

DRUG CONJUGATES AND METHODS OF PREPARING AND USING THE SAME

Priority Claims and Related Patent Applications

[0001] This application claims the benefit of priority to PCT/CN2023/073383, filed January 20, 2023, the entire content of which is incorporated herein by reference.

Technical Field of the Invention

[0002] The invention generally relates to novel compounds, methods of preparation, and therapeutic uses thereof. More particularly, the invention provides novel linkers, linker conjugates, and drug conjugates thereof, as well as methods of preparation and use thereof for treating various diseases or conditions.

Background of the Invention

[0003] Drug conjugation (*e.g.*, antibody drug conjugates (ADCs)) can provide an effective approach for delivering a drug to a targeted site in a tissue or organ. Twelve ADCs have been approved by the FDA to date, including gemtuzumab ozogamicin (Mylotarg™), the first ADC approved by the FDA in 2000. (See, *e.g.*, Drago *et al.* **2021** *Nature Reviews* 18, 327-344; Mckertish *et al.* **2021** *Biomedicines* 9, 872; Khongorzui *et al.* **2020** *Molecular Cancer Res.* 18:3–19; Bross *et al.* **2001** *Clin. Cancer Res.* 7, 1490–1496; Hamann *et al.* **2002** *Bioconjug. Chem.* 13, 47–58; Lamb, **2017** *Drugs* 77, 1603–1610.) Lessons learned from the development of these ADCs highlight the importance of optimizing the method for drug to protein attachment. Cysteine modification has gained popularity due to high nucleophilicity, selectivity towards electrophiles, and low natural abundance sulfhydryl group-bearing amino acid residues in naturally occurring proteins.

[0004] Maleimide-based linkers have been used in a number of ADCs currently under investigation. This is in part due to maleimide's high reactivity and selectivity towards cysteine residues. One conventional method employed in the design of ADCs includes the use of self-

hydrolyzing maleimides for cysteine modification, as maleimides react rapidly and selectively with thiols. While maleimide conjugation has led to stable drug-protein conjugation, the self-hydrolyzing maleimides generate acid species. The acid species can have unforeseen and deleterious effects on the properties of the resulting ADC. The maleimide moiety has been directly associated with instability in maleimide-based ADCs in plasma. In addition, ADCs have been shown to undergo reduction in drug antibody ratios (DARs) in the presence of glutathione. (See, *e.g.*, WIPO 2013/173337; Alley *et al.* **2008** *Bioconjugate Chem.* 19, 759.)

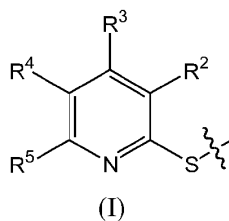
[0005] There remains a need for drug conjugates and conjugation methodologies that feature improved efficiency, stability and drug loading.

Summary of the Invention

[0006] Drug conjugates comprising a targeting moiety, a linker and a drug moiety, methods of preparing the same, and methods of treating and/or preventing a condition using the same are provided herein.

[0007] In particular, the invention provides linker strategies that significantly expand available cysteine-targeted electrophilic chemotypes with demonstrated high selectivity towards thiols (*e.g.*, cysteine) over other reactive functionalities such as amines. The linker compounds and methodologies disclosed herein allow efficient conjugation at low pH (*e.g.*, ~pH of 6.0), no retro-Michael deconjugation, and drug-linker conjugates with overall good stability at room temperature.

[0008] In one aspect, the invention generally relates to a drug conjugate comprising a targeting moiety, a linker moiety, and a drug moiety, wherein the drug moiety is conjugated to the linker which is conjugated to the targeting moiety, and wherein the linker moiety comprises the structural formula (I):



wherein:

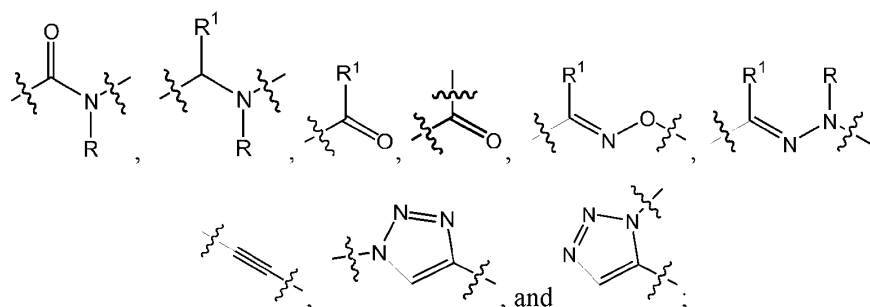
each R^2 , R^3 , R^4 is independently selected from the group consisting of H, NO_2 , CN, CF_3 , F, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne, C_{6-10} aryl, and W;

R^5 is H, Cl, SOR^6 , SO_2R^6 , OR^7 , NRR^7 or W;

R^6 is an unsubstituted or substituted C_{1-6} alkyl or C_{6-10} aryl;

R^7 is an unsubstituted or substituted C_{2-6} alkyne or C_{2-6} alkyne;

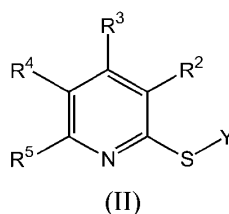
W is selected from the group consisting of:



R^1 is H, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne or C_{6-10} aryl;

R is H, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne or C_{6-10} aryl; or a pharmaceutically acceptable salt thereof.

[0009] In another aspect, the invention generally relates to a drug conjugate comprising a targeting moiety, a linker moiety, and a drug moiety, wherein the drug moiety is conjugated to the linker which is conjugated to the targeting moiety, and wherein the linker moiety comprises the structural formula (II):



wherein:

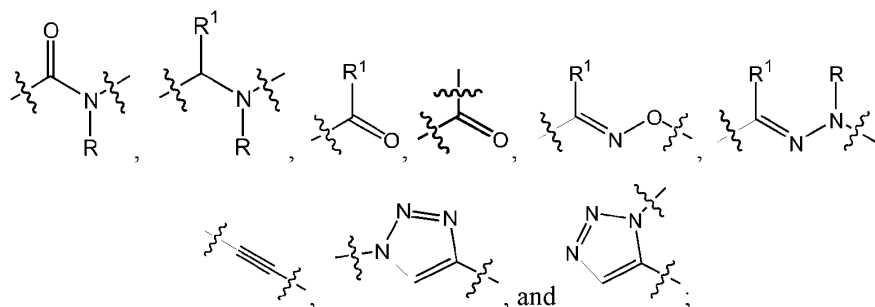
each R^2 , R^3 , R^4 is independently selected from the group consisting of H, NO_2 , CN, CF_3 , F, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne, C_{6-10} aryl, and W;

R^5 is H, Cl, SOR^6 , SO_2R^6 , OR^7 , NRR^7 or W;

R^6 is an unsubstituted or substituted C_{1-6} alkyl or C_{6-10} aryl;

R⁷ is an unsubstituted or substituted C₂₋₆ alkyne or C₂₋₆ alkyne;

W is selected from the group consisting of:



R¹ is H, an unsubstituted or substituted C₁₋₆ alkyl, C₂₋₆ alkyne, C₂₋₆ alkyne or C₆₋₁₀ aryl;

R is H, an unsubstituted or substituted C₁₋₆ alkyl, C₂₋₆ alkyne, C₂₋₆ alkyne or C₆₋₁₀ aryl;

and

each Y is independently a peptide or an antigen-binding moiety that comprises a cysteine moiety,

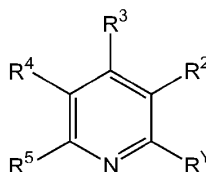
or a pharmaceutically acceptable salt thereof.

[0010] In yet another aspect, the invention generally relates to a composition comprising a drug conjugate disclosed herein.

[0011] In yet another aspect, the invention generally relates to a pharmaceutical composition comprising a drug conjugate disclosed herein, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient, carrier or diluent.

[0012] In yet another aspect, the invention generally relates to a unit dosage form comprising a pharmaceutical composition of the invention.

[0013] In yet another aspect, the invention generally relates to a compound useful for forming a linker moiety-drug moiety conjugate, a targeting moiety-linker moiety conjugate, or a targeting moiety-linker moiety-drug moiety conjugate, the compound having a structure comprising structural formula (III):



(III)

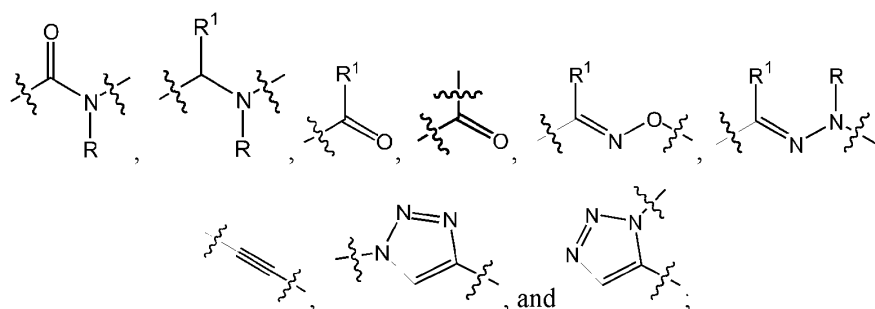
each R^2 , R^3 , R^4 is independently selected from the group consisting of H, NO_2 , CN, CF_3 , F, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne, C_{6-10} aryl, and W;

each R^5 and R^Y is independently selected from the group consisting of H, Cl, SOR^6 , SO_2R^6 , OR^7 , NRR^7 and W;

R^6 is an unsubstituted or substituted C_{1-6} alkyl or C_{6-10} aryl;

R^7 is an unsubstituted or substituted C_{2-6} alkyne or C_{2-6} alkyne;

W is selected from the group consisting of:



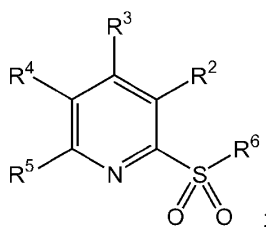
R^1 is H, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne or C_{6-10} aryl;
and

R is H, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne or C_{6-10} aryl.

[0014] In yet another aspect, the invention generally relates to a composition comprising a compound disclosed herein that is useful for forming a linker moiety-drug moiety conjugate, a targeting moiety-linker moiety conjugate, or a targeting moiety-linker moiety-drug moiety conjugate.

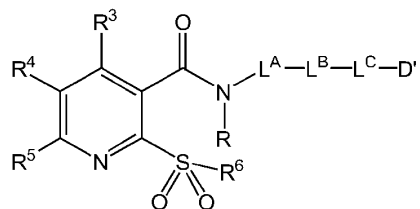
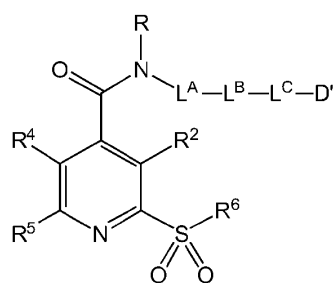
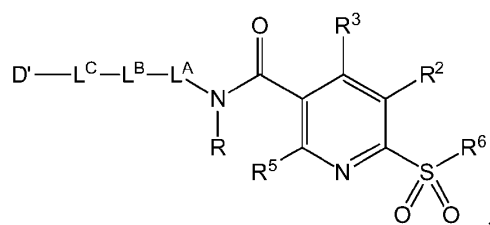
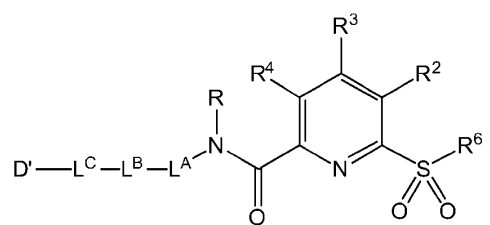
[0015] In yet another aspect, the invention generally relates to a method of preparing a linker-drug conjugate comprising:

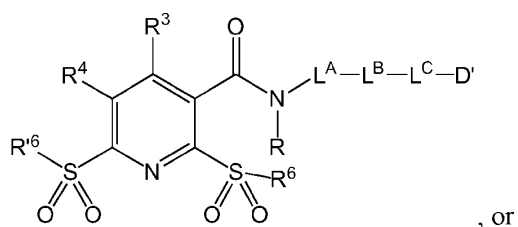
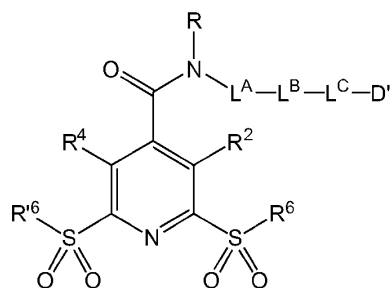
(a) providing a compound having the structural formula:



(III¹)

(b) reacting the compound with a precursor to a spacer, a precursor to a peptide, and/or a precursor to a self-immolative moiety, to form a conjugate having the structural formula:

(III²)(III³)(III⁴)

(III⁵)(III⁶)(III⁷)

wherein

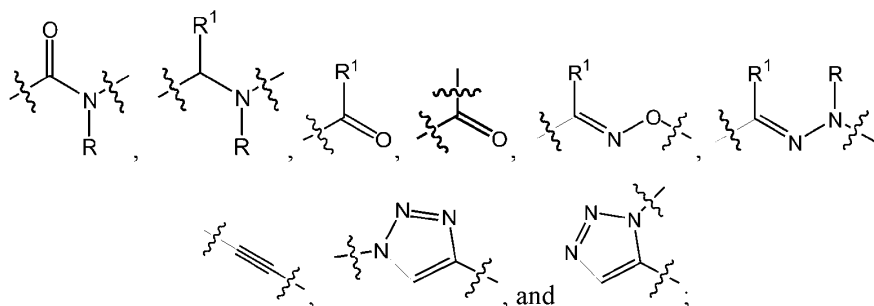
each R², R³, R⁴ is independently selected from the group consisting of H, NO₂, CN, CF₃, F, an unsubstituted or substituted C₁₋₆ alkyl, C₂₋₆ alkyne, C₂₋₆ alkyne, C₆₋₁₀ aryl, and W;

R⁵ is H, Cl, SOR⁶, SO₂R⁶, OR⁷, NRR⁷ or W;

R⁶ is an unsubstituted or substituted C₁₋₆ alkyl or C₆₋₁₀ aryl;

R⁷ is an unsubstituted or substituted C₂₋₆ alkyne or C₂₋₆ alkyne;

W is selected from the group consisting of:



R^1 is H, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyene, C_{2-6} alkyne or C_{6-10} aryl;
 R is H, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyene, C_{2-6} alkyne or C_{6-10} aryl;
 L^A is a spacer moiety or is absent;
 L^B is a peptide moiety or is absent;
 L^C is a self-immolative moiety or is absent; and
 D' is a drug moiety D or is absent.

[0016] In yet another aspect, the invention generally relates to a method of treating and/or preventing a condition in a subject in need thereof, the method comprising administering to the subject a drug conjugate of the invention.

[0017] In yet another aspect, the invention generally relates to use of a drug conjugate disclosed herein for the manufacture of a medicament.

[0018] In yet another aspect, the invention generally relates to use of a drug conjugate disclosed herein for treating one or more of cancer, an autoimmune disorder, or an infectious disease.

Brief Description of the Drawings

[0019] **FIG. 1** shows certain exemplary RP-HPLC data.

[0020] **FIG. 2** shows certain exemplary RP-HPLC and HIC data.

[0021] **FIG. 3** shows certain exemplary LCMS data.

Detailed Description of the Invention

[0022] As set forth herein, novel linkers, linking methodologies and conjugates, and drug conjugates have been developed that possess unexpected advantages over prior art.

[0023] More particularly, linkers, linker conjugates and conjugation strategies disclosed herein significantly expand available cysteine-targeted electrophilic chemotypes with demonstrated high selectivity towards thiols (*e.g.*, cysteine) over other reactive functionalities such as amines. The invention affords the ability to efficiently conduct conjugation at low pH (*e.g.*, ~pH of 6.0), without retro-Michael deconjugation thus leading to high stability. In addition, drug-linker conjugates of the invention show overall good stability at room temperature.

[0024] This disclosure further provides drug conjugates, methods of their preparation, kits comprising these drug conjugates and components thereof, as well as methods of using the drug conjugates and kits in the treatment of one or more diseases or conditions.

Definitions

[0025] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. General principles of organic chemistry, as well as specific functional moieties and reactivity, are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: 2006.

[0026] The following terms, unless indicated otherwise according to the context wherein the terms are found, are intended to have the following meanings.

[0027] Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 16 is understood to include any number, combination of numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16.

[0028] As used herein, "at least" a specific value is understood to be that value and all values greater than that value.

[0029] In this specification and the appended claims, the singular forms "a," "an," and "the" include plural reference, unless the context clearly dictates otherwise.

[0030] Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive.

[0031] Any compositions or methods disclosed herein can be combined with one or more of any of the other compositions and methods provided herein.

[0032] The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

[0033] The term "comprising", when used to define compositions and methods, is intended to mean that the compositions and methods include the recited elements, but do not exclude other elements. The term "consisting essentially of", when used to define compositions and

methods, shall mean that the compositions and methods include the recited elements and exclude other elements of any essential significance to the compositions and methods. For example, “consisting essentially of” refers to administration of the pharmacologically active agents expressly recited and excludes pharmacologically active agents not expressly recited. The term consisting essentially of does not exclude pharmacologically inactive or inert agents, *e.g.*, pharmaceutically acceptable excipients, carriers or diluents. The term “consisting of”, when used to define compositions and methods, shall mean excluding trace elements of other ingredients and substantial method steps. Embodiments defined by each of these transition terms are within the scope of this invention.

[0034] Certain compounds of the present invention may exist in particular geometric or stereoisomeric forms. The present invention contemplates all such compounds, including *cis*- and *trans*-isomers, atropisomers, *R*- and *S*-enantiomers, diastereomers, (D)-isomers, (L)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention. In certain embodiments, each asymmetric atom has at least 50 % enantiomeric excess, at least 60 % enantiomeric excess, at least 70 % enantiomeric excess, at least 80 % enantiomeric excess, at least 90 % enantiomeric excess, at least 95 % enantiomeric excess, or at least 99 % enantiomeric excess of either the *R*- or *S*-configuration. For optically active compounds, it is often preferred to use one enantiomer to the substantial exclusion of the other enantiomer.

[0035] Isomeric mixtures containing any of a variety of isomer ratios may be utilized in accordance with the present invention. For example, where only two isomers are combined, mixtures containing 50:50, 60:40, 70:30, 80:20, 90:10, 95:5, 96:4, 97:3, 98:2, 99:1, or 100:0 isomer ratios are contemplated by the present invention. Those of ordinary skill in the art will readily appreciate that analogous ratios are contemplated for more complex isomer mixtures.

[0036] If, for instance, a particular enantiomer of a compound of the present invention is desired, it may be prepared by asymmetric synthesis, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed by resolution of the

diastereomers thus formed by fractional crystallization or chromatographic methods well known in the art, and subsequent recovery of the pure enantiomers.

[0037] A mixture of isomers can be separated on the basis of the physicochemical differences of the constituents, into the pure or substantially pure geometric or optical isomers, diastereomers, racemates, for example, by chromatography and/or fractional crystallization.

[0038] Definitions of specific functional groups and chemical terms are described in more detail below. When a range of values is listed, it is intended to encompass each value and sub-range within the range. For example, "C₁₋₆ alkyl" is intended to encompass, C₁, C₂, C₃, C₄, C₅, C₆, C₁₋₆, C₁₋₅, C₁₋₄, C₁₋₃, C₁₋₂, C₂₋₆, C₂₋₅, C₂₋₄, C₂₋₃, C₃₋₆, C₃₋₅, C₃₋₄, C₄₋₆, C₄₋₅, and C₅₋₆ alkyl.

[0039] Where substituent groups are specified by their conventional chemical formulae, written from left to right, they equally encompass the chemically identical substituents that would result from writing the structure from right to left, *e.g.*, -C(=O)-O- is equivalent to -O-C(=O)-.

[0040] Structures of compounds of the invention are limited by principles of chemical bonding known to those skilled in the art. Accordingly, where a group may be substituted by one or more of a number of substituents, such substitutions are selected so as to comply with principles of chemical bonding and to give compounds that are not inherently unstable and/or would be known to one of ordinary skill in the art as likely to be unstable under ambient conditions (*e.g.*, aqueous, neutral, and several known physiological conditions).

[0041] The use of numerical values in the various quantitative values specified in this application, unless expressly indicated otherwise, are stated as approximations as though the minimum and maximum values within the stated ranges were both preceded by the word "about." It is to be understood, although not always explicitly stated, that all numerical designations are preceded by the term "about." It is to be understood that such range format is used for convenience and brevity and should be understood flexibly to include numerical values explicitly specified as limits of a range, but also to include all individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range is explicitly specified. For example, a ratio in the range of about 1 to about 200 should be understood to include the explicitly recited limits of about 1 and about 200, but also to include individual ratios such as about 2, about 3, and about 4, and sub-ranges such as about 10 to about 50, about 20 to about 100, and so forth. It also is to be understood, although not always explicitly stated, that the

reagents described herein are merely exemplary and that equivalents of such are known in the art.

[0042] Unless specifically stated or obvious from context, the term “about,” as used herein when referring to a measurable value such as an amount or concentration and the like, is meant to encompass variations of 20%, 10%, 5%, 1%, 0.5%, or even 0.1% of the specified amount.

[0043] The terms “treat,” “treating,” and “treatment” as used herein with regard to a condition refer to alleviating the condition partially or entirely; slowing the progression or development of the condition; eliminating, reducing, or slowing the development of one or more symptoms associated with the condition; or increasing progression-free or overall survival of the condition.

[0044] Treatment may be directed at one or more effects or symptoms of a disease and/or the underlying pathology. The treatment can be any reduction and can be, but is not limited to, the complete ablation of the disease or the symptoms of the disease. Treating or treatment thus refers to any indicia of success in the therapy or amelioration of an injury, disease, pathology or condition, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the injury, pathology or condition more tolerable to the patient; slowing in the rate of degeneration or decline; making the final point of degeneration less debilitating; improving a patient's physical or mental well-being. The treatment or amelioration of symptoms can be based on objective or subjective parameters, for example, the results of a physical examination, neuropsychiatric exams, and/or a psychiatric evaluation. As compared with an equivalent untreated control, such reduction or degree of amelioration may be at least 5%, 10%, 20%, 40%, 50%, 60%, 80%, 90%, 95%, or 100% as measured by any standard technique.

[0045] Treatment methods include administering to a subject a therapeutically effective amount of a compound described herein. The administering step may be a single administration or may include a series of administrations. The length of the treatment period depends on a variety of factors, such as the severity of the condition, the patient's age, the concentration of the compound, the activity of the compositions used in the treatment, or a combination thereof. It will also be appreciated that the effective dosage of an agent used for the treatment may increase or decrease over the course of a particular treatment regime. Changes in dosage may result and become apparent by standard diagnostic assays known in the art. In some instances, chronic

administration may be required. For example, the compositions are administered to the subject in an amount and for a duration sufficient to treat the patient.

[0046] The terms “prevent,” “preventing,” and “prevention” as used herein with regard to a condition refers to averting the onset of the condition or decreasing the likelihood of occurrence or recurrence of the condition, including in a subject that may be predisposed to the condition but has not yet been diagnosed as having the condition.

[0047] As used herein, the terms “disease,” “condition” or “disorder” are used interchangeably herein and refer to a pathological condition, for example, one that can be identified by symptoms or other identifying factors as diverging from a healthy or a normal state. The term “disease” includes disorders, syndromes, conditions, and injuries. Diseases include, but are not limited to, proliferative, inflammatory, immune, metabolic, infectious, and ischemic diseases.

[0048] The term “cancer” may refer to any accelerated proliferation of cells, including solid tumors, ascites tumors, blood or lymph or other malignancies; connective tissue malignancies; metastatic disease; minimal residual disease following transplantation of organs or stem cells; multi-drug resistant cancers, primary or secondary malignancies, angiogenesis related to malignancy, or other forms of cancer. Thus, the terms “cancer” and “cancerous” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma, lymphoma, sarcoma, blastoma and leukemia. More particular examples of such cancers include squamous cell carcinoma, lung cancer, pancreatic cancer, cervical cancer, bladder cancer, hepatoma, breast cancer, colon carcinoma, and head and neck cancer.

[0049] The term “autoimmune disorder” may refer to a set of sustained organ-specific or systemic clinical symptoms and signs associated with altered immune homeostasis that is manifested by qualitative and/or quantitative defects of expressed autoimmune repertoires.

[0050] The term “infectious disease” may refer to any disease caused by an infectious organism such as a virus, bacteria, parasite, and/or fungus.

[0051] As used herein, the term “in need of” a treatment refers to a subject that would benefit biologically, medically or in quality of life from such a treatment.

[0052] The term “alkyl” describes an aliphatic hydrocarbon including straight chain and branched chain groups.

[0053] The term “heteroalkyl” describes an aliphatic hydrocarbon including straight chain and branched chain groups substituted with one or more atoms such nitrogen, oxygen, and sulfur.

[0054] The term “amino acid” as used herein refers to a molecule of the general formula $\text{NH}_2\text{-CHR-COOH}$, wherein "R" is one of a number of different side chains, or a residue within a peptide bearing the parent amino acid. Amino acids include naturally occurring amino acids with "R" being a substituent found in naturally occurring amino acids. "R" can also be a substituent that is not found in naturally occurring amino acids. The term "amino acid residue" refers to the portion of the amino acid which remains after losing a water molecule when it is joined to another amino acid. The term "modified amino acid" refers to an amino acid bearing an "R" substituent that does not correspond to one of the twenty genetically coded amino acids.

[0055] The term “antibody” as used herein refers to an immunoglobulin molecule or an immunologically active portion thereof that binds to a specific antigen, e.g., a cancer cell antigen, viral antigen, or microbial antigen. In those embodiments where the targeting moiety is an antibody and the antibody is a full-length immunoglobulin molecule, the antibody comprises two heavy chains and two light chains, with each heavy and light chain containing three complementary determining regions (CDRs). In those embodiments where the targeting moiety is an antibody and the antibody is an immunologically active portion of an immunoglobulin molecule, the antibody may be, for example, a Fab, Fab', Fv, F(ab')₂, disulfide-linked Fv, scFv, single domain antibody (dAb), diabody, triabody, tetrabody, or linear antibody. Antibodies used as targeting moieties may be, for example, natural antibodies, synthetic antibodies, monoclonal antibodies, polyclonal antibodies, chimeric antibodies, humanized antibodies, multispecific antibodies, bispecific antibodies, dual-specific antibodies, anti-idiotypic antibodies, or fragments thereof that retain the ability to bind a specific antigen.

[0056] As used herein, the term “pharmaceutically acceptable salt” refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of subjects without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, Berge et al. describes pharmaceutically acceptable salts in detail in J. *Pharmaceutical Sciences* (1977) 66:1-19. Pharmaceutically acceptable salts of the compounds provided herein include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino

group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchlorate acid or with organic acids such as acetic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, besylate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, p-toluenesulfonate, undecanoate, valerate salts, and the like. In some embodiments, organic acids from which salts can be derived include, for example, acetic acid, propionic acid, glycolic acid, pyruvic acid, lactic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like.

[0057] The salts can be prepared *in situ* during the isolation and purification of the disclosed compounds, or separately, such as by reacting the free base or free acid of a parent compound with a suitable base or acid, respectively. Pharmaceutically acceptable salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium and $N^+(C_{1-4}alkyl)_4$ salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, iron, zinc, copper, manganese, aluminum, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate and aryl sulfonate. Organic bases from which salts can be derived include, for example, primary, secondary, and tertiary amines, substituted amines, including naturally occurring substituted amines, cyclic amines, basic ion exchange resins, and the like, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine. In some embodiments, the pharmaceutically acceptable base addition salt can be chosen from ammonium, potassium, sodium, calcium, and magnesium salts.

[0058] As used herein, the term “pharmaceutically acceptable” excipient, carrier, or diluent refers to a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject pharmaceutical agent from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations. Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate, magnesium stearate, and polyethylene oxide-polypropylene oxide copolymer as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

[0059] As used herein, the terms “protein” and “polypeptide” are used interchangeably to refer to a polymer of amino acid residues, and are not limited to a minimum length. Thus, peptides, oligopeptides, dimers, multimers, and the like, are included within the definition. Both full-length proteins and fragments thereof are encompassed by the definition. The terms also include post-expression modifications of the polypeptide, for example, glycosylation, acetylation, phosphorylation, and the like. Furthermore, a polypeptide may refer to a protein which includes modifications, such as deletions, additions, and substitutions (generally conservative in nature), to the native sequence, as long as the protein maintains the desired activity. These modifications may be deliberate or may be accidental. Amino acids can be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission.

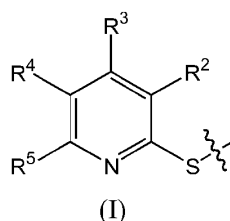
[0060] As used herein, the term “subject” refers to any animal (*e.g.*, a mammal), including, but not limited to humans, non-human primates, rodents, and the like, which is to be the recipient of a particular treatment. A subject to which administration is contemplated includes, but is not limited to, humans (*e.g.*, a male or female of any age group, *e.g.*, a pediatric subject (*e.g.*, infant, child, adolescent) or adult subject (*e.g.*, young adult, middle-aged adult or senior adult)) and/or other non-human animals, for example, non-human mammals (*e.g.*, primates (*e.g.*, cynomolgus monkeys, rhesus monkeys); commercially relevant mammals such as cattle, pigs, horses, sheep, goats, cats, and/or dogs), rodents (*e.g.*, rats and/or mice), etc. In certain embodiments, the non-human animal is a mammal. The non-human animal may be a male or female at any stage of development. A non-human animal may be a transgenic animal. Typically, the terms “subject” and “patient” are used interchangeably herein in reference to a human subject.

[0061] Ranges recited herein are intended as continuous ranges, including every value between the minimum and maximum values recited, as well as any ranges that can be formed by such values. Also disclosed herein are any and all ratios (and ranges of any such ratios) that can be formed by dividing a disclosed numeric value into any other disclosed numeric value. Accordingly, the skilled person will appreciate that many such ratios, ranges, and ranges of ratios can be unambiguously derived from the numerical values presented herein, and in all instances such ratios, ranges, and ranges of ratios represent various embodiments of the present disclosure.

Targeting Moiety-Linker-Drug Conjugates

[0062] Provided herein in certain embodiments are drug conjugates comprising a linker, a drug moiety, and a targeting moiety. Also provided herein are components of these drug conjugates, including for example linkers, linker-drug moiety complexes, and linker-targeting moiety complexes.

[0063] In one aspect, the invention generally relates to a drug conjugate comprising a targeting moiety, a linker moiety, and a drug moiety, wherein the drug moiety is conjugated to the linker which is conjugated to the targeting moiety, and wherein the linker moiety comprises the structural formula (I):



wherein:

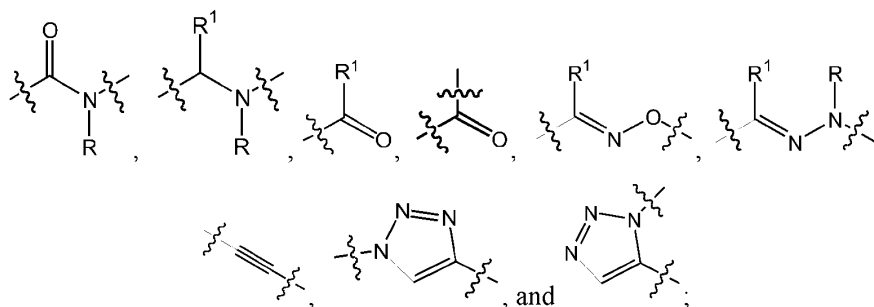
each R^2 , R^3 , R^4 is independently selected from the group consisting of H, NO_2 , CN, CF_3 , F, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkene, C_{6-10} aryl, and W;

R^5 is H, Cl, SOR^6 , SO_2R^6 , OR^7 , NRR^7 or W;

R^6 is an unsubstituted or substituted C_{1-6} alkyl or C_{6-10} aryl;

R^7 is an unsubstituted or substituted C_{2-6} alkyne or C_{2-6} alkene;

W is selected from the group consisting of:



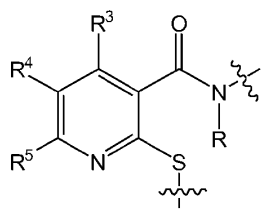
R^1 is H, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkene or C_{6-10} aryl;

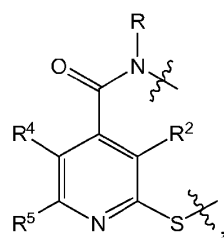
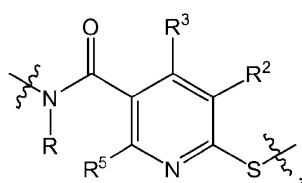
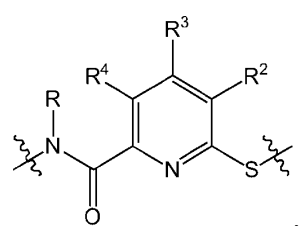
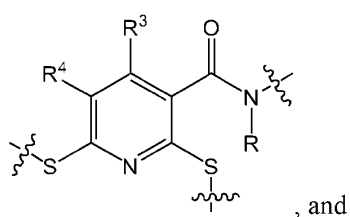
R is H, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkene or C_{6-10} aryl;

or a pharmaceutically acceptable salt thereof.

[0064] In certain embodiments, R^6 is an unsubstituted or substituted C_{1-6} alkyl.

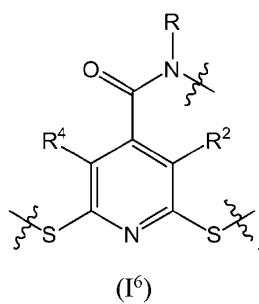
[0065] In certain embodiments, the drug conjugate of (I) has a structural formula selected from:



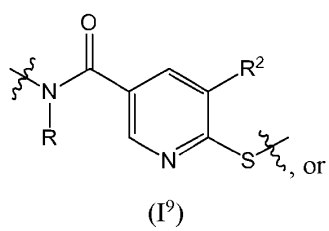
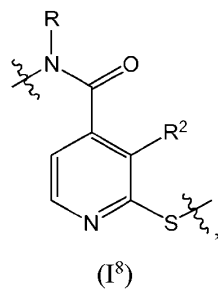
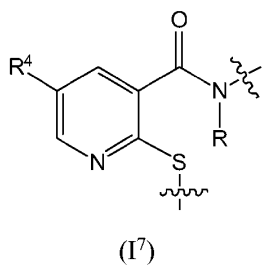
(I¹)(I²)(I³)(I⁴)

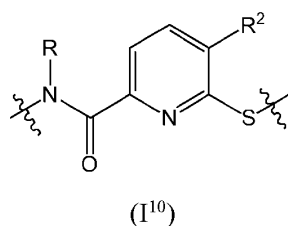
, and

(I⁵)



[0066] In certain embodiments, the drug conjugate of (I) has a structural formula selected from:





[0067] In certain embodiments of the drug conjugate of (I)-(I¹⁰), one of R², R³ and R⁴ is selected from the group consisting of NO₂, CN, CF₃ and F.

[0068] In certain embodiments, R is H. In certain embodiments, R is an unsubstituted or substituted C₁₋₆ alkyl.

[0069] In certain embodiments, one of R², R³ and R⁴ is NO₂.

[0070] In certain embodiments, one of R², R³ and R⁴ is CN.

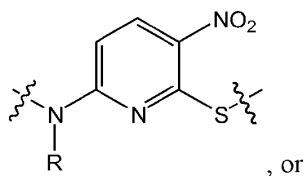
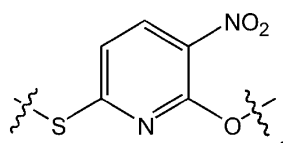
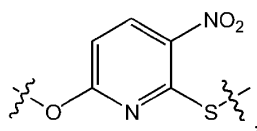
[0071] In certain embodiments, one of R² or R⁴ is CF₃.

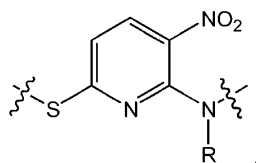
[0072] In certain embodiments, R⁴ is C(O)OR and R² is not CF₃.

[0073] In certain embodiments, R² is not CF₃.

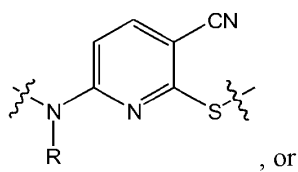
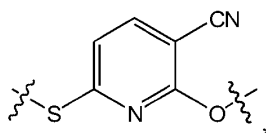
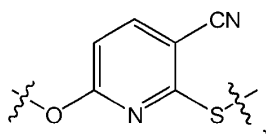
[0074] In certain embodiments, R² or R⁴ is F.

[0075] In certain embodiments of (I), the drug conjugate has a structural formula selected from:

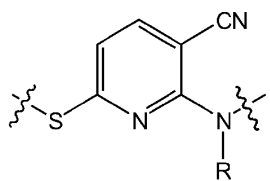




[0076] In certain embodiments of (I), the drug conjugate has a structural formula selected from:



, or



[0077] In certain embodiments of (I), the linker moiety has a structural formula of (I¹) or (I⁷).

[0078] In certain embodiments of (I), the linker moiety has a structural formula of (I²) or (I⁸).

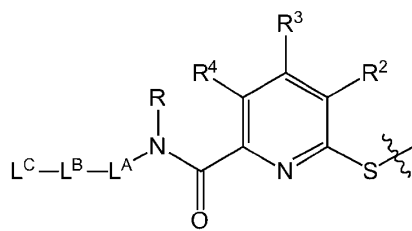
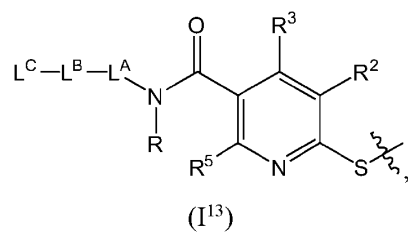
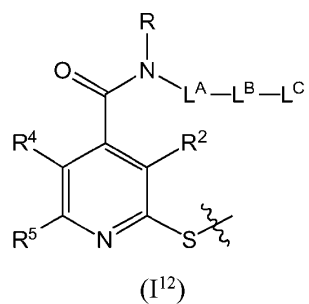
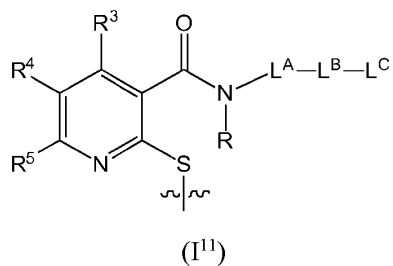
[0079] In certain embodiments of (I), the linker moiety has a structural formula of (I³) or (I⁹).

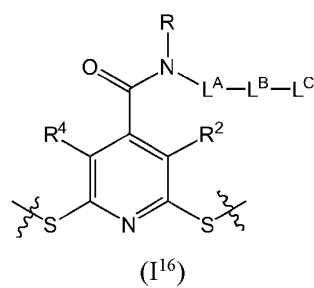
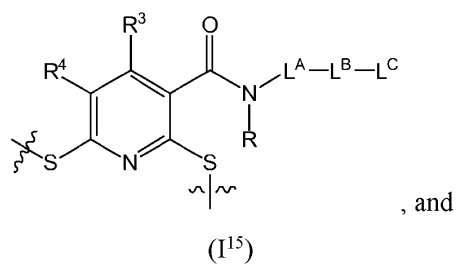
[0080] In certain embodiments of (I), the linker moiety has a structural formula of (I⁴) or (I¹⁰).

[0081] In certain embodiments of (I), the linker moiety has a structural formula of (I⁵) or (I⁶).

[0082] In certain embodiments of (I), the linker moiety further has a spacer, a peptide moiety, and/or a self-immolative moiety. In certain embodiments, the spacer, the peptide moiety, and/or the self-immolative moiety are conjugated to one of R^2 , R^3 , R^4 and R^5 .

[0083] In certain embodiments of (I), the drug conjugate has a structural formula selected from:



(I¹⁴)

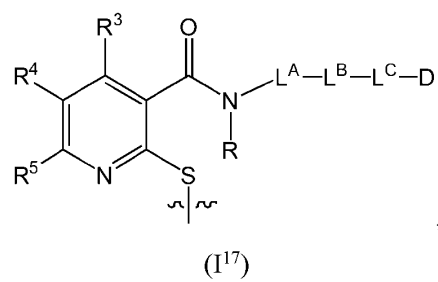
wherein

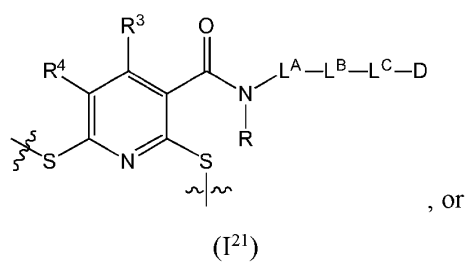
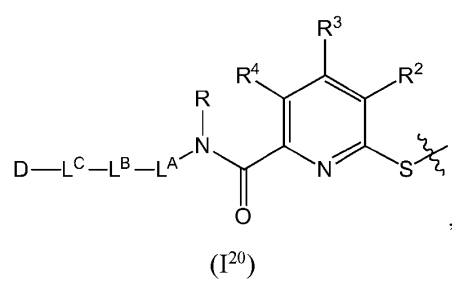
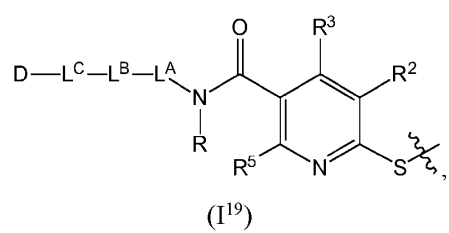
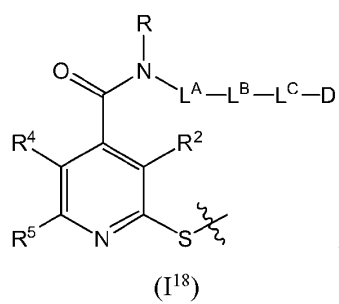
L^A is a spacer moiety or is absent;

L^B is a peptide moiety or is absent; and

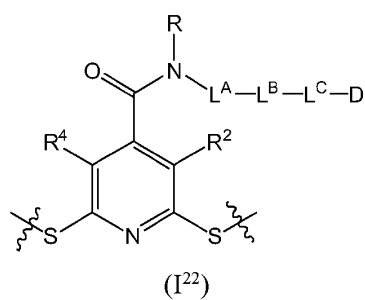
L^C is a self-immolative moiety or is absent.

[0084] In certain embodiments, the drug conjugate further has a drug moiety D:



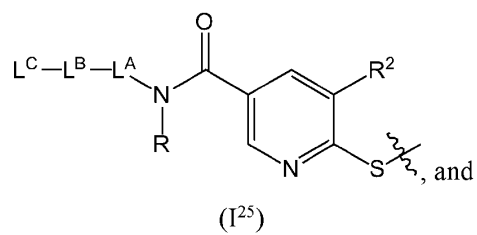
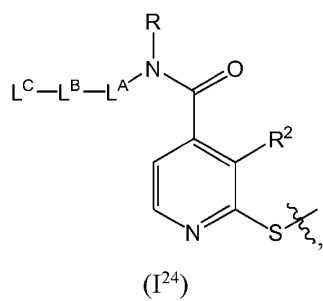
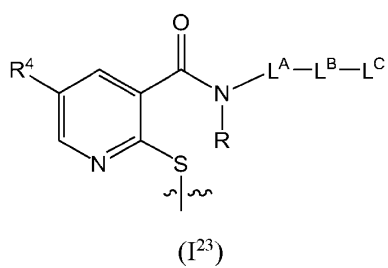


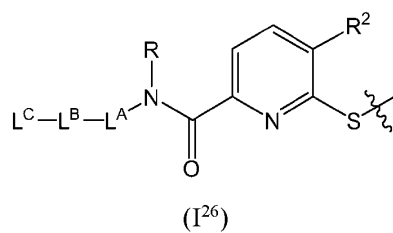
, or



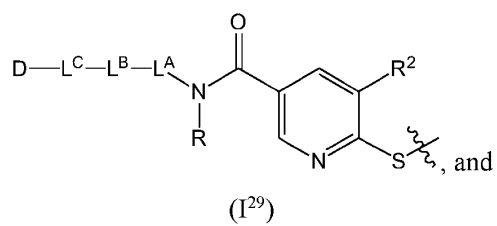
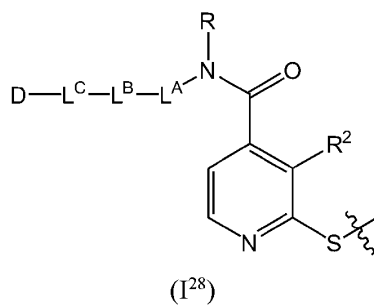
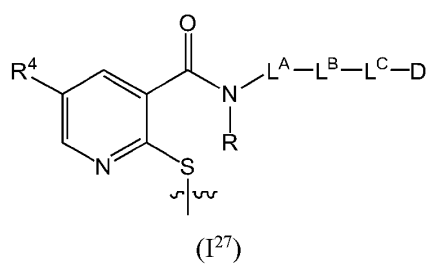
wherein D is a drug moiety.

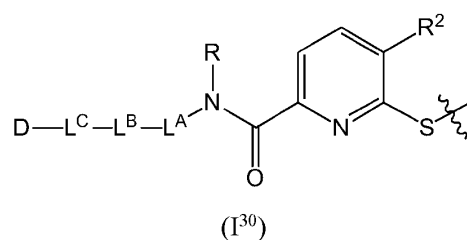
[0085] In certain embodiments of (I), the drug conjugate has a structural formula selected from:





[0086] In certain embodiments of (I), the drug conjugate has a structural formula selected from:



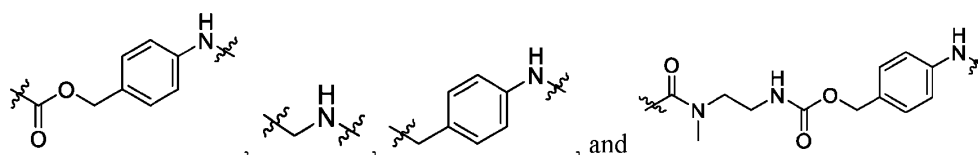


[0087] In certain embodiments of (I¹¹)-(I³⁰), the spacer moiety is selected from the group consisting of an alkyl, a heteroalkyl, polyethylene glycol (PEG), and a peptide.

[0088] In certain embodiments of (I¹¹)-(I³⁰), the peptide moiety comprises 1 to 6 (*e.g.*, 1, 2, 3, 4, 5 or 6) amino acid units.

[0089] In certain embodiments of (I¹¹)-(I³⁰), one or more of the amino acids are natural amino acids. In certain embodiments, one or more of the amino acids are unnatural amino acids.

[0090] In certain embodiments of (I¹¹)-(I³⁰), the self-immolative moiety is selected from the group consisting of:



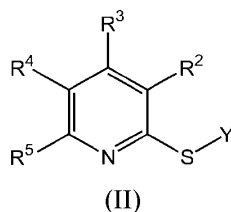
[0091] In certain embodiments, the drug moiety is a chemical agent selected from the group consisting of an antibiotic, an anti-cancer agent, a steroid, a TLR7/TLR9 antagonist, a peptide, a protein, and a nucleic acid.

