# HYDROPHILIC LINKERS FOR ANTIBODY DRUG CONJUGATES

**[01]** This PCT International application claims the benefit of priority to U.S. Provisional Application No. 62/669,034, filed on May 9, 2018; PCT International Application PCT/US17/60434 (published as WO 2018/089373), filed on November 7, 2017; and U.S. Nonprovisional Application No. 15/806,197 (published as US 2018/0155389), filed on November 7, 2017; the content of all said applications is incorporated herein by reference in its entirety for all purposes.

## FIELD

**[02]** Provided herein are novel protein, e.g., antibody, drug conjugates comprising hydrophilic solubilizing groups and/or linkers comprising hydrophilic solubilizing groups, and methods for treating diseases, disorders, and conditions comprising administering the protein drug conjugates comprising hydrophilic solubilizing groups and/or linkers thereof.

## BACKGROUND

**[03]** Antibody-drug conjugates (ADCs) are antibodies that are conjugated to a biologically active small molecule. ADCs deliver a potent drug selectively to target- expressing cells, leading to a potential reduction of off-target side effects and/or toxicity and improved therapeutic index. The lipophilic nature of many payloads (*i.e.*, drugs) can adversely affect the properties of the ADC to the extent that the payloads are not efficiently delivered to the target cells. Low bioavailability of lipophilic payloads can narrow therapeutic windows for ADC treatment. Furthermore, the hydropobic nature of payloads can present challenges to their conjugation to antibodies, a reaction performed in aqueous conditions. Thus there is an ongoing need for development of hydrophilic linkers for protein conjugates, e.g., ADCs, which would allow for the feasibility of conjugating lipophilc payloads, improved modulation of biological targets, improved bioavailability, and improved therapeutic window.

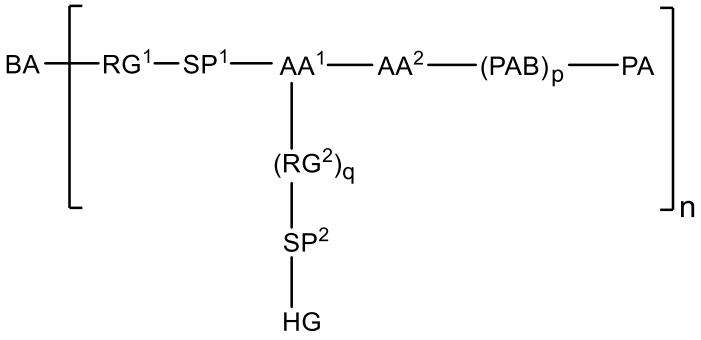
## SUMMARY

**[04]** Provided herein are compounds useful, for example, for the treatment of diseases, conditions, and disorders including, without limitation, metabolic diseases, proliferative diseases, and other diseases and conditions.

**[05]** In one example, set forth herein, is a compound or a pharmaceutically acceptable salt thereof, including: a protein binding agent linked to at least one payload moiety and linked to at least one hydrophilic moiety via a covalent linker, wherein said covalent linker is bonded directly or indirectly to each of the binding agent, the payload moiety, and the hydrophilic moiety.

**[05a]** In one aspect, set forth herein is a compound, or pharmaceutically acceptable salt, solvate, triazole linkage regioisomer, mixture of triazole linkage regioisomers, or stereoisomer thereof,

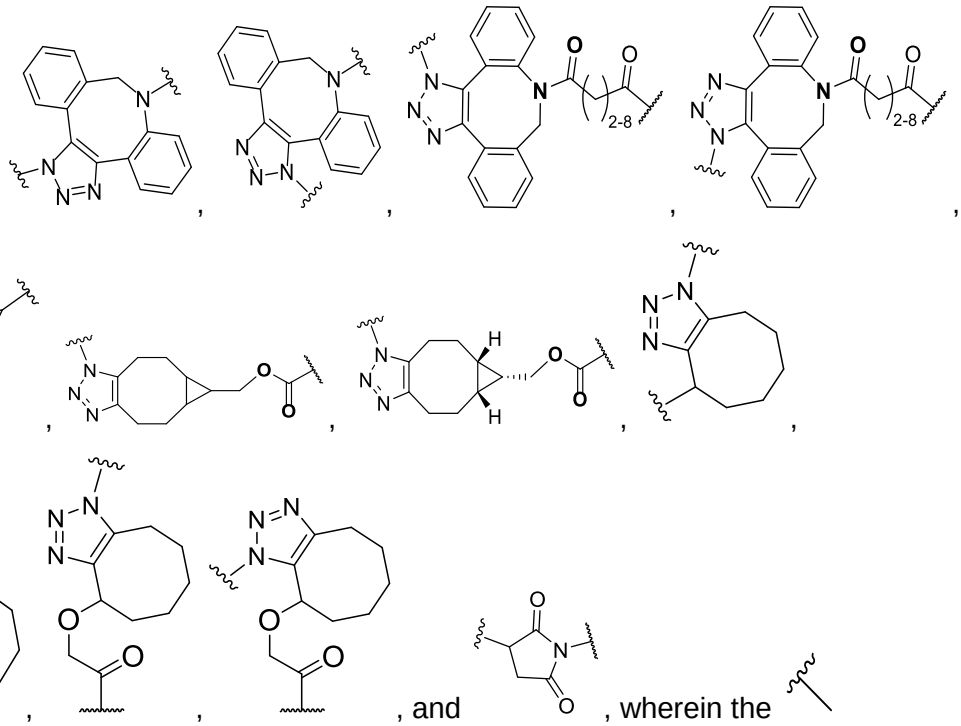
wherein the compound has a structure according to Formula (**IV**):




(**IV**)

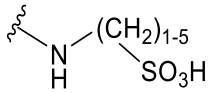
wherein:

**BA** is an antibody or antigen binding fragment thereof;

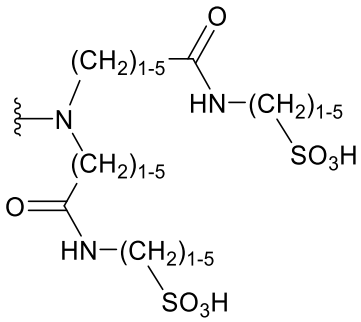
**RG¹** and **RG²** are reactive group residuesindependently selected from the group consisting of  , and , wherein the  indicates the atom through which the $RG^1$ or $RG^2$ is bonded to the adjacent groups in the formula;

**SP[1]** and **SP[2]** are independently, in each instance, absent, or a spacer group residue selected from the group consisting of $C_{1-6}$alkylene, -NH-, -C(O)-, -$(CH_2$-$CH_2$-$O)_e$, -NH-$(CH_2$-$CH_2$-$O)_e$-$CH_2$-$CH_2$-C(O)-, -C(O)-$(CH_2)_u$-C(O)-, -C(O)-NH-$(CH_2)_v$-, and combinations thereof wherein subscript e is an integer from 0 to 6, subscript u is an integer from 1 to 8, and subscript v is an integer from 1 to 8;

**HG** is a hydrophilic residue selected from



and

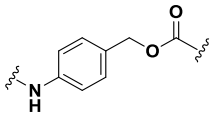


wherein the  indicates the atom through which the **HG** is bonded to the adjacent groups in the formula ;

**PA** is a payload residue;

**AA[1]** is an amino acid residue selected from tryptophan, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, and citrulline;

**AA$^2$** is a dipeptide, tripeptide, or tetrapeptide residuecomprising amino acid residues selected from alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, and citrulline;

**PAB** is  , wherein the  indicates the atom through which the **PAB** is bonded to the adjacent groups in the formula;

subscript **n** is an integer from 1 to 30;

subscript **p** is 0 or 1; and

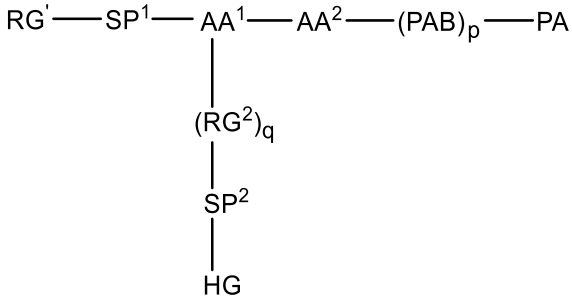
subscript **q** is 1.

**[05b]** In another aspect, set forth herein, is a pharmaceutical composition comprising a compound as described herein, or pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

**[05c]** In another aspect, set forth herein, is a compound as described herein, or a composition as described herein for use in treating a disease or disorder in a patient in need thereof.

**[06]** In another example, set forth herein, is a compound, including: a reactive group linked to at least one payload moiety and linked to at least one hydrophilic moiety via a covalent linker, wherein said covalent linker is bonded directly or indirectly to each of the reactive group, the payload moiety, and the hydrophilic moiety.

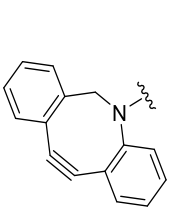
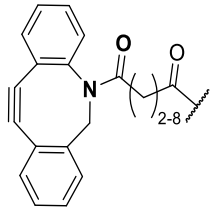
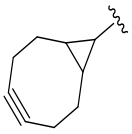
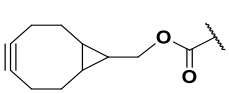
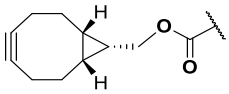
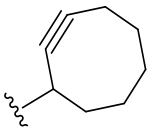
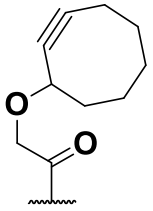
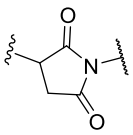
**[06a]** In another aspect, set forth herein, is a compound or stereoisomeric form, a triazole linkage regioisomer, or mixture of triazole linkage regioisomers thereof, according to Formula (**IX**):

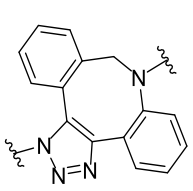
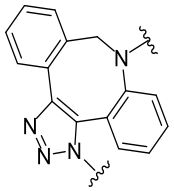
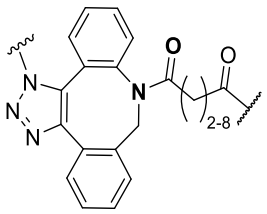
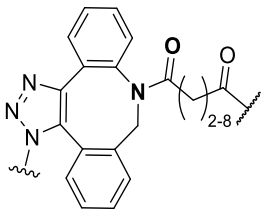
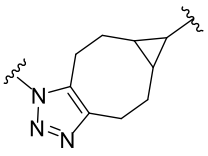
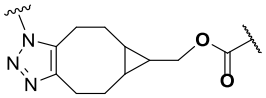
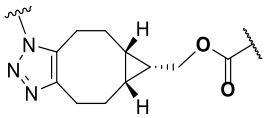
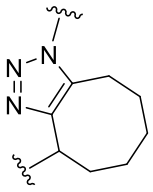
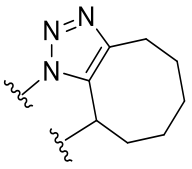
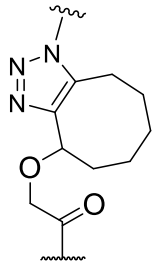
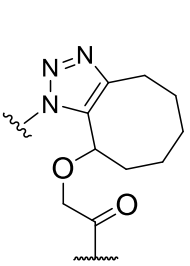
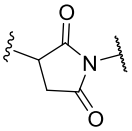



(**IX**)

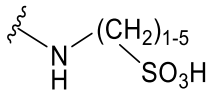
**AA$^1$** is an amino acid residue selected from tryptophan, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, and citrulline;

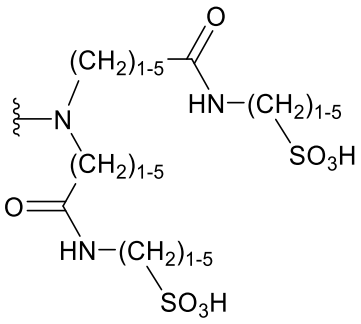
**AA$^2$** is a dipeptide, tripeptide or tetrapeptide residue comprising amino acid residues selected from alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, and citrulline;

**RG′** is a reactive group selected from  ,  ,  ,  ,  ,  ,  , and  , wherein the  indicates the atom through which the **RG′** is bonded to the adjacent groups in the formula;

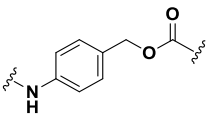
**RG$^2$** is a reactive group residue selected from  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  , and  , wherein the  indicates the atom through which the $RG^1$ or $RG^2$ is bonded to the adjacent groups in the formula;

**SP$^1$** and **SP$^2$** are independently, in each instance, absent, or a spacer group residue selected from the group consisting of $C_{1\text{-}6}$alkylene, -NH-, -C(O)-, -$(CH_2$-$CH_2$-$O)_e$, -NH-$(CH_2$-$CH_2$-$O)_e$-$CH_2$-$CH_2$-C(O)-, -C(O)-$(CH_2)_u$-C(O)-, -C(O)-NH-$(CH_2)_v$-, and combinations thereof wherein subscript e is an integer from 0 to 6, subscript u is an integer from 1 to 8, and subscript v is an integer from 1 to 8;

**HG** is a hydrophilic residue selected from  and



;

**PAB** is  , wherein the  indicates the atom through which the PAB is bonded to the adjacent groups in the formula;

subscript **p** is 0 or 1;

**PA** is a payload residue;

subscript **q** is 1; and

wherein the  indicates the atom through which the **HG** is bonded to the adjacent groups in the formula.

**[07]** In another example, set forth herein is an antibody-drug conjugate having a compound, described above and herein, bonded to an antibody or an antigen binding fragment thereof.

**[07a]** In another aspect, set forth herein is an antibody-drug-conjugate comprising the compound as described herein bonded to an antibody, or an antigen binding fragment thereof.

**[08]** In another example, set forth herein is a method of treating a disease, condition, or disorder in a patient in need thereof including administering to the patient a compound set forth herein. Also provided is the use of a compound set forth herein for treating a disease, condition, or disorder set forth herein. Further provided is the use of a compound set forth herein for the manufacture of a medicament for treating a disease, condition, or disorder set forth herein. In some examples, the compound is an antibody-drug conjugate.

**[08a]** In another aspect, set forth herein is a compound or pharmaceutical composition as described herein for use in treating a proliferative disease, a metabolic disease, inflammation, or a neurodegenerative disease in a subject.

**[08b]** In another aspect, set forth herein is a compound or pharmaceutical composition as described herein for use in the treatment of a proliferative disease in a subject.

**[08c]** In another aspect, set forth herein is a compound or pharmaceutical composition as described herein for use in the treatment of a metabolic disease in a subject.

**[08d]** In another aspect, set forth herein is a compound or pharmaceutical composition as described herein for use in the treatment of inflammation in a subject.

**[08e]** In another aspect, set forth herein is a compound or pharmaceutical composition as described herein for use in the treatment of a neurodegenerative disease in a subject.

**[08f]** In another aspect, set forth herein is use of a compound or pharmaceutical composition as described herein in the manufacture of a medicament for treating a proliferative disease, a metabolic disease, inflammation, or a neurodegenerative disease in a subject.

**[09]** In another example, set forth herein is a method of preparing an antibody-drug conjugate including the step of contacting a binding agent with a linker-payload compound set forth herein under conditions suitable for forming a bond between the binding agent and the linker-payload compound.

**[09a]** In another aspect, set forth herein is a method of preparing a compound as described herein comprising the step of contacting an antibody or antigen binding fragment thereof with a compound as described herein, under conditions suitable for forming a bond between the antibody or antigen binding fragment thereof and the compound.

## BRIEF DESCRIPTIONS OF THE DRAWINGS

**[010]** FIG. 1 shows three different types of payload compounds.

**[011]** FIG. 2 shows a synthetic process for preparing compounds 1a, 1c, 1e, 1f, 1i, 1j, 1k, 1m, 1q, Ib, Id, Ie-1, If-1, IIb, IIId, IIIe, and IVb.

**[012]** FIG. 3 shows shows a synthetic process for preparing compounds 1h, 1l, Ig, and Ih-1.

**[013]** FIG. 4 shows a synthetic process for preparing payloads B and C.

**[014]** FIG. 5 shows a synthetic process for preparing payload D.

**[015]** FIG. 6 shows a general synthetic procedure A for preparing intermediates 3a, 3b, 3c, and 3g.

**[016]** FIG. 7 shows a general synthethic procedure for preparing intermediates 3d, and 3f.

**[017]** FIG. 8 shows a synthethic process for preparation of intermediate 3e.

**[018]** FIG. 9 shows a synthethic process for preparation of intermediate 4a.

**[019]** FIG. 10 shows a synthetic process for preparation of intermediate 4b.

**[020]** FIG. 11 shows a synthetic process for preparation of intermediates 5a-g.

**[021]** FIG. 11A shows a synthetic process for preparation of intermediate 10a.

**[022]** FIG. 11B shows a synthetic process for preparation of intermediate 10b.

**[023]** FIG. 11C shows a synthetic process for preparation of intermediates 10a, 10b, 10c, and 10d.

**[024]** FIG. 12 shows a synthetic process for preparation of intermediate 6c.

**[025]** FIG. 13 shows a synthetic process for preparation of intermediate 6d.

**[026]** FIG. 13A shows a synthetic process for preparation of intermediate 6b.

**[027]** FIG. 13B shows a synthetic process for preparation of intermediate 6f

**[028]** FIG. 14 shows a general synthetic procedure E for preparing intermediates 7a, 7c, 7e, 7f, 7i, 7k, 7m, 7q, 7ab, 7ad, 7ae, 7bb, 7cb, and 7fb.

**[029]** FIG. 15 shows a general synthetic procedure F for preparing intermediates 7h and 7l.

**[030]** FIG. 15A shows a general synthetic procedure for preparing intermediate 7ah.

**[031]** FIG. 15B shows a synthetic procedure for preparing intermediate 8c.

**[032]** FIG. 16 shows a general synthetic procedure G for preparing compound 1a.

**[033]** FIG. 17 shows a general synthetic procedure H for preparing compounds 1a, 1c, 1e, 1f, 1h, 1i, 1j, 1k, 1l, 1m, 1q, Ib, Id, If-1, IIb, IIId, IIIe, and IVb.

**[034]** FIG. 18 shows cleavable pieces for Cathepsin B cleavage of linker-payload to release free payload.

**[035]** FIG. 18A shows a synthetic procedure for preparing Ig and Ih-1.

**[036]** FIG. 18B shows cleavable pieces for Cathepsin B cleavage of linker-payload to release free payload.

**[037]** FIG. 19 shows an example of a general synthetic procedure for antibody drug conjugations.

**[038]** FIG. 20 shows reagents that may be used in place of d-Lys in the methods set forth herein.

## DETAILED DESCRIPTION

**[039]** Provided herein are compounds, compositions, and methods useful for treating, for example, dyslipidemia, a metabolic disease, inflammation, or a neurodegenerative disease, in a subject.

**[040]** Before the present invention is described, it is to be understood that this invention is not limited to particular methods and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the

purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

**[041]** Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (e.g., 99.1, 99.2, 99.3, 99.4, etc.).

**[042]** Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All patents, applications and non-patent publications mentioned in this specification are incorporated herein by reference in their entireties.

**Definitions**

**[043]** When referring to the compounds provided herein, the following terms have the following meanings unless indicated otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art. In the event that there is a plurality of definitions for a term provided herein, these Definitions prevail unless stated otherwise.

**[044]** As used herein, "alkyl" refers to a monovalent and saturated hydrocarbon radical moiety. Alkyl is optionally substituted and can be linear, branched, or cyclic, *i.e.*, cycloalkyl. Alkyl includes, but is not limited to, those radicals having 1-20 carbon atoms, *i.e.*, $C_{1-20}$ alkyl; 1-12 carbon atoms, *i.e.*, $C_{1-12}$ alkyl; 1-8 carbon atoms, *i.e.*, $C_{1-8}$ alkyl; 1-6 carbon atoms, *i.e.*, $C_{1-6}$ alkyl; and 1-3 carbon atoms, *i.e.*, $C_{1-3}$ alkyl. Examples of alkyl moieties include, but are not limited to methyl, ethyl, n-propyl, i-propyl, n-butyl, s-butyl, t-butyl, i-butyl, a pentyl moiety, a hexyl moiety, cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl. A pentyl moiety includes, but is not limited to, n-pentyl and i-pentyl. A hexyl moiety includes, but is not limited to, n-hexyl.

**[045]** As used herein, "alkylene" refers to a divalent alkyl group. Unless specified otherwise, alkylene includes, but is not limited to, 1-20 carbon atoms. The alkylene group is optionally substitued as described herein for alkyl. In some embodiments, alkylene is unsubstituted.

**[046]** Designation of an amino acid or amino acid residue without specifying its stereochemistry is intended to encompass the L form of the amino acid, the D form of the amino acid, or a racemic mixture thereof.

**[047]** As used herein, "haloalkyl" refers to alkyl, as defined above, wherein the alkyl includes at least one substituent selected from a halogen, for example, fluorine (F), chlorine (Cl), bromine (Br), or iodine (I). Examples of haloalkyl include, but are not limited to, $-CF_3$, $-CH_2CF_3$, $-CCl_2F$, and $-CCl_3$.

**[048]** As used herein, "alkenyl" refers to a monovalent hydrocarbon radical moiety containing at least two carbon atoms and one or more non-aromatic carbon-carbon double bonds. Alkenyl is optionally substituted and can be linear, branched, or cyclic. Alkenyl includes, but is not limited to, those radicals having 2-20 carbon atoms, *i.e.*, $C_{2-20}$ alkenyl; 2-12 carbon atoms, *i.e.*, $C_{2-12}$ alkenyl; 2-8 carbon atoms, *i.e.*, $C_{2-8}$ alkenyl; 2-6 carbon atoms, *i.e.*, $C_{2-6}$ alkenyl; and 2-4 carbon atoms, *i.e.*, $C_{2-4}$ alkenyl. Examples of alkenyl moieties include, but are not limited to vinyl, propenyl, butenyl, and cyclohexenyl.

**[049]** As used herein, "alkynyl" refers to a monovalent hydrocarbon radical moiety containing at least two carbon atoms and one or more carbon-carbon triple bonds. Alkynyl is optionally substituted and can be linear, branched, or cyclic. Alkynyl includes, but is not limited to, those radicals having 2-20 carbon atoms, *i.e.*, $C_{2-20}$ alkynyl; 2-12 carbon atoms, *i.e.*, $C_{2-12}$ alkynyl; 2-8 carbon atoms, *i.e.*, $C_{2-8}$ alkynyl; 2-6 carbon atoms, *i.e.*, $C_{2-6}$ alkynyl; and 2-4 carbon atoms, *i.e.*, $C_{2-4}$ alkynyl. Examples of alkynyl moieties include, but are not limited to ethynyl, propynyl, and butynyl.

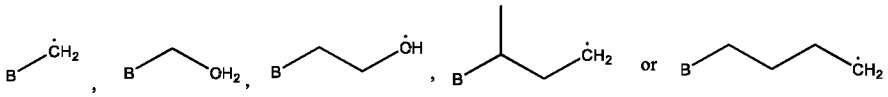
**[050]** As used herein, "alkoxy" refers to a monovalent and saturated hydrocarbon radical moiety wherein the hydrocarbon includes a single bond to an oxygen atom and wherein the radical is localized on the oxygen atom, *e.g.*, $CH_3CH_2$-O· for ethoxy. Alkoxy substituents bond to the compound which they substitute through this oxygen atom of the alkoxy substituent. Alkoxy is optionally substituted and can be linear, branched, or cyclic, *i.e.*, cycloalkoxy. Alkoxy includes, but is not limited to, those having 1-20 carbon atoms, *i.e.*, $C_{1-20}$ alkoxy; 1-12 carbon atoms, *i.e.*, $C_{1-12}$ alkoxy; 1-8 carbon atoms, *i.e.*, $C_{1-8}$ alkoxy; 1-6 carbon atoms, *i.e.*, $C_{1-6}$ alkoxy; and 1-3 carbon atoms, *i.e.*, $C_{1-3}$ alkoxy. Examples of alkoxy moieties include, but are not limited to methoxy, ethoxy, n-propoxy, i-propoxy, n-butoxy, s-butoxy, t-butoxy, i-butoxy, a pentoxy moiety, a hexoxy moiety, cyclopropoxy, cyclobutoxy, cyclopentoxy, and cyclohexoxy.

**[051]** As used herein, "haloalkoxy" refers to alkoxy, as defined above, wherein the alkoxy includes at least one substituent selected from a halogen, *e.g.*, F, Cl, Br, or I.

**[052]** As used herein, "aryl" refers to a monovalent moiety that is a radical of an aromatic compound wherein the ring atoms are carbon atoms. Aryl is optionally substituted and can be monocyclic or polycyclic, *e.g.*, bicyclic or tricyclic. Examples of aryl moieties include, but are not limited to, those having 6 to 20 ring carbon atoms, *i.e.*, $C_{6-20}$ aryl; 6 to 15 ring carbon atoms, *i.e.*, $C_{6-15}$ aryl, and 6 to 10 ring carbon atoms, *i.e.*, $C_{6-10}$ aryl. Examples of aryl moieties

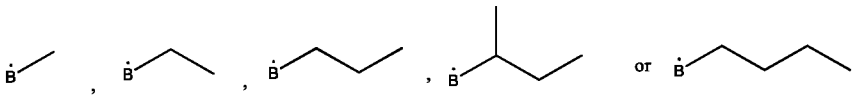
include, but are not limited to phenyl, naphthyl, fluorenyl, azulenyl, anthryl, phenanthryl, and pyrenyl.

**[053]** As used herein, "arylalkyl" refers to a monovalent moiety that is a radical of an alkyl compound, wherein the alkyl compound is substituted with an aromatic substituent, *i.e.*, the aromatic compound includes a single bond to an alkyl group and wherein the radical is localized on the alkyl group. An arylalkyl group bonds to the illustrated chemical structure via the alkyl group. An arylalkyl can be represented by the structure, *e.g.*,

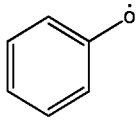



, wherein B is an aromatic moiety, *e.g.*, phenyl. Arylalkyl is optionally substituted, *i.e.*, the aryl group and/or the alkyl group, can be substituted as disclosed herein. Examples of arylalkyl include, but are not limited to, benzyl.

**[054]** As used herein, "alkylaryl" refers to a monovalent moiety that is a radical of an aryl compound, wherein the aryl compound is substituted with an alkyl substituent, *i.e.*, the aryl compound includes a single bond to an alkyl group and wherein the radical is localized on the aryl group. An alkylaryl group bonds to the illustrated chemical structure via the aryl group. An alkylaryl can be represented by the structure, *e.g.*,

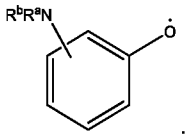
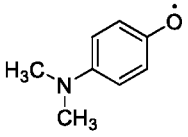



, wherein B is an aromatic moiety, *e.g.*, phenyl. Alkylaryl is optionally substituted, *i.e.*, the aryl group and/or the alkyl group, can be substituted as disclosed herein. Examples of alkylaryl include, but are not limited to, toluyl.

**[055]** As used herein, "aryloxy" refers to a monovalent moiety that is a radical of an aromatic compound wherein the ring atoms are carbon atoms and wherein the ring is substituted with an oxygen radical, *i.e.*, the aromatic compound includes a single bond to an oxygen atom and wherein the radical is localized on the oxygen atom, *e.g.*, 

for phenoxy. Aryloxy substituents bond to the compound which they substitute through this oxygen atom. Aryloxy is optionally substituted. Aryloxy includes, but is not limited to, those radicals having 6 to 20 ring carbon atoms, *i.e.*, $C_{6-20}$ aryloxy; 6 to 15 ring carbon atoms, *i.e.*, $C_{6-15}$ aryloxy, and 6 to 10 ring carbon atoms, *i.e.*, $C_{6-10}$ aryloxy. Examples of aryloxy moieties include, but are not limited to phenoxy, naphthoxy, and anthroxy.

**[056]** As used herein, "$R^aR^bN$-aryloxy" refers to a monovalent moiety that is a radical of an aromatic compound wherein the ring atoms are carbon atoms and wherein the ring is

substituted with at least one $R^aR^bN$ – substituent and at least one oxygen radical, *i.e.*, the aromatic compound includes a single bond to an $R^aR^bN$ - substituent and a single bond to an oxygen atom and wherein the radical is localized on the oxygen atom, *e.g.*,  . $R^aR^bN$ -aryloxy substituents bond to the compound which they substitute through this oxygen atom. $R^aR^bN$ -aryloxy is optionally substituted. $R^aR^bN$ -aryloxy includes, but is not limited to, those having 6 to 20 ring carbon atoms, for example, $C_{6-20}$ $(R^aR^bN)$n-aryloxy, 6 to 15 ring carbon atoms, for example, $C_{6-15}$ $(R^aR^bN)$n-aryloxy, and 6 to 10 ring carbon atoms, for example, $C_{6-10}$ $(R^aR^bN)$n-aryloxy, wherein n represents the number of $R^aR^bN$ – substituents. An example of an $R^aR^bN$ -aryloxy moiety includes, but is not limited to 4-(dimethylamino)-phenoxy,  .

**[057]** **As used herein, "arylene" refers to a divalent moiety** of an aromatic compound wherein the ring atoms are only carbon atoms. Arylene is optionally substituted and can be monocyclic or polycyclic, *e.g.*, bicyclic or tricyclic. Examples of arylene moieties include, but are not limited to those having 6 to 20 ring carbon atoms, *i.e.*, $C_{6-20}$ arylene; 6 to 15 ring carbon atoms, *i.e.*, $C_{6-15}$ arylene, and 6 to 10 ring carbon atoms, *i.e.*, $C_{6-10}$ arylene.

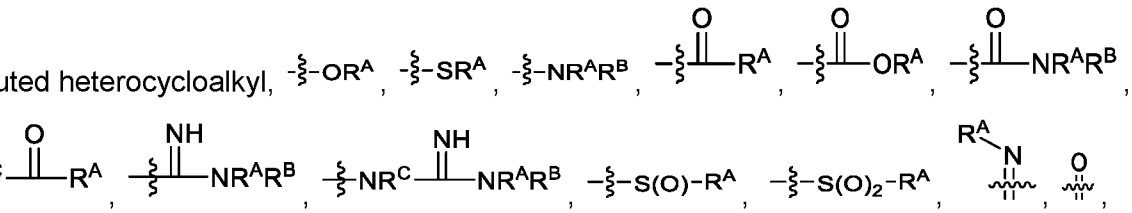
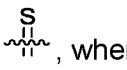
**[058]** **As used herein, "heteroalkyl" refers to an alkyl in which one or more carbon atoms are** replaced by heteroatoms. **As used herein, "heteroalkenyl" refers to an alkenyl in which one or more carbon atoms are replaced by heteroatoms. As used herein, "heteroalkynyl" refers to an** alkynyl in which one or more carbon atoms are replaced by heteroatoms. Suitable heteroatoms include, but are not limited to, nitrogen, oxygen, and sulfur atoms. Heteroalkyl is optionally substituted. Examples of heteroalkyl moieties include, but are not limited to, aminoalkyl, sulfonylalkyl, and sulfinylalkyl. Examples of heteroalkyl moieties also include, but are not limited to, methylamino, methylsulfonyl, and methylsulfinyl.

**[059]** **As used herein, "heteroaryl" refers to a monovalent moiety that is a radical of an** aromatic compound wherein the ring atoms contain carbon atoms and at least one oxygen, sulfur, nitrogen, or phosphorus atom. Examples of heteroaryl moieties include, but are not limited to those having 5 to 20 ring atoms; 5 to 15 ring atoms; and 5 to 10 ring atoms. Heteroaryl is optionally substituted.

**[060]** **As used herein, "heteroarylene" r**efers to an arylene in which one or more ring atoms of the aromatic ring are replaced with an oxygen, sulfur, nitrogen, or phosphorus atom. Heteroarylene is optionally substituted.

**[061]** As used herein, "heterocycloalkyl" refers to a cycloalkyl in which one or more carbon atoms are replaced by heteroatoms. Suitable heteroatoms include, but are not limited to, nitrogen, oxygen, and sulfur atoms. Heterocycloalkyl is optionally substituted. Examples of heterocycloalkyl moieties include, but are not limited to, morpholinyl, piperidinyl, tetrahydropyranyl, pyrrolidinyl, imidazolidinyl, oxazolidinyl, thiazolidinyl, dioxolanyl, dithiolanyl, oxanyl, or thianyl.

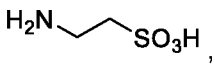
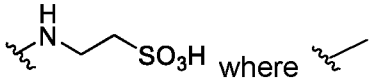
**[062]** As used herein, "N-containing heterocycloalkyl," refers to a cycloalkyl in which one or more carbon atoms are replaced by heteroatoms and wherein at least one heteroatom is a nitrogen atom. Suitable heteroatoms in addition to nitrogen, include, but are not limited to oxygen and sulfur atoms. N-containing heterocycloalkyl is optionally substituted. Examples of N-containing heterocycloalkyl moieties include, but are not limited to, morpholinyl, piperidinyl, pyrrolidinyl, imidazolidinyl, oxazolidinyl, or thiazolidinyl.

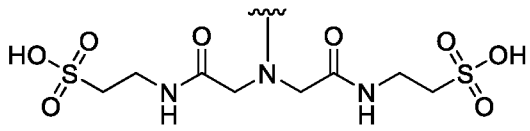
**[063]** As used herein, "optionally substituted," when used to describe a radical moiety, for example, optionally substituted alkyl, means that such moiety is optionally bonded to one or more substituents. Examples of such substituents include, but are not limited to, halo, cyano, nitro, optionally substituted haloalkyl, azido, epoxy, optionally substituted heteroaryl, optionally substituted heterocycloalkyl,  , or  , wherein $R^A$, $R^B$, and $R^C$ are, independently at each occurrence, a hydrogen atom, alkyl, alkenyl, alkynyl, aryl, alkylaryl, arylalkyl, heteroalkyl, heteroaryl, or heterocycloalkyl, or $R^A$ and $R^B$ together with the atoms to which they are bonded, form a saturated or unsaturated carbocyclic ring, wherein the ring is optionally substituted, and wherein one or more ring atoms is optionally replaced with a heteroatom. In certain embodiments, when a radical moiety is optionally substituted with an optionally substituted heteroaryl, optionally substituted heterocycloalkyl, or optionally substituted saturated or unsaturated carbocyclic ring, the substituents on the optionally substituted heteroaryl, optionally substituted heterocycloalkyl, or optionally substituted saturated or unsaturated carbocyclic ring, if they are substituted, are not substituted with substituents which are further optionally substituted with additional substituents. In some embodiments, when a group described herein is optionally substituted, the substituent bonded to the group is unsubstituted unless otherwise specified.

**[064]** As used herein, "binding agent" refers to any molecule, *e.g.*, protein, capable of binding with specificity to a given binding partner, *e.g.*, antigen.

**[065]** As used herein, "linker" refers to a divalent, trivalent, or multivalent moiety that covalently links the binding agent to one or more compounds described herein, for instance payload compounds and a hydrophilic group, as described herein.

**[066]** As used herein, "amide synthesis conditions" refers to reaction conditions suitable to effect the formation of an amide, *e.g.*, by the reaction of a carboxylic acid, activated carboxylic acid, or acyl halide with an amine. In some examples, amide synthesis conditions refer to reaction conditions suitable to effect the formation of an amide bond between a carboxylic acid and an amine. In some of these examples, the carboxylic acid is first converted to an activated carboxylic acid before the activated carboxylic acid reacts with an amine to form an amide. Suitable conditions to effect the formation of an amide include, but are not limited to, those utilizing reagents to effect the reaction between a carboxylic acid and an amine, including, but not limited to, dicyclohexylcarbodiimide (DCC), diisopropylcarbodiimide (DIC), (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP), (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP), (7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyAOP), bromotripyrrolidinophosphonium hexafluorophosphate (PyBrOP), O-(benzotriazol-1-yl)-**N,N,N′,N′**-tetramethyluronium hexafluorophosphate (HBTU), O-(benzotriazol-1-yl)-**N,N,N′,N′**-tetramethyluronium tetrafluoroborate (TBTU), 1–[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU), N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), N-ethyl-**N′**-(3-dimethylaminopropyl)carbodiimide (EDC), 2-chloro-1,3-dimethylimidazolidinium hexafluorophosphate (CIP), 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT), and carbonyldiimidazole (CDI). In some examples, a carboxylic acid is first converted to an activated carboxylic ester before treating the activated carboxylic ester with an amine to form an amide bond. In certain embodiments, the carboxylic acid is treated with a reagent. The reagent activates the carboxylic acid by deprotonating the carboxylic acid and then forming a product complex with the deprotonated carboxylic acid as a result of nucleophilic attack by the deprotonated carboxylic acid onto the protonated reagent. The activated carboxylic esters for certain carboxylic acids are subsequently more susceptible to nucleophilic attack by an amine than the carboxylic acid is before it is activated. This results in amide bond formation. As such, the carboxylic acid is described as activated. Exemplary reagents include DCC and DIC.

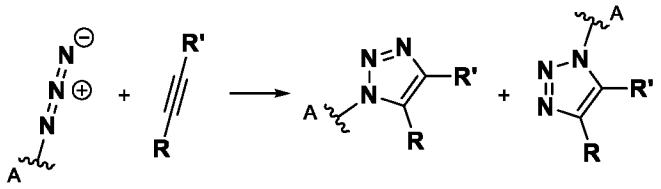
**[067]** As used herein, "taurine" refers to the reagent  , or the group  where  indicates the atom through which the taurine is bonded to the adjacent groups in the formula. As used herein, "dualtaurine" refers to the group



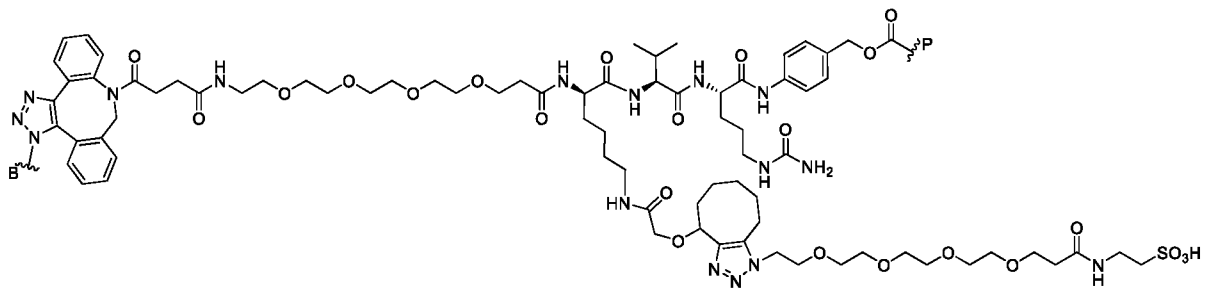
where  indicates the atom through which the dualtaurine is bonded to the adjacent groups in the formula.

**[068]** As used herein, "stereoisomeric form" refers to the relative spatial orientation of different groups in a compound. Stereoisomeric forms include enantiomers, diasteromers, and/or mixtures thereof.

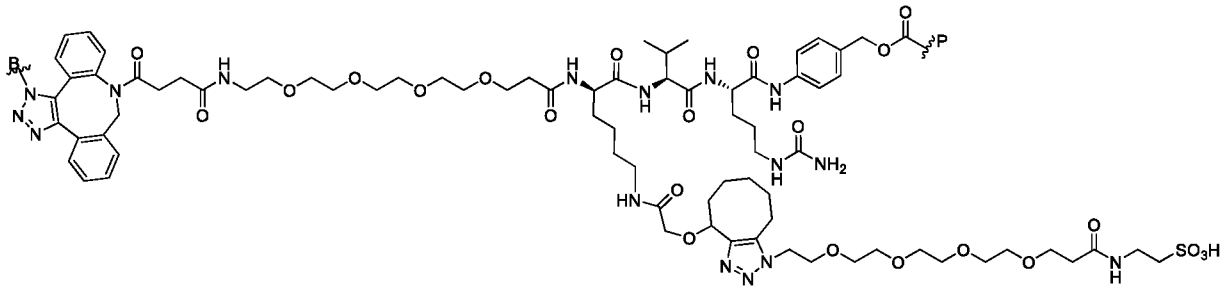
**[069]** As used herein, "regioisomer," "regioisomers," or "mixture of regioisomers" refers to the product(s) of 1,3-cycloadditions or strain-promoted alkyne-azide cycloadditions (SPAACs) – otherwise known as click reactions – that derive from suitable azides (*e.g.*, $-N_3$, or PEG-$N_3$ derivatized antibodies) treated with suitable alkynes. In certain embodiments, for example, regioisomers and mixtures of regioisomers are characterized by the click reaction products shown below:



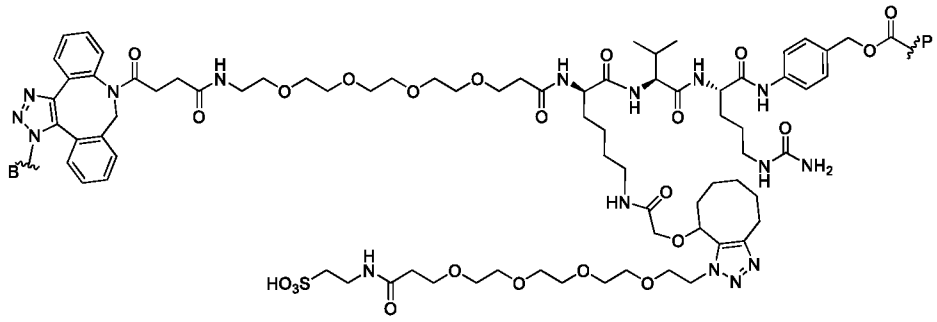
**[070]** By way of example only, regioisomers of compound A1', *i.e.*, compounds A2', A3', A4', are shown below, wherein each  is a bond to the binding agent; and each  is a bond to the payload:



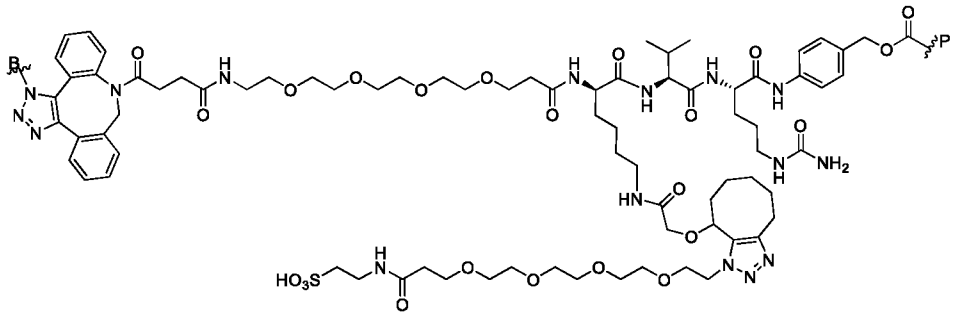
compound A1'



compound **A2′**;



compound **A3′**;



compound **A4′**.

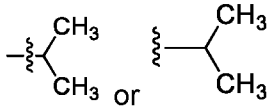
**[071]** In certain embodiments, more than one suitable azide and more than one suitable alkyne can be utilized within a synthetic process en route to a product, where each pair of azide-alkyne can participate in one or more independent click reactions to generate a mixture of regioisomeric click reaction products. For example, a person of skill will recognize that a first suitable azide may independently react with a first suitable alkyne, and a second suitable azide may independently react with a second suitable alkyne, en route to a product, resulting in the generation of four possible click reaction regioisomers or a mixture of the four possible click reaction regioisomers in a sample of an ADC described herein. For example, a person of skill will recognize that a first suitable azide may independently react with a first suitable alkyne, en route to a product, resulting in the generation of two possible click reaction regioisomers or a mixture of the two possible click reaction regioisomers in a sample of a linker-payload described herein.

