

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/CN2023/110989, filed August 3, 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 and conditions.

Background of the Invention

[0003] Drug conjugates (*e.g.*, antibody drug conjugates (ADCs) or immunoconjugates) can provide an effective means of delivering a drug to a targeted site in a tissue or organism. Thirteen 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 drug to protein attachment method.

[0004] There remains a need for drug conjugates and conjugation methodologies that feature improved efficiency, drug loading, stability and other properties.

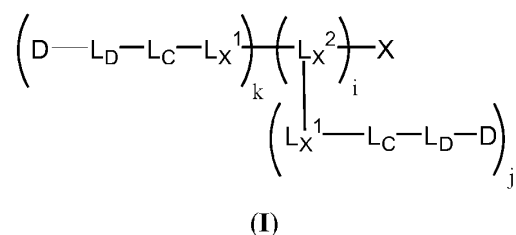
Summary of the Invention

[0005] Novel building blocks, drug-linker conjugates as well as conjugation methodologies are provided herein that enable improved conjugation efficiency, drug loading, product stability and other properties.

[0006] This present invention further provides novel immunoconjugates, methods of their preparation, kits comprising the drug conjugates and components thereof, and methods of using the drug conjugates and kits in the treatment of a disease or condition.

[0007] In particular, through novel building blocks that are modular and well-matched for click chemistry, the present invention allows significantly improved conjugation efficiency, increased drug loading, and more expansive electrophilic chemotypes and high selectivity towards thiols (*e.g.*, cysteine). A key feature of the invention, the introduction of phosphoryl choline (PC) groups on the backbone of the building scaffold or within a drug linker component, offers a convenient mechanism to efficiently adjust hydrophilicity of the building blocks and the resulting conjugates.

[0008] In one aspect, the invention generally relates to a drug-linker conjugate having the structural formula (I):



or a salt or ester thereof,

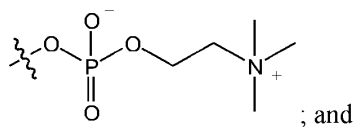
wherein

each D is independently a drug moiety;

X is a conjugation functional group capable of forming a covalent linkage with an antigen binding moiety;

each L_C is independently a moiety formed by a click chemistry reaction between an alkyne and an azide;

each of L_D , L_X^1 and L_X^2 is independently a linker or spacer moiety, wherein at least one of L_D , L_X^1 and L_X^2 comprises a PC group

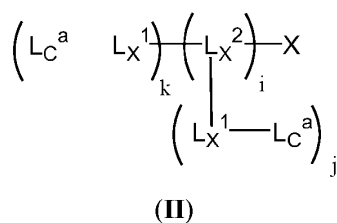


; and

i is an integer selected from 0-8; and

each of j and k is independently 0 or 1, provided that j and k are not both 0.

[0009] In another aspect, the invention generally relates to a compound having the structural formula (II):



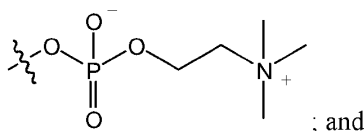
or a salt or ester thereof,

wherein

X is conjugation functional group capable of forming a covalent linkage with an antigen binding moiety;

each L_C^a is independently an alkyne or an azide group;

each of L_X^1 and L_X^2 is independently a linker or spacer moiety, wherein at least one of L_X^1 and L_X^2 comprises a PC group

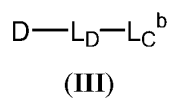


; and

i is an integer selected from 0-8; and

each of j and k is independently 0 or 1, provided that j and k are not both 0.

[0010] In yet another aspect, the invention generally relates to a compound having the structural formula (III):



or a salt or ester thereof,

wherein

D is a drug moiety;

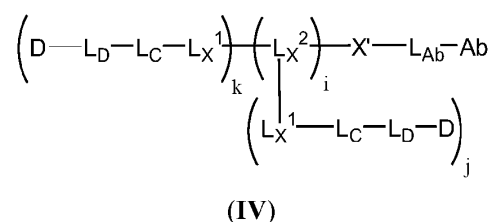
L_D is a linker or spacer moiety comprising a PC group; and

L_C^b is an alkyne or an azide group.

[0011] In yet another aspect, the invention generally relates to an immunoconjugate comprising a drug-linker conjugate or a building block disclosed herein, or a moiety or derivative thereof.

[0012] In yet another aspect, the invention generally relates to an immunoconjugate prepared from a drug-linker conjugate or a building block disclosed herein, or a moiety or derivative thereof.

[0013] In yet another aspect the invention generally relates to an immunoconjugate having the structural formula (IV):



or a pharmaceutically acceptable salt thereof,

wherein

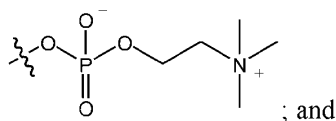
Ab represents an antigen binding moiety;

each D is independently a drug moiety;

X' is a single bond or a conjugation group;

each L_C is independently a moiety formed by a click chemistry reaction between an alkyne and an azide;

each of L_{Ab} , L_D , L_X^1 and L_X^2 is independently a linker or spacer moiety, wherein at least one of L_D , L_X^1 and L_X^2 comprises a PC group



i is an integer selected from 0-8; and

each of j and k is independently 0 or 1, provided that j and k are not both 0.

[0014] In yet another aspect, the invention generally relates to a pharmaceutical composition comprising an immunoconjugate disclosed herein and a pharmaceutically acceptable excipient, carrier or diluent.

[0015] In yet another aspect, the invention generally relates to a combination comprising a therapeutically effective amount of an immunoconjugate disclosed herein, and one or more therapeutically active co-agent(s) and/or adjuvant(s).

[0016] In yet another aspect, the invention generally relates to a composition comprising a compound disclosed herein that is useful for preparing a linker moiety-drug moiety conjugate, a targeting moiety-linker moiety conjugate, or a targeting moiety-linker moiety-drug moiety conjugate.

[0017] 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 therapeutically effective amount of an immunoconjugate of the invention.

[0018] In yet another aspect, the invention generally relates to a method of preparing a linker-drug conjugate under suitable conditions for click chemistry.

[0019] In yet another aspect, the invention generally relates to use of a compound or an immunoconjugate disclosed herein for the manufacture of a medicament.

Detailed Description of the Invention

[0020] As set forth herein, novel linkers, linking methodologies and conjugates, and drug conjugates have been developed that possess unexpected advantages over prior art.

[0021] More particularly, linkers, linker conjugates and conjugation strategies disclosed herein enable significantly increased drug loading with improved efficiency through modular building blocks and click chemistry. The disclosed invention helps to expand available cysteine-targeted electrophilic chemotypes with high selectivity towards thiols (*e.g.*, cysteine) over other reactive functionalities such as amines. The invention affords efficient adjustment of hydrophilicity through the introduction of PC groups on the backbone of the building scaffold or within a drug linker component. The drug-linker conjugates of the invention show overall good stability at room temperature.

[0022] This disclosure further provides drug conjugates, methods of their preparation, kits comprising these drug conjugates and components thereof, and methods of using the drug conjugates and kits in the treatment of a disease or condition.

Definitions

[0023] 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.

[0024] The following terms, unless indicated otherwise according to the context wherein the terms are found, are intended to have the following meanings.

[0025] 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.

[0026] As used herein, "at least" a specific value is understood to be that value and all values greater than that value.

[0027] In this specification and the appended claims, the singular forms "a," "an," and "the" include plural reference, unless the context clearly dictates otherwise.

[0028] Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive.

[0029] Any compositions or methods disclosed herein can be combined with one or more of any of the other compositions and methods provided herein.

[0030] 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.

[0031] 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.

[0032] 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.

[0033] 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.

[0034] 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.

[0035] 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.

[0036] 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.

[0037] 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)-.

[0038] 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).

[0039] 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.

[0040] 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.

[0041] 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.

[0042] 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.

[0043] 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.

[0044] 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.

[0045] 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.

[0046] 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.

[0047] 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.

[0048] The term “infectious disease” may refer to any disease caused by an infectious organism such as a virus, bacteria, parasite, and/or fungus.

[0049] 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.

[0050] The term “alkyl” describes an aliphatic hydrocarbon including straight chain and branched chain groups.

[0051] 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.

[0052] 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.

[0053] 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.

[0054] 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.

[0055] 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.

[0056] 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.

[0057] 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.

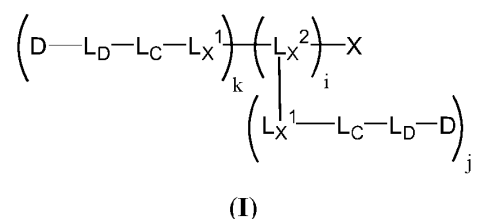
[0058] As used herein, the term “subject” refers to any animal (*e.g.*, a mammal), including, but not limited to humans, non-human primates, rodents, and the like, which is to be the recipient of a particular treatment. A subject to which administration is contemplated includes, but is not limited to, 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.

[0059] 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

[0060] Provided herein in certain embodiments are drug conjugates comprising a linker, a drug moiety, and a targeting moiety. Also provided herein are components or building blocks of these drug conjugates, including for example linkers, linker-drug moiety complexes, and linker-targeting moiety complexes.

[0061] In one aspect, the invention generally relates to a drug-linker conjugate having the structural formula (I):



or a salt or ester thereof,

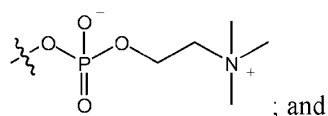
wherein

each D is independently a drug moiety;

X is a conjugation functional group capable of forming a covalent linkage with an antigen binding moiety;

each L_C is independently a moiety formed by a click chemistry reaction between an alkyne and an azide;

each of L_D, L_X¹ and L_X² is independently a linker or spacer moiety, wherein at least one of L_D, L_X¹ and L_X² comprises a PC group



i is an integer selected from 0-8 (e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8); and

each of *j* and *k* is independently 0 or 1, provided that *j* and *k* are not both 0.

[0062] In certain embodiments of (I), *j* is 0.

[0063] In certain embodiments of (I), *j* is 1.

[0064] In certain embodiments of (I), *k* is 1.

[0065] In certain embodiments of (I), *j* is 1 and *k* is 1.

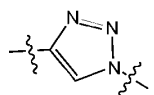
[0066] In certain embodiments of (I), *i* is 0.

[0067] In certain embodiments of (I), *i* is 1.

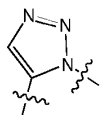
[0068] In certain embodiments of (I), *i* is 2.

[0069] In certain embodiments of (I), *i* is greater than 2.

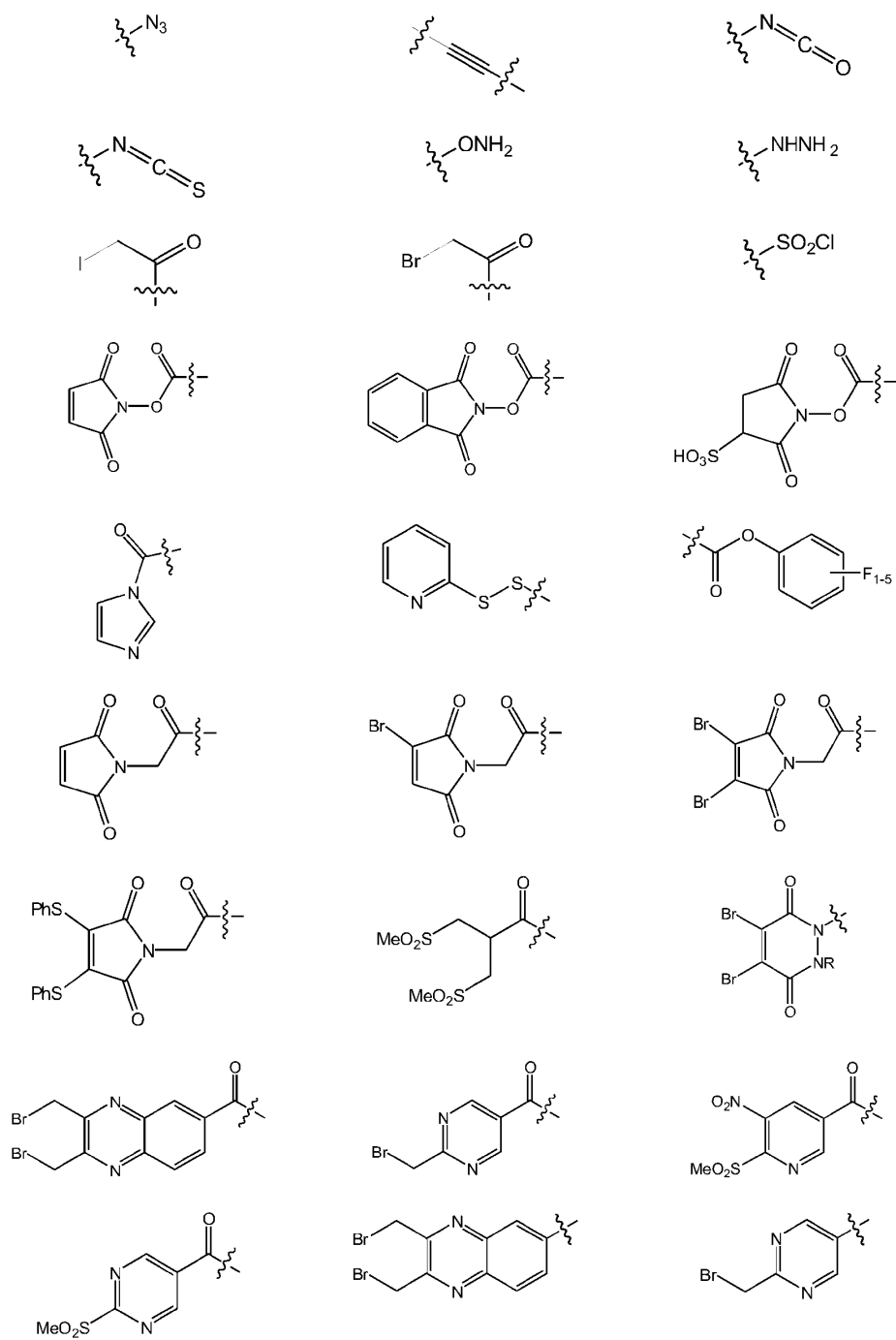
[0070] In certain embodiments of (I), L_C is

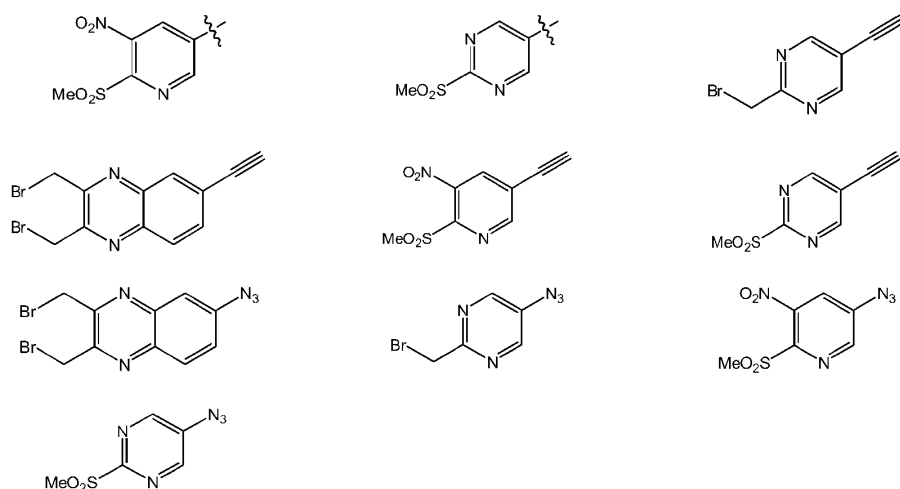


[0071] In certain embodiments of (I), L_C is



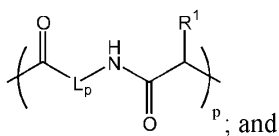
[0072] In certain embodiments of (I), X comprises a group selected from:



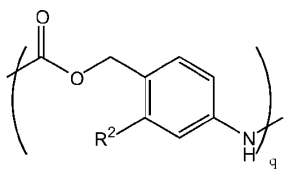


[0073] In certain embodiments of (I), $-\text{L}_\text{D}-$ is $-\text{L}_\text{D}^2-\text{L}_\text{D}^1-$, wherein

L_D^1 is



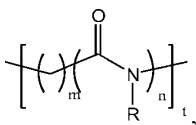
L_D^2 is



wherein

L_p is a di-, tri- or tetra peptide linker;

each of R^1 and R^2 is H, C_{1-3} alkyl, or $\text{L}_\text{PC}-(\text{PC})$, wherein L_PC is



each p and q is independently 0 or 1, provided that p and q are not both 0;

R is H or C_{1-3} alkyl;

m is 0, 1, 2 or 3;

n is 0 or 1, and

t is 0 or 1.

[0074] In certain embodiments, L_p is a di-peptide.

[0075] In certain embodiments, L_p is tri-peptide.

[0076] In certain embodiments, L_p is tetra peptide.

[0077] In certain embodiments, at least one of R^1 and R^2 is $L_{PC}-(PC)$.

[0078] In certain embodiments, each of R^1 and R^2 is $L_{PC}-(PC)$.

[0079] In certain embodiments, each R in L_{PC} is H.

[0080] In certain embodiments, p is 0 and q is 1.

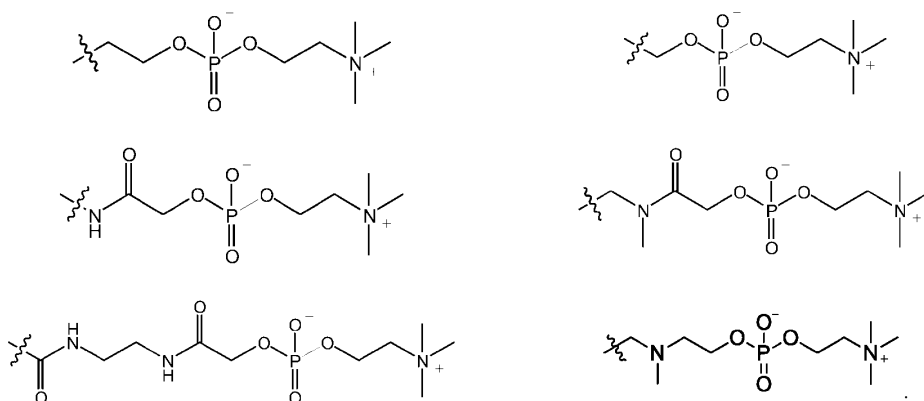
[0081] In certain embodiments, p is 1 and q is 0.

[0082] In certain embodiments, each of p and q is 1.

[0083] In certain embodiments, each of m and n is 1.

[0084] In certain embodiments, one of m and n is 0.

[0085] In certain embodiments, $L_{PC}-(PC)$ is selected from:

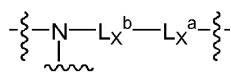


[0086] In certain embodiments of (I), L_X^1 is $(CH_2)_g$, wherein g is 0.

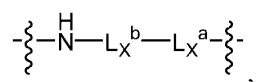
[0087] In certain embodiments of (I), L_X^1 is $(CH_2)_g$, wherein g is 1.

[0088] In certain embodiments of (I), L_X^1 is $(CH_2)_g$, wherein g is 2.

[0089] In certain embodiments of (I), L_X^2 is:



or

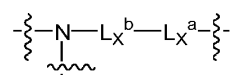


wherein

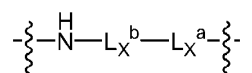
L_X^a is a substituted or unsubstituted C_{1-6} alkyl or a PEG group;

L_X^b is $-\text{C}(=\text{O})-$, CH or CH_2 .

[0090] In certain embodiments, L_X^2 is:



[0091] In certain embodiments, L_X^2 is:



[0092] In certain embodiments, L_X^a comprises a PEG group.

[0093] In certain embodiments, L_X^a comprises a C_{1-6} alkyl group.

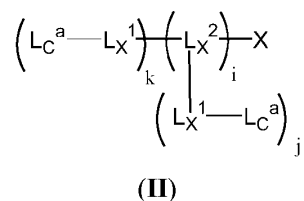
[0094] In certain embodiments, L_X^b is $-\text{C}(=\text{O})-$.

[0095] In certain embodiments, L_X^b is CH_2 .

[0096] In certain embodiments of (I), at least one of L_D , L_X^1 and L_X^2 comprises a spacer moiety selected from the group consisting of an alkyl, a heteroalkyl, polyethylene glycol (PEG), and a peptide. In certain embodiments, the spacer moiety is an alkyl group. In certain embodiments, the spacer moiety is a PEG. In certain embodiments, the spacer moiety is a peptide.

[0097] In certain embodiments of (I), a drug-linker conjugate of the invention comprises 1, 2, 3, 4, 5, 6, 7, 8 or more PC groups.

[0098] In another aspect, the invention generally relates to a compound having the structural formula (II):



or a salt or ester thereof,

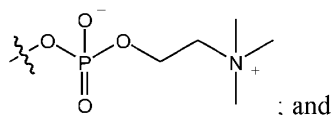
wherein

X is conjugation functional group capable of forming a covalent linkage with an antigen

binding moiety;

each L_C^a is independently an alkyne or an azide group;

each of L_X^1 and L_X^2 is independently a linker or spacer moiety, wherein at least one of L_X^1 and L_X^2 comprises a phosphoryl choline (PC) group



; and

i is an integer selected from 0-8 (*e.g.*, 0, 1, 2, 3, 4, 5, 6, 7, 8); and

each of j and k is independently 0 or 1, provided that j and k are not both 0.

[0099] In certain embodiments of (II), j is 0.

[0100] In certain embodiments of (II), j is 1.

[0101] In certain embodiments of (II), k is 1.

[0102] In certain embodiments of (II), j is 1 and k is 1.

[0103] In certain embodiments of (II), i is 0.

[0104] In certain embodiments of (II), i is 1.

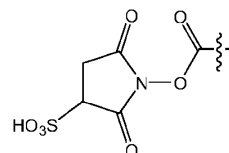
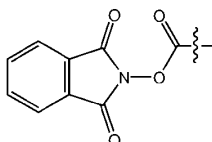
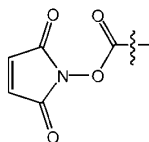
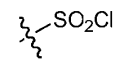
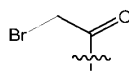
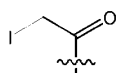
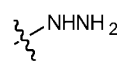
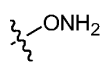
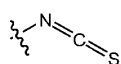
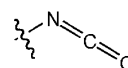
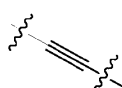
[0105] In certain embodiments of (II), i is 2.

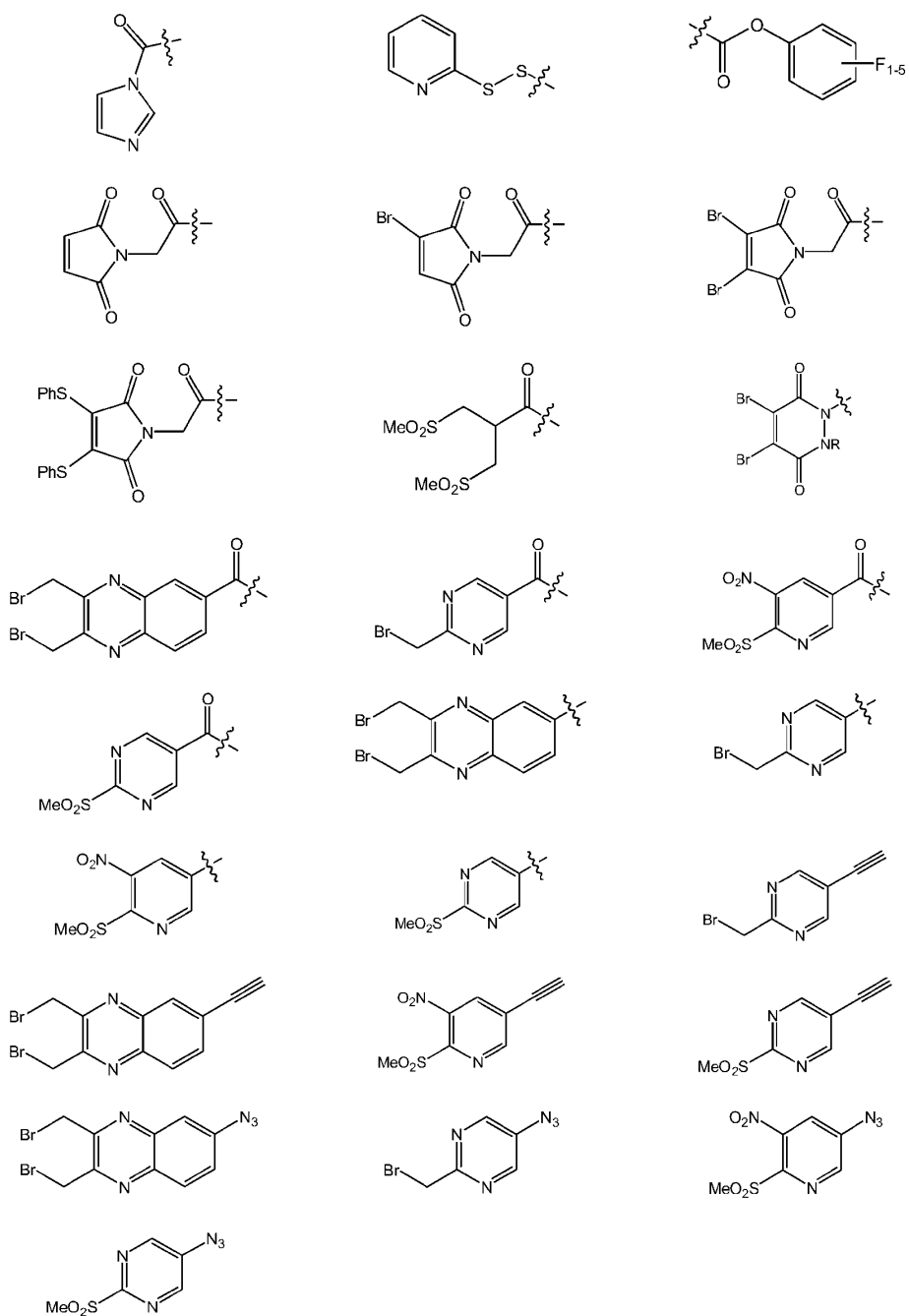
[0106] In certain embodiments of (II), i is greater than 2.

[0107] In certain embodiments of (II), each L_C^a is an alkyne group.

[0108] In certain embodiments of (II), each L_C^a is an azide group.

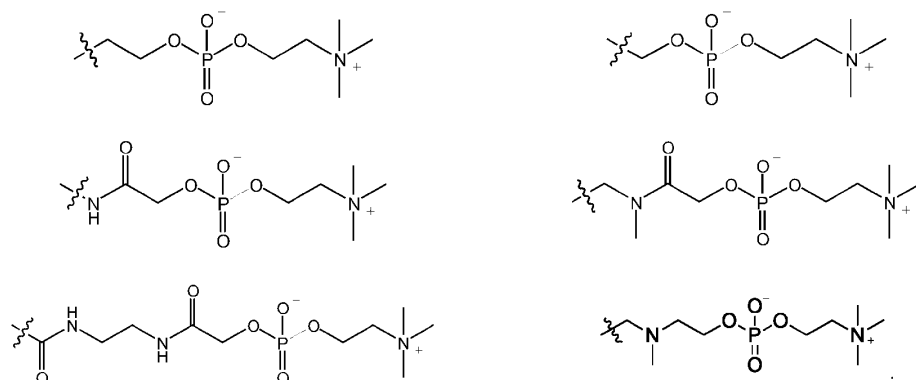
[0109] In certain embodiments of (II), X comprise a group selected from:





[0110] In certain embodiments of (II), each of L_X^1 and L_X^2 comprises $L_{PC}-(PC)$.

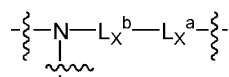
[0111] In certain embodiments of (II), $L_{PC}-(PC)$ is selected from:



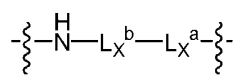
[0112] In certain embodiments of (II), L_X^1 is $(CH_2)_g$, wherein g is 1.

[0113] In certain embodiments of (II), L_X^1 is $(CH_2)_g$, wherein g is 2.

[0114] In certain embodiments of (II), L_X^2 is:



or

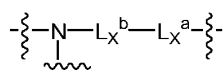


wherein

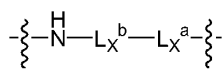
L_X^a is a substituted or unsubstituted C_{1-6} alkyl or a PEG group;

L_X^b is $-C(=O)-$, CH or CH_2 .

[0115] In certain embodiments, L_X^2 is:



[0116] In certain embodiments, L_X^2 is:



[0117] In certain embodiments, L_X^a comprises a PEG group.

[0118] In certain embodiments, L_X^a comprises a C_{1-6} alkyl group.

[0119] In certain embodiments, L_X^b is $-C(=O)-$.

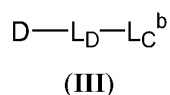
[0120] In certain embodiments, L_X^b is CH_2 .

[0121] In certain embodiments of (II), at least one of L_X^1 and L_X^2 comprises a spacer moiety selected from the group consisting of an alkyl, a heteroalkyl, polyethylene glycol (PEG), and a

peptide. In certain embodiments, the spacer moiety is an alkyl group. In certain embodiments, the spacer moiety is a PEG. In certain embodiments, the spacer moiety is a peptide.

[0122] In certain embodiments, a compound of (II) comprises 1, 2, 3, 4, 5, 6, 7, 8 or more PC groups.

[0123] In yet another aspect, the invention generally relates to a compound having the structural formula (III):



or a salt or ester thereof,

wherein

D is a drug moiety;

L_D is a linker or spacer moiety comprising a PC group; and

L_C^b is an alkyne or an azide group.

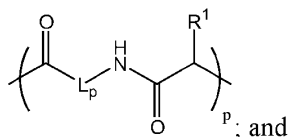
[0124] In certain embodiments of (III), L_C^b is an alkyne group.

[0125] In certain embodiments of (III), L_C^b is an azide group.

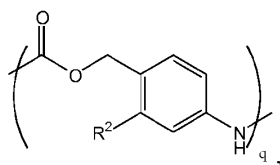
[0126] In certain embodiments of (III), -L_D- is -L_D²-L_D¹-,

wherein

L_D¹ is



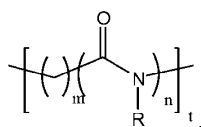
L_D² is



wherein

L_p is a di-, tri- or tetra peptide linker;

each of R¹ and R² is H, C₁₋₃ alkyl, or L_{PC}-(PC), wherein L_{PC} is



each p and q is independently 0 or 1, provided that p and q are not both 0;

R is H or C₁₋₃ alkyl;

m is 0, 1, 2 or 3;

n is 0 or 1, and

t is 0 or 1.

[0127] In certain embodiments, L_p is a di-peptide.

[0128] In certain embodiments, L_p is tri-peptide.

[0129] In certain embodiments, L_p is tetra peptide.

[0130] In certain embodiments, at least one of R¹ and R² is L_{PC}-(PC).

[0131] In certain embodiments, each of R¹ and R² is L_{PC}-(PC).

[0132] In certain embodiments, each R in L_{PC} is H.

[0133] In certain embodiments, p is 0 and q is 1.

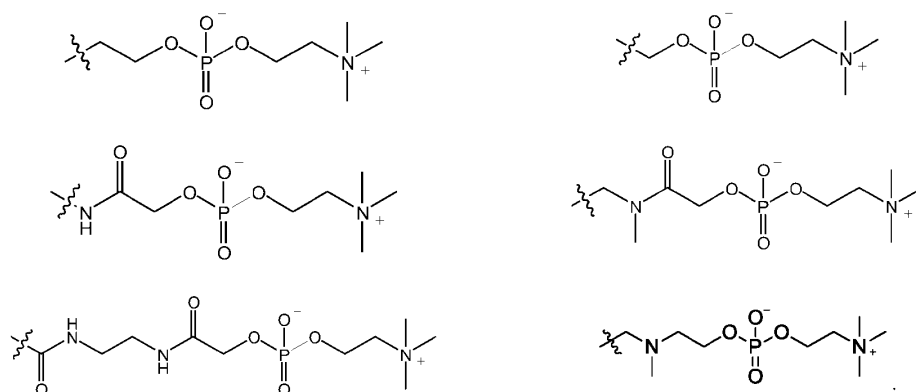
[0134] In certain embodiments, p is 1 and q is 0.

[0135] In certain embodiments, each of p and q is 1.

[0136] In certain embodiments, each of m and n is 1.

[0137] In certain embodiments, one of m and n is 0.

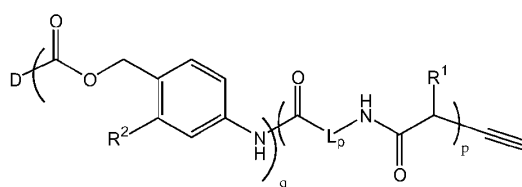
[0138] In certain embodiments, L_{PC}-(PC) is selected from:



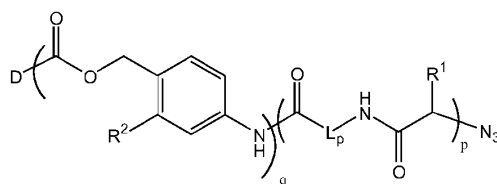
[0139] In certain embodiments of (I), at least one of L_D¹ and L_D² comprises a spacer moiety selected from the group consisting of an alkyl, a heteroalkyl, polyethylene glycol (PEG), and a

peptide. In certain embodiments, the spacer moiety is an alkyl group. In certain embodiments, the spacer moiety is a PEG. In certain embodiments, the spacer moiety is a peptide.

[0140] In yet another aspect, the invention generally relates to a compound having the structural formula (III^a) or (III^b):

(III^a)

or

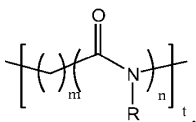
(III^b)

wherein

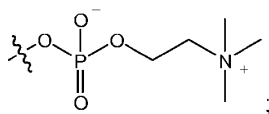
D is a drug moiety;

L_p is a di-, tri- or tetra peptide linker;

each of R¹ and R² is H, C₁₋₃ alkyl, or L_{PC}-(PC), wherein L_{PC} is



PC is phosphoryl choline group:



each *p* and *q* is independently 0 or 1, provided that *p* and *q* are not both 0;

R is H or C₁₋₃ alkyl;

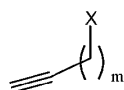
m is 0, 1, 2 or 3;

n is 0 or 1, and

t is 0 or 1.

[0141] Exemplary embodiments of (III^a) and (III^b) include those discussed in connection with (III).

[0142] In yet another aspect, the invention generally relates to a compound having the structural formula (III^c) or (III^d):

(III^c)

or

(III^d)

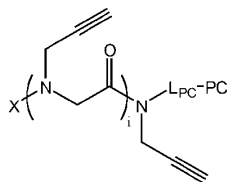
wherein

X is a conjugation functional group capable of forming a covalent linkage with an antigen binding moiety; and

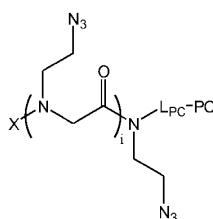
m is 1, 2, 3 or 4.

[0143] Exemplary embodiments of X in (III^c) or (III^d) include those discussed herein in connection with (I) and (II).

[0144] In yet another aspect, the invention generally relates to a compound having the structural formula (III^e) or (III^f):

(III^e)

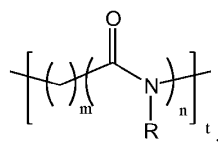
or

(III^f)

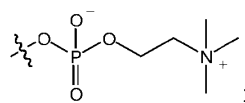
wherein

X is a conjugation functional group capable of forming a covalent linkage with an antigen binding moiety;

L_{PC} is



PC is phosphoryl choline group:



R is H or C₁₋₃ alkyl;

i is an integer selected from 0-8;

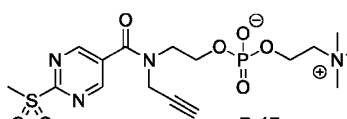
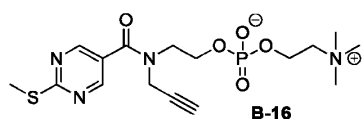
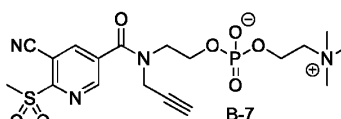
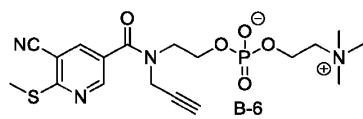
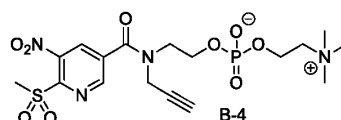
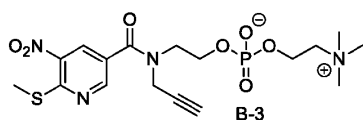
m is 0, 1, 2 or 3;

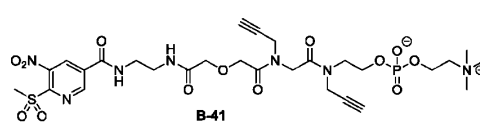
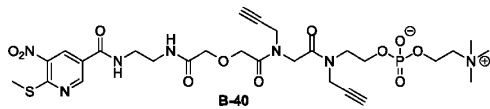
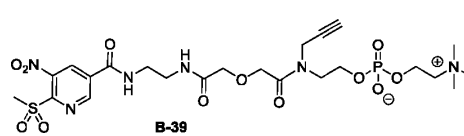
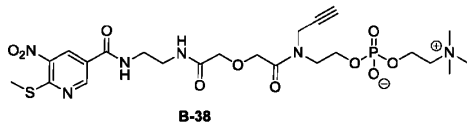
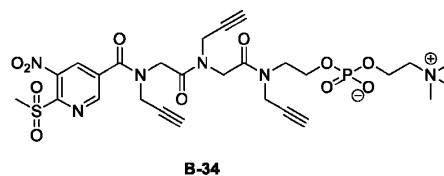
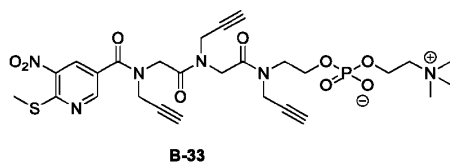
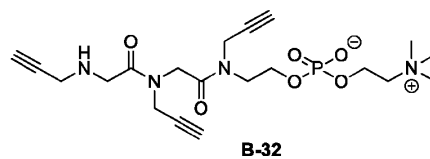
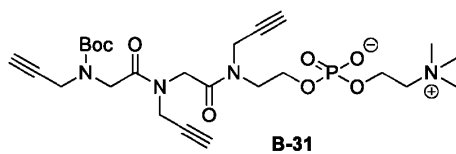
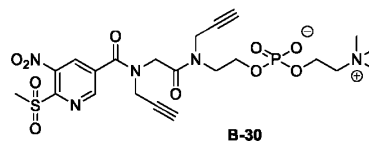
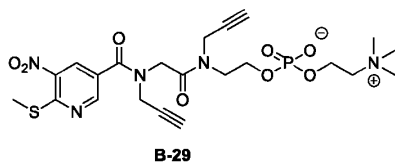
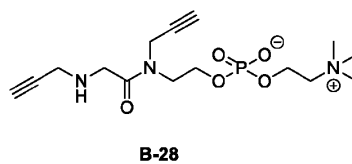
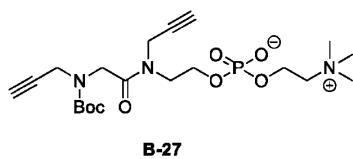
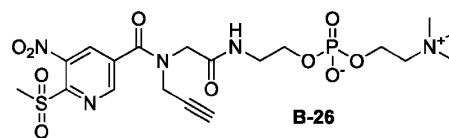
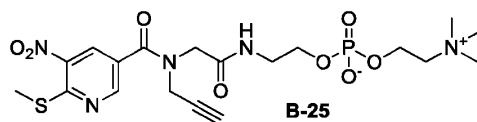
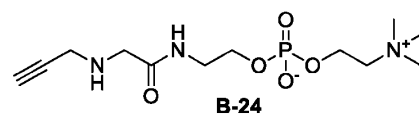
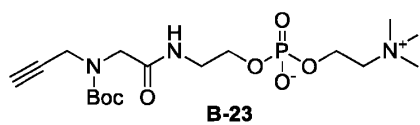
n is 0 or 1, and

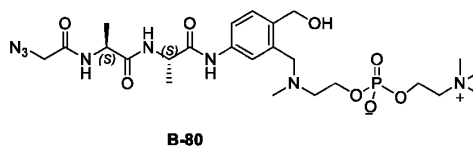
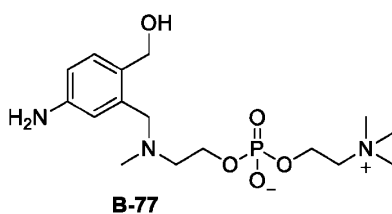
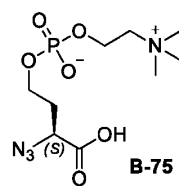
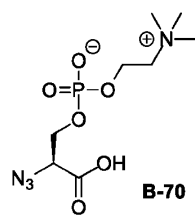
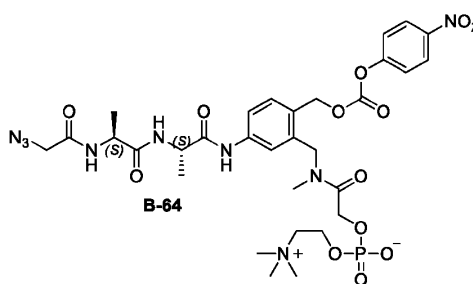
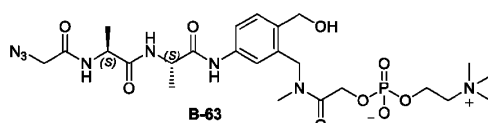
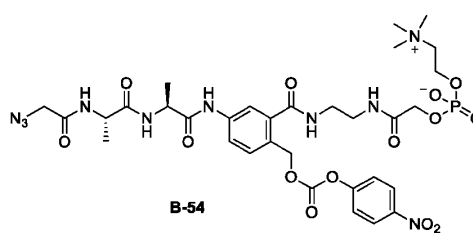
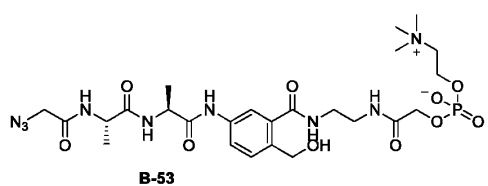
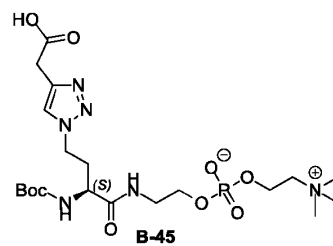
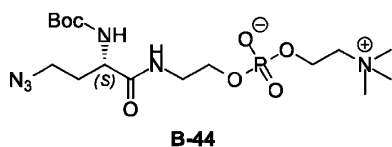
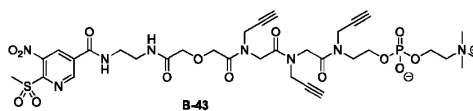
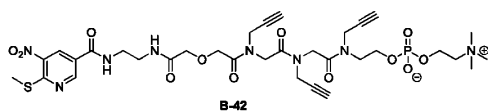
t is 0 or 1.

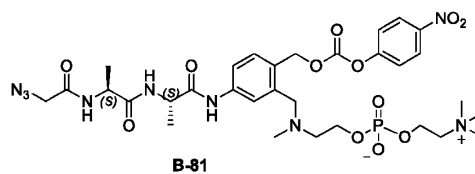
[0145] Exemplary embodiments of X and L_{PC}-(PC) in (III^e) or (III^f) include those discussed herein in connection with (I) and (II).

[0146] Non-limiting examples of compounds (*e.g.*, building blocks for ADCs) of the invention include:

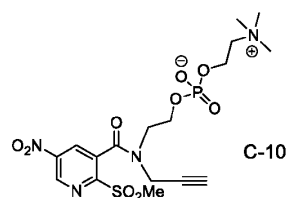
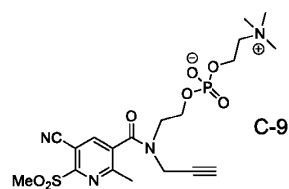
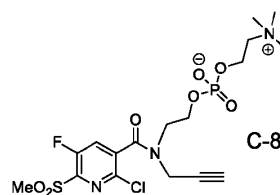
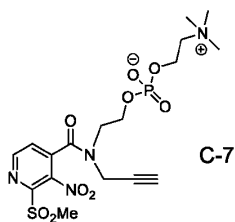
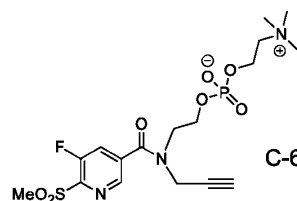
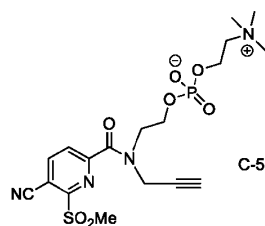
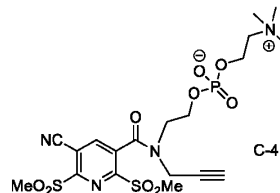
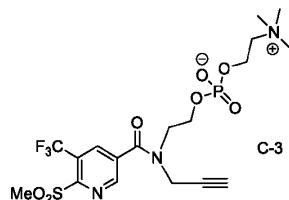
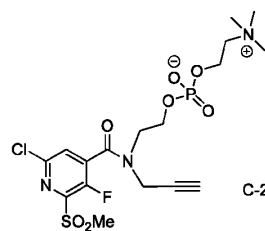
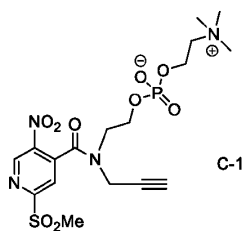


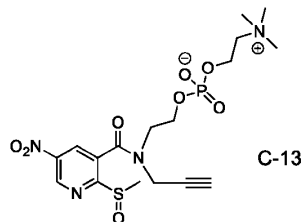
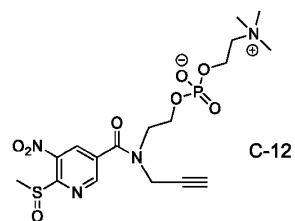
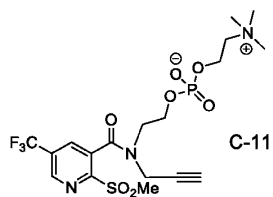




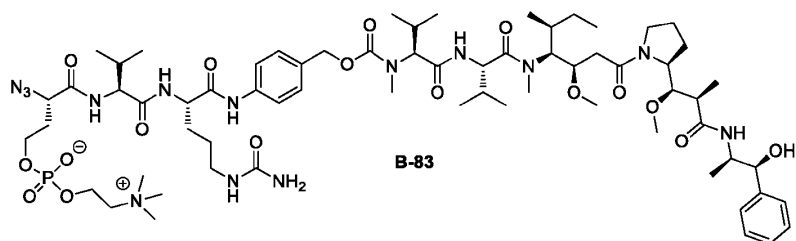
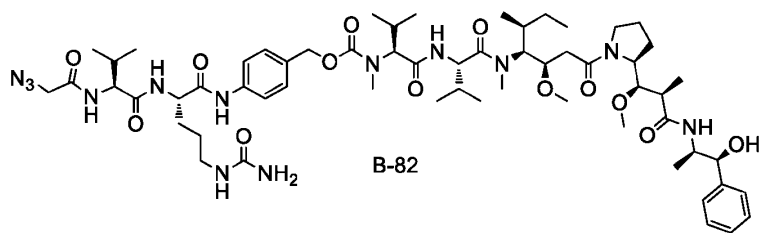


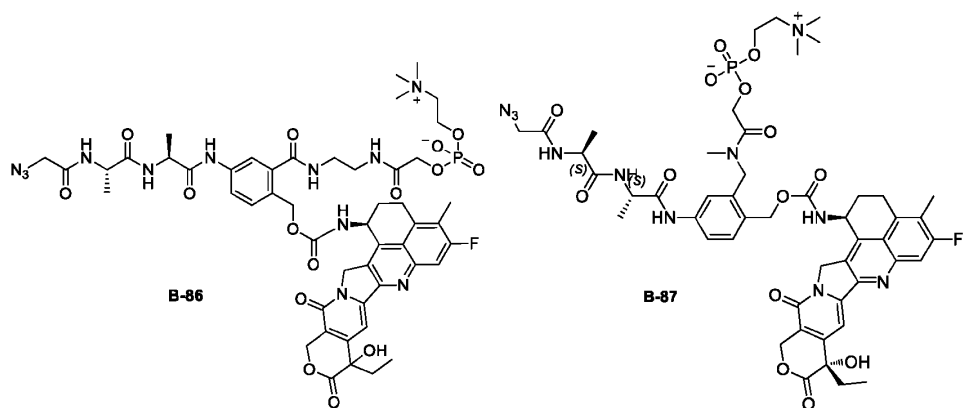
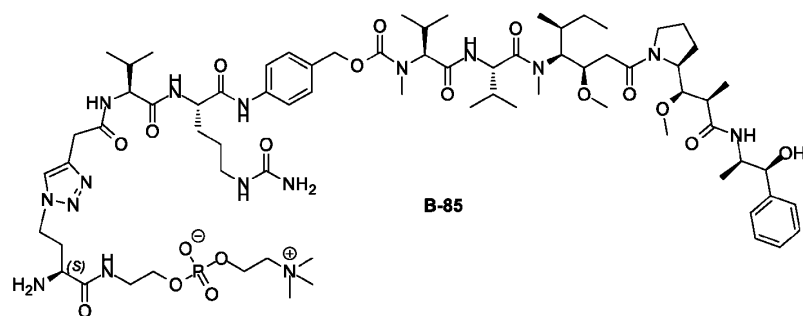
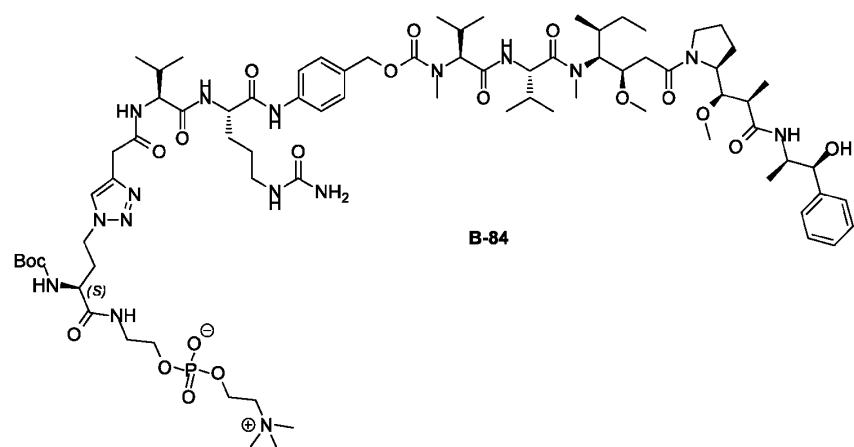
[0147] Non-limiting examples of compounds (*e.g.*, building blocks for ADCs) of the invention additionally include:

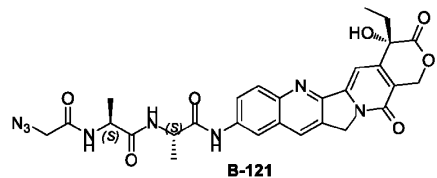
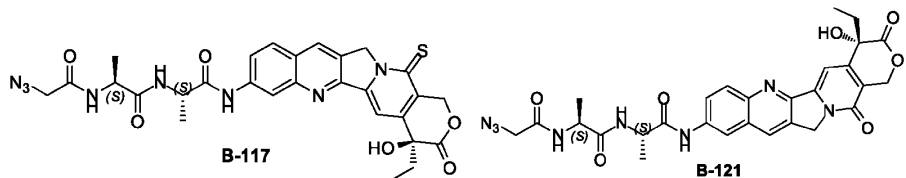
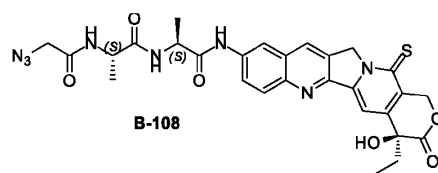
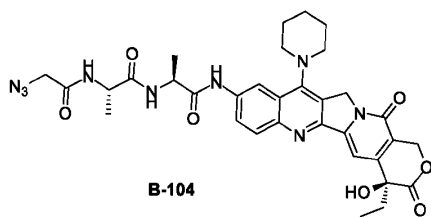
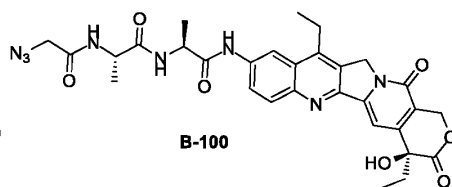
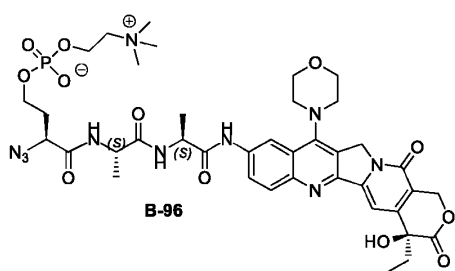
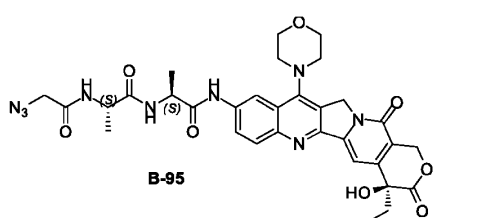
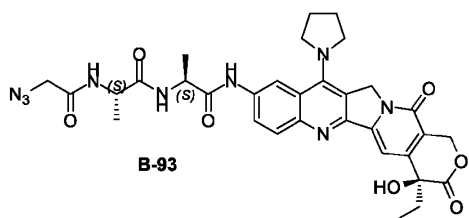
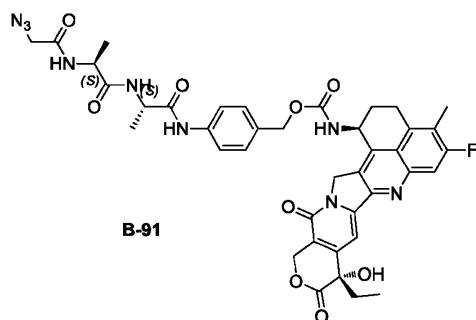
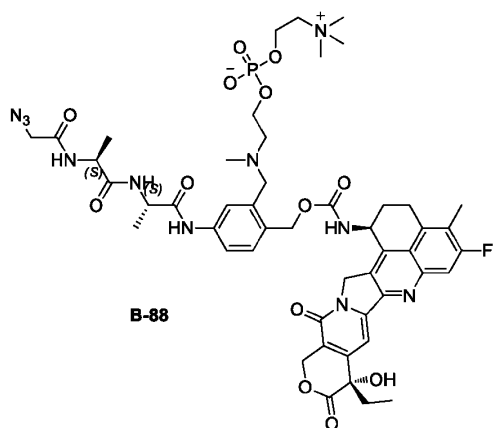




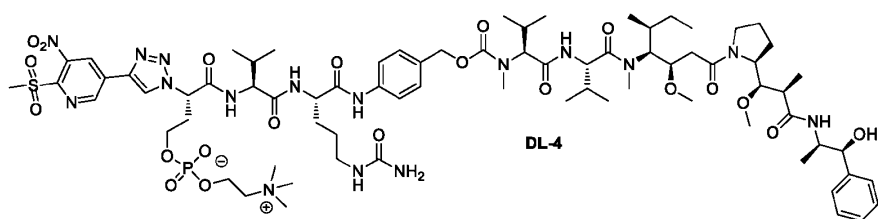
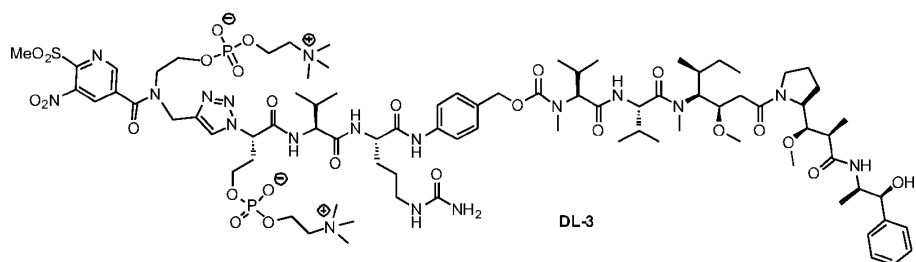
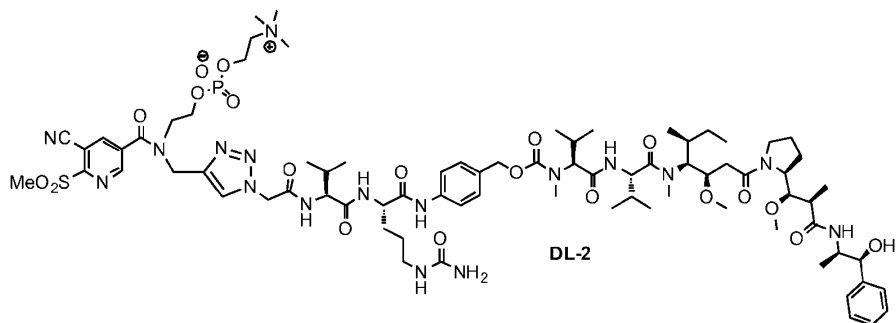
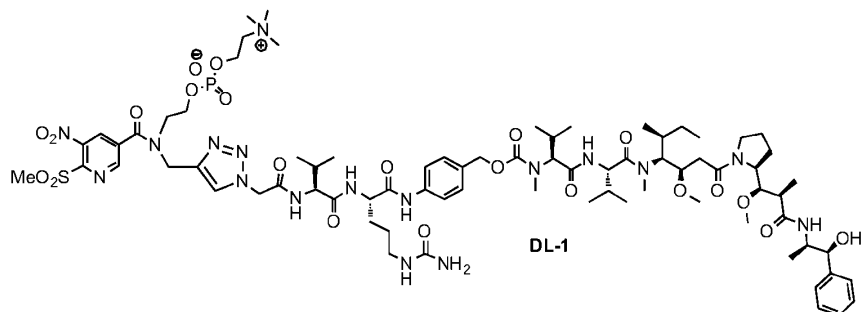
[0148] Non-limiting examples of compounds (*e.g.*, building blocks for ADCs) of the invention additionally include:

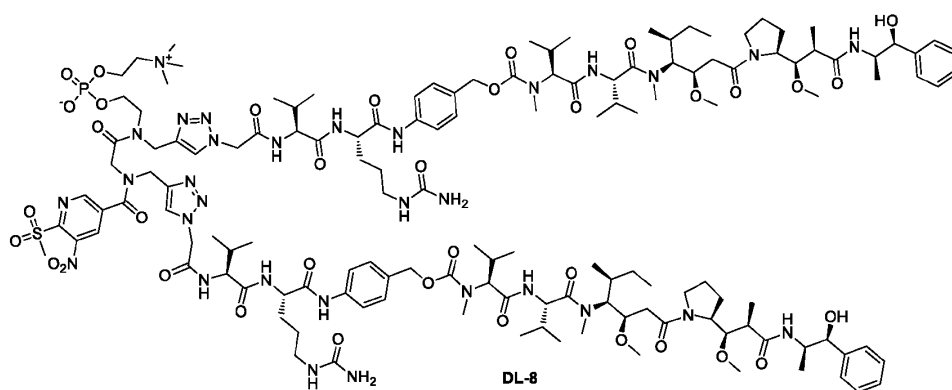
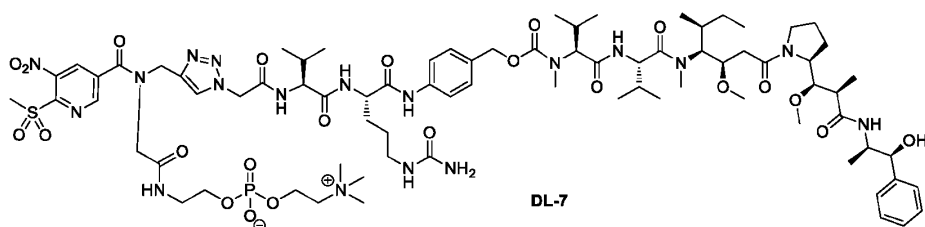
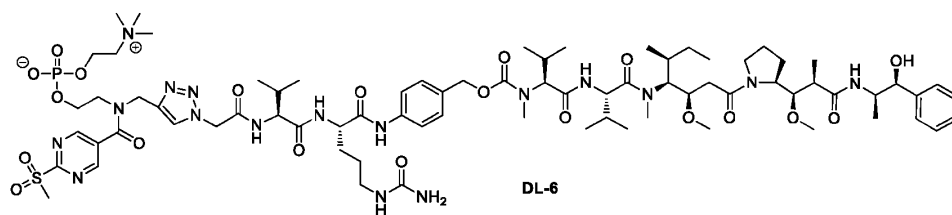
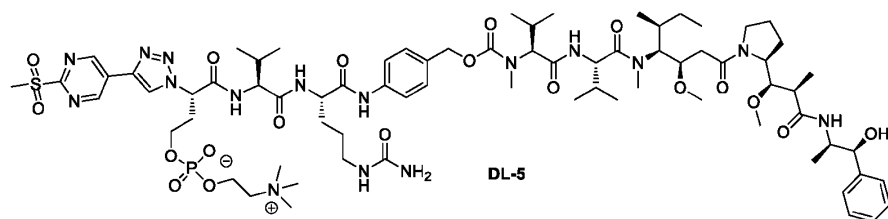


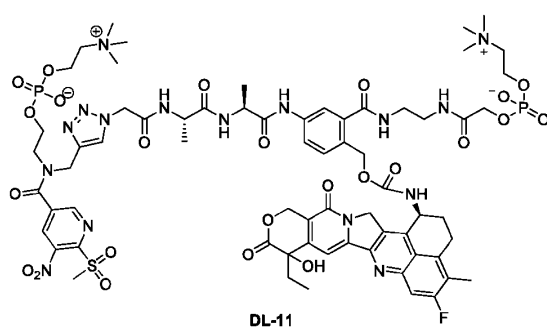
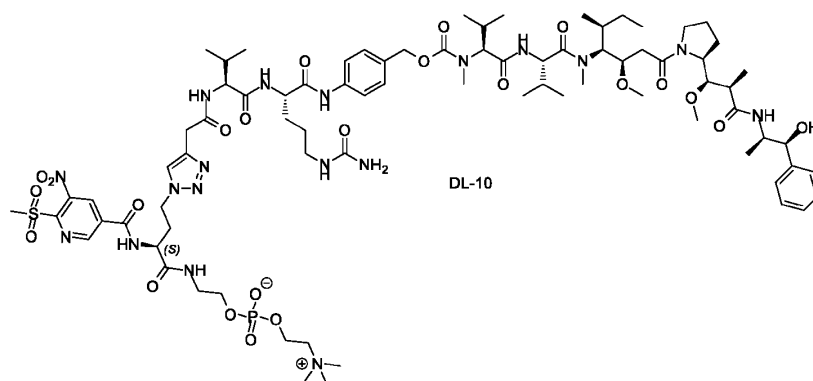
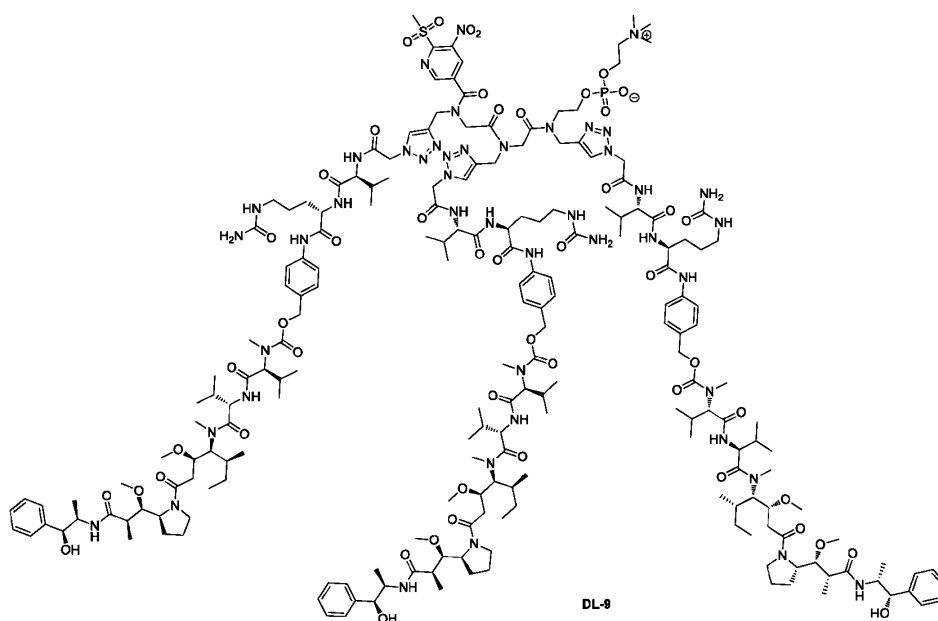


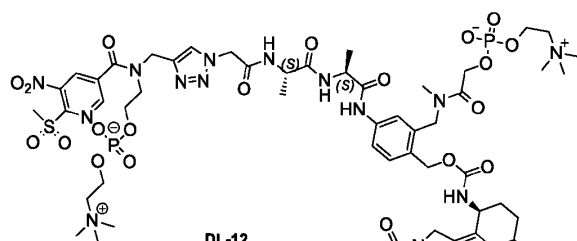


[0149] Non-limiting examples of compounds (*e.g.*, drug-linker conjugates) of the invention additionally include:

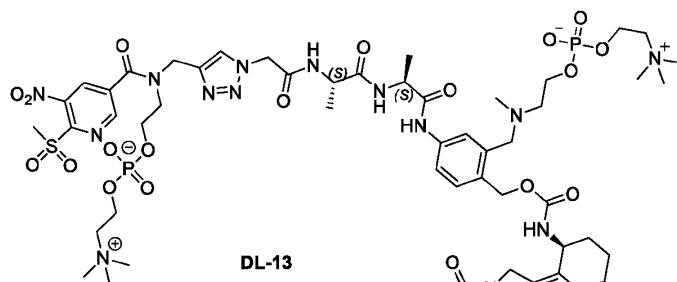
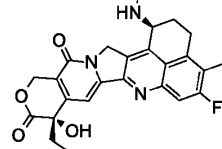




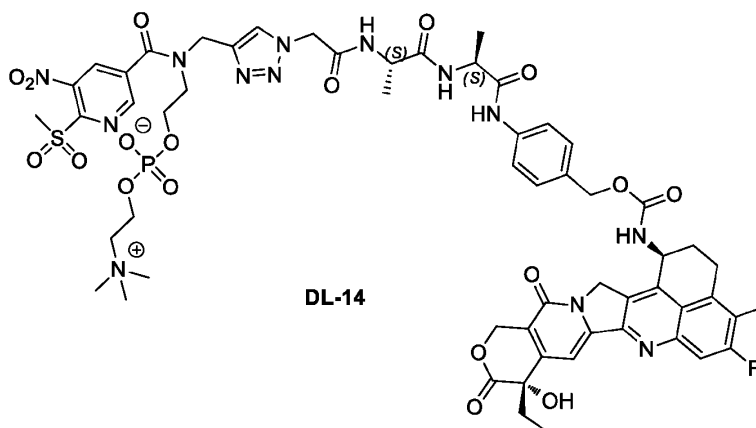
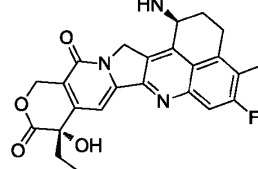




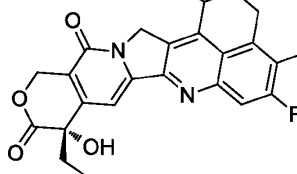
DL-12

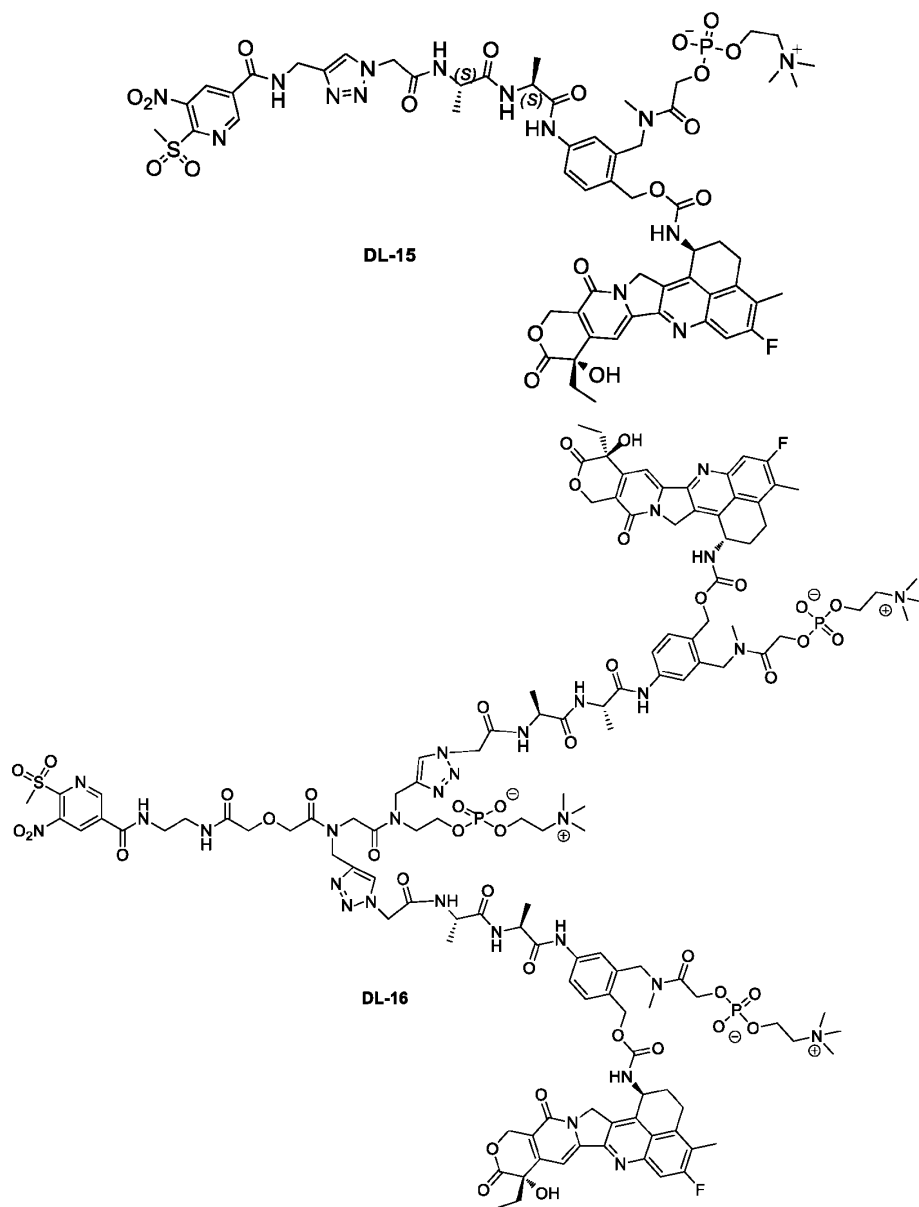


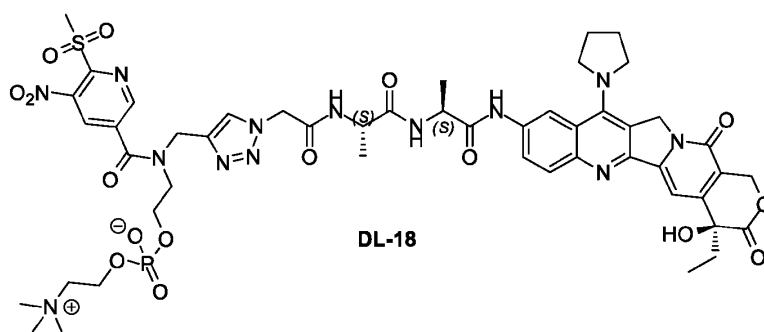
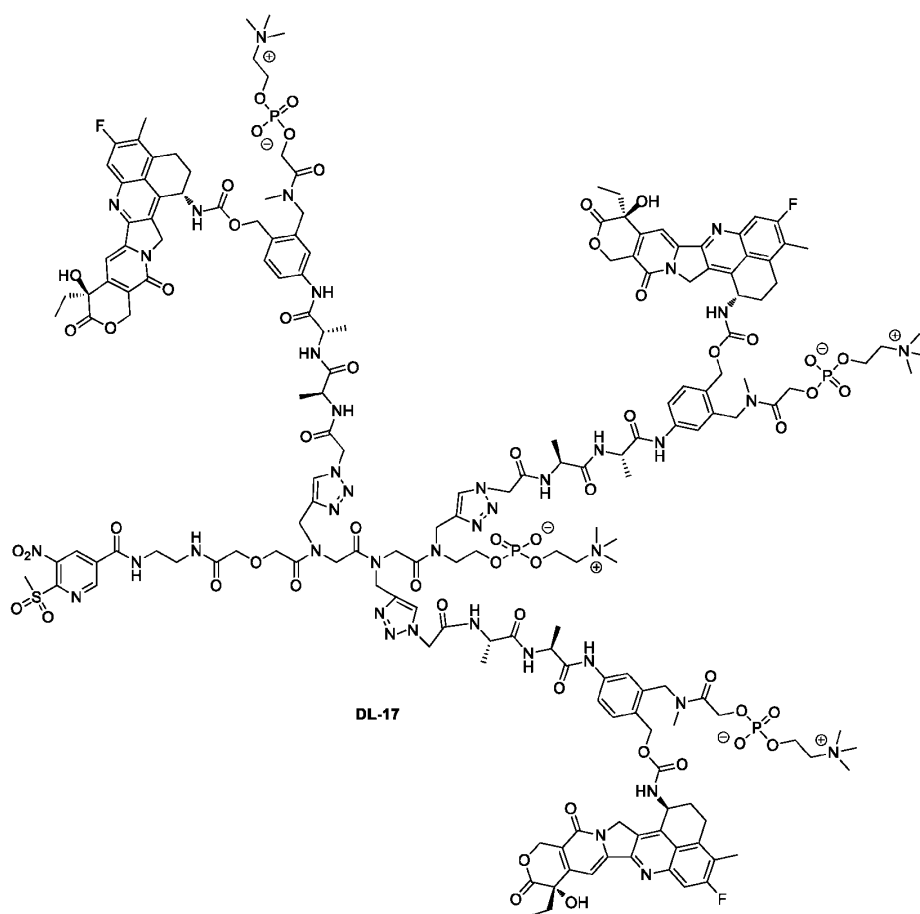
DL-13

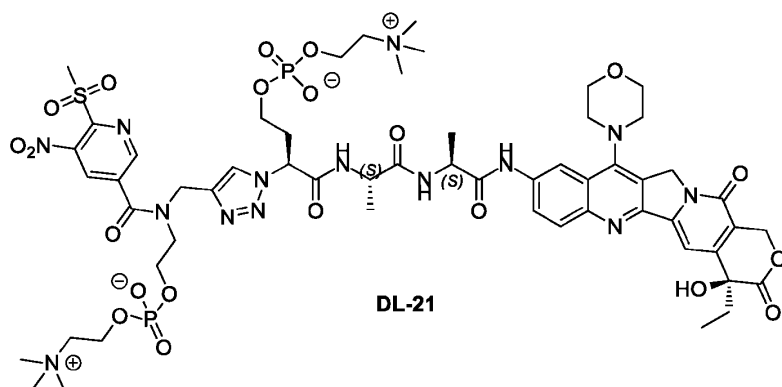
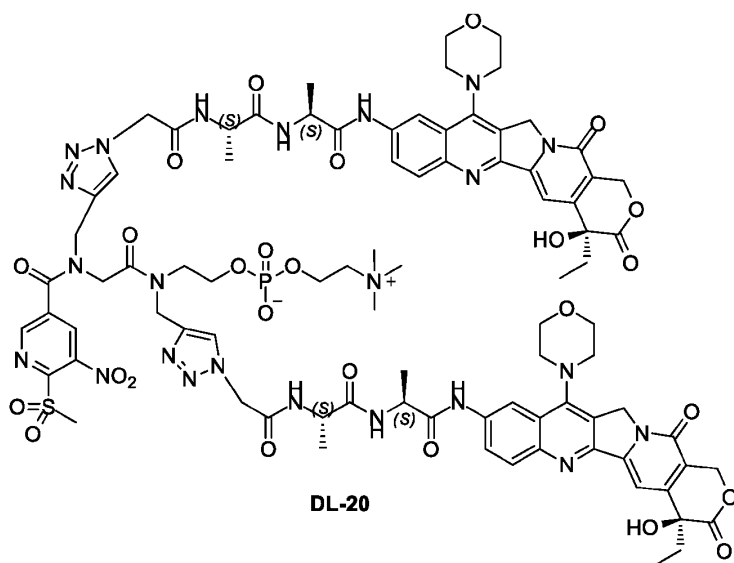
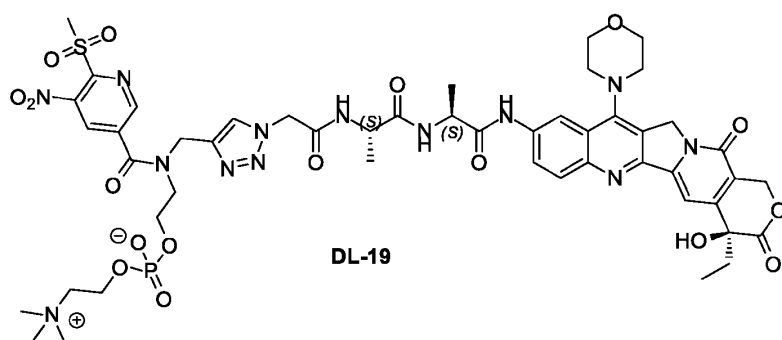


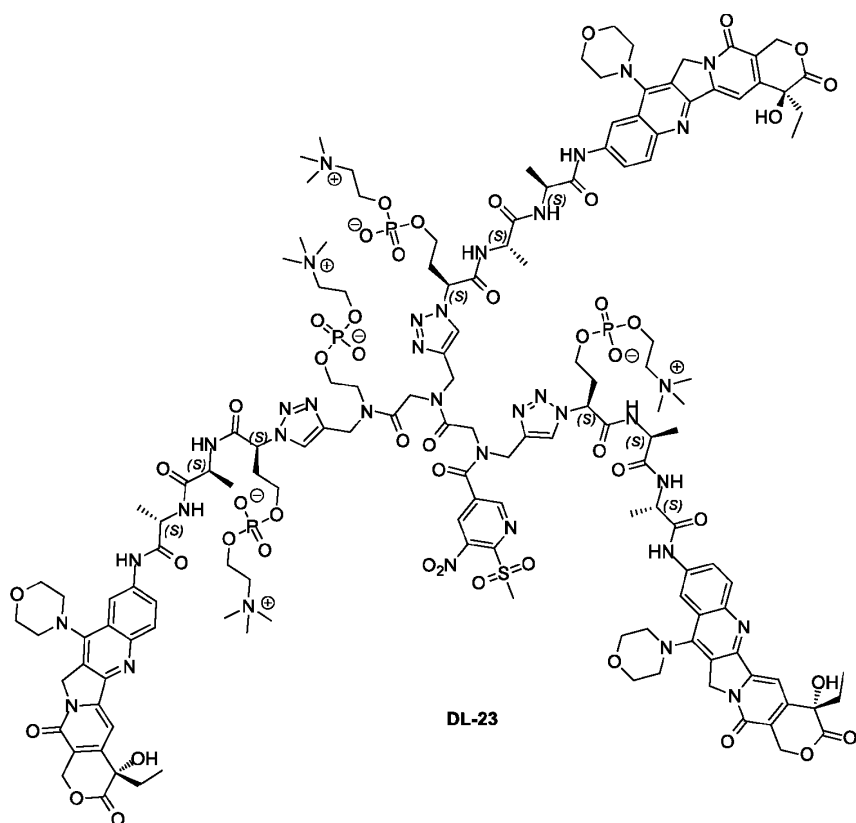
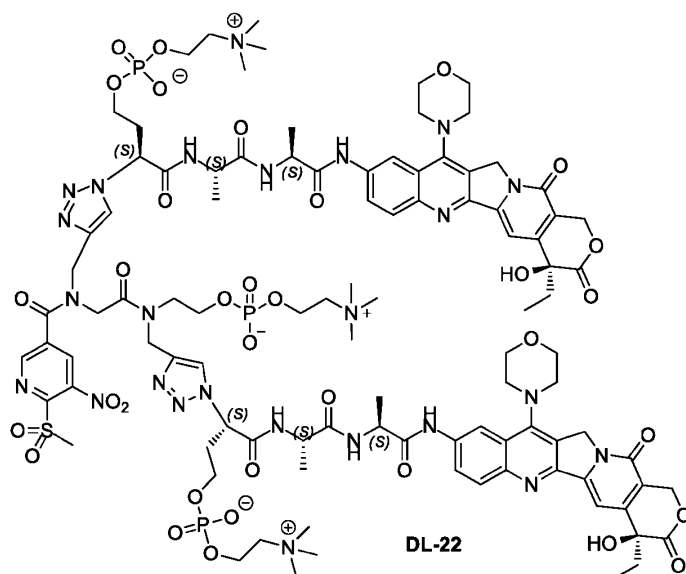
DL-14

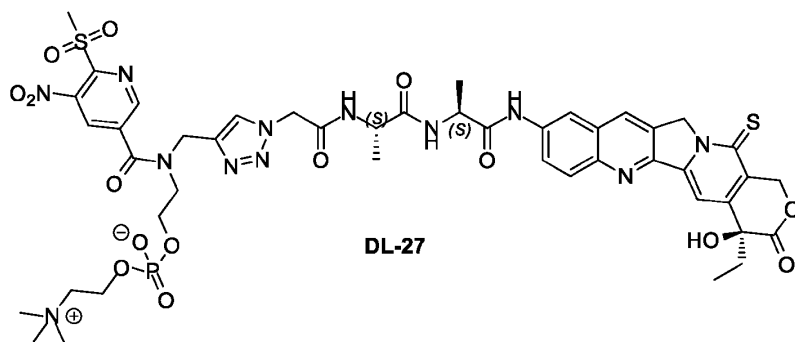
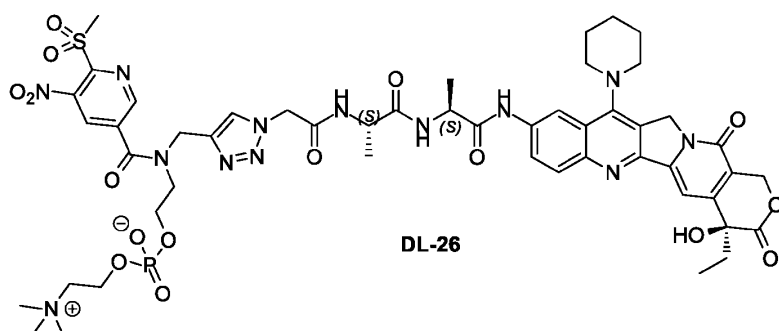
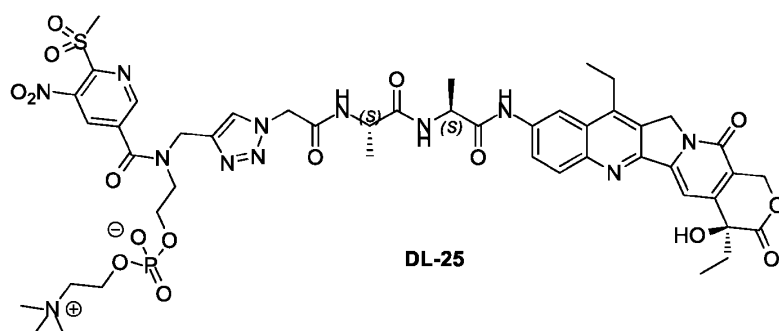
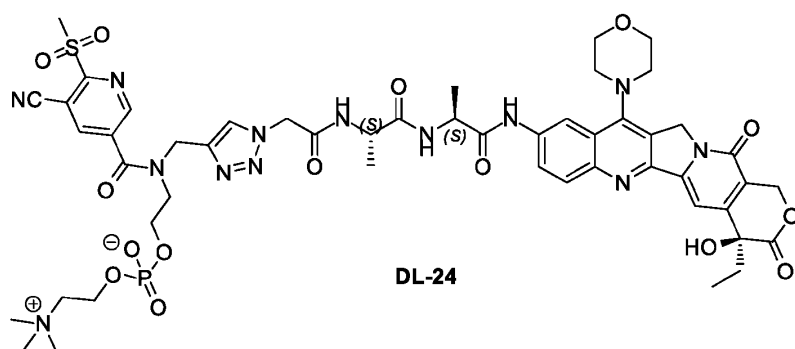


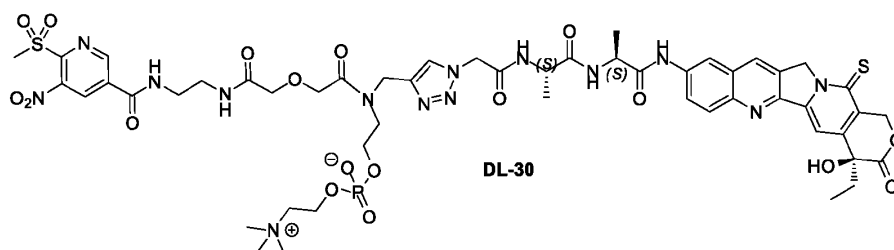
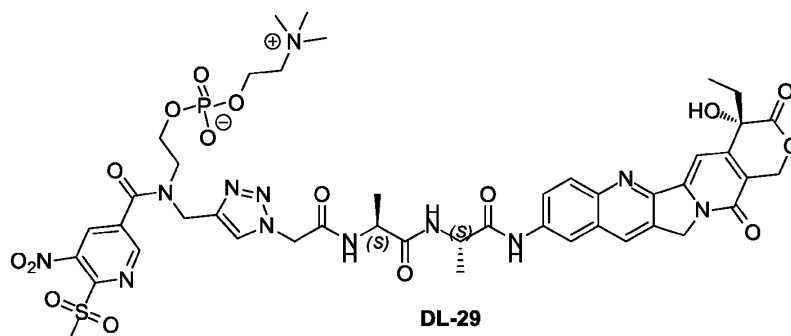
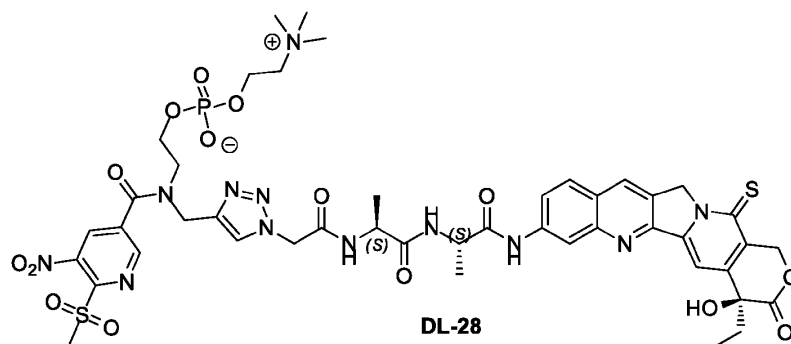




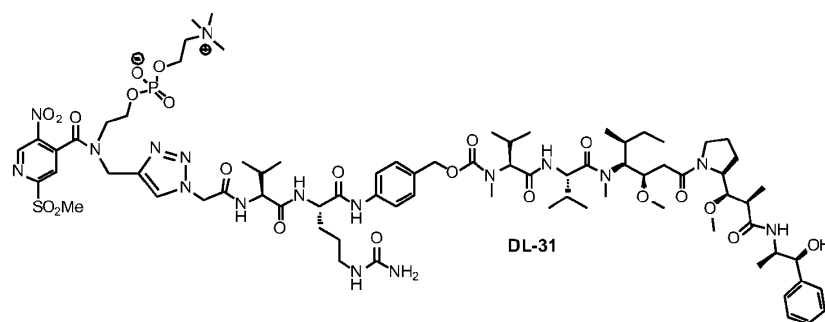


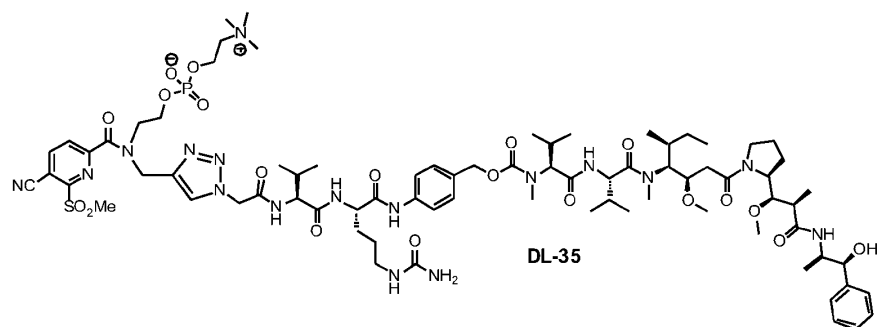
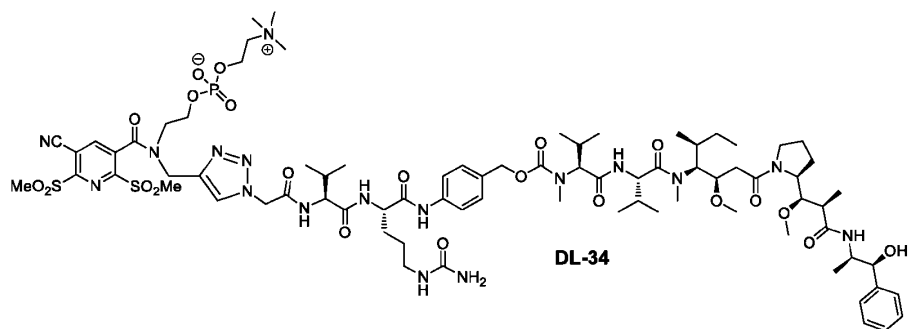
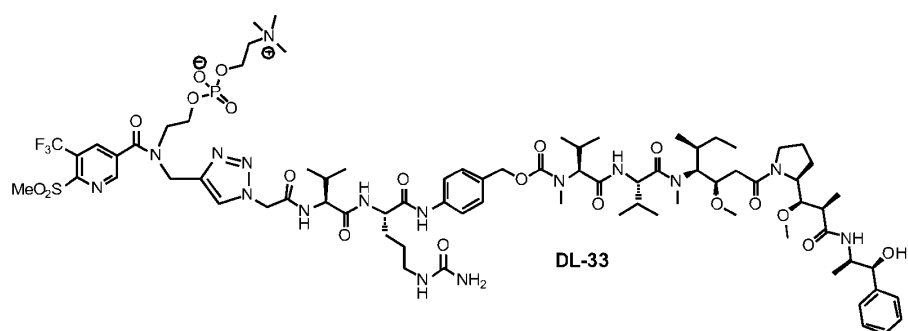
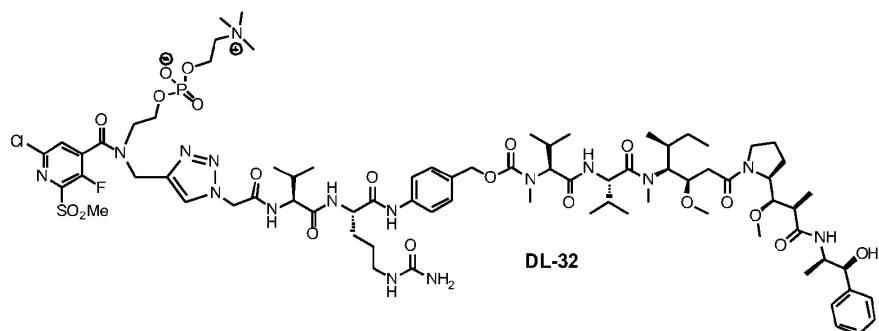


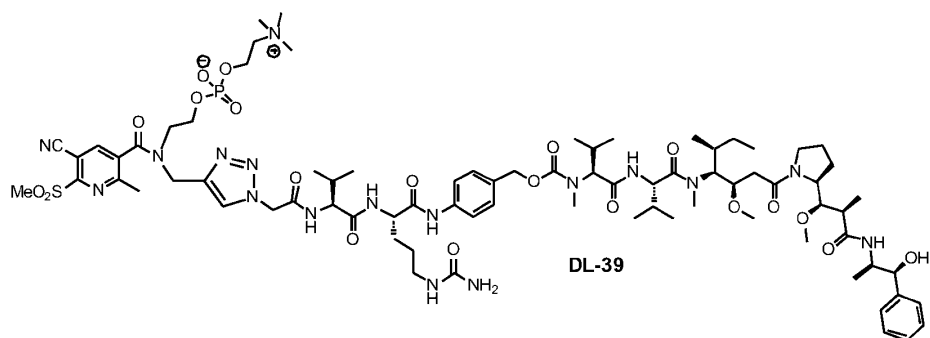
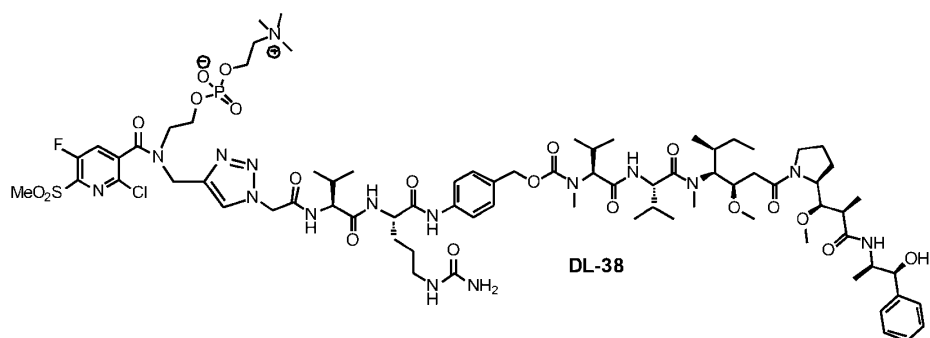
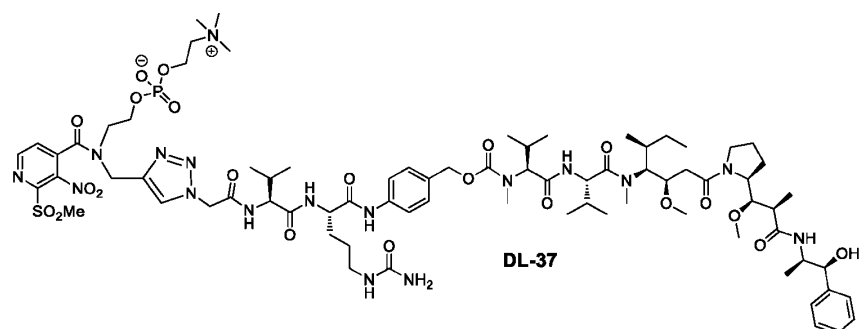
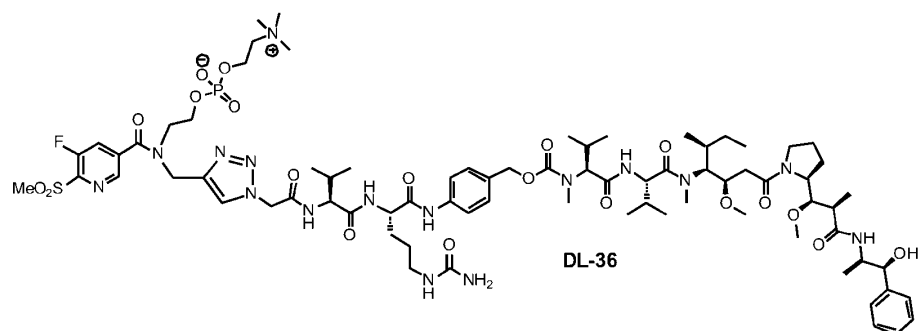


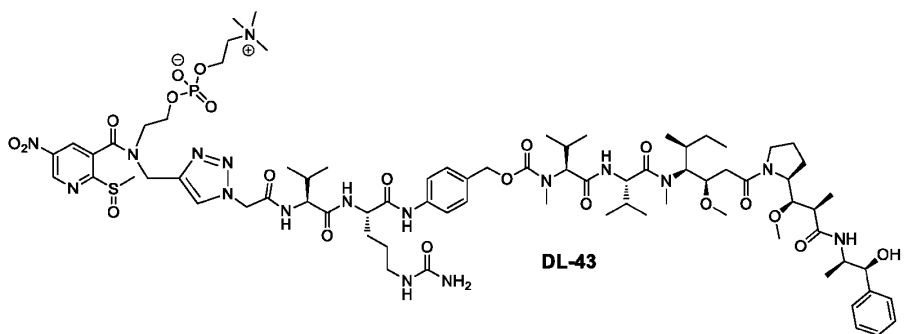
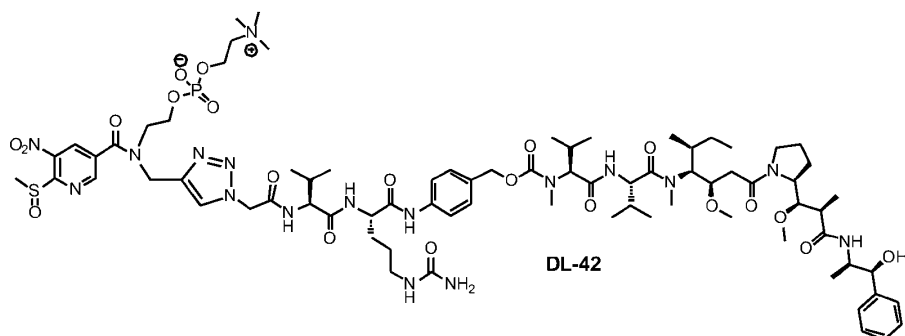
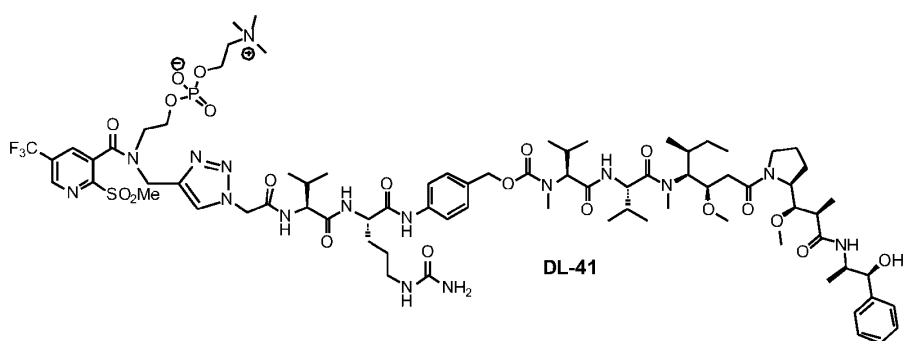
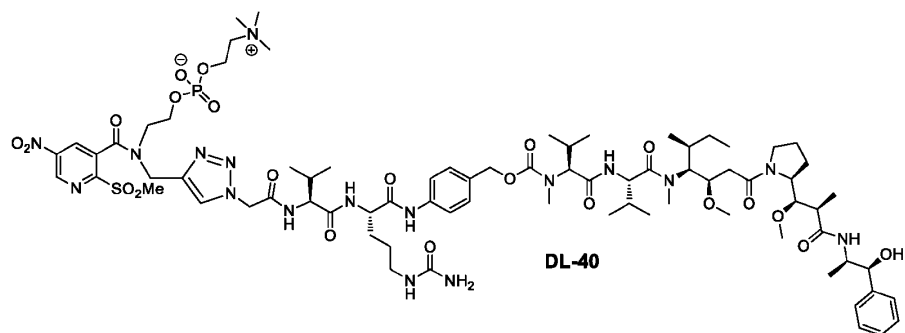


[0150] Non-limiting examples of compounds (*e.g.*, drug-linker conjugates) of the invention additionally include:







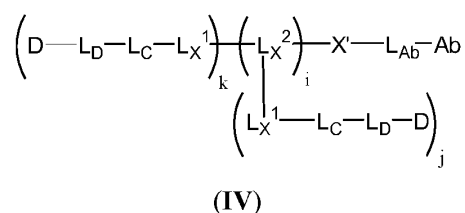


[0151] In yet another aspect, the invention generally relates to a composition comprising a compound disclosed herein.

[0152] In yet another aspect, the invention generally relates to an immunoconjugate comprising a drug-linker conjugate or a building block disclosed herein, or a moiety or derivative thereof.

[0153] In yet another aspect, the invention generally relates to an immunoconjugate prepared from a drug-linker conjugate or a building block disclosed herein, or a moiety or derivative thereof.

[0154] In yet another aspect the invention generally relates to an immunoconjugate having the structural formula (IV):



or a pharmaceutically acceptable salt thereof,

wherein

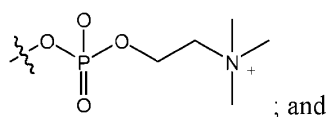
Ab represents an antigen binding moiety;

each D is independently a drug moiety;

X' is a single bond or a conjugation group;

each L_C is independently a moiety formed by a click chemistry reaction between an alkyne and an azide;

each of L_{Ab}, L_D, L_X¹ and L_X² is independently a linker or spacer moiety, wherein at least one of L_D, L_X¹ and L_X² comprises a PC group



i is an integer selected from 0-8 (*e.g.*, 0, 1, 2, 3, 4, 5, 6, 7, 8); and

each of *j* and *k* is independently 0 or 1, provided that *j* and *k* are not both 0.

[0155] In certain embodiments of (IV), *j* is 0.

[0156] In certain embodiments of (IV), *j* is 1.

[0157] In certain embodiments of (IV), *k* is 1.

[0158] In certain embodiments of (IV), j is 1 and k is 1.

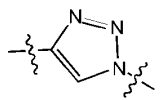
[0159] In certain embodiments of (IV), i is 0.

[0160] In certain embodiments of (IV), i is 1.

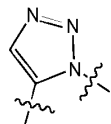
[0161] In certain embodiments of (IV), i is 2.

[0162] In certain embodiments of (IV), i is greater than 2.

[0163] In certain embodiments of (IV), L_C is



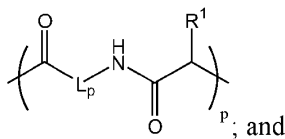
[0164] In certain embodiments of (IV), L_C is



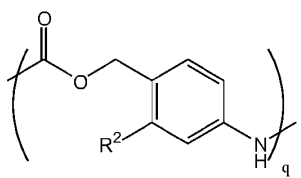
[0165] In certain embodiments of (IV), X' is a single bond.

[0166] In certain embodiments of (IV), $-L_D-$ is $-L_D^2-L_D^1-$, wherein

L_D^1 is



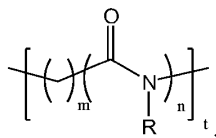
L_D^2 is



wherein

L_p is a di-, tri- or tetra peptide linker;

each of R^1 and R^2 is H, C_{1-3} alkyl, or $L_{PC}-(PC)$, wherein L_{PC} is



each p and q is independently 0 or 1, provided that p and q are not both 0;

R is H or C₁₋₃ alkyl;

m is 0, 1, 2 or 3;

n is 0 or 1, and

t is 0 or 1.

[0167] In certain embodiments, L_p is a di-peptide.

[0168] In certain embodiments, L_p is tri-peptide.

[0169] In certain embodiments, L_p is tetra peptide.

[0170] In certain embodiments, at least one of R¹ and R² is L_{PC}-(PC).

[0171] In certain embodiments, each of R¹ and R² is L_{PC}-(PC).

[0172] In certain embodiments, each R in L_{PC} is H.

[0173] In certain embodiments, *p* is 0 and *q* is 1.

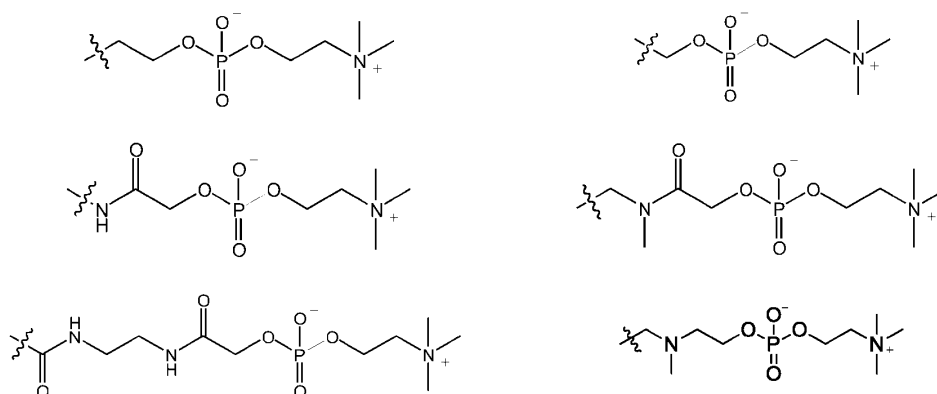
[0174] In certain embodiments, *p* is 1 and *q* is 0.

[0175] In certain embodiments, each of *p* and *q* is 1.

[0176] In certain embodiments, each of *m* and *n* is 1.

[0177] In certain embodiments, one of *m* and *n* is 0.

[0178] In certain embodiments, L_{PC}-(PC) is selected from:

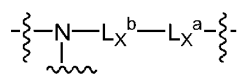


[0179] In certain embodiments of (IV), L_X¹ is (CH₂)_g, wherein *g* is 0.

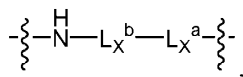
[0180] In certain embodiments of (IV), L_X¹ is (CH₂)_g, wherein *g* is 1.

[0181] In certain embodiments of (IV), L_X¹ is (CH₂)_g, wherein *g* is 2.

[0182] In certain embodiments of (IV), L_X² is:



or

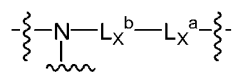


wherein

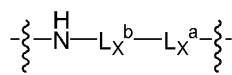
L_X^a is a substituted or unsubstituted C_{1-6} alkyl or a PEG group;

L_X^b is $-\text{C}(=\text{O})-$, CH or CH_2 .

[0183] In certain embodiments, L_X^2 is:



[0184] In certain embodiments, L_X^2 is:



[0185] In certain embodiments, L_X^a comprises a PEG group.

[0186] In certain embodiments, L_X^a comprises a C_{1-6} alkyl group.

[0187] In certain embodiments, L_X^b is $-\text{C}(=\text{O})-$.

[0188] In certain embodiments, L_X^b is CH_2 .

[0189] In certain embodiments of (IV), at least one of L_D , L_X^1 and L_X^2 comprises a spacer moiety selected from the group consisting of an alkyl, a heteroalkyl, polyethylene glycol (PEG), and a peptide. In certain embodiments, the spacer moiety is an alkyl group. In certain embodiments, the spacer moiety is a PEG. In certain embodiments, the spacer moiety is a peptide.

[0190] In certain embodiments of (IV), an immunoconjugate of the invention comprises 1, 2, 3, 4, 5, 6, 7, 8 or more PC groups.

[0191] 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.

[0192] In certain embodiments, the targeting moiety is selected from the group consisting of an antibody, small molecule, a peptide and a nucleic acid.

[0193] 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).

[0194] 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.

[0195] 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.

[0196] 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.

[0197] 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.

[0198] 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.

[0199] 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.

[0200] In certain embodiments, the drug conjugate is stable *in vivo* (e.g., does not undergo a deconjugation process).

[0201] In yet another aspect, the invention generally relates to a pharmaceutical composition comprising an immunoconjugate disclosed herein and a pharmaceutically acceptable excipient, carrier or diluent.

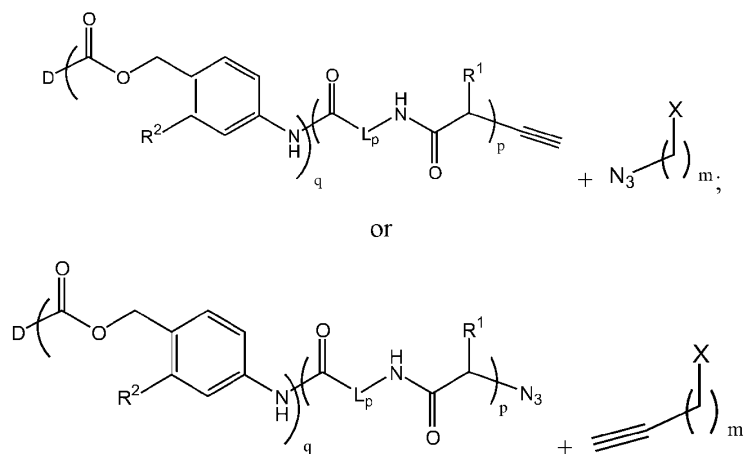
[0202] In yet another aspect, the invention generally relates to a combination comprising a therapeutically effective amount of an immunoconjugate disclosed herein, and one or more therapeutically active co-agent(s) and/or adjuvant(s).

Methods of Preparation

[0203] In yet another aspect, the invention generally relates to a method for preparing a linker moiety-drug moiety conjugate, a targeting moiety-linker moiety conjugate, or a targeting moiety-linker moiety-drug moiety conjugate, an immunoconjugate, or a precursor or intermediate compound thereof disclosed herein.

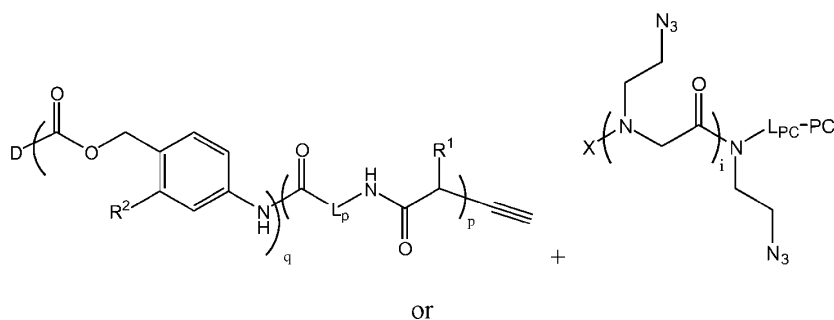
[0204] In yet another aspect, the invention generally relates to a composition comprising a compound disclosed herein that is useful for preparing a linker moiety-drug moiety conjugate, a targeting moiety-linker moiety conjugate, or a targeting moiety-linker moiety-drug moiety conjugate.

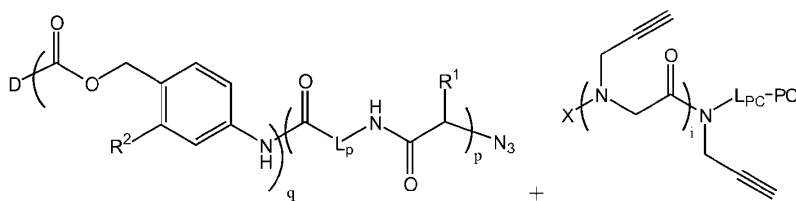
[0205] In yet another aspect, the invention generally relates to a method of preparing a linker-drug conjugate under suitable conditions for click chemistry:



wherein D, R¹, R², X, L_p, m, p and q are defined as discussed herein above.

[0206] In yet another aspect, the invention generally relates to a method of preparing a linker-drug conjugate under suitable conditions for click chemistry:





wherein D, R¹, R², X, L_p, L_{PC}-PC, *i*, *p* and *q* are defined as discussed herein above.

[0207] Additional methods of preparation and synthetic procedures are provided in the Examples section herein.

[0208] In yet another aspect, the invention generally relates to use of a compound or an immunoconjugate disclosed herein for the manufacture of a medicament.

Use of Compounds and Drug Conjugates

[0209] 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 therapeutically effective amount of an immunoconjugate of the invention.

[0210] In some embodiments, the condition is cancer, an autoimmune disorder, or an infectious disease.

[0211] 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.

[0212] 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.

[0213] 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.

[0214] 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).

[0215] 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).

[0216] 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.

[0217] 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.

[0218] 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.

[0219] 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.

[0220] 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.

[0221] 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.

[0222] 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.

[0223] 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.

[0224] 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.

[0225] 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.

Spacers

[0226] 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.

[0227] In some embodiments, the alkyl is a low alkyl, having 1 to 4 carbon atoms (e.g., methyl, ethyl, propyl and butyl).

[0228] 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.

[0229] 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.

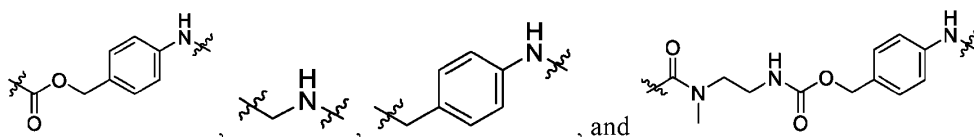
[0230] In some embodiments, the polypeptide moiety comprises 1 to 6 amino acids. For example, the polypeptide can include 1 amino acid, 2 amino acids, 3 amino acids, 4 amino acids, 5 amino acids, or 6 amino acids.

[0231] 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-acetylglucosaminy-L-serine, N-acetylglucosaminy-L-threonine, and O-phosphotyrosine.

Self-immolative Moiety

[0232] 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.

[0233] In some embodiments, the self-immolative moiety is selected from:



[0234] 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

[0235] 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, an anti-autoimmune disease agent, or an immune agonist. A drug moiety may also be a polypeptide or a nucleic acid. A drug moiety may also be a proteolysis targeting chimera (PROTAC) molecule, a molecular glue or degrader for targeted protein degradation.

[0236] 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.

[0237] 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.

[0238] 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., dimers of pyrrolbenzodiazepine (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).

[0239] 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.

[0240] 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).

[0241] 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).

[0242] 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).

[0243] 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.

[0244] 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, anthramycin, azaserine, bleomycins, cactinomycin, carabacin, 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.

[0245] 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.

[0246] In some embodiments, the anti-autoimmune disease drug is selected from one or more of polyketides (e.g., acetogenins such as 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.

[0247] In certain embodiments, the drug is an infectious disease drug. Non-limiting examples of infectious disease drugs include aminoglycosides, amphenicols, ansamycins, carbapenems, cepheids, glycopeptides, glycyclcyclines, β -lactamase inhibitors, lincosamides, lipopeptides, macrolides, monobactams, oxazolidinones, penicillin, polypeptides, quinolones, streptogramins, sulfonamides, steroid antibacterials, tetracyclines, and/or antibiotics.

[0248] In some embodiments, the aminoglycoside is one or more of amikacin, astromicin, 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 verdamicin.

[0249] In some embodiments, the amphenicol is one or more of azidamfenicol, chloramphenicol, florfenicol, and/or thiamphenicol.

[0250] In some embodiments, ansamycin is one or more of geldanamycin and/or herbimycin.

[0251] In some embodiments, carbapenems is one more of biapenem, doripenem, ertapenem, imipenem/cilastatin, meropenem, and/or panipenem.

[0252] 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, cefdalo xime, cefepime, cefminox, cefoxitin, cefprozil, cefroxadine, ceftazole, cefuroxime, cefixime, cefdinir, cefditoren, cefepime, cefetamet, cefmenoxime, cefodizime, cefonidicid, cefoperazone, ceforanide, cefotaxime, cefotiam, cefozopran, cephalixin, cefpimizole, cefpiramide, cefpirome, cefpodoxime, cefprozil, cefquinome, cefsulodin, ceftazidime, cefteram, ceftibuten, ceftiolene, ceftizoxime, ceftobiprole, ceftriaxone, cefuroxime, cefuzonam, cephamycin (e.g., cefoxitin, cefotetan, and cefmetazole), and/or oxacephem (e.g., flomoxef and latamoxef).

[0253] In some embodiments, the glycopeptide is one or more of bleomycin, vancomycin (oritavancin, telavancin), teicoplanin (dalbavancin), ramoplanin.

[0254] In some embodiments, the glycyclines is tigecycline.

[0255] 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).

[0256] In some embodiments, the lincosamide is one or more of clindamycin and/or lincomycin.

[0257] In some embodiments, the lipopeptide is one or more of daptomycin, A54145, and/or calcium-dependent antibiotics (CDA).

[0258] 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.

[0259] In some embodiments, the monobactams is selected from aztreonam and/or tigemonam.

[0260] In some embodiments, the oxazolidinones is linezolid.

[0261] 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.

[0262] In some embodiments, the polypeptide is one or more of bacitracin, colistin, and/or polymyxin B.

[0263] 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.

[0264] In some embodiments, streptogramins is pristnamycin such as quinupristin and/or dalbopristin.

[0265] In some embodiments, the sulfonamide is one or more of mafenide, prontosil, sulfacetamide, sulfamethizole, sulfanilimide, sulfasalazine, sulfisoxazole, trimethoprim, and/or trimethoprim-sulfamethoxazole (co-trimoxazole).

[0266] In some embodiments, the steroid antibacterial is fusidic acid.

[0267] 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).

[0268] 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

[0269] 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.)

[0270] 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.)

[0271] In certain embodiments, the targeting moiety is an antibody.

[0272] In certain embodiments, the targeting moiety is a monoclonal antibody.

[0273] In certain embodiments, the targeting moiety is a chimeric antibody.

[0274] In certain embodiments, the targeting moiety is a humanized antibody.

[0275] In certain embodiments, the targeting moiety is a bispecific antibody.

[0276] In certain embodiments, the targeting moiety is an antibody fragment.

[0277] In certain embodiments, the targeting moiety is a Fab fragment.

[0278] In certain embodiments, the targeting moiety is a peptide.

[0279] In certain embodiments, the targeting moiety is a small molecule ligand.

[0280] 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.

[0281] 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.

[0282] 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.

[0283] 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.)

[0284] 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.

[0285] 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.

[0286] 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.

[0287] 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.

[0288] 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.)

[0289] 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.)

[0290] 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.

[0291] 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.

[0292] In some embodiments, the targeting moiety comprises HuM195-Ac-225, HuM195-Bi-213, Anyara (naptumomab estafenatox; ABR-217620), AS 1409, Zevalin (ibritumomab tiuxetan), BIIB015, 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.

[0293] 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

[0294] 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.

[0295] 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.

[0296] 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.

[0297] 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.

[0298] 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.

[0299] 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.)

[0300] 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.

[0301] 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.

[0302] 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.

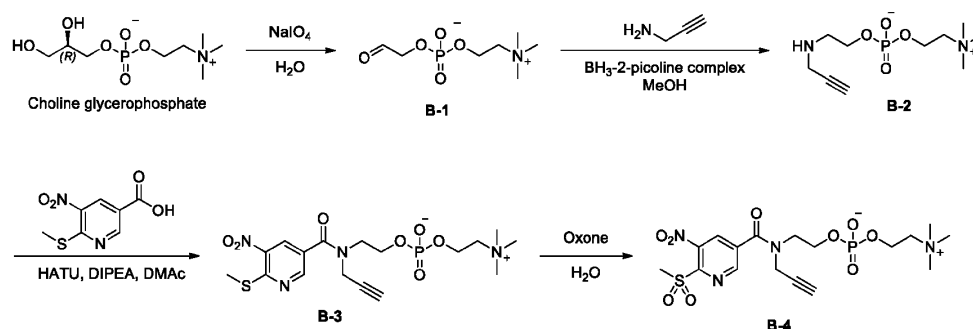
[0303] Solvates and polymorphs of the compounds of the invention are also contemplated herein. Solvates of the compounds of the present invention include, for example, hydrates.