[0092] In certain embodiments, the targeting moiety is selected from the group consisting of an antibody, small molecule, a peptide and a nucleic acid.

[0093] In certain embodiments, the drug conjugate has a targeting moiety to drug moiety ratio of about 1:1 to about 1:16 (*e.g.*, about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:7, about 1:8, about 1:9, about 1:10, about 1:11, about 1:12, about 1:13, about 1:14, about 1:15 or about 1:16).

[0094] In another aspect, the invention generally relates to a drug conjugate comprising a targeting moiety, a linker moiety, and a drug moiety, wherein the drug moiety is conjugated to

the linker which is conjugated to the targeting moiety, and wherein the linker moiety comprises the structural formula (II):



wherein:

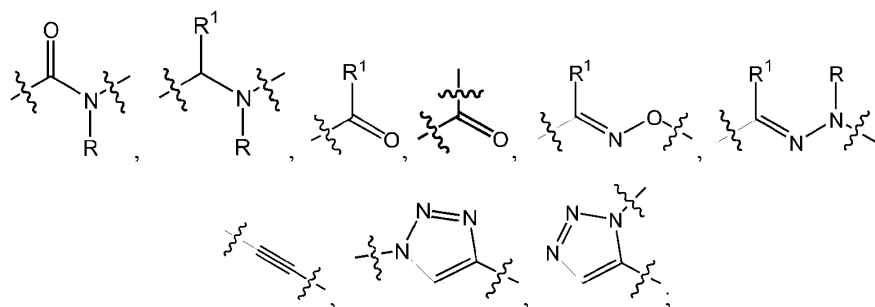
each R^2 , R^3 , R^4 is independently selected from H, NO_2 , CN, CF_3 , F, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne or C_{6-10} aryl, or W;

R^5 is H, Cl, SOR^6 , SO_2R^6 , OR^7 , NRR^7 or W;

R^6 is an unsubstituted or substituted C_{1-6} alkyl or C_{6-10} aryl;

R^7 is an unsubstituted or substituted C_{2-6} alkyne or C_{2-6} alkyne;

W is selected from:



R^1 is H, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne or C_{6-10} aryl;

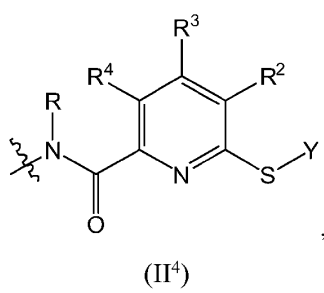
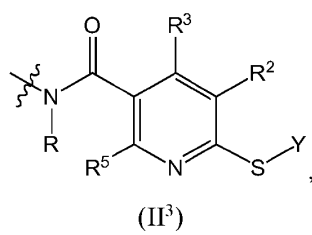
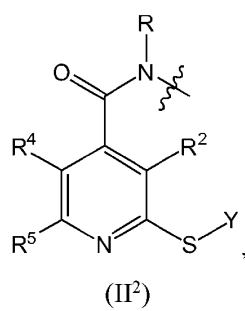
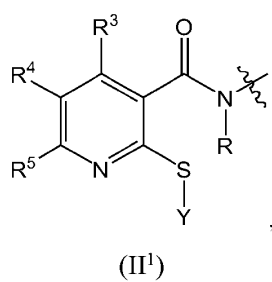
R is H, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne or C_{6-10} aryl;

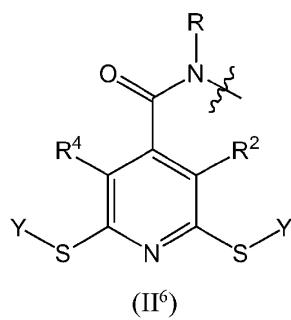
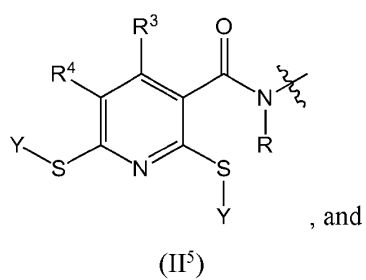
and

each Y is independently a peptide or an antigen-binding moiety that comprises a cysteine moiety,
or a pharmaceutically acceptable salt thereof.

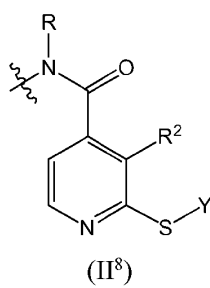
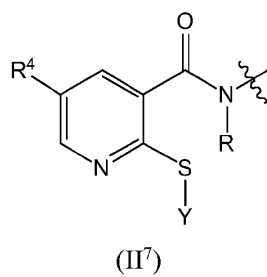
[0095] In certain embodiments, R^6 is an unsubstituted or substituted C_{1-6} alkyl.

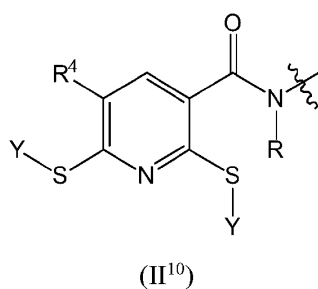
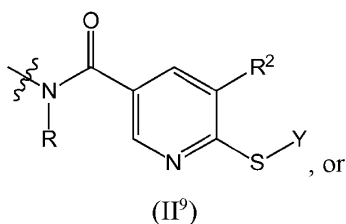
[0096] In certain embodiments of (II), the drug conjugate has a structural formula selected from:





[0097] In certain embodiments of (II), the drug conjugate has a structural formula selected from:





[0098] In certain embodiments of (II)-(II¹⁰), one of R², R³ and R⁴ is selected from the group consisting of NO₂, CN, CF₃ and F.

[0099] In certain embodiments of (II)-(II¹⁰), R is H. In certain embodiments, R is an unsubstituted or substituted C₁₋₆ alkyl.

[0100] In certain embodiments, one of R², R³ and R⁴ is NO₂.

[0101] In certain embodiments, one of R², R³ and R⁴ is CN.

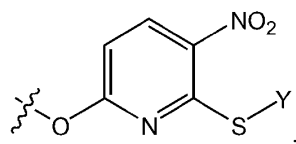
[0102] In certain embodiments, R² or R⁴ is CF₃.

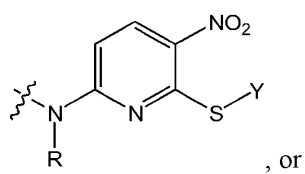
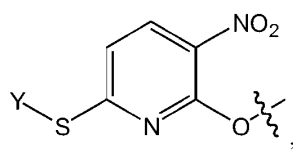
[0103] In certain embodiments, R⁴ is C(O)OR and R² is not CF₃.

[0104] In certain embodiments, R² is not CF₃.

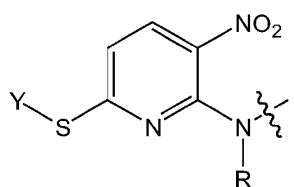
[0105] In certain embodiments, R² or R⁴ is F.

[0106] In certain embodiments of (II), the drug conjugate has a structural formula selected from:



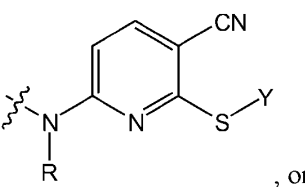
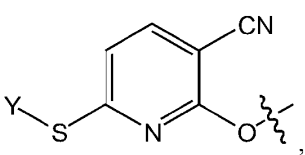
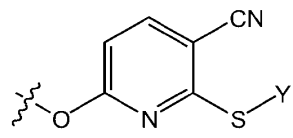


, or

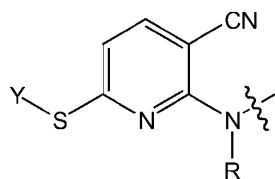


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[0107] In certain embodiments of (II), the drug conjugate has a structural formula selected from:

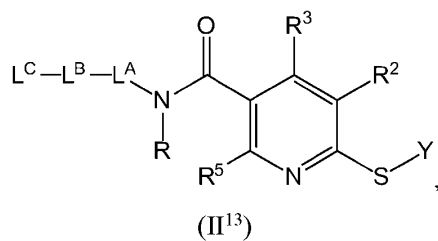
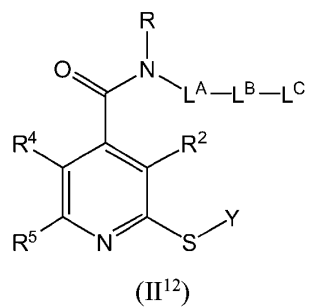
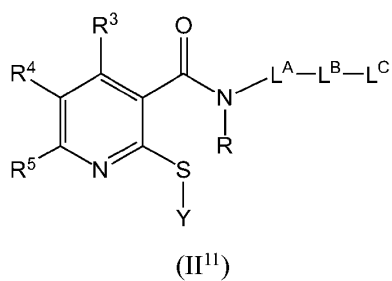


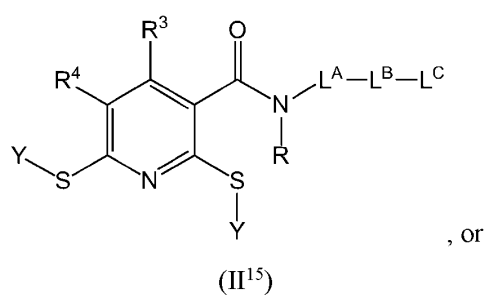
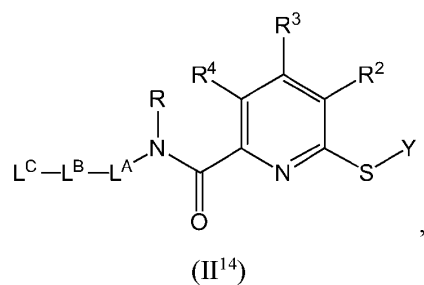
, or



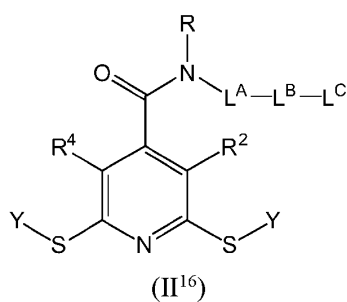
[0108] In certain embodiments of the drug conjugate, the linker moiety further has a spacer, a peptide moiety, and/or a self-immolative moiety.

[0109] In certain embodiments of (II), the drug conjugate has a structural formula selected from:





, or



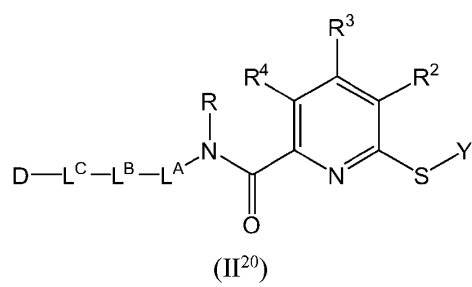
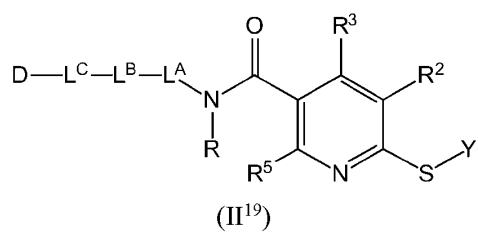
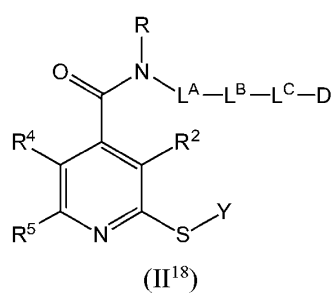
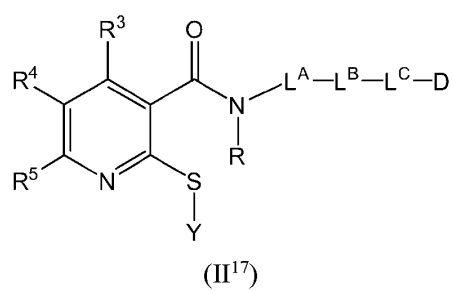
wherein

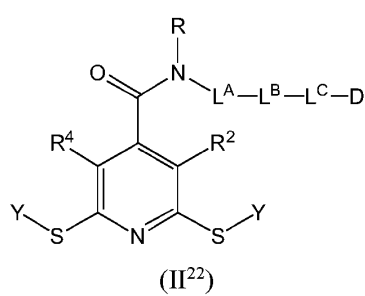
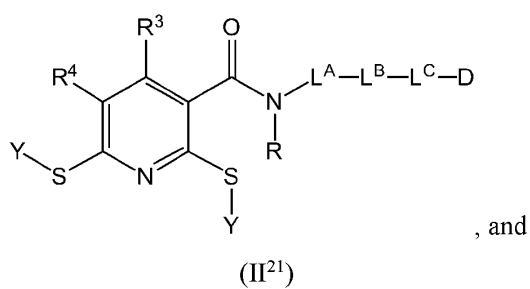
L^A is a spacer moiety or is absent;

L^B is a peptide moiety or is absent; and

L^C is a self-immolative moiety or is absent.

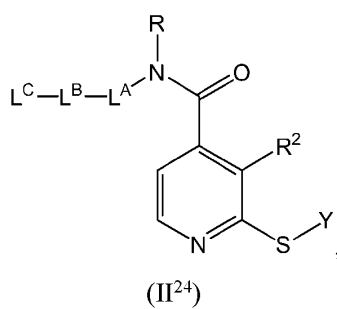
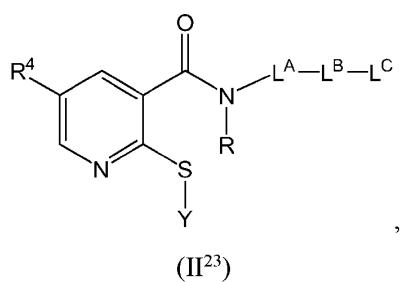
[0110] In certain embodiments, the drug conjugate further has a drug moiety, having a structural formula selected from:

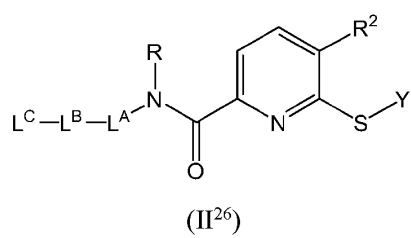
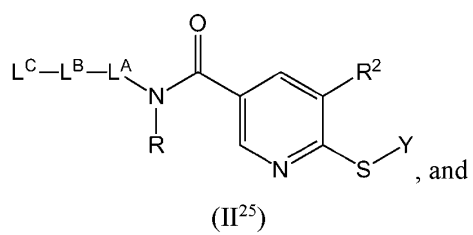




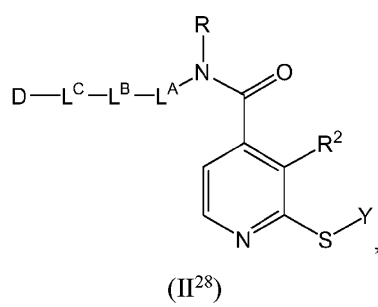
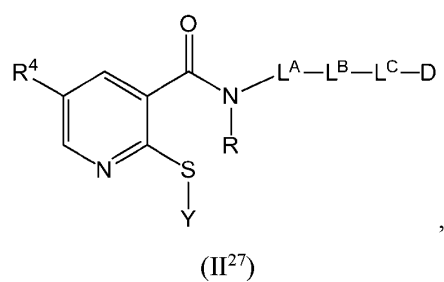
wherein D is a drug moiety.

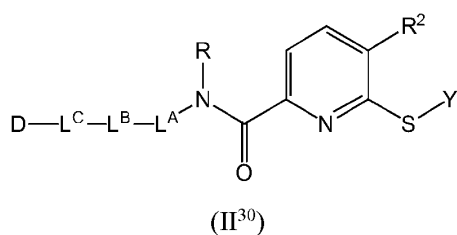
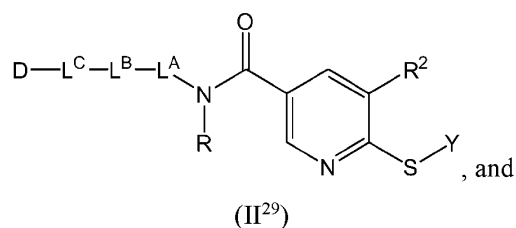
[0111] In certain embodiments, the drug conjugate has a structural formula selected from:





[0112] In certain embodiments, the drug conjugate further has a drug moiety, having a structural formula selected from:



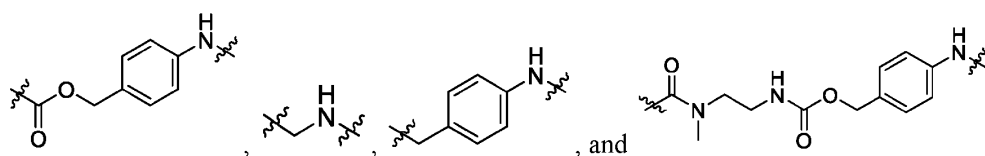


[0113] In certain embodiments of (II¹¹)-(II³⁰), the spacer moiety is selected from the group consisting of an alkyl, a heteroalkyl, PEG, and a peptide.

[0114] In certain embodiments of (II¹¹)-(II³⁰), the peptide moiety comprises 1 to 6 (*e.g.*, 1, 2, 3, 4, 5 or 6) amino acid units.

[0115] In certain embodiments of (II¹¹)-(II³⁰), one or more of the amino acids are natural amino acids. In certain embodiments, one or more of the amino acids are unnatural amino acids.

[0116] In certain embodiments of (II¹¹)-(II³⁰), the self-immolative moiety is selected from the group consisting of:



[0117] In certain embodiments, the drug moiety is a chemical agent selected from the group consisting of an antibiotic, an anti-cancer agent, a steroid, a TLR7/TLR9 antagonist, a peptide, a protein, and a nucleic acid.

[0118] In certain embodiments, the targeting moiety is selected from the group consisting of an antibody, small molecule, a peptide and a nucleic acid.

[0119] In certain embodiments, the drug conjugate has a targeting moiety to drug moiety ratio of about 1:1 to about 1:16 (*e.g.*, about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:7, about 1:8, about 1:9, about 1:10, about 1:11, about 1:12, about 1:13, about 1:14, about 1:15 or about 1:16).

[0120] In yet another aspect, the invention generally relates to a composition comprising a drug conjugate disclosed herein.

[0121] In yet another aspect, the invention generally relates to a pharmaceutical composition comprising a drug conjugate disclosed herein, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient, carrier or diluent.

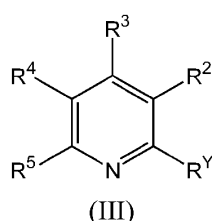
[0122] The invention thus provides a pharmaceutical preparation comprising a therapeutically effective amount of a compound or immunoconjugate according to the invention.

[0123] Examples of excipients that may be useful include, but not limited to, water, saline, dextrose, mannitol, lactose, lecithin, albumin, sodium glutamate, cysteine hydrochloride, starches, celluloses and gums. In a preferred embodiment, the pharmaceutical composition of the invention is formulated in a pharmaceutical form for administration as a solid (for example tablets, capsules, lozenges, granules, suppositories, crystalline or amorphous sterile solids that can be reconstituted to provide liquid forms, *etc.*), liquid (for example solutions, suspensions, emulsions, elixirs, lotions, unguents, *etc.*) or semi-solid (gels, ointments, creams and similar). The pharmaceutical compositions of the invention can be administered by any route, including, without limitation, oral, intravenous, intramuscular, intraarterial, intramedullary, intratecal, intraventricular, transdermic, subcutaneous, intraperitoneal, intranasal, enteric, topical, sublingual or rectal route. A revision of the different forms of administration of active principles, the excipients to be used and their manufacturing procedures can be found in Remington's Pharmaceutical Sciences (A. R. Gennaro, Ed.), 20th edition, Williams & Wilkins PA, USA (2000). Examples of pharmaceutically acceptable vehicles are known in the state of the technique and include saline solutions buffered with phosphate, water, emulsions, such as oil/water emulsions, different types of humidifying agents, sterile solutions, *etc.* The compositions comprising said vehicles can be formulated by conventional procedures known in the state of the technique. Preservatives, stabilizers, dyes and even flavoring agents, antioxidants and/or suspending agents can be provided in the pharmaceutical composition. For example, sodium benzoate, ascorbic acid and esters of p-hydroxybenzoic acid can be added as preservatives.

[0124] In yet another aspect, the invention generally relates to a unit dosage form comprising a pharmaceutical composition of the invention.

Linkers, Linker-Drug Conjugates, Targeting Moiety-Linker Conjugates

[0125] In yet another aspect, the invention generally relates to a compound useful for forming a linker moiety-drug moiety conjugate, a targeting moiety-linker moiety conjugate, or a targeting moiety-linker moiety-drug moiety conjugate, the compound having a structure comprising structural formula (III):



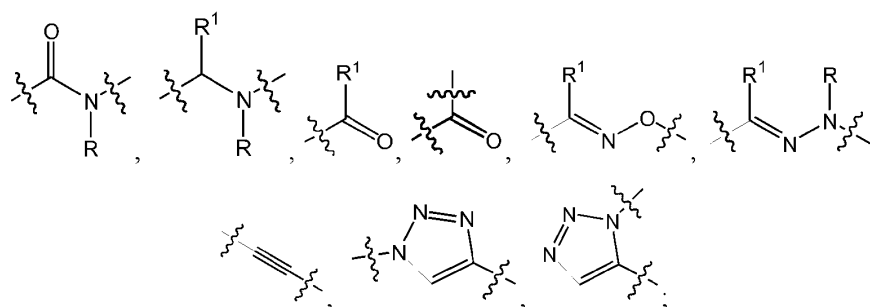
each R^2 , R^3 , R^4 is independently selected from the group consisting of H, NO_2 , CN, CF_3 , F, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne, C_{6-10} aryl, and W;

each R^5 and R^Y is independently selected from the group consisting of H, Cl, SOR^6 , SO_2R^6 , OR^7 , NRR^7 and W;

R^6 is an unsubstituted or substituted C_{1-6} alkyl or C_{6-10} aryl;

R^7 is an unsubstituted or substituted C_{2-6} alkyne or C_{2-6} alkyne;

W is selected from:

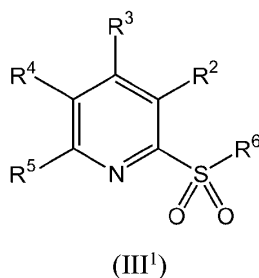


R^1 is H, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne or C_{6-10} aryl;
and

R is H, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne or C_{6-10} aryl.

[0126] In certain embodiments, R^6 is an unsubstituted or substituted C_{1-6} alkyl.

[0127] In certain embodiments of (III), R^Y is SO_2R^6 and the compound has the structural formula:



[0128] In certain embodiments of (III) or (III¹), one of R^2 , R^3 and R^4 is selected from the group consisting of NO_2 , CN , CF_3 and F .

[0129] In certain embodiments of (III) or (III¹), R is H . In certain embodiments of (III) or (III¹), R is an unsubstituted or substituted C_{1-6} alkyl.

[0130] In certain embodiments of (III) or (III¹), one of R^2 , R^3 and R^4 is NO_2 .

[0131] In certain embodiments of (III) or (III¹), one of R^2 , R^3 , R^4 and R^5 is CN .

[0132] In certain embodiments of (III) or (III¹), R^2 or R^4 is CF_3 .

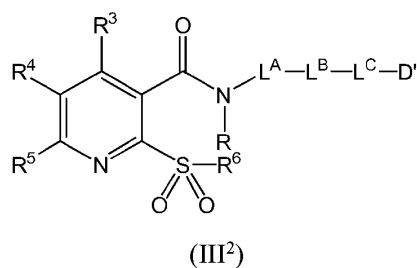
[0133] In certain embodiments of (III) or (III¹), R^4 is $C(O)OR$ and R^2 is not CF_3 .

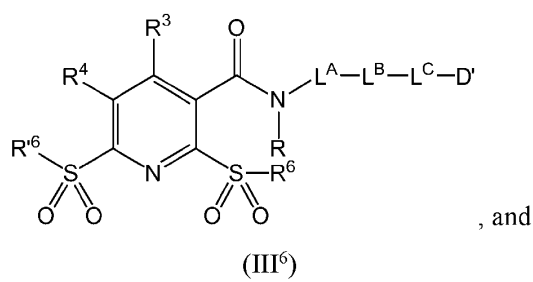
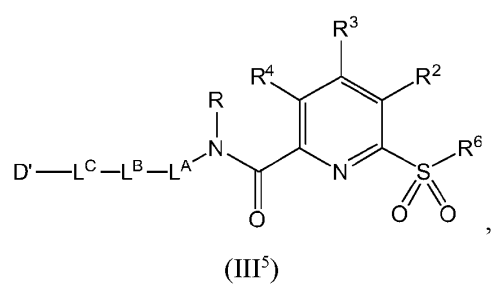
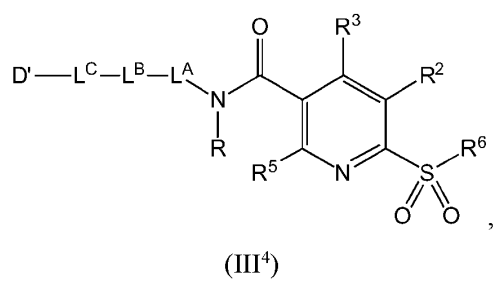
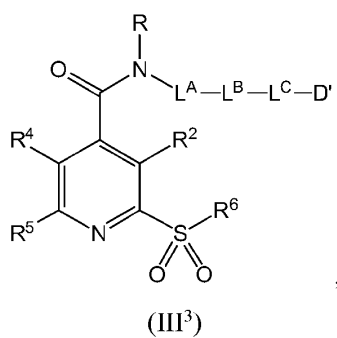
[0134] In certain embodiments of (III) or (III¹), R^2 is not CF_3 .

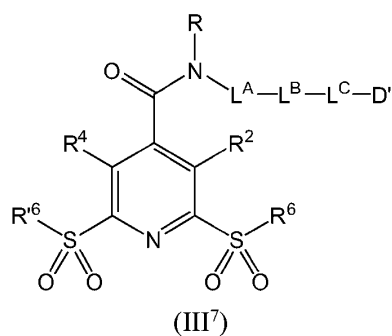
[0135] In certain embodiments of (III) or (III¹), R^2 or R^4 is F .

[0136] In certain embodiments of (III) or (III¹), one of R^2 , R^3 , R^4 and R^5 is $C(O)NHR'$.

[0137] In certain embodiments of (III), the drug conjugate has a structural formula selected from:







wherein

L^A is a spacer moiety or is absent;

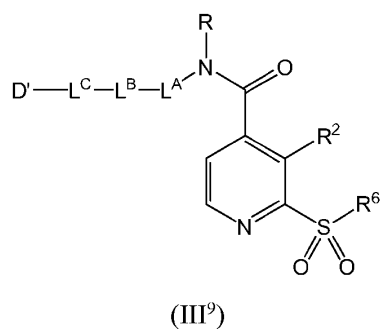
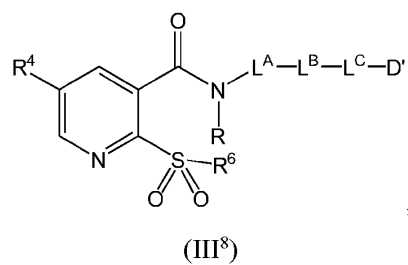
L^B is a peptide moiety or is absent;

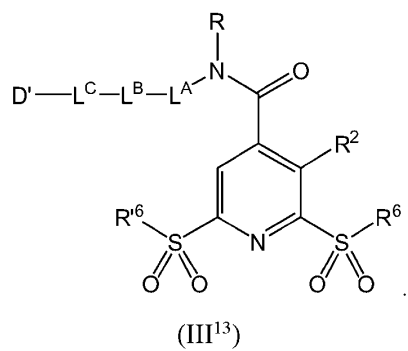
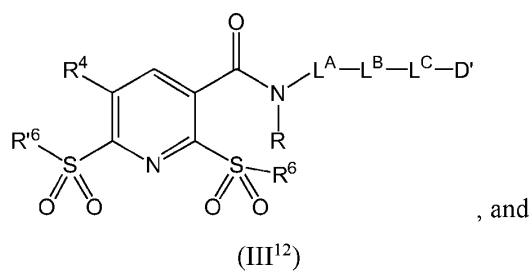
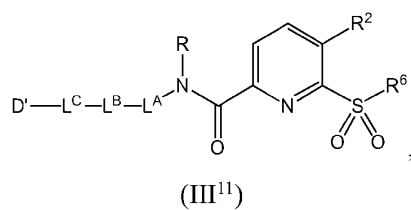
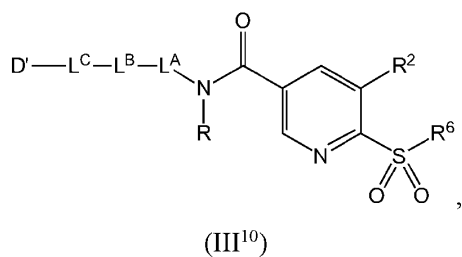
L^C is a self-immolative moiety or is absent;

R^6 and R'^6 may be the same or different and is an unsubstituted or substituted C_{1-6} alkyl or C_{6-10} aryl; and

D' is a drug moiety D or is absent.

[0138] In certain embodiments of (III), the drug conjugate has a structural formula selected from:





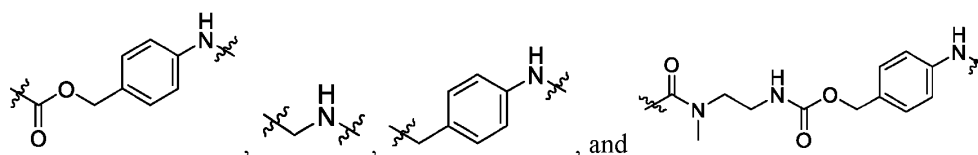
[0139] In certain embodiments, each of R⁶ and R'⁶ is methyl.

[0140] In certain embodiments of (III²)-(III¹³), the spacer moiety is selected from the group consisting of an alkyl, a heteroalkyl, PEG, and a peptide.

[0141] In certain embodiments of (III²)-(III¹³), the peptide moiety comprises 1 to 6 (*e.g.*, 1, 2, 3, 4, 5 or 6) amino acid units.

[0142] In certain embodiments of (III²)-(III¹³), one or more of the amino acids are natural amino acids. In certain embodiments, one or more of the amino acids are unnatural amino acids.

[0143] In certain embodiments of (III¹¹)-(III³⁰), the self-immolative moiety is selected from the group consisting of:



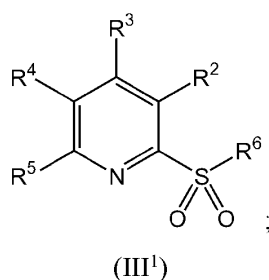
[0144] In certain embodiments, the drug moiety is a chemical agent selected from the group consisting of an antibiotic, an anti-cancer agent, a steroid, a TLR7/TLR9 antagonist, a peptide, a protein, and a nucleic acid.

[0145] In yet another aspect, the invention generally relates to a composition comprising a compound disclosed herein that is useful for forming a linker moiety-drug moiety conjugate, a targeting moiety-linker moiety conjugate, or a targeting moiety-linker moiety-drug moiety conjugate.

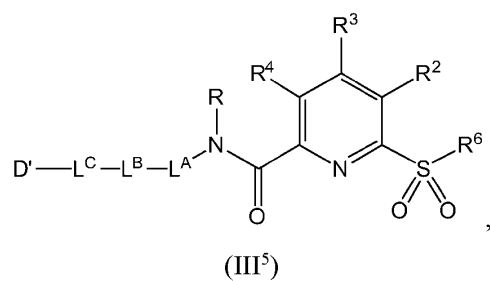
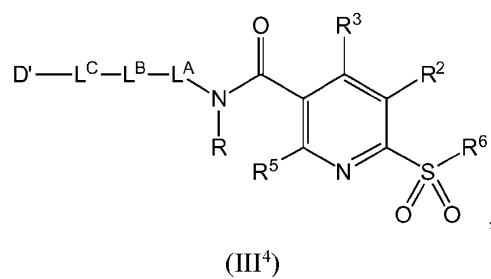
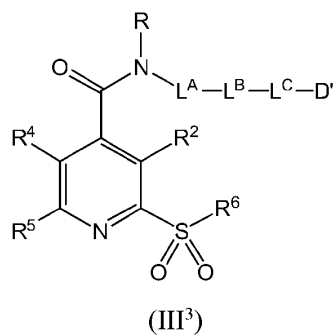
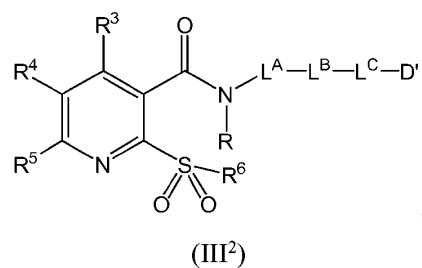
Methods of Preparation

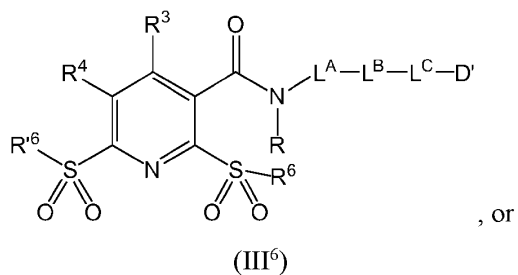
[0146] In yet another aspect, the invention generally relates to a method of preparing a linker-drug conjugate comprising:

(a) providing a compound having the structural formula:

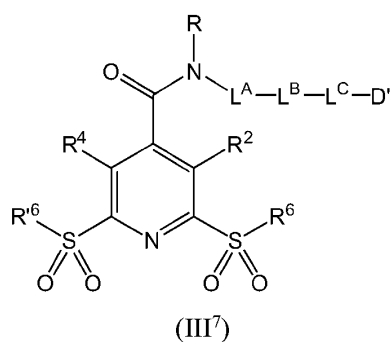


(b) reacting the compound with a precursor to a spacer, a precursor to a peptide, and/or a precursor to a self-immolative moiety, to form a conjugate having the structural formula:





, or



wherein

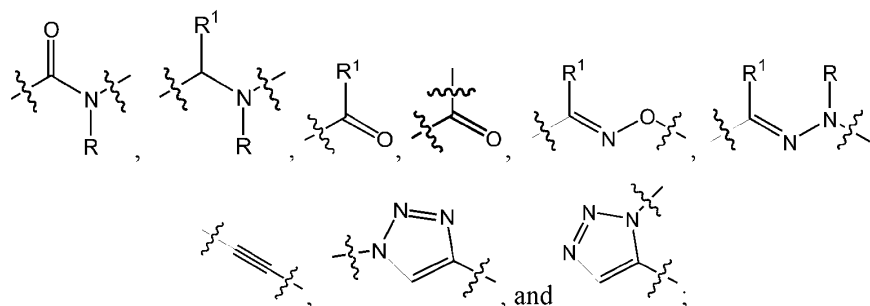
each R^2 , R^3 , R^4 is independently selected from the group consisting of H, NO_2 , CN, CF_3 , F, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{6-10} aryl, and W;

R^5 is H, Cl, SOR^6 , SO_2R^6 , OR^7 , NRR^7 or W;

R^6 is an unsubstituted or substituted C_{1-6} alkyl or C_{6-10} aryl;

R^7 is an unsubstituted or substituted C_{2-6} alkyne or C_{2-6} alkyne;

W is selected from the group consisting of:



R^1 is H, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyene, C_{2-6} alkyne or C_{6-10} aryl;

R is H, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyene, C_{2-6} alkyne or C_{6-10} aryl;

L^A is a spacer moiety or is absent;

L^B is a peptide moiety or is absent;

L^C is a self-immolative moiety or is absent; and

D' is a drug moiety D or is absent.

[0147] In certain embodiments, the conjugation step (b) is conducted at a pH in the range of 5.5 to 6.5 (*e.g.*, about 6.0).

[0148] In certain embodiments, L^A is a spacer moiety, L^B is a peptide moiety, L^C is a self-immolative moiety, and D' is a drug moiety.

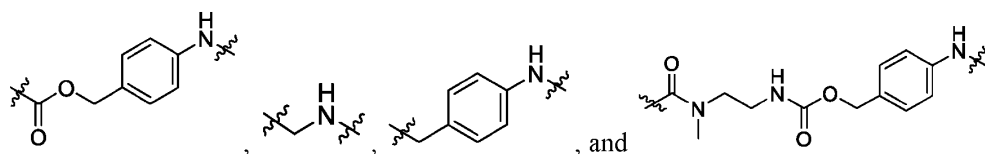
[0149] In certain embodiments, the method further includes reacting the resulting drug conjugate with a targeting moiety to form a targeting moiety-linker-drug conjugate.

[0150] In certain embodiments of the method, the spacer moiety is selected from the group consisting of an alkyl, a heteroalkyl, PEG, and a peptide.

[0151] In certain embodiments, the peptide moiety has 1 to 6 (*e.g.*, 1, 2, 3, 4, 5 or 6) amino acid units.

[0152] In certain embodiments, one or more of the amino acids are natural amino acids. In certain embodiments, one or more of the amino acids are unnatural amino acids.

[0153] In certain embodiments, the self-immolative moiety is selected from the group consisting of:



[0154] In certain embodiments, the drug moiety is a chemical agent selected from the group consisting of an antibiotic, an anti-cancer agent, a steroid, a TLR7/TLR9 antagonist, a peptide, a protein, and a nucleic acid.

[0155] In certain embodiments, the targeting moiety is selected from the group consisting of an antibody, small molecule, a peptide and a nucleic acid.

[0156] In certain embodiments, the drug conjugate has a targeting moiety to drug moiety ratio of about 1:1 to about 1:16 (e.g., about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:7, about 1:8, about 1:9, about 1:10, about 1:11, about 1:12, about 1:13, about 1:14, about 1:15 or about 1:16).

[0157] In some embodiments, the targeting moiety is an antibody comprising a cysteine residue, and the methods comprise reducing the cysteine residue to form a sulfhydryl group and reacting the sulfhydryl group with one or more linker portions described herein.

[0158] In certain embodiments, the targeting moiety is an antibody fragment comprising a cysteine residue, and the methods comprise reducing the cysteine residue to form a sulfhydryl group and reacting the sulfhydryl group with one or more linker portions described herein.

[0159] In certain embodiments, the targeting moiety is a protein ligand comprising a cysteine, and the methods comprise reducing the cysteine residue to form a sulfhydryl group and reacting the sulfhydryl group with one or more linker portions described herein.

[0160] In certain embodiments, the targeting moiety is a protein scaffold comprising a cysteine, and the methods comprise reducing the cysteine residue to form a sulfhydryl group and reacting the sulfhydryl group with one or more linker portions described herein.

[0161] In certain embodiments, the targeting moiety is a small molecule comprising a cysteine, and the methods comprise reducing the cysteine residue to form a sulfhydryl group and reacting the sulfhydryl group with one or more linker portions described herein.

[0162] In certain embodiments, conjugating the linker portion to the targeting moiety produces no deleterious side products. Non-limiting examples of deleterious side-products include acids, bases, or combination thereof.

[0163] In certain embodiments, the drug conjugate has a high drug loading. For example, in some embodiments, a molar ratio of the targeting moiety to the drug moiety is about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:7, about 1:8, about 1:9, about 1:10, about 1:11, about 1:12, about 1:13, about 1:14, about 1:15 or about 1:16.

[0164] In certain embodiments, the drug conjugate is stable *in vivo* (e.g., does not undergo a deconjugation process).

Use of Compounds and Drug Conjugates

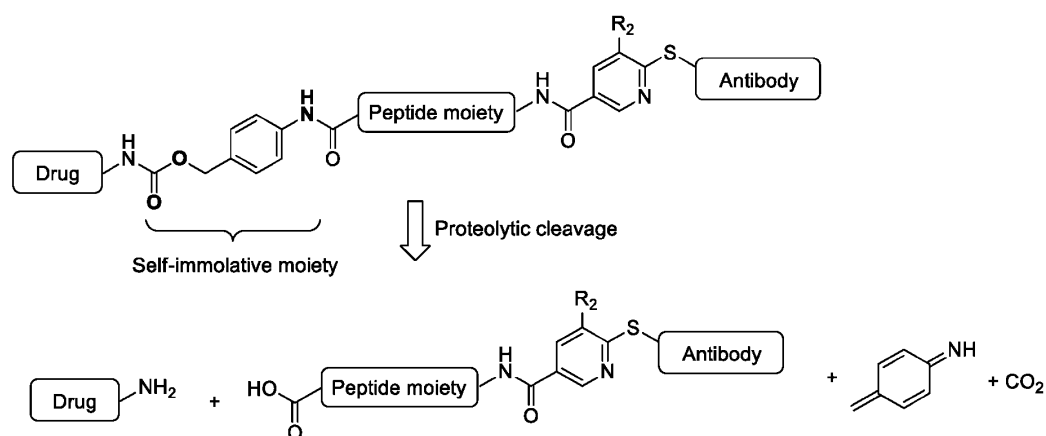
[0165] In yet another aspect, the invention generally relates to a method of treating and/or preventing a condition in a subject in need thereof, the method comprising administering to the subject a drug conjugate of the invention.

[0166] In some embodiments, the condition is cancer, an autoimmune disorder, or an infectious disease.

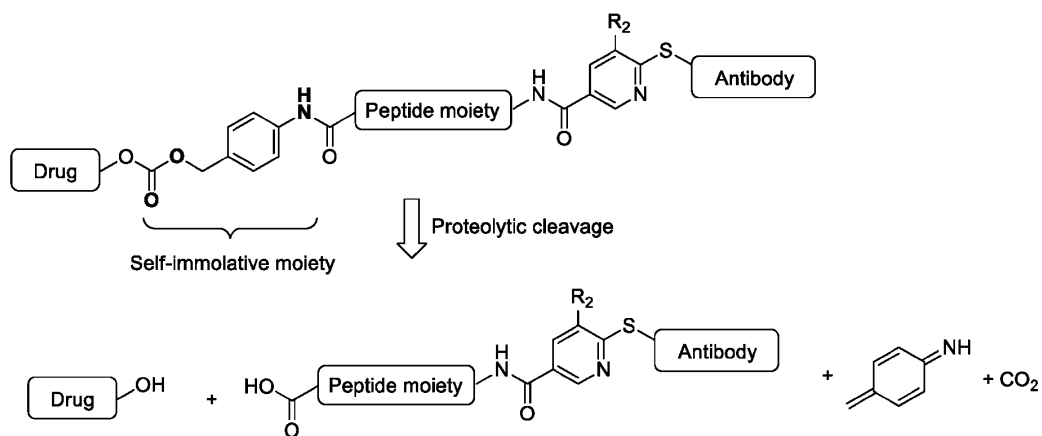
[0167] In some embodiments, the methods of treating and/or preventing a condition in a subject in need thereof comprise administering to the subject one or more drug conjugates of the present disclosure, where upon administration to the subject the drug moiety is released from the drug conjugate. In certain embodiments, the drug moiety is released from the drug conjugate by self-immolative cleavage of the self-immolative moiety.

[0168] In some embodiments, the methods for treating and/or preventing a condition comprise administering to the subject one or more drug conjugates of the present disclosure, where upon administration to the subject the drug moiety is released from the drug conjugate according to exemplary *Schemes 1-5*, each of which feature a different self-immolative moiety.

[0169] In some embodiments, proteolytic cleavage of the drug conjugate proceeds according to *Scheme 1*:

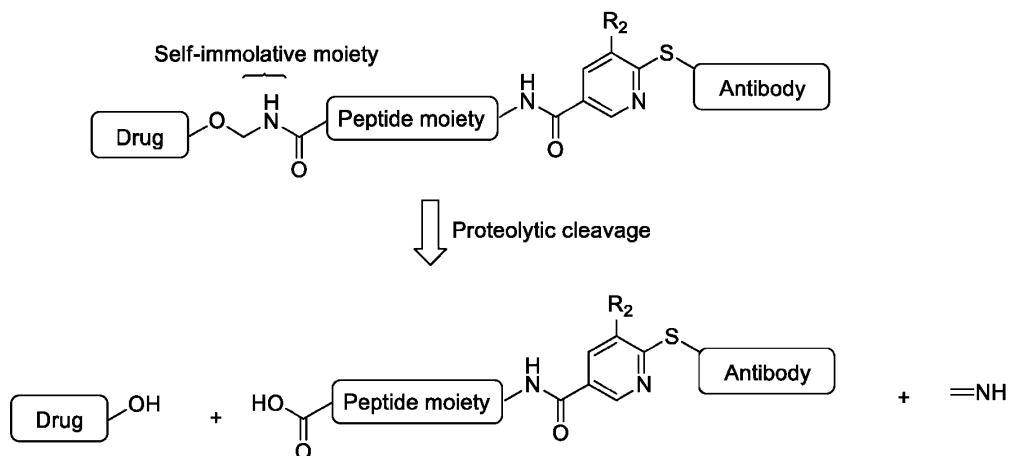


[0170] In some embodiments, the proteolytic cleavage of the drug conjugate proceeds according to *Scheme 2*:



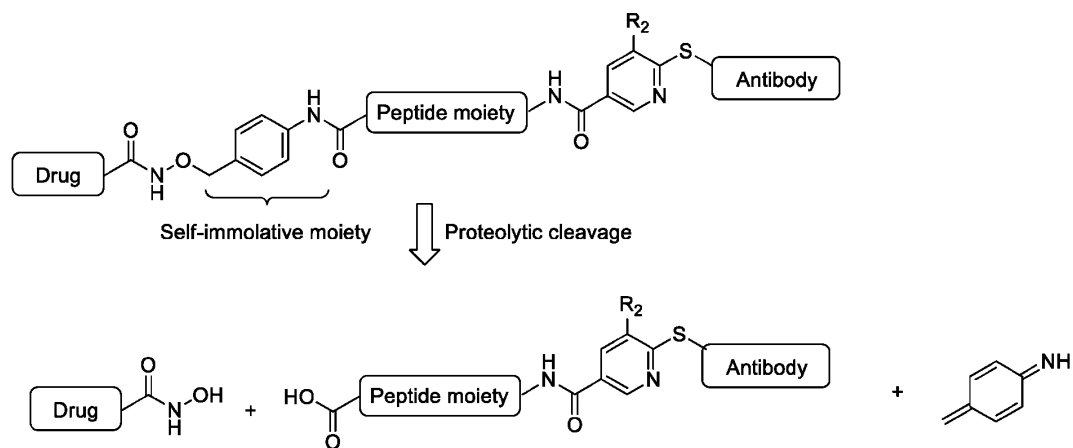
Scheme 2

[0171] In some embodiments, the proteolytic cleavage of the drug conjugate proceeds according to *Scheme 3*:

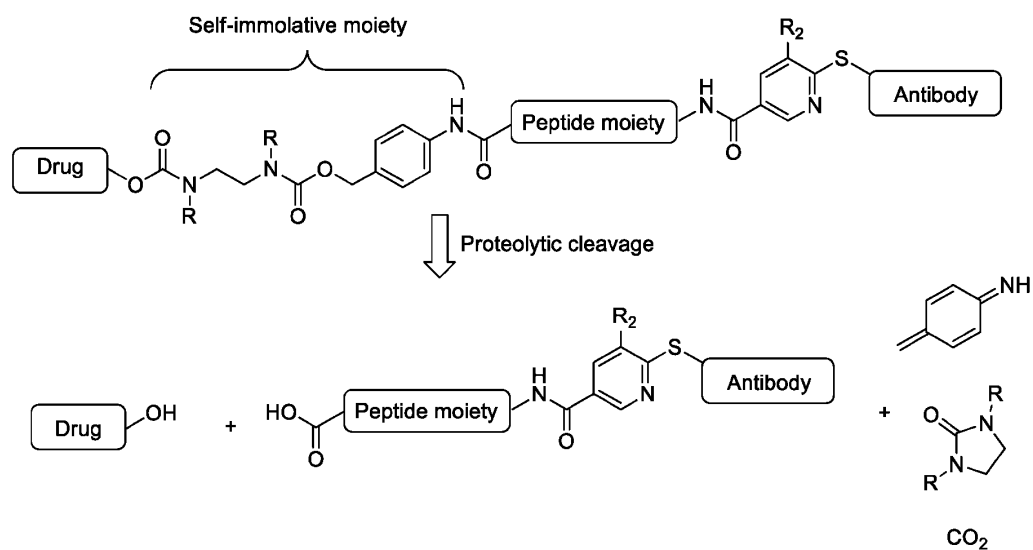


Scheme 3

[0172] In some embodiments, the proteolytic cleavage of the drug conjugate proceeds according to *Scheme 4*:



[0173] In some embodiments, the proteolytic cleavage of the drug conjugate proceeds according to *Scheme 5*:



[0174] In yet another aspect, the invention generally relates to a method for treating or reducing a disease or condition, comprising administering to a subject in need thereof a therapeutically effective amount of compound disclosed herein.