**[072]** As used herein, the term "residue" refers to the chemical moiety within a compound that remains after a chemical reaction. For example, the term "amino acid residue" or "N-alkyl amino acid residue" refers to the product of an amide coupling or peptide coupling of an amino acid or a N-alkyl amino acid to a suitable coupling partner; wherein, for example, a water molecule is expelled after the amide or peptide coupling of the amino acid or the N-alkylamino acid, resulting in the product having the amino acid residue or N-alkyl amino acid residue incorporated therein.

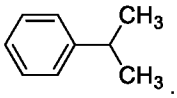
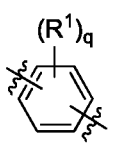
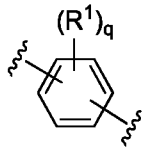
**[073]** As used herein, "therapeutically effective amount" refers to an amount (*e.g.*, of a compound) that is sufficient to provide a therapeutic benefit to a patient in the treatment or management of a disease or disorder, or to delay or minimize one or more symptoms associated with the disease or disorder.

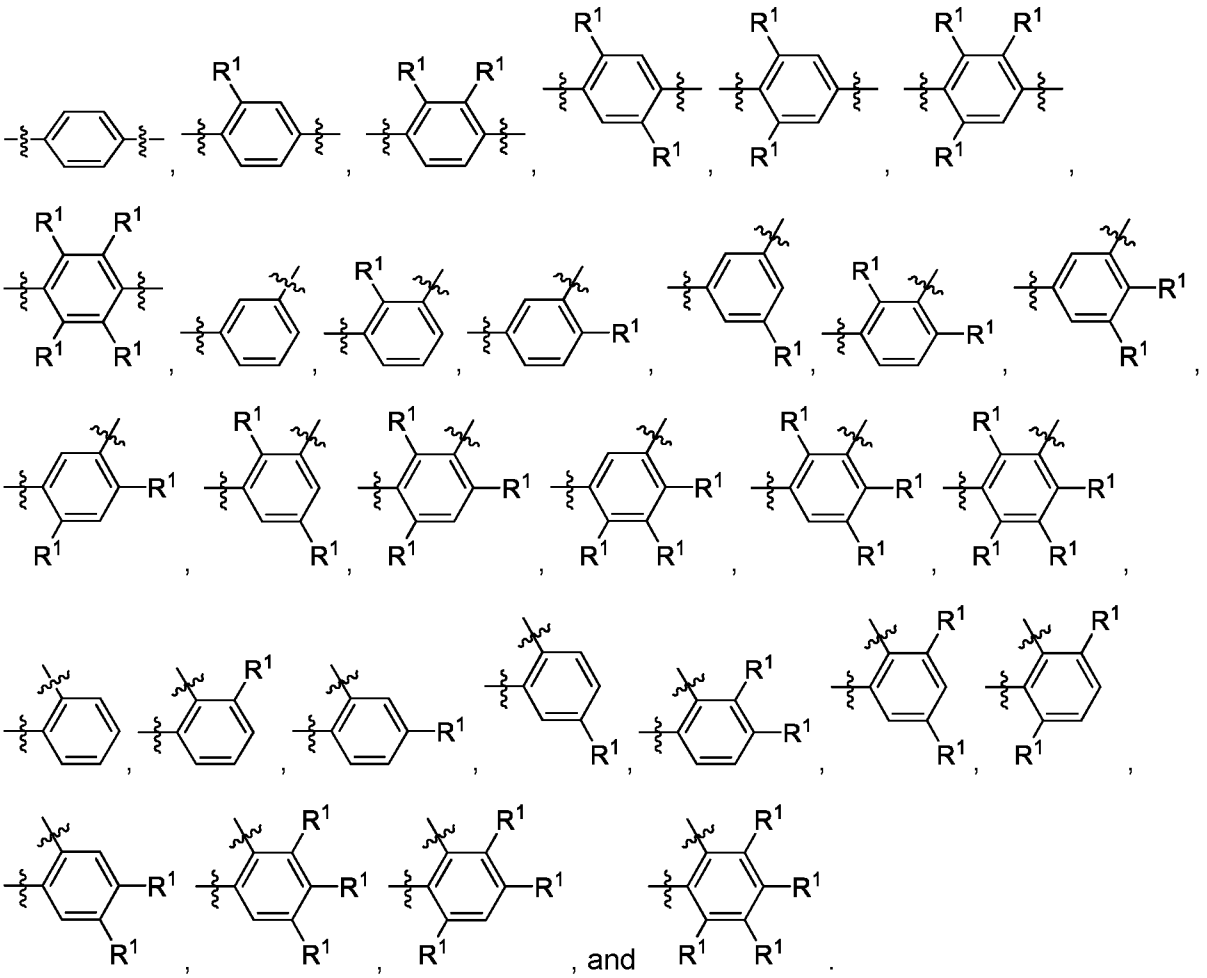
**[074]** As used herein, "sugar" or "sugar group" or "sugar residue" refers to a carbohydrate moiety which may comprise 3-carbon (triose) units, 4-carbon (tetrose) units, 5-carbon (pentose) units, 6-carbon (hexose) units, 7-carbon (heptose) units, or combinations thereof, and may be a monosaccharide, a disaccharide, a trisaccharide, a tetrasaccharide, a pentasaccharide, an oligosaccharide, or any other polysaccharide. In some instances, a "sugar" or "sugar group" or "sugar residue" comprises furanoses (e.g., ribofuranose, fructofuranose) or pyranoses (e.g., glucopyranose, galactopyranose), or a combination thereof. In some instances, a "sugar" or "sugar group" or "sugar residue" comprises aldoses or ketoses, or a combination thereof. Non-limiting examples of monosaccharides include ribose, deoxyribose, xylose, arabinose, glucose, mannose, galactose, and fructose. Non-limiting examples of disaccharides include sucrose, maltose, lactose, lactulose, and trehalose. Other "sugars" or "sugar groups" or "sugar residues" include polysaccharides and/or oligosaccharides, including, and not limited to, amylose, amylopectin, glycogen, inulin, and cellulose. In some instances a "sugar" or "sugar group" or "sugar residue" is an amino-sugar. In some instances a "sugar" or "sugar group" or "sugar residue" is a glucamine residue (1-amino-1-deoxy-D-glucitol) linked to the rest of molecule via its amino group to form an amide linkage with the rest of the molecule (i.e., a glucamide).

**[075]** Certain groups, moieties, substituents, and atoms are depicted with a wiggly line that intersects a bond or bonds to indicate the atom through which the groups, moieties, substituents, atoms are bonded. For example, a phenyl group that is substituted with a propyl group depicted as:




has the following structure:

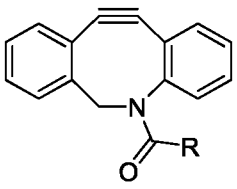
 . As used herein, illustrations showing substituents bonded to a cyclic group (*e.g.*, aromatic, heteroaromatic, fused ring, and saturated or unsaturated cycloalkyl or heterocycloalkyl) through a bond between ring atoms are meant to indicate, unless specified otherwise, that the cyclic group may be substituted with that substituent at any ring position in the cyclic group or on any ring in the fused ring group, according to techniques set forth herein or which are known in the field to which the instant disclosure pertains. For example, the group,  or  , wherein subscript q is an integer from 0 to 4 and in which the positions of substituent $R^1$ are described generically, *i.e.*, not directly attached to any vertex of the bond line structure, *i.e.*, specific ring carbon atom, includes the following, non-limiting examples of groups in which the substituent $R^1$ is bonded to a specific ring carbon atom:



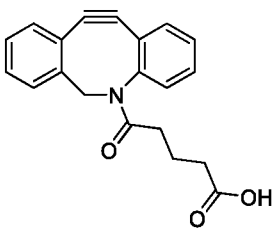
**[076]** As used herein, the phrase "hydrophilic linker (HL)" refers to a moiety comprising a hydrophilic group (**HG**) as defined herein, and a spacer **SP²** as defined herein.

**[077]** As used herein, the phrase "reactive linker" refers to a monovalent group that includes a reactive group and spacer group, depicted for example as $RG'—SP^1-\S$, wherein RG' is the reactive group and $SP^1$ is the spacer group. As described herein, a reactive linker may include more than one reactive group and more than one spacer group. The spacer group is any divalent or trivalent moiety that bridges the reactive group to another group, such as a payload or also to a binding agent. The reactive linkers (**L**, **LL**), together with the payloads to which they are bonded, provide intermediates ("linker-payloads") useful as synthetic precursors for the preparation of the antibody conjugates described herein. The reactive linker contains one or more than one reactive group **RG** (**$RG^1$**, **$RG^2$**, or **RG'**), which is a functional group or moiety that is capable of reacting with a reactive portion of another group, for instance, an antibody, modified antibody, or antigen binding fragment thereof, or a hydrophilic group, as described herein. The moiety resulting from the reaction of the reactive group with the antibody, modified antibody, or antigen binding fragment thereof, together with the linking group, includes the "binding agent linker" ("**BL**") portion of the conjugate, described herein. In certain embodiments, the "reactive group" is a functional group or moiety (*e.g.*, maleimide or N-hydroxysuccinimide (NHS) ester) that reacts with a cysteine or lysine residue of an antibody or antigen-binding fragment thereof.

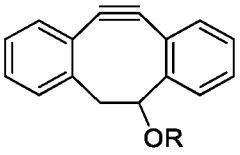
**[078]** In certain embodiments, the "reactive group" is a functional group or moiety that is capable of undergoing a click chemistry reaction (see, *e.g.*, click chemistry, Huisgen Proc. Chem. Soc. 1961, Wang et al. J. Am. Chem. Soc. 2003, and Agard et al. J. Am. Chem. Soc. 2004). In some embodiments of said click chemistry reaction, the reactive group is an alkyne that is capable of undergoing a 1,3-cycloaddition reaction with an azide. An alkyne that is capable of undergoing a 1,3-cycloaddition reaction with an azide is also referred to herein as a "click chemistry residue". Such suitable reactive groups include, but are not limited to, strained alkynes, *e.g.*, those suitable for strain-promoted alkyne-azide cycloadditions (SPAAC), cycloalkynes, *e.g.*, cyclooctynes, benzannulated alkynes, and alkynes capable of undergoing 1,3-cycloaddition reactions with alkynes in the absence of copper catalysts. Suitable alkynes also include, but are not limited to, dibenzoazacyclooctyne or



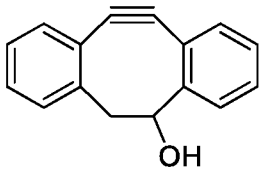

(DIBAC), for example,



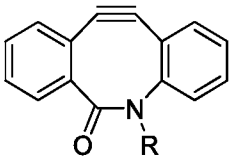

, dibenzocyclooctyne or



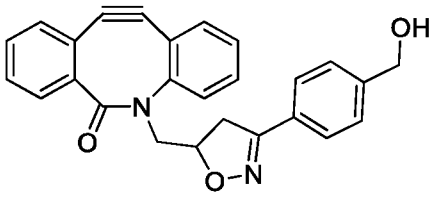

(DIBO), for example,



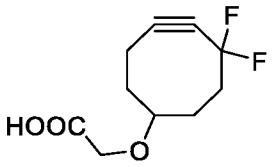

, biarylazacyclooctynone or



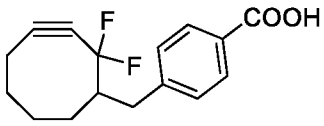

(BARAC), for example,



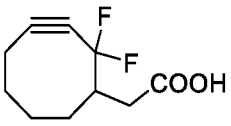

, difluorinated cyclooctyne or



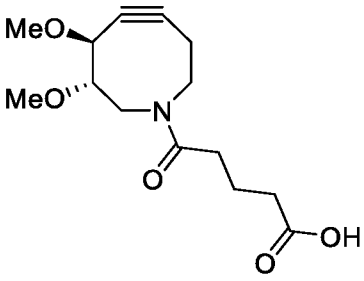

(DIFO), or



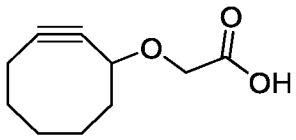

(DIFO2), or

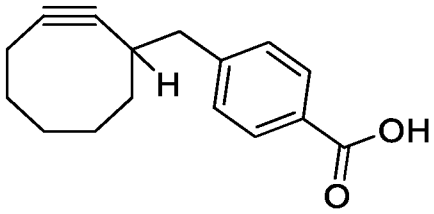



(DIFO3),

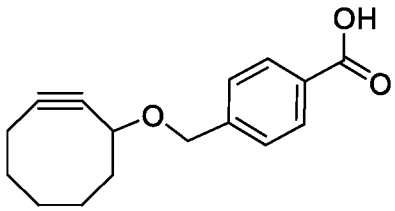



(DIMAC),



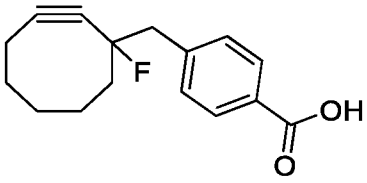




(ALO),

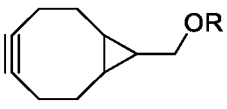



(NOFO),

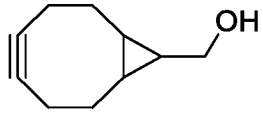
(OCT), and



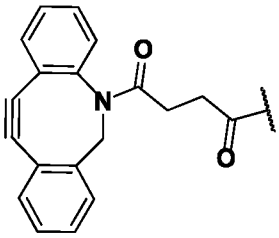

(MOFO), substituted, *e.g.*, fluorinated alkynes, aza-cycloalkynes, bicycle[6.1.0]nonyne or

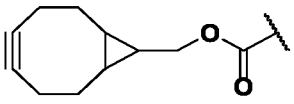



(BCN), where R is alkyl, alkoxy, or acyl, and derivatives thereof, for example,

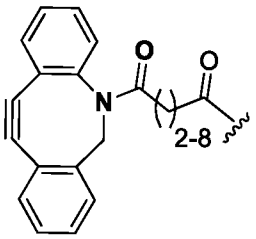


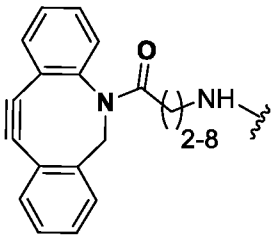

. Particularly useful alkynes include




and 


. Additional alkynes include




and 


.

**[079]** Linker-payloads including such reactive groups are useful for conjugating antibodies that have been functionalized with azido groups. Such functionalized antibodies include antibodies functionalized with azido-polyethylene glycol groups. In certain embodiments, such a functionalized antibody is derived by treating an antibody having at least one glutamine residue, *e.g.*, a heavy chain Gln295, with a compound bearing an amino group and an azide group, in the presence of the enzyme transglutaminase.

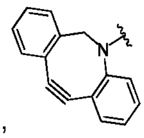
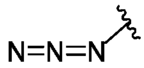
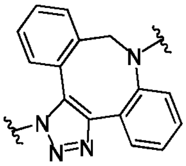
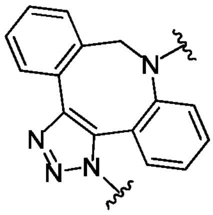
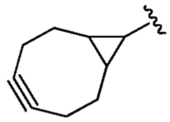
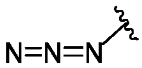
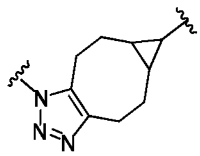
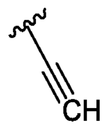
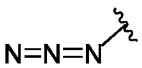
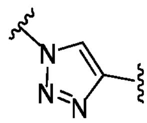
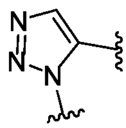
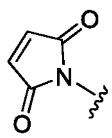
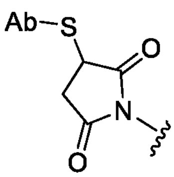
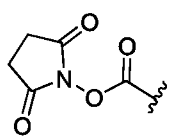
**[080]** In certain embodiments, the antibody or a glutaminyl-modified antibody or antigen binding molecule comprises at least one glutamine residue in at least one polypeptide chain sequence. In certain embodiments, the antibody or a glutaminyl-modified antibody or antigen binding molecule comprises two heavy chain polypeptides, each with one Gln295 residue. In further embodiments, the antibody or a glutaminyl-modified antibody or antigen binding molecule comprises one or more glutamine residues at a site other than a heavy chain Gln 295. Included herein are antibodies bearing N297Q mutation(s) described herein. Briefly, in some embodiments, an antibody including a glutamine residue is treated with a primary amine compound, described in more detail below, in the presence of the enzyme transglutaminase. In some embodiments, an antibody including a Asn297Gln (N297Q) residue is treated with a primary amine compound, described in more detail below, in the presence of the enzyme transglutaminase. In some embodiments, an antibody including Gln295 (Q295) residue is treated with a primary amine compound, described in more detail below, in the presence of the enzyme transglutaminase. In some embodiments, an antibody including Gln55 (Q55) residue is treated with a primary amine compound, described in more detail below, in the presence of the enzyme transglutaminase. For example, in some embodiments, such an antibody can be prepared by site-directed mutagenesis to remove or disable a sequence or to insert a glutamine residue at a site apart from any interfering structure. Such an antibody also can be isolated from natural or artificial sources.

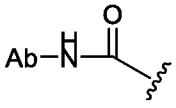
**[081]** The amino acid sequence of an antibody can be numbered using any known numbering schemes, including those described by Kabat et al., ("Kabat" numbering scheme); Al-Lazikani et al., 1997, *J. Mol. Biol.*, 273:927-948 ("Chothia" numbering scheme); MacCallum et al., 1996, *J. Mol. Biol.* 262:732-745 ("Contact" numbering scheme); Lefranc et al., *Dev. Comp. Immunol.*, 2003, 27:55-77 ("IMGT" numbering scheme); and Honegge and Plückthun, *J. Mol. Biol.*, 2001, 309:657-70 ("AHo" numbering scheme). Unless otherwise specified, the numbering scheme used herein is the Kabat numbering scheme. However, selection of a numbering scheme is not intended to imply differences in sequences where they do not exist, and one of skill in the art can readily confirm a sequence position by examining the amino acid sequence of one or more antibodies. Unless stated otherwise, the "EU numbering scheme" is generally used when referring to a residue in an antibody heavy chain constant region (e.g., as reported in Kabat et al., supra).

**[082]** The term "aglycosylated antibody" refers to an antibody that does not comprise a glycosylation sequence that might interfere with a transglutamination reaction, for instance an antibody that does not have saccharide group at N297 on one or more heavy chains. In particular embodiments, an antibody heavy chain has an N297 mutation. In other words, the antibody is mutated to no longer have an asparagine residue at position 297 according to the EU numbering system as disclosed by Kabat et al. In particular embodiments, an antibody heavy chain has an N297Q or an N297D mutation. Such an antibody can be prepared by site-directed mutagenesis to remove or disable a glycosylation sequence or by site-directed mutagenesis to insert a glutamine residue at site apart from any interfering glycosylation site or any other interfering structure. Such an antibody also can be isolated from natural or artificial sources.

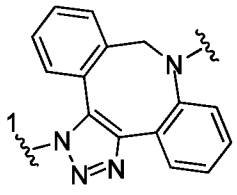
**[083]** The term "deglyosylated antibody" refers to an antibody in which a saccharide group at N297 was removed, thereby opening Q295 to transglutamination. In particular embodiments, provided herein are processes that encompass an additional step of deglycosylating an antibody, for instance an N297 antibody.

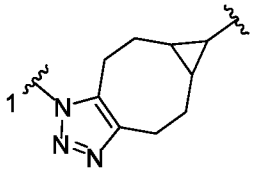
**[084]** In some examples, the alkyne used in the bioconjugation reaction is useful for Cu(I) click-chemistry conjugation reaction. In some examples, the alkyne used in the conjugation reaction reacts with 1,2 aminothiol in the 2-Cyanobenzothiazole (CBT) reaction. In some examples, the alkyne used is BCN, derivative of BCN, or trans-cyclooctene (TCOs) in an inverse electron demand Diels Alder reactions. See, for example, Wang et al., J. Am. Chem. Soc.; (Article), 2012, 134 (6), 2950-2953.

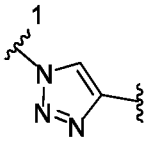
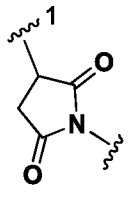
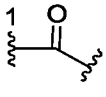
**[085]** In some examples, the reactive group is an alkyne, *e.g.*,  , which can react via click chemistry with an azide, *e.g.*,  , to form a click chemistry product, *e.g.*,  or  . In some examples, the group reacts with an azide on a modified antibody or antigen binding fragment thereof. In some examples, the reactive group is an alkyne, *e.g.*,  , which can react via click chemistry with an azide, *e.g.*,  to form a click chemistry product, *e.g.*,  . In some examples, the reactive group is an alkyne, *e.g.*,  , which can react via click chemistry with an azide, *e.g.*,  , to form a click chemistry product, *e.g.*,  or  . In some examples, the reactive group is a functional group, *e.g.*,  ,which reacts with a cysteine residue on an antibody or antigen-binding fragment thereof, to form a bond thereto, *e.g.*,  , wherein Ab refers to an antibody or antigen-binding fragment thereof and S refers to the S atom on a cysteine residue through which the functional group bonds to the Ab. In some examples, the reactive group is a functional group, *e.g.*,  ,which reacts with a lysine residue on an antibody or antigen-binding fragment thereof, to form a bond

thereto, *e.g.*,  , wherein Ab refers to an antibody or antigen-binding fragment thereof and NH refers to the NH atom on a lysine side chain residue through which the functional group bonds to the Ab.

**[086]** As used herein, the phrase "binding agent linker," or "BL" refers to any divalent, trivalent, or multi-valent group or moiety that links, connects, or bonds a binding agent (*e.g.*, an antibody or an antigen-binding fragment thereof) with a payload compound set forth herein (*e.g.*, MMAE, bis-octahydrophenanthrene carboxamides, steroids) and, optionally, with one or more side chain compounds. Generally, suitable binding agent linkers for the antibody conjugates described herein are those that are sufficiently stable to exploit the circulating half-life of the antibody and, at the same time, capable of releasing its payload after antigen-mediated internalization of the conjugate. Linkers can be cleavable or non-cleavable. Cleavable linkers are linkers that are cleaved by intracellular metabolism following internalization, *e.g.*, cleavage via hydrolysis, reduction, or enzymatic reaction. Non-cleavable linkers are linkers that release an attached payload via lysosomal degradation of the antibody following internalization. Suitable linkers include, but are not limited to, acid-labile linkers, hydrolysis-labile linkers, enzymatically cleavable linkers, reduction labile linkers, self-immolative linkers, and non-cleavable linkers. Suitable linkers also include, but are not limited to, those that are or comprise peptides, glucuronides, succinimide-thioethers, polyethylene glycol (PEG) units, hydrazones, mal-caproyl units, dipeptide units, valine-citrulline units, and para-aminobenzyl (PAB) units. In some embodiments, the binding agent linker (BL) includes a moiety that is formed by the reaction of the reactive group (RG) of a reactive linker (RL) and reactive portion of the binding agent, *e.g.*, antibody, modified antibody, or antigen binding fragment thereof.

**[087]** In some examples, the BL includes the following moiety:  , wherein  is the bond to the binding agent. In some examples, the BL includes the following moiety:

 , wherein  is the bond to the binding agent. In some examples, the BL

includes the following moiety:  , wherein  is the bond to the binding agent. In some examples, the BL includes the following moiety:  , wherein  is the bond to the cysteine of the antibody or antigen-binding fragment thereof. In some examples, the BL includes the following moiety:  , wherein  is the bond to the lysine of the antibody or antigen-binding fragment thereof.

**Conjugates and Payloads**

**[088]** In some examples, set forth herein is a compound, or a pharmaceutically acceptable salt thereof, comprising: a binding agent linked to at least one payload moiety and linked to at least one hydrophilic moiety via a covalent linker, wherein said covalent linker is bonded directly or indirectly to each of the binding agent, the payload moiety, and the hydrophilic moiety.

**[089]** In some other examples, set forth herein is a compound, or a pharmaceutically acceptable salt thereof, comprising: a protein linked to at least one payload moiety and linked to at least one hydrophilic moiety via a covalent linker, wherein said covalent linker is bonded directly or indirectly to each of the protein, the payload moiety, and the hydrophilic moiety. In some embodiments, the protein is an antibody or antigen binding fragment thereof.

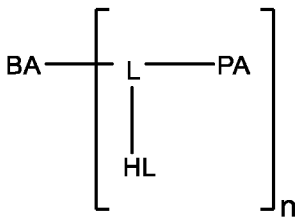
**[090]** As illustrated herein, in some examples, the binding agent is bonded directly to a covalent linker, such as a lysine amino acid. This means that the binding agent is one bond position away from the lysine amino acid covalent linker. In some of these examples, the covalent linker is also bonded directly to a payload moiety. This means that the covalent linker is one bond position away from a payload such as, but not limited to, a maytansinoid, MMAE, MMAF, a steroid, an LXR modulator, or any payload set forth herein. In some of these examples, the covalent linker is also bonded directly to a hydrophilic moiety. This means that the covalent linker is one bond position away from a hydrophilic residue, such as the hydrophilic residues set forth herein. In some of these examples, the covalent linker is a lysine amino acid or a derivative thereof.

**[091]** In other examples, the binding agent is bonded indirectly to a covalent linker. This means that the binding agent is more than one bond position away from the covalent linker.

This also means that the binding agent is bonded through another moiety to the covalent linker. For example, the binding agent may be bonded to a maleimide group which is bonded to a polyethylene glycol group which is bonded to the covalent linker. In some of these examples, the covalent linker is also bonded indirectly to a payload moiety. This means that the covalent linker is more than one bond position away from a payload such as, but not limited to, MMAE, a steroid, an LXR modulator, or any payload set forth herein. This also means that the covalent linker is bonded through another moiety to the payload. For example, the covalent linker may be bonded to a dipeptide, such as but not limited to Val-Ala or Val-Cit, which may be bonded to PAB which may be bonded to the payload. In some of these examples, the covalent linker is also bonded indirectly to a hydrophilic moiety. This means that the covalent linker is more than one bond position away from a hydrophilic moiety, such as the hydrophilic residues set forth herein. This also means that the covalent linker is bonded through another moiety to the hydrophilic moiety. For example, the covalent linker may be bonded to a polyethylene glycol group which may be bonded to reactive group which may be bonded to the hydrophilic residue. In some of these examples, the covalent linker is a lysine amino acid or a derivative thereof.

**[092]** In certain instances, the hydrophilic residue comprises a terminal hydrophilic group. In some instances, the hydrophilic residue comprises at least one taurine group. In some instances, the hydrophilic residue comprises a sulfonic acid group. In some cases, the hydrophilic reside comprises a terminal sulfonic acid group. In further instances, the hydrophilic residue comprises more than one sulfonic acid groups. In some cases, the hydrophilic reside comprises more than one terminal sulfonic acid groups.

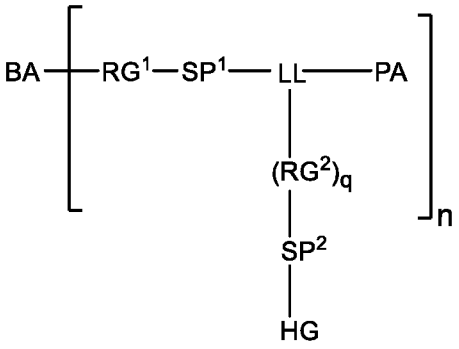
**[093]** Described herein are compounds according to Formula (**I**):




(**I**)

or pharmaceutically acceptable salts, solvates, stereoisomers, or derivatives thereof, wherein, in Formula (**I**), **BA** is a binding agent; **L** is a trivalent linker; **HL** is a hydrophilic residue; **PA** is a payload residue; and subscript **n** is an integer from 1 to 30. In some instances more than one trivalent linker **L** may be present. In some instances, **n** is an integer from 1 to 4. In some instances **n** is 1. In some instances **n** is 2. In some instances **n** is 3. In some instances **n** is 4.

**[094]** In one example, a compound of Formula (I) is according to Formula (II):

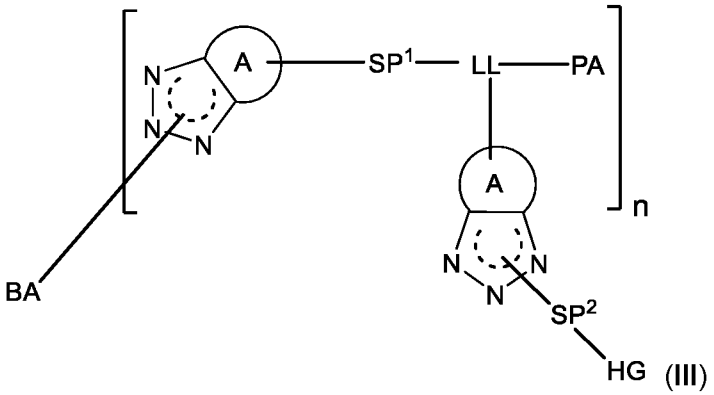



(II)

wherein, in Formula (II), **BA** is a binding agent; **LL** is a trivalent linker; $RG^1$ and $RG^2$ are reactive group residues; $SP^1$ and $SP^2$ are independently, in each instance, absent, or a spacer group residue; **HG** is a hydrophilic residue; **PA** is a payload residue; subscript **n** is an integer from 1 to 30; and subscript **q** is 0 or 1. In some instances more than one trivalent linker **LL** may be present. In some instances, **n** is an integer from 1 to 4. In some instances **n** is 1. In some instances **n** is 2. In some instances **n** is 3. In some instances **n** is 4. In some instances, **HG** is a terminal hydrophilic group. In some instances, **HG** comprises one terminal sulfonic acid group ($SO_3H$), or salts thereof. In other instances, **HG** comprises more than one terminal sulfonic acid groups, or salts thereof. In some instances, **HG** comprises one terminal taurine group or salts thereof. In other instances, **HG** comprises more than one terminal taurine groups or salts thereof. In some instances, **HG** comprises one terminal phosphonic acid group ($PO_3H$), or salt thereof. In other instances, **HG** comprises more than one terminal phosphonic acid groups, or salts thereof. In some instances, **HG** comprises one terminal amine group, or salt thereof. In other instances, **HG** comprises more than one terminal amine group, or salts thereof. In further instances, **HG** comprises one terminal quarternary amine group, or salts thereof. In further instances, **HG** comprises more than one terminal quarternary amine group, or salts thereof. In some instances, **HG** comprises one terminal sugar group, or salt thereof. In other instances, **HG** comprises more than one terminal sugar groups, or salts thereof.

**[095]** In some instances, the compound of Formula (I) or Formula (II) comprises a mixture of compounds wherein subscript **n** is 1, 2, 3 or 4. In some instances, the compound of Formula (I) or Formula (II) comprises a mixture of compounds wherein subscript **n** is 2. In some instances, the compound of Formula (I) or Formula (II) comprises a mixture of compounds wherein subscript **n** is 4.

**[096]** In one example, the compound of Formula (I) is according to Formula (III):



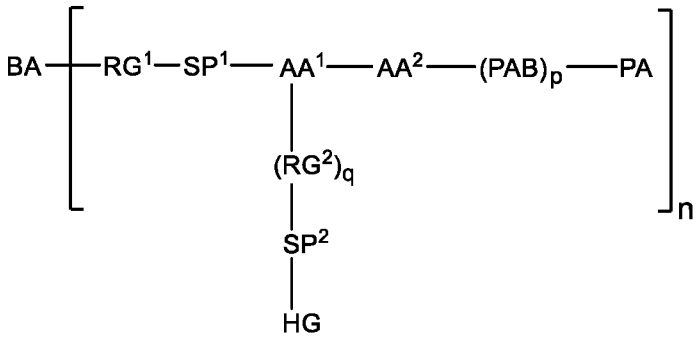

(III)

wherein

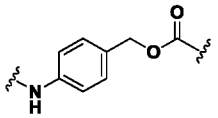
ring **A** is fused to the triazole and is selected from the group consisting of cycloalkyl, cycloalkenyl, heterocycloalkyl, and heterocycloalkenyl;

wherein cycloalkyl, cycloalkenyl, heterocycloalkyl, and heterocycloalkenyl are optionally substituted with alkyl, -OH, or -N$R^aR^b$, where each of $R^a$ and $R^b$ is alkyl or H.

**[097]** In another example, the compound of Formula (**I**) is according to Formula (**IV**):

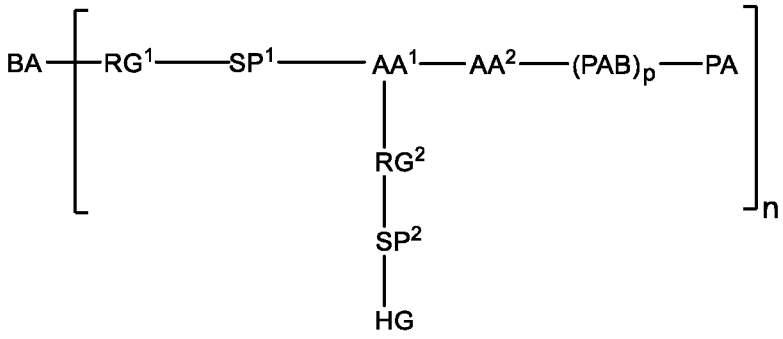



(**IV**).

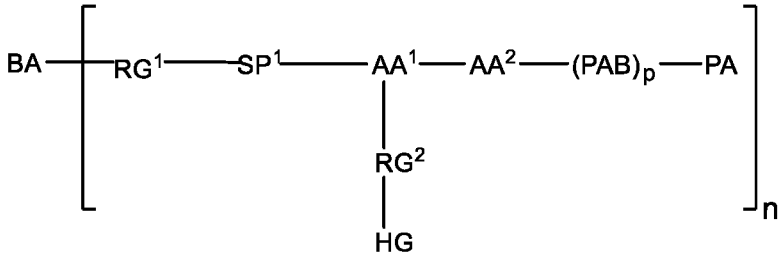
**[098]** In Formula (**IV**), **BA**, $RG^1$, $SP^1$, $RG^2$, $SP^2$ and **HG** are as defined above, $AA^1$ is a trivalent linker comprising an amino acid residue and is directly or indirectly linked to an antibody, a payload and a hydrophilic group; $AA^2$ is a dipeptide, tripeptide or tetrapeptide residue; and **PAB** is  , wherein the  indicates the atom through which the **PAB** is bonded to the adjacent groups in the formula; subscript **p** is 0 or 1; and subscript **q** is 0 or 1. In some instanes, subscript **p** is 0 and subscript **q** is 0. In some instances, subscript **p** is 1; and subscript **q** is 0. In some instances, subscript **p** is 0; and subscript **q** is 1. In some instances, subscript **p** is 1; and subscript **q** is 1. In some instances $SP^1$ comprises from 0-5 polyethylene glycol (**PEG**) residues. In some instances $SP^2$ comprises from 0-5 **PEG**

residues. In some examples, $SP^1$ is independently in each instance, selected from the group consisting of $C_{1-6}$ alkylene, -NH-, -C(O)-, $(-CH_2-CH_2-O)_e$, $-NH-CH_2-CH_2-(-O-CH_2-CH_2)_e-C(O)-$, $-C(O)-(CH_2)_u-C(O)-$, $-C(O)-NH-(CH_2)_v-$, polyglycine (e.g., $((glycine)_4-serine)_f$ wherein subscript **f** is an integer from 1 to 6), and combinations thereof, wherein subscript **e** is an integer from 0 to 4, subscript **u** is an integer from 1 to 8, and subscript **v** is an integer from 1 to 8. In some examples, $SP^2$ is independently in each instance, selected from the group consisting of $C_{1-6}$ alkylene, -NH-, -C(O)-, $(-CH_2-CH_2-O)_e$, $-NH-CH_2-CH_2-(-O-CH_2-CH_2)_e-C(O)-$, $-C(O)-(CH_2)_u-C(O)-$, $-C(O)-NH-(CH_2)_v-$, polyglycine (e.g., $((glycine)_4-serine)_f$ wherein subscript **f** is an integer from 1 to 6), and combinations thereof, wherein subscript **e** is an integer from 0 to 4, subscript **u** is an integer from 1 to 8, and subscript **v** is an integer from 1 to 8. In some examples, any one of $AA^1$ or $AA^2$ comprises, independently in each instance, an amino acid selected from alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, or citrulline, a derivative thereof, or a combination thereof. In certain embodiments, $AA^1$ is an amino acid selected from alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, or citrulline, a derivative thereof, or a combination thereof. In certain embodiments, $AA^1$ is an amino acid with three functional groups to link to a payload, to a binding agent (e.g., antibody or antigen binding fragment thereof), and to a linker comprising a hydrophilic group, e.g., lysine, aspargine, glutamic acid, aspartic acid, glutamine, cysteine, threonine, serine, or tyrosine. In certain embodiments, $AA^1$ is lysine. In certain embodiments, $AA^1$ is lysine or a derivative of lysine. In certain embodiments, $AA^1$ is L-lysine. In certain embodiments, the $AA^1$ is D-lysine. In certain embodiments, $AA^1$ is glutamine. In certain embodiments, $AA^1$ is glutamic acid. In certain embodiments, $AA^1$ is aspartic acid. In certain embodiments, the $AA^2$ is valine-citrulline. In some embodiments, the $AA^2$ is citrulline-valine. In some embodiments, the $AA^2$ is valine-alanine. In some embodiments, the $AA^2$ is alanine-valine. In some embodiments, the $AA^2$ is valine-glycine. In some embodiments, the $AA^2$ is glycine-valine. In some embodiments, the $AA^1$-$AA^2$ is glutamine-valine-citrulline. In some embodiments, the $AA^1$-$AA^2$ is lysine-valine-citrulline. In some embodiments, the $AA^1$-$AA^2$ is lysine-valine-alanine. In some embodiments, the $AA^1$-$AA^2$ is glutamine-valine-alanine. In certain embodiments, $((glycine)_4-serine)_f$ is $(glycine)_4-serine$.

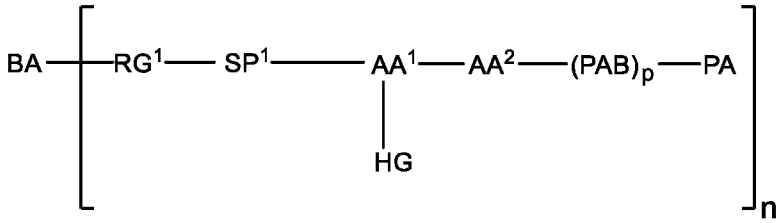
**[099]** In some examples, the compound of Formula (**I**) or Formula (**II**) or Formula (**III**) or Formula (**IV**) is according to Formula (**IVa**), Formula (**IVb**) or Formula (**IVc**):



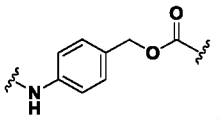

(IVa),




(IVb),

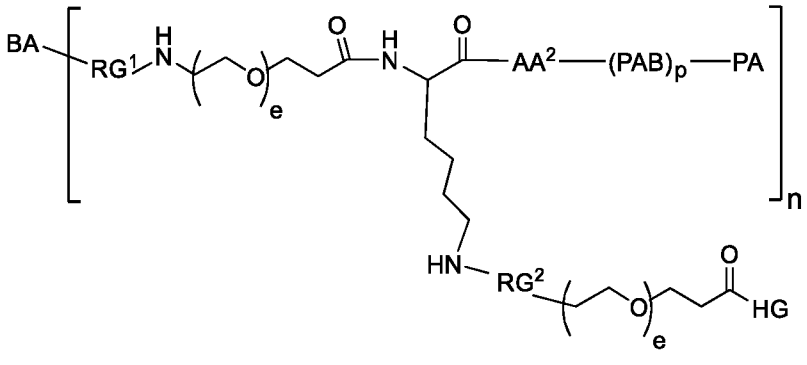



(IVc).

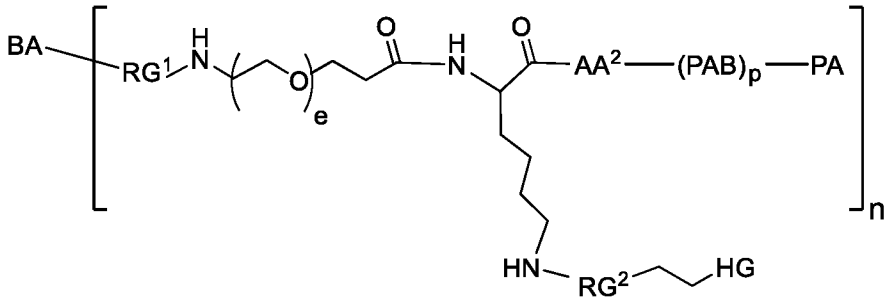
**[0100]** In Formulae (**IVa**), (**IVb**), and (**IVc**), **BA**, **RG**$^1$, **SP**$^1$, **RG**$^2$, **SP**$^2$, **HG** and subscript **p** are as defined above, **AA**$^1$ is a trivalent linker comprising an amino acid residue and is directly or indirectly linked to an antibody, a payload and a hydrophilic group; **AA**$^2$ is a dipeptide, tripeptide, or tetrapeptide residue; and **PAB** is  , wherein the  indicates the atom through which the **PAB** is bonded to the adjacent groups in the formula. In some instances, subscript **p** is 0. In some instances, subscript **p** is 1. In some instances **SP**$^1$ comprises from 0-5 polyethylene glycol (**PEG**) residues. In some instances **SP**$^2$ comprises from 0-5 **PEG** residues. In some examples, **SP**$^1$ is independently in each instance, selected from the group consisting of $C_{1-6}$ alkylene, -NH-, -C(O)-, $(-CH_2-CH_2-O)_e$, -NH-$CH_2$-$CH_2$-(-O-$CH_2$-$CH_2$)$_e$-C(O)-, -C(O)-$(CH_2)_u$-C(O)-, -C(O)-NH-$(CH_2)_v$-, polyglycine (e.g., ((glycine)$_4$-serine)$_f$ wherein subscript **f** is an integer from 1 to 6), and combinations thereof, wherein subscript **e** is an integer from 0 to 4, subscript **u** is an integer from 1 to 8, and subscript **v** is an integer from 1 to 8. In some examples, **SP**$^2$ is independently in each instance, selected from the group consisting of $C_{1-6}$ alkylene, -NH-, -C(O)-, $(-CH_2-CH_2-O)_e$, -NH-$CH_2$-$CH_2$-(-O-$CH_2$-$CH_2$)$_e$-C(O)-, -C(O)-$(CH_2)_u$-C(O)-, -C(O)-NH-$(CH_2)_v$-, polyglycine (e.g., ((glycine)$_4$-serine)$_f$ wherein subscript **f** is an integer from 1 to 6), and combinations thereof, wherein subscript **e** is an integer from 0

to 4, subscript **u** is an integer from 1 to 8, and subscript **v** is an integer from 1 to 8. In some examples, any one of $AA^1$ or $AA^2$ comprises, independently in each instance, an amino acid selected from alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, or citrulline, a derivative thereof, or a combination thereof. In certain embodiments, $AA^1$ is an amino acid selected from alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, or citrulline, a derivative thereof, or a combination thereof. In certain embodiments, $AA^1$ is lysine. In certain embodiments, $AA^1$ is an amino acid with three functional groups to link to a payload, to a binding agent (e.g., antibody or antigen binding fragment thereof), and to a linker comprising a hydrophilic group, e.g., lysine, aspargine, glutamic acid, aspartic acid, glutamine, cysteine, threonine, serine, or tyrosine. In certain embodiments, $AA^1$ is lysine or a derivative of lysine. In certain embodiments, $AA^1$ is L-lysine. In certain embodiments, the $AA^1$ is D-lysine. In certain embodiments, $AA^1$ is glutamine. In certain embodiments, $AA^1$ is glutamic acid. In certain embodiments, $AA^1$ is aspartic acid. In certain embodiments, the $AA^2$ is valine-citrulline. In some embodiments, the $AA^2$ is citrulline-valine. In some embodiments, the $AA^2$ is valine-alanine. In some embodiments, the $AA^2$ is alanine-valine. In some embodiments, the $AA^2$ is valine-glycine. In some embodiments, the $AA^2$ is glycine-valine. In some embodiments, the $AA^1$-$AA^2$ is glutamine-valine-citrulline. In some embodiments, the $AA^1$-$AA^2$ is lysine-valine-citrulline. In some embodiments, the $AA^1$-$AA^2$ is lysine-valine-alanine. In some embodiments, the $AA^1$-$AA^2$ is glutamine-valine-alanine. In certain embodiments, $((glycine)_4\text{-serine})_f$ is $(glycine)_4$-serine.