[0304] 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

Example 1: Synthesis of B-4



Step 1: Synthesis of 2-oxoethyl (2-(trimethylammonio)ethyl) phosphate (B-1)

[0305] To a solution of choline glycerophosphate (20.0 g, 77.75 mmol, 1.0 eq) in 100 mL water, cooling with ice water, was added NaIO₄ (17.627 g, 81.64 mmol, 1.05 eq) in portions. After addition, the mixture was stirred at r.t. overnight, then concentrated. To the residue methanol (200 mL) was added and stirred for 2h at r.t.. Solid was filtered off and the filtrate was concentrated. Ethanol (200 mL) was added to the residue, the mixture was stirred for another 2h at r.t., then filtered and concentrated to give the crude 2-oxoethyl (2-(trimethylammonio)ethyl) phosphate (**B-1**) (16.8 g, yield: 96.0%) as a pale white solid.

[0306] ¹H NMR (500 MHz, D₂O) δ 5.06 (t, 1H), 4.21 (m, 2H), 3.69 (dd, J = 6.7, 4.9 Hz, 2H), 3.59-3.46 (m, 2H), 3.10 (s, 9H).

Step 2: Synthesis of 2-(prop-2-yn-1-ylamino)ethyl (2-(trimethylammonio)ethyl) phosphate (B-2)

[0307] To a solution of the crude **B-1** (15.0 g, 66.6 mmol, 1.0 eq.) in MeOH (150 mL) was added propargylamine (4.04 g, 73.3 mmol, 1.1 eq.), followed by borane-2-picoline complex (7.48 g, 69.9mmol, 1.05 eq), and the mixture was allowed to stir at room temperature for 2 hours. The solution was concentrated, and the residue was redissolved in water (100 mL). This aqueous solution was extracted with ethyl acetate (100 mL*3) and the organic phase was discarded. The aqueous phase was concentrated under reduced pressure to give crude 2-(prop-2-yn-1-ylamino)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-2**) (11.6 g, yield: 65.9%).

[0308] LCMS[M+H]⁺: 265.24.

Step 3: Synthesis of 2-(6-(methylthio)-5-nitro-N-(prop-2-yn-1-yl)nicotinamido)ethyl (2-(trimethylammonio)ethyl) phosphate (B-3)

[0309] To a solution of the crude **B-2** (2.0 g, 7.57 mmol, 1.7 eq.) in DMAc (20 mL) was added 6-(methylthio)-5-nitronicotinic acid (973 mg, 4.54 mmol, 1.0 eq), DIPEA (1.97 g, 15.1 mmol, 3.4 eq) and HATU (2.88 g, 7.57 mmol, 1.7 eq) under N₂. The suspended reaction was stirred at room temperature for 16 h. The reaction mixture was purified by prep-HPLC to give 2-(6-(methylthio)-5-nitro-N-(prop-2-yn-1-yl)nicotinamido)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-3**) (1.62 g, 46.4%) as a yellow solid.

[0310] LCMS[M+H]⁺: 461.27.

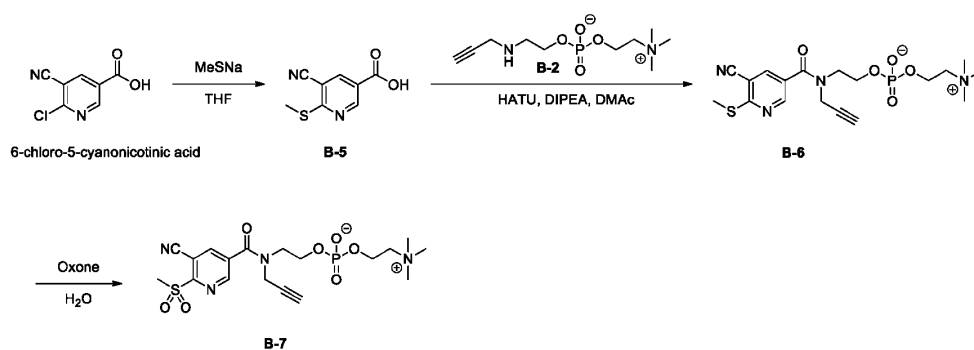
Step 4: Synthesis of 2-(6-(methylsulfonyl)-5-nitro-N-(prop-2-yn-1-yl)nicotinamido)ethyl (2-(trimethylammonio)ethyl) phosphate (B-4)

[0311] To a solution of **B-3** (1.30 g, 2.83 mmol, 1.0 eq.) in H₂O (10 mL) was added Oxone (2.60 g, 4.24 mmol, 1.5 eq.) at r.t., then stirred at that temperature for 2 hrs. The reaction was pre-purified by prep-HPLC to afford 2-(6-(methylsulfonyl)-5-nitro-N-(prop-2-yn-1-yl)nicotinamido)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-4**) (770 mg, yield: 55.3%) as a white solid.

[0312] LCMS[M+H]⁺: 493.27.

[0313] ¹H NMR (600 MHz, D₂O) δ 8.94 (dd, J = 53.3, 1.6 Hz, 1H), 8.62 (dd, J = 55.6, 1.6 Hz, 1H), 4.30 (d, J = 2.4 Hz, 1H), 4.18 (s, 1H), 4.06 (d, J = 8.0 Hz, 3H), 3.89 (dd, J = 10.0, 5.1 Hz, 1H), 3.61-3.39 (m, 4H), 3.06 (d, J = 12.9 Hz, 9H).

Example 2: Synthesis of B-7



Step 1: Synthesis of 5-cyano-6-(methylthio)nicotinic acid (**B-5**)

[0314] To a solution of 6-chloro-5-cyanonicotinic acid (1.07 g, 5.88 mmol, 1.0eq) in THF (25 mL) was added CH₃SNa (20% in H₂O) (3.98 mL, 13.94 mmol, 2.2eq) under N₂. The reaction mixture was stirred at room temperature for 2 hrs, then acidified with 3N hydrochloric acid to pH=3~4. Water (60 mL) was added, and precipitate was formed. The precipitated product was collected by filtration to get 5-cyano-6-(methylthio)nicotinic acid (**B-5**) (1.134 g, yield: 90.79%) as a white solid.

[0315] LCMS[M+H]⁺: 195.13.

Step 2: Synthesis of 2-(5-cyano-6-(methylthio)-N-(prop-2-yn-1-yl)nicotinamido)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-6**)

[0316] To a solution of **B-5** (1.10 g, 5.66 mmol, 0.6 eq) in DMAc (15 mL) was added DIPEA (3.3 mL, 18.88 mmol, 2.0 eq), **B-2** (2.50 g, 9.44 mmol, 1.0 eq) and HATU (3.59 g, 9.44 mmol,

1.0 eq) under N₂. The reaction mixture was stirred at room temperature for 2 h, Then purified by prep-HPLC to afford 2-(5-cyano-6-(methylthio)-N-(prop-2-yn-1-yl)nicotinamido)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-6**) (2.12 g, yield: 85%) as a pale solid.

[0317] LCMS[M+H]⁺: 441.12.

[0318] ¹H NMR (600 MHz, D₂O) δ 8.67 (d, J = 50.7 Hz, 1H), 8.14 (d, J = 36.8 Hz, 1H), 4.28 (d, J = 43.3 Hz, 2H), 4.20-4.07 (m, 3H), 3.96 (d, J = 4.8 Hz, 1H), 3.83 (s, 1H), 3.66 (s, 1H), 3.60-3.52 (m, 2H), 3.13 (d, J = 5.6 Hz, 9H), 2.72 (d, J = 57.5 Hz, 1H), 2.59 (s, 3H).

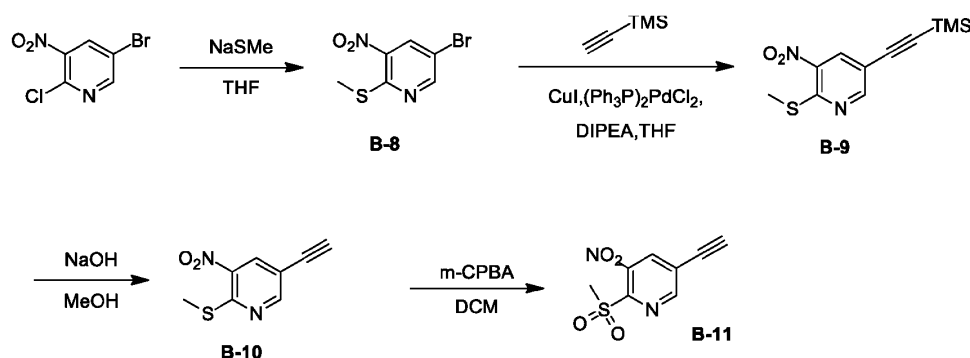
Step 3: Synthesis of 2-(5-cyano-6-(methylsulfonyl)-N-(prop-2-yn-1-yl)nicotinamido)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-7**)

[0319] To a solution of **B-6** (2.00 g, 4.54 mmol, 1.0 eq) in H₂O (20 mL) was added Oxone (4.18 g, 6.81 mmol, 1.5 eq) in portions at r.t.. the resulting mixture was stirred at RT for 16 hrs, then purified by prep- HPLC to afford 2-(5-cyano-6-(methylsulfonyl)-N-(prop-2-yn-1-yl)nicotinamido)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-7**) (1.89 g, yield: 88.1%) as a white solid.

[0320] LCMS[M+H]⁺: 473.12.

[0321] ¹H NMR (600 MHz, DMSO-d₆) δ 9.10-9.01 (m, 1H), 8.78 (d, J = 6.6 Hz, 1H), 4.43 (d, J = 1.7 Hz, 1H), 4.29-4.07 (m, 4H), 3.91 (d, J = 5.3 Hz, 1H), 3.77 (t, 1H), 3.64-3.51 (m, 4H), 3.49 (s, 3H), 3.13 (d, J = 5.9 Hz, 9H).

Example 3: Synthesis of **B-11**



Step 1: Synthesis of 5-Bromo-2-(methylthio)-3-nitropyridine (**B-8**)

[0322] 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 5-Bromo-2-(methylthio)-3-nitropyridine (**B-8**) (2.78 g, yield:88.7%, purity:100%) as a yellow solid.

[0323] 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).

Step 2: Synthesis of 2-(Methylthio)-3-nitro-5-((trimethylsilyl)ethynyl)pyridine (**B-9**)

[0324] To a solution of **B-8** (780 mg, 3.15 mmol, 1.0 eq) in DIPEA (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, 0.05 eq). The suspended reaction mixture was stirred at room temperature for 24h. Then evaporated and the residue was purified by the prep-HPLC to afford 2-(Methylthio)-3-nitro-5-((trimethylsilyl)ethynyl)pyridine (**B-9**) (47 mg, yield:5.6%, purity: 100%) as a brown solid .

[0325] LCMS[M+H]⁺:267.1.

[0326] 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).

Step 3: Synthesis of 5-Ethynyl-2-(methylthio)-3-nitropyridine (**B-10**)

[0327] To a solution of **B-9** (47 mg, 0.177 mmol, 1.0 eq) in MeOH (1 mL) was added KOH (20 mg, 0.35 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 5-Ethynyl-2-(methylthio)-3-nitropyridine (**B-10**) (18 mg, yield:52.3%, purity:100%) as a yellow solid.

[0328] LCMS[M+H]⁺:195.1.

[0329] 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).

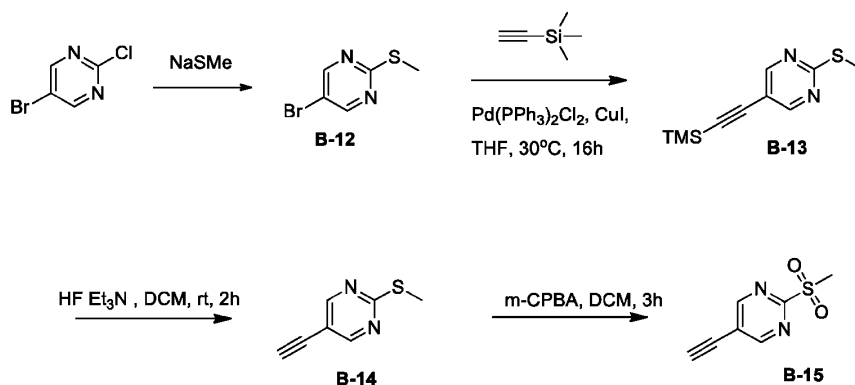
Step 4: Synthesis of 5-Ethynyl-2-(methylsulfonyl)-3-nitropyridine (**B-11**)

[0330] To a solution of **B-10** (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 5-Ethynyl-2-(methylsulfonyl)-3-nitropyridine (**B-11**) (2.3 mg, yield:11%, purity:96%) as a white solid.

[0331] LCMS[M+H]⁺:227.0.

[0332] 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).

Example 4: Synthesis of B-15



Step 1: Synthesis of 5-Bromo-2-(methylthio)pyrimidine (B-12)

[0333] To a solution of 5-bromo-2-chloropyrimidine (5.0 g, 26.18 mmol), NaSMe (2.4 g, 34.03 mmol) in EtOH (50 mL) was stirred at 25°C for 2h under nitrogen. The reaction was diluted with H₂O (50 mL), extracted with EA (50 mL*3). The organic layers were combined, washed with brine (50 mL), dried over Na₂SO₄, filtered and concentrated to give compound 5-Bromo-2-(methylthio)pyrimidine (**B-12**) (3.5 g, 65%) as a white solid.

[0334] LCMS: m/z = 205.0/207.0(M+H, ESI⁺).

Step 2: Synthesis of 2-(Methylthio)-5-((trimethylsilyl)ethynyl)pyrimidine (B-13)

[0335] To a mixture of **B-12** (1.8 g, 8.7 mmol), ethynyltrimethylsilane (3.5 g, 35.70 mmol), Bis(triphenylphosphine)palladium (II) chloride (501 mg, 0.71 mmol) and CuI (68 mg, 0.35 mmol) in THF (20 mL) was added Et₃N (2.89 g, 28.26 mmol). The resulting mixture was stirred at 30°C for 16h. The mixture was diluted with water (60 mL), extracted with EA (50 mL*3), dried over anhydrous Na₂SO₄, filtered and concentrated. The residue was purified by Flash Chromatography (0-15%, PE/EA) to give 2-(Methylthio)-5-((trimethylsilyl)ethynyl)pyrimidine (**B-13**) (1.30 g, 81%) as a black oil.

[0336] LCMS: m/z = 223.1(M+1, ESI⁺).

Step 3: Synthesis of 5-Ethynyl-2-(methylthio)pyrimidine (B-14)

[0337] To a solution of **B-13** (1.3 g, 5.86 mmol) in DCM (10 mL) was added HF/Et₃N (2 ml). The resulting mixture was stirred at 25°C for 2 h, diluted with water (10 mL), extracted with DCM (10 mL*3), The organic phase was dried, filtered, removed off under reduced pressure to give 5-Ethynyl-2-(methylthio)pyrimidine (**B-14**) (800 mg, 91%) as a brown oil.

[0338] LCMS: m/z = 151.1(M+1, ESI+).

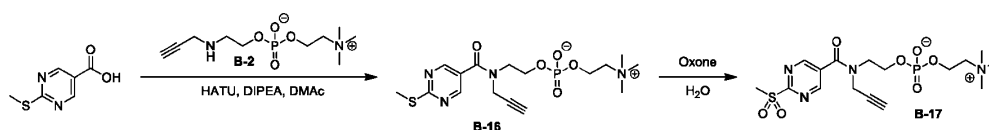
Step 4: Synthesis of 5-ethynyl-2-(methylsulfonyl)pyrimidine (**B-15**)

[0339] To a mixture of **B-14** (800 mg, 5.33mmol) and m-CPBA (2300 mg, 13.33 mmol) in DCM (10 ml) was stirred at 25°C for 3h . It was diluted with H₂O (30 mL), extracted with EA (30 mL*3). The organic layers were combined, washed with saturated sodium bicarbonate solution (30 mL), dried over Na₂SO₄, filtered and concentrated. The residue was purified by Flash Chromatography (0-15%, DCM/MeOH) to give 5-ethynyl-2-(methylsulfonyl)pyrimidine (**B-15**) (600 mg, 61%) as a yellow solid.

[0340] LCMS: m/z = 183.1(M+1, ESI+).

[0341] ¹H NMR (400 MHz, DMSO-d₆) δ 9.2 (s, 2H), 4.95 (s, 1H), 3.46 (s, 3H).

Example 5: Synthesis of **B-17**



Step 1: Synthesis of 2-(2-(methylthio)-N-(prop-2-yn-1-yl)pyrimidine-5-carboxamido)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-16**)

[0342] To a solution of 2-(methylthio)pyrimidine-5-carboxylic acid (500 mg, 2.94 mmol) in DMAc (15 mL) was added DIPEA (1.27 g, 9.79 mmol), **B-2** (1.29 g, 4.90 mmol) and HATU (1.265 g, 9.79 mmol) under N₂. Then the reaction mixture was stirred at room temperature for 2 h, purified by prep-HPLC directly to afford 2-(2-(methylthio)-N-(prop-2-yn-1-yl)pyrimidine-5-carboxamido)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-16**) (968 mg, yield: 47.5%) as a white solid.

[0343] LCMS[M+H]⁺: 417.18.

[0344] ¹H NMR (600 MHz, DMSO-d₆) δ 8.73 (s, 2H), 4.24 (s, 4H), 4.08 (d, J = 58.3 Hz, 2H), 3.75 (s, 1H), 3.19-3.05 (m, 9H), 2.69 (s, 2H), 2.56 (s, 2H), 1.25 (dt, J = 15.1, 7.6 Hz, 3H).

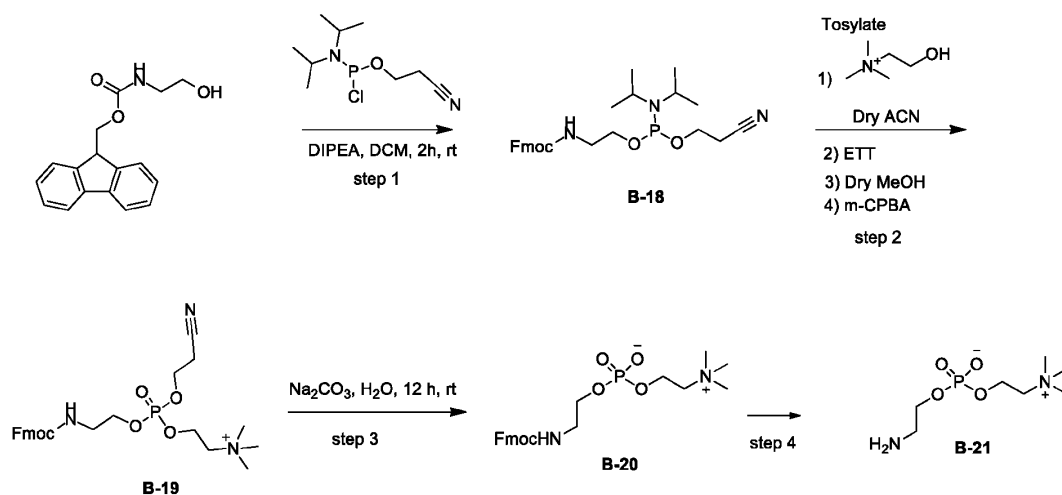
Step 2: Synthesis of 2-(2-(methylsulfonyl)-N-(prop-2-yn-1-yl)pyrimidine-5-carboxamido)ethyl (2-(trimethylammonio)ethyl) phosphate (B-17)

[0345] To a solution of **B-16** (750 mg, 0.934 mmol) in H₂O (10 mL) was added oxone (1.66 g, 2.7 mmol) at r.t., and stirred at that temperature for 16 hrs. The reaction was purified by prep-HPLC directly to afford 2-(2-(methylsulfonyl)-N-(prop-2-yn-1-yl)pyrimidine-5-carboxamido)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-17**) (367.82 mg, yield: 45.5%) as a white solid.

[0346] LCMS[M+H]⁺: 449.15.

[0347] ¹H NMR (600 MHz, DMSO-d₆) δ 9.22 (d, J = 6.5 Hz, 2H), 4.51-4.19 (m, 2H), 4.09-3.98 (m, 2H), 3.89-3.70 (m, 2H), 3.59-3.46 (m, 4H), 3.39-3.32 (m, 1H), 3.12 (dd, J = 14.3, 4.6 Hz, 9H), 1.25 (dt, J = 13.9, 7.0 Hz, 3H).

Example 6: Synthesis of B-21



Step 1: Synthesis of (9H-fluoren-9-yl)methyl 2-(((2-cyanoethoxy)(diisopropylamino)phosphino)oxy)ethylcarbamate (B-18)

[0348] (9H-fluoren-9-yl)methyl (2-hydroxyethyl)carbamate (7.1 g, 25.1 mmol) was first dried by co-evaporation with toluene (2*50mL). Dry DCM (100 mL) and DIPEA (5.53 g, 42.7 mmol) were added under argon, then 3-((chloro(diisopropylamino)phosphanyl)oxy)propanenitrile (7.74 g, 32.7 mmol) was added slowly via a syringe. The reaction mixture was stirred for 3 h at room temperature. After reaching completion, the reaction mixture was evaporated under

vacuum. The crude (9H-fluoren-9-yl)methyl 2-(((2-cyanoethoxy)(diisopropylamino)phosphino)oxy)ethyl carbamate (**B-18**) was obtained as a yellow solid (14 g).

Step 2: Synthesis of 2-(((2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)(2-cyanoethoxy)phosphoryl)oxy)-N,N,N-trimethylethanaminium (B-19**)**

[0349] (9H-fluoren-9-yl)methyl 2-(((2-cyanoethoxy)(diisopropylamino)phosphino)oxy)ethyl carbamate (**B-18**) was dried with dry toluene and dry ACN (2*50 mL). Choline p-toluenesulfonate (13.8 g, 50.2 mmol) was dried with toluene and dissolved in dry ACN (60 mL). This mixture was added to (9H-fluoren-9-yl)methyl 2-(((2-cyanoethoxy)(diisopropylamino)phosphino)oxy)ethyl carbamate (**B-18**) (14 g, crude) via a syringe. ETT (0.25 M in ACN) (7.3 g, 55.2 mmol) was added slowly with a syringe. The mixture was stirred at room temperature for 2 h. 3-Chloroperoxybenzoic acid (12.9 g, 75.3 mmol) was added portion wise to the mixture. After 4h of stirring, the mixture was concentrated under vacuum. The crude was purified by Flash Chromatography (C18, ACN-H₂O, 60%) to give 10 g of 2-(((2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)(2-cyanoethoxy)phosphoryl)oxy)-N,N,N-trimethylethanaminium (**B-19**) as a yellow solid.

[0350] LCMS (ESI): m/z 503.3 (M⁺)

Step 3: Synthesis of 2-(((2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl (2-(trimethylammonio)ethyl) phosphate (B-20**)**

[0351] To a solution of 2-(((2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)(2-cyanoethoxy)phosphoryl)oxy)-N,N,N-trimethylethanaminium (**B-19**) (10 g, 20 mmol) in H₂O (150 mL) stirred at 25°C was added a solution of sodium carbonate (5 g, 45 mmol) in H₂O (20 mL). The reaction mixture was stirred at 25°C for 12h. Adjust pH to 6 with HCl and filtered, the filtrate was purified by flash C18 ODS (ACN--H₂O 30%) to give 2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-20**) (5.1 g, 60%) as a white solid.

[0352] LCMS: m/z = 449.2 (M+1, ESI+).

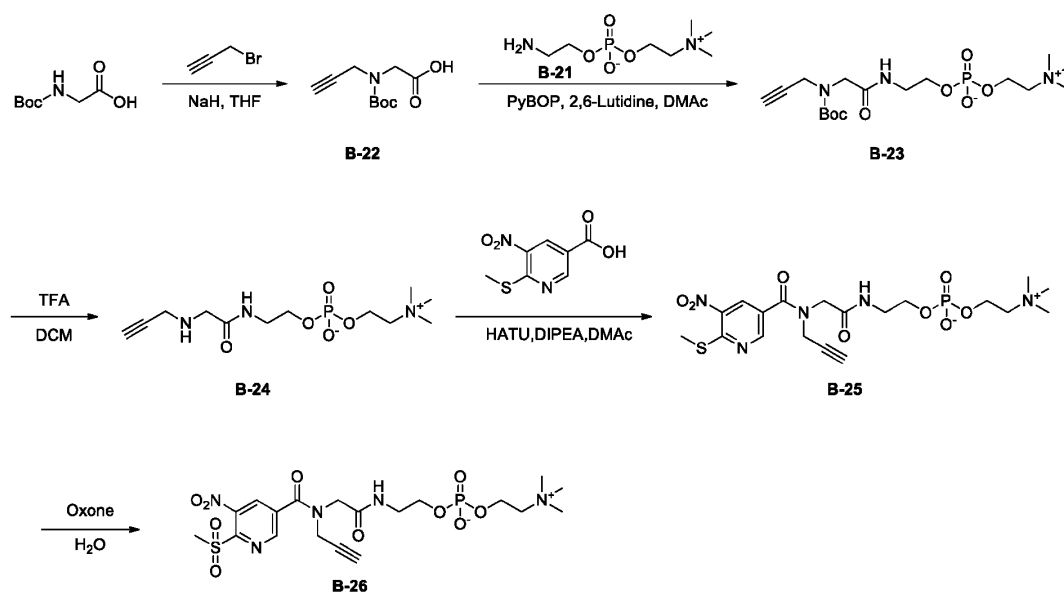
Step 4: Synthesis of 2-aminoethyl (2-(trimethylammonio)ethyl) phosphate (B-21**)**

[0353] To a solution of 2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-20**) (5g) in dry DCM (15 mL) was added diethyl amine

(9 g, 120 mmol). Volatiles were removed and the residue was dissolved in water, washed with hexane and dried with lyophilization to afford 2-aminoethyl (2-(trimethylammonio)ethyl) phosphate (**B-21**) as a white solid (1.7 g, 65%).

[0354] LCMS (ESI): m/z 227.2 ($M + H$)⁺.

Example 7: Synthesis of B-26



Step 1: Synthesis of 2-((tert-butoxycarbonyl)(prop-2-yn-1-yl)amino)acetic acid (**B-22**)

[0355] To a solution of 2-((tert-butoxycarbonyl)amino)acetic acid (Boc-Gly-OH) (1.0 g, 5.7 mmol, 1.0 eq) in anhydride THF (15 mL) was added NaH (0.50 g, 12.56 mmol, 2.2 eq) in portions at 0~5°C with ice-water bath under N₂ protected. The resulting mixture was stirred at 0~5°C for 1 h. 3-Bromopropyne (1.36 g, 11.42 mmol, 2.0 eq.) was added. The resulting mixture was stirred at r.t. for overnight at r.t., then quenched with water and acidified with 1N HCl until pH about 3, extracted with ethyl acetate, concentrated, and purified by prep-HPLC to give 2-((tert-butoxycarbonyl)(prop-2-yn-1-yl)amino)acetic acid (**B-22**) (0.562 g) as a white solid.

[0356] LCMS[M-H]⁻: 212.27.

[0357] ¹H NMR (600 MHz, DMSO-d₆) δ 12.69 (s, 1H), 4.08-4.02 (m, 2H), 3.89 (d, J = 13.0 Hz, 2H), 3.22 (d, J = 2.3 Hz, 1H), 1.39 (d, J = 30.3 Hz, 9H).

Step 2: Synthesis of 2-(2-((tert-butoxycarbonyl)(prop-2-yn-1-yl)amino)acetamido)ethyl (2-

(trimethylammonio)ethyl) phosphate (B-23)

[0358] To a solution of 2-aminoethyl (2-(trimethylammonio)ethyl) phosphate (**B-21**) (120 mg, 0.53 mmol, 1.0 eq) and **B-22** (226.2 mg, 1.06 mmol, 2.0 eq) in DMAc (13 mL) was added PyBOP (552 mg, 1.06 mmol, 2.0 eq), followed by 2,6-lutidine (267.2 μ L, 2.12 mmol, 4.0 eq), the resulting mixture was stirred at room temperature for overnight, and purified by prep-HPLC to give 2-(2-((tert-butoxycarbonyl)(prop-2-yn-1-yl)amino)acetamido)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-23**) (191 mg, yield: 85.4%) as a white solid.

[0359] LCMS[M+H]⁺:422.33.

[0360] ¹H NMR (600 MHz, DMSO-d₆) δ 8.37 (d, J = 21.0 Hz, 1H), 4.10 (s, 2H), 4.03 (d, J = 14.0 Hz, 2H), 3.80 (d, J = 24.0 Hz, 2H), 3.72 (dd, J = 13.9, 5.7 Hz, 2H), 3.56-3.51 (m, 2H), 3.24 (s, 1H), 3.23 (t, J = 2.4 Hz, 2H), 3.13 (s, 9H), 1.38 (d, J = 34.0 Hz, 9H).

Step 3: Synthesis of 2-(2-(prop-2-yn-1-ylamino)acetamido)ethyl (2-(trimethylammonio)ethyl) phosphate (B-24)

[0361] To a solution of **B-23** (161 mg, 0.048 mmol, 1.0 eq) in DCM (3 mL) was added TFA (1 mL), the resulting mixture was stirred at r.t. for 2h, then concentrated and purified by prep-HPLC to give 2-(2-(prop-2-yn-1-ylamino)acetamido)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-24**) (91 mg, yield:74.17%) as a white solid.

[0362] LCMS[M+H]⁺:322.21.

Step 4: Synthesis of 2-(2-(6-(methylthio)-5-nitro-N-(prop-2-yn-1-yl)nicotinamido)acetamido)ethyl (2-(trimethylammonio)ethyl) phosphate (B-25)

[0363] To a solution of **B-24** (91 mg, 0.28 mmol, 1.0 eq) and 6-(methylthio)-5-nitronicotinic acid (60.7 mg, 0.28 mmol, 1.0 eq) in DMAc (6 mL) was added DIPEA (99 μ L, 0.57 mmol, 2.0 eq) at r.t., followed by HATU (108 mg, 0.28 mmol, 1.0 eq). The resulting mixture was stirred at room temperature for overnight and purified by prep-HPLC to give 2-(2-(6-(methylthio)-5-nitro-N-(prop-2-yn-1-yl)nicotinamido)acetamido)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-25**) (107 mg, yield: 73.1%) as a yellow solid.

[0364] LCMS[M+H]⁺:518.29.

[0365] ^1H NMR (600 MHz, D_2O) δ 8.85 (d, J = 46.1 Hz, 1H), 8.70 (d, J = 57.0 Hz, 1H), 4.31 (s, 2H), 4.24 (s, 1H), 4.19 (d, J = 16.3 Hz, 3H), 3.87 (dd, J = 38.0, 5.7 Hz, 2H), 3.59 (s, 2H), 3.43 (dd, J = 23.8, 19.0 Hz, 2H), 3.14 (s, 9H), 2.75 (d, J = 72.8 Hz, 1H), 2.55 (d, J = 10.6 Hz, 3H).

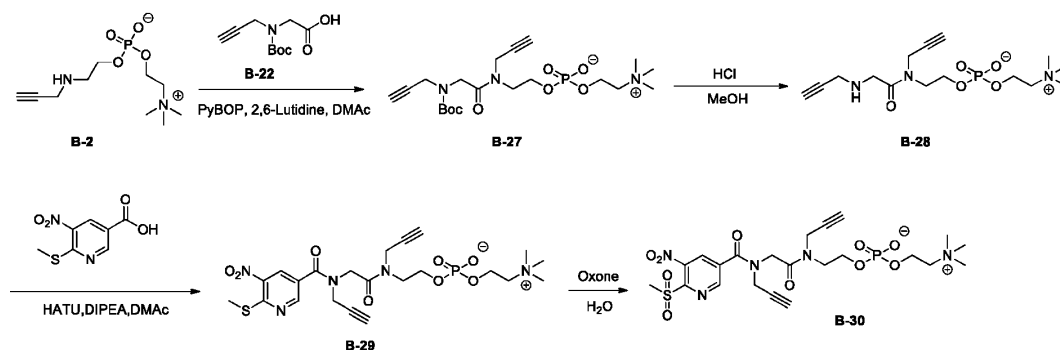
Step 5: Synthesis of 2-(2-(6-(methylsulfonyl)-5-nitro-N-(prop-2-yn-1-yl)nicotinamido)acetamido)ethyl (2-(trimethylammonio)ethyl) phosphate (B-26)

[0366] To a solution of **B-25** (8.3 mg, 0.016 mmol, 1.0 eq) in H_2O (2.0 mL) was added oxone (30 mg, 0.048 mmol, 3.0 eq), then stirred at r.t. for over weekend. The reaction mixture was purified by prep-HPLC to afford 2-(2-(6-(methylsulfonyl)-5-nitro-N-(prop-2-yn-1-yl)nicotinamido)acetamido)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-26**) (7.2 mg, yield: 82.10%) as a white solid.

[0367] LCMS[M+H] $^+$: 550.26.

[0368] ^1H NMR (600 MHz, D_2O) δ 9.02 (d, J = 80.4 Hz, 1H), 8.70 (d, J = 90.3 Hz, 1H), 4.35 (s, 2H), 4.22 (d, J = 20.2 Hz, 2H), 4.15 (s, 1H), 4.12 (d, J = 2.0 Hz, 1H), 3.90 (dd, J = 11.4, 5.6 Hz, 1H), 3.84 (dd, J = 11.9, 5.6 Hz, 1H), 3.61-3.56 (m, 2H), 3.52 (d, J = 8.9 Hz, 3H), 3.45 (t, J = 5.2 Hz, 1H), 3.37 (t, J = 5.3 Hz, 1H), 3.13 (d, J = 3.0 Hz, 9H), 2.76 (d, J = 54.8 Hz, 1H).

Example 8: Synthesis of B-30



Step 1: Synthesis of 2-(2-((tert-butoxycarbonyl)(prop-2-yn-1-yl)amino)-N-(prop-2-yn-1-yl)acetamido)ethyl (2-(trimethylammonio)ethyl) phosphate (B-27)

[0369] To a solution of **B-2** (393 mg, 1.49 mmol, 1.0 eq) and **B-22** (317 mg, 1.489 mmol, 1.0 eq) in DMAc (10 mL) was added 2,6-lutidine (520 μL , 4.46 mmol, 3.0 eq), followed by PyBOP (1.16 g, 2.23 mmol, 1.5 eq), the resulting mixture was stirred at room temperature for overnight, and purified by prep-HPLC to give 2-(2-((tert-butoxycarbonyl)(prop-2-yn-1-

yl)amino)-N-(prop-2-yn-1-yl)acetamido)ethyl 2-(trimethylammonio)ethyl) phosphate (**B-27**) (303 mg, yield: 44.35%) as a white solid.

[0370] LCMS[M+H]⁺:460.31.

Step 2: Synthesis of 2-(N-(prop-2-yn-1-yl)-2-(prop-2-yn-1-ylamino)acetamido)ethyl 2-(trimethylammonio)ethyl) phosphate (B-28)

[0371] Acetyl chloride (703 μ L, 9.9 mmol, 15.0 eq) was added to MeOH (10 mL), then to this solution was added **B-27** (303 mg, 0.66 mmol, 1.0 eq), the resulting mixture was stirred at r.t. for 2h, then concentrated and purified by prep-HPLC to offer 2-(N-(prop-2-yn-1-yl)-2-(prop-2-yn-1-ylamino)acetamido)ethyl 2-(trimethylammonio)ethyl) phosphate (**B-28**) (202 mg, yield: 85.24%) as a white solid.

[0372] LCMS[M+H]⁺:360.24.

Step 3: Synthesis of 2-(2-(6-(methylthio)-5-nitro-N-(prop-2-yn-1-yl)nicotinamido)-N-(prop-2-yn-1-yl)acetamido)ethyl 2-(trimethylammonio)ethyl) phosphate (B-29)

[0373] To a solution of **B-28** (191.6 mg, 0.53 mmol, 1.0 eq) and 6-(methylthio)-5-nitronicotinic acid (114.2 mg, 0.53 mmol, 1.0 eq) in DMAc (10 mL) was added DIPEA (186 μ L, 1.07 mmol, 2.0 eq.) at r.t., followed by HATU (202.7 mg, 0.53 mmol, 1.0 eq). The resulting mixture was stirred at room temperature for 2.5 h, and purified by prep-HPLC to give 2-(2-(6-(methylthio)-5-nitro-N-(prop-2-yn-1-yl)nicotinamido)-N-(prop-2-yn-1-yl)acetamido)ethyl 2-(trimethylammonio)ethyl) phosphate (**B-29**) (175 mg, yield: 59.14%) as a yellow powder.

[0374] LCMS[M+H]⁺:556.34.

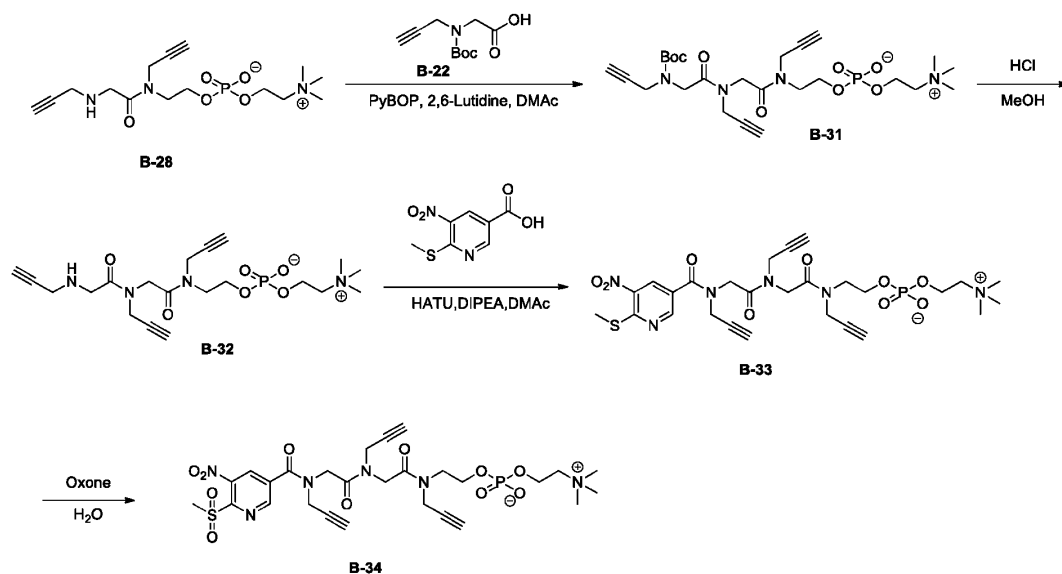
Step 4: Synthesis of 2-(2-(6-(methylsulfonyl)-5-nitro-N-(prop-2-yn-1-yl)nicotinamido)-N-(prop-2-yn-1-yl)acetamido)ethyl 2-(trimethylammonio)ethyl) phosphate (B-30)

[0375] To a solution of **B-29** (168 mg, 0.3 mmol, 1.0 eq) in H₂O (12.0 mL) was added oxone (558 mg, 0.9 mmol, 3.0 eq), then stirred at r.t. for 24 h. The reaction mixture was purified by prep-HPLC to afford 2-(2-(6-(methylsulfonyl)-5-nitro-N-(prop-2-yn-1-yl)nicotinamido)-N-(prop-2-yn-1-yl)acetamido)ethyl 2-(trimethylammonio)ethyl) phosphate (**B-30**) (165 mg, yield: 89.1%) as a white solid.

[0376] LCMS[M+H]⁺: 588.30.

[0377] ^1H NMR (600 MHz, DMSO- d_6) δ 9.07-8.86 (m, 1H), 8.72-8.53 (m, 1H), 4.50 (dd, J = 47.8, 23.9 Hz, 2H), 4.30 (dd, J = 43.9, 23.3 Hz, 2H), 4.23 (s, 1H), 4.17 (t, J = 11.3 Hz, 2H), 4.12-4.02 (m, 1H), 3.98-3.82 (m, 2H), 3.71 (s, 1H), 3.59 (d, J = 15.1 Hz, 2H), 3.56-3.47 (m, 6H), 3.40 (d, J = 56.1 Hz, 2H), 3.27 (d, J = 29.3 Hz, 1H), 3.17 (d, J = 8.3 Hz, 1H), 3.15-3.10 (m, 9H).

Example 9: Synthesis of B-34



Step 1: Synthesis of 2,2-dimethyl-4,7,10-trioxo-5,8,11-tri(prop-2-yn-1-yl)-3-oxa-5,8,11-triazatridecan-13-yl (2-(trimethylammonio)ethyl) phosphate (B-31)

[0378] To a solution of **B-28** (134.2 mg, 0.37 mmol, 1.0 eq) and **B-22** (95.6 mg, 0.44 mmol, 1.2 eq) in DMAc (9 mL) was added 2,6-lutidine (131 μL , 1.1 mmol, 3.0 eq), followed by PyBOP (291.5 mg, 0.56 mmol, 1.5 eq), the resulting mixture was stirred at room temperature for overnight, and purified by prep-HPLC to give 2,2-dimethyl-4,7,10-trioxo-5,8,11-tri(prop-2-yn-1-yl)-3-oxa-5,8,11-triazatridecan-13-yl (2-(trimethylammonio)ethyl) phosphate (**B-31**) (78 mg, yield: 37.68%) as a white solid.

[0379] LCMS[M+H]⁺:555.40.

Step 2: Synthesis of 2-(N-(prop-2-yn-1-yl)-2-(N-(prop-2-yn-1-yl)-2-(prop-2-yn-1-ylamino)acetamido)acetamido)ethyl (2-(trimethylammonio)ethyl) phosphate (B-32)

[0380] Acetyl chloride (150 μ L, 2.1 mmol, 15.0 eq) was added to MeOH (5 mL), to this solution was added **B-31** (78 mg, 0.14 mmol, 1.0 eq), the resulting mixture was stirred at r.t. for 5h, then concentrated and purified by prep-HPLC to offer 2-(N-(prop-2-yn-1-yl)-2-(N-(prop-2-yn-1-yl)-2-(prop-2-yn-1-ylamino)acetamido)acetamido)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-32**) (47 mg, yield: 73.53%) as a white solid.

[0381] LCMS[M+H]⁺:455.32.

Step 3: Synthesis of 2-(2-(2-(6-(methylthio)-5-nitro-N-(prop-2-yn-1-yl)nicotinamido)-N-(prop-2-yn-1-yl)acetamido)-N-(prop-2-yn-1-yl)acetamido)ethyl (2-(trimethylammonio)ethyl) phosphate (B-33)

[0382] To a solution of **B-32** (44.70 mg, 0.0984 mmol, 1.0 eq.) and **6-(methylthio)-5-nitronicotinic acid** (23.2 mg, 0.1 mmol, 1.1 eq) in DMAc (3.5 mL) was added DIPEA (36 μ L, 0.2 mmol, 2.1 eq.) at r.t., followed by HATU (41 mg, 0.1 mmol, 1.1 eq). The resulting mixture was stirred at room temperature for 18 h, and purified by prep-HPLC directly to give 2-(2-(2-(6-(methylthio)-5-nitro-N-(prop-2-yn-1-yl)nicotinamido)-N-(prop-2-yn-1-yl)acetamido)-N-(prop-2-yn-1-yl)acetamido)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-33**) (27.6 mg, yield: 43.25%) as a yellow powder.

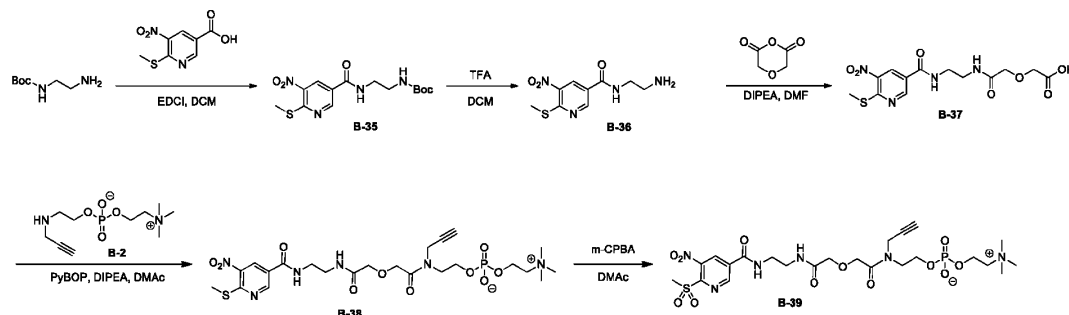
[0001] LCMS[M+H]⁺:651.4.

Step 4: Synthesis of 2-(2-(2-(6-(methylsulfonyl)-5-nitro-N-(prop-2-yn-1-yl)nicotinamido)-N-(prop-2-yn-1-yl)acetamido)-N-(prop-2-yn-1-yl)acetamido)ethyl (2-(trimethylammonio)ethyl) phosphate (B-34)

[0383] To a solution of **B-33** (29 mg, 0.045 mmol, 1.0 eq) in H₂O (4.0 mL) was added Oxone (110 mg, 0.18 mmol, 4.0 eq), then stirred at r.t. for 48 h. The reaction mixture was purified by prep-HPLC to afford 2-(2-(2-(6-(methylsulfonyl)-5-nitro-N-(prop-2-yn-1-yl)nicotinamido)-N-(prop-2-yn-1-yl)acetamido)-N-(prop-2-yn-1-yl)acetamido)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-34**) (21.6 mg, yield: 70.88%) as a white solid.

[0384] LCMS[M+H]⁺: 683.4.

[0385] ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.91 (dd, *J* = 68.9, 31.3 Hz, 1H), 8.72-8.44 (m, 1H), 4.58 (dd, *J* = 40.9, 21.7 Hz, 1H), 4.46-4.34 (m, 2H), 4.34-4.26 (m, 3H), 4.22 (dd, *J* = 22.6, 10.3 Hz, 2H), 4.13 (d, *J* = 39.6 Hz, 2H), 4.04 (d, *J* = 16.3 Hz, 3H), 3.81 (d, *J* = 31.4 Hz, 2H), 3.48 (t, *J* = 8.9 Hz, 7H), 3.26-3.16 (m, 2H), 3.16-3.09 (m, 9H).

Example 10: Synthesis of B-39**Step 1: Synthesis of tert-butyl (2-(6-(methylthio)-5-nitronicotinamido)ethyl)carbamate (B-35)**

[0386] To a solution of tert-butyl (2-aminoethyl)carbamate (6.67 g, 41.7 mmol) in DCM (100 mL), were added 6-(methylthio)-5-nitronicotinic acid (5.94 g, 28.0 mmol) and EDCI (8.05 g, 41.7 mmol) under N₂. The reaction suspension was stirred at room temperature for 16 h. The reaction was complete as detected by LC-MS. This reaction mixture was purified by prep. HPLC to afford tert-butyl (2-(6-(methylthio)-5-nitronicotinamido)ethyl)carbamate **B-35** (6.82 g, 45.9%) as a pale white solid.

[0387] LCMS[M+H]⁺: 355.4.

Step 2: Synthesis of N-(2-aminoethyl)-6-(methylthio)-5-nitronicotinamide (B-36)

[0388] To a solution of **B-35** (6.82 g, 19.2 mmol) in DCM (68 mL), was added TFA (13 mL), and the mixture was allowed to react at room temperature for 3 hours. The reaction was complete as detected by LC-MS. The solvent was removed to give N-(2-aminoethyl)-6-(methylthio)-5-nitronicotinamide **B-36** (4.9 g, yield 100%) as colorless oil.

[0389] LCMS[M+H]⁺: 257.2.

Step 3: Synthesis of 2-(2-((2-(6-(methylthio)-5-nitronicotinamido)ethyl)amino)-2-oxoethoxy)acetic acid (B-37)

[0390] To a solution of **B-36** (2.0 g, 7.8 mmol) in DMF (20 mL), were added 1,4-dioxane-2,6-dione (1.09 g, 9.36 mmol) and DIPEA (2.2 g, 17.2 mmol) under N₂. The reaction mixture was stirred at room temperature for 16 h. The reaction solution was added to 200 mL water, stirred and filtered to afford 2-(2-((2-(6-(methylthio)-5-nitronicotinamido)ethyl)amino)-2-oxoethoxy)acetic acid **B-37** (1.5 g, 51.7%) as a pale white solid.

[0002] LCMS[M+H]⁺: 373.3.

Step 4: Synthesis of 1-(6-(methylthio)-5-nitropyridin-3-yl)-1,6,10-trioxo-11-(prop-2-yn-1-yl)-8-oxa-2,5,11-triazatridecan-13-yl (2-(trimethylammonio)ethyl) phosphate (B-38)

[0391] To a solution of **B-37** (1.0 g, 2.68 mmol) in DMAc (15 mL) was added **B-2** (2.0 g, 5.38 mmol) and DIPEA (1.4 g, 10.8 mmol), followed by PyBoP (2.8 g, 5.38 mmol). The reaction mixture was stirred at R.T. for 16 hrs and purified by prep-HPLC to give 1-(6-(methylthio)-5-nitropyridin-3-yl)-1,6,10-trioxo-11-(prop-2-yn-1-yl)-8-oxa-2,5,11-triazatridecan-13-yl (2-(trimethylammonio)ethyl) phosphate **B-38** (1.1 g, 66%) as yellow solid.

[0392] LCMS[M+H]⁺: 619.19.

[0393] ¹H NMR (600 MHz, DMSO-*d*₆) δ: 9.54 (d, J= 55.1 Hz, 1H), 9.28 (s, 1H), 9.05-8.97 (m, 1H), 8.38 (dd, J= 28.2, 22.9 Hz, 1H), 4.41 (s, 1H), 4.24 (d, J= 23.3 Hz, 1H), 4.15 (t, J= 8.7 Hz, 2H), 4.07 (s, 1H), 3.93 (s, 2H), 3.87 (dd, J= 12.2, 5.2 Hz, 2H), 3.58-3.48 (m, 4H), 3.44-3.36 (m, 3H), 3.17 (t, J= 2.1 Hz, 1H), 3.11 (d, J= 7.3 Hz, 9H), 2.60 (s, 3H), 2.03-1.96 (m, 1H), 1.45 (s, 1H), 1.24 (s, 2H), 0.85 (t, J= 7.0 Hz, 1H)

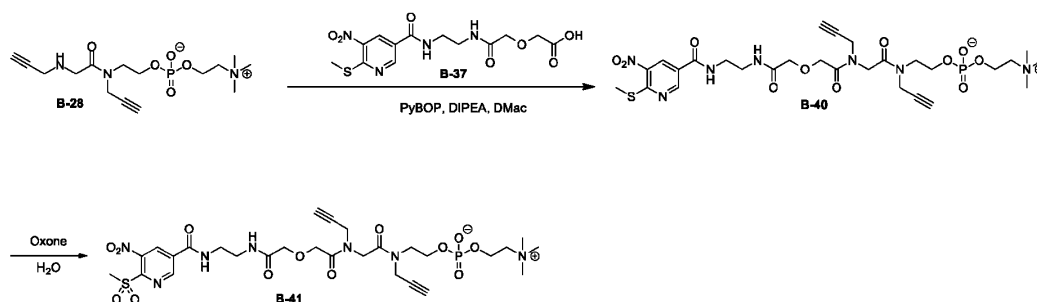
Step 5: Synthesis of 1-(6-(methylsulfonyl)-5-nitropyridin-3-yl)-1,6,10-trioxo-11-(prop-2-yn-1-yl)-8-oxa-2,5,11-triazatridecan-13-yl (2-(trimethylammonio)ethyl) phosphate (B-39)

[0394] To a solution of **B-38** (1.1 g, 1.78 mmol) in DMAc (15 mL), was added m-CPBA (80%) (1.35 g, 6.22 mmol). The reaction mixture was stirred at r.t. for 16 hrs and purified by prep-HPLC to give 1-(6-(methylsulfonyl)-5-nitropyridin-3-yl)-1,6,10-trioxo-11-(prop-2-yn-1-yl)-8-oxa-2,5,11-triazatridecan-13-yl (2-(trimethylammonio)ethyl) phosphate **B-39** (1.1 g, 90%) as yellow solid.

[0395] LCMS[M+H]⁺: 651.18.

[0396] ¹H NMR (600 MHz, DMSO-*d*₆) δ: 9.36-9.19 (m, 2H), 8.92 (s, 1H), 8.20 (dt, J= 17.8, 5.6 Hz, 1H), 4.36 (d, J= 20.7 Hz, 4H), 4.27 (d, J= 24.2 Hz, 5H), 4.18 (d, J= 14.3 Hz, 5H), 4.03 (d, J= 6.3 Hz, 2H), 4.01-3.97 (m, 2H), 3.95 (d, J= 5.9 Hz, 3H), 3.65-3.59 (m, 2H), 3.56 (t, J= 5.1 Hz, 2H), 3.44-3.38 (m, 2H), 3.35 (t, J= 9.7 Hz, 3H), 3.12 (d, J= 4.2 Hz, 10H), 2.95 (s, 3H), 2.78 (s, 3H), 1.96 (s, 3H)

Example 11: Synthesis of B-41



Step 1: Synthesis of 1-(6-(methylthio)-5-nitropyridin-3-yl)-1,6,10,13-tetraoxo-11,14-di(prop-2-yn-1-yl)-8-oxa-2,5,11,14-tetraazahexadecan-16-yl (2-(trimethylammonio)ethyl) phosphate (B-40)

[0397] To a solution of **B-28** (320 mg, 0.9 mmol, 1.0 eq) and **B-37** (330 mg, 0.9 mmol, 1.0 eq) in DMAc (3 mL) was added DIPEA (344 mg, 2.7 mmol, 3.0 eq) at r.t., followed by PyBOP (461 mg, 0.9 mmol, 1.0 eq). The resulting mixture was stirred at room temperature for 16 hours and purified by prep-HPLC to give 1-(6-(methylthio)-5-nitropyridin-3-yl)-1,6,10,13-tetraoxo-11,14-di(prop-2-yn-1-yl)-8-oxa-2,5,11,14-tetraazahexadecan-16-yl (2-(trimethylammonio)ethyl) phosphate **B-40** (300 mg, yield: 72%) as a white solid.

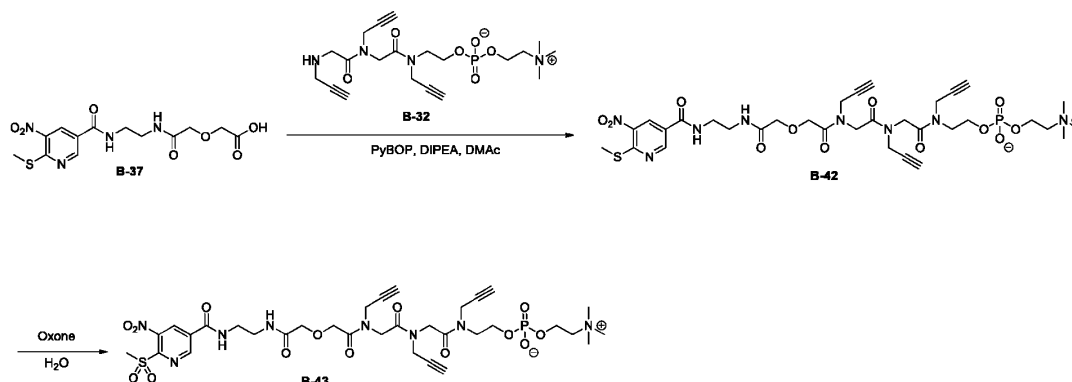
[0398] LCMS[M+H]⁺: 714.7.

Step 2: Synthesis of 1-(6-(methylsulfonyl)-5-nitropyridin-3-yl)-1,6,10,13-tetraoxo-11,14-di(prop-2-yn-1-yl)-8-oxa-2,5,11,14-tetraazahexadecan-16-yl (2-(trimethylammonio)ethyl) phosphate (B-41)

[0399] To a solution of **B-40** (300 mg, 0.42 mmol, 1.0 eq) in H₂O (12.0 mL) was added Oxone (1.033 g, 1.68 mmol, 4.0 eq), then stirred at r.t. for 24 h. The reaction mixture was purified by prep-HPLC to afford 1-(6-(methylsulfonyl)-5-nitropyridin-3-yl)-1,6,10,13-tetraoxo-11,14-di(prop-2-yn-1-yl)-8-oxa-2,5,11,14-tetraazahexadecan-16-yl (2-(trimethylammonio)ethyl) phosphate **B-41** (210.6 mg, yield: 60%) as a white solid.

[0400] LCMS[M+H]⁺: 746.6.

Example 12: Synthesis of B-43



Step 1: Synthesis of 1-(6-(methylthio)-5-nitropyridin-3-yl)-1,6,10,13,16-pentaoxo-11,14,17-tri(prop-2-yn-1-yl)-8-oxa-2,5,11,14,17-pentaazonadecan-19-yl (2-(trimethylammonio)ethyl) phosphate (B-42)

[0401] To a solution of **B-32** (983 mg, 2.16 mmol, 1.0 eq) and **B-37** (804 mg, 2.16 mmol, 1.0 eq) in DMAc (10 mL) was added DIPEA (836 mg, 6.48 mmol, 3.0 eq) at r.t., followed by PyBOP (1.12 g, 2.16 mmol, 1.0 eq). The resulting mixture was stirred at room temperature for 16 hours and purified by prep-HPLC to give 1-(6-(methylthio)-5-nitropyridin-3-yl)-1,6,10,13,16-pentaoxo-11,14,17-tri(prop-2-yn-1-yl)-8-oxa-2,5,11,14,17-pentaazonadecan-19-yl (2-(trimethylammonio)ethyl) phosphate (**B-42**) (830 mg, yield: 50%) as a white solid.

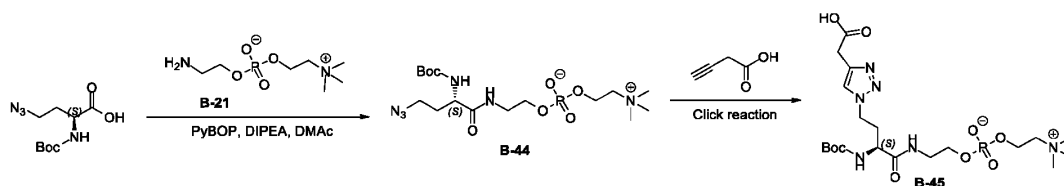
[0402] LCMS[M+H]⁺: 809.8.

Step 2: Synthesis of 1-(6-(methylsulfonyl)-5-nitropyridin-3-yl)-1,6,10,13,16-pentaoxo-11,14,17-tri(prop-2-yn-1-yl)-8-oxa-2,5,11,14,17-pentaazonadecan-19-yl (2-(trimethylammonio)ethyl) phosphate (B-43)

[0403] To a solution of **B-42** (830 mg, 1.03 mmol, 1.0 eq) in H₂O (12 mL) was added Oxone (2.52 g, 4.11 mmol, 4.0 eq), then stirred at r.t. for 24 h. The reaction mixture was purified by prep-HPLC to afford 1-(6-(methylsulfonyl)-5-nitropyridin-3-yl)-1,6,10,13,16-pentaoxo-11,14,17-tri(prop-2-yn-1-yl)-8-oxa-2,5,11,14,17-pentaazonadecan-19-yl (2-(trimethylammonio)ethyl) phosphate (**B-43**) (540 mg, yield: 78%) as a white solid.

[0404] LCMS[M+H]⁺: 841.7.

Example 13: Synthesis of B-45



Step 1: Synthesis of (S)-2-(4-azido-2-((tert-butoxycarbonyl)amino)butanamido)ethyl (2-(trimethylammonio)ethyl) phosphate (B-44)

[0405] To a solution of (S)-4-azido-2-((tert-butoxycarbonyl)amino)butanoic acid (215.9 mg, 0.88 mmol) in DMAc (2.0 mL) was added **B-19** (100 mg, 0.44 mmol), DIPEA (125.7 mg, 0.97 mmol) and PyBOP (460 mg, 0.88 mmol). The mixture was stirred at room temperature for overnight. The reaction was complete detected by LC-MS, then purified by prep-HPLC to afford (S)-2-(4-azido-2-((tert-butoxycarbonyl)amino)butanamido)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-44**) (165.0 mg, yield: 85%) as a white solid.

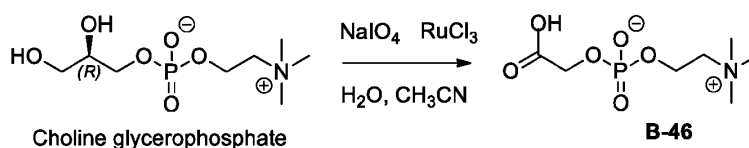
[0406] LCMS[M+H]⁺: m/z = 453.4.

