ANTIBODY-DRUG CONJUGATES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to United States Provisional Application Nos. 63/581,306, filed September 8, 2023, 63/591,078, filed June October 17, 2023, and 63/550,023, filed February 5, 2024, the entirety of each of which is incorporated herein by reference.

BACKGROUND

[0002] Antibody-drug conjugates (ADCs) can provide an effective means of delivering a drug to a targeted site in a tissue or organism. Recognition of a target, such as a tumor, by the antibody minimizes exposure of non-target tissues to toxic chemotherapeutic agents and limits adverse effects associated with the toxicity of "free" drugs (i.e., unbound to a carrier such as an antibody). Therefore, a need exists for the development of new therapeutic modalities, such as ADCs, optimized to deliver a drug to a targeted site in a tissue or organism and minimize adverse effects.

SUMMARY

[0003] Among other things, the present disclosure encompasses the recognition that conjugate designs, e.g., ADC designs, can impact their structures, properties, activities, etc. In some embodiments, the present disclosure provides conjugates that can provide various benefits and advantages, e.g., improved properties, improved activities, etc. For example, in some embodiments, provided conjugates have, among other things, improved solubility, reduced aggregation, improved activities, etc. In some embodiments, provided conjugates comprise moieties (e.g., carbohydrate moieties, PEG moieties, etc.) that can improve solubility and/or reduce aggregation. In some embodiments, provided conjugates comprise $-(CH_2CH_2O)n-$ as described herein. In some embodiments, n is 1-24. In some embodiments, $-(CH_2CH_2O)n-$ is in the chain of bonds from a target binding moiety to a payload moiety (compared to in a side chain off the chain of bonds from a target binding moiety to a payload moiety).

[0004] In some embodiments, the present disclosure provides conjugates comprising:

i) a target binding moiety;

ii) a payload moiety; and

iii) a linker that connects the target binding moiety and the payload moiety;

wherein:

the linker comprises $-(CH_2CH_2O)n-$ and L^C;

n is 0-24;

L^C is bonded to a payload moiety and is $-L^{Cl}-L^{CO}-$;

L^{Cl} is or comprises a cleavable unit; and

L^{CO} is a covalent bond, or a spacer moiety.

[0005] In some embodiments, the present disclosure provides conjugates comprising:

i) a target binding moiety;

ii) a payload moiety; and

iii) a linker that connects the target binding moiety and the payload moiety;

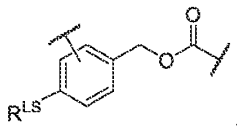
wherein:

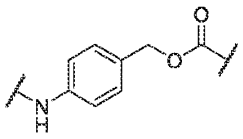
the linker comprises $-(CH_2CH_2O)n-$ and L^C;

n is 0-24;

L^C is bonded to a payload moiety and is $-L^{C1}-L^{CO}-$;

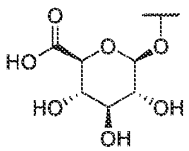
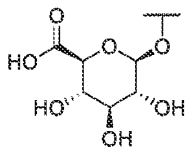
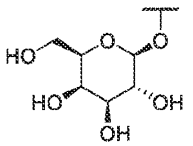
L^{C1} is –Gly–Gly–Phe–Gly–, –Val–Ala–, –Val–Cit–, L^{C1S}, –Gly–Gly–Phe–Gly–N(R')–L^{C1S}–, –Val–Ala–N(R')–L^{C1S}–, or –Val–Cit–N(R')–L^{C1S}–;

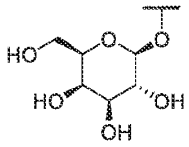
L^{C1S} is  , wherein the phenyl ring and the $-CH_2-$ is optionally substituted, and the carbonyl is bonded to L^{CO}, and R^{LS} is or comprises a sugar moiety;

L^{CO} is a covalent bond, or optionally substituted  wherein the carbonyl is bonded to a payload moiety;

each R' is independently –R, –C(O)R, $-CO_2R$, or $-S(O)_2R$, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

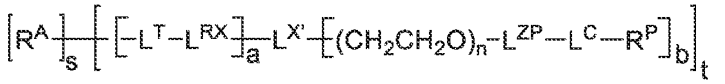
[0006] In some embodiments, R^{LS} is or comprises a sugar moiety so that L^C is cleavable, e.g., by an enzyme. In some embodiments, R^{LS} is or comprises a glucuronide moiety. In some embodiments, L^C can be cleaved by a β-glucuronidase. In some embodiments, R^{LS} is or comprises a galactoside moiety. In some embodiments, L^C can be cleaved by a β-galactosidase. In some embodiments, R^{LS} is or comprises  or a derivative thereof. In some embodiments, R^{LS} is  . In some embodiments, R^{LS} is or comprises  or a derivative thereof. In some embodiments, R^{LS}



is . In some embodiments, L^{Cl} is L^{ClS} as described herein. In some embodiments, L^{CO} is a covalent bond. In some embodiments, L^{C} is L^{Cl} as described herein. In some embodiments, L^{C} is L^{ClS} as described herein. In some embodiments, the phenyl ring the $-CH_2-$ in L^{ClS} is unsubstituted.

[0007] In some embodiments, n is 1-24.

[0008] In some embodiments, the present disclosure provides a conjugate, wherein the conjugate is a compound of formula A-1 or a salt thereof:



A-1

wherein:

each R^A is independently a target binding moiety;

s is 1-20;

each L^T is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^T are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each L^{RX} is independently a covalent bond, a moiety that results from a reaction between a group present on the unconjugated target binding moiety and a group present on an agent comprising $L^{X'}$, or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{RX} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each of a and b is independently 1-10;

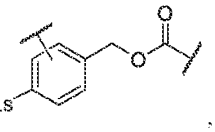
each $L^{X'}$ is independently a covalent bond or an optionally substituted bivalent or polyvalent straight or branched C_{1-30} aliphatic wherein 1-10 methylene units of $L^{X'}$ are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each n is independently 0-24;

each L^{ZP} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each L^{C} is independently $-L^{Cl}-L^{CO}-$;

each L^{Cl} is independently a cleavable unit, or each L^{Cl} is independently $-Gly-Gly-Phe-Gly-$, $-Val-Ala-$, $-Val-Cit-$, L^{ClS}, $-Gly-Gly-Phe-Gly-N(R')-L^{ClS}-$, $-Val-Ala-N(R')-L^{ClS}-$, or $-Val-Cit-N(R')-L^{ClS}-$;

L^{CLS} is  , wherein the phenyl ring and the $-CH_2-$ is optionally substituted, and the carbonyl is bonded to L^{CO}, and R^{LS} is or comprises a sugar moiety;

each L^{CO} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{CO} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each R^P is independently a payload moiety;

t is 1-20;

each $-Cy-$ is independently an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each R' is independently $-R$, $-C(O)R$, $-CO_2R$, or $-S(O)_2R$, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

[0009] In some embodiments, a is 1 and b is 1.

[0010] In some embodiments, the present disclosure provides a conjugate (e.g., a conjugate of formula A-1), wherein the conjugate is a compound of formula A-2 or a salt thereof:

$$R^A\left[-L^T-L^{RX}-L^X-(CH_2CH_2O)_n-L^{ZP}-L^C-R^P\right]_t,$$

A-2

wherein:

R^A is a target binding moiety;

each L^T is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^T are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each L^{RX} is independently a covalent bond, a moiety that results from a reaction between a group present on the unconjugated target binding moiety and a group present on an agent comprising $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$, or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{RX} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

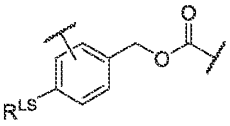
each L^X is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^X are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –S(O)$_2$–, –C(O)N(R')–, –S(O)$_2$(NR')–, and –Cy–;

each n is independently 0-24;

each L^{ZP} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –S(O)$_2$–, –C(O)N(R')–, –S(O)$_2$(NR')–, and –Cy–;

each L^C is independently $-L^{Cl}-L^{CO}-$;

each L^{Cl} is independently a cleavable unit, or each L^{Cl} is independently –Gly–Gly–Phe–Gly–, –Val–Ala–, –Val–Cit–, L^{ClS}, –Gly–Gly–Phe–Gly–N(R')–L^{ClS}–, –Val–Ala–N(R')–L^{ClS}–, or –Val–Cit–N(R')–L^{ClS}–;

L^{ClS} is  , wherein the phenyl ring and the –CH_2– is optionally substituted, and the carbonyl is bonded to L^{CO}, and R^{LS} is or comprises a sugar moiety;

each L^{CO} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{CO} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –S(O)$_2$–, –C(O)N(R')–, –S(O)$_2$(NR')–, and –Cy–;

each R^P is independently a payload moiety;

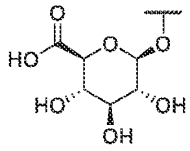
t is 1-20;

each –Cy– is independently an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

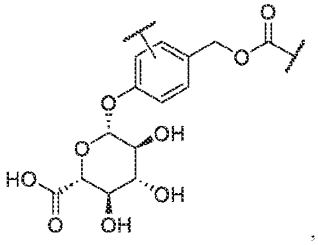
each R' is independently –R, –C(O)R, –CO_2R, or –S(O)$_2$R, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

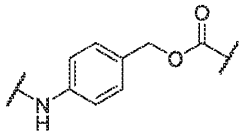
[0011] In some embodiments, each L^{Cl} is independently a cleavable unit. In some embodiments, each L^{Cl} is independently –Gly–Gly–Phe–Gly–, –Val–Ala–, –Val–Cit–, L^{ClS}, –Gly–Gly–Phe–Gly–N(R')–L^{ClS}–, –Val–Ala–N(R')–L^{ClS}–, or –Val–Cit–N(R')–L^{ClS}–, wherein each variable is independently as described herein. In some embodiments, each L^{Cl} is independently –Gly–Gly–Phe–Gly–, –Val–Ala–, –Val–Cit–, or L^{ClS}. In some embodiments, each L^{Cl} is independently –Val–Ala–, –Val–Cit–, or L^{ClS}. In some embodiments, each L^{Cl} is independently –Val–Ala–, –Val–Cit–, or L^{ClS}, wherein R^{LS} is or comprises a glucuronide moiety. In some embodiments, each L^{Cl} is



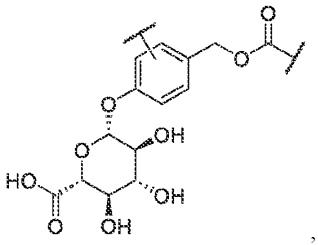
independently −Val−Ala−, −Val−Cit−, or L^{C1S}, wherein R^{LS} is . In some embodiments, each L^{C1} is independently −Gly−Gly−Phe−Gly−, −Val−Ala−, −Val−Cit−, or



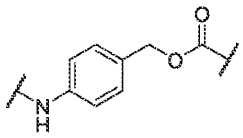
, and each L^{CO} is independently a covalent bond or optionally substituted



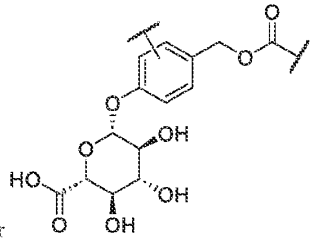
wherein the carbonyl is bonded to a payload moiety. In some embodiments, at least one occurrence of n is 1-24. In some embodiments, each occurrence of n is independently 1-24. In some embodiments, each L^{C1} is independently −Gly−Gly−Phe−Gly−, −Val−Ala−, −Val−Cit−, or



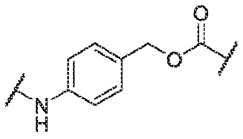
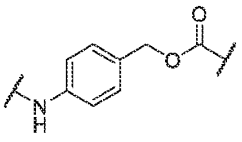
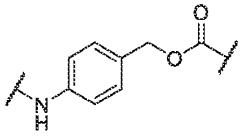
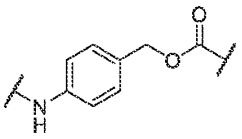
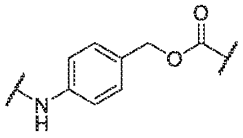
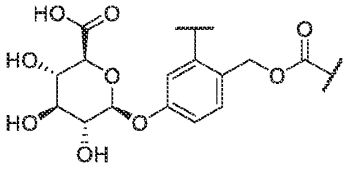
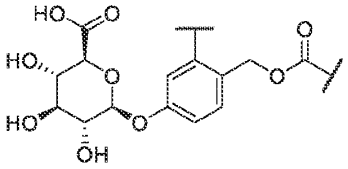
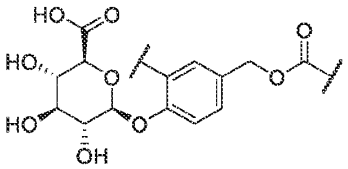
, each L^{CO} is independently a covalent bond or optionally substituted

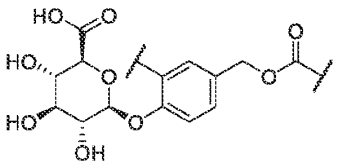


wherein the carbonyl is bonded to a payload moiety, and at least one occurrence of n is independently 1-24. In some embodiments, each L^{C1} is independently −Gly−Gly−Phe−Gly−, −Val−Ala−, −Val−Cit−, or



, each L^{CO} is independently a covalent bond or

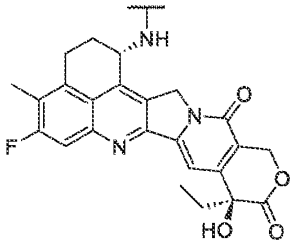
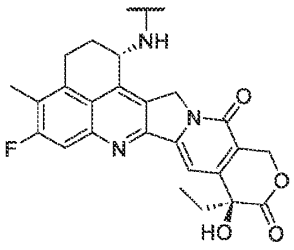
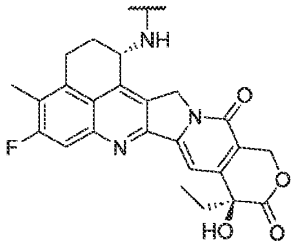
optionally substituted  wherein the carbonyl is bonded to a payload moiety, and each occurrence of n is independently 1-24. In some embodiments, each L^{C1} is independently –Val–Ala–. In some embodiments, each L^{C1} is independently –Val–Ala– and each L^{CO} is independently optionally substituted  . In some embodiments, each L^{C1} is independently –Val–Ala– and each L^{CO} is  . In some embodiments, each L^{C1} is independently –Val–Cit–. In some embodiments, each L^{C1} is independently –Val–Cit– and each L^{CO} is independently optionally substituted  . In some embodiments, each L^{C1} is independently –Val–Cit– and each L^{CO} is  . In some embodiments, each L^{C1} is independently  . In some embodiments, each L^{C1} is independently  and each L^{CO} is a covalent bond. In some embodiments, each L^{C1} is independently  . In some embodiments, each L^{C1} is independently



and each L^{CO} is a covalent bond.

[0012] In some embodiments, a target binding moiety binds to a polypeptide. For example, in some embodiments, a target binding moiety binds to HHLA2 or a fragment thereof.

[0013] In some embodiments, a target binding moiety is or comprises an antibody or an antigen-binding fragment thereof. For example, in some embodiments, a target binding moiety is or comprises an anti-HHLA2 antibody or an antigen-binding fragment thereof.

[0014] Those skilled in the art appreciate that various payload moieties, e.g., those reported for antibody-drug conjugates, can be utilized in accordance with the present disclosure. For example, in some embodiments, a payload moiety is or comprises camptothecin or a derivative or an analog thereof. In some embodiments, a payload moiety is or comprises  . In some embodiments, a payload is  . In some embodiments, each payload is  .

[0015] In some embodiments, the present disclosure provides various linkers. For example, in some embodiments, provided linkers comprise PEG moieties. In some embodiments, provided linkers comprise $-(CH_2CH_2O)n-$, wherein n is 1-24. In some embodiments, provided linkers comprise cleavable units, e.g., GGFG, that directly bonded to payload moieties. In some embodiments, a cleavable unit can be cleaved by an enzyme, e.g., an enzyme present at a target site. In some embodiments, a cleavable unit is or comprises a polypeptide, e.g., GGFG, Val-Ala, Val-Cit, etc. In some embodiments, a polypeptide is

bonded to a provided method at its C-terminus through −C(O)−. Among other things, the present disclosure provides antibody-drug conjugates in which there are no moieties (e.g., no spacer moieties) between cleavable units and payload moieties. In some embodiments, there are moieties, e.g., spacer moieties, between cleavable units and payload moieties. In some embodiments, a linker is or comprises $-L^{ZP}-L^{C}-$ wherein each variable is independently as described herein. In some embodiments, a linker is or comprises $-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ wherein each variable is independently as described herein. In some embodiments, a linker is or comprises $-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ wherein each variable is independently as described herein.

[0016] In some embodiments, the present disclosure provides pharmaceutical compositions comprising provided conjugates and pharmaceutically acceptable carriers. Among other things, provided technologies, e.g., compounds, conjugates, agents, compositions, etc., are useful for preventing or treating various conditions, disorders or diseases. In some embodiments, when utilized as therapeutics, provided compounds, conjugates, agents, e.g., are provided in pharmaceutical compositions. Compounds, conjugates, agents, etc., may be various forms, e.g., salt forms. For example, in some embodiments, they may exist in various pharmaceutically acceptable salt forms. In some embodiments, in a composition a compound, conjugate, agent, etc., may exist in one or more forms, e.g., in one or more pharmaceutically acceptable salt forms.

[0017] In some embodiments, the present disclosure provides methods for preventing or treating various conditions, disorders or diseases. In some embodiments, the present disclosure provides a method for preventing a condition, disorder or disease, comprising administering or delivering to a subject susceptible thereto an effective amount of a provided compound, conjugate, agent or composition. In some embodiments, the present disclosure provides a method for treating a condition, disorder or disease, comprising administering or delivering to a subject suffering therefrom an effective amount of a provided compound, conjugate, agent or composition. In some embodiments, a condition, disorder or disease is cancer. In some embodiments, a subject has or is at risk of developing a cancer. In some embodiments, a subject has a solid tumor or a hematological cancer. In some embodiments, a solid tumor is or comprises one or more of: a renal cancer, a bone cancer, a skin cancer, a breast cancer, a cervical cancer, a colorectal cancer, an endometrial cancer, a lung cancer, an ovarian cancer, a liver cancer, cholangiocarcinoma, or a thyroid cancer. In some embodiments, a hematological cancer comprises or is a leukemia or lymphoma. In some embodiments, a leukemia comprises or is acute lymphocytic leukemia, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, chronic leukemia, or acute leukemia. In some embodiments, a lymphoma comprises or is Hodgkin lymphoma (HL), non-Hodgkin's lymphoma, lymphocytic lymphoma, or diffuse large B cell lymphoma (DLBCL). In some embodiments, a condition, disorder or disease is associated with aberrant expression of an agent that a target binding moiety can bind, e.g., HHLA2. In some embodiments, a conjugate is administered parenterally. In some embodiments, parenteral administration is or comprises subcutaneous, intravenous, intramuscular, or intrasternal injection or infusion. In some embodiments, a conjugate is administered in combination with another therapeutic agent.

[0018] In some embodiments, the present disclosure provides technologies, e.g., methods, reagents, intermediates, etc., for manufacturing various compounds, e.g., conjugates described herein.

[0019] Various features, objects, and advantages of provided technologies are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating embodiments of provided technologies, is given by way of illustration only, not limitation. Various changes and modifications of provided technologies will become apparent to those skilled in the art from the present disclosure including the detailed description.

BRIEF DESCRIPTION OF DRAWINGS

[0020] Figures 1A, 1B and 1C. Provided conjugates can bind to targets. Figures 1A, 1B and 1C depict certain binding curves for anti-HHLA2 antibody drug conjugates binding to HHLA2+ A204 cells. Naked antibody, exatecan conjugates with DAR about 4 (Exatecan_DAR4), and exatecan conjugates with DAR about 8 (Exatecan_DAR8) for each of Hu2C4-H3L3 (1A), 65872 (1B), and 887-1DE (1C) were tested. Controls include human IgG1 isotype (IgG1 Control) and exatecan conjugates of human IgG1 isotype with DAR about 8 (IgG1-Exatecan_DAR8).

[0021] Figure 2. Provided conjugates can be internalized. Figure 2 depicts examples of induction of internalization in A204 cells by anti-HHLA2 antibodies: Hu2C4-H3L3, 65872, and 887-1DE.

[0022] Figures 3A and 3B. Provided conjugates can kill target cells. Figures 3A and 3B depict dose-response curves for data from example assessment of anti-HHLA2 antibody drug conjugates: Hu2C4-H3L3, 65872, and 887-1DE. 3A: DAR is about 4. 3B: DAR is about 8. Data for exatecan conjugates of human IgG1 isotype with DAR about 8 (IgG1-Exatecan_DAR8) and exatecan are also included.

[0023] Figure 4. Provided conjugates can bind to targets. Figure 4 depicts certain binding curves for anti-HHLA2 antibody drug conjugates binding to HEK cells overexpressing B7H7. Depicted are Hu2C4-H3L3 conjugates of LP-A, LP-B, and LP-C at DAR 8, and 65872 conjugates of LP-A, LP-B, and LP-C at DAR 8. Controls include Hu2C4-H3L3-PEG8-GGFG-Exatecan DAR8 and GGFG-Exatecan conjugates of human IgG1 isotype with DAR about 8. As demonstrated herein, provided conjugates can bind to targets.

[0024] Figure 5. Provided conjugates can kill target cells. Figure 5 depicts certain dose-response curves for data from example assessment of anti-HHLA2 antibody drug conjugates. Depicted are Hu2C4-H3L3 conjugates of LP-A, LP-B, and LP-C at DAR 8, and 65872 conjugates of LP-A, LP-B and LP-C at DAR 8. Controls include Hu2C4-H3L3-PEG8-GGFG-Exatecan DAR8 and GGFG-Exatecan conjugates of human IgG1 isotype with DAR about 8. As demonstrated herein, provided conjugates can effectively kill target cells.

DEFINITIONS

[0025] In order for provided technologies to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the specification. The publications (e.g., patents, patent application publications, non-patent publications,

etc.), websites and other reference materials referenced herein are each hereby incorporated by reference in its entirety; in the case of any conflict, the present disclosure will control.

Chemical Definitions

[0026] Compounds of the present disclosure include those described generally above, and are further illustrated by the classes, subclasses, and species disclosed herein. As used herein, the following definitions shall apply unless otherwise indicated. For purposes of this disclosure, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed. Additionally, general principles of organic chemistry are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: 1999, and "March's Advanced Organic Chemistry", 5th Ed., Ed.: Smith, M.B. and March, J., John Wiley & Sons, New York: 2001, the entire contents of which are hereby incorporated by reference.

[0027] Aliphatic: As used herein, "aliphatic" means a straight-chain (i.e., unbranched) or branched, substituted or unsubstituted hydrocarbon chain that is completely saturated or that contains one or more units of unsaturation (but not aromatic), or a substituted or unsubstituted monocyclic, bicyclic, or polycyclic hydrocarbon ring that is completely saturated or that contains one or more units of unsaturation (but not aromatic), or combinations thereof. In some embodiments, aliphatic groups contain 1-50 aliphatic carbon atoms. In some embodiments, aliphatic groups contain 1-20 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-10 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-9 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-8 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-7 aliphatic carbon atoms. In other embodiments, aliphatic groups contain 1-6 aliphatic carbon atoms. In still other embodiments, aliphatic groups contain 1-5 aliphatic carbon atoms, and in yet other embodiments, aliphatic groups contain 1, 2, 3, or 4 aliphatic carbon atoms. Certain aliphatic groups include linear or branched, substituted or unsubstituted alkyl, alkenyl, alkynyl groups and hybrids thereof such as (cycloalkyl)alkyl, (cycloalkenyl)alkyl or (cycloalkyl)alkenyl.

[0028] Alkyl: As used herein, the term "alkyl" is given its ordinary meaning in the art and refers to an aliphatic group, e.g., a straight-chain alkyl group, a branched-chain alkyl group, a cycloalkyl (alicyclic) group, an alkyl substituted cycloalkyl group, a cycloalkyl substituted alkyl group, etc., which is saturated. In some embodiments, alkyl has 1-100 carbon atoms. In some embodiments, alkyl has about 1-30 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 1-25, 1-20, 1-15, 1-10, 3-30, etc.) carbon atoms. In some embodiments, alkyl has 1-20 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 1-15, 1-10, etc.) carbon atoms. In certain embodiments, a straight chain or branched chain alkyl has about 1-20 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 1-15, 1-10, etc.) carbon atoms in its backbone (e.g., C_1-C_{20} for straight chain, C_2-C_{20} for branched chain, etc.), and alternatively, about 1-10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-5, 1-4, etc.) carbon atoms. In some embodiments, cycloalkyl rings have from about 3-10 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 3-8, 3-7, 3-6, 4-6, 5-6, 5-8, etc.) carbon atoms in their ring structure where such rings are monocyclic, bicyclic, or polycyclic, and alternatively about 5, 6 or 7 carbons in the ring structure. In some embodiments, an alkyl

group may be a lower alkyl group, wherein a lower alkyl group has 1-4 carbon atoms (e.g., C_1-C_4 for straight chain lower alkyls).

[0029] Aryl: The term "aryl", as used herein, used alone or as part of a larger moiety as in "aralkyl," "aralkoxy," or "aryloxyalkyl," refers to monocyclic, bicyclic or polycyclic ring systems having a total of, unless otherwise specified, 5-30 (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 5-25, 5-20, 5-15, 5-14, 5-10, 5-9, etc.) ring members, wherein at least one ring in the system is aromatic. In some embodiments, an aryl group is a monocyclic, bicyclic or polycyclic ring system having a total of five to fourteen (e.g., 5-10, 6-14, 6-10, 5, 6, 9, 10, 14, etc.) ring members, wherein at least one ring in the system is aromatic, and wherein each ring in the system contains 3 to 7 ring members. In some embodiments, each monocyclic ring unit is aromatic. In some embodiments, each aromatic ring is independently 6-membered. In some embodiments, each aromatic ring atom is carbon. In some embodiments, each ring atom is carbon. In some embodiments, an aryl group is a biaryl group. The term "aryl" may be used interchangeably with the term "aryl ring." In some embodiments, "aryl" refers to an aromatic ring system which includes phenyl, biphenyl, naphthyl, binaphthyl, anthracyl and the like, which may bear one or more substituents. Also included within the scope of the term "aryl," as it is used herein, is a group in which an aromatic ring is fused to one or more non-aromatic rings, such as indanyl, phthalimidyl, naphthylmethyl, phenanthridinyl, or tetrahydronaphthyl, and the like.

[0030] Halogen: The term "halogen" means F, Cl, Br, or I.

[0031] Heteroatom: The term "heteroatom", as used herein, means an atom that is not carbon or hydrogen. In some embodiments, a heteroatom is boron, oxygen, sulfur, nitrogen, phosphorus, or silicon (including oxidized forms of nitrogen, sulfur, phosphorus, or silicon; charged forms of nitrogen (e.g., quaternized forms, forms as in iminium groups, etc.), phosphorus, sulfur, oxygen; etc.). In some embodiments, a heteroatom is silicon, phosphorus, oxygen, sulfur or nitrogen. In some embodiments, a heteroatom is silicon, oxygen, sulfur or nitrogen. In some embodiments, a heteroatom is oxygen, sulfur or nitrogen.

[0032] Partially Unsaturated: As used herein, the term "partially unsaturated", as used herein, refers to a ring moiety that includes at least one double or triple bond. The term "partially unsaturated", as used herein, is intended to encompass rings having multiple sites of unsaturation, but is not intended to include aryl or heteroaryl moieties, as herein defined.

[0033] Unsaturated: The term "unsaturated", as used herein, means that a moiety has one or more units of unsaturation.

[0034] Heteroaryl: The terms "heteroaryl" and "heteroar-", as used herein, used alone or as part of a larger moiety, e.g., "heteroaralkyl," or "heteroaralkoxy," refer to monocyclic, bicyclic or polycyclic ring systems having a total of, unless otherwise specified, 5-30 (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 5-25, 5-20, 5-15, 5-14, 5-10, 5-9, etc.) ring members, wherein at least one ring in the system is aromatic and at least one aromatic ring atom is a heteroatom. In some embodiments, the number of ring heteroatoms in a heteroaryl group is about 1-10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-5, 1-4, 1-3, 1-2, etc.). In some embodiments, a heteroaryl group is a group having 5 to 10

ring atoms, in some embodiments 5, 6, 9, or 10 ring atoms. In some embodiments, each monocyclic ring unit is aromatic. In some embodiments, each aromatic monocyclic ring unit is independently 5- or 6-membered. In some embodiments, a heteroaryl ring is monocyclic and is 5-membered. In some embodiments, a heteroaryl ring is monocyclic and is 6-membered. In some embodiments, a heteroaryl ring is bicyclic and is 9-membered. In some embodiments, a heteroaryl ring is bicyclic and is 10-membered. In some embodiments, a heteroaryl group has 6, 10, or 14 π electrons shared in a cyclic array; and having, in addition to carbon atoms, from one to five heteroatoms. In some embodiments, each heteroatom is independently selected from nitrogen, oxygen and sulfur. Heteroaryl groups include, without limitation, thienyl, furanyl, pyrrolyl, imidazolyl, pyrazolyl, triazolyl, tetrazolyl, oxazolyl, isoxazolyl, oxadiazolyl, thiazolyl, isothiazolyl, thiadiazolyl, pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, indolizinyl, purinyl, naphthyridinyl, and pteridinyl. In some embodiments, a heteroaryl is a heterobiaryl group, such as bipyridyl and the like. The terms "heteroaryl" and "heteroar–", as used herein, also include groups in which a heteroaromatic ring is fused to one or more aryl, cycloaliphatic, or heterocyclyl rings, where the radical or point of attachment is on the heteroaromatic ring. Non-limiting examples include indolyl, isoindolyl, benzothienyl, benzofuranyl, dibenzofuranyl, indazolyl, benzimidazolyl, benzthiazolyl, quinolyl, isoquinolyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, 4H–quinolizinyl, carbazolyl, acridinyl, phenazinyl, phenothiazinyl, phenoxazinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, and pyrido[2,3–b]–1,4–oxazin–3(4H)–one. A heteroaryl group may be monocyclic, bicyclic or polycyclic. The term "heteroaryl" may be used interchangeably with the terms "heteroaryl ring," "heteroaryl group," or "heteroaromatic," any of which terms include rings that are optionally substituted. The term "heteroaralkyl" refers to an alkyl group substituted by a heteroaryl group, wherein the alkyl and heteroaryl portions independently are optionally substituted.

[0035] Additionally, it will be appreciated that, when two groups cyclize to form an optionally substituted heteroaryl ring having at least one nitrogen atom, the nitrogen atom in the ring can be, as valency permits, N or N-$R^{\dagger}$, as defined herein.

[0036] Heterocycle: As used herein, the terms "heterocycle," "heterocyclyl," "heterocyclic radical," and "heterocyclic ring", as used herein, are used interchangeably and refer to a monocyclic, bicyclic or polycyclic ring moiety (unless otherwise specified, 3-30 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 3-25, 3-20, 3-15, 3-14, 3-10, 5-10, 3-7, 3-6, etc.) membered) that is saturated or partially unsaturated and has one or more (e.g., 1-10, 1-5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, etc.) heteroatom ring atoms. In some embodiments, the number of ring heteroatoms in a heterocyclyl group is about 1-10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-5, 1-4, 1-3, 1-2, etc.). In some embodiments, a heterocyclyl group is a stable 3-7 membered monocyclic or 7-10 membered bicyclic heterocyclic moiety that is either saturated or partially unsaturated, and having, in addition to carbon atoms, one or more, preferably one to four, heteroatoms, as defined above. In some embodiments, each monocyclic ring is independently 3-10 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 3-8, 3-7, 3-6, 4-6, 5-6, 5-8, etc.) membered. In some embodiments, each monocyclic ring independently has 0-5 (e.g., 0, 1, 2, 3, 4, 5, 1-5, 1-4, 1-2, etc.) heteroatoms wherein at least one monocyclic ring independently has 1-5 (e.g., 1, 2, 3, 4, 5, 1-5, 1-4, 1-2, etc.) heteroatoms. In some

embodiments, each monocyclic ring is independently 3-7 membered. In some embodiments, each heteroatom is independently selected from nitrogen, oxygen and sulfur. When used in reference to a ring atom of a heterocycle, the term "nitrogen" includes substituted nitrogen. As an example, in a saturated or partially unsaturated ring having 0–3 heteroatoms selected from oxygen, sulfur and nitrogen, the nitrogen may be N (as in 3,4–dihydro–2H–pyrrolyl), NH (as in pyrrolidinyl), or $^{+}$NR (as in N–substituted pyrrolidinyl). A heterocyclic ring can be attached to its pendant group at any heteroatom or carbon atom that results in a stable structure and any of the ring atoms can be optionally substituted. Examples of such saturated or partially unsaturated heterocyclic radicals include, without limitation, tetrahydrofuranyl, tetrahydrothienyl, pyrrolidinyl, piperidinyl, pyrrolinyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, oxazolidinyl, piperazinyl, dioxanyl, dioxolanyl, diazepinyl, oxazepinyl, thiazepinyl, morpholinyl, and quinuclidinyl. The terms "heterocycle," "heterocyclyl," "heterocyclyl ring," "heterocyclic group," "heterocyclic moiety," and "heterocyclic radical," are used interchangeably herein, and also include groups in which a heterocyclyl ring is fused to one or more aryl, heteroaryl, or cycloaliphatic rings, such as indolinyl, 3H–indolyl, chromanyl, phenanthridinyl, or tetrahydroquinolinyl. A heterocyclyl group may be monocyclic, bicyclic or polycyclic. The term "heterocyclylalkyl" refers to an alkyl group substituted by a heterocyclyl, wherein the alkyl and heterocyclyl portions independently are optionally substituted.

[0037] Additionally, it will be appreciated that, when two groups cyclize to form an optionally substituted heterocyclic ring having at least one nitrogen atom, the nitrogen atom in the ring can be, as valency permits, N or N-$R^{\dagger}$, as defined infra.

[0038] Optionally Substituted: As described herein, compounds of the disclosure may contain optionally substituted, substituted and/or unsubstituted moieties. In general, the term "substituted," means that one or more hydrogens of the designated moiety are independently replaced with a substituent. Unless otherwise indicated, an "optionally substituted" group may independently have a substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with two or more substituents, the substituents may be either the same or different at every position. In some embodiments, an optionally substituted group is unsubstituted. In some embodiments, an optionally substituted group is substituted. Various substituents are described below.

[0039] Monovalent substituents are independently halogen; $–(CH_2)_{0–4}R^{\circ}$; $–(CH_2)_{0–4}OR^{\circ}$; $–O(CH_2)_{0–4}R^{\circ}$, $–O–(CH_2)_{0–4}C(O)OR^{\circ}$; $–(CH_2)_{0–4}CH(OR^{\circ})_2$; $–(CH_2)_{0–4}Ph$, which may be substituted with R°; $–(CH_2)_{0–4}O(CH_2)_{0–1}Ph$ which may be substituted with R°; –CH=CHPh, which may be substituted with R°; $–(CH_2)_{0–4}O(CH_2)_{0–1}$-pyridyl which may be substituted with R°; $–NO_2$; –CN; $–N_3$; $-(CH_2)_{0–4}N(R^{\circ})_2$; $–(CH_2)_{0–4}N(R^{\circ})C(O)R^{\circ}$; $–N(R^{\circ})C(S)R^{\circ}$; $–(CH_2)_{0–4}N(R^{\circ})C(O)N(R^{\circ})_2$; $–N(R^{\circ})C(S)N(R^{\circ})_2$; $–(CH_2)_{0–4}N(R^{\circ})C(O)OR^{\circ}$; $–N(R^{\circ})N(R^{\circ})C(O)R^{\circ}$; $–N(R^{\circ})N(R^{\circ})C(O)N(R^{\circ})_2$; $–N(R^{\circ})N(R^{\circ})C(O)OR^{\circ}$; $–(CH_2)_{0–4}C(O)R^{\circ}$; $–C(S)R^{\circ}$; $–(CH_2)_{0–4}C(O)OR^{\circ}$; $–(CH_2)_{0–4}C(O)SR^{\circ}$; $–(CH_2)_{0–4}C(O)OSi(R^{\circ})_3$; $–(CH_2)_{0–4}OC(O)R^{\circ}$; $–OC(O)(CH_2)_{0–4}SR^{\circ}$, $–SC(S)SR^{\circ}$; $–(CH_2)_{0–4}SC(O)R^{\circ}$; $–(CH_2)_{0–4}C(O)N(R^{\circ})_2$; $–C(S)N(R^{\circ})_2$; $–C(S)SR^{\circ}$; $–SC(S)SR^{\circ}$, $-(CH_2)_{0–4}OC(O)N(R^{\circ})_2$; $-C(O)N(OR^{\circ})R^{\circ}$; $–C(O)C(O)R^{\circ}$; $–C(O)CH_2C(O)R^{\circ}$;

–C(NOR°)R°; -$(CH_2)_{0-4}$SSR°; –$(CH_2)_{0-4}$S(O)$_2$R°; –$(CH_2)_{0-4}$S(O)$_2$OR°; –$(CH_2)_{0-4}$OS(O)$_2$R°; –S(O)$_2$N(R°)$_2$; -$(CH_2)_{0-4}$S(O)R°; –N(R°)S(O)$_2$N(R°)$_2$; –N(R°)S(O)$_2$R°; –N(OR°)R°; –C(NH)N(R°)$_2$; –Si(R°)$_3$; –OSi(R°)$_3$; –P(R°)$_2$; –P(OR°)$_2$; –OP(R°)$_2$; –OP(OR°)$_2$; –N(R°)P(R°)$_2$; –B(R°)$_2$; –OB(R°)$_2$; –P(O)(R°)$_2$; –OP(O)(R°)$_2$; –N(R°)P(O)(R°)$_2$; –(C_{1-4} straight or branched alkylene)O–N(R°)$_2$; or –(C_{1-4} straight or branched alkylene)C(O)O–N(R°)$_2$; wherein each R° may be independently substituted as defined below and is independently hydrogen, C_{1-10} (e.g., C_{1-6}, C_{1-5}, C_{1-4}, etc.) aliphatic, C_{1-10} (e.g., C_{1-6}, C_{1-5}, C_{1-4}, etc.) heteroaliphatic having 1-5 (e.g., 1, 2, 3, 4, 5, 1-5, 1-4, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen, sulfur, silicon and phosphorus, C_{6-10} (e.g., C_6, C_{10}, etc.) aryl, 5-10 (e.g., 5-9, 5-6, 5, 6, 9, 10, etc.) membered heteroaryl having 1-4 (e.g., 1, 2, 3, 4, 1-4, 1-3, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen and sulfur, –CH_2–(C_{6-10} (e.g., C_6, C_{10}, etc.) aryl), –O(CH_2)$_{0-1}$(C_{6-10} (e.g., C_6, C_{10}, etc.) aryl), –CH_2–(5-10 (e.g., 5-9, 5-6, 5, 6, 9, 10, etc.) membered heteroaryl having 1-4 (e.g., 1, 2, 3, 4, 1-4, 1-3, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen and sulfur), –O(CH_2)$_{0-1}$(5-10 (e.g., 5-9, 5-6, 5, 6, 9, 10, etc.) membered heteroaryl having 1-4 (e.g., 1, 2, 3, 4, 1-4, 1-3, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen and sulfur), a 3-10 (e.g., 3-6, 5-6, 3, 4, 5, 6, 7, 8, 9, 10, etc.) membered, monocyclic, bicyclic, or polycyclic, saturated, or partially unsaturated ring having 0-5 (e.g., 0, 1, 2, 3, 4, 5, 1-5, 1-4, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen, sulfur, silicon and phosphorus, or, notwithstanding the definition above, two independent occurrences of R°, taken together with their intervening atom(s), form a 3-10 (e.g., 3-6, 5-6, 3, 4, 5, 6, 7, 8, 9, 10, etc.) membered, monocyclic, bicyclic, or polycyclic, saturated, partially unsaturated or aromatic ring (for aromatic ring, 5-10 (e.g., 5-9, 5-6, 5, 6, 9, 10, etc.) membered) having, in addition to the intervening atom(s), 0-5 (e.g., 0, 1, 2, 3, 4, 5, 1-5, 1-4, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen, sulfur, silicon and phosphorus, which may be substituted as defined below.

[0040] Monovalent substituents on R° (or the ring formed by taking two independent occurrences of R° together with their intervening atoms), are independently halogen, –$(CH_2)_{0-2}$R•, –(haloR•), –$(CH_2)_{0-2}$OH, –$(CH_2)_{0-2}$OR•, –$(CH_2)_{0-2}$CH(OR•)$_2$; –O(haloR•), –CN, –N_3, –$(CH_2)_{0-2}$C(O)R•, –$(CH_2)_{0-2}$C(O)OH, –$(CH_2)_{0-2}$C(O)OR•, –$(CH_2)_{0-2}$SR•, –$(CH_2)_{0-2}$SH, –$(CH_2)_{0-2}$$NH_2$, –$(CH_2)_{0-2}$NHR•, –$(CH_2)_{0-2}$NR•$_2$, –$NO_2$, –SiR•$_3$, –OSiR•$_3$, -C(O)SR•, –($C_{1-4}$ straight or branched alkylene)C(O)OR•, or –SSR• wherein each R• is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently selected from C_{1-4} aliphatic, –CH_2Ph, –O(CH_2)$_{0-1}$Ph, or a 3-6 (e.g., 3, 4, 5, 6, 3-5, 5-6, etc.)-membered saturated, partially unsaturated, or aromatic ring (for aromatic ring, 5- or 6-membered) having 0-4 (e.g., 0, 1, 2, 3, 4, 1-4, 1-3, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen, and sulfur. Divalent substituents on a saturated carbon atom of R° are independently =O or =S.

[0041] Divalent substituents are independently the following: =O, =S, =NNR*$_2$, =NNHC(O)R*, =NNHC(O)OR*, =NNHS(O)$_2$R*, =NR*, =NOR*, –O(C(R*$_2$))$_{2-3}$O–, or –S(C(R*$_2$))$_{2-3}$S–, wherein each independent occurrence of R* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 3-6 (e.g., 3, 4, 5, 6, 3-5, 5-6, etc.)-membered saturated, partially unsaturated, or aromatic ring (for aromatic ring, 5- or 6-membered) having 0-4 (e.g., 0, 1, 2, 3, 4, 1-4, 1-3, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen, and sulfur. Divalent substituents

that are bound to vicinal substitutable carbons of an "optionally substituted" group are independently $-O(CR^*_2)_{2-3}O-$, wherein each independent occurrence of R^* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 3-6 (e.g., 3, 4, 5, 6, 3-5, 5-6, etc.)-membered saturated, partially unsaturated, or aromatic ring (for aromatic ring, 5- or 6-membered) having 0-4 (e.g., 0, 1, 2, 3, 4, 1-4, 1-3, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen, and sulfur.

[0042] Substituents on the aliphatic group of R^* are independently halogen, $-R^\bullet$, -(halo$R^\bullet$), $-OH$, $-OR^\bullet$, $-O$(halo$R^\bullet$), $-CN$, $-C(O)OH$, $-C(O)OR^\bullet$, $-NH_2$, $-NHR^\bullet$, $-NR^\bullet_2$, or $-NO_2$, wherein each $R^\bullet$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 3-6 (e.g., 3, 4, 5, 6, 3-5, 5-6, etc.)-membered saturated, partially unsaturated, or aromatic ring (for aromatic ring, 5- or 6-membered) having 0-4 (e.g., 0, 1, 2, 3, 4, 1-4, 1-3, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen, and sulfur.

[0043] Substituents on a substitutable nitrogen are independently $-R^\dagger$, $-NR^\dagger_2$, $-C(O)R^\dagger$, $-C(O)OR^\dagger$, $-C(O)C(O)R^\dagger$, $-C(O)CH_2C(O)R^\dagger$, $-S(O)_2R^\dagger$, $-S(O)_2NR^\dagger_2$, $-C(S)NR^\dagger_2$, $-C(NH)NR^\dagger_2$, or $-N(R^\dagger)S(O)_2R^\dagger$; wherein each $R^\dagger$ is independently hydrogen, C_{1-6} aliphatic which may be substituted as defined below, unsubstituted $-OPh$, or an unsubstituted 3-6 (e.g., 3, 4, 5, 6, 3-5, 5-6, etc.)-membered saturated, partially unsaturated, or aromatic ring (for aromatic ring, 5- or 6-membered) having 0-4 (e.g., 0, 1, 2, 3, 4, 1-4, 1-3, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen, and sulfur, or, notwithstanding the definition above, two independent occurrences of $R^\dagger$, taken together with their intervening atom(s) form an unsubstituted 3-12 (e.g., 3-10, 3-6, 5-10, 5-6, 3, 4, 5, 6, 7, 8, 9, 10, etc.) membered saturated, partially unsaturated, or aryl mono- or bicyclic ring having 0-4 (e.g., 0, 1, 2, 3, 4, 1-4, 1-3, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen, and sulfur.

[0044] Substituents on the aliphatic group of $R^\bullet$ are independently halogen, $-R^\bullet$, -(halo$R^\bullet$), $-OH$, $-OR^\bullet$, $-O$(halo$R^\bullet$), $-CN$, $-C(O)OH$, $-C(O)OR^\bullet$, $-NH_2$, $-NHR^\bullet$, $-NR^\bullet_2$, or $-NO_2$, wherein each $R^\bullet$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, $-CH_2Ph$, $-O(CH_2)_{0-1}Ph$, or a 3-6 (e.g., 3, 4, 5, 6, 3-5, 5-6, etc.)-membered saturated, partially unsaturated, or aromatic ring (for aromatic ring, 5- or 6-membered) having 0-4 (e.g., 0, 1, 2, 3, 4, 1-4, 1-3, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen, and sulfur.

[0045] Pharmaceutically acceptable salt: 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 humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge et al., describe pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 1977, 66, 1–19, incorporated herein by reference. Pharmaceutically acceptable salts 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 perchloric acid or with organic acids such as acetic acid, oxalic 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, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxyl-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2–naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3–phenylpropionate, phosphate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p–toluenesulfonate, undecanoate, valerate salts, and the like.

[0046] 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, 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.

[0047] Unless otherwise stated, structures depicted herein are meant to include all isomeric (e.g., enantiomeric, diastereomeric, and geometric (or conformational)) forms of the structures; for example, the R and S configurations for each asymmetric center, Z and E double bond isomers, etc. Therefore, unless otherwise stated, single stereochemical isomers as well as enantiomeric, diastereomeric, and geometric (or conformational) mixtures of the present compounds are within the scope of the present disclosure. Unless otherwise stated, all tautomeric forms are within the scope of the disclosure. As those skilled in the art appreciate, compounds and elements may exist in various isotopic forms. Unless otherwise stated, the present disclosure includes compounds that differ only in the presence of one or more isotopes at one or more positions, and compositions that are isotopically enriched at one or more positions of one or more compounds. For example, unless otherwise stated, in a compound H may be ^{1}H, ^{2}H or ^{3}H, and C may be ^{12}C, ^{13}C or ^{14}C. In some embodiments, compounds with certain isotopes, or compositions enriched for certain isotopes, are useful, for example, as analytical tools, as probes in biological assays, or as therapeutic agents in accordance with the present disclosure. In some embodiments, compounds of this disclosure comprise one or more deuterium atoms. In some embodiments, provided agent, compounds, conjugates, etc. are in salt forms, e.g., pharmaceutically acceptable salt forms.

[0048] In some embodiments, combinations of substituents and variables envisioned by this disclosure are only those that result in the formation of stable compounds, wherein "stable" refers to compounds which possess stability sufficient to allow manufacture and which maintains the integrity of the compound for a sufficient period of time to be useful for the purposes detailed herein (e.g., therapeutic or prophylactic administration to a subject).

Other Definitions

[0049] About: As used herein, the term "about," as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In some embodiments, the term "about" refers to a

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

[0050] Affinity matured: As used herein, the term "affinity matured" refers to an antibody with one or more alterations in one or more CDRs thereof, which result in an improvement in affinity of an antibody for an antigen, compared to a parent antibody that does not possess those one or more alterations. In some embodiments, affinity matured antibodies will have nanomolar or even picomolar affinities for a target antigen. Affinity matured antibodies may be produced by any of a variety of procedures known in the art. Affinity maturation by VH and VL domain shuffling is described in Marks et al., BioTechnology 10:779-783 (1992). Random mutagenesis of CDR and/or framework residues is described in: Barbas et al. Proc. Nat. Acad. Sci. U.S.A 91:3809-3813 (1994); Schier et al., Gene 169: 147-155 (1995); Yelton et al., J. Immunol. 155: 1994-2004 (1995); Jackson et al., J. Immunol. 154(7):3310-9 (1995); and Hawkins et al., J. Mol. Biol. 226:889-896 (1992).

[0051] Agent: As used herein, the term "agent" refers to a biological entity and/or compound including, for example, an antibody or antigen-binding fragment thereof, an organic molecule (e.g., a small molecule), a peptide (e.g., a fusion protein), an aptamer, a nucleic acid, a chimeric antigen receptor, a glycoprotein, a saccharide, a lipid, a growth factor, an enzyme, a synthetic molecule, a carbohydrate, a lipid, a hormone, a polymer, or a derivative, variation, complex, or any combination thereof. In appropriate circumstances, as will be clear from context to those skilled in the art, the term may be utilized to refer to an entity that is or comprises a cell or organism, or a fraction, extract, or component thereof. Alternatively or additionally, as context will make clear, the term may be used to refer to a natural product. In some instances, again as will be clear from context, the term may be used to refer to one or more entities that is man-made in that it is designed, engineered, and/or produced through human action and/or is not found in nature. In some embodiments, an agent may be utilized in isolated or pure form. In some embodiments, an agent may be utilized in crude form. In some embodiments, agents are provided as collections or libraries, which may be screened to identify or characterize active agents within them. An agent may bind any cell moiety, such as a receptor, an antigenic determinant, or other binding site present on a target or target cell. Various agents are useful in the compositions and methods described herein.

[0052] Antibody: As used herein, the term "antibody" refers to a polypeptide that includes canonical immunoglobulin sequence elements sufficient to confer specific binding to a particular target antigen. As is known in the art, intact antibodies as produced in nature are approximately 150 kD tetrameric agents comprising two identical heavy chain polypeptides (about 50 kD each) and two identical light chain polypeptides (about 25 kD each) that associate with each other into what is commonly referred to as a "Y-shaped" structure. Each heavy chain comprises at least four domains (each about 110 amino acids long) – an amino-terminal variable (VH) domain (located at the tips of the Y structure), followed by three constant domains: CH1, CH2, and the carboxy-terminal CH3 (located at the base of the Y's stem). A

short region, known as the "switch," connects the heavy chain variable and constant regions. The "hinge" connects CH2 and CH3 domains to the rest of the antibody. Two disulfide bonds in this hinge region connect the two heavy chain polypeptides to one another in an intact antibody. Each light chain comprises two domains – an amino-terminal variable (VL) domain, followed by a carboxy-terminal constant (CL) domain, separated from one another by another "switch". Intact antibody tetramers comprise two heavy chain-light chain dimers in which the heavy and light chains are linked to one another by a single disulfide bond; two other disulfide bonds connect the heavy chain hinge regions to one another so that the dimers are connected to one another and a tetramer is formed. Naturally-produced antibodies are also glycosylated, typically on the CH2 domain. Each domain in a natural antibody has a structure characterized by an "immunoglobulin fold" formed from two beta sheets (e.g., 3-, 4-, or 5-stranded sheets) packed against each other in a compressed antiparallel beta barrel. Each variable domain contains three hypervariable loops known as "complementarity determining regions" (CDR1, CDR2, and CDR3) and four somewhat invariant "framework" regions (FR1, FR2, FR3, and FR4). When natural antibodies fold, the FR regions form the beta sheets that provide the structural framework for the domains, and the CDR loop regions from both the heavy and light chains are brought together in three-dimensional space so that they create a single hypervariable antigen binding site located at the tip of the Y structure. The Fc region of naturally-occurring antibodies binds to elements of the complement system, and also to receptors on effector cells, including, for example, effector cells that mediate cytotoxicity. Affinity and/or other binding attributes of Fc regions for Fc receptors can be modulated through glycosylation or other modification. In some embodiments, antibodies produced and/or utilized in accordance with the present disclosure include glycosylated Fc domains, such as Fc domains with modified or engineered glycosylation. In some embodiments, any polypeptide or complex of polypeptides that includes sufficient immunoglobulin domain sequences as found in natural antibodies can be referred to and/or used as an "antibody", whether such polypeptide is naturally produced (e.g., generated by an organism reacting to an antigen) or produced by recombinant engineering, chemical synthesis, or other artificial system or methodology. In some embodiments, an antibody is polyclonal. In some embodiments, an antibody is monoclonal. In some embodiments, an antibody has constant region sequences characteristic of mouse, rabbit, primate, or human antibodies. In some embodiments, antibody sequence elements are humanized, primatized, or chimeric as is known in the art. Moreover, the term "antibody," as used herein, can refer in appropriate embodiments (unless otherwise stated or clear from context) to any of the art-known or developed constructs or formats for utilizing antibody structural and functional features in alternative presentation. For example, in some embodiments, an antibody utilized in accordance with provided technologies is in a format selected from, but not limited to, intact IgA, IgG, IgE or IgM antibodies; bispecific or multi- specific antibodies (e.g., Zybodies®, etc); and/or antibody fragments (preferably antibody fragments that exhibit desired antigen-binding activity). An antibody described herein can be an immunoglobulin, heavy chain antibody, light chain antibody, LRR-based antibody, or other protein scaffold with antibody-like properties, as well as any other immunological binding moiety known in the art, e.g., a Fab, Fab', Fab'2, Fab2, Fab3, F(ab')2 , Fd, Fv, Feb, scFv, SMIP, antibody, diabody, triabody,

tetrabody, minibody, maxibody, tandab, DVD, BiTe, TandAb, or any combination thereof. The subunit structures and three-dimensional configurations of different classes of antibodies are known in the art. In some embodiments, an antibody may lack a covalent modification (e.g., attachment of a glycan) that it would have if produced naturally. In some embodiments, an antibody may contain a covalent modification (e.g., attachment of a glycan, a payload (e.g., a detectable moiety, a therapeutic moiety, a catalytic moiety, etc), or other pendant group (e.g., poly-ethylene glycol, etc.).

[0053] Antibody agent: As used herein, the term "antibody agent" refers to an agent that specifically binds to a particular antigen. In some embodiments, the term encompasses any polypeptide or polypeptide complex that includes immunoglobulin structural elements sufficient to confer specific binding. Exemplary antibody agents include, but are not limited to monoclonal antibodies or polyclonal antibodies. In some embodiments, an antibody agent may include one or more constant region sequences that are characteristic of mouse, rabbit, primate, or human antibodies. In some embodiments, an antibody agent may include one or more sequence elements are humanized, primatized, or chimeric, as is known in the art. In many embodiments, the term "antibody agent" is used to refer to one or more of the art-known or developed constructs or formats for utilizing antibody structural and functional features in alternative presentation. For example, an antibody agent utilized in accordance with provided technologies is in a format including, but not limited to, intact IgA, IgG, IgE or IgM antibodies; bi- specific or multi-specific antibodies (e.g., Zybodies®, etc); antibody fragments such as Fab fragments, Fab' fragments, F(ab')2 fragments, Fd' fragments, Fd fragments, and isolated CDRs or sets thereof; single chain Fvs; polypeptide-Fc fusions; single domain antibodies (e.g., shark single domain antibodies such as IgNAR or fragments thereof); camelid antibodies; masked antibodies (e.g., Probodies®); Small Modular ImmunoPharmaceuticals ("SMIPs™"); single chain or Tandem diabodies (TandAb®); VHHs; Anticalins®; Nanobodies® minibodies; BiTE®s; ankyrin repeat proteins or DARPINs®; Avimers®; DARTs; TCR-like antibodies;, Adnectins®; Affilins®; Trans-bodies®; Affibodies®; TrimerX®; MicroProteins; Fynomers®, Centyrins®; or KALBITOR®s. In some embodiments, an antibody lacks a covalent modification (e.g., attachment of a glycan) that it would have if produced naturally. In some embodiments, an antibody contains a covalent modification (e.g., attachment of a glycan, a payload, e.g., a detectable moiety, a therapeutic moiety, or a catalytic moiety), or other pendant group (e.g., poly-ethylene glycol). In many embodiments, an antibody agent is or comprises a polypeptide whose amino acid sequence includes one or more structural elements recognized by those skilled in the art as a complementarity determining region (CDR); in some embodiments an antibody agent is or comprises a polypeptide whose amino acid sequence includes at least one CDR (e.g., at least one heavy chain CDR and/or at least one light chain CDR) that is substantially identical to one found in a reference antibody. In some embodiments, an included CDR substantially identical to a reference CDR in that it is either identical in sequence or contains between 1-5 amino acid substitutions as compared with the reference CDR. In some embodiments an included CDR is substantially identical to a reference CDR by at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the reference CDR. In some embodiments, an included CDR is substantially identical to a

reference CDR by at least 96%, 96%, 97%, 98%, 99%, or 100% sequence identity with the reference CDR. In some embodiments, an included CDR is substantially identical to a reference CDR in that at least one amino acid within the included CDR is deleted, added, or substituted as compared with the reference CDR, but the included CDR has an amino acid sequence that is otherwise identical with that of the reference CDR. In some embodiments an included CDR is substantially identical to a reference CDR in that 1-5 amino acids within the included CDR are deleted, added, or substituted as compared with the reference CDR, but the included CDR has an amino acid sequence that is otherwise identical to the reference CDR. In some embodiments, an included CDR is substantially identical to a reference CDR in that at least one amino acid within the included CDR is substituted as compared with the reference CDR, but the included CDR has an amino acid sequence that is otherwise identical with that of the reference CDR. In some embodiments, an included CDR is substantially identical to a reference CDR in that 1-5 amino acids within the included CDR are deleted, added, or substituted as compared with the reference CDR, but the included CDR has an amino acid sequence that is otherwise identical to the reference CDR. In some embodiments, an antibody agent is or comprises a polypeptide whose amino acid sequence includes structural elements recognized by those skilled in the art as an immunoglobulin variable domain. In some embodiments, an antibody agent is a polypeptide protein having a binding domain that is homologous or largely homologous to an immunoglobulin-binding domain.

[0054] Antibody heavy chain: As used herein, the term "antibody heavy chain" refers to the larger of the two types of polypeptide chains present in all antibodies in their naturally occurring conformations.

[0055] Antibody light chain: As used herein, the term "antibody light chain" refers to the smaller of the two types of polypeptide chains present in all antibodies in their naturally occurring conformations.

[0056] Antigen: As used herein, the term "antigen" or "Ag" refers to a molecule that is capable of provoking an immune response. This immune response may involve either antibody production, the activation of specific immunologically-competent cells, or both. A skilled artisan will understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen. Furthermore, antigens can be derived from recombinant or genomic DNA. A skilled artisan will understand that any DNA that comprises a nucleotide sequence or a partial nucleotide sequence encoding a protein that elicits an immune response encodes an "antigen" as that term is used herein. Furthermore, one skilled in the art will understand that an antigen need not be encoded solely by a full length nucleotide sequence of a gene. It is readily apparent that provided technologies include, but is not limited to, the use of partial nucleotide sequences of more than one gene and that these nucleotide sequences are arranged in various combinations to elicit the desired immune response. Moreover, a skilled artisan will understand that an antigen need not be encoded by a "gene" at all. It is readily apparent that an antigen can be generated synthesized or can be derived from a biological sample. Such a biological sample can include, but is not limited to a tissue sample, a tumor sample, a cell, or a biological fluid.

[0057] Antigen-binding fragment: As used herein, the term "antigen-binding fragment" refers to a portion of an intact antibody that binds the antigen to which the intact antibody binds. An antigen-binding fragment of an antibody includes any naturally occurring, enzymatically obtainable, synthetic, or

genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Exemplary antibody fragments include, but are not limited to, Fv, Fab, Fab', Fab'-SH, F(ab')2; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv, VHH, camelid, or VH or VL domains only); or multispecific antibodies formed from antibody fragments. In some embodiments, the antigen-binding fragments of the antibodies described herein are scFvs. In some embodiments, the antigen-binding fragments of the antibodies described herein are VHH domains only. As with full antibody molecules, antigen-binding fragments may be mono-specific or multispecific (e.g., bispecific). A multispecific antigen-binding fragment of an antibody may comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope of the same antigen. An antigen-binding fragment may be produced by any means. For example, in some embodiments, an antigen-binding fragment is enzymatically or chemically produced by fragmentation of an intact antibody or antibody agent. Alternatively, in some embodiments, an antigen-binding fragment is recombinantly produced. In some embodiments, an antigen-binding fragment is wholly or partially synthetically produced. In some embodiments, an antigen-binding fragment has a length of at least about 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 amino acids or more.

[0058] Antibody-Dependent Cellular Cytotoxicity: As used herein, the term "antibody-dependent cellular cytotoxicity" or "ADCC" refers to a phenomenon in which target cells bound by antibody are killed by immune effector cells. Without wishing to be bound by theory, ADCC is typically understood to involve Fc receptor (FcR)-bearing effector cells recognizing and subsequently killing antibody-coated target cells (e.g., cells that express on their surface specific antigens to which an antibody is bound). Effector cells that mediate ADCC include immune cells including, but not limited to, natural killer (NK) cells, macrophage, neutrophils, and eosinophils.

[0059] Aptamer: As used herein, the term "aptamer" refers to a macromolecule composed of nucleic acid (e.g., RNA, DNA) that binds tightly to a specific molecular target (e.g., an umbrella topology glycan). A particular aptamer may be described by a linear nucleotide sequence and is typically about 15-60 nucleotides in length. Without wishing to be bound by any theory, it is contemplated that the chain of nucleotides in an aptamer form intramolecular interactions that fold the molecule into a complex three-dimensional shape, and this three-dimensional shape allows the aptamer to bind tightly to the surface of its target molecule. Given the extraordinary diversity of molecular shapes that exist within the universe of all possible nucleotide sequences, aptamers may be obtained for a wide array of molecular targets, including proteins and small molecules. In addition to high specificity, aptamers typically have very high affinities for their targets (e.g., affinities in the picomolar to low nanomolar range for proteins). In many embodiments, aptamers are chemically stable and can be boiled or frozen without loss of activity. Because they are synthetic molecules, aptamers are amenable to a variety of modifications, which can optimize their function for particular applications. For example, aptamers can be modified to dramatically reduce their sensitivity to degradation by enzymes in the blood for use in in vivo applications. In addition, aptamers can be modified to alter their biodistribution or plasma residence time.

[0060] Associated: Two events or entities are "associated" with one another, as that term is used

herein, if the presence, level, degree, type and/or form of one is correlated with that of the other. For example, a particular entity (e.g., polypeptide, genetic signature, metabolite, or microbe) is considered to be associated with a particular disease, disorder, or condition, if its presence, level and/or form correlates with incidence of and/or susceptibility to the disease, disorder, or condition (e.g., across a relevant population). In some embodiments, two or more entities are physically "associated" with one another if they interact, directly or indirectly, so that they are and/or remain in physical proximity with one another. In some embodiments, two or more entities physically associated with one another are covalently linked to one another. In some embodiments, two or more entities physically associated with one another are not covalently linked to one another but are non-covalently associated, for example, by means of hydrogen bonds, van der Waals interaction, hydrophobic interactions, magnetism, and combinations thereof.

[0061] Binding: As used herein, the term "binding" refers to a non-covalent association between or among two or more entities. "Direct" binding involves physical contact between entities or moieties. Indirect binding involves physical interaction by way of physical contact with one or more intermediate entities. Binding between two or more entities can typically be assessed in any of a variety of contexts – including where interacting entities or moieties are studied in isolation or in the context of more complex systems (e.g., while covalently or otherwise associated with a carrier entity and/or in a biological system or cell).

[0062] Cancer: As used herein, the terms "cancer," "malignancy," "neoplasm," "tumor," and "carcinoma," refer to cells that exhibit relatively abnormal, uncontrolled, and/or autonomous growth, so that they exhibit an aberrant growth phenotype characterized by a significant loss of control of cell proliferation. Cancer cells can spread locally or through the bloodstream and lymphatic system to other parts of the body. In some embodiments, a tumor is or comprises cells that are precancerous (e.g., benign), malignant, pre-metastatic, metastatic, and/or non-metastatic. In some embodiments, cancer is or comprises a solid tumor. In some embodiments cancer is or comprises a hematologic tumor. Examples of various cancers are described herein and include, but are not limited to, hematopoietic cancers including leukemias, lymphomas (Hodgkin's and non-Hodgkin's), myelomas and myeloproliferative disorders; sarcomas, melanomas, adenomas, carcinomas of solid tissue, squamous cell carcinomas of the mouth, throat, larynx, and lung, liver cancer, genitourinary cancers, such as prostate, cervical, bladder, uterine, and endometrial cancer and renal cell carcinomas, bone cancer, pancreatic cancer, skin cancer, cutaneous or intraocular melanoma, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, head and neck cancers, breast cancer, gastro-intestinal cancers, nervous system cancers, or benign lesions, such as papillomas, as well as several other types including those as described elsewhere herein.

[0063] Carrier: as used herein, "carrier" refers to a diluent, adjuvant, excipient, and/or vehicle with which a composition is administered. In some exemplary embodiments, carriers include sterile liquids, such as, for example, water and oils, including oils of petroleum, animal, vegetable or synthetic origin, such as, for example, peanut oil, soybean oil, mineral oil, sesame oil and the like. In some embodiments, carriers are or include one or more solid components.

[0064] CDR: As used herein, "CDR" refers to a complementarity determining region within an antibody variable region. There are three CDRs in each of the variable regions of the heavy chain and the light chain, which are designated CDR1, CDR2 and CDR3, for each of the variable regions. A "set of CDRs" or "CDR set" refers to a group of three or six CDRs that occur in either a single variable region capable of binding the antigen or the CDRs of cognate heavy and light chain variable regions capable of binding the antigen. The exact definitional CDR boundaries and lengths are subject to different classification and numbering systems. Certain systems have been established in the art for defining CDR boundaries (e.g., Kabat, IMGT, Chothia, or a combination thereof). CDRs may therefore be referred to by Kabat, Chothia, IMGT, or any other boundary definitions known in the art. Despite differing boundaries, each of these systems has some degree of overlap in what constitutes the "hypervariable regions" within the variable sequences. CDR definitions according to these systems may therefore differ in length and boundary areas with respect to the adjacent framework region (see, e.g., Kabat et al., in "Sequences of Proteins of Immunological Interest," 5th Edition, U.S. Department of Health and Human Services, 1992; Chothia et al. (1987) J. Mol. Biol. 196, 901; and MacCallum et al., J. Mol. Biol. (1996) 262, 732, each of which is incorporated by reference in its entirety). Those skilled in the art appreciate the differences between and among these systems and are capable of understanding CDR boundaries to the extent required to understand and to practice the claims and disclosure herein.

[0065] Chemotherapeutic Agent: The term "chemotherapeutic agent," as used herein has its art-understood meaning referring to one or more pro-apoptotic, cytostatic and/or cytotoxic agents, for example specifically including agents utilized and/or recommended for use in treating one or more diseases, disorders or conditions associated with undesirable cell proliferation. In many embodiments, chemotherapeutic agents are useful in the treatment of cancer. In some embodiments, a chemotherapeutic agent may be or comprise one or more alkylating agents, one or more anthracyclines, one or more cytoskeletal disruptors (e.g. microtubule targeting agents such as taxanes, maytansine and analogs thereof, of), one or more epothilones, one or more histone deacetylase inhibitors HDACs), one or more topoisomerase inhibitors (e.g., inhibitors of topoisomerase I and/or topoisomerase II), one or more kinase inhibitors, one or more nucleotide analogs or nucleotide precursor analogs, one or more peptide antibiotics, one or more platinum-based agents, one or more retinoids, one or more vinca alkaloids, and/or one or more analogs of one or more of the following (i.e., that share a relevant anti-proliferative activity). In some embodiments, a chemotherapeutic agent may be or comprise one or more of Actinomycin, All-trans retinoic acid, an Auristatin, Azacitidine, Azathioprine, Bleomycin, Bortezomib, Carboplatin, Capecitabine, Cisplatin, Chlorambucil, Cyclophosphamide, Curcumin, Cytarabine, Daunorubicin, Docetaxel, Doxifluridine, Doxorubicin, Epirubicin, Epothilone, Etoposide, Fluorouracil, Gemcitabine, Hydroxyurea, Idarubicin, Imatinib, Irinotecan, Maytansine and/or analogs thereof (e.g. DM1) Mechlorethamine, Mercaptopurine, Methotrexate, Mitoxantrone, a Maytansinoid, Oxaliplatin, Paclitaxel, Pemetrexed, Teniposide, Tioguanine, Topotecan, Valrubicin, Vinblastine, Vincristine, Vindesine, Vinorelbine, or combinations thereof. In some embodiments, a chemotherapeutic agent may be utilized in the context of an antibody-drug conjugate. In some embodiments, a chemotherapeutic agent is an

antibody-drug conjugate comprising: hLL1-doxorubicin, hRS7-SN-38, hMN-14-SN-38, hLL2-SN-38, hA20-SN-38, hPAM4-SN-38, hLL1-SN-38, hRS7-Pro-2-P-Dox, hMN-14-Pro-2-P-Dox, hLL2-Pro-2-P-Dox, hA20-Pro-2-P-Dox, hPAM4-Pro-2-P-Dox, hLL1-Pro-2-P-Dox, P4/D10-doxorubicin, gemtuzumab ozogamicin, brentuximab vedotin, trastuzumab emtansine, inotuzumab ozogamicin, glembatumomab vedotin, SAR3419, SAR566658, BIIB015, BT062, SGN-75, SGN-CD19A, AMG-172, AMG-595, BAY-94-9343, ASG-5ME, ASG-22ME, ASG-16M8F, MDX-1203, MLN-0264, anti-PSMA ADC, RG-7450, RG-7458, RG-7593, RG-7596, RG-7598, RG-7599, RG-7600, RG-7636, ABT-414, IMGN-853, IMGN-529, vorsetuzumab mafodotin, and/or lorvotuzumab mertansine.

[0066] Chimeric antibody: as used herein, refers to an antibody whose amino acid sequence includes VH and VL region sequences that are found in a first species and constant region sequences that are found in a second species, different from the first species. In many embodiments, a chimeric antibody has murine VH and VL regions linked to human constant regions. In some embodiments, an antibody with human VH and VL regions linked to non-human constant regions (e.g., a mouse constant region) is referred to as a "reverse chimeric antibody."

[0067] Composition: Those skilled in the art will appreciate that the term "composition" may be used to refer to a discrete physical entity that comprises one or more specified components. In general, unless otherwise specified, a composition may be of any form – e.g., gas, gel, liquid, or solid.

[0068] Comprising: A composition or method described herein as "comprising" one or more named elements or steps is open-ended, meaning that the named elements or steps are essential, but other elements or steps may be added within the scope of the composition or method. To avoid prolixity, it is also understood that any composition or method described as "comprising" (or which "comprises") one or more named elements or steps also describes the corresponding, more limited composition or method "consisting essentially of" (or which "consists essentially of") the same named elements or steps, meaning that the composition or method includes the named essential elements or steps and may also include additional elements or steps that do not materially affect the basic and novel characteristic(s) of the composition or method. It is also understood that any composition or method described herein as "comprising" or "consisting essentially of" one or more named elements or steps also describes the corresponding, more limited, and closed-ended composition or method "consisting of" (or "consists of") the named elements or steps to the exclusion of any other unnamed element or step. In any composition or method disclosed herein, known or disclosed equivalents of any named essential element or step may be substituted for that element or step.

[0069] Conservative sequence modifications: As used herein, the term "conservative sequence modifications" refers to amino acid modifications that do not significantly affect or alter the binding characteristics of an antibody or antigen-binding fragment thereof containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions, and deletions. Modifications can be introduced into an antibody compatible with various embodiments by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions are ones in which an amino acid residue is replaced with an amino acid residue having a

similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within the CDR regions of an antibody can be replaced with other amino acid residues from the same side chain family and the altered antibody can be tested for the ability to bind antigens using the functional assays described herein.

[0070] Combination therapy: The term "combination therapy", as used herein, refers to those situations in which two or more different therapeutic agents are administered in overlapping regimens so that the subject is simultaneously exposed to both agents. When used in combination therapy, two or more different therapeutic agents may be administered simultaneously or separately. This administration in combination can include simultaneous administration of the two or more therapeutic agents in the same dosage form, simultaneous administration in separate dosage forms, and separate administration. That is, two or more therapeutic agents can be formulated together in the same dosage form and administered simultaneously. Alternatively, two or more therapeutic agents can be simultaneously administered, wherein the agents are present in separate formulations. In another alternative, a first therapeutic agent can be administered followed by one or more additional therapeutic agents. In the separate administration protocol, two or more therapeutic agents may be administered a few minutes apart, or a few hours apart, a few days apart, or a few weeks apart. In some embodiments, two or more therapeutic agents may be administered within hours (e.g., less than about 1 hour, about 2 hours, about 3 hours, about 4 hours, or about 5 hours) apart.

[0071] Effective amount: As used herein, an "effective amount" refers to a dose that is adequate to prevent or treat at least one sign and/or symptom of a disease, disorder or condition (e.g., cancer) in an individual. Amounts effective for a therapeutic or prophylactic use will depend on, for example, the stage and severity of the disease, disorder or condition being treated, the age, weight, and general state of health of the patient, and the judgment of the prescribing physician. The size of the dose will also be determined by the active selected, method of administration, timing and frequency of administration, the existence, nature, and extent of any adverse side-effects that might accompany the administration of a particular active, and the desired physiological effect. It will be appreciated by one of skill in the art that various diseases or disorders could require prolonged treatment involving multiple administrations. For purposes of the disclosure, the amount or dose of a therapeutic agent (e.g., at least one HHLA2 binding agent described herein) administered should be sufficient to effect a therapeutic or prophylactic response in a subject over a reasonable time frame (e.g., reduction or other lessening of severity or duration of at least one sign or symptom). For example, the dose should be sufficient to detect, treat, or prevent cancer in a period of from about 2 hours or longer, e.g., about 12 to about 24 or more hours, from the time of administration. In some embodiments, the time period is even longer. The dose will be determined by the

efficacy of one or more particular therapeutic agents and condition of a subject (e.g., a human) as well as body weight of a subject (e.g., a human) to be treated.

[0072] Encoding: As used herein, the term "encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (e.g., rRNA, tRNA or mRNA) or a defined sequence of amino acids and biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

[0073] Engineered: As used herein, the term "engineered" refers to the aspect of having been manipulated by the hand of man. For example, a polynucleotide is considered to be "engineered" when two or more sequences, that are not linked together in that order in nature, are manipulated by the hand of man to be directly linked to one another in the engineered polynucleotide. For example, in some embodiments, an engineered polynucleotide comprises or is a regulatory sequence that is found in nature in operative association with a first coding sequence but not in operative association with a second coding sequence, is linked by the hand of man so that it is operatively associated with the second coding sequence. Comparably, a cell or organism is considered to be "engineered" if it has been manipulated so that its genetic information is altered (e.g., new genetic material not previously present has been introduced, for example by transformation, mating, somatic hybridization, transfection, transduction, or other mechanism, or previously present genetic material is altered or removed, for example by substitution or deletion mutation, or by mating protocols). In some embodiments, engineered antibodies or antigen-binding fragments thereof (e.g., engineered monoclonal antibodies or antigen-binding fragments thereof) include VH and/or VL region sequences from a reference antibody raised in a non-human species (e.g., a mouse) and modifications in those sequences relative to a reference antibody intended to render them more "human-like" or more similar to human germline variable sequences.

[0074] As is common practice and is understood by those in the art, progeny of an engineered polynucleotide or cell are typically still referred to as "engineered" even though the actual manipulation was performed on a prior entity.

[0075] Epitope: As used herein, the term "epitope" refers to any moiety that is specifically recognized by an immunoglobulin (e.g., antibody or receptor) binding component. In some embodiments, an epitope is comprised of a plurality of chemical atoms or groups on an antigen. In some embodiments, such chemical atoms or groups are surface-exposed when the antigen adopts a relevant three-dimensional conformation. In some embodiments, such chemical atoms or groups are physically near to each other in space when the antigen adopts such a conformation. In some embodiments, at least some such chemical atoms are groups are physically separated from one another when the antigen adopts an alternative conformation (e.g., is linearized).

[0076] Expression: As used herein, the term "expression" of a nucleic acid sequence refers to generation of any gene product from a nucleic acid sequence (e.g., a nucleic acid sequence encoding an anti-HHLA2 antibody or antigen-binding fragment thereof described herein). In some embodiments, a gene product can be a transcript. In some embodiments, a gene product can be a polypeptide. In some embodiments, expression of a nucleic acid sequence involves one or more of the following: (1) production of an RNA template from a DNA sequence (e.g., by transcription); (2) processing of an RNA transcript (e.g., by splicing, editing, 5' cap formation, and/or 3' end formation); (3) translation of an RNA into a polypeptide or protein; and/or (4) post-translational modification of a polypeptide or protein.

[0077] Fragment: As used herein, the term "fragment" refers to a structure that includes a discrete portion of the whole, but lacks one or more moieties found in the whole structure. In some embodiments, a fragment consists of such a discrete portion. In some embodiments, a fragment consists of or comprises a characteristic structural element or moiety found in the whole. In some embodiments, an antigen-binding fragment comprises or consists of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, or more monomeric units (e.g., amino acids) as found in a whole antibody. In some embodiments, an antigen-binding fragment comprises or consists of at least about 5%, 10%, 15%, 20%, 25%, 30%, 25%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more of the monomeric units (e.g., residues) found in a whole antibody. In some embodiments, a nucleotide fragment comprises or consists of at least about 5%, 10%, 15%, 20%, 25%, 30%, 25%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more of the monomeric units (e.g., residues) found in the whole nucleotide.

[0078] Framework region: as used herein, the term "framework region" refers to the sequences of a variable region minus the CDRs. Because a CDR sequence can be determined by different systems, likewise a framework sequence is subject to correspondingly different interpretations. The six CDRs divide the framework regions on the heavy and light chains into four sub-regions (FR1, FR2, FR3, and FR4) on each chain, in which CDR1 is positioned between FR1 and FR2, CDR2 between FR2 and FR3, and CDR3 between FR3 and FR4. Without specifying the particular sub-regions as FR1, FR2, FR3 or FR4, a framework region, as referred by others, represents the combined FRs within the variable region of a single, naturally occurring immunoglobulin chain. As used herein, a FR represents one of the four sub-regions, FR1, for example, represents the first framework region closest to the amino terminal end of the variable region and 5' with respect to CDR1, and FRs represents two or more of the sub-regions constituting a framework region.

[0079] Gene: As used herein, the term "gene" refers to a DNA sequence in a chromosome that codes for a product (e.g., an RNA product and/or a polypeptide product). In some embodiments, a gene includes coding sequence (i.e., sequence that encodes a particular product); in some embodiments, a gene includes non-coding sequence. In some embodiments, a gene may include both coding (e.g., exonic) and non-coding (e.g., intronic) sequences. In some embodiments, a gene may include one or more regulatory

elements that, for example, may control or impact one or more aspects of gene expression (e.g., cell-type-specific expression and/or inducible expression).

[0080] Homology: As used herein, the term "homology" refers to the overall relatedness between polymeric molecules, e.g., between nucleic acids (e.g., DNA and/or RNA) and/or between polypeptides. In some embodiments, polymeric molecules are considered to be "homologous" to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identical. In some embodiments, polymeric molecules are considered to be "homologous" to one another if their sequences are at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% similar (e.g., containing residues with related chemical properties at corresponding positions). As will be understood by those skilled in the art, a variety of algorithms are available that permit comparison of sequences in order to determine their degree of homology, including by permitting gaps of designated length in one sequence relative to another when considering which residues "correspond" to one another in different sequences. Calculation of the percent homology between two nucleic acid sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second nucleic acid sequences for optimal alignment and non-corresponding sequences can be disregarded for comparison purposes). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or substantially 100% of the length of the reference sequence. The nucleotides at corresponding nucleotide positions are then compared. When a position in the first sequence is occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position; when a position in the first sequence is occupied by a similar nucleotide as the corresponding position in the second sequence, then the molecules are similar at that position. The percent homology between the two sequences is a function of the number of identical and similar positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences.

[0081] Host cell: as used herein, the term "host cell" refers to a cell into which exogenous DNA (recombinant or otherwise) has been introduced. Persons of skill upon reading this disclosure will understand that such terms refer not only to the particular subject cell, but also to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term host cell as used herein. In some embodiments, host cells include prokaryotic and eukaryotic cells selected from any of the Kingdoms of life that are suitable for expressing an exogenous DNA (e.g., a recombinant nucleic acid sequence). Exemplary cells include those of prokaryotes and eukaryotes (single-cell or multiple-cell), bacterial cells (e.g., strains of E. coli, Bacillus spp., or Streptomyces spp.), mycobacteria cells, fungal cells, yeast cells (e.g., S. cerevisiae, S. pombe, P. pastoris, or P. methanolica), plant cells, insect cells (e.g., SF-9, SF-21, baculovirus-infected insect cells, or Trichoplusia ni,), non-human animal cells, human cells, or cell fusions (e.g., hybridomas or quadromas).

In some embodiments, the cell comprises or is a human, monkey, ape, hamster, rat, or mouse cell. In some embodiments, the cell is a eukaryotic cell chosen from: CHO (e.g., CHO Kl, DXB-1 1 CHO, Veggie-CHO), COS (e.g., COS-7), retinal cell, Vero, CV1, kidney (e.g., HEK293, 293 EBNA, MSR 293, MDCK, HaK, BHK), HeLa, HepG2, WI38, MRC 5, Colo205, HB 8065, HL-60, (e.g., BHK21), Jurkat, Daudi, A431 (epidermal), CV-1, U937, 3T3, L cell, C127 cell, SP2/0, NS-0, MMT 060562, Sertoli cell, BRL 3 A cell, HT1080 cell, myeloma cell, tumor cell, or a cell line derived from an aforementioned cell. In some embodiments, a cell comprises one or more viral genes.

[0082] Human antibody: as used herein, the term "human antibody" refers to antibodies having variable and constant regions generated (or assembled) from human immunoglobulin sequences. Antibodies or antigen-binding fragments thereof may be considered "human" even though their amino acid sequences include residues or elements not encoded by human germline immunoglobulin sequences (e.g., sequence variations that may have been introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo), such as in one or more CDRs and in particular CDR3.

[0083] Humanized: as used herein, the term "humanized" refers to antibodies or antigen-binding fragments thereof whose amino acid sequence includes VH and/or VL region sequences from a reference antibody raised in a non-human species (e.g., a mouse), but also includes modifications in those sequences relative to the reference antibody intended to render them more "human-like" or more similar to human germline variable sequences. In some embodiments, a humanized antibody or antigen-binding fragment thereof is one that immunospecifically binds to an antigen of interest and has a FR region with substantially the amino acid sequence of a human antibody and a CDR with substantially the amino acid sequence of a non-human antibody. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', $F(ab')_2$, FabC, Fv) in which all or substantially all of the CDR regions correspond to a non-human immunoglobulin (e.g., a donor immunoglobulin) and all or substantially all of the framework regions correspond to a human immunoglobulin consensus sequence. In some embodiments, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin constant region. In some embodiments, a humanized antibody contains both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include a CH1, hinge, CH2, CH3, and, optionally, a CH4 region of a heavy chain constant region. In some embodiments, a humanized antibody only contains a humanized VL region. In some embodiments, a humanized antibody only contains a humanized VH region. In some certain embodiments, a humanized antibody contains humanized VH and VL regions.

[0084] Identity: As used herein, the term "identity" refers to the subunit sequence identity between two polymeric molecules, particularly between two amino acid molecules, such as between two polypeptide molecules. When two amino acid sequences have the same residues at the same positions; e.g., if a position in each of two polypeptide molecules is occupied by an Arginine, then they are identical at that position. The identity or extent to which two amino acid sequences have the same residues at the same positions in an alignment is often expressed as a percentage. The identity between two amino acid sequences is a direct function of the number of matching or identical positions; e.g., if half of the positions

(e.g., five positions in a polymer of 10 amino acids in length) in two sequences are identical, the two sequences are 50% identical; if 90% of the positions (e.g., nine positions in a polymer of 10 amino acids in length) are identical, the two amino acids sequences are 90% identical.

[0085] Immune cell: As used herein, the term "immune cell," refers to a cell that is involved in an immune response, e.g., promotion of an immune response. Examples of immune cells include, but are not limited to, T cells, natural killer (NK) cells, macrophages, monocytes, dendritic cells, neutrophils, eosinophils, mast cells, platelets, large granular lymphocytes, Langerhans' cells, or B-lymphocytes.

[0086] Immune checkpoint: As used herein, the term "immune checkpoint" refers to a group of molecules on the cell surface of CD4+ and/or CD8+ T cells as well as NK cells that fine-tune immune responses by down-modulating or inhibiting an anti-tumor immune response. Immune checkpoint proteins are well-known in the art and include, without limitation, HHLA2, KIR family receptors, CTLA-4, PD-1, VISTA, B7-H2, B7-H3, PD-L1, B7-H4, B7-H6, ICOS, HVEM, PD-L2, CD160, gp49B, PIR-B, TIM-1, TIM-3, TIM-4, LAG-3, GITR, 4-IBB, OX-40, BTLA, SIRPα, CD47, CD48, 2B4 (CD244), B7.1, B7.2, ILT-2, ILT-4, TIGIT, CD226, CD155, CD112, butyrophilins, and A2aR. In some embodiments, NK cells comprise TIGIT, CD226, and/or CD96. The term further encompasses biologically active protein fragment, as well as nucleic acids encoding full-length immune checkpoint proteins and biologically active protein fragments thereof. In some embodiment, the term further encompasses any fragment according to homology descriptions provided herein.

[0087] Immune response: As used herein the term "immune response" refers to a cellular and/or systemic response to an antigen that occurs when lymphocytes identify antigenic molecules as foreign and induce the formation of antibodies and/or activate lymphocytes to remove the antigen. In some embodiments, an immune cell response can include proliferation of an immune effector cell (e.g., a T cell), cytokine production by an immune effector cell (e.g., a T cell), and/or release of cytotoxic granules comprising perforin and/or granzymes by an immune effector cell (e.g., a T cell).

[0088] Immunoglobulin: As used herein, the term "immunoglobulin" or "Ig," refers to a class of proteins that function as antibodies. Antibodies expressed by B cells are sometimes referred to as a BCR (B cell receptor) or antigen receptor. The five members included in this class of proteins are IgA, IgG, IgM, IgD, and IgE. IgA is the primary antibody that is present in body secretions, such as saliva, tears, breast milk, gastrointestinal secretions and mucus secretions of the respiratory and genitourinary tracts. IgG is the most common circulating antibody. IgM is the main immunoglobulin produced in the primary immune response in most subjects. It is the most efficient immunoglobulin in agglutination, complement fixation, and other antibody responses, and is important in defense against bacteria and viruses. IgD is an immunoglobulin that has no known antibody function, but may serve as an antigen receptor. IgE is an immunoglobulin that mediates immediate hypersensitivity by causing release of mediators from mast cells and basophils upon exposure to allergen.

[0089] "Improve," "increase", "inhibit" or "reduce": As used herein, the terms "improve," "increase," "inhibit," "reduce," or grammatical equivalents thereof, indicate values that are relative to a baseline or other reference measurement. In some embodiments, an appropriate reference measurement is

or comprises a measurement in a particular system (e.g., in a single individual) under otherwise comparable conditions absent presence of (e.g., prior to and/or after) a particular agent or treatment, or in presence of an appropriate comparable reference agent. In some embodiments, an appropriate reference measurement is or comprises a measurement in comparable system known or expected to respond in a particular way, in presence of the relevant agent or treatment.

[0090] Isolated: As used herein, the term "isolated" refers to something altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not "isolated," but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is "isolated." An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

[0091] K_D: as used herein, the term "K_D" refers to the dissociation constant of a binding agent (e.g., an antibody or antigen-binding fragment thereof) from a complex with its partner (e.g., the epitope to which the antibody or antigen-binding fragment thereof binds). The term "K_D," as used herein, equals K_{off} divided by K_{on}.

[0092] K_{off}: as used herein, the term "K_{off}" refers to the off rate constant for dissociation of a binding agent (e.g., an antibody or antigen-binding fragment thereof) from a complex with its partner (e.g., the epitope to which the antibody or antigen-binding fragment thereof binds).

[0093] K_{on}: as used herein, the term "K_{on}" refers to the on rate constant for association of a binding agent (e.g., an antibody or antigen-binding fragment thereof) with its partner (e.g., the epitope to which the antibody or antigen-binding fragment thereof binds).

[0094] Modulating: As used herein the term "modulating," refers to mediating a detectable increase or decrease in a level of a response and/or change in nature of a response in a subject compared with a level and/or nature of a response in a subject without a treatment or an untreated subject. The term encompasses perturbing and/or affecting a native signal or response thereby mediating a beneficial therapeutic response in a subject, preferably, a human.

[0095] Monoclonal Antibody: A "monoclonal antibody" or "mAb" refers to an antibody obtained from a population of substantially homogeneous antibodies, such that individual antibodies of the population are identical and/or bind the same epitope, except for possible variant antibodies (e.g., containing naturally occurring mutations or arising during production of a monoclonal), such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen.

[0096] Operably linked: As used herein, the term "operably linked" refers to functional linkage between, for example, a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA

sequences are contiguous and, where necessary to join two protein coding regions, in the same reading frame.

[0097] Pharmaceutically acceptable: As used herein, the term "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0098] Pharmaceutically acceptable carrier: As used herein, the term "pharmaceutically acceptable carrier" means a pharmaceutically-acceptable material, composition, or vehicle, such as a liquid or solid filler, diluent, excipient, or solvent encapsulating material, involved in carrying or transporting the subject compound 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 subject. 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; pH buffered solutions; polyesters, polycarbonates and/or polyanhydrides; and other non-toxic compatible substances employed in pharmaceutical formulations.

[0099] Polynucleotide: As used herein, the term "polynucleotide" refers to a chain of nucleotides. Furthermore, nucleic acids are polymers of nucleotides. Thus, nucleic acids and polynucleotides as used herein are interchangeable. One skilled in the art has the general knowledge that nucleic acids are polynucleotides, which can be hydrolyzed into the monomeric "nucleotides." The monomeric nucleotides can be hydrolyzed into nucleosides. As used herein polynucleotides include, but are not limited to, all nucleic acid sequences which are obtained by any means available in the art, including, without limitation, recombinant means, i.e., the cloning of nucleic acid sequences from a recombinant library or a cell genome, using ordinary cloning technology and PCR™, and the like, and by synthetic means.

[0100] Polypeptide: As used herein, the terms "polypeptide" or "protein," as used interchangeably herein, refer to any polymeric chain of residues (e.g., amino acids) that are typically linked by peptide bonds. In some embodiments, a polypeptide has an amino acid sequence that occurs in nature. In some embodiments, a polypeptide has an amino acid sequence that does not occur in nature. In some embodiments, a polypeptide has an amino acid sequence that is engineered in that it is designed and/or produced through action of the hand of man. A polypeptide may comprise or consist of natural amino acids, non-natural amino acids, or both. A polypeptide may comprise or consist of only natural amino acids or only non-natural amino acids. A polypeptide may comprise D-amino acids, L-amino acids, or

both. A polypeptide may include one or more pendant groups or other modifications, e.g., modifying or attached to one or more amino acid side chains at the N-terminus, at the C-terminus, or both. In some embodiments, such pendant groups or modifications are chosen from acetylation, amidation, lipidation, methylation, or pegylation, including combinations thereof. A polypeptide may be cyclic and/or may comprise a cyclic portion. In some embodiments, a polypeptide is not cyclic and/or does not comprise any cyclic portion. In some embodiments, a polypeptide is linear. A polypeptide may be or comprise a stapled polypeptide. The term "polypeptide" may be appended to a name of a reference polypeptide, activity, or structure; in such instances, it is used herein to refer to polypeptides that share the relevant activity or structure and thus can be considered members of the same class or family of polypeptides. For each such class, the present specification provides and/or those skilled in the art will be aware of exemplary polypeptides within the class whose amino acid sequences and/or functions are known; in some embodiments, such exemplary polypeptides are reference polypeptides for the polypeptide class or family. In some embodiments, a member of a polypeptide class or family shows significant sequence homology or identity with, shares a common sequence motif (e.g., a characteristic sequence element) with, and/or shares a common activity (in some embodiments at a comparable level or within a designated range) with a reference polypeptide of the class. For example, a member polypeptide may have an overall degree of sequence homology or identity with a reference polypeptide that is at least about 30-40% and is often about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more and/or includes at least one region (e.g., a conserved region that may be or comprise a characteristic sequence element) that shows very high sequence identity, often greater than 90% or even 95%, 96%, 97%, 98%, or 99%. Such a conserved region usually encompasses at least 3-4 and often up to 20 or more amino acids; in some embodiments, a conserved region encompasses at least one stretch of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more contiguous amino acids. A useful polypeptide may comprise or consist of a fragment of a parent polypeptide. A useful polypeptide may comprise or consist of a plurality of fragments, each of which is found in the same parent polypeptide in a different spatial arrangement relative to one another than is found in the polypeptide of interest (e.g., fragments that are directly linked in the parent may be spatially separated in the polypeptide of interest or vice versa, and/or fragments may be present in a different order in the polypeptide of interest than in the parent) so that the polypeptide of interest is a derivative of its parent polypeptide.

[0101] Single chain antibodies: As used herein, the term "single chain antibodies" refers to antibodies formed by recombinant DNA techniques in which immunoglobulin heavy and light chain fragments are linked to the Fv region via an engineered span of amino acids. Various methods of generating single chain antibodies are known, including those described in U.S. Pat. No. 4,694,778; Bird (1988) Science 242:423-442; Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883; Ward et al. (1989) Nature 334:54454; and Skerra et al. (1988) Science 242:1038-1041.

[0102] Recombinant: as used herein, is intended to refer to polypeptides that are designed, engineered, prepared, expressed, created, manufactured, and/or or isolated by recombinant means, such as polypeptides expressed using a recombinant expression vector transfected into a host cell, polypeptides

isolated from a recombinant, combinatorial human polypeptide library (see, e.g., Hoogenboom, TIB Tech 15:62, 1997; Azzazy Clin. Biochem. 35:425, 2002; Gavilondo BioTechniques 29:128, 2002; Hoogenboom Immunology Today 21:371, 2000), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see, e.g., Taylor Nuc. Acids Res. 20:6287, 1992; Little Immunology Today 12:364, 2000; Kellermann Curr. Opin. Biotechnol 13:593, 2002; Murphy Proc. Natl Acad Sci USA 111:5153, 2104) or polypeptides prepared, expressed, created or isolated by any other means that involves splicing selected sequence elements to one another. In some embodiments, one or more of such selected sequence elements is found in nature. In some embodiments, one or more of such selected sequence elements is designed in silico. In some embodiments, one or more such selected sequence elements results from mutagenesis (e.g., in vivo or in vitro) of a known sequence element, e.g., from a natural or synthetic source. For example, in some embodiments, a recombinant antibody polypeptide is comprised of sequences found in the germline of a source organism of interest (e.g., human, mouse, etc.). In some embodiments, a recombinant antibody has an amino acid sequence that resulted from mutagenesis (e.g., in vitro or in vivo, for example in a transgenic animal), so that the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while originating from and related to germline VH and VL sequences, do not naturally exist within the germline antibody repertoire in vivo.

[0103] Subject: As used herein, the term "subject" refers to an organism, for example, a mammal (e.g., a human, a non-human mammal, a non-human primate, a primate, a laboratory animal, a mouse, a rat, a hamster, a gerbil, a cat, or a dog). In some embodiments, a human subject is an adult, adolescent, or pediatric subject. In some embodiments, a subject is suffering from a disease, disorder or condition, e.g., a disease, disorder, or condition that can be treated as provided herein, e.g., a cancer or a tumor listed herein. In some embodiments, a subject is susceptible to a disease, disorder, or condition. In some embodiments, a susceptible subject is predisposed to and/or shows an increased risk (as compared to the average risk observed in a reference subject or population) of developing a disease, disorder, or condition. In some embodiments, a subject displays one or more symptoms of a disease, disorder, or condition. In some embodiments, a subject does not display a particular symptom (e.g., clinical manifestation of disease) or characteristic of a disease, disorder, or condition. In some embodiments, a subject does not display any symptom or characteristic of a disease, disorder, or condition. In some embodiments, a subject is a patient. In some embodiments, a subject is an individual to whom diagnosis and/or therapy is and/or has been administered.

[0104] Substantial identity: As used herein, the term "substantial identity" refers to a comparison between amino acid or nucleic acid sequences. As will be appreciated by those of ordinary skill in the art, two sequences are generally considered to be substantially identical if they contain identical residues in corresponding positions. As is well known in this art, amino acid or nucleic acid sequences may be compared using any of a variety of algorithms, including those available in commercial computer programs such as BLASTN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-BLAST for amino acid sequences. In some embodiments, two sequences are considered to be substantially identical

if at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of their corresponding residues are identical over a relevant stretch of residues. In some embodiments, the relevant stretch is a complete sequence. In some embodiments, the relevant stretch is at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or more residues. In the context of a CDR, reference to "substantial identity" typically refers to a CDR having an amino acid sequence at least 80%, preferably at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to that of a reference CDR.

[0105] Substantially: As used herein, the term "substantially" refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term "substantially" is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[0106] Suffering from: An individual who is "suffering from" a disease, disorder, and/or condition has been diagnosed with and/or displays one or more symptoms of a disease, disorder, and/or condition.

[0107] Target: As used herein, the term "target" refers to an agent, cell, tissue, organ, or site within the body that is the subject of provided methods, systems, compounds, and /or compositions, for example, a cell, tissue, organ or site within a body that is in need of treatment or is preferentially bound by, for example, a HHLA2 binding agent described herein.

[0108] Therapeutic: As used herein, the term "therapeutic" refers to a treatment and/or prophylaxis. A therapeutic effect is obtained, for example, by suppression, remission, or eradication of a disease state.

[0109] Therapeutic agent: As used herein, the phrase "therapeutic agent" refers to any agent that, when administered to a subject, has a therapeutic effect and/or elicits a desired biological and/or pharmacological effect. In some embodiments, a therapeutic agent can be an agent that, when administered to a subject, can prevent an undesired side effect. In some embodiments, a therapeutic agent is any substance that can be used to alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of, and/or reduce incidence of one or more symptoms or features of a disease, disorder, and/or condition. A therapeutic agent includes, but is not limited to, at least one HHLA2 binding agent as described herein.

[0110] Therapeutically effective amount: As used herein, the term "therapeutically effective amount" means an amount of a substance (e.g., a therapeutic agent, composition, and/or formulation) that elicits a desired biological response when administered as part of a therapeutic regimen. In some embodiments, a therapeutically effective amount of a substance is an amount that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat, diagnose, prevent, and/or delay the onset of the disease, disorder, and/or condition. As will be appreciated by those of ordinary skill in this art, the effective amount of a substance may vary depending on such factors as the desired biological endpoint, the substance to be delivered, and/or the target cell or tissue.

For example, the effective amount of compound in a formulation to treat a disease, disorder, and/or condition is the amount that alleviates, ameliorates, relieves, inhibits, prevents, delays onset of, reduces severity of and/or reduces incidence of one or more symptoms or features of the disease, disorder, and/or condition. In some embodiments, a therapeutically effective amount is administered in a single dose. In some embodiments, multiple unit doses are required to deliver a therapeutically effective amount.

[0111] Treat: As used herein, the terms "treat," "treatment," or "treating" refer to partial or complete alleviation, amelioration, delay of onset of, inhibition, prevention, relief, and/or reduction in incidence and/or severity of one or more symptoms or features of a disease, disorder, and/or condition. In some embodiments, treatment is administered to a subject who does not exhibit signs or features of a disease, disorder, and/or condition (e.g., may be prophylactic). In some embodiments, treatment is administered to a subject who exhibits only early or mild signs or features of the disease, disorder, and/or condition, for example for the purpose of decreasing the risk of developing pathology associated with the disease, disorder, and/or condition. In some embodiments, treatment is administered to a subject who exhibits established, severe, and/or late-stage signs of the disease, disorder, or condition.

[0112] Tumor: As used herein, the term "tumor" refers to an abnormal growth of cells or tissue. A tumor may comprise cells that are precancerous (e.g., benign), malignant, pre-metastatic, metastatic, and/or non-metastatic. In some embodiments, a tumor is associated with or is a manifestation of a cancer. In some embodiments, a tumor is a disperse tumor or a liquid tumor. In some embodiments, a tumor is a solid tumor.

[0113] Throughout this disclosure, various aspects of provided technologies can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of a provided technology. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

[0114] As used herein in the present disclosure, unless otherwise clear from context, (i) the term "a" or "an" may be understood to mean "at least one"; (ii) the term "or" may be understood to mean "and/or"; (iii) the terms "comprising", "comprise", "including" (whether used with "not limited to" or not), and "include" (whether used with "not limited to" or not) may be understood to encompass itemized components or steps whether presented by themselves or together with one or more additional components or steps; (iv) the term "another" may be understood to mean at least an additional/second one or more; (v) the terms "about" and "approximately" may be understood to permit standard variation as would be understood by those of ordinary skill in the art; and (vi) where ranges are provided, endpoints are included.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[0115] In some embodiments, the present disclosure provides conjugates. In some embodiments, a conjugate comprises a target binding moiety, a payload moiety, and a linker connecting a target binding moiety and a payload moiety. In some embodiments, the present disclosure provides technologies for manufacturing conjugates. In some embodiments, the present disclosure provides technologies for assessing conjugates. In some embodiments, the present disclosure provides conjugates compositions, e.g., pharmaceutical compositions. In some embodiments, the present disclosure provides methods for using provided conjugates and compositions thereof. In some embodiments, the present disclosure provides technologies for preventing or treating various conditions, disorders or diseases utilizing provided conjugates and compositions thereof.

[0116] Certain embodiments, including those for target binding moieties, payload moieties, linkers, conjugates, etc., are described below as examples.

Target Binding Moieties

[0117] A target binding moiety binds to a target that can be or comprise a polypeptide, a small molecule, a carbohydrate, a lipid or a nucleic acid. In some embodiments, a target is on a surface of a cell, e.g., a cancer cell.

[0118] In some embodiments, a target binding moiety is or comprises an antibody or an antigen-binding fragment thereof. In some embodiments, a target binding moiety is an antibody or an antigen-binding fragment thereof. In some embodiments, a target binding moiety is an antibody. In some embodiments, a target binding moiety is a monoclonal antibody. In some embodiments, a target binding moiety is not trastuzumab. In some embodiments, a target binding moiety shares less than about 90%, 95%, or 99% sequence identity to trastuzumab. In some embodiments described herein, a target binding moiety shares less than about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to trastuzumab. In some embodiments, it is less than about 95%. In some embodiments, it is less than about 90%. In some embodiments, it is less than about 85%. In some embodiments, a target binding moiety is not an anti-HER2 antibody. In some embodiments, a target binding moiety is not an anti-HER2 antibody or an antigen-binding fragment thereof.

[0119] In some embodiments, a target binding moiety is a moiety of an antibody that without conjugation comprises four Cys residues that form two inter-heavy- and light-chain disulfide bonds. In some embodiments, each of the four Cys residues is independently conjugated to a payload moiety. In some embodiments, the target binding moiety is a moiety of an antibody that without conjugation comprises eight residues that form inter-chain disulfide bonds. In some embodiments, each of the eight Cys residues is independently conjugated to a payload moiety.

[0120] Those skilled in the art appreciate that various antibodies (e.g., those approved by FDA, those that can bind to the same antigens or entities (e.g., polypeptides, cells, etc.) as such antibodies, etc.) and antigen-binding fragments thereof can be utilized as target binding moieties in accordance with the present disclosure. For example, in some embodiments, a target binding moiety is an anti-HHLA2 antibody or antigen-binding fragment thereof.

[0121] In some embodiments, a target binding moiety is or comprises an antibody or an antigen-binding fragment thereof. In some embodiments, a target binding moiety comprises an antibody. In some embodiments, a target binding moiety is an antibody. In some embodiments, a target binding moiety comprises an antigen-binding fragment of an antibody. In some embodiments, a target binding moiety is an antigen-binding fragment of an antibody. In some embodiments, an antibody is a chimeric antibody.

[0122] In some embodiments, an antibody is a human antibody. In some embodiments, an antibody is a humanized antibody. In some embodiments, an antibody is a monospecific antibody. In some embodiments, an antibody is a bispecific antibody. In some embodiments, an antibody is a monoclonal antibody. In some embodiments, an antibody or an antigen-binding fragment thereof is or comprises a heavy chain constant region or a fragment thereof. In some embodiments, a heavy chain constant region is IgG1. In some embodiments, a heavy chain constant region is IgG2. In some embodiments, a heavy chain constant region is IgG3. In some embodiments, a heavy chain constant region is IgG4. Additionally or alternatively, an antibody or an antigen-binding fragment thereof is or comprises a light chain constant region. In some embodiments, a light chain constant region is a light chain constant regions of kappa. In some embodiments, a light chain constant region is a light chain constant regions of lambda.

[0123] In some embodiments, a target binding moiety is or comprises a chimeric antibody or an antigen-binding fragment thereof. In some embodiments, a target binding moiety independently is or comprises a human antibody or an antigen-binding fragment thereof. In some embodiments, a target binding moiety independently is or comprises a humanized antibody or an antigen-binding fragment thereof. In some embodiments, a target binding moiety independently is or comprises a monospecific antibody or an antigen-binding fragment thereof. In some embodiments, a target binding moiety independently is or comprises a bispecific antibody or an antigen-binding fragment thereof (e.g., an HHLA2 binding arm and at least one other non-HHLA2 binding arm). In some embodiments, a target binding moiety independently is or comprises an anti-EGFR x HER3 bispecific antibody or an antigen-binding fragment thereof. In some embodiments, a target binding moiety independently is or comprises a biparatopic antibody or an antigen-binding fragment thereof. In some embodiments, a target binding moiety is an anti-HHLA2 antibody or an antigen-binding fragment thereof. In some embodiments, an ntibody or an antigen-binding fragment thereof is an anti-HER2 x HHLA2 bispecific antibody or an antigen-binding fragment thereof. In some embodiments, such an antibody or antigen-binding fragment thereof comprises CDR sequences of trastuzumab. In some embodiments, such an antibody or antigen-binding fragment thereof comprises antigen-binding sequences of trastuzumab.

[0124] For example, in some embodiments, a target binding moiety is or comprises an anti-HHLA2 antibody or an antigen-binding fragment thereof.

[0125] In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises:

(a) a heavy chain variable region (VH) comprising one, two, or three VH CDR sequences each with at least about 90% identity to a VH CDR of Table 1; and/or

(b) a light chain variable region (VL) comprising one, two, or three VL CDR sequences each with at least

about 90% identity to a VL CDR of Table 1.

[0126] In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises:

(a) a VH comprising one, two, or three VH CDR sequences each with at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% identity to a VH CDR of Table 1; and/or

(b) a VL comprising one, two, or three VL CDR sequences each with at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% identity to a VL CDR of Table 1.

[0127] In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises:

(a) a VH comprising one, two, or three VH CDR sequences each comprising or consisting of a VH CDR of Table 1; and/or

(b) a VL comprising one, two, or three VL CDR sequences each comprising or consisting of a VL CDR of Table 1.

[0128] In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises:

(a) a VH with at least about 90% or more identity to a VH of Table 1; and/or

(b) a VL with at least about 90% or more identity to a VL of Table 1.

[0129] In some embodiments, an anti-HHLA2 antibody or antigen-binding fragment thereof is or comprises:

(a) a VH with at least about 95%, 96%, 97%, 98%, 99% or 99.5% identity to a VH of Table 1; and/or

(b) a VL with at least about 95%, 96%, 97%, 98%, 99% or 99.5% identity to a VL of Table 1.

[0130] In some embodiments, an anti-HHLA2 or antigen-binding fragment thereof is or comprises:

(a) a VH comprising or consisting of a VH of Table 1; and/or

(b) a VL comprising or consisting of a VL of Table 1.

[0131] In some embodiments, an anti-HHLA2 or antigen-binding fragment thereof is or comprises:

(a) a heavy chain with at least about 90% or more identity to a heavy chain of Table 1; and/or

(b) a light chain with at least about 90% or more identity to a light chain of Table 1.

[0132] In some embodiments, an anti-HHLA2 or antigen-binding fragment thereof is or comprises:

(a) a heavy chain with at least about 95%, 96%, 97%, 98%, 99% or 99.5% identity to a heavy chain of Table 1; and/or

(b) a light chain with at least about 95%, 96%, 97%, 98%, 99% or 99.5% identity to a light chain of Table 1.

[0133] In some embodiments, an anti-HHLA2 or antigen-binding fragment thereof is or comprises:

(a) a heavy chain comprising or consisting of a heavy chain of Table 1; and/or

(b) a light chain comprising or consisting of a light chain of Table 1.

[0134] In some embodiments, a target binding moiety comprises each of the CDRs of 887-1DE in Table 1. In some embodiments, a target binding moiety is or comprises a sequence with about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to VH of 887-1DE in

Table 1. In some embodiments, a target binding moiety is or comprises VH of 887-1DE in Table 1. In some embodiments, a target binding moiety is or comprises a sequence with about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to VL of 887-1DE in Table 1. In some embodiments, a target binding moiety is or comprises VL of 887-1DE in Table 1. In some embodiments, a target binding moiety is or comprises a sequence with about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to a heavy chain of 887-1DE in Table 1. In some embodiments, a target binding moiety is or comprises a heavy chain of 887-1DE in Table 1. In some embodiments, a target binding moiety is or comprises a sequence with about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to a light chain of 887-1DE in Table 1. In some embodiments, a target binding moiety is or comprises a light chain of 887-1DE in Table 1. In some embodiments, a target binding moiety comprises 887-1DE in Table 1 or antigen-binding fragments thereof. In some embodiments, a target binding moiety is 887-1DE in Table 1.

[0135] In some embodiments, a target binding moiety comprises each of the CDRs of 65889 in Table 1. In some embodiments, a target binding moiety is or comprises a sequence with about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to VH of 65889 in Table 1. In some embodiments, a target binding moiety is or comprises VH of 65889 in Table 1. In some embodiments, a target binding moiety is or comprises a sequence with about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to VL of 65889 in Table 1. In some embodiments, a target binding moiety is or comprises VL of 65889 in Table 1. In some embodiments, a target binding moiety is or comprises a sequence with about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to a heavy chain of 65889 in Table 1. In some embodiments, a target binding moiety is or comprises a heavy chain of 65889 in Table 1. In some embodiments, a target binding moiety is or comprises a sequence with about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to a light chain of 65889 in Table 1. In some embodiments, a target binding moiety is or comprises a light chain of 65889 in Table 1. In some embodiments, a target binding moiety comprises 65889 in Table 1 or antigen-binding fragments thereof. In some embodiments, a target binding moiety is 65889 in Table 1.

[0136] In some embodiments, a target binding moiety comprises each of the CDRs of 65872 in Table 1. In some embodiments, a target binding moiety is or comprises a sequence with about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to VH of 65872 in Table 1. In some embodiments, a target binding moiety is or comprises VH of 65872 in Table 1. In some embodiments, a target binding moiety is or comprises a sequence with about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to VL of 65872 in Table 1. In some embodiments, a target binding moiety is or comprises VL of 65872 in Table 1. In some embodiments, a target binding moiety is or comprises a sequence with about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to a heavy chain of 65872 in Table 1. In some embodiments, a target binding moiety is or comprises a heavy chain of 65872 in Table 1. In some embodiments, a target binding moiety is or comprises a sequence with about or at least about 90%, 91%,

92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to a light chain of 65872 in Table 1. In some embodiments, a target binding moiety is or comprises a light chain of 65872 in Table 1. In some embodiments, a target binding moiety comprises 65872 in Table 1 or antigen-binding fragments thereof. In some embodiments, a target binding moiety is 65872 in Table 1.

[0137] In some embodiments, a target binding moiety comprises each of the CDRs of Hu2C4-H3L3 in Table 1. In some embodiments, a target binding moiety is or comprises a sequence with about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to VH of Hu2C4-H3L3 in Table 1. In some embodiments, a target binding moiety is or comprises VH of Hu2C4-H3L3 in Table 1. In some embodiments, a target binding moiety is or comprises a sequence with about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to VL of Hu2C4-H3L3 in Table 1. In some embodiments, a target binding moiety is or comprises VL of Hu2C4-H3L3 in Table 1. In some embodiments, a target binding moiety is or comprises a sequence with about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to a heavy chain of Hu2C4-H3L3 in Table 1. In some embodiments, a target binding moiety is or comprises a heavy chain of Hu2C4-H3L3 in Table 1. In some embodiments, a target binding moiety is or comprises a sequence with about or at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to a light chain of Hu2C4-H3L3 in Table 1. In some embodiments, a target binding moiety is or comprises a light chain of Hu2C4-H3L3 in Table 1. In some embodiments, a target binding moiety comprises Hu2C4-H3L3 in Table 1 or antigen-binding fragments thereof. In some embodiments, a target binding moiety is Hu2C4-H3L3 in Table 1.

[0138] In some embodiments, conjugates or target binding moieties bind their targets, e.g., HHLA2, with a K_D of about or no more than about 1, 5, 10, 15, 20, 30, 50, 100 or 500 pM, or about or no more than about 1, 5, 10, 15, 20, 30, 50, 100 or 500 nM. In some embodiments, conjugates or target binding moieties bind their targets, e.g., HHLA2, with an affinity of about or at least about 10, 20, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000-fold over background or a reference agent (e.g., absence of the conjugates, immunoglobulins that target different targets, conjugates that target different targets, etc.).

[0139] In some embodiments, a conjugate comprises two or more target binding moieties. In some embodiments, each target binding moiety of a provided conjugate targets the same agent. In some embodiments, each target binding moiety targets the same agent, wherein the agent is or comprises a polypeptide, a nucleic acid, or a lipid. In some embodiments, each target binding moiety targets the same agent, wherein the agent is a polypeptide. In some embodiments, each target binding moiety targets the same antigen. In some embodiments, each target binding moiety is independently an antibody or an antigen-binding fragment thereof, and each target binding moiety shares about or at least about 90%, 95%, 96%, 97%, 98% or 99% sequence identity. In some embodiments, each target binding moiety is independently an antibody or an antigen-binding fragment thereof, and each target binding moiety shares the same amino acid sequence. In some embodiments, each target binding moiety is the same. In some embodiments, each target binding moiety is independently a target binding moiety described herein.

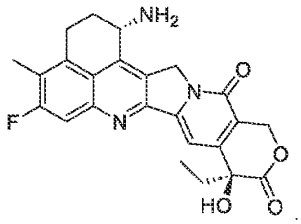
Payload Moieties

[0140] Various payload moieties including those reported for antibody-drug conjugates can be utilized in accordance with the present disclosure. In some embodiments, a payload moiety is moiety of a payload which is or comprises a cytotoxic agent.

[0141] In some embodiments, a payload is or comprises calicheamicin or a derivative or an analog thereof, or a pharmaceutically acceptable salt thereof. In some embodiments, a payload is or comprises N-acetyl gamma calicheamicin.

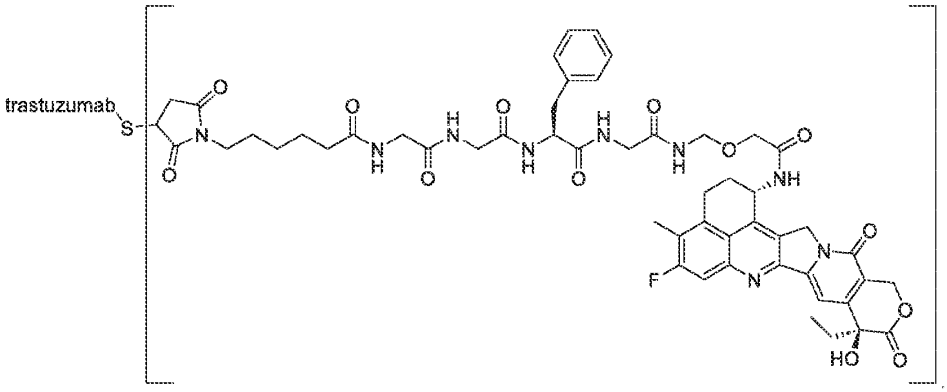
[0142] In some embodiments, a payload is or comprises a topoisomerase inhibitor. In some embodiments, a payload is or comprises camptothecin or a derivative or an analog thereof, or a pharmaceutically acceptable salt thereof. In some embodiments, a payload is or comprises exatecan or a derivative or an analog thereof, or pharmaceutically acceptable salt thereof.

[0143] Exatecan ((1S,9S)-1-Amino-9-ethyl-5-fluoro-9-hydroxy-4-methyl-1,2,3,9,12,15-hexahydro-10H,13H-benzo[de]pyrano[3′,4′:6,7]indolizino[1,2-*b*]quinoline-10,13-dione) is a structural analog of campothecin. Exatecan comprises a chemical structure:

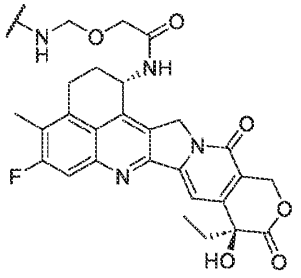


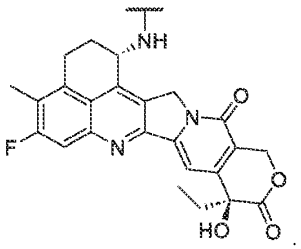
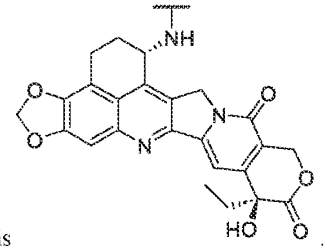
[0144] Among other things, the present disclosure provides various exatecan conjugates, e.g., various conjugates in the Examples. As shown herein, in various embodiments, exatecan is conjugated through its $-NH_2$ to a target binding moiety, e.g., an antibody, via a linker.

[0145] A derivative of exatecan is used in trastuzumab deruxtecan (sold under the trade name Enhertu), which is an antibody-drug conjugate consisting of the humanized monoclonal antibody trastuzumab (Herceptin) covalently linked to the topoisomerase I inhibitor deruxtecan (a derivative of exatecan). Trastuzumab binds to epidermal growth factor receptor 2 (HER2/neu), and once bound to HER2 receptors, the antibody is internalized by the cell, carrying the bound deruxtecan along with it. Trastuzumab deruxtecan has a chemical structure:



[0146] Without wishing to be bound to a particular theory, a conjugate binds to its target, e.g., HHLA2, and once bound, a conjugate can be internalized by the cell, carrying the bound payload (e.g., exatecan) along with it.

[0147] In some embodiments, a payload moiety is  or a pharmaceutically acceptable salt thereof.

[0148] In some embodiments, a payload moiety is  . In some embodiments, a payload moiety is  .

[0149] In some embodiments, a topoisomerase inhibitor is or comprises SN-38 or a pharmaceutically acceptable salt thereof.

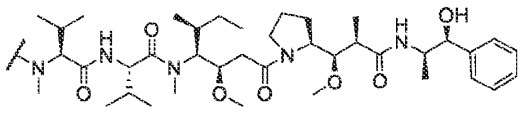
[0150] In some embodiments, a payload is or comprises a microtubule disrupting agent. In some embodiments, a payload is or comprises an anti-mitotic agent. In some embodiments, a payload is or

comprises a microtubule inhibitor. For example, in some embodiments, a payload is or comprises MMAE or a derivative or an analog thereof, or a pharmaceutically acceptable salt thereof. In some embodiments, a payload is or comprises MMAE or a pharmaceutically acceptable salt thereof. In some embodiments, a payload is or comprises MMAF or a derivative or an analog thereof, or a pharmaceutically acceptable salt thereof. In some embodiments, a payload is or comprises MMAF or a pharmaceutically acceptable salt thereof.

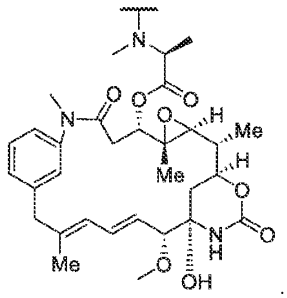
[0151] In some embodiments, a payload is or comprises an alkylating agent. In some embodiments, a payload is or comprises a DNA alkylating agent. In some embodiments, a payload is or comprises pyrrolobenzodiazepine. In some embodiments, a payload is or comprises a pyrrolobenzodiazepine dimer. In some embodiments, a payload is or comprises SG3199 or a derivative or an analog thereof, or a pharmaceutically acceptable salt thereof. In some embodiments, a payload is or comprises SG3199 or a pharmaceutically acceptable salt thereof.

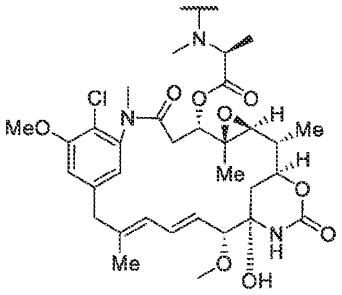
[0152] In some embodiments, a payload is or comprises a microtubule inhibitory agent, e.g., maytansine, or a derivative or an analog thereof (e.g., DM1, DM4, etc.), or a pharmaceutically acceptable salt thereof. In some embodiments, a payload is or comprises DM1 or a pharmaceutically acceptable salt thereof. In some embodiments, a payload is or comprises DM4 or a pharmaceutically acceptable salt thereof.

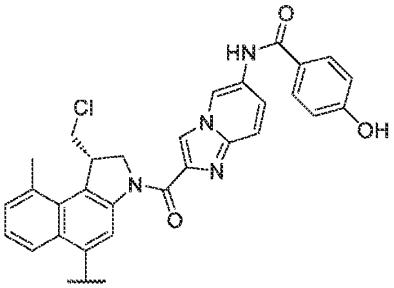
[0153] In some embodiments, a payload is or comprises an auristatin, a maytansinoid, a duocarmycin, a hemiasterlin, an eribulin, a N-myristoyl transferase inhibitor, an amanitin or a derivative or an analog thereof. In some embodiments, a payload is or comprises an auristatin or a derivative or an analog thereof. In some embodiments, a payload is or comprises a maytansinoid or a derivative or an analog thereof. In some embodiments, a payload is or comprises a duocarmycin or a derivative or an analog thereof. In some embodiments, a payload is or comprises a hemiasterlin or a derivative or an analog thereof. In some embodiments, a payload is or comprises an eribulin or a derivative or an analog thereof. In some embodiments, a payload is or comprises a N-myristoyl transferase inhibitor or a derivative or an analog thereof (see, e.g., WO 2022058745). In some embodiments, a payload is or comprises an anthracycline or a derivative or an analog thereof (see, e.g., US11833120). In some embodiments, a payload is or comprises nemorubicin or a metabolite (e.g., PNU-159682), or a derivative or an analog thereof, or a pharmaceutically acceptable salt thereof. In some embodiments, a payload is or comprises PNU-159682, or a derivative or an analog thereof, or a pharmaceutically acceptable salt thereof. See, e.g., Holte D. et al. Evaluation of PNU-159682 antibody drug conjugates (ADCs). Bioorg Med Chem Lett. 2020 Dec 15;30(24):127640. doi: 10.1016/j.bmcl.2020.127640. Epub 2020 Oct 28. PMID: 33127540. In some embodiments, a payload is or comprises PNU-159682 or pharmaceutically acceptable salt thereof. In some embodiments, a payload is or comprises an amanitin or a derivative or an analog thereof. For example, in some embodiments, a payload moiety is or comprises

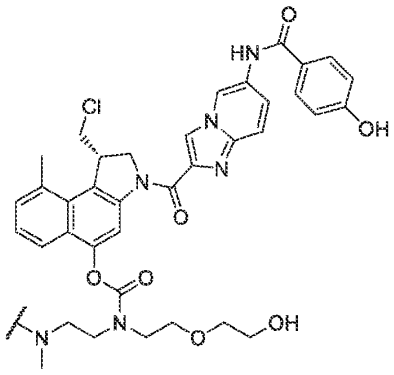


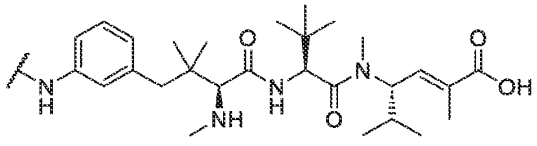
. In some embodiments, a payload moiety is or

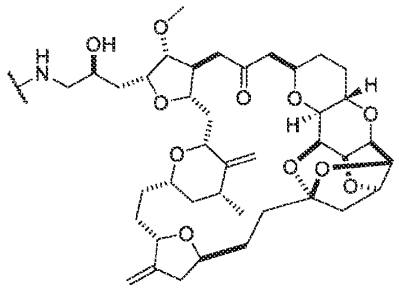
comprises  . In some embodiments, a payload moiety is or comprises

 . In some embodiments, a payload moiety is or comprises

 . In some embodiments, a payload moiety is or comprises

 . In some embodiments, a payload moiety is or comprises

 . In some embodiments, a payload moiety is or



comprises .

[0154] In some embodiments, a payload is or comprises a chemotherapeutic agent.

[0155] Among other things, conjugates can deliver payloads (e.g., one or more payloads). In some embodiments, a conjugate delivers at least one cytotoxic agent (e.g., 2, 3, 4, or more). In some embodiments, a conjugate delivers at least one chemotherapeutic agent (e.g., 2, 3, 4, or more). In some embodiments, a conjugate delivers a camptothecin, an auristatin, a maytansinoid, a duocarmycin, a hemiasterlin, an eribulin, or a N-myristoyl transferase inhibitor, or a derivative or an analog thereof, or a pharmaceutically acceptable salt thereof.

[0156] A payload can be conjugated at various positions, and often at a functional group such as amino, hydroxyl, etc. In some embodiments, a functional group is bonded to a secondary or tertiary carbon. In some embodiments, a functional group is bonded to a ring atom. In some embodiments, a functional group is bonded to a ring carbon atom. In some embodiments, a ring is a polycyclic ring system, e.g., those found in exatecan or derivates or analogs thereof. In some embodiments, provided technologies provide conjugates to functional groups, e.g., those bonded to secondary or tertiary carbon atoms or ring atoms (e.g., ring carbon atoms) without spacer moieties. In some embodiments, L^{C} is bonded to a functional group (e.g., an amino group) of a payload moiety at its –C(O)–. In some embodiments, a payload is bonded to a linker at an amino group. In some embodiments, for a payload moiety, H–payload moiety is the corresponding payload. In some embodiments, a payload moiety is R^{P} as described herein.

[0157] In some embodiments, a payload is a small molecule. For example, in some embodiments, its molecular weight is about or no more than about 5000, 4000, 3000, 2500, 2000, 1500, 1000, 900, 800, 700, 600 or 500 Da.

[0158] In some embodiments, in a conjugate one or more payload moieties are (e.g., exatecan or a derivative or an analog thereof, or pharmaceutically acceptable salt thereof) covalently bound to a single target binding moiety, e.g., an anti-HERV-H LTR-Associating 2 (HHLA2) antibody or antigen-binding fragment thereof. In some embodiments, the payload moiety to target binding moiety ratio, which equals the number of payload moieties (e.g., exatecan or a derivative thereof, or pharmaceutically acceptable salt thereof) covalently bound to a single target binding moiety in various conjugates, is referred to as the drug to antibody ratio (DAR). In some embodiments, provided conjugates have a DAR of between about 1 and about 20. In some embodiments, provided conjugates have a DAR of between about 1 and about 16. In some embodiments, provided conjugates have a DAR of between about 1 and about 12. In some

embodiments, provided conjugates have a DAR of between about 2 and about 15. In some embodiments, provided conjugates have a DAR of between about 2 and about 12. In some embodiments, provided conjugates have a DAR of between about 2 and about 10. In some embodiments, provided conjugates have a DAR of between about 2 and about 8. In some embodiments, provided conjugates have a DAR of between about 4 and about 8. In some embodiments, provided conjugates have a DAR of between about 7 and about 9. In some embodiments, provided conjugates have a DAR of between about 6 and about 10. In some embodiments, provided conjugates have a DAR of between about 4 and about 12. In some embodiments, provided conjugates have a DAR of about 1, 2, 3, 4, 5, 6, 7, or 8. In some embodiments, provided conjugates have a DAR of about 2, 4, 6, or 8. In some embodiments, provided conjugates have a DAR of about 2 or more. In some embodiments, provided conjugates have a DAR of about 2. In some embodiments, provided conjugates have a DAR of about 4. In some embodiments, provided conjugates have a DAR of about 8. In some embodiments, the payload moieties are the same.

Linkers

[0159] In some embodiments, a conjugate comprises one or more linkers that each connect a payload moiety, which may be in a pharmaceutically acceptable salt form thereof, to a target binding moiety, which may also be in a pharmaceutically acceptable salt form thereof. In some embodiments, a linker is bivalent, and connects a single payload moiety to a single target binding moiety. In some embodiments, a linker is polyvalent, and connects one or more payload moieties to one or more target binding moieties. In some embodiments, a linker is polyvalent, and connects one or more payload moieties to a single target binding moiety. In some embodiments, a linker is polyvalent, and connects a single target binding moiety to a single payload moiety.

[0160] In some embodiments, a linker comprises $-(CH_2CH_2O)n-$ described herein. In some embodiments, a linker comprises L^C as described herein. In some embodiments, a linker comprises $-(CH_2CH_2O)n-$ and L^C as described herein. In some embodiments, a linker comprises a PEG moiety. In some embodiments, a linker comprises $-(CH_2CH_2O)n-$, wherein n is at least 1. In some embodiments, a linker comprises $-(CH_2CH_2O)n-$, wherein n is 1-24. In some embodiments, n is 4. In some embodiments, n is 8.

[0161] In some embodiments, a linker comprises $-(CH_2CH_2O)n-L^{ZP}-L^C-$, wherein each variable is independently as described herein.

[0162] In some embodiments, a linker comprises $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$, wherein each variable is independently as described herein.

[0163] In some embodiments, a linker is comprises $-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$, wherein each variable is independently as described herein.

[0164] In some embodiments, a linker is comprises $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$, wherein each variable is independently as described herein.

[0165] In some embodiments, a linker is or comprises $\left[-L^{RX}\right]_a-L^{X'}-\left[(CH_2CH_2O)_n-L^{ZP}-L^C-\right]_b$

wherein each variable is independently as described herein.

L^{ZP}

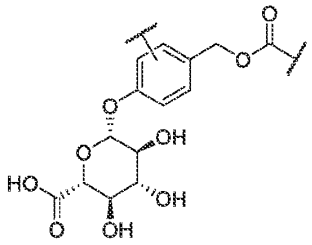
[0166] As described herein, in some embodiments, L^{ZP} connects $-(CH_2CH_2O)n-$ to L^C wherein n is not 0. In some embodiments, L^{ZP} connects L^X with L^C. In some embodiments, L^{ZP} is absent (a covalent bond). Various moieties including many utilized in antibody-drug conjugates can be utilized as L^{ZP} in accordance with the present disclosure.

[0167] In some embodiments, L^{ZP} is a covalent bond. In some embodiments, L^{ZP} is an optionally substituted bivalent straight or branched C_{1-20} (e.g., C_{1-6}, C_{1-10}, C_{1-15}, C_{2-8}, C_{5-10}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, C_{20}, etc.) aliphatic wherein 1-10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-5, 1-4, 1-3, 1-2, etc.) methylene units of L^{ZP} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$. In some embodiments, L^{ZP} is an optionally substituted bivalent straight or branched C_{1-10} (e.g., C_{1-6}, C_{2-8}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, etc.) aliphatic wherein 1-5 (e.g., 1, 2, 3, 4, 5, 1-5, 1-4, 1-2, etc.) methylene units of L^{ZP} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$. In some embodiments, L^{ZP} is optionally substituted bivalent C_{1-10} (e.g., C_{1-6}, C_{2-8}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, etc.) aliphatic. In some embodiments, L^{ZP} comprises $-C(O)-$ which is bonded to L^C. In some embodiments, L^{ZP} is $-(CH_2)_z-C(O)-$, wherein z is 1-19, and each of the $-CH_2-$ is independently optionally substituted. In some embodiments, L^{ZP} is $-(CH_2)_z-C(O)-$, wherein z is 1-19, each of the $-CH_2-$ is independently optionally substituted, and L^{C1} is or comprises a peptide moiety (e.g., GGFG, Val-Ala, Val-Cit, etc.). In some embodiments, L^{ZP} is $-(CH_2)_z-C(O)-$. In some embodiments, L^{ZP} is $-(CH_2)_z-N(R')C(O)-$, wherein z is 1-19, each of the $-CH_2-$ is independently optionally substituted, and R' is as described herein. In some embodiments, L^{ZP} is $-(CH_2)_z-N(R')C(O)-$, wherein z is 1-19, each of the $-CH_2-$ is independently optionally substituted, R' is as described herein, and L^{C1} is L^{C1S} (e.g., oGlu, mGlu, etc.). In some embodiments, L^{ZP} is $-(CH_2)_z-N(R')C(O)-$, wherein z is 1-19 and R' is as described herein. In some embodiments, L^{ZP} is $-(CH_2)_z-NHC(O)-$, wherein z is 1-19. In some embodiments, z is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 1-15, 1-10, etc. In some embodiments, z is 1-10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-5, 1-4, 1-3, 1-2, etc.). In some embodiments, z is 1-5 (e.g., 1, 2, 3, 4, 5, 1-5, 1-4, 1-2, etc.). In some embodiments, z is 1. In some embodiments, z is 2. In some embodiments, z is 3. In some embodiments, z is 4. In some embodiments, z is 5. In some embodiments L^{ZP} is $-(CH_2)_2-C(O)-$, wherein $-C(O)-$ is bonded to the amino group of the first amino acid in L^C. In some embodiments, L^{ZP} is $-(CH_2)_2-C(O)-$, wherein $-C(O)-$ is bonded to the amino group of the first amino acid in L^C, wherein L^C is $-L^{C1}-L^{CO}-$. In some embodiments, L^{ZP} is $-(CH_2)_2-N(R')-C(O)-$, wherein $-C(O)-$ is bonded to L^{C1}, each of the $-CH_2-$ is independently optionally substituted, R' is as described herein, and L^{C1} is L^{C1S}. In some embodiments, L^{ZP} is $-(CH_2)_2-N(R')-C(O)-$, wherein $-C(O)-$ is bonded to L^C, each of the $-CH_2-$ is independently optionally substituted, R' is as described herein, and L^{C1} is L^{C1S} (e.g., oGlu, mGlu, etc.). In some embodiments, L^{ZP} is $-(CH_2)_2-NH-C(O)-$, wherein $-C(O)-$ is bonded to

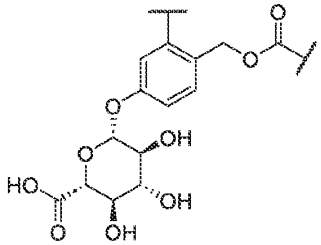
L^{C1}, and L^{C1} is L^{C1S}. In some embodiments, L^{ZP} is $-(CH_2)_2-NH-C(O)-$, wherein $-C(O)-$ is bonded to L^{C}, and L^{C1} is L^{C1S} (e.g., oGlu, mGlu, etc.). For example, as demonstrated in the Examples, in some embodiments, L^{ZP} is $-(CH_2)_2-NH-C(O)-$, and L^{C} is mGlu; in some embodiments, L^{ZP} is $-(CH_2)_2-NH-C(O)-$, and L^{C} is oGlu. In some embodiments, L^{ZP} is $-(CH_2)_2-C(O)-N(R')-$, wherein $-N(R')-$ is bonded to L^{C1}, each of the $-CH_2-$ is independently optionally substituted, R' is as described herein, and L^{C1} is L^{C1S}. In some embodiments, L^{ZP} is $-(CH_2)_2-C(O)-N(R')-$, wherein $-N(R')-$ is bonded to L^{C}, each of the $-CH_2-$ is independently optionally substituted, R' is as described herein, and L^{C1} is L^{C1S} (e.g., oGlu, mGlu, etc.). In some embodiments, L^{ZP} is $-(CH_2)_2-C(O)-NH-$, wherein $-NH-$ is bonded to L^{C1}, and L^{C1} is L^{C1S}. In some embodiments, L^{ZP} is $-(CH_2)_2-C(O)-NH$, wherein $-NH-$ is bonded to L^{C}, and L^{C1} is L^{C1S} (e.g., oGlu, mGlu, etc.). In some embodiments, L^{ZP} is $-(CH_2)_2-C(O)-NH-$, and L^{C} is mGlu; in some embodiments, L^{ZP} is $-(CH_2)_2-C(O)-NH-$, and L^{C} is oGlu.

L^{C1}

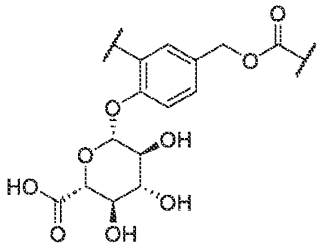
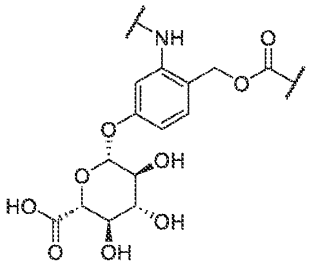
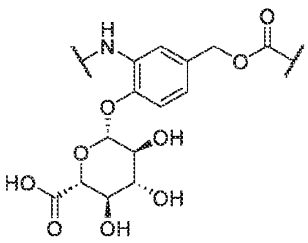
In some embodiments, each L^{C1} is independently a cleavable unit as described herein. Variable cleavable units can be utilized in accordance with the present disclosure. In some embodiments, certain cleavable units that when utilized as described herein can provide various advantages, e.g., improved property (e.g., solubility, stability, etc.), activity, etc. In some embodiments, L^{C1} is or comprises a polypeptide. In some embodiments, each L^{C1} is independently $-Gly-Gly-Phe-Gly-$, $-Val-Ala-$, $-Val-Cit-$, L^{C1S}, $-Gly-Gly-Phe-Gly-N(R')-L^{C1S}-$, $-Val-Ala-N(R')-L^{C1S}-$, or $-Val-Cit-N(R')-L^{C1S}-$ wherein each variable is independently as described herein. In some embodiments, L^{C1} is



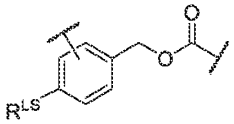
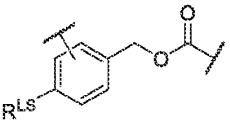
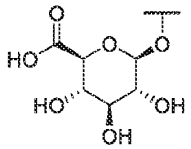
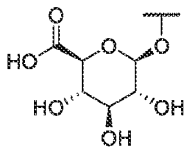
$-Gly-Gly-Phe-Gly-$, $-Val-Ala-$, $-Val-Cit-$, or . In some embodiments, L^{C1} is $-Gly-Gly-Phe-Gly-$. In some embodiments, L^{C1} is $-Val-Cit-$. In some embodiments, L^{C1} is $-Val-Ala$. In some embodiments, L^{C1} is L^{C1S} as described herein. In some embodiments, L^{C1} is $-Gly-Gly-Phe-Gly-N(R')-L^{C1S}-$, $-Val-Ala-N(R')-L^{C1S}-$, or $-Val-Cit-N(R')-L^{C1S}-$. In some embodiments, L^{C1} is $-Gly-Gly-Phe-Gly-NH-L^{C1S}-$, $-Val-Ala-NH-L^{C1S}-$, or

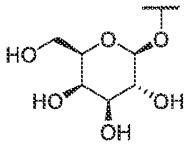
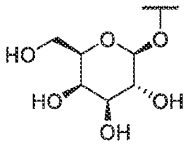


$-Val-Cit-NH-L^{C1S}-$. In some embodiments, L^{C1} is . In some

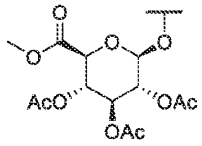
embodiments, L^{Cl} is  . In some embodiments, L^{Cl} is  . In some embodiments, L^{Cl} is  .

L^{ClS} and R^{LS}

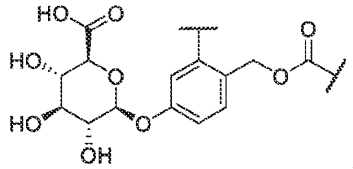
[0168] As used herein, L^{ClS} is  , wherein the phenyl ring and the $-CH_2-$ is optionally substituted, and the carbonyl is bonded to L^{CO}, and R^{LS} is or comprises a sugar moiety. In some embodiments, L^{ClS} is  , wherein the carbonyl is bonded to L^{CO}, and R^{LS} is or comprises a sugar moiety (the phenyl ring and the $-CH_2-$ is unsubstituted). In some embodiments, R^{LS} is or comprises a sugar moiety so that L^C is cleavable, e.g., by an enzyme. In some embodiments, R^{LS} is or comprises a glucuronide moiety. In some embodiments, L^C can be cleaved by a β-glucuronidase. In some embodiments, R^{LS} is or comprises a galactoside moiety. In some embodiments, L^C can be cleaved by a β-galactosidase. In some embodiments, R^{LS} is or comprises  or a derivative thereof. In some embodiments, R^{LS} is  . In some embodiments, R^{LS} is or comprises

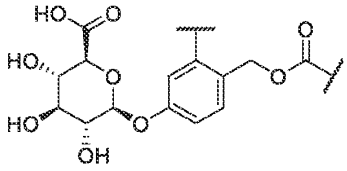
or a derivative thereof. In some embodiments, R^{LS} is . In some embodiments, a sugar moiety is protected, e.g., in a compound useful for preparing a conjugate. In some embodiments, one or more or all −OH is protected, e.g., as −OC(O)R wherein R is as described herein. In some embodiments, R is $-CH_3$. In some embodiments, −COOH is protected as −C(O)OR wherein R is as described herein and is not −H. In some embodiments, R is optionally substituted C_{1-6} alkyl. In some embodiments, each −OH is protected as −OAc and −COOH is protected as −C(O)OMe. In some



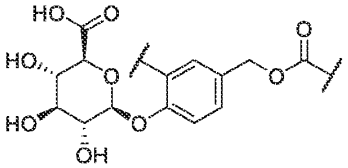
embodiments, R^{LS} is .



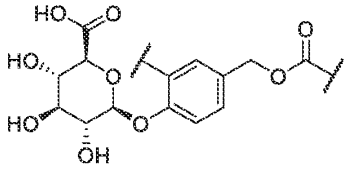
[0169] For example, in some embodiments, L^{CLS} is , wherein the phenyl ring and the $-CH_2-$ is optionally substituted, and the carbonyl is bonded to L^{CO}. In some



embodiments, L^{CLS} is wherein the carbonyl is bonded to L^{CO} or a



payload moiety ("oGlu"). In some embodiments, L^{CLS} is , wherein the phenyl ring and the $-CH_2-$ is optionally substituted, and the carbonyl is bonded to L^{CO}. In some

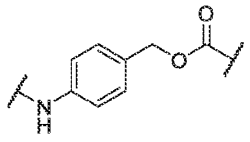
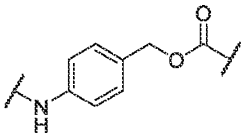


embodiments, L^{CLS} is wherein the carbonyl is bonded to L^{CO} or a payload moiety ("mGlu"). In some embodiments, L^{C} is such L^{CLS}. As those skilled in the art readily appreciate, various acidic and basic groups may exist in one or more forms. In some embodiments, a group, e.g., −COOH, is in its acidic form. In some embodiments, a group, e.g. −COOH, is in a salt form. In some embodiments, a group, e.g. −COOH, is in a pharmaceutically acceptable salt form. In some embodiments, in a preparation or composition, a group, e.g. −COOH, may exist in multiple forms, e.g., in

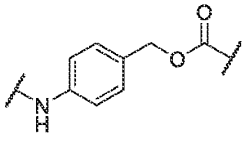
its acid form and/or one or more pharmaceutically acceptable salt forms.

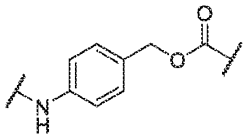
[0170] In some embodiments, L^{C1} is L^{C1S} as described herein. In some embodiments, L^{CO} is a covalent bond. In some embodiments, L^{C} is L^{C1} as described herein. In some embodiments, L^{C} is L^{C1S} as described herein.

L^{CO}

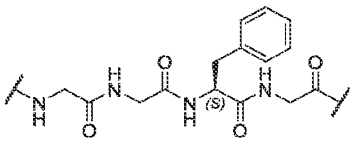
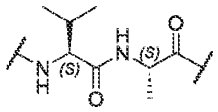
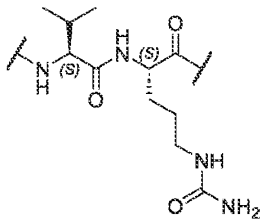
[0171] In some embodiments, L^{CO} is a covalent bond. In some embodiments, L^{CO} is a spacer moiety as described herein. In some embodiments, L^{CO} is a spacer. In some embodiments, L^{CO} is an optionally substituted bivalent straight or branched C_{1-20} (e.g., C_{1-6}, C_{1-10}, C_{1-15}, C_{2-8}, C_{5-10}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, C_{20}, etc.) aliphatic wherein 1-10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-5, 1-4, 1-3, 1-2, etc.) methylene units of L^{CO} are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, $-S(O)_2-$, −C(O)N(R')−, $-S(O)_2(NR')-$, and −Cy−. In some embodiments, L^{CO} is an optionally substituted bivalent straight or branched C_{1-10} (e.g., C_{1-6}, C_{2-8}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, etc.) aliphatic wherein 1-5 (e.g., 1, 2, 3, 4, 5, 1-5, 1-4, 1-2, etc.) methylene units of L^{CO} are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, $-S(O)_2-$, −C(O)N(R')−, $-S(O)_2(NR')-$, and −Cy−. For example, in some embodiments, L^{CO} is optionally substituted  wherein the carbonyl is bonded to the payload moiety, and the amino group is bonded to −C(O)− of L^{C1}. In some embodiments, L^{CO} is  (PABA). In some embodiments, L^{CO} is a covalent bond, and the payload moiety is bonded to −C(O)− of L^{C1}. In some embodiments, L^{ZP} is optionally substituted bivalent C_{1-10} (e.g., C_{1-6}, C_{2-8}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, etc.) aliphatic.

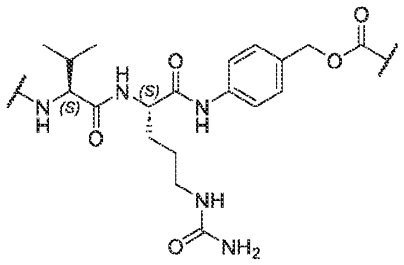
L^{C}

[0172] As described herein, L^{C} is bonded to a payload moiety and is $-L^{C1}-L^{CO}-$, wherein each of L^{C1} and L^{CO} is independently as described herein. In some embodiments, L^{C} is cleavable. In some embodiments, L^{C} is L^{C1}. In some embodiments, L^{C} is L^{C1S}. For example, in some embodiments, L^{C} is oGlu. In some embodiments, L^{C} is mGlu. In some embodiments, L^{C} is $-L^{C1}-L^{CO}-$ wherein L^{C1} is Val-Ala, and L^{CO} is optionally substituted  . In some embodiments, L^{C} is $-L^{C1}-L^{CO}-$

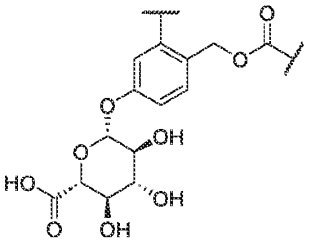
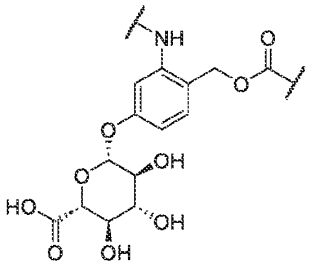
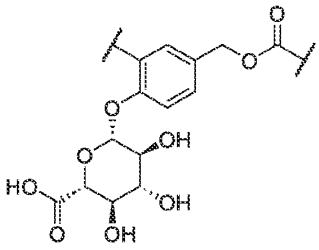
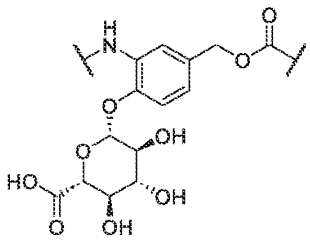
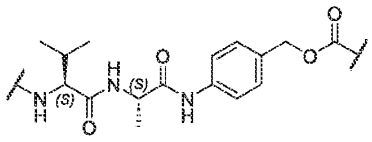


wherein L^{C1} is Val-Cit, and L^{CO} is optionally substituted . In some embodiments, L^{C} is –Val–Ala–NH–L^{C1S} wherein L^{C1S} is as described herein. In some embodiments, L^{C} is –Val–Ala–NH–L^{C1S} wherein L^{C1S} is as described herein (e.g., mGlu, oGlu, etc.). In some embodiments, L^{C} is –Val–Ala–NH–L^{C1S}–mGlu–. In some embodiments, L^{C} is –Val–Ala–NH–L^{C1S}–oGlu–. In some embodiments, L^{C} is –Val–Cit–NH–L^{C1S} wherein L^{C1S} is as described herein (e.g., mGlu, oGlu, etc.). In some embodiments, L^{C} is –Val–Cit–NH–L^{C1S}–mGlu–. In some embodiments, L^{C} is –Val–Cit–NH–L^{C1S}–oGlu–. In some embodiments, L^{C} is –GGFG–NH–L^{C1S} wherein L^{C1S} is as described herein (e.g., mGlu, oGlu, etc.). In some embodiments, L^{C} is –GGFG–NH–L^{C1S}–mGlu–. In some embodiments, L^{C} is –GGFG–NH–L^{C1S}–oGlu–.

[0173] In some embodiments, L^{C} is  , wherein the carbonyl is bonded to a payload moiety. In some embodiments, L^{C} is  , wherein the carbonyl is bonded to a payload moiety. In some embodiments, L^{C} is  , wherein the carbonyl is bonded to a payload moiety. In some embodiments, L^{C} is



(Val-Cit-PABA), wherein the carbonyl is bonded to a payload

moiety. In some embodiments, L^C is  (oGlc), wherein the carbonyl is bonded to a payload moiety. In some embodiments, L^C is  (oGlc-a), wherein the carbonyl is bonded to a payload moiety. In some embodiments, L^C is  (mGlc), wherein the carbonyl is bonded to a payload moiety. In some embodiments, L^C is  (mGlc-a), wherein the carbonyl is bonded to a payload moiety. In some embodiments, L^C is  (Val-Ala-PABA), wherein the carbonyl is bonded to a payload moiety. In some embodiments, L^C is Val–Cit–PABA, wherein the carbonyl of PABA is bonded to a payload moiety. In some embodiments, L^C is GGFG–PABA, wherein the carbonyl of PABA is bonded to a payload moiety. In some embodiments, L^C is Val–Ala–NH–oGlu, wherein the carbonyl of oGlu is bonded to a payload moiety. In some embodiments, L^C is Val–Cit–NH–oGlu, wherein the carbonyl of oGlu is bonded to a payload moiety. In some embodiments, L^C is Val–Ala–NH–mGlu, wherein the carbonyl of mGlu is bonded to a payload moiety. In some embodiments, L^C is Val–Cit–NH–mGlu, wherein the carbonyl of mGlu is bonded to a

payload moiety.

[0174] Unless otherwise specified, polypeptide sequences are from N to C.

[0175] In some embodiments, $-L^{ZP}-L^{C}-$ is $-(CH_2)_2C(O)-GGFG-$. In some embodiments, $-L^{ZP}-L^{C}-$ is $-(CH_2)_2C(O)-Val-Ala-$. In some embodiments, $-L^{ZP}-L^{C}-$ is $-(CH_2)_2C(O)-Val-Cit-$. In some embodiments, $-L^{ZP}-L^{C}-$ is $-(CH_2)_2C(O)-GGFG-PABA-$. In some embodiments, $-L^{ZP}-L^{C}-$ is $-(CH_2)_2C(O)-Val-Ala-PABA-$. In some embodiments, $-L^{ZP}-L^{C}-$ is $-(CH_2)_2C(O)-Val-Cit-PABA-$. In some embodiments, $-L^{ZP}-L^{C}-$ is $-(CH_2)_2NHC(O)oGlc-$. In some embodiments, $-L^{ZP}-L^{C}-$ is $-(CH_2)_2NHC(O)-mGlc-$. In some embodiments, $-L^{ZP}-L^{C}-$ is $-(CH_2)_2C(O)-(oGlc\text{-}a)-$. In some embodiments, $-L^{ZP}-L^{C}-$ is $-(CH_2)_2C(O)-(mGlc\text{-}a)-$. $-L^{ZP}-L^{C}-$ is $-(CH_2)_2NHC(O)-mGlu-$. In some embodiments, $-L^{ZP}-L^{C}-$ is $-(CH_2)_2NHC(O)-oGlu-$. In some embodiments, $-L^{ZP}-L^{C}-$ is bonded to a payload moiety at its $-C(O)-$.

n

[0176] In some embodiments, the linker comprises $-(CH_2CH_2O)n-$, wherein n is 0-24. In some embodiments, n is 1-24. In some embodiments, n is 6-24. In some embodiments, n is 7-20. In some embodiments, n is 8-15. In some embodiments, n is 5-10. In some embodiments, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. In some embodiments, n is 2. In some embodiments, n is 4. In some embodiments, n is 5, 6, 7, 8, 9, or 10. In some embodiments, n is 8. In some embodiments, n is 10. In some embodiments, n is 0.

[0177] In some embodiments, n is 0 and L^{C} comprises a carbohydrate moiety. In some embodiments, n is 0 and L^{C} comprises a glucuronic acid moiety. In some embodiments, n is 0 and L^{C} comprises a galactose moiety. In some embodiments, n is 0, and L^{C} is oGlc, oGlc-a, mGlc, or mGlc-a. In some embodiments, n is 0 and L^{C} is mGlu. In some embodiments, n is 0 and L^{C} is oGlu.

[0178] In some embodiments, $-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-(CH_2CH_2O)_8-(CH_2)_2C(O)-GGFG-$. In some embodiments, $-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Ala-$. In some embodiments, $-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Cit-$. In some embodiments, $-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-(CH_2CH_2O)_8-(CH_2)_2C(O)-GGFG-PABA-$. In some embodiments, $-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Ala-PABA-$. In some embodiments, $-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Cit-PABA-$. In some embodiments, $-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-oGlc-$. In some embodiments, $-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-mGlc-$. In some embodiments, $-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-(CH_2CH_2O)_8-(CH_2)_2C(O)-(oGlc\text{-}a)-$. In some embodiments, $-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-(CH_2CH_2O)_8-(CH_2)_2C(O)-(mGlc\text{-}a)-$. In some embodiments, $-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-L^{CIS}-$. In some embodiments, $-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-mGlu-$. In some embodiments, $-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-oGlu-$. In some embodiments, $-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is bonded to a payload moiety at its $-C(O)-$.

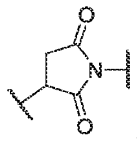
L^X

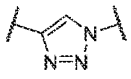
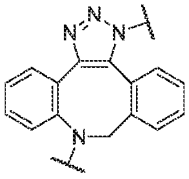
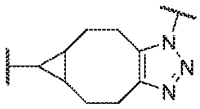
[0179] In some embodiments, L^X is a covalent bond. In some embodiments, L^X is an optionally substituted bivalent straight or branched C_{1-20} (e.g., C_{1-6}, C_{1-10}, C_{1-15}, C_{2-8}, C_{5-10}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, C_{20}, etc.) aliphatic wherein 1-10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-5, 1-4, 1-3, 1-2, etc.) methylene units of L^X are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –S(O)$_2$–, –C(O)N(R')–, –S(O)$_2$(NR')–, and –Cy–. In some embodiments, L^X is an optionally substituted bivalent straight or branched C_{1-10} (e.g., C_{1-6}, C_{2-8}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, etc.) aliphatic wherein 1-5 (e.g., 1, 2, 3, 4, 5, 1-5, 1-4, 1-2, etc.) methylene units of L^X are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –S(O)$_2$–, –C(O)N(R')–, –S(O)$_2$(NR')–, and –Cy–. In some embodiments, L^X is –(CH$_2$)$_X$–C(O)–N(R')–. In some embodiments, L^X is –(CH$_2$)$_X$–C(O)–N(R')–, wherein x is 1-19, and –N(R')– is bonded to –(CH$_2$CH$_2$O)n–. In some embodiments, L^X is –(CH$_2$)$_X$–C(O)–NH–, wherein x is 1-19, and –NH– is bonded to –(CH$_2$CH$_2$O)n–. In some embodiments, L^X is –(CH$_2$)$_X$–C(O)–N(R')– or –(CH$_2$)$_X$–C(O)–NH–, and x is 1-6. In some embodiments, L^X is –(CH$_2$)$_5$–C(O)–N(R')–, wherein –N(R')– is bonded to –(CH$_2$CH$_2$O)n–. In some embodiments, L^X is –(CH$_2$)$_5$–C(O)–NH–, wherein –NH– is bonded to –(CH$_2$CH$_2$O)n–. In some embodiments, L^X is –(CH$_2$)$_3$–C(O)–N(R')–, wherein –N(R')– is bonded to –(CH$_2$CH$_2$O)n–. In some embodiments, L^X is –(CH$_2$)$_3$–C(O)–NH–, wherein –NH– is bonded to –(CH$_2$CH$_2$O)n–. In some embodiments, x is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 1-15, 1-10, etc. In some embodiments, x is 1-10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-5, 1-4, 1-3, 1-2, etc.). In some embodiments, x is 1-5 (e.g., 1, 2, 3, 4, 5, 1-5, 1-4, 1-2, etc.). In some embodiments, x is 1. In some embodiments, x is 2. In some embodiments, x is 3. In some embodiments, x is 4. In some embodiments, x is 5.

[0180] In some embodiments, –L^X–(CH$_2$CH$_2$O)n–L^{ZP}–L^C– is –(CH$_2$)$_5$C(O)NH–(CH$_2$CH$_2$O)$_8$–(CH$_2$)$_2$C(O)–GGFG–. In some embodiments, –L^X–(CH$_2$CH$_2$O)n–L^{ZP}–L^C– is –(CH$_2$)$_5$C(O)NH–(CH$_2$CH$_2$O)$_8$–(CH$_2$)$_2$C(O)–Val–Ala–. In some embodiments, –L^X–(CH$_2$CH$_2$O)n–L^{ZP}–L^C– is –(CH$_2$)$_5$C(O)NH–(CH$_2$CH$_2$O)$_8$–(CH$_2$)$_2$C(O)–Val–Cit–. In some embodiments, –L^X–(CH$_2$CH$_2$O)n–L^{ZP}–L^C– is –(CH$_2$)$_5$C(O)NH–(CH$_2$CH$_2$O)$_8$–(CH$_2$)$_2$C(O)–GGFG–PABA–. In some embodiments, –L^X–(CH$_2$CH$_2$O)n–L^{ZP}–L^C– is –(CH$_2$)$_5$C(O)NH–(CH$_2$CH$_2$O)$_8$–(CH$_2$)$_2$C(O)–Val–Ala–PABA–. In some embodiments, –L^X–(CH$_2$CH$_2$O)n–L^{ZP}–L^C– is –(CH$_2$)$_5$C(O)NH–(CH$_2$CH$_2$O)$_8$–(CH$_2$)$_2$C(O)–Val–Cit–PABA–. In some embodiments, –L^X–(CH$_2$CH$_2$O)n–L^{ZP}–L^C– is –(CH$_2$)$_5$C(O)NH–(CH$_2$CH$_2$O)$_8$–(CH$_2$)$_2$NHC(O)–oGlc–. In some embodiments, –L^X–(CH$_2$CH$_2$O)n–L^{ZP}–L^C– is –(CH$_2$)$_5$C(O)NH–(CH$_2$CH$_2$O)$_8$–(CH$_2$)$_2$NHC(O)–mGlc–. In some embodiments, –L^X–(CH$_2$CH$_2$O)n–L^{ZP}–L^C– is –(CH$_2$)$_5$C(O)NH–(CH$_2$CH$_2$O)$_8$–(CH$_2$)$_2$C(O)–(oGlc-a)–. In some embodiments, –L^X–(CH$_2$CH$_2$O)n–L^{ZP}–L^C– is –(CH$_2$)$_5$C(O)NH–(CH$_2$CH$_2$O)$_8$–(CH$_2$)$_2$C(O)–(mGlc-a)–. In some embodiments, –L^X–(CH$_2$CH$_2$O)n–L^{ZP}–L^C– is –(CH$_2$)$_5$C(O)NH–(CH$_2$CH$_2$O)$_8$–(CH$_2$)$_2$NHC(O)–mGlu–. In some embodiments, –L^X–(CH$_2$CH$_2$O)n–L^{ZP}–L^C– is

−$(CH_2)_3C(O)NH$−$(CH_2CH_2O)_8$−$(CH_2)_2NHC(O)$−oGlu−. In some embodiments, −L^X−$(CH_2CH_2O)n$−L^{ZP}−L^C− is −$(CH_2)_3C(O)NH$−$(CH_2CH_2O)_8$−$(CH_2)_2C(O)$−GGFG−. In some embodiments, −L^X−$(CH_2CH_2O)n$−L^{ZP}−L^C− is −$(CH_2)_3C(O)NH$−$(CH_2CH_2O)_8$−$(CH_2)_2C(O)$−Val−Ala−. In some embodiments, −L^X−$(CH_2CH_2O)n$−L^{ZP}−L^C− is −$(CH_2)_3C(O)NH$−$(CH_2CH_2O)_8$−$(CH_2)_2C(O)$−Val−Cit−. In some embodiments, −L^X−$(CH_2CH_2O)n$−L^{ZP}−L^C− is −$(CH_2)_3C(O)NH$−$(CH_2CH_2O)_8$−$(CH_2)_2C(O)$−GGFG−PABA−. In some embodiments, −L^X−$(CH_2CH_2O)n$−L^{ZP}−L^C− is −$(CH_2)_3C(O)NH$−$(CH_2CH_2O)_8$−$(CH_2)_2C(O)$−Val−Ala−PABA−. In some embodiments, −L^X−$(CH_2CH_2O)n$−L^{ZP}−L^C− is −$(CH_2)_3C(O)NH$−$(CH_2CH_2O)_8$−$(CH_2)_2C(O)$−Val−Cit−PABA−. In some embodiments, −L^X−$(CH_2CH_2O)n$−L^{ZP}−L^C− is −$(CH_2)_3C(O)NH$−$(CH_2CH_2O)_8$−$(CH_2)_2NHC(O)$−oGlc−. In some embodiments, −L^X−$(CH_2CH_2O)n$−L^{ZP}−L^C− is −$(CH_2)_3C(O)NH$−$(CH_2CH_2O)_8$−$(CH_2)_2NHC(O)$−mGlc−. In some embodiments, −L^X−$(CH_2CH_2O)n$−L^{ZP}−L^C− is −$(CH_2)_3C(O)NH$−$(CH_2CH_2O)_8$−$(CH_2)_2C(O)$−(oGlc-a)−. In some embodiments, −L^X−$(CH_2CH_2O)n$−L^{ZP}−L^C− is −$(CH_2)_3C(O)NH$−$(CH_2CH_2O)_8$−$(CH_2)_2C(O)$−(mGlc-a)−. In some embodiments, −L^X−$(CH_2CH_2O)n$−L^{ZP}−L^C− is −$(CH_2)_3C(O)NH$−$(CH_2CH_2O)_8$−$(CH_2)_2C(O)$−mGlu−. In some embodiments. −L^X−$(CH_2CH_2O)n$−L^{ZP}−L^C− is −$(CH_2)_3C(O)NH$−$(CH_2CH_2O)_8$−$(CH_2)_2C(O)$−oGlu−. In some embodiments, −L^X−$(CH_2CH_2O)n$−L^{ZP}−L^C− is bonded to a payload moiety at its −C(O)−.

L^{RX}

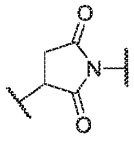
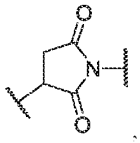
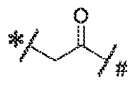
[0181] In some embodiments, L^X is a covalent bond. In some embodiments, L^X is an optionally substituted bivalent or polyvalent straight or branched C_{1-20} (e.g., C_{1-6}, C_{1-10}, C_{1-15}, C_{2-8}, C_{5-10}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, C_{20}, etc.) aliphatic wherein 1-10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-5, 1-4, 1-3, 1-2, etc.) methylene units of L^X are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −$S(O)_2$−, −C(O)N(R')−, −$S(O)_2$(NR')−, and −Cy−. In some embodiments, L^X is a covalent bond or an optionally substituted bivalent or polyvalent straight or branched C_{1-10} (e.g., C_{1-6}, C_{2-8}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, etc.) aliphatic wherein 1-5 (e.g., 1, 2, 3, 4, 5, 1-5, 1-4, 1-2, etc.) methylene units of L^X are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −$S(O)_2$−, −C(O)N(R')−, −$S(O)_2$(NR')−, and −Cy−. In some embodiments, at least one methylene unit is replaced with −Cy−. In some embodiments, −Cy− is  . In some embodiments, −Cy− is


. In some embodiments, –Cy– is 
. In some embodiments, –Cy– is 
. In some embodiments, at least one methylene unit is replaced with –S–. In some embodiments, at least one methylene unit is replaced with –C(O)–.

[0182] In some embodiments, each L^{RX} is independently $-L^{RX1}-L^{RX2}-$, wherein each of L^{RX1} and L^{RX2} is independently as described herein.

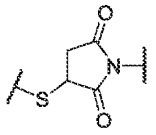
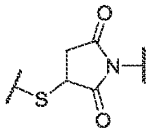
[0183] In some embodiments, $-L^{RX1}-$ is –S–. In some embodiments, $-L^{RX1}-$ is –S–, and the –S– is of a Cys residue of a target binding moiety. In some embodiments, $-L^{RX1}-$ is –S–, and the –S– is of a Cys residue in an antibody which Cys residue forms an interchain disulfide bonds in an unconjugated antibody. In some embodiments, $-L^{RX1}-$ is –S–, and the –S– is of a Cys residue of a target binding moiety, wherein the Cys residue forms an inter-heavy and light chain disulfide in an unconjugated target binding moiety. In some embodiments, $-L^{RX1}-$ is –S–, and the –S– is of a Cys residue of a target binding moiety, wherein the Cys residue is derived through partial or complete reduction of the interchain disulfide bonds.

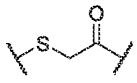
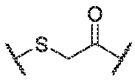
[0184] In some embodiments, L^{RX1} is –NH–. In some embodiments, L^{RX1} is –NH– which is of a Lys residue of a target binding moiety. In some embodiments, L^{RX1} is –NH– which is of a Lys residue of an antibody or an antigen-binding fragment thereof.

[0185] In some embodiments, $-L^{RX2}-$ is or comprises optionally substituted 
, wherein the nitrogen is bonded to L^X, and the carbon is bonded to L^{RX1}. In some embodiments, $-L^{RX2}-$ is or comprises 
, wherein the nitrogen is bonded to L^X, and the carbon is bonded to L^{RX1}. In some embodiments, $-L^{RX2}-$ is or comprises 
, wherein "#" represents a point of attachment to L^X, and "*" represents a point of attachment to L^{RX1}. In some embodiments, $-L^{RX2}-$ is –S–.

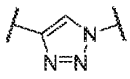
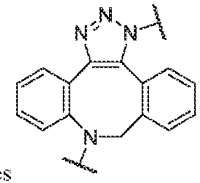
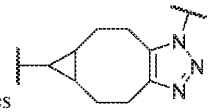
[0186] In some embodiments, L^{RX2} is or comprises –C(O)–. In some embodiments, the –C(O)– is bonded to –NH– of L^{RX1}.

[0187] In some embodiments, L^{RX} is or comprises –S–S–. In some embodiments, L^{RX} is or comprises $-S-CH_2-C(O)-$. In some embodiments, L^{RX} is or comprises optionally substituted

 wherein the nitrogen is bonded to L^X. In some embodiments, L^{RX} is  wherein the nitrogen is bonded to L^X ("SM").

[0188] In some embodiments, $-L^{RX}-$ is or comprises optionally substituted  wherein $-C(O)-$ is bonded to L^X. In some embodiments, $-L^{RX}-$ is or comprises  wherein $-C(O)-$ is bonded to L^X.

[0189] In some embodiments, L^{RX} is or comprises $-NH-C(O)-$.

[0190] In some embodiments, L^{RX} is or comprises a click chemistry product moiety. In some embodiments, L^{RX} is or comprises an optionally substituted triazole ring. In some embodiments, L^{RX} is or comprises  . In some embodiments, L^{RX} is or comprises  . In some embodiments, L^{RX} is or comprises  . In some embodiments, the nitrogen in the 5-membered ring is bonded to L^X. In some embodiments, the carbon or the nitrogen in the 8-membered ring is bonded to L^X.

[0191] In some embodiments, L^{RX} is a covalent bond. In some embodiments, L^{RX} is a moiety that results from a reaction between a group present on the unconjugated target binding moiety (e.g., L^{RXT}, L^{RX1}, etc.) and a group present on an agent comprising $L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ and a payload moiety (e.g., L^{RXP}, L^{RX2}, etc.). In some embodiments, L^{RX} is a moiety that results from a reaction between a group present on the unconjugated target binding moiety (e.g., L^{RXT}, L^{RX1}, etc.) and a group present on an agent comprising L^X and optionally a payload moiety (e.g., L^{RXP}, L^{RX2}, etc.).

[0192] In some embodiments, each L^{RX} is independently a moiety that results from a reaction between an unbound linker and a group present on an unconjugated target binding moiety.

[0193] In some embodiments, a linker comprises a moiety that results from a reaction between an unbound linker and a group present on an unconjugated target binding moiety. In some embodiments, a linker comprises a moiety that results from a reaction between an unbound linker and a group present on an antibody or antigen-binding fragment thereof.

[0194] In some embodiments, a linker comprises a moiety that results from a reaction between an unbound linker and a group present on an arginine, asparagine, aspartic acid, cysteine, glutamic acid,

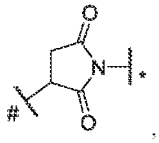
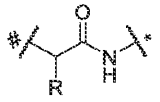
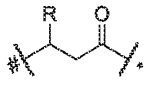
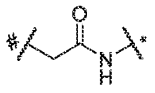
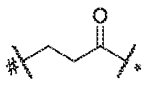
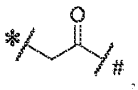
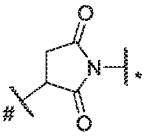
glutamine, histidine, lysine, serine, threonine, tryptophan, or tyrosine within a target binding moiety, e.g., an antibody or antigen-binding fragment thereof. In some embodiments, a linker comprises a moiety that results from a reaction between an unbound linker and a group present on a cysteine, lysine, serine, threonine, or tyrosine within a target binding moiety, e.g., an antibody or antigen-binding fragment thereof. In some embodiments, a linker comprises a moiety that results from a reaction between an unbound linker and a group present on a cysteine within a target binding moiety, e.g., an antibody or antigen-binding fragment thereof. In some embodiments, a linker comprises a moiety that results from a reaction between an unbound linker and a group present on a lysine within a target binding moiety, e.g., an antibody or antigen-binding fragment thereof. In some embodiments, a linker comprises a moiety that results from a reaction between an unbound linker and a thiol, amine, acid, or hydroxyl group within a target binding moiety, e.g., an antibody or antigen-binding fragment thereof. In some embodiments, a linker comprises a moiety that results from a reaction between an unbound linker and a thiol within a target binding moiety, e.g., an antibody or antigen-binding fragment thereof. In some embodiments, a linker comprises a moiety that results from a reaction between an unbound linker and an amine within a target binding moiety, e.g., an antibody or antigen-binding fragment thereof. In some embodiments, a linker comprises a moiety that results from a reaction between an unbound linker and a hydroxyl within a target binding moiety, e.g., an antibody or antigen-binding fragment thereof.

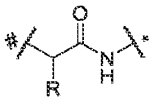
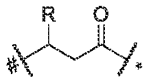
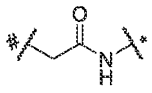
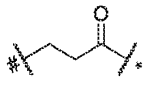
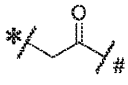
[0195] In some embodiments, a linker comprises a moiety that results from a reaction between an unbound linker and a group present on a target binding moiety, e.g., an antibody or antigen-binding fragment thereof, wherein the reaction is a conjugation with activated esters, sulfonyl chlorides, or isothiocyanates; reductive alkylation; aza-Michael addition; azetidinone chemistry; benzoyl fluoride-based chemistry; or click chemistry. Examples are provided in, e.g., Sletten and Bertozzi. Agew. Chem. Int. Ed. Engl. 2009, 48(38). 6974-6998.

[0196] In some embodiments, a linker comprises a moiety that results from a reaction between an unbound linker comprising a moiety capable of reacting with an arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, histidine, lysine, serine, threonine, tryptophan, or tyrosine; and a group present on a target binding moiety, e.g., an antibody or antigen-binding fragment thereof. In some embodiments, a linker comprises a moiety that results from a reaction between an unbound linker comprising a moiety capable of reacting with a cysteine, lysine, serine, threonine, or tyrosine; and a group present on a target binding moiety, e.g., an antibody or antigen-binding fragment thereof. In some embodiments, a linker comprises a moiety that results from a reaction between an unbound linker comprising a moiety capable of reacting with a cysteine and a group present on a target binding moiety, e.g., an antibody or antigen-binding fragment thereof. In some embodiments, a linker comprises a moiety that results from a reaction between an unbound linker comprising a moiety capable of reacting with a lysine and a group present on a target binding moiety, e.g., an antibody or antigen-binding fragment thereof.

[0197] In some embodiments, a moiety capable of reacting with a cysteine is or comprises a maleimide, haloacetamide (e.g., fluoroacetamide, chloroacetamide, bromoacetamide, or iodoacetamide),

or a Michael acceptor (e.g., α,β-unsaturated carbonyl, such as acrylamide or α,β-unsaturated ketone). In some embodiments, a moiety capable of reacting with a cysteine is a maleimide. In some embodiments, a moiety capable of reacting with a cysteine is a haloacetamide (e.g., fluoroacetamide, chloroacetamide, bromoacetamide, or iodoacetamide). In some embodiments, a moiety capable of reacting with a cysteine is a Michael acceptor (e.g., α,β-unsaturated carbonyl, such as acrylamide or α,β-unsaturated ketone). In some embodiments, a moiety capable of reacting with a cysteine is a maleimide, chloroacetamide, bromoacetamide, acrylamide, or an α,β-unsaturated ketone. In some embodiments, a moiety capable of reacting with a cysteine is a chloroacetamide. In some embodiments, a moiety capable of reacting with a cysteine is a bromoacetamide. In some embodiments, a moiety capable of reacting with a cysteine is an iodoacetamide. In some embodiments, a moiety capable of reacting with a cysteine is an acrylamide. In some embodiments, a moiety capable of reacting with a cysteine is an α,β-unsaturated ketone.

[0198] In some embodiments, a linker comprises  , wherein: # represents a point of attachment to a target binding moiety; and * represents a point of attachment to the remainder of the linker. In some embodiments, a linker comprises  , wherein R is as described herein, # represents a point of attachment to a target binding moiety; and * represents a point of attachment to the remainder of the linker. In some embodiments, a linker comprises  , wherein R is as described herein, # represents a point of attachment to a target binding moiety; and * represents a point of attachment to the remainder of the linker. In some embodiments, a linker comprises  , wherein # represents a point of attachment to a target binding moiety; and * represents a point of attachment to the remainder of the linker. In some embodiments, a linker comprises  , wherein # represents a point of attachment to a target binding moiety; and * represents a point of attachment to the remainder of the linker. In some embodiments, a linker comprises  , wherein * represents a point of attachment to a target binding moiety; and # represents a point of attachment to the remainder of the linker. In some embodiments, a linker comprises  .

wherein # represents a point of attachment to the anti-HHLA2 antibody or antigen-binding fragment thereof; and * represents a point of attachment to the remainder of the linker. In some embodiments, In some embodiments, a linker comprises  , wherein R is as described herein, # represents a point of attachment to an antibody or antigen-binding fragment thereof; and * represents a point of attachment to the remainder of the linker. In some embodiments, a linker comprises  , wherein R is as described herein, # represents a point of attachment to an antibody or antigen-binding fragment thereof; and * represents a point of attachment to the remainder of the linker. In some embodiments, a linker comprises  , wherein # represents a point of attachment to an antibody or antigen-binding fragment thereof; and * represents a point of attachment to the remainder of the linker. In some embodiments, a linker comprises  , wherein # represents a point of attachment to an antibody or antigen-binding fragment thereof; and * represents a point of attachment to the remainder of the linker. In some embodiments, a linker comprises  , wherein * represents a point of attachment to an antibody or antigen-binding fragment thereof; and # represents a point of attachment to the remainder of the linker.

[0199] In some embodiments, a target binding moiety is engineered to incorporate a reactive group. For example, in some embodiments, an antibody or antigen-binding fragment thereof is engineered to incorporate Cys, Lys, etc. at certain positions for conjugation. In some embodiments, unnatural amino acid residues, e.g., those comprising $-N_3$, alkyne, etc., are incorporated. In some embodiments, glycosylation is modified to introduce reactive groups for conjugation.

[0200] Various technologies including useful groups and reactions, e.g., L^{RX1}, L^{RX2}, L^{RX}, etc., are reported and can be utilized in accordance with the present disclosure, e.g., those described in Fu, Z., Li, S., Han, S. et al. Antibody drug conjugate: the "biological missile" for targeted cancer therapy. Sig Transduct Target Ther 7, 93 (2022). https://doi.org/10.1038/s41392-022-00947-7; Su Z, Xiao D, Xie F, Liu L, Wang Y, Fan S, Zhou X, Li S. Antibody-drug conjugates: Recent advances in linker chemistry. Acta Pharm Sin B. 2021 Dec;11(12):3889-3907. doi: 10.1016/j.apsb.2021.03.042. Epub 2021 Apr 6. PMID: 35024314; PMCID: PMC8727783; Sheyi R, de la Torre BG, Albericio F. Linkers: An Assurance for Controlled Delivery of Antibody-Drug Conjugate. Pharmaceutics. 2022 Feb 11;14(2):396. doi: 10.3390/pharmaceutics14020396. PMID: 35214128; PMCID: PMC8874516; etc.

[0201] In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-GGFG-$. In some embodiments,

$-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Ala-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Cit-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-GGFG-PABA-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Ala-PABA-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Cit-PABA-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-oGlc-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-mGlc-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-(oGlc-a)-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-(mGlc-a)-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-mGlu-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-oGlu-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-GGFG-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Ala-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Cit-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-GGFG-PABA-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Ala-PABA-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Cit-PABA-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-oGlc-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-mGlc-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-(oGlc-a)-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-(mGlc-a)-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-mGlu-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-oGlu-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is bonded to a payload moiety at its $-C(O)-$. In some embodiments, the --S-- in --SM is of a Cys residues as described herein.

[0202] In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-GGFG-$. In some embodiments,

$-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is

$-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Ala-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is

$-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Cit-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is

$-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-GGFG-PABA-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is

$-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Ala-PABA-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is

$-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Cit-PABA-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is

$-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-oGlc-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is

$-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-mGlc-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-(oGlc-a)-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is

$-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-(mGlc-a)-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is

$-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-mGlu-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is

$-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-oGlu-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is

$-S-CH_2C(O)NH-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-GGFG-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is

$-S-CH_2C(O)NH-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Ala-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is

$-S-CH_2C(O)NH-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Cit-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is

$-S-CH_2C(O)NH-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-GGFG-PABA-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is

$-S-CH_2C(O)NH-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Ala-PABA-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is

$-S-CH_2C(O)NH-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Cit-PABA-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is

$-S-CH_2C(O)NH-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-oGlc-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is

$-S-CH_2C(O)NH-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-mGlc-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-S-CH_2C(O)NH-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-(oGlc-$

a)–. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-S-CH_2C(O)NH-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-(mGlc\text{-}a)-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-S-CH_2C(O)NH-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-mGlu-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-S-CH_2C(O)NH-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-oGlu-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is bonded to a payload moiety at its $-C(O)-$. In some embodiments, the $-S-$ is of a Cys residues as described herein.

[0203] In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-GGFG-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Ala-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Cit-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-GGFG-PABA-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Ala-PABA-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Cit-PABA-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-oGlc-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-mGlc-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-(oGlc\text{-}a)-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-(mGlc\text{-}a)-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-mGlu-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-oGlu-$. In some embodiments, $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is bonded to a payload moiety at its $-C(O)-$. In some embodiments, the $-S-$ is of a Cys residues as described herein.

L^T

[0204] In some embodiments, L^T is a covalent bond. In some embodiments, L^T is an optionally substituted bivalent straight or branched C_{1-20} (e.g., C_{1-6}, C_{1-10}, C_{1-15}, C_{2-8}, C_{5-10}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, C_{20}, etc.) aliphatic wherein 1-10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-5, 1-4, 1-3, 1-2, etc.) methylene units of L^T are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, and $-S(O)_2(NR')-$. In some embodiments, L^T is an optionally substituted bivalent straight or branched C_{1-10} (e.g., C_{1-6}, C_{2-8}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, etc.) aliphatic wherein 1-5 (e.g., 1, 2, 3, 4, 5, 1-5, 1-4, 1-2, etc.) methylene units of L^T are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, and $-S(O)_2(NR')-$. In some embodiments, L^T is an

optionally substituted bivalent straight or branched C_{1-10} (e.g., C_{1-6}, C_{2-8}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, etc.) aliphatic group. In some embodiments, L^T is a bivalent straight or branched C_{1-20} (e.g., C_{1-6}, C_{1-10}, C_{1-15}, C_{2-8}, C_{5-10}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, C_{20}, etc.) aliphatic group. In some embodiments, L^T is an optionally substituted bivalent straight or branched C_{1-10} (e.g., C_{1-6}, C_{2-8}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, etc.) alkylene group. In some embodiments, L^T is an optionally substituted bivalent straight C_{1-10} (e.g., C_{1-6}, C_{2-8}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, etc.) alkylene group. In some embodiments, L^T is optionally substituted $-(CH_2)_{1-5}-$. In some embodiments, L^T is optionally substituted $-(CH_2)_{1-5}-$. In some embodiments, L^T is $-(CH_2)_{1-5}-$. In some embodiments, L^T is $-CH_2-$. In some embodiments, L^T is $-(CH_2)_4-$. In some embodiments, wherein the target binding moiety comprises an amino acid residue, L^T is bonded to the backbone atom of the amino acid residue. In some embodiments, wherein the target binding moiety comprises an amino acid residue, L^T is bonded to the alpha-carbon of the amino acid residue. In some embodiments, L^T is part of an amino acid residue (e.g., Cys, Lys, etc.) of a target binding moiety prior to conjugation.

[0205] In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-GGFG-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Ala-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Cit-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-GGFG-PABA-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Ala-PABA-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Cit-PABA-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-oGlc-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-mGlc-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-(oGlc-a)-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-(mGlc-a)-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-mGlu-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-oGlu-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-SM-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-GGFG-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Ala-$. In some embodiments,

$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is
$-CH_2-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Cit-$. In some embodiments,
$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is
$-CH_2-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-GGFG-PABA-$. In some embodiments,
$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is
$-CH_2-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Ala-PABA-$. In some embodiments,
$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is
$-CH_2-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Cit-PABA-$. In some embodiments,
$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is
$-CH_2-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-oGlc-$. In some embodiments,
$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is
$-CH_2-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-mGlc-$. In some embodiments,
$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-CH_2-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-(oGlc-a)-$. In some embodiments, $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is
$-CH_2-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-(mGlc-a)-$. In some embodiments,
$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-CH_2-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-mGlu-$. In some embodiments, $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is
$-CH_2-SM-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-oGlu-$. In some embodiments,
$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is bonded to a payload moiety at its $-C(O)-$. In some embodiments, the $-S-$ in $-SM-$ is of a Cys residues as described herein. In some embodiments, the $-CH_2-S-$ is of a Cys residues. In some embodiments, $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ bonds to a carbon atom (e.g., alpha carbon) of a polypeptide chain and a payload moiety.

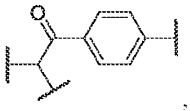
[0206] In some embodiments, $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is
$-CH_2-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-GGFG-$. In some embodiments,
$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is
$-CH_2-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Ala-$. In some embodiments,
$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is
$-CH_2-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Cit-$. In some embodiments,
$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is
$-CH_2-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-GGFG-PABA-$. In some embodiments, $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is
$-CH_2-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Ala-PABA-$. In some embodiments, $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is
$-CH_2-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Cit-PABA-$. In some embodiments, $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is
$-CH_2-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-oGlc-$. In some embodiments,
$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is
$-CH_2-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-mGlc-$. In some embodiments,

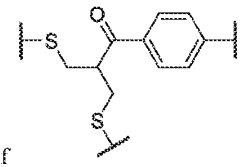
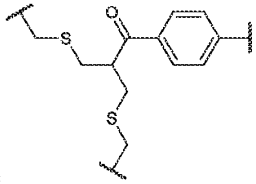
$-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is
$-CH_2-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-(oGlc\text{-}a)-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-(mGlc\text{-}a)-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-mGlu-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-oGlu-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-GGFG-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Ala-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Cit-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-GGFG-PABA-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Ala-PABA-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Cit-PABA-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-oGlc-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-S-CH_2C(O)NH-(CH_2)_5C(O)NH-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-mGlc-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-S-CH_2C(O)NH-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-(oGlc\text{-}a)-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-S-CH_2C(O)NH-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-(mGlc\text{-}a)-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-S-CH_2C(O)NH-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-mGlu-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-S-CH_2C(O)NH-(CH_2)_3C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-oGlu-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-GGFG-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Ala-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Cit-$. In some embodiments, $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ is $-CH_2-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-GGFG-PABA-$. In some embodiments,

$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-CH_2-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Ala-PABA-$. In some embodiments, $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-CH_2-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-Val-Cit-PABA-$. In some embodiments, $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-CH_2-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-oGlc-$. In some embodiments, $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-CH_2-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2NHC(O)-mGlc-$. In some embodiments, $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-CH_2-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-(oGlc-a)-$. In some embodiments, $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-CH_2-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-(mGlc-a)-$. In some embodiments, $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-CH_2-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-mGlu-$. In some embodiments, $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is $-CH_2-S-CH_2C(O)NH-(CH_2CH_2O)_8-(CH_2)_2C(O)-oGlu-$. In some embodiments, $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is bonded to a payload moiety at its $-C(O)-$. In some embodiments, the $-S-$ is of a Cys residues as described herein. In some embodiments, the $-CH_2-S-$ is of a Cys residues. In some embodiments, $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ bonds to a carbon atom (e.g., alpha carbon) of a polypeptide chain and a payload moiety.

$L^{X'}$

[0207] In some embodiments, a linker is or comprises $L^{X'}$. In some embodiments, $L^{X'}$ is a covalent bond. In some embodiments, $L^{X'}$ is an optionally substituted bivalent or polyvalent straight or branched C_{1-20} (e.g., C_{1-6}, C_{1-10}, C_{1-15}, C_{2-8}, C_{5-10}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, C_{20}, etc.) aliphatic wherein 1-10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-5, 1-4, 1-3, 1-2, etc.) methylene units of $L^{X'}$ are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;$-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$. In some embodiments, $L^{X'}$ is an optionally substituted bivalent or polyvalent straight or branched C_{1-20} (e.g., C_{1-6}, C_{1-10}, C_{1-15}, C_{2-8}, C_{5-10}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, C_{20}, etc.) aliphatic wherein 1-10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-5, 1-4, 1-3, 1-2, etc.) methylene units of $L^{X'}$ are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;$-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$. In some embodiments, $L^{X'}$ is an optionally substituted bivalent or polyvalent straight or branched C_{1-10} (e.g., C_{1-6}, C_{2-8}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, etc.) aliphatic wherein 1-5 (e.g., 1, 2, 3, 4, 5, 1-5, 1-4, 1-2, etc.) methylene units of $L^{X'}$ are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;$-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$. In some embodiments, $L^{X'}$ is bivalent. In some embodiments, $L^{X'}$ is polyvalent. For example, in some embodiments, $L^{X'}$ is optionally substituted



, wherein the phenyl ring is bonded to $-(CH_2CH_2O)n-$. In some embodiments, $-[L^{RX}]_a L^{X'}-$ has the structure of  . In some embodiments, $-[L^{T}-L^{RX}]_a L^{X'}-$ has the structure  . In some embodiments, each $-S-$ is independently of a Cys residue in the same target binding moiety. In some embodiments, each $-CH_2-S-$ is independently of a Cys residue in the same target binding moiety. In some embodiments, the two Cys residues form an inter-chain disulfide bond in an unconjugated target binding moiety.

[0208] In some embodiments, a is 1. In some embodiments, a is 2-10. In some embodiments, a is 2. In some embodiments, a is 3, 4, 5, 6, 7, 8, 9, or 10.

[0209] In some embodiments, b is 1. In some embodiments, b is 2-10. In some embodiments, b is 2. In some embodiments, b is 3, 4, 5, 6, 7, 8, 9, or 10.

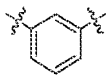
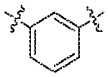
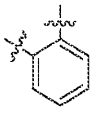
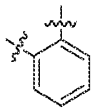
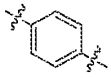
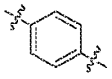
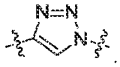
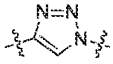
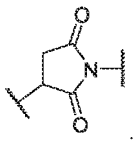
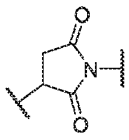
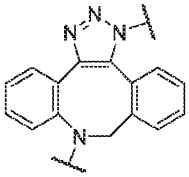
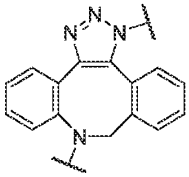
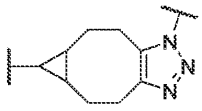
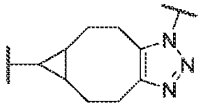
Cy

[0210] As used herein, Cy is an optionally substituted bivalent 3-20 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 3-15, 3-14, 3-10, 5-10, 3-7, 3-6, etc.) membered ring having 0-10 (e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-10, 0-5, 1-5, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. In some embodiments, each heteroatom is independently selected from nitrogen, oxygen and sulfur. In some embodiments, Cy is an optionally substituted bivalent 3-20 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 3-15, 3-14, 3-10, 5-10, 3-7, 3-6, etc.) membered ring having 0-10 (e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-10, 0-5, 1-5, etc.) heteroatoms independently selected from oxygen, nitrogen, and sulfur. In some embodiments, Cy is monocyclic. In some embodiments, Cy is bicyclic. In some embodiments, Cy is polycyclic. In some embodiments, each monocyclic ring unit is independently an optionally substituted 3-10 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 3-8, 3-7, 3-6, 5-8, etc.) membered ring having 0-5 (e.g., 1, 2, 3, 4, 5, 1-5, 1-4, 1-2, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur and 1-10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-5, 1-4, etc.) carbon atoms. In some embodiments, a monocyclic ring unit is an optionally substituted saturated ring. In some embodiments, a monocyclic ring unit is an optionally substituted partially unsaturated ring. In some embodiments, a monocyclic ring unit is an optionally substituted aromatic ring. In some embodiments, a monocyclic ring unit is an optionally substituted phenyl ring. In some

embodiments, a monocyclic ring unit is an optionally substituted 5-membered heteroaromatic having 1-4 (e.g., 1, 2, 3, 4, 1-3, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, a monocyclic ring unit is an optionally substituted 6-membered heteroaromatic having 1-4 (e.g., 1, 2, 3, 4, 1-3, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen and sulfur. Each monocyclic ring unit is independently optionally substituted. In some embodiments, a monocyclic ring unit is unsaturated. In some embodiments, a monocyclic ring unit is saturated. In some embodiments, Cy is an optionally substituted saturated ring. In some embodiments, Cy is an optionally substituted partially unsaturated ring. In some embodiments, Cy is an optionally substituted aromatic ring.

[0211] In some embodiments, Cy is an optionally substituted bivalent 3-10 (e.g., 3, 4, 5, 6, 7, 8, 9, or 10) membered monocyclic cycloaliphatic ring. In some embodiments, Cy is an optionally substituted 3-10 (e.g., 3, 4, 5, 6, 7, 8, 9, or 10) membered monocyclic cycloalkyl ring. In some embodiments, Cy is an optionally substituted 3-10 (e.g., 3, 4, 5, 6, 7, 8, 9, or 10) membered monocyclic heteroaliphatic ring having 1-5 heteroatoms. In some embodiments, Cy is an optionally substituted 3-10 (e.g., 3, 4, 5, 6, 7, 8, 9, or 10) membered monocyclic heteroalkyl ring having 1-5 heteroatoms. In some embodiments, Cy is an optionally substituted bivalent 5-15 (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) membered bicyclic or polycyclic cycloaliphatic group. In some embodiments, Cy is an optionally substituted bivalent 5-15 (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) membered bicyclic or polycyclic cycloalkyl group. In some embodiments, Cy is an optionally substituted 5-15 (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) membered bicyclic or polycyclic heteroaliphatic ring having 1-5 heteroatoms. In some embodiments, Cy is an optionally substituted 5-15 (e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15) membered bicyclic or polycyclic heterocyclyl ring having 1-5 heteroatoms. In some embodiments, a cycloaliphatic, cycloalkyl, heteroaliphatic or heteroalkyl ring is 3-membered. In some embodiments, it is 4-membered. In some embodiments, it is 5-membered. In some embodiments, it is 6-membered. In some embodiments, it is 7-membered. In some embodiments, it is 8-membered. In some embodiments, it is 9-membered. In some embodiments, it is 10-membered. In some embodiments, it is 11-membered. In some embodiments, it is 12-membered. In some embodiments, Cy is optionally substituted phenylene. In some embodiments, Cy is an optionally substituted bivalent 10-membered bicyclic aryl ring. In some embodiments, Cy is an optionally substituted 5-membered heteroaryl ring having 1-4 heteroatoms. In some embodiments, Cy is an optionally substituted 6-membered heteroaryl ring having 1-4 heteroatoms. In some embodiments, Cy is an optionally substituted 9-membered bicyclic heteroaryl ring having 1-5 heteroatoms. In some embodiments, Cy is an optionally substituted 10-membered bicyclic heteroaryl ring having 1-5 heteroatoms. In some embodiments, a heteroaliphatic, heterocyclyl or heteroaryl ring contains no more than 1 heteroatom. In some embodiments, each heteroatom is independently selected from nitrogen, oxygen and sulfur.

[0212] In some embodiments, Cy is an optionally substituted 4-7 membered ring having 0-3 heteroatoms. In some embodiments, Cy is an optionally substituted 6-membered aryl ring. In some embodiments, an aryl ring is substituted. In some embodiments, it is substituted with one or more

halogen. In some embodiments, it is substituted with one or more –F. In some embodiments, it is not substituted. In some embodiments, it is optionally substituted  . In some embodiments, it is  . In some embodiments, it is optionally substituted  . In some embodiments, it is  . In some embodiments, it is optionally substituted  . In some embodiments, it is  . In some embodiments, Cy is an optionally substituted 5-membered heteroaryl ring having 1-3 heteroatoms. In some embodiments, a heteroatom is nitrogen. In some embodiments, a heteroatom is oxygen. In some embodiments, a heteroatom is sulfur. In some embodiments, Cy is optionally substituted  . In some embodiments, Cy is  . In some embodiments, –Cy– is optionally substituted  . In some embodiments, –Cy– is  . In some embodiments, –Cy– is  . In some embodiments, –Cy– is optionally substituted  . In some embodiments, –Cy– is optionally substituted  . In some embodiments, –Cy– is  .

R'

[0213] Certain embodiments for R' are described below. Various variables can be R', and embodiments for R' can also be applied to such variables that can be R'.

[0214] In some embodiments, R' is R as described herein. In some embodiments, R' is –H. In some

embodiments, R' is not −H. In some embodiments, R' is optionally substituted C_{1-6} aliphatic.

[0215] In some embodiments, R' is −C(O)R wherein R is as described herein. In some embodiments, R' is $-CO_2R$ wherein R is as described herein. In some embodiments, R' is $-S(O)_2R$ wherein R is as described herein. In some embodiments, as described herein, R is not −H.

[0216] In some embodiments, two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 (e.g., 3-15, 3-10, 3-6, 5-10, 5-6, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etc.) membered ring having, in addition to the intervening atom(s), 0-10 (e.g., 0, 1-10, 1-5, 1-4, 1-3, 1-2, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. In some embodiments, two or more R' on the same atom are taken together with the atom to which they are attached to form an optionally substituted 3-20 (e.g., 3-15, 3-10, 3-6, 5-10, 5-6, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etc.) membered ring having, in addition to the intervening atom(s), 0-10 (e.g., 0, 1-10, 1-5, 1-4, 1-3, 1-2, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. In some embodiments, two R' on the same atom are taken together with the atom to which they are attached to form an optionally substituted 3-20 (e.g., 3-15, 3-10, 3-6, 5-10, 5-6, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etc.) membered ring having, in addition to the intervening atom(s), 0-10 (e.g., 0, 1-10, 1-5, 1-4, 1-3, 1-2, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

[0217] In some embodiments, each R' is independently −R, −C(O)R, $-CO_2R$, or $-S(O)_2R$, or two or more R' on the same atom are taken together with the atom to which they are attached to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

Ring

[0218] Compounds of the present disclosure (e.g., in a composition and/or a conjugate as described herein) may contain various rings. In some embodiments, a variable, e.g., R or a variable that can be R, can be of a ring as described herein. In some embodiments, two variables, e.g., two R' groups, may be taken together with their intervening atom(s) to form a ring as described herein. In some embodiments, a ring is monovalent. In some embodiments, a ring is bivalent. In some embodiments, a ring structure can be polyvalent. Rings are optionally substituted. In some embodiments, a ring is unsubstituted. In some embodiments, a ring is substituted.

[0219] Certain embodiments and features of rings are described below as examples.

[0220] In some embodiments, a ring is an optionally substituted 3-20 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 3-15, 3-14, 3-10, 5-10, 3-7, 3-6, etc.) membered ring having 0-10 (e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-10, 0-5, 1-5, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. In some embodiments, each heteroatom is independently selected from nitrogen, oxygen and sulfur. In some embodiments, a ring is an optionally substituted 3-20 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 3-15, 3-14, 3-10, 5-10, 3-7, 3-6, etc.)

membered ring having 0-10 (e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-10, 0-5, 1-5, etc.) heteroatoms independently selected from oxygen, nitrogen, and sulfur. In some embodiments, a ring is monocyclic In some embodiments, a ring is bicyclic. In some embodiments, a ring is polycyclic.

[0221] In some embodiments, each monocyclic ring unit is independently an optionally substituted 3-10 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 3-8, 3-7, 3-6, 5-8, etc.) membered ring having 0-5 (e.g., 1, 2, 3, 4, 5, 1-5, 1-4, 1-2, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur and 1-10 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1-5, 1-4, etc.) carbon atoms. In some embodiments, a monocyclic ring unit is an optionally substituted saturated ring. In some embodiments, a monocyclic ring unit is an optionally substituted partially unsaturated ring. In some embodiments, a monocyclic ring unit is an optionally substituted aromatic ring. In some embodiments, a monocyclic ring unit is an optionally substituted phenyl ring. In some embodiments, a monocyclic ring unit is an optionally substituted 5-membered heteroaromatic having 1-4 (e.g., 1, 2, 3, 4, 1-3, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, a monocyclic ring unit is an optionally substituted 6-membered heteroaromatic having 1-4 (e.g., 1, 2, 3, 4, 1-3, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen and sulfur. Each monocyclic ring unit is independently optionally substituted. In some embodiments, a monocyclic ring unit is unsaturated. In some embodiments, a monocyclic ring unit is saturated.

[0222] In some embodiments, a ring is an optionally substituted saturated ring. In some embodiments, a ring is an optionally substituted partially unsaturated ring. In some embodiments, a ring is an optionally substituted aromatic ring.

[0223] In some embodiments, a ring is an optionally substituted 3-10 membered, monocyclic, bicyclic or polycyclic ring having, in addition to the intervening atom(s), 0-4 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, a ring is 3-9, 3-8, 3-7, 3-6, 4-10, 4-9, 4-8, 4-7, 4-6, 5-10, 5-9, 5-8, 5-7, 5-6, or 3, 4, 5, 6, 7, 8, 9, or 10-membered. In some embodiments, a ring is 3-9 membered. In some embodiments, a ring is 3-7 membered. In some embodiments, a ring is 4-10 membered. In some embodiments, a ring is 4-7 membered. In some embodiments, a ring is 5-10 membered. In some embodiments, a ring is 5-7 membered. In some embodiments, a ring is 3-membered. In some embodiments, a ring is 4-membered. In some embodiments, a ring is 5-membered. In some embodiments, a ring is 6-membered. In some embodiments, a ring is 7-membered. In some embodiments, a ring is 8-membered. In some embodiments, a ring is 9-membered. In some embodiments, a ring is 10-membered. In some embodiments, a ring is monocyclic. In some embodiments, a ring is bicyclic. In some embodiments, a ring is polycyclic. In some embodiments, a ring has no heteroatoms. In some embodiments, a ring has 1-6, e.g., 1-5, 1-3, or 1, 2, 3, 4, 5, or 6 heteroatoms. In some embodiments, a ring formed by two or more groups taken together with their intervening atom(s) has no heteroatoms in addition to the intervening atom(s). In some embodiments, a ring formed by two or more groups taken together with their intervening atom(s) has 1-6, e.g., 1-5, 1-3, or 1, 2, 3, 4, 5, or 6 heteroatoms in addition to the intervening atom(s). In some embodiments, a ring is saturated. In some embodiments, a ring is partially unsaturated. In some embodiments, a ring comprises one or more

aromatic ring. In some embodiments, a ring is bicyclic or polycyclic, and each monocyclic ring unit is independently 3-10 (e.g., 3-9, 3-8, 3-7, 3-6, 4-10, 4-9, 4-8, 4-7, 4-6, 5-10, 5-9, 5-8, 5-7, 5-6, or 3, 4, 5, 6, 7, 8, 9, or 10) membered, saturated, partially unsaturated or aromatic and having 0-5 (e.g., 0, 1-5, 1-3, 1, 2, 3, 4, or 5) heteroatoms. In some embodiments, each heteroatom is independently selected from nitrogen, oxygen and sulfur. In some embodiments, a monocyclic ring unit is saturated. In some embodiments, a monocyclic ring unit is partially unsaturated. In some embodiments, a monocyclic ring unit is aromatic. In some embodiments, a monocyclic ring unit is heteroaromatic. Those skilled in the art appreciate that intervening atom(s), e.g., of groups taken together to form a ring, are typically atoms on the shortest path connecting such groups if multiple paths exist.

[0224] In some embodiments, a ring has one or more heteroatoms. In some embodiments, a ring comprises a nitrogen atom. In some embodiments, a ring comprises an oxygen atom. In some embodiments, a ring comprises a sulfur atom.

R

[0225] Various variables in the present disclosure can independently be R and embodiments for R can also be applied to such variables that can be R. Certain embodiments for R are described below as examples. Those skilled in the art reading the present disclosure appreciate that embodiments that are described for a variable that can be R and fall within the definition of R can be embodiments for R as well. Those skilled in the art reading the present disclosure further appreciate that embodiments for R, e.g., those described for R, those described for a variable that can be R and within the definition of R, etc., can be embodiments for a variable that can be R.