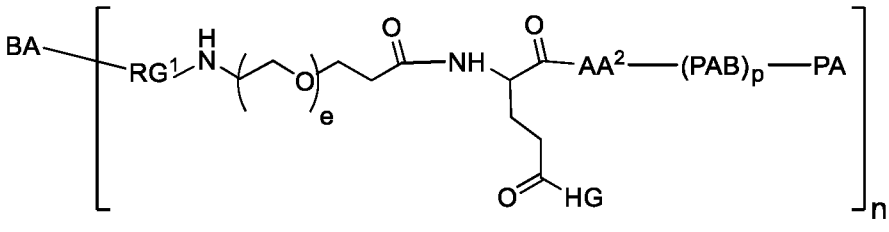
**[0101]** In some examples, the compound of Formula (**I**) or Formula (**II**) or Formula (**III**) or Formula (**IV**) is according to to Formula (**Va**), (**Vb**), (**Vc**) or (**Vd**) respectively:



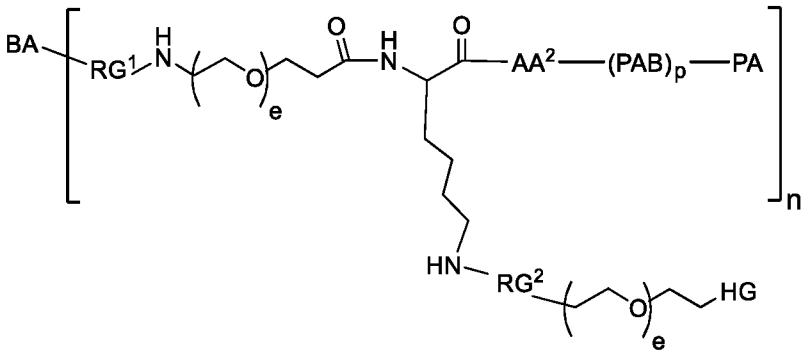

(**Va**),



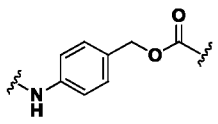
(**Vb**),



(**Vc**), or

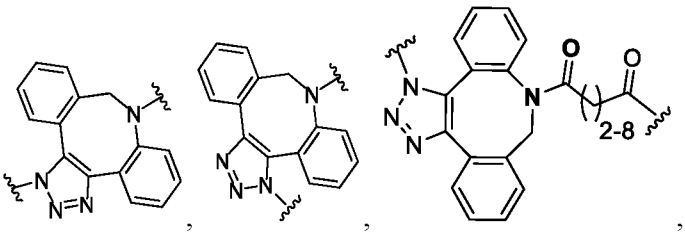


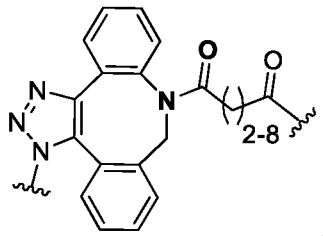
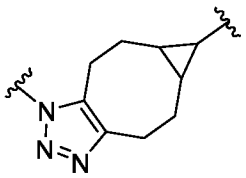
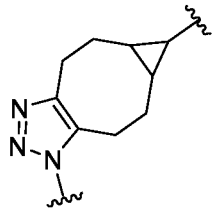
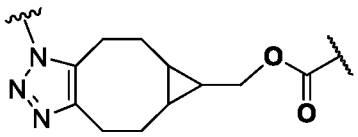
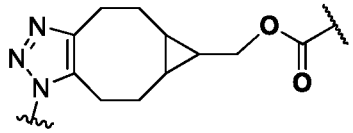
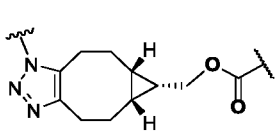
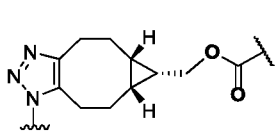
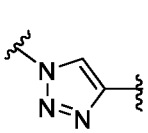
(**Vd**).

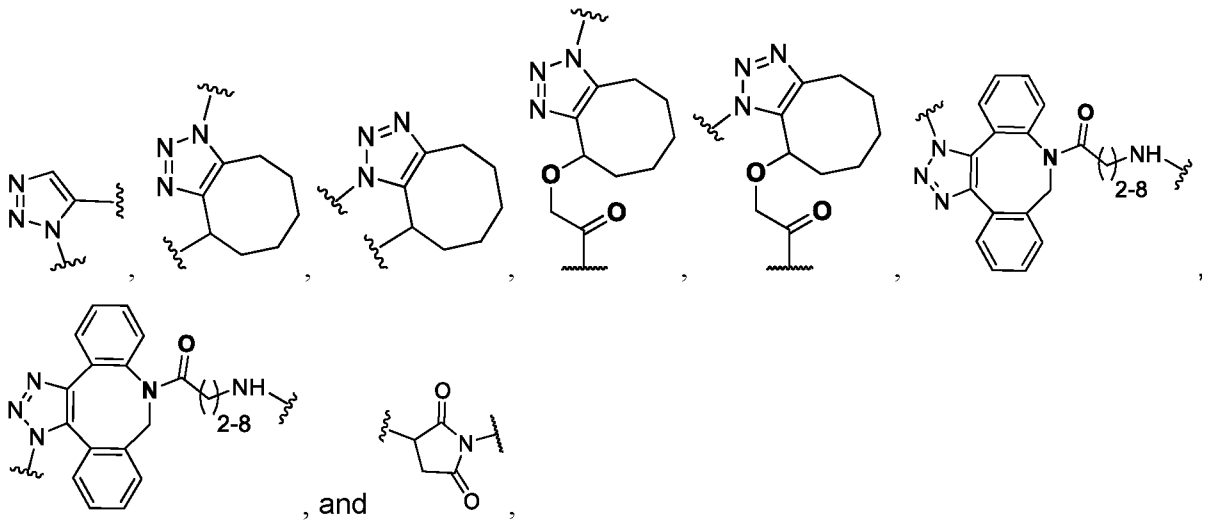
**[0102]** In Formulae (**Va**), (**Vb**), (**Vc**), and (**Vd**) **BA**, **RG**$^1$, **SP**$^1$, **RG**$^2$, **SP**$^2$ and **HG** are as defined above, **AA**$^2$ is a dipeptide, tripeptide or tetrapeptide residue; and **PAB** is  , wherein the  indicates the atom through which the **PAB** is bonded to the adjacent groups in the formula; subscript **p** is 0 or 1; and subscript **e** is independently, in each instance, an integer from 0 to 6, or an integer from 0 to 5. In some instances, subscript **p** is 0. In some instances, subscript **p** is 1. In any of these examples, subscript **e** is 1, 2, 3, or 4. In some examples, subscript **e** is 1. In some examples, subscript **e** is 2. In some examples, subscript **e** is 3. In some examples, subscript **e** is 4. In some examples, subscript **e** is 5. In some examples, subscript **e** is 6. In some examples, **SP**$^1$ is independently in each instance, selected from the group consisting of $C_{1\text{-}6}$ alkylene, -NH-, -C(O)-, $(-CH_2\text{-}CH_2\text{-}O)_e$, -NH-$CH_2$-$CH_2$-$(-O\text{-}CH_2\text{-}CH_2)_e$-C(O)-, -C(O)-$(CH_2)_u$-C(O)-, -C(O)-NH-$(CH_2)_v$-, polyglycine (e.g., $((glycine)_4\text{-}serine)_f$ wherein subscript **f** is an integer from 1 to 6), and combinations thereof, wherein subscript **e** is an integer from 0 to 4, subscript **u** is an integer from 1 to 8, and subscript **v** is an integer from 1 to 8. In some examples, **SP**$^2$ is independently in each

instance, selected from the group consisting of $C_{1-6}$ alkylene, -NH-, -C(O)-, $(-CH_2-CH_2-O)_e$, -NH-$CH_2$-$CH_2$-$(-O-CH_2-CH_2)_e$-C(O)-, -C(O)-$(CH_2)_u$-C(O)-, -C(O)-NH-$(CH_2)_v$-, polyglycine (e.g., $((glycine)_4\text{-serine})_f$ wherein subscript **f** is an integer from 1 to 6), and combinations thereof, wherein subscript **e** is an integer from 0 to 4, subscript **u** is an integer from 1 to 8, and subscript **v** is an integer from 1 to 8. In some examples, any $\mathbf{AA^2}$ comprises, independently in each instance, an amino acid selected from alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, or citrulline, a derivative thereof, or a combination thereof. In certain embodiments, the $\mathbf{AA^2}$ is valine-citrulline. In some embodiments, the $\mathbf{AA^2}$ is citrulline-valine. In some embodiments, the $\mathbf{AA^2}$ is valine-alanine. In some embodiments, the $\mathbf{AA^2}$ is alanine-valine. In some embodiments, the $\mathbf{AA^2}$ is valine-glycine. In some embodiments, the $\mathbf{AA^2}$ is glycine-valine. In certain embodiments, $((glycine)_4\text{-serine})_f$ is $(glycine)_4$-serine.

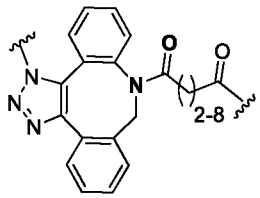
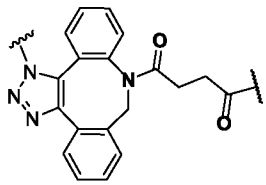
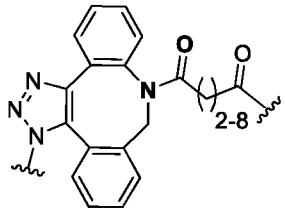
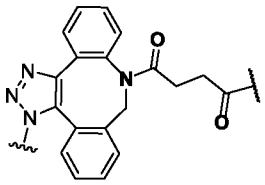
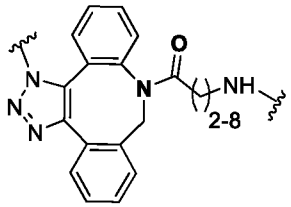
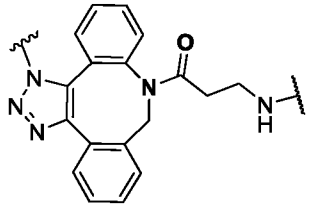
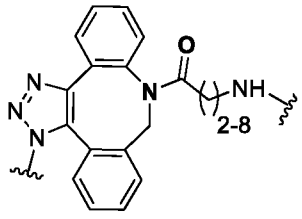
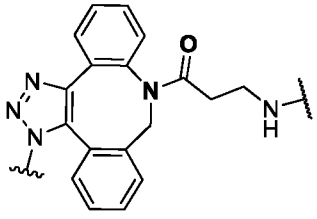
**[0103]** In any compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), Formula (**Vd**), $\mathbf{RG^1}$ and $\mathbf{RG^2}$ are independently in each instance, a click chemistry residue. In some examples, $\mathbf{RG^1}$ and $\mathbf{RG^2}$ independently in each instance, comprise a triazaole or a fused triazole. In some instances, $\mathbf{RG^1}$ and $\mathbf{RG^2}$ are independently, in each instance, selected from the group consisting of



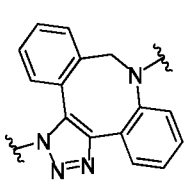
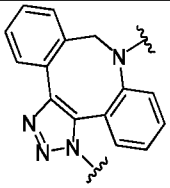
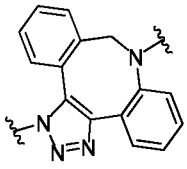
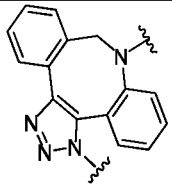
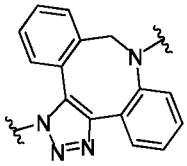
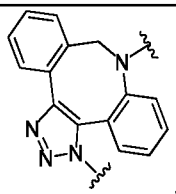
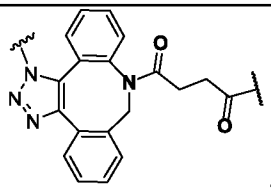
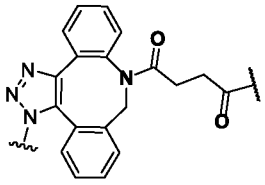
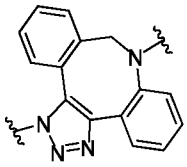
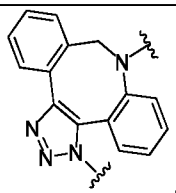
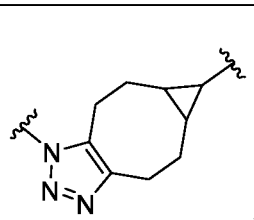
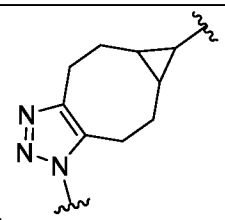
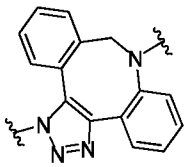
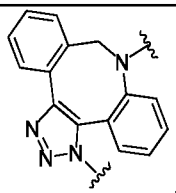
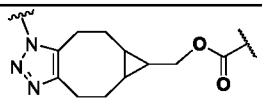
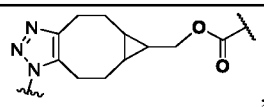
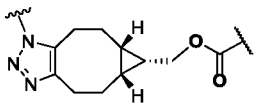
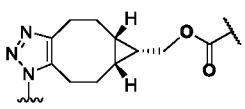
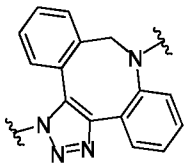
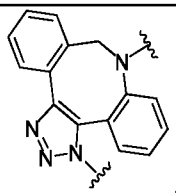
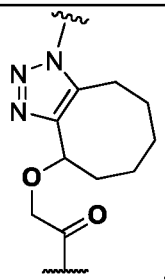
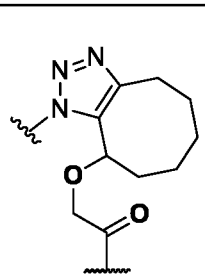


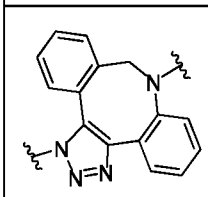
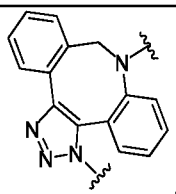
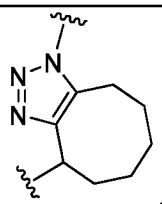
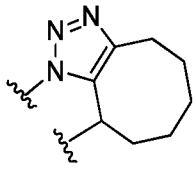
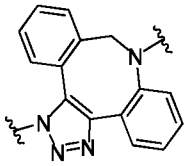
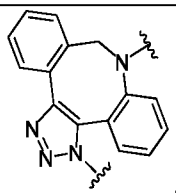
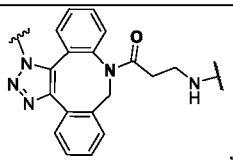
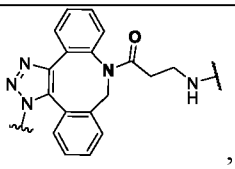
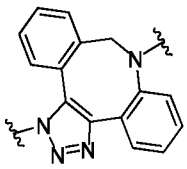
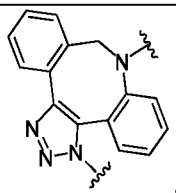
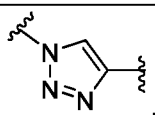
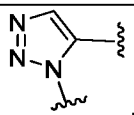
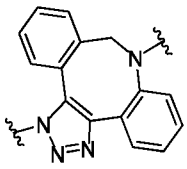
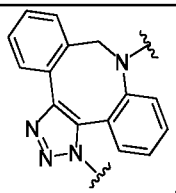
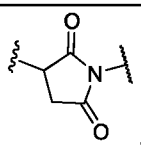
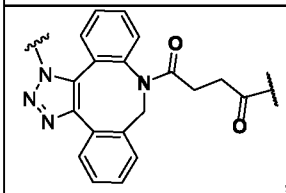
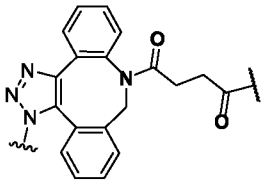
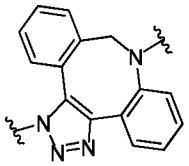
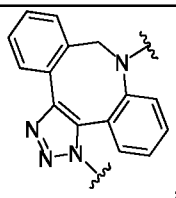
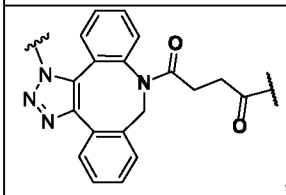
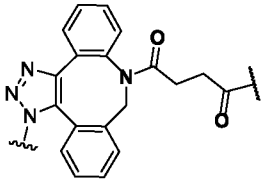
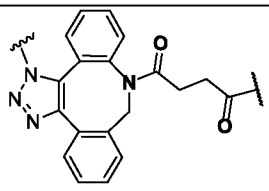
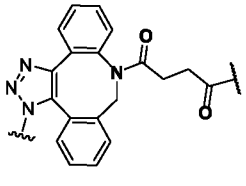
wherein the  indicates the atom through which the $RG^1$ or $RG^2$ is bonded to the adjacent groups in the formula.

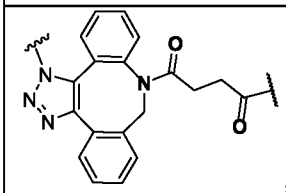
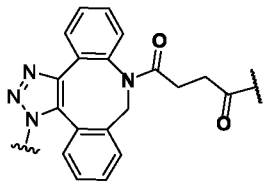
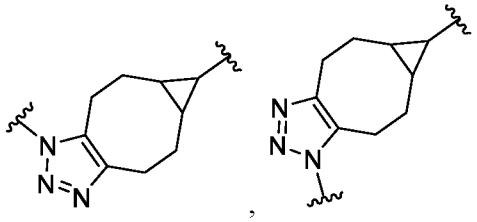
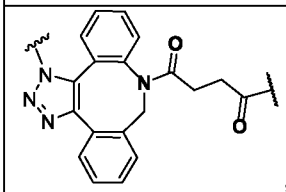
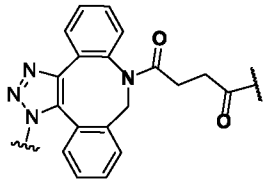
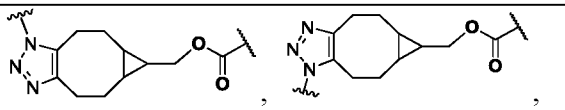
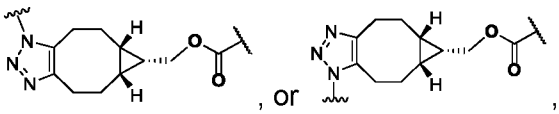
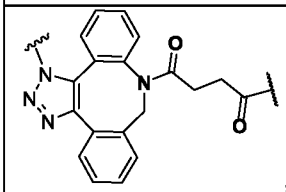
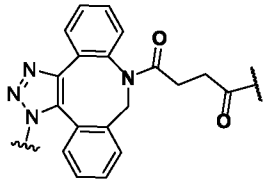
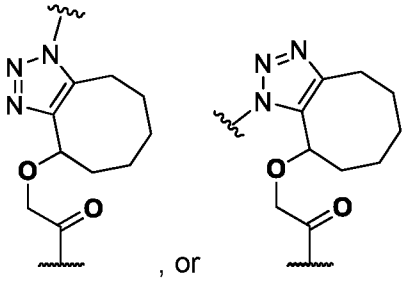
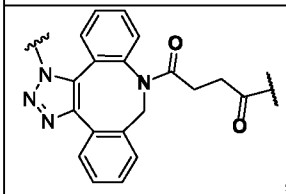
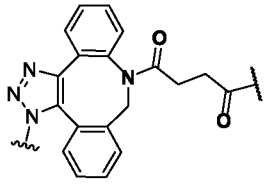
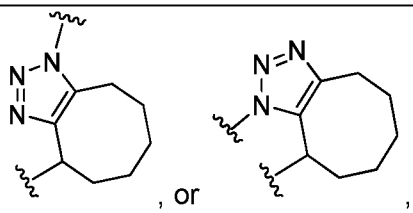
**[0104]** In certain embodiments,  is  . In certain embodiments,  is  . In certain embodiments,  is  . In some embodiments,  is  .

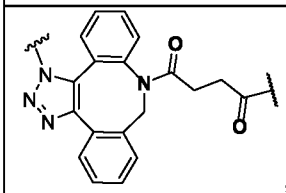
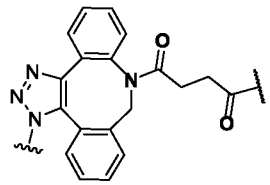
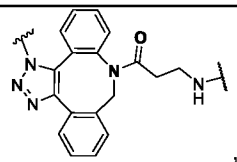
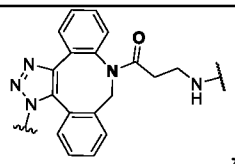
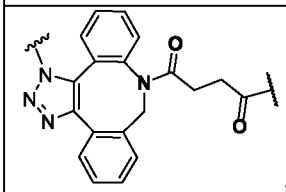
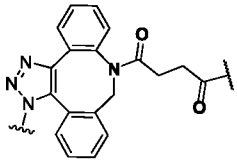
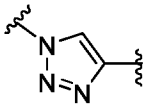
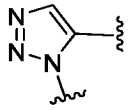
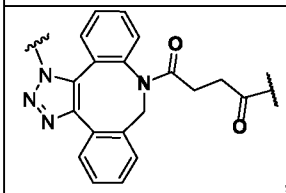
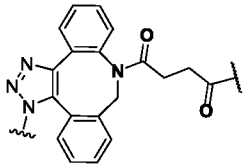
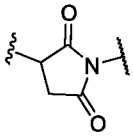
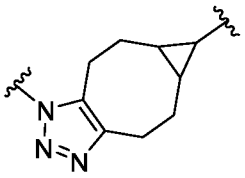
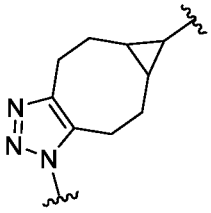
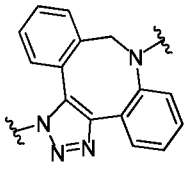
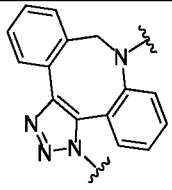
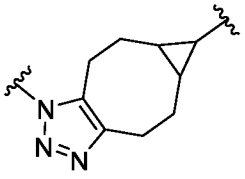
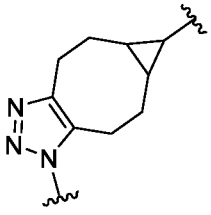
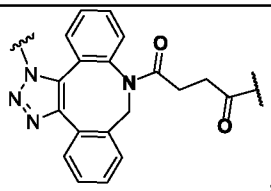
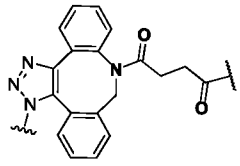
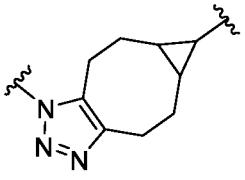
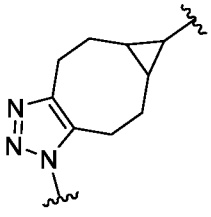
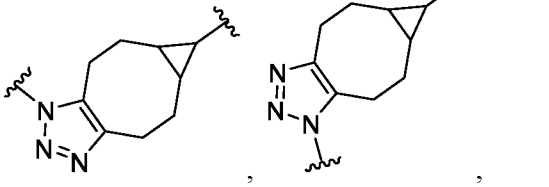
**[0105]** In certain instances, $RG^1$ and $RG^2$ are independently, in each instance, as shown in Table R.

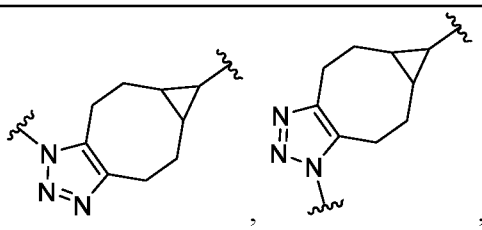
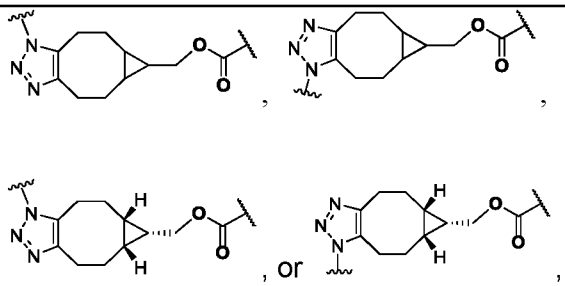
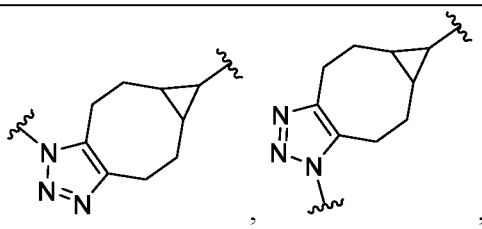
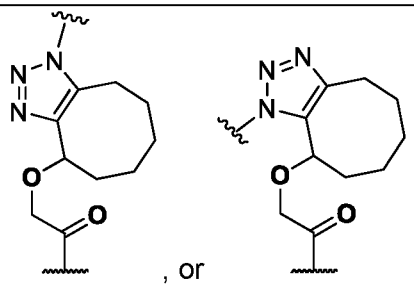
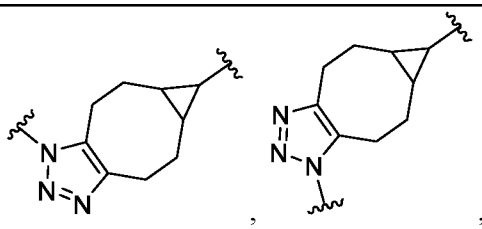
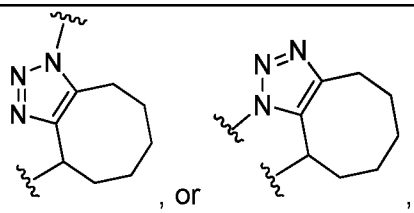
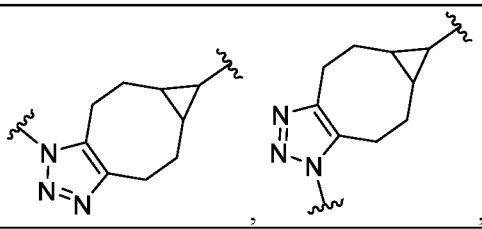
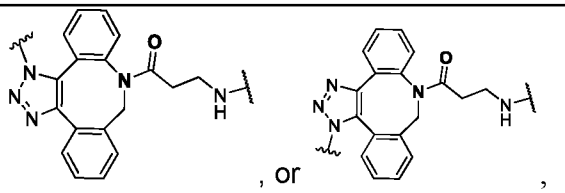
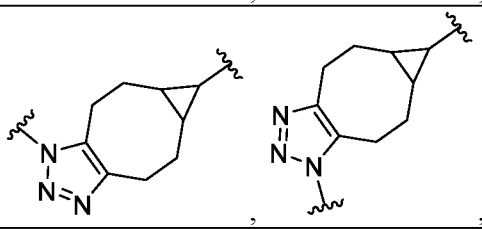
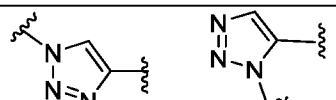
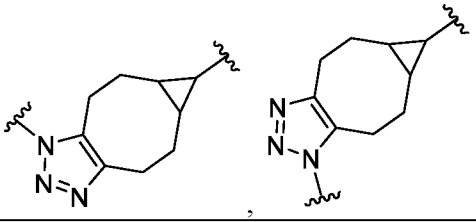
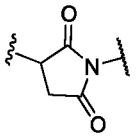
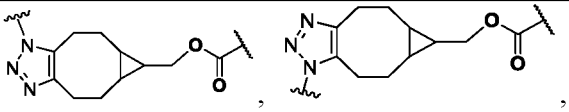
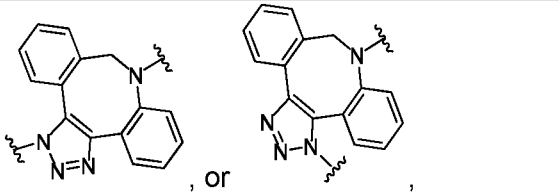
Table R

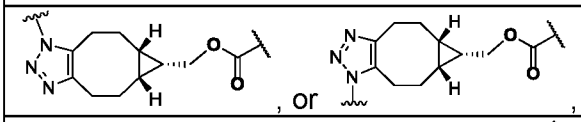
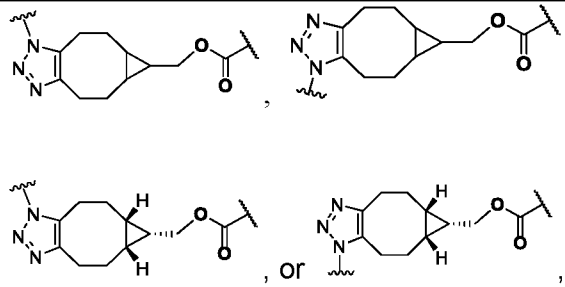
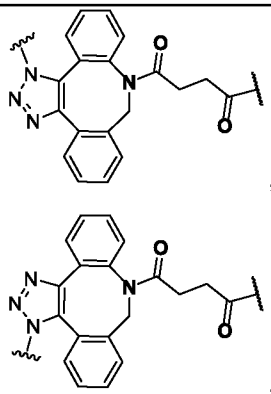
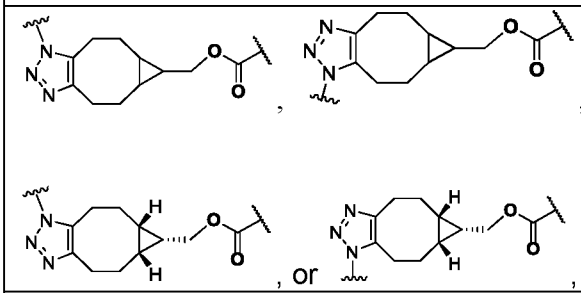
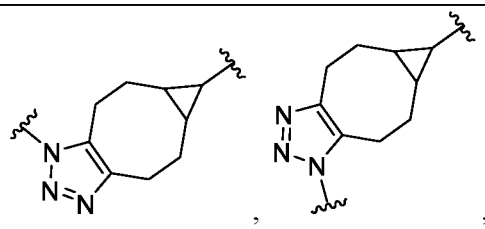
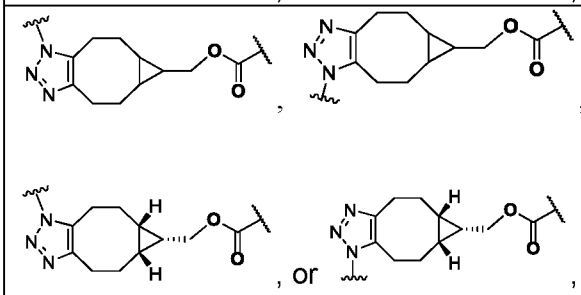
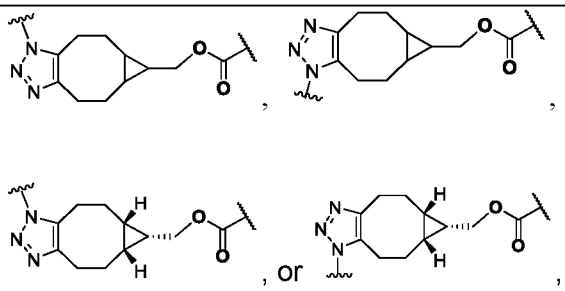
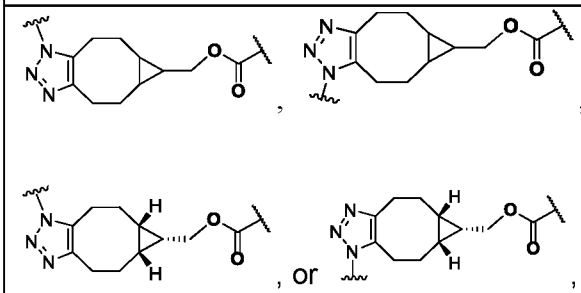
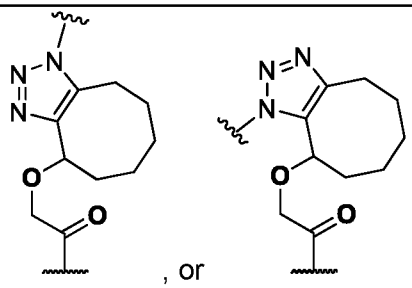
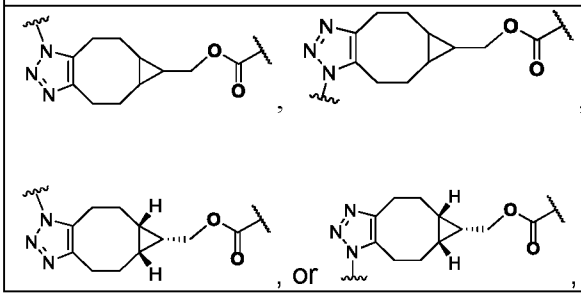
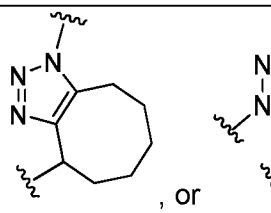
| $RG^1$ | $RG^2$ |
|---|---|
|  , or  , |  , or  , |
|  , or  , |  , or  , |
|  , or  , |  ,  ,, |
|  , or  , |  ,  ,  , or  , |
|  , or  , |  , or  |

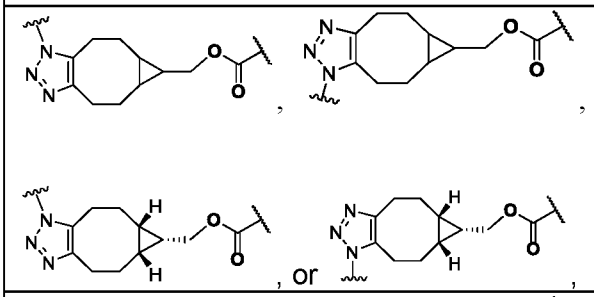
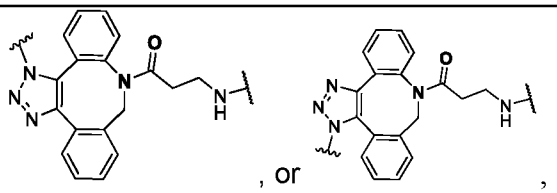
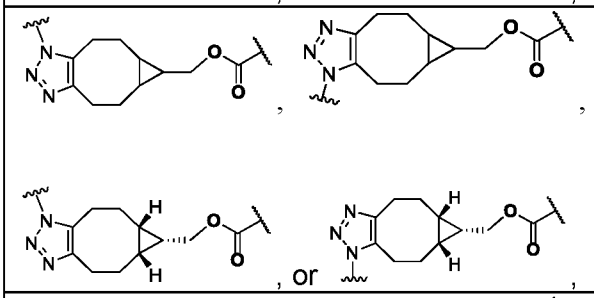
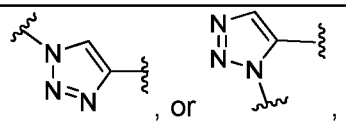
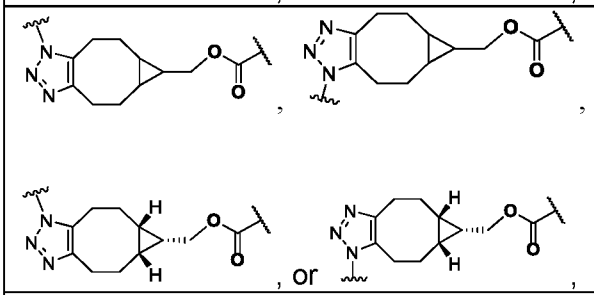
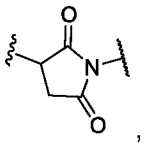
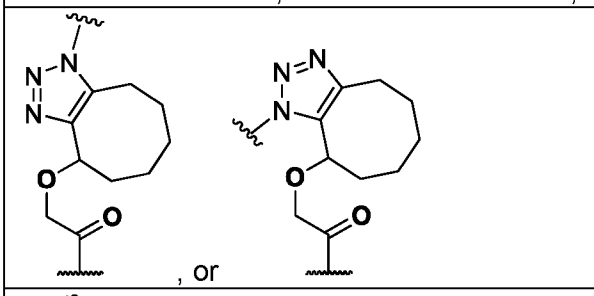
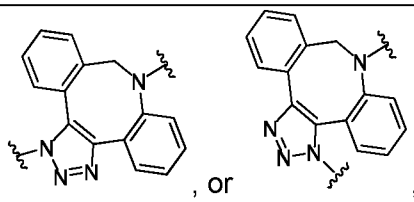
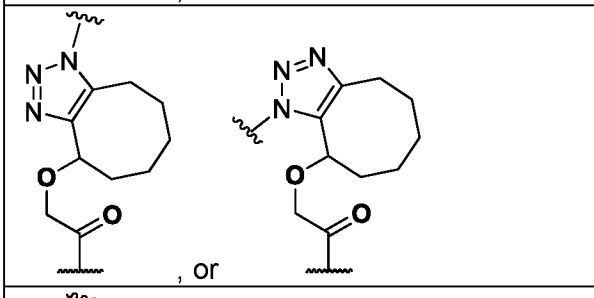
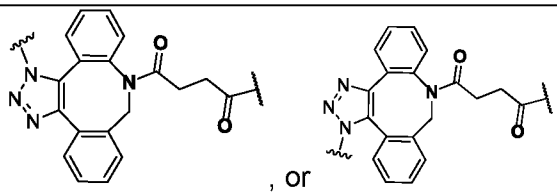
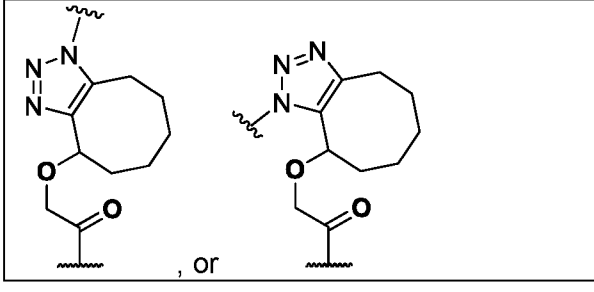
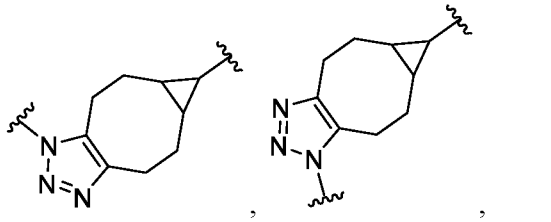
| $RG^1$ | $RG^2$ |
|---|---|
|  , or  , |  , or  , |
|  , or  , |  , or  , |
|  , or  , |  , or  , |
|  , or  , |  , |
|  , or  , |  , or  , |
|  , or  , |  , or  , |

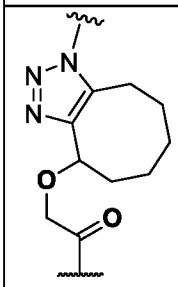
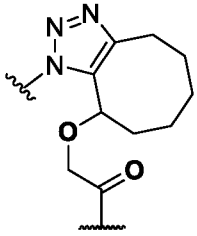
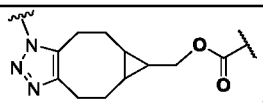
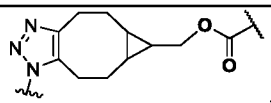
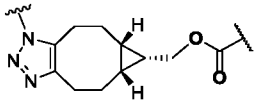
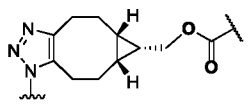
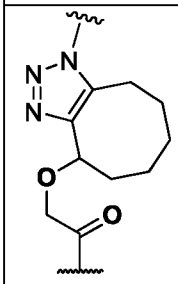
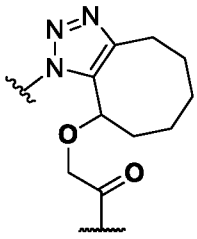
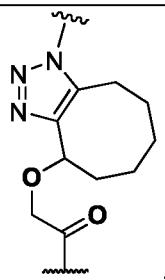
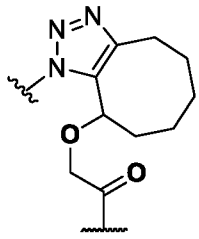
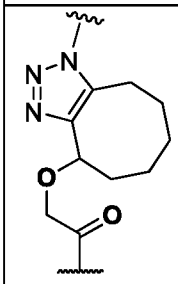
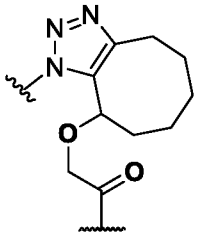
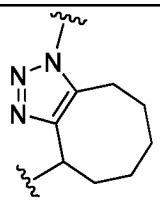
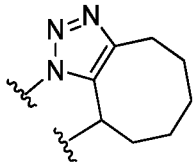
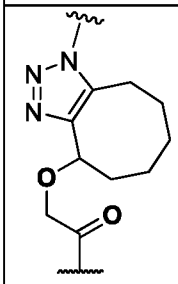
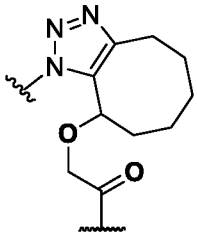
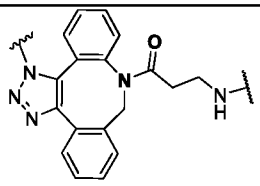
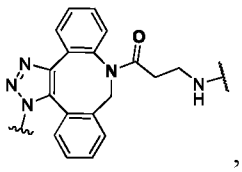
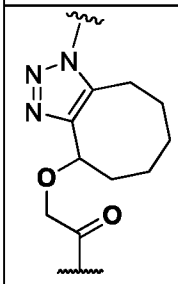
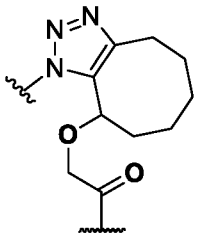
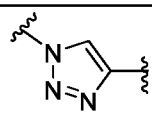
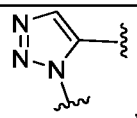
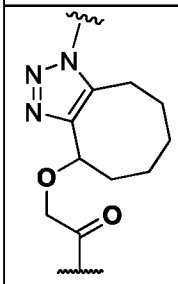
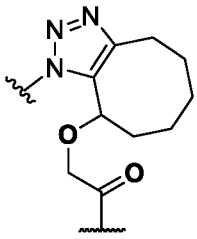
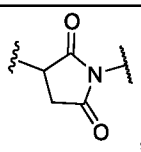
| $RG^1$ | $RG^2$ |
|---|---|
|  , or  , |  , , |
|  , or  , |  , ,  , or , |
|  , or  , |  , or |
|  , or  , |  , or , |