[0175] In some embodiments, the condition being treated and/or prevented is cancer. In some of these embodiments, the cancer is adrenal cancer, anal cancer, basal and squamous cell skin cancer, bile duct cancer, bladder cancer, bone cancer, brain and spinal cord tumors (e.g., astrocytoma, glioblastoma multiforme, meningioma), breast cancer, cervical cancer, colorectal cancer, endometrial cancer, esophagus cancer, Ewing family of tumors, eye cancer (ocular melanoma), gallbladder cancer, gastrointestinal neuroendocrine (carcinoid) tumors, gastrointestinal stromal tumor (gist), gestational trophoblastic disease, Kaposi sarcoma, kidney cancer, laryngeal and hypopharyngeal cancer, liver cancer, lung cancer, lung carcinoid tumor, malignant mesothelioma, melanoma skin cancer, Merkle cell skin cancer, nasal cavity and paranasal sinuses cancer, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, neoplasm of the central nervous system (CNS), oral cavity and oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, pancreatic neuroendocrine tumor (net), penile cancer, pituitary tumors, prostate cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, skin cancer, small cell lung cancer, small intestine cancer, soft tissue sarcoma, stomach cancer, testicular cancer, thymus cancer, thyroid cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenstrom macroglobulinemia, Wilms tumor, squamous cell cancer, cancers of unknown primary (CUP), environmentally induced cancers, combinations of the cancers, and metastatic lesions of the cancers. In some embodiments, the cancer is leukemia or lymphoma, for example, lymphoblastic lymphoma or B-cell Non-Hodgkin's lymphoma.

[0176] In some of these embodiments, the cancer is a hematologic malignancy. In some embodiments, the hematologic malignancy is chronic lymphocytic leukemia (CLL), acute leukemia, acute lymphoid leukemia (ALL), B-cell acute lymphoid leukemia (B-ALL), T-cell acute lymphoid leukemia (T-ALL), T-cell lymphoma, B-cell lymphoma, chronic myelogenous leukemia (CML), acute myelogenous leukemia, B-cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B-cell lymphoma, follicular lymphoma, hairy cell leukemia, small cell follicular lymphoma, large cell follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome,

non-Hodgkin's lymphoma, Hodgkin's lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, or preleukemia. In other embodiments, the cancer is a human hematologic malignancy such as myeloid neoplasm, acute myeloid leukemia (AML), AML with recurrent genetic abnormalities, AML with myelodysplasia-related changes, therapy-related AML, acute leukemias of ambiguous lineage, myeloproliferative neoplasm, essential thrombocythemia, polycythemia vera, myelofibrosis (MF), primary myelofibrosis, systemic mastocytosis, myelodysplastic syndromes (MDS), myeloproliferative/myelodysplastic syndromes, chronic myeloid leukemia, chronic neutrophilic leukemia, chronic eosinophilic leukemia, myelodysplastic syndromes (MDS), refractory anemia with ringed sideroblasts, refractory cytopenia with multilineage dysplasia, refractory anemia with excess blasts (type 1), refractory anemia with excess blasts (type 2), MDS with isolated del (5q), unclassifiable MDS, myeloproliferative/myelodysplastic syndromes, chronic myelomonocytic leukemia, atypical chronic myeloid leukemia, juvenile myelomonocytic leukemia, unclassifiable myeloproliferative/myelodysplastic syndromes, lymphoid neoplasms, precursor lymphoid neoplasms, B lymphoblastic leukemia, B lymphoblastic lymphoma, T lymphoblastic leukemia, T lymphoblastic lymphoma, mature B-cell neoplasms, diffuse large B-cell lymphoma, primary central nervous system lymphoma, primary mediastinal B-cell lymphoma, Burkitt lymphoma/leukemia, follicular lymphoma, chronic lymphocytic leukemia, small lymphocytic lymphoma, B-cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, mantle cell lymphoma, marginal zone lymphomas, post-transplant lymphoproliferative disorders, HIV-associated lymphomas, primary effusion lymphoma, intravascular large B-cell lymphoma, primary cutaneous B-cell lymphoma, hairy cell leukemia, multiple myeloma, monoclonal gammopathy of unknown significance (MGUS), smoldering multiple myeloma, or solitary plasmacytomas (solitary bone and extramedullary).

[0177] In some embodiments, the cancer comprises a solid tumor. In some embodiments, the solid tumor is lung cancer, colorectal cancer, breast cancer, pancreatic cancer, gallbladder cancer, brain and spinal cord cancer, head and neck cancer, skin cancers, testicular cancer, prostate cancer, ovarian cancer, renal cell carcinoma (RCC), bladder cancer and hepatocellular carcinoma (HCC).

[0178] Methods according to this disclosure may further include administering one or more drug conjugates provided herein to treat and/or prevent cancer in a combination therapy. For

example, in certain embodiments, a combination therapy comprises administering one or more drug conjugates (concurrently or sequentially) with a chemotherapeutic agent. In further embodiments, a combination therapy comprises administering one or more drug conjugates with a secondary therapy, such as chemotherapeutic agent, a radiation therapy, a surgery, an antibody, or any combination thereof. In some embodiments, administration one or more drug conjugates in combination with radiation therapy, antibody agent and/or chemotherapeutic agents results in an enhancement of said radiation therapy, antibody agent and/or chemotherapeutic agents such that, for example, a smaller dosage of the radiation, antibody therapy and/or chemotherapy may be effective for treatment and/or prevention.

[0179] In some embodiments, the condition being treated and/or prevented is an autoimmune disorder. In some of these embodiments, the autoimmune disorder is one or more of Th2 lymphocyte disorders, Th1 lymphocyte disorders, activated B lymphocyte disorders, active chronic hepatitis, Addison's disease, allergic alveolitis, allergic reaction, allergic rhinitis, Alport's syndrome, anaphylaxis, ankylosing spondylitis, anti-phospholipid syndrome, arthritis, ascariasis, aspergillosis, atopic allergy, atopic dermatitis, atopic rhinitis, Behcet's Disease, Bird fancier's lung, bronchial asthma, Caplan's Syndrome, cardiomyopathy, celiac disease, Chagas' Disease, chronic glomerulonephritis, Cogan's syndrome, cold agglutinin disease, congenital rubella infection, CREST Syndrome, Crohn's disease, cryoglobulinemia, Gushing's syndrome, dermatomyositis, discoid lupus, Dressler syndrome, Eaton-Lambert syndrome, echovirus infection, encephalomyelitis, endocrine ophthalmopathy, Epstein-Barr virus infection, equine heaves, erythematosis, Evans syndrome, Felty's syndrome, fibromyalgia, Fuchs heterochromatic iridocyclitis, gastric atrophy, gastrointestinal allergy, giant cell arteritis, glomerulonephritis, Goodpasture's syndrome, graft-versus-host disease, Graves' disease, Guillain-Barre disease, Hashimoto's thyroiditis, hemolytic anemia, Henoch-Schonlein purpura, idiopathic adrenal atrophy, idiopathic pulmonary fibrosis, IgA nephropathy, inflammatory bowel diseases, insulin-dependent diabetes mellitus, juvenile arthritis, juvenile diabetes mellitus (Type 1), Lambert-Eaton syndrome, laminitis, lichen planus, lupoid hepatitis, lupus, lymphopenia, Meniere's Disease, mixed connective tissue disease, multiple sclerosis, myasthenia gravis, pernicious anemia, polyglandular syndromes, presenile dementia, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Raynaud's phenomenon, recurrent abortion, Reiter's syndrome, rheumatic fever, rheumatoid arthritis, Samter's syndrome, schistosomiasis,

Schmidt's syndrome, scleroderma, Shulman's syndrome, Sjogren's syndrome, Stiff-person syndrome, sympathetic ophthalmia, systemic lupus erythematosus, Takayasu's arteritis, temporal arteritis, thyroiditis, thrombocytopenia, thyrotoxicosis, toxic epidermal necrolysis, type B insulin resistance, type I diabetes mellitus, ulcerative colitis, uveitis, vitiligo, Waldenstrom macroglobulinemia, and granulomatosis with polyangiitis.

[0180] Methods according to this disclosure may further include administering one or more drug conjugates provided herein to treat and/or prevent an autoimmune disorder in a combination therapy. For example, in certain embodiments, a combination therapy comprises administering one or more drug conjugates (concurrently or sequentially) with a therapeutic agent known to treatment and/or prevent an autoimmune disorder.

[0181] In some embodiments, the condition being treated and/or prevented is an infectious disease. In some of these embodiments, the infectious disease is a bacterial disease, systemic fungal disease, Rickettsial disease, parasitic disease, and/or viral disease.

[0182] In some embodiments, the one or more bacterial diseases include diphtheria, pertussis, occult bacteremia, urinary tract infection, gastroenteritis, cellulitis, epiglottitis, tracheitis, adenoid hypertrophy, retropharyngeal abscess, impetigo, ecthyma, pneumonia, endocarditis, septic arthritis, pneumococcal, peritonitis, bacteremia, meningitis, acute purulent meningitis, urethritis, cervicitis, proctitis, pharyngitis, salpingitis, epididymitis, gonorrhea, syphilis, listeriosis, anthrax, nocardiosis, salmonella, typhoid fever, dysentery, conjunctivitis, sinusitis, brucellosis, tularemia, cholera, bubonic plague, tetanus, necrotizing enteritis, actinomycosis, mixed anaerobic infections, syphilis, relapsing fever, leptospirosis, Lyme disease, rat bite fever, tuberculosis, lymphadenitis, leprosy, chlamydia, chlamydial pneumonia, trachoma, and/or inclusion conjunctivitis.

[0183] In some embodiments, the one or more systemic fungal diseases is selected from histoplasmosis, coccidioidomycosis, blastomycosis, sporotrichosis, cryptococcosis, systemic candidiasis, aspergillosis, mucormycosis, mycetoma, and/or chromomycosis.

[0184] In some embodiments, the one or more Rickettsial diseases is selected from typhus, Rocky Mountain spotted fever, ehrlichiosis, eastern tick-borne Rickettsioses, Rickettsialpox, Q fever, bartonellosis.

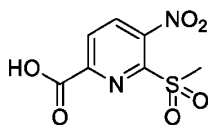
[0185] In some embodiments, the one or more parasitic diseases is selected from malaria, babesiosis, African sleeping sickness, chagas' disease, leishmaniasis, dum-dum fever,

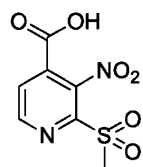
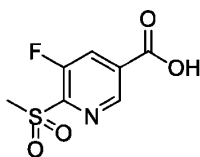
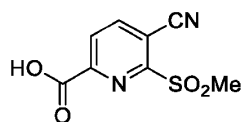
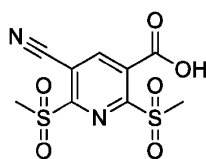
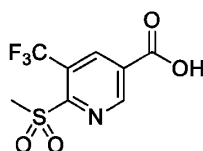
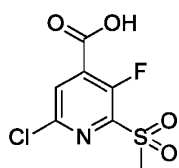
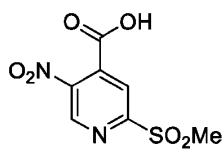
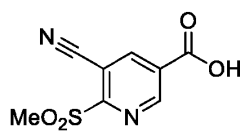
toxoplasmosis, meningoencephalitis, keratitis, amoebiasis, giardiasis, cryptosporidiosis, isosporiasis, cyclosporiasis, microsporidiosis, ascariasis, whipworm infection, hookworm infection, threadworm infection, ocular larva migrans, trichinosis, guinea worm disease, lymphatic filariasis, loiasis, river blindness, canine heartworm infection, schistosomiasis, swimmer's itch, oriental lung fluke, oriental liver fluke, fascioliasis, fasciolopsiasis, opisthorchiasis, tapeworm infections, hydatid disease, alveolar hydatid disease.

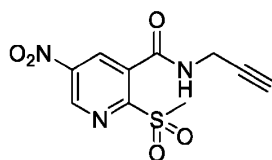
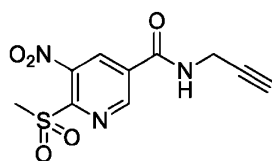
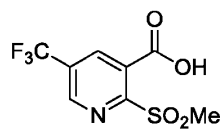
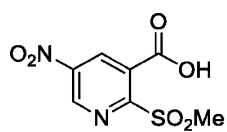
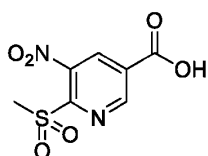
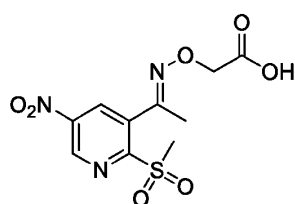
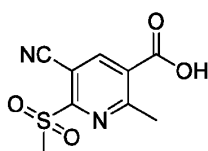
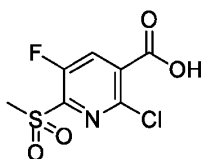
[0186] In some embodiments, the one or more viral diseases is selected from measles, subacute sclerosing panencephalitis, common cold, mumps, rubella, roseola, fifth disease, chickenpox, respiratory syncytial virus infection, croup, bronchiolitis, infectious mononucleosis, poliomyelitis, herpangina, hand-foot- and-mouth disease, Bornholm disease, genital herpes, genital warts, aseptic meningitis, myocarditis, pericarditis, gastroenteritis, acquired immunodeficiency Syndrome (AIDS), human immunodeficiency virus (HIV), Reye's syndrome, Kawasaki syndrome, influenza, bronchitis, viral "walking" pneumonia, acute febrile respiratory disease, acute pharyngoconjunctival fever, epidemic keratoconjunctivitis, herpes simplex virus 1 (hsv-1), herpes simplex virus 2 (hsv-2), shingles, cytomegalic inclusion disease, rabies, progressive multifocal leukoencephalopathy, kuru, fatal familial insomnia, Creutzfeldt- Jakob disease, Gerstraann-Straussler-Scheinker disease, tropical spastic paraparesis, western equine encephalitis, California encephalitis, St. Louis encephalitis, yellow fever, dengue, lymphocytic choriomeningitis, Lassa fever, hemorrhagic fever, hantavirus pulmonary syndrome, Marburg virus infections, Ebola virus infections, and/or smallpox.

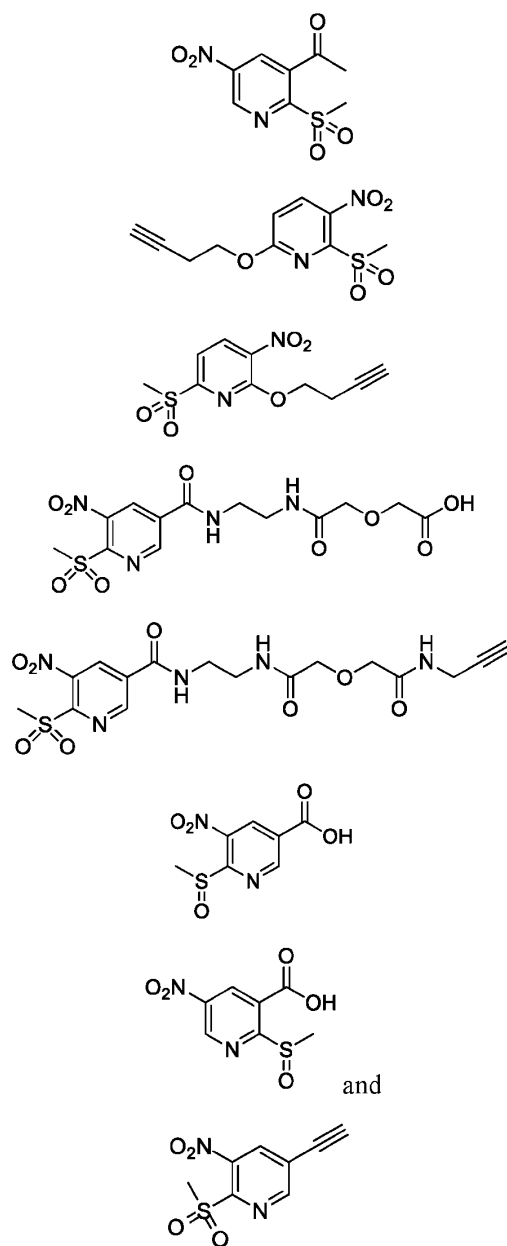
[0187] Methods according to this disclosure may further include administering one or more drug conjugates provided herein to treat and/or prevent an infectious disease in a combination therapy. For example, in certain embodiments, a combination therapy comprises administering one or more drug conjugates (concurrently or sequentially) with a therapeutic agent known to treatment and/or prevent an infectious disease.

[0188] Non-limiting examples of compounds of the invention include:

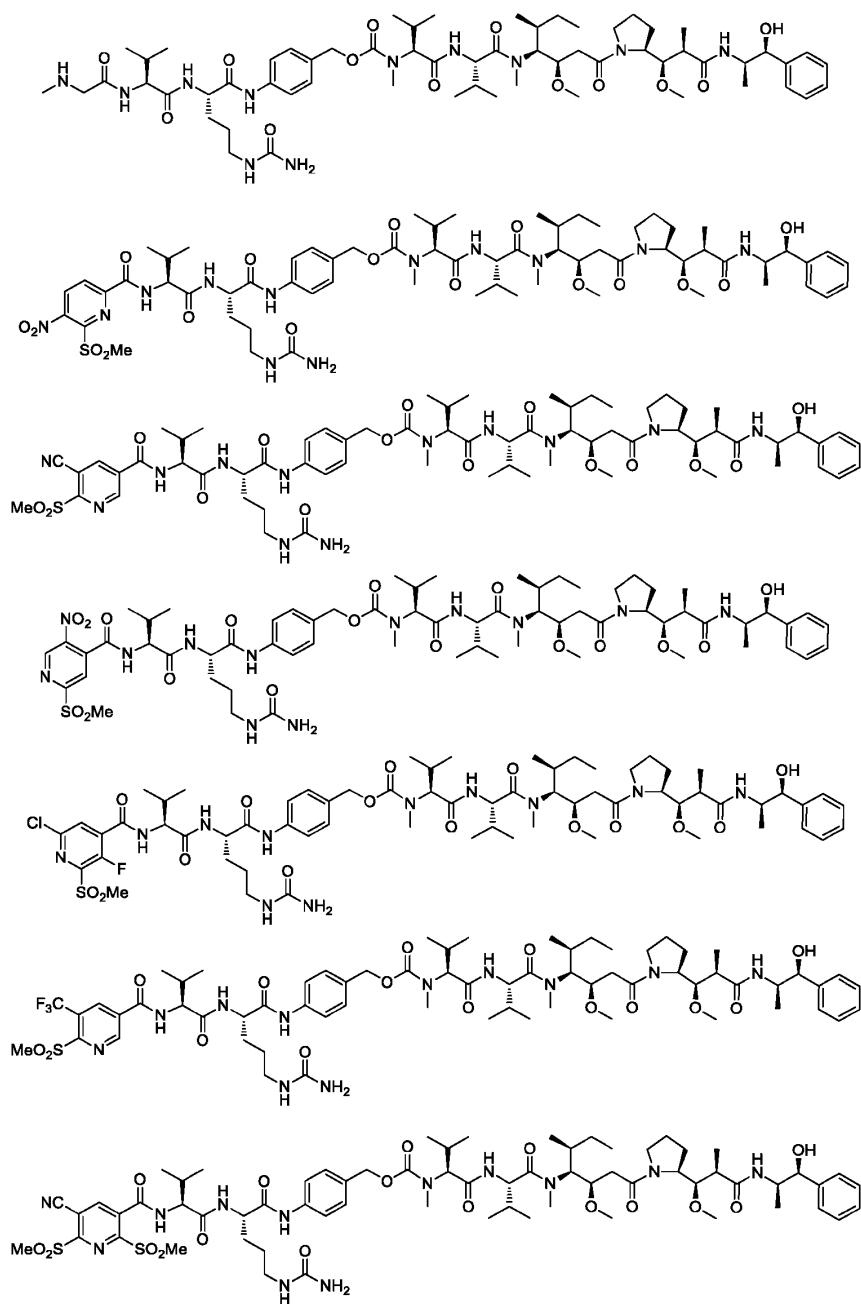


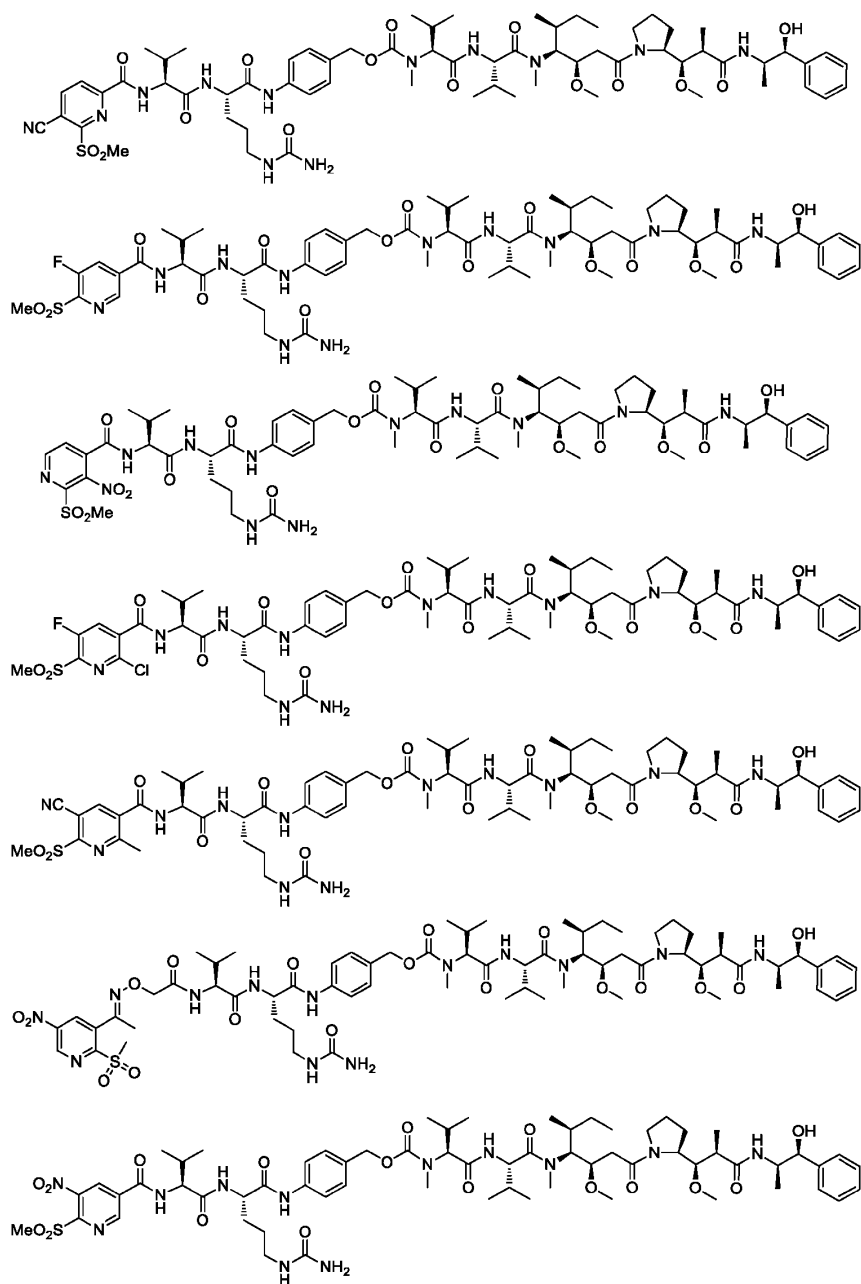


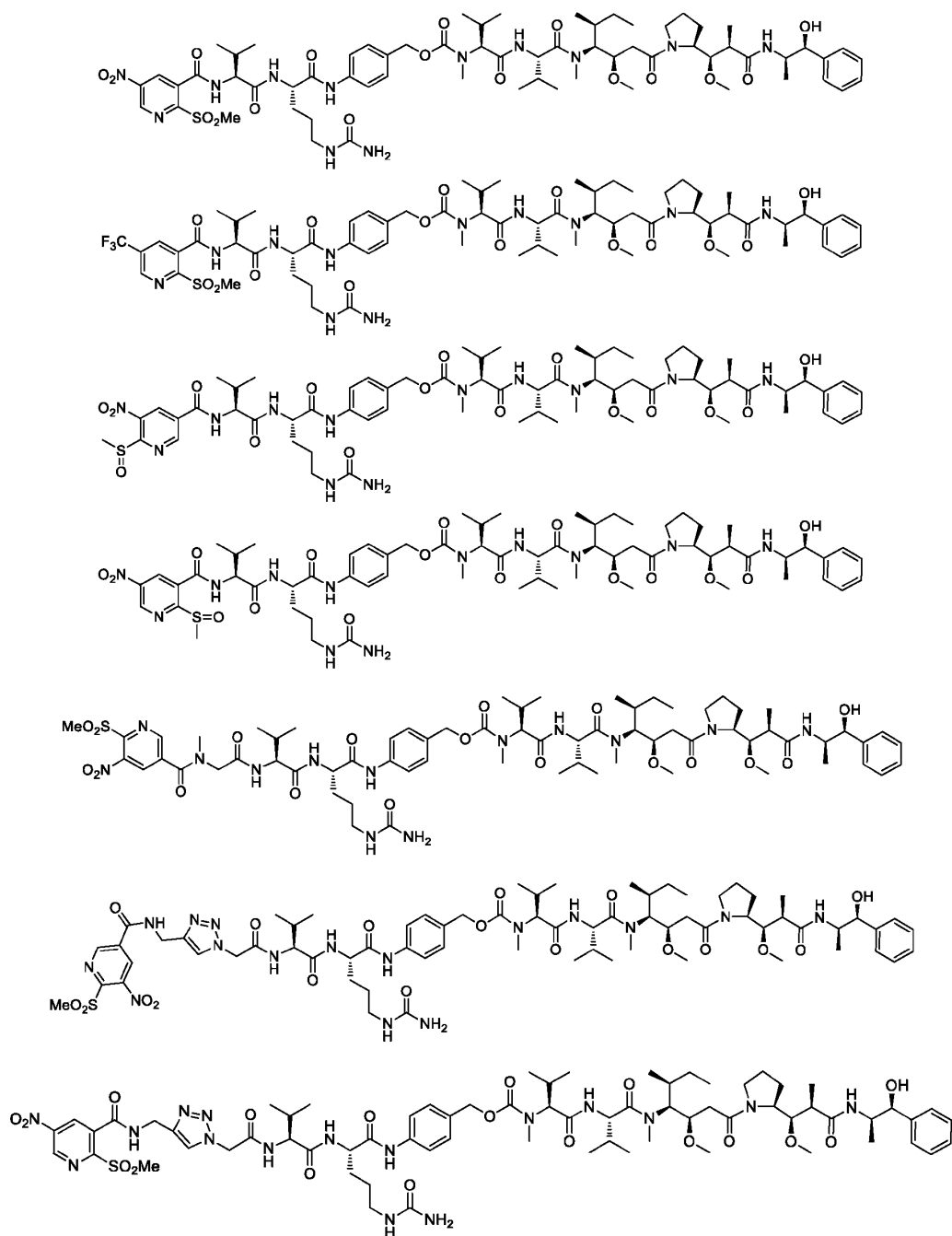


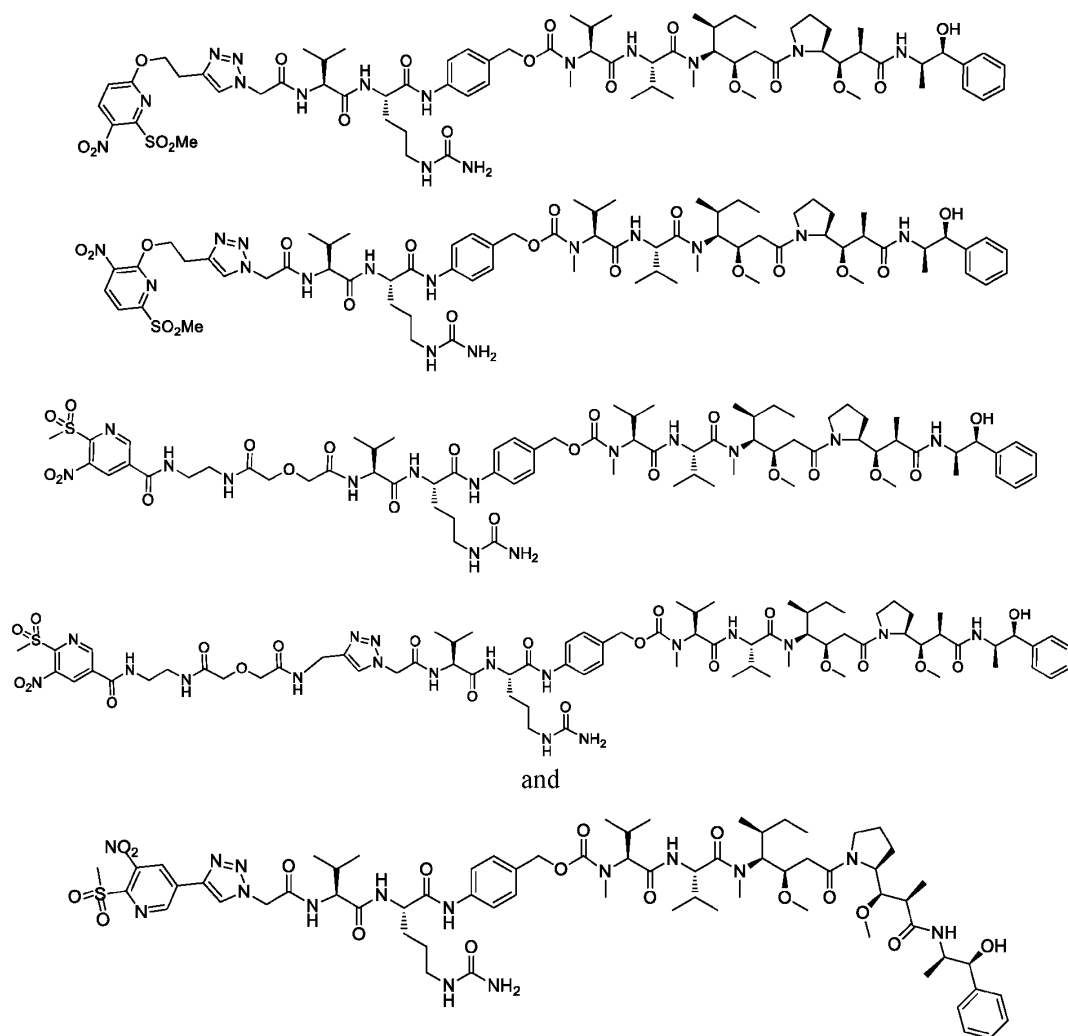


[0189] Non-limiting examples of compounds of the invention also include:









Spacers

[0190] In certain embodiments, the spacer moiety comprises an alkyl chain. In some embodiments, the spacer moiety has the following formula: $-(CH_2)_n$, where n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, the spacer moiety comprises a heteroalkyl chain. In some embodiments, the spacer moiety has the following formula: $-(CH_2CH_2O)_n$, where n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

[0191] In some embodiments, the alkyl is a low alkyl, having 1 to 4 carbon atoms (e.g., methyl, ethyl, propyl and butyl).

[0192] In certain embodiments, the spacer moiety comprises a peptide. In certain embodiments, the peptide comprises two or more amino acids for example, a dipeptide, a tripeptide, tetrapeptide, pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide, or decapeptide. In some of these embodiments, the spacer moiety comprises Val-Cit-PAB, Val-Ala-PAB, Val-Lys(Ac)-PAB, Phe-Lys-PAB, Phe-Lys(Ac)-PAB, D-Val-Leu-Lys, Gly-Gly-Arg, Ala-Ala-Asn-PAB, Ala-PAB, PAB, or combinations thereof.

[0193] In some embodiments, the spacer moiety comprises a combination of an alkyl, heteroalkyl, PEG, or a peptide. For example, the spacer moiety comprises $-(CH_2)_n$ and a peptide, the spacer moiety comprises $-(CH_2CH_2O)_n$ and a peptide, the spacer moiety comprises PEG and a peptide, the spacer moiety comprises $-(CH_2)_n$ and PEG, or the spacer moiety comprises $-(CH_2CH_2O)_n$ and PEG.

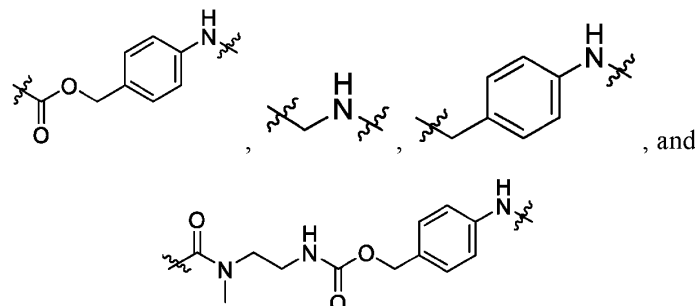
[0194] In some embodiments, the polypeptide moiety comprises 1 to 6 amino acids. For example, the polypeptide can include 1 amino acid, 2 amino acids, 3 amino acids, 4 amino acids, 5 amino acids, or 6 amino acids.

[0195] The polypeptide moiety may include one or more natural amino acids and/or one or more unnatural amino acids. In some embodiments, the natural amino acid is one or more of the 20 common amino acids selected from one or more of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. As used herein, the term "unnatural amino acid" refers to any amino acid, modified amino acid, and/or amino acid analogue that is not one of the 20 common naturally occurring amino acids. Non-limiting examples of unnatural amino acids include N-acetylglucosaminyl-L-serine, N-acetylglucosaminyl-L-threonine, and O-phosphotyrosine.

Self-immolative Moiety

[0196] The "self-immolative moiety" refers to a chemical moiety that is capable of covalently linking two chemical moieties, for example, a polypeptide moiety and a drug moiety. The self-immolative spacer is capable of spontaneously separating from the drug moiety if the bond to the polypeptide is cleaved, e.g., via proteolytic cleavage.

[0197] In some embodiments, the self-immolative moiety is selected from:



[0198] In some embodiments, a linker as provided herein is modified when conjugated to a drug moiety and/or targeting moiety, for example in a linker-drug moiety complex, linker-targeting moiety complex, or drug conjugate as provided herein. For example, where the linker comprises a hydroxyl group, that hydroxyl group may react with a functional group on the drug moiety or targeting moiety during the conjugation reaction, producing a conjugate wherein the linker no longer comprises the hydroxyl group.

Drug Moieties

[0199] The drug moiety in the drug conjugates and components thereof provided herein may be any compound or molecule that produces a therapeutic effect, including both small molecules and biologics. By way of example, a drug moiety may be a chemical agent, such as an antibiotic, anti-cancer agent, a polypeptide, or a nucleic acid.

[0200] In some embodiments, the drug moiety is a chemotherapeutic agent, an immune modulator, a tubulin-binder, a DNA-alkylating agent, an HSP90 inhibitor, a DNA topoisomerase, an anti-epigenetic agent, an HDAC inhibitor, an anti-metabolism agent, a proteasome inhibitor, a peptide, a peptidomimetic, an siRNA, and/or an antisense DNA.

[0201] In certain embodiments, the drug is a chemotherapeutic drug. Non-limiting examples of chemotherapeutic drugs include alkylating agents, plant alkaloids, DNA topoisomerase inhibitors, anti-metabolites, hormonal therapies, kinase inhibitors, and/or antibiotics.

[0202] In some embodiments, the alkylating agent is selected from one or more of chlorambucil, chlornaphazine, cyclophosphamide, dacarbazine, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, mannometrine, mitobronitol, melphalan, mitolactol, pipobroman, novembichin, phenesterine, prednimustine, thiotepa,

trofosfamide, uracil mustard; CC-1065 (e.g., adozelesin, carzelesin and bizelesin synthetic analogues); Duocarmycin (e.g., synthetic analogues, KW-2189 and CBI-TMI); Benzodiazepine dimers (e.g., dimmers of pyrrolobenzodiazepine (PBD) or tomaymycin, indolinobenzodiazepines, imidazobenzothiadiazepines, or oxazolidino-benzodiazepines), nitrosoureas (e.g., carmustine, lomustine, chlorozotocin, fotemustine, nimustine, ranimustine), alkylsulphonates (e.g., busulfan, treosulfan, improsulfan and piposulfan); triazenes (e.g., dacarbazine), platinum containing compounds (e.g., carboplatin, cisplatin, oxaliplatin), and/or aziridines (e.g., benzodopa, carboquone, meturedopa, and uredopa).

[0203] In some embodiments, the plant alkaloid is selected from one or more of vinca alkaloids (e.g., vincristine, vinblastine, vindesine, vinorelbine, navelbin), taxoids (e.g., paclitaxel and docetaxol), maytansinoids (e.g., DM1, DM2, DM3, DM4, maytansine and ansamitocins), cryptophycins (e.g., cryptophycin 1 and cryptophycin 8), epothilones, eleutherobin, discodermolide, bryostatins, dolostatins, auristatins, tubulysins, cephalostatins, pancratistatin, sarcodictyin, and/or spongistatin.

[0204] In some embodiments, the DNA topoisomerase inhibitor is selected from one or more of epipodophyllins (e.g., 9-aminocamptothecin, camptothecin, crisnatol, daunomycin, etoposide, etoposide phosphate, irinotecan, mitoxantrone, novantrone, retinoic acids (retinols), teniposide, topotecan, 9-nitrocamptothecin (RFS 2000)) and/or mitomycins (e.g., mitomycin C).

[0205] In some embodiments, the anti-metabolite is selected from one or more of anti-folate such DHFR inhibitors (e.g., methotrexate, trimetrexate, denopterin, pteropterin, aminopterin (4-aminopteroic acid) or the other folic acid analogues); IMP dehydrogenase inhibitors (e.g., mycophenolic acid, tiazofurin, ribavirin, EICAR); ribonucleotide reductase Inhibitors (e.g., hydroxyurea, deferoxamine), pyrimidine analogs such uracil analogs: (e.g., ancitabine, azacitidine, 6-azauridine, capecitabine (Xeloda), carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, 5-Fluorouracil, floxuridine, ratitrexed (e.g., tomudex), cytosine analogs (e.g., cytarabine, cytosine arabinoside, fludarabine), purine analogs (e.g., azathioprine, fludarabine, mercaptopurine, thiamiprine, thioguanine), and/or folic acid replenisher (e.g. frolinic acid).

[0206] In some embodiments, the hormonal therapy is one or more of receptor antagonists such anti-estrogens (e.g., megestrol, raloxifene, tamoxifen), LHRH agonists (e.g., goserelin, leuprolide), anti-androgens (e.g., bicalutamide, flutamide, calusterone, dromostanolone

propionate, epitiostanol, mepitiothane, nilutamide, testolactone, trilostane and other androgens inhibitors), retinoids/deltoids (e.g., Vitamin D3 analogs: CB 1093, EB 1089 KH 1060, cholecalciferol, ergocalciferol); photodynamic therapies (e.g., verteporfin, phthalocyanine, photosensitizer Pc4, demethoxy-hypocrellin A), and cytokines (e.g., interferon-alpha, interferon-gamma, tumor necrosis factor (TNFs), human proteins containing a TNF domain).

[0207] In some embodiments, the kinase inhibitor is one or more of BIBW 2992 (e.g., anti-EGFR/Erb2), imatinib, gefitinib, pegaptanib, sorafenib, dasatinib, sunitinib, erlotinib, nilotinib, lapatinib, axitinib, pazopanib, vandetanib, E7080 (e.g., anti-VEGFR2), mubritinib, ponatinib (e.g., AP24534), bafetinib (e.g., INNO-406), bosutinib (e.g., SKI-606), cabozantinib, vismodegib, iniparib, ruxolitinib, CYT387, axitinib, tivozanib, sorafenib, bevacizumab, cetuximab, Trastuzumab, Ranibizumab, Panitumumab, and/or ispinesib.

[0208] In some embodiments, the antibiotic is an enediyne antibiotic (e.g., calicheamicins, especially calicheamicin γ 1, δ 1, α 1 and β 1), dynemicin (e.g., dynemicin A and deoxydynemicin; esperamicin, kedarcidin, C-1027, maduropeptin, as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, aithramycin, azaserine, bleomycins, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, nitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, and/or zorubicin.

[0209] In some embodiments, the drug is an anti-autoimmune disease drug. Non-limiting examples of anti-autoimmune disease drugs include cyclosporine, cyclosporine A, aminocaproic acid, azathioprine, bromocriptine, chlorambucil, chloroquine, cyclophosphamide, corticosteroids (e.g., amcinonide, betamethasone, budesonide, hydrocortisone, flunisolide, fluticasone propionate, fluocortolone danazol, dexamethasone, Triamcinolone acetonide, beclometasone dipropionate), DHEA, enanercept, hydroxychloroquine, infliximab, meloxicam, methotrexate, mofetil, mycophenylate, prednisone, sirolimus, and tacrolimus.

[0210] In some embodiments, the anti-autoimmune disease drug is selected from one or more of polyketides (e.g., acetogenins such bullatacin and bullatacinone), gemcitabine, epoxomicins

(e. g. carfilzomib), bortezomib, thalidomide, lenalidomide, pomalidomide, tosedostat, zybrestat, PLX4032, STA-9090, stimuvax, allovectin-7, xegeva, provenge, yervoy, isoprenylation inhibitors (e.g., Lovastatin), dopaminergic neurotoxins (e.g., 1-methyl-4-phenylpyridinium ion), cell cycle inhibitors (e.g., staurosporine), actinomycins (e.g., actinomycin D, dactinomycin), bleomycins (e.g., bleomycin A2, bleomycin B2, peplomycin), anthracyclines (e.g., daunorubicin, doxorubicin, idarubicin, epirubicin, pirarubicin, zorubicin, mitoxantrone, MDR inhibitors), Ca^{2+} ATPase inhibitors (e.g., thapsigargin), histone deacetylase inhibitors (e.g., Vorinostat, Romidepsin, Panobinostat, Valproic acid, Mocetinostat (MGCD0103), Belinostat, PCI-24781, Entinostat, SB939, Resminostat, Givinostat, AR-42, CUDC-101, sulforaphane, Trichostatin A), thapsigargin, celecoxib, glitazones, epigallocatechin gallate, disulfiram, salinosporamide A., anti-adrenals, urethane, siRNA, antisense drugs, and/or a nucleolytic enzyme.

[0211] In certain embodiments, the drug is an infectious disease drug. Non-limiting examples of infectious disease drugs include aminoglycosides, amphenicols, ansamycins, carbapenems, cepheems, glycopeptides, glycyclcyclines, β -lactamase inhibitors, lincosamides, lipopeptides, macrolides, monobactams, oxazolidinones, penicillin, polypeptides, quinolones, streptogramins, sulfonamides, steroid antibacterials, tetracyclines, and/or antibiotics.

[0212] In some embodiments, the aminoglycoside is one or more of amikacin, astromycin, gentamicin (e.g., netilmicin, sisomicin, and isepamicin), hygromycin B, kanamycin (e.g., amikacin, arbekacin, bekanamycin, dibekacin, and tobramycin), neomycin (e.g., framycetin, paromomycin, and ribostamycin), netilmicin, spectinomycin, streptomycin, tobramycin, and/or verdamycin.

[0213] In some embodiments, the amphenicol is one or more of azidamfenicol, chloramphenicol, florfenicol, and/or thiamphenicol.

[0214] In some embodiments, ansamycin is one or more of geldanamycin and/or herbimycin.

[0215] In some embodiments, carbapenems is one more of biapenem, doripenem, ertapenem, imipenem/cilastatin, meropenem, and/or panipenem.

[0216] In some embodiments, the cephem is one or more of carbacephem (e.g., loracarbef), cefacetrile, cefaclor, cefradine, cefadroxil, cefalonium, cefaloridine, cefalotin or cefalothin, cefalexin, cefaloglycin, cefamandole, cefapirin, cefatrizine, cefazaflur, cefazedone, cefazolin, cefbuperazone, cefcapene, cefdaloxime, cefepime, cefminox, cefoxitin, cefprozil, cefroxadine, ceftezole, cefuroxime, cefixime, cefdinir, cefditoren, cefepime, cefetamet, cefmenoxime,

cefodizime, cefonicid, cefoperazone, ceforanide, cefotaxime, cefotiam, ceftazopran, cephalixin, cefpimizole, cefpiramide, cefpirome, cefpodoxime, cefprozil, cefquinome, cefsulodin, ceftazidime, cefteteram, ceftibuten, ceftiolene, ceftizoxime, ceftobiprole, ceftriaxone, cefuroxime, cefuzonam, cephamycin (e.g., cefoxitin, cefotetan, and cefmetazole), and/or oxacephem (e.g., flomoxef and latamoxef).

[0217] In some embodiments, the glycopeptide is one or more of bleomycin, vancomycin (oritavancin, telavancin), teicoplanin (dalbavancin), ramoplanin.

[0218] In some embodiments, the glycyclcyclines is tigecycline.

[0219] In some embodiments, the β -Lactamase inhibitor is one or more of a penam (e.g., sulbactam and tazobactam) and/or a clavam (e.g., clavulanic acid).

[0220] In some embodiments, the lincosamide is one or more of clindamycin and/or lincomycin.

[0221] In some embodiments, the lipopeptide is one or more of daptomycin, A54145, and/or calcium-dependent antibiotics (CDA).

[0222] In some embodiments, the macrolide is one or more of azithromycin, cethromycin, clarithromycin, dirithromycin, erythromycin, flurithromycin, josamycin, ketolide (telithromycin, cethromycin), midecamycin, miocamycin, oleandomycin, rifamycins (rifampicin, rifampin, rifabutin, rifapentine), rokitamycin, roxithromycin, spectinomycin, spiramycin, tacrolimus (FK506), troleandomycin, and/or telithromycin.

[0223] In some embodiments, the monobactams is selected from aztreonam and/or tigemonam.

[0224] In some embodiments, the oxazolidinones is linezolid.

