

MACROCYCLIC COMPOUNDS AS KRAS MUTANT INHIBITORS FOR THE TREATMENT OF CANCER

The present invention relates to organic compounds useful for therapy and/or prophylaxis in a mammal, and in particular to inhibition of KRAS mutant useful for treating cancers.

FIELD OF THE INVENTION

RAS is one of the most well-known proto-oncogenes. Approximately 30% of human
5 cancers contain mutations in three most notable members, KRAS, HRAS, and NRAS, making them the most prevalent oncogenic drivers. KRAS mutations are generally associated with poor prognosis especially in colorectal cancer, pancreatic cancer, lung cancers. As the most frequently mutated RAS isoform, KRAS has been intensively studied in the past years. Among the most commonly occurring KRAS alleles (including G12D, G12V, G12C, G13D, G12R, G12A, G12S,
10 Q61H, etc), G12C, G12D and G12V represent more than half of all K-RAS-driven cancers across colorectal cancer (CRC), pancreatic ductal adenocarcinoma (PDAC), lung adenocarcinoma (LUAD). Of note, KRAS wild-type amplifications are also found in around 7% of all KRAS-altered cancers (ovarian, esophagogastric, uterine), ranking among the top alterations.

15 All RAS proteins belong to a protein family of small GTPases that hydrolyze GTP to GDP. KRAS is structurally divided into an effector binding lobe followed by the allosteric lobe and a carboxy-terminal region that is responsible for membrane anchoring. The effector lobe comprises the P-loop, switch I, and switch II regions. The switch I/II loops play a critical role in KRAS downstream signaling through mediating protein-protein interactions with effector
20 proteins that include RAF in the mitogen-activated protein kinase (MAPK) pathway or PI3K in the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) pathway.

KRAS protein switches between an inactive to an active form via binding to GTP and GDP, respectively. Under physiological conditions, the transition between these two states is regulated by guanine nucleotide exchange factors (GEFs), such as Son Of Sevenless Homolog 1
25 (SOS1), or GTPase-activating proteins (GAPs) that involve catalyzing the exchange of GDP for GTP, potentiating intrinsic GTPase activity or accelerating RAS-mediated GTP hydrolysis. In response to extracellular stimuli, the inactive RAS-GDP is converted to active RAS-GTP which directly binds to RAF RAS binding domains (RAF^{RBD}), recruiting RAF kinase family from cytoplasm to membranes, where they dimerize and become active. The activated RAF

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subsequently carries out a chain of phosphorylation reactions to its downstream Mitogen-activated protein kinase (MEK) and extracellular signal-regulated kinase (ERK), and propagates the growth signal. Of the RAF family of protein kinases (three known isoforms ARAF, BRAF, CRAF/RAF1), BRAF is most frequently mutated and remains the most potent activator of MEK.

5 Despite that individual RAS and RAF family members revealed distinct binding preferences, all RAFs possess the conserved RBD for forward transmission of MAPK signaling, frequently used for characterize KRAS inhibition (e.g. KRAS-BRAF^{RBD} herein). For KRAS, mutations at positions 12, 13, 61, and 146 lead to a shift toward the active KRAS form through impairing nucleotide hydrolysis or activating nucleotide exchange, leading to hyper-activation of

10 the MAPK pathway that results in tumorigenesis.

Despite its well-recognized importance in cancer malignancy, continuous efforts in the past failed to develop approved therapies for KRAS mutant cancer until recently, the first selective drug AMG510 has fast approval as second line treatment in KRAS G12C driven non-small cell lung cancer (NSCLC). Nevertheless, the clinical acquired resistance to KRAS G12C inhibitors

15 emerge rigorously with disease progresses after around 6 month of treatment. All of the mutations converge to reactivate RAS–MAPK signaling, with secondary RAS mutants at oncogenic hotspots (e.g. G12/G13/Q61) and within the switch II pocket (e.g. H95, R68, and Y96) have been observed; moreover, over 85% of all KRAS-mutated or wild-type amplified driven cancers still lack novel agents. Altogether, both the myriad of escape mechanism and various

20 oncogenic alleles, highlight the urgent medical need for additional KRAS therapies. As such, we invented oral compounds that target and inhibit KRAS alleles for the treatment of KRAS mutant driven cancers.

SUMMARY OF THE INVENTION

The present invention relates to novel compounds:

- 25 1. A compound which is 1-[4-(dimethylamino)-4-methyl-pent-2-ynoyl]-N-[(1S)-1-[[[(6S,8S,14S)-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1S)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-9,15-dioxo-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]carbamoyl]-2-methyl-propyl]-4-fluoro-*N*-methyl-piperidine-4-carboxamide, or a pharmaceutically acceptable salt thereof.
- 30 2. A compound which is 1-[4-(dimethylamino)-4-methyl-pent-2-ynoyl]-4-fluoro-*N*-[(1S)-1-[[[(7S,13S)-24-fluoro-(20*M*)-20-[2-[(1S)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-17,17-dimethyl-8,14-dioxo-21-(2,2,2-trifluoroethyl)-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[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]carbamoyl]-2-methyl-propyl]-*N*-methyl-piperidine-4-carboxamide, or a pharmaceutically acceptable salt thereof.

3. A compound which is 1-[4-(dimethylamino)-4-methyl-pent-2-ynoyl]-*N*-[(1*S*)-1-[[[(7*S*,13*S*)-21-ethyl-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]-17,17-dimethyl-8,14-dioxo-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacos-1(25),2,5(28),19,22(26),23-hexaen-7-yl]carbamoyl]-2-methyl-propyl]-4-fluoro-*N*-methyl-piperidine-4-carboxamide, or a pharmaceutically acceptable salt thereof.

4. A compound which is 1-[4-(dimethylamino)-4-methyl-pent-2-ynoyl]-*N*-[(1*S*)-1-[[[(7*S*,13*S*)-21-ethyl-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-17,17-dimethyl-8,14-dioxo-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacos-1(25),2,5(28),19,22(26),23-hexaen-7-yl]carbamoyl]-2-methyl-propyl]-4-fluoro-*N*-methyl-piperidine-4-carboxamide, or a pharmaceutically acceptable salt thereof.

The above compounds show good KRAS inhibition for G12C. In one embodiment, compared with reference compound, the compounds of current invention had much improved pharmacokinetic properties and surprisingly better performance in anti-tumor activity in difficult KRAS mutated xenograft tumor model. In another embodiment, the compounds of this invention showed superior cancer cell inhibition and human hepatocyte stability. In addition, the compounds of this invention also show good or improved cytotoxicity and solubility profiles.

BRIEF DESCRIPTION OF THE FIGURE

Figure 1. X-ray crystallographic analysis of compound **G5**.

Figure 2. In vivo H2122 xenograft studies of Example 1 and RMC-6291.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

The term “pharmaceutically acceptable salts” denotes salts which are not biologically or otherwise undesirable. Pharmaceutically acceptable salts include both acid and base addition salts.

The term “pharmaceutically acceptable acid addition salt” denotes those pharmaceutically acceptable salts formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, carbonic acid, phosphoric acid, and organic acids selected from aliphatic, cycloaliphatic, aromatic, araliphatic, heterocyclic, carboxylic, and sulfonic classes of organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, gluconic acid, lactic

acid, pyruvic acid, oxalic acid, malic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, aspartic acid, ascorbic acid, glutamic acid, anthranilic acid, benzoic acid, cinnamic acid, mandelic acid, embonic acid, phenylacetic acid, methanesulfonic acid, ethanesulfonic acid, *p*-toluenesulfonic acid, and salicylic acid.

5 The term “pharmaceutically acceptable base addition salt” denotes those pharmaceutically acceptable salts formed with an organic or inorganic base. Examples of acceptable inorganic bases include sodium, potassium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, and aluminum salts. Salts derived from pharmaceutically acceptable organic nontoxic bases includes salts of primary, secondary, and *tertiary* amines, substituted amines
10 including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-diethylaminoethanol, trimethylamine, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, *N*-ethylpiperidine, and polyamine
15 resins.

 The term “A pharmaceutically active metabolite” denotes a pharmacologically active product produced through metabolism in the body of a specified compound or salt thereof. After entry into the body, most drugs are substrates for chemical reactions that may change their physical properties and biologic effects. These metabolic conversions, which usually affect the
20 polarity of the compounds of the invention, alter the way in which drugs are distributed in and excreted from the body. However, in some cases, metabolism of a drug is required for therapeutic effect.

 The term “therapeutically effective amount” denotes an amount of a compound or molecule of the present invention that, when administered to a subject, (i) treats or prevents the
25 particular disease, condition or disorder, (ii) attenuates, ameliorates or eliminates one or more symptoms of the particular disease, condition, or disorder, or (iii) prevents or delays the onset of one or more symptoms of the particular disease, condition or disorder described herein. The therapeutically effective amount will vary depending on the compound, the disease state being treated, the severity of the disease treated, the age and relative health of the subject, the route and
30 form of administration, the judgement of the attending medical or veterinary practitioner, and other factors.

 The term “pharmaceutical composition” denotes a mixture or solution comprising a therapeutically effective amount of an active pharmaceutical ingredient together with

pharmaceutically acceptable excipients to be administered to a mammal, e.g., a human in need thereof.

The terms “pharmaceutically acceptable excipient”, “pharmaceutically acceptable carrier” and “therapeutically inert excipient” can be used interchangeably and denote any

- 5 pharmaceutically acceptable ingredient in a pharmaceutical composition having no therapeutic activity and being non-toxic to the subject administered, such as disintegrators, binders, fillers, solvents, buffers, tonicity agents, stabilizers, antioxidants, surfactants, carriers, diluents or lubricants used in formulating pharmaceutical products.

10 INHIBITOR OF KRAS

The present invention relates to (i) a compound which is 1-[4-(dimethylamino)-4-methyl-pent-2-ynoyl]-*N*-[(1*S*)-1-[[[(6*S*,8*S*,14*S*)-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-9,15-dioxo-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]carbamoyl]-2-methyl-propyl]-4-fluoro-*N*-methyl-piperidine-4-carboxamide, or a pharmaceutically acceptable salt thereof.

Another embodiment of present invention is (ii) a compound which is 1-[4-(dimethylamino)-4-methyl-pent-2-ynoyl]-4-fluoro-*N*-[(1*S*)-1-[[[(7*S*,13*S*)-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-17,17-dimethyl-8,14-dioxo-21-(2,2,2-trifluoroethyl)-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[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]carbamoyl]-2-methyl-propyl]-*N*-methyl-piperidine-4-carboxamide, or a pharmaceutically acceptable salt thereof.

Another embodiment of present invention is (iii) a compound which is 1-[4-(dimethylamino)-4-methyl-pent-2-ynoyl]-*N*-[(1*S*)-1-[[[(7*S*,13*S*)-21-ethyl-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]-17,17-dimethyl-8,14-dioxo-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[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]carbamoyl]-2-methyl-propyl]-4-fluoro-*N*-methyl-piperidine-4-carboxamide, or a pharmaceutically acceptable salt thereof.

Another embodiment of present invention is (iv) a compound which is 1-[4-(dimethylamino)-4-methyl-pent-2-ynoyl]-*N*-[(1*S*)-1-[[[(7*S*,13*S*)-21-ethyl-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-17,17-dimethyl-8,14-dioxo-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[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]carbamoyl]-2-methyl-propyl]-4-fluoro-*N*-methyl-piperidine-4-carboxamide, or a pharmaceutically acceptable salt thereof.

Another embodiment of present invention is (v) a compound or pharmaceutically acceptable salt according to any one of (i) to (iv) for use as therapeutically active substance.

- 5 Another embodiment of present invention is (vi) a pharmaceutical composition comprising a compound in accordance with any one of (i) to (iv) and a pharmaceutically acceptable excipient.

Another embodiment of present invention is (vii) the use of a compound according to any one of (i) to (iv) for treating a KRAS G12C protein-related disease.

- 10 Another embodiment of present invention is (viii) the use of a compound according to any one of (i) to (iv) for inhibiting RAS interaction with downstream effectors, wherein the downstream effectors are RAF and PI3K.

Another embodiment of present invention is (ix) the use of a compound according to any one of (i) to (iv) for inhibiting the propagating oncogenic MAPK and PI3K signaling.

- 15 Another embodiment of present invention is (x) the use of a compound according to any one of (i) to (iv) for the treatment or prophylaxis of KRAS mutation driven cancers, wherein the cancer is selected from pancreatic cancer, colorectal cancer, lung cancer, esophageal cancer, gallbladder cancer, melanoma ovarian cancer and endometrial cancer.

- Another embodiment of present invention is (xi) the use of a compound according to any one of (i) to (iv) for the treatment or prophylaxis of KRAS mutation driven cancers, wherein the cancer is selected from pancreatic adenocarcinoma, colorectal cancer and non-small cell lung cancer.
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- Another embodiment of present invention is (xii) a compound or pharmaceutically acceptable salt according to any one of (i) to (iv) for the treatment or prophylaxis of KRAS mutation driven cancers, wherein the cancer is selected from pancreatic adenocarcinoma, colorectal cancer and non-small cell lung cancer.
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- Another embodiment of present invention is (xiii) the use of a compound according to any one of (i) to (iv) for the preparation of a medicament for the treatment or prophylaxis of KRAS mutation driven cancers, wherein the cancer is selected from pancreatic adenocarcinoma, colorectal cancer and non-small cell lung cancer.
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Another embodiment of present invention is (xiv) a method for the treatment or prophylaxis of KRAS mutation driven cancers, wherein the cancer is selected from pancreatic adenocarcinoma, colorectal cancer and non-small cell lung cancer, which method comprises

administering a therapeutically effective amount of a compound as defined in any one of (i) to (iv).

PHARMACEUTICAL COMPOSITIONS AND ADMINISTRATION

Another embodiment provides pharmaceutical compositions or medicaments containing the compounds of the invention and a therapeutically inert carrier, diluent or excipient, as well as methods of using the compounds of the invention to prepare such compositions and medicaments. In one example, compounds of formula (I) may be formulated by mixing at ambient temperature at the appropriate pH, and at the desired degree of purity, with physiologically acceptable carriers, i.e., carriers that are non-toxic to recipients at the dosages and concentrations employed into a galenical administration form. The pH of the formulation depends mainly on the particular use and the concentration of compound, but preferably ranges anywhere from about 3 to about 8. In one example, a compound of formula (I) is formulated in an acetate buffer, at pH 5. In another embodiment, the compounds of formula (I) are sterile. The compound may be stored, for example, as a solid or amorphous composition, as a lyophilized formulation or as an aqueous solution.

Compositions are formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The “effective amount” of the compound to be administered will be governed by such considerations, and is the minimum amount necessary to inhibit mutant RAS (e.g. KRAS G12C) interaction with RAF, blocking the oncogenic MAPK signaling. For example, such amount may be below the amount that is toxic to normal cells, or the mammal as a whole.

In one example, the pharmaceutically effective amount of the compound of the invention administered parenterally per dose will be in the range of about 0.1 to 1000 mg/kg, alternatively about 0.1 to 1000 mg/kg of patient body weight per day, with the typical initial range of compound used being 0.3 to 15 mg/kg/day. In another embodiment, oral unit dosage forms, such as tablets and capsules, preferably contain from about 1 to about 1000 mg of the compound of the invention.

The compounds of the invention may be administered by any suitable means, including oral, topical (including buccal and sublingual), rectal, vaginal, transdermal, parenteral, subcutaneous, intraperitoneal, intrapulmonary, intradermal, intrathecal and epidural and

intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration.

The compounds of the present invention may be administered in any convenient administrative form, e.g., tablets, powders, capsules, solutions, dispersions, suspensions, syrups, 5 sprays, suppositories, gels, emulsions, patches, etc. Such compositions may contain components conventional in pharmaceutical preparations, e.g., diluents, carriers, pH modifiers, sweeteners, bulking agents, and further active agents.

A typical formulation is prepared by mixing a compound of the present invention and a carrier or excipient. Suitable carriers and excipients are well known to those skilled in the art and 10 are described in detail in, e.g., Ansel, Howard C., et al., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems. Philadelphia: Lippincott, Williams & Wilkins, 2004; Gennaro, Alfonso R., et al. Remington: The Science and Practice of Pharmacy. Philadelphia: Lippincott, Williams & Wilkins, 2000; and Rowe, Raymond C. Handbook of Pharmaceutical Excipients. Chicago, Pharmaceutical Press, 2005. The formulations may also include one or more buffers, 15 stabilizing agents, surfactants, wetting agents, lubricating agents, emulsifiers, suspending agents, preservatives, antioxidants, opaquing agents, glidants, processing aids, colorants, sweeteners, perfuming agents, flavoring agents, diluents and other known additives to provide an elegant presentation of the drug (i.e., a compound of the present invention or pharmaceutical composition thereof) or aid in the manufacturing of the pharmaceutical product (i.e., 20 medicament).

An example of a suitable oral dosage form is a tablet containing about 1 to 1000 mg of the compound of the invention compounded with about 1 to 1000 mg anhydrous lactose, about 1 to 1000 mg sodium croscarmellose, about 1 to 1000 mg polyvinylpyrrolidone (PVP) K30, and about 1 to 1000 mg magnesium stearate. The powdered ingredients are first mixed together and 25 then mixed with a solution of the PVP. The resulting composition can be dried, granulated, mixed with the magnesium stearate and compressed to tablet form using conventional equipment. An example of an aerosol formulation can be prepared by dissolving the compound, for example 5 to 400mg, of the invention in a suitable buffer solution, e.g. a phosphate buffer, adding a tonicifier, e.g. a salt such sodium chloride, if desired. The solution may be filtered, e.g., using a 30 0.2 micron filter, to remove impurities and contaminants.

An embodiment, therefore, includes a pharmaceutical composition comprising a compound of formula (I), or a stereoisomer or pharmaceutically acceptable salt thereof. In a further embodiment includes a pharmaceutical composition comprising a compound of formula (I), or a

stereoisomer or pharmaceutically acceptable salt thereof, together with a pharmaceutically acceptable carrier or excipient.

Another embodiment includes a pharmaceutical composition comprising a compound of formula (I) for use in the treatment of mutant KRAS-driven cancers. Another embodiment includes a pharmaceutical composition comprising a compound of Formula (I) for use in the treatment of mutant KRAS-driven cancers.

The following composition A and B illustrate typical compositions of the present invention, but serve merely as representative thereof.