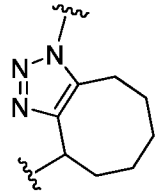
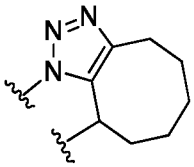
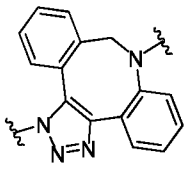
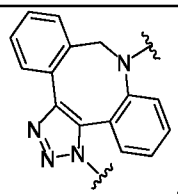
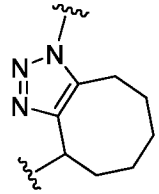
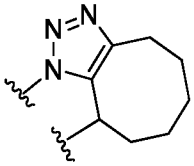
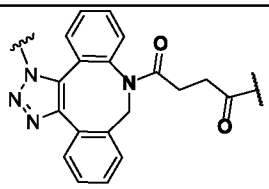
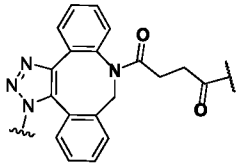
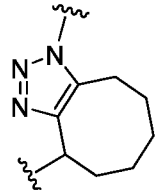
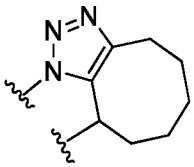
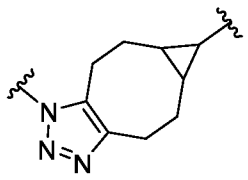
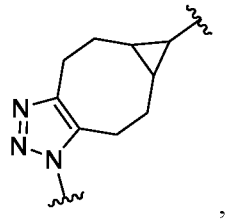
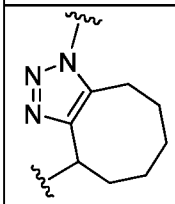
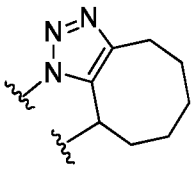
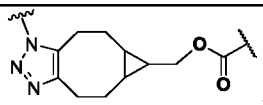
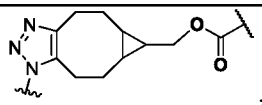
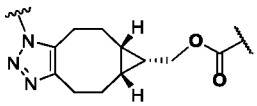
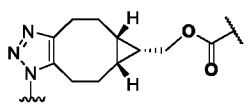
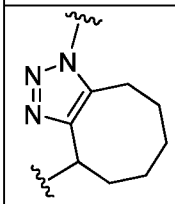
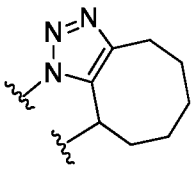
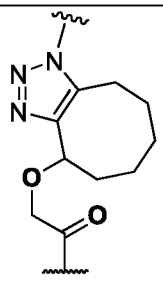
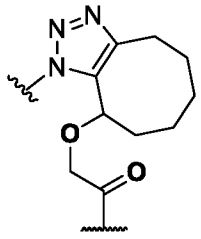
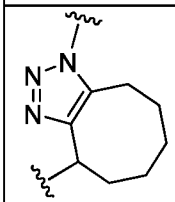
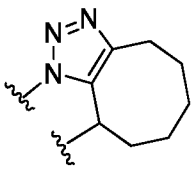
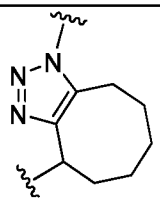
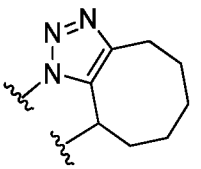
| $RG^1$ | $RG^2$ |
|---|---|
|  , or  , |  , or  , |
|  , or  , |  , or  , |
|  , or  , |  , |
|  ,  , |  , or  , |
|  ,  , |  , or  , |
|  ,  , |  , |

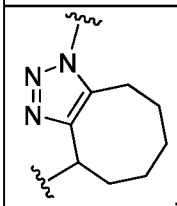
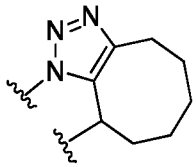
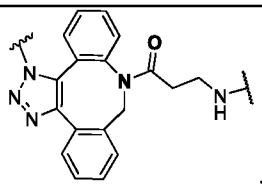
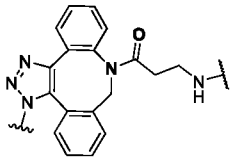
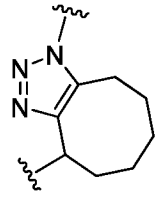
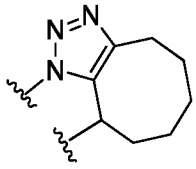
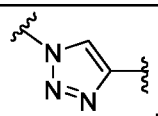
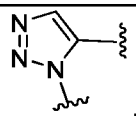
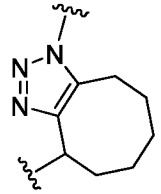
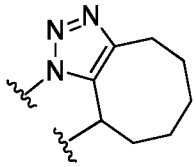
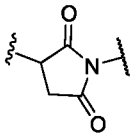
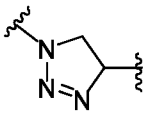
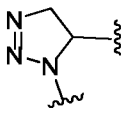
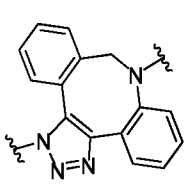
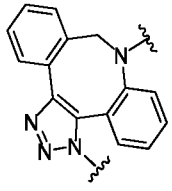
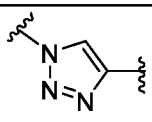
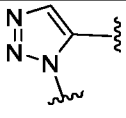
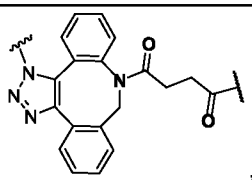
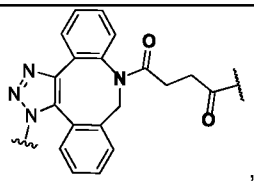
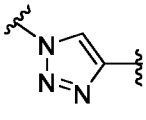
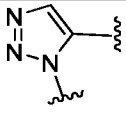
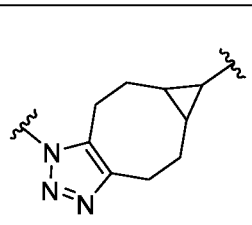
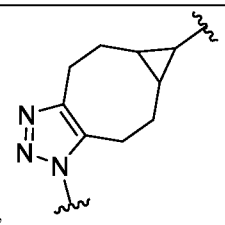
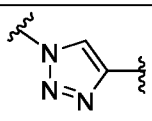
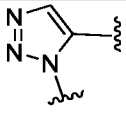
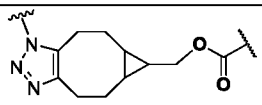
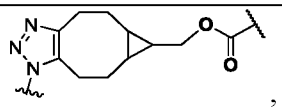
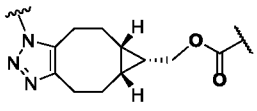
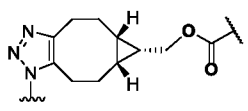
| $RG^1$ | $RG^2$ |
|---|---|
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |

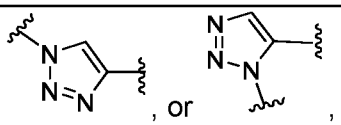
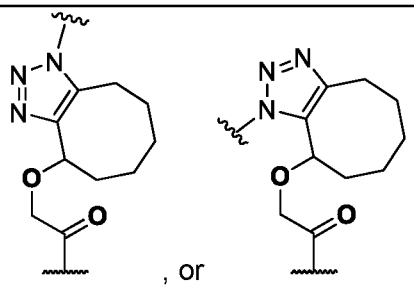
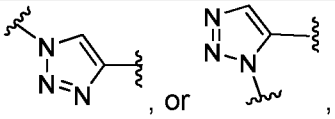
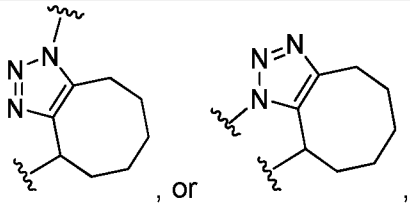
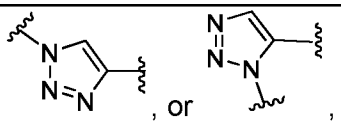
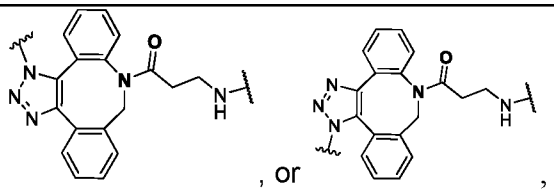
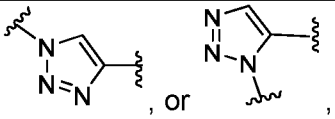
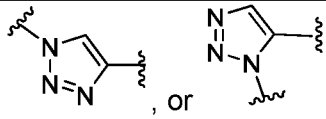
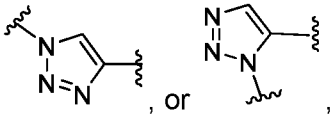
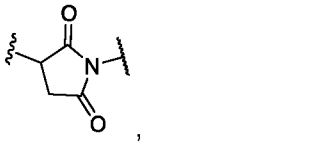
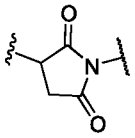
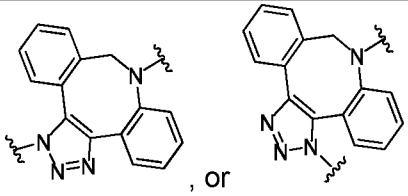
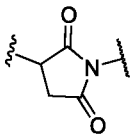
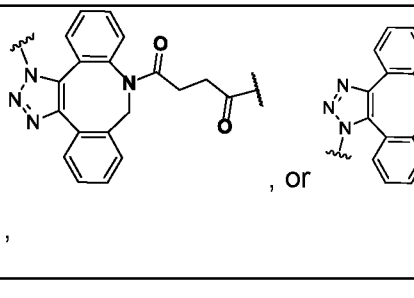
| $RG^1$ | $RG^2$ |
|---|---|
|  | |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |

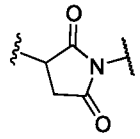
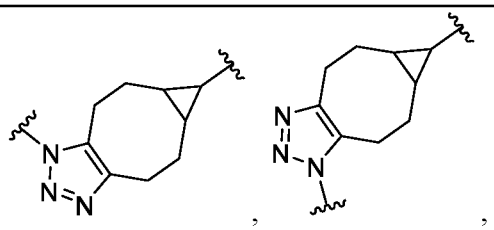
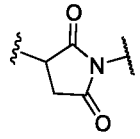
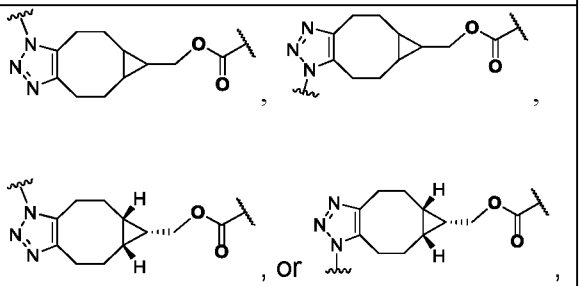
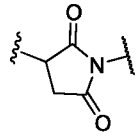
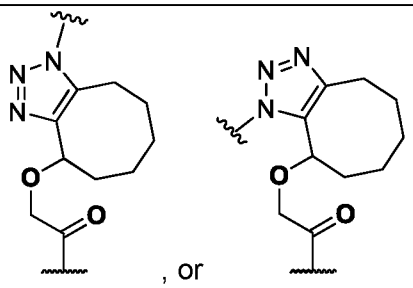
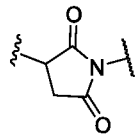
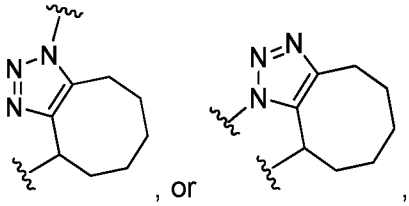
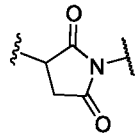
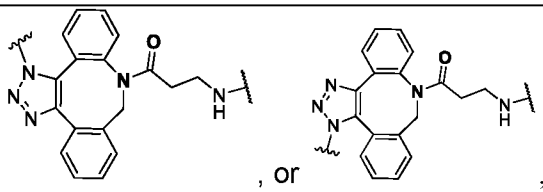
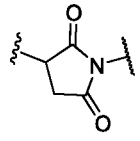
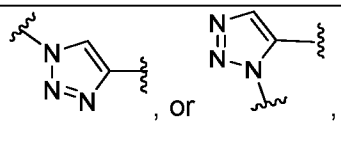
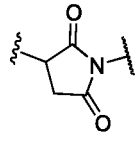
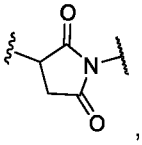
| $RG^1$ | $RG^2$ |
|---|---|
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |

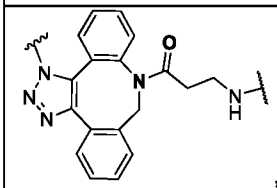
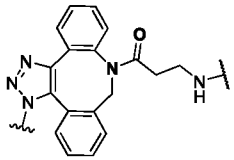
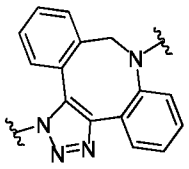
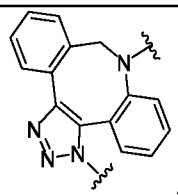
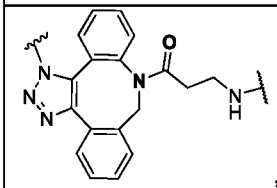
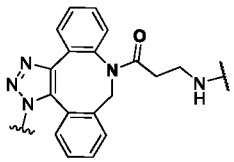
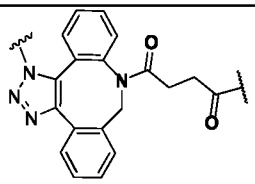
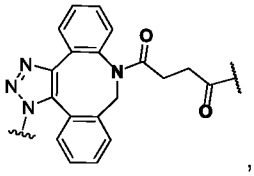
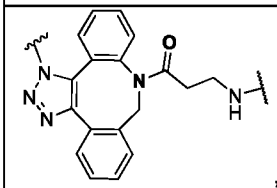
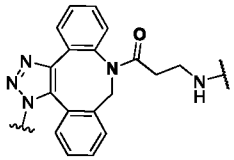
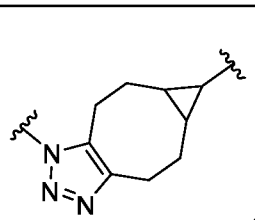
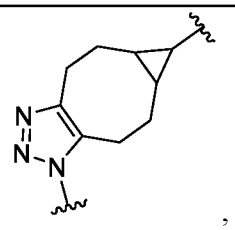
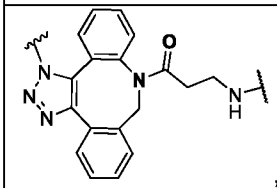
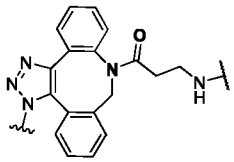
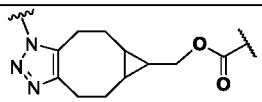
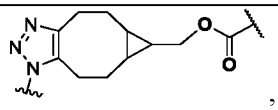
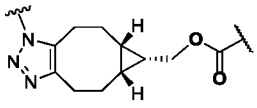
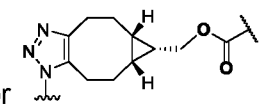
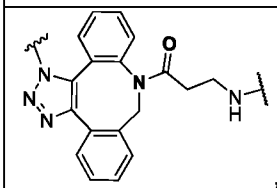
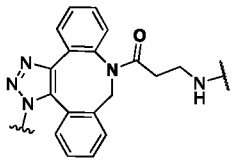
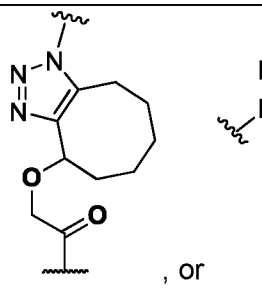
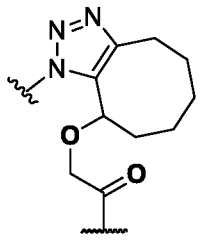
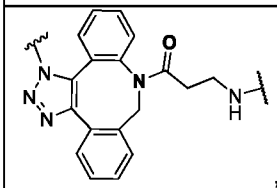
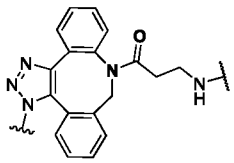
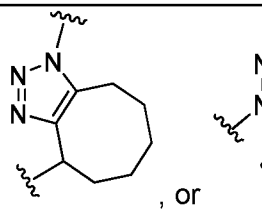
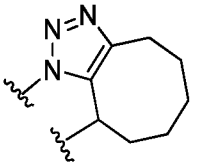
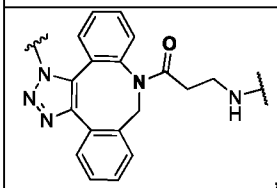
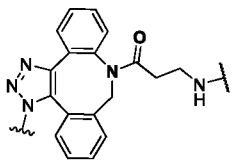
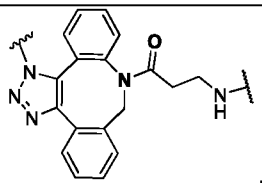
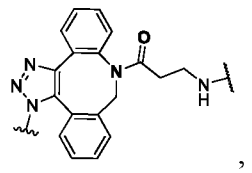
| $RG^1$ | $RG^2$ |
|---|---|
|  , or  |  ,  ,  , or  , |
|  , or  |  , or  |
|  , or  |  , or  , |
|  , or  |  , or  , |
|  , or  |  , or  , |
|  , or  |  , |

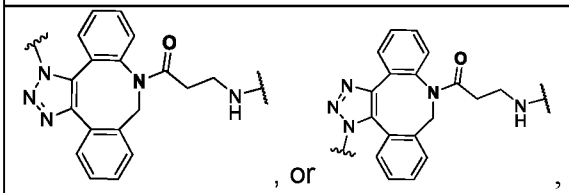
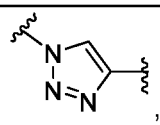
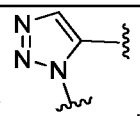
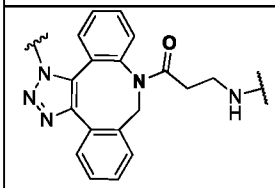
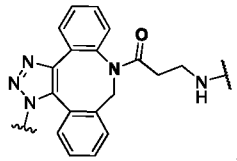
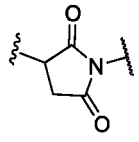
| $RG^1$ | $RG^2$ |
|---|---|
|  , or  , |  , or  , |
|  , or  , |  , or  , |
|  , or  , |  ,  , |
|  , or  , |  ,  ,  , or  , |
|  , or  , |  , or  |
|  , or  , |  , or  , |

| $RG^1$ | $RG^2$ |
|---|---|
|  , or  , |  , or  , |
|  , or  , |  , or  , |
|  , or  , |  , |
|  , or  , |  , or  , |
|  , or  , |  , or  , |
|  , or  , |  ,  , |
|  , or  , |  ,  ,  , or  , |

| $RG^1$ | $RG^2$ |
|---|---|
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |

| $RG^1$ | $RG^2$ |
|---|---|
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |

| $RG^1$ | $RG^2$ |
|---|---|
|  , or  , |  , or  , |
|  , or  , |  , or  , |
|  , or  , |  ,  , |
|  , or  , |  ,  ,  , or  , |
|  , or  , |  , or  |
|  , or  , |  , or  , |
|  , or  , |  , or  , |

| $RG^1$ | $RG^2$ |
|---|---|
|  , or  , |  , or  , |
|  , or  , |  , |

wherein the  indicates the atom through which the $RG^1$ or $RG^2$ is bonded to the adjacent groups in the formula.

**[0106]** In some instances, for any compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), **HG** is is $–(CH_2)_{1\text{-}5}SO_3H$, $–(CH_2)_n–NH\text{-}(CH_2)_{1\text{-}5}SO_3H$, $–(CH_2)_n–C(O)NH\text{-}(CH_2)_{1\text{-}5}SO_3H$, $–(CH_2CH_2O)_m–C(O)NH\text{-}(CH_2)_{1\text{-}5}SO_3H$, $–(CH_2)_n–N((CH_2)_{1\text{-}5}C(O)NH(CH_2)_{1\text{-}5}SO_3H)_2$, $–(CH_2)_n–C(O)N((CH_2)_{1\text{-}5}C(O)NH(CH_2)_{1\text{-}5}SO_3H)_2$, or $–(CH_2CH_2O)_m–C(O)N((CH_2)_{1\text{-}5}C(O)NH(CH_2)_{1\text{-}5}SO_3H)_2$, wherein n is 1, 2, 3, 4, or 5, and m is 1, 2, 3, 4, or 5. In one embodiment, **HG** is $–(CH_2)_{1\text{-}5}SO_3H$. In another embodiment, **HG** is $\text{-}(CH_2)_n–NH\text{-}(CH_2)_{1\text{-}5}SO_3H$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is $\text{-}(CH_2)_n–C(O)NH\text{-}(CH_2)_{1\text{-}5}SO_3H$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is $–(CH_2CH_2O)_m–C(O)NH\text{-}(CH_2)_{1\text{-}5}SO_3H$, wherein m is 1, 2, 3, 4, or 5. In another embodiment, **HG** is $–(CH_2)_n–N((CH_2)_{1\text{-}5}C(O)NH(CH_2)_{1\text{-}5}SO_3H)_2$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is $–(CH_2)_n–C(O)N((CH_2)_{1\text{-}5}C(O)NH(CH_2)_{1\text{-}5}SO_3H)_2$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is $–(CH_2CH_2O)_m–C(O)N((CH_2)_{1\text{-}5}C(O)NH(CH_2)_{1\text{-}5}SO_3H)_2$, wherein m is 1, 2, 3, 4, or 5.

**[0107]** In some instances, for any compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), **HG** is is $–(CH_2)_{1\text{-}5}PO_3H$, $–(CH_2)_n–NH\text{-}(CH_2)_{1\text{-}5}PO_3H$, $–(CH_2)_n–C(O)NH\text{-}(CH_2)_{1\text{-}5}PO_3H$, $–(CH_2CH_2O)_m–C(O)NH\text{-}(CH_2)_{1\text{-}5}PO_3H$, $–(CH_2)_n–N((CH_2)_{1\text{-}5}C(O)NH(CH_2)_{1\text{-}5}PO_3H)_2$, $–(CH_2)_n–C(O)N((CH_2)_{1\text{-}5}C(O)NH(CH_2)_{1\text{-}5}PO_3H)_2$, or $–(CH_2CH_2O)_m–C(O)N((CH_2)_{1\text{-}5}C(O)NH(CH_2)_{1\text{-}5}PO_3H)_2$, wherein n is 1, 2, 3, 4, or 5, and m is 1, 2, 3, 4, or 5. In one embodiment, **HG** is $–(CH_2)_{1\text{-}5}PO_3H$. In another embodiment, **HG**

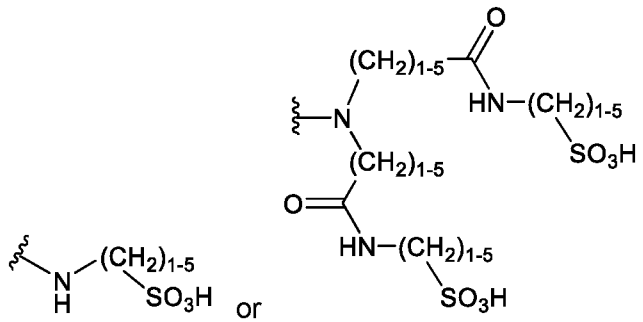
is -$(CH_2)_n$–NH-$(CH_2)_{1-5}PO_3H$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is -$(CH_2)_n$–C(O)NH-$(CH_2)_{1-5}PO_3H$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is –$(CH_2CH_2O)_m$–C(O)NH-$(CH_2)_{1-5}PO_3H$, wherein m is 1, 2, 3, 4, or 5. In another embodiment, **HG** is –$(CH_2)_n$–N($(CH_2)_{1-5}C(O)NH(CH_2)_{1-5}PO_3H)_2$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is –$(CH_2)_n$–C(O)N($(CH_2)_{1-5}C(O)NH(CH_2)_{1-5}PO_3H)_2$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is –$(CH_2CH_2O)_m$–C(O)N($(CH_2)_{1-5}C(O)NH(CH_2)_{1-5}PO_3H)_2$, wherein m is 1, 2, 3, 4, or 5.

**[0108]** In some instances, for any compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), **HG** is is –$(CH_2)_{1-5}N^+(\mathbf{R^M})_3$, –$(CH_2)_n$–NH-$(CH_2)_{1-5}N^+(\mathbf{R^M})_3$, –$(CH_2)_n$–C(O)NH-$(CH_2)_{1-5}N^+(\mathbf{R^M})_3$, –$(CH_2CH_2O)_m$–C(O)NH-$(CH_2)_{1-5}N^+(\mathbf{R^M})_3$, –$(CH_2)_n$–N($(CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+(\mathbf{R^M})_3)_2$, –$(CH_2)_n$–C(O)N($(CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+(\mathbf{R^M})_3)_2$, or –$(CH_2CH_2O)_m$–C(O)N($(CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+(\mathbf{R^M})_3)_2$, wherein n is 1, 2, 3, 4, or 5, m is 1, 2, 3, 4, or 5, and $\mathbf{R^M}$, at each occurrence, is independently H, $C_{1-6}$alkyl, $C_{3-7}$cycloalkyl or $C_{1-6}$alkyl-$C_{3-7}$cycloalkyl, or, two $\mathbf{R^M}$ together with the nitrogen atom to which they are attached, form a 3-7-membered heterocycloalkyl ring. In one embodiment, **HG** is –$(CH_2)_{1-5}N^+(\mathbf{R^M})_3$. In another embodiment, **HG** is -$(CH_2)_n$–NH-$(CH_2)_{1-5}N^+(\mathbf{R^M})_3$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is -$(CH_2)_n$–C(O)NH-$(CH_2)_{1-5}N^+(\mathbf{R^M})_3$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is –$(CH_2CH_2O)_m$–C(O)NH-$(CH_2)_{1-5}N^+(\mathbf{R^M})_3$, wherein m is 1, 2, 3, 4, or 5. In another embodiment, **HG** is –$(CH_2)_n$–N($(CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+(\mathbf{R^M})_3)_2$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is –$(CH_2)_n$–C(O)N($(CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+(\mathbf{R^M})_3)_2$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is –$(CH_2CH_2O)_m$–C(O)N($(CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+(\mathbf{R^M})_3)_2$, wherein m is 1, 2, 3, 4, or 5.

**[0109]** In some instances, for any compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), **HG** is is –$(CH_2)_{1-5}N^+Me_3$, –$(CH_2)_n$–NH-$(CH_2)_{1-5}N^+Me_3$, –$(CH_2)_n$–C(O)NH-$(CH_2)_{1-5}N^+Me_3$, –$(CH_2CH_2O)_m$–C(O)NH-$(CH_2)_{1-5}N^+Me_3$, –$(CH_2)_n$–N($(CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+Me_3)_2$, –$(CH_2)_n$–C(O)N($(CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+Me_3)_2$, or –$(CH_2CH_2O)_m$–C(O)N($(CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+Me_3)_2$, wherein n is 1, 2, 3, 4, or 5, and m is 1, 2, 3, 4, or 5. In one embodiment, **HG** is –$(CH_2)_{1-5}N^+Me_3$. In another embodiment, **HG** is -$(CH_2)_n$–NH-$(CH_2)_{1-5}N^+Me_3$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is -$(CH_2)_n$–C(O)NH-$(CH_2)_{1-5}N^+Me_3$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is –$(CH_2CH_2O)_m$–C(O)NH-$(CH_2)_{1-5}N^+Me_3$, wherein m is 1, 2, 3, 4, or 5. In another embodiment,

**HG** is $-(CH_2)_n-N((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+Me_3)_2$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is $-(CH_2)_n-C(O)N((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+Me_3)_2$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is $-(CH_2CH_2O)_m-C(O)N((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+Me_3)_2$, wherein m is 1, 2, 3, 4, or 5.

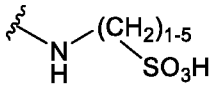
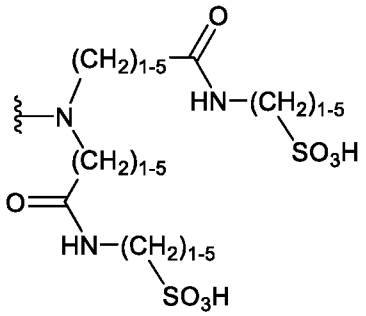
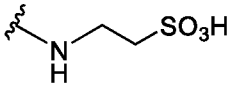
**[0110]** In some instances, for any compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), **HG** is

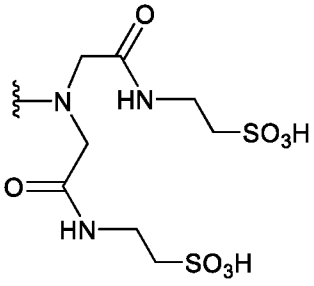


or



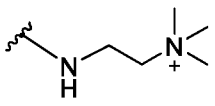
, or salts thereof,

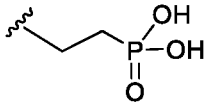
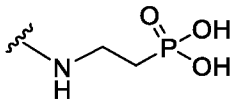
wherein the  indicates the atom through which the **HG** is bonded to the adjacent groups in the formula. In one instance **HG** is  , or salts thereof. In another instance, **HG** is  , or salts thereof. In one instance **HG** is  ,

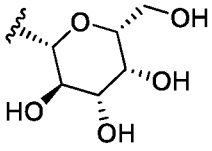
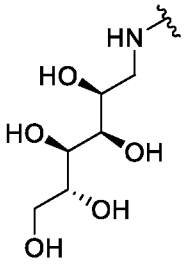
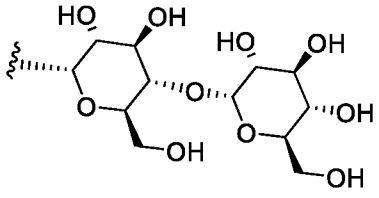


or salts thereof. In another instance, **HG** is , or salts thereof.

**[0111]** In some examples, for any compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), **HG** is an amine, or salts thereof, for instance, a quarternary

amine, e.g.,  , wherein the  indicates the atom through which the **HG** is bonded to the adjacent groups in the formula.

**[0112]** In other examples, for any compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), **HG** is a phosphonic acid, or salts thereof, e.g.,  wherein the  indicates the atom through which the **HG** is bonded to the adjacent groups in the formula. In other examples, for any compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), **HG** is a phosphonic acid, or salts thereof, e.g.,  wherein the  indicates the atom through which the **HG** is bonded to the adjacent groups in the formula.

**[0113]** In yet other examples, for any compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), **HG** is a sugar residue, e.g.,  (galactose),  (glucamine), or  (maltose), wherein the  indicates the atom through which the **HG** is bonded to the adjacent groups in the formula.

**[0114]** In some cases, for any compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), **SP**$^{1}$ and **SP**$^{2}$ are independently, in each instance, absent, or selected from the group consisting of $C_{1\text{-}6}$ alkylene, -NH-, -C(O)-, $(\text{-CH}_2\text{-CH}_2\text{-O})_e$, -NH-$\text{CH}_2$-$\text{CH}_2$-$(\text{-O-CH}_2\text{-CH}_2)_e$-C(O)-, -C(O)-$(\text{CH}_2)_u$-C(O)-, -C(O)-NH-$(\text{CH}_2)_v$-, polyglycine (e.g., $((\text{glycine})_4\text{-serine})_f$ wherein subscript **f** is an integer from 1 to 6), and combinations thereof, wherein subscript **e** is an integer from 0 to 4, subscript **u** is an integer from 1 to 8, and subscript **v** is an integer from

1 to 8. In some instances, $SP^1$ and $SP^2$ are independently, in each instance, as shown in Table S.

**TABLE S**

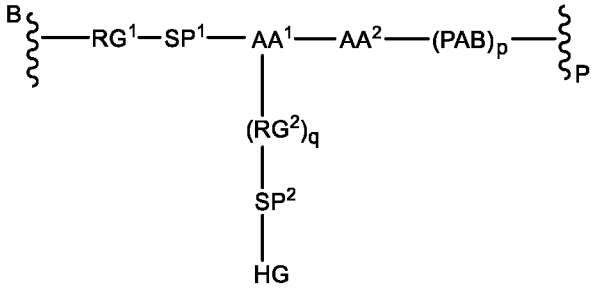
| $SP^1$ | $SP^2$ |
|---|---|
| absent | absent |
| absent | $C_{1-6}$ alkylene, |
| absent | -NH-, |
| absent | -C(O)-, |
| absent | $(-CH_2-CH_2-O)_e$, |
| absent | $-NH-CH_2-CH_2-(-O-CH_2-CH_2)_e-C(O)-$, |
| absent | $-C(O)-(CH_2)_u-C(O)-$, |
| absent | $-C(O)-NH-(CH_2)_v-$, |
| absent | $(glycine)_4$-serine |
| $C_{1-6}$ alkylene, | absent |
| $C_{1-6}$ alkylene, | $C_{1-6}$ alkylene, |
| $C_{1-6}$ alkylene, | -NH-, |
| $C_{1-6}$ alkylene, | -C(O)-, |
| $C_{1-6}$ alkylene, | $(-CH_2-CH_2-O)_e$, |
| $C_{1-6}$ alkylene, | $-NH-CH_2-CH_2-(-O-CH_2-CH_2)_e-C(O)-$, |
| $C_{1-6}$ alkylene, | $-C(O)-(CH_2)_u-C(O)-$, |
| $C_{1-6}$ alkylene, | $-C(O)-NH-(CH_2)_v-$, |
| $C_{1-6}$ alkylene, | $(glycine)_4$-serine |
| NH-, | absent |
| NH-, | $C_{1-6}$ alkylene, |
| NH-, | -NH-, |
| NH-, | -C(O)-, |
| NH-, | $(-CH_2-CH_2-O)_e$, |

| $SP^1$ | $SP^2$ |
|---|---|
| NH-, | -NH-$CH_2$-$CH_2$-(-O-$CH_2$-$CH_2$)$_e$-C(O)-, |
| NH-, | -C(O)-$(CH_2)_u$-C(O)-, |
| NH-, | -C(O)-NH-$(CH_2)_v$-, |
| NH-, | (glycine)$_4$-serine |
| NH-, | absent |
| -C(O)-, | $C_{1-6}$ alkylene, |
| -C(O)-, | -NH-, |
| -C(O)-, | -C(O)-, |
| -C(O)-, | (-$CH_2$-$CH_2$-O)$_e$, |
| -C(O)-, | -NH-$CH_2$-$CH_2$-(-O-$CH_2$-$CH_2$)$_e$-C(O)-, |
| -C(O)-, | -C(O)-$(CH_2)_u$-C(O)-, |
| -C(O)-, | -C(O)-NH-$(CH_2)_v$-, |
| -C(O)-, | (glycine)$_4$-serine |
| (-$CH_2$-$CH_2$-O)$_e$, | absent |
| (-$CH_2$-$CH_2$-O)$_e$, | $C_{1-6}$ alkylene, |
| (-$CH_2$-$CH_2$-O)$_e$, | -NH-, |
| (-$CH_2$-$CH_2$-O)$_e$, | -C(O)-, |
| (-$CH_2$-$CH_2$-O)$_e$, | (-$CH_2$-$CH_2$-O)$_e$, |
| (-$CH_2$-$CH_2$-O)$_e$, | -NH-$CH_2$-$CH_2$-(-O-$CH_2$-$CH_2$)$_e$-C(O)-, |
| (-$CH_2$-$CH_2$-O)$_e$, | -C(O)-$(CH_2)_u$-C(O)-, |
| (-$CH_2$-$CH_2$-O)$_e$, | -C(O)-NH-$(CH_2)_v$-, |
| (-$CH_2$-$CH_2$-O)$_e$, | (glycine)$_4$-serine |
| -NH-$CH_2$-$CH_2$-(-O-$CH_2$-$CH_2$)$_e$-C(O)-, | absent |
| -NH-$CH_2$-$CH_2$-(-O-$CH_2$-$CH_2$)$_e$-C(O)-, | $C_{1-6}$ alkylene, |
| -NH-$CH_2$-$CH_2$-(-O-$CH_2$-$CH_2$)$_e$-C(O)-, | -NH-, |
| -NH-$CH_2$-$CH_2$-(-O-$CH_2$-$CH_2$)$_e$-C(O)-, | -C(O)-, |

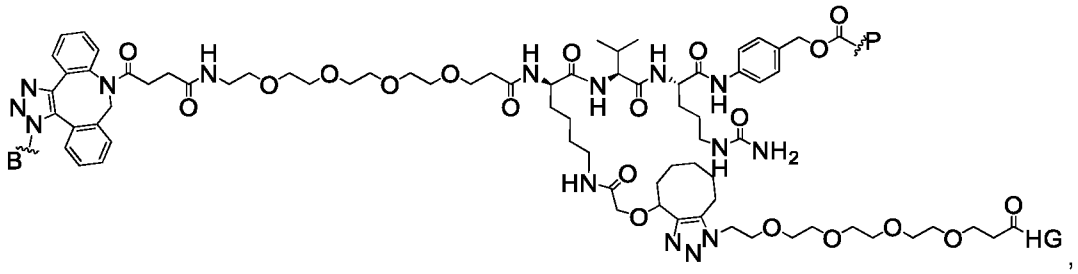
| $SP^1$ | $SP^2$ |
|---|---|
| -NH-$CH_2$-$CH_2$-(-O-$CH_2$-$CH_2$)$_e$-C(O)-, | (-$CH_2$-$CH_2$-O)$_e$, |
| -NH-$CH_2$-$CH_2$-(-O-$CH_2$-$CH_2$)$_e$-C(O)-, | -NH-$CH_2$-$CH_2$-(-O-$CH_2$-$CH_2$)$_e$-C(O)-, |
| -NH-$CH_2$-$CH_2$-(-O-$CH_2$-$CH_2$)$_e$-C(O)-, | -C(O)-($CH_2$)$_u$-C(O)-, |
| -NH-$CH_2$-$CH_2$-(-O-$CH_2$-$CH_2$)$_e$-C(O)-, | -C(O)-NH-($CH_2$)$_v$-, |
| -NH-$CH_2$-$CH_2$-(-O-$CH_2$-$CH_2$)$_e$-C(O)-, | (glycine)$_4$-serine |
| -C(O)-($CH_2$)$_u$-C(O)-, | absent |
| -C(O)-($CH_2$)$_u$-C(O)-, | $C_{1-6}$ alkylene, |
| -C(O)-($CH_2$)$_u$-C(O)-, | -NH-, |
| -C(O)-($CH_2$)$_u$-C(O)-, | -C(O)-, |
| -C(O)-($CH_2$)$_u$-C(O)-, | (-$CH_2$-$CH_2$-O)$_e$, |
| -C(O)-($CH_2$)$_u$-C(O)-, | -NH-$CH_2$-$CH_2$-(-O-$CH_2$-$CH_2$)$_e$-C(O)-, |
| -C(O)-($CH_2$)$_u$-C(O)-, | -C(O)-($CH_2$)$_u$-C(O)-, |
| -C(O)-($CH_2$)$_u$-C(O)-, | -C(O)-NH-($CH_2$)$_v$-, |
| -C(O)-($CH_2$)$_u$-C(O)-, | (glycine)$_4$-serine |
| -C(O)-NH-($CH_2$)$_v$-, | absent |
| -C(O)-NH-($CH_2$)$_v$-, | $C_{1-6}$ alkylene, |
| -C(O)-NH-($CH_2$)$_v$-, | -NH-, |
| -C(O)-NH-($CH_2$)$_v$-, | -C(O)-, |
| -C(O)-NH-($CH_2$)$_v$-, | (-$CH_2$-$CH_2$-O)$_e$, |
| -C(O)-NH-($CH_2$)$_v$-, | -NH-$CH_2$-$CH_2$-(-O-$CH_2$-$CH_2$)$_e$-C(O)-, |
| -C(O)-NH-($CH_2$)$_v$-, | -C(O)-($CH_2$)$_u$-C(O)-, |
| -C(O)-NH-($CH_2$)$_v$-, | -C(O)-NH-($CH_2$)$_v$-, |
| -C(O)-NH-($CH_2$)$_v$-, | (glycine)$_4$-serine |

**[0115]** Any combination of a row from Table R and a row from Table S may be present in a compound of Formula (I) described herein.