[0225] In some embodiments, the penicillin is one or more of amoxicillin, ampicillin (e.g., pivampicillin, hetacillin, bacampicillin, metampicillin, talampicillin), azidocillin, azlocillin, benzylpenicillin, benzathine benzylpenicillin, benzathine phenoxymethylpenicillin, clometocillin, procaine benzylpenicillin, carbenicillin (e.g., carindacillin), cloxacillin, dicloxacillin, epicillin, flucloxacillin, mecillinam (e.g., pivmecillinam), mezlocillin, meticillin, nafcillin, oxacillin, penamecillin, penicillin, pheneticillin, phenoxymethylpenicillin, piperacillin, propicillin, sulbenicillin, temocillin, and/or ticarcillin.

[0226] In some embodiments, the polypeptide is one or more of bacitracin, colistin, and/or polymyxin B.

[0227] In some embodiments, the quinolone is selected from one or more of alatrofloxacin, balofloxacin, ciprofloxacin, clinafloxacin, danofloxacin, difloxacin, enoxacin, enrofloxacin, floxin, garenoxacin, gatifloxacin, gemifloxacin, grepafloxacin, kano trovafloxacin, levofloxacin, lomefloxacin, marbofloxacin, moxifloxacin, nadifloxacin, norfloxacin, orbifloxacin, ofloxacin, pefloxacin, trovafloxacin, grepafloxacin, sitafloxacin, sparfloxacin, temafloxacin, tosufloxacin, and/or trovafloxacin.

[0228] In some embodiments, streptogramins is pristinamycin such as quinupristin and/or dalfopristin.

[0229] In some embodiments, the sulfonamide is one or more of mafenide, prontosil, sulfacetamide, sulfamethizole, sulfanilimide, sulfasalazine, sulfisoxazole, trimethoprim, and/or trimethoprim-sulfamethoxazole (co-trimoxazole).

[0230] In some embodiments, the steroid antibacterial is fusidic acid.

[0231] In some embodiments, the tetracyclines is one or more of doxycycline, chlortetracycline, clomocycline, demeclocycline, lymecycline, meclocycline, metacycline, minocycline, oxytetracycline, penimepicycline, rolitetracycline, tetracycline, and/or glycylcyclines (e.g., tigecycline).

[0232] In some embodiments, the anti-infectious disease drug is an antibiotic selected from one or more ofannonacin, arspenamine, bactoprenol inhibitors (e.g., bacitracin), DADAL/AR inhibitors (e.g., cycloserine), dictyostatin, discodermolide, eleutherobin, epothilone, ethambutol, etoposide, faropenem, fusidic acid, furazolidone, isoniazid, laulimalide, metronidazole, mupirocin, mycolactone, NAM synthesis inhibitors (e.g., fosfomycin), nitrofurantoin, paclitaxel, platensimycin, pyrazinamide, quinupristin/dalfopristin, rifampicin (e.g., rifampin), tazobactam, tinidazole, and/or uvaricin.

Targeting moieties

[0001] To date, numerous unique antigens have been identified and may be potentially used in antibody-based therapy as a target. Several factors are generally considered when selecting an antigen. First, the target antigen should have high expression in the tumor and no or low expression in the healthy cell. An example is the HER2 receptor, which is almost 100-fold higher expressed in the tumor cell compared to the healthy cell. Second, the target antigen should be displayed on the surface of the tumor cell to be available to the circulated monoclonal antibody.

In addition, the target antigen should possess internalization properties as it will facilitate the ADC to transport into the cell, which will in turn enhance the efficacy of cytotoxic agent. Though some studies have demonstrated that non-internalized ADC product directed against components of the tumor microenvironment can efficiently detach their drug in the extracellular space and arbitrate a potent therapeutic activity in some cases and that ADCs often induce a strong “bystander effect.” (Strohl WR **2018** *Protein & Cell*. 9(1):86-120; Damelin et al. **2015** *Pharma. Res.* 32(11):3494-507; Diamantis et al. **2016** *British J. Cancer* 114(4):362-7; Tipton et al. **2015** *Blood* 125(12):1901-9; Donaghy et al. **2016** *mAbs*. 8(4):659-71; Casi et al. **2015** *Molecular Pharmaceutics* 12(6):1880-4.)

[0002] A targeting moiety can be any moiety that selectively binds to a cell-surface marker found on a targeted cell type. In general, the antibody should preferably possess target specificity and deliver the cytotoxic drug to the tumor cell and possess target binding affinity, i.e., a high binding affinity to the tumor cell-surface antigens. Additionally, the antibody should preferably possess good retention, low immunogenicity, low cross-reactivity, and appropriate linkage binding properties. (Peters et al. **2015** *Bioscience Reports* 35(4); Hughes B **2010** *Nature Reviews Drug Discovery* 9(9):665-7.)

[0003] In certain embodiments, the targeting moiety is an antibody.

[0004] In certain embodiments, the targeting moiety is a monoclonal antibody.

[0005] In certain embodiments, the targeting moiety is a chimeric antibody.

[0006] In certain embodiments, the targeting moiety is a humanized antibody.

[0007] In certain embodiments, the targeting moiety is a bispecific antibody.

[0008] In certain embodiments, the targeting moiety is an antibody fragment.

[0009] In certain embodiments, the targeting moiety is a Fab fragment.

[0010] In certain embodiments, the targeting moiety is a peptide.

[0011] In certain embodiments, the targeting moiety is a small molecule ligand.

[0012] In some aspects, Ab is an antibody or antibody fragment (*e.g.*, antigen binding fragment of an antibody) that specifically binds to an antigen predominantly or preferentially found on the surface of cancer cells, *e.g.*, a tumor-associated antigen.

[0013] In some aspects, Ab is an antibody or antibody fragment (*e.g.*, antigen binding fragment) that specifically binds to a cell surface receptor protein or other cell surface molecules, a cell survival regulatory factor, a cell proliferation regulatory factor, a molecules associated

with, known or suspected to contribute functionally to, tissue development or differentiation, a lymphokine, a cytokine, a molecule involved in cell cycle regulation, a molecule involved in vasculogenesis or a molecule associated with, known or suspected to contribute functionally to, angiogenesis.

[0014] Thus, targeting moieties useful in immunoconjugates of the invention include, but not limited to, antibodies against cell surface receptors and tumor-associated or tumor-specific antigens, which are well known in the art and can be prepared for use in generating antibodies using methods and information known in the art.

[0015] In attempts to discover effective cellular targets for cancer diagnosis and therapy, researchers have sought to identify transmembrane or otherwise tumor-associated or tumor-specific polypeptides that are specifically expressed on the surface of one or more particular type(s) of cancer cell as compared to on one or more normal non-cancerous cell(s). Tumor-associated polypeptides are more abundantly expressed on the surface of the cancer cells as compared to on the surface of the non-cancerous cells, whereas tumor-specific polypeptides are specifically expressed on the surface of one or more particular type(s) of cancer cell but not on non-cancerous cell(s). The identification of such cell surface antigen polypeptides has given rise to the ability to specifically target cancer cells for destruction via antibody-based therapies. (See, *e.g.*, Liu et al. **2017** *Eur. J. Cancer Care (Engl)*. 2017 Sep; 26(5), doi: 10.1111/ecc.12446; WO 2016/192527 A1.)

[0016] A tumor-associated antigen may be a cluster differentiation factor (*e.g.*, a CD protein). In some aspects of the invention, the targeting moiety of the invention specifically binds to one antigen. In some aspects of the invention, the targeting moiety of the invention specifically binds to two or more antigens described herein, for example, the targeting moiety of the invention is a bispecific or multispecific antibody or antigen binding fragment thereof.

[0017] Non-limiting examples of antibodies or antigen binding fragments include anti-estrogen receptor antibody, anti-progesterone receptor antibody, anti-p53 antibody, anti-HER-2 antibody, anti-EGFR antibody, anti-cathepsin D antibody, anti-Bcl-2 antibody, anti-E-cadherin antibody, anti-CA125 antibody, anti-CA15-3 antibody, anti-CA19-9 antibody, anti-c-erbB-2 antibody, anti-P-glycoprotein antibody, anti-CEA antibody, anti-retinoblastoma protein antibody, anti-ras oncoprotein antibody, anti-Lewis X antibody, anti-Ki-67 antibody, anti-PCNA antibody, anti-CD3 antibody, anti-CD4 antibody, anti-CD5 antibody, anti-CD7 antibody, anti-CD8 antibody,

anti-CD9/p24 antibody, anti-CD1 - antibody, anti-CD11c antibody, anti-CD13 antibody, anti-CD14 antibody, anti-CD15 antibody, anti-CD19 antibody, anti-CD20 antibody, anti-CD22 antibody, anti-CD23 antibody, anti-CD30 antibody, anti-CD31 antibody, anti-CD33 antibody, anti-CD34 antibody, anti-CD35 antibody, anti-CD38 antibody, anti-CD39 antibody, anti-CD41 antibody, anti-LCA/CD45 antibody, anti-CD45RO antibody, anti-CD45RA antibody, anti-CD71 antibody, anti-CD95/Fas antibody, anti-CD99 antibody, anti-CD100 antibody, anti-S-100 antibody, anti-CD106 antibody, anti-ubiquitin antibody, anti-c-myc antibody, anti-cytokeratin antibody, anti-lambda light chains antibody, anti-melanosomes antibody, anti-prostate specific antigen antibody, anti-tau antigen antibody, anti-fibrin antibody, anti-keratins antibody, and anti-Tn-antigen antibody.

[0018] Antibodies and antibody fragments useful for the immunoconjugates of the invention include modified or engineered antibodies, such as an antibody modified to introduce a cysteine residue, or other reactive amino acid, including Pel, pyrrolysine, peptide tags, and non-natural amino acids, in place of at least one amino acid of the native sequence, thus providing a reactive site on the antibody or antigen binding fragment for conjugation to a cytotoxic agent.

[0019] The location of the drug moiety may be designed, controlled and known. For example, cysteine amino acids may be engineered at reactive sites in an antibody and which do not form intrachain or intermolecular disulfide linkages. (Junutula, et al. **2008** *Nature Biotech.* 26(8):925-932; Dornan et al. **2009** *Blood* 114(13):2721-2729; U.S. Pat. No. 7,521,541 B2; U.S. Pat. No. 7,723,485 B2; WO 2009/052249 A2.) The engineered cysteine thiols may react with linker reagents or the drug-linker reagents of the present invention which have thiol-reactive, electrophilic groups such as maleimide or alpha-halo amides to form ADC with cysteine engineered antibodies and the drug moieties.

[0020] Additionally, the antibodies or antibody fragments can be modified to incorporate Pel or pyrrolysine or unnatural amino acids as sites for conjugation to a drug. Peptide tags for enzymatic conjugation methods can be introduced into an antibody. (Junutula et al. **2008** *Nat. Biotechnol.* 26:925-932; Ou et al. **2011** *PNAS* 108 (26), 10437-10442; Axup et al. **2012** *Proc. Natl. Acad. Sci. USA*, 109, 16101-16106; Liu et al. **2010** *Annu. Rev. Biochem.* 79, 413-444; Kim et al. **2013** *Curr. Opin. Chem. Biol.* 17, 412-419; Strop et al. **2013** *Chem. Biol.* 20(2):161-7; Rabuka **2010** *Curr. Opin. Chem. Biol.* 14(6):790-6; Rabuka et al. **2012** *Nat. Protoc.* 7(6): 1052-67; WO 2015/095301 A2; WO 2013/184514 A2.)

[0233] Antibodies and antibody fragments can be readily produced by any methods known in the art, including but not limited to, recombinant expression, chemical synthesis, and enzymatic digestion of antibody tetramers, whereas full-length monoclonal antibodies can be obtained by, e.g., hybridoma or recombinant production. Recombinant expression can be from any appropriate host cells known in the art, for example, mammalian host cells, bacterial host cells, yeast host cells, insect host cells, etc. (See, e.g., Carvalho et al. **2016** “Production Processes for Monoclonal Antibodies”, DOI: 10.5772/64263 (<https://www.intechopen.com/chapters/51512>); Monoclonal Antibody Production, Committee on Methods of Producing Monoclonal Antibodies, Institute for Laboratory Animal Research, National Research Council, NATIONAL ACADEMY PRESS Washington, DC **1999**; Jakobovits **1998** *Adv. Drug Del. Rev.* 31:33-42; Marks et al. **1991** *J. Mol. Biol.* 222:581; Cole et al. **1985** *Monoclonal Antibodies And Cancer Therapy* 77-96; Teng et al. **1983** *Proc. Natl. Acad. Sci. USA.* 80:7308-7312; Kozbor et al., **1983** *Immunology Today* 4:72-79; Olsson et al. **1982** *Meth. Enzymol.* 92:3-16; U.S. Pat. No. 6,657,103 B2.)

[0234] The targeting moiety in the drug conjugates and components thereof provided herein may be any compound or molecule capable of specifically binding to a target. By way of example, a targeting moiety may be a small molecule, a peptide, a polypeptide, or a nucleic acid such as an aptamer.

[0235] In certain embodiments, the targeting moiety is a polypeptide, for example a protein ligand, protein scaffold, or antibody. In certain embodiments, the targeting moiety is a monoclonal antibody.

[0236] In some embodiments, the targeting moiety comprises HuM195-Ac-225, HuM195-Bi-213, Anyara (naptumomab estafenatox; ABR-217620), AS 1409, Zevalin (ibritumomab tiuxetan), BIIIB015, BT-062, Neuradiab, CDX-1307, CR011-vcMMAE, Trastuzumab- DMI (R3502), Bexxar (tositumomab), IMGN242, IMGN388, IMGN901, ¹³¹I- labetuzumab, IMMU-102 (⁹⁰Y-epratuzumab), IMMU-107 (⁹⁰Y-clivatuzumab tetraxetan), MDX-1203, CAT-8015, EMD 273063 (hul4.18-IL2), Tucotuzumab celmoleukin (EMD 273066; huKS-IL2), ¹⁸⁸Re-PTI-6D2, Cotara, L19-IL2, Teleukin (F16-IL2), Tenarad (F16- ¹³¹I), L19-¹³¹I, L19-TNF, PSMA-ADC, DI-Leul6-IL2, SAR3419, SGN-35, and/or CMC544, or a target-binding portion thereof.

[0237] In some embodiments, the targeting moiety comprises Brentuximab vedotin, Trastuzumab emtansine, Inotuzumab ozogamicin, Lorvotuzumab mertansine, Glembatumumab

vedotin, SAR3419, Moxetumomab pasudotox, AGS-16M8F, BIIB-015, BT-062, and/or IMGN-388, or a target-binding portion thereof.

Kits

[0238] Provided herein in certain embodiments are kits comprising one or more of the drug conjugates or components thereof provided herein. In certain embodiments, the kits further comprise instructions for use.

[0239] In some embodiments, the kits provided herein are for use in preparing a drug conjugate as disclosed herein. For example, the kit may comprise one or more of a linker, a drug moiety, and a targeting moiety, and may further comprise instructions for using the provided components to generate a drug conjugate.

[0240] In some embodiments, the kits provided herein are for use in a method of treatment as disclosed herein. For example, the kit may comprise a drug conjugate or all of the components of a drug conjugate, and may further comprise instructions for preparing and/or administering the drug conjugate.

[0021] Isotopically-labeled compounds are also within the scope of the present disclosure. As used herein, an "isotopically-labeled compound" refers to a presently disclosed compound including pharmaceutical salts and prodrugs thereof, each as described herein, in which one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into compounds presently disclosed include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, fluorine and chlorine, such as ^2H , ^3H , ^{13}C , ^{14}C , ^{15}N , ^{18}O , ^{17}O , ^{31}P , ^{32}P , ^{35}S , ^{18}F , and ^{36}Cl , respectively.

[0022] By isotopically-labeling the presently disclosed compounds, the compounds may be useful in drug and/or substrate tissue distribution assays. Tritiated (^3H) and carbon-14 (^{14}C) labeled compounds are particularly preferred for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium (^2H) can afford certain therapeutic advantages resulting from greater metabolic stability, for example increased *in vivo* half-life or reduced dosage requirements and, hence, may be preferred in some circumstances. Isotopically labeled compounds presently disclosed, including pharmaceutical salts, esters, and prodrugs

thereof, can be prepared by any means known in the art.

[0023] Further, substitution of normally abundant hydrogen (^1H) with heavier isotopes such as deuterium can afford certain therapeutic advantages, *e.g.*, resulting from improved absorption, distribution, metabolism and/or excretion (ADME) properties, creating drugs with improved efficacy, safety, and/or tolerability. Benefits may also be obtained from replacement of normally abundant ^{12}C with ^{13}C . (See, WO 2007/005643, WO 2007/005644, WO 2007/016361, and WO 2007/016431.)

[0024] Thus, isotope derivative compounds having one or more hydrogen atoms (*e.g.*, 1, 2, 4, 5, 6, 7, 8, 9, 10, *etc.*) replaced with deuterium atoms are contemplated in the presented invention. In certain embodiments, isotope derivative compounds of the invention have one hydrogen atom replaced with a deuterium atom.

[0025] Stereoisomers (*e.g.*, *cis* and *trans* isomers) and all optical isomers of a presently disclosed compound (*e.g.*, *R* and *S* enantiomers), as well as racemic, diastereomeric and other mixtures of such isomers are within the scope of the present disclosure.

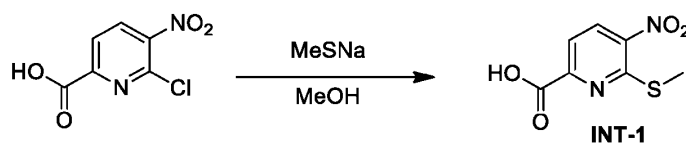
[0026] Compounds of the present invention are, subsequent to their preparation, preferably isolated and purified to obtain a composition containing an amount by weight equal to or greater than 95% (“substantially pure”), which is then used or formulated as described herein. In certain embodiments, the compounds of the present invention are more than 99% pure.

[0027] Solvates and polymorphs of the compounds of the invention are also contemplated herein. Solvates of the compounds of the present invention include, for example, hydrates.

[0241] As can be appreciated from the disclosure above, the present invention has a wide variety of applications. The invention is further illustrated by the following examples, which are only illustrative and are not intended to limit the definition and scope of the invention in any way.

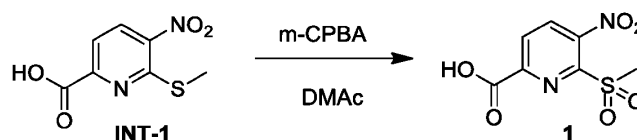
Examples

Synthesis



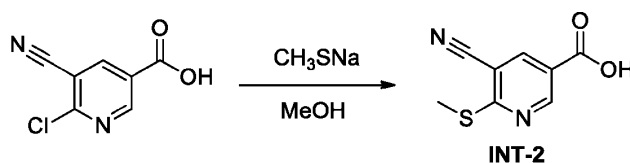
6-(Methylthio)-5-nitropicolinic acid INT-1

[0242] To a solution of 6-chloro-5-nitropicolinic acid (500 mg, 2.47 mmol, 1.0 eq.) in MeOH (20 mL) was added sodium methanethiolate (CH_3SNa , 1.9 mL 20% water solution, 5.43 mmol, 2.2 eq.). The reaction mixture was stirred at room temperature for 4 hrs. Methanol was removed under reduced pressure. Water (10 mL) was added, and the pH of the reaction mixture was adjusted to 4~5 with 3N HCl. The solid precipitate was filtered, washed with water and dried to give **INT-1** (528 mg, 99.8%) as a yellow solid. LCMS (ESI): m/z 215.12 $[\text{M} + \text{H}]^+$. ^1H NMR (DMSO- d_6 , 600 MHz): δ 8.67 (d, $J = 8.3$ Hz, 1H), 7.84 (d, $J = 8.3$ Hz, 1H), 2.59 (s, 3H).



6-(Methylsulfonyl)-5-nitropicolinic acid 1

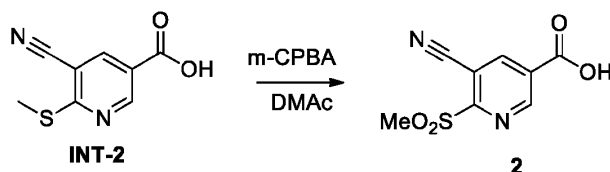
[0243] To a solution of **INT-1** (100 mg, 0.467 mmol, 1.0 eq.) in N,N-dimethylacetate (DMAc, 10 mL) was added 3-chloroperoxybenzoic acid (m-CPBA, 242 mg, 1.4 mmol, 3 eq.), and the reaction mixture was stirred at 50 °C for 18 hrs. DMAc was removed under reduced pressure, and the residue was purified by RP prep-HPLC to give **1** (53.25 mg, 46.33%) as a yellow solid. LCMS (ESI): m/z 247.08 $[\text{M} + \text{H}]^+$. ^1H NMR (DMSO- d_6 , 600 MHz): δ 14.27 (s, 1H), 8.75 (d, $J = 8.3$ Hz, 1H), 8.50 (d, $J = 8.3$ Hz, 1H), 3.50 (s, 3H).



5-Cyano-6-(methylthio)nicotinic acid INT-2

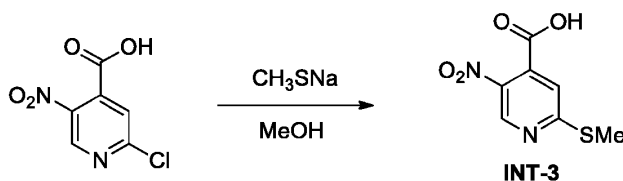
[0244] To a solution of 6-chloro-5-cyanonicotinic acid (150 mg, 0.82 mmol, 1.0 eq.) in MeOH (3 mL) was added CH_3SNa (0.58 mL 20% water solution, 1.65 mmol, 2.0 eq.). The

reaction mixture was stirred at room temperature for 2 hrs. The pH of the reaction mixture was adjusted to 4~5 with 3N HCl. Methanol was removed under reduced pressure. The precipitated solid was filtered, washed with water and dried to give **INT-2** (27 mg, 16.9%) as a white solid. LCMS (ESI): m/z 195.16 $[M + H]^+$



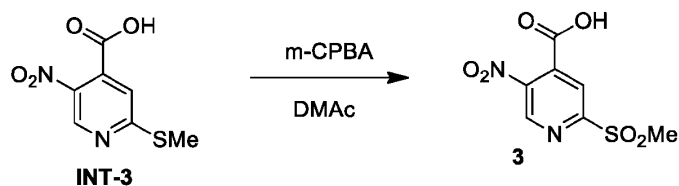
5-Cyano-6-(methylsulfonyl)nicotinic acid 2

[0245] To a solution of **INT-2** (19 mg, 0.1 mmol, 1.0 eq.) in DMAc (1 mL) was added m-CPBA (52 mg, 0.3 mmol, 3.0 eq.) at RT, and the reaction mixture was stirred at that temperature for 2 days. DMAc was removed under reduced pressure, and the residue was purified by RP prep-HPLC to give **2** (13 mg, 59%) as a white solid. LCMS (ESI): m/z 227.14 $[M + H]^+$



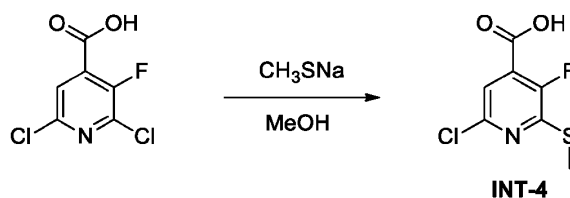
2-(Methylthio)-5-nitroisonicotinic acid INT-3

[0246] To a solution of 2-chloro-5-nitroisonicotinic acid (1000 mg, 4.94 mmol, 1.0 eq.) in MeOH (20 mL) was added CH_3SNa (5.2 mL 20% water solution, 14.81 mmol, 3.0 eq.). The reaction mixture was stirred at room temperature for 4 hrs. The pH of the reaction mixture was adjusted to 4~5 with 3N HCl. Methanol was removed under reduced pressure. The precipitated solid was filtered, washed with water and dried to give **INT-3** (874 mg, 82.7%) as a yellow solid. LCMS (ESI): m/z 215.15 $[M + H]^+$



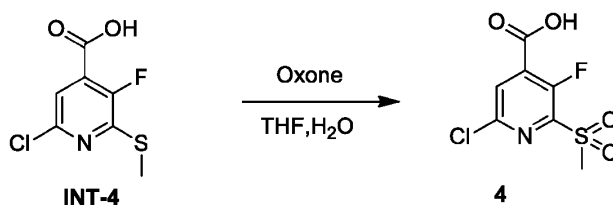
2-(Methylsulfonyl)-5-nitroisonicotinic acid 3

[0247] To a solution of **INT-3** (500 mg, 2.33 mmol, 1.0eq.) in DMAc (20 mL) was added m-CPBA (1420 mg, 7.0 mmol, 4 eq.), and the reaction mixture was stirred at RT for 16 hrs. DMAc was removed under reduced pressure and the residue was purified by RP prep-HPLC to give **3** (367 mg, 63.9%) as a yellow solid. LCMS (ESI): m/z 247.03 $[M + H]^+$ 1H NMR (DMSO- d_6 , 600 MHz): δ 9.44 (s, 1H), 8.37 (s, 1H), 3.41 (s, 3H).



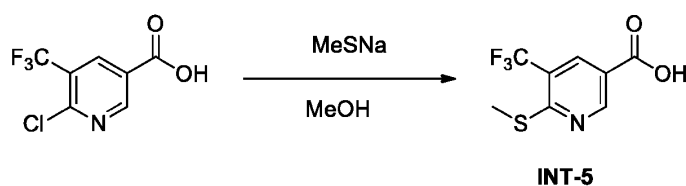
6-Chloro-3-fluoro-2-(methylthio)isonicotinic acid INT-4

[0248] To a solution of 2,6-dichloro-3-fluoroisonicotinic acid (500 mg, 2.38 mmol, 1.0 eq.) in MeOH (20 mL) was added CH_3SNa (2.5 mL 20% water solution, 7.14 mmol, 2.0 eq.). The reaction mixture was stirred at room temperature for 4 hrs. The pH of the reaction mixture was adjusted to 4~5 with 3N HCl. Methanol was removed under reduced pressure. The precipitated solid was filtered, washed with water and dried to give **INT-4** (422 mg, 80%) as a white solid. LCMS (ESI): m/z 222.35 $[M + H]^+$



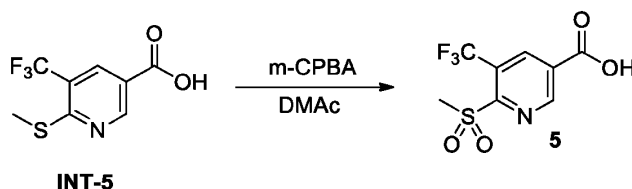
6-Chloro-3-fluoro-2-(methylsulfonyl)isonicotinic acid 4

[0249] To a solution of **INT-4** (90 mg, 0.41 mmol, 1.0 eq.) in THF (2 mL) was added Oxone (500 mg, 0.814 mmol, 2 eq.) in H₂O (2 mL) at RT, and the reaction mixture was stirred at that temperature for 16 hrs. THF was removed under reduced pressure and the residue was purified by RP prep-HPLC to give **4** (53.11 mg, 51.4%) as a pale white solid. LCMS (ESI): m/z 254.05 [M + H]⁺ ¹H NMR (DMSO-d₆, 600 MHz): δ 8.19 (s, 1H), 3.44 (s, 3H).



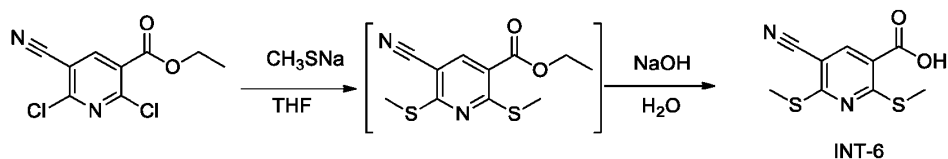
6-(Methylthio)-5-(trifluoromethyl)nicotinic acid INT-5

[0250] To a solution of 6-chloro-5-(trifluoromethyl)nicotinic acid (250 mg, 1.1 mmol, 1.0 eq.) in MeOH (3 mL) was added CH₃SNa (0.4 mL 20% water solution, 1.1 mmol, 1.0eq.). The reaction mixture was stirred at room temperature for 2 hrs. The pH of the reaction mixture was adjusted to 4~5 with 3N HCl. Methanol was removed under reduced pressure and the solid precipitate was filtered, washed with water and dried to give **INT-5** (286 mg, 99%) as a white solid. LCMS (ESI): m/z 238.06 [M + H]⁺



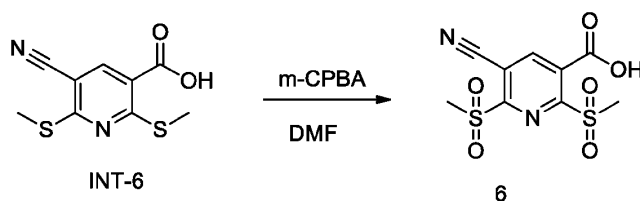
6-(Methylsulfonyl)-5-(trifluoromethyl)nicotinic acid 5

[0251] To a solution of **INT-5** (100 mg, 0.421 mmol, 1.0 eq.) in DMAc (2 mL) was added m-CPBA (256 mg, 1.265 mmol, 3 eq.) at RT, and the reaction mixture was stirred at that temperature for 16 hrs. DMAc was removed under reduced pressure and the residue was purified by RP prep-HPLC to give **5** (37 mg, 31%) as a pale white solid. LCMS (ESI): m/z 270.02 [M + H]⁺ ¹H NMR (DMSO-d₆, 600 MHz): δ 9.36 (s, 1H), 8.72 (s, 1H), 3.53 (s, 3H).



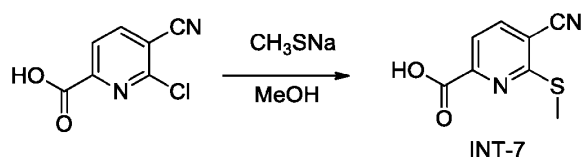
5-Cyano-2,6-bis(methylthio)nicotinic acid INT-6

[0252] To a solution of ethyl 2,6-dichloro-5-cyanonicotinate (200 mg, 0.816 mmol, 1.0eq.) in methanol (4 mL) was added CH_3SNa (0.86 mL 20% water solution, 2.45 mmol, 3.0 eq.). The reaction mixture was stirred at room temperature for 4 hrs. 1.5 mL 1N NaOH was added, and the solution was stirred at RT for 1 hr. The pH of the reaction mixture was adjusted to 4~5 with 3N HCl. Methanol was removed under reduced pressure and the solid precipitate was filtered, washed with water and dried to give **INT-6** (171mg, yield:87.2%) as a white solid. LCMS (ESI): m/z 241.22 $[\text{M} + \text{H}]^+$.



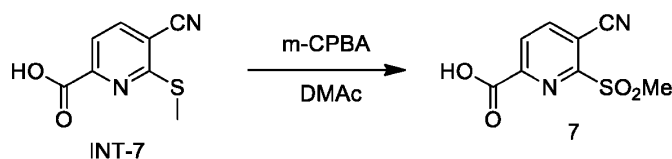
5-Cyano-2,6-bis(methylsulfonyl)nicotinic acid 6

[0253] To a solution of **INT-6** (171 mg, 0.712 mmol, 1.0 eq.) in DMF (6 mL) was added m-CPBA (6.14 mg, 3.56 mmol, 5.0 eq.) at RT, and the reaction mixture was stirred at that temperature for 24 hrs. DMF was removed under reduced pressure, and the crude product was purified by RP prep-HPLC to give **6** (104 mg, yield: 47.5%) as a white solid. LCMS (ESI): m/z 305.23 $[\text{M} + \text{H}]^+$. ^1H NMR ($\text{DMSO}-d_6$, 600 MHz): δ 9.11 (s, 1H, Ar-H), 3.55 (s, 3H, $-\text{CH}_3$), 3.47 (s, 3H, $-\text{CH}_3$).



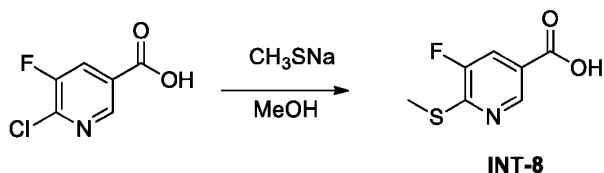
5-Cyano-6-(methylthio)picolinic acid INT-7

[0254] To a solution of 6-chloro-5-cyanopicolinic acid (500 mg, 2.74 mmol, 1.0 eq.) in CH₃OH (4 mL) was added CH₃SNa (1.9 mL 20% water solution, 5.48 mmol, 2.0 eq.). The reaction mixture was stirred at room temperature for 4h. The pH of the reaction mixture was adjusted to 4~5 with 3N HCl. Methanol was removed under the reduced pressure and the solid precipitate was filtered, washed with water and dried to give **INT-7** (393 mg, yield: 72.8%) as a white solid. LCMS (ESI): m/z 195.16 [M + H]⁺.



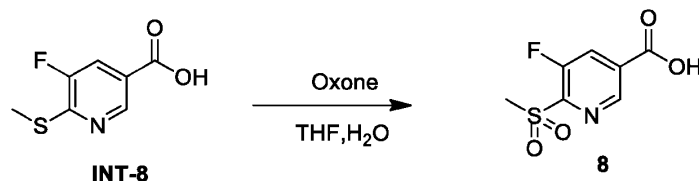
5-Cyano-6-(methylsulfonyl)picolinic acid 7

[0255] To a solution of **INT-7** (193 mg, 0.995 mmol, 1.0eq.) in DMAc (5 mL) was added m-CPBA (515 mg, 2.984 mmol, 3.0eq.) at RT, and the reaction mixture was stirred at that temperature for 2 days. DMAc was removed under reduced pressure, and the residue was purified by RP prep-HPLC to give **7** (132 mg, yield: 59.2%) as a white solid. LCMS (ESI): m/z 227.14 [M + H]⁺.



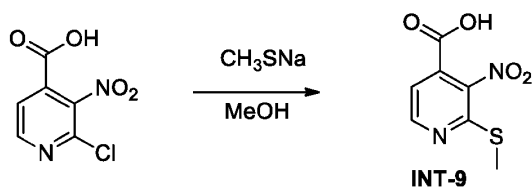
5-Fluoro-6-(methylthio)nicotinic acid INT-8

[0256] To a solution of 6-chloro-5-fluoronicotinic acid (500 mg, 2.85 mmol, 1.0 eq.) in CH₃OH (10 mL) was added CH₃SNa (2.0 mL 20% water solution, 5.7 mmol, 2.0 eq.). The reaction mixture was stirred at room temperature for 4h. The pH of the reaction mixture was adjusted to 4~5 with 3N HCl. Methanol was removed under reduced pressure and the solid precipitate was filtered, washed with water and dried to give **INT-8** (401 mg, yield: 85%) as a white solid. LCMS (ESI): m/z 188.15 [M + H]⁺.



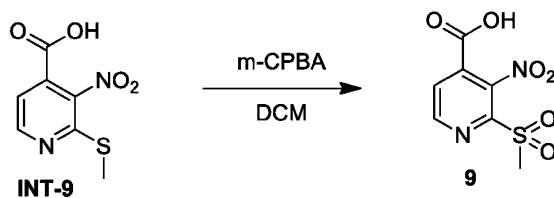
5-Fluoro-6-(methylsulfonyl)nicotinic acid 8

[0257] A solution of **INT-8** (100 mg, 0.535 mmol) in THF (3 mL) was added Oxone (657 mg, 1.0695 mmol) in H₂O (3 mL) at RT for 16 hrs. The reaction was complete as detected by LC-MS. Solvent was removed under reduced pressure. The crude product was purified by RP prep-HPLC to give **8** (79 mg, 67.4%) as a white solid. LCMS (ESI): *m/z* 220.14 [M + H]⁺. ¹H NMR (DMSO-*d*₆, 600 MHz): δ 14.23 (s, 1H), 9.00 (s, 1H), 8.45 (dd, *J* = 10.0, 1.3 Hz, 1H) 3.40 (d, *J* = 108.3 Hz, 3H)



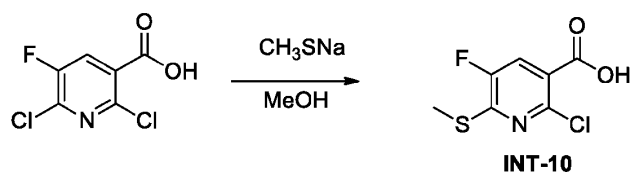
2-(Methylthio)-3-nitroisonicotinic acid INT-9

[0258] To a solution of 2-chloro-3-nitroisonicotinic acid (500 mg, 2.47 mmol) in MeOH (10 mL), was added CH₃SNa (2.5 mL 20% water solution, 7.2 mmol, 3 eq) under N₂. The reaction solution was stirred at room temperature for 2 hrs. The reaction was complete as monitored by LC-MS. The pH of the reaction mixture was adjusted to 4~5 with 3N HCl. Solvent was removed under reduced pressure. Water was added to the residue to make solid precipitate and filter to give **INT-9** (401 mg) as a white solid. LCMS (ESI): *m/z* 216.07 [M + H]⁺.



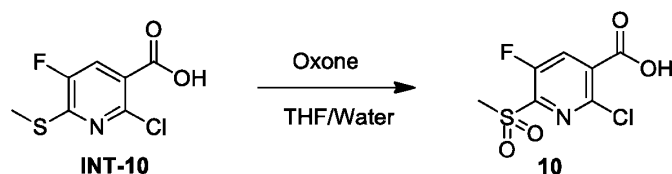
2-(Methylsulfonyl)-3-nitroisonicotinic acid 9

[0259] To a solution of **INT-9** (200 mg, 0.934 mmol) in DCM (10 mL), was added m-CPBA (322 mg, 1.868 mmol) at RT for 16 hrs. The reaction was complete as detected by LC-MS. Solvent was removed under reduced pressure. The crude product was purified by RP prep-HPLC to give **9** (117 mg, 50.9%) as a white solid. LCMS (ESI): m/z 247.13 $[M + H]^+$. 1H NMR (DMSO- d_6 , 600 MHz): δ 9.11 (s, 1H), 8.29 (s, 1H), 3.46 (s, 3H).



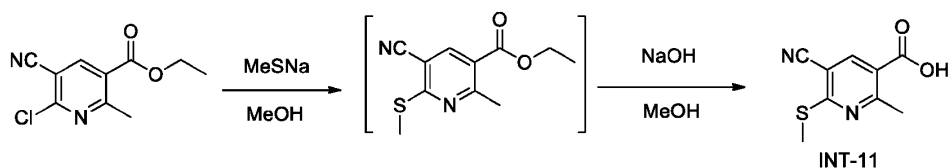
2-Chloro-5-fluoro-6-(methylthio)nicotinic acid INT-10

[0260] To a solution of 2,6-dichloro-5-fluoronicotinic acid (500 mg, 2.38 mmol, 1.0 eq.) in CH_3OH (4 mL) was added CH_3SNa (1.7 mL 20% water solution, 4.76 mmol, 2.0 eq.). The reaction mixture was stirred at room temperature for 4h. The pH of the reaction mixture was adjusted to 4~5 with 3N HCl. Methanol was removed under reduced pressure and the solid precipitate was filtered, washed with water and dried to give **INT-10** (392 mg, 74.3%) as a white solid. LCMS (ESI): m/z 222.15 $[M + H]^+$.



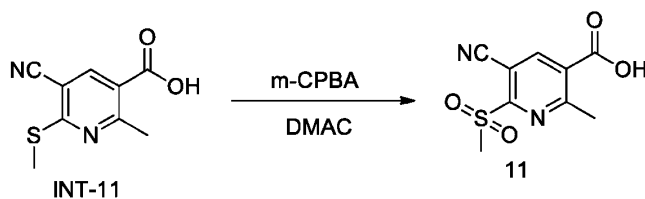
2-Chloro-5-fluoro-6-(methylsulfonyl)nicotinic acid 10

[0261] A solution of **INT-10** (100 mg, 0.45 mmol) in THF (3 mL) was added Oxone (553 mg, 0.9 mmol) in H_2O (3 mL) at RT for 16 hrs. The reaction was complete as detected by LC-MS. Solvent was removed under reduced pressure. The crude product was purified by RP prep-HPLC to give **10** (74.2 mg, 65%) as a white solid. LCMS (ESI): m/z 254.15 $[M + H]^+$.



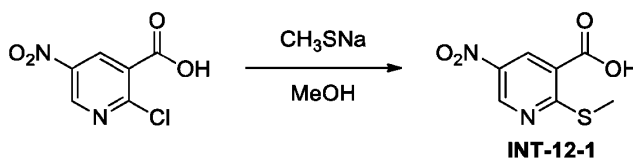
5-Cyano-2-methyl-6-(methylthio)nicotinic acid INT-11

[0262] To a solution of ethyl 6-chloro-5-cyano-2-methylnicotinate (1000 mg, 4.463 mmol, 1.0 eq.) in CH₃OH (20 mL) was added CH₃SNa (3.2 mL 20% water solution, 9.2 mmol, 2.0 eq.). The reaction mixture was stirred at room temperature for 4 hrs. LCMS showed the reaction was completed. 5 mL 1N NaOH was added to the reaction solution and stirred for 1 hr at RT. The pH of the reaction mixture was adjusted to 4~5 with 3N HCl. Methanol was removed under reduced pressure and the solid precipitate was filtered, washed with water and dried to give **INT-11** (200mg crude) as a white solid. LCMS (ESI): m/z 209.14 [M + H]⁺.



5-Cyano-2-methyl-6-(methylsulfonyl)nicotinic acid 11

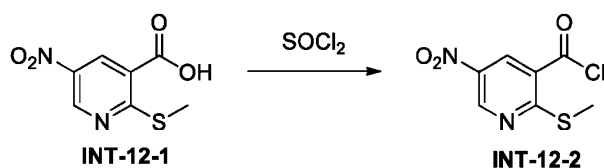
[0263] To a solution of **INT-11** (200 mg, 0.562 mmol, 1.0 eq.) in DMAc (5 mL) was added m-CPBA (330 mg, 1.912 mmol, 3.4 eq.) at RT, and the reaction mixture was stirred at that temperature for 2 days. Solvent was removed under reduced pressure and the residue was purified by RP prep-HPLC to give **11** (70 mg, yield: 51.9%) as a white solid. LCMS (ESI): m/z 241.10 [M + H]⁺. ¹H NMR (DMSO-d₆, 600 MHz): δ 8.85 (s, 1H), 3.49 (s, 3H), 2.86 (s, 3H).



2-(Methylthio)-5-nitronicotinic acid INT-12-1

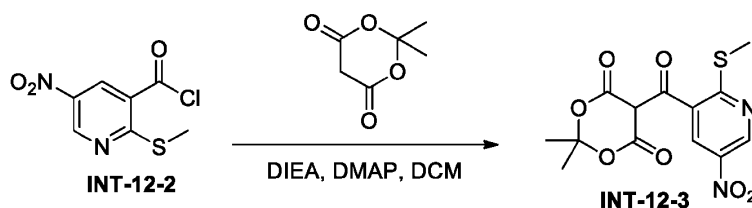
[0264] To a solution of 2-chloro-5-nitronicotinic acid (30.0 g, 0.148 mol, 1.0 eq.) in MeOH (650 ml) was added CH₃SNa (100.3 mL 20% water solution, 0.286 mol, 2 eq.). The reaction

mixture was stirred at room temperature for 18 hrs. Methanol was removed under reduced pressure. H₂O (600 ml) was added, and the pH of the reaction mixture was adjusted to 4~5 with 3N HCl. The solid precipitate was filtered, washed with water and dried to get **INT-12-1** (27.8 g, 87.7%) as a pale yellow solid. LCMS (ESI): m/z 215.07 [M + H]⁺. ¹H NMR (CD₃OD, 600 MHz): δ 9.30 (d, J = 2.4 Hz, 1H), 8.82 (d, J = 2.4 Hz, 1H), 2.58 (s, 3H)



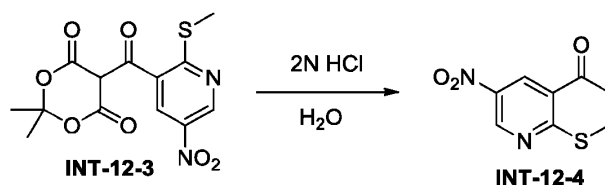
2-(Methylthio)-5-nitronicotinoyl chloride INT-12-2

[0265] **INT-12-1** (10.0 g, 0.0467 mol) was added to DCM (100 mL) and cooled to 0-5 °C in ice bath, oxalyl chloride (12 mL, 0.140 mol) was added dropwise, and followed by adding DMF (5 mL) dropwise. The reaction solution was stirred at room temperature for 4 hrs, remove solvent on roto-evaporator to get **INT-12-2**, DCM (50 mL) was added to make solution A



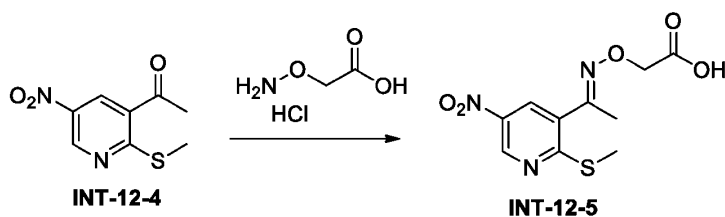
2,2-Dimethyl-5-(2-(methylthio)-5-nitronicotinoyl)-1,3-dioxane-4,6-dione INT-12-3

[0266] 2,2-Dimethyl-1,3-dioxane-4,6-dione (7.40 g, 0.0514 mol), DMAP (5.70 g, 0.047 mol) and TEA (14.17 g, 0.140 mol) were added to DCM (50 mL), cooled to 0-5 °C in ice bath, solution A (**INT-12-2** in DCM) was added dropwise and the mixture was stirred at room temperature for 16 hrs. The reaction solution was monitored by MS. Water (100 mL) was added, stirred, separated, collect the organic phase and remove solvent on roto-evaporator to give 20.1 g **INT-12-3**.



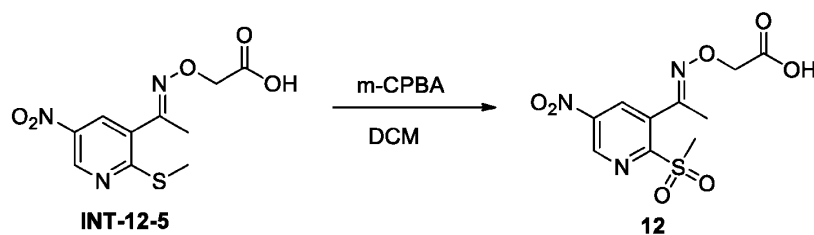
1-(2-(Methylthio)-5-nitropyridin-3-yl)ethan-1-one INT-12-4

[0267] Ethanol (150 mL) and 2N diluted hydrochloric acid (20 mL) were added to **INT-12-3**, and the solution was heated to 80 °C and stirred for 72 hrs. The reaction solution was monitored by MS. Remove solvent on roto-evaporator, added water (100 mL) and DCM (100 mL), stirred, separated, collect the organic phase, washed the organic phase with sodium hydroxide aqueous solution (80 mL), then washed with water (80 mL), dried with anhydrous sodium sulfate, filtered and remove solvent on roto-evaporator to give **INT-12-4** (5.98 g, 60%) as a yellow solid. LCMS (ESI): m/z 213.08 $[M + H]^+$.