Step 2: The synthesis of (S)-2-(2-((tert-butoxycarbonyl)amino)-4-(4-(carboxymethyl)-1H-1,2,3-triazol-1-yl)butanamido)ethyl (2-(trimethylammonio)ethyl) phosphate (B-45)

[0407] To a solution of **B-44** (130.0 mg, 0.287 mmol) in DMSO/H₂O (8 mL/8 mL) was added but-3-ynoic acid (26.6 mg, 0.316 mmol), TBTA (60.9 mg, 0.115 mmol), CuSO₄·5H₂O (28.7 mg, 0.115 mmol) and sodium ascorbate (45.0 mg, 0.23 mmol). The mixture was stirred at room temperature for 1 hour, then purified by prep-HPLC to afford (S)-2-(2-((tert-butoxycarbonyl)amino)-4-(4-(carboxymethyl)-1H-1,2,3-triazol-1-yl)butanamido)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-45**) (100 mg, yield: 66.7%) as a white solid.

[0408] LCMS[M+H]⁺: m/z = 537.4

Example 14: Synthesis of carboxymethyl (2-(trimethylammonio)ethyl) phosphate (B-46)



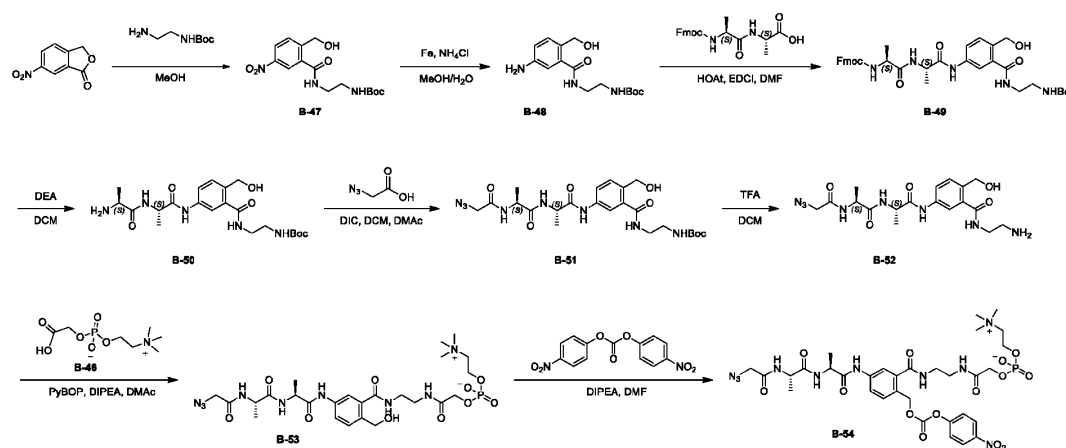
[0409] To a solution of choline glycerophosphate (10 g, 39 mmol, 1.0 eq) in 100 mL water, adding 45 mL acetonitrile, and ruthenium (III) chloride (129 mg, 0.62 mmol), followed

by NaIO₄ (35.7 g, 165.1 mmol) in portions, controlled the temperature below 50°C. After the addition, the mixture was stirred at r.t. for 3 hrs, then concentrated. To the residue was added methanol (200 mL) and stirred at r.t. for 2 h, filtered to remove solid and the organic phase was concentrated. Ethanol (200 mL) was added to the residue and stirred for another 30 min, filtered to remove solid, the organic phase was concentrated and dried to get carboxymethyl (2-(trimethylammonio)ethyl) phosphate (**B-46**) (8.8 g, yield 93.9%) as a pale white solid.

[0410] LCMS[M+H]⁺: 242.06.

[0411] ¹H NMR (600 MHz, DMSO-*d*₆) δ 4.17 (d, 2H), 4.13 (dd, 2H), 3.56-3.54 (m, 2H), 3.10 (s, 9H).

Example 15: Synthesis of B-54



Step 1: Synthesis of tert-butyl (2-(2-(hydroxymethyl)-5-nitrobenzamido)ethyl)carbamate (**B-47**)

[0412] To a solution of 6-nitroisobenzofuran-1(3H)-one (3.0 g, 16.75 mmol) in MeOH (30 mL) was added tert-butyl (2-aminoethyl)carbamate (5.37 g, 33.5 mmol). The reaction mixture was stirred at 50°C for 16 hrs. The reaction was completed as monitored by LC-MS. After removal of solvent by concentration, the residue was purified by silica gel chromatography with PE: EtOAc=10:1 to give tert-butyl (2-(2-(hydroxymethyl)-5-nitrobenzamido)ethyl)carbamate (**B-47**) (2.4 g, 42.2%) as yellow solid.

[0413] LCMS[M-H]⁻: 338.3.

[0414] ^1H NMR (600 MHz, DMSO- d_6) δ 8.64 (t, J = 5.5 Hz, 1H), 8.36-8.19 (m, 2H), 7.86 (d, J = 8.6 Hz, 1H), 6.94 (t, J = 5.8 Hz, 1H), 5.52 (t, J = 5.6 Hz, 1H), 4.72 (d, J = 5.6 Hz, 2H), 3.30 (d, J = 23.5 Hz, 1H), 3.27 (dd, J = 11.9, 6.0 Hz, 2H), 3.12 (q, J = 6.0 Hz, 2H), 1.38 (s, 9H).

Step 2: Synthesis of tert-butyl (2-(5-amino-2-(hydroxymethyl)benzamido)ethyl)carbamate (B-48)

[0415] To a solution of **B-47** (2.926 g, 8.6 mol) and Fe (2.405 g, 43.11 mol) in MeOH (71 mL), was added dropwise a solution of NH_4Cl (4.612 g, 1 mmol) in H_2O (70 mL) with stirring at r.t. under N_2 . The reaction mixture was stirred at 60 °C for 16 hrs. The reaction was complete detected by LC-MS. The suspension was filtered through a pad of diatomite and washed with MeOH (5mL $\times$ 2). The combined filtrates were concentrated to dryness to give crude. The crude product was purified by prep-HPLC to give tert-butyl (2-(5-amino-2-(hydroxymethyl)benzamido)ethyl)carbamate (**B-48**) (1.1 g, 41.2%) as white solid.

[0416] LCMS[M+H] $^+$: 292.2.

[0417] ^1H NMR (600 MHz, DMSO- d_6) δ 8.31 (t, J = 5.4 Hz, 1H), 7.06 (d, J = 8.2 Hz, 1H), 6.89-6.76 (m, 1H), 6.67 (d, J = 2.2 Hz, 1H), 6.58 (dd, J = 8.1, 2.4 Hz, 1H), 5.15 (s, 2H), 4.99 (t, J = 5.5 Hz, 1H), 4.35 (dd, J = 14.7, 5.5 Hz, 2H), 3.27-3.18 (m, 2H), 3.07 (dd, J = 12.5, 6.3 Hz, 2H), 1.38 (s, 9H).

Step 3: Synthesis of B-49

[0418] To a solution of **B-48** (1.1 g, 3.56 mmol) in DMF (71 mL) was added Fmoc-Ala-Ala-OH (1.359 g, 3.56 mmol), HOAt (508 mg, 3.73 mmol) and EDCI (715 mg, 3.73 mmol) under N_2 , and stirred at r.t. for 3 hrs. The reaction was complete detected by LC-MS. This reaction mixture was purified by prep-HPLC to give **B-49** (1.48 g, 61.8%) as white solid.

[0419] LCMS[M+H] $^+$: 674.6.

[0420] ^1H NMR (600 MHz, DMSO- d_6) δ 10.08 (d, J = 68.3 Hz, 1H), 8.38 (t, J = 5.6 Hz, 1H), 8.12 (d, J = 7.2 Hz, 1H), 7.89 (d, J = 7.5 Hz, 2H), 7.71 (dd, J = 20.7, 12.2 Hz, 3H), 7.63 (dd, J = 8.4, 1.9 Hz, 1H), 7.54 (d, J = 7.5 Hz, 1H), 7.46-7.39 (m, 3H), 7.33 (t, J = 7.4 Hz, 2H), 4.51 (s, 2H), 4.39 (dd, J = 14.3, 7.2 Hz, 1H), 4.32-4.15 (m, 4H), 4.12-4.07 (m, 1H), 3.25 (dd, J = 12.6, 6.3 Hz, 2H), 3.08 (dd, J = 12.7, 6.4 Hz, 2H), 1.37 (s, 9H), 1.31 (d, J = 7.1 Hz, 3H), 1.26-1.21 (m, 3H).

Step 4: Synthesis of tert-butyl (2-(5-((S)-2-((S)-2-aminopropanamido)propanamido)-2-

(hydroxymethyl)benzamido)ethyl)carbamate (B-50)

[0421] To a solution of **B-49** (1.480 g, 2.197 mmol) in DCM (10 mL) was added DEA (1.606 g, 21.97 mmol) in dropwise under N₂. The reaction mixture was stirred at r.t for 16 hrs. The reaction was complete detected by LC-MS. This reaction mixture was purified by prep-HPLC to give tert-butyl (2-(5-((S)-2-((S)-2-aminopropanamido)propanamido)-2-(hydroxymethyl)benzamido)ethyl)carbamate (**B-50**) (800 mg, 80.7%) as white solid.

[0422] LCMS[M+H]⁺: 452.4.

[0423] ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.12 (s, 1H), 8.38 (t, *J* = 5.6 Hz, 1H), 8.23 (s, 1H), 7.72-7.57 (m, 2H), 7.42 (d, *J* = 8.4 Hz, 1H), 6.85 (t, *J* = 5.6 Hz, 1H), 5.21 (t, *J* = 5.6 Hz, 1H), 4.49 (t, *J* = 20.4 Hz, 2H), 4.43 (s, 1H), 3.46-3.35 (m, 1H), 3.25 (dd, *J* = 12.6, 6.4 Hz, 2H), 3.08 (dd, *J* = 12.7, 6.4 Hz, 2H), 1.35 (d, *J* = 33.7 Hz, 9H), 1.30 (t, *J* = 14.6 Hz, 3H), 1.21-1.11 (m, 3H).

Step 5: Synthesis of tert-butyl (2-(5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(hydroxymethyl)benzamido)ethyl)carbamate (B-51)

[0424] To a solution of **B-50** (800 mg, 1.772 mmol) in DCM (10 mL) was added 2-azidoacetic acid (537 mg, 5.3 mmol) in dropwise, followed by DIC (447 mg, 3.54 mmol) and DMAc (1 mL) under N₂. The reaction mixture was stirred at r.t for 3 hrs. The reaction was complete detected by LC-MS. This reaction mixture was purified by prep-HPLC to give tert-butyl (2-(5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(hydroxymethyl)benzamido)ethyl)carbamate (**B-51**) (600 mg, 53.4%) as white solid.

[0425] LCMS[M+H]⁺: 533.5.

[0426] ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.99 (s, 1H), 8.41-8.20 (m, 3H), 7.73-7.55 (m, 2H), 7.42 (d, *J* = 8.4 Hz, 1H), 6.84 (t, *J* = 5.6 Hz, 1H), 5.20 (dd, *J* = 7.2, 4.1 Hz, 1H), 4.50 (d, *J* = 5.6 Hz, 2H), 4.38 (dd, *J* = 21.6, 7.2 Hz, 2H), 3.93-3.77 (m, 2H), 3.08 (dd, *J* = 12.7, 6.4 Hz, 2H), 1.38 (s, 9H), 1.31 (t, *J* = 5.8 Hz, 3H), 1.28-1.12 (m, 3H).

Step 6: Synthesis of N-(2-aminoethyl)-5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(hydroxymethyl)benzamide (B-52)

[0427] To a solution of **B-51** (400 mg, 0.75 mmol) in DCM (10 mL), was added dropwise a solution of TFA (2 mL) with stirring at r.t under N₂. The reaction mixture was stirred at r.t for 3 hrs. The reaction was complete detected by LC-MS. This reaction mixture was purified by prep.

HPLC to give N-(2-aminoethyl)-5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(hydroxymethyl)benzamide (**B-52**) (50 mg, 15.4%) as white solid.

[0428] LCMS[M-H]⁻: 435.5.

[0429] ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.00 (s, 1H), 8.55-8.46 (m, 1H), 8.30 (dd, *J* = 29.9, 7.2 Hz, 2H), 7.86-7.72 (m, 1H), 7.61 (m, *J* = 16.2, 8.3, 2.1 Hz, 1H), 7.43 (d, *J* = 8.4 Hz, 1H), 5.34 (s, 1H), 4.56 (d, *J* = 3.5 Hz, 1H), 4.37 (m, *J* = 25.0, 7.1 Hz, 1H), 3.93-3.78 (m, 2H), 3.56-3.38 (m, 2H), 3.00-2.82 (m, 2H), 1.31 (m, 2H), 1.26-1.19 (m, 8H).

Step 7: Synthesis of 2-((2-(5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(hydroxymethyl)benzamido)ethyl)amino)-2-oxoethyl (2-(trimethylammonio)ethyl) phosphate (B-53)

[0430] At 0 °C, to a solution of **B-52** (45 mg, 0.1 mmol) in DMAc (2 mL) was added carboxymethyl (2-(trimethylammonio)ethyl) phosphate (**B-40**) (37.5 mg, 0.15 mmol), PyBop (80.85 mg, 0.15 mmol) and DIPEA (40 mg, 0.31 mmol) under N₂. The reaction mixture was stirred at r.t for 16 hrs. The reaction was complete detected by LC-MS. This reaction mixture was purified by prep-HPLC to give 2-((2-(5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(hydroxymethyl)benzamido)ethyl)amino)-2-oxoethyl (2-(trimethylammonio)ethyl) phosphate (**B-53**) (48 mg, 70.1%) as white solid.

[0431] LCMS[M-H]⁻: 658.5.

[0432] ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.92 (s, 1H), 8.70-8.51 (m, 1H), 8.32 (t, *J* = 6.9 Hz, 3H), 8.01 (d, *J* = 6.7 Hz, 1H), 7.92 (dd, *J* = 15.4, 10.0 Hz, 1H), 7.65 (dd, *J* = 8.0, 2.2 Hz, 1H), 7.40 (d, *J* = 8.5 Hz, 1H), 4.51-4.41 (m, 1H), 4.36 (dd, *J* = 14.4, 7.2 Hz, 1H), 4.13 (dd, *J* = 30.5, 14.7 Hz, 4H), 3.92-3.78 (m, 2H), 3.62 (m, 2H), 3.57-3.53 (m, 2H), 3.16-3.06 (m, 13H), 1.37-1.27 (m, 6H).

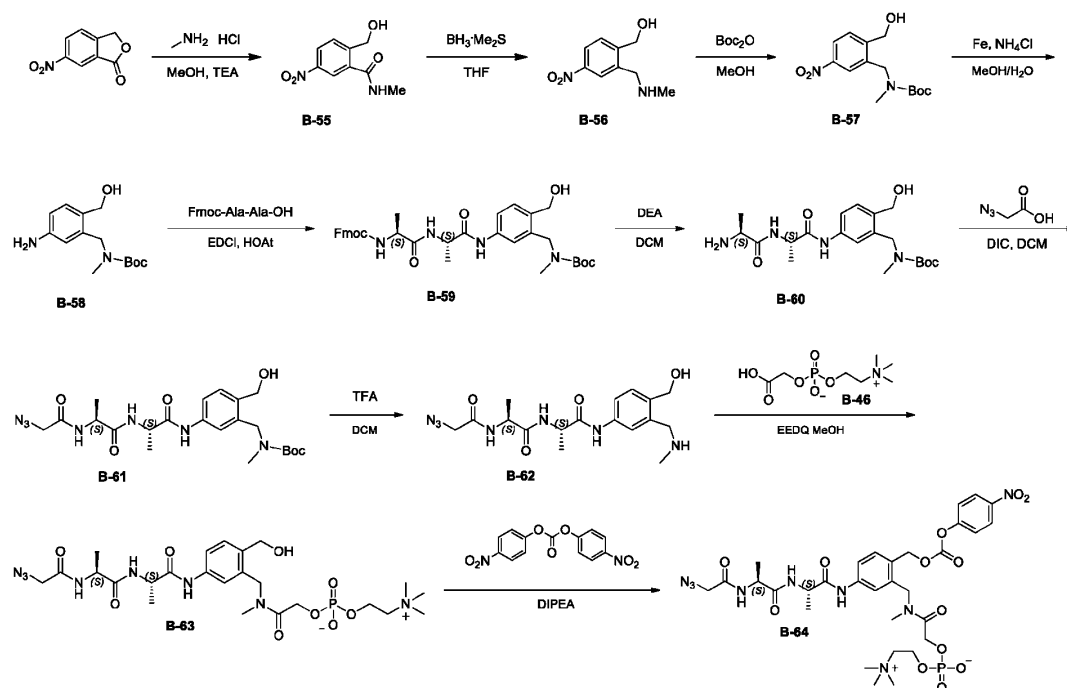
Step 8: Synthesis of 2-((2-(5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(((4-nitrophenoxy)carbonyl)oxy)methyl)benzamido)ethyl)amino)-2-oxoethyl (2-(trimethylammonio)ethyl) phosphate (B-54)

[0433] To a solution of **B-53** (28 mg, 0.043 mmol, 1.0 eq) in DMF (2 mL), were added dropwise a solution of **bis(4-nitrophenyl) carbonate** (51.8 mg, 0.17 mmol, 4.0 eq) and DIPEA (16.5 mg, 0.13 mmol, 3.0 eq) with stirring at r.t under N₂. The reaction mixture was stirred at r.t for 16 hrs. The reaction was complete detected by LC-MS. This reaction mixture was purified by

prep. HPLC to give 2-((2-(5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(((4-nitrophenoxy)carbonyl)oxy)methyl)benzamido)ethyl)amino)-2-oxoethyl (2-(trimethylammonio)ethyl) phosphate (**B-54**) (7.64 mg, 21.6%) as white solid.

[0434] LCMS[M+H]⁺: 823.5.

Example 16: Synthesis of B-64



Step 1: Synthesis of 2-(hydroxymethyl)-N-methyl-5-nitrobenzamide (**B-55**)

[0435] To a solution of 6-nitroisobenzofuran-1(3H)-one (5.0 g, 27.9 mmol) and methylamine hydrochloride (11.25 g, 167.4 mmol) in MeOH (40 mL) was added TEA (17.0 g, 167.4 mmol). The reaction mixture was stirred at 45°C for 16 hrs. The solvent was removed and added EA (100 mL) and PE (100 mL), then filtered to get 2-(hydroxymethyl)-N-methyl-5-nitrobenzamide (**B-55**) (4.4 g, 74.9%) as white solid.

[0436] LCMS[M+H]⁺: 211.1.

Step 2: Synthesis of 2-((methylamino)methyl)-4-nitrophenylmethanol (**B-56**)

[0437] To a solution of **B-55** (4.0 g, 18.8 mmol) in THF (40 mL) was added borane-dimethyl sulfide (2 M/L, 18.2 mL, 56.4 mmol) at 0°C under N₂. The reaction mixture was stirred at 70 °C

for 16 hrs. Then added HCl in MeOH (1M/L, 50 mL) at 0°C and stirred for 1 hour, then removed solvent and washed by EA, then filtered to get (2-((methylamino)methyl)-4-nitrophenyl)methanol (**B-56**) (3.0 g, 81.4%) as white solid.

[0438] LCMS[M+H]⁺: 197.2.

[0439] ¹H NMR (600 MHz, DMSO-*d*₆) δ 8.58 (d, *J* = 4.1 Hz, 1H), 8.32 (dd, *J* = 8.5, 2.4 Hz, 1H), 8.21 (d, *J* = 2.4 Hz, 1H), 7.87 (d, *J* = 8.6 Hz, 1H), 5.54 (s, 1H), 4.72 (s, 2H), 2.78 (d, *J* = 4.6 Hz, 3H).

Step 3: Synthesis of tert-butyl 2-(hydroxymethyl)-5-nitrobenzyl(methyl)carbamate (**B-57**)

[0440] To a solution of **B-56** (1.0 g, 5.1 mmol) in MeOH (10 mL) was added di-tert-butyl dicarbonate (1.3 g, 6.1 mmol) and DIPEA (1.3 g, 10.2 mmol). The reaction mixture was stirred at r.t. for 16 hrs. After removing solvent, EA (30 mL) was added and washed by sat. NaHCO₃ (10 mL*3), followed by brine (10 mL*2). The organic phase was dried over Na₂SO₄, filtered and concentrated to dryness to give tert-butyl 2-(hydroxymethyl)-5-nitrobenzyl(methyl)carbamate (**B-57**) (0.7 g, 46.4%) as white solid.

[0441] LCMS[M-100+H]⁺: 197.1.

[0442] ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.13 (d, *J* = 41.3 Hz, 2H), 8.46 (d, *J* = 2.5 Hz, 1H), 8.28 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.76 (d, *J* = 8.5 Hz, 1H), 4.77 (s, 2H), 4.31-4.25 (m, 2H), 2.64 (d, *J* = 2.8 Hz, 3H).

Step 4: Synthesis of tert-butyl 5-amino-2-(hydroxymethyl)benzyl(methyl)carbamate (**B-58**)

[0443] To a suspended solution of **B-57** (600 mg, 2.02 mmol) and Fe (1128 mg, 20.2 mmol) in MeOH (10 mL), was added dropwise a solution of NH₄Cl (2161.4 mg, 40.4 mmol) in H₂O (10 mL) with stirring at r.t. under N₂. The reaction mixture was stirred at 60°C for 16 hrs. The suspended solution was filtered through a pad of diatomite and washed with MeOH (5 mL*2). The combined filtrates were concentrated to dryness to give tert-butyl 5-amino-2-(hydroxymethyl)benzyl(methyl)carbamate (**B-58**) (510 mg, 94.9%) as white solid.

[0444] LCMS[M-117]⁺: 149.1.

Step 5: Synthesis of tert-butyl 5-((S)-2-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)propanamido)-2-(hydroxymethyl)benzyl(methyl)carbamate (**B-59**)

[0445] To a solution of **B-58** (510 mg, 1.92 mmol) in DMF (7 mL) was added Fmoc-Ala-Ala-OH (806.5 mg, 2.1 mmol), HOAt (313.2 mg, 2.3 mmol) and EDCI (357.2 mg, 2.3 mmol) at r.t. under N₂. The reaction mixture was stirred at r.t for 16 hrs. This reaction mixture was purified by prep-HPLC to give tert-butyl 5-((S)-2-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)propanamido)-2-(hydroxymethyl)benzyl(methyl)carbamate (**B-59**) (270 mg, 22.3%) as white solid.

[0446] LCMS[M-117]⁻: 513.5.

[0447] ¹H NMR (600 MHz, DMSO-*d*₆) δ 9.93 (s, 1H), 8.10 (d, *J* = 7.3 Hz, 1H), 7.89 (d, *J* = 7.5 Hz, 2H), 7.72 (dd, *J* = 13.1, 7.6 Hz, 2H), 7.54 (d, *J* = 7.6 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.36-7.26 (m, 4H), 4.44 (s, 2H), 4.40 (d, *J* = 10.5 Hz, 3H), 4.30-4.19 (m, 3H), 4.11-4.07 (m, 1H), 2.74 (s, 3H), 1.39 (d, *J* = 43.0 Hz, 9H), 1.29 (d, *J* = 7.1 Hz, 3H), 1.23 (d, *J* = 7.1 Hz, 3H).

Step 6: Synthesis of tert-butyl 5-((S)-2-((S)-2-aminopropanamido)propanamido)-2-(hydroxymethyl)benzyl(methyl)carbamate (B-60)

[0448] To a solution of **B-59** (270 mg, 0.43 mmol) in DCM (2.7 mL) was added DEA (0.94 mL, 3.5v/w) in dropwise with stirring at r.t. under N₂. The reaction mixture was stirred at r.t for 16 hrs. The reaction was complete detected by LC-MS. This reaction mixture was purified by prep-HPLC to give tert-butyl 5-((S)-2-((S)-2-aminopropanamido)propanamido)-2-(hydroxymethyl)benzyl(methyl)carbamate (**B-60**) (80 mg, 46%) as white solid.

[0449] LCMS[2M+H]⁺: 817.7.

Step 7: Synthesis of tert-butyl 5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(hydroxymethyl)benzyl(methyl)carbamate (B-61)

[0450] To a stirred solution of **B-60** (80 mg, 0.19 mmol) in DCM (0.3 mL) was added 2-azidoacetic acid (29 mg, 0.25 mmol), DIC (31 mg, 0.24 mmol) at r.t. under N₂. The reaction mixture was stirred at r.t for 16 hrs. The reaction was complete detected by LC-MS. This reaction mixture was purified by prep-HPLC to give tert-butyl 5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(hydroxymethyl)benzyl(methyl)carbamate (**B-61**) (44 mg, 43%) as white solid.

[0451] LCMS[M-17+H]⁺: 475.3.

Step 8: Synthesis of (S)-2-(2-azidoacetamido)-N-((S)-1-((4-(hydroxymethyl)-3-((methylamino)methyl)phenyl)amino)-1-oxopropan-2-yl)propanamide (B-62)

[0452] To a stirred solution of **B-61** (44 mg, 0.08 mmol) in DCM (2.0 mL), was added TFA (0.66 mL) at 0°C under N₂. The reaction mixture was stirred at r.t for 3 hrs. The reaction was completed as monitored by LC-MS. Then removed the solvent and the crude (S)-2-(2-azidoacetamido)-N-((S)-1-((4-(hydroxymethyl)-3-((methylamino)methyl)phenyl)amino)-1-oxopropan-2-yl)propanamide (**B-62**) is to use to **step 9** directly.

[0453] LCMS[M+H]⁺: 392.4.

Step 9: Synthesis of 2-((5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(hydroxymethyl)benzyl)(methyl)amino)-2-oxoethyl (2-(trimethylammonio)ethyl) phosphate (B-63)

[0454] To a stirred solution of **B-62** (33 mg, 0.08 mmol) in MeOH (2 mL) was added carboxymethyl (2-(trimethylammonio)ethyl) phosphate (**B-46**) (40.5 mg, 0.17 mmol), EEDQ (40.4 mg, 0.17 mmol) at r.t. under N₂. The reaction mixture was stirred at r.t for 16 hrs. The reaction was complete detected by LC-MS. This reaction mixture was purified by prep-HPLC to give 2-((5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(hydroxymethyl)benzyl)(methyl)amino)-2-oxoethyl (2-(trimethylammonio)ethyl) phosphate (**B-63**) (20 mg, 38.8%) as white solid.

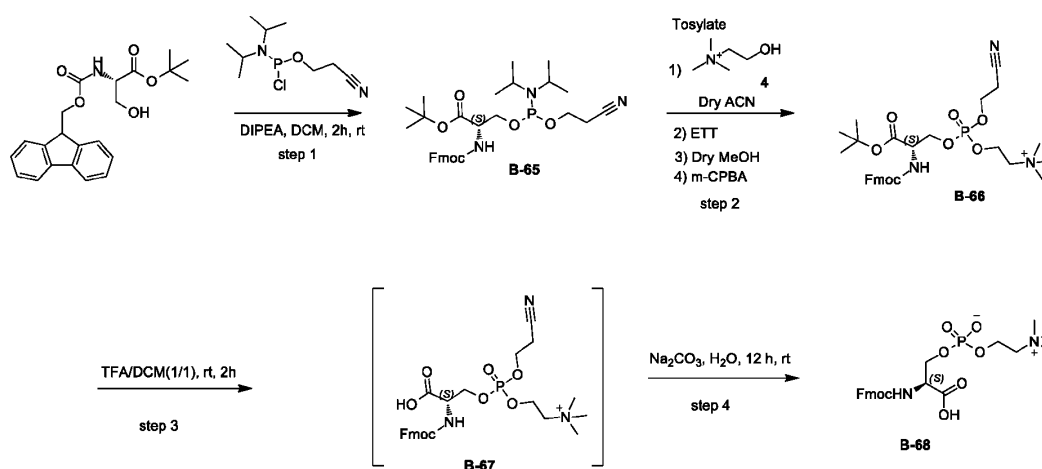
[0455] LCMS[M-H]⁺: 615.3.

Step 10: Synthesis of 2-((5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl)(methyl)amino)-2-oxoethyl (2-(trimethylammonio)ethyl) phosphate (B-64)

[0456] To a solution of **B-63** (20 mg, 0.03 mmol) in DMF (1.5 mL) was added dropwise a solution of bis(4-nitrophenyl) carbonate (267.2 mg, 0.17 mmol) and DIPEA (125 mg, 1 mmol) at r.t under N₂. The reaction mixture was stirred at r.t for 16 hrs. The reaction was complete detected by LC-MS. This reaction mixture was purified by prep-HPLC to give 2-((5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl)(methyl)amino)-2-oxoethyl (2-(trimethylammonio)ethyl) phosphate (**B-64**) (13.0 mg, 38%) as white solid.

[0457] LCMS[M+H]⁺: 780.4.

Example 17: Synthesis of B-68



Step 1: Synthesis of tert-butyl N-(((9H-fluoren-9-yl)methoxy)carbonyl)-O-((2cyanoethoxy)(diisopropylamino)phosphaneyl)-L-serinate (B-65)

[0458] tert-butyl (2S)-2-{[(9H-fluoren-9-ylmethoxy)carbonyl]amino}-3-hydroxypropanoate (22.4 g, 58.4 mmol) was first dried by co-evaporation with toluene (2*50 mL). Dry DCM (180 mL) and DIPEA (12.8 g, 99.2 mmol) were added under nitrogen and 3-{[chloro (diisopropylamino)phosphanyl]oxy}propanenitrile (17.97 g, 75.9 mmol) was added slowly via a syringe. The reaction mixture was stirred for 2h at room temperature. Quenched with methanol (30 mL) and was washed with a solution of sodium bicarbonate and brine. The aqueous phase was extracted with DCM. The organic phase was dried over Na₂SO₄, filtered, and evaporated under vacuum. The crude product was purified by Flash Chromatography (PE:EA=2:3) to give compound tert-butyl N-(((9H-fluoren-9-yl)methoxy)carbonyl)-O-((2cyanoethoxy)(diisopropylamino)phosphaneyl)-L-serinate (**B-65**) (27 g, 75.17%) as a colorless oil.

[0459] ¹H NMR (400 MHz, CDCl₃) δ 7.77 (d, *J* = 7.6 Hz, 2H); 7.62 (t, *J* = 6.4 Hz, 2H), 7.41 (t, *J* = 7.6 Hz, 2H), 7.32 (dd, *J* = 2.4 Hz, 7.6 Hz, 2H), 7.30 (d, *J* = 6.4 Hz, 2H), 5.74 (d, *J* = 8.4 Hz, 1H), 4.0 – 4.1 (m, 6H), 3.5-3.8 (m, 4H), 2.53-2.6 (m, 2H), 1.49 (s, 9H), 1.16-1.24 (m, 12H).

Step 2: Synthesis of 2-(((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tert-butoxy)-3-oxopropoxy)(2-cyanoethoxy)phosphoryl)oxy)-N,N,N-trimethylethan-1-aminium (B-66)

[0460] tert-butyl N-(((9H-fluoren-9-yl)methoxy)carbonyl)-O-((2cyanoethoxy) (diisopropylamino)phosphaneyl)-L-serinate (**B-65**) (27.5 g, 46.9 mmol) was dried with dry toluene and dry ACN (2*50 mL). Choline p-toluenesulfonate (14.2 g, 51.7 mmol) was dried with toluene and dissolved in dry ACN (500 mL). This solution was added to tert-butyl N-(((9H-fluoren-9-yl)methoxy)carbonyl)-O-((2cyanoethoxy) (diisopropylamino)phosphaneyl)-L-serinate (**B-65**) through a cannula. ETT (6.12 g, 46.9 mmol) was dissolved in dry CH₃CN (150 mL), which was added dropwise to the mixture. The mixture was stirred for 2h at room temperature. After reaching completion, the reaction mixture was quenched with methanol (20 mL). m-CPBA (19.0 g, 93.8 mmol, 85%) was added portion-wise to the mixture, the resulting mixture was stirred for 30 min. Evaporated and purified by flash chromatography (DCM:MeOH=5:1) to give compound 2-(((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tert-butoxy)-3-oxopropoxy)(2-cyanoethoxy)phosphoryl)oxy)-N,N,N-trimethylethan-1-aminium (**B-66**) (27.5 g, yield: 92.13%) as a yellow solid.

[0461] MS: m/z = 603.2(M⁺, ESI⁺).

Step 3: Synthesis of 2-(((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-carboxyethoxy)(2-cyanoethoxy)phosphoryl)oxy)-N,N,N-trimethylethan-1-aminium (B-67**)**

[0462] To a solution of 2-(((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tert-butoxy)-3-oxopropoxy)(2-cyanoethoxy)phosphoryl)oxy)-N,N,N-trimethylethan-1-aminium (**B-66**) (27.0 g, 44.7 mmol) and Triisopropylsilane (26.2 g, 165.3 mmol) in DCM (150 mL) stirred at 25°C under nitrogen was added TFA (150 mL) dropwise. The reaction mixture was stirred at 25°C for 2h. Evaporated to give 2-(((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-carboxyethoxy)(2-cyanoethoxy)phosphoryl)oxy)-N,N,N-trimethylethan-1-aminium (**B-67**) (19.5 g, crude product) as a yellow solid.

[0463] MS: m/z = 546.2 (M⁺, ESI⁺).

Step 4: Synthesis of (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-carboxyethyl (2-(trimethylammonio)ethyl) phosphate (B-68**)**

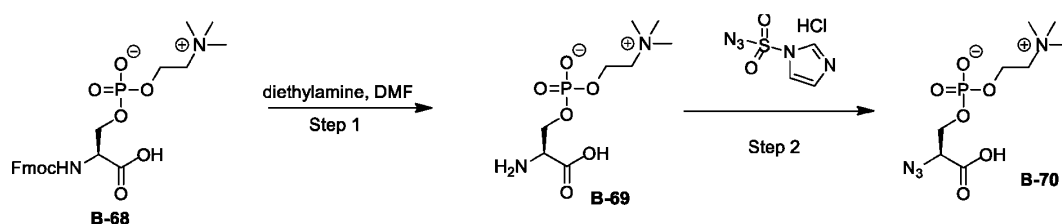
[0464] To a solution of 2-(((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-carboxyethoxy)(2-cyanoethoxy)phosphoryl)oxy)-N,N,N-trimethylethan-1-aminium (**B-67**) (5.0 g, 9.1 mmol) in H₂O (70 mL) stirred at 25°C was added a solution of sodium carbonate (2.4 g, 22.7 mmol) in H₂O (10 mL). The reaction mixture was stirred at 25°C for 12h. Adjust pH to 6

with HCl and filtered, the filtrate was purified by flash C18 ODS (ACN--H₂O 30%) to give (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-carboxyethyl (2-(trimethylammonio)ethyl) phosphate (**B-68**) (3.1 g, 68.13%) as a white solid.

[0465] MS: m/z = 493.1 (M+1, ESI+).

[0466] ¹H NMR (400 MHz, DMSO) δ 7.89 (d, J = 7.4 Hz, 2H), 7.70 (d, J = 7.3 Hz, 2H), 7.41 (t, J = 7.3 Hz, 2H), 7.35 (dd, J = 12.8, 6.6 Hz, 2H), 6.65 (s, 1H), 4.28 – 4.18 (m, 3H), 4.11 – 3.93 (m, 3H), 3.86 (s, 1H), 3.66 (s, 1H), 3.54 (s, 2H), 3.13 (s, 9H).

Example 18: Synthesis of B-70



Step 1: Synthesis of (S)-2-amino-2-carboxyethyl (2-(trimethylammonio)ethyl) phosphate (**B-69**)

[0467] To a solution of (2-(((2S)-2-carboxy-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethyl phosphonato)oxy)ethyl)trimethylazanium (**B-68**) (500 mg, 1.02 mmol) in dry DMF (6 mL) was added diethylamine (371 mg, 5.08 mmol). The mixture was stirred for 5 h at 25 °C. Diluted with water (30*3 mL), extracted with PE (100 mL). The aqueous phase was directly lyophilized and afford (S)-2-amino-2-carboxyethyl (2-(trimethylammonio)ethyl) phosphate (**B-69**) (260 mg, 85.3%) as a white solid.

[0468] LCMS (ESI): m/z 271.1 (M + H) +.

Step 2: Synthesis of (S)-2-azido-2-carboxyethyl (2-(trimethylammonio)ethyl) phosphate (**B-70**)

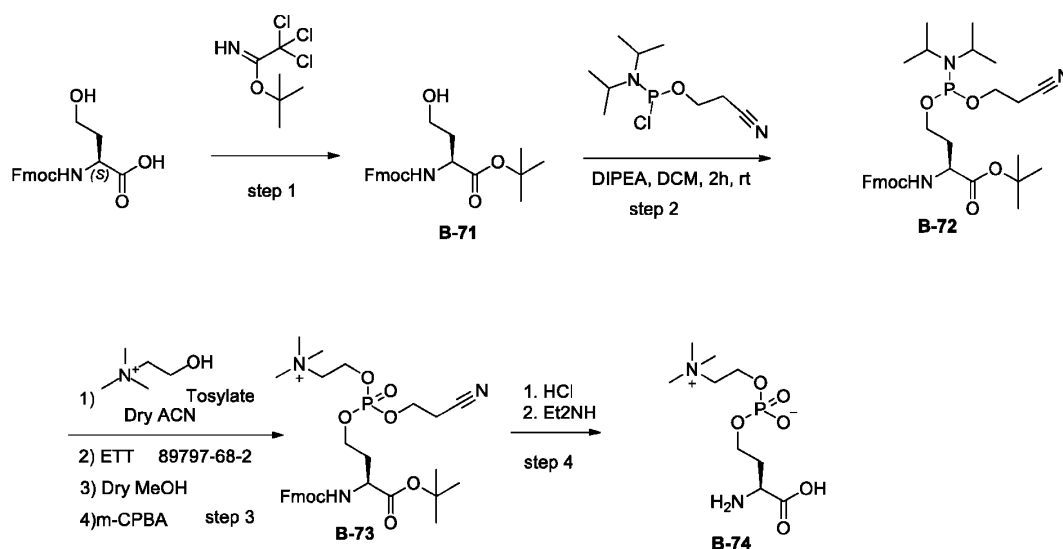
[0469] A solution of (2-(((2S)-2-amino-2-carboxyethyl (S)-2-amino-2-carboxyethyl (2-(trimethylammonio)ethyl) phosphate (**B-69**) (250 mg, 0.93 mmol), imidazole-1-sulfonyl azide hydrochloride (291 mg, 1.39 mmol), CuSO₄ 5H₂O (3 mg, 0.01 mmol) and K₂CO₃ (320 mg, 2.31 mmol) in dry MeOH (5 mL) was stirred at 25 °C for 16 h. The mixture was filtered, and the filtrate was concentrated under reduced pressure. MeCN and H₂O were added until the residue

all dissolved, purified by Flash Chromatography (ARG, ACN-H₂O, 40%) to give (S)-2-azido-2-carboxyethyl (2-(trimethylammonio)ethyl) phosphate (**B-70**) (170 mg, 58.9%) as a light yellow solid.

[0470] LCMS (ESI): m/z 297.1 (M + H)⁺.

[0471] ¹H NMR (400 MHz, D₂O) δ 4.33 – 4.25 (m, 2H), 4.16 (t, J = 4.4 Hz, 2H), 4.10 – 4.05 (m, 1H), 3.65 (t, J = 4.4 Hz, 2H), 3.20 (s, 9H).

Example 19: Synthesis of B-74



Step 1: Synthesis of tert-butyl (((9H-fluoren-9-yl)methoxy)carbonyl)-L-homoserinate (**B-71**)

[0472] A solution of (2S)-2-[(9H-fluoren-9-ylmethoxy)carbonyl]amino-4-hydroxybutanoic acid (35 g, 102.5 mmol) and tert-butyl 2,2,2-trichloroethanecarboximidate (13.4 g, 61.5 mmol) in dry DCM (500 mL) was stirred at 25 °C for 16 h. Then DCM was removed under reduced pressure and the residue was purified by flash chromatography (DCM/MeOH = 20:1 v/v) to afford tert-butyl (((9H-fluoren-9-yl)methoxy)carbonyl)-L-homoserinate (**B-71**) as a white solid (20 g, 49.8%).

[0473] LCMS (ESI): m/z 420.2 (M + Na)⁺.

Step 2: Synthesis of tert-butyl N-(((9H-fluoren-9-yl)methoxy)carbonyl)-O-((2-cyanoethoxy)(diisopropylamino)phosphaneyl)-L-homoserinate (**B-72**)

[0474] Tert-butyl (((9H-fluoren-9-yl)methoxy)carbonyl)-L-homoserinate (**B-71**) (10 g, 25.1 mmol) was first dried by co-evaporation with toluene (2*50mL). Dry DCM (100 mL) and DIPEA (5.53 g, 42.7 mmol) were added under argon, then 3-((chloro(diisopropylamino)phosphaneyl)oxy)propanenitrile (7741 mg, 32.7 mmol) was added slowly via a syringe. The reaction mixture was stirred for 3 h at room temperature. After reaching completion, the reaction mixture was evaporated under vacuum. The crude tert-butyl N-(((9H-fluoren-9-yl)methoxy)carbonyl)-O-((2-cyanoethoxy)(diisopropylamino)phosphaneyl)-L-homoserinate (**B-72**) was obtained as a yellow solid (20 g).

Step 3: Synthesis of 2-(((S)-3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(tert-butoxy)-4-oxobutoxy)(2-cyanoethoxy)phosphoryl)oxy)-N,N,N-trimethylethan-1-aminium (B-73**)**

[0475] Tert-butylN-(((9H-fluoren-9-yl)methoxy)carbonyl)-O-((2-cyanoethoxy)(diisopropylamino)phosphaneyl)-L-homoserinate (**B-72**) was dried with dry toluene and dry ACN (2*50 mL). Choline p-toluenesulfonate (13.8 g, 50.2 mmol) was dried with toluene and dissolved in dry ACN (60 mL). This mixture was added to tert-butylN-(((9H-fluoren-9-yl)methoxy)carbonyl)-O-((2-cyanoethoxy)(diisopropylamino)phosphaneyl)-L-homoserinate (**B-72**) (20 g, crude) via a syringe. ETT (0.25 M in ACN) (7.3 g, 55.2 mmol) was added slowly with a syringe. The mixture was stirred at room temperature for 2 h. 3-Chloroperoxybenzoic acid (12.9 g, 75.3 mmol) was added portion-wise to the mixture. After 4h of stirring, the mixture was concentrated under vacuum. The crude was purified by Flash Chromatography (C18, ACN-H₂O, 60%) to give 15 g of 2-(((S)-3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(tert-butoxy)-4-oxobutoxy)(2-cyanoethoxy)phosphoryl)oxy)-N,N,N-trimethylethan-1-aminium (**B-73**) as a yellow solid.

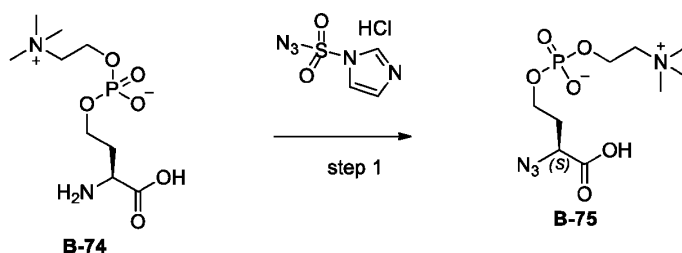
[0476] LCMS (ESI): m/z 616.3 (M⁺)

Step 4: Synthesis of (S)-3-amino-3-carboxypropyl (2-(trimethylammonio)ethyl) phosphate (B-74**)**

[0477] To a solution of [2-({[(3S)-4-(tert-butoxy)-3-{{(9H-fluoren-9-yl)methoxy}carbonyl}amino}-4-oxobutoxy](2-cyanoethoxy)phosphoryl}oxy)ethyl]trimethylazanium (**B-73**) (15 g, 24.32 mmol) in dry DCM (30 mL) was added HCl/dioxane (30 mL). The mixture was stirred at 25°C for 2h. DCM was removed off under reduced pressure to afford the crude. Then diethyl

amine (17.79 g, 243.24 mmol) and DCM (30 mL) were added to the crude. The mixture was stirred at 25°C for 2h. Diluted by water (30*3 mL), extracted with PE (100 mL). The aqueous was purified by flash chromatography (H₂O/MeCN = 10:1 v/v) to afford (S)-3-amino-3-carboxypropyl (2-(trimethylammonio)ethyl) phosphate (**B-74**) as a white solid (2.4 g, 33.0%).
 [0478] LCMS (ESI): m/z 285.2 (M + H)⁺.

Example 20: Synthesis of B-75



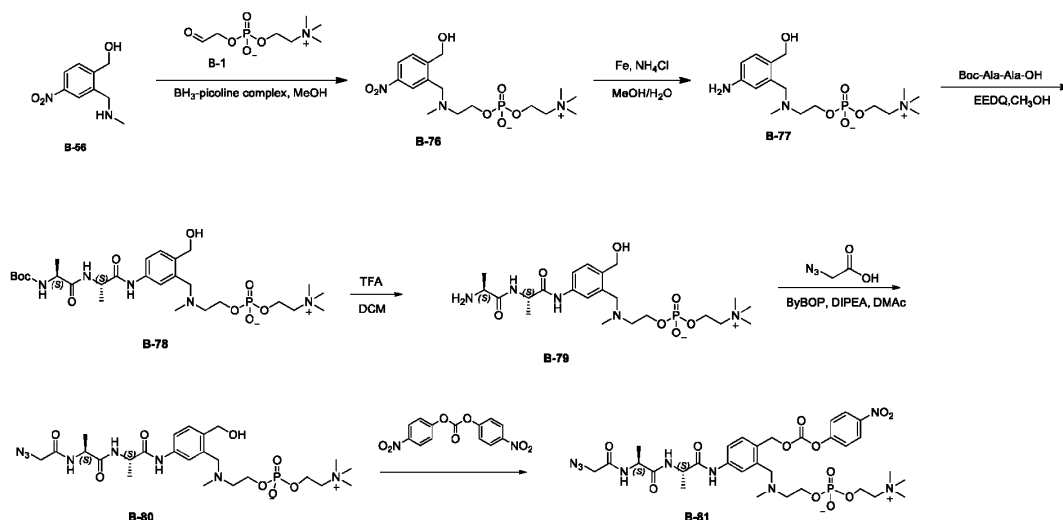
Step 1: Synthesis of (S)-3-azido-3-carboxypropyl (2-(trimethylammonio)ethyl) phosphate (B-75)

[0479] A solution of (2-{[(3S)-3-amino-3-carboxypropyl phosphonato]oxy}ethyl) trimethylazanium (**B-74**) (1.5 g, 3.33 mmol), imidazole-1-sulfonyl azide hydrochloride (1.66 g, 7.92 mmol), CuSO₄·5H₂O (13 mg, 0.05 mmol) and K₂CO₃ (1.82 g, 13.2 mmol) in dry MeOH (30 mL) was stirred for 16 h at 25 °C. The mixture was filtered and the filtrate was concentrated under reduced pressure to give the crude. ACN and H₂O were added until the crude all dissolved, purified by Flash Chromatography (ARG, ACN-H₂O, 60%) to give (S)-3-azido-3-carboxypropyl (2-(trimethylammonio)ethyl) phosphate (**B-75**) (1.5 g, 87.0%) as a light yellow solid.

[0480] LCMS (ESI): m/z 311.0 (M + H)⁺.

[0481] ¹H NMR (400 MHz, D₂O) δ 4.19 (d, J = 1.7, 2H), 4.93 – 4.84(m, 3H), 4.16 (t, J = 4.4 Hz, 2H), 3.57 – 4.32 (m, 2H), 3.10 (s, 9 H), 2.07-1.98 (m, 1H), 1.85-1.77 (m, 1H).

Example 21: Synthesis of 2-((5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl)(methyl)amino)ethyl (2-(trimethylammonio)ethyl) phosphate (B-81)



Step 1: Synthesis of 2-((2-(hydroxymethyl)-5-nitrobenzyl)(methyl)amino)ethyl 2-(trimethylammonio)ethyl phosphate (B-76)

[0482] To a solution of 2-((methylamino)methyl)-4-nitrophenyl)methanol (**B-56**) (800 mg, 4.08 mmol) in MeOH (20 mL) was added 2-oxoethyl 2-(trimethylammonio)ethyl phosphate (**B-1**) (1.38 g, 6.12 mmol), followed by BH_3 -picoline complex (857 mg, 8.01 mmol). The reaction mixture was stirred at r.t. for 5 hrs. The reaction mixture was purified by prep-HPLC to give 2-((2-(hydroxymethyl)-5-nitrobenzyl)(methyl)amino)ethyl 2-(trimethylammonio)ethyl phosphate (**B-76**) (1.03 g, 62.4%) as a colorless oil.

[0483] LCMS[M+H]⁺: 406.4;

[0484] ¹H NMR (600 MHz, D₂O) δ 8.36 (s, 1H), 8.29 (dd, 1H), 7.65 (d, 1H), 4.09-4.21 (m, 8H), 3.45-3.57 (m, 4H), 3.12 (s, 9H), 2.84 (s, 3H).

Step 2: Synthesis of 2-((5-amino-2-(hydroxymethyl)benzyl)(methyl)amino)ethyl 2-(trimethylammonio)ethyl phosphate (B-77)

[0485] To a suspended solution of 2-((2-(hydroxymethyl)-5-nitrobenzyl)(methyl)amino)ethyl 2-(trimethylammonio)ethyl phosphate (**B-76**) (1.03 g, 2.52 mmol) and Fe (704 mg, 12.6 mmol) in MeOH (20 mL), was added dropwise a solution of NH_4Cl (675 mg, 12.6 mmol) in H₂O (20 mL) with stirring at r.t. under N₂. The reaction mixture was stirred at 60°C for 2 hrs. The suspended solution was filtered through a pad of diatomite and washed with MeOH (20 mL×2).

The combined filtrates were concentrated to dryness to give the crude 2-((5-amino-2-(hydroxymethyl)benzyl)(methylamino)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-77**) (1.5 g) as a yellow solid.

[0486] LCMS[M+H]⁺: 376.5;

Step 3: Synthesis of 2-((5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)propanamido)propanamido)-2-(hydroxymethyl)benzyl)(methylamino)ethyl (2-(trimethylammonio)ethyl) phosphate (B-78**)**

[0487] To a solution of 2-((5-amino-2-(hydroxymethyl)benzyl)(methylamino)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-77**) (1.0 g, 2.66 mmol) in MeOH (10 mL) was added Boc-Ala-Ala-OH (1.04 g, 4.0 mmol), followed by EEDQ (1.32 g, 5.33 mmol) at r.t. under N₂. The reaction mixture was stirred at r.t for 30 mins. The reaction mixture was purified by prep-HPLC to give 2-((5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)propanamido)propanamido)-2-(hydroxymethyl)benzyl)(methylamino)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-78**) (210 mg) as a yellow solid.

[0488] LCMS[M+H]⁺: 618.7;

[0489] ¹H NMR (600 MHz, DMSO-d₆) δ 10.18 (s, 1H), 8.04 (d, 1H), 7.72 (s, 1H), 7.63 (dd, 1H), 7.39 (d, 1H), 6.98 (d, 1H), 4.60 (s, 2H), 4.44 (s, 2H), 4.41 (s, 2H), 4.36-4.40 (m, 1H), 4.08-4.12 (m, 4H), 3.98-4.01 (m, 1H), 3.53-3.55 (m, 4H), 3.34-3.38 (m, 2H), 3.11 (s, 9H), 2.75 (s, 3H), 3.11 (s, 9H), 1.38 (s, 9H), 1.31 (d, 3H), 1.19 (d, 3H).

Step 4: Synthesis of 2-((5-((S)-2-((S)-2-aminopropanamido)propanamido)-2-(hydroxymethyl)benzyl)(methylamino)ethyl (2-(trimethylammonio)ethyl) phosphate (B-79**)**

[0490] To a solution of 2-((5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)propanamido)propanamido)-2-(hydroxymethyl)benzyl)(methylamino)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-78**) (140 mg, 0.23 mmol) in DCM (2.4 mL) was added TFA (0.8 mL). The reaction mixture was stirred at r.t for 1 hr. The reaction was concentrated to give the crude 2-((5-((S)-2-((S)-2-aminopropanamido)propanamido)-2-(hydroxymethyl)benzyl)(methylamino)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-79**) (97 mg, 82.9%) as a yellow solid.

[0491] LCMS[M+H]⁺: 518.4

Step 5: Synthesis of 2-((5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(hydroxymethyl)benzyl)(methyl)amino)ethyl (2-(trimethylammonio)ethyl) phosphate (B-80)

[0492] To a stirred solution of the crude 2-((5-((S)-2-((S)-2-((tert-butoxycarbonyl)amino)propanamido)propanamido)-2-(hydroxymethyl) benzyl)(methyl)amino) ethyl (2-(trimethylammonio)ethyl) phosphate (B-79) (80 mg, 0.15 mmol) in DMAc (2 mL) was added azidoacetic acid (23 mg, 0.23 mmol), followed by PyBOP (161 mg, 0.31 mmol) and DIPEA (60 mg, 0.46 mmol) at r.t. under N₂. The reaction mixture was stirred at r.t for 1 h. The reaction was complete as detected by LC-MS. This reaction mixture was purified by prep-HPLC to give 2-((5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(hydroxymethyl)benzyl)(methyl)amino)ethyl (2-(trimethylammonio)ethyl) phosphate (B-80) (18 mg, 15.9%) as a white solid.

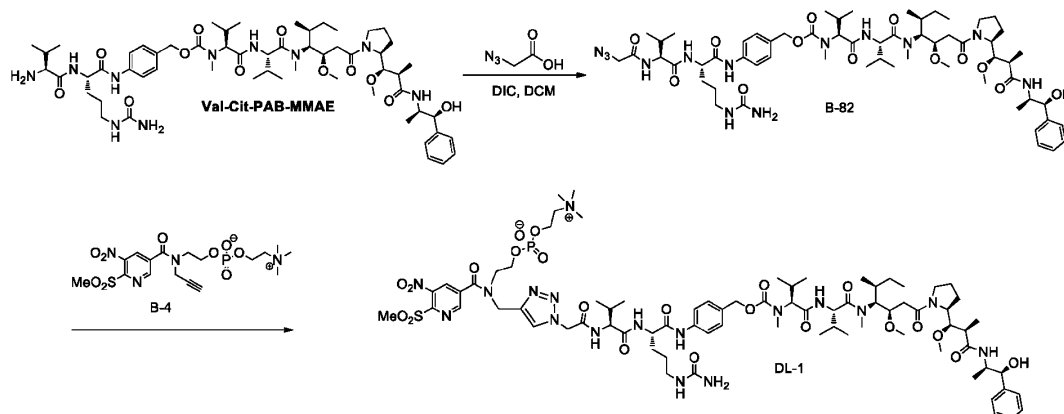
[0493] LCMS[M+H]⁺: 601.7

[0494] ¹H NMR (600 MHz, DMSO-d₆) δ 10.06 (s, 1H), 8.33 (d, 1H), 8.28 (d, 1H), 7.85 (s, 1H), 7.58 (dd, 1H), 7.40 (d, 1H), 4.61 (s, 2H), 4.32-4.46 (m, 4H), 4.21-4.26 (m, 3H), 3.82-3.92 (m, 2H), 3.57-3.63 (m, 3H), 3.42-3.44 (m, 2H), 3.11 (s, 9H), 2.77 (s, 3H), 1.34 (d, 3H), 1.24 (d, 3H).

Step 6: Synthesis of 2-((5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl)(methyl)amino)ethyl (2-(trimethylammonio)ethyl) phosphate (B-81)

[0495] To a solution of 2-((5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(hydroxymethyl)benzyl)(methyl)amino)ethyl (2-(trimethylammonio)ethyl) phosphate (B-80) (20.0 mg, 0.033 mmol) in DMF (1.5 mL) was added dropwise a solution of bis(4-nitrophenyl) carbonate (267.2 mg, 0.17 mmol) and DIPEA (125 mg, 1 mmol) at r.t under N₂. The reaction mixture was stirred at r.t for 16 hrs. The reaction was complete as detected by LC-MS. This reaction mixture was purified by prep-HPLC to give 2-((5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl)(methyl)amino)ethyl (2-(trimethylammonio)ethyl) phosphate (B-81) (13.2 mg, 38%) as white solid.

[0496] LCMS[M+H]⁺: 766.4.

Example 22: Synthesis of DL-1**Step 1: Synthesis of Azido-Ac-Val-Cit-PAB-MMAE (B-82)**

[0497] To a solution of Val-Cit-PAB-MMAE (200 mg, 0.18 mmol) in DCM (2 mL) was added azido acetic acid (54 mg, 0.54 mmol) and DIC (45 mg, 0.36 mmol) at 0°C under N_2 . The reaction suspension was stirred at that temperature for 2 h, then concentrated, and the residue was purified by prep-HPLC to afford Azido-Ac-Val-Cit-PAB-MMAE (**B-82**) (171 mg, 79.5%) as a white solid.

[0498] LCMS $[\text{M}+\text{H}]^+$: 1207.2.

[0499] ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 10.00 (d, J = 11.8 Hz, 1H), 8.30 (d, J = 7.5 Hz, 1H), 8.13 (d, J = 8.6 Hz, 1H), 8.05 (s, 1H), 7.89 (d, J = 8.5 Hz, 1H), 7.69-7.54 (m, 2H), 7.38-7.23 (m, 6H), 7.17 (dd, J = 14.7, 7.3 Hz, 1H), 6.05 (s, 1H), 5.04 (m, J = 46.9, 26.7, 13.3 Hz, 2H), 4.74 (s, 1H), 4.48 (t, J = 7.5 Hz, 1H), 4.46-4.32 (m, 2H), 4.27 (dt, J = 18.2, 9.8 Hz, 3H), 3.95-3.88 (m, 7H), 3.63-2.92 (m, 10H), 2.90-2.80 (m, 2H), 2.41 (d, J = 16.7 Hz, 1H), 2.26 (dd, J = 15.4, 9.5 Hz, 1H), 2.18-2.04 (m, 2H), 2.02-1.87 (m, 2H), 1.86-1.61 (m, 4H), 1.62-1.40 (m, 5H), 1.41-1.24 (m, 4H), 1.03-0.95 (m, 6H), 0.91-0.67 (m, 24H).

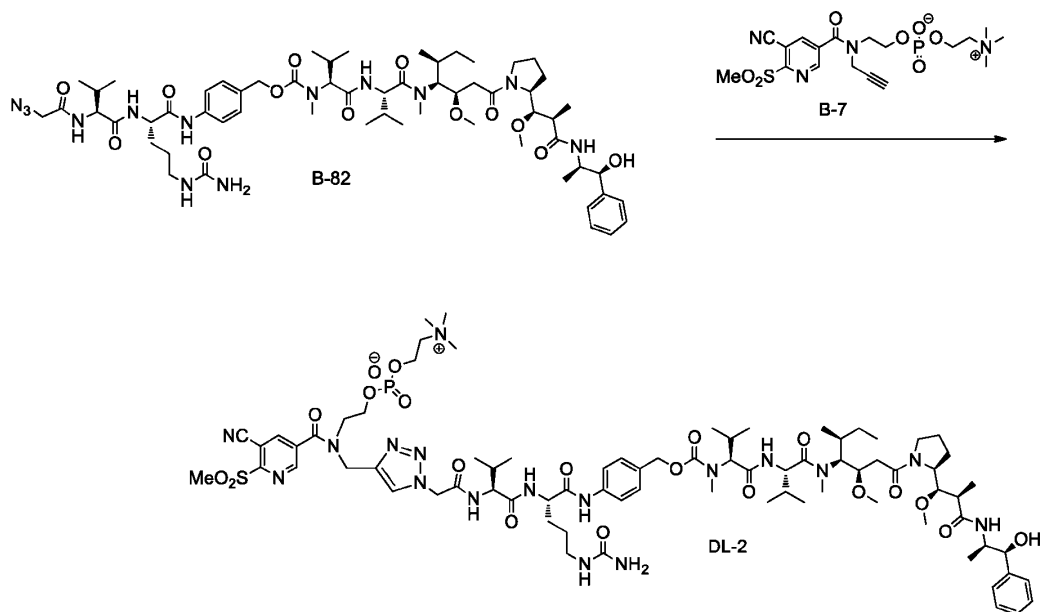
Step 2: Synthesis of 2-(N-((1-(2-(((S)-1-(((S)-1-((4-((5S,8S,11S,12R)-11-((S)-sec-butyl)-12-(2-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-5,8-diisopropyl-4,10-dimethyl-3,6,9-trioxo-2,13-dioxo-4,7,10-triazatetradecyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-6-(methylsulfonyl)-5-nitronicotinamido)ethyl (2-(trimethylammonio)ethyl) phosphate (DL-1)

[0500] To a solution of **B-82** (10 mg, 0.008 mmol, 1.0 eq) in H₂O/DMSO (2 mL, v/v=1:1) was added **B-4** (4 mg, 0.009 mmol, 1.1 eq), TBTA (0.8 mg, 0.003 mmol, 0.4 eq), CuSO₄·5H₂O (0.8 mg, 0.003 mmol, 0.4 eq) and sodium ascorbate (1.3 mg, 0.006 mmol, 0.8 eq) at r.t., the reaction mixture was stirred at r.t. for 1.5 hrs, then purified by prep-HPLC to afford **DL-1** (6.5 mg, yield: 46.2%) as a white solid.