10 **Composition A**

A compound of the present invention can be used in a manner known per se as the active ingredient for the production of tablets of the following composition:

	<u>Per tablet</u>
Active ingredient	200 mg
15 Microcrystalline cellulose	155 mg
Corn starch	25 mg
Talc	25 mg
Hydroxypropylmethylcellulose	<u>20 mg</u>
	425 mg

20

Composition B

A compound of the present invention can be used in a manner known per se as the active ingredient for the production of capsules of the following composition:

	<u>Per capsule</u>
25 Active ingredient	100.0 mg
Corn starch	20.0 mg
Lactose	95.0 mg
Talc	4.5 mg
Magnesium stearate	<u>0.5 mg</u>
30	220.0 mg

INDICATIONS AND METHODS OF TREATMENT

The compounds of the invention induce a new binding pocket in KRAS by driving formation of a high affinity tri-complex between KRAS protein and the widely expressed cyclophilin A (CYPA), which inhibit KRAS interaction with downstream effectors, such as RAF and PI3K. Accordingly, the compounds of the invention are useful for inhibiting the propagating
5 oncogenic MAPK and PI3K signaling, reducing cell proliferation, in particular cancer cells. Compounds of the invention are useful for termination of RAS signaling in cells that express RAS mutant, e.g. KRAS mutation driven pancreatic cancer, colorectal cancer, lung cancer, esophageal cancer, gallbladder cancer, melanoma ovarian cancer, endometrial cancer, etc. Alternatively, compounds of the invention are useful for termination of RAS signaling in
10 malignant solid tumor where the oncogenic role of KRAS mutation is reinforced by dysregulation or mutation of effector pathways as MAPK, PI3K-AKT-mTOR (Mammalian target of rapamycin) driven signaling, for targeted therapy in pancreatic adenocarcinoma, colorectal cancer, non-small cell lung cancer, etc.

Another embodiment includes a method of treating or preventing cancer in a mammal in
15 need of such treatment, wherein the method comprises administering to said mammal a therapeutically effective amount of a compound of formula (I), a stereoisomer, tautomer or pharmaceutically acceptable salt thereof.

EXAMPLES

20 The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention.

ABBREVIATIONS

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention.

25 Abbreviations used herein are as follows:

ACN	acetonitrile
aq.	Aqueous
(Boc) ₂ O	Di- <i>tert</i> -butyldicarbonate
(<i>R</i>)-binap	(<i>R</i>)-(+)-2,2'-Bis(diphenylphosphino)-1,1'-binaphthyl
30 CDCl ₃ :	deuterated chloroform
CMPI	2-Chloro-1-methylpyridinium iodide
Cs ₂ CO ₃	Cesium carbonate
DMF:	dimethyl formamide

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	DMSO:	dimethyl sulfoxide
	EDCI:	N-Ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride
	EtOAc or EA:	ethyl acetate
5	HATU:	(1-[Bis(dimethylamino)methylene]-1 <i>H</i> -1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate)
	hr(s):	hour(s)
	HPLC:	high performance liquid chromatography
	HOBt:	<i>N</i> -hydroxybenzotriazole
	[Ir(OMe)(COD)] ₂	(1,5-Cyclooctadiene)(methoxy)iridium(I) dimer
10	MS: (ESI):	mass spectroscopy (electron spray ionization)
	min(s)	minute(s)
	NMM:	<i>N</i> -Methylmorpholine
	NMR:	nuclear magnetic resonance
	NPLC:	Normal phase liquid chromatography
15	Pd(dppf)Cl ₂ :	[1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium(II)
	Pd(dtbpf)Cl ₂ :	[1,1'-Bis(di-tert-butylphosphino)ferrocene]dichloropalladium(II)
	Pd(OH) ₂ :	Palladium hydroxide
	Pd-PESSI-IPent Cl:	[1,3-bis[2,6-bis(1-ethylpropyl)phenyl]-4,5-dichloro-imidazol-2-yl]-dichloro-(3-chloro-1-pyridyl)palladium
20	prep-HPLC:	preparative high performance liquid chromatography
	RT or rt:	room temperature
	sat.:	saturated
	SFC:	supercritical fluid chromatography
	TBAF:	Tetrabutylammonium fluoride
25	TEA:	triethylamine
	TFA:	trifluoroacetic acid
	THF:	tetrahydrofuran
	T ₄ P:	1,3,5,2,4,6-Trioxatriphosphorinane, 2,4,6-tributyl-, 2,4,6-trioxide

30 GENERAL EXPERIMENTAL CONDITIONS

Intermediates and final compounds were purified by flash chromatography using one of the following instruments: i) Biotage SP1 system and the Quad 12/25 Cartridge module. ii) ISCO combi-flash chromatography instrument. Silica gel brand and pore size: i) KP-SIL 60 Å, particle

size: 40-60 μm ; ii) CAS registry NO: Silica Gel: 63231-67-4, particle size: 47-60 micron silica gel; iii) ZCX from Qingdao Haiyang Chemical Co., Ltd, pore: 200-300 or 300-400.

Intermediates and final compounds were purified by preparative HPLC on reversed phase column using XBridgeTM Prep-C18 (5 μm , OBDTM 30 $\times$ 100 mm) column, SunFireTM Prep-C18 (5 μm , OBDTM 30 $\times$ 100 mm) column, Phenomenex Synergi-C18 (10 μm , 25 $\times$ 150 mm) or Phenomenex Gemini-C18 (10 μm , 25 $\times$ 150 mm). Waters AutoP purification System (Sample Manager 2767, Pump 2525, Detector: Micromass ZQ and UV 2487, solvent system: acetonitrile and 0.1% ammonium hydroxide in water; acetonitrile and 0.1% FA in water or acetonitrile and 0.1% TFA in water). Or Gilson-281 purification System (Pump 322, Detector: UV 156, solvent system: acetonitrile and 0.05% ammonium hydroxide in water; acetonitrile and 0.225% FA in water; acetonitrile and 0.05% HCl in water; acetonitrile and 0.075% TFA in water; or acetonitrile and water).

For SFC chiral separation, intermediates were separated by chiral column (Daicel chiralpak IC, 5 μm , 30 $\times$ 250 mm), AS (10 μm , 30 $\times$ 250 mm) or AD (10 μm , 30 $\times$ 250 mm) using Mettler Toledo Multigram III system SFC, Waters 80Q preparative SFC or Thar 80 preparative SFC, solvent system: CO₂ and IPA (0.5% TEA in IPA) or CO₂ and MeOH (0.1% NH₃·H₂O in MeOH), back pressure 100bar, detection UV @ 254 or 220 nm.

LC/MS spectra of compounds were obtained using a LC/MS (WatersTM Alliance 2795-Micromass ZQ, Shimadzu Alliance 2020-Micromass ZQ or Agilent Alliance 6110-Micromass ZQ), LC/MS conditions were as follows (running time 3 or 1.5 mins):

Acidic condition I: A: 0.1% TFA in H₂O; B: 0.1% TFA in acetonitrile;

Acidic condition II: A: 0.0375% TFA in H₂O; B: 0.01875% TFA in acetonitrile;

Basic condition I: A: 0.1% NH₃·H₂O in H₂O; B: acetonitrile;

Basic condition II: A: 0.025% NH₃·H₂O in H₂O; B: acetonitrile;

Neutral condition: A: H₂O; B: acetonitrile.

Mass spectra (MS): generally only ions which indicate the parent mass are reported, and unless otherwise stated the mass ion quoted is the positive mass ion (MH)⁺.

NMR Spectra were obtained using Bruker Avance 400 MHz or 500MHz.

The microwave assisted reactions were carried out in a Biotage Initiator Sixty microwave synthesizer. All reactions involving air-sensitive reagents were performed under an argon or nitrogen atmosphere. Reagents were used as received from commercial suppliers without further purification unless otherwise noted.

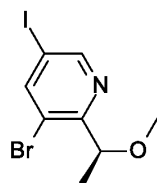
PREPARATIVE EXAMPLES

The following examples are intended to illustrate the meaning of the present invention but should by no means represent a limitation within the meaning of the present invention:

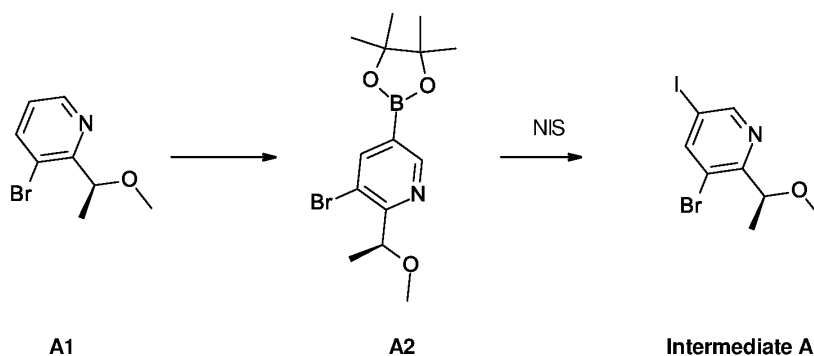
Preparation of Intermediate

5

Intermediate A

3-bromo-5-iodo-2-[(1*S*)-1-methoxyethyl]pyridine

The title intermediate **A** was prepared according to the following scheme:



10 **Step 1: Preparation of 3-bromo-2-[(1*S*)-1-methoxyethyl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (compound A2)**

To a solution of 3-bromo-2-[(1*S*)-1-methoxyethyl]pyridine (compound **A1**, 2.0 g, 9.26 mmol) and bis(pinacolato)diboron (3.5 g, 13.9 mmol) in THF (30 mL) were added 4,4'-di-*tert*-butyl-2,2'-bipyridin (372.7 mg, 1.39 mmol) and [Ir(OMe)(COD)]₂ (306.3 mg, 0.460 mmol). The mixture was stirred at 75 °C for 16 hours under N₂ protection. The mixture was filtrated and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel chromatography (EA/PE: 0-20%) to afford 3-bromo-2-[(1*S*)-1-methoxyethyl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (compound **A2**, 2.4 g) as yellow oil. ¹H NMR (400 MHz, CDCl₃) δ ppm 8.91 (d, *J* = 1.4 Hz, 1 H), 8.21 (d, *J* = 1.4 Hz, 1 H), 4.95 (q, *J* = 6.5 Hz, 1 H), 3.30 (s, 3 H), 1.49 (d, *J* = 6.5 Hz, 3 H), 1.35 (s, 12 H).

15

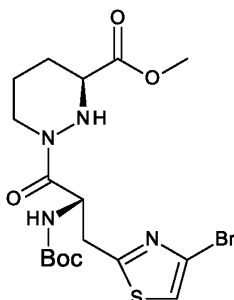
20

Step 2: Preparation of 3-bromo-5-iodo-2-[(1S)-1-methoxyethyl]pyridine (Intermediate A)

To a solution of 3-bromo-2-[(1S)-1-methoxyethyl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (compound **A2**, 2.5 g, 7.3 mmol) in ACN (40 mL) was added N-iodosuccinimide (4.1 g, 18.27 mmol). The mixture was stirred at 90 °C for 40 hrs under N₂ protection. The reaction was quenched with saturated solution of Na₂SO₃ (40 mL) and the reaction mixture was extracted with EtOAc (30 mL, twice). The combined organic layer was washed with brine (50 mL), filtered and the filtrate was concentrated under vacuum. The residue was purified by silica gel chromatography (EA/PE: 0-20%) to afford 3-bromo-5-iodo-2-[(1S)-1-methoxyethyl]pyridine (intermediate **A**, 660 mg) as yellow oil. MS calc'd 342 (MH⁺), measured 341.8 (MH⁺).

Intermediate B

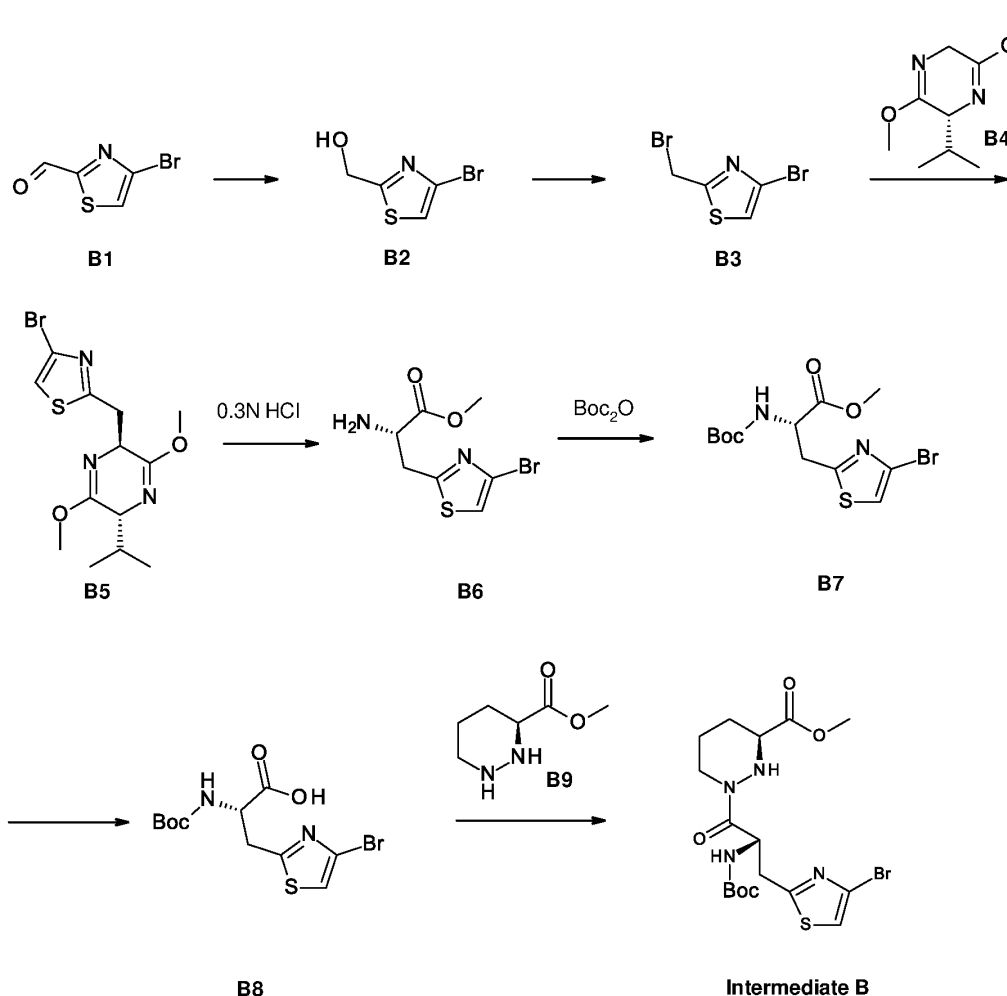
Methyl (3S)-1-[(2S)-3-(4-bromothiazol-2-yl)-2-(*tert*-butoxycarbonylamino)propanoyl]hexahydropyridazine-3-carboxylate



15

The intermediate **B** was prepared according to the following scheme:

-15-



Step 1: Preparation of (4-bromothiazol-2-yl)methanol (compound **B2**)

To a solution of 4-bromothiazole-2-carboxaldehyde (compound **B1**, 6.0 g, 31.25 mmol) in methanol (70 mL) was added sodium borohydride (1.7 g, 46.87 mmol) at 0 °C. The mixture was stirred at 25 °C for 1 hour. The reaction was quenched with water (300 mL) at 0 °C and the reaction mixture was extracted by ethyl acetate (200 mL, three times). The combined organic phase was washed with brine (150 mL, twice), dried over anhydrous sodium sulfate, filtered and the filtrate was concentrated under vacuum to afford (4-bromothiazol-2-yl)methanol (compound **B2**, 6g) as colorless oil.

Step 2: Preparation of 4-bromo-2-(bromomethyl)thiazole (compound **B3**)

To a solution of (4-bromothiazol-2-yl)methanol (compound **B2**, 6.0 g, 30.92 mmol) in DCM (80 mL) was added CBr_4 (15.4 g, 46.38 mmol) and triphenylphosphine (12.1 g, 46.38 mmol) at 0 °C. After being stirred at 25 °C for 1 hour, the mixture was filtered and the filtrate

was concentrated under vacuum. The residue was purified by silica gel column, eluted with ethyl acetate in petroleum ether (0~10%) to afford (4-bromothiazol-2-yl)methanol (compound **B3**, 6.0 g) as yellow oil. MS calc'd 255.9 (MH⁺), measured 255.9 (MH⁺).

- Step 3: Preparation of 4-bromo-2-[(2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl]methylthiazole (compound **B5**)**
- To a mixture of (*R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine (compound **B4**, 4.3 g, 23.45 mmol) in THF (60 mL) was added n-butyllithium (10 mL, 25.22 mmol, 2.5 M) at -78 °C slowly. After addition, the mixture was stirred for 0.5 hour at -78 °C. 4-bromo-2-(bromomethyl)thiazole (compound **B3**, 5.4 g, 21.02 mmol) was added into above mixture at -78 °C which was stirred for another hour. The reaction was quenched with saturated solution of NH₄Cl (100 mL) and the reaction mixture was extracted with EtOAc (100 mL, twice). The combined organic layer was washed with brine (150 mL), dried over anhydrous sodium sulfate, filtered and the filtrate was concentrated under vacuum. The residue was purified by reversed-phase chromatography to afford 4-bromo-2-[(2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl]methylthiazole (compound **B5**, 3.6 g) as yellow oil. MS calc'd 360 (MH⁺), measured 359.9 (MH⁺).

Step 4: Preparation of methyl (2*S*)-2-amino-3-(4-bromothiazol-2-yl)propanoate (compound **B6)**

- To a solution of 4-bromo-2-[(2*S*,5*R*)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl]methylthiazole (compound **B5**, 3.6 g, 10 mmol) in ACN (20 mL) was added hydrochloric acid (66.6 mL, 0.3 M). The mixture was stirred at 25 °C for 2 hours. The mixture was basified by saturated solution of NaHCO₃ until pH=8. The mixture was extracted with EtOAc (80 mL, six times). The combined organic layer was dried over anhydrous sodium sulfate, filtered and the filtrate was concentrated under vacuum to afford methyl (2*S*)-2-amino-3-(4-bromothiazol-2-yl)propanoate (compound **B6**, 3.1 g) as yellow oil. MS calc'd 264.9 (MH⁺), measured 264.9 (MH⁺).

Step 5: Preparation of methyl (2*S*)-3-(4-bromothiazol-2-yl)-2-(*tert*-butoxycarbonylamino)propanoate (compound **B7)**

- To a solution of methyl (2*S*)-2-amino-3-(4-bromothiazol-2-yl)propanoate (compound **B6**, 3.1 g, 11.69 mmol) in DCM (40 mL) were added triethylamine (2.9 g, 29.23 mmol) and (Boc)₂O (3.8 g, 17.54 mmol). After being stirred at 30 °C for 12 hours, the mixture was concentrated under vacuum. The residue was purified by silica gel column, eluted with ethyl acetate in petroleum ether (0~30%) to afford methyl (2*S*)-3-(4-bromothiazol-2-yl)-2-(*tert*-

butoxycarbonylamino)propanoate (compound **B7**, 3.2 g) as yellow oil. MS calc'd 387(MNa⁺), measured 386.9 (MNa⁺).