[0226] In some embodiments, R is –H. In some embodiments, R is not –H. In some embodiments, R is optionally substituted C_{1-10} (e.g., C_{1-6}, C_{2-8}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, etc.) aliphatic. In some embodiments, R is optionally substituted C_{1-8} aliphatic. In some embodiments, R is optionally substituted C_{1-6} aliphatic. In some embodiments, R is optionally substituted C_{1-10} (e.g., C_{1-6}, C_{2-8}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, etc.) alkyl. In some embodiments, R is optionally substituted C_{1-8} alkyl. In some embodiments, R is optionally substituted C_{1-6} aliphatic. In some embodiments, R is optionally substituted C_{1-6} alkyl. In some embodiments, R is optionally substituted $-CH_2-C_{3-6}$ cycloaliphatic. In some embodiments, R is optionally substituted $-CH_2-C_{3-6}$ cycloaliphatic. In some embodiments, R is optionally substituted $-CH_2-C_{3-6}$ cycloalkyl. In some embodiments, R is methyl. In some embodiments, R is ethyl. In some embodiments, R is isopropyl. In some embodiments, R is $-CF_3$. In some embodiments, R is $-CH_2CF_3$. In some embodiments, R is butyl. In some embodiments, R is t-butyl.

[0227] In some embodiments, R is optionally substituted 3-10 membered (e.g., 3-9, 3-8, 3-7, 3-6, 5-7, 4, 5, 6, 7, 8, 9, 10, etc.) cycloaliphatic. In some embodiments, R is optionally substituted C_{3-10} cycloalkyl. In some embodiments, R is optionally substituted cyclopropyl. In some embodiments, R is optionally substituted cyclobutyl. In some embodiments, R is optionally substituted cyclopentyl. In some embodiments, R is optionally substituted cyclohexyl.

[0228] In some embodiments, R is optionally substituted C_{1-10} (e.g., C_{1-6}, C_{2-8}, C_1, C_2, C_3, C_4, C_5, C_6,

C_7, C_8, C_9, C_{10}, etc.) heteroaliphatic having 1-5 (e.g., 1-4, 1-3, 1-2, 1, 2, 3, 4, 5, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. In some embodiments, R is optionally substituted C_{1-6} (e.g., C_{1-6}, C_{2-8}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, etc.) heteroaliphatic having 1-5 (e.g., 1-4, 1-3, 1-2, 1, 2, 3, 4, 5, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. In some embodiments, R is optionally substituted C_{1-10} (e.g., C_{1-6}, C_{2-8}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, etc.) heteroaliphatic having 1-5 (e.g., 1-4, 1-3, 1-2, 1, 2, 3, 4, 5, etc.) heteroatoms independently selected from oxygen, nitrogen and sulfur. In some embodiments, R is optionally substituted C_{1-6} (e.g., C_{1-6}, C_{2-8}, C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, etc.) heteroaliphatic having 1-5 (e.g., 1-4, 1-3, 1-2, 1, 2, 3, 4, 5, etc.) heteroatoms independently selected from oxygen, nitrogen and sulfur. In some embodiments, R is optionally substituted C_{1-6} heteroaliphatic having 1-3 heteroatoms independently selected from nitrogen, oxygen and sulfur C_{1-6} heteroaliphatic having 1-3 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted C_{1-6} heteroaliphatic having 1-3 heteroatoms independently selected from nitrogen, oxygen and sulfur C_{1-6} heteroaliphatic having 1 heteroatom independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is heteroalkyl.

[0229] In some embodiments, R is optionally substituted 3-15 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 3-10, 3-7, 4-10, 4-6, etc.) membered heterocyclic ring having 1-5 (e.g., 1-4, 1-3, 1-2, 1, 2, 3, 4, 5, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. In some embodiments, R is optionally substituted 3-15 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 3-10, 3-7, 4-10, 4-6, etc.) membered heterocyclic ring having 1-5 (e.g., 1-4, 1-3, 1-2, 1, 2, 3, 4, 5, etc.) heteroatoms independently selected from oxygen, nitrogen and sulfur. In some embodiments, R is optionally substituted 3-10 (e.g., 3-9, 3-8, 3-7, 3-6, 5-7, 4, 5, 6, 7, 8, 9, 10, etc.) membered heterocyclyl having 1-4 (e.g., 1, 2, 3, 4, 1-2, etc.) heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 3-membered heterocyclyl having 1-2 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 3-membered heterocyclyl having one heteroatom independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 4-membered heterocyclyl having 1-2 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 5-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 6-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 7-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 8-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 9-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 10-membered heterocyclyl having 1-3 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, there is one carbon atom in a heterocyclyl ring. In some embodiments, there are two or more (e.g., 2-14, 2-9, 2-6, 2-4, 3-10, 3-5, 2,

3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, etc.) carbon atoms in a heterocyclyl ring.

[0230] In some embodiments, R is optionally substituted phenyl. In some embodiments, R is phenyl. In some embodiments, R is optionally substituted naphthyl. In some embodiments, R is optionally substituted 1-naphthyl. In some embodiments, R is optionally substituted 2-naphthyl. In some embodiments, R is naphthyl.

[0231] In some embodiments, R is optionally substituted 5-14 (e.g., 5, 6, 9, 10, 14, etc.) membered heteroaryl having 1-6 (e.g., 1-5, 1-4, 1, 2, 3, 4, 5, 6, etc.) heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur. In some embodiments, R is optionally substituted 5-14 (e.g., 5, 6, 9, 10, 14, etc.) membered heteroaryl having 1-6 (e.g., 1-5, 1-4, 1, 2, 3, 4, 5, 6, etc.) heteroatoms independently selected from oxygen, nitrogen and sulfur. In some embodiments, R is optionally substituted 5-10 (e.g., 5, 6, 9, 10, etc.) membered heteroaryl having 1-6 (e.g., 1-5, 1-4, 1, 2, 3, 4, 5, 6, etc.) heteroatoms independently selected from oxygen, nitrogen and sulfur. In some embodiments, R is optionally substituted 5-10 membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, a heteroaryl ring is monocyclic and is 5-membered. In some embodiments, a heteroaryl ring is monocyclic and is 6-membered. In some embodiments, a heteroaryl ring is bicyclic and is 9-membered. In some embodiments, a heteroaryl ring is bicyclic and is 10-membered. In some embodiments, a heteroaryl ring is tricyclic and is 14-membered. In some embodiments, R is optionally substituted 5-membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 5-membered heteroaryl having 1 heteroatom selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 6-membered heteroaryl having 1-4 heteroatoms independently selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted 6-membered heteroaryl having 1 heteroatom selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted bicyclic 8-10 membered aromatic ring having 1-6 heteroatoms nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted bicyclic 9-membered aromatic ring having 1-6 heteroatoms nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted bicyclic 10-membered aromatic ring having 1-6 heteroatoms nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted bicyclic 9-membered aromatic ring having 1 heteroatom selected from nitrogen, oxygen and sulfur. In some embodiments, R is optionally substituted bicyclic 10-membered aromatic ring having 1 heteroatom nitrogen, oxygen and sulfur. In some embodiments, at least one heteroatom is nitrogen. In some embodiments, at least one heteroatom is oxygen. In some embodiments, at least one heteroatom is sulfur. In some embodiments, each heteroatom is the same. In some embodiments, at least one heteroatom is different from another heteroatom.

[0232] In some embodiments, there are multiple bivalent moieties between two connected moieties. As appreciated by those skilled in the art, when each of these bivalent moieties is a covalent bond, there is a single covalent bond between the connected moieties unless otherwise specified.

[0233] As described herein, various groups may be optionally substituted. Substituents are routinely utilized in chemistry including in development of various therapeutics. Many substituents can be utilized

in accordance with the present disclosure. In some embodiments, an optionally substituted group is unsubstituted. In some embodiments, an optionally substituted group is substituted. Substituents are preferably those that result in the formation of compounds for a desired property, activity, use, etc., as described herein. In some embodiments, compounds are stable for therapeutic use as described herein. The term "stable," as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain embodiments, their recovery, purification, and use for one or more of the purposes disclosed herein. In some embodiments, a substituent is a hydrocarbon group. In some embodiments, a substituent comprises a heteroatom. In some embodiments, a substituent comprises multiple heteroatoms. In some embodiments, each atom in a substituent is independently selected from hydrogen, carbon, halogen, nitrogen, oxygen, sulfur, phosphorus and silicon. In some embodiments, each atom in a substituent is independently selected from hydrogen, carbon, halogen, nitrogen, oxygen, and sulfur. In some embodiments, each atom in a substituent is independently selected from hydrogen, carbon, fluorine, chlorine, bromine, iodine, nitrogen, oxygen, and sulfur. In some embodiments, the total number of carbon and non-halogen heteroatom(s) in a substituent is about or no more than about 1; in some embodiments, it is no more than about 2; in some embodiments, it is no more than about 3; in some embodiments, it is no more than about 4; in some embodiments, it is no more than about 5; in some embodiments, it is no more than about 6; in some embodiments, it is no more than about 7; in some embodiments, it is no more than about 8; in some embodiments, it is no more than about 9; in some embodiments, it is no more than about 10; in some embodiments, it is no more than about 11; in some embodiments, it is no more than about 12; in some embodiments, it is no more than about 13; in some embodiments, it is no more than about 14; in some embodiments, it is no more than about 15; in some embodiments, it is no more than about 20. In some embodiments, the total number of carbon and non-halogen heteroatom(s) in each substituent is independently no more than about 20. In some embodiments, the total number of carbon and non-halogen heteroatom(s) in each substituent is independently no more than about 15. In some embodiments, the total number of carbon and non-halogen heteroatom(s) in each substituent is independently no more than about 10. In some embodiments, the total number of carbon and non-halogen heteroatom(s) in each substituent is independently no more than about 6.

Cleavable Unit

[0234] In some embodiments, a linker comprises a cleavable unit. In some embodiments, L^{Cl} is a cleavable unit.

[0235] In some embodiments, a cleavable unit is a chemically cleavable unit. Such a cleavable unit is cleavable under certain chemical conditions without catalysis of an enzyme. In some embodiments, a cleavable unit an acid cleavable unit. In some embodiments, a cleavable unit is or comprises a hydrazone group. In some embodiments, a cleavable unit is or comprises a carbonate group. In some embodiments, a cleavable unit is or comprises a silyl group. In some embodiments, a cleavable unit is or comprises —S—S—. In some embodiments, a cleavable unit is cleavable in the presence of GSH. In some

embodiments, a cleavable unit is cleavable in the presence of Fe (II). In some embodiments, a cleavable unit is or comprises 1,2,4-trioxolane moiety.

[0236] In some embodiments, a cleavable unit is an enzyme cleavable unit (i.e., a unit or a portion thereof that is cleaved by an enzyme). In some embodiments, an enzyme cleavable unit comprises a sequence of amino acids that is cleaved by a protease, e.g., a protease within a cell. In some embodiments, a protease breaks an amide bond between a sequence of amino acids within a cleavable unit. In some embodiments, an enzyme cleavable unit comprises an amino acid sequence that is cleaved by a protease (e.g., a human protease within a cell). In some embodiments, an enzyme cleavable unit comprises an amino acid sequence that is cleaved by a cathepsin (e.g., human cathepsin L, B, F, or S).

[0237] In some embodiments, an enzyme cleavable unit comprises between 1 and 12 amino acids. In some embodiments, an enzyme cleavable unit comprises between 2 and 6 amino acids. In some embodiments, an enzyme cleavable unit comprises between 2 and 5 amino acids. In some embodiments, an enzyme cleavable unit comprises between 2 and 4 amino acids. In some embodiments, an enzyme cleavable unit comprises 2 or 3 amino acids. In some embodiments, an enzyme cleavable unit comprises 3 or 4 amino acids. In some embodiments, an enzyme cleavable unit comprises 2 amino acids. In some embodiments, an enzyme cleavable unit comprises 3 amino acids. In some embodiments, an enzyme cleavable unit comprises 4 amino acids. In some embodiments, an enzyme cleavable unit comprises 5 amino acids. In some embodiments, an enzyme cleavable unit comprises 6 amino acids.

[0238] In some embodiments, an enzyme cleavable unit is –DGGFG–, –KGGFG–, –GGFG–, –GGFGG–, –GGFGGG–, –KGGF–, –DGGF–, –V–Cit–, –VA–, –FK–, –AA–, –VQ–, –LQ–, –FQ–, –TT–, –AAA–, –EA–, –YR–, –FR–, –YM–, –VR–, –MT–, –VT–, –VM–, –LM–, –AN–, –GGR–, –D–Val–D–Gln–, –D–Ala–D–Ala–, –DVA–, or –FM–. In some embodiments, an enzyme cleavable unit is an amino acid sequence selected from: –V–Cit–, –VA–, or –AA–. In some embodiments, an enzyme cleavable unit is an amino acid sequence selected from: –GGFG–, –V–Cit–, –AAA–, or –DVA–. In some embodiments, an enzyme cleavable unit is an amino acid sequence selected from: –DGGFG–, –KGGFG–, –GGFG–, –GGFGG–, –GGFGGG–, –KGGF–, or –DGGF–. In some embodiments, an enzyme cleavable unit is an amino acid sequence selected from: –V–Cit–, –VA–, VQ–, –VR–, –VT–, –VM–, or –D–Val–D–Gln–. In some embodiments, an enzyme cleavable unit is an amino acid sequence selected from: –GGFG– or –VA–. In some embodiments, an enzyme cleavable unit is an amino acid sequence selected from: –VQ–, or –LQ. In some embodiments, an enzyme cleavable unit is an amino acid sequence selected from: –LQ–, –FQ–, –TT–, –AAA–, or –EA–.

[0239] In some embodiments, an enzyme cleavable unit is –DGGFG–. In some embodiments, an enzyme cleavable unit is –KGGFG–. In some embodiments, an enzyme cleavable unit is –GGFG–. In some embodiments, an enzyme cleavable unit is –GGFGG–. In some embodiments, an enzyme cleavable unit is –GGFGGG–. In some embodiments, an enzyme cleavable unit is –KGGF–. In some embodiments, an enzyme cleavable unit is –DGGF–. In some embodiments, an enzyme cleavable unit is –V–Cit–. In some embodiments, an enzyme cleavable unit is –VA–. In some embodiments, an enzyme cleavable unit is –FK–. In some embodiments, an enzyme cleavable unit is –AA–. In some embodiments, an enzyme

cleavable unit is −VQ−. In some embodiments, an enzyme cleavable unit is −YR−. In some embodiments, an enzyme cleavable unit is −FR−. In some embodiments, an enzyme cleavable unit is −YM−. In some embodiments, an enzyme cleavable unit is −VR−. In some embodiments, an enzyme cleavable unit is −MT−. In some embodiments, an enzyme cleavable unit is −VT−. In some embodiments, an enzyme cleavable unit is −VM−. In some embodiments, an enzyme cleavable unit is −LM−. In some embodiments, an enzyme cleavable unit is −AN−. In some embodiments, an enzyme cleavable unit is −GGR−. In some embodiments, an enzyme cleavable unit is −D−Val−D−Gln−. In some embodiments, an enzyme cleavable unit is −D−Ala−D−Ala−. In some embodiments, an enzyme cleavable unit is −FM−. In some embodiments, an enzyme cleavable unit is −GGFG− or −VA−.

[0240] In some embodiments, a cleavable unit is cleavable in the presence of a glycosidase. In some embodiments, a cleavable unit is cleavable in the presence of beta-glucuronidase (e.g., in lysosomes). In some embodiments, a cleavable unit is or comprises a beta-glucuronide moiety. In some embodiments, a cleavable unit is cleavable in the presence of beta-galactosidase (e.g., in lysosomes). In some embodiments, a cleavable unit is or comprises a galactoside moiety.

[0241] In some embodiments, a cleavable unit is a phosphatase cleavable unit. In some embodiments, a cleavable unit is a pyrophosphates cleavable unit. In some embodiments, a cleavable unit is or comprises pyrophosphate. In some embodiments, a cleavable unit is cleavable in the presence of a phosphatase or pyrophosphates (e.g., in lysosomes).

[0242] In some embodiments, a cleavable unit is a sulfatase cleavable unit. In some embodiments, a cleavable unit is or comprises an arylsulfate moiety. In some embodiments, a cleavable unit is cleavable in the presence of a sulfatase (e.g., in lysosomes).

[0243] In some embodiments, a cleavable unit is a photo-responsive cleavable unit. In some embodiments, a cleavable unit is or comprises a heptamethine cyanine fluorophore moiety. In some embodiments, it is cleavable by irradiation with NIR light (λ = 650–900 nm). In some embodiments, a cleavable unit is or comprises an *o*-nitrobenzyl moiety. In some embodiments, it is cleavable by irradiation with UV light (λ = 365 nm). In some embodiments, a cleavable unit is or comprises PC4AP moiety. In some embodiments, it is cleavable by irradiation with near-infrared (NIR) light (λ = 365 nm) and intramolecular addition reaction with nearby amine.

[0244] In some embodiments, a cleavable unit is a bioorthogonal cleavable unit. In some embodiments, it is or comprises a dsProc moiety. In some embodiments, it is cleavable by the bioorthogonal cleavage pair: Cu(I)-BTTAA/dsProc.

[0245] Various other cleavable units are reported and can be utilized in accordance with the present disclosure, e.g., those described in Fu, Z., Li, S., Han, S. et al. Antibody drug conjugate: the "biological missile" for targeted cancer therapy. Sig Transduct Target Ther 7, 93 (2022). https://doi.org/10.1038/s41392-022-00947-7; Su Z, Xiao D, Xie F, Liu L, Wang Y, Fan S, Zhou X, Li S. Antibody-drug conjugates: Recent advances in linker chemistry. Acta Pharm Sin B. 2021 Dec;11(12):3889-3907. doi: 10.1016/j.apsb.2021.03.042. Epub 2021 Apr 6. PMID: 35024314; PMCID: PMC8727783; Sheyi R, de la Torre BG, Albericio F. Linkers: An Assurance for Controlled Delivery of

Antibody-Drug Conjugate. Pharmaceutics. 2022 Feb 11;14(2):396. doi: 10.3390/pharmaceutics14020396. PMID: 35214128; PMCID: PMC8874516; etc.

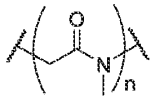
Spacer Moieties

[0246] In some embodiments, a linker comprises a spacer moiety (e.g., at least one spacer moiety). In some embodiments, a spacer moiety comprises an optionally substituted bivalent C1-12 aliphatic, aryl, heteroaryl, polyether, polyester, poly(N-(2-hydroxypropyl)methacrylamide) (pHPMA), polylactic-co-glycolic acid (PLGA), polymers of alkylene diamines, polysarcosine, saccharide, or combinations thereof. In some embodiments, a spacer moiety comprises an optionally substituted bivalent C1-12 aliphatic, polyether, polyester, poly(N-(2-hydroxypropyl)methacrylamide) (pHPMA), polylactic-co-glycolic acid (PLGA), polymers of alkylene diamines, polysarcosine, saccharide, or combinations thereof. In some embodiments, a spacer moiety comprises an optionally substituted bivalent polyether, polysarcosine, saccharide, or combinations thereof.

[0247] In some embodiments, a spacer moiety comprises an optionally substituted bivalent C_{1-10} aliphatic. In some embodiments, a spacer moiety comprises an optionally substituted bivalent C_{1-8} aliphatic. In some embodiments, a spacer moiety is an optionally substituted bivalent C_{1-6} aliphatic. In some embodiments, a spacer moiety comprises an optionally substituted bivalent C_{2-6} aliphatic. In some embodiments, a spacer moiety comprises an optionally substituted bivalent C_{3-6} aliphatic. In some embodiments, a spacer moiety comprises an optionally substituted bivalent C_{4-6} aliphatic. In some embodiments, a spacer moiety comprises an optionally substituted bivalent C_5 or C_6 aliphatic. In some embodiments, a spacer moiety comprises an optionally substituted bivalent C_3 aliphatic.

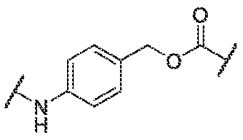
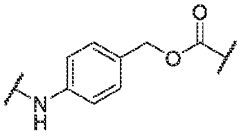
[0248] In some embodiments, a spacer moiety comprises polyethylene glycol having a structure $-(CH_2CH_2O)_n-$, wherein n is an integer between 0 and 24. In some embodiments, n is an integer between 1 and 24. In some embodiments, n is an integer between 1 and 12. In some embodiments, n is an integer between 1 and 11. In some embodiments, n is an integer between 1 and 10. In some embodiments, n is an integer between 1 and 9. In some embodiments, n is an integer between 1 and 8. In some embodiments, n is an integer between 2 and 8. In some embodiments, n is an integer between 3 and 8. In some embodiments, n is an integer between 4 and 8. In some embodiments, n is an integer between 6 and 8. In some embodiments, n is 7 or 8. In some embodiments, n is 1. In some embodiments, n is 2. In some embodiments, n is 2. In some embodiments, n is 3. In some embodiments, n is 4. In some embodiments, n is 5. In some embodiments, n is 6. In some embodiments, n is 7. In some embodiments, n is 8. In some embodiments, n is 9. In some embodiments, n is 10. In some embodiments, n is 11. In some embodiments, n is 12.

[0249] In some embodiments, a spacer moiety comprises polysarcosine having a structure:

[0250] 

[0251] wherein n is as described herein.

[0252] In some embodiments, a spacer moiety comprises a monosaccharide, disaccharide, or oligosaccharides. In some embodiments, a spacer moiety comprises a monosaccharide. In some embodiments, a spacer moiety comprises a glycoside. In some embodiments, a spacer moiety comprises a uronic acid or derivative thereof. In some embodiments, a spacer moiety comprises glucuronic acid or derivative thereof. In some embodiments, a spacer moiety comprises a glucuronide.

[0253] In some embodiments, a spacer moiety is optionally substituted  . In some embodiments, a spacer moiety is  .

[0254] In some embodiments, a linker is or comprises a structure of formulae A, B, or C:



A B C

or a pharmaceutically acceptable salt thereof, wherein:

represents a point of attachment to a target binding moiety, e.g., anti-HHLA2 antibody or antigen-binding fragment;

% represents a point of attachment to a payload moiety, e.g., exatecan or a derivative or an analog thereof, or pharmaceutically acceptable salt thereof;

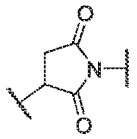
X is a moiety that results from the reaction of the unbound linker and a group present on the anti-HHLA2 antibody or antigen-binding fragment thereof (e.g., as described herein);

Y is a cleavable unit (e.g., an enzyme cleavable unit) as described herein; and

Z is a spacer moiety as described herein.

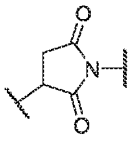
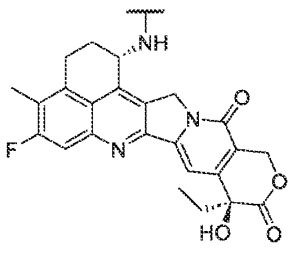
[0255] Various other spacer moieties are reported and can be utilized in accordance with the present disclosure, e.g., those described in Fu, Z., Li, S., Han, S. et al. Antibody drug conjugate: the "biological missile" for targeted cancer therapy. Sig Transduct Target Ther 7, 93 (2022). https://doi.org/10.1038/s41392-022-00947-7; Su Z, Xiao D, Xie F, Liu L, Wang Y, Fan S, Zhou X, Li S. Antibody-drug conjugates: Recent advances in linker chemistry. Acta Pharm Sin B. 2021 Dec;11(12):3889-3907. doi: 10.1016/j.apsb.2021.03.042. Epub 2021 Apr 6. PMID: 35024314; PMCID: PMC8727783; Sheyi R, de la Torre BG, Albericio F. Linkers: An Assurance for Controlled Delivery of Antibody-Drug Conjugate. Pharmaceutics. 2022 Feb 11;14(2):396. doi: 10.3390/pharmaceutics14020396. PMID: 35214128; PMCID: PMC8874516; etc.

[0256] In some embodiments, a linker is or comprises $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ wherein each variable is independently as described herein. In some embodiments, a linker is or comprises $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ wherein each variable is independently as described herein. In some embodiments, a linker is or comprises $-SM1-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ wherein SM1 is



wherein the carbon atom is bonded to a target binding moiety (e.g., an antibody), and each variable is independently as described herein. In some embodiments, a linker is or comprises $-SM1-(CH_2)_x-C(O)-N(R')-(CH_2CH_2O)n-(CH_2)_z-C(O)-L^C-$, wherein each variable is independently as described herein. In some embodiments, a linker is or comprises $-SM1-(CH_2)_x-C(O)-NH-(CH_2CH_2O)n-(CH_2)_z-C(O)-L^C-$, wherein each variable is independently as described herein. In some embodiments, a linker is or comprises $-SM1-(CH_2)_x-C(O)-N(R')-(CH_2CH_2O)n-(CH_2)_z-C(O)-GGFG-$, wherein each variable is independently as described herein. In some embodiments, a linker is or comprises $-SM1-(CH_2)_x-C(O)-NH-(CH_2CH_2O)n-(CH_2)_z-C(O)-GGFG-$, wherein each variable is independently as described herein. In some embodiments, a linker is or comprises $-SM1-(CH_2)_x-C(O)-N(R')-(CH_2CH_2O)n-(CH_2)_z-C(O)-Val-Ala-PABA-$, wherein each variable is independently as described herein. In some embodiments, a linker is or comprises $-SM1-(CH_2)_x-C(O)-NH-(CH_2CH_2O)n-(CH_2)_z-C(O)-Val-Ala-PABA-$, wherein each variable is independently as described herein. In some embodiments, a linker is or comprises $-SM1-(CH_2)_x-C(O)-N(R')-(CH_2CH_2O)n-(CH_2)_z-C(O)-Val-Cit-PABA-$, wherein each variable is independently as described herein. In some embodiments, a linker is or comprises $-SM1-(CH_2)_x-C(O)-NH-(CH_2CH_2O)n-(CH_2)_z-C(O)-Val-Cit-PABA-$, wherein each variable is independently as described herein. In some embodiments, a linker is or comprises $-SM1-(CH_2)_x-C(O)-N(R')-(CH_2CH_2O)n-(CH_2)_z-N(R')-C(O)-oGlu-$, wherein each variable is independently as described herein. In some embodiments, a linker is or comprises $-SM1-(CH_2)_x-C(O)-NH-(CH_2CH_2O)n-(CH_2)_z-NH-C(O)-oGlu-$, wherein each variable is independently as described herein. In some embodiments, a linker is or comprises $-SM1-(CH_2)_x-C(O)-N(R')-(CH_2CH_2O)n-(CH_2)_z-N(R')-C(O)-mGlu-$, wherein each variable is independently as described herein. In some embodiments, a linker is or comprises $-SM1-(CH_2)_x-C(O)-NH-(CH_2CH_2O)n-(CH_2)_z-NH-C(O)-mGlu-$, wherein each variable is independently as described herein. In some embodiments, x is 2-10. In some embodiments, n is 2-10. In some embodiments, z is 2-10. In some embodiments, x is 2, n is 8 and z is 2. In some embodiments, x is 3, n is 8, and z is 2. In some embodiments, x is 5, n is 8 and z is 2. In some embodiments, SM1 is attached to −S− of a cysteine residue. Various conjugates comprising such linkers are described herein.

[0257] In some embodiments, a linker−payload moiety is or comprises $-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ wherein each variable is independently as described herein. In some embodiments, a linker−payload moiety is or comprises $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ wherein each variable is independently as described herein. In some embodiments, a linker-payload moiety is or

comprises –SM1–L^X–$(CH_2CH_2O)_n$–L^{ZP}–L^C–R^P, wherein SM1 is  wherein the carbon atom is bonded to a target binding moiety (e.g., an antibody), and each variable is independently as described herein. In some embodiments, a linker-payload moiety is or comprises –SM1–$(CH_2)_x$–C(O)–N(R')–$(CH_2CH_2O)_n$–$(CH_2)_z$–C(O)–L^C–R^P, wherein each variable is independently as described herein. In some embodiments, a linker-payload moiety is or comprises –SM1–$(CH_2)_x$–C(O)–NH–$(CH_2CH_2O)_n$–$(CH_2)_z$–C(O)–L^C–R^P, wherein each variable is independently as described herein. In some embodiments, a linker-payload moiety is or comprises –SM1–$(CH_2)_x$–C(O)–N(R')–$(CH_2CH_2O)_n$–$(CH_2)_z$–C(O)–GGFG–R^P, wherein each variable is independently as described herein. In some embodiments, a linker-payload moiety is or comprises –SM1–$(CH_2)_x$–C(O)–NH–$(CH_2CH_2O)_n$–$(CH_2)_z$–C(O)–GGFG–R^P, wherein each variable is independently as described herein. In some embodiments, a linker-payload moiety is or comprises –SM1–$(CH_2)_x$–C(O)–N(R')–$(CH_2CH_2O)_n$–$(CH_2)_z$–C(O)–Val–Ala–PABA–R^P, wherein each variable is independently as described herein. In some embodiments, a linker-payload moiety is or comprises –SM1–$(CH_2)_x$–C(O)–NH–$(CH_2CH_2O)_n$–$(CH_2)_z$–C(O)–Val–Ala–PABA–R^P, wherein each variable is independently as described herein. In some embodiments, a linker-payload moiety is or comprises –SM1–$(CH_2)_x$–C(O)–N(R')–$(CH_2CH_2O)_n$–$(CH_2)_z$–C(O)–Val–Cit–PABA–R^P, wherein each variable is independently as described herein. In some embodiments, a linker-payload moiety is or comprises –SM1–$(CH_2)_x$–C(O)–NH–$(CH_2CH_2O)_n$–$(CH_2)_z$–C(O)–Val–Cit–PABA–R^P, wherein each variable is independently as described herein. In some embodiments, a linker-payload moiety is or comprises –SM1–$(CH_2)_x$–C(O)–N(R')–$(CH_2CH_2O)_n$–$(CH_2)_z$–N(R')–C(O)–oGlu–R^P, wherein each variable is independently as described herein. In some embodiments, a linker-payload moiety is or comprises –SM1–$(CH_2)_x$–C(O)–NH–$(CH_2CH_2O)_n$–$(CH_2)_z$–NH–C(O)–oGlu–R^P, wherein each variable is independently as described herein. In some embodiments, a linker-payload moiety is or comprises –SM1–$(CH_2)_x$–C(O)–N(R')–$(CH_2CH_2O)_n$–$(CH_2)_z$–N(R')–C(O)–mGlu–R^P, wherein each variable is independently as described herein. In some embodiments, a linker-payload moiety is or comprises –SM1–$(CH_2)_x$–C(O)–NH–$(CH_2CH_2O)_n$–$(CH_2)_z$–NH–C(O)–mGlu–R^P, wherein each variable is independently as described herein. In some embodiments, x is 2-10. In some embodiments, n is 2-10. In some embodiments, z is 2-10. In some embodiments, x is 2, n is 8 and z is 2. In some embodiments, x is 3, n is 8, and z is 2. In some embodiments, x is 5, n is 8 and z is 2. In some embodiments, R^P is a exatecan payload moiety. In some embodiments, R^P is  . Various

conjugates comprising such linker-payload moieties are described herein. For example, in some embodiments, a target binding moiety is or comprises an antibody or an antigen-binding fragment thereof. In some embodiments, a target binding moiety is or comprises an anti-HHLA2 antibody or an antigen-binding fragment thereof. In some embodiments, a target binding moiety is or comprises Hu2C4-H3L3, 65872, 65889, or 887-1DE, or an antigen-binding fragment thereof. In some embodiments, a target binding moiety comprises 1, 2, 3, 4, 5, or 6 CDRs of an antibody, e.g., Hu2C4-H3L3, 65872, 65889, or 887-1DE. In some embodiments, a target binding moiety is Hu2C4-H3L3, 65872, 65889, or 887-1DE.

Conjugates

[0258] In some embodiments, the present disclosure provides a conjugate comprising a target binding moiety, a payload moiety, and a linker that connects the target binding moiety and the payload moiety. In some embodiments, a linker moiety comprises a PEG moiety. In some embodiments, a linker is or comprises $-(CH_2CH_2O)n-$ wherein n is 1-24. In some embodiments, n is 4. In some embodiments, n is 8. In some embodiments, $-(CH_2CH_2O)n-$ in a linker of a conjugate is $-(CH_2CH_2O)_4-$. In some embodiments, $-(CH_2CH_2O)n-$ in a linker of a conjugate is $-(CH_2CH_2O)_8-$. In some embodiments, the PEG moiety in a linker or conjugate is PEG4. In some embodiments, the PEG moiety in a linker or conjugate is PEG8. In some embodiments, a payload moiety is or comprises a moiety of exatecan or a derivative or an analog thereof, or a pharmaceutically acceptable salt thereof.

[0259] In some embodiments, the present disclosure provides a conjugate comprising:

i) a target binding moiety;

ii) a payload moiety; and

iii) a linker that connects the target binding moiety and the payload moiety;

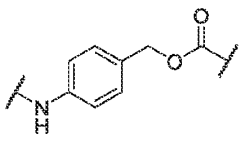
wherein:

the linker comprises $-(CH_2CH_2O)n-$ and L^C;

n is 0-24;

L^C is bonded to a payload moiety and is $-L^{Cl}-L^{CO}-$;

L^{Cl} is a cleavable unit; and

L^{CO} is a covalent bond, or a spacer moiety (e.g., optionally substituted  wherein the carbonyl is bonded to a payload moiety).

[0260] For example, in some embodiments, the present disclosure provides conjugates comprising:

i) a target binding moiety;

ii) a payload moiety; and

iii) a linker that connects the target binding moiety and the payload moiety;

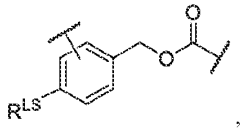
wherein:

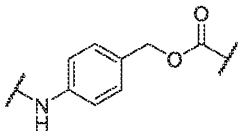
the linker comprises $-(CH_2CH_2O)n-$ and L^C;

n is 0-24;

L^C is bonded to a payload moiety and is $-L^{Cl}-L^{CO}-$;

L^{Cl} is –Gly–Gly–Phe–Gly–, –Val–Ala–, –Val–Cit–, L^{ClS}, –Gly–Gly–Phe–Gly–N(R')–L^{ClS}–, –Val–Ala–N(R')–L^{ClS}–, or –Val–Cit–N(R')–L^{ClS}–;

L^{ClS} is  , wherein the phenyl ring and the $-CH_2-$ is optionally substituted, and the carbonyl is bonded to L^{CO}, and R^{LS} is or comprises a sugar moiety;

L^{CO} is a covalent bond, or optionally substituted  wherein the carbonyl is bonded to a payload moiety;

each R' is independently –R, –C(O)R, $-CO_2R$, or $-S(O)_2R$, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

[0261] In some embodiments, the present disclosure provides a conjugate, comprising:

i) a target binding moiety;

ii) a payload moiety; and

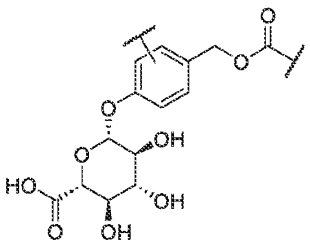
iii) a linker that connects the target binding moiety and the payload moiety;

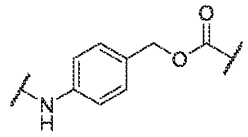
wherein:

the linker comprises $-(CH_2CH_2O)n-$ and L^C;

n is 0-24;

L^C is bonded to a payload moiety and is $-L^{Cl}-L^{CO}-$;

L^{Cl} is –Gly–Gly–Phe–Gly–, –Val–Ala–, –Val–Cit–, or  ; and

L^{CO} is a covalent bond, or optionally substituted  wherein the carbonyl is bonded to a payload moiety.

[0262] In some embodiments, a linker comprises a PEG moiety. In some embodiments, a linker comprises –$(CH_2CH_2O)n$– wherein n is 1-24. In some embodiments, a PEG moiety is PEG4. In some embodiments, a PEG moiety is PEG8. Among other things, the present disclosure demonstrates that antibody-drug conjugates comprising linkers that comprise PEG moieties can provide various benefits and advantages. For example, in some embodiments, with such linkers payload moieties can be directly connected to cleavable units, e.g., GGFG, without spacers as utilized in reported products, to provide antibody-drug conjugates with comparable or improved properties and/or activities. In some embodiments, conjugates with such linkers have reduced aggregation compared to reference conjugates without such linkers. For example, in some embodiments, the present disclosure provides a conjugate, comprising:

i) a target binding moiety;

ii) a payload moiety; and

iii) a linker that connects the target binding moiety and the payload moiety;

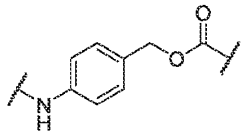
wherein:

the linker comprises –$(CH_2CH_2O)n$– and L^C;

n is 1-24;

L^C is bonded to a payload moiety and is –L^{Cl}–L^{CO}–;

L^{Cl} is a cleavable unit; and

L^{CO} is a covalent bond, or a spacer moiety (e.g., optionally substituted  wherein the carbonyl is bonded to a payload moiety).

[0263] For example, in some embodiments, the present disclosure provides a conjugate, comprising:

i) a target binding moiety;

ii) a payload moiety; and

iii) a linker that connects the target binding moiety and the payload moiety;

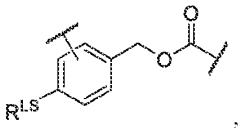
wherein:

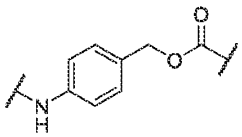
the linker comprises –$(CH_2CH_2O)n$– and L^C;

n is 1-24;

L^C is bonded to a payload moiety and is –L^{Cl}–L^{CO}–;

L^{Cl} is –Gly–Gly–Phe–Gly–, –Val–Ala–, –Val–Cit–, L^{ClS}, –Gly–Gly–Phe–Gly–N(R')–L^{ClS}–, –Val–Ala–N(R')–L^{ClS}–, or –Val–Cit–N(R')–L^{ClS}–;

L^{C1S} is  , wherein the phenyl ring and the $-CH_2-$ is optionally substituted, and the carbonyl is bonded to L^{CO}, and R^{LS} is or comprises a sugar moiety;

L^{CO} is a covalent bond, or optionally substituted  wherein the carbonyl is bonded to a payload moiety;

each R' is independently –R, –C(O)R, –CO_2R, or –$S(O)_2$R, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

[0264] In some embodiments, the present disclosure provides a conjugate, comprising:

i) a target binding moiety;

ii) a payload moiety; and

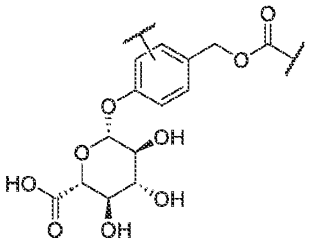
iii) a linker that connects the target binding moiety and the payload moiety;

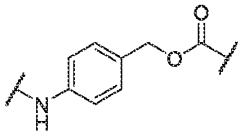
wherein:

the linker comprises –(CH_2CH_2O)n– and L^C;

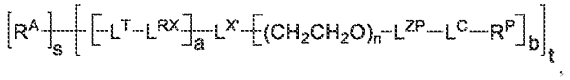
n is 1-24;

L^C is bonded to a payload moiety and is $-L^{C1}-L^{CO}-$;

L^{C1} is –Gly–Gly–Phe–Gly–, –Val–Ala–, –Val–Cit–, or  ; and

L^{CO} is a covalent bond, or optionally substituted  wherein the carbonyl is bonded to a payload moiety.

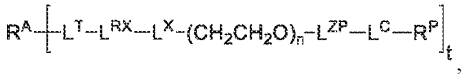
[0265] In some embodiments, a conjugate is a compound having a structure of Formula A-1 or a salt thereof:



A-1

wherein each variable is independently as described herein.

[0266] In some embodiments, a conjugate is a compound having a structure of Formula A-1 or a salt thereof:



A-2

wherein each variable is independently as described herein.

[0267] Various embodiments of certain variables are described, e.g., in Linkers section, as examples. Additional embodiments for variables are described below as examples.

R^A

[0268] In some embodiments, each R^A is independently a target binding moiety as described herein. In some embodiments, each R^A is independently a target binding moiety comprising an antibody or an antigen-binding fragment thereof. In some embodiments, each occurrence of a target binding moiety is an antibody or an antigen-binding fragment thereof. In some embodiments, each occurrence of a target binding moiety is an antibody. In some embodiments, each occurrence of a target binding moiety is a monoclonal antibody. In some embodiments, each occurrence of a target binding moiety is not trastuzumab. In some embodiments, each occurrence of a target binding moiety shares less than about 90%, 95%, or 99% sequence identity to trastuzumab. In some embodiments, each occurrence of a target binding moiety shares less than about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to trastuzumab. In some embodiments, each occurrence of a target binding moiety is not an anti-HER2 antibody. In some embodiments, each occurrence of a target binding moiety is not an anti-HER2 antibody or an antigen-binding fragment thereof.

[0269] In some embodiments, each target binding moiety of a provided conjugate targets the same agent. In some embodiments, each target binding moiety targets the same agent, wherein the agent is or comprises a polypeptide, a nucleic acid, or a lipid. In some embodiments, each target binding moiety targets the same agent, wherein the agent is a polypeptide. In some embodiments, each target binding moiety targets the same antigen. In some embodiments, each target binding moiety is independently an antibody or an antigen-binding fragment thereof, and each target binding moiety shares about 90%, 95%, 96%, 97%, 98% or 99% sequence identity. In some embodiments, each target binding moiety targets the same antigen. In some embodiments, each target binding moiety is independently an antibody or an antigen-binding fragment thereof, and each target binding moiety shares the same amino acid sequence. In some embodiments, each target binding moiety is the same.

[0270] In some embodiments, a target binding moiety is or comprises an antibody or an antigen-binding fragment thereof. In some embodiments, a target binding moiety is or comprises an anti-HHLA2 antibody or an antigen-binding fragment thereof. In some embodiments, a target binding moiety is or comprises Hu2C4-H3L3, 65872, 65889, or 887-1DE, or an antigen-binding fragment thereof. In some embodiments, a target binding moiety comprises 1, 2, 3, 4, 5, or 6 CDRs of an antibody, e.g., Hu2C4-H3L3, 65872, 65889, or 887-1DE. In some embodiments, a target binding moiety is Hu2C4-H3L3, 65872, 65889, or 887-1DE.

s

[0271] In some embodiments, a conjugate contains a single target binding moiety. In some embodiments, a conjugate contains two or more target binding moieties.

[0272] In some embodiments, s is 1. In some embodiments, s is 2-20, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20.

a

[0273] In some embodiments, a linker is bonded to a single site on a target binding moiety. In some embodiments, an occurrence of a is 1. In some embodiments, each occurrence of a is 1.

[0274] In some embodiments, a linker is bonded to two or more target binding moieties. In some embodiments, a linker bonds is bonded to two or more sites on a target binding moiety (e.g., two or more Cys residues of an antibody or an antigen-binding fragment thereof). In some embodiments, a is greater than 1. In some embodiments, an occurrence of a is 2-10, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10. In some embodiments, an occurrence of a is 2. In some embodiments, an occurrence of a is 3-10.

[0275] In some embodiments, each a is the same.

[0276] For each $\left[-L^{T}-L^{RX}\right]_{a}-L^{X'}-\left[(CH_2CH_2O)_n-L^{ZP}-L^{C}-R^{P}\right]_{b}$, in some embodiments, each L^{T} is the same; in some embodiments, each L^{RX} is the same; in some embodiments, each $-L^{T}-L^{RX}-$ is the same.

[0277] In some embodiments, each L^{T} is the same. In some embodiments, each L^{RX} is the same. In some embodiments, each $-L^{T}-L^{RX}-$ is the same.

b

[0278] In some embodiments, a linker is bonded to a single site on a payload moiety. In some embodiments, an occurrence of b is 1. In some embodiments, each occurrence of b is 1.

[0279] In some embodiments, a linker is bonded to two or more payload moieties. In some embodiments, a linker bonds is bonded to two or more sites on a payload moiety. In some embodiments, b is greater than 1. In some embodiments, an occurrence of b is 2-10, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10. In some embodiments, an occurrence of b is 2. In some embodiments, an occurrence of b is 3-10.

[0280] In some embodiments, b is the same.

[0281] In some embodiments, each $L^{X'}$ is the same.

[0282] In some embodiments, at least one occurrence of n is independently 1-24. In some embodiments, each occurrence of n is independently 1-24. In some embodiments, each n is 8. For each $\left[-L^{T}-L^{RX}\right]_{a}-L^{X}-\left[(CH_2CH_2O)_n-L^{ZP}-L^{C}-R^{P}\right]_{b}$, in some embodiments, each n is the same; in some embodiments, each L^{ZP} is the same; in some embodiments, each L^{C} is the same; in some embodiments, each R^{P} is the same; in some embodiments, each $-(CH_2CH_2O)n-L^{ZP}-$ is the same; in some embodiments, each $-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is the same; in some embodiments, each $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is the same; in some embodiments, each $-L^{C}-R^{P}$ is the same; in some embodiments, $-L^{ZP}-L^{C}-R^{P}$ is the same.

[0283] In some embodiments, each n is the same. In some embodiments, each L^{ZP} is the same. In some embodiments, each L^{C} is the same. In some embodiments, each R^{P} is the same. In some embodiments, each $-(CH_2CH_2O)n-L^{ZP}-$ is the same. In some embodiments, each $-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ is the same. In some embodiments, each $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is the same. In some embodiments, each $-L^{C}-R^{P}$ is the same. In some embodiments, $-L^{ZP}-L^{C}-R^{P}$ is the same.

t

[0284] In some embodiments, a conjugate contains a single linker moiety. In some embodiments, a conjugate contains two or more linker moieties.

[0285] In some embodiments, t is 1. In some embodiments, t is 2-20 (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 2-15, 2-10, 2-8, etc.). In some embodiments, t is about 1-8. In some embodiments, t is about 2-8. In some embodiments, t is about between 1 and 16. In some embodiments, t is about between 1 and 12. In some embodiments, t is about between 2 and 10. In some embodiments, t is about between 4 and 8. In some embodiments, t is about 1. In some embodiments, t is about 2. In some embodiments, t is about 3. In some embodiments, t is about 4. In some embodiments, t is about 5. In some embodiments, t is about 6. In some embodiments, t is about 7. In some embodiments, t is about 8. In some embodiments, t is about an integer between about 7 and about 9. In some embodiments, t is about an integer between about 6 and about 10.

[0286] In some embodiments, s is 1, a is 1 and b is 1, and t is a DAR as described herein. In some embodiments, for a conjugate of formula A-2 or a salt thereof, t is a DAR as described herein.

[0287] For example, in some embodiments, each occurrence of L^{X} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic, wherein 1-10 methylene units of L^{X} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –S(O)$_2$–, –C(O)N(R')–, –S(O)$_2$(NR')–, and –Cy–. In some embodiments, each occurrence of L^{X} is $-(CH_2)_X-C(O)-N(R')-$, wherein x is 1-19, and –N(R')– is bonded to $-(CH_2CH_2O)n-$. In some embodiments, each occurrence of L^{X} is $-(CH_2)_X-C(O)-NH-$, wherein x is 1-19, and –NH– is bonded to $-(CH_2CH_2O)n-$. In some embodiments, each occurrence of L^{X} is $-(CH_2)_X-C(O)-N(R')-$ or $-(CH_2)_X-C(O)-NH-$, and x is 1-6. In some embodiments, each occurrence of L^{X} is $-(CH_2)_5-C(O)-N(R')-$, wherein –N(R')– is bonded to $-(CH_2CH_2O)n-$. In some embodiments, each occurrence of L^{X} is $-(CH_2)_5-C(O)-NH-$, wherein –NH– is bonded to $-(CH_2CH_2O)n-$. In some

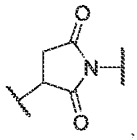
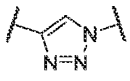
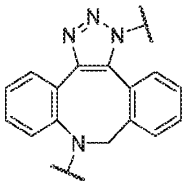
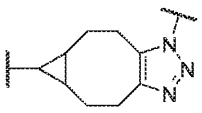
embodiments, each occurrence of L^X is $-(CH_2)_3-C(O)-N(R')-$, wherein $-N(R')-$ is bonded to $-(CH_2CH_2O)n-$. In some embodiments, each occurrence of L^X is $-(CH_2)_3-C(O)-NH-$, wherein $-NH-$ is bonded to $-(CH_2CH_2O)n-$. In some embodiments, each occurrence of L^X is the same.

[0288] In some embodiments, an occurrence of L^T is a covalent bond. In some embodiments, an occurrence of L^T is an optionally substituted bivalent straight or branched C_{1-20} aliphatic, wherein 1-10 methylene units of L^T are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, and $-S(O)_2(NR')-$. In some embodiments, an occurrence of L^T is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic group. In some embodiments, an occurrence of L^T is a bivalent straight or branched C_{1-20} aliphatic group. In some embodiments, an occurrence of L^T is an optionally substituted $-(CH_2)_{1-5}-$. In some embodiments, an occurrence of L^T is $-(CH_2)_{1-5}-$. In some embodiments, an occurrence of L^T is $-CH_2-$. In some embodiments, an occurrence of L^T is bonded to a backbone atom of an amino acid residue in a target binding moiety. In some embodiments, an occurrence of L^T is independently bonded to a backbone atom of an amino acid residue in the same target binding moiety. In some embodiments, an occurrence of L^T is bonded to a different backbone atom of an amino acid residue in the same target binding moiety. In some embodiments, an occurrence of L^T is bonded to an alpha atom of an amino acid residue in a target binding moiety. In some embodiments, an occurrence of L^T is bonded to an alpha atom of an amino acid residue in the same target binding moiety. In some embodiments, an occurrence of L^T is bonded to a different alpha atom of an amino acid residue in the same target binding moiety. In some embodiments, each occurrence of L^T is the same.

[0289] In some embodiments, an occurrence of L^{RX} is a covalent bond. In some embodiments, an occurrence of L^{RX} is a moiety that results from a reaction between a group present on the unconjugated target binding moiety and a group present on an agent comprising $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ and a payload moiety. In some embodiments, an occurrence of L^{RX} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{RX} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$, wherein each $-Cy-$ is independently an optionally substituted 3-12 membered ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur.

[0290] In some embodiments, an occurrence of L^{RX} is $-L^{RX1}-L^{RX2}-$, wherein each of L^{RX1} and L^{RX2} is independently as described herein.

[0291] In some embodiments, an occurrence of $-L^{RX1}-$ is $-S-$. In some embodiments, an occurrence of $-L^{RX1}-$ is $-S-$, and the $-S-$ is of a Cys residue of the target binding moiety. In some embodiments, an occurrence of $-L^{RX1}-$ is $-S-$, and the $-S-$ is of a Cys residue in an antibody wherein the Cys residue forms an interchain disulfide bonds in an unconjugated antibody. In some embodiments, an occurrence of $-L^{RX1}-$ is $-S-$, and the $-S-$ is of a Cys residue in an antibody wherein the Cys residue forms an inter-heavy- and light-chain disulfide bond in an unconjugated antibody. In some embodiments, an occurrence of $-L^{RX1}-$ is $-S-$, and the $-S-$ is of a Cys residue of the target binding moiety, wherein the Cys residue is derived through partial or complete reduction of the interchain disulfide bonds.

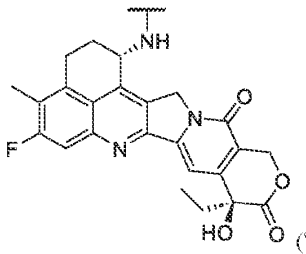
[0292] In some embodiments, an occurrence of $-L^{RX2}-$ is  , wherein the nitrogen is bonded to L^X, and the carbon is bonded to L^{RX1}. In some embodiments, an occurrence of $-L^{RX2}-$ is $-S-$. In some embodiments, an occurrence of L^{RX} is or comprises a click chemistry product moiety. In some embodiments, L^{RX} is or comprises a click chemistry product moiety. In some embodiments, an occurrence of L^{RX} is or comprises  . In some embodiments, an occurrence of wherein L^{RX} is or comprises  . In some embodiments, an occurrence of L^{RX} is or comprises  . In some embodiments, the nitrogen in the 5-membered ring is bonded to L^X. In some embodiments, the carbon or the nitrogen in the 8-membered ring is bonded to L^X.

[0293] In some embodiments, an occurrence of n is 1-24. In some embodiments, an occurrence of n is 4-24. In some embodiments, an occurrence of n is 7-20. In some embodiments, each occurrence of n is independently 4-24. In some embodiments, each occurrence of n is independently 7-20. In some embodiments, an occurrence of n is 8. In some embodiments, each n is the same. In some embodiments, each n is 8.

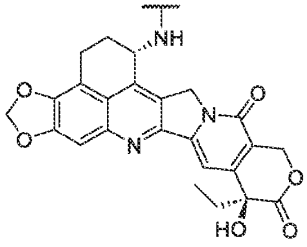
[0294] In some embodiments, an occurrence of L^{ZP} is a covalent bond. In some embodiments, an occurrence of L^{ZP} is an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^Z are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$. In some embodiments, an occurrence of L^{ZP} is $-(CH_2)z-C(O)-$, wherein z is 1-19. In some embodiments, an occurrence of L^{ZP} is $-(CH_2)_2-C(O)-$, wherein $-C(O)-$ is bonded to the amino group of the first amino acid in $-L^C-$. In some embodiments, each L^{ZP} is the same.

R^P

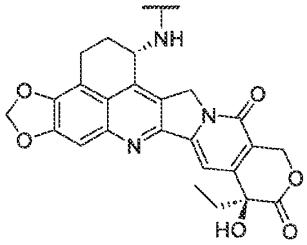
[0295] As used herein, each R^P is independently payload moiety. Various payload moieties are available and can be utilized in accordance with the present disclosure. In some embodiments, each R^P is the same. In some embodiments, an occurrence of R^P is a cytotoxic agent. In some embodiments, each R^P is independently a cytotoxic agent. In some embodiments, an occurrence of R^P is exatecan or a derivative thereof. In some embodiments, each R^P is independently exatecan or a derivative thereof. In some



embodiments, an occurrence of R^P is ("R^P-Ex" or "R^{EX}"). In some embodiments, each R^P is R^P-Ex. In some embodiments, an occurrence of R^P is



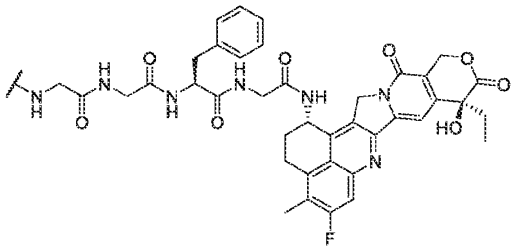
. In some embodiments, each occurrence of R^P is



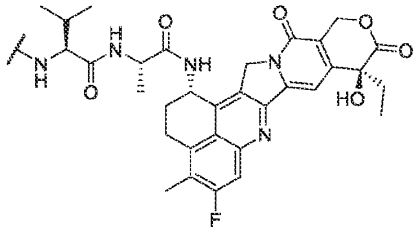
.

[0296] In some embodiments, a conjugate comprises L^C–R^P. In some embodiments, an occurrence of –L^C–R^P is –GGFG–R^P. In some embodiments, it is –Val–Ala–PABA–R^P. In some embodiments, it is –Val–Cit–PABA–R^P. In some embodiments, it is –Val–Ala–NH–oGlu–R^P. In some embodiments, it is –Val–Ala–NH–mGlu–R^P. In some embodiments, it is –Val–Cit–NH–oGLu–R^P. In some embodiments, it is –Val–Cit–NH–mGLu–R^P. In some embodiments, it is –oGlu–R^P. In some embodiments, it is –mGlu–R^P. In some embodiments, R^P comprises a nitrogen atom and is bonded at the nitrogen atom, e.g., to the rest of a conjugate or other compound. In some embodiments, R^P is R^{EX}. In some embodiments, each occurrence of –L^C–R^P is the same.

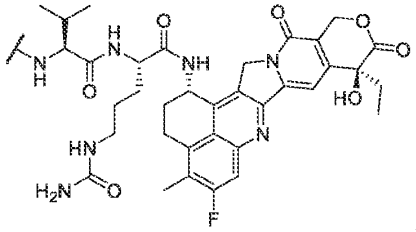
[0297] In some embodiments, an occurrence of –L^C–R^P is



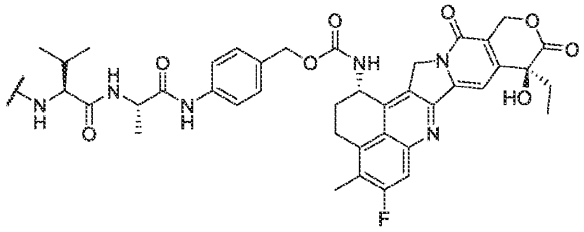
,



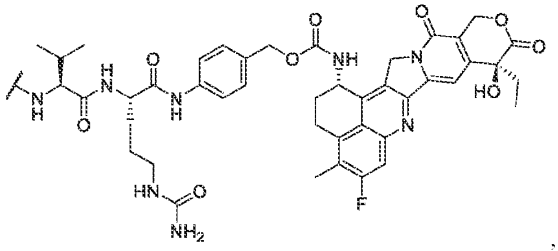
.



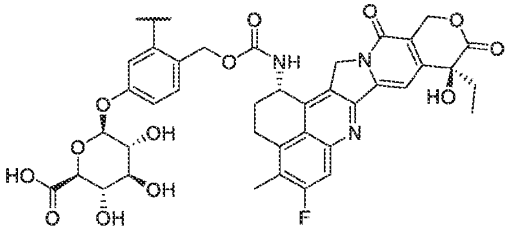
,



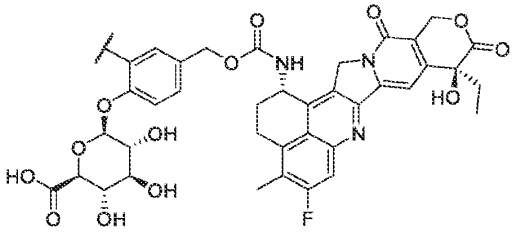
,



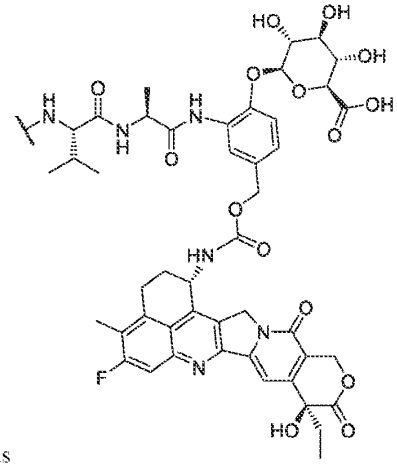
,



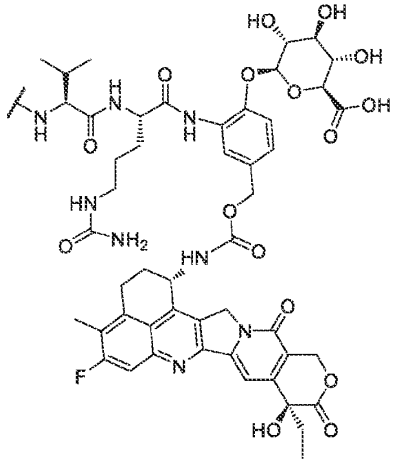
, or



. In some embodiments, each $-L^C-R^P$ is the same.

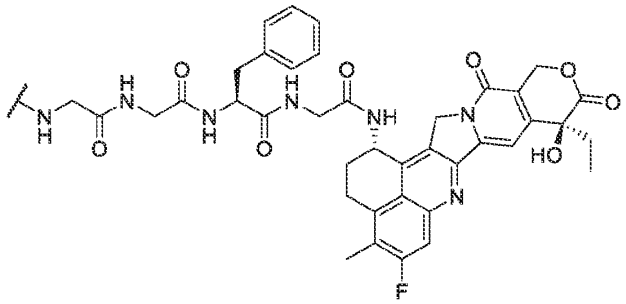


[0298] In some embodiments, an occurrence of $-L^C-R^P$ is

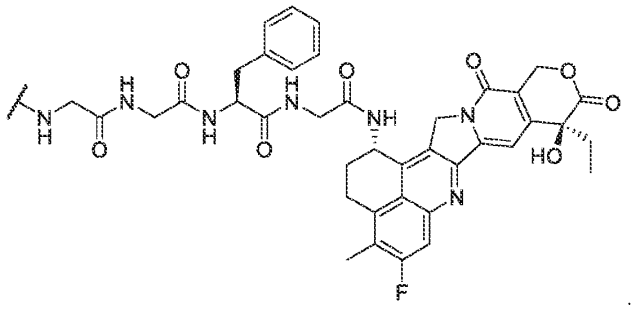


or . In some embodiments, each $-L^C-R^P$ is the same.

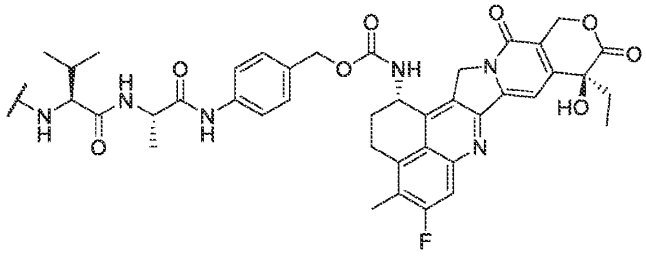
[0299] In some embodiments, an occurrence of $-L^C-R^P$ is



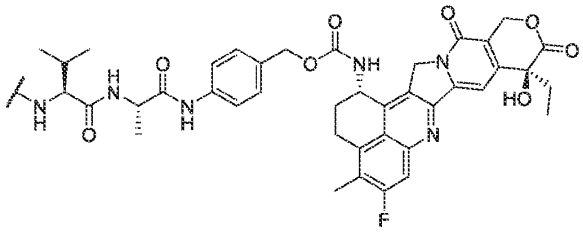
[0300] In some embodiments, each occurrence of $-L^C-R^P$ is independently



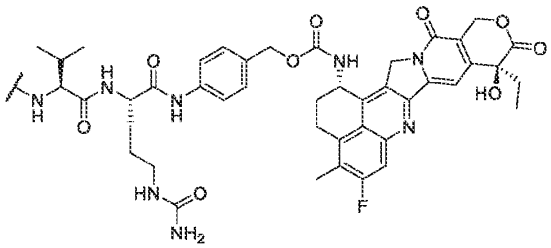
[0301] In some embodiments, an occurrence of $-L^C-R^P$ is



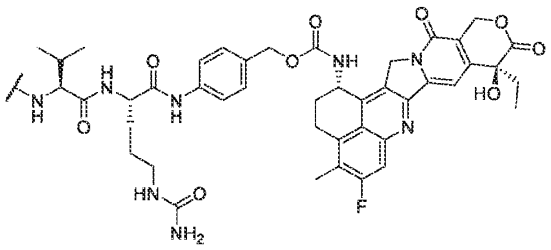
[0302] In some embodiments, each occurrence of $-L^C-R^P$ is



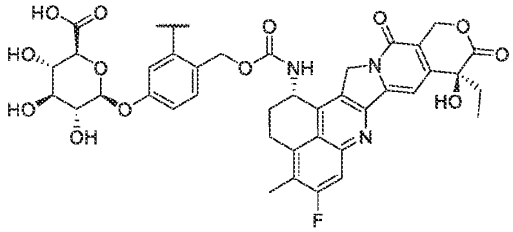
[0303] In some embodiments, an occurrence of $-L^C-R^P$ is



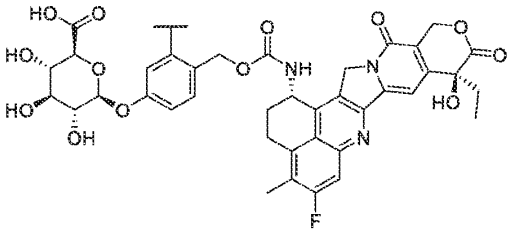
[0304] In some embodiments, each occurrence of $-L^C-R^P$ is



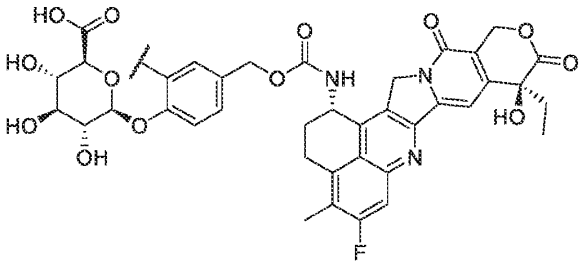
[0305] In some embodiments, an occurrence of $-L^C-R^P$ is



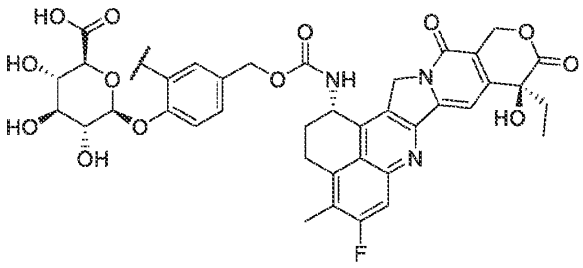
. In some embodiments, each occurrence of



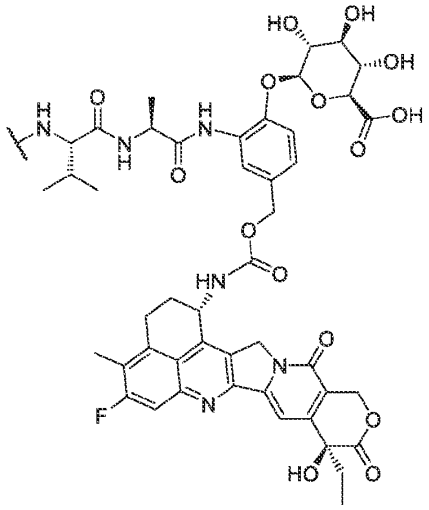
$-L^C-R^P$ is . In some embodiments, an occurrence



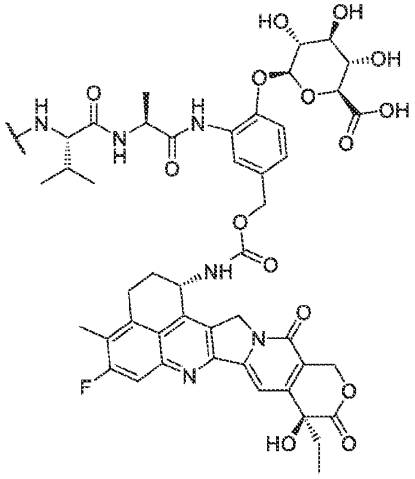
of $-L^C-R^P$ is . In some embodiments, each



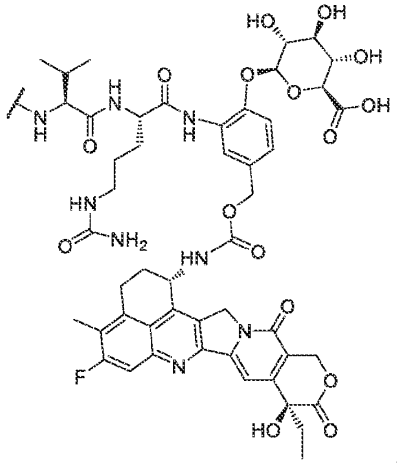
occurrence of $-L^C-R^P$ is .In some



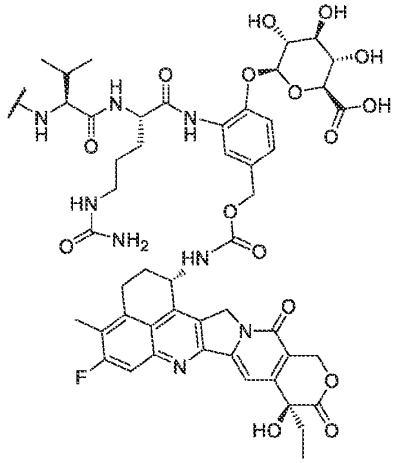
embodiments, an occurrence of $-L^C-R^P$ is . In some



embodiments, each occurrence of $-L^C-R^P$ is . In some



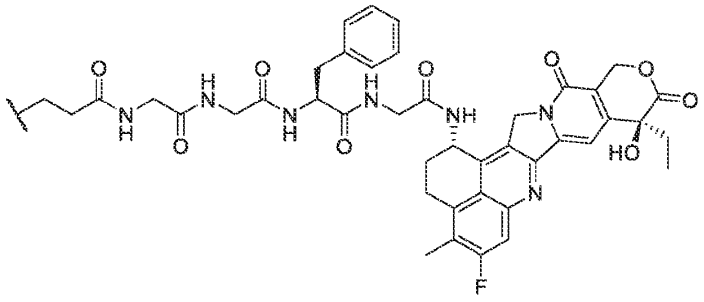
embodiments, an occurrence of $-L^C-R^P$ is . In some

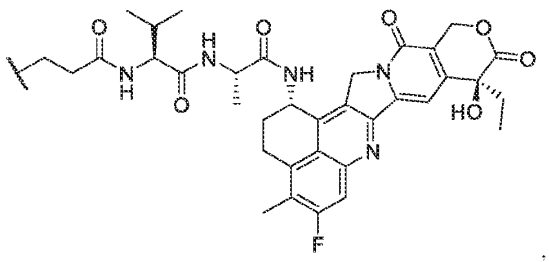


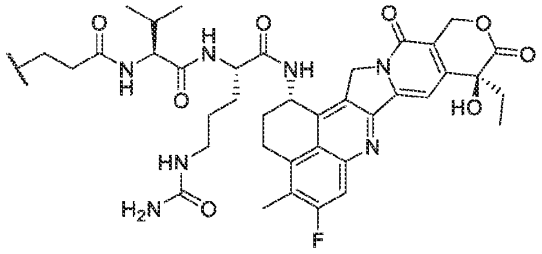
embodiments, each occurrence of $-L^C-R^P$ is

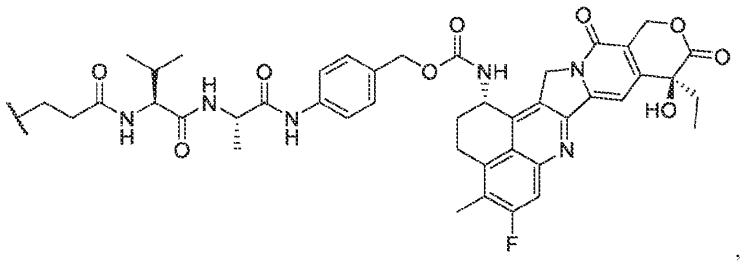
[0306] In some embodiments, a conjugate comprises $-L^{ZP}-L^C-R^P$. In some embodiments, an occurrence of $-L^{ZP}-L^C-R^P$ is $-(CH_2)_zC(O)-GGFG-R^P$. In some embodiments, it is $-(CH_2)_zC(O)-Val-Ala-PABA-R^P$. In some embodiments, it is $-(CH_2)_zC(O)-Val-Cit-PABA-R^P$. In some embodiments, it is $-(CH_2)_zC(O)-Val-Ala-NH-oGlu-R^P$. In some embodiments, it is $-(CH_2)_zC(O)-Val-Ala-NH-mGlu-R^P$. In some embodiments, it is $-(CH_2)_zC(O)-Val-Cit-NH-oGLu-R^P$. In some embodiments, it is $-(CH_2)_zC(O)-Val-Cit-NH-mGLu-R^P$. In some embodiments, it is $-(CH_2)_zNHC(O)-oGlu-R^P$. In some embodiments, it is $-(CH_2)_zNHC(O)-mGlu-R^P$. In some embodiments, z is 2. In some embodiments, R^P comprises a nitrogen atom and is bonded at the nitrogen atom, e.g., to the rest of a conjugate or other compound. In some embodiments, R^P is R^{EX}. In some embodiments, z is 2 and R^P is R^{EX}. In some embodiments, each occurrence of $-L^{ZP}-L^C-R^P$ is the same.

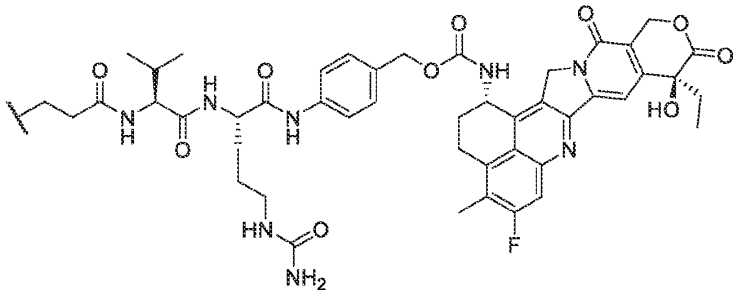
[0307] In some embodiments, an occurrence of $-L^{ZP}-L^C-R^P$ is

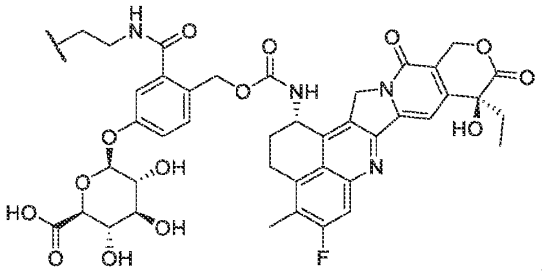


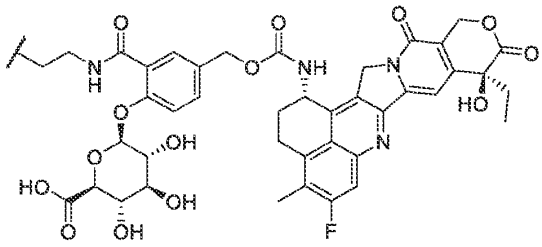


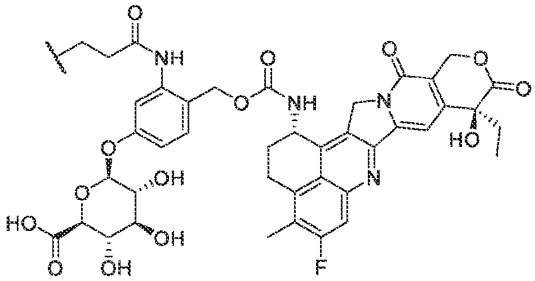




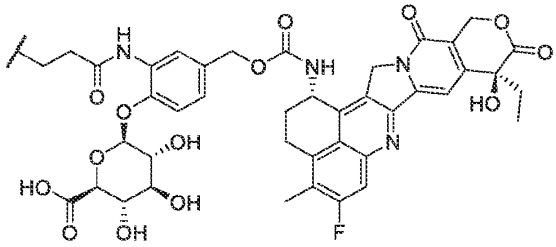






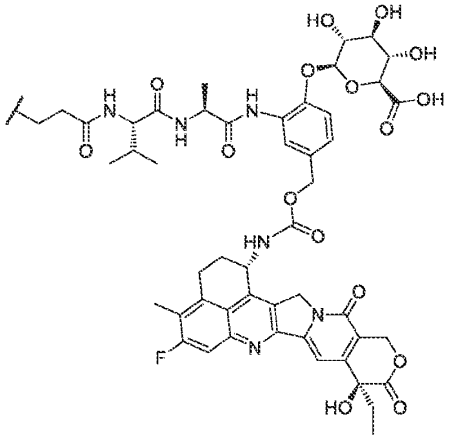


, or

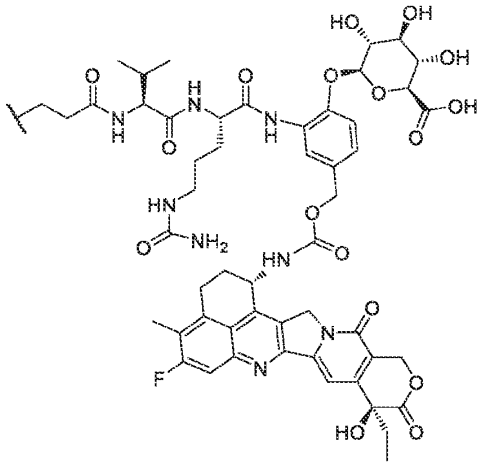


. In some embodiments, each occurrence is the same.

[0308] In some embodiments, an occurrence of $-L^{ZP}-L^{C}-R^{P}$ is

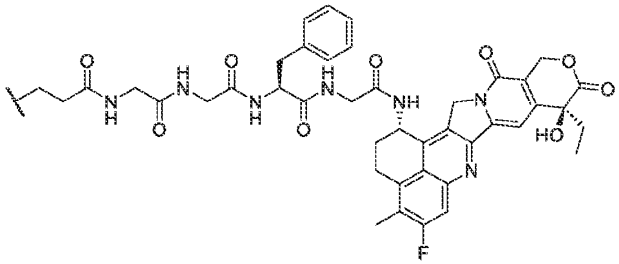


or



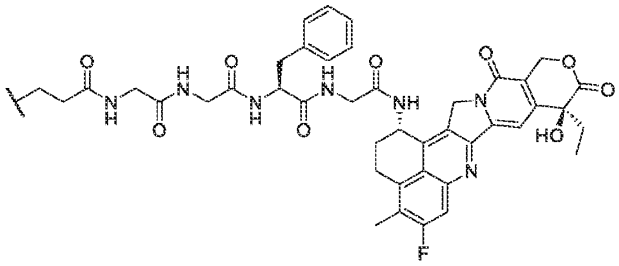
. In some embodiments, each occurrence is the same.

[0309] In some embodiments, an occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises

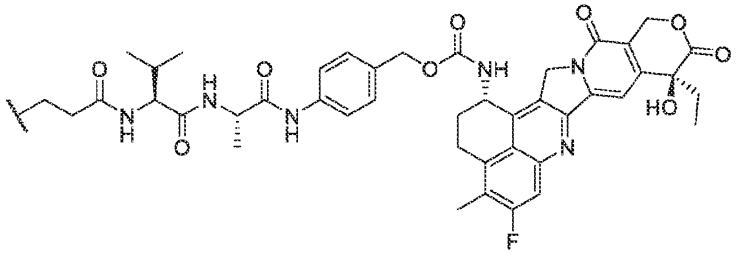


.

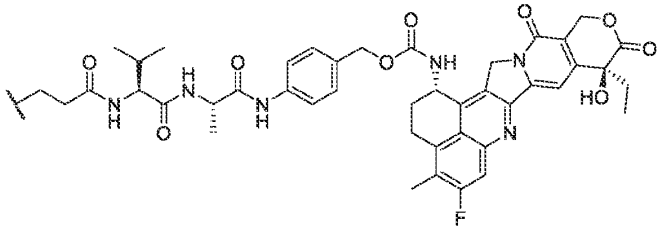
[0310] In some embodiments, each occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises



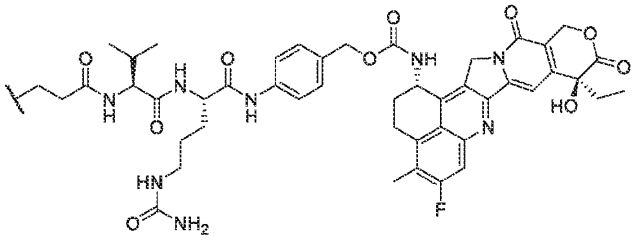
[0311] In some embodiments, an occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises



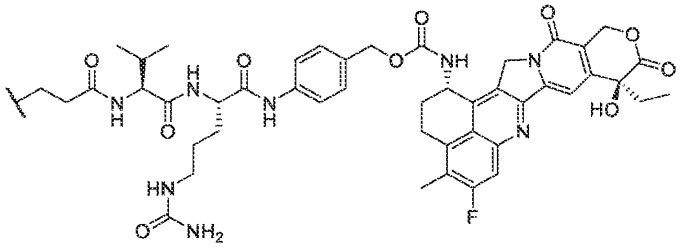
[0312] In some embodiments, each occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises



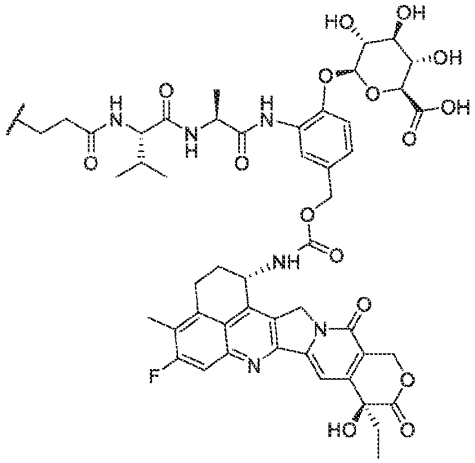
[0313] In some embodiments, an occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises



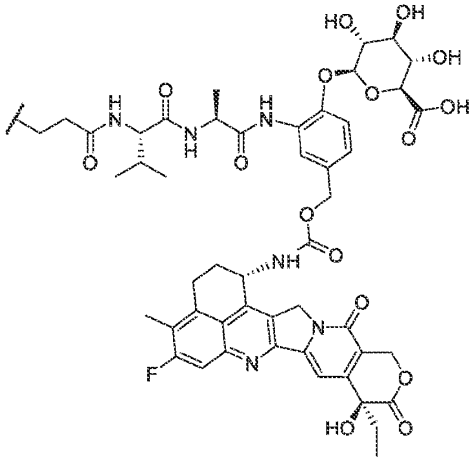
[0314] In some embodiments, each occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises



[0315] In some embodiments, an occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises



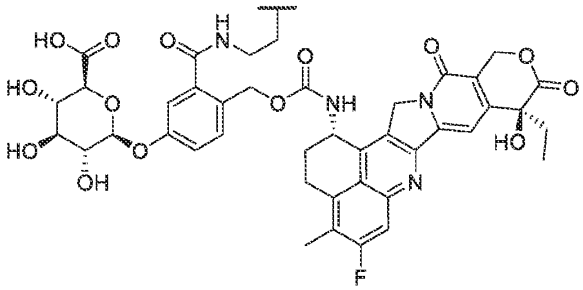
. In some embodiments, each occurrence of



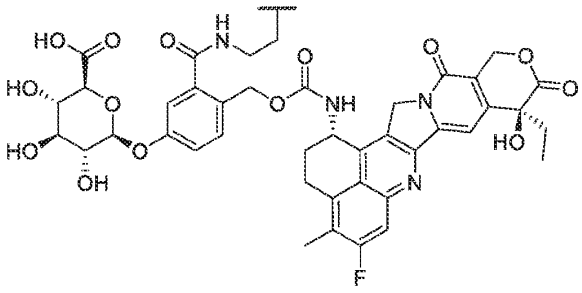
$-L^{ZP}-L^{C}-R^{P}$ is or comprises

.

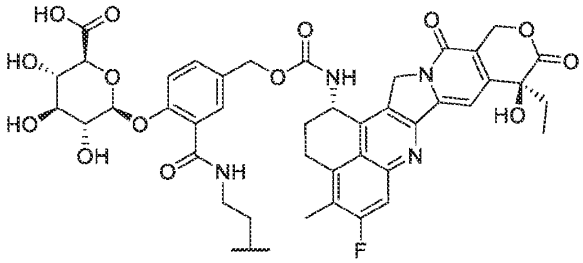
[0316] In some embodiments, an occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises



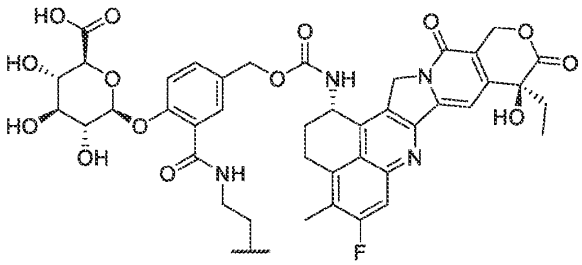
. In some embodiments, each occurrence of



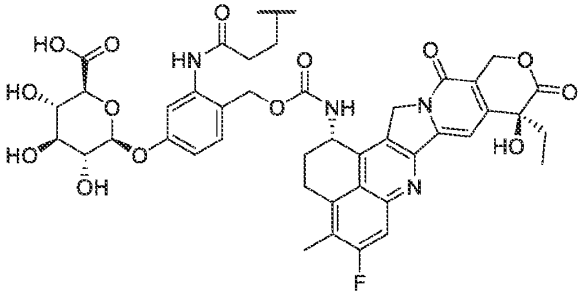
$-L^{ZP}-L^{C}-R^{P}$ is or comprises . In some embodiments, an occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises



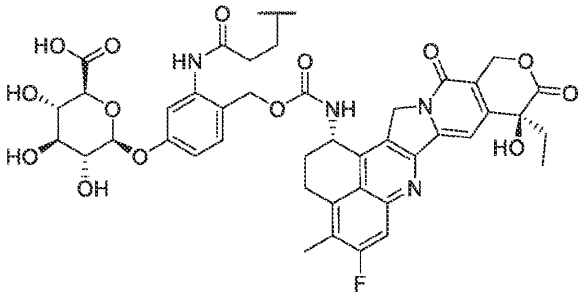
. In some embodiments, each occurrence of



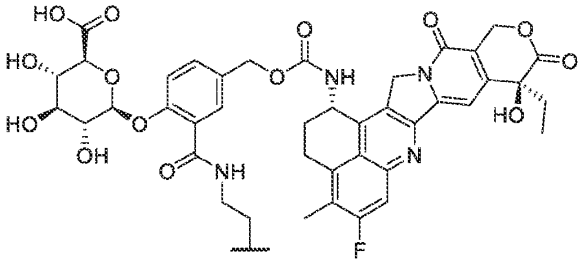
$-L^{ZP}-L^{C}-R^{P}$ is or comprises . In some embodiments, an occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises



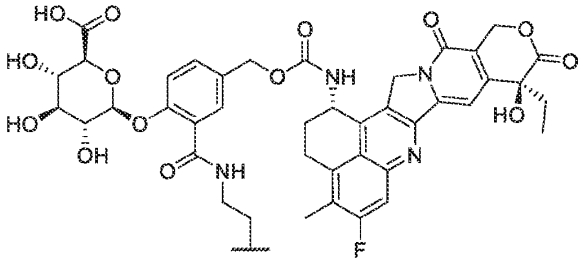
. In some embodiments, each occurrence of



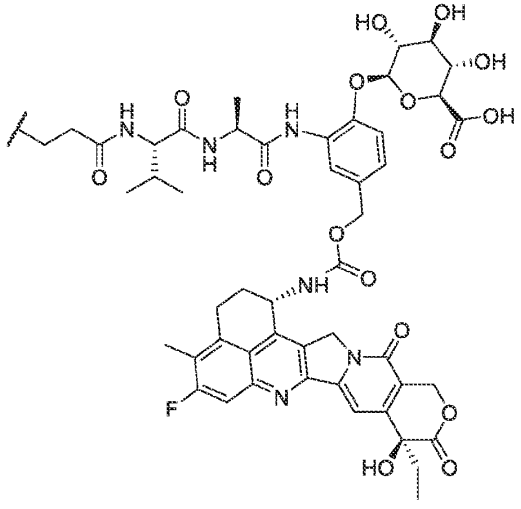
$-L^{ZP}-L^{C}-R^{P}$ is or comprises . In some embodiments, an occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises



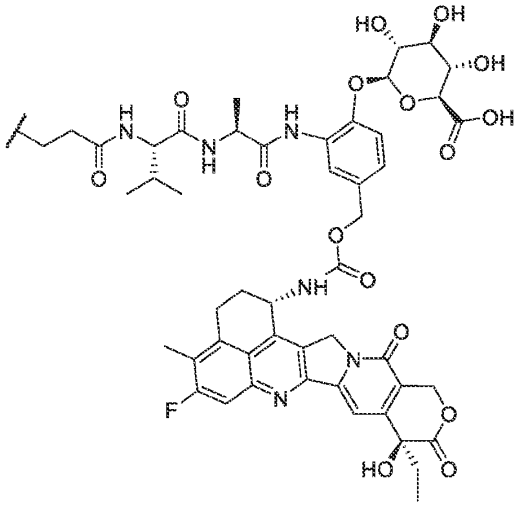
. In some embodiments, each occurrence of



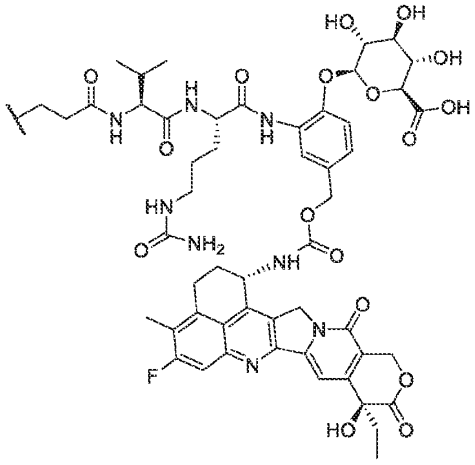
$-L^{ZP}-L^{C}-R^{P}$ is or comprises . In some embodiments, an occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises



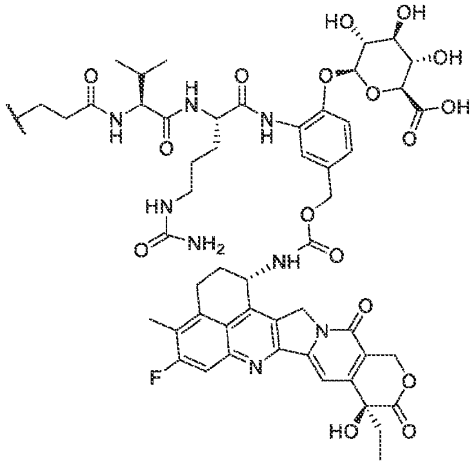
. In some embodiments, each occurrence of



$-L^{ZP}-L^{C}-R^{P}$ is or comprises . In some embodiments, an occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises



.In some embodiments, each occurrence of $-L^{ZP}-L^{C}-R^{P}$

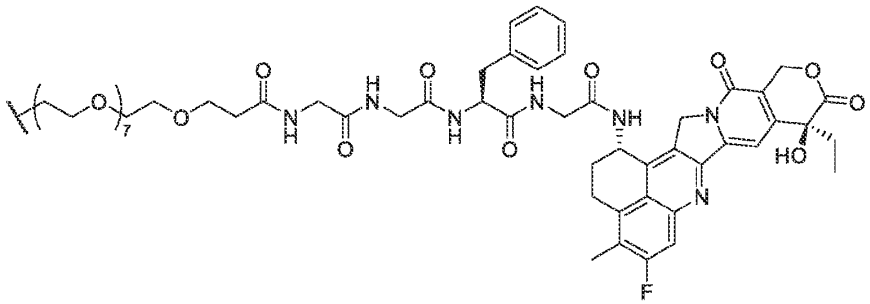


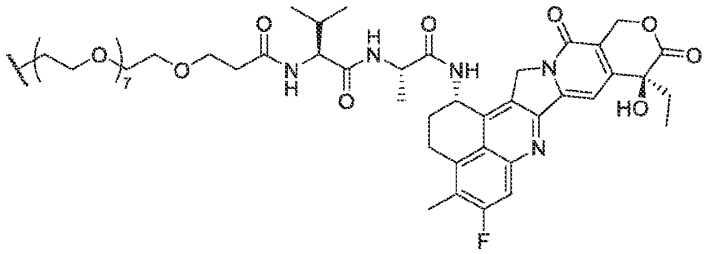
is or comprises .

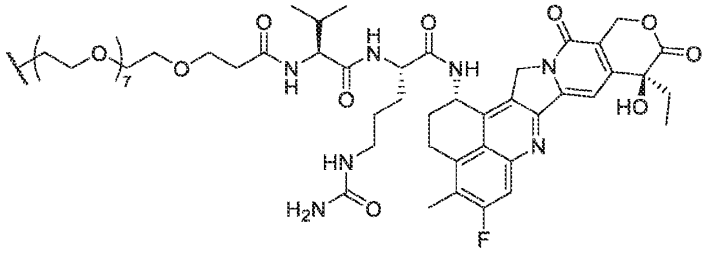
[0317] In some embodiments, a conjugate comprises $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$. In some

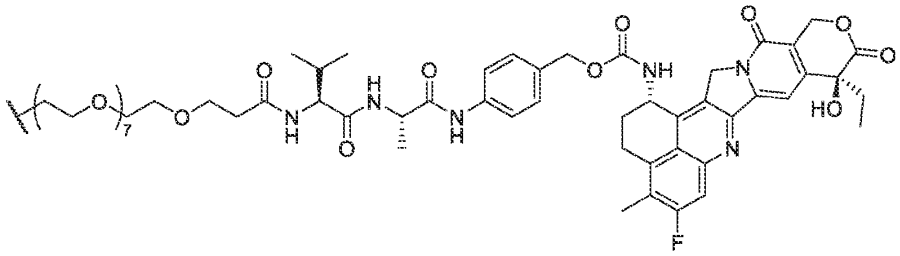
embodiments, an occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is $-(CH_2CH_2O)n-(CH_2)_zC(O)-GGFG-R^{P}$. In some embodiments, it is $-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Ala-PABA-R^{P}$. In some embodiments, it is $-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-PABA-R^{P}$. In some embodiments, it is $-(CH_2CH_2O)n-(CH_2)_zC(O)Val-Ala-NH-oGlu-R^{P}$. In some embodiments, it is $-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Ala-NH-mGlu-R^{P}$. In some embodiments, it is $-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-NH-oGLu-R^{P}$. In some embodiments, it is $-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-NH-mGLu-R^{P}$. In some embodiments, it is $-(CH_2CH_2O)n-(CH_2)_zNHC(O)-oGlu-R^{P}$. In some embodiments, it is $-(CH_2CH_2O)n-(CH_2)_zNHC(O)-mGlu-R^{P}$. In some embodiments, n is 8. In some embodiments, z is 2. In some embodiments, R^{P} comprises a nitrogen atom and is bonded at the nitrogen atom, e.g., to the rest of a conjugate or other compound. In some embodiments, R^{P} is R^{EX}. In some embodiments, z is 2 and R^{P} is R^{EX}. In some embodiments, n is 8, z is 2 and R^{P} is R^{EX}. In some embodiments, each occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is the same.

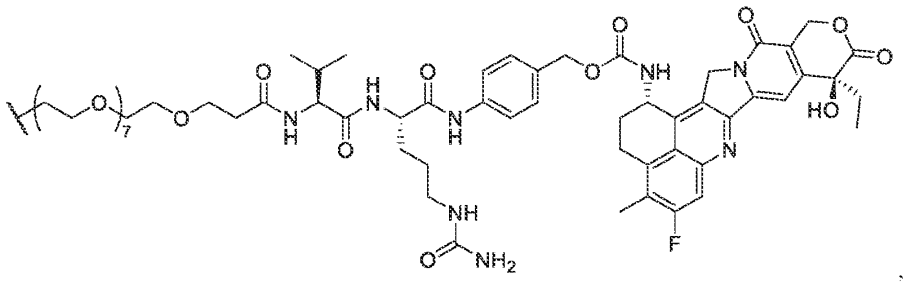
[0318] In some embodiments, an occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

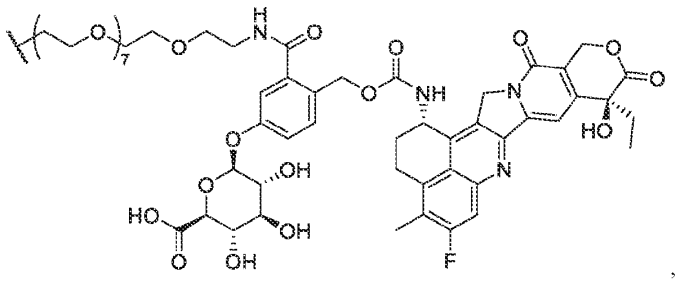


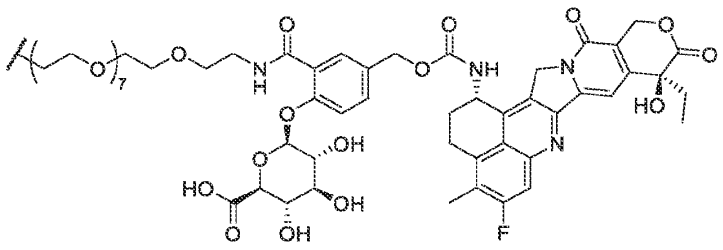


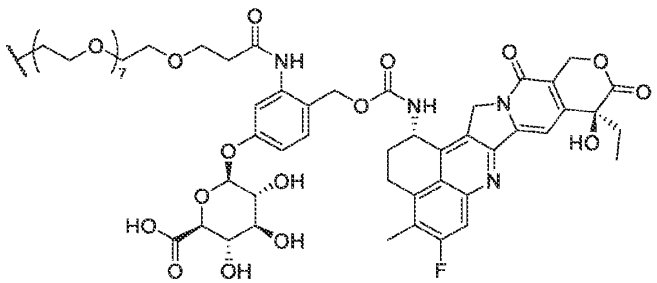




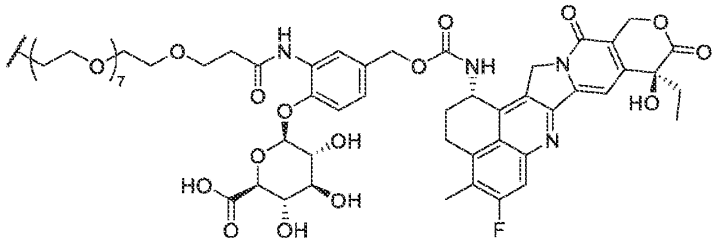






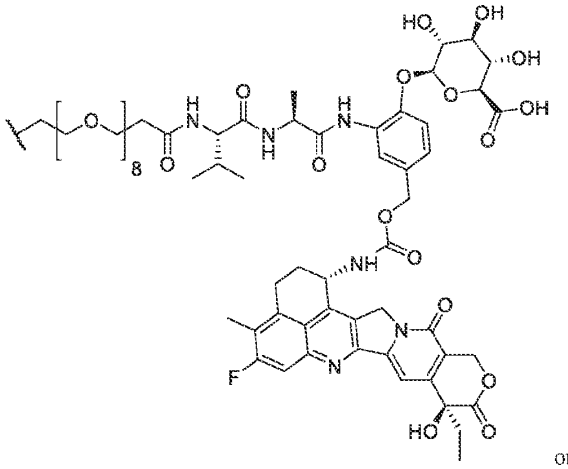


, or

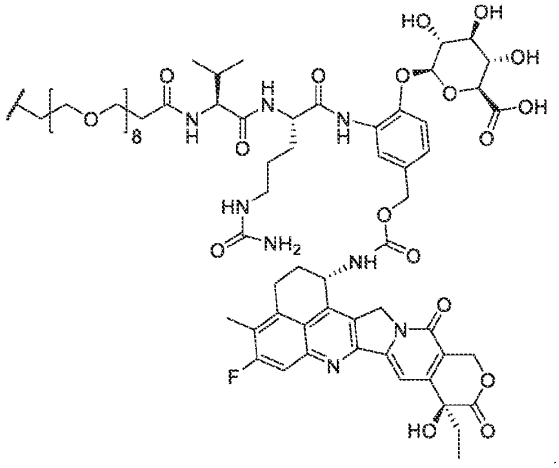


. In some embodiments, each occurrence is the same.

[0319] In some embodiments, an occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

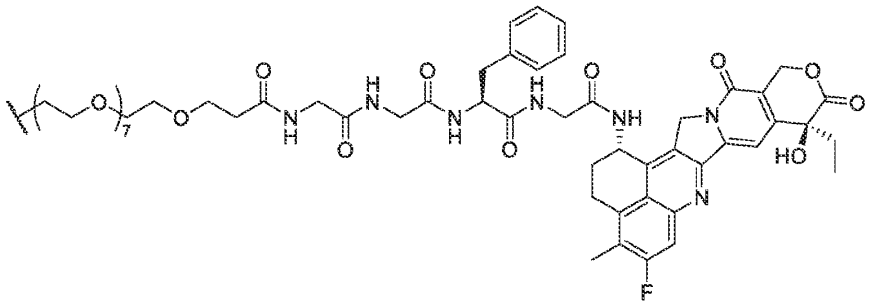


or

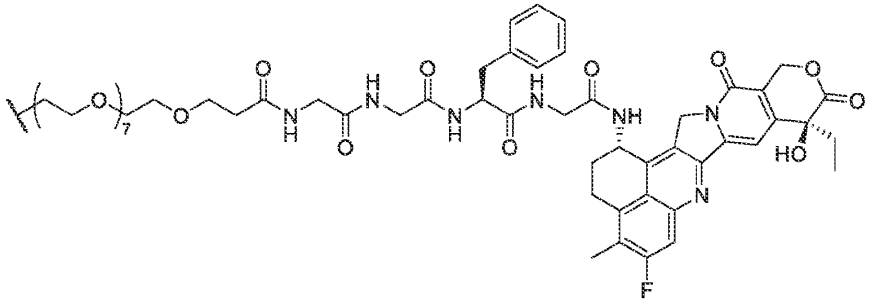


. In some embodiments, each occurrence is the same.

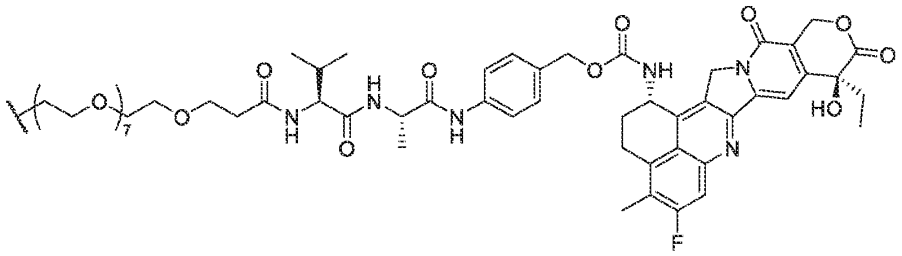
[0320] In some embodiments, an occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



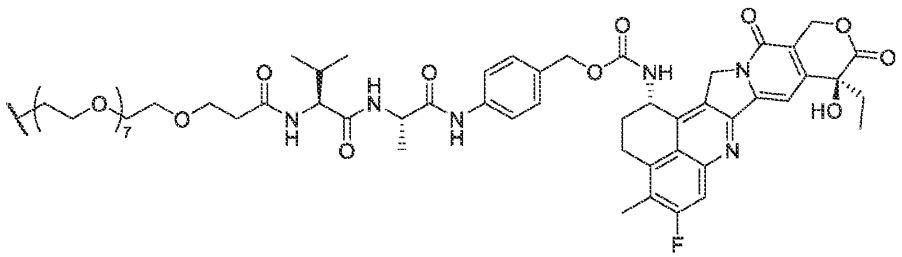
[0321] In some embodiments, each occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



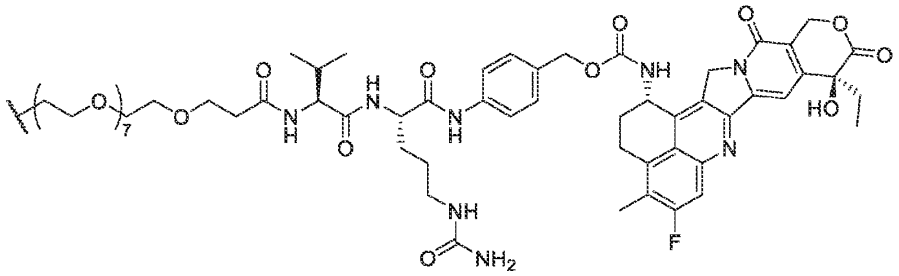
[0322] In some embodiments, an occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



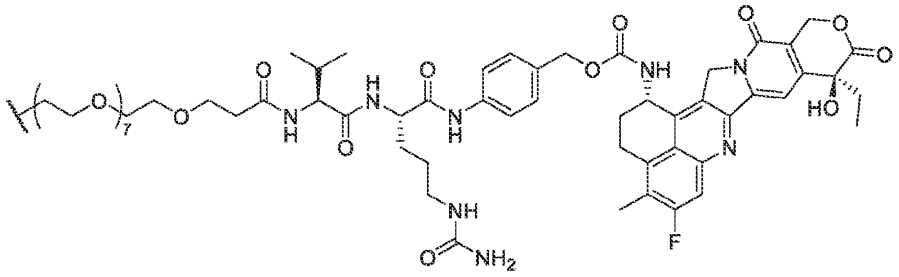
[0323] In some embodiments, each occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



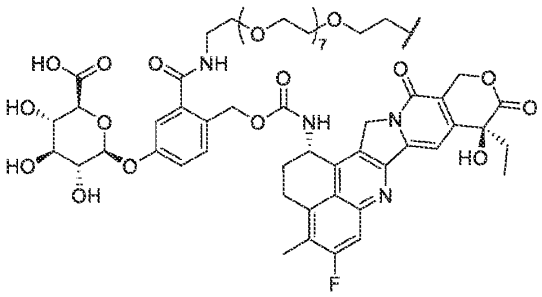
[0324] In some embodiments, an occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



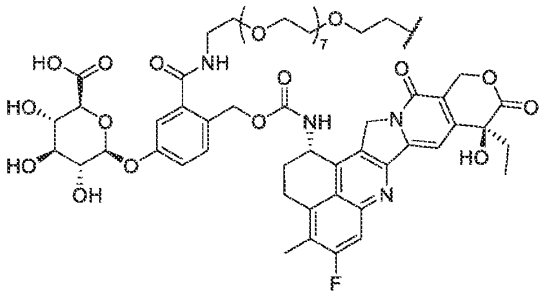
[0325] In some embodiments, each occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



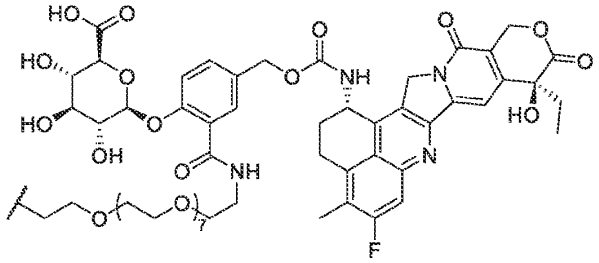
[0326] In some embodiments, an occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



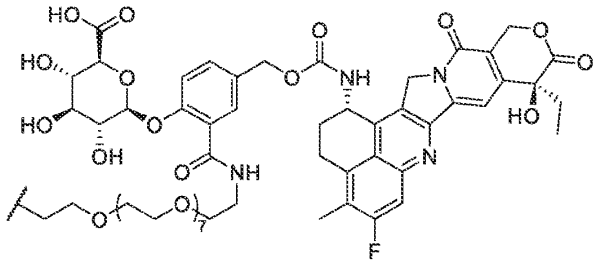
. In some embodiments, each occurrence of



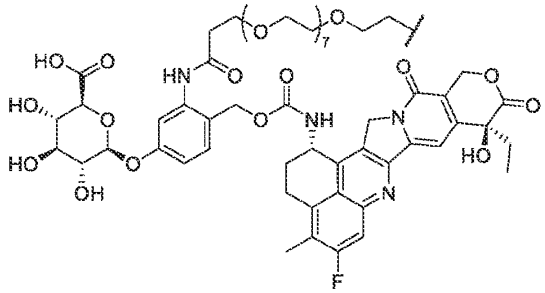
$-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises . In some embodiments, an occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



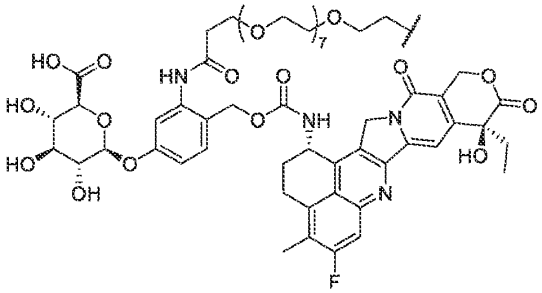
. In some embodiments, each occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



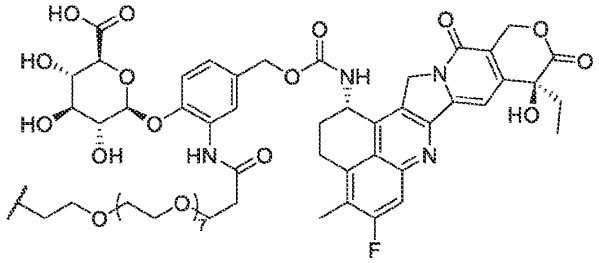
. In some embodiments, an occurrence of



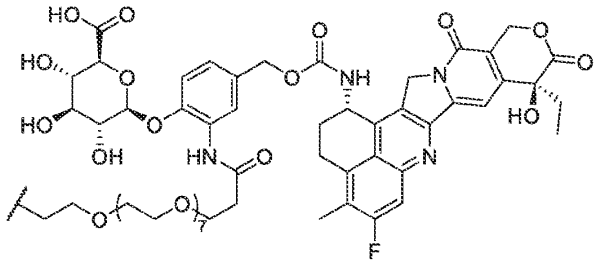
$-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises . In some embodiments, each occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



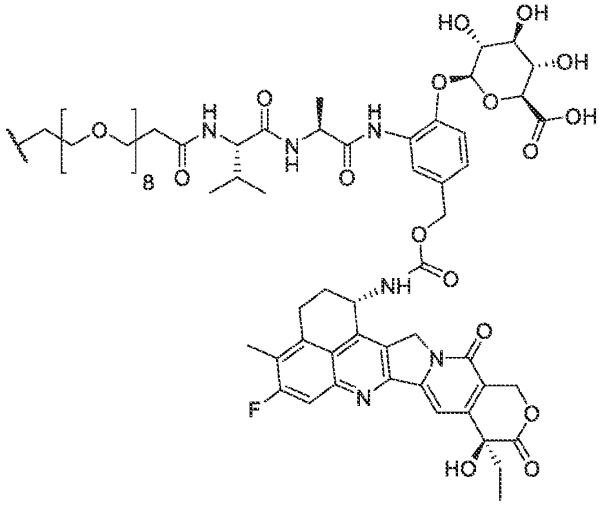
. In some embodiments, an occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



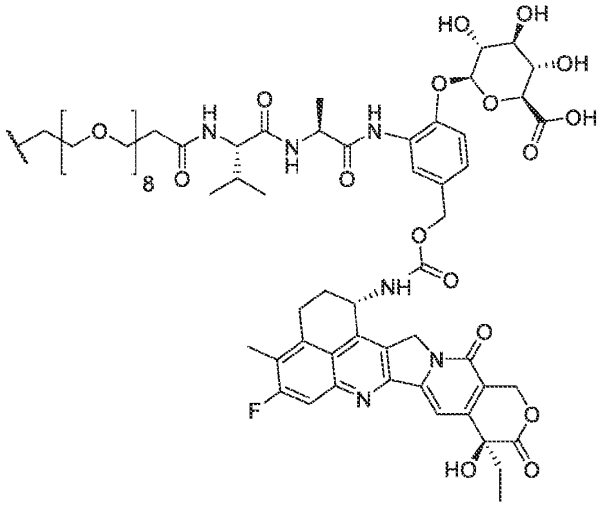
. In some embodiments, each occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



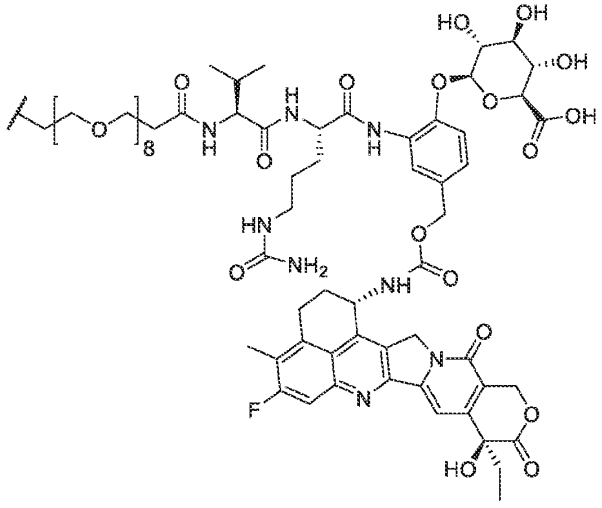
. In some embodiments, an occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



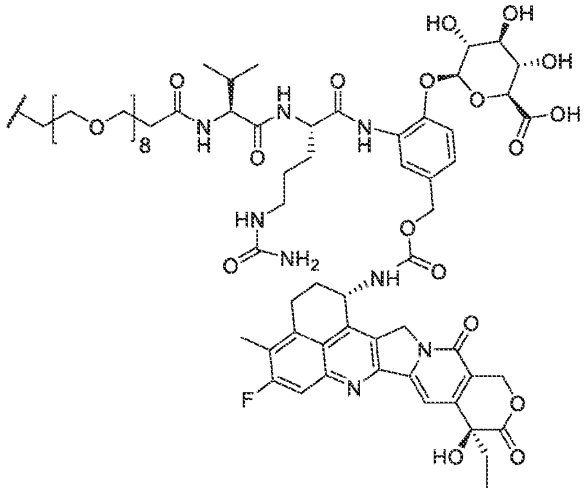
. In some embodiments, each occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



. In some embodiments, an occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

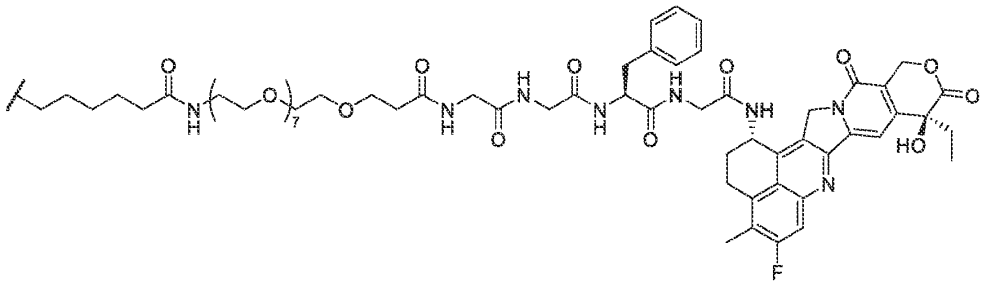


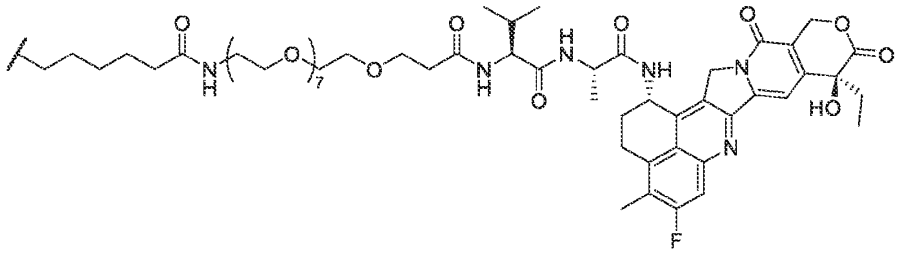
. In some embodiments, each occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

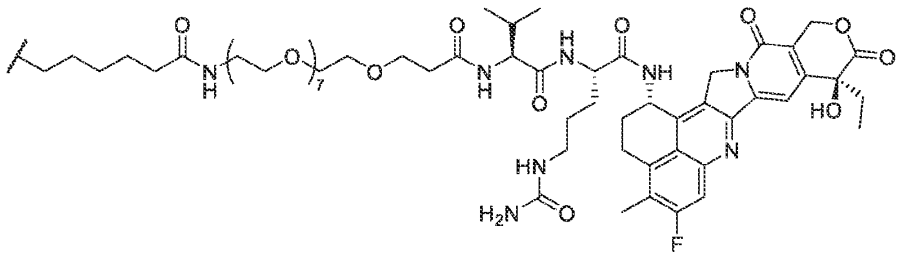


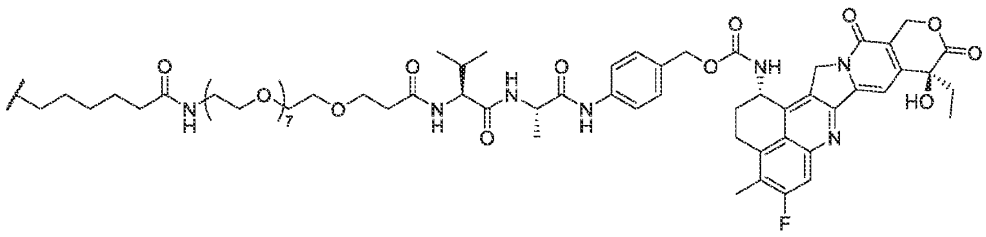
[0327] In some embodiments, a conjugate comprises $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$. In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is $-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-GGFG-R^P$. In some embodiments, it is $-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Ala-PABA-R^P$. In some embodiments, it is $-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-PABA-R^P$. In some embodiments, it is $-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)Val-Ala-NH-oGlu-R^P$. In some embodiments, it is $-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Ala-NH-mGlu-R^P$. In some embodiments, it is $-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-NH-oGLu-R^P$. In some embodiments, it is $-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-NH-mGLu-R^P$. In some embodiments, it is $-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zNHC(O)-oGlu-R^P$. In some embodiments, it is $-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zNHC(O)-mGlu-R^P$. In some embodiments, x is 2. In some embodiments, x is 3. In some embodiments, x is 5. In some embodiments, n is 8. In some embodiments, z is 2. In some embodiments, R^P comprises a nitrogen atom and is bonded at the nitrogen atom, e.g., to the rest of a conjugate or other compound. In some embodiments, R^P is R^{EX}. In some embodiments, z is 2 and R^P is R^{EX}. In some embodiments, n is 8, z is 2 and R^P is R^{EX}. In some embodiments, x is 2, n is 8, z is 2 and R^P is R^{EX}. In some embodiments, x is 3, n is 8, z is 2 and R^P is R^{EX}. In some embodiments, x is 5, n is 8, z is 2 and R^P is R^{EX}. In some embodiments, each occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is the same.

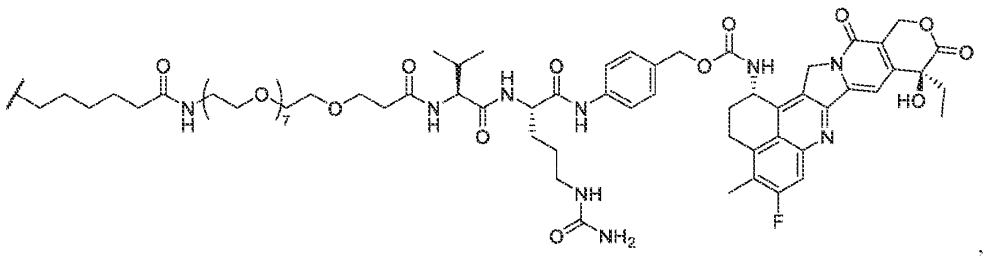
[0328] In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

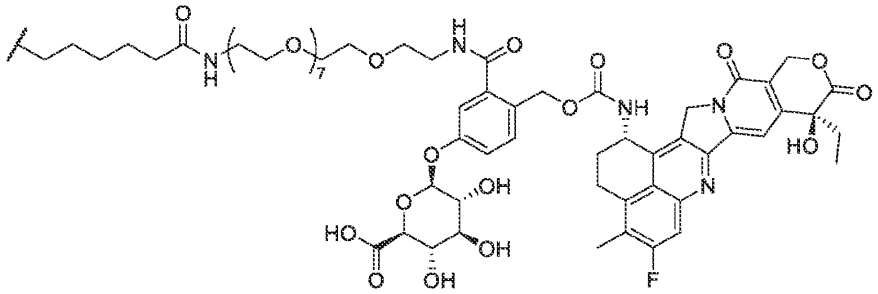




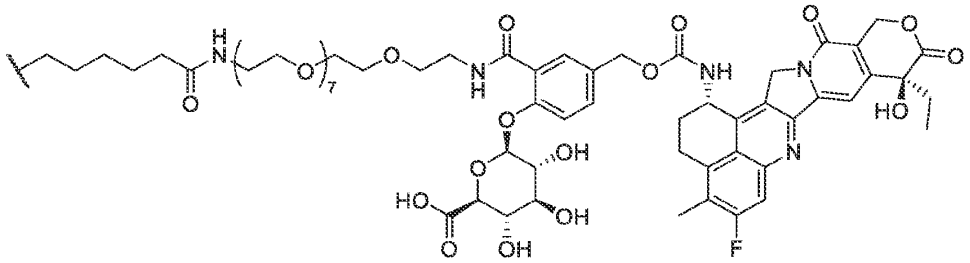




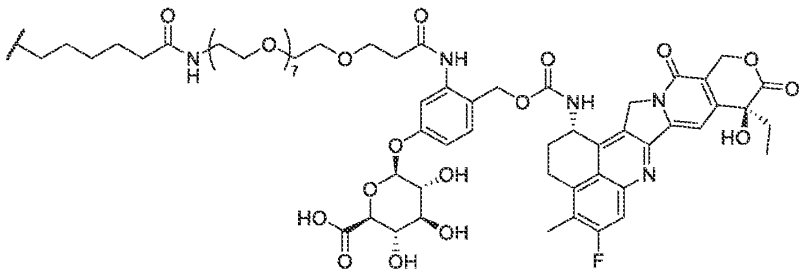




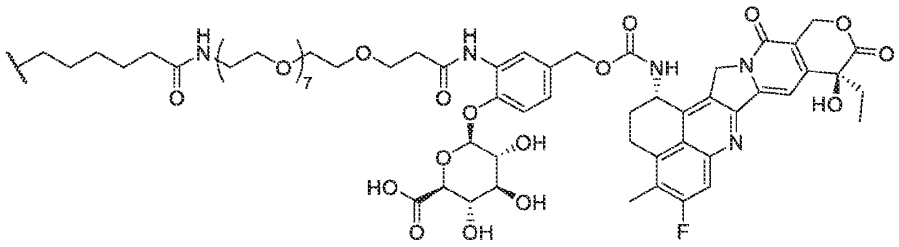
,



,

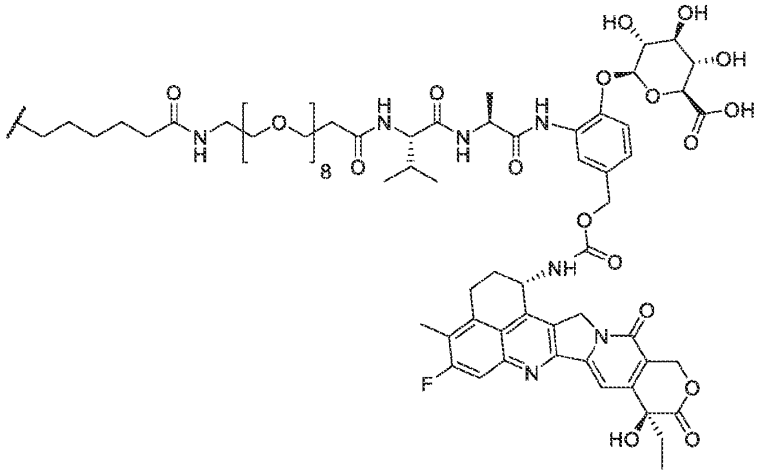


, or

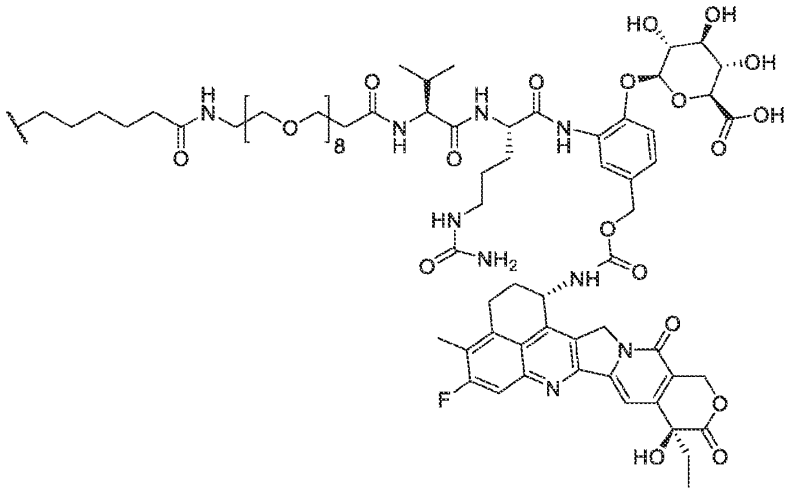


. In some embodiments, each occurrence is the same.

[0329] In some embodiments, an occurrence of $-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

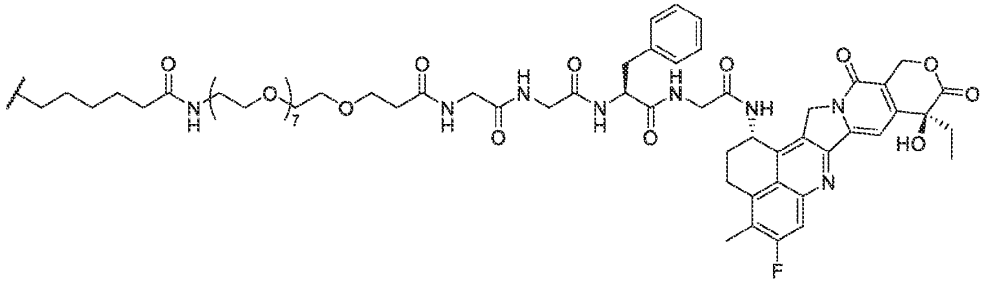


or



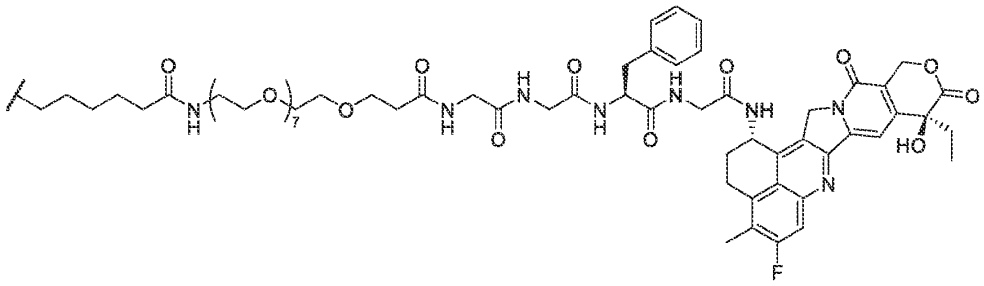
. In some embodiments, each occurrence is the same.

[0330] In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

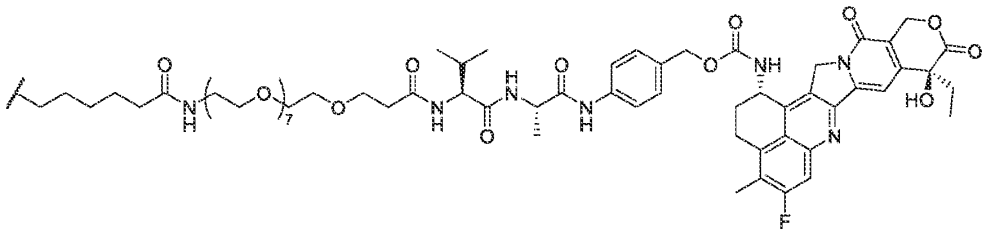


.

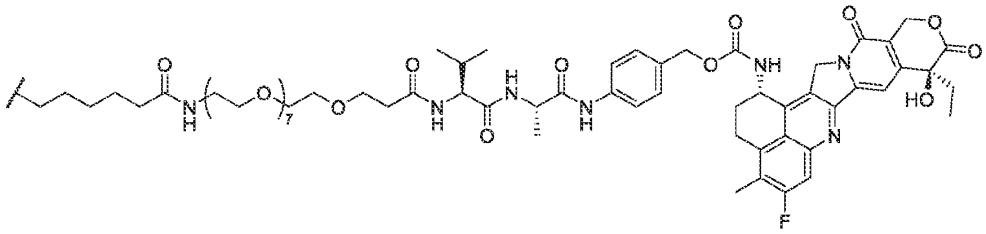
[0331] In some embodiments, each occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



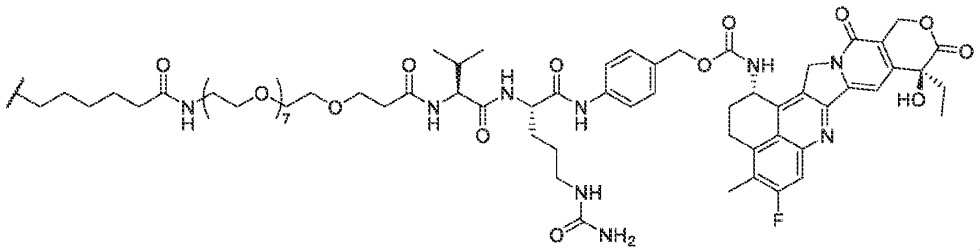
[0332] In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



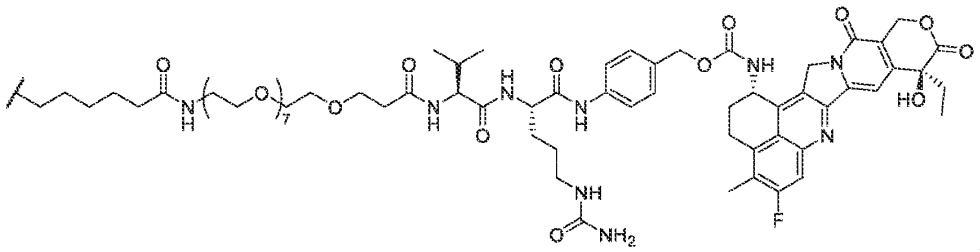
[0333] In some embodiments, each occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



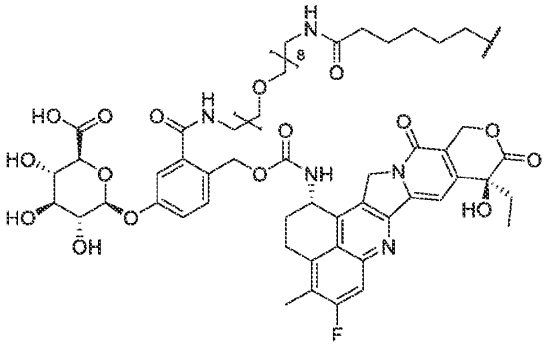
[0334] In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



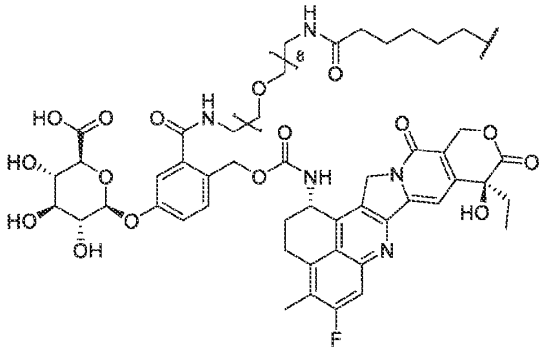
[0335] In some embodiments, each occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



[0336] In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

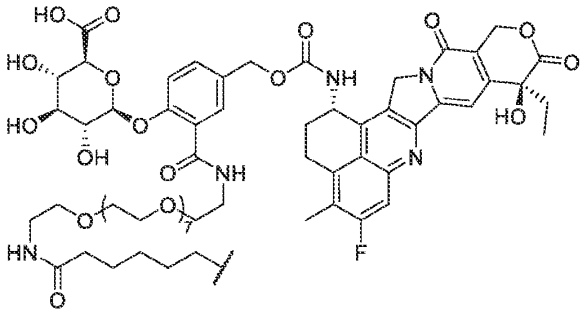


. In some embodiments, each occurrence of



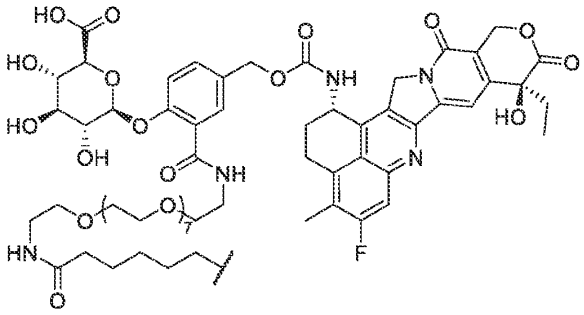
$-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

. In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

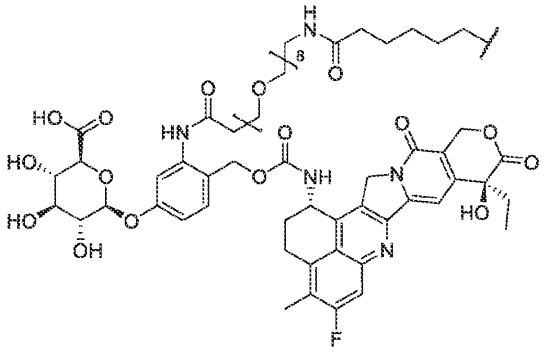


. In some embodiments, each occurrence of

$-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

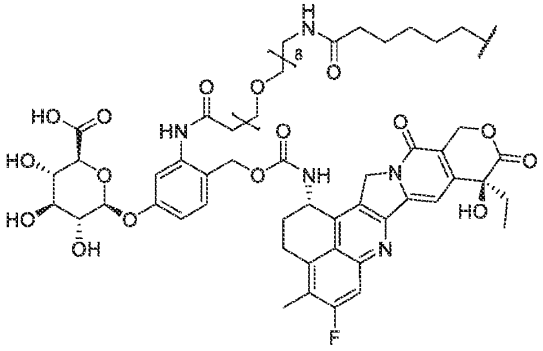


. In some embodiments, an occurrence of



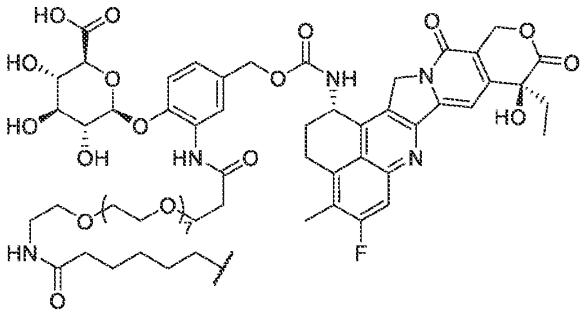
$-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises .

In some embodiments, each occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



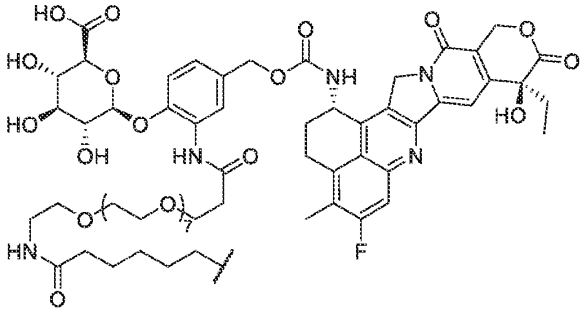
. In some embodiments, an occurrence of

$-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



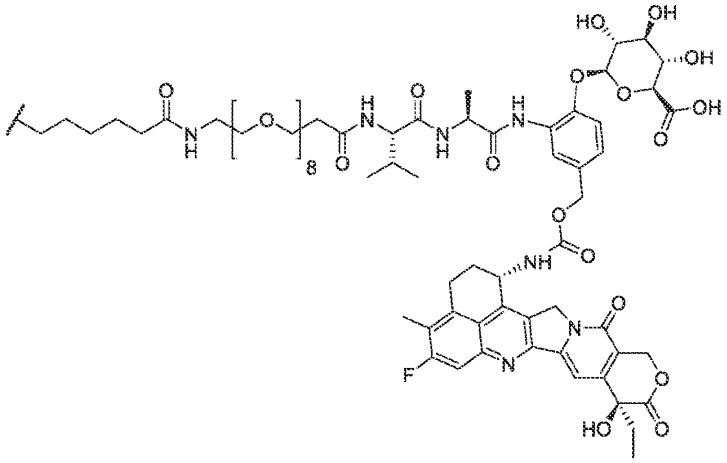
. In some embodiments, each occurrence of

$-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

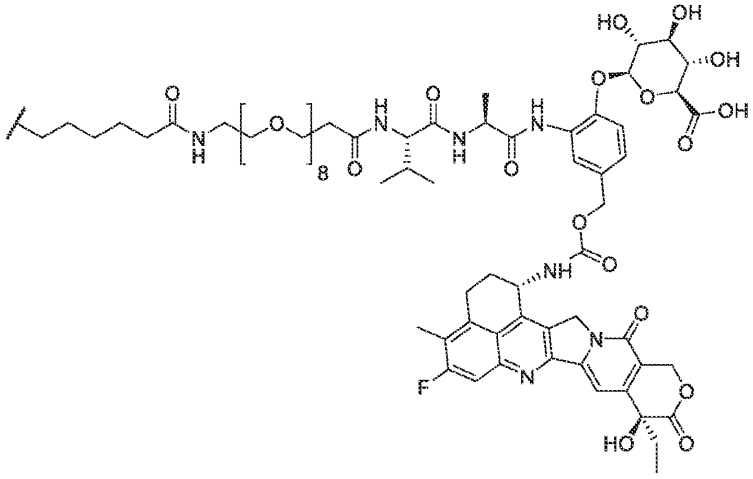


. In some embodiments, an occurrence of

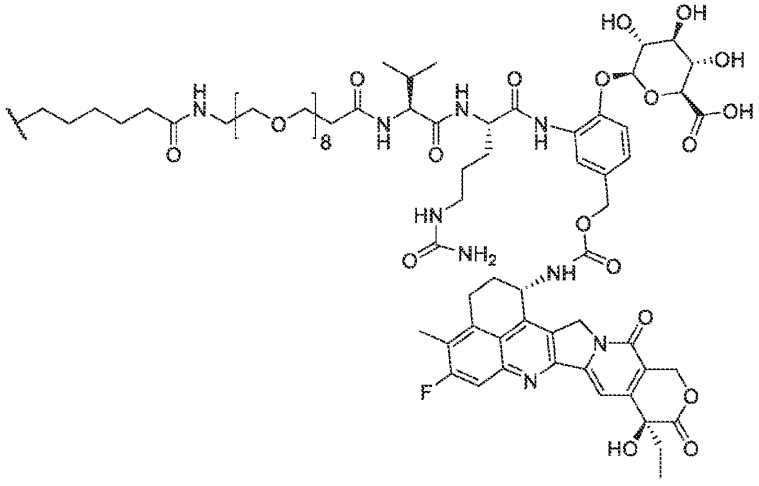
$-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



. In some embodiments, each occurrence of –L^X–$(CH_2CH_2O)n$–L^{ZP}–L^C–R^P is or comprises

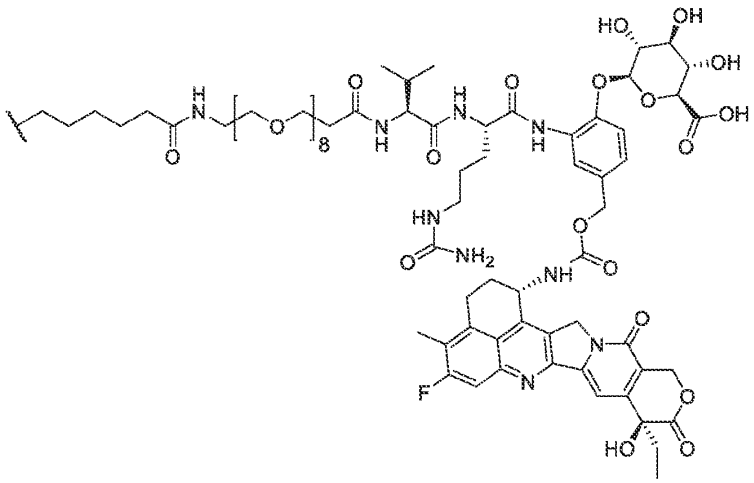


.In some embodiments, an occurrence of –L^X–$(CH_2CH_2O)n$–L^{ZP}–L^C–R^P is or comprises

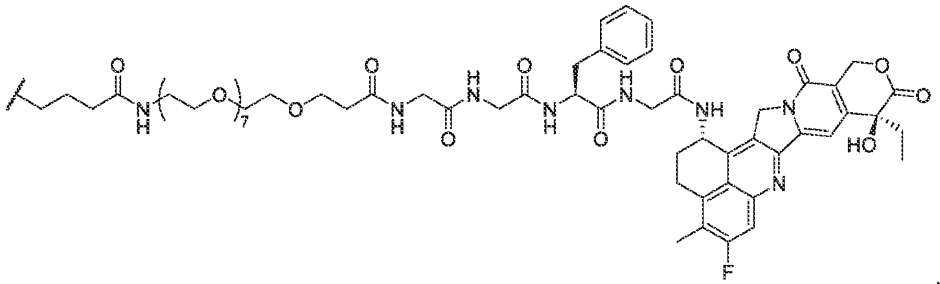


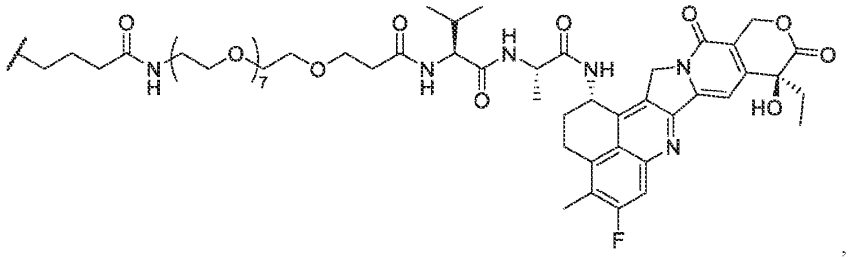
. In some embodiments,

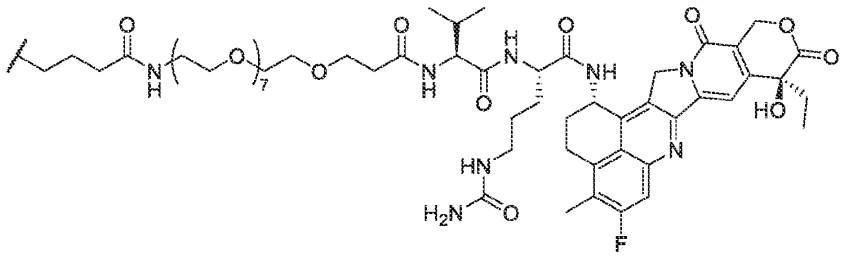
each occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

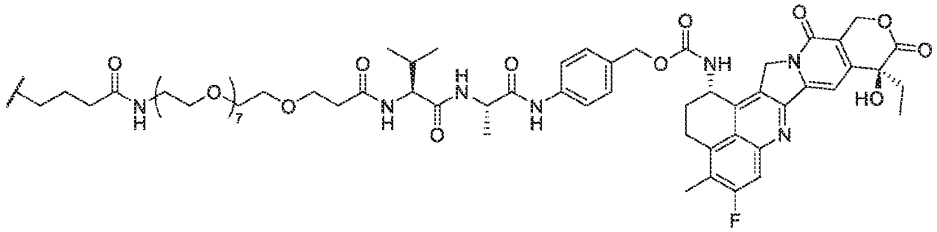


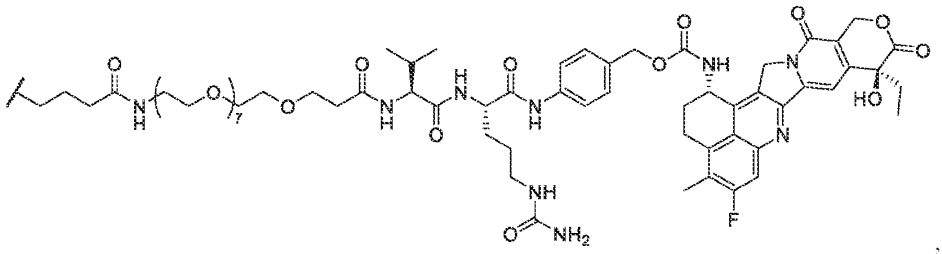
[0337] In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

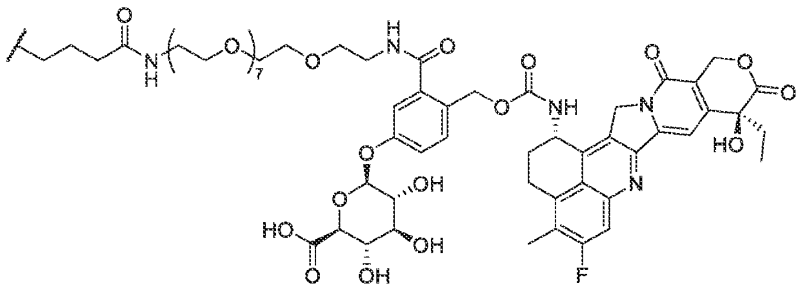


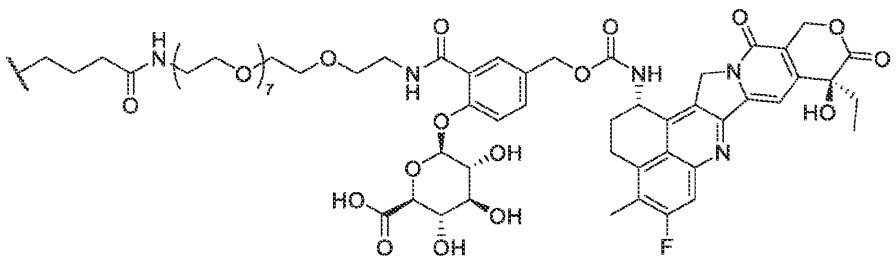


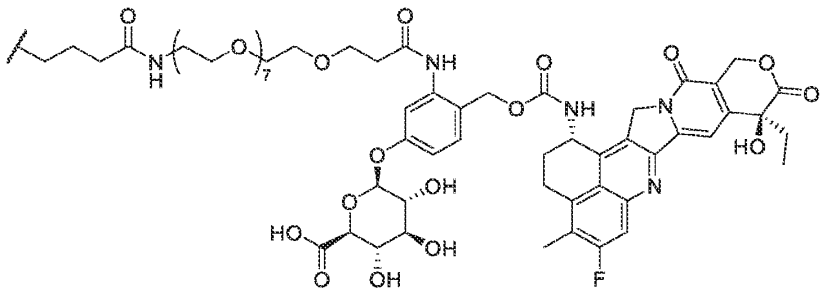




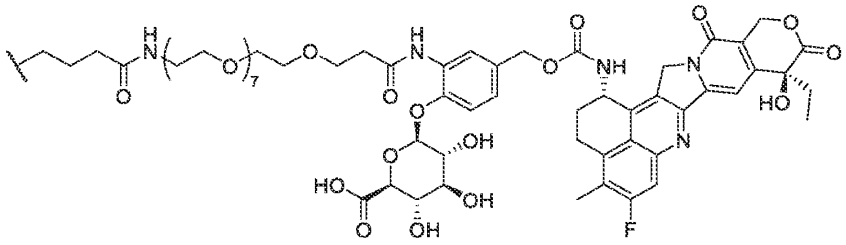






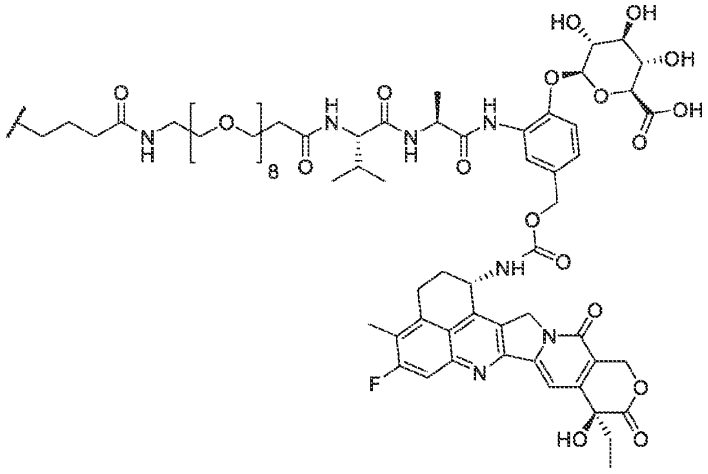


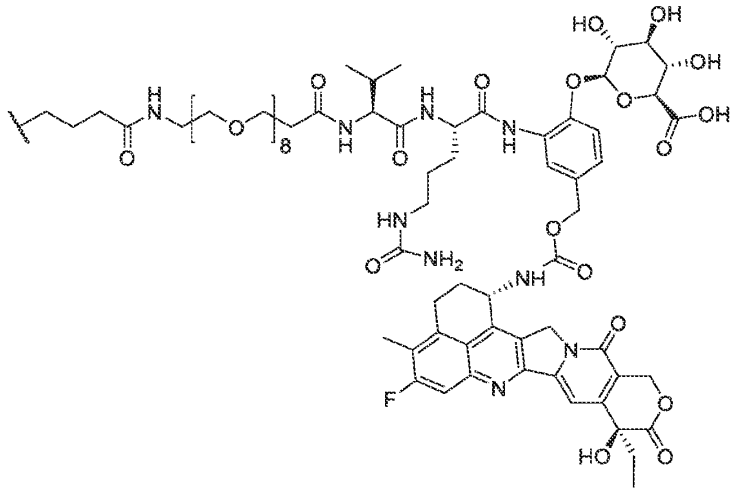
, or



. In some embodiments, each occurrence is the same.

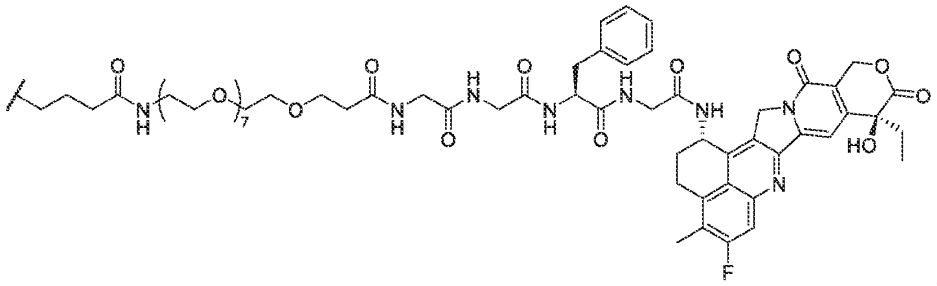
[0338] In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



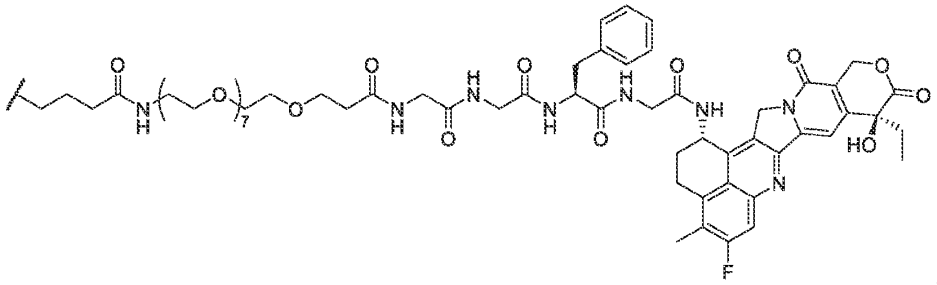


or . In some embodiments, each occurrence is the same.

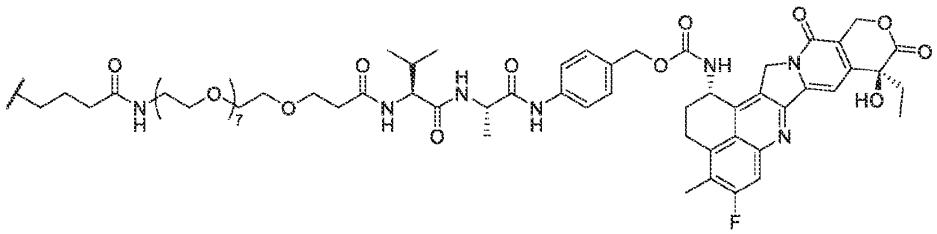
[0339] In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



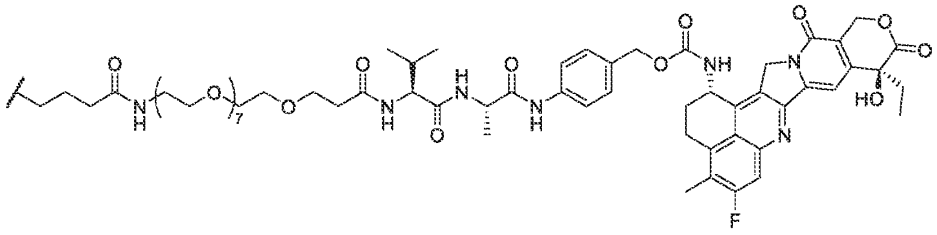
[0340] In some embodiments, each occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



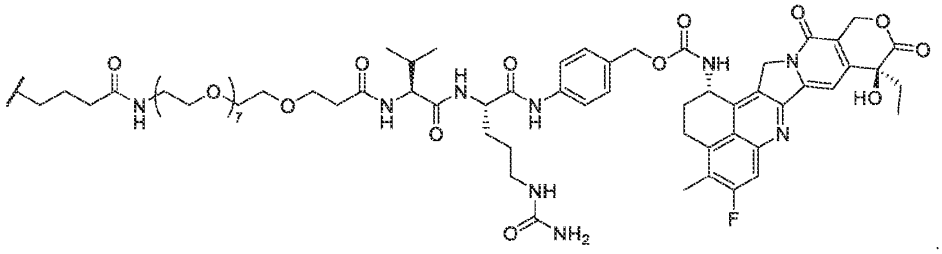
[0341] In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



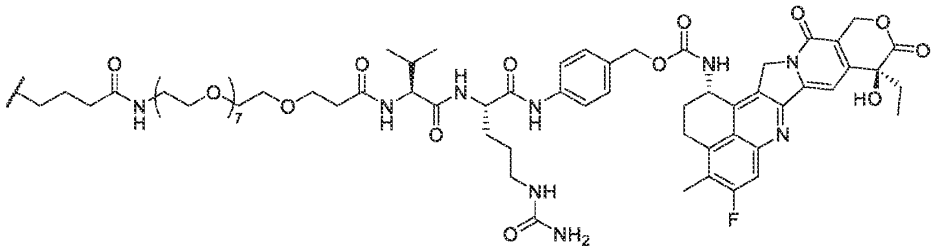
[0342] In some embodiments, each occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



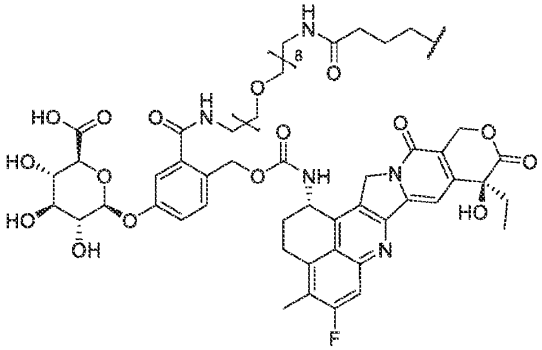
[0343] In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



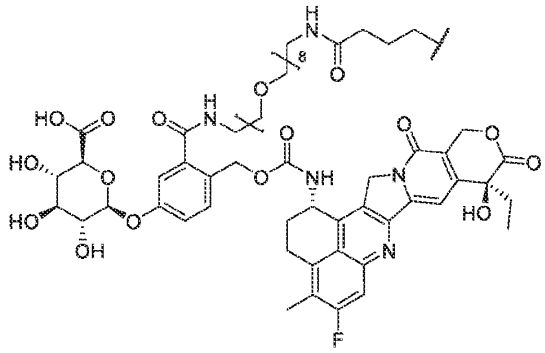
[0344] In some embodiments, each occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



[0345] In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

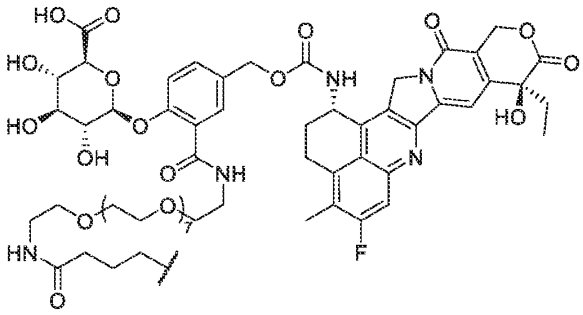


. In some embodiments, each occurrence of



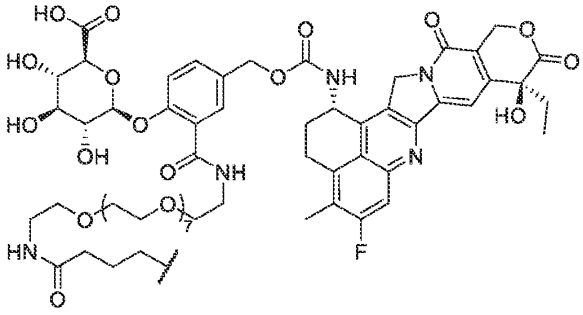
$-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises .

In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

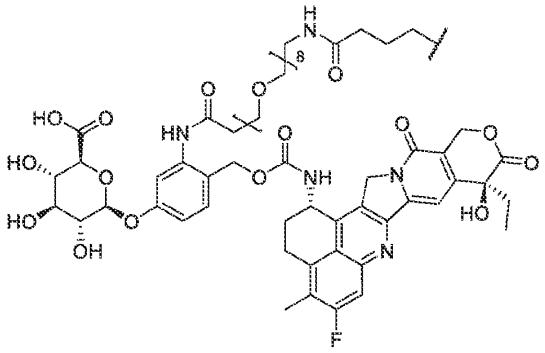


. In some embodiments, each occurrence of

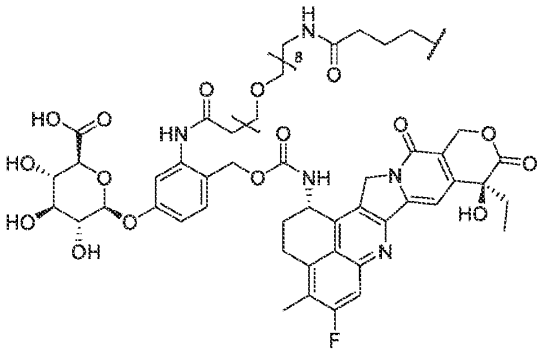
$-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



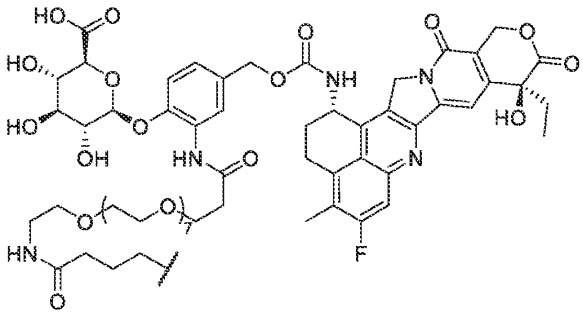
. In some embodiments, an occurrence of



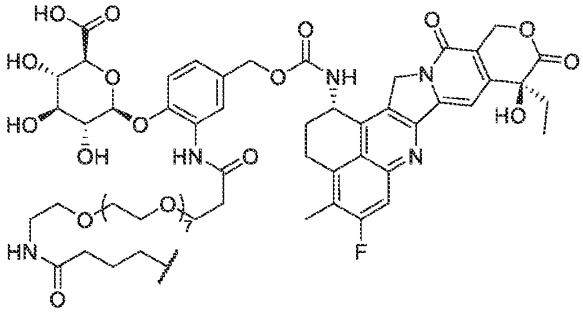
$-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises . In some embodiments, each occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



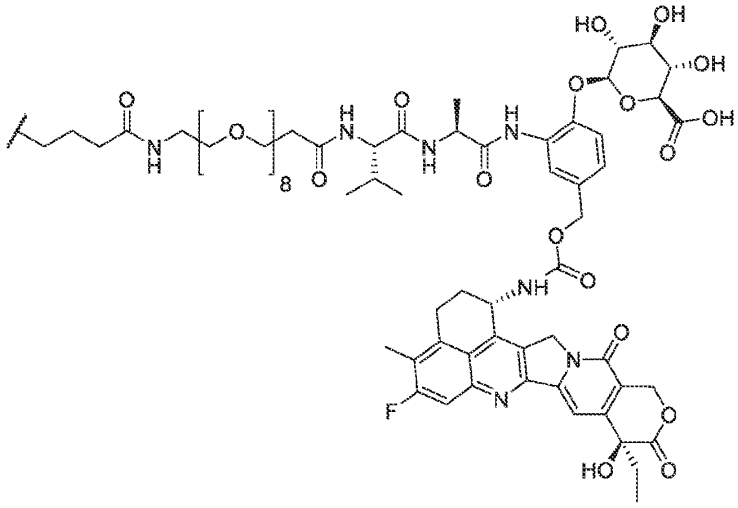
. In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



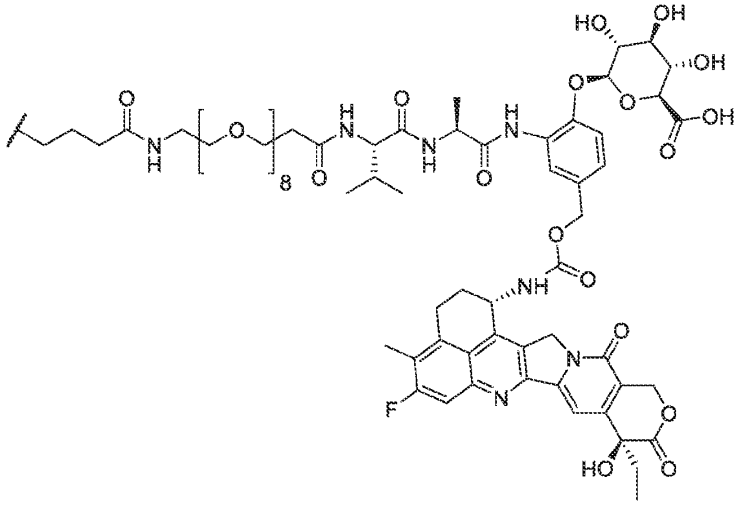
. In some embodiments, each occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



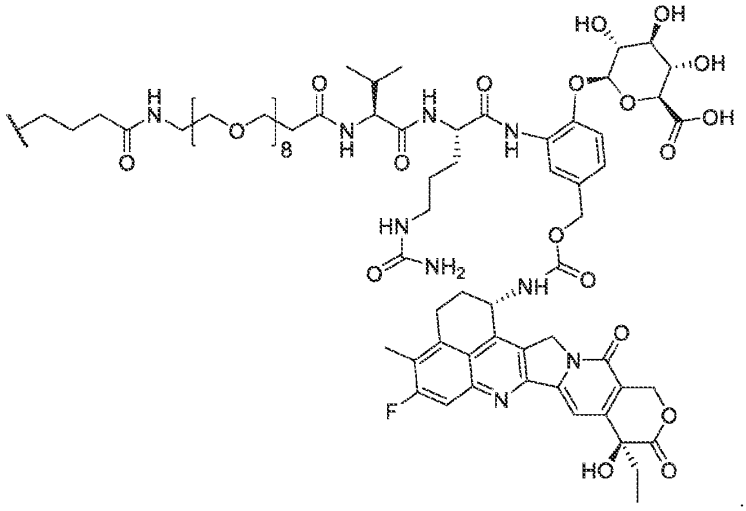
. In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



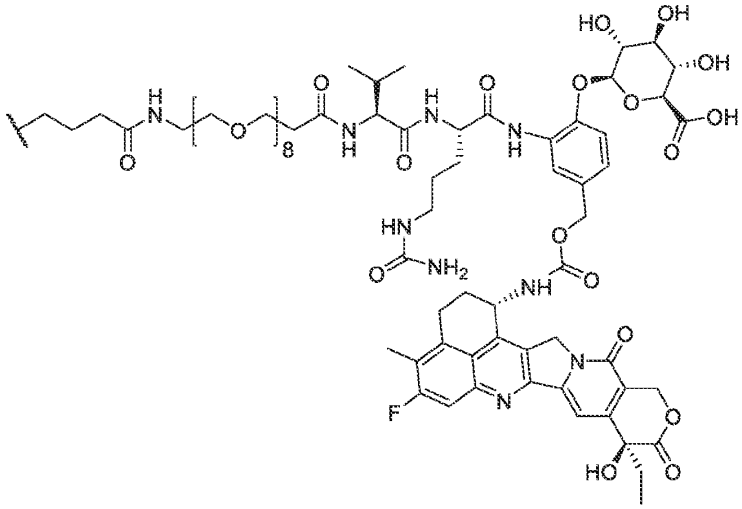
. In some embodiments, each occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



.In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



. In some embodiments, each occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

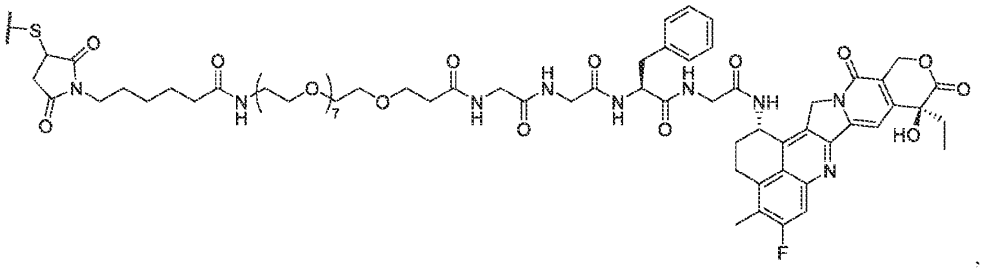


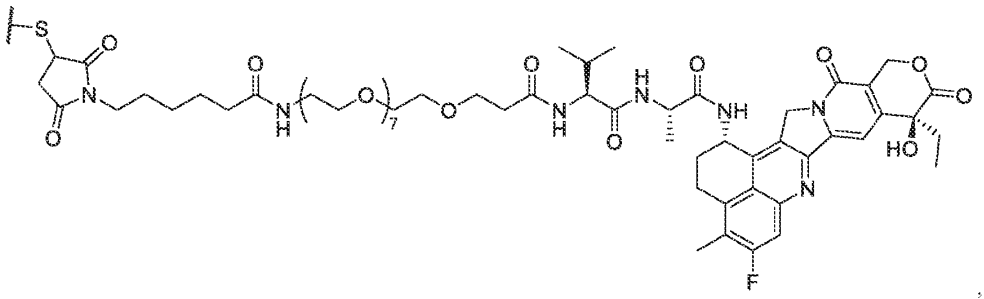
.

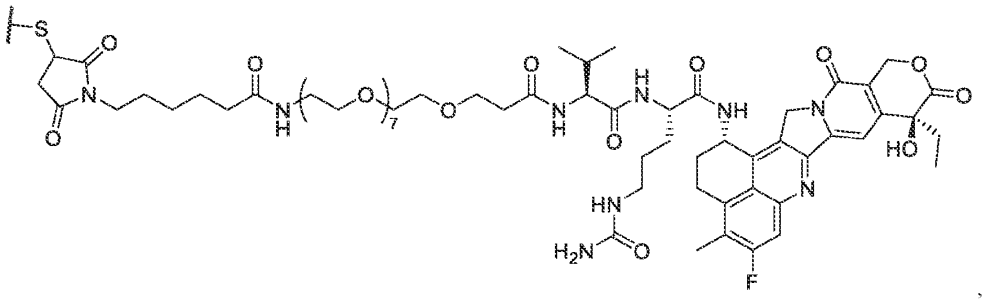
[0346] In some embodiments, a conjugate comprises $-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$. In some embodiments, an occurrence of $-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is $-S-SM-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-GGFG-R^P$. In some embodiments, it is $-S-SM-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Ala-PABA-R^P$. In some embodiments, it is $-S-SM-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-PABA-R^P$. In some embodiments, it is $-S-SM-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)Val-Ala-NH-oGlu-R^P$. In some embodiments, it is $-S-SM-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Ala-NH-mGlu-R^P$. In some embodiments, it is $-S-SM-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-NH-oGLu-R^P$. In some embodiments, it is $-S-SM-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-NH-mGLu-R^P$. In some embodiments, it is $-S-SM-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zNHC(O)-oGlu-R^P$. In some embodiments, it is $-S-SM-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zNHC(O)-mGlu-R^P$. In some

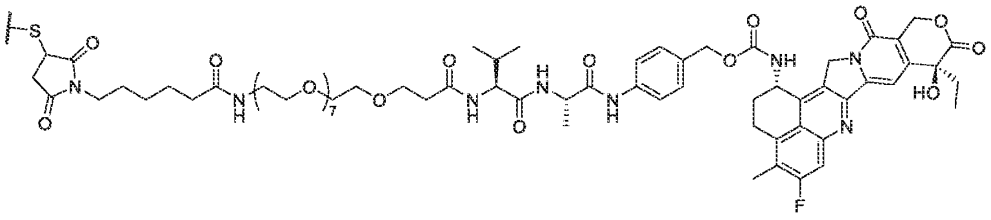
embodiments, x is 2. In some embodiments, x is 3. In some embodiments, x is 5. In some embodiments, n is 8. In some embodiments, z is 2. In some embodiments, R^P comprises a nitrogen atom and is bonded at the nitrogen atom, e.g., to the rest of a conjugate or other compound. In some embodiments, R^P is R^{EX}. In some embodiments, z is 2 and R^P is R^{EX}. In some embodiments, n is 8, z is 2 and R^P is R^{EX}. In some embodiments, x is 2, n is 8, z is 2 and R^P is R^{EX}. In some embodiments, x is 3, n is 8, z is 2 and R^P is R^{EX}. In some embodiments, x is 5, n is 8, z is 2 and R^P is R^{EX}. In some embodiments, each occurrence of $-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is the same.

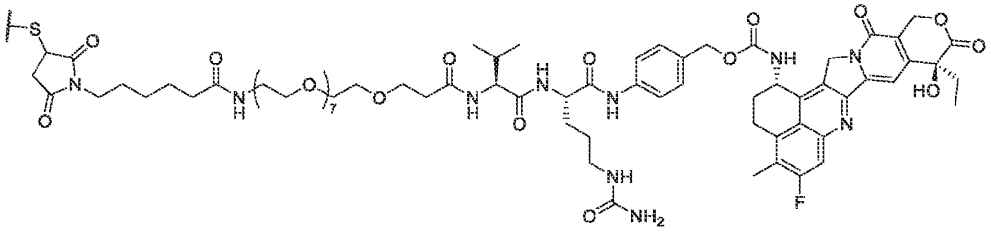
[0347] In some embodiments, an occurrence of $-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

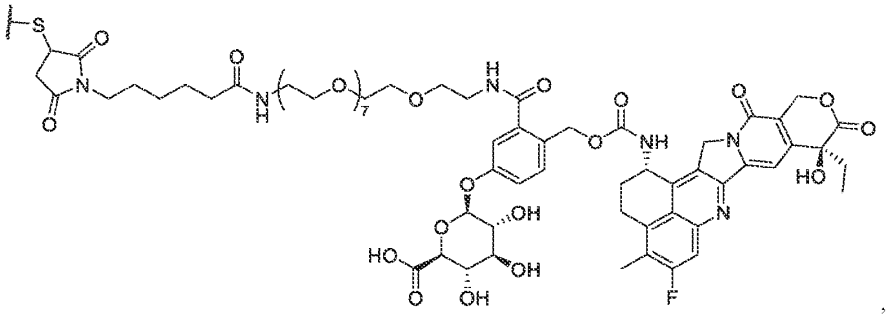


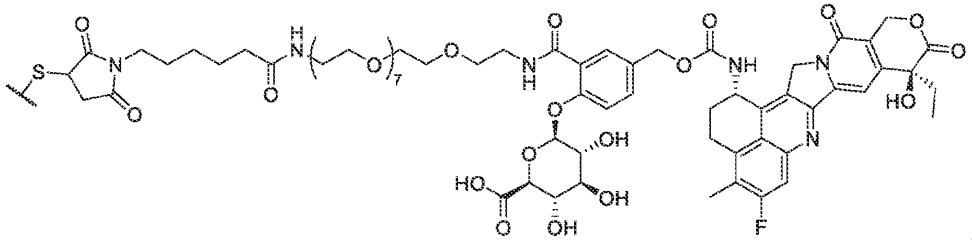


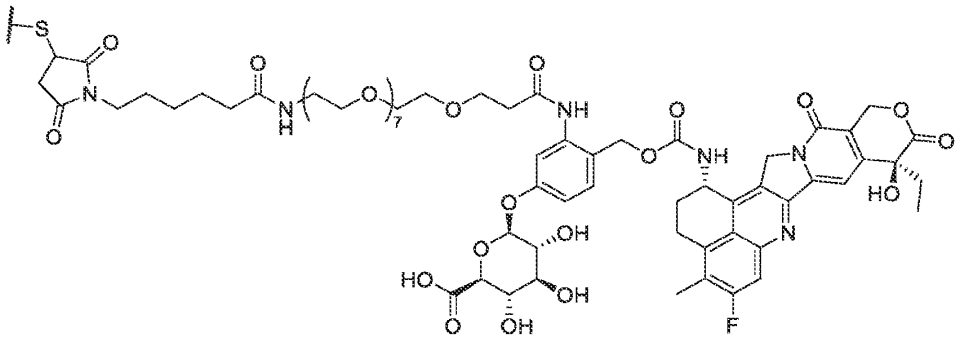




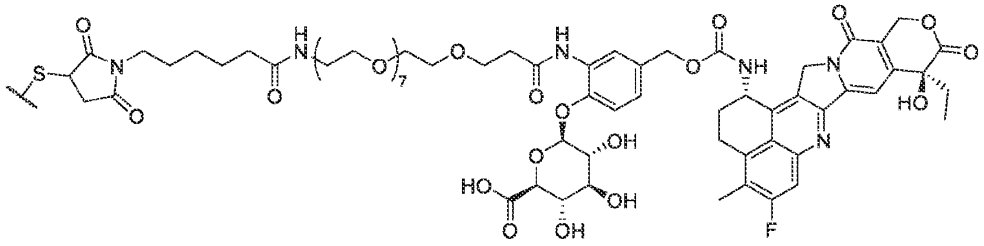






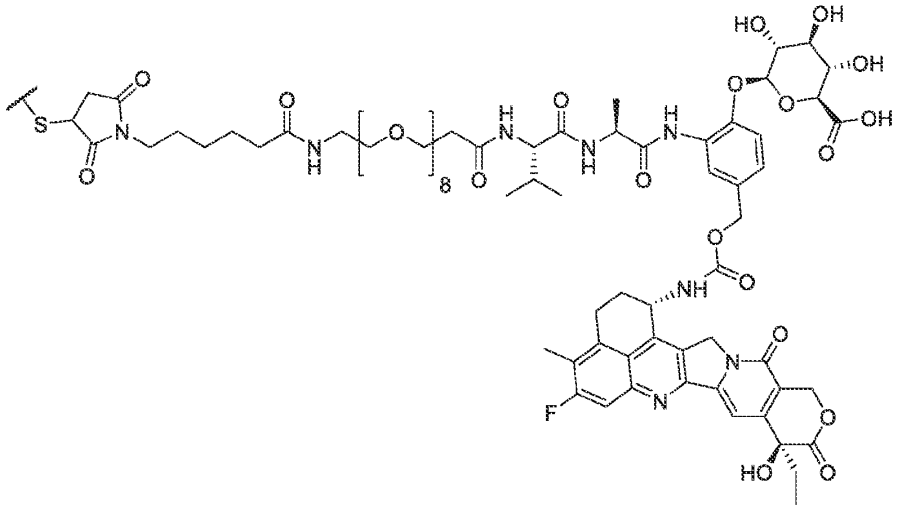


. or

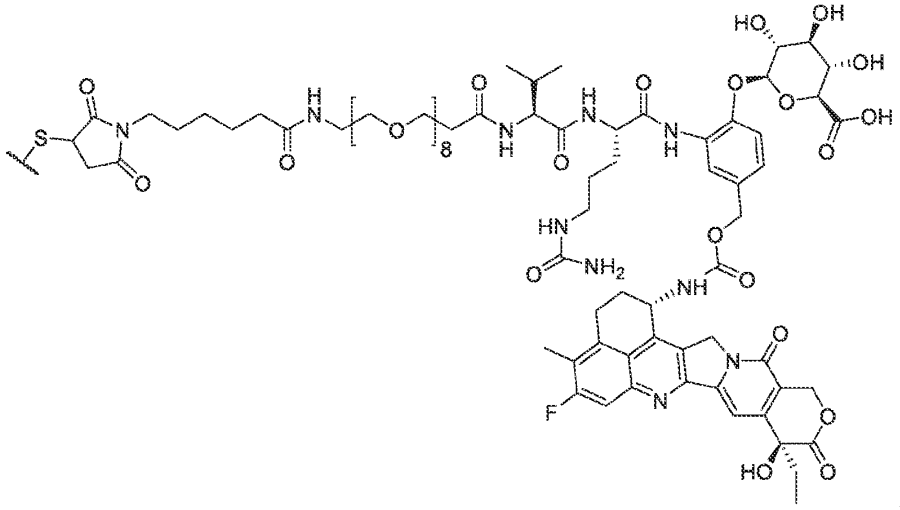


In some embodiments, each occurrence is the same.

[0348] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

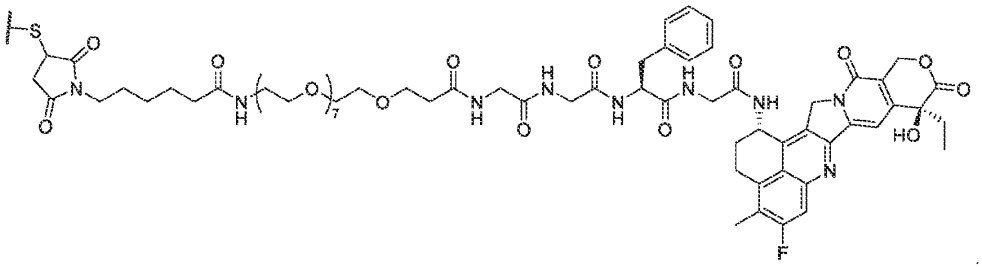


or

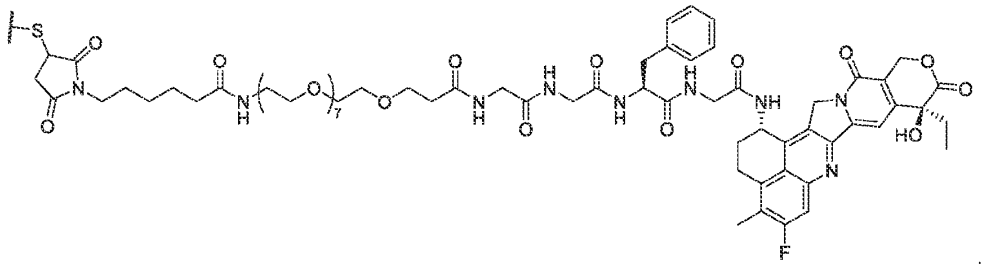


. In some embodiments, each occurrence is the same.

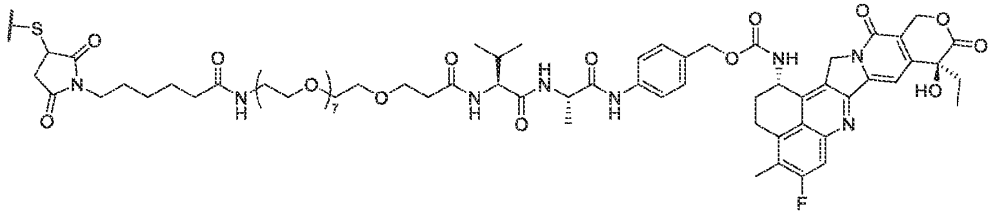
[0349] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



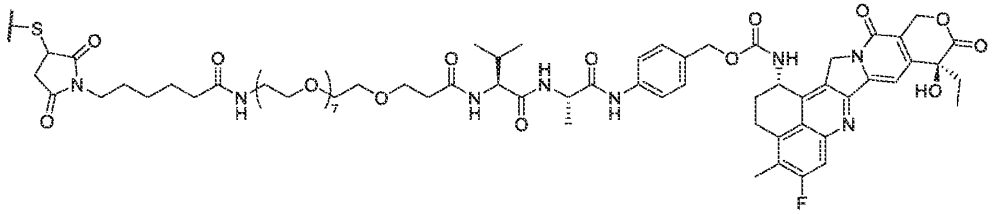
[0350] In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



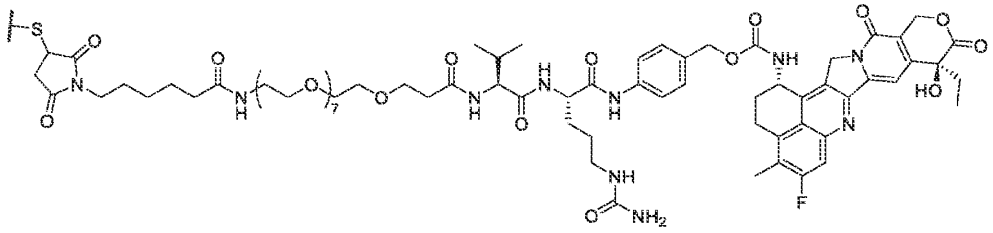
[0351] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



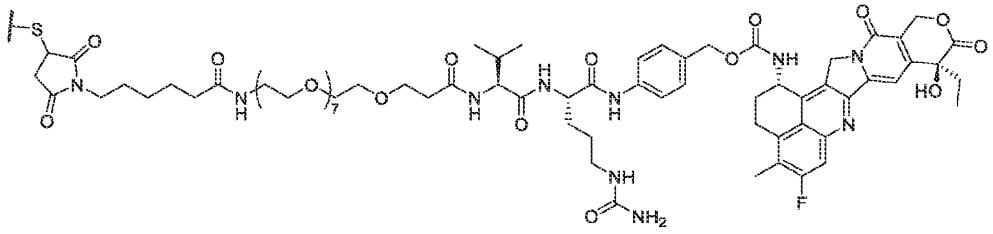
[0352] In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



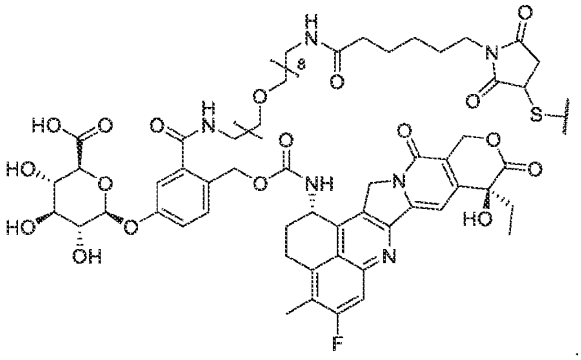
[0353] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



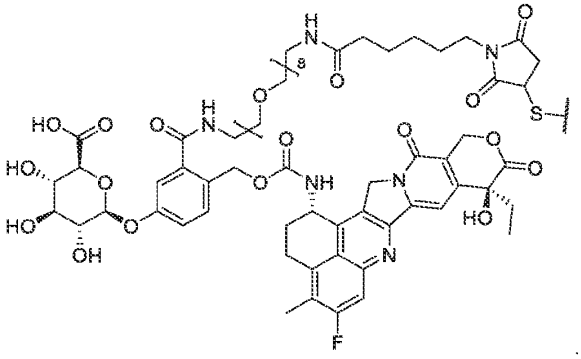
[0354] In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



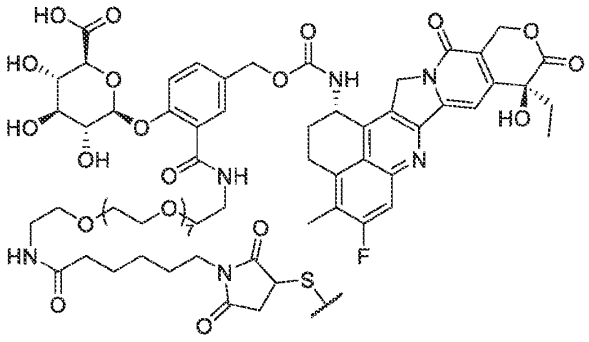
[0355] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



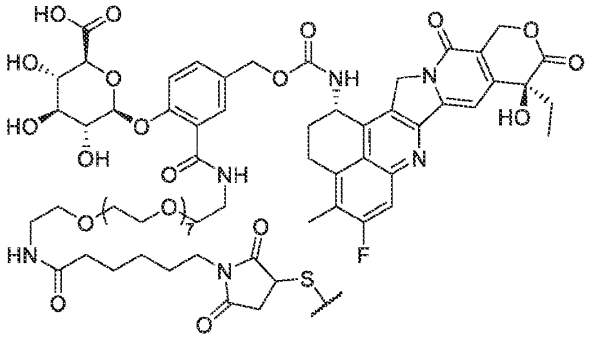
. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



. In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

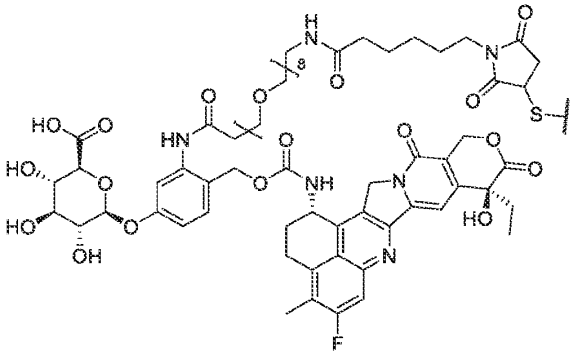


. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



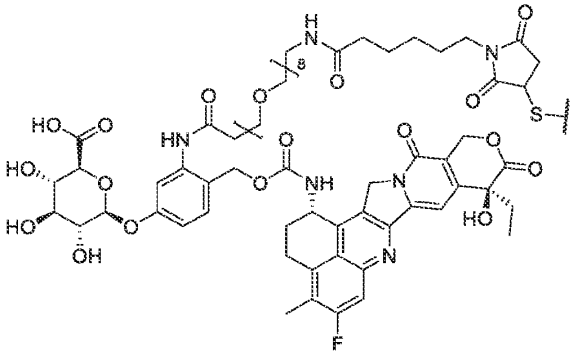
. In some embodiments, an occurrence of

$-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



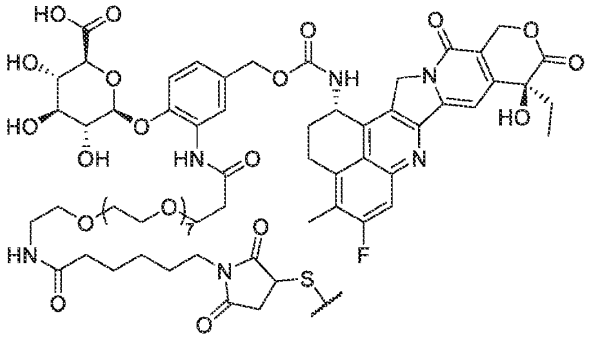
. In some embodiments, each occurrence of

$-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

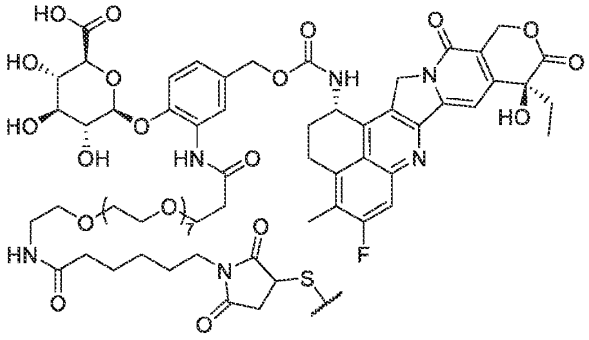


. In some embodiments, an occurrence of

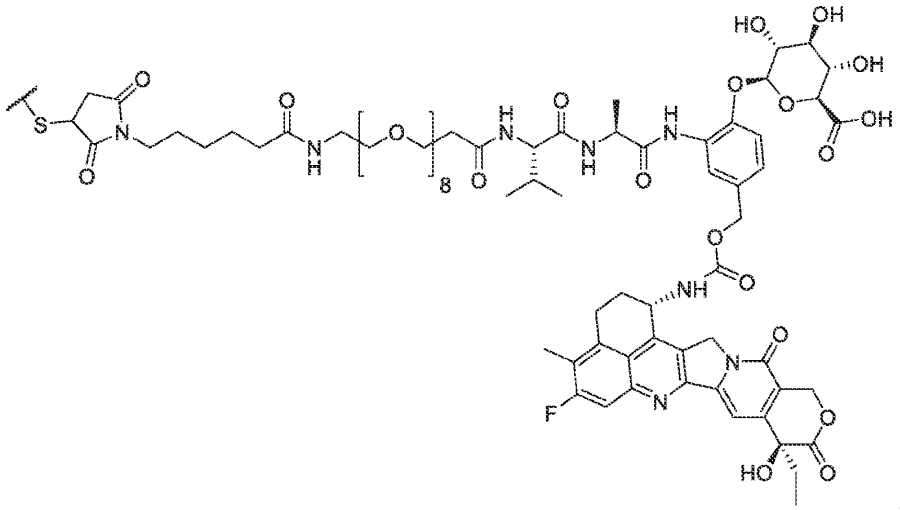
$-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



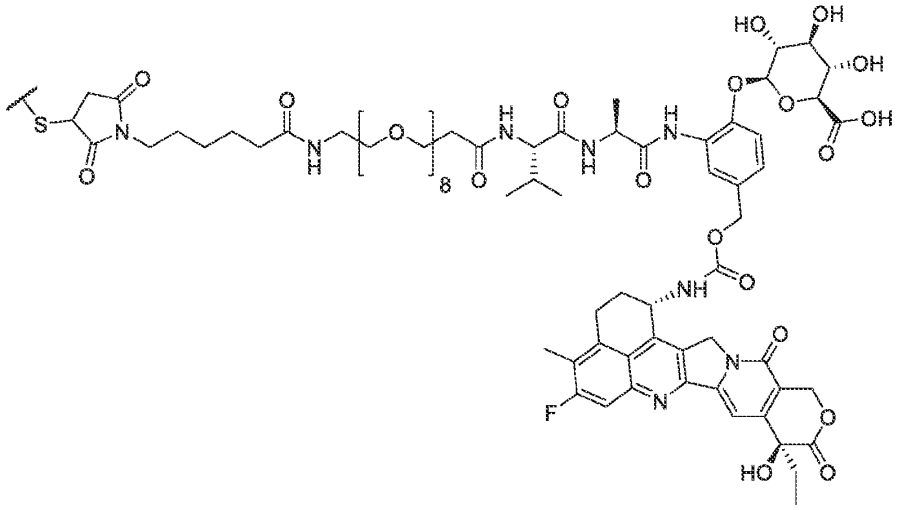
. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



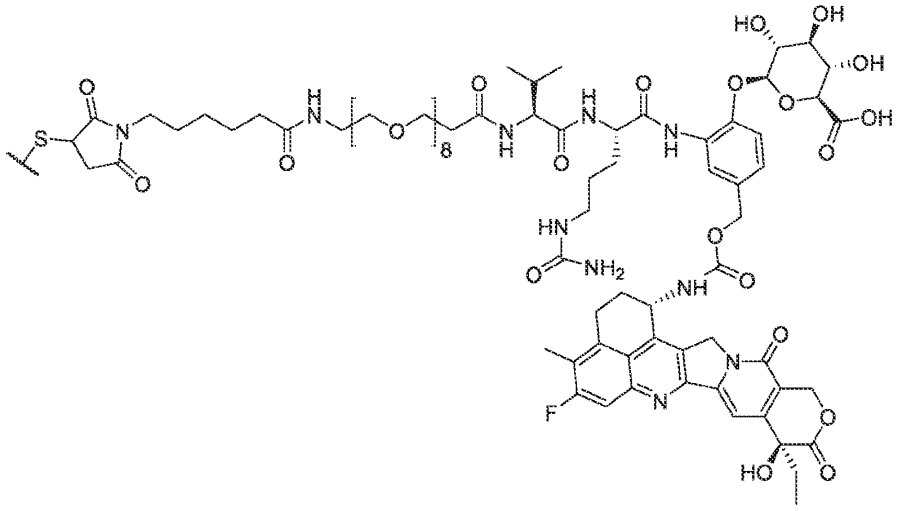
. In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



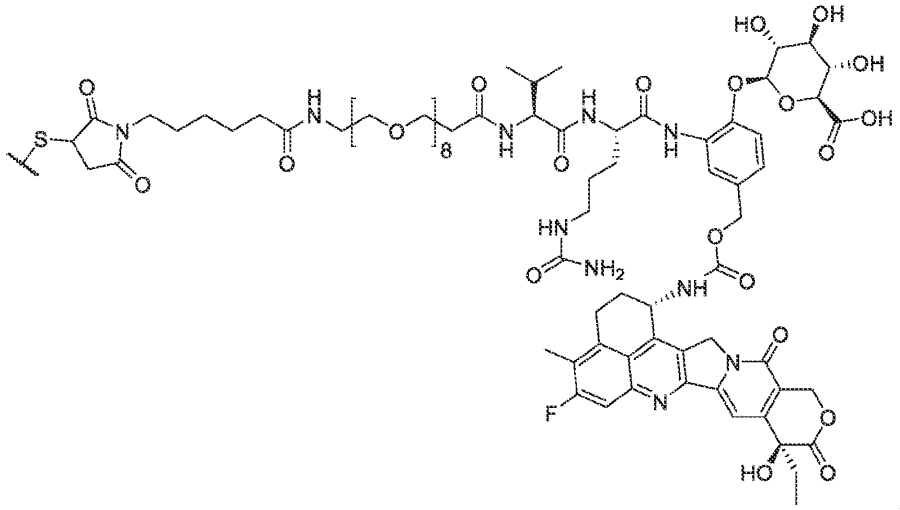
. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



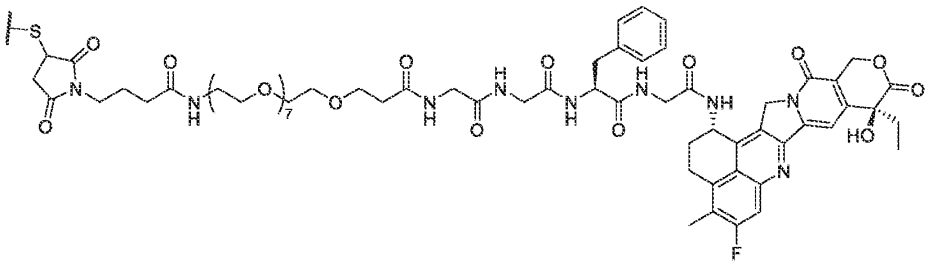
.In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

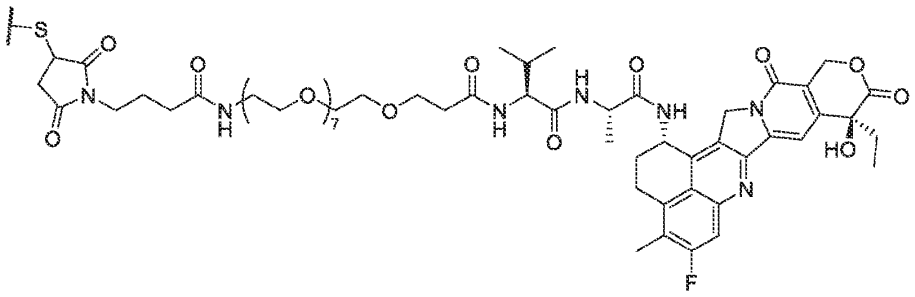


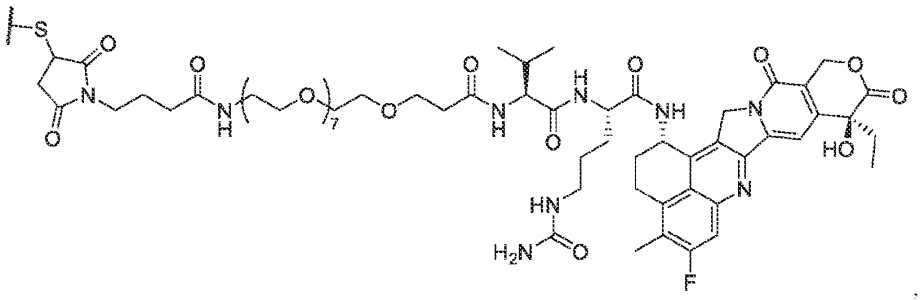
. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

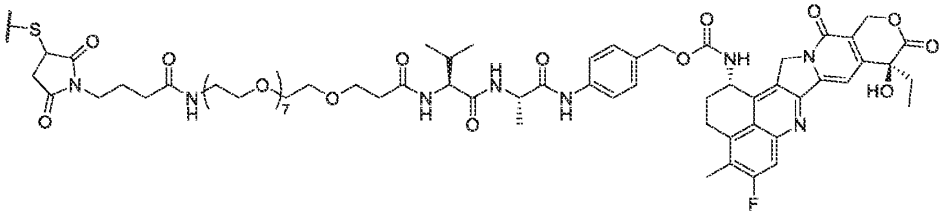


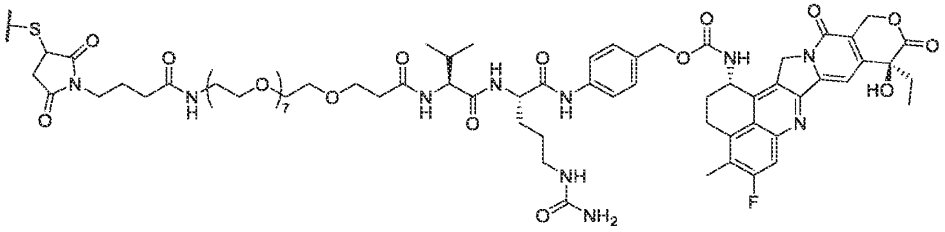
[0356] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

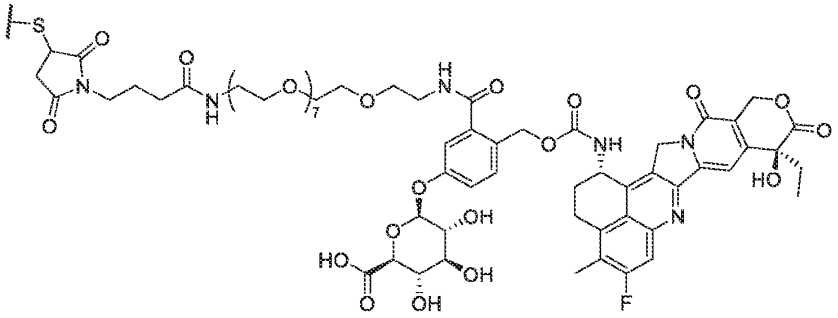


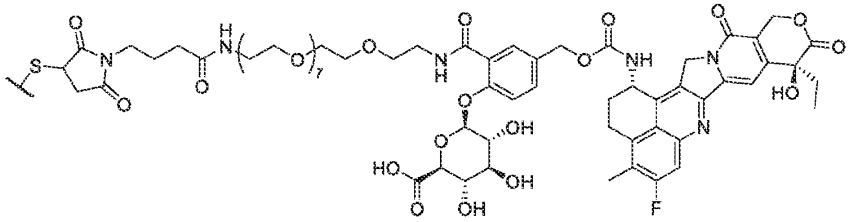


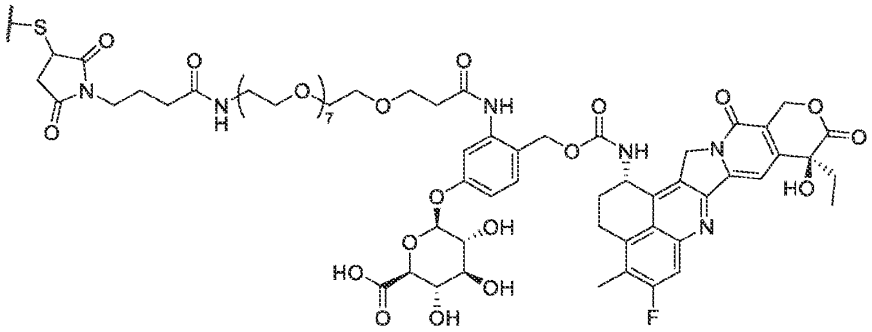




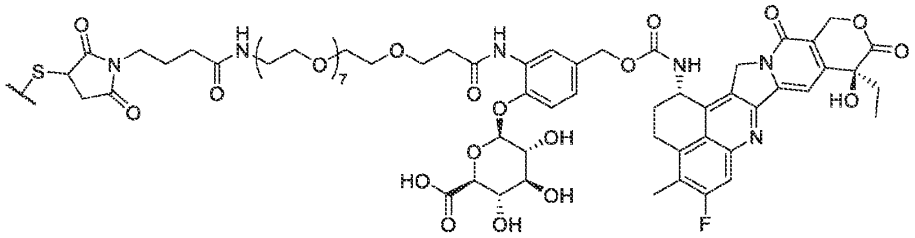






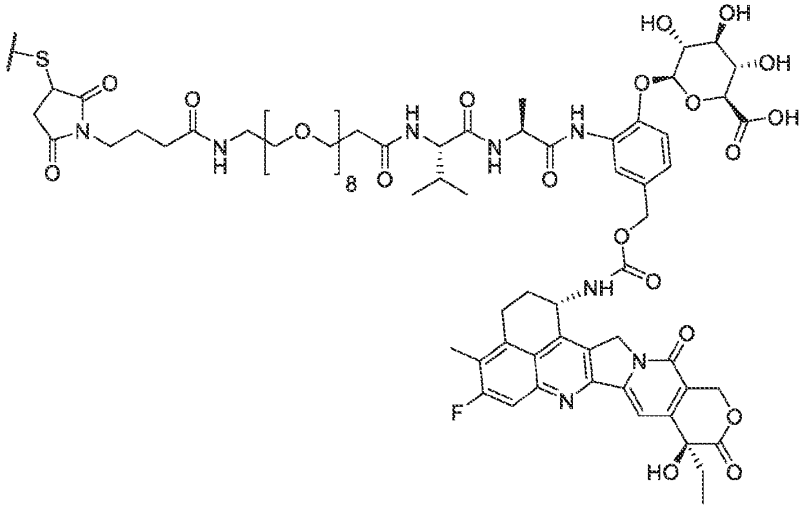


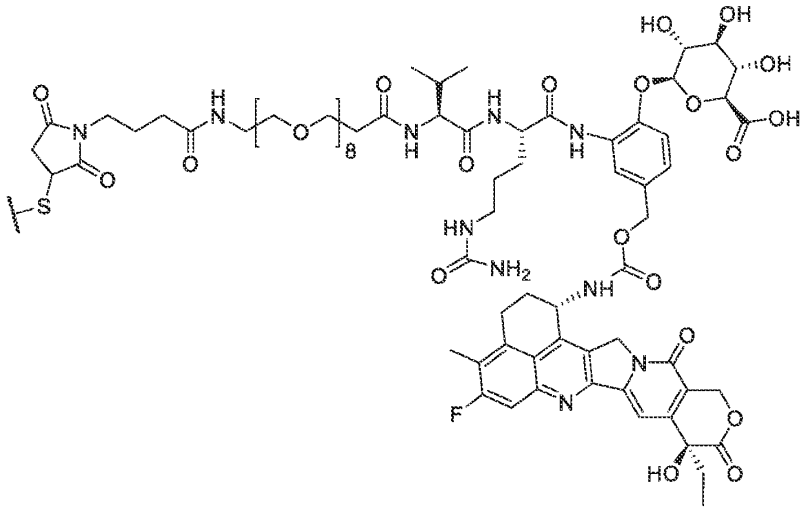
, or



. In some embodiments, each occurrence is the same.

