atmosphere of N_2 was added BH_3-Me_2S (2.48 g, 32.6 mmol). To the above mixture was added *tert*-butyl (2*S*)-2-acetylazetidine-1-carboxylate [*Org. Lett.* **2019**, *22*, 9981-9984 – see Supporting Information] (2.6 g, 13.0 mmol) dropwise over 10 min. The resulting mixture was stirred for additional 2 h at room temperature. The reaction was monitored by LCMS. The reaction was quenched with MeOH at 0 °C and was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give *tert*-butyl (*S*)-2-((*R*)-1-hydroxyethyl)azetidine-1-carboxylate (1.1 g, 42% yield) as an oil. LCMS (ESI): m/z [M-C_4H_8+H]$^+$ calc'd for $C_6H_{11}NO_3$ 145.1; found 146.1.

Step 2.

To a mixture of *tert*-butyl (*S*)-2-((*R*)-1-hydroxyethyl)azetidine-1-carboxylate (300 mg, 1.49 mmol) in DCM (5 mL) at 0 °C was added 4M HCl in 1,4-dioxane (5 mL). The mixture was stirred until completion, then concentrated under reduced pressure to give (*R*)-1-((*S*)-azetidin-2-yl)ethan-1-ol (310 mg) as a solid, which was used directly in the next step without further purification. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_5H_{11}NO$ 101.1; found 102.2.

Step 3.

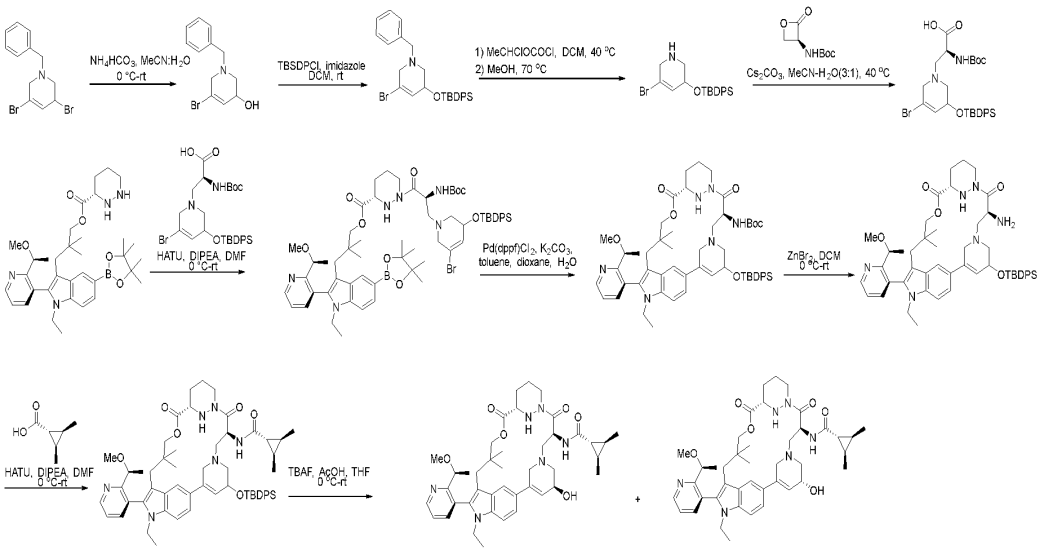
To a mixture of (6^3S,3*S*,4*S*,*Z*)-4-amino-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (150 mg, 0.19 mmol), pyridine (1 mL), and DCM (2 mL) at 0 °C was added 4-nitrophenyl chloroformate (65 mg, 0.39 mmol). The mixture was warmed to room temperature and stirred for 4 h, then concentrated under reduced pressure to give 4-nitrophenyl ((6^3S,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (crude) as an oil, which was used directly in the next step without further purification. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{48}H_{59}N_9O_9S$ 937.4; found 938.3.

Step 4.

To a mixture of 4-nitrophenyl ((6^3S,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (195 mg, 0.21 mmol) and (*R*)-1-((*S*)-azetidin-2-yl)ethan-1-ol (285 mg, 2.8 mmol) in MeCN (2 mL) at 0 °C was added DIPEA (403 mg, 3.1 mmol) dropwise. The mixture was warmed to room temperature and stirred for 1 h. The residue was purified by preparative-HPLC to give (2*S*)-*N*-((6^3S,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-((*R*)-1-hydroxyethyl)azetidine-1-carboxamide (4.8 mg, 3%) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{47}H_{65}N_9O_7S$ 899.5; found 900.9; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 - 8.45 (m, 2H), 7.91 (s, 1H), 7.76 - 7.72 (m, 1H), 7.58 - 7.53 (m, 1H), 7.22 (s, 1H), 6.73 - 6.68 (m, 1H), 5.54 - 5.50 (m, 1H), 5.31 - 5.26 (m, 1H), 5.16 - 5.12 (m, 1H), 4.91 (s, 1H), 4.30 - 4.24 (m, 3H), 4.16 - 4.04 (m, 3H), 3.84 - 3.67 (m, 2H), 3.69 - 3.41 (m, 5H), 3.28 - 3.23 (m, 4H), 3.16 (s, 3H), 2.84 - 2.79 (m, 2H), 2.48 - 2.44 (m, 4H), 2.23 - 2.21 (m, 4H), 2.08 - 2.05 (m, 1H), 1.93 - 1.89 (m, 1H), 1.79 - 1.77 (m, 2H), 1.54 - 1.52 (m, 1H), 1.36 - 1.33 (m, 3H), 1.26 - 1.16 (m, 4H), 1.13 - 1.10 (m, 3H), 1.00 - 0.73 (m, 6H), 0.43 (s, 2H).

Examples A441 and A424. Synthesis of (1*R*,2*R*,3*S*)-*N*-((2^3S,6^3S,4*S*)-1^1-ethyl-2^3-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-

pyridinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide and (1*R*,2*R*,3*S*)-*N*-((2^3*R*,6^3*S*,4*S*)-1^1-ethyl-2^3-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide



Step 1.

To a mixture of 1-benzyl-3,5-dibromo-1,2,3,6-tetrahydropyridine (40.0 g, 120.8 mmol) in MeCN (900 mL) and H_2O (600 mL) at 0 °C was added NH_4HCO_3 (14.33 g, 181.2 mmol). The mixture was warmed to room temperature and stirred for 16 h, then concentrated under reduced pressure and the residue was purified by reverse-phase silica gel column chromatography to give 1-benzyl-5-bromo-1,2,3,6-tetrahydropyridin-3-ol (16.0 g, 49% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{12}H_{14}BrNO$ 267.0; found 268.1.

Step 2.

To a mixture of 1-benzyl-5-bromo-1,2,3,6-tetrahydropyridin-3-ol (15.0 g, 55.9 mmol) in DCM (150 mL) was added TBDPSCl (23.1 g, 83.9 mmol) and imidazole (7.62 g, 111.9 mmol) under an atmosphere of N_2 for 16 h. H_2O was added and the mixture and was extracted with EtOAc (3 x 300 mL). The combined organic layers were washed with brine (3 x 300 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by reverse-phase silica gel column chromatography to give 1-benzyl-5-bromo-3-((*tert*-butyldiphenylsilyl)oxy)-1,2,3,6-tetrahydropyridine (15.0 g, 53% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{28}H_{32}BrNOSi$ 505.1; found 506.2.

Step 3.

To a mixture of 1-benzyl-5-bromo-3-((*tert*-butyldiphenylsilyl)oxy)-1,2,3,6-tetrahydropyridine (15.0 g, 29.6 mmol) in DCM (150 mL) at 0 °C was added 2-chloroethyl chloroformate (16.93 g, 118.5 mmol). The mixture was warmed to 40 °C and was stirred for 4 h, then diluted with H_2O and the mixture was extracted with EtOAc (2 x 300 mL). The combined organic layers were washed with brine (2 x 300 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was dissolved in MeOH (150 mL). The mixture was heated to 70 °C and stirred for 2 h, then the combined organic layers were washed with $NaHCO_3$ (2 x 300 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by reverse-phase silica gel column chromatography to give 5-bromo-3-((*tert*-butyldiphenylsilyl)oxy)-1,2,3,6-tetrahydropyridine (9.0 g, 73% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{21}H_{26}BrNOSi$ 417.1; found 418.0 [for ^{81}Br].

Step 4.

To a mixture of 5-bromo-3-((*tert*-butyldiphenylsilyl)oxy)-1,2,3,6-tetrahydropyridine (6.0 g, 14.4 mmol) in MeCN (60 mL) and H_2O (60 mL) at 0 °C was added *tert*-butyl (*S*)-(2-oxooxetan-3-yl)carbamate (2.97 g, 15.9 mmol) and Cs_2CO_3 (11.74 g, 36.0 mmol). The mixture was warmed to 40 °C and stirred for 16 h, then extracted with EtOAc (2 x 100 mL). The combined organic layers were washed with brine (3 x 200 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by reverse-phase silica gel column chromatography to give (2*S*)-3-(5-bromo-3-((*tert*-butyldiphenylsilyl)oxy)-3,6-dihydropyridin-1(2*H*)-yl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (4.0 g, 46% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{29}H_{39}BrN_2O_5Si$ 602.2; found 603.1.

Step 5.

To a mixture of 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl (*S*)-hexahydropyridazine-3-carboxylate (1.0 g, 1.7 mmol) in DMF (10 mL) at 0 °C was added DIPEA (1.07 g, 8.3 mmol), (2*S*)-3-(5-bromo-3-((*tert*-butyldiphenylsilyl)oxy)-3,6-dihydropyridin-1(2*H*)-yl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (749 mg, 1.24 mmol) and HATU (1.26 g, 3.3 mmol). The mixture was stirred at 0 °C for 2 h, then diluted with H_2O, and the mixture was extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine (3 x 100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl-(3*S*)-1-((2*S*)-3-(5-bromo-3-((*tert*-butyldiphenylsilyl)oxy)-3,6-dihydropyridin-1(2*H*)-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (1.2 g, 61% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{63}H_{86}BBrN_6O_9Si$ 1188.6; found 1189.4.

Step 6.

To a mixture of 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl-(3*S*)-1-((2*S*)-3-(5-bromo-3-((*tert*-butyldiphenylsilyl)oxy)-3,6-dihydropyridin-1(2*H*)-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (1.2 g, 1.0 mmol) in toluene (30 mL), 1,4-dioxane (10 mL) and H_2O (10 mL) was added K_2CO_3 (418 mg, 3.0 mmol) and Pd(dppf)Cl_2 (74 mg, 0.1 mmol). The mixture was heated to 65 °C and stirred for 2 h, then diluted with H_2O, and the mixture was extracted with EtOAc (2 x 100 mL). The combined organic layers were washed with brine (2 x 100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give *tert*-butyl ((6^3*S*,4*S*)-2^3-((*tert*-butyldiphenylsilyl)oxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)carbamate (420 mg, 42% yield) as an oil. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{57}H_{74}N_6O_7Si$ 982.5; found 984.1.

Step 7.

To a mixture of *tert*-butyl ((6^3*S*,4*S*)-2^3-((*tert*-butyldiphenylsilyl)oxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)carbamate (420 mg, 0.43 mmol) in DCM (10 mL) at 0 °C was added $ZnBr_2$ (481 mg, 2.14 mmol). The mixture was warmed to room temperature and stirred for 16 h, then filtered, and the filter cake was washed with EtOAc (3 x 20 mL). The filtrate was concentrated under reduced pressure to give (6^3*S*,4*S*)-4-amino-2^3-((*tert*-butyldiphenylsilyl)oxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-5,7-dione (400 mg) which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{52}H_{66}N_6O_5Si$ 882.5; found 883.6.

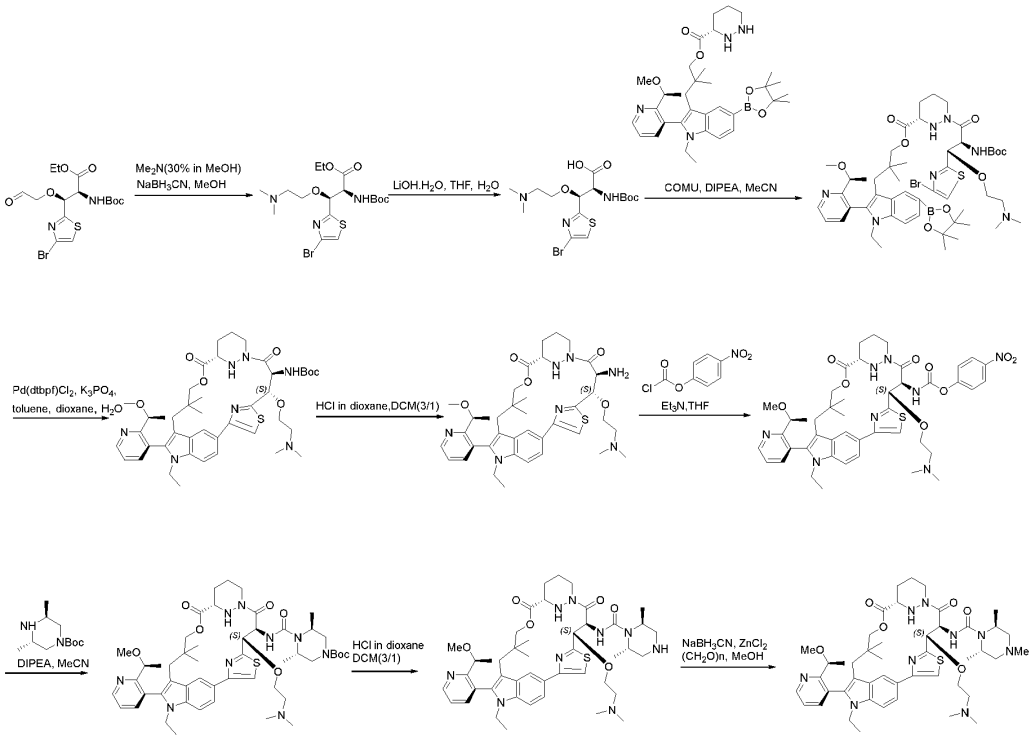
Step 8.

To a mixture of (6^3*S*,4*S*)-4-amino-2^3-((*tert*-butyldiphenylsilyl)oxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-5,7-dione (380 mg, 0.43 mmol) in DMF (4 mL) at 0 °C was added DIPEA (556 mg, 4.3 mmol), (1*R*,2*R*,3*S*)-2,3-dimethylcyclopropane-1-carboxylic acid (74 mg, 0.65 mmol) and HATU (327 mg, 0.86 mmol). The mixture was stirred at 0 °C for 2 h, then diluted with H_2O, and the mixture was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (3 x 50 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give (1*R*,2*R*,3*S*)-*N*-((6^3*S*,4*S*)-2^3-((*tert*-butyldiphenylsilyl)oxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide (190 mg, 45% yield) as a solid. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{58}H_{74}N_6O_6Si$ 978.5; found 979.7.

Step 9.

To a mixture of (1*R*,2*R*,3*S*)-*N*-((6^3*S*,4*S*)-2^3-((*tert*-butyldiphenylsilyl)oxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide (190 mg, 0.19 mmol) in THF (4 mL) at 0 °C was added TBAF (811 mg, 3.1 mmol) and AcOH (1 mg, 0.02 mmol). The mixture was warmed to room temperature and stirred for 16 h, then diluted with H_2O, and the mixture was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (3 x 50 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (1*R*,2*R*,3*S*)-*N*-((2^3*S*,6^3*S*,4*S*)-1^1-ethyl-2^3-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide (26 mg, 13% yield) and (1*R*,2*R*,3*S*)-*N*-((2^3*R*,6^3*S*,4*S*)-1^1-ethyl-2^3-hydroxy-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1*H*-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide (11 mg, 8% yield) both as solids. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{42}H_{56}N_6O_6$ 740.4; found 741.3; ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.76 (dd, *J* = 4.8, 1.7 Hz, 1H), 8.07 (d, *J* = 8.4 Hz, 1H), 7.88 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.61 - 7.55 (m, 1H), 7.55 - 7.47 (m, 2H), 7.42 (s, 1H), 6.24 (s, 1H), 5.66 (d, *J* = 12.2 Hz, 1H), 5.59 (t, *J* = 9.1 Hz, 1H), 4.91 (d, *J* = 6.7 Hz, 1H), 4.40 - 4.26 (m, 2H), 4.13 (q, *J* = 6.2 Hz, 1H), 4.08 - 3.96 (m, 2H), 3.88 - 3.78 (m, 1H), 3.72 (t, *J* = 11.5 Hz, 1H), 3.64 (q, *J* = 11.0 Hz, 2H), 3.11 (d, *J* = 14.2 Hz, 1H), 2.96 (dt, *J* = 14.4, 7.1 Hz, 2H), 2.80 (d, *J* = 23.8 Hz, 6H), 2.15 (t, *J* = 9.5 Hz, 1H), 2.06 (d, *J* = 8.6 Hz, 1H), 1.94 (d, *J* = 11.0 Hz, 1H), 1.84 - 1.73 (m, 1H), 1.66 - 1.51 (m, 2H), 1.41 (d, *J* = 6.2 Hz, 3H), 1.24 (s, 1H), 1.19 - 1.05 (m, 7H), 1.05 - 0.96 (m, 6H), 0.88 (s, 3H), 0.48 (s, 3H) and LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{42}H_{56}N_6O_6$ 740.4; found 741.4; ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.76 (dd, *J* = 4.7, 1.8 Hz, 1H), 8.12 (d, *J* = 8.5 Hz, 1H), 7.85 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.51 (q, *J* = 4.5 Hz, 3H), 7.45 (s, 1H), 6.19 (s, 1H), 5.70 (t, *J* = 8.7 Hz, 1H), 5.49 (s, 1H), 4.72 (d, *J* = 6.4 Hz, 1H), 4.31 (d, *J* = 12.7 Hz, 1H), 4.18 (d, *J* = 6.2 Hz, 2H), 4.14 - 4.06 (m, 1H), 3.99 - 3.68 (m, 3H), 3.61 (d, *J* = 11.0 Hz, 1H), 3.53 (d, *J* = 10.9 Hz, 1H), 3.14 (d, *J* = 15.8 Hz, 1H), 2.94 (s, 3H), 2.92 - 2.83 (m, 2H), 2.73 (d, *J* = 14.1 Hz, 3H), 2.33 (q, *J* = 1.8 Hz, 1H), 1.95 (d, *J* = 10.5 Hz, 1H), 1.77 (d, *J* = 10.6 Hz, 1H), 1.64 - 1.48 (m, 2H), 1.40 (d, *J* = 6.2 Hz, 3H), 1.24 (s, 1H), 1.19 - 0.95 (m, 12H), 0.85 (s, 1H), 0.72 (s, 3H), 0.58 (s, 3H).

Example A437. Synthesis of (2*S*,6*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-(2-(dimethylamino)ethoxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,4,6-trimethylpiperazine-1-carboxamide



Step 1.

To a mixture of ethyl (2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-(2-oxoethoxy)propanoate (0.31 g, 6.9 mmol) and dimethylamine, 30% in MeOH (865 mg, 8.5 mmol) in MeOH (20 mL) at 0 °C was added $NaBH_3CN$ (1.08 g, 17.2 mmol) over 2 min. The mixture was stirred at rt, then diluted with H_2O (30 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with H_2O (4 x 10 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give ethyl (2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-(2-(dimethylamino)ethoxy)propanoate (1.2 g, 45% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{17}H_{28}BrN_3O_5S$ 467.1; found 468.2.

Step 2.

To a mixture of ethyl (2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-(2-(dimethylamino)ethoxy)propanoate (1.25 g, 2.68 mmol) in THF (9 mL) at 0 °C under an atmosphere of N_2 was added 1M LiOH (8.0 mL, 8.0 mmol) dropwise. The mixture was stirred at 0 °C for 2 h, then acidified to pH ~6 with 1M HCl, then extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (3 x 10 mL), dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to give (2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-(2-(dimethylamino)ethoxy)propanoic acid (600 mg, 51% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{15}H_{24}BrN_3O_5S$ 439.1; found 440.3 [for ^{81}Br].

Step 3.

A mixture of (2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-(2-(dimethylamino)ethoxy)propanoic acid (570 mg, 1.3 mmol), 2-{[(2*M*)-1-ethyl-2-{2-[(1*S*)-1-methoxyethyl]pyridin-3-yl}-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-3-yl]methyl}-2-methylpropyl (3*S*)-1,2-diazinane-3-carboxylate (1.18 g, 1.95 mmol) and DIPEA (5.04 g, 39.0 mmol) in MECN (6 mL) at 0 °C under an atmosphere of N_2 was stirred for 5 min, then HATU (593 mg, 1.56 mmol) was added in portions over 2 min. The mixture was warmed to room temperature and stirred for 4 h, then diluted with H_2O (30 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with H_2O (3 x 10 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl-(*S*)-1-((2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-(2-(dimethylamino)ethoxy)propanoyl)hexahydropyridazine-3-carboxylate (580 mg, 44% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{49}H_{71}BBrN_7O_9S$ 1025.4; found 1026.5 [for ^{81}Br].

Step 4.

To a mixture of 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl-(*S*)-1-((2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-(2-(dimethylamino)ethoxy)propanoyl)hexahydropyridazine-3-carboxylate (580 mg, 0.57 mmol) and K_3PO_4 (300 mg, 1.42 mmol) in toluene (6 mL), 1,4-dioxane (2 mL) and H_2O (2 mL) under an atmosphere of Ar was added Pd(dtbpf)Cl_2. The mixture was heated to 60 °C and stirred for 4 h, then diluted with H_2O (30 mL), and the mixture was extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (3 x 10 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give (2*S*,6*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-(2-(dimethylamino)ethoxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,4,6-trimethylpiperazine-1-carboxamide (230 mg, 50% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{43}H_{59}N_7O_7S$ 817.4; found 818.4.

Step 5.

To a mixture of *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-3-(2-(dimethylamino)ethoxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (230 mg, 0.28 mmol) in DCM (3 mL) at 0 °C under an atmosphere of N_2 was added HCl in 1,4-dioxane (1 mL). The mixture was warmed to room temperature and stirred for 2 h, then concentrated under reduced pressure, toluene (30 mL) was added to the residue, and the mixture was concentrated under reduced pressure to give (6^3*S*,3*S*,4*S*,*Z*)-4-amino-3-(2-(dimethylamino)ethoxy)-1^1-ethyl-1^2-(2-((*S*)-1-

methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (200 mg) as a solid, which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{38}H_{51}N_7O_5S$ 717.4; found 718.7.

Step 6.

To a mixture of (6^3*S*,3*S*,4*S*,*Z*)-4-amino-3-(2-(dimethylamino)ethoxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (200 mg, 0.28 mmol) in THF (2 mL) at 0 °C was added NEt_3 (85 mg, 0.84 mmol) dropwise over 1 min. The mixture was stirred at 0 °C for 5 min, then 4-nitrophenyl chloroformate (56 mg, 0.28 mmol) was added over 1 min. The mixture was warmed to room temperature and stirred for 4 h and the mixture was concentrated under reduced pressure to give 4-nitrophenyl ((6^3*S*,3*S*,4*S*,*Z*)-3-(2-(dimethylamino)ethoxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (250 mg) as a solid, that was used directly in the next step without further purification.

Step 7.

To a mixture of 4-nitrophenyl ((6^3*S*,3*S*,4*S*,*Z*)-3-(2-(dimethylamino)ethoxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (200 mg, 0.23 mmol) in MeCN (2 mL) at 0 °C was added DIPEA (88 mg, 0.68 mmol) dropwise. The mixture was stirred at 0 °C for 2 min, then *tert*-butyl (3*S*,5*S*)-3,5-dimethylpiperazine-1-carboxylate (97 mg, 0.45 mmol) was added dropwise. The mixture was warmed to room temperature and stirred overnight, then diluted with H_2O (3 mL), and the mixture was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (3 x 10 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give *tert*-butyl (3*S*,5*S*)-4-(((6^3*S*,3*S*,4*S*,*Z*)-3-(2-(dimethylamino)ethoxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamoyl)-3,5-dimethylpiperazine-1-carboxylate (50 mg, 23% yield) as a solid. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{50}H_{71}N_9O_8S$ 957.5; found 958.9.

Step 8.

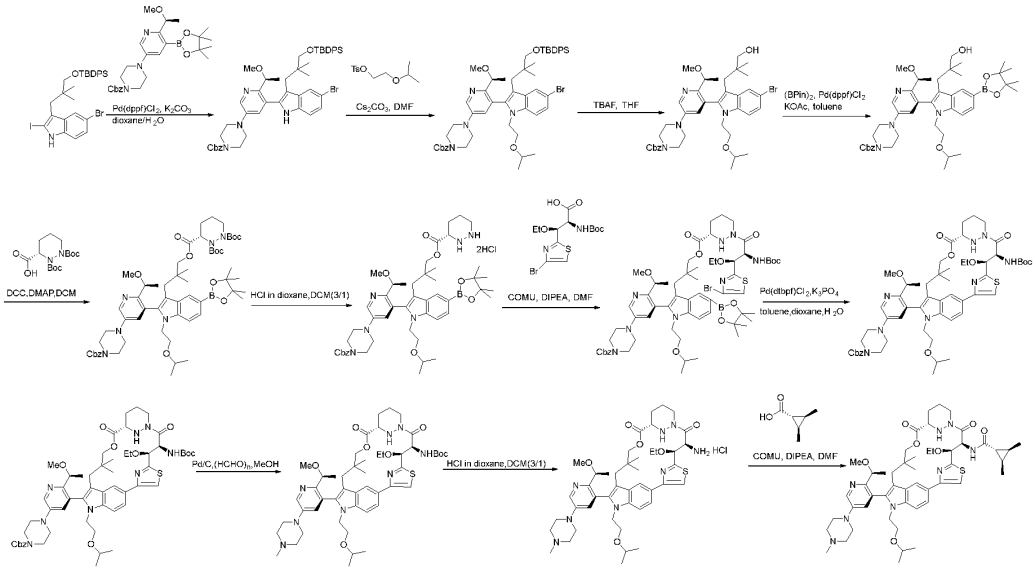
To a mixture of *tert*-butyl (3*S*,5*S*)-4-(((6^3*S*,3*S*,4*S*,*Z*)-3-(2-(dimethylamino)ethoxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamoyl)-3,5-dimethylpiperazine-1-carboxylate (50 mg, 0.05 mmol) in DCM (3 mL) at 0 °C under an atmosphere of N_2 was added HCl in 1,4-dioxane (1 mL) dropwise. The mixture was warmed to room temperature and stirred for 2 h, then concentrated under reduced pressure, toluene (30 mL) was added to the

residue and the mixture was concentrated under reduced pressure to give (2*S*,6*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-(2-(dimethylamino)ethoxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,6-dimethylpiperazine-1-carboxamide (50 mg) as a solid, that was used directly in the next step without further purification.

Step 9.

To a mixture of (2*S*,6*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-(2-(dimethylamino)ethoxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,6-dimethylpiperazine-1-carboxamide (40 mg, 0.05 mmol) and MeOH at 0 °C was added paraformaldehyde (11 mg, 0.24 mmol) in portions, followed by $NaBH_3CN$ (4.4 mg, 0.07 mmol) in portions. The mixture was warmed to room temperature and stirred overnight, then diluted with H_2O (5 mL) and extracted with EtOAc (3 x 10mL). The combined organic layers were washed with H_2O (3 x 10 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (2*S*,6*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-(2-(dimethylamino)ethoxy)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,4,6-trimethylpiperazine-1-carboxamide (3.3 mg, 8% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{46}H_{65}N_9O_6S$ 871.5; found 872.3; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.74 - 8.64 (dd, *J* = 4.7, 1.6 Hz, 1H), 8.49 - 8.36 (s, 1H), 7.91 - 7.81 (s, 1H), 7.79 - 7.71 (d, *J* = 7.7 Hz, 1H), 7.71 - 7.63 (d, *J* = 8.6 Hz, 1H), 7.56 - 7.47 (s, 1H), 7.47 - 7.36 (dd, *J* = 7.7, 4.7 Hz, 1H), 6.32 - 6.21 (d, *J* = 9.9 Hz, 1H), 5.55 - 5.46 (s, 1H), 5.19 - 5.11 (s, 1H), 5.04 - 4.97 (s, 1H), 4.45 - 3.94 (m, 6H), 3.67 - 3.36 (m, 11H), 3.23 (s, 3H), 3.11 (s, 3H), 2.78 - 2.75 (m, 2H), 2.40 - 2.34 (m, 3H), 2.15 (s, 6H), 2.11 - 2.04 (m, 6H), 1.75 (s, 2H), 1.59 - 1.41 (m, 1H), 1.30 (d, *J* = 6.0 Hz, 3H), 1.13 (s, 1H), 1.12 (d, *J* = 6.1 Hz, 6H), 0.80 (d, *J* = 28.4 Hz, 6H), 0.57 - 0.12 (s, 3H).

Example A438. Synthesis of (1*r*,2*R*,3*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-(2-isopropoxyethyl)-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide



Step 1.

To a mixture of 5-bromo-3-{3-[(*tert*-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl}-2-iodo-1*H*-indole (22.0 g, 34.0 mmol) and benzyl 4-[6-(methoxymethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl]piperazine-1-carboxylate (19.1 g, 40.8 mmol) in 1,4-dioxane (400 mL) and H_2O (80 mL) under an atmosphere of N_2 was added $Pd(dppf)Cl_2$ (2.49 g, 3.4 mmol) and K_2CO_3 (11.76 g, 85.1 mmol) in portions. The mixture was heated to 70 °C and stirred for 16 h, then H_2O added, and the mixture was extracted with EtOAc (3 x 200 mL). The combined organic layers were washed with brine (3 x 100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give benzyl (*S*)-4-(5-(5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (20 g, 67% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{49}H_{57}BrN_4O_4Si$ 874.3; found 875.5.

Step 2.

To a mixture of benzyl (*S*)-4-(5-(5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (23.0 g, 26.3 mmol) and 2-isopropoxyethyl 4-methylbenzenesulfonate (13.6 g, 52.6 mmol) in DMF (300 mL) under an atmosphere of N_2 was added Cs_2CO_3 (25.72 g, 79.0 mmol) in portions. The mixture was heated to 60 °C and stirred for 2, then diluted with brine (100 mL) and extracted with EtOAc (3 x 200 mL). The combined organic layers were washed with brine (3 x 200 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give benzyl (*S*)-4-(5-(5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1-(2-isopropoxyethyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (19.2 g, 76% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{54}H_{67}BrN_4O_5Si$ 960.4; found 961.4 [for ^{81}Br].

Step 3.

To a mixture of benzyl (*S*)-4-(5-(5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1-(2-isopropoxyethyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (3.0 g, 3.1 mmol) in THF (30 mL) at 0 °C under an atmosphere of N_2 was added TBAF, 1M in THF (15.6 mL, 15.6 mmol) in portions. The mixture was heated to 45 °C and stirred overnight, then diluted with brine (30 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine (3 x 50 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give benzyl (*S*)-4-(5-(5-bromo-3-(3-hydroxy-2,2-dimethylpropyl)-1-(2-isopropoxyethyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (1.19 g, 53% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{38}H_{49}BrN_4O_5$ 720.3; found 721.3.

Step 4.

To a mixture of benzyl (*S*)-4-(5-(5-bromo-3-(3-hydroxy-2,2-dimethylpropyl)-1-(2-isopropoxyethyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (1.09 g, 1.51 mmol) and bis(pinacolato)diboron (0.58 g, 2.27 mmol) in toluene (11 mL) under an atmosphere of N_2 was added $Pd(dppf)Cl_2$ (0.11 g, 0.15 mmol) and KOAc (0.37 g, 3.78 mmol) in portions. The mixture was heated to 80 °C and stirred for 2 h, then diluted with brine (10 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (3 x 20 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give benzyl (*S*)-4-(5-(3-(3-hydroxy-2,2-dimethylpropyl)-1-(2-isopropoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (1.0 g, 86% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{44}H_{61}BN_4O_7$ 768.5; found 769.7.

Step 5.

To a mixture of benzyl (*S*)-4-(5-(3-(3-hydroxy-2,2-dimethylpropyl)-1-(2-isopropoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (1.0 g, 1.3 mmol) and (3*S*)-1,2-bis(*tert*-butoxycarbonyl)-1,2-diazinane-3-carboxylic acid (0.64 g, 2.0 mmol) in DCM (10 mL) at 0 °C under an atmosphere of N_2 was added DMAP (0.24 g, 2.0 mmol) and DCC (0.40 g, 2.0 mmol) in portions. The mixture was warmed to room temperature and stirred overnight, then diluted with brine (20 mL) and extracted with DCM (3 x 50 mL). The combined organic layers were washed with brine (3 x 20 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-(3-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2-isopropoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl)1,2-di-*tert*-butyl-(*S*)-tetrahydropyridazine-1,2,3-tricarboxylate (1.08 g, 77% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{59}H_{85}BN_6O_{12}$ 1080.6; found 1081.7.

Step 6.

To a mixture of 3-(3-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2-isopropoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl)1,2-di-*tert*-butyl-(*S*)-tetrahydropyridazine-1,2,3-tricarboxylate (1.0 g, 0.9 mmol) in DCM (3 mL) at 0 °C under an atmosphere of N_2 was added HCl in 1,4-dioxane (9 mL) in portions. The mixture was warmed to room temperature and stirred for 6 h, then diluted with toluene (30 mL) and concentrated under reduced pressure to give 3-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2-isopropoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl-(*S*)-hexahydropyridazine-3-carboxylate bis hydrochloride (1.0 g) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{49}H_{69}BN_6O_8$ 880.5; found 881.5.

Step 7.

To a mixture of 3-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2-isopropoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl-(*S*)-hexahydropyridazine-3-carboxylate bis hydrochloride (1.0 g, 1.1 mmol) and (2*S*,3*S*)-3-(4-bromo-1,3-thiazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]-3-ethoxypropanoic acid (0.54 g, 1.36 mmol) in DMF (10 mL) at 0 °C under an atmosphere of N_2 was added DIPEA (4.40 g, 34.1 mmol) and (*Z*)-(ethyl cyano({[(dimethyliminiumyl)(morpholin-4-yl)methoxy]imino})formate); hexafluorophosphate (0.53 g, 1.3 mmol) in portions. The mixture was stirred at 0 °C for 1 h, then diluted with brine (30 mL) and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (3 x 30 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give 3-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2-isopropoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl-(*S*)-1-((2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-ethoxypropanoyl)hexahydropyridazine-3-carboxylate (1.0 g, 70% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{62}H_{86}BBrN_8O_{12}S$ 1258.5; found 1259.5.

Step 8.

To a mixture of 3-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2-isopropoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl-(*S*)-1-((2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-ethoxypropanoyl)hexahydropyridazine-3-carboxylate (1.0 g, 0.8 mmol) in toluene (30 mL), 1,4-dioxane (10 mL) and H_2O (10 mL) under an atmosphere of N_2 was added Pd(dtbpf)Cl_2 (0.16 g, 0.24 mmol) and K_3PO_4 (0.42 g, 2.0 mmol) in portions. The mixture was heated to 65 °C and stirred for 2 h, then diluted with H_2O and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (3 x 50 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give benzyl 4-(5-((6^3*S*,3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-3-ethoxy-1^1-(2-isopropoxyethyl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate

(370 mg, 44% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{56}H_{74}N_8O_{10}S$ 1050.5; found 1051.9.

Step 9.

To a mixture of benzyl 4-(5-((6^3*S*,3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-3-ethoxy-1^1-(2-isopropoxyethyl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (340 mg, 0.32 mmol) in MeOH (5 mL) was added paraformaldehyde (49 mg, 1.6 mmol) and Pd/C (34 mg, 0.32 mmol) in portions. The mixture was stirred under an atmosphere of H_2 overnight, then filtered through a pad of Celite and the filter cake was washed with MeOH (3 x 20 mL). The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-(2-isopropoxyethyl)-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (240 mg, 80% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{49}H_{70}N_8O_8S$ 930.5; found 931.5.

Step 10.

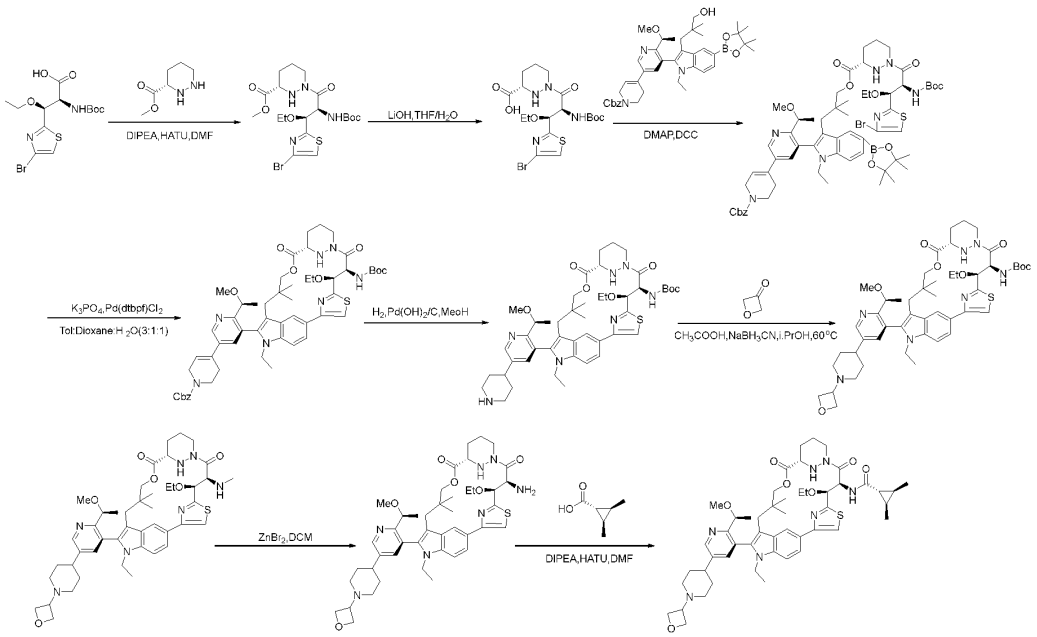
To a mixture of *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-(2-isopropoxyethyl)-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (240 mg, 0.26 mmol) in DCM (2 mL) at 0 °C under an atmosphere of N_2 was added HCl in 1,4-dioxane (6 mL) in portions. The mixture was stirred at 0 °C for 1 h, then diluted with toluene (20 mL) and concentrated under reduced pressure to give (6^3*S*,3*S*,4*S*,*Z*)-4-amino-3-ethoxy-1^1-(2-isopropoxyethyl)-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione HCl salt (240 mg) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{44}H_{62}N_8O_6S$ 830.5; found 831.4.

Step 11.