Step 6: Preparation of (2S)-3-(4-bromothiazol-2-yl)-2-(tert-butoxycarbonylamino)-propanoic acid (compound B8)

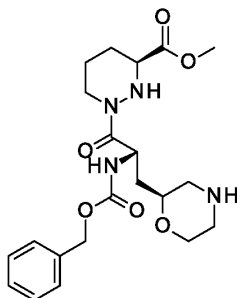
- 5 To a solution of methyl (2S)-3-(4-bromothiazol-2-yl)-2-(tert-butoxycarbonylamino)propanoate (compound **B7**, 3.2 g, 8.76 mmol) in THF (30 mL), methanol (2 mL) and water (10 mL) was added lithium hydroxide (0.4 mL, 43.81 mmol). After being stirred at 25 °C for 1 hour, the reaction mixture was acidified by 1 M solution of HCl until pH=5. The mixture was extracted with EtOAc (40 mL, twice). The combined organic layer was washed
10 with brine (100 mL), dried over anhydrous sodium sulfate, filtered and the filtrate was concentrated under vacuum to afford (2S)-3-(4-bromothiazol-2-yl)-2-(tert-butoxycarbonylamino)propanoic acid (compound **B8**, 3.1 g) as yellow oil. MS calc'd 373(MNa⁺), measured 372.9 (MNa⁺).

- Step 7: Preparation of methyl (3S)-1-[(2S)-3-(4-bromothiazol-2-yl)-2-(tert-butoxycarbonylamino)propanoyl]hexahydropyridazine-3-carboxylate (Intermediate B)**
15 To a solution of (2S)-3-(4-bromothiazol-2-yl)-2-(tert-butoxycarbonylamino)propanoic acid (compound **B8**, 3.1 g, 8.83 mmol) in DCM (50 mL) was added methyl (3S)-hexahydropyridazine-3-carboxylate;hydrochloride (compound **B9**, 2.4 g, 13.24 mmol), EDCI (3.4 g, 17.65 mmol), 1-hydroxybenzotriazole (238.5 mg, 1.77 mmol) and NMM (9.92 mL, 88.26
20 mmol) at 0 °C. After being stirred at 25 °C for 1 hour, the reaction mixture was diluted with water (60 mL) and extracted with EtOAc (60 mL, three times). The combined organic layer was washed with brine (100 mL), dried over anhydrous sodium sulfate, filtered and the filtrate was concentrated under vacuum. The residue was purified by silica gel column and eluted with ethyl acetate in petroleum ether (10~30%) to afford methyl (3S)-1-[(2S)-3-(4-bromothiazol-2-yl)-2-
25 (tert-butoxycarbonylamino)propanoyl]hexahydropyridazine-3-carboxylate (intermediate **B**, 2.4 g). MS calc'd 477(MH⁺), measured 476.9 (MH⁺).

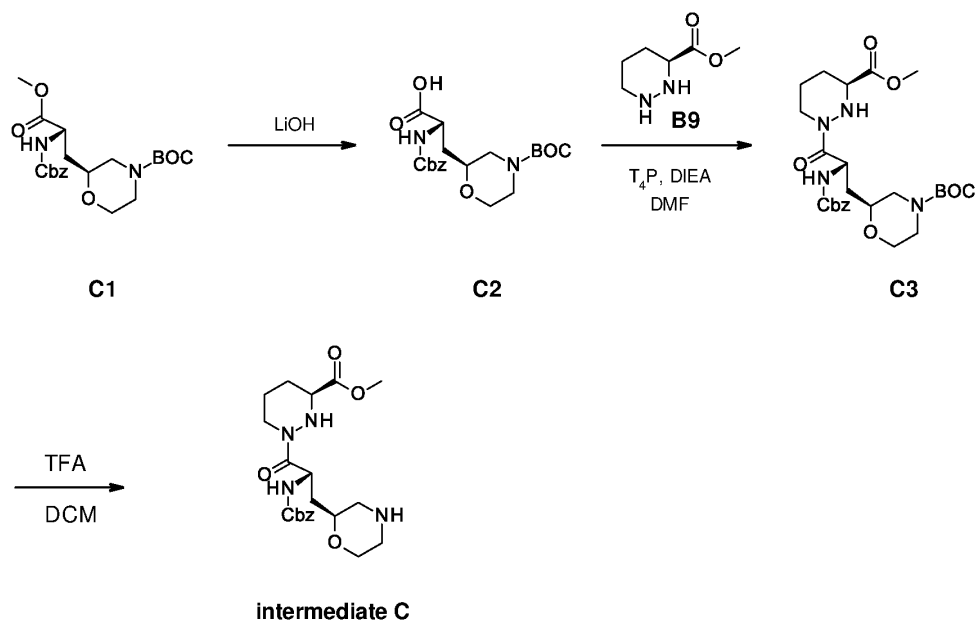
Intermediate C

Methyl (3S)-1-[(2S)-2-(benzyloxycarbonylamino)-3-[(2S)-morpholin-2-yl]propanoyl]hexahydropyridazine-3-carboxylate

-18-



The compound was prepared according to the following scheme:



Step 1: Preparation of (2S)-2-(benzyloxycarbonylamino)-3-[(2S)-4-tert-

5 butoxycarbonylmorpholin-2-yl]propanoic acid (compound C2)

To a solution of *tert*-butyl (2S)-2-[(2S)-2-(benzyloxycarbonylamino)-3-methoxy-3-oxopropyl]morpholine-4-carboxylate (CAS: 2641824-60-2, 20.0 g, 47.34 mmol) in THF (250 mL)/water (50 mL) was added LiOH·H₂O (4.0 g, 94.68 mmol) at 0°C. After being stirred at 25 °C for 1.5 hrs, the reaction mixture was acidified by citric acid aq. until pH=6. The mixture was extracted with EtOAc (200 mL, three times). The combined organic layer was washed with brine (200 mL), dried over anhydrous sodium sulfate, filtered and the filtrate was concentrated under vacuum to afford (2S)-2-(benzyloxycarbonylamino)-3-[(2S)-4-tert-butoxycarbonylmorpholin-2-yl]propanoic acid (compound **C2**, 20.0 g) as a yellow solid. MS calc'd 409.2 (MH⁺), measured 409.2 (MH⁺).

Step 2: Preparation of *tert*-butyl (2*S*)-2-[(2*S*)-2-(benzyloxycarbonylamino)-3-[(3*S*)-3-methoxycarbonylhexahydropyridazin-1-yl]-3-oxo-propyl]morpholine-4-carboxylate (compound C3)

To a mixture of (2*S*)-2-(benzyloxycarbonylamino)-3-[(2*S*)-4-*tert*-
5 butoxycarbonylmorpholin-2-yl]propanoic acid (compound C2, 20.0 g, 48.97 mmol) and methyl (3*S*)-hexahydropyridazine-3-carboxylate; dihydrochloride (compound B9, 21.3 g, 97.93 mmol,) in DMF (300 mL) was added DIEA (42.6 mL, 244.83 mmol) and T4P (70.6 g, 97.93 mmol) at 0 °C. After being stirred at 25 °C for 0.5 h, the reaction mixture was poured into water (300 mL) and extracted with EtOAc (150 mL, three times). The combined organic layer was washed with
10 brine (150 mL, three times), dried over anhydrous Na₂SO₄, filtered and concentrated under vacuum to get a residue. The residue was purified by silica gel chromatography to *tert*-butyl (2*S*)-2-[(2*S*)-2-(benzyloxycarbonylamino)-3-[(3*S*)-3-methoxycarbonylhexahydropyridazin-1-yl]-3-oxo-propyl]morpholine-4-carboxylate (compound C3, 22.7 g) as yellow oil. MS calc'd 535.3 (MH⁺), measured 535.3 (MH⁺).

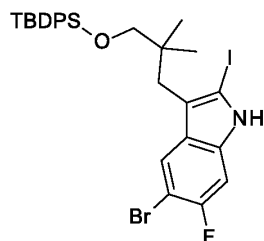
Step 3: Preparation of methyl (3*S*)-1-[(2*S*)-2-(benzyloxycarbonylamino)-3-[(2*S*)-morpholin-2-yl]propanoyl]hexahydropyridazine-3-carboxylate (intermediate C)

To a solution of *tert*-butyl (2*S*)-2-[(2*S*)-2-(benzyloxycarbonylamino)-3-[(3*S*)-3-methoxycarbonylhexahydropyridazin-1-yl]-3-oxo-propyl]morpholine-4-carboxylate (compound C3, 5.0 g, 9.35 mmol) in DCM (20 mL) was added TFA (19.8 mL) at 0 °C. The mixture was
20 stirred at 25 °C for 1 hour. After the reaction was completed, the reaction mixture was concentrated under vacuum to give a residue. Sat. NaHCO₃ aq. (20 mL) was added and the mixture was extracted with EtOAc (30 mL, three times). The combined organic layer was washed with brine (60 mL), dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo* to afford methyl (3*S*)-1-[(2*S*)-2-(benzyloxycarbonylamino)-3-[(2*S*)-morpholin-2-
25 yl]propanoyl]hexahydropyridazine-3-carboxylate (intermediate C, 4.0 g) as a brown solid. MS calc'd 435.2 (MH⁺), measured 435.2 (MH⁺).

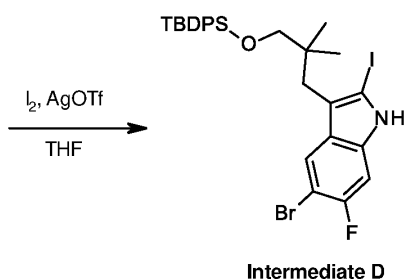
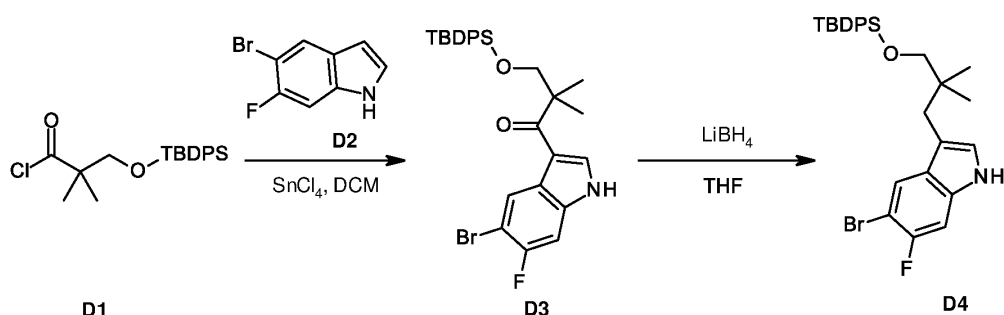
Intermediate D

[3-(5-bromo-6-fluoro-2-iodo-1*H*-indol-3-yl)-2,2-dimethyl-propoxy]-*tert*-butyl-diphenyl-silane

-20-



The compound was prepared according to the following scheme:



Step 1: Preparation of 1-(5-bromo-6-fluoro-1H-indol-3-yl)-3-((tert-butyldiphenylsilyl)

5 oxy)-2,2-dimethylpropan-1-one (compound D3)

To a mixture of 3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropanoyl chloride (compound **D1**, 35.0 g, 116.8 mmol) in DCM (400 mL) at 0 °C was added a solution of SnCl₄ (97.2 mL, 121.5 mmol) slowly. After the mixture was stirred at -40 °C for 0.5 hour, 5-bromo-6-fluoro-1H-indole (compound **D2**, 25.0 g, 116.8 mmol) in DCM (200 mL) was added dropwise and the mixture was stirred at -40 °C for another 15 min. After the reaction was completed, it was quenched with sat. NaHCO₃ aq. (800 mL), and the reaction mixture was extracted with EtOAc (900 mL, twice). The combined organic layer was washed with brine (700 mL), dried over Na₂SO₄, filtered and concentrated *in vacuo*. The residue was triturated in a mixed solvent (100 mL, Petroleum ether: Ethyl acetate = 8:1) and filtered. The collected solid was dried *in*

vacuo to afford 1-(5-bromo-6-fluoro-1*H*-indol-3-yl)-3-((*tert*butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one (compound **D3**, 50.0 g) as a yellow solid. MS calc'd 552.1 (MH⁺), measured 552.1 (MH⁺).

Step 2: Preparation of [3-(5-bromo-6-fluoro-1*H*-indol-3-yl)-2,2-dimethyl-propoxy]-*tert*-butyl-diphenyl-silane (compound **D4)**

To a mixture of 1-(5-bromo-6-fluoro-1*H*-indol-3-yl)-3-((*tert*butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one (compound **D3**, 50.0 g, 90.49 mmol) in THF (600 mL) was added LiBH₄ (48.4 mL, 193.49 mmol, 4 M in THF) dropwise at 0 °C. The mixture was stirred at 70 °C for 24 hrs under nitrogen atmosphere. After the reaction was completed, it was quenched by addition of water (600 mL) at 0 °C slowly and the reaction mixture was extracted with EtOAc (600 mL, twice). The combined organic layer was washed with brine (600 mL), dried over Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by silica column chromatography (EtOAc in PE = 20% ~ 33%) to afford [3-(5-bromo-6-fluoro-1*H*-indol-3-yl)-2,2-dimethyl-propoxy]-*tert*-butyl-diphenyl-silane (compound **D4**, 46.0 g) as a white solid. MS calc'd 538.1 (MH⁺), measured 538.2 (MH⁺).

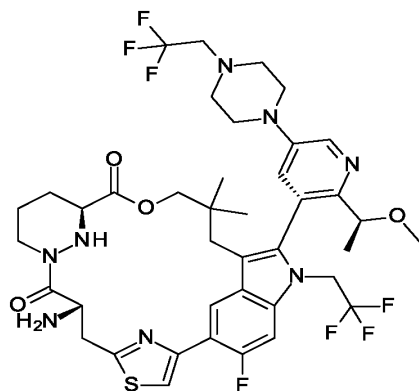
Step 3: Preparation of [3-(5-bromo-6-fluoro-2-iodo-1*H*-indol-3-yl)-2,2-dimethyl-propoxy]-*tert*-butyl-diphenyl-silane (intermediate **D)**

To a mixture of [3-(5-bromo-6-fluoro-1*H*-indol-3-yl)-2,2-dimethyl-propoxy]-*tert*-butyl-diphenyl-silane (compound **D4**, 35.4 g, 65.73 mmol) and iodine (18.4 g, 72.3 mmol) in THF (400 mL) was added silver trifluoromethanesulfonate (20.3 g, 78.88 mmol) at 0 °C. The mixture was stirred at 0 °C for 10 min. After the reaction was completed, it was quenched by sat. Na₂SO₃ aq. (400 mL) and EtOAc (400 mL) and the reaction mixture was filtered. The organic layer was washed with brine (100 mL), dried over Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by silica column chromatography (EtOAc in PE = 0% ~ 2.5%) to afford [3-(5-bromo-6-fluoro-2-iodo-1*H*-indol-3-yl)-2,2-dimethyl-propoxy]-*tert*-butyl-diphenyl-silane (intermediate **D**, 43.0 g) as a yellow solid. MS calc'd 664.0 (MH⁺), measured 664.1 (MH⁺).

Intermediate E

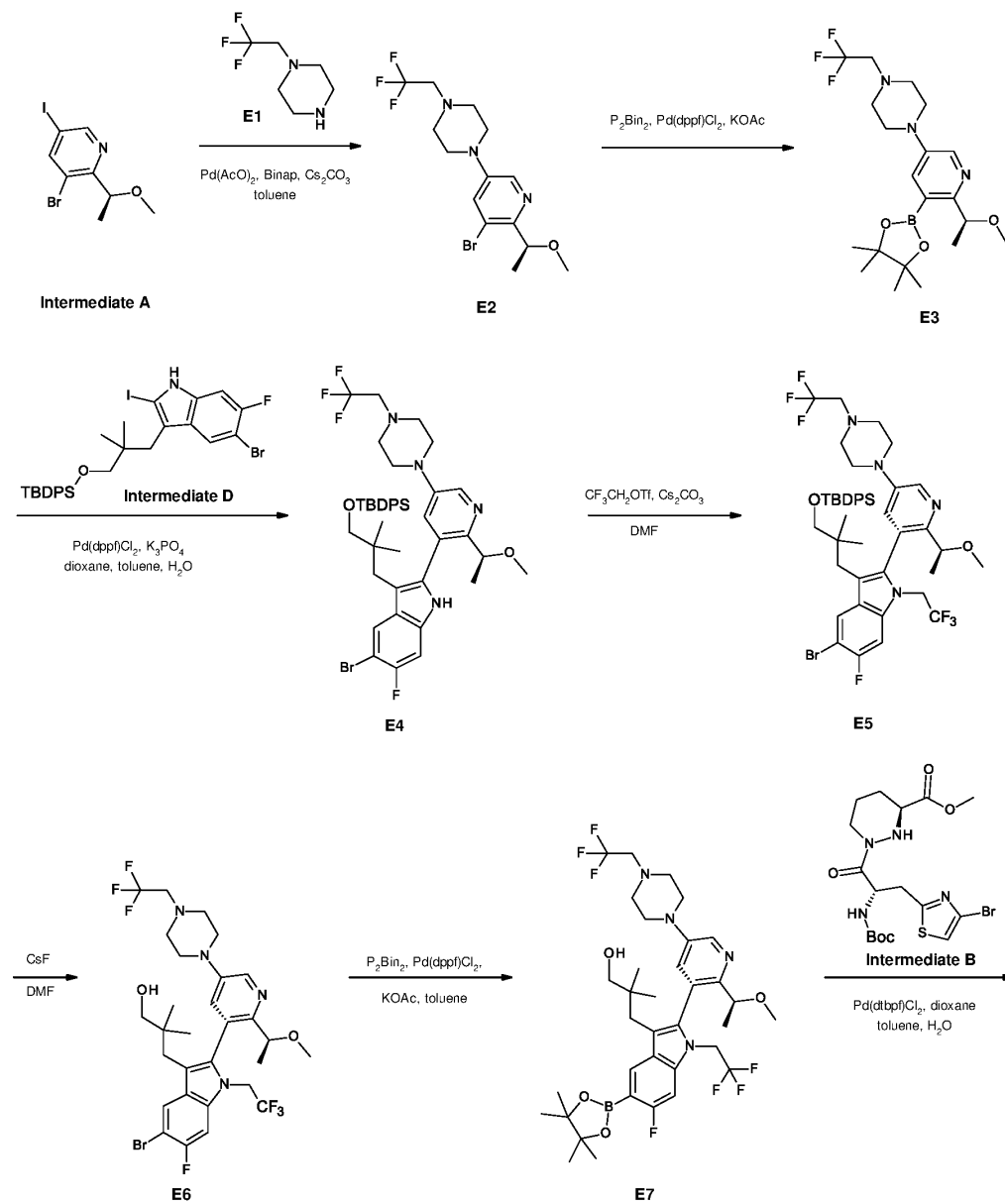
(7*S*,13*S*)-7-amino-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-17,17-dimethyl-21-(2,2,2-trifluoroethyl)-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacos-1(25),2,5(28),19,22(26),23-hexaene-8,14-dione

-22-

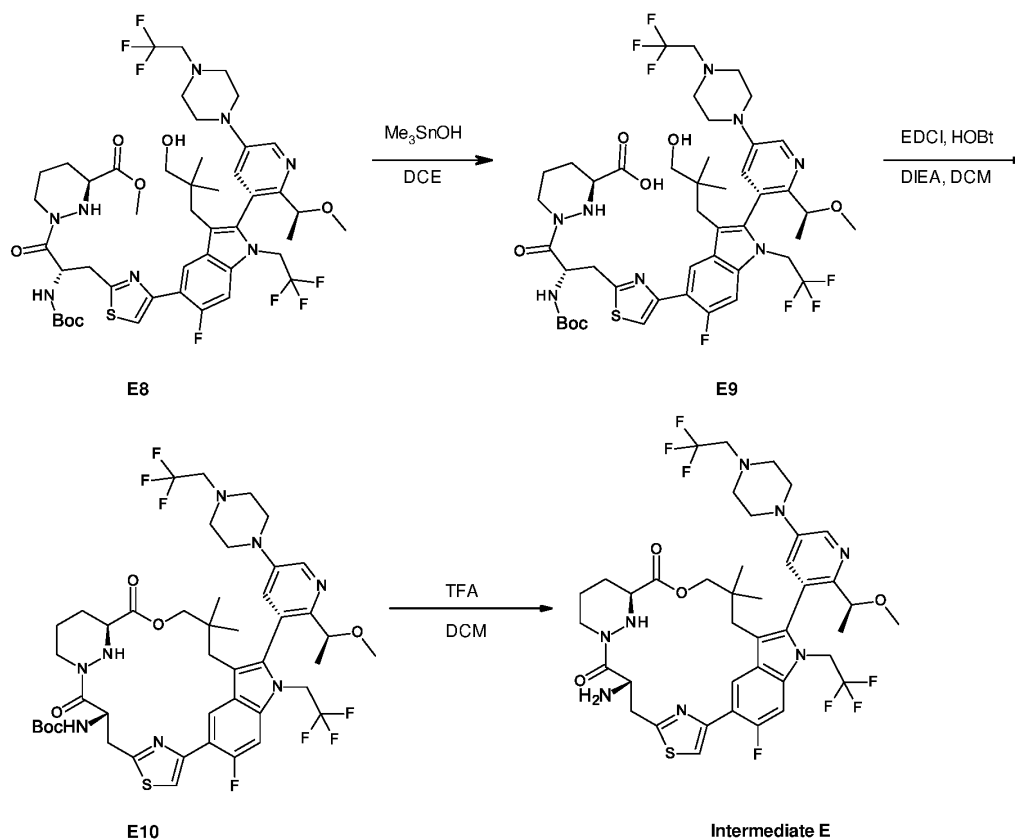


The compound was prepared according to the following scheme:

-23-



-24-



Step 1: Preparation of 1-[5-bromo-6-[(1S)-1-methoxyethyl]-3-pyridyl]-4-(2,2,2-trifluoroethyl)piperazine (compound E2).