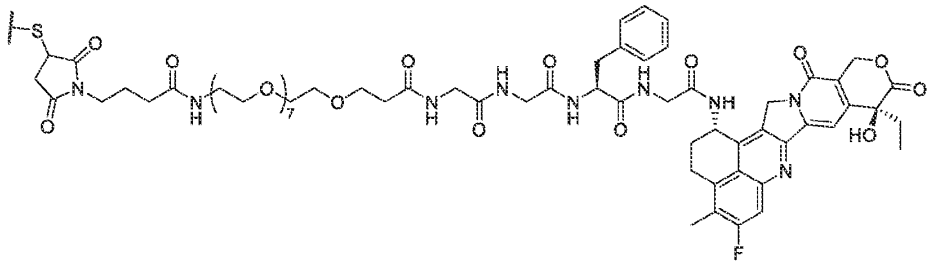
[0357] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



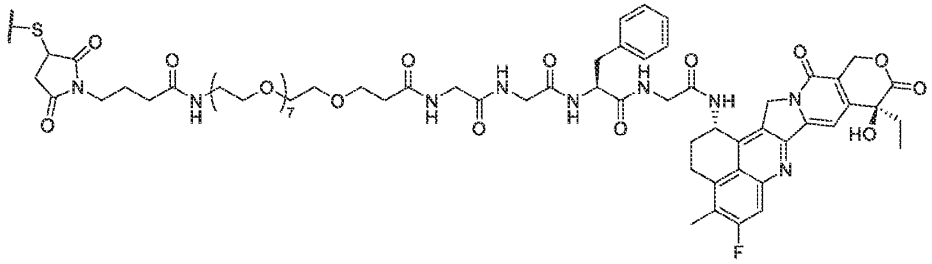


or . In some embodiments, each occurrence is the same.

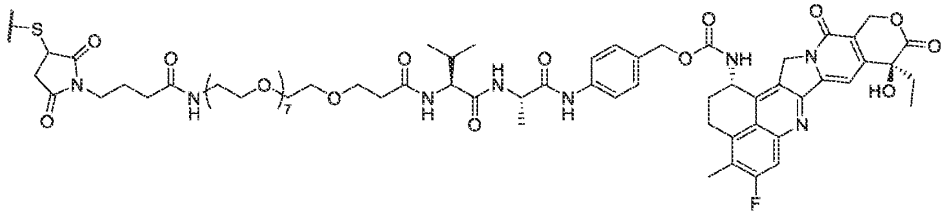
[0358] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



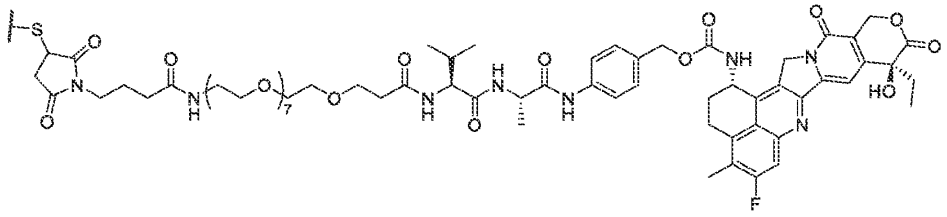
[0359] In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



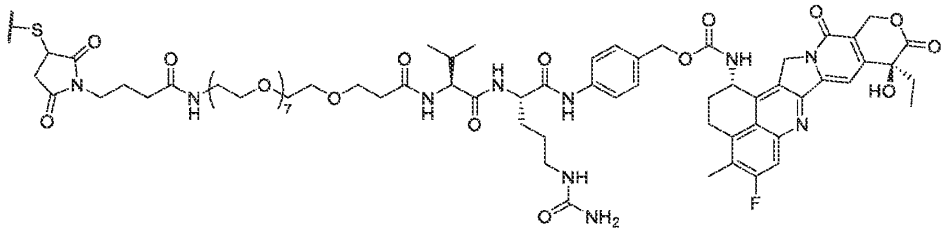
[0360] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



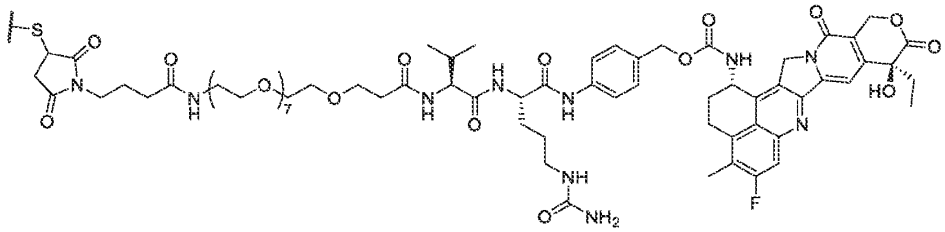
[0361] In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



[0362] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

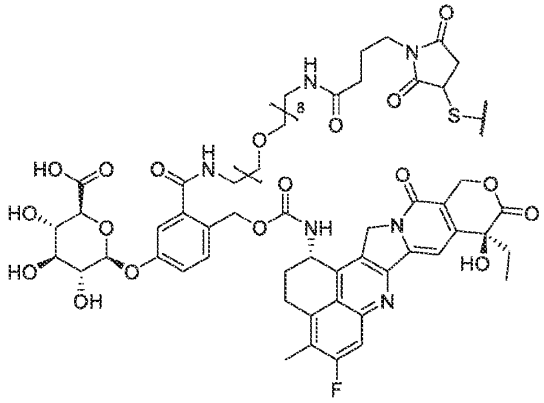


[0363] In some embodiments, each occurrence of $-L^{PX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

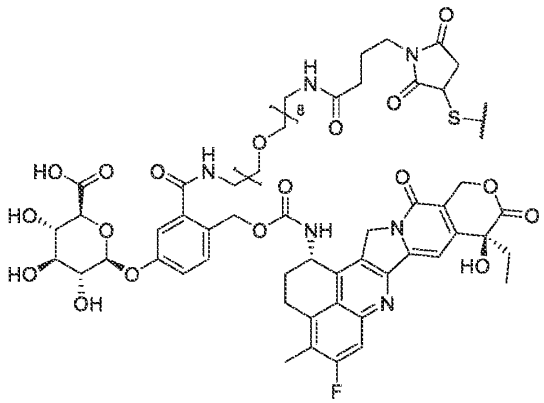


.

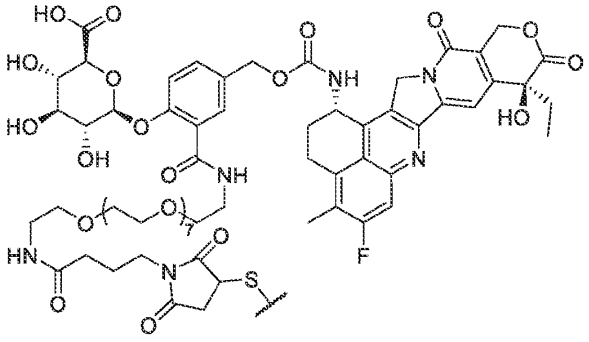
[0364] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

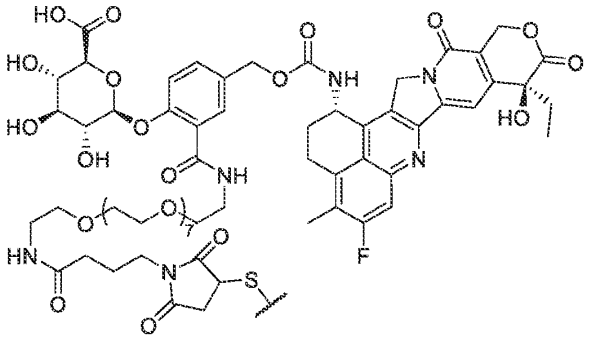


. In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



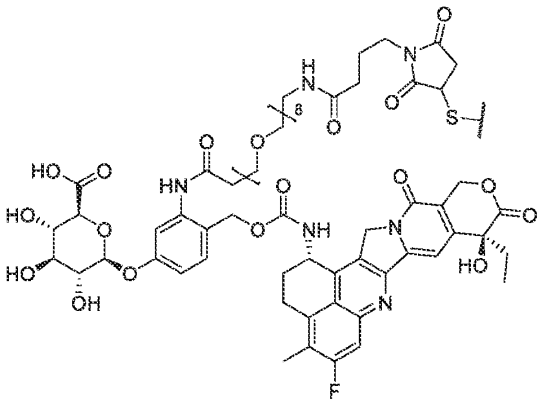
. In some embodiments, each occurrence of

$-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



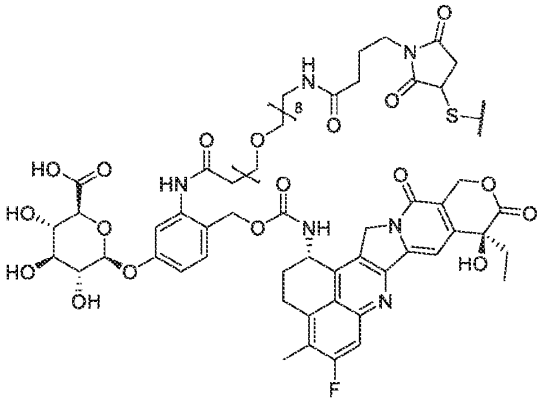
. In some embodiments, an occurrence of

$-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

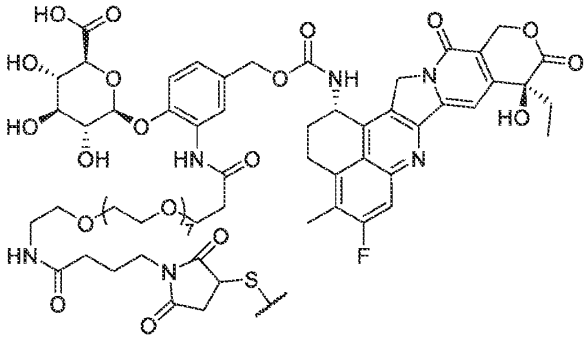


. In some embodiments, each occurrence of

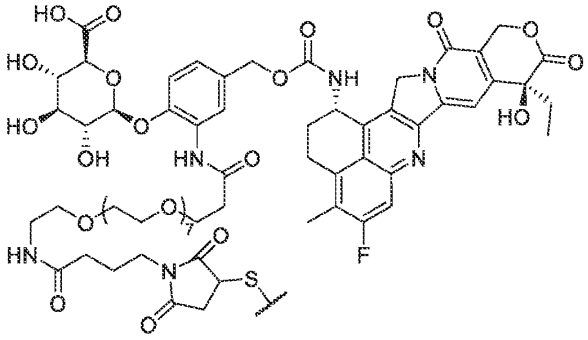
$-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



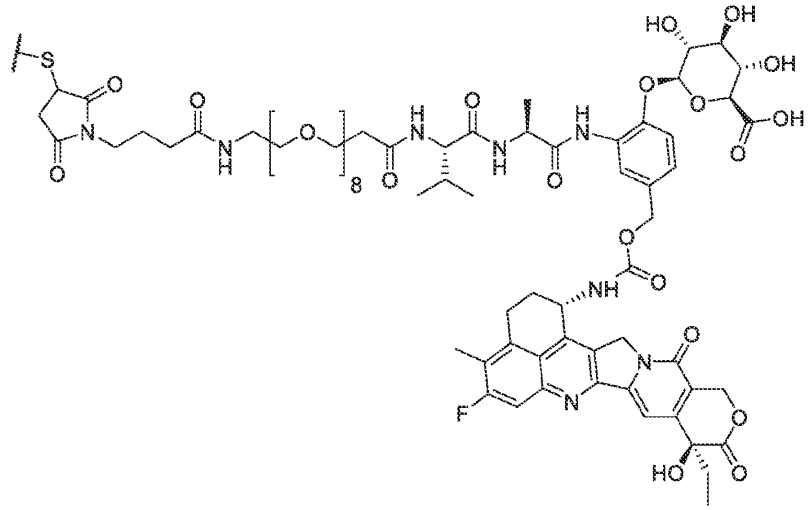
. In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



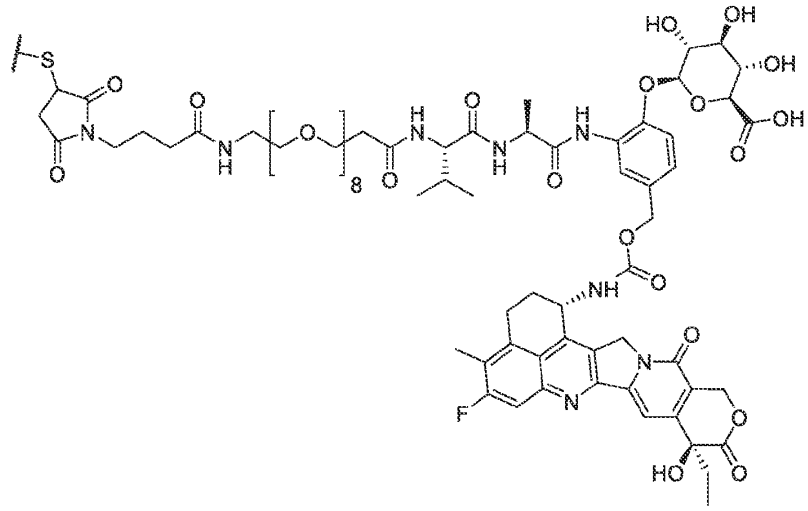
. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



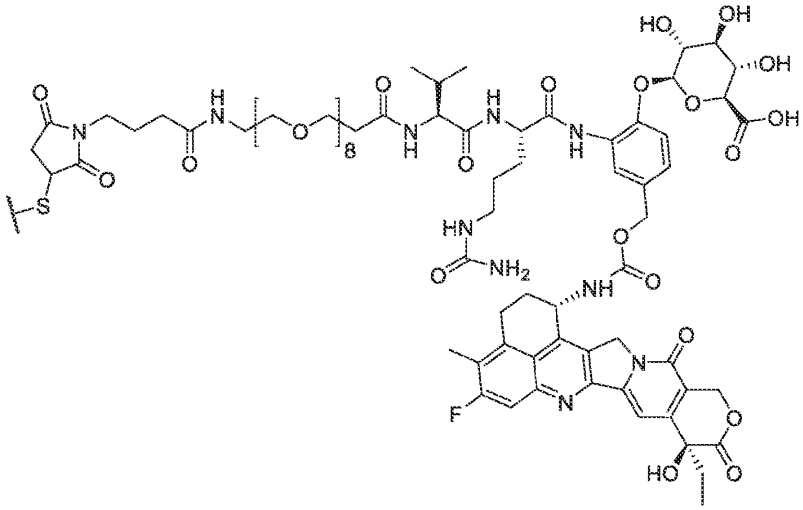
. In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



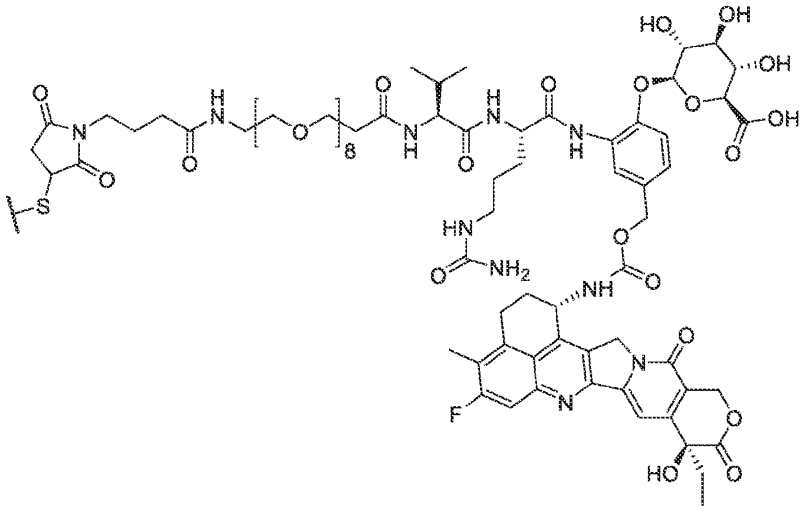
. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



. In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

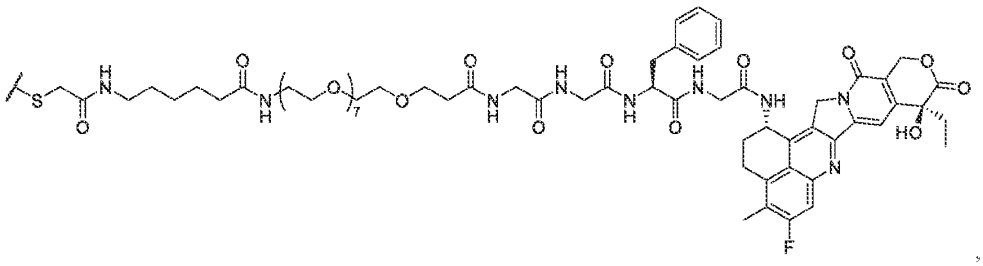


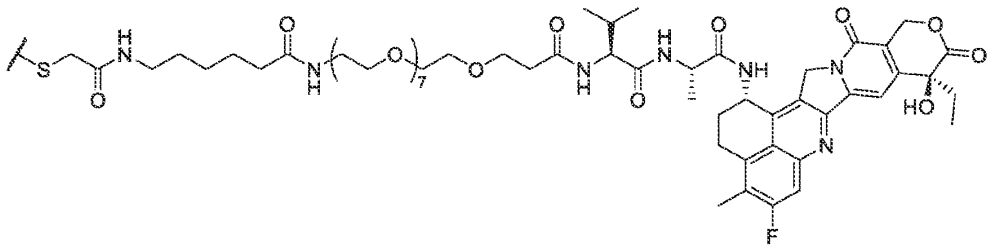
.

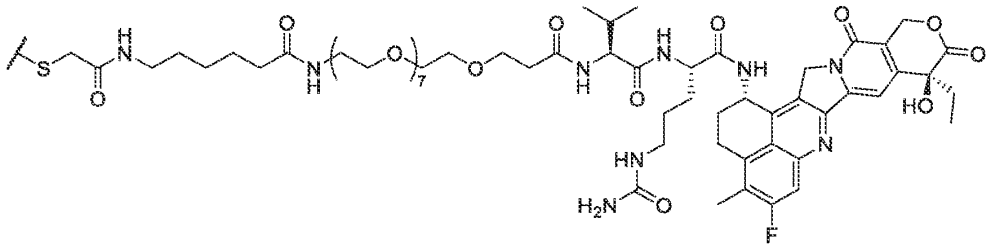
[0365] In some embodiments, a conjugate comprises $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$. In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is $-S-CH_2-C(O)NH-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-GGFG-R^{P}$. In some embodiments, it is $-S-CH_2-C(O)NH-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Ala-PABA-R^{P}$. In some embodiments, it is $-S-CH_2-C(O)NH-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-PABA-R^{P}$. In some embodiments, it is $-S-CH_2-C(O)NH-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)Val-Ala-NH-oGlu-R^{P}$. In some embodiments, it is $-S-CH_2-C(O)NH-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Ala-NH-mGlu-R^{P}$. In some embodiments, it is $-S-CH_2-C(O)NH-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-NH-oGLu-R^{P}$. In some

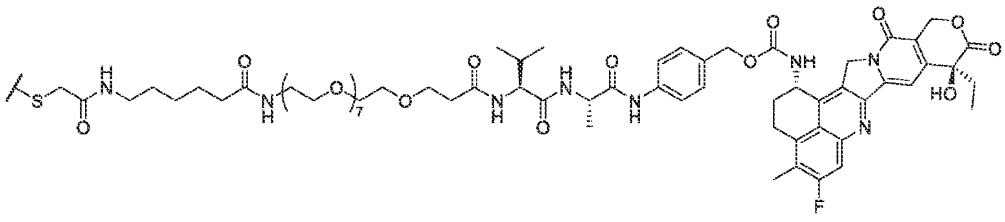
embodiments, it is $-S-CH_2-C(O)NH-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-NH-mGLu-R^P$. In some embodiments, it is $-S-CH_2-C(O)NH-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zNHC(O)-oGlu-R^P$. In some embodiments, it is $-S-CH_2-C(O)NH-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zNHC(O)-mGlu-R^P$. In some embodiments, x is 2. In some embodiments, x is 3. In some embodiments, x is 5. In some embodiments, n is 8. In some embodiments, z is 2. In some embodiments, R^P comprises a nitrogen atom and is bonded at the nitrogen atom, e.g., to the rest of a conjugate or other compound. In some embodiments, R^P is R^{EX}. In some embodiments, z is 2 and R^P is R^{EX}. In some embodiments, n is 8, z is 2 and R^P is R^{EX}. In some embodiments, x is 2, n is 8, z is 2 and R^P is R^{EX}. In some embodiments, x is 3, n is 8, z is 2 and R^P is R^{EX}. In some embodiments, x is 5, n is 8, z is 2 and R^P is R^{EX}. In some embodiments, each occurrence of $-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is the same.

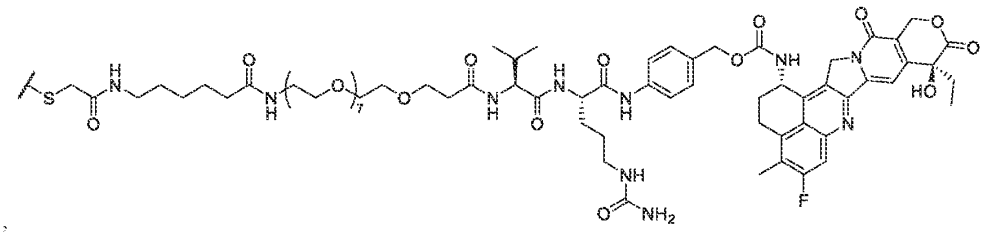
[0366] In some embodiments, an occurrence of $-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

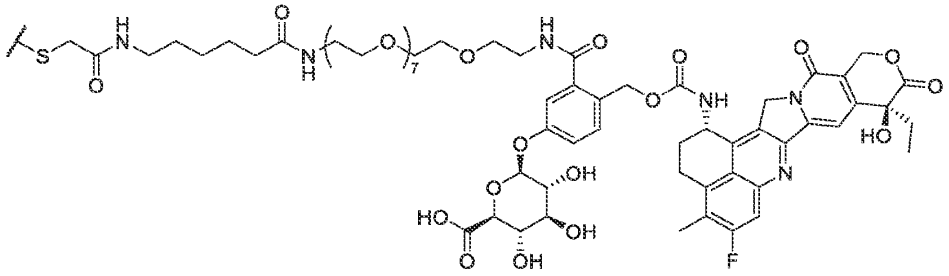


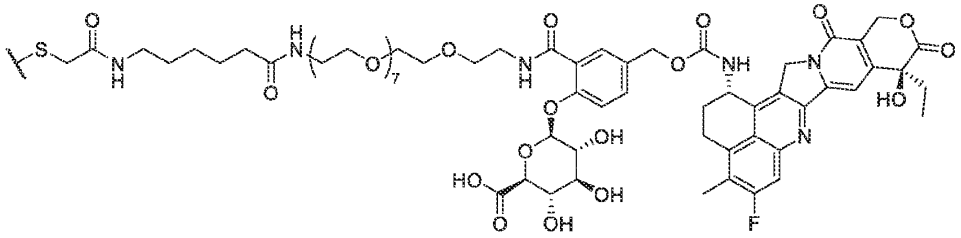


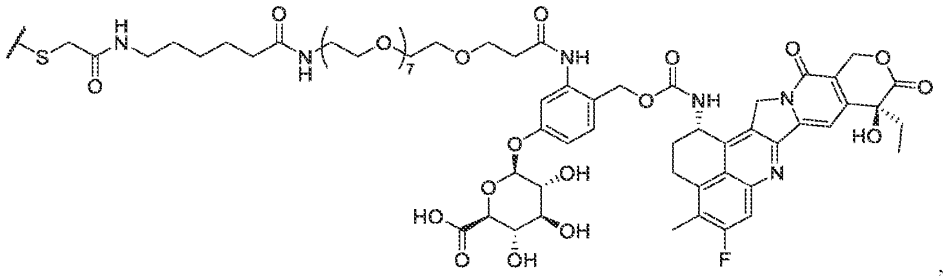




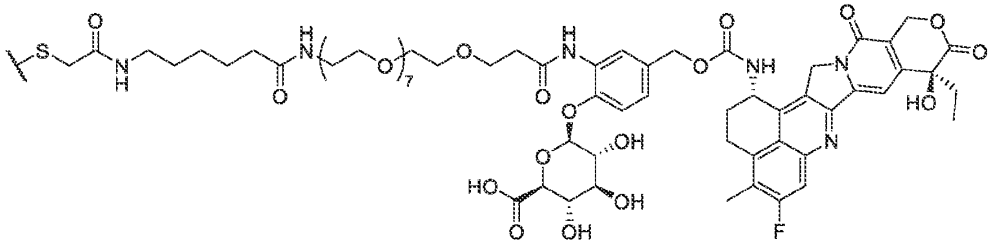






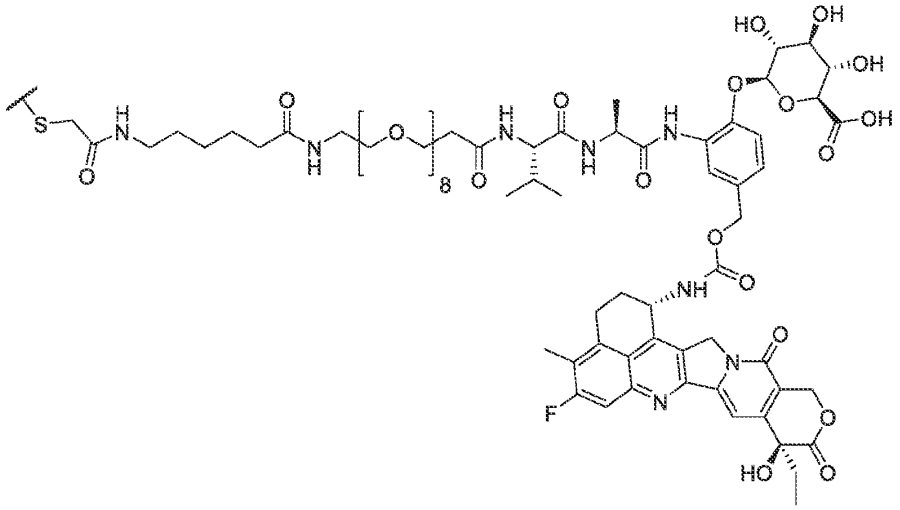


, or

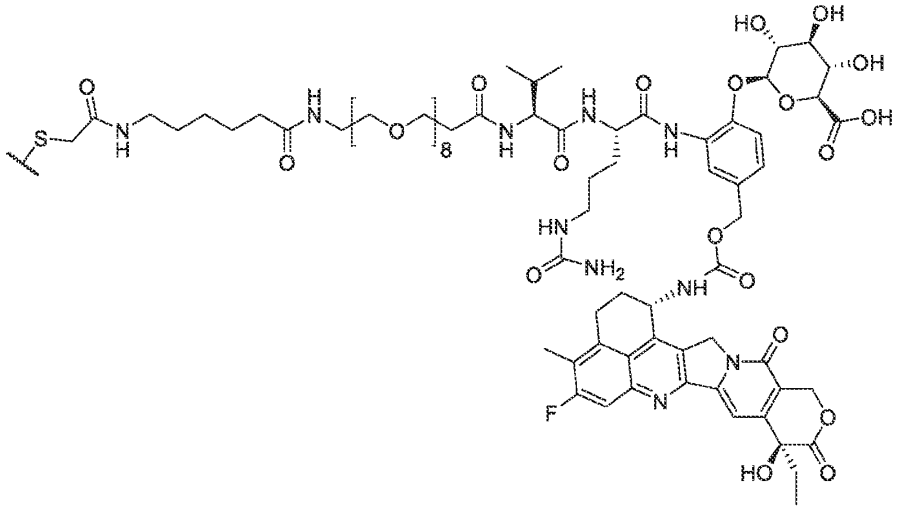


In some embodiments, each occurrence is the same.

[0367] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

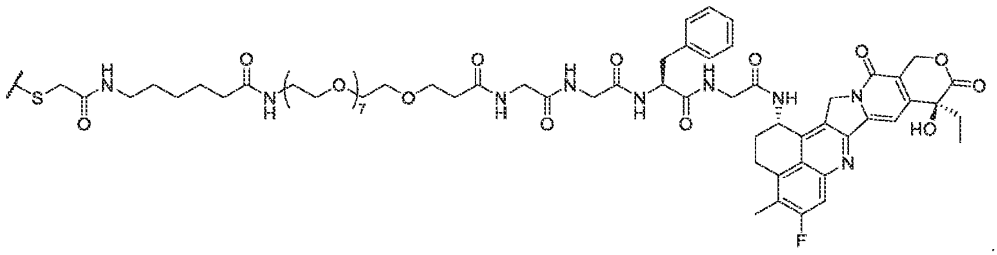


or



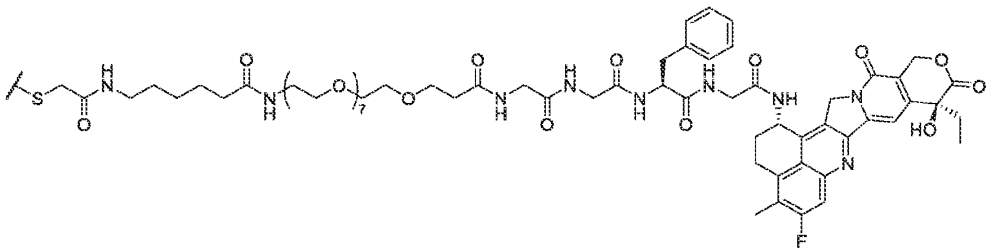
. In some embodiments, each occurrence is the same.

[0368] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

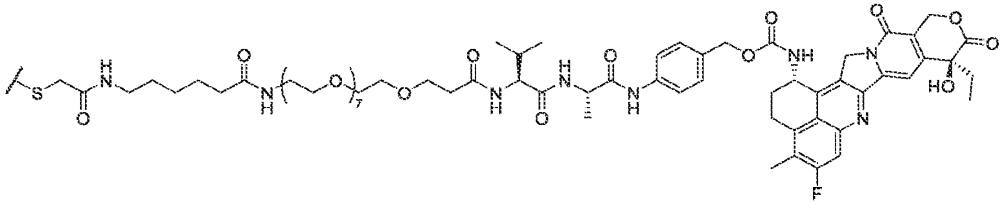


.

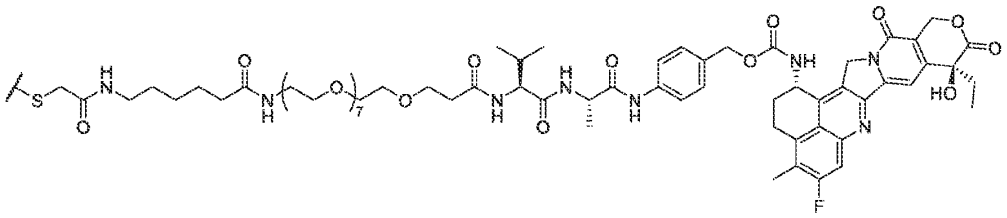
[0369] In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



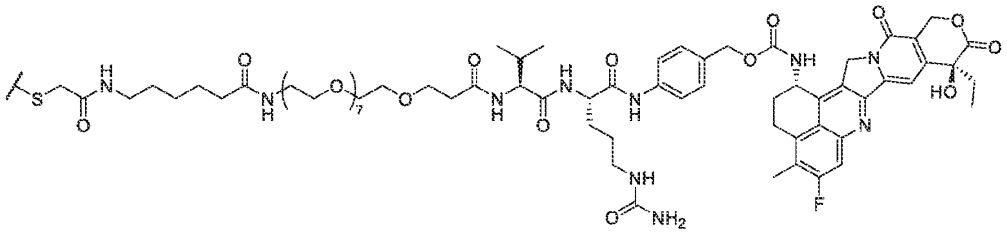
[0370] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



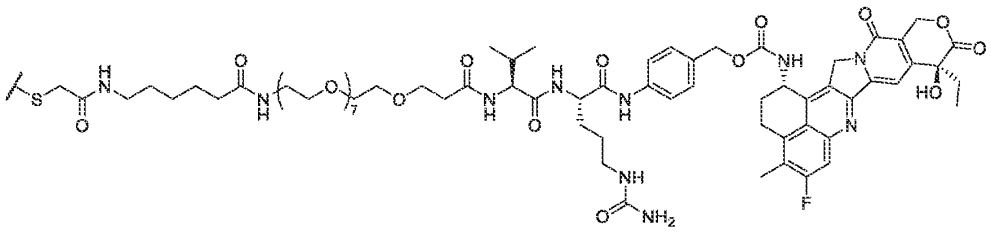
[0371] In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



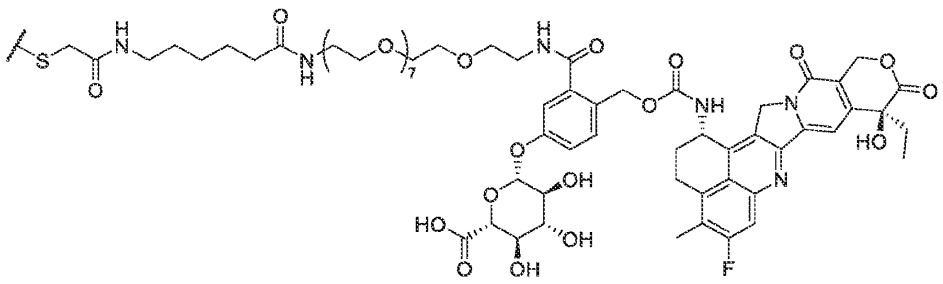
[0372] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



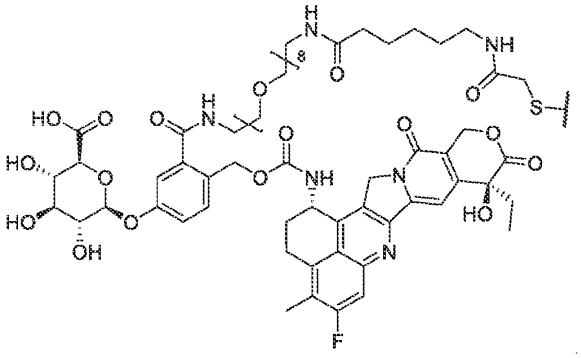
[0373] In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



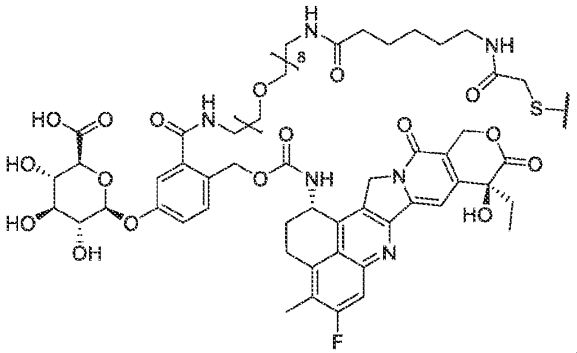
[0374] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



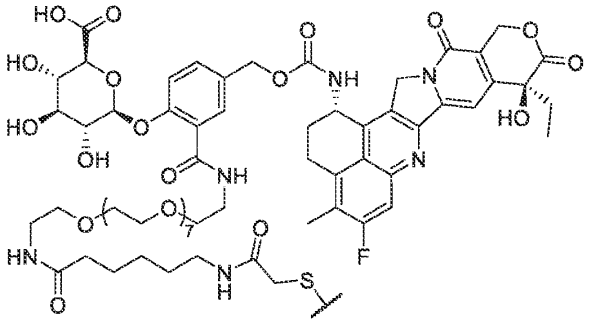
[0375] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



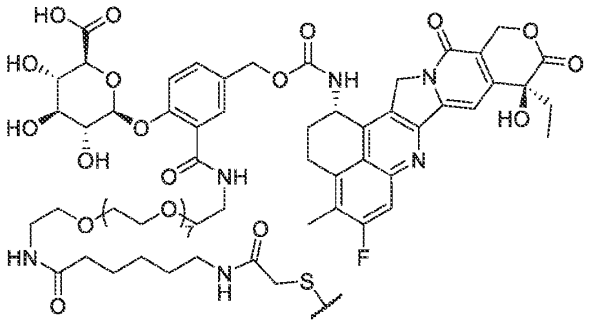
. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



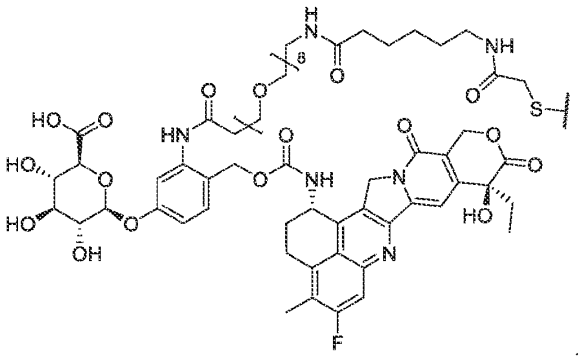
. In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



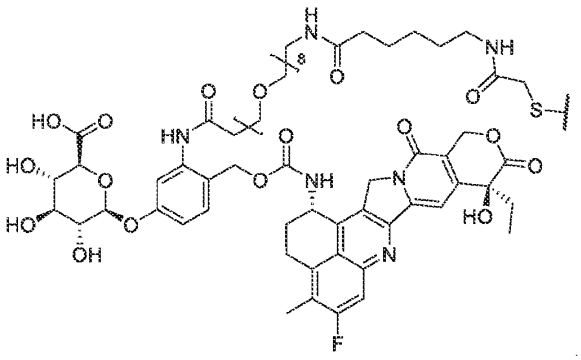
. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



. In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

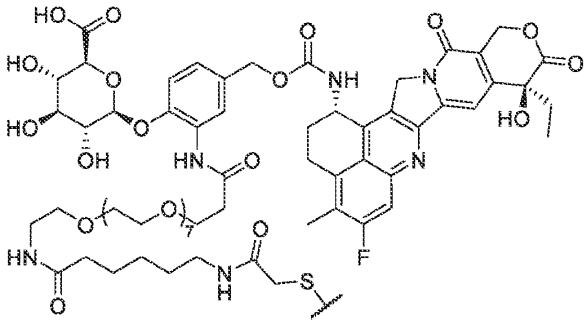


. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

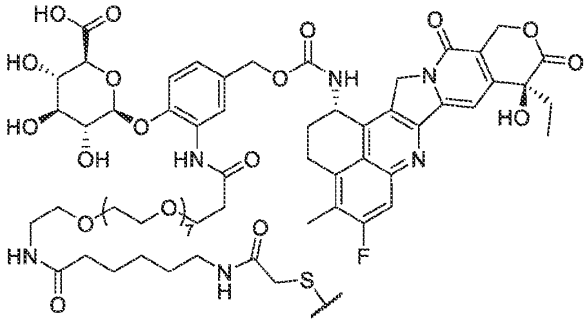


. In some embodiments, an occurrence of

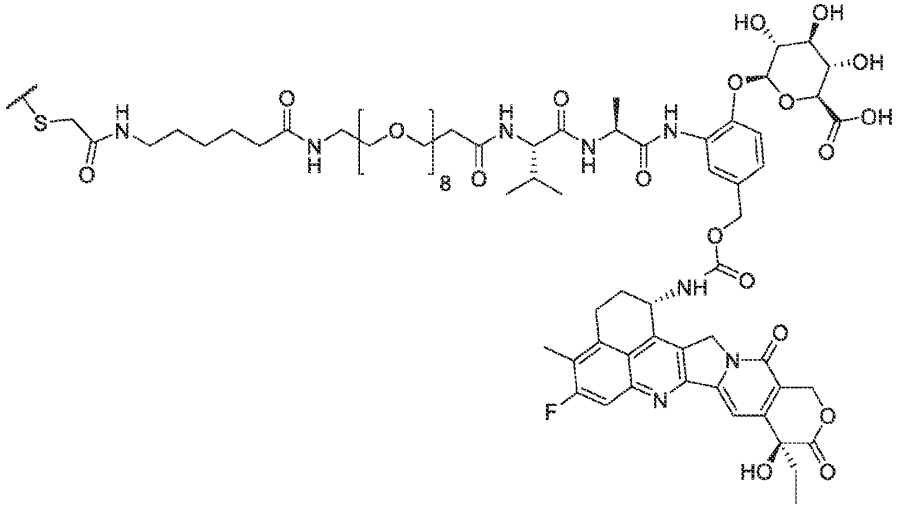
$-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



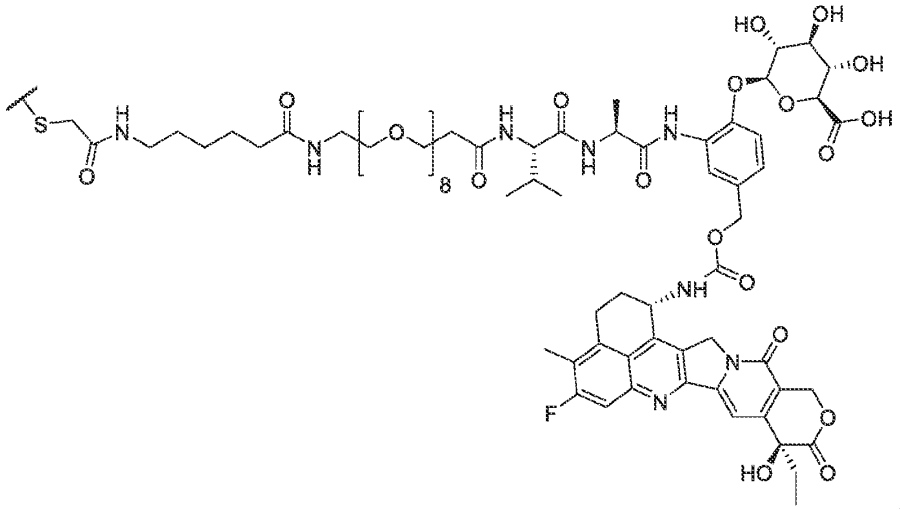
. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



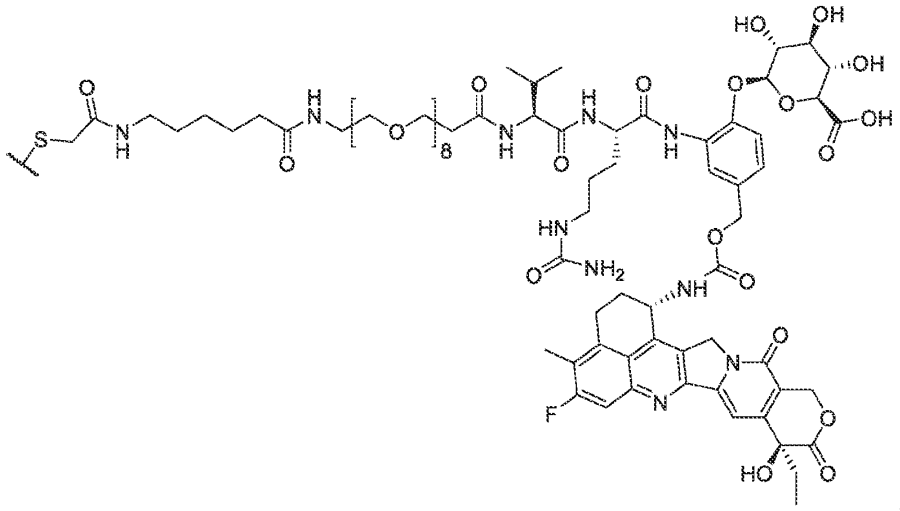
. In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



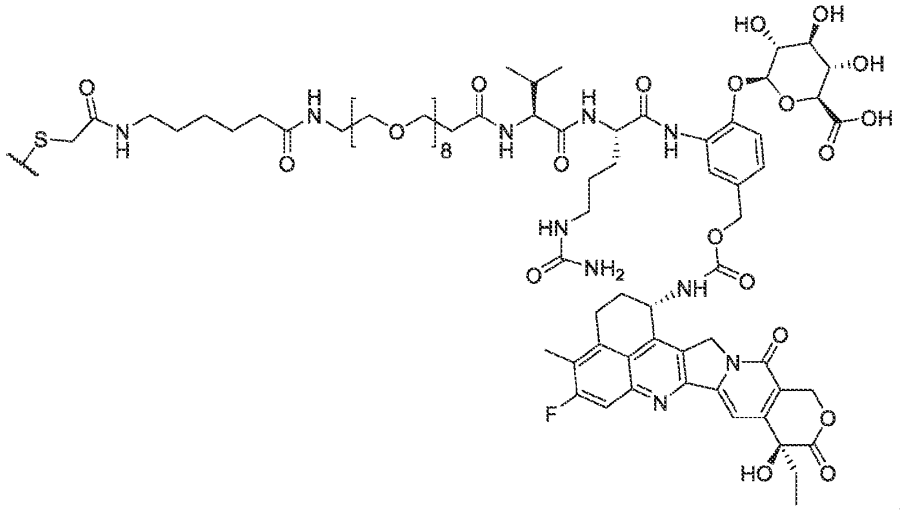
. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



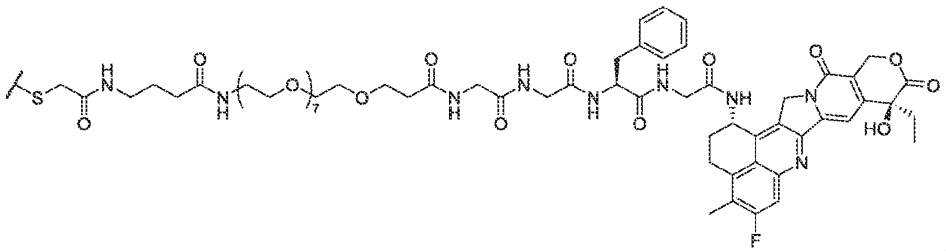
.In some embodiments, an occurrence of –L^{RX}–L^{X}–$(CH_2CH_2O)n$–L^{ZP}–L^{C}–R^{P} is or comprises

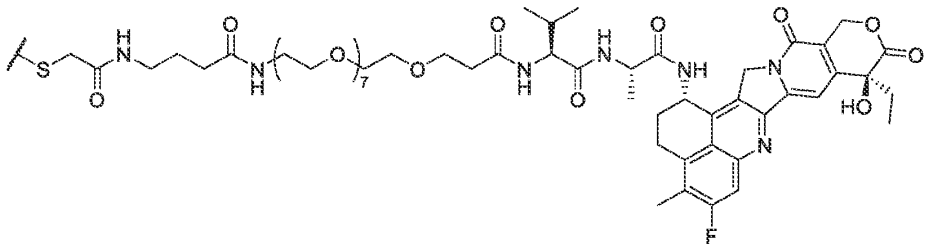


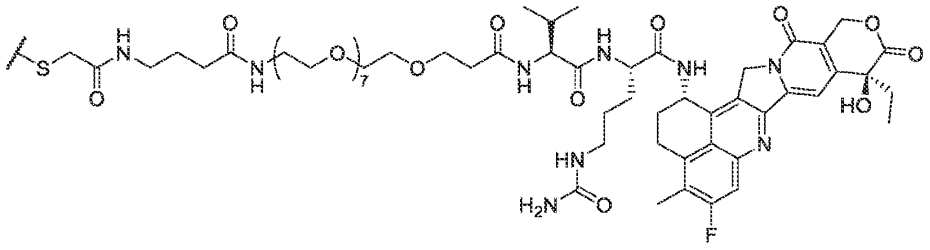
. In some embodiments, each occurrence of –L^{RX}–L^{X}–$(CH_2CH_2O)n$–L^{ZP}–L^{C}–R^{P} is or comprises

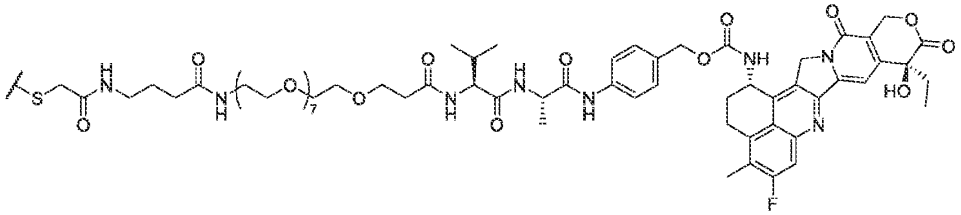


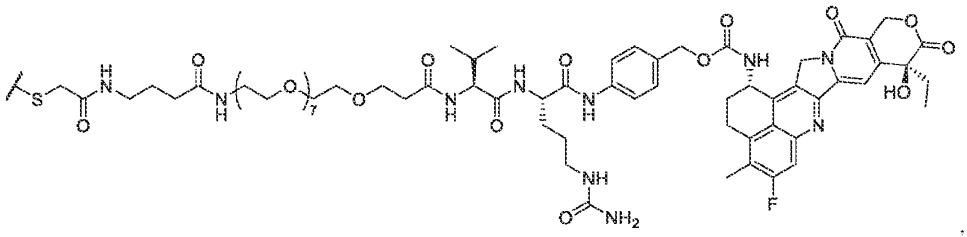
[0376] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

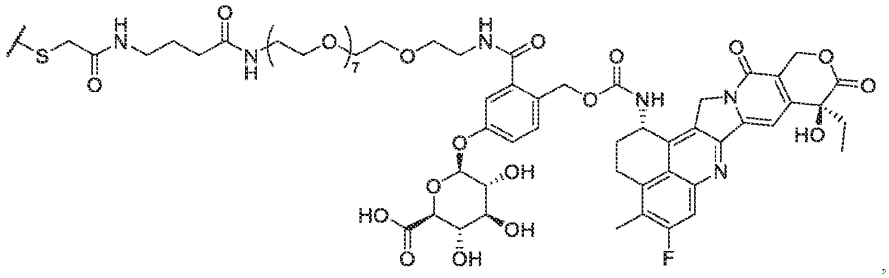


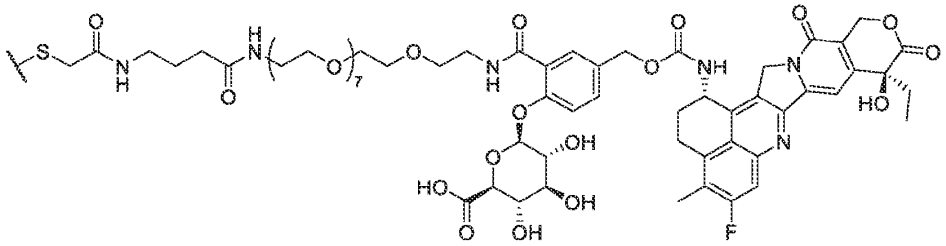


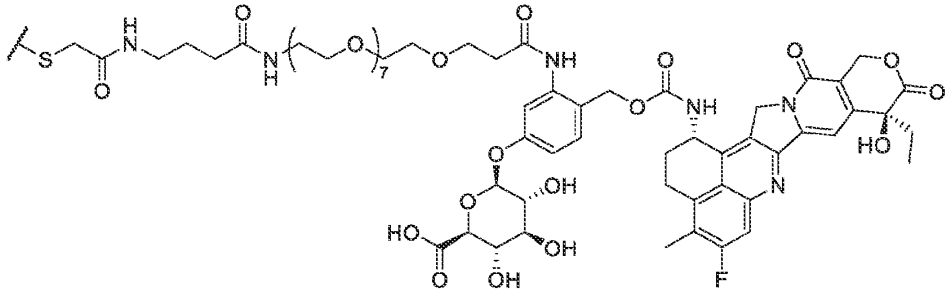




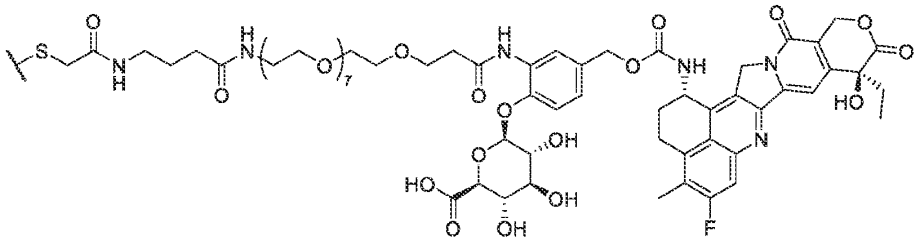






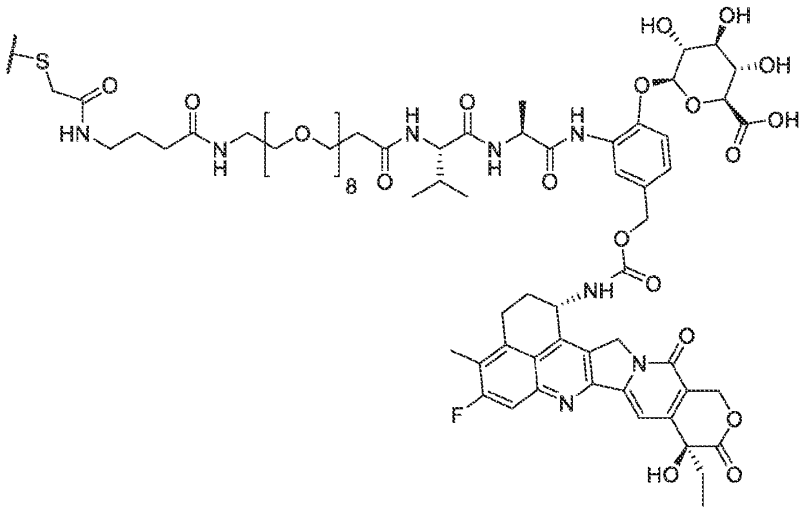


, or

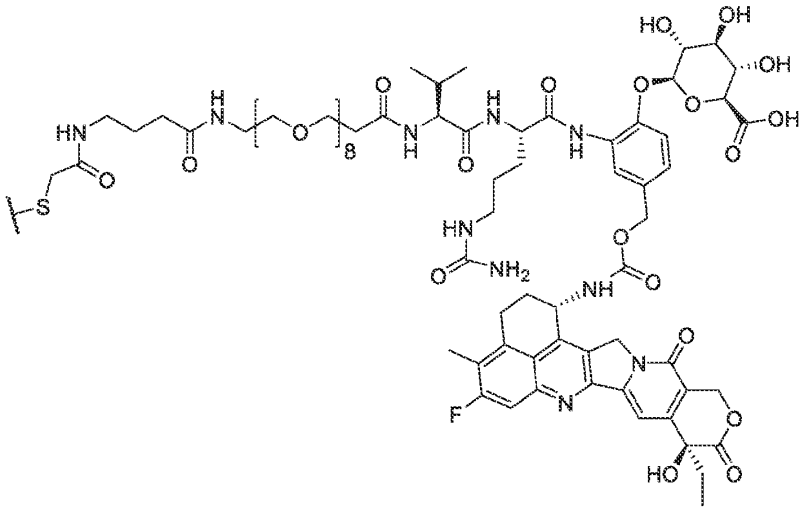


. In some embodiments, each occurrence is the same.

[0377] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

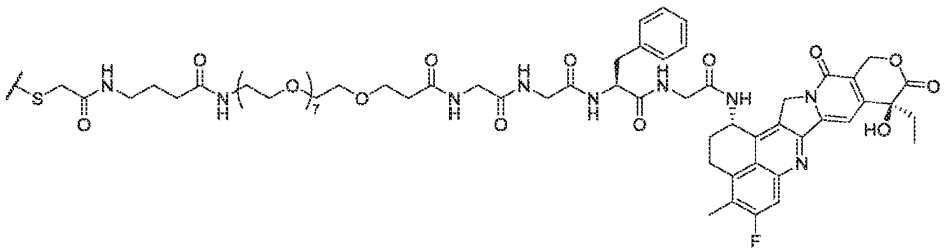


or

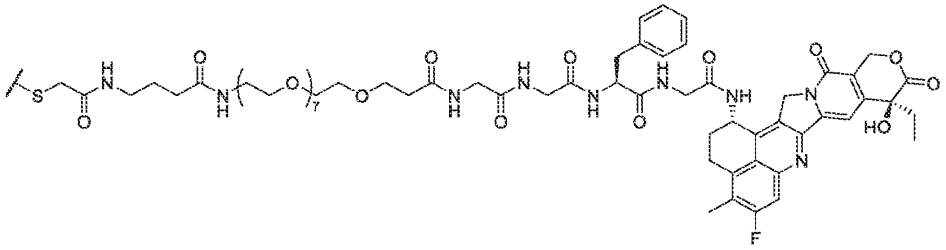


. In some embodiments, each occurrence is the same.

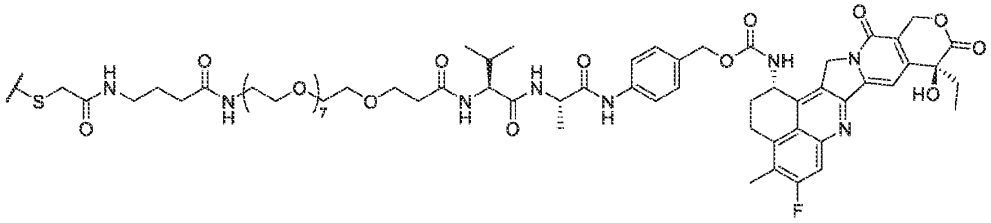
[0378] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



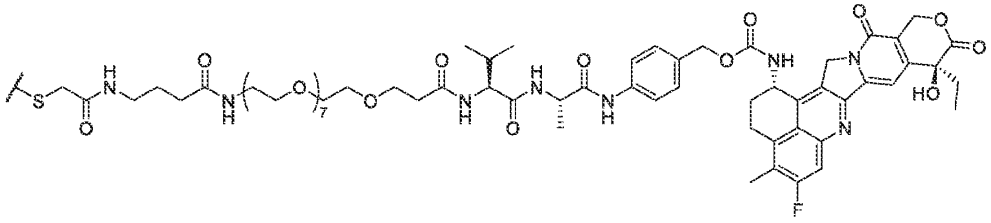
[0379] In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



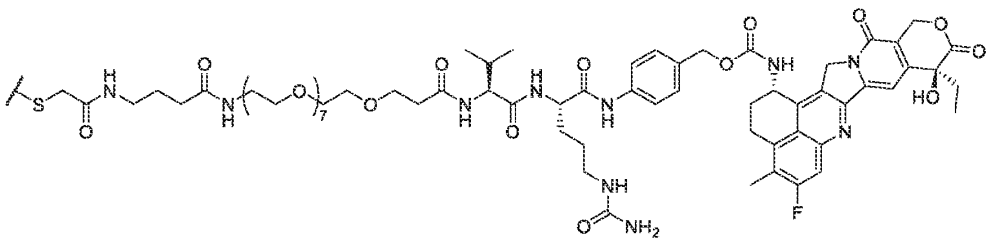
[0380] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



[0381] In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

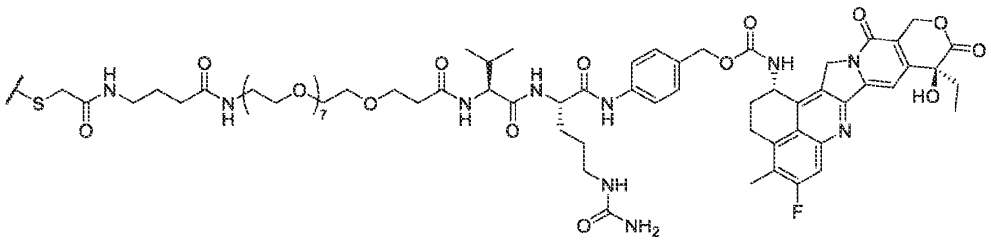


[0382] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



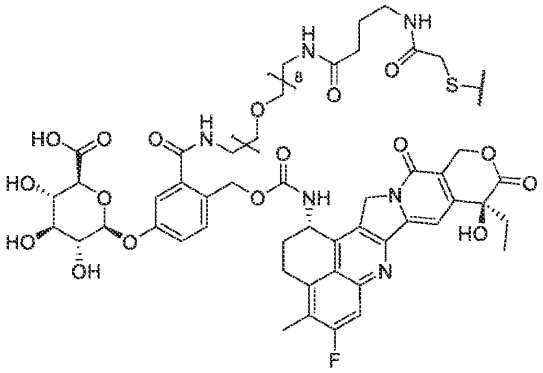
[0383] In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or

comprises

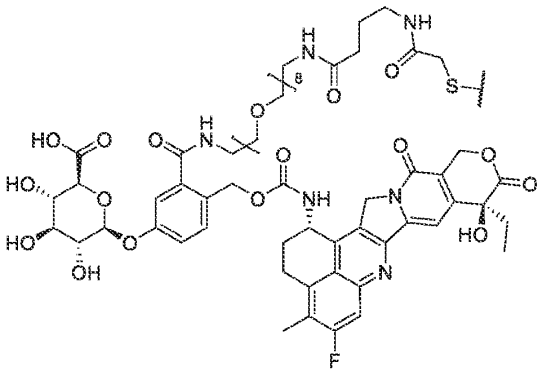


.

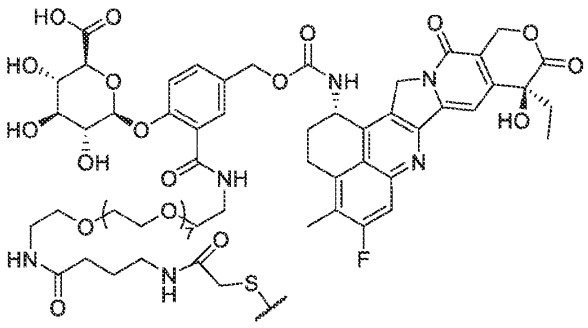
[0384] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

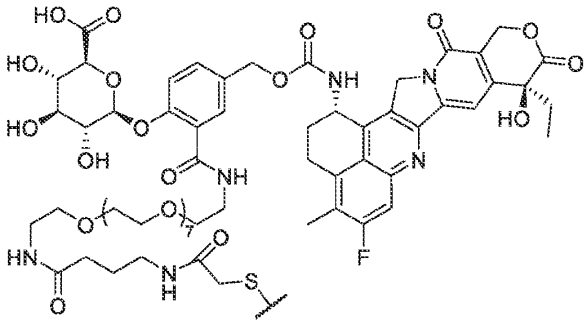


. In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

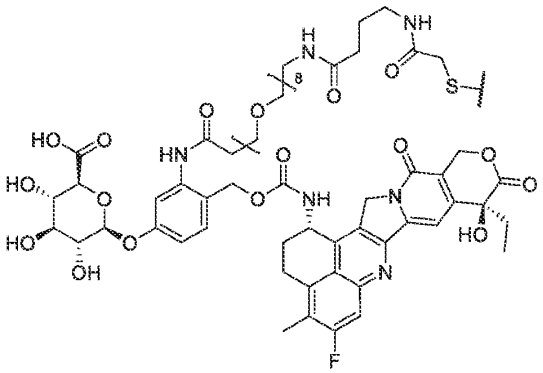


. In some embodiments, each occurrence of

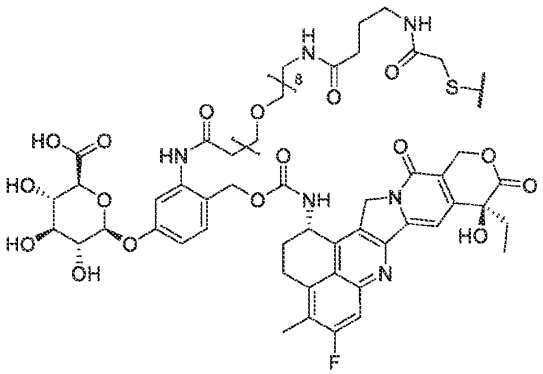
$-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



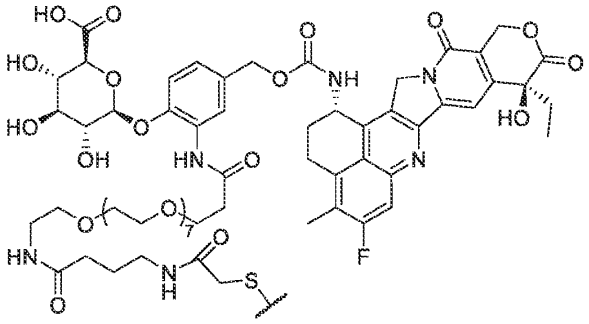
. In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



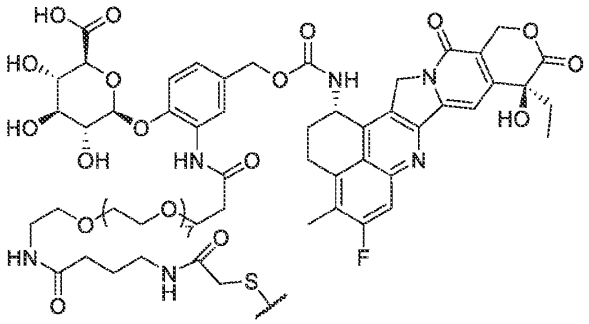
. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



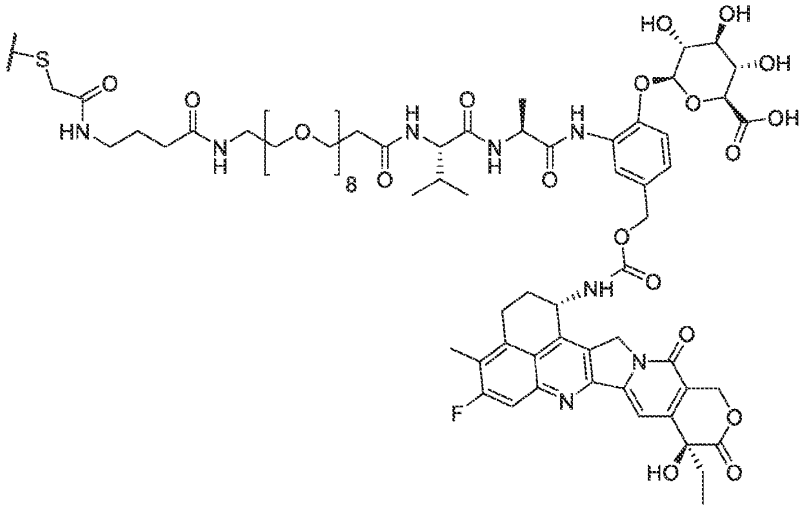
. In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



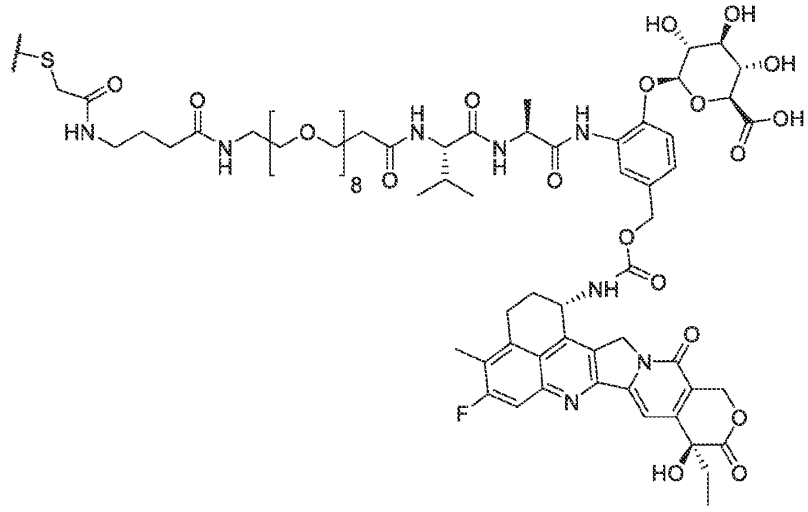
. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



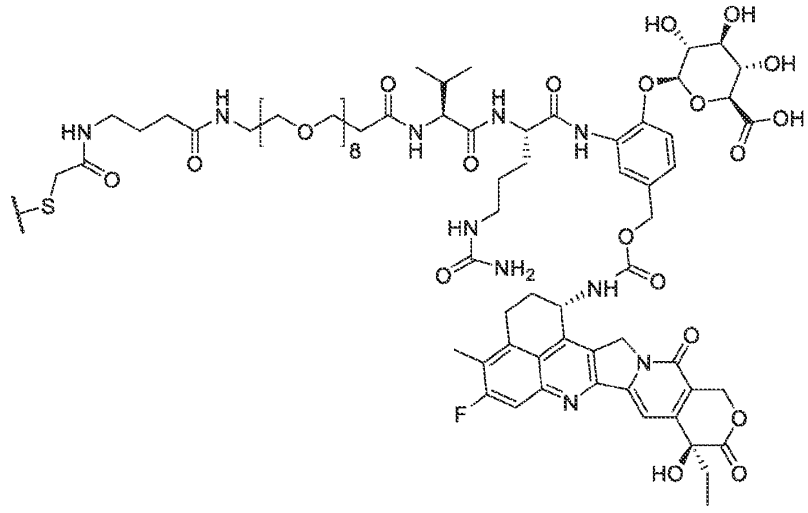
. In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



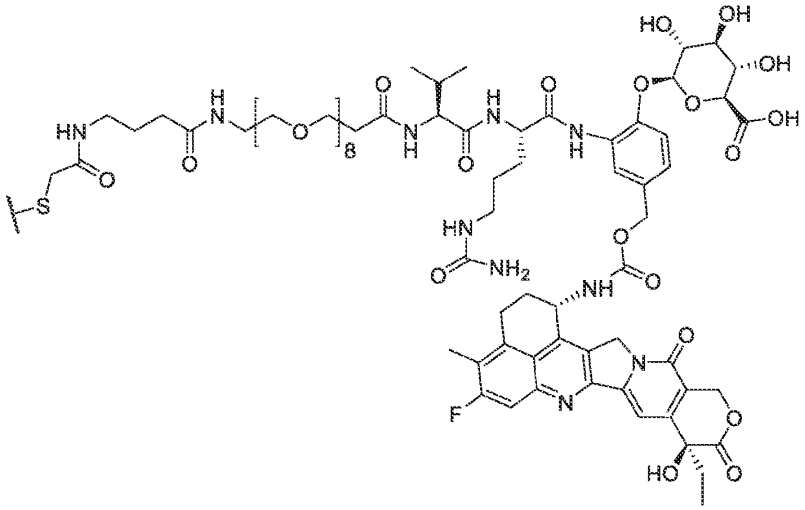
. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



. In some embodiments, an occurrence of –L^{RX}–L^{X}–$(CH_2CH_2O)_n$–L^{ZP}–L^{C}–R^{P} is or comprises

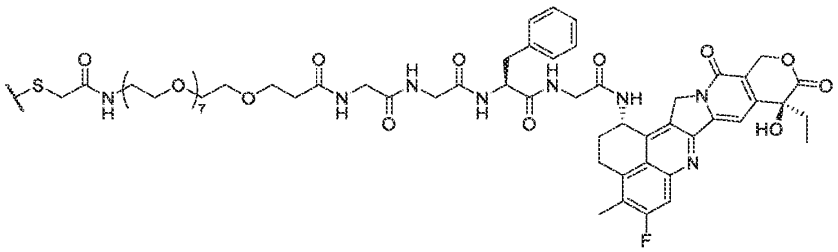


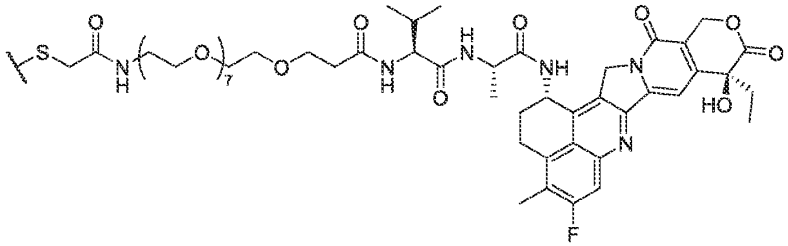
. In some embodiments, each occurrence of –L^{RX}–L^{X}–$(CH_2CH_2O)_n$–L^{ZP}–L^{C}–R^{P} is or comprises

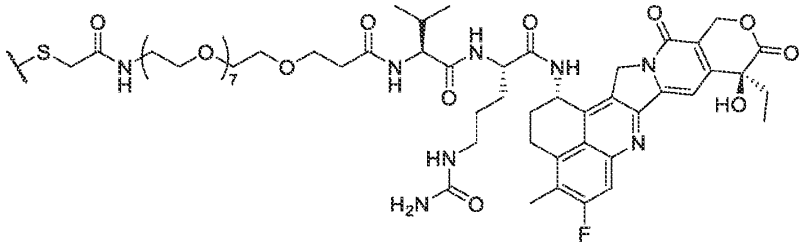


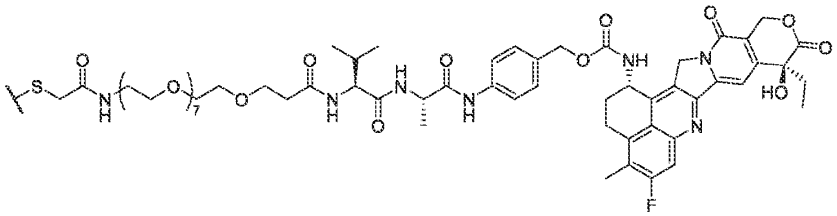
[0385] In some embodiments, a conjugate comprises $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$. In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is $-S-CH_2-C(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-GGFG-R^{P}$. In some embodiments, it is $-S-CH_2-C(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Ala-PABA-R^{P}$. In some embodiments, it is $-S-CH_2-C(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-PABA-R^{P}$. In some embodiments, it is $-S-CH_2-C(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)Val-Ala-NH-oGlu-R^{P}$. In some embodiments, it is $-S-CH_2-C(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Ala-NH-mGlu-R^{P}$. In some embodiments, it is $-S-CH_2-C(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-NH-oGLu-R^{P}$. In some embodiments, it is $-S-CH_2-C(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-NH-mGLu-R^{P}$. In some embodiments, it is $-S-CH_2-C(O)NH-(CH_2CH_2O)n-(CH_2)_zNHC(O)-oGlu-R^{P}$. In some embodiments, it is $-S-CH_2-C(O)NH-(CH_2CH_2O)n-(CH_2)_zNHC(O)-mGlu-R^{P}$. In some embodiments, n is 8. In some embodiments, z is 2. In some embodiments, R^{P} comprises a nitrogen atom and is bonded at the nitrogen atom, e.g., to the rest of a conjugate or other compound. In some embodiments, R^{P} is R^{EX}. In some embodiments, z is 2 and R^{P} is R^{EX}. In some embodiments, n is 8, z is 2 and R^{P} is R^{EX}. In some embodiments, x is 5, n is 8, z is 2 and R^{P} is R^{EX}. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is the same.

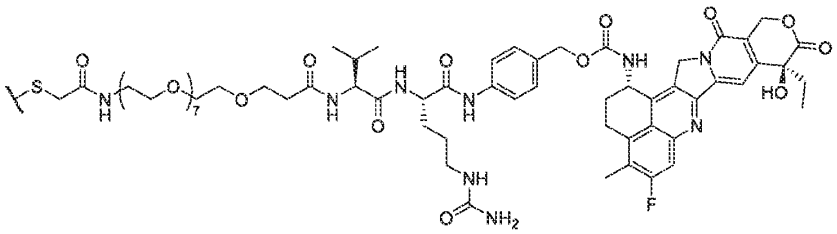
[0386] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

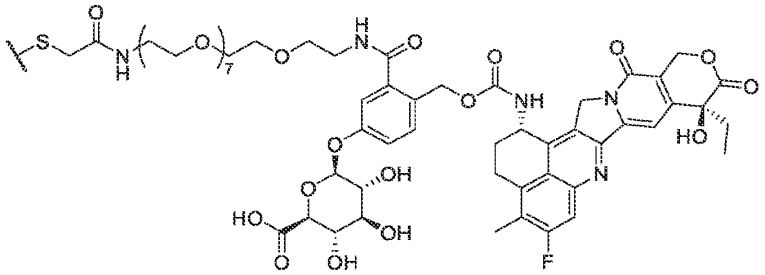


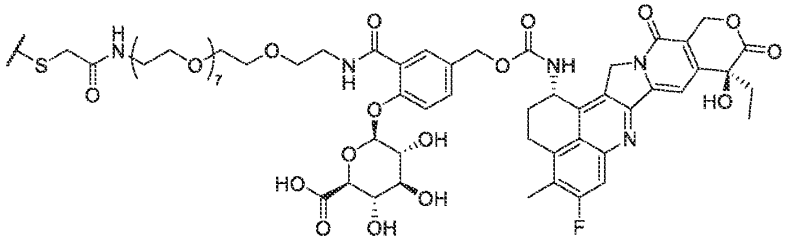


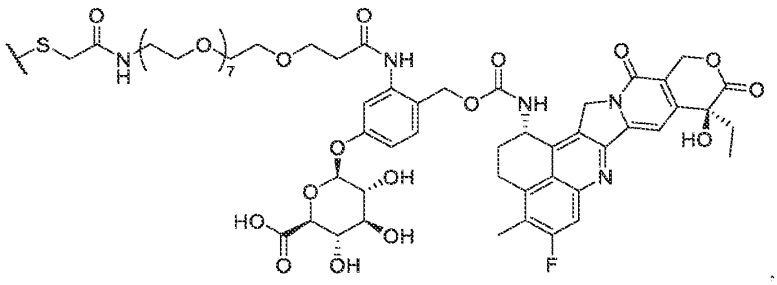




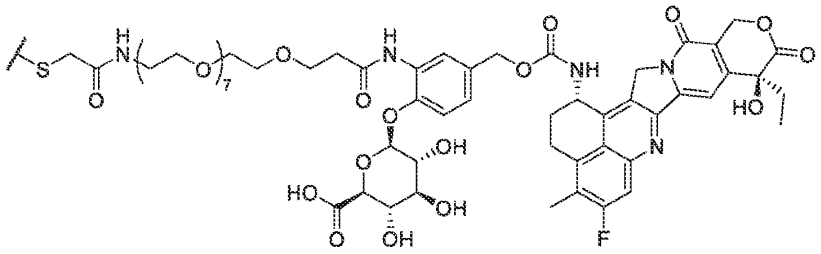






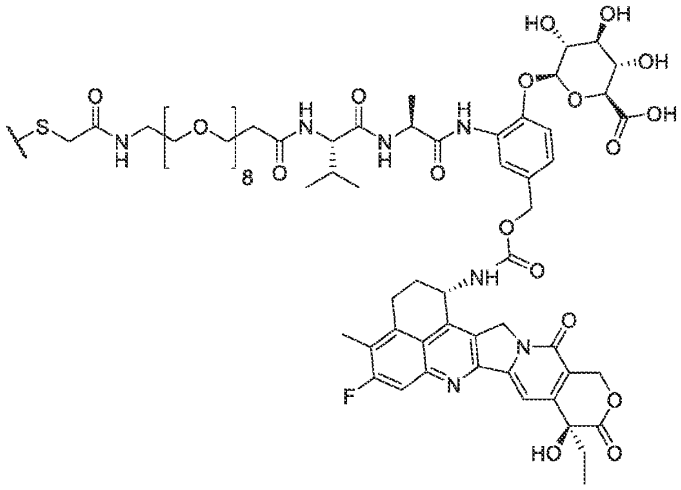


, or

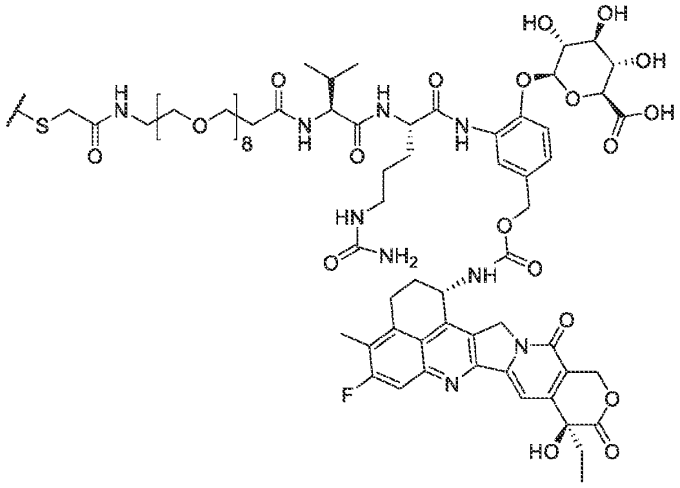


. In some embodiments, each occurrence is the same.

[0387] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

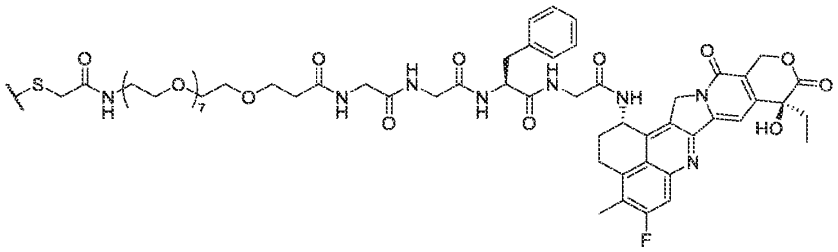


or

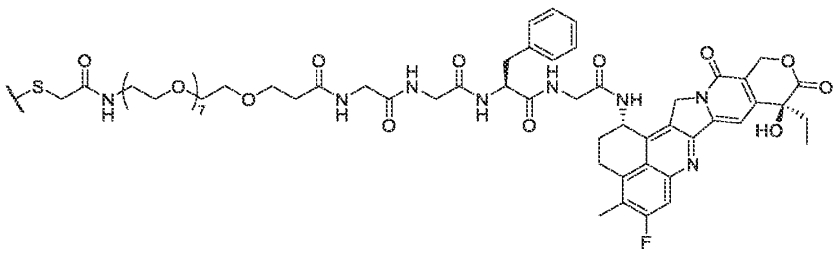


. In some embodiments, each occurrence is the same.

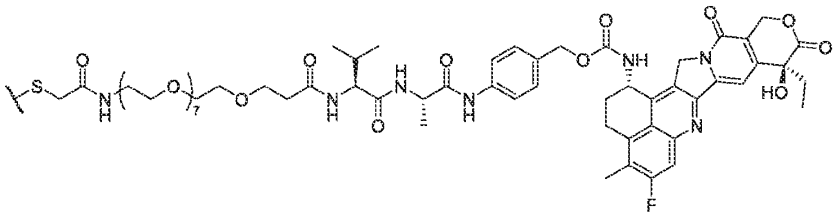
[0388] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



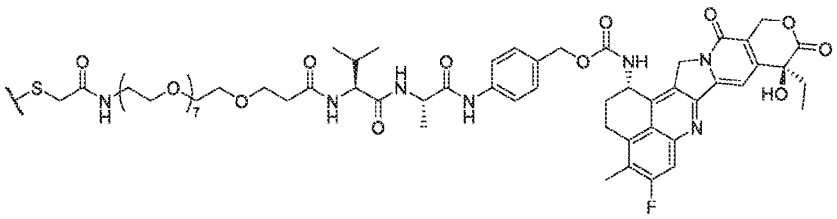
[0389] In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



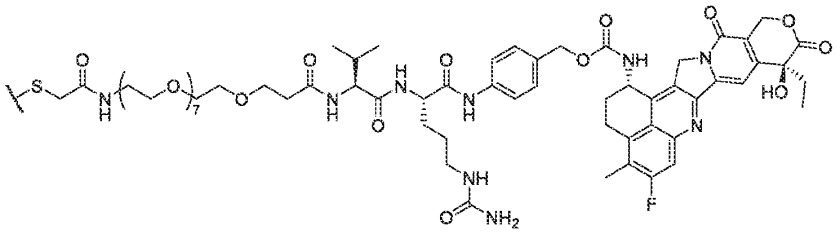
[0390] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



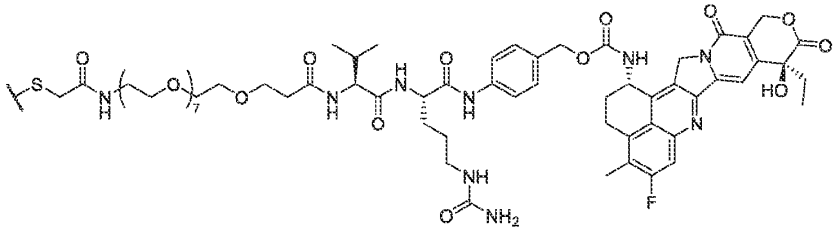
[0391] In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



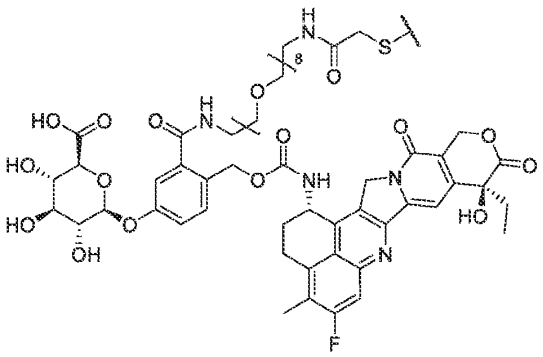
[0392] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



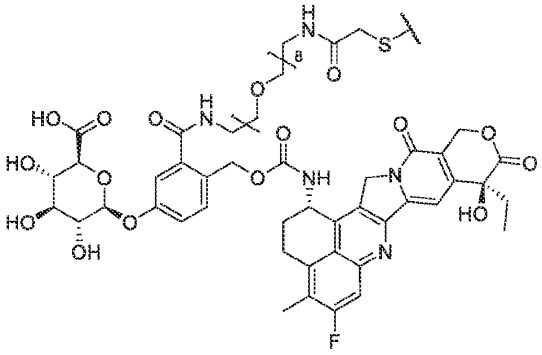
[0393] In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



[0394] In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

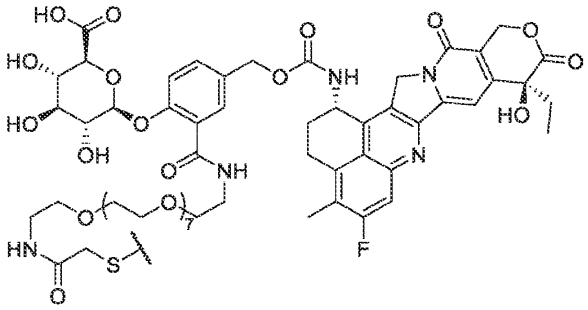


. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



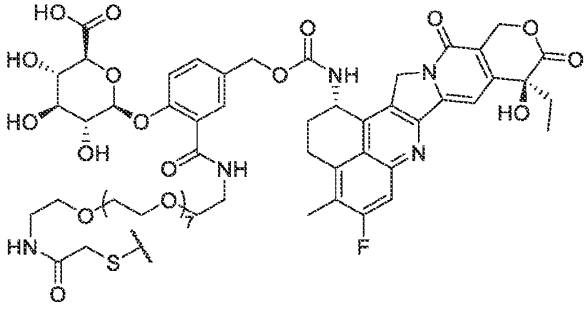
. In some embodiments, an occurrence of

$-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



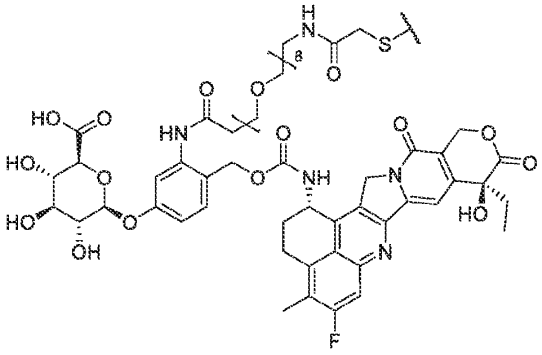
. In some embodiments, each occurrence of

$-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



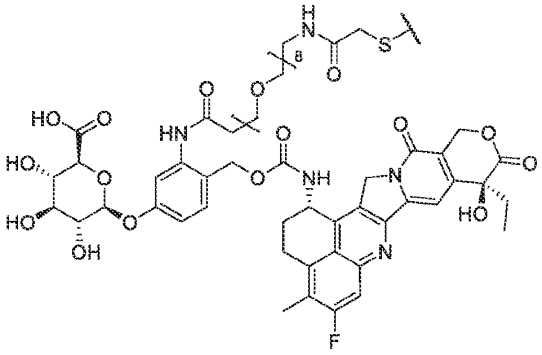
. In some embodiments, an occurrence of

$-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



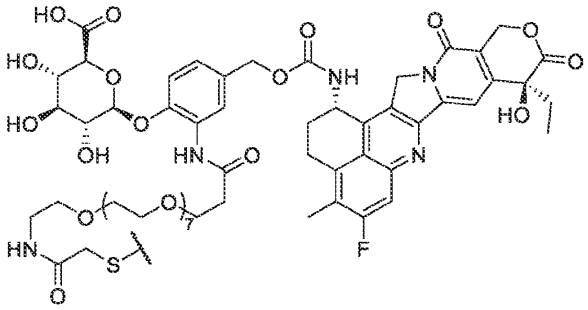
. In some embodiments, each occurrence of

$-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



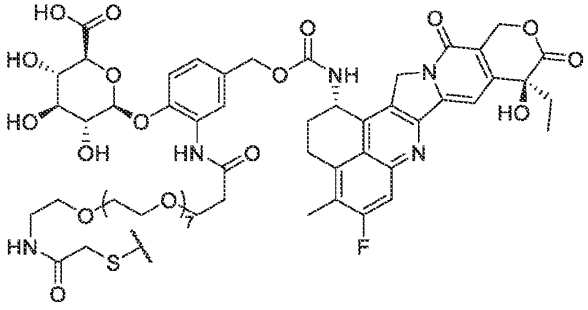
. In some embodiments, an occurrence of

$-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



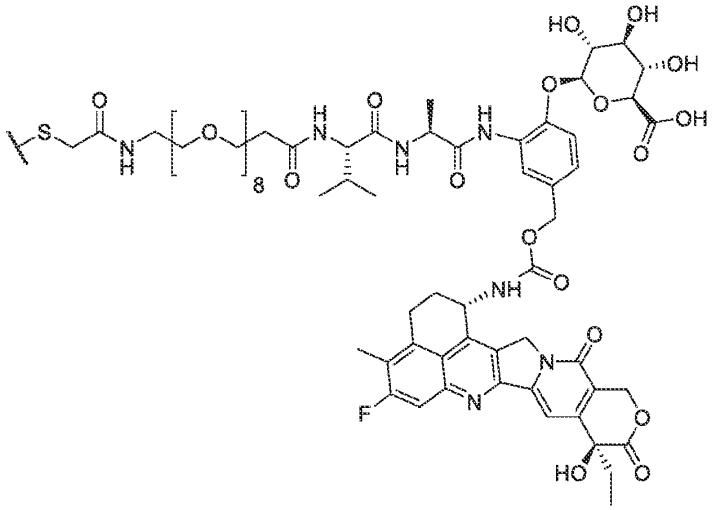
. In some embodiments, each occurrence of

$-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

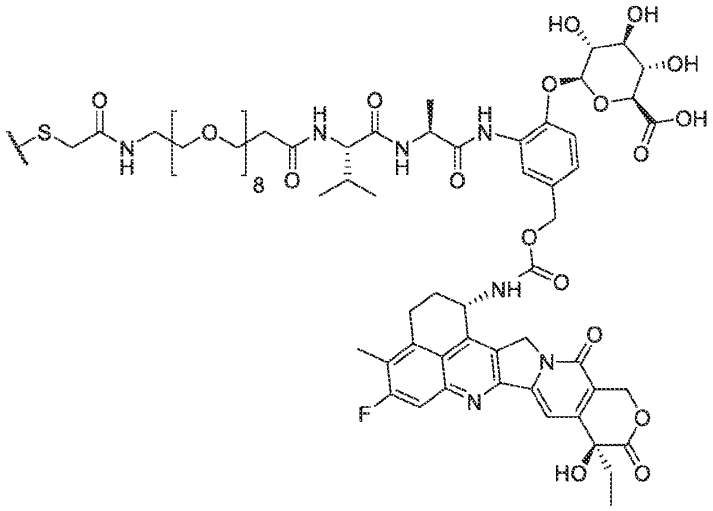


. In some embodiments, an occurrence of

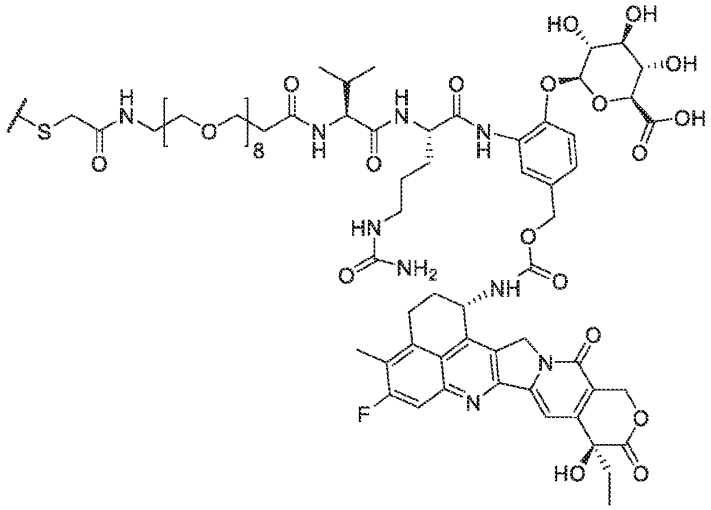
$-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



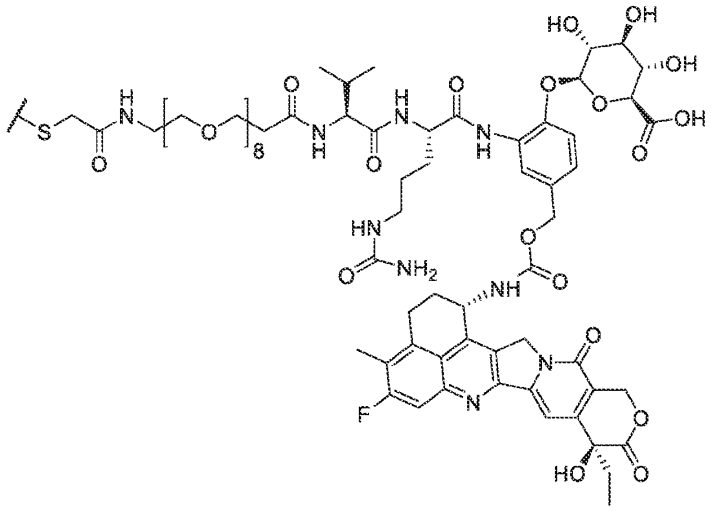
. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



. In some embodiments, an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

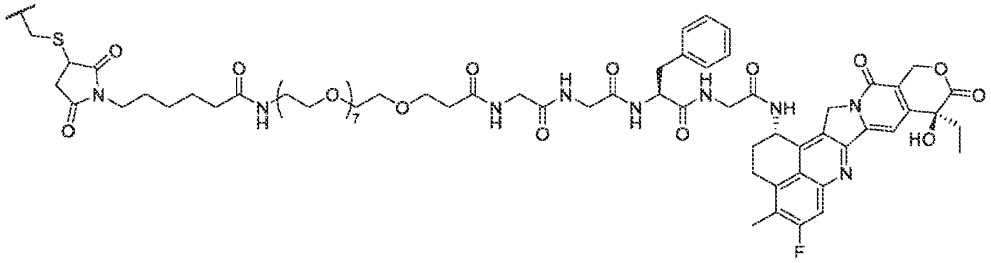


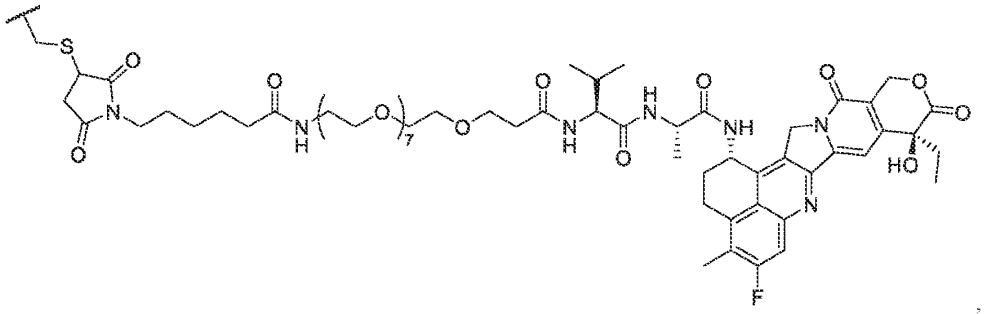
.

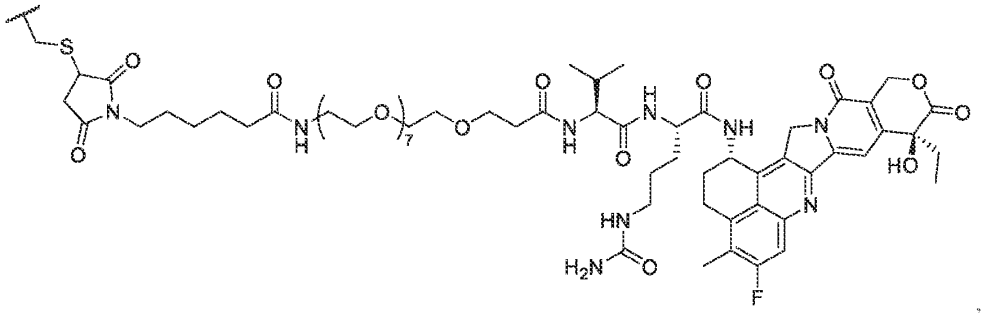
[0395] In some embodiments, a conjugate comprises $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$. In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is $-CH_2-S-SM-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-GGFG-R^{P}$. In some embodiments, it is $-CH_2-S-SM-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Ala-PABA-R^{P}$. In some embodiments, it is $-CH_2-S-SM-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-PABA-R^{P}$. In some embodiments, it is $-CH_2-S-SM-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)Val-Ala-NH-oGlu-R^{P}$. In some embodiments, it is $-CH_2-S-SM-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Ala-NH-mGlu-R^{P}$. In some embodiments, it is $-CH_2-S-SM-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-NH-oGLu-R^{P}$. In some embodiments, it is $-CH_2-S-SM-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-NH-mGLu-R^{P}$. In some

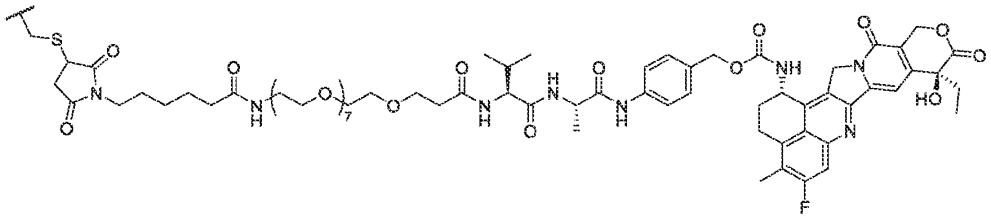
embodiments, it is $-CH_2-S-SM-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zNHC(O)-oGlu-R^P$. In some embodiments, it is $-CH_2-S-SM-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zNHC(O)-mGlu-R^P$. In some embodiments, x is 2. In some embodiments, x is 3. In some embodiments, x is 5. In some embodiments, n is 8. In some embodiments, z is 2. In some embodiments, R^P comprises a nitrogen atom and is bonded at the nitrogen atom, e.g., to the rest of a conjugate or other compound. In some embodiments, R^P is R^{EX}. In some embodiments, z is 2 and R^P is R^{EX}. In some embodiments, n is 8, z is 2 and R^P is R^{EX}. In some embodiments, x is 2, n is 8, z is 2 and R^P is R^{EX}. In some embodiments, x is 3, n is 8, z is 2 and R^P is R^{EX}. In some embodiments, x is 5, n is 8, z is 2 and R^P is R^{EX}. In some embodiments, each occurrence of $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is the same.

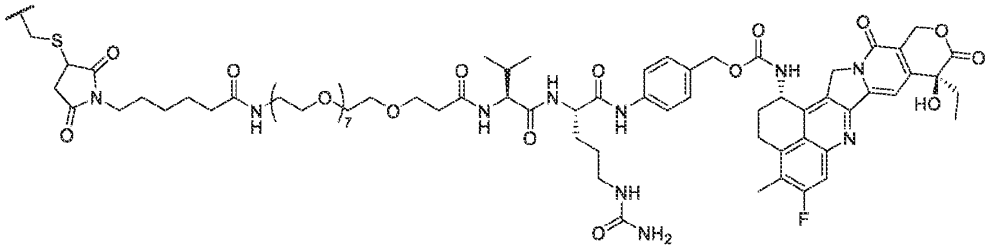
[0396] In some embodiments, an occurrence of $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

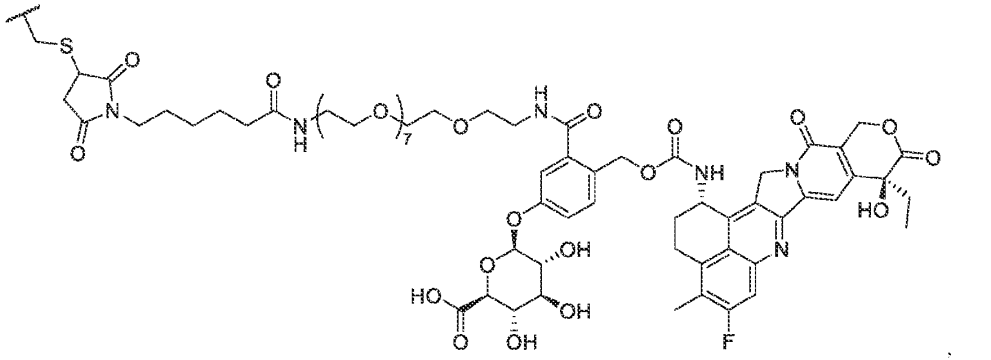


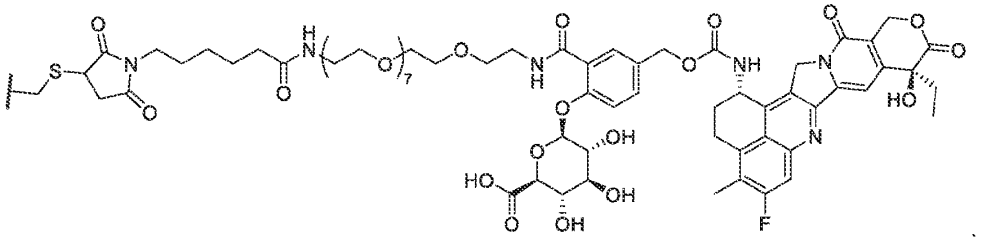


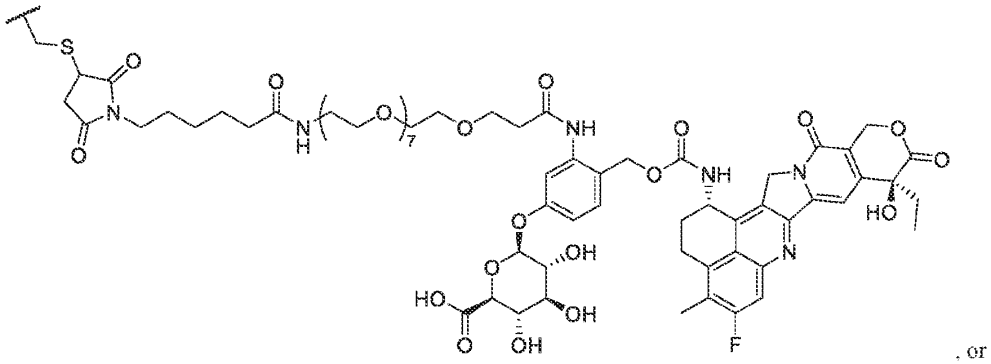




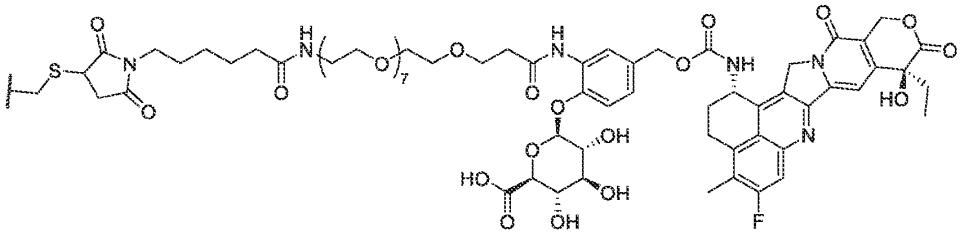






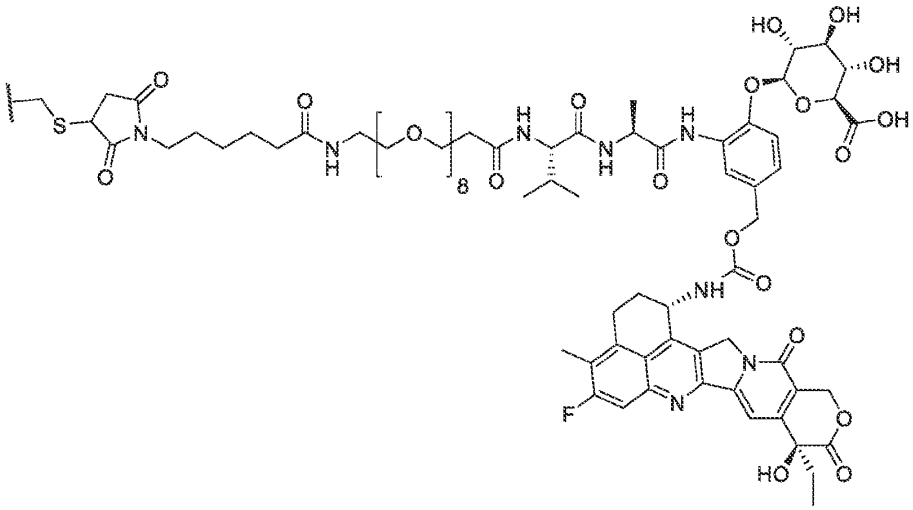


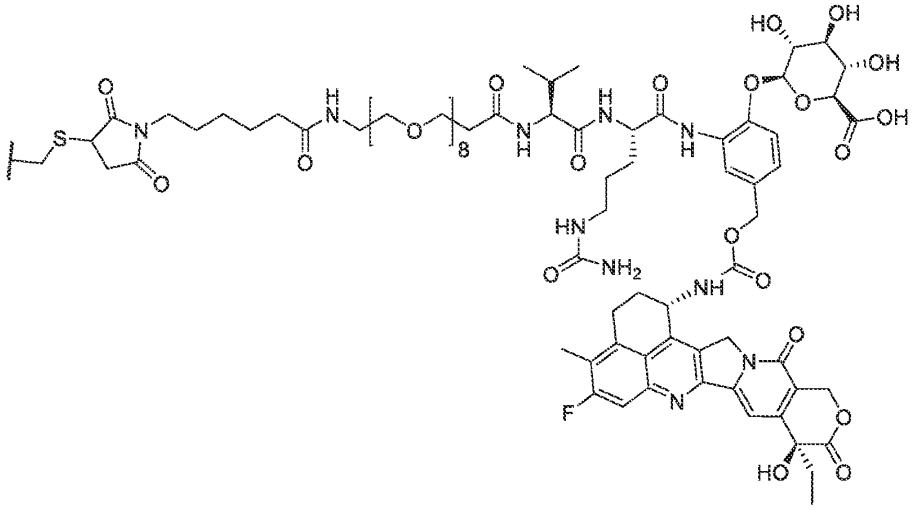
, or



. In some embodiments, each occurrence is the same.

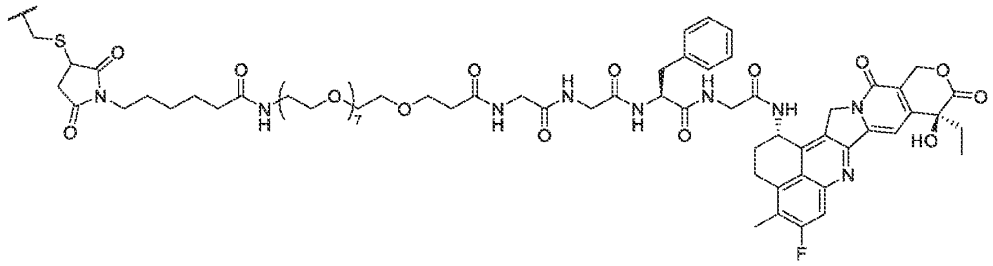
[0397] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



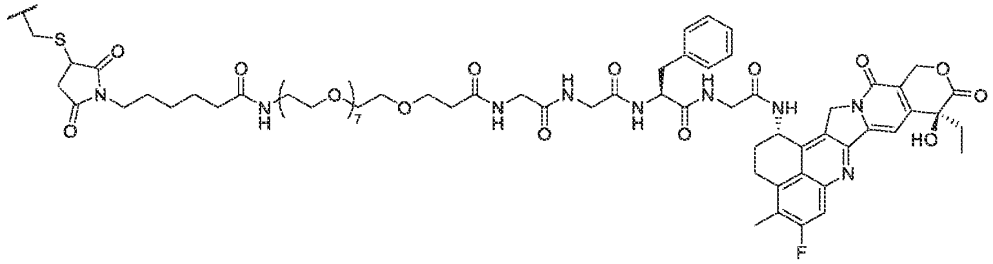


or . In some embodiments, each occurrence is the same.

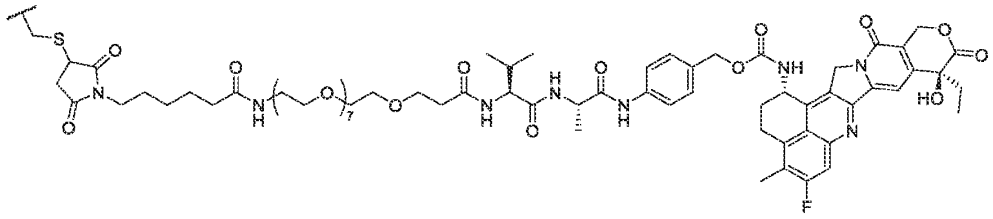
[0398] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



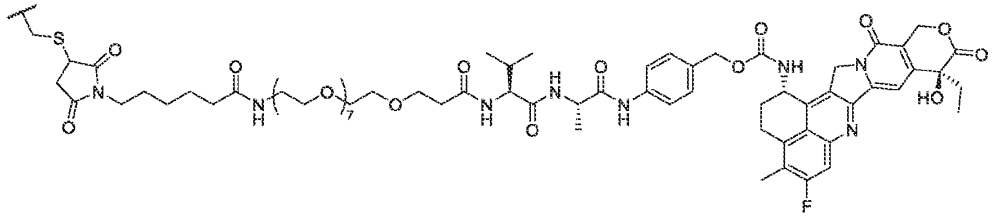
[0399] In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



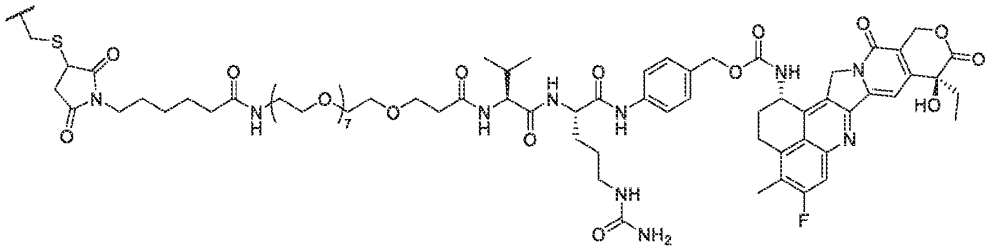
[0400] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



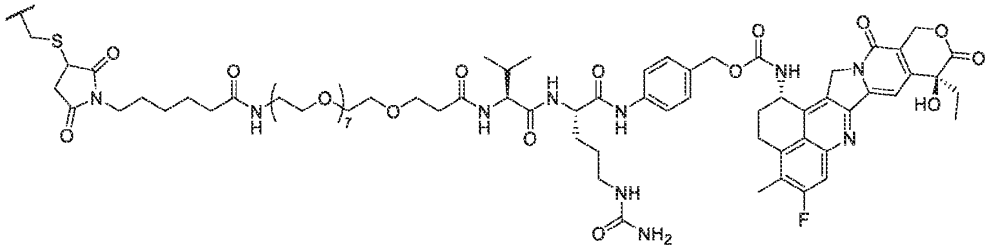
[0401] In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



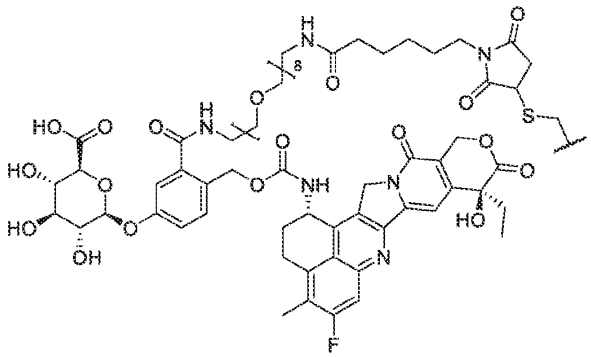
[0402] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



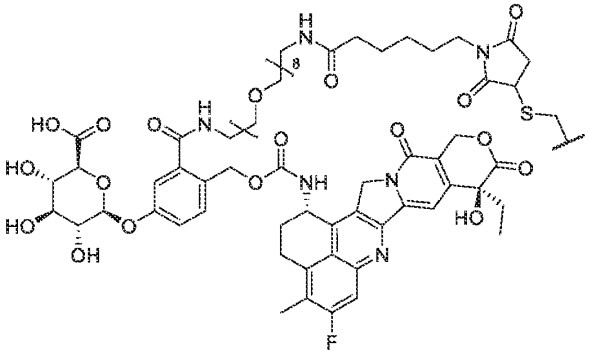
[0403] In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



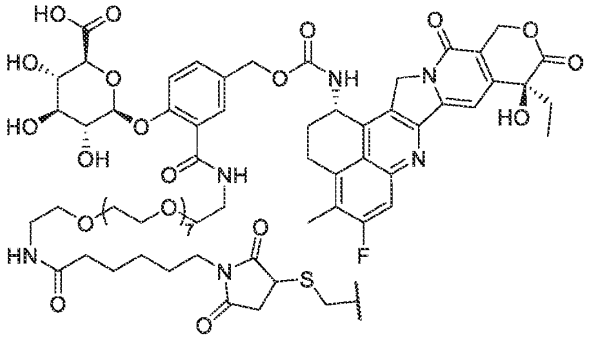
[0404] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or



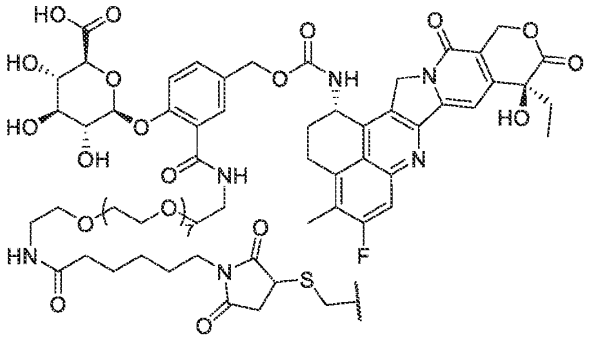
comprises . In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



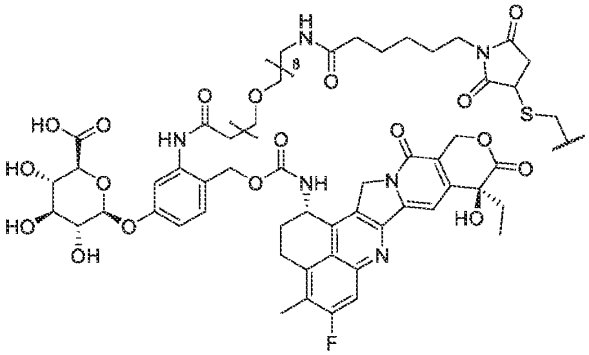
. In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



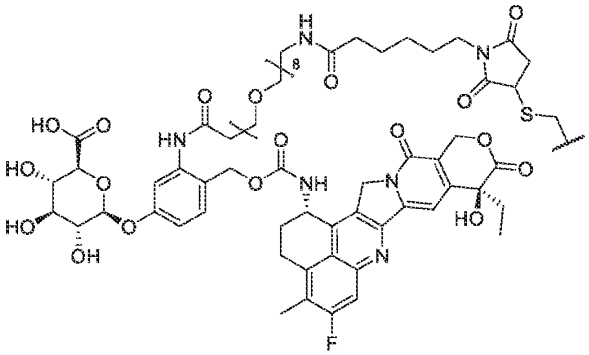
. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



. In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

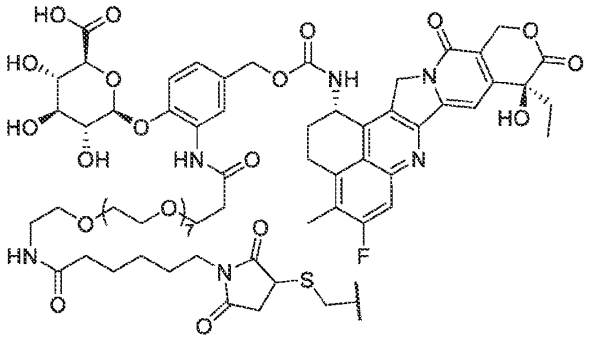


. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



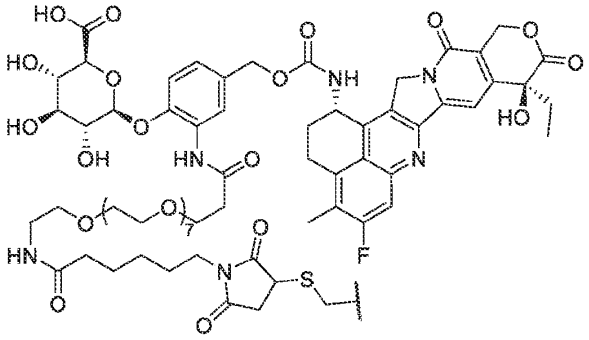
. In some embodiments, an occurrence of

$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



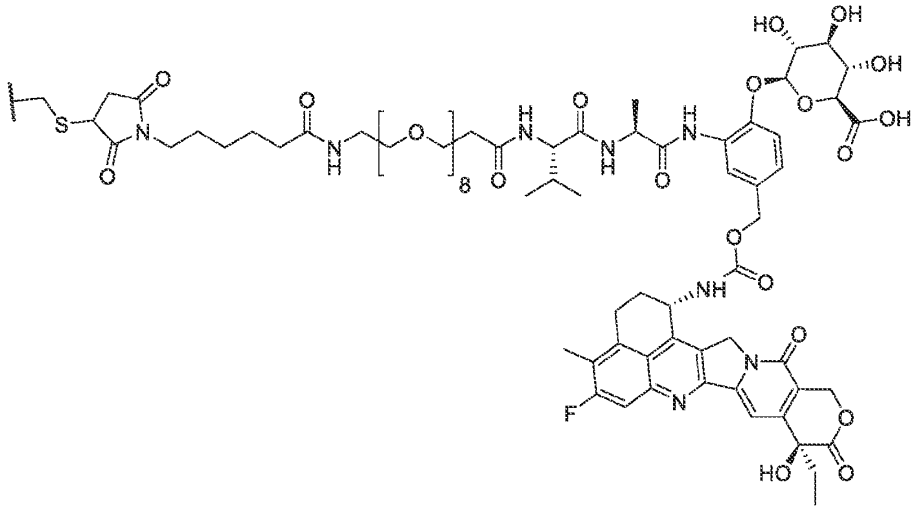
. In some embodiments, each occurrence of

$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

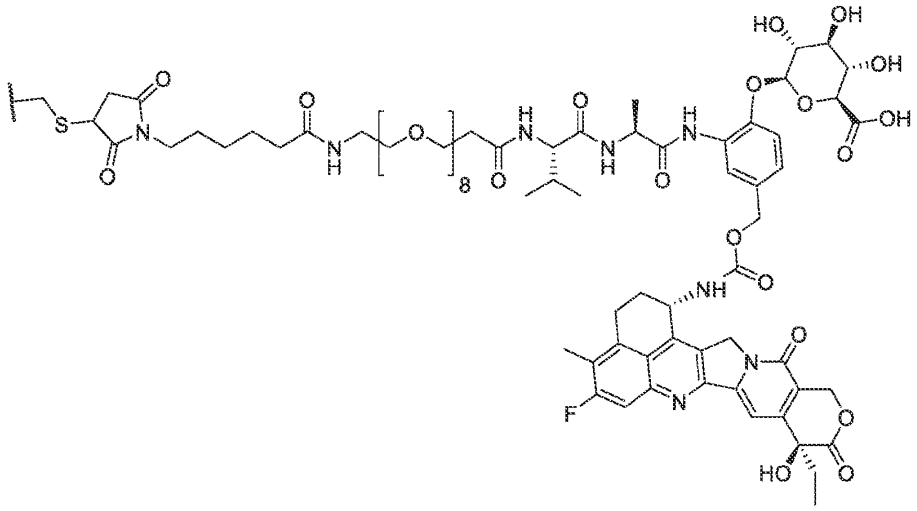


. In some embodiments, an occurrence of

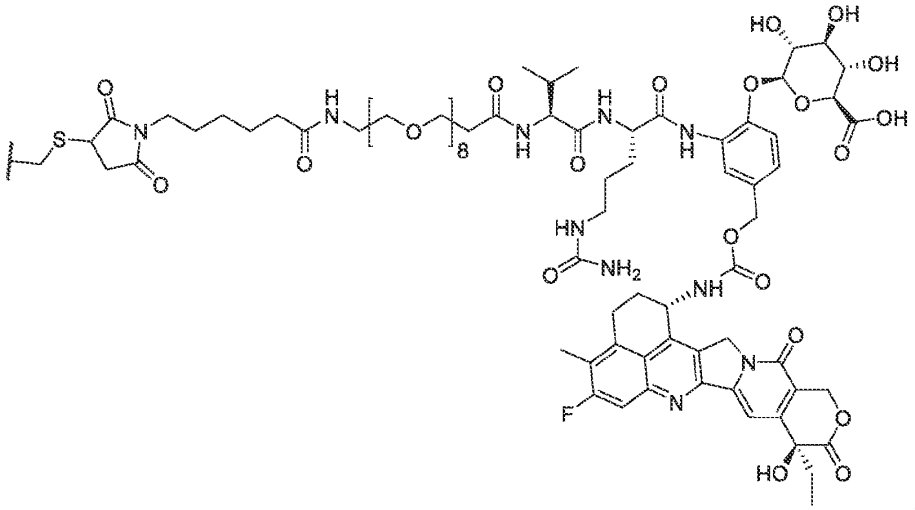
$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



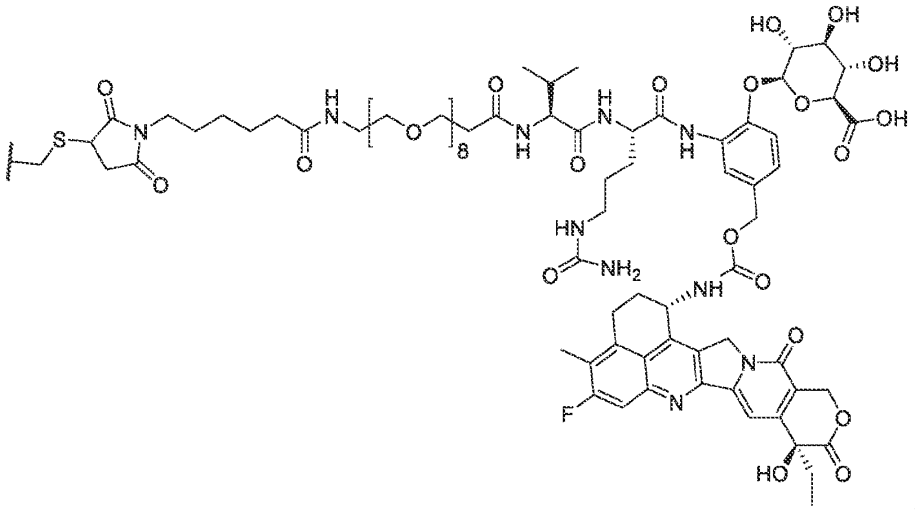
. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



.In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

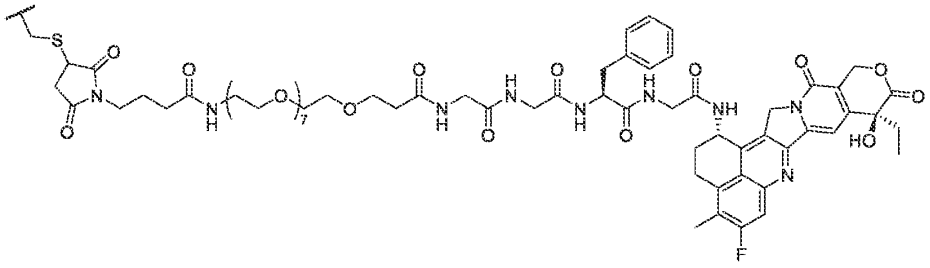


. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

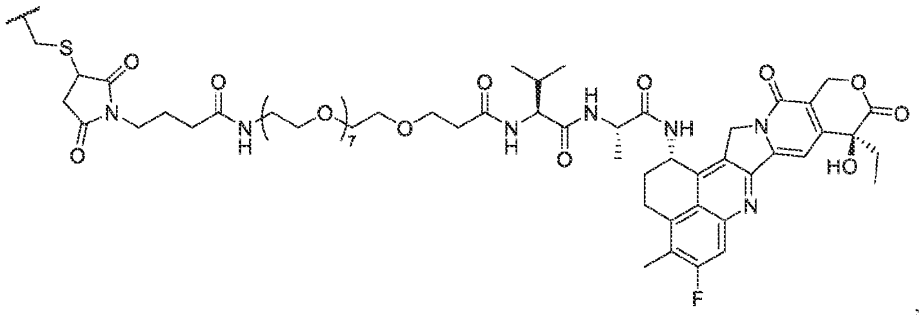


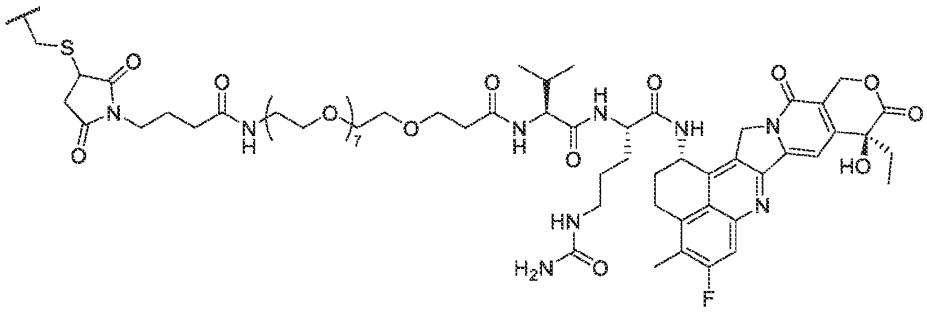
.

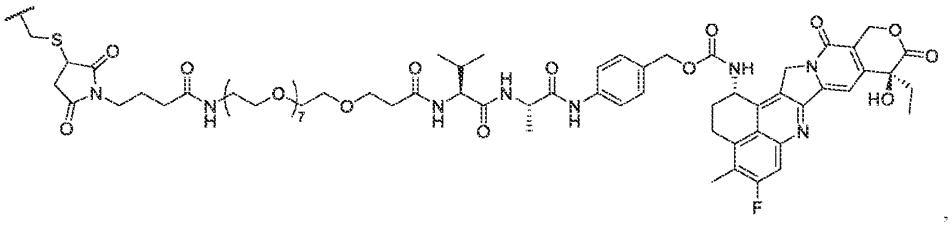
[0405] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

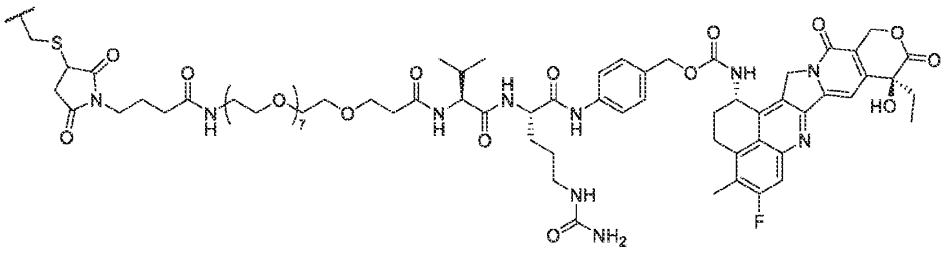


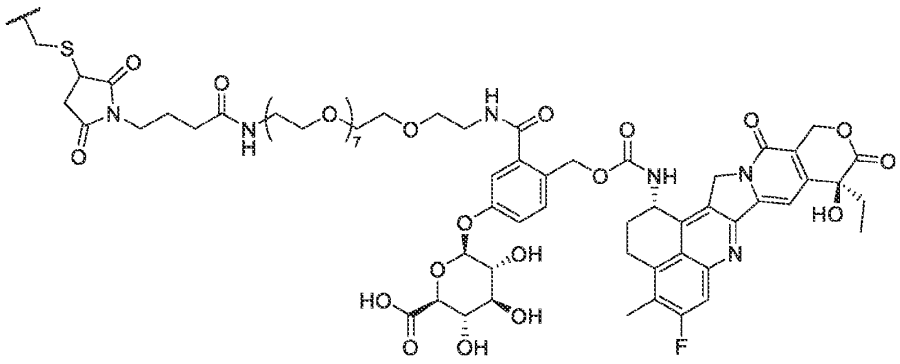
.

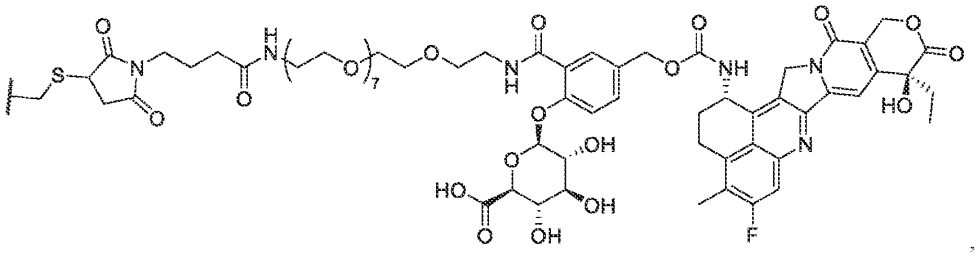




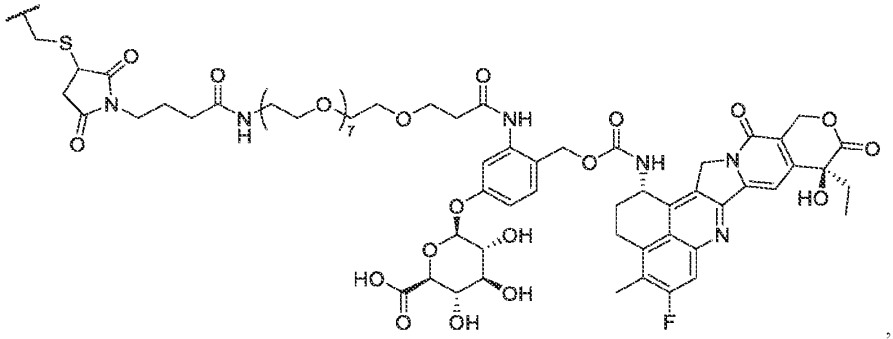




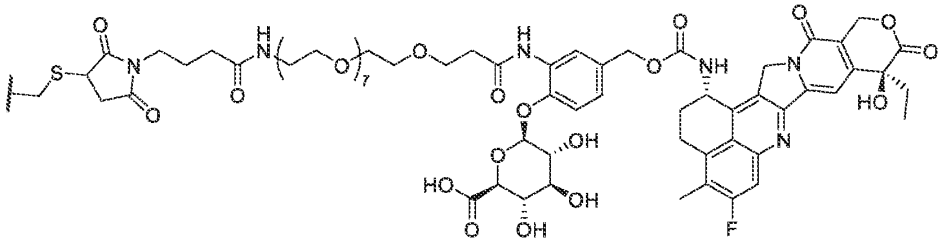




,

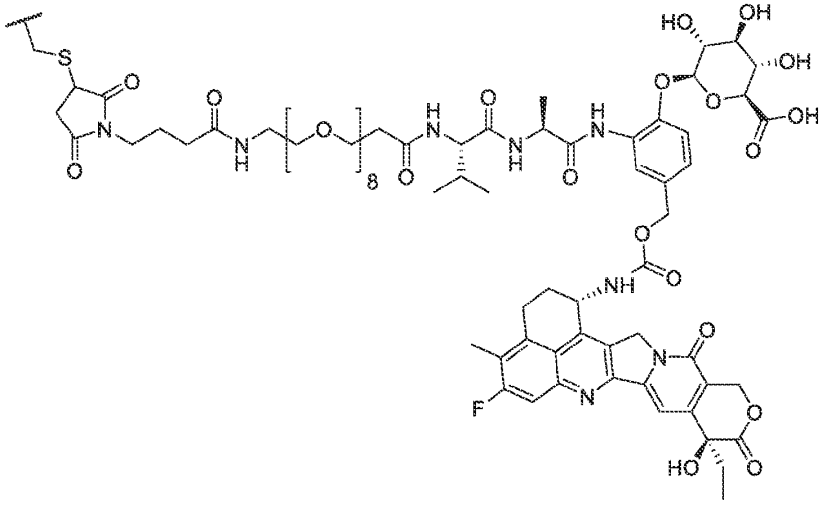


, or

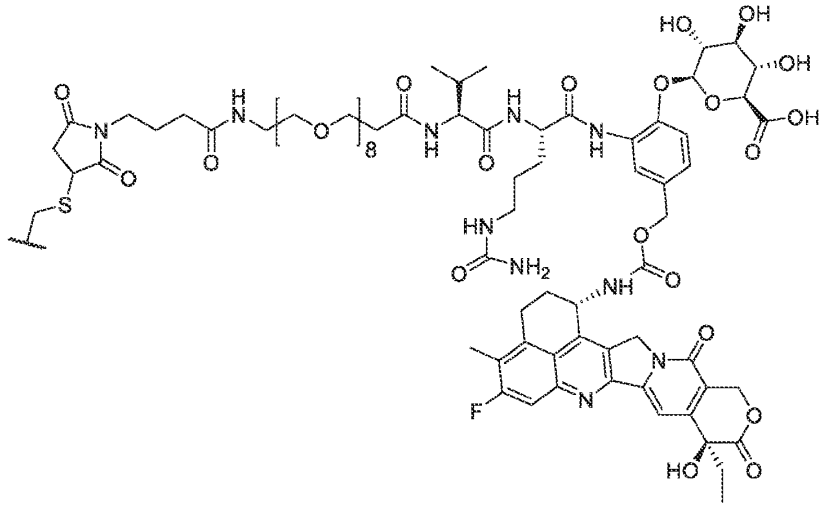


. In some embodiments, each occurrence is the same.

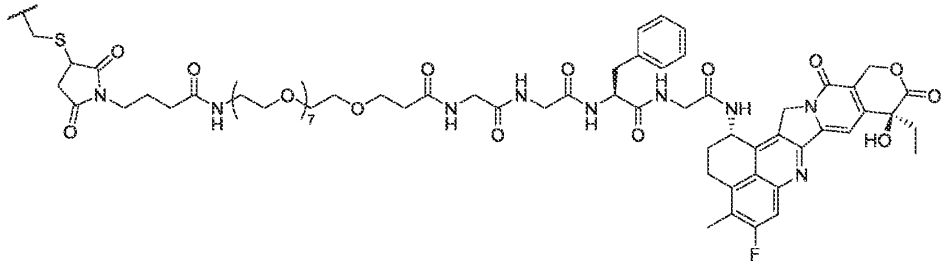
[0406] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



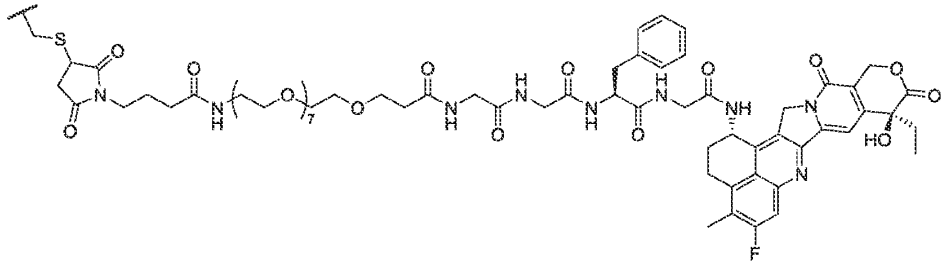
or



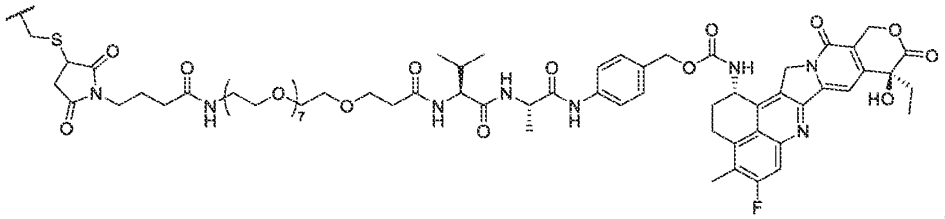
[0407] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



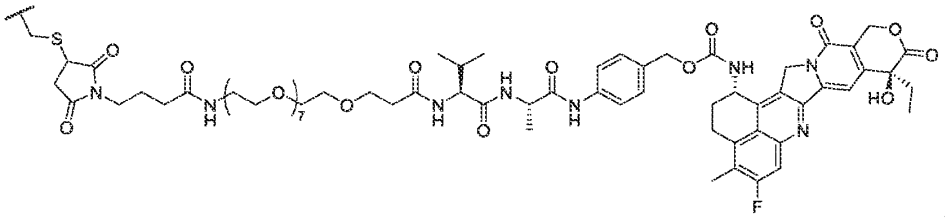
[0408] In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



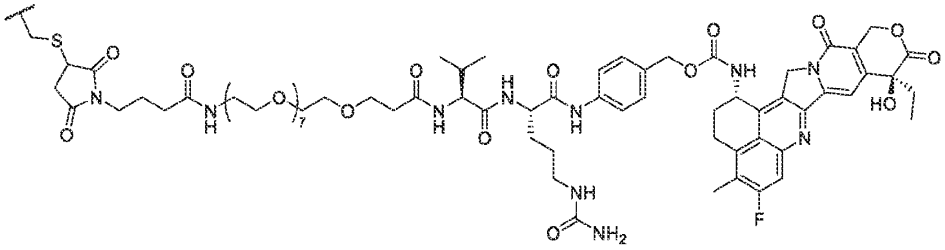
[0409] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



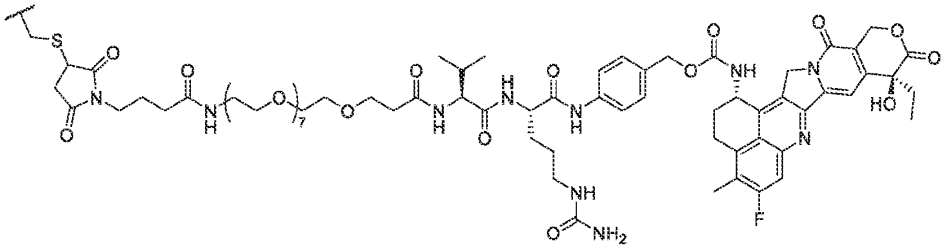
[0410] In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



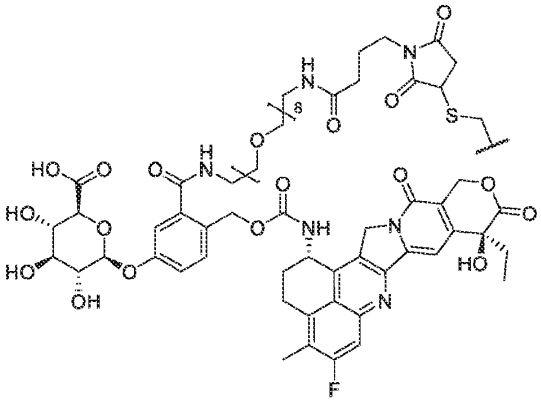
[0411] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



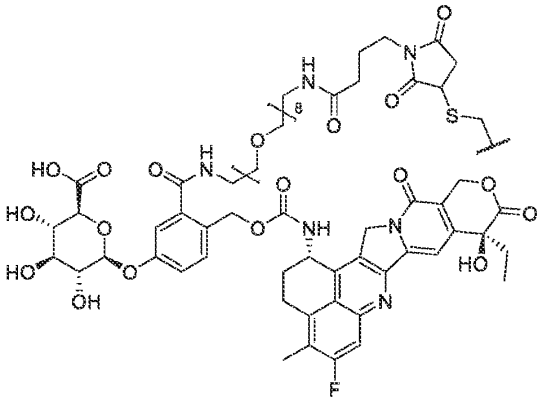
[0412] In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



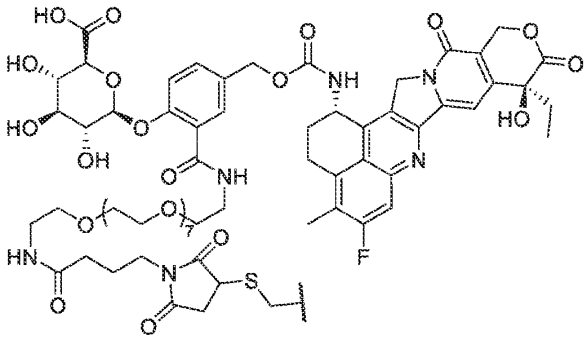
[0413] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or



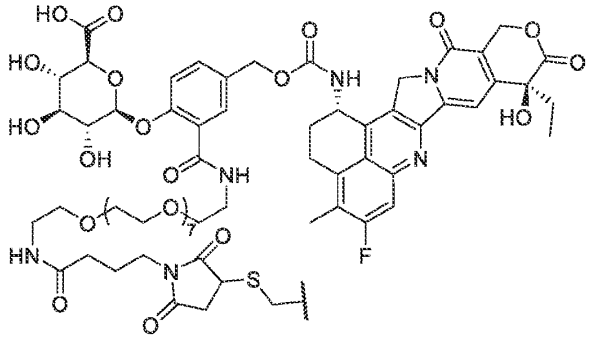
comprises . In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



. In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

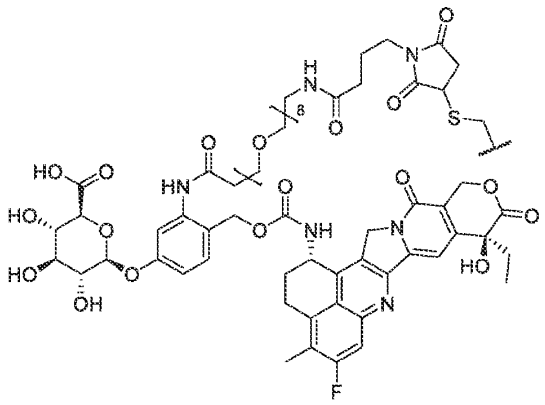


. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



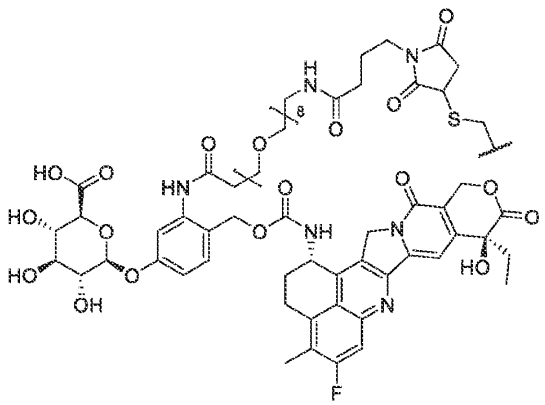
. In some embodiments, an occurrence of

$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



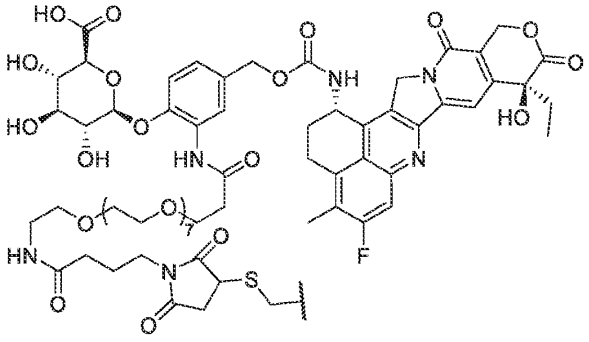
. In some embodiments, each occurrence of

$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

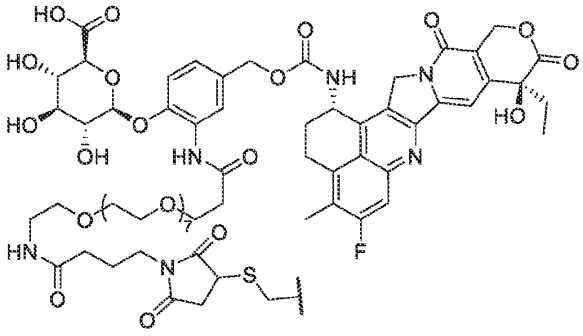


. In some embodiments, an occurrence of

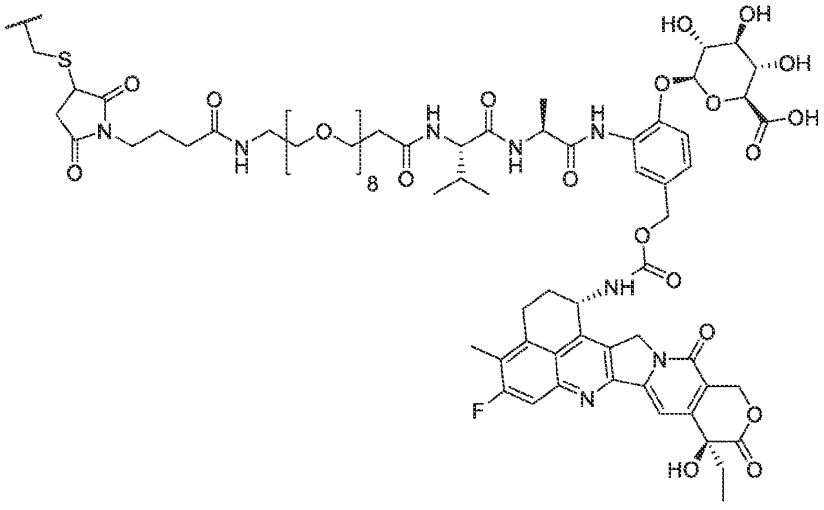
$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



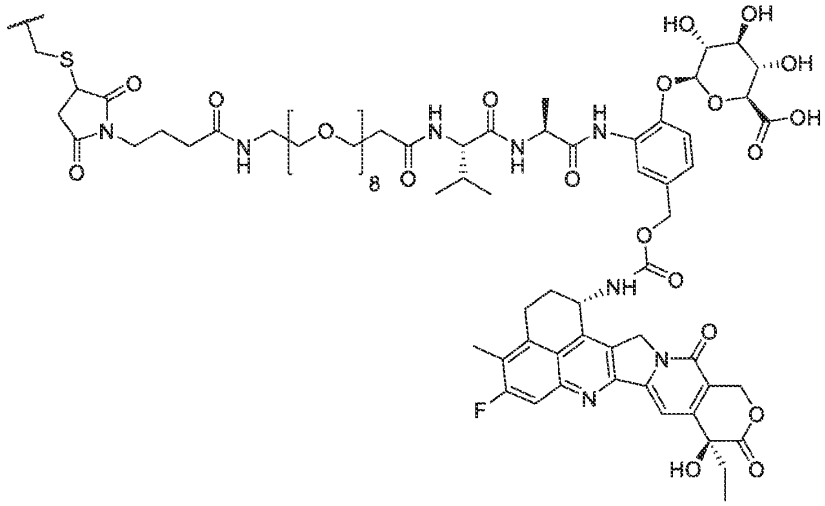
. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



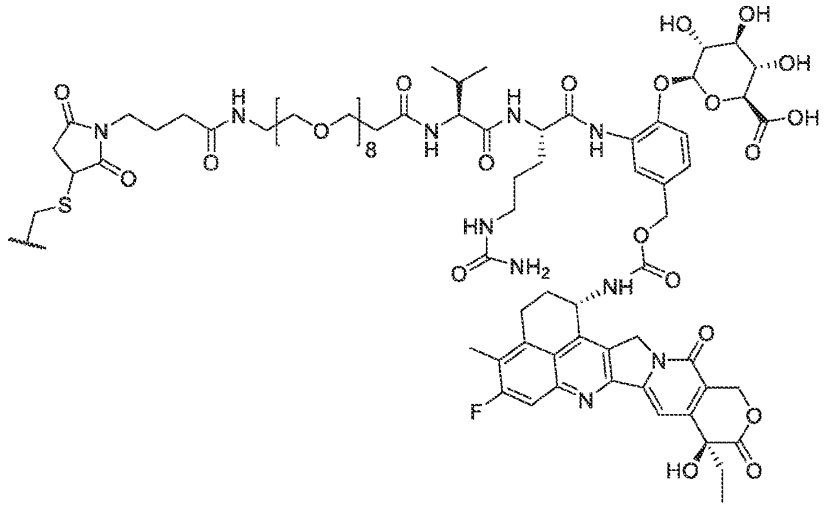
. In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



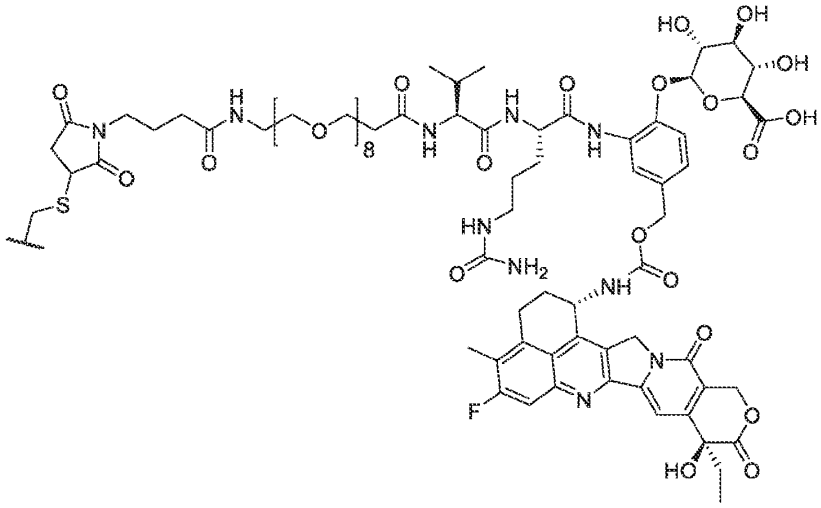
. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



.In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



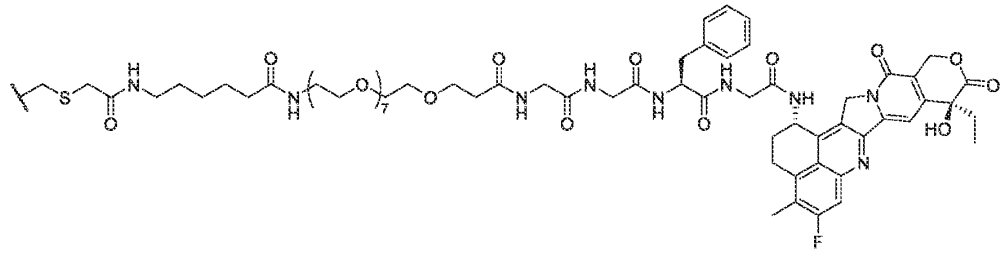
. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



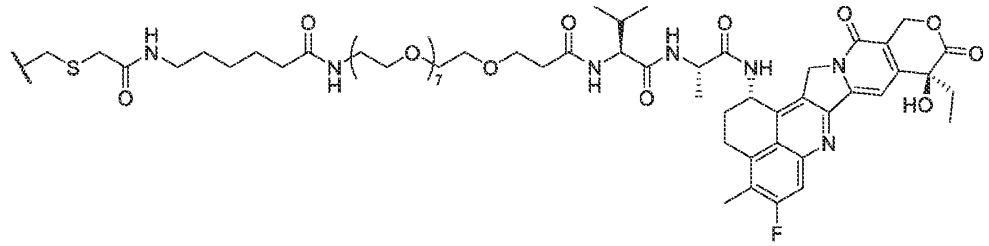
[0414] In some embodiments, a conjugate comprises $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$. In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is $-CH_2-S-CH_2-C(O)NH-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-GGFG-R^{P}$. In some embodiments, it is $-CH_2-S-CH_2-C(O)NH-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Ala-PABA-R^{P}$. In some embodiments, it is $-CH_2-S-CH_2-C(O)NH-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-PABA-R^{P}$. In some embodiments, it is $-CH_2-S-CH_2-C(O)NH-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)Val-Ala-NH-oGlu-R^{P}$. In some embodiments, it is $-CH_2-S-CH_2-C(O)NH-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Ala-NH-mGlu-R^{P}$. In some embodiments, it is $-CH_2-S-CH_2-C(O)NH-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-NH-oGLu-R^{P}$. In some embodiments, it is $-CH_2-S-CH_2-C(O)NH-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-NH-mGLu-R^{P}$. In some embodiments, it is $-CH_2-S-CH_2-C(O)NH-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zNHC(O)-oGlu-R^{P}$. In some embodiments, it is $-CH_2-S-CH_2-C(O)NH-(CH_2)_xC(O)NH-(CH_2CH_2O)n-(CH_2)_zNHC(O)-mGlu-R^{P}$. In some embodiments, x is 2. In some embodiments, x is 3. In some embodiments, x is 5. In some embodiments, n is 8. In some embodiments, z is 2. In some embodiments, R^{P} comprises a nitrogen atom and is bonded at the nitrogen atom, e.g., to the rest of a conjugate or other compound. In some embodiments, R^{P} is R^{EX}. In some embodiments, z is 2 and R^{P} is R^{EX}. In some embodiments, n is 8, z is 2 and R^{P} is R^{EX}. In some embodiments, x is 2, n is 8, z is 2 and R^{P} is R^{EX}. In some embodiments, x is 3, n is 8, z is 2 and R^{P} is R^{EX}. In some embodiments, x is 5, n is 8, z is 2 and R^{P} is R^{EX}. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is the same.

[0415] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or

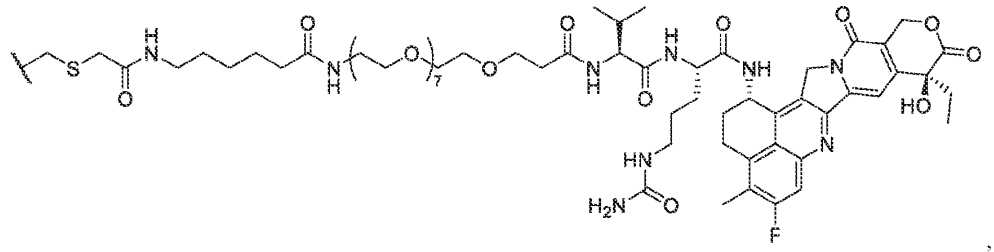
comprises



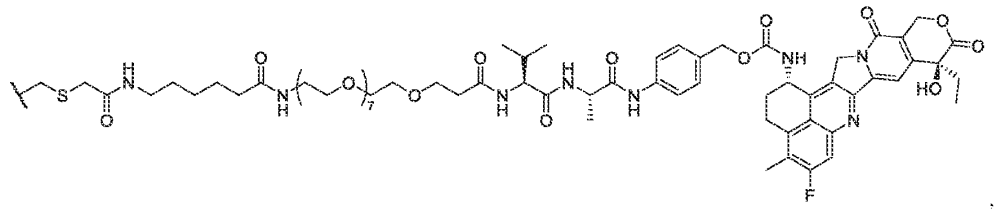
,



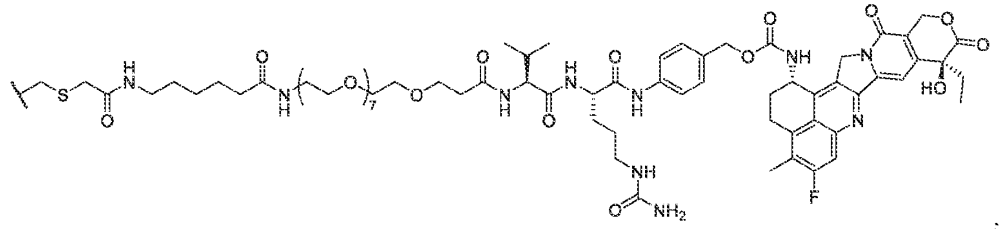
,



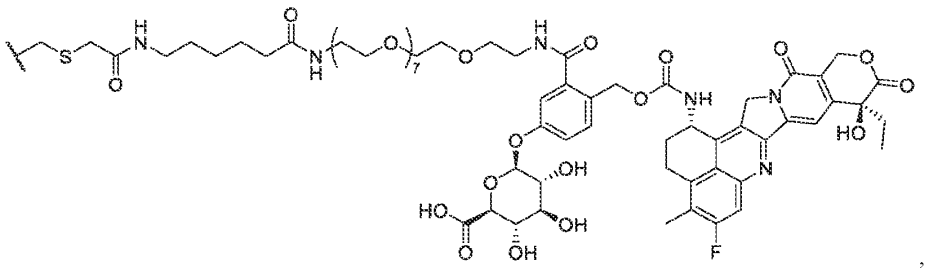
,

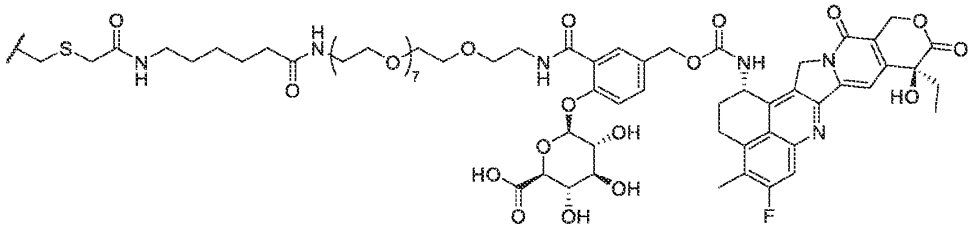


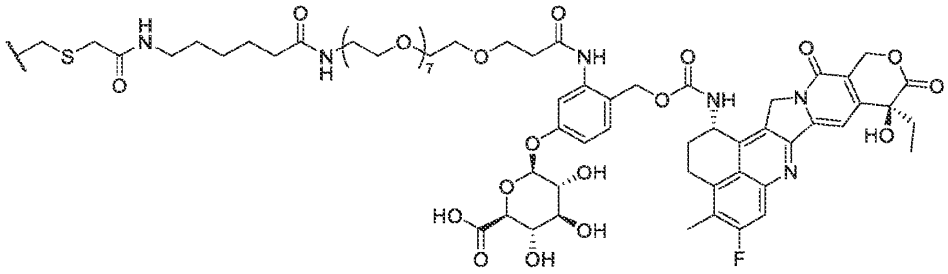
,



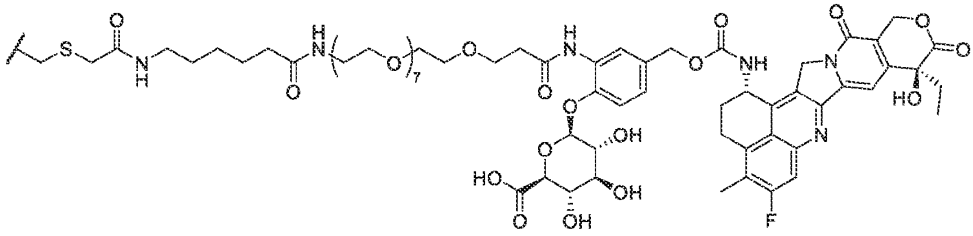
.





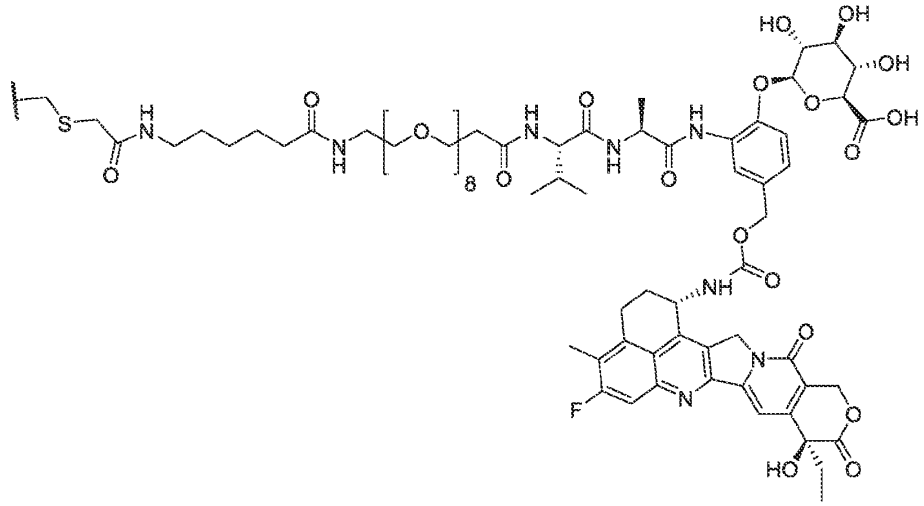


, or

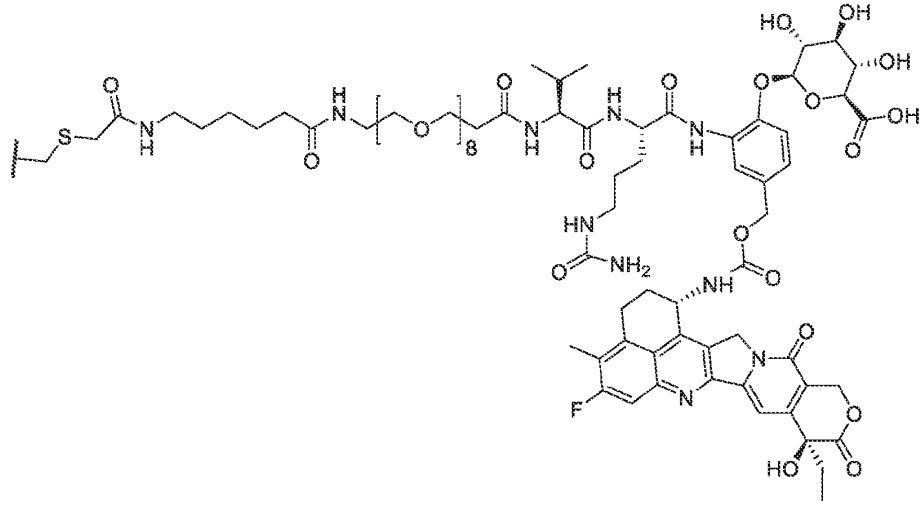


In some embodiments, each occurrence is the same.

[0416] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

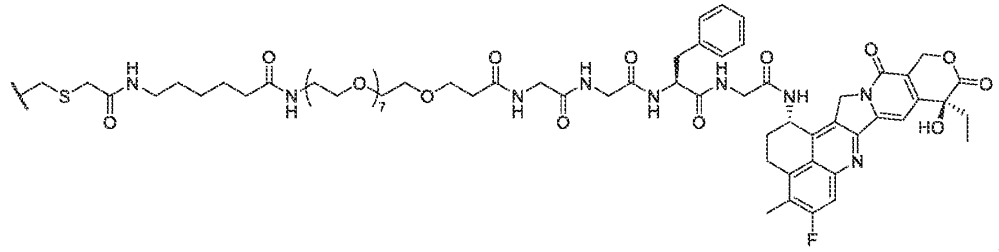


or



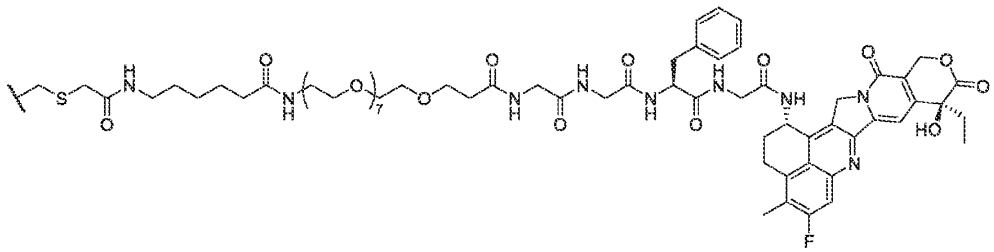
. In some embodiments, each occurrence is the same.

[0417] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

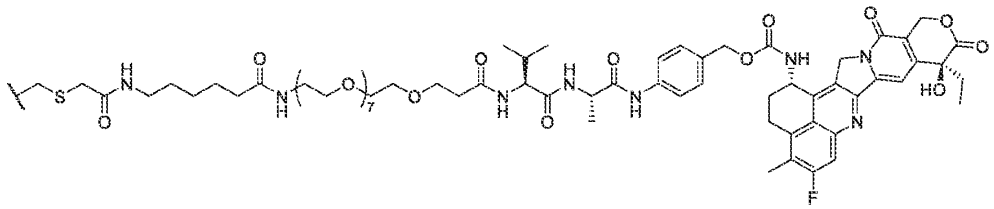


.

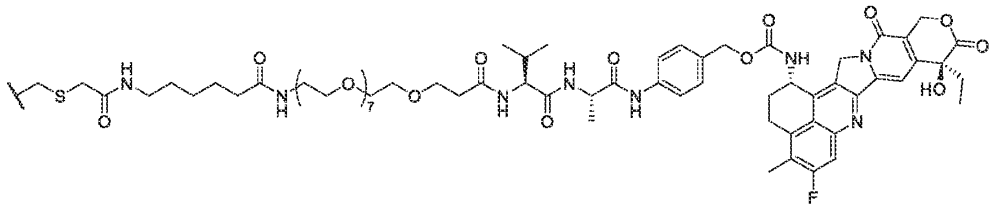
[0418] In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



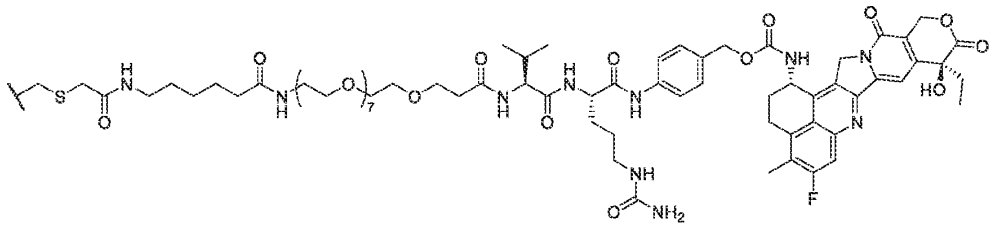
[0419] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



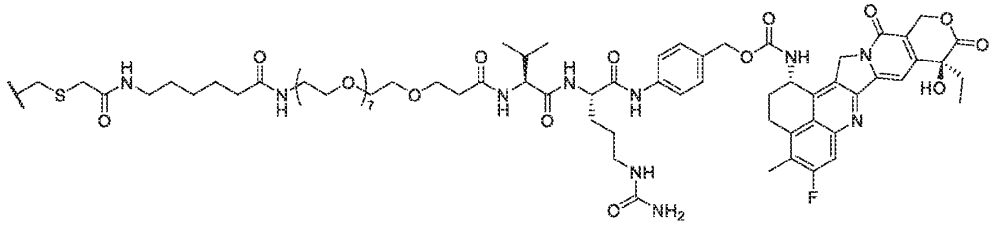
[0420] In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



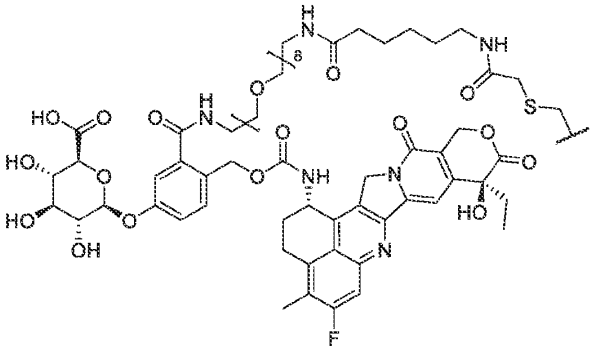
In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



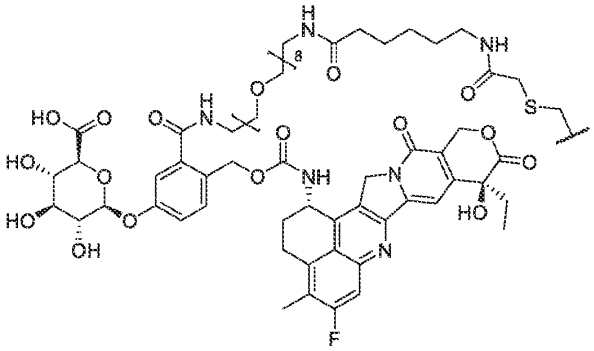
[0421] In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



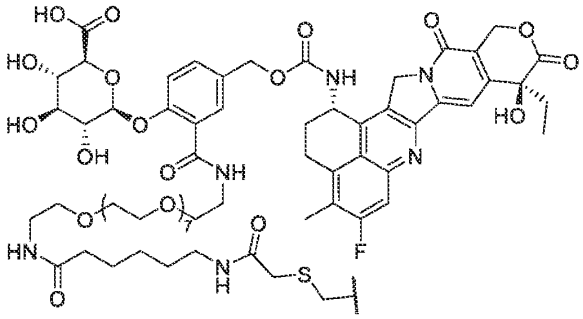
[0422] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or



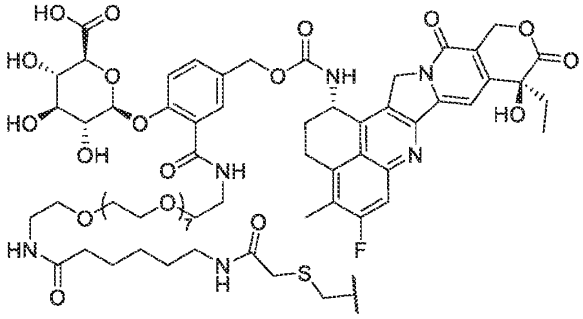
comprises . In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



. In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

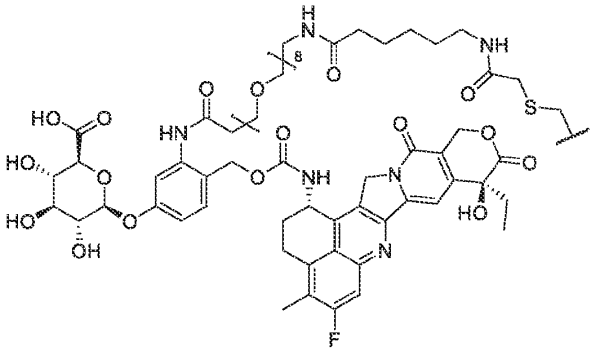


. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

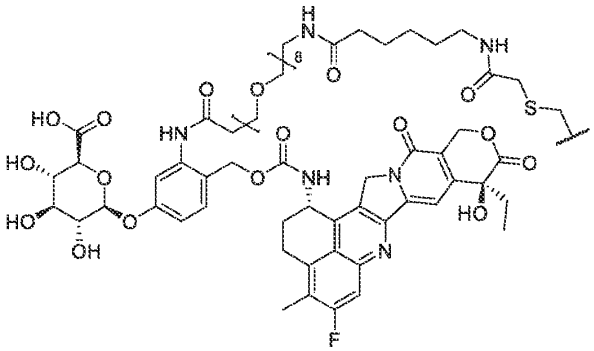


. In some embodiments, an occurrence of

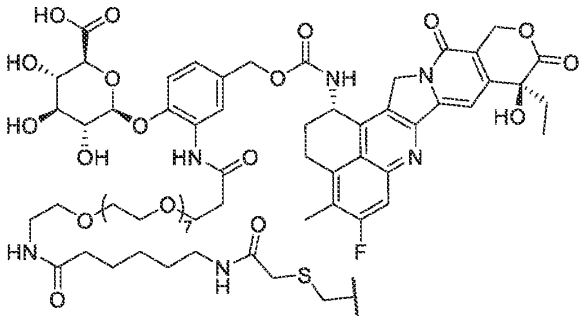
$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



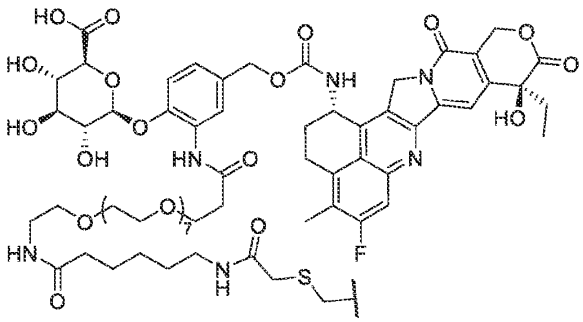
. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



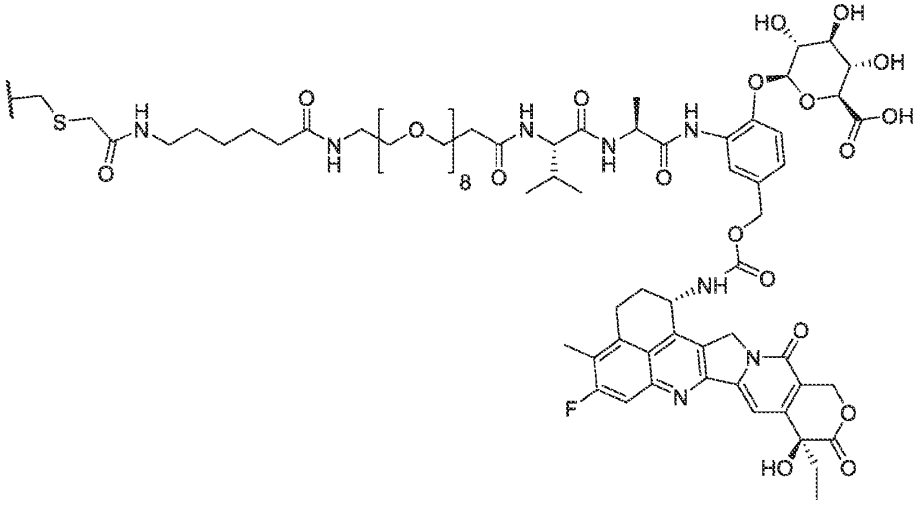
. In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



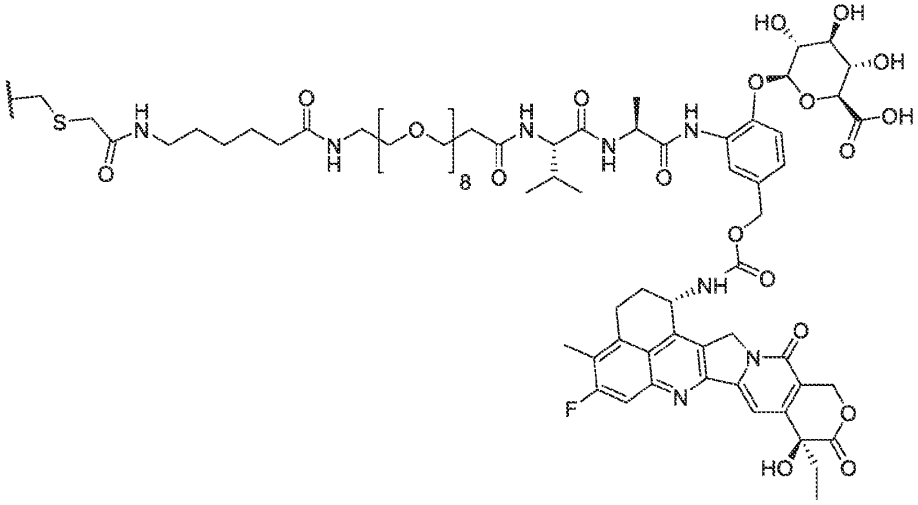
. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



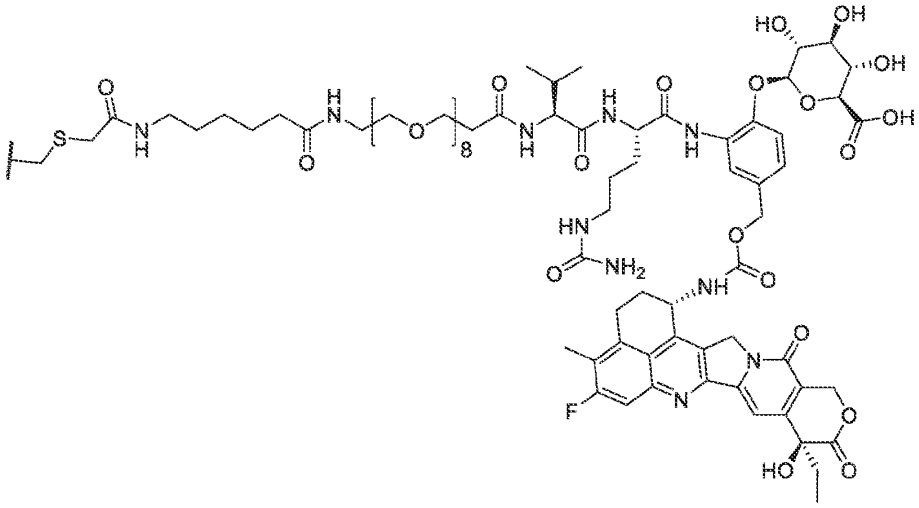
. In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



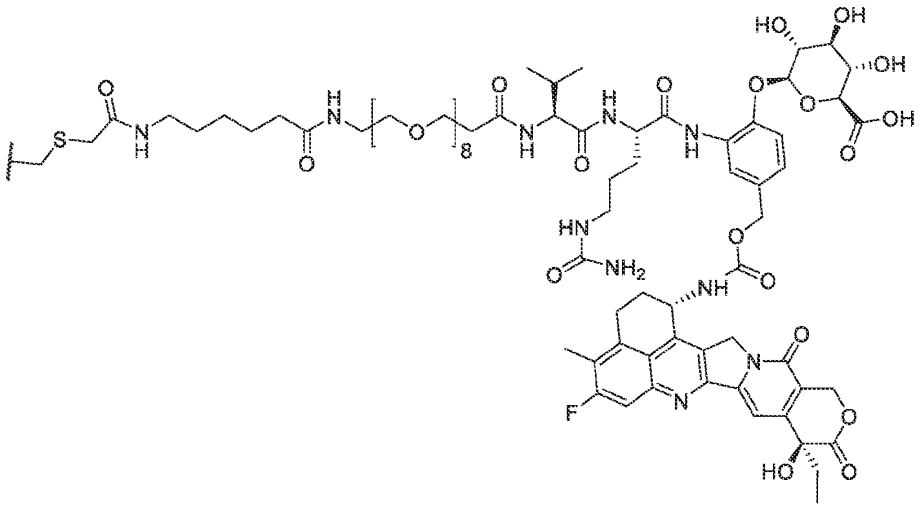
. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



. In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

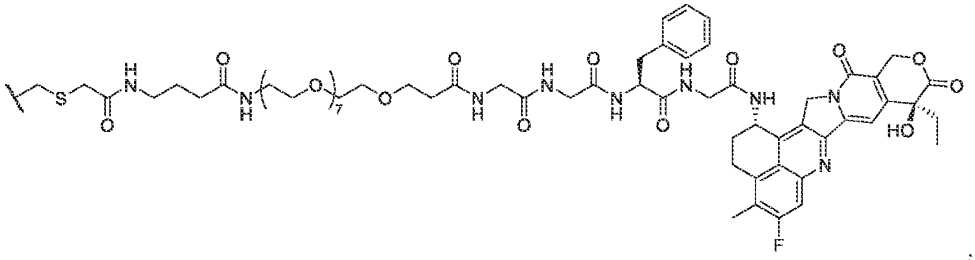


. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

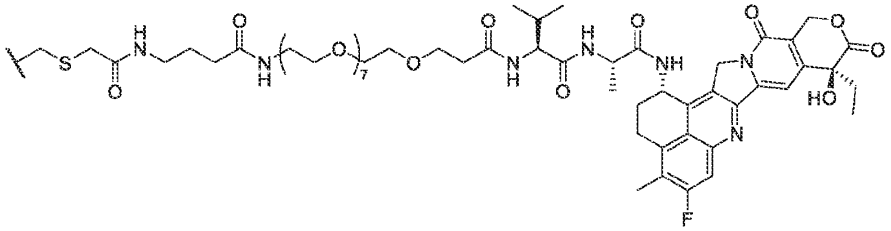


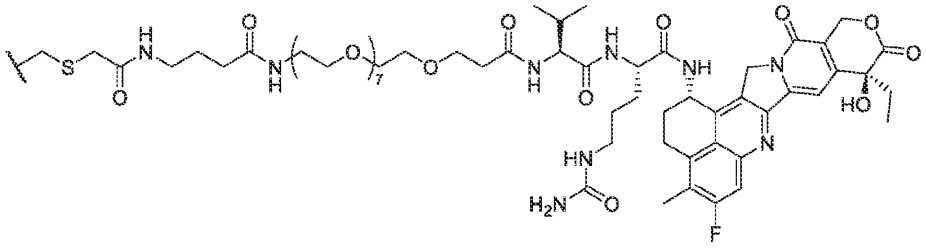
.

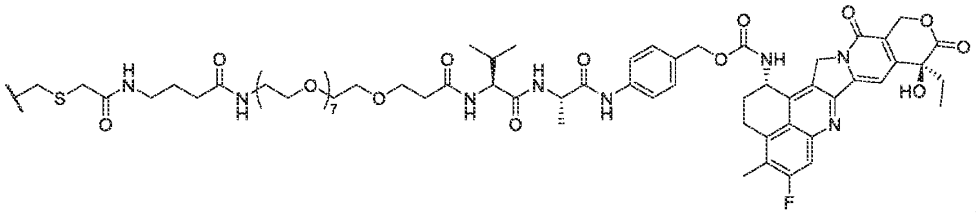
[0423] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

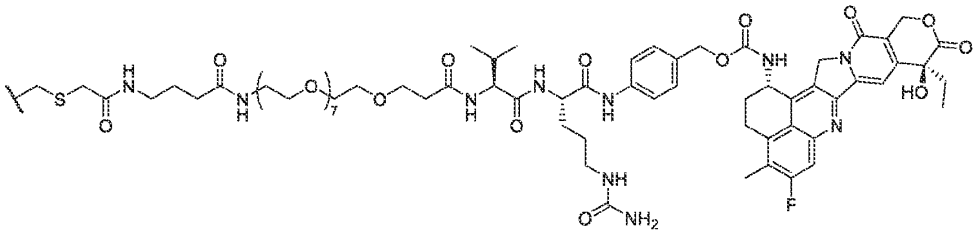


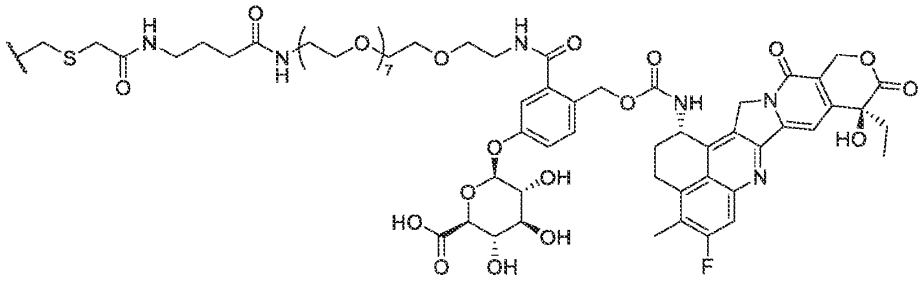
.

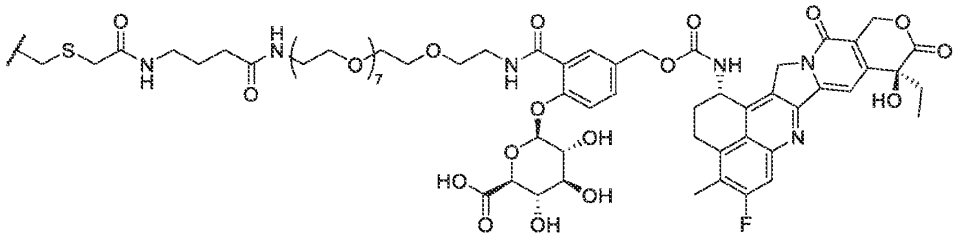


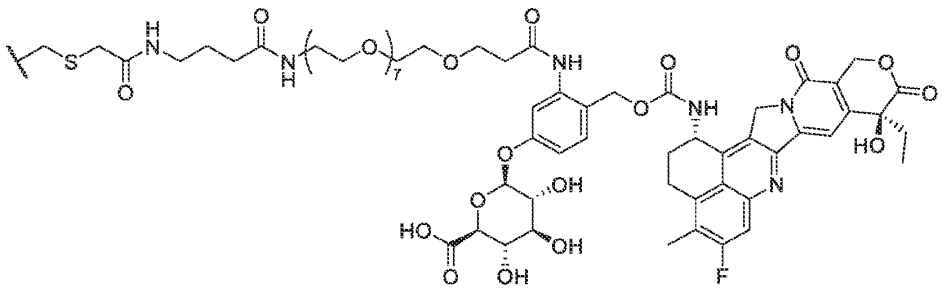




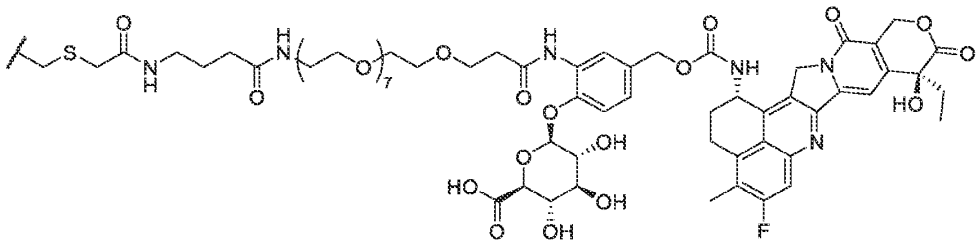






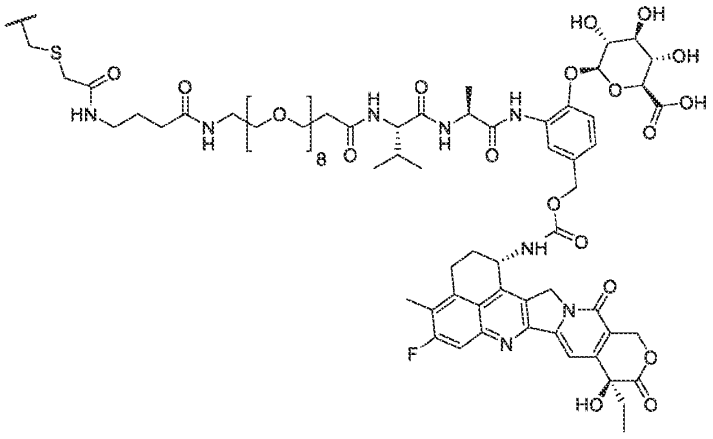


, or

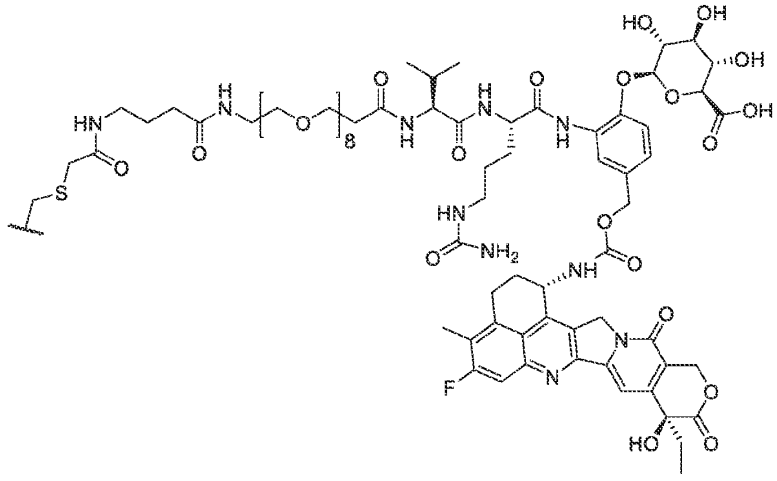


In some embodiments, each occurrence is the same.

[0424] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

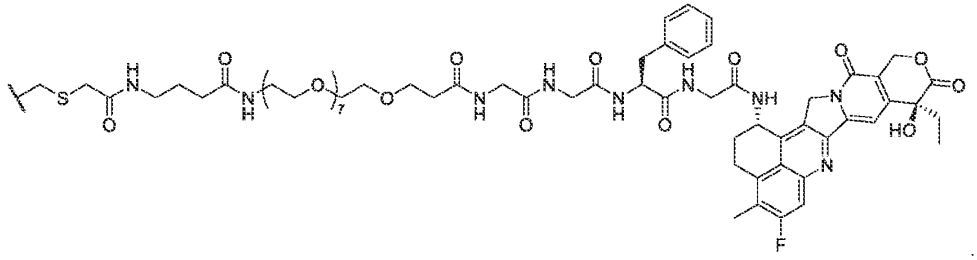


or

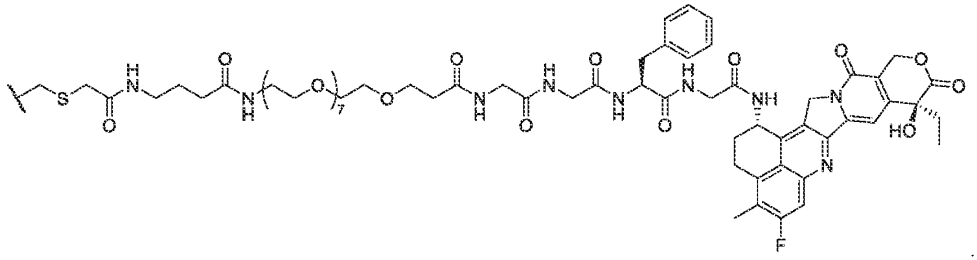


. In some embodiments, each occurrence is the same.

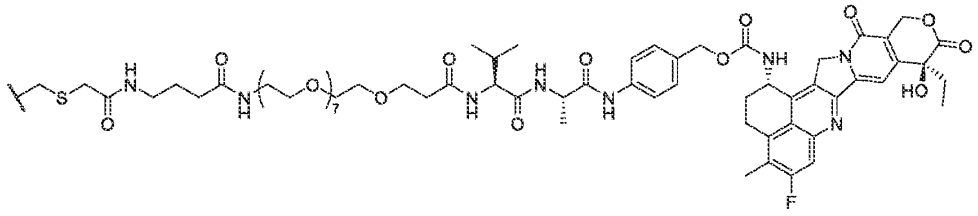
[0425] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



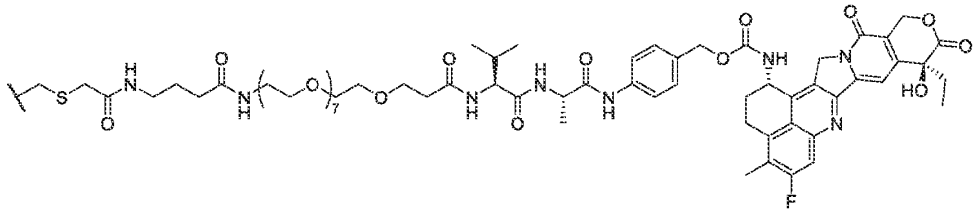
[0426] In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



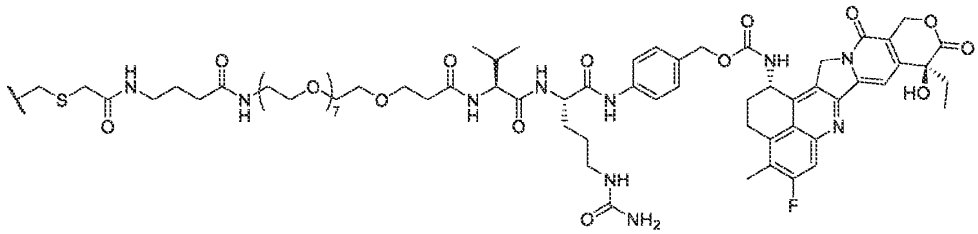
[0427] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



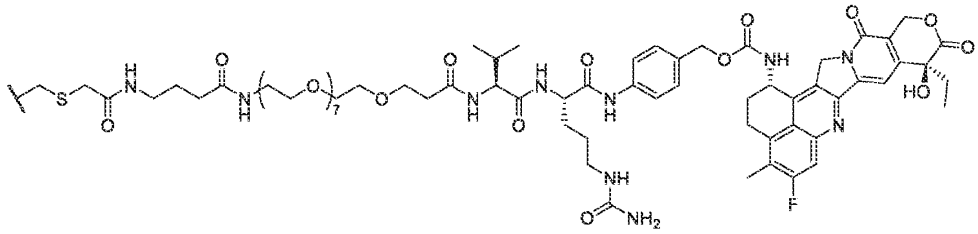
[0428] In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



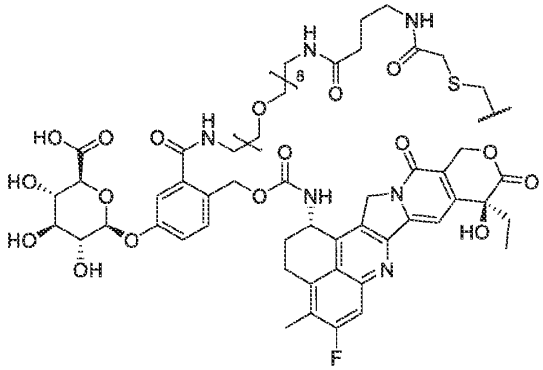
[0429] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



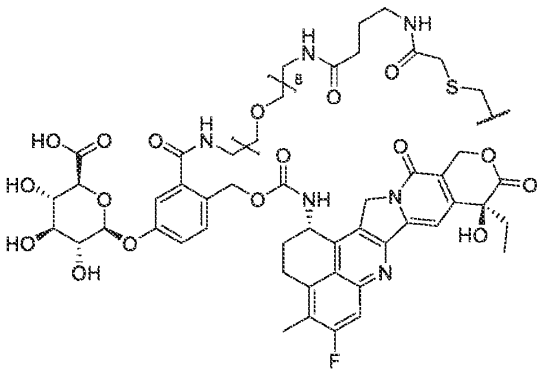
[0430] In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



[0431] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or

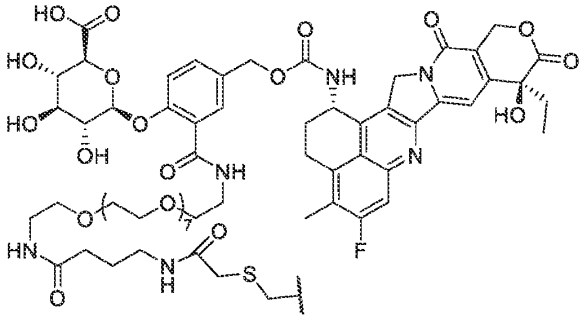


comprises . In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



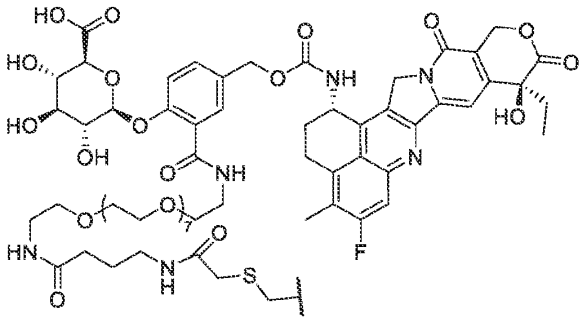
. In some embodiments, an occurrence of

$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



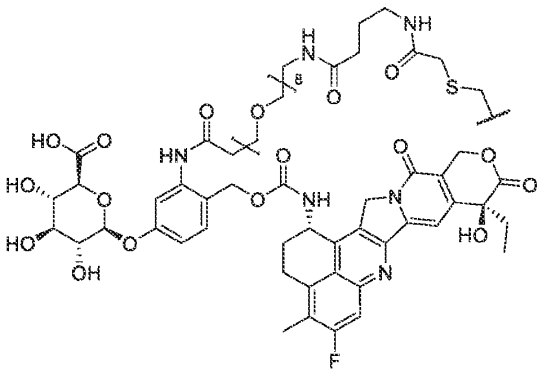
. In some embodiments, each occurrence of

$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

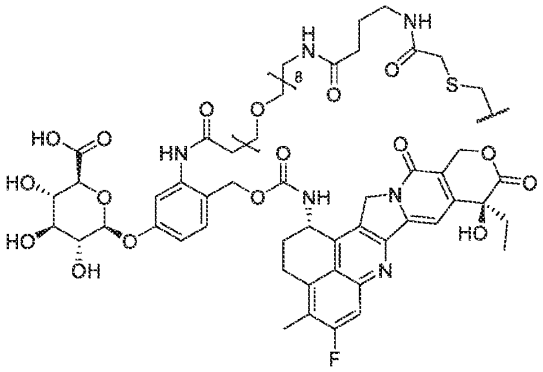


. In some embodiments, an occurrence of

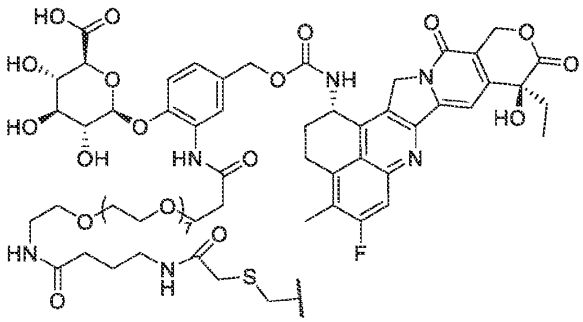
$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



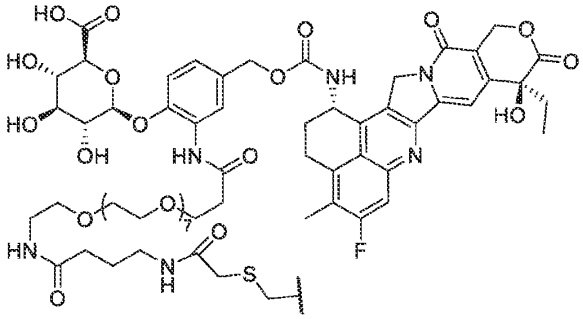
. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



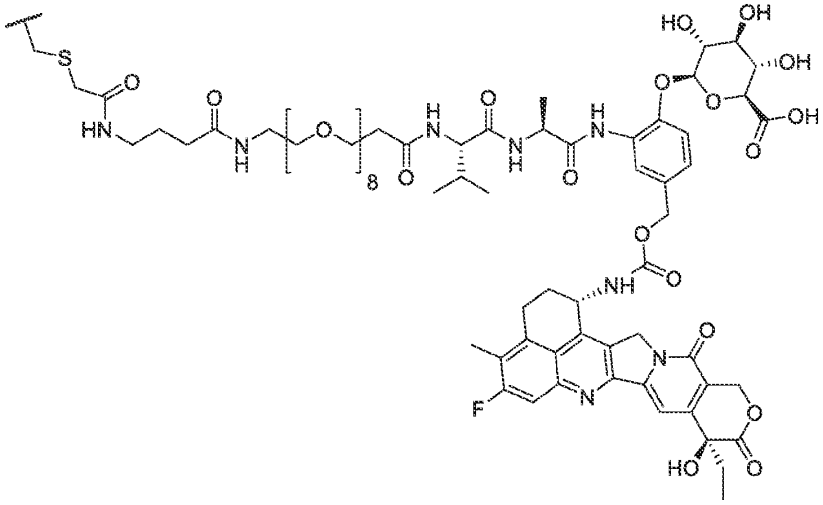
. In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



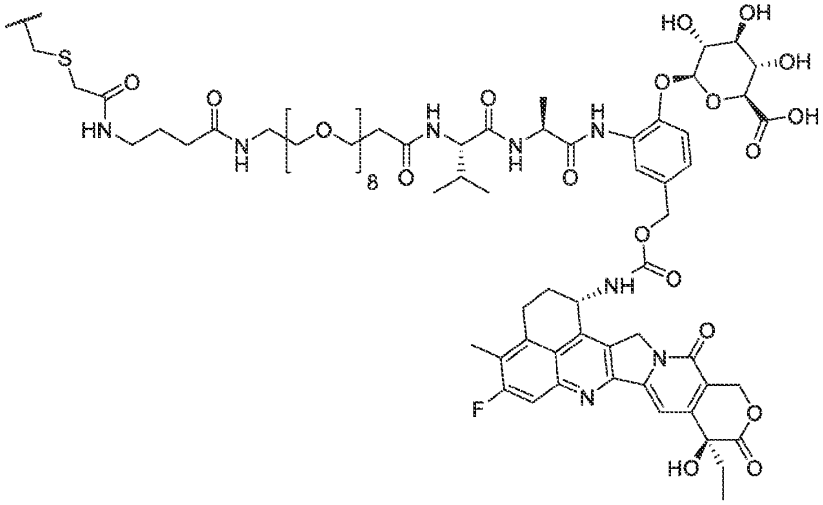
. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



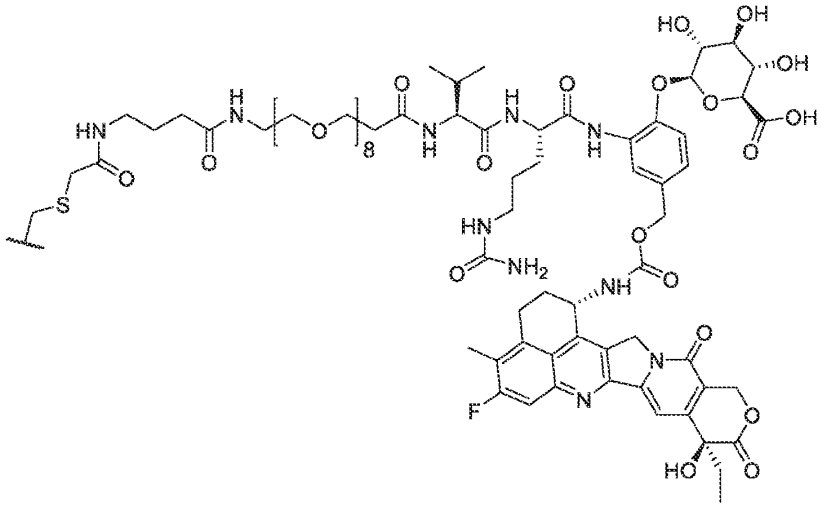
. In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



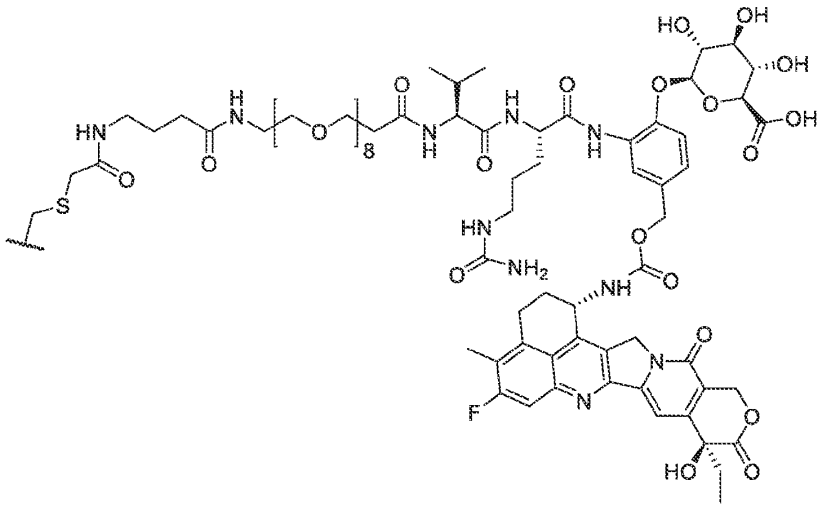
. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



.In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

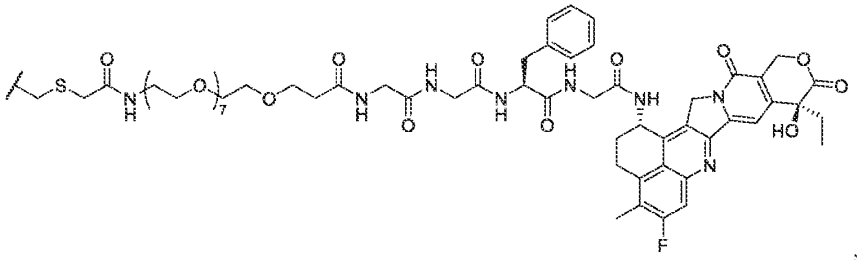


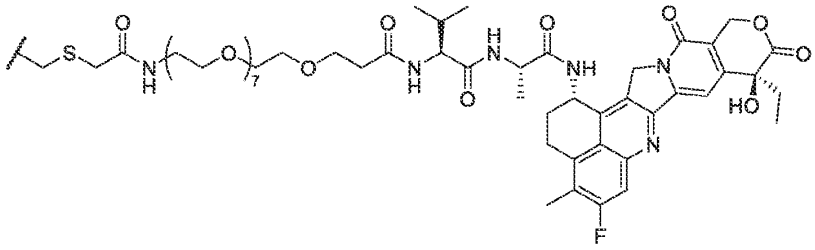
.