To a mixture of (6^3*S*,3*S*,4*S*,*Z*)-4-amino-3-ethoxy-1^1-(2-isopropoxyethyl)-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione HCl salt (100 mg, 0.12 mmol) and (1*R*,2*R*,3*S*)-2,3-dimethylcyclopropane-1-carboxylic acid (21 mg, 0.18 mmol) in DMF (5 mL) at 0 °C under an atmosphere of N_2 was added DIPEA (467 mg, 3.6 mmol) and (*Z*)-(ethyl cyano({[(dimethyliminiumyl)(morpholin-4-yl)methoxy]imino})formate); hexafluorophosphate (62 mg, 0.14 mmol) in portions. The mixture was stirred at 0 °C for 1 h, then diluted with brine (5 mL) and extracted with EtOAc (3 x 5 mL). The combined organic layers were washed with brine (3 x 15 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by (1*r*,2*R*,3*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-(2-isopropoxyethyl)-1^2-(2-((*S*)-

1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide (38 mg, 34% yield) as a solid. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{50}H_{70}N_8O_7S$ 926.5; found 927.5; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 - 8.42 (m, 2H), 7.91 (s, 1H), 7.72 (dd, *J* = 8.5, 1.6 Hz, 1H), 7.61 (dd, *J* = 18.7, 9.2 Hz, 2H), 7.21 (d, *J* = 2.9 Hz, 1H), 5.88 (d, *J* = 9.8 Hz, 1H), 5.19 (d, *J* = 12.2 Hz, 1H), 4.92 (s, 1H), 4.41 - 4.02 (m, 5H), 3.73 - 3.47 (m, 4H), 3.25 (q, *J* = 4.1, 2.7 Hz, 8H), 3.13 (s, 3H), 2.78 (t, *J* = 11.3 Hz, 2H), 2.49 - 2.39 (m, 6H), 2.22 (s, 4H), 2.11 - 1.96 (m, 1H), 1.78 (d, *J* = 29.7 Hz, 3H), 1.51 (s, 3H), 1.32 (d, *J* = 6.1 Hz, 4H), 1.24 (d, *J* = 2.8 Hz, 1H), 1.20 - 0.97 (m, 12H), 0.84 (dd, *J* = 23.4, 6.0 Hz, 10H), 0.44 (s, 3H).

Example A449. Synthesis of (1*r*,2*R*,3*S*)-N-((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(1-(oxetan-3-yl)piperidin-4-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide



Step 1.

A mixture of (2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-ethoxypropanoic acid (1.11 g, 7.7 mmol) and DIPEA (4.95 g, 38.3 mmol) in DMF (20 mL) was stirred at room temperature for 5 min, then (2S,3S)-3-(4-bromo-1,3-thiazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]-3-ethoxypropanoic acid (1.52 g, 3.8 mmol) and HATU (2.91 g, 7.7 mmol) were added. When the reaction was complete by LCMS, it was cooled to 0 °C, diluted with H_2O and extracted with EtOAc (50 mL). The organic layer was washed with brine (3 x 100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (*S*)-1-((2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-ethoxypropanoyl) hexahydropyridazine-3-

carboxylate (1.56 g, 78% yield). LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{19}H_{29}BrN_4O_6S$ 522.1; found 523.1 [for ^{81}Br].

Step 2.

A mixture of methyl (3*S*)-1-[(2*S*,3*S*)-3-(4-bromo-1,3-thiazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]-3-ethoxypropanoyl]-1,2-diazinane-3-carboxylate (1.56 g, 3.0 mmol), $LiOH.H_2O$ (0.63 g, 15.0 mmol), THF (5 mL) and H_2O (5 mL) at room temperature was stirred until complete by LCMS. The mixture was acidified to pH ~7 with 1N HCl was extracted with EtOAc (3 x 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure to give (3*S*)-1-[(2*S*,3*S*)-3-(4-bromo-1,3-thiazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]-3-ethoxypropanoyl]-1,2-diazinane-3-carboxylic acid (1.3 g), which was used directly in the next step without further purification. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{19}H_{29}BrN_4O_6S$ 506.1; found 507.1.

Step 3.

A mixture of (3*S*)-1-[(2*S*,3*S*)-3-(4-bromo-1,3-thiazol-2-yl)-2-[(tert-butoxycarbonyl)amino]-3-ethoxypropanoyl]-1,2-diazinane-3-carboxylic acid (600 mg, 1.18 mmol,), benzyl (5M)-5-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-2-yl]-6-[(1*S*)-1-methoxyethyl]-3',6'-dihydro-2'*H*-[3,4'-bipyridine]-1'-carboxylate (754 mg, 1.1 mmol), DMAP (29 mg, 0.24 mmol), DCC (488 mg, 2.37 mmol) and DCM (15 mL) at room temperature until deemed complete by LCMS. The mixture was diluted with DCM (100 mL) and washed with H_2O (100 mL), then the aqueous layer was extracted with DCM (3 x 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give benzyl (5M)-5-(3-{3-[(3*S*)-1-[(2*S*,3*S*)-3-(4-bromo-1,3-thiazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]-3-ethoxypropanoyl]-1,2-diazinane-3-carbonyloxy]-2,2-dimethylpropyl}-1-ethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-2-yl)-6-[(1*S*)-1-methoxyethyl]-3',6'-dihydro-2'*H*-[3,4'-bipyridine]-1'-carboxylate (735 mg, 52% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{60}H_{79}BBrN_7O_{11}S$ 1195.5; found 1196.4.

Step 4.

A mixture of benzyl (5M)-5-(3-{3-[(3*S*)-1-[(2*S*,3*S*)-3-(4-bromo-1,3-thiazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]-3-ethoxypropanoyl]-1,2-diazinane-3-carbonyloxy]-2,2-dimethylpropyl}-1-ethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-2-yl)-6-[(1*S*)-1-methoxyethyl]-3',6'-dihydro-2'*H*-[3,4'-bipyridine]-1'-carboxylate (725 mg, 0.61 mmol), K_3PO_4 (322 mg, 1.52 mmol) , Pd(DtBPF)Cl_2 (79 mg, 0.12 mmol), toluene (9 mL), 1,4-dioxane (3 mL) and H_2O (3 mL) under an atmosphere of N_2 was heated to 70 °C and stirred for 1 h at 70 °C. The mixture was diluted with H_2O (50 mL) and extracted with EtOAc (3 x 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give benzyl 5-((6^3*S*,3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-3-ethoxy-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-

8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-1^2-yl)-6-((S)-1-methoxyethyl)-3',6'-dihydro-[3,4'-bipyridine]-1'(2'*H*)-carboxylate (233 mg, 39% yield) as a an oil. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{54}H_{67}N_7O_9S$ 989.5; found 990.6.

Step 5.

A mixture of benzyl 5-((6^3*S*,3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-3-ethoxy-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-1^2-yl)-6-((S)-1-methoxyethyl)-3',6'-dihydro-[3,4'-bipyridine]-1'(2'*H*)-carboxylate (223 mg, 0.23 mmol) and $Pd(OH)_2/C$ (200 mg, 1.4 mmol) in MeOH (2 mL) was stirred under an atmosphere of H_2 until deemed complete by LCMS. The mixture was filtered, and the filter cake was washed with MeOH (3 x 20 mL). The filtrate was concentrated under reduced pressure to give *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(piperidin-4-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (192 mg) as a solid, that was used in the next step without further purification. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{46}H_{63}N_7O_7S$ 875.5; found 858.4.

Step 6.

A mixture of *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(piperidin-4-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (80 mg, 0.09 mmol), 3-oxetanone (134 mg, 1.86 mmol), AcOH (56 mg, 0.93 mmol) and $NaBH_3CN$ (59 mg, 0.93 mmol) in ^{i}PrOH (2 mL) at room temperature was stirred until deemed complete by LCMS. The mixture was cooled to 0 °C quenched with saturated $NaHCO_3$ and extracted with EtOAc (3 x 30 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(1-(oxetan-3-yl)piperidin-4-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (30 mg, 35% yield) as a an oil. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{49}H_{67}N_7O_8S$ 913.5; found 914.4.

Step 7.

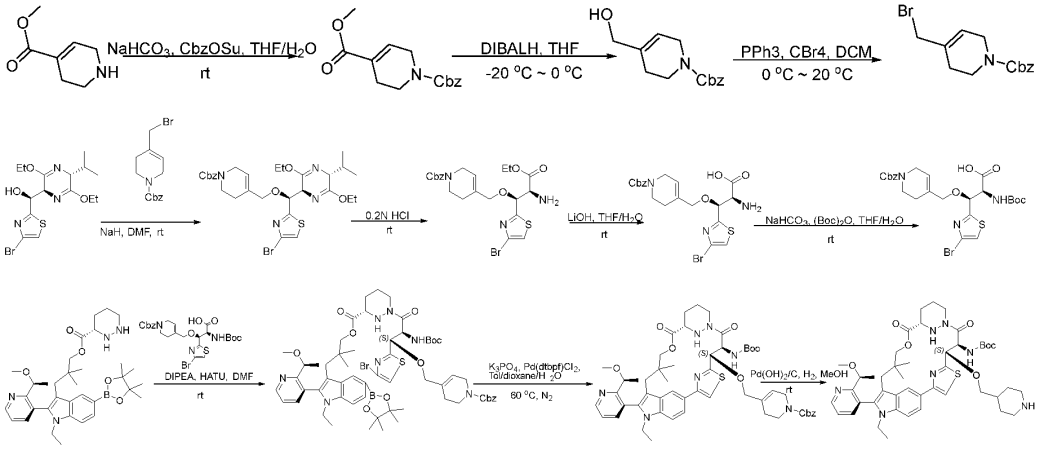
A mixture of *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(1-(oxetan-3-yl)piperidin-4-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (40 mg, 0.05 mmol), $ZnBr_2$ (55 mg, 0.24 mmol) and DCM (1 mL) was stirred at room temperature until deemed complete by LCMS. The mixture was filtered, the filter cake was washed with DCM (3 x 10 mL), and the filtrate was concentrated under reduced pressure to give (6^3*S*,3*S*,4*S*,*Z*)-4-amino-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(1-(oxetan-3-yl)piperidin-4-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-

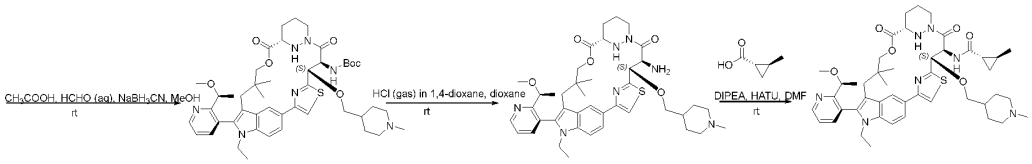
pyridazinacycloundecaphane-5,7-dioneas(crude) an oil, which was used directly in the next step without further purification. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{44}H_{59}N_7O_6S$ 813.4; found 814.8.

Step 8.

To a mixture of (6^3S,3*S*,4*S*,*Z*)-4-amino-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(1-(oxetan-3-yl)piperidin-4-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dioneas (40 mg, 0.05 mmol) in DMF (2 mL) at room temperature was added DIPEA (64 mg, 0.49 mmol), (1*R*,2*R*,3*S*)-2,3-dimethylcyclopropane-1-carboxylic acid (6 mg, 0.05 mmol) and HATU (37 mg, 0.1 mmol) in portions. The mixture was stirred until deemed complete by LCMS. The mixture was diluted with H_2O (10 mL), extracted with EtOAc (50 mL), the organic layer was washed with brine (3 x 50 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (1*r*,2*R*,3*S*)-N-((6^3S,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-(1-(oxetan-3-yl)piperidin-4-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide (3.5 mg, 8% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{50}H_{67}N_7O_7S$ 909.5; found 910.8; ^{1}H NMR (400 MHz, CD_3OD) δ 8.66 -8.52 (m, 2H), 7.82 - 7.72 (m, 2H), 7.67 (s, 1H), 7.53 - 7.44 (m, 1H), 5.99 (s, 1H), 4.78 - 4.68 (m, 4H), 4.71 - 4.54 (m, 10H), 4.48 - 4.39 (m, 1H), 4.36 - 4.02 (m, 4H), 3.75 - 3.68 (m, 1H), 3.68 - 3.64 (m, 3H), 3.02 - 2.97 (m, 3H), 2.91 - 2.80 (m, 2H), 2.65 - 2.63 (m, 1H), 2.24 - 2.12 (m, 3H), 2.03 - 2.00 (m, 4H), 1.93 - 1.72 (m, 3H), 1.69 - 1.55 (m, 1H), 1.47 - 1.23 (m, 6H), 1.16 - 1.13 (m, 5H), 1.06 - 0.85 (m, 5H), 0.50 (s, 3H).

Example A451. Synthesis of (1*S*,2*S*)-*N*-((6^3S,3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-3-((1-methylpiperidin-4-yl)methoxy)-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methylcyclopropane-1-carboxamide





Step 1.

To a mixture of methyl 1,2,3,6-tetrahydropyridine-4-carboxylate (10.0 g, 70.8 mmol) and $NaHCO_3$ (29.75 g, 354.2 mmol) in THF (50 mL) and H_2O (50 mL) was added CbzOSu (26.48 g, 106.3 mmol) in portions. The mixture was stirred at room temperature for 2 h, then washed with H_2O (3 x 100 mL) and the combined aqueous layers were extracted with EtOAc (3 x 100 mL). The combined organic layers were concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 1-benzyl 4-methyl 3,6-dihydropyridine-1,4(2*H*)-dicarboxylate as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{15}H_{17}NO_4$ 275.1; found 276.1.

Step 2.

To a mixture of 1-benzyl 4-methyl 3,6-dihydropyridine-1,4(2*H*)-dicarboxylate (8.0 g, 29.1 mmol) in THF at -20 °C under an atmosphere of N_2 was added DIBAL-H (80 mL, 80 mmol) dropwise. The mixture was stirred at -20 °C for 2 h, then warmed to room temperature and quenched with saturated NH_4Cl. The aqueous layer was extracted with EtOAc (2 x 100 mL) and the combined organic layers were concentrated under reduced pressure. The residue was purified by reverse-phase silica gel column chromatography to give benzyl 4-(hydroxymethyl)-3,6-dihydropyridine-1(2*H*)-carboxylate (2.3 g, 32% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{14}H_{17}NO_3$ 247.1; found 248.2.

Step 3.

To a mixture of benzyl 4-(hydroxymethyl)-3,6-dihydropyridine-1(2*H*)-carboxylate (2.3 g, 9.3 mmol) and PPh_3 (2.93 g, 11.2 mmol) in DCM at 0 °C was added CBr_4 (3.70 g, 11.2 mmol) dropwise. The mixture was stirred at room temperature until completion, then quenched with saturated NH_4Cl. The aqueous layer was extracted with EtOAc (2 x 30 mL), the combined organic layers were concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give benzyl 4-(bromomethyl)-3,6-dihydropyridine-1(2*H*)-carboxylate (2.1 g, 73% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{14}H_{16}BrNO_2$ 309.0; found 310.0.

Step 4.

To a mixture of (*S*)-(4-bromothiazol-2-yl)((2*S*,5*R*)-3,6-diethoxy-5-isopropyl-2,5-dihydropyrazin-2-yl)methanol (1.37 g, 3.39 mmol) in DMF (20 mL) at 0 °C was added NaH (0.16 g, 6.78 mmol) in portions. The mixture was stirred at 0 °C for 1 h, then benzyl 4-(bromomethyl)-3,6-dihydropyridine-1(2*H*)-carboxylate (2.10 g, 6.78 mmol) was added. The mixture was warmed to room temperature and stirred for 2 h, then diluted with saturated NH_4Cl (100 mL), and the mixture was extracted with EtOAc (2 x 30 mL). The combined organic layers were concentrated under reduced

pressure and the residue was purified by silica gel column chromatography to give benzyl 4-(((*S*)-(4-bromothiazol-2-yl)((2*S*,5*R*)-3,6-diethoxy-5-isopropyl-2,5-dihydropyrazin-2-yl)methoxy)methyl)-3,6-dihydropyridine-1(2*H*)-carboxylate (1.75 g, 82% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{29}H_{27}BrN_4O_5S$ 632.2; found 633.2.

Step 5.

To a mixture of benzyl 4-(((*S*)-(4-bromothiazol-2-yl)((2*S*,5*R*)-3,6-diethoxy-5-isopropyl-2,5-dihydropyrazin-2-yl)methoxy)methyl)-3,6-dihydropyridine-1(2*H*)-carboxylate (1.75 g, 2.76 mmol) in THF (40 mL) and MECN (16 mL) at 0 °C was added 0.02M HCl (35 mL, 0.7 mmol) dropwise. The mixture was warmed to room temperature and stirred overnight, then quenched with saturated $NaHCO_3$ and extracted with EtOAc (2 x 100 mL). The combined organic layers were concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give benzyl 4-(((1*S*,2*S*)-2-amino-1-(4-bromothiazol-2-yl)-3-ethoxy-3-oxopropoxy)methyl)-3,6-dihydropyridine-1(2*H*)-carboxylate (1.15 g, 79% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{22}H_{26}BrN_3O_5S$ 523.1; found 524.2.

Step 6.

A mixture of benzyl 4-(((1*S*,2*S*)-2-amino-1-(4-bromothiazol-2-yl)-3-ethoxy-3-oxopropoxy)methyl)-3,6-dihydropyridine-1(2*H*)-carboxylate (1.15 g, 2.19 mmol) and LiOH (0.21 g, 8.77 mmol) in THF (50 mL) and H_2O (50 mL) was stirred at room temperature for 1 h, then acidified to pH ~4 with 1M HCl. The mixture was then used directly in the next step without further purification. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{20}H_{22}BrN_3O_5S$ 497.0; found 497.9 [for ^{81}Br].

Step 7.

To the above mixture was added $NaHCO_3$ (0.97 g, 11.59 mmol) and $(Boc)_2O$ (1.01 g, 4.63 mmol). The mixture was stirred at room temperature overnight, then extracted with DCM (3 x 20 mL). The aqueous layer was acidified to pH ~4 with 1M HCl and extracted with EtOAc (3 x 30 mL). The combined organic layers were concentrated under reduced pressure to give (2*S*,3*S*)-3-((1-((benzyloxy)carbonyl)-1,2,3,6-tetrahydropyridin-4-yl)methoxy)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (1.1 g, 79% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{25}H_{30}BrN_3O_7S$ 597.1; found 598.0 [for ^{81}Br].

Step 8.

To a mixture of 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl (*S*)-hexahydropyridazine-3-carboxylate (1.5 g, 2.5 mmol) and (2*S*,3*S*)-3-((1-((benzyloxy)carbonyl)-1,2,3,6-tetrahydropyridin-4-yl)methoxy)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (1.48 g, 2.5 mmol) in DMF was added DIPEA (3.21 g, 24.8 mmol) and HATU (1.89 g, 4.96 mmol) in portions. The mixture was stirred at room temperature for 2 h, then washed with H_2O (3 x 30 mL). The combined aqueous layers were extracted with EtOAc (3 x 30 mL), the combined organic layers were concentrated under reduced

pressure and the residue was purified by silica gel column chromatography to give 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl-(*S*)-1-((2*S*,3*S*)-3-((1-((benzyloxy)carbonyl)-1,2,3,6-tetrahydropyridin-4-yl)methoxy)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (1.0 g, 34% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{59}H_{77}BBrN_7O_{11}S$ 1183.5; found 1184.3 [for ^{81}Br].

Step 9.

To a mixture of 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl-(*S*)-1-((2*S*,3*S*)-3-((1-((benzyloxy)carbonyl)-1,2,3,6-tetrahydropyridin-4-yl)methoxy)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (1.0 g, 0.85 mmol) and K_3PO_4 (0.45 g, 2.11 mmol) in toluene, dioxane and H_2O under an atmosphere of N_2 was added Pd(dtbpf)Cl_2 (0.11 g, 0.17 mmol) in portions. The mixture was heated to 60 °C and stirred for 2 h, then washed with H_2O (3 x 30 mL) and the combined aqueous layers extracted with EtOAc (3 x 30 mL). The combined organic layers were concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give benzyl 4-((((6^3*S*,3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-3-yl)oxy)methyl)-3,6-dihydropyridine-1(2*H*)-carboxylate (260 mg, 32% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{53}H_{65}N_7O_9S$ 975.5; found 976.6.

Step 10.

A mixture of benzyl 4-((((6^3*S*,3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-3-yl)oxy)methyl)-3,6-dihydropyridine-1(2*H*)-carboxylate (260 mg, 0.27 mmol) and $Pd(OH)_2$, 20% on carbon (0.26 g) in MeOH (3 mL) was stirred under an atmosphere of H_2 (balloon) at room temperature for 1 h. The mixture was filtered through a pad of Celite pad and the filtrate was concentrated under reduced pressure to give *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-3-(piperidin-4-ylmethoxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (230 mg, 72% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{45}H_{61}N_7O_7S$ 843.4; found 844.3.

Step 11.

To a mixture of *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-3-(piperidin-4-ylmethoxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (230 mg, 0.27 mmol) and AcOH (49 mg, 0.82 mmol) in MeOH was added paraformaldehyde (49 mg, 1.6 mmol) and $NaBH_3CN$ (86 mg, 1.36 mmol) in portions. The mixture was stirred at room temperature for 2 h, then diluted with

H_2O and extracted with EtOAc (3 x 20 mL). The combined organic layers were concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl ((6^3*S*,3*S*,4*S*, *Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-3-((1-methylpiperidin-4-yl)methoxy)-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (280 mg, 81% yield) as a an oil. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{46}H_{63}N_7O_7S$ 857.5; found 858.4.

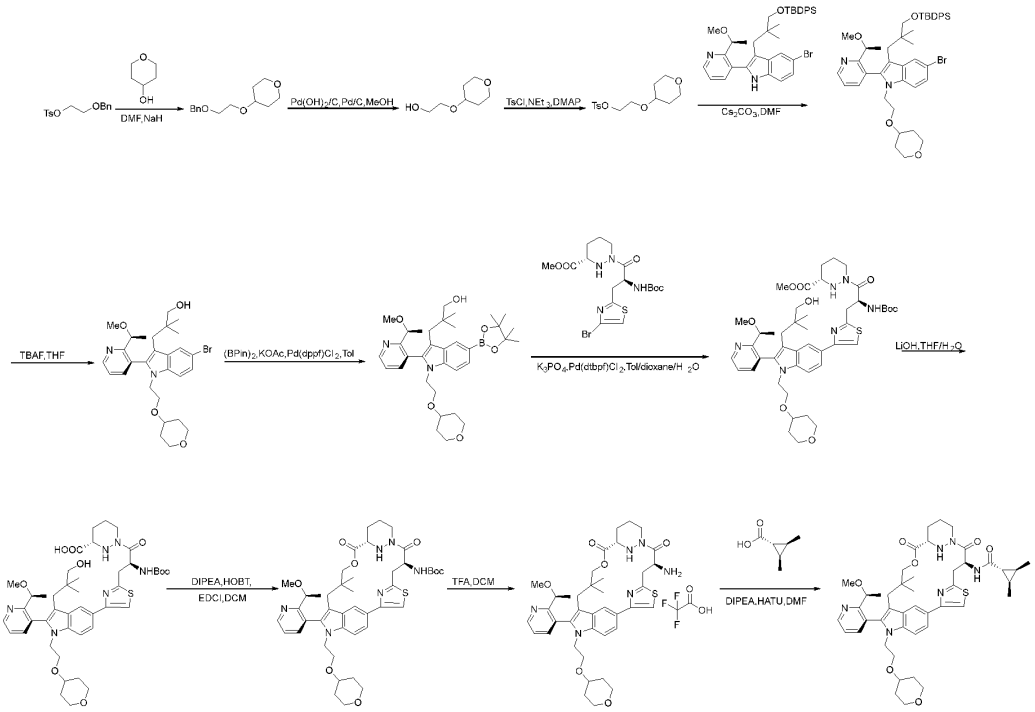
Step 12.

A mixture of *tert*-butyl ((6^3*S*,3*S*,4*S*, *Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-3-((1-methylpiperidin-4-yl)methoxy)-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (280 mg, 0.33 mmol) and HCl in 1,4-dioxane (3 mL) in 1,4-dioxane was stirred at room temperature for 1 h. The mixture was concentrated under reduced pressure to give (6^3*S*,3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-3-((1-methylpiperidin-4-yl)methoxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione as an oil. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{41}H_{55}N_7O_5S$ 757.4; found 758.5.

Step 13.

To a mixture of (6^3*S*,3*S*,4*S*,*Z*)-4-amino-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-3-((1-methylpiperidin-4-yl)methoxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (265 mg, 0.35 mmol) and (1*S*,2*S*)-2-methylcyclopropane-1-carboxylic acid (35 mg, 0.35 mmol) in DMF was added DIPEA (904 mg, 7.0 mmol) in portions. The mixture was stirred at room temperature for 2 h, then washed with H_2O (3 x 20 mL). The combined aqueous layers were extracted with EtOAc (2 x 20 mL) and the combined organic layers were concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (1*S*,2*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-3-((1-methylpiperidin-4-yl)methoxy)-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methylcyclopropane-1-carboxamide (24 mg, 8% yield) as a solid. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{46}H_{61}N_7O_6S$ 839.4; found 840.4; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.83 - 8.64 (m, 1H), 8.56 - 8.43 (m, 1H), 8.00 - 7.89 (m, 1H), 7.81 - 7.73 (m, 3H), 7.63 - 7.53 (m, 2H), 5.93 - 5.85 (m, 1H), 5.20 - 5.12 (m, 1H), 4.90 (s, 1H), 4.32 - 4.13 (m, 5H), 3.68 - 3.60 (m, 2H), 3.26 - 3.22 (m, 5H), 2.95 - 2.83 (m, 1H), 2.79 - 2.72 (m, 3H), 2.49 - 2.46 (m, 1H), 2.20 - 2.10 (s, 3H), 2.09 - 2.01 (m, 1H), 1.93 - 1.72 (m, 6H), 1.70 - 1.45 (m, 3H), 1.41 - 1.30 (m, 3H),1.21 - 0.99(m, 7H), 0.98 - 0.87(m, 8H),0.60 - 0.50 (m, 1H), 0.35 (s, 3H).

Example A457. Synthesis of (1*r*,2*R*,3*S*)-*N*-((6^3*S*,4*S*,*Z*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2-((tetrahydro-2*H*-pyran-4-yl)oxy)ethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide



Step 1.

To a mixture of (*S*)-5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1*H*-indole (3.0 g, 4.6 mmol) and 2-((tetrahydro-2*H*-pyran-4-yl)oxy)ethyl 4-methylbenzenesulfonate (2.06 g, 6.9 mmol) in DMF (20 mL) at 0 °C under an atmosphere of N_2 was added Cs_2CO_3 (3.73 g, 11.4 mmol) in portions. The mixture was heated to 65 °C and stirred overnight, then diluted with H_2O (50 mL) and extracted with EtOAc (3 x 50mL). The combined organic layers were washed with brine (3 x 50 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (*S*)-5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2-((tetrahydro-2*H*-pyran-4-yl)oxy)ethyl)-1*H*-indole (2.3 g, 64% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{44}H_{55}BrN_2O_4Si$ 784.3; found 785.2.

Step 2.

To a mixture of (*S*)-5-bromo-3-(3-((*tert*-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2-((tetrahydro-2*H*-pyran-4-yl)oxy)ethyl)-1*H*-indole (2.3 g, 2.9 mmol) in THF (20 mL) at 0 °C under an atmosphere of N_2 was added TBAF, 1M in THF (14.67 mL, 14.7 mmol) in portions. The mixture was heated to 45 °C and stirred for 6 h, then diluted with H_2O and extracted with EtOAc (3 x 30mL). The combined organic layers were washed with brine (3 x 30 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give (*S*)-3-(5-bromo-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2-((tetrahydro-2*H*-pyran-4-yl)oxy)ethyl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (690 mg, 43% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{28}H_{37}BrN_2O_4$ 546.2; found 546.9.

Step 3.

To a mixture of (*S*)-3-(5-bromo-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2-((tetrahydro-2*H*-pyran-4-yl)oxy)ethyl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (690 mg, 1.3 mmol) and $(Bpin)_2$ (643 mg, 2.5 mmol) in toluene (7 mL) under an atmosphere of Ar was added KOAc (372 mg, 3.8 mmol) and $Pd(dppf)Cl_2$ (93 mg, 0.13 mmol) in portions. The mixture was heated to 80 °C and stirred for 2.5 h, then diluted with H_2O (20 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (3 x 30 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give (*S*)-3-(2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2-((tetrahydro-2*H*-pyran-4-yl)oxy)ethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (700 mg, 93% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{34}H_{49}BN_2O_6$ 592.4; found 593.1.

Step 4.

To a mixture of (*S*)-3-(2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2-((tetrahydro-2*H*-pyran-4-yl)oxy)ethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (670 mg, 1.13 mmol) and $Pd(DtBPF)Cl_2$ (147 mg, 0.23 mmol) in toluene (3 mL), 1,4-dioxane (1 mL) and H_2O (1 mL) under an atmosphere of Ar was added methyl (*S*)-1-((*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (648 mg, 1.36 mmol) and K_3PO_4 (720 mg, 3.39 mmol) in portions. The mixture was heated to 60 °C and stirred for 3 h, then diluted with H_2O (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (3 x 10 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2-((tetrahydro-2*H*-pyran-4-yl)oxy)ethyl)-1*H*-indol-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (680 mg, 70% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{45}H_{62}N_6O_9S$ 862.4; found 863.1.

Step 5.

To a mixture of methyl (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2-((tetrahydro-2*H*-pyran-4-yl)oxy)ethyl)-1*H*-indol-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (300 mg, 0.35 mmol) in THF (6 mL) at 0 °C under an atmosphere of N_2 was added 1M LiOH (1.74 mL, 1.74 mmol) in portions. When the reaction was deemed complete by LCMS the mixture was acidified to pH ~6 with 1M HCl, then extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (2 x 20 mL), dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to give (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2-((tetrahydro-2*H*-pyran-4-yl)oxy)ethyl)-1*H*-indol-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (280 mg) as a solid, which was used directly in

the next step without further purification. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{44}H_{60}N_8O_9S$ 848.4; found 849.4.

Step 6.

To a mixture of (*S*)-1-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(4-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2-((tetrahydro-2*H*-pyran-4-yl)oxy)ethyl)-1*H*-indol-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (300 mg, 0.35 mmol) and DIPEA (457 mg, 3.5 mmol) in DCM (50 mL) at 0 °C under an atmosphere of N_2 was added HOBT (477 mg, 3.5 mmol) and EDCI (2.03 g, 10.6 mmol) in portions. The mixture was warmed to room temperature and stirred overnight, then washed with H_2O (50 mL) and the aqueous layer was extracted with DCM (3 x 50 mL). The combined organic layers were washed with brine (3 x 100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give t*ert*-butyl ((6^3*S*,4*S*,*Z*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2-((tetrahydro-2*H*-pyran-4-yl)oxy)ethyl)-6^1,6^2,6^3,6^4,65,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (200 mg, 68% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{44}H_{58}N_6O_8S$ 830.4; found 831.3.

Step 7.

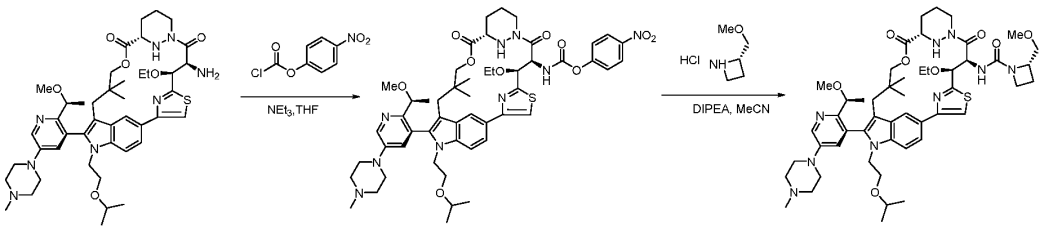
To a mixture of t*ert*-butyl ((6^3*S*,4*S*,*Z*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2-((tetrahydro-2*H*-pyran-4-yl)oxy)ethyl)-6^1,6^2,6^3,6^4,65,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (90 mg, 0.11 mmol) in DCM (1 mL) at 0 °C under an atmosphere of N_2 was added TFA (1 mL) in portions. The mixture was stirred at 0 °C for 1.5 h, then concentrated under reduced pressure to give (6^3*S*,4*S*,*Z*)-4-amino-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-1^1-(2-((tetrahydro-2*H*-pyran-4-yl)oxy)ethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione TFA salt (80 mg) as an oil, that was used directly in the next step without further purification. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{39}H_{50}N_6O_6S$ 730.4; found 731.4.

Step 8.

To a mixture of (6^3*S*,4*S*,*Z*)-4-amino-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-1^1-(2-((tetrahydro-2*H*-pyran-4-yl)oxy)ethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione TFA salt (90 mg, 0.12 mmol) and (1*r*,2*R*,3*S*)-2,3-dimethylcyclopropane-1-carboxylic acid (17 mg, 0.15 mmol) at 0 °C under an atmosphere of N_2 was added DIPEA (318 mg, 2.5 mmol) and HATU (56 mg, 0.15 mmol) in portions. The mixture was stirred at 0 °C for 1 h, then washed with H_2O (1 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (30 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (1*r*,2*R*,3*S*)-*N*-((6^3*S*,4*S*,*Z*)-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2-((tetrahydro-2*H*-pyran-4-yl)oxy)ethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-

oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide (17 mg, 17% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{45}H_{58}N_6O_7S$ 826.4; found 827.6; ^1H NMR (400 MHz, DMSO-d_6) δ 8.74 (dd, J = 4.7, 1.8 Hz, 1H), 8.48 (d, J = 1.6 Hz, 1H), 8.39 (d, J = 9.0 Hz, 1H), 7.81 (s, 1H), 7.74 (ddd, J = 10.5, 8.2, 1.8 Hz, 2H), 7.61 (d, J = 8.7 Hz, 1H), 7.51 (dd, J = 7.7, 4.7 Hz, 1H), 5.56 (t, J = 9.0 Hz, 1H), 5.07 (d, J = 12.1 Hz, 1H), 4.46 (dt, J = 14.9, 5.3 Hz, 1H), 4.32 - 4.09 (m, 4H), 3.62 - 3.47 (m, 4H), 3.34 - 3.30 (m, 1H), 3.30 - 3.28 (m, 1H), 3.26 (s, 3H), 3.21 - 3.04 (m, 4H), 2.94 (d, J = 14.3 Hz, 1H), 2.83 - 2.68 (m, 1H), 2.46 - 2.43 (m, 1H), 2.16 - 2.05 (m, 1H), 1.76 (d, J = 20.9 Hz, 2H), 1.51 (d, J = 13.5 Hz, 3H), 1.36 (d, J = 6.1 Hz, 3H), 1.30 - 1.09 (m, 3H), 1.13 - 0.98 (m, 8H), 0.88 (s, 3H), 0.32 (s, 3H).

Example A459. Synthesis of (2*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-(2-isopropoxyethyl)-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-(methoxymethyl)azetidine-1-carboxamide



Step 1.

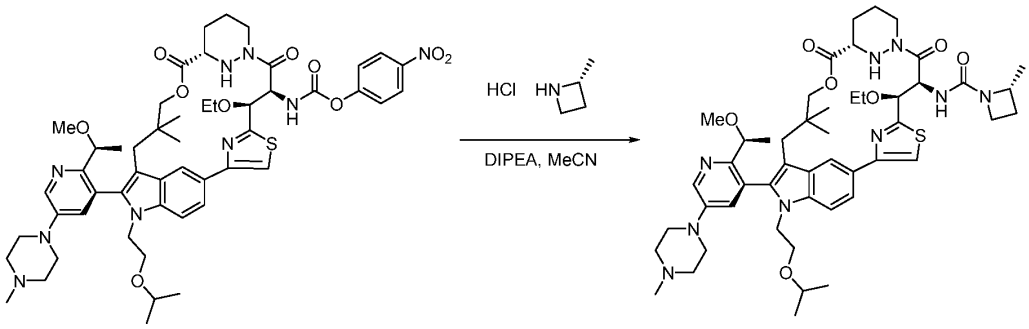
A mixture of (6^3*S*,3*S*,4*S*,*Z*)-4-amino-3-ethoxy-1^1-(2-isopropoxyethyl)-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (170 mg, 0.21 mmol) in THF (6 mL) at 0 °C under an atmosphere of N_2 was added TEA (62 mg, 0.62 mmol) and 4-nitrophenyl chloroformate (62 mg, 0.31 mmol) in portions. The mixture was warmed to room temperature and stirred for 4 h, then concentrated under reduced pressure to give 4-nitrophenyl ((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-(2-isopropoxyethyl)-12-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (200 mg) as a solid, which was used directly in the next step without further purification.

Step 2.

To a mixture of 4-nitrophenyl ((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-(2-isopropoxyethyl)-12-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (150 mg, 0.15 mmol) in MeCN (8 mL) at 0 °C under an atmosphere of N_2 was added DIPEA (78 mg, 0.60 mmol) and (2*S*)-2-(methoxymethyl)azetidine HCl salt (46 mg, 0.45 mmol) in portions. The mixture was warmed to room temperature and stirred overnight, then the mixture was diluted with brine (10 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were

washed with brine (3 x 20 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (2*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-(2-isopropoxyethyl)-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-(methoxymethyl)azetidine-1-carboxamide (56 mg, 39% yield) as a solid. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{50}H_{71}N_9O_8S$ 957.5; found 958.4; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.47 (dd, *J* = 14.9, 2.2 Hz, 2H), 7.91 (s, 1H), 7.74 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.59 (d, *J* = 8.7 Hz, 1H), 7.21 (s, 1H), 6.83 (d, *J* = 10.2 Hz, 1H), 5.51 (d, *J* = 10.2 Hz, 1H), 5.16 (d, *J* = 12.0 Hz, 1H), 4.90 (s, 1H), 4.37 (s, 2H), 4.27 - 4.00 (m, 4H), 3.71 (q, *J* = 8.4 Hz, 1H), 3.65 - 3.54 (m, 5H), 3.50 (q, *J* = 9.4, 8.4 Hz, 2H), 3.43 (s, 3H), 3.40 (s, 2H), 3.30 (s, 1H), 3.29 - 3.20 (m, 4H), 3.13 (s, 3H), 2.90 - 2.72 (m, 2H), 2.67 (s, 1H), 2.43 (s, 2H), 2.23 (s, 4H), 2.05 (d, *J* = 12.0 Hz, 1H), 1.82 (dd, *J* = 23.8, 13.9 Hz, 3H), 1.52 (d, *J* = 12.1 Hz, 1H), 1.33 (d, *J* = 6.1 Hz, 3H), 1.13 (t, *J* = 7.0 Hz, 3H), 0.93 - 0.84 (m, 6H), 0.81 (d, *J* = 6.1 Hz, 3H), 0.44 (s, 3H).