To a mixture of 3-bromo-5-iodo-2-[(1S)-1-methoxyethyl]pyridine (intermediate A, 2.03 g, 5.95 mmol) and 1-(2,2,2-trifluoroethyl)piperazine (compound E1, 1.0 g, 5.95 mmol) in toluene (15 mL) were added Cs_2CO_3 (4.85 g, 14.88 mmol), (*R*)-binap (92.6 mg, 0.15 mmol) and $\text{Pd}(\text{OAc})_2$ (66.8 mg, 0.3 mmol). The reaction mixture was degassed and purged with nitrogen for 3 times and the mixture was stirred at 100 °C for 12 hrs under nitrogen atmosphere. After being cooled to room temperature, the reaction mixture was filtered and the filtrate was concentrated *in vacuo* to give a residue. The residue was purified by column chromatography to afford 1-[5-bromo-6-[(1S)-1-methoxyethyl]-3-pyridyl]-4-(2,2,2-trifluoroethyl)piperazine (compound E2, 2.0 g) as yellow oil. MS calc'd 382.2 (MH^+), measured 382.1 (MH^+).

Step 2: 1-[6-[(1S)-1-methoxyethyl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-pyridyl]-4-(2,2,2-trifluoroethyl)piperazine (compound E3).

To a solution of 1-[5-bromo-6-[(1S)-1-methoxyethyl]-3-pyridyl]-4-(2,2,2-trifluoroethyl)piperazine (compound E2, 3.2 g, 8.37 mmol), bis(pinacolato)diboron (3.19 g, 12.56 mmol) and KOAc (2.1 g, 20.93 mmol) in toluene (50 mL) was added $\text{Pd}(\text{dppf})\text{Cl}_2$ (306.3

mg, 0.42 mmol). The mixture was degassed and purged with nitrogen for 3 times and the mixture was stirred at 90 °C for 12 hrs under nitrogen atmosphere. After being cooled to the room temperature, the reaction mixture was filtered, the filtrate was concentrated *in vacuo* to give a residue, which was purified by reversed phase column to afford 1-[6-[(1*S*)-1-methoxyethyl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-pyridyl]-4-(2,2,2-trifluoroethyl)piperazine (compound **E3**, 1.9 g) as a yellow gum. MS calc'd 430.2 (MH⁺), measured 348.4 (M-C₆H₁₀+H⁺).

Step 3: Preparation of [3-[5-bromo-6-fluoro-2-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-1*H*-indol-3-yl]-2,2-dimethyl-propoxy]-*tert*-butyl-diphenyl-silane (compound **E4).**

To a solution of 1-[6-[(1*S*)-1-methoxyethyl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-pyridyl]-4-(2,2,2-trifluoroethyl)piperazine (compound **E3**, 1.9 g, 4.41 mmol), [3-(5-bromo-6-fluoro-2-iodo-1*H*-indol-3-yl)-2,2-dimethyl-propoxy]-*tert*-butyl-diphenyl-silane (intermediate **D**, 2.1 g, 3.15 mmol) in 1,4-dioxane (24 mL), water (8 mL) and toluene (8 mL) was added K₃PO₄ (2.1 g, 9.5 mmol) and Pd(dppf)Cl₂ (231 mg, 0.37 mmol). The mixture was degassed and purged with nitrogen, then stirred at 70 °C for 12 hrs. After being cooled to room temperature, the reaction mixture was filtered. The filtrate was concentrated *in vacuo* to give a residue. The residue was purified by column chromatography (EtOAc in PE : 30% - 60%) to afford [3-[5-bromo-6-fluoro-2-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-1*H*-indol-3-yl]-2,2-dimethyl-propoxy]-*tert*-butyl-diphenyl-silane (compound **E4**, 960.0 mg) as a yellow gum. MS calc'd 839.3 (MH⁺), measured 839.3 (MH⁺).

Step 4: Preparation of [3-[5-bromo-6-fluoro-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-1-(2,2,2-trifluoroethyl)indol-3-yl]-2,2-dimethyl-propoxy]-*tert*-butyl-diphenyl-silane (compound **E5).**

To a solution of [3-[5-bromo-6-fluoro-2-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-1*H*-indol-3-yl]-2,2-dimethyl-propoxy]-*tert*-butyl-diphenyl-silane (compound **E4**, 1 g, 1.14 mmol) in DMF (35 mL) was added Cs₂CO₃ (1.1 g, 3.44 mmol) and 2,2,2-trifluoroethyl trifluoromethanesulfonate (2.7 g, 11.63 mmol) at 0 °C. After being stirred at 20 °C for 15 hrs, the reaction mixture was poured into water (100 mL), and extracted with EtOAc (50 mL, three times). The combined organic was washed with brine (50 mL, three times), dried over Na₂SO₄, filtered and concentrated under vacuum to give a residue which was purified by column chromatography (EtOAc in PE: 30% - 40%) to afford [3-[5-bromo-6-fluoro-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-1-(2,2,2-trifluoroethyl)indol-3-yl]-2,2-dimethyl-propoxy]-*tert*-butyl-diphenyl-silane

(compound **E5**, 640.0 mg, 0.69 mmol, faster eluted) as a white solid. MS calc'd 921.3 (MH⁺), measured 921.4 (MH⁺).

Step 5: Preparation of 3-[5-bromo-6-fluoro-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-1-(2,2,2-trifluoroethyl)indol-3-yl]-2,2-dimethyl-propan-1-ol (compound **E6).**

To a solution of [3-[5-bromo-6-fluoro-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-1-(2,2,2-trifluoroethyl)indol-3-yl]-2,2-dimethyl-propoxy]-*tert*-butyl-diphenyl-silane (compound **E5**, 640.0 mg, 0.69 mmol) in DMF (7 mL) was added cesium fluoride (421.8 mg, 2.78 mmol). The mixture was stirred at 60 °C for 16 hrs. After being cooled to room temperature, the reaction mixture was filtered and the filtrate was concentrated *in vacuo* to give a residue. The residue was purified by column chromatography (EtOAc in PE : 30% - 60%) to afford 3-[5-bromo-6-fluoro-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-1-(2,2,2-trifluoroethyl)indol-3-yl]-2,2-dimethyl-propan-1-ol (compound **E6**, 360.0 mg) as yellow oil. MS calc'd 683.2 (MH⁺), measured 683.1 (MH⁺).

Step 6: Preparation of 3-[5-bromo-6-fluoro-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-1-(2,2,2-trifluoroethyl)indol-3-yl]-2,2-dimethyl-propan-1-ol (compound **E7).**

To a solution of 3-[5-bromo-6-fluoro-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-1-(2,2,2-trifluoroethyl)indol-3-yl]-2,2-dimethyl-propan-1-ol (compound **E6**, 360.0 mg, 0.53 mmol), bis(pinacolato)diboron (200.6 mg, 0.79 mmol) in toluene (6 mL) was added potassium acetate (129 mg, 1.32 mmol) and Pd(dppf)Cl₂ (40 mg, 0.1 mmol). The reaction mixture was degassed by bubbling nitrogen for 5 min then stirred at 80 °C for 15 hrs. After being cooled to room temperature, the reaction mixture was filtered and the filtrate was concentrated *in vacuo* to give a residue. The residue was purified by column chromatography (EtOAc in PE : 30% - 50%) to afford 3-[5-bromo-6-fluoro-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-1-(2,2,2-trifluoroethyl)indol-3-yl]-2,2-dimethyl-propan-1-ol (compound **E7**, 300.0 mg) as yellow gum. MS calc'd 731.4 (MH⁺), measured 731.4 (MH⁺).

Step 7: Preparation of methyl (3*S*)-1-[(2*S*)-2-(*tert*-butoxycarbonylamino)-3-[4-[6-fluoro-3-(3-hydroxy-2,2-dimethyl-propyl)-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-1-(2,2,2-trifluoroethyl)indol-5-yl]thiazol-2-yl]propanoyl]hexahydropyridazine-3-carboxylate (compound **E8).**

To a mixture of 3-[5-bromo-6-fluoro-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-1-(2,2,2-trifluoroethyl)indol-3-yl]-2,2-dimethyl-propan-1-ol (compound **E7**, 0.3 g, 0.41 mmol) and methyl (3*S*)-1-[(2*S*)-3-(4-bromothiazol-2-yl)-2-(*tert*-butoxycarbonylamino)propanoyl]hexahydropyridazine-3-carboxylate (intermediate **B**, 196.7 mg, 0.41 mmol) in toluene (3 mL), 1,4-dioxane (1 mL) and water (1 mL) were added K₃PO₄ (221.3 mg, 1.04 mmol) and Pd(dtbpf)Cl₂ (27.05 mg, 0.04 mmol). The mixture was stirred at 70 °C for 12 hrs under nitrogen atmosphere. After being cooled to room temperature, the reaction mixture was filtered and the filtrate was concentrated *in vacuo* to give a residue. The residue was purified by column chromatography (EtOAc in PE : 60% - 80%) to afford methyl (3*S*)-1-[(2*S*)-2-(*tert*-butoxycarbonylamino)-3-[4-[6-fluoro-3-(3-hydroxy-2,2-dimethyl-propyl)-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-1-(2,2,2-trifluoroethyl)indol-5-yl]thiazol-2-yl]propanoyl]hexahydropyridazine-3-carboxylate (compound **E8**, 200.0 mg) as yellow gum. MS calc'd 1001.4 (MH⁺), measured 1001.4 (MH⁺).

Step 8: Preparation of (3*S*)-1-[(2*S*)-2-(*tert*-butoxycarbonylamino)-3-[4-[6-fluoro-3-(3-hydroxy-2,2-dimethyl-propyl)-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-1-(2,2,2-trifluoroethyl)indol-5-yl]thiazol-2-yl]propanoyl]hexahydropyridazine-3-carboxylic acid (compound **E9).**

To a mixture of methyl (3*S*)-1-[(2*S*)-2-(*tert*-butoxycarbonylamino)-3-[4-[6-fluoro-3-(3-hydroxy-2,2-dimethyl-propyl)-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-1-(2,2,2-trifluoroethyl)indol-5-yl]thiazol-2-yl]propanoyl]hexahydropyridazine-3-carboxylate (compound **E8**, 200.0 mg, 0.2 mmol) in DCE (5 mL) was added Me₃SnOH (200.0 mg, 1.11 mmol). The mixture was stirred at 60 °C for 12 hrs, and then concentrated under vacuum to give a residue. EtOAc (10 mL) and water (10 mL) were added to the residue and the layers were separated. The aqueous phase was extracted with EtOAc (15 mL, twice). The combined organic layer was washed with brine (20 mL), dried over Na₂SO₄, filtered, and concentrated under vacuum to afford (3*S*)-1-[(2*S*)-2-(*tert*-butoxycarbonylamino)-3-[4-[6-fluoro-3-(3-hydroxy-2,2-dimethyl-propyl)-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-1-(2,2,2-trifluoroethyl)indol-5-yl]thiazol-2-yl]propanoyl]hexahydropyridazine-3-carboxylic acid (compound **E9**, 188.0 mg) as a brown solid. MS calc'd 987.4 (MH⁺), measured 987.4 (MH⁺).

Step 9: Preparation of *tert*-butyl *N*-[(7*S*,13*S*)-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-17,17-dimethyl-8,14-dioxo-21-(2,2,2-trifluoroethyl)-15-oxa-4-thia-9,21,27,28-

tetrazapentacyclo[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]carbamate (compound E10).

To a mixture of (3*S*)-1-[(2*S*)-2-(*tert*-butoxycarbonylamino)-3-[4-[6-fluoro-3-(3-hydroxy-2,2-dimethyl-propyl)-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-1-(2,2,2-trifluoroethyl)indol-5-yl]thiazol-2-yl]propanoyl]hexahydropyridazine-3-carboxylic acid (compound **E9**, 188.0 mg, 0.19 mmol) in DCM (20 mL) were added DIEA (0.7 mL, 3.81 mmol), EDCI (550.0 mg, 2.87 mmol) and HOBt (65.0 mg, 0.48 mmol) at 0 °C. After being stirred at 20 °C for 12 hrs, the reaction mixture was poured into water (20 mL) and extracted with EtOAc (20 mL, three times). The combined organic layer was washed with brine (30 mL), dried over Na₂SO₄, filtered and concentrated under vacuum to give a residue which was purified by column chromatography (EtOAc in PE : 50% - 70%) to afford *tert*-butyl *N*-[(7*S*,13*S*)-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-17,17-dimethyl-8,14-dioxo-21-(2,2,2-trifluoroethyl)-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[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]carbamate (compound **E10**, 110.0 mg) as a yellow solid. MS calc'd 969.4 (MH⁺), measured 969.5 (MH⁺).

Step 10: Preparation of (7*S*,13*S*)-7-amino-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-17,17-dimethyl-21-(2,2,2-trifluoroethyl)-15-oxa-4-thia-9,21,27,28-

tetrazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacosa-1(25),2,5(28),19,22(26),23-hexaene-8,14-dione (Intermediate E).

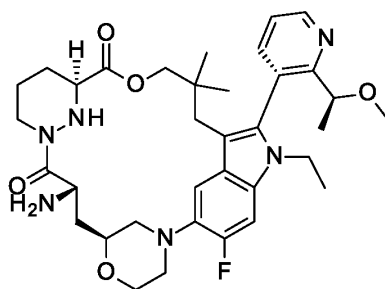
To a solution of *tert*-butyl *N*-[(7*S*,13*S*)-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-17,17-dimethyl-8,14-dioxo-21-(2,2,2-trifluoroethyl)-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacosa-1(25),2,5(28),19,22(26),23-hexaen-7-yl]carbamate (compound **E10**, 110.0 mg, 0.11 mmol) in DCM (1 mL) was added TFA (1.0 mL, 12.98 mmol). The mixture was stirred at 20 °C for 1 h. After the reaction was completed, the reaction mixture was concentrated under vacuum to give a residue. Sat. NaHCO₃ aq. (20 mL) was added and the mixture was extracted with EtOAc (15 mL, three times). The combined organic layer was washed with brine (20 mL), dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo* to afford (7*S*,13*S*)-7-amino-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-17,17-dimethyl-21-(2,2,2-trifluoroethyl)-15-oxa-4-thia-9,21,27,28-

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tetrazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacos-1(25),2,5(28),19,22(26),23-hexaene-8,14-dione (Intermediate E, 98.0 mg) as a yellow solid. MS calc'd 869.4 (MH⁺), measured 869.2 (MH⁺).