[0501] LCMS[M+H]⁺: 1699.9.

[0502] ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.07 (s, 1H), 9.08 (d, *J* = 74.4 Hz, 1H), 8.77 (d, *J* = 84.2 Hz, 1H), 8.47 (d, *J* = 8.8 Hz, 1H), 8.34 (s, 2H), 8.12 (d, *J* = 36.6 Hz, 2H), 7.89 (s, 1H), 7.68-7.52 (m, 2H), 7.35-7.22 (m, 6H), 7.18 (t, 1H), 6.04 (s, 1H), 5.21 (s, 2H), 5.01 (d, *J* = 38.5 Hz, 2H), 4.83 (s, 1H), 4.62 (s, 1H), 4.45 (dd, *J* = 42.8, 7.7 Hz, 3H), 4.34-3.87 (m, 9H), 3.67-3.55 (m, 9H), 3.21 (dd, *J* = 29.3, 12.6 Hz, 11H), 2.86 (dd, *J* = 22.6, 10.9 Hz, 3H), 2.28-1.21 (m, 24H), 1.10-0.67 (m, 36H).

Example 23: Synthesis of DL-2



Step 1: Synthesis of 2-(N-((1-(2-(((S)-1-(((S)-1-((4-((5S,8S,11S,12R)-11-((S)-sec-butyl)-12-(2-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-5,8-diisopropyl-4,10-dimethyl-3,6,9-trioxo-2,13-dioxo-4,7,10-triazatetradecyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-

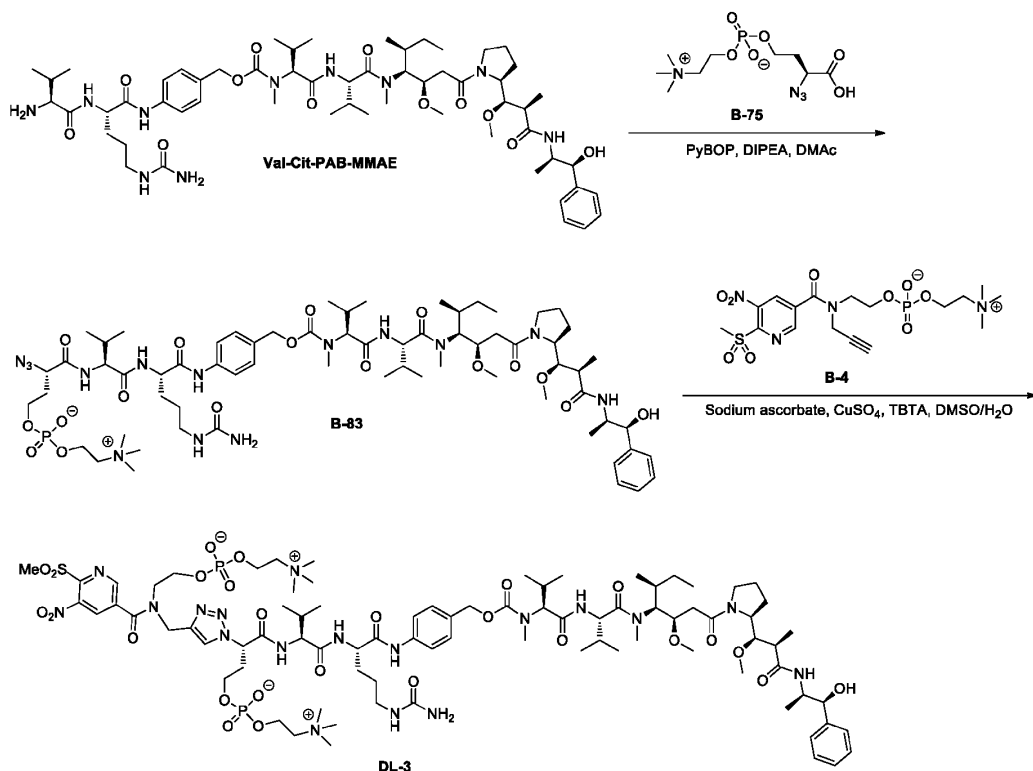
oxobutan-2-yl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-5-cyano-6-(methylsulfonyl)nicotinamido)ethyl (2-(trimethylammonio)ethyl) phosphate (DL-2)

[0503] To a solution of **B-82** (10 mg, 0.008 mmol) in H₂O/DMSO (2 mL, v/v=1:1) was added **B-7** (4 mg, 0.009 mmol), TBTA (0.8 mg 0.003 mmol), CuSO₄·5H₂O (0.8 mg, 0.003 mmol) and sodium ascorbate (1.3 mg, 0.006 mmol) at r.t., and stirred at r.t. for 1 hrs. The reaction solution was purified by prep-HPLC directly to afford **DL-2** (5.5 mg, 39.5%) as a white solid.

[0504] LCMS[M-H]⁺: 1677.8

[0505] ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.07 (s, 1H), 8.47 (d, *J* = 8.8 Hz, 1H), 8.34 (d, *J* = 6.6 Hz, 1H), 8.09 (s, 1H), 7.57 (s, 2H), 7.38-7.20 (m, 6H), 7.19-7.12 (m, 1H), 6.04 (s, 1H), 5.21 (s, 2H), 5.00 (dd, *J* = 39.1, 13.2 Hz, 2H), 4.59 (s, 1H), 4.48 (d, *J* = 5.9 Hz, 1H), 4.44-4.36 (m, 2H), 4.35-4.17 (m, 5H), 4.12 (s, 1H), 4.05-3.91 (m, 4H), 3.69-3.52 (m, 10H), 3.28-3.07 (m, 21H), 3.07-2.79 (m, 8H), 2.45-1.88 (m, 5H), 1.87-1.23 (m, 11H), 1.08-0.71 (m, 33H).

Example 24: Synthesis of DL-3



Step 1: Synthesis of (S)-3-azido-4-(((S)-1-(((S)-1-((4-((5S,8S,11S,12R)-11-((S)-sec-butyl)-12-(2-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-5,8-diisopropyl-4,10-dimethyl-3,6,9-trioxo-2,13-dioxo-4,7,10-triazatetradecyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-4-oxobutyl (2-(trimethylammonio)ethyl) phosphate (B-83)

[0506] To a solution of Val-Cit-PAB-MMAE (13 mg, 0.012 mmol, 1.0 eq) and **B-75** (5.4 mg, 0.017 mmol, 1.5 eq) in DMAc (1.5 mL) was added DIPEA (6 μ L, 0.035 mmol, 3.0 eq) at r.t., followed by PyBOP (9 mg, 0.017 mmol, 1.5 eq). The resulting mixture was stirred at room temperature for 4h and purified by prep-HPLC to give **B-83** (11.2 mg, yield: 68.68%) as a white powder.

[0507] LCMS[M+H]⁺: 1416.3.

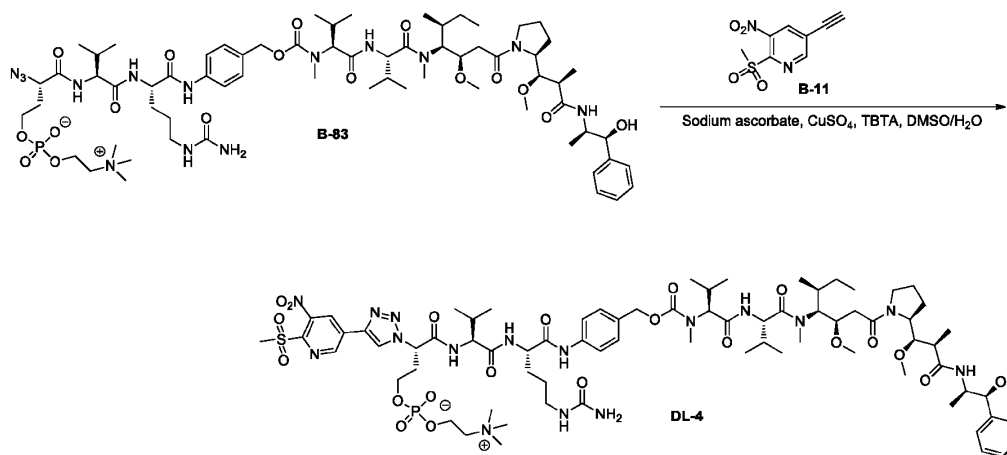
Step 2: Synthesis of DL-3

[0508] To a solution of **B-83** (11.3 mg, 0.08 mmol, 1.0 eq) in H₂O/DMSO (2 mL, v/v=1/1) was added **B-4** (4.3 mg, 0.009 mmol, 1.1 eq), CuSO₄·5H₂O (0.8 mg, 0.003 mmol, 0.4 eq), TBTA (1.7 mg 0.003 mmol, 0.4 eq) and sodium ascorbate (1.3 mg, 0.006 mmol, 0.8 eq) at r.t., the reaction mixture was stirred at r.t. for 1.5 hrs, then purified by prep-HPLC to afford **DL-3** (10.8 mg, yield: 71.29%) as a white powder.

[0509] LCMS[M+H]⁺: 1908.6.

[0510] ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.08 (s, 1H), 8.99 (dd, *J* = 183.0, 81.8 Hz, 2H), 8.35 (s, 1H), 8.26 (s, 1H), 8.06 (s, 1H), 7.90 (d, *J* = 8.8 Hz, 1H), 7.63 (d, *J* = 8.6 Hz, 1H), 7.58 (s, 2H), 7.36-7.24 (m, 7H), 7.19-7.14 (m, 1H), 6.13 (s, 1H), 5.76 (s, 3H), 5.66 (dd, *J* = 35.5, 6.2 Hz, 1H), 5.14-4.89 (m, 2H), 4.75 (d, *J* = 14.9 Hz, 1H), 4.62 (q, *J* = 16.5 Hz, 1H), 4.48 (d, *J* = 5.4 Hz, 1H), 4.46-4.35 (m, 2H), 4.26 (dd, *J* = 18.1, 11.0 Hz, 1H), 4.19 (s, 5H), 3.98 (ddd, *J* = 21.2, 15.2, 6.6 Hz, 3H), 3.83 (s, 1H), 3.78 (d, *J* = 9.4 Hz, 1H), 3.69 (s, 2H), 3.58 (d, *J* = 7.2 Hz, 4H), 3.50 (s, 2H), 3.48 (s, 3H), 3.24 (s, 3H), 3.23 (s, 2H), 3.20 (s, 2H), 3.17 (s, 2H), 3.13 (s, 6H), 3.12 (s, 6H), 3.10 (d, *J* = 2.1 Hz, 9H), 2.97 (s, 3H), 2.86 (dd, *J* = 21.9, 11.0 Hz, 4H), 2.44-2.38 (m, 3H), 2.30-2.23 (m, 2H), 2.15-2.09 (m, 2H), 2.03-1.98 (m, 2H), 1.81 (s, 2H), 1.70 (d, *J* = 11.5 Hz, 3H), 1.55 (d, *J* = 6.7 Hz, 1H), 1.47 (s, 2H), 1.37 (s, 1H), 1.31 (s, 1H), 1.04 (d, *J* = 6.6 Hz, 2H), 1.01 (dd, *J* = 6.5, 2.4 Hz, 4H), 0.98 (d, *J* = 6.7 Hz, 2H), 0.89-0.73 (m, 30H).

Example 25: Synthesis of DL-4



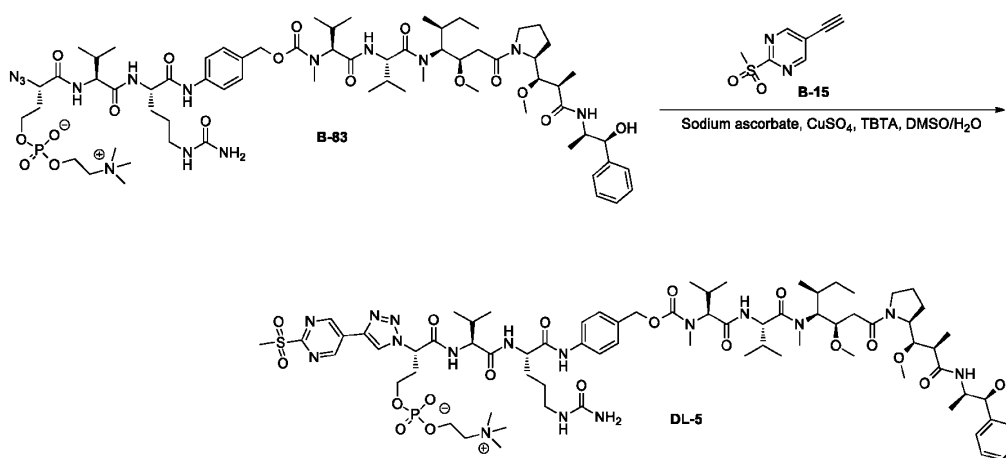
Step 1: Synthesis of (S)-4-(((S)-1-(((S)-1-((4-((5S,8S,11S,12R)-11-((S)-sec-butyl)-12-(2-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-5,8-diisopropyl-4,10-dimethyl-3,6,9-trioxo-2,13-dioxo-4,7,10-triazatetradecyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-(4-(6-(methylsulfonyl)-5-nitropyridin-3-yl)-1H-1,2,3-triazol-1-yl)-4-oxobutyl (2-(trimethylammonio)ethyl) phosphate (DL-4)

[0511] To a solution of **B-83** (11 mg, 0.008 mmol, 1.0 eq) in DMSO/H₂O (2 mL, V/V=1/1) was added **B-11** (2.1 mg, 0.01 mmol, 1.2 eq), TBTA (1.6 mg, 0.003 mmol, 0.4 eq), CuSO₄•5H₂O (0.7 mg, 0.003 mmol, 0.4 eq), sodium ascorbate (1.6 mg, 0.008 mmol, 1.0 e.) at r.t., and the reaction mixture was stirred at r.t. for 30 mins. Then the reaction mixture was purified by the prep-HPLC to afford the **DL-4** (7.14 mg, yield: 54.4%, purity: 95.53%) as a white solid.

[0512] LCMS[M+H]⁺: 1642.2.

[0513] ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.09 (s, 1H), 9.46 (d, J = 1.8 Hz, 1H), 9.23 (s, 1H), 9.03 (d, J = 1.8 Hz, 1H), 7.59 (s, 2H), 7.28 (dd, J = 24.5, 7.7 Hz, 7H), 7.17 (s, 1H), 5.84 (t, J = 7.5 Hz, 1H), 5.05 (s, 1H), 4.42 (s, 2H), 4.26 (dd, J = 19.5, 9.0 Hz, 6H), 4.02 (s, 5H), 3.21 (dd, J = 28.6, 12.5 Hz, 14H), 3.11 (s, 3H), 2.86 (dd, J = 22.3, 12.7 Hz, 4H), 2.64-2.56 (m, 1H), 2.41-2.35 (m, 4H), 2.27 (s, 1H), 2.12 (s, 2H), 2.04 (s, 2H), 1.70 (s, 4H), 1.48 (s, 4H), 1.24 (s, 3H), 1.01 (m, 9H), 0.89-0.74 (m, 37H).

Example 26: Synthesis of DL-5



Step 1: Synthesis of (S)-4-(((S)-1-(((S)-1-((4-((5S,8S,11S,12R)-11-((S)-sec-butyl)-12-(2-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-5,8-diisopropyl-4,10-dimethyl-3,6,9-trioxo-2,13-dioxo-4,7,10-triazatetradecyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-(4-(2-(methylsulfonyl)pyrimidin-5-yl)-1H-1,2,3-triazol-1-yl)-4-oxobutyl (2-(trimethylammonio)ethyl) phosphate (DL-5)

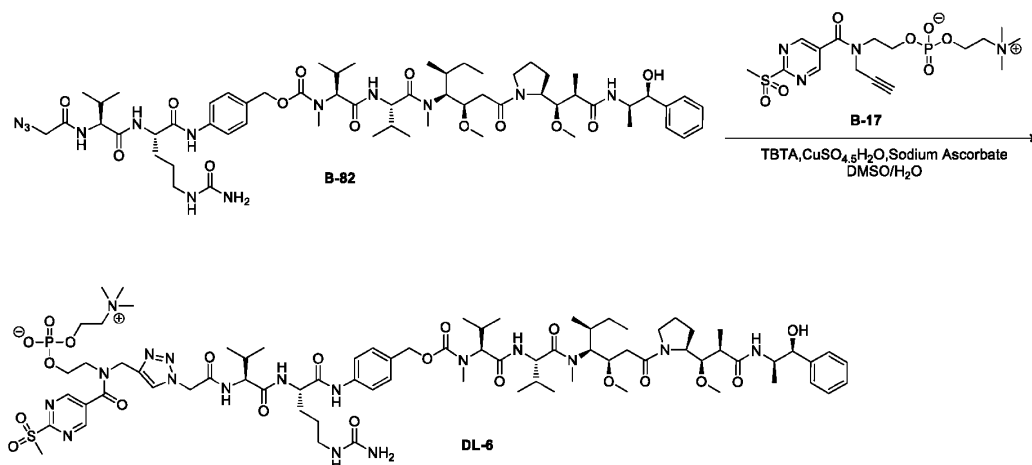
[0514] To a solution of **B-83** (10 mg, 0.007 mmol, 1.0 eq) in H₂O/DMSO (2 mL, v/v=1/1), was added **B-15** (2.2 mg, 0.008 mmol, 1.2 eq), CuSO₄·5H₂O (0.7 mg, 0.003 mmol, 0.4 eq), TBTA (1.5 mg 0.003 mmol, 0.4 eq) and sodium ascorbate (1.1 mg, 0.006 mmol, 0.8 eq) at r.t., the reaction mixture was stirred at that temperature for 1.5 hrs, then purified by prep-HPLC directly to afford **DL-5** (5.5 mg, 48.76%) as a white powder.

[0515] LCMS[M+H]⁺: 1598.6.

[0516] ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.10 (d, *J* = 13.8 Hz, 1H), 9.53 (s, 2H), 9.17 (s, 1H), 9.05 (s, 1H), 8.42 (d, *J* = 7.0 Hz, 1H), 8.18 (d, *J* = 137.5 Hz, 1H), 7.76 (dd, *J* = 159.5, 8.7 Hz, 1H), 7.59 (s, 2H), 7.33 (d, *J* = 8.1 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 3H), 7.26 (td, *J* = 7.7, 2.9 Hz, 2H), 7.17 (t, *J* = 7.0 Hz, 1H), 5.85 (t, *J* = 7.6 Hz, 1H), 5.13-4.95 (m, 2H), 4.68 (d, *J* = 58.2 Hz, 1H), 4.48 (d, *J* = 5.6 Hz, 1H), 4.41 (dd, *J* = 19.4, 6.8 Hz, 2H), 4.28 (d, *J* = 11.0 Hz, 1H), 4.25-4.19 (m, 3H), 4.08-3.92 (m, 3H), 3.78 (d, *J* = 9.3 Hz, 2H), 3.60-3.54 (m, 4H), 3.44 (s, 6H), 3.24 (s, 3H), 3.23 (s, 2H), 3.20 (s, 2H), 3.17 (s, 2H), 3.11 (s, 2H), 3.08 (s, 9H), 3.00 (dd, *J* = 17.3, 11.5 Hz, 4H), 2.86 (dd, *J* = 22.2, 12.1 Hz, 3H), 2.16-1.99 (m, 5H), 1.73 (t, *J* = 33.4 Hz, 6H), 1.57-1.36

(m, 5H), 1.04 (d, $J = 6.7$ Hz, 2H), 1.01 (dd, $J = 6.6, 3.1$ Hz, 3H), 0.98 (d, $J = 6.7$ Hz, 1H), 0.89-0.73 (m, 24H).

Example 27: Synthesis of DL-6



Step 1: Synthesis of 2-(N-((1-(2-(((S)-1-(((S)-1-((4-((5S,8S,11S,12R)-11-((S)-sec-butyl)-12-(2-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-5,8-diisopropyl-4,10-dimethyl-3,6,9-trioxo-2,13-dioxo-4,7,10-triazatetradecyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-2-(methylsulfonyl)pyrimidine-5-carboxamido)ethyl (2-(trimethylammonio)ethyl) phosphate (DL-6)

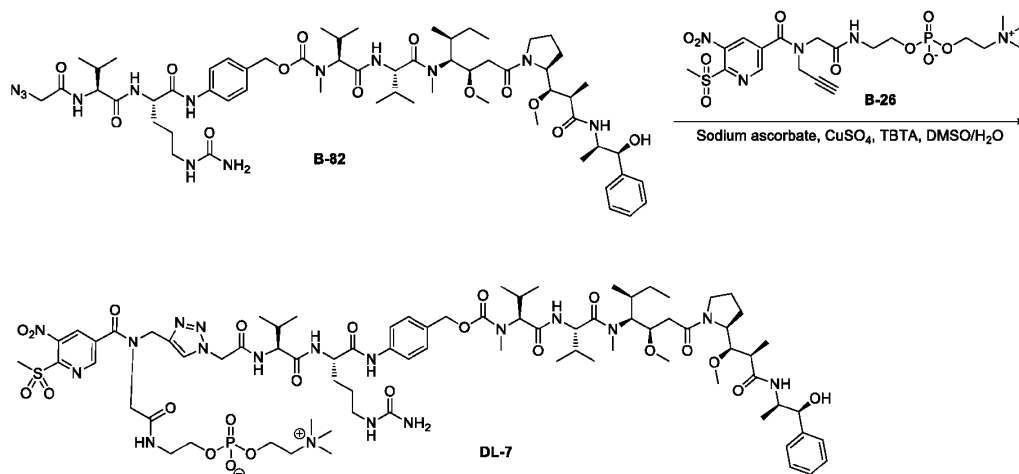
[0517] To a solution of **B-82** (10 mg, 0.008 mmol) in H₂O/DMSO (2 mL, v/v=1:1) was added **B-17** (4 mg, 0.009 mmol), TBTA (0.8 mg 0.003 mmol), CuSO₄·5H₂O (0.8 mg, 0.003 mmol) and sodium ascorbate (1.3 mg, 0.006 mmol) at r.t., the reaction mixture was stirred at r.t. for 1.5 hrs, then purified by prep-HPLC to afford **DL-6** (5.5 mg, yield: 39.5%) as a white solid.

[0518] LCMS[M+H]⁺: 1655.4.

[0519] ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.10 (d, $J = 20.9$ Hz, 1H), 9.29 (s, 1H), 9.18 (s, 1H), 8.47 (d, $J = 7.9$ Hz, 1H), 8.35 (d, $J = 7.3$ Hz, 1H), 8.14 (d, $J = 11.3$ Hz, 1H), 7.57 (s, 2H), 7.37-7.21 (m, 5H), 7.21-7.12 (m, 1H), 6.09 (d, $J = 33.8$ Hz, 1H), 5.43 (s, 2H), 5.20 (s, 2H), 5.00 (dd, $J = 37.6, 12.7$ Hz, 2H), 4.82 (s, 1H), 4.74 (s, 1H), 4.64 (s, 1H), 4.48 (d, $J = 6.0$ Hz, 1H), 4.41

(d, $J = 12.9$ Hz, 2H), 4.33-4.19 (m, 3H), 4.16-4.02 (m, 2H), 4.03-3.87 (m, 3H), 3.78 (d, $J = 9.4$ Hz, 1H), 3.58 (d, $J = 25.1$ Hz, 6H), 3.46 (d, $J = 16.6$ Hz, 8H), 3.24 (d, $J = 9.4$ Hz, 6H), 3.21-3.15 (m, 5H), 3.13 (d, $J = 3.5$ Hz, 9H), 2.86 (dd, $J = 22.4, 10.3$ Hz, 3H), 2.44-2.35 (m, 1H), 2.26 (s, 1H), 2.16-1.90 (m, 4H), 1.50 (m, 11H), 1.08-0.95 (m, 6H), 0.92-0.69 (m, 24H).

Example 28: Synthesis of DL-7



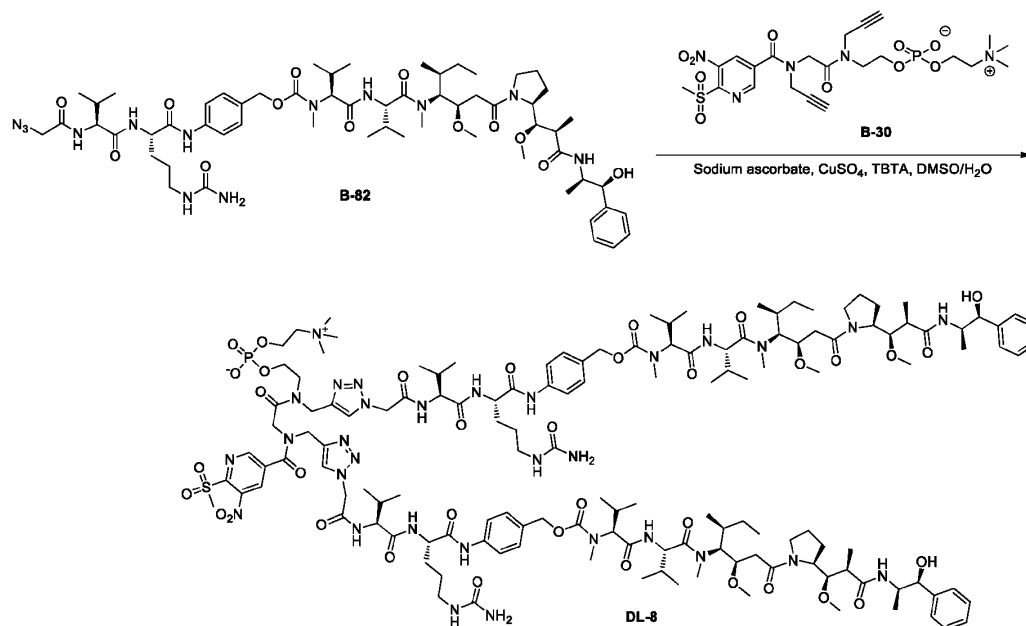
Step 1: Synthesis of 2-(2-(N-((1-(2-(((S)-1-(((S)-1-((4-((5S,8S,11S,12R)-11-((S)-sec-butyl)-12-(2-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-5,8-diisopropyl-4,10-dimethyl-3,6,9-trioxo-2,13-dioxo-4,7,10-triazatetradecyl)phenyl)amino)-1-oxo-5-ureidopentane-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-6-(methylsulfonyl)-5-nitronicotinamido)acetamido)ethyl (2-(trimethylammonio)ethyl) phosphate (DL-7)

[0520] To a solution of **B-82** (11 mg, 0.009 mmol) in H₂O/DMSO (2 mL, v/v=1/1) was added **B-26** (5.3 mg, 0.01 mmol), CuSO₄·5H₂O (0.9 mg, 0.0035 mmol), TBTA (1.9 mg, 0.0035 mmol) and sodium ascorbate (1.4 mg, 0.007 mmol) at r.t., and stirred at r.t. for 1 hrs. The reaction mixture was purified by prep-HPLC directly to afford **DL-7** (10.4 mg, 67.08%) as a white solid.

[0521] LCMS[M+H]⁺: 1755.8.

[0522] ^1H NMR (600 MHz, DMSO- d_6) δ 10.10-9.98 (m, 1H), 9.24 (d, J = 62.4 Hz, 1H), 8.89 (dd, J = 193.1, 32.8 Hz, 2H), 8.61-8.45 (m, 1H), 8.25 (dt, J = 61.1, 29.2 Hz, 3H), 7.78 (dd, J = 155.2, 8.4 Hz, 1H), 7.56 (s, 2H), 7.34-7.23 (m, 6H), 7.20-7.14 (m, 1H), 7.05 (d, J = 51.1 Hz, 1H), 6.55 (s, 1H), 6.13 (d, J = 44.9 Hz, 1H), 5.45 (d, J = 17.7 Hz, 2H), 5.39-5.19 (m, 3H), 5.01 (d, J = 47.3 Hz, 2H), 4.72 (d, J = 13.7 Hz, 1H), 4.64 (s, 1H), 4.58 (s, 1H), 4.48 (s, 1H), 4.45-4.35 (m, 2H), 4.26 (dd, J = 17.5, 10.8 Hz, 2H), 4.12 (s, 2H), 4.01 (dd, J = 15.1, 6.9 Hz, 1H), 3.94 (s, 2H), 3.82 (s, 1H), 3.78 (d, J = 9.5 Hz, 1H), 3.56 (d, J = 24.1 Hz, 3H), 3.49 (s, 2H), 3.47 (s, 1H), 3.24 (d, J = 9.5 Hz, 7H), 3.20 (s, 2H), 3.17 (s, 2H), 3.13 (s, 6H), 3.12 (s, 6H), 2.97 (s, 3H), 2.88 (d, J = 9.7 Hz, 2H), 2.84 (d, J = 10.4 Hz, 1H), 2.62 (s, 1H), 2.40 (d, J = 19.7 Hz, 2H), 2.27 (dd, J = 21.4, 13.0 Hz, 1H), 2.16-1.96 (m, 4H), 1.76 (d, J = 58.5 Hz, 4H), 1.49 (d, J = 54.6 Hz, 3H), 1.33 (d, J = 37.3 Hz, 2H), 1.04 (d, J = 6.6 Hz, 2H), 1.01 (d, J = 6.1 Hz, 3H), 0.98 (d, J = 6.7 Hz, 2H), 0.92-0.72 (m, 25H).

Example 29: Synthesis of DL-8



Step 1: Synthesis of 2-(N-((1-(2-(((S)-1-(((S)-1-(((4-((5S,8S,11S,12R)-11-(((S)-sec-butyl)-12-(2-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-5,8-diisopropyl-4,10-dimethyl-3,6,9-trioxo-2,13-dioxo-4,7,10-triazatetradecyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-

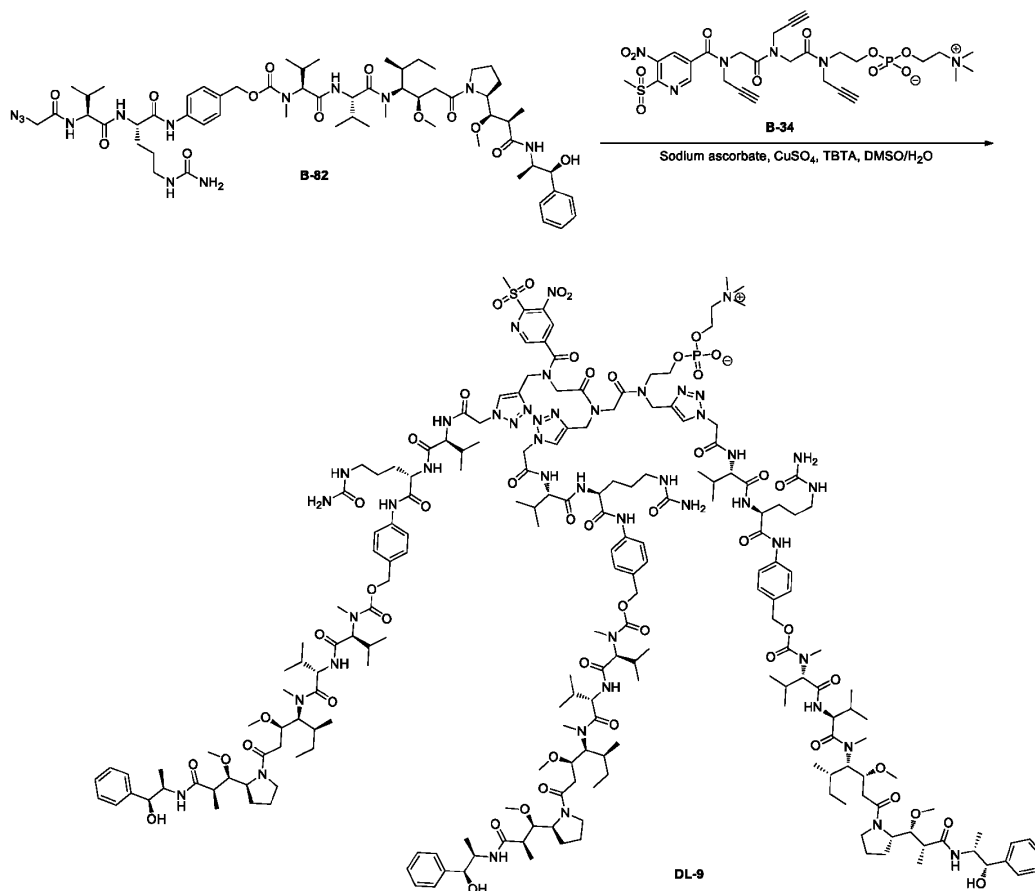
oxobutan-2-yl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-2-(N-((1-(2-(((S)-1-(((S)-1-(((4-((5S,8S,11S,12R)-11-((S)-sec-butyl)-12-(2-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-5,8-diisopropyl-4,10-dimethyl-3,6,9-trioxo-2,13-dioxo-4,7,10-triazatetradecyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-6-(methylsulfonyl)-5-nitronicotinamido)acetamido)ethyl (2-(trimethylammonio)ethyl) phosphate (DL-8)

[0523] To a solution of **B-82** (13 mg, 0.01 mmol, 2.1 eq) in H₂O/DMSO (2 mL, v/v=1/1) was added **B-30** (3 mg, 0.005 mmol, 1.0 e.), CuSO₄·5H₂O (1 mg, 0.004 mmol, 0.8 eq), TBTA (2 mg, 0.004 mmol, 0.8 e.) and sodium ascorbate (1.6 mg, 0.008 mmol, 1.6 eq) at r.t., and stirred at r.t. for 1 hrs. The reaction mixture was purified by prep-HPLC directly to afford **DL-8** (9.23 mg, 60.09%) as a white solid.

[0524] LCMS[M/2+H]⁺: 1499.4.

[0525] ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.06 (s, 2H), 8.85 (dd, *J* = 29.5, 21.9 Hz, 1H), 8.50 (d, *J* = 14.7 Hz, 2H), 8.35 (d, *J* = 21.9 Hz, 3H), 8.11 (dd, *J* = 18.1, 13.0 Hz, 2H), 7.99-7.87 (m, 2H), 7.64 (d, *J* = 8.2 Hz, 1H), 7.57 (s, 4H), 7.36-7.22 (m, 13H), 7.17 (t, *J* = 7.3 Hz, 3H), 6.07 (s, 2H), 5.21 (d, *J* = 15.9 Hz, 4H), 5.05 (t, *J* = 47.1 Hz, 6H), 4.78-4.54 (m, 7H), 4.48 (d, *J* = 6.0 Hz, 4H), 4.44-4.36 (m, 5H), 4.28 (d, *J* = 11.2 Hz, 7H), 4.03-3.92 (m, 6H), 3.78 (d, *J* = 9.7 Hz, 1H), 3.63 (s, 2H), 3.57 (d, *J* = 13.3 Hz, 6H), 3.51-3.49 (m, 4H), 3.48 (s, 3H), 3.23 (d, *J* = 9.5 Hz, 10H), 3.20 (s, 4H), 3.17 (s, 4H), 3.12 (dd, *J* = 13.8, 5.0 Hz, 14H), 2.97 (s, 4H), 2.86 (dd, *J* = 22.7, 11.1 Hz, 7H), 2.61 (s, 1H), 2.44-2.37 (m, 4H), 2.27 (d, *J* = 7.6 Hz, 2H), 2.16-2.08 (m, 3H), 2.00 (s, 4H), 1.76 (d, *J* = 58.0 Hz, 9H), 1.48 (d, *J* = 68.3 Hz, 9H), 1.35 (s, 5H), 1.04 (d, *J* = 6.7 Hz, 3H), 1.01 (d, *J* = 6.4 Hz, 6H), 0.98 (d, *J* = 6.7 Hz, 3H), 0.90-0.74 (m, 50H).

Example 30: Synthesis of DL-9



Step 1: Synthesis of 2-(N-((1-(2-(((S)-1-(((S)-1-((4-((5S,8S,11S,12R)-11-((S)-sec-butyl)-12-(2-(((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-5,8-diisopropyl-4,10-dimethyl-3,6,9-trioxo-2,13-dioxa-4,7,10-triazatetradecyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-2-(N-((1-(2-(((S)-1-(((S)-1-((4-((5S,8S,11S,12R)-11-((S)-sec-butyl)-12-(2-(((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-5,8-diisopropyl-4,10-dimethyl-3,6,9-trioxo-2,13-dioxa-4,7,10-triazatetradecyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-2-(N-((1-(2-(((S)-1-(((S)-1-((4-((5S,8S,11S,12R)-11-((S)-sec-butyl)-12-(2-(((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-5,8-diisopropyl-4,10-dimethyl-3,6,9-trioxo-2,13-dioxa-4,7,10-

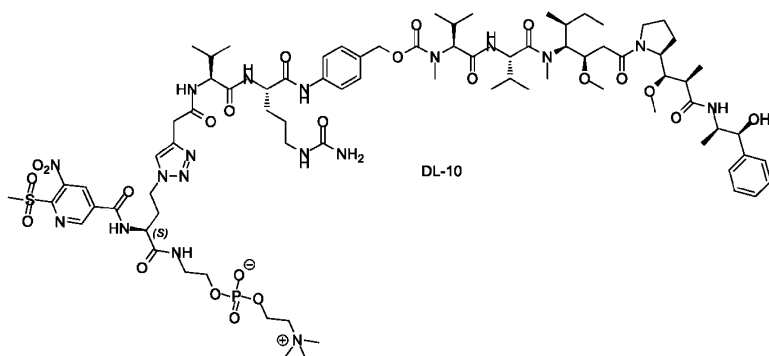
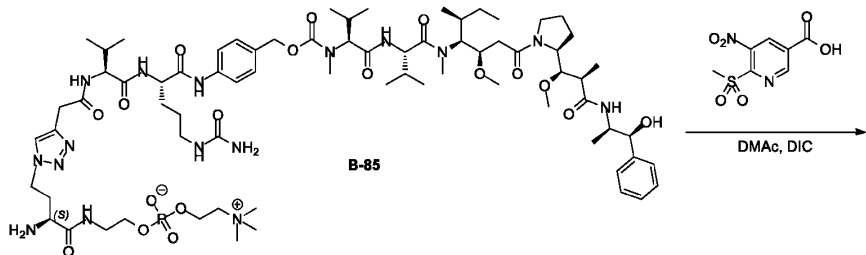
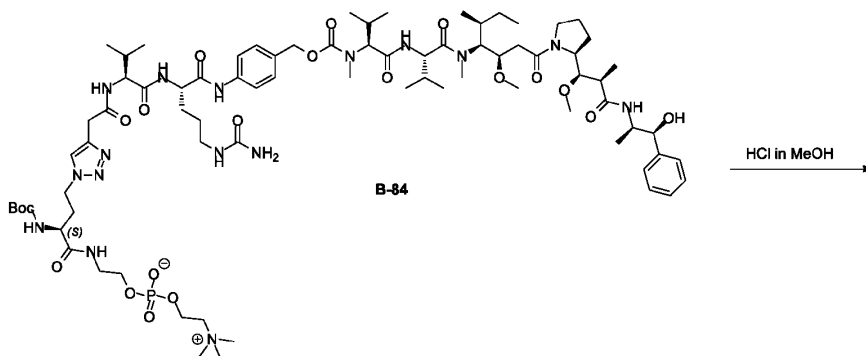
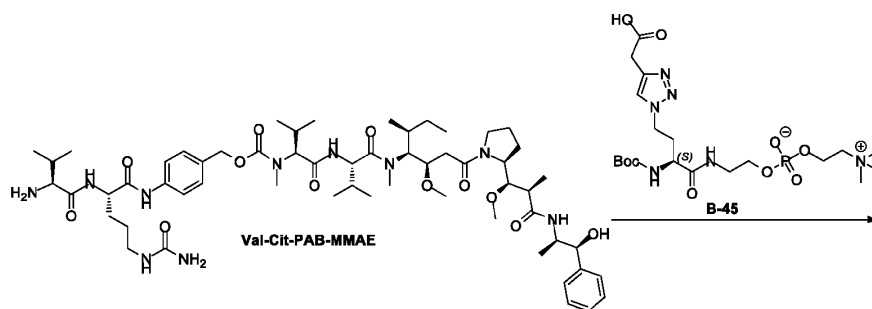
5,8-diisopropyl-4,10-dimethyl-3,6,9-trioxo-2,13-dioxo-4,7,10-triazatetradecyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)-6-(methylsulfonyl)-5-nitronicotinamido)acetamido)ethyl (2-(trimethylammonio)ethyl) phosphate (DL-9)

[0526] To a solution of **B-82** (13.7 mg, 0.01 mmol, 3.1 eq) in H₂O/DMSO (2 mL, v/v=1/1) was added **B-34** (2.5 mg, 0.004 mmol, 1.0 eq), CuSO₄·5H₂O (1.1 mg, 0.004 mmol, 1.2 eq), TBTA (2.3 mg, 0.004 mmol, 1.2 eq) and sodium ascorbate (1.7 mg, 0.009 mmol, 2.4 eq) at r.t., the reaction mixture was stirred at r.t. for 1.5 hrs, then purified by prep-HPLC to afford **DL-9** (10.5 mg, 66.8%) as a white solid.

[0527] LCMS[M/2+H]⁺: 2151.5.

[0528] ¹H NMR (600 MHz, DMSO-*d*₆) δ 10.08 (s, 3H), 8.87 (dd, *J* = 38.3, 26.4 Hz, 2H), 8.49 (s, 5H), 8.36 (s, 4H), 8.11 (d, *J* = 27.2 Hz, 3H), 7.92 (dt, *J* = 43.6, 28.2 Hz, 3H), 7.64 (d, *J* = 9.0 Hz, 2H), 7.57 (s, 6H), 7.37-7.23 (m, 18H), 7.21-7.13 (m, 3H), 7.06 (d, *J* = 51.1 Hz, 1H), 6.05 (s, 3H), 5.44 (s, 3H), 5.22 (s, 6H), 5.04 (t, *J* = 37.6 Hz, 6H), 4.68 (d, *J* = 61.3 Hz, 6H), 4.48 (d, *J* = 5.8 Hz, 3H), 4.44-4.35 (m, 6H), 4.28 (d, *J* = 10.4 Hz, 7H), 3.98 (ddd, *J* = 39.3, 15.0, 6.8 Hz, 4H), 3.77 (d, *J* = 9.2 Hz, 1H), 3.56 (d, *J* = 11.7 Hz, 2H), 3.23 (d, *J* = 9.6 Hz, 18H), 3.20 (s, 8H), 3.17 (s, 8H), 3.12 (dd, *J* = 9.8, 5.9 Hz, 17H), 2.97 (s, 6H), 2.86 (dd, *J* = 22.9, 11.4 Hz, 12H), 2.39 (d, *J* = 1.8 Hz, 5H), 2.26 (s, 6H), 2.16-2.08 (m, 6H), 2.00 (s, 6H), 1.85-1.63 (m, 16H), 1.61-1.40 (m, 15H), 1.29 (s, 8H), 1.23 (s, 5H), 1.04 (d, *J* = 6.6 Hz, 5H), 1.01 (d, *J* = 5.3 Hz, 9H), 0.98 (d, *J* = 6.7 Hz, 4H), 0.90-0.71 (m, 74H).

Example 31: Synthesis of DL-10



Step 1: Synthesis of 2-((S)-2-((tert-butoxycarbonyl)amino)-4-(4-(2-(((S)-1-(((S)-1-((4-((5S,8S,11S,12R)-11-((S)-sec-butyl)-12-(2-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-5,8-diisopropyl-4,10-dimethyl-3,6,9-trioxo-2,13-dioxo-4,7,10-triazatetradecyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-2-oxoethyl)-1H-1,2,3-triazol-1-yl)butanamido)ethyl (2-(trimethylammonio)ethyl) phosphate (B-84)

[0529] To a solution of **B-45** (19.1 mg, 0.035 mmol) in DMAc (0.6 mL) was added **Val-Cit-PAB-MMAE** (20.0 mg, 0.017 mmol), Lutidine (2.0 mg 0.053 mmol), HOPO (3.2 mg, 0.028 mmol) and EDCI (5.4 mg, 0.035 mmol). The mixture was stirred at room temperature for 3 hours. The reaction was purified by prep-HPLC to afford **B-84** (20.0 mg, yield: 68%) as a white solid.

[0530] LCMS[M+H]⁺: m/z = 1642.8.

Step 2: Synthesis of 2-((S)-2-amino-4-(4-(2-(((S)-1-(((S)-1-((4-((5S,8S,11S,12R)-11-((S)-sec-butyl)-12-(2-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-5,8-diisopropyl-4,10-dimethyl-3,6,9-trioxo-2,13-dioxo-4,7,10-triazatetradecyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-2-oxoethyl)-1H-1,2,3-triazol-1-yl)butanamido)ethyl (2-(trimethylammonio)ethyl) phosphate (B-85)

[0531] To a solution of **B-84** (19 mg, 0.011 mmol) in MeOH (0.2 mL) was added HCl (4M/L, 0.2 mL). The mixture was stirred at room temperature for 3 hours. The reaction was complete detected by LC-MS, then removed the solvent to get **B85** (17 mg, yield: 95%) as colorless solid.

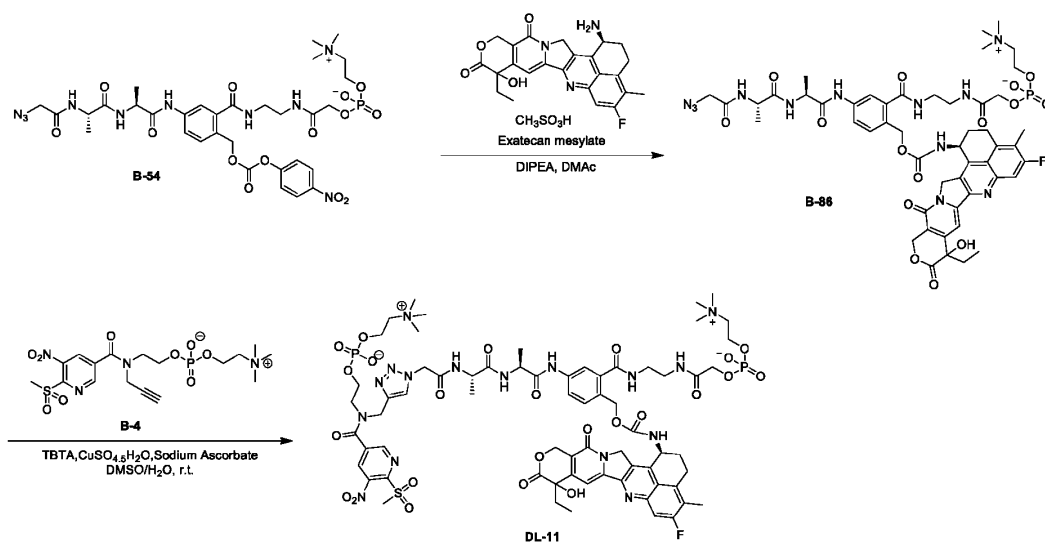
[0532] LCMS[M+H]⁺: m/z = 1542.8.

Step 3: Synthesis of 2-((S)-4-(4-(2-(((S)-1-(((S)-1-((4-((5S,8S,11S,12R)-11-((S)-sec-butyl)-12-(2-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-5,8-diisopropyl-4,10-dimethyl-3,6,9-trioxo-2,13-dioxo-4,7,10-triazatetradecyl)phenyl)amino)-1-oxo-5-ureidopentan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-2-oxoethyl)-1H-1,2,3-triazol-1-yl)-2-(6-(methylsulfonyl)-5-nitronicotinamido)butanamido)ethyl (2-(trimethylammonio)ethyl) phosphate (DL-10)

[0533] To a solution of **6-(methylsulfonyl)-5-nitronicotinic acid** (8.1 mg, 0.033 mmol) in DMAc (0.2 mL) was added DIC (3.9 mg, 0.031 mmol), then stirred at room temperature for 10 min. Then added **B-85** (17 mg, 0.011 mmol) to this mixture and stirred at room temperature for overnight. The reaction was complete detected by LC-MS, then purified by prep-HPLC to afford **DL-10** (2 mg, yield: 3%) as yellow solid.

[0534] LCMS[M+H]⁺: m/z = 1770.5.

Example 32: Synthesis of DL-11



Step 1: Synthesis of 2-((2-(5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-((((1S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamoyl)oxy)methyl)benzamido)ethyl)amino)-2-oxoethyl (2-(trimethylammonio)ethyl)phosphate (B-86**)**

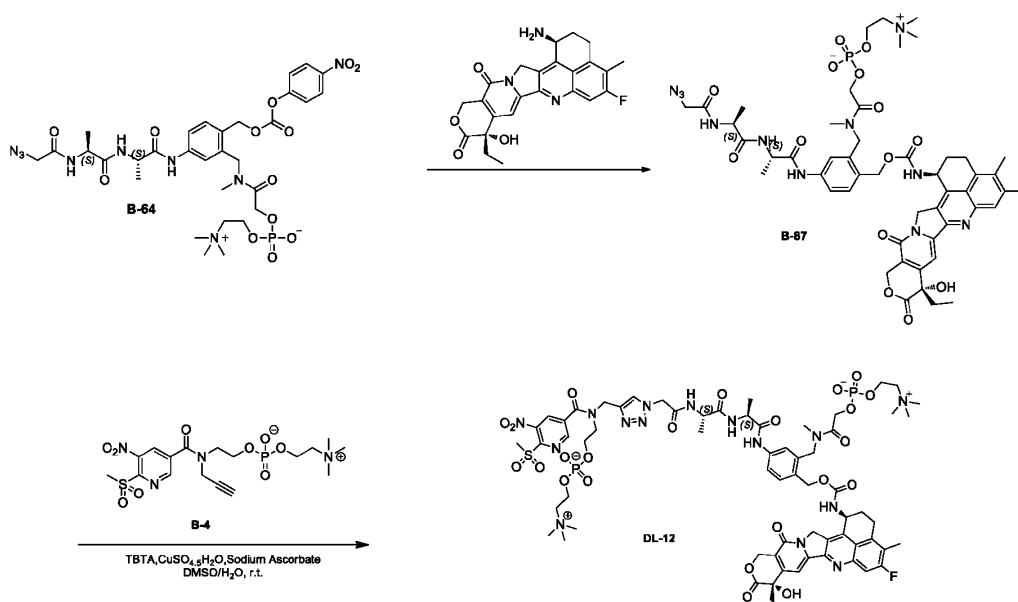
[0535] To a solution of **B-54** (3.2 mg, 0.004 mmol, 1.0 eq) in **DMAc** (0.5 mL), was added a solution of exatecan mesylate (3.1 mg, 0.006 mmol, 1.5 eq) in DMAc (31 μ L) and **DIPEA** (2.3 mg, 0.018 mmol, 3.0 eq). The reaction mixture was stirred at r.t for 16 hrs. The reaction was complete as detected by LC-MS. This reaction mixture was purified by prep-HPLC to give **B-86** (2.9 mg, 61.3%) as white solid.

[0536] LCMS[M+H]⁺: 1119.9.

Step 2: Synthesis of DL-11

[0537] To a solution of **B-86** (2.9 mg, 0.002 mmol, 1.0eq) in H₂O/DMSO (1V/1V, 1mL) was added **B-4** (2.6 mg, 0.005 mmol, 2.2 eq), TBTA (0.25 mg, 0.001 mmol, 0.4eq), CuSO₄·5H₂O (0.24 mg, 0.001 mmol, 0.4eq) and sodium ascorbate (0.38 mg, 0.0002 mmol, 0.8eq) at r.t. for 6 hrs. The reaction was complete as detected by LC-MS. This reaction mixture was purified by prep-HPLC to afford **DL-11** (3.2 mg, 82.1%) as a white solid.

[0538] LCMS[M-H]⁻: 1609.7.

Example 33: Synthesis of DL-12

Step 1: Synthesis of 2-((5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamoyl)oxy)methyl)benzyl)(methylamino)-2-oxoethyl (2-(trimethylammonio)ethyl) phosphate (B-87**)**

[0539] To a solution of **B-64** (4.0 mg, 0.0052 mmol) in DMAc (0.5 mL) was added a solution of exatecan mesylate (3.3 mg, 0.008 mmol) and DIPEA (3.3 mg, 0.03 mmol) at r.t., the reaction mixture was stirred at r.t for 16 hrs. The reaction was complete detected by LC-MS. This reaction mixture was purified by prep-HPLC to give **B-87** (5.2 mg, 93.0%) as white solid.

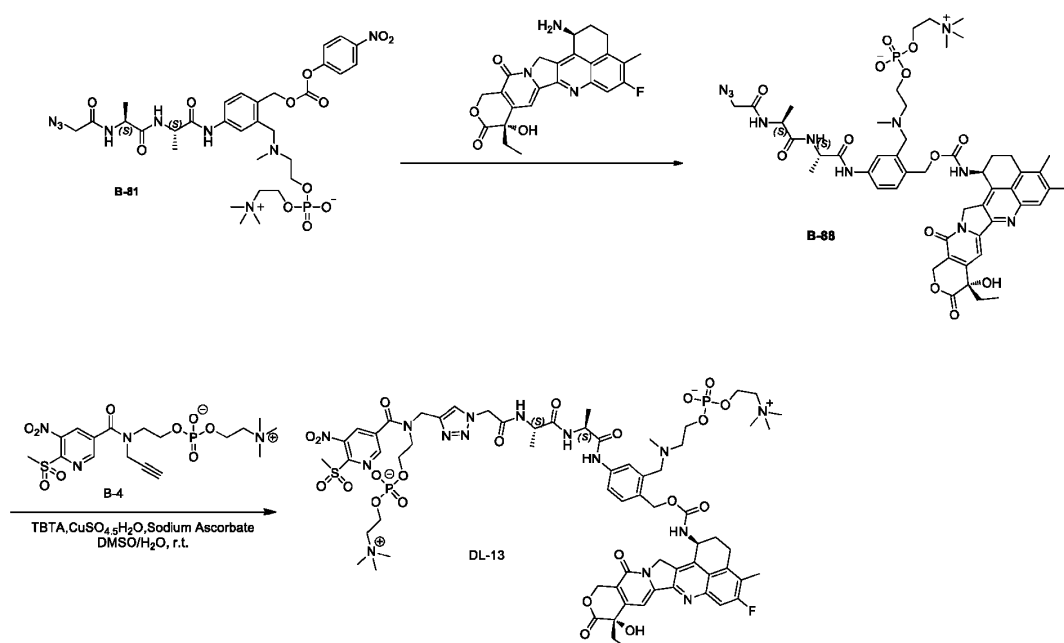
[0540] LCMS[M+H]⁺: 1076.8.

Step 2: Synthesis of DL-12

[0541] To a solution of **B-87** (5.2 mg, 0.005 mmol) in H₂O/DMSO (1mL, v/v=1:1,) was added **B-4** (2.6 mg, 0.005 mmol), TBTA (1 mg, 0.002 mmol), CuSO₄·5H₂O (0.47 mg, 0.002 mmol) and sodium ascorbate (0.75 mg, 0.004 mmol) at r.t. for 1.5 hrs. The reaction was complete as detected by LC-MS. This reaction mixture was purified by prep-HPLC to afford **DL-12** (4.2 mg, 56%) as a white solid.

[0542] LCMS[M+H]⁺: 1569.6.

Example 34: Synthesis of DL-13



Step 1: Synthesis of 2-((5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamoyl)oxy)methyl)benzyl)(methylamino)ethyl 2-(trimethylammonio)ethyl phosphate (B-88)

[0543] To a solution of 2-((5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-(((4-nitrophenoxy)carbonyl)oxy)methyl)benzyl)(methylamino)ethyl 2-(trimethylammonio)

ethyl) phosphate (**B-81**) (4.0 mg, 0.0052 mmol) in DMAc (0.5 mL) was added a solution of exatecan mesylate (3.3 mg, 0.008 mmol) and DIPEA (3.3 mg, 0.03 mmol) at r.t., the reaction mixture was stirred at r.t for 16 hrs. The reaction was complete as detected by LC-MS. This reaction mixture was purified by prep-HPLC to give 2-((5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-((((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamoyl)oxy)methyl)benzyl)(methylamino)ethyl (2-(trimethylammonio)ethyl) phosphate (**B-88**) (5.1 mg, 93.0%) as white solid.

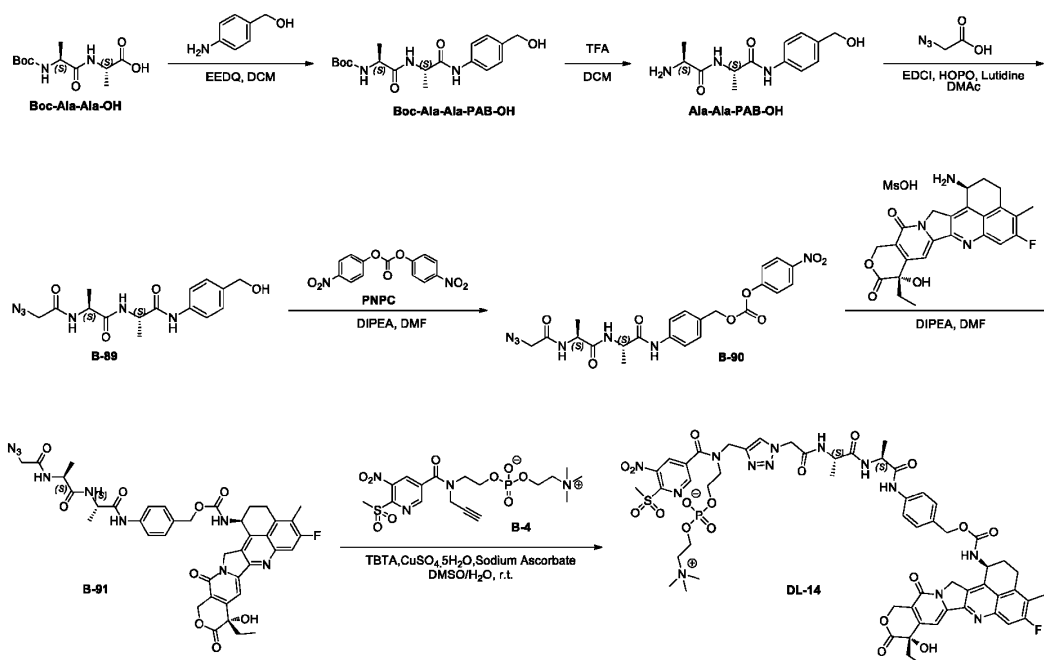
[0544] LCMS[M+H]⁺: 1062.4

Step 2: Synthesis of DL-13

[0545] To a solution of 2-((5-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)-2-((((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamoyl)oxy)methyl)benzyl)(methylamino)ethyl (2-(trimethylammonio)ethyl) phosphate **B-88** (5.1 mg, 0.005 mmol) in H₂O/DMSO (1mL, v/v=1:1,) was added **B-4** (2.6 mg, 0.005 mmol), TBTA (1 mg, 0.002 mmol), CuSO₄·5H₂O (0.47 mg, 0.002 mmol) and sodium ascorbate (0.75 mg, 0.004 mmol) at r.t. for 1.5 hrs. The reaction was complete as detected by LC-MS. This reaction mixture was purified by prep-HPLC to afford **DL-13** (4.0 mg, 56%) as a white solid.