Example A460. Synthesis of (2*R*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-(2-isopropoxyethyl)-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methylazetidine-1-carboxamide

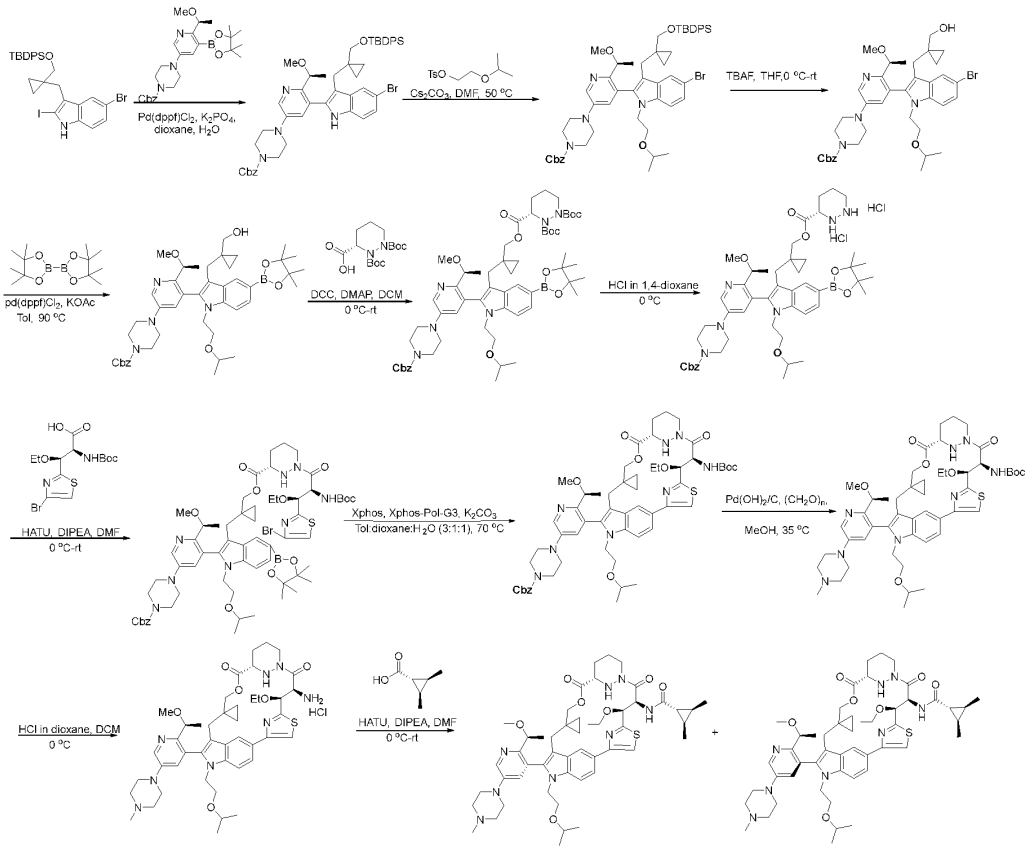


Step 1.

To a mixture of 4-nitrophenyl ((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-(2-isopropoxyethyl)-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (150 mg, 0.15 mmol) in MECN (8 mL) at 0 °C under an atmosphere of N_2 was added DIPEA (78 mg, 0.60 mmol) and (2*R*)-2-methylazetidine (32 mg, 0.45 mmol) in portions. The mixture was warmed to room temperature and stirred overnight, then diluted with brine (10 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (3 x 20 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (2*R*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-(2-isopropoxyethyl)-1^2-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methylazetidine-1-carboxamide (57 mg, 41% yield) as a solid.

LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{49}H_{69}N_9O_7S$ 927.5; found 928.4; ^1H NMR (400 MHz, DMSO-d_6) δ 8.53 - 8.40 (m, 2H), 7.93 (s, 1H), 7.78 - 7.69 (m, 1H), 7.59 (d, *J* = 8.6 Hz, 1H), 7.21 (s, 1H), 5.56 (d, *J* = 10.2 Hz, 1H), 5.21 (d, *J* = 12.1 Hz, 1H), 5.12 (d, *J* = 10.2 Hz, 1H), 4.91 (s, 1H), 4.35 (d, *J* = 13.9 Hz, 1H), 4.27 - 4.12 (m, 4H), 4.08 (d, *J* = 6.1 Hz, 1H), 3.75 (t, *J* = 7.4 Hz, 2H), 3.65 - 3.46 (m, 4H), 3.30 (s, 2H), 3.29 (s, 1H), 3.26 (d, *J* = 5.9 Hz, 5H), 3.13 (s, 3H), 2.79 (d, *J* = 13.5 Hz, 2H), 2.60 (s, 2H), 2.45 (p, *J* = 1.9 Hz, 4H), 2.22 (s, 3H), 2.06 (d, *J* = 12.1 Hz, 1H), 1.82 (s, 3H), 1.54 - 1.49 (m, 1H), 1.34 (dd, *J* = 14.9, 6.2 Hz, 6H), 1.13 (t, *J* = 7.0 Hz, 3H), 0.87 (t, *J* = 6.8 Hz, 6H), 0.81 (d, *J* = 6.0 Hz, 3H), 0.44 (s, 3H).

Example A476. Synthesis of (1*R*,2*R*,3*S*)-*N*-((3'*S*,3'*S*,4'*S*,*Z*)-3'-ethoxy-1'-(2-isopropoxyethyl)-2'-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-5',7'-dioxospiro[cyclopropane-1,10'-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphan]-4'-yl)-2,3-dimethylcyclopropane-1-carboxamide and (1*R*,2*R*,3*S*)-*N*-((3'*S*,3'*S*,4'*S*,*Z*)-3'-ethoxy-1'-(2-isopropoxyethyl)-2'-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-5',7'-dioxospiro[cyclopropane-1,10'-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphan]-4'-yl)-2,3-dimethylcyclopropane-1-carboxamide



Step 1.

To a mixture of 5-bromo-3-((1-(((*tert*-butyldiphenylsilyl)oxy)methyl)cyclopropyl)methyl)-2-iodo-1*H*-indole (6.1 g, 9.5 mmol) and benzyl (*S*)-4-(6-(1-methoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl)piperazine-1-carboxylate (4.56 g, 9.5 mmol) in 1,4-dioxane (120 mL) and H_2O (20 mL) under an atmosphere of N_2 was added Pd(dppf)Cl_2 (1.04 g, 1.4 mmol) and K_3PO_4 (4.02 g, 18.9 mmol) in portions. The mixture was heated to 65°C and stirred for 3 h, then extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine (3 x 100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give benzyl (*S*)-4-(5-(5-bromo-3-((1-(((*tert*-butyldiphenylsilyl)oxy)methyl)cyclopropyl)methyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (4.7 g, 57% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{49}H_{55}BrN_4O_4Si$ 870.3; found 871.5.

Step 2.

To a mixture of benzyl (*S*)-4-(5-(5-bromo-3-((1-(((*tert*-butyldiphenylsilyl)oxy)methyl)cyclopropyl)methyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (4.6 g, 5.3 mmol) in DMF (50 mL) at 0 °C was added 2-isopropoxyethyl 4-methylbenzenesulfonate (2.04 g, 7.9 mmol) and Cs_2CO_3 (5.16 g, 15.8 mmol). The mixture was heated to 50 °C and stirred for 2 h, then diluted with H_2O and extracted with EtOAc (2 x 100 mL). The combined organic layers were washed with brine (3 x 100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by reverse-phase silica gel column chromatography to give benzyl (*S*)-4-(5-(5-bromo-3-((1-(((*tert*-butyldiphenylsilyl)oxy)methyl)cyclopropyl)methyl)-1-(2-isopropoxyethyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (4.5 g, 89% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{54}H_{65}BrN_4O_5Si$ 956.4; found 957.2.

Step 3.

To a mixture of benzyl (*S*)-4-(5-(5-bromo-3-((1-(((*tert*-butyldiphenylsilyl)oxy)methyl)cyclopropyl)methyl)-1-(2-isopropoxyethyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (3.2 g, 3.3 mmol) in THF (32 mL) at 0 °C was added TBAF (13.1 g, 50.1 mmol). The mixture was warmed to room temperature and stirred for 16 h, then diluted with H_2O and extracted with EtOAc (2 x 100 mL). The combined organic layers were washed with brine (3 x 100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by reverse-phase silica gel column chromatography to give benzyl (*S*)-4-(5-(5-bromo-3-((1-(hydroxymethyl)cyclopropyl)methyl)-1-(2-isopropoxyethyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (1.84 g, 77% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{38}H_{47}BrN_4O_5$ 718.3; found 719.2.

Step 4.

To a mixture of benzyl (*S*)-4-(5-(5-bromo-3-((1-(hydroxymethyl)cyclopropyl)methyl)-1-(2-isopropoxyethyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (1.7 g, 2.4

mmol) in toluene (20 mL) at 0 °C under an atmosphere of Ar was added 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (1.80 g, 7.1 mmol), KOAc (580 mg, 5.9 mmol) and Pd(dppf)Cl_2 (346 mg, 0.47 mmol). The mixture was heated to 90 °C and stirred for 3 h, then diluted with H_2O and extracted with EtOAc (2 x 50 mL). The combined organic layers were washed with brine (2 x 50 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by reverse-phase silica gel column chromatography to give benzyl (*S*)-4-(5-(3-((1-(hydroxymethyl)cyclopropyl)methyl)-1-(2-isopropoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (1.4 g, 77% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{44}H_{59}BN_4O_7$ 766.5; found 767.4.

Step 5.

To a mixture of benzyl (*S*)-4-(5-(3-((1-(hydroxymethyl)cyclopropyl)methyl)-1-(2-isopropoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (1.4 g, 1.8 mmol) in DCM (20 mL) at 0 °C was added (*S*)-1,2-bis(*tert*-butoxycarbonyl)hexahydropyridazine-3-carboxylic acid (664 mg, 2.0 mmol), DCC (490 mg, 2.4 mmol) and DMAP (45 mg, 0.37 mmol). The mixture was warmed to room temperature and stirred for 16 h, then diluted with H_2O and extracted with EtOAc (2 x 100 mL). The combined organic layers were washed with brine (2 x 100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by reverse-phase silica gel column chromatography to give 3-((1-((2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2-isopropoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)methyl)cyclopropyl)methyl) 1,2-di-*tert*-butyl (*S*)-tetrahydropyridazine-1,2,3-tricarboxylate (1.2 g, 61% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{59}H_{83}BN_6O_{12}$ 1078.6; found 1079.5.

Step 6.

A mixture of 3-((1-((2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2-isopropoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)methyl)cyclopropyl)methyl) 1,2-di-*tert*-butyl (*S*)-tetrahydropyridazine-1,2,3-tricarboxylate (1.2 g, 1.1 mmol) in HCl in 1,4-dioxane (15 mL) at 0 °C was stirred for 2 h, then concentrated under reduced pressure to give (1-((2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2-isopropoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)methyl)cyclopropyl)methyl (*S*)-hexahydropyridazine-3-carboxylate (1.25 g), which was used directly in the next step without further purification. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{49}H_{67}BN_6O_8$ 878.5; found 879.4.

Step 7.

To a mixture of (1-((2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2-isopropoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)methyl)cyclopropyl)methyl (*S*)-hexahydropyridazine-3-carboxylate (1.0 g, 1.1 mmol) in

DMF (15 mL) at 0 °C was added DIPEA (1.47 g, 11.4 mmol) and (2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-ethoxypropanoic acid (675 mg, 1.7 mmol), followed by HATU (865 mg, 2.3 mmol). The mixture was stirred at 0 °C for 2 h, then diluted with H_2O and extracted with EtOAc (2 x 100 mL). The combined organic layers were washed with brine (2 x 100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give (1-((2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2-isopropoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)methyl)cyclopropyl)methyl (*S*)-1-((2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-ethoxypropanoyl)hexahydropyridazine-3-carboxylate (730 mg, 51% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{62}H_{84}BBrN_8O_{12}S$ 1254.5; found 1255.4.

Step 8.

To a mixture of (1-((2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-(2-isopropoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)methyl)cyclopropyl)methyl (*S*)-1-((2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-ethoxypropanoyl)hexahydropyridazine-3-carboxylate (700 mg, 0.56 mmol) in toluene (12 mL), 1,4-dioxane (4 mL) and H_2O (4 mL) at 0 °C under an atmosphere of Ar was added XPhos (53 mg, 0.11 mmol), K_3PO_4 (296 mg, 1.39 mmol) and XPho-Pd-G3 (47 mg, 0.06 mmol). The mixture was heated to 65 °C and stirred for 2 h, then diluted with H_2O and extracted with EtOAc (2 x 50 mL). The combined organic layers were washed with brine (2 x 50 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give benzyl 4-(5-((3'*S*,3'*S*,4'*S*,*Z*)-4'-((*tert*-butoxycarbonyl)amino)-3'-ethoxy-1'-(2-isopropoxyethyl)-5',7'-dioxospiro[cyclopropane-1,10'-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphan]-2'-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (160 mg, 27% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{56}H_{72}N_8O_{10}S$ 1048.5; found 1049.3.

Step 9.

To a mixture of benzyl 4-(5-((3'*S*,3'*S*,4'*S*,*Z*)-4'-((*tert*-butoxycarbonyl)amino)-3'-ethoxy-1'-(2-isopropoxyethyl)-5',7'-dioxospiro[cyclopropane-1,10'-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphan]-2'-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (160 mg, 0.15 mmol) in MeOH (3 mL) at 0 °C was added methoxymethanol amine (34 mg, 0.76 mmol) and $Pd(OH)_2/C$ (171 mg, 1.2 mmol). The mixture was placed under an atmosphere of H_2, heated to 35 °C and stirred for 4 h, then filtered, and the filter cake was washed with MeOH (2 x 50 mL). The filtrate was concentrated under reduced pressure to give *tert*-butyl ((3'*S*,3'*S*,4'*S*,*Z*)-3'-ethoxy-1'-(2-isopropoxyethyl)-2'-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-5',7'-dioxospiro[cyclopropane-1,10'-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphan]-4'-yl)carbamate (100 mg), which was used directly in the next step without further purification. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{49}H_{68}N_8O_8S$ 928.5; found 929.4.

Step 10.

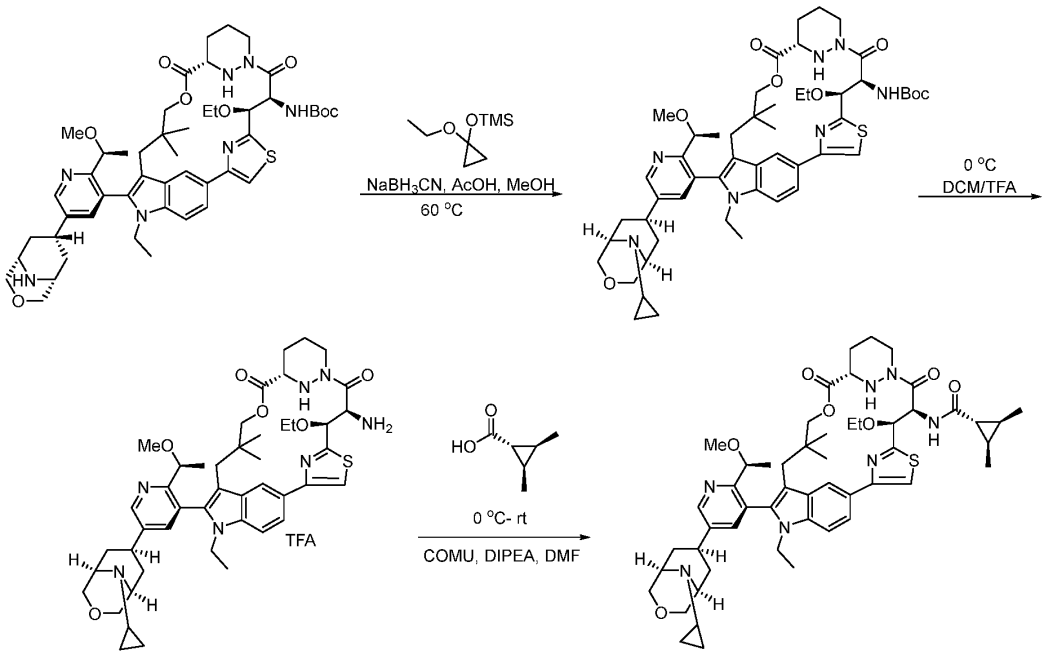
To a mixture of *tert*-butyl ((3'*S*,3'*S*,4'*S*,*Z*)-3'-ethoxy-1'-(2-isopropoxyethyl)-2'-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-5',7'-dioxospiro[cyclopropane-1,10'-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphan]-4'-yl)carbamate (100 mg, 0.11 mmol) in DCM (1 mL) at 0 °C was added HCl in 1,4-dioxane (1 mL). The mixture was stirred at 0 °C for 2 h, then concentrated under reduced pressure to give (3'*S*,3'*S*,4'*S*,*Z*)-4'-amino-3'-ethoxy-1'-(2-isopropoxyethyl)-2'-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)spiro[cyclopropane-1,10'-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane]-5',7'-dione (110 mg), which was used directly in the next step without further purification. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{44}H_{60}N_8O_6S$ 828.4; found 829.4.

Step 11.

To a mixture of (3'*S*,3'*S*,4'*S*,*Z*)-4'-amino-3'-ethoxy-1'-(2-isopropoxyethyl)-2'-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)spiro[cyclopropane-1,10'-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane]-5',7'-dione (100 mg, 0.12 mmol) in DMF (2 mL) at 0 °C was added DIPEA (78 mg, 0.61 mmol), (1*R*,2*R*,3*S*)-2,3-dimethylcyclopropane-1-carboxylic acid (21 mg, 0.18 mmol) and HATU (92 mg, 0.24 mmol). The mixture was stirred at 0 °C for 2 h, then diluted with H_2O and extracted with EtOAc (2 x 30 mL). The combined organic layers were washed with brine (2 x 30 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (1*R*,2*R*,3*S*)-*N*-((3'*S*,3'*S*,4'*S*,*Z*)-3'-ethoxy-1'-(2-isopropoxyethyl)-2'-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-5',7'-dioxospiro[cyclopropane-1,10'-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphan]-4'-yl)-2,3-dimethylcyclopropane-1-carboxamide (7 mg, 6% yield) and (1*R*,2*R*,3*S*)-*N*-((3'*S*,3'*S*,4'*S*,*Z*)-3'-ethoxy-1'-(2-isopropoxyethyl)-2'-(2-((*S*)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-5',7'-dioxospiro[cyclopropane-1,10'-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphan]-4'-yl)-2,3-dimethylcyclopropane-1-carboxamide (13 mg, 12% yield), both as solids. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{50}H_{68}N_8O_7S$ 924.5; found 925.5; ^1H NMR (400 MHz, DMSO-d_6) δ 8.50 (d, *J* = 1.5 Hz, 1H), 8.42 (d, *J* = 3.0 Hz, 1H), 7.91 (s, 1H), 7.71 (d, *J* = 8.6 Hz, 1H), 7.64 (d, *J* = 10.1 Hz, 1H), 7.58 (d, *J* = 8.6 Hz, 1H), 7.25 (d, *J* = 2.8 Hz, 1H), 6.55 (s, 1H), 5.77 (d, *J* = 10.1 Hz, 1H), 5.04 (d, *J* = 12.3 Hz, 1H), 4.92 (s, 1H), 4.37 - 4.28 (m, 1H), 4.22 (d, *J* = 10.9 Hz, 1H), 4.18 - 4.08 (m, 3H), 3.98 (dd, *J* = 12.2, 6.1 Hz, 3H), 3.65 - 3.58 (m, 6H), 3.36 - 3.20 (m, 6H), 3.17 (d, *J* = 5.2 Hz, 2H), 2.98 (s, 3H), 2.45 (s, 6H), 2.39 - 2.30 (m, 3H), 2.10 - 1.89 (m, 3H), 1.86 - 1.66 (m, 3H), 1.51 (d, *J* = 3.8 Hz, 2H), 1.36 (d, *J* = 6.2 Hz, 3H), 1.24 (s, 1H), 1.16 (t, *J* = 7.0 Hz, 5H), 1.08 (d, *J* = 5.5 Hz, 3H), 1.04 (d, *J* = 5.5 Hz, 3H), 0.91 (d, *J* = 6.1 Hz, 3H), 0.86 (d, *J* = 6.1 Hz, 4H) and LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{50}H_{68}N_8O_7S$ 924.5; found 925.5; ^1H NMR (400 MHz, DMSO-d_6) δ 8.52 (s, 1H), 8.47 (d, *J* = 3.1 Hz, 1H), 7.76 - 7.71 (m, 1H), 7.67 (d, *J* = 10.2 Hz, 1H), 7.57 (d, *J* = 8.5 Hz, 1H), 7.37 (d, *J* = 3.2 Hz, 1H), 5.76 (d, *J* = 10.1 Hz, 1H), 5.03 (d, *J* = 12.3 Hz, 1H), 4.92 (s, 1H), 4.15 (dd, *J* = 27.1, 12.3 Hz, 1H), 3.83 (d, *J* = 6.2 Hz, 3H), 3.54 (dd, *J* = 17.3, 6.9 Hz, 1H), 3.44 (d, *J* = 6.7 Hz, 3H), 3.34 - 3.26 (m, 9H), 2.92 (s, 3H), 2.47 - 2.41 (m, 7H), 2.37 - 2.31 (m, 3H), 2.23 (s, 1H), 1.94 - 1.77 (m, 3H), 1.55 (s, 2H), 1.24 (d, *J* = 6.4 Hz, 4H), 1.15 (t, *J* = 6.9 Hz, 5H), 1.09

(d, *J* = 5.7 Hz, 3H), 1.04 (d, *J* = 5.7 Hz, 3H), 0.95 (d, *J* = 6.1 Hz, 3H), 0.86 (d, *J* = 6.1 Hz, 3H), 0.50 - 0.42 (m, 1H), 0.33 (s, 2H), 0.14 - 0.06 (m, 1H).

Example A483. Synthesis of (1*r*,2*R*,3*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-1^2-(5-((1*R*,5*S*,7*s*)-9-cyclopropyl-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-ethoxy-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide



Step 1.

To a mixture of *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-1^2-(5-((1*R*,5*S*,7*s*)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-ethoxy-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (50% purity; 500 mg, 0.28 mmol) and (1-ethoxycyclopropoxy)trimethylsilane (968 mg, 5.6 mmol) in MeOH (2 mL) at 0 °C was added AcOH (83 mg, 1.39 mmol) and $NaBH_3CN$ (87 mg, 1.39 mmol) in portions. The mixture was warmed to 60 °C and stirred for 2 h, then concentrated under reduced pressure. The residue purified by silica gel column chromatography to give *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-1^2-(5-((1*R*,5*S*,7*s*)-9-cyclopropyl-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-ethoxy-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (80 mg, 30% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{51}H_{69}N_7O_8S$ 939.5; found 940.6.

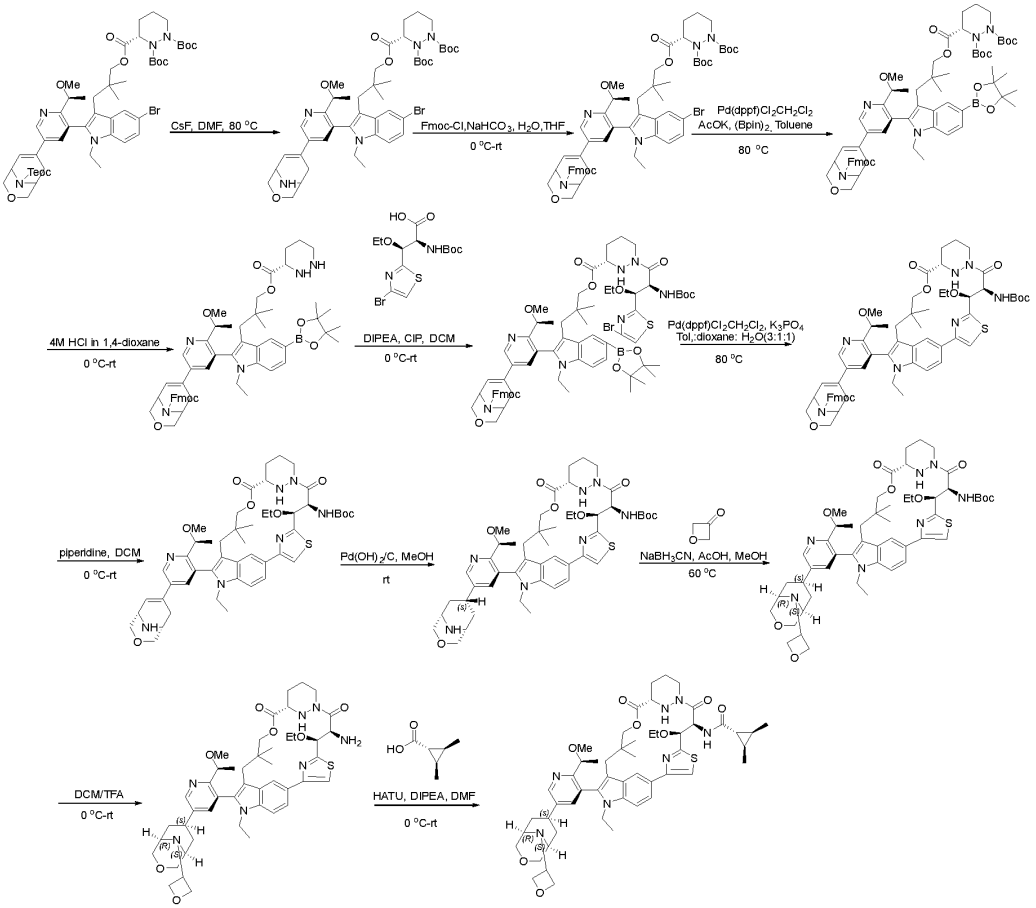
Step 2.

A mixture of *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-1^2-(5-((1*R*,5*S*,7*s*)-9-cyclopropyl-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-ethoxy-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (80 mg, 0.09 mmol) and TFA (0.4 mL) in DCM (2 mL) at 0 °C was stirred for 1 h, then concentrated under reduced pressure to give (6^3*S*,3*S*,4*S*,*Z*)-4-amino-1^2-(5-((1*R*,5*S*,7*s*)-9-cyclopropyl-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-ethoxy-1^1-ethyl-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione as a TFA salt (180 mg) as an oil. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{46}H_{61}N_7O_6S$ 839.4; found 840.4.

Step 3.

To a mixture of (6^3*S*,3*S*,4*S*,*Z*)-4-amino-1^2-(5-((1*R*,5*S*,7*s*)-9-cyclopropyl-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-ethoxy-1^1-ethyl-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (180 mg, 0.21 mmol) and (1*R*,2*R*,3*S*)-2,3-dimethylcyclopropane-1-carboxylic acid (49 mg, 0.43 mmol) in DMF (3 mL) at 0 °C was added DIPEA (277 mg, 2.14 mmol) and COMU (184 mg, 0.43 mmol) in portions. The mixture was warmed to room temperature and stirred for 2 h. The residue was purified by preparative-HPLC to give (1*r*,2*R*,3*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-1^2-(5-((1*R*,5*S*,7*s*)-9-cyclopropyl-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-ethoxy-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide (23 mg, 11% yield) as a solid. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{52}H_{69}N_7O_7S$ 935.5; found 936.4; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.74 (d, *J* = 2.2 Hz, 1H), 8.56 (s, 1H), 7.98 (s, 1H), 7.81 (d, *J* = 8.6 Hz, 1H), 7.74 - 7.56 (m, 3H), 5.94 (d, *J* = 9.7 Hz, 1H), 5.23 (d, *J* = 12.4 Hz, 1H), 5.00 (s, 1H), 4.42 - 4.08 (m, 5H), 3.92 (d, *J* = 10.9 Hz, 2H), 3.90 - 3.65 (m, 6H) 3.57 - 3.46 (m, 2H), 3.26 (s, 4H), 3.02 (d, *J* = 10.1 Hz, 2H), 2.89 (m, 2H), 2.67 (m, 1H), 2.35 (s, 2H), 2.13 (d, *J* = 10.5 Hz, 1H), 1.85 (d, *J* = 13.6 Hz, 3H), 1.59 (s, 2H), 1.43 (d, *J* = 6.1 Hz, 3H), 1.42 - 1.30 (m, 2H), 1.28 - 1.08 (m, 12H), 1.10 - 0.98 (m, 7H), 0.55 (d, *J* = 6.0 Hz, 2H), 0.45 (s, 2H), 0.39 (s, 2H).

Example A484. Synthesis of (6^3*S*,3*S*,4*S*,*Z*)-4-amino-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-((1*R*,5*S*,7*s*)-9-(oxetan-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione



Step 1.

A mixture of 3-(3-(5-bromo-1-ethyl-2-(2-((*S*)-1-methoxyethyl)-5-(9-((2,2,2-trichloroethoxy)carbonyl)-3-oxa-9-azabicyclo[3.3.1]non-6-en-7-yl)pyridin-3-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl)-1,2-di-*tert*-butyl-(3*S*)-tetrahydropyridazine-1,2,3-tricarboxylate (6.57 g, 6.22 mmol) and CsF (4.72 g, 31.1 mmol) in DMF (50 mL) was heated to 80 °C and stirred for 2 h, then diluted with H_2O (3 x 200 mL) and extracted with EtOAc (200 mL). The organic layer was concentrated under reduced pressure to give 3-(3-(2-(5-(3-oxa-9-azabicyclo[3.3.1]non-6-en-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-bromo-1-ethyl-1*H*-indol-3-yl)-2,2-dimethylpropyl)-1,2-di-*tert*-butyl-(3*S*)-tetrahydropyridazine-1,2,3-tricarboxylate (5.83 g) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{45}H_{62}BrN_5O_8$ 879.4 & 881.4; found 880.1 & 882.1.

Step 2.

To a mixture of 3-(3-(2-(5-(3-oxa-9-azabicyclo[3.3.1]non-6-en-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-bromo-1-ethyl-1*H*-indol-3-yl)-2,2-dimethylpropyl)-1,2-di-*tert*-butyl-(3*S*)-tetrahydropyridazine-1,2,3-tricarboxylate (5.83 g, 6.62 mmol) and $NaHCO_3$ (2.78 g, 33.1 mmol) in H_2O (20 mL) and THF (20 mL) at 0 °C was added 9*H*-fluoren-9-ylmethyl chloroformate (2.57 g, 9.93 mmol) in portions. The mixture was warmed to room temperature and stirred overnight, then extracted

with EtOAc (3 x 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-(3-(2-(5-(9-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-3-oxa-9-azabicyclo[3.3.1]non-6-en-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-bromo-1-ethyl-1*H*-indol-3-yl)-2,2-dimethylpropyl)1,2-di-*tert*-butyl(3*S*)-tetrahydropyridazine-1,2,3-tricarboxylate (6.63 g, 90% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{60}H_{72}BrN_5O_{10}$ 1101.5 & 1103.4; found 1102.4 & 1104.4.

Step 3.

To a mixture of 3-(3-(2-(5-(9-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-3-oxa-9-azabicyclo[3.3.1]non-6-en-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-bromo-1-ethyl-1*H*-indol-3-yl)-2,2-dimethylpropyl)1,2-di-*tert*-butyl(3*S*)-tetrahydropyridazine-1,2,3-tricarboxylate (6.4 g, 5.8 mmol) and bis(pinacolato)diboron (2.21 g, 8.7 mmol) in toluene (25 mL) under an atmosphere of N_2 was added AcOK (1.42 g, 14.5 mmol) and $Pd(dppf)Cl_2CH_2Cl_2$ (0.47 g, 0.58 mmol) in portions. The mixture was heated to 80 °C and stirred for 3 h, then concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-(3-(2-(5-(9-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-3-oxa-9-azabicyclo[3.3.1]non-6-en-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-ethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl) 1,2-di-*tert*-butyl (3*S*)-tetrahydropyridazine-1,2,3-tricarboxylate (4.9 g, 73% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{66}H_{84}BN_5O_{12}$ 1149.6; found 1150.8.

Step 4.

To 3-(3-(2-(5-(9-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-3-oxa-9-azabicyclo[3.3.1]non-6-en-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-ethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl) 1,2-di-*tert*-butyl (3*S*)-tetrahydropyridazine-1,2,3-tricarboxylate (4.9 g, 4.3 mmol) was added HCl in 1,4-dioxane (15 mL) at 0°C. The mixture was warmed to room temperature and stirred for 5 h, then concentrated under reduced pressure to give (9*H*-fluoren-9-yl)methyl-7-(5-(1-ethyl-3-(3-(((*S*)-hexahydropyridazine-3-carbonyl)oxy)-2,2-dimethylpropyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-oxa-9-azabicyclo[3.3.1]non-6-ene-9-carboxylate (4.9 g) as a solid, that was used directly in the next step without further purification. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{56}H_{68}BN_5O_8$ 949.5; found 950.5.

Step 5.

To a mixture of (9*H*-fluoren-9-yl)methyl-7-(5-(1-ethyl-3-(3-(((*S*)-hexahydropyridazine-3-carbonyl)oxy)-2,2-dimethylpropyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-oxa-9-azabicyclo[3.3.1]non-6-ene-9-carboxylate (4.8 g, 5.1 mmol) and (2*S*,3*S*)-3-(4-bromo-1,3-thiazol-2-yl)-2-[(*tert*-butoxycarbonyl)amino]-3-ethoxypropanoic acid (3.00 g, 7.6 mmol) in DCM (30 mL) at 0 °C was added DIPEA (6.53 g, 50.5 mmol) and CIP (4.22 g, 15.2 mmol) in portions. The mixture was warmed to room temperature and stirred for 3 h, then concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (9*H*-fluoren-9-yl)methyl 7-(5-(3-(3-(((*S*)-1-((2*S*,3*S*)-3-(4-bromothiazol-2-yl)-

2-((*tert*-butoxycarbonyl)amino)-3-ethoxypropanoyl)hexahydropyridazine-3-carbonyl)oxy)-2,2-dimethylpropyl)-1-ethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-oxa-9-azabicyclo[3.3.1]non-6-ene-9-carboxylate (5.5 g, 82% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{69}H_{85}BBrN_7O_{12}S$ 1325.5 & 1327.5; found 1326.5 & 1328.5.

Step 6.

To a mixture of (9*H*-fluoren-9-yl)methyl 7-(5-(3-(3-(((*S*)-1-((2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-ethoxypropanoyl)hexahydropyridazine-3-carbonyl)oxy)-2,2-dimethylpropyl)-1-ethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-oxa-9-azabicyclo[3.3.1]non-6-ene-9-carboxylate (5.5 g, 4.1 mmol) and K_3PO_4 (2.20 g, 10.4 mmol) in toluene (12 mL), dioxane (4 mL) and H_2O (4 mL) under an atmosphere of N_2 was added Pd(dppf)Cl_2.DCM (0.34 g, 0.41 mmol) in portions. The mixture was heated to 80 °C and stirred for 3 h, then filtered and the filter cake was washed with EtOAc (3 x 10 mL) and H_2O (10 mL). The filtrate was partioned and the aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (9*H*-fluoren-9-yl)methyl-7-(5-((6^3*S*,3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-3-ethoxy-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-12-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-oxa-9-azabicyclo[3.3.1]non-6-ene-9-carboxylate (2.3 g, 49% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{63}H_{73}N_7O_{10}S$ 1119.5; found 1120.3.

Step 7.

To a mixture of (9*H*-fluoren-9-yl)methyl-7-(5-((6^3*S*,3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-3-ethoxy-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-12-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-oxa-9-azabicyclo[3.3.1]non-6-ene-9-carboxylate (2.3 g, 2.1 mmol) and piperidine (0.87 g, 10.3 mmol) in DCM (10 mL) was stirred at room temperature for 3 h, then concentrated under reduced pressure to give *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-1^2-(5-((1*R*,5*S*)-3-oxa-9-azabicyclo[3.3.1]non-6-en-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-ethoxy-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (2.3 g) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{48}H_{63}N_7O_8S$ 897.5; found 898.9.

Step 8.

A mixture of *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-1^2-(5-((1*R*,5*S*)-3-oxa-9-azabicyclo[3.3.1]non-6-en-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-ethoxy-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (2.3 g, 2.6 mmol) and Pd(OH)$_2$, 20% on carbon (2.30 g, 3.3 mmol) in MeOH (10 mL) was hydrogenated (balloon) at room temperature for 2 h. The mixture was filtered through a pad of Celite and the filtrate was concentrated under reduced pressure to give *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-1^2-

(5-((1*R*,5*S*,7*s*)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-ethoxy-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (2.3 g) as an oil, which was used directly in the next step without further purification. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{48}H_{65}N_7O_8S$ 899.5; found 901.0.

Step 9.

To a mixture of *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-1^2-(5-((1*R*,5*S*,7*s*)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-ethoxy-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (480 mg, 0.53 mmol) and 3-oxetanone (154 mg, 2.1 mmol) in MeOH (4 mL) at 0 °C was added AcOH (320 mg, 5.3 mmol) dropwise. The mixture was warmed to room temperature and stirred for 30 min, then the mixture was re-cooled to 0 °C and $NaBH_3CN$ (101 mg, 1.6 mmol) was added in portions. The mixture was heated to 60°C and stirred for 2 h, then concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-((1*R*,5*S*,7*s*)-9-(oxetan-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (200 mg, 39% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{51}H_{69}N_7O_9S$ 955.5; found 956.7.

Step 10.

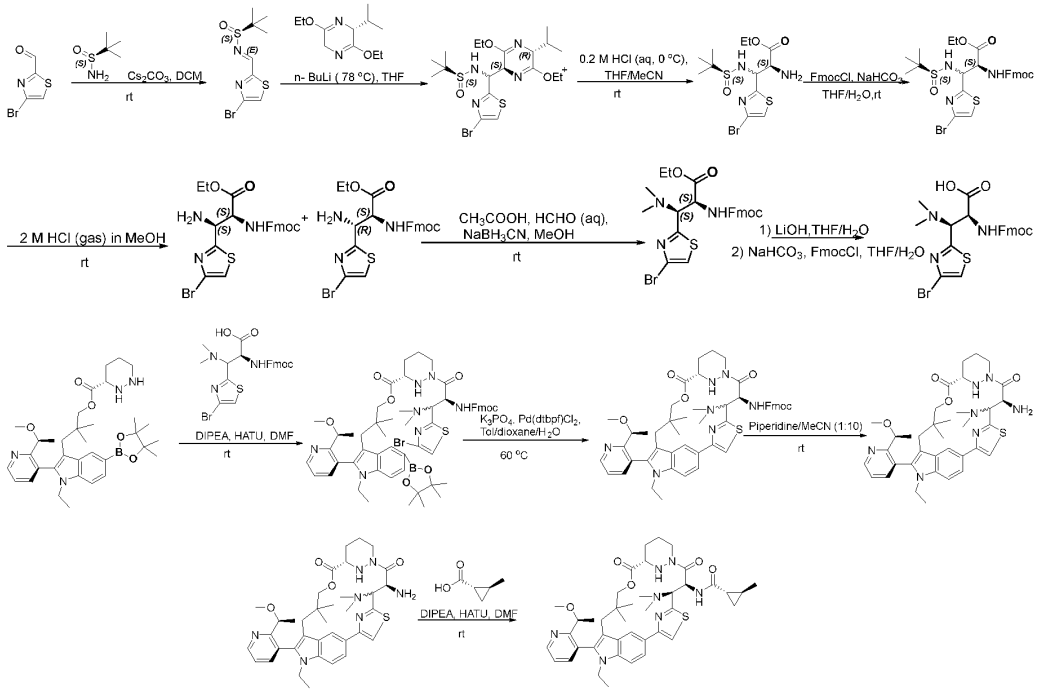
To a mixture of *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-((1*R*,5*S*,7*s*)-9-(oxetan-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (200 mg, 0.21 mmol) in DCM (2 mL) at 0 °C was added TFA (0.4 mL) dropwise. The mixture was warmed to room temperature and stirred for 1 h, then concentrated under reduced pressure to give (6^3*S*,3*S*,4*S*,*Z*)-4-amino-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-((1*R*,5*S*,7*s*)-9-(oxetan-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (220 mg) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{46}H_{61}N_7O_7S$ 855.4; found 856.6.

Step 11.

To a mixture of (6^3*S*,3*S*,4*S*,*Z*)-4-amino-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-((1*R*,5*S*,7*s*)-9-(oxetan-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (220 mg, 0.26 mmol) and (1*r*,2*R*,3*S*)-2,3-dimethylcyclopropane-1-carboxylic acid (59 mg, 0.51 mmol) in DMF (3 mL) at 0 °C under an atmosphere of N_2 was added DIPEA (332 mg, 2.57 mmol) and HATU (293 mg, 0.77 mmol) in portions.