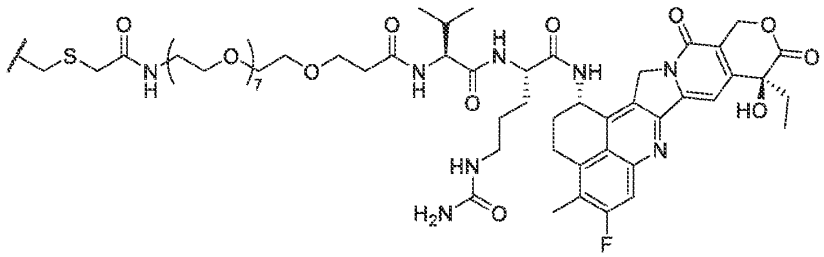
[0432] In some embodiments, a conjugate comprises $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$. In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is $-CH_2-S-CH_2-C(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-GGFG-R^{P}$. In some embodiments, it is $-CH_2-S-CH_2-C(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Ala-PABA-R^{P}$. In some embodiments, it is $-CH_2-S-CH_2-C(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-PABA-R^{P}$. In some embodiments, it is $-CH_2-S-CH_2-C(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)Val-Ala-NH-oGlu-R^{P}$. In some embodiments, it is $-CH_2-S-CH_2-C(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Ala-NH-mGlu-R^{P}$. In some embodiments, it is $-CH_2-S-CH_2-C(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-NH-oGLu-R^{P}$. In some embodiments, it is $-CH_2-S-CH_2-C(O)NH-(CH_2CH_2O)n-(CH_2)_zC(O)-Val-Cit-NH-mGLu-R^{P}$. In some embodiments, it is $-CH_2-S-CH_2-C(O)NH-(CH_2CH_2O)n-(CH_2)_zNHC(O)-oGlu-R^{P}$. In some embodiments, it is $-CH_2-S-CH_2-C(O)NH-(CH_2CH_2O)n-(CH_2)_zNHC(O)-mGlu-R^{P}$. In some

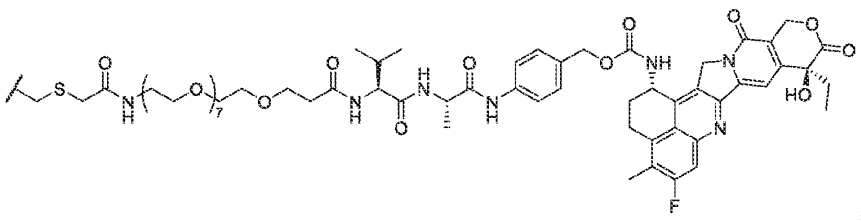
embodiments, n is 8. In some embodiments, z is 2. In some embodiments, R^P comprises a nitrogen atom and is bonded at the nitrogen atom, e.g., to the rest of a conjugate or other compound. In some embodiments, R^P is R^{EX}. In some embodiments, z is 2 and R^P is R^{EX}. In some embodiments, n is 8, z is 2 and R^P is R^{EX}. In some embodiments, x is 5, n is 8, z is 2 and R^P is R^{EX}. In some embodiments, each occurrence of $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is the same.

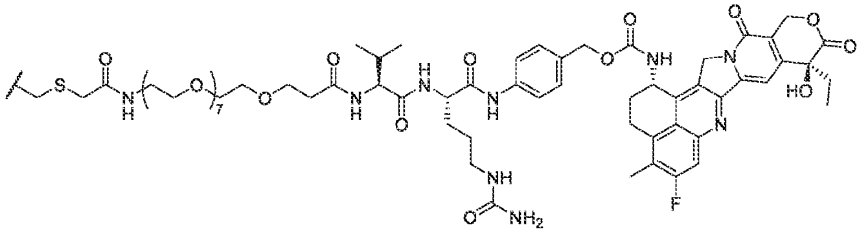
[0433] In some embodiments, an occurrence of $-L^T-L^{RX}-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

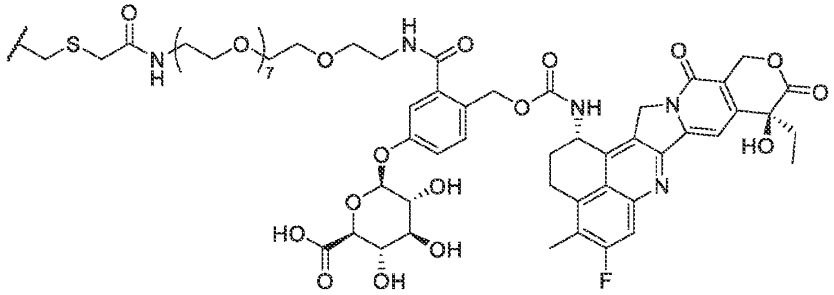


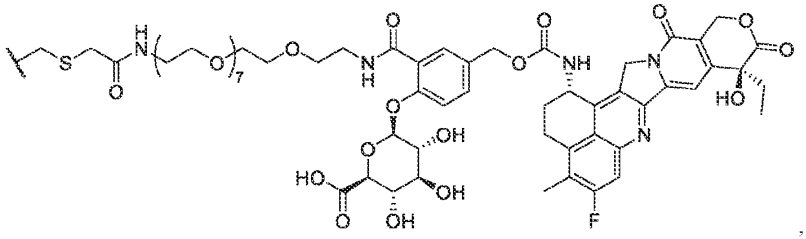


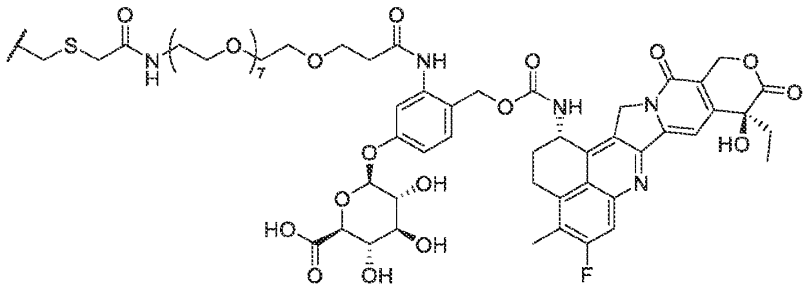




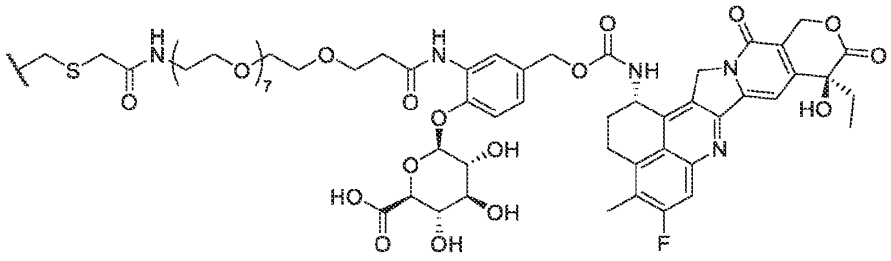






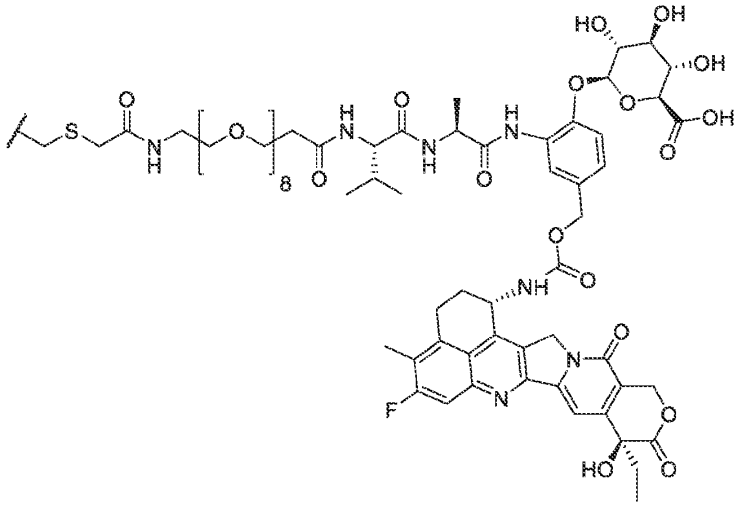


, or

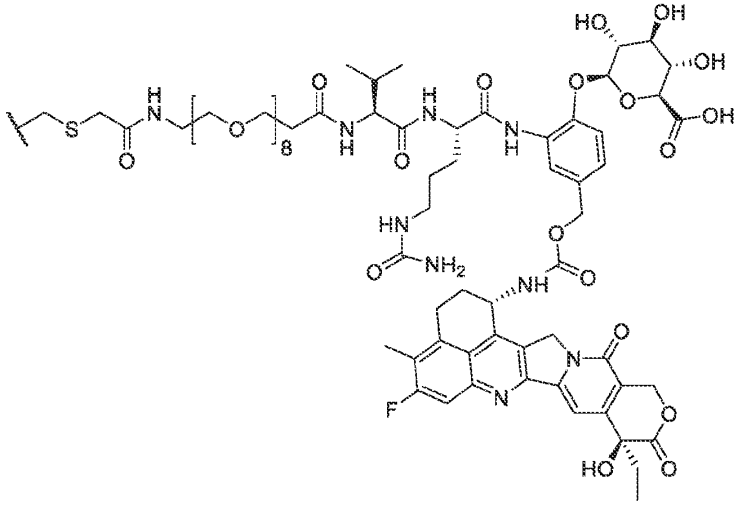


. In some embodiments, each occurrence is the same.

[0434] In some embodiments, an occurrence of $-L^{T}-L^{PK}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

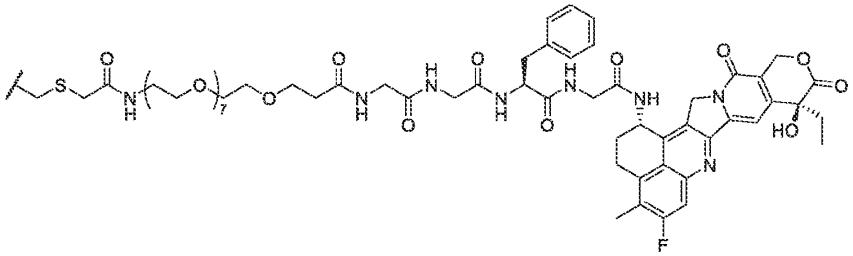


or

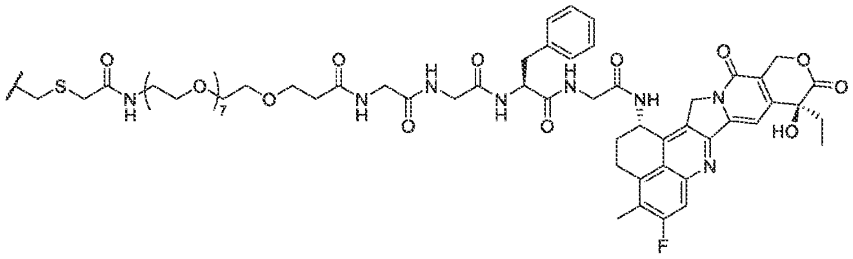


. In some embodiments, each occurrence is the same.

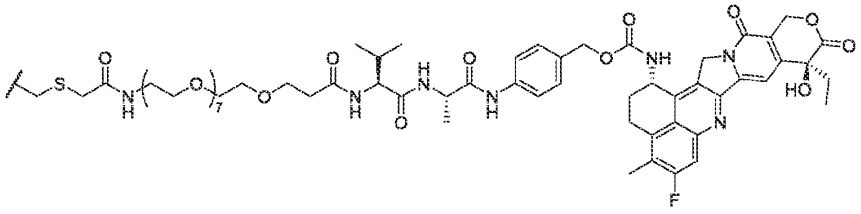
[0435] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



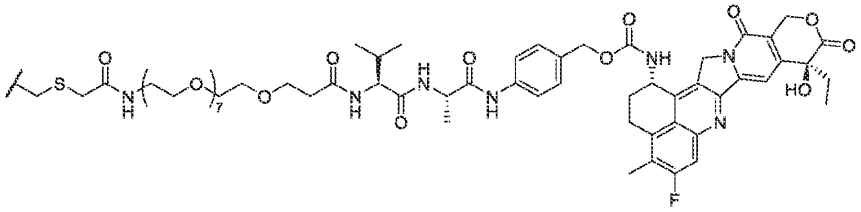
[0436] In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



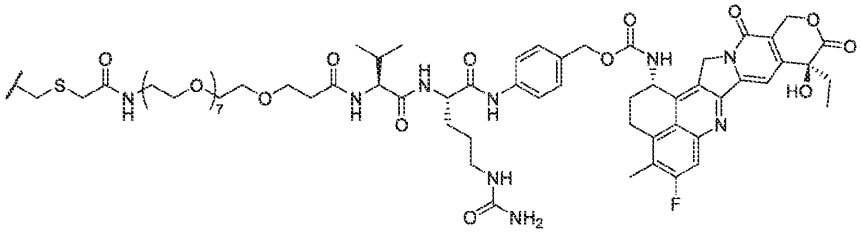
[0437] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



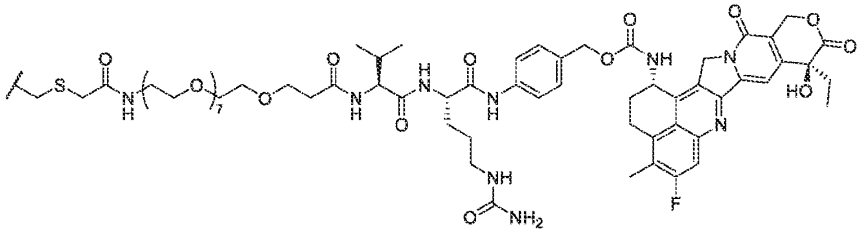
[0438] In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



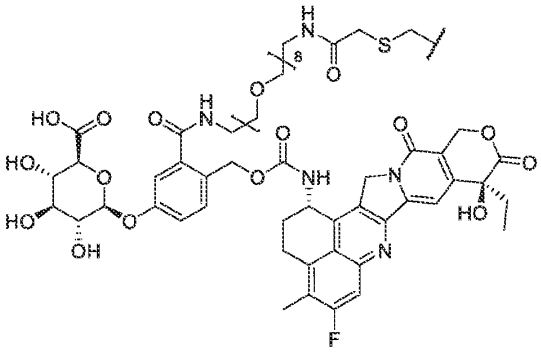
[0439] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



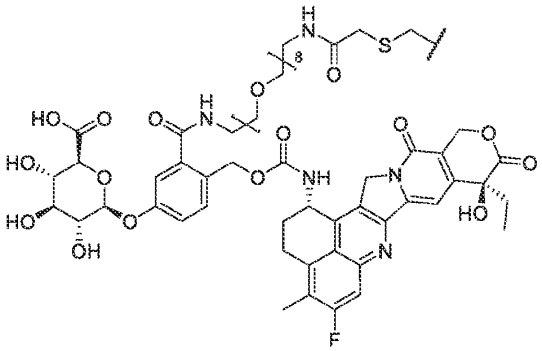
[0440] In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



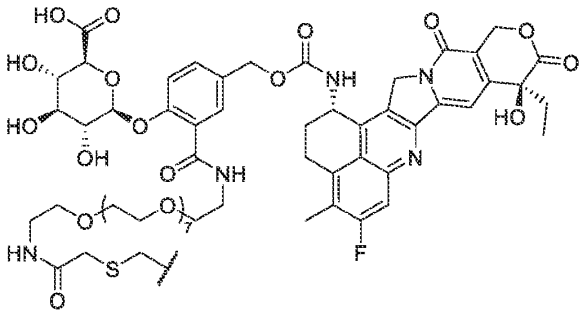
[0441] In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or



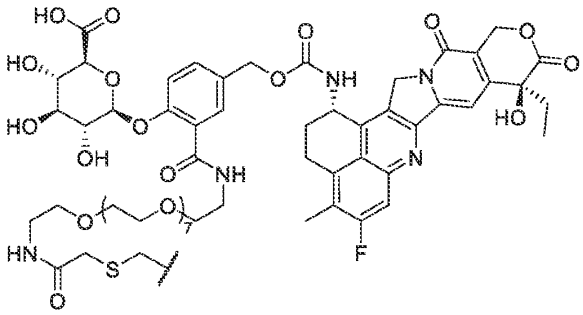
comprises . In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



. In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

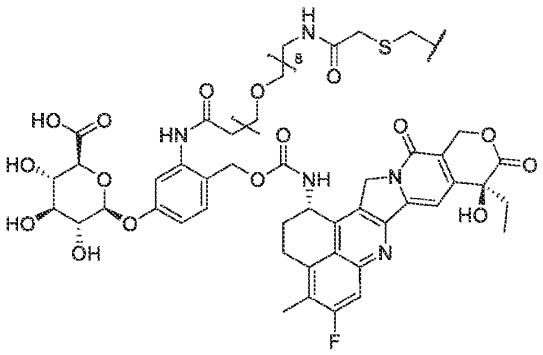


. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

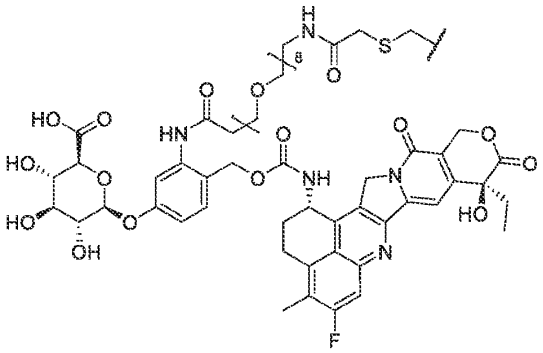


. In some embodiments, an occurrence of

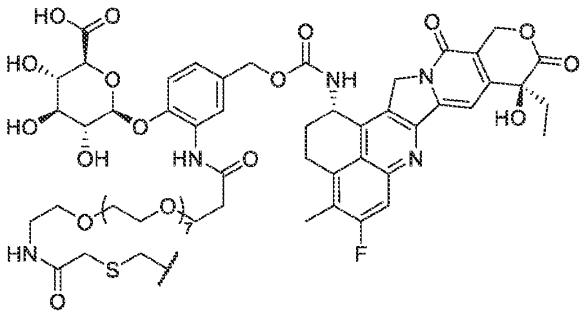
–L^{T}–L^{RX}–L^{X}–$(CH_2CH_2O)n$–L^{ZP}–L^{C}–R^{P} is or comprises



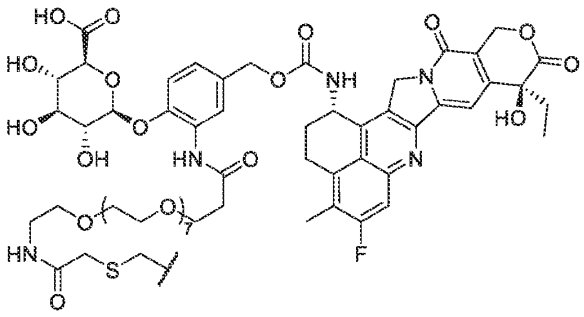
. In some embodiments, each occurrence of –L^{T}–L^{RX}–L^{X}–$(CH_2CH_2O)n$–L^{ZP}–L^{C}–R^{P} is or comprises



. In some embodiments, an occurrence of –L^{T}–L^{RX}–L^{X}–$(CH_2CH_2O)n$–L^{ZP}–L^{C}–R^{P} is or comprises

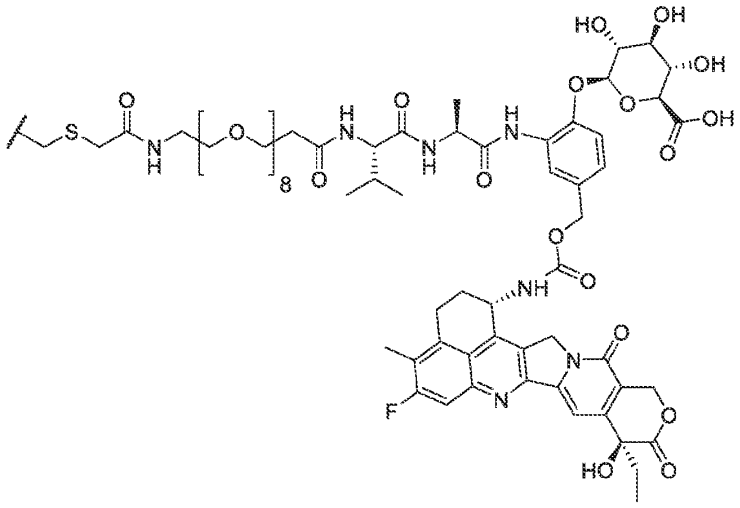


. In some embodiments, each occurrence of –L^{T}–L^{RX}–L^{X}–$(CH_2CH_2O)n$–L^{ZP}–L^{C}–R^{P} is or comprises

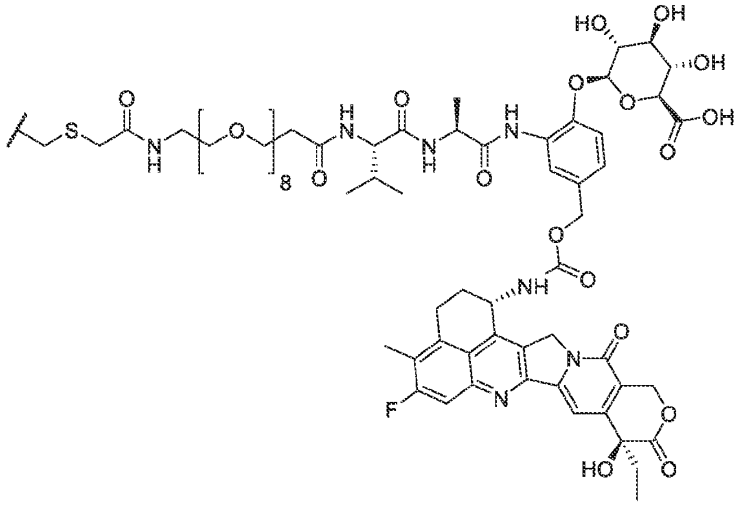


. In some embodiments, an occurrence of

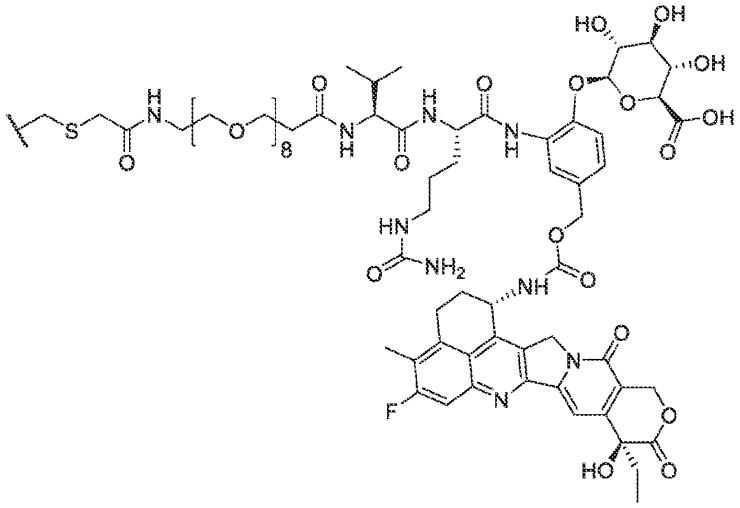
$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



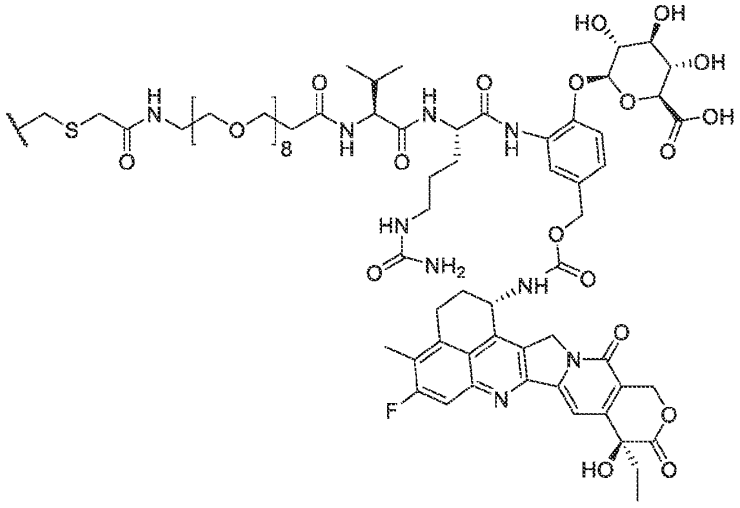
. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



. In some embodiments, an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



.

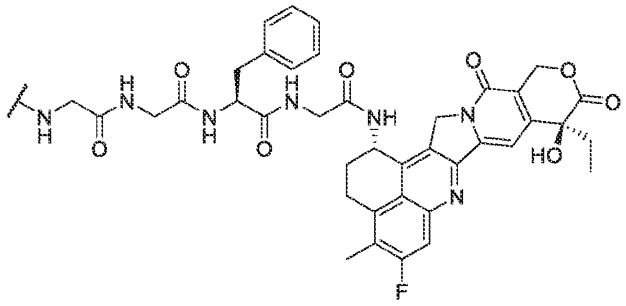
[0442] As used in the present disclosure, in some embodiments, "is or comprises" is "is"; in some embodiments, "is or comprises" is "comprises".

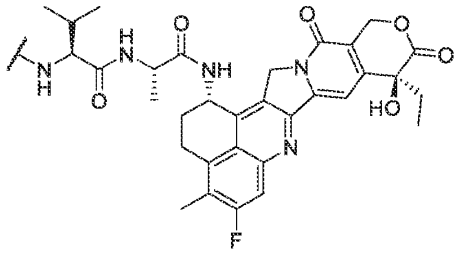
[0443] In some embodiments, a target binding moiety is or comprises an amino acid residue, and $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is bonded to a backbone atom of an amino acid residue. In some embodiments, a target binding moiety is or comprises an amino acid residue, and $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is bonded to an alpha-carbon of an amino acid residue. In some embodiments, each target binding moiety is independently an antibody or an antigen-binding fragment thereof. In some embodiments, each target binding moiety is independently an antibody. In some embodiments, each target binding moiety is independently a monoclonal antibody. In some embodiments, the target binding moiety is not trastuzumab. In some embodiments, the antibody shares less than about 90%, 95% or 99% sequence identity to trastuzumab. In some embodiments, the target binding moiety is

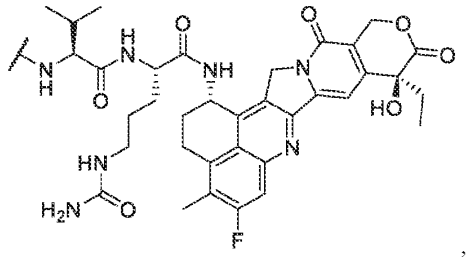
not an anti-HER2 antibody. In some embodiments, the target binding moiety is not an anti-HER2 antibody or an antigen-binding fragment thereof.

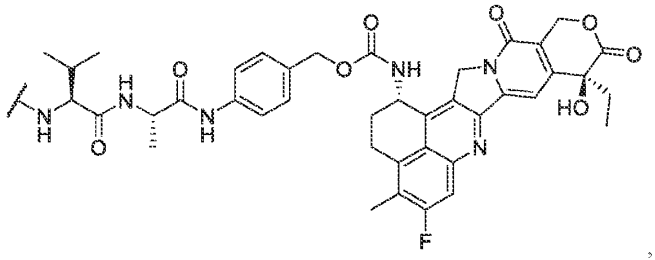
[0444] In some embodiments, t is 1-10. In some embodiments, t is 2-8. In some embodiments, t is 3-5. In some embodiments, t is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, t is 1. In some embodiments, t is about 2. In some embodiments, t is about 4. In some embodiments, t is about 6. In some embodiments, t is about 8.

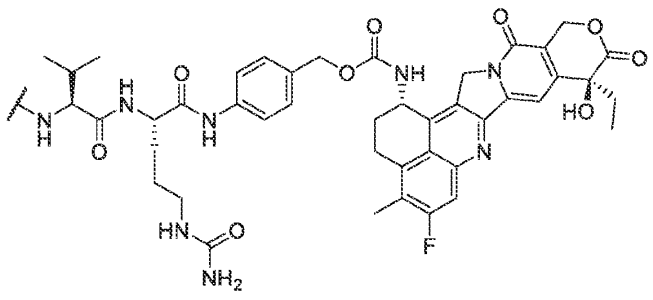
[0445] In some embodiments, a conjugate comprises

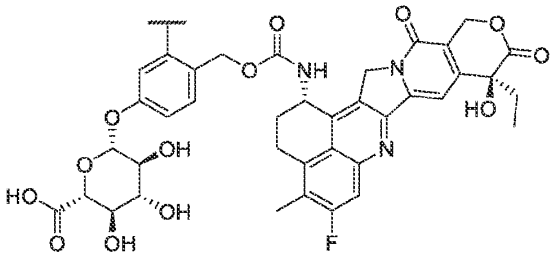




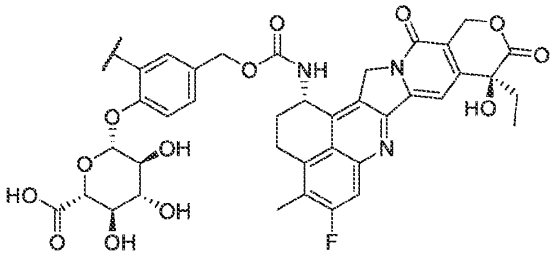




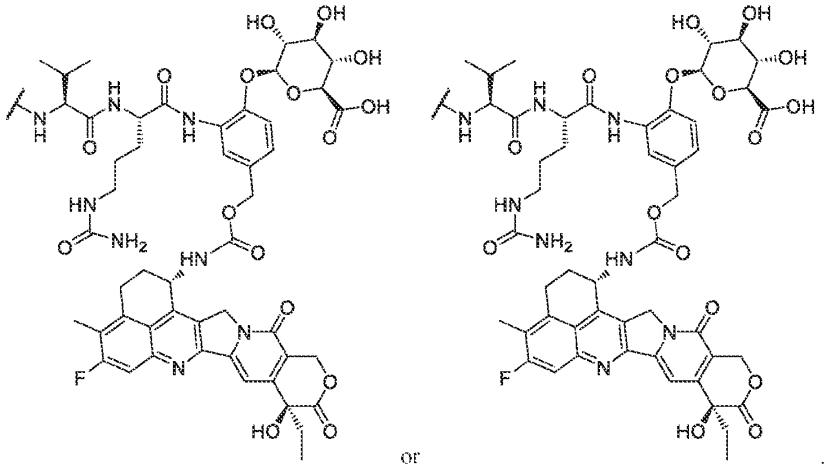




. or

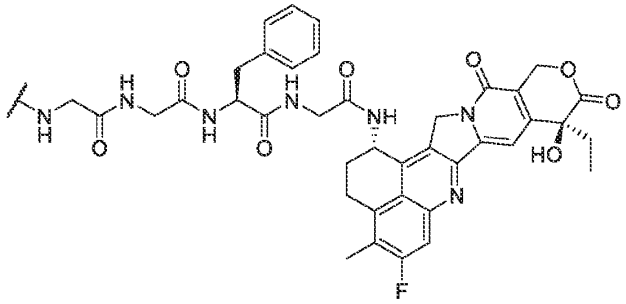


[0446] In some embodiments, a conjugate comprises

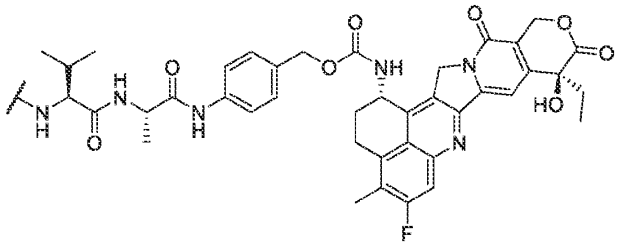


or

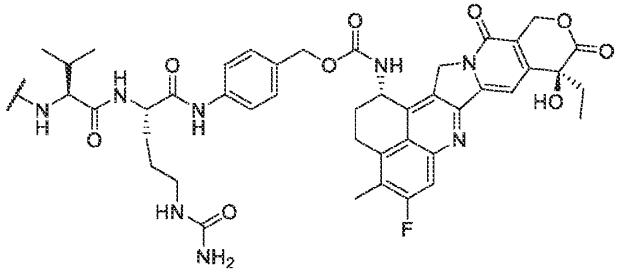
[0447] In some embodiments, a conjugate comprises

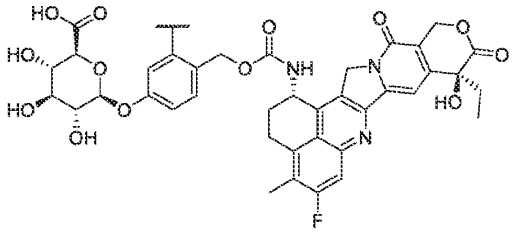


[0448] In some embodiments, a conjugate comprises

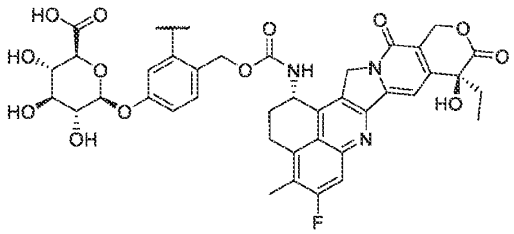


[0449] In some embodiments, a conjugate comprises



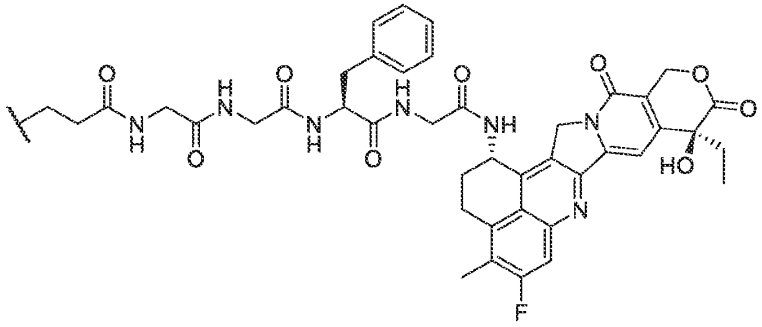


In some embodiments, a conjugate comprises . In some embodiments, a conjugate comprises

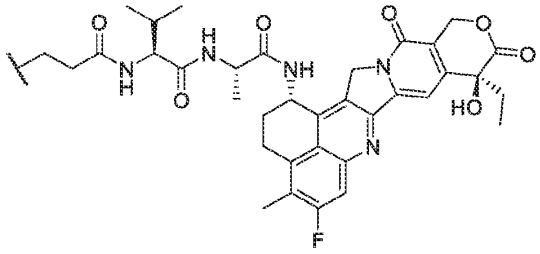


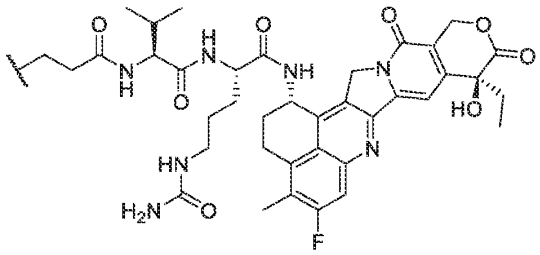
.

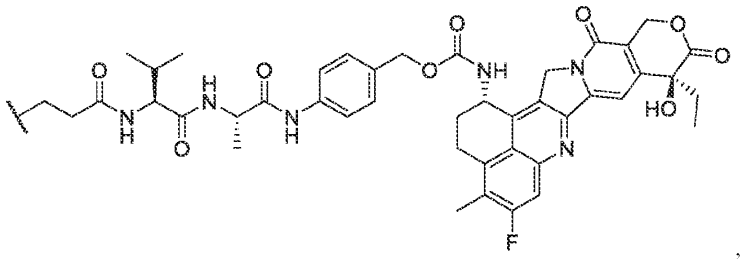
[0450] In some embodiments, a conjugate comprises

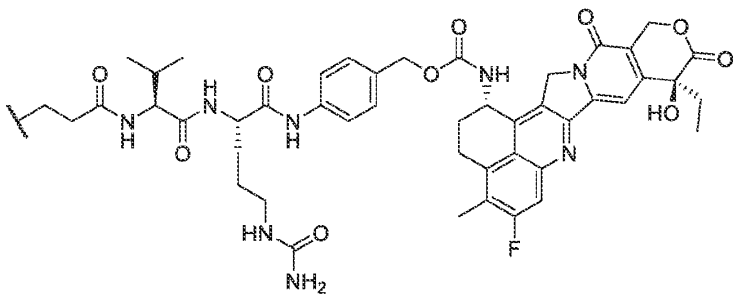


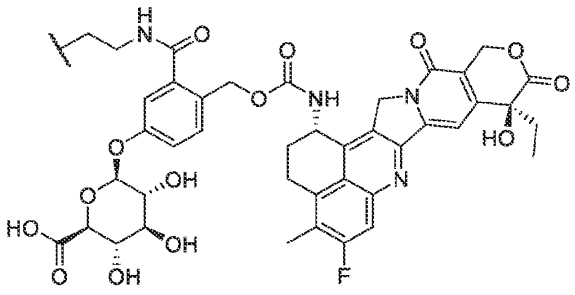
.

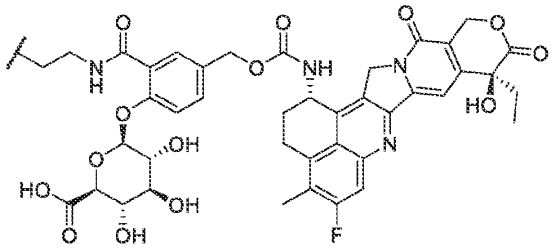


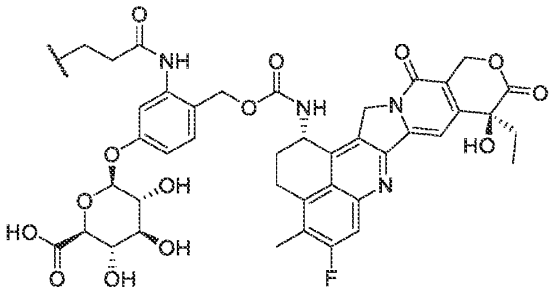




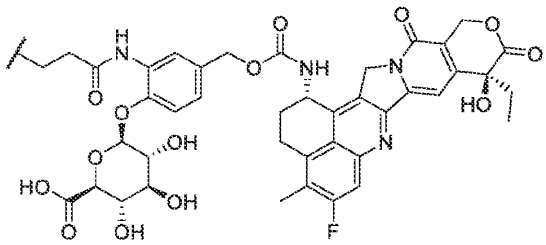


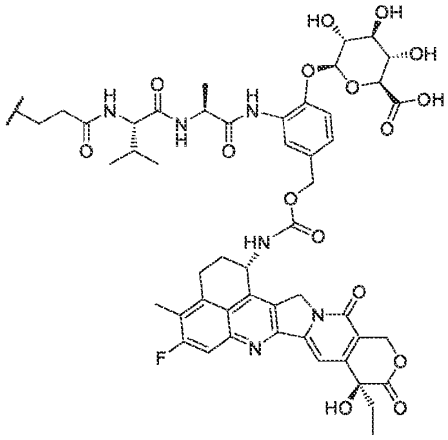




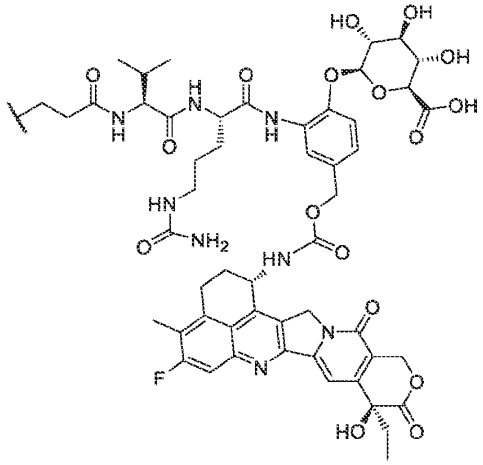


, or



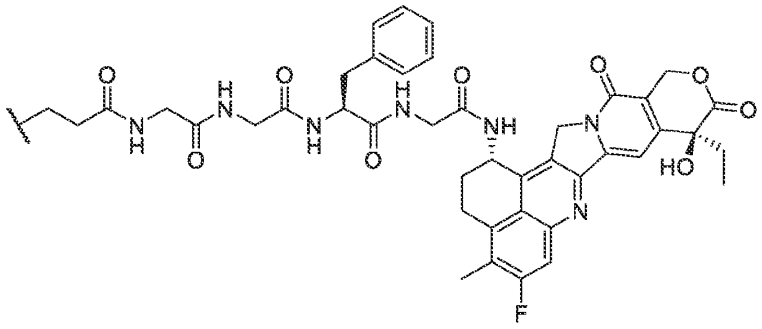


[0451] In some embodiments, a conjugate comprises

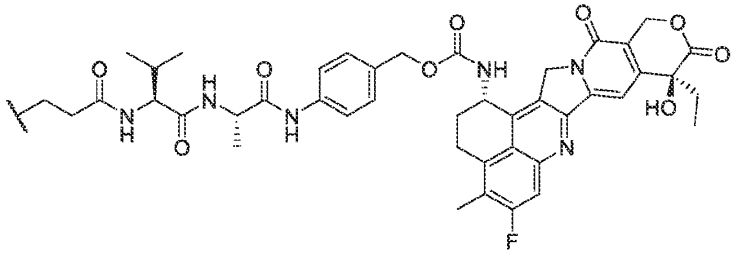


or

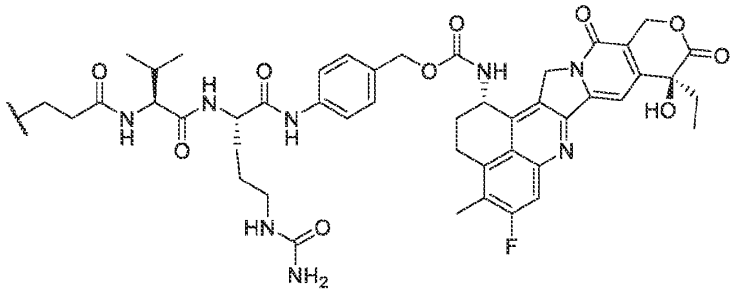
[0452] In some embodiments, a conjugate comprises



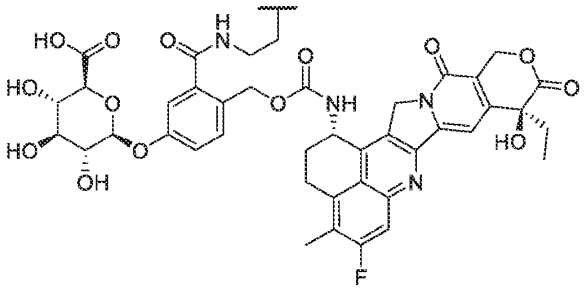
[0453] In some embodiments, a conjugate comprises



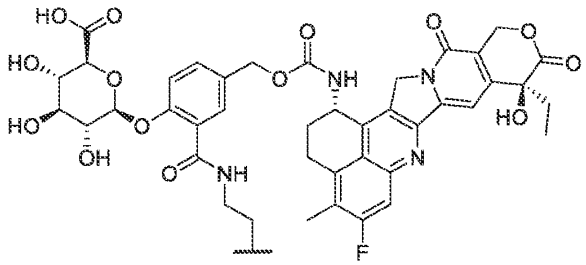
[0454] In some embodiments, a conjugate comprises



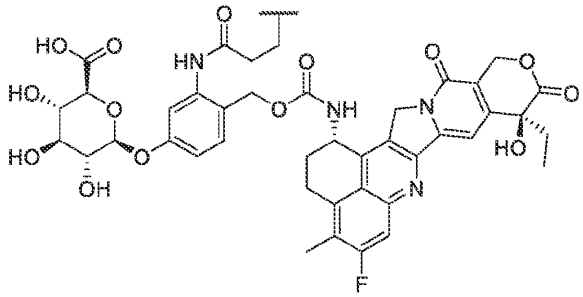
[0455] In some embodiments, a conjugate comprises



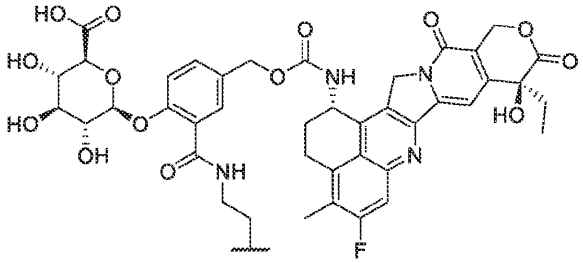
. In some embodiments, a conjugate comprises



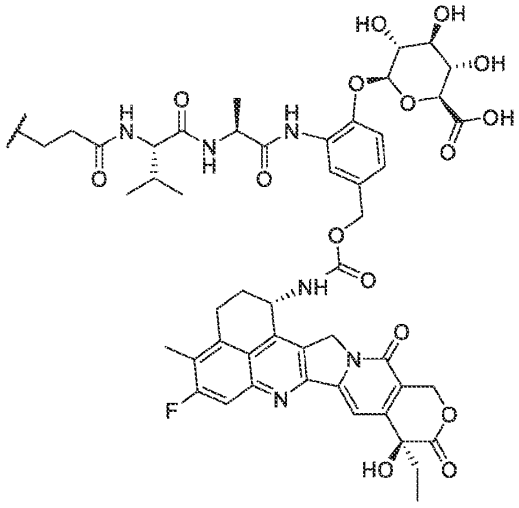
. In some embodiments, a conjugate comprises



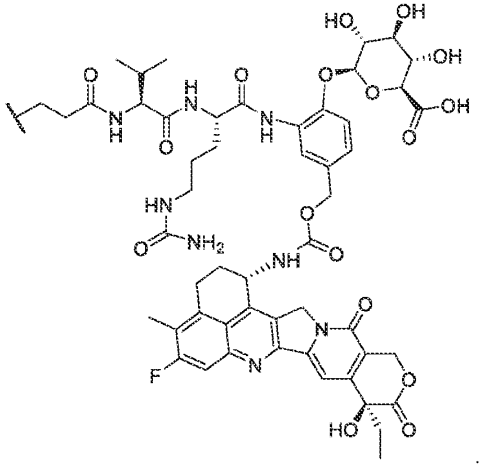
. In some embodiments, a conjugate comprises



. In

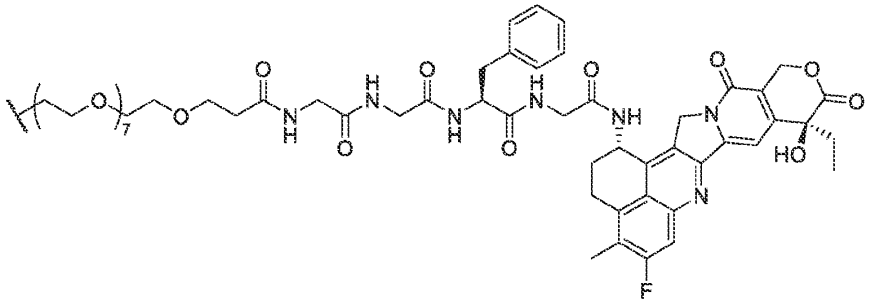


some embodiments, a conjugate comprises . In

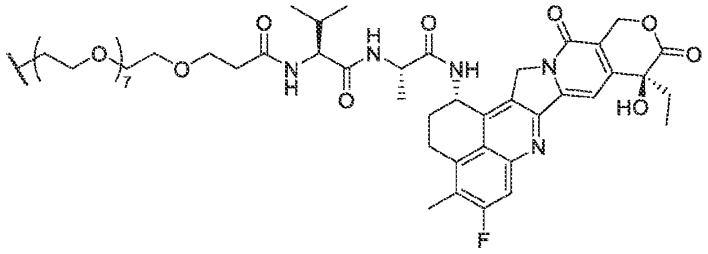


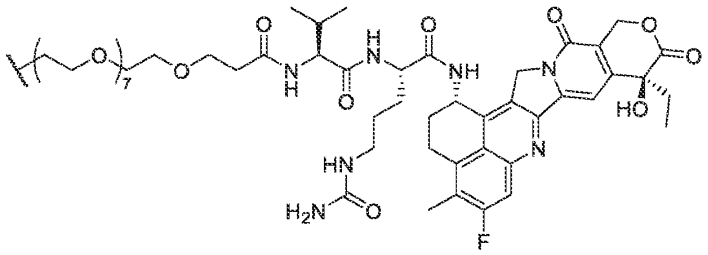
some embodiments, a conjugate comprises .

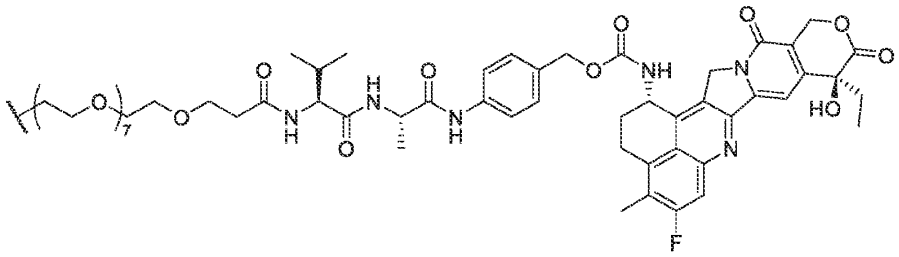
[0456] In some embodiments, a conjugate comprises

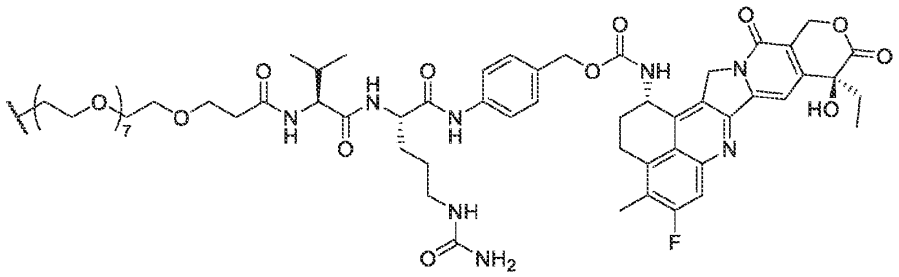


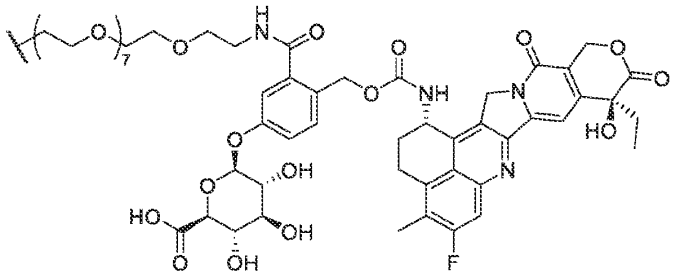
.

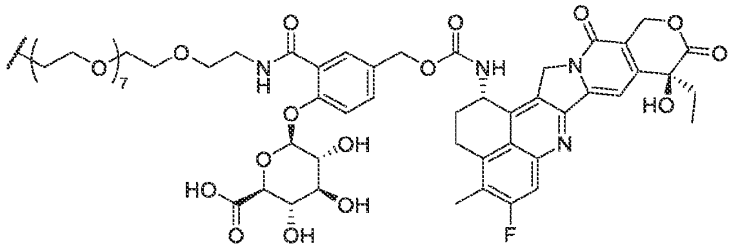


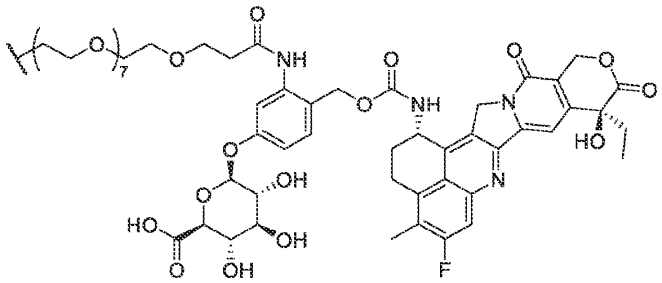




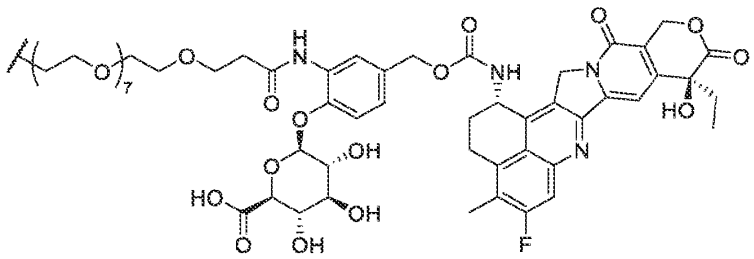




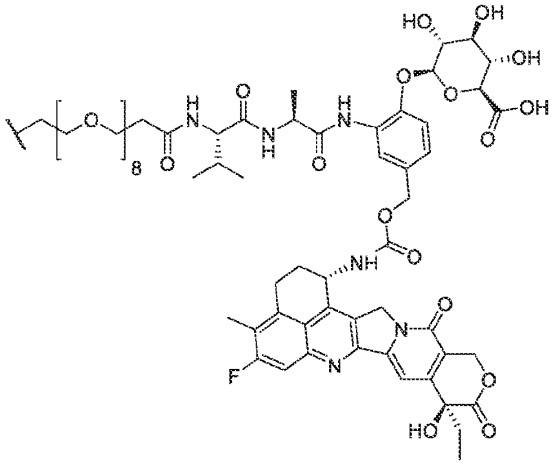


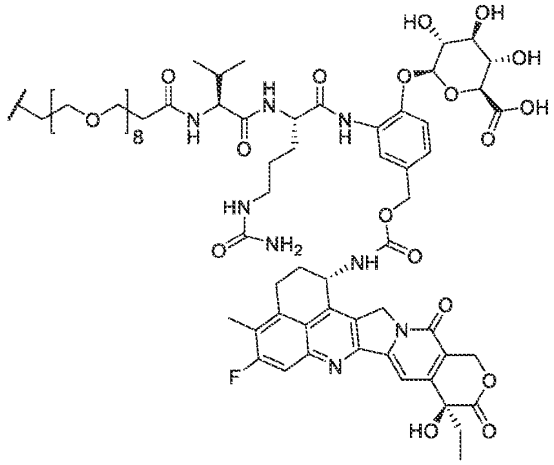


, or



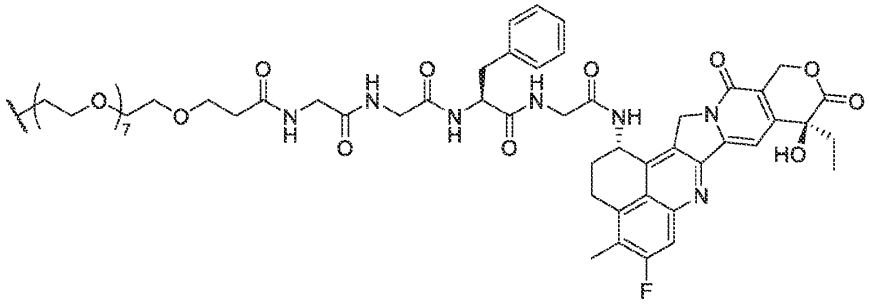
[0457] In some embodiments, a conjugate comprises.



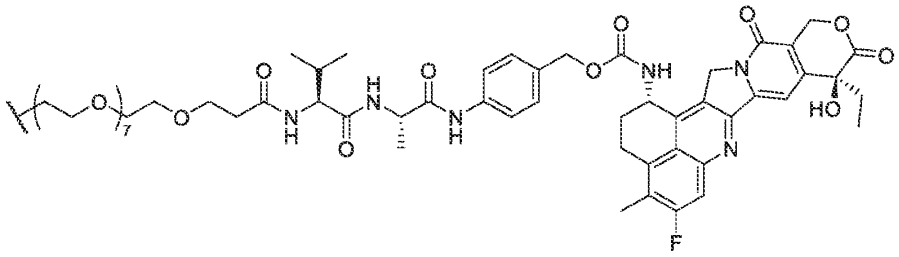


or

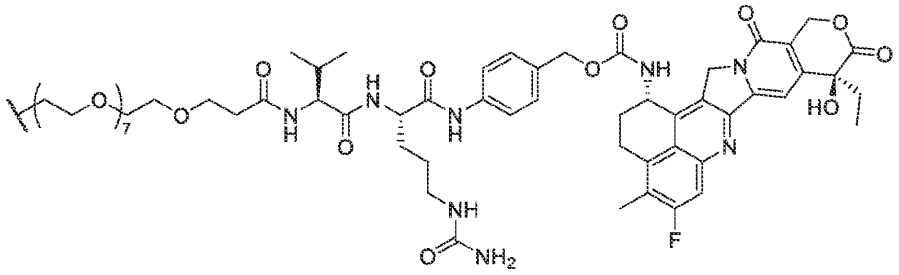
[0458] In some embodiments, a conjugate comprises



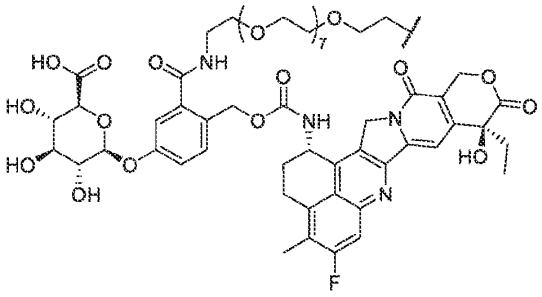
[0459] In some embodiments, a conjugate comprises



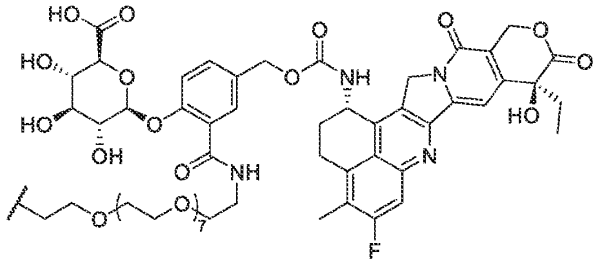
[0460] In some embodiments, a conjugate comprises



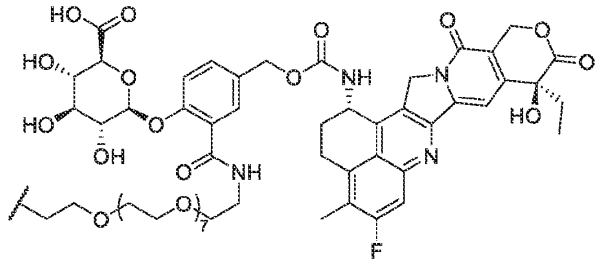
[0461] In some embodiments, a conjugate comprises



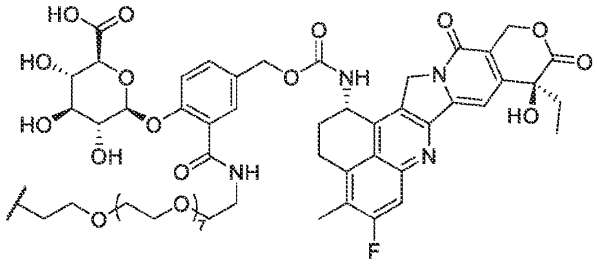
. In some embodiments, a conjugate comprises



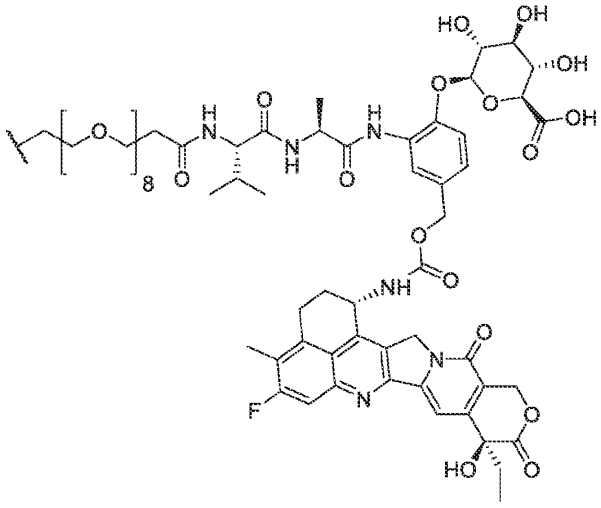
. In some embodiments, a conjugate



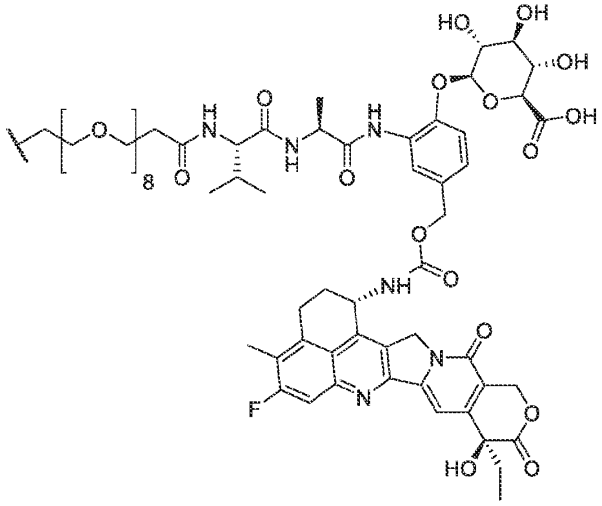
comprises . In some embodiments, a



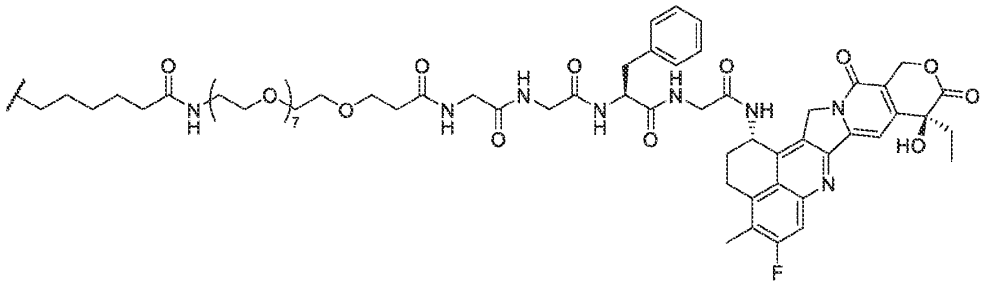
conjugate comprises . In some

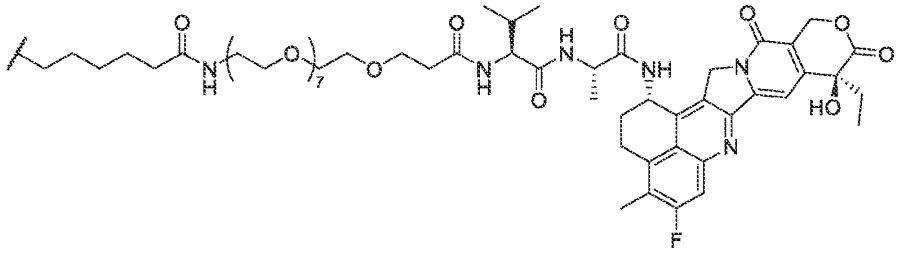


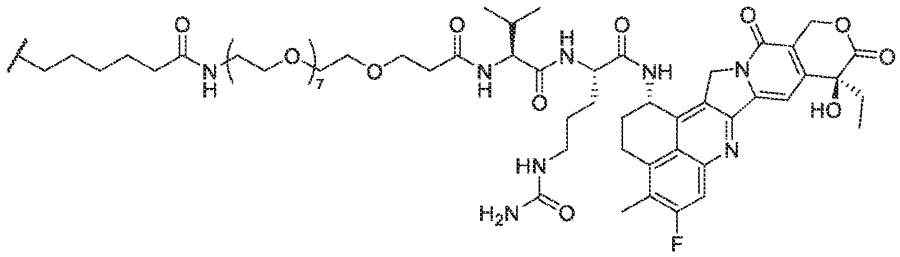
embodiments, a conjugate comprises . In some embodiments, a conjugate comprises

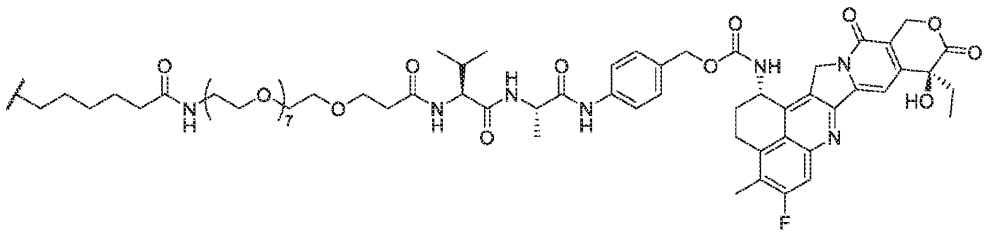


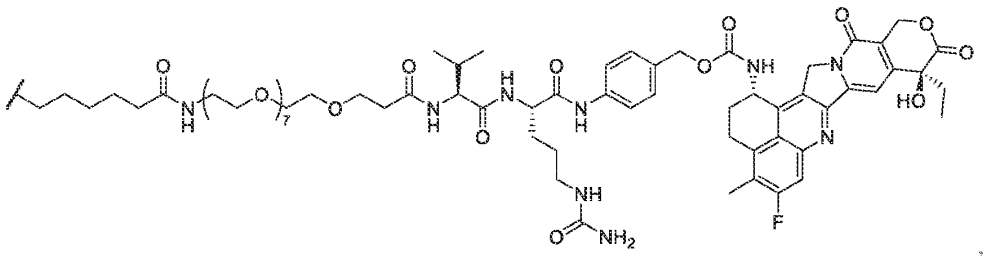
[0462] In some embodiments, a conjugate comprises

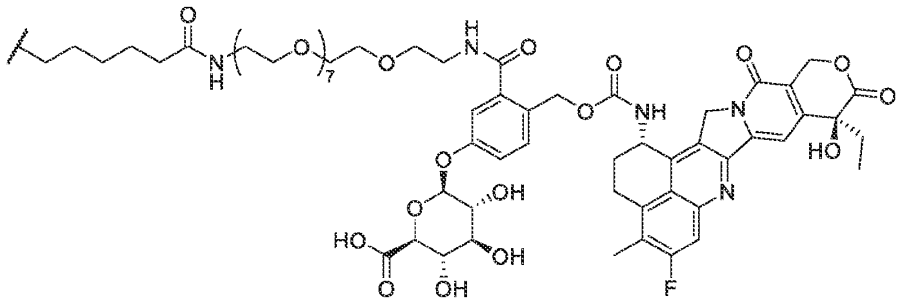


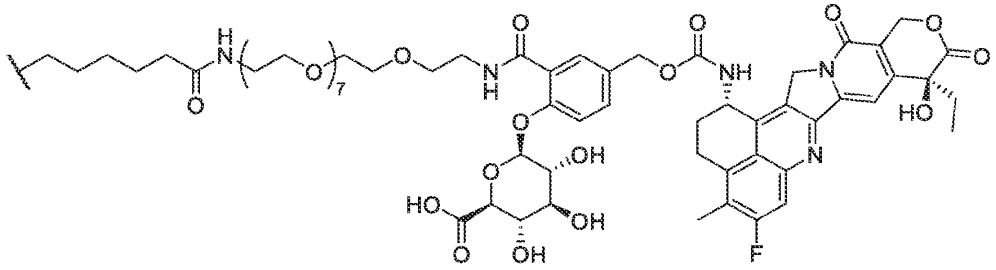


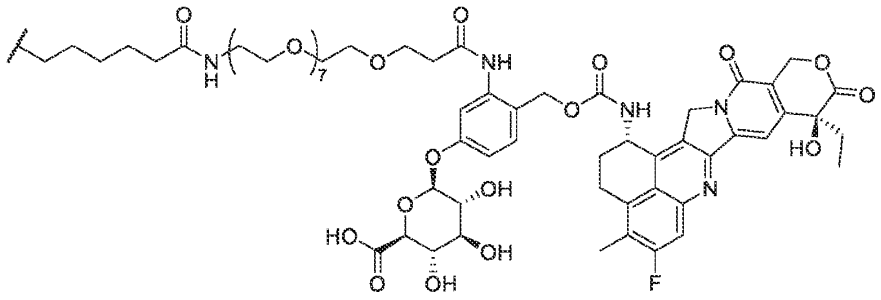




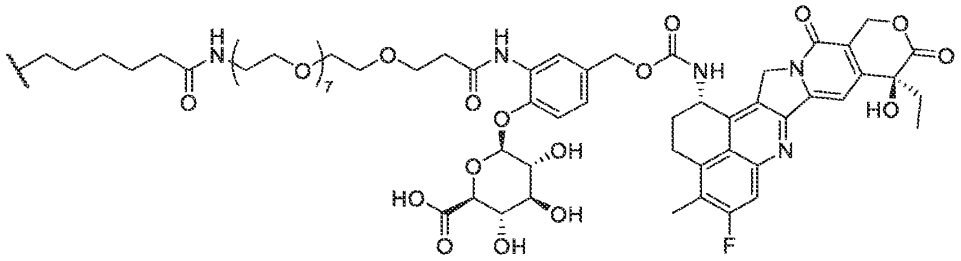




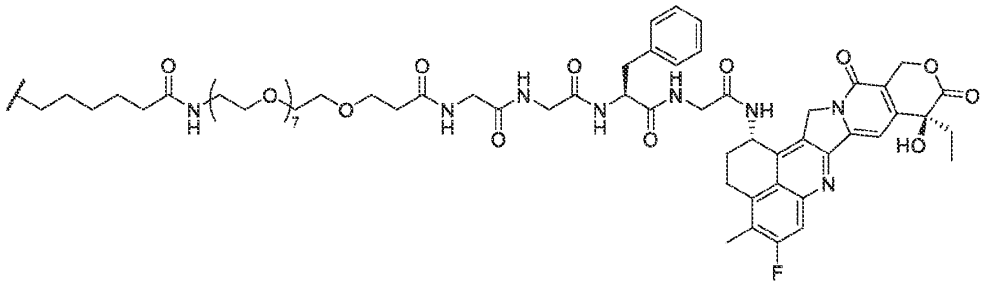




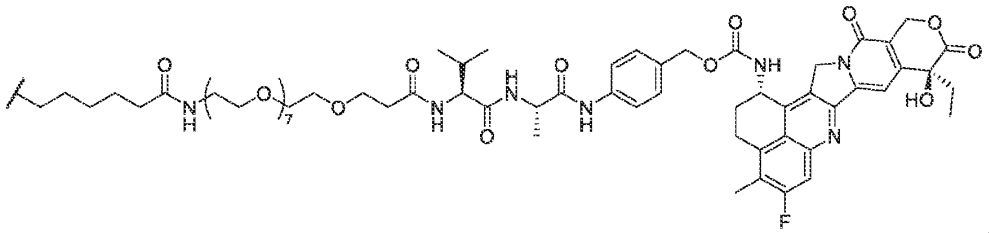
, or



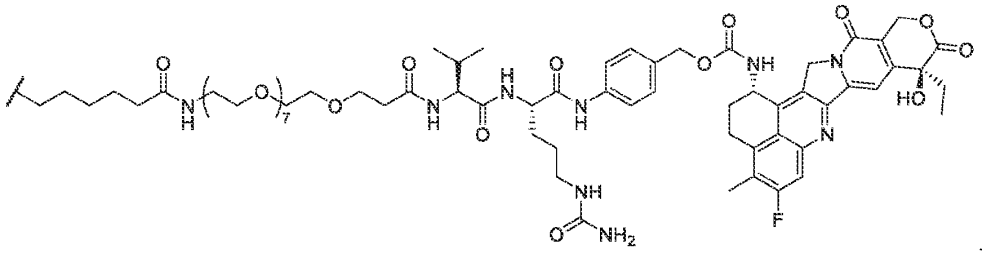
[0463] In some embodiments, a conjugate comprises



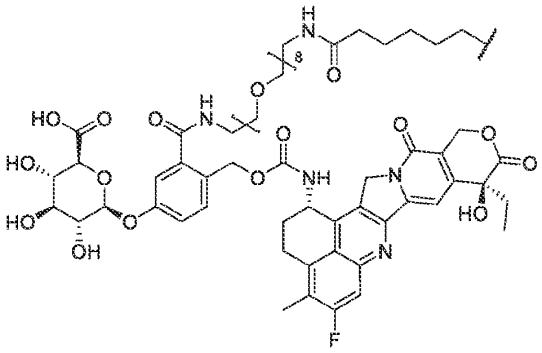
[0464] In some embodiments, a conjugate comprises



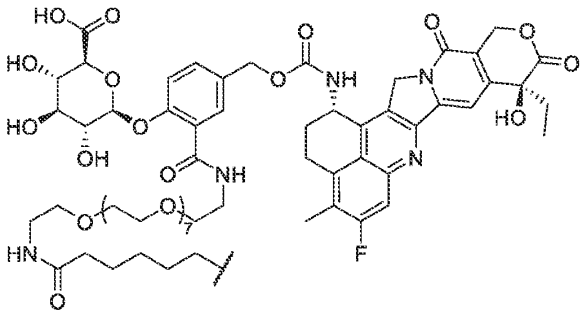
[0465] In some embodiments, a conjugate comprises



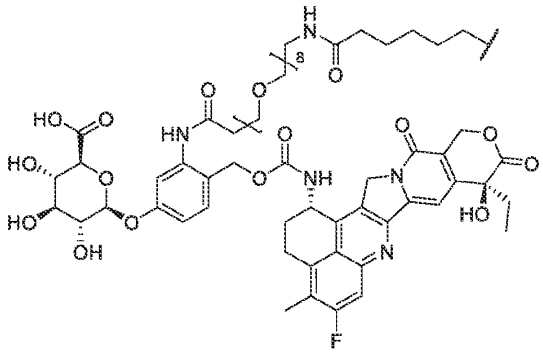
[0466] In some embodiments, a conjugate comprises



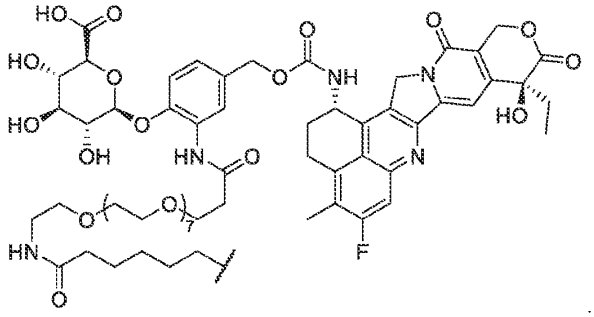
. In some embodiments, a conjugate comprises



. In some embodiments, a conjugate

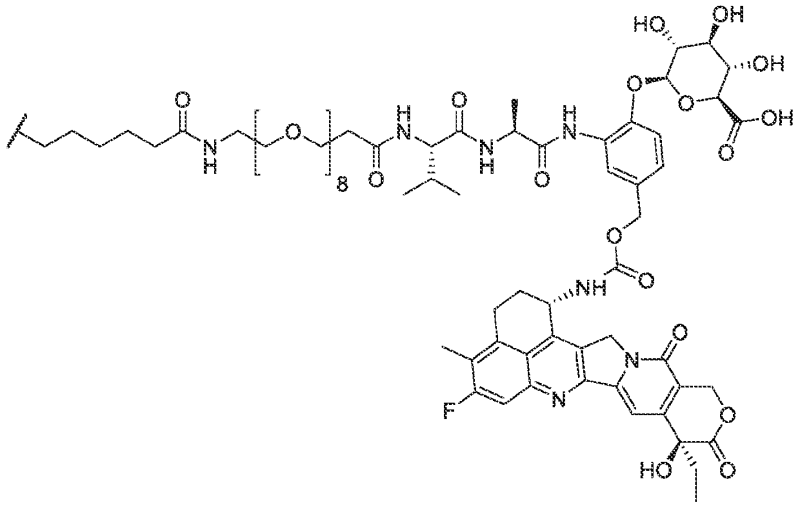


comprises . In some embodiments, a conjugate

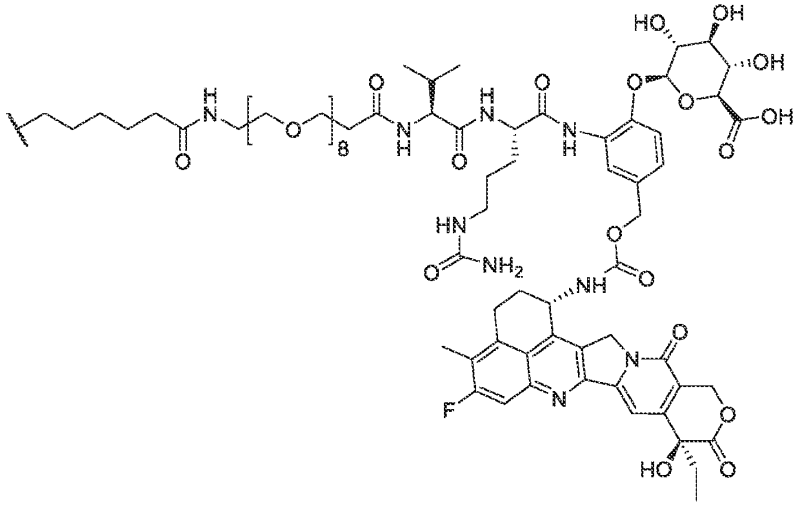


comprises .

[0467] In some embodiments, a conjugate comprises

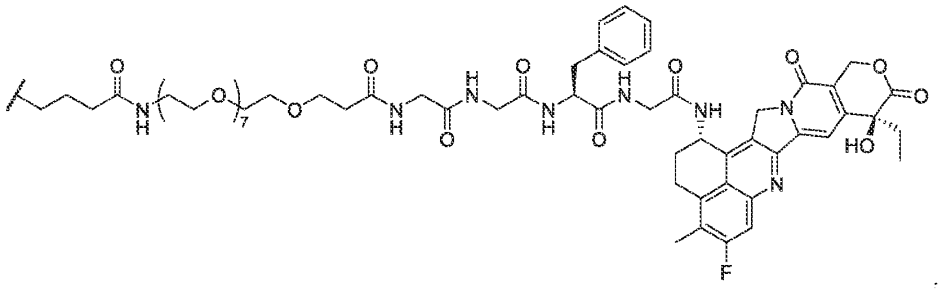


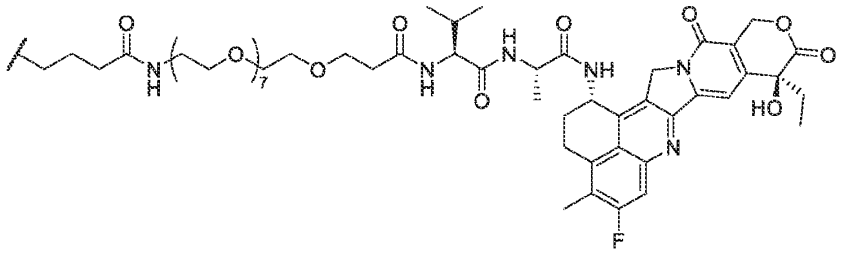
. In some embodiments, a conjugate comprises

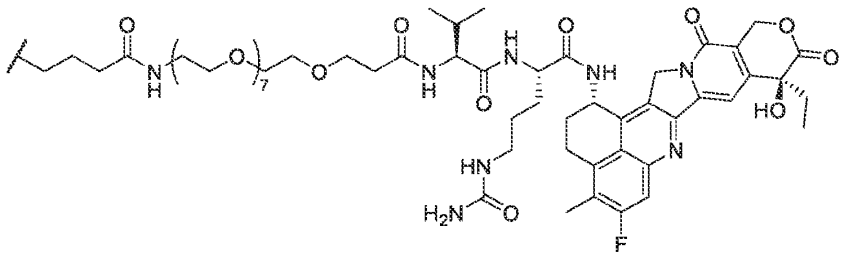


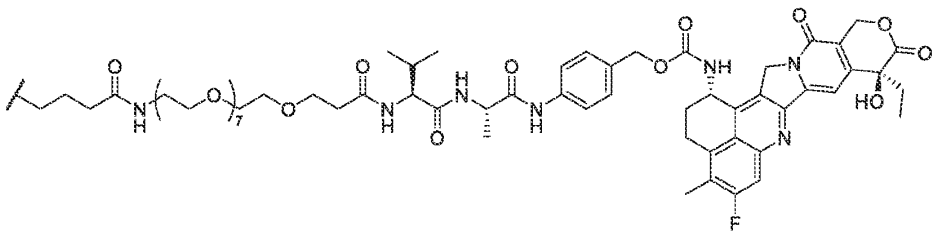
.

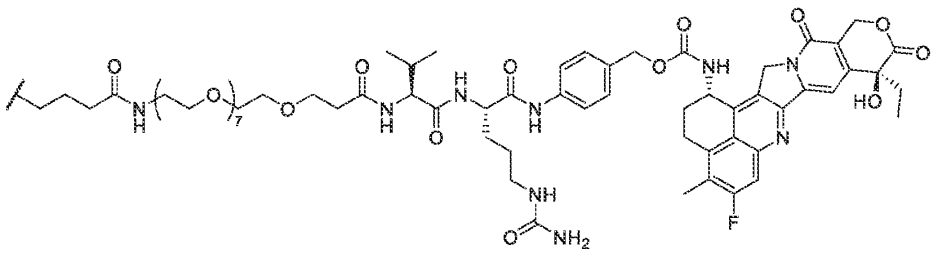
[0468] In some embodiments, a conjugate comprises

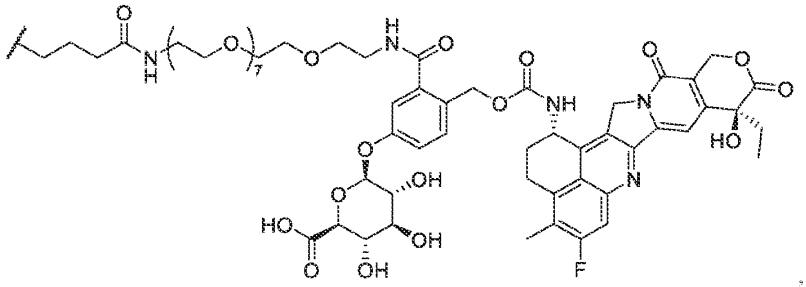


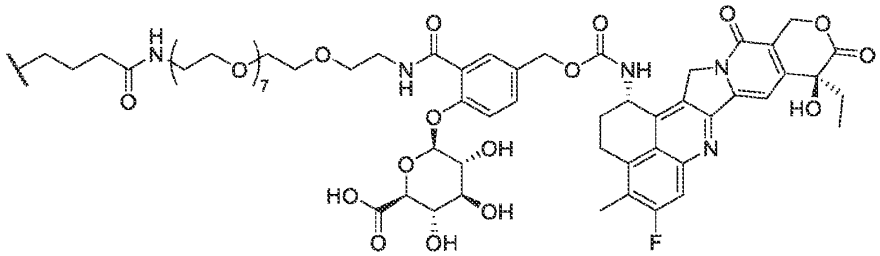


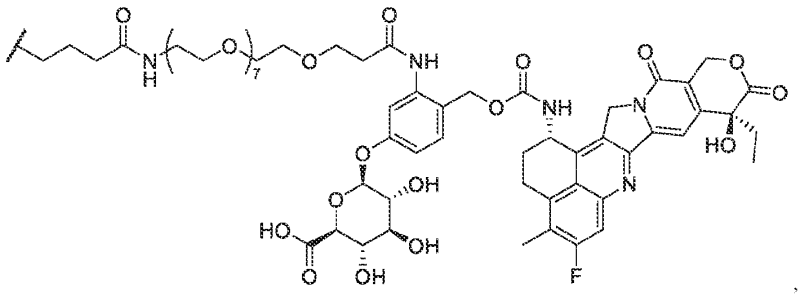




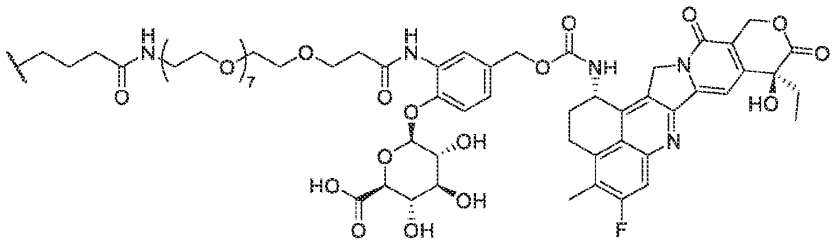




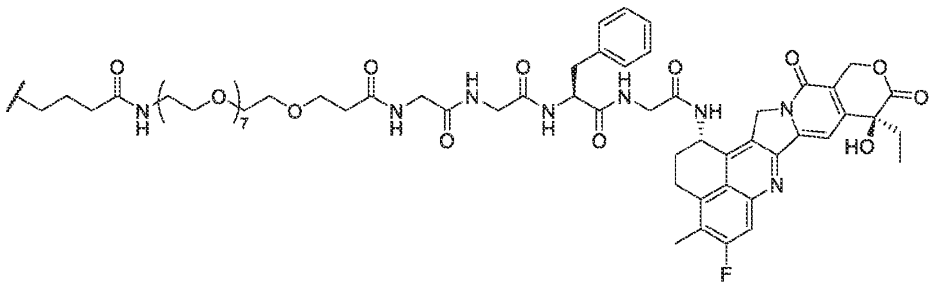




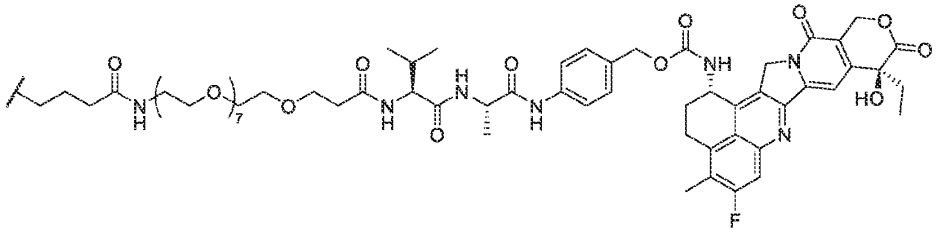
, or



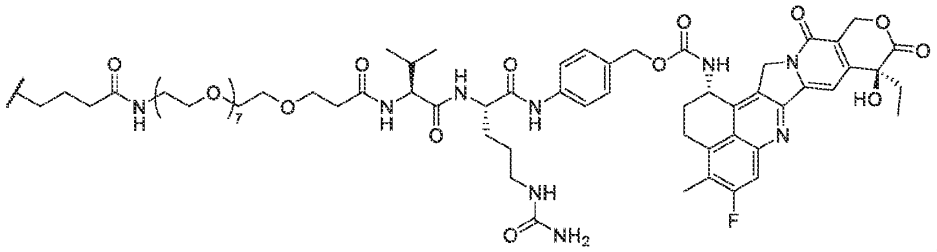
[0469] In some embodiments, a conjugate comprises



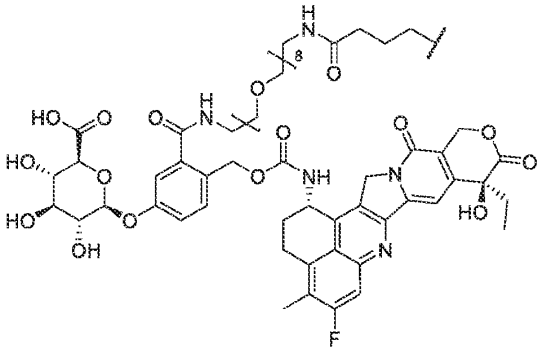
[0470] In some embodiments, a conjugate comprises



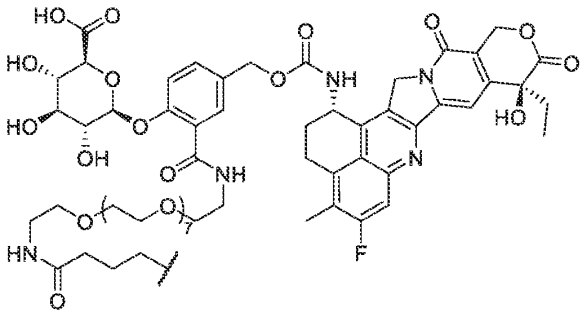
[0471] In some embodiments, a conjugate comprises



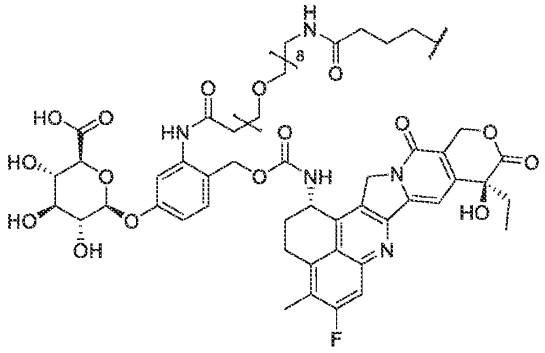
[0472] In some embodiments, a conjugate comprises



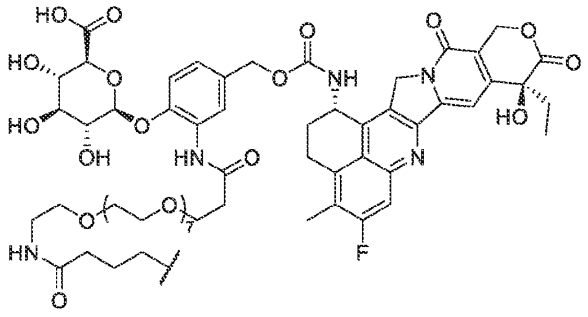
. In some embodiments, a conjugate comprises



. In some embodiments, a conjugate

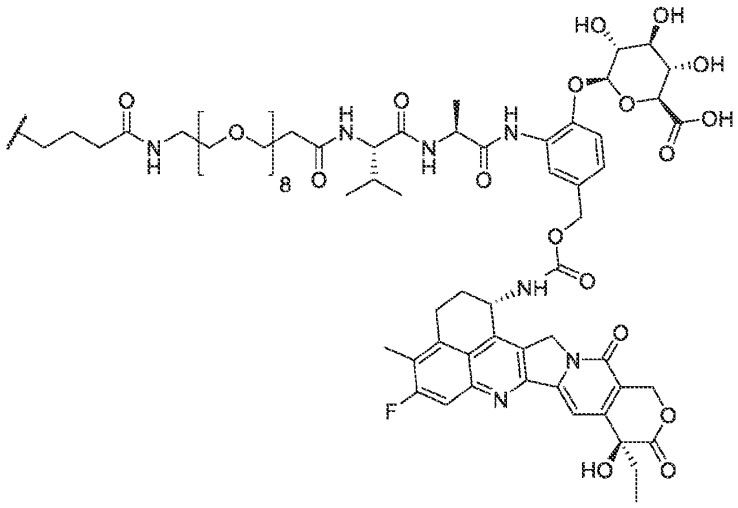


comprises . In some embodiments, a conjugate

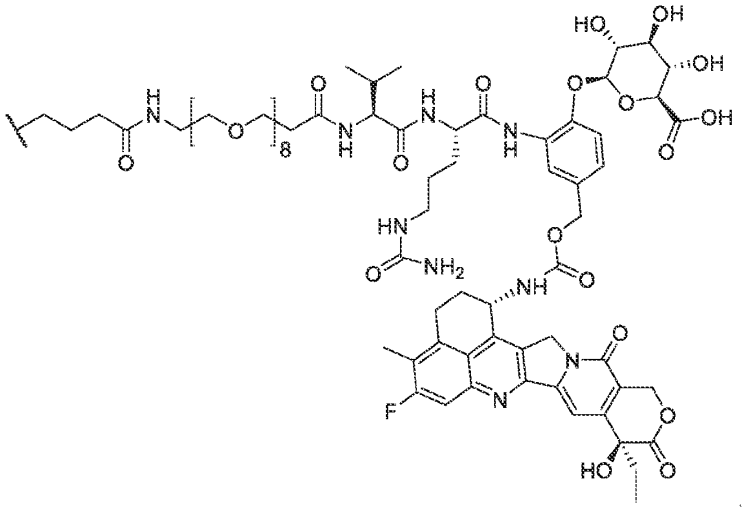


comprises .

[0473] In some embodiments, a conjugate comprises

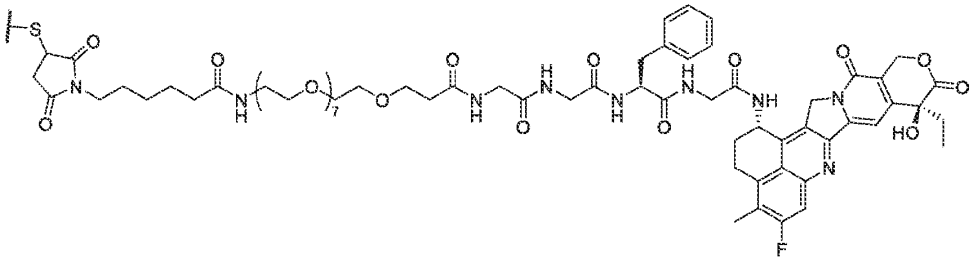


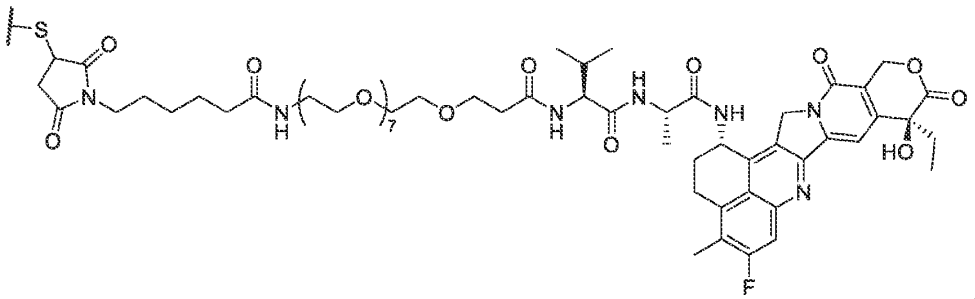
. In some embodiments, a

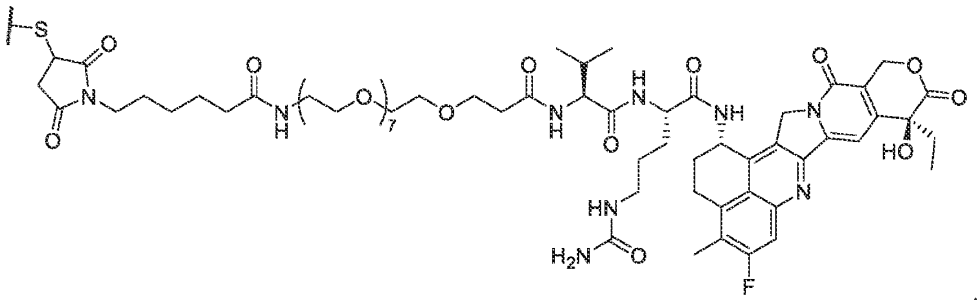


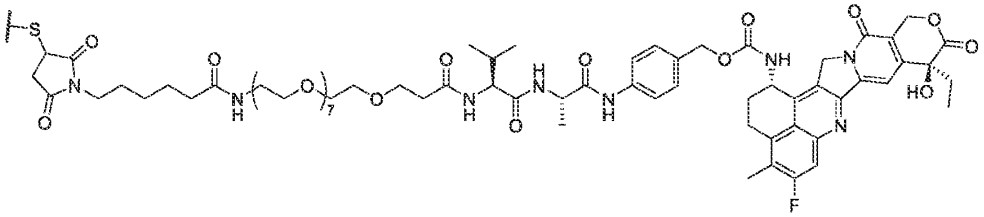
conjugate comprises

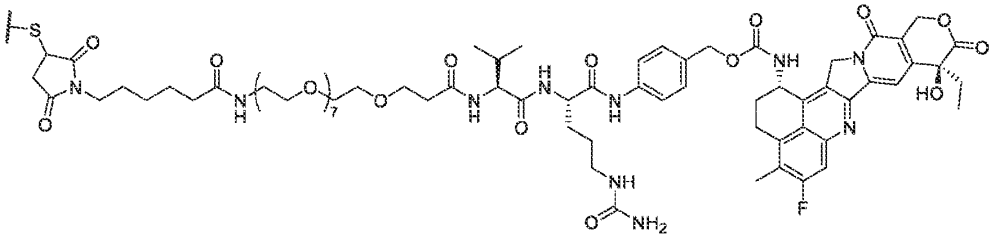
[0474] In some embodiments, a conjugate comprises

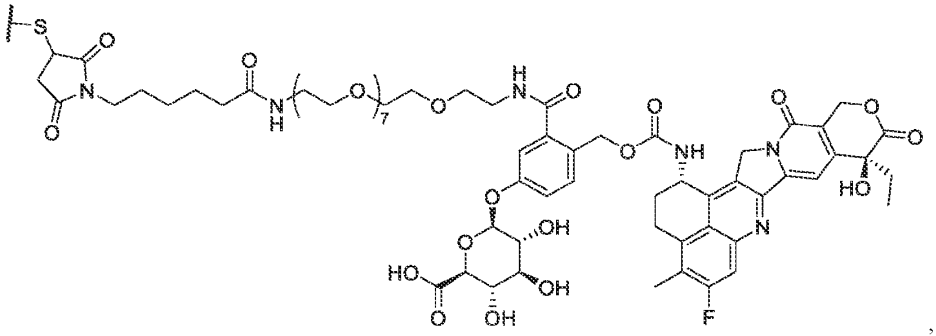


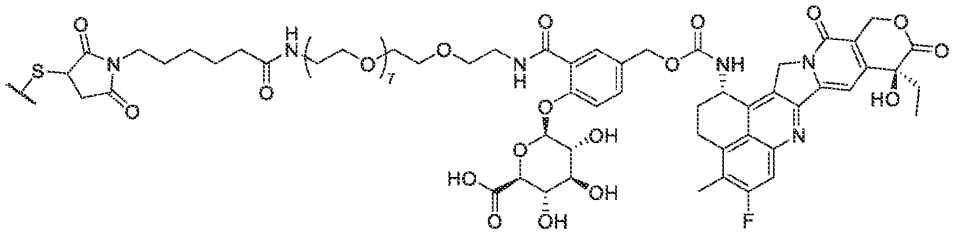


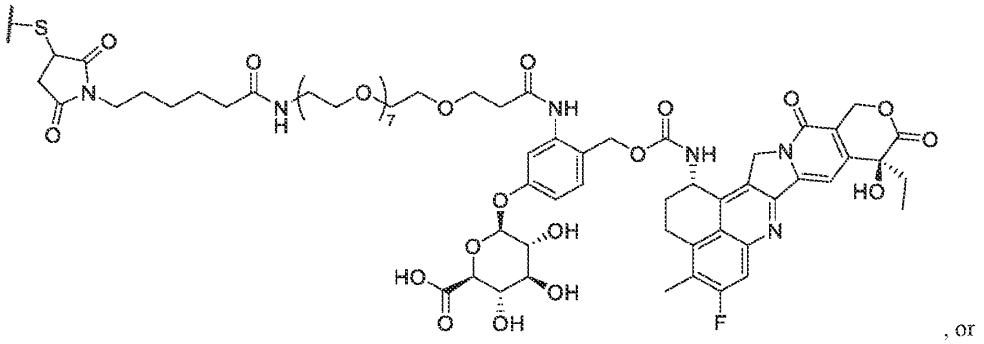




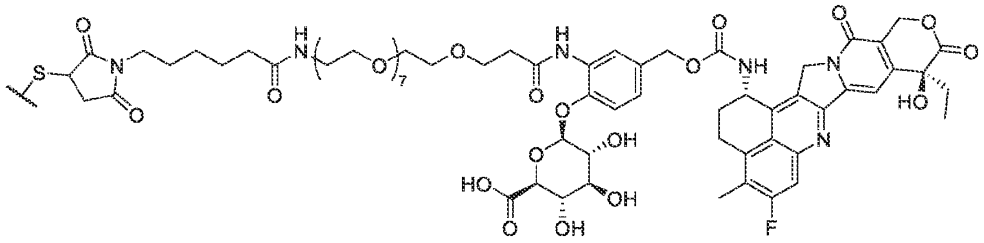




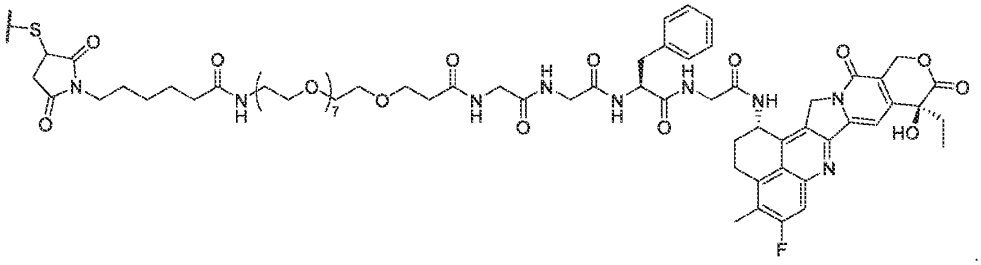




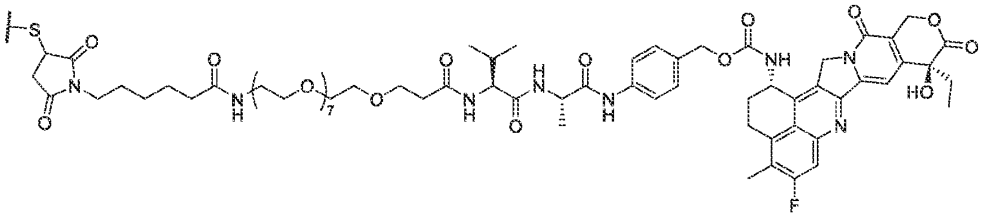
, or



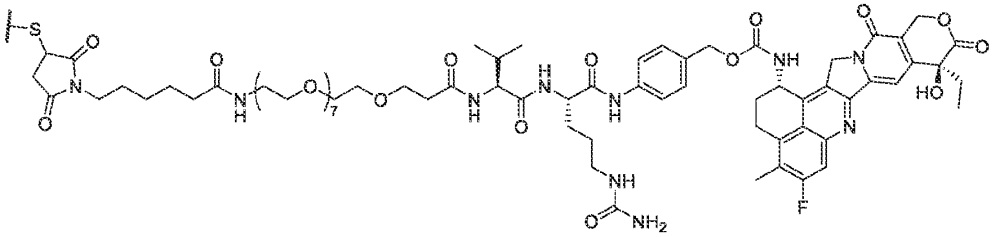
[0475] In some embodiments, a conjugate comprises



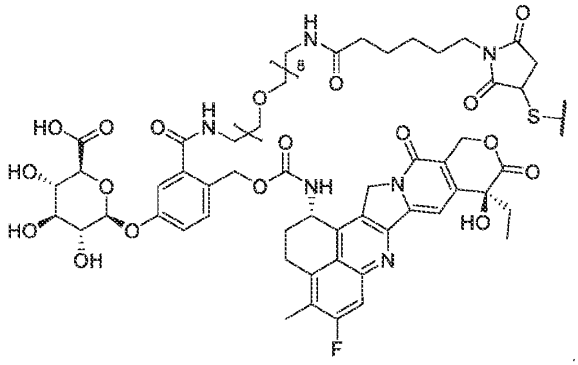
[0476] In some embodiments, a conjugate comprises



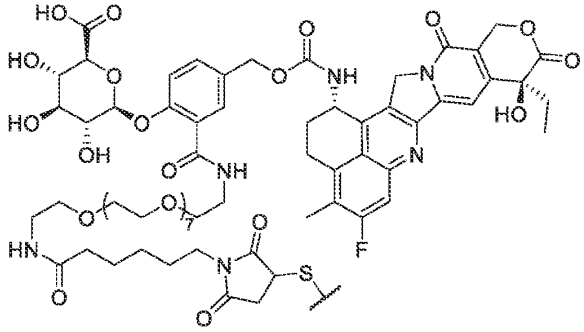
[0477] In some embodiments, a conjugate comprises



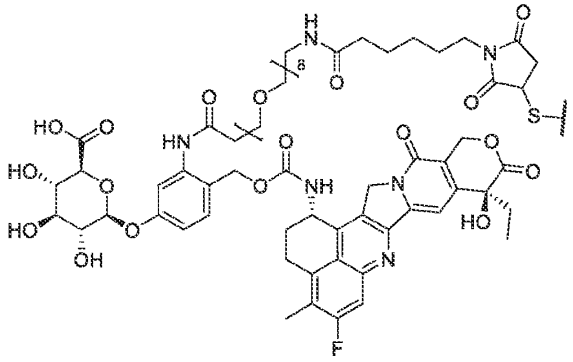
[0478] In some embodiments, a conjugate comprises



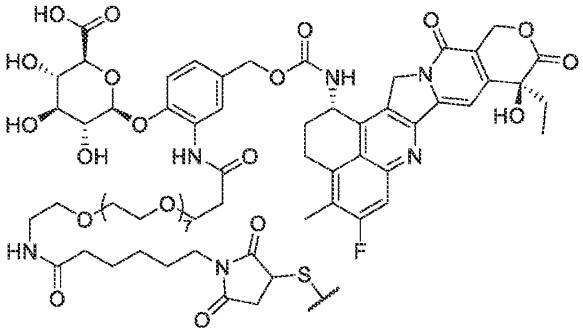
. In some embodiments, a conjugate



comprises . In some embodiments, a

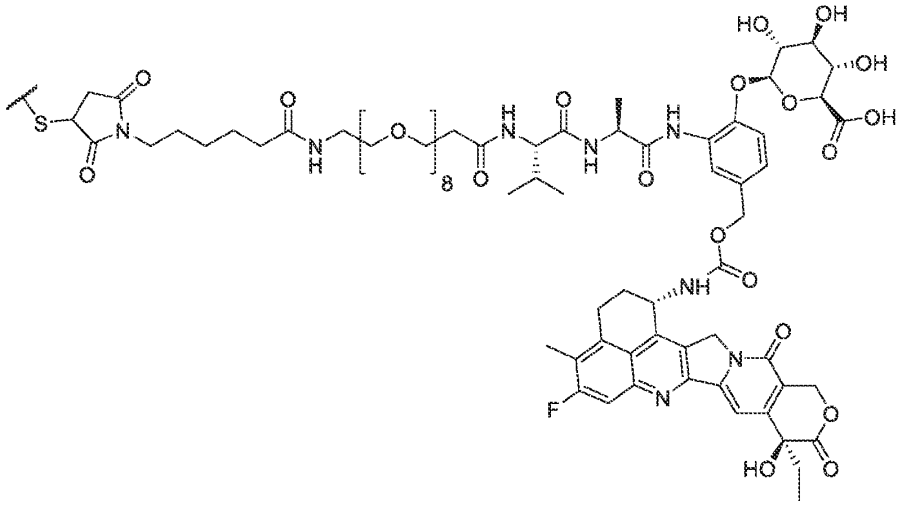


conjugate comprises . In some embodiments,

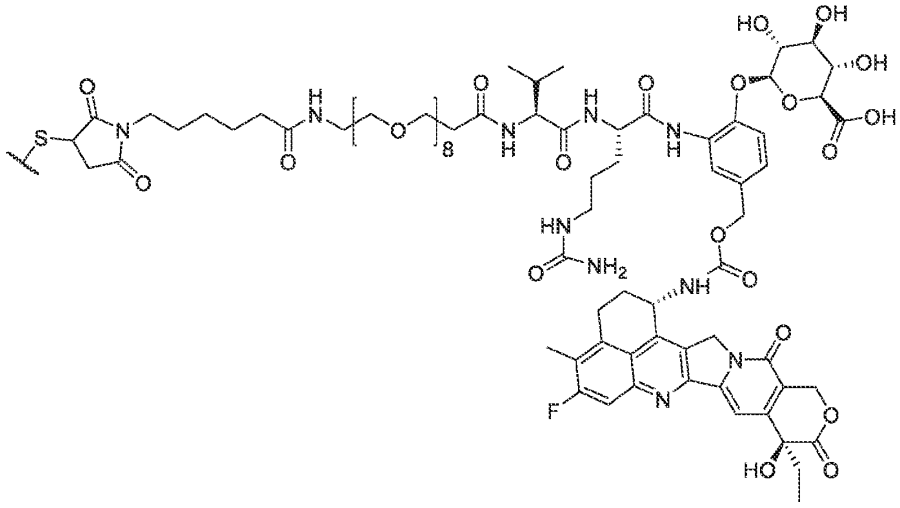


a conjugate comprises .

[0479] In some embodiments, a conjugate comprises

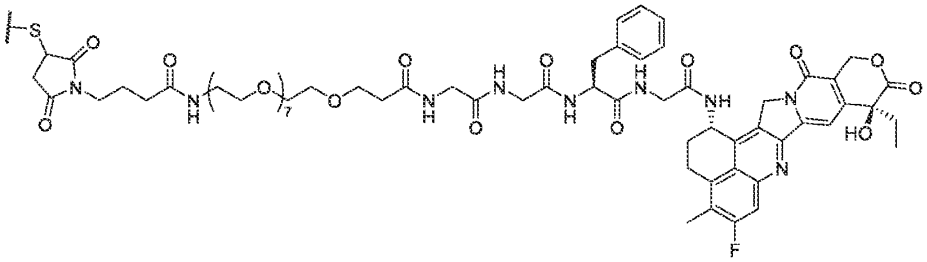


. In some embodiments, a conjugate comprises

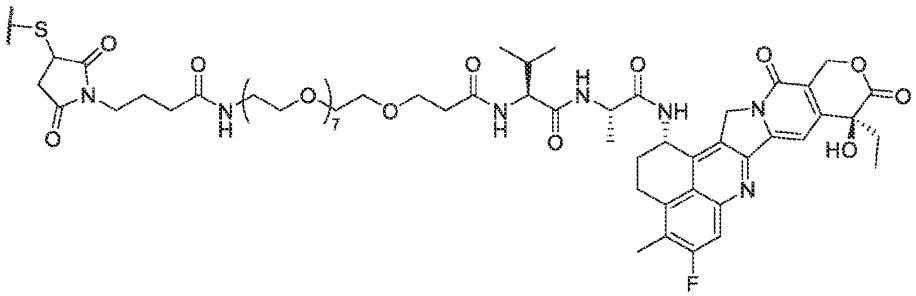


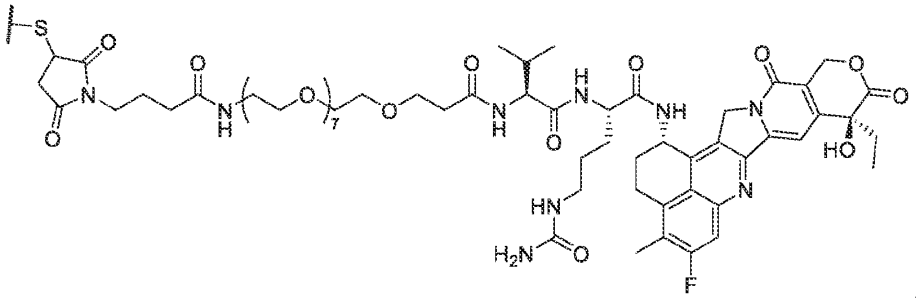
.

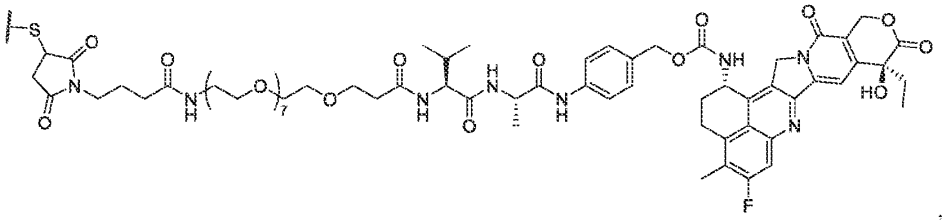
[0480] In some embodiments, a conjugate comprises

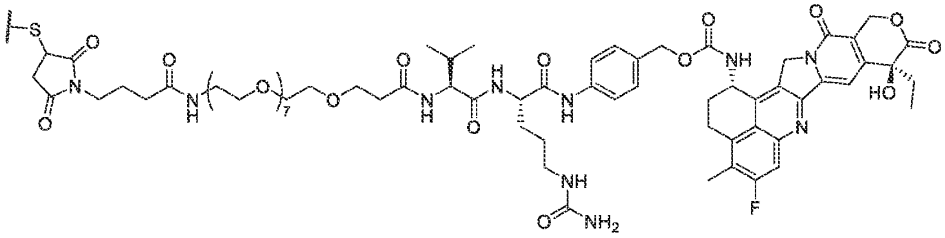


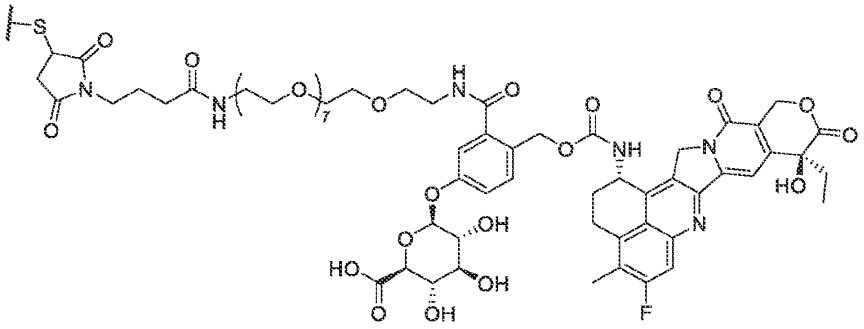
.

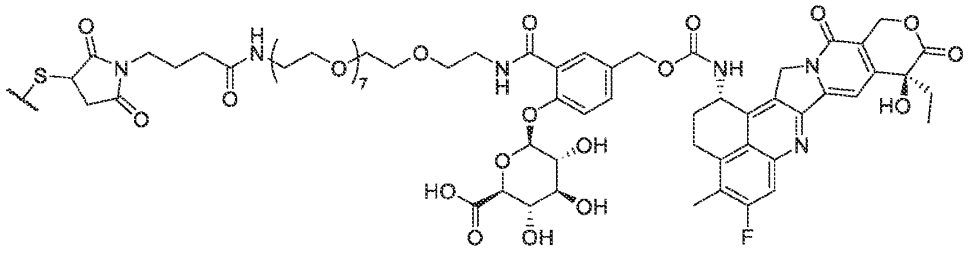


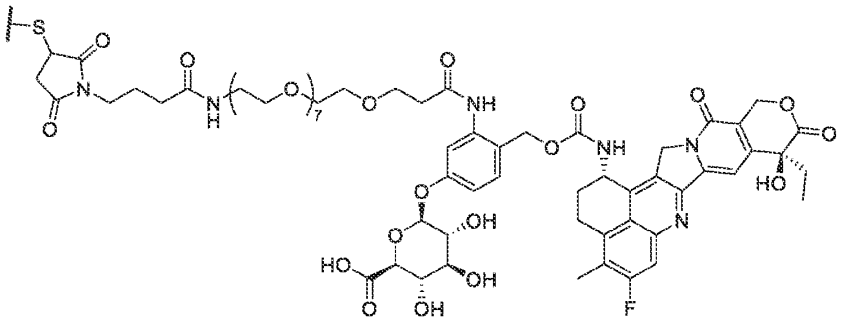




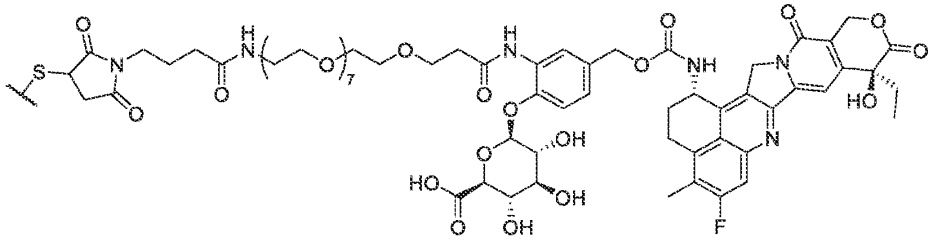




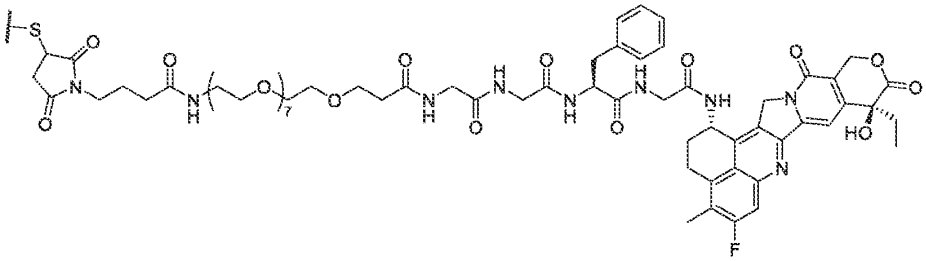




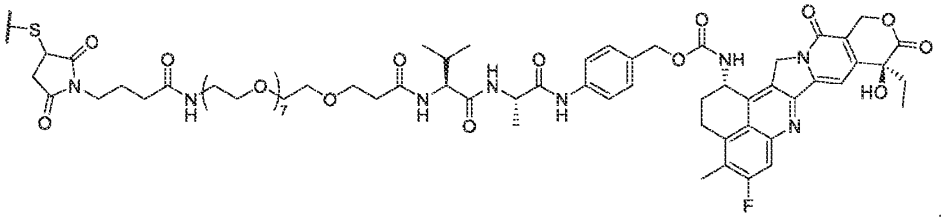
, or



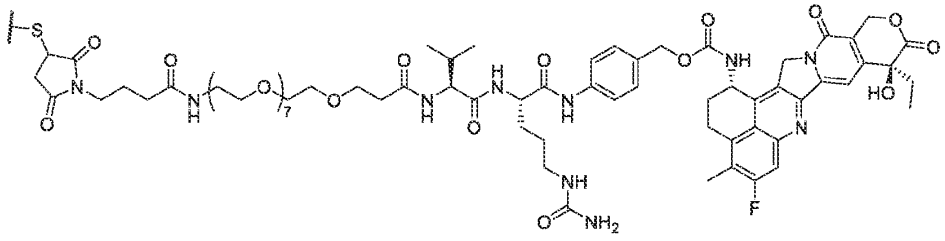
[0481] In some embodiments, a conjugate comprises



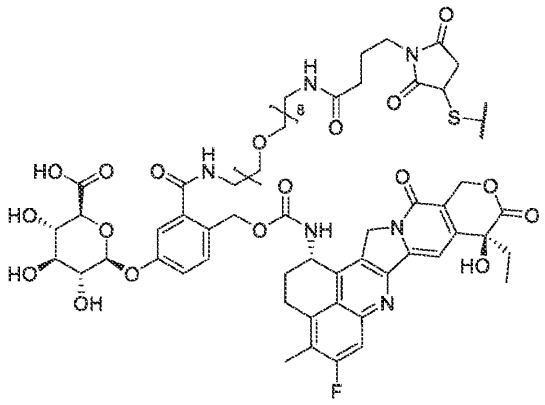
[0482] In some embodiments, a conjugate comprises



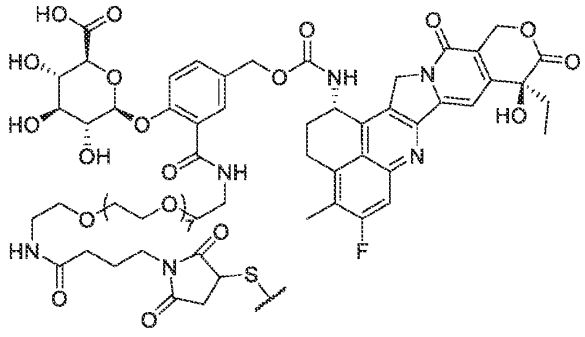
[0483] In some embodiments, a conjugate comprises



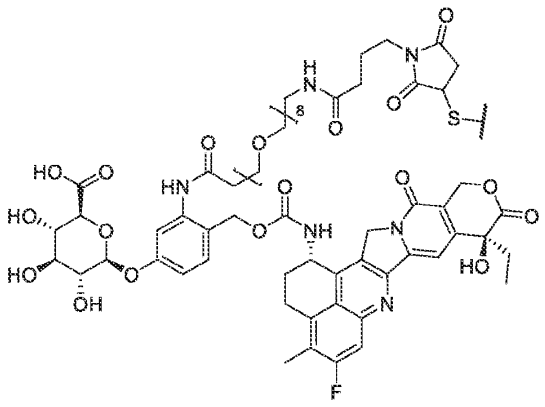
[0484] In some embodiments, a conjugate comprises



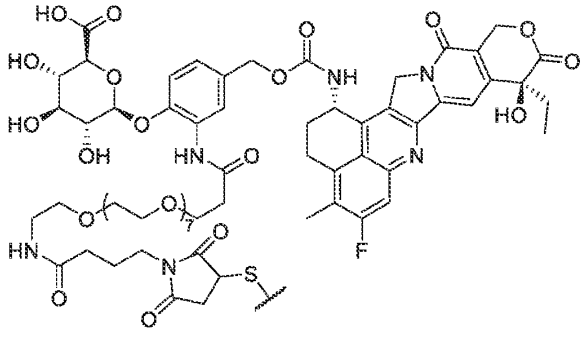
. In some embodiments, a conjugate comprises



. In some embodiments, a conjugate

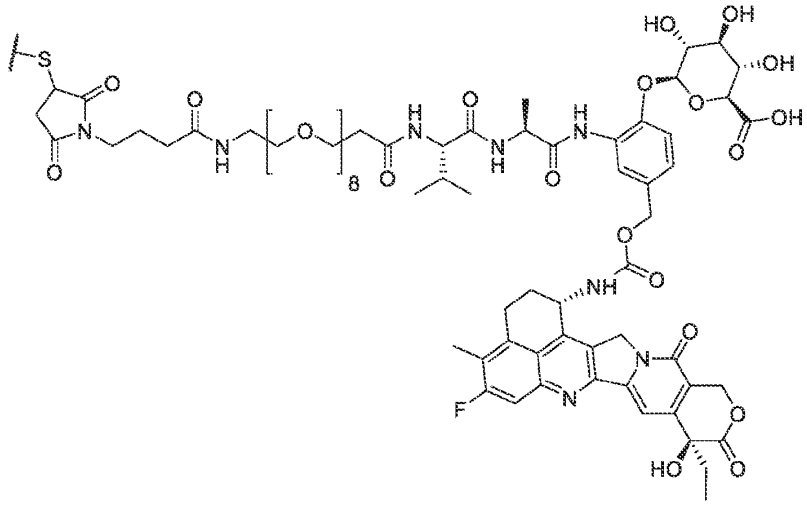


comprises . In some embodiments, a conjugate

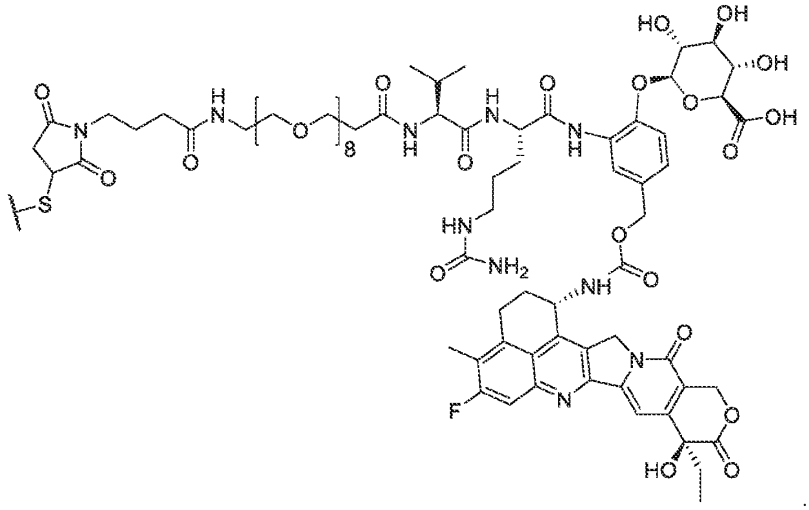


comprises .

[0485] In some embodiments, a conjugate comprises

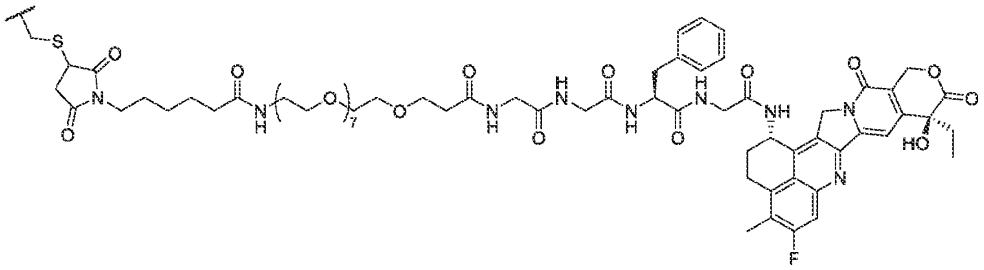


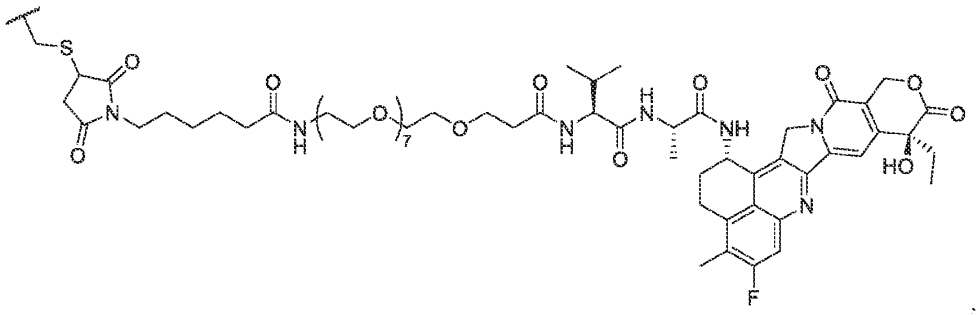
. In some embodiments, a conjugate comprises

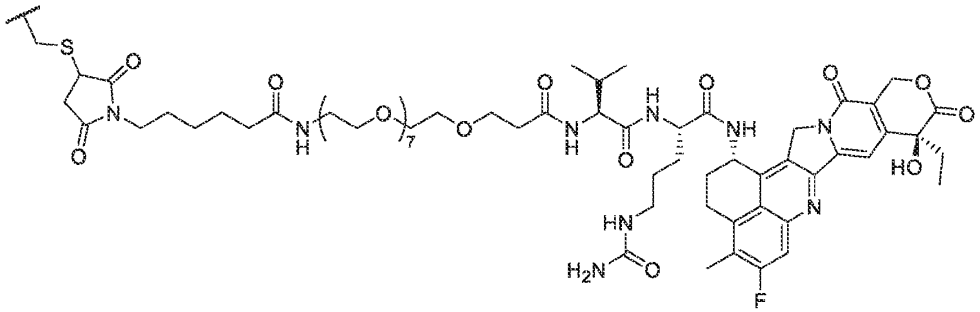


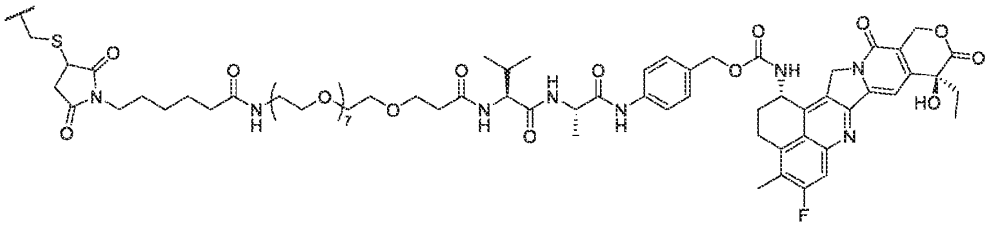
.

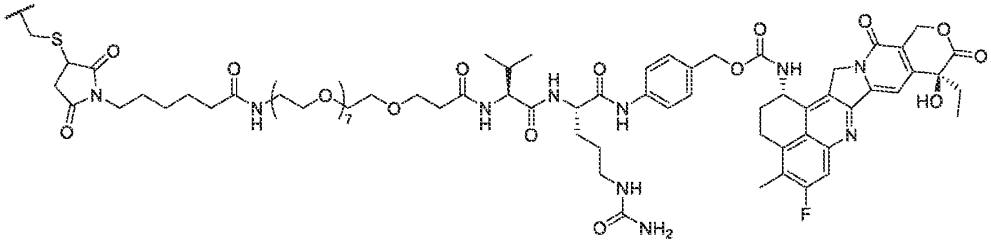
[0486] In some embodiments, a conjugate comprises

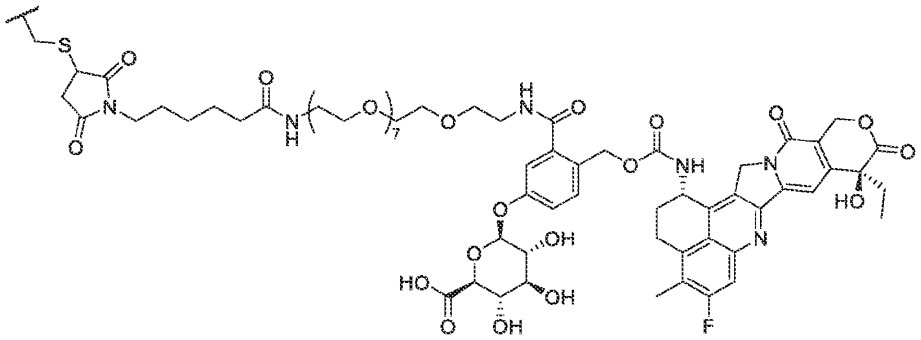


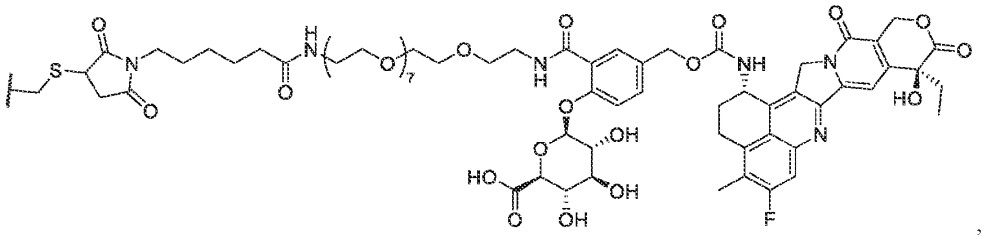


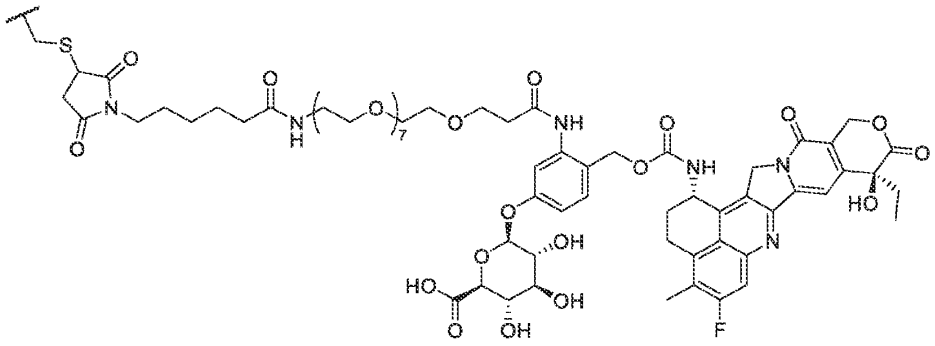




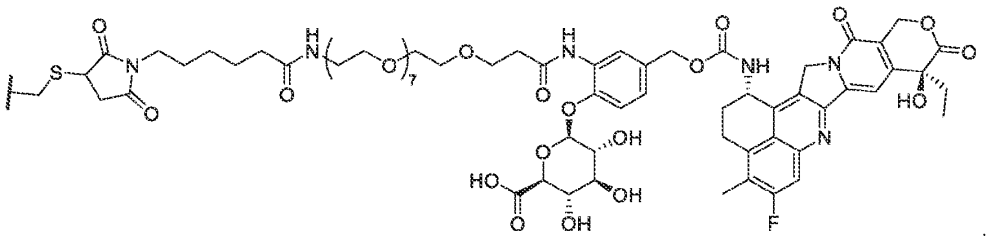




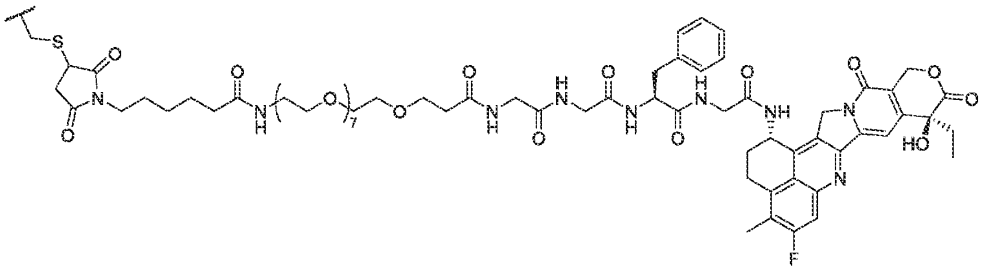




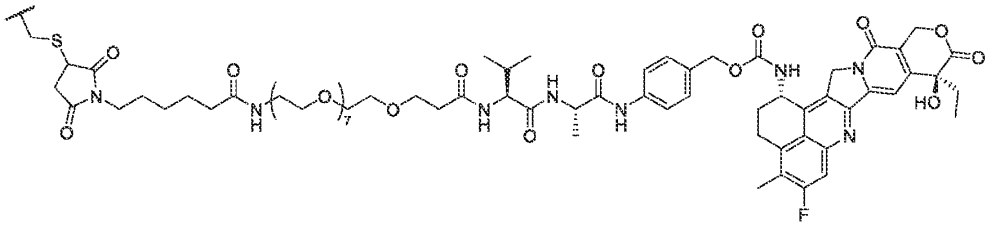
, or



[0487] In some embodiments, a conjugate comprises

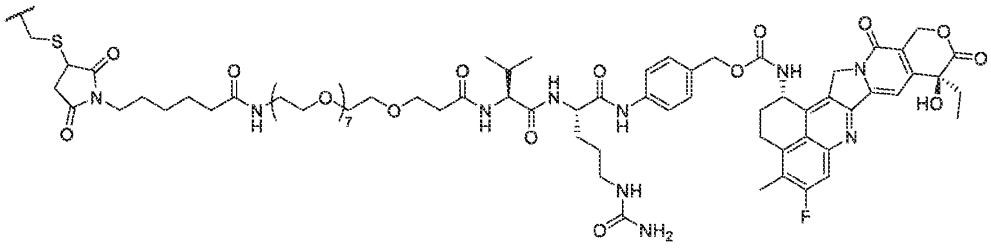


[0488] In some embodiments, a conjugate comprises



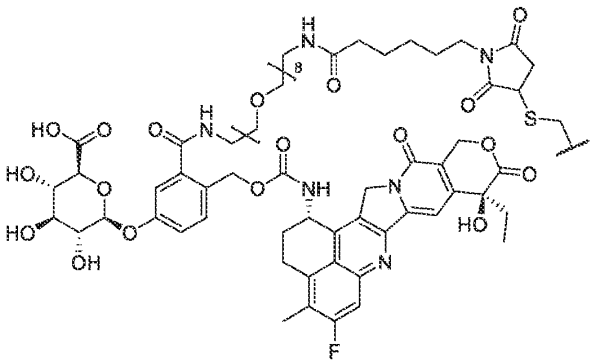
.

[0489] In some embodiments, a conjugate comprises

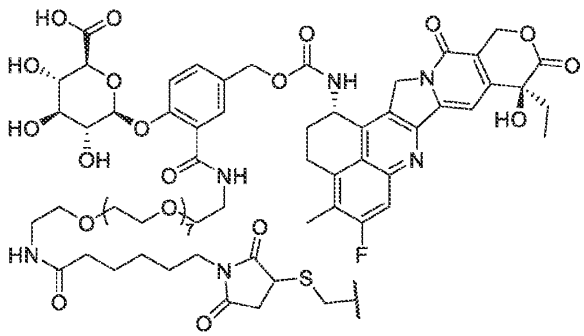


.

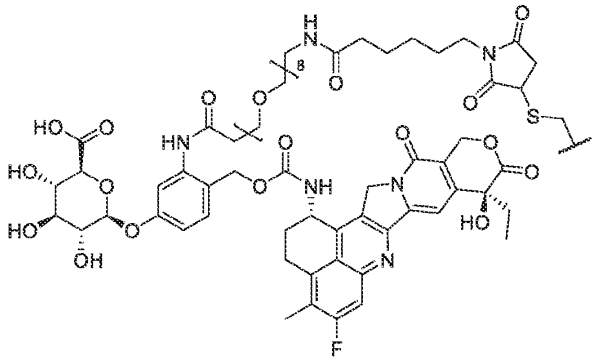
[0490] In some embodiments, a conjugate comprises



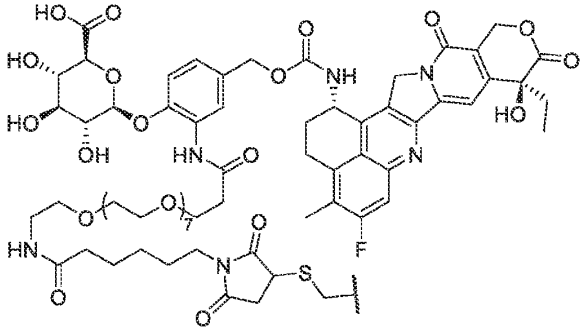
. In some embodiments, a conjugate



comprises . In some embodiments, a

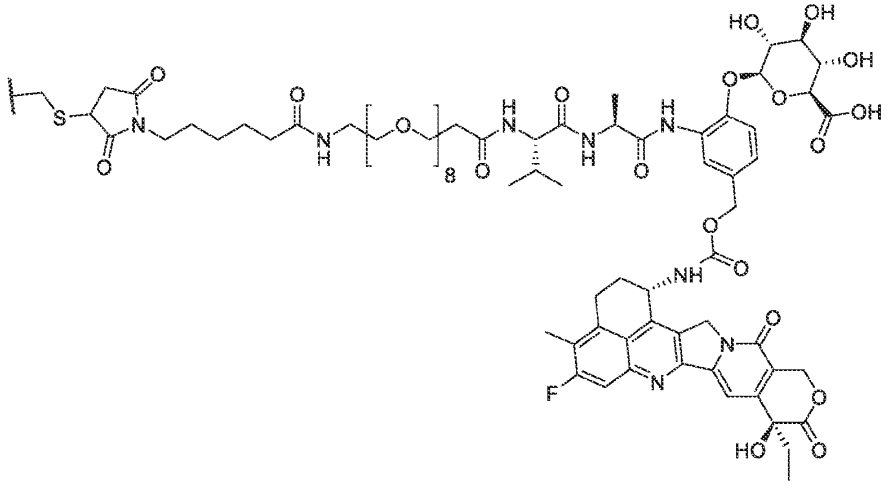


conjugate comprises . In some



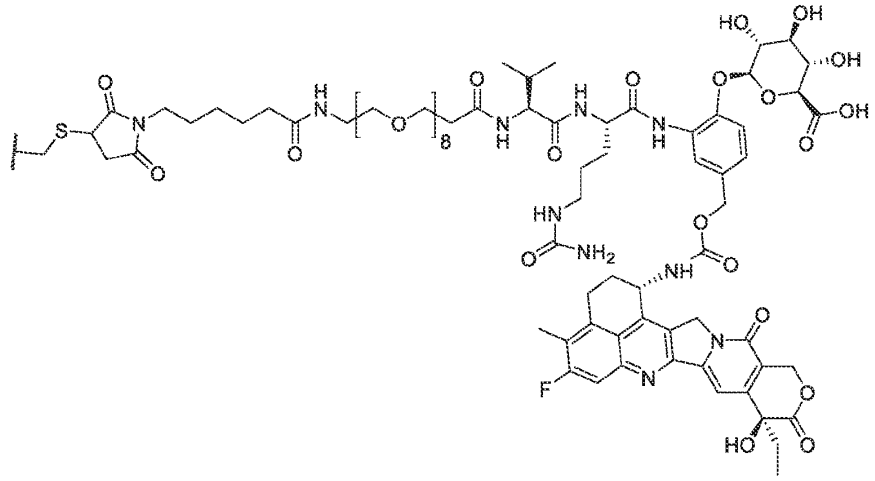
embodiments. a conjugate comprises .

[0491] In some embodiments, a conjugate comprises

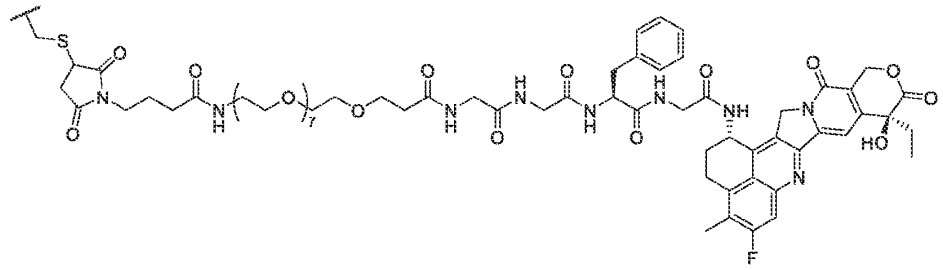


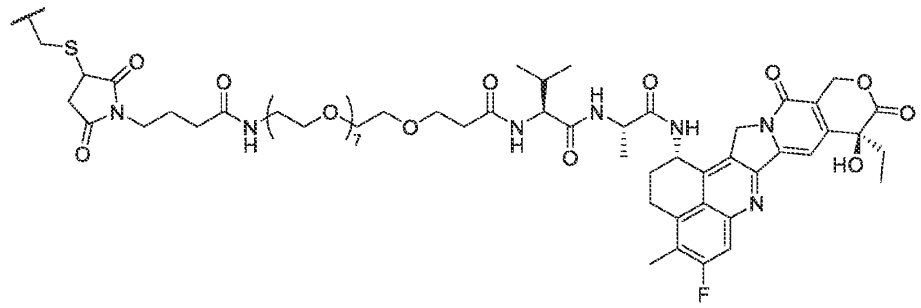
. In some

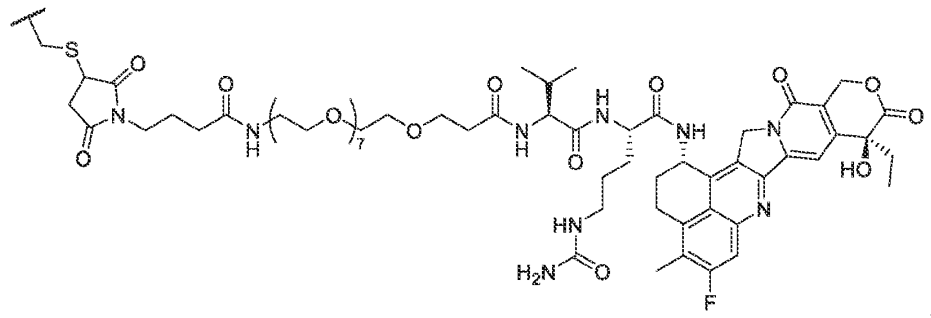
embodiments, a conjugate comprises

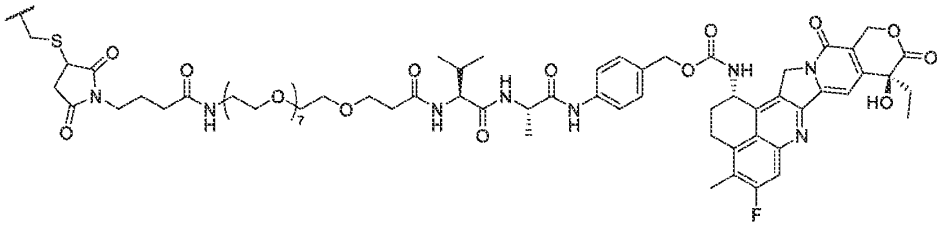


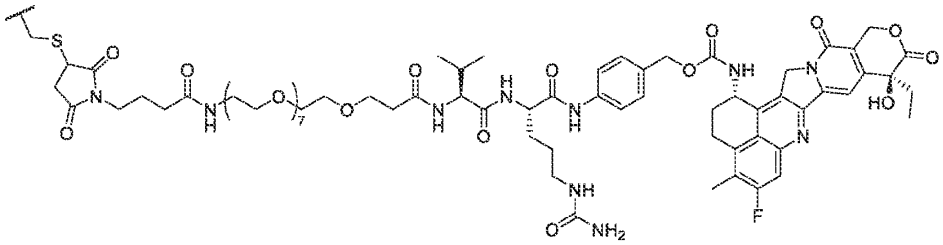
[0492] In some embodiments, a conjugate comprises

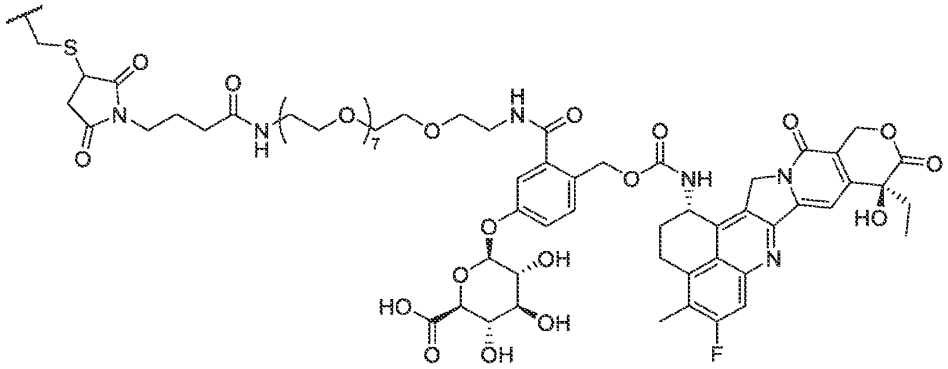


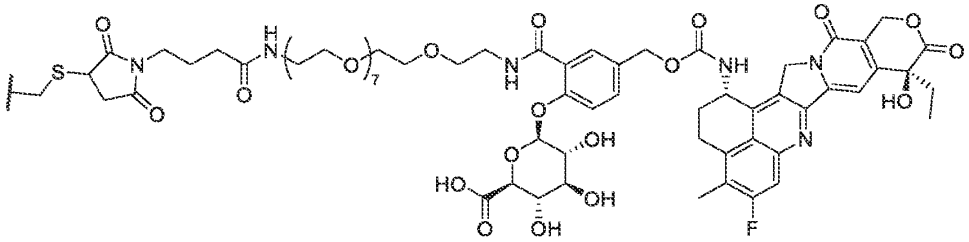


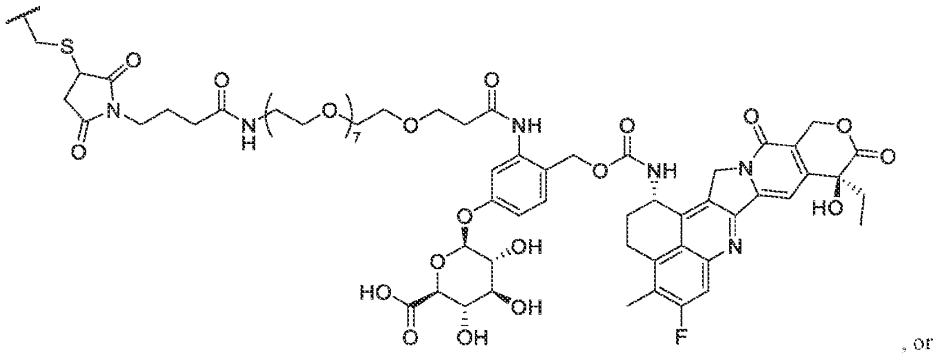




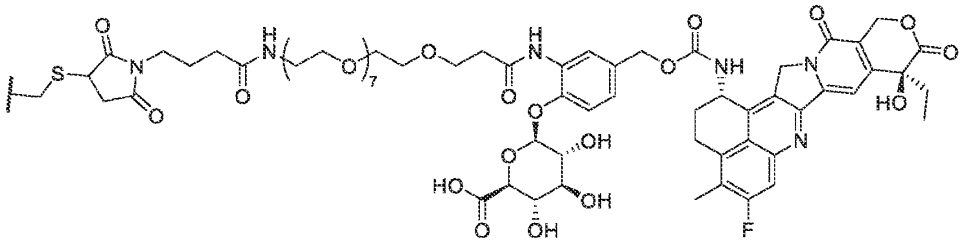




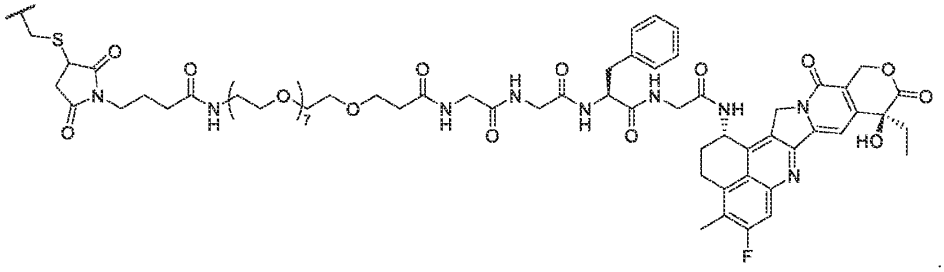




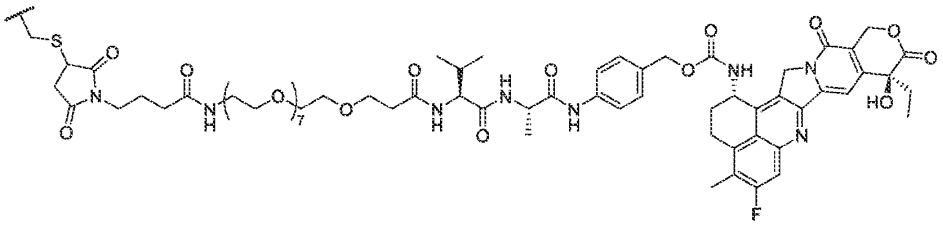
, or



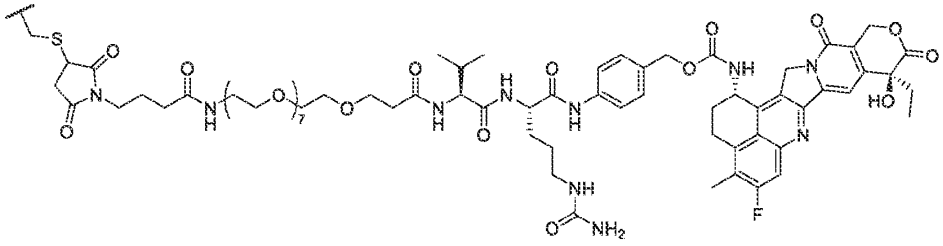
[0493] In some embodiments, a conjugate comprises



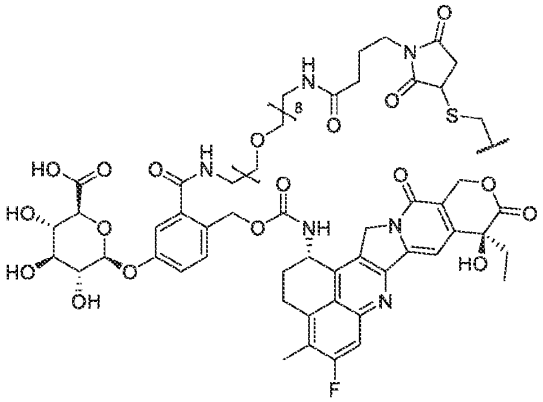
[0494] In some embodiments, a conjugate comprises



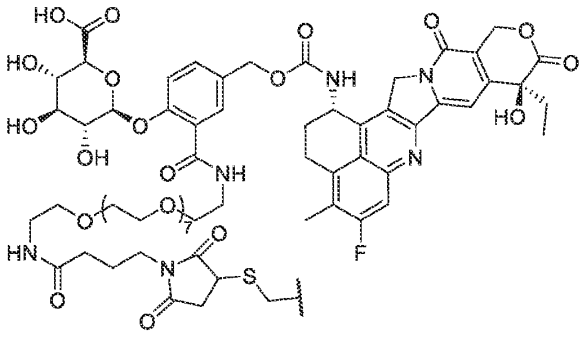
[0495] In some embodiments, a conjugate comprises



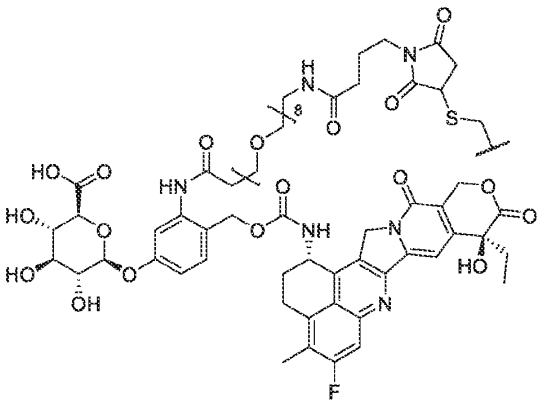
[0496] In some embodiments, a conjugate comprises



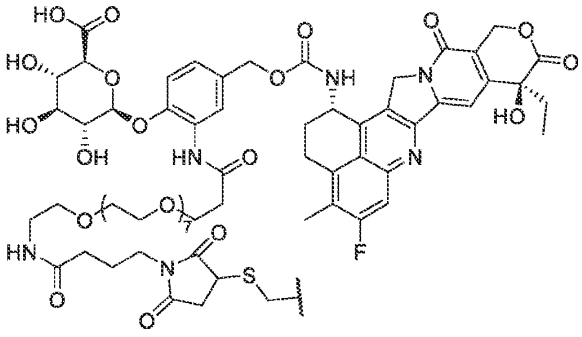
. In some embodiments, a conjugate comprises



. In some embodiments, a conjugate comprises

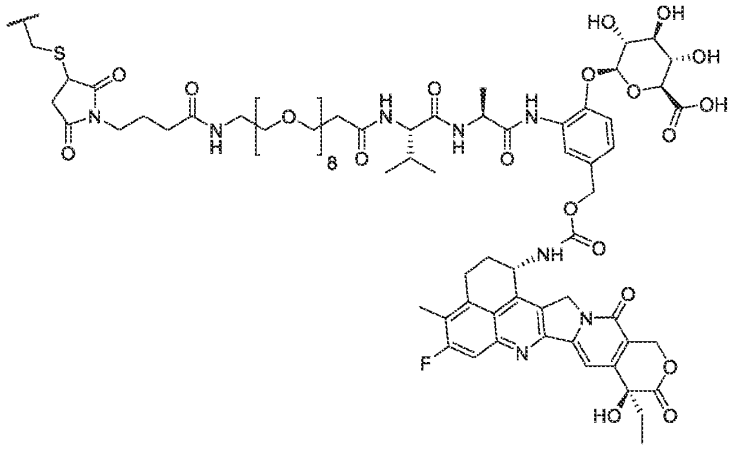


. In some embodiments, a conjugate comprises

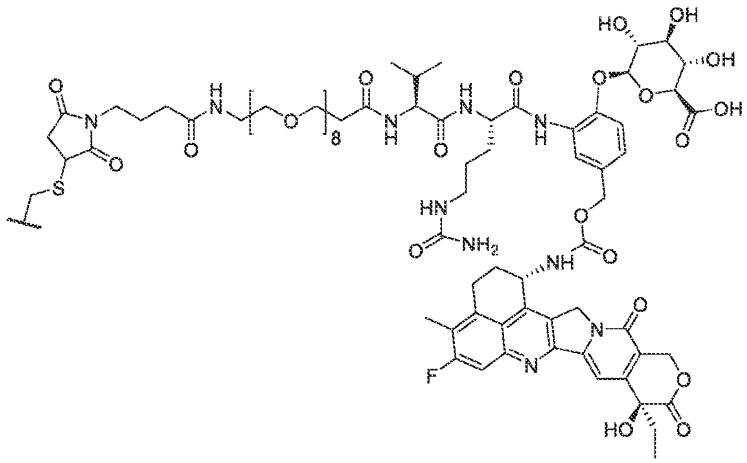


.

[0497] In some embodiments, a conjugate comprises

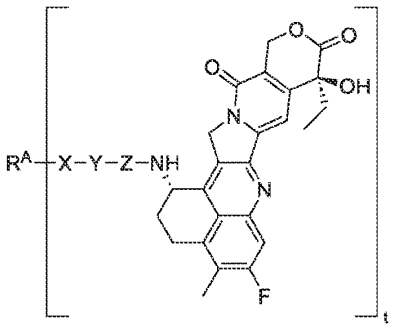


. In some embodiments, a

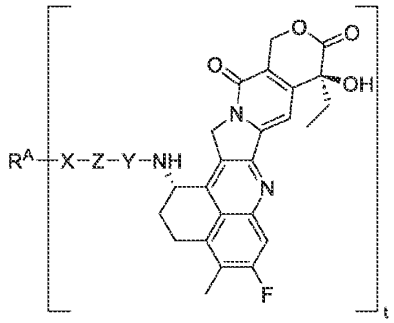


conjugate comprises

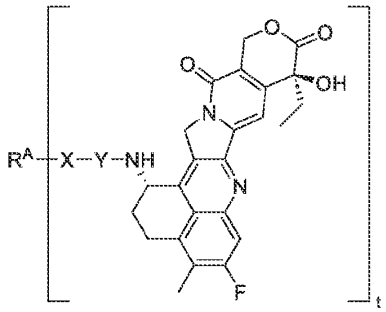
[0498] In some embodiments, a conjugate has a structure of formula I-A, I-B, or I-C:



I-A



I-B



I-C

or a pharmaceutically acceptable salt thereof, wherein:

R^A is a target binding moiety;

t is an integer between 1 and 16;

X is a moiety that results from the reaction of the unbound linker and a group present on R^A;

Y is or comprises $-L^C-$; and

Z is or comprises $-(CH_2CH_2O)n-$ wherein n is 0-24.

[0499] As described herein, in some embodiments, n is 1-24.

[0500] In some embodiments, X is or comprises L^{RX} as described herein. In some embodiments, X is $-L^I-L^{RX}-$ wherein each of L^I and L^{RX} is independently as described herein. In some embodiments, Y is L^C as described herein. In some embodiments, Z is $-(CH_2CH_2O)n-$ wherein n is as described herein. In some embodiments, Z is $-L^X-(CH_2CH_2O)n-L^{ZP}-$, wherein each of L^X, n and L^{ZP} is independently as described herein. In some embodiments, X is a moiety that results from a reaction of an unbound linker and a group present on R^A. In some embodiments, X is a moiety that results from a reaction of an unbound linker and a group present on an antibody or antigen-binding fragment thereof.

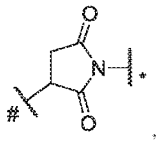
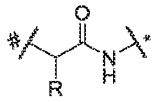
[0501] In some embodiments, X is a moiety that results from a reaction between an unbound linker and a group present on an arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, histidine, lysine, serine, threonine, tryptophan, or tyrosine within an antibody or antigen-binding fragment thereof. In some embodiments, X is a moiety that results from a reaction between an unbound linker and a group present on a cysteine, lysine, serine, threonine, or tyrosine within an antibody or antigen-binding fragment thereof. In some embodiments, X is a moiety that results from a reaction between an unbound linker and a group present on a cysteine within an antibody or antigen-binding fragment thereof. In some embodiments, X is a moiety that results from a reaction between an unbound linker and a group present on a lysine within an antibody or antigen-binding fragment thereof. In some embodiments, X is a moiety that results from a reaction between an unbound linker and a thiol, amine, acid, or hydroxyl group within an antibody or antigen-binding fragment thereof. In some embodiments, X is a moiety that results from a reaction between an unbound linker and a thiol within an antibody or antigen-binding fragment thereof. In some embodiments, X is a moiety that results from a reaction between an unbound linker and an amine within an antibody or antigen-binding fragment thereof. In some embodiments, X is a moiety that results from a reaction between an unbound linker and a hydroxyl within an antibody or antigen-binding

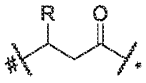
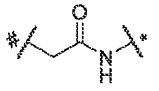
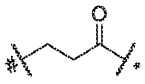
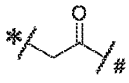
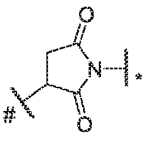
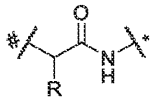
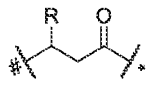
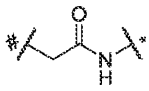
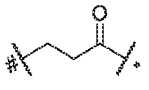
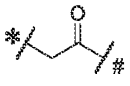
fragment thereof.

[0502] In some embodiments, X is a moiety that results from a reaction between an unbound linker and a group present on an antibody or antigen-binding fragment thereof, wherein the reaction is a conjugation with activated esters, sulfonyl chlorides, or isothiocyanates; reductive alkylation; aza-Michael addition; azetidinone chemistry; benzoyl fluoride-based chemistry; or click chemistry. Examples are provided in, e.g., Sletten and Bertozzi. Agew. Chem. Int. Ed. Engl. 2009, 48(38), 6974-6998.

[0503] In some embodiments, X is a moiety that results from a reaction between an unbound linker comprising a moiety capable of reacting with an arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, histidine, lysine, serine, threonine, tryptophan, or tyrosine; and a group present on an antibody or antigen-binding fragment thereof. In some embodiments, X is a moiety that results from a reaction between an unbound linker comprising a moiety capable of reacting with a cysteine, lysine, serine, threonine, or tyrosine; and a group present on an antibody or antigen-binding fragment thereof. In some embodiments X is a moiety that results from a reaction between an unbound linker comprising a moiety capable of reacting with a cysteine; and a group present on an antibody or antigen-binding fragment thereof. In some embodiments, X is a moiety that results from a reaction between an unbound linker comprising a moiety capable of reacting with a lysine; and a group present on an antibody or antigen-binding fragment thereof.

[0504] In some embodiments, a moiety capable of reacting with a cysteine is a maleimide, haloacetamide (e.g., fluoroacetamide, chloroacetamide, bromoacetamide, or iodoacetamide), or a Michael acceptor (e.g., α,β-unsaturated carbonyl, such as acrylamide or α,β-unsaturated ketone). In some embodiments, a moiety capable of reacting with a cysteine is a maleimide. In some embodiments, a moiety capable of reacting with a cysteine is a haloacetamide (e.g., fluoroacetamide, chloroacetamide, bromoacetamide, or iodoacetamide). In some embodiments, a moiety capable of reacting with a cysteine is a Michael acceptor (e.g., α,β-unsaturated carbonyl, such as acrylamide or α,β-unsaturated ketone). In some embodiments, a moiety capable of reacting with a cysteine is a chloroacetamide. In some embodiments, a moiety capable of reacting with a cysteine is a bromoacetamide. In some embodiments, a moiety capable of reacting with a cysteine is an iodoacetamide. In some embodiments, a moiety capable of reacting with a cysteine is acrylamide. In some embodiments, a moiety capable of reacting with a cysteine is an α,β-unsaturated ketone.

[0505] In some embodiments, a linker comprises  , wherein # represents a point of attachment to a target binding moiety, and * represents a point of attachment to the remainder of the linker. In some embodiments, a linker comprises  , wherein: R is as described herein, # represents a point of attachment to a target binding moiety, and * represents a point of attachment to the

remainder of the linker. In some embodiments, a linker comprises  , wherein R is as defined herein, # represents a point of attachment to a target binding moiety, * represents a point of attachment to the remainder of the linker. In some embodiments, a linker comprises  , wherein # represents a point of attachment to a target binding moiety, and * represents a point of attachment to the remainder of the linker. In some embodiments, a linker  , wherein # represents a point of attachment to a target binding moiety, and * represents a point of attachment to the remainder of the linker. In some embodiments, a linker comprises  , wherein * represents a point of attachment to a target binding moiety, and # represents a point of attachment to the remainder of the linker. In some embodiments, X comprises  , wherein # represents a point of attachment to an antibody or antigen-binding fragment thereof, and * represents a point of attachment to the remainder of X. In some embodiments, a linker comprise  , wherein R is as defined herein, # represents a point of attachment to an antibody or antigen-binding fragment thereof, and * represents a point of attachment to the remainder of X. In some embodiments, a linker comprise  , wherein R is as defined herein, # represents a point of attachment to an antibody or antigen-binding fragment thereof, and * represents a point of attachment to the remainder of X. In some embodiments, X comprises  , wherein # represents a point of attachment to an antibody or antigen-binding fragment thereof, and * represents a point of attachment to the remainder of X. In some embodiments, X comprises  , wherein # represents a point of attachment to an antibody or antigen-binding fragment thereof, and * represents a point of attachment to the remainder of X. In some embodiments, a linker comprises  , wherein * represents a point of attachment to an antibody

or antigen-binding fragment thereof, and # represents a point of attachment to the remainder of the linker.

[0506] In some embodiments, t is an integer between 1 and 16. In some embodiments, t is an integer between 1 and 12. In some embodiments, t is an integer between 2 and 10. In some embodiments, t is an integer between 4 and 8. In some embodiments, t is 1. In some embodiments, t is 2. In some embodiments, t is 3. In some embodiments, t is 4. In some embodiments, t is 5. In some embodiments, t is 6. In some embodiments, t is 7. In some embodiments, t is 8. In some embodiments, t is an integer between about 7 and about 9. In some embodiments, t is an integer between about 6 and about 10. In some embodiments, provided t is an integer between about 4 and about 12.

[0507] In some embodiments, Y is $-L^C-$. In some embodiments, Y is or comprises a cleavable unit as described herein. In some embodiments, Y is a chemically cleavable unit. In some embodiments, Y is an enzyme cleavable unit. In some embodiments, Y is or comprises a sequence of amino acids that is cleaved by protease within a cell. In some embodiments, a protease breaks an amide bond between a sequence of amino acids within the linker. In some embodiments, Y is or comprises an amino acid sequence that is cleaved by a protease (e.g., a human protease within a cell). In some embodiments, Y is or comprises an amino acid sequence that is cleaved by a cathepsin (e.g., human cathepsin L, B, F, or S).

[0508] In some embodiments, Y comprises between 1 and 12 amino acids. In some embodiments, Y comprises between 2 and 6 amino acids. In some embodiments, Y comprises between 2 and 5 amino acids. In some embodiments, Y comprises between 2 and 4 amino acids. In some embodiments, Y comprises 2 or 3 amino acids. In some embodiments, Y comprises 3 or 4 amino acids. In some embodiments, Y comprises 2 amino acids. In some embodiments, Y comprises 3 amino acids. In some embodiments, Y comprises 4 amino acids. In some embodiments, Y comprises 5 amino acids. In some embodiments, Y comprises 6 amino acids.

[0509] In some embodiments, Y comprises natural amino acids. In some embodiments, Y comprises amino acids selected from citrulline, phenylalanine, leucine, threonine, tryptophan, glutamine, proline, glutamic acid, tyrosine, lysine, valine, isoleucine, alanine, aspartic acid, asparagine, arginine, methionine, glycine, histidine, serine, or cysteine. In some embodiments, Y comprises amino acids selected from glycine, phenylalanine, alanine, valine, and citrulline. In some embodiments, Y comprises amino acids selected from glycine or phenylalanine. In some embodiments, Y comprises amino acids selected from alanine or valine. In some embodiments, Y comprises amino acids selected from citrulline or valine.

[0510] In some embodiments, Y is or comprises&-DGGFG-, &-KGGFG-, &-GGFG-, &-GGFGG-, &-GGFGGG-, &-KGGF-, &-DGGF-, &-V-Cit-, &-VA-, &-FK-, &-AA-, &-VQ-, &-LQ-, &-FQ-, &-TT-, &-AAA-, &-EA-, &-YR-, &-FR-, &-YM-, &-VR-, &-MT-, &-VT-, &-VM-, &-LM-, &-AN-, &-GGR-, &-D-Val-D-Gln-, &-D-Ala-D-Ala-, &-DVA-, or &-FM-, wherein & represents a point of attachment to:

(i) X in formulae A, C, I-A, and I-C;

(ii) Z in formulae B and I-B;

(iii) L^X in formulae II-A, II-C, and V-A; or

(iv) the carbon atom of the adjacent C=O in formulae II-B, III-A, III-B, III-C, V-B, VI-A, and VI-B.

[0511] In some embodiments, Y is or comprises&-V-Cit-, &-VA-, or &-AA-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-GGFG-, &-V-Cit-, &-AAA-, or &-DVA-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-DGGFG-, &-KGGFG-, &-GGFG-, &-GGFGG-, &-GGFGGG-, &-KGGF-, or &-DGGF-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-V-Cit-, &-VA-, &-VQ-, &-VR-, &-VT-, &-VM-, or &-D-Val-D-Gln-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-GGFG- or &-VA-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-VQ-, or &-LQ, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-LQ-, &-FQ-, &-TT-, &-AAA-, &-EA-, wherein & is as defined and described herein.

[0512] In some embodiments, Y is or comprises&-DGGFG-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-KGGFG-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-GGFG-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-GGFGG-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-GGFGGG-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-KGGF-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-DGGF-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-V-Cit-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-VA-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-FK-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-AA-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-VQ-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-YR-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-FR-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-YM-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-VR-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-MT-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-VT-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-VM-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-LM-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-AN-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-GGR-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-D-Val-D-Gln-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-D-Ala-D-Ala-, wherein & is as defined and described herein. In some embodiments, Y is or comprises&-FM-, wherein & is as defined and described herein. In some embodiments, an enzyme cleavable unit of a linker is &-GGFG- or &-VA-, wherein & is as defined and described herein.

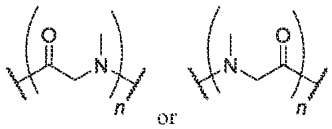
[0513] In some embodiments, Z is L^{CO} as described herein. In some embodiments, a Z comprises an

optionally substituted bivalent C_{1-12} aliphatic, aryl, heteroaryl, polyether, polyester, poly(N-(2-hydroxypropyl)methacrylamide) (pHPMA), polylactic-co-glycolic acid (PLGA), polymers of alkylene diamines, polysarcosine, saccharide, or combinations thereof. In some embodiments, a Z comprises an optionally substituted bivalent C_{1-12} aliphatic, polyether, polyester, poly(N-(2-hydroxypropyl)methacrylamide) (pHPMA), polylactic-co-glycolic acid (PLGA), polymers of alkylene diamines, polysarcosine, saccharide, or combinations thereof. In some embodiments, Z comprises an optionally substituted bivalent polyether, polysarcosine, saccharide, or combinations thereof.

[0514] In some embodiments, Z comprises an optionally substituted bivalent C_{1-10} aliphatic. In some embodiments, Z comprises an optionally substituted bivalent C_{1-8} aliphatic. In some embodiments, Z comprises an optionally substituted bivalent C_{1-6} aliphatic. In some embodiments, Z comprises an optionally substituted bivalent C_{2-6} aliphatic. In some embodiments, Z comprises an optionally substituted bivalent C_{3-6} aliphatic. In some embodiments, Z comprises an optionally substituted bivalent C_{4-6} aliphatic. In some embodiments, Z comprises an optionally substituted bivalent C_5 or C_6 aliphatic.

[0515] In some embodiments, Z comprises polyethylene glycol having a structure –(OCH2CH2)n–, wherein n is as described herein. In some embodiments, Z is or comprises $-(CH_2CH_2O)n-$ wherein n is 1-24. In some embodiments, Z is or comprises $-(CH_2CH_2O)n-$ wherein n is 8.

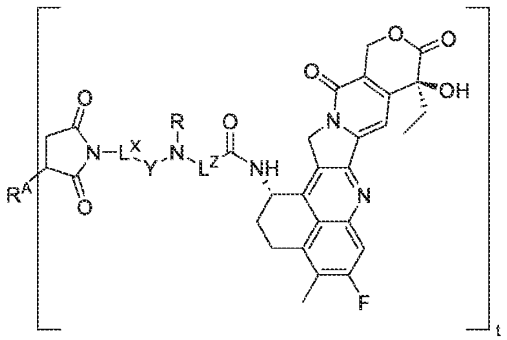
[0516] In some embodiments, Z comprises polysarcosine having a structure:

[0517]  or

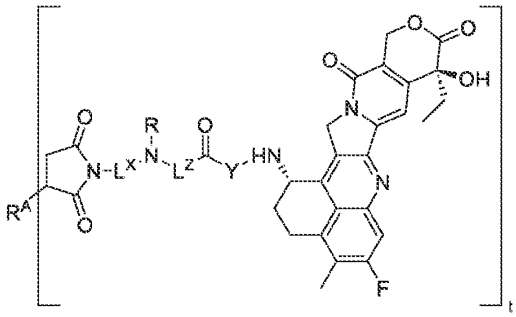
[0518] wherein n is as described herein.

[0519] In some embodiments, Z comprises a monosaccharide, disaccharide, or oligosaccharides. In some embodiments, Z comprises a monosaccharide. In some embodiments, Z comprises a glycoside. In some embodiments, Z comprises a uronic acid or derivative thereof. In some embodiments, Z comprises glucuronic acid or derivative thereof. In some embodiments, Z comprises a glucuronide.

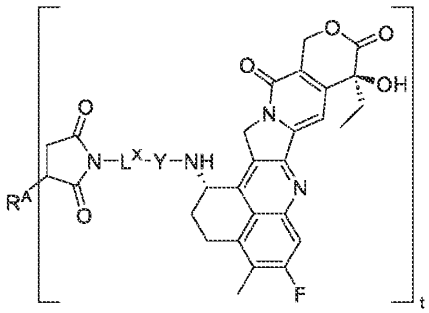
[0520] In some embodiments, a conjugate has a structure of formula II-A, II-B, or II-C:



II-A



II-B

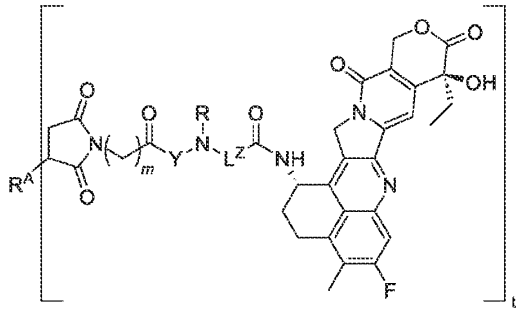


II-C

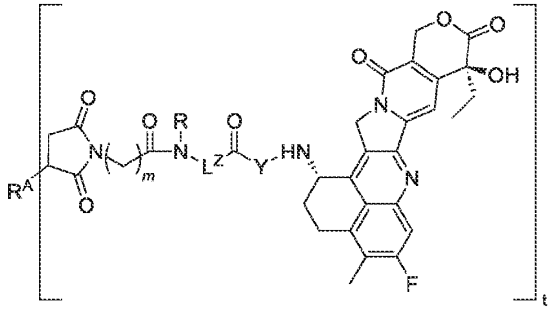
[0521] or a pharmaceutically acceptable salt thereof, wherein L^Z is or comprises $-(CH_2CH_2O)n-$ wherein n is 0-24 and each other variable is independently as described herein. In some embodiments, L^X is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene unites of L^X are optionally and independently replaced by a group selected from $-O-$, $-N(R)-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, or a 3-12 membered saturated, partially unsaturated, or aromatic monocyclic or bicyclic ring comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, and R in L^X is hydrogen or an optionally substituted C_{1-6} aliphatic.

[0522] Each of R^A, t, Y, L^Z, R' and R is independently as described herein. For example, in some embodiments, each R is independently hydrogen or an optionally substituted C_{1-6} aliphatic. In some embodiments, R is hydrogen. In some embodiments, R is optionally substituted C_{1-6} aliphatic. In some embodiments, R is C_{1-6} aliphatic. In some embodiments, R is hydrogen, methyl, ethyl, n-propyl, isopropyl, n-butyl, s-butyl, or t-butyl.

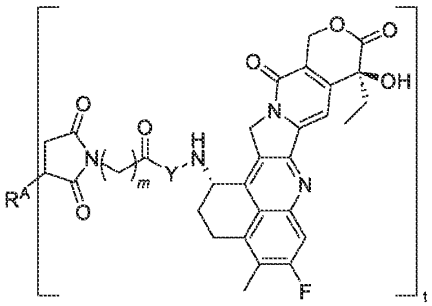
[0523] In some embodiments, a conjugate has a structure of formula III-A, III-B, or III-C:



III-A



III-B



III-C

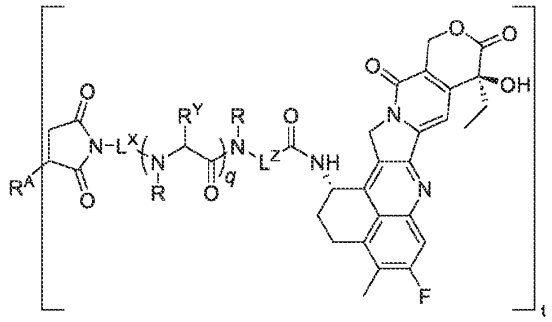
or a pharmaceutically acceptable salt thereof, wherein:

each of R^A, t, Y, L^Z, and R is independently as described herein; and

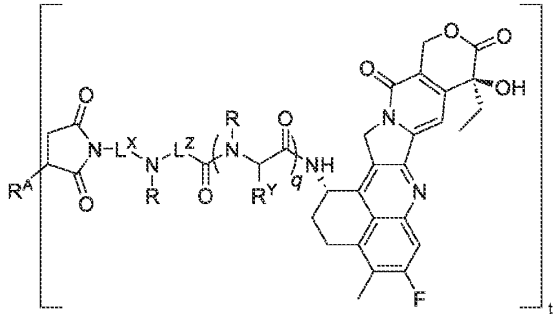
m is an integer between 1-12.

[0524] In some embodiments, m is an integer between 1-8. In some embodiments, m is an integer between 1-6. In some embodiments, m is an integer between 2-6. In some embodiments, m is an integer between 3-6. In some embodiments, m is an integer between 4-6. In some embodiments, m is an integer between 5-6. In some embodiments, m is 2. In some embodiments, m is 3. In some embodiments, m is 4. In some embodiments, m is 5. In some embodiments, m is 6. In some embodiments, m is 7. In some embodiments, m is 8.

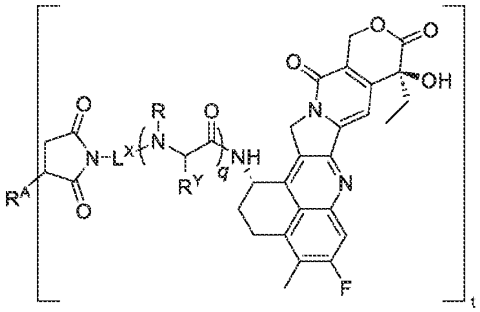
[0525] In some embodiments, a conjugate has a structure of formula IV-A, IV-B, or IV-C:



IV-A



IV-B



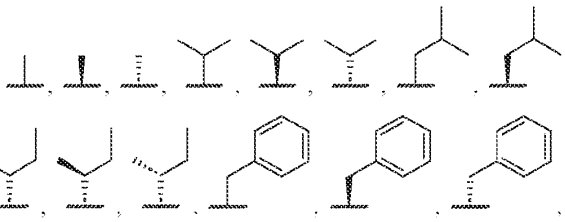
IV-C

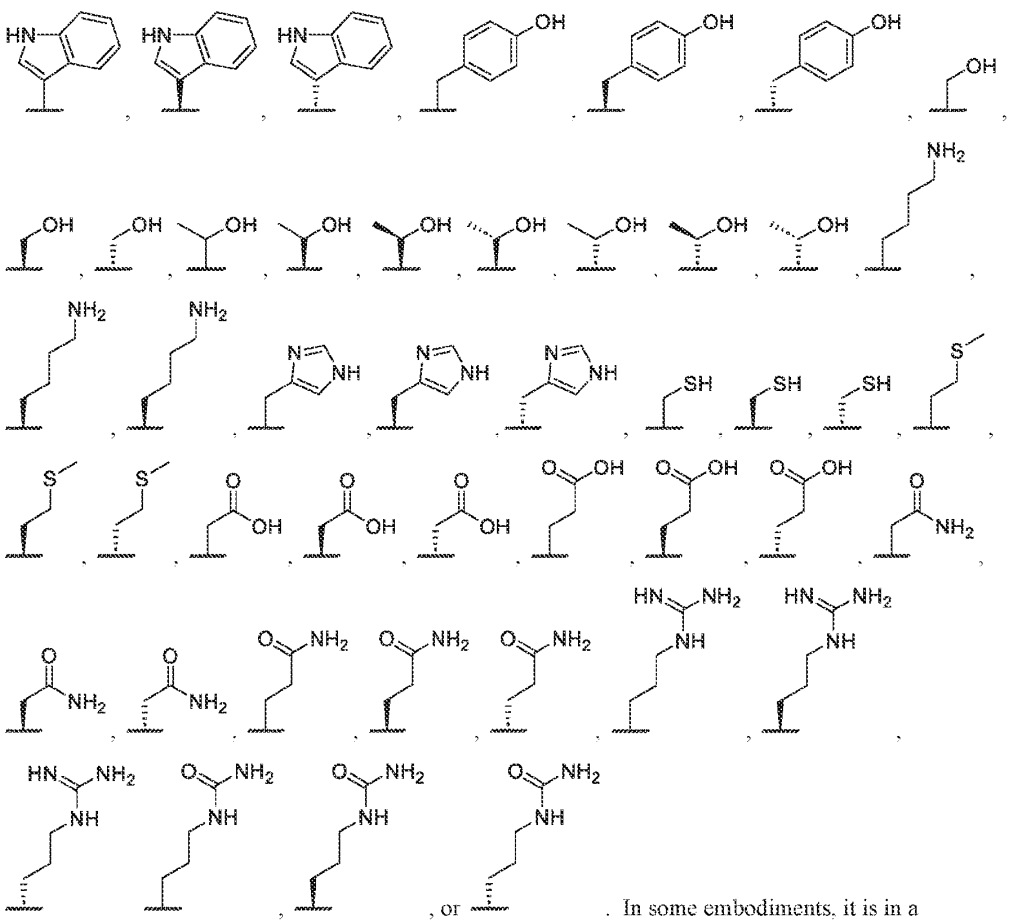
or a pharmaceutically acceptable salt thereof, wherein;

each of R^A, t, L^X, L^Z, and R is independently as described herein;

each R^Y is independently a side chain of an amino acid; and

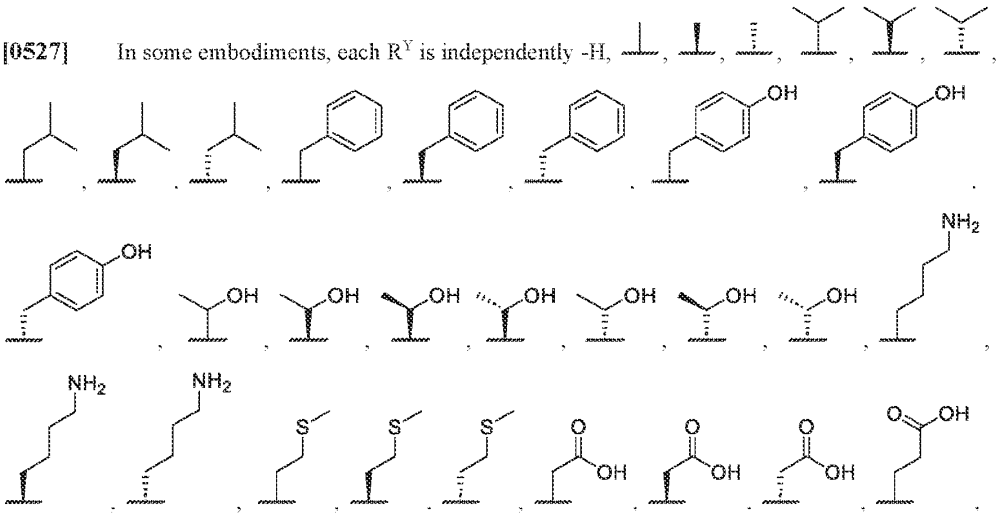
q is an integer between 1 and 12.

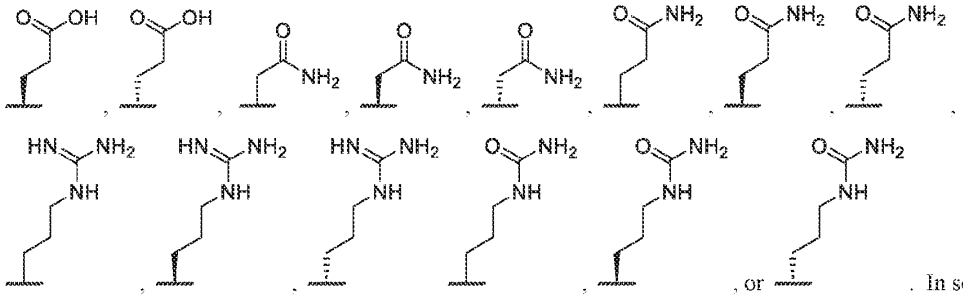
[0526] As defined above, each R^Y is independently a side chain of an amino acid. In some embodiments, each R^Y is independently -H, 

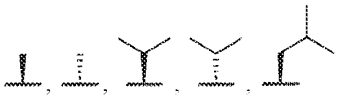
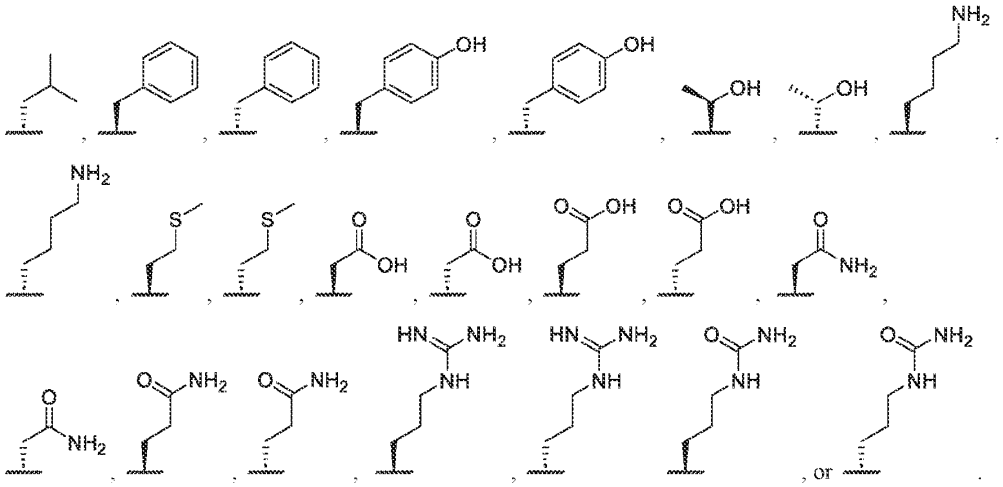


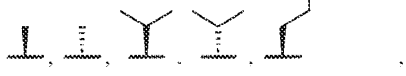
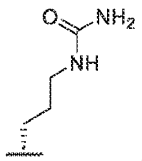
pharmaceutically acceptable salt form thereof.

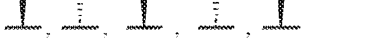
[0527] In some embodiments, each R^Y is independently -H,

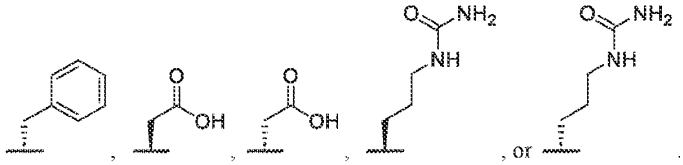


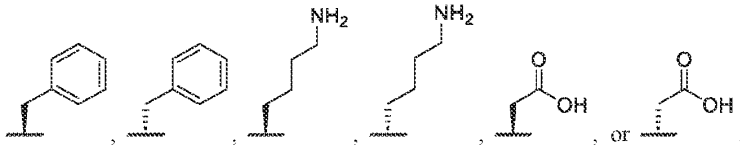

, or . In some embodiments, it is in a pharmaceutically acceptable salt form thereof.

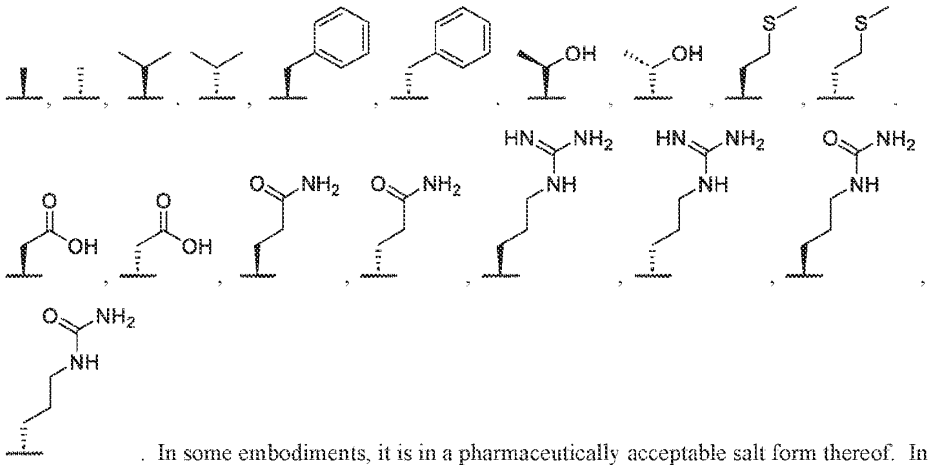
[0528] In some embodiments, each R^Y is independently -H, 

, or . In some embodiments, it is in a pharmaceutically acceptable salt form thereof.

[0529] In some embodiments, each R^Y is independently 
, or 
. In some embodiments, it is in a pharmaceutically acceptable salt form thereof.

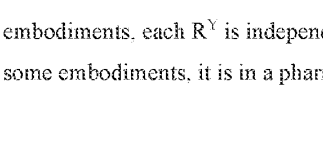
[0530] In some embodiments, each R^Y is independently -H, 
,


. In some embodiments, it is in a pharmaceutically acceptable salt form thereof. In some embodiments, each R^Y is independently -H,

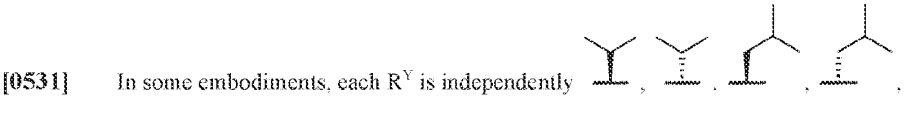

. In some embodiments, it is in a pharmaceutically acceptable salt form thereof. In some embodiments, each R^Y is independently -H,

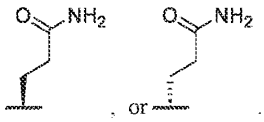

. In some embodiments, it is in a pharmaceutically acceptable salt form thereof. In some embodiments, each R^Y is independently -H,

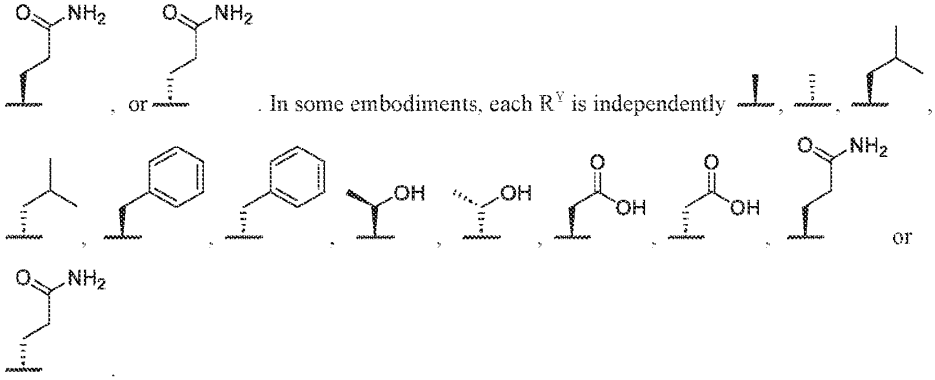
, or


. In some embodiments, it is in a pharmaceutically acceptable salt form thereof.

[0531] In some embodiments, each R^Y is independently


, or

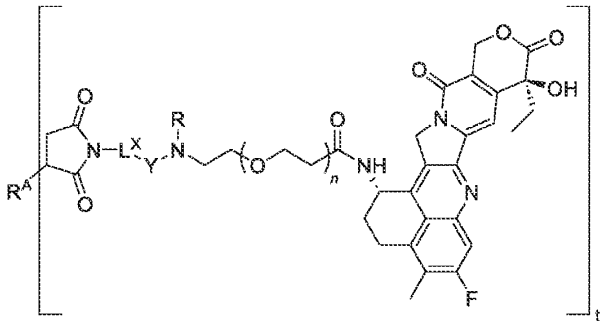

. In some embodiments, each R^Y is independently


.

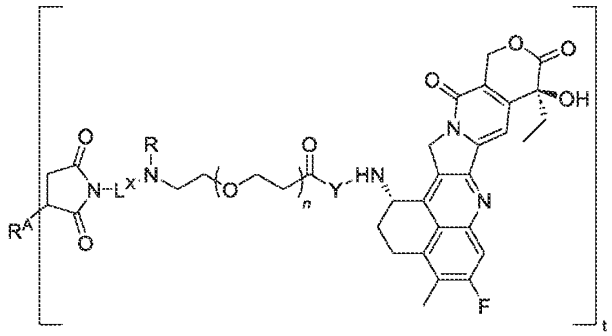
[0532] In some embodiments R^Y results in an L-amino acid. In some embodiments, R^Y results in a D-amino acid.

[0533] As defined herein, q is an integer between 1 and 12. In some embodiments, q is an integer between 2 and 8. In some embodiments, q is an integer between 2 and 6. In some embodiments, q is an integer between 2 and 5. In some embodiments, q is an integer between 2 and 4. In some embodiments, q is an integer 2 or 3. In some embodiments, q is 3 or 4. In some embodiments, q is 2. In some embodiments, q is 3. In some embodiments, q is 4. In some embodiments, q is 5. In some embodiments, q is 6.

[0534] In some embodiments, a conjugate has a structure of formula V-A or V-B:



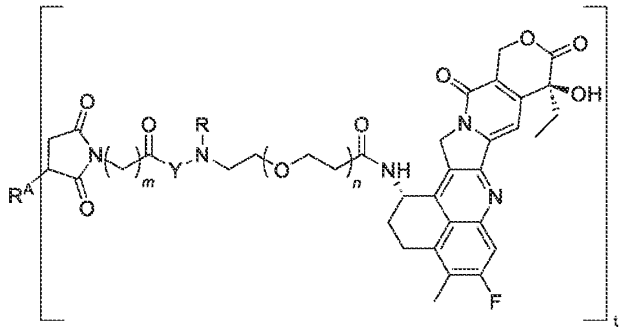
V-A



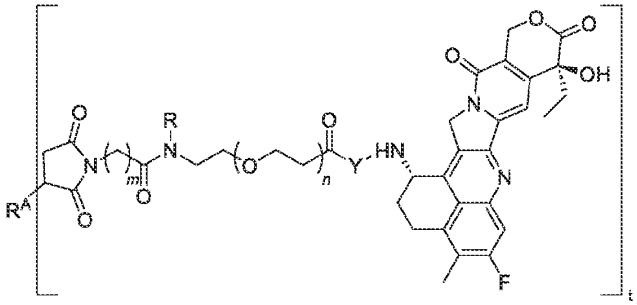
V-B

or a pharmaceutically acceptable salt thereof, wherein each of R^A, t, L^X, L^Z, n and R is independently as described herein.

[0535] In some embodiments, a conjugate has a structure of formula VI-A or VI-B:



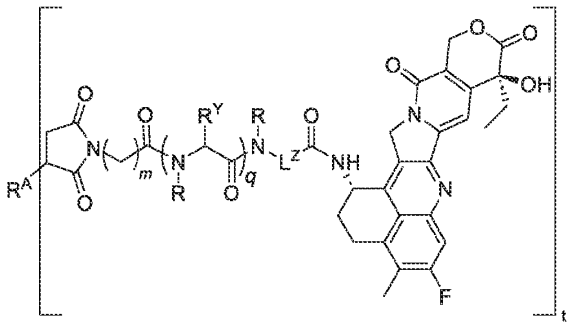
VI-A



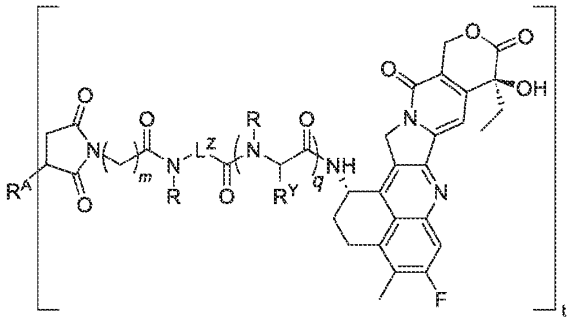
VI-B

or a pharmaceutically acceptable salt thereof, wherein each of R^A, m, Y, n and R is independently as described herein.

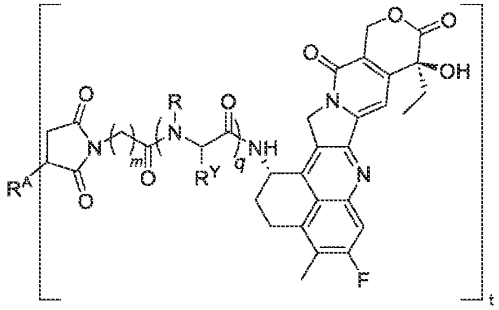
[0536] In some embodiments, a conjugate has a structure of formula VII-A, VII-B, or VII-C:



VII-A



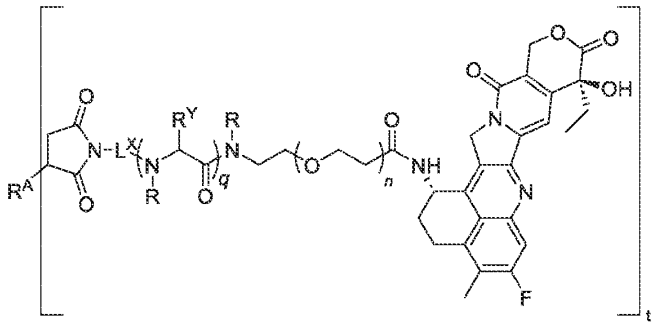
VII-B



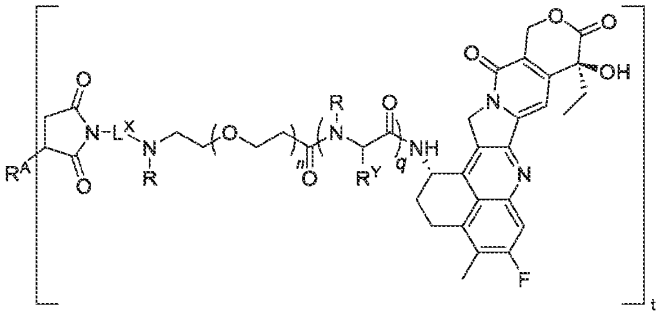
VII-C

or a pharmaceutically acceptable salt thereof, wherein each of R^A, t, m, R^Y, q, L^Z, and R is independently as described herein

[0537] In some embodiments, a conjugate has a structure of formula VIII-A or VIII-B:



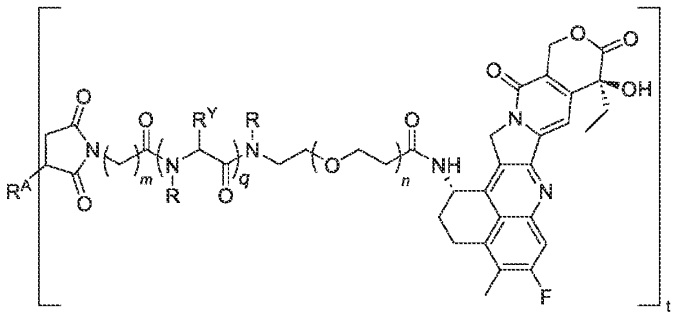
VIII-A



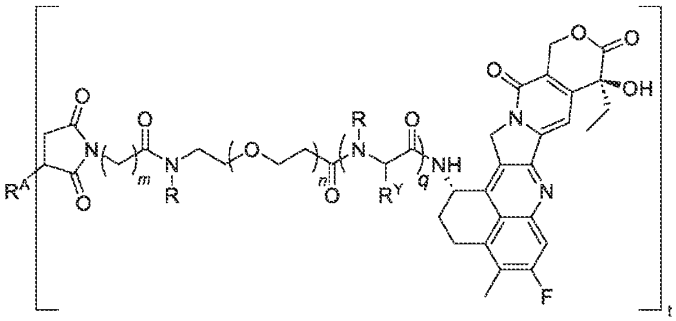
VIII-B

or a pharmaceutically acceptable salt thereof, wherein each of R^A, t, L^X, R^Y, n, and R is independently as described herein.

[0538] In some embodiments, a conjugate has a structure of formula IX-A or IX-B:



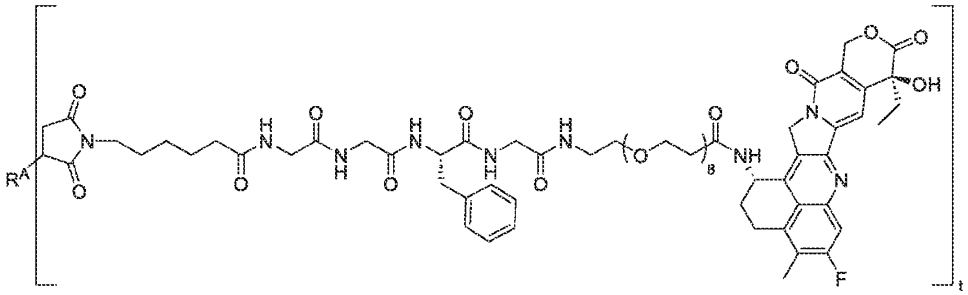
IX-A



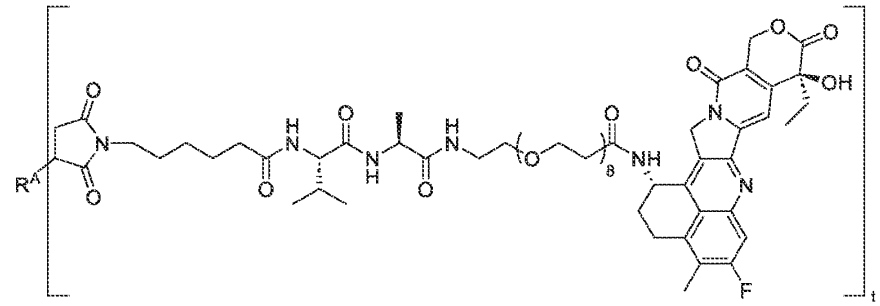
IX-B

or a pharmaceutically acceptable salt thereof, wherein each of R^A, t, m, R^Y, q, n, and R is independently as described herein.