[0546] LCMS[M+H]⁺: 1554.6.

Example 35: Synthesis of DL-14



Step 1: Synthesis of Boc-Ala-Ala-PAB-OH

[0547] To a solution of p-aminobenzyl alcohol (500 mg, 4.07 mmol, 1.0 eq) in DCM (20 mL) and MeOH (10 mL) was added Boc-Ala-Ala-OH (1058 mg, 4.07 mmol, 1.0 eq) and EEDQ (2011 mg, 8.13 mmol, 2.0 eq). The reaction mixture was stirred at r.t. for 24 hrs. Solvents were evaporated and the residue was retreated with MTBE. The mixture was filtered to afford Boc-Ala-Ala-PAB-OH (1142 mg, 76.9%) as a white solid.

[0548] LCMS [M+H]⁺: 366.4

Step 2: Synthesis of Ala-Ala-PAB-OH

[0549] To a solution of Boc-Ala-Ala-PAB-OH (1124.8 mg, 3.08 mmol, 1.0 eq) in DCM (12 mL) was added TFA (4 mL). After stirred at r.t. for 4 hrs, the reaction mixture was evaporated and the residue was added to MeOH (20 mL) and H₂O (5 mL), followed by Na₂CO₃ (3.47 g, 32.7 mmol, 10.6 eq) and stirred at r.t. overnight. Methanol was evaporated, water (20 mL) was added, the precipitate was filtered and dried to afford crude Ala-Ala-PAB-OH (2606 mg) as a white solid.

[0550] LCMS [M+H]⁺: 266.3

Step 3: Synthesis of (S)-2-(2-azidoacetamido)-N-((S)-1-((4-(hydroxymethyl)phenyl)amino)-1-oxopropan-2-yl)propanamide (B-89)

[0551] To a solution of 2-azidoacetic acid (461 uL, 6.16 mmol, 2.0 eq) in DMAc (10.0 mL) was added HOPO (854.9 mg, 7.70 mmol, 2.5 eq) and EDCI (1121 mg, 5.85 mmol, 1.9 eq), and stirred at r.t. for 15 min. The resulting mixture was added to a solution of Ala-Ala-PAB-OH (816.6 mg, 3.08 mmol, 1.0 eq) and 2,6-Lutidine (1792 uL, 15.39 mmol, 5.0 eq) in DMAc (10.0 mL). The reaction mixture was stirred at r.t. for 3 hrs, then purified by prep-HPLC to afford (S)-2-(2-azidoacetamido)-N-((S)-1-((4-(hydroxymethyl)phenyl)amino)-1-oxopropan-2-yl)propanamide (**B-89**) (639 mg, 59.6%) as a white solid.

[0552] LCMS [M+H]⁺: 349.3

Step 4: Synthesis of 4-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)benzyl (4-nitrophenyl) carbonate (B-90)

[0553] **B-89** (500 mg, 1.44 mmol, 1.0 eq) and PNPC (1310 mg, 4.31 mmol, 3.0 eq) were added to DMF (25 mL), followed by DIPEA (750 uL, 4.31 mmol, 3.0 eq). The reaction mixture was stirred at r.t. for 6 hrs, then purified by prep-HPLC to afford 4-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)benzyl (4-nitrophenyl) carbonate (**B-90**) (593 mg, 80.5%) as a white powder.

[0554] LCMS [M+H]⁺: 514.4

[0555] ¹H NMR (600 MHz, DMSO) δ 9.99 (s, 1H), 8.35-8.28 (m, 4H), 7.67 (d, J= 8.6 Hz, 2H), 7.59-7.55 (m, 2H), 7.42 (d, J= 8.6 Hz, 2H), 5.25 (s, 2H), 4.44-4.32 (m, 2H), 3.91-3.81 (m, 2H), 1.33 (d, J= 7.1 Hz, 3H), 1.24 (d, J= 7.1 Hz, 3H).

Step 5: Synthesis of 4-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)benzyl ((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamate (B-91)

[0556] To a solution of **B-90** (284 mg, 0.55 mmol, 1.0 eq) in DMAc (10 mL) was added exatecan mesylate (361 mg, 0.83 mmol, 1.5 eq), followed by DIPEA (482 uL, 2.77 mmol, 5.0 eq). The reaction mixture was stirred at r.t. for 18 hrs, purified by prep-HPLC to afford 4-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)benzyl ((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7]indolizino[1,2-b]quinolin-1-yl)carbamate (**B-91**) (423 mg, 94.5%) as a yellow powder.

[0557] LCMS [M+H]⁺: 810.7

[0558] ¹H NMR (600 MHz, DMSO) δ 9.91 (s, 1H), 8.32 (d, J= 7.2 Hz, 1H), 8.27 (d, J= 7.2 Hz, 1H), 8.06 (d, J= 8.9 Hz, 1H), 7.78 (d, J= 10.9 Hz, 1H), 7.62 (d, J= 8.4 Hz, 2H), 7.37 (d, J= 8.4 Hz, 2H), 7.31 (s, 1H), 5.45 (s, 2H), 5.33-5.24 (m, 3H), 5.11-5.05 (m, 2H), 4.42-4.32 (m, 2H), 3.89-

3.81 (m, 2H), 3.24 (d, J= 17.0 Hz, 1H), 3.15-3.08 (m, 1H), 2.38 (s, 3H), 2.18 (dd, J= 33.3, 6.2 Hz, 2H), 1.94-1.81 (m, 2H), 1.31 (d, J= 7.1 Hz, 3H), 1.23 (d, J= 7.1 Hz, 3H), 0.88 (t, J= 7.3 Hz, 3H).

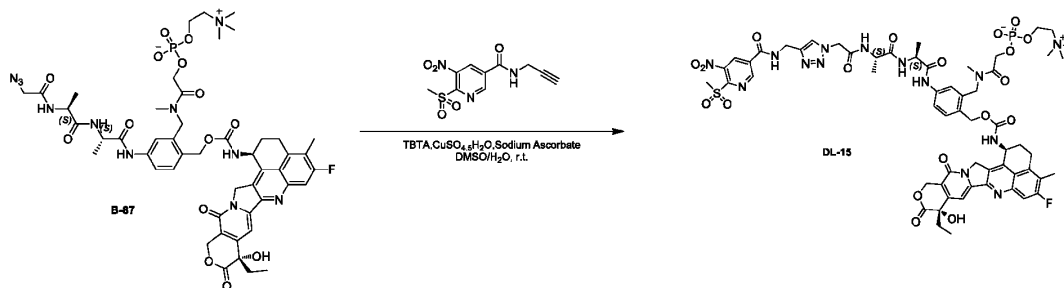
Step 6: Synthesis of DL-14

[0559] To a solution of 4-((S)-2-((S)-2-(2-azidoacetamido)propanamido)propanamido)benzyl ((1S,9S)-9-ethyl-5-fluoro-9-hydroxy-4-methyl-10,13-dioxo-1,2,3,9,10,12,13,15-octahydrobenzo[de]pyrano[3',4':6,7] indolizino[1,2-b]quinolin-1-yl)carbamate (**B-91**) (7.77 mg, 0.01 mmol, 1.0 eq) in H₂O/DMSO (0.5 mL/0.5 mL) was added **B-4** (4.96 mg, 0.01 mmol, 1 eq), CuSO₄·5H₂O (0.48 mg, 0.002 mmol, 0.2 eq), TBTA (1 mg 0.002 mmol, 0.2 eq) and sodium ascorbate (0.76 mg, 0.003 mmol, 0.4 eq) and stirred at r.t. for 1.5 hrs. The reaction mixture was purified by prep-HPLC to afford **DL-14** (8.05 mg, 64.5%) as a yellow powder.

[0560] LCMS[M+H]⁺: 1302.9.

[0561] ¹H NMR (600 MHz, DMSO) δ 9.96 (d, J= 49.5 Hz, 1H), 9.20-9.04 (m, 1H), 8.90-8.74 (m, 1H), 8.67 (dd, J= 28.9, 7.1 Hz, 1H), 8.34-8.30 (m, 1H), 8.19-8.08 (m, 2H), 7.78 (d, J= 10.9 Hz, 1H), 7.58 (d, J= 8.2 Hz, 2H), 7.35 (d, J= 8.3 Hz, 2H), 7.31 (s, 1H), 6.52 (s, 1H), 5.45 (s, 2H), 5.29 (q, J= 18.9 Hz, 3H), 5.19-5.11 (m, 2H), 5.07 (s, 2H), 4.84-4.76 (m, 1H), 4.62 (s, 1H), 4.37 (ddd, J= 19.2, 13.1, 6.8 Hz, 2H), 4.13 (s, 1H), 4.02 (s, 2H), 3.79 (s, 1H), 3.54 (s, 2H), 3.50 (s, 2H), 3.48 (s, 3H), 3.25-3.22 (m, 1H), 3.12 (d, J= 6.0 Hz, 9H), 2.38 (s, 3H), 2.25-2.13 (m, 2H), 1.87 (ddd, J= 32.7, 14.1, 7.1 Hz, 2H), 1.34-1.29 (m, 3H), 1.26 (dd, J= 6.9, 4.8 Hz, 3H), 0.88 (t, J= 7.4 Hz, 3H).

Example 36: Synthesis of DL-15

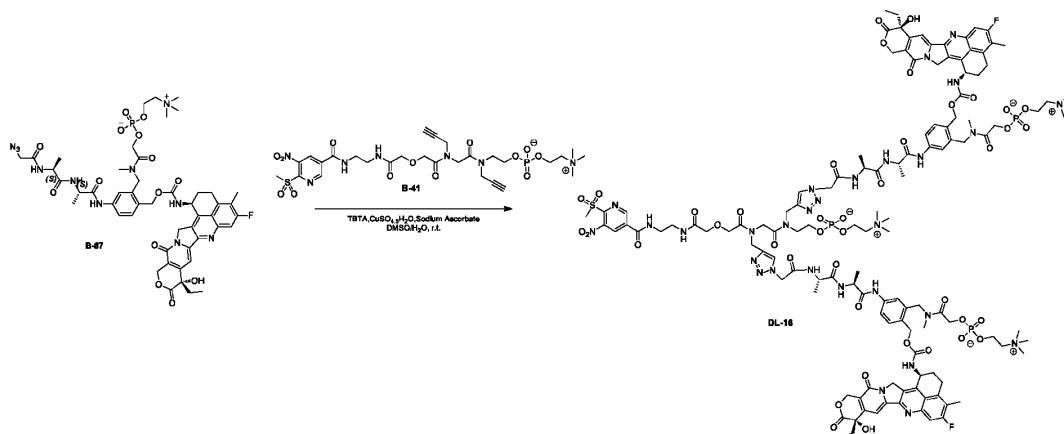


[0562] To a stirred solution of **B-87** (380 mg, 0.35 mmol, 1.0 eq) in H₂O/DMSO (1V/1V, 4 mL) was added 6-(methylsulfonyl)-5-nitro-N-(prop-2-yn-1-yl)nicotinamide (72 mg, 0.28 mmol, 0.8 eq), TBTA (60 mg, 0.11 mmol, 0.4 eq), CuSO₄·5H₂O (28 mg, 0.11 mmol, 0.4 eq) and sodium ascorbate (45 mg, 0.23 mmol, 0.8 eq) at r.t.. The resulting mixture was stirred at RT for 3 hrs, purified by prep-HPLC to afford **DL-15** (340 mg, 70.8%) as a yellow solid.

[0563] LCMS [M+H]⁺: 1360.3

[0564] ^1H NMR (600 MHz, DMSO) δ 10.02 (d, J = 32.9 Hz, 1H), 9.80 (d, J = 34.4 Hz, 1H), 9.32 (d, J = 4.5 Hz, 1H), 8.96 (d, J = 8.2 Hz, 1H), 8.71 (m, J = 78.2, 7.0 Hz, 1H), 8.31 (m, J = 26.8, 7.2 Hz, 1H), 8.01 (d, J = 4.7 Hz, 1H), 7.76 (m, J = 11.7 Hz, 1H), 7.66 (m, J = 42.2, 8.4 Hz, 1H), 7.44-7.22 (m, 3H), 6.52 (d, J = 16.5 Hz, 1H), 5.51-5.36 (m, 3H), 5.34-5.17 (m, 3H), 5.17-5.01 (m, 4H), 4.81 (dd, J = 45.6, 18.3 Hz, 1H), 4.66 (d, J = 16.0 Hz, 1H), 4.55 (t, J = 18.8 Hz, 2H), 4.53-4.23 (m, 4H), 4.14 (d, J = 35.9 Hz, 2H), 3.79 (s, 1H), 3.51 (d, J = 14.4 Hz, 4H), 3.10 (s, 4H), 3.01-2.84 (m, 9H), 2.37 (s, 4H), 2.19 (s, 2H), 2.05-1.78 (m, 2H), 1.48-1.11 (m, 6H), 0.94-0.81 (m, 3H).

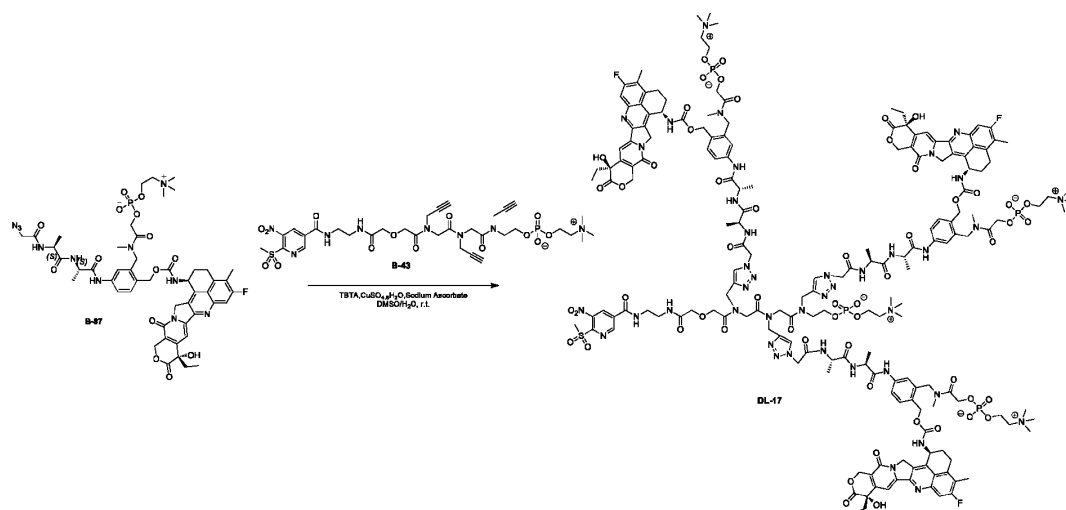
Example 37: Synthesis of DL-16



[0565] To a solution of **B-41** (100 mg, 0.13 mmol, 0.8 eq) in $\text{H}_2\text{O}/\text{DMSO}$ (1 mL/1 mL), was added **B-87** (361 mg, 0.33 mmol, 2.0 eq), TBTA (68.9 mg, 0.13 mmol, 0.8 eq), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (32.3 mg, 0.13 mmol, 0.8 eq) and sodium ascorbate (51.4 mg, 0.26 mmol, 1.6 eq) at r.t. for 1.5 hrs. The reaction mixture was purified by prep-HPLC to afford **DL-16** (214 mg, 22%) as a white solid.

[0566] LCMS $[1/3\text{M}+\text{H}]^+$: 967.2

Example 38: Synthesis of DL-17

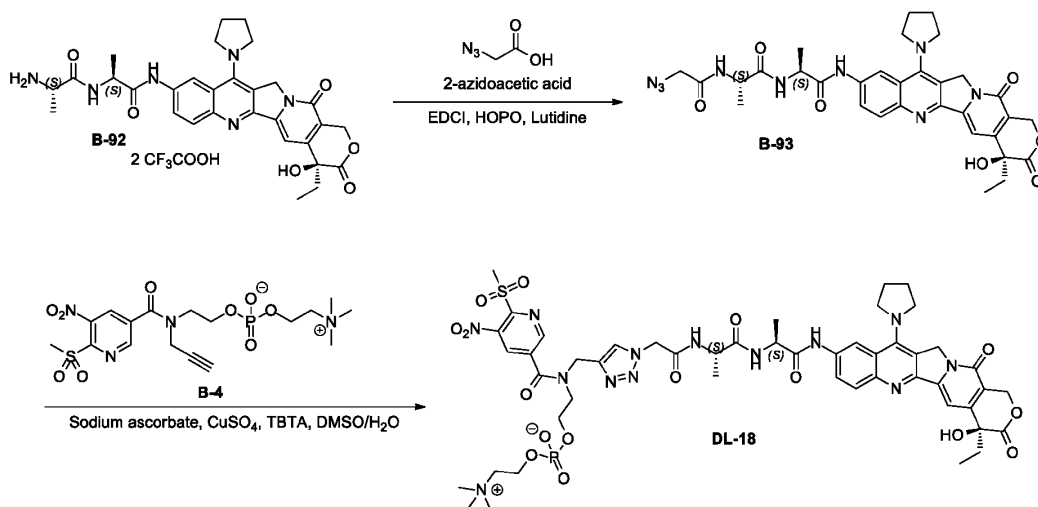


[0567] To a solution of **B-87** (60 mg, 0.06 mmol, 4.0 eq) and **B-43** (11.72 mg, 0.014 mmol, 1.0 eq) in DMSO/H₂O (0.35 mL/0.35 mL) was added CuSO₄·5H₂O (4.18 mg, 0.017 mmol, 1.2 eq) and TBTA (8.88 mg, 0.017 mmol, 1.2 eq), followed by sodium ascorbate (6.63 mg, 0.033 mmol, 2.4 eq), the resulting mixture was stirred at r.t. for 1h and purified prep-HPLC to give the **DL-17** (26.84 mg).

[0568] LCMS[1/4M+H]⁺:1018.5

[0569] ¹H NMR (DMSO-d₆, 600MHz) δ 10.18 (brs, 3H), 9.29-9.55 (m, 2H), 8.72-8.93 (m, 4H), 9.91-8.43 (m, 9H), 7.63-7.72 (m, 5H), 7.11-7.44 (m, 9H), 6.51-6.67 (m, 3H), 5.04-5.43 (m, 25H), 3.80-4.78 (m, 41H), 3.48-3.59 (m, 12H), 3.07 (s, 27H), 2.94 (s, 9H), 2.90 (s, 6H), 2.34 (s, 9H), 2.13-2.18(m, 6H), 1.81-1.89 (m, 6H), 1.23-1.28 (m, 20H), 0.86 (t, 9H).

Example 39: Synthesis of DL-18



Step 1: Synthesis of (S)-2-(2-azidoacetamido)-N-(((S)-1-(((S)-4-ethyl-4-hydroxy-3,14-dioxo-11-(pyrrolidin-1-yl)-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)amino)-1-oxopropan-2-yl)propanamide (B-93)

[0570] To a solution of 2-azidoacetic acid (26 μL , 0.35 mmol, 2.0 eq) in DMAc (1.0 mL) was added HOPO (39 mg, 0.35 mmol, 2.0 eq) and EDCI (63.9 mg, 0.33 mmol, 1.9 eq). After stirred at rt for 15 min, the reaction mixture was added to the solution of **B-92** (WO2024059236) (140.9 mg, 0.18 mmol, 1.0 eq) and 2,6-Lutidine (102 μL , 0.88 mmol, 5.0 eq) in DMAc (1.0 mL). The resulting mixture was stirred at r.t. for 2 hrs and purified by prep-HPLC to afford **B-93** (115 mg, 85.0%) as a yellow powder

[0571] LCMS[M+H]⁺: 658.7

Step 2: Synthesis of DL-18

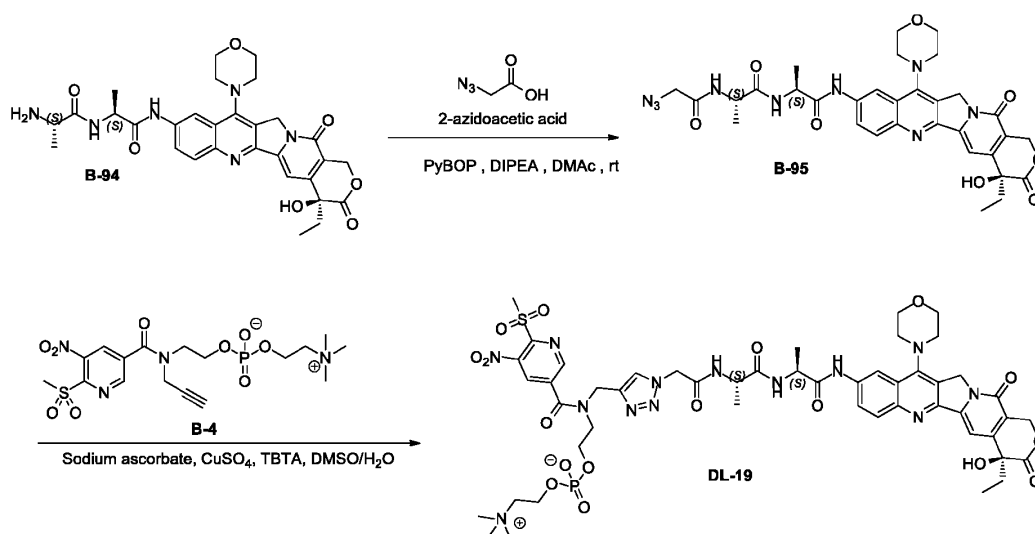
[0572] To a solution of **B-93** (115 mg, 0.18 mmol, 1.0 eq) in H₂O/DMSO (2.0 mL/2.0 mL) was added **B-4** (90.4 mg, 0.18 mmol, 1.05 eq), CuSO₄·5H₂O (2.2 mg, 0.009 mmol, 0.05 eq), TBTA (4.64 mg 0.009 mmol, 0.05 eq) and sodium ascorbate (6.9 mg, 0.035 mmol, 0.2 eq). After stirred at r.t. for 1h. The reaction mixture was purified by prep-HPLC to afford **DL-18** (158.47 mg, 84.17%) as a yellow powder.

[0573] LCMS[M+H]⁺: 1151.2

[0574] ¹H NMR (DMSO-d₆, 600 MHz) δ 9.98 (d, J= 28.9 Hz, 1H), 9.09 (d, J= 1.5 Hz, 1H), 9.06-8.99 (m, 1H), 8.84 (s, 1H), 8.74 (dd, J= 49.9, 1.5 Hz, 1H), 8.45-8.40 (m, 1H), 8.14 (d, J=

49.9 Hz, 1H), 7.99 (dd, J= 43.9, 9.0 Hz, 1H), 7.73 (s, 1H), 7.62 (d, J= 36.8 Hz, 1H), 6.62 (s, 1H), 5.71-5.62 (m, 2H), 5.43 (d, J= 2.8 Hz, 2H), 5.25 (dt, J= 29.8, 11.9 Hz, 2H), 4.83 (s, 1H), 4.65 (s, 1H), 4.46 (dd, J= 15.1, 7.5 Hz, 1H), 4.32 (s, 1H), 4.28-4.18 (m, 2H), 4.15 (d, J= 6.3 Hz, 1H), 4.11 (s, 4H), 4.05-3.96 (m, 1H), 3.64 (d, J= 4.8 Hz, 2H), 3.61-3.58 (m, 2H), 3.47 (d, J= 1.9 Hz, 3H), 3.14 (d, J= 11.2 Hz, 9H), 2.03 (s, 4H), 1.92-1.81 (m, 2H), 1.39 (d, J= 7.1 Hz, 3H), 1.31 (d, J= 7.1 Hz, 3H), 0.87 (t, J= 7.3 Hz, 3H).

Example 40: Synthesis of DL-19



Step 1: Synthesis of (S)-2-(2-azidoacetamido)-N-(((S)-1-(((S)-4-ethyl-4-hydroxy-11-morpholino-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]-indolizino[1,2-b]quinolin-9-yl)amino)-1-oxopropan-2-yl)propenamide (B-95)

[0575] To a solution of **B-94** (WO2024059236) (16.7 mg, 0.028 mmol, 1.0 eq) and 2-azidoacetic acid (4.5 uL, 0.06 mmol, 2.1 eq) in DMAc (3.0 mL) was added PyBOP (31 mg, 0.06 mmol, 2.1 eq), followed by DIPEA (20.7 uL, 0.119 mmol, 4.2 eq). The reaction mixture was stirred at r.t. for 4 hrs, then purified by prep-HPLC to afford **B-95** (6.97 mg, 36.7%) as a powder.

[0576] LCMS[M+H]⁺: 674.69

Step 2: Synthesis of DL-19

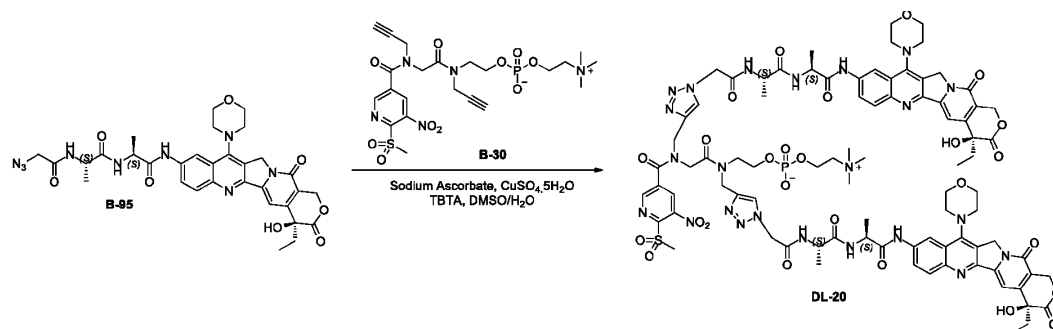
[0577] To a solution of **B-95** (5.0 mg, 0.0074 mmol, 1.0 eq) in H₂O/DMSO (1.6 mL/1.6 mL) was added **B-4** (3.6 mg, 0.0074 mmol, 1.0 eq.), CuSO₄·5H₂O (0.74 mg, 0.003 mmol, 0.4 eq), TBTA (1.6 mg, 0.003 mmol, 0.4 eq) and sodium ascorbate (1.2 mg, 0.006 mmol, 0.8 eq) and

stirred at r.t. for 1h. Then the reaction mixture was purified by prep-HPLC to afford **DL-19** (5.67 mg, 65.9%) as a yellow powder.

[0578] LCMS[M+H]⁺: 1166.97

[0579] ¹H NMR (600 MHz, DMSO-d₆) δ 10.42 (s, 1H), 10.36 (s, 1H), 9.12 (s, 1H), 9.00 (s, 1H), 8.80 (s, 1H), 8.67 (s, 1H), 8.65 (s, 2H), 8.43 (s, 1H), 8.13 (s, 1H), 8.07 (s, 1H), 8.02 (s, 1H), 8.01 (d, J= 4.0 Hz, 1H), 7.85 (s, 1H), 7.26 (s, 1H), 5.53 (s, 2H), 5.41 (s, 2H), 5.15 (s, 2H), 4.80 (s, 1H), 4.61 (s, 1H), 4.49 (s, 1H), 4.39 (s, 1H), 4.30 (s, 2H), 4.18 (s, 2H), 4.11 (s, 2H), 3.91 (s, 3H), 3.61 (s, 3H), 3.57 (s, 2H), 3.47 (s, 3H), 3.11 (d, J= 3.8 Hz, 9H), 2.63-2.61 (m, 1H), 2.35-2.33 (m, 1H), 1.90-1.79 (m, 4H), 1.36 (d, J= 4.9 Hz, 2H), 1.29-1.26 (m, 2H), 1.22 (s, 1H), 0.85 (t, J= 7.4 Hz, 3H)

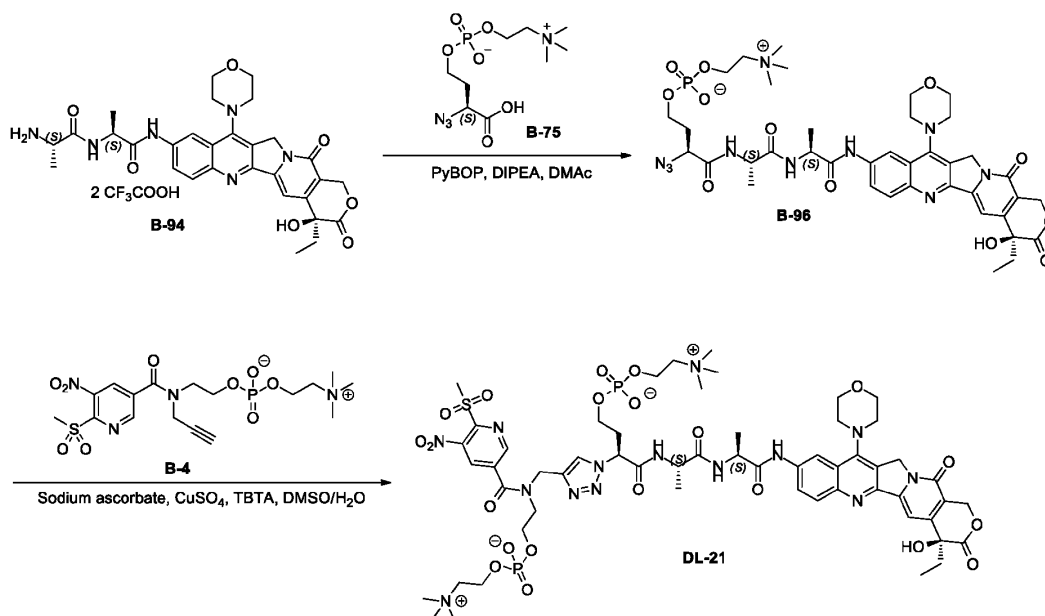
Example 41: Synthesis of DL-20



[0580] To a solution of **B95** (2.0 mg, 0.003 mmol, 1.0 eq) in H₂O/DMSO (1.0 mL/1.0 mL) was added **B-30** (0.9 mg, 0.0015 mmol, 0.5 eq), CuSO₄·5H₂O (0.3 mg, 0.001 mmol, 0.4 eq), TBTA (0.6 mg, 0.001 mmol, 0.4 eq) and sodium ascorbate (0.5 mg, 0.0024 mmol, 0.8 eq). After stirred at r.t. for 5 hrs, the reaction mixture was purified by prep-HPLC to afford **DL-20** (1.32 mg, 45.1%) as a yellow powder.

[0581] LCMS[M/2+H]⁺: 968.22

Example 42: Synthesis of DL-21



Step 1: Synthesis of (S)-3-azido-4-(((S)-1-(((S)-1-(((S)-4-ethyl-4-hydroxy-11-morpholino-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)amino)-4-oxobutyl (2-(trimethylammonio)ethyl) phosphate (B-96)

[0582] To a solution of **B-94** (60 mg, 0.073 mmol, 1.0 eq) in DMAc (6.0 mL) was added **B-75** (34 mg, 0.1 mmol, 1.5 eq) and DIPEA (51 μ L, 0.3 mmol, 4.0 eq), followed by PyBOP (57 mg, 0.1 mmol, 1.5 eq). The reaction was stirred at r.t. for 3 hrs, then purified by prep-HPLC to afford **B-96** (26.3 mg, 40.68%) as a yellow powder.

[0583] LCMS[M+H]⁺: 883.6

Step 2: Synthesis of DL-21

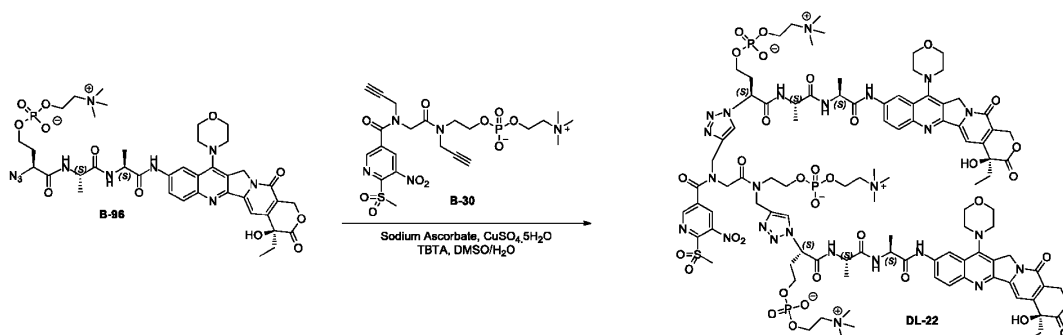
[0584] To a solution of **B-96** (6.4 mg, 0.007 mmol, 1.0 eq) in H₂O/DMSO (1.0 mL/1.0 mL) was added **B-4** (4 mg, 0.008 mmol, 1.1 eq), CuSO₄·5H₂O (0.7 mg, 0.003 mmol, 0.4 eq) and TBTA (1.5 mg, 0.003 mmol, 0.4 eq), followed by sodium ascorbate (1.2 mg, 0.006 mmol, 0.8 eq). The resulting mixture was stirred at r.t. for 1.5 hrs and then purified by prep-HPLC to afford **DL-21** (6.5 mg, 65.7%) as a yellow powder.

[0585] LCMS[M+H]⁺: 1376.0

[0586] ¹H NMR (DMSO-d₆, 600 MHz): δ 10.51 (d, J= 9.4 Hz, 1H), 9.16 (d, J= 70.8 Hz, 1H), 8.86 (d, J= 63.7 Hz, 1H), 8.70 (d, J= 7.6 Hz, 1H), 8.53 (s, 1H), 8.30 (d, J= 43.0 Hz, 1H), 8.05 (d,

J= 9.0 Hz, 1H), 7.94 (t, J= 10.2 Hz, 1H), 7.28 (s, 1H), 6.52 (s, 1H), 5.62-5.56 (m, 1H), 5.55 (s, 2H), 5.43 (s, 2H), 4.84 (dd, J= 86.8, 15.0 Hz, 1H), 4.63 (q, J= 16.5 Hz, 1H), 4.51-4.43 (m, 1H), 4.29 (s, 1H), 4.15 (s, 3H), 4.01 (s, 1H), 3.94 (s, 4H), 3.75 (s, 2H), 3.70 (s, 2H), 3.63 (s, 3H), 3.56 (d, J= 10.2 Hz, 5H), 3.51 (s, 2H), 3.49 (d, J= 5.1 Hz, 5H), 3.47 (s, 7H), 3.14 (s, 6H), 3.11 (d, J= 5.8 Hz, 9H), 3.10 (s, 5H), 2.36 (s, 1H), 1.87 (ddt, J= 21.5, 14.2, 7.1 Hz, 2H), 1.42 (t, J= 7.0 Hz, 3H), 1.27 (d, J= 5.5 Hz, 3H), 0.87 (t, J= 7.3 Hz, 3H).

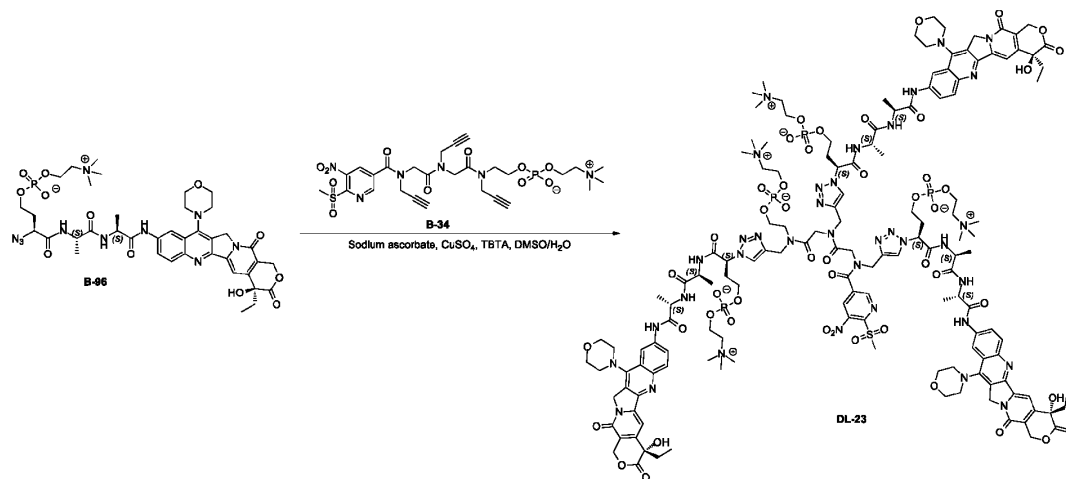
Example 43: Synthesis of DL-22



[0587] To a solution of **B-96** (7 mg, 0.008 mmol, 2.1 eq) in H₂O/DMSO (1.0 mL/1.0 mL) was added **B-30** (2 mg, 0.004 mmol, 1.0 eq), CuSO₄·5H₂O (0.8 mg, 0.003 mmol, 0.8 eq), TBTA (1.6 mg, 0.003 mmol, 0.8 eq) and sodium ascorbate (1.2 mg, 0.006 mmol, 1.6 eq). After stirred at rt for 1.5 hrs, the reaction mixture was purified by prep-HPLC to afford **DL-22** (6.5 mg, 73.0%) as a yellow powder.

[0588] LCMS[M+H]⁺: 2353.8

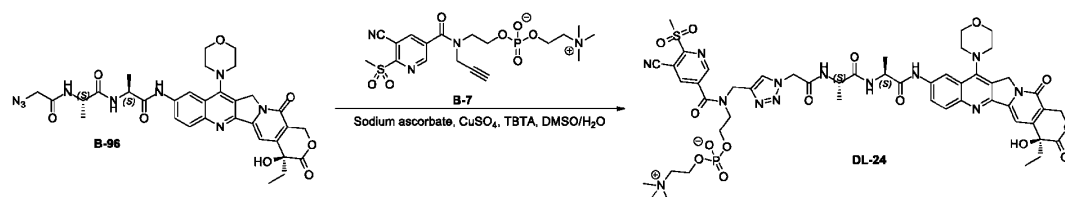
[0589] ¹H NMR (DMSO-d₆, 600 MHz): δ 10.50 (d, J= 12.6 Hz, 3H), 9.26 (d, J= 10.3 Hz, 1H), 9.24-9.20 (m, 1H), 9.15 (s, 2H), 9.07-8.98 (m, 1H), 8.94 (s, 1H), 8.92 (s, 1H), 8.88 (s, 1H), 8.67 (s, 2H), 8.58 (d, J= 27.0 Hz, 1H), 8.49 (s, 2H), 8.34 (s, 1H), 8.31 (s, 1H), 8.22 (d, J= 24.6 Hz, 1H), 8.10 (d, J= 10.0 Hz, 1H), 8.04 (d, J= 8.9 Hz, 2H), 7.92 (d, J= 9.1 Hz, 2H), 7.26 (s, 2H), 6.51 (s, 2H), 5.61 (s, 3H), 5.54 (s, 4H), 5.43 (s, 4H), 4.69 (s, 2H), 4.66-4.61 (m, 2H), 4.58 (s, 2H), 4.47 (s, 3H), 4.33 (s, 1H), 4.29 (s, 3H), 4.18 (s, 7H), 4.12-4.07 (m, 1H), 3.93 (s, 10H), 3.82 (s, 2H), 3.68 (s, 7H), 3.61 (s, 4H), 3.56 (s, 9H), 3.52 (s, 3H), 3.50 (d, J= 2.7 Hz, 4H), 3.49 (s, 3H), 3.46 (s, 15H), 3.14 (s, 2H), 3.13 (s, 4H), 3.12 (s, 3H), 3.12-3.11 (m, 1H), 3.10 (s, 1H), 3.09 (s, 7H), 3.08 (s, 4H), 3.06 (s, 4H), 1.86 (ddd, J= 21.8, 14.0, 7.0 Hz, 5H), 1.40 (s, 6H), 1.27 (t, J= 7.5 Hz, 6H), 0.87 (t, J= 7.3 Hz, 6H).

Example 44: Synthesis of DL-23

[0590] To a solution of **B-96** (7.5 mg, 0.008 mmol, 3.1 eq) in H₂O/DMSO (1.0 mL/1.0 mL) was added **B-34** (1.9 mg, 0.003 mmol, 1.0 eq), CuSO₄·5H₂O (0.8 mg, 0.003 mmol, 1.2 eq), TBTA (1.8 mg, 0.003 mmol, 1.2 eq) and sodium ascorbate (1.3 mg, 0.0066 mmol, 2.4 eq). After stirred at r.t. for 1.5 hrs, the reaction mixture was purified by prep-HPLC to afford **DL-23** (7 mg, 75.8%) as a yellow powder.

[0591] LCMS[M+2H]²⁺: 1666.1

[0592] ¹H NMR (DMSO-d₆, 600 MHz): δ 10.56 (s, 4H), 9.37-9.10 (m, 5H), 9.01-8.80 (m, 4H), 8.66 (s, 5H), 8.59-8.42 (m, 5H), 8.35-8.14 (m, 5H), 8.02 (s, 5H), 7.93 (s, 3H), 7.26 (s, 4H), 6.50 (s, 5H), 5.62 (s, 4H), 5.53 (s, 6H), 5.42 (s, 7H), 4.74-4.54 (m, 7H), 4.47 (s, 7H), 4.17 (s, 18H), 3.92 (s, 17H), 3.60 (s, 14H), 3.57-3.44 (m, 30H), 3.15 (d, J= 15.3 Hz, 14H), 3.10-3.01 (m, 24H), 1.94-1.78 (m, 7H), 1.39 (s, 9H), 1.27 (s, 10H), 0.86 (t, J= 7.4 Hz, 9H).

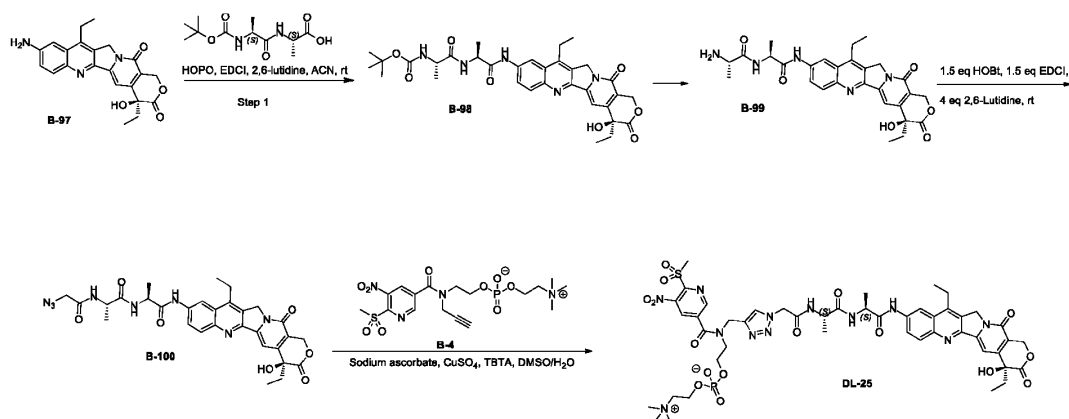
Example 45: Synthesis of DL-24

[0593] To a solution of **B-96** (1.0 mg, 0.0016 mmol, 1.0 eq) in H₂O/DMSO (0.25 mL/0.25 mL) was added **B-7** (0.7 mg, 0.0016 mmol, 1.0 eq), CuSO₄·5H₂O (0.16 mg, 0.0006 mmol, 0.4 eq), TBTA (0.33 mg, 0.0006 mmol, 0.4 eq) and sodium ascorbate (0.25 mg, 0.0013 mmol, 0.8

eq). After stirred at r.t. for 7 hrs, the reaction mixture was purified by prep-HPLC to afford **DL-24** (0.92 mg, 51.1%) as a yellow powder.

[0594] LCMS[M+H]⁺: 1146.85

Example 46: Synthesis of DL-25



Step 1: Synthesis of tert-butyl ((S)-1-(((S)-1-(((S)-4,11-diethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (**B-98**)

[0595] To a solution of Boc-Ala-Ala-OH (159.6 mg, 0.61 mmol), HOPO (85 mg, 0.76 mmol), EDCI (146.9 mg, 0.76 mmol) in ACN (10 mL) was added 2,6-Lutidine (328 mg, 3.1 mmol) to stir for 0.5h, then (S)-9-amino-4,11-diethyl-4-hydroxy-1,12-dihydro-14H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H)-dione (**B-97**) (200 mg, 0.5 mmol) at 25°C for 24h. Quenched by water (3 mL), filtered, purified by prep HPLC to give **B-98** (180 mg, yield: 73%) as a yellow solid

[0596] LCMS[M+H]⁺: 634.3

Step 2: Synthesis of (S)-2-amino-N-((S)-1-(((S)-4,11-diethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)amino)-1-oxopropan-2-yl)propanamide (**B-99**)

[0597] (2-{[(2S)-2-azido-2-{(1S)-1-{[(2S)-1-(tert-butoxy)-1-oxopropan-2-yl]carbamoyl}ethyl]carbamoyl}ethyl phosphonate]oxy}ethyl)trimethylazanium (**B-98**) (180 mg, 0.28 mmol) was added anisole (153 mg, 1.4 mmol) and CF₃COOH (2 mL) to stirred at 25°C for 5

min. Quenched by water (3 mL), filtered, purified by prep HPLC to give **B-99** (140 mg, yield: 73%) as a yellow solid.

[0598] LCMS[M+H]⁺: 534.3

[0599] ¹H NMR (400 MHz, DMSO) δ 10.66 (s, 1H), 8.80 (d, J = 7.0 Hz, 1H), 8.65 (s, 1H), 8.15 (d, J = 9.1 Hz, 1H), 8.07 (s, 2H), 7.98 (d, J = 9.2 Hz, 1H), 7.29 (s, 1H), 6.54 (s, 1H), 5.44 (s, 2H), 5.33 (s, 2H), 4.63 – 4.51 (m, 1H), 3.95 – 3.87 (m, 1H), 3.16 – 3.04 (m, J = 7.5 Hz, 2H), 1.93 – 1.79 (m, 2H), 1.45 – 1.37 (m, J = 13.4, 7.0 Hz, 6H), 1.34 (t, J = 7.5 Hz, 3H), 0.87 (t, J = 7.2 Hz, 3H).

Step 3: Synthesis of (S)-2-(2-azidoacetamido)-N-(((S)-1-(((S)-4,11-diethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)amino)-1-oxopropan-2-yl)propanamide (B-100)

[0600] To a solution of 2-azidoacetic acid (7 mg, 0.06 mmol), **B-99** (30 mg, 0.056 mmol), 2,6-Lutidine (24 mg 0.22 mmol) and 1-hydroxybenzotriazole (13 mg, 0.08 mmol) in DCM (10 mL), EDCI (13 mg, 0.08 mmol) was added and stirred at 25 °C for 45 min. The residue was purified by prep-HPLC to give the intermediate **B-100** (30 mg, yield: 92%) as a white solid.

[0601] LCMS[M+H]⁺: 617.3

[0602] ¹H NMR (400 MHz,) δ 8.64 (s, 1H), 7.87 (d, J = 9.1 Hz, 1H), 7.69 (dd, J = 9.1, 1.9 Hz, 1H), 7.52 (s, J = 20.8 Hz, 1H), 5.52 (d, J = 16.2 Hz, 1H), 5.31 (d, J = 16.2 Hz, 1H), 5.11 (s, 2H), 4.57 (q, J = 7.0 Hz, 1H), 4.44 (q, J = 7.1 Hz, 1H), 3.94 (s, 2H), 3.18 – 3.05 (m, 2H), 1.99 – 1.86 (m, 2H), 1.54 – 1.45 (m, 6H), 1.37 (t, J = 7.6 Hz, 3H), 0.99 (t, J = 7.5 Hz, 3H).

Step 4: Synthesis of DL-25

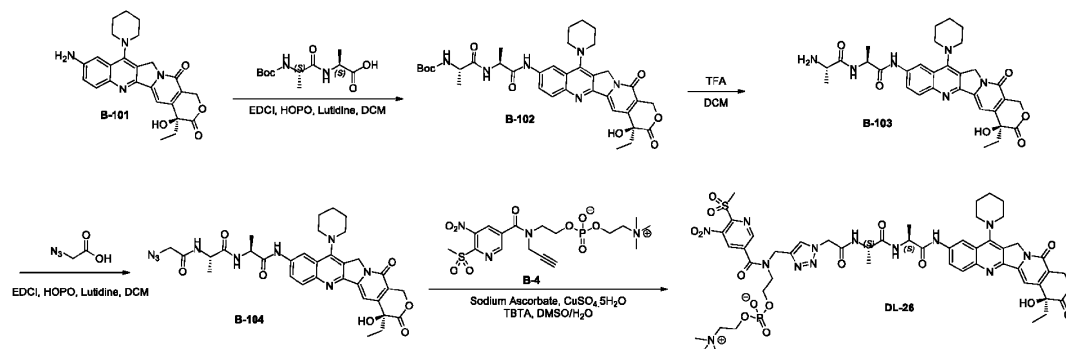
[0603] To a solution of **B-100** (20 mg, 0.03 mmol, 1.0 eq.) in H₂O/DMSO (2.3 mL/2.3 mL) was added B-4 (16 mg, 0.03 mmol, 1.0 eq), CuSO₄·5H₂O (3.2 mg, 0.013 mmol, 0.4 eq), TBTA (7 mg 0.013 mmol, 0.4 eq) and sodium ascorbate (5.1 mg, 0.03 mmol, 0.8 eq) and stirred at r.t. for 1h. Then the reaction mixture was purified by prep-HPLC to afford **DL-25** (26.3 mg, 73.1%) as a yellow powder.

[0604] LCMS[M+H]⁺: 1109.87

[0605] ¹H NMR (600 MHz, DMSO-d₆) δ 10.50 (s, 1H), 10.41 (s, 1H), 9.14 (d, J = 1.5 Hz, 1H), 9.02 (d, J = 1.6 Hz, 1H), 8.83 (s, 1H), 8.70 (dd, J = 8.7, 4.6 Hz, 2H), 8.63 (s, 2H), 8.43-8.39 (m, 1H), 8.14 (s, 1H), 8.12-8.08 (m, 2H), 7.98-7.93 (m, 2H), 7.28 (s, 1H), 7.28 (s, 1H), 6.50 (s, 2H), 5.41 (s, 2H), 5.31 (s, 2H), 5.15 (s, 2H), 4.80 (s, 1H), 4.61 (s, 1H), 4.48 (dd, J = 12.7, 6.9 Hz,

2H), 4.39 (dd, $J = 12.7, 7.1$ Hz, 2H), 4.23 (s, 2H), 4.10 (s, 4H), 3.88 (s, 2H), 3.59 (d, $J = 4.9$ Hz, 3H), 3.53 (s, 2H), 3.47 (s, 3H), 3.11 (d, $J = 4.7$ Hz, 9H), 2.62 (dd, $J = 3.6, 1.8$ Hz, 1H), 2.35 (dd, $J = 3.6, 1.8$ Hz, 1H), 1.85 (ddd, $J = 26.2, 14.1, 7.3$ Hz, 4H), 1.37 (d, $J = 6.9$ Hz, 3H), 1.32 (t, $J = 7.6$ Hz, 3H), 1.27 (dd, $J = 6.9, 4.5$ Hz, 3H), 0.86 (t, $J = 7.3$ Hz, 3H).

Example 47: Synthesis of DL-26



Step 1: Synthesis of tert-butyl ((S)-1-(((S)-1-(((S)-4-ethyl-4-hydroxy-3,14-dioxo-11-(piperidin-1-yl)-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (B-102)

[0606] To a solution of **B-101** (WO2024059236) (200 mg, 0.45 mmol, 1.0 eq) and Boc-Ala-Ala-OH (179 mg, 0.69 mmol, 1.5 eq) in DCM (10 mL) was added lutidine (167 mg, 1.56 mmol, 3.5 eq) at r.t., followed by EDCI (174 mg, 0.6 mmol, 1.3 eq) and HOPO (66.4 mg, 0.6 mmol, 1.3 eq). The resulting mixture was stirred at room temperature for 16 hours and purified by prep-HPLC to give intermediate **B-102** (130 mg, yield: 42%) as a white solid.

[0607] LCMS[M-H]⁻: 689.3

Step 2: Synthesis of (S)-2-amino-N-((S)-1-(((S)-4-ethyl-4-hydroxy-3,14-dioxo-11-(piperidin-1-yl)-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)amino)-1-oxopropan-2-yl)propenamide (B-103)

[0608] To a solution of **B-102** (130 mg, 0.19 mmol, 1.0 eq) in DCM (6 mL) was added TFA (1.0 mL) at 0°C, and stirred at r.t. for 4 h, solvent was removed to give crude **B-103** which was used for next step directly.

[0609] LCMS[M+H]⁺: 588.3

Step 3: Synthesis of (S)-2-(2-azidoacetamido)-N-(((S)-1-(((S)-4-ethyl-4-hydroxy-3,14-dioxo-11-(piperidin-1-yl)-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)amino)-1-oxopropan-2-yl)propenamide (B-104)

[0610] To a solution of **B-103** (112 mg, 0.19 mmol, 1.0 eq) and 2-azidoacetic acid (26 mg, 0.26 mmol, 1.4 eq) in DCM (10 mL) was added lutidine (70 mg, 0.66 mmol, 3.5 eq) at r.t., followed by EDCI (45 mg, 0.24 mmol, 1.2 eq) and HOPO (26 mg, 0.24 mmol, 1.2 eq.). The resulting mixture was stirred at room temperature for 16 hours and purified by prep-HPLC to give **B-104** (90 mg, yield: 70%) as a white solid.

[0611] LCMS[M-H]⁻: 672.3

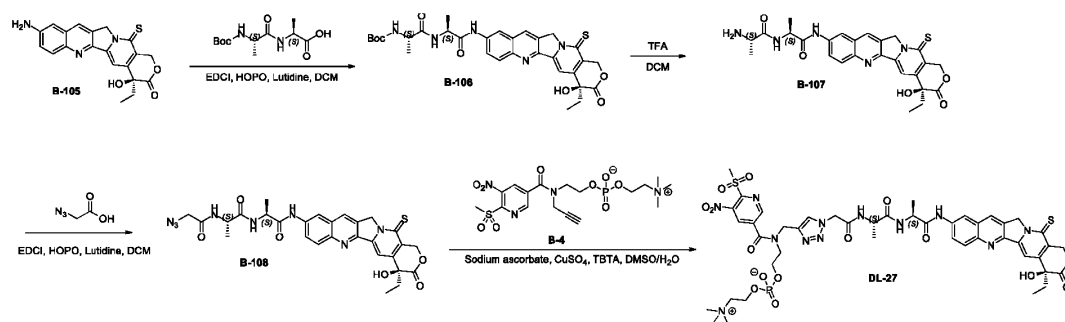
Step 4: Synthesis of DL-26

[0612] To a solution of **B-104** (19.6 mg, 0.025 mmol, 1.0 eq) in H₂O/DMSO (1.6 mL/1.6 mL) was added **B-4** (12.3 mg, 0.025 mmol, 1.0 eq), CuSO₄·5H₂O (2.5 mg, 0.01 mmol, 0.4 eq), TBTA (5.3 mg 0.01 mmol, 0.4 eq) and sodium ascorbate (4 mg, 0.02 mmol, 0.8 eq) and stirred at r.t. for 1h. Then the reaction mixture was purified by prep-HPLC to afford **DL-26** (22.33 mg, 73.1%) as a yellow powder.

[0613] LCMS[M+H]⁺: 1164.78

[0614] ¹H NMR (600 MHz, DMSO-d₆) δ 10.37 (d, J= 22.8 Hz, 1H), 9.07 (d, J= 69.4 Hz, 1H), 8.82 (s, 1H), 8.67 (d, J= 14.4 Hz, 2H), 8.45 (s, 1H), 8.12 (d, J= 41.7 Hz, 1H), 8.03-7.99 (m, 1H), 7.82 (s, 1H), 7.30-7.27 (m, 1H), 5.49 (s, 2H), 5.42 (s, 2H), 5.17 (s, 2H), 4.83 (s, 1H), 4.63 (s, 1H), 4.54-4.49 (m, 1H), 4.43-4.39 (m, 1H), 4.33 (s, 1H), 4.23 (s, 1H), 4.16 (s, 1H), 4.01 (s, 1H), 3.64 (s, 3H), 3.60 (s, 2H), 3.49 (s, 9H), 3.13 (d, J= 4.6 Hz, 9H), 2.63-2.60 (m, 1H), 2.40-2.37 (m, 2H), 2.22 (s, 1H), 2.03-1.98 (m, 1H), 1.90-1.80 (m, 5H), 1.71 (s, 1H), 1.38 (d, J= 4.9 Hz, 3H), 1.32-1.26 (m, 3H), 0.87 (t, J= 7.4 Hz, 3H).

Example 48: Synthesis of DL-27



Step 1: Synthesis of tert-butyl ((S)-1-(((S)-1-(((S)-4-ethyl-4-hydroxy-3-oxo-14-thioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (B-106)

[0615] To a solution of **B-105** (200 mg, 0.53 mmol, 1.0 eq.) and Boc-Ala-Ala-OH (179 mg, 0.69 mmol, 1.3 eq.) in DCM (10 mL) was added lutidine (167 mg, 1.56 mmol, 3.0 eq.) at r.t., followed by EDCI (174 mg, 0.6 mmol, 1.2 eq.) and HOPO (66.4 mg, 0.6 mmol, 1.2 eq.). The resulting mixture was stirred at room temperature for 16 hours and purified by prep-HPLC to give **B-106** (140 mg, yield: 43%) as a white solid..

[0616] LCMS[M-H]⁻: 620.3

Step 2: Synthesis of (S)-2-amino-N-((S)-1-(((S)-4-ethyl-4-hydroxy-3-oxo-14-thioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)amino)-1-oxopropan-2-yl)propanamide (B-107)

[0617] To a solution of **B-106** (140 mg, 0.22 mmol, 1.0 eq) in DCM (6 mL) was added TFA (1.0 mL) at 0°C, and stirred at r.t. for 4 h, solvent was removed to give crude **B-107** which was used for next step directly.

[0618] LCMS[M+H]⁺: 522.3

Step 3: Synthesis of (S)-2-(2-azidoacetamido)-N-((S)-1-(((S)-4-ethyl-4-hydroxy-3-oxo-14-thioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)amino)-1-oxopropan-2-yl)propanamide (B-108)

[0619] To a solution of **B-107** (117 mg, 0.22 mmol, 1.0 eq) and 2-azidoacetic acid (26 mg, 0.26 mmol, 1.2 eq) in DCM (10 mL) was added lutidine (70 mg, 0.66 mmol, 3.0 eq) at r.t., followed by EDCI (45 mg, 0.24 mmol, 1.1 eq) and HOPO (26 mg, 0.24 mmol, 1.1 eq). The resulting mixture was stirred at room temperature for 16 hours and purified by prep-HPLC to give **B-108** (86 mg, yield: 65%) as a white solid.