The mixture was warmed to room temperature and stirred for 2 h, then purified by preparative-HPLC to give (1*r*,2*R*,3*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-((1*R*,5*S*,7*s*)-9-(oxetan-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide (67 mg, 27% yield) as a solid. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{52}H_{69}N_7O_8S$ 951.5; found 952.5; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.79 (s, 1H), 8.50 (d, *J* = 1.6 Hz, 1H), 7.92 (s, 1H), 7.79 - 7.72 (m, 2H), 7.65 (d, *J* = 9.9 Hz, 1H), 7.58 (d, *J* = 8.7 Hz, 1H), 5.86 (d, *J* = 9.9 Hz, 1H), 5.15 (d, *J* = 12.3 Hz, 2H), 4.91 (s, 1H), 4.74 (s, 4H), 4.37 - 4.20 (m, 3H), 4.18 - 4.05 (m, 3H), 3.92 (s, 3H), 3.57 (d, *J* = 3.5 Hz, 5H), 3.51 - 3.44 (m, 7H), 3.22 (s, 3H), 2.90 - 2.74 (m, 2H), 2.55 (s, 2H), 2.39 (s, 2H), 2.06 (d, *J* = 12.3 Hz, 1H), 1.78 (d, *J* = 29.9 Hz, 2H), 1.56 - 1.47 (m, 2H), 1.37 (d, *J* = 6.1 Hz, 3H), 1.16 (t, *J* = 7.0 Hz, 5H), 1.11 - 0.94 (m, 7H), 0.93 - 0.77 (m, 6H), 0.36 (s, 3H).

Example A496. Synthesis of (1*S*,2*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-(dimethylamino)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methylcyclopropane-1-carboxamide



Step 1.

To a mixture of 4-bromothiazole-2-carbaldehyde (15.0 g, 78.1 mmol) and (*S*)-2-methylpropane-2-sulfinamide (9.47 g, 78.1mmol) in DCM was added Cs_2CO_3 (50.90 g, 156.2 mmol) in portions. The mixture was stirred at room temperature for 2 h, then filtered and the filter cake was washed with DCM (3 x 20 mL). The filtrate was concentrated under reduced pressure and the residue

was purified by silica gel column chromatography to give (*S*,*E*)-*N*-((4-bromothiazol-2-yl)methylene)-2-methylpropane-2-sulfinamide (26 g, 97% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_8H_{11}BrN_2OS_2$ 294.0; found 294.8.

Step 2.

To a mixture of (*R*)-3,6-diethoxy-2-isopropyl-2,5-dihydropyrazine (20.57 g, 96.9 mmol) in THF at -78 °C under an atmosphere of N_2 was treated with n-BuLi in hexanes (20.5 mL, 105.7 mmol). The mixture was stirred at -78 °C for 30 min, then (*S*,*E*)-*N*-((4-bromothiazol-2-yl)methylene)-2-methylpropane-2-sulfinamide (26.0 g, 88.1 mmol) was added dropwise. The mixture was stirred at -78 °C for 2 h, then warmed to 0 °C and quenched with saturated $NaHCO_3$. The aqueous layer was extracted with EtOAc (3 x 500 mL), the combined organic layers were concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (*S*)-*N*-((*S*)-(4-bromothiazol-2-yl)((2*S*,5*R*)-3,6-diethoxy-5-isopropyl-2,5-dihydropyrazin-2-yl)methyl)-2-methylpropane-2-sulfinamide (32.0 g, 72% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{19}H_{31}BrN_4O_3S_2$ 506.1; found 507.0.

Step 3.

To a mixture of (*S*)-*N*-((*S*)-(4-bromothiazol-2-yl)((2*S*,5*R*)-3,6-diethoxy-5-isopropyl-2,5-dihydropyrazin-2-yl)methyl)-2-methylpropane-2-sulfinamide (32.0 g, 63.0 mmol) in THF (1 L) and MECN (640 mL) at 0 °C was added 0.2M HCl (790 mL) dropwise. The mixture was warmed to room temperature and stirred overnight, then quenched by the addition of saturated $NaHCO_3$ and extracted with EtOAc (3 x 500 mL). The combined organic layers were concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give ethyl (2*S*,3*S*)-2-amino-3-(4-bromothiazol-2-yl)-3-(((*S*)-*tert*-butylsulfinyl)amino)propanoate (18.0 g, 72% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{12}H_{20}BrN_3O_3S_2$ 397.0; found 398.1.

Step 4.

To a mixture of ethyl (2*S*,3*S*)-2-amino-3-(4-bromothiazol-2-yl)-3-(((*S*)-*tert*-butylsulfinyl)amino)propanoate (15.0 g, 37.7 mmol) and $NaHCO_3$ (15.82 g, 188.3 mmol) in THF (100 mL) and H_2O (100 mL) was added FmocCl (11.69 g, 45.2 mmol) in portions. The mixture was stirred at room temperature for 2 h, then washed with H_2O (3 x 100 mL). The aqueous layer was extracted with EtOAc (3 x 100 mL), the combined organic layers were concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give ethyl (2*S*,3*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-bromothiazol-2-yl)-3-(((*S*)-*tert*-butylsulfinyl)amino)propanoate (20.0 g, 86% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{27}H_{30}BrN_3O_5S_2$ 619.1; found 620.0.

Step 5.

A mixture of ethyl (2*S*,3*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-bromothiazol-2-yl)-3-(((*S*)-*tert*-butylsulfinyl)amino)propanoate (20.0 g, 32.2 mmol) and 4M HCl in MeOH (150 mL)

was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure and the residue was purified by reverse-phase silica gel column chromatography to give ethyl (2*S*,3*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-amino-3-(4-bromothiazol-2-yl)propanoate (7.5 g, 45% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{23}H_{22}BrN_3O_4S$ 515.1; found 516.0.

Step 6.

To a mixture of ethyl (2*S*,3*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-amino-3-(4-bromothiazol-2-yl)propanoate (1.2 g, 2.3 mmol) and AcOH (419 mg, 7.0 mmol) in MeOH (20 mL) was added HCHO, 37% aqueous solution (419 mg, 13.9 mmol) and $NaBH_3CN$ (730 mg, 11.6 mmol) in portions. The mixture was stirred at room temperature for 2 h, then diluted with H_2O and extracted with EtOAc (3 x 200 mL). The combined organic layers were concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give ethyl (2*S*,3*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-bromothiazol-2-yl)-3-(dimethylamino)propanoate (990 mg, 78% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{25}H_{26}BrN_3O_4S$ 543.1; found 543.8.

Step 7.

A mixture of ethyl (2*S*,3*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-bromothiazol-2-yl)-3-(dimethylamino)propanoate (990 mg, 1.8 mmol) and $LiOH.H_2O$ (174 mg, 7.3 mmol) in THF (50 mL) and H_2O (50 mL) was stirred at room temperature for 1 h, then acidified to pH ~5 with 1M HCl. The mixture was used directly in the next step without further purification.

Step 8.

To the above mixture was added $NaHCO_3$ (764 mg, 9.1 mmol) and FmocCl (565 mg, 9.1 mmol) in portions. The mixture was stirred at room temperature overnight, then washed with H_2O (3 x 300 mL) and the combined aqueous layers were extracted with EtOAc (3 x 30 mL). The combined organic layers were concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (2*S*,3*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-bromothiazol-2-yl)-3-(dimethylamino)propanoic acid (320 mg, 60% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{23}H_{22}BrN_3O_4S$ 515.1; found 516.0.

Step 9.

To a mixture of 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl (*S*)-hexahydropyridazine-3-carboxylate (450 mg, 0.74 mmol) and DIPEA (1.60 g, 12.4 mmol) in DMF (30 mL) was added (2*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-bromothiazol-2-yl)-3-(dimethylamino)propanoic acid (320 mg, 0.62 mmol) and HATU (471 mg, 1.24 mmol) in portions. The mixture was stirred at room temperature for 1 h, then washed with H_2O (3 x 30 mL) and the combined aqueous layers were extracted with EtOAc (3 x 30 mL). The combined organic layers were concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl-(3*S*)-1-((2*S*)-

2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-bromothiazol-2-yl)-3-(dimethylamino)propanoyl)hexahydropyridazine-3-carboxylate (450 mg, 66% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{57}H_{69}BBrN_7O_8S$ 1101.4; found 1102.5.

Step 10.

To a mixture of 3-(1-ethyl-2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl-(3*S*)-1-((2*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-bromothiazol-2-yl)-3-(dimethylamino)propanoyl)hexahydropyridazine-3-carboxylate (450 mg, 0.41 mmol) and K_3PO_4 (217 mg, 1.0 mmol) in toluene (9 mL), 1,4-dioxane (3 mL) and H_2O (3mL) under an atmosphere of N_2 was added Pd(dtbpf)Cl_2 (53 mg, 0.08 mmol) in portions. The mixture was heated to 60 °C and stirred for 1 h, then washed with H_2O (3 x 20 mL) and the combined aqueous layers extracted with EtOAc (3 x 20 mL). The combined organic layers were concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (9*H*-fluoren-9-yl)methyl((6^3*S*,4*S*,*Z*)-3-(dimethylamino)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (70 mg, 19% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{51}H_{57}N_7O_6S$ 895.4; found 896.3.

Step 11.

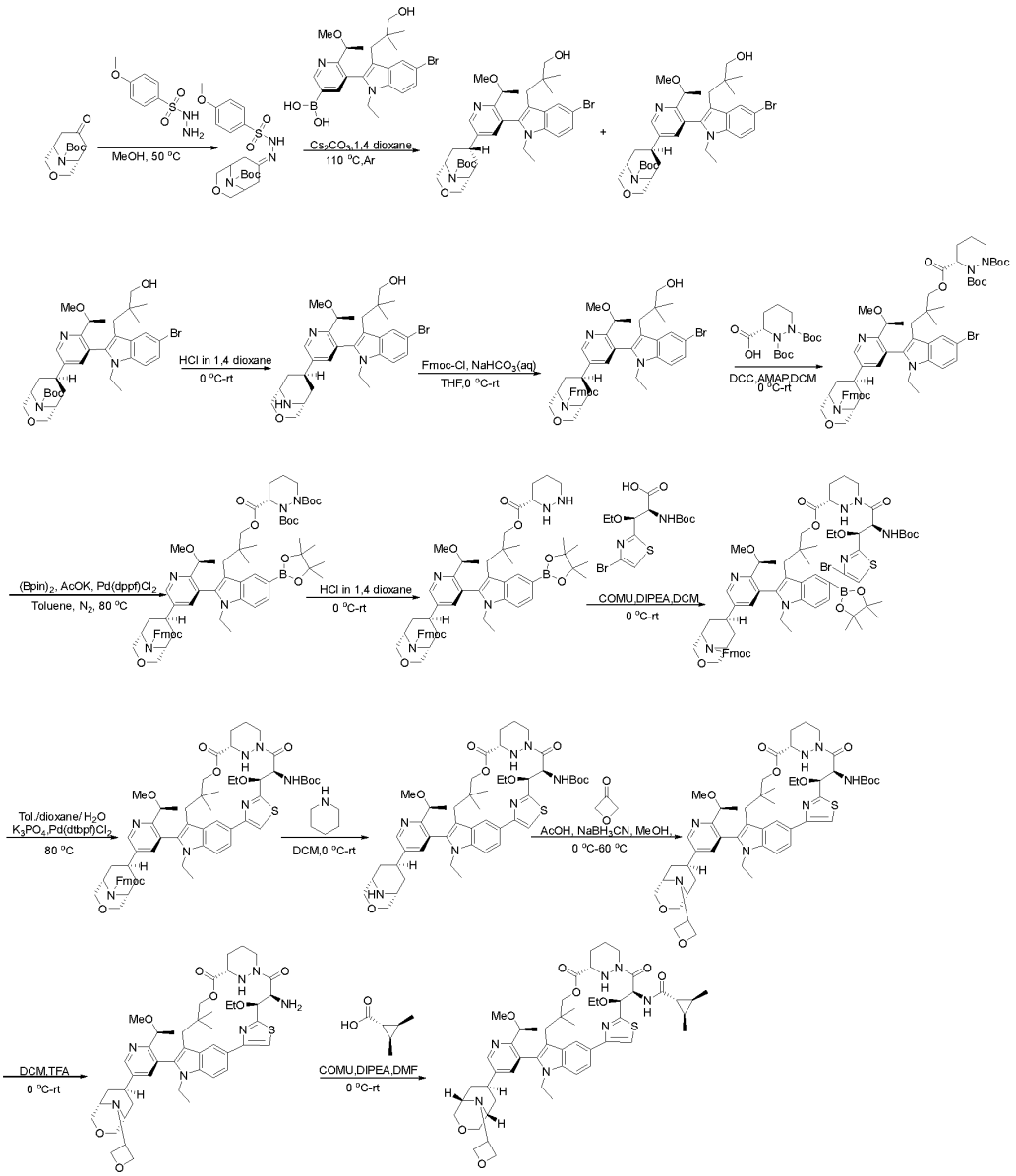
A mixture of (9*H*-fluoren-9-yl)methyl((6^3*S*,4*S*,*Z*)-3-(dimethylamino)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (70 mg, 0.08 mmol) and piperidine (0.2 mL) in MECN (2 mL) was stirred at room temperature for 1 h. The mixture was concentrated under reduced pressure to give (6^3*S*,4*S*,*Z*)-4-amino-3-(dimethylamino)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (60 mg, 71% yield) as an oil, which was used directly in the next step without further purification. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{36}H_{47}N_7O_4S$ 673.3; found 674.1.

Step 12.

To a mixture of (6^3*S*,4*S*,*Z*)-4-amino-3-(dimethylamino)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (60 mg, 0.09 mmol) and (1*S*,2*S*)-2-methylcyclopropane-1-carboxylic acid (9 mg, 0.09 mmol) in DMF (5 mL) was added DIPEA (230 mg, 1.78 mmol) and HATU (68 mg, 0.18 mmol) in portions. The mixture was stirred at room temperature for 1 h, then diluted with H_2O and extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with H_2O (3 x 20 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (1*S*,2*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-(dimethylamino)-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-

dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methylcyclopropane-1-carboxamide (3.6 mg, 5% yield) as a solid. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{41}H_{53}N_7O_5S$ 755.4; found 756.4; ^{1}H NMR (400 MHz, CD_3OD) δ 8.66 - 8.62 (m, 1H), 8.44 - 8.39 (m, 1H), 7.86 - 7.79 (m, 1H), 7.63 - 7.54 (m, 2H), 7.48 - 7.43 (m, 1H), 7.41 - 7.37 (m, 1H), 6.01 - 5.94 (m, 1H), 4.26 - 3.95 (m, 6H), 3.94 - 3.81 (m, 2H), 3.47 - 3.38 (m, 1H), 3.07 (s, 3H), 2.80 - 2.72 (m, 1H), 2.67 - 2.58 (m, 1H), 2.55 - 2.45 (m, 1H), 2.38 - 2.13 (s, 6H), 1.91 (s, 2H), 1.63 - 1.40 (m, 3H), 1.39 - 1.32(m, 3H), 1.29 - 1.08 (m, 7H), 1.06 - 0.91 (m, 8H), 0.84 - 0.75 (m, 1H), 0.69 - 0.41 (m, 7H).

Example A502. Synthesis of (1*r*,2*R*,3*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-((1*R*,5*S*,7*r*)-9-(oxetan-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,66-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide



Step 1.

To a mixture of *tert*-butyl (1*R*,5*S*)-7-oxo-3-oxa-9-azabicyclo[3.3.1]nonane-9-carboxylate (9.00 g, 37.3 mmol) in MeOH (90 mL) was added 4-methoxybenzenesulfonohydrazide (9.05 g, 44.8 mmol). The mixture was heated to 50 °C and stirred for 16 h, then concentrated under reduced pressure to give *tert*-butyl 7-(2-((4-methoxyphenyl)sulfonyl)hydrazineylidene)-3-oxa-9-azabicyclo[3.3.1]nonane-9-carboxylate (15.0 g) as a solid, which was used directly in the next step without further purification. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{19}H_{27}N_3O_6S$ 425.2; found 426.3.

Step 2.

A mixture of *tert*-butyl 7-(2-((4-methoxyphenyl)sulfonyl)hydrazineylidene)-3-oxa-9-azabicyclo[3.3.1]nonane-9-carboxylate (12.2 g, 28.7 mmol), (*S*)-(5-(5-bromo-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)boronic acid (21.0 g, 43.0 mmol) and Cs_2CO_3 (14.0 g, 43.0 mmol) in 1,4-dioxane (120 mL) under an atmosphere of Ar was heated to 110 °C and stirred for 16 h. The mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography and chiral-HPLC to give *tert*-butyl (1*R*,5*S*,7*s*)-7-(5-(5-bromo-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonane-9-carboxylate (3.3 g, 17% yield; RT = 1.98 min) as solid and *tert*-butyl (1*R*,5*S*,7*r*)-7-(5-(5-bromo-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonane-9-carboxylate (4.5 g, 23% yield, RT = 2.16 min) as a solid.

Step 3.

To *tert*-butyl (1*R*,5*S*)-7-(5-(5-bromo-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-2-yl)-6-((S)-1-methoxyethyl)pyridin-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonane-9-carboxylate (4.49 g, 6.7 mmol) was added HCl in 1,4-dioxane (40 mL, 10.0 mmol) at 0 °C. The mixture was warmed to room temperature and stirred for 1 h, then concentrated under reduced pressure to give 3-(2-(5-((1*R*,5*S*,7*r*)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-bromo-1-ethyl-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (4.5 g, 92% yield) as a solid, which was used directly in the next step without further purification. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{30}H_{40}BrN_3O_3$ 569.2; found 570.3.

Step 4.

To a mixture of 3-(2-(5-((1*R*,5*S*,7*r*)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-5-bromo-1-ethyl-1*H*-indol-3-yl)-2,2-dimethylpropan-1-ol (4.49 g, 7.87 mmol) in THF (20 mL) at 0 °C was added 9*H*-fluoren-9-ylmethyl chloroformate (3.05 g, 11.8 mmol) in aqueous $NaHCO_3$ (20 mL) dropwise. The mixture was warmed to room temperature and stirred for 2 h, then extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with H_2O (2 x 20 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (9*H*-fluoren-9-yl)methyl (1*R*,5*S*,7*r*)-7-(5-(5-bromo-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonane-9-carboxylate (5.8 g, 92% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{45}H_{50}BrN_3O_5$ 793.3; found 794.3 [for ^{81}Br].

Step 5.

To a mixture of (9*H*-fluoren-9-yl)methyl (1*R*,5*S*,7*r*)-7-(5-(5-bromo-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-1*H*-indol-2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonane-9-carboxylate (5.8 g, 7.3 mmol) and (*S*)-1,2-bis(*tert*-butoxycarbonyl)hexahydropyridazine-3-carboxylic acid (4.83 g, 14.6 mmol) in DCM (50 mL) at 0 °C was added DMAP (0.38 g, 3.1 mmol) and DCC (2.55 g, 12.4 mmol) in portions. The mixture was warmed to room temperature and stirred

for 2 h, then filtered and the filter cake was washed with DCM (3 x 30 mL). The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-(3-(2-(5-((1*R*,5*S*,7*r*)-9-(((9*H*-fluoren-9-yl) methoxy)carbonyl)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((S)-1-methoxyethyl)pyridin-3-yl)-5-bromo-1-ethyl-1*H*-indol-3-yl)-2,2-dimethylpropyl)1,2-di-*tert*-butyl-(*S*)-tetrahydropyridazine-1,2,3-tricarboxylate (7.6 g) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{60}H_{74}BrN_5O_{10}$ 1105.5; found 1106.4 [for ^{81}Br].

Step 6.

To a mixture of 3-(3-(2-(5-((1*R*,5*S*,7*r*)-9-(((9*H*-fluoren-9-yl) methoxy)carbonyl)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((S)-1-methoxyethyl)pyridin-3-yl)-5-bromo-1-ethyl-1*H*-indol-3-yl)-2,2-dimethylpropyl)1,2-di-*tert*-butyl-(*S*)-tetrahydropyridazine-1,2,3-tricarboxylate (7.4 g, 6.7 mmol) and bis(pinacolato)diboron (8.50 g, 33.5 mmol) in toluene (70 mL) under an atmosphere of N_2 was added AcOK (2.63 g, 26.8 mmol) and $Pd(dppf)Cl_2CH_2Cl_2$ (1.09 g, 1.34 mmol). The mixture was heated to 80 °C and stirred for 3 h, then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give 3-(3-(2-(5-((1*R*,5*S*,7*r*)-9-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-ethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl)1,2-di-*tert*-butyl-(*S*)-tetrahydropyridazine-1,2,3-tricarboxylate (6.9 g, 89% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{66}H_{86}BN_5O_{12}$ 1151.6; found 1152.6.

Step 7.

To 3-(3-(2-(5-((1*R*,5*S*,7*r*)-9-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-1-ethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-3-yl)-2,2-dimethylpropyl)1,2-di-*tert*-butyl-(*S*)-tetrahydropyridazine-1,2,3-tricarboxylate (6.9 g, 6.0 mmol) was added HCl in 1,4-dioxane (60 mL) at 0 °C. The mixture was warmed to room temperature and was stirred for 4 h, then concentrated under reduced pressure to give (9*H*-fluoren-9-yl)methyl(1*R*,5*S*,7*r*)-7-(5-(1-ethyl-3-(3-(((*S*)-hexahydropyridazine-3-carbonyl)oxy)-2,2-dimethylpropyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonane-9-carboxylate (6.47 g) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{56}H_{70}BN_5O_8$ 951.5; found 952.6.

Step 8.

To a mixture of (9*H*-fluoren-9-yl)methyl(1*R*,5*S*,7*r*)-7-(5-(1-ethyl-3-(3-(((*S*)-hexahydropyridazine-3-carbonyl)oxy)-2,2-dimethylpropyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonane-9-carboxylate (6.45 g, 6.78 mmol) and (2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((*tert*-butoxycarbonyl)amino)-3-ethoxypropanoic acid (3.21 g, 8.13 mmol) in DCM (60 mL) at 0 °C was added DIPEA (8.76 g, 67.8 mmol) and COMU (3.48 g, 8.1 mmol) in portions. The mixture was warmed to room temperature and stirred for 2 h, then concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (9*H*-fluoren-9-yl)methyl (1*R*,5*S*,7*r*)-

7-(5-(3-(3-(((*S*)-1-((2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((tert-butoxycarbonyl)amino)-3-ethoxypropanoyl)hexahydropyridazine-3-carbonyl)oxy)-2,2-dimethylpropyl)-1-ethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonane-9-carboxylate (6.9 g, 76% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{69}H_{87}BBrN_7O_{12}S$ 1329.5; found 1330.5.

Step 9.

To a mixture of (9*H*-fluoren-9-yl)methyl (1*R*,5*S*,7*r*)-7-(5-(3-(3-(((*S*)-1-((2*S*,3*S*)-3-(4-bromothiazol-2-yl)-2-((tert-butoxycarbonyl)amino)-3-ethoxypropanoyl)hexahydropyridazine-3-carbonyl)oxy)-2,2-dimethylpropyl)-1-ethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indol-2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonane-9-carboxylate (6.9 g, 5.2 mmol) in toluene (300 mL), 1,4-dioxane (100 mL) and H_2O (100 mL) under an atmosphere of N_2 was added K_3PO_4 (3.31 g, 15.6 mmol) and Pd(DtBPF)Cl_2 (0.42 g, 0.52 mmol). The mixture was heated to 80 °C and stirred for 3 h, then concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (9*H*-fluoren-9-yl)methyl(1*R*,5*S*,7*r*)-7-(5-((63*S*,3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-3-ethoxy-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonane-9-carboxylate (3.8 g, 65% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{63}H_{75}N_7O_{10}S$ 1121.5; found 1123.3.

Step 10.

To a mixture of (9*H*-fluoren-9-yl)methyl(1*R*,5*S*,7*r*)-7-(5-((63*S*,3*S*,4*S*,*Z*)-4-((*tert*-butoxycarbonyl)amino)-3-ethoxy-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-1^2-yl)-6-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonane-9-carboxylate (3.8 g, 3.4 mmol) in DCM (40 mL) at 0 °C was added piperidine (1.44 g, 16.9 mmol) dropwise. The mixture was warmed to room temperature and stirred for 5 h, then concentrated under reduced pressure to give *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-1^2-(5-((1*R*,5*S*,7*r*)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-ethoxy-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (4.7 g) as a solid, which was used directly in the next step without further purification. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{48}H_{65}N_7O_8S$ 899.5; found 900.6.

Step 11.

To a mixture of *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-1^2-(5-((1*R*,5*S*,7*r*)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-ethoxy-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (1.12 g, 15.6 mmol) in MeOH (2 mL) at 0 °C was added AcOH (467 mg, 7.8 mmol). The mixture was stirred for 30 min, then $NaBH_3CN$ (147 mg, 2.33 mmol) was added, and the mixture was heated to 60 °C and stirred for 3 h. The mixture was concentrated under reduced pressure and the

residue was purified by silica gel column chromatography to give *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-((1*R*,5*S*,7*r*)-9-(oxetan-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (251 mg, 33% yield) as a solid. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{51}H_{69}N_7O_9S$ 955.5; found 956.5.

Step 12.

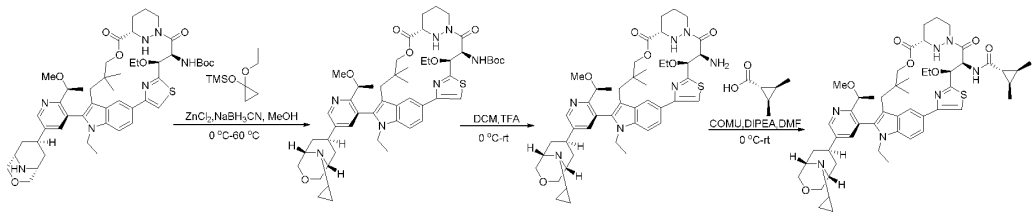
To a mixture of *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-((1*R*,5*S*,7*r*)-9-(oxetan-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (250 mg, 0.26 mmol) in DCM (2 mL) at 0°C was added TFA (0.4 mL). The mixture was warmed to room temperature and stirred for 30 min, then concentrated under reduced pressure to give (6^3*S*,3*S*,4*S*,*Z*)-4-amino-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-((1*R*,5*S*,7*r*)-9-(oxetan-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (310 mg) as a solid, that was used directly in the next step without further purification. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{46}H_{61}N_7O_7S$ 855.4; found 856.5.

Step 13.

To a mixture of (6^3*S*,3*S*,4*S*,*Z*)-4-amino-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-((1*R*,5*S*,7*r*)-9-(oxetan-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (310 mg, 0.36 mmol) and (1*r*,2*R*,3*S*)-2,3-dimethylcyclopropane-1-carboxylic acid (62 mg, 0.54 mmol) in DMF (3 mL) at 0 °C was added DIPEA (468 mg, 3.62 mmol) and COMU (155 mg, 0.36 mmol) in portions. The mixture was warmed to room temperature and stirred for 1 h, then purified by preparative-HPLC to give (1*r*,2*R*,3*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)-5-((1*R*,5*S*,7*r*)-9-(oxetan-3-yl)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,66-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide (113 mg, 32% yield) as a solid. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{52}H_{69}N_7O_8S$ 951.5; found 952.5; ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 8.66 (d, *J* = 2.1 Hz, 1H), 8.51 (s, 1H), 7.92 (s, 1H), 7.76 (d, *J* = 8.4 Hz, 2H), 7.61 (dd, *J* = 15.3, 9.2 Hz, 2H), 5.88 (d, *J* = 9.9 Hz, 1H), 5.18 (d, *J* = 11.6 Hz, 1H), 4.93 (s, 1H), 4.59 - 4.41 (m, 3H), 4.32 (s, 2H), 4.24 (d, *J* = 6.2 Hz, 2H), 4.13 (s, 3H), 3.82 (d, *J* = 11.0 Hz, 4H), 3.69 - 3.49 (m, 4H), 3.19 (s, 3H), 2.84 - 2.74 (s, 2H), 2.64 (s, 2H), 2.10 - 1.94 (m, 3H), 1.76 (s, 4H), 1.53 (s, 1H), 1.37 (d, *J* = 6.1 Hz, 3H), 1.24 (s, 1H), 1.19 (d, *J* = 7.0 Hz, 3H), 1.15 (s, 2H), 1.07 (d, *J* = 7.2 Hz, 6H), 0.93 (t, *J* = 7.0 Hz, 3H), 0.84 (s, 3H), 0.41 (s, 3H).

Example A503. Synthesis of (1*r*,2*R*,3*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-12-(5-((1*R*,5*S*,7*r*)-9-cyclopropyl-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-ethoxy-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11H-8-oxa-2(4,2)-

thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide



Step 1.

To a mixture of *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-12-(5-((1*R*,5*S*,7*r*)-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((S)-1-methoxyethyl)pyridin-3-yl)-3-ethoxy-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (300 mg, 0.33 mmol) and (1-ethoxycyclopropoxy)trimethylsilane (1.16 g, 6.6 mmol) in MeOH (1.5 mL) at 0 °C was added AcOH (200 mg, 3.3 mmol) dropwise. The mixture was stirred at 0 °C for 30 min, then $NaBH_3CN$ (105 mg, 1.67 mmol) was added, the mixture was heated to 60 °C and stirred for 2 h. The residue was purified by silica gel column chromatography to give *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-12-(5-((1*R*,5*S*,7*r*)-9-cyclopropyl-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-ethoxy-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (235 mg, 75% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{51}H_{69}N_7O_8S$ 939.5; found 940.3.

Step 2.

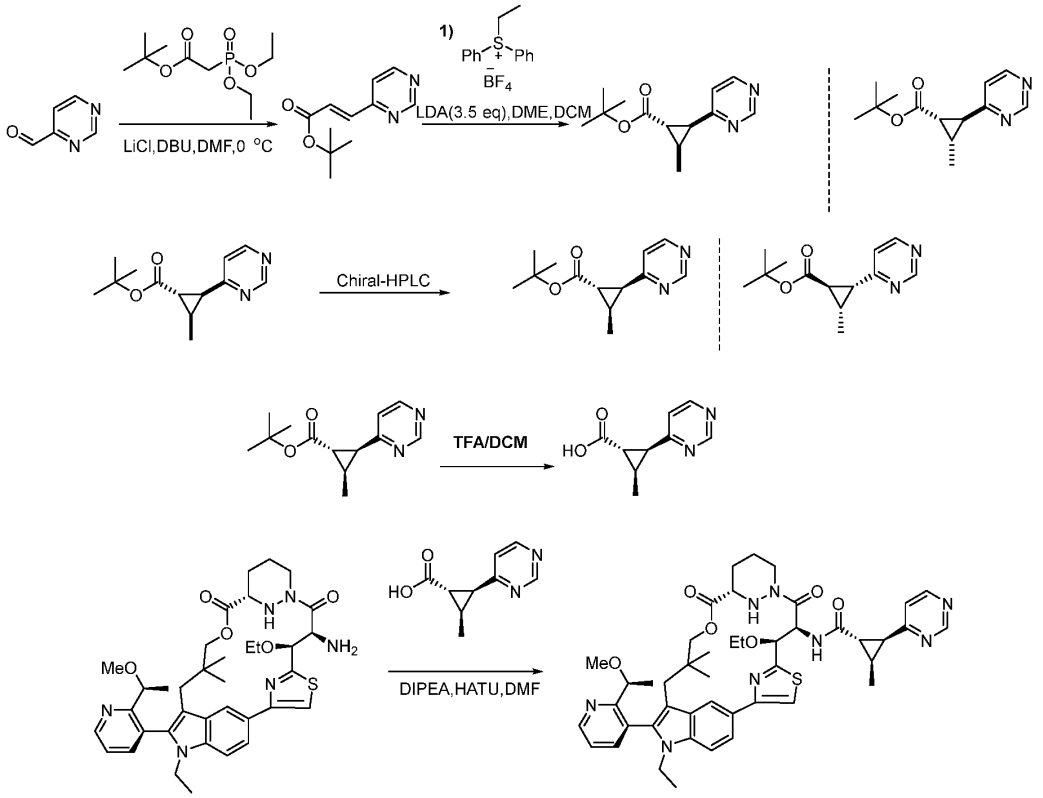
To a mixture of *tert*-butyl ((6^3*S*,3*S*,4*S*,*Z*)-12-(5-((1*R*,5*S*,7*r*)-9-cyclopropyl-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-ethoxy-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (230 mg, 0.25 mmol) in DCM (2 mL) at 0 °C was added TFA (0.4 mL) dropwise. The mixture was warmed to room temperature and stirred for 1 h, then concentrated under reduced pressure to give (6^3*S*,3*S*,4*S*,*Z*)-4-amino-1^2-(5-((1*R*,5*S*,7*r*)-9-cyclopropyl-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-ethoxy-1^1-ethyl-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (340 mg) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{48}H_{61}N_7O_6S$ 839.4; found 840.5.

Step 3.

To a mixture of (6^3*S*,3*S*,4*S*,*Z*)-4-amino-12-(5-((1*R*,5*S*,7*r*)-9-cyclopropyl-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-ethoxy-1^1-ethyl-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (200 mg, 0.24 mmol) and (1*r*,2*R*,3*S*)-2,3-

dimethylcyclopropane-1-carboxylic acid (41 mg, 0.36 mmol) in DMF (2 mL) at 0 °C was added DIPEA (308 mg, 2.4 mmol) and COMU (102 mg, 0.24 mmol) in portions. The mixture was warmed to room temperature and stirred for 1 h, then purified by preparative-HPLC to give (1*r*,2*R*,3*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-12-(5-((1*R*,5*S*,7*r*)-9-cyclopropyl-3-oxa-9-azabicyclo[3.3.1]nonan-7-yl)-2-((*S*)-1-methoxyethyl)pyridin-3-yl)-3-ethoxy-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-11H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2,3-dimethylcyclopropane-1-carboxamide (78 mg, 35% yield) as a solid. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{52}H_{69}N_7O_7S$ 935.5; found 936.4; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.73 (m, 1H), 8.50 (m, 1H), 7.93 (s, 1H), 7.82 - 7.53 (m, 3H), 5.88 (d, *J* = 9.8 Hz, 1H), 5.22 (d, *J* = 9.7 Hz, 1H), 4.92 (s, 2H), 4.55 - 3.86 (m, 14H), 3.84 - 3.37 (m, 7H), 3.21 (s, 3H), 2.81 (d, *J* = 12.3 Hz, 2H), 2.32 (s, 3H), 2.02 (d, 1H), 1.82 (s, 2H), 1.53 (t, *J* = 3.9 Hz, 2H), 1.38 (d, *J* = 6.0 Hz, 3H), 1.12 (dt, *J* = 34.8, 6.3 Hz, 13H), 0.86 (s, 7H), 0.55 (s, 3H).

Example A504. Synthesis of presumed (1*S*,2*R*,3*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methyl-3-(pyrimidin-4-yl)cyclopropane-1-carboxamide



Step 1.

To a mixture of LiCl (0.31 g, 7.4 mmol) and pyrimidine-4-carbaldehyde (1.0 g, 9.3 mmol) in DMF at 0 °C was added DBU (1.69 g, 11.1 mmol) and *tert*-butyl 2-(diethoxyphosphoryl)acetate (2.80 g, 11.1 mmol) dropwise. The mixture was warmed to room temperature and stirred for 1 h at room temperature, then cooled to 0 °C, quenched with saturated NH_4Cl and extracted with EtOAc (200 mL). The organic layer was washed with brine (3 x 200 mL) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give *tert*-butyl (*E*)-3-(pyrimidin-4-yl)acrylate (1.0 g, 52% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{11}H_{14}N_2O_2$ 206.1; found 207.1.

Step 2.

To a mixture ethyldiphenylsulfanium tetrafluoroborate (2.2 g, 10.2 mmol) in DME and DCM (10 : 1) at -60°C under an atmosphere of N_2 was treated with LDA, 2M in THF (6.0 mL, 12.0 mmol) for 0.5 h. The mixture was warmed to room temperature and *tert*-butyl (*E*)-3-(pyrimidin-4-yl)acrylate (700 mg, 3.4 mmol) was added dropwise. The mixture was stirred at room temperature 1 h, then quenched with saturated NH_4Cl and extracted with EtOAc (3 x 100 mL). The combined organic layers were concentrated under reduced pressure and the residue was purified by preparative-HPLC to give 2-methyl-3-(pyrimidin-4-yl)cyclopropane-1-carboxylic acid (160mg, 20% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{13}H_{18}N_2O_2$ 234.1; found 235.1; ^1H NMR (300 MHz, $CDCl_3$) δ 9.10 (s, 1H), 8.63 (s, 1H), 7.33 (s, 1H), 2.66 (dd, *J* = 9.7, 4.7 Hz, 1H), 2.46 (dd, *J* = 5.7, 4.7 Hz, 1H), 2.06 - 1.78 (m, 1H), 1.47 (s, 9H), 1.13 (d, *J* = 6.4 Hz, 3H).

A racemic mixture of the above compound was separated by chiral-HPLC to give (55 mg, single diastereomer of unknown absolute configuration, RT = 6.2 min) as a solid and (61 mg, single diastereomer of unknown absolute configuration, RT = 7.3 min).

Step 3.

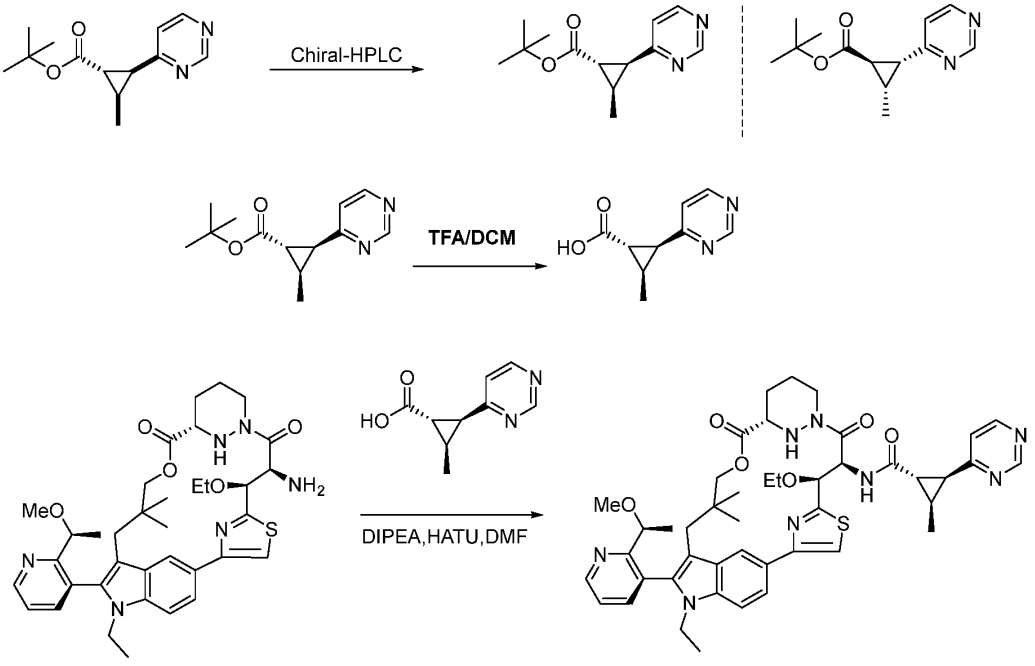
A mixture of *tert*-butyl (1*S*,2*R*,3*S*)-2-methyl-3-(pyrimidin-4-yl)cyclopropane-1-carboxylate (50 mg, 0.21 mmol, single diastereomer of unknown absolute configuration; RT = 6.2 min) and TFA (5 mL, 67.3 mmol) in DCM was stirred at room temperature for 1 h, then concentrated under reduced pressure to give (1*S*,2*R*,3*S*)-2-methyl-3-(pyrimidin-4-yl)cyclopropane-1-carboxylic acid, that was used directly in the next step without further purification (single diastereomer of unknown absolute configuration). LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_9H_{10}N_2O_2$ 178.0; found 179.1.