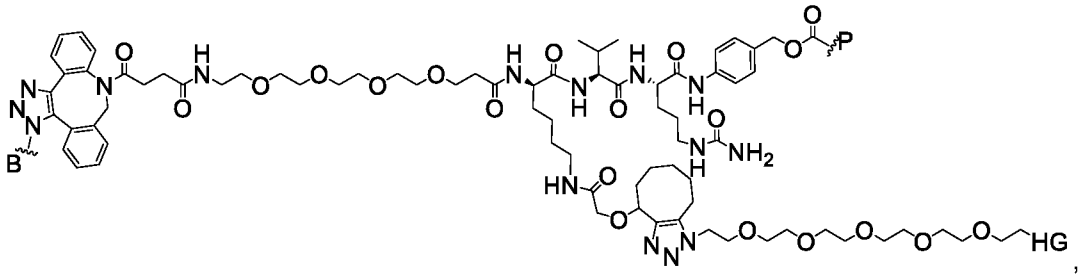
**[0116]** In some examples, for any compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**),



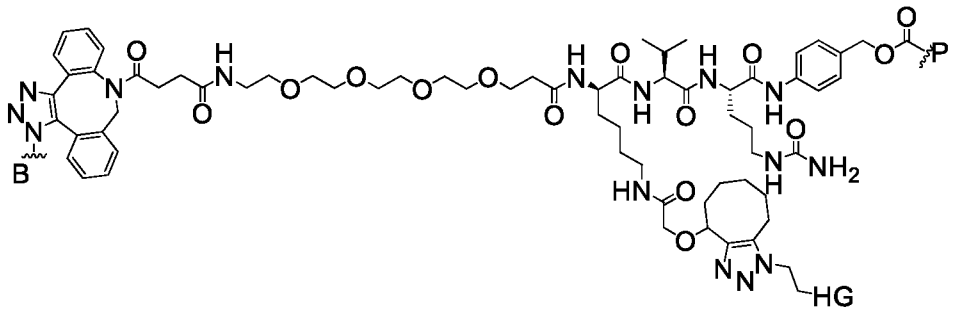

is selected from the group consisting of:



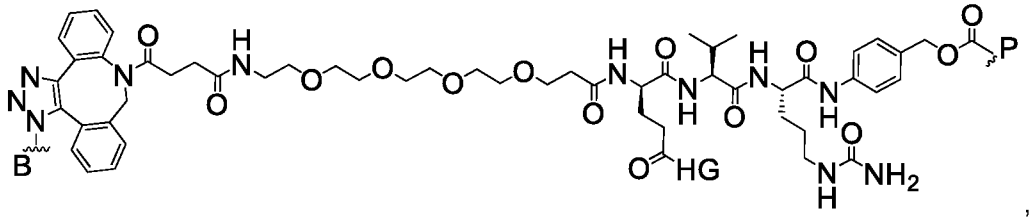

,



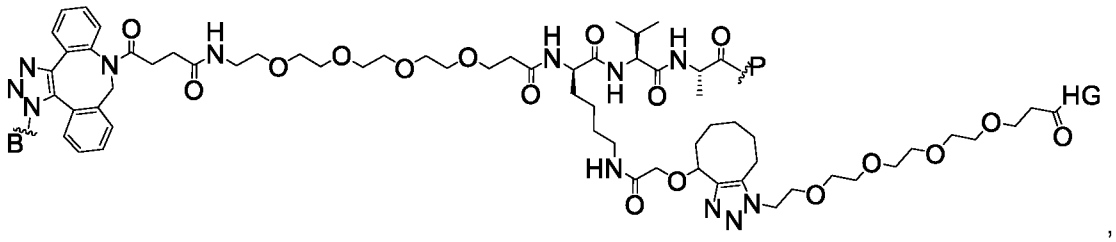

,



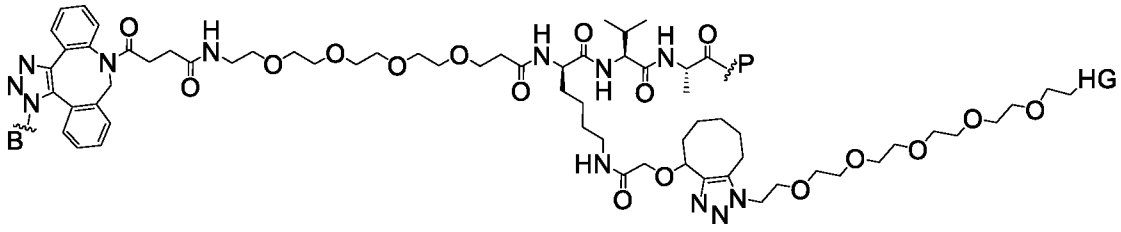

,



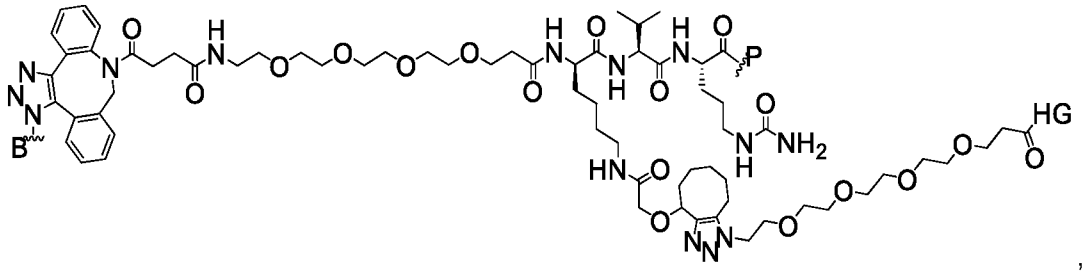

,



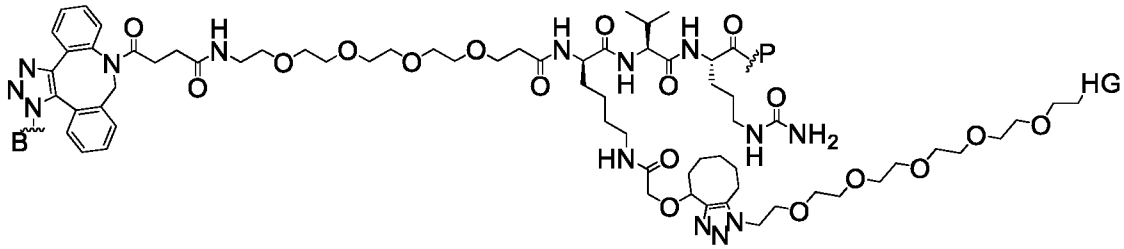
,



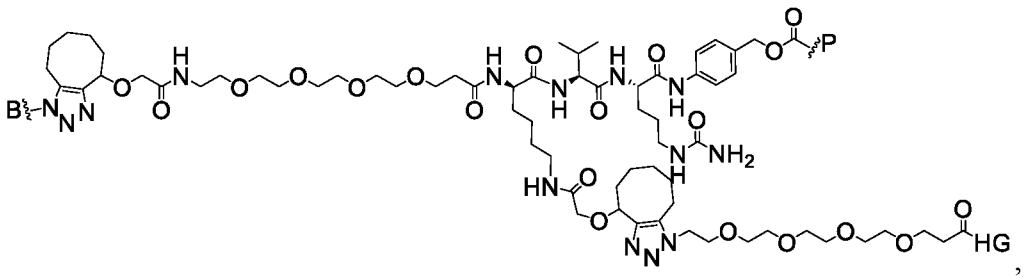
,



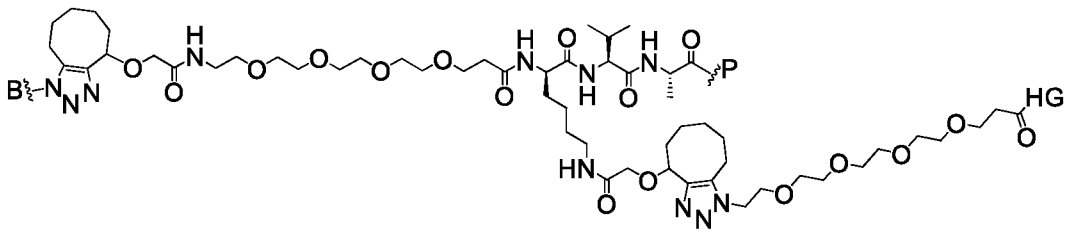
,



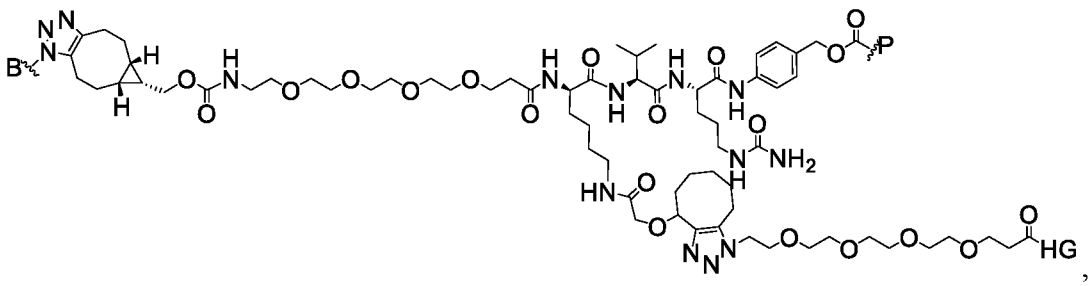
,



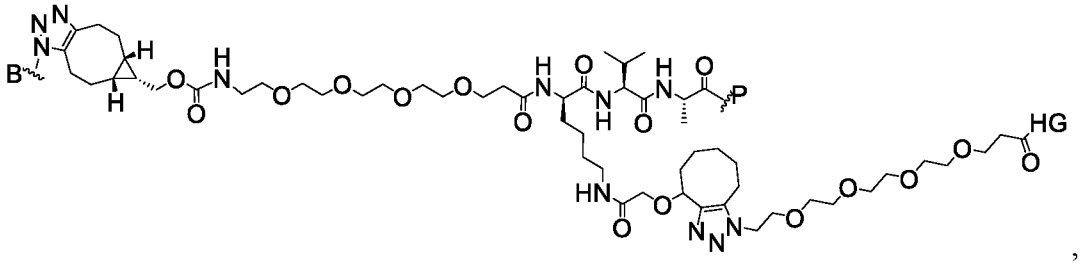
,



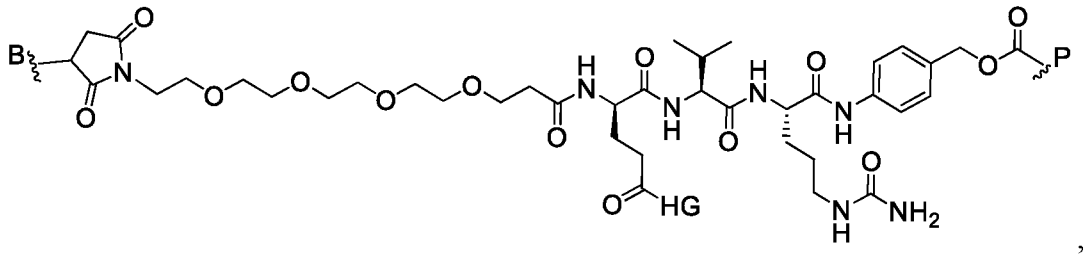
,



,

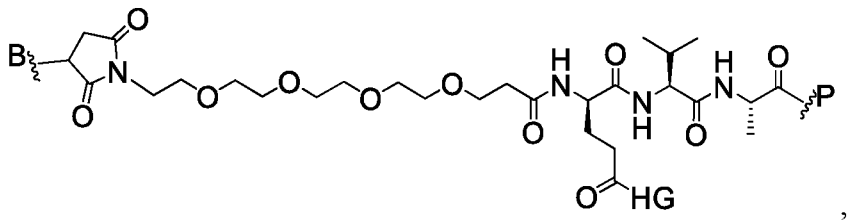


,



,

and



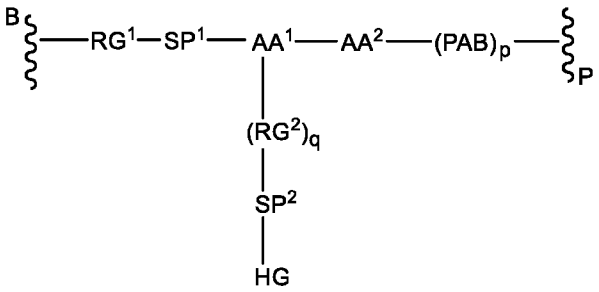
,

or a stereoisomeric form thereof, or a regioisomer thereof, or a mixture of regioisomers thereof, wherein

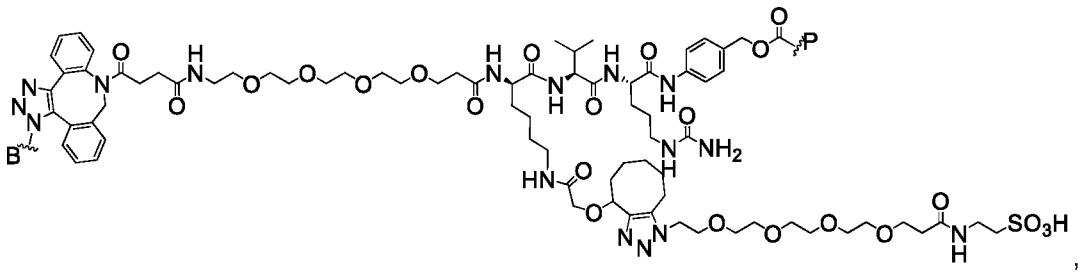
each  is a bond to the binding agent; and

each  is a bond to the payload residue.

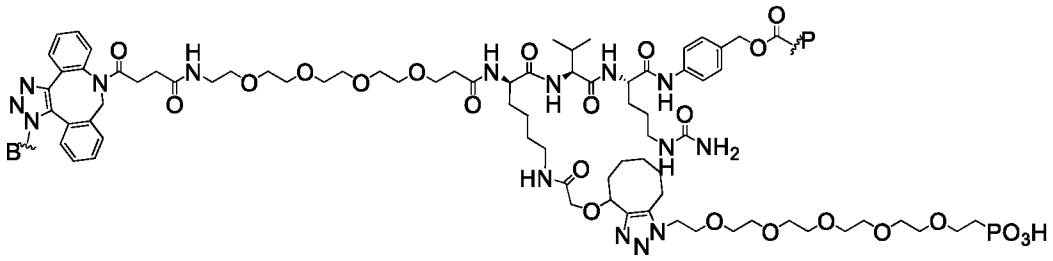
**[0117]** In some examples, for any compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**),

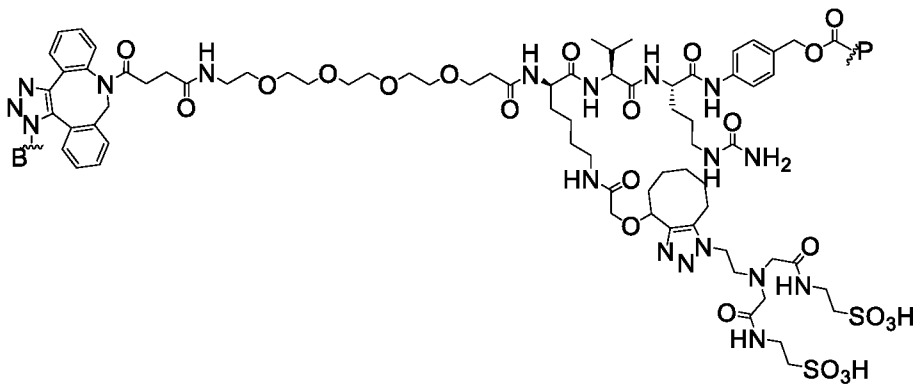


is selected from the group consisting of:

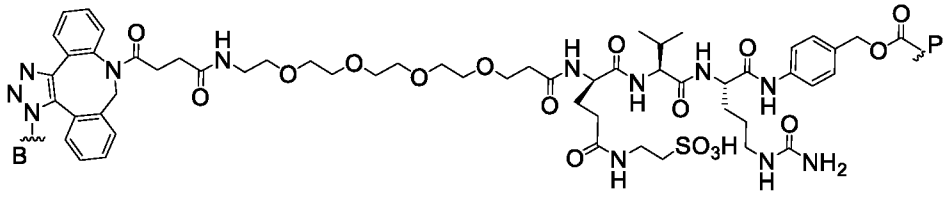


,

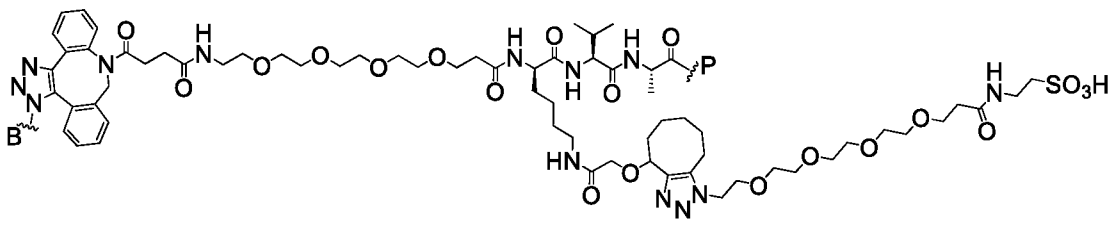




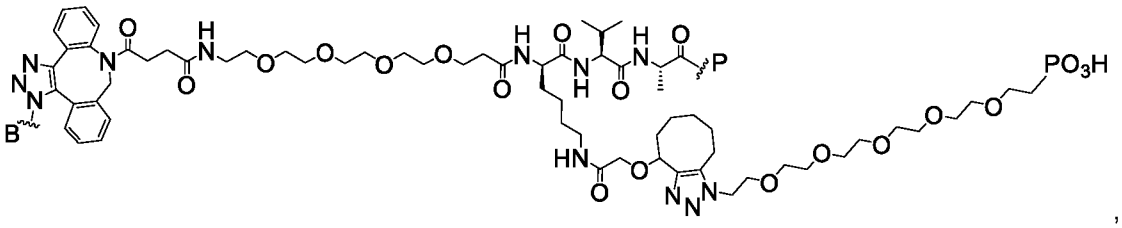
,



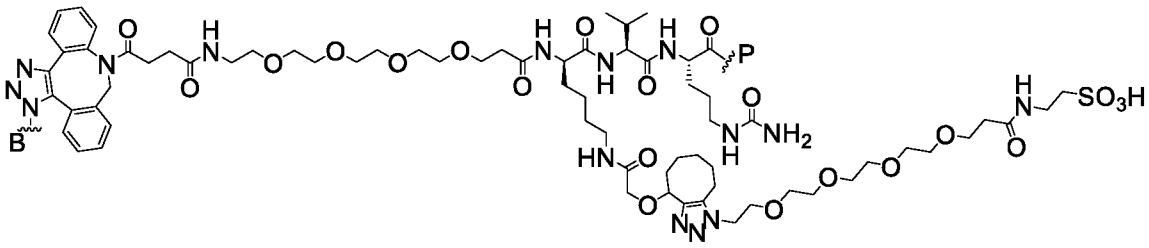
,



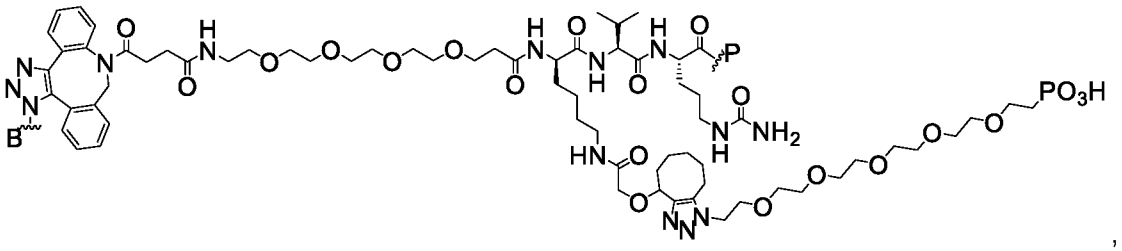
,



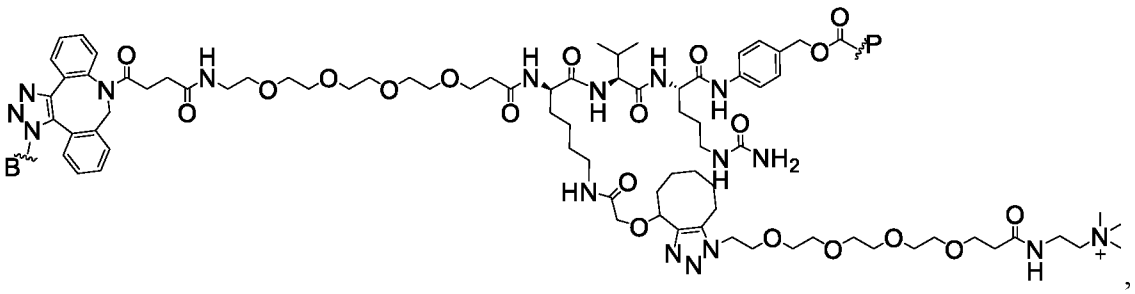
,



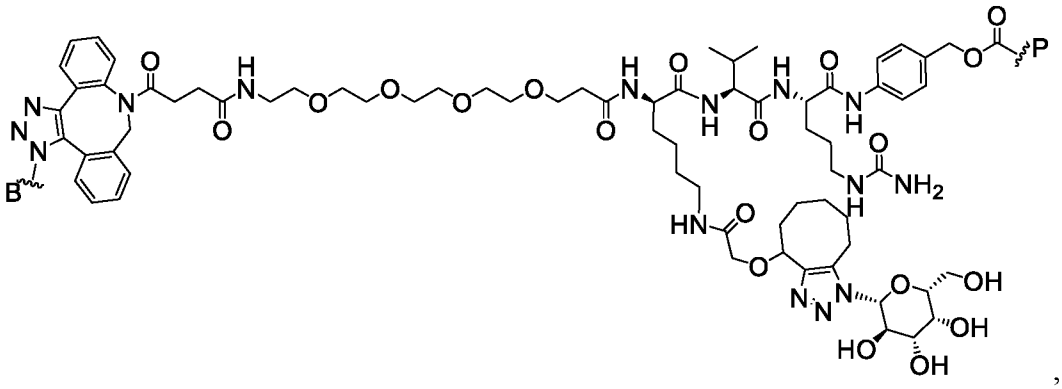
,



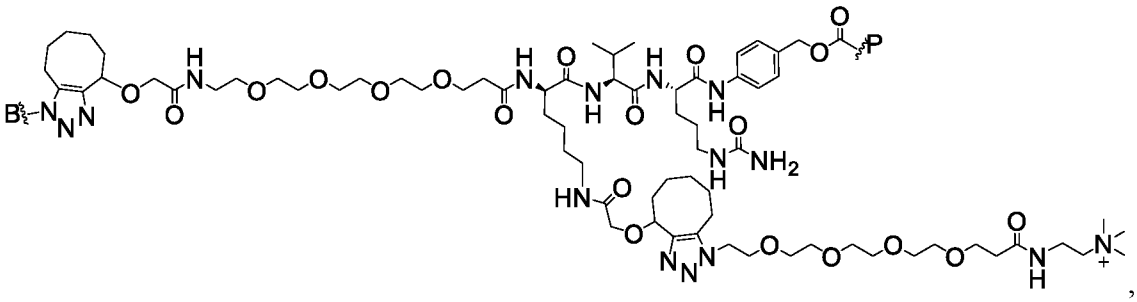
,



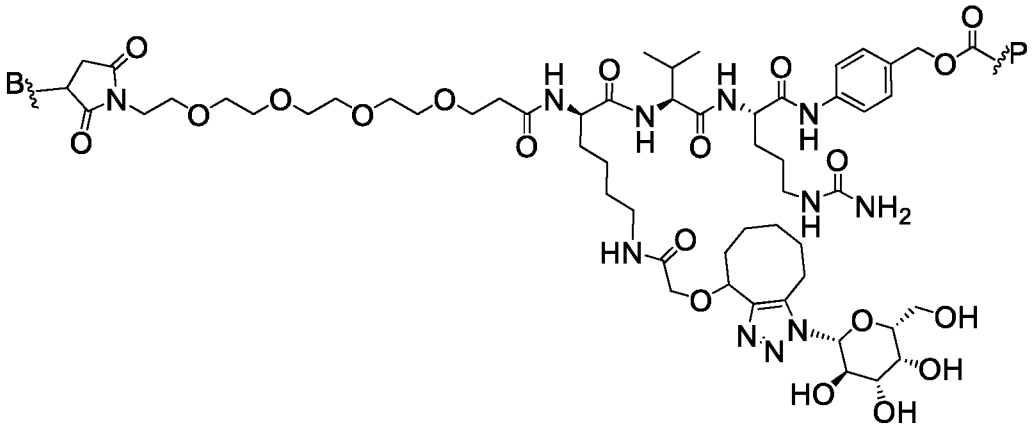
,



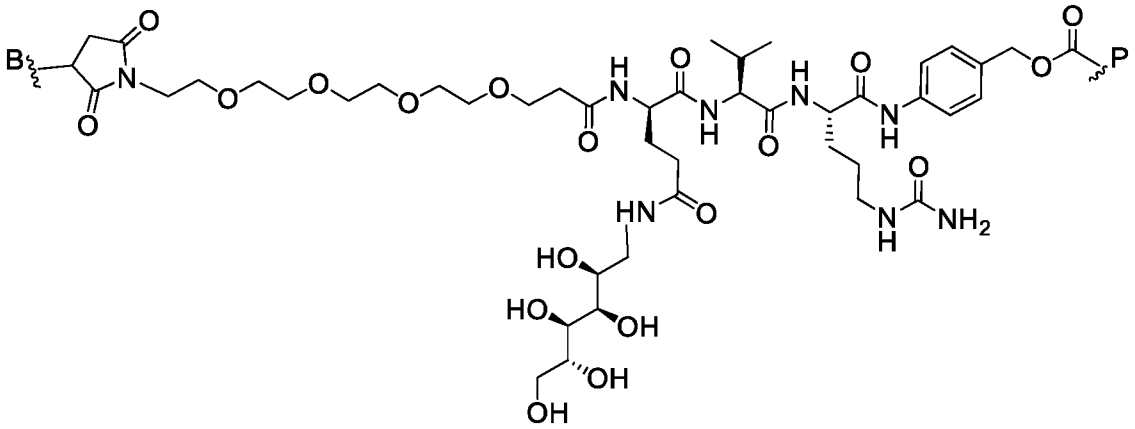
,



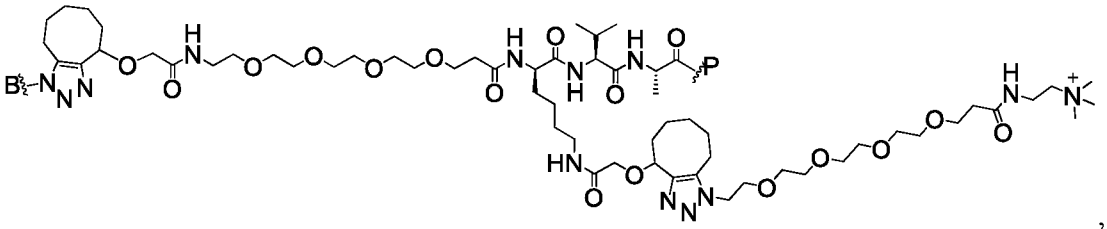
,



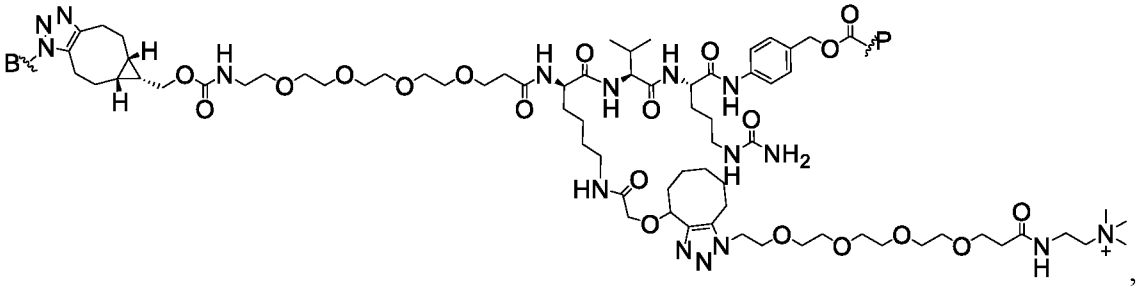
,



,

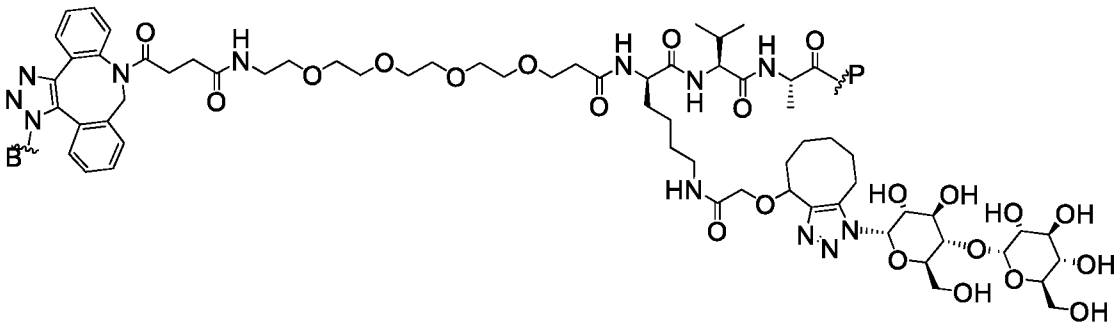


,



,

and



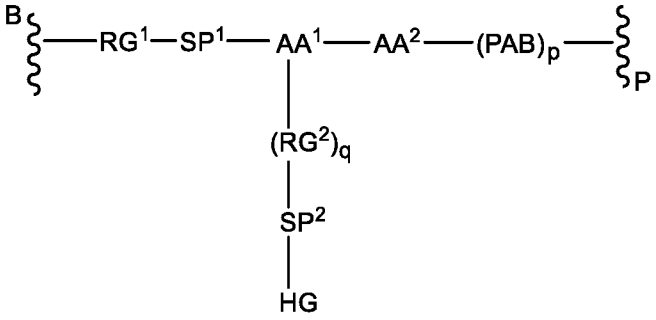
,

or a stereoisomeric form thereof, or a regioisomer thereof, or a mixture of regioisomers thereof, wherein

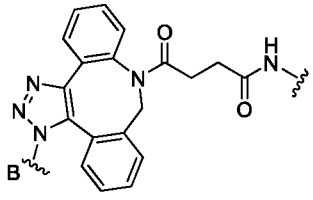
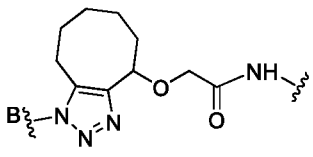
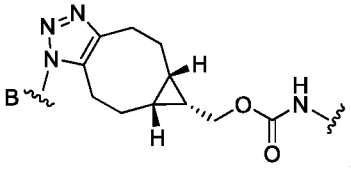
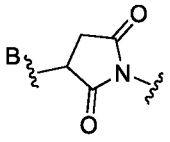
each  is a bond to the binding agent; and

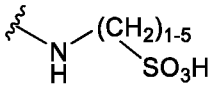
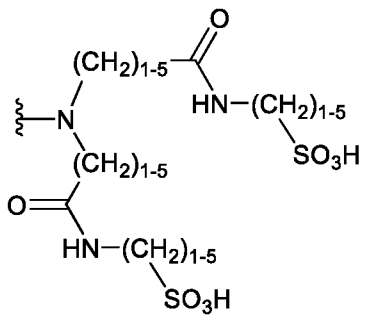
each  is a bond to the payload residue.

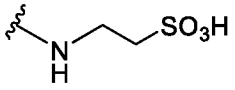
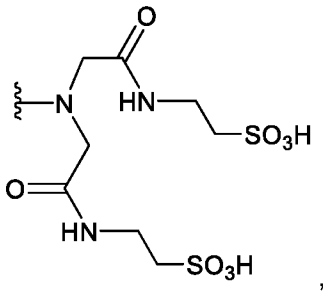
**[0118]** In some examples, for any compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), in the structure



, or a stereoisomeric form thereof, or a regioisomer thereof, or a mixture of regioisomers thereof,

**RG$^1$** is  ,  ,  , or  ,

**HG** is  , or  , or

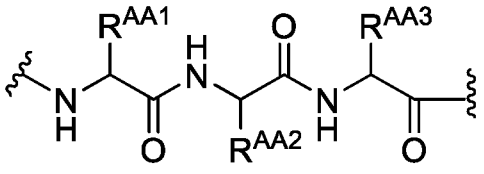
HG is  , or  ,

each  is a bond to the binding agent;

each  is a bond to the payload residue; and

each  indicates the atom through which the group is attached to the rest of the molecule.

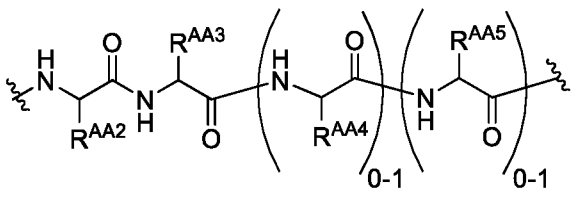
**[0119]** In some examples, for any compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), **LL** is according to Formula (**LL1**):


(**LL1**)

wherein $R^{AA1}$, $R^{AA2}$, and $R^{AA3}$ are each, independently, amino acid side chains, at least one of which is bonded directly or indirectly to **–(RG$^2$)$_q$-SP$^2$-HG**

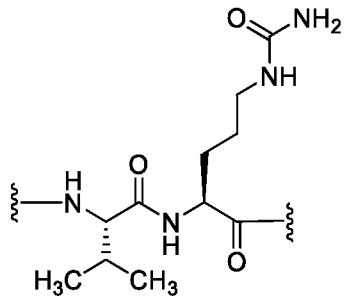
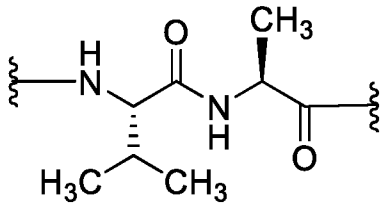
**[0120]** In some cases, for Formula **LL1**, $R^{AA1}$ is a lysine, glutamine, glutamic acid, or aspartic acid side chain bonded directly or indirectly to **HG**, and $R^{AA2}$ and $R^{AA3}$ are either valine and alanine or valine and citrulline sidechains respectively.

**[0121]** In some cases, for any compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), **AA$^2$** is

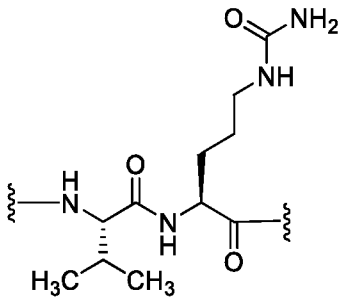


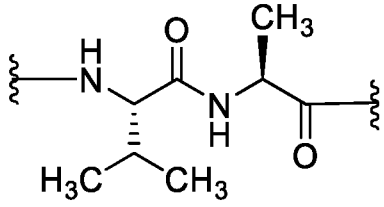
wherein $R^{AA2}$, $R^{AA3}$, $R^{AA4}$, and $R^{AA5}$ are each, independently, amino acid side chains, at least one of which is bonded directly or indirectly to **–(RG²)$_q$-SP²-HG**, wherein the  indicates the atom through which **AA²** is bonded to the adjacent groups in the formula. In some examples, $R^{AA2}$, $R^{AA3}$, $R^{AA4}$, and $R^{AA5}$, are independently in each instance, an amino acid side chain selected from the sidechains of alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, or citrulline, a derivative thereof, or a combination thereof.

**[0122]** In some cases, for any compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), **AA²** is


or

,

wherein the  indicates the atom through which **AA²** is bonded to the adjacent groups in the formula.

In one instance **AA²** is  . In another instance, **AA²** is


.

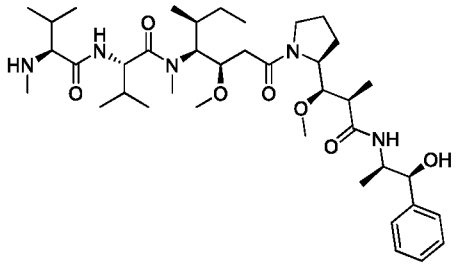
**[0123]** In some cases, for any compound of Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), subscript e is 4. In some cases, for any compound of Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), subscript e is 5.

**[0124]** In some embodiments, for any compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), the binding agent (**BA**) is an antibody or antigen-binding fragment thereof. In some instances, the antibody or antigen-binding fragment thereof binds a tumor-expressed antigen. In some instances, the antibody or antigen-binding fragment thereof binds a macrophage expressed antigen. In some instances, the binding agent (**BA**) is an antibody, or an antigen-binding fragment thereof, selective for an antigen selected from the group consisting of AXL, BAFFR, BCMA, BCR-list components, BDCA2, BDCA4, BTLA, BTNL2, BTNL3, BTNL8, BTNL9, C10 or f54, CCR1, CCR3, CCR4, CCR5, CCR6, CCR7, CCR9, CCR10, CD11c, CD137, CD138, CD14, CD168, CD177, CD19, CD20, CD209, CD209L, CD22, CD226, CD248, CD25, CD27, CD274, CD276, CD28, CD30, CD300A, CD33, CD37, CD38, CD4, CD40, CD44, CD45, CD46, CD48, CD5, CD52, CD55, CD56, CD59, CD62E, CD68, CD69, CD70, CD74, CD79a, CD79b, CD8, CD80, CD86, CD90.2, CD96, CLEC12A, CLEC12B, CLEC7A, CLEC9A, CR1, CR3, CRTAM, CSF1R, CTLA4, CXCR1/2, CXCR4, CXCR5, DDR1, DDR2, DEC-205, DLL4, DR6, FAP, EGFR, EGFRVIII, FCamR, FCMR, FcR's, Fire, GITR, HHLA2, HLA class II, HVEM, ICOSLG, IFNLR1, IL10R1, IL10R2, IL12R, IL13RA1, IL13RA2, IL15R, IL17RA, IL17RB, IL17RC, IL17RE, IL20R1, IL20R2, IL21R, IL22R1, IL22RA, IL23R, IL27R, IL29R, IL2Rg, IL31R, IL36R, IL3RA, IL4R, IL6R, IL5R, IL7R, IL9R, Integrins, LAG3, LIFR, MAG/Siglec-4, MET, MMR, MSR1, NCR3LG1, NKG2D, NKp30, NKp46, PDCD1, PRLR, PROKR1, PVR, PVRIG, PVRL2, PVRL3, RELT, SIGIRR, Siglec-1, Siglec-10, Siglec-5, Siglec-6, Siglec-7, Siglec-8, Siglec-9, SIRPA, SLAMF7, TACI, TCR-list components/assoc, PTCRA, TCRb, CD3z, CD3, TEK, TGFBR1, TGFBR2, TGFBR3, TIGIT, TLR2, TLR4, TROY, TSLPR, TYRO, VLDLR, VSIG4, and VTCN1.

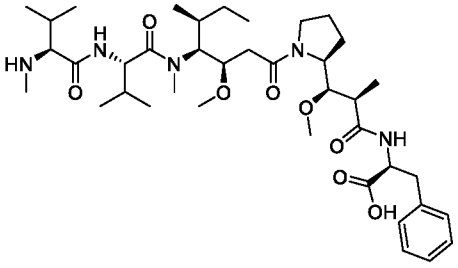
**[0125]** In some instances, for any compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), **PA** is the residue of a group selected from the group consisting of a dolastatin, an auristatin, a maytansinoid, a plant alkaloid, a taxane, a vinca alkaloid, a steroid, and a liver X receptor (LXR) modulator. In some cases, **PA** is a dolastatin. In some cases, **PA** isn auristatin. In some cases, **PA** is a maytansinoid. In some cases, **PA** is a plant alkaloid. In some cases, **PA** is a taxane. In some cases, **PA** is a vinca alkaloid. In some cases, **PA** is a steroid. In some cases, **PA** is a LXR modulator. In some cases, a LXR modulator is a LXR agonist. In some embodiments, a LXR modulator is a LXR antagonist. In some examples, **PA** is any compound set forth in FIG. 1. In some cases **PA** is a steroid, *e.g.*,

a glucocorticoid. Other suitable payloads include those that are highly hydrophobic, e.g., those that are not amenable to Ab conjugation conditions due to their hydrophilic nature, e.g., payloads such as pyrrolobenzodiazepines (PBDs), SN38 (7-Ethyl-10-hydroxy-camptothecin), etc. In certain embodiments, provided herein are antibody-drug conjugates according to any formula herein wherein **PA** is a hydrophobic payload moiety.

**[0126]** In some embodiments a payload in Formula (**I**) is a dolastatin or a synthetic analog thereof. In certain embodiments, the payloads in compounds of Formula (**I**) are auristatins that have the structure of monomethyl auristatin D (MMAD), (MMAE monomethyl auristatin E (MMAE), or monomethyl auristatin F (MMAF), or stereoisomers thereof.



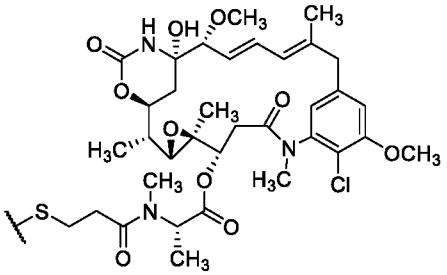
(MMAE)



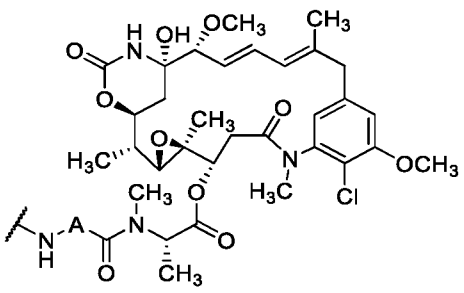
(MMAF)

**[0127]** In certain aspects, the compounds of Formula (**I**) described herein are protein-drug conjugates, *e.g.*, antibody-drug conjugates, comprising an antigen-binding protein, *e.g.*, antibody, an auristatin payload, and a hydrophilic moiety.

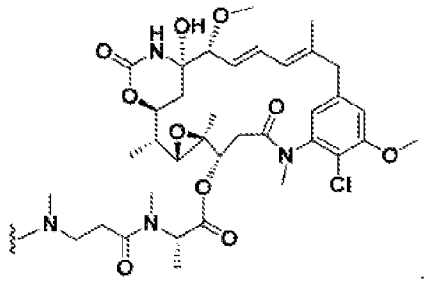
**[0128]** In certain embodiments, the payloads in compounds of Formula (**I**) are maytansinoids. Maytansinoid payloads disclosed in U.S. Non-Provisional Application No. 15/081,759 filed on March 25, 2016, titled "MAYTANSINOID DERIVATIVES, CONJUGATES THEREOF, AND METHODS OF USE," published as U.S. Patent Application Publication No. 2016/0375147, and in U.S. Non-Provisional Application No. 15/414,537 filed on January 24, 2017, titled "MAYTANSINOID DERIVATIVES, CONJUGATES THEREOF, AND METHODS OF USE," issued as U.S. Patent No. 9,950,076, are incorporated herein by reference. In certain embodiments, **PA** is DM1, DM3, or DM4. In certain embodiments, **PA** is



. In certain embodiments, **PA** is



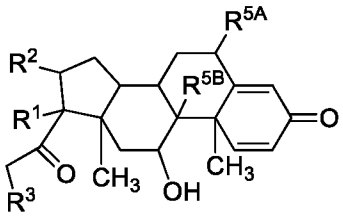
, where **A** is optionally substituted arylene or heteroarylene. In certain embodiments, **PA** is



. In particular embodiments, the wavy line indicates a bond to **LL**.

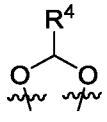
**[0129]** In certain aspects, the compounds of Formula (**I**) described herein are protein-drug conjugates, *e.g.*, antibody-drug conjugates, comprising an antigen-binding protein, *e.g.*, antibody, a maytansinoid payload, and a hydrophilic moiety.

**[0130]** In certain embodiments, the payloads in compounds of Formula (**I**) are glucocorticoids according to Formula (**A**):



(**A**)

wherein

$R^1$ and $R^2$ are, independently, –H, alkyl, alkyl-C(O)-O–, –OH, or halo; or $R^1$ and $R^2$ together form  ,

wherein $R^4$ is alkyl, aryl, arylalkyl, or an N-containing heterocycloalkyl,

wherein the alkyl, aryl, arylalkyl, and N-containing heterocycloalkyl are, independently in each instance, optionally substituted with -$NR^{Aa}R^{Ab}$;

$R^3$ is –OH, $R^Z$-C(O)-**X**–, heteroalkyl, piperidinyl, $–NR^{Aa}R^{Ab}$, -oxyaryl–$NR^{Aa}R^{Ab}$ or -**Z**-**A'**$(R^P)_t$;

$R^Z$ is alkyl;

**X** is O or $NR^{Aa}$;

**Z** is S, S(O), $S(O)_2$, $SO_2NR^{Aa}$, O, $C(O)NR^{Aa}$, C(O), or $NR^{Aa}$;

**A'** is aryl, arylalkyl, or heteroaryl;

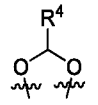
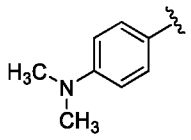
$R^P$ is, independently in each instance, halo, optionally substituted alkyl, –OH, or $-NR^{Aa}R^{Ab}$;

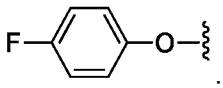
$R^{Aa}$ and $R^{Ab}$ are, independently in each instance, –H, optionally substituted alkyl, or optionally subtitued aryl;

subscript **a** is an integer from 0-19; and

**t** is an integer from 1-3;

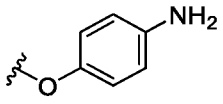
with the proviso that:

(1) $R^3$ is not –OH (a) when $R^1$ is –OH or (b) when $R^1$ and $R^2$ together form  , wherein $R^4$ is $C_{1\text{-}9}$alkyl or  and

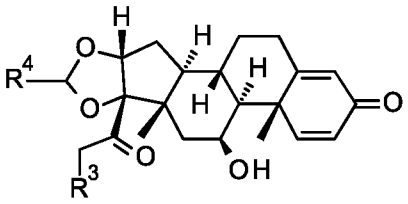
(2) $R^3$ is not  .

and

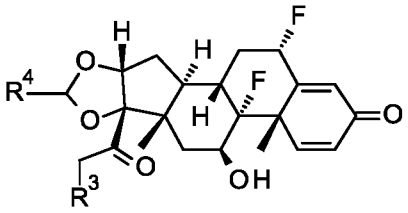
$R^{5A}$ and $R^{5B}$ are each, independently, halo or a hydrogen atom.