[0620] LCMS[M-H]⁻: 603.5

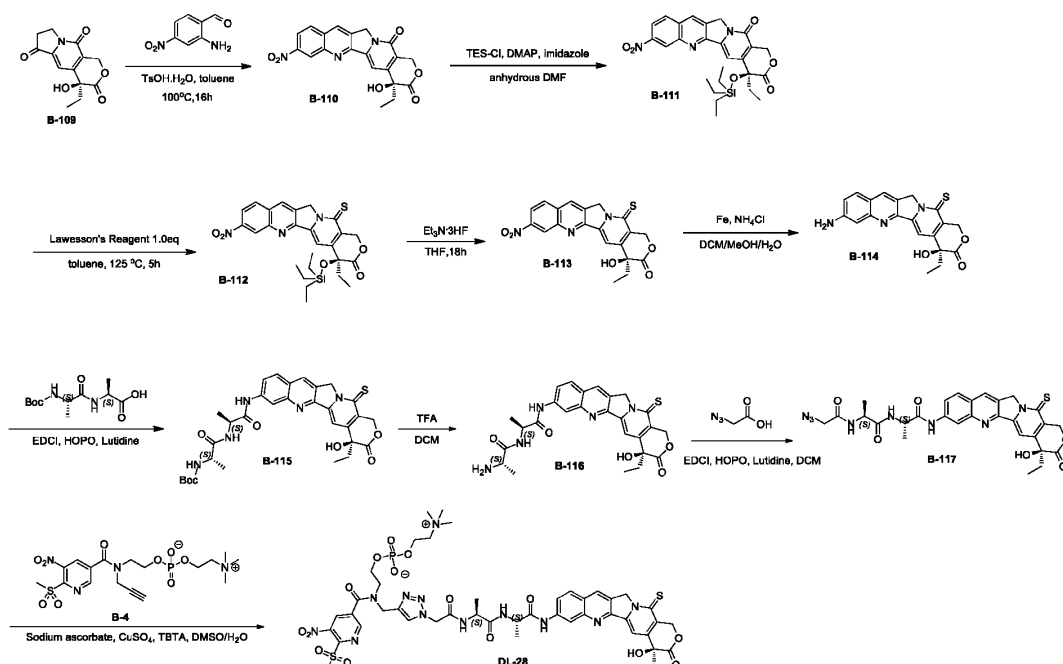
Step 4: Synthesis of DL-27

[0621] To a solution of **B-108** (6.5 mg, 0.01 mmol, 1.0 eq) in H₂O/DMSO (1 mL/1mL) was added **B-4** (6.3 mg, 0.013 mmol, 1.2 eq), TBTA (5.8 mg, 0.011 mmol, 1.0 eq), CuSO₄·5H₂O (2.7 mg, 0.01 mmol, 1.0 eq) and sodium ascorbate (4.3 mg, 0.022 mmol, 2.0 eq), and stirred at r.t. for 1.5 hrs. Then the reaction mixture was purified by prep-HPLC to afford **DL-27** (5.4 mg, 44%) as a white solid.

[0622] LCMS[M+H]⁺: 1097.6

[0623] ¹H NMR (600 MHz, DMSO-d₆) δ 10.8 (s, 0.5H), 10.4 (s, 0.5H), 9.12 (d, 1H), 8.93-8.65 (m, 3H), 8.55 (s, 1H), 8.45 (t, 1H), 8.18-8.10 (m, 2H), 7.92 (dd, 1H), 7.80 (s, 1H), 6.74 (d, 1H), 5.93 (d, 1H), 5.51 (d, 3H), 5.18 (d, 2H), 4.81 (s, 1H), 4.64 (s, 1H), 4.55-4.33 (m, 2H), 4.16 (s, 1H), 4.03 (d, 2H), 3.82 (s, 1H), 3.64-3.46 (m, 8H), 3.13 (s, 9H), 1.95-1.88 (m, 2H), 1.39 (d, 3H), 1.30 (d, 3H), 0.87 (t, 3H).

Example 49: Synthesis of DL-28



Step 1: Synthesis of (S)-4-ethyl-4-hydroxy-8-nitro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione (**B-110**)

[0624] To the mixture of **B-109** (1.0 g, 3.8 mmol) and 2-amino-4-nitrobenzaldehyde (757 mg, 4.6 mmol) in toluene (100 mL) was added p-TsOH (274 mg, 1.4 mmol), and then heated to 100°C for 4h. Toluene was evaporated, and the residue was retreated with DCM and filtered to obtain brown solid as **B-110** (868 mg, yield: 58%).

[0625] LCMS[M+H]⁺: 394.3

Step 2: Synthesis of (S)-4-ethyl-8-nitro-4-((triethylsilyl)oxy)-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione (**B-111**)

[0626] To the solution of **B-110** (3.0 g, 7.63 mmol) and TES-Cl (9.2 g, 61.0 mmol) in DMF (60 mL) was added DMAP (932 mg, 7.63 mmol) and imidazole (2.6 g, 38.2 mmol) under N₂. The reaction was stirred at r.t. for overnight and purified by prep-MPLC to give **B-111** (2.46 g, 63.6%) as a yellow solid.

[0627] LCMS[M-H]⁻: 506.3

Step 3: Synthesis of (S)-4-ethyl-8-nitro-14-thioxo-4-((triethylsilyl)oxy)-12,14-dihydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-3(4H)-one (B-112)

[0628] To a solution of **B-111** (2.46 g, 4.85 mmol) in Toluene (100 mL) at 20°C was added Lawesson's Reagent (2.35 g, 5.82 mmol). The reaction mixture was stirred at 125°C for 16h. Solvent was evaporated, the crude product was purified by Flash Chromatography (PE/DCM=3/7) to give **B-112** (715 mg, 28.3%) as a yellow solid.

[0629] LCMS[M+H]⁺: 524.2

Step 4: Synthesis of (S)-4-ethyl-4-hydroxy-8-nitro-14-thioxo-12,14-dihydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-3(4H)-one (B-113)

[0630] To a solution of **B-112** (475 mg, 0.91 mol) in THF (12 mL) was added Et₃N·3HF (1.1 mL, 6.81 mmol) under N₂. The reaction suspension was stirred at room temperature for 16hrs. The reaction mixture was poured into H₂O (20 mL) and stirred at r.t for 0.5 h. Precipitate was filtered, the filter cake was washed with H₂O (2 mL*3) to give **B-113** (256 mg, yield: 69.0%) as a yellow solid.

[0631] LCMS[M-H]⁻: 408.2

Step 5: Synthesis of (S)-8-amino-4-ethyl-4-hydroxy-14-thioxo-12,14-dihydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-3(4H)-one (B-114)

[0632] To a solution of **B-113** (256 mg, 0.63 mol) in MeOH/DCM/H₂O (13 mL/13 mL/8 mL) was added Fe (349 mg, 6.3 mmol) and NH₄Cl (402 mg, 7.5 mmol) under N₂. The reaction suspension was stirred at room temperature for 16 hrs, then filtered and concentrated. The crude product was purified by prep-HPLC to give **B-114** (236 mg, 99.2%) as a yellow solid.

[0633] LCMS[M+H]⁺: 380.4

[0634] ¹H NMR (600 MHz, DMSO) δ 8.48 (s, 1H), 7.82 (d, 1H), 7.78 (s, 1H), 7.15 (dd, 1H), 7.07 (s, 1H), 5.92 (d, 1H), 5.50 (d, 1H), 5.42 (s, 2H), 1.88-1.90 (m, 2H), 0.87 (t, 3H).

Step 6: Synthesis of tert-butyl ((S)-1-(((S)-1-(((S)-4-ethyl-4-hydroxy-3-oxo-14-thioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-8-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (B-115)

[0635] To a solution of **B-114** (50 mg, 0.13 mmol) in DMAc (3 mL) was added Boc-Ala-Ala-OH (70 mg, 0.26 mmol), HOPO (45 mg, 0.4 mmol), lutidine (60 mg, 0.53 mmol) and EDCI (45 mg, 0.25 mmol) under N₂, and stirred at r.t for 24 hrs. The reaction mixture was poured into water (10 mL) and extracted with DCM (10 mL*3), the organic phase was concentrated to give the crude **B-115** as yellow oil and used for the next step directly.

Step 7: Synthesis of (S)-2-amino-N-((S)-1-(((S)-4-ethyl-4-hydroxy-3-oxo-14-thioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-8-yl)amino)-1-oxopropan-2-yl)propenamide (B-116)

[0636] The crude **B-115** from the previous step was dissolved in DCM (4 mL), TFA (1 mL) was added to the solution and stirred at r.t. under N₂ for 3h. This reaction mixture was purified by prep-HPLC to give **B-116** (21.5 mg, 31.3%) as yellow white solid.

[0637] LCMS[M+H]⁺: 522.4

Step 8: Synthesis of (S)-2-(2-azidoacetamido)-N-((S)-1-(((S)-4-ethyl-4-hydroxy-3-oxo-14-thioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-8-yl)amino)-1-oxopropan-2-yl)propenamide (B-117)

[0638] To a solution of **B-116** (21.3 mg, 0.04 mmol) in DCM/DMAc (1v/1v, 2 mL) was added 2-azidoacetic acid (6 µL, 0.082 mmol), followed by EDCI (15 mg, 0.078 mmol) and lutidine (19 µL, 0.16 mmol) under N₂. The reaction mixture was stirred at r.t for 2h and purified by prep-HPLC to give **B-117** (12.9 mg, 52.0%) as yellow solid.

[0639] LCMS[M+H]⁺: 605.3

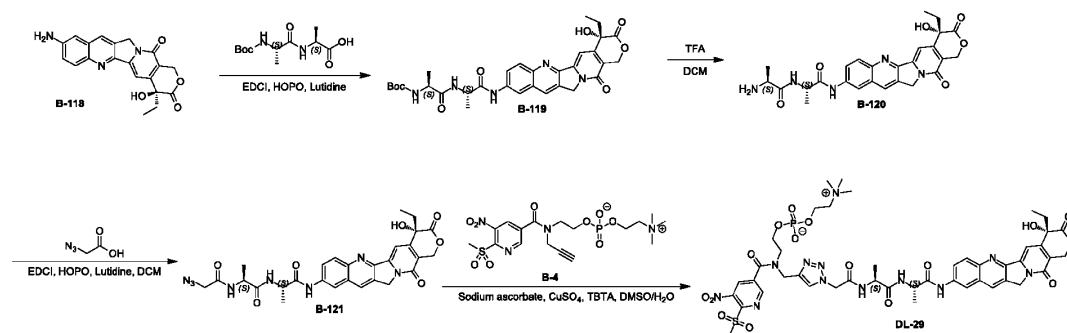
Step 9: Synthesis of DL-28

[0640] To a solution of **B-117** (12.9 mg, 0.02 mmol, 1.0 eq) in H₂O/DMSO (1V/1V, 2mL) was added **B-4** (15.8 mg, 0.032 mmol, 1.5 eq), TBTA (4.3 mg, 0.008 mmol, 0.4 eq), CuSO₄.5H₂O (2.1 mg, 0.008 mmol, 0.4 eq) and sodium ascorbate (3.3 mg, 0.017 mmol, 0.8 eq) at r.t. for 2 hrs. The reaction mixture was purified by prep-HPLC to afford **DL-28** (12.8 mg, 55.4%) as yellow solid.

[0641] LCMS[M+H]⁺: 1097.8

[0642] ^1H NMR (600 MHz, DMSO) δ 10.55 (s, 0.5H), 10.48 (s, 0.5H), 9.17 (s, 0.5H), 9.05 (s, 0.5H), 8.87 (s, 0.5H), 8.69-8.75 (m, 2.5H), 8.61 (s, 1H), 8.46 (t, 0.5H), 8.08-8.17 (m, 2H), 7.82 (t, 1H), 6.75 (brs, 1H), 5.93 (d, 1H), 5.58-5.44 (m, 3H), 5.26-5.09 (m, 2H), 4.83 (s, 1H), 4.64 (s, 1H), 4.51 (m, 1H), 4.40 (m, 1H), 4.20 (s, 1H), 4.12-3.97 (m, 2H), 3.86 (d, 1H), 3.61-3.51 (m, 3H), 3.48 (s, 3H), 3.13 (s, 9H), 1.99-1.81 (m, 2H), 1.47-1.20 (m, 6H), 0.88 (t, 3H).

Example 50: Synthesis of DL-29



Step 1: Synthesis of tert-butyl ((S)-1-(((S)-1-(((S)-4-ethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)amino)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (B-119)

[0643] To a solution of **B-118** (47 mg, 0.13 mmol) in DCM (2 mL) was added Boc-Ala-Ala-OH (67 mg, 0.26 mmol), HOPO (43 mg, 0.39 mmol), lutidine (55 mg, 0.52 mmol) and EDCI (47 mg, 0.25 mmol) under N_2 , and stirred at r.t for 3 hrs. The reaction mixture was poured into 10 mL of water and the organic layer was separated and concentrated to give the crude **B-119** as yellow oil which was used for the next step directly.

Step 2: Synthesis of (S)-2-amino-N-((S)-1-(((S)-4-ethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)amino)-1-oxopropan-2-yl)propenamide (B-120)

[0644] The crude **B-119** from previous step was dissolved DCM (3 mL) and added TFA (1 mL) at r.t under N_2 . The reaction mixture was stirred at r.t for 3h and purified by prep-HPLC to give **B-120** (45 mg, 69.0%) as yellow white solid.

[0645] LCMS[M+H] $^+$: 506.4

Step 3: Synthesis of (S)-2-(2-azidoacetamido)-N-((S)-1-(((S)-4-ethyl-4-hydroxy-3,14-dioxo-

3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)amino)-1-oxopropan-2-yl)propenamide (B-121)

[0646] To a solution of **B-120** (45 mg, 0.09 mmol) in DCM (2 mL) was added 2-azidoacetic acid (18 mg, 0.18 mmol), followed by EDCI (32 mg, 0.17 mmol) and lutidine (38 mg, 0.36 mmol) under N₂. The reaction mixture was stirred at r.t for 5h and purified by prep-HPLC to give **B-121** (20 mg, 38.2%) as yellow solid.

[0647] LCMS[M+H]⁺: 589.4

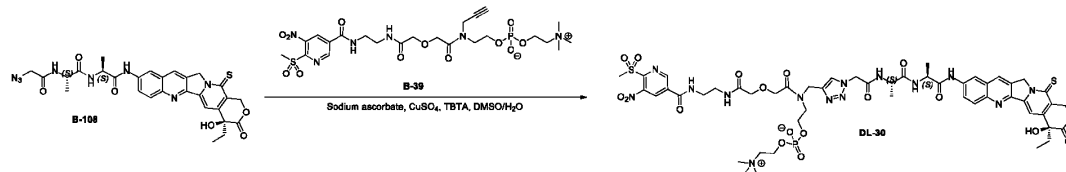
Step 4: Synthesis of DL-29

[0648] To a solution of **B-121** (20 mg, 0.034 mmol, 1.0 eq) in H₂O/DMSO (1V/1V, 2mL) was added **B-4** (25 mg, 0.05 mmol, 1 eq), TBTA (7.2 mg, 0.014 mmol, 0.4 eq), CuSO₄·5H₂O (3.4 mg, 0.014 mmol, 0.4 eq) and sodium ascorbate (5.4 mg, 0.03 mmol, 0.8 eq) at r.t for 3 hrs. This reaction mixture was purified by prep-HPLC to afford **DL-29** (14.1mg, yield: 38.4%) as a yellow solid.

[0649] LCMS[M+H]⁺: 1081.9

[0650] ¹H NMR (600 MHz, DMSO-d₆) δ 10.45 (d, J = 60.1 Hz, 1H), 9.10 (d, J = 71.6 Hz, 1H), 8.92-8.34 (m, 5H), 8.24-8.05 (m, 2H), 7.91 (m, 1H), 7.31 (s, 1H), 6.53 (s, 1H), 5.42 (s, 2H), 5.27 (s, 2H), 5.17 (s, 2H), 4.82 (s, 1H), 4.63 (s, 1H), 4.40-4.49 (m, 2H), 4.07-4.21 (m, 5H), 3.53-3.87 (m, 7H), 3.48 (s, 3H), 3.13 (s, 9H), 1.84-1.89 (m, 2H), 1.38 (dd, 3H), 1.29 (dd, 3H), 0.88 (t, 3H).

Example 51: Synthesis of DL-30



[0651] To a solution of **B-108** (60 mg, 0.1 mmol, 1.0 eq) in H₂O/DMSO (1 mL/1mL) was added **B-39** (60 mg, 0.1 mmol, 1.0 eq), TBTA (53 mg, 0.1 mmol, 1.0 eq), CuSO₄·5H₂O (24.9 mg, 0.1 mmol, 1.0 eq) and sodium ascorbate (40 mg, 0.2 mmol, 2.0 eq), and stirred at r.t. for 3.0 hrs. The reaction mixture was purified by prep-HPLC to afford **DL-30** (20.1 mg, 16%) as a white solid.

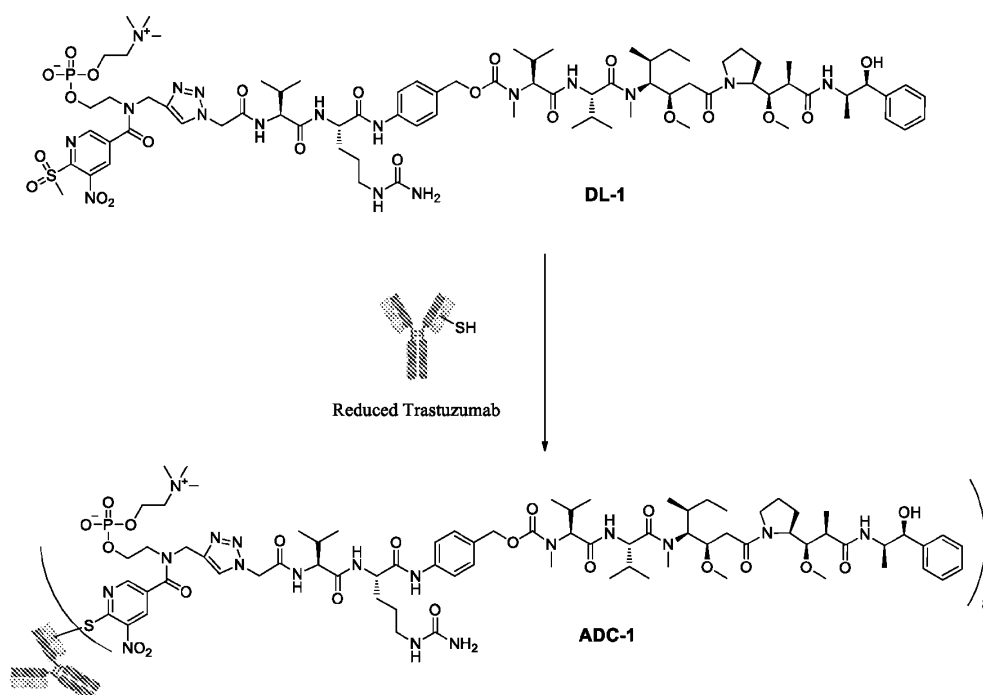
[0652] LCMS[M+H]⁺: 1255.7

[0653] ¹H NMR (600 MHz, DMSO-d₆) δ 10.48 (d, J= 9.9 Hz, 1H), 9.38-9.28 (m, 1H), 8.97 (s,

1H), 8.71 (s, 1H), 8.68-8.63 (m, 1H), 8.55 (s, 1H), 8.42 (d, J= 6.6 Hz, 1H), 8.33 (s, 1H), 8.20-8.14 (m, 1H), 8.09 (s, 1H), 7.99-7.89 (m, 1H), 7.80 (d, J= 2.2 Hz, 1H), 5.92 (d, J= 16.4 Hz, 1H), 5.51 (d, J= 14.0 Hz, 3H), 5.22-5.05 (m, 2H), 4.62-4.33 (m, 6H), 4.16 (d, J= 52.1 Hz, 2H), 3.95 (d, J= 25.6 Hz, 4H), 3.61-3.43 (m, 11H), 3.12 (d, J= 6.6 Hz, 9H), 1.96-1.83 (m, 2H), 1.38 (d, J= 7.1 Hz, 3H), 1.29 (d, J= 7.0 Hz, 3H), 0.87 (t, J= 7.3 Hz, 3H).

Conjugation Methodology

[0654] Exemplary conjugation methodology is illustrated below with compound **DL-1** to Trastuzumab (DAR 8):



[0655] General conjugation protocols:

- o Prep Antibody into 20mM Histidine pH 6.0 to approx. 15 mg/mL
- o Adjust pH between 6.5 to 7.8 and adjust concentration of antibody to approx. 10 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
- o Reduction of antibody by addition of 10 molar equivalence of TCEP (stock: 10 mM in

water) at 37 °C for 2 hours

- o Monitor by RP-HPLC
- o Bring to room temperature (20 – 25 °C) for 10 minutes
- o 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
- o Prepare 10 mM stock of drug-linker in DMSO
- o Add 12 molar equivalence of drug-linker
- o Adding DMSO to make total DMSO 10%, V/V
- o Conjugate at RT and monitor by RP-HPLC
- o After completion (within 1-16 h) quench by addition of 12 equivalence of N-acetylcysteine and left standing for 1 hour
- o Remove excess DL by desalting through Zeba Spin desalting column and buffer exchange into 20 mM Histidine*HAc pH 6.0 using 30 kD amicon filters.

[0656] General method for RP-HPLC:

➤ RP

Detector	DAD							
Wavelength	280 nm with BW = 4 nm. Ref WL 360 nm, BW= 100 nm							
Temperature	80 °C							
Column	PLRP column, 1000 angstrom, 8 micron, 4.6*150mm							
Injection volume	~50 µg							
Flow rate	0.5 mL/min							
Mobile phase	A: 0.1% TFA in water B: 0.1% TFA in ACN							
Gradient	Time	0	5	36	36.2	37.2	37.5	42
	B%	10	32	48	98	98	10	10

[0657] General method for SEC:

➤ SEC

Detector	DAD
Wavelength	280 nm
Column	Agilent, AdvanceBio, SEC 200A, 1.9µm, 4.6*150mm
Temperature	25 °C
Flow rate	0.3 mL/min
Mobile phase	0.2M K ₂ HPO ₄ , 0.25M KCl, 10% MeOH, pH7.7
Isocratic	10 min

[0658] General method for HIC:

➤ HIC

Detector	DAD						
Wavelength	280 nm						
Temperature	25°C						
Flow rate	0.75 mL/min						
Column	Tosoh Butyl NPR (4.6mm X 3.5cm, 2.5 micron)						
Mobile phase	A: 1.5 M Ammonium Sulfate, 40 mM Sodium Phosphate, pH7.0 B: 40 mM Sodium Phosphate, 25% IPA, pH7.0						
Gradient	Time	0	5	20	25	26	30
	B%	0	0	95	95	0	0

[0659] Characterization data of exemplary ADCs are provided in **Table 1** below.

Table 1: Characterization Data of Exemplary ADCs

ADC	DL	Light chain observed m/z	Heavy chain observed m/z	Drug Antibody Ratio (DAR)	SEC Monomer %
1	1	25059	55451	8	97.70%
2	2	25039	55392	8	97.60%
3	3	25268	56079	8	97.50%
4	4	25001	55280	8	96.40%
5	5	24957	55148	8	97.30%

6	6	25015	55319	8	97.60%
7	7	25116	55622	8	97.30%
10	11	24971	55189	8	99.30%
11	12	24928	55060	8	99.30%

Cytotoxicity Data for Representative Conjugates

[0660] *In vitro* cytotoxicity data of certain exemplary ADCs in SK-BR-3 cells are provided in **Table 2** below.

Table 2: *In vitro* Cytotoxicity of Exemplary ADCs in SK-BR-3 Cells

ADC	IC50 (nM)
1	0.019
2	0.013
3	0.014
4	0.013
5	0.015
6	0.021
7	0.017
10	0.330
11	0.281

Assay Protocol:

[0661] 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 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.

[0662] 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.

[0663] 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.

[0664] 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

[0665] 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.

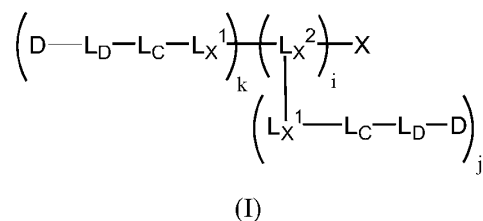
Equivalents

[0666] 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-linker conjugate having the structural formula (I):



or a salt or ester thereof,

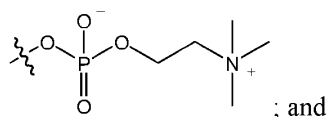
wherein

each D is independently a drug moiety;

X is a conjugation functional group capable of forming a covalent linkage with an antigen binding moiety;

each L_C is independently a moiety formed by a click chemistry reaction between an alkyne and an azide;

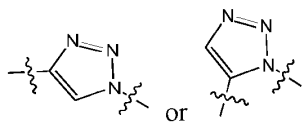
each of L_D, L_X¹ and L_X² is independently a linker or spacer moiety, wherein at least one of L_D, L_X¹ and L_X² comprises a phosphoryl choline (PC) group



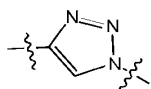
i is an integer selected from 0-8; and

each of *j* and *k* is independently 0 or 1, provided that *j* and *k* are not both 0.

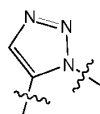
2. The drug-linker conjugate of claim 1, wherein *j* is 0.
3. The drug-linker conjugate of claim 1, wherein *j* is 1.
4. The drug-linker conjugate of claim 3, wherein *k* is 1.
5. The drug-linker conjugate of any one of claims 1-4, wherein *i* is 1.
6. The drug-linker conjugate of any one of claims 1-4, wherein *i* is 2.
7. The drug-linker conjugate of any one of claims 1-4, wherein *i* is greater than 2.
8. The drug-linker conjugate of any one of claims 1-7, wherein each L_C is



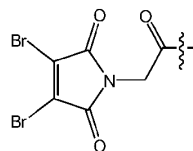
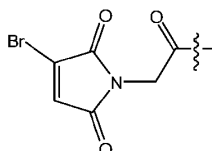
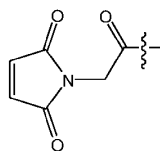
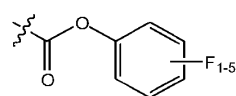
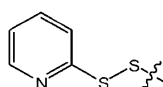
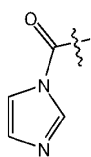
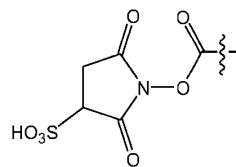
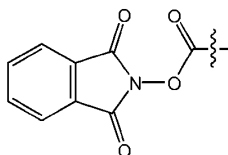
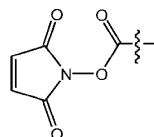
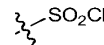
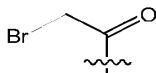
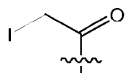
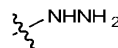
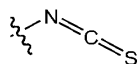
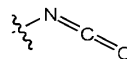
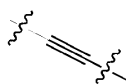
9. The drug-linker conjugate of claim 8, wherein L_C is

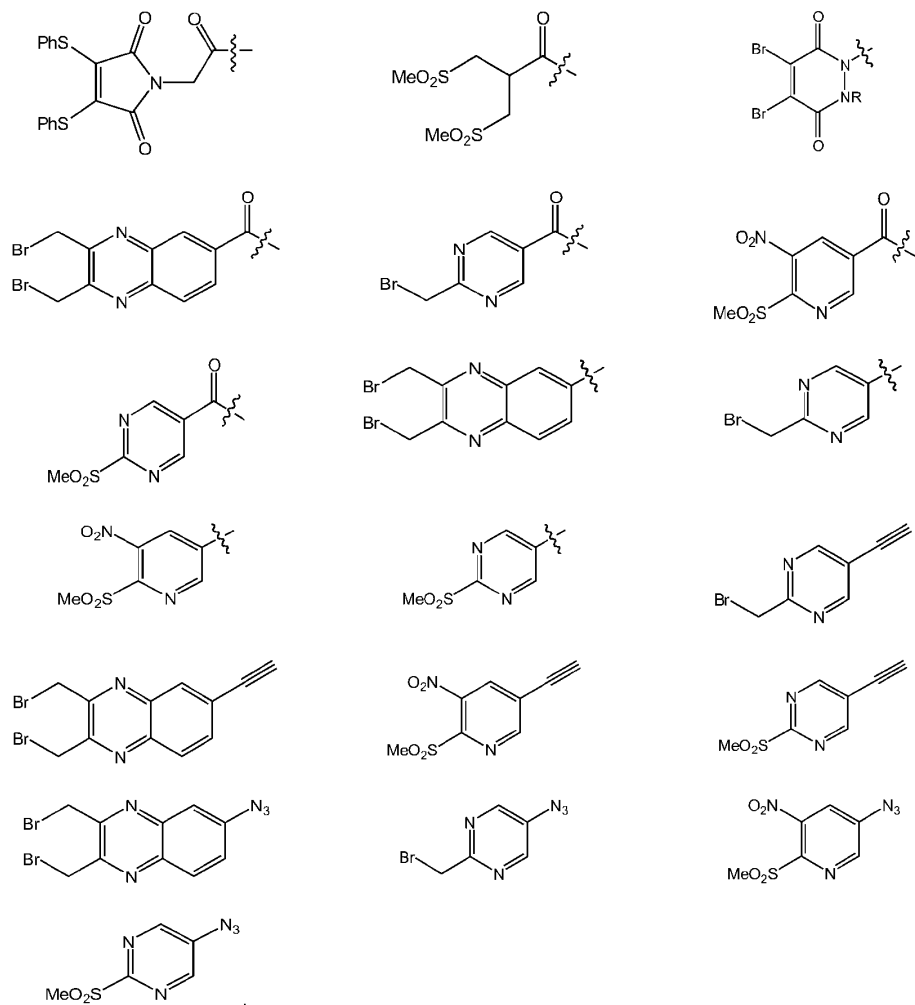


10. The drug-linker conjugate of claim 8, wherein L_C is



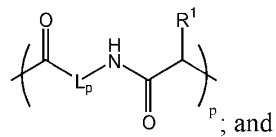
11. The drug-linker conjugate of any one of claims 1-10, wherein X is selected from:



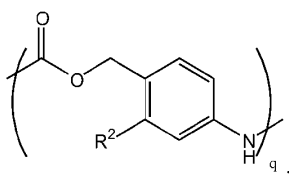


12. The drug-linker conjugate of any of claims 1-11, wherein $-L_D-$ is $-L_D^2-L_D^1-$, wherein

L_D^1 is



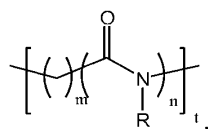
L_D^2 is



wherein

L_p is a di-, tri- or tetra peptide linker;

each of R^1 and R^2 is H, C_{1-3} alkyl, or $L_{PC}-(PC)$, wherein L_{PC} is



each p and q is independently 0 or 1, provided that p and q are not both 0;

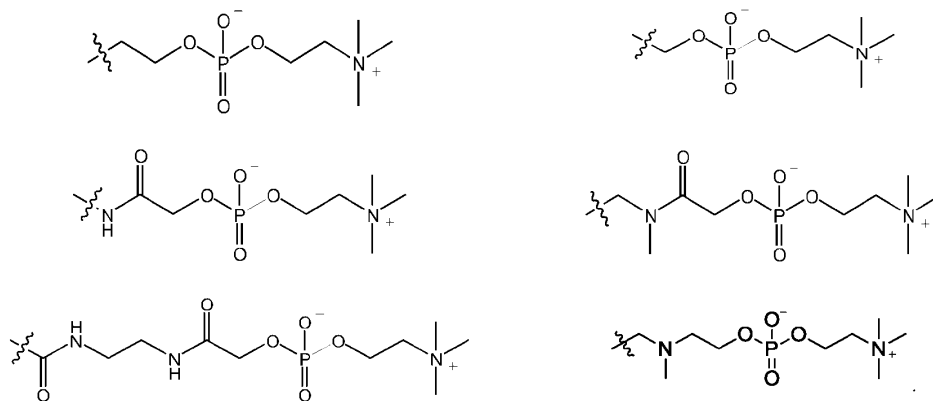
R is H or C_{1-3} alkyl;

m is 0, 1, 2 or 3;

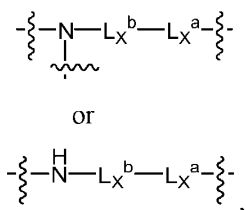
n is 0 or 1, and

t is 0 or 1.

13. The drug-linker conjugate of claim 12, wherein each of R^1 and R^2 is $L_{PC}-(PC)$.
14. The drug-linker conjugate of claim 12 or 13, wherein $L_{PC}-(PC)$ is selected from:



15. The drug-linker conjugate of any of claims 1-14, wherein L_X^1 is $(CH_2)_g$, wherein g is an integer selected from 0-6.
16. The drug-linker conjugate of claim 15, wherein g is 1.
17. The drug-linker conjugate of any of claims 1-16, wherein L_X^2 is:

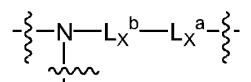


wherein

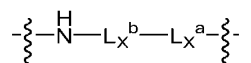
L_X^a is a substituted or unsubstituted C₁₋₆ alkyl or a PEG group;

L_X^b is $-C(=O)-$, CH or CH_2 .

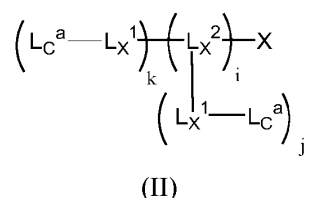
18. The drug-linker conjugate of claim 17, wherein L_X^2 is:



19. The drug-linker conjugate of claim 17, wherein L_X^2 is:



20. The drug-linker conjugate of any of claims 1-19, wherein the spacer moiety is selected from the group consisting of an alkyl, a heteroalkyl, polyethylene glycol (PEG), and a peptide.
21. The drug-linker conjugate of claim 20, wherein the spacer moiety is a PEG.
22. The drug-linker conjugate of claim 20, wherein the spacer moiety is a peptide.
23. A compound having the structural formula (II):



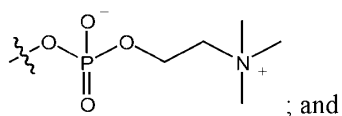
or a salt or ester thereof,

wherein

X is conjugation functional group capable of forming a covalent linkage with an antigen binding moiety;

each L^C_a is independently an alkyne or an azide group;

each of L_X^1 and L_X^2 is independently a linker or spacer moiety, wherein at least one of L_X^1 and L_X^2 comprises a phosphoryl choline (PC) group

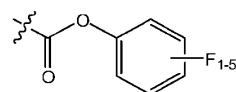
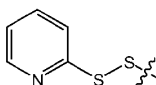
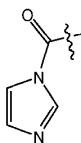
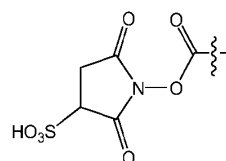
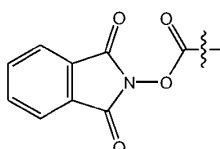
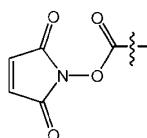
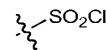
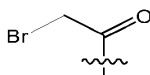
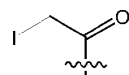
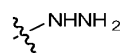
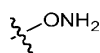
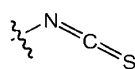
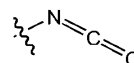
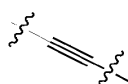


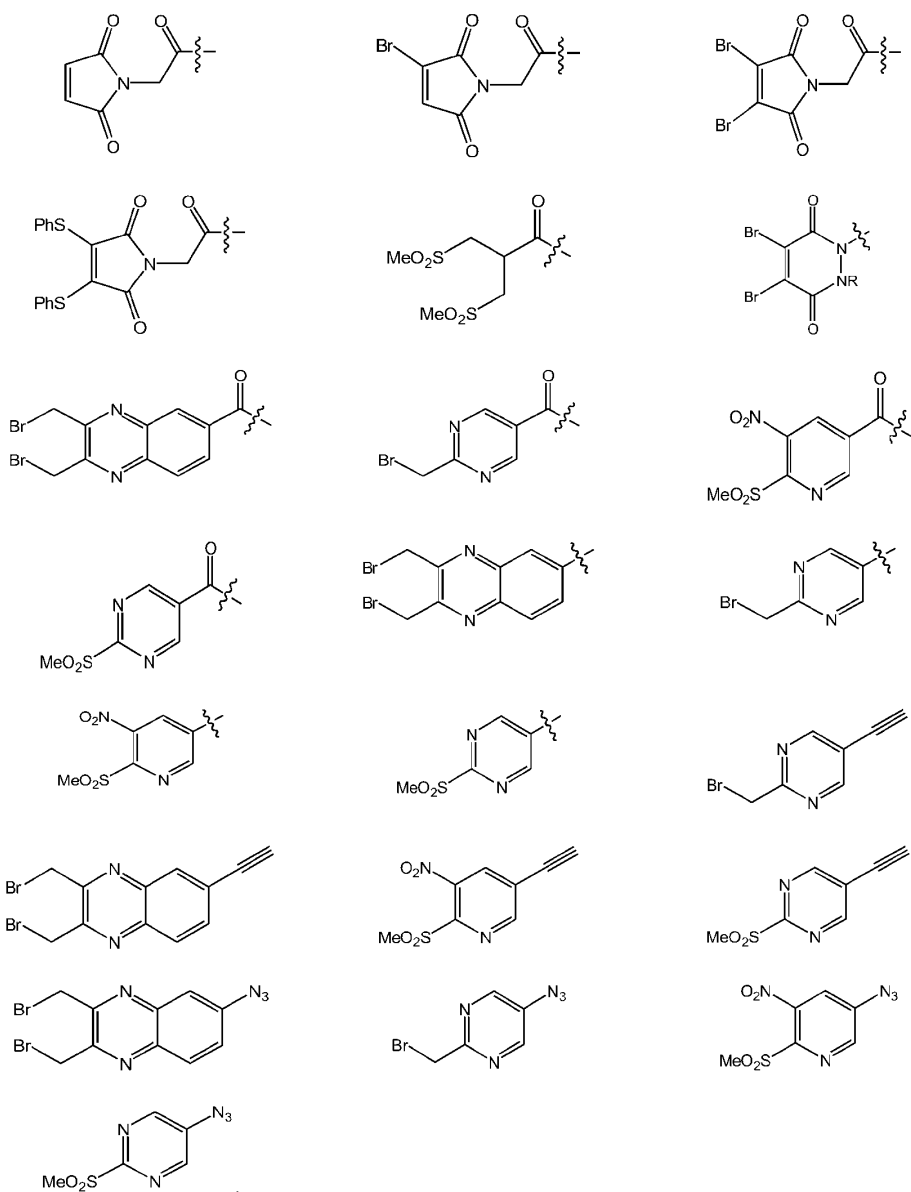
; and

i is an integer selected from 0-8; and

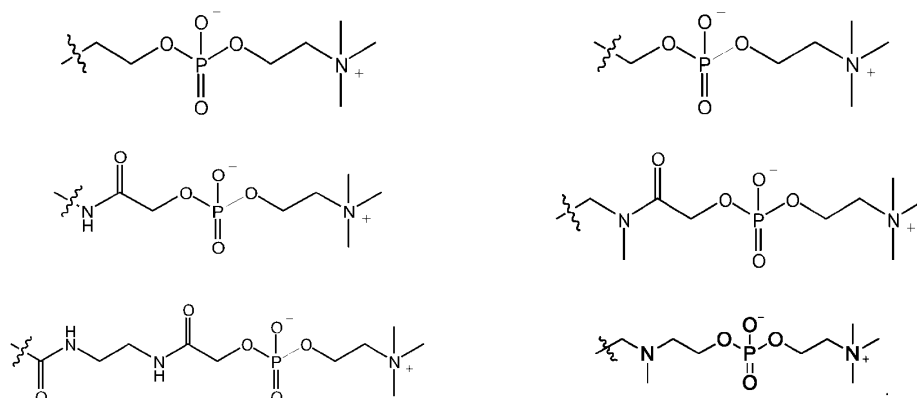
each of j and k is independently 0 or 1, provided that j and k are not both 0.

24. The compound of claim 23, wherein j is 0.
25. The compound of claim 23, wherein j is 1.
26. The compound of claim 25, wherein k is 1.
27. The compound of any one of claims 23-26, wherein i is 1.
28. The compound of any one of claims 23-26, wherein i is 2.
29. The compound of any one of claims 23-26, wherein i is greater than 2.
30. The compound of any one of claims 23-29, wherein each L_C^a is an alkyne group.
31. The compound of any one of claims 23-29, wherein each L_C^a is an azide group.
32. The compound of any one of claims 23-31, wherein X is selected from:



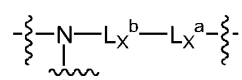


33. The compound of any one of of claim 23-32, wherein each of L_X^1 and L_X^2 comprises L_{PC} -(PC).
34. The compound of claim 33, wherein L_{PC} -(PC) is selected from:

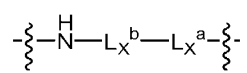


35. The compound of any of claims 23-34, wherein L_X^1 is $(CH_2)_g$, wherein g is an integer selected from 0-6.

36. The compound of any of claims 23-35, wherein L_X^2 is:



or

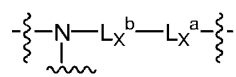


wherein

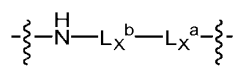
L_X^a is a substituted or unsubstituted C_{1-6} alkyl or a PEG group;

L_X^b is $-C(=O)-$, CH or CH_2 .

37. The compound of claim 36, wherein L_X^2 is:



38. The compound of claim 36, wherein L_X^2 is:

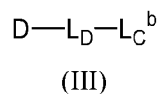


39. The compound of any of claims 23-38, wherein the spacer moiety is selected from the group consisting of an alkyl, a heteroalkyl, polyethylene glycol (PEG), and a peptide.

40. The compound of claim 39, wherein the spacer moiety is a PEG.

41. The compound of claim 39, wherein the spacer moiety is a peptide.

42. A compound having the structural formula (III):



or a salt or ester thereof,

wherein

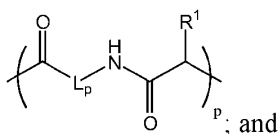
D is a drug moiety;

L_D is a linker or spacer moiety comprising a phosphoryl choline (PC) group; and

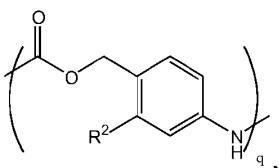
L_C^b is an alkyne or an azide group.

43. The compound of claim 42, wherein L_C^b is an alkyne group.
44. The compound of claim 42, wherein L_C^b is an azide group.
45. The compound of any of claims 42-44, wherein -L_D- is -L_D²-L_D¹-,
wherein

L_D¹ is



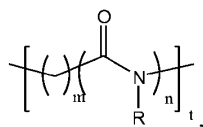
L_D² is



wherein

L_p is a di-, tri- or tetra peptide linker;

each of R¹ and R² is H, C₁₋₃ alkyl, or L_{PC}-(PC), wherein L_{PC} is



each *p* and *q* is independently 0 or 1, provided that *p* and *q* are not both 0;

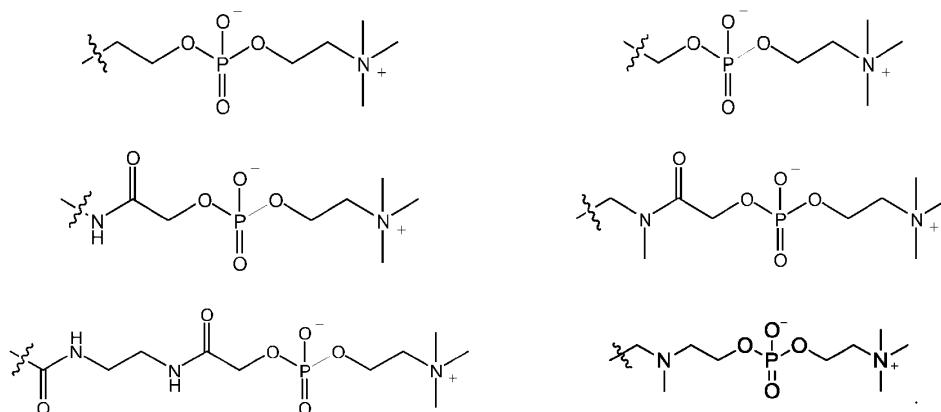
R is H or C₁₋₃ alkyl;

m is 0, 1, 2 or 3;

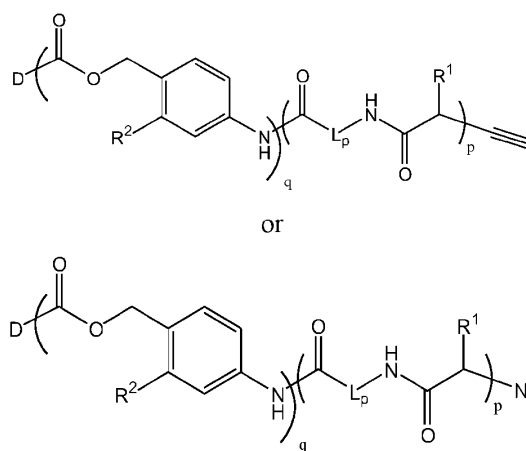
n is 0 or 1, and

t is 0 or 1.

46. The compound of any of claim 45, wherein each of R^1 and R^2 is $L_{PC}-(PC)$,
 47. The compound of any of claim 45 or 36, wherein $L_{PC}-(PC)$ is selected from:



48. The compound of any of claims 42-47, wherein the spacer moiety is selected from the group consisting of an alkyl, a heteroalkyl, polyethylene glycol (PEG), and a peptide.
 49. The compound of claim 48, wherein the spacer moiety is a PEG.
 50. The compound of claim 48, wherein the spacer moiety is a peptide.
 51. A compound having the structural formula:

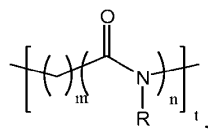


wherein

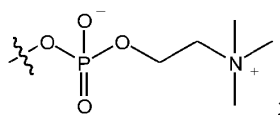
D is a drug moiety;

L_p is a di-, tri- or tetra peptide linker;

each of R^1 and R^2 is H, C_{1-3} alkyl, or $L_{PC}-(PC)$, wherein L_{PC} is



PC is phosphoryl choline group:



each p and q is independently 0 or 1, provided that p and q are not both 0;

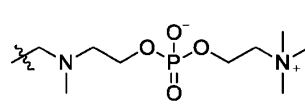
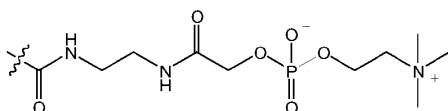
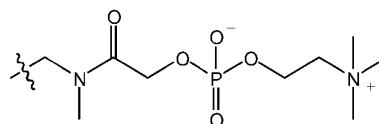
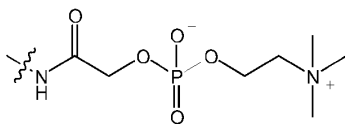
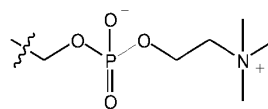
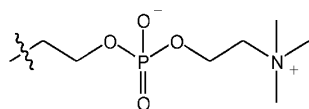
R is H or C₁₋₃ alkyl;

m is 0, 1, 2 or 3;

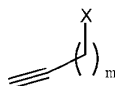
n is 0 or 1, and

t is 0 or 1.

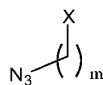
52. The compound of any of claim 51, wherein each of R¹ and R² is L_{PC}-(PC),
53. The compound of claim 51 or 52, wherein L_{PC}-(PC) is selected from:



54. The compound of any of claims 51-53, wherein the spacer moiety is selected from the group consisting of an alkyl, a heteroalkyl, polyethylene glycol (PEG), and a peptide.
55. The compound of claim 54, wherein the spacer moiety is a PEG.
56. The compound of claim 54, wherein the spacer moiety is a peptide.
57. A compound having the structural formula:



or

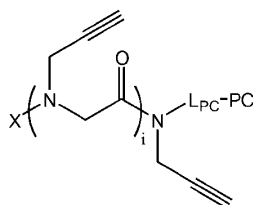


wherein

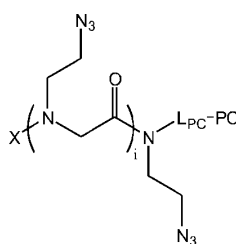
X is a conjugation functional group capable of forming a covalent linkage with an antigen binding moiety; and

m is 1, 2, 3 or 4.

58. The compound of any one of claims 1-19, wherein D is selected from the group consisting of an anticancer agent, an antibiotic, an immune agonist, a steroid, and an anti-autoimmune disease agent.
59. The compound of any one of claims 1-19, wherein D is a peptide or protein.
60. The compound of any one of claims 1-19, wherein D is a PROTAC molecule, molecular glue, or degrader.
61. The compound of any one of claims 1-19, wherein D is a nucleic acid.
62. A compound having the structural formula:



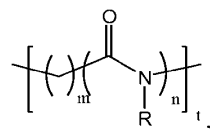
or



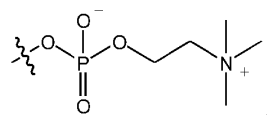
wherein

X is a conjugation functional group capable of forming a covalent linkage with an antigen binding moiety;

L_{PC} is



PC is phosphoryl choline group:



R is H or C₁₋₃ alkyl;

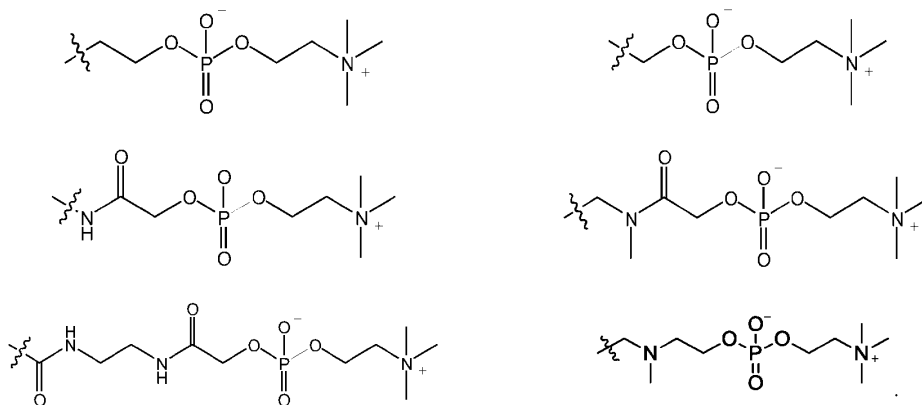
i is an integer selected from 0-8;

m is 0, 1, 2 or 3;

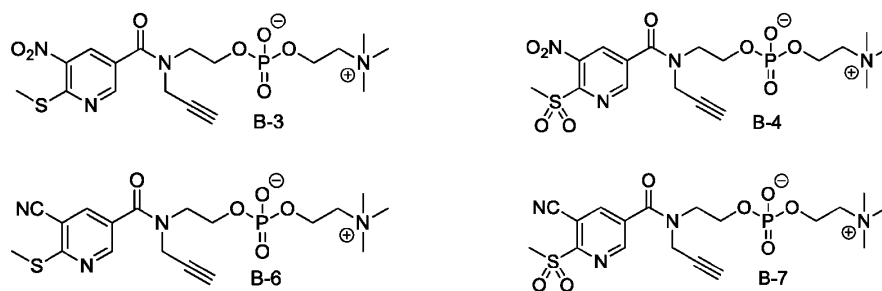
n is 0 or 1, and

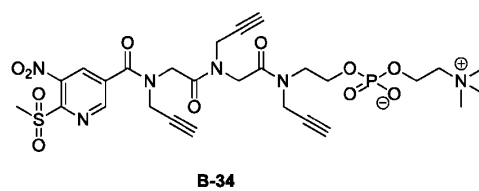
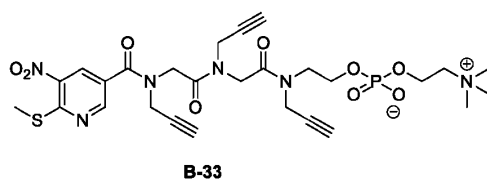
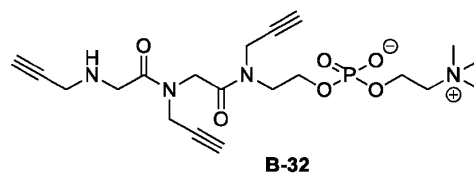
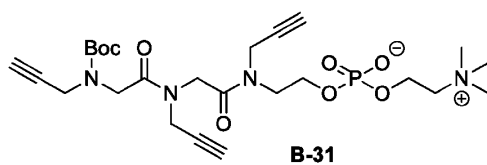
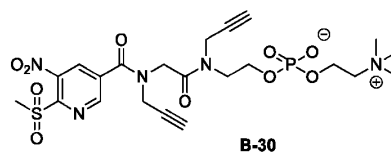
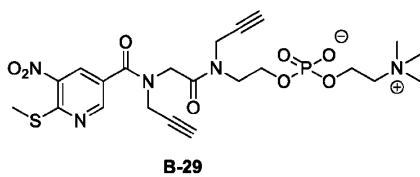
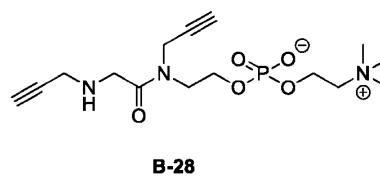
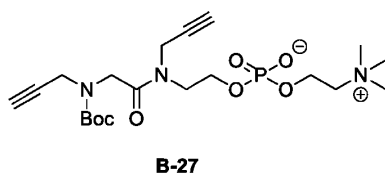
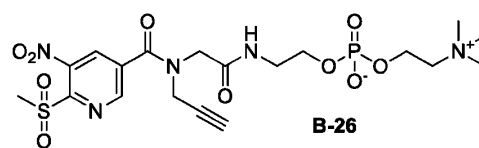
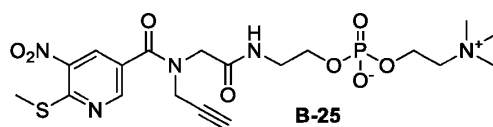
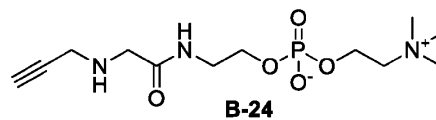
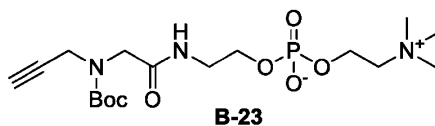
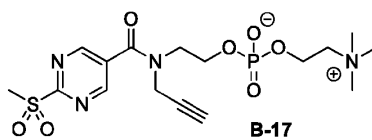
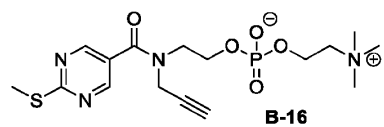
t is 0 or 1.