Step 4.

To a mixture of (1*S*,2*R*,3*S*)-2-methyl-3-(pyrimidin-4-yl)cyclopropane-1-carboxylic acid (53 mg, 0.3 mmol) and DIPEA (192 mg, 1.48 mmol) in DMF at room temperature under an atmosphere of N_2 was added (6^3*S*,3*S*,4*S*,*Z*)-4-amino-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (100 mg, 0.15 mmol) and HATU (113 mg, 0.3 mmol). The mixture was stirred at room temperature for 1 h, then diluted with EtOAc (100 mL) and washed with

brine (3 x 100 mL). The organic layer was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (1*S*,2*R*,3*S*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methyl-3-(pyrimidin-4-yl)cyclopropane-1-carboxamide (19 mg, 15% yield; single diastereomer of unknown absolute configuration) as a solid. LCMS (ESI): m/z [M+H]$^+$ calc'd for $C_{45}H_{54}N_8O_6S$ 834.4; found 835.2; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.09 (s, 1H), 8.82 - 8.71 (m, 1H), 8.66 (m, 1H), 8.51 (s, 1H), 8.23 - 8.22 (m, 1H), 7.94 (s, 1H), 7.87 - 7.65 (m, 2H), 7.63 - 7.30 (m, 3H), 5.89 - 5.76 (m, 1H), 5.19 - 5.15 (m,1H), 4.95 (s, 1H), 4.28 - 4.13 (m, 5H), 3.79 - 3.52 (m, 7H), 3.42 - 3.31 (m, 3H), 2.83 (s, 2H), 2.08 (s, 1H), 1.78 (s, 3H), 1.38 - 1.34 (m,3H), 1.31 - 1.20 (m, 6H), 0.88 - 0.78 (m, 6H), 0.41 (s, 3H).

Example A505. Synthesis of presumed (1*R*,2*S*,3*R*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methyl-3-(pyrimidin-4-yl)cyclopropane-1-carboxamide



Step 1.

A racemic mixture of the starting cyclopropane was separated by chiral HPLC [condition (Column: CHIRALPAK IF, 2x25 cm, 5 μM; Mobile Phase A: Hexane (10mM NH_3-MeOH), Mobile Phase B: IPA--HPLC; Flow rate: 20 mL/min; Gradient: 5% B to 5% B in 10 min; Wave Length: 252/220 nm; RT1 (min): 6.2; RT2 (min): 7.3; Sample Solvent: MeOH: DCM=1 : 1; Injection Volume: 0.2 mL; Number Of Runs: 11)]. **Product A** (single diastereomer of unknown absolute configuration, 55 mg, RT = 6.2 min) as white solid; **Product B** (single diastereomer of unknown absolute configuration, 61 mg, RT = 7.3 min).

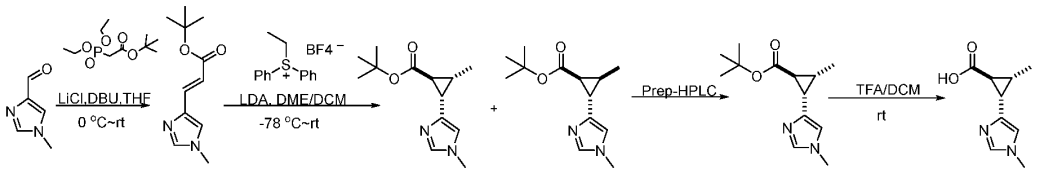
Step 2.

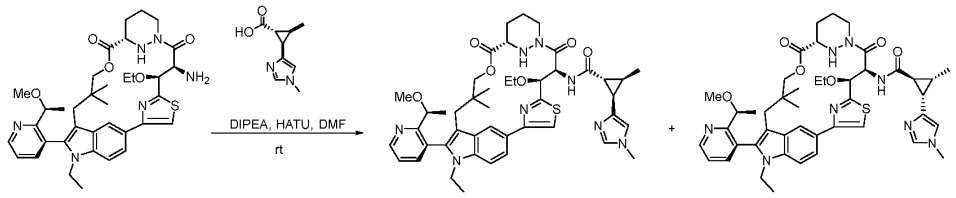
A mixture of presumed *tert*-butyl (1*R*,2*S*,3*R*)-2-methyl-3-(pyrimidin-4-yl)cyclopropane-1-carboxylate (single diastereomer of unknown absolute configuration, 50 mg, 0.21 mmol) and TFA (5 mL, 67.3 mmol) in DCM was stirred at room temperature for 1 h, then concentrated under reduced pressure to give presumed (1*R*,2*S*,3*R*)-2-methyl-3-(pyrimidin-4-yl)cyclopropane-1-carboxylic acid (single diastereomer of unknown absolute configuration). LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_9H_{10}N_2O_2$ 178.0; found 179.1.

Step 3.

To a mixture of (1*R*,2*S*,3*R*)-2-methyl-3-(pyrimidin-4-yl)cyclopropane-1-carboxylic acid (53 mg, 0.3 mmol) and DIPEA (192 mg, 1.48 mmol) in DMF at room temperature under an atmosphere of N_2 was added (6^3S,3*S*,4*S*,*Z*)-4-amino-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (100 mg, 0.15 mmol) and HATU (113 mg, 0.3 mmol). The mixture was stirred at room temperature for 1 h, then diluted with EtOAc (100mL) and washed with brine (3 x 100 mL). The organic layer was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (1*R*,2*S*,3*R*)-*N*-((6^3S,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methyl-3-(pyrimidin-4-yl)cyclopropane-1-carboxamide (single diastereomer of unknown absolute configuration, 29 mg, 19% yield) as a solid. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{45}H_{54}N_8O_6S$ 834.4; found 835.2; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.10 (s, 1H), 8.83 - 8.78 (m, 1H), 8.71 (m, 1H), 8.54 (s, 1H), 8.29 - 8.24 (m, 1H), 7.98 (s, 1H), 7.88 - 7.78 (m, 2H), 7.74 - 7.69 (m, 1H), 7.67 - 7.35 (m, 3H), 5.85 - 5.72 (m, 1H), 5.19 - 5.12 (m,1H), 4.93 (s, 1H), 4.30 - 4.22 (m, 5H), 3.80 - 3.60 (m, 7H), 3.46 - 3.38 (m, 3H), 2.88 (s, 1H), 2.75 - 2.56 (m, 1H), 2.18 (s, 1H), 1.88 (s, 1H), 1.71 - 1.56 (m, 2H), 1.48 - 1.24 (m,3H), 1.28 - 1.22 (m, 3H), 1.19 - 1.02 (m, 3H), 0.98 - 0.88 (m,6H), 0.44 (s, 3H).

Examples A506 and A507. Synthesis of presumed (1*R*,2*S*,3*R*)-*N*-((6^3S,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methyl-3-(1-methyl-1*H*-imidazol-4-yl)cyclopropane-1-carboxamide and (1*R*,2*S*,3*R*)-*N*-((6^3S,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methyl-3-(1-methyl-1*H*-imidazol-4-yl)cyclopropane-1-carboxamide





Step 1.

To a mixture of 1-methyl-1*H*-imidazole-4-carbaldehyde (1.9 g, 17.3 mmol) and LiCl (0.95 g, 22.4 mmol) in THF (10 mL) at 0 °C were added *tert*-butyl 2-(diethoxyphosphoryl)acetate (5.66 g, 22.4 mmol) and DBU (2.63 g, 17.3 mmol) in portions. The mixture was allowed to warm to room temperature and stirred for 2 h then the mixture was washed H_2O (2 x 30 mL). The combined aqueous layers were extracted with EtOAc (3 x 30 mL) and the combined organic layers were dried and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give *tert*-butyl (*E*)-3-(1-methyl-1*H*-imidazol-4-yl)acrylate (3 g, 84% yield) as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{11}H_{16}N_2O_2$ 208.1; found 209.1.

Step 2.

To a mixture of ethyldiphenylsulfonium tetrafluoroborate (2.18 g, 7.2 mmol) in DCM : DME (1 : 10) at -60 °C under an atmosphere of N_2 was treated with 2M LDA in THF (12 mL, 24 mmol) for 30 min, followed by the addition of *tert*-butyl (*E*)-3-(1-methyl-1*H*-imidazol-4-yl)acrylate (500 mg, 2.4 mmol) in portions. The mixture was warmed to room temperature and stirred for 2 h, then quenched with saturated NH_4Cl and extracted with EtOAc (3 x 50 mL). The combined organic layers were dried. The residue was purified by preparative-HPLC to give *tert*-butyl (1*S*,2*R*,3*S*)-2-methyl-3-(1-methyl-1*H*-imidazol-4-yl)cyclopropane-1-carboxylate (200 mg, 35% yield of a single diastereomer of unknown absolute configuration) and *tert*-butyl (1*S*,2*S*,3*S*)-2-methyl-3-(1-methyl-1*H*-imidazol-4-yl)cyclopropane-1-carboxylate (110 mg, 19% yield of a single diastereomer of unknown absolute configuration), as an oil. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{13}H_{20}N_2O_2$ 236.2; found 236.9.

Step 3.

A mixture of presumed *tert*-butyl (1*S*,2*R*,3*S*)-2-methyl-3-(1-methyl-1*H*-imidazol-4-yl)cyclopropane-1-carboxylate (70 mg, 0.3 mmol) and TFA (1 mL) in DCM was stirred at room temperature for 2 h, then concentrated under reduced pressure to give (1*S*,2*R*,3*S*)-2-methyl-3-(1-methyl-1*H*-imidazol-4-yl)cyclopropane-1-carboxylic acid as an oil, which was used directly in the next step without further purification. LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_9H_{12}N_2O_2$ 180.1; found 181.3.

Step 4.

To a mixture of (6^3*S*,3*S*,4*S*, *Z*)-4-amino-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (122 mg, 0.18 mmol) and *tert*-butyl (1*S*,2*R*,3*S*)-2-methyl-3-(1-methyl-1*H*-imidazol-4-yl)cyclopropane-1-carboxylate (65 mg, 0.36 mmol) in DMF (10 mL) at

room temperature was added DIPEA (467 mg, 3.6 mmol) and HATU (76 mg, 0.2 mmol) in portions. The mixture was irradiated under microwave radiation for 2 h at room temperature. The mixture was extracted with EtOAc (2 x 20 mL) and the combined organic layers were washed with H_2O (3 x 20 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (1*R*,2*S*,3*R*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methyl-3-(1-methyl-1*H*-imidazol-4-yl)cyclopropane-1-carboxamide (34 mg, 21%, diastereomer of unknown absolute configuration RT = 1.00 min) as a solid and (1*R*,2*S*,3*R*)-*N*-((6^3*S*,3*S*,4*S*,*Z*)-3-ethoxy-1^1-ethyl-1^2-(2-((*S*)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1*H*-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-2-methyl-3-(1-methyl-1*H*-imidazol-4-yl)cyclopropane-1-carboxamide (36 mg, 22%, single diastereomer of unknown absolute configuration RT = 1.07 min) as a solid. Data for first diastereomer (RT = 1.00 min): LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{45}H_{56}N_8O_6S$ 836.4; found 837.1; ^{1}H NMR (400 MHz, CD_3OD) δ 8.74 - 8.69 (m, 1H), 8.63 - 8.60 (m, 1H), 7.86 - 7.80 (m, 1H), 7.73 - 7.68 (m, 1H), 7.63 (s, 1H), 7.56 - 7.45 (m, 3H), 6.94 (s, 1H), 6.01 (s, 1H), 5.04 - 5.01 (m, 1H), 4.50 - 4.41 (m, 1H), 4.38 - 4.33 (m, 1H), 4.29 - 4.20 (m, 2H), 4.15 - 4.06 (m, 1H), 3.76 - 3.73 (m, 2H), 3.72 - 3.68 (m, 3H), 3.66 - 3.58 (m, 2H), 3.32 - 3.27 (m, 3H), 3.05 - 2.95 (m, 1H), 2.86 - 2.78 (m, 1H), 2.66 - 2.61 (m, 1H), 2.49 - 2.41 (m, 1H), 2.24 - 2.16 (m, 1 H), 2.11 - 2.06 (m, 1H), 2.02 - 1.92 (m, 1H), 1.87 - 1.77 (m, 1H), 1.69 - 1.52 (m, 3H), 1.46 - 1.41 (m, 3H), 1.38 - 1.15 (m, 11H), 1.04 - 0.81 (m, 13H), 0.50 (s, 3H). Data for second diastereomer (RT = 1.07 min): LCMS (ESI): m/z $[M+H]^+$ calc'd for $C_{45}H_{56}N_8O_6S$ 836.4; found 837.1; ^{1}H NMR (400 MHz, CD_3OD) δ 8.74 - 8.67 (m, 1H), 8.63 - 8.60 (m, 1H), 7.86 - 7.80 (m, 1H), 7.73 - 7.68 (m, 1H), 7.63 (s, 1H), 7.56 - 7.45 (m, 3H), 6.94 (s, 1H), 6.01 (s, 1H), 5.04 - 5.01 (m, 1H), 4.50 - 4.41 (m, 1H), 4.38 - 4.33 (m, 1H), 4.29 - 4.20 (m, 2H), 4.15 - 4.06 (m, 1H), 3.76 - 3.73 (m, 2H), 3.72 - 3.68 (m, 3H), 3.66 - 3.58 (m, 2H), 3.32 - 3.27 (m, 3H), 3.05 - 2.95 (m, 1H), 2.86 - 2.78 (m, 1H), 2.66 - 2.61 (m, 1H), 2.49 - 2.41 (m, 1H), 2.24 - 2.16 (m, 1 H), 2.11 - 2.06 (m, 1H), 2.02 - 1.92 (m, 3H), 1.87 - 1.77 (m, 3H), 1.68 - 1.53 (m, 4H), 1.46 - 1.41 (m, 11H), 1.38 - 1.15 (m, 3H), 1.04 - 0.81 (m, 10H), 0.50 (s, 3H).

Biological Assays

All compounds herein exhibit an IC50 of 2 μM or less in an AsPC-1 (K-Ras G12D) pERK potency assay and/or a Capan-1 (K-Ras G12V) pERK potency assay.

Potency assay: pERK

The purpose of this assay was to measure the ability of test compounds to inhibit K-Ras in cells. Activated K-Ras induces increased phosphorylation of ERK at Threonine 202 and Tyrosine 204 (pERK). This procedure measures a decrease in cellular pERK in response to test compounds. The procedure described below in NCI-H358 cells is applicable to K-Ras G12C.

Note: this protocol may be executed substituting other cell lines to characterize inhibitors of other RAS variants, including, for example, AsPC-1 (K-Ras G12D), Capan-1 (K-Ras G12V), NCI-

H1355 (K-Ras G13C), Hs 766T (K-Ras Q61H), NCI-H2347 (N-Ras Q61R), or SK-MEL-30 (N-Ras Q61K).

NCI-H358 cells were grown and maintained using media and procedures recommended by the ATCC. On the day prior to compound addition, cells were plated in 384-well cell culture plates (40 µl/well) and grown overnight in a 37°C, 5% CO2 incubator. Test compounds were prepared in 10, 3-fold dilutions in DMSO, with a high concentration of 10 mM. On day of assay, 40 nl of test compound was added to each well of cell culture plate using an Echo550 liquid handler (LabCyte®). Concentrations of test compound were tested in duplicate. After compound addition, cells were incubated 4 hours at 37°C, 5% CO2. Following incubation, culture medium was removed and cells were washed once with phosphate buffered saline.

In some experiments, cellular pERK level was determined using the AlphaLISA SureFire Ultra p-ERK1/2 Assay Kit (PerkinElmer). Cells were lysed in 25 µl lysis buffer, with shaking at 600 RPM at room temperature. Lysate (10 µl) was transferred to a 384-well Opti-plate (PerkinElmer) and 5 µl acceptor mix was added. After a 2-hour incubation in the dark, 5 µl donor mix was added, plate was sealed and incubated 2 hours at room temperature. Signal was read on an Envision plate reader (PerkinElmer) using standard AlphaLISA settings. Analysis of raw data was carried out in Excel (Microsoft) and Prism (GraphPad). Signal was plotted vs. the decadal logarithm of compound concentration, and IC_{50} was determined by fitting a 4-parameter sigmoidal concentration response model.

In other experiments, cellular pERK was determined by In-Cell Western. Following compound treatment, cells were washed twice with 200 µl tris buffered saline (TBS) and fixed for 15 minutes with 150 µl 4% paraformaldehyde in TBS. Fixed cells were washed 4 times for 5 minutes with TBS containing 0.1% Triton X-100 (TBST) and then blocked with 100 µl Odyssey blocking buffer (LI-COR) for 60 minutes at room temperature. Primary antibody (pERK, CST-4370, Cell Signaling Technology) was diluted 1:200 in blocking buffer, and 50 µl was added to each well and incubated overnight at 4°C. Cells were washed 4 times for 5 minutes with TBST. Secondary antibody (IR-800CW rabbit, LI-COR, diluted 1:800) and DNA stain DRAQ5 (LI-COR, diluted 1:2000) were added and incubated 1-2 hours at room temperature. Cells were washed 4 times for 5 minutes with TBST. Plates were scanned on a Li-COR Odyssey CLx Imager. Analysis of raw data was carried out in Excel (Microsoft) and Prism (GraphPad). Signal was plotted vs. the decadal logarithm of compound concentration, and IC_{50} was determined by fitting a 4-parameter sigmoidal concentration response model.

Compound A, a Representative Inhibitor of the Present Invention, Drives Regressions of $KRAS^{G12D}$ Tumors in Vivo

Methods: Effects of Compound A on tumor cell growth in vivo were evaluated in the human pancreatic adenocarcinoma HPAC $KRAS^{G12D/wt}$ xenograft model using female BALB/c nude mice (6-8 weeks old). Mice were implanted with HPAC tumor cells in PBS (3 x 106 cells/mouse) subcutaneously in the flank. Once tumors reached an average size of ~150 mm^3, mice were randomized to treatment groups to start the administration of test articles or vehicle. Compound A

was administered by oral gavage once every other day (po q2d). Body weight and tumor volume (using calipers) was measured twice weekly until study endpoints.

Results: Single-agent Compound A administered at 50 mg/kg po and 100 mg/kg po every other day led to complete regression of all tumors in each group (complete regression defined as >85% tumor regression from baseline) at the end of treatment (Day 38 after treatment started) in the HPAC CDX model with heterozygous $KRAS^{G12D}$ (FIG. 1A). The anti-tumor activity of both tested doses of Compound A was statistically significant compared with control group (***p<0.001, ordinary One-way ANOVA with multiple comparisons via a post-hoc Tukey's test).

Compound A, a Representative Inhibitor of the Present Invention, Regulates RAS Pathway and Drives Regressions of $KRAS^{G12V}$ Tumors in Vivo

Methods: Effects of Compound A on blood and tumor pharmacokinetics (PK), pharmacodynamics (PD), and tumor cell growth were evaluated *in vivo* in the human non-small cell lung cancer (NSCLC) NCI-H441 $KRAS^{G12V/wt}$ xenograft model using female BALB/c nude mice (6-8 weeks old). Mice were implanted with NCI-H441 tumor cells (2 x 106 cells/mouse) in 50% media, 50% Matrigel, subcutaneously in the flank. For PK/PD, animals were grouped out when tumors were ~400 mm^3 and animals were treated with a single dose of Compound A at 10, 25 or 50 mg/kg by oral gavage. For PK/PD n=3 measurements per timepoint.

Once tumors reached an average size of ~155 mm^3, mice were randomized to treatment groups to start the administration of test articles or vehicle. In NCI-H441 Compound A was administered by oral gavage once daily (po qd) at 10 or 25 mg/kg. Body weight and tumor volume (using calipers) was measured twice weekly until study endpoints.

Results: Pharmacokinetics were analyzed based on total concentration (nM) of Compound A in tumors or blood, following a single oral gavage dose of Compound A at 10, 25 or 50 mg/kg, monitored through 72 hours following dose. Compound A exhibited dose-dependent exposure in blood and tumor samples. Compound A treated at 25 mg/kg or 50 mg/kg doses was detectable in tumors through 72 hours following treatment (FIG. 1B). PK from naïve animals treated with a single dose of Compound A delivered at 10 mg/kg demonstrates maximum exposure of at 2 hours (FIG. 1C). Tumor DUSP6 demonstrates modulation of DUSP6, a marker of RAS pathway activity for 72 hours following single dose administration (FIG. 1C).

Single-agent Compound A administered to NCI-H441 tumor bearing animals, treated at 10 mg/kg po qd led to regressions (reductions in tumor volume >10% from initial) in all animals. Treated at 25 mg/kg po qd, Compound A led to complete regression of all tumors (complete regression defined as >85% tumor regression from baseline) at the end of treatment (Day 38 after treatment started) in the NCI-H441 CDX model with heterozygous $KRAS^{G12V}$ (FIG. 1D, FIG. 1E). The anti-tumor activity of Compound A was statistically significant compared with control group (***p<0.0001, ordinary One-way ANOVA with multiple comparisons via a post-hoc Tukey's test). Treatments were well tolerated by body weight measurements (FIG. 1F).

Compound A, a Representative Inhibitor of the Present Invention, Drives Regressions of KRAS^{G12V} Pancreatic Ductal Adenocarcinoma and Colorectal Tumors in Vivo

Methods: Effects of Compound A on tumor cell growth in vivo were evaluated in the human pancreatic adenocarcinoma Capan-2 KRAS$^{G12V/wt}$ and colorectal SW403 KRAS$^{G12V/wt}$ xenograft models using female BALB/c nude mice (6-8 weeks old). Mice were implanted with Capan-2 tumor cells (4 x 106 (media/Matrigel) cells/mouse), or SW403 tumor cells (1 x 107 cells/mouse) in 50% PBS, 50% Matrigel, subcutaneously in the flank. Once tumors reached an average size of ~160-170 mm^3, mice were randomized to treatment groups to start the administration of test articles or vehicle. In Capan-2, Compound A was administered by oral gavage once daily (po qd) at 10 or 25 mg/kg. In SW403 CDX, Compound A was administered by oral gavage once daily (po qd) at 25 mg/kg or every other day (q2d) at 50mg/kg. Body weight and tumor volume (using calipers) was measured twice weekly until study endpoints.

Results: Single-agent Compound A administered to Capan-2 tumor bearing animals, treated at 10 mg/kg po qd led to 5/8 regressions, and 25 mg.kg po qd led to 8/8 regressions at the end of treatment (Day 38 after treatment started) in the Capan-2 CDX model with heterozygous KRAS^{G12V} (FIG. 2A, FIG. 2B). The anti-tumor activity of Compound A was statistically significant compared with control group (**p<0.01, ***p<0.0001, ordinary One-way ANOVA with multiple comparisons via a post-hoc Tukey's test). Treatments were well tolerated by body weight measurements (FIG. 2C). In SW403 CDX model, single-agent Compound A administered at 25 mg/kg po daily or 50 mg/kg po q2d led to significant tumor growth inhibition in all tumors through the end of treatment (Day 35 after treatment started). Compound A treatment at 25 mg/kg po qd drove regressions in 8/8 tumors, and 2/8 CR's (FIG. 2D, FIG. 2E). Compound A treatment at 50 mg/kg po q2d drove regressions in 8/8 tumors. The anti-tumor activity of Compound A was statistically significant compared with control group (***p<0.0001, ordinary One-way ANOVA with multiple comparisons via a post-hoc Tukey's test). Treatments were well tolerated by body weight measurements (FIG. 2F).

Compound A Exhibits Potent in Vivo Inhibition of Multiple RAS-Driven Cancer Cell Lines

Methods: Potency of in vitro cell proliferation inhibition of Capan-1 (KRAS^{G12V}), AsPC-1 (KRAS^{G12D}), HCT116 (KRAS^{G13D}), SK-MEL-30 (NRAS^{Q61K}), NCI-H1975 (EGFR$^{T790M/L858R}$), and A375 (BRAF^{V600E}) cells exposed to Compound A for 120 hours. Data represent the mean of multiple experiments. Cells were seeded in growth medium in 384-well assay plates and incubated overnight in a humidified atmosphere of 5% CO_2 at 37°C. The following day, cells were exposed to a 9-concentration 3-fold serial dilution of Compound A at a starting assay concentration of 1 µM (or 10 µM for A375). After 5 days of incubation, CellTiter-Glo® 2.0 Reagent was added to assay plates and luminescence measured. Data were normalized to the mean signal of DMSO-treated cells, and IC_{50} values were estimated using a four-parameter concentration response model.

Results: Compound A inhibited cell proliferation in RAS-driven lines (FIG. 3). IC50 for RAS-Driven cancer cell lines as follows: Capan-1 (KRAS^{G12V}) = 1 nM, AsPC-1 (KRAS^{G12D}) = 3 nM,

HCT116 ($KRAS^{G13D}$) = 27 nM, SK-MEL-30 ($NRAS^{Q61K}$) = 13 nM, NCI-H1975 ($EGFR^{T790M/L858R}$) = 1 nM. RAS WT-Independent cell line A375 ($BRAF^{V600E}$) was not sensitive to Compound A treatment with IC50 >8700 nM.

Compound A, a Representative Inhibitor of the Present Invention, Drives Regressions of $KRAS^{G12D}$ Tumors in Vivo

Methods: Effects of Compound A on tumor cell growth in vivo were evaluated in the human pancreatic adenocarcinoma HPAC $KRAS^{G12D/wt}$ and colorectal GP2d $KRAS^{G12D/wt}$ xenograft models using female BALB/c nude mice (6-8 weeks old). Mice were implanted with HPAC tumor cells (3 x 106 cells/mouse), or GP2d tumor cells (2 x 106 cells/mouse) in 50% PBS, 50% Matrigel, subcutaneously in the flank. Once tumors reached an average size of ~150 mm^3, mice were randomized to treatment groups to start the administration of test articles or vehicle. Compound A was administered by oral gavage once daily (po qd) at 25 mg/kg. Body weight and tumor volume (using calipers) was measured twice weekly until study endpoints.

Results: Single-agent Compound A administered at 25 mg/kg po daily led to complete regression of all tumors (complete regression defined as >85% tumor regression from baseline) at the end of treatment (Day 38 after treatment started) in the HPAC CDX model with heterozygous $KRAS^{G12D}$ (FIG. 4A, FIG. 4B). The anti-tumor activity of Compound A was statistically significant compared with control group (***p<0.0001, ordinary One-way ANOVA with multiple comparisons via a post-hoc Tukey's test). Treatments were well tolerated by body weight measurements (FIG. 4C). In Gp2d CDX model, single-agent Compound A administered at 25 mg/kg po daily led to significant tumor growth inhibition in all tumors through the end of treatment (Day 35 after treatment started) (FIG. 4D, FIG. 4E). The anti-tumor activity of Compound A was statistically significant compared with control group (***p<0.0001, ordinary One-way ANOVA with multiple comparisons via a post-hoc Tukey's test). Treatments were well tolerated by body weight measurements (FIG. 4F).

Compound A Down-Regulates Immune Checkpoint Proteins in NCI-H358, SW900, and Capan-2 Cells in Vitro

Methods: To assess the effect of Compound A on checkpoint molecule expression in vitro, NCI-H358, SW900 or Capan-2 cells (5e4 cells/well) were seeded in a 96-well plate and after 24 hours treated with a five-fold dilution of Compound A in the presence of 250 pg/ml IFNγ. The plates were incubated for 48 hours at 37°C and 5% CO_2. The cells were detached with 0.25% Trypsin, incubated for 15 minutes in PBS containing Fixable Blue Dead Cell Stain (Invitrogen) and subsequently incubated with FITC anti-human CD274 (PD-L1), PerCP/Cyanine5.5 anti-human CD155 (PVR) and Brilliant Violet 605 anti-human CD73 (Biolegend) for 30 minutes on ice. The cells were washed twice with staining buffer (PBS/2% FCS) before flow cytometric acquisition on a

Cytek Aurora instrument. The analysis was performed using the SpectroFlo and FlowJo v10 software.

Results: Compound A produced a concentration-dependent 2- to 5-fold decrease of PD-L1, PVR and CD73 on NCI-H358 (FIG. 5A), SW900 (FIG. 5B), or Capan-2 (FIG. 5C) cells in vitro. Down-regulation of these proteins is predicted to transform the immuno-suppressive tumor immune microenvironment in favor of anti-tumor immunity (Rothlin *et al* JITC 2020).

Compound A, a KRAS^{MULTI}(ON) inhibitor disclosed herein, is active against RAS oncogene switching mutations

FIG. 6A is a heatmap representing cellular RAS/RAF disruption assay results regarding various KRAS mutations in the presence of different RAS inhibitors (Compound A, a KRAS^{MULTI}(ON) inhibitor disclosed herein, and KRAS^{G12C}(OFF) inhibitors MRTX849 and AMG 510).

Plasmids expressing nanoluciferase-tagged mutant KRAS4B and halo-tagged RAF1(residues 51-149) were co-transfected into U2OS cells and incubated for 24 hours. Plasmids encoding the relevant mutation were generated by New England Biolabs Q5 site-directed mutagenesis. Transfected cells were reseeded at 25000 cells/well in 96-well plates in assay media (OptiMEM + 4% FBS + 100 nM HaloTag NanoBRET 618 Ligand) and incubated overnight. Promega Vivazine Nano-Glo substrate was added according to manufacturer's instructions. Compounds were added at concentrations ranging from 0 to 10 µM and incubated for 1 hour. The luminescence signal was measured at 460 nm and 618 nm and the BRET ratio was calculated as the 618 nm signal divided by the 460 nm signal. The BRET ratios were fit to a standard sigmoidal dose response function and the IC50 values were used to calculate the Log2(Fold-Change) relative to KRAS^{G12C}. FIG. 6B shows the IC50 value associated with each colored bar of the heatmap.

The following four assays may be conducted to further characterize properties of compounds of the present invention.

Determination of Cell Viability in RAS Mutant Cancer Cell Lines

Protocol: CellTiter-Glo® Cell Viability Assay

Note – The following protocol describes a procedure for monitoring cell viability of K-Ras mutant cancer cell lines in response to a compound of the invention. Other RAS isoforms may be employed, though the number of cells to be seeded will vary based on cell line used.

The purpose of this cellular assay is to determine the effects of test compounds on the proliferation of three human cancer cell lines (NCI-H358 (K-Ras G12C), AsPC-1 (K-Ras G12D), Capan-1 (K-Ras G12V)) over a 5-day treatment period by quantifying the amount of ATP present at endpoint using the CellTiter-Glo® 2.0 Reagent (Promega).

Cells are seeded at 250 cells/well in 40 µl of growth medium in 384-well assay plates and incubated overnight in a humidified atmosphere of 5% CO_2 at 37 °C. On the day of the assay, 10 mM stock solutions of test compounds are first diluted into 3 mM solutions with 100% DMSO. Well-mixed compound solutions (15 µl) are transferred to the next wells containing 30 µl of 100% DMSO,

and repeated until a 9-concentration 3-fold serial dilution is made (starting assay concentration of 10 µM). Test compounds (132.5 nl) are directly dispensed into the assay plates containing cells. The plates are shaken for 15 seconds at 300 rpm, centrifuged, and incubated in a humidified atmosphere of 5% CO_2 at 37 °C for 5 days. On day 5, assay plates and their contents are equilibrated to room temperature for approximately 30 minutes. CellTiter-Glo® 2.0 Reagent (25 µl) is added, and plate contents are mixed for 2 minutes on an orbital shaker before incubation at room temperature for 10 minutes. Luminescence may be measured using the PerkinElmer Enspire. Data may be normalized by the following: (Sample signal/Avg. DMSO)*100. The data may be fit using a four-parameter logistic fit.

Disruption of B-Raf Ras-binding Domain ($BRAF^{RBD}$) Interaction with K-Ras by Compounds of the Invention

Note – The following protocol describes a procedure for monitoring disruption of K-Ras G12C (GMP-PNP) binding to $BRAF^{RBD}$ by a compound of the invention. This protocol may also be executed substituting other Ras proteins or nucleotides.

The purpose of this biochemical assay is to measure the ability of test compounds to facilitate ternary complex formation between a nucleotide-loaded K-Ras isoform and Cyclophilin A; the resulting ternary complex disrupts binding to a $BRAF^{RBD}$ construct, inhibiting K-Ras signaling through a RAF effector. Data may be reported as IC50 values.

In assay buffer containing 25 mM HEPES pH 7.3, 0.002% Tween20, 0.1% BSA, 100 mM NaCl and 5 mM $MgCl_2$, tagless Cyclophilin A, His6-K-Ras-GMPPNP, and GST-$BRAF^{RBD}$ are combined in a 384-well assay plate at final concentrations of 25 µM, 12.5 nM and 50 nM, respectively. Compound is present in plate wells as a 10-point 3-fold dilution series starting at a final concentration of 30 µM. After incubation at 25 °C for 3 hours, a mixture of Anti-His Eu-W1024 and anti-GST allophycocyanin is then added to assay sample wells at final concentrations of 10 nM and 50 nM, respectively, and the reaction incubated for an additional 1.5 hours. TR-FRET signal is read on a microplate reader (Ex 320 nm, Em 665/615 nm). Compounds that facilitate disruption of a K-Ras:RAF complex are identified as those eliciting a decrease in the TR-FRET ratio relative to DMSO control wells.

Determination of Cell Viability in RAS Mutant Cancer Cell Lines

Protocol: CellTiter-Glo® Cell Viability Assay

Note – The following protocol describes a procedure for monitoring cell viability of KRAS mutant cancer cell lines in response to a compound of the invention. Other RAS isoforms may be employed, though the number of cells to be seeded will vary based on cell line used.

The purpose of this cellular assay is to determine the effects of test compounds on the proliferation of three human cancer cell lines (NCI-H358 (KRAS G12C), AsPC-1 (KRAS G12D),

Capan-1 (KRAS G12V)) over a 5-day treatment period by quantifying the amount of ATP present at endpoint using the CellTiter-Glo® 2.0 Reagent (Promega).

Cells are seeded at 250 cells/well in 40 µl of growth medium in 384-well assay plates and incubated overnight in a humidified atmosphere of 5% CO_2 at 37 °C. On the day of the assay, 10 mM stock solutions of test compounds are first diluted into 3 mM solutions with 100% DMSO. Well-mixed compound solutions (15 µl) are transferred to the next wells containing 30 µl of 100% DMSO, and repeated until a 9-concentration 3-fold serial dilution is made (starting assay concentration of 10 µM). Test compounds (132.5 nl) are directly dispensed into the assay plates containing cells. The plates are shaken for 15 seconds at 300 rpm, centrifuged, and incubated in a humidified atmosphere of 5% CO_2 at 37°C for 5 days. On day 5, assay plates and their contents are equilibrated to room temperature for approximately 30 minutes. CellTiter-Glo® 2.0 Reagent (25 µl) is added, and plate contents are mixed for 2 minutes on an orbital shaker before incubation at room temperature for 10 minutes. Luminescence may be measured using the PerkinElmer Enspire. Data is normalized by the following: (Sample signal/Avg. DMSO)*100. The data may be fit using a four-parameter logistic fit.

Determination of Oral Bioavailability

Oral bioavailability may be determined in BALB/c mice. Following intravenous (IV) bolus and oral gavage (PO) administration of a test compound, about 30 µL of whole blood samples are collected at designated time points into tubes containing K_2EDTA. The blood samples are centrifuged at 4600 rpm at 4 °C for about 5 minutes and plasma samples are stored at -80 °C prior to bioanalysis. Plasma samples are extracted by protein precipitation and analyzed by tandem mass spectrometry (LC MS/MS) on, for example, an API 5500 system using electrospray positive ionization.

All PK parameters may be derived from plasma concentration over time data with noncompartment analysis using WinNonlin. The bioavailability (F%) was estimated using the following equation:

$$F\% = \frac{AUC_{inf,PO}}{AUC_{inf,IV}} \cdot \frac{Dose_{IV}}{Dose_{PO}}$$

$AUC_{inf,PO}$ is the area under the plasma concentration over time from time zero to infinity following PO administration.

$AUC_{inf,IV}$ is the area under the plasma concentration over time from time zero to infinity following IV administration.

$Dose_{IV}$ is the total dose of IV administration

$Dose_{PO}$ is the total dose of PO administration

In general, F% values of over 30% are preferred, with values over 50% being more preferred.

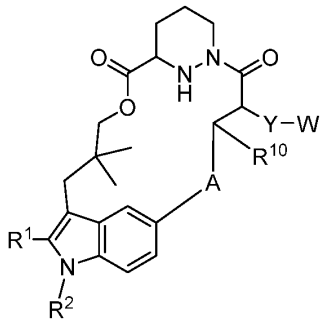
While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover

any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure come within known or customary practice within the art to which the invention pertains and may be applied to the essential features set forth herein.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

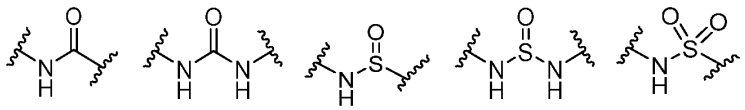
Numbered Embodiments

[1] A compound, or pharmaceutically acceptable salt thereof, having the structure of Formula I:

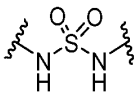


Formula I

wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;



Y is -NHC(O)-, -NHC(O)NH-, -NHC(S)-, -NHC(S)NH-, -NHS(O)$_2$-, or



-NHS(O)$_2$NH-;

W is hydrogen, C_1-C_4 alkyl, optionally substituted 3 to 10-membered heterocycloalkyl, optionally substituted 3 to 10-membered cycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^1 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; and

R^{10} is hydrogen or optionally substituted C_1-C_6 heteroalkyl.

[2] The compound of paragraph [1], or pharmaceutically acceptable salt thereof, wherein R^1 is optionally substituted 6 to 10-membered aryl or optionally substituted 5 to 10-membered heteroaryl.

[3] The compound of paragraph [2], or pharmaceutically acceptable salt thereof, wherein R^1 is optionally substituted phenyl or optionally substituted pyridine.

[4] The compound of any one of paragraphs [1] to [3], or pharmaceutically acceptable salt thereof, wherein A is optionally substituted thiazole, optionally substituted triazole, optionally substituted morpholino, or phenyl.

[5] The compound of any one of paragraphs [1] to [3], or pharmaceutically acceptable salt thereof, wherein A is not an optionally substituted phenyl or benzimidazole.

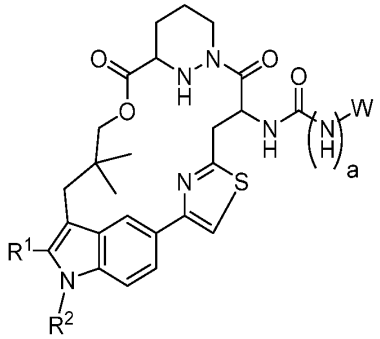
[6] The compound of paragraph [5], or pharmaceutically acceptable salt thereof, wherein A is not hydroxyphenyl.