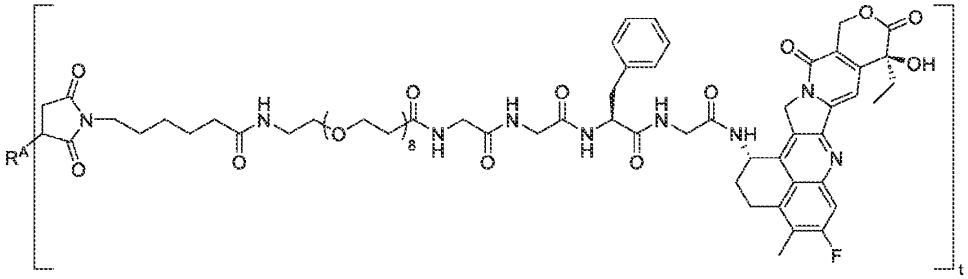
[0539] In some embodiments, a conjugate has a structure of formula X-A, X-B, X-C, X-D, X-E, or X-F:



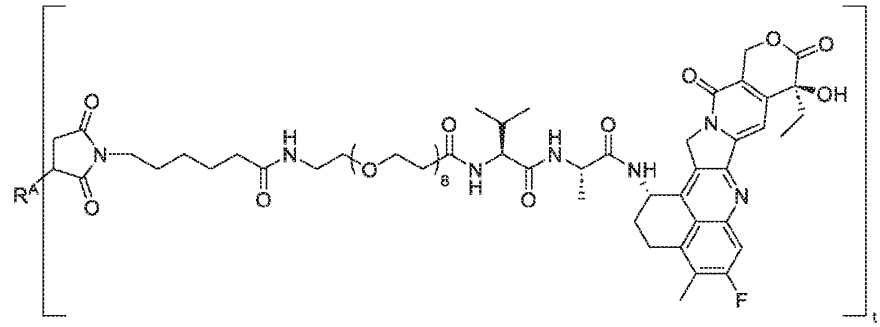
X-A



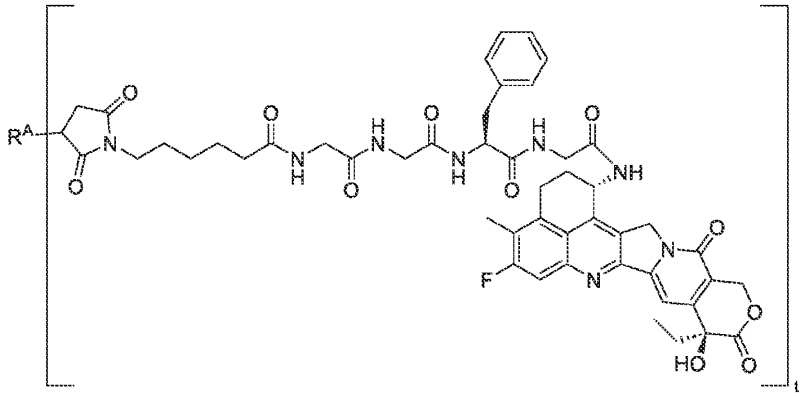
X-B



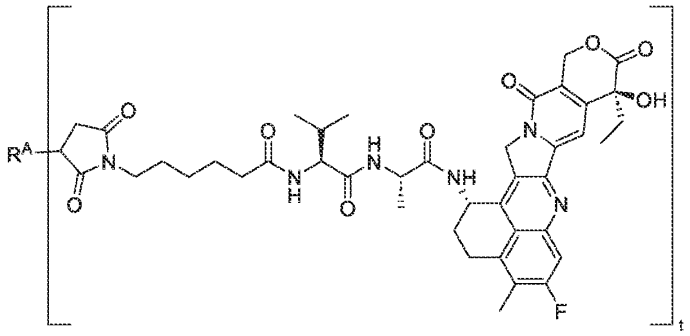
X-C



X-D



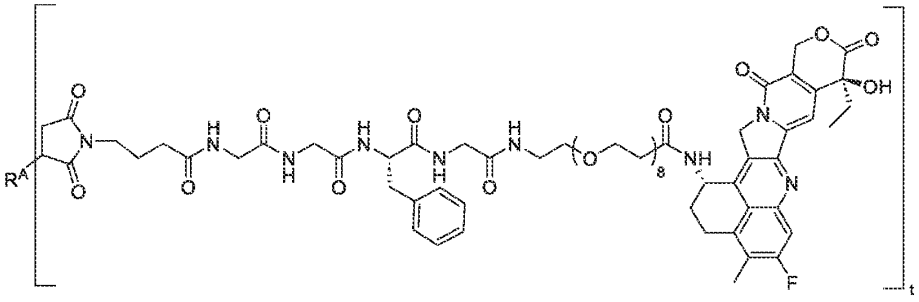
X-E



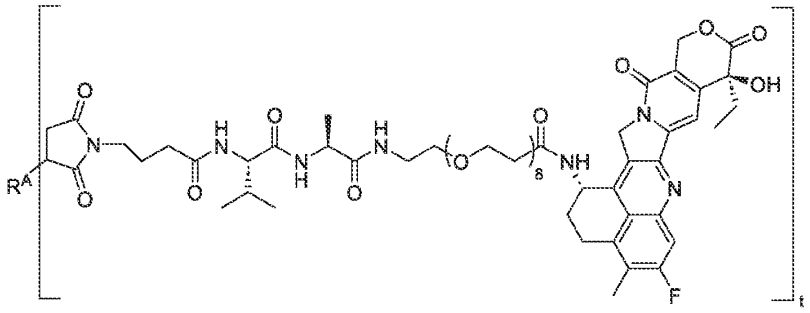
X-F

or a pharmaceutically acceptable salt thereof, wherein each of R^A and t is independently as described herein.

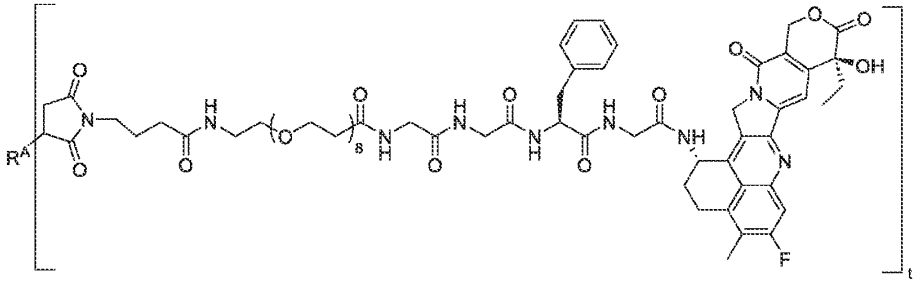
[0540] In some embodiments, a conjugate has a structure of formula X-A', X-B', X-C', X-D', X-E', X-F', X-G', X-H', X-I', X-J', X-K' or X-L':



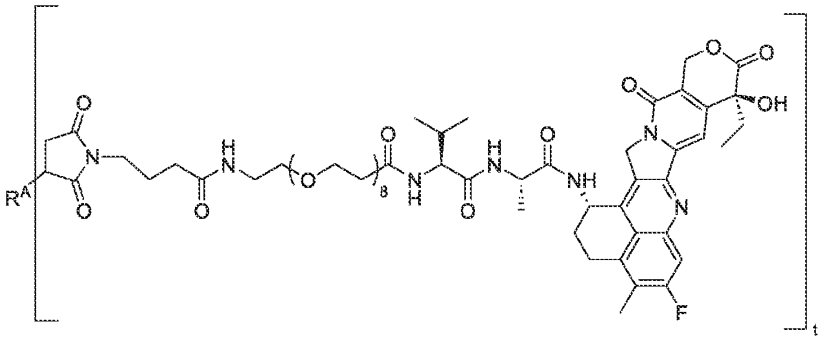
X-A'



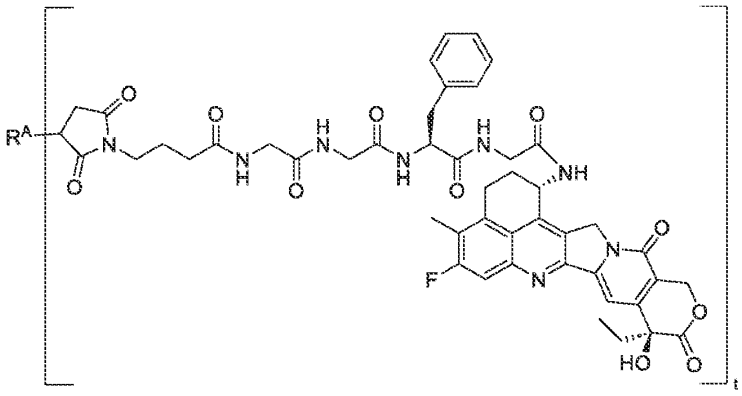
X-B'



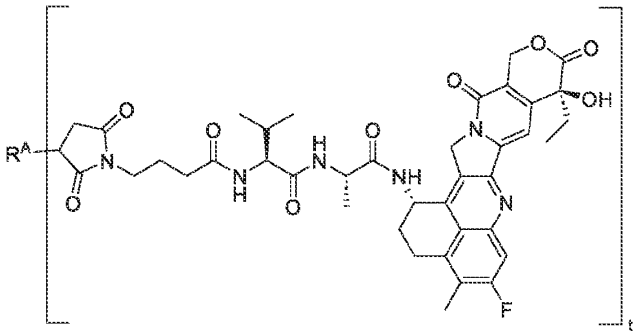
X-C'



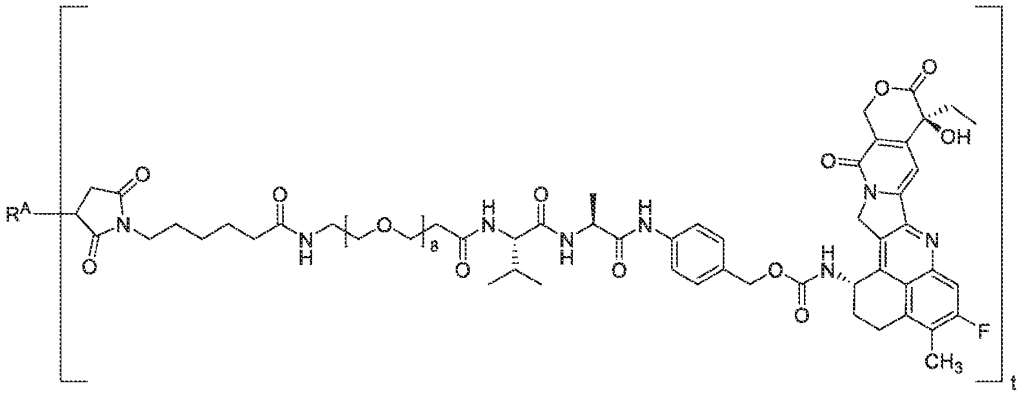
X-D'



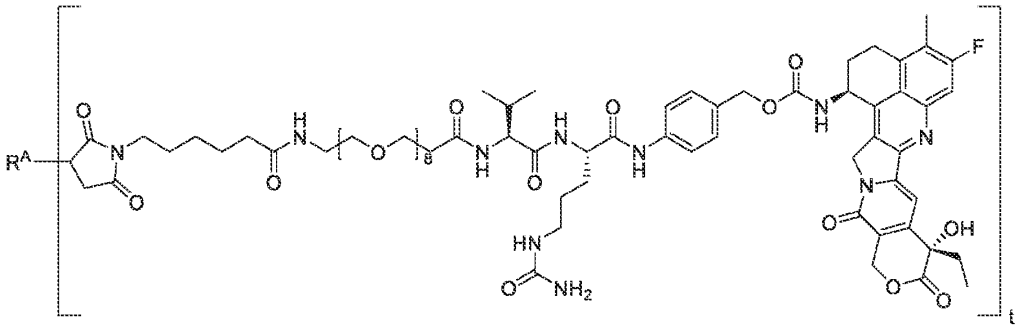
X-E'



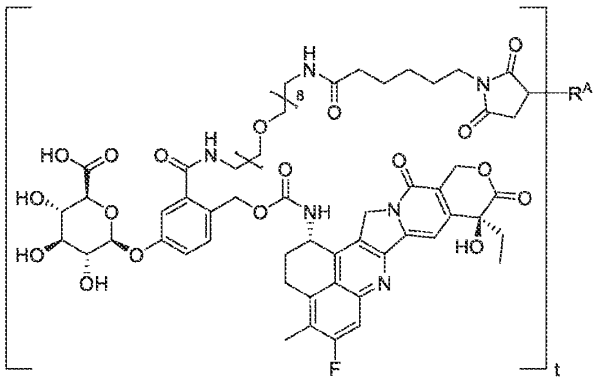
X-F'



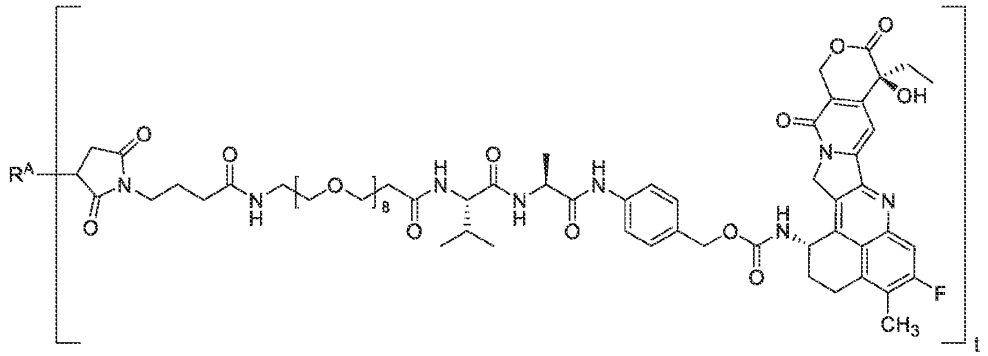
X-G'



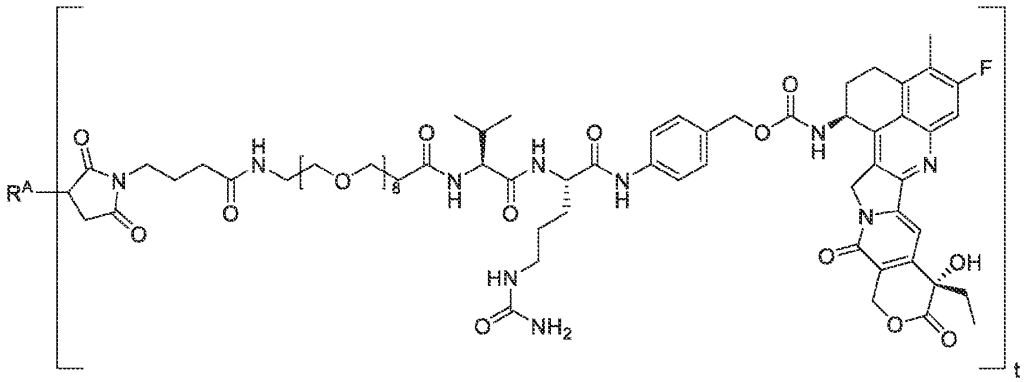
X-H'



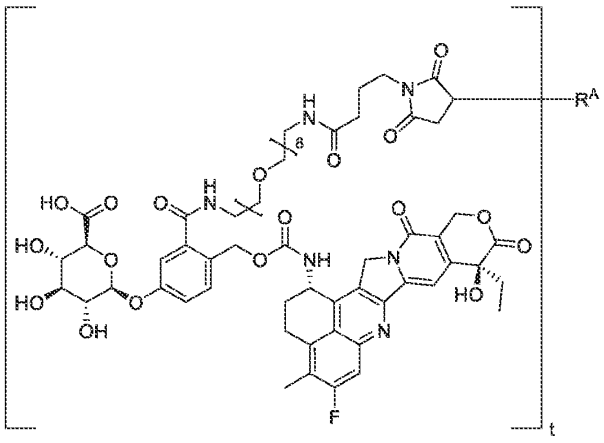
X-I'



X-J'



X-K'



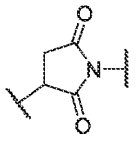
X-L'

or a pharmaceutically acceptable salt thereof, wherein each of R^A and t is independently as described herein.

[0541] In some embodiments, a conjugate has a structure of formula X-A or a pharmaceutically acceptable salt thereof. In some embodiments, a conjugate has a structure of formula X-B or a pharmaceutically acceptable salt thereof. In some embodiments, a conjugate has a structure of formula X-

C or a pharmaceutically acceptable salt thereof. In some embodiments, a conjugate has a structure of formula X-D or a pharmaceutically acceptable salt thereof. In some embodiments, a conjugate has a structure of formula X-E or a pharmaceutically acceptable salt thereof. In some embodiments, a conjugate has a structure of formula X-F or a pharmaceutically acceptable salt thereof. In some embodiments, a conjugate has a structure of formula X-A' or a pharmaceutically acceptable salt thereof. In some embodiments, a conjugate has a structure of formula X-B' or a pharmaceutically acceptable salt thereof. In some embodiments, a conjugate has a structure of formula X-C' or a pharmaceutically acceptable salt thereof. In some embodiments, a conjugate has a structure of formula X-D' or a pharmaceutically acceptable salt thereof. In some embodiments, a conjugate has a structure of formula X-E' or a pharmaceutically acceptable salt thereof. In some embodiments, a conjugate has a structure of formula X-F' or a pharmaceutically acceptable salt thereof. In some embodiments, a conjugate has a structure of formula X-G' or a pharmaceutically acceptable salt thereof. In some embodiments, a conjugate has a structure of formula X-H' or a pharmaceutically acceptable salt thereof. In some embodiments, a conjugate has a structure of formula X-I' or a pharmaceutically acceptable salt thereof. In some embodiments, a conjugate has a structure of formula X-J' or a pharmaceutically acceptable salt thereof. In some embodiments, a conjugate has a structure of formula X-K' or a pharmaceutically acceptable salt thereof. In some embodiments, a conjugate has a structure of formula X-L' or a pharmaceutically acceptable salt thereof. In some embodiments, R^A is or comprises an anti-HHLA2 antibody or an antigen-binding fragment thereof, e.g., those described in Table 1. In some embodiments, R^A is or comprises 887-1DE in Table 1 or an antigen-binding fragment thereof. In some embodiments, R^A is 887-1DE in Table 1. In some embodiments, R^A is or comprises 65889 in Table 1 or an antigen-binding fragment thereof. In some embodiments, R^A is 65889 in Table 1. In some embodiments, R^A is or comprises 65872 in Table 1 or an antigen-binding fragment thereof. In some embodiments, R^A is 65872 in Table 1. In some embodiments, R^A is or comprises Hu2C4-H3L3 in Table 1 or an antigen-binding fragment thereof. In some embodiments, R^A is Hu2C4-H3L3 in Table 1. In some embodiments, t is about 2. In some embodiments, t is about 4. In some embodiments, t is about 8. In some embodiments, for a composition comprising a conjugate, its DAR is about 4; in some embodiments, its DAR is about 8. In some embodiments, a composition comprises a conjugation of 887-1DE in Table 1. In some embodiments, a composition comprises a conjugation of 65889 in Table 1. In some embodiments, a composition comprises a conjugation of 65872 in Table 1. In some embodiments, a composition comprises a conjugation of Hu2C4-H3L3 in Table 1. In some embodiments, a composition comprises a conjugation of human IgG (e.g., human IgG1). For example, various conjugates are described in the Examples.

[0542] In some embodiments, a conjugate has the structure of $Ab-(-SM1-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P)t$ or a pharmaceutically acceptable salt thereof, wherein Ab is

a target binding moiety, SM1 is  wherein the carbon atom is bonded to Ab, and each variable is independently as described herein. In some embodiments, a conjugate has the structure of Ab–(–SM1–$(CH_2)_X$–C(O)–N(R')–(CH_2CH_2O)n–$(CH_2)_z$–C(O)–L^C–R^P)t or a pharmaceutically acceptable salt thereof, wherein each variable is independently as described herein. In some embodiments, a conjugate has the structure of Ab–(–SM1–$(CH_2)_X$–C(O)–NH–(CH_2CH_2O)n–$(CH_2)_z$–C(O)–L^C–R^P)t or a pharmaceutically acceptable salt thereof, wherein each variable is independently as described herein. In some embodiments, a conjugate has the structure of Ab–(–SM1–$(CH_2)_X$–C(O)–N(R')–(CH_2CH_2O)n–$(CH_2)_z$–C(O)–GGFG–R^P)t or a pharmaceutically acceptable salt thereof, wherein each variable is independently as described herein. In some embodiments, a conjugate has the structure of Ab–(–SM1–$(CH_2)_X$–C(O)–NH–(CH_2CH_2O)n–$(CH_2)_z$–C(O)–GGFG–R^P)t or a pharmaceutically acceptable salt thereof, wherein each variable is independently as described herein. In some embodiments, a conjugate has the structure of Ab–(–SM1–$(CH_2)_X$–C(O)–N(R')–(CH_2CH_2O)n–$(CH_2)_z$–C(O)–Val–Ala–PABA–R^P)t or a pharmaceutically acceptable salt thereof, wherein each variable is independently as described herein. In some embodiments, a conjugate has the structure of Ab–(–SM1–$(CH_2)_X$–C(O)–NH–(CH_2CH_2O)n–$(CH_2)_z$–C(O)–Val–Ala–PABA–R^P)t or a pharmaceutically acceptable salt thereof, wherein each variable is independently as described herein. In some embodiments, a conjugate has the structure of Ab–(–SM1–$(CH_2)_X$–C(O)–N(R')–(CH_2CH_2O)n–$(CH_2)_z$–C(O)–Val–Cit–PABA–R^P)t or a pharmaceutically acceptable salt thereof, wherein each variable is independently as described herein. In some embodiments, a conjugate has the structure of Ab–(–SM1–$(CH_2)_X$–C(O)–NH–(CH_2CH_2O)n–$(CH_2)_z$–C(O)–Val–Cit–PABA–R^P)t or a pharmaceutically acceptable salt thereof, wherein each variable is independently as described herein. In some embodiments, a conjugate has the structure of Ab–(–SM1–$(CH_2)_X$–C(O)–N(R')–(CH_2CH_2O)n–$(CH_2)_z$–N(R')–C(O)–oGlu–R^P)t or a pharmaceutically acceptable salt thereof, wherein each variable is independently as described herein. In some embodiments, a conjugate has the structure of Ab–(–SM1–$(CH_2)_X$–C(O)–NH–(CH_2CH_2O)n–$(CH_2)_z$–NH–C(O)–oGlu–R^P)t or a pharmaceutically acceptable salt thereof, wherein each variable is independently as described herein. In some embodiments, a conjugate has the structure of Ab–(–SM1–$(CH_2)_X$–C(O)–N(R')–(CH_2CH_2O)n–$(CH_2)_z$–N(R')–C(O)–mGlu–R^P)t or a pharmaceutically acceptable salt thereof, wherein each variable is independently as described herein. In some embodiments, a conjugate has the structure of Ab–(–SM1–$(CH_2)_X$–C(O)–NH–(CH_2CH_2O)n–$(CH_2)_z$–NH–C(O)–mGlu–R^P)t or a pharmaceutically

acceptable salt thereof, wherein each variable is independently as described herein. In some embodiments, Ab is or comprises an antibody or an antigen-binding fragment thereof. In some embodiments, Ab is or comprises an anti-HHLA2 antibody or an antigen-binding fragment thereof, e.g., those described in Table 1. In some embodiments, Ab is or comprises 887-1DE in Table 1 or an antigen-binding fragment thereof. In some embodiments, Ab is 887-1DE in Table 1. In some embodiments, Ab is or comprises 65889 in Table 1 or an antigen-binding fragment thereof. In some embodiments, Ab is 65889 in Table 1. In some embodiments, Ab is or comprises 65872 in Table 1 or an antigen-binding fragment thereof. In some embodiments, Ab is 65872 in Table 1. In some embodiments, Ab is or comprises Hu2C4-H3L3 in Table 1 or an antigen-binding fragment thereof. In some embodiments, Ab is Hu2C4-H3L3 in Table 1. In some embodiments, x is 2-10. In some embodiments, x is 2. In some embodiments, x is 3. In some embodiments, x is 5. In some embodiments, n is 2-20. In some embodiments, n is 6-10. In some embodiments, n is 8. In some embodiments, z is 1-10. In some embodiments, z is 1-5. In some embodiments, z is 2-5. In some embodiments, z is 2. In some embodiments, x is 3, n is 8, and z is 2. In some embodiments, x is 5, n is 8, and z is 2. In some embodiments, R^P is or comprises a nitrogen atom and is bonded to the rest of a conjugate at the nitrogen atom. In some embodiments, R^P is or comprises an exatecan moiety or a derivative thereof. In some embodiments, R^P is R^{EX}. In some embodiments, SM1 is conjugated to Ab at a cysteine residue of Ab. In some embodiments, SM1 is bonded to –S– of a cysteine residue. In some embodiments, a cysteine residue is that one forms an interchain disulfide bond without conjugation. In some embodiments, t is about 2. In some embodiments, t is about 4. In some embodiments, t is about 8. In some embodiments, for a composition comprising a conjugate, its DAR is about 4; in some embodiments, its DAR is about 8. In some embodiments, a composition comprises a conjugation of 887-1DE in Table 1. In some embodiments, a composition comprises a conjugation of 65889 in Table 1. In some embodiments, a composition comprises a conjugation of 65872 in Table 1. In some embodiments, a composition comprises a conjugation of Hu2C4-H3L3 in Table 1. In some embodiments, a composition comprises a conjugation of human IgG (e.g., human IgG1). Various conjugates are described in the Examples as examples.

[0543] In some embodiments, a linker is or comprises , wherein each variable is independently as described herein. In some embodiments, a linker is or comprises , wherein each variable is independently as described herein. In some embodiments, a linker is or comprises , wherein each variable is independently as described herein. In some embodiments, a linker is or comprises , wherein each variable is independently as described herein. In some embodiments, a linker is or comprises , wherein each variable is independently as described herein. In some embodiments, a linker is or comprises , wherein each variable is independently as described herein. In some embodiments, a linker is or comprises , wherein each variable is independently as described herein. In some embodiments, a linker is or comprises , wherein each variable is independently as described herein. In some embodiments, a linker is or comprises , wherein each variable is independently as described herein. In some embodiments, x is 2-10. In some embodiments, n is 2-10. In some embodiments, z is 2-10. In some embodiments, x is 2, n is

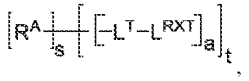
8 and z is 2. In some embodiments, x is 3, n is 8, and z is 2. In some embodiments, x is 5, n is 8 and z is 2. In some embodiments, SM1 is attached to –S– of a cysteine residue. Various conjugates comprising such linkers are described herein.

[0544]

Certain Compounds

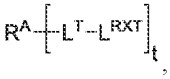
[0545] In some embodiments, the present disclosure provides various compound. In some embodiments, provided compounds, upon reaction with a target binding moiety, afford a disclosed conjugate. For example, in some embodiments, the present disclosure provides compounds that, upon reaction with an anti-HERV-H LTR-Associating 2 (HHLA2) antibody or antigen-binding fragment thereof, provide a conjugate comprising an anti-HERV-H LTR-Associating 2 (HHLA2) antibody or antigen-binding fragment thereof.

[0546] In some embodiments, the present disclosure provides a compound, e.g., a targeting binding agent, has the structure of formula T-1 or a slat thereof:

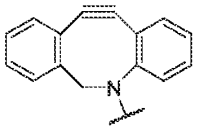
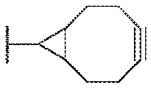


T-1

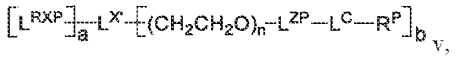
[0547] wherein each L^{RXT} is independently a target binding agent reactive group, and each other variable is independently as described herein. In some embodiments, the present disclosure provides a compound, e.g., a targeting binding agent, has the structure of formula T-2 or a slat thereof:



T-2

[0548] wherein each variable is independently as described herein. For example, in some embodiments, L^{RXT} is or comprises a nucleophilic group, e.g., –SH. In some embodiments, L^{RXT} is or comprises –SH (e.g., of a Cys residue for conjugation). In some embodiments, L^{RXT} is or comprises $–NH_2$ (e.g., of a Lys residue for conjugation). In some embodiments, L^{RXT} is or comprises $–N_3$. In some embodiments, L^{RXT} is or comprises alkyne. In some embodiments, an alkyne is activated. In some embodiments, L^{RXT} is or comprises –C≡CH,  , or  . In some embodiments, each L^{RXT} is independently L^{RX1} as described herein. In some embodiments, each L^{RXT} is independently L^{RX2} as described herein.

[0549] In some embodiments, the present disclosure provides a compound, e.g., a payload agent, has the structure of formula P-1 or a slat thereof:

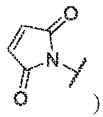
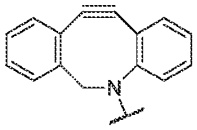
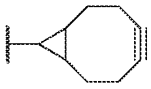


P-1

wherein each L^{RXP} is independently a payload agent reactive group, and each other variable is independently as described. In some embodiments, the present disclosure provides a compound, e.g., a payload agent, has the structure of formula P-2 or a slat thereof:

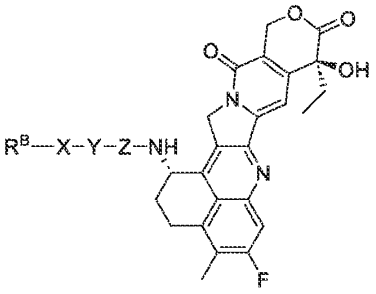
$$L^{RXP}-L^{X}-(CH_2CH_2O)_n-L^{ZP}-L^{C}-R^{P}$$

P-2

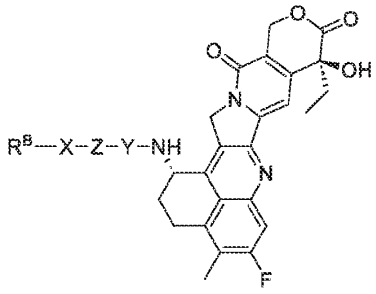
wherein each variable is independently as described. For example, in some embodiments, L^{RXP} is or comprises −SH (e.g., for reaction with −SH of a target binding agent). In some embodiments, L^{RXP} is or comprises an electrophilic group (e.g., for reaction with a nucleophilic group of a target binding agent). In some embodiments, an electrophilic group is or comprises a double bond conjugated with an electron-withdrawing group, e.g., −C(O)−. In some embodiments, an electrophilic group is or comprises ). In some embodiments, an electrophilic group is or comprises LG−CH_2−EWG− as described herein. In some embodiments, an electrophilic group is or comprises LG−CH_2−C(O)−. In some embodiments, LG is −Cl, −Br, or −I. In some embodiments, L^{RXP} is or comprises −C(O)OH or an activated form thereof (e.g., for reaction with $-NH_2$ of a target binding agent). In some embodiments, L^{RXP} is or comprises $-N_3$ (e.g., for reaction with alkyne of a target binding agent). In some embodiments, L^{RXP} is or comprises alkyne (e.g., for reaction with $-N_3$ of a target binding agent). In some embodiments, an alkyne is activated. In some embodiments, L^{RXP} is or comprises −C≡CH,  , or  . In some embodiments, each L^{RXP} is independently R^B as described herein. In some embodiments, each L^{RXT} is independently L^{RX1} as described herein. In some embodiments, each L^{RXT} is independently L^{RX2} as described herein.

[0550] In some embodiments, one of L^{RXP} and one of L^{RXT} is L^{RX1} as described herein, and the other is L^{RX2} as described herein. In some embodiments, each L^{RXP} is independently L^{RX1} as described herein, and each L^{RXT} is independently L^{RX2} as described herein. In some embodiments, each L^{RXP} is independently L^{RX2} as described herein, and each L^{RXT} is independently L^{RX1} as described herein. In some embodiments, L^{RXP} and L^{RXT} react to form L^{RX} as described herein.

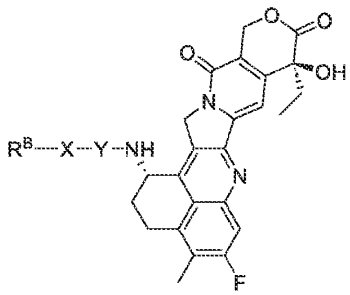
[0551] In some embodiments, the present disclosure provides compounds of formula I-AA, I-BB, or I-CC:



I-AA



I-BB



I-CC

or a pharmaceutically acceptable salt thereof, wherein:

each of X, Y, and Z is independently as described herein; and

R^B is a reactive moiety.

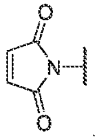
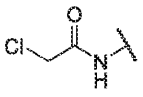
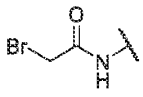
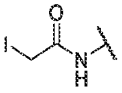
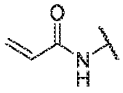
[0552] In some embodiments, R^B is a moiety that is capable of reacting with a group present on an arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, histidine, lysine, serine, threonine, tryptophan, or tyrosine within a target binding moiety. In some embodiments, R^B is a moiety that is capable of reacting with a group present on an arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, histidine, lysine, serine, threonine, tryptophan, or tyrosine within an antibody or antigen-binding fragment thereof. In some embodiments, R^B is a moiety that is capable of reacting with a group present on a cysteine, lysine, serine, threonine, or tyrosine within an antibody or antigen-binding fragment thereof. In some embodiments, R^B is a moiety that is capable of reacting with a group present on a cysteine within an antibody or antigen-binding fragment thereof. In some embodiments, R^B is a moiety that is capable of reacting with a group present on a lysine within an antibody or antigen-binding fragment thereof. In some embodiments, R^B is a moiety that is capable of reacting with a thiol, amine, acid, or hydroxyl group within an antibody or antigen-binding fragment thereof. In some embodiments, R^B is a moiety that is capable of reacting with a thiol within an antibody or antigen-binding fragment thereof. In some embodiments, R^B is a moiety that is capable of reacting with an amine within an antibody or antigen-binding fragment thereof. In some embodiments, R^B is a moiety that is capable of reacting with a hydroxyl within an antibody or antigen-binding fragment thereof.

[0553] In some embodiments, R^B is a moiety that is capable of reacting with a group present on an antibody or antigen-binding fragment thereof, wherein the reaction between R^B and the group present on

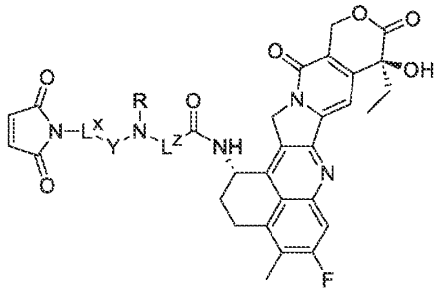
an antibody or antigen-binding fragment thereof is a conjugation with activated esters, sulfonyl chlorides, or isothiocyanates; reductive alkylation; aza-Michael addition; azetidinone chemistry; benzoyl fluoride-based chemistry; or click chemistry. Examples are provided in, e.g., Sletten and Bertozzi. Agew. Chem. Int. Ed. Engl. 2009, 48(38), 6974-6998.

[0554] In some embodiments, R^B is a moiety that is capable of reacting with an arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, histidine, lysine, serine, threonine, tryptophan, or tyrosine; and a group present on a target binding moiety. In some embodiments, R^B is a moiety that is capable of reacting with an arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, histidine, lysine, serine, threonine, tryptophan, or tyrosine; and a group present on an antibody or antigen-binding fragment thereof. In some embodiments, R^B is a moiety that is capable of reacting with a cysteine, lysine, serine, threonine, or tyrosine; and a group present on an antibody or antigen-binding fragment thereof. In some embodiments, R^B is a moiety that is capable of reacting with a cysteine present on an antibody or antigen-binding fragment thereof. In some embodiments, R^B is a moiety that is capable of reacting with a lysine present on an antibody or antigen-binding fragment thereof.

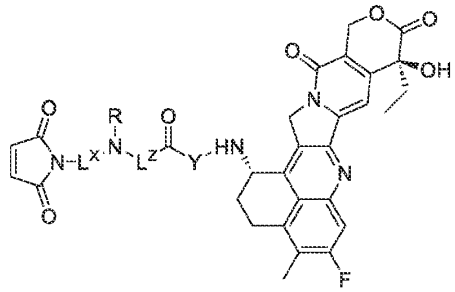
[0555] In some embodiments, R^B is a maleimide, haloacetamide (e.g., fluoroacetamide, chloroacetamide, bromoacetamide, or iodoacetamide), or a Michael acceptor (e.g., α,β-unsaturated carbonyl, such as acrylamide or α,β-unsaturated ketone). In some embodiments, RB a maleimide. In some embodiments, R^B is a haloacetamide (e.g., fluoroacetamide, chloroacetamide, bromoacetamide, or iodoacetamide). In some embodiments, the moiety capable of reacting with a cysteine is a Michael acceptor (e.g., α,β-unsaturated carbonyl, such as acrylamide or α,β-unsaturated ketone). In some embodiments, R^B is a maleimide, chloroacetamide, bromoacetamide, acrylamide, or an α,β-unsaturated ketone. In some embodiments, R^B is a chloroacetamide. In some embodiments, R^B is a bromoacetamide. In some embodiments, R^B is a acrylamide. In some embodiments, R^B is an α,β-unsaturated ketone.

[0556] In some embodiments, R^B is or comprises  . In some embodiments, R^B is or comprises  . In some embodiments, R^B is or comprises  . In some embodiments R^B is or comprises  . In some embodiments R^B is or comprises  . In some embodiments a linker comprises  .

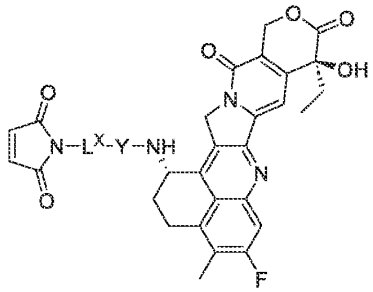
[0557] In some embodiments, the present disclosure provides compounds of formula II-AA, II-BB, or II-CC:



II-AA



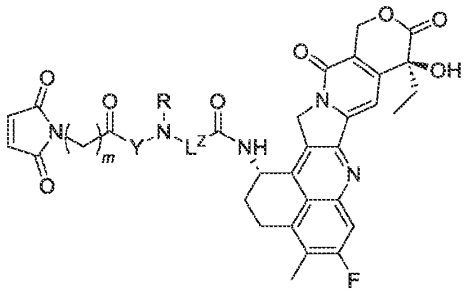
II-BB



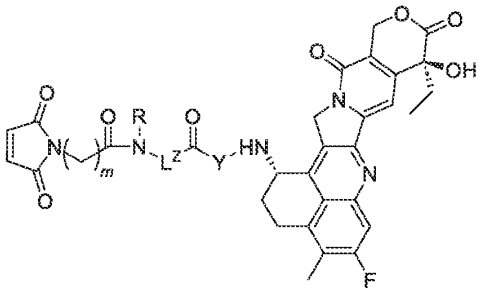
II-CC

or a pharmaceutically acceptable salt thereof, wherein each L^X, Y, L^Z, and R is independently as described herein.

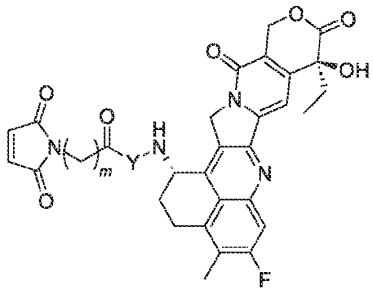
[0558] In some embodiments, the present disclosure provides compounds of formula III-AA, III-BB, or III-CC:



III-AA



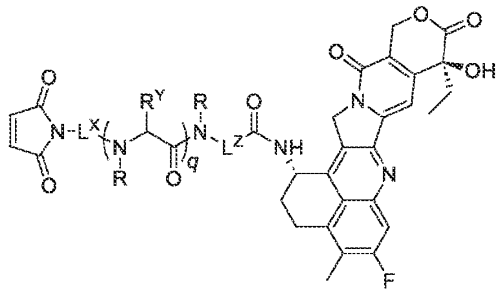
III-BB



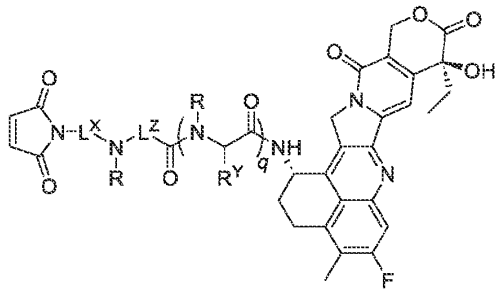
III-CC

or a pharmaceutically acceptable salt thereof, wherein each m, Y, L^Z, and R is independently as described herein.

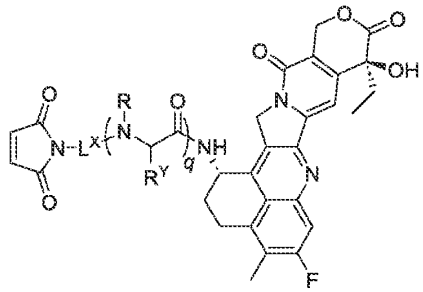
[0559] In some embodiments, the present disclosure provides compounds of formula IV-AA, IV-BB, or IV-CC:



IV-AA



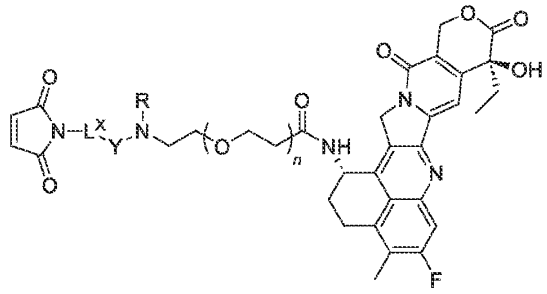
IV-BB



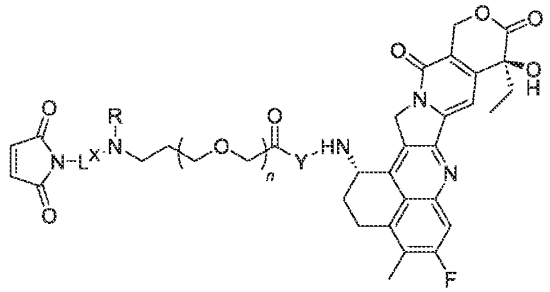
IV-CC

[0560] or a pharmaceutically acceptable salt thereof, wherein each L^X, L^Z, R^Y, q, and R is independently as described herein.

[0561] In some embodiments, the present disclosure provides compounds of formula V-AA or V-BB:



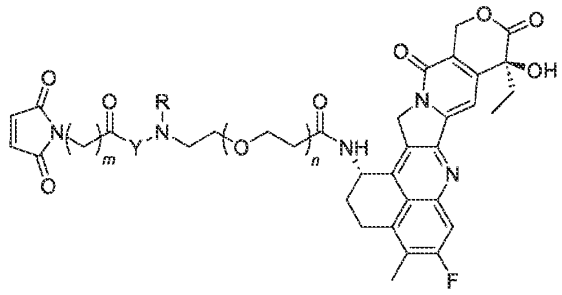
V-AA



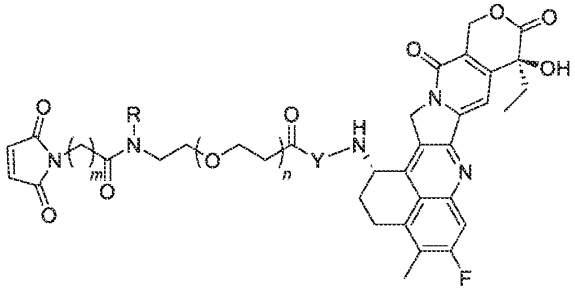
V-BB

or a pharmaceutically acceptable salt thereof, wherein each L^X, Y, n, or R is independently as described herein.

[0562] In some embodiments, the present disclosure provides compounds of formula VI-AA or VI-BB:



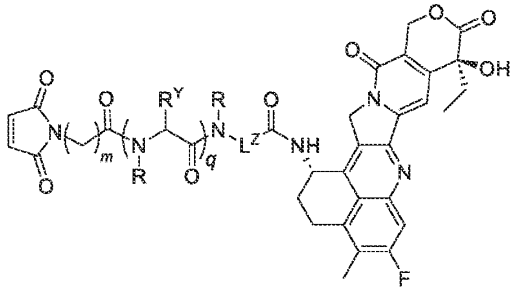
VI-AA



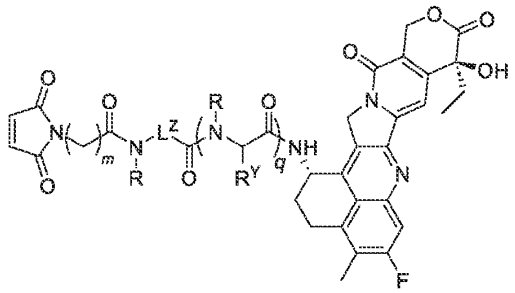
VI-BB

or a pharmaceutically acceptable salt thereof, wherein each Y, m, n, or R is independently as described herein.

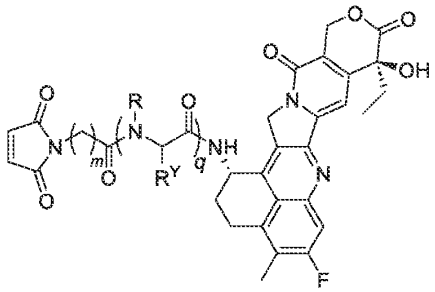
[0563] In some embodiments, the present disclosure provides compounds of formula VII -AA, VII -BB, or VII -CC:



VII-AA



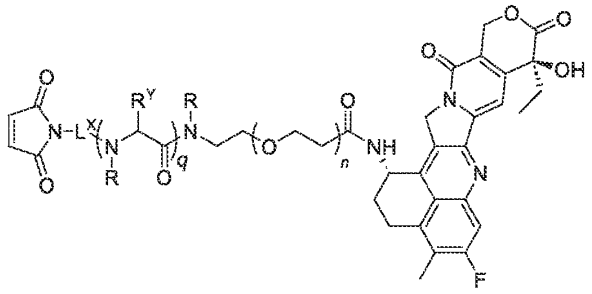
VII-BB



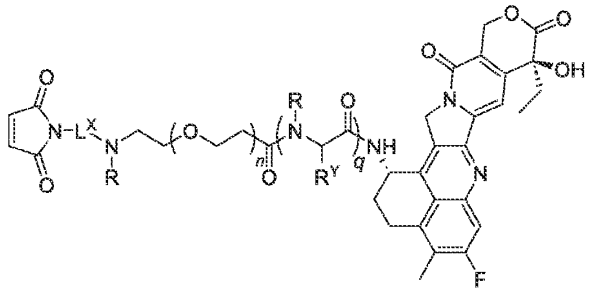
VII-CC

or a pharmaceutically acceptable salt thereof, wherein each L^Z, R^Y, m, q, or R is independently as described herein.

[0564] In some embodiments, the present disclosure provides compounds of formula VIII-AA or VIII-BB:



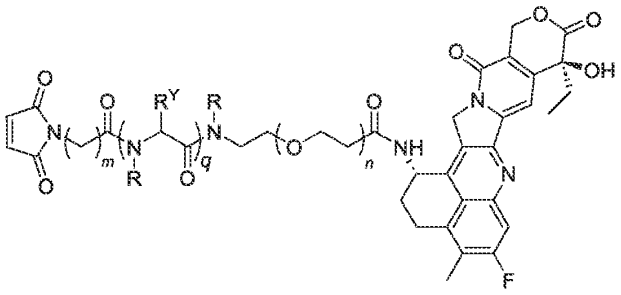
VIII-AA



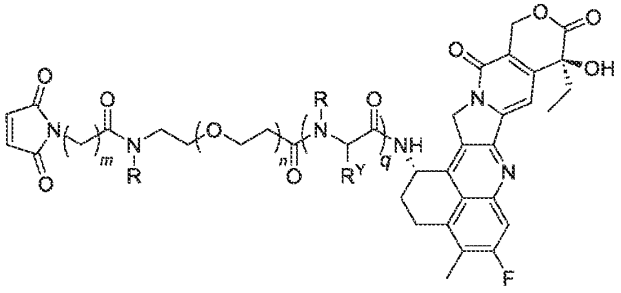
VIII-BB

or a pharmaceutically acceptable salt thereof, wherein each L^X, R^Y, n, q, or R is independently as described herein.

[0565] In some embodiments, the present disclosure provides compounds of formula IX-AA or IX-BB:



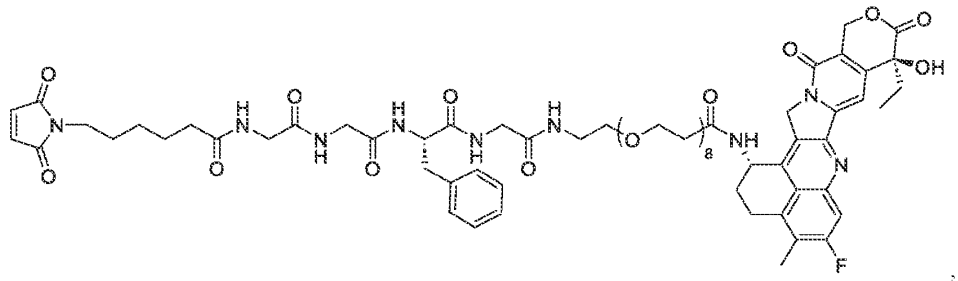
IX-AA

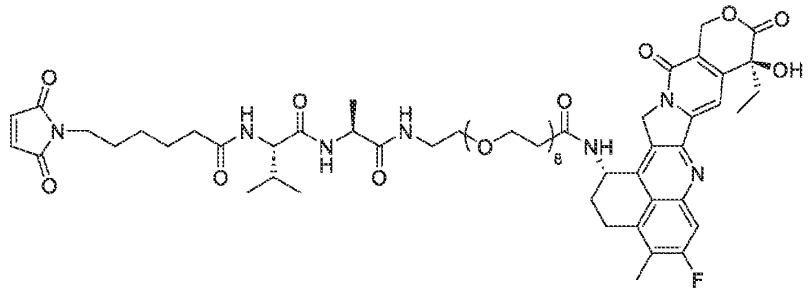


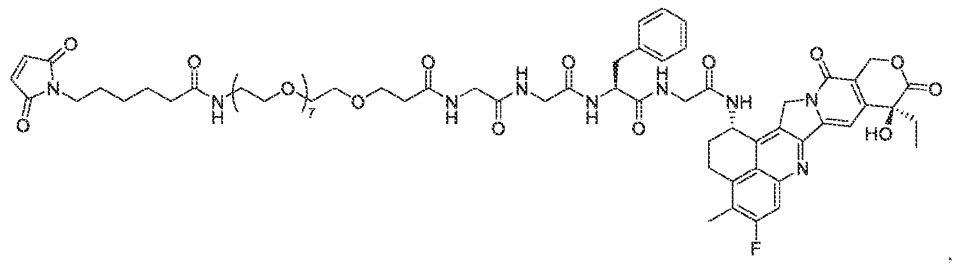
IX-BB

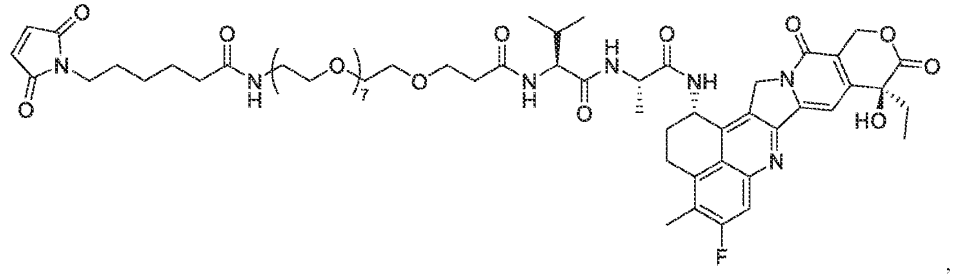
or a pharmaceutically acceptable salt thereof, wherein each R^Y, m, n, q, or R is independently as described herein.

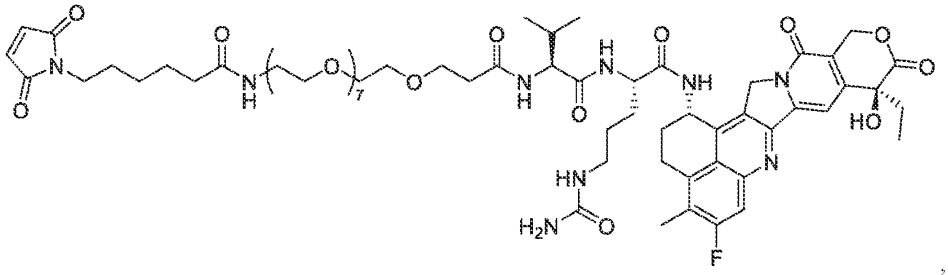
[0566] In some embodiments, a provided compound is

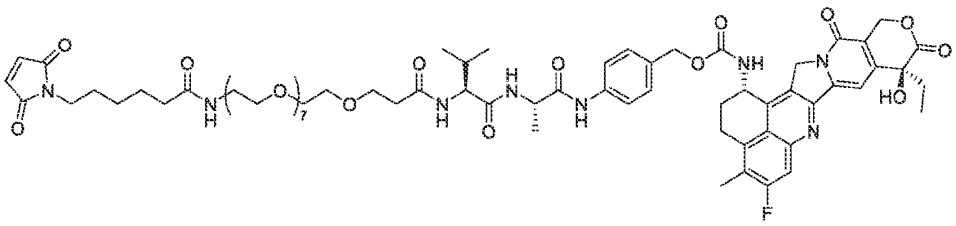


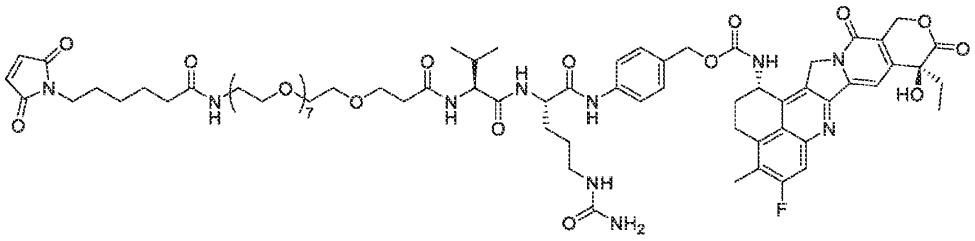


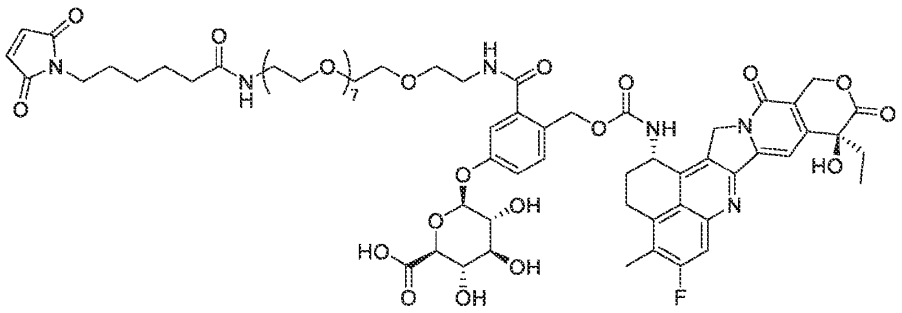


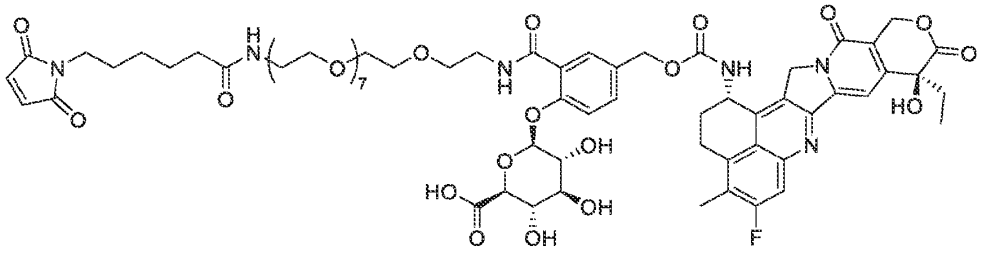


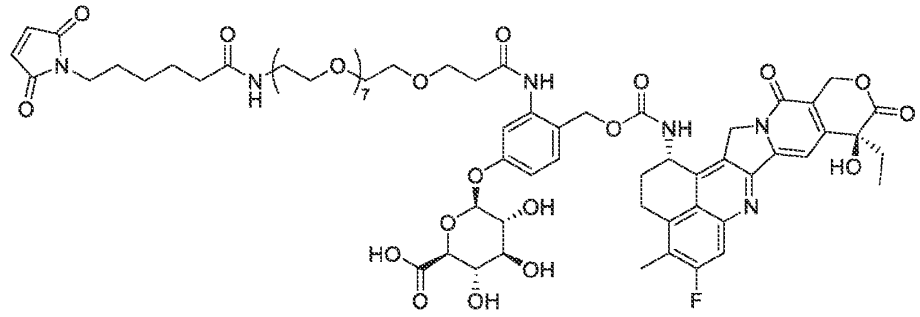




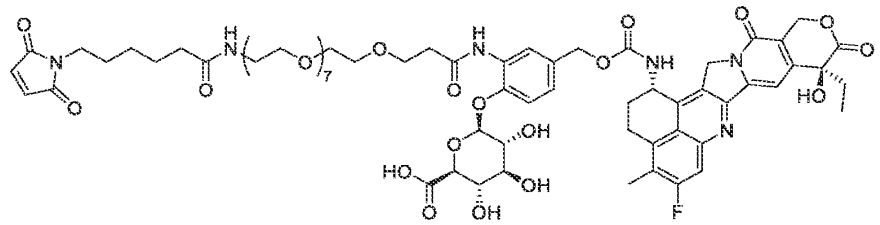






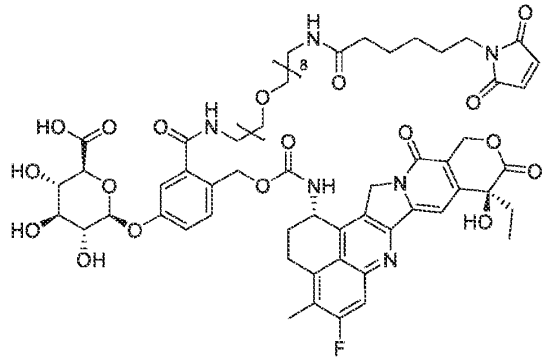


, or

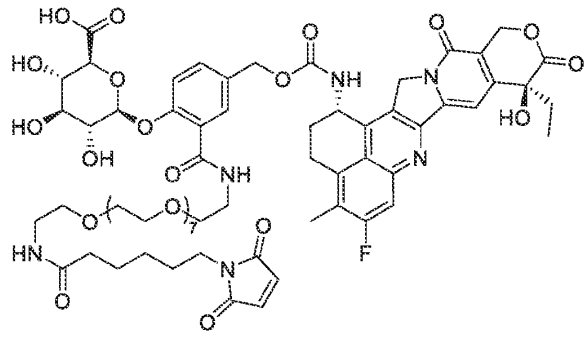


, or a salt,

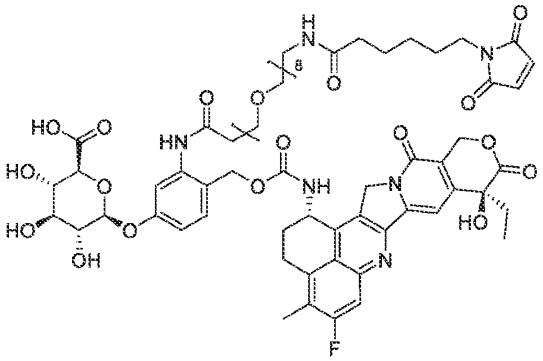
e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, a compound is

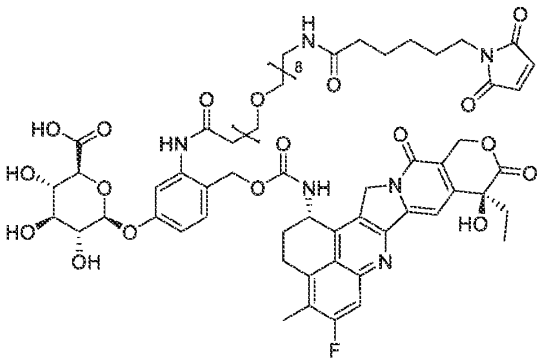


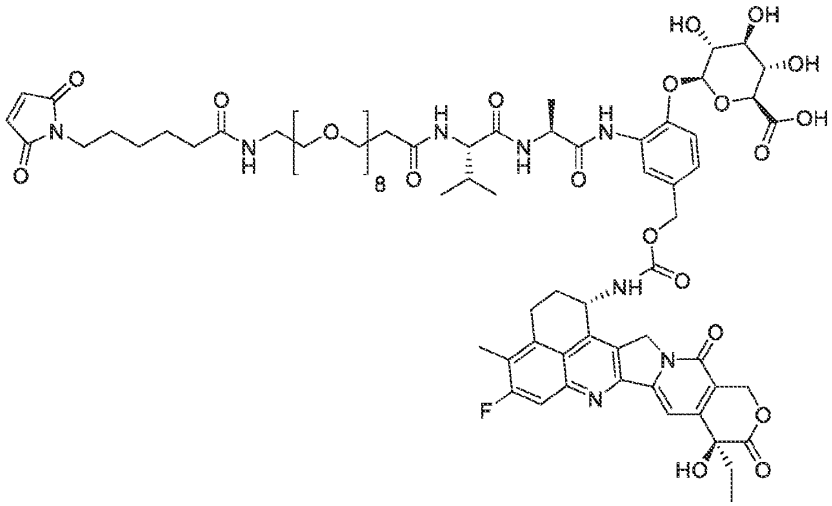
,



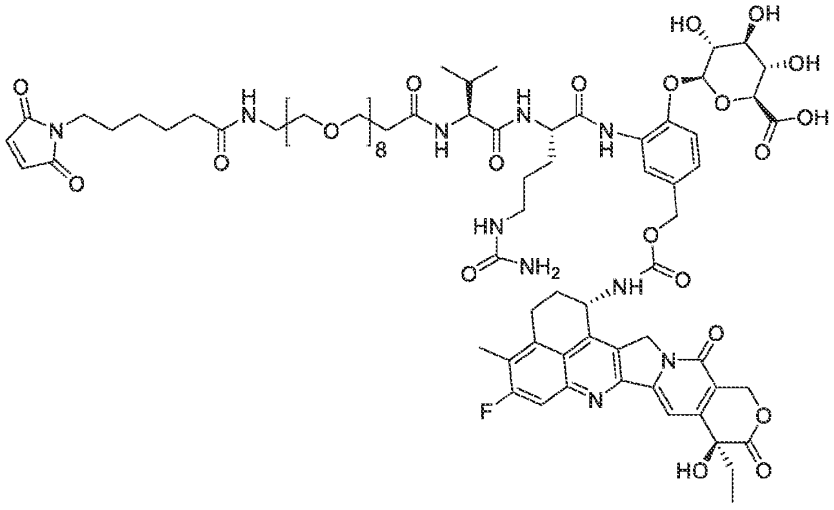
.





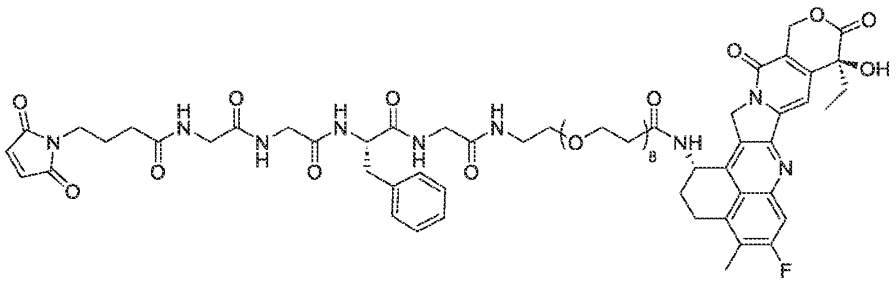


, or

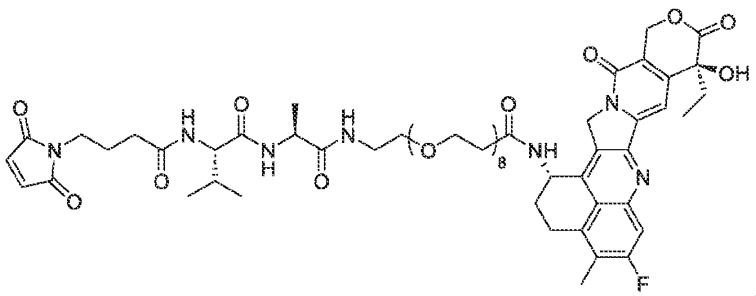


, or a salt, e.g., a pharmaceutically acceptable salt, thereof.

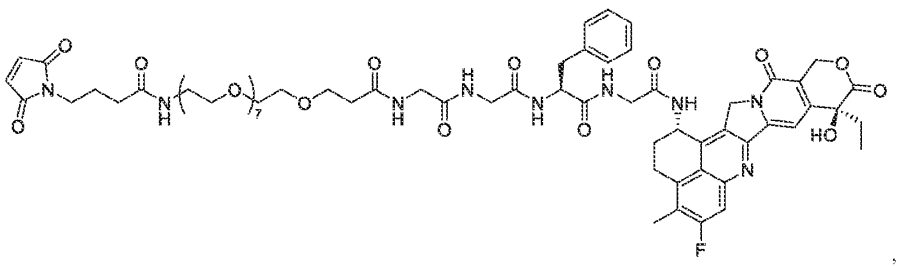
[0567] In some embodiments, a compound is



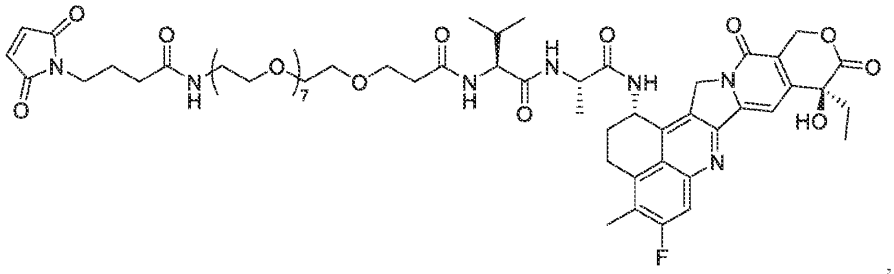
,

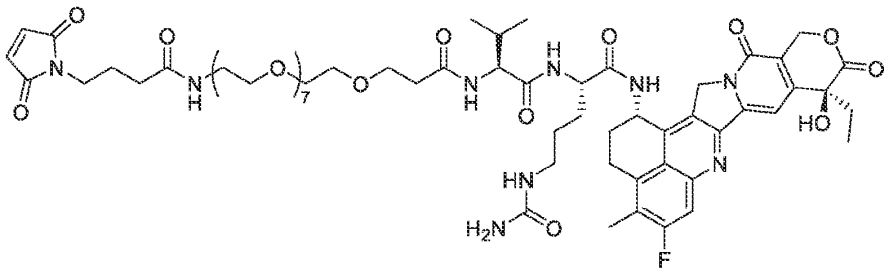


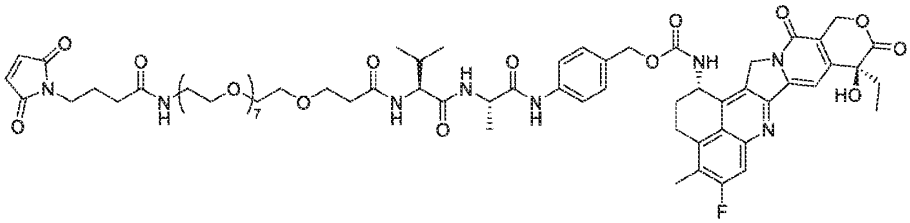
,

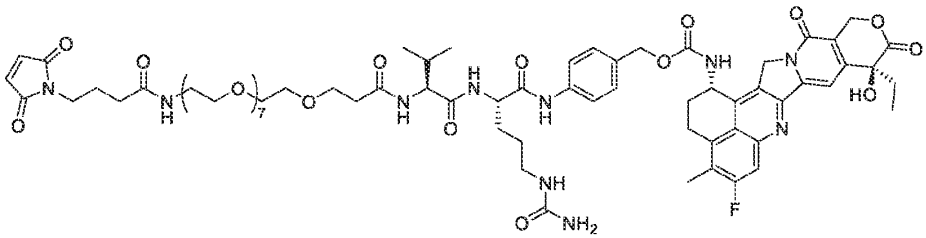


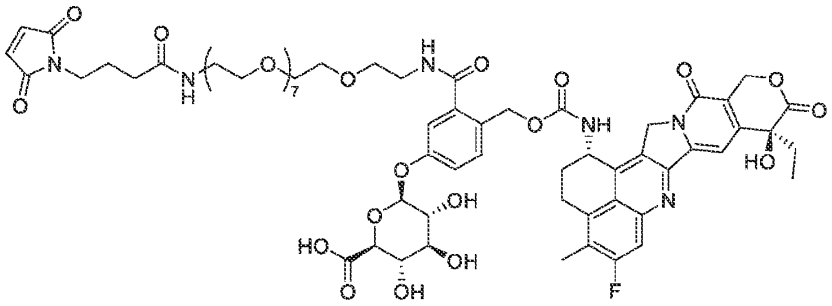
,

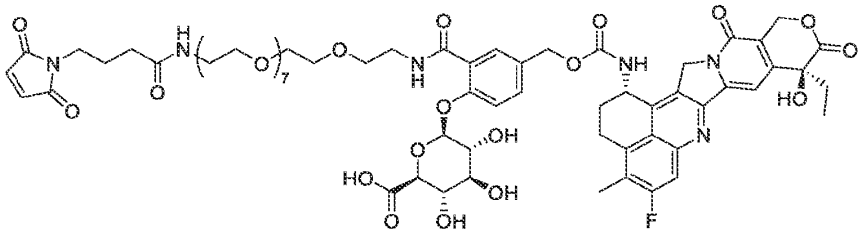




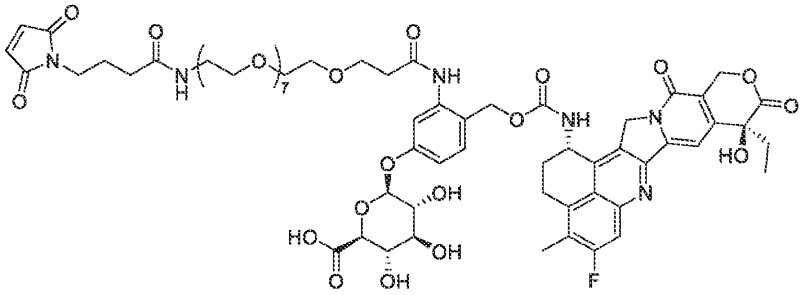




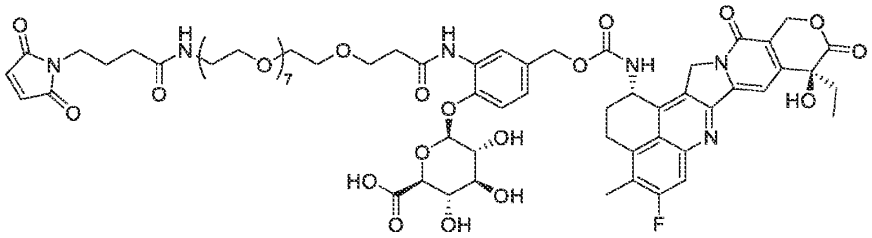




,

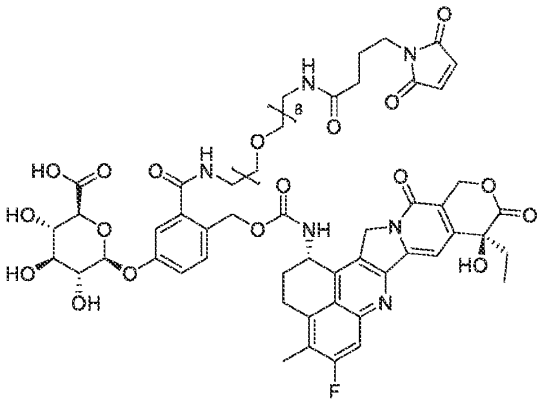


, or

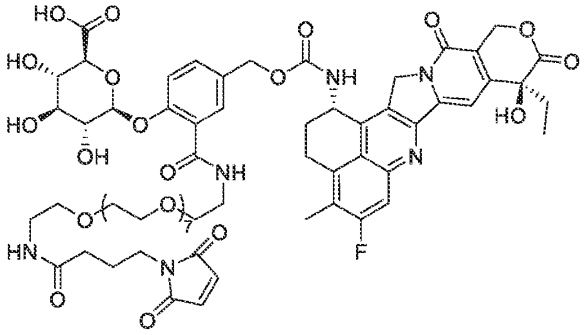


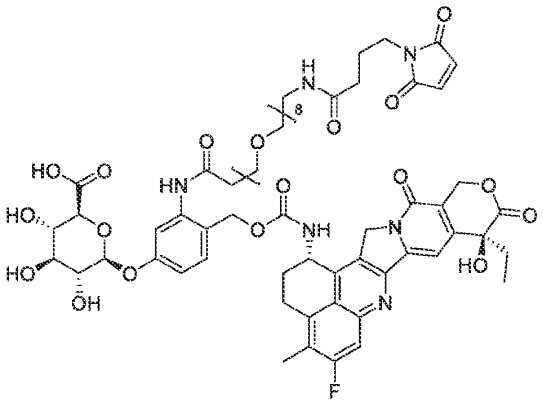
, or a salt,

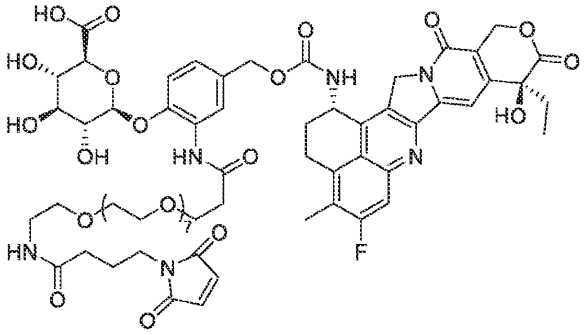
e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, a compound is

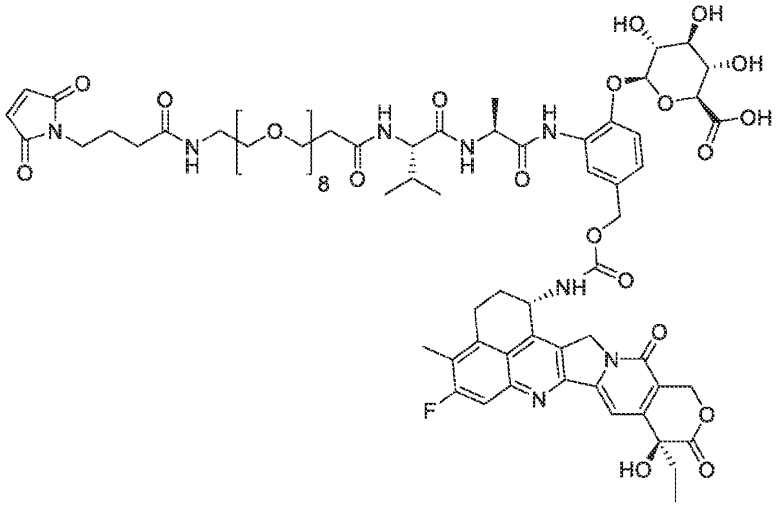


,

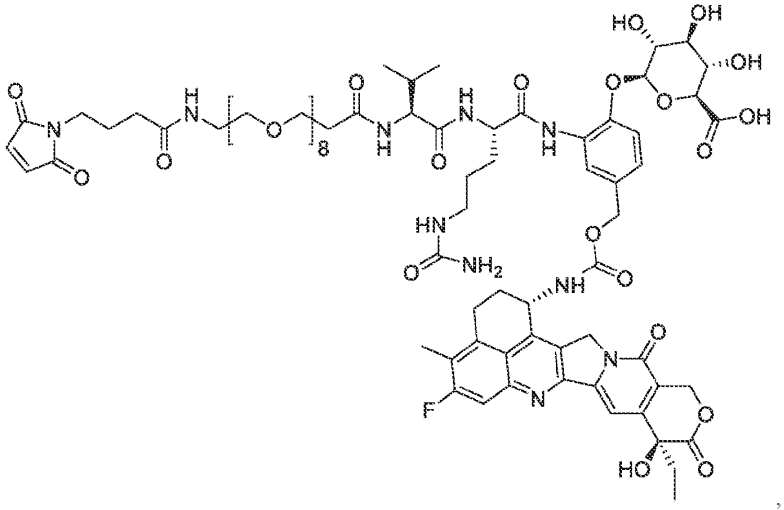








, or



, or a salt, e.g., a pharmaceutically acceptable salt thereof. In some embodiments, a compound is Compound 1 or a salt thereof. In some embodiments, a compound is Compound 2 or a salt thereof. In some embodiments, a compound is Compound 3 or a salt thereof. In some embodiments, a compound is Compound 4 or a salt thereof. In some embodiments, a compound is Compound 5 or a salt thereof. In some embodiments, a compound is Compound 6 or a salt thereof. In some embodiments, a compound is LP-A or a salt thereof. In some embodiments, a compound is LP-B or a salt thereof. In some embodiments, a compound is LP-C or a salt thereof. In some embodiments, a compound is LP-D or a salt thereof.

HHLA2 Binding Agents

[0568] Various target binding agents are reported or can be developed for use in accordance with the present disclosure. In some embodiments, a target binding agent is or comprises an antibody or an antigen-binding fragment thereof. In some embodiments, a target binding agent comprises a target binding

moiety and a target binding agent reactive group (e.g., L^{RXT} as described herein). Various HHLA2 binding agents are described herein as examples.

[0569] The present disclosure, among other things, provides conjugates comprising an HHLA2 binding moiety. In some embodiments, an HHLA2 binding agent exhibits the ability to: (i) inhibit HHLA2 binding to one or more receptors that inhibit an immune response (e.g. KIR3DL3), and/or (ii) enhance HHLA2 binding to one or more receptors that promote an immune response (e.g. TMIGD2). In some embodiments, HHLA2 binding agents described herein, e.g., conjugates comprising HHLA2 binding moieties, are particularly useful for treating a variety of cancers, including solid and hematological tumors, as well as modulating an immune response in a subject.

[0570] HHLA2 is a member of the B7 family. HHLA2 is also reported as HERV-H LTR-associating 2, B7y, B7H7, or B7-H7. It is reported to be broadly expressed in a variety of tumors and antigen presenting cells. According to some reports, HHLA2 protein has limited expression in normal human tissues, but is widely expressed in human cancers. It is reported that HHLA2 is a membrane protein with three Ig-like domains (IgV-IgC-IgV) whereas other members of the B7 family generally have only two Ig domains (IgV-IgC). According to some reports, HHLA2 in normal human tissues is expressed in the epithelium of kidney, gut, gallbladder, and breast as well as placental trophoblast cells. In the immune system, HHLA2 is reported to be constitutively expressed on human monocytes and macrophages. It is reported that HHLA2 regulates human T cell functions including, for example, T cell proliferation and cytokine production. According to some reports, HHLA2 is expressed in higher levels in a wide range of human cancers from the colorectal, renal, lung, pancreas, ovary, and prostate. HHLA2 is also reported to be expressed in human cancers of thyroid, melanoma, liver, bladder, colon, kidney, breast, and esophagus. HHLA2 is reported to interact with both inhibitory and stimulatory receptors to regulate T-cell functions. It is reported that killer-cell immunoglobulin-like receptor (KIR) proteins include either two (KIR2D) or three (KIR3D) immunoglobulin-like extracellular domains, and KIR3DL3 is an inhibitory HHLA2 receptor found on T cells and NK cells. HHLA2 binding to KIR3DL3 has been reported to inhibit the immune response of activated T cells and the cytotoxic activity of NK cells. In contrast, transmembrane and immunoglobulin domain containing 2 (TMIGD2) is reported to be an activating receptor for HHLA2. Concomitant with T cell receptor (TCR) signaling, TMIGD2 on naïve T cells is reported to interact with HHLA2 and co-stimulates T cell activation via a pathway involving AKT phosphorylation. It is reported that with repetitive T cell activation, expression of stimulatory receptor TMIGD2 is gradually lost, allowing expression of the inhibitory receptor KIR3DL3 to become dominant. While HHLA2 binding agents have been investigated as immunotherapies (see, e.g., WO 2022/165258 A1), the present disclosure provides conjugates in which HHLA2 binding agents are conjugated to payloads. Such conjugates, in some embodiments are "antibody drug conjugates" or "ADC" for short, are composed of target binding moieties (e.g., an HHLA2 binding agent that binds its antigen), payload moieties (e.g., a drug that acts intracellularly), and linkers that connects the targeting moiety to the payload.

[0571] Unless otherwise specified, the term "HHLA2" includes HHLA2 and fragments, variants (e.g., allelic variants), and derivatives thereof. Representative human HHLA2 cDNA and human HHLA2

protein sequences are publicly available from the National Center for Biotechnology Information (NCBI). Human HHLA2 variants include variant 1 (NM_007072.3 and NP_009003.1, which represents the longest transcript and encodes the longest isoform a), variant 2 (NM_001282556.1 and NP_001269485.1, which represents the use of an alternate promoter and differs in the 5' UTR, compared to variant 1), variant 3 (NM_001282557.1 and NP_001269486.1, which represents the use of an alternate promoter and differs in the 5' UTR, compared to variant 1), variant 4 (NM_001282558.1 and NP_001269487.1, which encodes isoform b, represents the use of an alternate promoter, differs in the 5' UTR and lacks an alternate in-frame exon in the 3' coding region, compared to variant 1, resulting a shorter isoform than isoform a), and variant 5 (NM_001282559.1 and NP_001269488.1, which encodes isoform c, represents the use of an alternate promoter, and has multiple differences compared to variant 2, resulting in a distinct 5' UTR and causing translation initiation at an alternate start codon, compared to variant 1, resulting in a distinct N-terminus and a shorter isoform than isoform a).

[0572] In some embodiments, binding of an HHLA2 binding agent (e.g., an HHLA2 antibody, a conjugate comprising a target binding moiety targeting HHLA2, etc.) described herein to HHLA2 is assessed using an assay, such as bio-layer interferometry (BLI), immunohistochemical (IHC), western blot, intercellular flow, ELISA, surface plasmon resonance (SPR), isothermal titration calorimetry (ITC), or any other known method in the art). In some embodiments, an HHLA2 binding agent described herein binds to HHLA2 with a K_D of about 20 nM to about 0.1 nM, e.g., about 10 nM to about 0.1 nM, e.g., about 5 nM to about 0.5 nM. In some embodiments, an HHLA2 binding agent described herein binds to HHLA2 with a K_D of about 20 nM or less, about 15 nM or less, about 10 nM or less, about 9 nM or less, about 8 nM or less, about 7 nM or less, about 6 nM or less, about 5 nM or less, about 4nM or less, about 3 nM or less, about 2 nM or less, about 1 nM or less, about 0.5 nM or less, or about 0.1 nM or less. As those skilled in the art appreciate, such technologies including levels can be utilized for assessing agents binding to other targets.

[0573] In some embodiments, HHLA2 binding agents described herein inhibit HHLA2 binding to KIR3DL3. The terms "KIR3DL3" or "Killer cell immunoglobulin-like receptor 3DL3," as used herein, refer to a member of the Killer cell immunoglobulin-like receptor transmembrane glycoprotein family expressed by NK cells and T cells. KIR3DL3 is also reported as KIRC1, CD158Z, KIR3DL7, and KIR44. The killer cell immunoglobulin-like receptor (KIR) genes are polymorphic and highly homologous genes found in a cluster on chromosome 19q13.4 within the 1 Mb leukocyte receptor complex (LRC). The gene content of the KIR gene cluster varies among haplotypes, although several "framework" genes are found in all haplotypes (KIR3DL3, KIR3DL1, KIR3DL4, and KIR3DL2). The KIR proteins are classified by the number of extracellular immunoglobulin domains (2D or 3D) and by whether they have a long (L) or short (S) cytoplasmic domain. KIR proteins with the long cytoplasmic domain transduce inhibitory signals upon ligand binding via an immune tyrosine-based inhibitory motif (ITIM), while KIR proteins with the short cytoplasmic domain lack the ITIM motif and instead associate with the TYRO protein tyrosine kinase binding protein to transduce activating signals. The ligands for several KIR proteins are subsets of HLA class I molecules, thus, KIR proteins are thought to play an

important role in regulation of the immune response. The KIR3DL3 protein has an N-terminal signal sequence, 3 Ig domains, a transmembrane region lacking a positively charged residue, and a long cytoplasmic tail containing an ITIM. KIR3DL3 lacks the stalk region found in other KIRs.

[0574] Unless otherwise specified, the term "KIR3DL3" includes KIR3DL3 and fragments, variants (e.g., allelic variants), and derivatives thereof. Representative human KIR3DL3 cDNA and human KIR3DL3 polypeptide sequences are publicly available from NCBI. For example, at least one human KIR3DL3 isoform is known: human KIR3DL3 (NM_153443.4) encoded by the transcript (NP_703144.3). Nucleic acid and polypeptide sequences of KIR3DL3 orthologs in organisms other than humans are also known including, but not limited to, chimpanzee KIR3DL3 (XM_003316679.3 and XP_003316727.3), Rhesus monkey KIR3DL3 (NM_001104552.2 and NP_001098022.1), mouse KIR3DL3 (NM_001310690.1 and NP_001297619.1, NM_177749.4 and NP_808417.2, NM_177748.2 and NP_808416.1), and rat KIR3DL3 (NM_181479.2 and NP_852144.1).

[0575] In some embodiments, inhibition of HHLA2 binding to KIR3DL3 by an HHLA2 binding agent described herein is assessed using an assay, such as a cell binding competition assay (e.g., an assay of soluble HHLA2 binding to KIR3DL3-expressing cells (e.g., KIR3DL3-expressing 300.19 mouse pre-B leukemic cells)), surface plasmon resonance (SPR), or any other known method in the art. In some embodiments, an HHLA2 binding agent described herein inhibits binding of HHLA2 to KIR3DL3 at a ratio of about 0.8 to about 0.0, e.g., relative to an isotope control. In some embodiments, an HHLA2 binding agent described herein inhibits binding of HHLA2 to KIR3DL3 at a ratio of about 0.8, about 0.7, about 0.6, about 0.5, about 0.4, about 0.3, about 0.2, about 0.1, or about 0.0, e.g., relative to an isotope control. In some embodiments, an HHLA2 binding agent described herein abolishes binding of HHLA2 to KIR3DL3. As those skilled in the art appreciate, such technologies including levels can be utilized for assessing agents binding to other targets.

[0576] In some embodiments, HHLA2 binding agents described herein enhance HHLA2 binding to TMIGD2. The terms "TMIGD2" or "transmembrane and immunoglobulin domain containing 2 (TMIGD2)," as used herein, refer to a membrane protein having an extracellular IgV-like domain, a transmembrane region, and a proline-rich cytoplasmic domain with two tyrosine signaling motifs. TMIGD2 is constitutively expressed on naïve T cells and natural killer (NK) cells, but not on T regulatory cells or B cells. TMIGD2 expression is slowly lost with repetitive stimulation of T cells. Consistent with this, TMIGD2 is expressed on only about half of memory T cells, and TMIGD2-negative T cells have a terminally-differentiated, senescent phenotype. TMIGD2 is also expressed in endothelial and epithelial cells and functions to reduce cell migration and promote capillary tube formation during angiogenesis. In some embodiments, HHLA2 binding agents described herein inhibit HHLA2 binding to TMIGD2.

[0577] Unless otherwise specified, the term "TMIGD2" includes TIMIGD2 and fragments, variants (e.g., allelic variants), and derivatives thereof. Representative human TMIGD2 cDNA and human TMIGD2 protein sequences are publicly available from NCBI. Human TMIGD2 isoforms include isoform 1 (NM_144615.2 and NP 653216.2), isoform 2 (NM_001169126.1 and NP 001162597.1; which uses an alternate in-frame splice site in the 3' coding region, compared to variant 1, resulting a shorter

isoform, compared to isoform 1), and isoform 3 (NM_001308232.1 and NP 001295161.1, which lacks an alternate in-frame exon in the 5' coding region compared to variant 1, resulting a shorter isoform, compared to isoform 1). Nucleic acid and polypeptide sequences of TMIGD2 orthologs in organisms other than humans are also known including, for example, chimpanzee TMIGD2 (XM_009434393.2 and XP _009432668.2, and XM_OOI 138228.4 and XP _001138228.3), and cattle TMIGD2 (XM_005208980.3 and XP 005209037.1, XM_005208979.3 and XP _005209036.1, and XM_002688933.5 and XP _002688979.1).

[0578] In some embodiments, enhancement of HHLA2 binding to TMIGD2 by an HHLA2 binding agent described herein is assessed using an assay, such as a cell binding competition assay (e.g., an assay of soluble TMIGD2 binding to HHLA2-expressing cells (e.g., HHLA2-expressing 300.19 mouse pre-B leukemic cells)), or any other known method in the art. In some embodiments, an HHLA2 binding agent described herein enhances binding of HHLA2 to TMIGD2 at a ratio of about 2.0 to about 8.0, e.g., relative to an isotope control. In some embodiments, an HHLA2 binding agent described herein enhances binding of HHLA2 to TMIGD2 at a ratio of about 2.0, about 2.5, about 3.0, about 3.5, about 4.0, about 4.5, about 5.0, about 5.5, about 6.0, about 6.5, about 7.0, about 7.5, about 8.0, or greater, e.g., relative to an isotope control. As those skilled in the art appreciate, such technologies including levels can be utilized for assessing agents binding to other targets.

[0579] In some embodiments, an HHLA2 binding agent is or comprises an antibody or antigen-binding fragment thereof. In some embodiments, an HHLA2 binding agent is or comprises an organic molecule (e.g., a small molecule). In some embodiments, an HHLA2 binding agent is or comprises a polypeptide (e.g., a fusion polypeptide). In some embodiments, an HHLA2 binding agent is or comprises an aptamer. In some embodiments, an HHLA2 binding agent is or comprises a nucleic acid. In some embodiments, an HHLA2 binding agent is or comprises a chimeric antigen receptor (e.g., a CAR comprising an anti-HHLA2 antigen-binding fragment described herein, such as an scFv).

Anti-HHLA2 antibodies and fragments thereof

[0580] Various antibodies and fragments thereof (e.g., antigen-binding fragments thereof) can be utilized in accordance with the present disclosure. Certain examples of anti-HHLA2 antibodies and fragments thereof are described below as examples.

[0581] The present disclosure, among other things, provides anti-HHLA2 antibodies or antigen-binding fragments thereof. In some embodiments, an anti-HHLA2 antibody or antigen-binding fragment thereof described herein binds specifically to an epitope on HHLA2. In some embodiments, an anti-HHLA2 antibody or antigen-binding fragment thereof described herein can be or comprise an immunoglobulin, heavy chain antibody, light chain antibody, or other protein scaffold with antibody-like properties, as well as other immunological binding moiety known in the art, including a Fab fragment, a Fab' fragment, a F(ab')2 fragment, a Fv fragment, a disulfide-bonded Fv fragment, a scFv fragment, a diabody, a triabody, a tetrabody, a minibody, a maxibody, a tandab, BiTe, or any combination thereof. In some embodiments, an anti-HHLA2 antibody or antigen-binding fragment thereof described herein

inhibits binding of HHLA2 to KIR3DL3. In some embodiments, an anti-HHLA2 antibody or antigen-binding fragment thereof described herein enhances binding of HHLA2 to TIMGD2.

[0582] In some embodiments, an anti-HHLA2 antibody or antigen-binding fragment thereof described herein comprises or is a monoclonal antibody. In some embodiments, an anti-HHLA2 antibody or antigen-binding fragment thereof described herein comprises or is a full length antibody, e.g., comprising an immunoglobulin Fc region. In some embodiments, an anti-HHLA2 antibody or antigen-binding fragment thereof described herein comprises or is a multispecific antibody, e.g., comprising a plurality of immunoglobulin variable domain sequences, wherein a first immunoglobulin variable domain sequence of the plurality has binding specificity for a first epitope and a second immunoglobulin variable domain sequence of the plurality has binding specificity for a second epitope. In some embodiments, an anti-HHLA2 antibody or antigen-binding fragment thereof described herein comprises or is a bispecific antibody molecule. In some embodiments, an anti-HHLA2 antibody or antigen-binding fragment thereof described herein is or has been affinity matured.

[0583] An anti-HHLA2 antibody or antigen-binding fragment thereof can include a heavy chain variable domain sequence (VH), and a light chain variable domain sequence (VL). In some embodiments, an anti-HHLA2 antibody or antigen-binding fragment thereof comprises an immunoglobulin molecule of four polypeptide chains, e.g., two heavy chains and two light chains. A heavy chain can include a VH and a heavy chain constant domain. A heavy chain constant domain can include CH1, hinge, CH2, CH3, and optionally, a CH4 region. A light chain can include a VL and a light chain constant domain. A light chain constant domain can include a CL domain.

[0584] A VH and/or a VL can be further subdivided into regions of variability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Such VH and/or VL domains can each include three CDRs and four framework regions, arranged from amino-terminus to carboxyl-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4, one or more of which can be engineered as described herein. In general, there are three CDRs in each VH (HCDR1, HCDR2, and HCDR3) and three CDRs in each VL (LCDR1, LCDR2, and LCDR3). The extent of the framework region and CDRs can be defined using a number of well-known schemes (see, e.g., Kabat, E. A., et al. (1991) Sequences of Proteins of Immunological Interest. Fifth Edition, U.S. Department of Health and Human Services. NIH Publication No. 91-3242; Chothia, C. et al. (1987) J. Mol. Biol. 196:901-917; and the AbM definition used by Oxford Molecular's AbM antibody modeling software, each of which is hereby incorporated by reference in its entirety).

[0585] An anti-HHLA2 antibody or antigen-binding fragment thereof described herein can be from any class of antibodies including, but not limited to, IgG, IgA, IgM, IgD, and IgE, and from any subclass (e.g., IgG1, IgG2, IgG3, and IgG4) of antibodies. An anti-HHLA2 antibody or antigen-binding fragment thereof described herein can be or comprise a human, humanized, CDR-grafted, or in vitro generated antibody. An anti-HHLA2 antibody or antigen-binding fragment thereof described herein can have or comprise a heavy chain constant region chosen from, e.g., IgG1, IgG2, IgG3, or IgG4. An anti-HHLA2 antibody or fragment can have or comprise a light chain chosen from, e.g., kappa or lambda.

[0586] In some embodiments, an anti-HHLA2 antibody or antigen-binding fragment thereof described herein is or comprises a monoclonal antibody. Typically, monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, such that the individual antibodies comprising the population are substantially identical, except for possible naturally occurring mutations that may be present in minor amounts. Thus, the modifier "monoclonal" as used herein, indicates the character of the antibody as not being a mixture of discrete antibodies. In some embodiments, monoclonal antibodies directed to a particular epitope are derived from a single cell line (e.g., a B cell line).

[0587] In some embodiments, an anti-HHLA2 antibody or antigen-binding fragment thereof described herein is or comprises a polyclonal antibody. In contrast to monoclonal antibodies, polyclonal antibodies are typically obtained from a population of heterogeneous antibodies, such that the antibodies in a particular population include structural variation, for example, affinity for different epitopes on a particular target (e.g., HHLA2). Several methods of producing polyclonal antibodies are known in the art, including use of multiple subcutaneous and/or intraperitoneal injections of the relevant antigen into an animal, optionally including co-administration of one or more adjuvants.

[0588] In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises: (a) a VH comprising one, two, or three VH CDR sequences each with at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more identity to a VH CDR in Table 1; and/or (b) a VL comprising one, two, or three VL CDR sequences each with at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more identity to a VL CDR in Table 1. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises: (a) a VH with at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more identity to a VH in Table 1; and/or (a) a VL with at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more identity to a VL in Table 1. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises: (a) a heavy chain with at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more identity to a heavy chain in Table 1; and/or (a) a light chain with at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% or more identity to a light chain in Table 1.

[0589] In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises: (a) a VH comprising a VH CDR1 amino acid sequence of SEQ ID NO: 1, a VH CDR2 amino acid sequence of SEQ ID NO: 2, and a VH CDR3 amino acid sequence of SEQ ID NO: 3; and (b) a VL comprising a VL CDR1 amino acid sequence of SEQ ID NO: 14, a VL CDR2 amino acid sequence of SEQ ID NO: 15, and a VL CDR3 amino acid sequence of SEQ ID NO: 16. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises: (a) a VH comprising a VH CDR1 amino acid sequence of SEQ ID NO: 4, a VH CDR2 amino acid sequence of SEQ ID NO: 5, and a VH CDR3 amino acid sequence of SEQ ID NO: 6; and (b) a VL comprising a VL CDR1 amino acid sequence of SEQ ID NO: 17, a VL CDR2 amino acid sequence of

SEQ ID NO: 18, and a VL CDR3 amino acid sequence of SEQ ID NO: 19, each disclosed in Table 1. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises: (a) a VH comprising a VH CDR1 amino acid sequence of SEQ ID NO: 7, a VH CDR2 amino acid sequence of SEQ ID NO: 8, and a VH CDR3 amino acid sequence of SEQ ID NO: 9; and (b) a VL comprising a VL CDR1 amino acid sequence of SEQ ID NO: 20, a VL CDR2 amino acid sequence of SEQ ID NO: 21, and a VL CDR3 amino acid sequence of SEQ ID NO: 22, each disclosed in Table 1.

[0590] In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a VH comprising an amino acid sequence of SEQ ID NO: 10, or an amino acid sequence at least 85%, 90%, 95%, or 99% identical or higher to SEQ ID NO: 10. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a VL comprising an amino acid sequence of SEQ ID NO: 23, or an amino acid sequence at least 85%, 90%, 95%, or 99% identical or higher to SEQ ID NO: 23. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a VH comprising an amino acid sequence of SEQ ID NO: 10 and a VL comprising an amino acid sequence of SEQ ID NO: 23.

[0591] In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 12, or an amino acid sequence at least 85%, 90%, 95%, or 99% identical or higher to SEQ ID NO: 12. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a light chain comprising an amino acid sequence of SEQ ID NO: 25, or an amino acid sequence at least 85%, 90%, 95%, or 99% identical or higher to SEQ ID NO: 25. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 12 and a light chain comprising an amino acid sequence of SEQ ID NO: 25.

[0592] In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises: (a) a VH comprising a VH CDR1 amino acid sequence of SEQ ID NO: 27, a VH CDR2 amino acid sequence of SEQ ID NO: 28, and a VH CDR3 amino acid sequence of SEQ ID NO: 29; and (b) a VL comprising a VL CDR1 amino acid sequence of SEQ ID NO: 40, a VL CDR2 amino acid sequence of SEQ ID NO: 41, and a VL CDR3 amino acid sequence of SEQ ID NO: 42. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises: (a) a VH comprising a VH CDR1 amino acid sequence of SEQ ID NO: 30, a VH CDR2 amino acid sequence of SEQ ID NO: 31, and a VH CDR3 amino acid sequence of SEQ ID NO: 32; and (b) a VL comprising a VL CDR1 amino acid sequence of SEQ ID NO: 43, a VL CDR2 amino acid sequence of SEQ ID NO: 44, and a VL CDR3 amino acid sequence of SEQ ID NO: 45. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises: (a) a VH comprising a VH CDR1 amino acid sequence of SEQ ID NO: 33, a VH CDR2 amino acid sequence of SEQ ID NO: 34, and a VH CDR3 amino acid sequence of SEQ ID NO: 35; and (b) a VL comprising a VL CDR1 amino acid sequence of SEQ ID NO: 46, a VL CDR2 amino acid sequence of SEQ ID NO: 47, and a VL CDR3 amino acid sequence of SEQ ID NO: 48.

[0593] In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a VH comprising an amino acid sequence of SEQ ID NO: 36, or an amino acid sequence at least 85%, 90%, 95%, or 99% identical or higher to SEQ ID NO: 36. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a VL comprising an amino acid sequence of SEQ ID NO: 49, or an amino acid sequence at least 85%, 90%, 95%, or 99% identical or higher to SEQ ID NO: 49. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a VH comprising an amino acid sequence of SEQ ID NO: 36 and a VL comprising an amino acid sequence of SEQ ID NO: 49.

[0594] In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 38, or an amino acid sequence at least 85%, 90%, 95%, or 99% identical or higher to SEQ ID NO: 38. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a light chain comprising an amino acid sequence of SEQ ID NO: 51, or an amino acid sequence at least 85%, 90%, 95%, or 99% identical or higher to SEQ ID NO: 51. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 38, and a light chain comprising an amino acid sequence of SEQ ID NO: 51.

[0595] In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises: (a) a VH comprising a VH CDR1 amino acid sequence of SEQ ID NO: 53, a VH CDR2 amino acid sequence of SEQ ID NO: 54, and a VH CDR3 amino acid sequence of SEQ ID NO: 55; and (b) a VL comprising a VL CDR1 amino acid sequence of SEQ ID NO: 66, a VL CDR2 amino acid sequence of SEQ ID NO: 67, and a VL CDR3 amino acid sequence of SEQ ID NO: 68. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises: (a) a VH comprising a VH CDR1 amino acid sequence of SEQ ID NO: 56, a VH CDR2 amino acid sequence of SEQ ID NO: 57, and a VH CDR3 amino acid sequence of SEQ ID NO: 58; and (b) a VL comprising a VL CDR1 amino acid sequence of SEQ ID NO: 69, a VL CDR2 amino acid sequence of SEQ ID NO: 70, and a VL CDR3 amino acid sequence of SEQ ID NO: 71. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises: (a) a VH comprising a VH CDR1 amino acid sequence of SEQ ID NO: 59, a VH CDR2 amino acid sequence of SEQ ID NO: 60, and a VH CDR3 amino acid sequence of SEQ ID NO: 61; and (b) a VL comprising a VL CDR1 amino acid sequence of SEQ ID NO: 72, a VL CDR2 amino acid sequence of SEQ ID NO: 73, and a VL CDR3 amino acid sequence of SEQ ID NO: 74.

[0596] In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a VH comprising an amino acid sequence of SEQ ID NO: 62, or an amino acid sequence at least 85%, 90%, 95%, or 99% identical or higher to SEQ ID NO: 62. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a VL comprising an amino acid sequence of SEQ ID NO: 75, or an amino acid sequence at least 85%, 90%, 95%, or 99% identical or higher to SEQ ID NO: 75. In some embodiments, an anti-

HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a VH comprising an amino acid sequence of SEQ ID NO: 62 and a VL comprising an amino acid sequence of SEQ ID NO: 75.

[0597] In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 64, or an amino acid sequence at least 85%, 90%, 95%, or 99% identical or higher to SEQ ID NO: 64. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a light chain comprising an amino acid sequence of SEQ ID NO: 77, or an amino acid sequence at least 85%, 90%, 95%, or 99% identical or higher to SEQ ID NO: 77. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 64, and a light chain comprising an amino acid sequence of SEQ ID NO: 77.

[0598] In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises: (a) a VH comprising a VH CDR1 amino acid sequence of SEQ ID NO: 79, a VH CDR2 amino acid sequence of SEQ ID NO: 80, and a VH CDR3 amino acid sequence of SEQ ID NO: 81; and (b) a VL comprising a VL CDR1 amino acid sequence of SEQ ID NO: 92, a VL CDR2 amino acid sequence of SEQ ID NO: 93, and a VL CDR3 amino acid sequence of SEQ ID NO: 94. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises: (a) a VH comprising a VH CDR1 amino acid sequence of SEQ ID NO: 82, a VH CDR2 amino acid sequence of SEQ ID NO: 83, and a VH CDR3 amino acid sequence of SEQ ID NO: 84; and (b) a VL comprising a VL CDR1 amino acid sequence of SEQ ID NO: 95, a VL CDR2 amino acid sequence of SEQ ID NO: 96, and a VL CDR3 amino acid sequence of SEQ ID NO: 97, each disclosed in Table 1. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises: (a) a VH comprising a VH CDR1 amino acid sequence of SEQ ID NO: 85, a VH CDR2 amino acid sequence of SEQ ID NO: 86, and a VH CDR3 amino acid sequence of SEQ ID NO: 87; and (b) a VL comprising a VL CDR1 amino acid sequence of SEQ ID NO: 98, a VL CDR2 amino acid sequence of SEQ ID NO: 99, and a VL CDR3 amino acid sequence of SEQ ID NO: 100, each disclosed in Table 1.

[0599] In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a VH comprising an amino acid sequence of SEQ ID NO: 88, or an amino acid sequence at least 85%, 90%, 95%, or 99% identical or higher to SEQ ID NO: 88. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a VL comprising an amino acid sequence of SEQ ID NO: 101, or an amino acid sequence at least 85%, 90%, 95%, or 99% identical or higher to SEQ ID NO: 101. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a VH comprising an amino acid sequence of SEQ ID NO: 88 and a VL comprising an amino acid sequence of SEQ ID NO: 101.

[0600] In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 90, or an amino acid sequence at least 85%, 90%, 95%, or 99% identical or higher to SEQ ID NO: 90. In some

embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a light chain comprising an amino acid sequence of SEQ ID NO: 103, or an amino acid sequence at least 85%, 90%, 95%, or 99% identical or higher to SEQ ID NO: 103. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 90 and a light chain comprising an amino acid sequence of SEQ ID NO: 103.

[0601] Table 1. Amino acid and nucleotide sequences of exemplary anti-HHLA2 antibodies.

Clone	Sequences	SEQ ID NO
887-1DE	Heavy Chain	
	HCDR1 (IMGT) GYTFEQYA	SEQ ID NO: 1
	HCDR2 (IMGT) IPAGTGNT	SEQ ID NO: 2
	HCDR3 (IMGT) ARDRGGYWYDSWDD	SEQ ID NO: 3
	HCDR1 (Kabat) QYAMH	SEQ ID NO: 4
	HCDR2 (Kabat) FIPAGTGNTKYSQKFQG	SEQ ID NO: 5
	HCDR3 (Kabat) DRGGYWYDSWDD	SEQ ID NO: 6
	HCDR1 (Combined Kabat/IMGT) GYTFEQYAMH	SEQ ID NO: 7
	HCDR2 (Combined Kabat/IMGT) FIPAGTGNTKYSQKFQG	SEQ ID NO: 8
	HCDR3 (Combined Kabat/IMGT) DRGGYWYDSWDD	SEQ ID NO: 9
	VH - Amino acid sequence QVQLVQSGAEVKKPGASVKVSCKASGYTFEQYAMHWVRQAPGQRL EWMGFIPAGTGNTKYSQKFQGRVTITRDTSASTAYMELSSLRSEDTA VYYCARDRGGYWYDSWDDWGQGTLVTVSS	SEQ ID NO: 10
	VH - DNA sequence CAGGTCCAGCTTGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG GCCTCAGTGAAGGTTTCCTGCAAGGCTTCTGGATACACCTTCGAAC AATATGCTATGCATTGGGTGCGCCAGGCCCCCGGACAAAGGCTTG AGTGGATGGGATTCATCCCCGCTGGCACTGGTAACACAAAATATT	SEQ ID NO: 11

Clone	Sequences	SEQ ID NO
	CACAGAAGTTCCAGGGCAGAGTCACCATTACCAGGGACACATCCG CGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCTGAAGACA CGGCGGTGTACTACTGCGCCAGAGACCGTGGCGGATACTGGTACG ACAGTTGGGATGATTGGGGACAGGGTACATTGGTCACCGTCTCCTC A	
	Heavy chain - Amino acid sequence QVQLVQSGAEVKKPGASVKVSCKASGYTFEQYAMHWVRQAPGQRL EWMGFIPAGTGNTKYSQKFQGRVTITRDTSASTAYMELSSLRSEDTA VYYCARDRGGYWYDSWDDWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPEEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK	SEQ ID NO: 12
	Heavy chain - DNA sequence CAGGTGCAGCTGGTGCAGAGCGGCGCGGAAGTGAAAAAACCGGG CGCGAGCGTGAAAGTGAGCTGCAAAGCGAGCGGCTATACCTTTGA ACAGTATGCGATGCATTGGGTGCGCCAGGCGCCGGGCCAGCGCCT GGAATGGATGGGCTTTATTCCGGCGGGCACCGGCAACACCAAATA TAGCCAGAAATTTCAGGGCCGCGTGACCATTACCCGCGATACCAG CGCGAGCACCGCGTATATGGAACTGAGCAGCCTGCGCAGCGAAGA TACCGCGGTGTATTATTGCGCGCGCGATCGCGGCGGCTATTGGTAT GATAGCTGGGATGATTGGGGCCAGGGCACCCTGGTGACCGTGAGC AGCGCGAGCACCAAAGGCCCGAGCGTGTTTCCGCTGGCGCCGAGC AGCAAAAGCACCAGCGGCGGCACCGCGGCGCTGGGCTGCCTGGTG AAAGATTATTTTCCGGAACCGGTGACCGTGAGCTGGAACAGCGGC GCGCTGACCAGCGGCGTGCATACCTTTCCGGCGGTGCTGCAGAGC AGCGGCCTGTATAGCCTGAGCAGCGTGGTGACCGTGCCGAGCAGC AGCCTGGGCACCCAGACCTATATTTGCAACGTGAACCATAAACCG AGCAACACCAAAGTGGATAAAAAAGTGGAACCGAAAAGCTGCGA TAAAACCCATACCTGCCCGCCGTGCCCGGCGCCGGAACTGCTGGG CGGCCCGGATGTGTTTCTGTTTCCGCCGAAACCGAAAGATACCCTG ATGATTAGCCGCACCCCGGAAGTGACCTGCGTGGTGGTGGATGTG AGCCATGAAGATCCGGAAGTGAAATTTAACTGGTATGTGGATGGC	SEQ ID NO: 13

Clone	Sequences	SEQ ID NO
	GTGGAAGTGCATAACGCGAAAACCAAACCGCGCGAAGAACAGTA TAACAGCACCTATCGCGTGGTGAGCGTGCTGACCGTGCTGCATCA GGATTGGCTGAACGGCAAAGAATATAAATGCAAAGTGAGCAACA AAGCGCTGCCGGCGCCGGAAGAAAAAACCATTAGCAAAGCGAAA GGCCAGCCGCGCGAACCGCAGGTGTATACCCTGCCGCCGAGCCGC GATGAACTGACCAAAAACCAGGTGAGCCTGACCTGCCTGGTGAAA GGCTTTTATCCGAGCGATATTGCGGTGGAATGGGAAAGCAACGGC CAGCCGGAAAACAACTATAAAACCACCCCGCCGGTGCTGGATAGC GATGGCAGCTTTTTTCTGTATAGCAAACTGACCGTGGATAAAAGCC GCTGGCAGCAGGGCAACGTGTTTAGCTGCAGCGTGATGCATGAAG CGCTGCATAACCATTATACCCAGAAAAGCCTGAGCCTGAGCCCGG GCAAA	
	Light Chain	
	LCDR1 (IMGT) QSISSY	SEQ ID NO: 14
	LCDR2 (IMGT) GA	SEQ ID NO: 15
	LCDR3 (IMGT) QQVVFTPPT	SEQ ID NO: 16
	LCDR1 (Kabat) RASQSISSYLN	SEQ ID NO: 17
	LCDR2 (Kabat) GASSLQS	SEQ ID NO: 18
	LCDR3 (Kabat) QQVVFTPPT	SEQ ID NO: 19
	LCDR1 (Combined Kabat/IMGT) RASQSISSYLN	SEQ ID NO: 20
	LCDR2 (Combined Kabat/IMGT) GASSLQS	SEQ ID NO: 21
	LCDR3 (Combined Kabat/IMGT) QQVVFTPPT	SEQ ID NO: 22
	VL - Amino acid sequence DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY GASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQVVFTPPTF GGGTKVEIK	SEQ ID NO: 23

Clone	Sequences	SEQ ID NO
	VL - DNA sequence GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAG GAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCA GCTATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGC TCCTGATCTATGGTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAG GTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGC AGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAGCAAGTAG TGTTCACGCCTCCTACTTTTGGCGGAGGGACCAAGGTTGAGATCAA A	SEQ ID NO: 24
	Light chain - Amino acid sequence DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY GASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQVVFTPPTF GGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 25
	Light chain - DNA sequence GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAG GAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCA GCTATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGC TCCTGATCTATGGTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAG GTTCAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGC AGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAGCAAGTAG TGTTCACGCCTCCTACTTTTGGCGGAGGGACCAAGGTTGAGATCAA ACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGAT GAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATA ACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACG CCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACA GCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCA AAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCC ATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAG AGTGT	SEQ ID NO: 26
65889	Heavy Chain	
	HCDR1 (IMGT) GYTFASYK	SEQ ID NO: 27
	HCDR2 (IMGT) IPAGTGNT	SEQ ID NO: 28

Clone	Sequences	SEQ ID NO
	HCDR3 (IMGT) ARDRGGYYYDSWDD	SEQ ID NO: 29
	HCDR1 (Kabat) SYKMH	SEQ ID NO: 30
	HCDR2 (Kabat) DIPAGTGNTHYSQKFQG	SEQ ID NO: 31
	HCDR3 (Kabat) DRGGYYYDSWDD	SEQ ID NO: 32
	HCDR1 (Combined Kabat/IMGT) GYTFASYKMH	SEQ ID NO: 33
	HCDR2 (Combined Kabat/IMGT) DIPAGTGNTHYSQKFQG	SEQ ID NO: 34
	HCDR3 (Combined Kabat/IMGT) DRGGYYYDSWDD	SEQ ID NO: 35
	VH - Amino acid sequence QVQLVQSGAEVKKPGASVKVSCKASGYTFASYKMHWVRQAPGQRL EWMGDIPAGTGNTHYSQKFQGRVTITRDTSASTAYMELSSLRSEDTA VYYCARDRGGYYYDSWDDWGQGTLVTVSS	SEQ ID NO: 36
	VH - DNA SEQUENCE CAGGTGCAGCTGGTGCAGAGCGGCGCGGAAGTGAAAAAACCGGG CGCGAGCGTGAAAGTGAGCTGCAAAGCGAGCGGCTATACCTTTGC GAGCTATAAAATGCATTGGGTGCGCCAGGCGCCGGGCCAGCGCCT GGAATGGATGGGCGATATTCCGGCGGGCACCGGCAACACCCATTA TAGCCAGAAATTTCAGGGCCGCGTGACCATTACCCGCGATACCAG CGCGAGCACCGCGTATATGGAACTGAGCAGCCTGCGCAGCGAAGA TACCGCGGTGTATTATTGCGCGCGCGATCGCGGCGGCTATTATTAT GATAGCTGGGATGATTGGGGCCAGGGCACCCTGGTGACCGTGAGC AGC	SEQ ID NO: 37
	Heavy chain - Amino acid sequence QVQLVQSGAEVKKPGASVKVSCKASGYTFASYKMHWVRQAPGQRL EWMGDIPAGTGNTHYSQKFQGRVTITRDTSASTAYMELSSLRSEDTA VYYCARDRGGYYYDSWDDWGQGTLVTVSSASTKGPSVFPLAPSSKS TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK	SEQ ID NO: 38

Clone	Sequences	SEQ ID NO
	VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK	
	Heavy chain - DNA sequence CAGGTGCAGCTGGTGCAGAGCGGCGCGGAAGTGAAAAAACCGGG CGCGAGCGTGAAAGTGAGCTGCAAAGCGAGCGGCTATACCTTTGC GAGCTATAAAATGCATTGGGTGCGCCAGGCGCCGGGCCAGCGCCT GGAATGGATGGGCGATATTCCGGCGGGCACCGGCAACACCCATTA TAGCCAGAAATTTCAGGGCCGCGTGACCATTACCCGCGATACCAG CGCGAGCACCGCGTATATGGAACTGAGCAGCCTGCGCAGCGAAGA TACCGCGGTGTATTATTGCGCGCGCGATCGCGGCGGCTATTATTAT GATAGCTGGGATGATTGGGGCCAGGGCACCCTGGTGACCGTGAGC AGCGCGAGCACCAAAGGCCCGAGCGTGTTTCCGCTGGCGCCGAGC AGCAAAAGCACCAGCGGCGGCACCGCGGCGCTGGGCTGCCTGGTG AAAGATTATTTTCCGGAACCGGTGACCGTGAGCTGGAACAGCGGC GCGCTGACCAGCGGCGTGCATACCTTTCCGGCGGTGCTGCAGAGA GCGGCCTGTATAGCCTGAGCAGCGTGGTGACCGTGCCGAGCAGCA GCCTGGGCACCCAGACCTATATTTGCAACGTGAACCATAAACCGA GCAACACCAAAGTGGATAAAAAAGTGGAACCGAAAAGCTGCGAT AAAACCCATACCTGCCCGCCGTGCCCGGCGCCGGAACTGCTGGGC GGCCCGAGCGTGTTTCTGTTTCCGCCGAAACCGAAAGATACCCTGA TGATTAGCCGCACCCCGGAAGTGACCTGCGTGGTGGTGGATGTGA GCCATGAAGATCCGGAAGTGAAATTTAACTGGTATGTGGATGGCG TGGAAGTGCATAACGCGAAAACCAAACCGCGCGAAGAACAGTAT AACAGCACCTATCGCGTGGTGAGCGTGCTGACCGTGCTGCATCAG GATTGGCTGAACGGCAAAGAATATAAATGCAAAGTGAGCAACAA AGCGCTGCCGGCGCCGATTGAAAAAACCATTAGCAAAGCGAAAGG CCAGCCGCGCGAACCGCAGGTGTATACCCTGCCGCCGAGCCGCGA TGAACTGACCAAAAACCAGGTGAGCCTGACCTGCCTGGTGAAAGG CTTTTATCCGAGCGATATTGCGGTGGAATGGGAAAGCAACGGCCA GCCGGAAAACAACTATAAAACCACCCCGCCGGTGCTGGATAGCGA TGGCAGCTTTTTTCTGTATAGCAAACTGACCGTGGATAAAAGCCGC TGGCAGCAGGGCAACGTGTTTAGCTGCAGCGTGATGCATGAAGCG CTGCATAACCATTATACCCAGAAAAGCCTGAGCCTGAGCCCGGGC AAA	SEQ ID NO: 39
	Light Chain	

Clone	Sequences	SEQ ID NO
	LCDR1 (IMGT) QSISSY	SEQ ID NO: 40
	LCDR2 (IMGT) GA	SEQ ID NO: 41
	LCDR3 (IMGT) QQVPFEPPT	SEQ ID NO: 42
	LCDR1 (Kabat) RASQSISSYLN	SEQ ID NO: 43
	LCDR2 (Kabat) GASSLQS	SEQ ID NO: 44
	LCDR3 (Kabat) QQVPFEPPT	SEQ ID NO: 45
	LCDR1 (Combined Kabat/IMGT) RASQSISSYLN	SEQ ID NO: 46
	LCDR2 (Combined Kabat/IMGT) GASSLQS	SEQ ID NO: 47
	LCDR3 (Combined Kabat/IMGT) QQVPFEPPT	SEQ ID NO: 48
	VL - Amino acid sequence DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY GASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQVPFEPPTF GGGTKVEIK	SEQ ID NO: 49
	VL - DNA sequence GATATTCAGATGACCCAGAGCCCGAGCAGCCTGAGCGCGAGCGTG GGCGATCGCGTGACCATTACCTGCCGCGCGAGCCAGAGCATTAGC AGCTATCTGAACTGGTATCAGCAGAAACCGGGCAAAGCGCCGAAA CTGCTGATTTATGGCGCGAGCAGCCTGCAGAGCGGCGTGCCGAGC CGCTTTAGCGGCAGCGGCAGCGGCACCGATTTTACCCTGACCATTA GCAGCCTGCAGCCGGAAGATTTTGCGACCTATTATTGCCAGCAGGT GCCGTTTGAACCGCCGACCTTTGGCGGCGGCACCAAAGTGGAAAT TAAA	SEQ ID NO: 50
	Light chain - Amino acid sequence DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIY GASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQVPFEPPTF GGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV	SEQ ID NO: 51

Clone	Sequences	SEQ ID NO
	QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC	
	Light chain - DNA sequence GATATTCAGATGACCCAGAGCCCGAGCAGCCTGAGCGCGAGCGTG GGCGATCGCGTGACCATTACCTGCCGCGCGAGCCAGAGCATTAGC AGCTATCTGAACTGGTATCAGCAGAAACCGGGCAAAGCGCCGAAA CTGCTGATTTATGGCGCGAGCAGCCTGCAGAGCGGCGTGCCGAGC CGCTTTAGCGGCAGCGGCAGCGGCACCGATTTTACCCTGACCATTA GCAGCCTGCAGCCGGAAGATTTTGCGACCTATTATTGCCAGCAGGT GCCGTTTGAACCGCCGACCTTTGGCGGCGGCACCAAAGTGGAAAT TAAACGCACCGTGGCGGCGCCGAGCGTGTTTATTTTTCCGCCGAGC GATGAACAGCTGAAAAGCGGCACCGCGAGCGTGGTGTGCCTGCTG AACAACTTTTATCCGCGCGAAGCGAAAGTGCAGTGGAAAGTGGAT AACGCGCTGCAGAGCGGCAACAGCCAGGAAAGCGTGACCGAACA GGATAGCAAAGATAGCACCTATAGCCTGAGCAGCACCCTGACCCT GAGCAAAGCGGATTATGAAAAACATAAAGTGTATGCGTGCGAAGT GACCCATCAGGGCCTGAGCAGCCCGGTGACCAAAAGCTTTAACCG CGGCGAATGC	SEQ ID NO: 52
65872	Heavy Chain	
	HCDR1 (IMGT) GFTFDDNA	SEQ ID NO: 53
	HCDR2 (IMGT) ISWSSNSI	SEQ ID NO: 54
	HCDR3 (IMGT) AKGSIIAGIWLGYFDL	SEQ ID NO: 55
	HCDR1 (Kabat) DNAMH	SEQ ID NO: 56
	HCDR2 (Kabat) GISWSSNSIAYADSVKG	SEQ ID NO: 57
	HCDR3 (Kabat) GSIIAGIWLGYFDL	SEQ ID NO: 58
	HCDR1 (Combined Kabat/IMGT) GFTFDDNAMH	SEQ ID NO: 59
	HCDR2 (Combined Kabat/IMGT) ISWSSNSIAYADSVKG	SEQ ID NO: 60

Clone	Sequences	SEQ ID NO
	HCDR3 (Combined Kabat/IMGT) GSIIAGIWLGYFDL	SEQ ID NO: 61
	VH - Amino acid sequence EVQLVESGGGLVQPGRSLRLSCAASGFTFDDNAMHWVRQAPGKGLE WVSGISWSSNSIAYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAV YYCAKGSIIAGIWLGYFDLWGRGTLVTVSS	SEQ ID NO: 62
	VH - DNA sequence GAAGTGCAGCTGGTGGAAAGCGGCGGCGGCCTGGTGCAGCCGGGC CGCAGCCTGCGCCTGAGCTGCGCGGCGAGCGGCTTTACCTTTGATG ATAACGCGATGCATTGGGTGCGCCAGGCGCCGGGCAAAGGCCTGG AATGGGTGAGCGGCATTAGCTGGAGCAGCAACAGCATTGCGTATG CGGATAGCGTGAAAGGCCGCTTTACCATTAGCCGCGATAACGCGA AAAACAGCCTGTATCTGCAGATGAACAGCCTGCGCGCGGAAGATA CCGCGGTGTATTATTGCGCGAAAGGCAGCATTATTGCGGGCATTTG GCTGGGCTATTTTGATCTGTGGGGCCGCGGCACCCTGGTGACCGTG AGCAGC	SEQ ID NO: 63
	Heavy chain - Amino acid sequence EVQLVESGGGLVQPGRSLRLSCAASGFTFDDNAMHWVRQAPGKGLE WVSGISWSSNSIAYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAV YYCAKGSIIAGIWLGYFDLWGRGTLVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK	SEQ ID NO: 64
	Heavy chain - DNA sequence GAAGTGCAGCTGGTGGAAAGCGGCGGCGGCCTGGTGCAGCCGGGC CGCAGCCTGCGCCTGAGCTGCGCGGCGAGCGGCTTTACCTTTGATG ATAACGCGATGCATTGGGTGCGCCAGGCGCCGGGCAAAGGCCTGG AATGGGTGAGCGGCATTAGCTGGAGCAGCAACAGCATTGCGTATG CGGATAGCGTGAAAGGCCGCTTTACCATTAGCCGCGATAACGCGA AAAACAGCCTGTATCTGCAGATGAACAGCCTGCGCGCGGAAGATA CCGCGGTGTATTATTGCGCGAAAGGCAGCATTATTGCGGGCATTTG GCTGGGCTATTTTGATCTGTGGGGCCGCGGCACCCTGGTGACCGTG	SEQ ID NO: 65

Clone	Sequences	SEQ ID NO
	AGCAGCGCGAGCACCAAAGGCCCGAGCGTGTTTCCGCTGGCGCCG AGCAGCAAAAGCACCAGCGGCGGCACCGCGGCGCTGGGCTGCCTG GTGAAAGATTATTTTCCGGAACCGGTGACCGTGAGCTGGAACAGC GGCGCGCTGACCAGCGGCGTGCATACCTTTCCGGCGGTGCTGCAG AGCAGCGGCCTGTATAGCCTGAGCAGCGTGGTGACCGTGCCGAGC AGCAGCCTGGGCACCCAGACCTATATTTGCAACGTGAACCATAAA CCGAGCAACACCAAAGTGGATAAAAAAGTGGAACCGAAAAGCTG CGATAAAACCCATACCTGCCCGCCGTGCCCGGCGCCGGAACTGCT GGGCGGCCCGAGCGTGTTTCTGTTTCCGCCGAAACCGAAAGATAC CCTGATGATTAGCCGCACCCCGGAAGTGACCTGCGTGGTGGTGGA TGTGAGCCATGAAGATCCGGAAGTGAAATTTAACTGGTATGTGGA TGGCGTGGAAGTGCATAACGCGAAAACCAAACCGCGCGAAGAAC AGTATAACAGCACCTATCGCGTGGTGAGCGTGCTGACCGTGCTGC ATCAGGATTGGCTGAACGGCAAAGAATATAAATGCAAAGTGAGCA ACAAAGCGCTGCCGGCGCCGATTGAAAAAACCATTAGCAAAGCGA AAGGCCAGCCGCGCGAACCGCAGGTGTATACCCTGCCGCCGAGCC GCGATGAACTGACCAAAAACCAGGTGAGCCTGACCTGCCTGGTGA AAGGCTTTTATCCGAGCGATATTGCGGTGGAATGGGAAAGCAACG GCCAGCCGGAAAACAACTATAAAACCACCCCGCCGGTGCTGGATA GCGATGGCAGCTTTTTTCTGTATAGCAAACTGACCGTGGATAAAAG CCGCTGGCAGCAGGGCAACGTGTTTAGCTGCAGCGTGATGCATGA AGCGCTGCATAACCATTATACCCAGAAAAGCCTGAGCCTGAGCCC GGGCAAA	
	Light Chain	
	LCDR1 (IMGT) QSVSSY	SEQ ID NO: 66
	LCDR2 (IMGT) DA	SEQ ID NO: 67
	LCDR3 (IMGT) QQVSISPPWT	SEQ ID NO: 68
	LCDR1 (Kabat) RASQSVSSYLA	SEQ ID NO: 69
	LCDR2 (Kabat) DASNRAT	SEQ ID NO: 70
	LCDR3 (Kabat) QQVSISPPWT	SEQ ID NO: 71

Clone	Sequences	SEQ ID NO
	LCDR1 (Combined Kabat/IMGT) RASQSVSSYLA	SEQ ID NO: 72
	LCDR2 (Combined Kabat/IMGT) DASNRAT	SEQ ID NO: 73
	LCDR3 (Combined Kabat/IMGT) QQVSISPPWT	SEQ ID NO: 74
	VL - Amino acid sequence EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIY DASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQVSISPPWT FGGGTKVEIK	SEQ ID NO: 75
	VL - DNA sequence GAAATTGTGCTGACCCAGAGCCCGGCGACCCTGAGCCTGAGCCCG GGCGAACGCGCGACCCTGAGCTGCCGCGCGAGCCAGAGCGTGAGC AGCTATCTGGCGTGGTATCAGCAGAAACCGGGCCAGGCGCCGCGC CTGCTGATTTATGATGCGAGCAACCGCGCGACCGGCATTCCGGCG CGCTTTAGCGGCAGCGGCAGCGGCACCGATTTTACCCTGACCATTA GCAGCCTGGAACCGGAAGATTTTGCGGTGTATTATTGCCAGCAGG TGAGCATTAGCCCGCCGTGGACCTTTGGCGGCGGCACCAAAGTGG AAATTAAA	SEQ ID NO: 76
	Light chain - Amino acid sequence EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIY DASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQVSISPPWT FGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 77
	Light chain - DNA sequence GAAATTGTGCTGACCCAGAGCCCGGCGACCCTGAGCCTGAGCCCG GGCGAACGCGCGACCCTGAGCTGCCGCGCGAGCCAGAGCGTGAGC AGCTATCTGGCGTGGTATCAGCAGAAACCGGGCCAGGCGCCGCGC CTGCTGATTTATGATGCGAGCAACCGCGCGACCGGCATTCCGGCG CGCTTTAGCGGCAGCGGCAGCGGCACCGATTTTACCCTGACCATTA GCAGCCTGGAACCGGAAGATTTTGCGGTGTATTATTGCCAGCAGG TGAGCATTAGCCCGCCGTGGACCTTTGGCGGCGGCACCAAAGTGG AAATTAAACGCACCGTGGCGGCGCCGAGCGTGTTTATTTTTCCGCC GAGCGATGAACAGCTGAAAAGCGGCACCGCGAGCGTGGTGTGCCT GCTGAACAACTTTTATCCGCGCGAAGCGAAAGTGCAGTGGAAAGT	SEQ ID NO: 78

Clone	Sequences	SEQ ID NO
	GGATAACGCGCTGCAGAGCGGCAACAGCCAGGAAAGCGTGACCG AACAGGATAGCAAAGATAGCACCTATAGCCTGAGCAGCACCCTGA CCCTGAGCAAAGCGGATTATGAAAAACATAAAGTGTATGCGTGCG AAGTGACCCATCAGGGCCTGAGCAGCCCGGTGACCAAAAGCTTTA ACCGCGGCGAATGC	
Hu2C4-H3L3	Heavy Chain	
	HCDR1 (IMGT) GFSFTDYN	SEQ ID NO: 79
	HCDR2 (IMGT) IDPYYGRI	SEQ ID NO: 80
	HCDR3 (IMGT) ATGAYTSGYSWFAY	SEQ ID NO: 81
	HCDR1 (Kabat) DYNMN	SEQ ID NO: 82
	HCDR2 (Kabat) NIDPYYGRINYNQKFKG	SEQ ID NO: 83
	HCDR3 (Kabat) GAYTSGYSWFAY	SEQ ID NO: 84
	HCDR1 (Combined Kabat/IMGT) GFSFTDYNMN	SEQ ID NO: 85
	HCDR2 (Combined Kabat/IMGT) NIDPYYGRINYNQKFKG	SEQ ID NO: 86
	HCDR3 (Combined Kabat/IMGT) GAYTSGYSWFAY	SEQ ID NO: 87
	VH - Amino acid sequence EVQLVQSGAEVKKPGATVKISCKVSGFSFTDYNMNWVQQAPGKGLE WIGNIDPYYGRINYNQKFKGRATLTVDKSTDTAYMELSSLRSEDTAV YYCATGAYTSGYSWFAYWGQGTTVTVSS	SEQ ID NO: 88
	VH - DNA SEQUENCE GAAGTGCAGCTGGTGCAGAGCGGCGCGGAAGTGAAAAAACCGGG CGCGACCGTGAAAATTAGCTGCAAAGTGAGCGGCTTTAGCTTTAC CGATTATAACATGAACTGGGTGCAGCAGGCGCCGGGCAAAGGCCT GGAATGGATTGGCAACATTGATCCGTATTATGGCCGCATTAACTAT AACCAGAAATTTAAAGGCCGCGCGACCCTGACCGTGGATAAAAGC ACCGATACCGCGTATATGGAACTGAGCAGCCTGCGCAGCGAAGAT	SEQ ID NO: 89

Clone	Sequences	SEQ ID NO
	ACCGCGGTGTATTATTGCGCGACCGGCGCGTATACCAGCGGCTAT AGCTGGTTTGCGTATTGGGGCCAGGGCACCACCGTGACCGTGAGC AGC	
	Heavy chain - Amino acid sequence EVQLVQSGAEVKKPGATVKISCKVSGFSFTDYNMNWVQQAPGKGLE WIGNIDPYYGRINYNQKFKGRATLTVDKSTDTAYMELSSLRSEDTAV YYCATGAYTSGYSWFAYWGQGTTVTVSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK	**[0602]** SEQ ID NO: 90
	Heavy chain - DNA sequence GAAGTGCAGCTGGTGCAGAGCGGCGCGGAAGTGAAAAAACCGGG CGCGACCGTGAAAATTAGCTGCAAAGTGAGCGGCTTTAGCTTTAC CGATTATAACATGAACTGGGTGCAGCAGGCGCCGGGCAAAGGCCT GGAATGGATTGGCAACATTGATCCGTATTATGGCCGCATTAACTAT AACCAGAAATTTAAAGGCCGCGCGACCCTGACCGTGGATAAAAGC ACCGATACCGCGTATATGGAACTGAGCAGCCTGCGCAGCGAAGAT ACCGCGGTGTATTATTGCGCGACCGGCGCGTATACCAGCGGCTAT AGCTGGTTTGCGTATTGGGGCCAGGGCACCACCGTGACCGTGAGC AGCGCGAGCACCAAAGGCCCGAGCGTGTTTCCGCTGGCGCCGAGC AGCAAAAGCACCAGCGGCGGCACCGCGGCGCTGGGCTGCCTGGTG AAAGATTATTTTCCGGAACCGGTGACCGTGAGCTGGAACAGCGGC GCGCTGACCAGCGGCGTGCATACCTTTCCGGCGGTGCTGCAGAGC AGCGGCCTGTATAGCCTGAGCAGCGTGGTGACCGTGCCGAGCAGC AGCCTGGGCACCCAGACCTATATTTGCAACGTGAACCATAAACCG AGCAACACCAAAGTGGATAAAAAAGTGGAACCGAAAAGCTGCGA TAAAACCCATACCTGCCCGCCGTGCCCGGCGCCGGAACTGCTGGG CGGCCCGAGCGTGTTTCTGTTTCCGCCGAAACCGAAAGATACCCTG ATGATTAGCCGCACCCCGGAAGTGACCTGCGTGGTGGTGGATGTG AGCCATGAAGATCCGGAAGTGAAATTTAACTGGTATGTGGATGGC GTGGAAGTGCATAACGCGAAAACCAAACCGCGCGAAGAACAGTA TAACAGCACCTATCGCGTGGTGAGCGTGCTGACCGTGCTGCATCA	**[0603]** SEQ ID NO: 91

Clone	Sequences	SEQ ID NO
	GGATTGGCTGAACGGCAAAGAATATAAATGCAAAGTGAGCAACA AAGCGCTGCCGGCGCCGATTGAAAAAACCATTAGCAAAGCGAAAG GCCAGCCGCGCGAACCGCAGGTGTATACCCTGCCGCCGAGCCGCG ATGAACTGACCAAAAACCAGGTGAGCCTGACCTGCCTGGTGAAAG GCTTTTATCCGAGCGATATTGCGGTGGAATGGGAAAGCAACGGCC AGCCGGAAAACAACTATAAAACCACCCCGCCGGTGCTGGATAGCG ATGGCAGCTTTTTTCTGTATAGCAAACTGACCGTGGATAAAAGCCG CTGGCAGCAGGGCAACGTGTTTAGCTGCAGCGTGATGCATGAAGC GCTGCATAACCATTATACCCAGAAAAGCCTGAGCCTGAGCCCGGG CAAA	
	Light Chain	
	LCDR1 (IMGT) SSVSSSY	SEQ ID NO: 92
	LCDR2 (IMGT) RT	SEQ ID NO: 93
	LCDR3 (IMGT) QQWSGYPFT	SEQ ID NO: 94
	LCDR1 (Kabat) SASSSVSSSYLH	SEQ ID NO: 95
	LCDR2 (Kabat) RTSNLAS	SEQ ID NO: 96
	LCDR3 (Kabat) QQWSGYPFT	SEQ ID NO: 97
	LCDR1 (Combined Kabat/IMGT) SASSSVSSSYLH	SEQ ID NO: 98
	LCDR2 (Combined Kabat/IMGT) RTSNLAS	SEQ ID NO: 99
	LCDR3 (Combined Kabat/IMGT) QQWSGYPFT	SEQ ID NO: 100
	VL - Amino acid sequence DNQLTQSPSFLSASVGDRVTITCSASSSVSSSYLHWYQQKPGASPKPLI HRTSNLASGVPSRFSGSGSGTEYTLTISSLQPEDFATYYCQQWSGYPF TFGQGTKLEIK	SEQ ID NO: 101
	VL - DNA sequence GATAACCAGCTGACCCAGAGCCCGAGCTTTCTGAGCGCGAGCGTG	SEQ ID NO: 102

Clone	Sequences	SEQ ID NO
	GGCGATCGCGTGACCATTACCTGCAGCGCGAGCAGCAGCGTGAGC AGCAGCTATCTGCATTGGTATCAGCAGAAACCGGGCGCGAGCCCG AAACCGCTGATTCATCGCACCAGCAACCTGGCGAGCGGCGTGCCG AGCCGCTTTAGCGGCAGCGGCAGCGGCACCGAATATACCCTGACC ATTAGCAGCCTGCAGCCGGAAGATTTTGCGACCTATTATTGCCAGC AGTGGAGCGGCTATCCGTTTACCTTTGGCCAGGGCACCAAACTGG AAATTAAA	
	Light chain - Amino acid sequence DNQLTQSPSFLSASVGDRVTITCSASSSVSSSYLHWYQQKPGASPKPLI HRTSNLASGVPSRFSGSGSGTEYTLTISSLQPEDFATYYCQQWSGYPF TFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKV YACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 103
	Light chain - DNA sequence GATAACCAGCTGACCCAGAGCCCGAGCTTTCTGAGCGCGAGCGTG GGCGATCGCGTGACCATTACCTGCAGCGCGAGCAGCAGCGTGAGC AGCAGCTATCTGCATTGGTATCAGCAGAAACCGGGCGCGAGCCCG AAACCGCTGATTCATCGCACCAGCAACCTGGCGAGCGGCGTGCCG AGCCGCTTTAGCGGCAGCGGCAGCGGCACCGAATATACCCTGACC ATTAGCAGCCTGCAGCCGGAAGATTTTGCGACCTATTATTGCCAGC AGTGGAGCGGCTATCCGTTTACCTTTGGCCAGGGCACCAAACTGG AAATTAAACGCACCGTGGCGGCGCCGAGCGTGTTTATTTTTCCGCC GAGCGATGAACAGCTGAAAAGCGGCACCGCGAGCGTGGTGTGCCT GCTGAACAACTTTTATCCGCGCGAAGCGAAAGTGCAGTGGAAAGT GGATAACGCGCTGCAGAGCGGCAACAGCCAGGAAAGCGTGACCG AACAGGATAGCAAAGATAGCACCTATAGCCTGAGCAGCACCCTGA CCCTGAGCAAAGCGGATTATGAAAAACATAAAGTGTATGCGTGCG AAGTGACCCATCAGGGCCTGAGCAGCCCGGTGACCAAAAGCTTTA ACCGCGGCGAATGC	SEQ ID NO: 104

Antigen-Binding Fragments

[0604] The present disclosure, among other things, provides conjugates comprising anti-HHLA2 antigen-binding fragments. As used herein, an "anti-HHLA2 antigen-binding fragment" comprises or is any protein or peptide-containing molecule comprising at least a portion of an immunoglobulin molecule containing at least one complementarity determining region (CDR) of a VH or a VL or an HHLA2 binding portion derived from any of the antibodies described herein. Antibody fragments can be obtained

using conventional techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as intact antibodies. Such functional antibody fragments can retain the ability to selectively bind with HHLA2.

[0605] Examples of anti-HHLA2 antigen-binding fragments described herein can include: (i) a Fab fragment, a monovalent fragment comprising VL, VH, CL, and CH1 domains; (ii) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at a hinge region; (iii) a Fd fragment comprising VH and CH1 domains; (iv) a Fv fragment comprising VL and VH domains of a single arm of an antibody, (v) a diabody (dAb) fragment comprising a VH domain; (vi) a camelid or camelized variable domain; (vii) a scFv, a fusion protein of VH and VL regions; or (viii) a single domain antibody. In some embodiments, an anti-HHLA2 antigen-binding fragment thereof described herein comprises or is a heavy chain and a light chain (e.g., a half antibody).

[0606] In some embodiments, the present invention provides conjugates comprising an HHLA2 binding agent moiety and a payload. In some embodiments, the present invention provides conjugates comprising an HHLA2 antibody or antigen-binding fragment thereof and a payload. In some embodiments, a payload is exatecan or a derivative thereof, or pharmaceutically acceptable salt thereof. In some embodiments, such conjugates are internalized by a cell, and the payload is released (e.g., through cleavage of a moiety that links a payload to a HHLA2 binding agent.

[0607] In some embodiments, the present disclosure provides conjugates comprising:

i) an anti-HERV-H LTR-Associating 2 (HHLA2) antibody or antigen-binding fragment thereof;

ii) exatecan or a derivative thereof, or pharmaceutically acceptable salt thereof; and

iii) a linker that connects the anti-HHLA2 antibody or antigen-binding fragment thereof and the exatecan or a derivative thereof.

Manufacturing

Target Binding Agents

[0608] Various technologies, e.g., various antibody manufacturing technologies, can be utilized to manufacture target binding agents that comprise antibodies or fragments thereof. Certain technologies for manufacturing HHLA2 binding agents are described below as examples.

[0609] In some embodiments, methods of making anti-HHLA2 antibodies or antigen-binding fragments thereof are described in WO 2022/165258 A1. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein is identified using a display technology, such as yeast display, phage display, or ribosome display. In some embodiments, an anti-HHLA2 antibody or an antigen-binding fragment thereof described herein is identified using a hybridoma library (e.g., a mammalian hybridoma library, e.g., a mouse hybridoma library), followed by supernatant screening.

[0610] Combinatorial methods for generating antibodies are known in the art (as described in, e.g., Ladner et al. U.S. Patent No. 5,223,409; Kang et al. International Publication No. WO 92/18619; Dower et al. International Publication No. WO 91/17271; Winter et al. International Publication WO 92/20791;

Markland et al. International Publication No. WO 92/15679; Breitling et al. International Publication WO 93/01288; McCafferty et al. International Publication No. WO 92/01047; Garrard et al. International Publication No. WO 92/09690; Ladner et al. International Publication No. WO 90/02809; Fuchs et al. (1991) Bio/Technology 9:1370-1372; Hay et al. (1992) Hum Antibody Hybridomas 3:81-85; Huse et al. (1989) Science 246:1275-1281; Griffths et al. (1993) EMBO J 12:725-734; Hawkins et al. (1992) J Mol Biol 226:889-896; Clackson et al. (1991) Nature 352:624-628; Gram et al. (1992) PNAS 89:3576-3580; Garrad et al. (1991) Bio/Technology 9:1373-1377; Hoogenboom et al. (1991) Nuc Acid Res 19:4133-4137; and Barbas et al. (1991) PNAS 88:7978-7982, each of which his hereby incorporated by reference in its entirety).

[0611] In some embodiments, an anti-HHLA2 antibody or antigen-binding fragment thereof described herein may be derived from other species. A humanized antibody is an antibody produced by recombinant DNA technology, in which some or all amino acids of a human immunoglobulin light chain or heavy chain that are not required for antigen binding (e.g., constant regions and/or framework regions of variable domains) are used to substitute for the corresponding amino acids from light chain or heavy chain of the cognate, nonhuman antibody. By way of example, a humanized version of a murine antibody to a given antigen has on both heavy and light chains: (1) constant regions of a human antibody; (2) FRs from the variable domains of a human antibody; and (3) CDRs from the murine antibody. Human FRs may be selected based on their highest sequence homology to mouse FR sequence. When necessary, one or more residues in human FRs can be changed to residues at corresponding positions in a murine antibody so as to preserve binding affinity of the humanized antibody to a target. This change is sometimes called "back mutation." Similarly, forward mutations may be made to revert back to murine sequence for a desired reason, e.g. stability or affinity to a target. Humanized antibodies generally are less likely to elicit an immune response in humans as compared to chimeric human antibodies because the former contain considerably fewer non-human components.

[0612] Methods for humanizing non-human antibodies are well known in the art. Suitable methods for making humanized antibodies in accordance with the present disclosure are described in, e.g., Winter EP 0 239 400; Jones et al., Nature 321:522-525 (1986); Riechmann et al., Nature 332:323-327 (1988); Verhoeyen et al., Science 239: 1534-1536 (1988); Queen et al., Proc. Nat. Acad. ScL USA 86:10029 (1989); U.S. Patent 6,180,370, and Orlandi et al., Proc. Natl. Acad. Sd. USA 86:3833 (1989); the disclosures of each of which are incorporated herein by reference in their entireties. Generally, transplantation of non-human (e.g., murine) CDRs onto a human antibody is achieved as follows. cDNAs encoding VH and VL are isolated from a hybridoma, and nucleic acid sequences encoding VH and VL including CDRs are determined by sequencing. Nucleic acid sequences encoding CDRs are inserted into corresponding regions of a human antibody VH or VL coding sequences and attached to human constant region gene segments of a desired isotype (e.g., γl for CH and κ for CL). Humanized heavy and light chain genes are co-expressed in mammalian host cells (e.g., CHO or NSO cells) to produce soluble humanized antibody. To facilitate large-scale production of antibodies, it is often desirable to select for a high expressor using, for example, a DHFR gene or GS gene in the producer line.

[0613] In some embodiments, an anti-HHLA2 antibody or antigen-binding fragment thereof described herein comprises or is a human antibody. Completely human antibodies may be particularly desirable for therapeutic treatment of human subjects. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences (see, e.g., U.S. Pat. Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/60433, WO 98/24893, WO 98/16664, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety). Techniques are also available for the preparation of human monoclonal antibodies in, e.g., Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Riss, (1985); and Boerner et al., J. Immunol., 147(1):86-95, (1991), each of which is incorporated herein by reference in its entirety.

Conjugates

[0614] In some embodiments, manufacturing of payloads, payload agents and conjugates utilizes chemical synthesis. Various chemical technologies can be utilized optionally with biological technologies in accordance with the present disclosure for manufacturing.

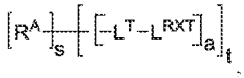
[0615] In some embodiments, the disclosure provides methods of making a conjugate, or a pharmaceutically composition comprising a conjugate, the method comprising reacting a target binding agent and a payload agent, wherein:

(i) the target binding agent comprises a target binding moiety and a target binding agent reactive group;

(ii) the payload agent comprises a payload moiety and a payload agent reactive group; and

(iii) the target binding agent reactive group reacts with the payload agent reactive group to conjugate the payload moiety to the target binding moiety optionally through a linker.

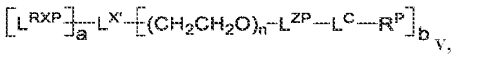
[0616] In some embodiments, a target binding agent is a compound of formula T-1 or a salt thereof:



T-1

wherein each variable is independently as described herein.

[0617] In some embodiments, the payload agent is a compound of formula P-1 or a salt thereof:

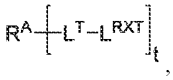


P-1

wherein each variable is independently as described herein.

[0618] In some embodiments, a in formula T-1 and a in formula P-1 are the same. In some embodiments, each a is independently as described herein.

[0619] In some embodiments, the target binding agent is a compound of formula T-2 or a salt thereof:



T-2

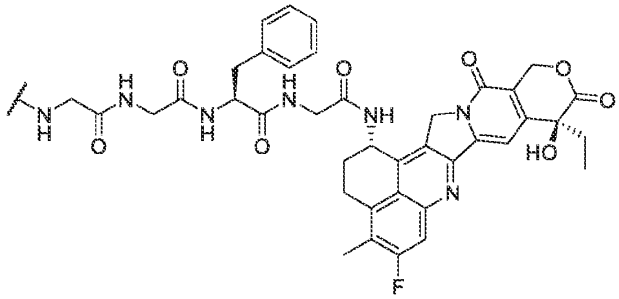
wherein each variable is independently as described herein. In some embodiments, the payload agent is a compound of formula P-2 or a salt thereof:

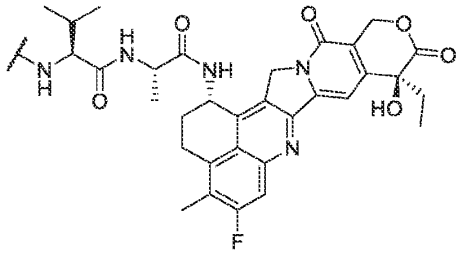
$$L^{RXP}-L^{X}-(CH_2CH_2O)_n-L^{ZP}-L^{C}-R^{P}$$

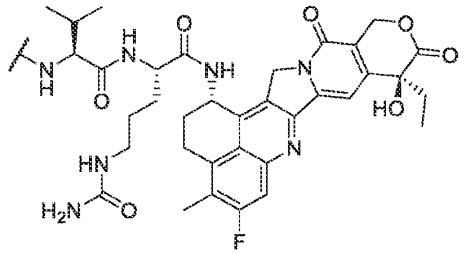
P-2

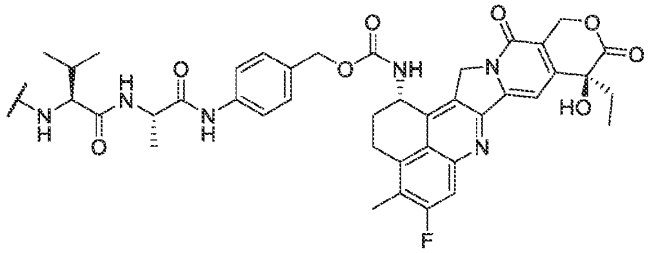
wherein each variable is independently other variable is independently as described herein.

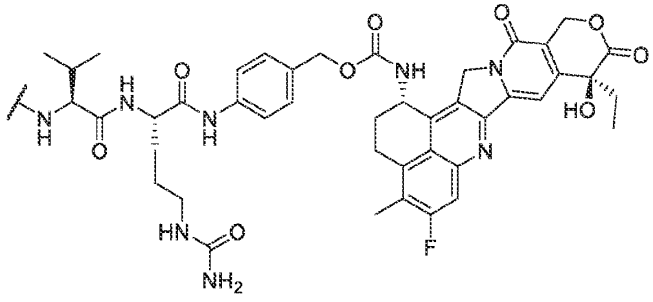
[0620] Various embodiments for R^P, L^C, L^{ZP}, $-(CH_2CH_2O)_n-$, L^X, L^{RX}, L^T, and combinations thereof are described herein. For example, in some embodiments, an occurrence of $-L^C-R^P$ is

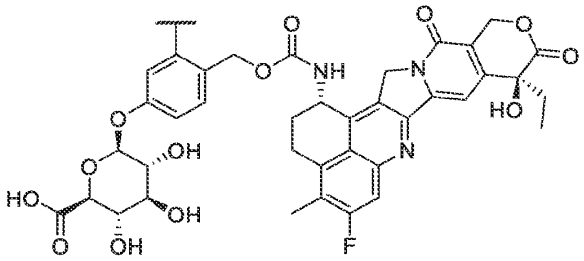




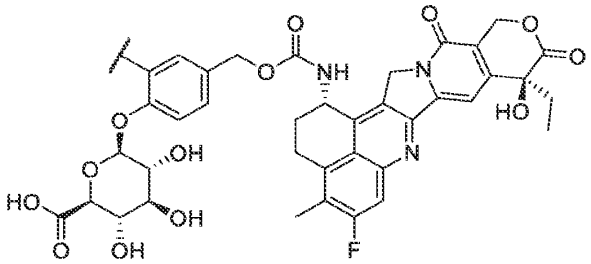




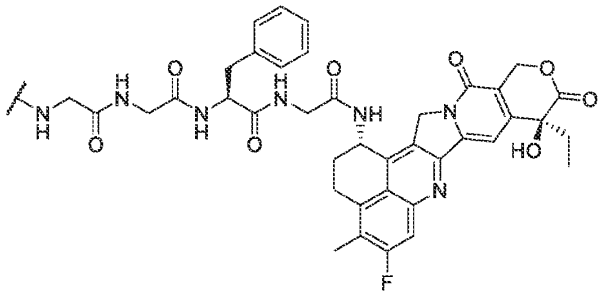




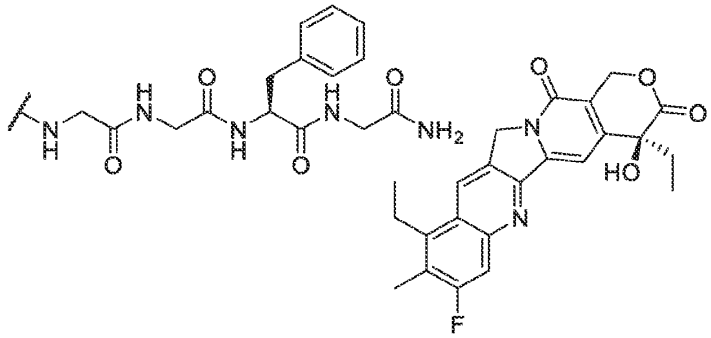
, or



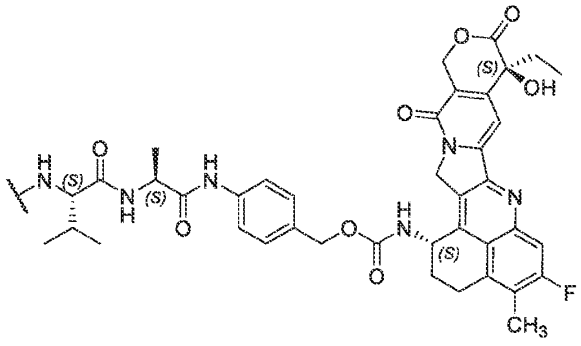
. In some embodiments, an occurrence of



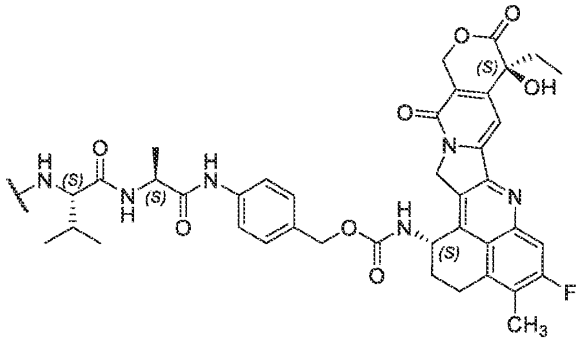
$-L^C-R^P$ is . In some embodiments, each occurrence of $-L^C-R^P$ is independently



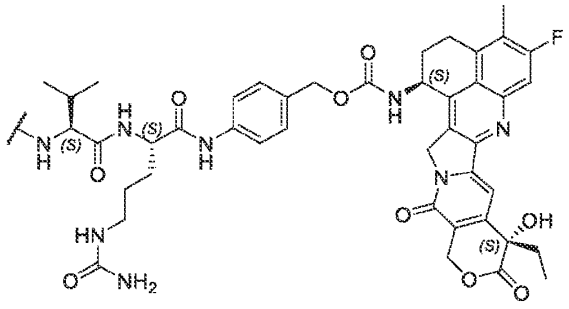
. In some embodiments, an



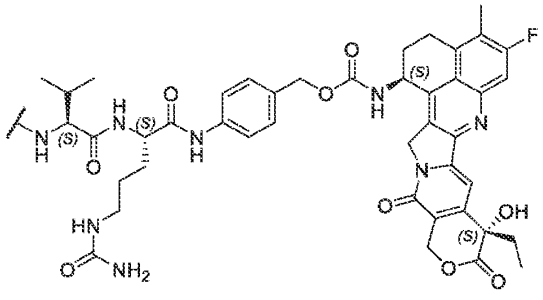
occurrence of $-L^C-R^P$ is . In some embodiments, each occurrence of $-L^C-R^P$ is



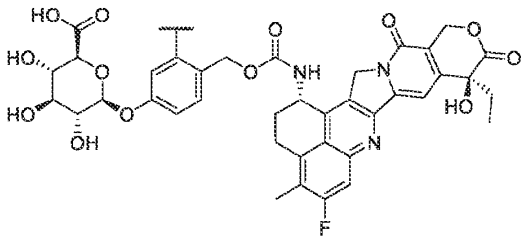
. In some embodiments, an occurrence of $-L^C-R^P$ is



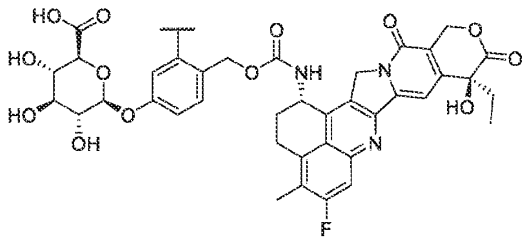
. In some embodiments, each occurrence of $-L^C-R^P$ is



. In some embodiments,



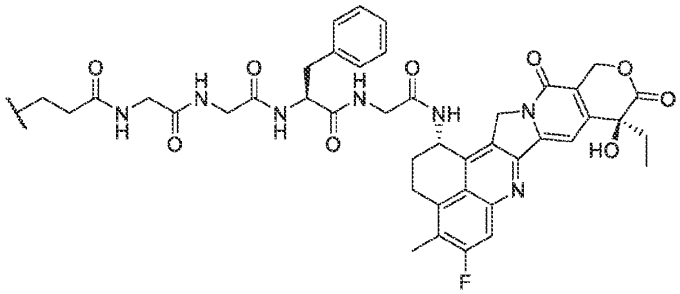
an occurrence of $-L^C-R^P$ is . In some

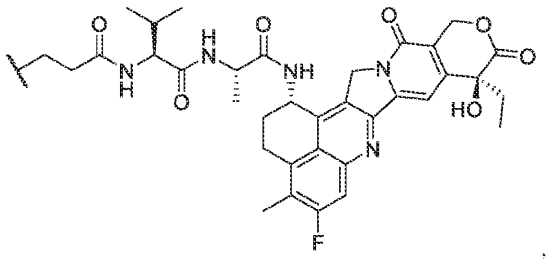


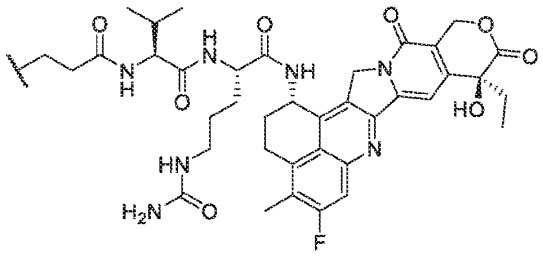
embodiments, each occurrence of $-L^C-R^P$ is .

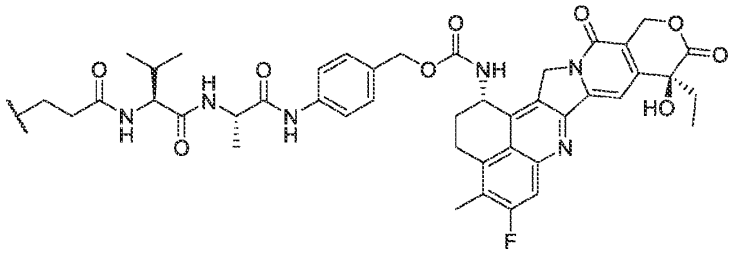
Those skilled in the art reading the present desclosure appreciate that other embodiments of $-L^C-R^P$ described herein can be also utilized.

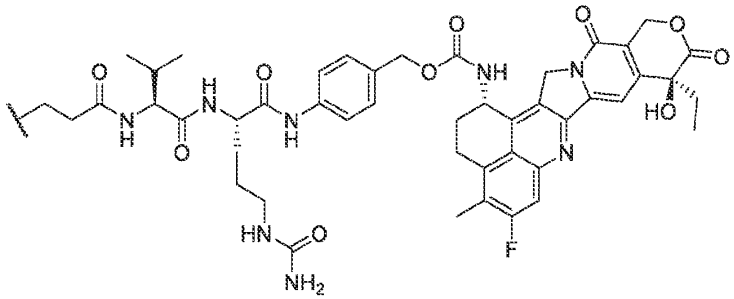
[0621] In some embodiments, an occurrence of $-L^{ZP}-L^C-R^P$ is

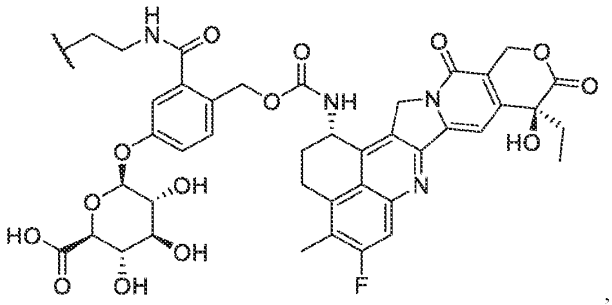


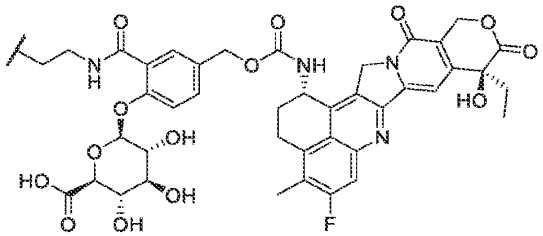


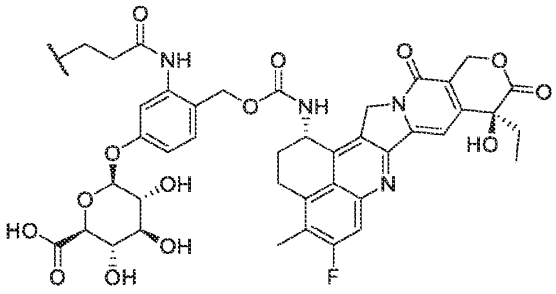




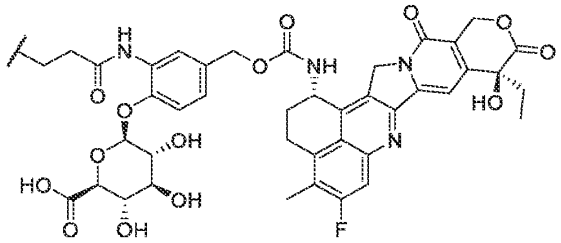




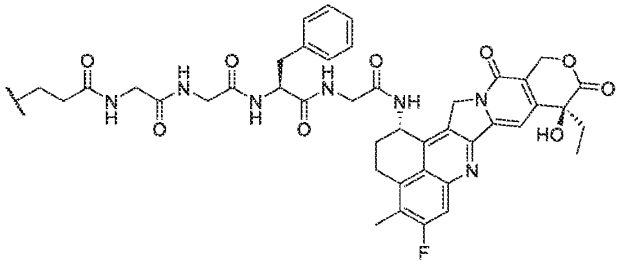




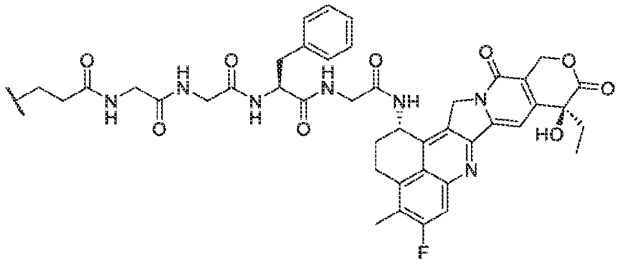
, or



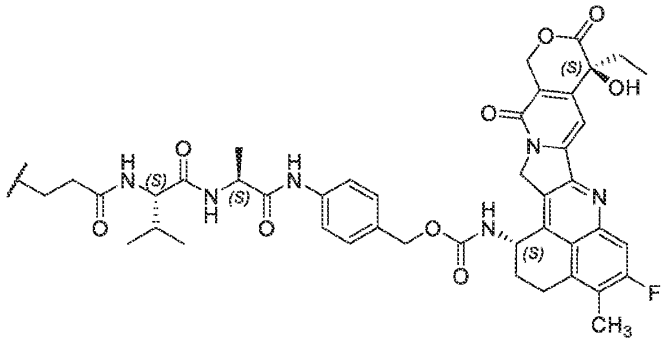
. In some embodiments, an occurrence of



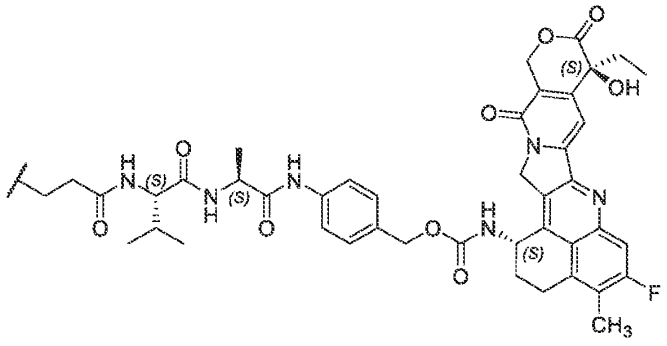
$-L^{ZP}-L^{C}-R^{P}$ is or comprises . In some embodiments, each occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises



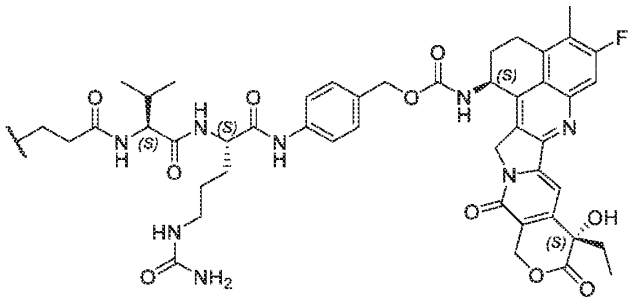
. In some embodiments, an occurrence



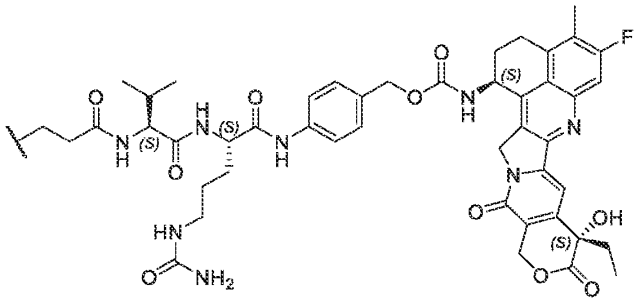
of $-L^{ZP}-L^{C}-R^{P}$ is or comprises . In some embodiments, each occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises



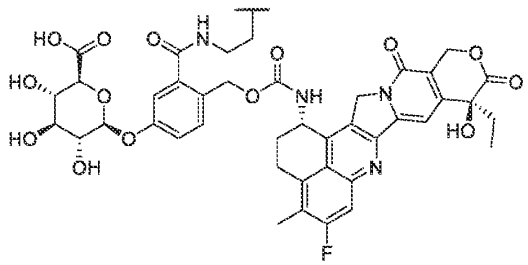
. In some embodiments, an occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises



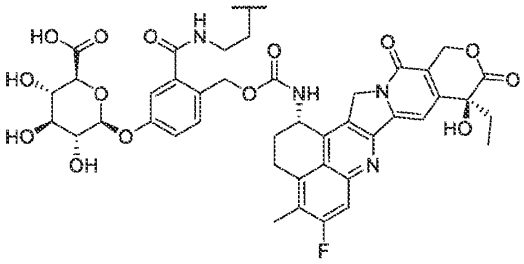
. In some embodiments, each occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises



. In some embodiments, an occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises

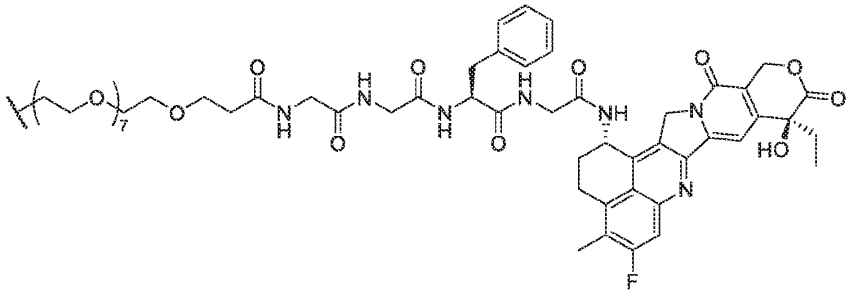


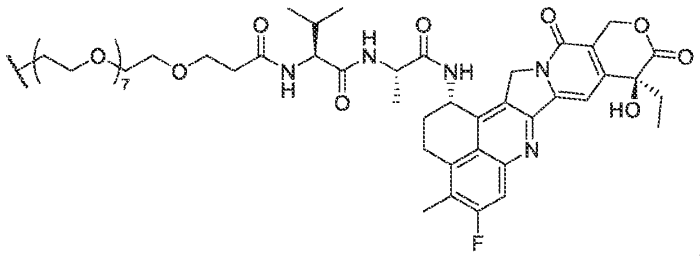
. In some embodiments, each occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises

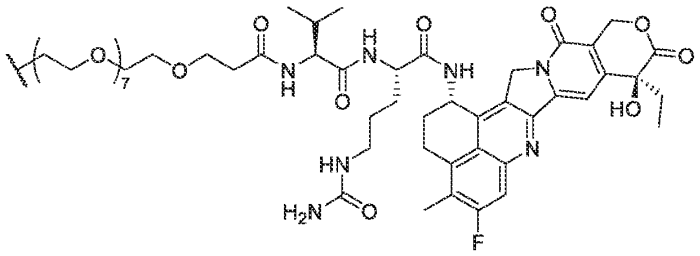


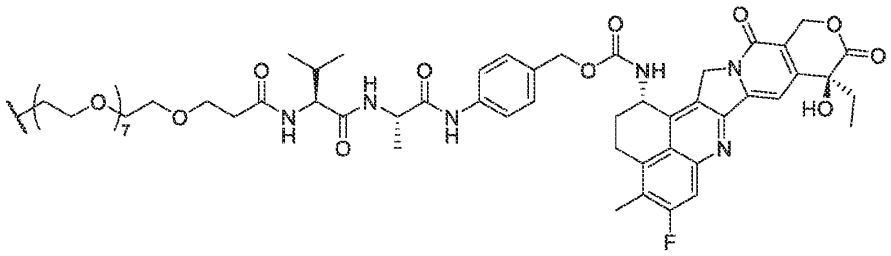
. Those skilled in the art reading the present desclosure appreciate that other embodiments of $-L^{ZP}-L^{C}-R^{P}$ described herein can be also utilized.

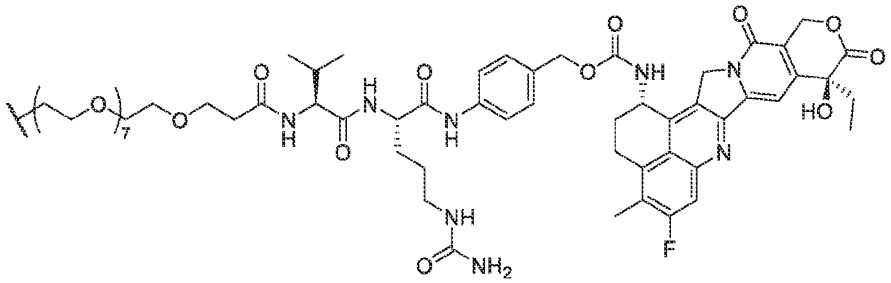
[0622] In some embodiments, an occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

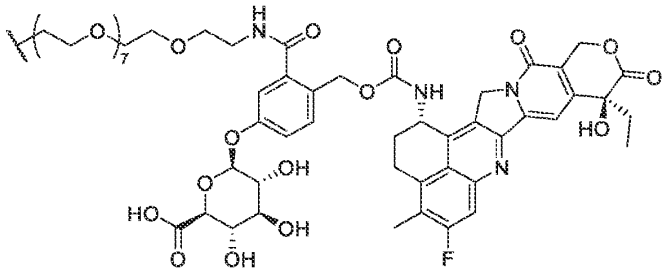


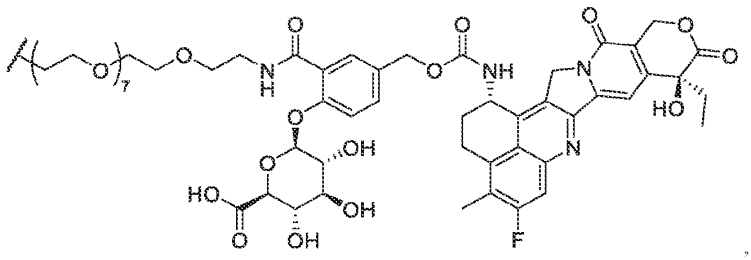


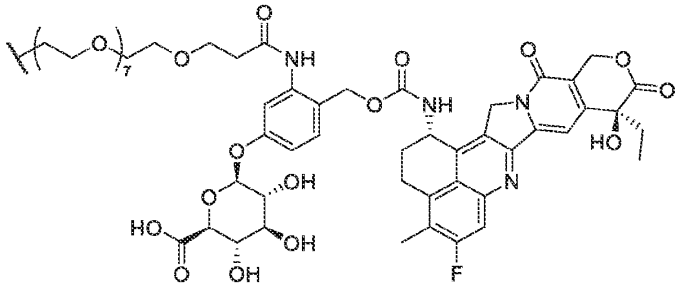




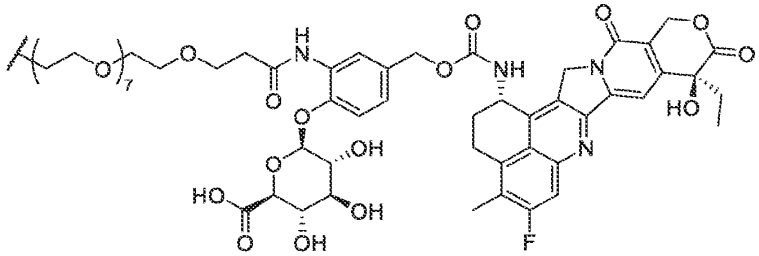




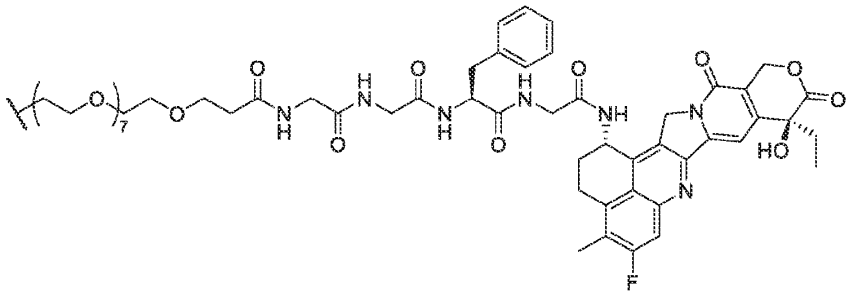




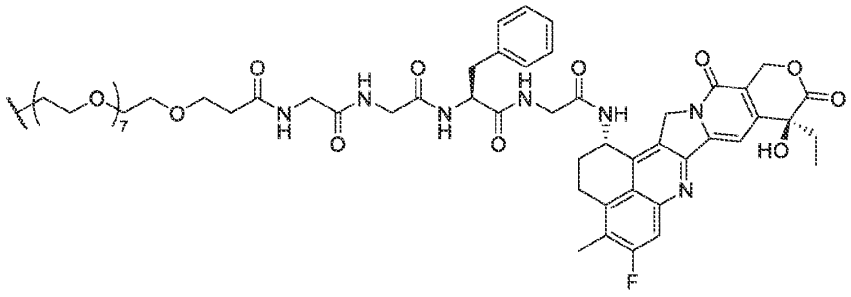
, or



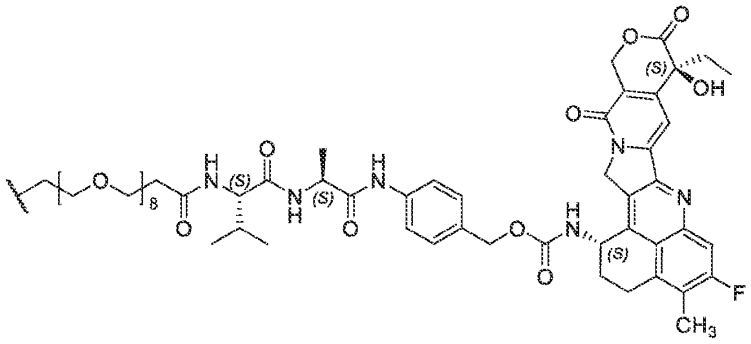
. In some embodiments, an occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



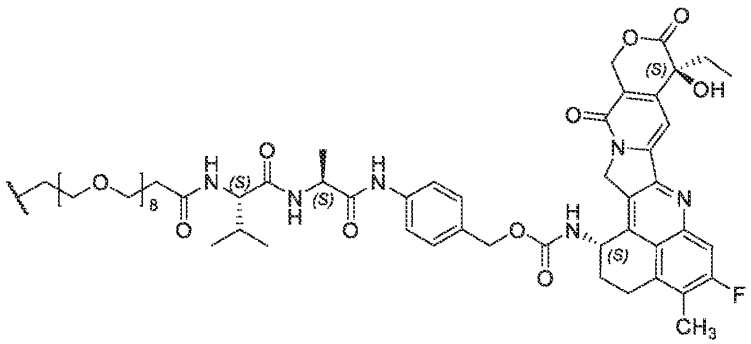
. In some embodiments, each occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



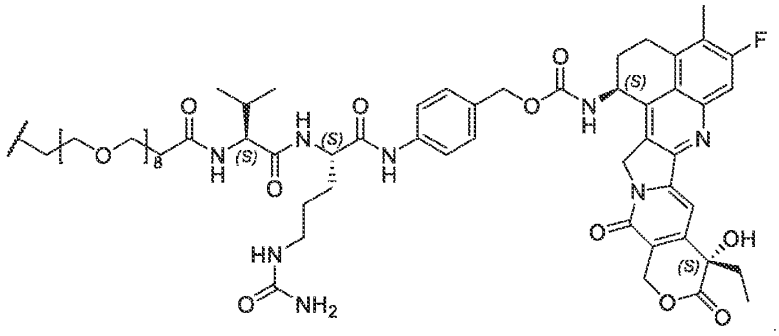
. In some embodiments, an occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



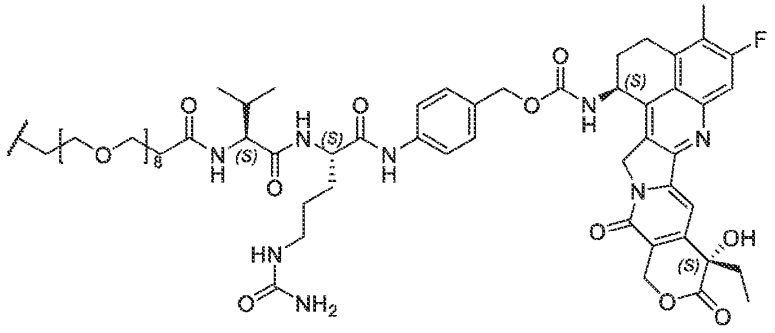
. In some embodiments, each occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



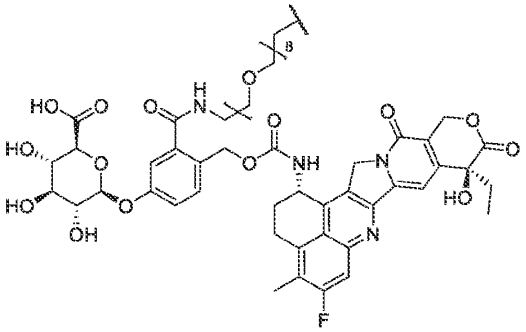
. In some embodiments, an occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



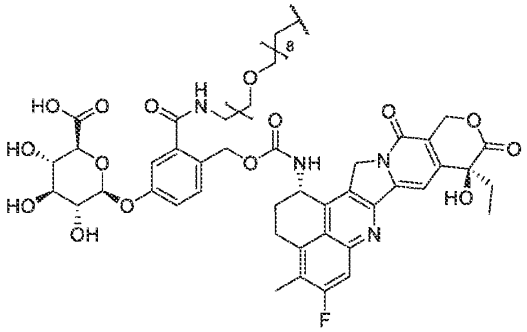
. In some embodiments, each occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



. In some embodiments, an occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



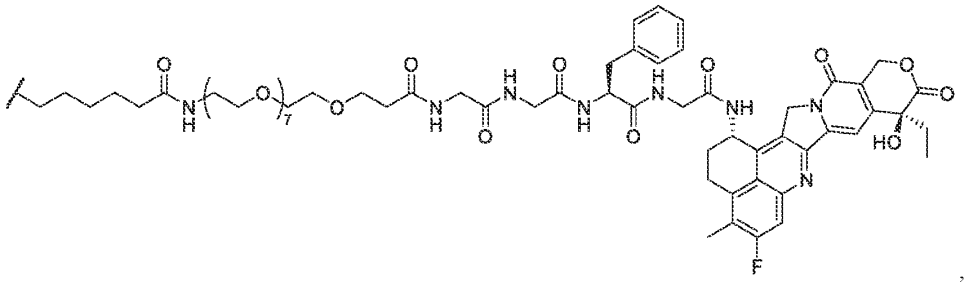
. In some embodiments, each occurrence of

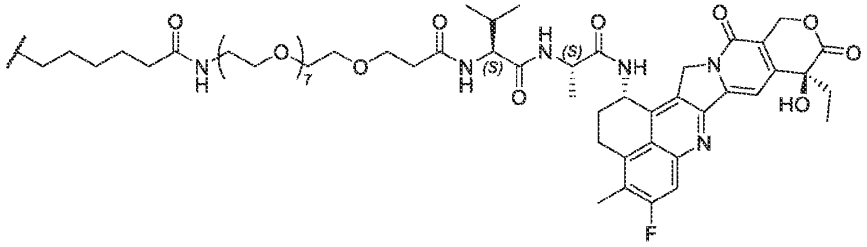


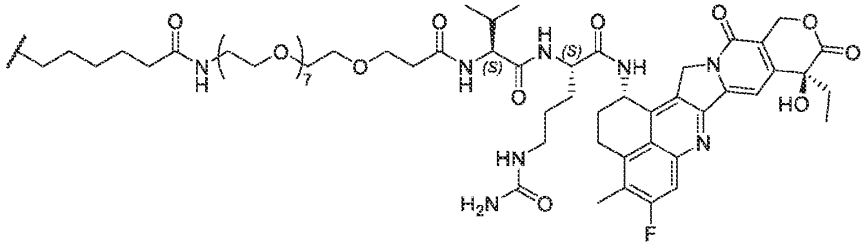
$-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises .

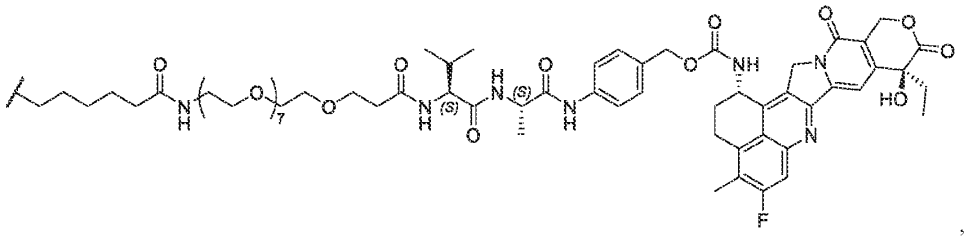
Those skilled in the art reading the present desclosure appreciate that other embodiments of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ described herein can be also utilized.

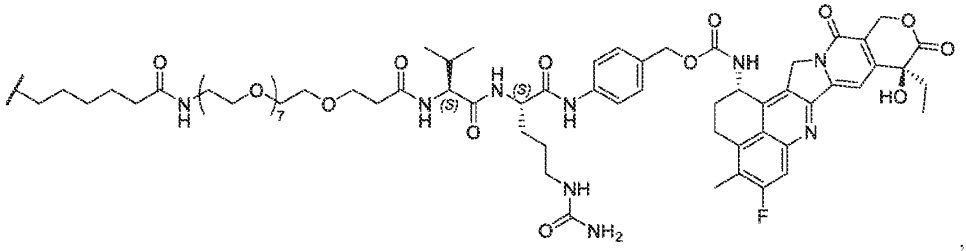
[0623] In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

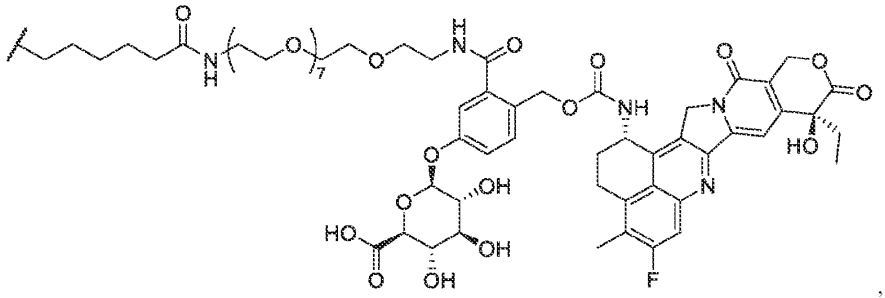




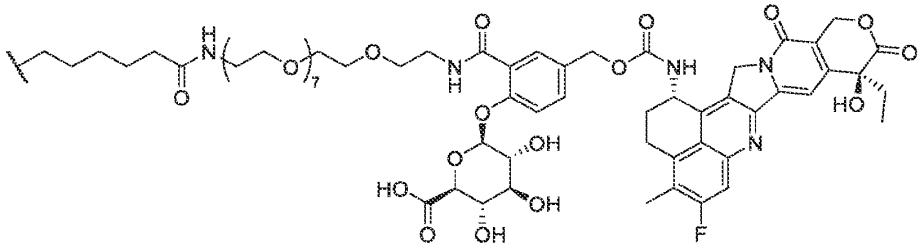




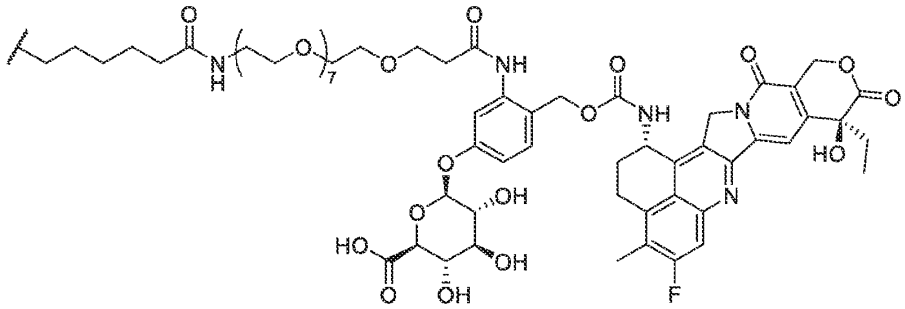




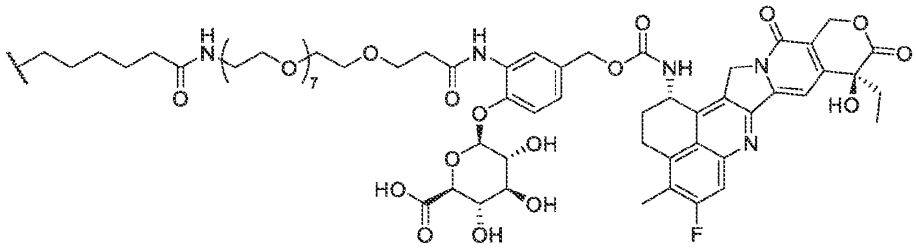
,



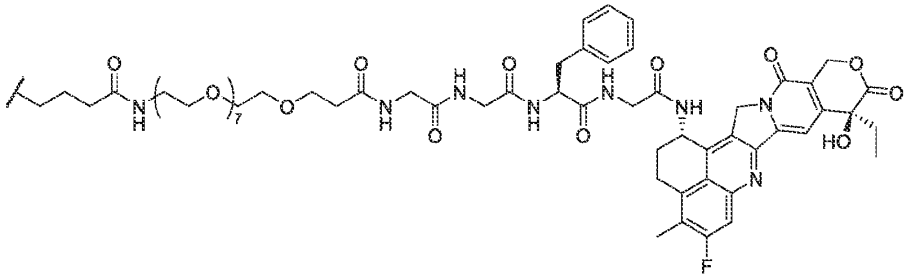
,



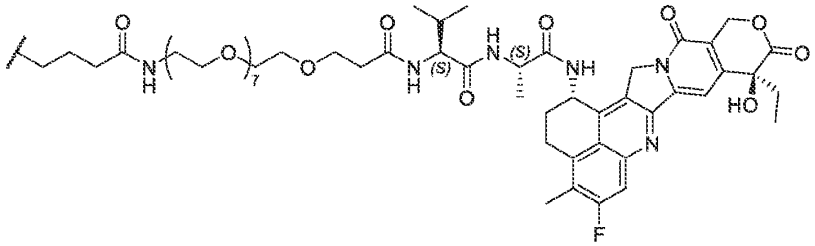
, or

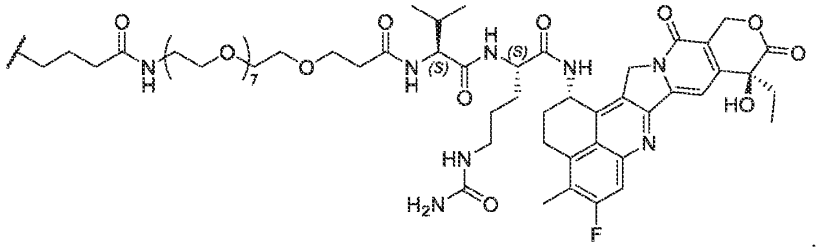


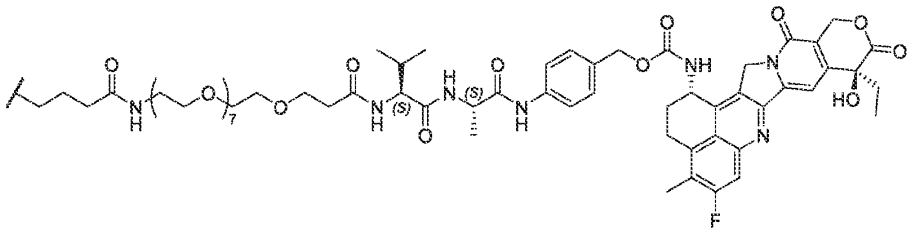
. In some embodiments, an occurrence of $-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

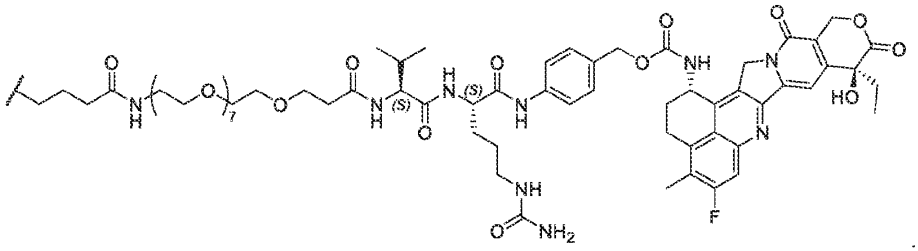


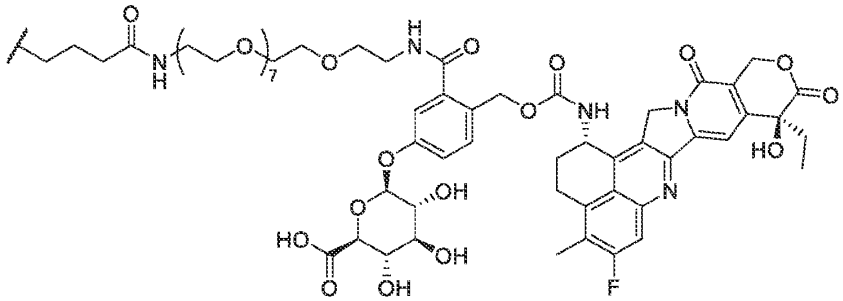
.

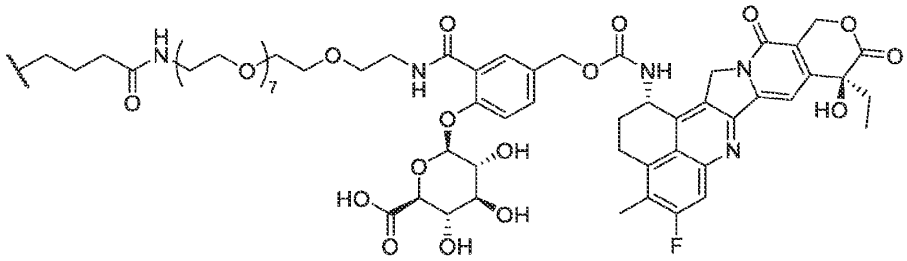




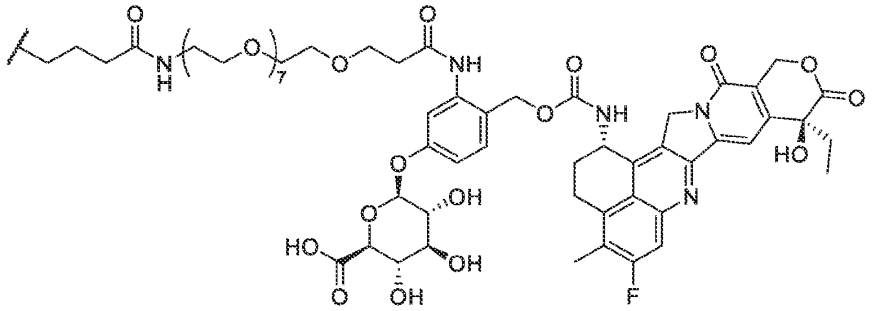




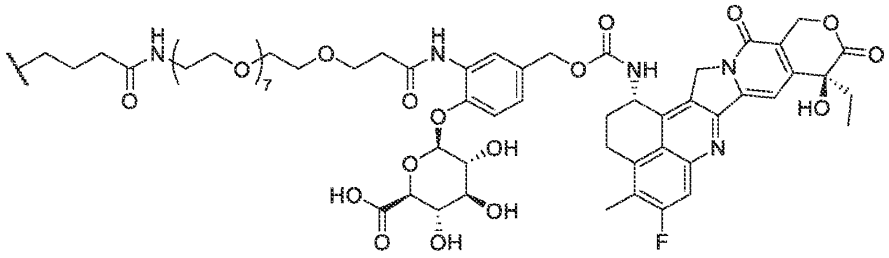




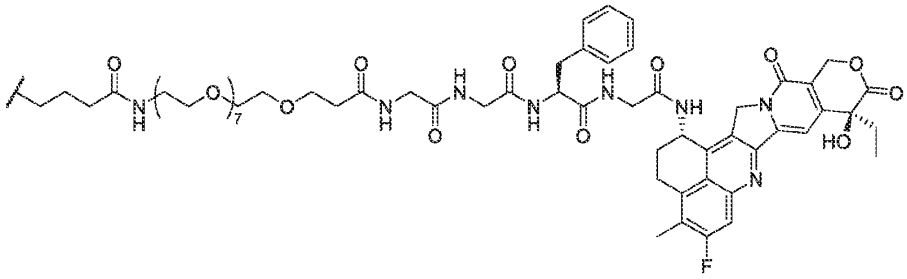
,



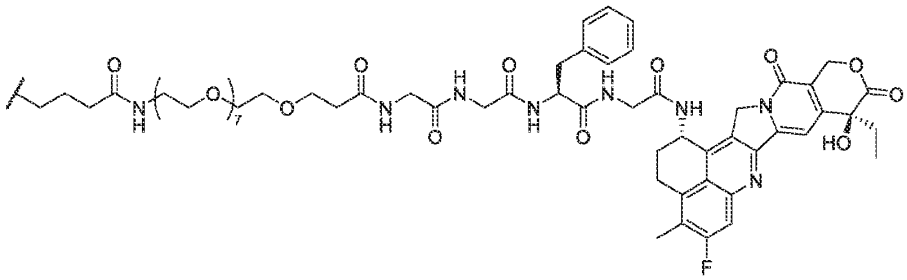
, or



. In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

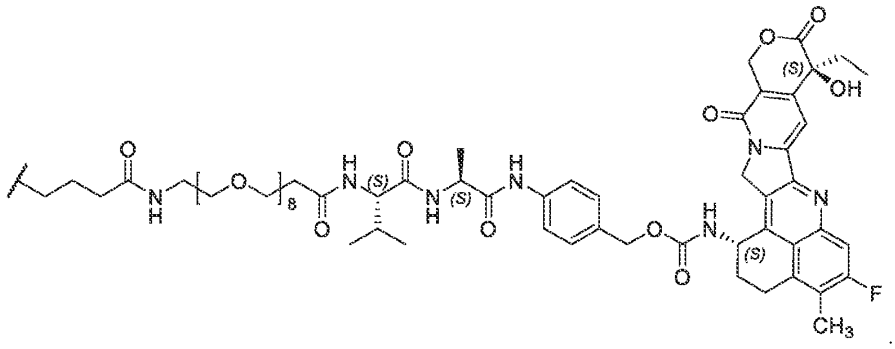


. In some embodiments, each occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

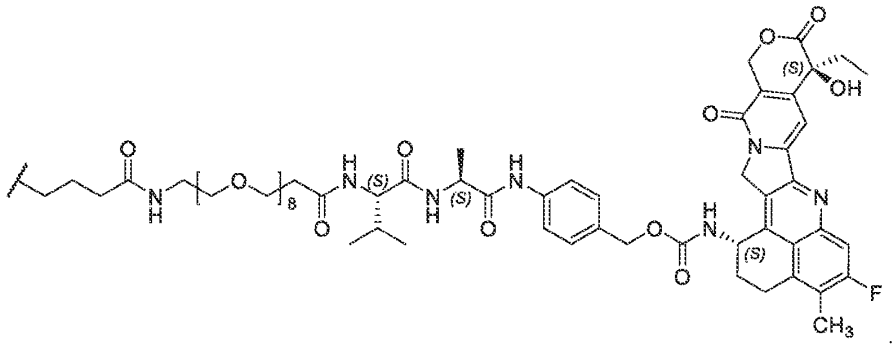


. In some

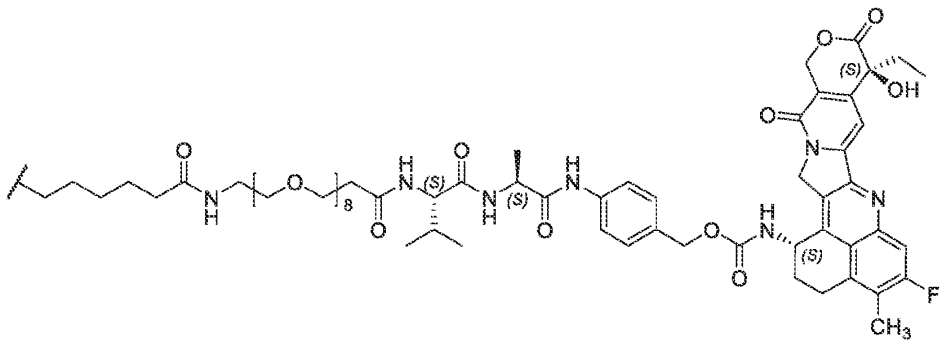
embodiments, an occurrence of $-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



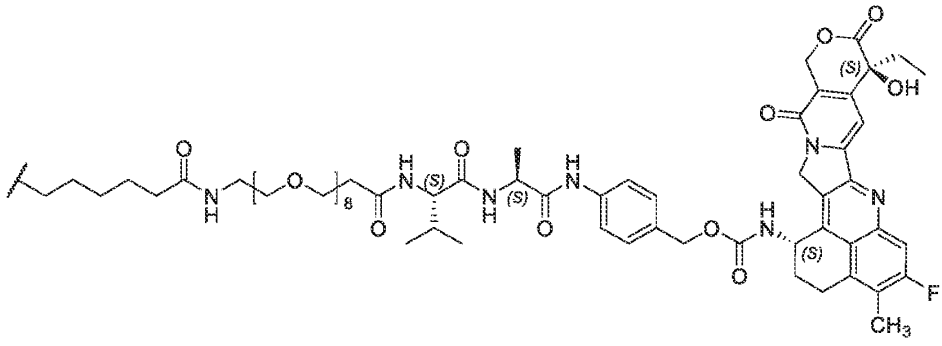
. In some embodiments, each occurrence of $-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



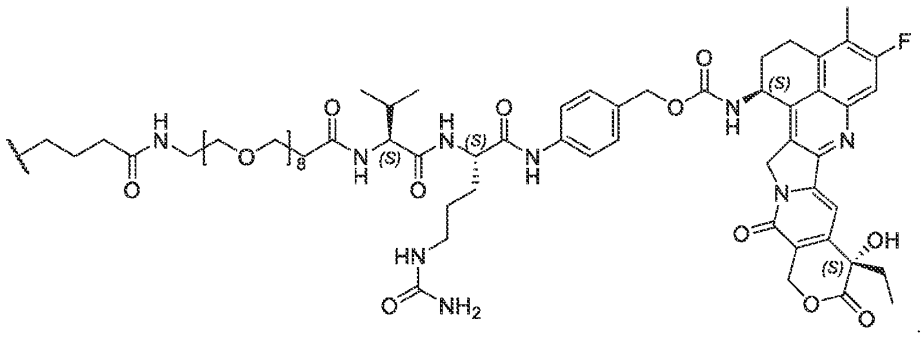
. In some embodiments, an occurrence of $-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



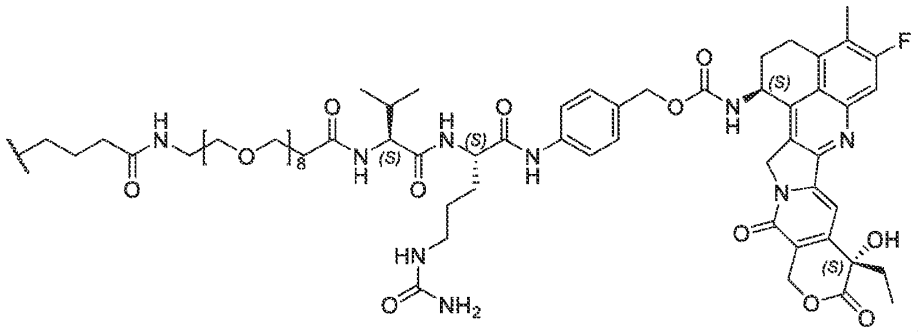
. In some embodiments, each occurrence of $-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



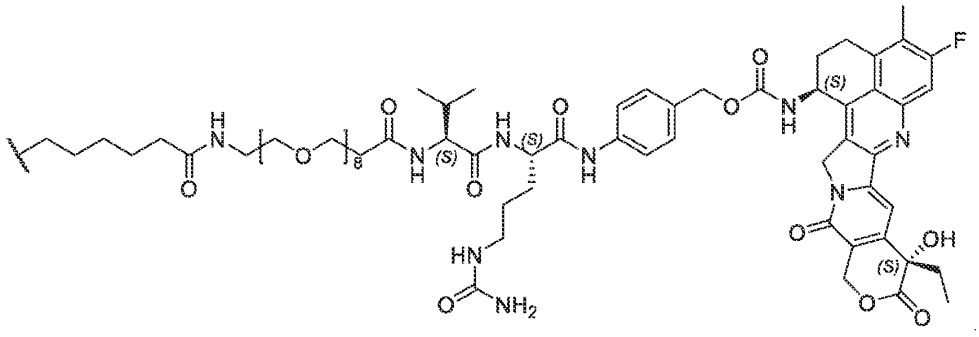
. In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



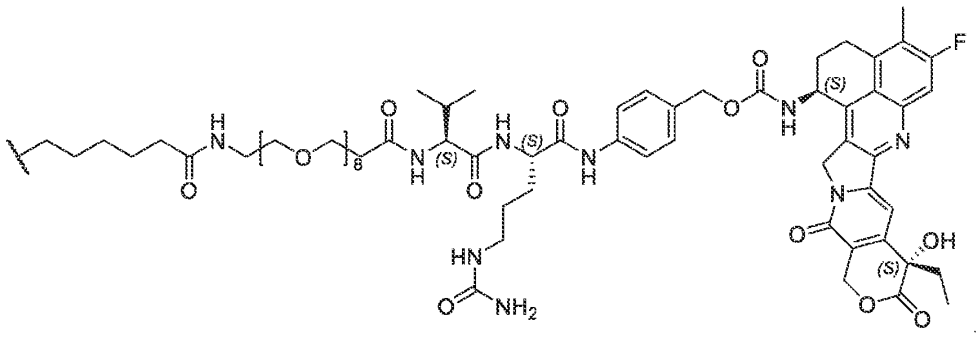
. In some embodiments, each occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



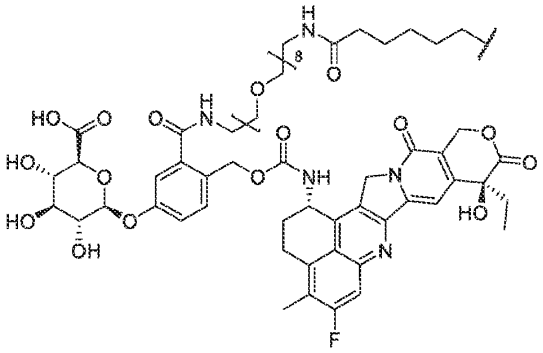
. In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



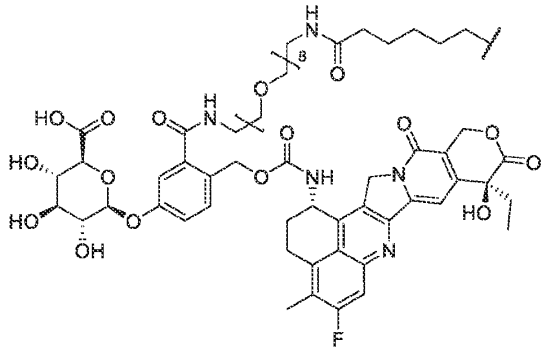
. In some embodiments, each occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

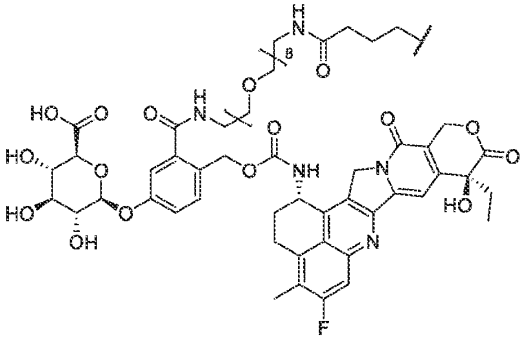


. In some embodiments, each occurrence of

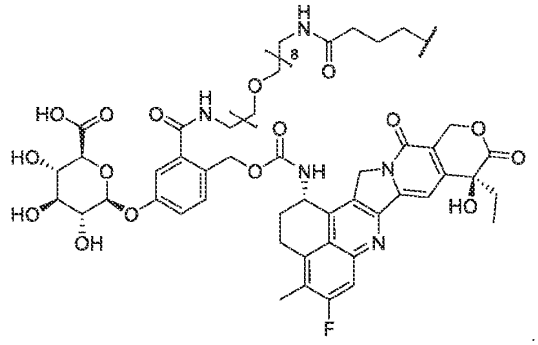


$-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises .

In some embodiments, an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



. In some embodiments, each occurrence of

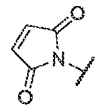


$-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises .

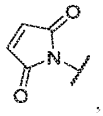
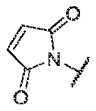
Those skilled in the art reading the present desclosure appreciate that other embodiments of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ described herein can be also utilized.

[0624] In some embodiments, a target binding agent reactive group is or comprises a nucleophilic group, e.g., −SH. In some embodiments, a target binding agent reactive group is or comprises −SH of a cysteine residue. In some embodiments, a method comprises reducing a −S−S− moiety in a target binding agent to provide −SH. In some embodiments, a target binding moiety is or comprises an antibody or an antigen-binding fragment thereof.

[0625] In some embodiments, a payload agent reactive group is or comprises −SH. In some embodiments, a payload agent reactive group is or comprises an electrophilic group as described herein,

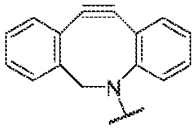
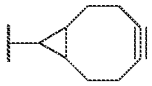


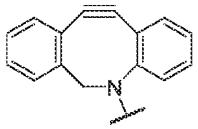
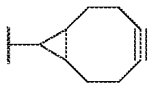
e.g., .

[0626] In some embodiments, one of a target binding agent reactive group and a payload agent reactive group is or comprises an electrophilic group (e.g., a double bond conjugated to an electron-withdrawing group (EWG) (e.g., −C(O)−, $-NO_2$, etc.) such as  , $LG-CH_2-EWG$ (wherein LG is a leaving group such as −Cl, −Br, −I, −OTs, etc. and the $-CH_2-$ is optionally substituted; in some embodiments, EWG is or comprises −C(O)−) etc.) and the other is or comprises a nucleophilic group (e.g., −SH). In some embodiments, a target binding moiety reactive group is or comprises −SH and a payload agent reactive group is or comprises  . In some embodiments, a target binding moiety reactive group is or comprises −SH and a payload agent reactive group is or comprises $LG-CH_2-EWG$ wherein the $-CH_2-$ is optionally substituted. In some embodiments, a target binding moiety reactive group is or comprises −SH and a payload agent reactive group is or comprises $LG-CH_2-C(O)-$ wherein the $-CH_2-$ is optionally substituted. In some embodiments, the $-CH_2-$ is substituted. In some embodiments, the $-CH_2-$ is unsubstituted. Various leaving groups can be utilized in accordance with the present disclosure. In some embodiments, LG is −Cl. In some embodiments, LG is −Br. In some

embodiments, LG is –I. In some embodiments, LG is –OTs.

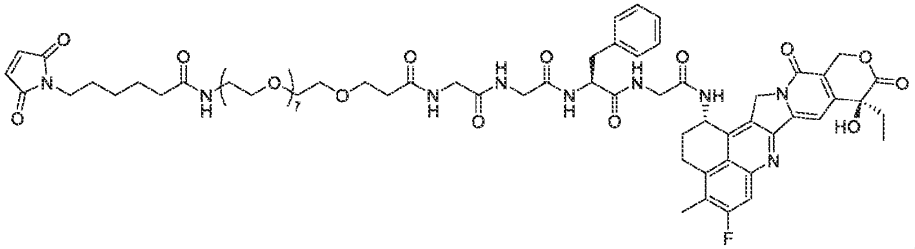
[0627] In some embodiments, one of a target binding agent reactive group and a payload agent reactive group is or comprises an amino group and the other is or comprises an acid group (e.g., –C(O)OH, –$S(O)_2OH$, etc.) or an activated form thereof.

[0628] In some embodiments, a payload agent reactive group comprises –C≡C–. In some embodiments, a payload agent reactive group is or comprises –C≡CH. In some embodiments, a payload agent reactive group is or comprises  . In some embodiments, a payload agent reactive group is or comprises  . In some embodiments, a payload agent reactive group is or comprises $–N_3$.

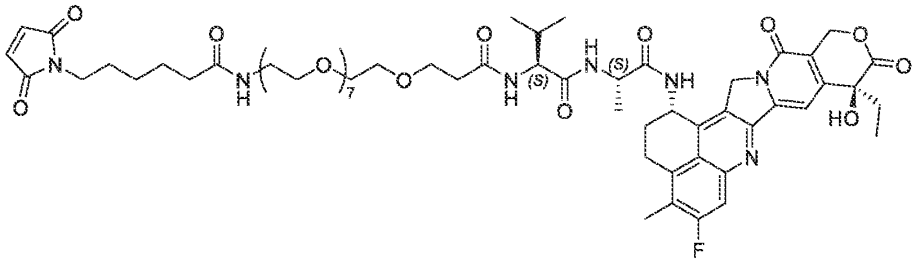
[0629] In some embodiments, a target binding agent reactive group is or comprises $–N_3$. In some embodiments, a target binding agent reactive group comprises –C≡C–. In some embodiments, a target binding agent reactive group is or comprises –C≡CH. In some embodiments, a target binding agent reactive group is or comprises  . In some embodiments, a target binding agent reactive group is or comprises  .

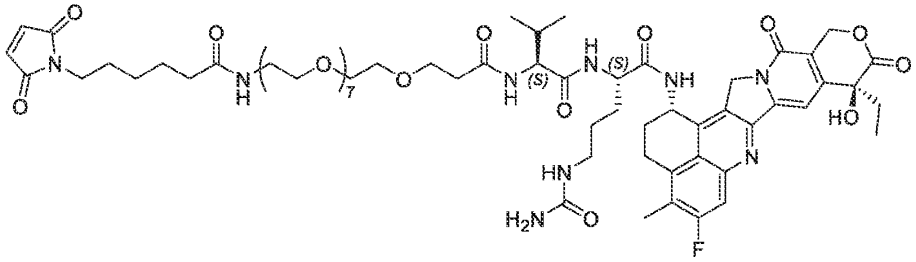
[0630] In some embodiments, one of a target binding agent reactive group and a payload agent reactive group is or comprises –C≡C– and the other is or comprises $–N_3$.

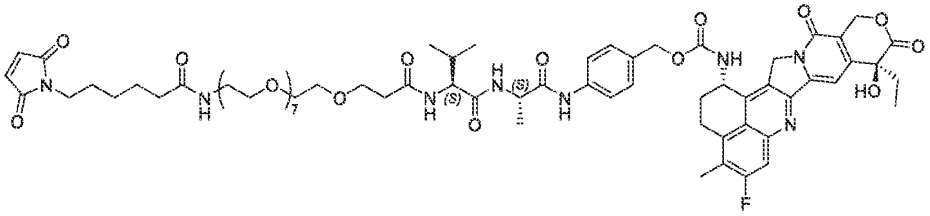
[0631] In some embodiments, a compound of formula P-1 or P-2 is

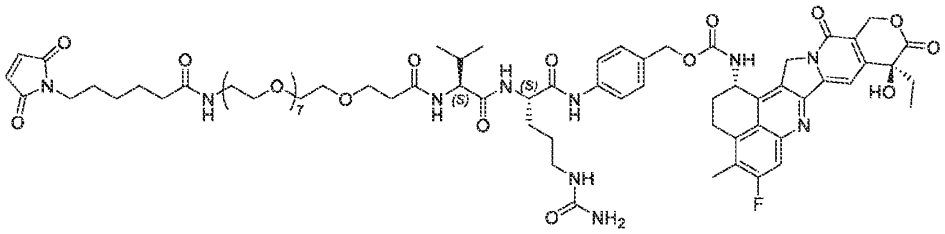


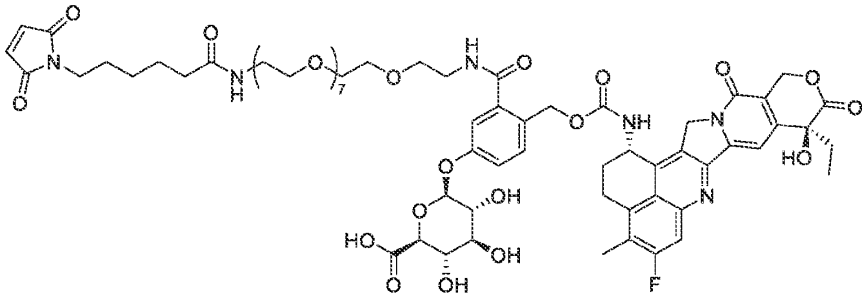
,

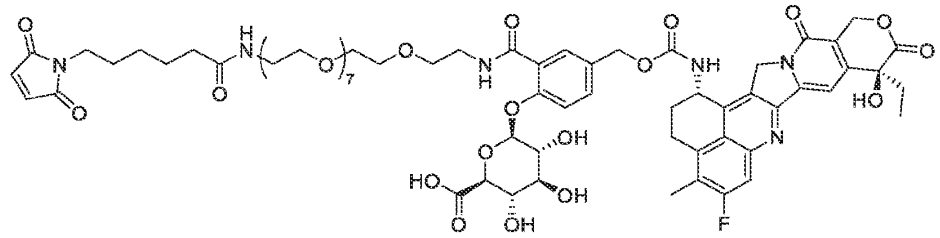


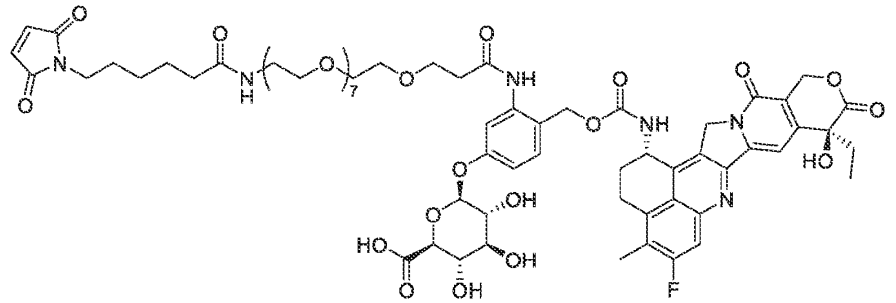




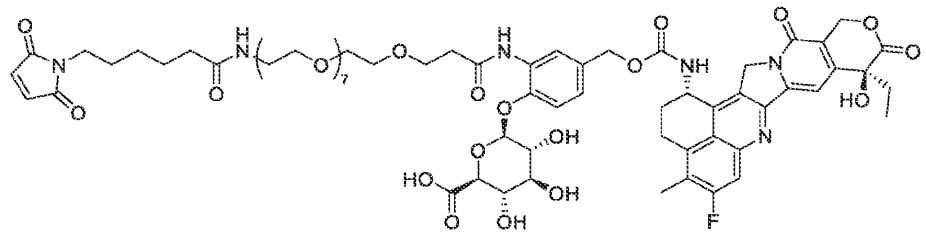




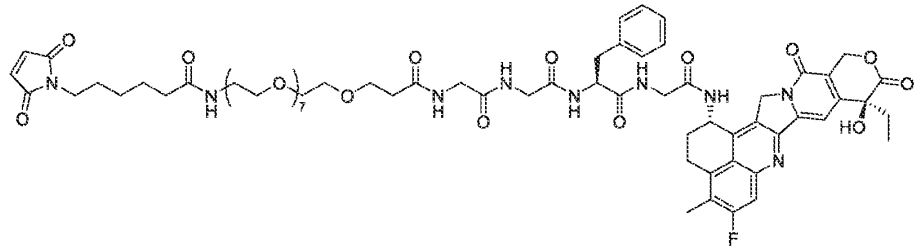




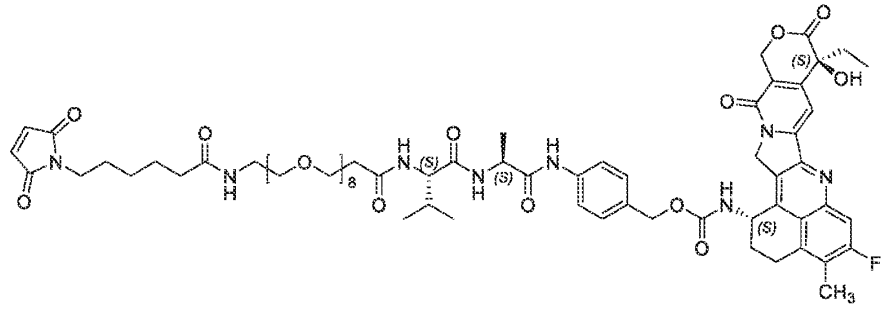
or



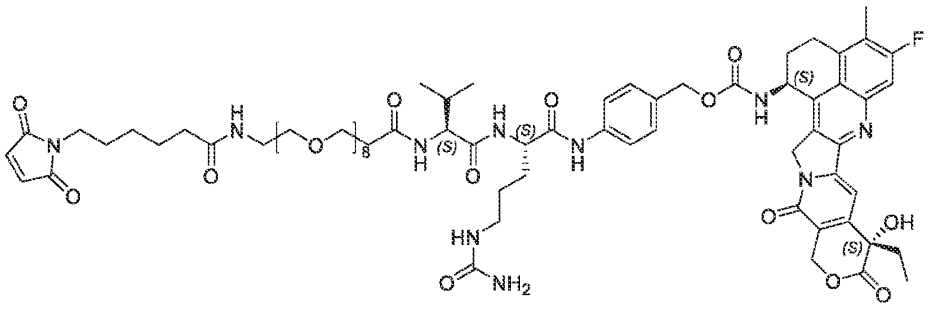
, or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is



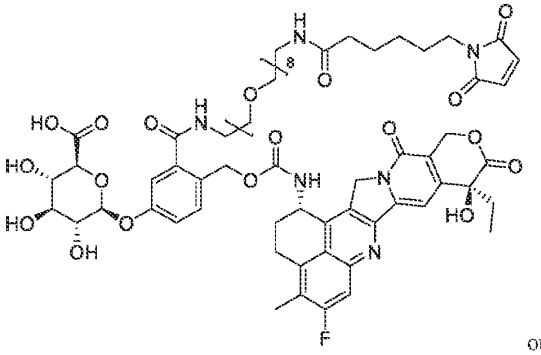
or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is



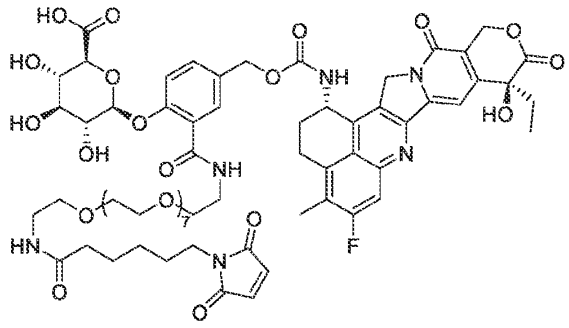
or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is



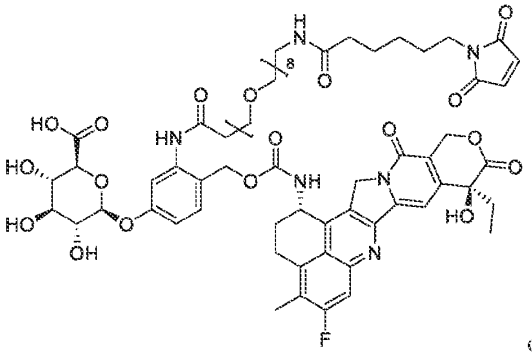
or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is



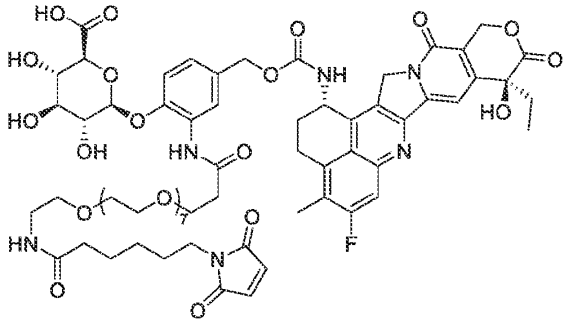
or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is



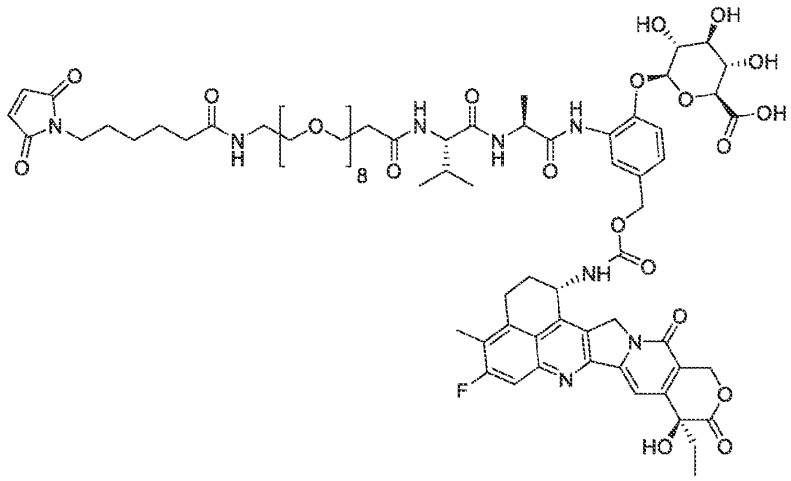
or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is



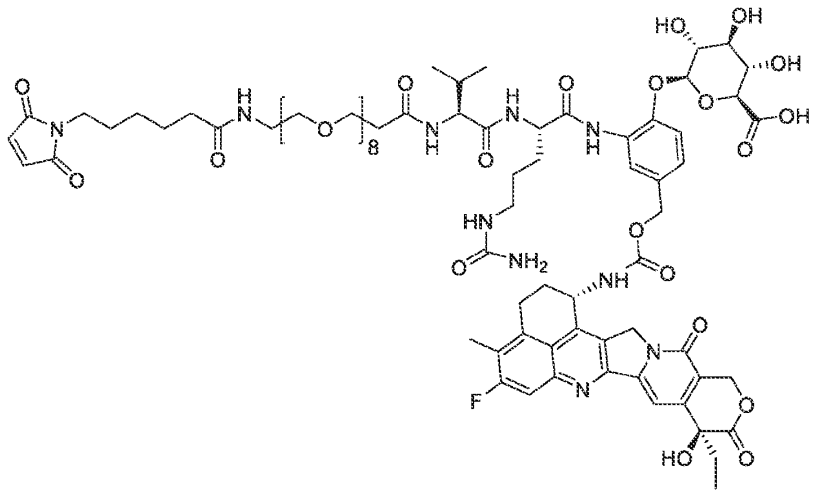
or a salt, e.g., a pharmaceutically acceptable salt,



thereof. In some embodiments, it is or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is

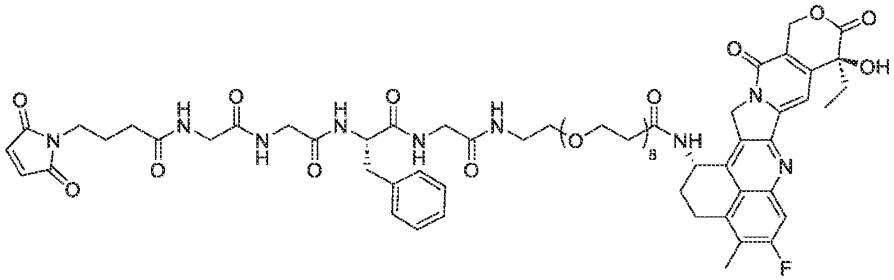


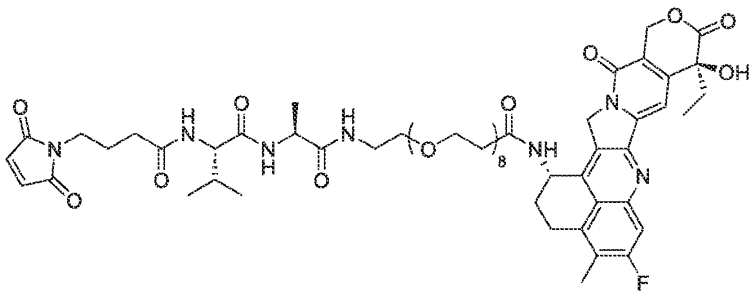
or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is

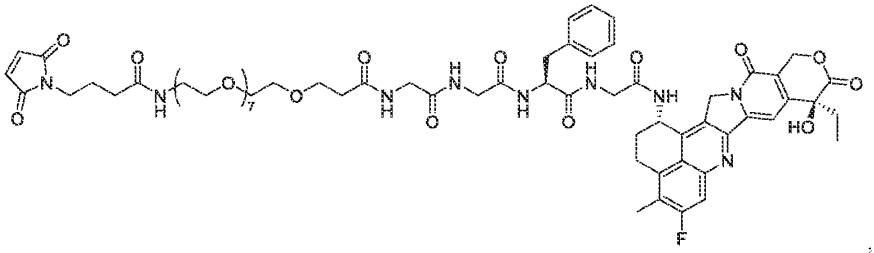


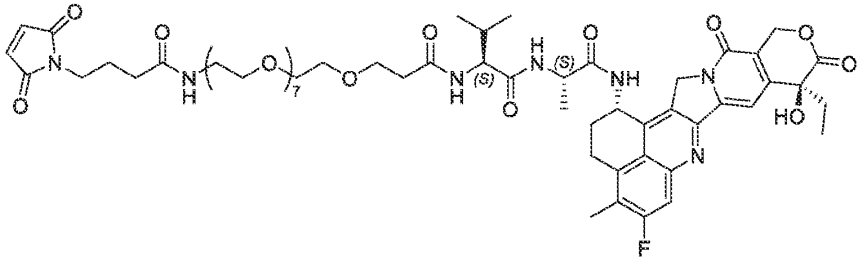
or a salt, e.g., a pharmaceutically acceptable salt, thereof.

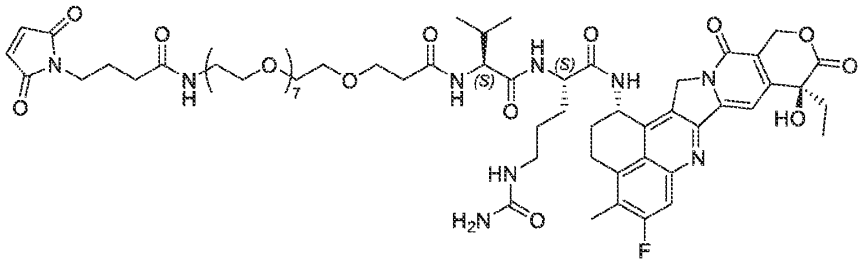
[0632] In some embodiments, a compound of formula P-1 or P-2 is

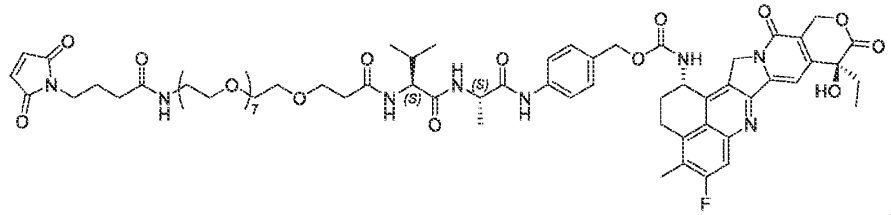


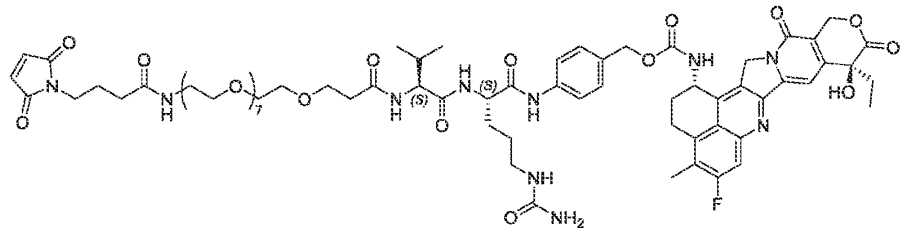


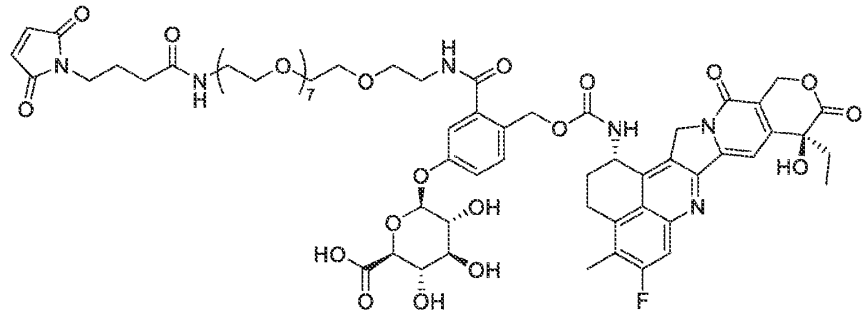


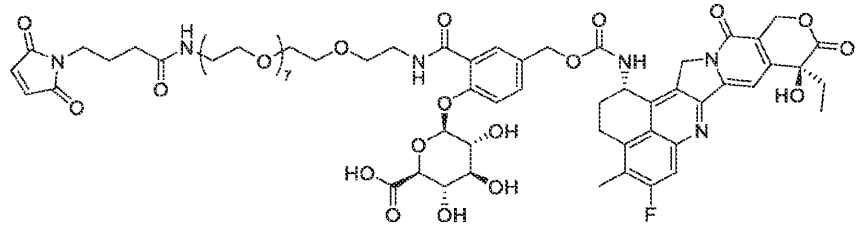


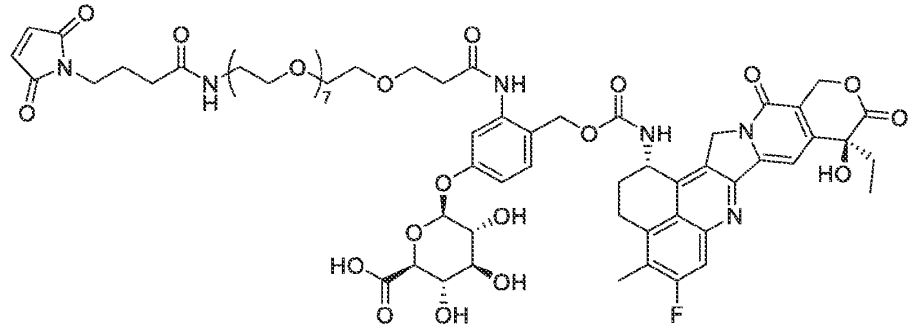




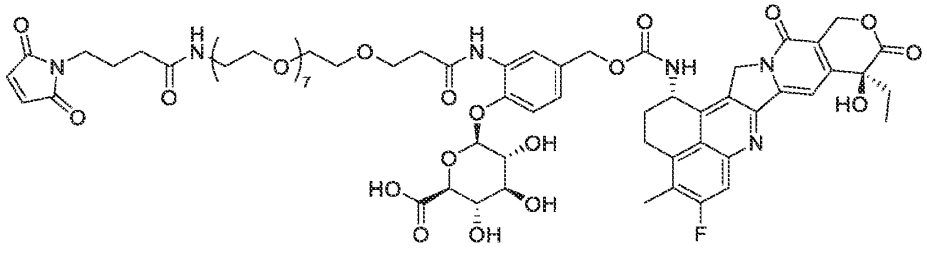




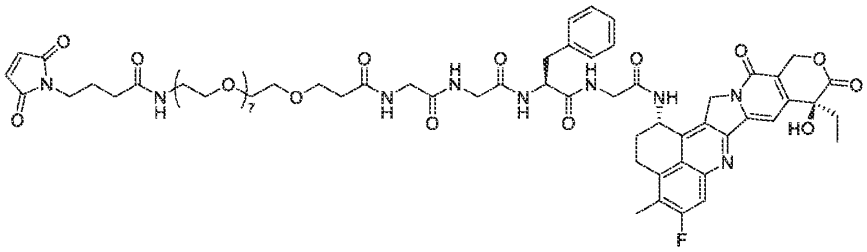


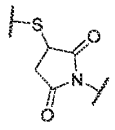


, or



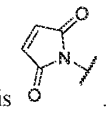
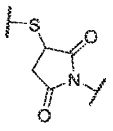
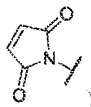
, or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is



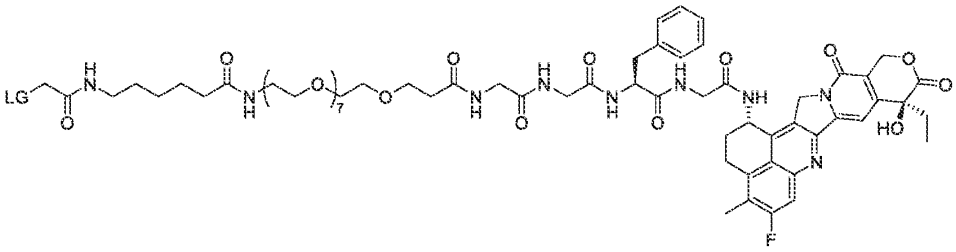
or a salt, e.g., a pharmaceutically acceptable salt thereof. In some embodiments, a compound is Compound 1 or a salt thereof. In some embodiments, a compound is Compound 2 or a salt thereof. In some embodiments, a compound is Compound 3 or a salt thereof. In some embodiments, a compound is Compound 4 or a salt thereof. In some embodiments, a compound is Compound 5 or a salt thereof. In some embodiments, a compound is Compound 6 or a salt thereof. In some embodiments, a compound is LP-A or a salt thereof. In some embodiments, a compound is LP-B or a salt thereof. In some embodiments, a compound is LP-C or a salt thereof. In some embodiments, a compound is LP-D or a salt thereof. In some embodiments, a conjugate is a conjugate of such a compound with a target binding moiety, e.g., an antibody or an antigen-binding fragment thereof, wherein the compound is conjugated with nucleophiles, e.g., −SH of Cys, at its maleimide moiety, forming  . In some embodiments, a target binding moiety is or comprises an antibody (e.g., 887-1DE, 65889, 65872, Hu2C4-H3L3, etc.) or an antigen-binding fragment thereof as described herein.

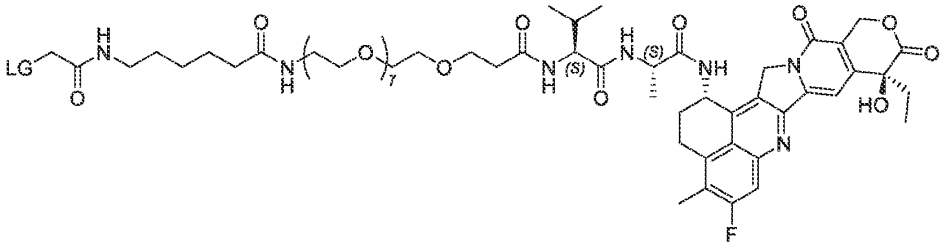
[0633] In some embodiments, a compound, e.g., a compound of formula P-1 or P-2, has the structure of L^{RXP}−$(CH_2)_xC(O)NH$−$(CH_2CH_2O)n$−$(CH_2)_zC(O)$−GGFG−R^P. In some embodiments, a compound, e.g., a compound of formula P-1 or P-2, has the structure of L^{RXP}−$(CH_2)_xC(O)NH$−$(CH_2CH_2O)n$−$(CH_2)_zC(O)$−Val−Ala−PABA−R^P. In some embodiments, a compound, e.g., a compound of formula P-1 or P-2, has the structure of L^{RXP}−$(CH_2)_xC(O)NH$−$(CH_2CH_2O)n$−$(CH_2)_zC(O)$−Val−Cit−PABA−R^P. In some embodiments, a compound, e.g., a compound of formula P-1 or P-2, has the structure of L^{RXP}−$(CH_2)_xC(O)NH$−$(CH_2CH_2O)n$−$(CH_2)_zC(O)$Val−Ala−NH−oGlu−R^P. In some embodiments, a compound, e.g., a compound of formula P-1 or P-2, has the structure of L^{RXP}−$(CH_2)_xC(O)NH$−$(CH_2CH_2O)n$−$(CH_2)_zC(O)$−Val−Ala−NH−mGlu−R^P. In some embodiments, a

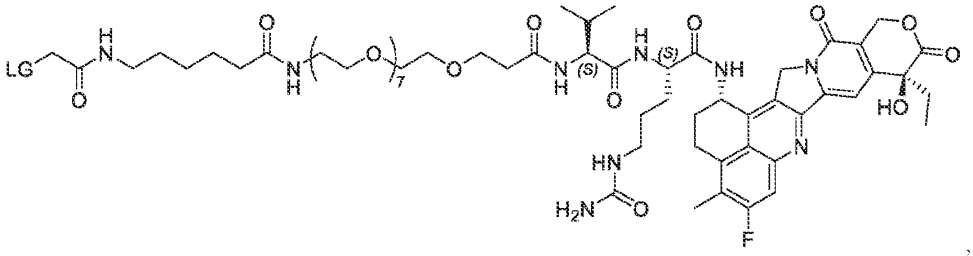
compound, e.g., a compound of formula P-1 or P-2, has the structure of L^{RXP}–$(CH_2)_xC(O)NH$–$(CH_2CH_2O)n$–$(CH_2)_zC(O)$–Val–Cit–NH–oGlu–R^P. In some embodiments, a compound, e.g., a compound of formula P-1 or P-2, has the structure of L^{RXP}–$(CH_2)_xC(O)NH$–$(CH_2CH_2O)n$–$(CH_2)_zC(O)$–Val–Cit–NH–mGlu–R^P. In some embodiments, a compound, e.g., a compound of formula P-1 or P-2, has the structure of L^{RXP}–$(CH_2)_xC(O)NH$–$(CH_2CH_2O)n$–$(CH_2)_zNHC(O)$–oGlu–R^P. In some embodiments, a compound, e.g., a compound of formula P-1 or P-2, has the structure of

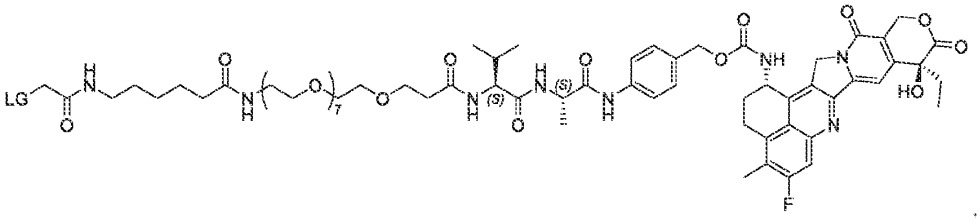
L^{RXP}–$(CH_2)_xC(O)NH$–$(CH_2CH_2O)n$–$(CH_2)_zNHC(O)$–mGlu–R^P. In some embodiments, L^{RXP} is  . In some embodiments, L^{RXP} is LG–CH_2–C(O)– wherein LG is a leaving group as described herein. In some embodiments, LG is halogen. In some embodiments, LG is –Cl, –Br, or –I. In some embodiments, x is 2. In some embodiments, x is 3. In some embodiments, x is 5. In some embodiments, n is 8. In some embodiments, z is 2. In some embodiments, R^P comprises a nitrogen atom and is bonded at the nitrogen atom, e.g., to the rest of a conjugate or other compound. In some embodiments, R^P is R^{EX}. In some embodiments, z is 2 and R^P is R^{EX}. In some embodiments, n is 8, z is 2 and R^P is R^{EX}. In some embodiments, x is 2, n is 8, z is 2 and R^P is R^{EX}. In some embodiments, x is 3, n is 8, z is 2 and R^P is R^{EX}. In some embodiments, x is 5, n is 8, z is 2 and R^P is R^{EX}. In some embodiments, a conjugate is a conjugate of such a compound with a target binding moiety, e.g., an antibody or an antigen-binding fragment thereof, wherein the compound is conjugated with nucleophiles, e.g., –SH of Cys, e.g., to form  (e.g., whein L^{RXP} is ), to form –S–CH_2–C(O)– (e.g., when L^{RXP} is LG–CH_2–C(O)–), etc. In some embodiments, a target binding moiety is or comprises an antibody (e.g., 887-1DE, 65889, 65872, Hu2C4-H3L3, etc.) or an antigen-binding fragment thereof as described herein.

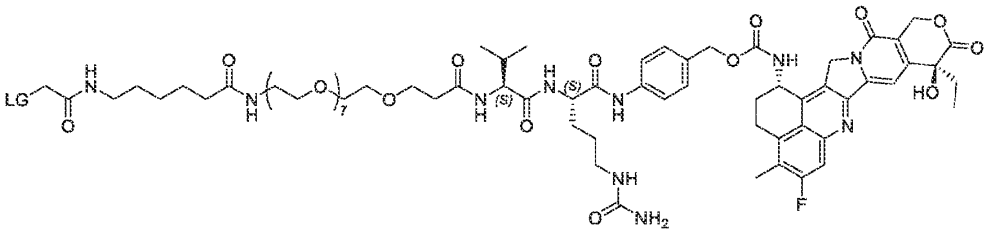
[0634] In some embodiments, a compound of formula P-1 or P-2 is

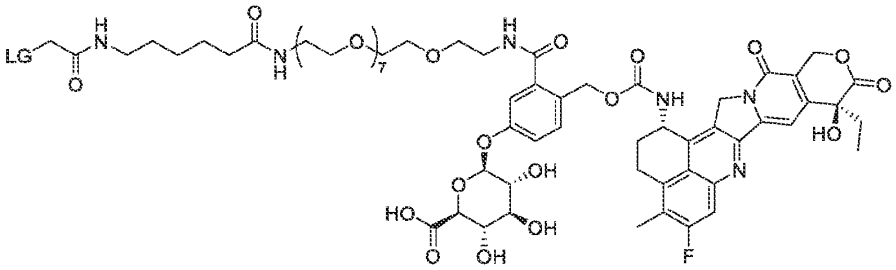


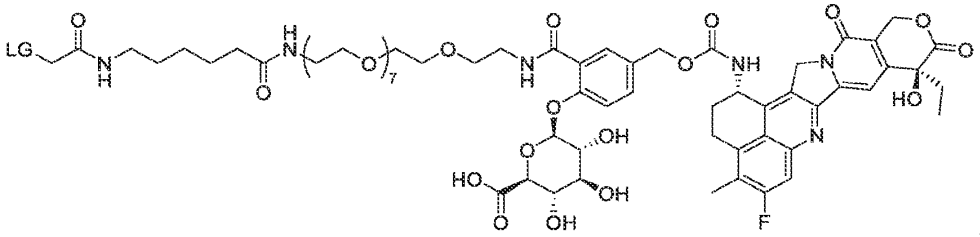


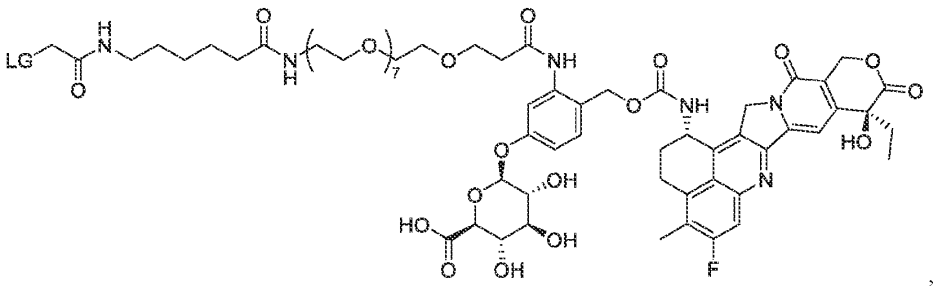




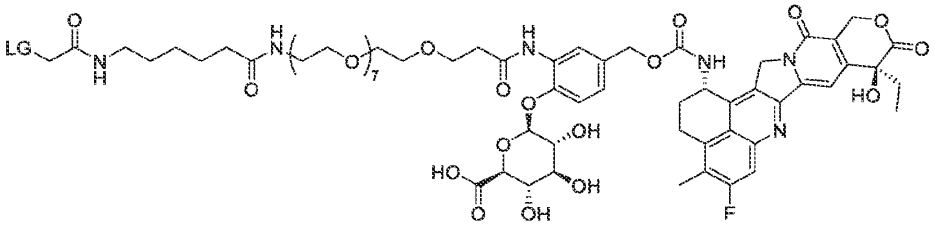




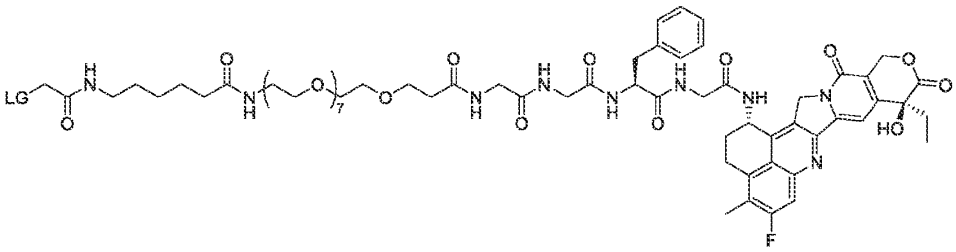




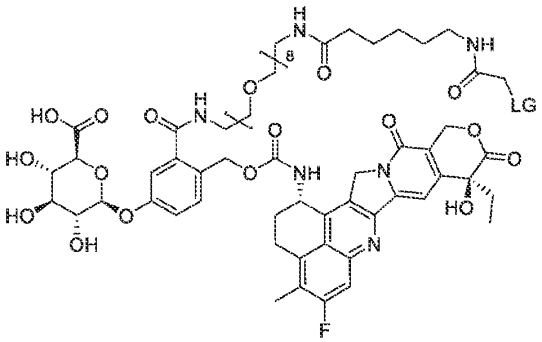
, or



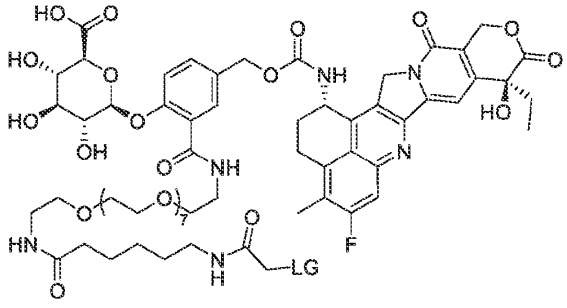
, or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is



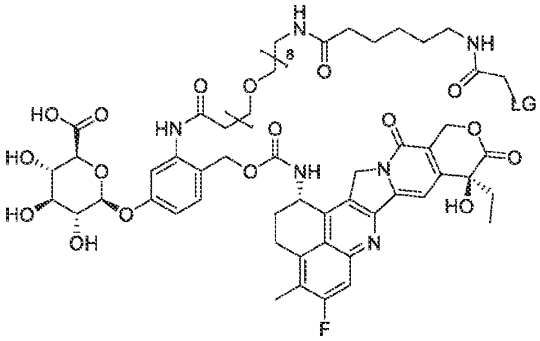
or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is



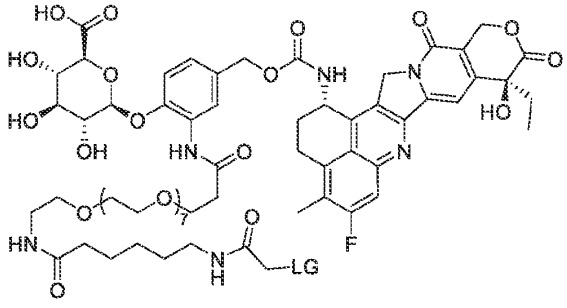
or a salt, e.g., a pharmaceutically acceptable salt.



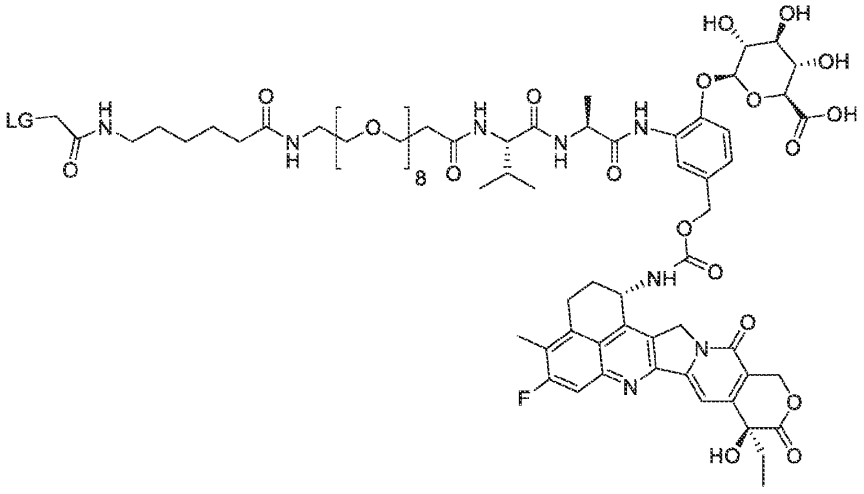
thereof. In some embodiments, it is or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is



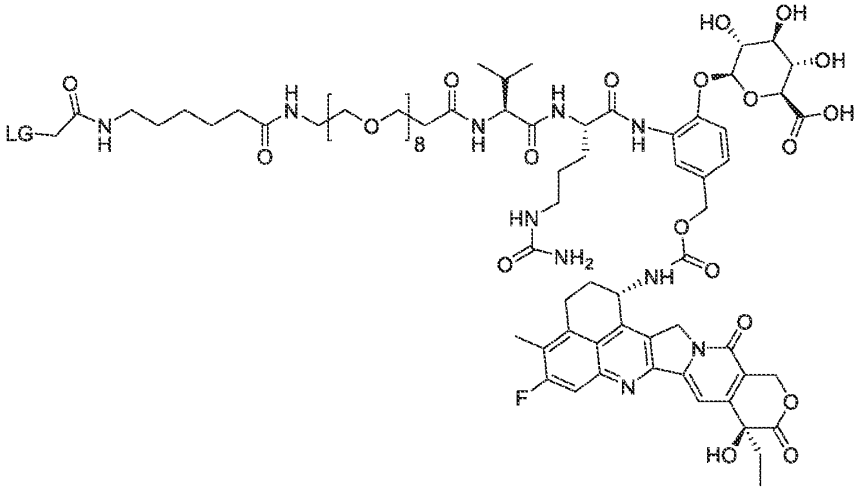
or a salt, e.g., a pharmaceutically acceptable salt,



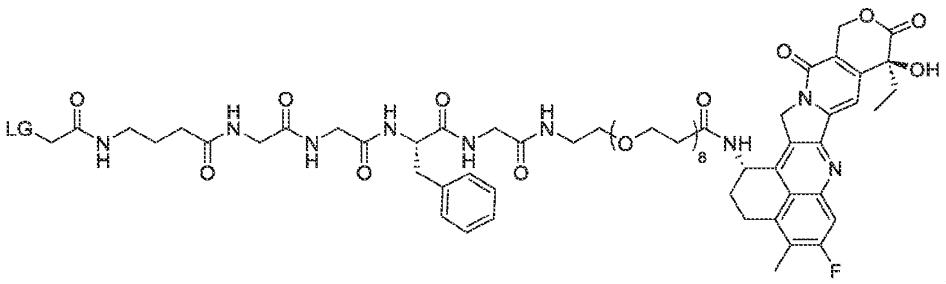
thereof. In some embodiments, it is or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is



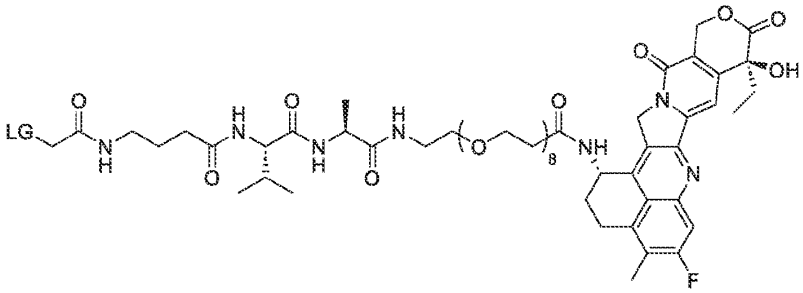
or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is

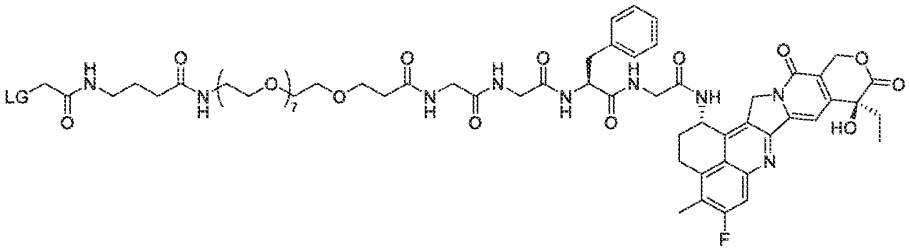


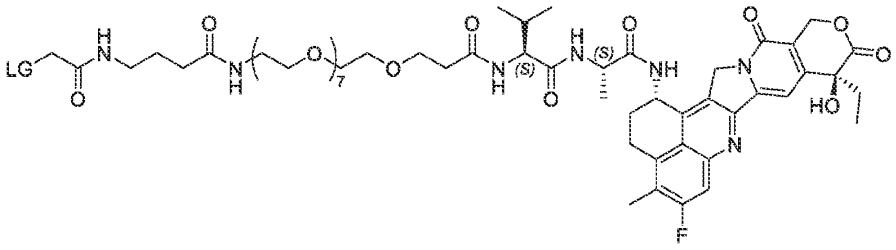
or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, a compound of formula P-1 or P-2 is

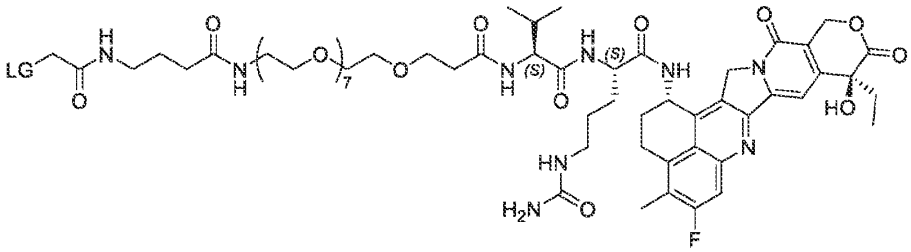


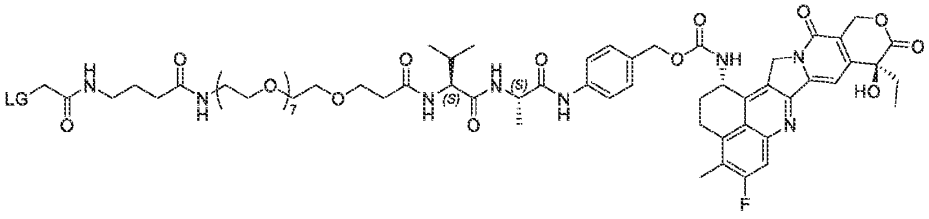
.

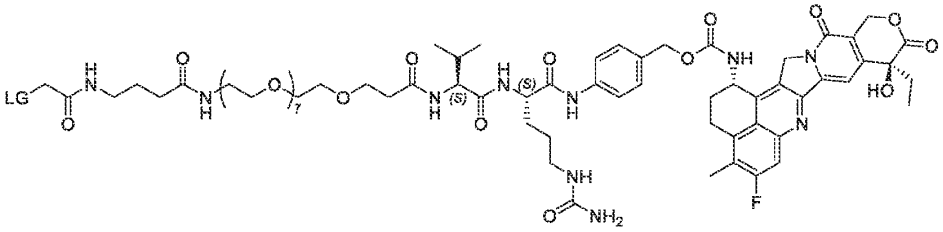


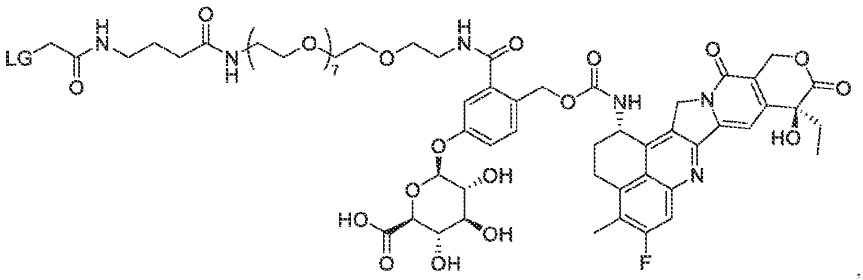


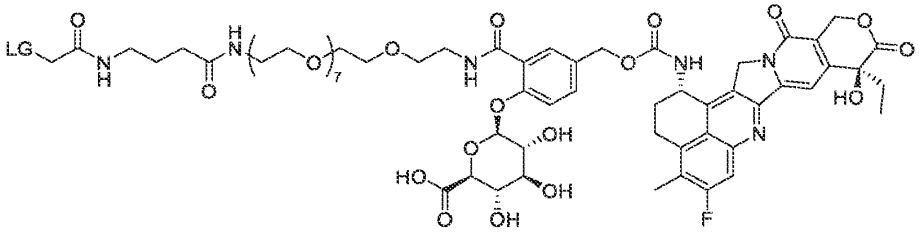


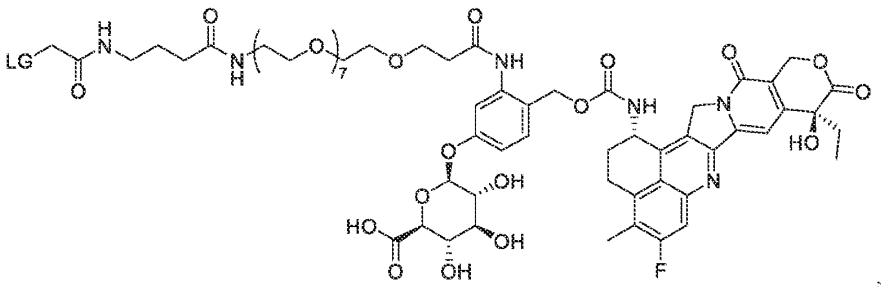




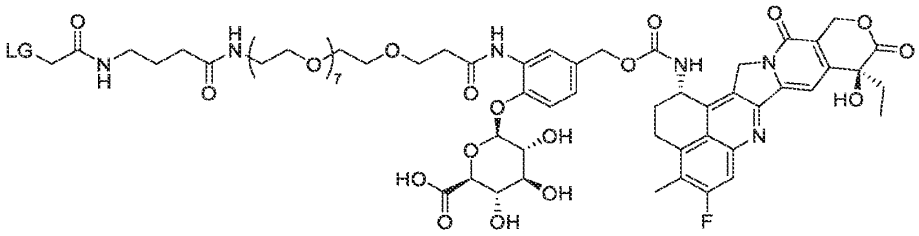




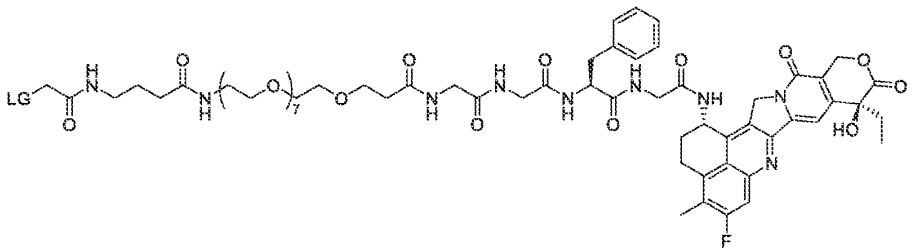




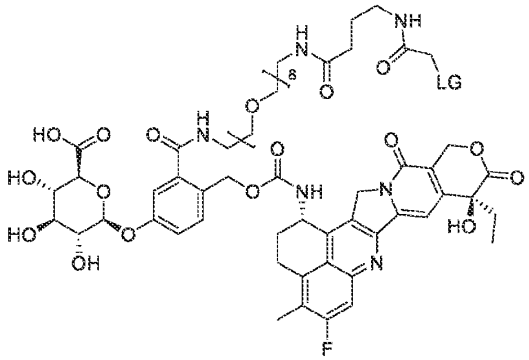
, or



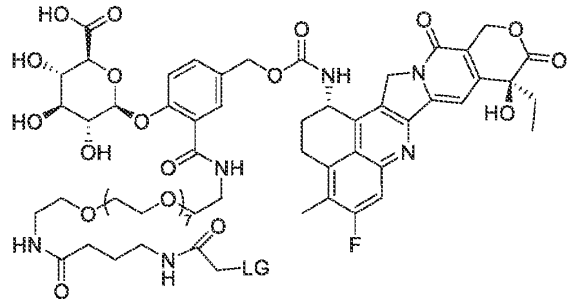
, or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is



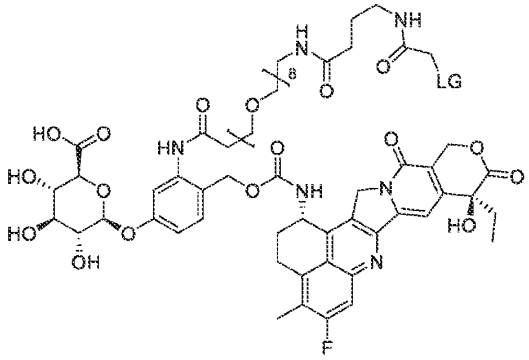
or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is



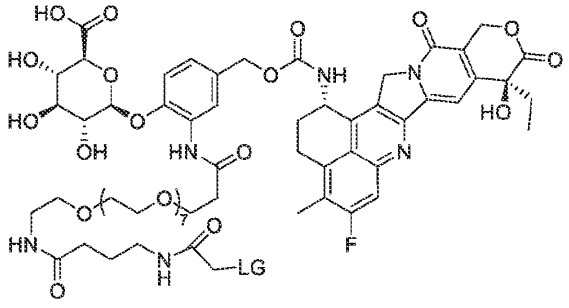
or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is



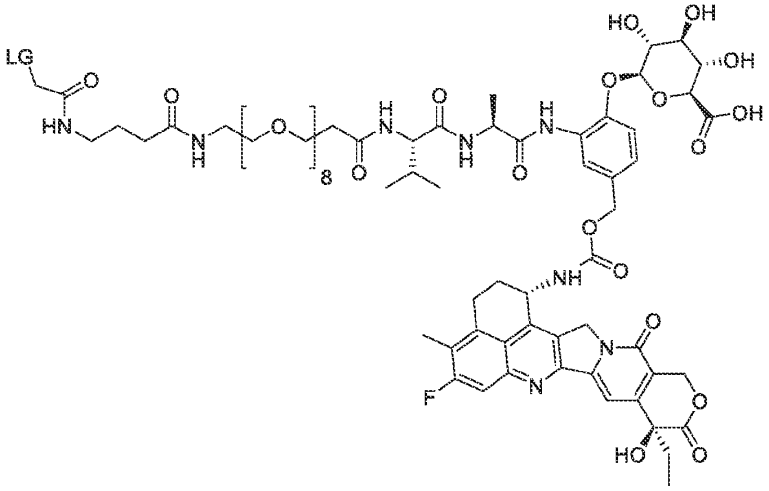
or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is



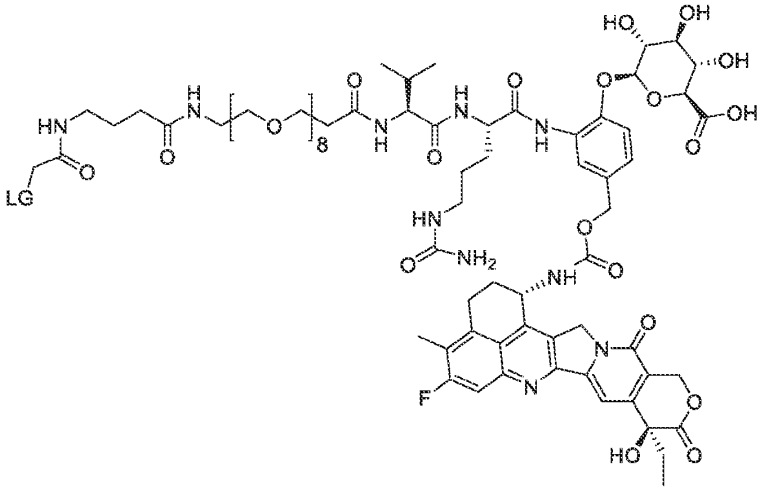
or a salt, e.g., a pharmaceutically acceptable salt,



thereof. In some embodiments, it is or a salt, e.g., pharmaceutically acceptable salt thereof. In some embodiments, it is

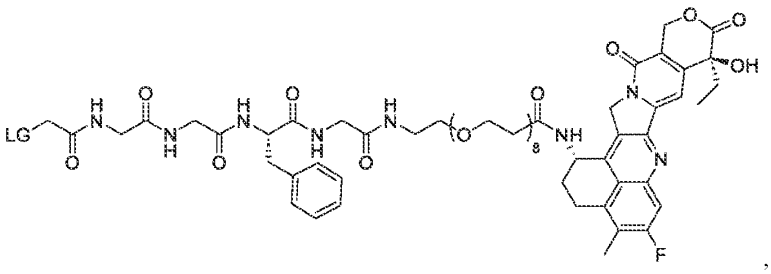


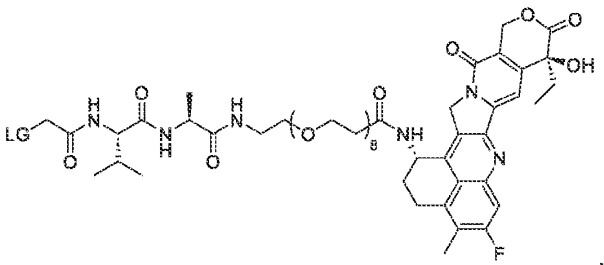
or a salt, e.g., pharmaceutically acceptable salt, thereof. In some embodiments, it is

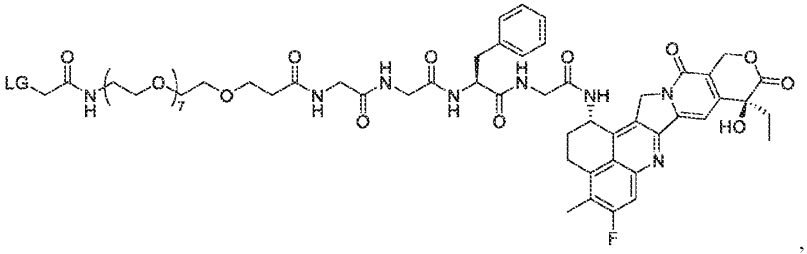


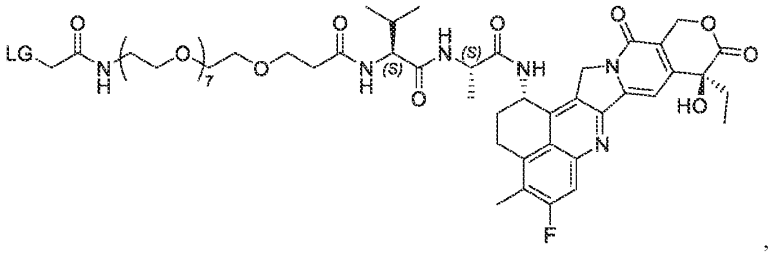
or a salt, e.g., pharmaceutically acceptable salt, thereof.

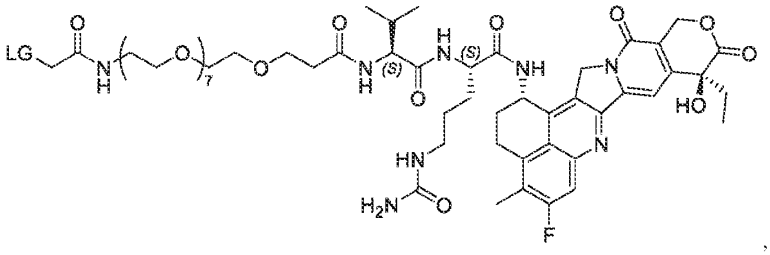
[0635] In some embodiments, a compound of formula P-1 or P-2 is

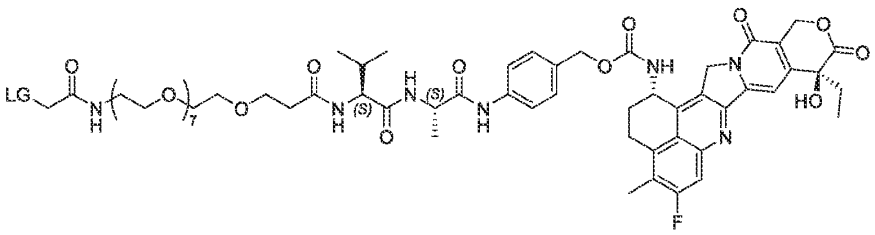


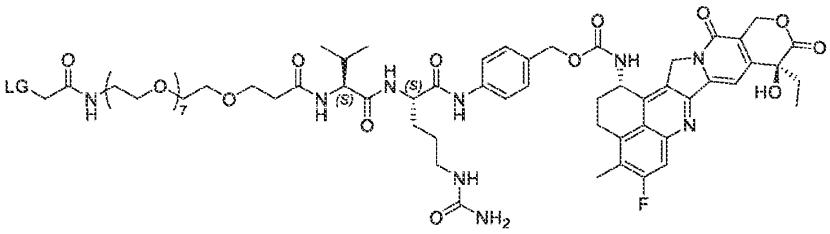


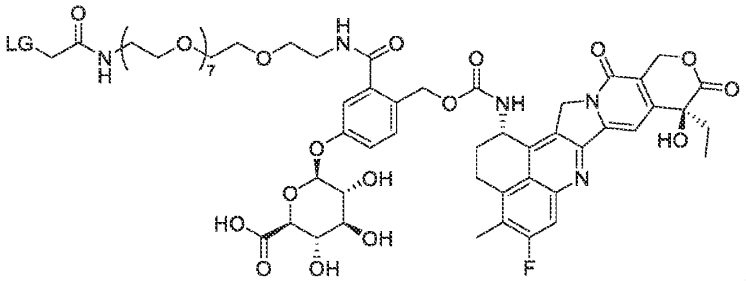


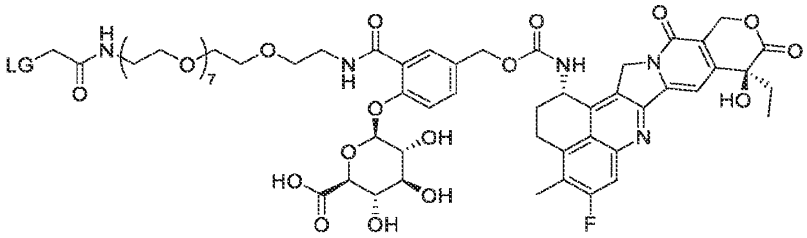


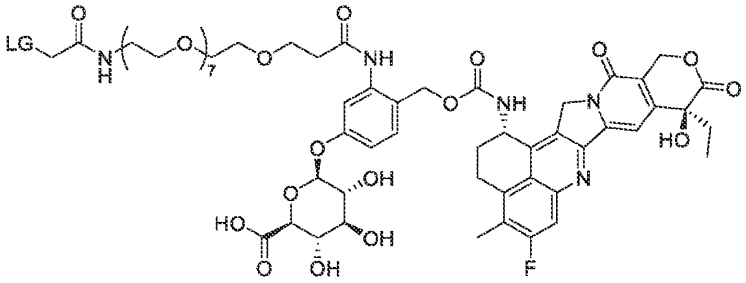




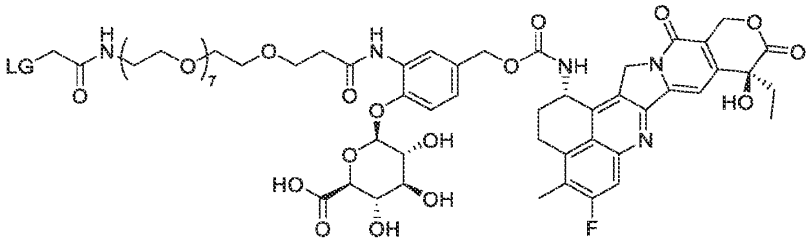




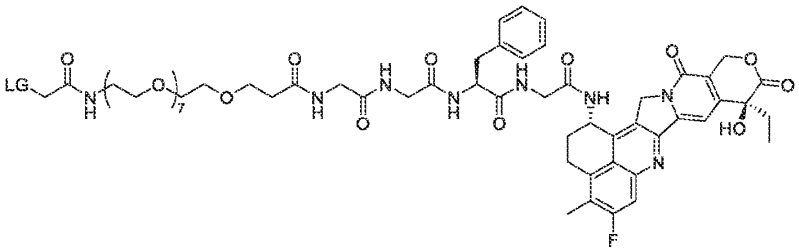




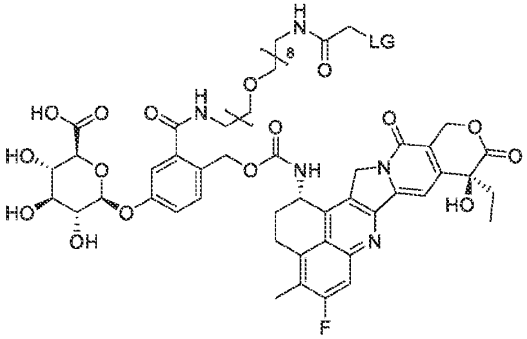
, or



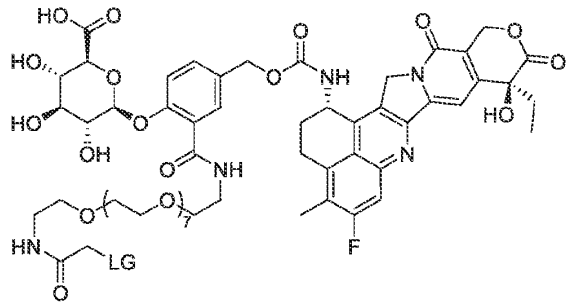
, or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is



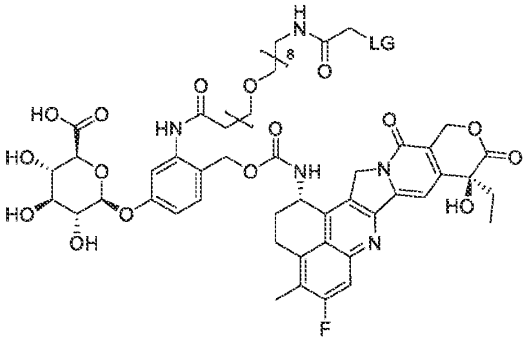
or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is



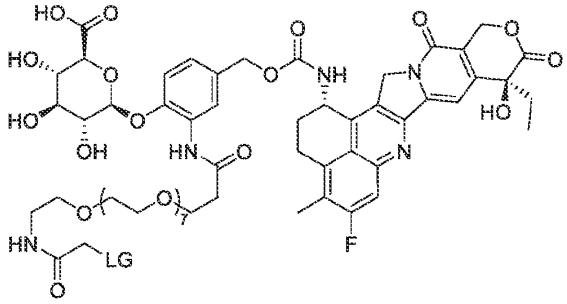
or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is



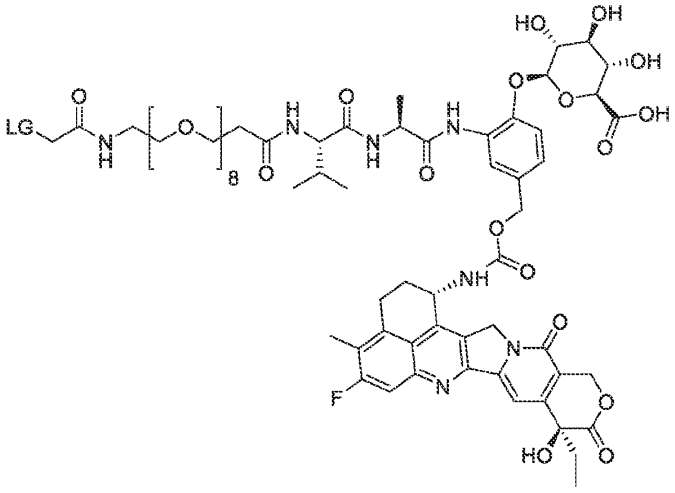
or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is



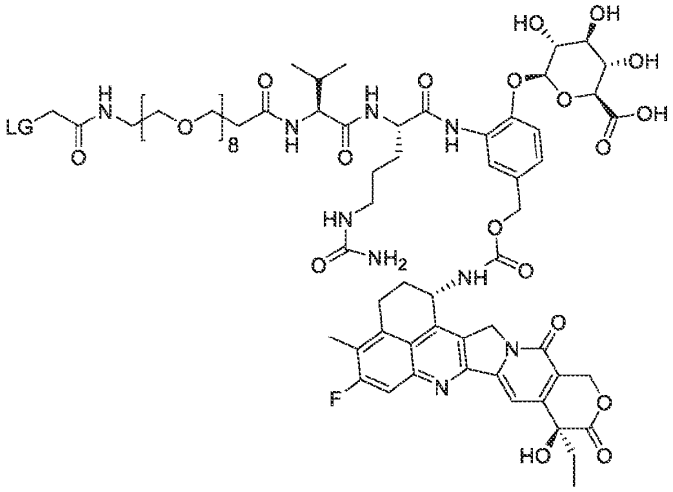
or a salt, e.g., a pharmaceutically acceptable salt,



thereof. In some embodiments, it is or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is



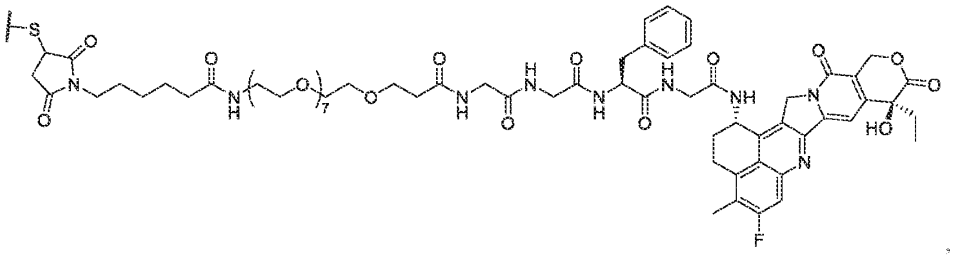
or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, it is

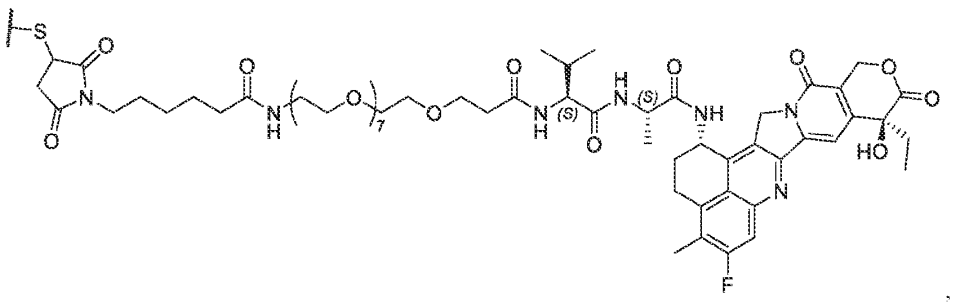


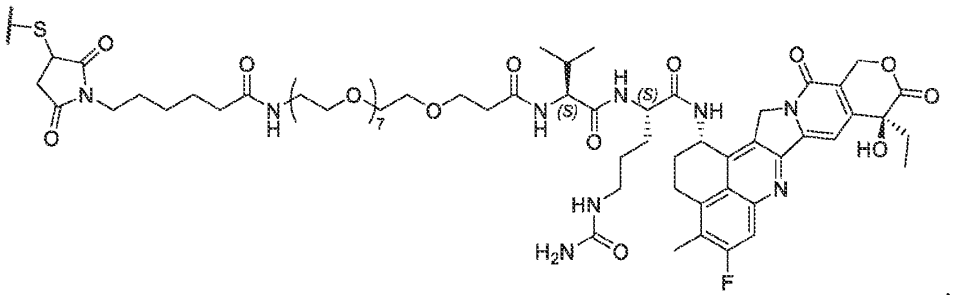
or a salt, e.g., a pharmaceutically acceptable salt, thereof. In some embodiments, a conjugate is a conjugate of such a compound with a target binding moiety, e.g., an antibody or an antigen-binding fragment thereof, wherein the compound is conjugated with nucleophiles, e.g., −SH of Cys, to form $-S-CH_2-C(O)-$. In some embodiments, a target binding moiety is or comprises an antibody (e.g., 887-1DE, 65889, 65872, Hu2C4-H3L3, etc.) or an antigen-binding fragment thereof as described herein.

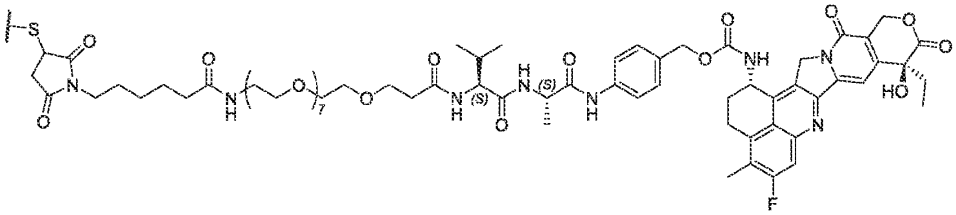
[0636] In some embodiments, L^{RXT} and L^{RXP} react to form L^{RX}, wherein L^{RX} is as described herein.

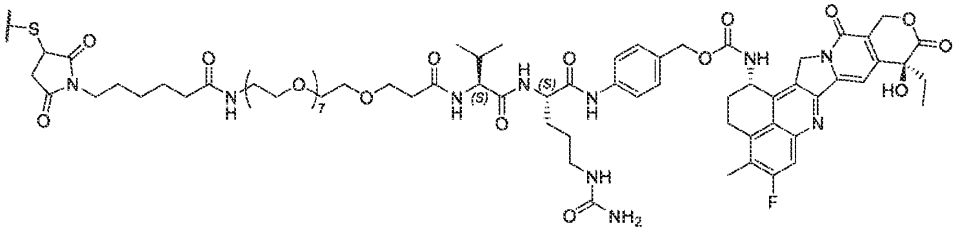
[0637] In some embodiments, a method prepares a compound comprising $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

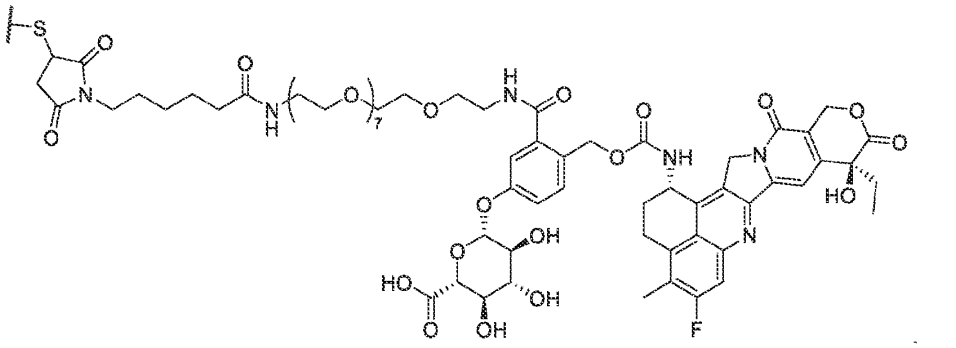


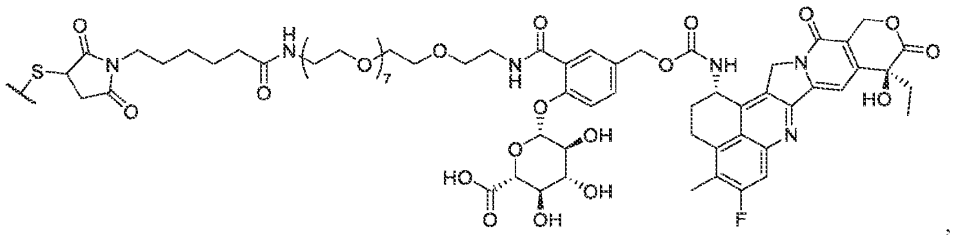


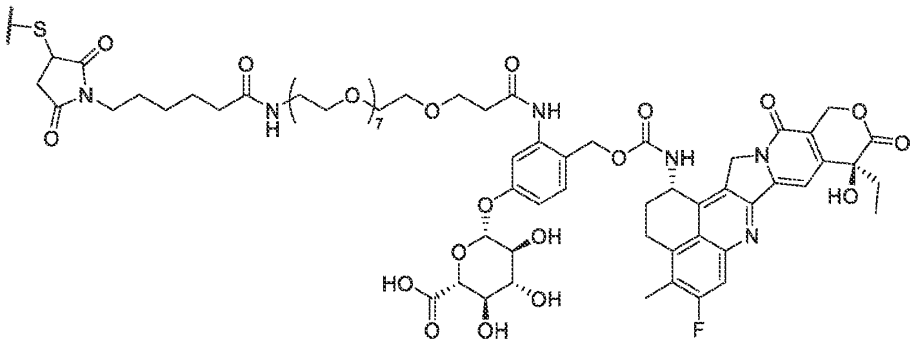




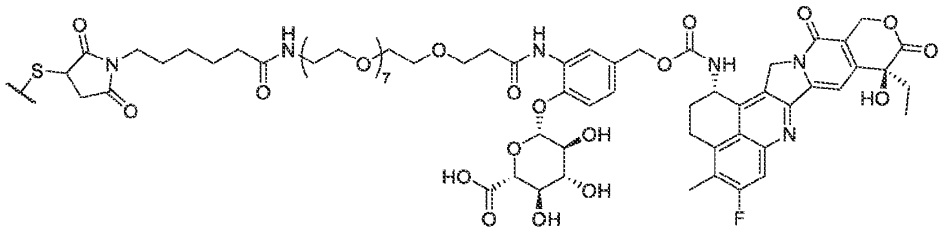






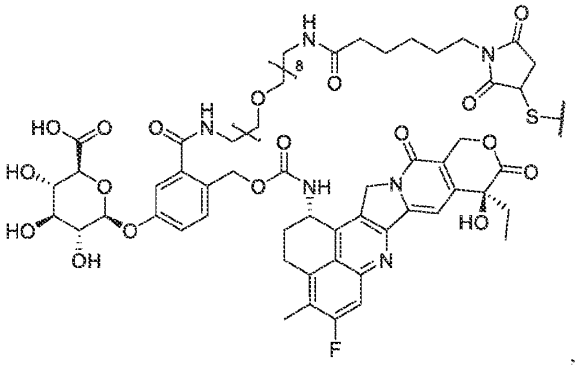


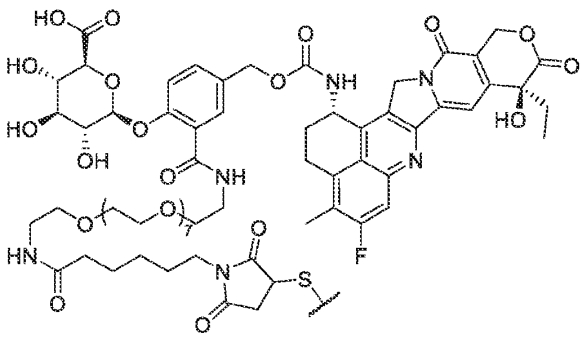
, or

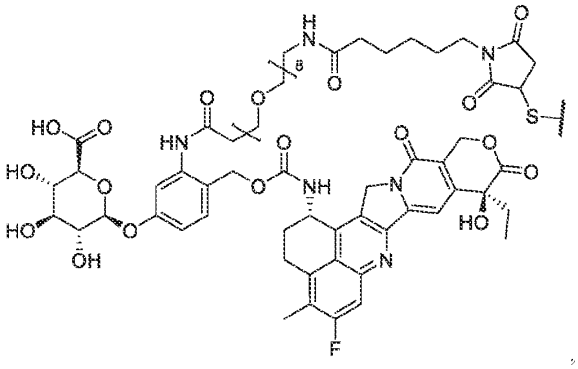


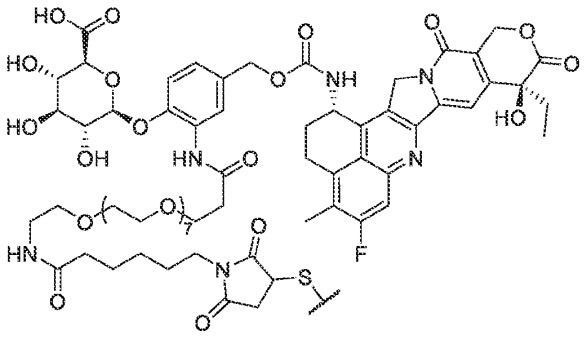
. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is the same.

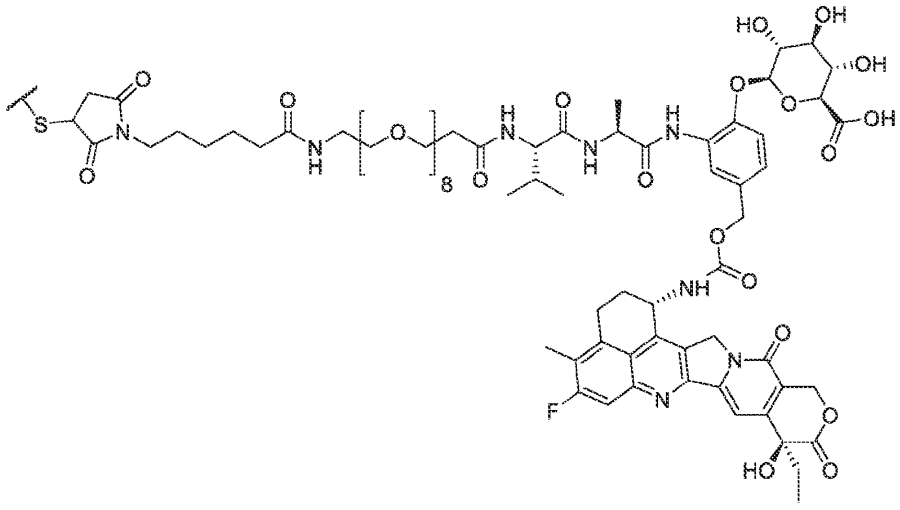
[0638] In some embodiments, a method prepares a compound comprising $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



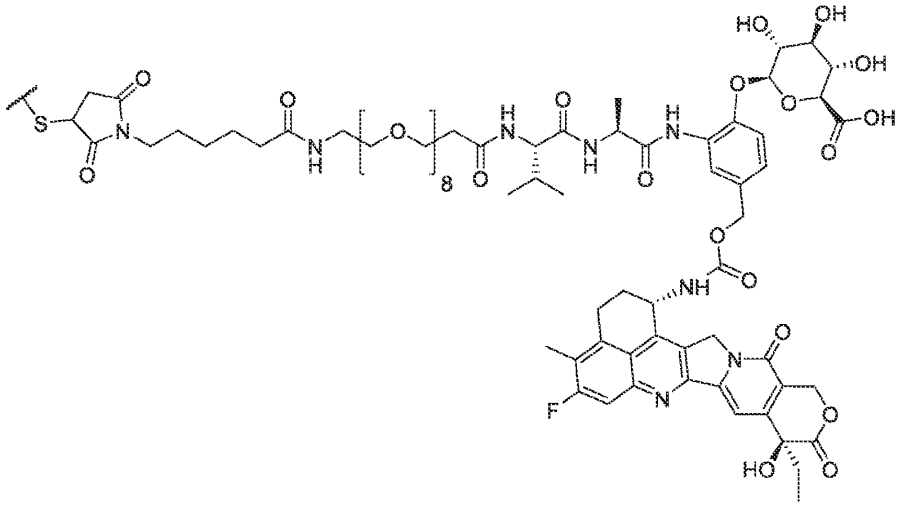






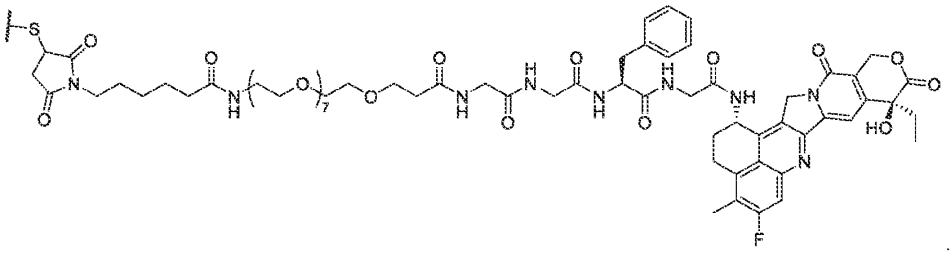


, or



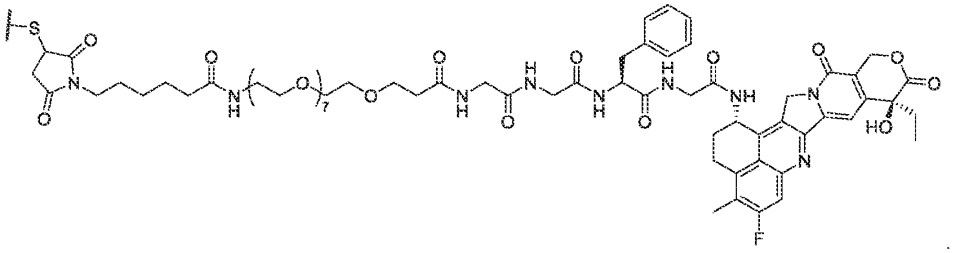
. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is the same.

[0639] In some embodiments, a method prepares a compound comprising $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



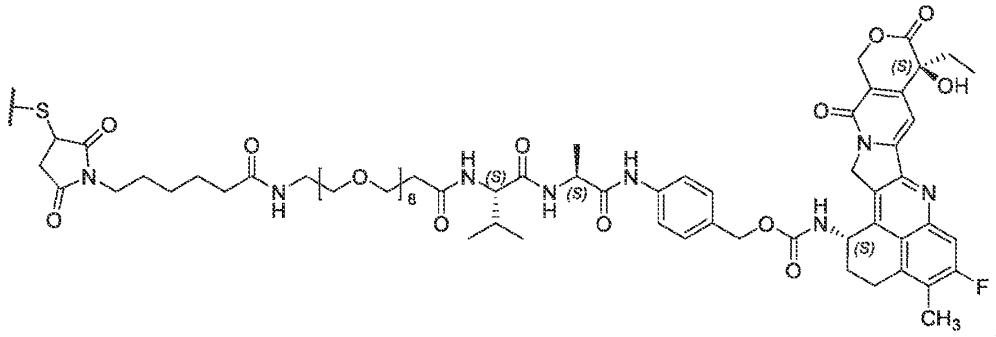
.

[0640] In some embodiments, a method prepares a compound comprising $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein each $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is or comprises



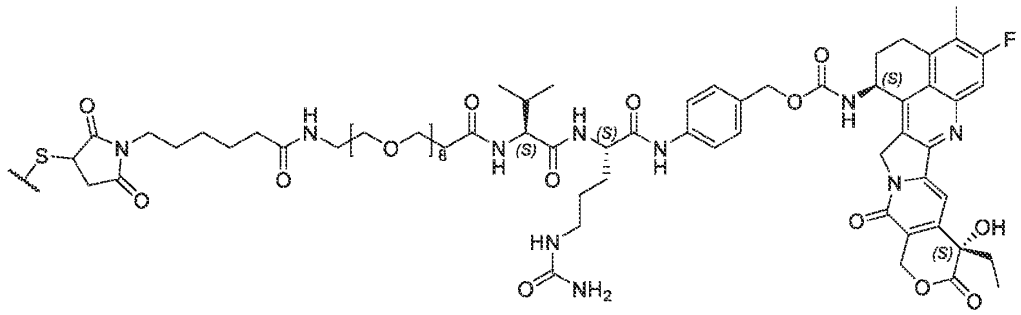
.

[0641] In some embodiments, a method prepares a compound comprising $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is or comprises



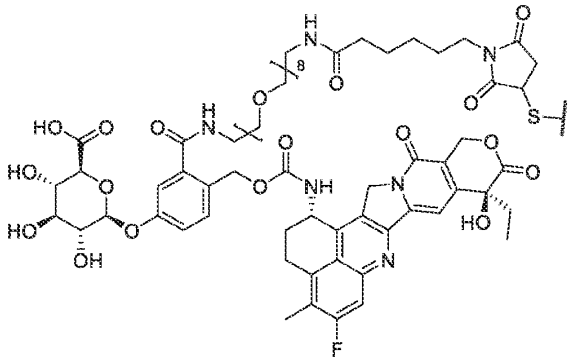
In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is the same.

[0642] In some embodiments, a method prepares a compound comprising $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is or comprises



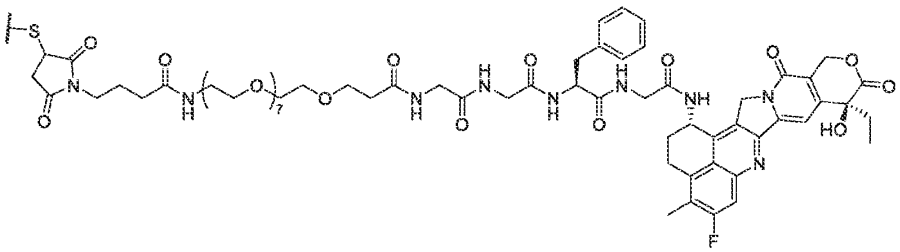
. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is the same.

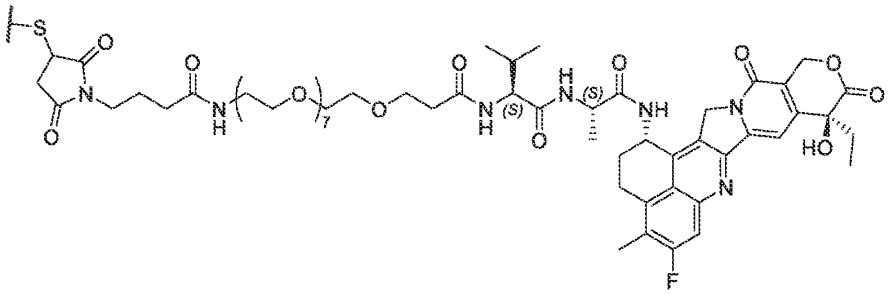
[0643] In some embodiments, a method prepares a compound comprising $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is or

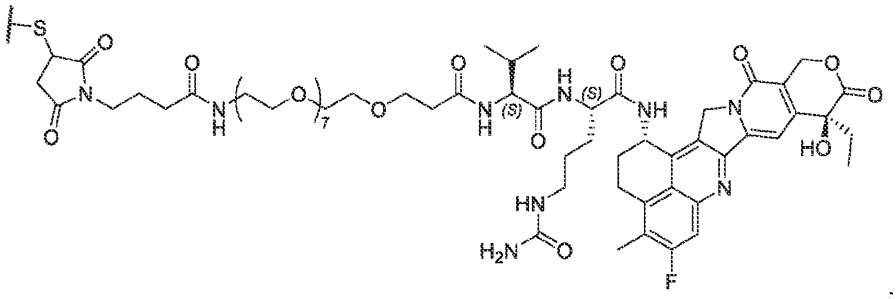


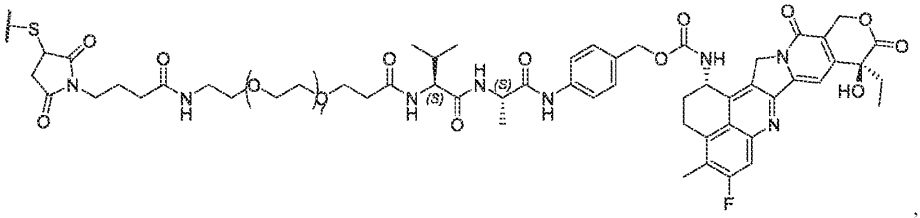
comprises . In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is the same.

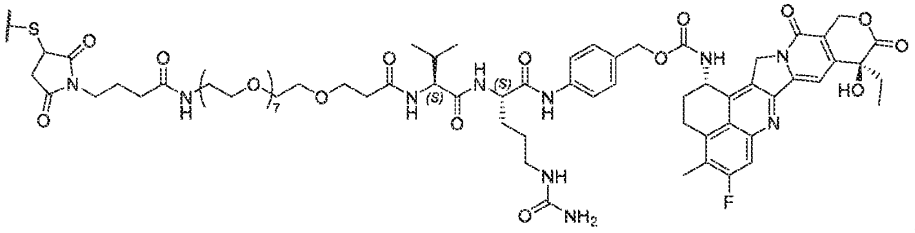
[0644] In some embodiments, a method prepares a compound comprising $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

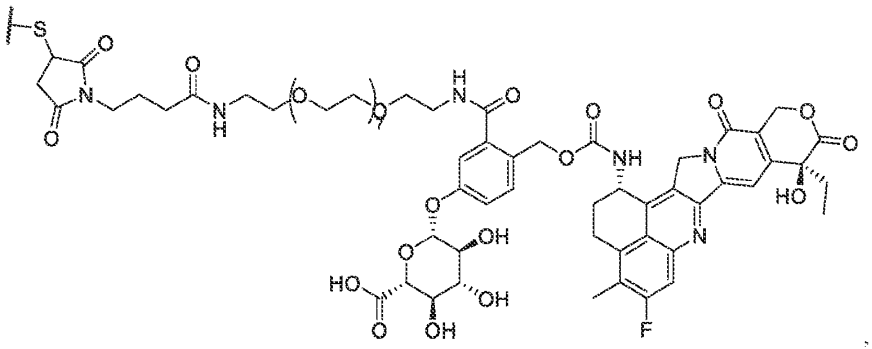




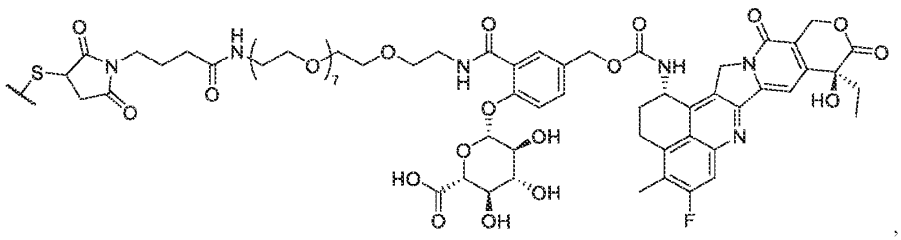




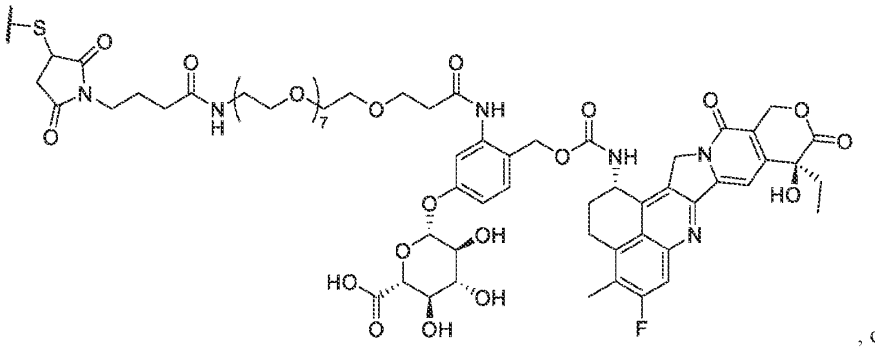




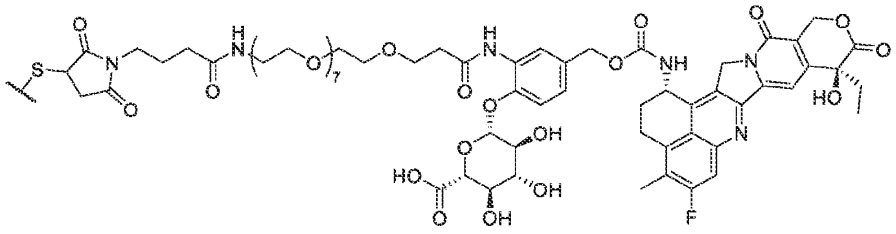
,



,

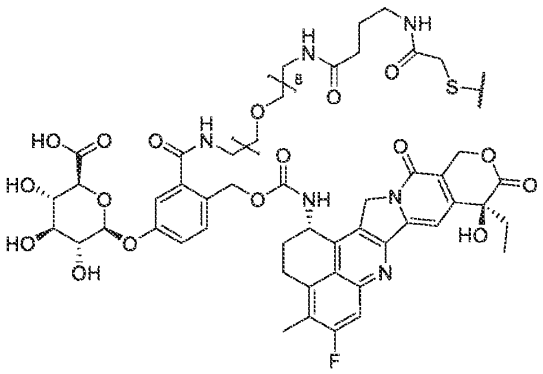


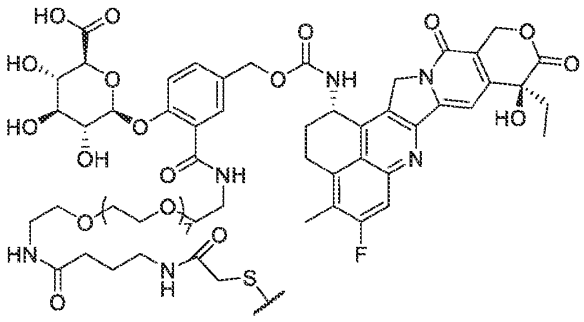
, or

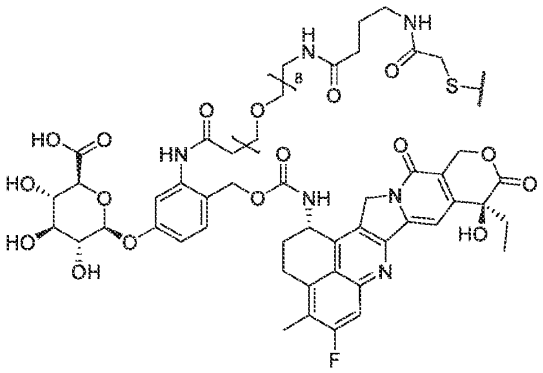


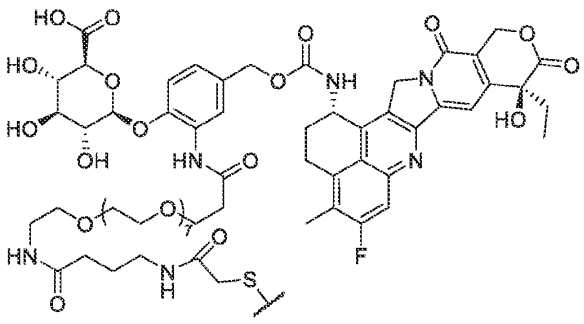
. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is the same.

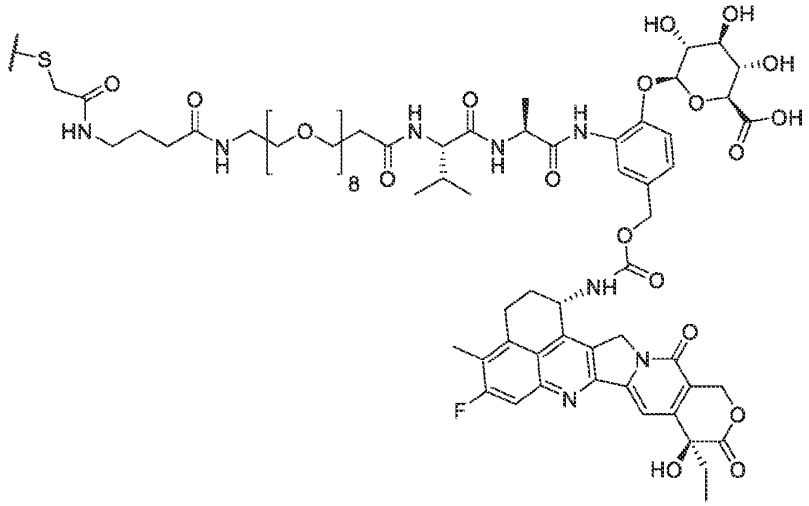
[0645] In some embodiments, a method prepares a compound comprising $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



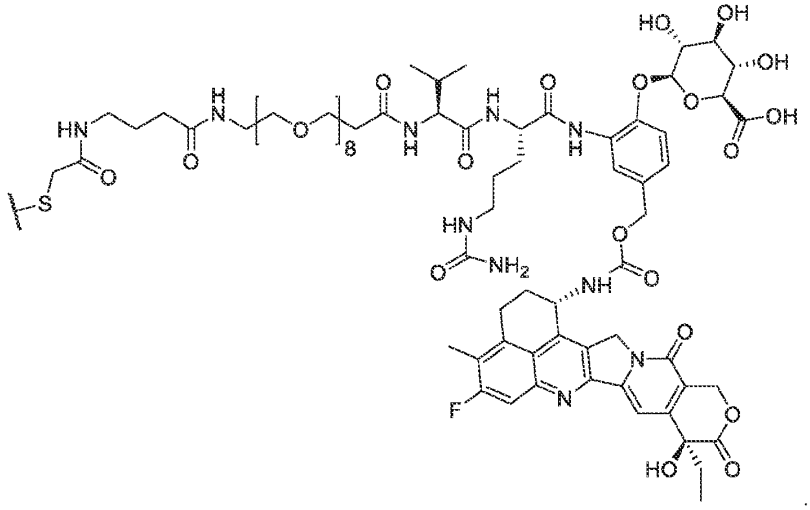






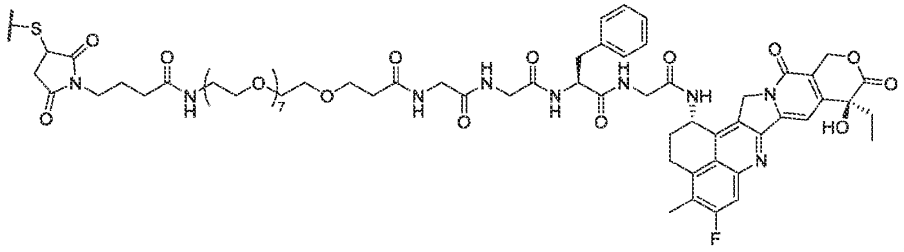


, or



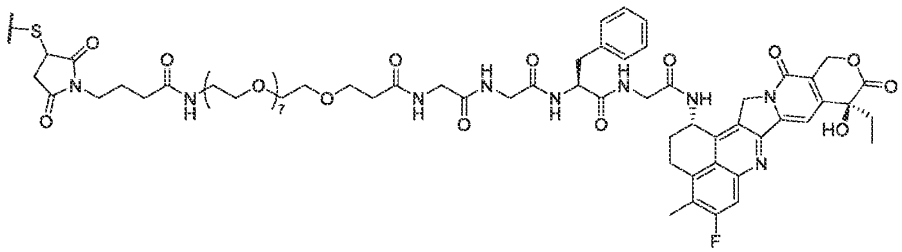
. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is the same.

[0646] In some embodiments, a method prepares a compound comprising $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

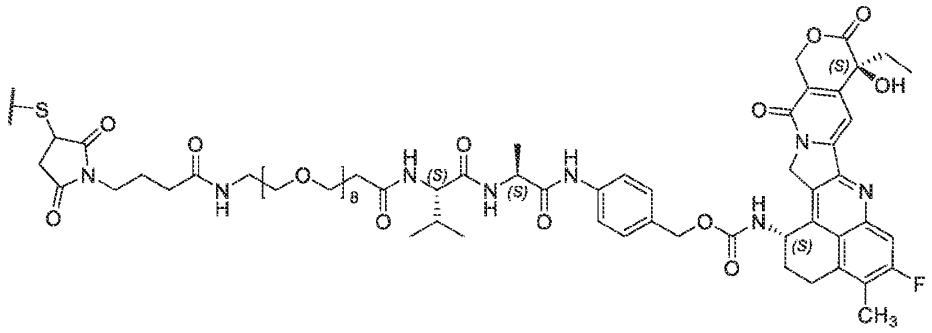


.

[0647] In some embodiments, a method prepares a compound comprising $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein each $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is or comprises

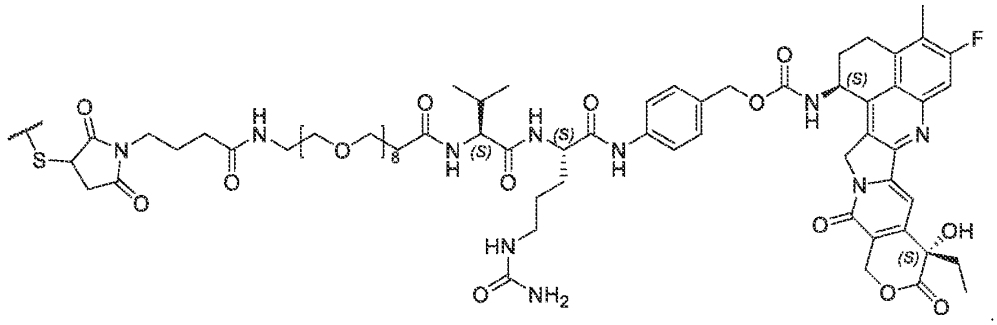


[0648] In some embodiments, a method prepares a compound comprising $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



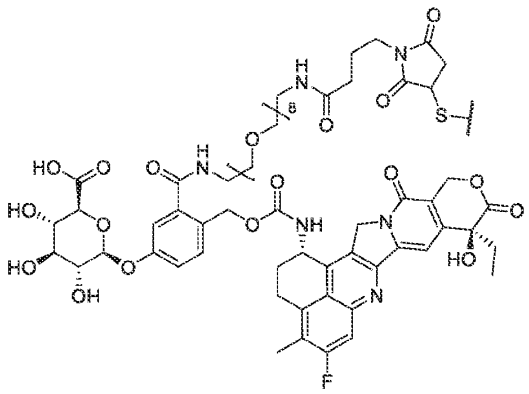
. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is the same.

[0649] In some embodiments, a method prepares a compound comprising $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



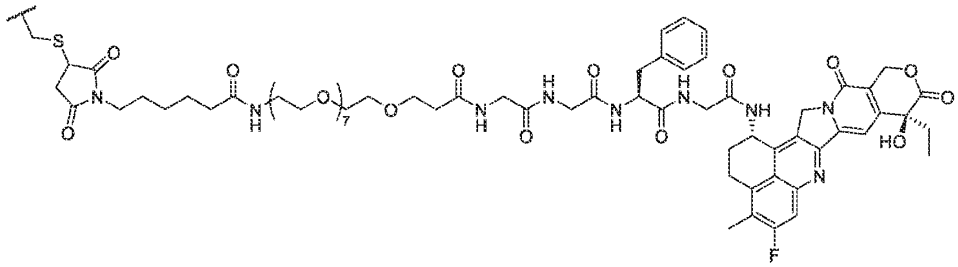
. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is the same.

[0650] In some embodiments, a method prepares a compound comprising $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

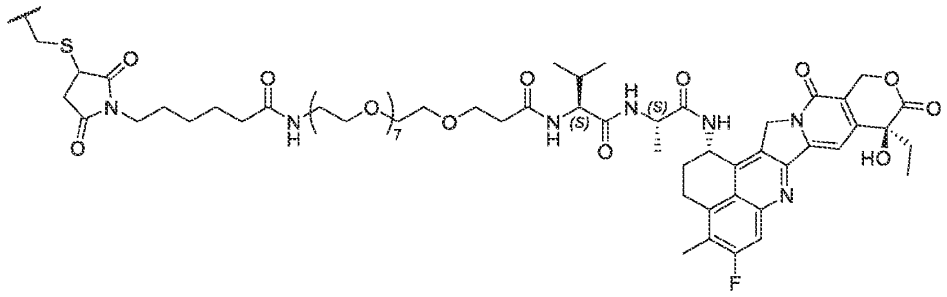


. In some embodiments, each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is the same.

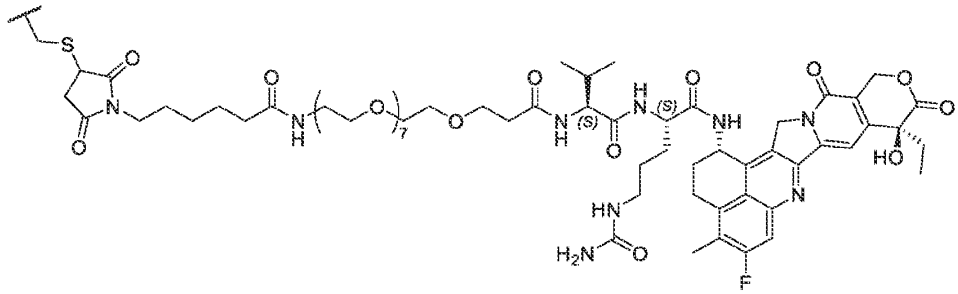
[0651] In some embodiments, a method prepares a compound comprising $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



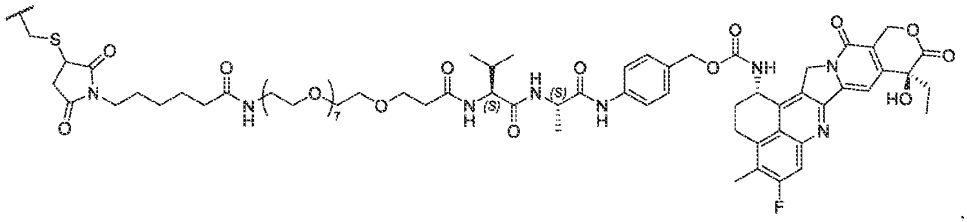
.

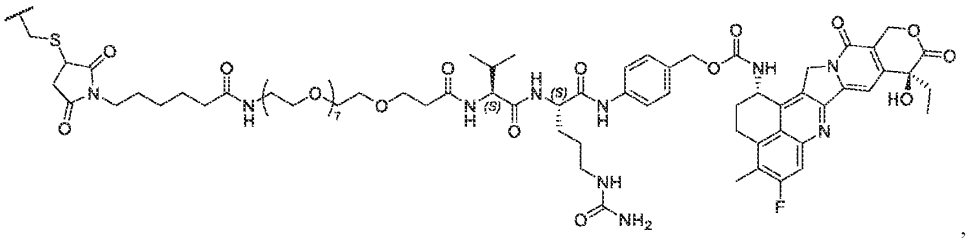


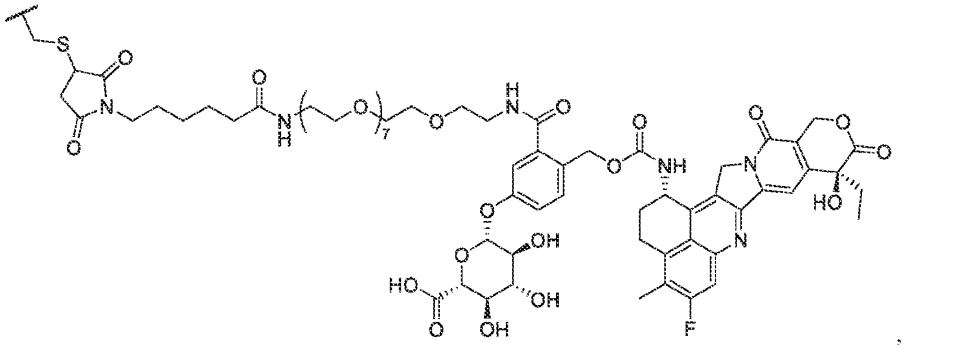
.

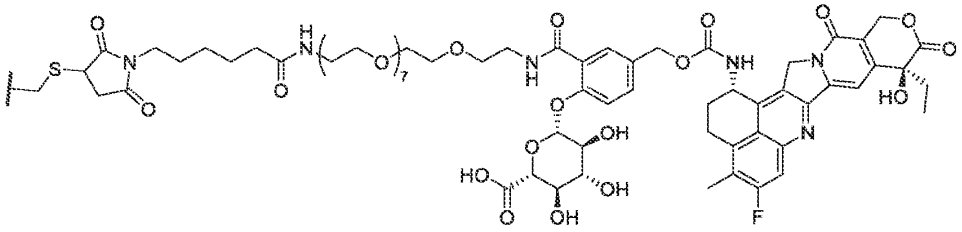


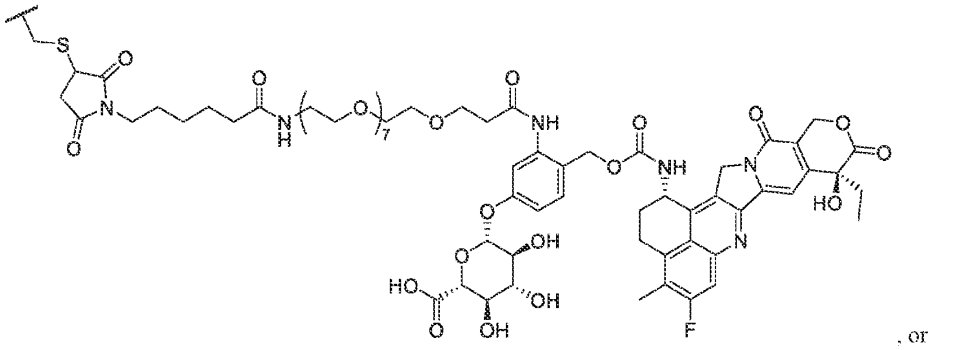
.



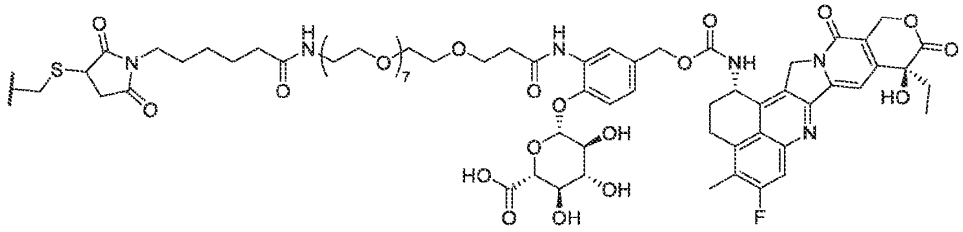






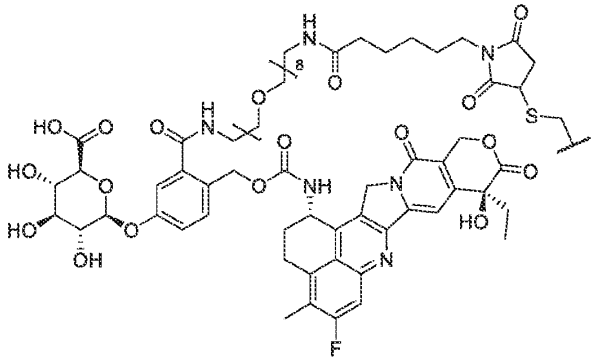


, or

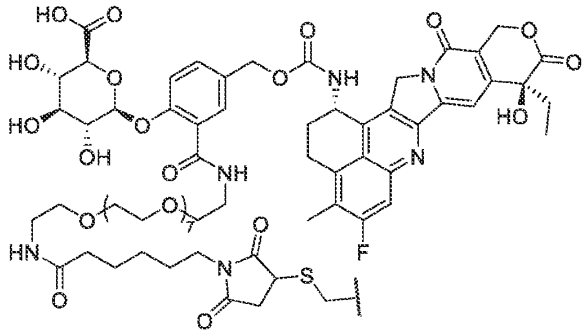


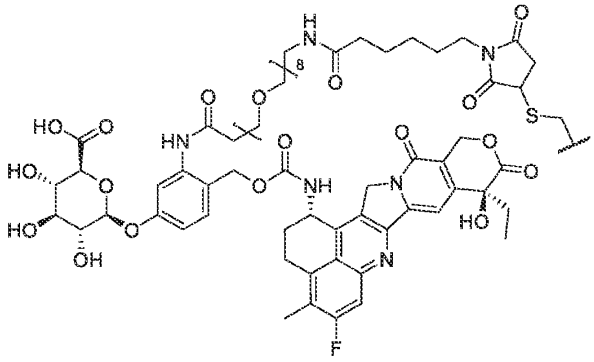
. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is the same.

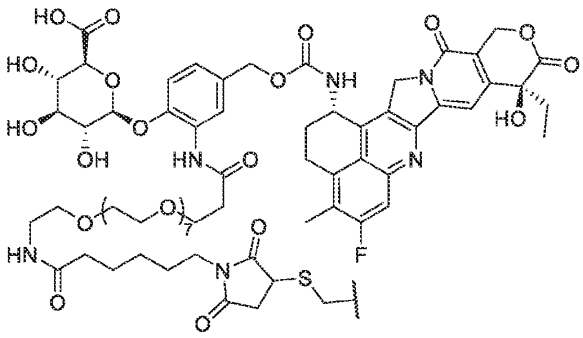
[0652] In some embodiments. a method prepares a compound comprising $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or

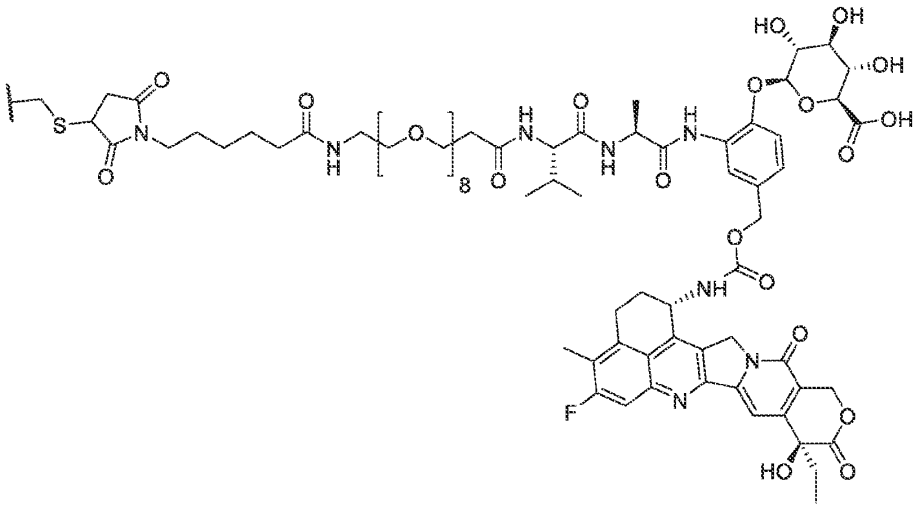


comprises

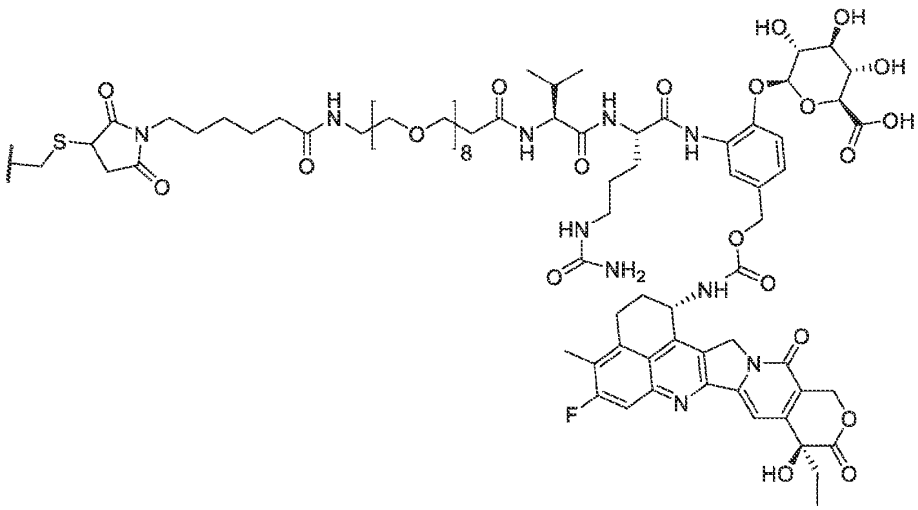








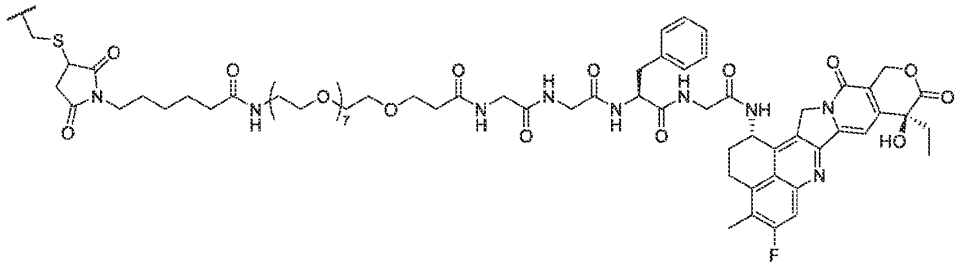
, or



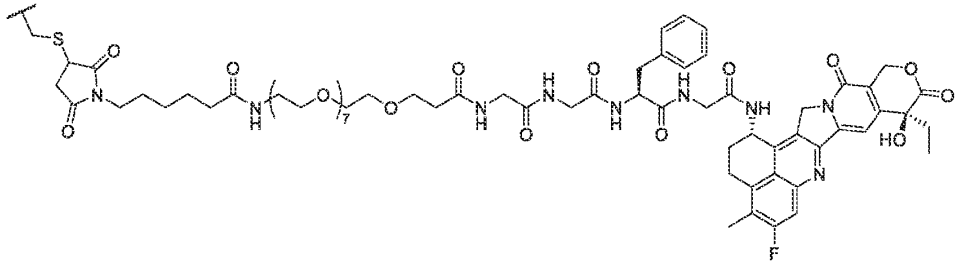
. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is the same.

[0653] In some embodiments, a method prepares a compound comprising $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or

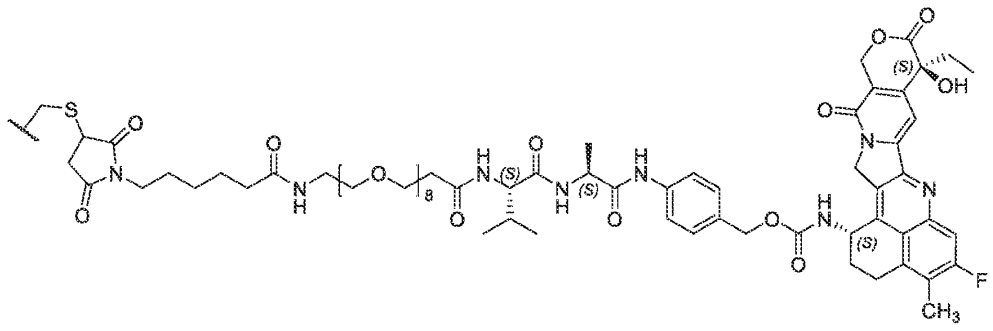
comprises



[0654] In some embodiments, a method prepares a compound $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is or comprises

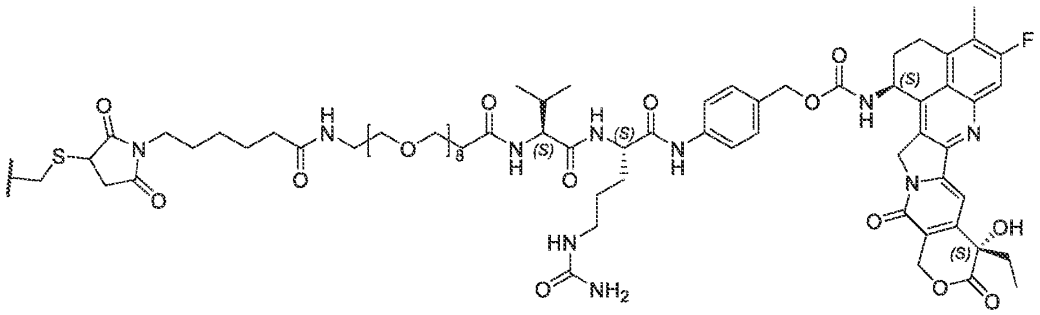


[0655] In some embodiments, a method prepares a compound comprising $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



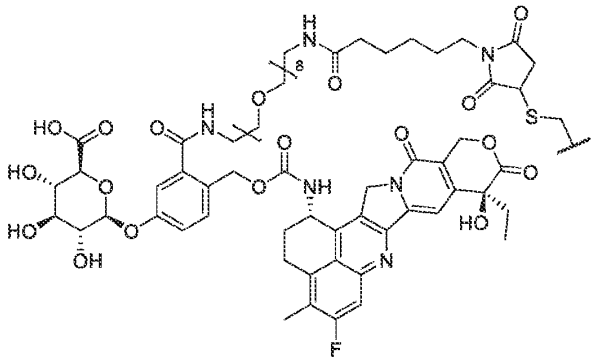
In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is the same.

[0656] In some embodiments, a method prepares a compound comprising $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



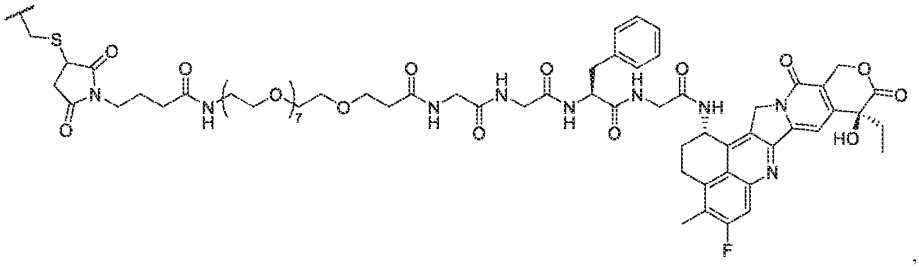
. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is the same.

[0657] In some embodiments, a method prepares a compound comprising $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or

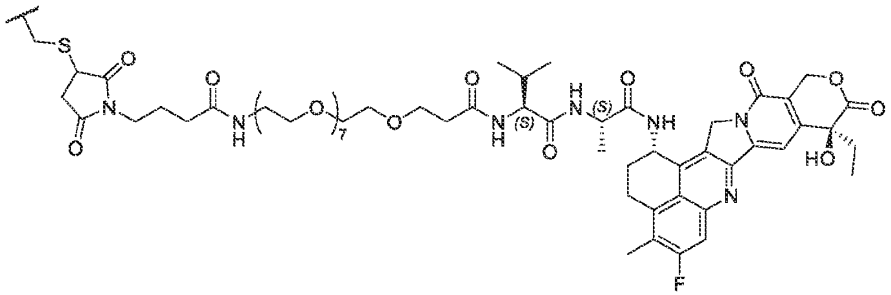


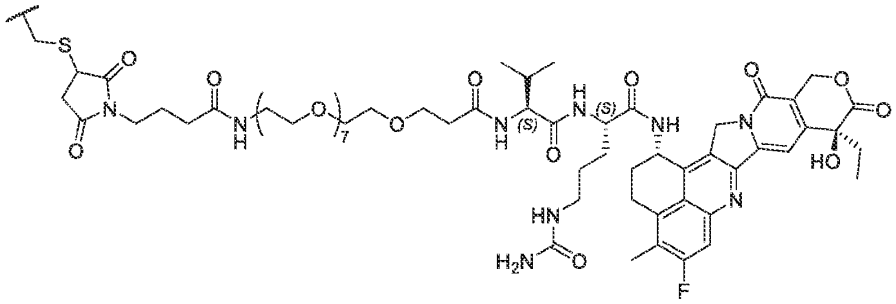
comprises . In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is the same.

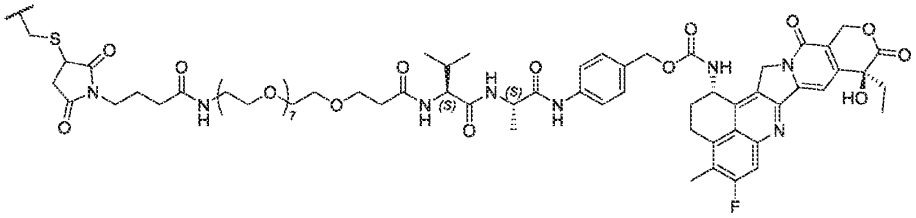
[0658] In some embodiments, a method prepares a compound comprising $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

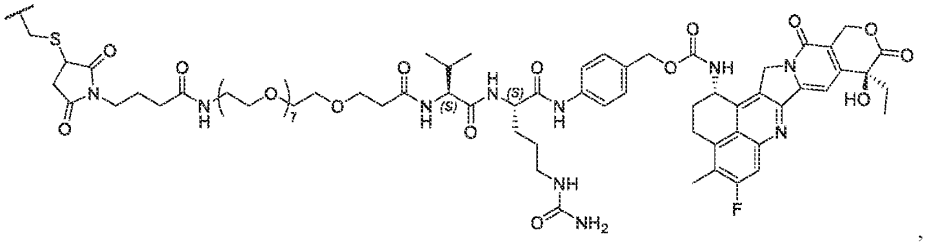


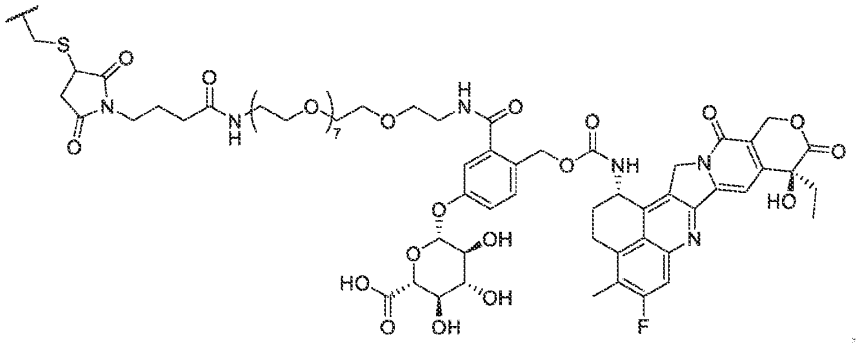
.

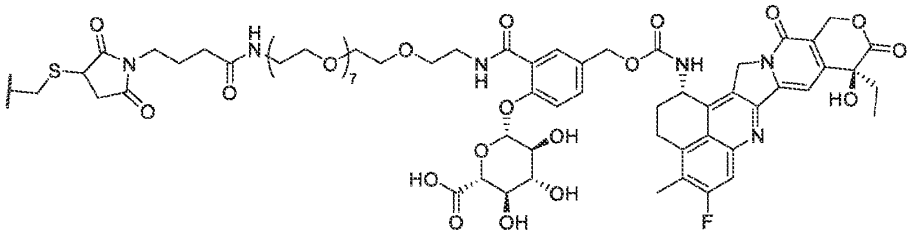




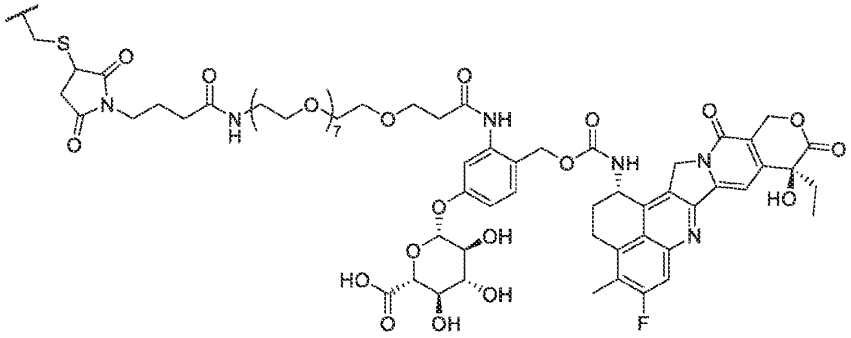




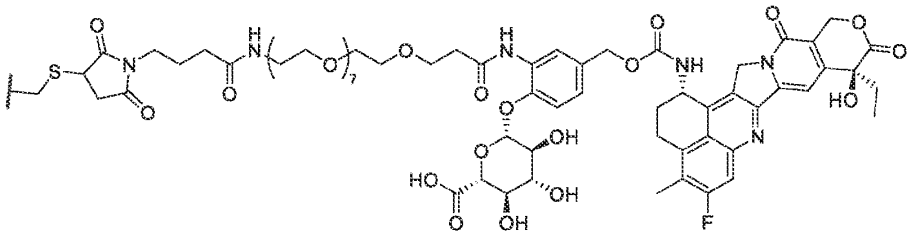




,

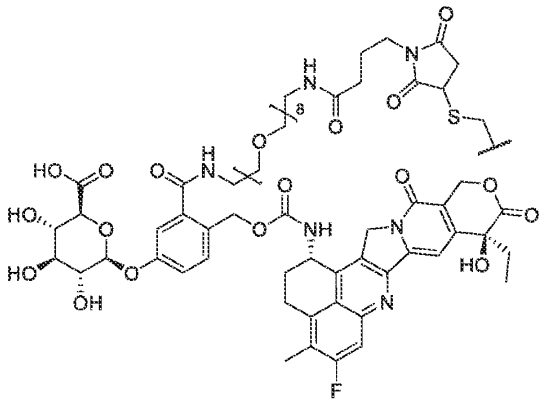


, or

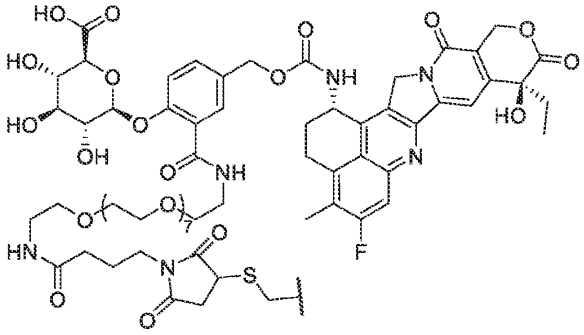


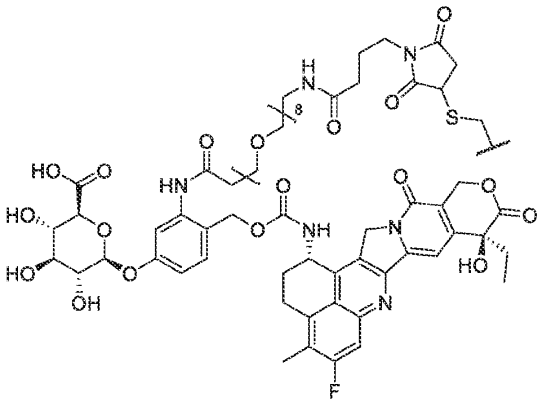
. In some embodiments, each occurrence of $--L^{T}--L^{RX}--L^{X}--(CH_2CH_2O)n--L^{ZP}--L^{C}--R^{P}$ in the compound is the same.

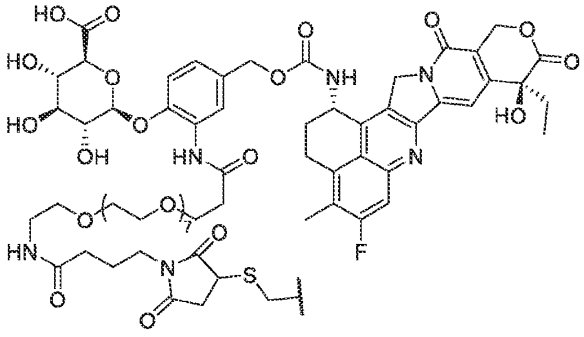
[0659] In some embodiments, a method prepares a compound comprising $--L^{T}--L^{RX}--L^{X}--(CH_2CH_2O)n--L^{ZP}--L^{C}--R^{P}$, wherein $--L^{T}--L^{RX}--L^{X}--(CH_2CH_2O)n--L^{ZP}--L^{C}--R^{P}$ is or

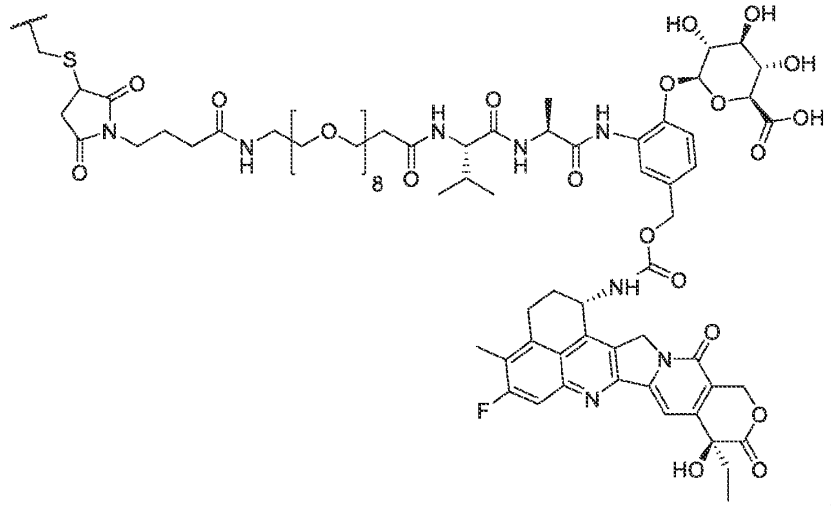


comprises .

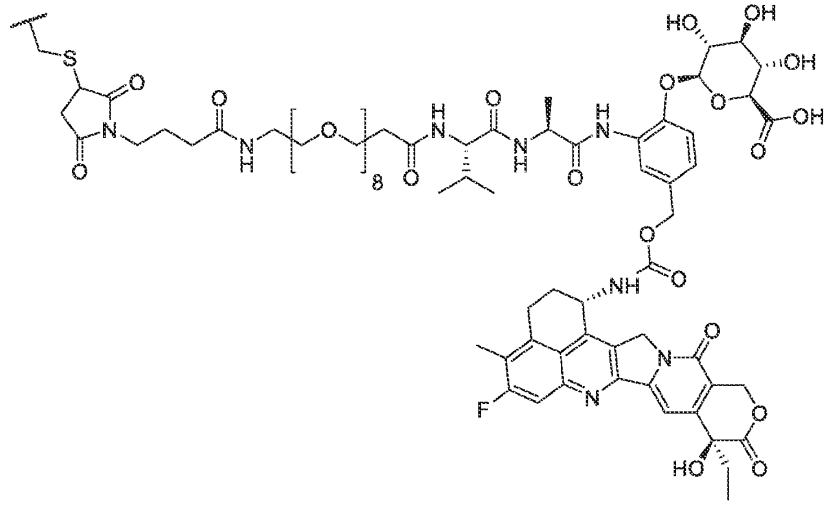






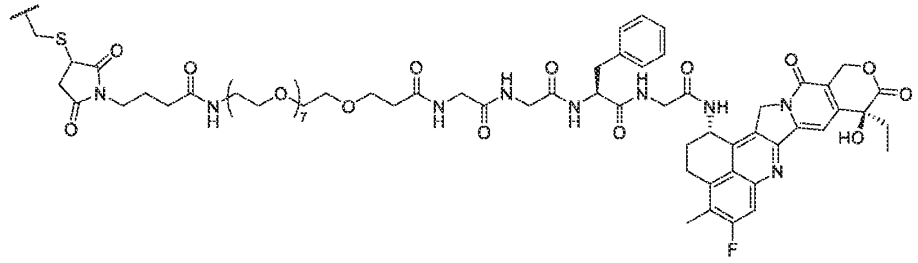


, or



. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is the same.

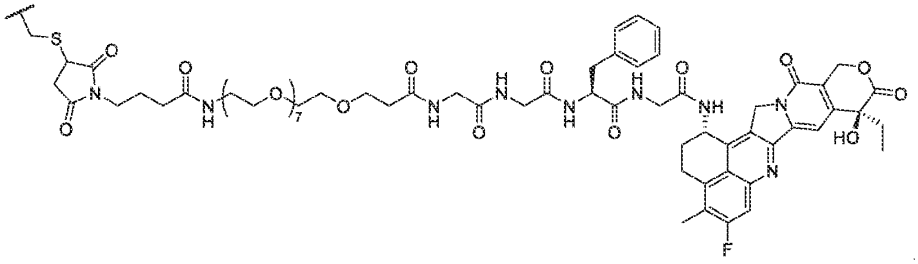
[0660] In some embodiments, a method prepares a compound comprising $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



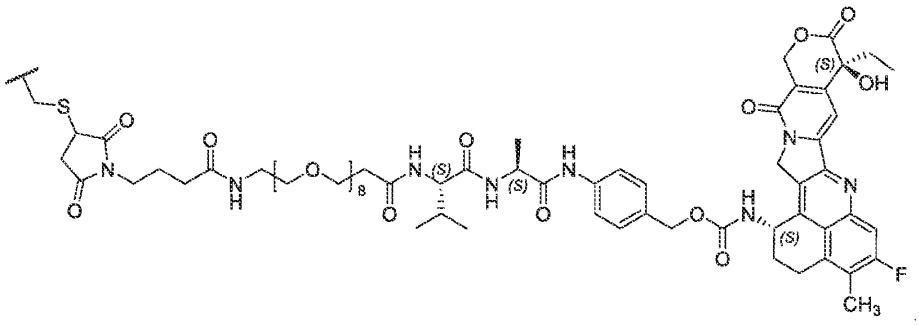
.

[0661] In some embodiments, a method prepares a compound

$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is or comprises

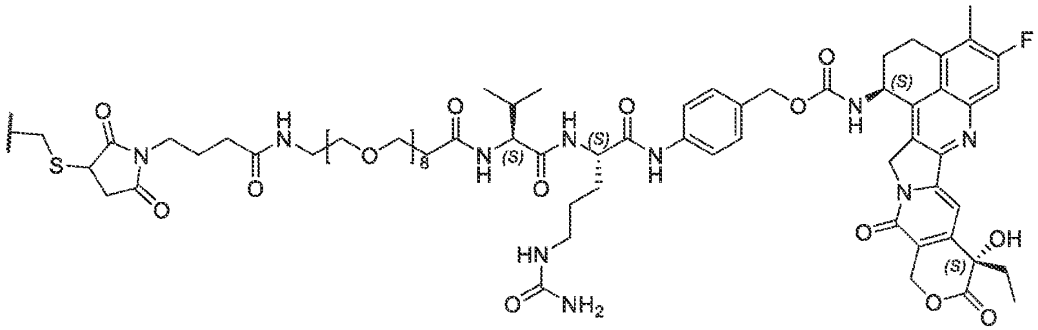


[0662] In some embodiments, a method prepares a compound comprising $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



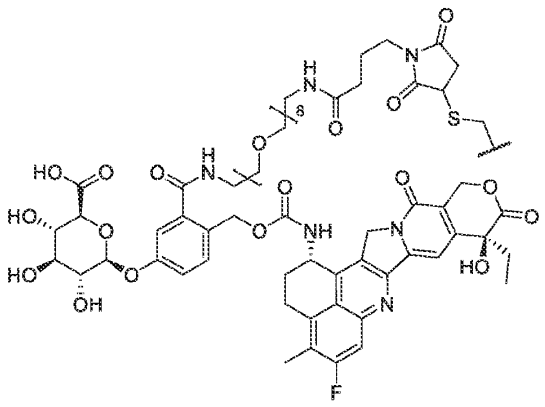
. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is the same.

[0663] In some embodiments, a method prepares a compound comprising $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



. In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is the same.

[0664] In some embodiments, a method prepares a compound comprising $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, wherein $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or



comprises . In some embodiments, each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ in the compound is the same.

[0665] Many structures in the present disclosure contain chiral centers. In certain structures, configuration of one or more or all chiral centers are specified, typically using ━ and ····. In some embodiments, stereochemistry labels (*(R)* or *(S)*) of one or more or all chiral centers whose configurations are specified are provided. Those skilled in the art can readily determine configurations of chiral centers whose configurations are specified in structures, irrespective of whether stereochemistry labels are provided.

[0666] In some embodiments, the disclosure provides methods of making a conjugate, comprising: (i) providing a target binding agent, e.g., an HHLA2 binding agent (e.g., as described in WO 2022/165258 A1 and above and herein), and (ii) reacting a payload agent, e.g., a linker-payload with the target binding agent afford the conjugate. In some embodiments, provided methods comprising reacting a linker-payload with a target binding agent under conditions that afford the provided conjugates (e.g., as described in Example 3 herein). In some embodiments, a method comprises reduction of a target binding agent, e.g., an antibody or antigen-binding fragment thereof (e.g., conditions where cysteine residues are reduced to thiols, or salts thereof). In some embodiments, a method comprises conjugating a reduced antibody or antigen-binding fragment thereof to a linker-payload (e.g., exatecan). Additional reaction conditions to make provided conjugates are disclosed in, e.g., US 10,195,288.

[0667] In some embodiments, a composition comprising a plurality of conjugates, each of which is independently as described herein. In some embodiments, conjugates in a composition independently have different DARs. In some embodiments, DAR of a composition is the ratio between the number of payload moieties and the number of target binding moieties. In some embodiments, DAR for a composition is about 1-20. In some embodiments, DAR for a composition is about 1-8. In some embodiments, it is about 2-8. In some embodiments, it is about or at least about 1, 2, 3, 4, 5, 6, 7, or 8. In some embodiments, it is about 1. In some embodiments, it is about 2. In some embodiments, it is about 3. In some embodiments, it is about 4. In some embodiments, it is about 5. In some embodiments, it is about 6. In some embodiments, it is about 7. In some embodiments, it is about 8.

[0668] In some embodiments, about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% of all conjugates in a composition have the same DAR. In some embodiments, about 10%, 20%, 30%, 40%,

50%, 60%, 70%, 80%, 90% or 95% of all agents that share a common target binding moiety in a composition have the same DAR. In some embodiments, about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% of all agents that comprises a target binding moiety in a composition have the same DAR. In some embodiments, the percentage is about or at least about 10%. In some embodiments, the percentage is about or at least about 20%. In some embodiments, the percentage is about or at least about 30%. In some embodiments, the percentage is about or at least about 40%. In some embodiments, the percentage is about or at least about 50%. In some embodiments, the percentage is about or at least about 60%. In some embodiments, the percentage is about or at least about 70%. In some embodiments, the percentage is about or at least about 80%. In some embodiments, the percentage is about or at least about 90%. In some embodiments, the percentage is about or at least about 95%.

[0669] In some embodiments, about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% of all conjugates in a composition independently have a DAR that within ±5%, ±10%, ±15%, ±20%, ±25% of the DAR of the composition. In some embodiments, about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% of all conjugates in a composition independently have a DAR that within ±5%, ±10%, ±15%, ±20%, ±25%, ±30%, ±35%, ±40%, ±45% or ±50% of the DAR of the composition. In some embodiments, about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% of all conjugates in a composition independently have a DAR that within ±5%, ±10%, ±15%, ±20%, ±25% of the DAR of the composition. In some embodiments, the percentage of conjugates is about or at least about 10%. In some embodiments, the percentage is about or at least about 20%. In some embodiments, the percentage is about or at least about 30%. In some embodiments, the percentage is about or at least about 40%. In some embodiments, the percentage is about or at least about 50%. In some embodiments, the percentage is about or at least about 60%. In some embodiments, the percentage is about or at least about 70%. In some embodiments, the percentage is about or at least about 80%. In some embodiments, the percentage is about or at least about 90%. In some embodiments, the percentage is about or at least about 95%. In some embodiments, such conjugates in a composition independently have a DAR that within ±5% of that of the composition. In some embodiments, such conjugates in a composition independently have a DAR that within ±10% of that of the composition. In some embodiments, such conjugates in a composition independently have a DAR that within ±15% of that of the composition. In some embodiments, such conjugates in a composition independently have a DAR that within ±20% of that of the composition. In some embodiments, such conjugates in a composition independently have a DAR that within ±25% of that of the composition. In some embodiments, such conjugates in a composition independently have a DAR that within ±30% of that of the composition. In some embodiments, such conjugates in a composition independently have a DAR that within ±40% of that of the composition. In some embodiments, such conjugates in a composition independently have a DAR that within ±50% of that of the composition.

[0670] Various technologies can be utilized in accordance with the present disclosure to select and control conjugate sites, chemistry and DARs, e.g., those described in US11478553; Fu, Z., Li, S., Han, S. et al. Antibody drug conjugate: the "biological missile" for targeted cancer therapy. Sig Transduct Target Ther 7, 93 (2022). https://doi.org/10.1038/s41392-022-00947-7; Su Z, Xiao D, Xie F, Liu L, Wang Y, Fan

S, Zhou X, Li S. Antibody-drug conjugates: Recent advances in linker chemistry. Acta Pharm Sin B. 2021 Dec;11(12):3889-3907. doi: 10.1016/j.apsb.2021.03.042. Epub 2021 Apr 6. PMID: 35024314; PMCID: PMC8727783; Sheyi R, de la Torre BG, Albericio F. Linkers: An Assurance for Controlled Delivery of Antibody-Drug Conjugate. Pharmaceutics. 2022 Feb 11;14(2):396. doi: 10.3390/pharmaceutics14020396. PMID: 35214128; PMCID: PMC8874516; etc.

[0671] In some embodiments, conjugation sites are or comprise Cys residues of target binding moieties, e.g., antibodies or antigen-binding fragments thereof. In some embodiments, all eight Cys residues that form disulfide bonds in an antibody or fragments thereof are utilized for conjugation in one or more conjugates in a composition. In some embodiments, the four Cys residues that form inter-heavy chain and light chain disulfide bonds in an antibody or fragments thereof are selectively utilized for conjugation in one or more conjugates in a composition. In some embodiments, target binding agents, e.g., antibodies, agents comprising antibodies or fragments thereof, are engineered to have Cys residues at certain locations to facilitate conjugation, control DAR, increase DAR, etc. In some embodiments, target binding agents are engineered to incorporated amino acid residues at certain positions to facilitate conjugation, control DAR, increase DAR, etc. In some embodiments, one or more Lys residues are incorporated. In some embodiments, one or more Cys residues are incorporated. In some embodiments, one or more unnatural amino acid residues bearing certain reactive groups for certain reactions (e.g., click reactions) are incorporated.

Applications

[0672] Among other things, provided technologies can deliver payloads to targets targeted by target binding moieties. In some embodiments, the present disclosure provides a method for delivering a payload to a target in a system, comprising administering to or delivering to the system a conjugate or composition provided herein, wherein a payload moiety of a conjugate is a moiety of the payload, and the target expresses or comprises an agent that a target binding moiety of the conjugate can bind. In some embodiments, delivery to a target is about or at least about 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000 or 5000-fold of that to a non-target. In some embodiments, it is at least 10-fold. In some embodiments, it is at least 100-fold. In some embodiments, it is at least 500-fold. In some embodiments, it is at least 1000-fold. In some embodiments, a target is or comprises diseased cells expressing or comprising an agent (e.g., a polypeptide, a nucleic acid, a chemical, etc.) that a target binding moiety can bind. In some embodiments, a target is or comprises cancer cells. In some embodiments, provided conjugates can selectively target cells that highly express an agent targeted by a target binding moiety, e.g., HHLA2 when a target binding moiety is or comprises an anti-HHLA2 antibody or an antigen-binding fragment thereof, be internalized, and release a payload. In some embodiments, the present disclosure provides conjugates that are particularly useful for treating a variety of cancers expressing agents that target binding moieties can bind, including solid tumors, such as non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), cholangiocarcinoma, or breast cancer, and hematological tumors, as well as modulating an immune response in a subject.

[0673] In some embodiments, a payload is or comprises a cytotoxic agent. In some embodiments, a payload can inhibit cell proliferation or leads to cell death.

[0674] In some embodiments, the present disclosure provides a method for inhibiting cell proliferation in a system, comprising administering to or delivering to the system a provided conjugate or composition. In some embodiments, the present disclosure provides a method for inhibiting proliferation of diseased cells in a system, comprising administering to or delivering to the system a provided conjugate or composition. In some embodiments, the present disclosure provides a method for promoting cell death in a system, comprising administering to or delivering to the system a provided conjugate or composition. In some embodiments, the present disclosure provides a method for reducing number of cells in a system, comprising administering to or delivering to the system a provided conjugate or composition. In some embodiments, the present disclosure provides a method for reducing number of diseased cells in a system, comprising administering to or delivering to the system a provided conjugate or composition. In some embodiments, cells are or comprise cancer cells. In some embodiments, cells express or comprise an agent that a target binding moiety of the conjugate can bind.

[0675] In some embodiments, an agent that a target binding moiety of the conjugate can bind is or comprises a cancer antigen or a fragment thereof.

[0676] In some embodiments, a system is or comprises a cell. In some embodiments, a system is or comprises a population of cell. In some embodiments, a system is or comprises a tissue. In some embodiments, a system is or comprises an organ. In some embodiments, a system is or comprises an organism. In some embodiments, a system is or comprises an animal (e.g., of an animal model of a condition, disorder or disease). In some embodiments, a system is a human.

[0677] In some embodiments, an agent that a target binding moiety can bind has one or more functions in an immune system.

[0678] In some embodiments, the present disclosure provides a method of modulating an immune response in a subject comprising administering or delivering to a subject in need thereof an effective amount of a provided conjugate or composition.

[0679] In some embodiments, provided technologies, e.g., conjugates, methods, etc., are useful for preventing or treating conditions, disorders or diseases. In some embodiments, the present disclosure provides a method for preventing a condition, disorder or disease, comprising administering or delivering to a subject susceptible thereto an effective amount of a provided conjugate or composition. In some embodiments, the present disclosure provides a method for treating a condition, disorder or disease, comprising administering or delivering to a subject suffering therefrom an effective amount of a provided conjugate or composition. In some embodiments, the present disclosure provides methods of treating a disease, disorder or condition (e.g., a disease, disorder or condition described herein) in a subject comprising administering a pharmaceutical composition comprising at least conjugate described herein. In some embodiments, a therapeutically effective amount of at least one pharmaceutical composition described herein is administered to a subject having a disease, disorder, or condition. In some embodiments, a condition, disorder or disease is cancer.

[0680] Pharmaceutical compositions comprising at least one conjugate described herein can be for use in the manufacture of a medicament for treating a disease, disorder, or condition in a subject or stimulating an immune response in a subject. Pharmaceutical compositions at least one conjugate described herein can be administered to a subject in accordance with a dosage regimen described herein, alone or in combination with one or more therapeutic agents, procedures, or modalities.

[0681] A subject to be treated with methods described herein can be a mammal, e.g., a primate, e.g., a human (e.g., a patient having, or at risk of having, a disease, disorder or condition described herein). A method of treating (e.g., one or more of reducing, inhibiting, or delaying progression of) a cancer or a tumor in a subject with a pharmaceutical composition comprising at least one conjugate described herein is provided. A subject can have an adult or pediatric form of cancer. A cancer may be at an early, intermediate, or late stage, or a metastatic cancer.

[0682] A method of treating (e.g., one or more of reducing, inhibiting, or delaying progression of) a sign or symptom of cancer in a subject with a pharmaceutical composition comprising at least one conjugate described herein is provided. In some embodiments, pharmaceutical compositions described herein are useful to delay the onset of, slow the progression of, or ameliorate one or more signs or symptoms of cancer. In some embodiments, a physiological sign or symptom of cancer comprises or is an increase in tumor volume, an increase in number of cancer cells, an increase in number of metastases, a decrease in life expectancy, an increase in cancer cell proliferation, and/or an increase in cancer cell survival. In some embodiments, a physical sign or symptom of cancer comprises or is a skin lesion (e.g., a lump or mole), weight loss, digestive problems, discomfort, fatigue, pain, trouble swallowing, cough, unusual bleeding and/or discharge, changes in bowel and/or bladder habits, and/or mental confusion.

[0683] A cancer can include, but is not limited to, a solid tumor, a hematological cancer (e.g., leukemia, lymphoma, or myeloma, e.g., multiple myeloma), or a metastatic lesion. Examples of solid tumors include malignancies, e.g., sarcomas and carcinomas, e.g., adenocarcinomas of the various organ systems, such as those affecting the lung, breast, ovarian, lymphoid, gastrointestinal (e.g., colon), anal, genitals and genitourinary tract (e.g., renal, urothelial, bladder cells, prostate), pharynx, CNS (e.g., brain, neural or glial cells), head and neck, skin (e.g., melanoma, e.g., a cutaneous melanoma), pancreas, and bones (e.g., a chordoma).

[0684] In some embodiments, a cancer is chosen from a lung cancer (e.g., a non-small cell lung cancer (NSCLC) (e.g., a non-small cell lung cancer (NSCLC) with squamous and/or non-squamous histology, or a NSCLC adenocarcinoma), or a small cell lung cancer (SCLC)), a skin cancer (e.g., a Merkel cell carcinoma or a melanoma (e.g., an advanced melanoma)), an ovarian cancer, a mesothelioma, a bladder cancer, a soft tissue sarcoma (e.g., a hemangiopericytoma (HPC)), a bone cancer (a bone sarcoma), a kidney cancer (e.g., a renal cancer (e.g., a renal cell carcinoma)), a liver cancer (e.g., a hepatocellular carcinoma), a cholangiocarcinoma, a sarcoma, a myelodysplastic syndrome (MDS), a prostate cancer, a breast cancer (e.g., a breast cancer that does not express one, two or all of estrogen receptor, progesterone receptor, or Her2/neu, e.g., a triple negative breast cancer), a colorectal cancer (e.g., a relapsed colorectal cancer or a metastatic colorectal cancer, e.g., a microsatellite unstable

colorectal cancer, a microsatellite stable colorectal cancer, a mismatch repair proficient colorectal cancer, or a mismatch repair deficient colorectal cancer), a nasopharyngeal cancer, a duodenal cancer, an endometrial cancer, a pancreatic cancer, a head and neck cancer (e.g., head and neck squamous cell carcinoma (HNSCC)), an anal cancer, a gastro-esophageal cancer, a thyroid cancer (e.g., anaplastic thyroid carcinoma), a cervical cancer (e.g., a squamous cell carcinoma of the cervix), a neuroendocrine tumor (NET) (e.g., an atypical pulmonary carcinoid tumor), a lymphoproliferative disease (e.g., a post-transplant lymphoproliferative disease), a lymphoma (e.g., T-cell lymphoma, B-cell lymphoma, or a non-Hogdkin lymphoma), a myeloma (e.g., a multiple myeloma), or a leukemia (e.g., a myeloid leukemia or a lymphoid leukemia). In some embodiments, a subject has renal cell carcinoma.

[0685] In some embodiments, a cancer is a brain tumor, e.g., a glioblastoma, a gliosarcoma, or a recurrent brain tumor. In some embodiments, a cancer is a pancreatic cancer, e.g., an advanced pancreatic cancer. In some embodiments, a cancer is a skin cancer, e.g., a melanoma (e.g., a stage II-IV melanoma, an HLA-A2 positive melanoma, an unresectable melanoma, or a metastatic melanoma), or a Merkel cell carcinoma. In some embodiments, a cancer is a renal cancer, e.g., a renal cell carcinoma (RCC) (e.g., a metastatic renal cell carcinoma). In some embodiments, a cancer is a breast cancer, e.g., a metastatic breast carcinoma or a stage IV breast carcinoma, e.g., a triple negative breast cancer (TNBC). In some embodiments, a cancer is a virus-associated cancer. In some embodiments, a cancer is an anal canal cancer (e.g., a squamous cell carcinoma of the anal canal). In some embodiments, a cancer is a cervical cancer (e.g., a squamous cell carcinoma of the cervix). In some embodiments, a cancer is a gastric cancer (e.g., an Epstein Barr Virus (EBV) positive gastric cancer, or a gastric or gastro-esophageal junction carcinoma). In some embodiments, a cancer is a head and neck cancer (e.g., an HPV positive and negative squamous cell cancer of the head and neck (SCCHN)). In some embodiments, a cancer is a nasopharyngeal cancer (NPC). In some embodiments, a cancer is a colorectal cancer, e.g., a relapsed colorectal cancer, a metastatic colorectal cancer, e.g., a microsatellite unstable colorectal cancer, a microsatellite stable colorectal cancer, a mismatch repair proficient colorectal cancer, or a mismatch repair deficient colorectal cancer.

[0686] In some embodiments, a cancer is a hematological cancer. In some embodiments, a cancer is a leukemia, e.g., acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, chronic leukemia, or acute leukemia. In some embodiments, a cancer is a lymphoma, e.g., Hodgkin lymphoma (HL), non-Hodgkin's lymphoma, lymphocytic lymphoma, or diffuse large B cell lymphoma (DLBCL) (e.g., a relapsed or refractory HL or DLBCL). In some embodiments, a cancer is a myeloma, e.g., multiple myeloma.

[0687] Administration of pharmaceutical compositions comprising a conjugate described herein may be carried out in any convenient manner (e.g., injection, ingestion, transfusion, inhalation, implantation, or transplantation). In some embodiments, a pharmaceutical compositions described herein is administered by injection or infusion. Pharmaceutical compositions described herein may be administered to a patient transarterially, subcutaneously, intravenously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, or intraperitoneally. In some embodiments, a pharmaceutical composition described

herein is administered parenterally (e.g., intravenously, subcutaneously, intraperitoneally, or intramuscularly). In some embodiments, a pharmaceutical composition described herein is administered by subcutaneous, intravenous, intramuscular, or intrasternal infusion or injection. In some embodiments, a pharmaceutical composition described herein is administered by intramuscular or subcutaneous injection. Pharmaceutical compositions described herein may be injected directly into a site of inflammation, a local disease site, a lymph node, an organ, a tumor, or site of infection in a subject.

[0688] In some embodiments, a conjugate is utilized in combination with one or more other therapeutic agents or modalities. In some embodiments, the one or more other therapeutic agents or modalities is an anti-cancer agent or modality. In some embodiments, a combination shows a synergistic effect in treating a condition, disorder or disease such as cancer. Therapeutic agents or treatments that show therapeutic efficacy in treating cancer may include, for example, one or more chemotherapeutic agents, alkylating agents, anti-metabolites, anti-microtubule agents, topoisomerase inhibitors, cytotoxic antibiotics, angiogenesis inhibitors, immunomodulators, vaccines, cell-based therapies (e.g. allogeneic or autologous stem cell transplantation), organ transplantation, radiation therapy, and/or surgery. In some embodiments, a conjugate is administered simultaneously with, prior to or subsequent to administration or delivery of another therapeutic agent or modality. In some embodiments, in a combination therapy, a subject is exposed to effect of both a conjugate and another therapeutic agent or modality.

Pharmaceutical Compositions

[0689] The present disclosure, among other things, provides pharmaceutical compositions comprising at least one conjugate in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents, or excipients. In some embodiments, a present disclosure provides a pharmaceutical composition, comprising a provided conjugate and a pharmaceutically acceptable carrier (which in some embodiments, is a diluent; in some embodiments, is an excipient, etc.). In some embodiments, a composition comprises a population of conjugates which share target binding moieties targeting the same agent or target or share a common target binding moiety, and each independently have a DAR as described herein. In some embodiments, a pharmaceutical composition has a DAR as described herein. In some embodiments, a conjugate in a pharmaceutical composition is in a pharmaceutically acceptable salt form.

[0690] When "an effective amount," "a therapeutically effective amount," "an immunologically effective amount," "an anti-immune response effective amount," or "an immune response-inhibiting effective amount" is indicated, a precise amount of a pharmaceutical composition comprising a conjugate described herein can be determined by a physician with consideration of purposes, individual differences in ages, weights, immune responses, conditions of a patient (subject), etc.

[0691] Pharmaceutical compositions described herein may comprise buffers including neutral buffered saline or phosphate buffered saline (PBS); carbohydrates, such as glucose, mannose, sucrose, dextrans, or mannitol; proteins, polypeptides, or amino acids (e.g., glycine); antioxidants; chelating agents, such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives. In some

embodiments, a pharmaceutical composition is substantially free of contaminants, e.g., there are no detectable levels of a contaminant (e.g., an endotoxin).

[0692] Pharmaceutical compositions described herein may be administered in a manner appropriate to the disease, disorder, or condition to be treated or prevented. Quantity and frequency of administration will be determined by such factors as condition of a patient, and type and severity of a patient's disease, disorder, or condition, although appropriate dosages may be determined by clinical trials.

[0693] Pharmaceutical compositions described herein may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, liposomes, and suppositories. Preferred compositions may be injectable or infusible solutions. Pharmaceutical compositions described herein can be formulated for administration intravenously, subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, transarterially, or intraperitoneally.

[0694] In some embodiments, a pharmaceutical composition described herein is formulated for parenteral (e.g., intravenous, subcutaneous, intraperitoneal, or intramuscular) administration. In some embodiments, a pharmaceutical composition described herein is formulated for subcutaneous, intravenous, intramuscular, or intrasternal injection or infusion. In preferred embodiments, a pharmaceutical composition described herein is formulated for subcutaneous or intravenous injection of infusion. Pharmaceutical compositions described herein can be formulated for administered by using infusion techniques that are commonly known in immunotherapy (See, e.g., Rosenberg et al., New Eng. J. of Med. 319:1676, 1988, which is hereby incorporated by reference in its entirety).

[0695] As used herein, the terms "parenteral administration" and "administered parenterally" refer to modes of administration other than enteral and topical administration, usually by injection or infusion, and include, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural, intratumoral, and intrasternal injection and infusion.

[0696] In some embodiments, pharmaceutical compositions described herein are administered in combination with (e.g., before, simultaneously, or following) bone marrow transplantation or lymphocyte ablative therapy using a chemotherapy agent (e.g., fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or Rituxan). In certain embodiments, subjects undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain embodiments, following transplant, subjects receive one or more pharmaceutical compositions described herein. In some embodiments, pharmaceutical compositions described herein may be administered before or following surgery.

[0697] A dosage of any aforementioned therapy to be administered to a subject will vary with a disease, disorder, or condition being treated and based on a specific subject. Scaling of dosages for human administration can be performed according to art-accepted practices.

Kits

[0698] The present disclosure, among other things, provides kits comprising at least one conjugate described herein, and instructions for use and/or administration. In some embodiments, a kit comprises least one conjugate described herein and a pharmaceutically acceptable carrier, and instructions for use and/or administration.

[0699] In some embodiments, a kit comprises instructions for use in a method described herein. Instructions can comprise a description of administration of a pharmaceutical composition or a first and second pharmaceutical compositions to a subject to achieve an intended activity or effect in a subject. A kit may further comprise a description of selecting a subject suitable for treatment based on identifying whether a subject is in need of or will likely be responsive to a treatment. In some embodiments, instructions comprise a description of administering a first and second pharmaceutical compositions to a subject who is in need of the treatment.

[0700] Instructions relating to a first and second pharmaceutical compositions described herein generally include information as to dosage, dosing schedule, and route of administration for an intended treatment. Containers may be unit doses, bulk packages (e.g., multi-dose packages) or sub-unit doses. Instructions supplied in kits are typically written instructions on a label or package insert. A label or package insert indicates that the pharmaceutical compositions are used for treating, delaying the onset, and/or alleviating a disease, disorder or condition in a subject.

[0701] Kits provided herein are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging, and the like. Also contemplated are packages for use in combination with a specific device, such as an infusion device. A kit may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierce able by a hypodermic injection needle). The container may also have a sterile access port.

[0702] Kits optionally may provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container. In some embodiment, the disclosure provides articles of manufacture comprising contents of the kits described above.

[0703] Among other things, the present disclosure provides the following Embodiments:

1. A conjugate, comprising:

i) a target binding moiety;

ii) a payload moiety; and

iii) a linker that connects the target binding moiety and the payload moiety;

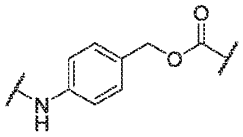
wherein:

the linker comprises –$(CH_2CH_2O)n$– and L^C;

n is 0-24;

L^C is bonded to a payload moiety and is –L^{Cl}–L^{CO}–;

L^{Cl} is a cleavable unit; and

L^{CO} is a covalent bond, or a spacer moiety (e.g., optionally substituted  wherein the carbonyl is bonded to a payload moiety).

2. A conjugate, comprising:

i) a target binding moiety;

ii) a payload moiety; and

iii) a linker that connects the target binding moiety and the payload moiety;

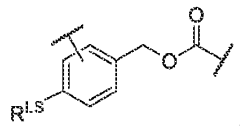
wherein:

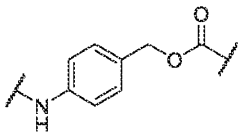
the linker comprises –$(CH_2CH_2O)n$– and L^{C};

n is 0-24;

L^{C} is bonded to a payload moiety and is –L^{Cl}–L^{CO}–;

L^{Cl} is –Gly–Gly–Phe–Gly–, –Val–Ala–, –Val–Cit–, L^{ClS}, –Gly–Gly–Phe–Gly–N(R')–L^{ClS}–, –Val–Ala–N(R')–L^{ClS}–, or –Val–Cit–N(R')–L^{ClS}–;

L^{ClS} is  , wherein the phenyl ring and the –CH_2– is optionally substituted, and the carbonyl is bonded to L^{CO}, and R^{LS} is or comprises a sugar moiety;

L^{CO} is a covalent bond, or optionally substituted  wherein the carbonyl is bonded to a payload moiety;

each R' is independently –R, –C(O)R, –CO_2R, or –$S(O)_2$R, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

3. A conjugate, comprising:

i) a target binding moiety;

ii) a payload moiety; and

iii) a linker that connects the target binding moiety and the payload moiety;

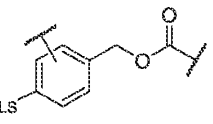
wherein:

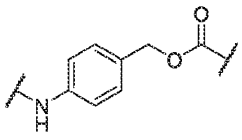
the linker comprises –$(CH_2CH_2O)n$– and L^C;

n is 1-24;

L^C is bonded to a payload moiety and is $-L^{C1}-L^{CO}-$;

L^{C1} is –Gly–Gly–Phe–Gly–, –Val–Ala–, –Val–Cit–, L^{C1S}, –Gly–Gly–Phe–Gly–N(R')–L^{C1S}–, –Val–Ala–N(R')–L^{C1S}–, or –Val–Cit–N(R')–L^{C1S}–;

L^{C1S} is  , wherein the phenyl ring and the $-CH_2-$ is optionally substituted, and the carbonyl is bonded to L^{CO}, and R^{LS} is or comprises a sugar moiety;

L^{CO} is a covalent bond, or optionally substituted  wherein the carbonyl is bonded to a payload moiety;

each R' is independently –R, –C(O)R, –CO_2R, or –$S(O)_2$R, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

4. The conjugate of any one of Embodiments 1-3, wherein the target binding moiety comprises an antibody or an antigen-binding fragment thereof.

5. The conjugate of any one of Embodiments 1-3, wherein the target binding moiety is an antibody or an antigen-binding fragment thereof.

6. The conjugate of any one of Embodiments 1-3, wherein the target binding moiety is an antibody.

7. The conjugate of any one of Embodiments 1-6, wherein the target binding moiety is a monoclonal antibody.

8. The conjugate of any one of Embodiments 1-7, wherein the target binding moiety is not trastuzumab.

9. The conjugate of any one of Embodiments 3-7, wherein the antibody shares less than about 90%, 95% or 99% sequence identity to trastuzumab.

10. The conjugate of any one of Embodiments 1-9, wherein the target binding moiety is not an anti-HER2 antibody.

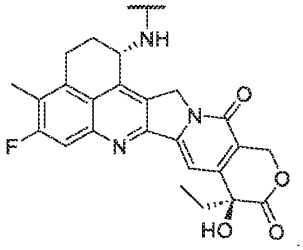
11. The conjugate of any one of Embodiments 1-10, wherein the target binding moiety is not an anti-HER2 antibody or an antigen-binding fragment thereof.

12. The conjugate of any one of the preceding Embodiments, wherein the payload moiety is a cytotoxic agent.

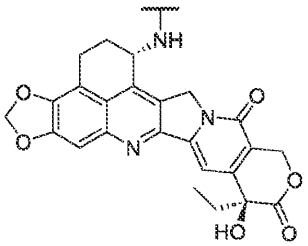
13. The conjugate of any one of the preceding Embodiments, wherein the payload moiety is a topoisomerase inhibitor.

14. The conjugate of any one of the preceding Embodiments, wherein the payload moiety is or comprises camptothecin or a derivative or an analog thereof.

15. The conjugate of any one of the preceding Embodiments, wherein the payload moiety is

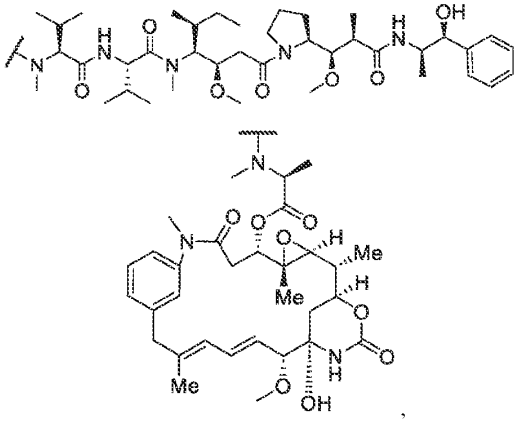


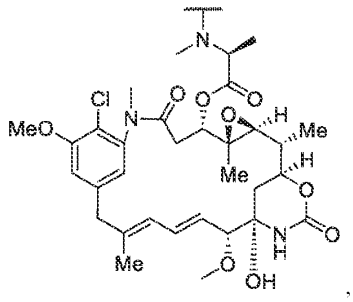
16. The conjugate of any one of Embodiments 1-12, wherein the payload moiety is

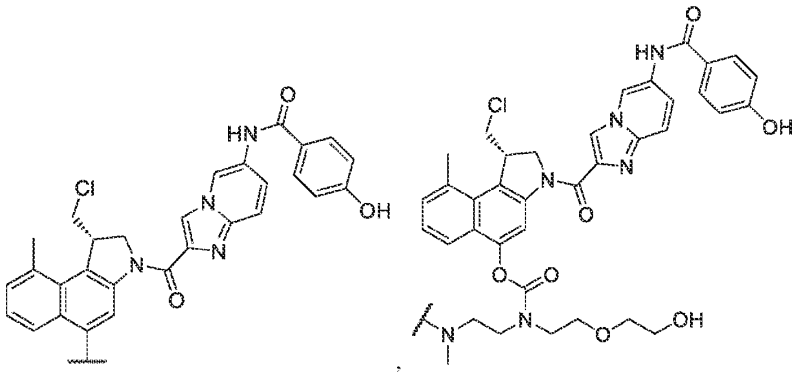


17. The conjugate of any one of Embodiments 1-12, wherein the payload moiety is an auristatin, a maytansinoid, a duocarmycin, a hemiasterlin, an eribulin, a N-myristoyl transfersse inhibitor, an amanitin or a derivative or an analog thereof.

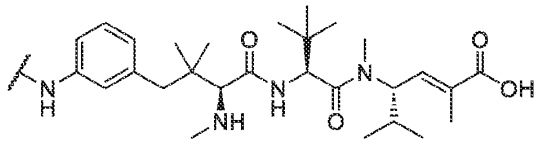
18. The conjugate of any one of Embodiments 1-12, wherein the payload is or comprises



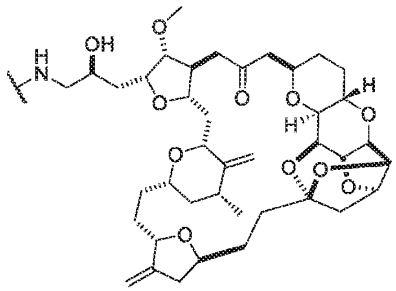




(Duocarmycin),



(hemiasterlin), or



(eribulin), or a derivative or an analog thereof.

19. The conjugate of any one of Embodiments 1-12, wherein the conjugate delivers a camptothecin, an auristatin, a maytansinoid, a duocarmycin, a hemiasterlin, an eribulin, or a N-myristoyl transferase inhibitor, or a derivative or an analog thereof.

20. The conjugate of any one of the preceding Embodiments, wherein the payload moiety to target binding moiety ratio of the conjugate (DAR) is about 1-20.

21. The conjugate of any one of the preceding Embodiments, wherein the DAR is about 2-15.

22. The conjugate of any one of the preceding Embodiments, wherein the DAR is about 2-12.

23. The conjugate of any one of the preceding Embodiments, wherein the DAR is about 2-8.

24. The conjugate of any one of the preceding Embodiments, wherein the DAR is about 2, 4, 6, or 8.

25. The conjugate of any one of the preceding Embodiments, wherein the DAR is about 2 or more.

26. The conjugate of any one of the preceding Embodiments, wherein the DAR is about 4.

27. The conjugate of any one of the preceding Embodiments, wherein the DAR is about 8.

28. The conjugates of any one of Embodiments 20-27, wherein the payload moieties are the same.

29. The conjugate of any one of Embodiments 1-28, wherein the target binding moiety is a moiety of an antibody that without conjugation comprises four Cys residues that form two inter-heavy and light chain disulfide bonds, wherein each of the four Cys residues is independently conjugated to a payload moiety.

30. The conjugate of any one of Embodiments 1-28, wherein the target binding moiety is a moiety of an antibody that without conjugation comprises eight Cys residues that form four inter-chain disulfide bonds, wherein each of the eight Cys residues is independently conjugated to a payload moiety.

31. The conjugate of any one of the preceding Embodiments, wherein the linker comprises $-(CH_2CH_2O)n-L^{ZP}-L^{C}-$, wherein:

L^{ZP} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each $-Cy-$ is independently an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

R is optionally substituted C_{1-6} aliphatic.

32. The conjugate of any one of the preceding Embodiments, wherein the linker comprises $-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$, wherein:

L^{X} is a covalent bond or an optionally substituted bivalent or polyvalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{X} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

L^{ZP} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each $-Cy-$ is independently an optionally substituted 3-12 membered ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and

each R is independently hydrogen or optionally substituted C_{1-6} aliphatic.

33. The conjugate of any one of the preceding Embodiments, wherein the linker is or comprises $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$, wherein:

L^{RX} is a covalent bond or a moiety that results from a reaction between a group present on the unconjugated target binding moiety and a group present on an agent comprising $-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ and a payload moiety, wherein $-L^{C}-$ is bonded to the payload moiety;

L^{X} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{X} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

L^{ZP} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic

wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –$S(O)_2$–, –C(O)N(R')–, –$S(O)_2$(NR')–, and –Cy–;

each –Cy– is independently an optionally substituted 3-12 membered ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and

each R is independently hydrogen or optionally substituted C_{1-6} aliphatic.

34. The conjugate of any one of the preceding Embodiments, wherein the linker is or comprises –L^{RX}–L^{X}–$(CH_2CH_2O)n$–L^{ZP}–L^{C}–, wherein:

L^{RX} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{RX} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –$S(O)_2$–, –C(O)N(R')–, –$S(O)_2$(NR')–, and –Cy–;

L^{X} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{X} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –$S(O)_2$–, –C(O)N(R')–, –$S(O)_2$(NR')–, and –Cy–;

L^{ZP} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –$S(O)_2$–, –C(O)N(R')–, –$S(O)_2$(NR')–, and –Cy–;

each –Cy– is independently an optionally substituted 3-12 membered ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and

each R is independently hydrogen or optionally substituted C_{1-6} aliphatic.

35. The conjugate of any one of the preceding Embodiments, wherein the linker is or comprises –L^{T}–L^{RX}–L^{X}–$(CH_2CH_2O)n$–L^{ZP}–L^{C}–, wherein:

L^{T} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{T} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –$S(O)_2$–, –C(O)N(R')–, –$S(O)_2$(NR')–, and –Cy–;

L^{RX} is a covalent bond or a moiety that results from a reaction between a group present on the unconjugated target binding moiety and a group present on an agent comprising –L^{X}–$(CH_2CH_2O)n$–L^{ZP}–L^{C}– and a payload moiety, wherein –L^{C}– is bonded to the payload moiety;

L^{X} is a covalent bond or an optionally substituted bivalent or polyvalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{X} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –$S(O)_2$–, –C(O)N(R')–, –$S(O)_2$(NR')–, and –Cy–;

L^{ZP} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –$S(O)_2$–, –C(O)N(R')–, –$S(O)_2$(NR')–, and –Cy–;

each –Cy– is independently an optionally substituted 3-12 membered ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and

each R is independently hydrogen or optionally substituted C_{1-6} aliphatic.

36. The conjugate of any one of the preceding Embodiments, wherein the linker is or comprises –L^{T}–L^{RX}–L^{X}–$(CH_2CH_2O)n$–L^{ZP}–L^{C}–, wherein:

L^T is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^T are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)$_2$−, −C(O)N(R')−, −S(O)$_2$(NR')−, and −Cy−;

L^{RX} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{RX} are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)$_2$−, −C(O)N(R')−, −S(O)$_2$(NR')−, and −Cy−;

L^X is a covalent bond or an optionally substituted bivalent or polyvalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^X are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)$_2$−, −C(O)N(R')−, −S(O)$_2$(NR')−, and −Cy−;

L^{ZP} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)$_2$−, −C(O)N(R')−, −S(O)$_2$(NR')−, and −Cy−;

each −Cy− is independently an optionally substituted 3-12 membered ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and

each R is independently hydrogen or optionally substituted C_{1-6} aliphatic.

37. The conjugate of any one of Embodiments 31-36, wherein L^{ZP} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^Z are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)$_2$−, −C(O)N(R')−, and −S(O)$_2$(NR')−.

38. The conjugate of any one of Embodiments 31-37, wherein L^{ZP} is –(CH$_2$)z−N(R')C(O)−, wherein z is 1-19.

39. The conjugate of any one of Embodiments 31-37, wherein L^{ZP} is –(CH$_2$)z−NHC(O)−, wherein z is 1-19.

40. The conjugate of any one of Embodiments 31-37, wherein L^{ZP} is–(CH$_2$)$_2$−NHC(O)−, wherein −C(O)− is bonded to the amino group of the first amino acid in L^C.

41. The conjugate of any one of Embodiments 31-37, wherein L^{ZP} is –(CH$_2$)z−C(O)−, wherein z is 1-19.

42. The conjugate of Embodiment 41, wherein L^{ZP} is –(CH$_2$)$_2$−C(O)−, wherein −C(O)− is bonded to the amino group of the first amino acid in L^C.

43. The conjugate of any one of Embodiments 31-37, wherein L^{ZP} is –(CH$_2$)z−C(O)N(R')−, wherein z is 1-19.

44. The conjugate of any one of Embodiments 31-37, wherein L^{ZP} is –(CH$_2$)z−C(O)NH−, wherein z is 1-19.

45. The conjugate of any one of Embodiments 31-37, wherein L^{ZP} is–(CH$_2$)$_2$−C(O)NH−, wherein −NH− is bonded to L^{CO}.

46. The conjugate of any one of Embodiments 1-43, wherein L^{C1} is −Gly−Gly−Phe−Gly−.

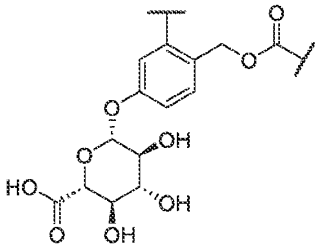
47. The conjugate of any one of Embodiments 1-43, wherein L^{C1} is L^{C1S}.

48. The conjugate of any one of Embodiments 1-43, wherein L^{C1} is mGlu.

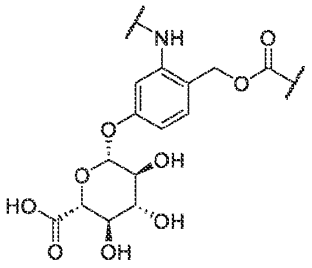
49. The conjugate of any one of Embodiments 1-43, wherein L^{C1} is oGlu.

50. The conjugate of any one of Embodiments 1-43, wherein L^{C1} is –Val–Cit–.

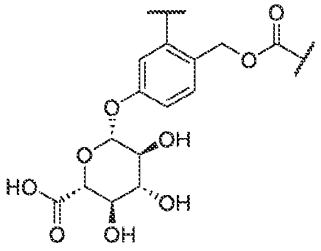
51. The conjugate of any one of the preceding Embodiments, wherein L^{C1} is



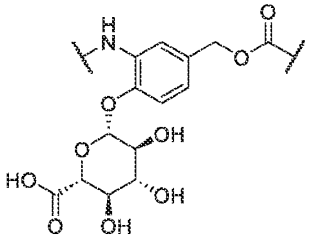
52. The conjugate of any one of the preceding Embodiments, wherein L^{C1} is



53. The conjugate of any one of the preceding Embodiments, wherein L^{C1} is

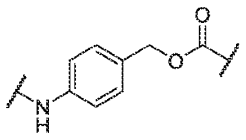


54. The conjugate of any one of the preceding Embodiments, wherein L^{C1} is



55. The conjugate of any one of the preceding Embodiments, wherein L^{C1} is –Val–Ala–.

56. The conjugate of any one of the preceding Embodiments, wherein L^{CO} is optionally substituted

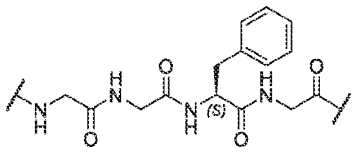


wherein the carbonyl is bonded to the payload moiety, and the amino group is

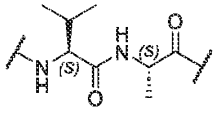
bonded to −C(O)− of L^{C1}.

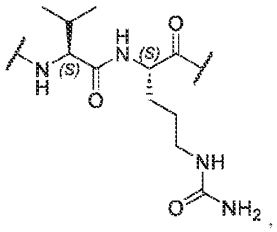
57. The conjugate of any one of Embodiments 1-55, wherein L^{CO} is a covalent bond and the payload moiety is bonded to −C(O)− of L^{C1}.

58. The conjugate of any one of Embodiments 1-42, wherein L^{C} is



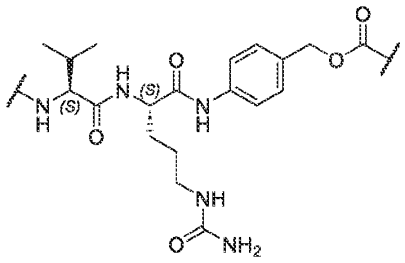
, wherein the carbonyl is bonded to the payload moiety.

59. The conjugate of any one of Embodiments 1-42, wherein L^{C} is  , wherein the carbonyl is bonded to the payload moiety.

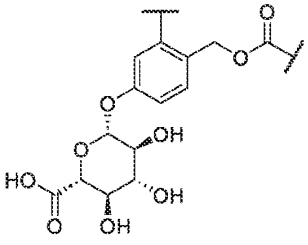


60. The conjugate of any one of Embodiments 1-42, wherein L^{C} is , wherein the carbonyl is bonded to the payload moiety.

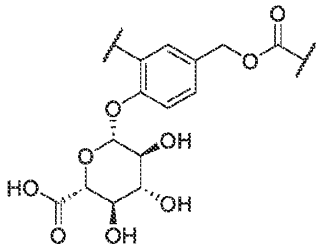
61. The conjugate of any one of Embodiments 1-42, wherein L^{C} is



, wherein the carbonyl is bonded to the payload moiety.

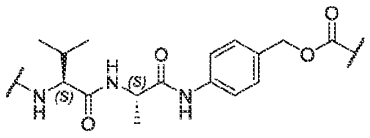


62. The conjugate of any one of Embodiments 1-42, wherein L^{C} is , wherein the carbonyl is bonded to the payload moiety.



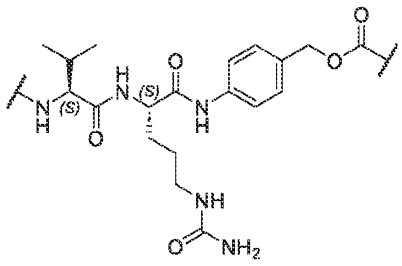
63. The conjugate of any one of Embodiments 1-42, wherein L^C is , wherein the carbonyl is bonded to the payload moiety.

64. The conjugate of any one of Embodiments 1-42, wherein L^C is



, wherein the carbonyl is bonded to the payload moiety.

65. The conjugate of any one of Embodiments 1-42, wherein L^C is

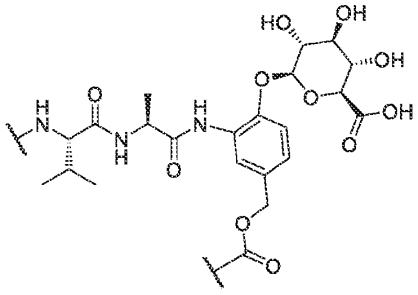


, wherein the carbonyl is bonded to the payload moiety..

66. The conjugate of any one of Embodiments 1-42, wherein L^C is oGlu, wherein the carbonyl is bonded to the payload moiety.

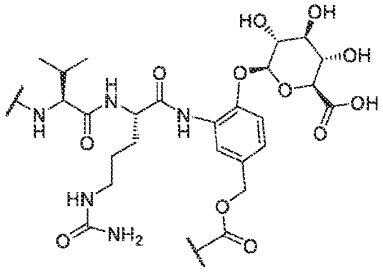
67. The conjugate of any one of Embodiments 1-42, wherein L^C is mGlu, wherein the carbonyl is bonded to the payload moiety.

68. The conjugate of any one of Embodiments 1-42, wherein L^C is



, wherein the carbonyl is bonded to the payload moiety.

69. The conjugate of any one of Embodiments 1-42, wherein L^C is



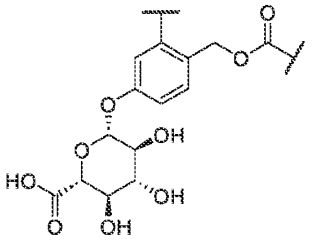
, wherein the carbonyl is bonded to the payload moiety.

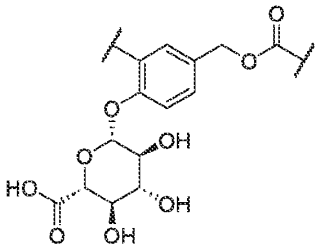
70. The conjugate of any one of the preceding Embodiments, wherein n is 4-24.

71. The conjugate of any one of the preceding Embodiments, wherein n is 7-20.

72. The conjugate of any one of the preceding Embodiments, wherein n is 8.

73. The conjugate of any one of Embodiments 1 and 4-69, wherein n is 0.

74. The conjugate of Embodiment 73, wherein L^C is  or



75. The conjugate of any one of Embodiments 32-74, wherein L^X is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^X are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)$_2$−, −C(O)N(R')−, −S(O)$_2$(NR')−, and −Cy−.

76. The conjugate of Embodiment 75, wherein L^X is –(CH$_2$)x−C(O)−N(R')−, wherein x is 1-19, and −N(R')− is bonded to −(CH$_2$CH$_2$O)n−.

77. The conjugate of Embodiment 75, wherein L^X is –(CH$_2$)x−C(O)−NH−, wherein x is 1-19, and −NH− is bonded to −(CH$_2$CH$_2$O)n−.

78. The conjugate of Embodiment 76 or 77, wherein x is 1-6.

79. The conjugate of Embodiment 76, wherein L^X is –(CH$_2$)$_5$−C(O)−N(R')−, wherein −N(R')− is bonded to −(CH$_2$CH$_2$O)n−.

80. The conjugate of Embodiment 77, wherein L^X is –(CH$_2$)$_5$−C(O)−NH−, wherein −NH− is bonded to −(CH$_2$CH$_2$O)n−.

81. The conjugate of Embodiment 76, wherein L^X is –(CH$_2$)$_3$−C(O)−N(R')−, wherein −N(R')− is

bonded to −(CH_2CH_2O)n−.

82. The conjugate of Embodiment 77, wherein L^X is $-(CH_2)_3$−C(O)−NH−, wherein −NH− is bonded to −(CH_2CH_2O)n−.

83. The conjugate of any one of Embodiments 33-82, wherein each L^{RX} is independently $-L^{RX1}-L^{RX2}-$, wherein:

each L^{RX1} is independently a covalent bond or an optionally substituted bivalent or polyvalent straight or branched C_{1-10} aliphatic wherein 1-5 methylene units of L^{RX1} are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, $-S(O)_2-$, −C(O)N(R')−, $-S(O)_2(NR')-$, and −Cy−;

each L^{RX2} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-10} aliphatic wherein 1-5 methylene units of L^{RX2} are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, $-S(O)_2-$, −C(O)N(R')−, $-S(O)_2(NR')-$, and −Cy−;

each −Cy− is independently an optionally substituted 3-12 membered ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

each L^{RX2} is bonded to L^X; and

each R is independently hydrogen or optionally substituted C_{1-6} aliphatic.

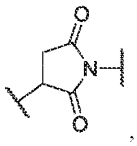
84. The conjugate of Embodiment 83, wherein $-L^{RX1}-$ is −S−.

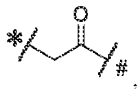
85. The conjugate of Embodiment 84, wherein the −S− is of a Cys residue of the target binding moiety.

86. The conjugate of Embodiment 84, wherein the −S− is of a Cys residue in an antibody which Cys residue forms an interchain disulfide bonds in an unconjugated antibody.

87. The conjugate of Embodiment 84, wherein the −S− is of a Cys residue in an antibody which Cys residue forms an inter-heavy and light chain disulfide bond in an unconjugated antibody.

88. The conjugate of Embodiment 84, wherein the −S− is of a Cys residue of the target binding moiety, wherein the Cys residue is derived through partial or complete reduction of the interchain disulfide bonds.

89. The conjugate of any one of Embodiments 83-88, wherein $-L^{RX2}-$ is  , wherein the nitrogen is bonded to L^X, and the carbon is bonded to L^{RX1}.

90. The conjugate of any one of Embodiments 83-88, wherein $-L^{RX2}-$ is  , wherein "#" represents the point of attachment to L^X, and "*" represents the point of attachment to L^{RX1}.

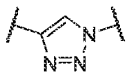
91. The conjugate of any one of Embodiments 83-85, wherein $-L^{RX2}-$ is −S−.

92. The conjugate of any one of Embodiments 33-82, wherein L^{RX} is or comprises a click chemistry

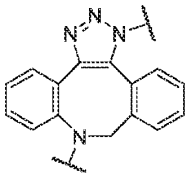
product moiety.

93. The conjugate of any one of Embodiments 33-82, wherein L^{RX} is or comprises an optionally substituted triazole ring.

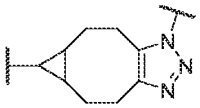
94. The conjugate of any one of Embodiments 33-34 and 92-93, wherein L^{RX} is or comprises



95. The conjugate of any one of Embodiments 33-34 and 92-93, wherein L^{RX} is or comprises



96. The conjugate of any one of Embodiments 33-34 and 92-93, wherein L^{RX} is or comprises



97. The conjugate of anyone of Embodiments 94-96, wherein the nitrogen in the 5-membered ring is bonded to L^X.

98. The conjugate of anyone of Embodiments 94-96, wherein the carbon or the nitrogen in the 8-membered ring is bonded to L^X.

99. The conjugate of any one of Embodiments 35-98, wherein L^T is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^T are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –$S(O)_2$–, –C(O)N(R')–, and –$S(O)_2$(NR')–.

100. The conjugate of any one of Embodiments 35-98, wherein L^T is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic group.

101. The conjugate of any one of Embodiments 35-98, wherein L^T is a bivalent straight or branched C_{1-20} aliphatic group.

102. The conjugate of any one of Embodiments 35-98, wherein L^T is optionally substituted –$(CH_2)_{1-5}$–.

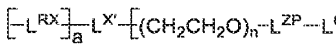
103. The conjugate of any one of Embodiments 35-98, wherein L^T is –$(CH_2)_{1-5}$–.

104. The conjugate of any one of Embodiments 35-103, wherein L^T is –CH_2–.

105. The conjugate of any one of Embodiments 35-104, wherein the target binding moiety comprises an amino acid residue, and L^T is bonded to the backbone atom of the amino acid residue.