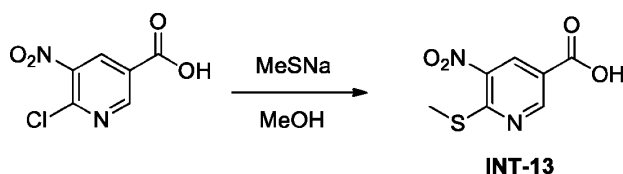
(E)-2-(((1-(2-(methylthio)-5-nitropyridin-3-yl)ethylidene)amino)oxy)acetic acid INT-12-5

[0268] To a solution of **INT-12-4** (50 mg, 0.2 mmol, 1.0 eq.) and 2-(aminooxy)acetic acid HCl salt (77 mg, 0.353 mmol, 1.7 eq) in MeOH (5 mL) was added AcONa (58 mg, 0.707 mmol, 3.5 eq.). The resulting mixture was stirred at 50°C for 3 hrs. The pH of the reaction mixture was adjusted to 4~5 with 3N HCl. Methanol was removed under reduced pressure and the solid precipitate was filtered, washed with water and dried to give **INT-12-5** (35 mg, 52.2%) as an off-white solid. LCMS (ESI): m/z 286.25 $[M + H]^+$.



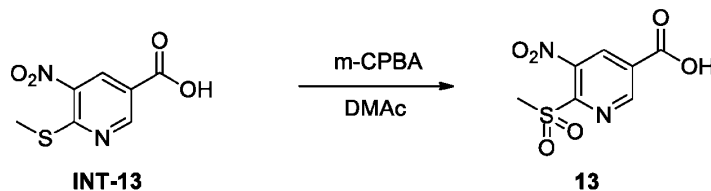
(E)-2-(((1-(2-(methylsulfonyl)-5-nitropyridin-3-yl)ethylidene)amino)oxy)acetic acid 12

[0269] To a solution of **INT-12-5** (135 mg, 0.47 mmol, 1.0 eq.) in DCM (5 mL) was added m-CPBA (224 mg, 1.18 mmol, 2.5eq.) at RT, and the reaction mixture was stirred at that temperature for 16 hrs. DCM was removed under reduced pressure and the residue was purified by RP prep-HPLC to give **12** (85 mg, 57.0%) as a pale white solid. LCMS (ESI): m/z 318.26 $[M + H]^+$.



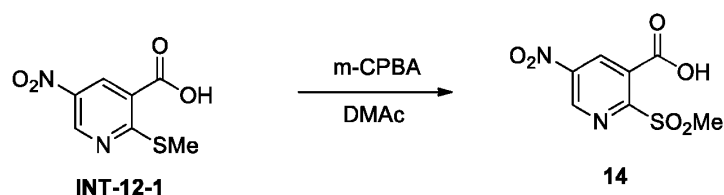
6-(Methylthio)-5-nitronicotinic acid INT-13

[0270] To a solution of 6-chloro-5-nitronicotinic acid (2.865 g, 14.10 mmol, 1.0eq.) in MeOH (20 mL) was added CH_3SNa (74 mL 20% water solution, 21.20 mmol, 1.5 eq.). The reaction mixture was stirred at room temperature for 4h. The pH of the reaction mixture was adjusted to 4~5 with 3N HCl. Methanol was removed under reduced pressure and the solid precipitate was filtered, washed with water and dried to give **INT-13** (2000 mg, 64.5%) as a yellow solid. LCMS (ESI): m/z 215.22 $[M + H]^+$.



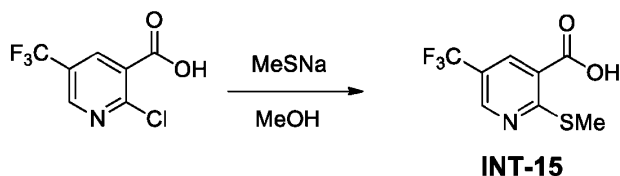
6-(Methylsulfonyl)-5-nitronicotinic acid 13

[0271] To a solution of **INT-13** (100 mg, 0.47 mmol, 1.0 eq) in DMAc (5 mL), was added m-CPBA (243 mg, 3.450 mmol, 3.0eq) at RT for 16 hrs. The reaction was complete as detected by LC-MS. Solvent was removed under reduced pressure and the crude was purified by RP prep-HPLC to give **13** (24.6 mg, 21.7%) as a yellow solid. LCMS (ESI): m/z 247.18 $[M + H]^+$. 1H NMR ($CDCl_3$, 600 MHz): δ 14.35 (s, 1H), 9.34 (d, 1H), 8.94 (d, 1H), 3.51 (s, 3H).



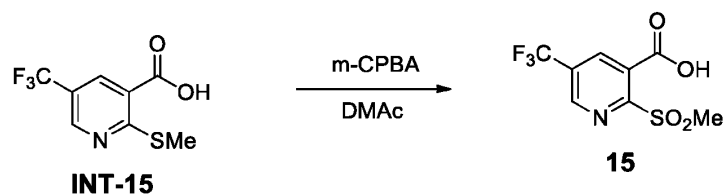
2-(Methylsulfonyl)-5-nitronicotinic acid 14

[0272] To a solution of **INT-14** (100 mg, 0.47 mmol, 1.0eq) in DMAc (5 mL), was added m-CPBA (243 mg, 3.45 mmol, 3.0 eq) at RT for 16 hrs. The reaction was complete as detected by LC-MS. Solvent was removed under reduced pressure and the crude was purified RP prep-HPLC to give **14** (35.5mg, 31.3%) as a yellow solid. LCMS (ESI): m/z 247.18 $[M + H]^+$.



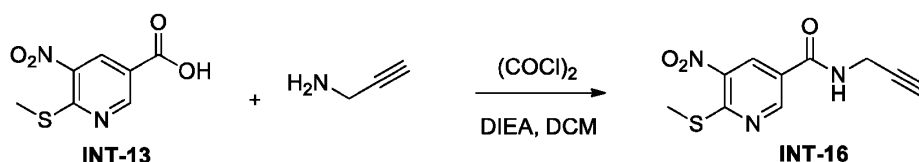
2-(Methylthio)-5-(trifluoromethyl)nicotinic acid INT-15

[0273] To a solution of 2-chloro-5-(trifluoromethyl)nicotinic acid (250 mg, 1.1 mmol, 1.0eq.) in MeOH (3 mL) was added CH_3SNa (0.4 mL 20% water solution, 1.1 mmol, 1.0 eq.). The reaction mixture was stirred at room temperature for 2 hrs. The pH of the reaction mixture was adjusted to 4~5 with 3N HCl. Methanol was removed under reduced pressure and the solid precipitate was filtered, washed with water and dried to give **INT-15** (255 mg, 89%) as a white solid. LCMS (ESI): m/z 238.06 $[M + H]^+$.



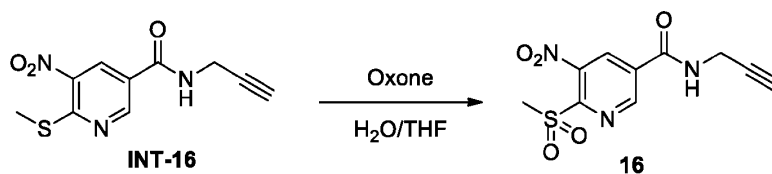
2-(Methylsulfonyl)-5-(trifluoromethyl)nicotinic acid 15

[0274] To a solution of **INT-15** (100 mg, 0.421 mmol, 1.0 eq.) in DMAc (2 mL) was added m-CPBA (256 mg, 1.26 mmol, 3 eq.) at RT, and the reaction mixture was stirred at that temperature for 16 hrs. DMAc was removed under reduced pressure and the residue was purified by RP prep-HPLC to give **15** (35 mg, 29%) as a pale white solid. LCMS (ESI): m/z 270.02 $[M + H]^+$.



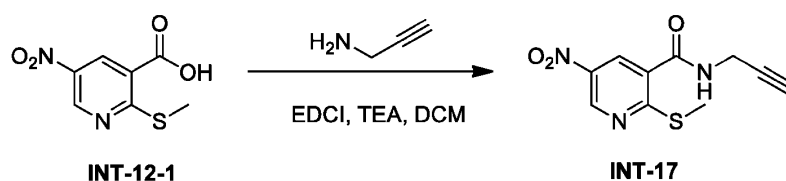
6-(Methylthio)-5-nitro-N-(prop-2-yn-1-yl)nicotinamide INT-16

[0275] To a solution of 6-(methylthio)-5-nitronicotinic acid **INT-13** (2.0 g, 9.3 mmol, 1.0 eq.) in DCM (5 mL) was added prop-2-yn-1-amine (610 mg, 11.2 mmol, 1.2 eq), followed by oxalyl dichloride (11.8 g, 93.0 mmol, 10.0eq.) and diisopropylethylamine (DIEA, 3.6 g, 27.9 mmol, 3.0 eq) under N_2 . The reaction mixture was stirred at room temperature for 2 hrs. Volatile was removed under reduced pressure and the crude product was purified by RP prep-HPLC to give **INT-16** (2.07 g, 86.7%) as a yellow solid. LCMS (ESI): m/z 252.25 $[M + H]^+$.



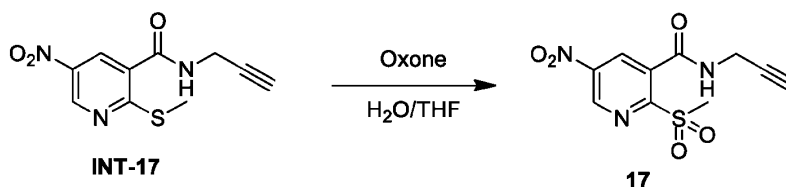
6-(Methylsulfonyl)-5-nitro-N-(prop-2-yn-1-yl)nicotinamide 16

[0276] To a solution of **INT-16** (2.07g, 8.2 mmol, 1 eq) in THF/Water (1:1, V/V) (100 mL) was added Oxone (30.4 g, 49.5 mmol, 6.0eq.) at RT, and the reaction mixture was stirred at that temperature for 16 hrs. THF was removed under reduced pressure and the residue was purified by RP-HPLC to give **16** (1.18 g, 50.6%) as a yellow solid. LCMS (ESI): m/z 284.25 $[M + H]^+$. 1H NMR (DMSO- d_6 , 600 MHz): δ 9.13 (s, 1H), 8.56 (s, 1H), 8.06 (s, 1H), 4.27 (s, 2H), 3.37 (s, 3H), 2.37 (s, 1H).



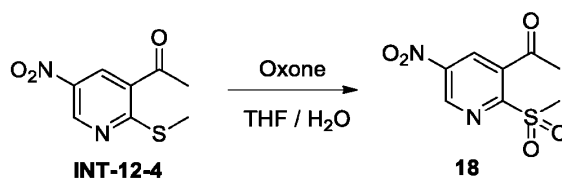
2-(Methylthio)-5-nitro-N-(prop-2-yn-1-yl)nicotinamide INT-17

[0277] To a solution of **INT-12-1** (200 mg, 0.93 mmol, 1.0eq.) in DCM (6 mL) was added the prop-2-yn-1-amine (61 mg, 1.12 mmol, 1.2 eq), EDCI (530 mg) and DIEA (240 mg, 1.86 mmol, 2.0 eq) under N_2 . The reaction mixture was stirred at room temperature for 2 hrs. Solvent was removed under reduced pressure and the crude product was purified by RP prep-HPLC to give **INT-17** (197 mg, 84.5%) as a yellow solid. LCMS (ESI): m/z 252.22 $[M + H]^+$.



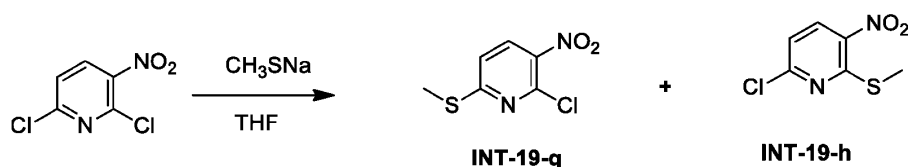
2-(Methylsulfonyl)-5-nitro-N-(prop-2-yn-1-yl)nicotinamide 17

[0278] To a solution of **INT-17** (197 mg, 0.78 mmol, 1.0 eq.) in THF:H₂O (6 mL) was added Oxone (1.44 mg, 2.34 mmol, 3 eq.) at RT, and the reaction mixture was stirred at RT for 16 hrs. THF was removed under reduced pressure and the residue was purified by RP prep-HPLC to give **17** (204 mg, 92.1%) as a yellow solid. LCMS (ESI): m/z 284.22 $[M + H]^+$.



1-(2-(Methylsulfonyl)-5-nitropyridin-3-yl)ethan-1-one 18

[0279] To a solution of **INT-12-4** (0.3 g, 1.41 mmol, 1.0 eq.) in THF (15 mL) and H₂O (15 mL) was added Oxone (4.35 g, 7.068 mmol, 5.0 eq.). The reaction mixture was stirred at room temperature for 18 hrs. Solid was filtered from the solution. THF was removed from the filtrate and the aqueous solution was extracted with ethyl acetate (10 mL x 3). After concentration, the crude product was purified by Silica gel column to give **18** (0.15 g, 43.5%) as a pale yellow solid. LCMS (ESI): *m/z* 245.17 [M + H]⁺.

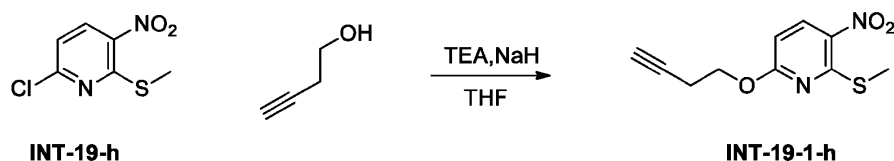


2-Chloro-6-(methylthio)-3-nitropyridine INT-19-q and 6-chloro-2-(methylthio)-3-nitropyridine INT-19-h

[0280] To a solution of 2,6-dichloro-3-nitropyridine (2.0 g, 10.4 mmol, 1.0 eq.) in THF (40 mL) was added CH₃SNa (5.5 mL 20% aq solution, 15.6 mmol, 1.5 eq.) under N₂. The reaction mixture was stirred at room temperature for 2 hrs. The reaction was complete as detected by LC-MS. Added 3N hydrochloric acid to the reaction solution and adjust pH to 4-5. Solvent was removed under reduced pressure. Added water to the residue and filter to get crude product, which was purified by RP prep-HPLC to get two isomers as yellow solid. The late eluted peak is named as **INT-19-h** (625 mg) and the early eluted peak is named as **INT-19-q** (683 mg). Combined yield 61.7%. Structure was assigned tentatively.

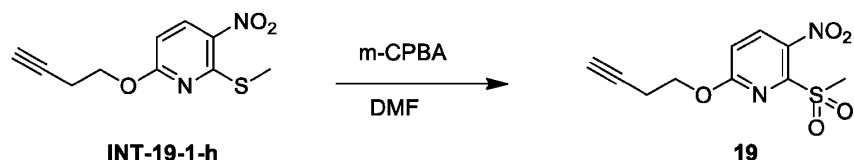
INT-19-h: LCMS (ESI): *m/z* 205.07 [M + H]⁺ (5.85 min)

INT-19-q: LCMS (ESI): *m/z* 205.07 [M + H]⁺ (5.44 min)



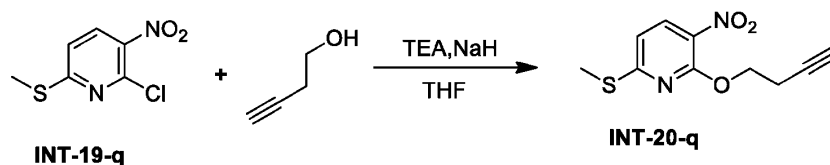
6-(But-3-yn-1-yloxy)-2-(methylthio)-3-nitropyridine INT-19-1-h

[0281] To a solution of but-3-yn-1-ol (125 mg, 1.77 mmol, 1.2 eq.) in THF (12 mL) was added NaH (87 mg, 2.2 mmol, 1.5 eq), followed by **INT-19-h** (300 mg, 1.47 mmol, 1.0 eq) under N₂. The reaction mixture was stirred at room temperature for 2 hrs. Solvent was removed under reduced pressure, and the crude product was purified by RP prep-HPLC to **INT-19-1-h** (134 mg, 38.4%) as a yellow solid. LCMS (ESI): m/z 239.20 [M + H]⁺



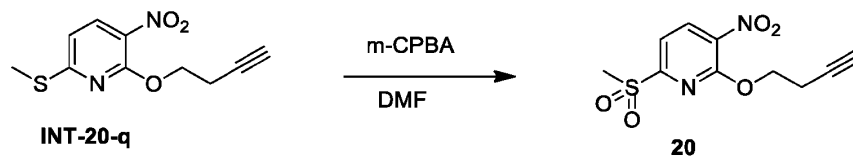
6-(But-3-yn-1-yloxy)-2-(methylsulfonyl)-3-nitropyridine 19

[0282] To a solution of **INT-19-1-h** (80 mg, 0.32 mmol, 1.0 eq.) in DMF (10 mL) was added m-CPBA (160 mg, 0.96 mmol, 3eq.) at RT., and the reaction mixture was stirred at that temperature for 16 hrs. DMF was removed under reduced pressure and the residue was purified RP prep-HPLC to give **19** (17.8 mg, 19.6%) as a white solid. LCMS (ESI): m/z 271.24 [M + H]⁺.



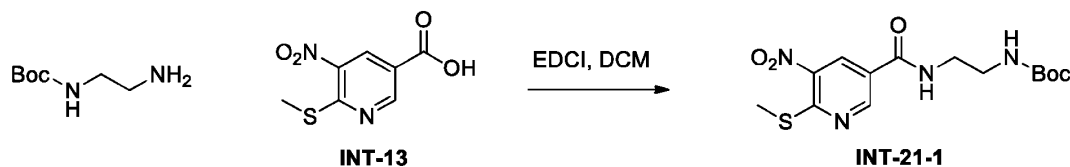
2-(But-3-yn-1-yloxy)-6-(methylthio)-3-nitropyridine INT-20-q

[0283] To a solution of but-3-yn-1-ol (44 mg, 0.63 mmol, 1.2 eq.) in THF (5 mL) was added NaH (31 mg, 0.78 mmol, 1.5eq), followed by **INT-19-q** (107 mg, 0.52 mmol, 1.0 eq) under N₂. The reaction mixture was stirred at room temperature for 2 hrs. Solvent was removed under reduced pressure, and the crude product was purified by RP prep-HPLC to give **INT-20-q** (80 mg, 65.0%) as a yellow solid. LCMS (ESI): m/z 239.19 [M + H]⁺.



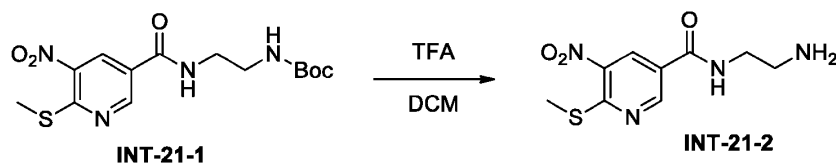
2-(But-3-yn-1-yloxy)-6-(methylsulfonyl)-3-nitropyridine 20

[0284] To a solution of **INT-20-q** (80 mg, 0.32 mmol, 1.0 eq.) in DMF (10 mL) was added m-CPBA (160 mg, 0.96 mmol, 3eq.) at RT, and the reaction mixture was stirred at RT for 16 hrs. DMF was removed under reduced pressure and the residue was purified RP prep-HPLC to give **20** (38 mg, 41.3%) as a yellow solid. LCMS (ESI): m/z 271.19 $[M + H]^+$.



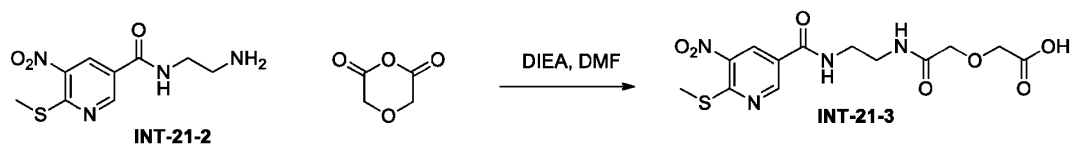
tert-Butyl (2-(6-(methylthio)-5-nitronicotinamido)ethyl)carbamate (INT-21-1)

[0285] To a solution of tert-butyl (2-aminoethyl)carbamate (6.670 g, 41.7 mmol) in DCM (100 mL) was added **INT-13** (5.940 g, 28.0 mmol) and EDCI (8.050 g, 41.7 mmol) under N₂. The reaction suspension was stirred at room temperature for 16 hrs. The reaction is complete as monitored by LC-MS and purified by prep-HPLC to afford **INT-21-1** (6.820 g, 45.9%) as a pale white solid. LCMS $[M-H]^-$: 355.4



N-(2-Aminoethyl)-6-(methylthio)-5-nitronicotinamide (INT-21-2)

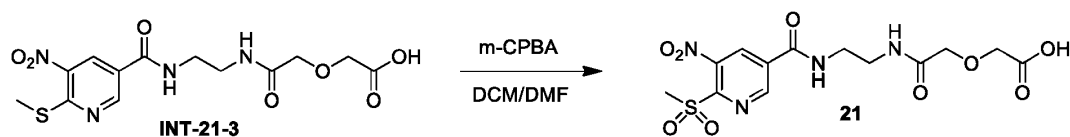
[0286] To a solution of **INT-12-1** (6.820 g, 19.2 mmol) in DCM (68 mL) was added TFA (13 mL), and the mixture was allowed to stir at room temperature for 3 hours. The reaction was complete as detected by LC-MS and concentrated to give **INT-21-2** as colorless solid (4.9 g, yield 100%). LCMS (ESI): m/z 257.2 $[M + H]^+$.



2-((2-((6-(Methylthio)-5-nitronicotinamido)ethyl)amino)-2-oxoethoxy)acetic acid (INT-21-3)

[0287] To a solution of **INT-12-2** (2.0 g, 7.804 mmol) in DMF (20 mL) was added **1,4-dioxane-2,6-dione** (1.086 g, 9.365 mmol) and DIEA (2.215 g, 17.168 mmol) under N₂ and stirred at room temperature for 16 hrs. The reaction is complete as detected by LC-MS. Adding 200 mL water to the solution and stir until precipitate formed, filtered, dried to afford **INT-21-34** (1.5 g, 51.7%) as a pale white solid. LCMS (ESI): m/z 373.3 [M + H]⁺.

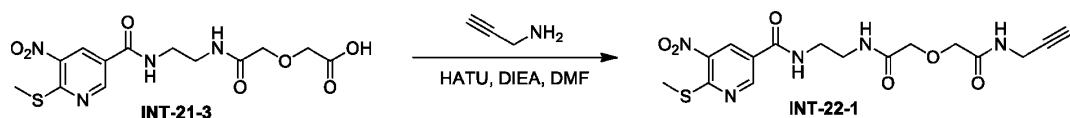
[0288] ¹H NMR (DMSO-*d*₆, 600 MHz): δ 12.77 (brs, 1H), 9.19 (ds, 1H), 8.99 (t, 1H), 8.96 (ds, 1H), 8.07 (t, 1H), 4.10 (s, 2H), 3.96 (s, 2H), 3.41-3.48 (m, 2H), 3.34-3.30 (m, 2H), 2.60 (s, 3H).



2-((2-((6-(Methylsulfonyl)-5-nitronicotinamido)ethyl)amino)-2-oxoethoxy)acetic acid (21)

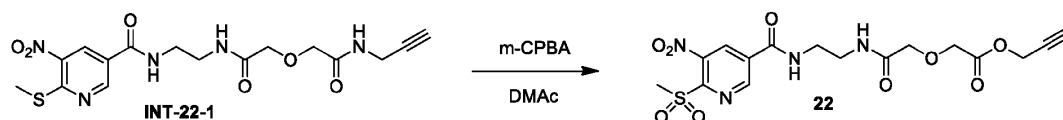
[0289] To a solution of **INT-21-3** (100.0 mg, 0.269 mmol, 1.0 eq.) in DCM/DMAc (2 mL / 2 mL) was added m-CPBA (139.0 mg, 0.807 mmol, 3.0 eq.). The reaction mixture was stirred at room temperature for 24 hrs. The reaction mixture was purified by the prep-HPLC to afford **21** (38.4 mg, yield:35.36%, purity:97.30%) as a white powder. LCMS [M+H]⁺: 405.1.

[0290] ¹H NMR (DMSO-*d*₆, 600 MHz): δ 12.83 (s, 1H), 9.28 (s, 1H), 9.15 (t, 1H), 8.90 (s, 1H), 8.08 (t, 1H), 4.11 (s, 2H), 3.97 (s, 2H), 3.51 (s, 3H), 3.42-3.88 (m, 2H), 3.35-3.31 (m, 2H).



6-(Methylthio)-5-nitro-N-(2-((2-((2-oxo-2-(prop-2-yn-1-ylamino)ethoxy)acetamido)ethyl)nicotinamide (INT-22-1)

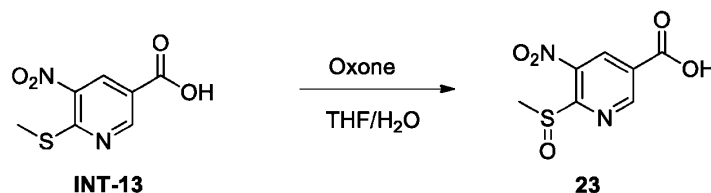
[0291] To a solution of **INT-21-3** (1.5 g, 4.028 mmol) in DMF (15 mL) was added prop-2-yn-1-amine (332 mg, 6.043 mmol), followed by HATU (2.298 g, 6.043 mmol) and DIEA (1.562 g, 12.086 mmol). It was stirred at r.t. for 2 hrs. The reaction mixture was purified by prep-HPLC to afford **INT-22-1** (1.0 g, 60.6%) as a white solid. LCMS [M+H]⁺: 410.2.



Prop-2-yn-1-yl 2-((2-((6-(methylsulfonyl)-5-nitronicotinamido)ethyl)amino)-2-oxoethoxy)acetate (22)

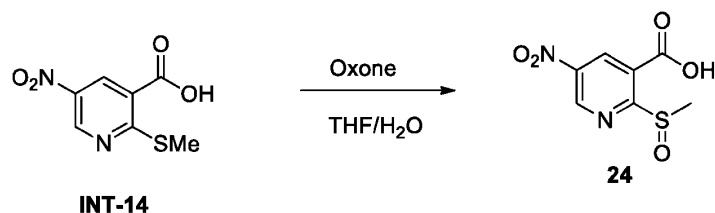
[0292] To solution of **INT-22-1** (900 mg, 2.198 mmol) in DMAc (10 mL) was added m-CPBA (1.327 g, 7.694 mmol), and stirred at r.t. for 2 hrs. This reaction mixture was purified by prep-HPLC to afford **22** (841 mg, 86.7%) as a white solid. LCMS [M+H]⁺: 422.2.

[0293] ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.27 (s, 1H), 9.18 (t, 1H), 8.89 (s, 1H), 8.43 (t, 1H), 8.23 (t, 1H), 3.98 (s, 2H), 3.95 (s, 2H), 3.91 (dd, 2H), 3.51 (s, 3H), 3.45-3.38 (m, 2H), 3.37-3.33 (m, 2H), 3.10 (d, 1H).



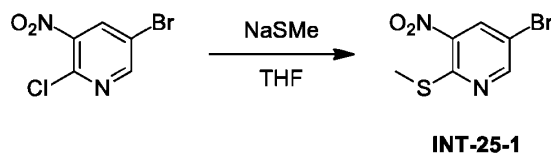
6-(Methylsulfinyl)-5-nitronicotinic acid 23

[0294] To a solution of **INT-13** (150 mg, 0.7 mmol) in THF (3 mL) and H₂O (3 mL) was added Oxone (646 mg, 1.05 mmol) and stirred at RT for 2 hrs. The reaction was completed as detected by LC-MS. Solvent was removed reduced pressure and the residue was purified by RP prep-HPLC to give **23** (101 mg, 61%) as a yellow solid. LCMS (ESI): m/z 231.19 [M + H]⁺.



2-(Methylsulfinyl)-5-nitronicotinic acid 24

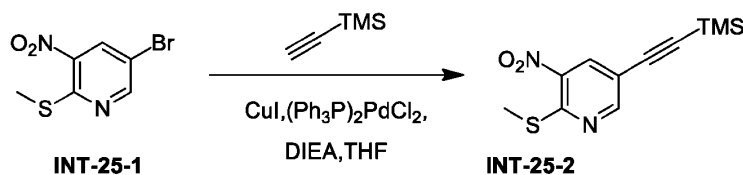
[0295] To a solution of **INT-14** (150 mg, 0.7 mmol) in THF (3 mL) and H₂O (3 mL) was added Oxone (646 mg, 1.05 mmol) and stirred at RT for 2 hrs. The reaction was completed as detected by LC-MS. Solvent was removed reduced pressure and the residue was purified by RP prep-HPLC to give **24** (95 mg, 59%) as a yellow solid. LCMS (ESI): m/z 231.19 [M + H]⁺.



5-Bromo-2-(methylthio)-3-nitropyridine (INT-25-1)

[0296] To a solution of 5-bromo-2-chloro-3-nitropyridine (3.00 g, 12.6 mmol, 1.0 eq.) in THF (30 mL) was added CH₃SNa (4.7 mL in 20% water solution, 15.2 mmol, 1.2eq.). The suspended reaction mixture was stirred at room temperature for 1h. The THF was removed under the reduced pressure, filtered, washed with H₂O (20 mL), dried to get INT-25-1 (2.78 g, yield: 88.7%, purity: 100%) as a yellow solid.

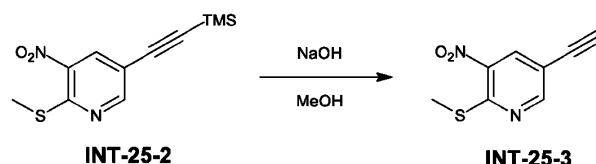
[0297] ¹H NMR (600 MHz, DMSO-d₆) δ 9.01 (d, J = 2.1 Hz, 1H), 8.82 (d, J = 2.1 Hz, 1H), 2.54 (s, 3H).



2-(Methylthio)-3-nitro-5-((trimethylsilyl)ethynyl)pyridine (INT-25-2)

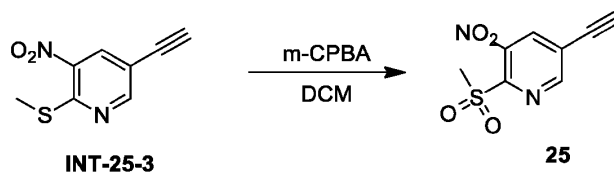
[0298] To a solution of INT-25-1 (780 mg, 3.15 mmol, 1.0eq.) in DIEA (15 mL) was added ethynyltrimethylsilane (464 mg, 4.72 mmol, 1.5 eq.), (Ph₃P)₂PdCl₂ (110 mg, 0.16 mmol, 0.05eq.), CuI (30 mg, 0.16 mmol, 0.05eq.). The suspended reaction mixture was stirred at room

temperature for 24h. Then evaporated and the residue was purified by the prep-HPLC to afford INT-21-2 (47 mg, yield:5.6%, purity: 100%) as a brown solid. LCMS[M+H]⁺:267.1; ¹H NMR (600 MHz, DMSO-d₆) δ 8.89 (d, J = 1.9 Hz, 1H), 8.57 (d, J = 1.9 Hz, 1H), 2.56 (s, 3H), 0.27 (d, J = 3.5 Hz, 9H).



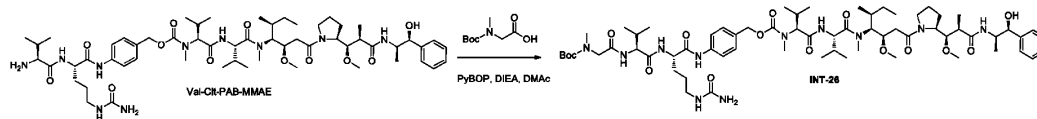
5-Ethynyl-2-(methylthio)-3-nitropyridine (INT-25-3)

[0299] To a solution of INT-25-2 (47 mg, 0.177 mmol, 1.0 eq.) in MeOH (1 mL) was added KOH (20 mg, 0.353 mmol, 2.0 eq.) at r.t., and the reaction mixture was stirred at that temperature for 2 hrs. Then the reaction mixture was purified by the prep-HPLC to afford INT-21-3 (18 mg, yield:52.3%, purity:100%) as a yellow solid. LCMS[M+H]⁺:195.1; ¹H NMR (600 MHz, DMSO-d₆) δ 8.94 (d, J = 1.9 Hz, 1H), 8.63 (d, J = 1.9 Hz, 1H), 4.64 (s, 1H), 2.56 (s, 3H).



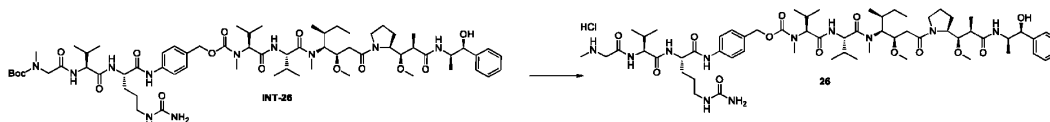
5-Ethynyl-2-(methylsulfonyl)-3-nitropyridine (25)

[0300] To a solution of INT-25-3 (18 mg, 0.093 mmol, 1.0 eq.) in DMAc (2 mL) was added m-CPBA (48 mg, 0.278 mmol, 3.0 eq.) at r.t., and the reaction mixture was stirred at that temperature for 2 hrs. Then the reaction mixture was purified by the prep-HPLC to afford **25** (2.3 mg, yield:11%, purity:96%) as a white solid. LCMS[M+H]⁺:227.0; ¹H NMR (600 MHz, DMSO-d₆) δ 9.08 (d, J = 1.7 Hz, 1H), 8.79 (d, J = 1.7 Hz, 1H), 4.98 (s, 1H), 3.46 (s, 3H).



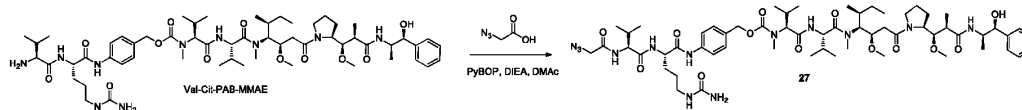
4-((9*S*,12*S*)-9-isopropyl-2,2,5-trimethyl-4,7,10-trioxo-12-(3-ureidopropyl)-3-oxa-5,8,11-triazatridecanamido)benzyl ((*S*)-1-(((*S*)-1-(((3*R*,4*S*,5*S*)-1-((*S*)-2-((1*R*,2*R*)-3-(((1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate INT-26

[0301] To a solution of 2-((tert-butoxycarbonyl)(methyl)amino)acetic acid (35 mg, 0.187 mmol, 2.1 eq.) in DMAc (5 mL) was added PyBOP (97 mg, 0.187 mmol, 2.1 eq.), followed by DIEA (65 μ L, 0.374 mmol, 4.2 eq.) and stirred at room temperature for 20 min. Val-Cit-PAB-MMAE (CAS No. 644981-35-1) (100 mg, 0.0890 mmol, 1.0 eq.) was added. The reaction mixture was stirred at room temperature for 1 hr. The reaction mixture was purified by RP prep-HPLC to give **INT-26** (93.3 mg, 81.3%) as a pale white solid. LCMS (ESI): m/z 1296.14 $[M + H]^+$.



4-(((*S*)-2-((*S*)-3-methyl-2-(2-(methylamino)acetamido)butanamido)-5-ureidopentanamido)benzyl ((*S*)-1-(((*S*)-1-(((3*R*,4*S*,5*S*)-1-((*S*)-2-((1*R*,2*R*)-3-(((1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate 26

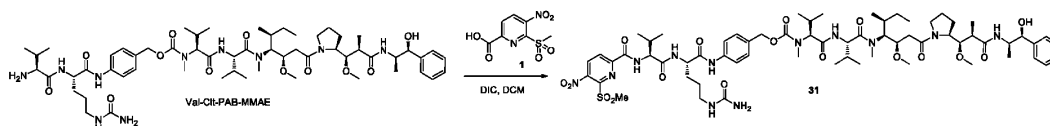
[0302] To a solution of **INT-26** (50 mg, 0.062 mmol, 1.0 eq.) in DCM (1 mL) was added 4N HCl/Dioxane (1 mL) and the mixture was stirred at RT for 1 hr. Solvent was removed to give a white solid **26** as HCl salt (76 mg, 100%). LCMS (ESI): m/z 1195.94 $[M + H]^+$.



4-(((*S*)-2-((*S*)-2-(2-azidoacetamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl ((*S*)-1-(((*S*)-1-(((3*R*,4*S*,5*S*)-1-((*S*)-2-((1*R*,2*R*)-3-(((1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-

yl)(methylamino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate 27

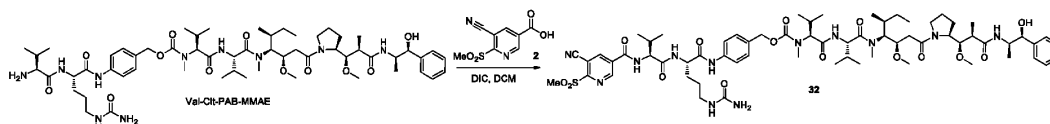
[0303] To a solution of Val-Cit-PAB-MMAE (100.05 mg, 0.0891 mmol, 1.0 eq.) in DMAc (2 mL) was added 2-azidoacetic acid (13.33 uL, 0.178 mmol, 2.0 eq.) and DIEA (46.53 uL, 0.267 mmol, 3.0 eq.), and PyBOP (92.69 mg, 0.178 mmol, 2.0 eq.) was added. The reaction mixture was stirred at room temperature for 1.5 hrs. Then the reaction mixture was purified by the prep-HPLC to afford **27** (88 mg, 81.9%) as a white powder. LCMS (ESI): m/z 1207.1 $[M + H]^+$. 1H NMR (DMSO- d_6 , 600 MHz): δ 10.01 (d, $J = 12.9$ Hz, 1H), 8.32 (d, $J = 7.5$ Hz, 1H), 8.14 (d, $J = 8.7$ Hz, 1H), 8.09 (d, $J = 7.6$ Hz, 1H), 7.79-7.72 (m, 1H), 7.59 (s, 2H), 7.37-7.23 (m, 6H), 7.17 (dd, $J = 15.0, 7.7$ Hz, 1H), 6.02 (s, 1H), 5.08-5.01 (m, 3H), 4.74 (s, 1H), 4.63 (s, 1H), 4.48 (t, $J = 7.8$ Hz, 1H), 4.43 (d, $J = 6.6$ Hz, 1H), 4.42-4.35 (m, 1H), 4.29-4.22 (m, 2H), 4.04-3.91 (m, 2H), 3.90 (d, $J = 1.1$ Hz, 2H), 3.81-3.74 (m, 1H), 3.61-3.53 (m, 1H), 3.47 (s, 1H), 3.31 (d, $J = 10.1$ Hz, 1H), 3.24 (d, $J = 9.6$ Hz, 4H), 3.20 (s, 2H), 3.17 (s, 1H), 3.12 (s, 2H), 3.08-2.99 (m, 2H), 2.99-2.92 (m, 2H), 2.88-2.81 (m, 3H), 2.41 (d, $J = 17.7$ Hz, 1H), 2.29-2.21 (m, 1H), 2.16-2.08 (m, 2H), 2.01-1.93 (m, 2H), 1.85-1.66 (m, 4H), 1.59-1.46 (m, 4H), 1.38-1.31 (m, 2H), 1.06-0.96 (m, 6H), 0.90-0.73 (m, 24H).



4-((S)-2-((S)-3-methyl-2-(6-(methylsulfonyl)-5-nitropicolinamido)butanamido)-5-ureidopentanamido)benzyl ((S)-1-(((S)-1-(((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methylamino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate 31

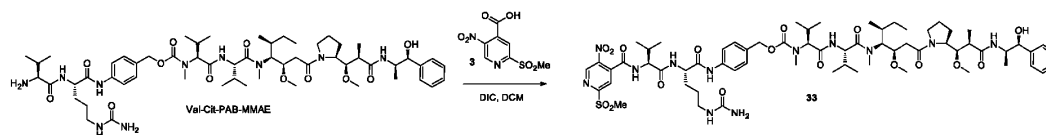
[0304] To a solution of **1** (8.77 mg, 0.036 mmol, 2.0 eq.) in dichloromethane (DCM, 1 mL) was added N,N-diisopropylcarbodiimide (DIC, 4.49 mg, 0.036 mmol, 2.0 eq.), followed Val-Cit-PAB-MMAE (20 mg, 0.018 mmol, 1.0 eq.) under N_2 . The reaction mixture was stirred at room temperature for 3 hrs. Solvent was removed under reduced pressure, and the crude product was purified by RP prep-HPLC to give **31** (14.49 mg, 60.22%) as a pale white solid. LCMS (ESI): m/z 1351.99 $[M + H]^+$. 1H NMR (DMSO- d_6 , 600 MHz): δ 10.08 (s, 1H), 8.76 (d, 1H),

8.66 (d, 1H), 8.48 (s, 1H), 8.46 (d, 1H), 8.27 (s, 1H), 8.03 (s, 1H), 7.87 (d, 1H), 7.60 (d, 1H), 7.56 (s, 2H), 7.32 (s, 1H), 7.31-7.22 (m, 4H), 7.16 (d, 1H), 5.97 (s, 1H), 5.00 (d, 4H), 4.72 (s, 1H), 4.49-4.38 (m, 4H), 4.26 (d, 1H), 4.22 (s, 1H), 3.98 (s, 4H), 3.60 (s, 3H), 3.48-3.42 (m, 2H), 3.30 (d, 1H), 3.22 (d, 3H), 3.18 (s, 2H), 3.16 (s, 1H), 3.10 (s, 2H), 2.98 (s, 2H), 2.95 (s, 1H), 2.87 (s, 2H), 2.83 (s, 1H), 2.63-2.60 (m, 1H), 2.41 (s, 1H), 2.38 (s, 1H), 2.36-2.33 (m, 1H), 2.29-2.21 (m, 2H), 2.18 (dd, 1H), 2.14-2.07 (m, 2H), 1.95 (d, 2H), 1.70 (s, 5H), 1.65-1.40 (m, 6H), 1.37 (s, 2H), 1.03 (d, 2H), 0.99 (d, 2H), 0.96 (d, 1H), 0.93 (d, 2H), 0.87 (d, 3H), 0.83 (d, 2H), 0.80 (d, 3H), 0.78-0.71 (m, 6H).



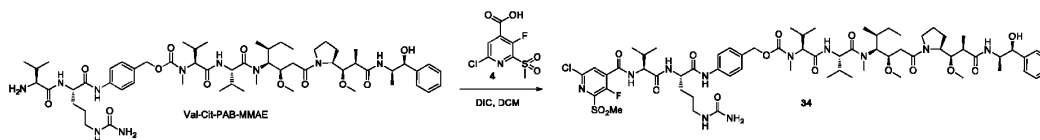
4-(((S)-2-((S)-2-(5-cyano-6-(methylsulfonyl)nicotinamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl ((S)-1-(((S)-1-(((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate 32

[0305] To a solution of **2** (7 mg, 0.036 mmol, 2.0eq.) in DCM (1 mL) was added Val-Cit-PAB-MMAE (20 mg, 0.018 mmol, 1.0eq.), followed by DIC (5 mg, 0.036 mmol, 2.0 eq.) under N₂. The reaction mixture was stirred at room temperature for 2 hrs. Then concentrated, and the crude product was purified by RP prep-HPLC to give **32** (13 mg, 55.6%) as a pale white solid. LCMS (ESI): m/z 1331.99 [M + H]⁺. ¹H NMR (DMSO-d₆, 600 MHz): δ 9.76 (s, 1H), 9.29 (s, 1H), 8.93 (d, 1H), 8.36 (m, 3H), 8.28 (m, 1H), 7.59 (s, 2H), 7.42 – 7.22 (m, 7H), 6.00 (s, 1H), 5.45 (m, 3H), 5.03 (s, 1H), 4.64 – 4.08 (m, 8H), 4.03 – 3.29 (m, 13H), 3.28 – 3.11 (m, 6H), 3.02 (s, 2H), 2.95 (m, 4H), 2.52 – 2.44 (m, 2H), 2.35 – 1.70 (d, 7H), 1.65 (m, 4H), 1.55 (m, 2H), 1.38 (m, 3H), 1.33 (m, 3H), 1.33 – 0.79 (m, 24H).



4-((S)-2-((S)-3-methyl-2-(2-(methylsulfonyl)-5-nitroisonicotinamido)butanamido)-5-ureidopentanamido)benzyl ((S)-1-(((S)-1-(((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate 33

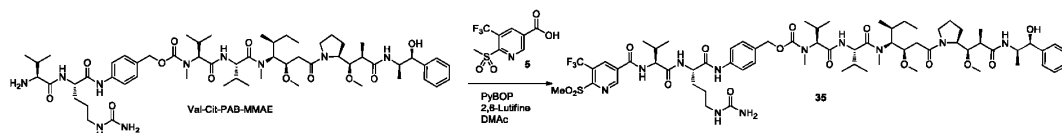
[0306] To a solution of **3** (9 mg, 0.036 mmol, 2.0 eq.) in DCM (1 mL) was added Val-Cit-PAB-MMAE (20 mg, 0.018 mmol, 1.0eq.), followed by DIC (5 mg, 0.036 mmol, 2.0 eq.) under N₂. The reaction mixture was stirred at room temperature for 2 hrs. Solvent was removed under reduced pressure, and the crude product was purified by RP prep-HPLC to give **33** (14 mg, 57.5%) as a pale white solid. LCMS (ESI): m/z 1352.27 [M + H]⁺. ¹H NMR (DMSO-d₆, 600 MHz): δ 10.10 (s, 1H), 9.41 (s, 1H), 9.17 (d, 1H), 8.38 (d, 1H), 8.07 (d, 2H), 7.87 (d, 2H), 7.68 – 7.03 (m, 7H), 6.00 (s, 1H), 5.45 (m, 3H), 5.03 (s, 1H), 4.64 – 4.08 (m, 8H), 4.03 – 3.29 (m, 13H), 3.28 – 3.11 (m, 6H), 3.02 (s, 2H), 2.95 (m, 4H), 2.52 – 2.44 (m, 2H), 2.35 – 1.70 (d, 7H), 1.65 (m, 4H), 1.55 (m, 2H), 1.38 (m, 3H), 1.33 (m, 3H), 1.33 – 0.79 (m, 24H).