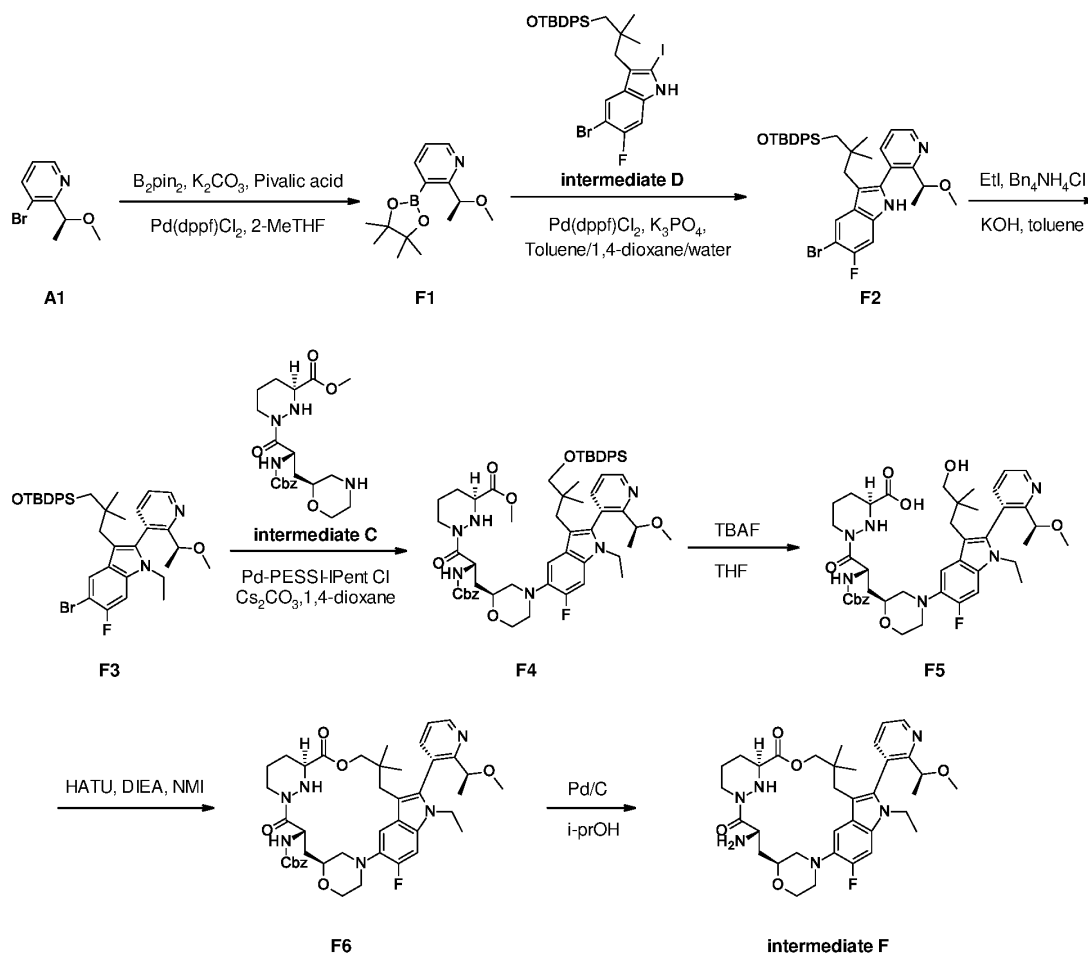
Intermediate F

(6*S*,8*S*,14*S*)-8-amino-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-
 5 18,18-dimethyl-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacos-
 1(26),20,23(27),24-tetraene-9,15-dione



The compound was prepared according to the following scheme:

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Step 1: Preparation of 2-[(1*S*)-1-methoxyethyl]-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (compound **F1)**

To a solution of 3-bromo-2-[(1*S*)-1-methoxyethyl]pyridine (compound **A1**, 46.0 g, 212.88 mmol) and bis(pinacolato)diboron (108.1 g, 425.77 mmol) in 2-methyltetrahydrofuran (1 L) were added potassium carbonate (88.3 g, 638.65 mmol), $Pd(dppf)Cl_2$ (15.6 g, 21.29 mmol) and pivalic acid (10.87 g, 106.44 mmol). Then the mixture was degassed with N_2 (this sequence was repeated three times). After being stirred at 80 °C for 3 hrs under N_2 , the reaction mixture was filtered and the collected solid was washed with EtOAc (100 mL). The filtrate was poured into water (400 mL), extracted with EA (400 mL, three times). The combined organic layer was washed with brine (300 mL, twice), dried over Na_2SO_4 , filtered, and concentrated under vacuum to give the crude product which was purified by silica gel chromatography to afford 2-[(1*S*)-1-methoxyethyl]-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (compound **F1**, 75.0 g) as brown oil. MS calc'd 264.1 (MH^+), measured 264.0 (MH^+).

Step 2: Preparation of [3-[5-bromo-6-fluoro-2-[2-[(1S)-1-methoxyethyl]-3-pyridyl]-1H-indol-3-yl]-2,2-dimethyl-propoxy]-tert-butyl-diphenyl-silane (compound F2)

To a mixture of 2-[(1S)-1-methoxyethyl]-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (compound **F1**, 18.0 g, 30.78 mmol) and [3-(5-bromo-6-fluoro-2-iodo-1H-indol-3-yl)-2,2-dimethyl-propoxy]-tert-butyl-diphenyl-silane (intermediate **D**, 20.5 g, 30.78 mmol) in 1,4-dioxane (200 mL)/water (70 mL)/toluene (70 mL) were added potassium phosphate (19.6 g, 92.35 mmol) and Pd(dppf)Cl₂ (2.3 g, 3.08 mmol). The mixture was stirred at 70 °C for 16 hrs under N₂. After the reaction completed, the cooling reaction mixture was filtered and the filtrate was concentrated under vacuum to remove the solvent. The resultant mixture was extracted with EtOAc (200 mL, three times). The combined organic layer was washed with brine (100 mL, twice), dried over Na₂SO₄, filtered and concentrated *in vacuo*. The residue was purified by column chromatography to afford [3-[5-bromo-6-fluoro-2-[2-[(1S)-1-methoxyethyl]-3-pyridyl]-1H-indol-3-yl]-2,2-dimethyl-propoxy]-tert-butyl-diphenyl-silane (compound **F2**, 22.0 g) as a brown solid. MS calc'd 674.7 (MH⁺), measured 675.3 (MH⁺).

Step 3: Preparation of [3-[5-bromo-1-ethyl-6-fluoro-(2M)-2-[2-[(1S)-1-methoxyethyl]-3-pyridyl]indol-3-yl]-2,2-dimethyl-propoxy]-tert-butyl-diphenyl-silane (compound F3)

To a solution of [3-[5-bromo-6-fluoro-2-[2-[(1S)-1-methoxyethyl]-3-pyridyl]-1H-indol-3-yl]-2,2-dimethyl-propoxy]-tert-butyl-diphenyl-silane (compound **F2**, 7 g, 10.39 mmol), in toluene (20 mL) were added benzyl(triethyl)azanium;bromide (5.6 g, 20.78 mmol), molecular sieve (4Å) (7.5 g, 31.17 mmol) and KOH (1.2 g, 20.78 mmol). The reaction mixture was added with a solution of iodoethane (1.1 mL, 13.51 mmol) in toluene (2 mL). After being stirred at 25 °C for 6 hrs, the reaction mixture was filtered and the filtrate was concentrated under vacuum to give a residue. The residue was purified by NPLC (Column: Welch Ultimate XB-SiOH 250 mm × 100 mm × 10 um, Hexane-EtOH) and concentrated to give [3-[5-bromo-1-ethyl-6-fluoro-(2M)-2-[2-[(1S)-1-methoxyethyl]-3-pyridyl]indol-3-yl]-2,2-dimethyl-propoxy]-tert-butyl-diphenyl-silane (compound **F3**, 7.0 g) as yellow oil. MS calc'd 702.8 (MH⁺), measured 703.2 (MH⁺).

Step 4: Preparation of methyl (3S)-1-[(2S)-2-(benzyloxycarbonylamino)-3-[(2S)-4-[3-[3-tert-butyl(diphenyl)silyl]oxy-2,2-dimethyl-propyl]-1-ethyl-6-fluoro-(2M)-2-[2-[(1S)-1-methoxyethyl]-3-pyridyl]indol-5-yl]morpholin-2-yl]propanoyl]hexahydropyridazine-3-carboxylate (compound F4)

To the mixture of [3-[5-bromo-1-ethyl-6-fluoro-(2M)-2-[2-[(1S)-1-methoxyethyl]-3-pyridyl]indol-3-yl]-2,2-dimethyl-propoxy]-tert-butyl-diphenyl-silane (compound **F3**, 2.1 g, 2.99 mmol) in 1,4-dioxane (20 mL) was added methyl (3S)-1-[(2S)-2-(benzyloxycarbonylamino)-3-

[(2*S*)-morpholin-2-yl]propanoyl]hexahydropyridazine-3-carboxylate (intermediate **C**, 1.6 g, 3.59 mmol), Pd-PESSI-IPent Cl (251.4 mg, 0.3 mmol) and cesium carbonate (2.9 g, 8.98 mmol). The mixture was stirred at 115 °C for 16 hrs. The cooling reaction mixture was added with EtOAc (40 mL) and water (40 mL) and layers were separated. The aqueous phase was extracted with EtOAc (30 mL, twice). The combined organic layer was washed with brine (50 mL), dried over Na₂SO₄, filtered, and purified by column chromatography to afford methyl (3*S*)-1-[(2*S*)-2-(benzyloxycarbonylamino)-3-[(2*S*)-4-[3-[3-*tert*-butyl(diphenyl)silyl]oxy-2,2-dimethyl-propyl]-1-ethyl-6-fluoro-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]indol-5-yl]morpholin-2-yl]propanoyl]hexahydropyridazine-3-carboxylate (compound **F4**, 1.0 g) as a yellow solid. MS calc'd 1055.5 (MH⁺), measured 1055.6 (MH⁺).

Step 5: Preparation of (3*S*)-1-[(2*S*)-2-(benzyloxycarbonylamino)-3-[(2*S*)-4-[1-ethyl-6-fluoro-3-(3-hydroxy-2,2-dimethyl-propyl)-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]indol-5-yl]morpholin-2-yl]propanoyl]hexahydropyridazine-3-carboxylic acid (compound **F5)**

A solution of methyl (3*S*)-1-[(2*S*)-2-(benzyloxycarbonylamino)-3-[(2*S*)-4-[3-[3-*tert*-butyl(diphenyl)silyl]oxy-2,2-dimethyl-propyl]-1-ethyl-6-fluoro-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]indol-5-yl]morpholin-2-yl]propanoyl]hexahydropyridazine-3-carboxylate (compound **F4**, 1.0 g, 0.95 mmol) in TBAF (7.6 mL, 7.6 mmol, 1 M in THF) was stirred at 40 °C for 12 hrs. After the reaction completed, the mixture was concentrated under vacuum to remove most solvent. The residue was diluted with 1N HCl aqueous solution (15 mL) and extracted with EtOAc (50 mL, three times). The combined organic layer was washed by aq. NH₄Cl (30 mL, twice), brine (40 mL, twice), dried over anhydrous sodium sulfate, filtered and concentrated under vacuum to give a crude product. The crude product was purified by reversed phase chromatography to afford (3*S*)-1-[(2*S*)-2-(benzyloxycarbonylamino)-3-[(2*S*)-4-[1-ethyl-6-fluoro-3-(3-hydroxy-2,2-dimethyl-propyl)-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]indol-5-yl]morpholin-2-yl]propanoyl]hexahydropyridazine-3-carboxylic acid (compound **F5**, 500.0 mg) as a yellow solid. MS calc'd 803.4 (MH⁺), measured 803.4 (MH⁺).

Step 6: Preparation of benzyl *N*-[(6*S*,8*S*,14*S*)-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-9,15-dioxo-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]carbamate (compound **F6)**

To a solution of (3*S*)-1-[(2*S*)-2-(benzyloxycarbonylamino)-3-[(2*S*)-4-[1-ethyl-6-fluoro-3-(3-hydroxy-2,2-dimethyl-propyl)-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]indol-5-yl]morpholin-2-yl]propanoyl]hexahydropyridazine-3-carboxylic acid (compound **F5**, 700.0 mg, 0.87 mmol) in

-33-

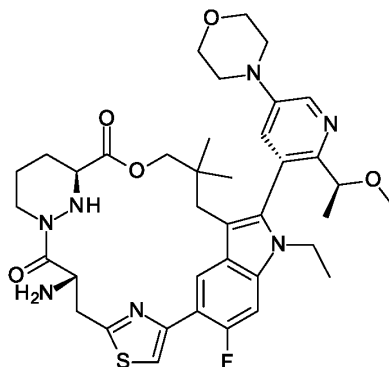
DCM (70 mL) were added DIEA (1.1 g, 8.72 mmol), HATU (1.7 g, 4.36 mmol) and 1-methylimidazole (715.8 mg, 8.72 mmol) at 25 °C. After being stirred at rt for 1 h, the mixture was concentrated under vacuum and purified by reversed phase chromatography to give benzyl *N*-[(6*S*,8*S*,14*S*)-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-9,15-dioxo-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]carbamate (compound **F6**, 320.0 mg) as a light yellow solid. MS calc'd 785.5 (MH⁺), measured 785.4(MH⁺).

Step 7: Preparation of (6*S*,8*S*,14*S*)-8-amino-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraene-9,15-dione (intermediate **F)**

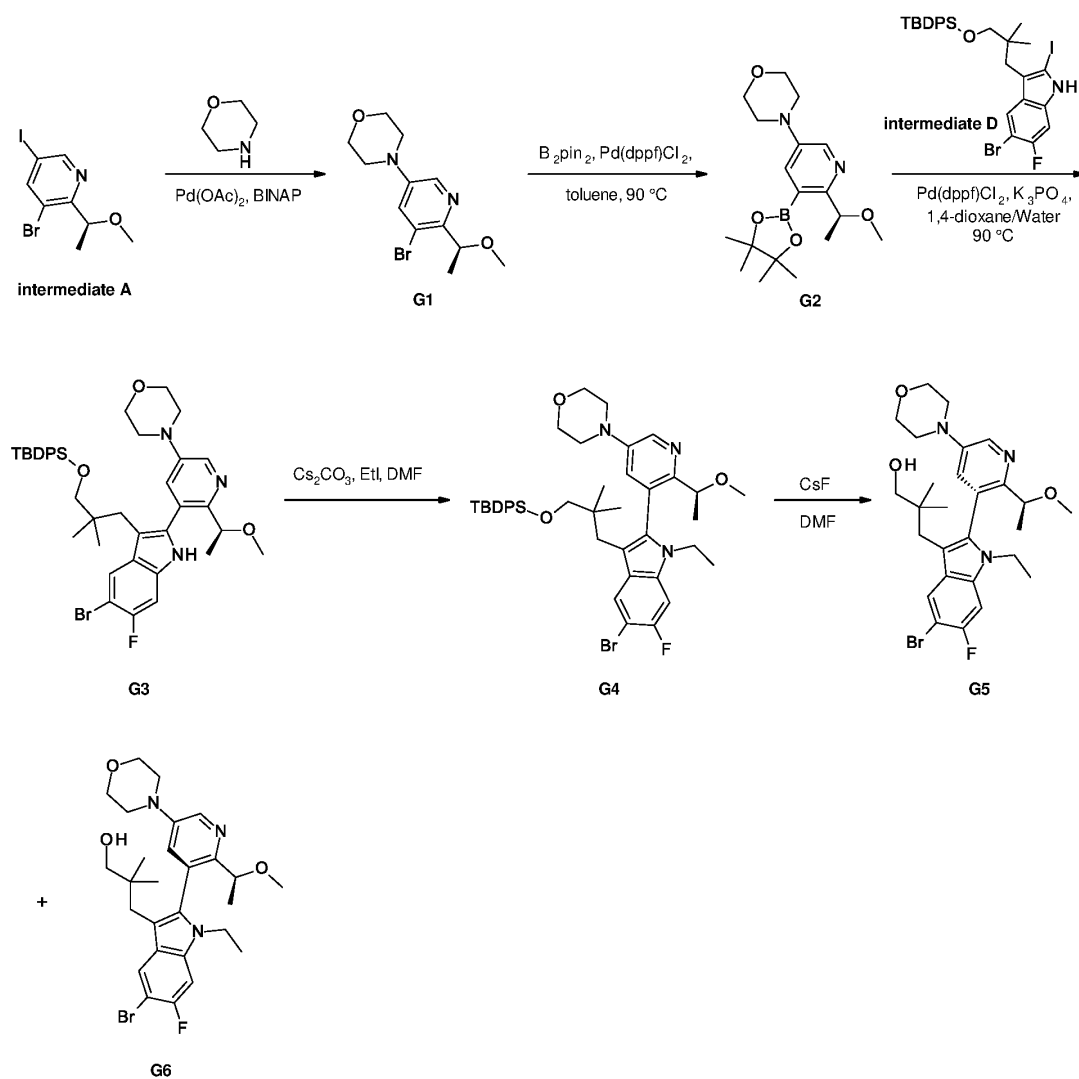
To a solution of benzyl *N*-[(6*S*,8*S*,14*S*)-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-9,15-dioxo-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]carbamate (compound **F6**, 300.0 mg, 0.38 mmol) in THF (3 mL) was added Pd(OH)₂ on activated carbon (200 mg). The mixture reaction was stirred at 25 °C for 2 hrs. The mixture was filtered and the filtrate was concentrated under vacuum to afford (6*S*,8*S*,14*S*)-8-amino-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraene-9,15-dione (intermediate **F**, 270.0 mg) as a yellow solid. MS calc'd 651.4 (MH⁺), measured 651.4 (MH⁺).

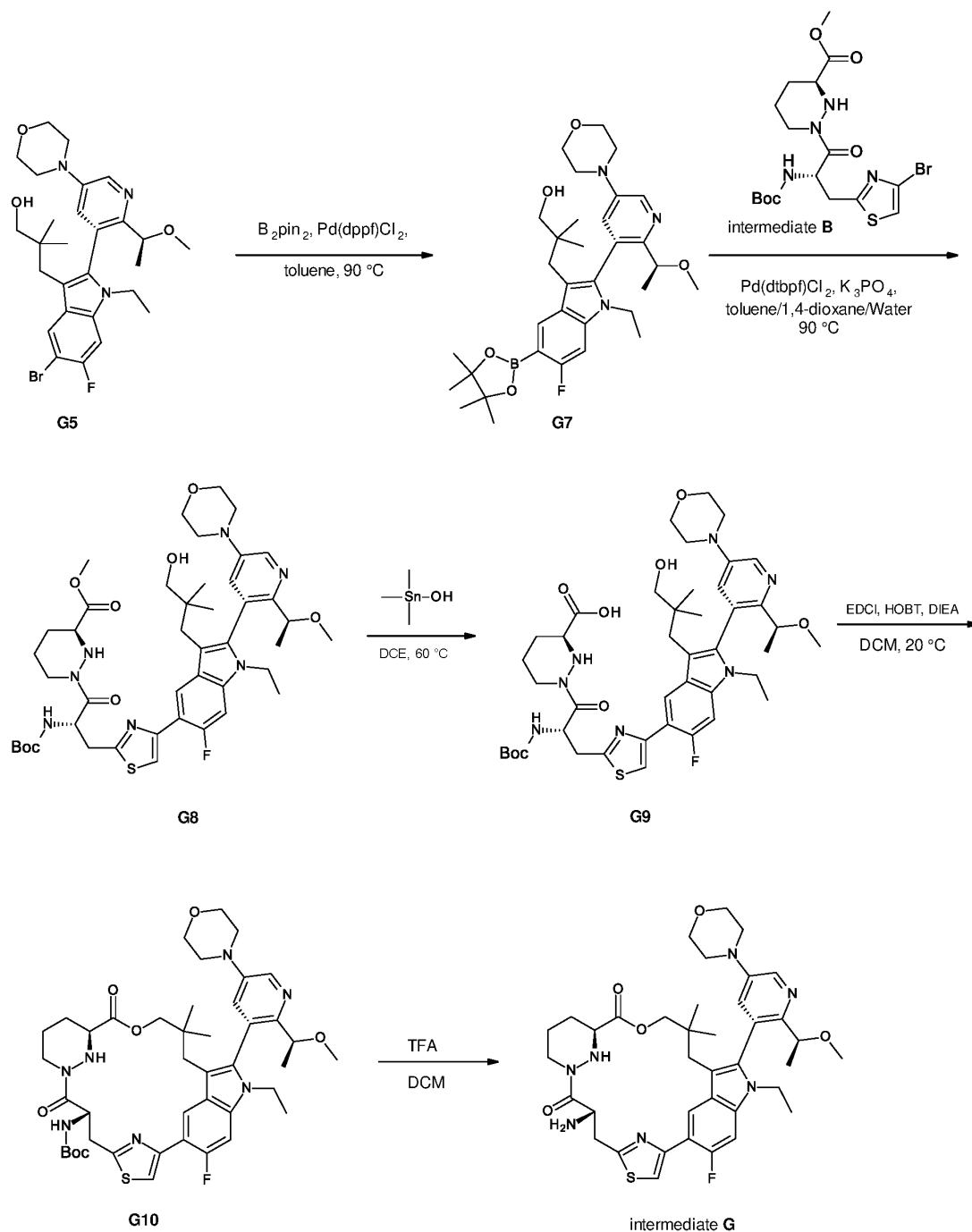
Intermediate **G**

(7*S*,13*S*)-7-amino-21-ethyl-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]-17,17-dimethyl-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacosa-1(25),2,5(28),19,22(26),23-hexaene-8,14-dione



The compound was prepared according to the following scheme:





Step 1: Preparation of 4-[5-bromo-6-[(1*S*)-1-methoxyethyl]-3-pyridyl]morpholine (compound G1)

To a mixture of 3-bromo-5-iodo-2-[(1*S*)-1-methoxyethyl]pyridine (intermediate A, 30 g, 5 87.73 mmol) and morpholine (7.6 g, 87.73 mmol) in toluene (450 mL) were added Cs_2CO_3 (57.2 g, 175.45 mmol), (*R*)-binap (2.7 g, 4.39 mmol) and $Pd(OAc)_2$ (0.98 g, 4.39 mmol). The reaction

mixture was degassed and purged with nitrogen for 3 times and the mixture was stirred at 90 °C for 12 hrs under nitrogen atmosphere. After being cooled to room temperature, the reaction mixture was filtered and the filtrate was concentrated *in vacuo* to give a residue. The residue was purified by column chromatography to afford 4-[5-bromo-6-[(1*S*)-1-methoxyethyl]-3-

- 5 pyridyl]morpholine (compound **G1**, 21 g) as yellow oil. MS calc'd 301.1 (MH⁺), measured 301.1 (MH⁺).