[7] The compound of any one of paragraphs [1] to [6], or pharmaceutically acceptable salt thereof, wherein the compound is not a compound of Table 2.

[8] The compound of any one of paragraphs [1] to [7], or pharmaceutically acceptable salt thereof, wherein the compound is not a compound of Table 3.

[9] The compound of any one of paragraphs [1] to [8], or pharmaceutically acceptable salt thereof, wherein Y is -NHC(O)- or -NHC(O)NH-.

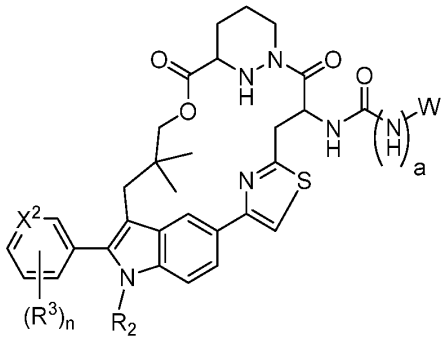
[10] The compound of paragraph [9], or pharmaceutically acceptable salt thereof, having the structure of Formula II:



Formula II,

wherein a is 0 or 1.

[11] The compound of paragraph [10], or pharmaceutically acceptable salt thereof, having the structure of Formula II-1:



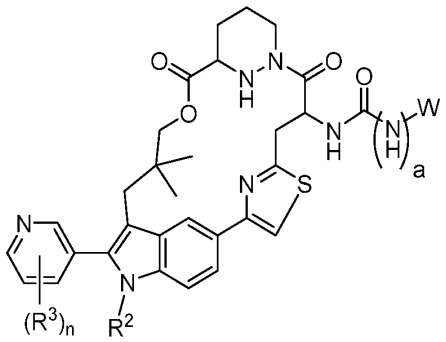
Formula II-1,

wherein X^2 is N or CH;

each R^3 is independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; and

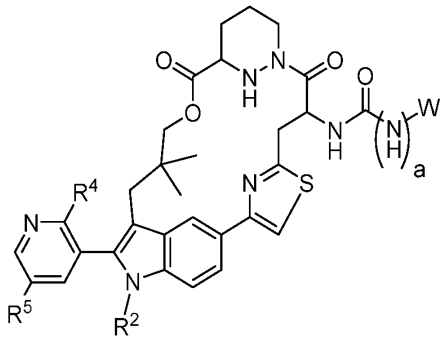
n is an integer from 1 to 4.

[12] The compound of paragraph [11], or pharmaceutically acceptable salt thereof, having the structure of Formula II-2:



Formula II-2.

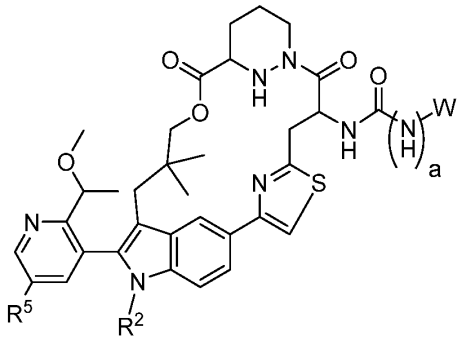
[13] The compound of paragraph [12], or pharmaceutically acceptable salt thereof, having the structure of Formula II-3:



Formula II-3,

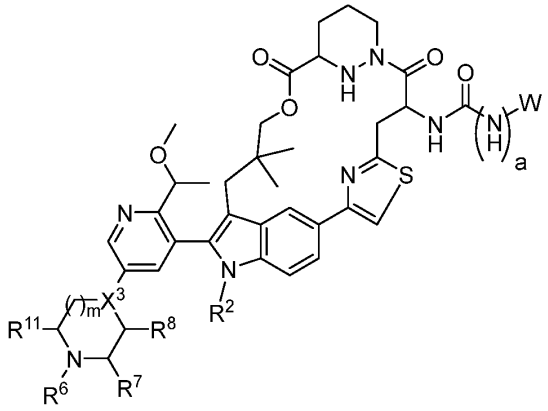
wherein R^4 and R^5 are each independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

[14] The compound of paragraph [13], or pharmaceutically acceptable salt thereof, having the structure of Formula II-4:



Formula II-4.

[15] The compound of paragraph [14], or pharmaceutically acceptable salt thereof, having the structure of Formula II-5:



Formula II-5,

wherein X^3 is N or CH;

m is 1 or 2;

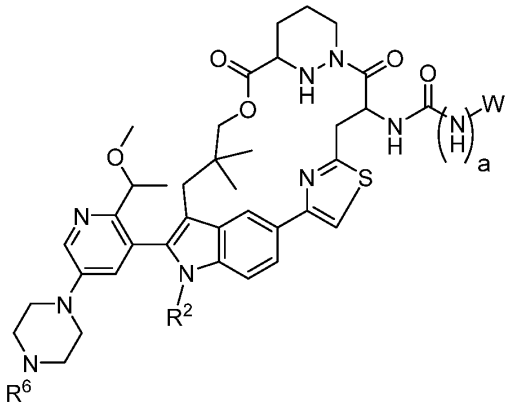
R^6, R^7, R^8, and R^{11} are each independently selected from optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; or

R^6 and R^7 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^8 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered heterocycloalkyl; or

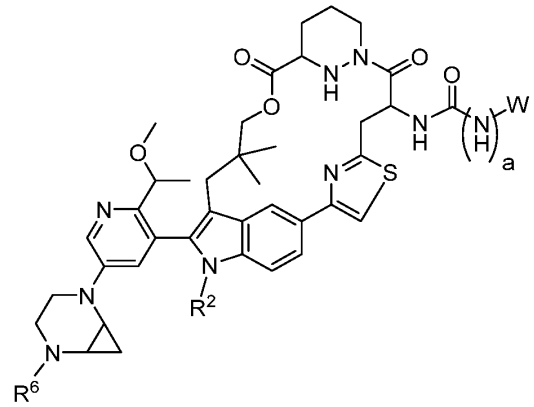
R^7 and R^{11} combine with the atoms to which they are attached to form an optionally substituted 4 to 8-membered heterocycloalkyl.

[16] The compound of paragraph [15], or pharmaceutically acceptable salt thereof, having the structure of Formula II-6:



Formula II-6.

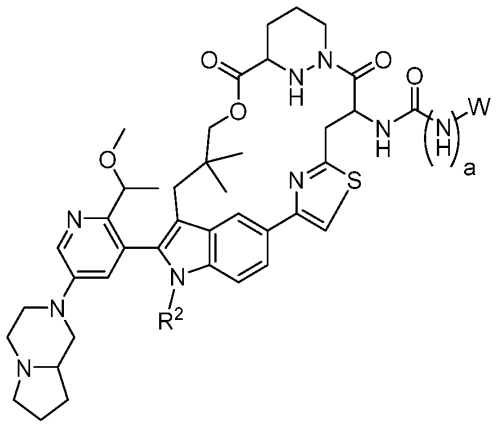
[17] The compound of paragraph [15], or pharmaceutically acceptable salt thereof, having the structure of Formula II-7:



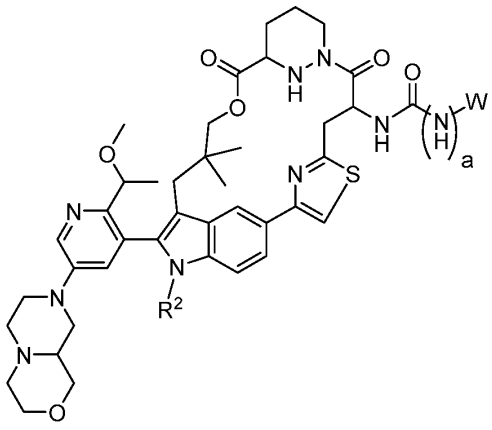
Formula II-7.

[18] The compound of paragraph [16] or [17], wherein R^6 is methyl.

[19] The compound of paragraph [15], or pharmaceutically acceptable salt thereof, having the structure of Formula II-8 or Formula II-9:

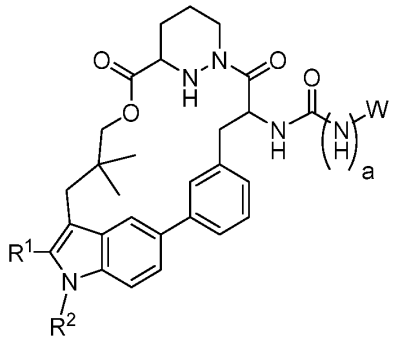


Formula II-8,



Formula II-9.

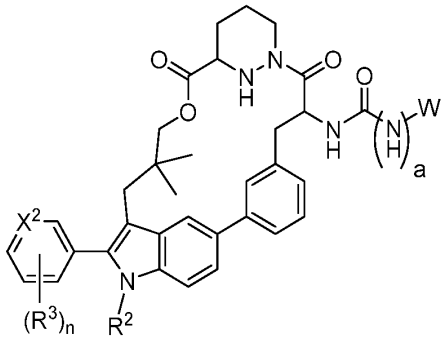
[20] The compound of paragraph [9], or pharmaceutically acceptable salt thereof, having the structure of Formula III:



Formula III,

wherein a is 0 or 1.

[21] The compound of paragraph [20], or pharmaceutically acceptable salt thereof, having the structure of Formula III-1:



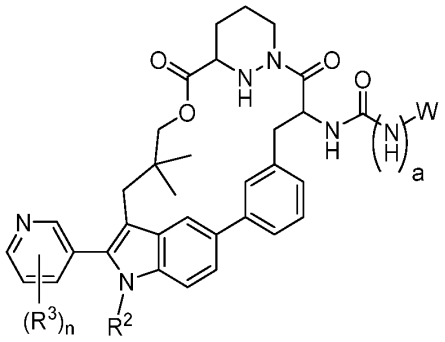
Formula III-1,

wherein X^2 is N or CH;

each R^3 is independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; and

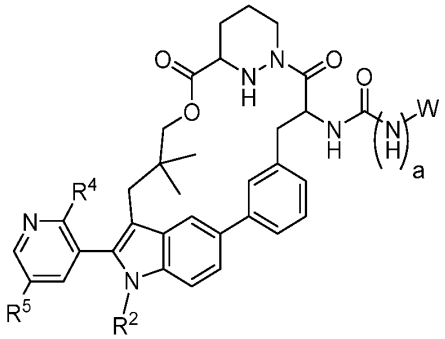
n is an integer from 1 to 4.

[22] The compound of paragraph [21], or pharmaceutically acceptable salt thereof, having the structure of Formula III-2:



Formula III-2.

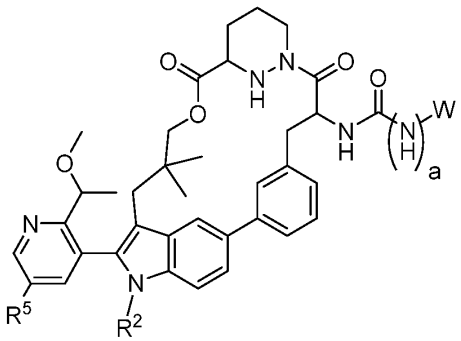
[23] The compound of paragraph [22], or pharmaceutically acceptable salt thereof, having the structure of Formula III-3:



Formula III-3,

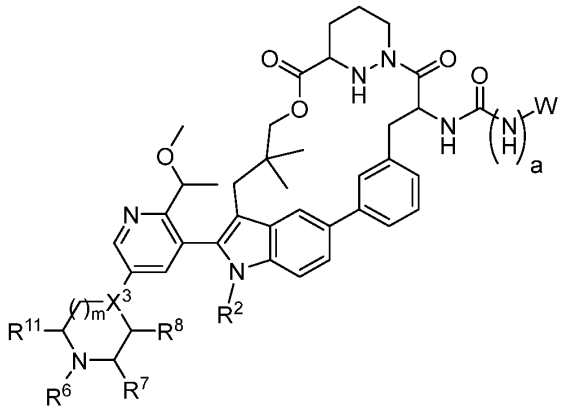
wherein R^4 and R^5 are each independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

[24] The compound of paragraph [23], or pharmaceutically acceptable salt thereof, having the structure of Formula III-4:



Formula III-4.

[25] The compound of paragraph [24], or pharmaceutically acceptable salt thereof, having the structure of Formula III-5:



Formula III-5,

wherein X^3 is N or CH;

m is 1 or 2;

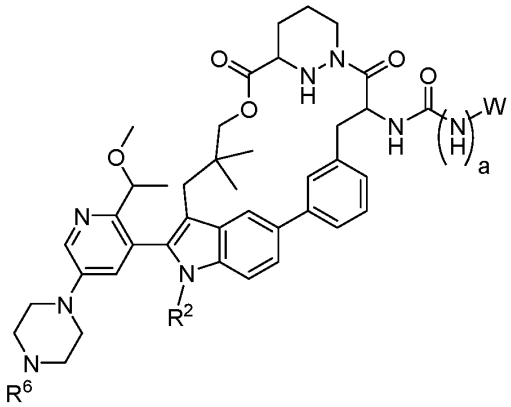
R^6, R^7, R^8, and R^{11} are each independently selected from optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; or

R^6 and R^7 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^8 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered heterocycloalkyl; or

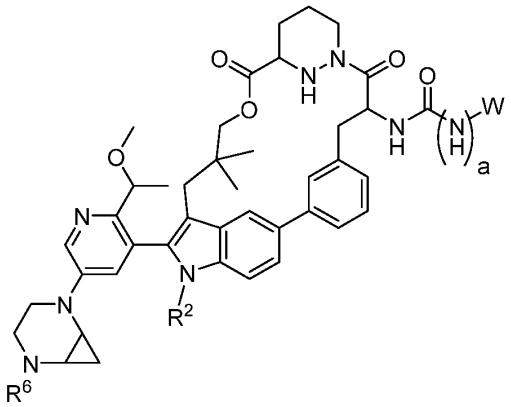
R^7 and R^{11} combine with the atoms to which they are attached to form an optionally substituted 4 to 8-membered heterocycloalkyl.

[26] The compound of paragraph [25], or pharmaceutically acceptable salt thereof, having the structure of Formula III-6:



Formula III-6.

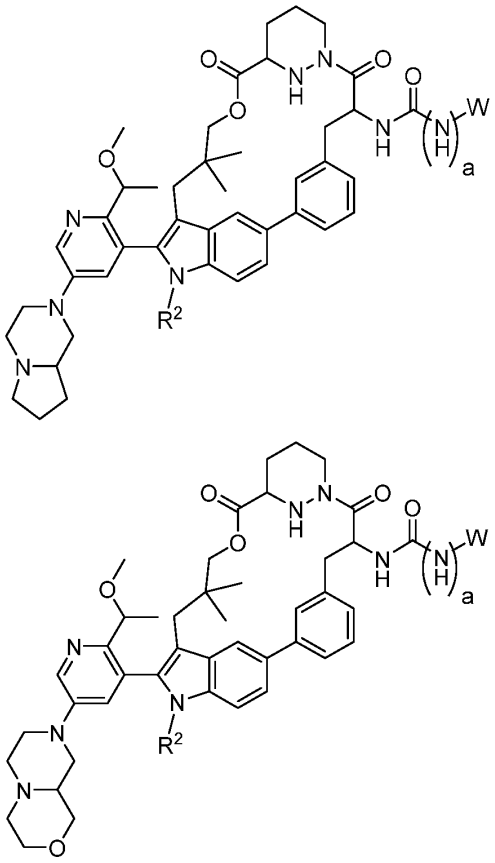
[27] The compound of paragraph [25], or pharmaceutically acceptable salt thereof, having the structure of Formula III-7:



Formula III-7.

[28] The compound of paragraph [26] or [27], wherein R^6 is methyl.

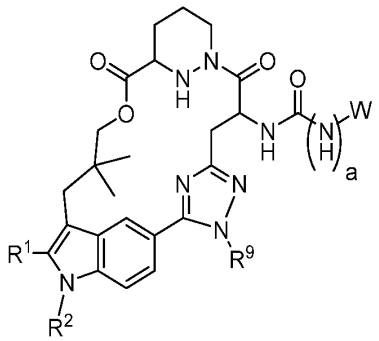
[29] The compound of paragraph [25], or pharmaceutically acceptable salt thereof, having the structure of Formula III-8 or Formula III-9:



Formula III-8,

Formula III-9.

[30] The compound of paragraph [9], or pharmaceutically acceptable salt thereof, having the structure of Formula IV:

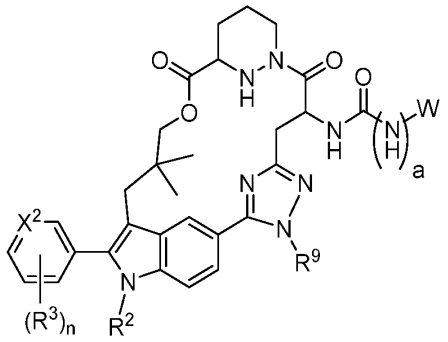


Formula IV,

wherein R^9 is H or C_1-C_6 alkyl; and

a is 0 or 1.

[31] The compound of paragraph [30], or pharmaceutically acceptable salt thereof, having the structure of Formula IV-1:



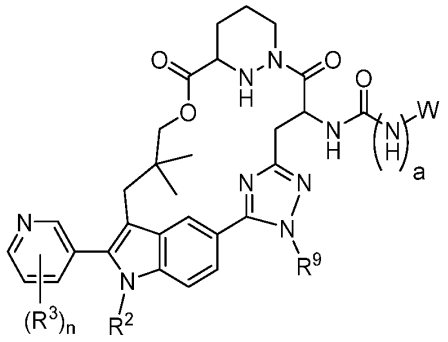
Formula IV-1,

wherein X^2 is N or CH;

each R^3 is independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; and

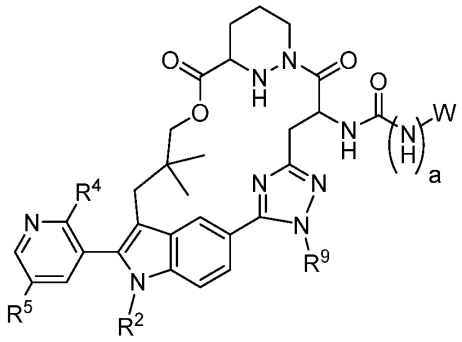
n is an integer from 1 to 4.

[32] The compound of paragraph [31], or pharmaceutically acceptable salt thereof, having the structure of Formula IV-2:



Formula IV-2.

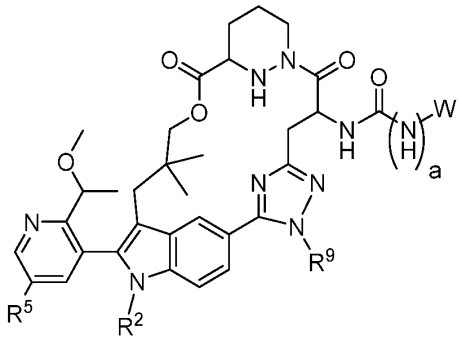
[33] The compound of paragraph [32], or pharmaceutically acceptable salt thereof, having the structure of Formula IV-3:



Formula IV-3,

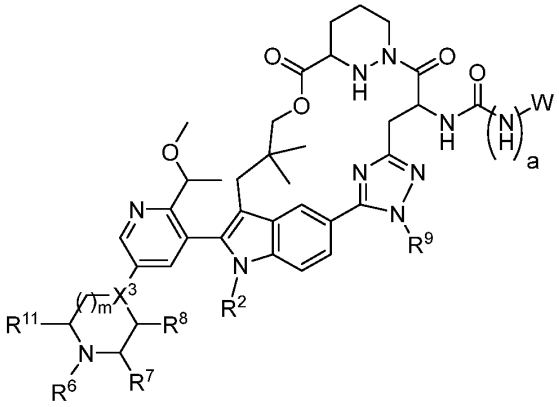
wherein R^4 and R^5 are each independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

[34] The compound of paragraph [33], or pharmaceutically acceptable salt thereof, having the structure of Formula IV-4:



Formula IV-4.

[35] The compound of paragraph [34], or pharmaceutically acceptable salt thereof, having the structure of Formula IV-5:



Formula IV-5,

wherein X^3 is N or CH;

m is 1 or 2;

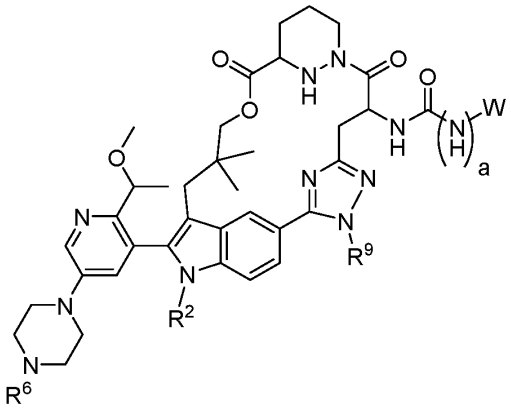
R^6, R^7, R^8, and R^{11} are each independently selected from optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; or

R^6 and R^7 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^8 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered heterocycloalkyl; or

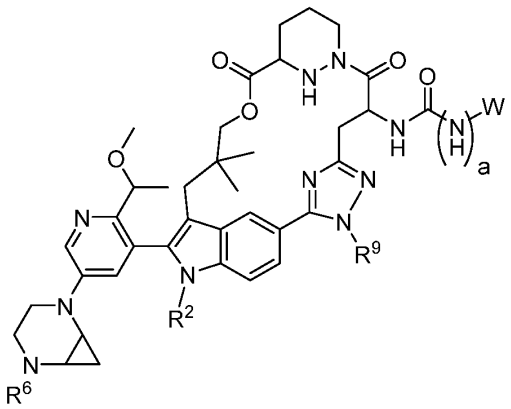
R^7 and R^{11} combine with the atoms to which they are attached to form an optionally substituted 4 to 8-membered heterocycloalkyl.

[36] The compound of paragraph [35], or pharmaceutically acceptable salt thereof, having the structure of Formula IV-6:



Formula IV-6.

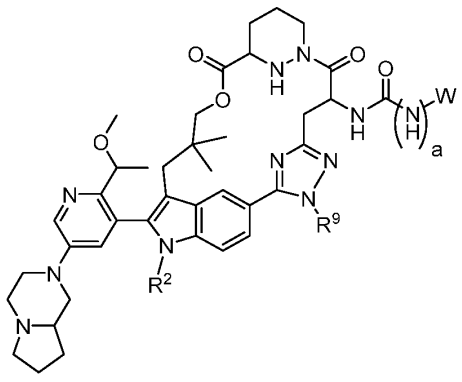
[37] The compound of paragraph [35], or pharmaceutically acceptable salt thereof, having the structure of Formula IV-7:



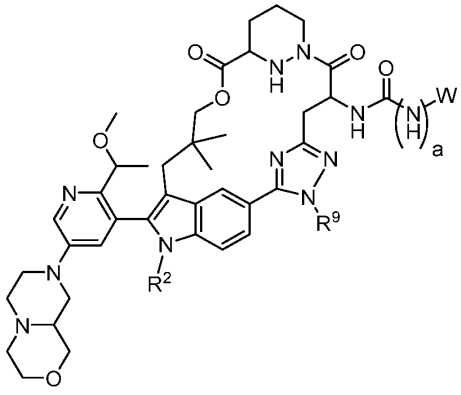
Formula IV-7.

[38] The compound of paragraph [36] or [37], wherein R^6 is methyl.

[39] The compound of paragraph [35], or pharmaceutically acceptable salt thereof, having the structure of Formula IV-8 or Formula IV-9:



Formula IV-8,

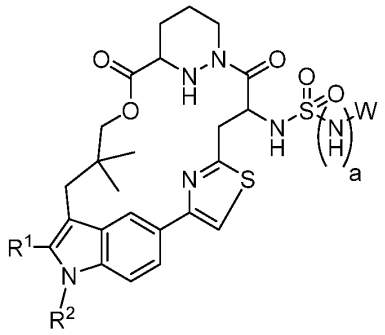


Formula IV-9.

[40] The compound of any one of paragraphs [30] to [39], or pharmaceutically acceptable salt thereof, wherein R^9 is methyl.

[41] The compound of any one of paragraphs [1] to [8], or pharmaceutically acceptable salt thereof, wherein Y is $-NHS(O)_2-$ or $-NHS(O)_2NH-$.

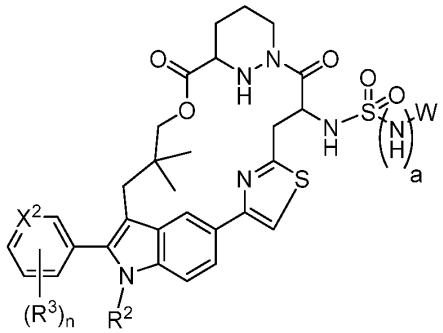
[42] The compound of paragraph [41], or pharmaceutically acceptable salt thereof, having the structure of Formula V:



Formula V,

wherein a is 0 or 1.

[43] The compound of paragraph [42], or pharmaceutically acceptable salt thereof, having the structure of Formula V-1:



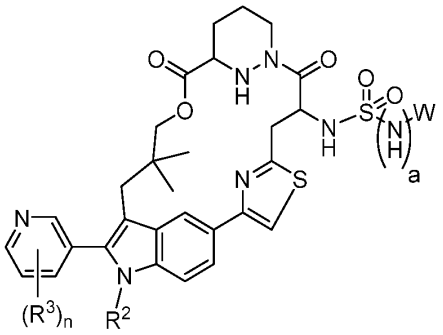
Formula V-1,

wherein X^2 is N or CH;

each R^3 is independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; and

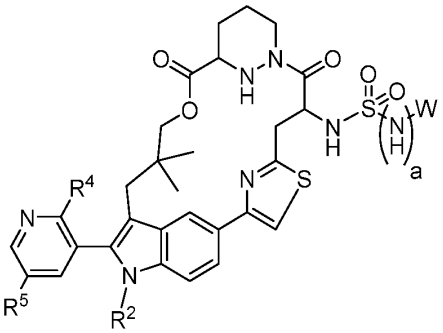
n is an integer from 1 to 4.

[44] The compound of paragraph [43], or pharmaceutically acceptable salt thereof, having the structure of Formula V-2:



Formula V-2.

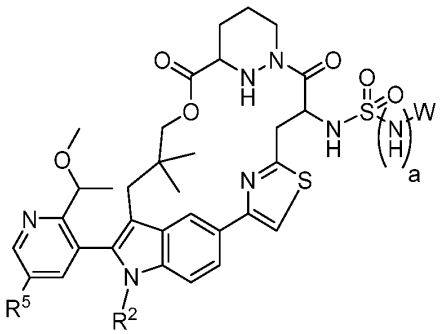
[45] The compound of paragraph [44], or pharmaceutically acceptable salt thereof, having the structure of Formula V-3:



Formula V-3,

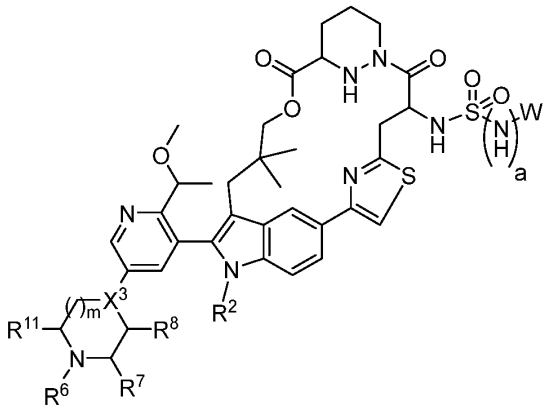
wherein R^4 and R^5 are each independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

[46] The compound of paragraph [45], or pharmaceutically acceptable salt thereof, having the structure of Formula V-4:



Formula V-4.

[47] The compound of paragraph [46], or pharmaceutically acceptable salt thereof, having the structure of Formula V-5:



Formula V-5,

wherein X^3 is N or CH;

m is 1 or 2;

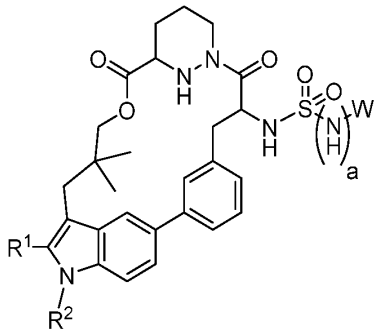
R^6, R^7, R^8, and R^{11} are each independently selected from optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; or

R^6 and R^7 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^8 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^{11} combine with the atoms to which they are attached to form an optionally substituted 4 to 8-membered heterocycloalkyl.

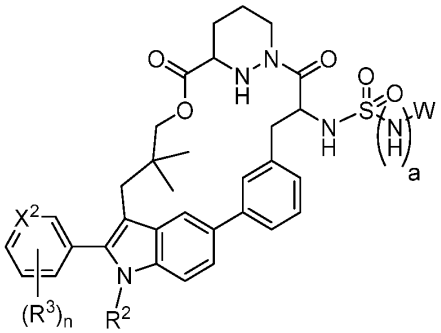
[48] The compound of paragraph [41], or pharmaceutically acceptable salt thereof, having the structure of Formula VI:



Formula VI,

wherein a is 0 or 1.

[49] The compound of paragraph [48], or pharmaceutically acceptable salt thereof, having the structure of Formula VI-1:



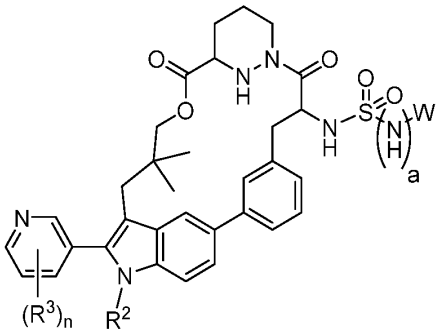
Formula VI-1,

wherein X^2 is N or CH;

each R^3 is independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; and

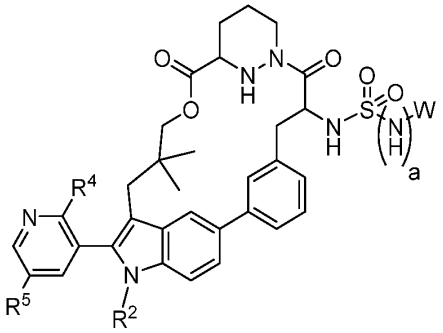
n is an integer from 1 to 4.

[50] The compound of paragraph [49], or pharmaceutically acceptable salt thereof, having the structure of Formula VI-2:



Formula VI-2.

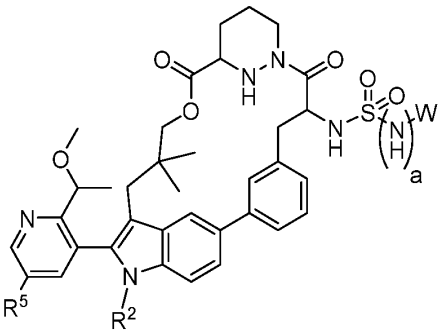
[51] The compound of paragraph [50], or pharmaceutically acceptable salt thereof, having the structure of Formula VI-3:



Formula VI-3,

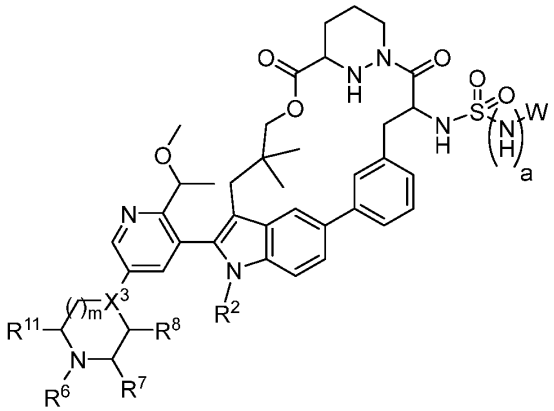
wherein R^4 and R^5 are each independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

[52] The compound of paragraph [51], or pharmaceutically acceptable salt thereof, having the structure of Formula VI-4:



Formula VI-4.

[53] The compound of paragraph [52], or pharmaceutically acceptable salt thereof, having the structure of Formula VI-5:



Formula VI-5,

wherein X^3 is N or CH;

m is 1 or 2;

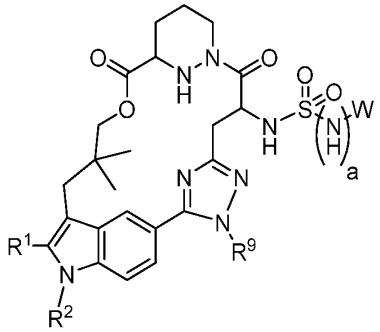
R^6, R^7, R^8, and R^{11} are each independently selected from optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; or

R^6 and R^7 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^8 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^{11} combine with the atoms to which they are attached to form an optionally substituted 4 to 8-membered heterocycloalkyl.

[54] The compound of paragraph [41], or pharmaceutically acceptable salt thereof, having the structure of Formula VII:

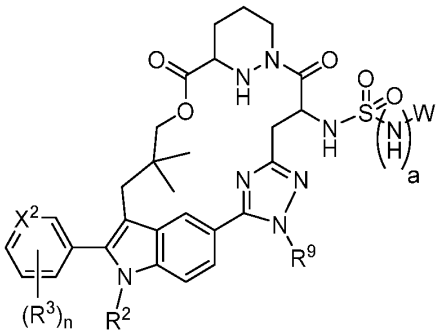


Formula VII,

wherein R^9 is H or C_1-C_6 alkyl; and

a is 0 or 1.

[55] The compound of paragraph [54], or pharmaceutically acceptable salt thereof, having the structure of Formula VII-1:



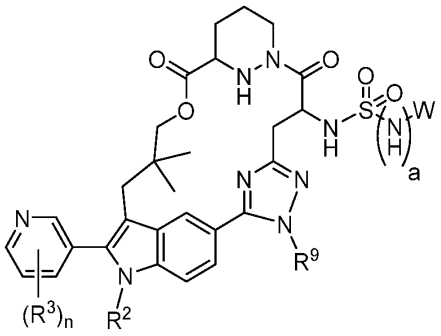
Formula VII-1,

wherein X^2 is N or CH;

each R^3 is independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; and

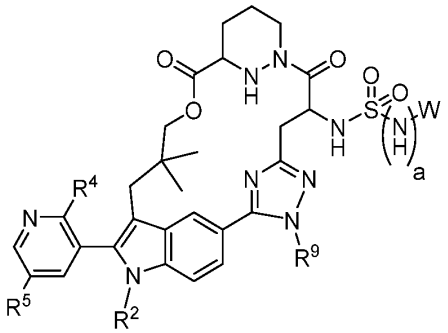
n is an integer from 1 to 4.

[56] The compound of paragraph [55], or pharmaceutically acceptable salt thereof, having the structure of Formula VII-2:



Formula VII-2.

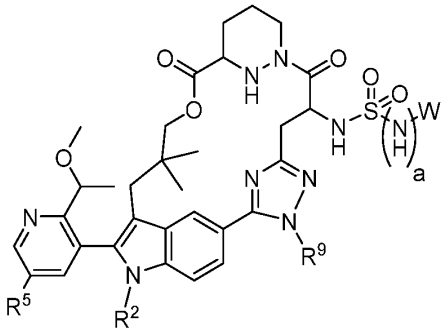
[57] The compound of paragraph [56], or pharmaceutically acceptable salt thereof, having the structure of Formula VII-3:



Formula VII-3,

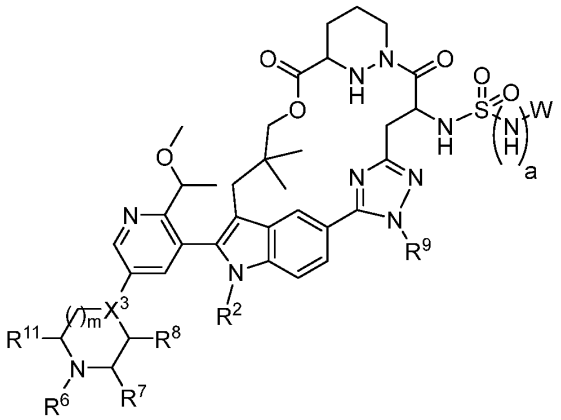
wherein R^4 and R^5 are each independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

[58] The compound of paragraph [57], or pharmaceutically acceptable salt thereof, having the structure of Formula VII-4:



Formula VII-4.

[59] The compound of paragraph [58], or pharmaceutically acceptable salt thereof, having the structure of Formula VII-5:



Formula VII-5,

wherein X^3 is N or CH;

m is 1 or 2;

R^6, R^7, R^8, and R^{11} are each independently selected from optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; or

R^6 and R^7 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or an optionally substituted 3 to 8-membered heterocycloalkyl; or

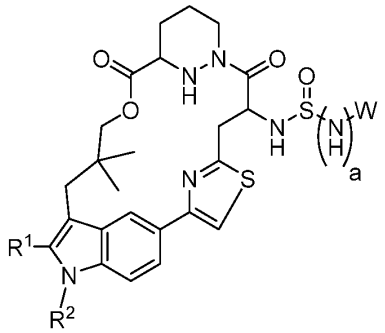
R^7 and R^8 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^{11} combine with the atoms to which they are attached to form an optionally substituted 4 to 8-membered heterocycloalkyl.

[60] The compound of any one of paragraphs [54] to [59], or pharmaceutically acceptable salt thereof, wherein R^9 is methyl.

[61] The compound of any one of paragraphs [1] to [8], or pharmaceutically acceptable salt thereof, wherein Y is -NHS(O)- or -NHS(O)NH-.

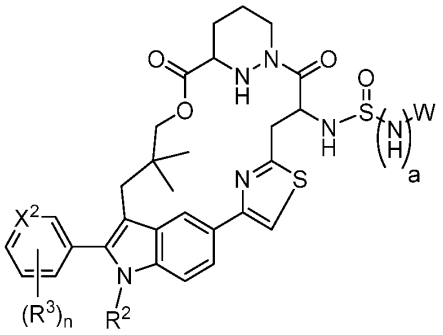
[62] The compound of paragraph [61], or pharmaceutically acceptable salt thereof, having the structure of Formula VIII:



Formula VIII,

wherein a is 0 or 1.

[63] The compound of paragraph [62], or pharmaceutically acceptable salt thereof, having the structure of Formula VIII-1:



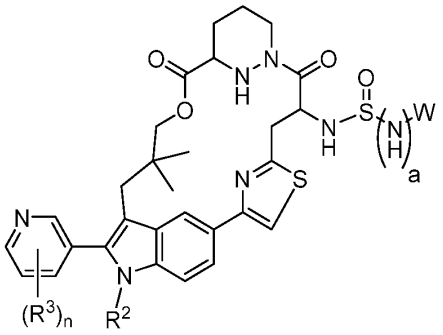
Formula VIII-1,

wherein X^2 is N or CH;

each R^3 is independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; and

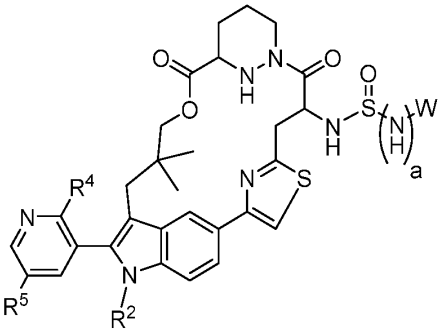
n is an integer from 1 to 4.