4-((S)-2-((S)-2-(6-chloro-3-fluoro-2-(methylsulfonyl)isonicotinamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl ((S)-1-(((S)-1-(((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate 34

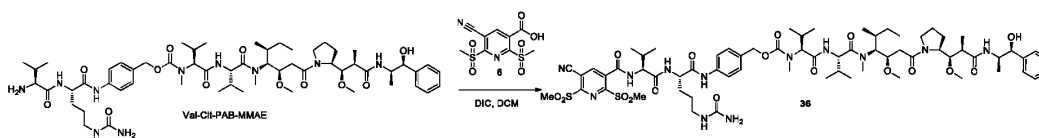
[0307] To a solution of **4** (9 mg, 0.036 mmol, 2.0 eq.) in DCM (1 mL) was added Val-Cit-PAB-MMAE (20 mg, 0.018 mmol, 1.0 eq.), followed by DIC (5 mg, 0.036 mmol, 2.0 eq.) under N₂. The reaction mixture was stirred at room temperature for 2 hrs. Solvent was removed under reduced pressure, and the crude product was purified by RP prep-HPLC to give **34** (15 mg, 61.3%) as a pale white solid. LCMS (ESI): m/z 1360.59 [M + H]⁺. ¹H NMR (DMSO-d₆, 600 MHz): δ 10.08 (s, 1H), 8.96 (s, 1H), 8.41 (m, 2H), 8.06 (m, 1H), 7.69 – 7.51 (m, 2H), 7.43 – 7.10 (m, 7H), 6.00 (s, 1H), 5.45 (m, 3H), 5.03 (s, 1H), 4.64 – 4.08 (m, 8H), 4.03 – 3.29 (m, 13H),

3.28 – 3.11 (m, 6H), 3.02 (s, 2H), 2.95 (m, 4H), 2.52 – 2.44 (m, 2H), 2.35 -1.70 (d, 7H), 1.65 (m, 4H), 1.55(m, 2H), 1.38 (m, 3H), 1.33 (m, 3H), 1.33 - 0.79 (m, 24H).



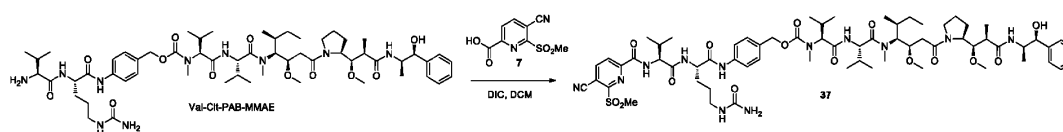
4-((S)-2-((S)-3-methyl-2-(6-(methylsulfonyl)-5-(trifluoromethyl)nicotinamido)butanamido)-5-ureidopentanamido)benzyl ((S)-1-(((S)-1-(((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate 35

[0308] To a solution of **5** (9 mg, 0.032 mmol, 2.0 eq.) in DMAc (1 mL) was added Val-Cit-PAB-MMAE (20 mg, 0.016 mmol, 1.0 eq.), followed by (Benzotriazole-1-yloxy)-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP, 13 mg, 0.024 mmol, 2.0 eq.) and 2,6-Lutidine (3.5 mg, 0.032 mmol) under N₂. The reaction mixture was stirred at room temperature for 2 hrs. DMAc was removed under reduced pressure, and the crude product was purified by RP prep-HPLC to give **35** (10.11 mg, 46.5%) as a pale white solid. LCMS (ESI): m/z 1375.93 [M + H]⁺. ¹H NMR (DMSO-d₆, 600 MHz): δ 9.41 (s, 1H), 9.17 (d, 1H), 8.38 (d, 2H), 8.07 (d, 2H), 7.87 (d, 2H), 7.68 – 7.03 (m, 7H), 6.00 (s, 1H), 5.45 (m, 3H), 5.03 (s, 1H), 4.64 – 4.08 (m, 8H), 4.03 – 3.29 (m, 13H), 3.28 – 3.11 (m, 6H), 3.02 (s, 2H), 2.95 (m, 4H), 2.52 – 2.44 (m, 2H), 2.35 -1.70 (d, 7H), 1.65 (m, 4H), 1.55(m, 2H), 1.38 (m, 3H), 1.33 (m, 3H), 1.33 - 0.79 (m, 24H).



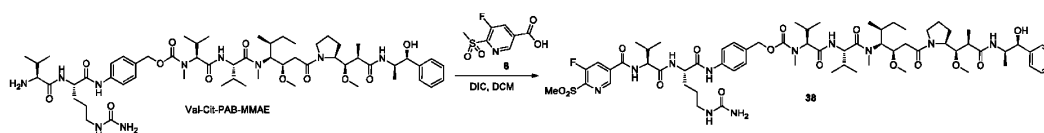
4-((S)-2-((S)-2-(5-cyano-2,6-bis(methylsulfonyl)nicotinamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl ((S)-1-(((S)-1-(((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate 36

[0309] To a solution of **6** (16 mg, 0.010 mmol, 1.0 eq.) in DCM (3.0 mL) was added DIC (8.6 μ L, 0.054 mmol, 2 eq.), Val-Cit-PAB-MMAE (30 mg, 0.027 mmol, 1 eq.), at RT, and the reaction mixture was stirred at that temperature for 1 hr. DCM was removed under reduced pressure, and the crude product was purified by RP prep-HPLC to give **36** (13.0 mg, yield: 47.5%) as a white solid. LCMS (ESI): m/z 1410.70 $[M + H]^+$.



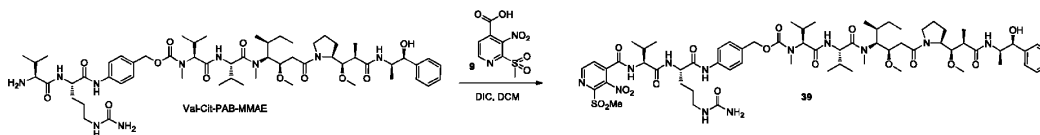
4-(((S)-2-(((S)-2-(5-cyano-6-(methylsulfonyl)picolinamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl ((S)-1-(((S)-1-(((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate 37

[0310] To a solution of **7** (20 mg, 0.090 mmol, 2.0 eq.) in DCM (2 mL) was added DIC (11 μ L, 0.090 mmol, 2 eq.), Val-Cit-PAB-MMAE (50mg, 0.045 mmol, 1 eq.) at RT, and the reaction mixture was stirred at that temperature for 1 hr. DCM was removed under reduced pressure, and the residue was purified by RP prep-HPLC to give **37** (33 mg, yield: 55.1%) as a white solid. LCMS (ESI): m/z 1332.11 $[M + H]^+$. 1H NMR (MeOH- d_4 , 600 MHz): δ 8.73 – 8.65 (m, 2H), 8.58 (dd, J = 7.3, 4.2 Hz, 1H), 8.46 (dd, J = 8.1, 2.1 Hz, 1H), 7.92 (d, J = 9.3 Hz, 1H), 7.86 (d, J = 8.7 Hz, 1H), 7.73 (d, J = 8.9 Hz, 1H), 7.57 (d, J = 6.2 Hz, 2H), 7.40 – 7.26 (m, 5H), 7.19 (dd, J = 15.0, 7.7 Hz, 2H), 5.36 – 5.32 (m, 1H), 5.18 (dd, J = 21.7, 10.9 Hz, 3H), 5.10 – 5.04 (m, 1H), 4.57 – 4.48 (m, 3H), 4.26 – 4.14 (m, 3H), 4.09 – 4.03 (m, 1H), 3.86 (dd, J = 9.1, 2.1 Hz, 1H), 3.75 – 3.63 (m, 3H), 3.53 (d, J = 2.4 Hz, 3H), 3.45 – 3.38 (m, 2H), 3.22 – 3.06 (m, 6H), 2.93 (dd, J = 12.9, 6.3 Hz, 3H), 2.53 – 2.43 (m, 3H), 2.29 – 2.16 (m, 4H), 1.64 – 1.51 (m, 5H), 1.38 – 1.25 (m, 10H), 1.21 – 0.66 (m, 30H).



4-((S)-2-((S)-2-(5-fluoro-6-(methylsulfonyl)nicotinamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl ((S)-1-(((S)-1-(((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate 38

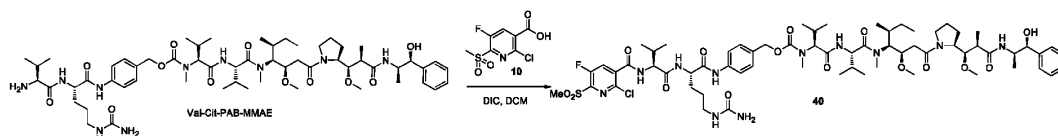
[0311] To a solution of **8** (7.8 mg, 0.036 mmol, 2.0 eq.) in DCM (2 mL) was added DIC (5.3 μ L, 0.036 mmol, 2.0 eq.), Val-Cit-PAB-MMAE (20mg, 0.018 mmol, 1eq.) at RT, and the reaction mixture was stirred at that temperature for 1 hr. DCM was removed under reduced pressure, and the residue was purified by RP-HPLC to give **38** (13 mg, yield: 54.6%) as a white solid. LCMS (ESI): m/z 1325.20 $[M + H]^+$. 1H NMR (DMSO- d_6 , 600 MHz): δ 10.04 (d, J = 10.1 Hz, 1H), 8.96 (s, 1H), 8.85 (d, J = 8.3 Hz, 1H), 8.48 (d, J = 10.5 Hz, 1H), 8.36 (d, J = 7.2 Hz, 1H), 8.30 (t, J = 16.0 Hz, 1H), 8.05 (d, J = 6.3 Hz, 1H), 7.89 (d, J = 8.3 Hz, 1H), 7.63 (d, J = 8.4 Hz, 1H), 7.58 (s, 2H), 7.30 (tt, J = 19.6, 10.0 Hz, 6H), 7.20 – 7.14 (m, 1H), 5.99 (s, 1H), 5.40 (s, 2H), 5.13 – 4.95 (m, 2H), 4.54 – 4.38 (m, 4H), 4.31 – 3.93 (m, 5H), 3.78 (d, J = 7.2 Hz, 1H), 3.32 (d, J = 10.7 Hz, 1H), 3.22 (dd, J = 39.3, 16.1 Hz, 8H), 3.12 (s, 2H), 3.07 – 2.93 (m, 4H), 2.91 – 2.81 (m, 3H), 2.41 (d, J = 15.3 Hz, 1H), 2.27 (dd, J = 15.5, 10.9 Hz, 1H), 2.16 – 1.93 (m, 4H), 1.86 – 1.28 (m, 10H), 1.08 – 0.71 (m, 33H).



4-((S)-2-((S)-3-methyl-2-(2-(methylsulfonyl)-3-nitroisonicotinamido)butanamido)-5-ureidopentanamido)benzyl ((S)-1-(((S)-1-(((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate 39

[0312] To a solution of **9** (8.8 mg, 0.036 mmol, 2.0 eq.) in DCM (2 mL) was added DIC (5.3 μ L, 0.036 mmol, 2.0 eq.), Val-Cit-PAB-MMAE (20mg, 0.018 mmol, 1eq.) at RT, and the reaction mixture was stirred at that temperature for 1 hr. Solvent was removed under reduced pressure and the residue was purified by RP prep-HPLC to give **39** (13.3 mg, yield: 54.1%) as a yellow solid. LCMS (ESI): m/z 1352.600 $[M + H]^+$. 1H NMR (DMSO- d_6 , 600 MHz): δ 10.08 (d,

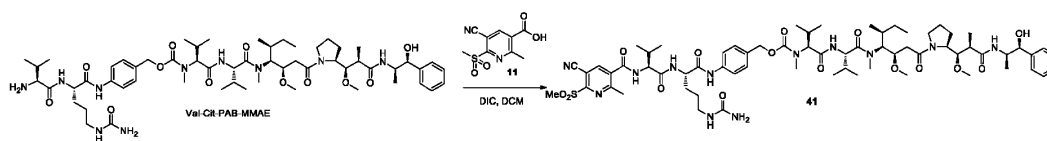
J = 16.3 Hz, 1H), 9.21 (d, J = 8.9 Hz, 1H), 9.08 – 9.04 (m, 1H), 8.38 (d, J = 7.3 Hz, 1H), 8.06 (t, J = 9.2 Hz, 1H), 8.02 (dd, J = 4.8, 1.7 Hz, 1H), 7.89 (d, J = 8.3 Hz, 1H), 7.59 (s, 2H), 7.37 – 7.24 (m, 6H), 7.17 (dt, J = 14.9, 7.1 Hz, 1H), 6.00 (s, 1H), 5.13 – 4.96 (m, 2H), 4.68 (d, J = 77.0 Hz, 1H), 4.54 – 4.38 (m, 4H), 4.27 (dd, J = 23.2, 10.8 Hz, 1H), 3.97 (ddd, J = 15.8, 14.8, 9.3 Hz, 3H), 3.32 (d, J = 10.8 Hz, 1H), 3.22 (dd, J = 39.6, 16.1 Hz, 8H), 3.12 (s, 2H), 3.05 (dt, J = 13.9, 7.0 Hz, 2H), 2.98 (s, 2H), 2.87 (dd, J = 29.5, 13.0 Hz, 3H), 2.44 – 2.39 (m, 1H), 2.28 (dt, J = 15.5, 8.2 Hz, 1H), 2.16 – 2.04 (m, 3H), 1.99 (ddt, J = 32.0, 20.7, 7.3 Hz, 1H), 1.86 – 1.68 (m, 4H), 1.66 – 1.22 (m, 9H), 1.07 – 0.73 (m, 33H).



4-((S)-2-((S)-2-(2-chloro-5-fluoro-6-(methylsulfonyl)nicotinamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl ((S)-1-(((S)-1-(((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate 40

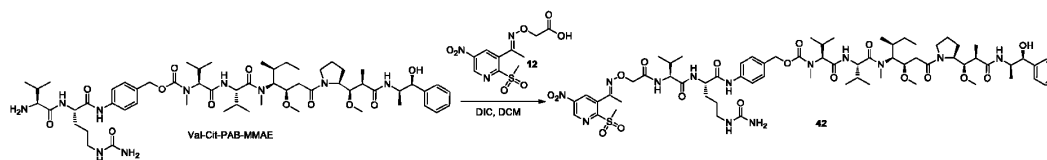
[0313] To a solution of **10** (9.1 mg, 0.036 mmol, 2.0 eq.) in DCM (2 mL) was added DIC (5.3 μ L, 0.036 mmol, 2.0eq.), Val-Cit-PAB-MMAE (20mg, 0.018 mmol, 1eq.) at RT, and the reaction mixture was stirred at that temperature for 1 hr. Solvent was removed under reduced pressure and the residue was purified by RP prep-HPLC to give **40** (14 mg, yield: 57.6%) as a white solid.

LCMS (ESI): m/z 1359.21 [M + H]⁺. ¹H NMR (DMSO-d₆, 600 MHz): δ 10.08 (d, J = 15.9 Hz, 1H), 8.83 (d, J = 10.0 Hz, 1H), 8.34 (d, J = 7.3 Hz, 1H), 8.30 (dd, J = 8.7, 1.5 Hz, 1H), 8.05 (d, J = 7.6 Hz, 1H), 7.89 (d, J = 8.8 Hz, 1H), 7.59 (s, 2H), 7.36 – 7.25 (m, 6H), 7.20 – 7.14 (m, 1H), 6.00 (s, 1H), 5.43 (s, 2H), 5.13 – 4.96 (m, 2H), 4.52 – 4.42 (m, 4H), 4.27 (dd, J = 23.5, 10.7 Hz, 1H), 4.05 – 3.93 (m, 3H), 3.32 (d, J = 10.6 Hz, 1H), 3.27 – 2.81 (m, 19H), 2.27 (dd, J = 14.9, 9.8 Hz, 1H), 2.15 – 2.06 (m, 3H), 2.02 – 1.93 (m, 1H), 1.84 – 1.69 (m, 4H), 1.65 – 1.37 (m, 6H), 1.06 – 0.74 (m, 34H).



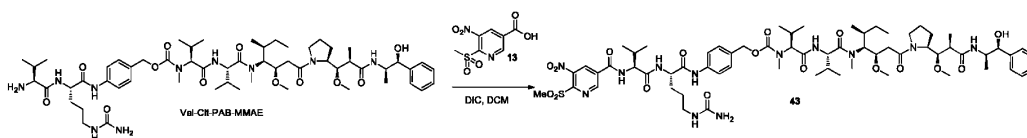
4-((*S*)-2-((*S*)-2-(5-cyano-2-methyl-6-(methylsulfonyl)nicotinamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl ((*S*)-1-(((*S*)-1-(((3*R*,4*S*,5*S*)-1-((*S*)-2-((1*R*,2*R*)-3-(((1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate **41**

[0314] To a solution of **11** (12.8 mg, 0.053 mmol, 2.0 eq.) in DCM (2 mL) was added DIC (8.3 μ L, 0.053 mmol, 2eq.), Val-Cit-PAB-MMAE (30 mg, 0.027 mmol, 1eq.) at RT, and the reaction mixture was stirred at that temperature for 1 hr. Solvent was removed under reduced pressure and the residue was purified by RP-HPLC to give **41** (19 mg, yield: 52.3%) as a white solid. LCMS (ESI): m/z 1347.33 $[M + H]^+$. 1H NMR ($CDCl_3$, 600 MHz): δ 8.08 – 7.92 (m, 12H), 7.26 (d, J = 7.2 Hz, 2H), 4.62 (d, J = 4.1 Hz, 8H), 4.44 (d, J = 5.0 Hz, 1H), 4.37 – 4.24 (m, 4H), 3.85 – 3.77 (m, 1H), 3.55 – 1.92 (m, 33H), 1.63 – 0.50 (m, 33H).



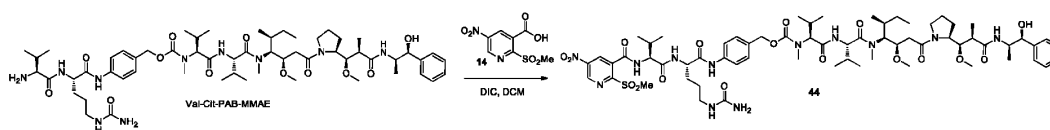
4-((*8S*,11*S*,*E*)-8-isopropyl-2-(2-(methylsulfonyl)-5-nitropyridin-3-yl)-6,9-dioxo-11-(3-ureidopropyl)-4-oxa-3,7,10-triazadodec-2-en-12-amido)benzyl ((*S*)-1-(((*S*)-1-(((3*R*,4*S*,5*S*)-1-((*S*)-2-((1*R*,2*R*)-3-(((1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate **42**

[0315] To a solution of **12** (7.5 mg, 0.036 mmol, 2.0eq.) in DCM (1 mL) was added DIC (5 mg, 0.036 mmol, 2.0 eq.) under N_2 . After stirred at RT for 30 min, Val-Cit-PAB-MMAE (20 mg, 0.018 mmol, 1.0 eq.) was added. The reaction mixture was stirred at room temperature for 2 hrs. DCM was removed under reduced pressure and the residue was purified by RP prep-HPLC to give **42** (16.5 mg, 64.4%) as an off-white solid. LCMS (ESI): m/z 1423.79 $[M + H]^+$.



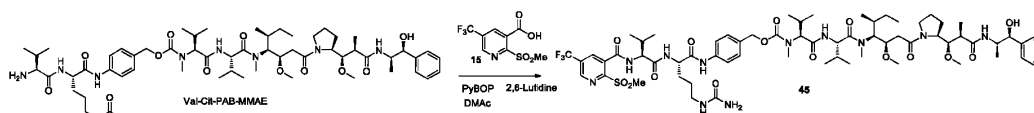
4-((S)-2-((S)-3-methyl-2-(6-(methylsulfonyl)-5-(trifluoromethyl)nicotinamido)butanamido)-5-ureidopentanamido)benzyl ((S)-1-(((S)-1-(((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate 43

[0316] To a solution of **13** (8.9 mg, 0.036 mmol, 2.0 eq.) in DCM (1 mL) was added DIC (5 mg, 0.036 mmol, 2.0eq.) under N₂. After stirred at RT for 30 min, Val-Cit-PAB-MMAE (20 mg, 0.018 mmol, 1.0 eq.) was added. The reaction mixture was stirred at room temperature for 2 hrs. DCM was removed under reduced pressure and the crude product was purified by RP prep-HPLC to give **43** (14.7 mg, 60%) as a yellow solid. LCMS (ESI): m/z 1354.15 [M + H]⁺.



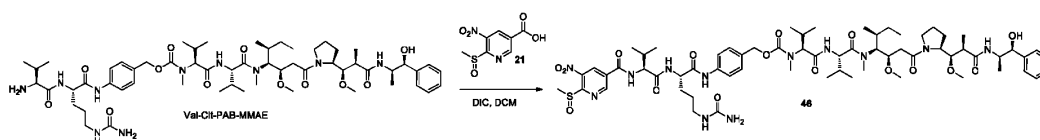
4-((S)-2-((S)-3-methyl-2-(2-(methylsulfonyl)-5-nitronicotinamido)butanamido)-5-ureidopentanamido)benzyl ((S)-1-(((S)-1-(((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate 44

[0317] To a solution of **14** (8.9 mg, 0.036 mmol, 2.0 eq.) in DCM (1 mL) was added DIC (5 mg, 0.036 mmol, 2.0eq.) under N₂. After stirred at RT for 30 min, Val-Cit-PAB-MMAE (20 mg, 0.018 mmol, 1.0 eq.) was added. The reaction mixture was stirred at room temperature for 2 hrs. DCM was removed under reduced pressure and the crude product was purified by RP prep-HPLC to give **44** (18.5 mg, 75.5%) as a yellow solid. LCMS (ESI): m/z 1354.15 [M + H]⁺.



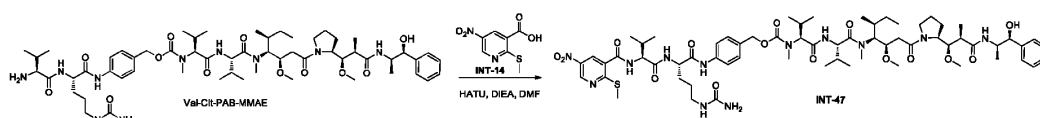
4-((S)-2-((S)-3-methyl-2-(2-(methylsulfonyl)-5-(trifluoromethyl)nicotinamido)butanamido)-5-ureidopentanamido)benzyl ((S)-1-(((S)-1-(((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate 45

[0318] To a solution of **15** (9 mg, 0.0324 mmol, 2.0eq.) in DMAc (1 mL) was added Val-Cit-PAB-MMAE (20 mg, 0.016 mmol, 1.0 eq.), followed by (PyBOP, 13 mg, 0.024 mmol, 2.0eq.) and 2,6-Lutidine (3.5 mg, 0.032 mmol) under N₂. The reaction mixture was stirred at room temperature for 2 hrs. DMAc was removed under reduced pressure, and the crude product was purified by RP prep-HPLC to give **45** (12.2 mg, 55.7%) as a pale white solid. LCMS (ESI): m/z 1375.35 [M + H]⁺.



4-((2S)-2-((2S)-3-methyl-2-(6-(methylsulfinyl)-5-nitronicotinamido)butanamido)-5-ureidopentanamido)benzyl ((S)-1-(((S)-1-(((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate 46

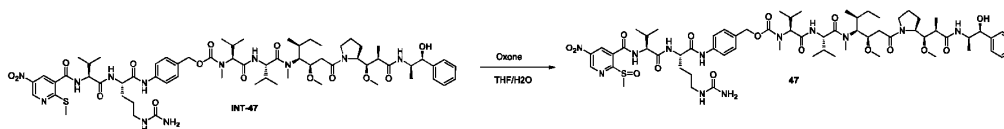
[0319] To a solution of **21** (22 mg, 0.09 mmol, 1.1 eq.) and Val-Cit-PAB-MMAE (100mg, 0.089 mmol, 1.0 eq.) in DMF (2.2 mL) was added HATU (50 mg, 0.133 mmol, 1.5 eq.) and DIEA (49 ul, 0.267 mmol, 3.0 eq.), and the reaction mixture was stirred at RT for 3 hr. The solution was purified by RP prep-HPLC to give **46** (35 mg, 30%) as a yellow solid. LCMS (ESI): m/z 1336.85 [M + H]⁺.



4-((S)-2-((S)-3-methyl-2-(2-(methylthio)-5-nitronicotinamido)butanamido)-5-ureidopentanamido)benzyl ((S)-1-(((S)-1-(((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-

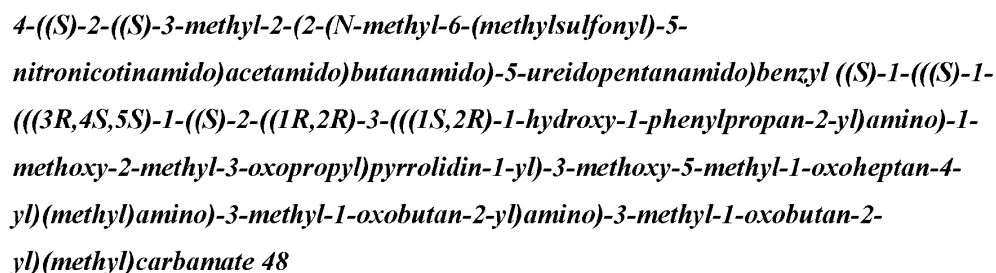
hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate INT-47

[0320] To a solution of **INT-12-1** (9.5 mg, 0.045 mmol, 1.0 eq.) in DMF (5 mL) was added HATU (25 mg, 0.0133 mmol, 1.5 eq.), DIEA (25 μ L, 0.0267 mmol, 3.0 eq.) and Val-Cit-PAB-MMAE (50mg, 0.045 mmol, 1.0 eq.), and the reaction mixture was stirred at RT for 4 hr. The solution was purified by RP prep-HPLC to afford **INT-47** (41 mg, 69%) as a white solid. LCMS (ESI): m/z 1319.75 $[M + H]^+$.

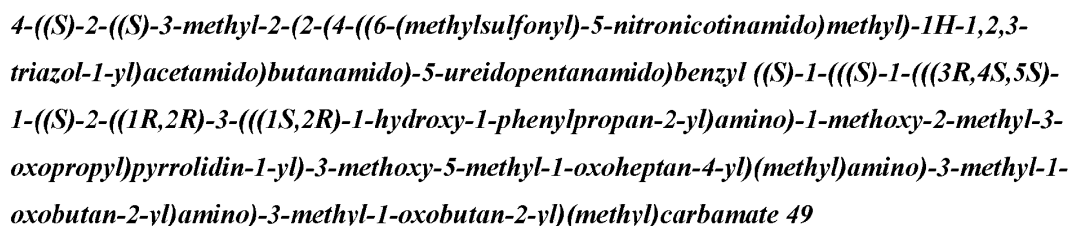


4-((2S)-2-((2S)-3-methyl-2-(2-(methylsulfinyl)-5-nitronicotinamido)butanamido)-5-ureidopentanamido)benzyl ((S)-1-(((S)-1-(((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate 47

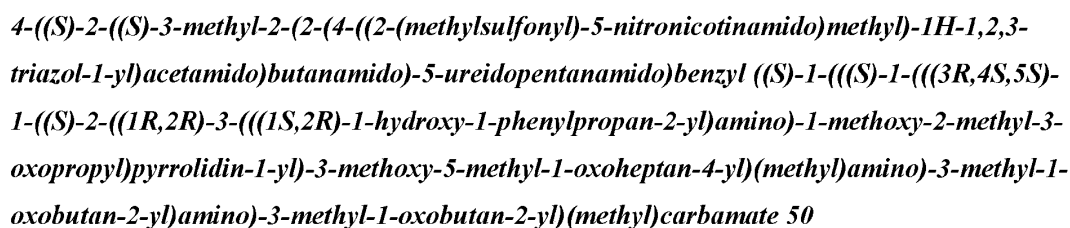
[0321] To a solution of **INT-47** (10.0 mg, 0.0076 mmol) in THF (0.2 mL) and H₂O (0.2 mL) was added Oxone (7.0 mg, 0.0114 mmol) in at rt for 10 hrs. Then the solution was purified by RP prep-HPLC to give **47** (2.8 mg, 27%) as a yellow solid. LCMS (ESI): m/z 1335.89 $[M + H]^+$.



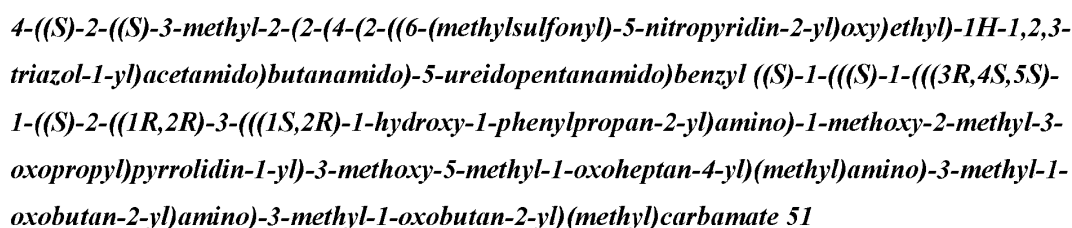
113



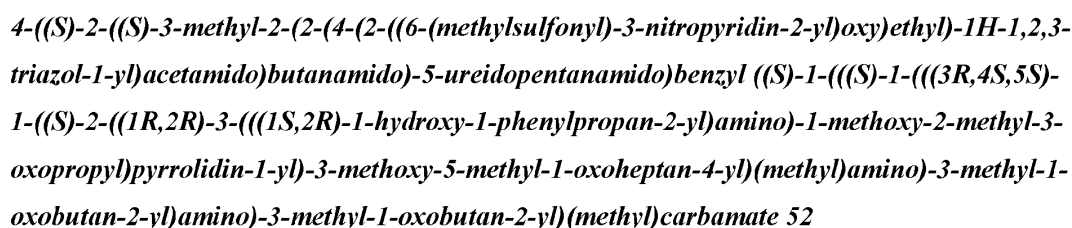
114



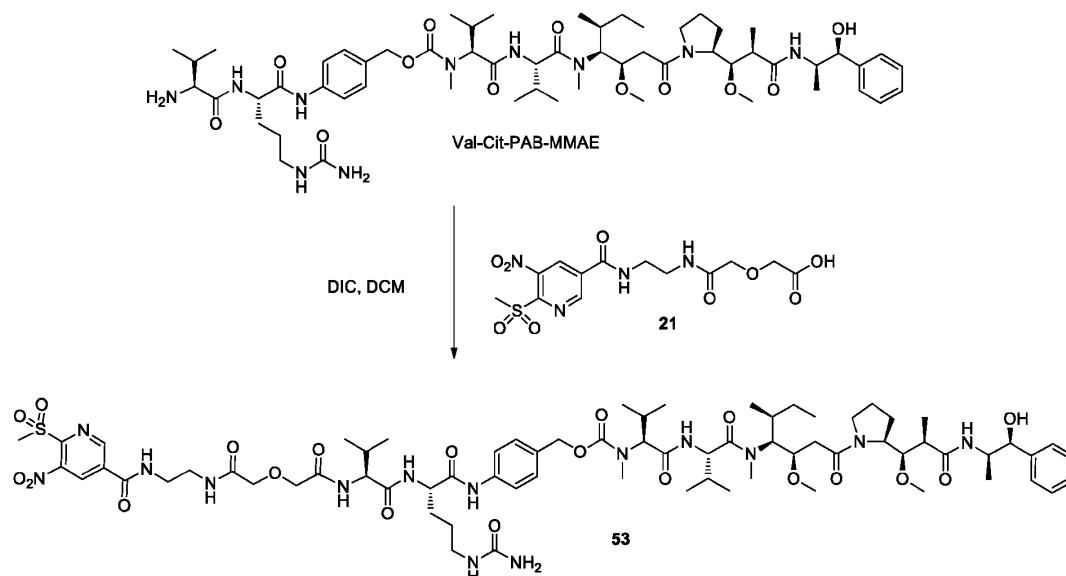
115



116



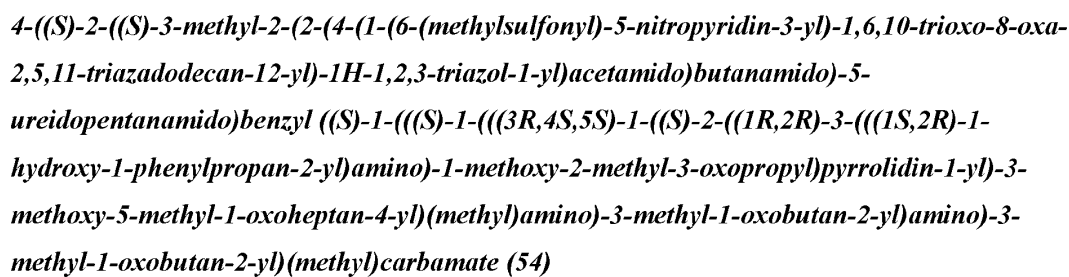
117



4-((12*S*,15*S*)-12-isopropyl-1-(6-(methylsulfonyl)-5-nitropyridin-3-yl)-1,6,10,13-tetraoxo-15-(3-ureidopropyl)-8-oxa-2,5,11,14-tetraazahexadecanamido)benzyl ((*S*)-1-(((*S*)-1-(((3*R*,4*S*,5*S*)-1-((*S*)-2-((1*R*,2*R*)-3-(((1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (53**)**

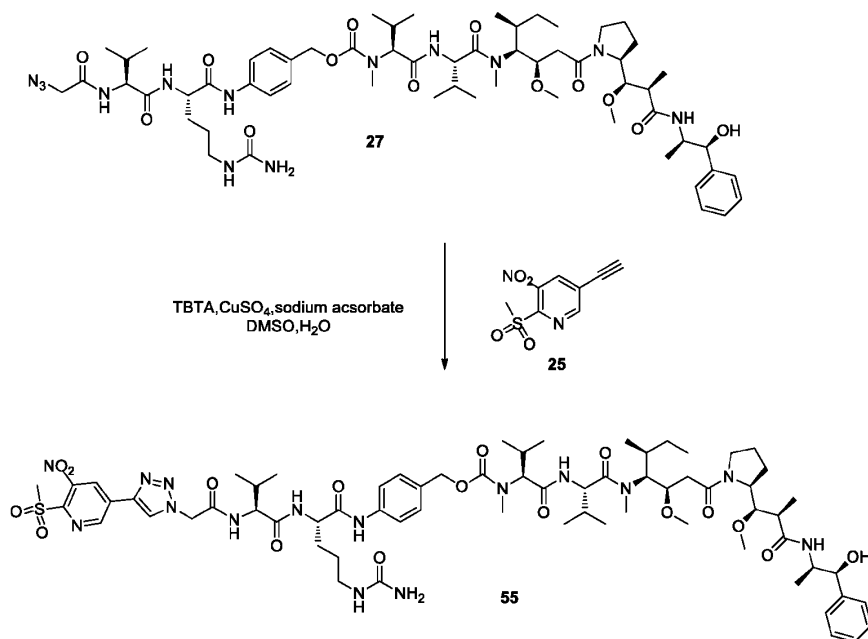
[0327] To a solution of **21** (20.0 mg, 0.049 mmol, 1.0 eq.) in DCM/DMAc (2 mL /2 mL) was added Val-Cit-PAB-MMAE (67.0 mg, 0.059 mmol, 1.2 eq.), followed by DIC (12.0 mg, 0.098 mmol, 2.0 eq.). The reaction mixture was stirred at room temperature for 1 hr. Then the reaction mixture was purified by the prep-HPLC to afford **53** (14.5 mg, yield: 19.59%, purity: 97.30%) as a white powder. LCMS (ESI): m/z : 1510.1 $[M + H]^+$.

[0328] ^1H NMR (DMSO- d_6 , 600 MHz): δ 10.04 (d, 1H), 9.27 (d, 1H), 9.16 (t, 1H), 8.89 (d, 1H), 8.36-8.23 (m, 2H), 8.14-8.01 (m, 1H), 7.91-7.82 (m, 2H), 7.57 (s, 2H), 7.36-7.23 (m, 6H), 7.19-7.15 (m, 1H), 6.00 (s, 1H), 5.13-4.94 (m, 3H), 4.74 (s, 1H), 4.63 (s, 1H), 4.52-4.45 (m, 2H), 4.43-4.36 (m, 3H), 4.30-4.22 (m, 3H), 4.05-3.93 (m, 7H), 3.78 (d, 1H), 3.58-3.54 (m, 1H), 3.49-3.47 (m, 3H), 3.42-3.37 (m, 2H), 3.34-3.30 (m, 2H), 3.24-3.16 (m, 5H), 3.12 (s, 1H), 3.07-2.91 (m, 4H), 2.89-2.81 (m, 2H), 2.42-2.38 (m, 1H), 2.30-2.22 (m, 1H), 2.17-1.89 (m, 4H), 1.85-1.65 (m, 4H), 1.62-1.41 (m, 4H), 1.39-1.18 (m, 4H), 1.07-0.72 (m, 30H).



[0330] ¹H NMR (DMSO-*d*₆, 600 MHz): δ 10.05 (s, 1H), 9.28 (d, *J* = 1.5 Hz, 1H), 9.20 (t, *J* = 5.5 Hz, 1H), 8.90 (d, *J* = 1.5 Hz, 1H), 8.57 (t, *J* = 6.0 Hz, 1H), 8.43 (d, *J* = 8.5 Hz, 1H), 8.34 (d, *J* = 7.2 Hz, 1H), 8.26 (t, *J* = 5.9 Hz, 1H), 8.10 (s, 1H), 7.94-7.62 (m, 2H), 7.57 (s, 2H), 7.32-7.25 (m, 6H), 7.22-7.13 (m, 1H), 5.99 (s, 1H), 5.43 (s, 2H), 5.21-5.13 (m, 2H), 5.11-4.95 (m, 2H), 4.74 (s, 1H), 4.63 (s, 1H), 4.48 (d, *J* = 6.1 Hz, 1H), 4.44-4.39 (m, 2H), 4.36 (d, *J* = 5.9 Hz, 2H), 4.32-4.23 (m, 2H), 3.98 (s, 3H), 3.95 (s, 3H), 3.50 (s, 4H), 3.43-3.38 (m, 3H), 3.37-3.33 (m, 3H), 3.23 (d, *J* = 9.6 Hz, 4H), 3.20 (s, 2H), 3.17 (s, 1H), 3.12 (s, 2H), 3.03-2.98 (m, 4H), 2.91-2.83

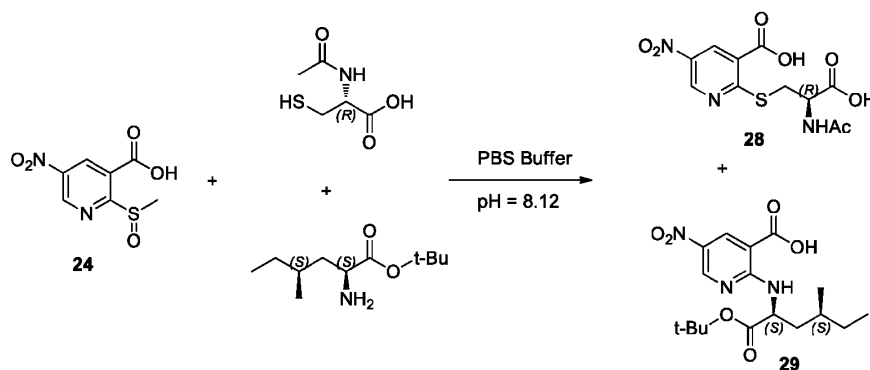
(m, 3H), 2.41 (d, $J = 19.1$ Hz, 1H), 2.30-2.22 (m, 1H), 2.16-2.07 (m, 2H), 2.03-1.95 (m, 2H), 1.76-1.73 (m, 4H), 1.61-1.40 (m, 4H), 1.35 (s, 2H), 1.06-0.96 (m, 6H), 0.91-0.72 (m, 24H).



4-((*S*)-2-((*S*)-3-methyl-2-(2-(4-(6-(methylsulfonyl)-5-nitropyridin-3-yl)-1*H*-1,2,3-triazol-1-yl)acetamido)butanamido)-5-ureidopentanamido)benzyl ((*S*)-1-(((*S*)-1-(((3*R*,4*S*,5*S*)-1-((*S*)-2-((1*R*,2*R*)-3-(((1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (**55**)

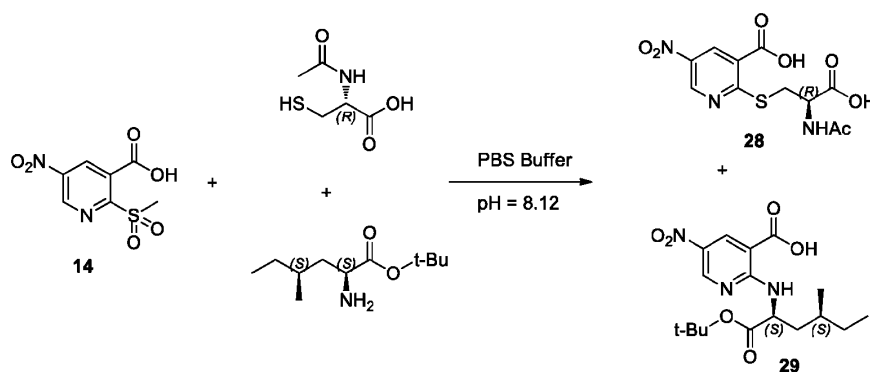
[0331] To a solution of **25** (1.9 mg, 0.008 mmol, 1.0 eq.) in DMSO/H₂O (2 mL, v/v=1:1) was added **27** (10 mg, 0.008 mmol, 1.0 eq.), TBTA (1.7 mg, 0.003 mmol, 0.4 eq.), CuSO₄•5H₂O (0.8 mg, 0.003 mmol, 0.4 eq.), sodium ascorbate (1.6mg, 0.008mmol, 1eq.) at r.t, and the reaction mixture was stirred at that temperature for 2 hrs. Then the residue was purified by the prep-HPLC to afford compound **53** (6.60 mg, yield: 57.4%, purity:95.8%) as a white solid. LCMS[M+H]⁺:1433.1; ¹H NMR (600 MHz, DMSO-d₆) δ 10.02 (s, 1H), 9.45 (d, $J = 1.8$ Hz, 1H), 9.02 (s, 1H), 8.96 (s, 1H), 8.56 (d, $J = 8.8$ Hz, 1H), 8.35 (d, $J = 7.5$ Hz, 1H), 8.05 (d, $J = 5.2$ Hz, 1H), 7.89 (d, $J = 8.0$ Hz, 1H), 7.62 (d, $J = 9.1$ Hz, 1H), 7.57 (t, $J = 7.0$ Hz, 2H), 7.27 (m, 7H), 7.18 (s, 2H), 7.09 (s, 1H), 7.01 (s, 1H), 6.01-5.95 (m, 1H), 5.40 (d, $J = 16.6$ Hz, 5H), 5.12-4.90

(m, 2H), 4.49 (d, J = 6.1 Hz, 1H), 4.38 (m, 3H), 4.28 (d, J = 10.4 Hz, 1H), 3.78 (dd, J = 9.8, 2.3 Hz, 1H), 3.61-3.53 (m, 2H), 3.49 (s, 4H), 3.24 (d, J = 9.2 Hz, 6H), 3.20 (s, 3H), 3.17 (s, 2H), 3.11 (s, 2H), 2.97 (s, 2H), 2.86 (dd, J = 22.0, 8.0 Hz, 4H), 2.44-2.37 (m, 3H), 2.31-2.22 (m, 1H), 2.15-1.99 (m, 4H), 1.75 (s, 1H), 1.35 (s, 1H), 1.24 (s, 4H), 0.92-0.74 (m, 28H).



(R)-2-((2-acetamido-2-carboxyethyl)thio)-5-nitronicotinic acid 28 and/or 2-(((2S,4S)-1-(tert-butoxy)-4-methyl-1-oxohexan-2-yl)amino)-5-nitronicotinic acid 29

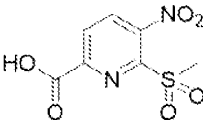
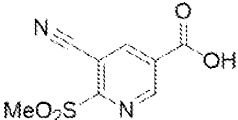
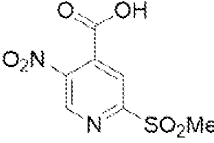
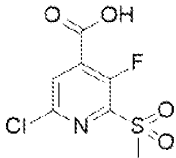
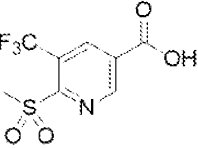
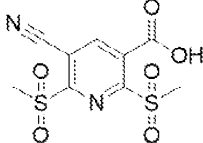
[0332] To a solution of N-acetyl-L-cysteine (21 mg, 0.13 mmol, 1.0 eq) and tert-butyl (2S,4S)-2-amino-4-methylhexanoate (29 mg, 0.13 mmol, 1.0 eq) in PBS buffer (6 mL, pH = 8.12) was added **24** (30 mg, 0.13 mmol, 1.0 eq) in DMSO (0.5 mL) dropwise at RT and stirred at RT for one hour. **24** was consumed, the main product is **28**. **29** was not detected.

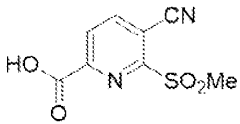
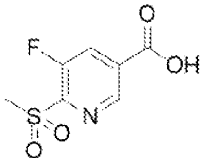
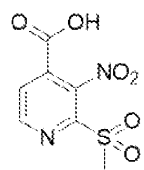
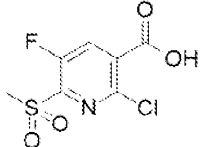
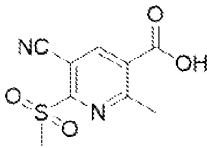
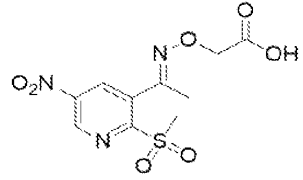
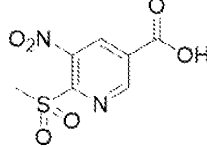
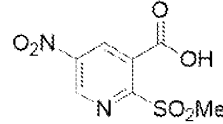


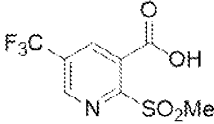
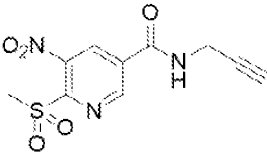
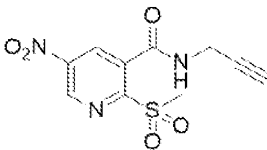
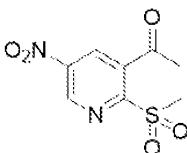
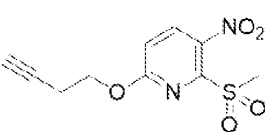
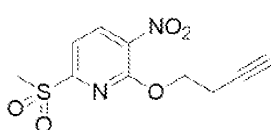
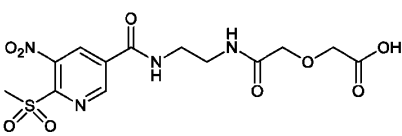
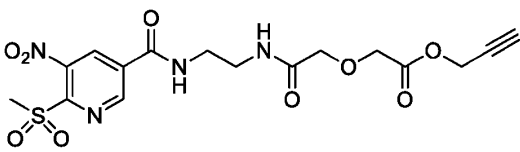
(R)-2-((2-acetamido-2-carboxyethyl)thio)-5-nitronicotinic acid 28 and/or 2-(((2S,4S)-1-(tert-butoxy)-4-methyl-1-oxohexan-2-yl)amino)-5-nitronicotinic acid 29

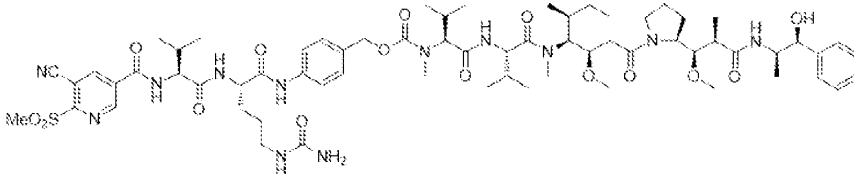
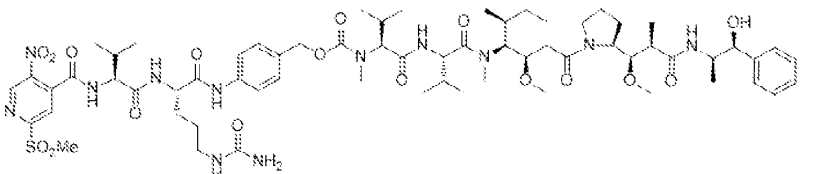
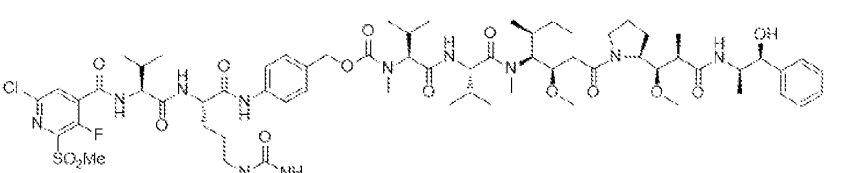
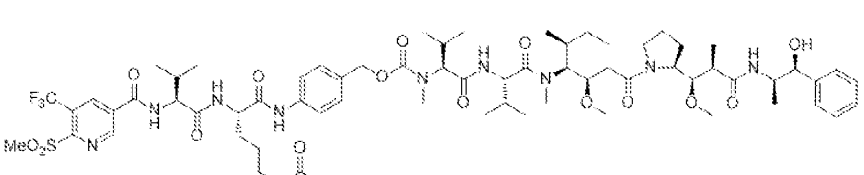
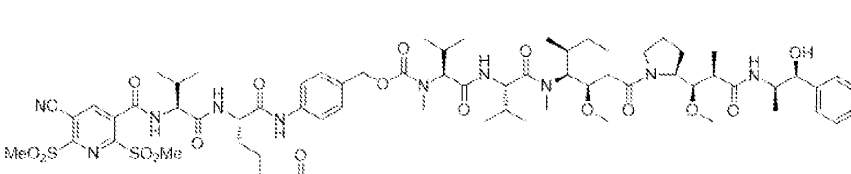
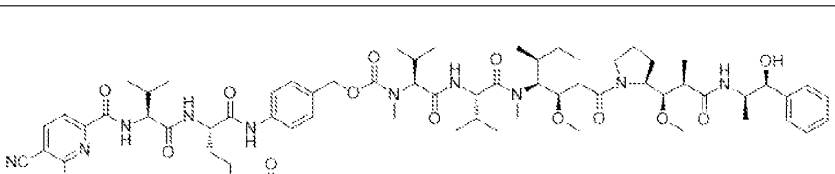
[0333] To a solution of N-acetyl-L-cysteine (21 mg, 0.13 mmol, 1.0 eq) and tert-butyl (2S,4S)-2-amino-4-methylhexanoate (29 mg, 0.13 mmol, 1.0 eq) in PBS buffer (6 mL, pH = 8.12) was added **14** (32 mg, 0.13 mmol, 1.0 eq) in DMSO (0.5 mL) dropwise at RT and stirred at RT for one hour. **14** was consumed, the main product is **28**. **29** was not detected. Both reactions indicating the specificity of this chemotype for reaction with thiol nucleophiles.