Step 2: Preparation of 4-[6-[(1*S*)-1-methoxyethyl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-pyridyl]morpholine (compound **G2)**

- To a solution of 4-[5-bromo-6-[(1*S*)-1-methoxyethyl]-3-pyridyl]morpholine (compound **G1**, 21 g, 63.3 mmol), bis(pinacolato)diboron (24.0 g, 94.63 mmol) and KOAc (13.6 g, 138.79 mmol) in toluene (500 mL) was added Pd(dppf)Cl₂ (4.4 g, 6.31 mmol). The mixture was degassed and purged with nitrogen for 3 times and the mixture was stirred at 90 °C for 12 hrs under nitrogen atmosphere. After being cooled to the room temperature, the reaction mixture was filtered, the filtrate was concentrated *in vacuo* to give crude product 4-[6-[(1*S*)-1-methoxyethyl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-pyridyl]morpholine (compound **G2**, 45 g) as a yellow gum, which was used in the next step directly. MS calc'd 349.2 (MH⁺), measured 349.2 (MH⁺).

Step 3: Preparation of [3-[5-bromo-6-fluoro-2-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]-1*H*-indol-3-yl]-2,2-dimethyl-propoxy]-*tert*-butyl-diphenyl-silane

- 20 (compound **G3**)

- To a solution of 4-[6-[(1*S*)-1-methoxyethyl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-pyridyl]morpholine (compound **G2**, 40.6 g, 46.65 mmol), [3-(5-bromo-6-fluoro-2-iodo-1*H*-indol-3-yl)-2,2-dimethyl-propoxy]-*tert*-butyl-diphenyl-silane (intermediate **C5**, 31 g, 46.65 mmol) in 1,4-dioxane (420 mL) and water (80 mL) was added K₃PO₄ (29.7 g, 2.33 mmol) and Pd(dppf)Cl₂ (1.7 g, 0.29 mmol). The mixture was degassed and purged with nitrogen, and the reaction mixture was stirred at 90 °C for 18 hrs. After being cooled to room temperature, the reaction mixture was extracted with EA (200 mL, three times). The combined organic layer was washed with brine (200 mL), dried over Na₂SO₄, filtered and the filtrate was concentrated *in vacuo* to give a residue. The residue was purified by column chromatography to afford [3-[5-bromo-6-fluoro-2-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]-1*H*-indol-3-yl]-2,2-dimethyl-propoxy]-*tert*-butyl-diphenyl-silane (compound **G3**, 17.2 g) as yellow oil. MS calc'd 758.3 (MH⁺), measured 758.3 (MH⁺).

Step 4: Preparation of [3-[5-bromo-1-ethyl-6-fluoro-2-[2-[(1S)-1-methoxyethyl]-5-morpholino-3-pyridyl]indol-3-yl]-2,2-dimethyl-propoxy]-tert-butyl-diphenyl-silane (compound G4)

To a solution of [3-[5-bromo-6-fluoro-2-[2-[(1S)-1-methoxyethyl]-5-morpholino-3-pyridyl]-1*H*-indol-3-yl]-2,2-dimethyl-propoxy]-tert-butyl-diphenyl-silane (compound **G3**, 15 g, 19.77 mmol) in DMF (300 mL) was added Cs₂CO₃ (19.3 g, 59.3 mmol) and iodoethane (6.16 g, 39.53 mmol) at 0 °C. After being stirred at 20 °C for 16 hrs, the reaction mixture was poured into water (200 mL), and extracted with EtOAc (200 mL, three times). The combined organic layer was washed with brine (10 mL, three times), dried over Na₂SO₄, filtered and concentrated under vacuum to give a residue. The residue was purified by column chromatography to afford [3-[5-bromo-1-ethyl-6-fluoro-2-[2-[(1S)-1-methoxyethyl]-5-morpholino-3-pyridyl]indol-3-yl]-2,2-dimethyl-propoxy]-tert-butyl-diphenyl-silane (compound **G4**, 14.7 g) as yellow oil. MS calc'd 786.3 (MH⁺), measured 786.4 (MH⁺).

Step 5: Preparation of 3-[5-bromo-1-ethyl-6-fluoro-(2*M*)-2-[2-[(1S)-1-methoxyethyl]-5-morpholino-3-pyridyl]indol-3-yl]-2,2-dimethyl-propan-1-ol (compound G5) and 3-[5-bromo-1-ethyl-6-fluoro-(2*P*)-2-[2-[(1S)-1-methoxyethyl]-5-morpholino-3-pyridyl]indol-3-yl]-2,2-dimethyl-propan-1-ol (compound G6)

To a solution of [3-[5-bromo-1-ethyl-6-fluoro-2-[2-[(1S)-1-methoxyethyl]-5-morpholino-3-pyridyl]indol-3-yl]-2,2-dimethyl-propoxy]-tert-butyl-diphenyl-silane (compound **G4**, 14.7 g, 18.68 mmol) in DMF (160 mL) was added cesium fluoride (14.2 g, 93.41 mmol). The mixture was stirred at 60 °C for 48 hrs. After being cooled to room temperature, the reaction mixture were added with EtOAc (300 mL) and water (300 mL) and the layers were separated. The aqueous phase was extracted with EtOAc (200 mL, three times). The combined organic layer was washed with brine (200 mL, four times), dried over Na₂SO₄, filtered, and concentrated under vacuum to give a residue. The residue was purified by column chromatography to afford 3-[5-bromo-1-ethyl-6-fluoro-(2*M*)-2-[2-[(1S)-1-methoxyethyl]-5-morpholino-3-pyridyl]indol-3-yl]-2,2-dimethyl-propan-1-ol (compound **G5**, 6 g, faster eluted) as colorless foam and 3-[5-bromo-1-ethyl-6-fluoro-(2*P*)-2-[2-[(1S)-1-methoxyethyl]-5-morpholino-3-pyridyl]indol-3-yl]-2,2-dimethyl-propan-1-ol (compound **G6**, 4.5 g, slower eluted) as colorless foam. Compound **G5**: MS calc'd 548.2 (MH⁺), measured 548.2 (MH⁺). ¹H NMR (400MHz, Methanol-*d*₄) δ = 8.41 (d, *J* = 2.4 Hz, 1H), 7.92 (d, *J* = 6.8 Hz, 1H), 7.37 - 7.33 (m, 2H), 4.58 (s, 1H), 4.05 - 3.98 (m, 2H), 3.87-3.82 (m, 5H), 3.27 - 3.23 (m, 4H), 3.15 - 3.13 (m, 1H), 3.00 (s, 3H), 2.75-2.71 (m, 1H), 2.24 - 2.22 (m, 1H), 1.42 (d, *J* = 6.4 Hz, 3H), 1.22 (t, *J* = 7.2 Hz, 3H), 0.76 (s, 3H), 0.76 (s, 3H).

X-ray crystallographic analysis of compound G5

Absolute configuration structure of compound **G5** was confirmed by X-ray crystallographic analysis of its single crystal. (Figure 1).

5 **Step 6: Preparation of 3-[1-ethyl-6-fluoro-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-3-yl]-2,2-dimethyl-propan-1-ol (compound G7)**

To a solution of 3-[5-bromo-1-ethyl-6-fluoro-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]indol-3-yl]-2,2-dimethyl-propan-1-ol (compound **G5**, 6 g, 10.94 mmol),
10 bis(pinacolato)diboron (4.2 g, 16.41 mmol) in toluene (60 mL) was added potassium acetate (2.7 g, 27.35 mmol) and Pd(dppf)Cl₂ (0.8 g, 1.09 mmol). The reaction mixture was degassed by bubbling nitrogen for 5 min then stirred at 90 °C for 15 hrs. After being cooled to room temperature, the reaction mixture was filtered and the filtrate was concentrated *in vacuo* to give a residue. The residue was purified by column chromatography to afford 3-[1-ethyl-6-fluoro-(2*M*)-
15 2-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-3-yl]-2,2-dimethyl-propan-1-ol (compound **G7**, 4.5 g) as colorless gum. MS calc'd 596.4 (MH⁺), measured 596.4 (MH⁺).

20 **Step 7: Preparation of methyl (3*S*)-1-[(2*S*)-2-(tert-butoxycarbonylamino)-3-[4-[1-ethyl-6-fluoro-3-(3-hydroxy-2,2-dimethyl-propyl)-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]indol-5-yl]thiazol-2-yl]propanoyl]hexahydropyridazine-3-carboxylate (compound G8)**

To a mixture of 3-[1-ethyl-6-fluoro-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-3-yl]-2,2-dimethyl-propan-1-ol (compound **G7**, 4.5 g, 7.56 mmol) and methyl (3*S*)-1-[(2*S*)-3-(4-bromothiazol-2-yl)-2-(tert-butoxycarbonylamino)propanoyl]hexahydropyridazine-3-carboxylate (intermediate **B**, 3.6 g, 7.56 mmol) in toluene (45 mL), 1,4-dioxane (15 mL) and water (15 mL) were added K₃PO₄ (4.0 g, 18.89 mmol) and Pd(dtbpf)Cl₂ (492.5 mg, 0.75 mmol). The mixture was stirred at 70 °C for 12 hrs under nitrogen atmosphere. After being cooled to room temperature, the reaction mixture was filtered and the filtrate was concentrated *in vacuo* to give a residue. The residue was purified by
25 column chromatography to afford methyl (3*S*)-1-[(2*S*)-2-(tert-butoxycarbonylamino)-3-[4-[1-ethyl-6-fluoro-3-(3-hydroxy-2,2-dimethyl-propyl)-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]indol-5-yl]thiazol-2-yl]propanoyl]hexahydropyridazine-3-carboxylate (compound **G8**, 3.8 g) as colorless gum. MS calc'd 866.4 (MH⁺), measured 866.4 (MH⁺).

Step 8: Preparation of (3*S*)-1-[(2*S*)-2-(*tert*-butoxycarbonylamino)-3-[4-[1-ethyl-6-fluoro-3-(3-hydroxy-2,2-dimethyl-propyl)-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]indol-5-yl]thiazol-2-yl]propanoyl]hexahydropyridazine-3-carboxylic acid (compound **G9)**

To a mixture of methyl (3*S*)-1-[(2*S*)-2-(*tert*-butoxycarbonylamino)-3-[4-[1-ethyl-6-fluoro-3-(3-hydroxy-2,2-dimethyl-propyl)-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]indol-5-yl]thiazol-2-yl]propanoyl]hexahydropyridazine-3-carboxylate (compound **G8**, 3.8 g, 4.39 mmol) in DCE (76 mL) was added Me₃SnOH (3.2 g, 17.55 mmol). The mixture was stirred at 60 °C for 48 hrs. The reaction mixture was concentrated under vacuum to give a residue. EtOAc (200 mL) and water (100 mL) were added to the residue and the layers were separated. The aqueous phase was extracted with EtOAc (150 mL, twice). The combined organic layer was washed with brine (200 mL), dried over Na₂SO₄, filtered, and concentrated under vacuum to afford (3*S*)-1-[(2*S*)-2-(*tert*-butoxycarbonylamino)-3-[4-[1-ethyl-6-fluoro-3-(3-hydroxy-2,2-dimethyl-propyl)-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]indol-5-yl]thiazol-2-yl]propanoyl]hexahydropyridazine-3-carboxylic acid (compound **G9**, 3.7 g) as a brown solid. MS calc'd 852.4 (MH⁺), measured 852.4 (MH⁺).

Step 9: Preparation of *tert*-butyl *N*-[(7*S*,13*S*)-21-ethyl-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]-17,17-dimethyl-8,14-dioxo-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacos-1(25),2,5(28),19,22(26),23-hexaen-7-yl]carbamate (compound **G10)**

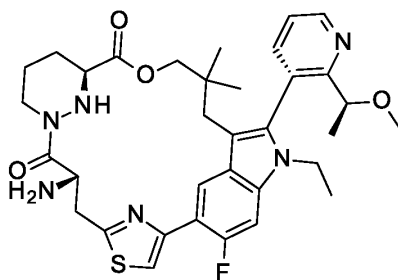
To a mixture of (3*S*)-1-[(2*S*)-2-(*tert*-butoxycarbonylamino)-3-[4-[1-ethyl-6-fluoro-3-(3-hydroxy-2,2-dimethyl-propyl)-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]indol-5-yl]thiazol-2-yl]propanoyl]hexahydropyridazine-3-carboxylic acid (compound **G9**, 2.5 g, 2.93 mmol) in DCM (250 mL) were added DIEA (7.58 mL, 58.68 mmol), EDCI (8.4 g, 44.01 mmol) and HOBt (991.2 mg, 0.91 mmol) at 0 °C. After being stirred at 20 °C for 12 hrs, the reaction mixture was poured into water (100 mL) and extracted with EtOAc (100 mL, three times). The combined organic layer was washed with brine (30 mL), dried over Na₂SO₄, filtered and concentrated under vacuum to give a residue which was purified by column chromatography to afford *tert*-butyl *N*-[(7*S*,13*S*)-21-ethyl-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]-17,17-dimethyl-8,14-dioxo-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacos-1(25),2,5(28),19,22(26),23-hexaen-7-yl]carbamate (compound **G10**, 1.2 g) as a yellow oil. MS calc'd 834.4 (MH⁺), measured 834.4 (MH⁺).

Step 10: Preparation of (7*S*,13*S*)-7-amino-21-ethyl-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]-17,17-dimethyl-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacos-1(25),2,5(28),19,22(26),23-hexaene-8,14-dione (Intermediate G)

- 5 To a solution of *tert*-butyl *N*-[(7*S*,13*S*)-21-ethyl-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]-17,17-dimethyl-8,14-dioxo-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacos-1(25),2,5(28),19,22(26),23-hexaen-7-yl]carbamate (compound **G10**, 1.2 g, 1.44 mmol) in DCM (12 mL) was added TFA (6.0 mL). The mixture was stirred at 20 °C for 3 hrs. After the reaction was completed, the reaction
- 10 mixture was concentrated under vacuum to give a residue. Sat. NaHCO₃ aq. (60 mL) was added and the mixture was extracted with EtOAc (80 mL, three times). The combined organic layer was washed with brine (100 mL), dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo* to afford (7*S*,13*S*)-7-amino-21-ethyl-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]-17,17-dimethyl-15-oxa-4-thia-9,21,27,28-
- 15 tetrazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacos-1(25),2,5(28),19,22(26),23-hexaene-8,14-dione (intermediate **G**, 1 g) as a yellow solid. MS calc'd 734.3 (MH⁺), measured 734.3 (MH⁺).

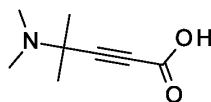
Intermediate H

- (7*S*,13*S*)-7-amino-21-ethyl-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-17,17-dimethyl-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacos-1(25),2,5(28),19,22(26),23-hexaene-8,14-dione
- 20

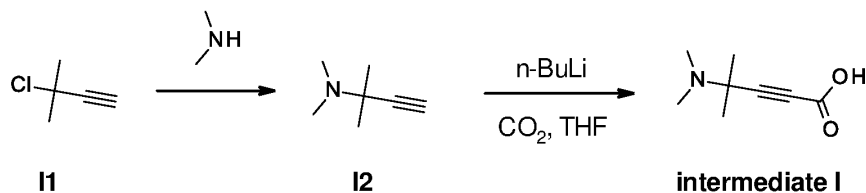


- The title compound was prepared in analogy to the preparation of Intermediate **G** by using [3-[5-bromo-1-ethyl-6-fluoro-(2*M*)-2-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]indol-3-yl]-2,2-dimethyl-propoxy]-*tert*-butyl-diphenyl-silane (compound **F3**) instead of [3-[5-bromo-1-ethyl-6-fluoro-2-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]indol-3-yl]-2,2-dimethyl-propoxy]-*tert*-butyl-diphenyl-silane (compound **G4**).
- 25

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Intermediate I**4-(dimethylamino)-4-methyl-pent-2-ynoic acid**

The compound was prepared according to the following scheme:

**Step 1: Preparation of *N,N*,2-trimethylbut-3-yn-2-amine (compound **I2**)**

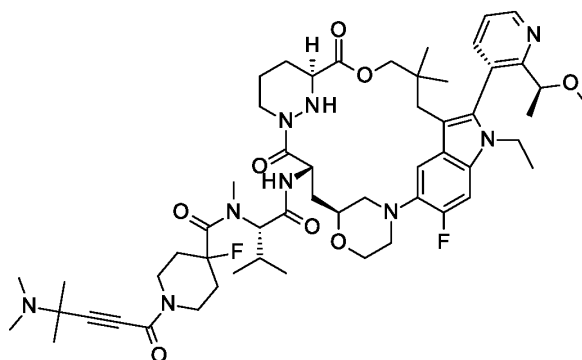
A mixture of 3-chloro-3-methyl-1-butyne (20.0 g, 195.01 mmol) and dimethylamine (22.0 g, 195.01 mmol) was stirred at 25 °C for 12 hrs. After the reaction completed, the suspension was filtered. The collected solid was washed with ice-water (50 mL, three times) and dissolved in cold HCl (3 N, 10 mL). The acidic solution was extracted with methyl *tert*-butyl ether (50 mL, twice). The resulting aqueous phase was cooled at 0 °C and made strongly alkaline with cold NH₃.H₂O. The yellow precipitate was collected, washed with NH₃.H₂O (50 mL, three times) and H₂O (50 mL, three times). The collected solid was concentrated *in vacuo* to afford *N,N*,2-trimethylbut-3-yn-2-amine (compound **I2**, 4.0 g) as a yellow solid. MS calc'd 112.1 (MH⁺), measured 112.1 (MH⁺).