[64] The compound of paragraph [63], or pharmaceutically acceptable salt thereof, having the structure of Formula VIII-2:



Formula VIII-2.

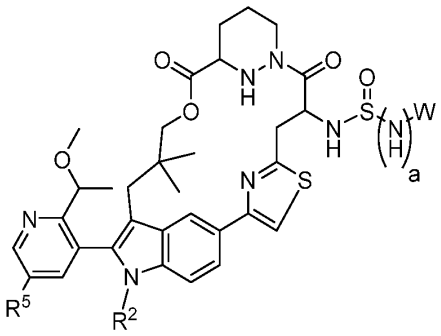
[65] The compound of paragraph [64], or pharmaceutically acceptable salt thereof, having the structure of Formula VIII-3:



Formula VIII-3,

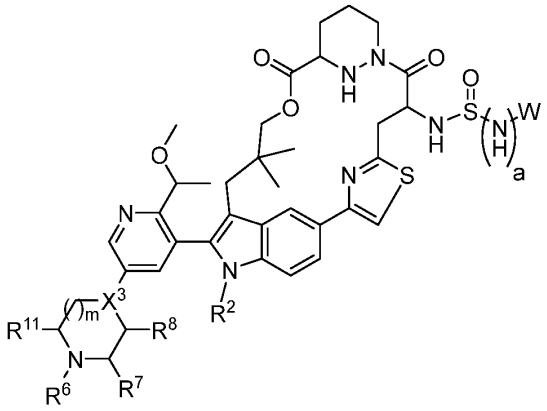
wherein R^4 and R^5 are each independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

[66] The compound of paragraph [65], or pharmaceutically acceptable salt thereof, having the structure of Formula VIII-4:



Formula VIII-4.

[67] The compound of paragraph [66], or pharmaceutically acceptable salt thereof, having the structure of Formula VIII-5:



Formula VIII-5,

wherein X^3 is N or CH;

m is 1 or 2;

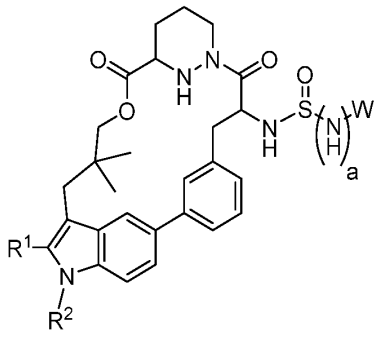
R^6, R^7, R^8, and R^{11} are each independently selected from optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; or

R^6 and R^7 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^8 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^{11} combine with the atoms to which they are attached to form an optionally substituted 4 to 8-membered heterocycloalkyl.

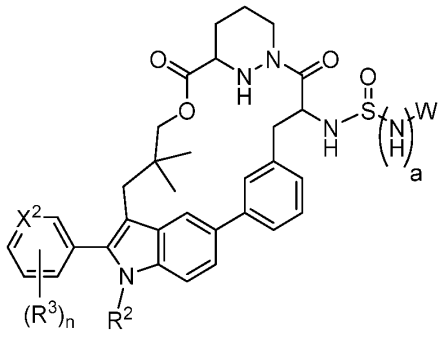
[68] The compound of paragraph [61], or pharmaceutically acceptable salt thereof, having the structure of Formula IX:



Formula IX,

wherein a is 0 or 1.

[69] The compound of paragraph [68], or pharmaceutically acceptable salt thereof, having the structure of Formula IX-1:



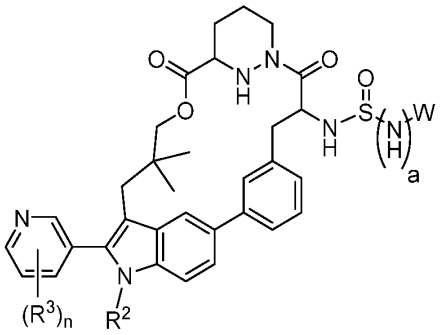
Formula IX-1,

wherein X^2 is N or CH;

each R^3 is independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; and

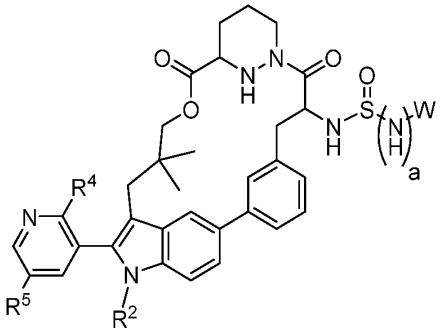
n is an integer from 1 to 4.

[70] The compound of paragraph [69], or pharmaceutically acceptable salt thereof, having the structure of Formula IX-2:



Formula IX-2.

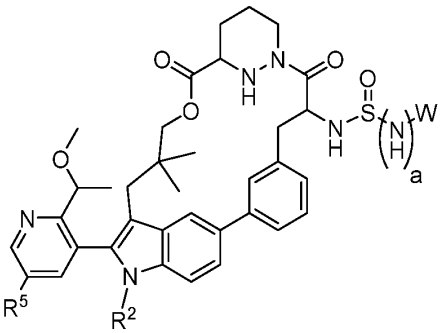
[71] The compound of paragraph [70], or pharmaceutically acceptable salt thereof, having the structure of Formula IX-3:



Formula IX-3,

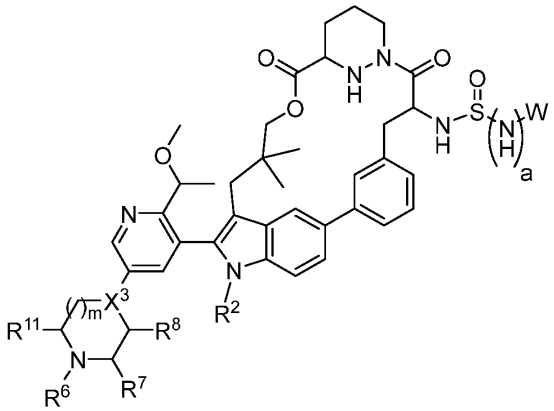
wherein R^4 and R^5 are each independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

[72] The compound of paragraph [71], or pharmaceutically acceptable salt thereof, having the structure of Formula IX-4:



Formula IX-4.

[73] The compound of paragraph [72], or pharmaceutically acceptable salt thereof, having the structure of Formula IX-5:



Formula IX-5,

wherein X^3 is N or CH;

m is 1 or 2;

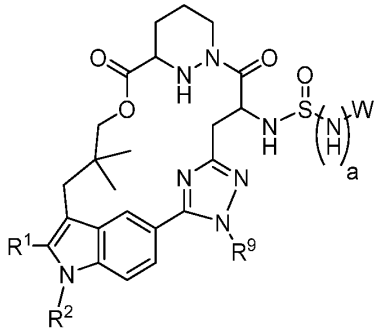
R^6, R^7, R^8, and R^{11} are each independently selected from optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; or

R^6 and R^7 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^8 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^{11} combine with the atoms to which they are attached to form an optionally substituted 4 to 8-membered heterocycloalkyl.

[74] The compound of paragraph [61], or pharmaceutically acceptable salt thereof, having the structure of Formula X:

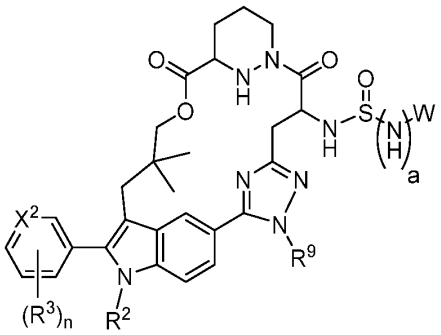


Formula X,

wherein R^9 is H or C_1-C_6 alkyl; and

a is 0 or 1.

[75] The compound of paragraph [74], or pharmaceutically acceptable salt thereof, having the structure of Formula X-1:



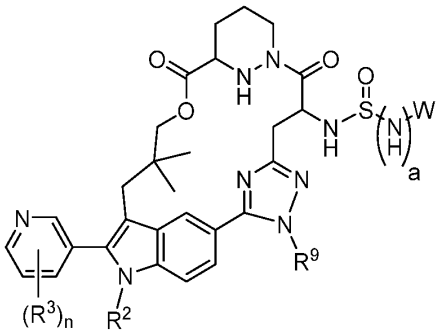
Formula X-1,

wherein X^2 is N or CH;

each R^3 is independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; and

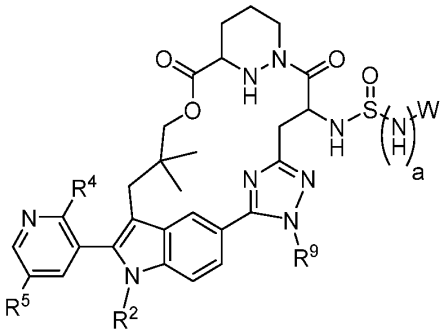
n is an integer from 1 to 4.

[76] The compound of paragraph [75], or pharmaceutically acceptable salt thereof, having the structure of Formula X-2:



Formula X-2.

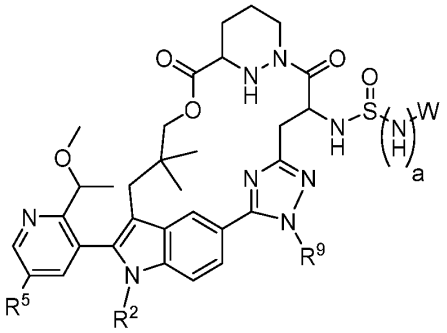
[77] The compound of paragraph [76], or pharmaceutically acceptable salt thereof, having the structure of Formula X-3:



Formula X-3,

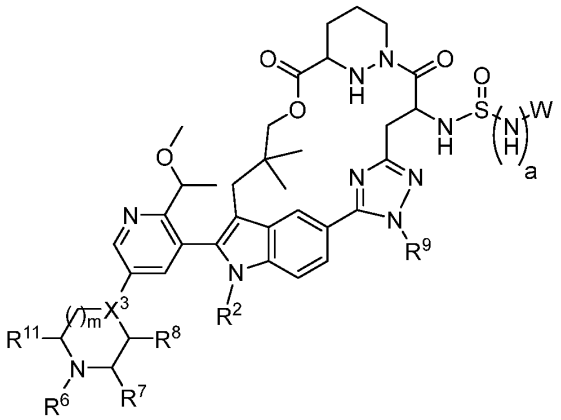
wherein R^4 and R^5 are each independently selected from halogen, cyano, hydroxy, optionally substituted amine, optionally substituted amido, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 11-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

[78] The compound of paragraph [77], or pharmaceutically acceptable salt thereof, having the structure of Formula X-4:



Formula X-4.

[79] The compound of paragraph [78], or pharmaceutically acceptable salt thereof, having the structure of Formula X-5:



Formula X-5,

wherein X^3 is N or CH;

m is 1 or 2;

R^6, R^7, R^8, and R^{11} are each independently selected from optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl; or

R^6 and R^7 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^8 combine with the atoms to which they are attached to form an optionally substituted 3 to 8-membered heterocycloalkyl; or

R^7 and R^{11} combine with the atoms to which they are attached to form an optionally substituted 4 to 8-membered heterocycloalkyl.

[80] The compound of any one of paragraphs [74] to [79], or pharmaceutically acceptable salt thereof, wherein R^9 is methyl.

[81] The compound of any one of paragraphs [10] to [40], [42] to [60], and [62] to [80], or pharmaceutically acceptable salt thereof, wherein a is 0.

[82] The compound of any one of paragraphs [10] to [40], [42] to [60], and [62] to [80], or pharmaceutically acceptable salt thereof, wherein a is 1.

[83] The compound of any one of paragraphs [1] to [82], or pharmaceutically acceptable salt thereof, wherein R^2 is optionally substituted C_1-C_6 alkyl.

[84] The compound of paragraph [83], or pharmaceutically acceptable salt thereof, wherein R^2 is selected from $-CH_2CH_3$ or $-CH_2CF_3$.

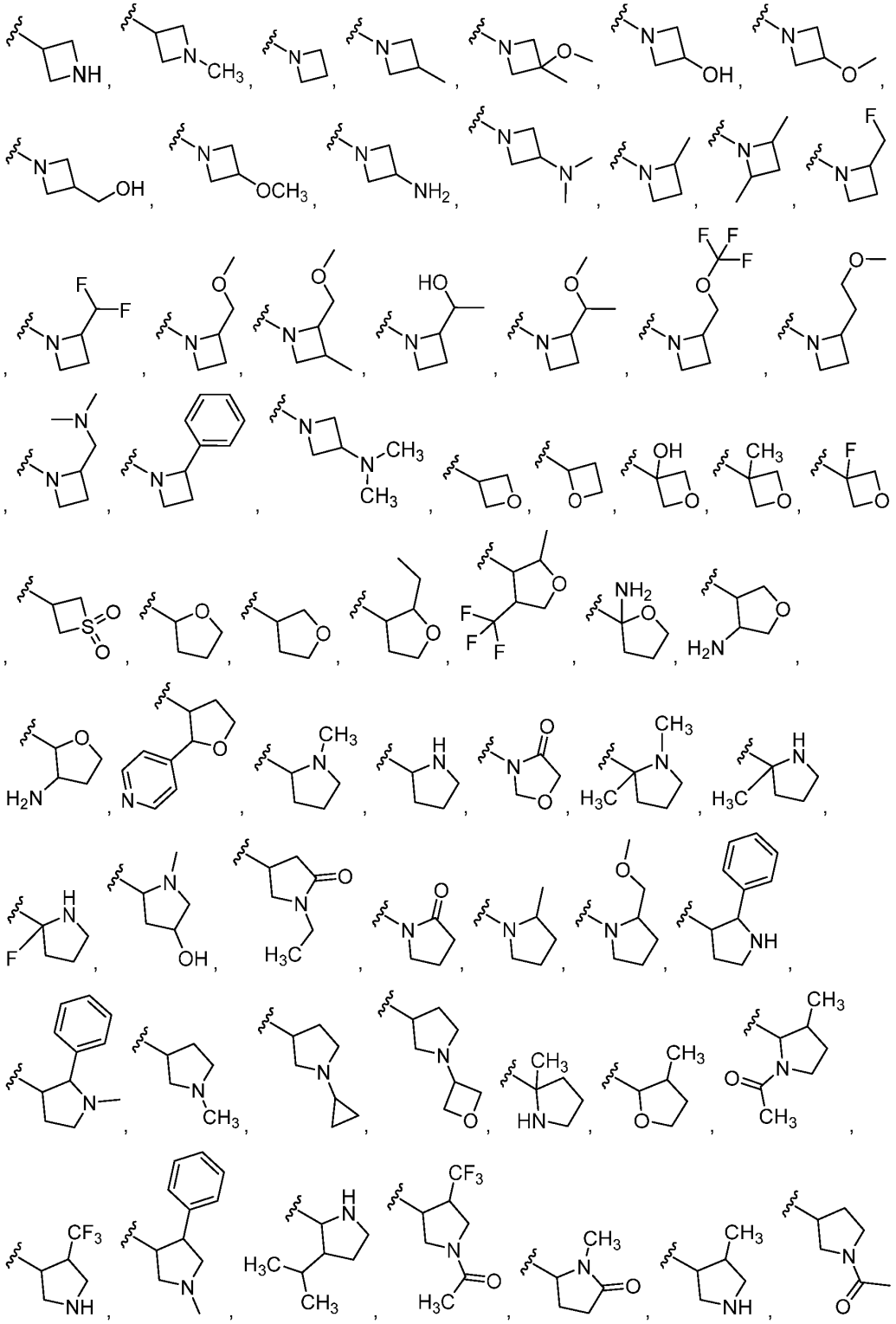
[85] The compound of any one of paragraphs [1] to [84], or pharmaceutically acceptable salt thereof, wherein W is C_1-C_4 alkyl.

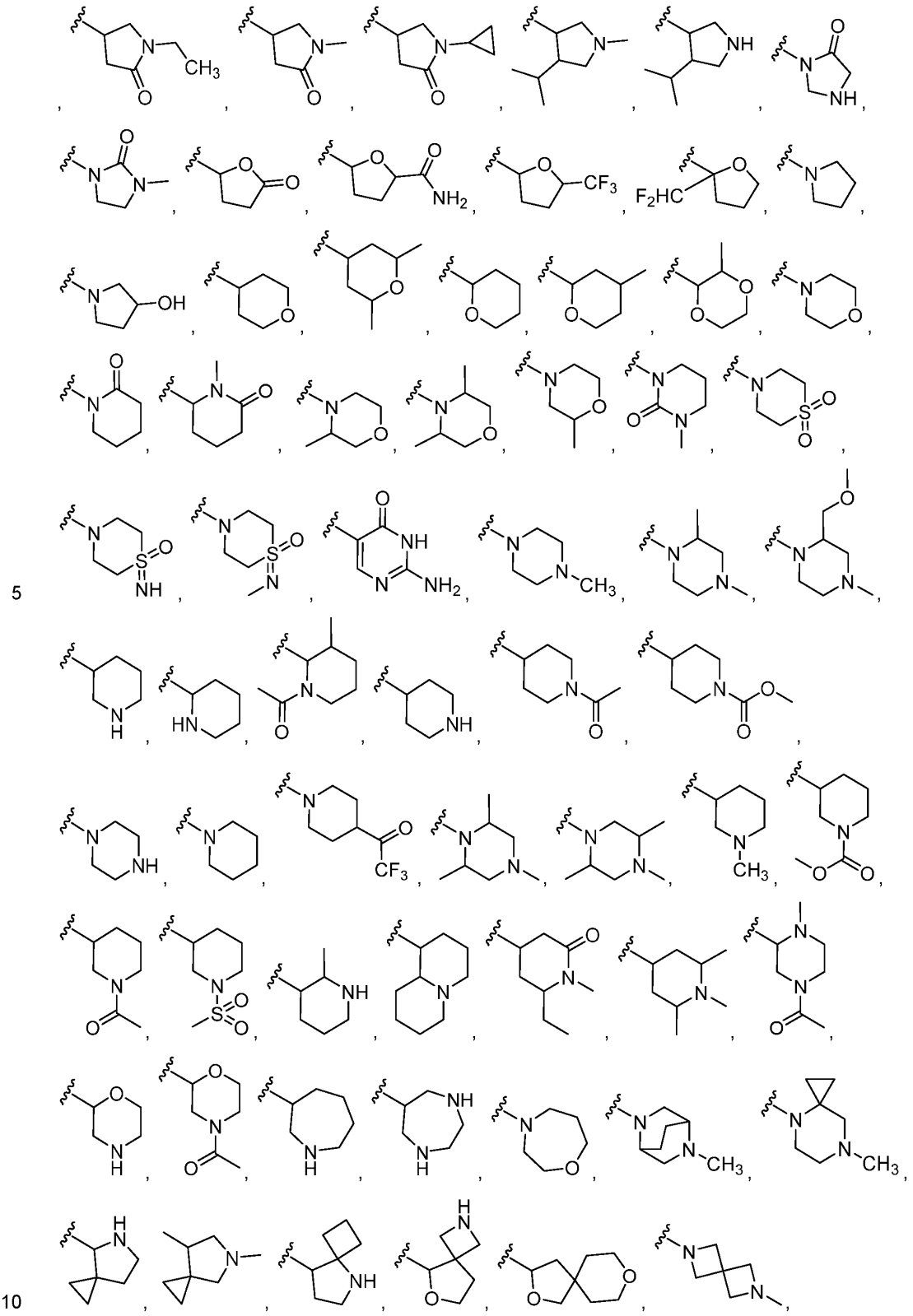
[86] The compound of any one of paragraphs [1] to [84], or pharmaceutically acceptable salt thereof, wherein W is optionally substituted cyclopropyl, optionally substituted cyclobutyl, optionally substituted cyclopentyl, or optionally substituted cyclohexyl, optionally substituted piperidine, optionally substituted piperazine, optionally substituted pyridine, or optionally substituted phenyl.

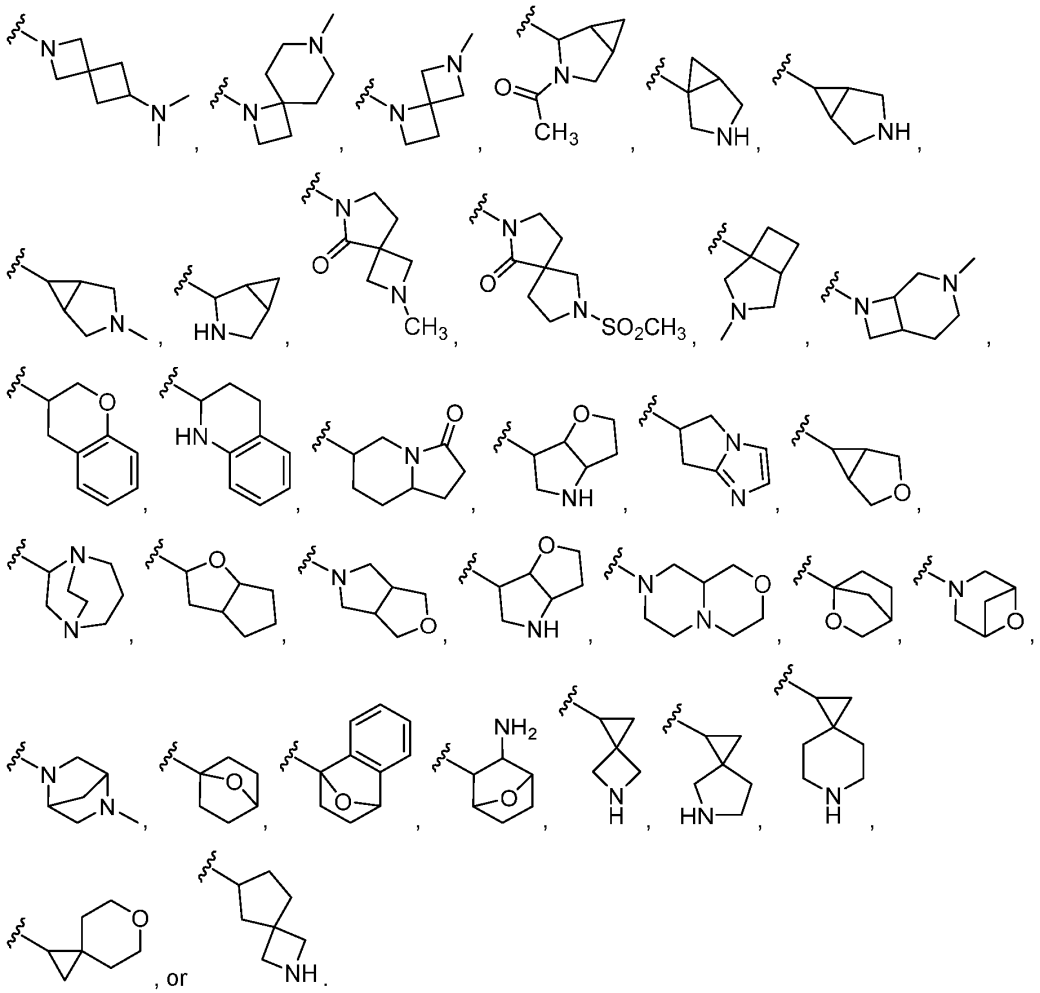
[87] The compound of any one of paragraphs [1] to [84], or pharmaceutically acceptable salt thereof, wherein W is optionally substituted 3 to 10-membered heterocycloalkyl, optionally substituted 3 to 10-membered cycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl.

[88] The compound of any one of paragraphs [1] to [84], or pharmaceutically acceptable salt thereof, wherein W is optionally substituted 3 to 10-membered heterocycloalkyl.

[89] The compound of paragraph [88], or pharmaceutically acceptable salt thereof, wherein W is selected from the following, or a stereoisomer thereof:

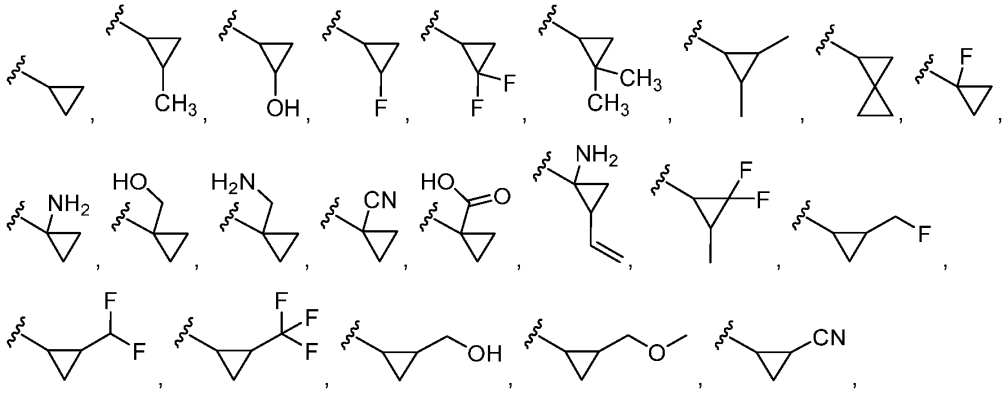


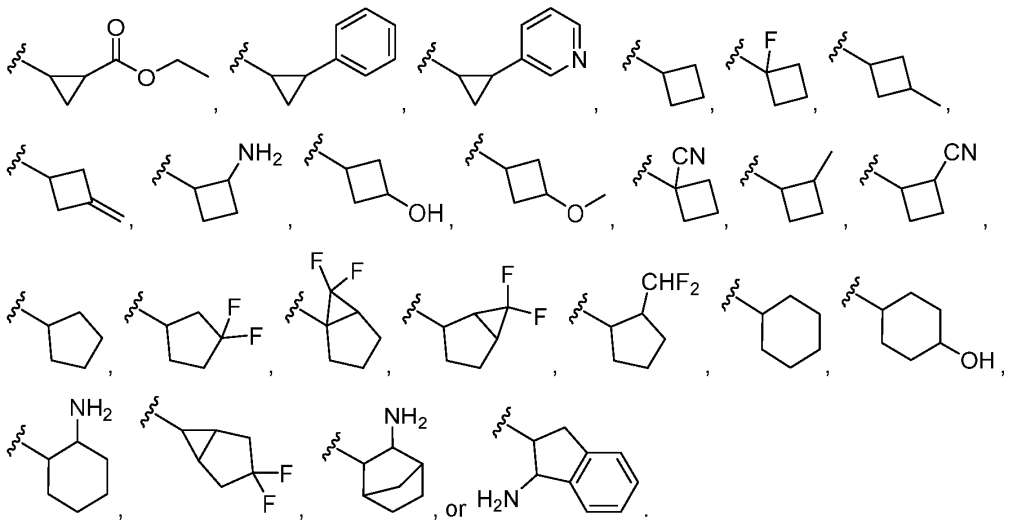




[90] The compound of any one of paragraphs [1] to [84], or pharmaceutically acceptable salt thereof, wherein W is optionally substituted 3 to 10-membered cycloalkyl.

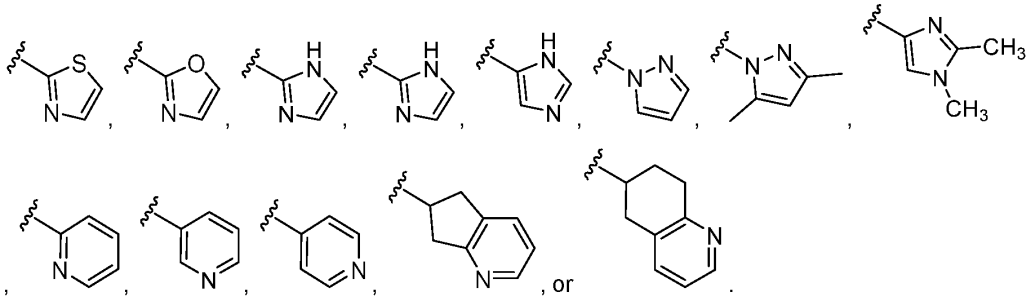
[91] The compound of paragraph [90], or pharmaceutically acceptable salt thereof, wherein W is selected from the following, or a stereoisomer thereof:





[92] The compound of any one of paragraphs [1] to [84], or pharmaceutically acceptable salt thereof, wherein W is optionally substituted 5 to 10-membered heteroaryl.

[93] The compound of paragraph [92], or pharmaceutically acceptable salt thereof, wherein W is selected from the following, or a stereoisomer thereof:



[94] The compound of any one of paragraphs [1] to [84], or pharmaceutically acceptable salt thereof, wherein W is optionally substituted 6 to 10-membered aryl.

[95] The compound of paragraph [94], or pharmaceutically acceptable salt thereof, wherein W is optionally substituted phenyl.

[96] A compound, or a pharmaceutically acceptable salt thereof, of Table 1.

[97] A compound, or a pharmaceutically acceptable salt thereof, of Table 1-1.

[98] A pharmaceutical composition comprising a compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [97] and a pharmaceutically acceptable excipient.

FIG. 1A

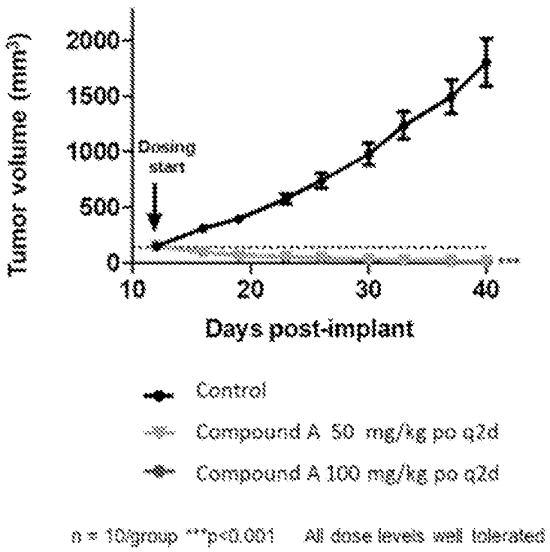

Tumor volume (mm³)
2000
1500
1000
500
0
Dosing start
10
20
30
40
Days post-implant
Control
Compound A 50 mg/kg po q2d
Compound A 100 mg/kg po q2d
n = 10/group ***p<0.001 All dose levels well tolerated

FIG. 1B

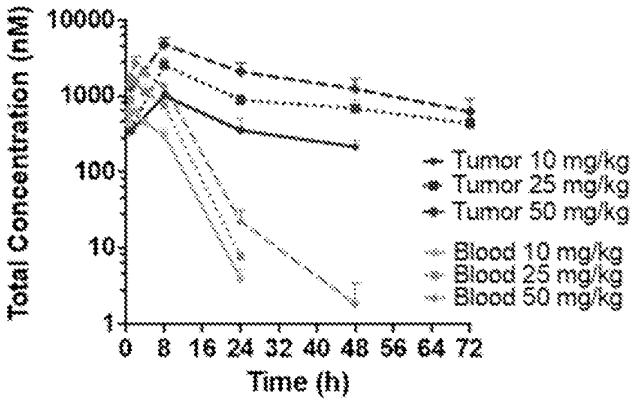

Total Concentration (nM)
10000
1000
100
10
1
0 8 16 24 32 40 48 56 64 72
Time (h)
Tumor 10 mg/kg
Tumor 25 mg/kg
Tumor 50 mg/kg
Blood 10 mg/kg
Blood 25 mg/kg
Blood 50 mg/kg

FIG. 1C

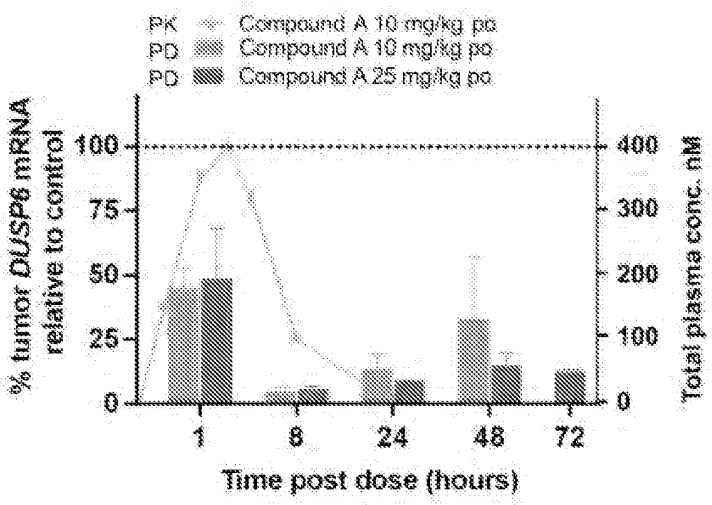

PK Compound A 10 mg/kg po
PD Compound A 10 mg/kg po
PD Compound A 25 mg/kg po
% tumor DUSP6 mRNA relative to control
Total plasma conc. nM
100
75
50
25
0
400
300
200
100
0
1
8
24
48
72
Time post dose (hours)

FIG. 1D

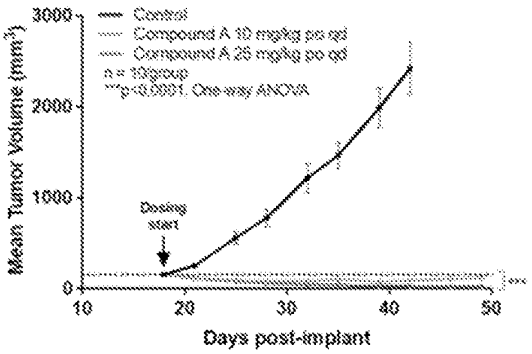

Control
Compound A 10 mg/kg po qd
Compound A 25 mg/kg po qd
n = 10/group
***p<0.0001, One-way ANOVA
Dosing start
Mean Tumor Volume (mm3)
3000
2000
1000
0
10
20
30
40
50
Days post-implant

FIG. 1E

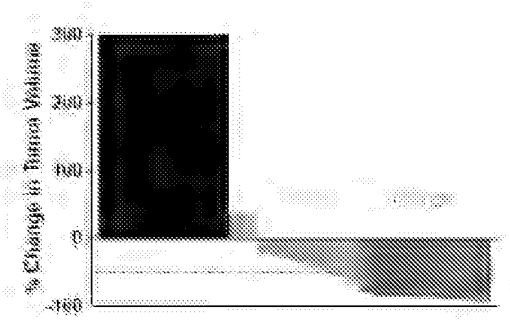

% Change in Tumor Volume
300
200
100
0
-100

FIG. 1F

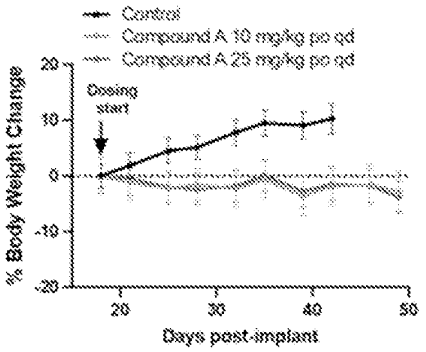

Control
Compound A 10 mg/kg po qd
Compound A 25 mg/kg po qd
Dosing start
% Body Weight Change
20
10
0
-10
-20
20
30
40
50
Days post-implant

FIG. 2A

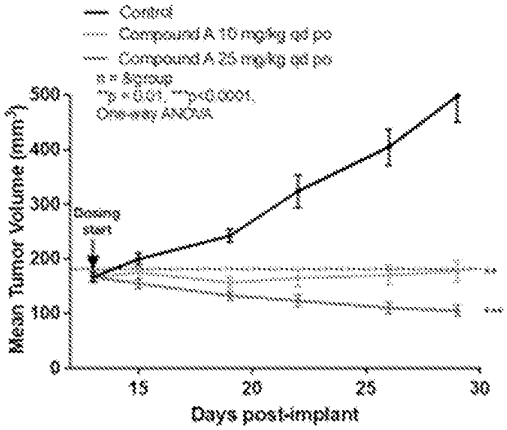

Control
Compound A 10 mg/kg qd po
Compound A 25 mg/kg qd po
n = 8/group
*p = 0.01, **p<0.0001,
One-way ANOVA
Mean Tumor Volume (mm³)
500
400
300
200
100
0
Dosing start
15
20
25
30
Days post-implant

FIG. 2B

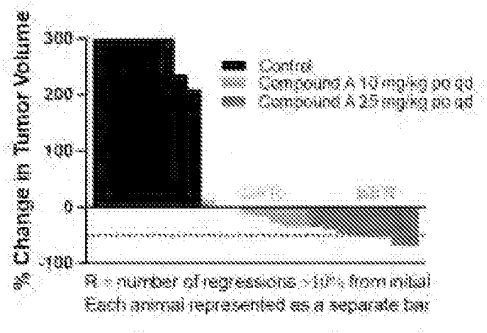

% Change in Tumor Volume
300
200
100
0
-100
Control
Compound A 10 mg/kg po qd
Compound A 25 mg/kg po qd
R = number of regressions >(0%) from initial
Each animal represented as a separate bar

FIG. 2C

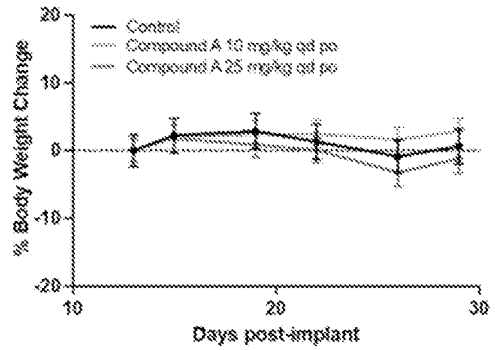

Control
Compound A 10 mg/kg qd po
Compound A 25 mg/kg qd po
% Body Weight Change
20
10
0
-10
-20
10
20
30
Days post-implant

FIG. 2D

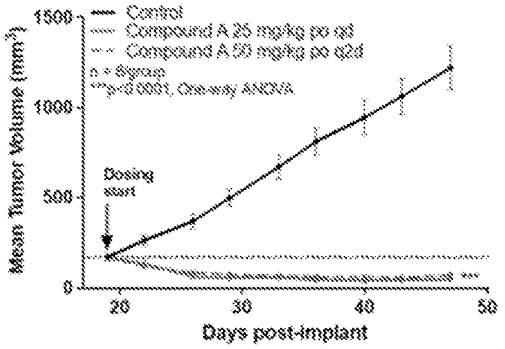

Control
Compound A 25 mg/kg po qd
Compound A 50 mg/kg po q2d
n = 8/group
***p<0.0001, One-way ANOVA
Mean Tumor Volume (mm³)
1500
1000
500
0
Dosing start
20
30
40
50
Days post-implant

FIG. 2E

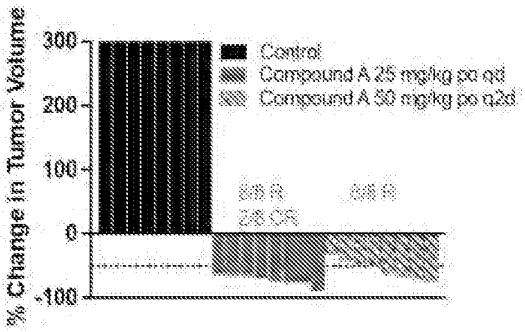

% Change in Tumor Volume
300
200
100
0
-100
Control
Compound A 25 mg/kg po qd
Compound A 50 mg/kg po q2d
6/8 R
2/8 CR
6/8 R