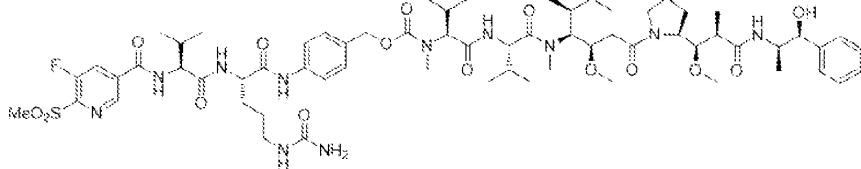
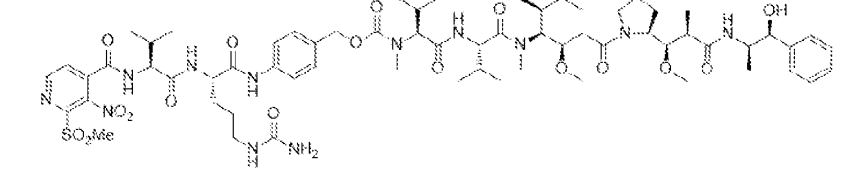
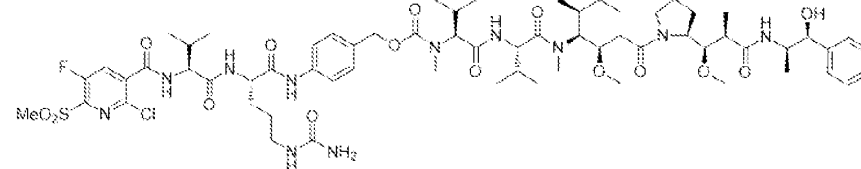
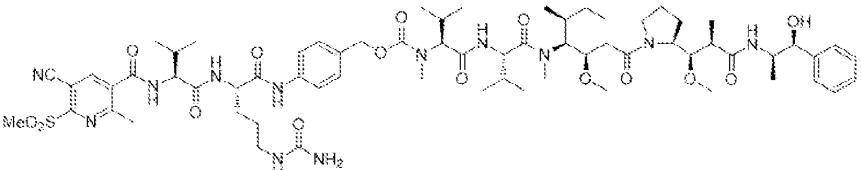
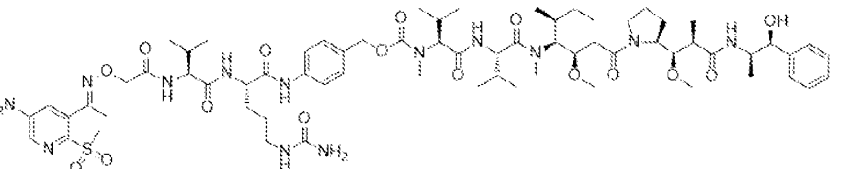
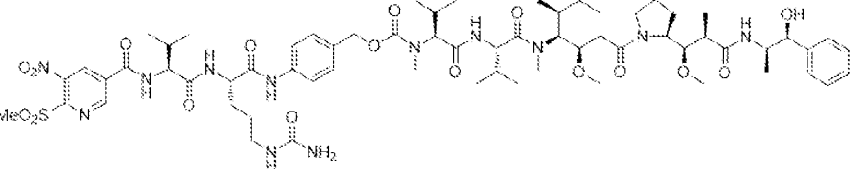
Exemplary Compounds

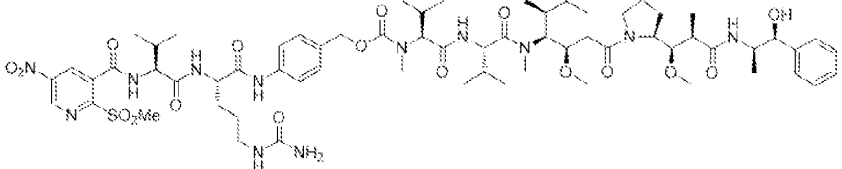
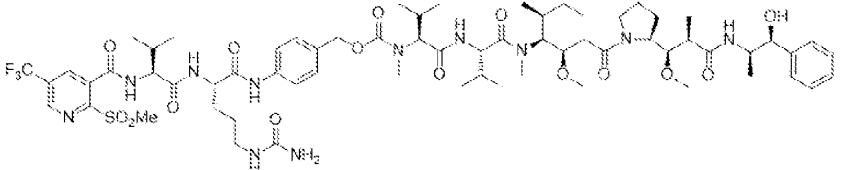
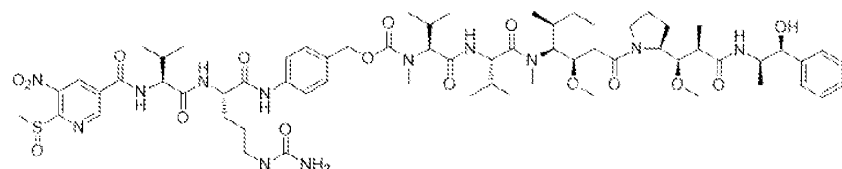
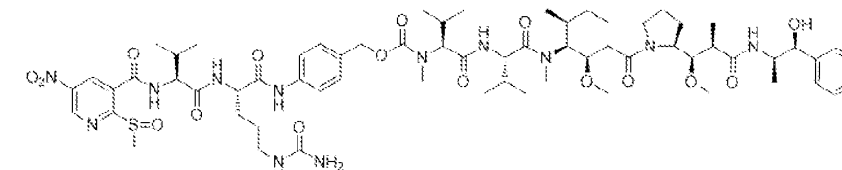
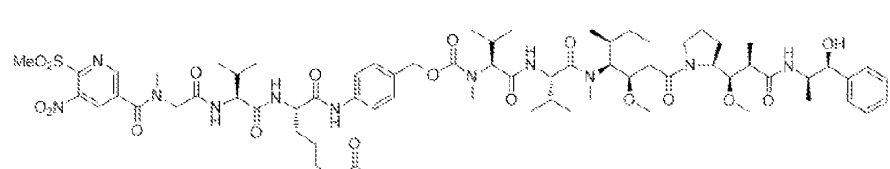
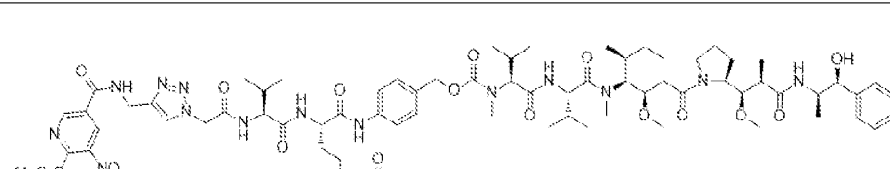
Compound	Structure
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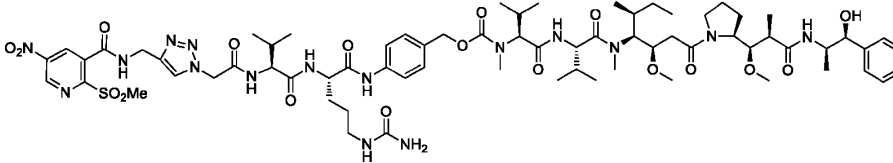
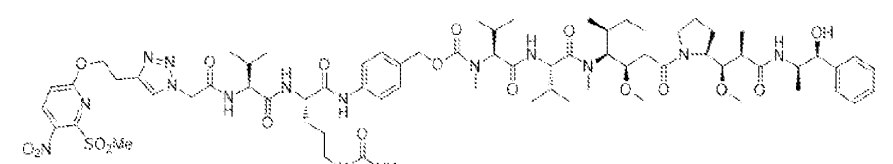
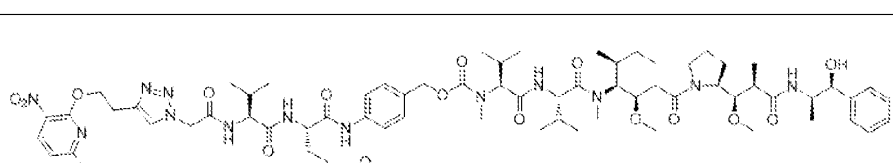
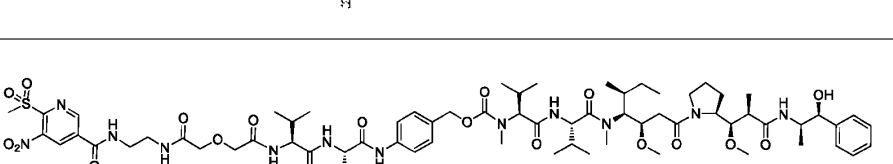
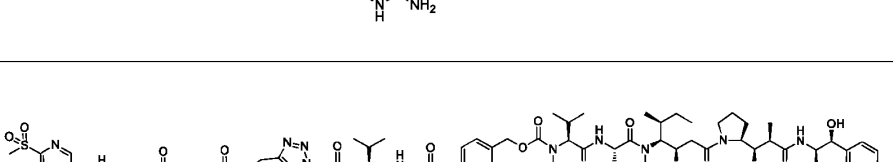
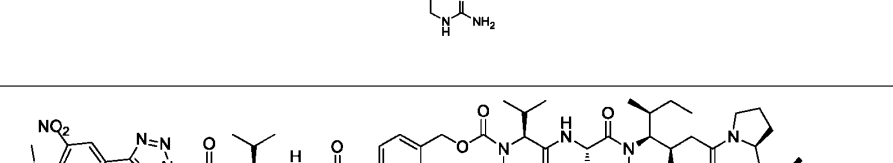
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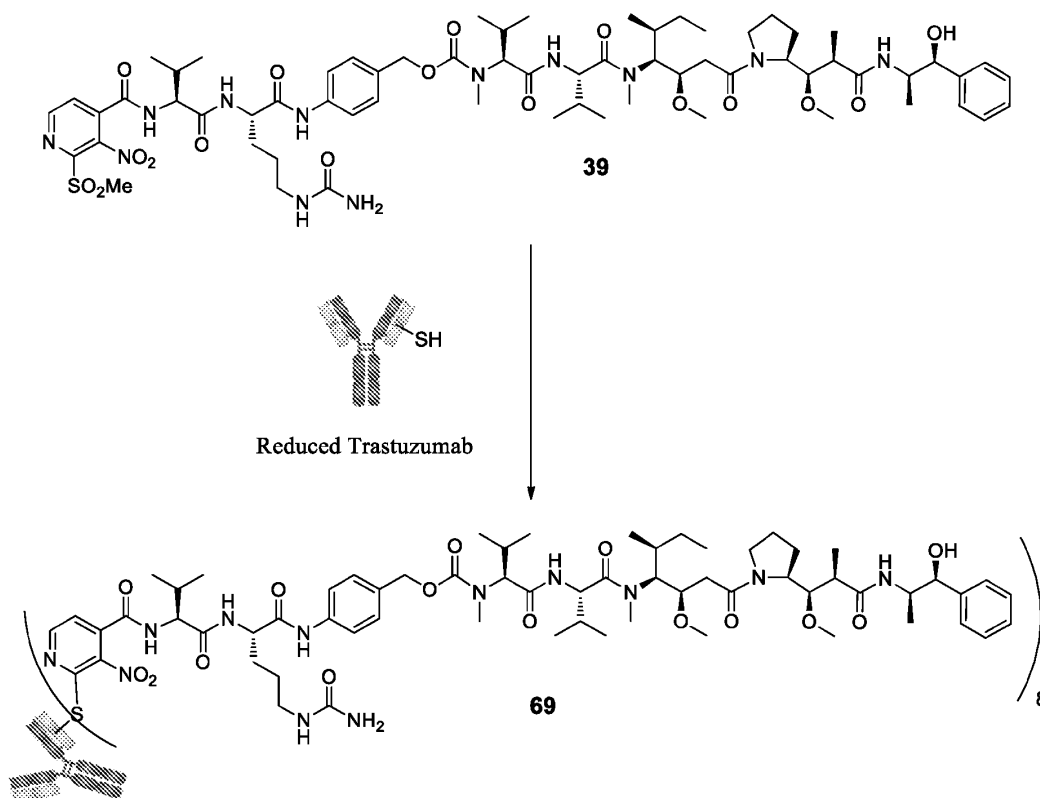
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Conjugation Methodology

[0334] Exemplary conjugation methodology is illustrated below with compound **39** to Trastuzumab (DAR8):



Reaction scheme

[0335] General conjugation protocols:

- Prep Antibody into 20mM Histidine pH 6.0 to approx. 15 mg/mL
- Adjust pH to 7.2 and adjust concentration of antibody to approx. 12 mg/mL with 0.5 M Sodium phosphate buffer + 50 mM EDTA to get a final phosphate buffer concentration to 100mM Sodium Phosphate + 10mM EDTA
- Reduction of antibody by addition of 8~10 molar equivalence of TCEP (stock: 10 mM in water) at 37 °C for 2 hours
- Monitor by RP-HPLC

- Bring to room temperature (20 – 25 °C) for 10 minutes
- Buffer exchange into 20 mM Histidine pH 6.5 ~8.7 (pH can be any point between 6.5 to 8.7 as optimized for each drug linker) by desalting through Zeba Spin desalting column
- Prepare 10 mM stock of drug-linker in DMSO
- Add 11-14 molar equivalence of drug-linker
- Conjugate at RT and monitor by RP-HPLC
- After completion (within 1-16 h) quench by addition of 20 equivalence of N-acetylcysteine and left standing for 1 hour
- Remove excess DL by desalting through Zeba Spin desalting column and buffer exchange into 20 mM Histidine pH 6.0 using 30 kD amicon filters.

[0336] Exemplary RP-HPLC results are shown in **FIGs. 1-2**.

DAR Stability

[0337] Exemplary DAR stability study of Trastuzumab-32 and Trastuzumab-38 human plasma were carried out. DAR loss was minimal with Trastuzumab-conjugates.

Table X. DAR stability (Trastuzumab-32 and Trastuzumab-38)

Time (h)	DAR (% from T0)	
	Trastuzumab-32	Trastuzumab-38
0	100	100
24	101	103
72	101	99
168	98	93

[0338] Protocol for DAR stability assay:

- *Plasma IgG Depletion*: endogenous IgG's were removed by Recombinant Protein A-Sepharose gel filtration. 10 mL's of Sepharose-A were used per 10mL's plasma. Sepharose-A was washed 3x with PBS, centrifugation used to separate wash buffer from Sepharose. Plasma was mixed with Sepharose-A for 2 hours at 4 ° C before plasma was removed from Sepharose by centrifugation.

- *Incubation*: ADC's were spiked into depleted plasma from each test species and PBS to achieve 1mg/mL concentration ADC. Samples were incubated at 37 ° C. Aliquots containing 20 ug's of ADC were removed at designated time points. Aliquots were frozen at -80 ° C until analysis.
- *Capture and Elution*: Protein-A beads were washed 2x with PBS and resuspended in initial volume. On a 96 well ultra low attachment plate, 15 uL of beads were added per well with 35uL PBS and 10 uL of ADC incubated samples. Plate is mixed for 1 hour at room temp. Beads were separated and the supernatant was collected and frozen at -80° C. Beads were washed with PBS-T 3x with 250 uL PBS-T. ADC is eluted from beads with 50 uL of 100mM Acetic acid, neutralized with 10uL of 1.5M Tris-HCL pH 8.5. ADC's are reduced with addition 2uL of 100mM DTT, incubated 30 minutes at 37 ° C. Bead capture supernatant is diluted 1:3 with Acetonitrile and centrifuged at 17G for 10 minutes, supernatant is removed from the pelleted protein and injected on MS.
- *Data Analysis*: DAR was determined by RP-MS, Free Payload was determined by MRM.

Cytotoxicity Data for Representative Conjugates

[0339] The tested compounds included MC-VC-PABC-MMAE Seagen drug-linker, exemplary compounds, and MMAE.

Table Y. *In Vitro* Potency of Representative Conjugates of the Present Disclosure

ADC	DAR	IC50 (nM)	
		HCC1954	SK-BR-3
Trastuzumab-MC-VC-PABC-MMAE	4	0.004	0.002
Trastuzumab- 31	4	0.004	0.002
Trastuzumab- 33	4	0.004	0.002
Trastuzumab- 42	4	0.005	0.003
MMAE	-	0.003	0.02

Assay protocol:

[0340] HCC1954 breast ductal carcinoma or SK-BR-3 cells (ATCC, Manassas, VA, USA) were seeded into 384-well white-walled culture plates and allowed to adhere for 2-4 hours. Cells were then treated with test articles at least in duplicate by addition of 5-fold serially diluted test articles prepared at 2X final concentration and incubated at 37° C for 120 hours. Cell viability following treatment was determined by Cell Titer Glo 2.0 Assay (Promega, Madison, WI, USA) and normalized to non-treated controls. Dose-response relationships were analyzed using GraphPad Prism (La Jolla, CA, USA), and IC50 values were derived from non-linear regression analyses using a 4-parameter logistic equation.

[0341] Applicant's disclosure is described herein in preferred embodiments with reference to the Figures, in which like numbers represent the same or similar elements. Reference throughout this specification to "one embodiment," "an embodiment," or similar language means that a particular feature, structure, or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, appearances of the phrases "in one embodiment," "in an embodiment," and similar language throughout this specification may, but do not necessarily, all refer to the same embodiment.

[0342] The described features, structures, or characteristics of Applicant's disclosure may be combined in any suitable manner in one or more embodiments. In the description, herein, numerous specific details are recited to provide a thorough understanding of embodiments of the invention. One skilled in the relevant art will recognize, however, that Applicant's composition and/or method may be practiced without one or more of the specific details, or with other methods, components, materials, and so forth. In other instances, well-known structures, materials, or operations are not shown or described in detail to avoid obscuring aspects of the disclosure.

[0343] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure, the preferred methods and materials are now described. Methods recited herein may be carried out in any order that is logically possible, in addition to a particular order disclosed.

Incorporation by Reference

[0344] References and citations to other documents, such as patents, patent applications, patent publications, journals, books, papers, manuscripts, web contents, have been made in this disclosure. All such documents are hereby incorporated herein by reference in their entirety for all purposes. Any material, or portion thereof, that is said to be incorporated by reference herein, but which conflicts with existing definitions, statements, or other disclosure material explicitly set forth herein is only incorporated to the extent that no conflict arises between that incorporated material and the present disclosure material. In the event of a conflict, the conflict is to be resolved in favor of the present disclosure as the preferred disclosure.

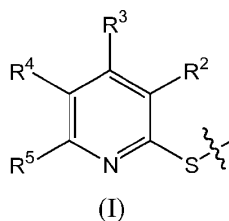
Equivalents

[0345] The representative examples are intended to help illustrate the invention, and are not intended to, nor should they be construed to, limit the scope of the invention. Indeed, various modifications of the invention and many further embodiments thereof, in addition to those shown and described herein, will become apparent to those skilled in the art from the full contents of this document, including the examples and the references to the scientific and patent literature included herein. The examples contain important additional information, exemplification and guidance that can be adapted to the practice of this invention in its various embodiments and equivalents thereof.

What is claimed is:

CLAIMS

1. A drug conjugate comprising a targeting moiety, a linker moiety, and a drug moiety, wherein the drug moiety is conjugated to the linker which is conjugated to the targeting moiety, and wherein the linker moiety comprises the structural formula (I):



wherein:

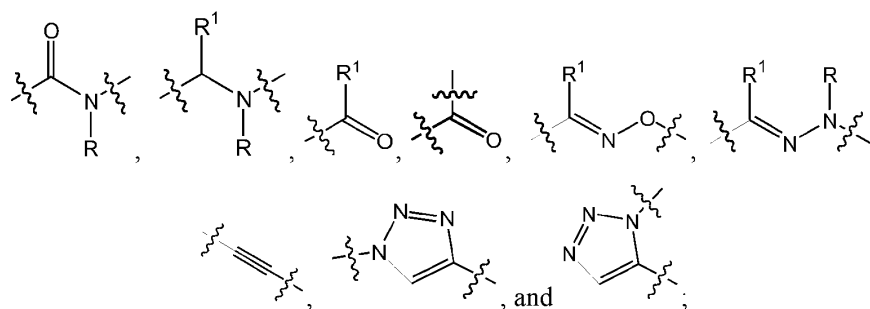
each R^2 , R^3 , R^4 is independently selected from the group consisting of H, NO_2 , CN, CF_3 , F, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne, C_{6-10} aryl, and W;

R^5 is H, Cl, SOR^6 , SO_2R^6 , OR^7 , NRR^7 or W;

R^6 is an unsubstituted or substituted C_{1-6} alkyl or C_{6-10} aryl;

R^7 is an unsubstituted or substituted C_{2-6} alkyne or C_{2-6} alkyne;

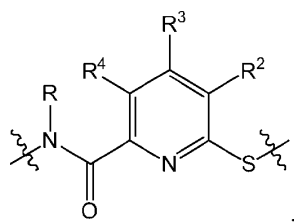
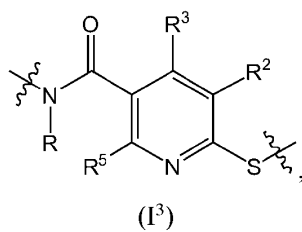
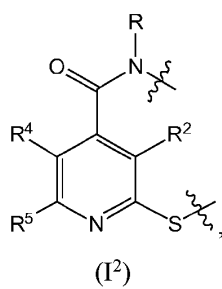
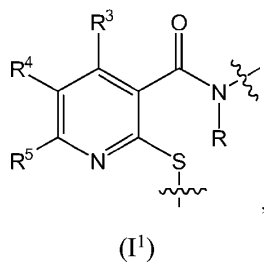
W is selected from the group consisting of:

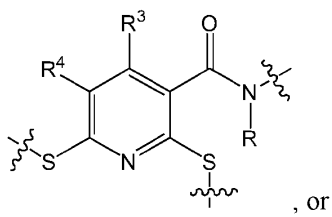
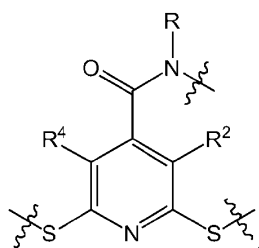


R^1 is H, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne or C_{6-10} aryl;

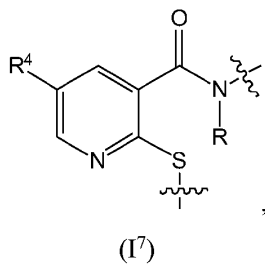
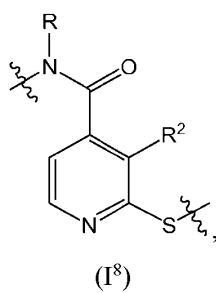
R is H, an unsubstituted or substituted C₁₋₆ alkyl, C₂₋₆ alkyene, C₂₋₆ alkyne or C₆₋₁₀ aryl;
or a pharmaceutically acceptable salt thereof.

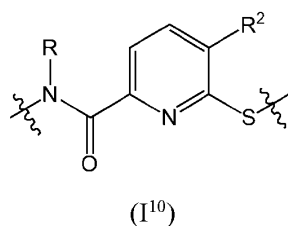
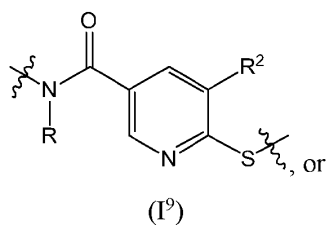
2. The drug conjugate of claim 1, comprising a structural formula:



(I⁴)(I⁵)(I⁶)

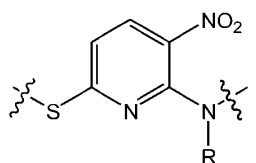
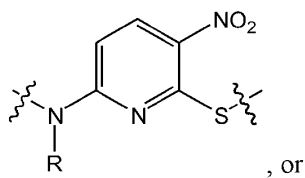
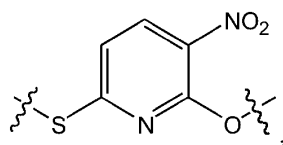
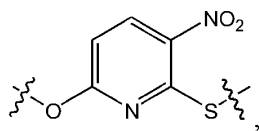
3. The drug conjugate of claim 1 or 2, comprising the structural formula:

(I⁷)(I⁸)

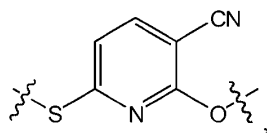
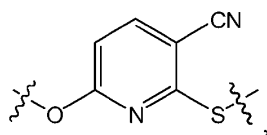


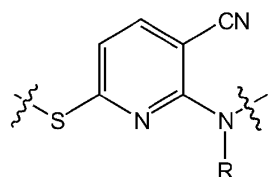
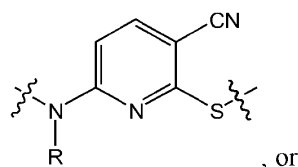
4. The drug conjugate of any one of claims 1-3, wherein one of R², R³ and R⁴ is selected from the group consisting of NO₂, CN, CF₃ and F.
5. The drug conjugate of any one of claims 1-4, wherein R is H.
6. The drug conjugate of any one of claims 1-4, wherein R is an unsubstituted or substituted C₁₋₆ alkyl.
7. The drug conjugate of claim 4 or 5, wherein one of R², R³ and R⁴ is NO₂.
8. The drug conjugate of claim 4 or 5, wherein one of R², R³ and R⁴ is CN.
9. The drug conjugate of claim 4 or 5, wherein R² or R⁴ is CF₃.
10. The drug conjugate of claim 4 or 5, wherein R² is not CF₃.
11. The drug conjugate of claim 4 or 5, wherein R⁴ is C(O)OR and R² is not CF₃.
12. The drug conjugate of claim 4 or 5, wherein R² or R⁴ is F.

13. The drug conjugate of any one of claims 1-4, comprising the structural formula:



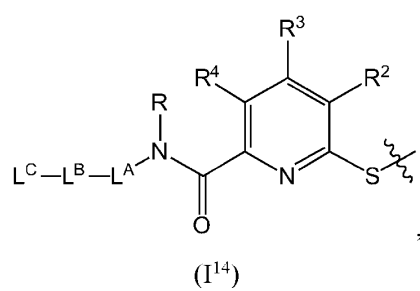
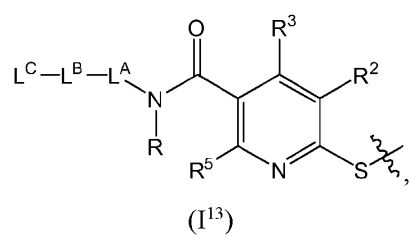
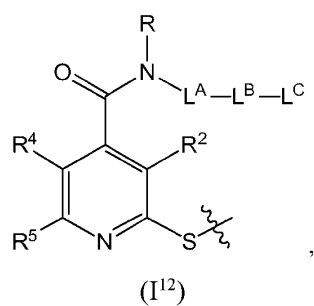
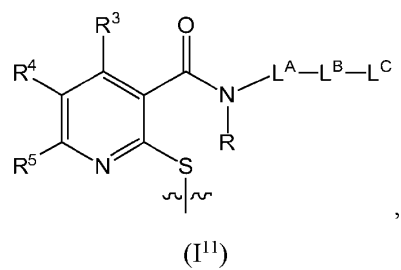
14. The drug conjugate of any one of claims 1-4, comprising the structural formula:

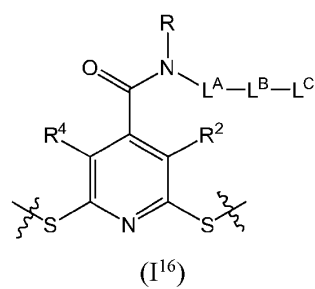
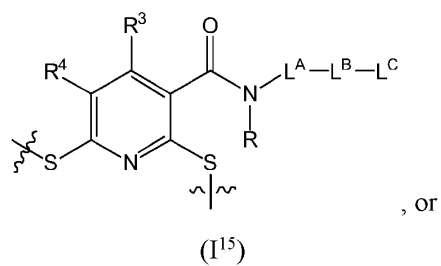




15. The drug conjugate of any one of claims 1-12, wherein the linker moiety comprises the structural formula (I¹) or (I⁷).
16. The drug conjugate of any one of claims 1-12, wherein the linker moiety comprises the structural formula (I²) or (I⁸).
17. The drug conjugate of any one of claims 1-12, wherein the linker moiety comprises the structural formula (I³) or (I⁹).
18. The drug conjugate of any one of claims 1-12, wherein the linker moiety comprises the structural formula (I⁴) or (I¹⁰).
19. The drug conjugate of any one of claims 1-12, wherein the linker moiety comprises the structural formula (I⁵) or (I⁶).
20. The drug conjugate of any one of claims 1-19, wherein the linker moiety further comprises a spacer, a peptide moiety, and/or a self-immolative moiety.
21. The drug conjugate of claim 20, wherein the spacer, the peptide moiety, and/or the self-immolative moiety are conjugated to one of R², R³, R⁴ and R⁵.

22. The drug conjugate of claim 1 or 2, comprising a structural formula:





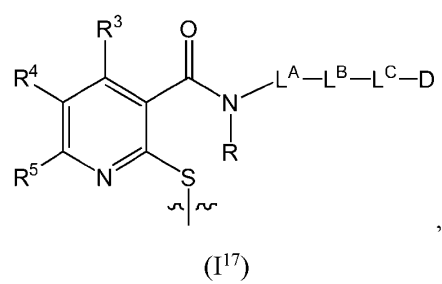
wherein

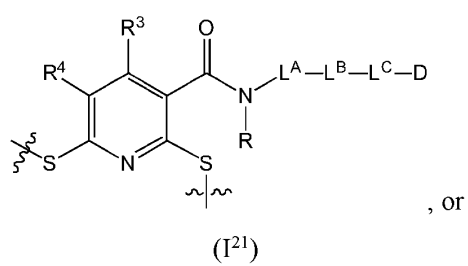
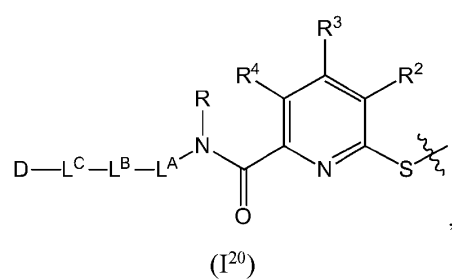
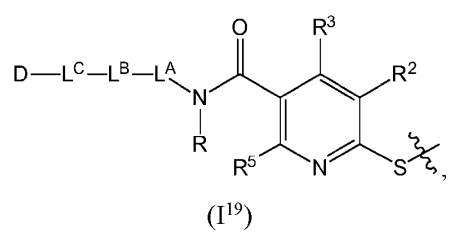
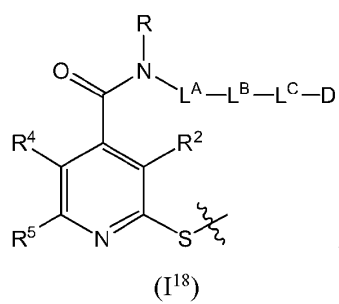
L^A is a spacer moiety or is absent;

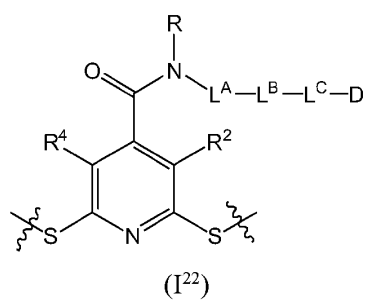
L^B is a peptide moiety or is absent; and

L^C is a self-immolative moiety or is absent.

23. The drug conjugate of claim 22, further comprising a drug moiety D, comprising the structural formula:

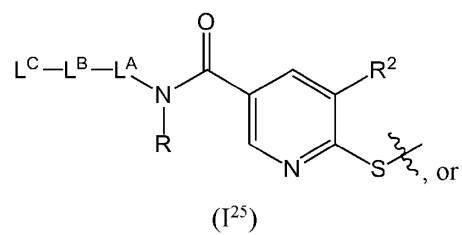
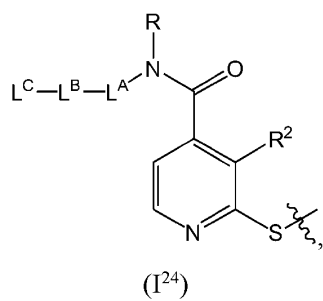
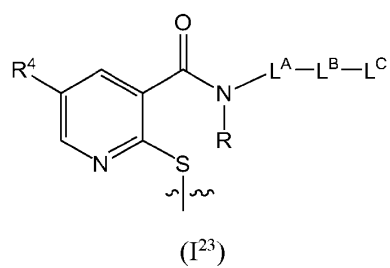


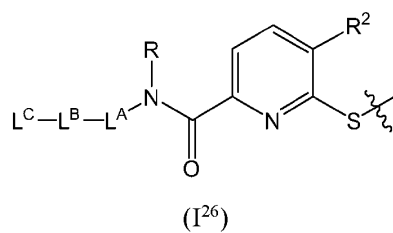




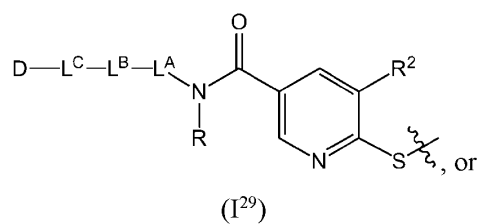
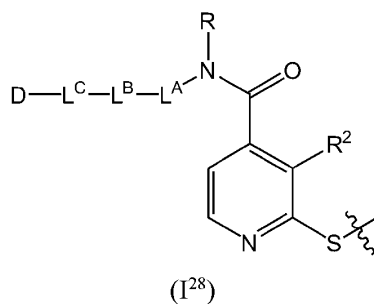
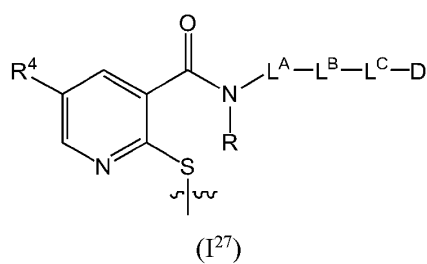
wherein D is a drug moiety.

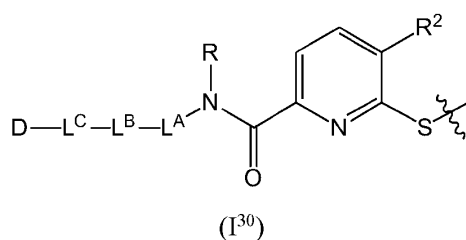
24. The drug conjugate of claim 3, comprising the structural formula:



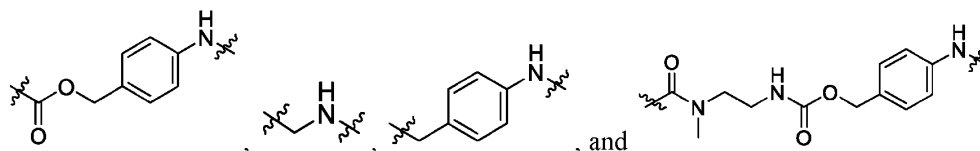


25. The drug conjugate of claim 24, further comprising drug moiety, comprising the structural formula:



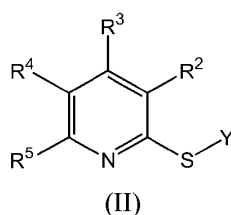


26. The drug conjugate of any one of claims 22-25, wherein the spacer moiety is selected from the group consisting of an alkyl, a heteroalkyl, polyethylene glycol (PEG), and a peptide.
27. The drug conjugate of any one of claims 22-25, wherein the peptide moiety comprises 1 to 6 amino acids.
28. The drug conjugate of claim 27, wherein the amino acids are natural and/or unnatural amino acids.
29. The drug conjugate of any one of claims 22-29, wherein the self-immolative moiety is selected from the group consisting of:



30. The drug conjugate of any one of claims 1-29, wherein the drug moiety is a chemical agent selected from the group consisting of an antibiotic, an anti-cancer agent, a steroid, a TLR7/TLR9 antagonist, a peptide, a protein, and a nucleic acid.
31. The drug conjugate of any one of claims 1-30, wherein the targeting moiety is selected from the group consisting of an antibody, small molecule, a peptide and a nucleic acid.

32. The drug conjugate of claim 31, wherein the drug conjugate has a targeting moiety to drug moiety ratio of about 1:1 to about 1:16.
33. A drug conjugate comprising a targeting moiety, a linker moiety, and a drug moiety, wherein the drug moiety is conjugated to the linker which is conjugated to the targeting moiety, and wherein the linker moiety comprises the structural formula (II):



wherein:

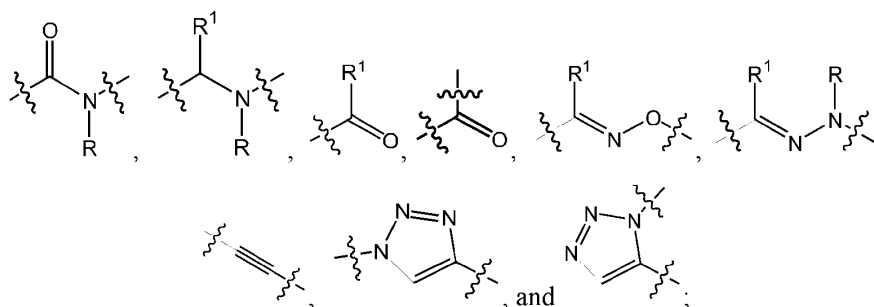
each R^2 , R^3 , R^4 is independently selected from the group consisting of H, NO_2 , CN, CF_3 , F, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne, C_{6-10} aryl, and W;

R^5 is H, Cl, SOR^6 , SO_2R^6 , OR^7 , NRR^7 or W;

R^6 is an unsubstituted or substituted C_{1-6} alkyl or C_{6-10} aryl;

R^7 is an unsubstituted or substituted C_{2-6} alkyne or C_{2-6} alkyne;

W is selected from the group consisting of:

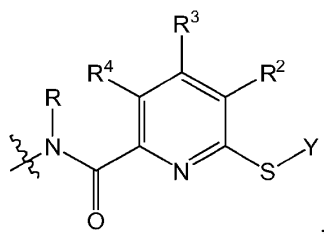
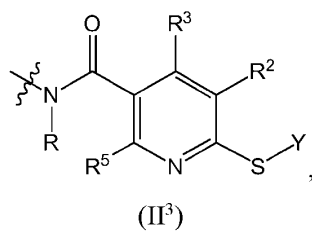
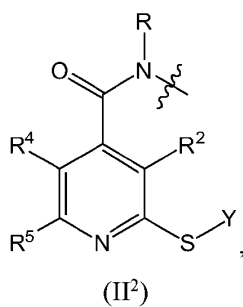
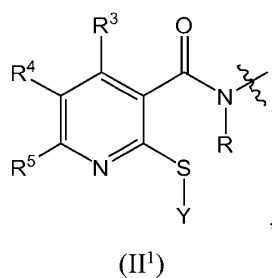


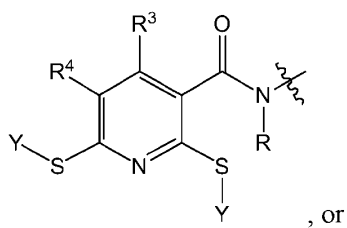
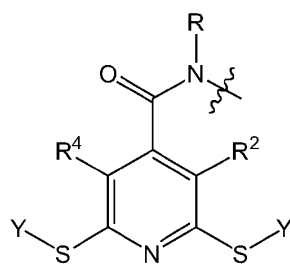
R^1 is H, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne or C_{6-10} aryl;

R is H, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne or C_{6-10} aryl; and

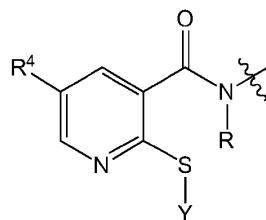
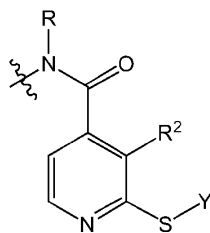
each Y is independently a peptide or an antigen-binding moiety that comprises a cysteine moiety,
or a pharmaceutically acceptable salt thereof.

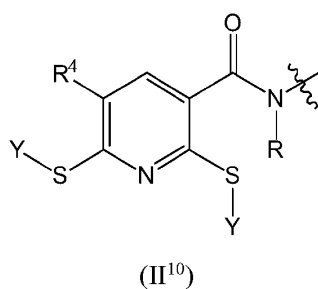
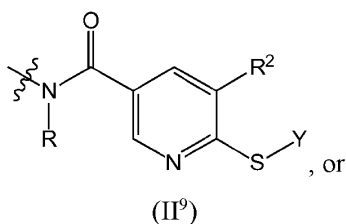
34. The drug conjugate of claim 33, comprising the structural formula:



$$(\text{II}^4)$$

$$(\text{II}^5)$$

$$(\text{II}^6)$$

35. The drug conjugate of claim 34, comprising the structural formula:

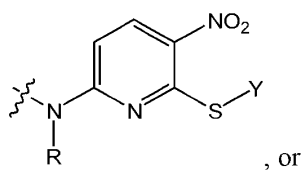
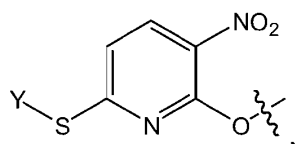
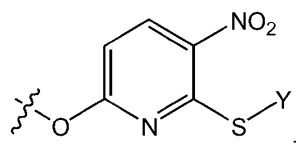
(II⁷)
$$(\text{II}^8)$$



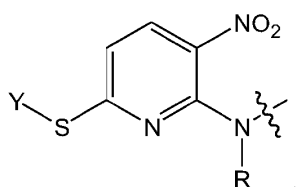
36. The drug conjugate of any one of claims 33-35, wherein one of R², R³ and R⁴ is selected from the group consisting of NO₂, CN, CF₃ and F.
37. The drug conjugate of any one of claims 33-36, wherein R is H.
38. The drug conjugate of any one of claims 33-36, wherein R is an unsubstituted or substituted C₁₋₆ alkyl.
39. The drug conjugate of claim 37 or 38, wherein one of R², R³ and R⁴ is NO₂.
40. The drug conjugate of claim 37 or 38, wherein one of R², R³ and R⁴ is CN.
41. The drug conjugate of claim 37 or 38, wherein R² or R⁴ is CF₃.
42. The drug conjugate of claim 37 or 38, wherein R² is not CF₃.
43. The drug conjugate of claim 37 or 38, wherein R⁴ is C(O)OR and R² is not CF₃.