Step 2: Preparation of 4-(dimethylamino)-4-methyl-pent-2-ynoic acid (intermediate **I)**

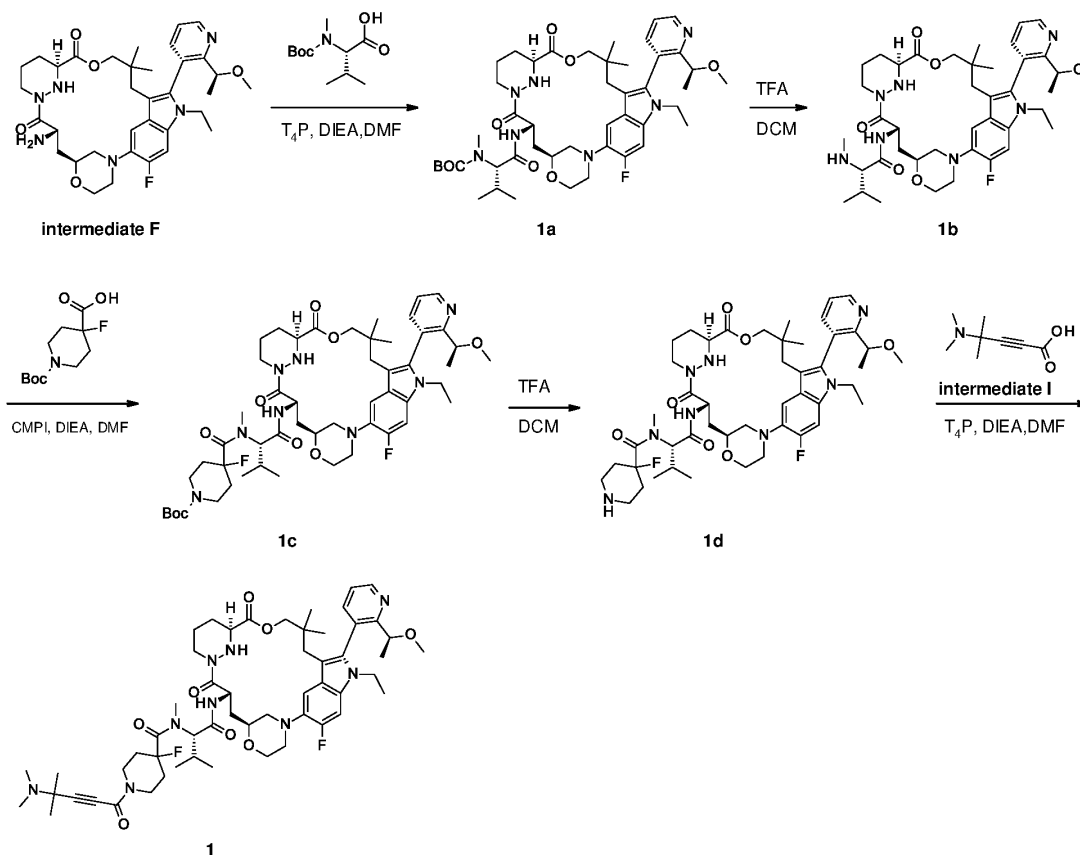
To a solution of *N,N*,2-trimethylbut-3-yn-2-amine (compound **I2**, 3.2 g, 28.78 mmol) in THF (120 mL) was added dropwise *n*-BuLi (12.7 mL, 31.66 mmol, 2M in hexane) at -70 °C. After being stirred at -70 °C for 5 min, the reaction mixture was warmed to 0 °C and stirred for another 10 min. After being cooled to -70 °C, the reaction mixture was bubbled with carbon dioxide for 30 min. The reaction mixture was stirred at 0 °C for 2 hrs. After the reaction completed, the reaction mixture was carefully hydrolyzed at 0 °C by water (40 mL). Two phases were separated and the aqueous layer was acidified pH to 1-2 with 6N HCl. The aqueous was concentrated under vacuum to afford 4-(dimethylamino)-4-methyl-pent-2-ynoic acid (intermediate **I**, 4.3 g) as a white solid, which was used in the next step without purification. MS calc'd 156.1 (MH⁺), measured 156.1 (MH⁺). ¹H NMR (400 MHz, DEUTERIUM OXIDE-D₂O) δ = 2.99 - 2.91 (m, 6H), 1.77 - 1.68 (m, 6H).

Example 1

1-[4-(dimethylamino)-4-methyl-pent-2-ynoyl]-*N*-[(1*S*)-1-[[*(6S,8S,14S)*-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-9,15-dioxo-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]carbamoyl]-2-methyl-propyl]-4-fluoro-*N*-methyl-piperidine-4-carboxamide



The compound was prepared according to the following scheme:



Step 1: Preparation of *tert*-butyl *N*-[(1*S*)-1-[(6*S*,8*S*,14*S*)-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-9,15-dioxo-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (compound **1a)**

5 To a mixture of (6*S*,8*S*,14*S*)-8-amino-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraene-9,15-dione (intermediate **F**, 270.0 mg, 0.41 mmol) and (2*S*)-2-[*tert*-butoxycarbonyl(methyl)amino]-3-methyl-butanoic acid (191.9 mg, 0.83 mmol) in DMF (3 mL) were added DIEA (0.36 mL, 2.07 mmol) and T₄P (597.9 mg, 0.83 mmol) at 0 °C. The mixture was stirred at 25 °C for 1h. The mixture was concentrated under vacuum to get a residue, which was purified by reversed phase chromatography to afford *tert*-butyl *N*-[(1*S*)-1-[(6*S*,8*S*,14*S*)-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-9,15-dioxo-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (compound **1a**, 210.0 mg) as a brown solid. MS calc'd 864.5 (MH⁺), measured 864.5(MH⁺).

Step 2: Preparation of (2*S*)-*N*-[(6*S*,8*S*,14*S*)-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-9,15-dioxo-5,16-dioxo-2,10,22,28-tetrazapentacyclo[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-(methylamino)butanamide (compound **1b)**

20 To a solution of *tert*-butyl *N*-[(1*S*)-1-[(6*S*,8*S*,14*S*)-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-9,15-dioxo-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]carbamoyl]-2-methyl-propyl]-*N*-methyl-carbamate (compound **1a**, 210.0 mg, 0.24 mmol) in DCM (2 mL) was added TFA (1.0 mL, 12.98 mmol). The mixture was stirred at 25 °C for 1 h. After the reaction completed, the mixture was concentrated under vacuum to remove most solvent, then poured into sat. NaHCO₃ aq. (5 mL) and extracted with EtOAc (30 mL, five times). The combined organic layer was washed by brine (50 mL), dried over anhydrous sodium sulfate, filtered and concentrated under vacuum to afford (2*S*)-*N*-[(6*S*,8*S*,14*S*)-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-9,15-dioxo-5,16-dioxo-2,10,22,28-tetrazapentacyclo[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-(methylamino)butanamide (compound **1b**, 225.0 mg) as a brown solid. MS calc'd 764.4 (MH⁺), measured 764.4 (MH⁺).

Step 3: Preparation of *tert*-butyl 4-[[[(1*S*)-1-[[[(6*S*,8*S*,14*S*)-22-ethyl-25-fluoro-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-9,15-dioxo-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]carbamoyl]-2-methyl-propyl]-methyl-carbamoyl]-4-fluoro-piperidine-1-carboxylate

5 (compound 1c)

To a solution of (2*S*)-*N*-[(6*S*,8*S*,14*S*)-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-9,15-dioxo-5,16-dioxo-2,10,22,28-tetrazapentacyclo[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-(methylamino)butanamide (compound **1b**, 200.0 mg, 0.26 mmol) in DMF (2.5 mL) were added
10 DIEA (0.5 mL, 2.62 mmol), 1-*tert*-butoxycarbonyl-4-fluoro-piperidine-4-carboxylic acid (194.2 mg, 0.79 mmol) and CMPI (200.6 mg, 0.79 mmol) at 0 °C. The mixture was stirred at 25 °C for 1 h. The mixture was poured into water (30 mL) and extracted with EtOAc (30 mL, three times). The combined organic layer was washed by brine (30 mL, twice), dried over Na₂SO₄, filtered and concentrated under vacuum to give a residue. The residue was purified by reversed phase
15 chromatography to afford *tert*-butyl 4-[[[(1*S*)-1-[[[(6*S*,8*S*,14*S*)-22-ethyl-25-fluoro-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-9,15-dioxo-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]carbamoyl]-2-methyl-propyl]-methyl-carbamoyl]-4-fluoro-piperidine-1-carboxylate (compound **1c**, 200.0 mg) as yellow oil. MS calc'd 993.6 (MH⁺), measured 993.6 (MH⁺).

20 Step 4: Preparation of *N*-[(1*S*)-1-[[[(6*S*,8*S*,14*S*)-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-9,15-dioxo-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]carbamoyl]-2-methyl-propyl]-4-fluoro-*N*-methyl-piperidine-4-carboxamide (compound 1d)

To a solution of *tert*-butyl 4-[[[(1*S*)-1-[[[(6*S*,8*S*,14*S*)-22-ethyl-25-fluoro-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-9,15-dioxo-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]carbamoyl]-2-methyl-propyl]-methyl-carbamoyl]-4-fluoro-piperidine-1-carboxylate (compound **1c**, 200.0 mg, 0.2 mmol) in DCM (2 mL) was added TFA (1.1 mL, 14.35 mmol) at 0°C. The mixture was
25 stirred at 25°C for 1 hour. After the reaction completed, the mixture was concentrated under vacuum to remove most solvent, then poured into sat. NaHCO₃ aq. (5 mL) and extracted with EtOAc (20 mL, three times). The combined organic layer was washed by brine (20 mL), dried over anhydrous sodium sulfate, filtered and concentrated under vacuum to afford *N*-[(1*S*)-1-

[[[(6*S*,8*S*,14*S*)-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-9,15-dioxo-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]carbamoyl]-2-methyl-propyl]-4-fluoro-*N*-methyl-piperidine-4-carboxamide (compound **1d**, 200.0 mg) as yellow oil. MS calc'd 893.5 (MH⁺), measured 893.5 (MH⁺).

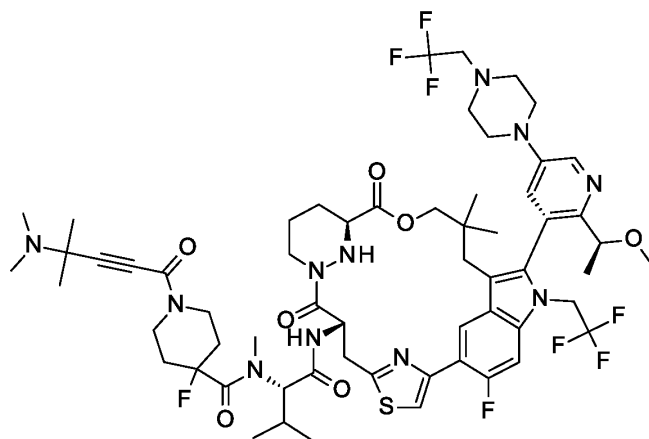
Step 5: Preparation of 1-[4-(dimethylamino)-4-methyl-pent-2-ynoyl]-*N*-[(1*S*)-1-[[[(6*S*,8*S*,14*S*)-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-9,15-dioxo-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]carbamoyl]-2-methyl-propyl]-4-fluoro-*N*-methyl-piperidine-4-carboxamide (Example 1)

To a mixture of *N*-[(1*S*)-1-[[[(6*S*,8*S*,14*S*)-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-9,15-dioxo-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]carbamoyl]-2-methyl-propyl]-4-fluoro-*N*-methyl-piperidine-4-carboxamide (compound **1d**, 100.0 mg, 0.11 mmol) and 4-(dimethylamino)-4-methyl-pent-2-ynoic acid (intermediate **I**, 34.7 mg, 0.22 mmol) in DMF (2 mL) were added DIEA (0.1 mL, 0.56 mmol) and T₄P (161.4 mg, 0.22 mmol) at 0 °C. The mixture was stirred at 25 °C for 1h, then purified by Prep-HPLC to afford 1-[4-(dimethylamino)-4-methyl-pent-2-ynoyl]-*N*-[(1*S*)-1-[[[(6*S*,8*S*,14*S*)-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-9,15-dioxo-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]carbamoyl]-2-methyl-propyl]-4-fluoro-*N*-methyl-piperidine-4-carboxamide (Example **1**, 45.0 mg) as a yellow solid. MS calc'd 1030.6 (MH⁺), measured 1030.6 (MH⁺). ¹H NMR (400 MHz, METHANOL-*d*₄) δ = 8.82 (s, 1H), 8.25 - 8.13 (m, 1H), 7.75 - 7.65 (m, 1H), 7.33 - 7.23 (m, 2H), 5.74 - 5.64 (m, 1H), 4.65 - 4.60 (d, *J* = 10.8 Hz, 1H), 4.55 - 4.44 (m, 1H), 4.40 - 4.30 (m, 1H), 4.30 - 4.17 (m, 2H), 4.16 - 4.06 (m, 1H), 3.98 - 3.89 (m, 2H), 3.89 - 3.84 (m, 1H), 3.80 (dd, *J* = 4.0, 11.2 Hz, 2H), 3.65-3.55 (m, 2H), 3.50 - 3.37 (m, 1H), 3.26 - 3.18 (m, 4H), 3.16 (d, *J* = 5.2 Hz, 2H), 3.10 - 3.08 (m, 1H), 3.02 (s, 5H), 2.98 - 2.94 (m, 1H), 2.84 - 2.76 (m, 1H), 2.71 - 2.58 (m, 1H), 2.38 - 2.25 (m, 2H), 2.23 - 2.03 (m, 5H), 1.99 - 1.85 (m, 3H), 1.80 (s, 6H), 1.75 - 1.56 (m, 4H), 1.51 - 1.39 (m, 5H), 1.11-1.01 (m, 3H), 1.00 - 0.89 (m, 6H), 0.85 (d, *J* = 6.4 Hz, 2H), 0.82 - 0.73 (m, 4H).

Example 2

1-[4-(dimethylamino)-4-methyl-pent-2-ynoyl]-4-fluoro-*N*-[(1*S*)-1-[[[(7*S*,13*S*)-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-

17,17-dimethyl-8,14-dioxo-21-(2,2,2-trifluoroethyl)-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacos-1(25),2,5(28),19,22(26),23-hexaen-7-yl]carbamoyl]-2-methyl-propyl]-N-methyl-piperidine-4-carboxamide

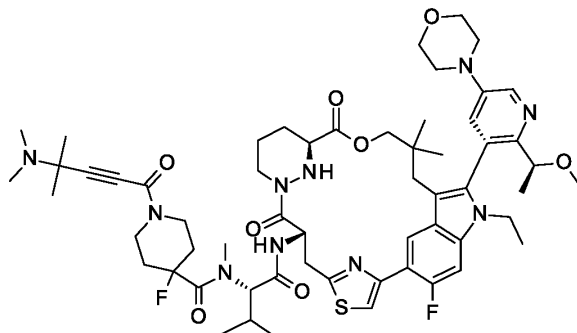


5 The title compound was prepared in analogy to the preparation of Example 1 by using (7*S*,13*S*)-7-amino-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-17,17-dimethyl-21-(2,2,2-trifluoroethyl)-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacos-1(25),2,5(28),19,22(26),23-hexaene-8,14-dione (intermediate **E**) instead of (6*S*,8*S*,14*S*)-8-amino-22-ethyl-25-fluoro-(21*M*)-21-[2-10 [(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacos-1(26),20,23(27),24-tetraene-9,15-dione (intermediate **F**). Example 2 (38.5 mg) was obtained as a yellow solid. MS calc'd 1248.6 (MH⁺), measured 1248.6 (MH⁺). ¹H NMR (400 MHz, METHANOL-*d*₄) δ = 8.69 (dd, *J* = 2.0, 7.6 Hz, 1H), 8.51 - 8.45 (m, 1H), 8.42 (d, *J* = 2.0 Hz, 1H), 7.68 - 7.66 (m, 1H), 7.52 - 7.44 (m, 1H), 5.78 - 5.67 (m, 1H), 5.22 - 5.12 (m, 1H), 4.76 (dd, *J* = 2.4, 11.2 Hz, 1H), 4.47 - 4.35 (m, 2H), 4.27 - 4.17 (m, 3H), 3.82 - 3.75 (m, 1H), 3.73 - 3.59 (m, 2H), 3.51 - 3.43 (m, 1H), 3.42 - 3.34 (m, 7H), 3.28 - 3.19 (m, 5H), 3.18 - 3.10 (m, 3H), 3.01 (d, *J* = 7.2 Hz, 6H), 2.88 (t, *J* = 5.2 Hz, 4H), 2.84 - 2.77 (m, 1H), 2.68 - 2.58 (m, 1H), 2.39 - 2.12 (m, 6H), 2.00 - 1.92 (m, 1H), 1.79 (d, *J* = 4.8 Hz, 7H), 1.71 - 1.58 (m, 1H), 1.45 (d, *J* = 6.4 Hz, 3H), 1.15 - 1.04 (m, 1H), 1.03 - 0.94 (m, 6H), 0.89 - 0.87 (d, *J* = 6.8 Hz, 3H), 0.47 (d, *J* = 6.0 Hz, 3H).