FIG. 2F

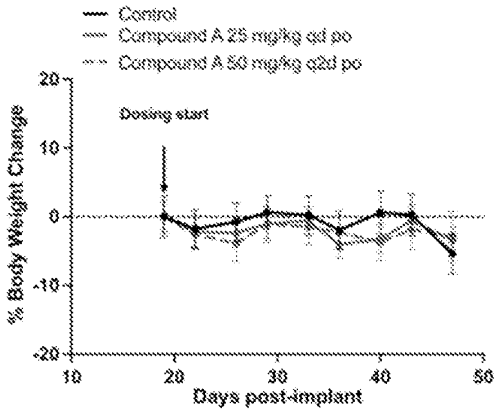

Control
Compound A 25 mg/kg qd po
Compound A 50 mg/kg q2d po
Dosing start
% Body Weight Change
20
10
0
-10
-20
10
20
30
40
50
Days post-implant

FIG. 3

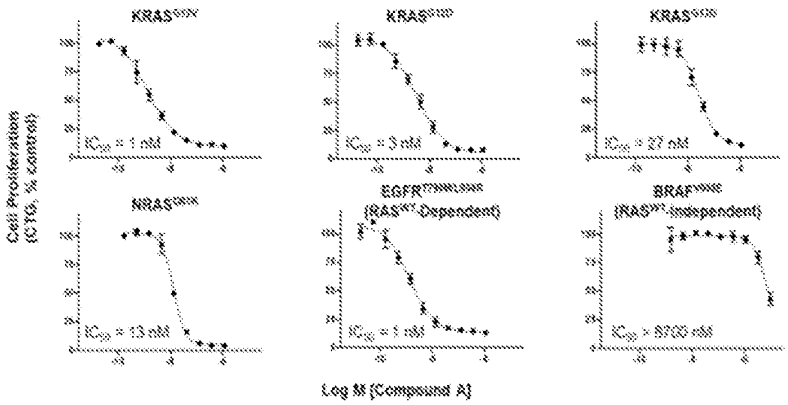

Cell Proliferation (CTG, % control)
Log M [Compound A]

FIG. 4A

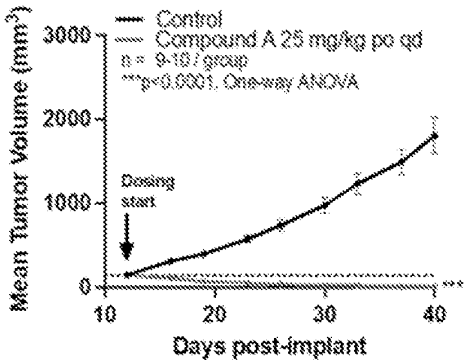

Control
Compound A 25 mg/kg po qd
n = 9-10 / group
***p<0.0001, One-way ANOVA
Dosing start
Mean Tumor Volume (mm³)
Days post-implant
3000
2000
1000
0
10
20
30
40

FIG. 4B

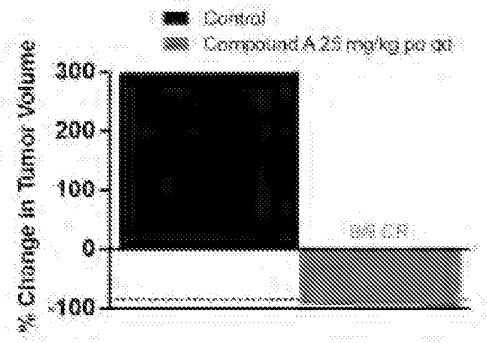

Control
Compound A 25 mg/kg po qd
% Change in Tumor Volume
300
200
100
0
-100

FIG. 4C

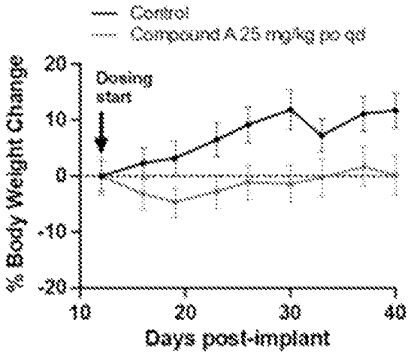

Control
Compound A 25 mg/kg po qd
% Body Weight Change
Dosing start
20
10
0
-10
-20
10
20
30
40
Days post-implant

FIG. 4D

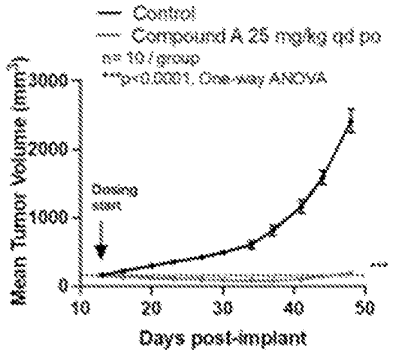

Control
Compound A 25 mg/kg qd po
n= 10 / group
**p<0.0001, One-way ANOVA
Mean Tumor Volume (mm3)
3000
2000
1000
0
Dosing start
10
20
30
40
50
Days post-implant

FIG. 4E

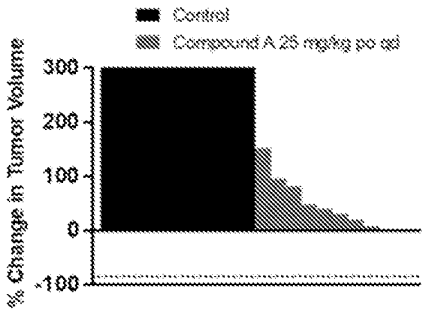

Control
Compound A 25 mg/kg po qd
% Change in Tumor Volume
300
200
100
0
-100

FIG. 4F

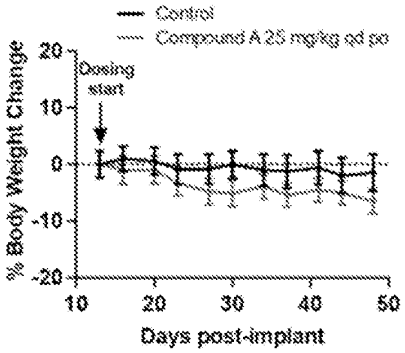

Control
Compound A 25 mg/kg qd po
Dosing start
% Body Weight Change
20
10
0
-10
-20
10
20
30
40
50
Days post-implant

FIG. 5A

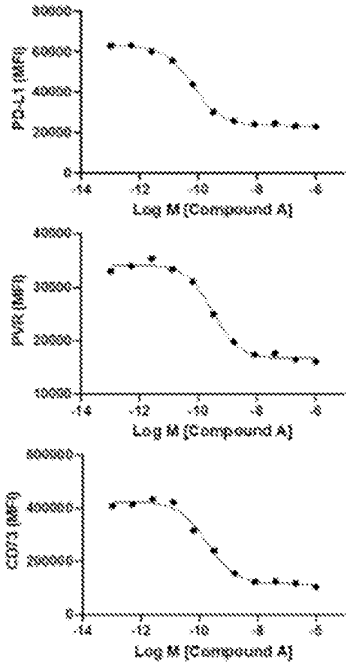

PD-L1 (MFI)
Log M [Compound A]
PVR (MFI)
Log M [Compound A]
CD73 (MFI)
Log M [Compound A]

FIG. 5B

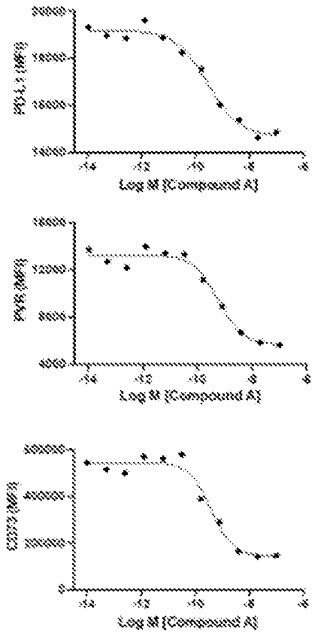

Log M [Compound A]
Log M [Compound A]
Log M [Compound A]

FIG. 5C

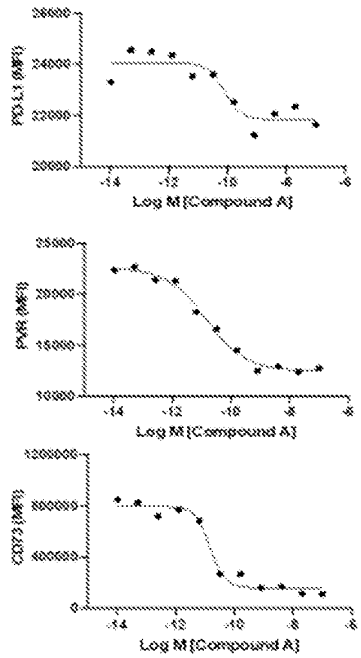

Log M [Compound A]
Log M [Compound A]
Log M [Compound A]

FIG. 6A

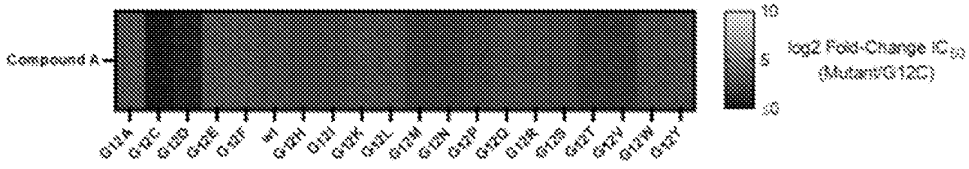


FIG. 6B

	G12A	G12C	G12D	G12E	G12F	wt	G12H	G12I	G12K	G12L	G12M	G12N	G12P	G12Q	G12R	G12S	G12T	G12V	G12W	G12Y
Compound A	1.72	0.00	0.23	1.02	1.50	1.44	1.02	1.20	1.47	1.40	0.87	0.88	1.50	1.38	1.33	1.04	0.78	0.70	1.38	1.54

We Claim:

1. A method of treating a subject having a disease or disorder associated with cells having a SHP2 mutation, the method comprising:

administering to the subject a therapeutically effective amount of a SOS1 inhibitor.

2. The method of claim 1, wherein the SHP2 mutation induces an activated form of SHP2.

3. The method of claim 1, wherein the subject expressed the SHP2 mutation after prior treatment with a SHP2 inhibitor.

4. The method of claim 1, wherein the subject expressed the SHP2 mutation after prior treatment with an allosteric SHP2 inhibitor.

5. The method of claim 3 or claim 4, wherein the SHP2 inhibitor or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof is selected from those disclosed in WO 2021149817, WO 2021148010, WO 2021147879, WO 2021143823, WO 2021143701, WO 2021143680, WO2021121397, WO 2021119525, WO 2021115286, WO 2021110796, WO 2021088945, WO 2021073439, WO 2021061706, WO 2021061515, WO 2021043077, WO 2021033153, WO 2021028362, WO 2021033153, WO 2021028362, WO 2021018287, WO 2020259679, WO 2020249079, WO 2020210384, WO 2020201991, WO 2020181283, WO 2020177653, WO 2020165734, WO 2020165733, WO 2020165732, WO 2020156243, WO 2020156242, WO 2020108590, WO 2020104635, WO 2020094104, WO 2020094018, WO 2020081848, WO 2020073949, WO 2020073945, WO 2020072656, WO 2020065453, WO 2020065452, WO 2020063760, WO 2020061103, WO 2020061101, WO 2020033828, WO 2020033286, WO 2020022323, WO 2019233810, WO 2019213318, WO 2019183367, WO 2019183364, WO 2019182960, WO 2019167000, WO 2019165073, WO 2019158019, WO 2019152454, WO 2019051469, WO 2019051084, WO 2018218133, WO 2018172984, WO 2018160731, WO 2018136265, WO 2018136264, WO 2018130928, WO 2018129402, WO 2018081091, WO 2018057884, WO 2018013597, WO 2017216706, WO 2017211303, WO 2017210134, WO 2017156397, WO 2017100279, WO 2017079723, WO 2017078499, WO 2016203406, WO 2016203405, WO 2016203404, WO 2016196591, WO 2016191328,

WO 2015107495, WO 2015107494, WO 2015107493, WO 2014176488, WO 2014113584, US 20210085677, US 10858359, US 10934302 and US 10954243.

6. The method of claim 3 or claim 4, wherein the SHP2 inhibitor or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof is selected from the group consisting of ERAS-601, BBP-398, RLY-1971, JAB-3068, JAB-3312, TNO155, SHP099, RMC-4550, and RMC-4630.

7. The method of claim 3 or 4, wherein the SHP2 inhibitor is TNO155 or RMC-4630, or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof.

8. The method of claim 7, wherein the SHP2 inhibitor is RMC-4630, or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof.

9. The method of claim 1, wherein the SHP2 mutation confers resistance to a SHP2 inhibitor or an allosteric SHP2 inhibitor.

10. The method of any one of claims 1 through 9, wherein SHP2 mutation is at a position selected from the group consisting of T52, I56, G60, D61, Y62, Y63, E69, K70, A72, T73, E76, E123, E139, Y197, S189, T253, Q257, L261, L262, R265, F285, N308, V428, A461, T468, P491, S502, G503, M504, Q506, Q510, T507, and a combination thereof.

11. The method of any one of claims 1 through 10, wherein the SHP2 mutation is at a position selected from the group consisting of A72, E76 and G503, and a combination thereof.

12. The method of any one of claims 1 through 9, wherein SHP2 mutation is selected from the group consisting of T52I, I56V, G60V, D61G, D61V, D61Y, Y62D, Y63D, Y63C, E69K, E69Q, K70R, A72S, A72T, A72V, T73I, E76A, E76G, E76K, E76Q, E123D, E139D, S189A, T253M, Q257L, L261F, L261H, L262R, R265Q, F285S, N308D, V428M, A461T, A461G, T468M, P491S, S502L, S502P, G503A, G503V, M504V, Q506P, T507K, Q510P, Q510H, and a combination thereof.

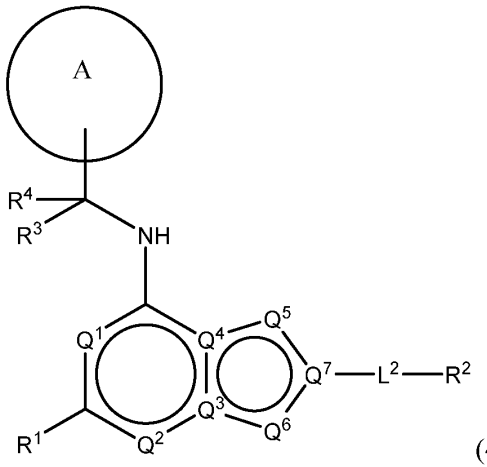
13. The method of any one of claims 1 through 12, wherein SHP2 mutation is selected from the group consisting of G60V, D61G, D61V, E69K, A72S, A72T, A72V,

T73I, E76A, E76G, E76K, E76Q, S189A, L262R, F285S, N308D, T468M, P491S, S502P, G503V, Q506P, T507K, T253M/Q257L, and a combination thereof.

14. The method of any one of claims 1 through 13, wherein SHP2 mutation is selected from the group consisting of A72V, E76K, or G503V, and a combination thereof.

15. The method of any one of claims 1 through 14, wherein the SOS1 inhibitor or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof is selected from those disclosed in WO 2018/115380, WO 2018/172250, WO 2019/122129, and WO 2019/201848.

16. The method of any one of claims 1 through 14, wherein the SOS1 inhibitor is a compound having the structure of Formula (41-I),



(41-I)

or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof, wherein:

Q^1 and Q^2 are independently CH or N;

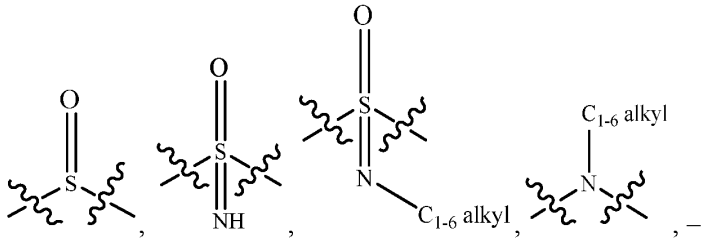
Q^3, Q^4, and Q^7 are independently C or N, wherein at least one of Q^3 and Q^4 is C and wherein Q^3, Q^4, and Q^7 are not all N;

Q^5 is CH, N, NH, O, or S;

Q^6 is CH, N, NH, N-C_{1-6} alkyl, N-C_{1-6} heteroalkyl, N-(3-7 membered cycloalkyl), N-(3-7 membered heterocyclyl), O, or S;

wherein at least one of Q^1, Q^2, Q^3, Q^4, Q^5, Q^6, and Q^7 is N, NH, O, or S;

R^1 is selected from the group consisting of H, C_{1-6} alkyl, halogen, -NHR^{1a}, –OR^{1a}, cyclopropyl, and –CN; wherein C_{1-6} alkyl is optionally substituted with halogen, -NHR^{1a}, or –OR^{1a}; wherein R^{1a} is H, C_{1-6} alkyl, 3-6 membered heterocyclyl, or C_{1-6} haloalkyl;

L^2 is selected from the group consisting of a bond, –C(O)–, –C(O)O–, –C(O)NH$(CH_2)_o$–, –$S(O)_2$–,  , –C(O)$(CH_2)_p$–, –$(CH_2)_p$–, and –O–; wherein o is 0, 1, or 2; and wherein p is a number from 1 to 6;

R^2 is selected from the group consisting of H, C_{1-6} alkyl, C_{2-6} alkenyl, -$NR^{2b}R^{2c}$, -OR^{2a}, 3-14 membered cycloalkyl, 3-14 membered cycloalkenyl, 3-14 membered heterocyclyl, 6-10 membered aryl, and 5-10 membered heteroaryl; wherein each C_{1-6} alkyl, C_{2-6} alkenyl, 3-14 membered cycloalkyl, 3-14 membered cycloalkenyl, 3-14 membered heterocyclyl, 6-10 membered aryl, and 5-10 membered heteroaryl are independently optionally substituted with C_{1-6} alkyl, C_{1-6} haloalkyl, –OH, -OR^{2a}, oxo, halogen, –C(O)R^{2a}, –C(O)OR^{2a}, –C(O)$NR^{2b}R^{2c}$, –CN, -$NR^{2b}R^{2c}$, 3-6 membered cycloalkyl, 3-7 membered heterocyclyl, 6-10 membered aryl, or 5-10 membered heteroaryl;

wherein R^{2a} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, 3-7 membered heterocyclyl, or –$(CH_2)_rOCH_3$, wherein r is 1, 2, or 3;

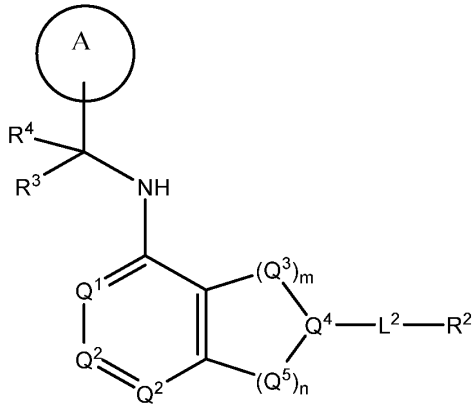
wherein R^{2b} is H or C_{1-6} alkyl;

wherein R^{2c} is H or C_{1-6} alkyl;

R^3 and R^4 are independently H or C_{1-6} alkyl optionally substituted with halo or -OH; wherein at least one of R^3 and R^4 is H or wherein R^3 and R^4 together with the atom to which they are attached combine to form a 3-6 membered cycloalkyl; and

A is an optionally substituted 6-membered aryl or an optionally substituted 5-6 membered heteroaryl.

17. The method of any one of claims 1 through 14, wherein the SOS1 inhibitor is a compound having the structure of Formula (42-I),



(42-I)

or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof, wherein:

Q^1 is CH or N;

Q^4 is CH, C, or N;

each Q^2 is independently C-R^1 or N, wherein one Q^2 is N and the other Q^2 is C-R^1;

each Q^3 and Q^5 are independently $C(R^{QC})_2$, NR^{QN}, CO, O, S, or SO_2, wherein each R^{QC} is independently H, F, Cl, Br, or 6-10 membered aryl, and wherein each R^{QN} is independently H, C_{1-6} alkyl, or 6-10 membered aryl;

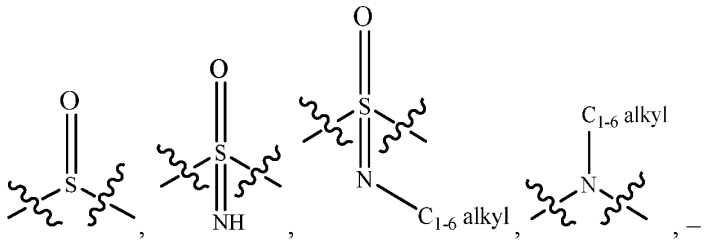
wherein at least one of Q^1, Q^2, Q^3, Q^4, and Q^5 is N, NR^{QN}, O, or SO_2;

m is 0, 1, 2, or 3;

n is 0, 1, 2, or 3;

wherein when m is 0, then n is not 0;

R^1 is selected from the group consisting of H, C_{1-6} alkyl, halogen, -$CONHR^{1a}$, -NHR^{1a}, –OR^{1a}, cyclopropyl, azetidinyl, and –CN; wherein each C_{1-6} alkyl and azetidinyl is optionally substituted with halogen, R^{1a}, -NHR^{1a}, or –OR^{1a}; wherein R^{1a} is H, C_{1-6} alkyl, cyclopropyl, 3-6 membered heterocyclyl, or C_{1-6} haloalkyl;

L^2 is selected from the group consisting of a bond, –C(O)–, –C(O)O–, –C(O)NH(CH_2)$_o$–, –S(O)$_2$–,  , –C(O)(CH_2)$_p$–, –(CH_2)$_p$–, and –O–; wherein o is 0, 1, or 2; and wherein p is a number from 1 to 6;

R^2 is selected from the group consisting of H, C_{1-6} alkyl, -$NR^{2b}R^{2c}$, -OR^{2a}, 3-14 membered cycloalkyl, 3-14 membered cycloalkenyl, 3-14 membered heterocyclyl, 6-10 membered aryl, and 5-10 membered heteroaryl; wherein each C_{1-6} alkyl, 3-14 membered cycloalkyl, 3-14 membered cycloalkenyl, 3-14 membered heterocyclyl, 6-10 membered aryl, and 5-10 membered heteroaryl are independently optionally substituted with C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} hydroxyalkyl, C_{1-6} methoxyalkyl, –OH, –OR^{2a}, oxo, =N, halogen, –C(O)R^{2a}, –C(O)OR^{2a}, –C(O)$NR^{2b}R^{2c}$, -SO_2R^{2a}, –CN, -$NR^{2b}R^{2c}$, 3-6 membered cycloalkyl, 3-7 membered heterocyclyl, 6-10 membered aryl, or 5-10 membered heteroaryl;

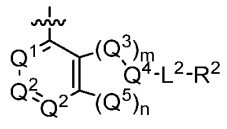
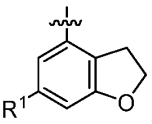
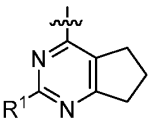
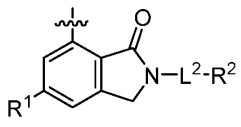
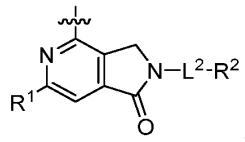
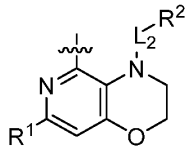
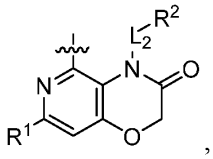
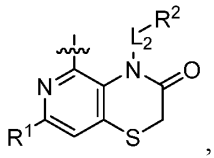
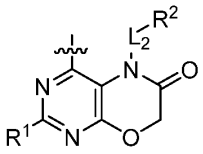
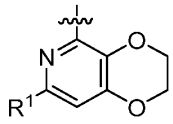
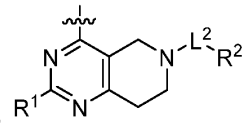
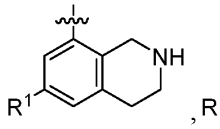
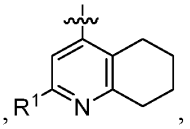
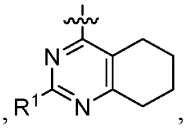
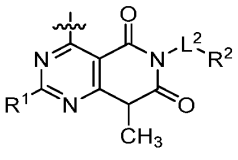
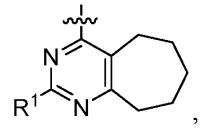
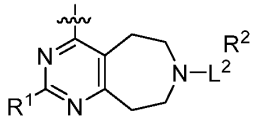
wherein R^{2a} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, 3-7 membered heterocyclyl, or –(CH_2)$_r$$OCH_3$, wherein r is 1, 2, or 3;

wherein R^{2b} is H or C_{1-6} alkyl;

wherein R^{2c} is H or C_{1-6} alkyl;

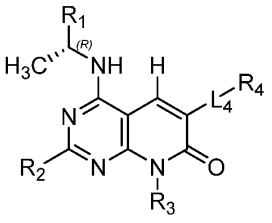
R^3 and R^4 are independently H or C_{1-6} alkyl optionally substituted with halo or -OH; wherein at least one of R^3 and R^4 is H or wherein R^3 and R^4 together with the atom to which they are attached combine to form a 3-6 membered cycloalkyl; and

A is an optionally substituted 6-membered aryl or an optionally substituted 5-6 membered heteroaryl;

with the proviso that when  is  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  , or  ;

then R^1 is not H.

18. The method of any one of claims 1 through 14, wherein the SOS1 inhibitor is a compound having the structure of Formula (48-I),



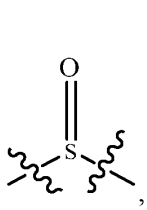
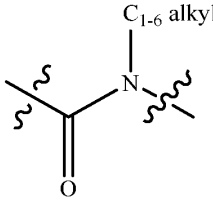
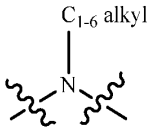
(48-I)

or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof, wherein:

R_1 is selected from the group consisting of optionally substituted 3-6 membered cycloalkyl, optionally substituted 3-6 membered heterocyclyl, optionally substituted 6-membered aryl, and optionally substituted 5-6 membered heteroaryl;

R_2 is selected from the group consisting of H, C_{1-6} alkyl, halogen, -NHR_{2a}, –OR_{2a}, cyclopropyl, and –CN; wherein C_{1-6} alkyl is optionally substituted with halogen, -NHR_{2a}, –OR_{2a}, or 5-6 membered heterocyclyl, and further wherein R_{2a} is selected from the group consisting of H, C_{1-6} alkyl, 3-6 membered heterocyclyl, and C_{1-6} haloalkyl;

R_3 is selected from the group consisting of H, C_{1-3} alkyl, –OR_{3a}, cyclopropyl, and 3-6 membered heterocyclyl, wherein each of C_{1-3} alkyl, cyclopropyl, and 3-6 membered heterocyclyl is optionally substituted with R_{3a}, and further wherein R_{3a} is selected from the group consisting of C_{1-3} alkyl, halogen, –OH, and –CN;

L_4 is selected from the group consisting of bond, –C(O)–, –C(O)O–, –C(O)$NH(CH_2)_o$–, –NH–, –S–, –$S(O)_2$–,  ,  ,  , –$(CH_2)_p$–, and –O–; wherein o is 0, 1, or 2; and wherein p is a number from 1 to 6; and

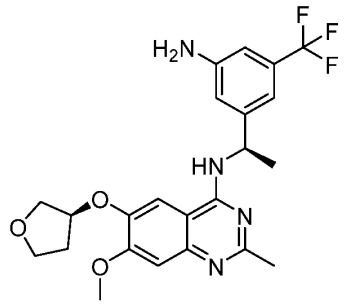
R_4 is selected from the group consisting of H, C_{1-6} alkyl, 3-14 membered cycloalkyl, 3-14 membered cycloalkenyl, 3-14 membered heterocyclyl, 6-10 membered aryl, and 5-10 membered heteroaryl; wherein each C_{1-6} alkyl, 3-14 membered cycloalkyl, 3-14 membered cycloalkenyl, 3-14 membered heterocyclyl, 6-10 membered aryl, and 5-10 membered heteroaryl is optionally substituted with C_{1-6} alkyl, –R_{4a}, –OR_{4a}, –O–C_{1-6} alkyl–R_{4a}, =O, halogen, –C(O)R_{4a}, –C(OO)R_{4a}, –C(O)$NR_{4b}R_{4c}$, –NR_{4b}C(O)R_{4c}, –CN, =NR_{4a}, –$NR_{4b}R_{4c}$, –SO_2R_{4a}, 3-6 membered cycloalkyl, 3-7 membered heterocyclyl, 6-10 membered aryl, or 5-10 membered heteroaryl;

wherein R_{4a} is H, C_{1-6} alkyl, C_{1-6} haloalkyl, –C(O)$NR_{4b}R_{4c}$, 3-6 membered cycloalkyl, 6-10 membered aryl optionally substituted with –OR_{4b}, –CN, 3-7 membered heterocyclyl, –$(CH_2)_rOCH_3$, or –$(CH_2)_rOH$, wherein r is 1, 2, or 3;

wherein each R_{4b} is independently H, C_{1-6} alkyl; and

wherein each R_{4c} is independently H or C_{1-6} alkyl.

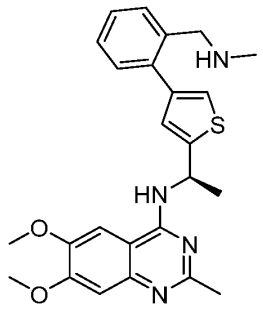
19. The method of any one of claims 1 through 14, wherein the SOS1 inhibitor is BI-3406, having the structure:



or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof.

20. The method of any one of claims 1 through 14, wherein the SOS1 inhibitor is BI-1701963 or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof.

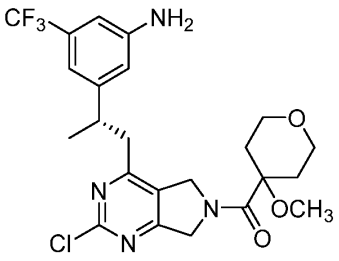
21. The method of any one of claims 1 through 9, wherein the SOS1 inhibitor is BAY-293, having the structure:



or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof.

22. The method of any one of claims 1 through 14, wherein the SOS1 inhibitor is SDGR5 or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof.

23. The method of any one of claims 1 through 14, wherein the SOS1 inhibitor is Compound SOS1-(A) (also called RMC-0331), having the structure:



or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof.

24. The method of any one of claims 1 through 14, wherein the SOS1 inhibitor is Compound SOS1-(B) or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof.

25. The method of any one of claims 1 through 24 further comprising administering to the subject a therapeutically effective amount of a RAS inhibitor.

26. The method of claim 25, wherein the RAS inhibitor is selected from the group consisting of a RAS(ON) inhibitor, a RAS(OFF) inhibitor, MRTX1133 or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof, and a combination thereof.

27. The method of claim 25, wherein the RAS inhibitor is selective for a mutation at position 12 or 13 of a RAS protein.

28. The method of any one of claims 25 through 27, wherein the RAS inhibitor is a RAS(ON) inhibitor.

29. The method of claim 28, wherein the RAS(ON) inhibitor is an inhibitor selective for RAS G12C, RAS G13D, or RAS G12D.

30. The method of claim 28, wherein the RAS(ON) inhibitor is a $RAS(ON)^{MULTI}$ inhibitor.

31. The method of claim 28, wherein the RAS(ON) inhibitor is selected from a compound of any one of Appendices A, B, C, or D, or a compound described by a Formula of any one of Appendices A, B, C, or D, or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof.

32. The method of claim 31, wherein the RAS(ON) inhibitor is a compound described by Formula I in Appendix A, or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof.

33. The method of claim 32, wherein the RAS(ON) inhibitor is selected from a compound of Table 1 or Table 2 of Appendix A, or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof.

34. The method of claim 31, wherein the RAS(ON) inhibitor is a compound described by Formula I in Appendix B, or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof.

35. The method of claim 34, wherein the RAS(ON) inhibitor is selected from a compound of Table 1 or Table 2 of Appendix B, or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof.

36. The method of claim 31, wherein the RAS(ON) inhibitor is a compound described by Formula I in Appendix C, or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof.

37. The method of claim 36, wherein the RAS(ON) inhibitor is selected from a compound of Table 1 or Table 2 of Appendix C, or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof.

38. The method of claim 31, wherein the RAS(ON) inhibitor is a compound described by Formula I in Appendix D, or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof.

39. The method of claim 38, wherein the RAS(ON) inhibitor is selected from a compound of Table 1 or Table 1-1 of Appendix D, or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof.

40. The method of claim 31, wherein the RAS(ON) inhibitor is selected from the group consisting of RAS-(D), RAS-(E), or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof, and a combination thereof.

41. The method of any one of claims 25 through 27, wherein the RAS inhibitor is a RAS(OFF) inhibitor.

42. The method of claim 41, wherein the RAS(OFF) inhibitor selectively targets RAS G12C.

43. The method of claim 42, wherein the RAS(OFF) inhibitor is selected from the group consisting of sotorasib (AMG 510), adagrasib (MRTX849), MRTX1257, JNJ-74699157 (ARS-3248), LY3537982, ARS-853, ARS-1620, GDC-6036, BPI-421286, JDQ443, and JAB-21000, or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof.

44. The method of any one of claims 25 through 27, wherein the RAS inhibitor selectively targets RAS G12D.

45. The method of claim 44, wherein the RAS inhibitor is MRTX1133 or a pharmaceutically acceptable salt, solvate, stereoisomer, prodrug, or tautomer thereof.

46. The method of any one of claims 1 through 24 further comprising administering to the subject a therapeutically effective amount of a MEK inhibitor.

47. The method of claim 46, wherein the MEK inhibitor is pimasertib, selumetinib, cobimetinib, trametinib or binimetinib.

48. The method of any one of claims 1 through 24 further comprising administering to the subject a therapeutically effective amount of a CDK4/6 inhibitor.

49. The method of claim 48, wherein the CDK4/6 inhibitor is abemaciclib, ribociclib, or Palbociclib.

50. The method of any one of claims 1 through 24 further comprising administering to the subject a therapeutically effective amount of a PD-1 inhibitor.

51. The method of claim 50, wherein the PD-1 inhibitor is pembrolizumab, nivolumab, or cemiplimab.

52. The method of any one of claims 1 through 51, wherein the disease or disorder is selected from the group consisting of tumors of hematopoietic and lymphoid system; a myeloproliferative syndrome; a myelodysplastic syndrome; leukemia; acute myeloid leukemia; acute B-lymphoblastic leukemia-lymphoma, juvenile myelomonocytic leukemia; esophageal cancer; breast cancer; lung cancer; colon cancer; gastric cancer;

neuroblastoma; bladder cancer; prostate cancer; glioblastoma; urothelial carcinoma; uterine carcinoma; adenoid and ovarian serous cystadenocarcinoma; paraganglioma; pheochromocytoma; pancreatic cancer; adrenocortical carcinoma; stomach adenocarcinoma; sarcoma; rhabdomyosarcoma; lymphoma; head and neck cancer; skin cancer; peritoneum cancer; intestinal cancer; thyroid cancer; endometrial cancer; cancer of the biliary tract; soft tissue cancer; ovarian cancer; central nervous system cancer; stomach cancer; pituitary cancer; genital tract cancer; urinary tract cancer; salivary gland cancer; cervical cancer; liver cancer; eye cancer; cancer of the adrenal gland; cancer of autonomic ganglia; cancer of the upper aerodigestive tract; bone cancer; testicular cancer; pleura cancer; kidney cancer; penis cancer; parathyroid cancer; cancer of the meninges; vulvar cancer; and melanoma.

53. The method of any one of claims 1 through 51, wherein the disease or disorder is selected from brain glioblastoma, lung adenocarcinoma, colon adenocarcinoma, bone marrow leukemia, acute myelocytic leukemia (AML), breast carcinoma, unknown primary melanoma, non-small cell lung carcinoma, skin melanoma, breast invasive ductal carcinoma, lung squamous cell carcinoma, unknown primary adenocarcinoma, bone marrow multiple myeloma, gastroesophageal junction adenocarcinoma, bone marrow myelodysplastic syndrome, prostate acinar adenocarcinoma, bladder urothelial (transitional cell) carcinoma, uterus endometrial adenocarcinoma, acute B-lymphoblastic leukemia-lymphoma, stomach adenocarcinoma, and unknown primary carcinoma.

54. The method of any one of claims 1 through 51, wherein the disease or disorder is acute myelocytic leukemia (AML).

55. The method of claim 54 further comprising administering to the subject a therapeutically effective amount of an AML therapeutic agent.

56. The method of claim 54 or claim 55, wherein the SHP2 mutation is at a position selected from the group consisting of G60, D61, A72, E76, G503 and S502, and a combination thereof.

57. The method of any one of claims 1 through 51, wherein the disease or disorder is selected from the group consisting of acute myeloid leukemia (AML), lung adenocarcinoma, non-small cell lung carcinoma, brain glioblastoma, a myelodysplastic

syndrome, skin melanoma, breast carcinoma, stomach adenocarcinoma, acute B-lymphoblastic leukemia-lymphoma, and colon adenocarcinoma.

58. The method of any one of claims 1-53 and 57, wherein the SHP2 mutation is at a position selected from the group consisting of G60, D61, E69, A72, E123, Y197, N308, V428, A461, T468, S502, G503, T507, and a combination thereof.

59. The method of any one of claims 1-53 and 58, wherein the SHP2 mutation is selected from the group consisting of G60V, D61G, D61V, D61Y, E69K, E69Q, A72S, A72T, A72V, E123D, N308D, V428M, A461T, A461G, T468M, S502L, S502P, G503A, G503V, T507K, and a combination thereof.

60. The method of any one of claims 1 through 51, wherein the disease or disorder is a RASopathy.

61. The method of any one of claims 1-51 and 60, wherein the SHP2 mutation is at a position selected from the group consisting of T52, I56, Y62, Y63, E69, K70, E139, L261, R265, N308, T468, M504, Q510, and a combination thereof.

62. The method of any one of claims 1-51 and 61, wherein the SHP2 mutation is selected from the group consisting of T52I, I56V, Y62D, Y63D, Y63C, E69K, E69Q, K70R, E139D, L261F, L261H, R265Q, N308D, T468M, M504V, Q510P, Q510H, and a combination thereof.

63. The method of any one of claims 60 through 62, wherein the RASopathy is selected from the group consisting of Neurofibromatosis type 1, Noonan Syndrome, Noonan Syndrome with Multiple Lentigines, Capillary Malformation-Arteriovenous Malformation Syndrome, Costello Syndrome, Cardio-Facio-Cutaneous Syndrome, Legius Syndrome, and Hereditary gingival fibromatosis.

64. The method of any one of claims 1 through 63, further comprising performing a diagnostic test to determine whether the subject has a SHP2 mutation that induces an activated form of SHP2.

65. The method of any one of claims 25 through 64, wherein the RAS inhibitor targets a wild-type RAS protein.

66. The method of any one of claims 25 through 64, wherein the RAS inhibitor targets a RAS protein mutation.

67. The method of claim 66, wherein the RAS protein mutation is at a position selected from the group consisting of G12, G13, Q61, A146, K117, L19, Q22, V14, A59, and a combination thereof.

68. The method of claim 67, wherein the mutation is at a position selected from the group consisting of G12, G13, and Q61, and a combination thereof.

69. The method of claim 68, wherein the mutation is selected from the group consisting of G12C, G12D, G12A, G12S, G12V, G13C, G13D, Q61K, and Q61L, and a combination thereof.

70. The method of any one of claims 25 through 69, wherein the RAS protein is KRAS.