44. The drug conjugate of claim 37 or 38, wherein R^2 or R^4 is F.

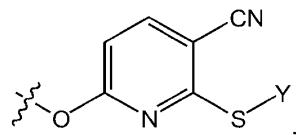
45. The drug conjugate of any one of claims 33-36, comprising the structural formula:

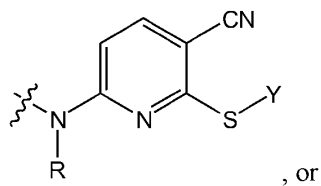
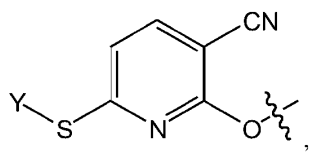


, or

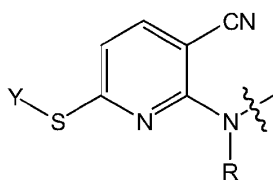


46. The drug conjugate of any one of claims 33-36, comprising the structural formula:

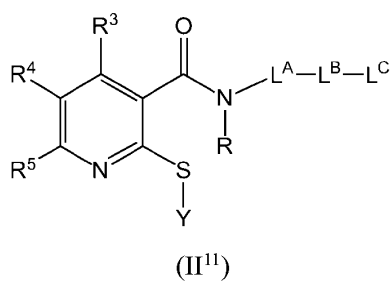


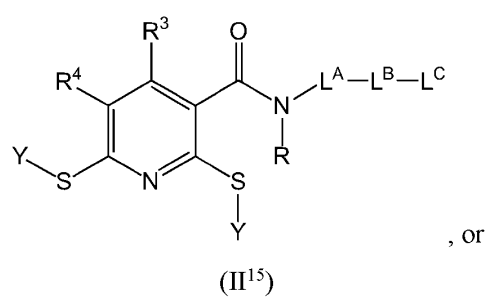
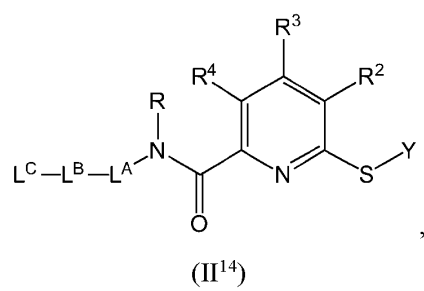
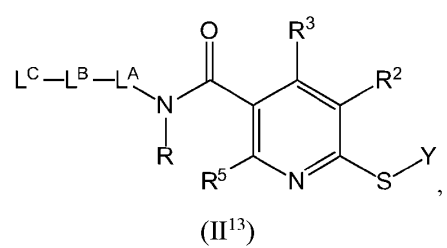
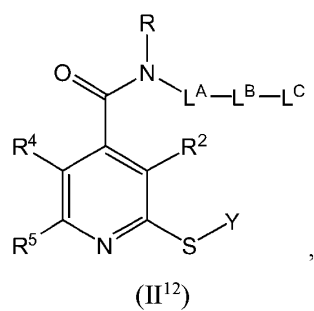


, or

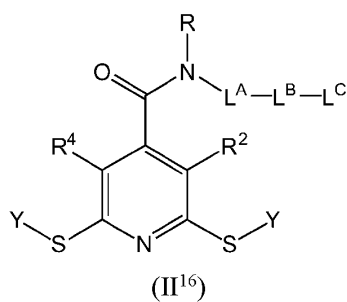


47. The drug conjugate of any one of claims 33-46, wherein the linker moiety further comprises a spacer, a peptide moiety, and/or a self-immolative moiety.
48. The drug conjugate of claim 33 or 34, comprising a structural formula:





, or



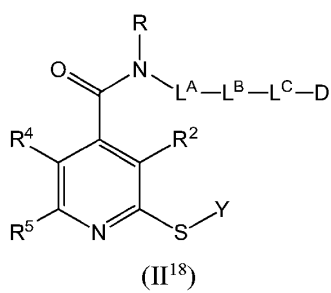
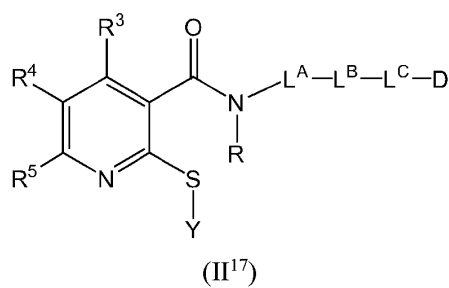
wherein

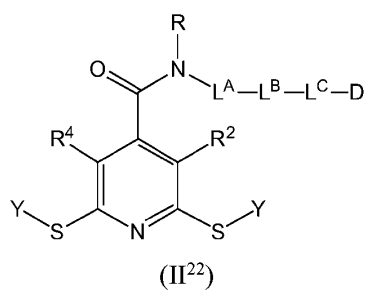
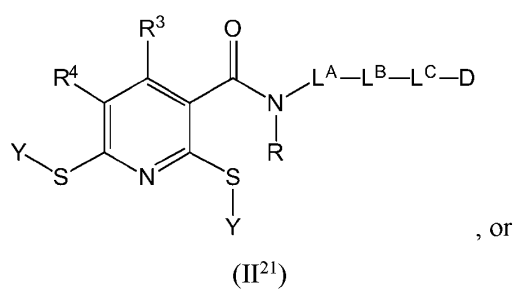
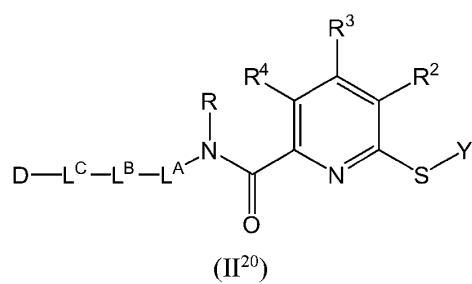
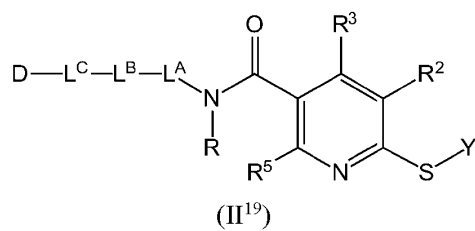
L^A is a spacer moiety or is absent;

L^B is a peptide moiety or is absent; and

L^C is a self-immolative moiety or is absent.

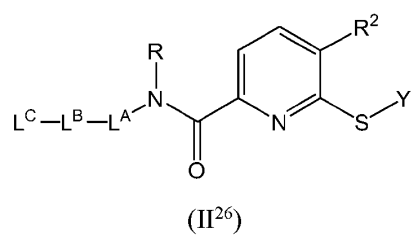
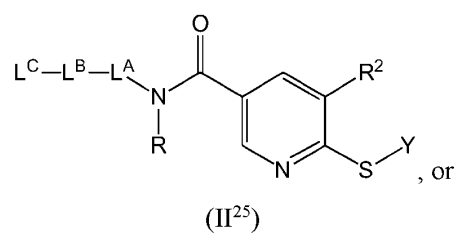
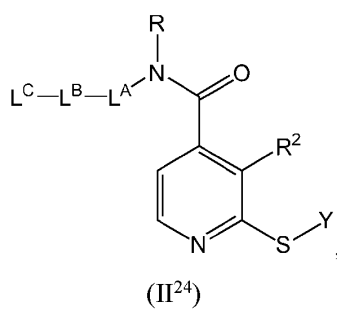
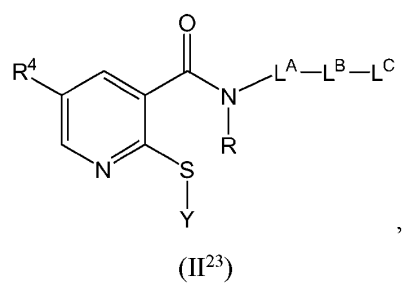
49. The drug conjugate of claim 48, further comprising a drug moiety, comprising the structural formula:



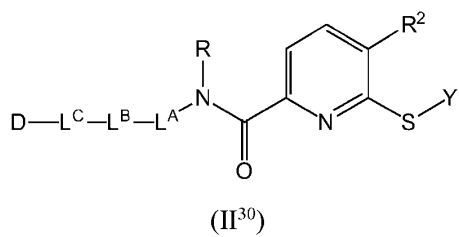
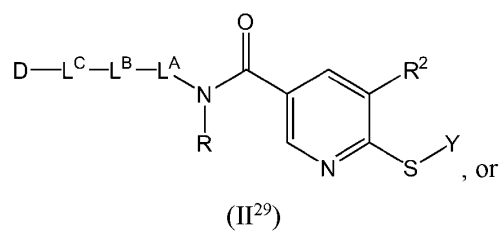
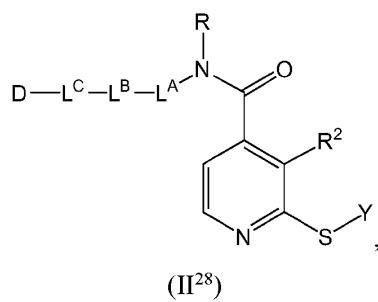
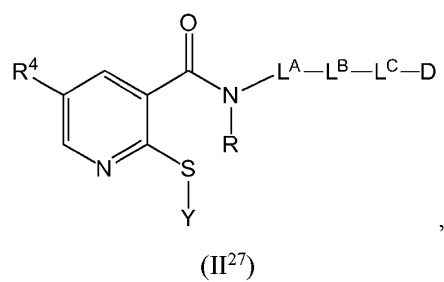


wherein D is a drug moiety.

50. The drug conjugate of claim 48, comprising the structural formula:



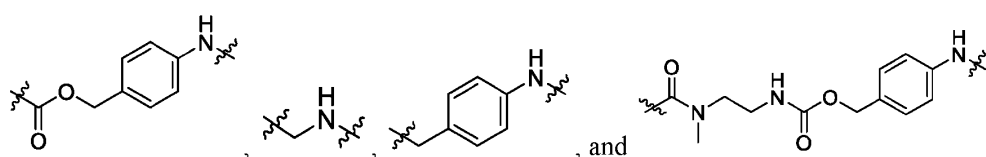
51. The drug conjugate of claim 50, further comprising a drug moiety, comprising the structural formula:



52. The drug conjugate of any one of claims 48-51, wherein the spacer moiety is selected from the group consisting of an alkyl, a heteroalkyl, polyethylene glycol (PEG), and a peptide.

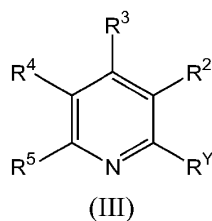
53. The drug conjugate of any one of claims 48-52, wherein the peptide moiety comprises 1 to 6 amino acids.
54. The drug conjugate of claim 53, wherein the amino acids are natural and/or unnatural amino acids.

55. The drug conjugate of any one of claims 48-54, wherein the self-immolative moiety is selected from the group consisting of:



56. The drug conjugate of any one of claims 48-55, wherein the drug moiety is a chemical agent selected from the group consisting of an antibiotic, an anti-cancer agent, a steroid, a TLR7/TLR9 antagonist, a peptide, a protein, and a nucleic acid.
57. The drug conjugate of any one of claims 48-56, wherein the targeting moiety is selected from the group consisting of an antibody, small molecule, a peptide and a nucleic acid.
58. The drug conjugate of claim 57, wherein the drug conjugate has a targeting moiety to drug moiety ratio of about 1:1 to about 1:16.
59. A composition comprising a drug conjugate of any one of claims 1-58.
60. A pharmaceutical composition comprising a drug conjugate of any one of claims 1-58, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient, carrier or diluent.
61. A unit dosage form comprising a pharmaceutical composition of claim 60.

62. A compound useful for forming a linker moiety-drug moiety conjugate, a targeting moiety-linker moiety conjugate, or a targeting moiety-linker moiety-drug moiety conjugate, the compound having a structure comprising structural formula (III):



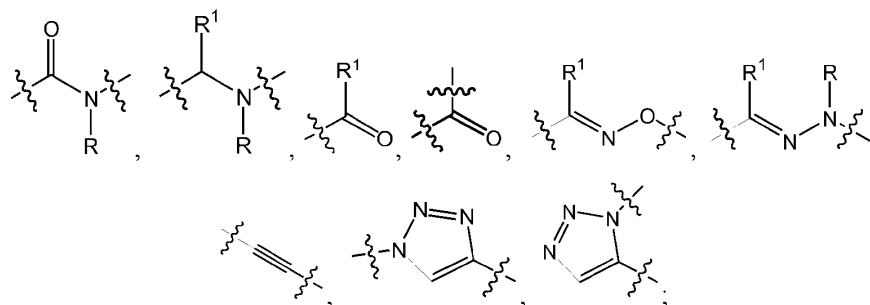
each R^2 , R^3 , R^4 is independently selected from the group consisting of H, NO_2 , CN, CF_3 , F, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne, C_{6-10} aryl, and W;

each R^5 and R^Y is independently H, Cl, SOR^6 , SO_2R^6 , OR^7 , NRR^7 or W;

R^6 is an unsubstituted or substituted C_{1-6} alkyl or C_{6-10} aryl;

R^7 is an unsubstituted or substituted C_{2-6} alkyne or C_{2-6} alkyne;

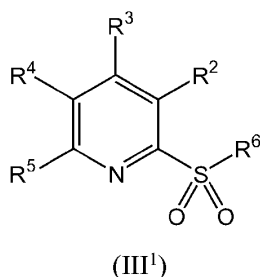
W is selected from:



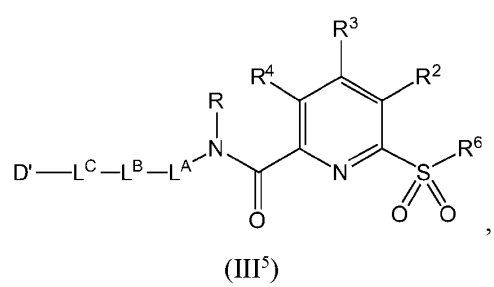
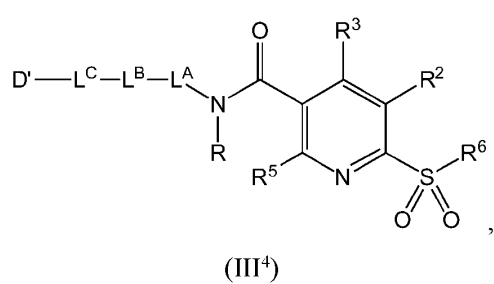
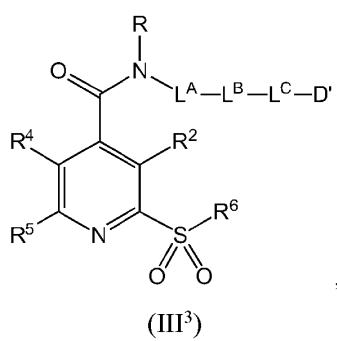
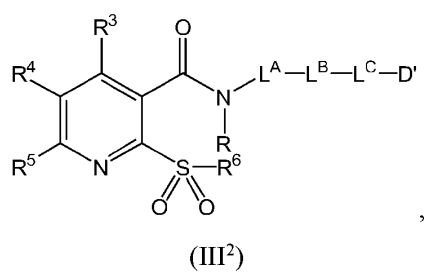
R^1 is H, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne or C_{6-10} aryl; and

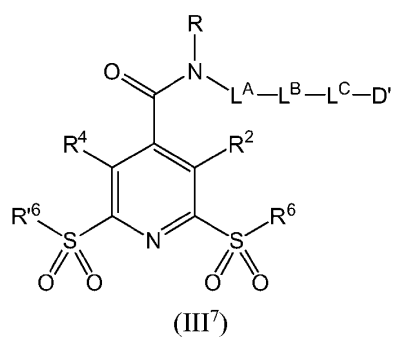
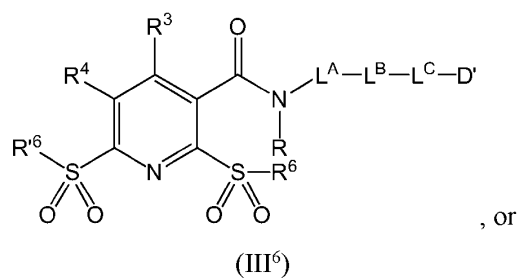
R is H, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne or C_{6-10} aryl.

63. The compound of claim 62, wherein R^Y is SO_2R^6 , having the structural formula:



64. The compound of claim 62 or 63, wherein one of R², R³ and R⁴ is selected from the group consisting of NO₂, CN, CF₃ and F.
65. The compound of any one of claims 62-64, wherein R is H.
66. The compound of any one of claims 62-64, wherein R is an unsubstituted or substituted C₁₋₆ alkyl.
67. The compound of claim 65 or 66, wherein one of R², R³ and R⁴ is NO₂.
68. The compound of claim 65 or 66, wherein one of R², R³ and R⁴ is CN.
69. The compound of claim 65 or 66, wherein R² or R⁴ is CF₃.
70. The compound of claim 65 or 66, wherein R² is not CF₃.
71. The compound of claim 65 or 66, wherein R⁴ is C(O)OR and R² is not CF₃.
72. The compound of claim 65 or 66, wherein R² or R⁴ is F.
73. The compound of claim 66, wherein one of R², R³, R⁴ and R⁵ is C(O)NHR'.
74. The compound of claim 64 or 65, having the structural formula:





wherein

L^A is a spacer moiety or is absent;

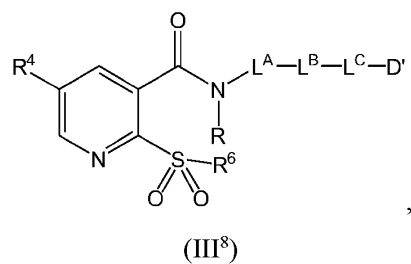
L^B is a peptide moiety or is absent;

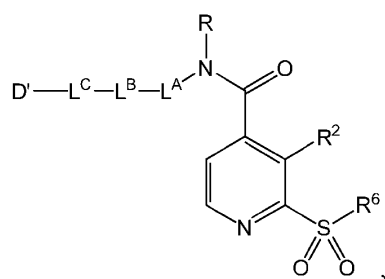
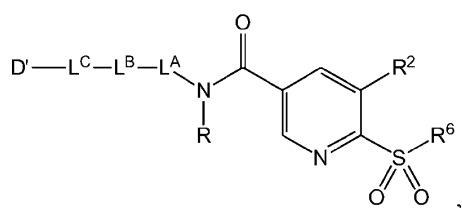
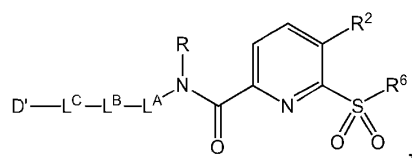
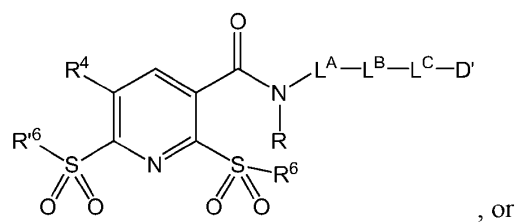
L^C is a self-immolative moiety or is absent;

R^6 and R'^6 may be the same or different and is an unsubstituted or substituted C_{1-6} alkyl or C_{6-10} aryl; and

D' is a drug moiety D or is absent.

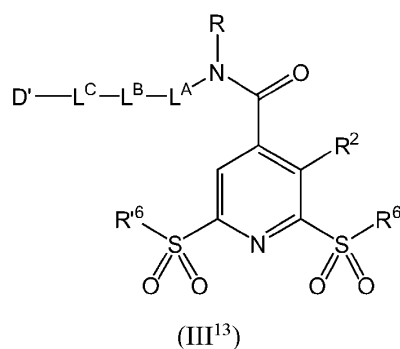
75. The compound of claim 74, having the structural formula:



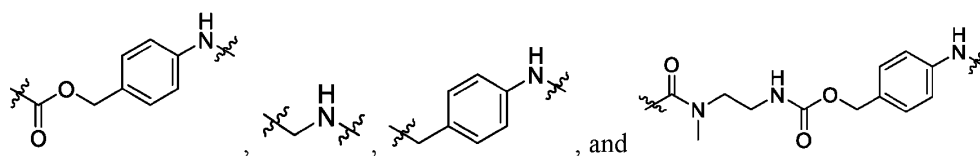
(III⁹)(III¹⁰)(III¹¹)

, or

(III¹²)



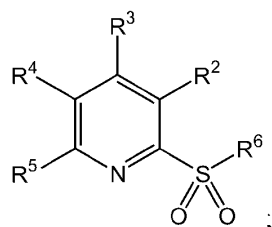
76. The compound of claim 74 or 75, wherein each of R⁶ and R'⁶ is methyl.
77. The compound of any one of claims 74-76, wherein the spacer moiety is selected from the group consisting of an alkyl, a heteroalkyl, polyethylene glycol (PEG), and a peptide.
78. The compound of any one of claims 74-77, wherein the peptide moiety comprises 1 to 6 amino acids.
79. The compound of claim 78, wherein the amino acids are natural and/or unnatural amino acids.
80. The compound of any one of claims 74-79, wherein the self-immolative moiety is selected from the group consisting of:



81. The compound of any one of claims 74-80, wherein the drug moiety is a chemical agent selected from the group consisting of an antibiotic, an anti-cancer agent, a steroid, a TLR7/TLR9 antagonist, a peptide, a protein, and a nucleic acid.
82. A composition comprising a compound of any one of claims 62-81.

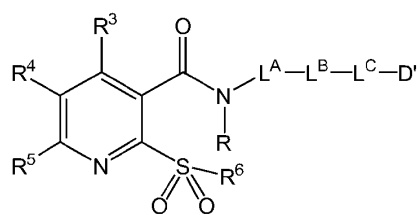
83. A method of preparing a linker-drug conjugate comprising:

(a) providing a compound having the structural formula:

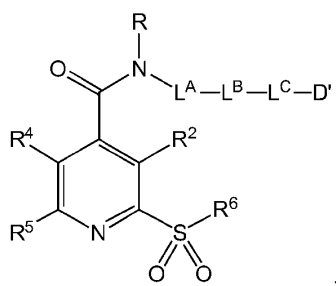


(III¹)

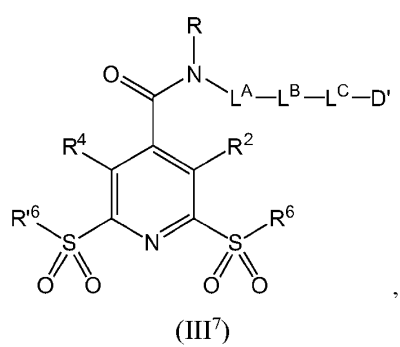
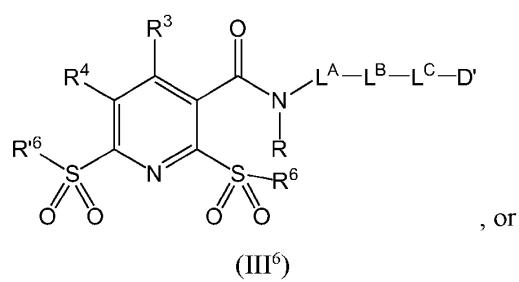
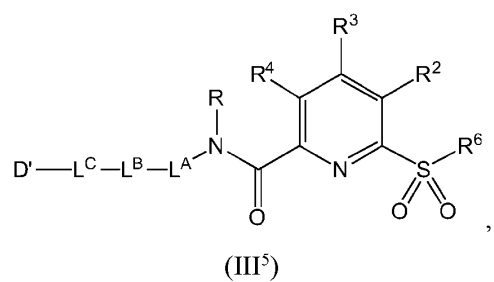
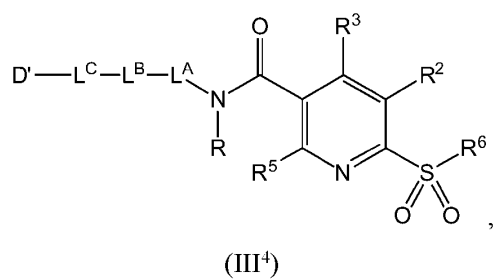
(b) reacting the compound with a precursor to a spacer, a precursor to a peptide, and/or a precursor to a self-immolative moiety, to form a conjugate having the structural formula:



(III²)



(III³)



wherein

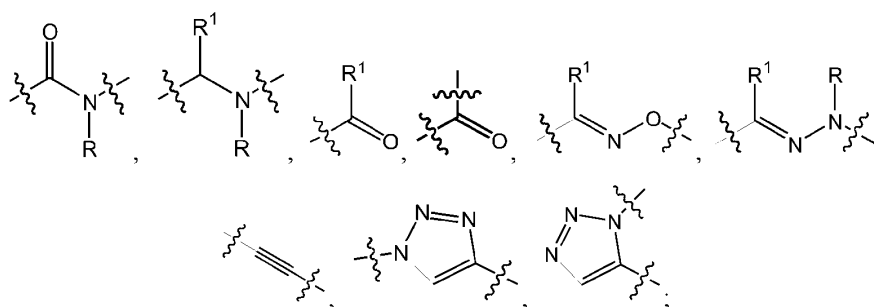
each R^2 , R^3 , R^4 is independently selected from the group consisting of H, NO_2 , CN, CF_3 , F, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne, C_{6-10} aryl, and W;

R^5 is H, Cl, SOR^6 , SO_2R^6 , OR^7 , NRR^7 or W;

R^6 is an unsubstituted or substituted C_{1-6} alkyl or C_{6-10} aryl;

R^7 is an unsubstituted or substituted C_{2-6} alkyne or C_{2-6} alkyne;

W is selected from:



R^1 is H, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne or C_{6-10} aryl;

R is H, an unsubstituted or substituted C_{1-6} alkyl, C_{2-6} alkyne, C_{2-6} alkyne or C_{6-10} aryl;

L^A is a spacer moiety or is absent;

L^B is a peptide moiety or is absent;

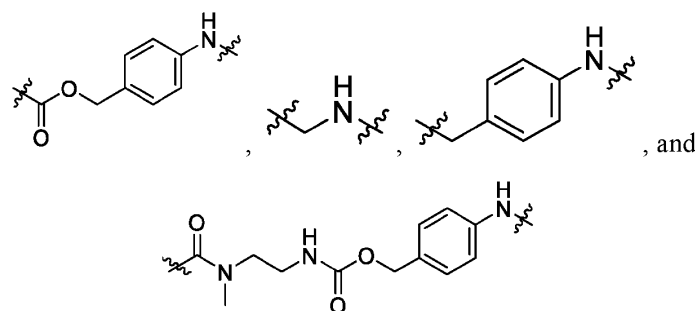
L^C is a self-immolative moiety or is absent; and

D' is a drug moiety D or is absent.

84. The method of claim 83, wherein L^A is a spacer moiety, L^B is a peptide moiety, L^C is a self-immolative moiety, and D' is a drug moiety.

85. The method of claim 83 or 84, further comprising reacting the resulting drug conjugate with a targeting moiety to form a targeting moiety-linker-drug conjugate.

86. The method of any one of claims 83-85, wherein the spacer moiety is selected from the group consisting of an alkyl, a heteroalkyl, polyethylene glycol (PEG), and a peptide.
87. The method of any one of claims 83-86, wherein the peptide moiety comprises about 1 to about 6 amino acids.
88. The method of claim 87, wherein the amino acids are natural and/or unnatural amino acids.
89. The method of any one of claims 83-88, wherein the self-immolative moiety is selected from the group consisting of:

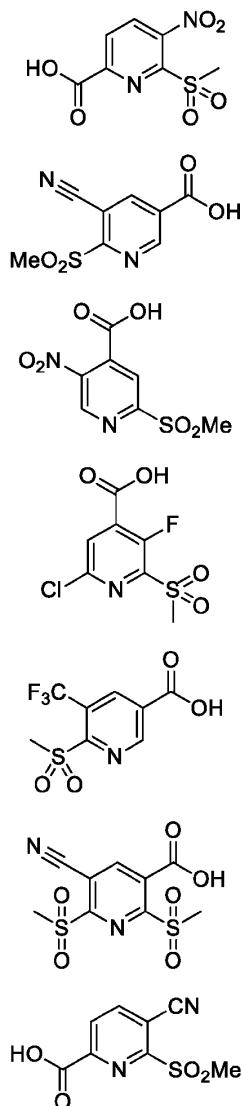


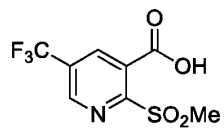
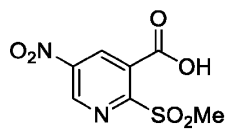
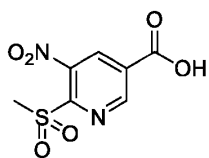
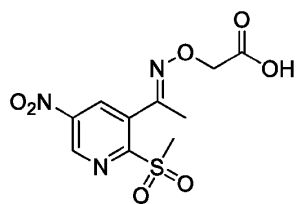
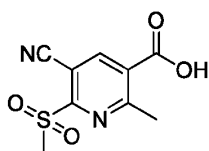
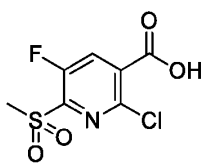
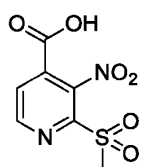
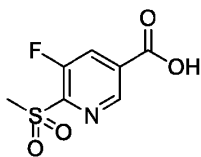
90. The method of any one of claims 83-89, wherein step (b) is conducted at a pH in the range of 5.5 to 6.5.
91. The method of any one of claims 83-89, wherein step (b) is conducted at a pH of about 6.0.
92. The method of any one of claims 83-91, wherein the drug moiety is a chemical agent selected from the group consisting of an antibiotic, an anti-cancer agent, a steroid, a TLR7/TLR9 antagonist, a peptide, a protein, and a nucleic acid.
93. The method of any one of claims 83-92, wherein the targeting moiety is selected from the group consisting of an antibody, small molecule, a peptide and a nucleic acid.

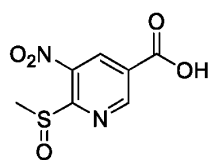
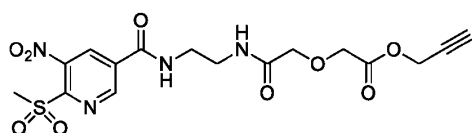
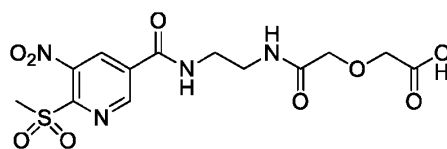
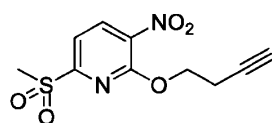
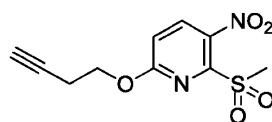
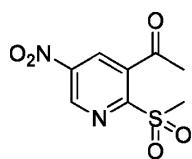
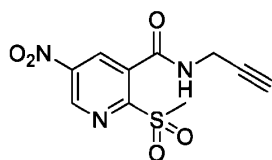
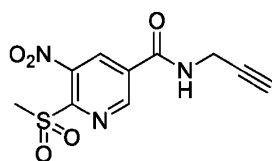
94. The method of claim 93, wherein the drug conjugate has a targeting moiety to drug moiety ratio of about 1:1 to about 1:16.
95. A method of treating and/or preventing a condition in a subject in need thereof, the method comprising administering to the subject a drug conjugate of any one of claims 1-58.
96. The method of claim 95, wherein the condition is one or more of cancer, an autoimmune disorder, or an infectious disease.
97. The method of claim 96, wherein the cancer is one or more of adrenal cancer, anal cancer, basal and squamous cell skin cancer, bile duct cancer, bladder cancer, bone cancer, brain and spinal cord tumors (astrocytoma, glioblastoma multiforme, meningioma), breast cancer, cervical cancer, colorectal cancer, endometrial cancer, esophagus cancer, Ewing family of tumors, eye cancer (ocular melanoma), gallbladder cancer, gastrointestinal neuroendocrine (carcinoid) tumors, gastrointestinal stromal tumor (gist), gestational trophoblastic disease, Kaposi sarcoma, kidney cancer, laryngeal and hypopharyngeal cancer, liver cancer, lung cancer, lung carcinoid tumor, malignant mesothelioma, melanoma skin cancer, Merkle cell skin cancer, nasal cavity and paranasal sinuses cancer, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, neoplasm of the central nervous system (CNS), oral cavity and oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, pancreatic neuroendocrine tumor (net), penile cancer, pituitary tumors, prostate cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, skin cancer, small cell lung cancer, small intestine cancer, soft tissue sarcoma, stomach cancer, testicular cancer, thymus cancer, thyroid cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenstrom macroglobulinemia, Wilms tumor, squamous cell cancer, cancers of unknown primary (CUP), environmentally induced cancers, combinations of the cancers, and/or metastatic lesions of the cancers.

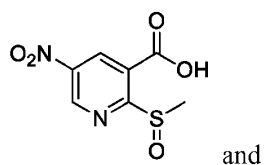
98. The method of claim 96, wherein the autoimmune disorder is one or more of Th2 lymphocyte disorders, Th1 lymphocyte disorders, activated B lymphocyte disorders, active chronic hepatitis, Addison's disease, allergic alveolitis, allergic reaction, allergic rhinitis, Alport's syndrome, anaphylaxis, ankylosing spondylitis, anti-phospholipid syndrome, arthritis, ascariasis, aspergillosis, atopic allergy, atopic dermatitis, atopic rhinitis, Behcet's Disease, Bird fancier's lung, bronchial asthma, Caplan's syndrome, cardiomyopathy, celiac disease, Chagas' Disease, chronic glomerulonephritis, Cogan's syndrome, cold agglutinin disease, congenital rubella infection, CREST Syndrome, Crohn's disease, cryoglobulinemia, Gushing's syndrome, dermatomyositis, discoid lupus, Dressler syndrome, Eaton-Lambert syndrome, echovirus infection, encephalomyelitis, endocrine ophthalmopathy, Epstein-Barr virus infection, equine heaves, erythematosis, Evans syndrome, Felty's syndrome, fibromyalgia, Fuchs heterochromatic iridocyclitis, gastric atrophy, gastrointestinal allergy, giant cell arteritis, glomerulonephritis, Goodpasture's syndrome, graft-versus-host disease, Graves' disease, Guillain-Barre disease, Hashimoto's thyroiditis, hemolytic anemia, Henoch-Schonlein purpura, idiopathic adrenal atrophy, idiopathic pulmonary fibrosis, IgA nephropathy, inflammatory bowel diseases, insulin-dependent diabetes mellitus, juvenile arthritis, juvenile diabetes mellitus (Type 1), Lambert-Eaton syndrome, laminitis, lichen planus, lupoid hepatitis, lupus, lymphopenia, Meniere's Disease, mixed connective tissue disease, multiple sclerosis, myasthenia gravis, pernicious anemia, polyglandular syndromes, presenile dementia, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Raynaud's phenomenon, recurrent abortion, Reiter's syndrome, rheumatic fever, rheumatoid arthritis, Samter's syndrome, schistosomiasis, Schmidt's syndrome, scleroderma, Shulman's syndrome, Sjogren's syndrome, Stiff-person syndrome, sympathetic ophthalmia, systemic lupus erythematosus, Takayasu's arteritis, temporal arteritis, thyroiditis, thrombocytopenia, thyrotoxicosis, toxic epidermal necrolysis, type B insulin resistance, type I diabetes mellitus, ulcerative colitis, uveitis, vitiligo, Waldenstrom macroglobulinemia, and/or granulomatosis with polyangiitis.

99. The method of claim 96, wherein the infectious disease one or more of a bacterial disease, systemic fungal disease, Rickettsial disease, parasitic disease, and/or viral disease.
100. A compound selected from the group consisting of:

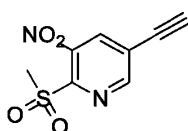




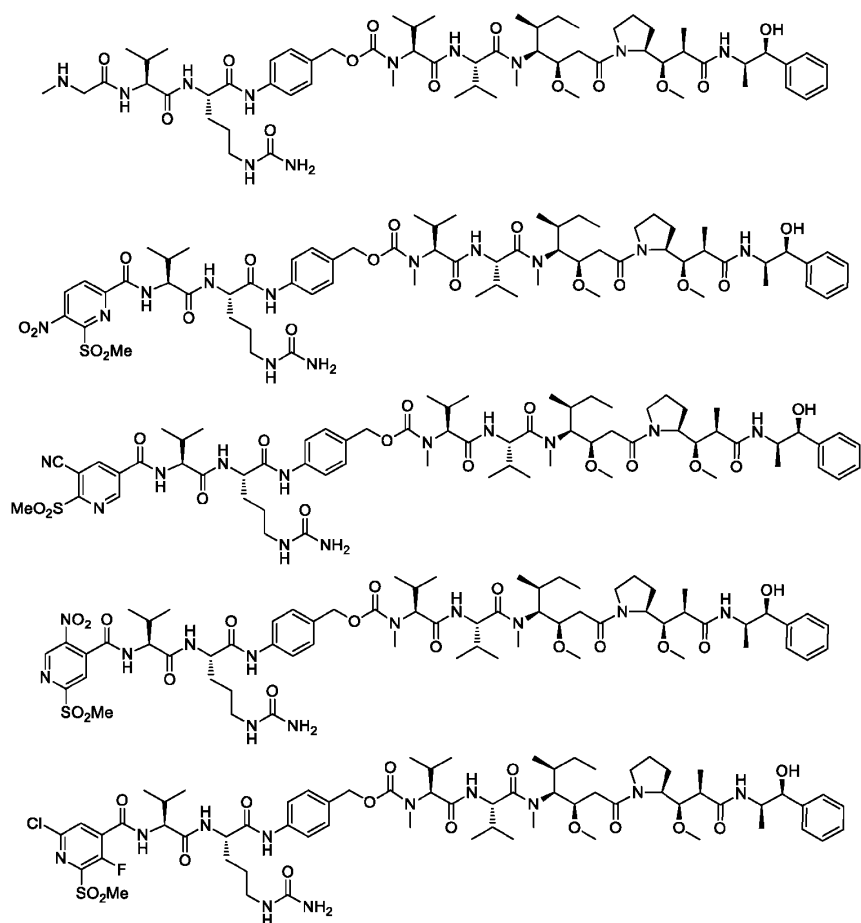


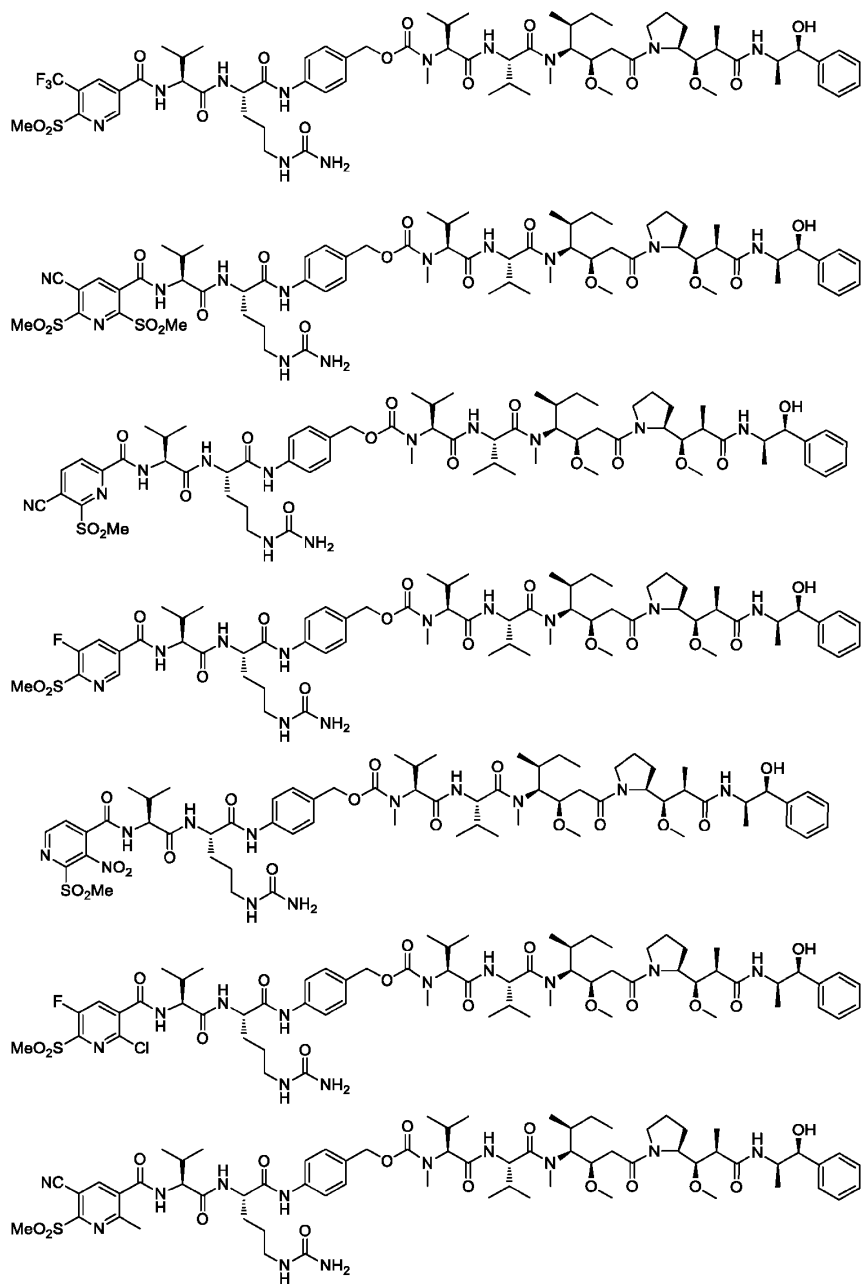


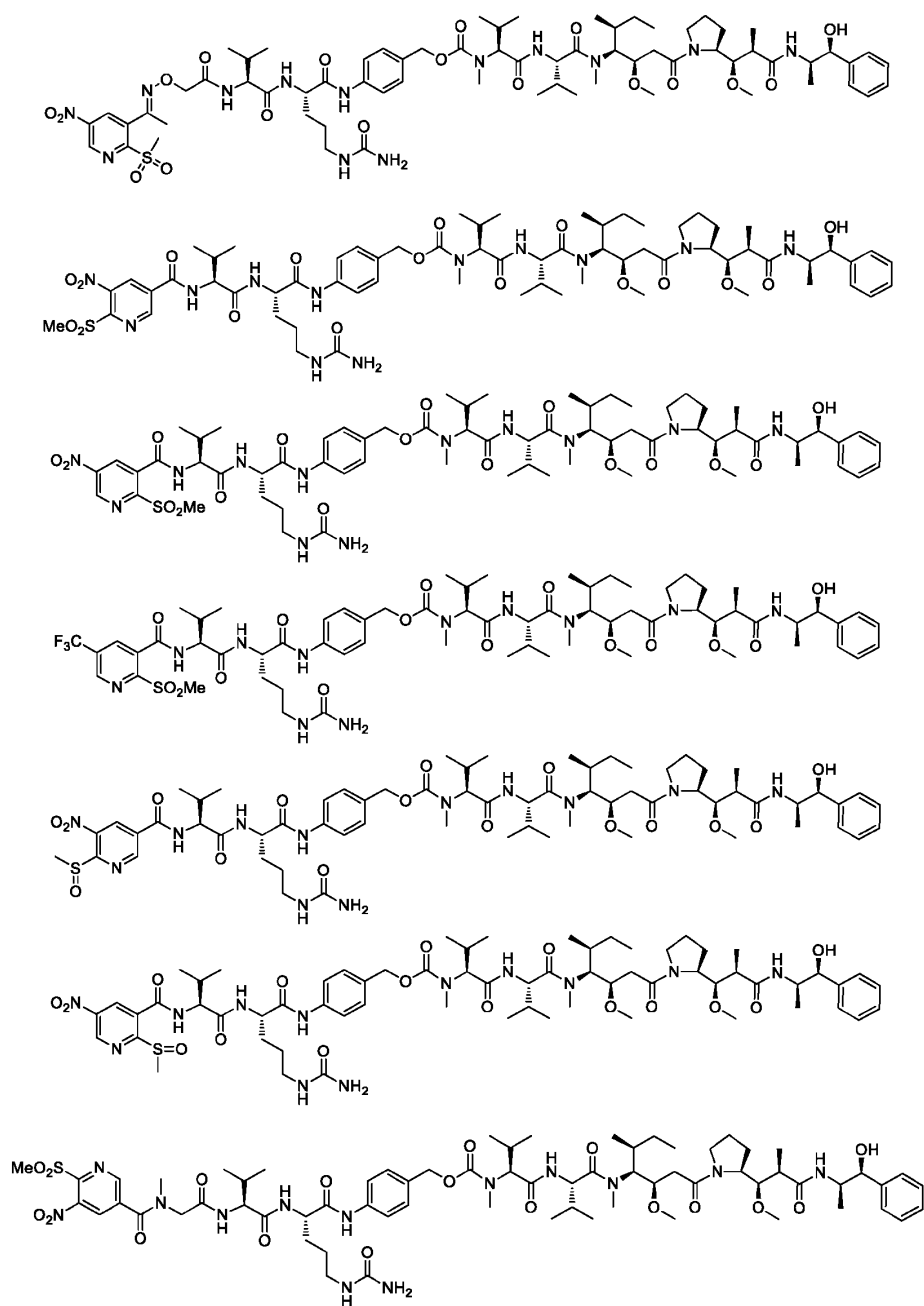
and

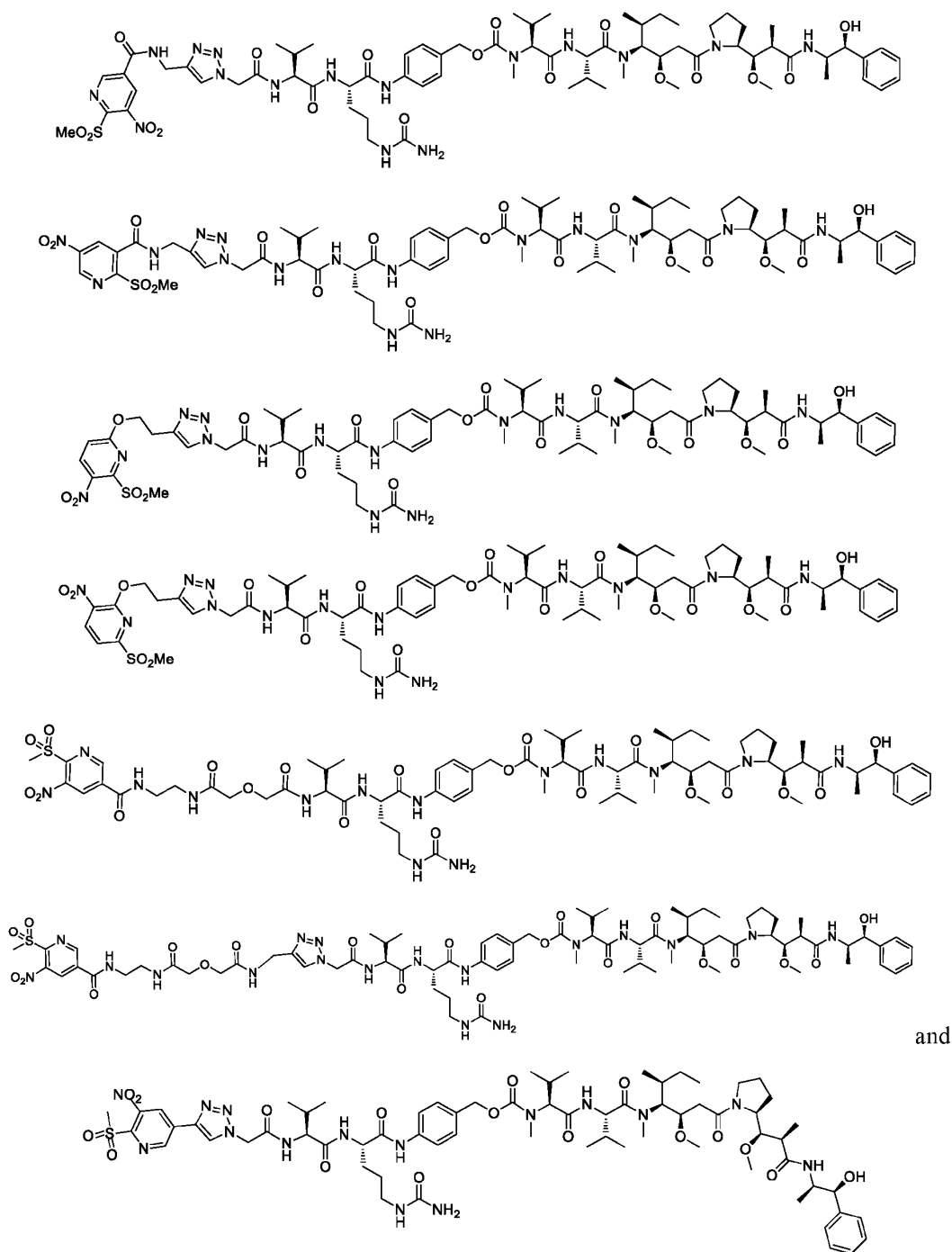


101. A compound selected from the group consisting of:









102. A composition comprising a compound of claim 100 or 101.

- 103. Use of a drug conjugate of any one of claims 1-58 for the manufacture of a medicament.
- 104. Use of a drug conjugate of any one of claims 1-58 for treating cancer.
- 105. Use of a drug conjugate of any one of claims 1-58 for treating an autoimmune disorder.
- 106. Use of a drug conjugate of any one of claims 1-58 for treating an infectious disease.