106. The conjugate of any one of Embodiments 35-104, wherein the target binding moiety comprises an amino acid residue, and L^T is bonded to the alpha-carbon of the amino acid residue.

107. The conjugate of any one of Embodiments 1-31, wherein the linker is or comprises


, wherein:

each L^{RX} is independently a covalent bond or a moiety that results from a reaction between a group present on the unconjugated target binding moiety and a group present on an agent comprising $L^{X'}$ and optionally a payload moiety, wherein $-L^{C}-$ is bonded to the payload moiety;

each of a and b is independently 1-10;

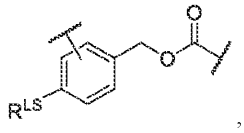
$L^{X'}$ is a covalent bond or an optionally substituted bivalent or polyvalent straight or branched C_{1-30} aliphatic wherein 1-10 methylene units of $L^{X'}$ are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;$-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

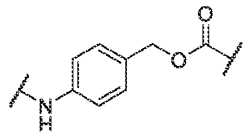
each n is independently 0-24;

each L^{ZP} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each L^{C} is independently $-L^{Cl}-L^{CO}-$;

each L^{Cl} is independently $-Gly-Gly-Phe-Gly-$, $-Val-Ala-$, $-Val-Cit-$, L^{ClS}, $-Gly-Gly-Phe-Gly-N(R')-L^{ClS}-$, $-Val-Ala-N(R')-L^{ClS}-$, or $-Val-Cit-N(R')-L^{ClS}-$;

each L^{ClS} is independently 
, wherein the phenyl ring and the $-CH_2-$ is optionally substituted, and the carbonyl is bonded to L^{CO}, and R^{LS} is or comprises a sugar moiety;

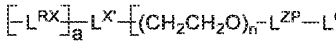
each L^{CO} is independently absent or optionally substituted 
wherein the carbonyl is bonded to a payload moiety;

each L^{C} is independently bonded to a payload moiety;

each $-Cy-$ is independently an optionally substituted 3-12 membered ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

each R' is independently $-R$, $-C(O)R$, $-CO_2R$, or $-S(O)_2R$, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen or optionally substituted C_{1-6} aliphatic.

108. The conjugate of any one of Embodiments 1-31, wherein the linker is or comprises 
, wherein:

each L^{RX} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{RX} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –$S(O)_2$–, –C(O)N(R')–, –$S(O)_2$(NR')–, and –Cy–;

each of a and b is independently 1-10;

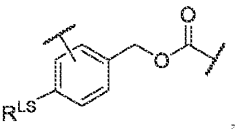
$L^{X'}$ is a covalent bond or an optionally substituted bivalent or polyvalent straight or branched C_{1-30} aliphatic wherein 1-10 methylene units of $L^{X'}$ are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –$S(O)_2$–, –C(O)N(R')–, –$S(O)_2$(NR')–, and –Cy–;

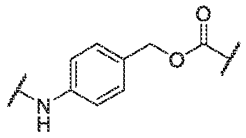
each n is independently 0-24;

each L^{ZP} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –$S(O)_2$–, –C(O)N(R')–, –$S(O)_2$(NR')–, and –Cy–;

each L^{C} is independently $-L^{C1}-L^{CO}-$;

each L^{C1} is independently –Gly–Gly–Phe–Gly–, –Val–Ala–, –Val–Cit–, L^{C1S}, –Gly–Gly–Phe–Gly–N(R')–L^{C1S}–, –Val–Ala–N(R')–L^{C1S}–, or –Val–Cit–N(R')–L^{C1S}–;

each L^{C1S} is independently  , wherein the phenyl ring and the –CH_2– is optionally substituted, and the carbonyl is bonded to L^{CO}, and R^{LS} is or comprises a sugar moiety;

each L^{CO} is independently absent or optionally substituted  wherein the carbonyl is bonded to a payload moiety;

each L^{C} is independently bonded to a payload moiety;

each –Cy– is independently an optionally substituted 3-12 membered ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

each R' is independently –R, –C(O)R, –CO_2R, or –$S(O)_2$R, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen or optionally substituted C_{1-6} aliphatic.

109. The conjugate of any one of Embodiments 1-31, wherein the linker is or comprises $\left[-L^{T}-L^{RX}-\right]_a-L^{X'}-\left[-(CH_2CH_2O)_n-L^{ZP}-L^{C}-\right]_b$, wherein:

each L^{T} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{T} are optionally and independently replaced by a group

selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –S(O)$_2$–, –C(O)N(R')–, –S(O)$_2$(NR')–, and –Cy–;

each L^{RX} is independently a covalent bond or a moiety that results from a reaction between a group present on the unconjugated target binding moiety and a group present on an agent comprising $L^{X'}$ and optionally a payload moiety;

each of a and b is independently 1-10;

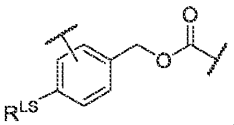
$L^{X'}$ is a covalent bond or an optionally substituted bivalent or polyvalent straight or branched C_{1-30} aliphatic wherein 1-10 methylene units of $L^{X'}$ are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –S(O)$_2$–, –C(O)N(R')–, –S(O)$_2$(NR')–, and –Cy–;

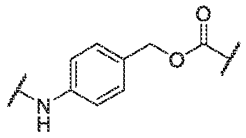
each n is independently 0-24;

each L^{ZP} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –S(O)$_2$–, –C(O)N(R')–, –S(O)$_2$(NR')–, and –Cy–;

each L^{C} is independently $-L^{C1}-L^{CO}-$;

each L^{C1} is independently –Gly–Gly–Phe–Gly–, –Val–Ala–, –Val–Cit–, L^{C1S}, –Gly–Gly–Phe–Gly–N(R')–L^{C1S}–, –Val–Ala–N(R')–L^{C1S}–, or –Val–Cit–N(R')–L^{C1S}–;

each L^{C1S} is independently  , wherein the phenyl ring and the –CH$_2$– is optionally substituted, and the carbonyl is bonded to L^{CO}, and R^{LS} is or comprises a sugar moiety;

each L^{CO} is independently absent or optionally substituted  wherein the carbonyl is bonded to a payload moiety;

each L^{C} is independently bonded to a payload moiety;

each –Cy– is independently an optionally substituted 3-12 membered ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

each R' is independently –R, –C(O)R, –CO$_2$R, or –S(O)$_2$R, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen or optionally substituted C_{1-6} aliphatic.

110. The conjugate of any one of Embodiments 1-31, wherein the linker is or comprises $\left[-L^{T}-L^{RX}-\right]_a-L^{X'}-\left[-(CH_2CH_2O)_n-L^{ZP}-L^{C}-\right]_b$, wherein:

each L^{T} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{T} are optionally and independently replaced by a group

selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –S(O)$_2$–, –C(O)N(R')–, –S(O)$_2$(NR')–, and –Cy–;

each L^{RX} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{RX} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –S(O)$_2$–, –C(O)N(R')–, –S(O)$_2$(NR')–, and –Cy–;

each of a and b is independently 1-10;

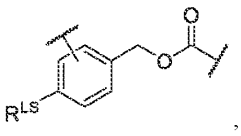
L^{X} is a covalent bond or an optionally substituted bivalent or polyvalent straight or branched C_{1-30} aliphatic wherein 1-10 methylene units of L^{X} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –S(O)$_2$–, –C(O)N(R')–, –S(O)$_2$(NR')–, and –Cy–;

each n is independently 0-24;

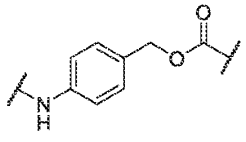
each L^{ZP} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –S(O)$_2$–, –C(O)N(R')–, –S(O)$_2$(NR')–, and –Cy–;

each L^{C} is independently $-L^{C1}-L^{CO}-$;

each L^{C1} is independently –Gly–Gly–Phe–Gly–, –Val–Ala–, –Val–Cit–, L^{C1S}, –Gly–Gly–Phe–Gly–N(R')–L^{C1S}–, –Val–Ala–N(R')–L^{C1S}–, or –Val–Cit–N(R')–L^{C1S}–;

each L^{C1S} is independently  , wherein the phenyl ring and the –CH$_2$– is optionally substituted, and the carbonyl is bonded to L^{CO}, and R^{LS} is or comprises a sugar moiety;

each R' is independently –R, –C(O)R, –CO$_2$R, or –S(O)$_2$R, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each L^{CO} is independently absent or optionally substituted  wherein the carbonyl is bonded to a payload moiety;

each L^{C} is independently bonded to a payload moiety;

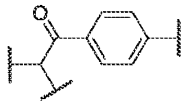
each –Cy– is independently an optionally substituted 3-12 membered ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and

each R is independently hydrogen or optionally substituted C_{1-6} aliphatic.

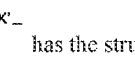
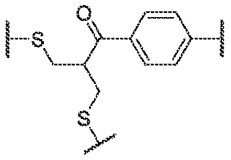
111. The conjugate of any one of Embodiments 107-110, wherein each L^{RX} is independently of any one of Embodiments 83-98.

112. The conjugate of any one of Embodiments 107-111, wherein each L^{RX} is the same.

113. The conjugate of any one of Embodiments 107-112, wherein a is 2.

114. The conjugate of any one of Embodiments 107-113, wherein $L^{X'}$ is  , wherein the phenyl ring is bonded to –$(CH_2CH_2O)n$–.

115. The conjugate of any one of Embodiments 107-112, wherein a is 3-10.

116. The conjugate of Embodiment 114, wherein  has the structure of 

117. The conjugate of any one of Embodiments 109-116, wherein each L^T is the same.

118. The conjugate of any one of Embodiments 109-117, wherein each L^T is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^T are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –$S(O)_2$–, –C(O)N(R')–, and –$S(O)_2$(NR')–.

119. The conjugate of any one of Embodiments 109-117, wherein each L^T is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic group.

120. The conjugate of any one of Embodiments 109-119, wherein each L^T is independently a bivalent straight or branched C_{1-20} aliphatic group.

121. The conjugate of any one of Embodiments 109-120, wherein each occurrence of L^T is independently optionally substituted –$(CH_2)_{1-5}$–.

122. The conjugate of any one of Embodiments 109-121, wherein each occurrence of L^T is independently –$(CH_2)_{1-5}$–.

123. The conjugate of any one of Embodiments 109-122, wherein each occurrence of L^T is –CH_2–.

124. The conjugate of any one of Embodiments 109-123, wherein each occurrence of L^T is independently bonded to a backbone atom of an amino acid residue in a target binding moiety.

125. The conjugate of any one of Embodiments 109-123, wherein each occurrence of L^T is independently bonded to a backbone atom of an amino acid residue in the same target binding moiety.

126. The conjugate of any one of Embodiments 109-123, wherein each occurrence of L^T is independently bonded to a different backbone atom of an amino acid residue in the same target binding moiety.

127. The conjugate of any one of Embodiments 109-123, wherein each occurrence of L^T is independently bonded to an alpha atom of an amino acid residue in a target binding moiety.

128. The conjugate of any one of Embodiments 109-123, wherein each occurrence of L^T is independently bonded to an alpha atom of an amino acid residue in the same target binding moiety.

129. The conjugate of any one of Embodiments 109-123, wherein each occurrence of L^T is independently bonded to a different alpha atom of an amino acid residue in the same target binding

moiety.

130. The conjugate of Embodiment 116, wherein each –S– is independently of a Cys residue in the same target binding moiety.

131. The conjugate of Embodiment 130, wherein the two Cys residues form an inter-chain disulfide bond in an unconjugated target binding moiety.

132. The conjugate of any one of Embodiments 109-117, wherein each L^T is independently of any one of Embodiments 99-106.

133. The conjugate of any one of Embodiments 107-110, wherein each occurrence of $-L^T-L^{RX}-$ is independently of any one of Embodiments 83-106.

134. The conjugate of any one of Embodiments 107-133, wherein each occurrence of $-L^T-L^{RX}-$ is the same.

135. The conjugate of any one of Embodiments 107-134, wherein each occurrence of n is independently of any one of Embodiments 71-74.

136. The conjugate of any one of Embodiments 107-135, wherein each occurrence of n is the same.

137. The conjugate of any one of Embodiments 107-135, wherein each occurrence of n is 8.

138. The conjugate of any one of Embodiments 107-137, wherein each occurrence of L^{ZP} is independently of any one of Embodiments 37-42.

139. The conjugate of any one of Embodiments 107-138, wherein each occurrence of L^{ZP} is the same.

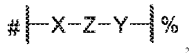
140. The conjugate of any one of Embodiments 107-139, wherein each occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^C-$ is independently of any one of Embodiments 37-74.

141. The conjugate of any one of Embodiments 107-140, wherein each occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^C-$ is the same.

142. The conjugate of any one of the preceding Embodiments, wherein the linker comprises a moiety that results from a reaction between an unbound linker and a group present on the unconjugated target binding moiety.

143. The conjugate of any one of the preceding Embodiments, wherein the linker comprises a spacer moiety.

144. The conjugate of any one of the preceding Embodiments, wherein the linker is or comprises a structure of formula B:



B

wherein:

represents the point of attachment to the target binding moiety;

% represents the point of attachment to a payload moiety;

X is a moiety that results from the reaction of an unbound linker and a group present on the target binding moiety;

Y is L^C; and

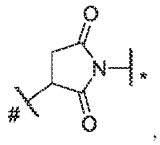
Z is or comprises $-(CH_2CH_2O)n-$.

145. The conjugate of Embodiment 144, wherein the group present on the unbound linker comprises a maleimide moiety.

146. The conjugate of any one of Embodiments 144-145, wherein the group present on the target binding moiety is a thiol.

147. The conjugate of any one of Embodiments 1-146, wherein the linker comprises a moiety that results from the reaction of the unbound linker and a thiol present on the target binding moiety.

148. The conjugate of any one of Embodiments 1-147, wherein the linker comprises a structure:



or a pharmaceutically acceptable salt form thereof, wherein:

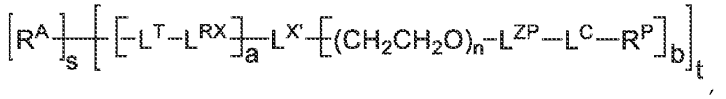
represents the point of attachment to the target binding moiety; and

* represents the point of attachment to the remainder of the linker.

149. The conjugate of any one of Embodiments 143-148, wherein the spacer moiety comprises an optionally substituted bivalent C_{1-12} aliphatic, aryl, heteroaryl, polyether, polyester, poly(*N*-(2-hydroxypropyl)methacrylamide) (pHPMA), polylactic-co-glycolic acid (PLGA), polymers of alkylene diamines, polysarcosine, saccharide, or combinations thereof.

150. The conjugate of Embodiment 149, wherein the spacer moiety is or comprises $-(CH_2CH_2O)_n-$, wherein n is an integer between 1 and 24.

151. A conjugate, wherein the conjugate is a compound of formula A-1 or a salt thereof:



A-1

wherein:

each R^A is independently a target binding moiety;

s is 1-20;

each L^T is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^T are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each L^{RX} is independently a covalent bond, a moiety that results from a reaction between a group present on the unconjugated target binding moiety and a group present on an agent comprising $L^{X'}$, or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{RX} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each of a and b is independently 1-10;

each $L^{X'}$ is independently a covalent bond or an optionally substituted bivalent or polyvalent straight or branched C_{1-30} aliphatic wherein 1-10 methylene units of $L^{X'}$ are optionally and independently

replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −$S(O)_2$−, −C(O)N(R')−, −$S(O)_2$(NR')−, and −Cy−;

each n is independently 0-24;

each L^{ZP} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −$S(O)_2$−, −C(O)N(R')−, −$S(O)_2$(NR')−, and −Cy−;

each L^{C} is independently $-L^{C1}-L^{CO}-$;

each L^{C1} is independently a cleavable unit;

each L^{CO} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{CO} are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −$S(O)_2$−, −C(O)N(R')−, −$S(O)_2$(NR')−, and −Cy−;

each R^{P} is independently a payload moiety;

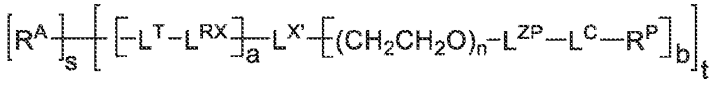
t is 1-20;

each −Cy− is independently an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each R' is independently −R, −C(O)R, −CO_2R, or −$S(O)_2$R, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

152. A conjugate, wherein the conjugate is a compound of formula A-1 or a salt thereof:



A-1

wherein:

each R^{A} is independently a target binding moiety;

s is 1-20;

each L^{T} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{T} are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −$S(O)_2$−, −C(O)N(R')−, −$S(O)_2$(NR')−, and −Cy−;

each L^{RX} is independently a covalent bond or an optionally substituted bivalent straight or

branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{RX} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –$S(O)_2$–, –C(O)N(R')–, –$S(O)_2$(NR')–, and –Cy–;

each of a and b is independently 1-10;

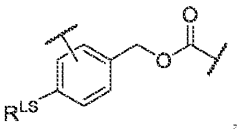
each $L^{X'}$ is independently a covalent bond or an optionally substituted bivalent or polyvalent straight or branched C_{1-30} aliphatic wherein 1-10 methylene units of $L^{X'}$ are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –$S(O)_2$–, –C(O)N(R')–, –$S(O)_2$(NR')–, and –Cy–;

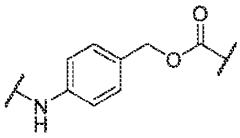
each n is independently 0-24;

each L^{ZP} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –$S(O)_2$–, –C(O)N(R')–, –$S(O)_2$(NR')–, and –Cy–;

each L^{C} is independently –L^{C1}–L^{CO}–;

each L^{C1} is independently –Gly–Gly–Phe–Gly–, –Val–Ala–, –Val–Cit–, L^{C1S}, –Gly–Gly–Phe–Gly–N(R')–L^{C1S}–, –Val–Ala–N(R')–L^{C1S}–, or –Val–Cit–N(R')–L^{C1S}–;

each L^{C1S} is independently  , wherein the phenyl ring and the –CH_2– is optionally substituted, and the carbonyl is bonded to L^{CO}, and R^{LS} is or comprises a sugar moiety;

each L^{CO} is independently a covalent bond or optionally substituted  wherein the carbonyl is bonded to a payload moiety;

each R^{P} is independently a payload moiety;

t is 1-20;

each –Cy– is independently an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each R' is independently –R, –C(O)R, –CO_2R, or –$S(O)_2$R, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

153. The conjugate of any one of Embodiments 151-152, wherein each R^A is independently a target binding moiety of any one of Embodiments 1-11.

154. The conjugate of any one of Embodiments 151-153, wherein each target binding moiety targets the same agent.

155. The conjugate of any one of Embodiments 151-153, wherein each target binding moiety targets the same agent, wherein the agent is or comprises a polypeptide, a nucleic acid or a lipid.

156. The conjugate of any one of Embodiments 151-153, wherein each target binding moiety targets the same agent, wherein the agent is a polypeptide.

157. The conjugate of any one of Embodiments 151-156, wherein each target binding moiety targets the same antigen.

158. The conjugate of any one of Embodiments 151-157, wherein each target binding moiety is independently an antibody or an antigen-binding fragment thereof, and each target binding moiety shares about or at least about 90%, 95%, 96%, 97%, 98% or 99% sequence identity.

159. The conjugate of any one of Embodiments 151-157, wherein each target binding moiety is independently an antibody or an antigen-binding fragment thereof, and each target binding moiety shares the same amino acid sequence.

160. The conjugate of any one of Embodiments 151-159, wherein each target binding moiety is the same.

161. The conjugate of any one of Embodiments 151-160, wherein s is 1.

162. The conjugate of any one of Embodiments 151-161, wherein an occurrence of a is 1.

163. The conjugate of any one of Embodiments 151-162, wherein an occurrence of a is 2.

164. The conjugate of any one of Embodiments 151-163, wherein an occurrence of a is 3-10.

165. The conjugate of any one of Embodiments 151-164, wherein an occurrence of b is 1.

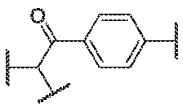
166. The conjugate of any one of Embodiments 151-165, wherein an occurrence of b is 2-10.

167. The conjugate of any one of Embodiments 151-166, wherein an occurrence of $L^{X'}$ is a covalent bond.

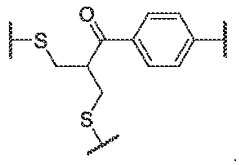
168. The conjugate of any one of Embodiments 151-167, wherein an occurrence of $L^{X'}$ is a covalent bond or an optionally substituted bivalent straight or branched C_{1-30} aliphatic wherein 1-10 methylene units of $L^{X'}$ are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –$S(O)_2$–, –C(O)N(R')–, –$S(O)_2$(NR')–, and –Cy–.

169. The conjugate of any one of Embodiments 151-168, wherein an occurrence of $L^{X'}$ is a covalent bond or an optionally substituted polyvalent straight or branched C_{1-30} aliphatic wherein 1-10 methylene units of $L^{X'}$ are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –$S(O)_2$–, –C(O)N(R')–, –$S(O)_2$(NR')–, and –Cy–.

170. The conjugate of any one of Embodiments 151-169, wherein an occurrence of $L^{X'}$ is



, wherein the phenyl ring is bonded to –$(CH_2CH_2O)n$–.

171. The conjugate of any one of Embodiments 151-170, wherein an occurrence of $-[-L^{RX}-]_a-L^{X}-$ has the structure of  .

172. The conjugate of any one of Embodiments 151-161, wherein each a is the same.

173. The conjugate of any one of Embodiments 151-161, wherein each a is 1.

174. The conjugate of any one of Embodiments 151-161, wherein each a is 2.

175. The conjugate of any one of Embodiments 151 and 172-174, wherein each b is the same.

176. The conjugate of any one of Embodiments 151 and 172-174, wherein each b is 1.

177. The conjugate of any one of Embodiments 151 and 172-176, wherein each L^{X} is the same.

178. A conjugate, wherein the conjugate is a compound of formula A-2 or a salt thereof:

$$R^A-\left[-L^T-L^{RX}-L^X-(CH_2CH_2O)_n-L^{ZP}-L^C-R^P\right]_t,$$

A-2

wherein:

R^A is a target binding moiety;

each L^T is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^T are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each L^{RX} is independently a covalent bond, a moiety that results from a reaction between a group present on the unconjugated target binding moiety and a group present on an agent comprising $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$, or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{RX} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each L^X is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^X are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each n is independently 0-24;

each L^{ZP} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each L^C is independently $-L^{Cl}-L^{CO}-$;

each L^{Cl} is independently a cleavable unit;

each L^{CO} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{CO} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –S(O)$_2$–, –C(O)N(R')–, –S(O)$_2$(NR')–, and –Cy–;

each R^P is independently a payload moiety;

t is 1-20;

each –Cy– is independently an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each R' is independently –R, –C(O)R, –CO_2R, or –S(O)$_2$R, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

179. A conjugate, wherein the conjugate is a compound of formula A-2 or a salt thereof:

$$R^A\left[-L^T-L^{RX}-L^X-(CH_2CH_2O)_n-L^{ZP}-L^C-R^P\right]_t,$$

A-2

wherein:

R^A is a target binding moiety;

each L^T is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^T are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –S(O)$_2$–, –C(O)N(R')–, –S(O)$_2$(NR')–, and –Cy–;

each L^{RX} is independently a covalent bond, a moiety that results from a reaction between a group present on the unconjugated target binding moiety and a group present on an agent comprising –L^X–(CH$_2$CH$_2$O)n–L^{ZP}–L^C–R^P, or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{RX} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –S(O)$_2$–, –C(O)N(R')–, –S(O)$_2$(NR')–, and –Cy–;

each L^X is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^X are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –S(O)$_2$–, –C(O)N(R')–, –S(O)$_2$(NR')–, and –Cy–;

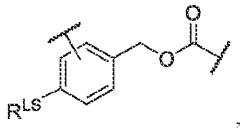
each n is independently 0-24;

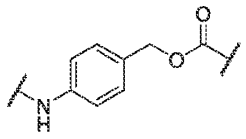
each L^{ZP} is independently a covalent bond or an optionally substituted bivalent straight or

branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –$S(O)_2$–, –C(O)N(R')–, –$S(O)_2$(NR')–, and –Cy–;

each L^{C} is independently –L^{C1}–L^{CO}–;

each L^{C1} is independently –Gly–Gly–Phe–Gly–, –Val–Ala–, –Val–Cit–, L^{C1S}, –Gly–Gly–Phe–Gly–N(R')–L^{C1S}–, –Val–Ala–N(R')–L^{C1S}–, or –Val–Cit–N(R')–L^{C1S}–;

each L^{C1S} is independently  , wherein the phenyl ring and the –CH_2– is optionally substituted, and the carbonyl is bonded to L^{CO}, and R^{LS} is or comprises a sugar moiety;

each L^{CO} is independently absent or optionally substituted  wherein the carbonyl is bonded to a payload moiety;

each R^{P} is independently a payload moiety;

t is 1-20;

each –Cy– is independently an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each R' is independently –R, –C(O)R, –CO_2R, or –$S(O)_2$R, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

180. The conjugate of any one of Embodiments 178-179, wherein R^{A} is a target binding moiety of any one of Embodiments 1-11.

181. The conjugate of any one of Embodiments 178-180, wherein an occurrence of L^{X} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{X} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –$S(O)_2$–, –C(O)N(R')–, –$S(O)_2$(NR')–, and –Cy–.

182. The conjugate of any one of Embodiments 178-181, wherein an occurrence of L^{X} is –(CH_2)x–C(O)–N(R')–, wherein x is 1-18, and –N(R')– is bonded to –(CH_2CH_2O)n–.

183. The conjugate of any one of Embodiments 178-181, wherein an occurrence of L^{X} is –(CH_2)x–C(O)–NH–, wherein x is 1-18, and –NH– is bonded to –(CH_2CH_2O)n–.

184. The conjugate of Embodiments 182-183, wherein x is 1-6.

185. The conjugate of Embodiment 182, wherein an occurrence of L^X is $-(CH_2)_5-C(O)-N(R')-$, wherein $-N(R')-$ is bonded to $-(CH_2CH_2O)n-$.

186. The conjugate of Embodiment 183, wherein an occurrence of L^X is $-(CH_2)_5-C(O)-NH-$, wherein $-NH-$ is bonded to $-(CH_2CH_2O)n-$.

187. The conjugate of Embodiment 182, wherein an occurrence of L^X is $-(CH_2)_3-C(O)-N(R')-$, wherein $-N(R')-$ is bonded to $-(CH_2CH_2O)n-$.

188. The conjugate of Embodiment 183, wherein an occurrence of L^X is $-(CH_2)_3-C(O)-NH-$, wherein $-NH-$ is bonded to $-(CH_2CH_2O)n-$.

189. The conjugate of any one of Embodiments 178-186, wherein each occurrence of L^X is independently of any one of Embodiments 75-82.

190. The conjugate of any one of Embodiments 178-189, wherein each L^X is the same.

191. The conjugate of any one of Embodiments 151-190, wherein an occurrence of L^T is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^T are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$.

192. The conjugate of any one of Embodiments 151-190, wherein an occurrence of L^T is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic group.

193. The conjugate of any one of Embodiments 151-190, wherein an occurrence of L^T is a bivalent straight or branched C_{1-20} aliphatic group.

194. The conjugate of any one of Embodiments 151-193, wherein an occurrence of L^T is optionally substituted $-(CH_2)_{1-5}-$.

195. The conjugate of any one of Embodiments 151-194, wherein an occurrence of L^T is $-(CH_2)_{1-5}-$.

196. The conjugate of any one of Embodiments 151-195, wherein an occurrence of L^T is $-CH_2-$.

197. The conjugate of any one of Embodiments 151-196, wherein the target binding moiety comprises an amino acid residue, and an occurrence of L^T is bonded to the backbone atom of the amino acid residue.

198. The conjugate of any one of Embodiments 151-196, wherein the target binding moiety comprises an amino acid residue, and an occurrence of L^T is bonded to the alpha-carbon of the amino acid residue.

199. The conjugate of any one of Embodiments 151-196, wherein each occurrence of L^T is independently of any one of Embodiments 88-95.

200. The conjugate of any one of Embodiments 151-199, wherein each L^T is the same.

201. The conjugate of any one of Embodiments 151-200, wherein an occurrence of L^{RX} is a covalent bond or a moiety that results from a reaction between a group present on the unconjugated target binding moiety and a group present on an agent comprising $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ and a payload moiety.

202. The conjugate of any one of Embodiments 151-200, wherein an occurrence of L^{RX} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{RX} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$, wherein each $-Cy-$ is independently an

optionally substituted 3-12 membered ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur.

203. The conjugate of any one of Embodiments 151-200, wherein an occurrence of L^{RX} is $-L^{RX1}-L^{RX2}-$, wherein:

each L^{RX1} is independently a covalent bond or an optionally substituted bivalent or polyvalent straight or branched C_{1-10} aliphatic wherein 1-5 methylene units of L^{RX1} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each L^{RX2} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-10} aliphatic wherein 1-5 methylene units of L^{RX2} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each L^{RX2} is bonded to L^X;

each $-Cy-$ is independently an optionally substituted 3-12 membered ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and

each R is independently hydrogen or optionally substituted C_{1-6} aliphatic.

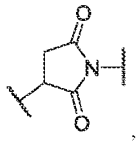
204. The conjugate of Embodiment 203, wherein $-L^{RX1}-$ is $-S-$.

205. The conjugate of Embodiment 204, wherein the $-S-$ is of a Cys residue of the target binding moiety.

206. The conjugate of Embodiment 205, wherein the $-S-$ is of a Cys residue in an antibody wherein the Cys residue forms an interchain disulfide bonds in an unconjugated antibody.

207. The conjugate of Embodiment 205, wherein the $-S-$ is of a Cys residue in an antibody wherein the Cys residue forms an inter-heavy and light chain disulfide bond in an unconjugated antibody.

208. The conjugate of Embodiment 205, wherein the $-S-$ is of a Cys residue of the target binding moiety, wherein the Cys residue is derived through partial or complete reduction of the interchain disulfide bonds.

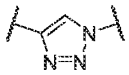
209. The conjugate of any one of Embodiments 203-208, wherein $-L^{RX2}-$ is  , wherein the nitrogen is bonded to L^X, and the carbon is bonded to L^{RX1}.

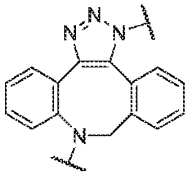
210. The conjugate of any one of Embodiments 203-208, wherein $-L^{RX2}-$ is $-S-$.

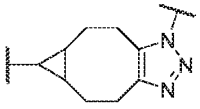
211. The conjugate of any one of Embodiments 151-200, wherein an occurrence of L^{RX} is or comprises a click chemistry product moiety.

212. The conjugate of any one of Embodiments 151-200, wherein L^{RX} is or comprises a click chemistry product moiety.

213. The conjugate of any one of Embodiments 151-200, wherein an occurrence of L^{RX} is or comprises



214. The conjugate of any one of Embodiments 151-200, wherein an occurrence of wherein L^{RX} is or comprises  .

215. The conjugate of any one of Embodiments 151-200, wherein an occurrence of L^{RX} is or comprises  .

216. The conjugate of anyone of Embodiments 213-215, wherein the nitrogen in the 5-membered ring is bonded to L^{X}.

217. The conjugate of anyone of Embodiments 213-215, wherein the carbon or the nitrogen in the 8-membered ring is bonded to L^{X}.

218. The conjugate of any one of Embodiments 151-200, wherein each occurrence of L^{RX} is independently of any one of Embodiments 83-98.

219. The conjugate of any one of Embodiments 151-218, wherein each L^{RX} is the same.

220. The conjugate of any one of Embodiments 151-219, wherein an occurrence of n is 1-24.

221. The conjugate of any one of Embodiments 151-219, wherein an occurrence of n is 4-24.

222. The conjugate of any one of Embodiments 151-219, wherein an occurrence of n is 7-20.

223. The conjugate of any one of Embodiments 151-219, wherein each occurrence of n is independently 4-24.

224. The conjugate of any one of Embodiments 151-219, wherein each occurrence of n is independently 7-20.

225. The conjugate of any one of Embodiments 151-219, wherein each occurrence of n is independently of any one of Embodiments 71-74.

226. The conjugate of any one of Embodiments 151-219, wherein an occurrence of n is 8.

227. The conjugate of any one of Embodiments 151-226, wherein each n is the same.

228. The conjugate of any one of Embodiments 151-227, wherein an occurrence of L^{ZP} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{Z} are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)$_2$−, −C(O)N(R')−, −S(O)$_2$(NR')−, and −Cy−.

229. The conjugate of any one of Embodiments 151-227, wherein an occurrence of L^{ZP} is −$(CH_2)_z$−C(O)−, wherein z is 1-19.

230. The conjugate of any one of Embodiments 151-227, wherein an occurrence of L^{ZP} is −$(CH_2)_2$−C(O)−, wherein −C(O)− is bonded to the amino group of the first amino acid in −L^{C}−.

231. The conjugate of any one of Embodiments 151-227, wherein each occurrence of L^{ZP} is independently of any one of Embodiments 37-45.

232. The conjugate of any one of Embodiments 151-231, wherein each L^{ZP} is the same.

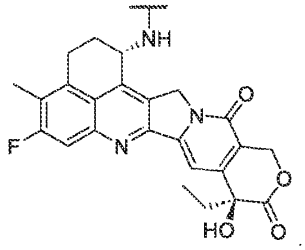
233. The conjugate of any one of Embodiments 151-232, wherein an occurrence of R^P is a cytotoxic agent.

234. The conjugate of any one of Embodiments 151-232, wherein each R^P is independently a cytotoxic agent.

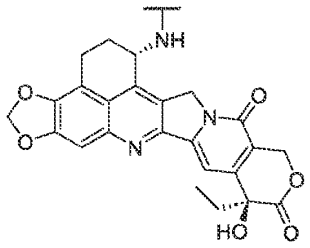
235. The conjugate of any one of Embodiments 151-232, wherein an occurrence of R^P is exatecan or a derivative thereof.

236. The conjugate of any one of Embodiments 151-232, wherein each R^P is independently exatecan or a derivative thereof.

237. The conjugate of any one of Embodiments 151-232, wherein an occurrence of R^P is



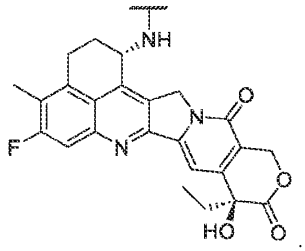
238. The conjugate of any one of Embodiments 151-232, wherein an occurrence of R^P is



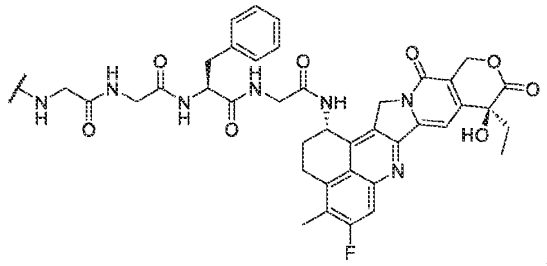
239. The conjugate of any one of Embodiments 151-232, wherein each occurrence of R^P is independently a payload moiety of any one of Embodiments 12-18.

240. The conjugate of any one of Embodiments 151-239, wherein each R^P is the same.

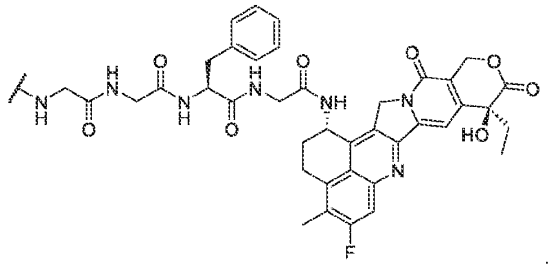
241. The conjugate of any one of Embodiments 151-232, wherein each R^P is



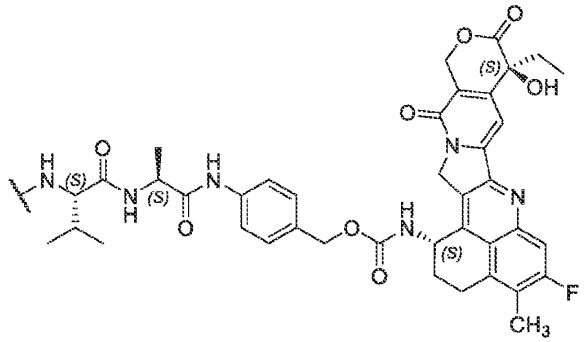
242. The conjugate of any one of Embodiments 151-239, wherein an occurrence of $-L^C-R^P$ is



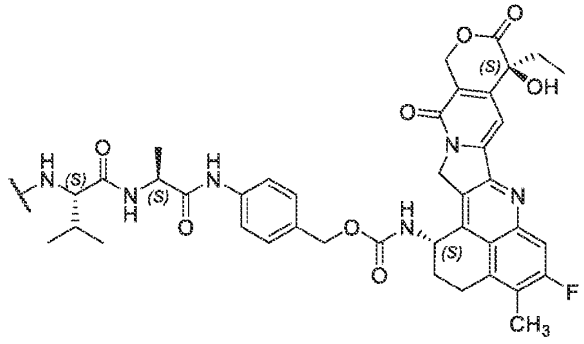
243. The conjugate of any one of Embodiments 151-239, wherein each $-L^C-R^P$ is independently



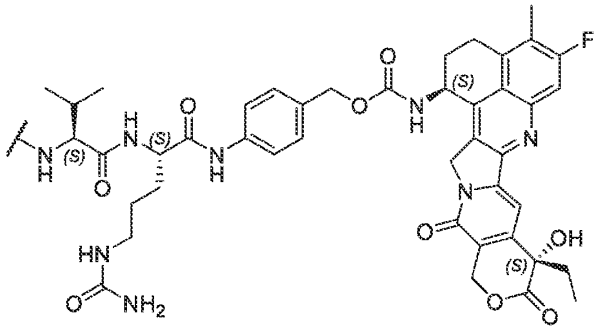
244. The conjugate of any one of Embodiments 151-239, wherein an occurrence of $-L^C-R^P$ is



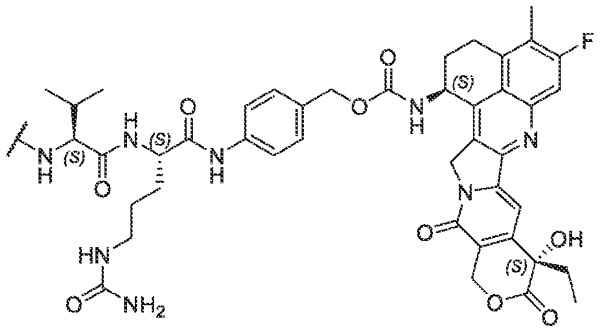
245. The conjugate of any one of Embodiments 151-239, wherein each $-L^C-R^P$ is independently



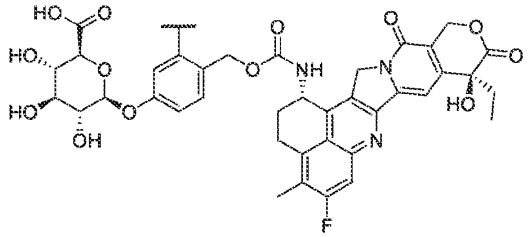
246. The conjugate of any one of Embodiments 151-239, wherein an occurrence of $-L^C-R^P$ is



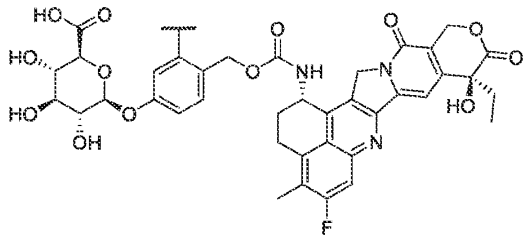
247. The conjugate of any one of Embodiments 151-239, wherein each $-L^C-R^P$ is independently



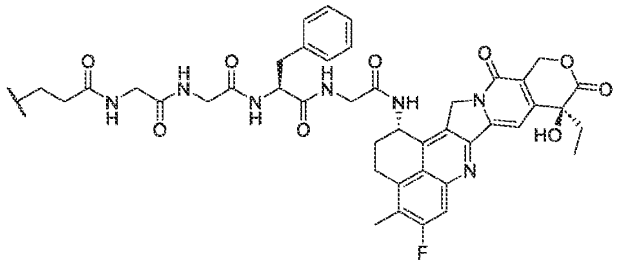
248. The conjugate of any one of Embodiments 151-239, wherein an occurrence of $-L^C-R^P$ is



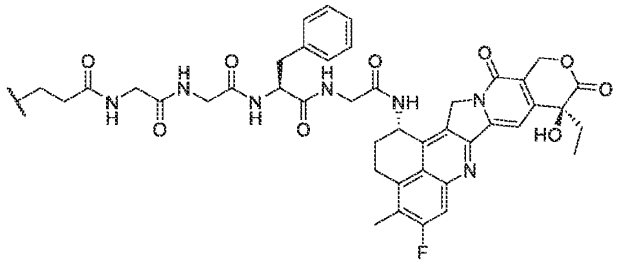
249. The conjugate of any one of Embodiments 151-239, wherein each $-L^C-R^P$ is independently



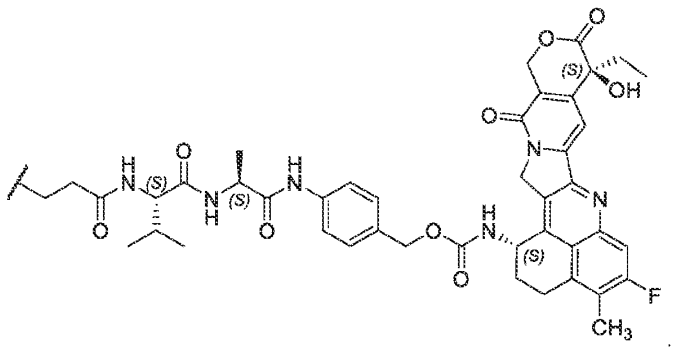
250. The conjugate of any one of Embodiments 151-227, wherein an occurrence of $-L^{ZP}-L^C-R^P$ is or comprises



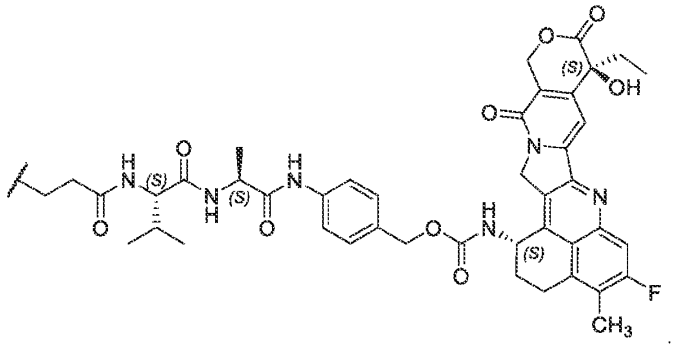
251. The conjugate of any one of Embodiments 151-227, wherein each $-L^{ZP}-L^{C}-R^{P}$ is or comprises



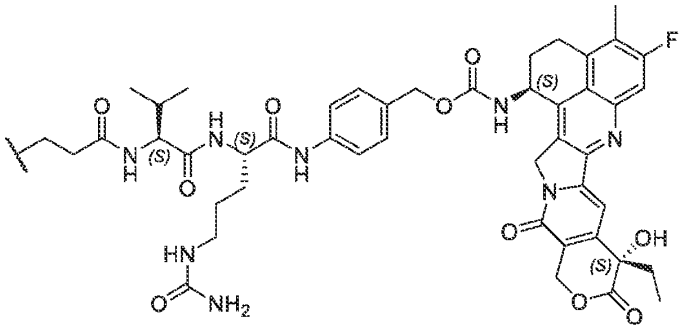
252. The conjugate of any one of Embodiments 151-227, wherein an occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises



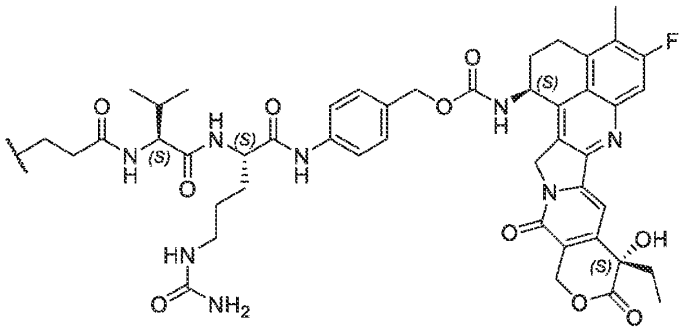
253. The conjugate of any one of Embodiments 151-227, wherein each $-L^{ZP}-L^{C}-R^{P}$ is or comprises



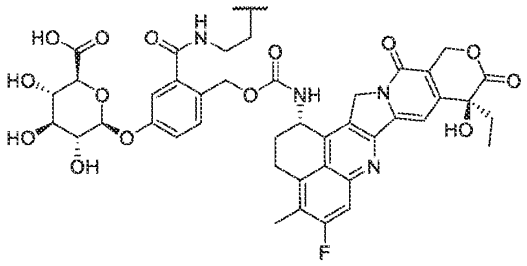
254. The conjugate of any one of Embodiments 151-227, wherein an occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises



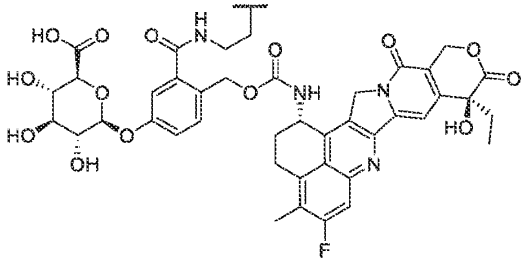
255. The conjugate of any one of Embodiments 151-227, wherein each $-L^{ZP}-L^{C}-R^{P}$ is or comprises



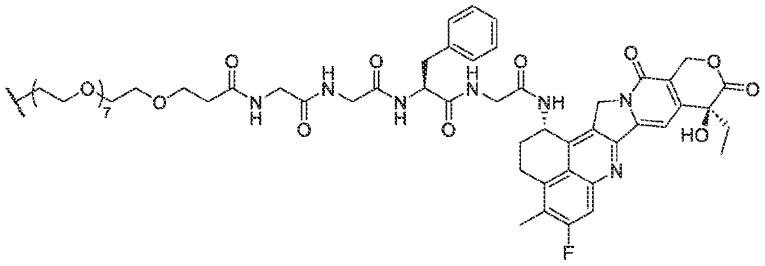
256. The conjugate of any one of Embodiments 151-227, wherein an occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises



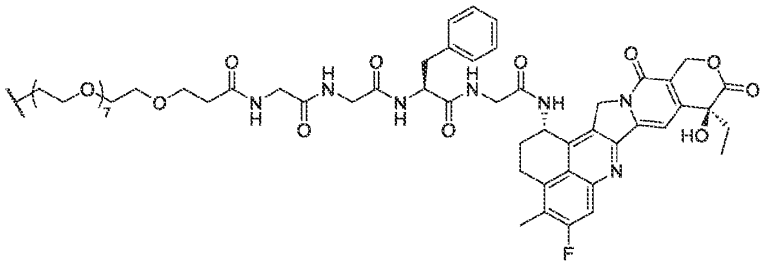
257. The conjugate of any one of Embodiments 151-227, wherein each $-L^{ZP}-L^{C}-R^{P}$ is or comprises



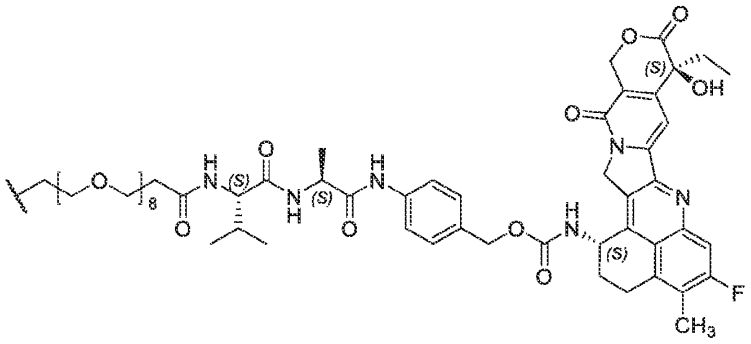
258. The conjugate of any one of Embodiments 151-227, wherein an occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



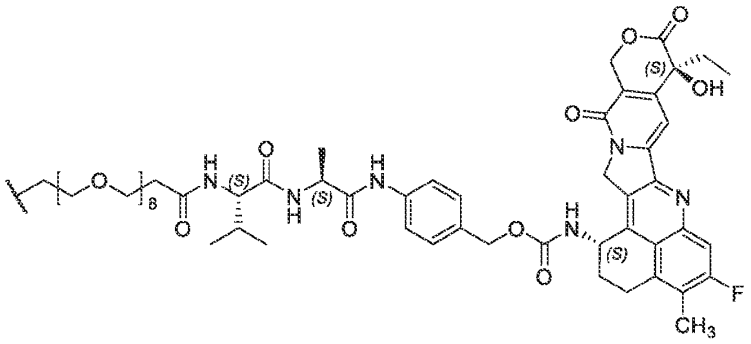
259. The conjugate of any one of Embodiments 151-227, wherein each $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



260. The conjugate of any one of Embodiments 151-227, wherein an occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

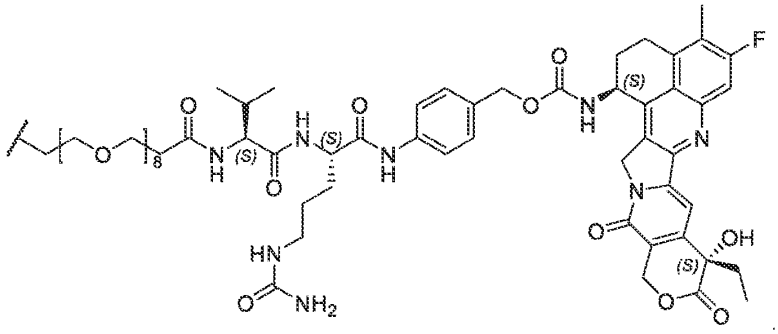


261. The conjugate of any one of Embodiments 151-227, wherein each $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

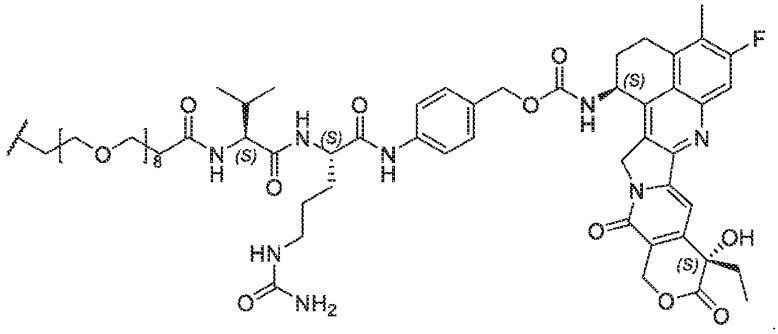


262. The conjugate of any one of Embodiments 151-227, wherein an occurrence of

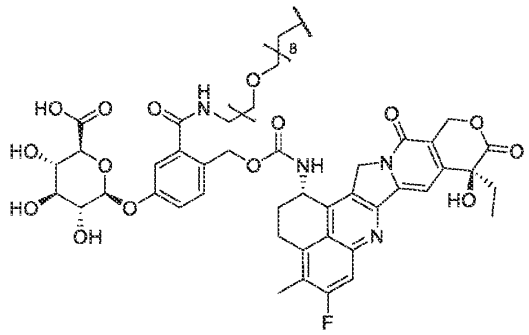
$-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



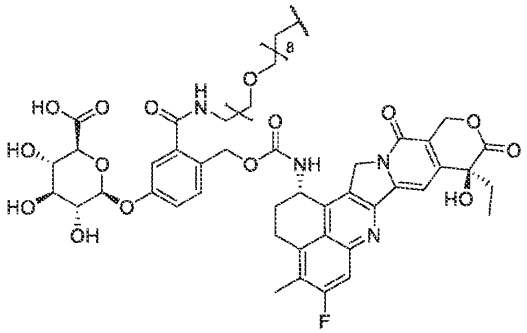
263. The conjugate of any one of Embodiments 151-227, wherein each $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



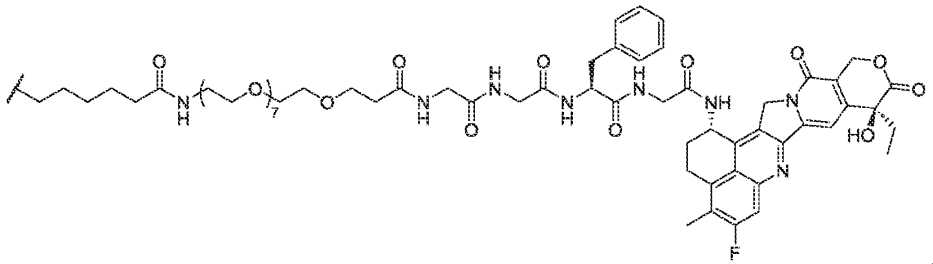
264. The conjugate of any one of Embodiments 151-227, wherein an occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



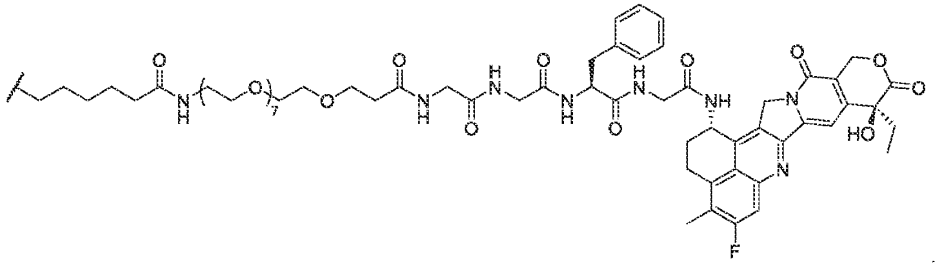
265. The conjugate of any one of Embodiments 151-227, wherein each $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



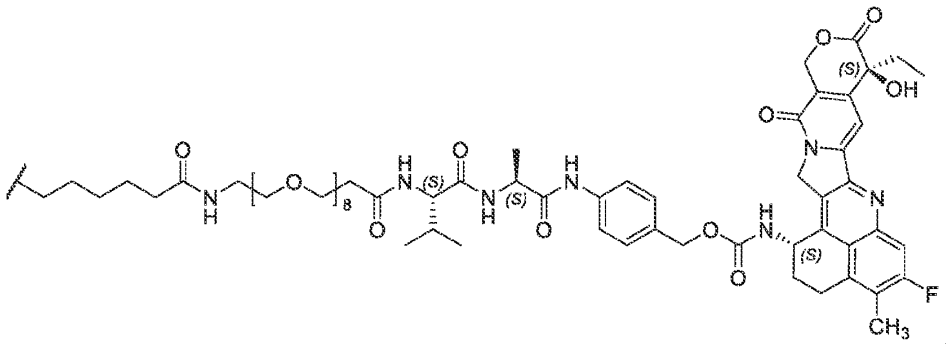
266. The conjugate of any one of Embodiments 178-265, wherein an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



267. The conjugate of any one of Embodiments 178-265, wherein each $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

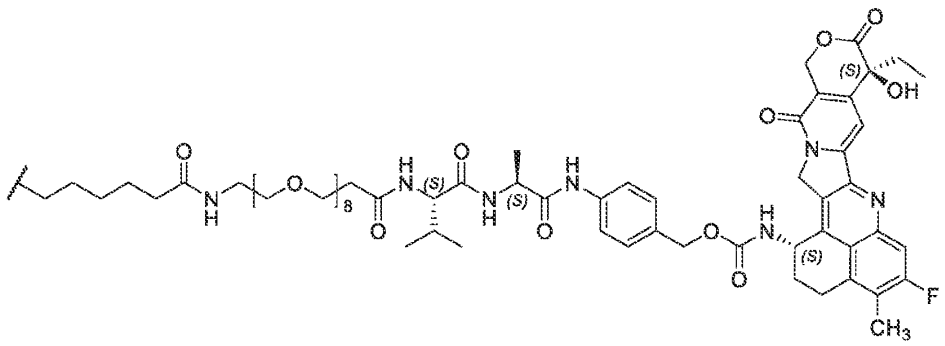


268. The conjugate of any one of Embodiments 178-265, wherein an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

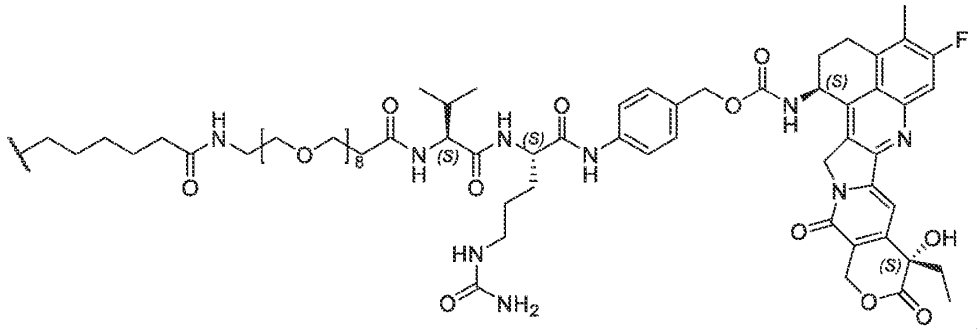


269. The conjugate of any one of Embodiments 178-265, wherein each $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$

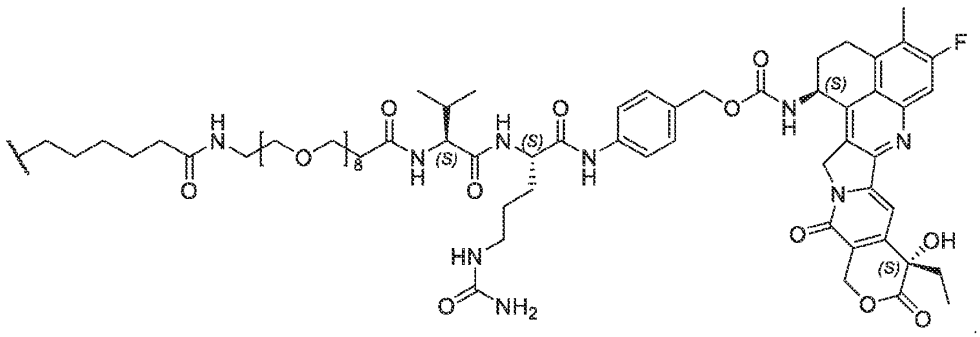
is or comprises



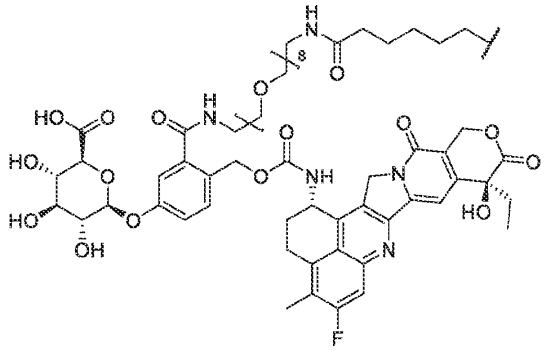
270. The conjugate of any one of Embodiments 178-265, wherein an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



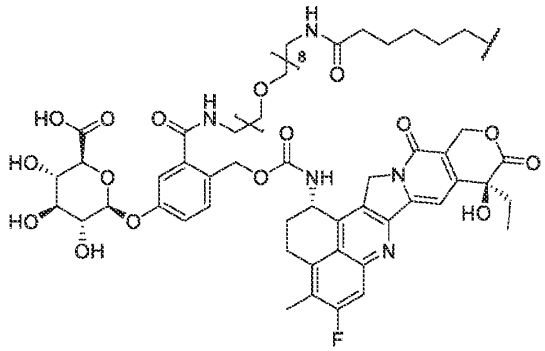
271. The conjugate of any one of Embodiments 178-265, wherein each $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



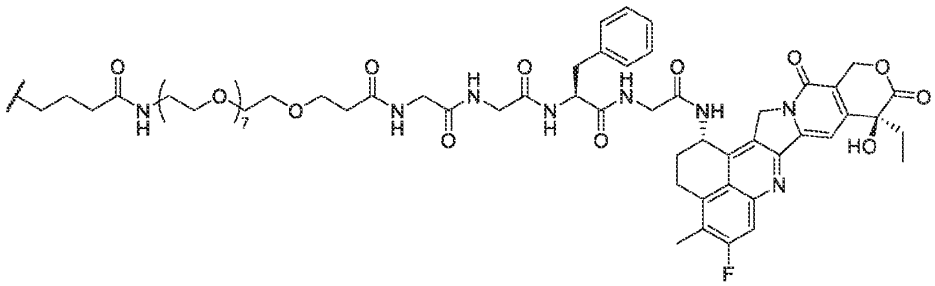
272. The conjugate of any one of Embodiments 178-265, wherein an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



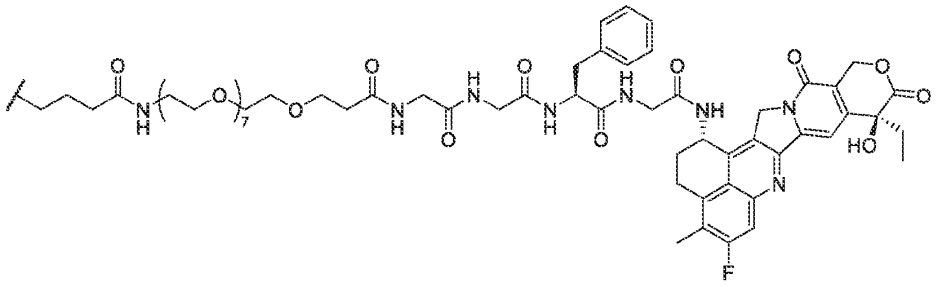
273. The conjugate of any one of Embodiments 178-265, wherein each $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



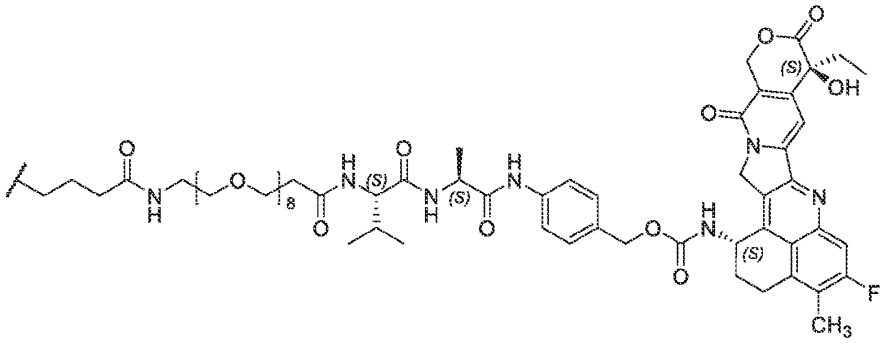
274. The conjugate of any one of Embodiments 178-265, wherein an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



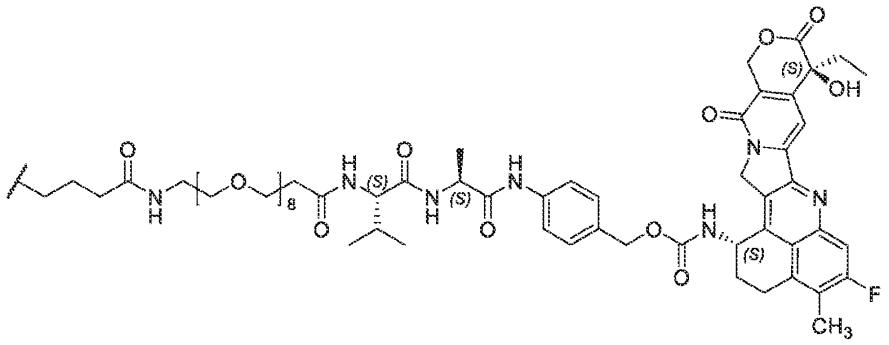
275. The conjugate of any one of Embodiments 178-265, wherein each $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



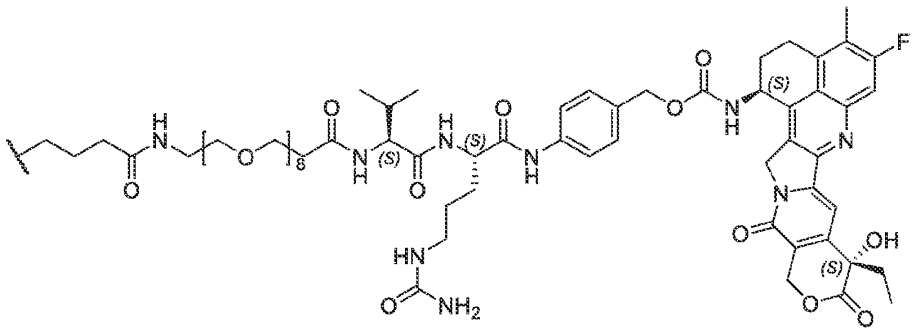
276. The conjugate of any one of Embodiments 178-265, wherein an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



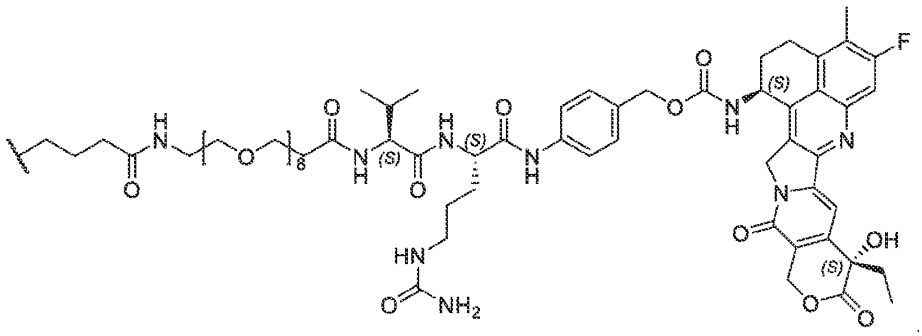
277. The conjugate of any one of Embodiments 178-265, wherein each $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



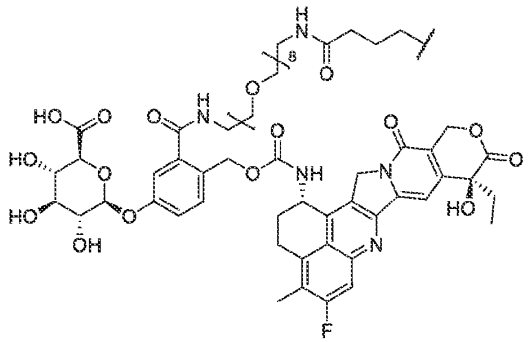
278. The conjugate of any one of Embodiments 178-265, wherein an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



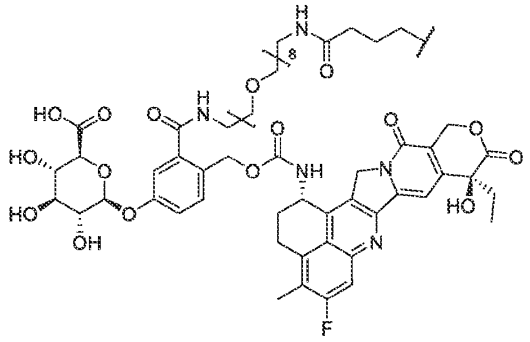
279. The conjugate of any one of Embodiments 178-265, wherein each $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



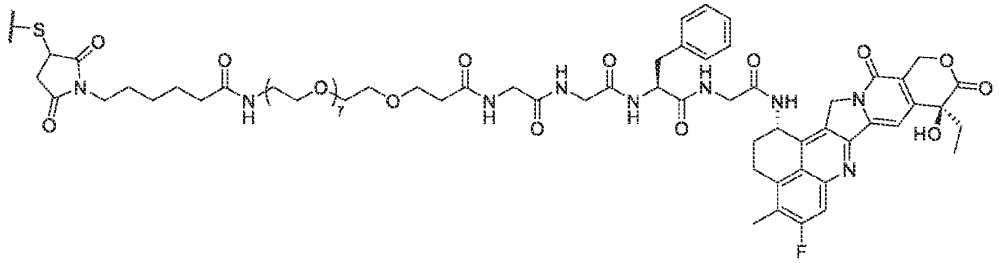
280. The conjugate of any one of Embodiments 178-265, wherein an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



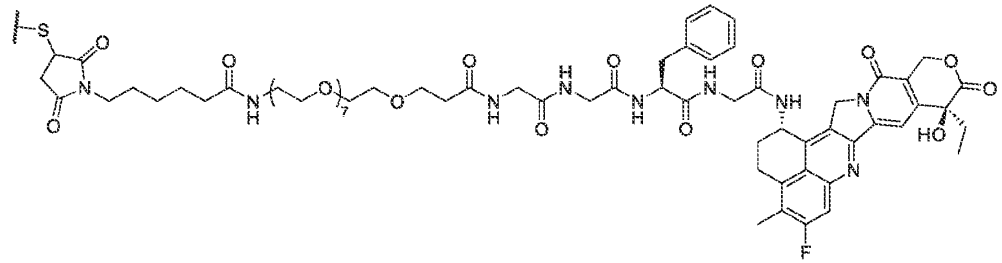
281. The conjugate of any one of Embodiments 178-265, wherein each $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



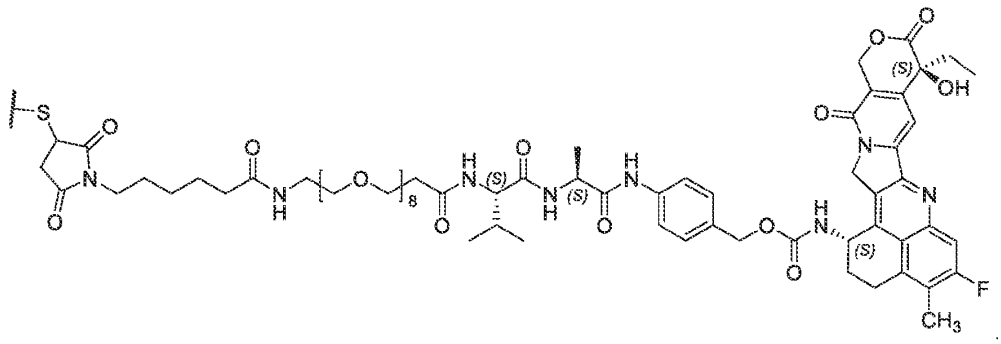
282. The conjugate of any one of Embodiments 178-273, wherein an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



283. The conjugate of any one of Embodiments 178-273, wherein each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

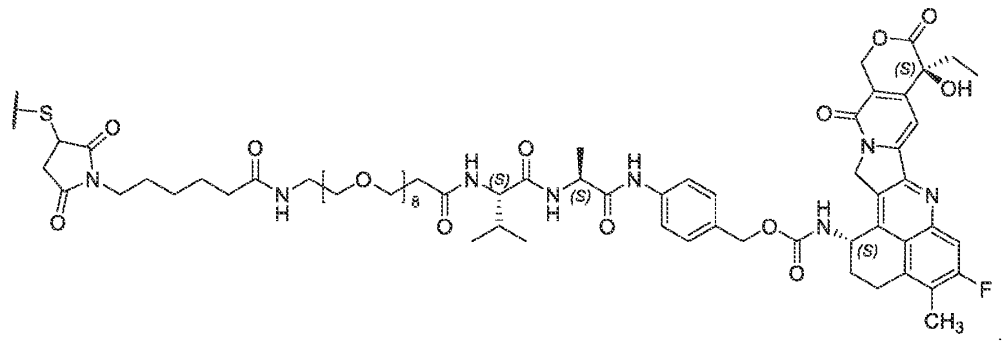


284. The conjugate of any one of Embodiments 178-273, wherein an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

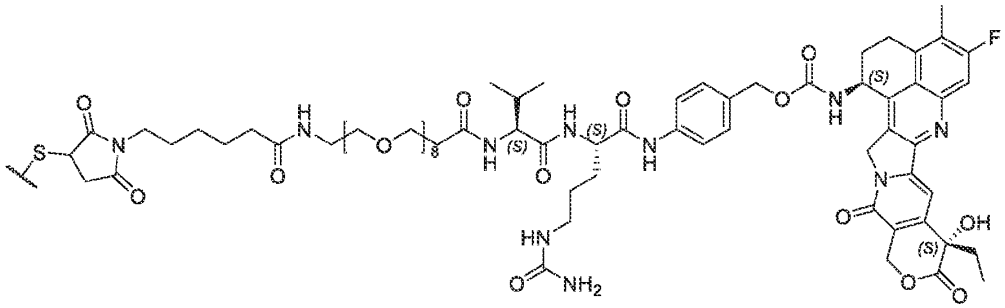


285. The conjugate of any one of Embodiments 178-273, wherein each occurrence of

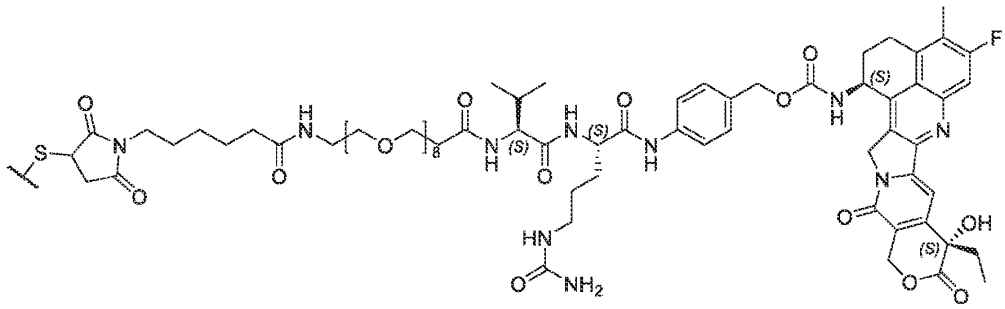
$-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



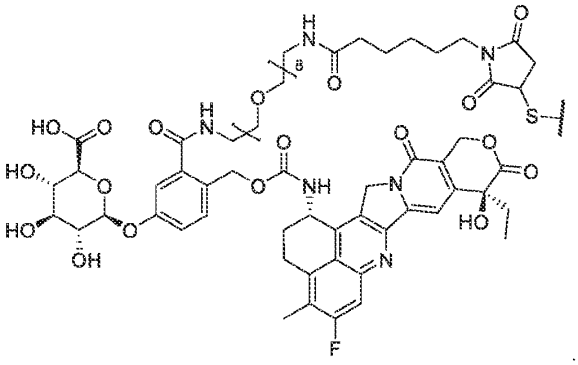
286. The conjugate of any one of Embodiments 178-273, wherein an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



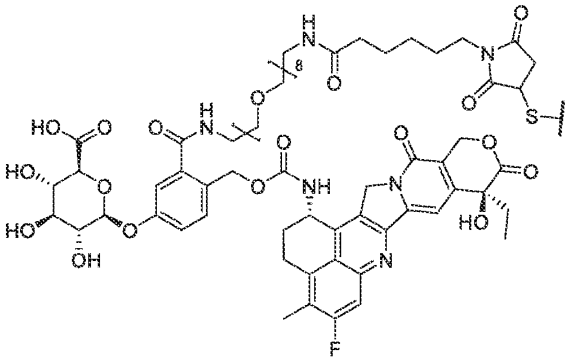
287. The conjugate of any one of Embodiments 178-273, wherein each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



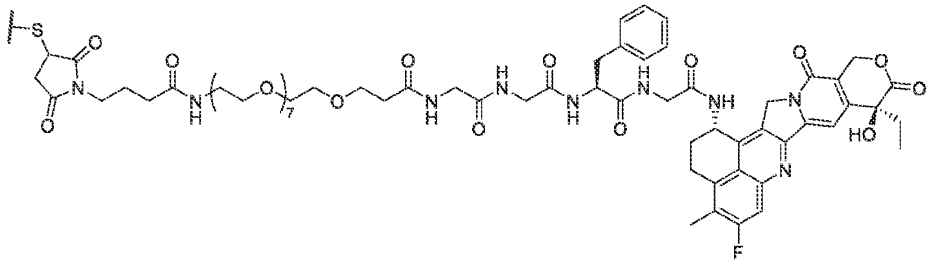
288. The conjugate of any one of Embodiments 178-273, wherein an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



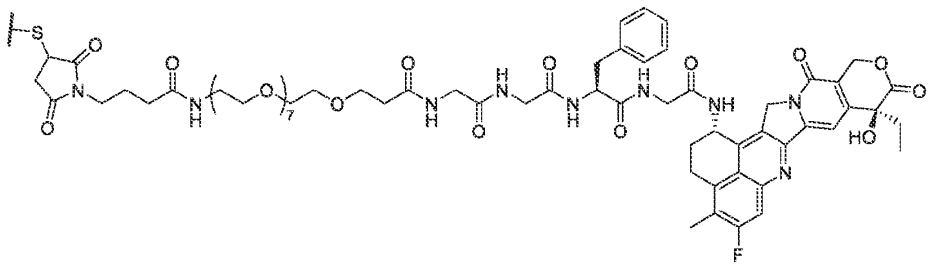
289. The conjugate of any one of Embodiments 178-273, wherein each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



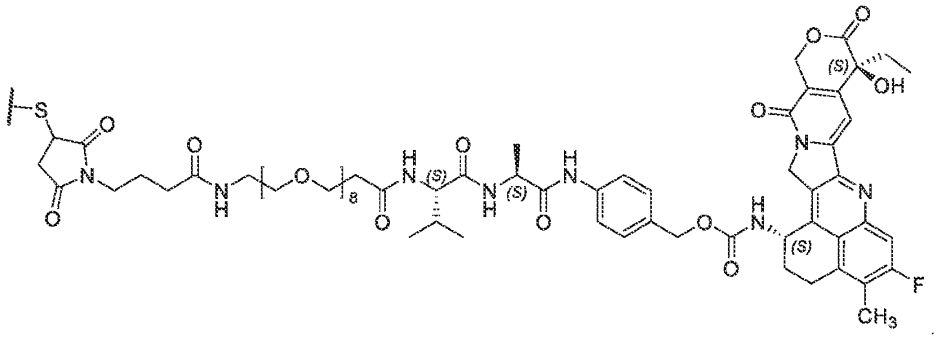
290. The conjugate of any one of Embodiments 178-273, wherein an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



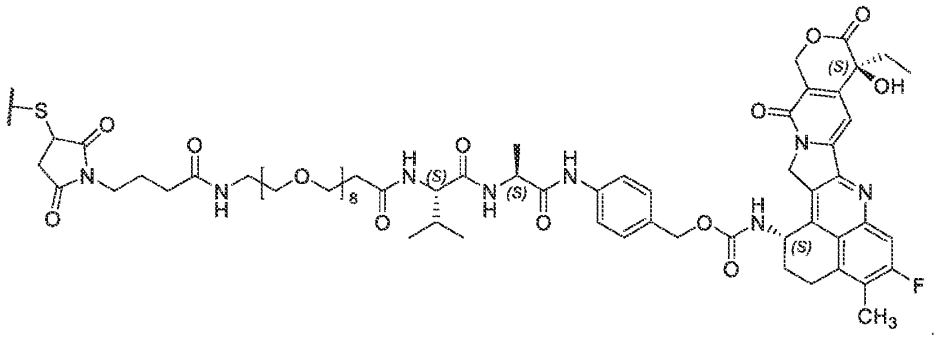
291. The conjugate of any one of Embodiments 178-273, wherein each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



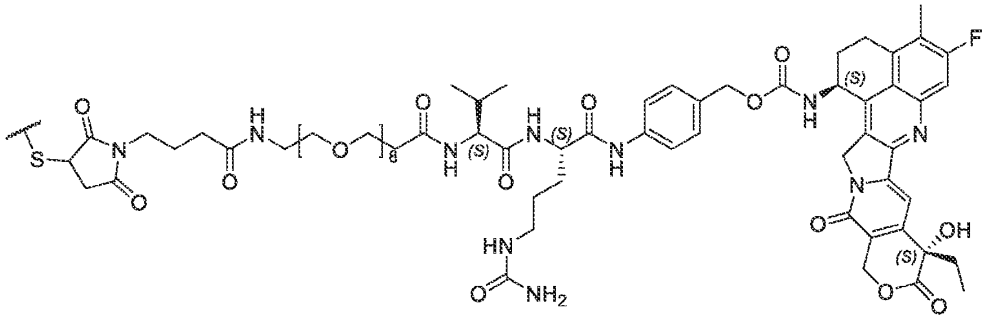
292. The conjugate of any one of Embodiments 178-273, wherein an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



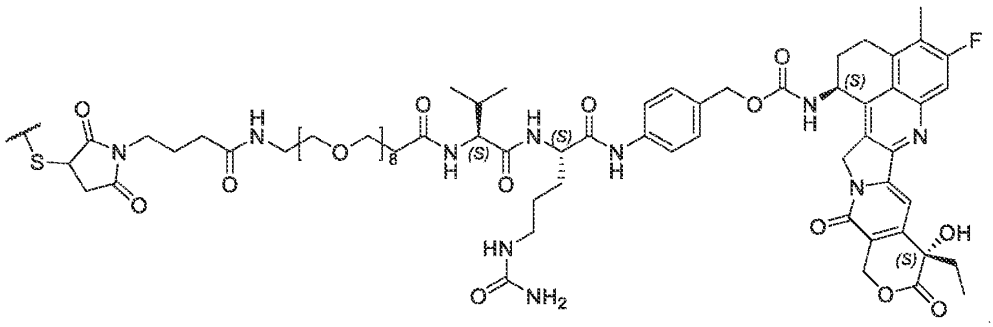
293. The conjugate of any one of Embodiments 178-273, wherein each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



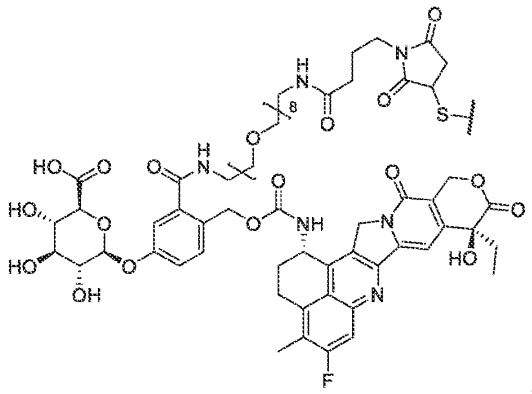
294. The conjugate of any one of Embodiments 178-273, wherein an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



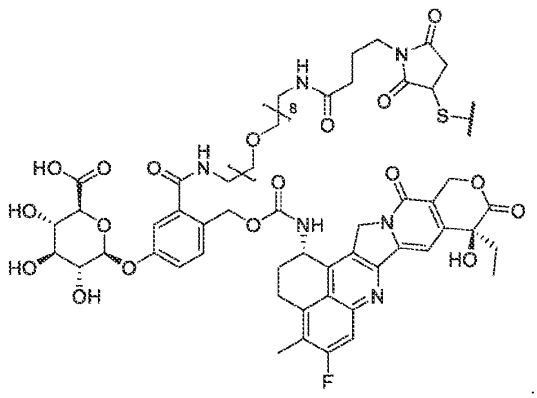
295. The conjugate of any one of Embodiments 178-273, wherein each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



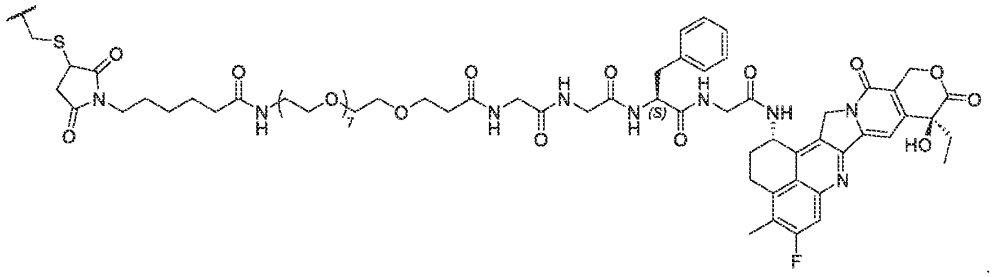
296. The conjugate of any one of Embodiments 178-273, wherein an occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



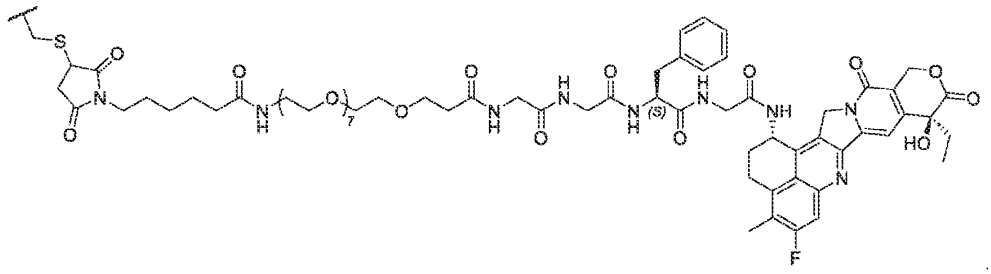
297. The conjugate of any one of Embodiments 178-273, wherein each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



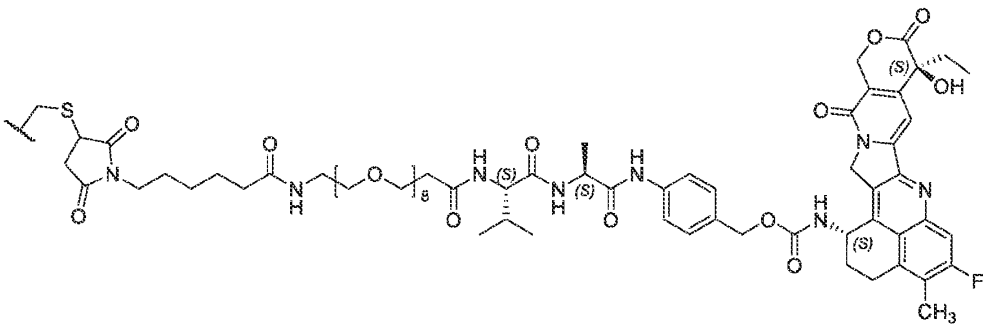
298. The conjugate of any one of Embodiments 178-297, wherein an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



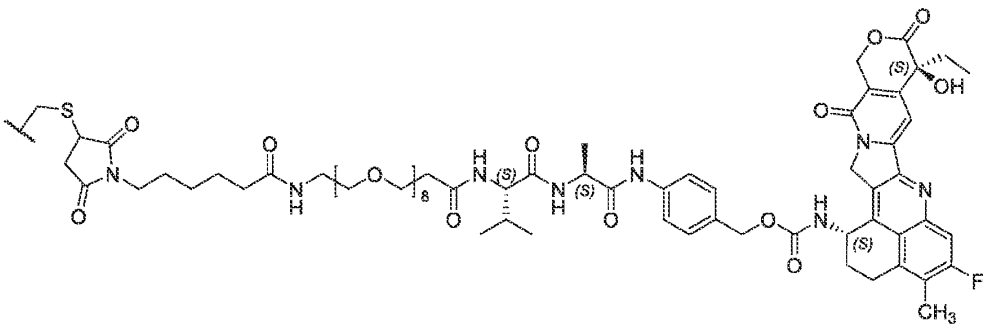
299. The conjugate of any one of Embodiments 178-297, wherein each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



300. The conjugate of any one of Embodiments 178-297, wherein an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

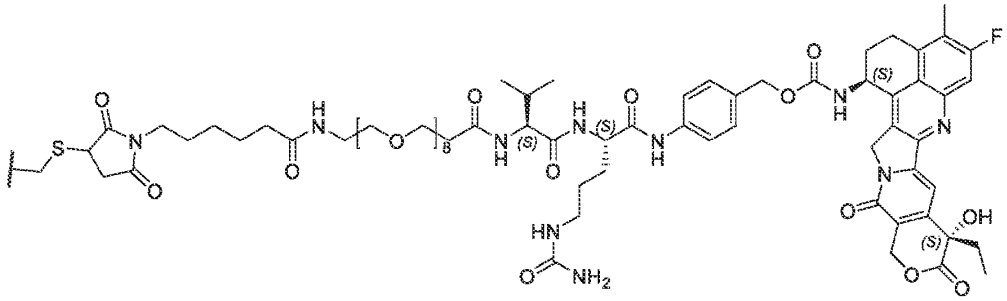


301. The conjugate of any one of Embodiments 178-297, wherein each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

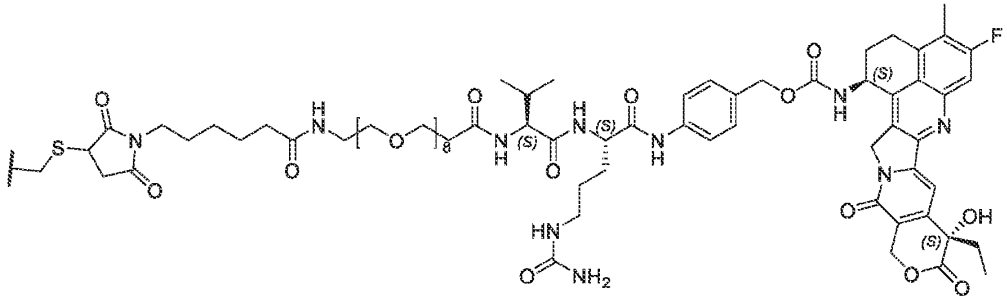


302. The conjugate of any one of Embodiments 178-297, wherein an occurrence of

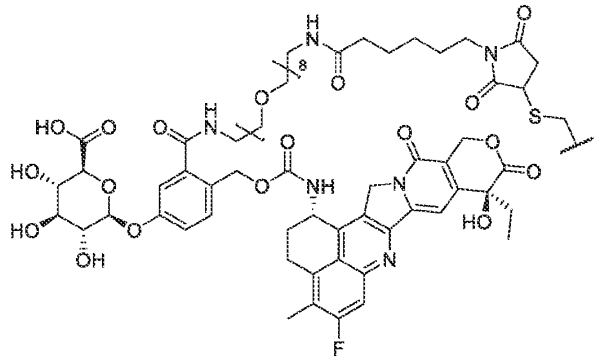
$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



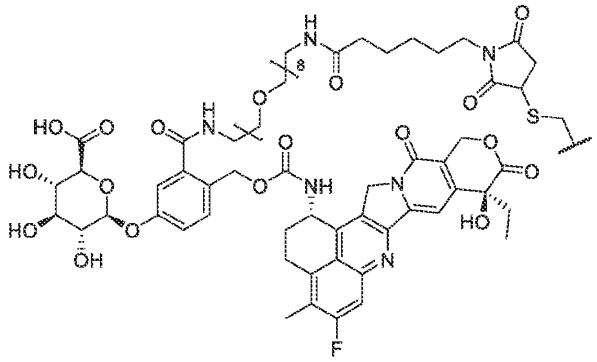
303. The conjugate of any one of Embodiments 178-297, wherein each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



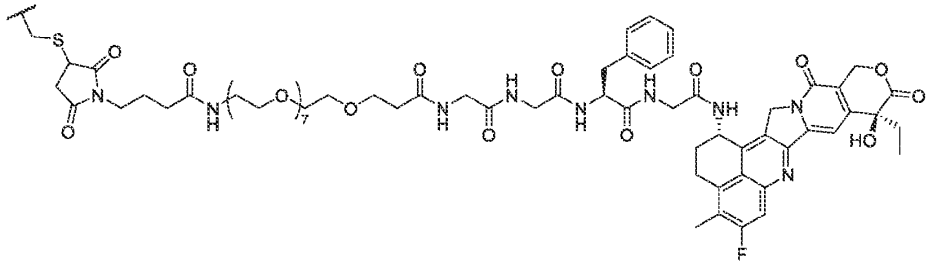
304. The conjugate of any one of Embodiments 178-297, wherein an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



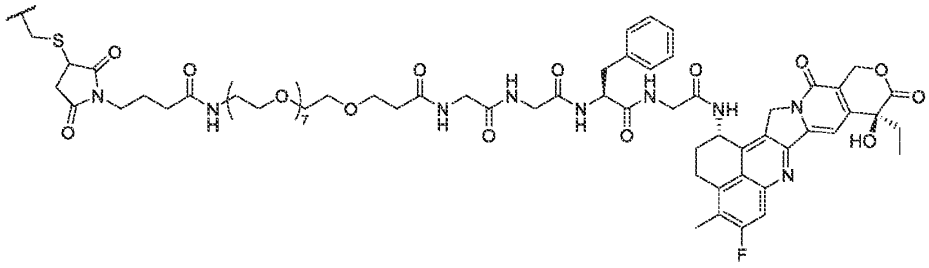
305. The conjugate of any one of Embodiments 178-297, wherein each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



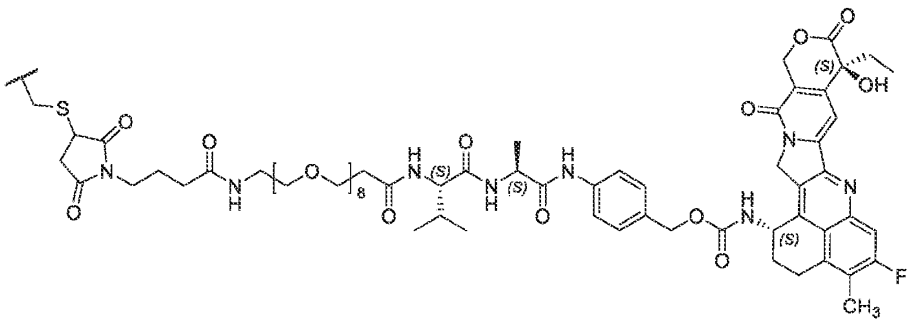
306. The conjugate of any one of Embodiments 178-297, wherein an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



307. The conjugate of any one of Embodiments 178-297, wherein each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

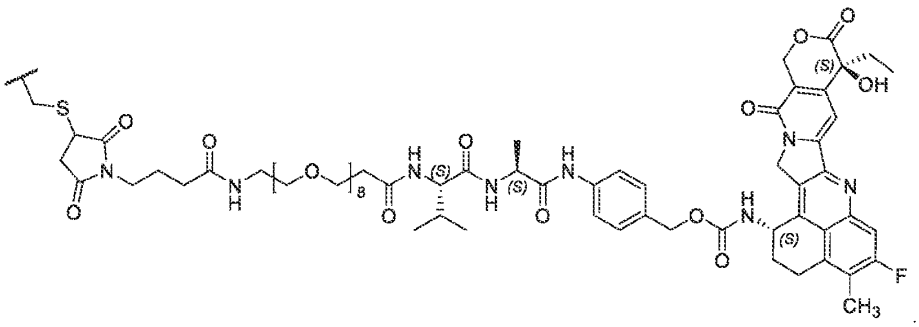


308. The conjugate of any one of Embodiments 178-297, wherein an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

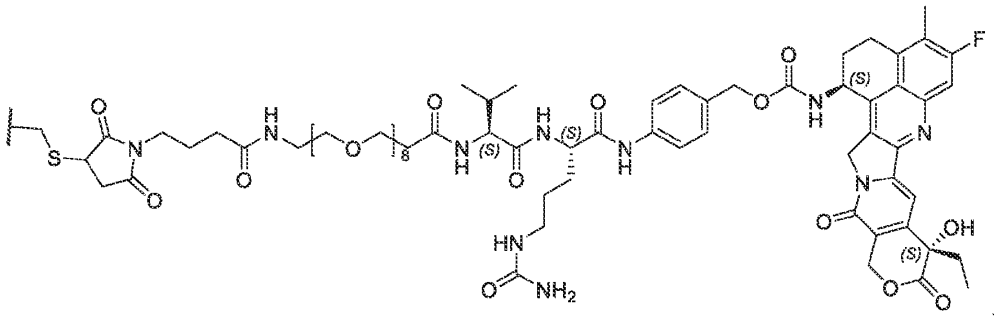


309. The conjugate of any one of Embodiments 178-297, wherein each occurrence of

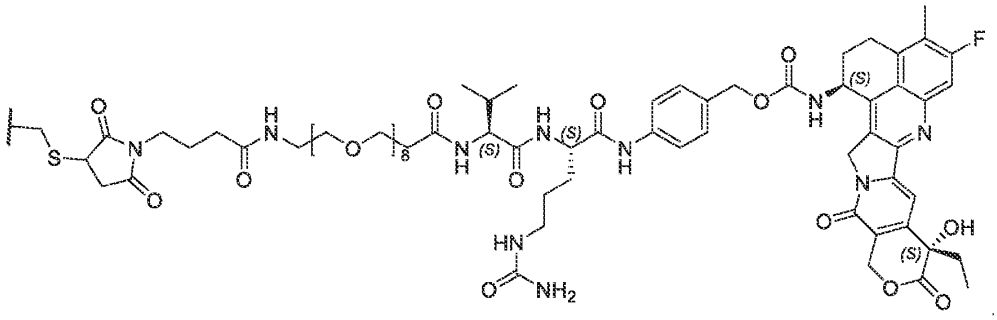
$-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



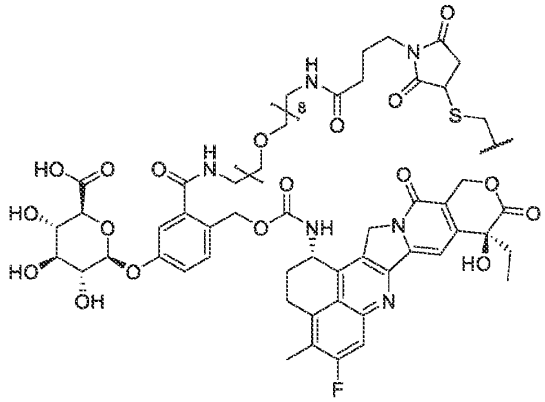
310. The conjugate of any one of Embodiments 178-297, wherein an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



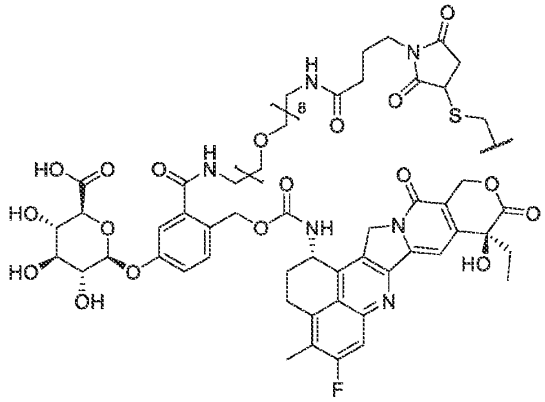
311. The conjugate of any one of Embodiments 178-297, wherein each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



312. The conjugate of any one of Embodiments 178-297, wherein an occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



313. The conjugate of any one of Embodiments 178-297, wherein each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



314. The conjugate of any one of Embodiments 304-313, wherein the target binding moiety comprises an amino acid residue, and $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is bonded to the backbone atom of the amino acid residue.

315. The conjugate of any one of Embodiments 304-313, wherein the target binding moiety comprises an amino acid residue, and $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is bonded to the alpha-carbon of the amino acid residue.

316. The conjugate of any one of Embodiments 151-315, wherein each target binding moiety is independently an antibody or an antigen-binding fragment thereof.

317. The conjugate of any one of Embodiments 151-315, wherein each target binding moiety is independently an antibody.

318. The conjugate of any one of Embodiments 151-315, wherein each target binding moiety is independently a monoclonal antibody.

319. The conjugate of any one of Embodiments 316-318, wherein the target binding moiety is not trastuzumab.

320. The conjugate of any one of Embodiments 316-318, wherein the antibody shares less than about

90%, 95% or 99% sequence identity to trastuzumab.

321. The conjugate of any one of Embodiments 316-318, wherein the target binding moiety is not an anti-HER2 antibody.

322. The conjugate of any one of Embodiments 316-318, wherein the target binding moiety is not an anti-HER2 antibody or an antigen-binding fragment thereof.

323. The conjugate of any one of Embodiments 151-322, wherein t is 1.

324. The conjugate of any one of Embodiments 151-322, wherein t is about 1-10.

325. The conjugate of any one of Embodiments 151-322, wherein t is about 2-8.

326. The conjugate of any one of Embodiments 151-322, wherein t is about 4-8.

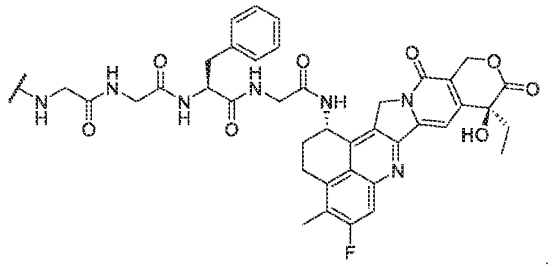
327. The conjugate of any one of Embodiments 151-322, wherein t is about 2.

328. The conjugate of any one of Embodiments 151-322, wherein t is about 4.

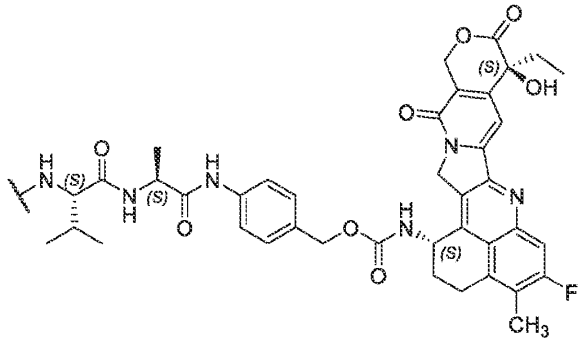
329. The conjugate of any one of Embodiments 151-322, wherein t is about 6.

330. The conjugate of any one of Embodiments 151-322, wherein t is about 8.

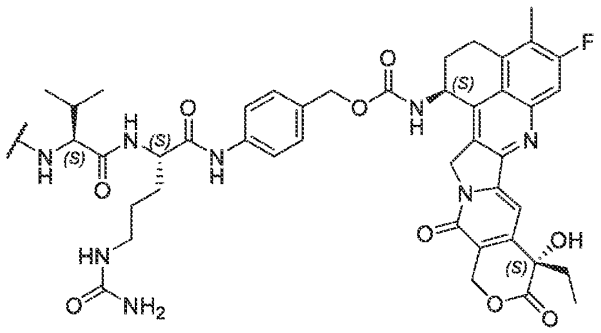
331. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



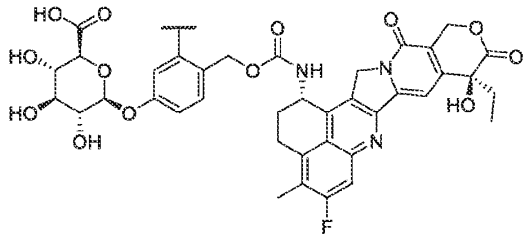
332. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



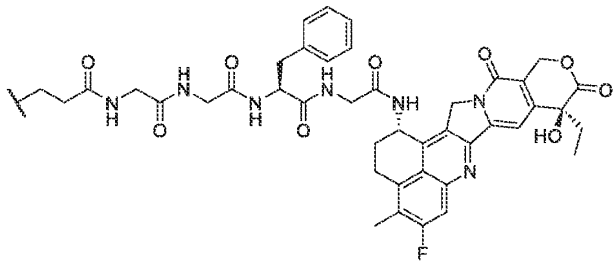
333. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



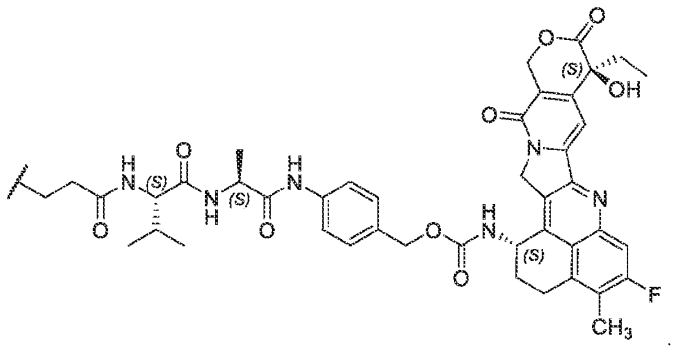
334. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



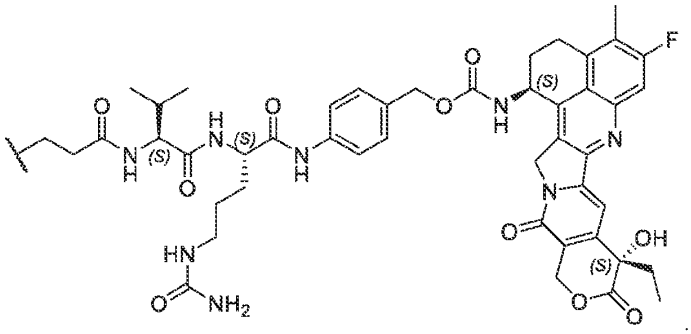
335. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



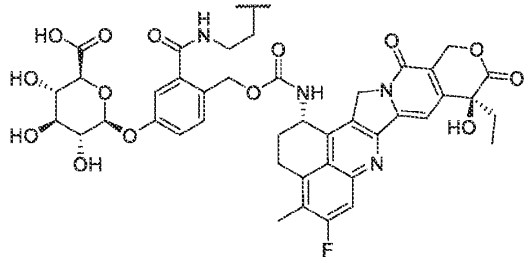
336. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



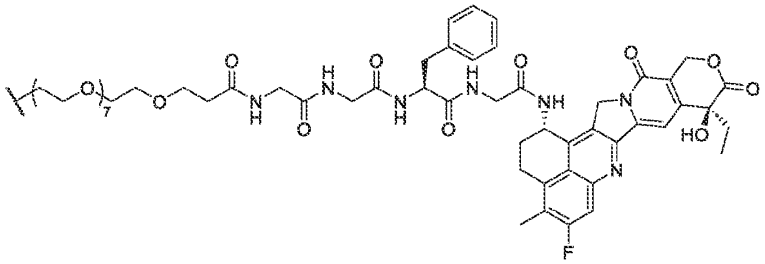
337. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



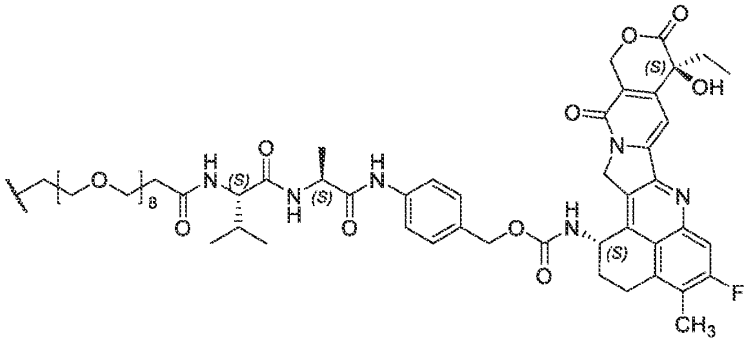
338. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



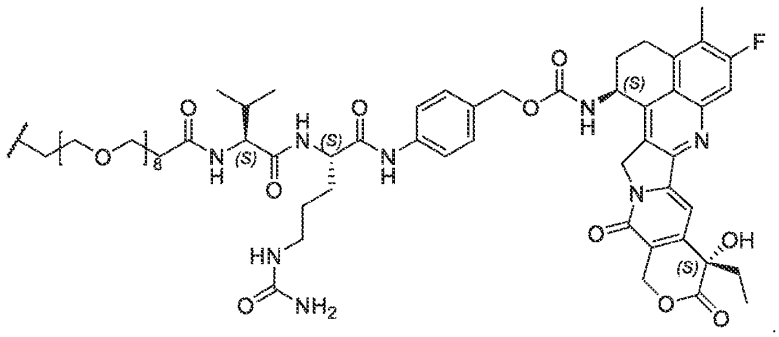
339. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



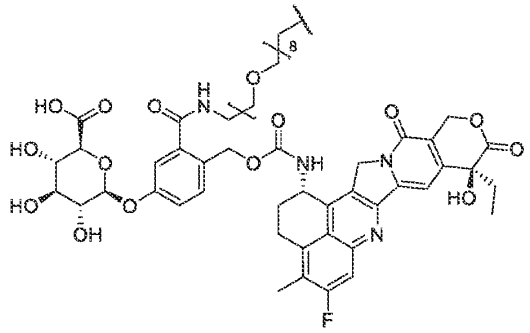
340. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



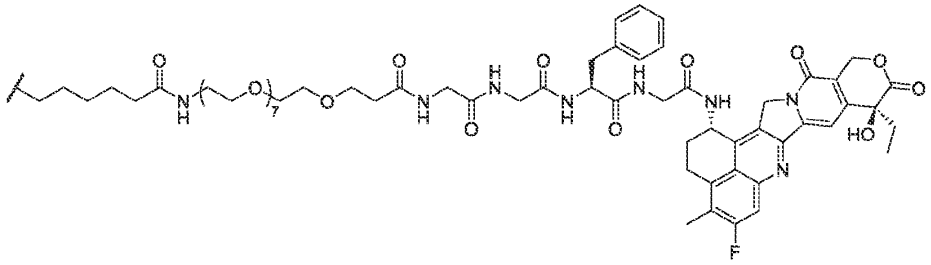
341. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



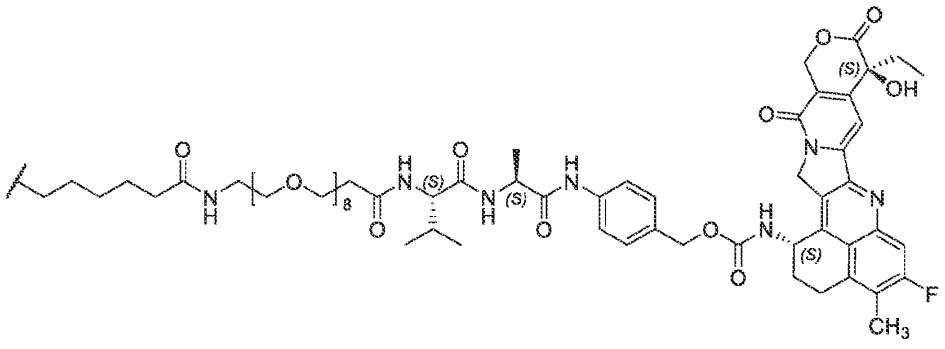
342. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



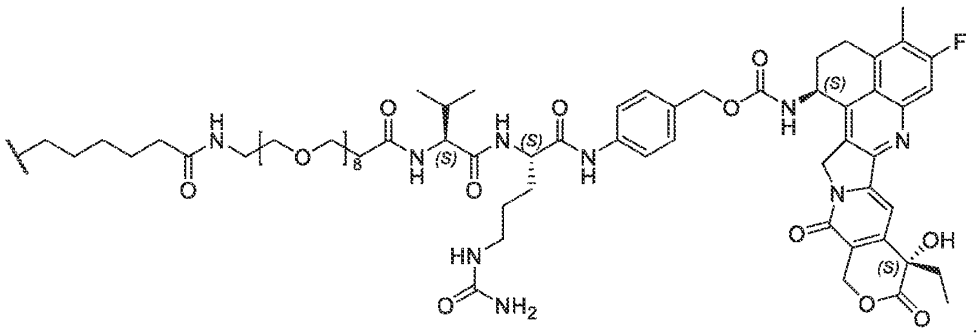
343. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



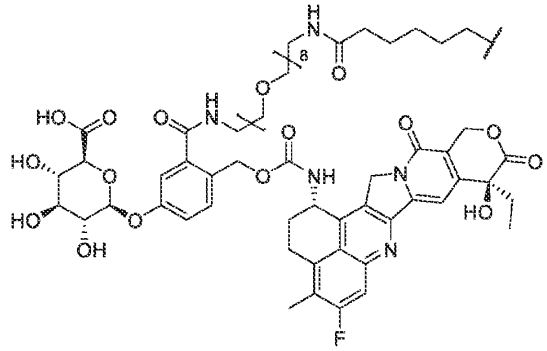
344. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



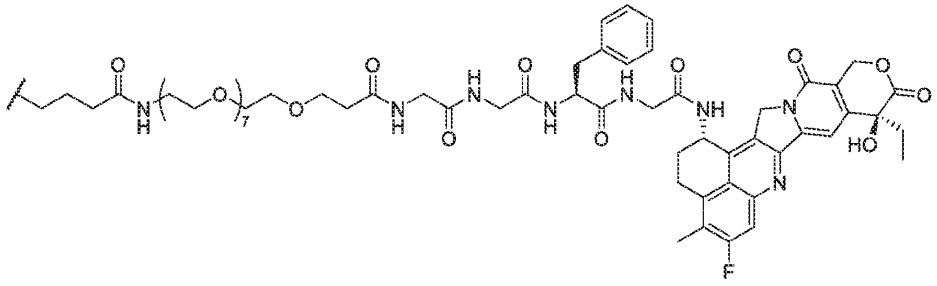
345. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



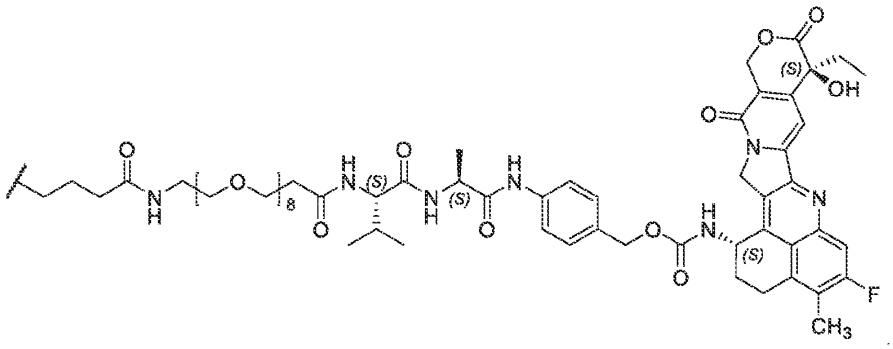
346. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



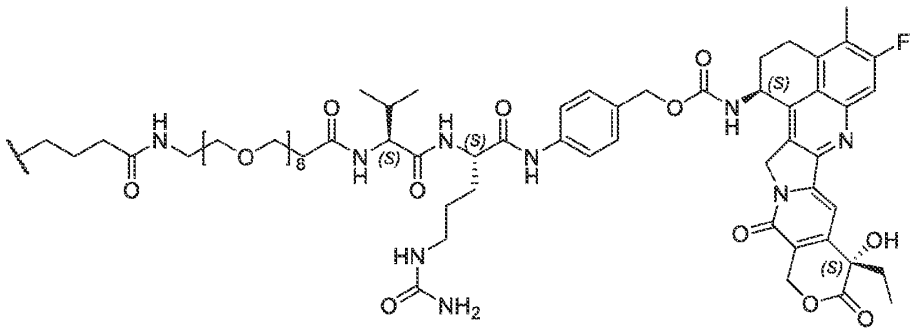
347. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



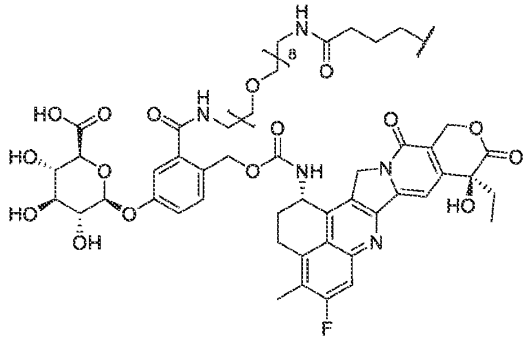
348. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



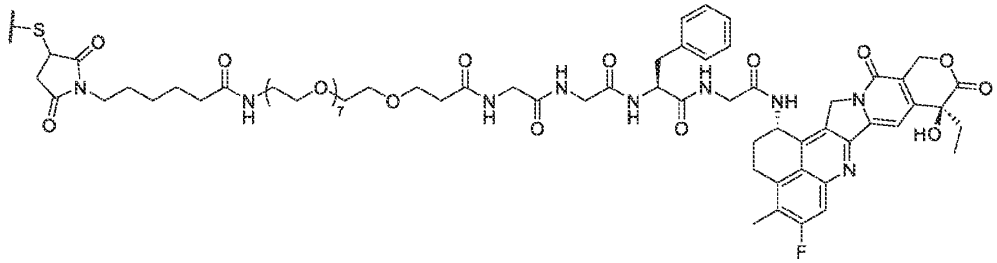
349. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



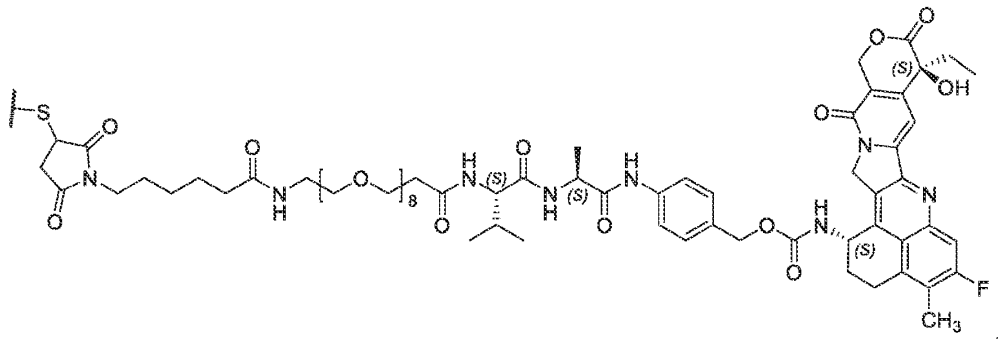
350. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



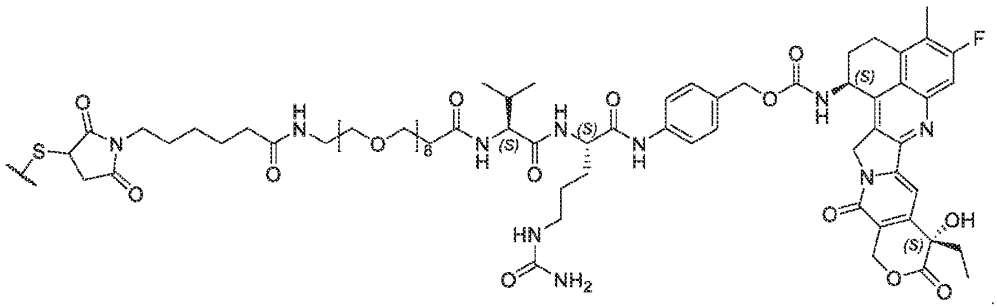
351. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



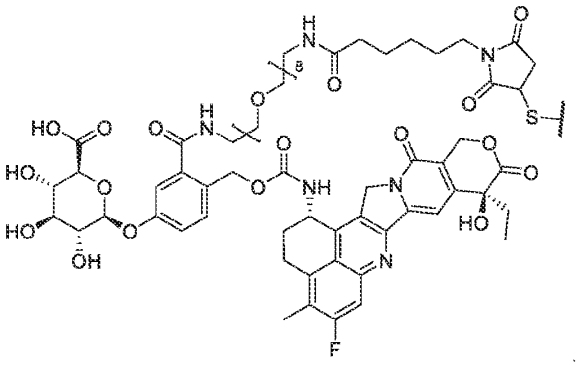
352. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



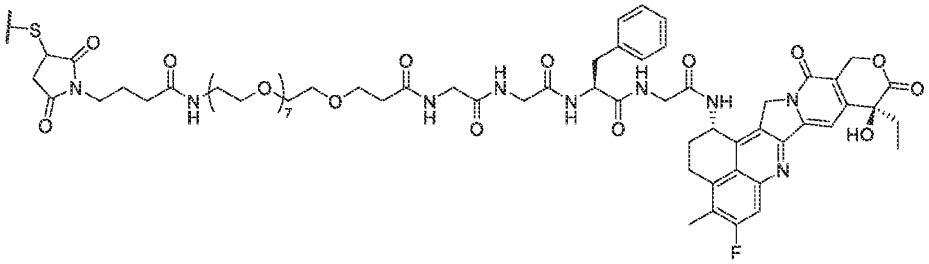
353. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



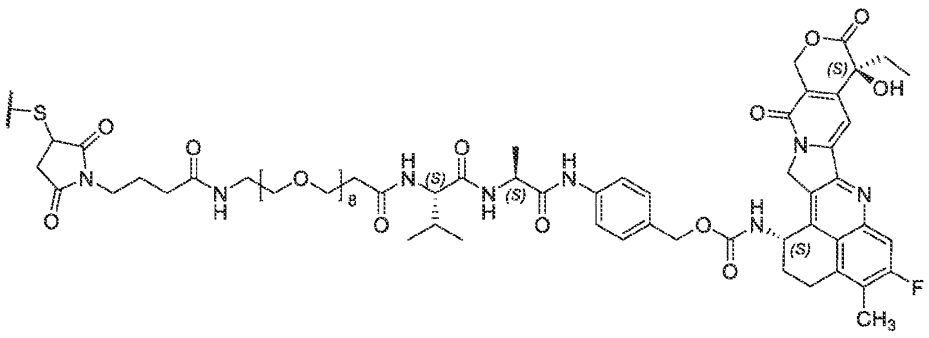
354. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



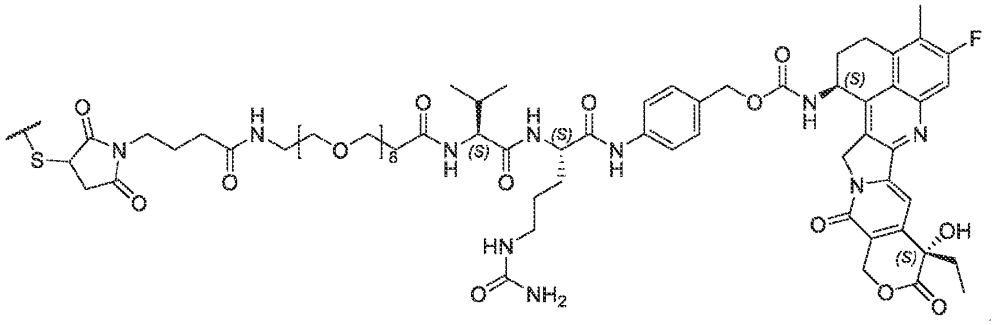
355. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



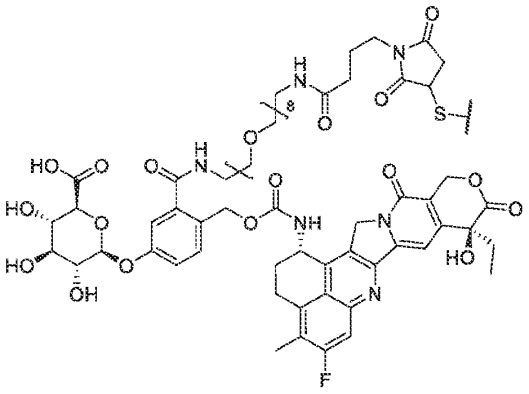
356. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



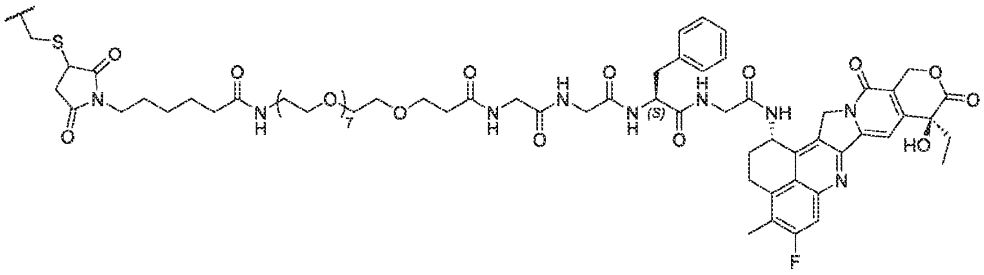
357. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



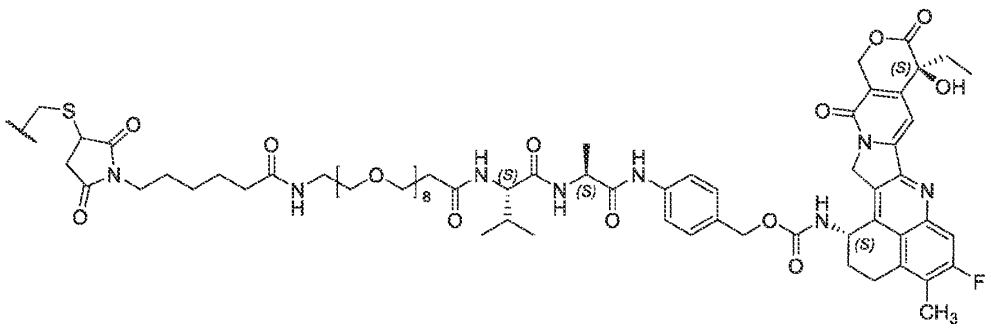
358. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



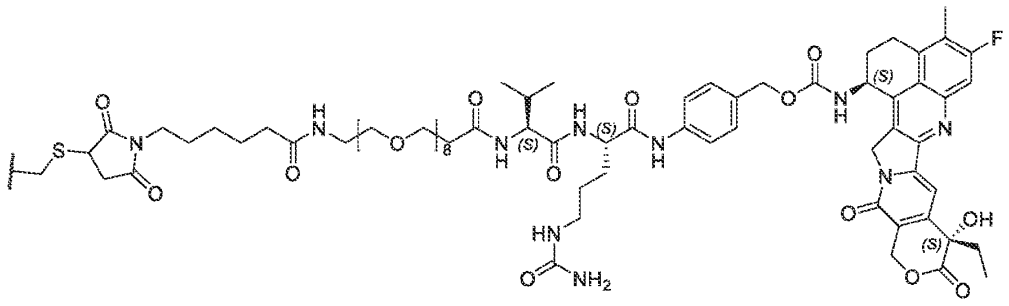
359. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



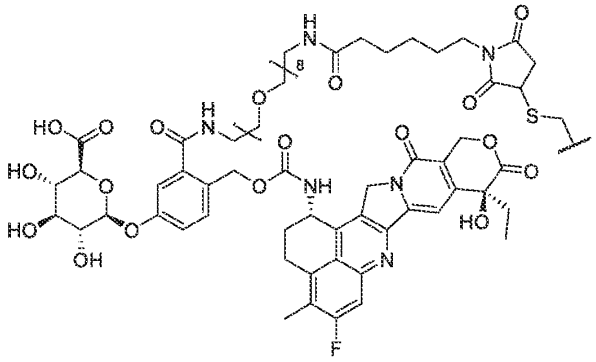
360. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



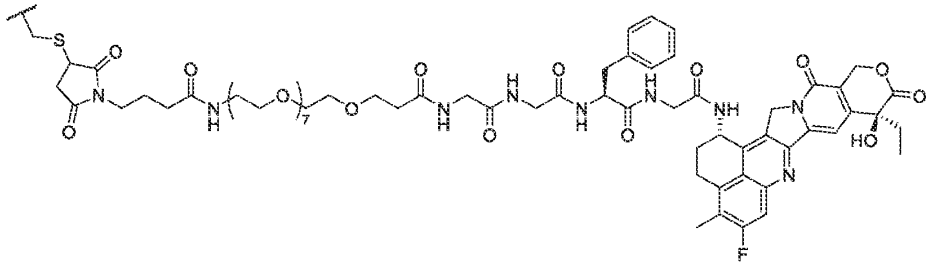
361. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



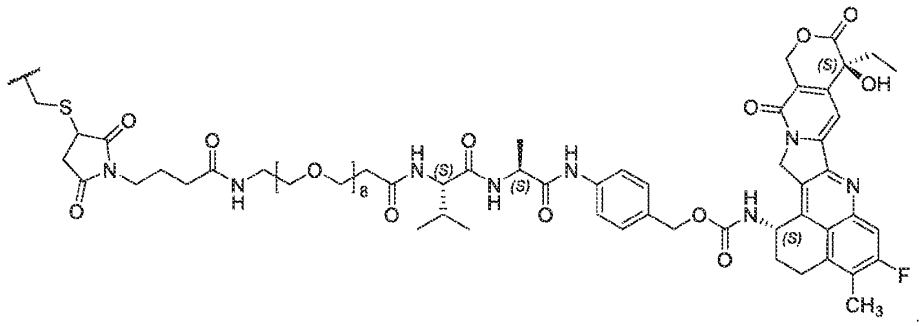
362. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



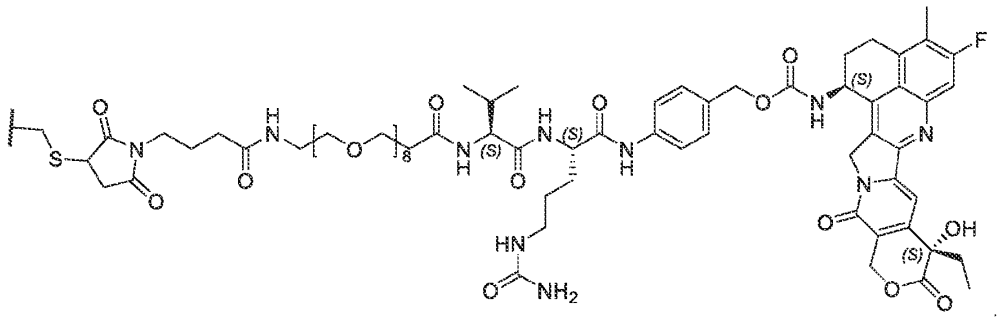
363. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



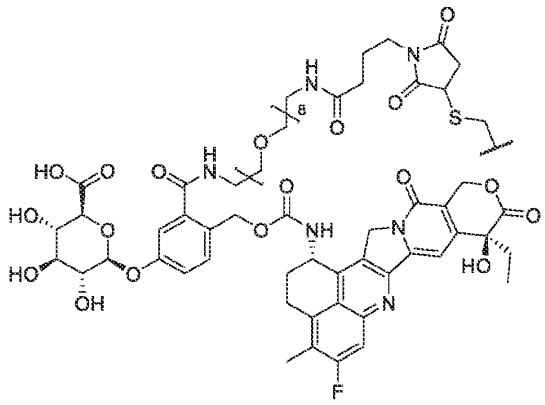
364. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



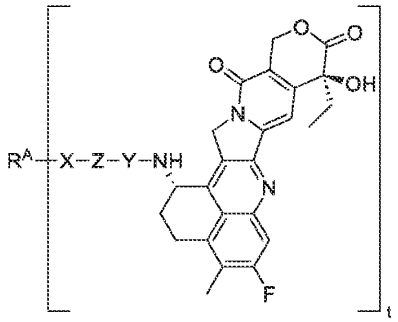
365. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



366. The conjugate of any one of Embodiments 1-15 and 20-30, comprising



367. The conjugate of Embodiment 1, wherein the conjugate has a structure of formula I-B:



I-B

or a pharmaceutically acceptable salt thereof, wherein:

R^A is a target binding moiety;

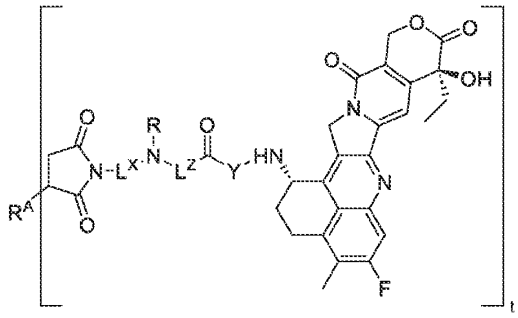
t is an integer between 1 and 16;

X is a moiety that results from the reaction of the unbound linker and a group present on R^A;

Y is $-L^C-$; and

Z is or comprises $-(CH_2CH_2O)n-$ wherein n is 0-24.

368. The conjugate of Embodiment 1, wherein the conjugate has a structure of formula II-B:



II-B

or a pharmaceutically acceptable salt thereof, wherein:

R^A is a target binding moiety;

t is an integer between 1 and 16;

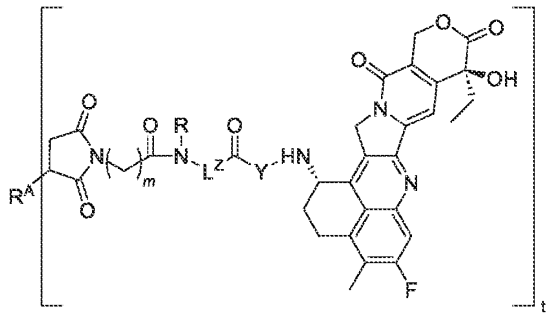
L^X is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^X are optionally and independently replaced by a group selected from -O-, -N(R)-, -C(O)-, -S-, -S(O)-, -$S(O)_2$-, or a 3-12 membered saturated, partially unsaturated, or aromatic monocyclic or bicyclic ring comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Y is $-L^C-$;

L^Z is or comprises $-(CH_2CH_2O)n-$ wherein n is 0-24; and

each R is independently hydrogen or an optionally substituted C_{1-6} aliphatic.

369. The conjugate of Embodiment 1, wherein the conjugate has a structure of formula III-B:



III-B

or a pharmaceutically acceptable salt thereof, wherein:

R^A is a target binding moiety;

t is an integer between 1 and 16;

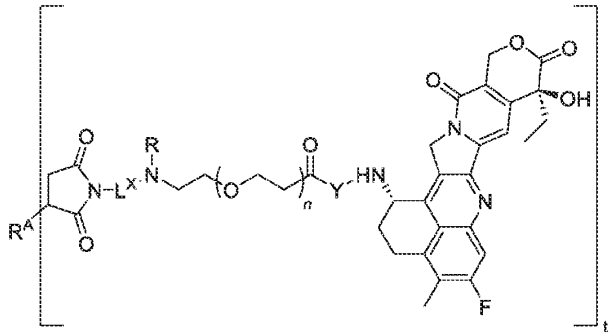
m is an integer between 1-12;

Y is $-L^C-$;

L^Z is or comprises $-(CH_2CH_2O)n-$ wherein n is 0-24; and

each R is independently hydrogen or an optionally substituted C_{1-6} aliphatic.

370. The conjugate of Embodiment 1, wherein the conjugate has a structure of formula V-B:



V-B

or a pharmaceutically acceptable salt thereof, wherein:

R^A is a target binding moiety;

t is an integer between 1 and 16;

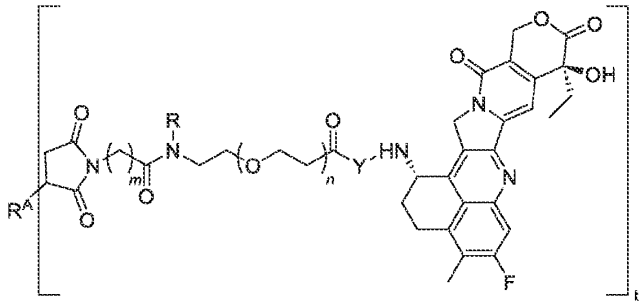
L^X is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^X are optionally and independently replaced by a group selected from -O-, -N(R)-, -C(O)-, -S-, -S(O)-, -$S(O)_2$-, or a 3-12 membered saturated, partially unsaturated, or aromatic monocyclic or bicyclic ring comprising 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur;

Y is $-L^C-$;

n is an integer between 1 and 24; and

each R is independently hydrogen or an optionally substituted C_{1-6} aliphatic.

371. The conjugate of Embodiment 1, wherein the conjugate has a structure of formula VI-B:



VI-B

or a pharmaceutically acceptable salt thereof, wherein:

R^A is a target binding moiety;

t is an integer between 1 and 16;

m is an integer between 1-12;

Y is $-L^C-$;

n is an integer between 1 and 24; and

each R is independently hydrogen or an optionally substituted C_{1-6} aliphatic.

372. The conjugate of Embodiment 368 or 370, wherein L^X is an optionally substituted bivalent

straight or branched C_{1-20} aliphatic wherein 1-4 methylene units of L^X are optionally and independently replaced by a group selected from –O–, –N(R')–, and –C(O)–.

373. The conjugate of Embodiment 368 or 370, wherein L^X is an optionally substituted bivalent straight or branched C_{1-12} aliphatic wherein 1-4 methylene units of L^X are optionally and independently replaced by a group selected from –O–, –N(R')–, and –C(O)–.

374. The conjugate of Embodiment 368 or 370, wherein L^X is an optionally substituted bivalent straight or branched C_{1-6} aliphatic wherein 1-4 methylene units of L^X are optionally and independently replaced by a group selected from –O–, –N(R')–, and –C(O)–.

375. The conjugate of any one of Embodiments 369 and 371, wherein m is an integer between 1-8.

376. The conjugate of any one of Embodiments 369 and 371, wherein m is an integer between 1-6.

377. The conjugate of any one of Embodiments 368 and 369, wherein L^Z has a structure **-L^{Z1}-L^{Z2}-L^{Z3}-, wherein:

** represents the point of attachment to the adjacent nitrogen atom;

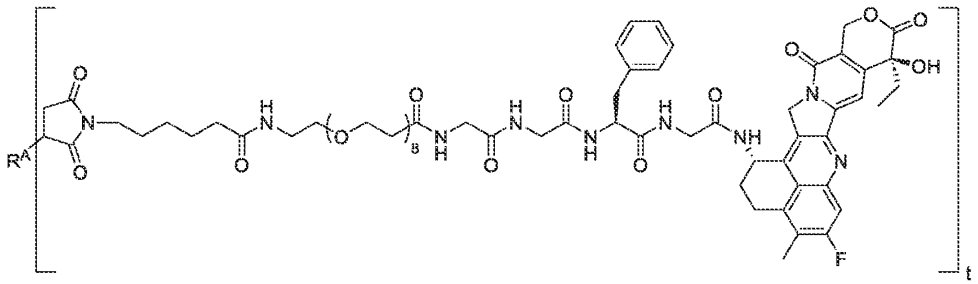
each L^{Z1} and L^{Z3} is independently a covalent bond or optionally substituted bivalent C_{1-4} aliphatic; and

L^{Z2} is –$(CH_2CH_2O)n$– wherein n is 0-24.

378. The conjugate of any one of Embodiment 366-377, wherein n is 1-24.

379. The conjugate of Embodiment 377, wherein n is between 2-8.

380. The conjugate of Embodiment 1, having a structure of formula X-C:



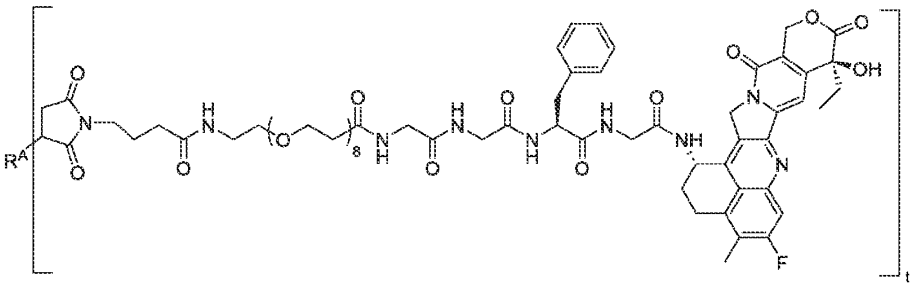
X-C

or a pharmaceutically acceptable salt thereof, wherein:

R^A is a target binding moiety; and

t is an integer between 1 and 16.

381. The conjugate of Embodiment 1, having a structure of formula X-C':



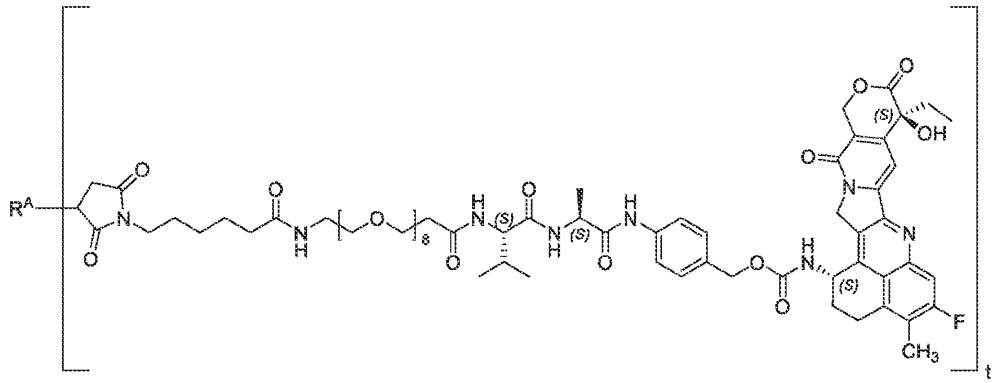
X-C'

or a pharmaceutically acceptable salt thereof, wherein:

R^A is a target binding moiety; and

t is an integer between 1 and 16.

382. The conjugate of Embodiment 1, having a structure of formula X-G':



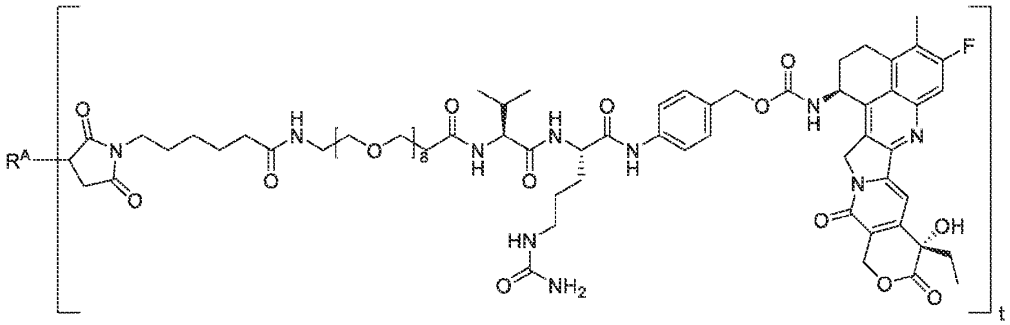
X-G'

or a pharmaceutically acceptable salt thereof, wherein:

R^A is a target binding moiety; and

t is an integer between 1 and 16.

383. The conjugate of Embodiment 1, having a structure of formula X-H':



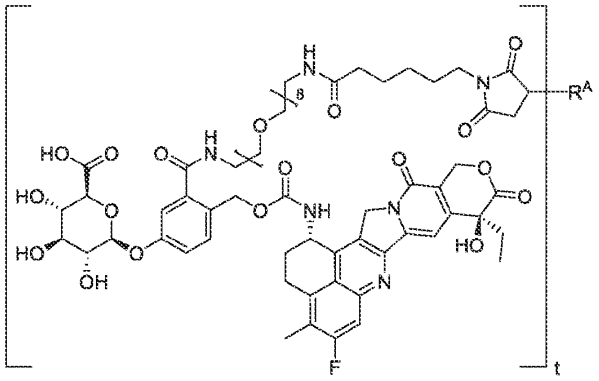
X-H'

or a pharmaceutically acceptable salt thereof, wherein:

R^A is a target binding moiety; and

t is an integer between 1 and 16.

384. The conjugate of Embodiment 1, having a structure of formula X-I':



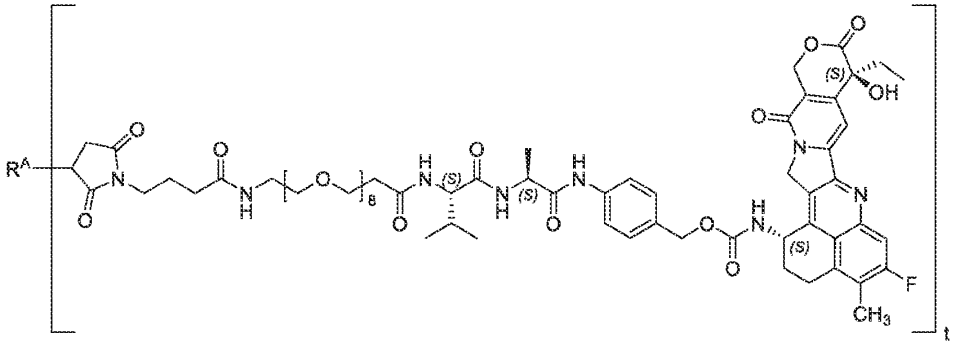
X-I'

or a pharmaceutically acceptable salt thereof, wherein:

R^A is a target binding moiety; and

t is an integer between 1 and 16.

385. The conjugate of Embodiment 1, having a structure of formula X-J':



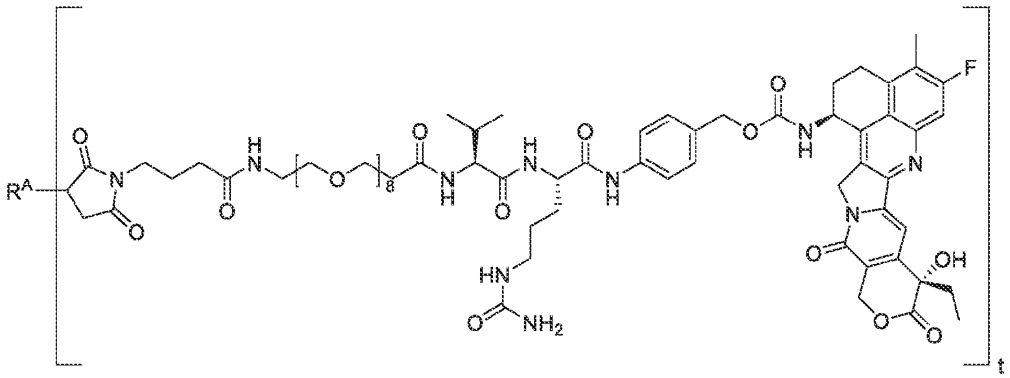
X-J'

or a pharmaceutically acceptable salt thereof, wherein:

R^A is a target binding moiety; and

t is an integer between 1 and 16.

386. The conjugate of Embodiment 1, having a structure of formula X-K':



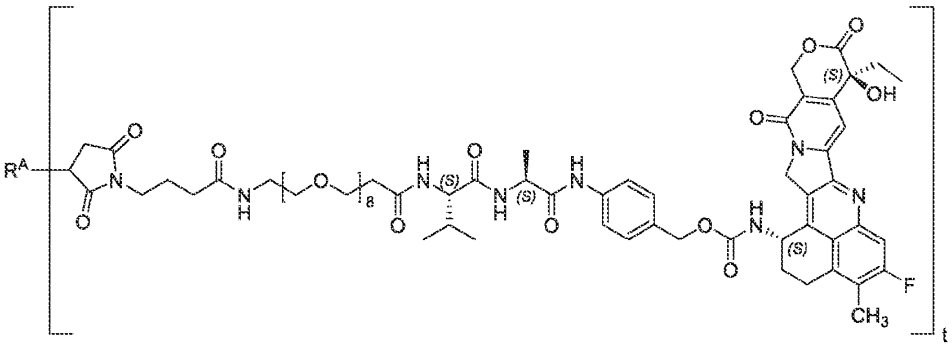
X-K'

or a pharmaceutically acceptable salt thereof, wherein:

R^A is a target binding moiety; and

t is an integer between 1 and 16.

387. The conjugate of Embodiment 1, having a structure of formula X-L':



X-L'

or a pharmaceutically acceptable salt thereof, wherein:

R^A is a target binding moiety; and

t is an integer between 1 and 16.

388. The conjugate of any one of Embodiments 366-387, wherein t is an integer between about 4 and about 8.

389. The conjugate of any one of Embodiments 366-387, wherein t is an integer between about 4 and about 12.

390. The conjugate of any one of the preceding Embodiments, wherein each target binding moiety is independently an antibody or an antigen-binding fragment thereof that is or comprises:

(i) a chimeric antibody, a human antibody, or a humanized antibody, or an antigen-binding fragment thereof;

(ii) a monospecific antibody or a bispecific antibody, or an antigen-binding fragment thereof; and/or

(iii) a monoclonal antibody, or an antigen-binding fragment thereof.

391. The conjugate of any one of the preceding Embodiments, wherein each target binding moiety is independently an antibody or an antigen-binding fragment thereof that is or comprises:

(i) a heavy chain constant region chosen from IgG1, IgG2, IgG3, or IgG4; and/or

(ii) a light chain constant region chosen from the light chain constant regions of kappa or lambda.

392. The conjugate of any one of Embodiments 1-387, wherein each target binding moiety independently is or comprises a chimeric antibody or an antigen-binding fragment thereof.

393. The conjugate of any one of Embodiments 1-387, wherein each target binding moiety independently is or comprises a human antibody or an antigen-binding fragment thereof.

394. The conjugate of any one of Embodiments 1-387, wherein each target binding moiety

independently is or comprises a humanized antibody or an antigen-binding fragment thereof.

395. The conjugate of any one of Embodiments 1-387, wherein each target binding moiety independently is or comprises a monospecific antibody or an antigen-binding fragment thereof.

396. The conjugate of any one of Embodiments 1-387, wherein each target binding moiety independently is or comprises a bispecific antibody or an antigen-binding fragment thereof.

397. The conjugate of any one of Embodiments 1-387, wherein each target binding moiety independently is or comprises an anti-EGFR x HER3 bispecific antibody or an antigen-binding fragment thereof.

398. The conjugate of any one of Embodiments 1-387, wherein each target binding moiety independently is or comprises a biparatopic antibody or an antigen-binding fragment thereof.

399. The conjugate of any one of Embodiments 1-387, wherein each target binding moiety is an anti-HHLA2 antibody or an antigen-binding fragment thereof.

400. The conjugate of Embodiment 399, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises:

(a) a heavy chain variable region (VH) comprising one, two, or three VH CDR sequences each with at least about 90% identity to a VH CDR of Table 1; and/or

(b) a light chain variable region (VL) comprising one, two, or three VL CDR sequences each with at least about 90% identity to a VL CDR of Table 1.

401. The conjugate of Embodiment 400, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises:

(a) a VH comprising one, two, or three VH CDR sequences each with at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% identity to a VH CDR of Table 1; and/or

(b) a VL comprising one, two, or three VL CDR sequences each with at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% identity to a VL CDR of Table 1.

402. The conjugate of Embodiment 401, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises:

(a) a VH comprising one, two, or three VH CDR sequences each comprising or consisting of a VH CDR of Table 1; and/or

(b) a VL comprising one, two, or three VL CDR sequences each comprising or consisting of a VL CDR of Table 1.

403. The conjugate of any one of Embodiments 399-402, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises:

(a) a VH with at least about 90% or more identity to a VH of Table 1; and/or

(b) a VL with at least about 90% or more identity to a VL of Table 1.

404. The conjugate of Embodiment 403, wherein the anti-HHLA2 antibody or antigen-binding fragment thereof is or comprises:

(a) a VH with at least about 95%, 96%, 97%, 98%, 99% or 99.5% identity to a VH of Table 1; and/or

(b) a VL with at least about 95%, 96%, 97%, 98%, 99% or 99.5% identity to a VL of Table 1.

405. The conjugate of Embodiment 404, wherein the anti-HHLA2 or antigen-binding fragment thereof is or comprises:

(a) a VH comprising or consisting of a VH of Table 1; and/or

(b) a VL comprising or consisting of a VL of Table 1.

406. The conjugate of any one of Embodiments 399-405, wherein the anti-HHLA2 or antigen-binding fragment thereof is or comprises:

(a) a heavy chain with at least about 90% or more identity to a heavy chain of Table 1; and/or

(b) a light chain with at least about 90% or more identity to a light chain of Table 1.

407. The conjugate of Embodiment 406, wherein the anti-HHLA2 or antigen-binding fragment thereof is or comprises:

(a) a heavy chain with at least about 95%, 96%, 97%, 98%, 99% or 99.5% identity to a heavy chain of Table 1; and/or

(b) a light chain with at least about 95%, 96%, 97%, 98%, 99% or 99.5% identity to a light chain of Table 1.

408. The conjugate of Embodiment 407, wherein the anti-HHLA2 or antigen-binding fragment thereof is or comprises:

(a) a heavy chain comprising or consisting of a heavy chain of Table 1; and/or

(b) a light chain comprising or consisting of a light chain of Table 1.

409. The conjugate of any one of Embodiments 399-408, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR1 of 887-1DE in Table 1.

410. The conjugate of any one of Embodiments 399-408, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to HCDR1 of 887-1DE in Table 1.

411. The conjugate of any one of Embodiments 399-408, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence that is HCDR1 of 887-1DE in Table 1.

412. The conjugate of any one of Embodiments 409-411, wherein the HCDR1 is SEQ ID NO: 1.

413. The conjugate of any one of Embodiments 409-411, wherein the HCDR1 is SEQ ID NO: 4.

414. The conjugate of any one of Embodiments 409-411, wherein the HCDR1 is SEQ ID NO: 7.

415. The conjugate of any one of Embodiments 399-414, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR2 of 887-1DE in Table 1.

416. The conjugate of any one of Embodiments 399-414, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%

identity to HCDR2 of 887-1DE in Table 1.

417. The conjugate of any one of Embodiments 399-414, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence that is HCDR2 of 887-1DE in Table 1.

418. The conjugate of any one of Embodiments 415-417, wherein the HCDR2 is SEQ ID NO: 2.

419. The conjugate of any one of Embodiments 415-417, wherein the HCDR2 is SEQ ID NO: 5.

420. The conjugate of any one of Embodiments 415-417, wherein the HCDR2 is SEQ ID NO: 8.

421. The conjugate of any one of Embodiments 399-420, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR3 of 887-1DE in Table 1.

422. The conjugate of any one of Embodiments 399-420, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to HCDR3 of 887-1DE in Table 1.

423. The conjugate of any one of Embodiments 399-420, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence that is HCDR3 of 887-1DE in Table 1.

424. The conjugate of any one of Embodiments 421-423, wherein the HCDR3 is SEQ ID NO: 3.

425. The conjugate of any one of Embodiments 421-423, wherein the HCDR3 is SEQ ID NO: 6.

426. The conjugate of any one of Embodiments 421-423, wherein the HCDR3 is SEQ ID NO: 9.

427. The conjugate of any one of Embodiments 399-426, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VH with about or at least about 90% identity to SEQ ID NO: 10.

428. The conjugate of any one of Embodiments 399-426, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VH with about or at least about 95%, 96%, 97%, 98%, 99%, 99.5% identity to SEQ ID NO: 10.

429. The conjugate of any one of Embodiments 399-426, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VH that is or comprises SEQ ID NO: 10.

430. The conjugate of any one of Embodiments 399-429, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain with about or at least about 90% identity to SEQ ID NO: 12.

431. The conjugate of any one of Embodiments 399-429, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain with about or at least about 95%, 96%, 97%, 98%, 99%, 99.5% identity to SEQ ID NO: 12.

432. The conjugate of any one of Embodiments 399-429, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises heavy chain that is or comprises SEQ ID NO: 12.

433. The conjugate of any one of Embodiments 399-432, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR

sequence with about or at least about 90% identity to LCDR1 of 887-1DE in Table 1.

434. The conjugate of any one of Embodiments 399-432, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to LCDR1 of 887-1DE in Table 1.

435. The conjugate of any one of Embodiments 399-432, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence that is LCDR1 of 887-1DE in Table 1.

436. The conjugate of any one of Embodiments 433-435, wherein the LCDR1 is SEQ ID NO: 14.

437. The conjugate of any one of Embodiments 433-435, wherein the LCDR1 is SEQ ID NO: 17.

438. The conjugate of any one of Embodiments 433-435, wherein the LCDR1 is SEQ ID NO: 20.

439. The conjugate of any one of Embodiments 399-438, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 90% identity to LCDR2 of 887-1DE in Table 1.

440. The conjugate of any one of Embodiments 399-438, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to LCDR2 of 887-1DE in Table 1.

441. The conjugate of any one of Embodiments 399-438, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence that is LCDR2 of 887-1DE in Table 1.

442. The conjugate of any one of Embodiments 439-441, wherein the LCDR2 is SEQ ID NO: 15.

443. The conjugate of any one of Embodiments 439-441, wherein the LCDR2 is SEQ ID NO: 18.

444. The conjugate of any one of Embodiments 439-441, wherein the LCDR2 is SEQ ID NO: 21.

445. The conjugate of any one of Embodiments 399-444, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 90% identity to LCDR3 of 887-1DE in Table 1.

446. The conjugate of any one of Embodiments 399-444, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to LCDR3 of 887-1DE in Table 1.

447. The conjugate of any one of Embodiments 399-444, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence that is LCDR3 of 887-1DE in Table 1.

448. The conjugate of any one of Embodiments 445-447, wherein the LCDR3 is SEQ ID NO: 16.

449. The conjugate of any one of Embodiments 445-447, wherein the LCDR3 is SEQ ID NO: 19.

450. The conjugate of any one of Embodiments 445-447, wherein the LCDR3 is SEQ ID NO: 22.

451. The conjugate of any one of Embodiments 399-450, wherein the anti-HHLA2 antibody or an

antigen-binding fragment thereof is or comprises a VL with about or at least about 90% identity to SEQ ID NO: 23.

452. The conjugate of any one of Embodiments 399-450, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VL with about or at least about 95%, 96%, 97%, 98%, 99%, 99.5% identity to SEQ ID NO: 23.

453. The conjugate of any one of Embodiments 399-450, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VL that is or comprises SEQ ID NO: 23.

454. The conjugate of any one of Embodiments 399-453, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain with about or at least about 90% identity to SEQ ID NO: 25.

455. The conjugate of any one of Embodiments 399-453, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain with about or at least about 95%, 96%, 97%, 98%, 99%, 99.5% identity to SEQ ID NO: 25.

456. The conjugate of any one of Embodiments 399-453, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises light chain that is or comprises SEQ ID NO: 25.

457. The conjugate of any one of Embodiments 399-408, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR1 of 65889 in Table 1.

458. The conjugate of any one of Embodiments 399-408, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to HCDR1 of 65889 in Table 1.

459. The conjugate of any one of Embodiments 399-408, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence that is HCDR1 of 65889 in Table 1.

460. The conjugate of any one of Embodiments 457-459, wherein the HCDR1 is SEQ ID NO: 27.

461. The conjugate of any one of Embodiments 457-459, wherein the HCDR1 is SEQ ID NO: 30.

462. The conjugate of any one of Embodiments 457-459, wherein the HCDR1 is SEQ ID NO: 33.

463. The conjugate of any one of Embodiments 399-408 and 457-462, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR2 of 65889 in Table 1.

464. The conjugate of any one of Embodiments 399-408 and 457-462, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to HCDR2 of 65889 in Table 1.

465. The conjugate of any one of Embodiments 399-408 and 457-462, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence that is HCDR2 of 65889 in Table 1.

466. The conjugate of any one of Embodiments 463-465, wherein the HCDR2 is SEQ ID NO: 28.

467. The conjugate of any one of Embodiments 463-465, wherein the HCDR2 is SEQ ID NO: 31.

468. The conjugate of any one of Embodiments 463-465, wherein the HCDR2 is SEQ ID NO: 34.

469. The conjugate of any one of Embodiments 399-408 and 457-468, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR3 of 65889 in Table 1.

470. The conjugate of any one of Embodiments 399-408 and 457-468, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to HCDR3 of 65889 in Table 1.

471. The conjugate of any one of Embodiments 399-408 and 457-468, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence that is HCDR3 of 65889 in Table 1.

472. The conjugate of any one of Embodiments 469-471, wherein the HCDR3 is SEQ ID NO: 29.

473. The conjugate of any one of Embodiments 469-471, wherein the HCDR3 is SEQ ID NO: 32.

474. The conjugate of any one of Embodiments 469-471, wherein the HCDR3 is SEQ ID NO: 35.

475. The conjugate of any one of Embodiments 399-408 and 457-474, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VH with about or at least about 90% identity to SEQ ID NO: 36.

476. The conjugate of any one of Embodiments 399-408 and 457-474, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VH with about or at least about 95%, 96%, 97%, 98%, 99%, 99.5% identity to SEQ ID NO: 36.

477. The conjugate of any one of Embodiments 399-408 and 457-474, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VH that is or comprises SEQ ID NO: 36.

478. The conjugate of any one of Embodiments 399-408 and 457-477, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain with about or at least about 90% identity to SEQ ID NO: 38.

479. The conjugate of any one of Embodiments 399-408 and 457-477, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain with about or at least about 95%, 96%, 97%, 98%, 99%, 99.5% identity to SEQ ID NO: 38.

480. The conjugate of any one of Embodiments 399-408 and 457-477, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises heavy chain that is or comprises SEQ ID NO: 38.

481. The conjugate of any one of Embodiments 399-408 and 457-480, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 90% identity to LCDR1 of 65889 in Table 1.

482. The conjugate of any one of Embodiments 399-408 and 457-480, wherein the anti-HHLA2

antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to LCDR1 of 65889 in Table 1.

483. The conjugate of any one of Embodiments 399-408 and 457-480, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence that is LCDR1 of 65889 in Table 1.

484. The conjugate of any one of Embodiments 481-483, wherein the LCDR1 is SEQ ID NO: 40.

485. The conjugate of any one of Embodiments 481-483, wherein the LCDR1 is SEQ ID NO: 43.

486. The conjugate of any one of Embodiments 481-483, wherein the LCDR1 is SEQ ID NO: 46.

487. The conjugate of any one of Embodiments 399-408 and 457-486, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 90% identity to LCDR2 of 65889 in Table 1.

488. The conjugate of any one of Embodiments 399-408 and 457-486, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to LCDR2 of 65889 in Table 1.

489. The conjugate of any one of Embodiments 399-408 and 457-486, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence that is LCDR2 of 65889 in Table 1.

490. The conjugate of any one of Embodiments 487-489, wherein the LCDR2 is SEQ ID NO: 41.

491. The conjugate of any one of Embodiments 487-489, wherein the LCDR2 is SEQ ID NO: 44.

492. The conjugate of any one of Embodiments 487-489, wherein the LCDR2 is SEQ ID NO: 47.

493. The conjugate of any one of Embodiments 399-408 and 457-492, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 90% identity to LCDR3 of 65889 in Table 1.

494. The conjugate of any one of Embodiments 399-408 and 457-492, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to LCDR3 of 65889 in Table 1.

495. The conjugate of any one of Embodiments 399-408 and 457-492, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence that is LCDR3 of 65889 in Table 1.

496. The conjugate of any one of Embodiments 493-495, wherein the LCDR3 is SEQ ID NO: 42.

497. The conjugate of any one of Embodiments 493-495, wherein the LCDR3 is SEQ ID NO: 45.

498. The conjugate of any one of Embodiments 493-495, wherein the LCDR3 is SEQ ID NO: 48.

499. The conjugate of any one of Embodiments 399-408 and 457-498, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VL with about or at least about 90% identity to SEQ ID NO: 49.

500. The conjugate of any one of Embodiments 399-408 and 457-498, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VL with about or at least about 95%, 96%, 97%, 98%, 99%, 99.5% identity to SEQ ID NO: 49.

501. The conjugate of any one of Embodiments 399-408 and 457-498, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VL that is or comprises SEQ ID NO: 49.

502. The conjugate of any one of Embodiments 399-408 and 457-501, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain with about or at least about 90% identity to SEQ ID NO: 51.

503. The conjugate of any one of Embodiments 399-408 and 457-501, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain with about or at least about 95%, 96%, 97%, 98%, 99%, 99.5% identity to SEQ ID NO: 51.

504. The conjugate of any one of Embodiments 399-408 and 457-501, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises light chain that is or comprises SEQ ID NO: 51.

505. The conjugate of any one of Embodiments 399-408, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR1 of 65872 in Table 1.

506. The conjugate of any one of Embodiments 399-408, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to HCDR1 of 65872 in Table 1.

507. The conjugate of any one of Embodiments 399-408, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence that is HCDR1 of 65872 in Table 1.

508. The conjugate of any one of Embodiments 505-507, wherein the HCDR1 is SEQ ID NO: 53.

509. The conjugate of any one of Embodiments 505-507, wherein the HCDR1 is SEQ ID NO: 56.

510. The conjugate of any one of Embodiments 505-507, wherein the HCDR1 is SEQ ID NO: 59.

511. The conjugate of any one of Embodiments 399-408 and 505-510, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR2 of 65872 in Table 1.

512. The conjugate of any one of Embodiments 399-408 and 505-510, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to HCDR2 of 65872 in Table 1.

513. The conjugate of any one of Embodiments 399-408 and 505-510, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence that is HCDR2 of 65872 in Table 1.

514. The conjugate of any one of Embodiments 511-513, wherein the HCDR2 is SEQ ID NO: 54.

515. The conjugate of any one of Embodiments 511-513, wherein the HCDR2 is SEQ ID NO: 57.

516. The conjugate of any one of Embodiments 511-513, wherein the HCDR2 is SEQ ID NO: 60.

517. The conjugate of any one of Embodiments 399-408 and 505-516, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR3 of 65872 in Table 1.

518. The conjugate of any one of Embodiments 399-408 and 505-516, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to HCDR3 of 65872 in Table 1.

519. The conjugate of any one of Embodiments 399-408 and 505-516, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence that is HCDR3 of 65872 in Table 1.

520. The conjugate of any one of Embodiments 517-519, wherein the HCDR3 is SEQ ID NO: 55.

521. The conjugate of any one of Embodiments 517-519, wherein the HCDR3 is SEQ ID NO: 58.

522. The conjugate of any one of Embodiments 517-519, wherein the HCDR3 is SEQ ID NO: 61.

523. The conjugate of any one of Embodiments 399-408 and 505-522, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VH with about or at least about 90% identity to SEQ ID NO: 62.

524. The conjugate of any one of Embodiments 399-408 and 505-522, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VH with about or at least about 95%, 96%, 97%, 98%, 99%, 99.5% identity to SEQ ID NO: 62.

525. The conjugate of any one of Embodiments 399-408 and 505-522, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VH that is or comprises SEQ ID NO: 62.

526. The conjugate of any one of Embodiments 399-408 and 505-525, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain with about or at least about 90% identity to SEQ ID NO: 64.

527. The conjugate of any one of Embodiments 399-408 and 505-525, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain with about or at least about 95%, 96%, 97%, 98%, 99%, 99.5% identity to SEQ ID NO: 64.

528. The conjugate of any one of Embodiments 399-408 and 505-525, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises heavy chain that is or comprises SEQ ID NO: 64.

529. The conjugate of any one of Embodiments 399-408 and 505-528, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 90% identity to LCDR1 of 65872 in Table 1.

530. The conjugate of any one of Embodiments 399-408 and 505-528, wherein the anti-HHLA2

antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to LCDR1 of 65872 in Table 1.

531. The conjugate of any one of Embodiments 399-408 and 505-528, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence that is LCDR1 of 65872 in Table 1.

532. The conjugate of any one of Embodiments 529-531, wherein the LCDR1 is SEQ ID NO: 66.

533. The conjugate of any one of Embodiments 529-531, wherein the LCDR1 is SEQ ID NO: 69.

534. The conjugate of any one of Embodiments 529-531, wherein the LCDR1 is SEQ ID NO: 72.

535. The conjugate of any one of Embodiments 399-408 and 505-534, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 90% identity to LCDR2 of 65872 in Table 1.

536. The conjugate of any one of Embodiments 399-408 and 505-534, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to LCDR2 of 65872 in Table 1.

537. The conjugate of any one of Embodiments 399-408 and 505-534, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence that is LCDR2 of 65872 in Table 1.

538. The conjugate of any one of Embodiments 535-537, wherein the LCDR2 is SEQ ID NO: 67.

539. The conjugate of any one of Embodiments 535-537, wherein the LCDR2 is SEQ ID NO: 70.

540. The conjugate of any one of Embodiments 535-537, wherein the LCDR2 is SEQ ID NO: 73.

541. The conjugate of any one of Embodiments 399-408 and 505-540, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 90% identity to LCDR3 of 65872 in Table 1.

542. The conjugate of any one of Embodiments 399-408 and 505-540, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to LCDR3 of 65872 in Table 1.

543. The conjugate of any one of Embodiments 399-408 and 505-540, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence that is LCDR3 of 65872 in Table 1.

544. The conjugate of any one of Embodiments 541-543, wherein the LCDR3 is SEQ ID NO: 68.

545. The conjugate of any one of Embodiments 541-543, wherein the LCDR3 is SEQ ID NO: 71.

546. The conjugate of any one of Embodiments 541-543, wherein the LCDR3 is SEQ ID NO: 74.

547. The conjugate of any one of Embodiments 399-408 and 505-546, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VL with about or at least about 90% identity to SEQ ID NO: 75.

548. The conjugate of any one of Embodiments 399-408 and 505-546, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VL with about or at least about 95%, 96%, 97%, 98%, 99%, 99.5% identity to SEQ ID NO: 75.

549. The conjugate of any one of Embodiments 399-408 and 505-546, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VL that is or comprises SEQ ID NO: 75.

550. The conjugate of any one of Embodiments 399-408 and 505-549, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain with about or at least about 90% identity to SEQ ID NO: 77.

551. The conjugate of any one of Embodiments 399-408 and 505-549, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain with about or at least about 95%, 96%, 97%, 98%, 99%, 99.5% identity to SEQ ID NO: 77.

552. The conjugate of any one of Embodiments 399-408 and 505-549, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises light chain that is or comprises SEQ ID NO: 77.

553. The conjugate of any one of Embodiments 399-408, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR1 of Hu2C4-H3L3 in Table 1.

554. The conjugate of any one of Embodiments 399-408, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to HCDR1 of Hu2C4-H3L3 in Table 1.

555. The conjugate of any one of Embodiments 399-408, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence that is HCDR1 of Hu2C4-H3L3 in Table 1.

556. The conjugate of any one of Embodiments 553-555, wherein the HCDR1 is SEQ ID NO: 79.

557. The conjugate of any one of Embodiments 553-555, wherein the HCDR1 is SEQ ID NO: 82.

558. The conjugate of any one of Embodiments 553-555, wherein the HCDR1 is SEQ ID NO: 85.

559. The conjugate of any one of Embodiments 399-408 and 553-558, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR2 of Hu2C4-H3L3 in Table 1.

560. The conjugate of any one of Embodiments 399-408 and 553-558, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to HCDR2 of Hu2C4-H3L3 in Table 1.

561. The conjugate of any one of Embodiments 399-408 and 553-558, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH)

comprising a VH CDR sequence that is HCDR2 of Hu2C4-H3L3 in Table 1.

562. The conjugate of any one of Embodiments 559-561, wherein the HCDR2 is SEQ ID NO: 80.

563. The conjugate of any one of Embodiments 559-561, wherein the HCDR2 is SEQ ID NO: 83.

564. The conjugate of any one of Embodiments 559-561, wherein the HCDR2 is SEQ ID NO: 86.

565. The conjugate of any one of Embodiments 399-408 and 553-564, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR3 of Hu2C4-H3L3 in Table 1.

566. The conjugate of any one of Embodiments 399-408 and 553-564, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to HCDR3 of Hu2C4-H3L3 in Table 1.

567. The conjugate of any one of Embodiments 399-408 and 553-564, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain variable region (VH) comprising a VH CDR sequence that is HCDR3 of Hu2C4-H3L3 in Table 1.

568. The conjugate of any one of Embodiments 565-567, wherein the HCDR3 is SEQ ID NO: 81.

569. The conjugate of any one of Embodiments 565-567, wherein the HCDR3 is SEQ ID NO: 84.

570. The conjugate of any one of Embodiments 565-567, wherein the HCDR3 is SEQ ID NO: 87.

571. The conjugate of any one of Embodiments 399-408 and 553-570, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VH with about or at least about 90% identity to SEQ ID NO: 88.

572. The conjugate of any one of Embodiments 399-408 and 553-570, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VH with about or at least about 95%, 96%, 97%, 98%, 99%, 99.5% identity to SEQ ID NO: 88.

573. The conjugate of any one of Embodiments 399-408 and 553-570, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VH that is or comprises SEQ ID NO: 88.

574. The conjugate of any one of Embodiments 399-408 and 553-573, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain with about or at least about 90% identity to SEQ ID NO: 90.

575. The conjugate of any one of Embodiments 399-408 and 553-573, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a heavy chain with about or at least about 95%, 96%, 97%, 98%, 99%, 99.5% identity to SEQ ID NO: 90.

576. The conjugate of any one of Embodiments 399-408 and 553-573, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises heavy chain that is or comprises SEQ ID NO: 90.

577. The conjugate of any one of Embodiments 399-408 and 553-576, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL)

comprising a VL CDR sequence with about or at least about 90% identity to LCDR1 of Hu2C4-H3L3 in Table 1.

578. The conjugate of any one of Embodiments 399-408 and 553-576, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to LCDR1 of Hu2C4-H3L3 in Table 1.

579. The conjugate of any one of Embodiments 399-408 and 553-576, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence that is LCDR1 of Hu2C4-H3L3 in Table 1.

580. The conjugate of any one of Embodiments 577-579, wherein the LCDR1 is SEQ ID NO: 92.

581. The conjugate of any one of Embodiments 577-579, wherein the LCDR1 is SEQ ID NO: 95.

582. The conjugate of any one of Embodiments 577-579, wherein the LCDR1 is SEQ ID NO: 98.

583. The conjugate of any one of Embodiments 399-408 and 553-582, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 90% identity to LCDR2 of Hu2C4-H3L3 in Table 1.

584. The conjugate of any one of Embodiments 399-408 and 553-582, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to LCDR2 of Hu2C4-H3L3 in Table 1.

585. The conjugate of any one of Embodiments 399-408 and 553-582, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence that is LCDR2 of Hu2C4-H3L3 in Table 1.

586. The conjugate of any one of Embodiments 583-585, wherein the LCDR2 is SEQ ID NO: 93.

587. The conjugate of any one of Embodiments 583-585, wherein the LCDR2 is SEQ ID NO: 96.

588. The conjugate of any one of Embodiments 583-585, wherein the LCDR2 is SEQ ID NO: 99.

589. The conjugate of any one of Embodiments 399-408 and 553-588, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 90% identity to LCDR3 of Hu2C4-H3L3 in Table 1.

590. The conjugate of any one of Embodiments 399-408 and 553-588, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5% identity to LCDR3 of Hu2C4-H3L3 in Table 1.

591. The conjugate of any one of Embodiments 399-408 and 553-588, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain variable region (VL) comprising a VL CDR sequence that is LCDR3 of Hu2C4-H3L3 in Table 1.

592. The conjugate of any one of Embodiments 589-591, wherein the LCDR3 is SEQ ID NO: 94.

593. The conjugate of any one of Embodiments 589-591, wherein the LCDR3 is SEQ ID NO: 97.

594. The conjugate of any one of Embodiments 589-591, wherein the LCDR3 is SEQ ID NO: 100.

595. The conjugate of any one of Embodiments 399-408 and 553-594, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VL with about or at least about 90% identity to SEQ ID NO: 101.

596. The conjugate of any one of Embodiments 399-408 and 553-594, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VL with about or at least about 95%, 96%, 97%, 98%, 99%, 99.5% identity to SEQ ID NO: 101.

597. The conjugate of any one of Embodiments 399-408 and 553-594, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a VL that is or comprises SEQ ID NO: 101.

598. The conjugate of any one of Embodiments 399-408 and 553-597, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain with about or at least about 90% identity to SEQ ID NO: 103.

599. The conjugate of any one of Embodiments 399-408 and 553-597, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises a light chain with about or at least about 95%, 96%, 97%, 98%, 99%, 99.5% identity to SEQ ID NO: 103.

600. The conjugate of any one of Embodiments 399-408 and 553-597, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises light chain that is or comprises SEQ ID NO: 103.

601. The conjugate of Embodiment 399, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises 887-1DE in Table 1.

602. The conjugate of Embodiment 399, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises 65889 in Table 1.

603. The conjugate of Embodiment 399, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises 65872 in Table 1.

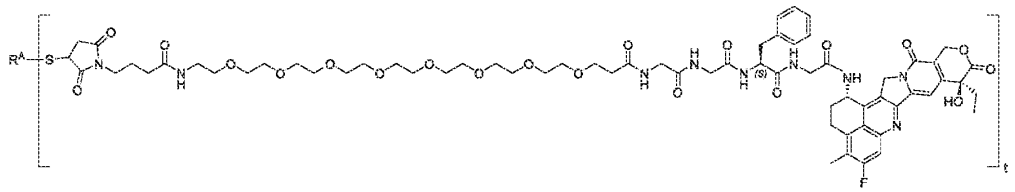
604. The conjugate of Embodiment 399, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises Hu2C4H3L3in Table 1.

605. The conjugate of any one of Embodiments 392-604, which binds HHLA2 with a K_D of about 15 nM or less.

606. The conjugate of any one of Embodiments 399-605, which binds human HHLA2 with an affinity of at least about 50-fold to about 800-fold over background.

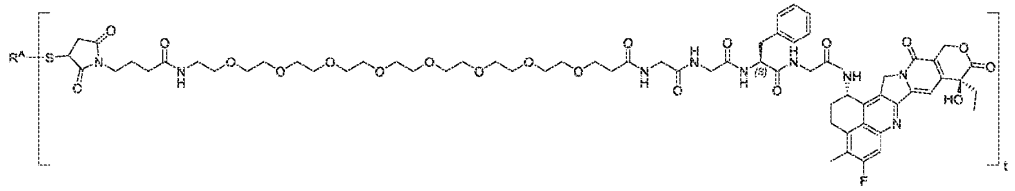
607. The conjugate of any one of Embodiments 1-391, wherein the antibody or an antigen-binding fragment thereof is an anti-HER2 x HHLA2 bispecific antibody or an antigen-binding fragment thereof.

608. A conjugate, wherein the conjugate is a compound having the structure of



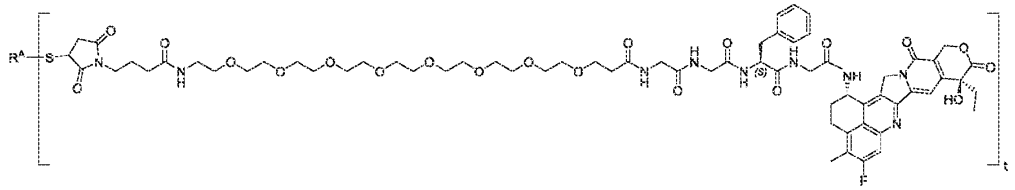
or a salt thereof, wherein R^A is 887-1DE, and t is about 1-8.

609. A conjugate, wherein the conjugate is a compound having the structure of



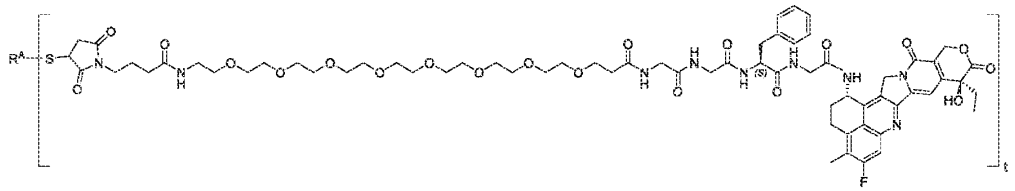
or a salt thereof, wherein R^A is 65889, each −S− is independently of a Cys residue, and t is about 1-8.

610. A conjugate, wherein the conjugate is a compound having the structure of



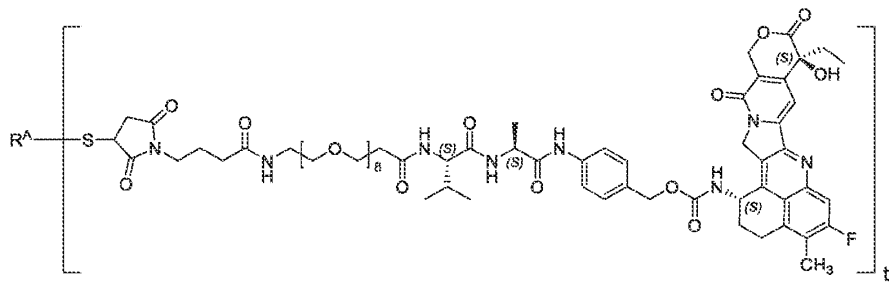
or a salt thereof, wherein R^A is 65872, each −S− is independently of a Cys residue, and t is about 1-8.

611. A conjugate, wherein the conjugate is a compound having the structure of



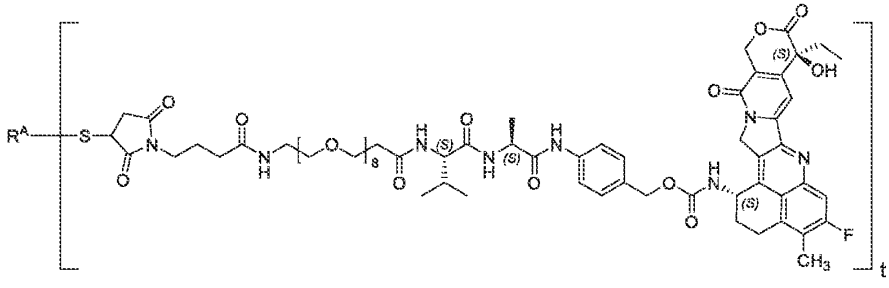
or a salt thereof, wherein R^A is Hu2C4-H3L3, each −S− is independently of a Cys residue, and t is about 1-8.

612. A conjugate, wherein the conjugate is a compound having the structure of



or a salt thereof, wherein R^A is 887-1DE, and t is about 1-8.

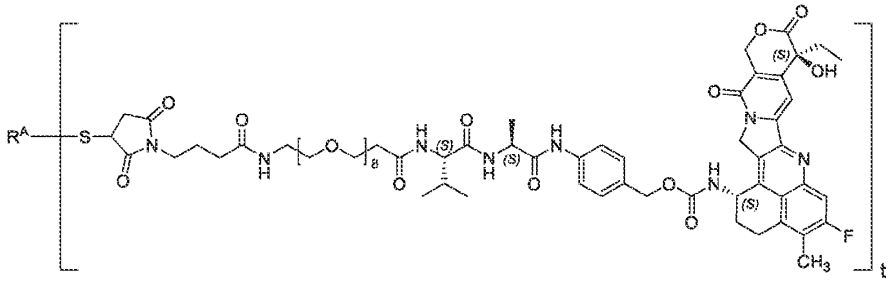
613. A conjugate, wherein the conjugate is a compound having the structure of



t or a salt

thereof, wherein R^A is 65889, each −S− is independently of a Cys residue, and t is about 1-8.

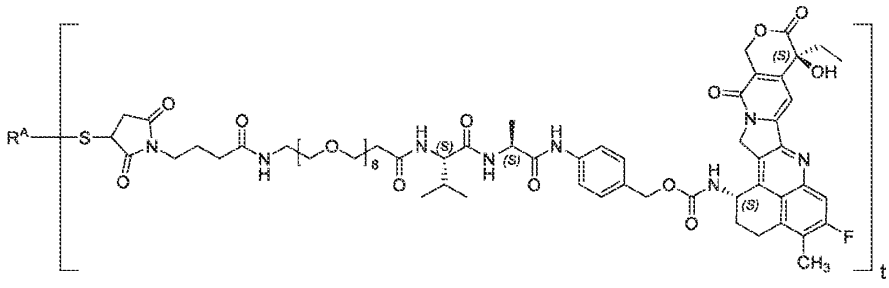
614. A conjugate, wherein the conjugate is a compound having the structure of



t or a salt

thereof, wherein R^A is 65872, each −S− is independently of a Cys residue, and t is about 1-8.

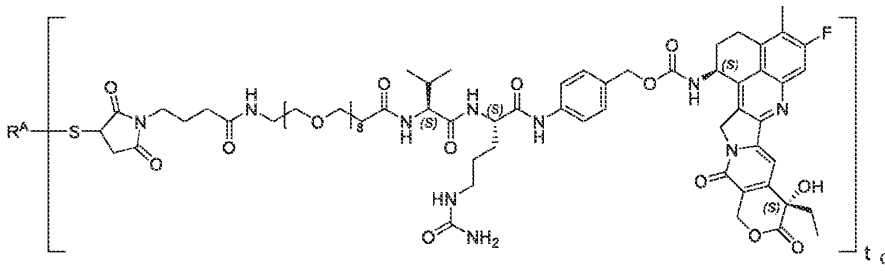
615. A conjugate, wherein the conjugate is a compound having the structure of



t or a salt

thereof, wherein R^A is Hu2C4-H3L3, each −S− is independently of a Cys residue, and t is about 1-8.

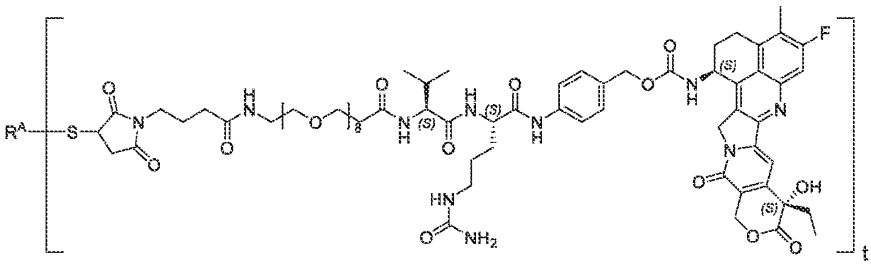
616. A conjugate, wherein the conjugate is a compound having the structure of



t or a salt

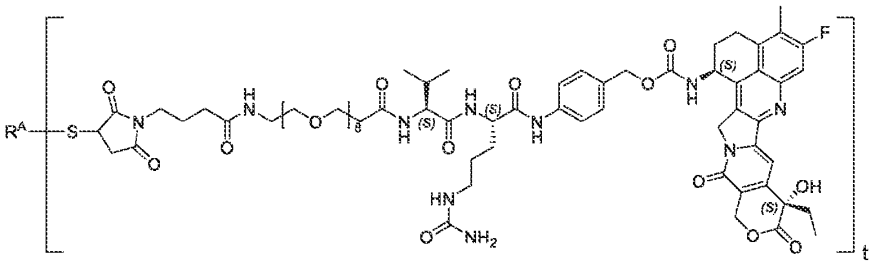
thereof, wherein R^A is 887-1DE, and t is about 1-8.

617. A conjugate, wherein the conjugate is a compound having the structure of



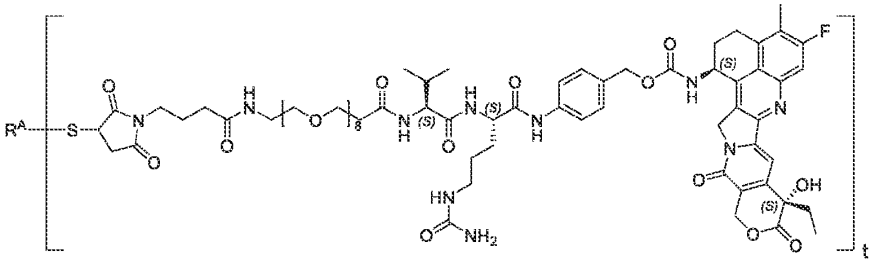
or a salt thereof, wherein R^A is 65889, each –S– is independently of a Cys residue, and t is about 1-8.

618. A conjugate, wherein the conjugate is a compound having the structure of



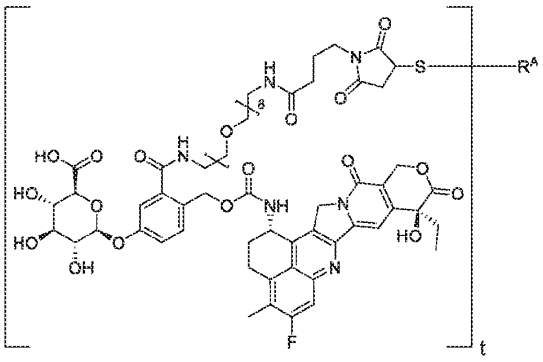
or a salt thereof, wherein R^A is 65872, each –S– is independently of a Cys residue, and t is about 1-8.

619. A conjugate, wherein the conjugate is a compound having the structure of



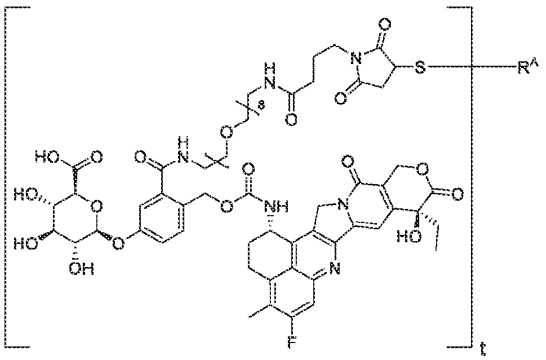
or a salt thereof, wherein R^A is Hu2C4-H3L3, each –S– is independently of a Cys residue, and t is about 1-8.

620. A conjugate, wherein the conjugate is a compound having the structure of



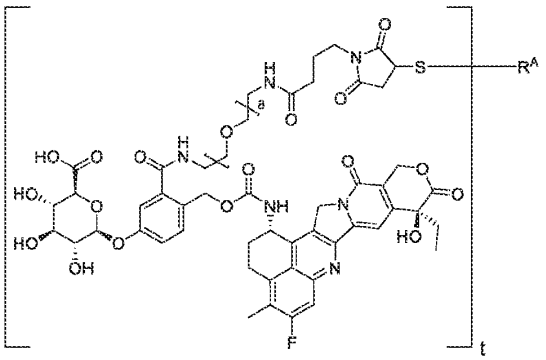
or a salt thereof, wherein R^A is 887-1DE, and t is about 1-8.

621. A conjugate, wherein the conjugate is a compound having the structure of



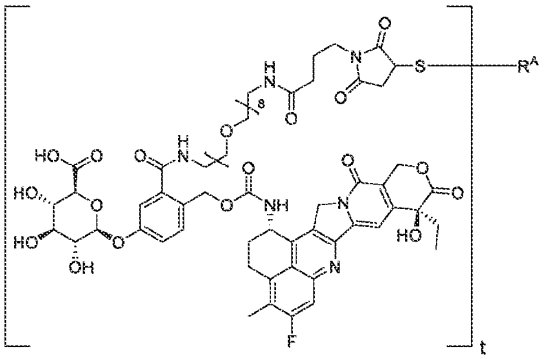
or a salt thereof, wherein R^A is 65889, each −S− is independently of a Cys residue, and t is about 1-8.

622. A conjugate, wherein the conjugate is a compound having the structure of



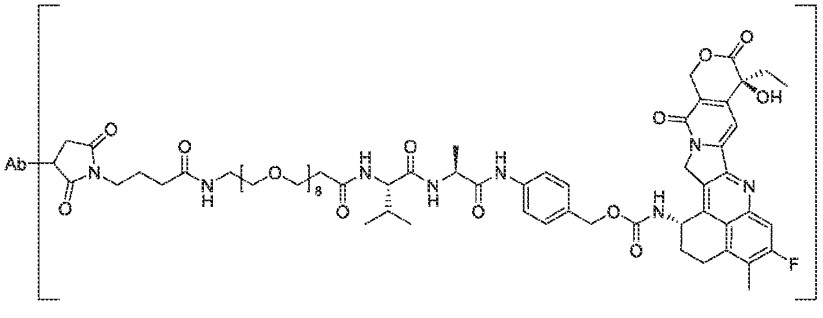
or a salt thereof, wherein R^A is 65872, each −S− is independently of a Cys residue, and t is about 1-8.

623. A conjugate, wherein the conjugate is a compound having the structure of



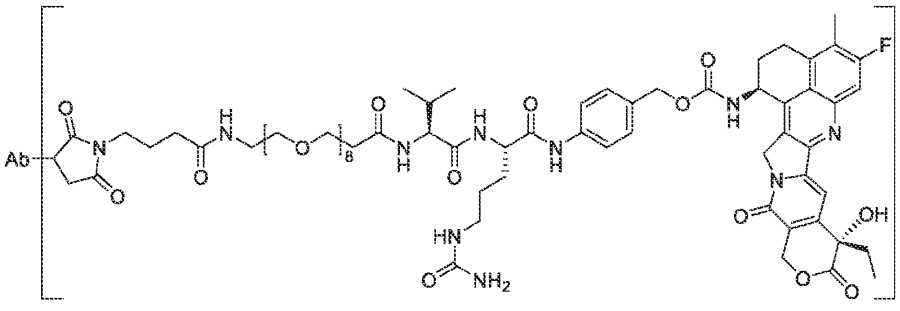
or a salt thereof, wherein R^A is Hu2C4-H3L3, each −S− is independently of a Cys residue, and t is about 1-8.

624. A conjugate, wherein the conjugate is a compound having the structure of



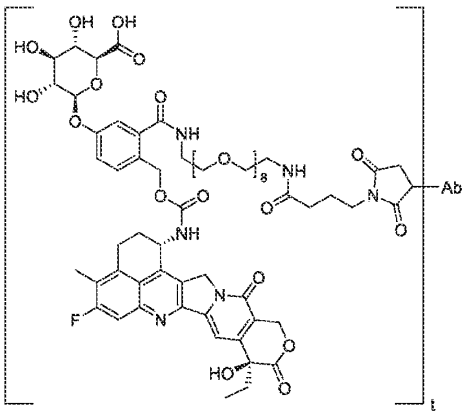
or a salt thereof, wherein Ab is or comprises an antibody or an antigen-binding fragment thereof, and t is about 1-8.

625. A conjugate, wherein the conjugate is a compound having the structure of



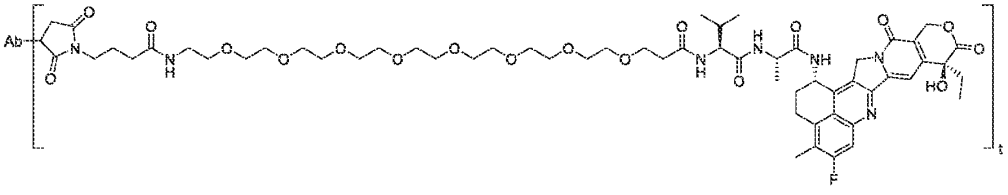
or a salt thereof, wherein Ab is or comprises an antibody or an antigen-binding fragment thereof, and t is about 1-8.

626. A conjugate, wherein the conjugate is a compound having the structure of



or a salt thereof, wherein Ab is or comprises an antibody or an antigen-binding fragment thereof, and t is about 1-8.

627. A conjugate, wherein the conjugate is a compound having the structure of



or a salt thereof, wherein Ab is or comprises an antibody or an antigen-binding fragment thereof, and t is about 1-8.

628. The conjugate of any one of Embodiments 624-627, wherein Ab is or comprises an anti-HHLA2 antibody or an antigen-binding fragment thereof.

629. The conjugate of any one of Embodiments 624-627, wherein Ab is Hu2C4-H3L3.

630. The conjugate of any one of Embodiments 624-627, wherein Ab is 65872.

631. The conjugate of any one of Embodiments 624-627, wherein Ab is 65889.

632. The conjugate of any one of Embodiments 624-627, wherein Ab is 887-1DE .

633. The conjugate of any one of Embodiments 624-632, wherein Ab is conjugated at one or more cysteine residues.

634. The conjugate of any one of Embodiments 608-633, wherein t is about 1.

635. The conjugate of any one of Embodiments 608-633, wherein t is about 2.

636. The conjugate of any one of Embodiments 608-633, wherein t is about 3.

637. The conjugate of any one of Embodiments 608-633, wherein t is about 4.

638. The conjugate of any one of Embodiments 608-633, wherein t is about 5.

639. The conjugate of any one of Embodiments 608-633, wherein t is about 6.

640. The conjugate of any one of Embodiments 608-633, wherein t is about 7.

641. The conjugate of any one of Embodiments 608-633, wherein t is about 8.

642. The conjugate of any one of the preceding Embodiments, wherein the conjugate is in a pharmaceutically acceptable salt form.

643. A composition comprising one or more conjugates each of which is independently a conjugate of any one of the preceding Embodiments.

644. The composition of Embodiment 643, wherein each target binding moiety of the one or more conjugates is independently a target binding moiety of any one of Embodiments 1-11.

645. The composition of any one of Embodiments 643-644, wherein each target binding moiety of the one or more conjugates targets the same agent.

646. The composition of any one of Embodiments 643-644, wherein each target binding moiety of the one or more conjugates targets the same agent, wherein the agent is or comprises a polypeptide, a nucleic acid or a lipid.

647. The composition of any one of Embodiments 643-644, wherein each target binding moiety of the one or more conjugates targets the same agent, wherein the agent is a polypeptide.

648. The composition of any one of Embodiments 643-647, wherein each target binding moiety of the one or more conjugates targets the same antigen.

649. The composition of any one of Embodiments 643-648, wherein each target binding moiety of the one or more conjugates is independently an antibody or an antigen-binding fragment thereof, and each target binding moiety of the one or more conjugates shares about 90%, 95%, 96%, 97%, 98% or 99% sequence identity.

650. The composition of any one of Embodiments 643-648, wherein each target binding moiety of the

one or more conjugates is independently an antibody or an antigen-binding fragment thereof, and each target binding moiety of the one or more conjugates shares the same amino acid sequence.

651. The composition of any one of Embodiments 643-648, wherein each target binding moiety of the one or more conjugates is the same.

652. The composition of any one of Embodiments 643-651, wherein the composition comprises unconjugated target binding moiety.

653. The composition of any one of Embodiments 643-652, wherein the ratio of the payload moiety to the target binding moiety of the one or more conjugates is about 1-20.

654. The composition of any one of Embodiments 643-654, wherein the ratio of the payload moiety to the target binding moiety of all conjugates in the composition is about 1-20.

655. The composition of any one of Embodiments 643-655, wherein the ratio of the payload moiety to the target binding moiety of the composition is about 1-20.

656. The composition of any one of Embodiments 653-655, wherein the ratio is about 1-8.

657. The composition of any one of Embodiments 653-655, wherein the ratio is about 2-4.

658. The composition of any one of Embodiments 653-655, wherein the ratio is about 2.

659. The composition of any one of Embodiments 653-655, wherein the ratio is about 4.

660. The composition of any one of Embodiments 653-655, wherein the ratio is about 6.

661. The composition of any one of Embodiments 653-655, wherein the ratio is about 8.

662. The composition of any one of Embodiments 643-655, wherein each antibody molecule in the composition, if any, carries an average of about 1-20 payload moieties.

663. The composition of any one of Embodiments 643-655, wherein each antibody molecule in the composition, if any, carries an average of about 2-8 payload moieties.

664. The composition of any one of Embodiments 643-655, wherein each antibody molecule in the composition, if any, carries an average of about 2-6 payload moieties.

665. The composition of any one of Embodiments 643-655, wherein each antibody molecule in the composition, if any, carries an average of about 2-4 payload moieties.

666. The composition of any one of Embodiments 643-655, wherein each antibody molecule in the composition, if any, carries an average of about 2 payload moieties.

667. The composition of any one of Embodiments 643-655, wherein each antibody molecule in the composition, if any, carries an average of about 2 payload moieties.

668. The composition of any one of Embodiments 643-655, wherein each antibody molecule in the composition, if any, carries an average of about 6 payload moieties.

669. .The composition of any one of Embodiments 643-655, wherein each antibody molecule in the composition, if any, carries an average of about 8 payload moieties.

670. The composition of any one of Embodiments 643-669, wherein the composition is a pharmaceutical composition.

671. The composition of any one of Embodiments 643-670, wherein the composition comprises a pharmaceutically acceptable carrier.

672. A pharmaceutical composition comprising at least one conjugate of any of the above Embodiments, and a pharmaceutically acceptable carrier.

673. The composition of any one of Embodiments 643-672, wherein the composition is a solution.

674. The composition of any one of Embodiments 643-672, wherein the composition is a solution for injection.

675. A kit comprising a conjugate of any one of Embodiments 1-642 or a composition of any one of Embodiments 643-674, and optionally instructions for use and/or administration.

676. A method of modulating an immune response in a subject comprising administering or delivering to a subject in need thereof a therapeutically effective amount of a conjugate of any one of Embodiments 1-642.

677. A method of modulating an immune response in a subject comprising administering to a subject in need thereof a therapeutically effective amount of a composition of any one of Embodiments 643-674.

678. A method of treating a subject having a disease, disorder, or condition comprising administering or delivering to a subject in need thereof a therapeutically effective amount of a conjugate of any one of Embodiments 1-642.

679. A method of treating a subject having a disease, disorder, or condition comprising administering to a subject in need thereof a therapeutically effective amount of a composition of any one of Embodiments 643-674.

680. The method of any one of Embodiments 676-679, wherein the subject has cancer.

681. The method of any one of Embodiments 676-679, wherein the subject has a solid tumor.

682. The method of any one of Embodiments 676-679, wherein the subject has a renal cancer, a bone cancer, a skin cancer, a breast cancer, a cervical cancer, a colorectal cancer, an endometrial cancer, a lung cancer, an ovarian cancer, a liver cancer, a prostate cancer, a pancreatic cancer, cholangiocarcinoma, or a thyroid cancer.

683. The method of any one of Embodiments 676-679, wherein the subject has a hematological tumor.

684. The method of any one of Embodiments 676-679, wherein the subject has leukemia.

685. The method of any one of Embodiments 676-679, wherein the subject has acute lymphocytic leukemia, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, chronic leukemia, or acute leukemia.

686. The method of any one of Embodiments 676-679, wherein the subject has lymphoma.

687. The method of any one of Embodiments 676-679, wherein the subject has Hodgkin lymphoma (HL), non-Hodgkin's lymphoma, lymphocytic lymphoma, or diffuse large B cell lymphoma (DLBCL).

688. The method of any one of Embodiments 676-679, wherein the subject is at risk of developing a cancer.

689. The method of any one of Embodiments 678-688, wherein the disease, disorder, or condition is associated with aberrant HHLA2 expression.

690. The method of any one of Embodiments 676-689, wherein the conjugate is administered parenterally.

691. The method of Embodiment 690, wherein the parenteral administration is or comprises subcutaneous, intravenous, intramuscular, or intrasternal injection or infusion.

692. The method of any one of Embodiments 676-691, wherein the conjugate is administered in combination with a second agent.

693. A method for delivering a payload to a target in a system, comprising administering to or delivering to the system a conjugate of any one of Embodiments 1-642 or a composition of any one of Embodiments 643-674, wherein:

a payload moiety of the conjugate is a moiety of the payload; and

the target expresses or comprises an agent that a target binding moiety of the conjugate can bind.

694. The method of Embodiment 694, wherein the target is or comprises a cell.

695. The method of Embodiment 694, wherein the target is or comprises a cancer cell.

696. A method for inhibiting proliferation of diseased cells in a system, comprising administering to or delivering to the system a conjugate of any one of Embodiments 1-642 or a composition of any one of Embodiments 643-674, wherein the diseased cells expresses or comprise an agent that a target binding moiety of the conjugate can bind.

697. A method for reduce number of diseased cells in a system, comprising administering to or delivering to the system a conjugate of any one of Embodiments 1-642 or a composition of any one of Embodiments 643-674, wherein the diseased cells expresses or comprise an agent that a target binding moiety of the conjugate can bind.

698. The method of any one of Embodiments 696-697, wherein the diseased cells are or comprise cancer cells.

699. The method of any one of Embodiments 693-695, wherein the system is or comprises a cell.

700. The method of any one of Embodiments 693-695, wherein the system is or comprises a tissue.

701. The method of any one of Embodiments 693-695, wherein the system is or comprises a tissue.

702. The method of any one of Embodiments 693-695, wherein the system is or comprises an organism.

703. The method of any one of Embodiments 693-695, wherein the system is an animal.

704. The method of any one of Embodiments 693-695, wherein the system is a human.

705. A conjugate, composition or kit of any one of Embodiments 1-675 for use in the treatment of a condition, disease or disorder.

706. A conjugate, composition or kit of any one of Embodiments 1-675 for use in a method of any one of the preceding Embodiments.

707. Use of a conjugate, composition or kit of any one of Embodiments 1-675 for the manufacture of a medicament for the treatment of a condition, disease or disorder.

708. Use of a conjugate, composition or kit of any one of Embodiments 1-675 for the manufacture of a medicament for a method of any one of the preceding Embodiments.