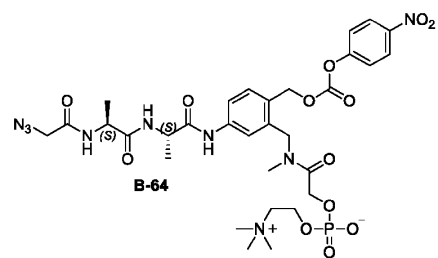
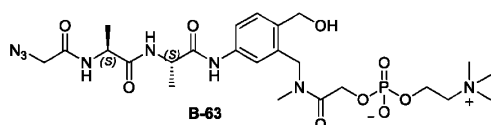
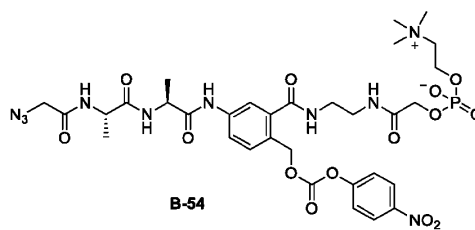
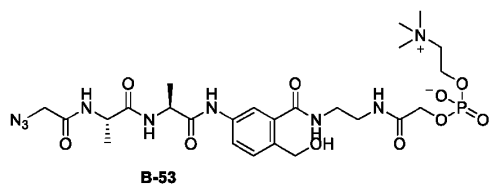
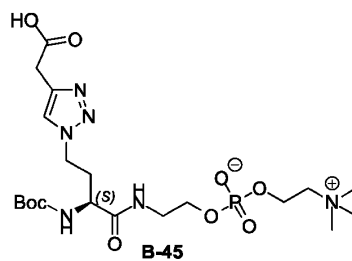
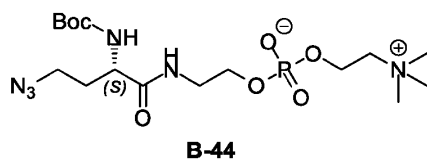
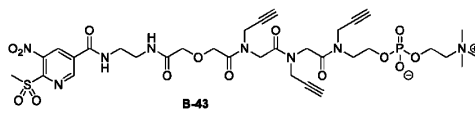
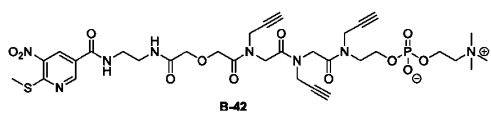
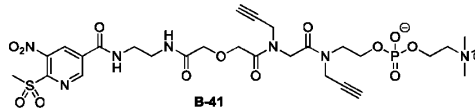
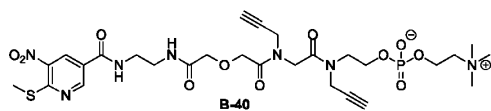
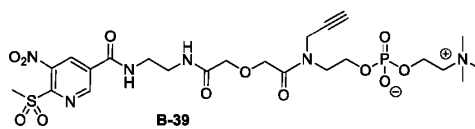
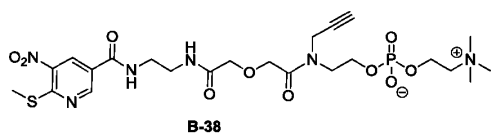
63. The compound of claim 62, wherein L_{PC}-(PC) is selected from:

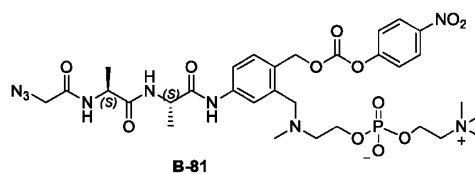
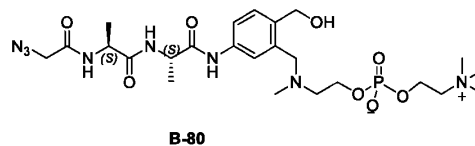
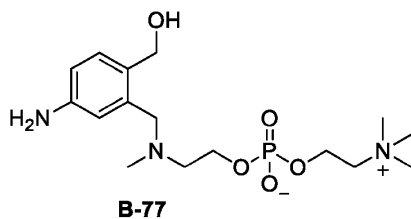
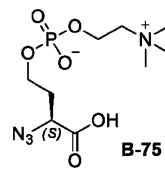
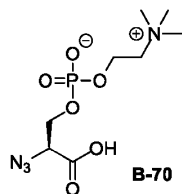


64. A compound selected from:

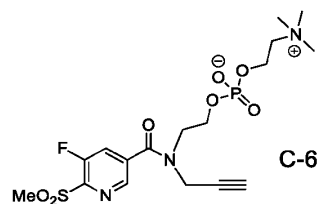
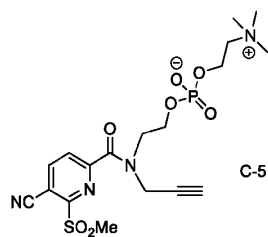
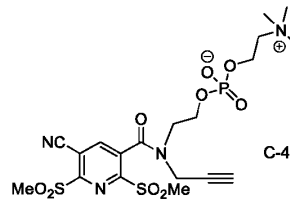
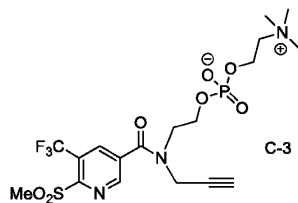
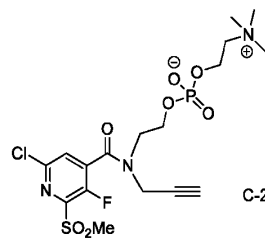
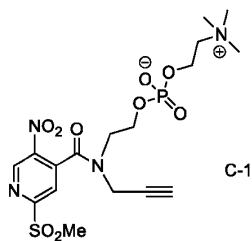


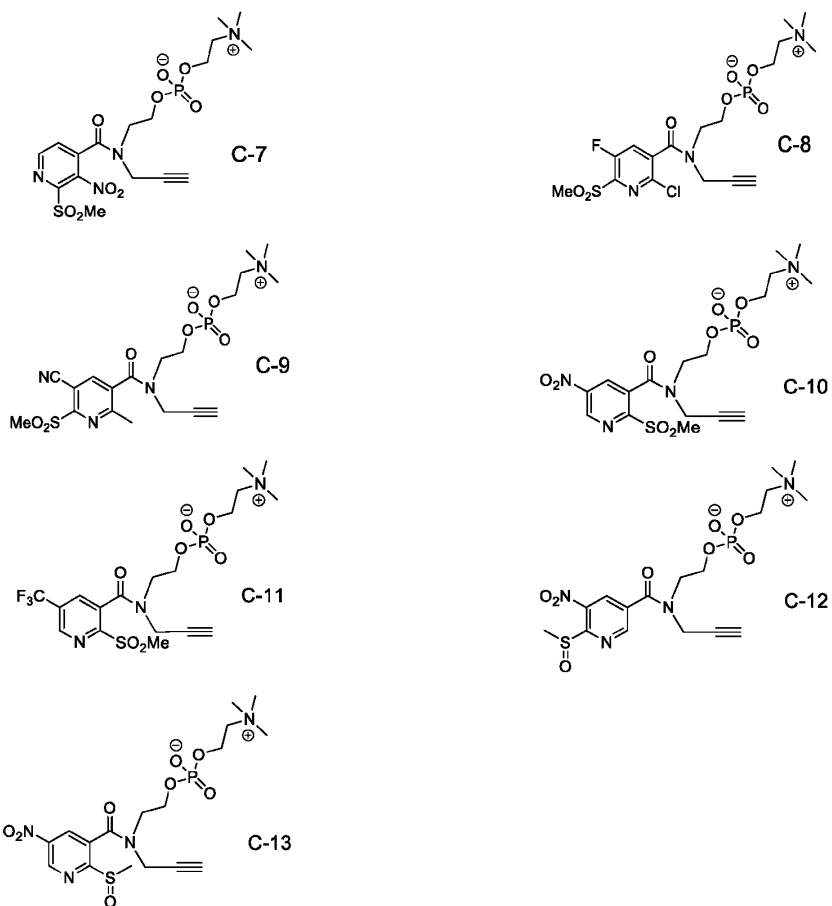




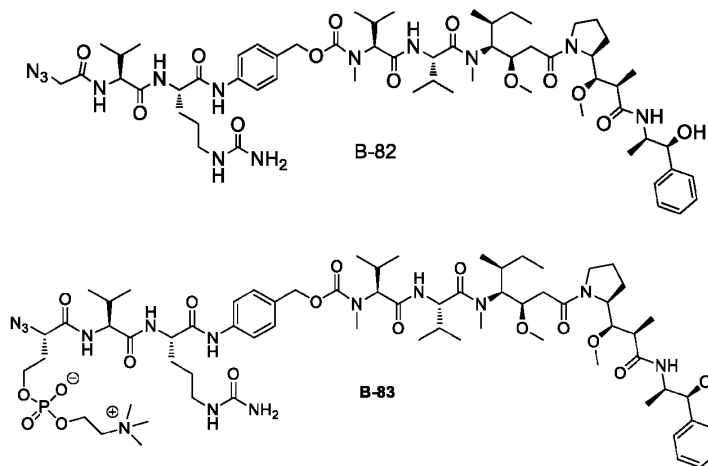


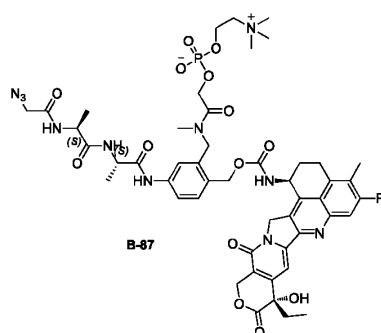
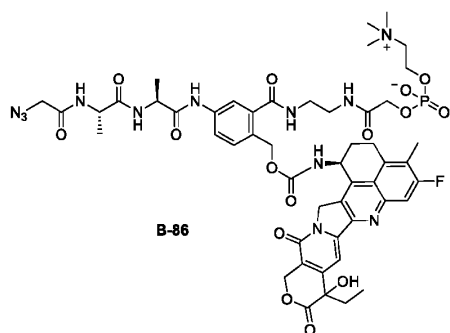
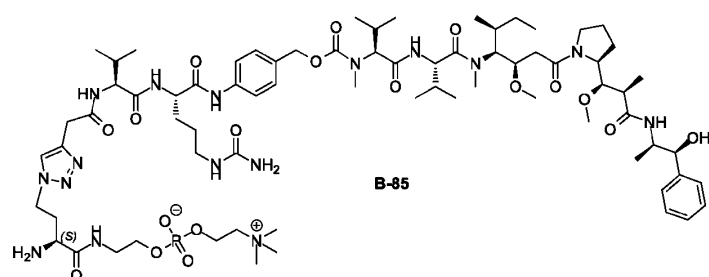
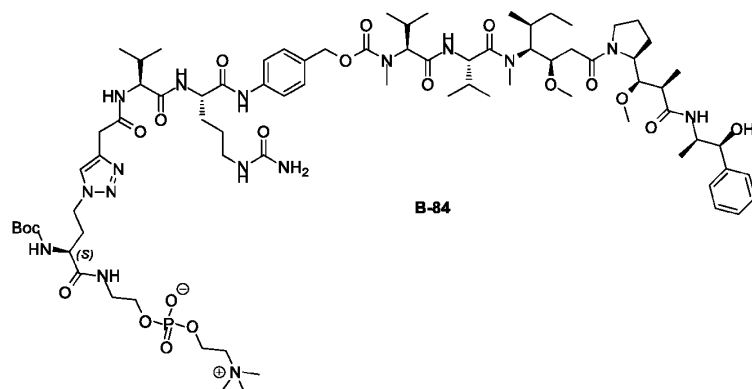
65. A compound selected from:

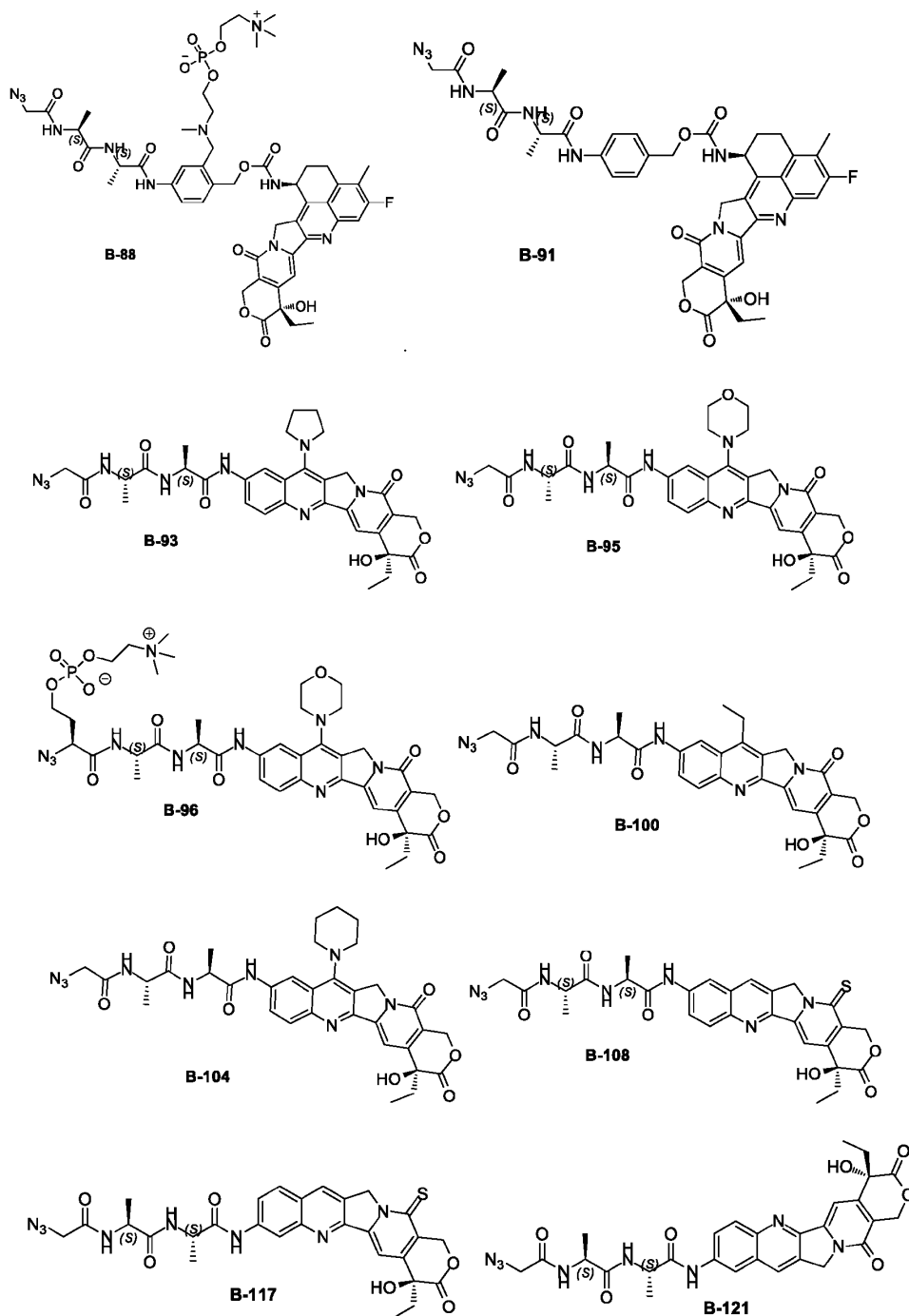




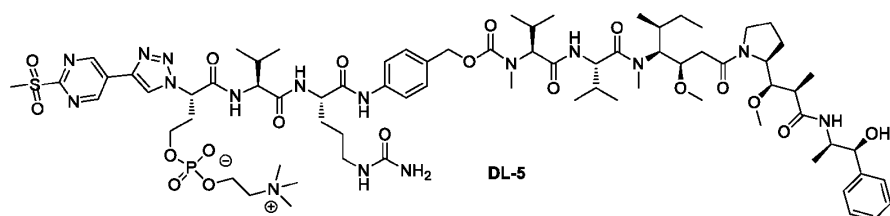
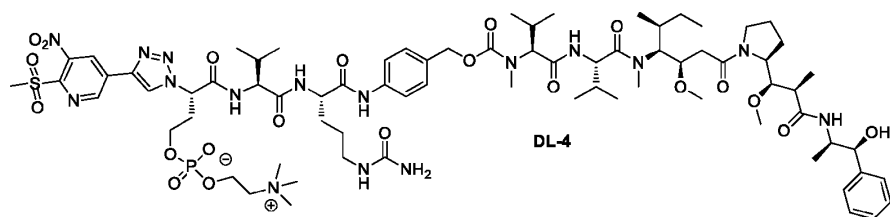
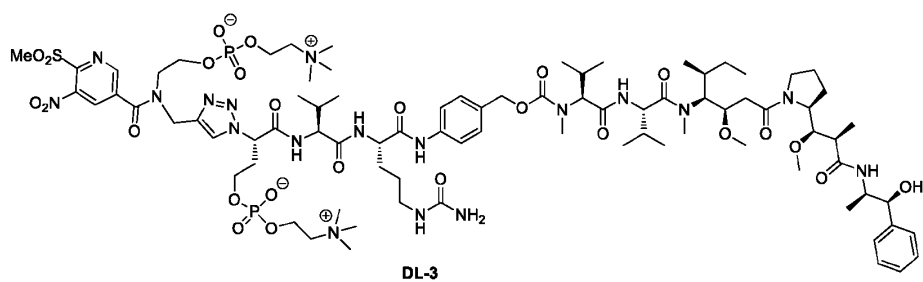
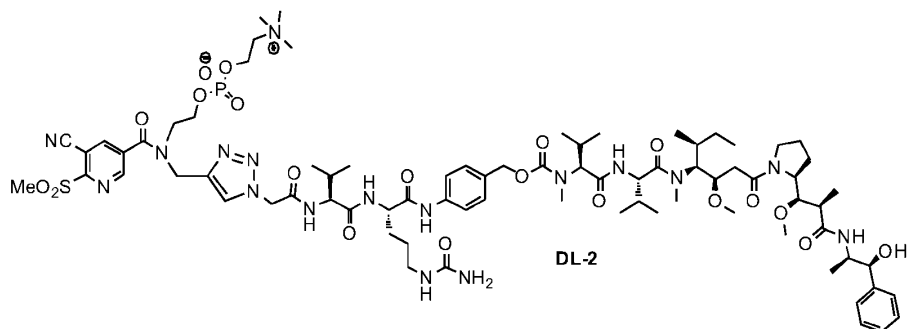
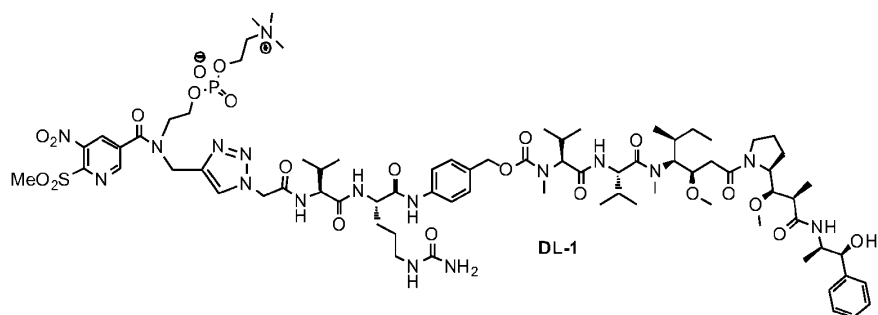
66. A compound selected from:

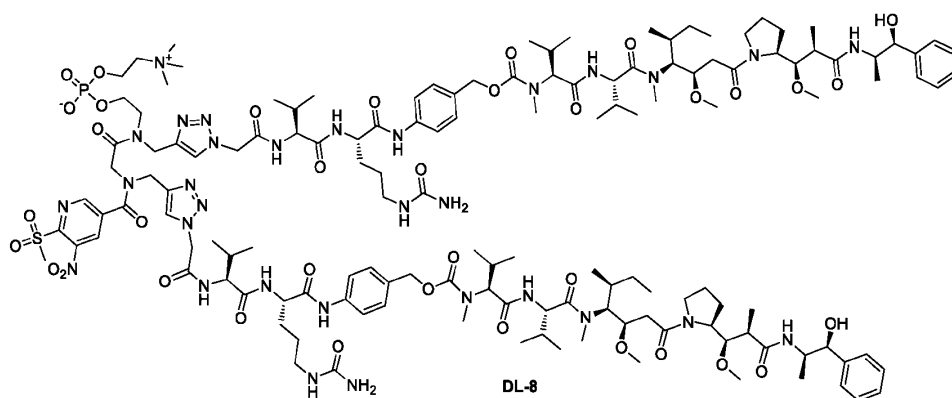
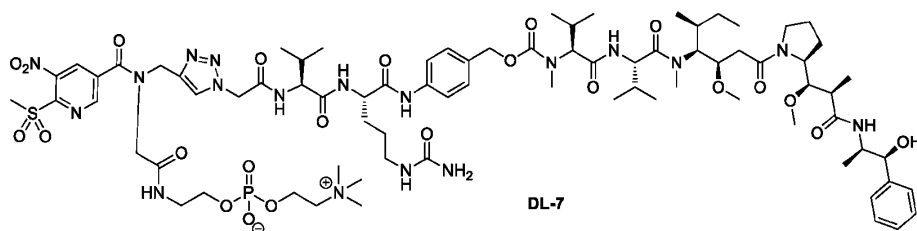
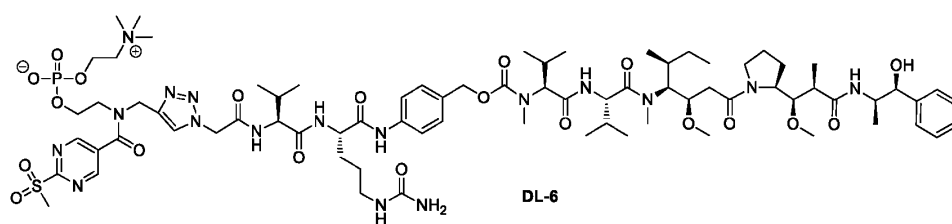


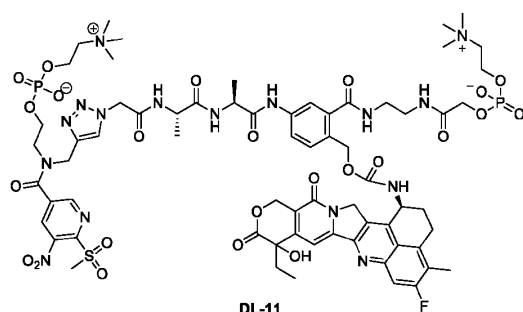
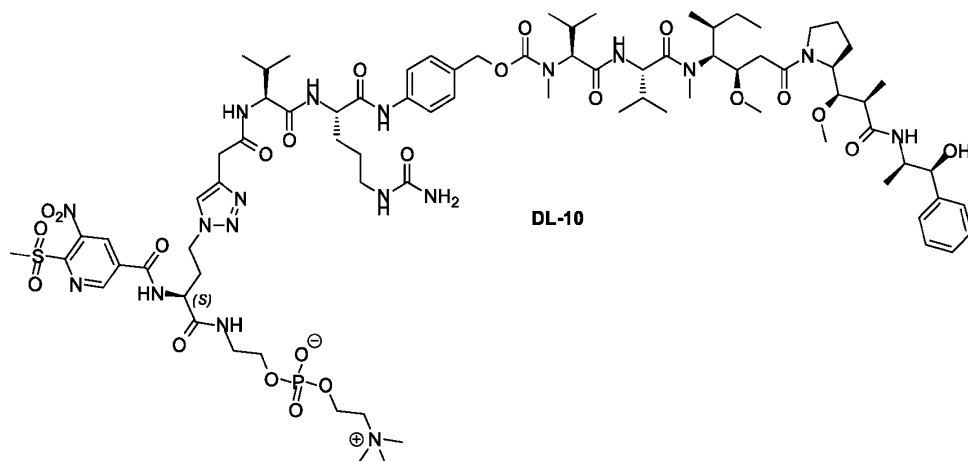
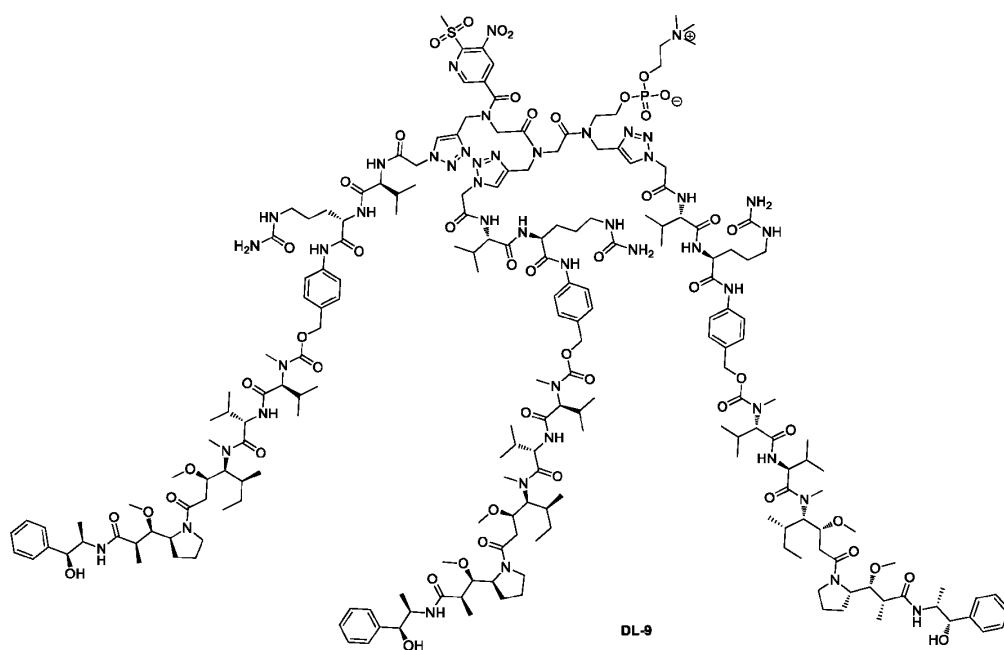


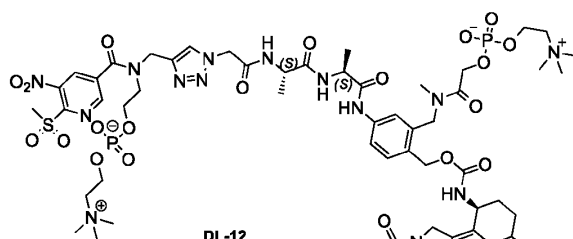


67. A compound selected from:

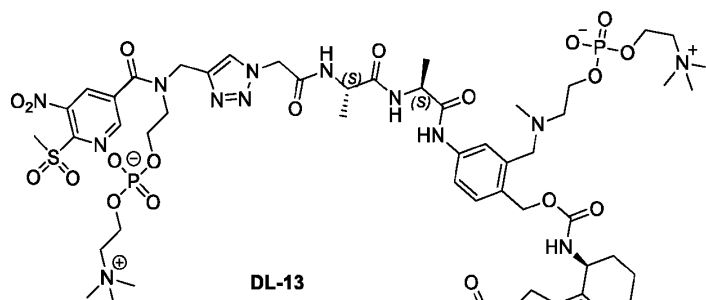
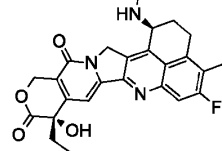




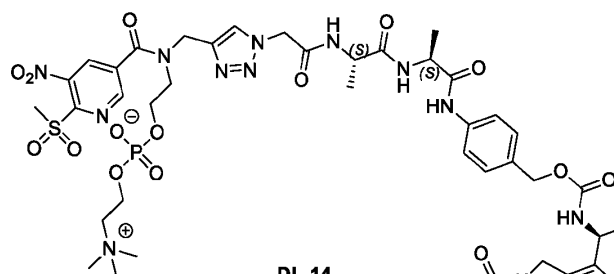
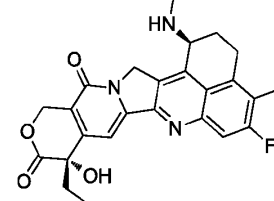




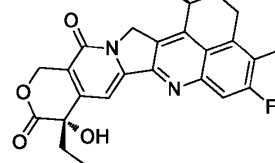
DL-12

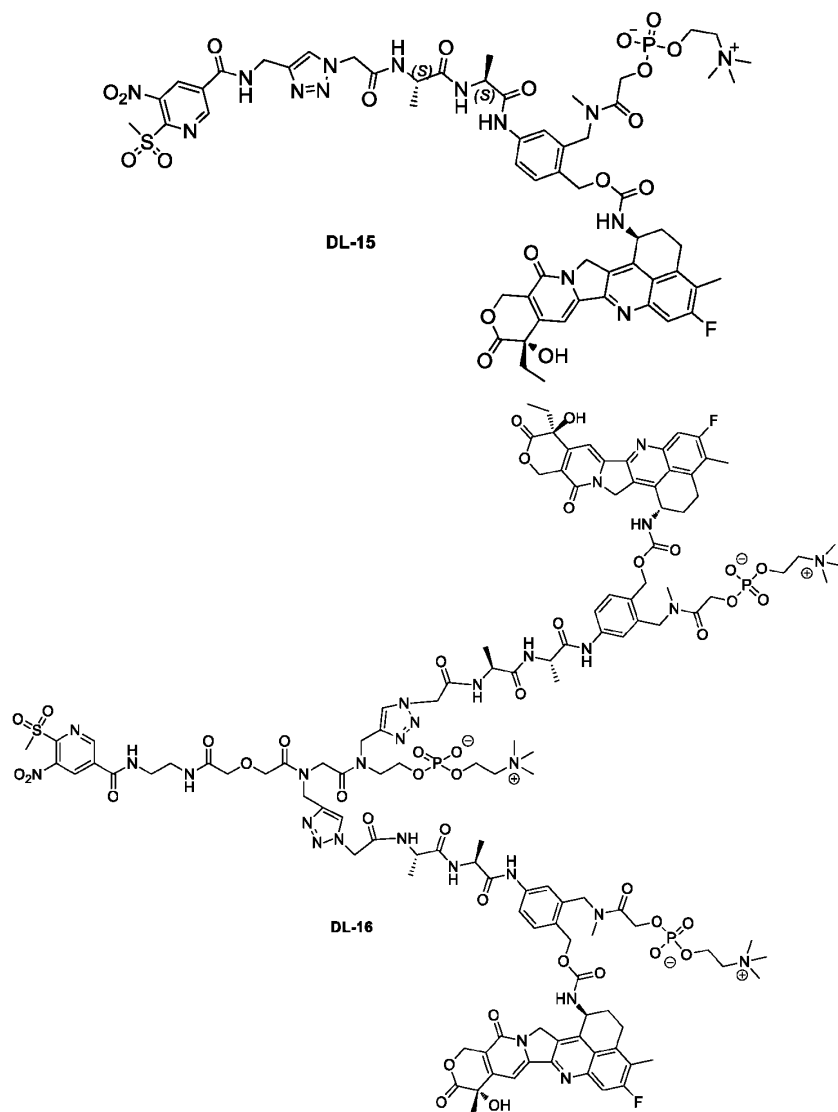


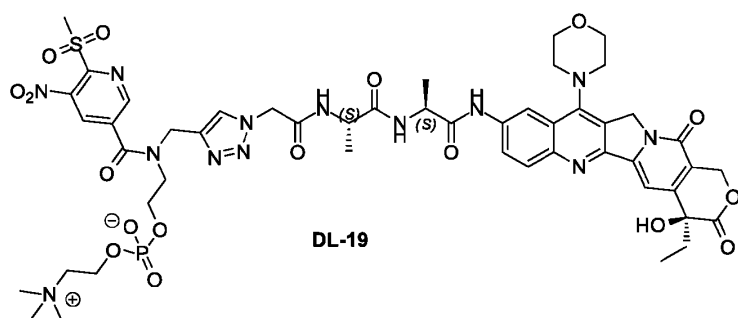
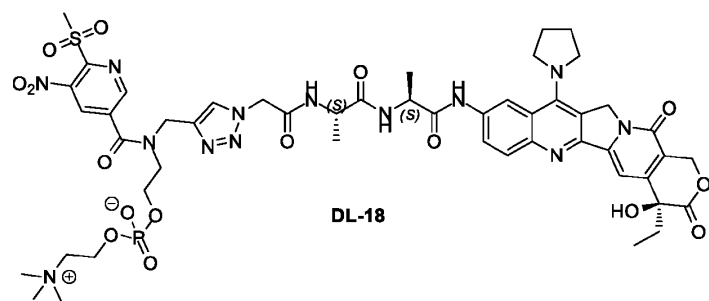
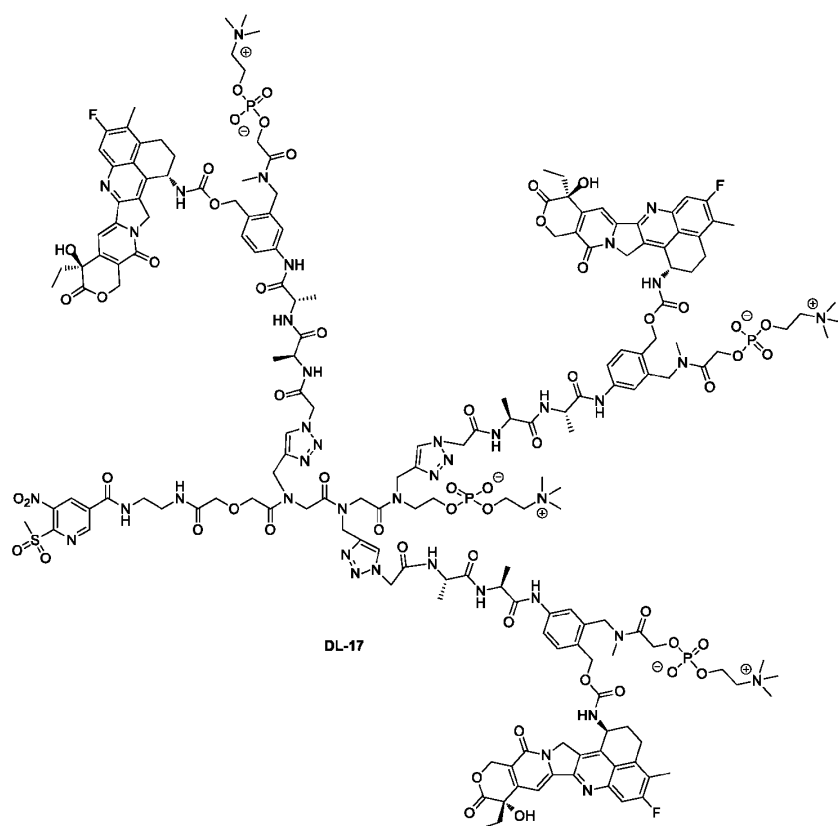
DL-13

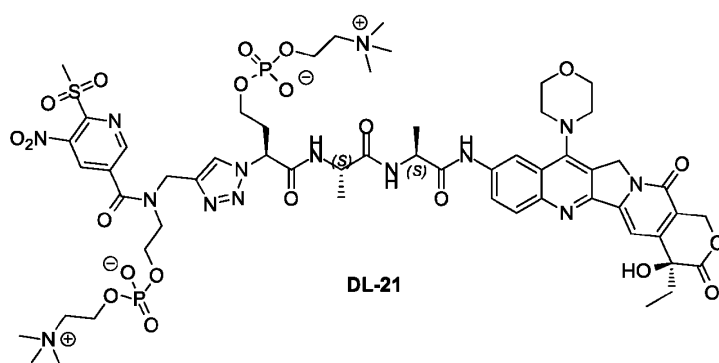
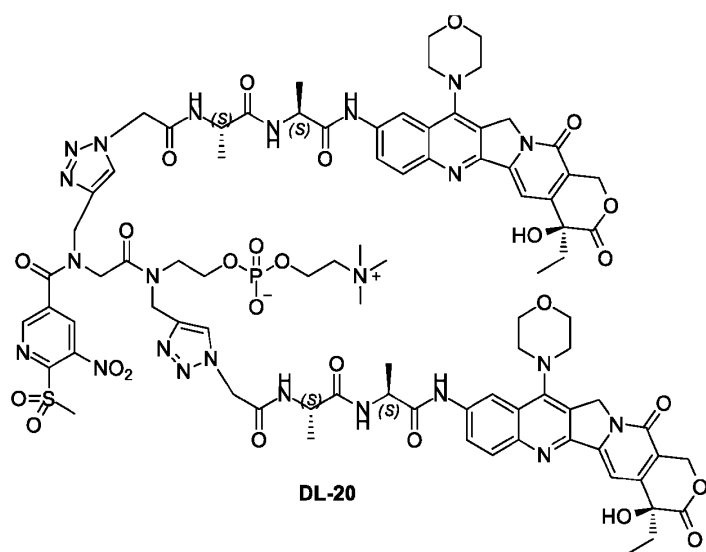


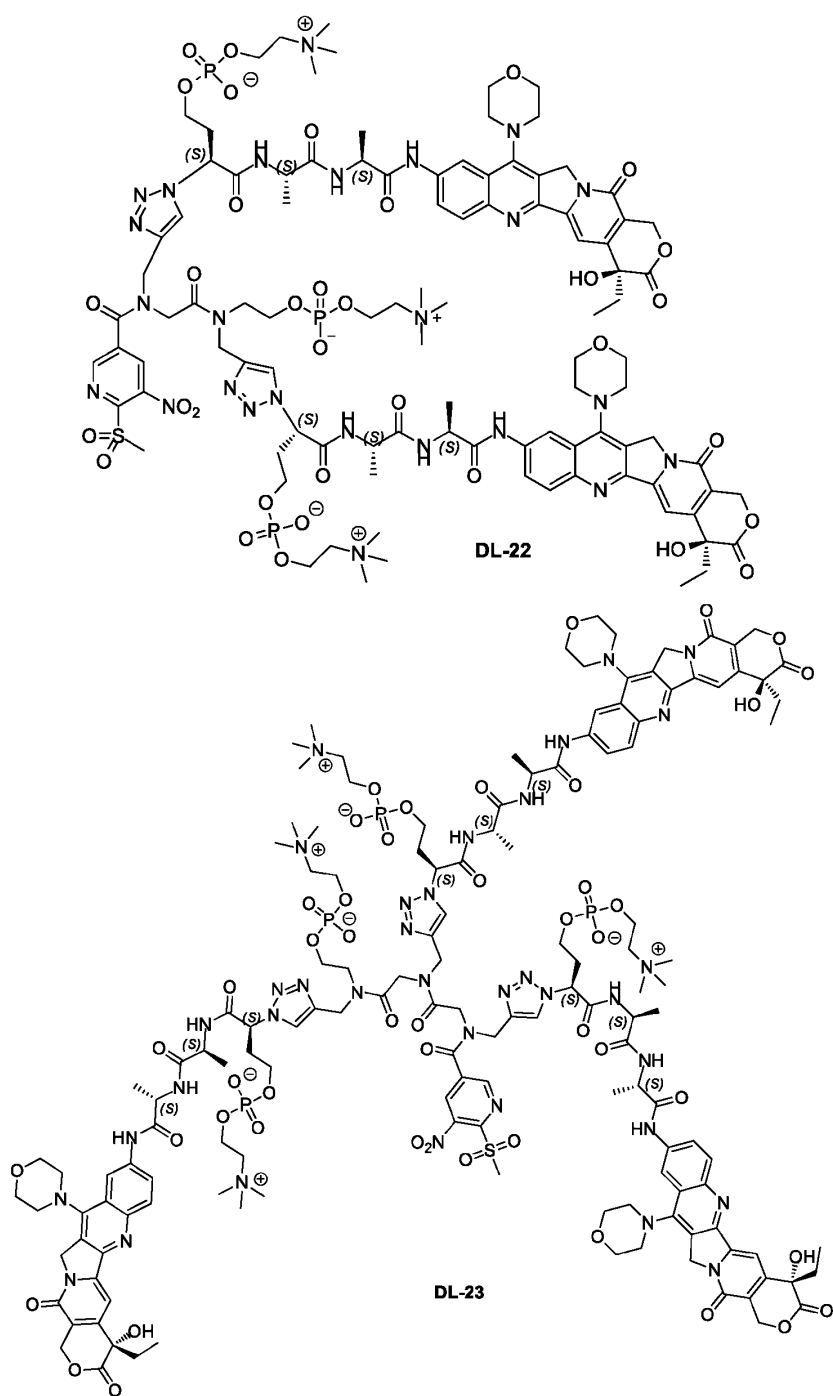
DL-14

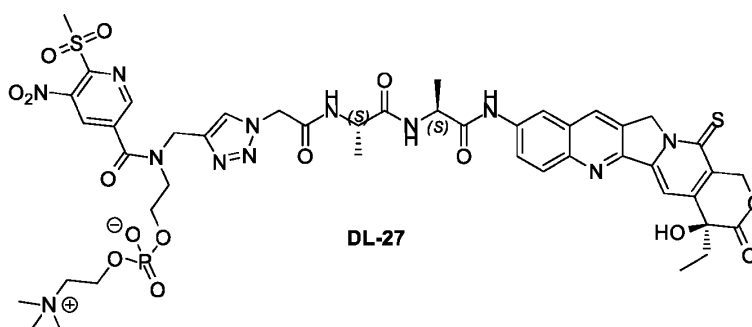
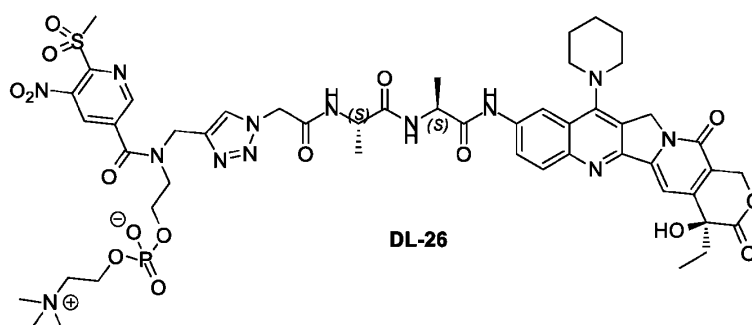
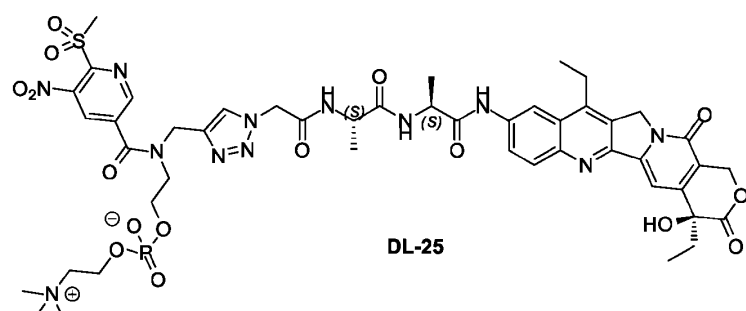
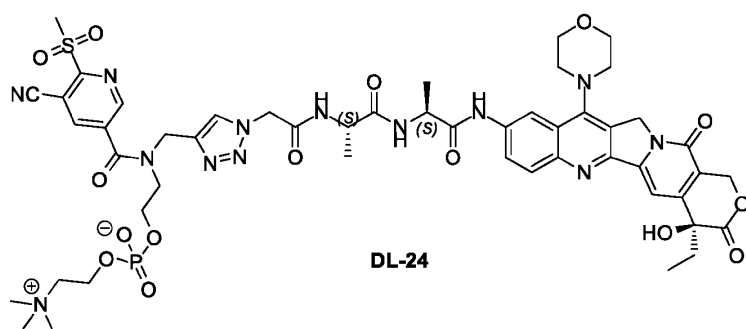


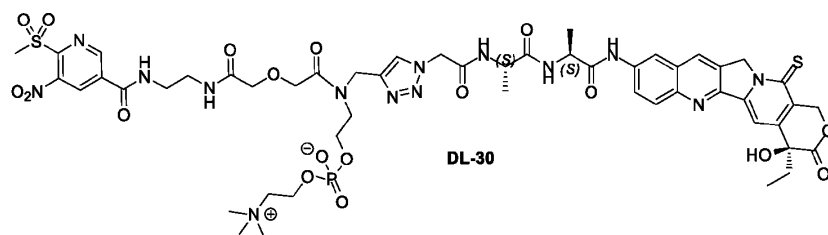
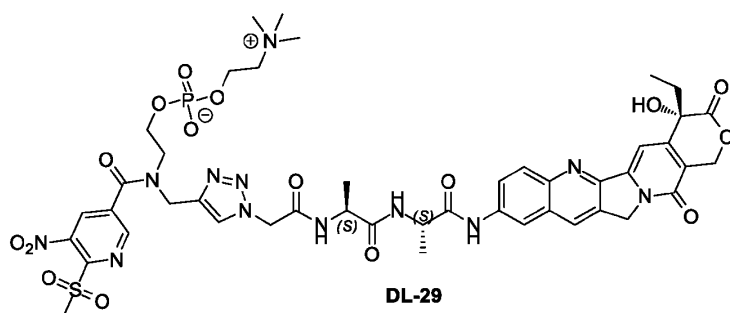
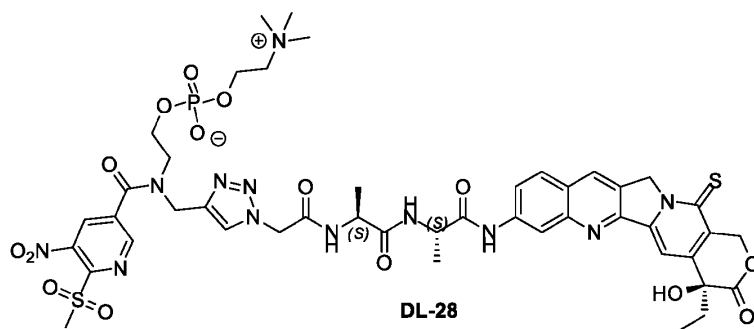




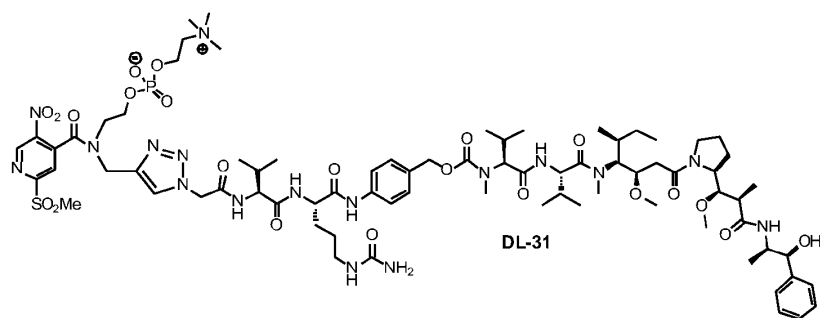


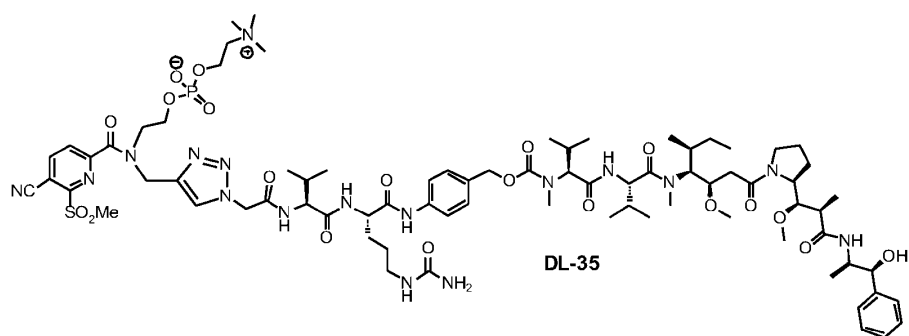
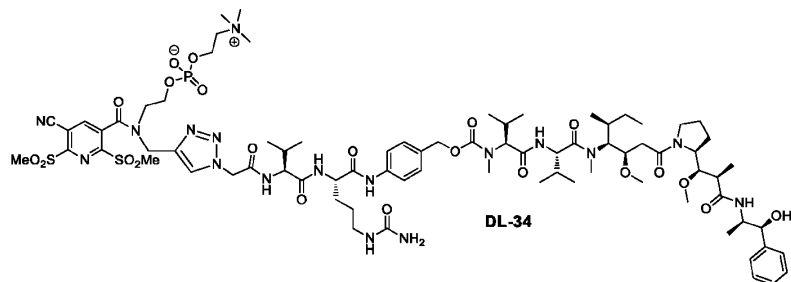
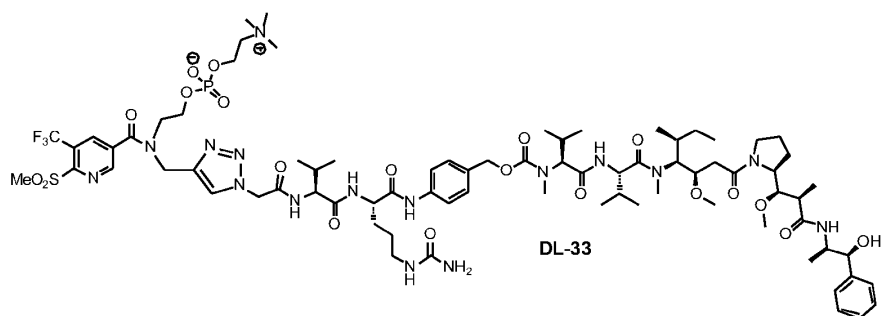
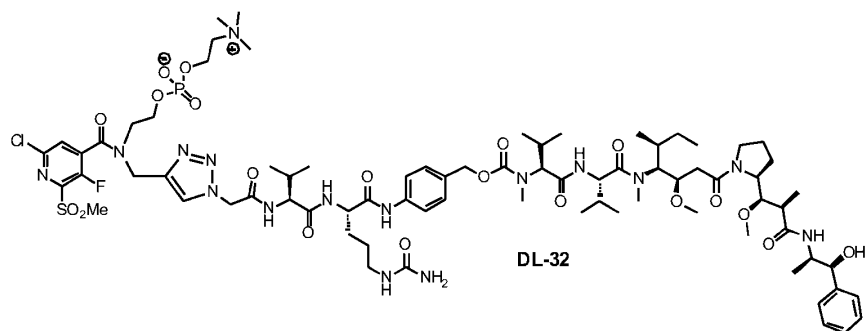


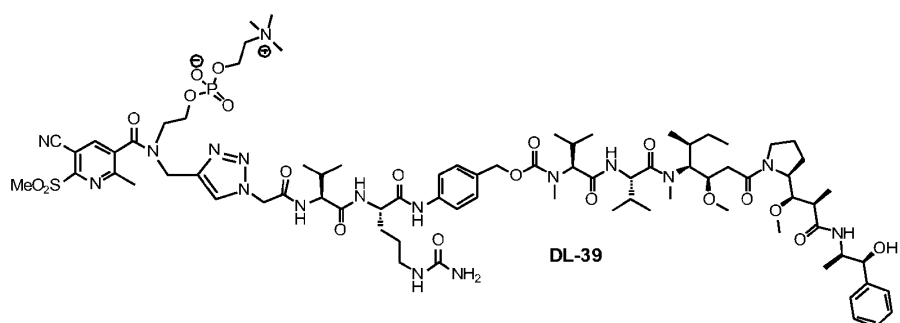
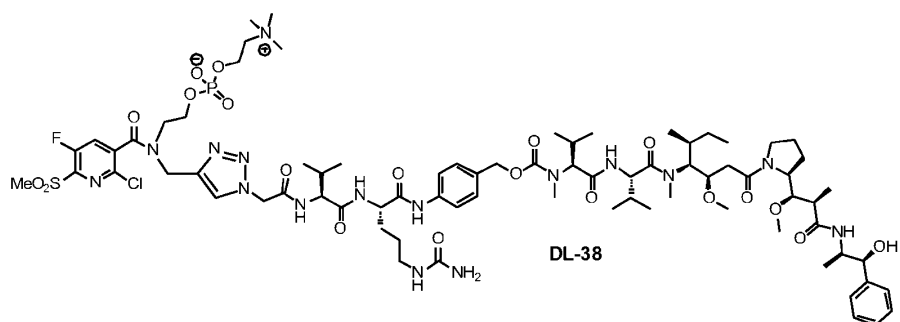
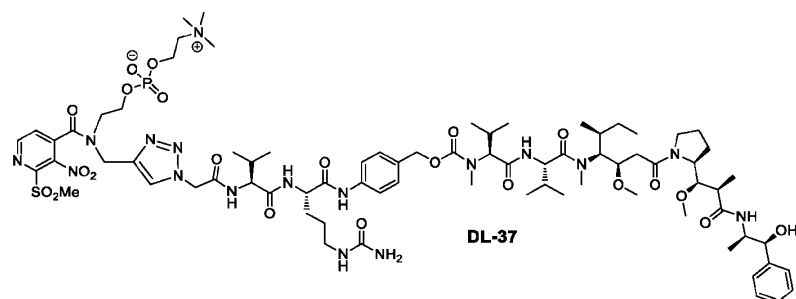
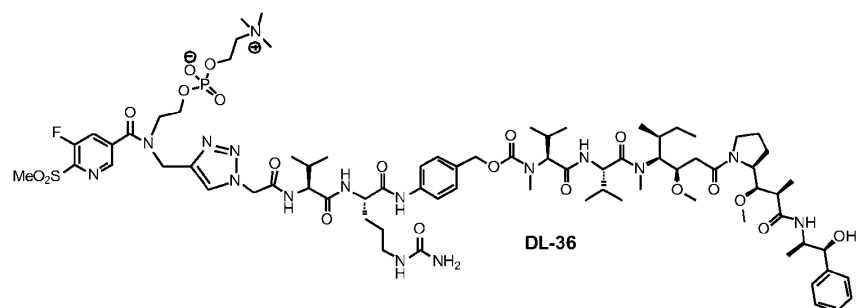


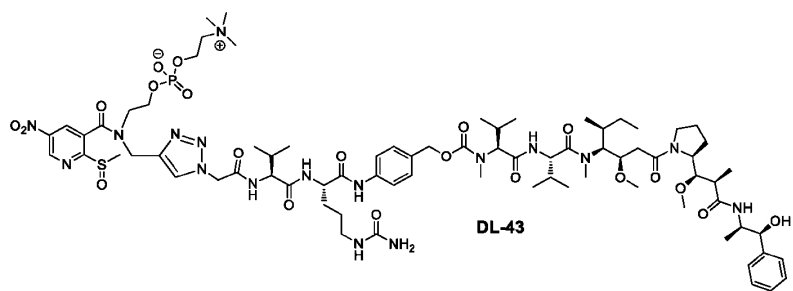
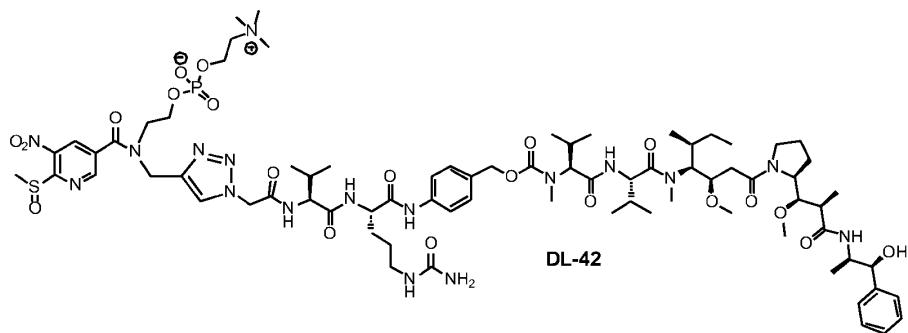
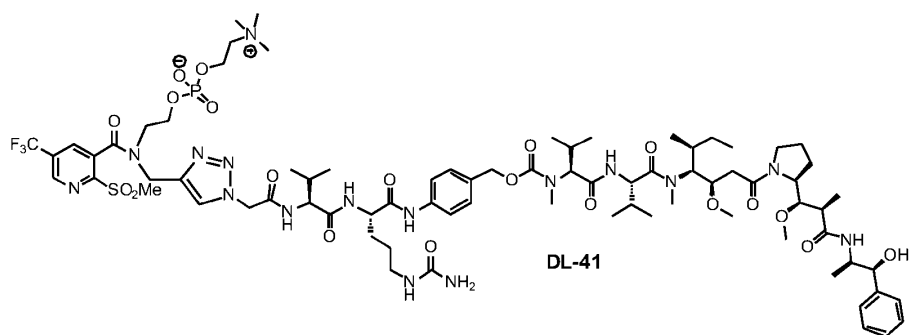
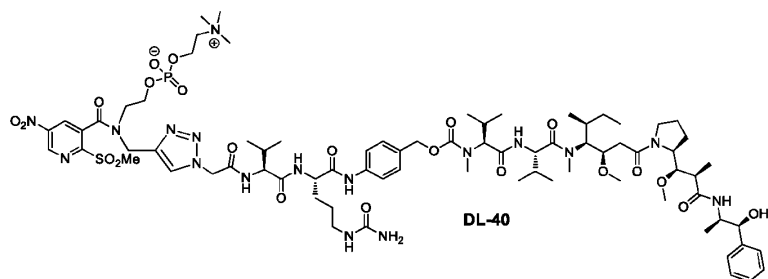


68. A compound selected from:





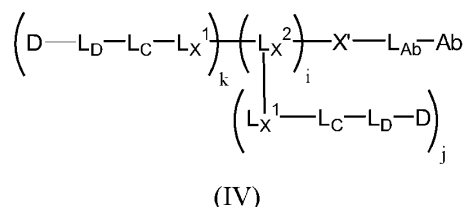




69. A composition comprising a compound of claims 1-68.
70. An immunoconjugate comprising a drug-linker conjugate or a building block, or a moiety

or derivative thereof, according to any one of claims 1-68.

71. An immunoconjugate prepared from a drug-linker conjugate or a building block, or a moiety or derivative thereof, according to any one of claims 1-68.
72. An immunoconjugate having the structural formula (IV)



or a pharmaceutically acceptable salt thereof,

wherein

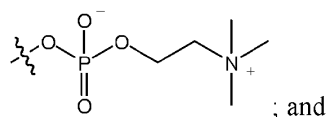
Ab represents an antigen binding moiety;

each D is independently a drug moiety;

X' is a single bond or a conjugation group;

each L_C is independently a moiety formed by a click chemistry reaction between an alkyne and an azide;

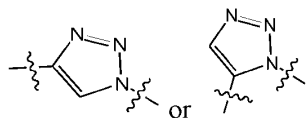
each of L_{Ab}, L_D, L_X¹ and L_X² is independently a linker or spacer moiety, wherein at least one of L_D, L_X¹ and L_X² comprises a phosphoryl choline (PC) group



i is an integer selected from 0-8; and

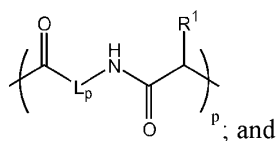
each of *j* and *k* is independently 0 or 1, provided that *j* and *k* are not both 0.

73. The immunoconjugate of claim 72, wherein *j* is 0.
74. The immunoconjugate of claim 72, wherein *j* is 1.
75. The immunoconjugate of claim 74, wherein *k* is 1.
76. The immunoconjugate of any one of claims 72-75, wherein *i* is 1.
77. The immunoconjugate of any one of claims 72-75, wherein *i* is 2.
78. The immunoconjugate of any one of claims 72-75, wherein *i* is greater than 2.
79. The immunoconjugate of any one of claims 72-78, wherein each L_C is

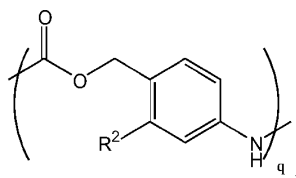


80. The immunoconjugate of any one of claims 72-79, wherein X' is a single bond.
81. The immunoconjugate of any of claims 72-80, wherein -L_D- is -L_D²-L_D¹-, wherein

L_D¹ is



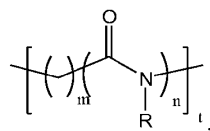
L_D² is



wherein

L_p is a di-, tri- or tetra peptide linker;

each of R¹ and R² is H, C₁₋₃ alkyl, or L_{PC}-(PC), wherein L_{PC} is



each *p* and *q* is independently 0 or 1, provided that *p* and *q* are not both 0;

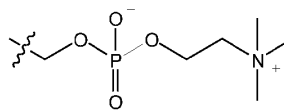
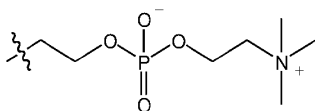
R is H or C₁₋₃ alkyl;

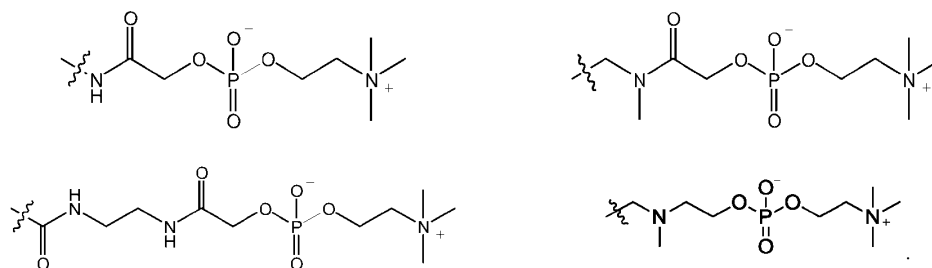
m is 0, 1, 2 or 3;

n is 0 or 1, and

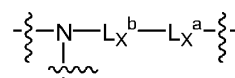
t is 0 or 1.

82. The immunoconjugate of claim 81, wherein each of R¹ and R² is H, C₁₋₃ alkyl, or L_{PC}-(PC).
83. The immunoconjugate of claim 81 or 82, wherein L_{PC}-(PC) is selected from:

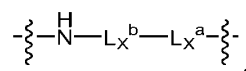




84. The immunoconjugate of any of claims 72-83, wherein L_X^1 is $(CH_2)_g$, wherein g is an integer selected from 0-6.
85. The immunoconjugate of claim 84, wherein g is 1.
86. The immunoconjugate of any of claims 72-85, wherein L_X^2 is:



or

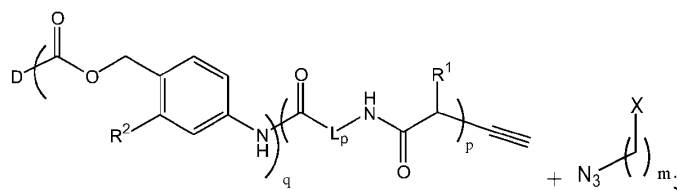


wherein

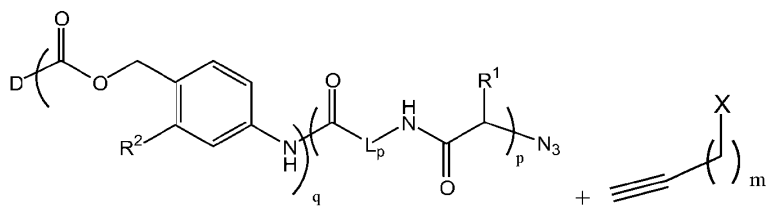
L_X^a is a substituted or unsubstituted C_{1-6} alkyl or a PEG group;

L_X^b is $-C(=O)-$, CH or CH_2 .

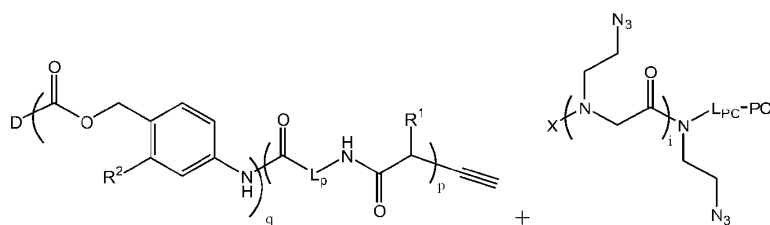
87. The immunoconjugate of any of claims 72-86, wherein the antigen-binding moiety is a monoclonal antibody or a fragment thereof.
88. The immunoconjugate of any of claims 72-87, wherein the immunoconjugate is in a substantially pure form.
89. A pharmaceutical composition comprising the immunoconjugate of any of claims 72-88 and a pharmaceutically acceptable excipient, carrier or diluent.
90. A combination comprising a therapeutically effective amount of an immunoconjugate of any one of claims 72-88, and one or more therapeutically active co-agent(s) and/or adjuvant(s).
91. The combination of claim 90, comprising a therapeutically active co-agent.
92. The combination of claim 90, comprising an adjuvant.
93. A method of preparing a linker-drug conjugate comprising reacting a compound of claim 51 with a compound of claim 57 under suitable conditions for click chemistry:



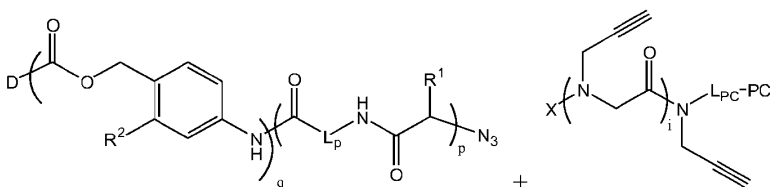
or



94. A method of preparing a linker-drug conjugate comprising reacting a compound of claim 51 with a compound of claim 62 under suitable conditions for click chemistry:



or



95. A method for treating or reducing a disease or condition, comprising administering to a subject in need thereof a therapeutically effective amount of an immunoconjugate of any one of claims 72-89.
96. The method of claim 94, wherein the disease or condition is cancer.
97. The method of claim 94 or 95, further comprising administering one or more of chemotherapy and radiotherapy on the subject.
98. A method for making a drug-linker conjugate of any one of claims 1-22.
99. A method for making a compound of any one of claims 23-41.
100. A method for making a compound of any one of claims 42-50.

101. A method for making a compound of any one of claims 23-41.
102. A method for making a compound of any one of claims 62-65.
103. A method for making a compound of any one of claims 66-68.
104. A method for making an immunoconjugate any one of claims 72-87.
105. Use of an immunoconjugate of any one of claims 72-89 for the manufacture of a medicament.
106. Use of an immunoconjugate of any one of claims 72-89 for treating cancer.
107. An immunoconjugate of any one of claims 72-89 for use in treating cancer.
108. A composition comprising an immunoconjugate of any one of claims 72-89.