Example 3

1-[4-(dimethylamino)-4-methyl-pent-2-ynoyl]-N-[(1*S*)-1-[(7*S*,13*S*)-21-ethyl-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]-17,17-dimethyl-8,14-dioxo-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacos-

1(25),2,5(28),19,22(26),23-hexaen-7-yl]carbamoyl]-2-methyl-propyl]-4-fluoro-*N*-methyl-piperidine-4-carboxamide



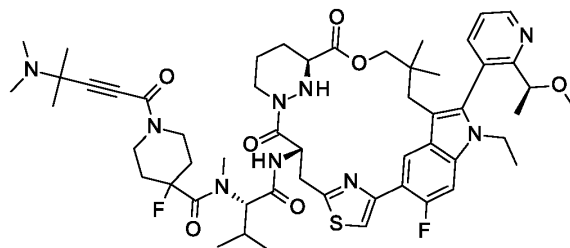
The title compound was prepared in analogy to the preparation of Example 1 by using
 5 (7*S*,13*S*)-7-amino-21-ethyl-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]-17,17-dimethyl-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacos-1(25),2,5(28),19,22(26),23-hexaene-8,14-dione (intermediate **G**) instead of (6*S*,8*S*,14*S*)-8-amino-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-5,16-dioxa-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacos-1(26),20,23(27),24-tetraene-9,15-dione
 10 (intermediate **F**). Example 3 (16.5 mg) was obtained as a white solid. MS calc'd 1113.6 (MH⁺), measured 1113.6 (MH⁺). ¹H NMR (400 MHz, METHANOL-*d*₄) δ = 8.66 (dd, *J* = 7.6 Hz, 3.2 Hz, 1H), 8.42 (d, *J* = 2.8 Hz, 1H), 7.62 (dd, *J* = 12.4 Hz, 0.8 Hz, 1H), 7.39 - 7.28 (m, 2H), 5.84 - 5.66 (m, 1H), 4.80 - 4.75 (m, 1H), 4.49 - 4.32 (m, 3H), 4.30 - 4.14 (m, 4H), 3.88 (t, *J* = 4.4 Hz, 4H),
 15 3.81 - 3.70 (m, 2H), 3.69 - 3.57 (m, 1H), 3.50 - 3.41 (m, 1H), 3.36 - 3.30 (m, 10H), 3.22 (t, *J* = 6.4 Hz, 3H), 3.10 - 3.01 (m, 1H), 2.89 - 2.76 (m, 1H), 2.70 - 2.58 (m, 1H), 2.39 (s, 3H), 2.36 (s, 3H), 2.33 - 2.26 (m, 2H), 2.25 - 2.13 (m, 3H), 2.01 - 1.92 (m, 1H), 1.87 - 1.75 (m, 1H), 1.71 - 1.57 (m, 1H), 1.50 (s, 5H), 1.45 (d, *J* = 3.0 Hz, 3H), 1.38 - 1.27 (m, 1H), 1.07 - 0.93 (m, 9H), 0.89 (d, *J* = 3.2 Hz, 3H), 0.51 (d, *J* = 4.2 Hz, 3H).

20

Example 4

1-[4-(dimethylamino)-4-methyl-pent-2-ynoyl]-*N*-[(1*S*)-1-[(7*S*,13*S*)-21-ethyl-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-17,17-dimethyl-8,14-dioxo-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacos-1(25),2,5(28),19,22(26),23-hexaen-7-yl]carbamoyl]-2-methyl-propyl]-4-fluoro-*N*-methyl-piperidine-4-carboxamide

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The title compound was prepared in analogy to the preparation of Example **1** by using (7*S*,13*S*)-7-amino-21-ethyl-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-17,17-dimethyl-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacos-

5 1(25),2,5(28),19,22(26),23-hexaene-8,14-dione (intermediate **H**) instead of (6*S*,8*S*,14*S*)-8-amino-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-5,16-dioxa-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraene-9,15-dione (intermediate **F**). Example **4** (44.0 mg) was obtained as a white solid. MS calc'd 1028.5 (MH⁺), measured 1028.5 (MH⁺). ¹H NMR (400 MHz, METHANOL-*d*₄) δ = 8.75 (dd, *J* = 4.8 Hz, 1.6 Hz, 1H), 8.67 (dd, *J* = 7.2 Hz, 2.4 Hz, 1H), 7.85 (dd, *J* = 7.6 Hz, 1.2 Hz, 1H), 7.62 (dd, *J* = 12.8 Hz, 2.4 Hz, 1H), 7.55 (dd, *J* = 8.0 Hz, 5.2 Hz, 1H), 7.35 (dd, *J* = 11.6 Hz, 5.2 Hz, 1H), 5.80 - 5.65 (m, 1H), 4.99 - 4.93 (m, 1H), 4.81 - 4.73 (m, 1H), 4.49 - 4.31 (m, 4H), 4.28 - 4.10 (m, 3H), 3.80 - 3.70 (m, 2H), 3.68 - 3.57 (m, 1H), 3.49 - 3.40 (m, 1H), 3.39 - 3.34 (m, 3H), 3.30 - 3.25 (m, 1H), 3.23 - 3.18 (m, 3H), 3.10 - 3.02 (m, 1H), 2.89 - 2.77 (m, 1H), 2.66 - 2.55 (m,

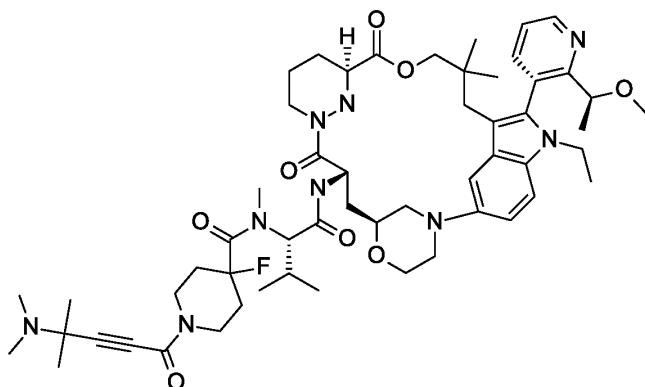
15 1H), 2.39 (*s*, 3H), 2.36 (*s*, 3H), 2.33 - 2.27 (m, 2H), 2.27 - 2.11 (m, 4H), 2.01 - 1.90 (m, 1H), 1.87 - 1.74 (m, 1H), 1.69 - 1.59 (m, 1H), 1.53 - 1.41 (m, 9H), 1.04 - 0.83 (m, 12H), 0.48 (*d*, *J* = 6.8 Hz, 3H).

BIOLOGICAL EXAMPLE

Compound **A647** (page 176 of Table.1, or referred as "**RMC-6291**"), from

20 WO2021091982 was cited as reference compound for this invention.

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(RMC-6291)

Example 5

5 Single dose pharmacokinetics (PK) study in female BALB/c mice

The purpose of this study was to determine the pharmacokinetics of selected compounds following single intravenous bolus or oral gavage administration in female BALB/c mice. Briefly, two groups of female BALB/c mice (available from Shanghai Lingchang Biotechnology Co., Ltd) (N=3/group) were treated with a single dose of compound intravenously at 3 mg/kg (IV) or orally at 30 mg/kg (PO). Blood samples were collected at 5 min (only for IV), 15 min, 30 min, 1 h, 2 h, 4 h, 7 h and 24 h post-dose. Blood samples were placed on ice until centrifugation to obtain plasma samples. The concentration of compound in plasma samples was determined using LC-MS/MS method. The pharmacokinetic parameters were calculated by non-compartmental analysis.

15 **Table 1. Results of SDPK**

Compound	3 mg/kg, iv	30 mg/kg, po	
	CL (mL/min/kg)	C _{max} (ng/mL)	AUC _{0-last} (h×ng/mL)
RMC-6291	44.2	2706	4116
Example 1	8.4	9919	24959

From Table 1, it can be seen that Example **1** has excellent pharmacokinetic properties in mouse model. Especially Example **1** has almost 3.7 folds of $C_{\max}$, 6 folds of $AUC_{0-\text{last}}$ and significant lower clearance compared with compound RMC-6291, which makes Example **1** more

suitable for treating cancers with RAS mutation as an orally therapeutic active ingredient in clinic.

Example 6

5 In vivo xenograft studies

The purpose of the study was to assess the anti-tumor activity in difficult KRAS mutated xenograft tumor model, NCI-H2122 which is a non-small cell lung cancer (NSCLC), by examining the tumor suppression post-administration of Example 1 and RMC-6291.

Studies were conducted at Wuxi AppTec (Nantong, China). All CDX mouse studies and
10 procedures related to animal handling, care and treatment were conducted in compliance with all applicable regulations and guidelines of the relevant Institutional Animal Care and Use Committee (IACUC). Mice were maintained under pathogen-free conditions, and food and water was provided ad libitum.

Female BALB/c nude mice at 6-8 weeks old from Vital River Co., LTD. were used for
15 these studies. In order to generate subcutaneous xenograft tumors, each mouse was inoculated at the right flank with tumor cells in 200 μ L of PBS and 50% matrigel matrix in the right hind flank with 5×10^6 cells. Mouse health was monitored daily, and caliper measurements began when tumors were palpable. Tumor volume measurements were determined utilizing the formula $0.5 \times L \times W^2$ in which L refers to length and W refers to width of each tumor. When tumors reached
20 an average tumor volume of approximately 350 mm^3 , mice were randomized into treatment groups.

Mice were treated by oral gavage at 10 mg/kg with either vehicle consisting of DMSO: Solutol HS15 :water=10:10:80 (%v/v/v) or compounds (Example 1 or RMC-6291) in vehicle at indicated doses. For efficacy studies, animals were orally administered daily, tumor volumes and
25 body weights were measured 3 times per week. Study day on efficacy plots indicates the day after which treatment was initiated.

$$\text{TGI (tumor growth inhibition)\%} = (\text{Final treated tumor volume} - \text{Initial treated tumor volume}) / (\text{Vehicle final treated tumor volume} - \text{Vehicle initial treated tumor volume}) \times 100\%$$

$$\text{Tumor regression} = (-100\%) \times (1 - (\text{Final treated tumor volume}) / (\text{Initial treated tumor volume}))$$

30

$$\text{Tumor growth} = 100\% \times (\text{Final treated tumor volume} - \text{Initial treated tumor volume}) / (\text{Initial treated tumor volume})$$

Daily administration of Example 1 at 100 mg/kg was well-tolerated based on body weights and resulted in 108% mean tumor growth inhibition (TGI) or -32% tumor regression following 21 days treatment in comparison to RMC-6291 that leads to only 92% TGI or unwanted 35% tumor growth at same dose regiments. (Figure 2)

5 **Table 2. Summary of in vivo efficacy in NCI-H2122 xenograft study**

	Tumor Volume (mm ³ , Mean/SEM)		TGI (Day 21)	Tumor regression (Day 21)	Tumor growth (Day 21)
	Day 0	Day 21			
Vehicle, QD	348±26	1799±223			
RMC-6291 100mpk,QD	348±29	470±49	92%		35%
Example 1 100mpk, QD	348±29	237±11	108%	-32%	

Example 7

Cell viability assay

10 The purpose of this cellular assay was to determine the effects of test compounds on the proliferation of human cancer cell lines NCI-H358 (ATCC-CRL5807) cells over a 3-day treatment period by quantifying the amount of NADPH present at endpoint using Cell Counting Kit-8.

15 Cells were seeded at 5,000 cells/well (NCI-H358) in 96-well assay plates (Corning-3699) and incubated overnight. On the day of the assay, diluted compounds were then added in a final concentration of 0.5% DMSO. After 72 hrs incubation, a tenth of the volume of cell counting kit 8(Dnjindo-CK04) was added into each well. Read the signal (OD450 minus OD650) using EnVision after 2 hrs incubation. IC₅₀ was determined by fitting a 4-parameter sigmoidal concentration response model.

20 **Table 3. Activity of Examples and Compounds of present invention in KRAS Cell viability assay**

Example	G12C IC ₅₀ (nM)
Example 1	0.42
Example 2	0.44
Example 3	0.62
Example 4	0.41

Example 8

KRAS-BRAF with CYPA (500 nM) interaction assay

In this example, TR-FRET was also used to measure the compound or compound-CYPA dependent disruption of the KRAS G12C-BRAF complex. In assay buffer containing 25mM HEPES PH=7.4 (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, Thermo, 15630080), 0.002% Tween20, 0.1% BSA, 100mM NaCl, 5mM MgCl₂, 10 μM GMPPNP (Guanosine 5'-[β,γ-imido]triphosphate trisodium salt hydrate, Sigma, G0635), tagless CYPA, GMPPNP loaded 6His-KRAS proteins, and GST-BRAF^{RBD} were mixed in a well of a 384-well assay plate at final concentrations of 50 nM, 6.25 nM and 1nM, respectively. Compound was present in plate wells as a 16-point 3-fold dilution series starting at a final concentration of 10 μM and incubated for 3 hours. A mixture of Mab Anti-6His-XL665 (Cisbio, 61HISXLB) and Mab anti-GST-TB cryptate (Cisbio, 61GSTTLB) was then added at a final concentration of 6.67 nM and 0.21 nM, respectively, and the plate was incubated for an additional 1.5 hours. TR-FRET signal was read on a PHERstar FSX microplate reader (Ex320 nm, Em 665/615 nm). Compounds that facilitate disruption of the KRAS-BRAF complex were identified as those eliciting a decrease in the TR-FRET ratio relative to DMSO control wells.

Table 4. Activity of Examples and Compounds of present invention in KRAS-BRAF with CYPA (500 nM) interaction assay

Example	G12C IC ₅₀ (nM)
Example 1	0.50
Example 2	0.60
Example 3	0.50
Example 4	0.70

Example 9

pERK inhibition assay

This assay is to measure the ability of test compounds in inhibiting the phosphorylation of ERK, the downstream signaling of KRAS G12C in NCI-H358 cells. NCI-H358 (ATCC-CRL5807) cells was grown and maintained using RPMI-1640 medium (Thermo Fisher Scientific) with 10% fetal bovine serum and 1% penicillin/streptomycin. On the day prior to compound

addition, cells were plated in tissue culture-treated 96 well plates (Corning-3699) at a density of 30,000 cell/well for NCI-H358 respectively, and allowed for attachment overnight. Diluted compounds were then added in a final concentration of 0.5% DMSO. After 4 hours of incubation, the medium was removed, 100 μ L of 4% formaldehyde was added, and the assay plates were
5 incubated at room temperature for 20 minutes. The plates were then washed once with phosphate buffered saline (PBS), and permeabilized with 100 μ L of chilled methanol for 10 minutes. Non-specific antibody binding to the plates was blocked using 50 μ L 1X BSA blocking buffer (Thermo-37520, 10-fold dilution by Phosphate-Buffered Saline Tween (PBST) for at least 1 hour at room temperature.

10 The amount of phosphor-ERK was determined using an antibody specific for phosphorylated form of ERK. Primary antibody (pERK, CST-4370, Cell Signaling Technology) was diluted 1:300 in blocking buffer, with 50 μ L aliquoted to each well, and incubated overnight at 4 $^{\circ}$ C. Cells was washed five times for 5 minutes with PBST. Secondary antibody (HRP-linked anti-rabbit IgG, CST-7074, Cell Signaling Technology) was diluted 1:1000 in blocking buffer,
15 and 50 μ L was added to each well and incubated 1-2 hrs at room temperature. Cells was washed 5 times for 5 minutes with PBST, 100 μ L TMB ELISA substrate (abcam-ab171523) were added and gently shake for 20 minutes. 50 μ L stop solution (abcam-ab171529) were added, and then read the signal (OD450) by EnVision.

IC₅₀ was determined by fitting a 4-parameter sigmoidal concentration response model.

20 **Table 5. Activity of Examples and Compounds of present invention in KRAS pERK inhibition assay**

Example	G12C IC ₅₀ (nM)
Example 1	1.54
Example 2	0.96
Example 3	2.31
Example 4	1.29

CLAIMS

1. A compound which is 1-[4-(dimethylamino)-4-methyl-pent-2-ynoyl]-*N*-[(1*S*)-1-[(6*S*,8*S*,14*S*)-22-ethyl-25-fluoro-(21*M*)-21-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-18,18-dimethyl-9,15-dioxo-5,16-dioxo-2,10,22,28-tetrazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]carbamoyl]-2-methyl-propyl]-4-fluoro-*N*-methyl-piperidine-4-carboxamide, or a pharmaceutically acceptable salt thereof.
2. A compound which is 1-[4-(dimethylamino)-4-methyl-pent-2-ynoyl]-4-fluoro-*N*-[(1*S*)-1-[[[(7*S*,13*S*)-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-5-[4-(2,2,2-trifluoroethyl)piperazin-1-yl]-3-pyridyl]-17,17-dimethyl-8,14-dioxo-21-(2,2,2-trifluoroethyl)-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[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]carbamoyl]-2-methyl-propyl]-*N*-methyl-piperidine-4-carboxamide, or a pharmaceutically acceptable salt thereof.
3. A compound which is 1-[4-(dimethylamino)-4-methyl-pent-2-ynoyl]-*N*-[(1*S*)-1-[(7*S*,13*S*)-21-ethyl-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-5-morpholino-3-pyridyl]-17,17-dimethyl-8,14-dioxo-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[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]carbamoyl]-2-methyl-propyl]-4-fluoro-*N*-methyl-piperidine-4-carboxamide, or a pharmaceutically acceptable salt thereof.
4. A compound which is 1-[4-(dimethylamino)-4-methyl-pent-2-ynoyl]-*N*-[(1*S*)-1-[(7*S*,13*S*)-21-ethyl-24-fluoro-(20*M*)-20-[2-[(1*S*)-1-methoxyethyl]-3-pyridyl]-17,17-dimethyl-8,14-dioxo-15-oxa-4-thia-9,21,27,28-tetrazapentacyclo[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]carbamoyl]-2-methyl-propyl]-4-fluoro-*N*-methyl-piperidine-4-carboxamide, or a pharmaceutically acceptable salt thereof.
5. A compound or pharmaceutically acceptable salt according to any one of claims 1 to 4 for use as therapeutically active substance.
6. A pharmaceutical composition comprising a compound in accordance with any one of claims 1 to 4 and a pharmaceutically acceptable excipient.

7. The use of a compound according to any one of claims 1 to 4 for treating a KRAS G12C protein-related disease.
8. The use of a compound according to any one of claims 1 to 4 for inhibiting RAS interaction
5 with downstream effectors, wherein the downstream effectors are RAF and PI3K.
9. The use of a compound according to any one of claims 1 to 4 for inhibiting the propagating oncogenic MAPK and PI3K signaling.
- 10 10. The use of a compound according to any one of claims 1 to 4 for the treatment or prophylaxis of KRAS mutation driven cancers, wherein the cancer is selected from pancreatic cancer, colorectal cancer, lung cancer, esophageal cancer, gallbladder cancer, melanoma ovarian cancer and endometrial cancer.
- 15 11. The use of a compound according to any one of claims 1 to 4 for the treatment or prophylaxis of KRAS mutation driven cancers, wherein the cancer is selected from pancreatic adenocarcinoma, colorectal cancer and non-small cell lung cancer.
12. A compound or pharmaceutically acceptable salt according to any one of claims 1 to 4 for
20 the treatment or prophylaxis of KRAS mutation driven cancers, wherein the cancer is selected from pancreatic adenocarcinoma, colorectal cancer and non-small cell lung cancer.
13. The use of a compound according to any one of claims 1 to 4 for the preparation of a medicament for the treatment or prophylaxis of KRAS mutation driven cancers, wherein the
25 cancer is selected from pancreatic adenocarcinoma, colorectal cancer and non-small cell lung cancer.
14. A method for the treatment or prophylaxis of KRAS mutation driven cancers, wherein the cancer is selected from pancreatic adenocarcinoma, colorectal cancer and non-small cell lung
30 cancer, which method comprises administering a therapeutically effective amount of a compound as defined in any one of claims 1 to 4.