709. A method for preparing a conjugate of any one of Embodiments 1-642 or a composition of any one of Embodiments 643-674, comprising reacting a target binding agent and a payload agent, wherein:

the target binding agent comprises a target binding moiety and a target binding agent reactive group;

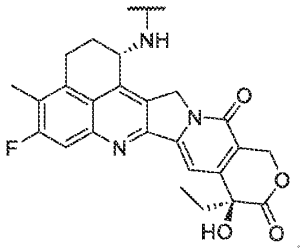
the payload agent comprises a payload moiety and a payload agent reactive group; and

the target binding agent reactive group reacts with the payload agent reactive group to conjugate the payload moiety to the target binding moiety optionally through a linker.

710. The method of Embodiment 709, wherein the target binding moiety is a target binding moiety of any one of Embodiments 1-11.

711. The method of any one of Embodiments 709-710, wherein the payload moiety is a payload moiety of any one of Embodiments 12-17.

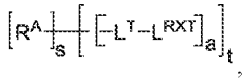
712. The method of any one of Embodiments 709-711, wherein the payload moiety is



713. The method of any one of Embodiments 709-712, wherein the linker comprises $-(CH_2CH_2O)n-$, wherein n is 1-24; and $-L^C-$.

714. The method of any one of Embodiments 709-713, wherein the linker is a linker of any one of Embodiments 31-150.

715. The method of any one of Embodiments 709-713, wherein the target binding agent is a compound of formula T-1 or a salt thereof:



T-1

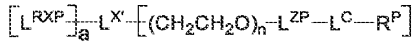
wherein each L^{RXT} is independently a target binding agent reactive group, and each other variable is independently as described in any one of Embodiments 151-642.

716. The method of Embodiment 715, wherein each R^A is independently as described in any one of Embodiments 151-159.

717. The method of any one of Embodiments 715-716, wherein s is 1.

718. The method of any one of Embodiments 715-717, wherein each a is independently as described in any one of Embodiments 162-164.

719. The method of any one of Embodiments 709-718, wherein the payload agent is a compound of formula P-1 or a salt thereof:



P-1

wherein each L^{RXP} is independently a payload agent reactive group, and each other variable is

independently as described in any one of Embodiments 151-642.

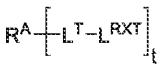
720. The method of any one of Embodiments 709-719, wherein a in formula T-1 and a in formula P-1 are the same.

721. The method of any one of Embodiments 715-720, wherein each a is independently as described in any one of Embodiments 162-164 and 172-174.

722. The method of any one of Embodiments 719-721, wherein b is as described in any one of Embodiments 165-166 and 175-176.

723. The method of any one of Embodiments 719-722, wherein $L^{X'}$ is as described in any one of Embodiments 167-171 and 177.

724. The method of any one of Embodiments 709-713, wherein the target binding agent is a compound of formula T-2 or a salt thereof:

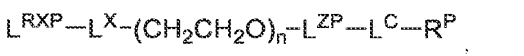


T-2

wherein each L^{RXT} is a target binding agent reactive group, and each other variable is independently as described in any one of Embodiments 178-642.

725. The conjugate of Embodiment 724, wherein R^A is a target binding moiety of any one of Embodiments 1-11.

726. The method of any one of Embodiments 724-725, wherein the payload agent is a compound of formula P-2 or a salt thereof:



P-2

wherein each L^{RXP} is a payload agent reactive group, and each other variable is independently as described in any one of Embodiments 178-642.

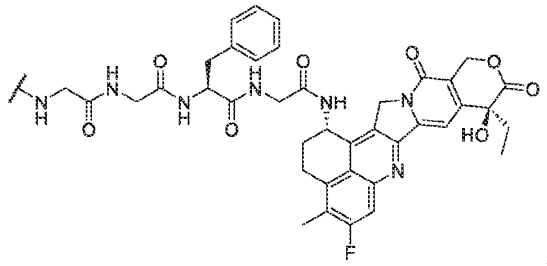
727. The method of Embodiment 726, wherein L^X is as described in any one of Embodiments 181-190.

728. The method of any one of Embodiments 715-727, wherein L^T is as described in any one of Embodiments 191-200.

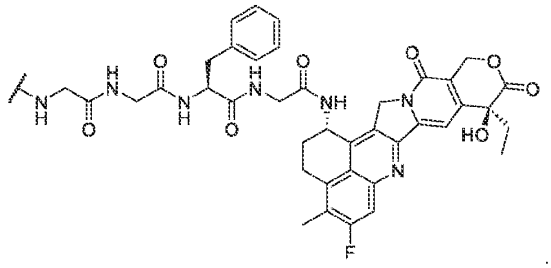
729. The method of any one of Embodiments 715-728, wherein L^{ZP} is as described in any one of Embodiments 228-232.

730. The method of any one of Embodiments 715-729, wherein R^P is as described in any one of Embodiments 233-241.

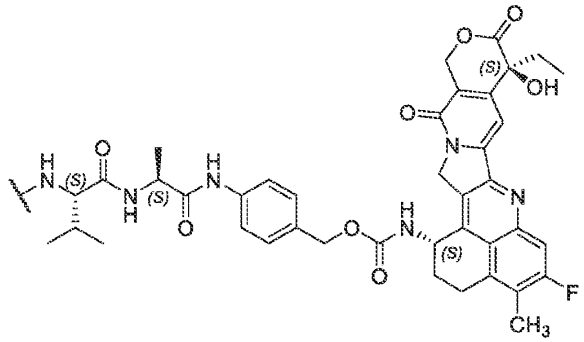
731. The method of any one of Embodiments 715-729, wherein an occurrence of $-L^C-R^P$ is



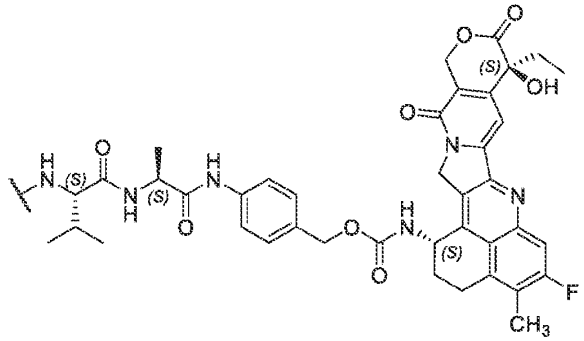
732. The method of any one of Embodiments 715-729, wherein each $-L^C-R^P$ is



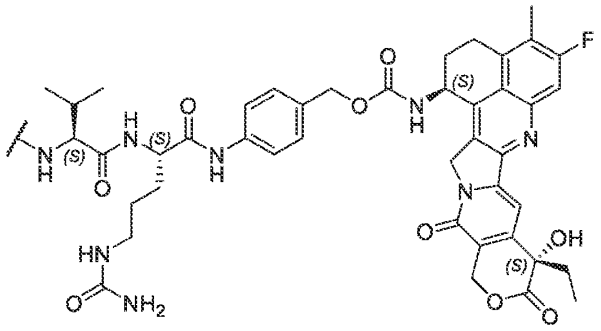
733. The method of any one of Embodiments 715-729, wherein an occurrence of $-L^C-R^P$ is



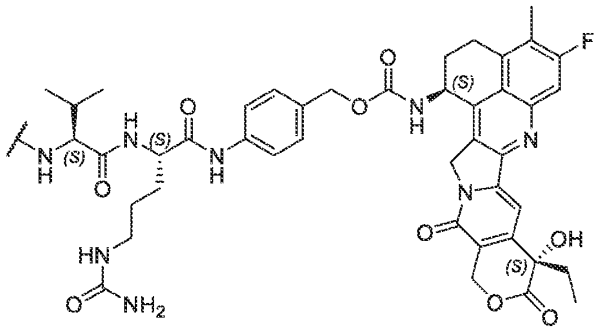
734. The method of any one of Embodiments 715-729, wherein each $-L^C-R^P$ is



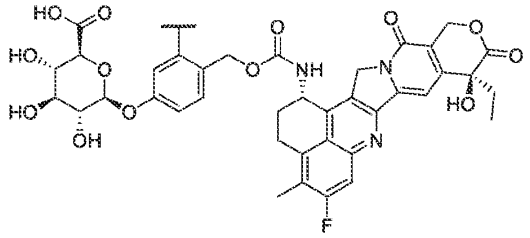
735. The method of any one of Embodiments 715-729, wherein an occurrence of $-L^C-R^P$ is



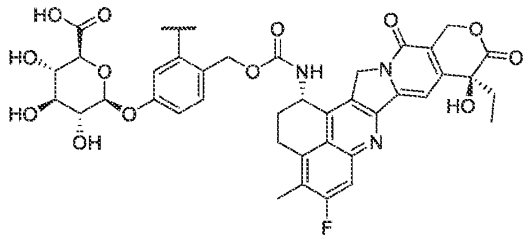
736. The method of any one of Embodiments 715-729, wherein each $-L^C-R^P$ is



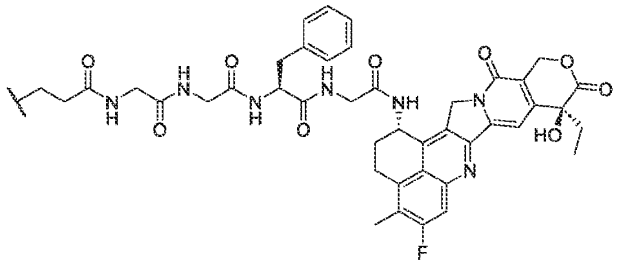
737. The method of any one of Embodiments 715-729, wherein an occurrence of $-L^C-R^P$ is



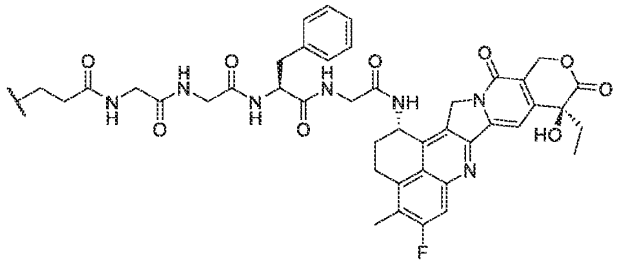
738. The method of any one of Embodiments 715-729, wherein each $-L^C-R^P$ is



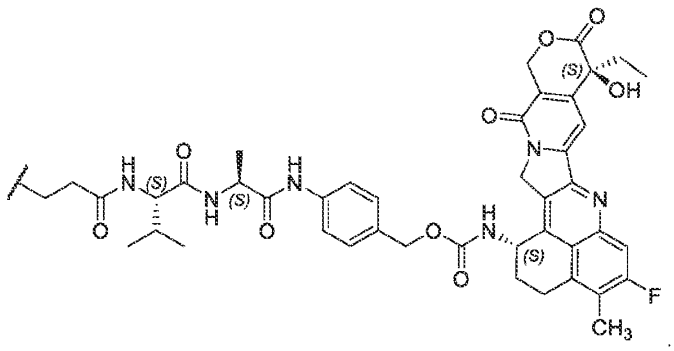
739. The method of any one of Embodiments 715-729, wherein an occurrence of $-L^{ZP}-L^C-R^P$ is or comprises



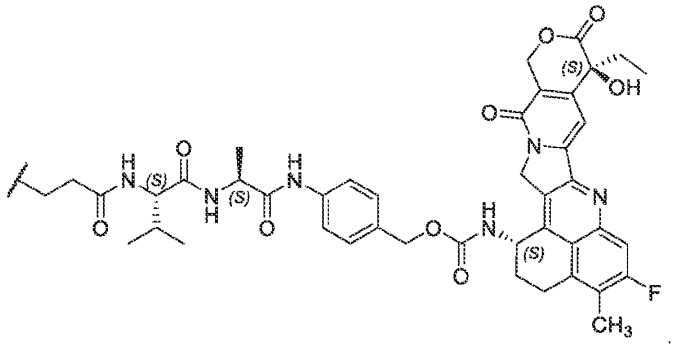
740. The method of any one of Embodiments 715-729, wherein each $-L^{ZP}-L^{C}-R^{P}$ is or comprises



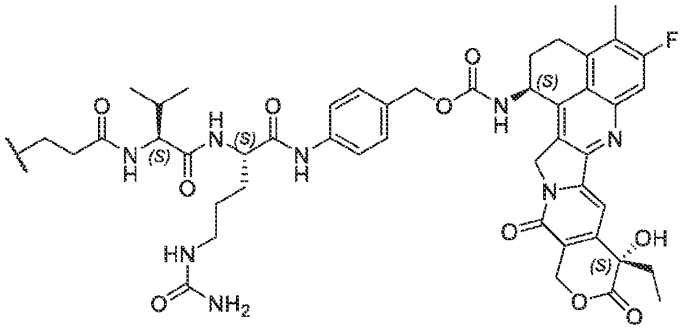
741. The method of any one of Embodiments 715-729, wherein an occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises



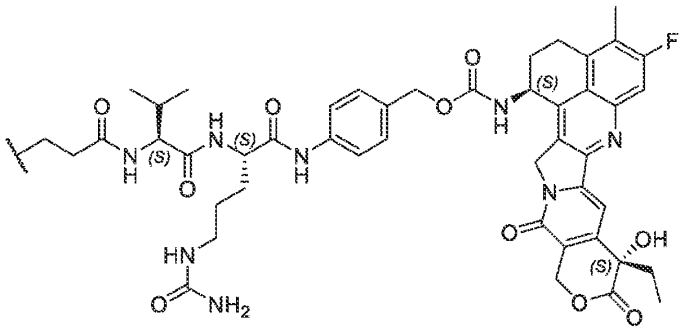
742. The method of any one of Embodiments 715-729, wherein each $-L^{ZP}-L^{C}-R^{P}$ is or comprises



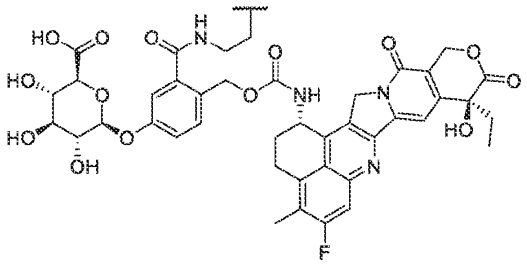
743. The method of any one of Embodiments 715-729, wherein an occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises



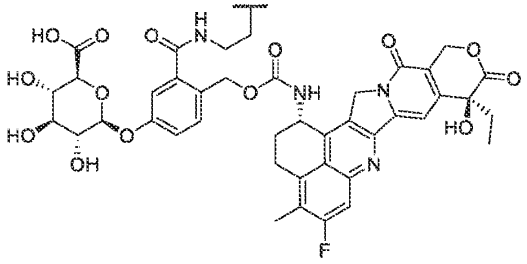
744. The method of any one of Embodiments 715-729, wherein each $-L^{ZP}-L^{C}-R^{P}$ is or comprises



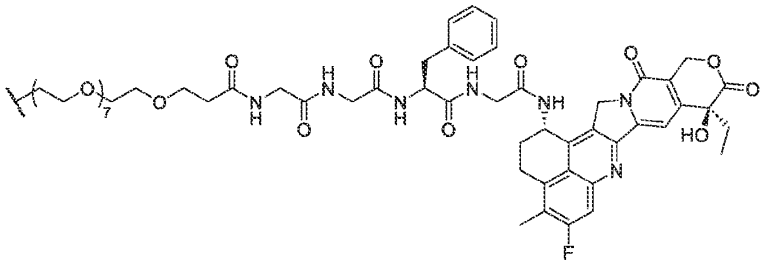
745. The method of any one of Embodiments 715-729, wherein an occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or comprises



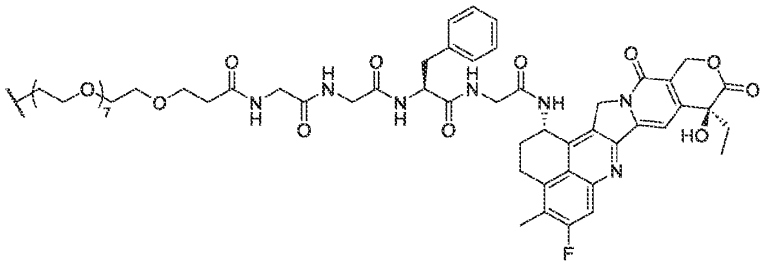
746. The method of any one of Embodiments 715-729, wherein each $-L^{ZP}-L^{C}-R^{P}$ is or comprises



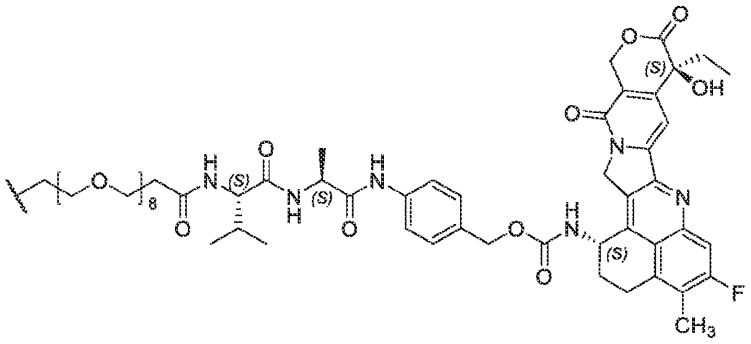
747. The method of any one of Embodiments 715-729, wherein an occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



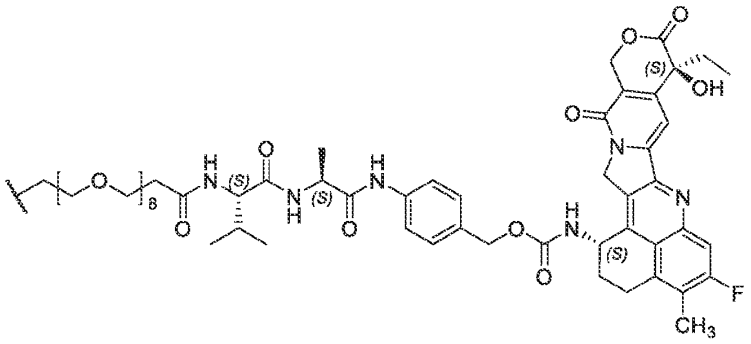
748. The method of any one of Embodiments 715-729, wherein each $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



749. The method of any one of Embodiments 715-729, wherein an occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

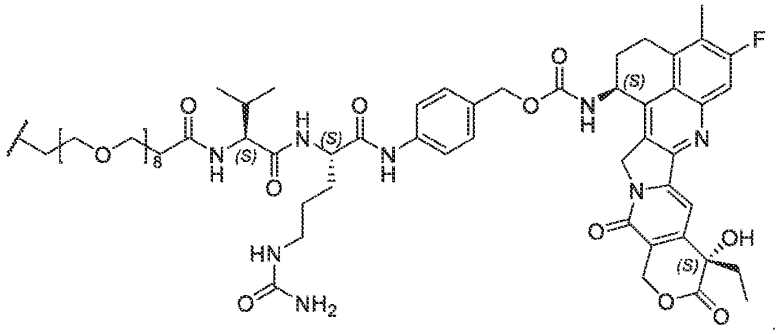


750. The method of any one of Embodiments 715-729, wherein each $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises

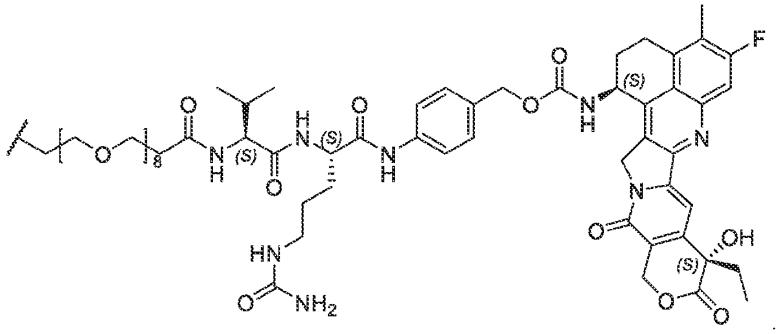


751. The method of any one of Embodiments 715-729, wherein an occurrence of

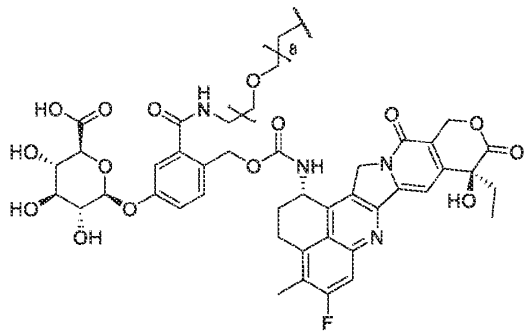
$-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



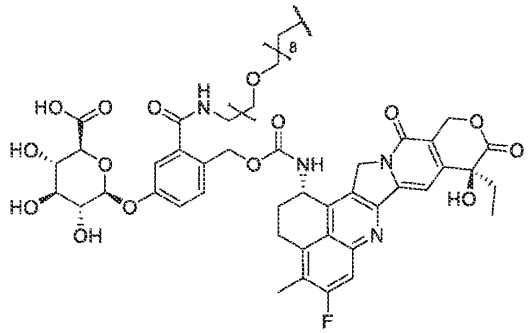
752. The method of any one of Embodiments 715-729, wherein each $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



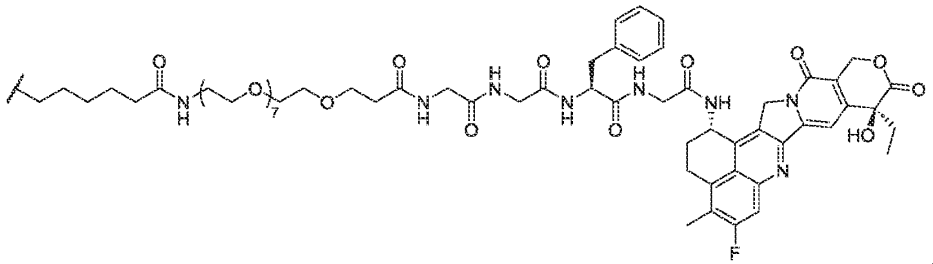
753. The method of any one of Embodiments 715-729, wherein an occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



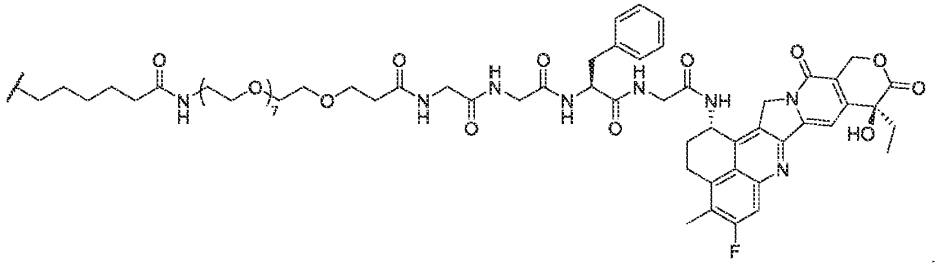
754. The method of any one of Embodiments 715-729, wherein each $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



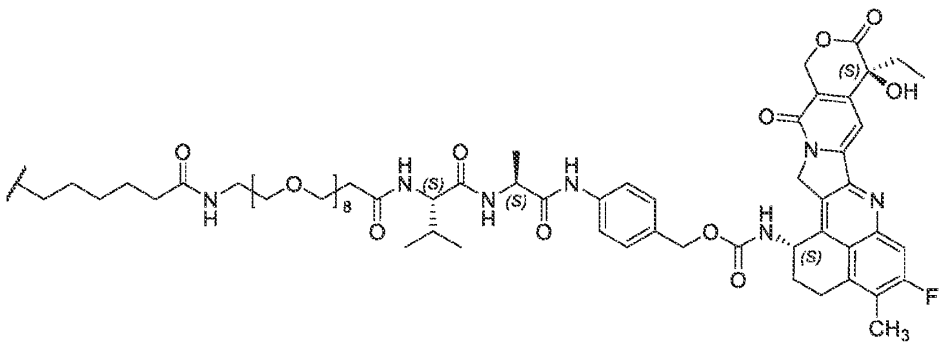
755. The method of any one of Embodiments 715-729, wherein an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



756. The method of any one of Embodiments 715-729, wherein each $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

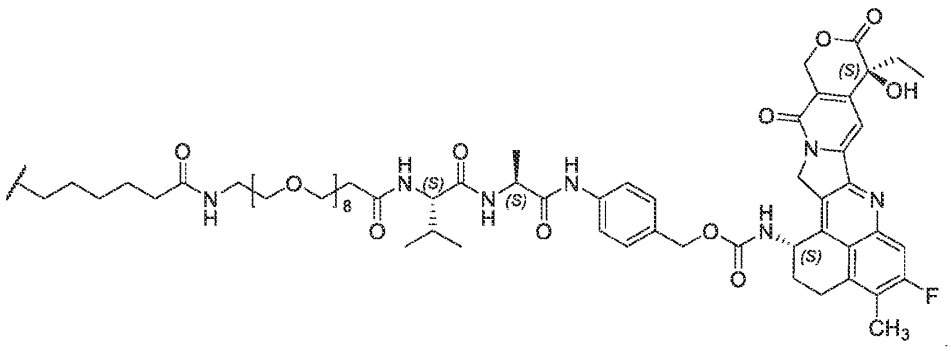


757. The method of any one of Embodiments 715-729, wherein an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises

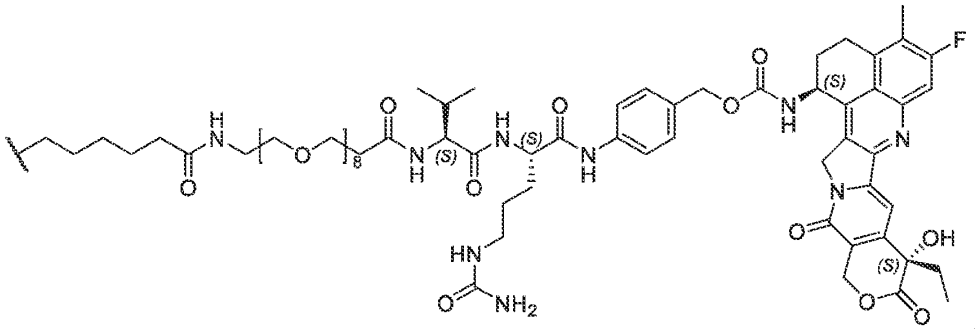


758. The method of any one of Embodiments 715-729, wherein each $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is

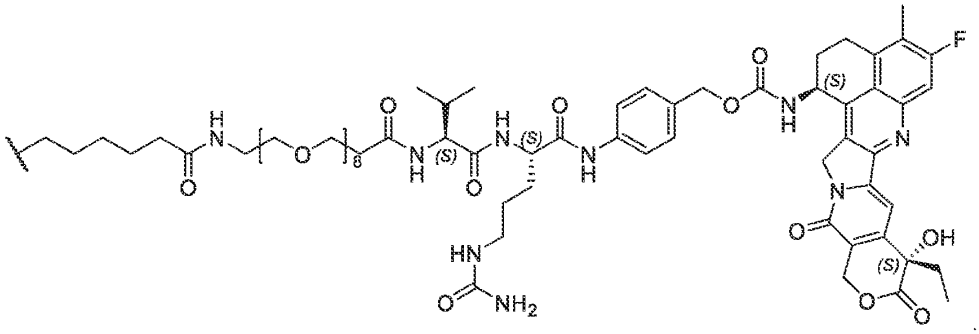
or comprises



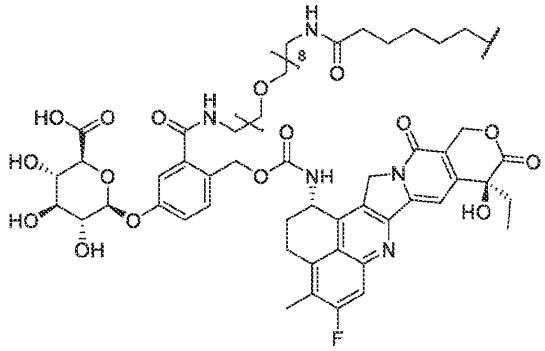
759. The method of any one of Embodiments 715-729, wherein an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



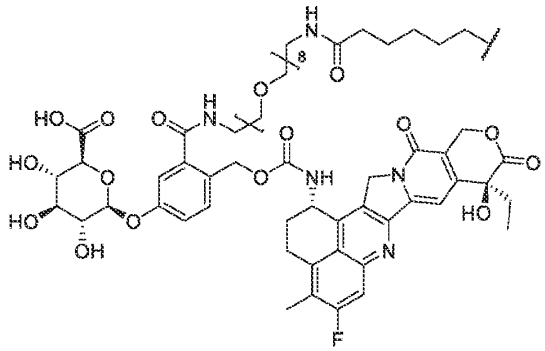
760. The method of any one of Embodiments 715-729, wherein each $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



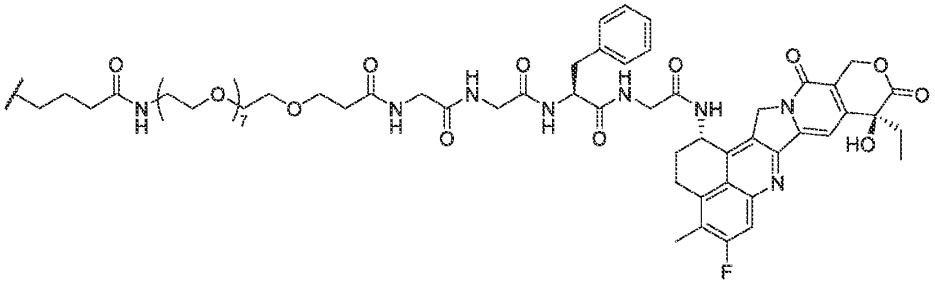
761. The method of any one of Embodiments 715-729, wherein an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



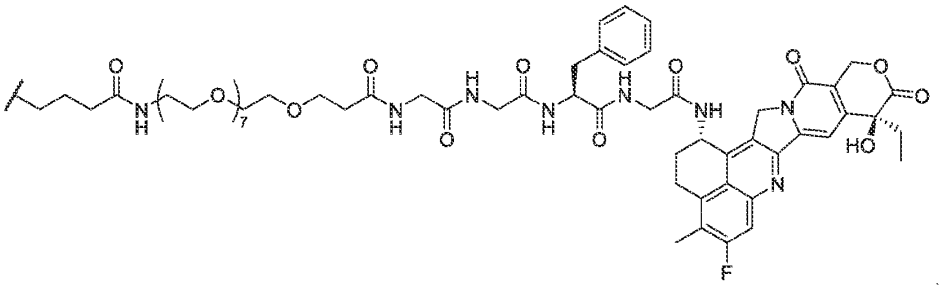
762. The method of any one of Embodiments 715-729, wherein each $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



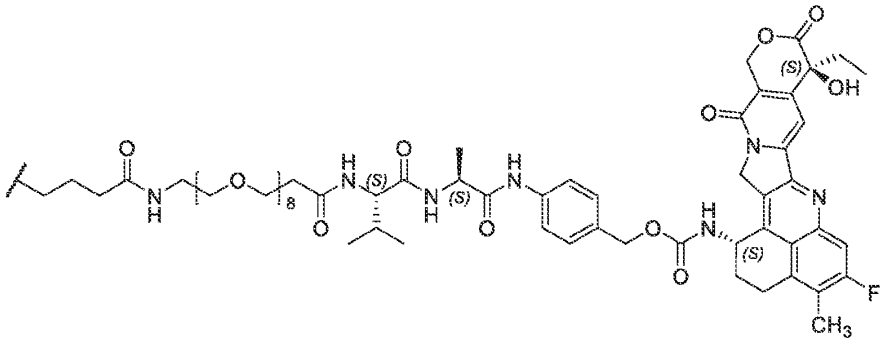
763. The method of any one of Embodiments 715-729, wherein an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



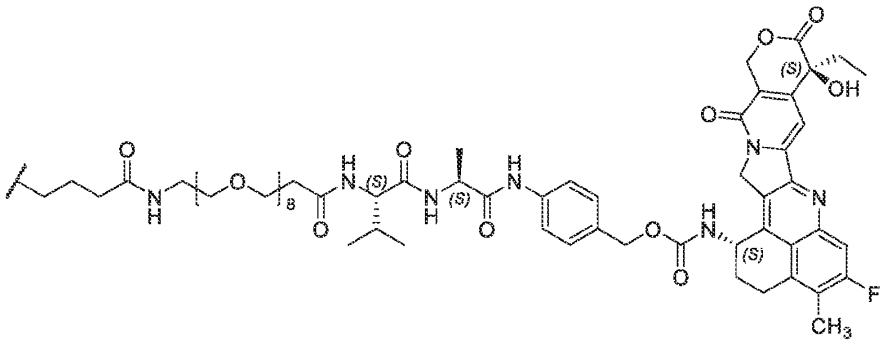
764. The method of any one of Embodiments 715-729, wherein each $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



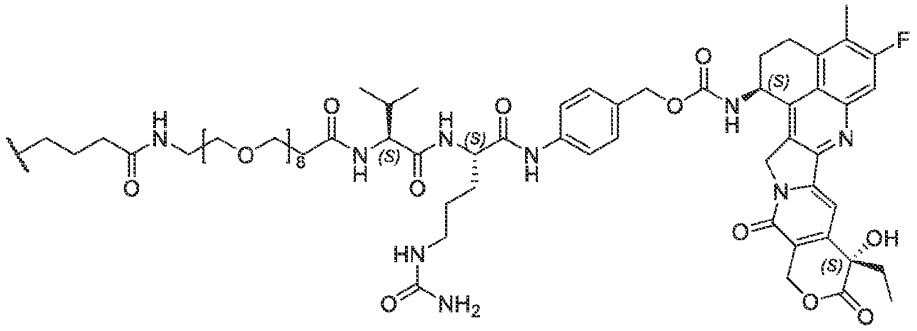
765. The method of any one of Embodiments 715-729, wherein an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



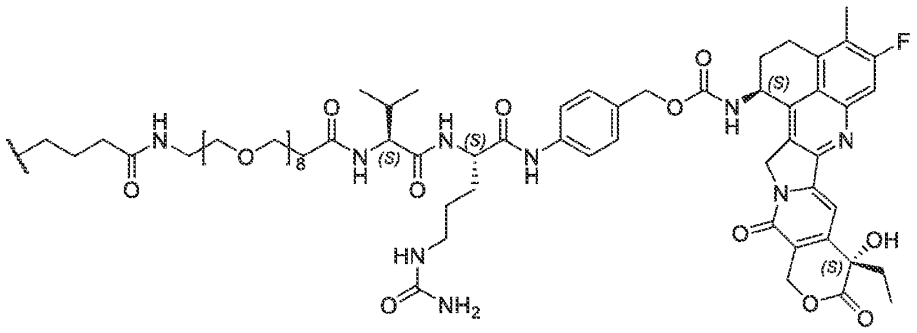
766. The method of any one of Embodiments 715-729, wherein each $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



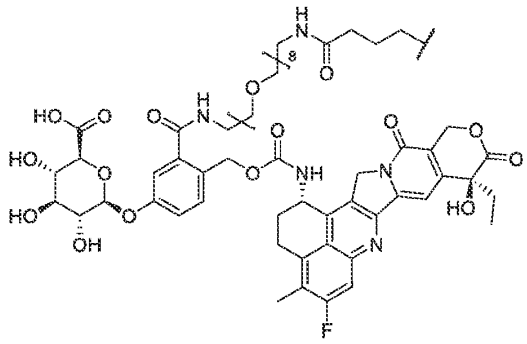
767. The method of any one of Embodiments 715-729, wherein an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



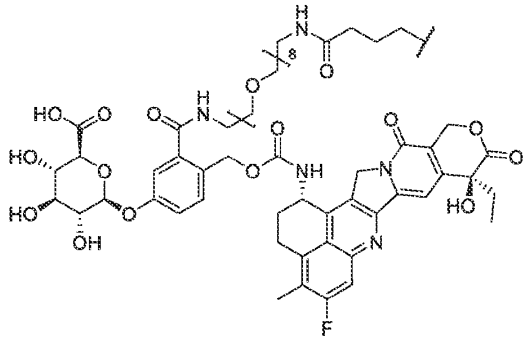
768. The method of any one of Embodiments 715-729, wherein each $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



769. The method of any one of Embodiments 715-729, wherein an occurrence of $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



770. The method of any one of Embodiments 715-729, wherein each $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-R^P$ is or comprises



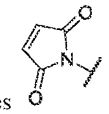
771. The method of any one of Embodiments 709-770, wherein the target binding agent reactive group is or comprises −SH.

772. The method of any one of Embodiments 709-770, wherein the target binding agent reactive group is or comprises −SH of a cysteine residue.

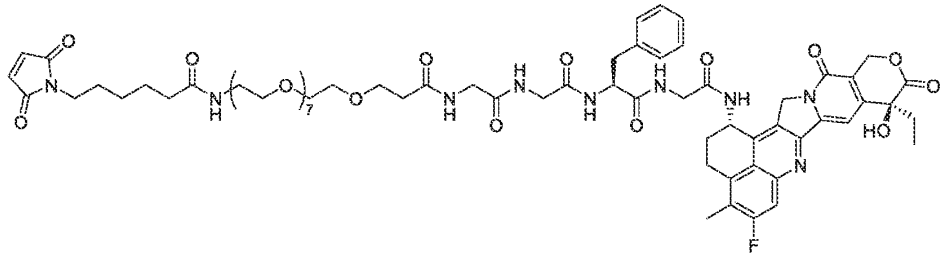
773. The method of any one of Embodiments 769-772, comprising reducing a −S−S− moiety in the target binding agent to provide −SH.

774. The method of any one of Embodiments 769-773, wherein the target binding moiety is or comprises an antibody or an antigen-binding fragment thereof.

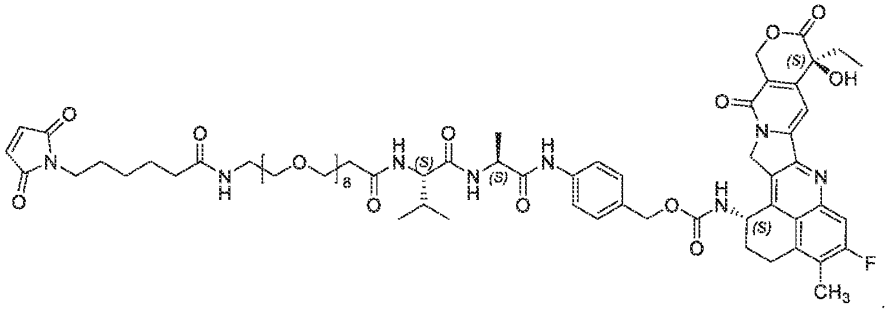
775. The method of any one of Embodiments 709-774, wherein the payload agent reactive group is or comprises −SH.

776. The method of any one of Embodiments 709-775, wherein the payload agent reactive group is or comprises  .

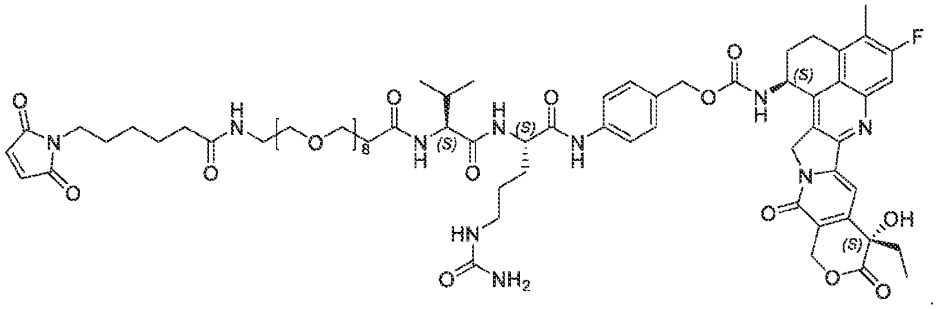
777. The method of Embodiment 776, wherein the payload agent is



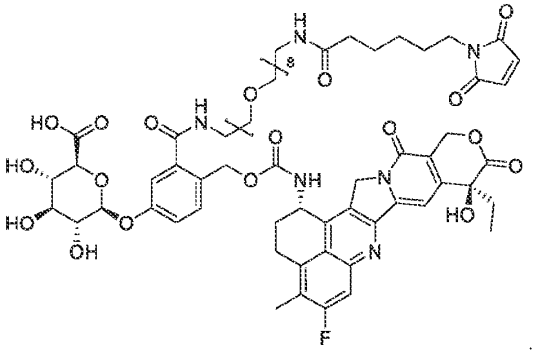
778. The method of Embodiment 776, wherein the payload agent is



779. The method of Embodiment 776, wherein the payload agent is



780. The method of Embodiment 776, wherein the payload agent is



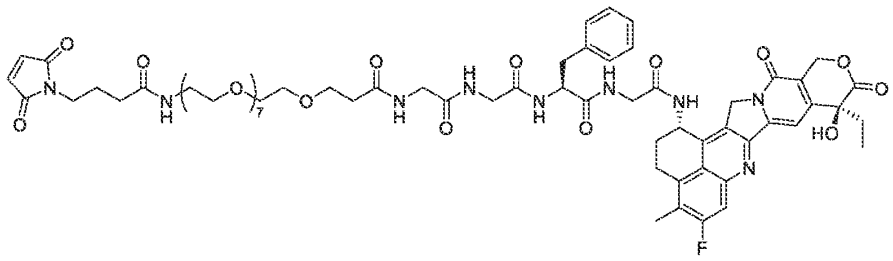
781. The method of Embodiment 776, wherein the payload agent is LP-A.

782. The method of Embodiment 776, wherein the payload agent is LP-B.

783. The method of Embodiment 776, wherein the payload agent is LP-C.

784. The method of Embodiment 776, wherein the payload agent is LP-D.

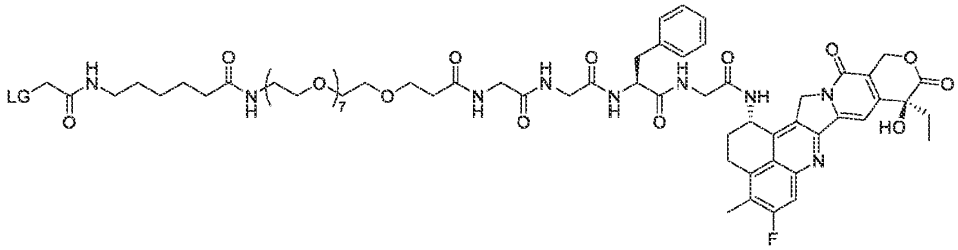
785. The method of Embodiment 776, wherein the payload agent is



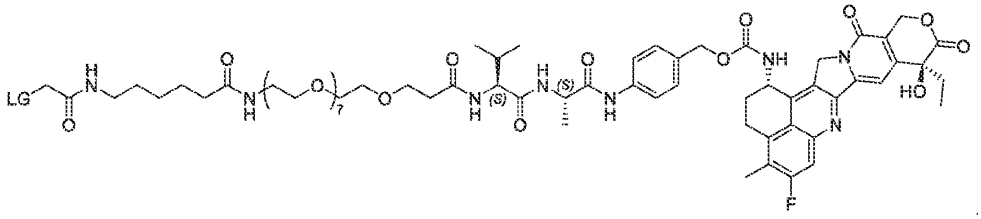
786. The method of any one of Embodiments 709-775, wherein the payload agent reactive group is or comprises LG–CH_2–EWG–, wherein LG is a leaving group, the –CH_2– is optionally substituted, EWG is an electron-withdrawing group.

787. The method of Embodiment 786, wherein the payload agent reactive group is or comprises LG–CH_2–C(O)–.

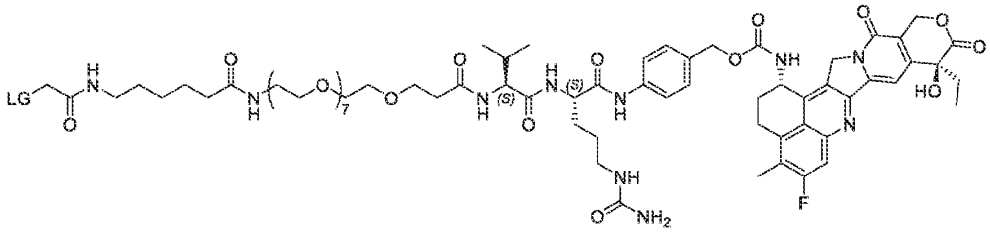
788. The method of any one of Embodiments 786-799, wherein the payload agent is



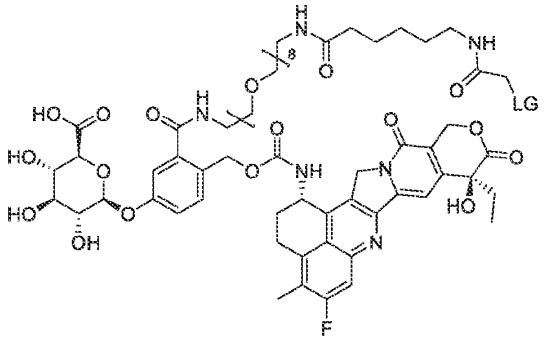
789. The method of any one of Embodiments 786-799, wherein the payload agent is



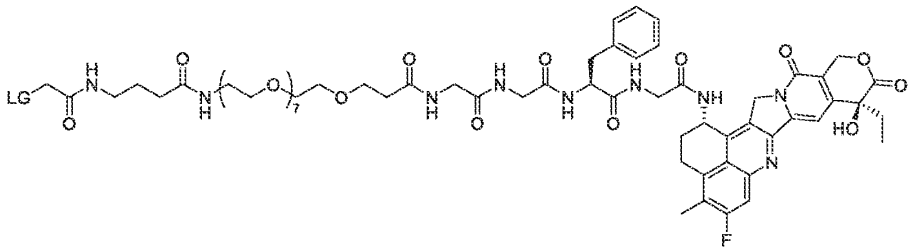
790. The method of any one of Embodiments 786-799, wherein the payload agent is



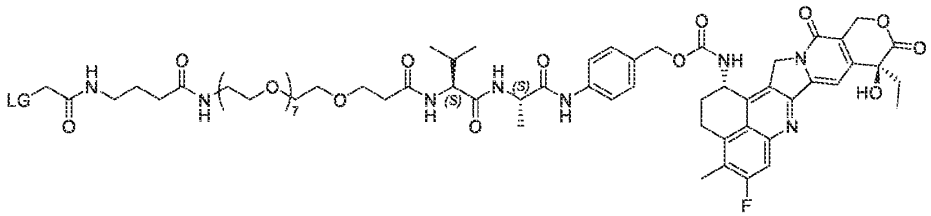
791. The method of any one of Embodiments 786-799, wherein the payload agent is



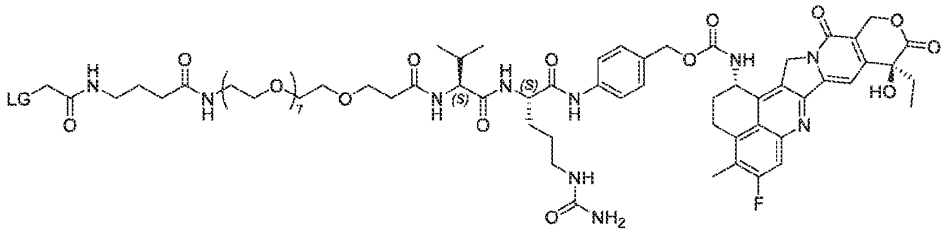
792. The method of any one of Embodiments 786-799, wherein the payload agent is



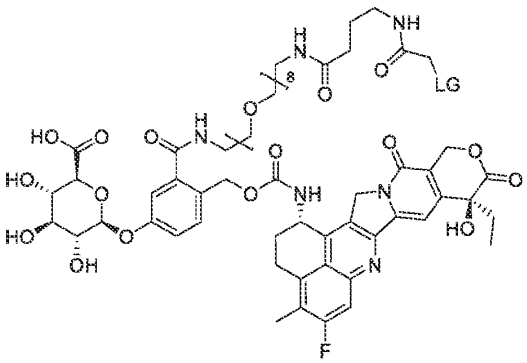
793. The method of any one of Embodiments 786-799, wherein the payload agent is



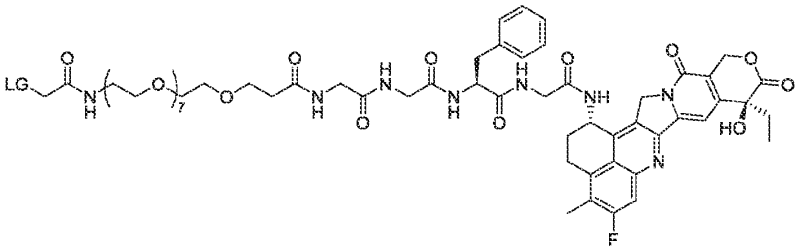
794. The method of any one of Embodiments 786-799, wherein the payload agent is



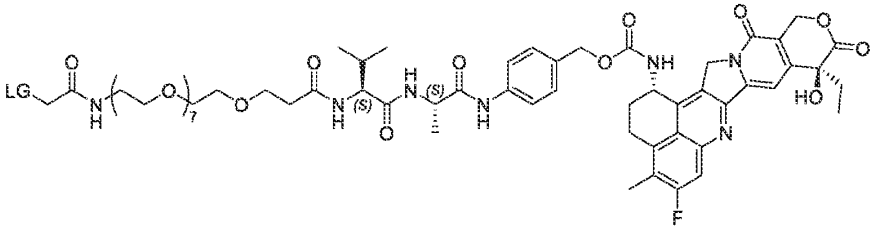
795. The method of any one of Embodiments 786-799, wherein the payload agent is



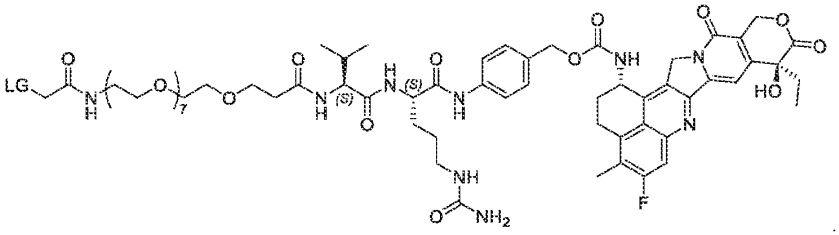
796. The method of any one of Embodiments 786-799, wherein the payload agent is



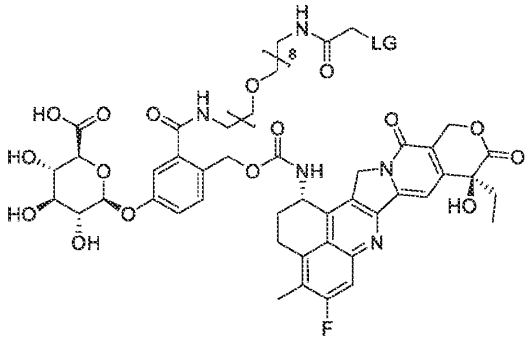
797. The method of any one of Embodiments 786-799, wherein the payload agent is



798. The method of any one of Embodiments 786-799, wherein the payload agent is



799. The method of any one of Embodiments 786-799, wherein the payload agent is



800. The method of any one of Embodiments 786-799, wherein LG is –Cl.

801. The method of any one of Embodiments 786-799, wherein LG is –Br.

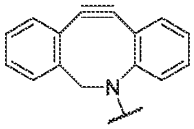
802. The method of any one of Embodiments 786-799, wherein LG is –I.

803. The method of any one of Embodiments 709-770, wherein the target binding agent reactive group is or comprises $-N_3$.

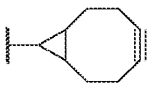
804. The method of Embodiment 803, wherein the payload agent reactive group comprises –C≡C–.

805. The method of Embodiment 803, wherein the payload agent reactive group is or comprises –C≡CH.

806. The method of Embodiment 803, wherein the payload agent reactive group is or comprises



807. The method of Embodiment 803, wherein the payload agent reactive group is or comprises

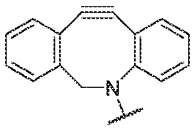


808. The method of any one of Embodiments 709-770, wherein the payload agent reactive group is or comprises $-N_3$.

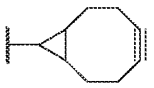
809. The method of Embodiment 808, wherein the target binding agent reactive group comprises –C≡C–.

810. The method of Embodiment 808, wherein the target binding agent reactive group is or comprises –C≡CH.

811. The method of Embodiment 808, wherein the target binding agent reactive group is or comprises



812. The method of Embodiment 808, wherein the target binding agent reactive group is or comprises



813. The method of any one of Embodiments 709-812, wherein L^{RXT} and L^{RXP} react to form L^{RX}, wherein L^{RX} is as described in any one of Embodiments 201-227.

INCORPORATION BY REFERENCE

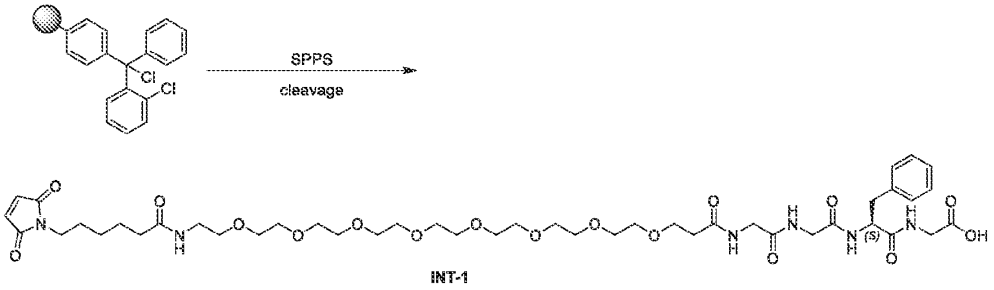
[0704] All publications, patent applications, patents, and other references mentioned herein, including GenBank Accession Numbers, are incorporated by reference in their entirety; in case of a conflict, the present disclosure will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which a provided technology belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present technologies, certain suitable methods and materials are described herein as examples.

[0705] The disclosure is further illustrated by the following example. An example is provided for illustrative purposes only. It is not to be construed as limiting the scope or content of the disclosure in any way.

EXAMPLES

[0706] The following examples are provided so as to describe to the skilled artisan how to make and use methods and compositions described herein and are not intended to limit the scope of the present disclosure.

[0707] Example 1-1. Procedure for preparation of INT-1.



INT-1

[0708] Peptide Synthesis. The peptide was synthesized using standard Fmoc chemistry.

[0709] Resin preparation: To a solution of 2-CTC Resin (1.00 mmol, 1.00 eq, Sub 1.03 mmol/g) and Fmoc-Gly -OH (1.00 eq) in DCM (10.0 mL) was added DIEA (6.00 eq), the mixture was agitated with N2 at 25 °C for 2 hr. The MeOH (0.97 mL) was added in the resin and agitated with N2 at 25 °C for 0.5 hr. Then the mixture was filtered, and the resin was washed with DMF (100 mL x 5).

[0710] Deprotection: 20% piperidine in DMF (50.0 mL) was added, and the resin was agitated with N2 at 25 °C for 30 mins. The resin was washed with DMF (100 mL x 5) and filtered.

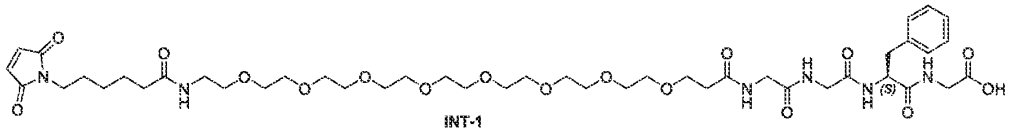
[0711] Coupling: A solution of HATU (2.85 eq) and Fmoc-Phe-OH (3.00 eq) in DMF (10.0 mL) was added to the resin, then the DIEA (6.00 eq) was added, the mixture was agitated with N2 at 25 °C for 30 mins. The resin was washed with DMF (50 mL x 5).

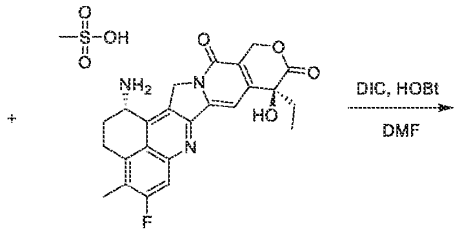
[0712] Repeat above step 2 to 3 for the coupling of following amino acids: (2-5)

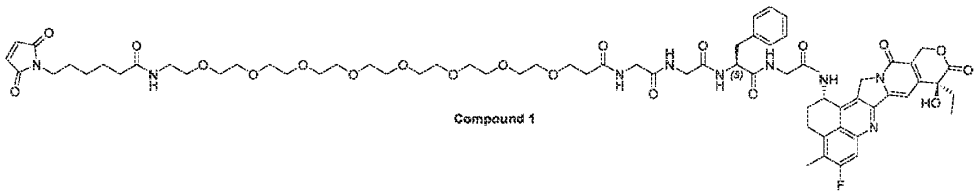
#	Materials	Coupling reagents
1	Fmoc-Gly-OH (1.00 eq)	DIEA (6.00 eq)
2	Fmoc-Phe -OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)
3	Fmoc-Gly-Gly-OH (3.00 eq)	HATU (2.85 eq) and DIEA (6.00 eq)
4	Fmoc-PEG8-OH (2.00eq.)	HATU (1.9 eq) and DIEA (4.00 eq)
5	6-Maleimidocaproic acid (2.00 eq.)	HOAT (2.00 eq.) and DIC (2.00 eq.)

[0713] Peptide Cleavage and Purification. Cleavage buffer (20% HFIP/DCM) was added to the flask containing the side chain protected peptide at room temperature, and the flask was stirred for 0.5 hr x 3. The resin was filtered, and the filtrate was collected. The solvent was removed under vacuum to afford the crude peptide (1.80 g), which was confirmed via LC/MS. The crude peptide was purified by prep-HPLC (Gradient of Solvent A: 0.075 % TFA in H_2O; and Solvent B: ACN) to give the final product INT-1 (240 mg, 250 μmol, 25.0% yield) as a colorless oil.

[0714] Example 1-2. Procedure for preparation of Compound 1.



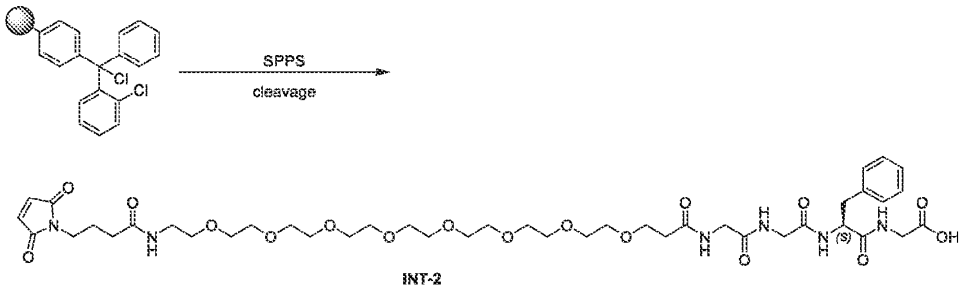




[0715] To a solution of INT-1 (220 mg, 231 μmol), Exatecan (123 mg, 231 μmol) in DMF (3.0 mL) was added HOBt (37.4 mg, 277 μmol) and DIC (233 mg, 1.85 mmol, 286 μL). The mixture was stirred at 25 °C for 24 hrs. LC-MS showed INT-1 was consumed completely and one main peak with desired mass was detected. H_2O/MeCN was added to the reaction, and it was centrifuged to give a solution. The

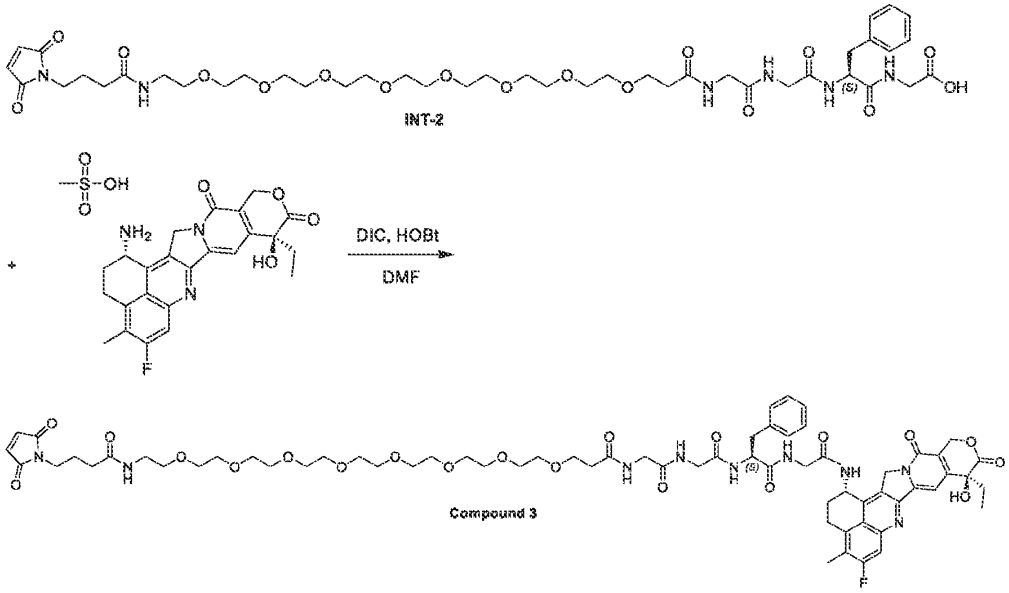
solution was purified by prep-HPLC (Gradient of Solvent A: 0.075 % TFA in H_2O; and Solvent B: ACN) to give Compound 1 (110 mg, 79.0 μmol, 34.2% yield) as a pale yellow solid.

[0716] Example 1-3. Int-2.



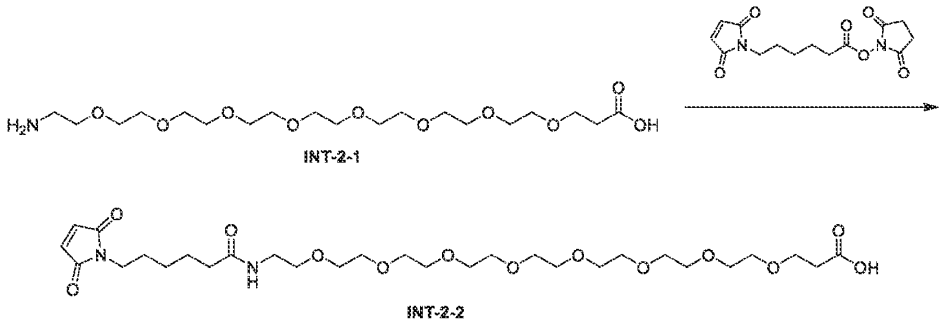
[0717] INT-2 was prepared using the protocol described in Example 1-1.

[0718] Example 1-4. Compound 3.



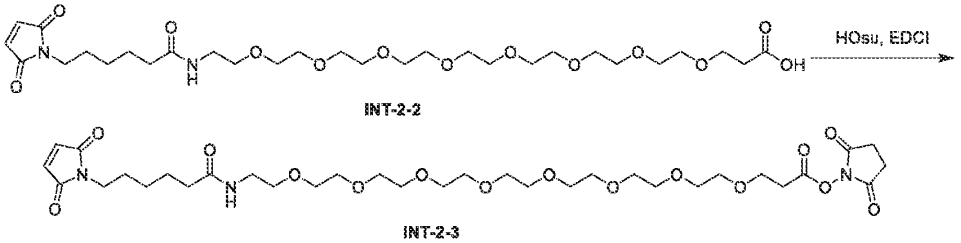
[0719] Compound 2 was prepared using the protocol described in Example 1-2.

[0720] Example 2-1. Procedure for preparation of INT-2-2.



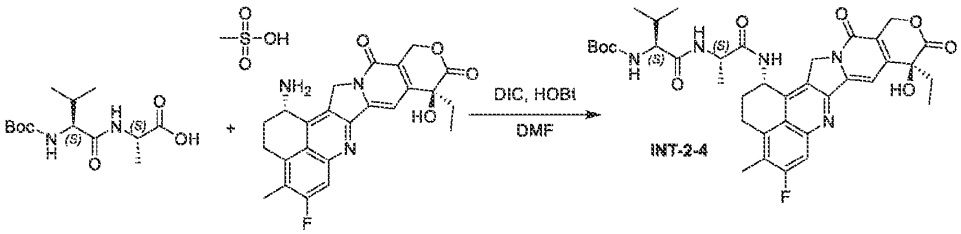
[0721] To a solution of INT-2-1 (1.00 g, 2.26 mmol) in DMF (5.0 mL) was added DIEA (878 mg, 6.79 mmol, 1.18 mL) and 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (698 mg, 2.26 mmol). The mixture was stirred at 25 °C for 2 hrs. LC-MS showed INT-2-1 was consumed completely and one main peak with desired mass was detected. The reaction mixture was purified by prep-HPLC (Gradient of Solvent A: 0.1 % TFA in H_2O; and Solvent B: ACN) to give INT-2-2 (500 mg, 766 μmol, 33.8% yield) as a brown oil.

[0722] Example 2-2. Procedure for preparation of INT-2-3.



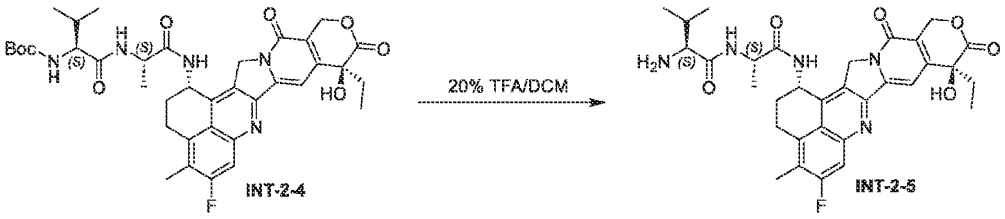
[0723] To a solution of INT-2-2 (500 mg, 788 μmol), HOSu (118 mg, 1.02 mmol8) in DCM (5.0 mL) was added EDCI (3028 mg, 1.58 mmol8). The mixture was stirred at 25 °C for 16 hrs. LC-MS showed few INT-2-2 remained, and one main peak with desired mass was detected. The reaction mixture was washed with HCl (2.0 mL) and sat. brine (3.0 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to give INT-2-3 (540 mg, 629 μmol, 79.9% yield) as a brown oil.

[0724] Example 2-3. Procedure for preparation of INT-2-4.



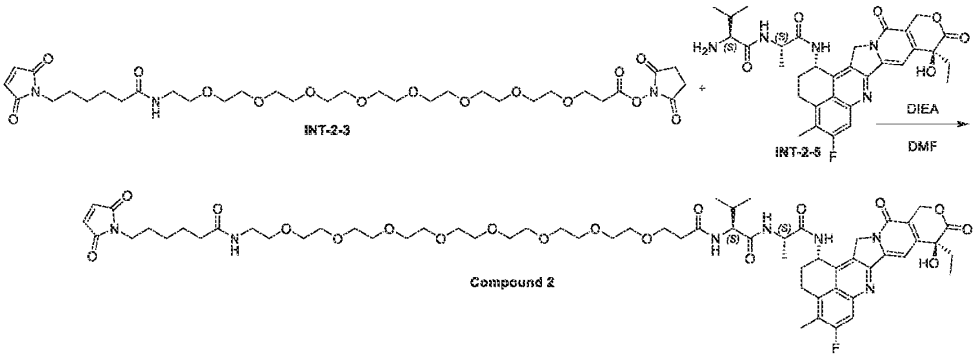
[0725] To a solution of (tert-butoxycarbonyl)-L-valyl-L-alanine (200 mg, 694 μmol), Exatecan (294.96 mg, 554.90 μmol, 0.8 eq) in DMF (4.5 mL) was added HOBt (112 mg, 832 μmol) and DIC (525 mg, 4.16 mmol, 644 μL). The mixture was stirred at 25 °C for 2 hrs. LC-MS showed that the (tert-butoxycarbonyl)-L-valyl-L-alanine was consumed completely, and one main peak with desired mass was detected. H2O/MeCN was added to the reaction, and it was centrifuged to give a solution. The solution was purified by prep-HPLC (Gradient of Solvent A: 0.1 % TFA in H2O; and Solvent B: ACN) to give INT-2-4 (200 mg, 242 μmol, 34.9% yield, contained 13.4% of diastereomer) as a yellow solid.

[0726] Example 2-4. Procedure for preparation of INT-2-5.



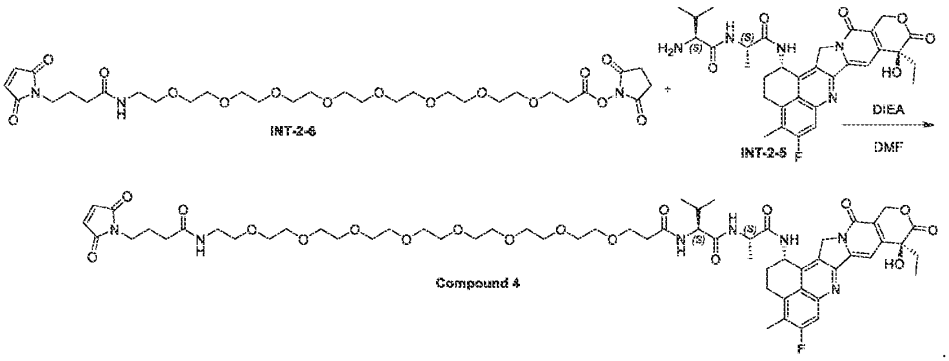
[0727] To a solution of INT-2-4 (200 mg, 283 μmol) in DCM (3.2 mL) was added TFA (1.23 g, 10.8 mmol, 800 μL). The mixture was stirred at 25 °C for 1 hr. LC-MS showed INT-2-4 was consumed completely, and one main peak with desired mass was detected. The reaction mixture was concentrated under reduced pressure to give INT-2-5 (170 mg, crude) as a yellow oil.

[0728] Example 2-5. Procedure for preparation of Compound 2.



[0729] To a solution of INT-2-3 (205 mg, 281 μmol), INT-2-5 (170 mg, 281 μmol) in DMF (4.0 mL) was added DIEA (218 mg, 1.68 mmol, 293 μL). The mixture was stirred at 25 °C for 1 hr. LC-MS showed INT-2-3 was consumed completely, and one main peak with desired mass was detected. The reaction mixture was purified by prep-HPLC (Gradient of Solvent A: 0.1 % TFA in H2O; and Solvent B: ACN) twice to give Compound 2 as a white solid.

[0730] Example 2-6. Compound 4.



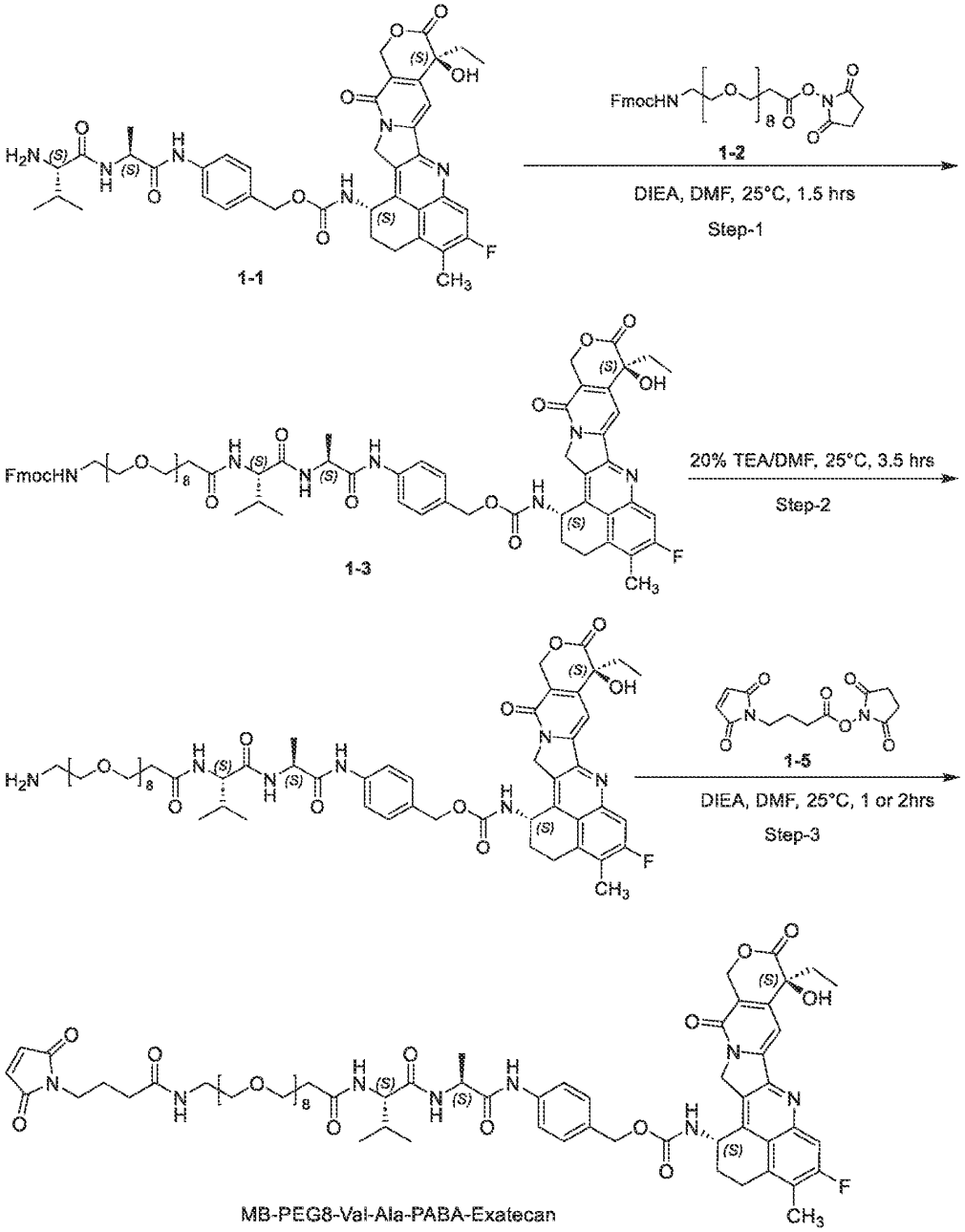
[0731] Compound 4 was prepared using protocol described in Examples 2-1 to 2-5.

[0732] Example 2-7. Preparation of additional compounds.

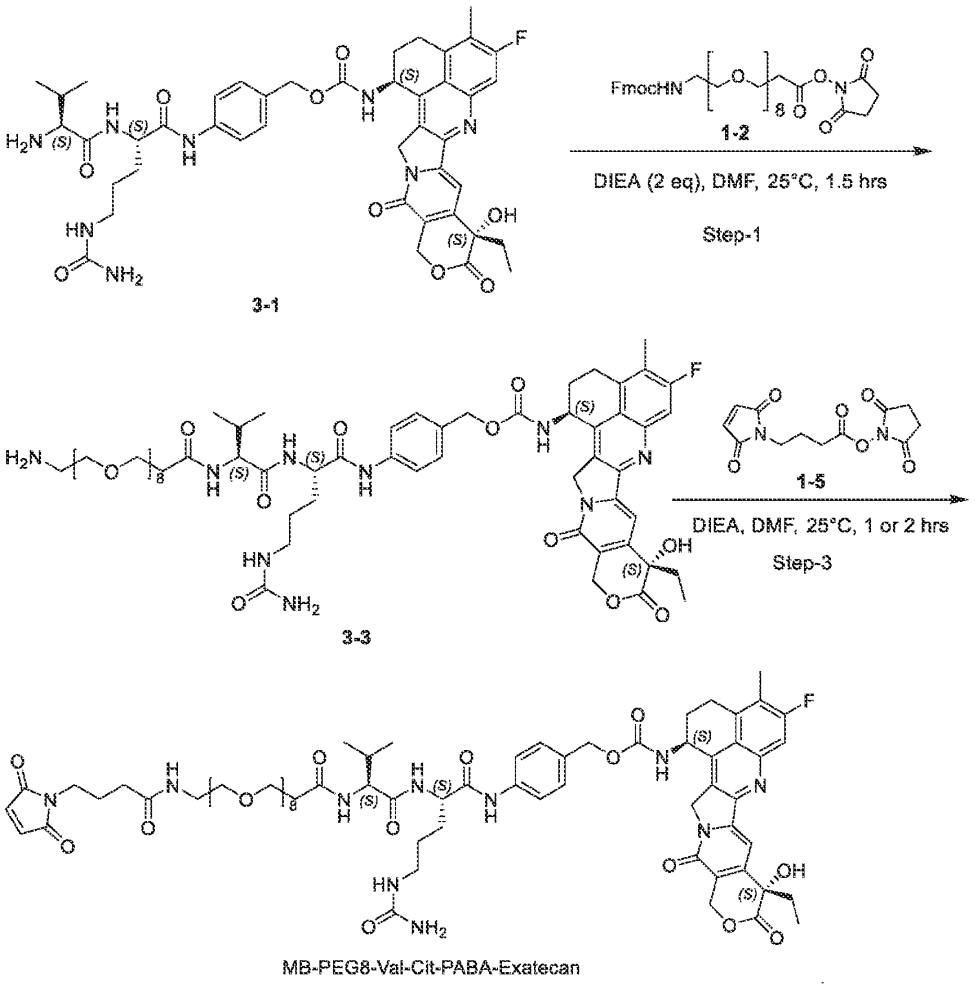
[0733] Additional compounds were prepared utilizing various technologies including certain

technologies described above. Several preparations are presented below as examples.

[0734] MB-PEG8-Val-Ala-PABA-Exatecan (Compound 5; prepared with about 95% or more purity):



[0735] MB-PEG8-Val-Cit-PABA-Exatecan (Compound 6; prepared with about 95% or more purity):

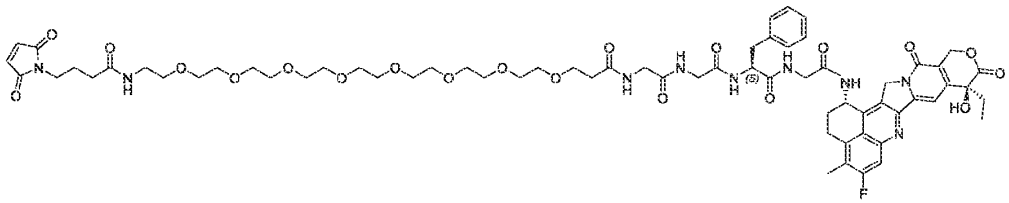


[0736] Example 3. General Procedure for Producing Conjugates.

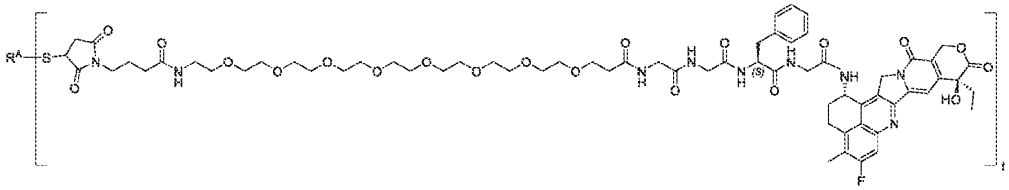
[0737] To a solution of an anti-HERV-H LTR-Associating 2 (HHLA2) antibody or antigen-binding fragment thereof, TCEP is added, and the resulting mixture is incubated at 22 °C for 3 h. Linker-payload reagent (e.g., Compound 1 or Compound 2) is dissolved in DMA (10 mM) and is added to the reduced anti-HERV-H LTR-Associating 2 (HHLA2) antibody or antigen-binding fragment thereof solution in excess. The resulting conjugation reaction is carefully mixed and incubated at 22 °C for 1 h. The reaction mixture is then rapidly purified by desalting column, and then buffer exchanged to formulation buffer to remove free linker-payload reagent.

[0738] As those skilled in the art appreciate, TCEP amount relative to antibodies can be adjusted to achieve conjugates and compositions thereof with different DARs.

[0739] Among others, antibodies Hu2C4-H3L3, 65872, and 887-1DE, and human IgG1 were conjugated with the compound below ("LP-D")to provide compositions with DAR about 4 and/or DAR about 8, respectively, and were utilized in various Examples below.



[0740] In some embodiments, a conjugate has the structure of



or a pharmaceutically acceptable salt thereof. In some embodiments, R^A is Hu2C4-H3L3, each −S− is independently of a Cys residue, and t is about 4. In some embodiments, R^A is Hu2C4-H3L3, each −S− is independently of a Cys residue, and t is about 8. In some embodiments, R^A is 65872, each −S− is independently of a Cys residue, and t is about 4. In some embodiments, R^A is 65872, each −S− is independently of a Cys residue, and t is about 8. In some embodiments, R^A is 887-1DE, each −S− is independently of a Cys residue, and t is about 4. In some embodiments, R^A is 887-1DE, each −S− is independently of a Cys residue, and t is about 8. In some embodiments, the present disclosure provides a composition, e.g., a pharmaceutical composition, comprising such a conjugate. In some embodiments, DAR of a composition is about 4 (e.g., about 3-5, 3.5-4.5, 3.6-4.4, 3.7-4.3, 3.8-4.2, etc.). In some embodiments, DAR of a composition is about 8 (e.g., about 7-9, 7.5-8.5, 7.6-8.4, 7.7-8.3, 7.8-8.2, 7.5-8.4, 7.6-8.3, 7.5-8.3, 7.6-8.2, 7.7-8.2, 7.7-8.1, etc.). In some embodiments, a composition comprises an unconjugated target binding moiety, e.g., an antibody such as Hu2C4-H3L3, 65872, 887-1DE, etc., and a conjugate. In some embodiments, in a composition there are various conjugate with different t values. For example, for a composition with DAR about 4, there may be compounds with t being 0 (unconjugated), 1, 2, 3 and 4.

[0741] Example 4. Provided conjugates bind to target cells.

[0742] Among other things, the present disclosure confirms that provided conjugates can bind to targets. For example, as described herein, anti-HHLA2 antibody ADCs can bind to HHLA2+ A 204 cells. $5x10^5$ A204 cells were treated with the anti-HHLA2 ADC Hu2C4-H3L3, 65872, and 887-1DE antibodies and conjugates at concentrations ranging from 20 to 0.000256 nM in 100 µL PBS containing 1% BSA for 1 hour at 4°C. For each antibody, the unconjugated antibody, and Exatecan_DAR4 (DAR about 4) and Exatecan_DAR8 (DAR about 8) conjugates were tested. Cells were then washed once in PBS + 1% BSA and stained with PE-conjugated goat anti-human IgG for 30 minutes at 4°C. Cells were then washed once in PBS + 1% BSA and antibody binding was analyzed by flow cytometry. As seen in Figures 1A, 1B and 1C, all the antibody drug conjugates (Hu2C4-H3L3, 65872, and 887-1DE) bound to HHLA2 endogenously expressed on A204 cells with similar EC_{50}s to the naked antibodies. Binding EC_{50}s are listed in Table 2.

[0743] Table 2. On-cell binding EC_{50}s of anti-HHLA2 antibody drug conjugates to HHLA2+ A204 Cells.

Variant	EC_{50} (nM)		
	887-1DE	Hu2C4-H3L3	65872
---	0.059	0.14	0.018
DAR-4	0.049	0.11	0.011
DAR-8	0.047	0.12	0.016

[0744] Example 5. Provided conjugates can be internalized.

[0745] Among other things, the present disclosure confirms that provided conjugates can be internalized. For example, as demonstrated in this Example, anti-HHLA2 antibodies induce HHLA2 internalization on HHLA2+ A204 tumor cells. In an example, Hu2C4-H3L3, 65872, 887-1DE, and human IgG1 isotype control antibodies were conjugated with Fabfluor-pH at 20 ug/mL. A204 cells were plated at $1.5x10^4$ cells per well for 4h at 37 °C. Cells were then treated with conjugated antibodies at a concentration of 0.1 ug/mL. Antibody internalization was monitored by Incucyte scanner and recorded every 15 min during 48h. Internalization was determined by intensity of fluorescence on the cell surface over time. As shown in Figure 2, all anti-HHLA2 antibodies induced internalization.

[0746] Example 6. Provided technologies can kill target cells.

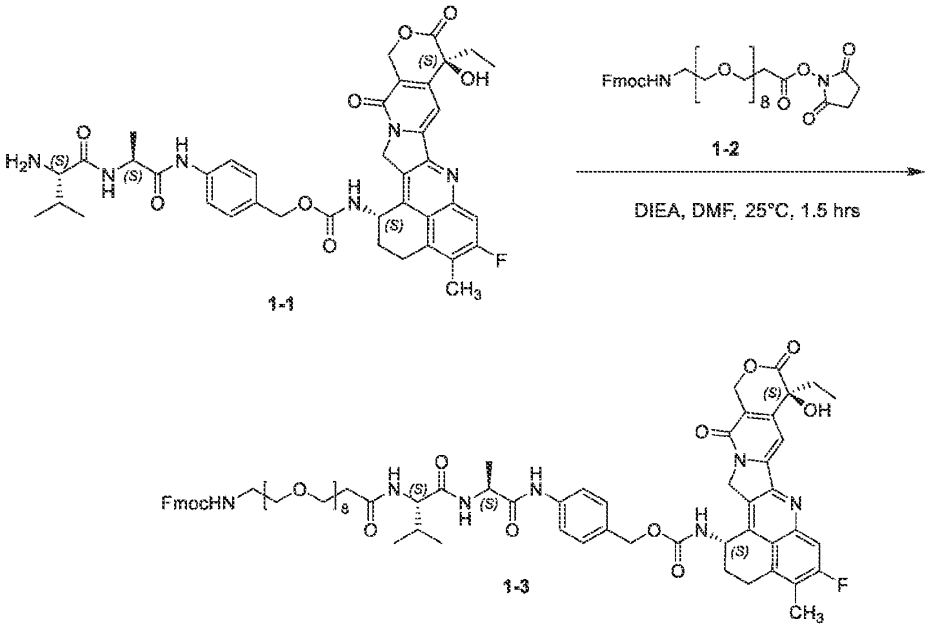
[0747] Among other things, the present disclosure confirms that provided technologies can deliver payloads to target cells and, in case payloads are cytotoxic agents, kill target cells. In an example, HEK293 cells engineered to express HHLA2 were seeded into the wells of flat-bottom 96-well plates at a density of $1x10^4$ cells/well and allowed to adhere overnight. The next day HEK293-HHLA2 cells were treated with anti-HHLA2 antibody drug conjugates (Hu2C4-H3L3, 65872, and 887-1DE) at concentrations ranging from 500 to 0.0064 nM for 5 days at 37°C. For each antibody, Exatecan_DAR4 (DAR about 4) and Exatecan_DAR8 (DAR about 8) conjugates were tested. Some cells were also treated with Exatecan_DAR8 (DAR about 8) human IgG1 isotype control. After 5 days, cell killing was assessed using the CellTiter Glo Luminescent Cell Viability Assay. As shown in Figures 3A and 3B, all anti-HHLA2 antibody drug conjugates induce dose dependent killing of HHLA2+ HEK293 cells. Cell killing IC_{50}s are listed in Table 3. Provided technologies can also effectively reduce toxicity of payloads (see, e.g., IgG1-Exatecan_DAR8 and Exatecan in Figures 3A and 3B).

[0748] Table 3. In Vitro Cell Killing IC_{50}s of Anti-HHLA2 Antibody Drug Conjugates to HHLA2+ HEK293 Cells

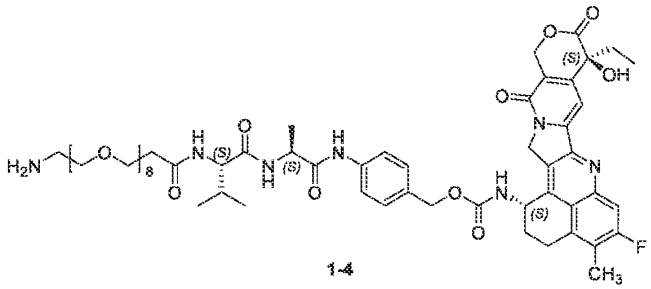
Variant	IC_{50} (nM)		
Exatecan	0.29		
	887-1DE	Hu2C4-H3L3	65872
DAR-4	0.044	0.039	0.157
DAR-8	0.036	0.035	0.086

[0749] Example 7. Procedure for synthesis of Additional Linker-Payload Compounds

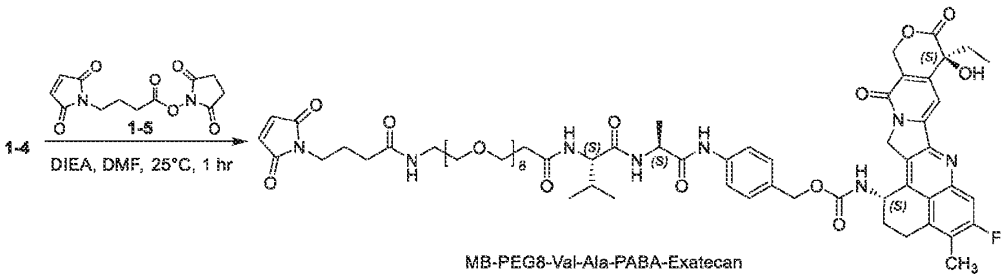
[0750] Compound LP-A: MB-PEG8-Val-Ala-PABA-Exatecan.



[0751] To a solution of compound 1-1 (70 mg, 92.74 μmol, 1 *eq.*) and compound 1-2 (70.56 mg, 92.74 μmol, 1 *eq.*) in DMF (1 mL) DIEA (23.97 mg, 185.48 μmol, 32.31 μL, 2 *eq.*) was added. The mixture was stirred at 25 °C for 1.5 hrs. The reaction was monitored by LCMS (MS cal.: 1399.6, MS observed: $[M+H]^+$ = 1400.7). The reaction mixture was slowly dropped into the isopropyl ether. The compound 1-3 (129 mg, crude) was obtained as a gray solid and it was confirmed by LCMS and HPLC.



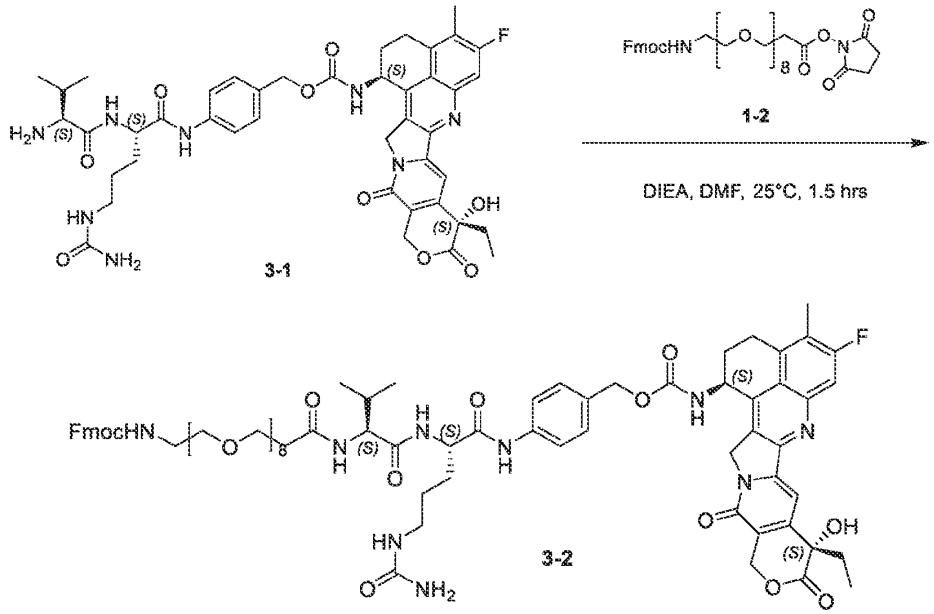
[0752] 20% TEA was added to a solution of compound 1-3 (129 mg, 92.11 μmol, 1 *eq.*) in DMF (1 mL). The mixture was stirred at 25 °C for 3.5 hrs. The reaction was monitored by LCMS 1 (MS cal.: 1178.5, MS observed: $[M+H]^+$ = 1179.0) and HPLC. The pH was adjusted and the resultant was directly purified by prep-HPLC. The compound 1-4 (59 mg, 50.1 μmol, 54.4% yield) was obtained as a yellow solid and confirmed by LCMS and HPLC (purity: 93.6% purity).



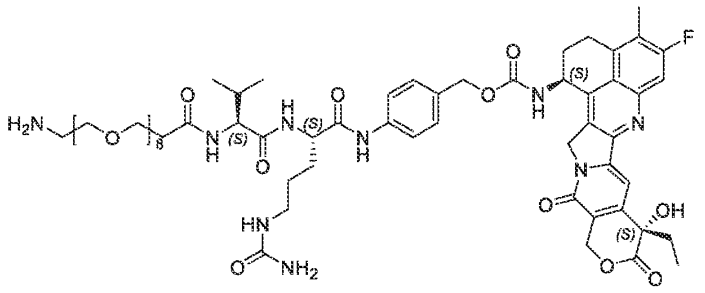
[0753] DIEA (12.9 mg, 100 μmol, 17.4 μL, 2 *eq.*) was added to a solution of compound 1-4 (59 mg, 50.1 μmol, 1 *eq.*) and compound 1-5 (21.1 mg, 75.1 μmol, 1.5 *eq.*) in DMF (0.5 mL). The mixture was stirred at 25 °C for 1 hr. The reaction was monitored by LCMS (MS cal.:1343.4, MS observed: $[M+H]^+$ = 1344.2). The resultant was purified by prep-HPLC. MB-PEG8-Val-Ala-PABA-Exatecan (9 mg, 6.7 μmol, 13.3% yield, 99.5% purity) was obtained as a yellow solid.

[0754] Example 8. Procedure for synthesis of Additional Linker-Payload Compounds.

[0755] Compound LP-B: MB-PEG8-Val-Cit-PABA-Exatecan.

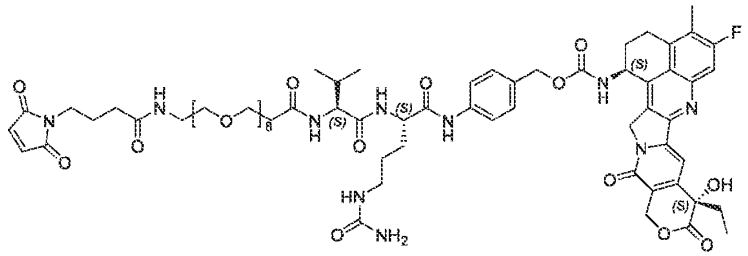


[0756] To a solution of compound 3-1 (70 mg, 83.2 μmol, 1 *eq.*) and compound 1-2 (60.2 mg, 79.1 μmol, 0.95 *eq.*) in DMF (1 mL) DIEA (21.5 mg, 166 μmol, 29.0 μL, 2 *eq.*) was added. The mixture was stirred at 25 °C for 1.5 hrs. The reaction was monitored by LCMS (MS cal.: 1485.6, MS observed: $[M+H]^+$ = 1486.9). The resultant mixture was dropped into isopropyl ether to get compound 3-2 (123 mg, crude). The crude was obtained as a gray solid and confirmed by LCMS and HPLC (purity: 92.4%.).



3-3

[0757] 20% TEA was added to a solution of compound 3-2 (123 mg, 82.7 μmol, 1 *eq.*) in DMF (1 mL). The mixture was stirred at 25 °C for 3.5 hrs. The reaction was monitored by LCMS (MS cal.: 1263.6, MS observed: $[M+H]^+$ = 1264.7). The pH of the resultant was adjusted by TFA, and it was purified by prep-HPLC. The compound 3-2 (64.7 mg, 51.2 μmol, 61.9% yield) was obtained as a yellow solid and confirmed by LCMS and HPLC (purity: 87.5%.).

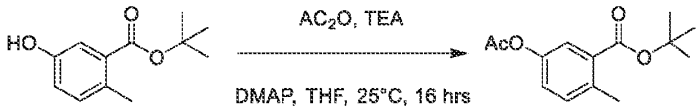


MB-PEG8-Val-Cit-PABA-Exatecan

[0758] DIEA (13.2 mg, 102 μmol, 17.8 μL, 2 *eq.*) was added to a solution of compound 3-3 (64.7 mg, 51.2 μmol, 1 *eq.*) and compound 1-5 (21.5 mg, 76.8 μmol, 1.5 *eq.*) in DMF (0.5 mL). The mixture was stirred at 25 °C for 1 hr. The reaction was monitored by LCMS (MS cal.:1428.6, MS observed: $[M+H]^+$ = 1429.7). The residue was directly purified by prep-HPLC. The compound MB-PEG8-Val-Cit-PABA-Exatecan (LP-B) (6 mg, 4.2 μmol, 7.6% yield, 96.0% purity, TFA) was obtained as a yellow solid and confirmed by LCMS.

[0759] Example 9. Procedure for synthesis of Additional Linker-Payload Compounds.

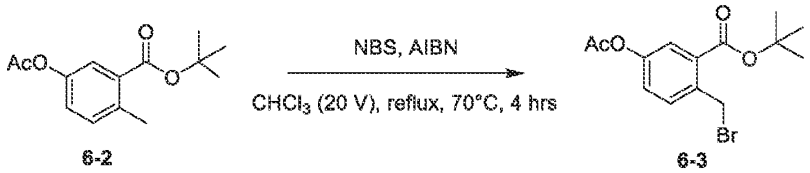
[0760] LP-C. Exatecan glucuonide.



6-1 6-2

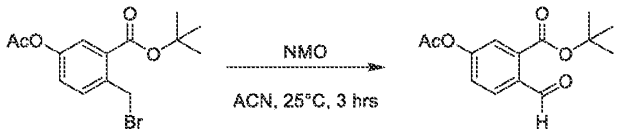
[0761] To a solution of compound 6-1 (5 g, 24.0 mmol, 1 *eq.*) in THF (100 mL, 20 Vol) TEA (7.29 g, 72.0 mmol, 10.0 mL, 3 *eq.*), DMAP (293 mg, 2.40 mmol, 0.1 *eq.*) and Ac_2O (3.68 g, 36.0 mmol, 3.38 mL, 1.5 *eq.*) were addred succesively. The mixture was stirred at 25 °C for 16 hrs. The reaction was monitored by TLC (DCM: MeOH= 10:1, R_f=0.8). The reaction mixture was quenched by addition of 1M

HCl (50 mL), and extracted with EtOAc 200 mL (100 mL * 2). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give compound 6-2 (6.137 g, crude) as a colorless oil. The structure was confirmed by LCMS (MS cal.: 250.2, MS observed: $[M+H_2O]+ = 268.1$) and ^{1}HNMR (400 MHz, DMSO δ: 7.44 (d, *J*=2.50 Hz, 1 H), 7.27 - 7.34 (m, 1 H), 7.19 (dd, *J*=8.32, 2.56 Hz, 1 H), 2.45 (s, 3 H), 2.25 (s, 3 H), 1.52 (s, 9 H)). The crude material was carried on without further purification.



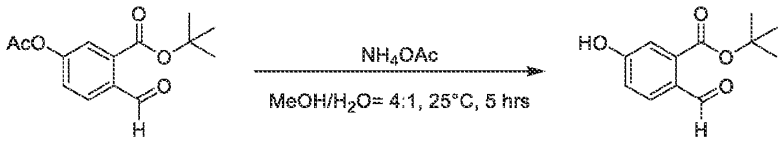
6-2 6-3

[0762] To a solution of compound 6-2 (5.53 g, 22.1 mmol, 1 *eq.*) in $CHCl_3$ (100 mL, 20 Vol.) NBS (6.49 g, 36.5 mmol, 1.65 *eq.*) and AIBN (544 mg, 3.31 mmol, 0.15 *eq.*) were added at 25°C. The resultant was refluxed for 4 hrs at 70°C and under N2 atmosphere. The reaction mixture was monitored by TLC (PE: EtOAc = 5:1, R_f=0.56). The reaction was quenched by addition of H_2O (100 mL). The layers were separated, and aq. layer was extracted with EtOAc (50 mL * 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate=100/1 to 9/1) to afford compound 6-3 (4.47 g, 13.6 mmol, 61.4% yield) as a yellow oil. The structure was further confirmed by LCMS (MS cal.: 328.0, MS observed: $[M+H_2O]+ = 346.0$), and ^{1}HNMR (400 MHz, DMSO, δ: 7.58 - 7.64 (m, 1 H), 7.54 (d, *J*=2.50 Hz, 1 H), 7.31 - 7.37 (m, 1 H), 4.98 (s, 2 H), 2.29 (s, 3 H), 1.51 - 1.64 (m, 9 H)).



6-3 6-4

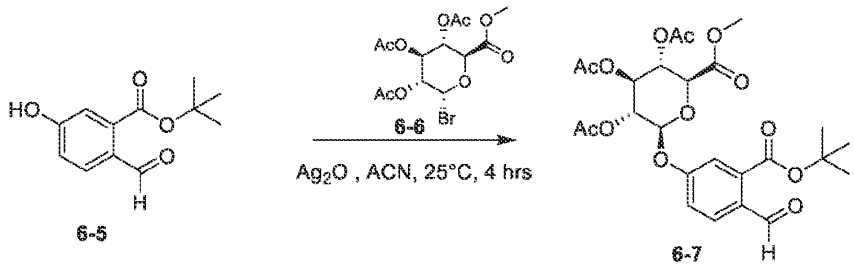
[0763] To a solution of compound 6-3 (4.47 g, 13.6 mmol, 1 *eq.*) in ACN (40 mL) 4Å MS (2 g). NMO (3.18 g, 27.1 mmol, 2.86 mL, 2 *eq.*) were added. The mixture was stirred at 25 °C for 3 hrs. The reaction was monitored by TLC (PE: EtOAc= 5:1, R_f=0.5). The reaction mixture was filtered, and diluted with 1M HCl (40 mL). Thereafter it was extracted with 50 ml of EtOAc (25 mL * 2). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give compound 6-4 (2.64 g, 9.99 mmol, 73.6% yield) as a yellow oil. The structure was further confirmed by ^{1}H NMR (400 MHz, DMSO, δ: 10.32 (s, 1 H), 7.89 (d, *J*=8.38 Hz, 1 H), 7.60 (d, *J*=2.25 Hz, 1 H), 7.48 - 7.54 (m, 1 H), 2.32 (s, 3 H), 1.56 (s, 9 H)).



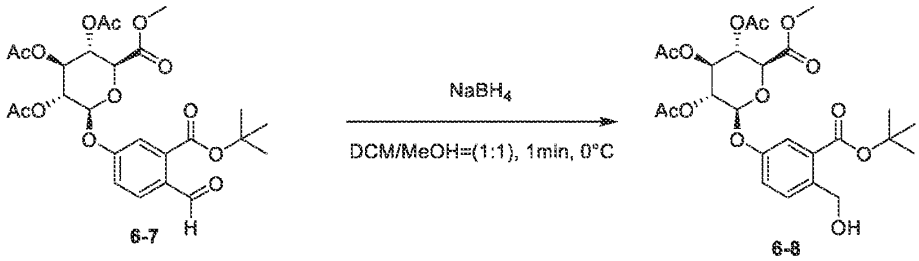
6-4 6-5

[0764] NH_4OAc (19.3 g, 250 mmol, 25 *eq.*) was added to a solution of compound 6-4 (2.64 g, 9.99 mmol, 1 *eq.*) in MeOH (20 mL) and H_2O (5 mL). The mixture was stirred at 25 °C for 4.5 hrs. LC-MS (MS cal.:222.0, MS observed: [M+ Na]+ = 245.0) showed the reactant 6-4 was consumed completely, and identified the main peak contained the desired mass. The reaction mixture was quenched by adding $NaHCO_3$, and extracted with 40 ml of EtOAc (20 mL * 2). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a compound 6-5 (2.22 g, crude) as a yellow oil. LCMS: MS cal.:222.0, MS observed: [M+ Na]+ = 245.0. HPLC: purity 71.1%.

[0765] ^1H NMR: 400 MHz, DMSO, δ: 10.15 (s, 1 H), 7.77 (d, *J*=8.51 Hz, 1 H), 7.10 (d, *J*=2.25 Hz, 1 H), 6.99 - 7.06 (m, 1 H), 1.53 - 1.58 (m, 9 H).

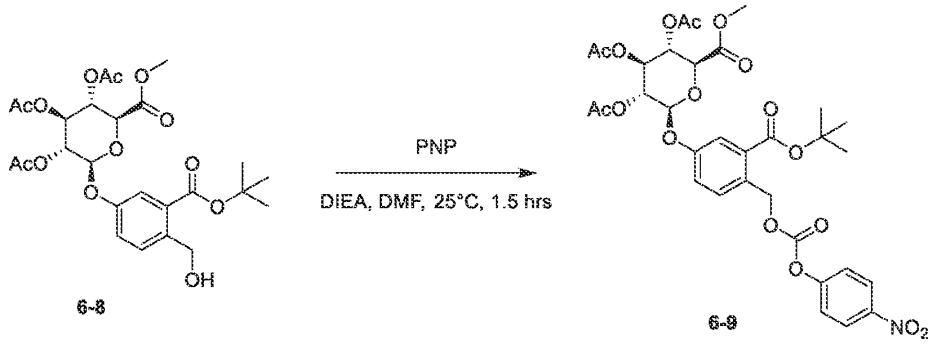


[0766] To a solution of compound 6-5 (2.22 g, 9.99 mmol, 1 *eq.*) and compound 6-6 (7.93 g, 20.0 mmol, 2 *eq.*) in ACN (20 mL) 4Å MS (1.5 g) and Ag_2O (5.79 g, 25.0 mmol, 2.5 *eq.*) were added. The mixture was stirred at 25 °C for 4 hrs. LC-MS (MS cal.:538.1, MS observed: [M+ Na]+ = 561.1) showed reactant 6-5 was consumed completely, and identified the desired mass. The reaction mixture was filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate=100/1 to 3/1). Compound 6-7 (4.25 g, 7.89 mmol, 79.0% yield) was obtained as a white solid, and confirmed by LCMS and HPLC (purity: 90.0%).

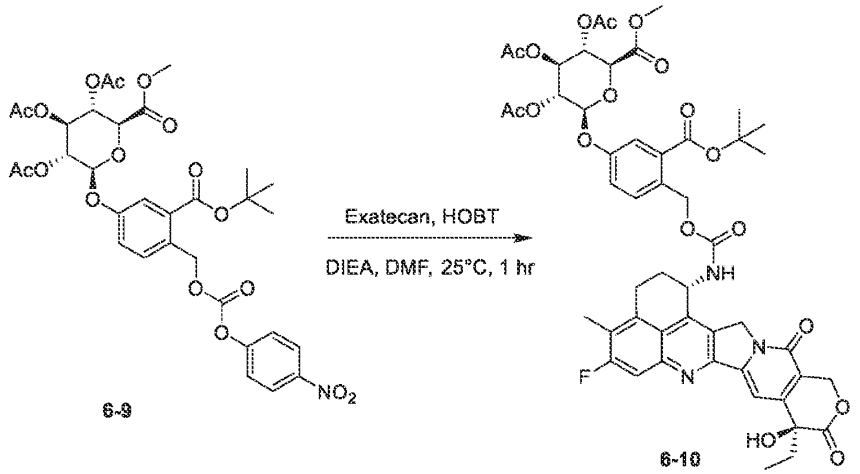


[0767] $NaBH_4$ (328 mg, 8.68 mmol, 1.1 *eq.*) in MeOH (20 mL) was added to a solution of compound 6-7 (4.25 g, 7.89 mmol, 1 *eq.*) in DCM (20 mL). The mixture was stirred at 0 °C for 1 min. The reaction mixture was monitored by TLC (PE: EtOAc= 1:1, R_f=0.3). The reaction mixture was quenched by adding NH_4Cl at 0 °C. Subsequently, it was extracted with DCM 100 mL (50 mL * 2). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate=100/1 to 5/1). Compound 6-8 (2.17 g, 4.01 mmol, 50.8% yield) was obtained as a white solid, and confirmed by LCMS

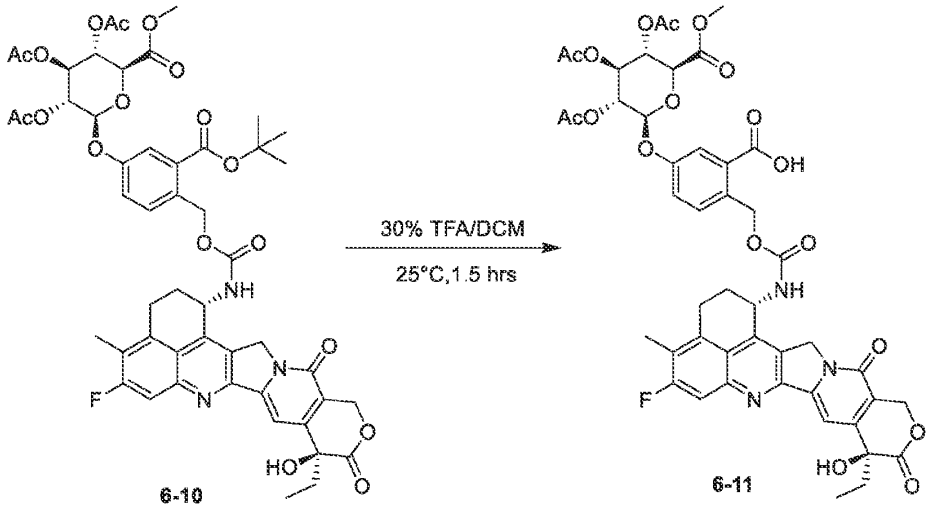
(MS cal.: 540.1, MS observed: [M+ Na]+ = 563.1) and HPLC (purity: 96.5%).



[0768] DIEA (909 mg, 7.03 mmol, 1.22 mL, 2 *eq.*) was added to a solution of compound 6-8 (1.9 g, 3.52 mmol, 1 *eq.*) and PNP (2.14 g, 7.03 mmol, 2 *eq.*) in DMF (20 mL). The mixture was stirred at 25 °C for 1.5 hrs. LC-MS (MS cal.:705.6, MS observed: [M+ Na]+ = 728.1) showed reactant 6-8 was consumed completely and one main peak with desired mass was detected. The residue was purified by prep-HPLC. Compound 6-9 (2.24 g, 3.18 mmol, 90.5% yield) was obtained as a white solid, confirmed by LCMS and HPLC (purity: 99.3%).

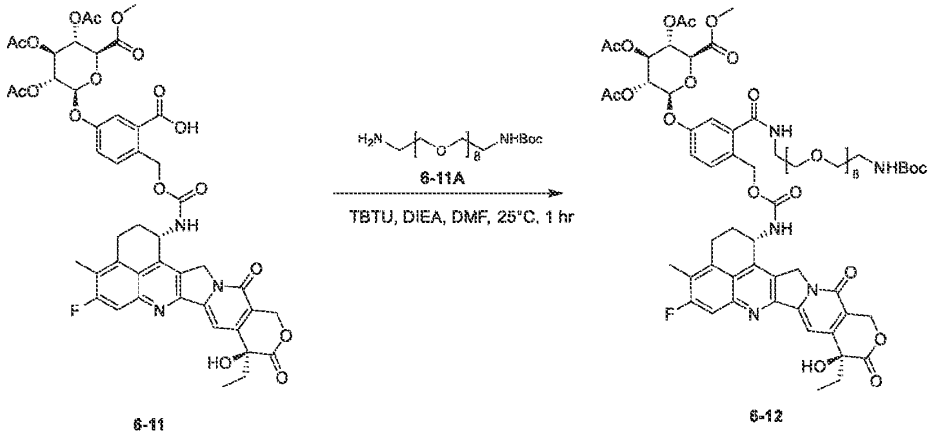


[0769] To a solution of compound 6-9 (2.24 g, 3.18 mmol, 1 *eq.*) and exatecan (1.35 g, 2.54 mmol, 0.8 *eq.*) in DMF (20 mL) HOBt (516 mg, 3.82 mmol, 1.2 *eq.*) and DIEA (822 mg, 6.36 mmol, 1.11 mL, 2 *eq.*) were added. The mixture was stirred at 25 °C for 1 hr. LC-MS (MS cal.: 1001.9, MS observed: [M+ H]$^+$ = 1002.2) showed the completion of reactant 6-9 and identified the main peak with desired mass. The reaction mixture was the added dropwise into isopropyl ether, and stirred for 30 min. Further it was filtrate, and concentrated to give compound 6-10 (2.49 g, 2.48 mmol, 78.0% yield) as a green solid.



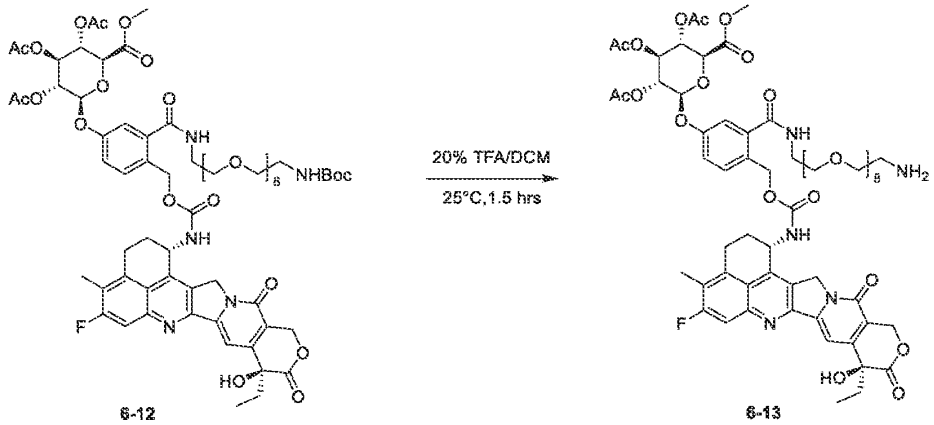
6-10 6-11

[0770] TFA (6 mL) was added to a solution of compound 6-10 (2.49 g, 2.48 mmol, 1 *eq.*) in DCM (14 mL). The mixture was stirred at 0 °C for 1.5 hrs. LC-MS (MS cal.: 945.8, MS observed: $[M+H]^+$ = 946.1) showed the completion of reactant 6-10, and main peak was identified with desired mass. The resultant was added dropwise into isopropyl ether and stirred for 30 min. Then it was filtered and concentrated to get compound 6-11 (500 mg, crude) as a yellow solid. Further it was confirmed by LCMS and HPLC (purity: 95.6%).



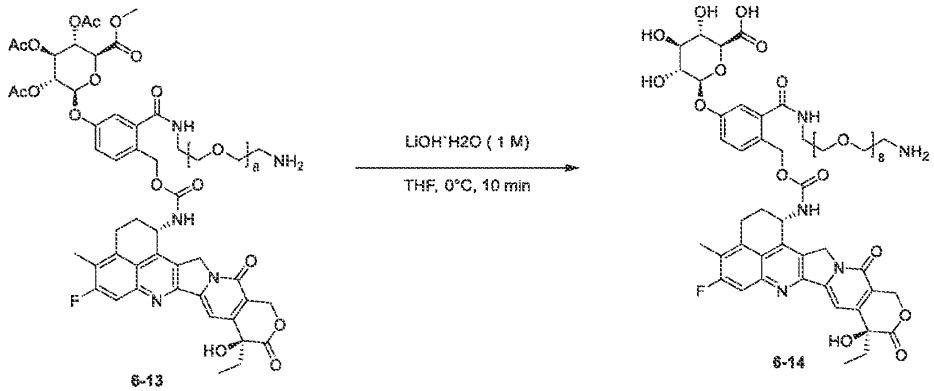
6-11 6-12

[0771] To a solution of compound 6-11 (795 mg, 840 µmol, 1 *eq.*) and compound 6-11A (431 mg, 840 µmol, 1 *eq.*) in DMF (12 mL) TBTU (324 mg, 1.01 mmol, 1.2 *eq.*) and DIEA (326 mg, 2.52 mmol, 3 *eq.*) were added. The mixture was stirred at 25 °C for 1 hr. LC-MS showed the completion of reactant 6-11, and identified the desired peak. The residue was purified by prep-HPLC. The compound 6-12 (360 mg, 250 µmol, 29.7% yield) was obtained as a yellow solid. Further it was confirmed by LCMS and HPLC (90.7% purity).



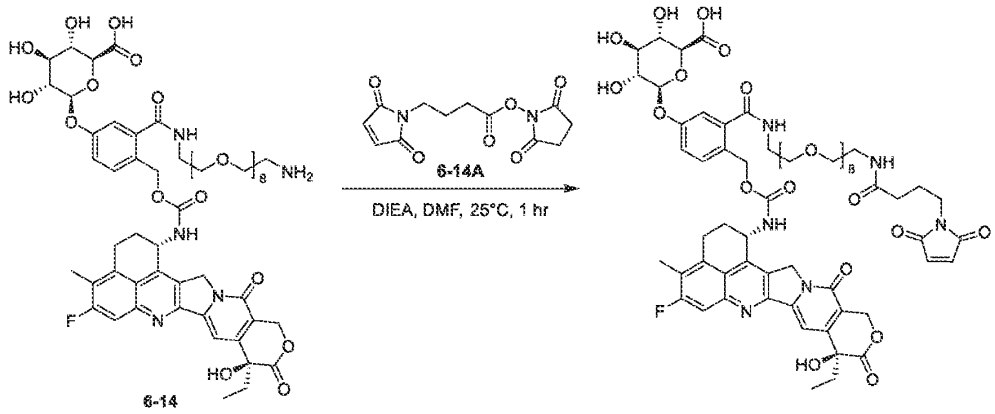
6-12 6-13

[0772] To a solution of compound 6-12 (360 mg, 250 μmol, 1 *eq.*) in DCM (4 mL), TFA (1 mL) was added. The mixture was stirred at 25 °C for 1 hr. LC-MS (MS cal.: 1340.3, MS observed: $[M+H]^+$ = 1340.5) showed reactant 6-12 was consumed, and identified the desired mass. The resultant was added dropwise into isopropyl ether, and stirred for 30 min. Then it was filtered and concentrated to give a residue. The residue was purified by prep-HPLC. Compound 6-13 (227 mg, 169 μmol, 67.9% yield) was obtained as a yellow solid, and further confirmed by LCMS and HPLC (purity: 98.8%).



6-13 6-14

[0773] $LiOH \cdot H_2O$ (1.69 mL, 1M, 10 eq.) was added to a solution of compound 6-13 (227 mg, 169 μmol, 1 *eq.*) in THF (1 mL). The mixture was stirred at 0 °C for 10 min. LC-MS (MS cal.: 1200.2, MS observed: $[M+H]^+$ = 1200.5) showed reactant 6-13 was consumed and detected the desired mass. TFA was addd to adjusted the pH under at 0°C. The residue was purified by prep-HPLC. Compound 6-14 (56 mg, 46.7 μmol, 27.5% yield) was obtained as a yellow solid and it was further confirmed by LCMS and HPLC (purity: 98.5%).



[0774] DIEA (12.1 mg, 93.4 μmol, 16.3 μL, 2 *eq.*) was added to a solution of compound 6-14 (56 mg, 46.7 μmol, 1 *eq.*) and compound 6-14A (13.1 mg, 46.7 μmol, 1 *eq.*) in DMF (0.5 mL). The mixture was stirred at 25 °C for 1 hr. LC-MS (MS cal.: 1365.3, MS observed: $[M+H]^+$ = 1365.5) showed reactant 6-14 was consumed completely, and detected the desired mass. TFA was added to adjusted the pH at 0°C. The residue was purified by prep-HPLC (TFA condition). Compound exatecan glucuronide (LP-C) (24 mg, 17.6 μmol, 38.1% yield) was obtained as a yellow solid, and further confirmed by LCMS and HPLC (purity: >99.9%).

[0775] Example 10. Preparation of Additional Conjugates.

[0776] Various technologies are available for conjugate compounds, e.g., LP-A, LP-B, LP-C, etc., with antibodies. For example, in some embodiments, compounds were conjugated with antibodies at one or more cysteine residues (e.g., Example 3). In some embodiments, LP-A was conjugated with antibodies Hu2C4-H3L3, 65872, or 887-1DE. In some embodiments, LP-B was conjugated with antibodies Hu2C4-H3L3, 65872, or 887-1DE. In some embodiments, LP-C was conjugated with antibodies Hu2C4-H3L3, 65872, or 887-1DE. In some embodiments, a compound was conjugated with human IgG1. In some embodiments, a provided composition has a DAR of about 4. In some embodiments, a provided composition has a DAR of about 8.

[0777] A useful protocol for preparing conjugates is described below as an example.

[0778] Step 1: Reduction of disulfide bonds of an antibody.

[0779] An antibody is in or is first buffer exchanged into an appropriate buffer (e.g., PBS, about pH 7, containing EDTA, typically at about 2 mM, final concentration) for reduction of antibody disulfide bonds (e.g., the 4 interchain disulfides). Buffer exchange can be achieved either through dialysis into a desired buffer, or passage through a column, e.g., a Sephadex G25 column, such as a commercially available NAP25 column, that has been pre-equilibrated in a desired buffer. An antibody can be concentrated, e.g., using an Amicon centrifugal filter to provide a final concentration of 10 mg/mL. A antibody solution is subsequently treated with a small molar excess (e.g., between about 1.2 to 3 molar equivalents per disulfide reduced) of tris(2-carboxyethyl)phosphine (TCEP) for a period of time, e.g., 2 hours, at a suitable temperature, e.g., 37 °C.

[0780] Step 2: Conjugation.

[0781] In some embodiments, conjugation can be achieved through reactions of linker-payload compounds with cysteine residues in antibodies. In some embodiments, conjugation with LP-A, LP-B, LP-C or LP-D is achieved as follows. To an reduced antibody, an excess (e.g., between about 10-20 molar equivalents/antibody (e.g., about 14 molar equivalents)) of a linker-payload compound is added (e.g., LP-A, LP-B, LP-C, LP-D, etc.), e.g., from a stock solution. In some embodiments, a stock solution is in DMA, and the final DMA concentration in the buffer is below about 10% (v/v). The mixture is incubated at a suitable temperature, e.g., about room temperature, for a period of time for conjugation, e.g., about 1 hour followed by purification, e.g., by passage through a Sephadex G25 desalting column to remove any unreacted reagents, solvent (e.g., DMA), and other small molecules. In this step, the resulting conjugates can also be buffer exchanged into another buffer, e.g., a formulation buffer (e.g., typically about 20 mM histidine, about 150 mM NaCl, about pH 5.5, often containing sucrose). The resulting conjugate solution can be concentrated, e.g., using an Amicon centrifugal concentrator if desired. In some embodiments, a low concentration of polysorbate 80 is added to a conjugate solution, which can be useful for preventing or reducing aggregation.

[0782] Step 3: Biochemical and Analytical Characterization of Conjugates.

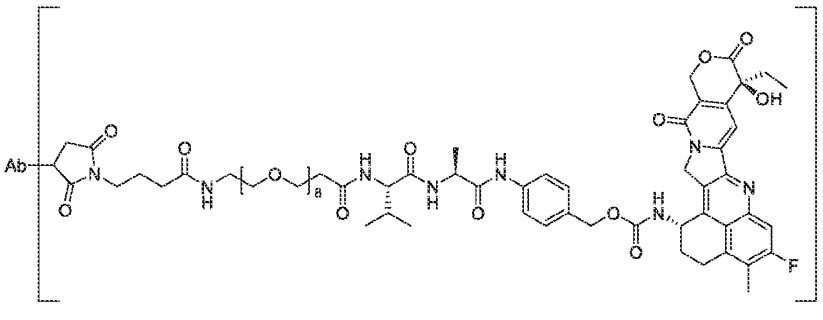
[0783] Conjugates can be characterized and assessed using various biochemical and analytical technologies in accordance with the present disclosure. In some embodiments, concentrations of a conjugate and drug (payload, e.g., exatecan): antibody ratios (DAR) are assessed by measuring UV absorbance of an aqueous solution of the conjugate at two wavelengths - 280 nm and 370 nm. At 370 nm, contribution to absorbance is solely or primarily by a payload, e.g., exatecan. The concentration of the linked payload can be determined using the absorbance and a separately determined extinction coefficient of the linker-payload. The antibody (typical extinction coefficient of the antibody ε_{280nM} = 201,400 $cm^{-1}(mol/L)^{-1}$) absorbs only or primarily at 280 nM. The contribution to the absorbance by the linker-payload at 280 nM is determined and subtracted from the absorbance of the conjugate at 280 nM to provide the absorbance of the antibody component. Using this value and the extinction coefficient of the antibody, the antibody concentration in a conjugate preparation can be calculated. The DAR value can be determined using the payload and antibody concentrations. Other technologies are also available and can be utilized to assess DARs in accordance with the present disclosure.

[0784] In some embodiments, TCEP was removed by de-salting, e.g., using a column. In some embodiments, charcoal was utilized to remove certain impurities, e.g., free drug and related impurities. In some embodiments, buffer change was performed.

[0785] In some embodiments, a conjugate is a conjugate of LP-A and 65872. In some embodiments, a conjugate is a conjugate of LP-A and 65889. In some embodiments, a conjugate is a conjugate of LP-A and 887-1DE. In some embodiments, a conjugate is a conjugate of LP-B and Hu2C4-H3L3. In some embodiments, a conjugate is a conjugate of LP-B and 65872. In some embodiments, a conjugate is a conjugate of LP-B and 65889. In some embodiments, a conjugate is a conjugate of LP-B and 887-1DE. In some embodiments, a conjugate is a conjugate of LP-C and Hu2C4-H3L3. In some embodiments, a

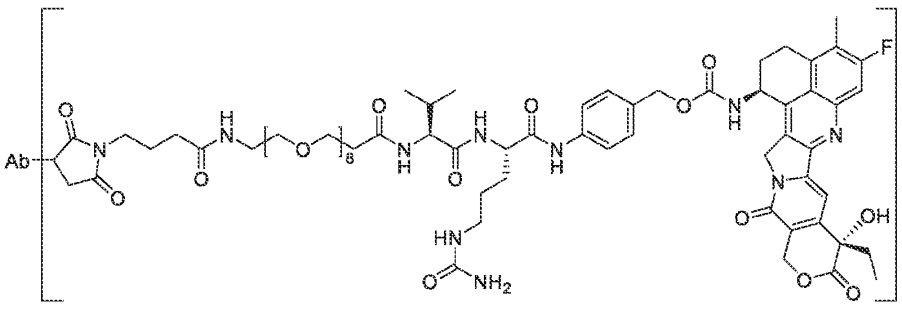
conjugate is a conjugate of LP-C and 65872. In some embodiments, a conjugate is a conjugate of LP-C and 65889. In some embodiments, a conjugate is a conjugate of LP-C and 887-1DE. In some embodiments, a conjugate is a conjugate of LP-D and Hu2C4-H3L3. In some embodiments, a conjugate is a conjugate of LP-D and 65872. In some embodiments, a conjugate is a conjugate of LP-D and 65889. In some embodiments, a conjugate is a conjugate of LP-D and 887-1DE.

[0786] In some embodiments, a conjugate has the structure of



t or a pharmaceutically acceptable salt thereof, wherein Ab is or comprises an antibody or an antigen-binding fragment thereof, and t is as described herein. In some embodiments, Ab is or comprises an anti-HHLA2 antibody or an antigen-binding fragment thereof. In some embodiments, t is about 1-8. In some embodiments, t is about 2. In some embodiments, t is about 4. In some embodiments, t is about 8. In some embodiments, Ab is Hu2C4-H3L3 and t is about 8. In some embodiments, Ab is Hu2C4-H3L3 and t is about 4. In some embodiments, Ab is 65872 and t is about 8. In some embodiments, Ab is 65872 and t is about 4. In some embodiments, Ab is 65889 and t is about 8. In some embodiments, Ab is 65889 and t is about 4. In some embodiments, Ab is 887-1DE and t is about 8. In some embodiments, Ab is 887-1DE and t is about 4.

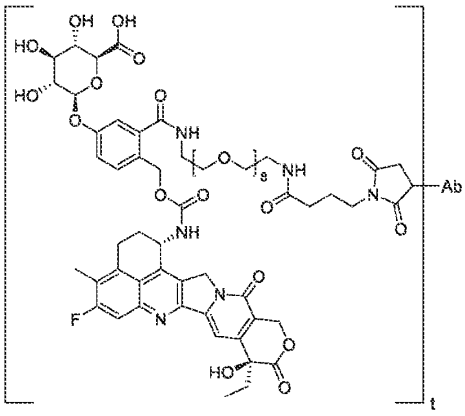
[0787] In some embodiments, a conjugate has the structure of



t or a pharmaceutically acceptable salt thereof, wherein Ab is or comprises an antibody or an antigen-binding fragment thereof, and t is as described herein. In some embodiments, Ab is or comprises an anti-HHLA2 antibody or an antigen-binding fragment thereof. In some embodiments, t is about 1-8. In some embodiments, t is about 2. In some embodiments, t is about 4. In some embodiments, t is about 8. In some embodiments, Ab is Hu2C4-H3L3 and t is about 8. In some embodiments, Ab is Hu2C4-H3L3 and t is about 4. In some embodiments, Ab is 65872 and t is about 8. In some embodiments, Ab is 65872 and

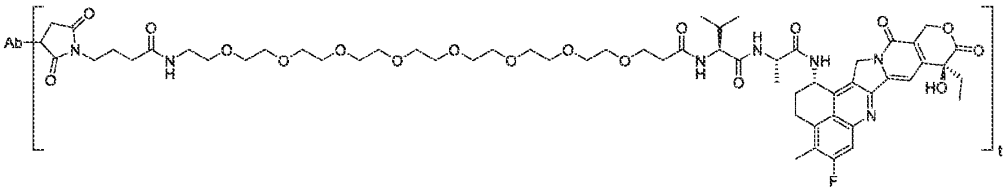
t is about 4. In some embodiments, Ab is 65889 and t is about 8. In some embodiments, Ab is 65889 and t is about 4. In some embodiments, Ab is 887-1DE and t is about 8. In some embodiments, Ab is 887-1DE and t is about 4.

[0788] In some embodiments, a conjugate has the structure of



or a pharmaceutically acceptable salt thereof, wherein Ab is or comprises an antibody or an antigen-binding fragment thereof, and t is as described herein. In some embodiments, Ab is or comprises an anti-HHLA2 antibody or an antigen-binding fragment thereof. In some embodiments, t is about 1-8. In some embodiments, t is about 2. In some embodiments, t is about 4. In some embodiments, t is about 8. In some embodiments, Ab is Hu2C4-H3L3 and t is about 8. In some embodiments, Ab is Hu2C4-H3L3 and t is about 4. In some embodiments, Ab is 65872 and t is about 8. In some embodiments, Ab is 65872 and t is about 4. In some embodiments, Ab is 65889 and t is about 8. In some embodiments, Ab is 65889 and t is about 4. In some embodiments, Ab is 887-1DE and t is about 8. In some embodiments, Ab is 887-1DE and t is about 4.

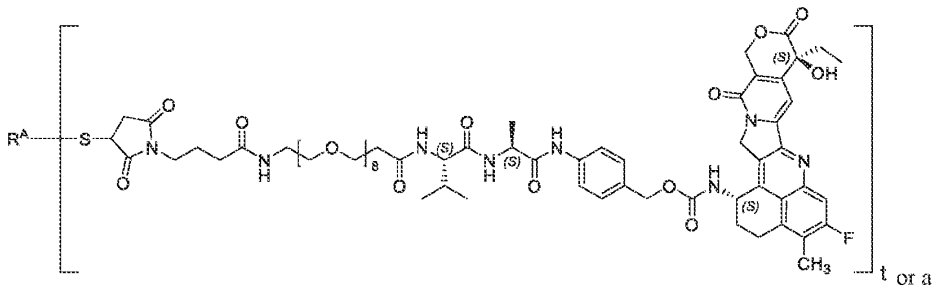
[0789] In some embodiments, a conjugate has the structure of



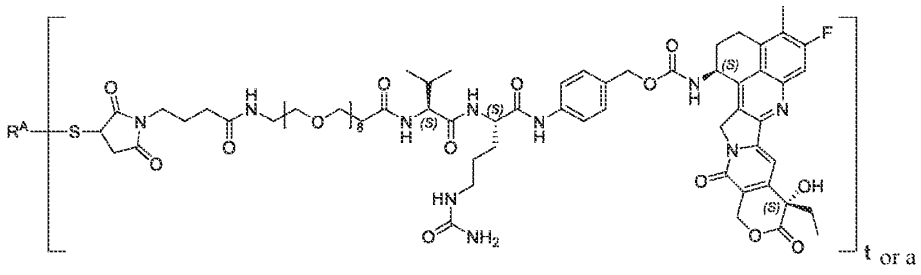
or a pharmaceutically acceptable salt thereof, wherein Ab is or comprises an antibody or an antigen-binding fragment thereof, and t is as described herein. In some embodiments, Ab is or comprises an anti-HHLA2 antibody or an antigen-binding fragment thereof. In some embodiments, t is about 1-8. In some embodiments, t is about 2. In some embodiments, t is about 4. In some embodiments, t is about 8. In some embodiments, Ab is Hu2C4-H3L3 and t is about 8. In some embodiments, Ab is Hu2C4-H3L3 and t is about 4. In some embodiments, Ab is 65872 and t is about 8. In some embodiments, Ab is 65872 and t is about 4. In some embodiments, Ab is 65889 and t is about 8. In some embodiments, Ab is 65889 and t is about 4. In some embodiments, Ab is 887-1DE and t is about 8. In some embodiments, Ab is 887-1DE and t is about 4.

[0790] In some embodiments, a conjugate is prepared as described herein. In some embodiments,

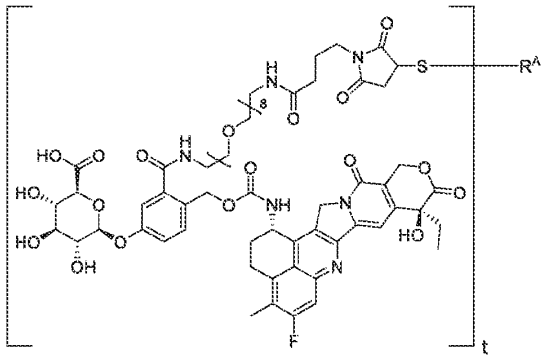
LP-A, LP-B, LP-C, and LP-D are conjugated at one or more cysteine residues, e.g., about 1-8 (e.g., about 4, about 8, etc.) cysteine residues that typically form interchain disulfide bonds without conjugation. For example, in some embodiments, a conjugate has the structure of



or a pharmaceutically acceptable salt thereof. In some embodiments, a conjugate has the structure of



or a pharmaceutically acceptable salt thereof. In some embodiments, a conjugate has the structure of



or a pharmaceutically acceptable salt thereof. In some embodiments, R^A is Hu2C4-H3L3, each –S– is independently of a Cys residue, and t is about 4. In some embodiments, R^A is Hu2C4-H3L3, each –S– is independently of a Cys residue, and t is about 8. In some embodiments, R^A is 65872, each –S– is independently of a Cys residue, and t is about 4. In some embodiments, R^A is 65872, each –S– is independently of a Cys residue, and t is about 8. In some embodiments, R^A is 65889, each –S– is independently of a Cys residue, and t is about 4. In some embodiments, R^A is 65889, each –S– is independently of a Cys residue, and t is about 8. In some embodiments, R^A is 887-1DE, each –S– is independently of a Cys residue, and t is about 4. In some embodiments, R^A is 887-1DE, each –S– is independently of a Cys residue, and t is about 8. In some embodiments, a conjugate is a conjugate of LP-A and Hu2C4-H3L3. In some embodiments, the present disclosure provides a composition, e.g., a pharmaceutical composition, comprising a conjugate as

described herein. In some embodiments, DAR of a composition is about 4 (e.g., about 3-5, 3.5-4.5, 3.6-4.4, 3.7-4.3, 3.8-4.2, etc.). In some embodiments, DAR of a composition is about 8 (e.g., about 7-9, 7.5-8.5, 7.6-8.4, 7.7-8.3, 7.8-8.2, 7.5-8.4, 7.6-8.3, 7.5-8.3, 7.6-8.2, 7.7-8.2, 7.7-8.1, etc.). In some embodiments, a composition comprises an unconjugated target binding moiety, e.g., an antibody such as Hu2C4-H3L3, 65872, 887-1DE, etc., and a conjugate. In some embodiments, in a composition there are various conjugate with different t values. For example, for a composition with DAR about 4, there may be compounds with t being 0 (unconjugated), 1, 2, 3 and 4. In some embodiments, DAR of light chain is about 2. In some embodiments, DAR of heavy chain is about 6.

[0791] Various technologies can be utilized to characterize and assess provided conjugates in accordance with the present disclosure. For example, conjugates were characterized and assessed using various available technologies in accordance with the present disclosure, e.g., mass spectrometry, chromatography, etc. Among other things, mass, DAR, etc. were assessed and confirmed. In some embodiments, conjugates were characterized by BCA concentration, SEC, LC-MS (e.g., reduced), endotoxin, free drug assay, etc.

[0792] In some embodiments, conjugates are provided in a buffer. Various buffers can be utilized to formulate conjugates in accordance with the present disclosure. In some embodiments, a formulation buffer is 20 mM Histidine-HCl, 8% sucrose, 0.02% PS80, pH 5.5.

[0793] Among other things, technologies of the present disclosure can provide various advantages. For example, in some embodiments, certain provided conjugates have high solubility. In some embodiments, certain provided conjugates have high stability, e.g., when subjected to storage conditions, freeze-thaw cycles, etc. In some embodiments, certain provided conjugates, e.g., those of Hu2C4-H3L3 and 65872, can be formulated at concentrations of about 1.5, 2, 2.5, 3, 3.5, 4 or more mg/mL, without significant aggregation (e.g., about 1%, 5%, 10% or less). In some embodiments, certain provided conjugates, e.g., those of Hu2C4-H3L3 and 65872, are less prone to aggregation than conjugates of other antibodies.

[0794] Example 11. Provided conjugates bind to target cells.

[0795] Among other things, the present disclosure confirms that provided conjugates can bind to targets. For example, as described herein, anti-HHLA2 antibodies conjugated with LP-A, LP-B, and LP-C compounds can bind to HEK cells engineered to over-express B7H7 (HHLA2). $5x10^4$ cells were treated with the anti-B7-H7 antibody drug conjugates at increasing concentrations in 100 µL PBS containing 1% BSA for 1 hour at 4°C. Cells were then washed once in PBS + 1% BSA and stained with PE-conjugated goat anti-human IgG for 30 minutes at 4°C. For each antibody (Hu2C4-H3L3 and 65872), LP-A (PEG8-Val-Ala-PABA-Exatecan), LP-B (PEG8-Val-Cit-PABA-Exatecan), and LP-C (Glucuronide-Exatecan) linker payload moieties at DAR 8 were tested. Human IgG1 conjugated with GGFG-Exatecan at DAR 8 was utilized as isotype control. Cells were then washed once in PBS + 1% BSA and antibody binding was analyzed by flow cytometry (FACS). All the antibody drug conjugates bound to B7-H7. Certain results are presented in Figure 4. Certain binding EC_{50}s are listed in Table 4 below.

[0796] Table 4. EC_{50}s of anti-HHLA2 ADCs to HEK cells overexpressing B7H7.

Compound	B7H7 Antibody	DAR	EC_{50} (nM)
LP-A (PEG8-Val-Ala-PABA-Exatecan)	Hu2C4-H3L3	8	2.6
	65872	8	2.6
LP-B (PEG8-Val-Cit-PABA-Exatecan)	Hu2C4-H3L3	8	2.3
	65872	8	2
LP-C (Glucuronide-Exatecan)	Hu2C4-H3L3	8	1.3
	65872	8	1.4

[0797] Example 12. Provided technologies can kill target cells.

[0798] Among other things, the present disclosure confirms that provided technologies can deliver payloads to target cells and, in case payloads are cytotoxic agents, kill target cells. In an example, HEK293 cells engineered to express HHLA2 were seeded into the wells of flat-bottom 96-well plates at a density of $1x10^4$ cells/well and allowed to adhere overnight. The next day HEK293-HHLA2 cells were treated with anti-HHLA2 antibody drug conjugates (Hu2C4-H3L3 and 65872 conjugated with LP-A, LP-B, or LP-C) at 20 nM and subsequent 5x dilutions for 6 days at 37 °C. For each antibody (Hu2C4-H3L3and 65872), LP-A (PEG8-Val-Ala-PABA-Exatecan), LP-B (PEG8-Val-Cit-PABA-Exatecan), and LP-C (Glucuronide-Exatecan) at DAR 8 were tested. Human IgG1 conjugated with GGFG-Exatecan at DAR 8 was used as isotype control. Certain results are presented in Figure 4. Certain cytotoxic IC_{50}s are listed in Table 5 below.

[0799] Table 5. In Vitro Cytotoxic Activity of Certain Conjugates.

Compound	B7H7 Antibody	DAR	IC_{50} (nM)
LP-A (PEG8-Val-Ala-PABA-Exatecan)	Hu2C4-H3L3	8	0.15
	65872	8	0.31
LP-B (PEG8-Val-Cit-PABA-Exatecan)	Hu2C4-H3L3	8	0.25
	65872	8	0.25
LP-C (Glucuronide-Exatecan)	Hu2C4-H3L3	8	0.19
	65872	8	0.35

[0800] Example 13. Provided conjugates are thermally stable.

[0801] Among other things, the present disclosure confirms that provided conjugates are thermally stable for various purposes. In an example, various conjugates were incubated at 25 °C for 5 and 7 days. Stability of the conjugates was determined via SEC to assess the percentage of monomeric conjugate. SEC purity measurements were conducted at 0, 5, and 7 days. Certain thermostability data is presented in Table 6 below as examples.

[0802] Table 6. Thermal Stability of Certain Provided Conjugates.

Compound	B7H7 Antibody	DAR	25 °C (0 Days)	25 °C (5 Days)	25 °C (7 Days)
LP-A (PEG8-Val-Ala-PABA-Exatecan)	Hu2C4-H3L3	8	97.32	97.45	97.43
	65872	8	96.98	96.95	96.94
LP-B (PEG8-Val-Cit-PABA-Exatecan)	Hu2C4-H3L3	8	97.03	96.24	96.59
	65872	8	95.95	95.47	95.36
LP-C (Glucuronide-Exatecan)	Hu2C4-H3L3	8	99.29	99.14	99.1
	65872	8	98.05	97.87	97.75

[0803] Example 14. Provided conjugates are stable over multiple freeze thaw cycles.

[0804] Among other things, the present disclosure confirms that provided conjugates are stable when subjected to freeze-thaw cycles. In an example, various conjugates were incubated at subjected to 1, 3, or 5 freeze thaw cycles. Stability of the conjugates was determined via SEC to assess the percentage of monomeric conjugate. The SEC purity measurements were conducted after 1, 3, or 5 freeze thaw cycles. Certain stability data is presented in Table 7 below.

[0805] Table 7. Stability of various conjugates.

Compound	B7H7 Antibody	DAR	1X	3X	5X
LP-A (PEG8-Val-Ala-PABA-Exatecan)	Hu2C4-H3L3	8	97.14	97.07	97.27
	65872	8	95.82	96.27	96.76
LP-B (PEG8-Val-Cit-PABA-Exatecan)	Hu2C4-H3L3	8	96.71	96.99	96.64
	65872	8	96.71	96.99	96.64
LP-C (Glucuronide-Exatecan)	Hu2C4-H3L3	8	99.28	99.31	99.3
	65872	8	98.28	98.12	98.07

[0806] As demonstrated herein, provided conjugates are stable under various conditions. In some embodiments, percentages of monomers of provided conjugates in compositions are about or at least about 90%, 91%, 92%, 93%, 94% or 95%.

[0807] Example 15. Additional technologies for assessing provided conjugates.

[0808] Many technologies are useful for characterizing and assessing technologies including conjugates in accordance with the present disclosure. Certain useful technologies for demonstrating and confirming properties, activities, benefits, advantages, etc., of provided techs are described below as examples.

[0809] Stability: In some embodiments, conjugate stability in serum, e.g., human serum, is assessed. In some embodiments, stability may be assessed using the following protocol. Conjugates are mixed with human serum at a ratio (e.g., of at least 1:9) and incubated at 37 °C for a period of time, e.g., 14 days. Samples are collected on certain time points, e.g., on days 0, 1, 4, 7, and 14. If needed, samples can be

frozen immediately for storage. The collected samples may be assessed, e.g., in a dose-dependent on-cell binding assay such as the assay described in Example 11. The amount of released payload from each sample may be assessed using, e.g., mass spectrometry.

[0810] In some embodiments, PK of conjugates is assessed, e.g., in an animal (e.g., mouse, rat, etc.). In some embodiments, PK is assessed in rat. For example, the pharmacokinetic profile of a conjugate may be determined following single intravenous bolus administration in rats. In an example protocol, groups of 5 rats are each dosed intravenously with a dose, e.g., a single 10 mg/kg dose, of a conjugate. Each rat is bled at various time points, e.g., pre-dose (day -1), 0.5 hours, 4 hours, 24 hours (day 1), 72 hours (day 3), 120 hours (day 5), 168 hours (day 7), 240 hours (day 10), 288 hours (day 12), 336 hours (day 14), and 504 hours (day 21), post dose. In some embodiments, pharmacokinetic profiles are determined using PK ELISA.

[0811] In some embodiments, efficacy of provided technologies are assessed and confirmed in animal models, e.g. in mice. In some embodiments, an animal model is a cancer model. For example, efficacy of a conjugate may be assessed in mice carrying K562-HHLA2-NPTI tumor cells. In an example protocol, these mice are treated with initial once a day intravenous administration of a conjugate for a period of time, e.g., 5 days, and then biweekly through the end of the study. The size of the tumors are measured biweekly to assess effect of tested ADCs on tumor growth.

EQUIVALENTS

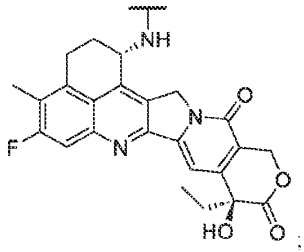
[0812] It is to be appreciated by those skilled in the art that various alterations, modifications, and improvements to the provided technologies will readily occur to those skilled in the art. Such alterations, modifications, and improvements are intended to be part of provided technologies including claimed technologies, and are intended to be within the spirit and scope of provided technologies including claimed technologies. The foregoing description and drawing are by way of example.

[0813] Those skilled in the art will appreciate typical standards of deviation or error attributable to values obtained in assays or other processes described herein. Various publications, websites and other reference materials referenced herein to describe the background of provided technologies or to provide additional detail regarding its practice are hereby incorporated by reference in their entireties; in the case of any conflict, the present disclosure will control.

CLAIMS

1. A conjugate, comprising:

i) a target binding moiety targeting HHLA2;

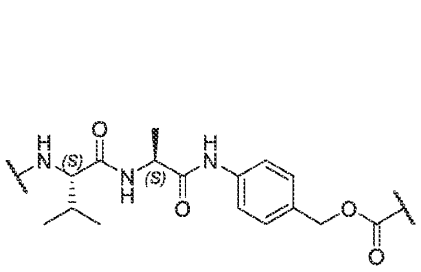
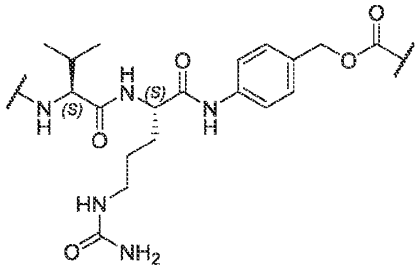
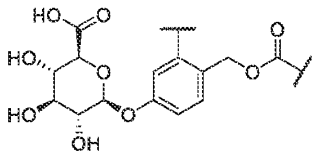
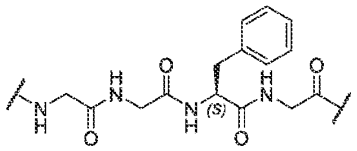
ii) a payload moiety having the structure of  ; and

iii) a linker that connects the target binding moiety and the payload moiety;

wherein:

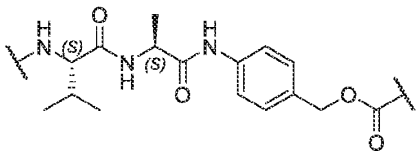
the linker comprises $-(CH_2CH_2O)n-$ and L^C;

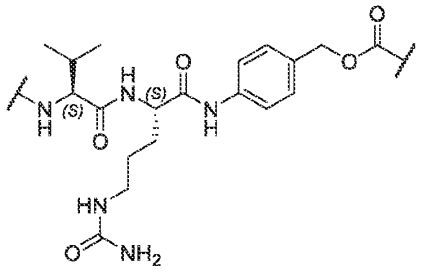
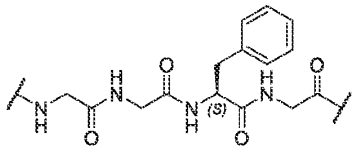
n is 0-24;

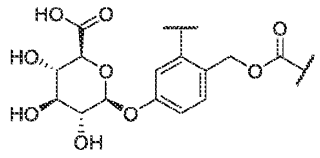
L^C is  ,  ,  , or  , wherein the carbonyl is bonded to the payload moiety.

2. The conjugate of claim 1, wherein the targeting binding moiety is an antibody selected from Hu2C4-H3L3, 65872, 65889, or 887-1DE.

3. The conjugate of claim 2, wherein n is 1-24.

4. The conjugate of claim 3, wherein L^C is  .


, or 



5. The conjugate of claim 2, wherein n is 0 and L^C is .

6. A conjugate, comprising:

i) a target binding moiety;

ii) a payload moiety; and

iii) a linker that connects the target binding moiety and the payload moiety;

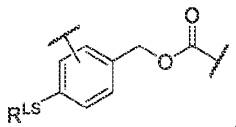
wherein:

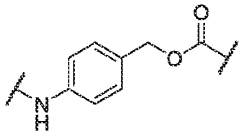
the linker comprises $-(CH_2CH_2O)n-$ and L^C;

n is 0-24;

L^C is bonded to a payload moiety and is $-L^{C1}-L^{CO}-$;

L^{C1} is a cleavable unit; or L^{C1} is $-Gly-Gly-Phe-Gly-$, $-Val-Ala-$, $-Val-Cit-$, L^{C1S}, $-Gly-Gly-Phe-Gly-N(R')-L^{C1S}-$, $-Val-Ala-N(R')-L^{C1S}-$, or $-Val-Cit-N(R')-L^{C1S}-$;

L^{C1S} is  , wherein the phenyl ring and the $-CH_2-$ is optionally substituted. and the carbonyl is bonded to L^{CO}, and R^{LS} is or comprises a sugar moiety;

L^{CO} is a covalent bond, or a spacer moiety (e.g., optionally substituted  wherein the carbonyl is bonded to a payload moiety);

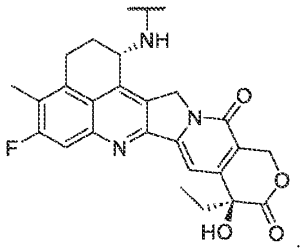
each R' is independently $-R$, $-C(O)R$, $-CO_2R$, or $-S(O)_2R$, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently

selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

7. The conjugate of claim 6, wherein the target binding moiety comprises an antibody or an antigen-binding fragment thereof.

8. The conjugate of claim 6 or 7, wherein the payload moiety is



.

9. The conjugate of any one of the preceding claims, wherein the payload moiety to target binding moiety ratio of the conjugate (DAR) is about 2-8.

10. The conjugate of any one of the preceding claims, wherein the linker comprises $-(CH_2CH_2O)n-L^{ZP}-L^{C}-$, wherein:

L^{ZP} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each $-Cy-$ is independently an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

R is optionally substituted C_{1-6} aliphatic.

11. The conjugate of any one of the preceding claims, wherein the linker comprises $-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$, wherein:

L^{X} is a covalent bond or an optionally substituted bivalent or polyvalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{X} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

L^{ZP} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each $-Cy-$ is independently an optionally substituted 3-12 membered ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and

each R is independently hydrogen or optionally substituted C_{1-6} aliphatic.

12. The conjugate of any one of the preceding claims, wherein the linker is or comprises $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$, wherein:

L^{RX} is a covalent bond or a moiety that results from a reaction between a group present on the unconjugated target binding moiety and a group present on an agent comprising $-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-$ and a payload moiety, wherein $-L^{C}-$ is bonded to the payload moiety; or L^{RX} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10

methylene units of L^{RX} are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)$_2$−, −C(O)N(R')−, −S(O)$_2$(NR')−, and −Cy−;

L^X is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^X are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)$_2$−, −C(O)N(R')−, −S(O)$_2$(NR')−, and −Cy−;

L^{ZP} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)$_2$−, −C(O)N(R')−, −S(O)$_2$(NR')−, and −Cy−;

each −Cy− is independently an optionally substituted 3-12 membered ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and

each R is independently hydrogen or optionally substituted C_{1-6} aliphatic.

13. The conjugate of any one of the preceding claims, wherein the linker is or comprises −L^T−L^{RX}−L^X−$(CH_2CH_2O)n$−L^{ZP}−L^C−, wherein:

L^T is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^T are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)$_2$−, −C(O)N(R')−, −S(O)$_2$(NR')−, and −Cy−;

L^{RX} is a covalent bond or a moiety that results from a reaction between a group present on the unconjugated target binding moiety and a group present on an agent comprising −L^X−$(CH_2CH_2O)n$−L^{ZP}−L^C− and a payload moiety, wherein −L^C− is bonded to the payload moiety; or L^{RX} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{RX} are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)$_2$−, −C(O)N(R')−, −S(O)$_2$(NR')−, and −Cy−;

L^X is a covalent bond or an optionally substituted bivalent or polyvalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^X are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)$_2$−, −C(O)N(R')−, −S(O)$_2$(NR')−, and −Cy−;

L^{ZP} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)$_2$−, −C(O)N(R')−, −S(O)$_2$(NR')−, and −Cy−;

each −Cy− is independently an optionally substituted 3-12 membered ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur; and

each R is independently hydrogen or optionally substituted C_{1-6} aliphatic.

14. The conjugate of any one of claims 10-13, wherein L^{ZP} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^Z are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)$_2$−, −C(O)N(R')−, and −S(O)$_2$(NR')−.

15. The conjugate of claim 14, wherein L^{ZP} is −(CH_2)z−N(R')C(O)−, −(CH_2)z−C(O)−, or −(CH_2)z−C(O)N(R')−, wherein z is 1-19.

16. The conjugate of any one of claims 6-15, wherein L^C is −Gly−Gly−Phe−Gly−,

−Val−Ala−PABA−, −Val−Cit−PABA−, mGlu or oGlu.

17. The conjugate of any one of the preceding claims, wherein n is 8.

18. The conjugate of any one of claims 11-17, wherein L^X is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^X are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)$_2$−, −C(O)N(R')−, −S(O)$_2$(NR')−, and −Cy−.

19. The conjugate of any one of claims 12-18, wherein each L^{RX} is independently $-L^{RX1}-L^{RX2}-$, wherein:

each L^{RX1} is independently a covalent bond or an optionally substituted bivalent or polyvalent straight or branched C_{1-10} aliphatic wherein 1-5 methylene units of L^{RX1} are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)$_2$−, −C(O)N(R')−, −S(O)$_2$(NR')−, and −Cy−;

each L^{RX2} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-10} aliphatic wherein 1-5 methylene units of L^{RX2} are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)$_2$−, −C(O)N(R')−, −S(O)$_2$(NR')−, and −Cy−;

each −Cy− is independently an optionally substituted 3-12 membered ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

each L^{RX2} is bonded to L^X; and

each R is independently hydrogen or optionally substituted C_{1-6} aliphatic.

20. The conjugate of any one of claims 13-19, wherein L^T is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^T are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)$_2$−, −C(O)N(R')−, and −S(O)$_2$(NR')−.

21. The conjugate of any one of claims 13-20, wherein L^T is $-CH_2-$.

22. The conjugate of any one of claims 13-21, wherein the target binding moiety comprises an amino acid residue, and L^T is bonded to the backbone atom of the amino acid residue.

23. The conjugate of any one of claims 6-10, wherein the linker is or comprises $\left[-L^{RX}\right]_a-L^{X'}-\left[(CH_2CH_2O)_n-L^{ZP}-L^C-\right]_b$, wherein:

each L^{RX} is independently a covalent bond or a moiety that results from a reaction between a group present on the unconjugated target binding moiety and a group present on an agent comprising $L^{X'}$ and optionally a payload moiety, wherein $-L^C-$ is bonded to the payload moiety; or each L^{RX} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{RX} are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)$_2$−, −C(O)N(R')−, −S(O)$_2$(NR')−, and −Cy−;

each of a and b is independently 1-10;

$L^{X'}$ is a covalent bond or an optionally substituted bivalent or polyvalent straight or branched C_{1-30}

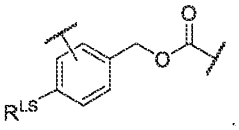
aliphatic wherein 1-10 methylene units of $L^{X'}$ are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –S(O)$_2$–, –C(O)N(R')–, –S(O)$_2$(NR')–, and –Cy–;–C(O)N(R')–, –S(O)$_2$(NR')–, and –Cy–;

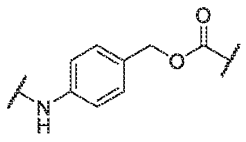
each n is independently 0-24;

each L^{ZP} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –S(O)$_2$–, –C(O)N(R')–, –S(O)$_2$(NR')–, and –Cy–;

each L^{C} is independently $-L^{C1}-L^{CO}-$;

each L^{C1} is independently –Gly–Gly–Phe–Gly–, –Val–Ala–, –Val–Cit–, L^{C1S}, –Gly–Gly–Phe–Gly–N(R')–L^{C1S}–, –Val–Ala–N(R')–L^{C1S}–, or –Val–Cit–N(R')–L^{C1S}–;

each L^{C1S} is independently  , wherein the phenyl ring and the $-CH_2-$ is optionally substituted, and the carbonyl is bonded to L^{CO}, and R^{LS} is or comprises a sugar moiety;

each L^{CO} is independently absent or optionally substituted  wherein the carbonyl is bonded to a payload moiety;

each L^{C} is independently bonded to a payload moiety;

each –Cy– is independently an optionally substituted 3-12 membered ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

each R' is independently –R, –C(O)R, –CO$_2$R, or –S(O)$_2$R, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen or optionally substituted C_{1-6} aliphatic.

24. The conjugate of any one of claims 6-10, wherein the linker is or comprises $\left[-L^{T}-L^{RX}\right]_a-L^{X'}-\left[(CH_2CH_2O)_n-L^{ZP}-L^{C}\right]_b$, wherein:

each L^{T} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{T} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, –S(O)$_2$–, –C(O)N(R')–, –S(O)$_2$(NR')–, and –Cy–;

each L^{RX} is independently a covalent bond or a moiety that results from a reaction between a group present on the unconjugated target binding moiety and a group present on an agent comprising $L^{X'}$ and optionally a payload moiety, wherein $-L^{C}-$ is bonded to the payload moiety; or each L^{RX} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic

wherein 1-10 methylene units of L^{PX} are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −$S(O)_2$−, −C(O)N(R')−, −$S(O)_2$(NR')−, and −Cy−;

each of a and b is independently 1-10;

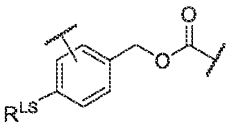
L^X is a covalent bond or an optionally substituted bivalent or polyvalent straight or branched C_{1-30} aliphatic wherein 1-10 methylene units of L^X are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −$S(O)_2$−, −C(O)N(R')−, −$S(O)_2$(NR')−, and −Cy−;

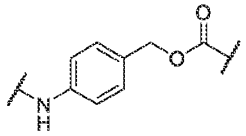
each n is independently 0-24;

each L^{ZP} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −$S(O)_2$−, −C(O)N(R')−, −$S(O)_2$(NR')−, and −Cy−;

each L^C is independently −L^{C1}−L^{CO}−;

each L^{C1} is independently −Gly−Gly−Phe−Gly−, −Val−Ala−, −Val−Cit−, L^{C1S}, −Gly−Gly−Phe−Gly−N(R')−L^{C1S}−, −Val−Ala−N(R')−L^{C1S}−, or −Val−Cit−N(R')−L^{C1S}−;

each L^{C1S} is independently  , wherein the phenyl ring and the −CH_2− is optionally substituted, and the carbonyl is bonded to L^{CO}, and R^{LS} is or comprises a sugar moiety;

each L^{CO} is independently absent or optionally substituted  wherein the carbonyl is bonded to a payload moiety;

each L^C is independently bonded to a payload moiety;

each −Cy− is independently an optionally substituted 3-12 membered ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur;

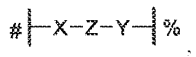
each R' is independently −R, −C(O)R, −CO_2R, or −$S(O)_2$R, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen or optionally substituted C_{1-6} aliphatic.

25. The conjugate of claim 24, wherein each L^T is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^T are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −$S(O)_2$−, −C(O)N(R')−, and −$S(O)_2$(NR')−.

26. The conjugate of any one of claims 24-25, wherein each occurrence of L^T is −CH_2−.

27. The conjugate of any one of the preceding claims, wherein the linker is or comprises a structure of formula B:



B

wherein:

represents the point of attachment to the target binding moiety;

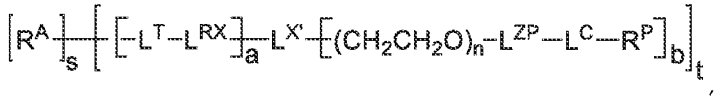
% represents the point of attachment to a payload moiety;

X is a moiety that results from the reaction of an unbound linker and a group present on the target binding moiety;

Y is L^C; and

Z is or comprises $-(CH_2CH_2O)n-$.

28. A conjugate, wherein the conjugate is a compound of formula A-1 or a salt thereof:



A-1

wherein:

each R^A is independently a target binding moiety;

s is 1-20;

each L^T is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^T are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each L^{RX} is independently a covalent bond, a moiety that results from a reaction between a group present on the unconjugated target binding moiety and a group present on an agent comprising $L^{X'}$, or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{RX} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$; or each L^{RX} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{RX} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each of a and b is independently 1-10;

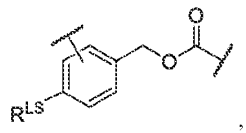
each $L^{X'}$ is independently a covalent bond or an optionally substituted bivalent or polyvalent straight or branched C_{1-30} aliphatic wherein 1-10 methylene units of $L^{X'}$ are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each n is independently 0-24;

each L^{ZP} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each L^C is independently $-L^{Cl}-L^{CO}-$;

each L^{Cl} is independently a cleavable unit, or each L^{Cl} is independently –Gly–Gly–Phe–Gly–, –Val–Ala–, –Val–Cit–, L^{ClS}, –Gly–Gly–Phe–Gly–N(R')–L^{ClS}–, –Val–Ala–N(R')–L^{ClS}–, or –Val–Cit–N(R')–L^{ClS}–;

L^{ClS} is  , wherein the phenyl ring and the $-CH_2-$ is optionally substituted, and the carbonyl is bonded to L^{CO}, and R^{LS} is or comprises a sugar moiety;

each L^{CO} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{CO} are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, $-S(O)_2-$, –C(O)N(R')–, $-S(O)_2(NR')-$, and –Cy–;

each R^P is independently a payload moiety;

t is 1-20;

each –Cy– is independently an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each R' is independently –R, $-C(O)R$, $-CO_2R$, or $-S(O)_2R$, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

29. The conjugate of claim 28, wherein s is 1.

30. The conjugate of any one of claims 28-29, wherein an occurrence of a is 2-10.

31. The conjugate of any one of claims 28-30, wherein an occurrence of b is 2-10.

32. A conjugate, wherein the conjugate is a compound of formula A-2 or a salt thereof:

$$R^A-\left[-L^T-L^{RX}-L^X-(CH_2CH_2O)_n-L^{ZP}-L^C-R^P\right]_t,$$

A-2

wherein:

R^A is a target binding moiety;

each L^T is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^T are optionally and independently replaced by a group selected from –O–, –N(R')–, –C(O)–, –S–, –S(O)–, $-S(O)_2-$, –C(O)N(R')–, $-S(O)_2(NR')-$, and –Cy–;

each L^{RX} is independently a covalent bond, a moiety that results from a reaction between a group present on the unconjugated target binding moiety and a group present on an agent comprising $-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$, or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{RX} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each L^{X} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{X} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each n is independently 0-24;

each L^{ZP} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{ZP} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each L^{C} is independently $-L^{Cl}-L^{CO}-$;

each L^{Cl} is independently a cleavable unit;

each L^{CO} is independently a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{CO} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$, $-S(O)_2-$, $-C(O)N(R')-$, $-S(O)_2(NR')-$, and $-Cy-$;

each R^{P} is independently a payload moiety;

t is 1-20;

each $-Cy-$ is independently an optionally substituted bivalent 3-20 membered ring having 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur;

each R' is independently $-R$, $-C(O)R$, $-CO_2R$, or $-S(O)_2R$, or two or more R' are taken together with their intervening atom(s) to form an optionally substituted 3-20 membered ring having, in addition to the intervening atom(s), 0-10 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur; and

each R is independently hydrogen, or an optionally substituted group selected from C_{1-10} aliphatic, C_{1-10} heteroaliphatic having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, C_{6-14} aryl, a 5-14 membered heteroaryl ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur, and a 3-15 membered heterocyclic ring having 1-5 heteroatoms independently selected from silicon, nitrogen, oxygen, phosphorus and sulfur.

33. The conjugate of claim 32, wherein R^{A} is or comprises an antibody or an antigen-binding fragment thereof.

34. The conjugate of any one of claims 32-33, wherein an occurrence of L^{X} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{X} are optionally and independently replaced by a group selected from $-O-$, $-N(R')-$, $-C(O)-$, $-S-$, $-S(O)-$,

−S(O)2−, −C(O)N(R')−, −S(O)2(NR')−, and −Cy−.

35. The conjugate of any one of claims 28-34, wherein an occurrence of L^T is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^T are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)2−, −C(O)N(R')−, −S(O)2(NR')−, and −Cy−.

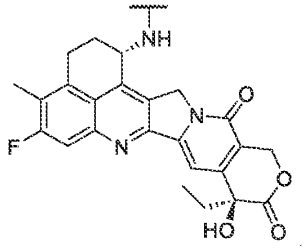
36. The conjugate of any one of claims 28-35, wherein an occurrence of L^T is $-CH_2-$.

37. The conjugate of any one of claims 28-36, wherein an occurrence of L^{RX} is a covalent bond or a moiety that results from a reaction between a group present on the unconjugated target binding moiety and a group present on an agent comprising $-L^X-(CH_2CH_2O)n-L^{ZP}-L^C-$ and a payload moiety, or wherein an occurrence of L^{RX} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^{RX} are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)2−, −C(O)N(R')−, −S(O)2(NR')−, and −Cy−, wherein each −Cy− is independently an optionally substituted 3-12 membered ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, and sulfur.

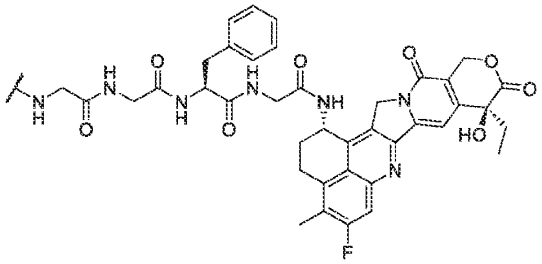
38. The conjugate of any one of claims 28-37, wherein an occurrence of n is 8.

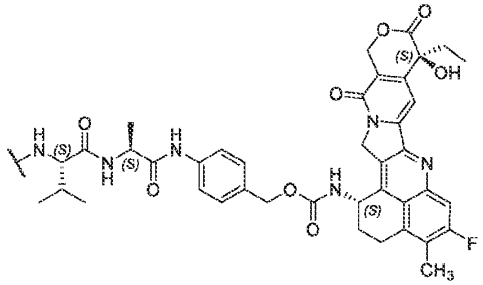
39. The conjugate of any one of claims 28-38, wherein an occurrence of L^{ZP} is a covalent bond or an optionally substituted bivalent straight or branched C_{1-20} aliphatic wherein 1-10 methylene units of L^Z are optionally and independently replaced by a group selected from −O−, −N(R')−, −C(O)−, −S−, −S(O)−, −S(O)2−, −C(O)N(R')−, −S(O)2(NR')−, and −Cy−.

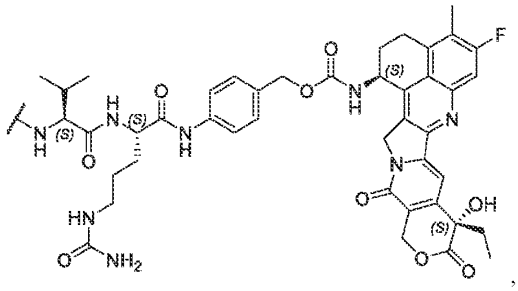
40. The conjugate of any one of claims 28-39, wherein each R^P is

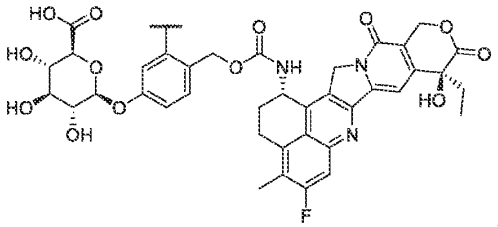


41. The conjugate of any one of claims 28-33, wherein each $-L^C-R^P$ is independently



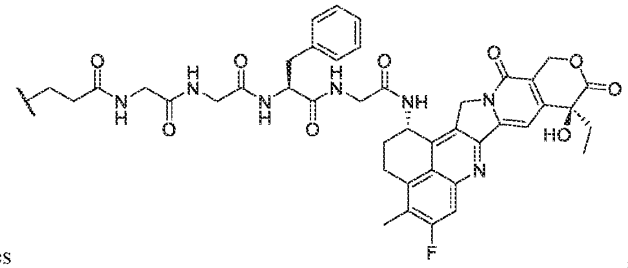




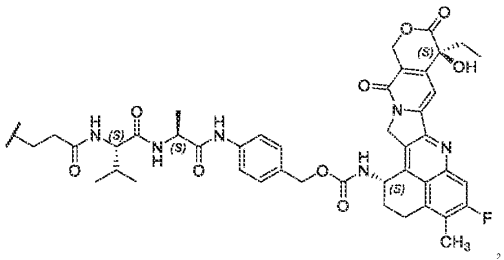


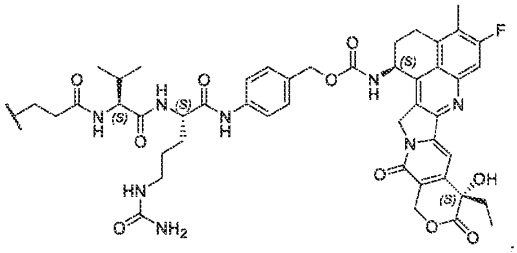
or

42. The conjugate of any one of claims 28-33, wherein each occurrence of $-L^{ZP}-L^{C}-R^{P}$ is or

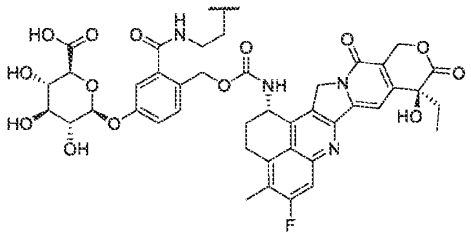


comprises

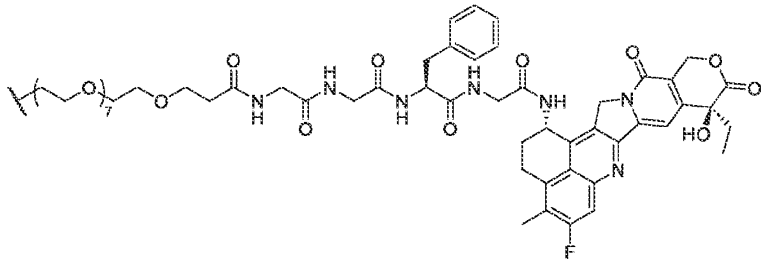


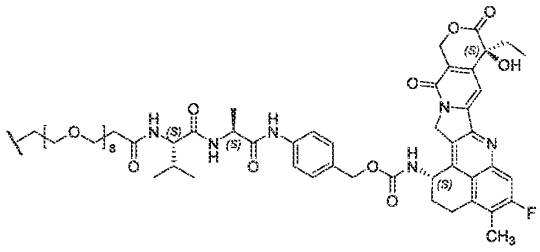


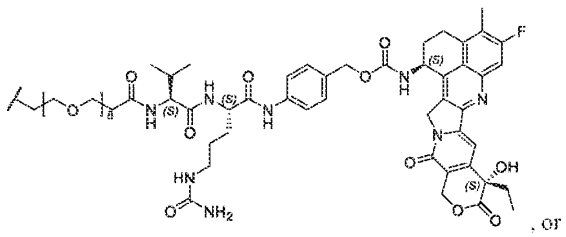
. or



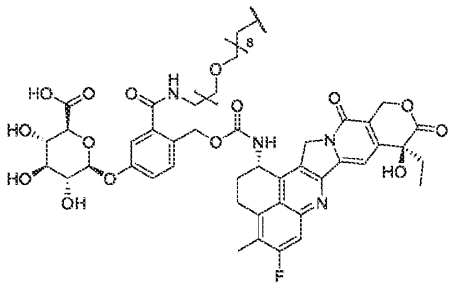
43. The conjugate of any one of claims 28-33, wherein each occurrence of $-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



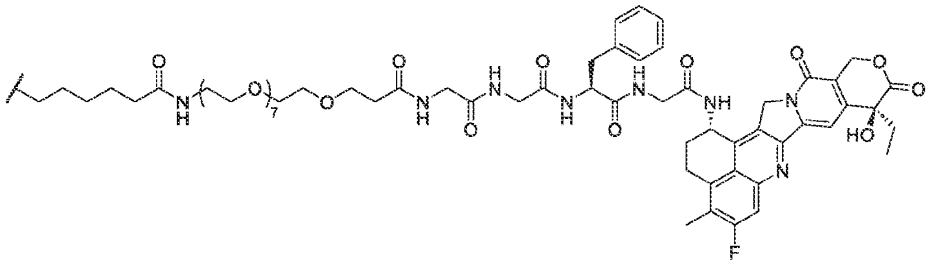


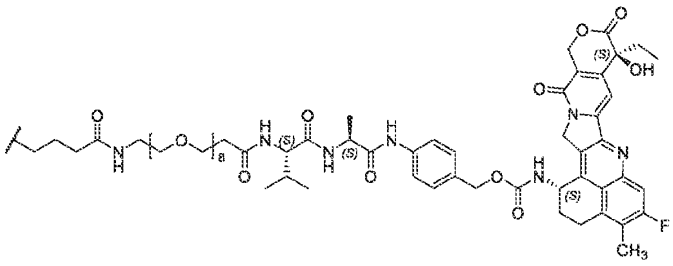


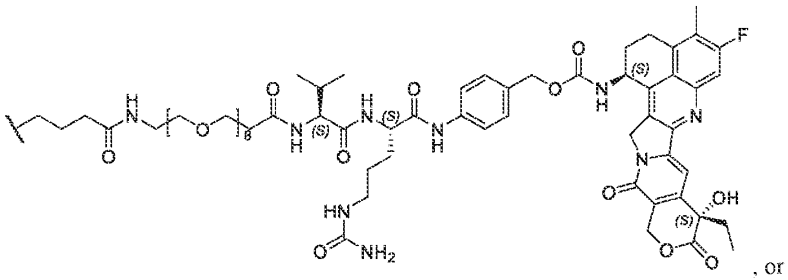
, or



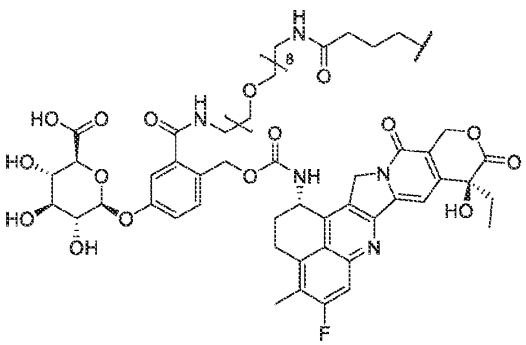
44. The conjugate of any one of claims 28-33, wherein an occurrence of $-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



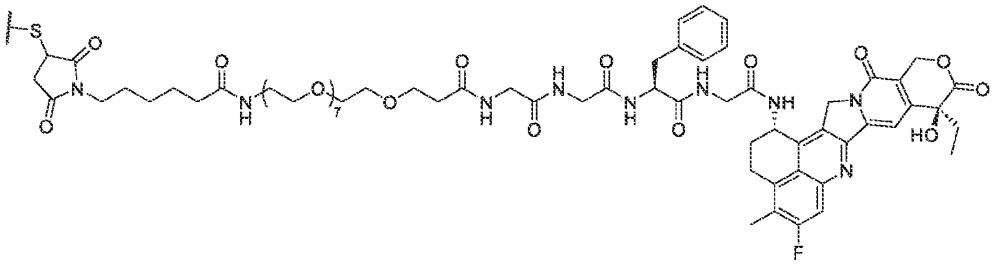


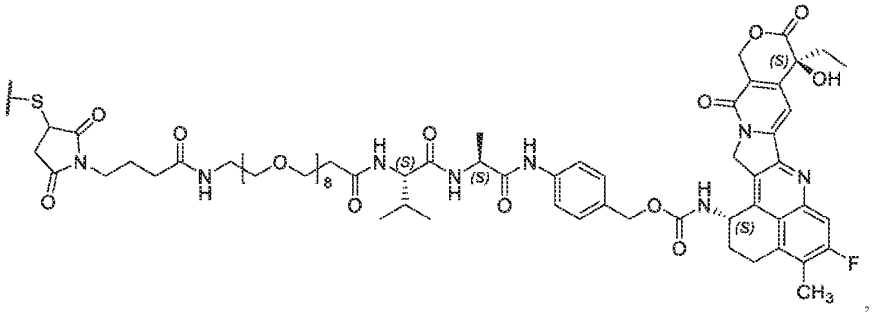


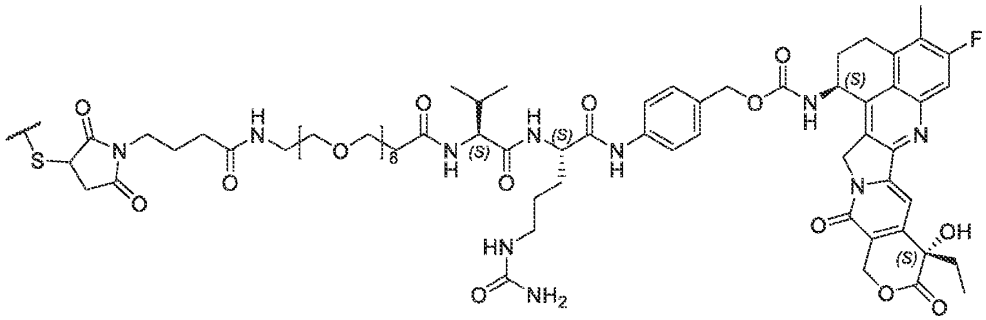
, or

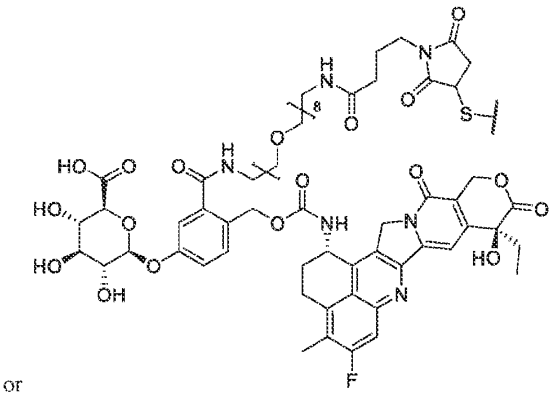


45. The conjugate of any one of claims 28-33, wherein each occurrence of $-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises



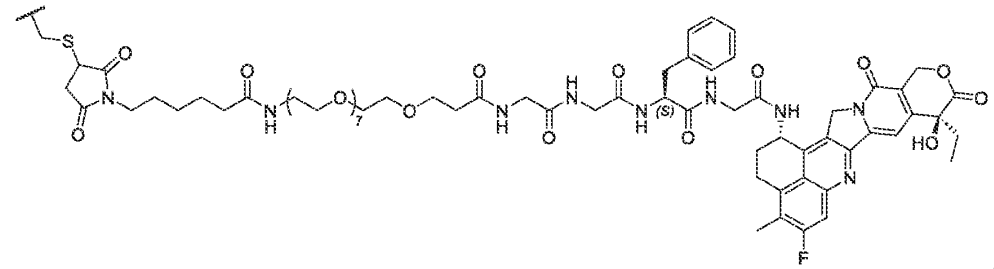


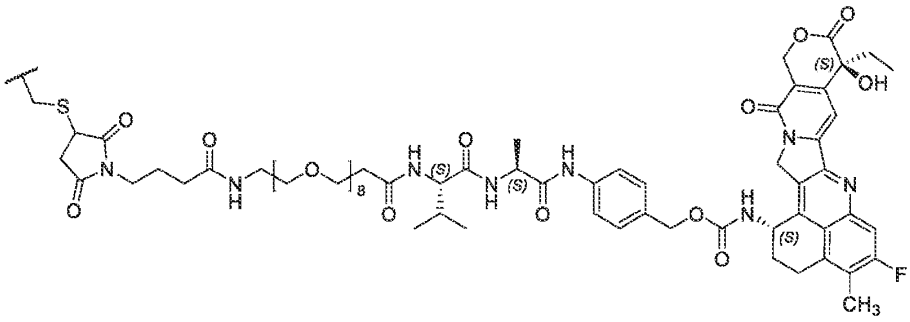


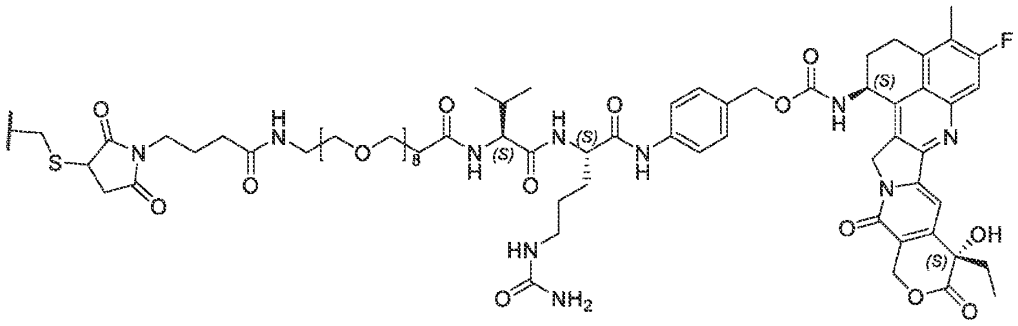


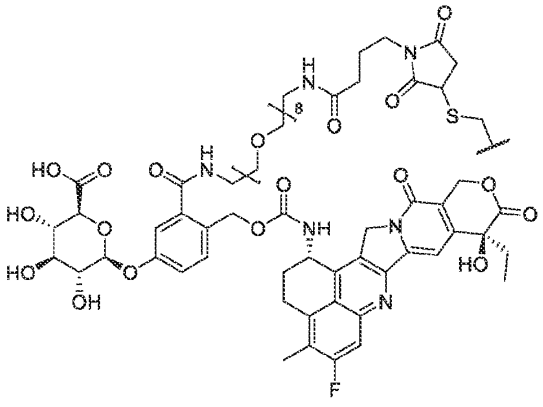
or

46. The conjugate of any one of claims 28-33, wherein each occurrence of $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is or comprises





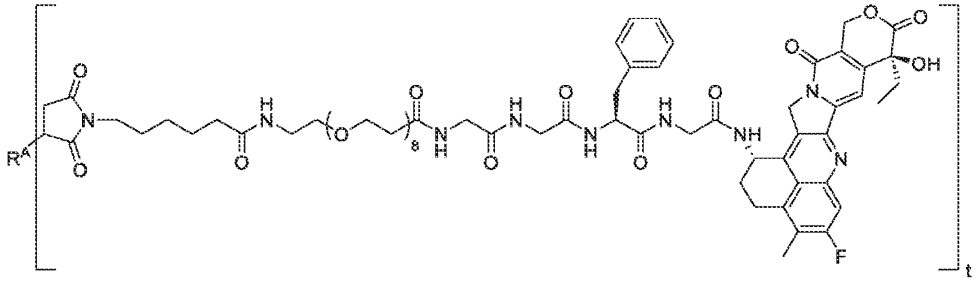




or

47. The conjugate of any one of claims 28-46, wherein the target binding moiety comprises an amino acid residue, and $-L^{T}-L^{RX}-L^{X}-(CH_2CH_2O)n-L^{ZP}-L^{C}-R^{P}$ is bonded to the backbone atom of the amino acid residue.

48. The conjugate of claim 6, having a structure of formula X-C:



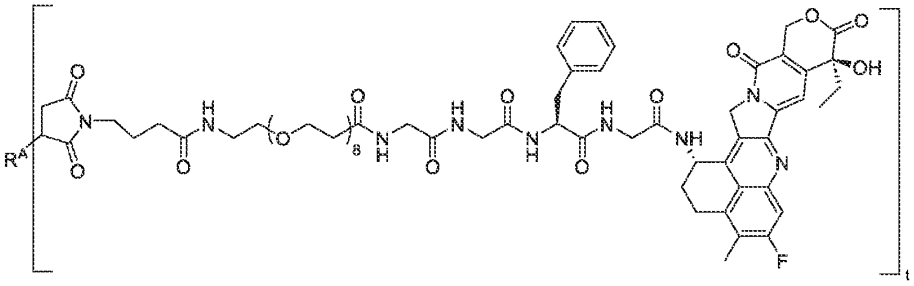
X-C

or a pharmaceutically acceptable salt thereof, wherein:

R^A is a target binding moiety; and

t is an integer between 1 and 16.

49. The conjugate of claim 6, having a structure of formula X-C':



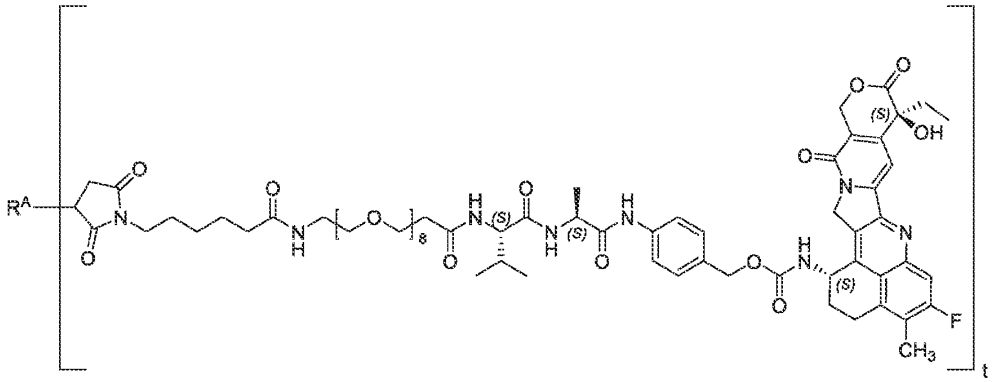
X-C'

or a pharmaceutically acceptable salt thereof, wherein:

R^A is a target binding moiety; and

t is an integer between 1 and 16.

50. The conjugate of claim 6, having a structure of formula X-G':



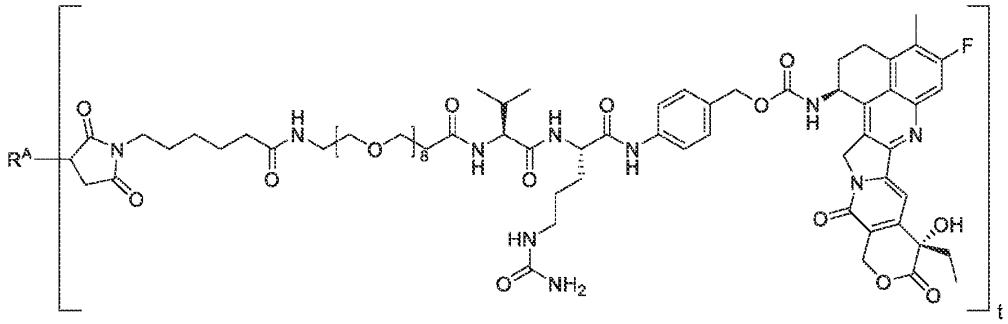
X-G'

or a pharmaceutically acceptable salt thereof, wherein:

R^A is a target binding moiety; and

t is an integer between 1 and 16.

51. The conjugate of claim 6, having a structure of formula X-H':



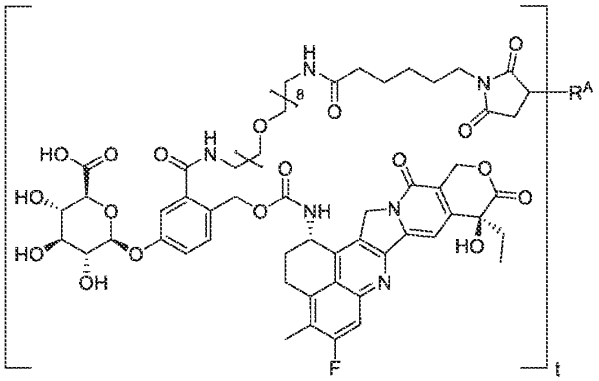
X-H'

or a pharmaceutically acceptable salt thereof, wherein:

R^A is a target binding moiety; and

t is an integer between 1 and 16.

52. The conjugate of claim 6, having a structure of formula X-I':



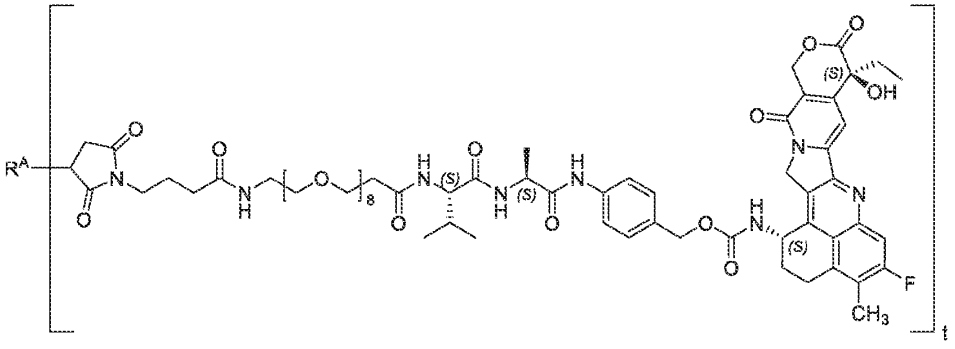
X-I'

or a pharmaceutically acceptable salt thereof, wherein:

R^A is a target binding moiety; and

t is an integer between 1 and 16.

53. The conjugate of claim 6, having a structure of formula X-J':



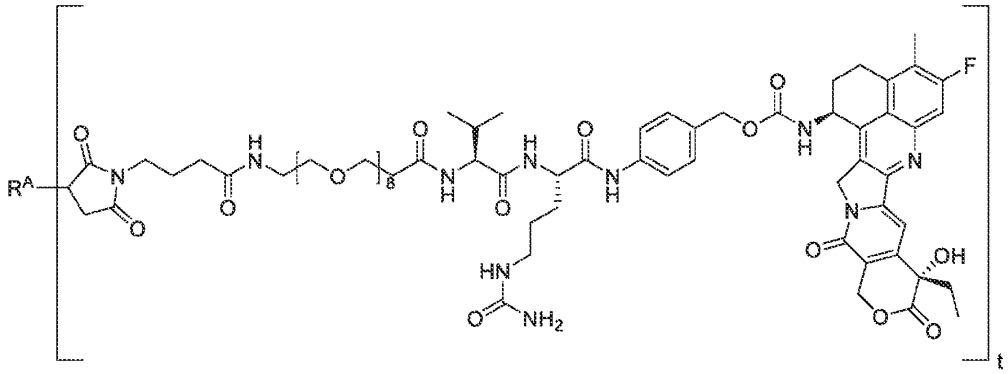
X-J'

or a pharmaceutically acceptable salt thereof, wherein:

R^A is a target binding moiety; and

t is an integer between 1 and 16.

54. The conjugate of claim 6, having a structure of formula X-K':



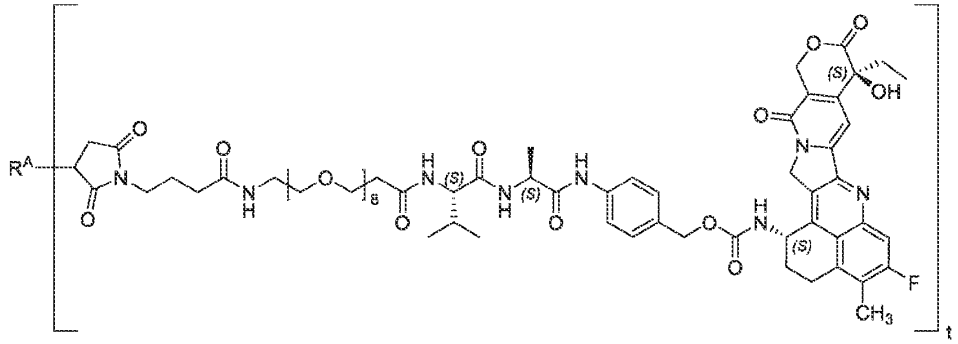
X-K'

or a pharmaceutically acceptable salt thereof, wherein:

R^A is a target binding moiety; and

t is an integer between 1 and 16.

55. The conjugate of claim 6, having a structure of formula X-L':



X-L'

or a pharmaceutically acceptable salt thereof, wherein:

R^A is a target binding moiety; and

t is an integer between 1 and 16.

56. The conjugate of any one of claims 28-55, wherein t is about 2-8.

57. The conjugate of any one of the preceding claims, wherein each target binding moiety is independently an antibody or an antigen-binding fragment thereof.

58. The conjugate of any one of claims 6-56, wherein each target binding moiety is an anti-HHLA2 antibody or an antigen-binding fragment thereof.

59. The conjugate of claim 58, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises:

(a) a heavy chain variable region (VH) comprising one, two, or three VH CDR sequences each with at least about 90% identity to a VH CDR of Table 1; and/or

(b) a light chain variable region (VL) comprising one, two, or three VL CDR sequences each with at least about 90% identity to a VL CDR of Table 1.

60. The conjugate of claim 58, wherein the anti-HHLA2 or antigen-binding fragment thereof is or comprises:

(a) a VH comprising or consisting of a VH of Table 1; and/or

(b) a VL comprising or consisting of a VL of Table 1.

61. The conjugate of any one of claims 58-60, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises:

a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR1 of 887-1DE in Table 1;

a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR2 of 887-1DE in Table 1;

a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR3 of 887-1DE in Table 1;

a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 90% identity to LCDR1 of 887-1DE in Table 1;

a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 90% identity to LCDR2 of 887-1DE in Table 1; and/or

a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 90% identity to LCDR3 of 887-1DE in Table 1.

62. The conjugate of any one of claims 58-60, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises:

a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR1 of 65889 in Table 1;

a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR2 of 65889 in Table 1;

a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR3 of 65889 in Table 1;

a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 90% identity to LCDR1 of 65889 in Table 1;

a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 90% identity to LCDR2 of 65889 in Table 1; and/or

a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 90% identity to LCDR3 of 65889 in Table 1.

63. The conjugate of any one of claims 58-60, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises:

a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR1 of 65872 in Table 1;

a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR2 of 65872 in Table 1;

a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR3 of 65872 in Table 1;

a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 90% identity to LCDR1 of 65872 in Table 1;

a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 90% identity to LCDR2 of 65872 in Table 1; and/or

a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 90% identity to LCDR3 of 65872 in Table 1.

64. The conjugate of any one of claims 58-60, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises:

a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR1 of Hu2C4-H3L3 in Table 1;

a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR2 of Hu2C4-H3L3 in Table 1;

a heavy chain variable region (VH) comprising a VH CDR sequence with about or at least about 90% identity to HCDR3 of Hu2C4-H3L3 in Table 1;

a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 90% identity to LCDR1 of Hu2C4-H3L3 in Table 1;

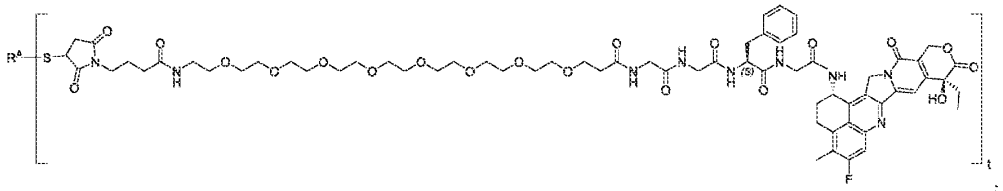
a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 90% identity to LCDR2 of Hu2C4-H3L3 in Table 1; and/or

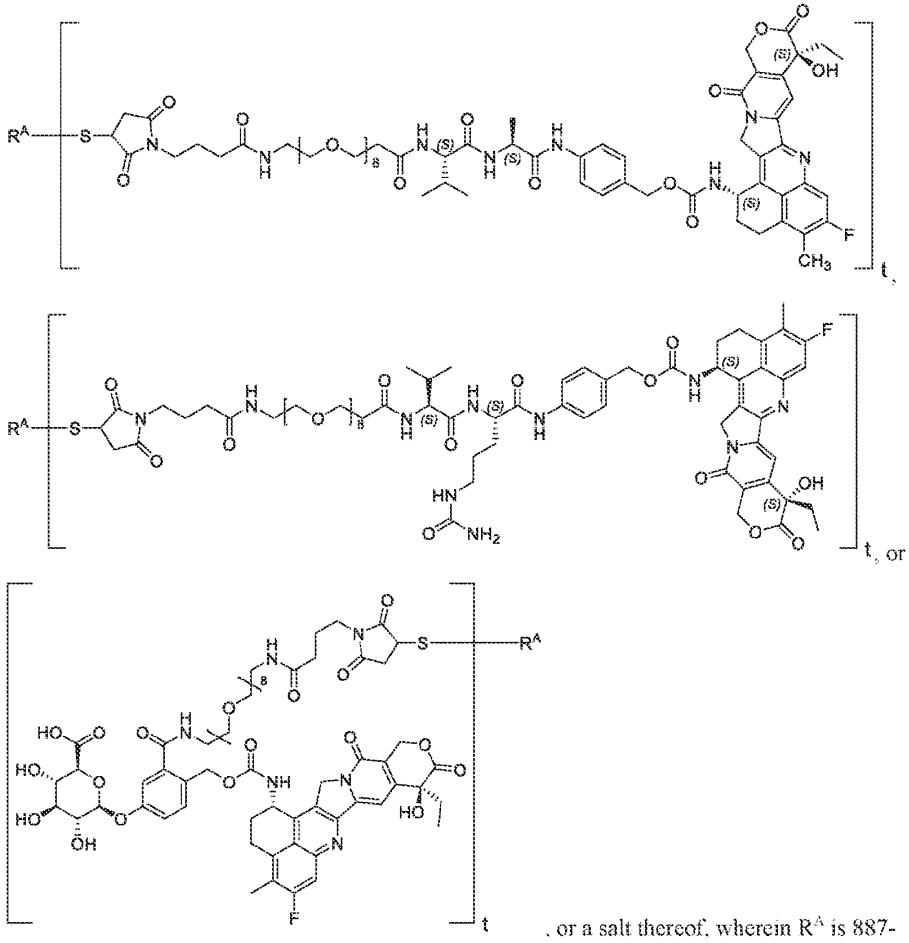
a light chain variable region (VL) comprising a VL CDR sequence with about or at least about 90% identity to LCDR3 of Hu2C4-H3L3 in Table 1.

65. The conjugate of any one of claims 58-60, wherein the anti-HHLA2 antibody or an antigen-binding fragment thereof is or comprises 887-1DE, 65889, 65872 or Hu2C4H3L3 in Table 1.

66. The conjugate of any one of claims 58-60, which binds HHLA2 with a K_D of about 15 nM or less.

67. A conjugate, wherein the conjugate is a compound having the structure of





, or a salt thereof, wherein R^A is 887-1DE, 65889, 65872 or Hu2C4H3L3 in Table 1, each –S– is independently of a Cys residue, and t is about 1-8.

68. The conjugate of claim 67, wherein t is about 2-8.

69. The conjugate of any one of the preceding claims, wherein the conjugate is in a pharmaceutically acceptable salt form.

70. A composition comprising one or more conjugates each of which is independently a conjugate of any one of the preceding claims.

71. The composition of claim 70, wherein the ratio of the payload moiety to the target binding moiety of the composition is about 4-8.

72. The composition of any one of claims 70-71, wherein the composition is a pharmaceutical composition.

73. The composition of any one of claims 70-72, wherein the composition comprises a pharmaceutically acceptable carrier.

74. A kit comprising a conjugate of any one of claims 1-69 or a composition of any one of claims 70-73, and optionally instructions for use and/or administration.

75. A method of modulating an immune response in a subject comprising administering or delivering to a subject in need thereof a therapeutically effective amount of a conjugate or composition of any one of claims 1-73.

76. A method of treating a subject having a disease, disorder, or condition comprising administering or delivering to a subject in need thereof a therapeutically effective amount of a conjugate or composition of any one of claims 1-73.

77. The method of any one of claims 75-76, wherein the subject has cancer.

78. The method of any one of claims 75-77, wherein the subject has a solid tumor, or wherein the subject has a renal cancer, a bone cancer, a skin cancer, a breast cancer, a cervical cancer, a colorectal cancer, an endometrial cancer, a lung cancer, an ovarian cancer, a liver cancer, a prostate cancer, a pancreatic cancer, cholangiocarcinoma, or a thyroid cancer, or wherein the subject has a hematological tumor, or wherein the subject has leukemia, or wherein the subject has acute lymphocytic leukemia, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, chronic leukemia, or acute leukemia, or wherein the subject has lymphoma, or wherein the subject has Hodgkin lymphoma (HL), non-Hodgkin's lymphoma, lymphocytic lymphoma, or diffuse large B cell lymphoma (DLBCL), or wherein the subject is at risk of developing a cancer.

79. The method of any one of claims 76-78, wherein the disease, disorder, or condition is associated with aberrant HHLA2 expression.

80. The method of any one of claims 75-79, wherein the conjugate is administered in combination with a second agent.

81. A method for inhibiting proliferation of diseased cells in a system, comprising administering to or delivering to the system a conjugate or composition of any one of claims 1-73, wherein the diseased cells expresses or comprise an agent that a target binding moiety of the conjugate can bind.

82. A method for reduce number of diseased cells in a system, comprising administering to or delivering to the system a conjugate or composition of any one of claims 1-73, wherein the diseased cells expresses or comprise an agent that a target binding moiety of the conjugate can bind.

83. A method for delivering a payload to a target in a system, comprising administering to or delivering to the system a conjugate or composition of any one of claims 1-73, wherein:

a payload moiety of the conjugate is a moiety of the payload; and

the target expresses or comprises an agent that a target binding moiety of the conjugate can bind.

84. A conjugate, composition or kit of any one of claims 1-74 for use in the treatment of a condition, disease or disorder, or for use in a method of any one of the preceding claims.

85. Use of a conjugate, composition or kit of any one of claims 1-74 for the manufacture of a medicament for the treatment of a condition, disease or disorder, or for the manufacture of a medicament for a method of any one of the preceding claims.

86. A method for preparing a conjugate or composition of any one of claims 1-73, comprising reacting a target binding agent and a payload agent, wherein:

the target binding agent comprises a target binding moiety and a target binding agent reactive

group;

the payload agent comprises a payload moiety and a payload agent reactive group; and

the target binding agent reactive group reacts with the payload agent reactive group to conjugate the payload moiety to the target binding moiety optionally through a linker.

87. The method of claim 84, wherein the payload agent is Compound 1, Compound 2, Compound 3, Compound 4, Compound 5, Compound 6, LP-A, LP-B, LP-C or LP-D.

88. A conjugate, composition, kit, method, or compound of any one of Embodiments 1-813.