**[0131]** In some of such embodiments, $R^3$ is $NH_2$. In some of such embodiments, $R^3$ is  wherein  indicates the atom through which $R^3$ is attached to the adjacent groups in Formula (**I**).

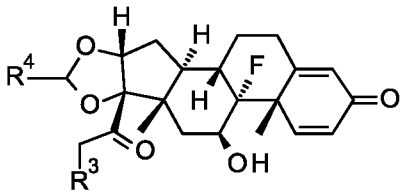
**[0132]** In certain embodiments, **PA** is selected from



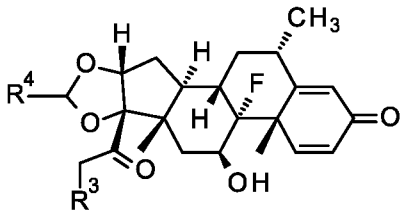
1110



1120

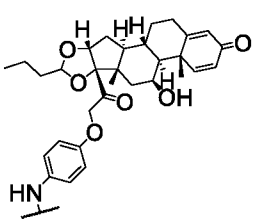


1130

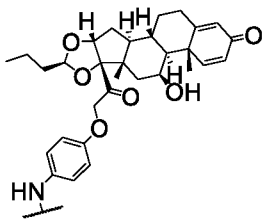


1140

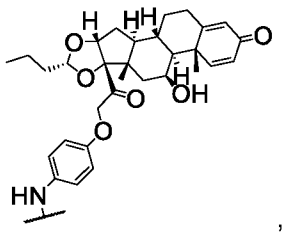
or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof. In certain embodiments according to any of Formulas 1110-1140, $R^3$ is -O-aryl, -$NR^{Aa}R^{Ab}$, -alkylene-$NR^{Aa}R^{Ab}$, -X-arylene-Y-$NR^{Aa}R^{Ab}$, -X-heteroarylene-Y-$NR^{Aa}R^{Ab}$, or N-containing heterocycloalkyl; wherein X is absent, -N-, -$CH_2$-, or -O-; wherein Y is absent or -$CH_2$-; and $R^4$ is alkyl, aryl, alkylaryl, or arylalkyl. In certain embodiments, $R^3$ is -O-arylene-$NR^{Aa}R^{Ab}$, -O-heteroarylene-$NR^{Aa}R^{Ab}$; wherein aryl or heteroaryl is optionally substituted with halogen, deuterium, hydroxyl, or methoxyl. In certain embodiments, $R^3$ is -O-phenyl-$NR^{Aa}R^{Ab}$, -O-heteroarylene-$NR^{Aa}R^{Ab}$; wherein phenyl or heteroaryl is optionally substituted with halogen or deuterium. In certain embodiments, $R^4$ is n-propyl. In certain embodiments, $R^{Aa}$ and $R^{Ab}$ are each independently hydrogen or alkyl. In particular embodiments, one of $R^{Aa}$ and $R^{Ab}$ is substituted with a bond to **LL**. In certain embodiments, **PA** is



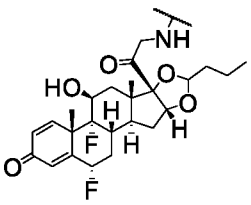
,



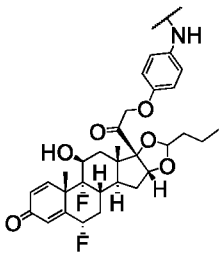
,



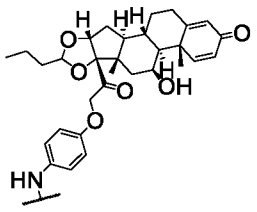
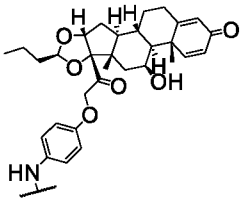
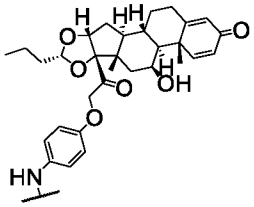
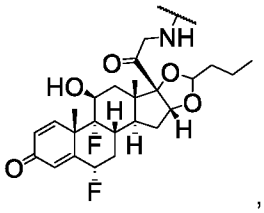
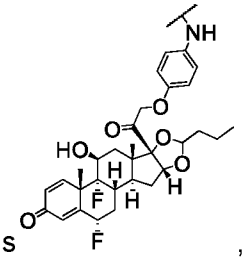
,



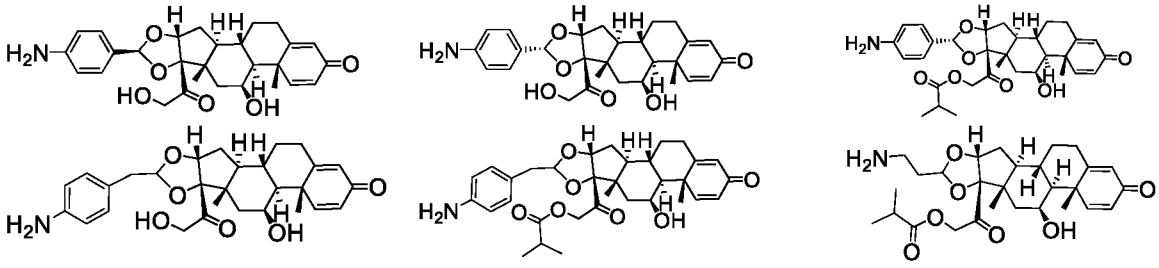
, or

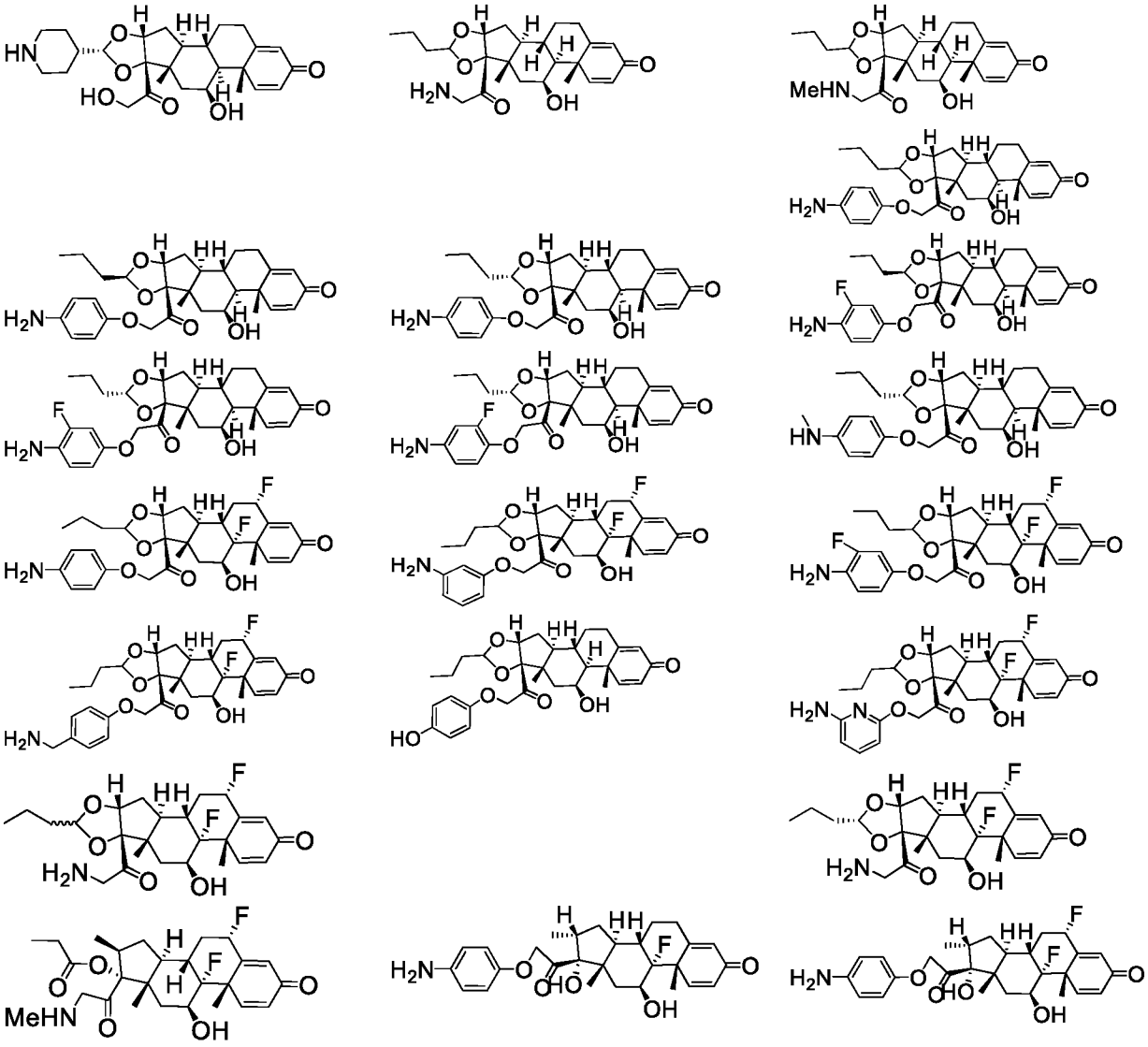


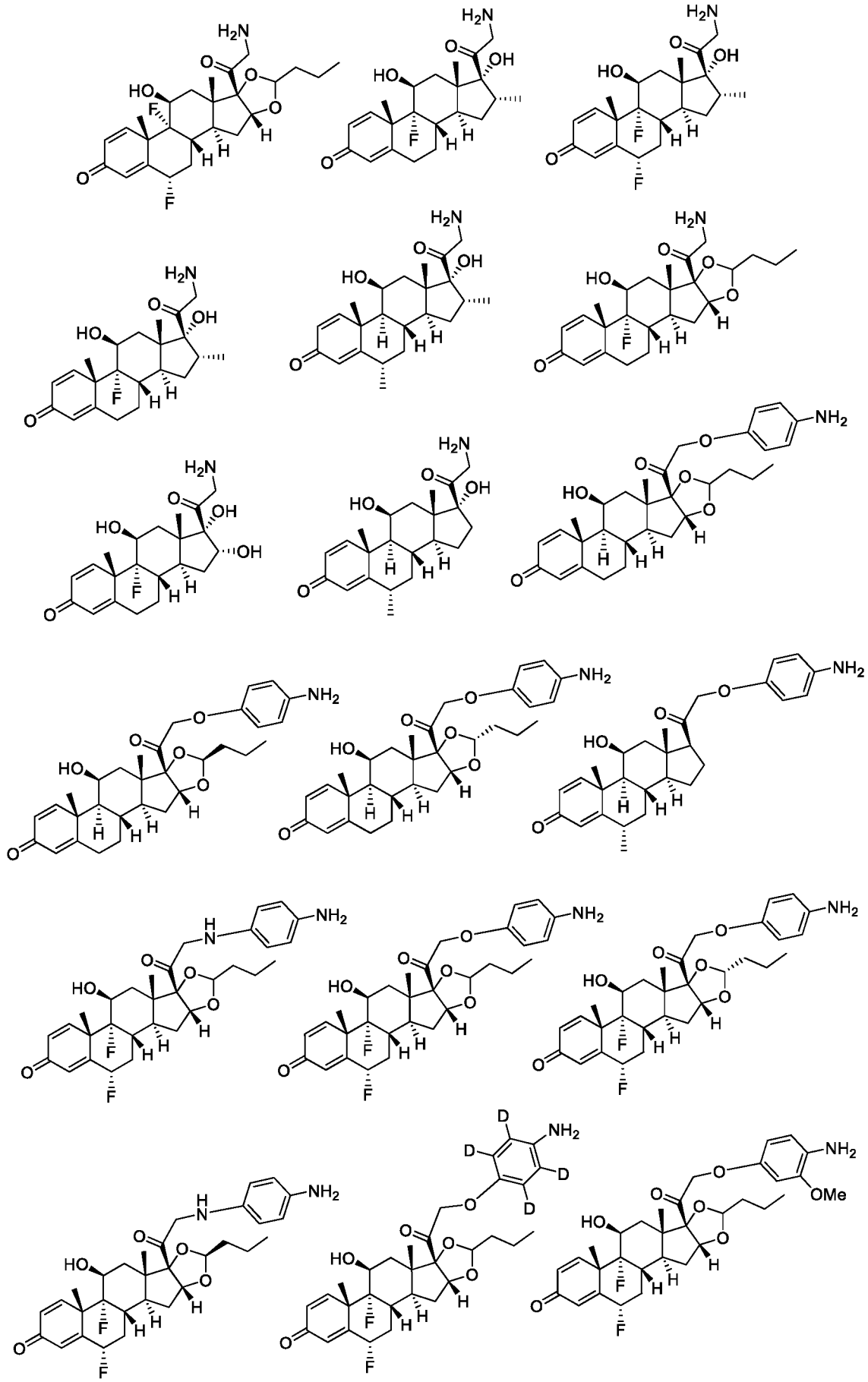
,

or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof. In certain embodiments, **PA** is  , or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof In certain embodiments, **PA** is  , or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof. In certain embodiments, **PA** is  , or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof In certain embodiments, **PA** is  , or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof. In certain embodiments, **PA** is  , or a pharmaceutically acceptable salt, solvate, or stereoisomer thereof. In such embodiments, the wavy line indicates a bond to **LL**.

**[0133]** In some embodiments, **PA** is selected from the group consisting of:





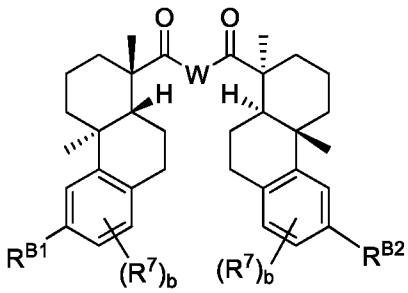


or a pharmaceutically acceptable stereoisomer thereof, wherein the **PA** is linked as a residue through the $R^3$ group, e.g., the conjugates comprise a payload described above linked to a linker via a bond to a residue of a primary or secondary amine of the payload.

**[0134]** In certain aspects, the compounds of Formula (**I**) described herein are protein-drug conjugates, *e.g.*, antibody-drug conjugates, comprising an antigen-binding protein, *e.g.*, antibody, a payload of Formula (**A**), and a hydrophilic moiety.

**[0135]** Steroid payloads disclosed in U.S. Provisional Application No. 62/614,905 filed on January 8, 2018, titled "STEROIDS AND ANTIBODY CONJUGATES THEREOF," and in U.S. Non-Provisional Application No. 15/806,197 filed on November 7, 2017, titled "STEROIDS AND PROTEIN-CONJUGATES THEREOF" are incorporated herein by reference.

**[0136]** In certain embodiments, the payloads in compounds of Formula (**I**) are LXR modulators that have the structure according to Formula (**B**):




Formula (**B**)

or a pharmaceutically acceptable stereoisomeric form thereof, wherein

**W** is $–CH_2–$, –N(H)–, or –O–;

$R^{B1}$ is –H, –OH, $–NH_2$, alkyl, or $–OP(O)(OR^6)(OH)–OP(O)(OR^6)_2$;

$R^{B2}$ is –H, –OH, $–CH_2NH_2$, $R^{B3}$, $R^{B4}$, $R^{B5}$, or $–O–R^{B5}$, wherein $R^{B1}$ and $R^{B2}$ are not simultaneously –H;

$R^{B3}$ is $–N(R^6)_2$;

$R^{B4}$ is **–X–Y–Z**;

**X** is selected from the group consisting of –O– and –N(H)–;

**Y** is selected from the group consisting of alkylene, substituted alkylene (including, without limitation, oxo substitution, *i.e.*, =O), heteroalkylene, and substituted heteroalkylene (including, without limitation, oxo substitution (*i.e.*, =O));

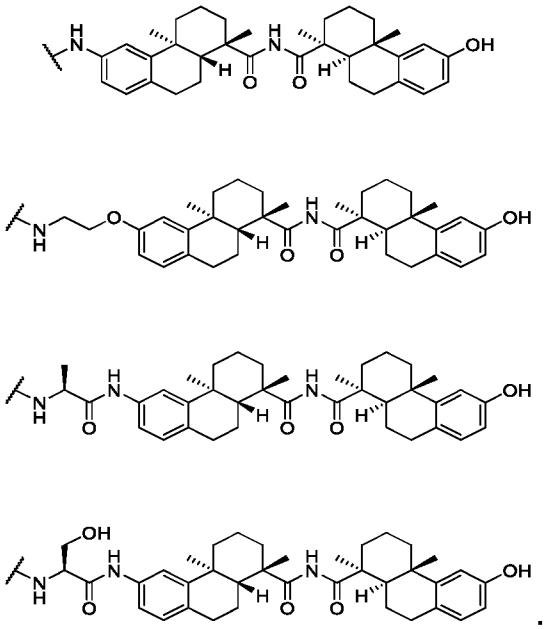
**Z** is selected from the group consisting of –OH and $–NH_2$;

$R^{B5}$ is alkyl, heterocycloalkyl, or substituted heterocycloalkyl, wherein each heterocycloalkyl or substituted heterocycloalkyl includes one, two, or three heteroatoms selected from nitrogen and oxygen, and includes at least one –OH and –$CH_2OH$ substituent, or at least one primary or secondary nitrogen, for instance, O-glucose;

each $R^6$ is in each instance, –H, an amino acid residue, an *N*-alkyl amino acid residue, a peptide, or alkyl; and

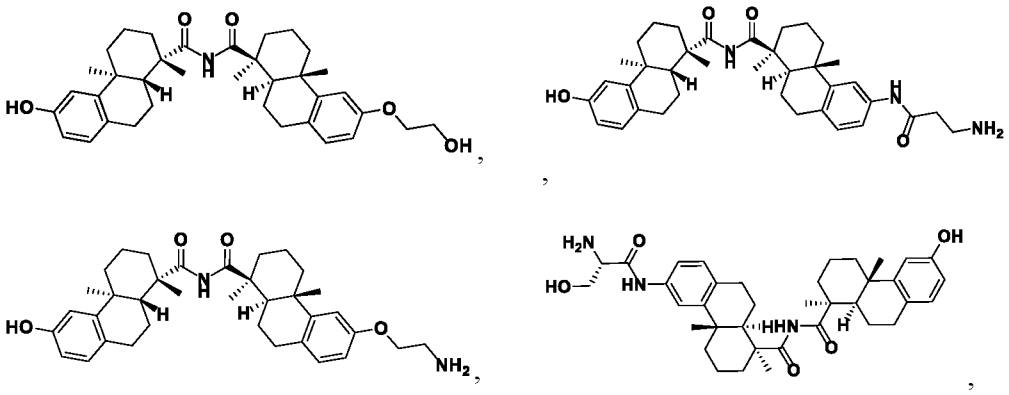
each $R^7$ is, independently, halo, $C_{1-6}$ alkyl, $C_{1-6}$ alkoxy, –CN, O-glucose, O-amino acid residue, and O-$PEG_b$, wherein each subscript **b** is an integer from 0-3.

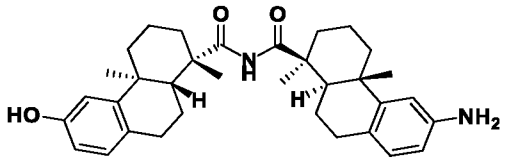
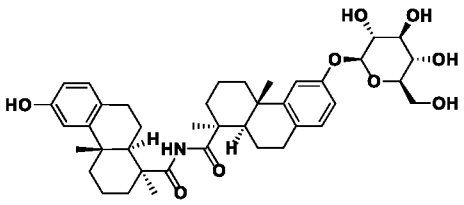
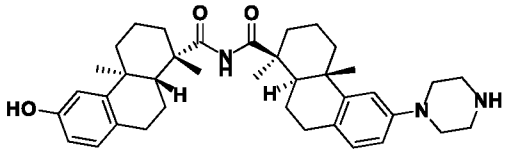
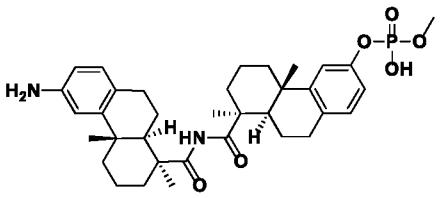
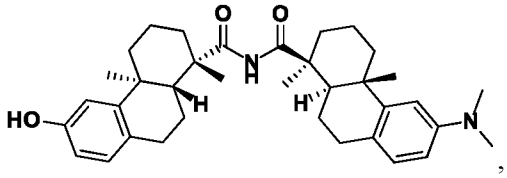
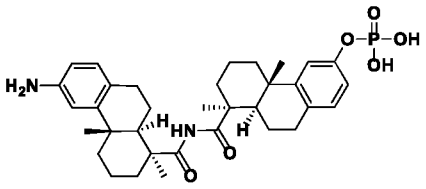
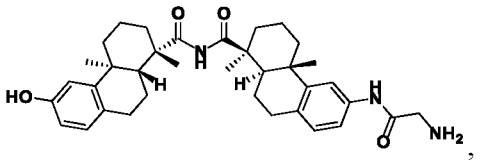
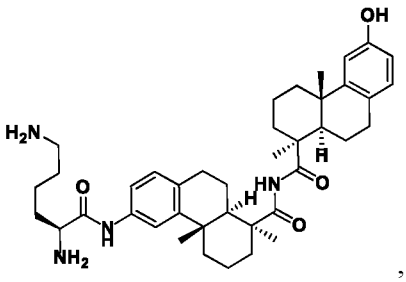
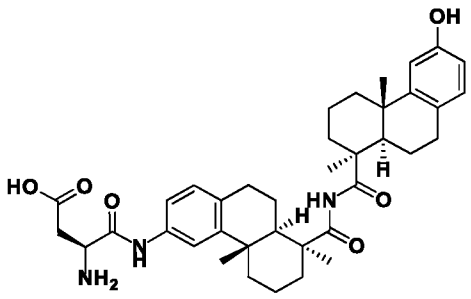
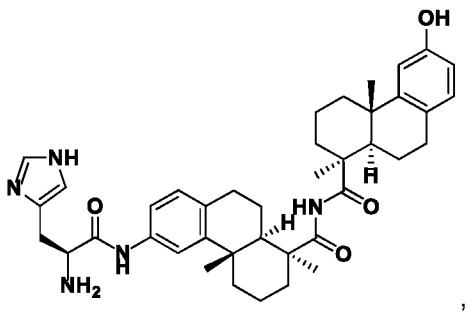
**[0137]** In particular embodiments, $R^{B1}$ or $R^{B2}$ is substituted with a bond to **LL**. In certain embodiments, **PA** is selected from:



**[0138]** In particular embodiments, the wavy line indicates a bond to **LL**.

**[0139]** In certain embodiments, **PA** is selected from the group consisting of:




,

,

,

,

,

,

,

,

, and

,

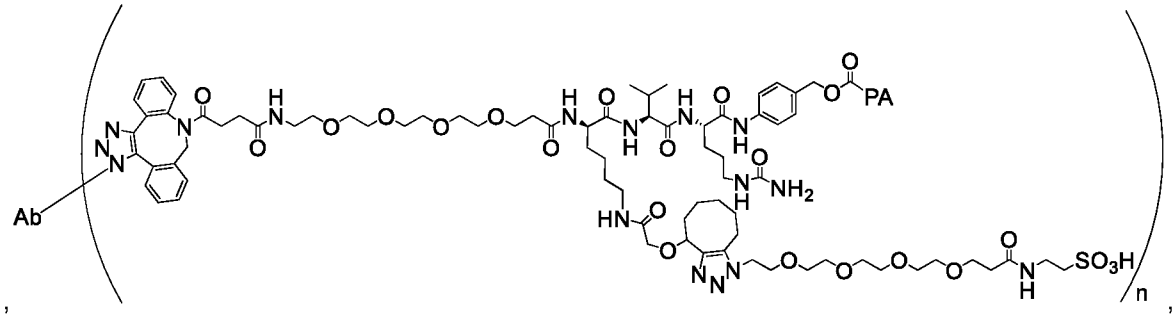
or a pharmaceutically acceptable stereoisomeric form thereof, wherein the **PA** is linked as a residue through the $R^{B1}$ or $R^{B2}$ group, e.g., the conjugates comprise a payload described above linked to a linker via a bond to a residue of a primary or secondary amine of the payload.

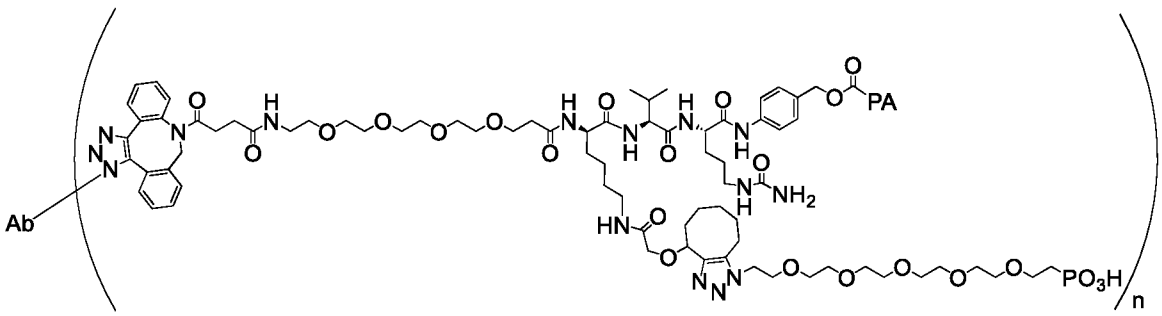
**[0140]** In certain aspects, the compounds of Formula (**I**) described herein are protein-drug conjugates, *e.g.*, antibody-drug conjugates, comprising an antigen-binding protein, *e.g.*, antibody, a payload of Formula (**B**), and a hydrophilic moiety.

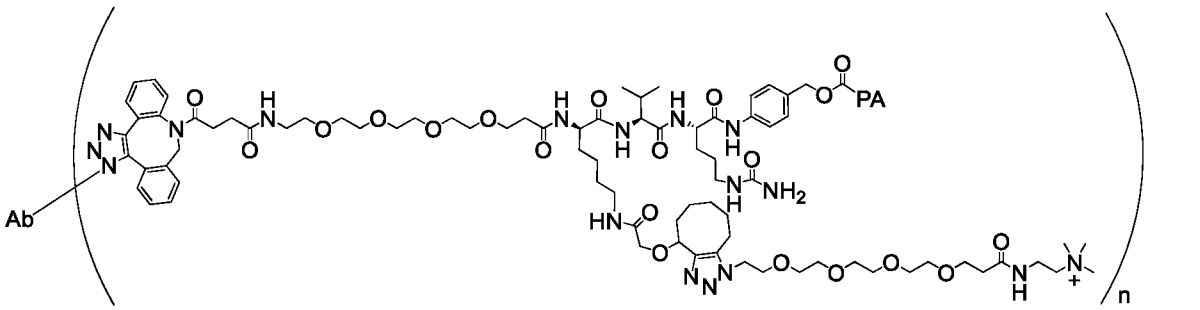
**[0141]** LXR modulator payloads disclosed in U.S. Provisional Application No. 62/508,327 filed on May 18, 2017, titled "BIS-OCTAHYDROPHENANTHRENE CARBOXAMIDES AND PROTEIN CONJUGATES THEREOF" are incorporated herein by reference.

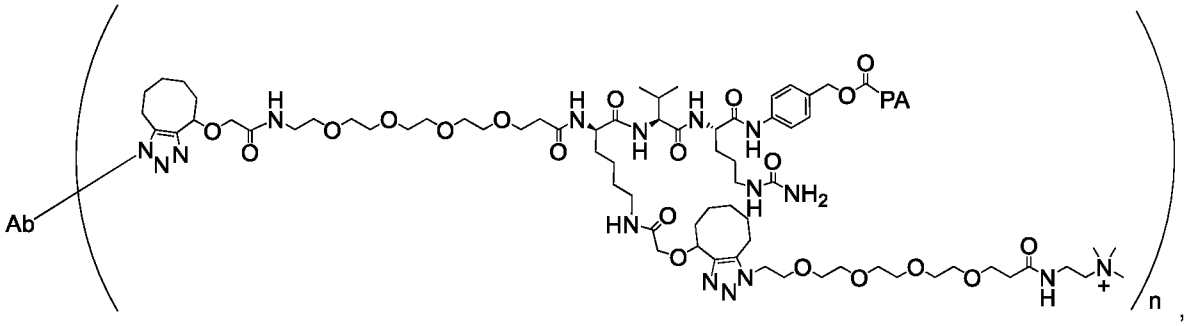
**[0142]** Also contemplated within the scope of embodiments presented herein are payloads disclosed in U.S. Non-Provisional Application No. 14/776,668 filed on September 14, 2015, titled "BIOLOGICALLY ACTIVE MOLECULES, CONJUGATES THEREOF, AND THERAPEUTIC USES," published as U.S. Patent Application Publication No. 2016/0030591, and U.S. Non-Provisional Application No. 14/913,965 filed on February 23, 2016, titled "PHARMACEUTICAL COMPOSITIONS COMPRISING MACROLIDE DIASTEREOMERS, METHODS OF THEIR SYNTHESIS AND THERAPEUTIC USES," published as U.S. Patent Application Publication No. 2016/0354482, the disclosure of said payloads is incorporated herein by reference.

**[0143]** In some instances, a compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), is an antibody drug conjugate (ADC) wherein **BA** is Ab, and Ab is an antibody or antigen-binding fragment thereof. In some instances, a compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), is a conjugate selected from the group consisting of:

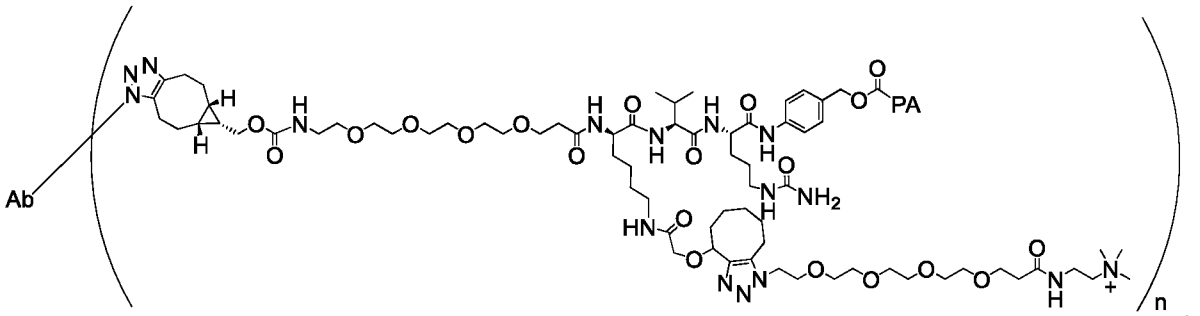


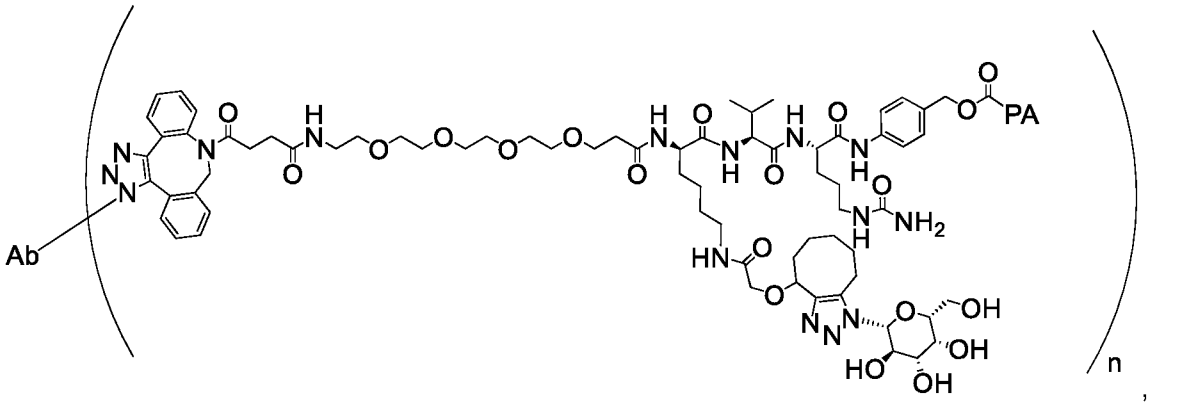

Ab
PA
NH2
HN
n


Ab
PA
NH2
HN
n

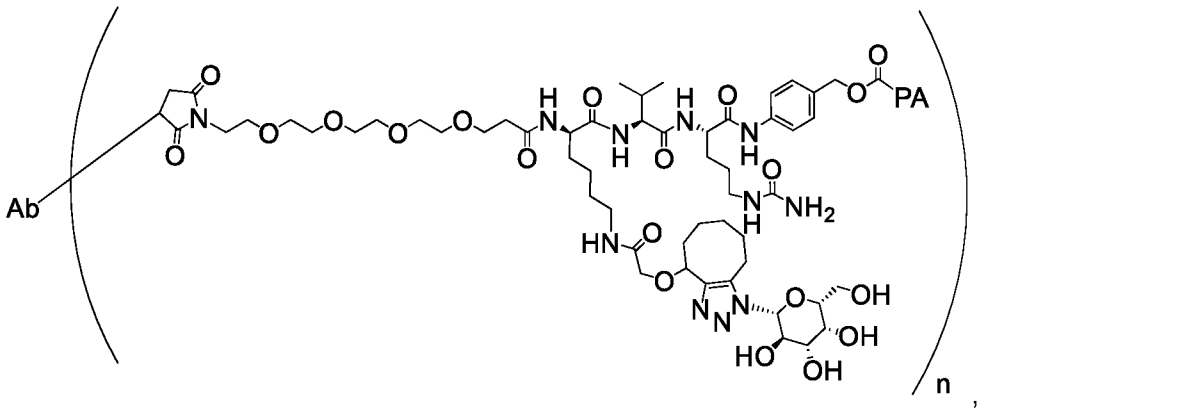
,


Ab
PA
NH2
HN
n

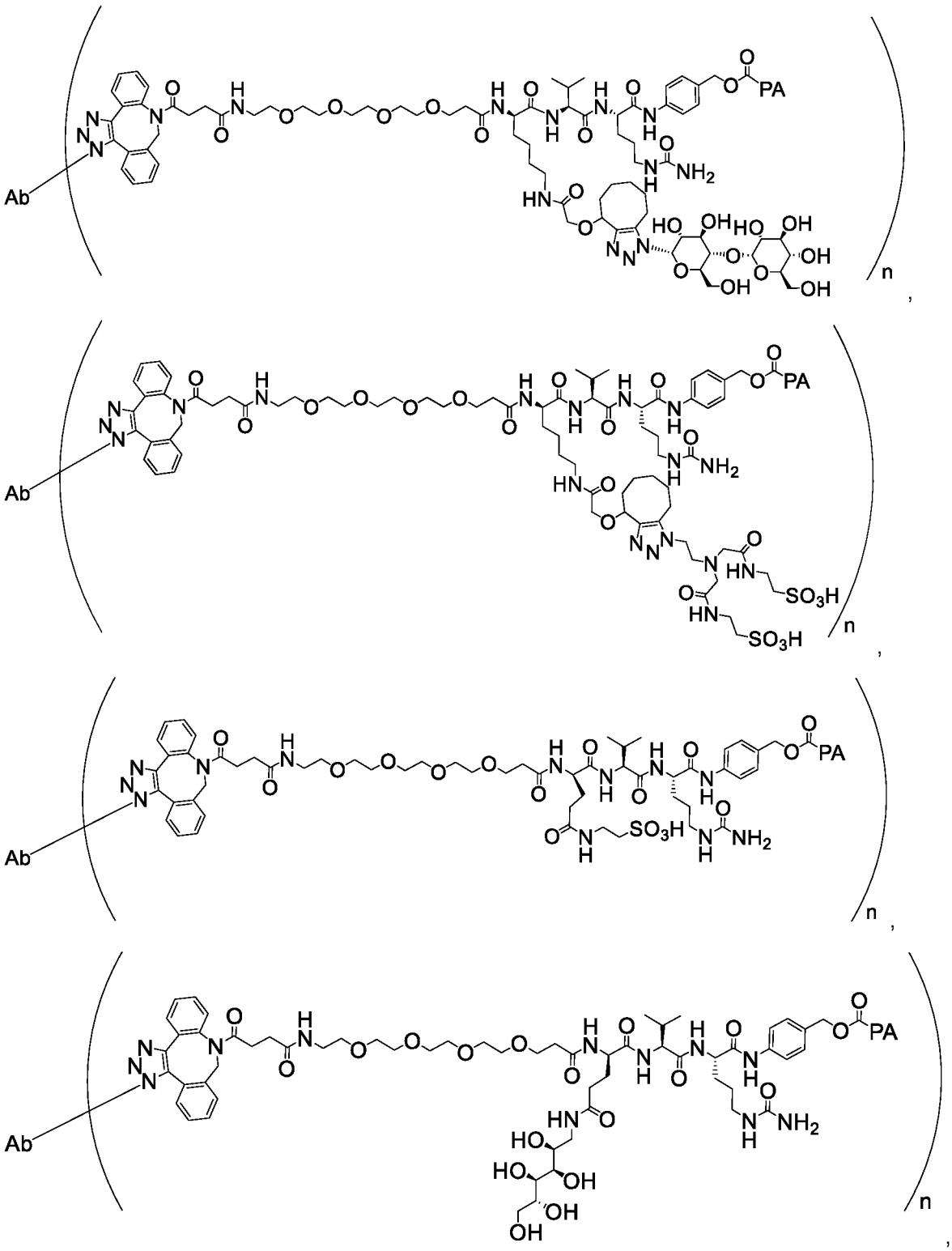
,

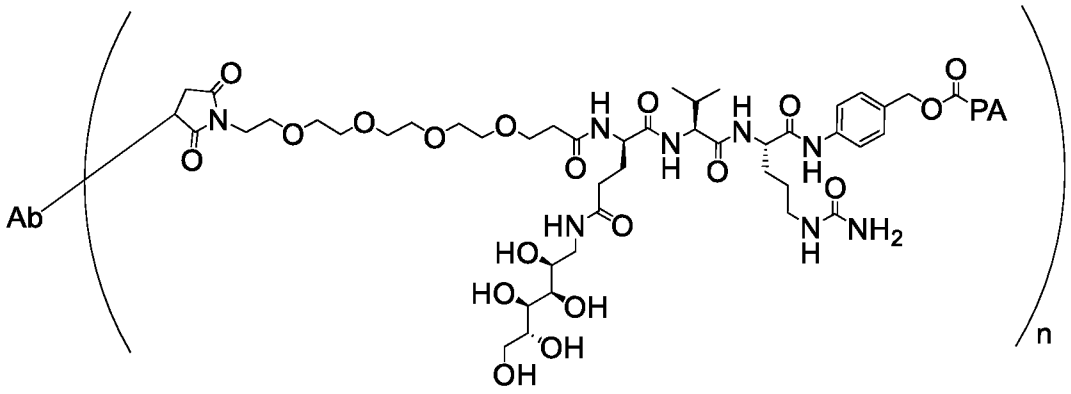

Ab
PA
NH2
HN
OH
HO
n

,

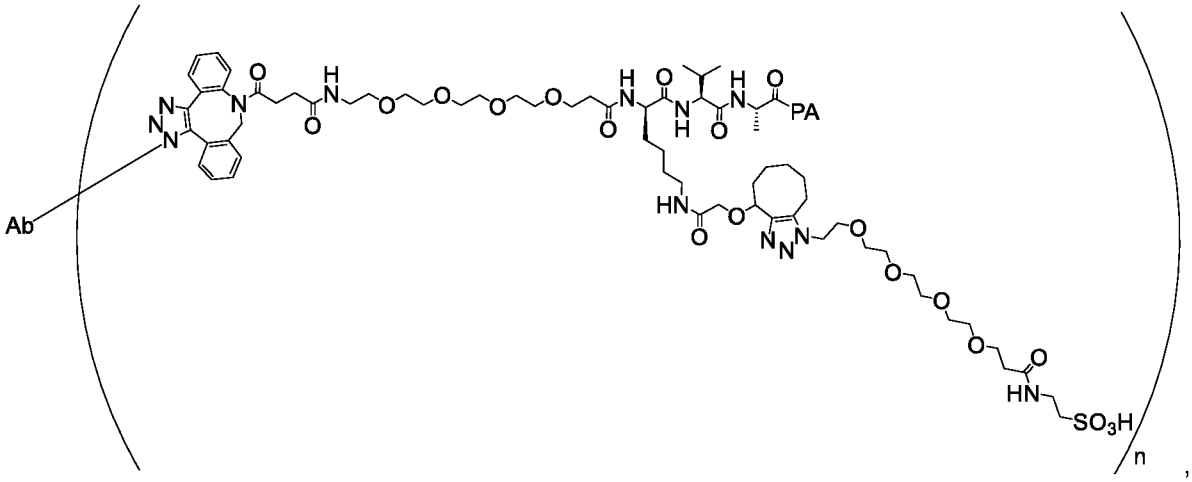

Ab
PA
NH2
HN
OH
HO
n

,

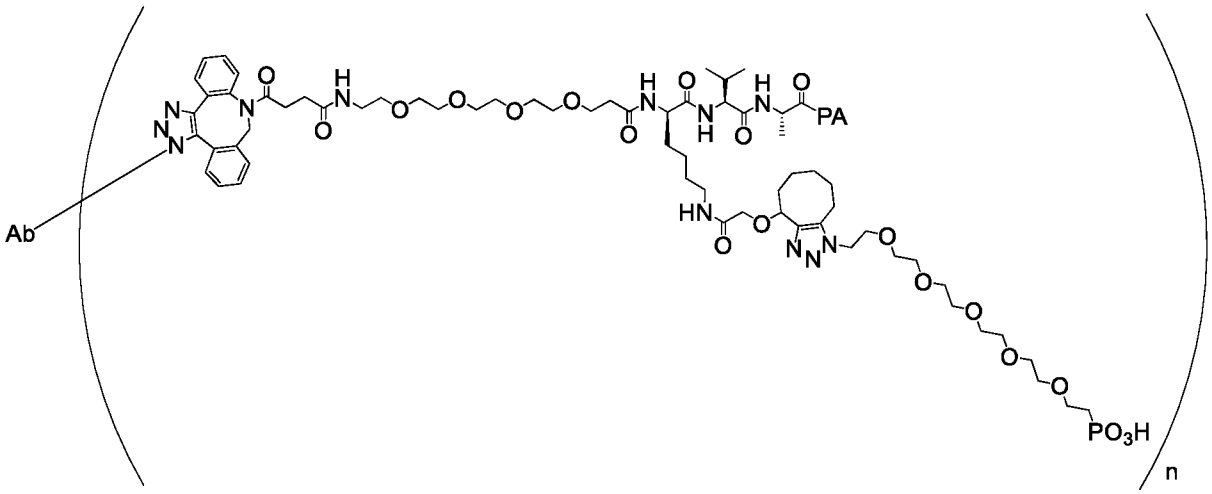




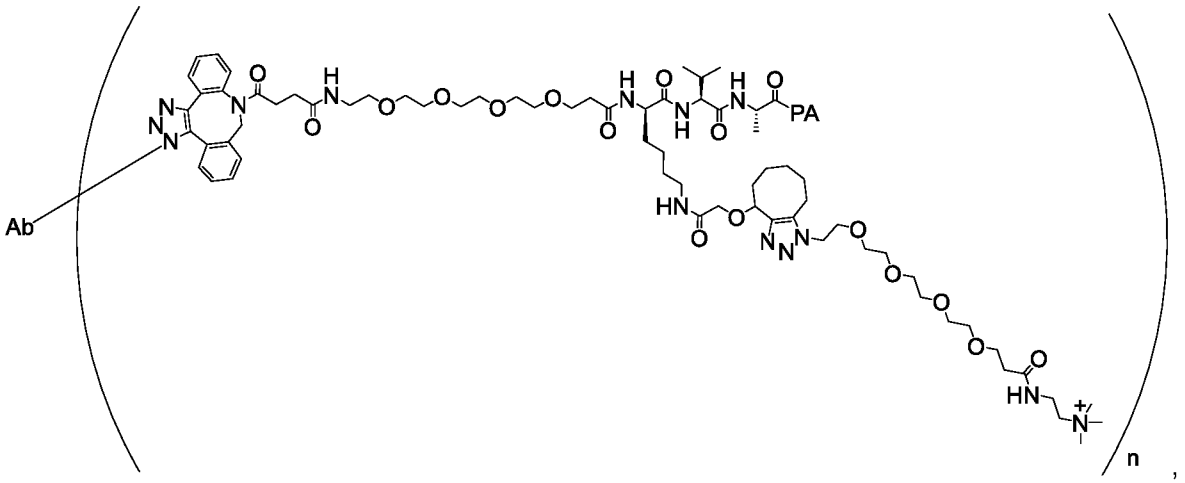
,



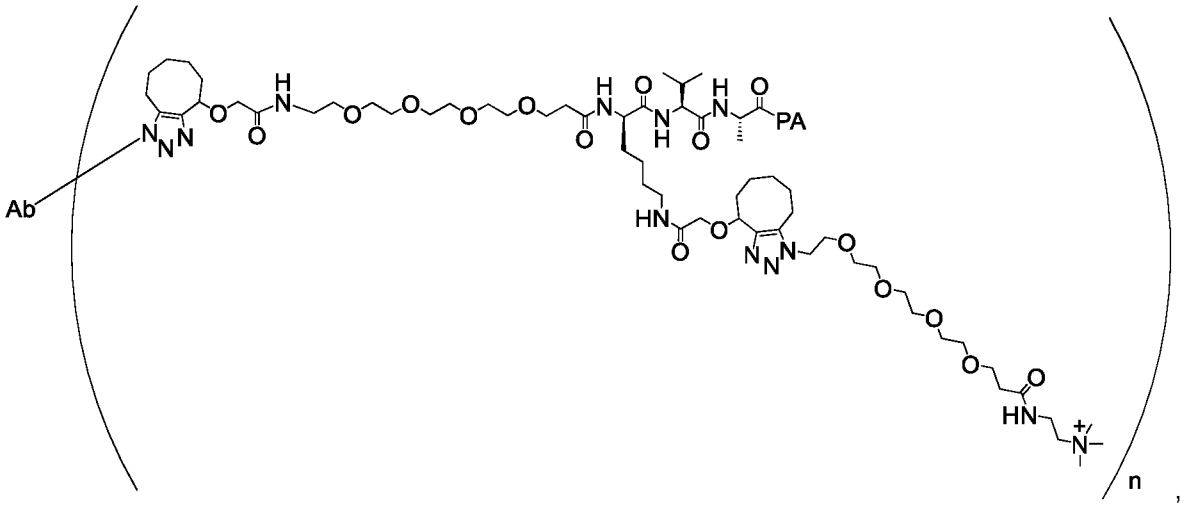
,



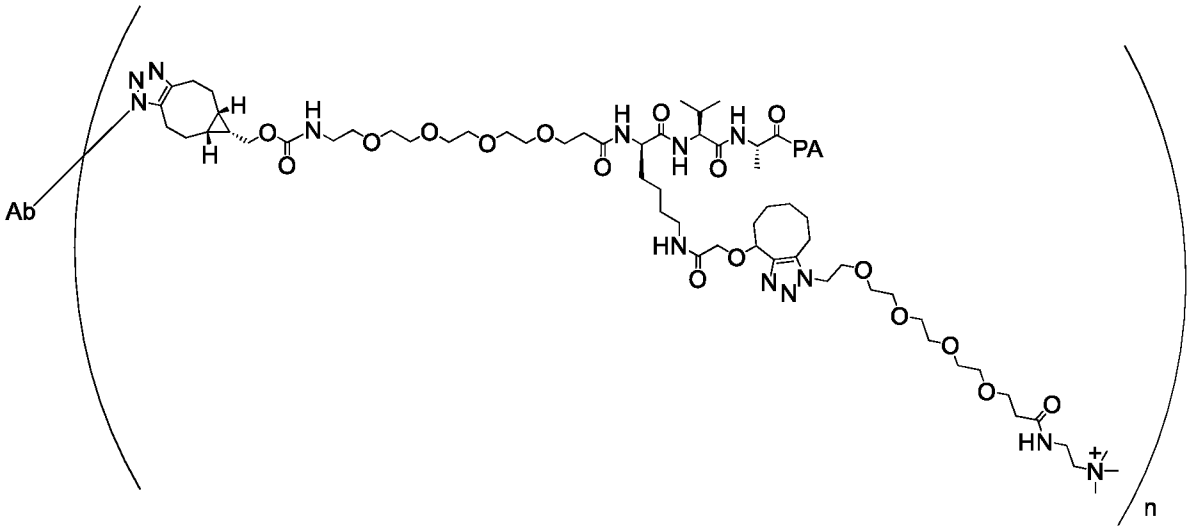
,


Ab
PA
HN
n

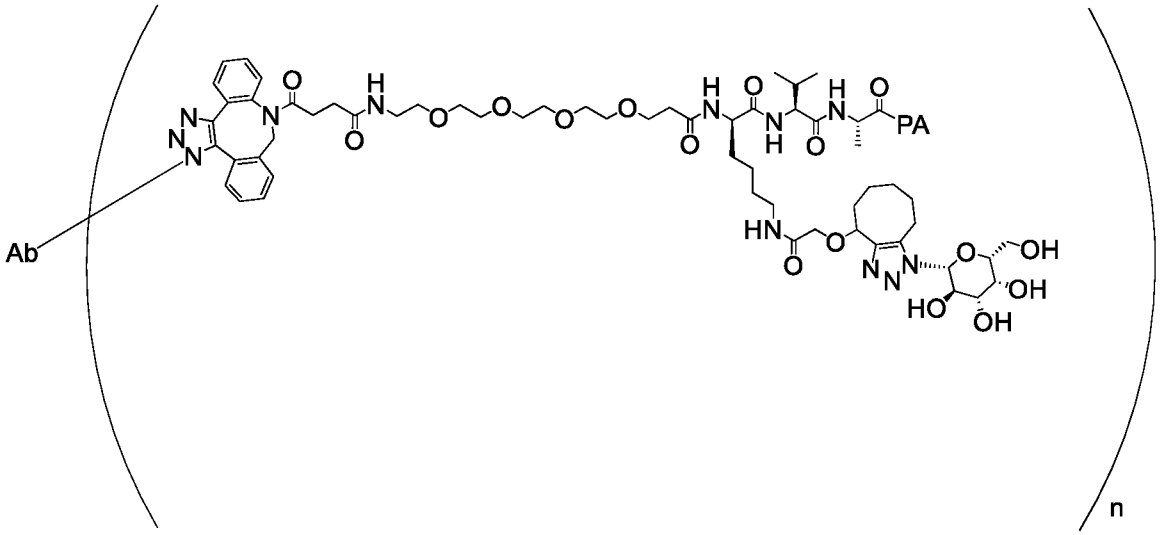
,

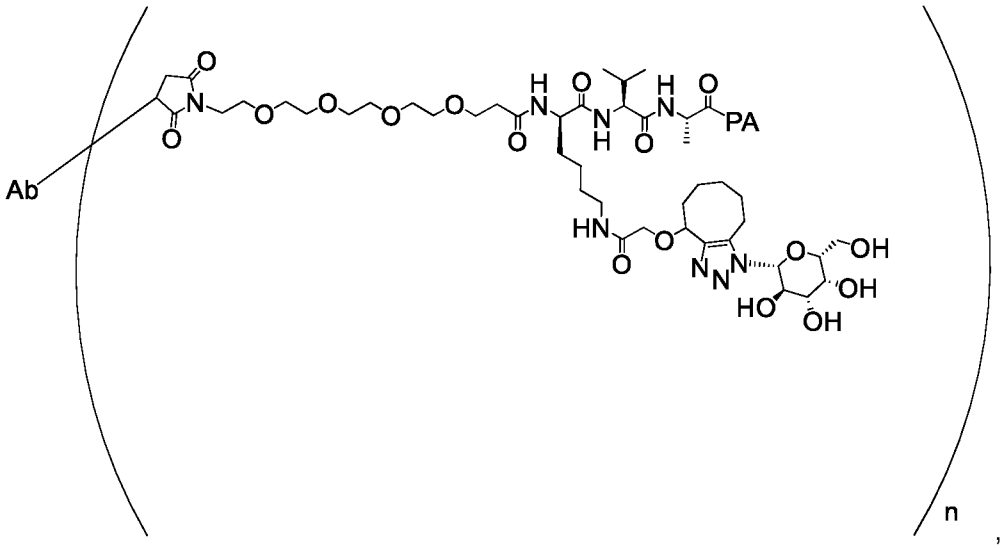

Ab
PA
HN
n

,

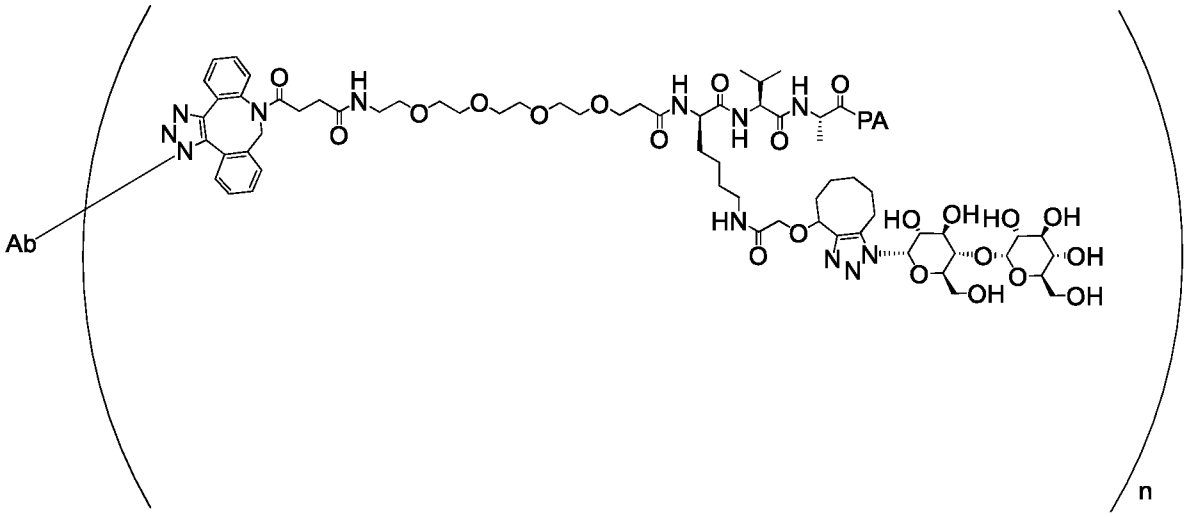

Ab
PA
HN
n

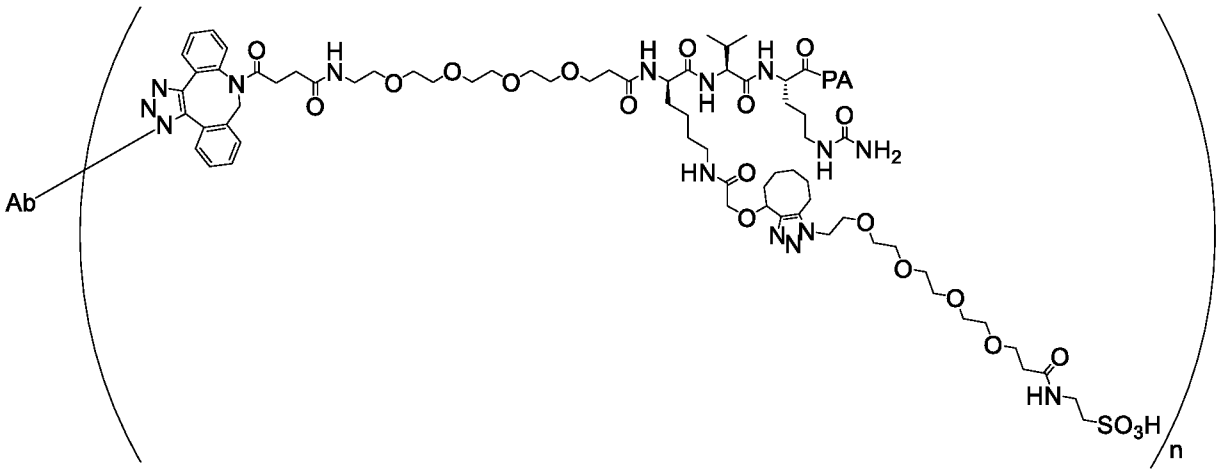
,


Ab
PA
HN
OH
HO
n

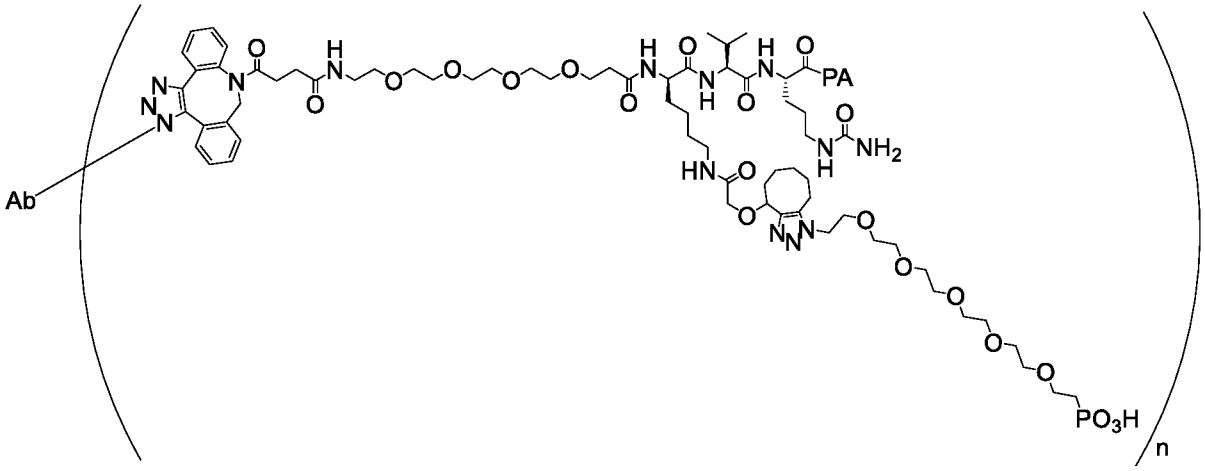

Ab
PA
HN
OH
HO
n

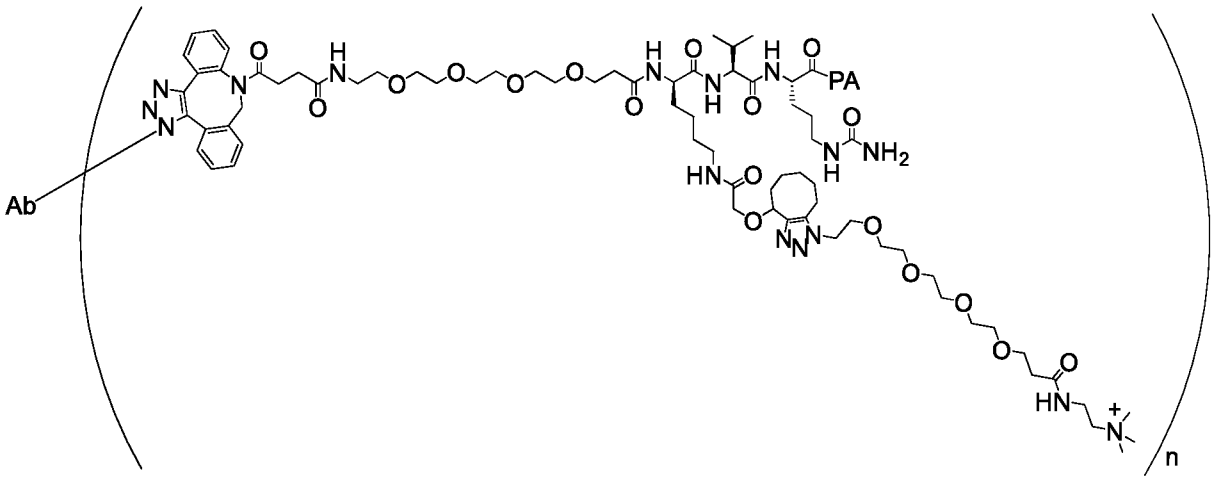
,


Ab
PA
HN
HO
OH
n

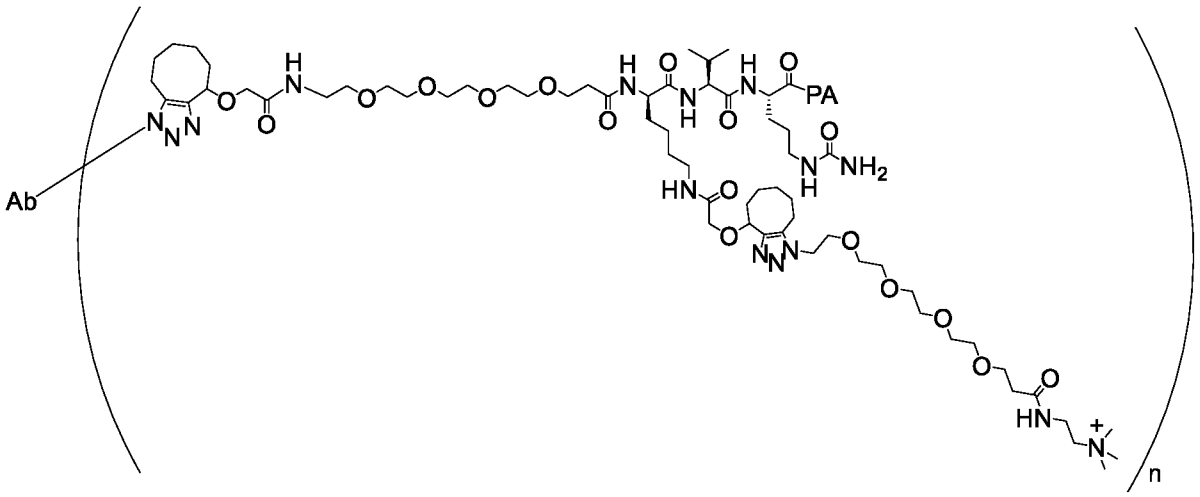

Ab
PA
NH2
HN
SO3H
n

,

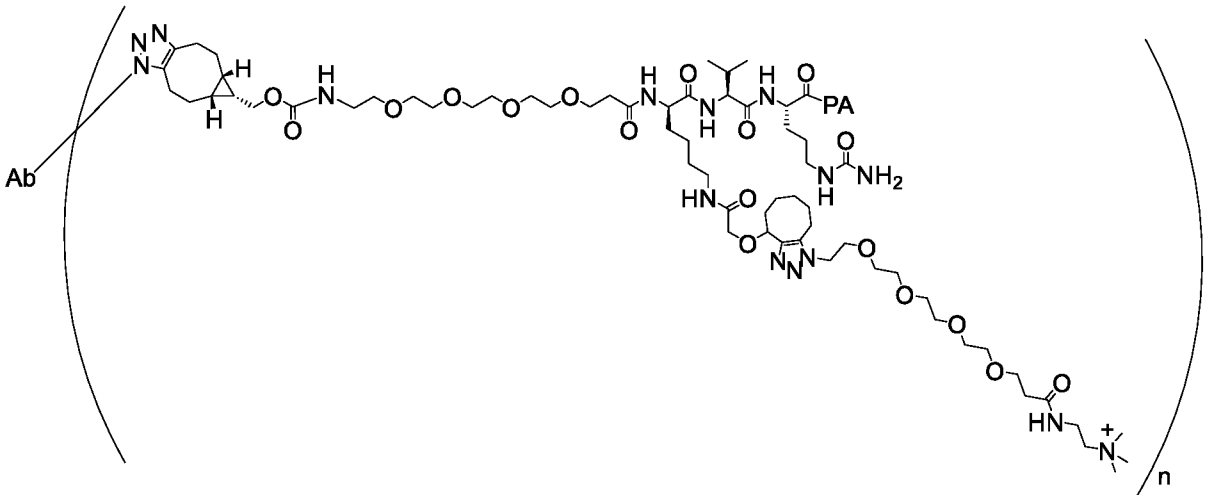

Ab
PA
NH2
HN
PO3H
n


Ab
PA
NH2
HN
n

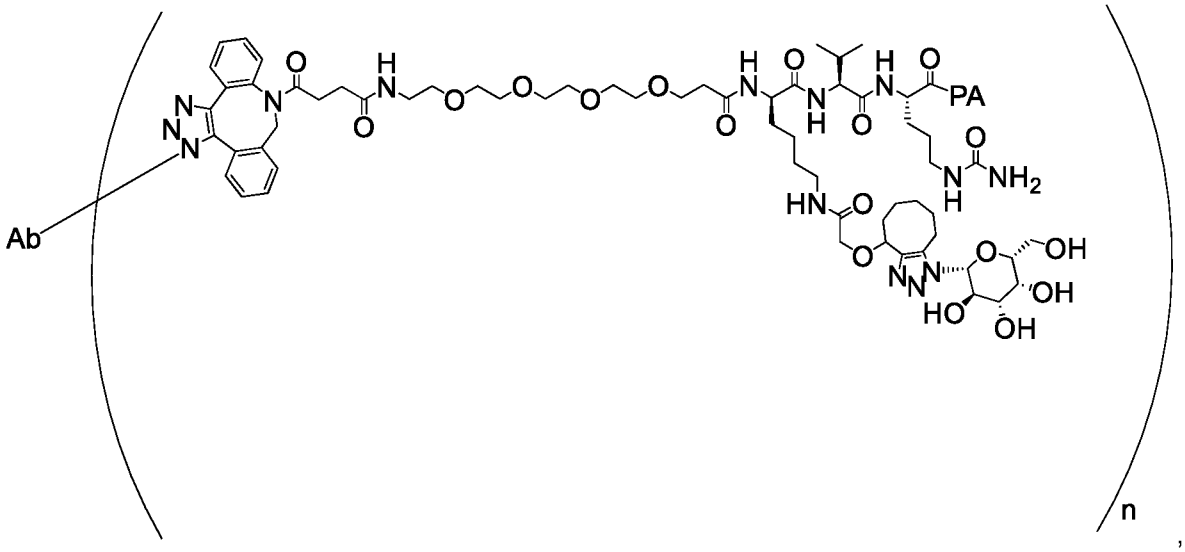
,



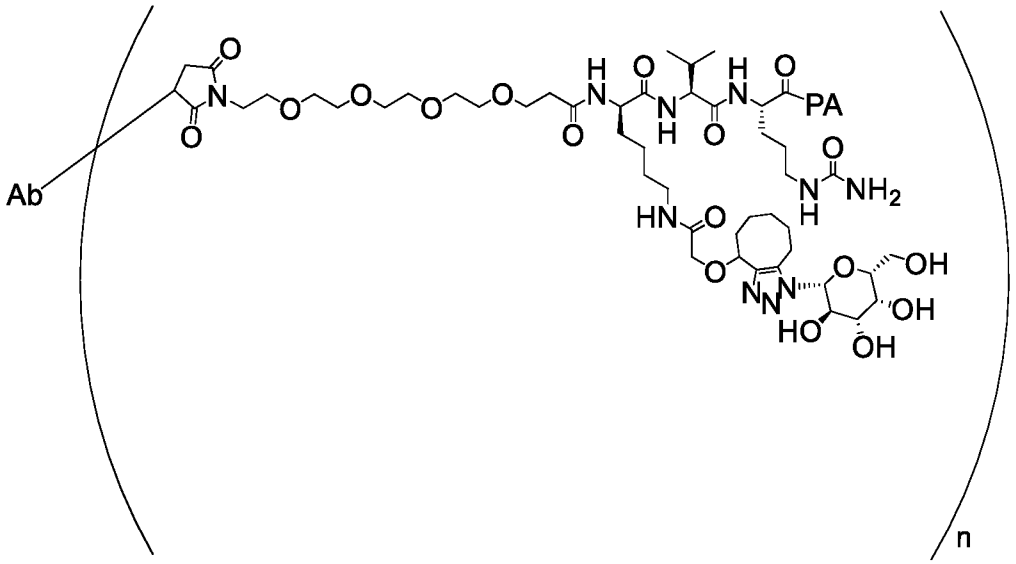
,



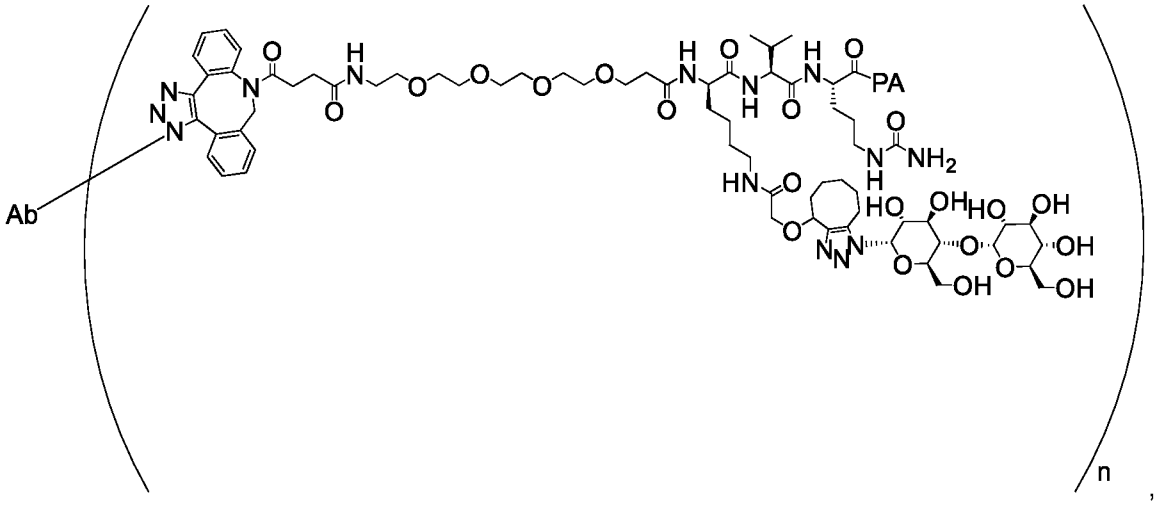
,



,



and



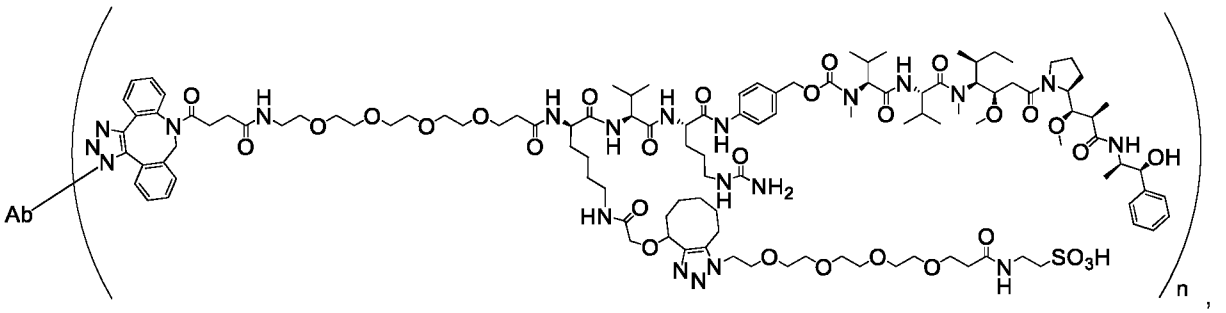
or a stereoisomeric form thereof, or a regioisomer thereof, or a mixture of regioisomers thereof, wherein

each **Ab** is an antibody, or an antigen-binding fragment thereof;

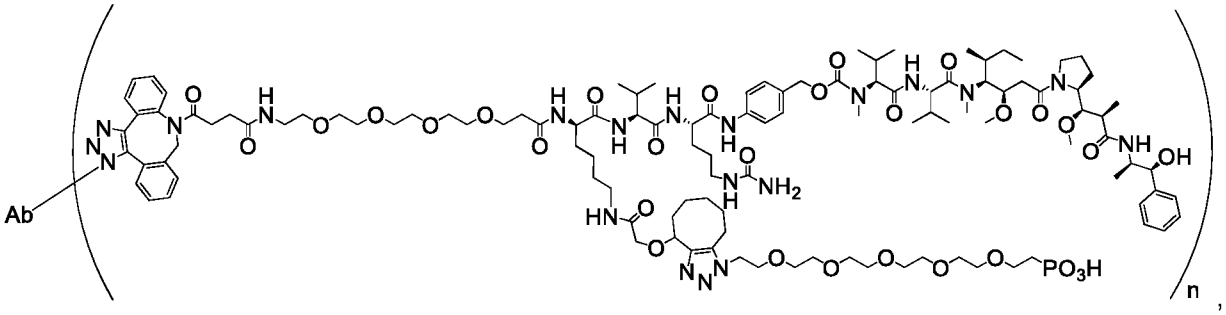
**PA** is a payload residue; and

subscript **n** is an integer from 1 to 30.

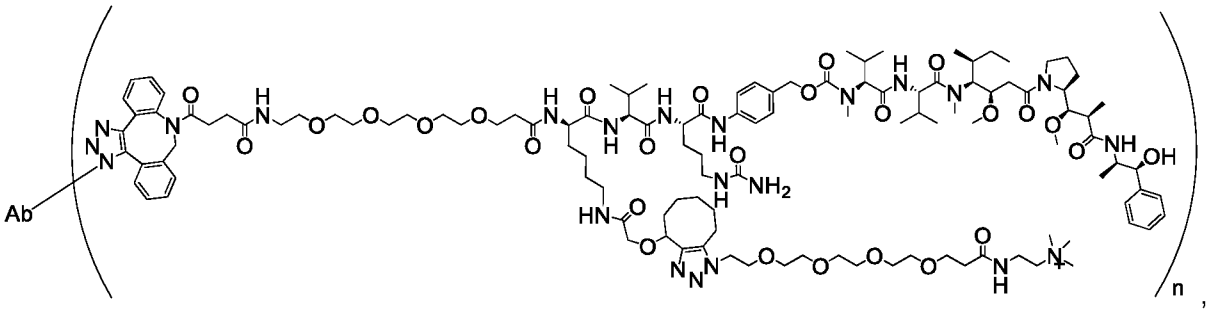
**[0144]** In some instances, a compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), is an antibody drug conjugate (ADC), wherein BA is Ab and Ab is an antigen or antigen-binding fragment thereof, selected from the group consisting of:



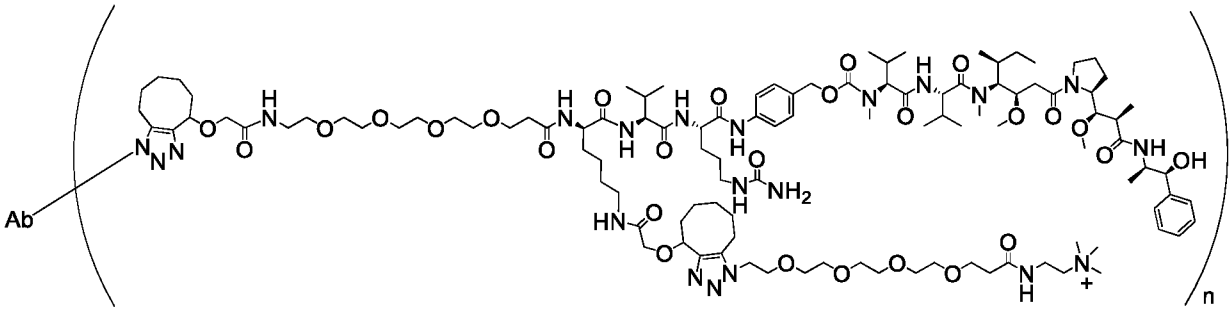
,



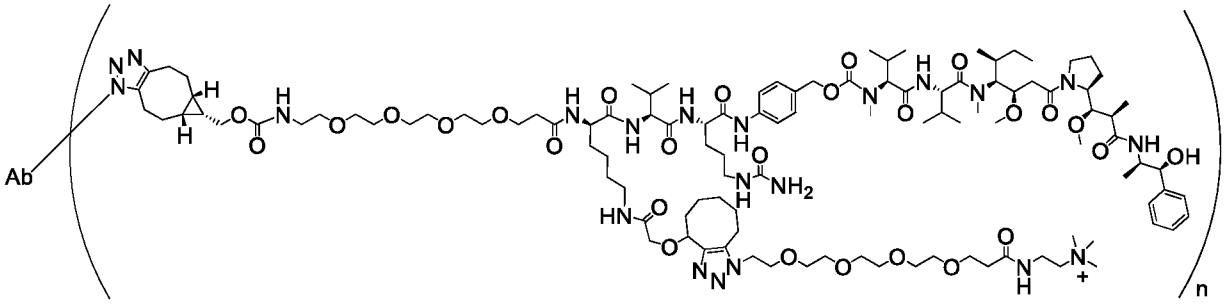
,



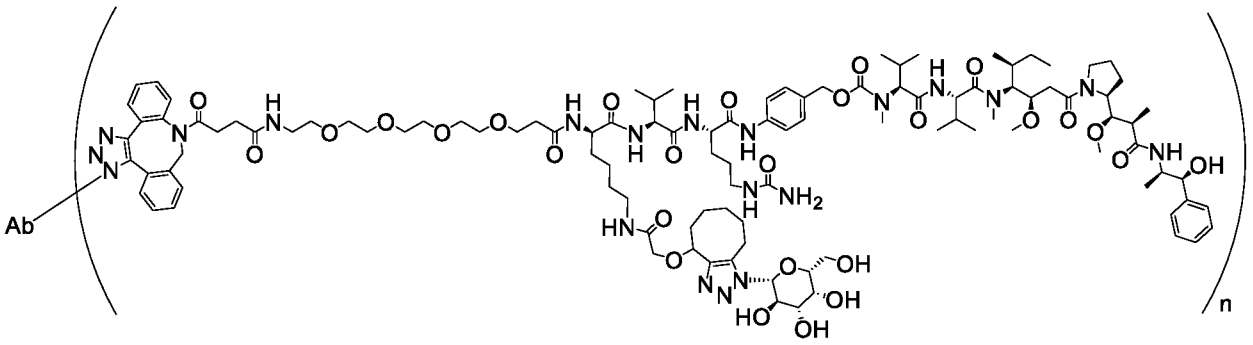
,



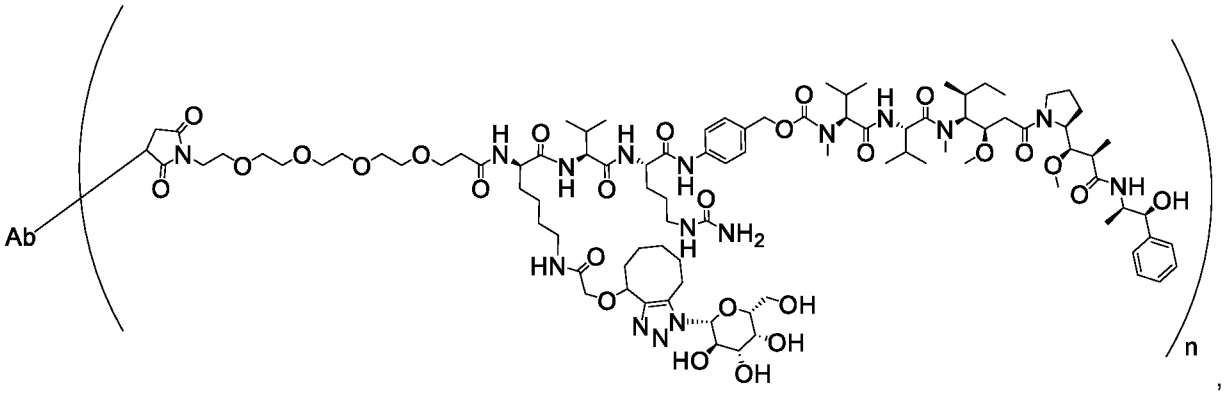
,



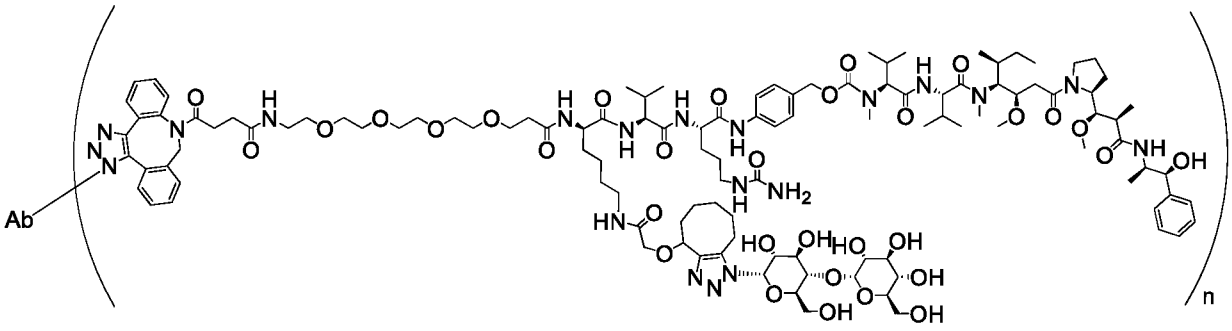
,



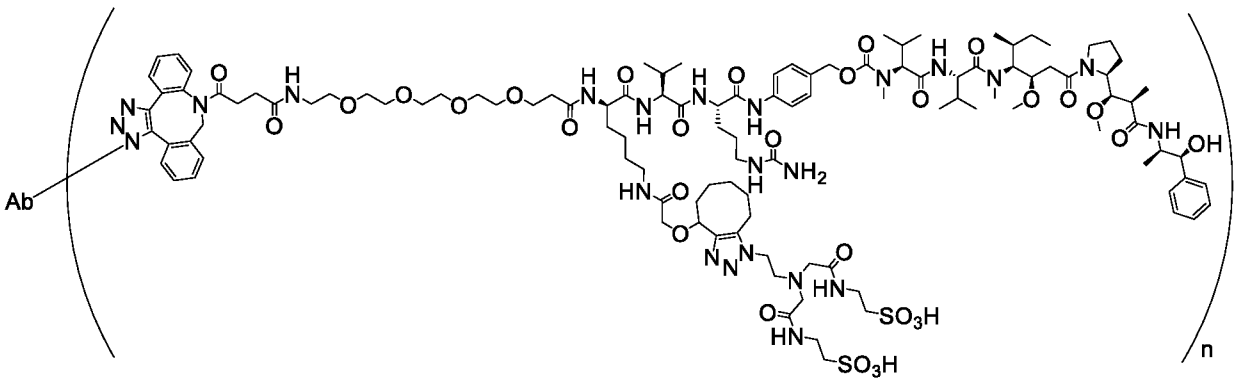
,



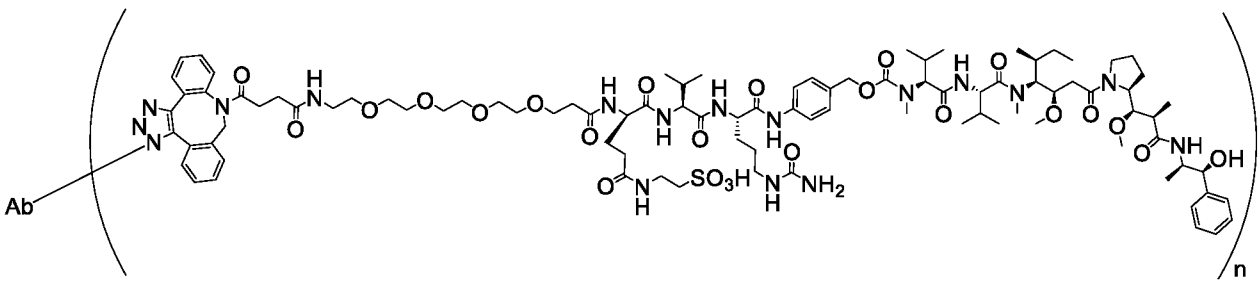
,



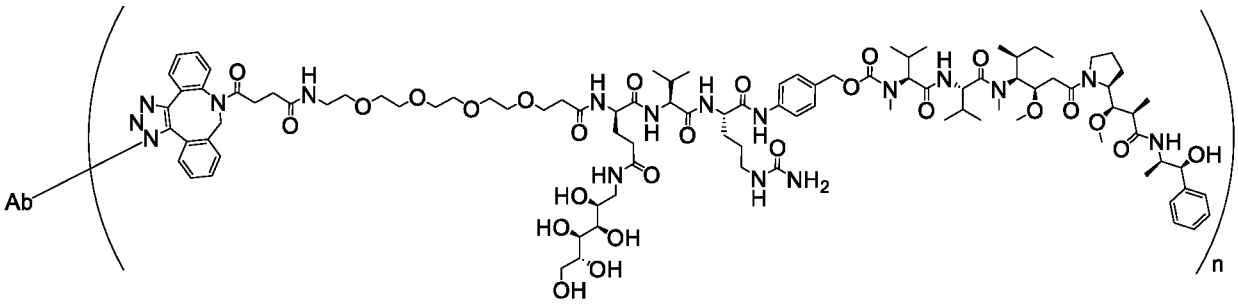
,



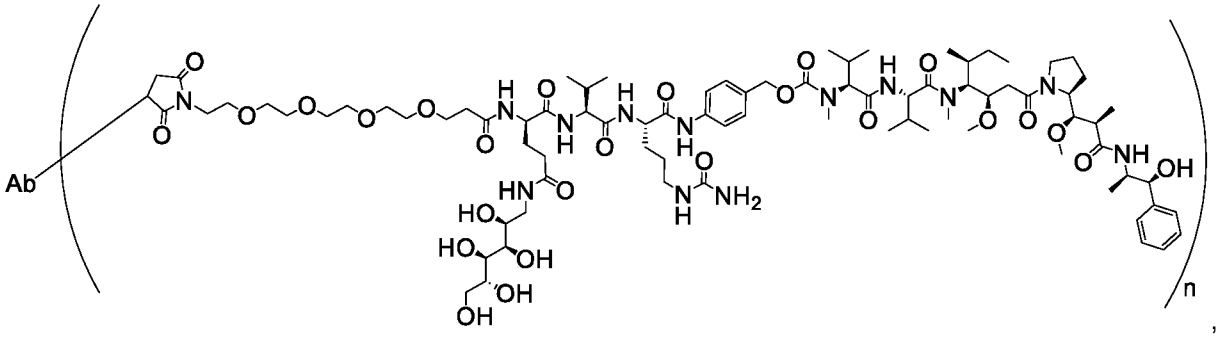
,



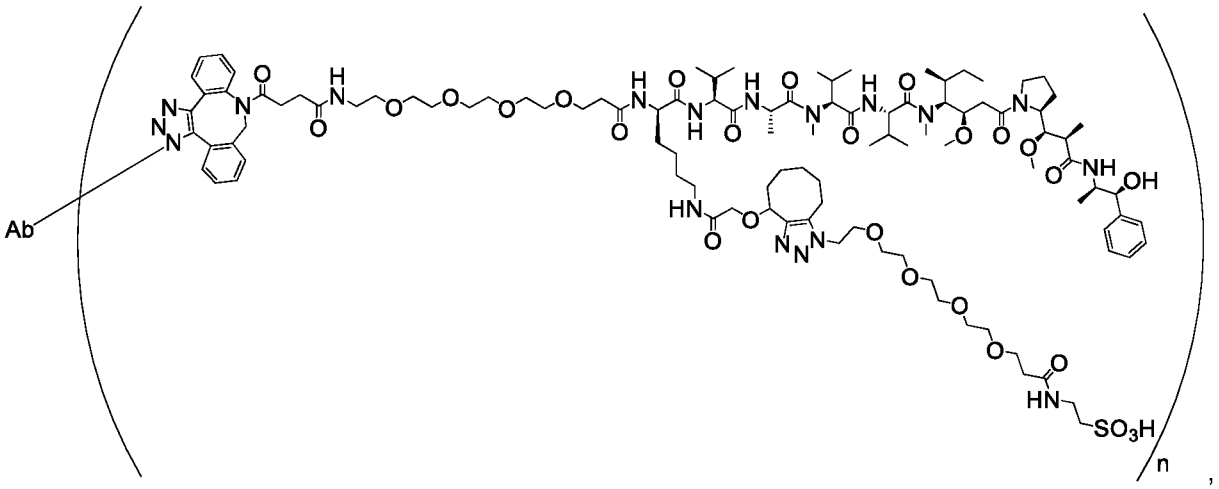
,


Ab
n

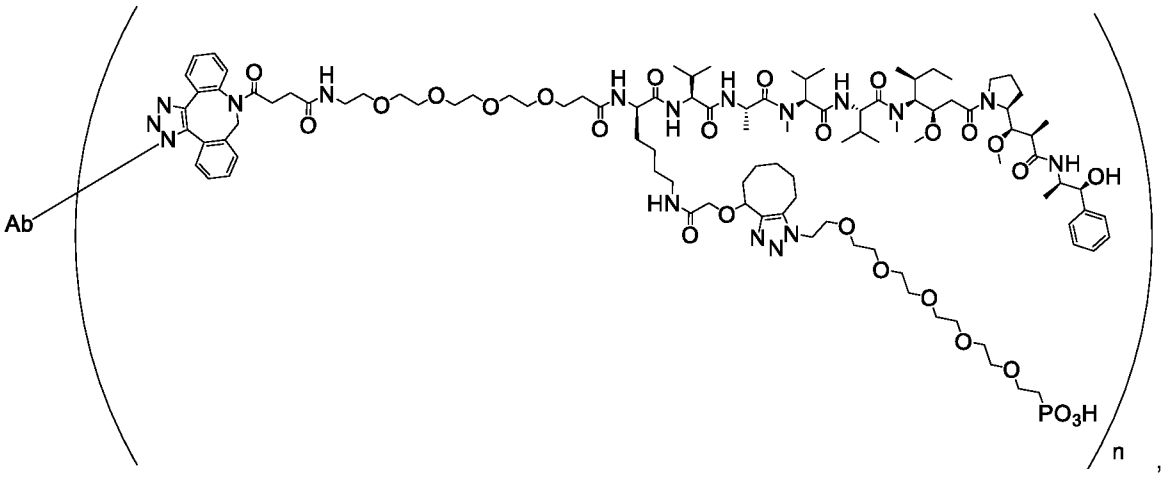
,


Ab
n

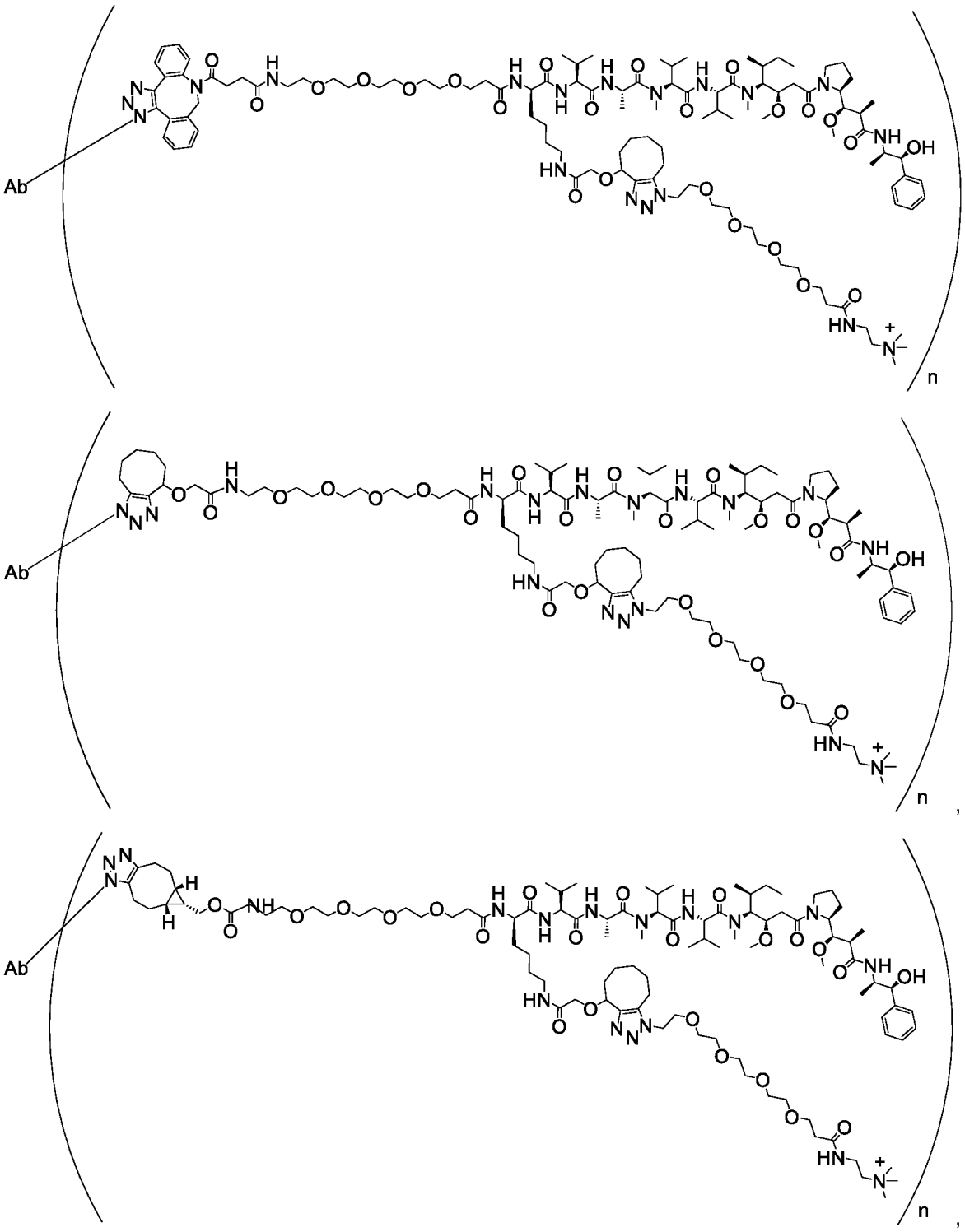
,

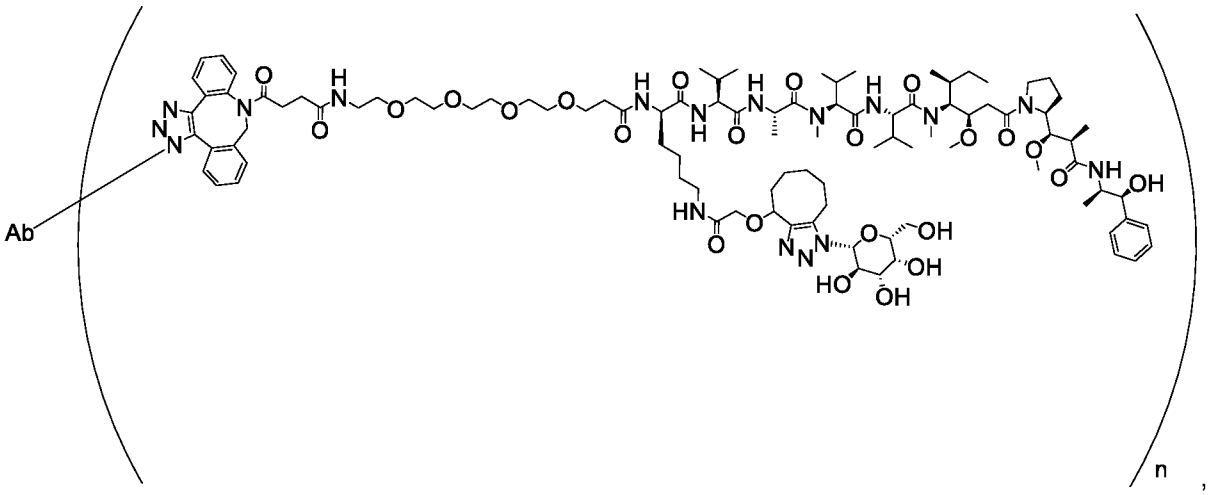

Ab
n

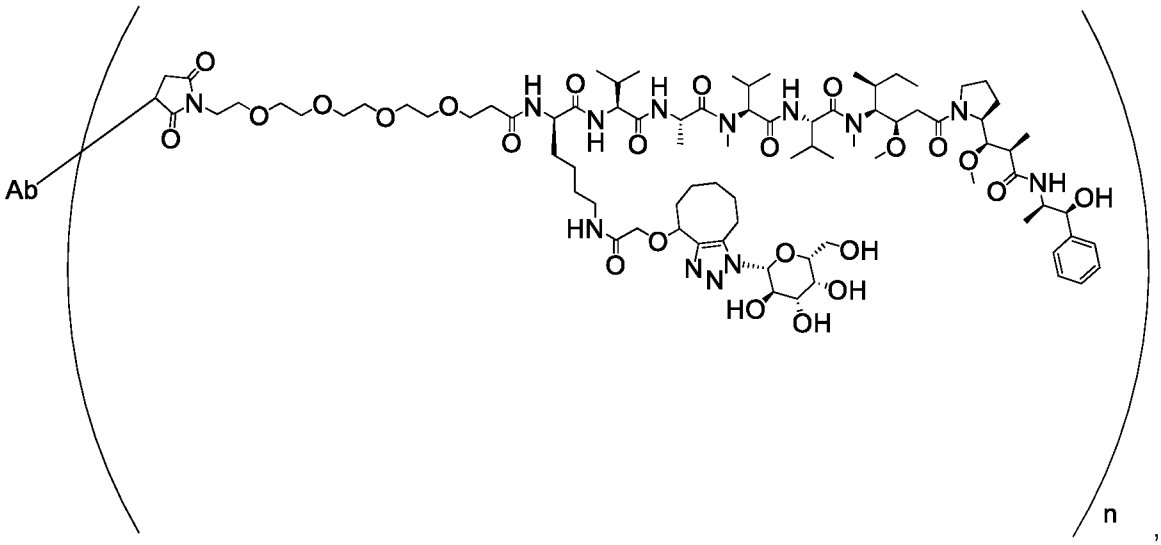
,

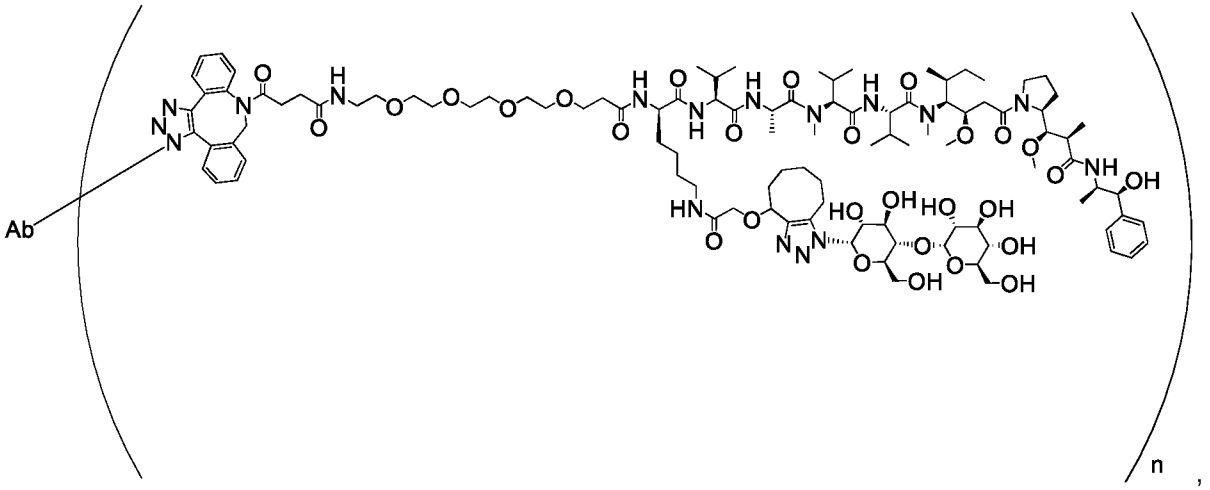

Ab
n

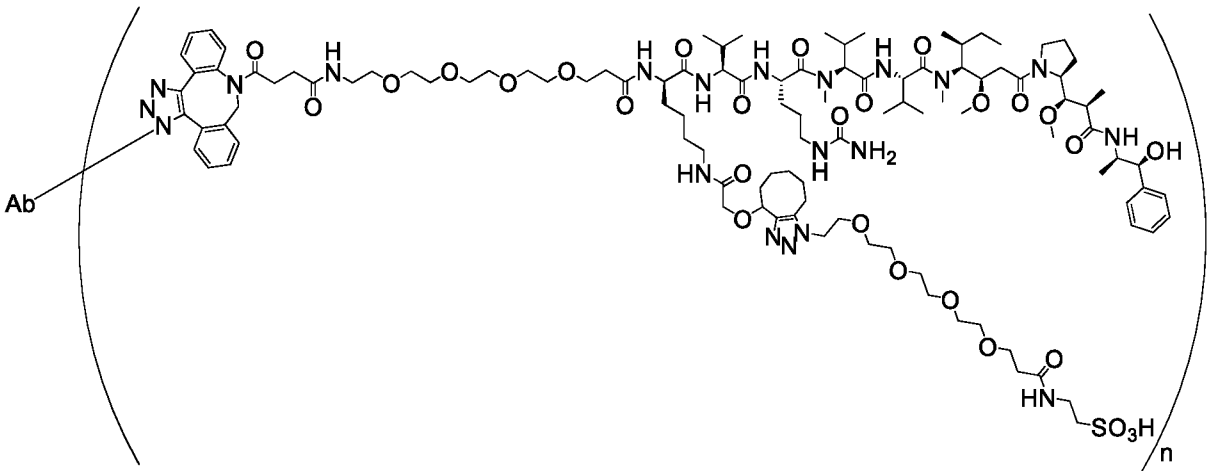
,

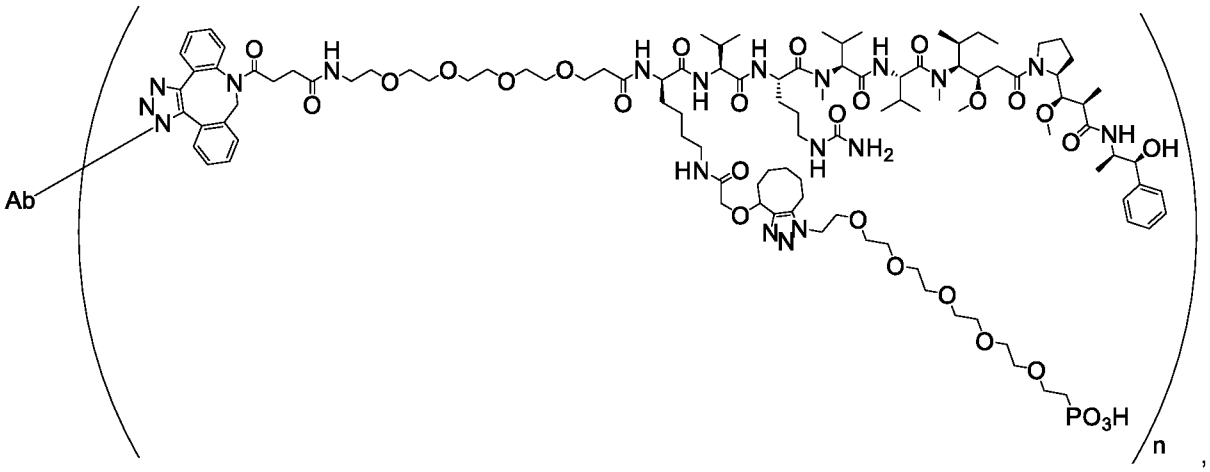


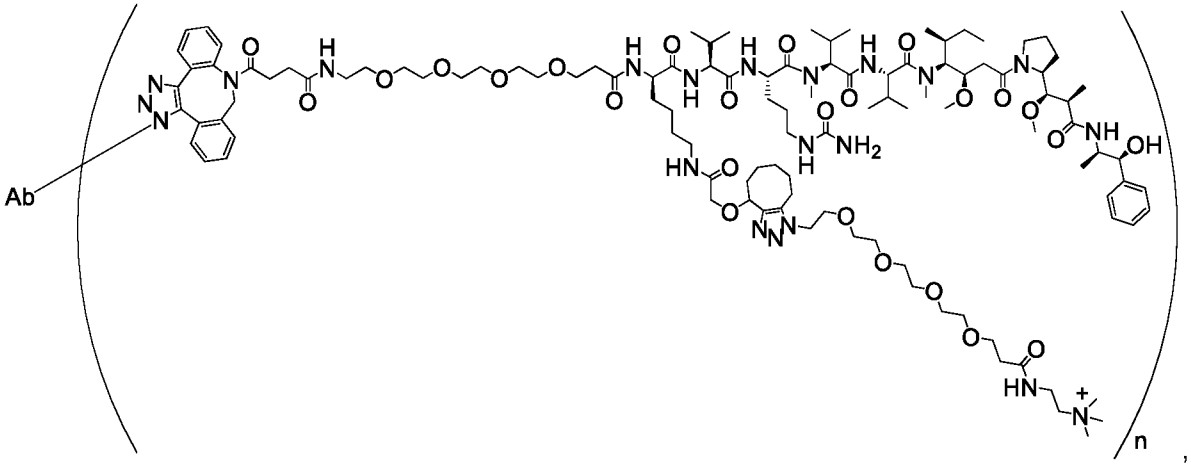


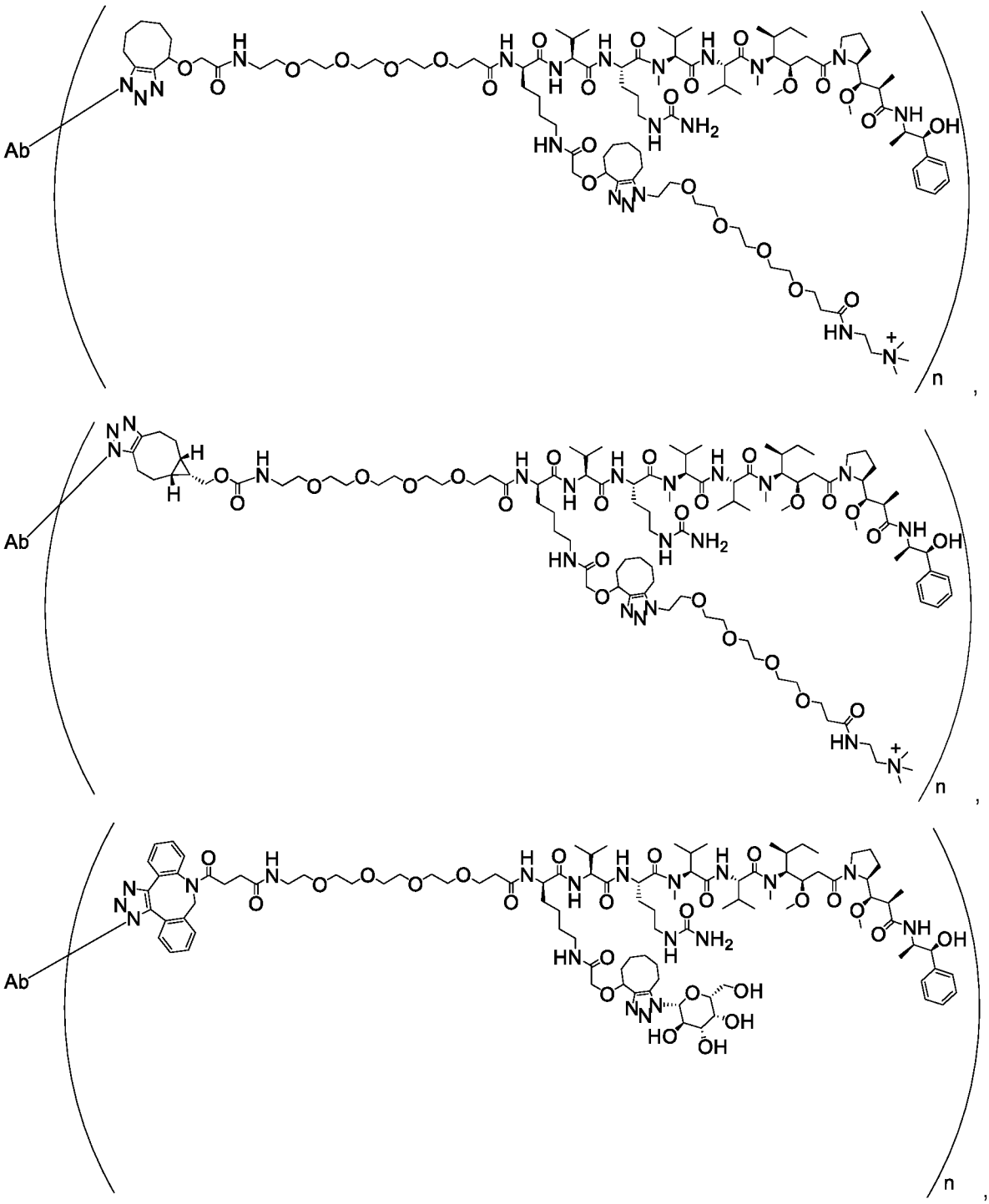


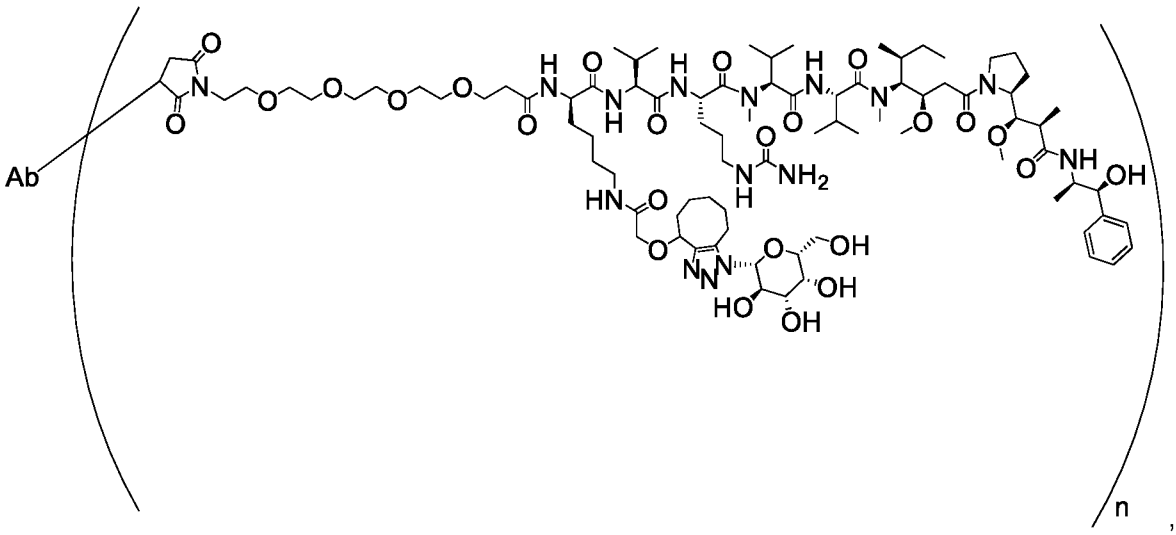


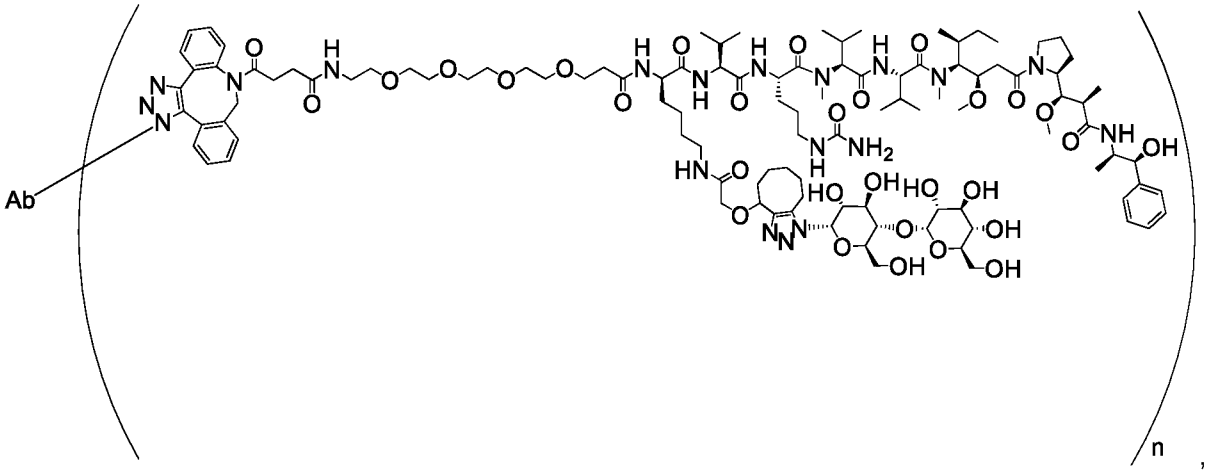


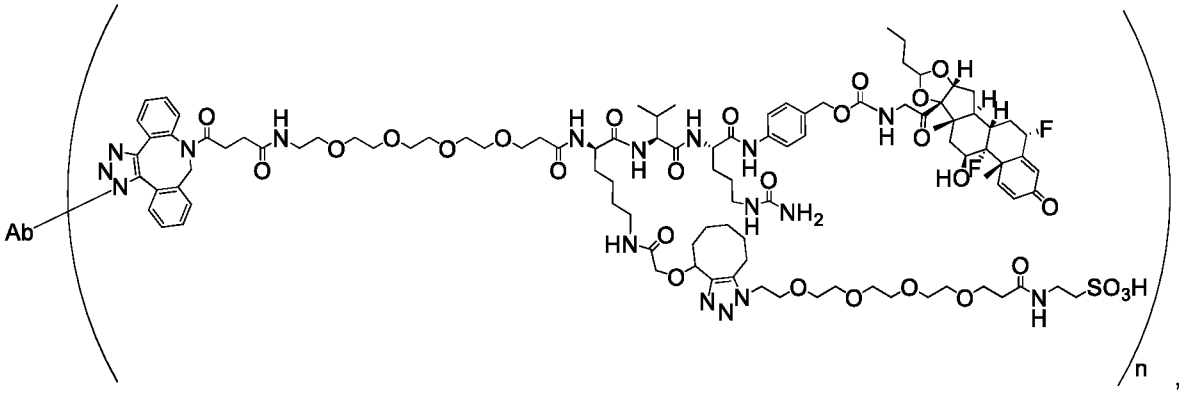


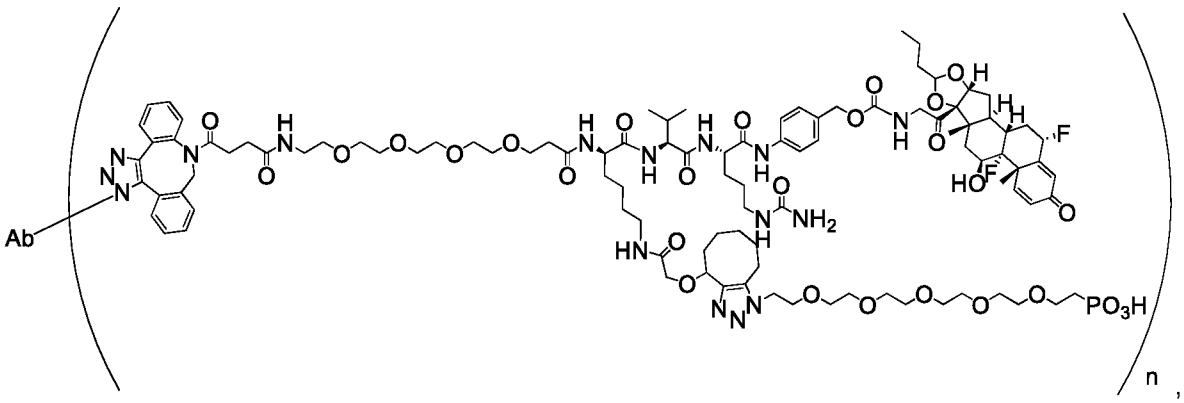


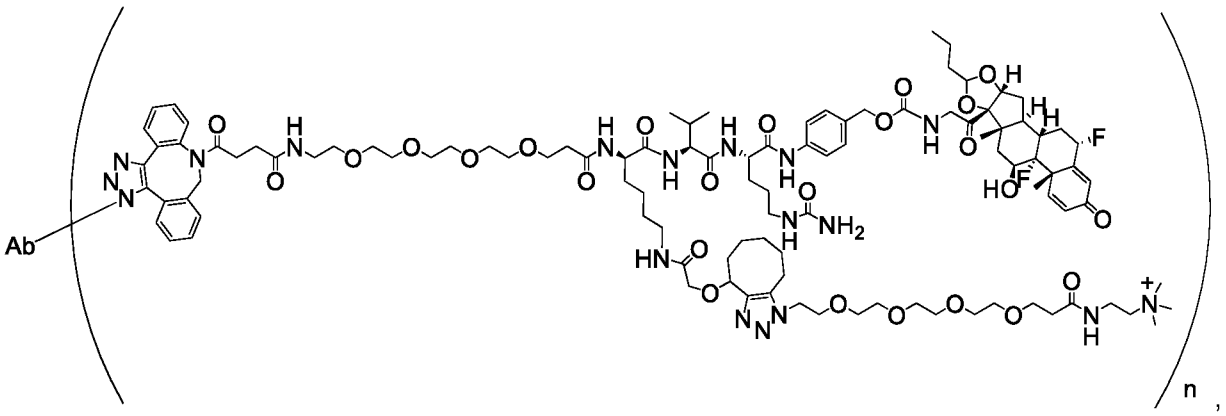




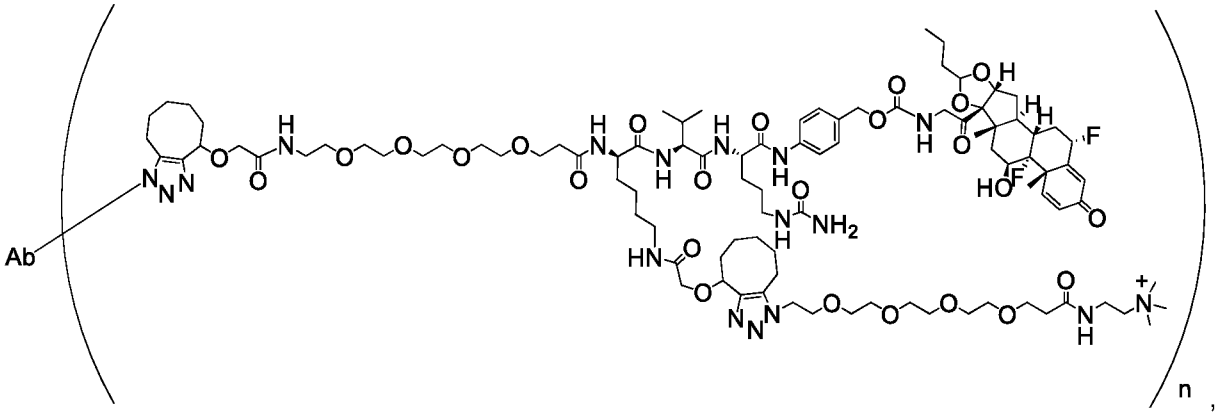




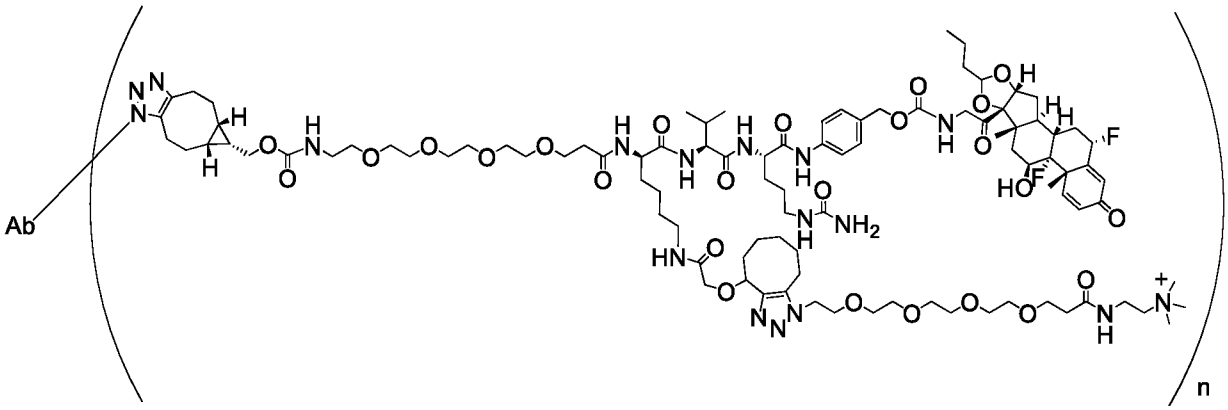




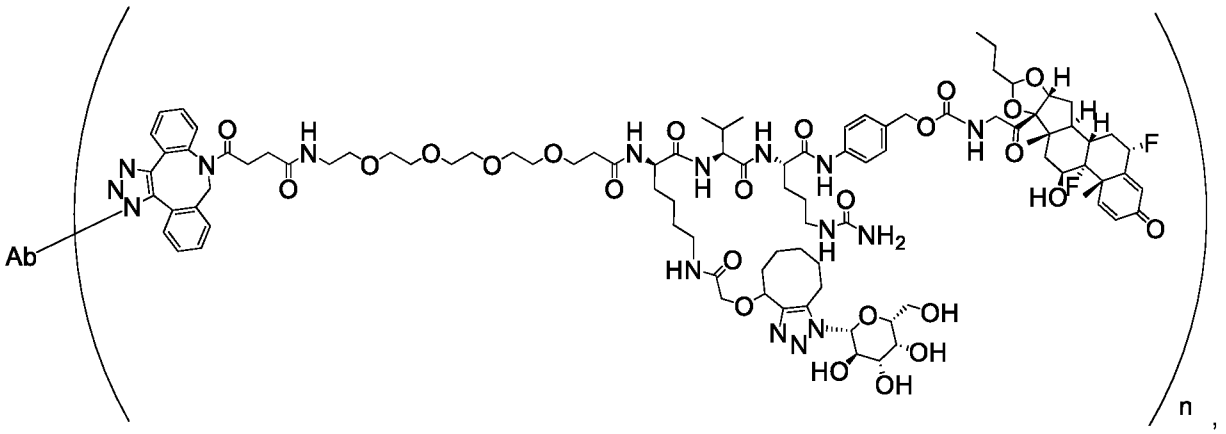
,



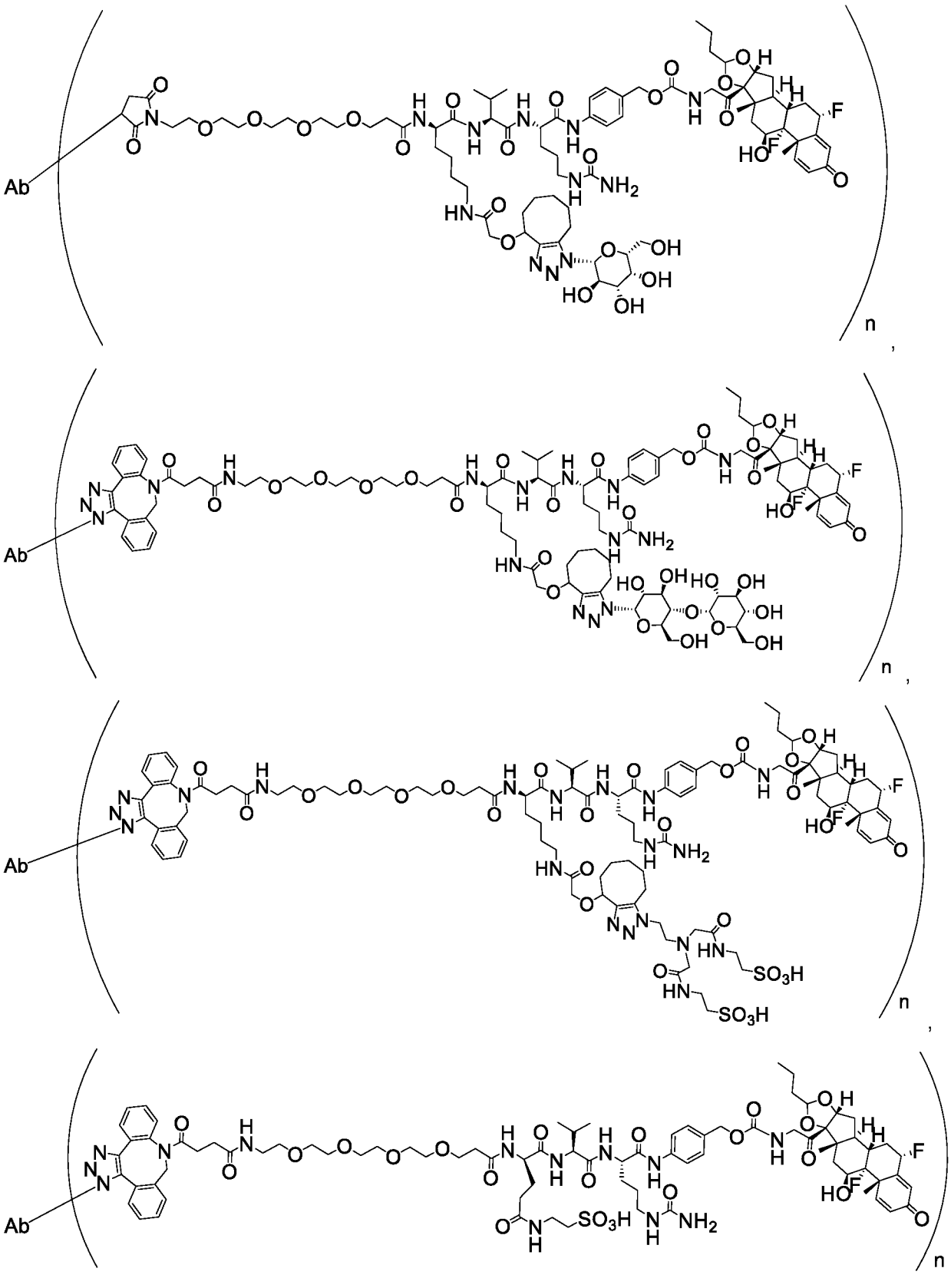
,

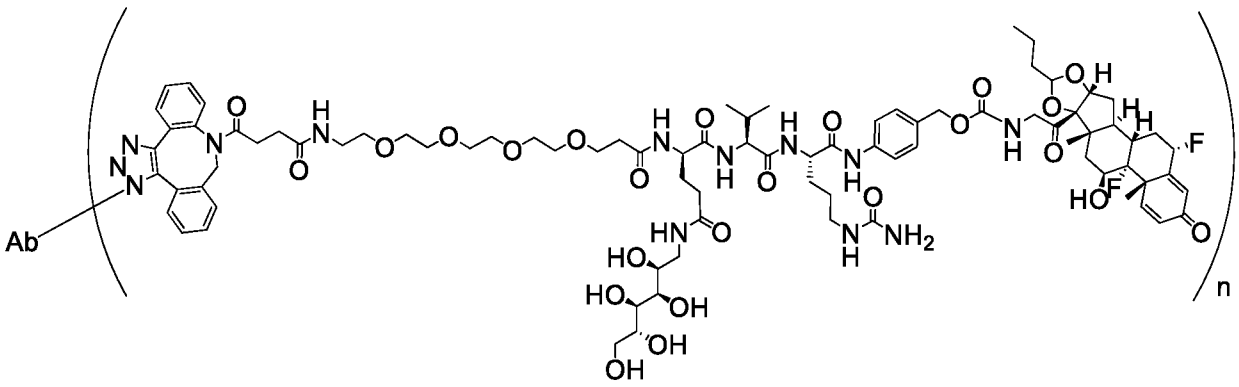


,

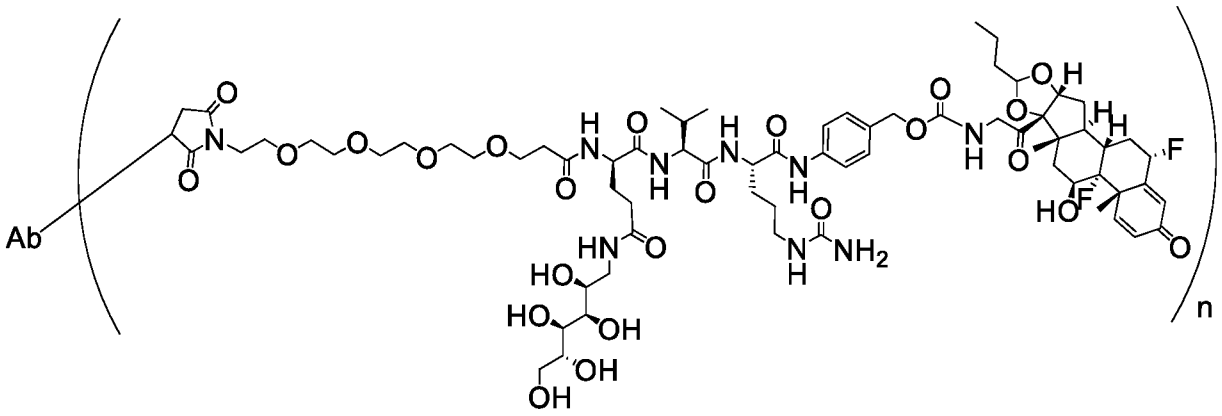


,

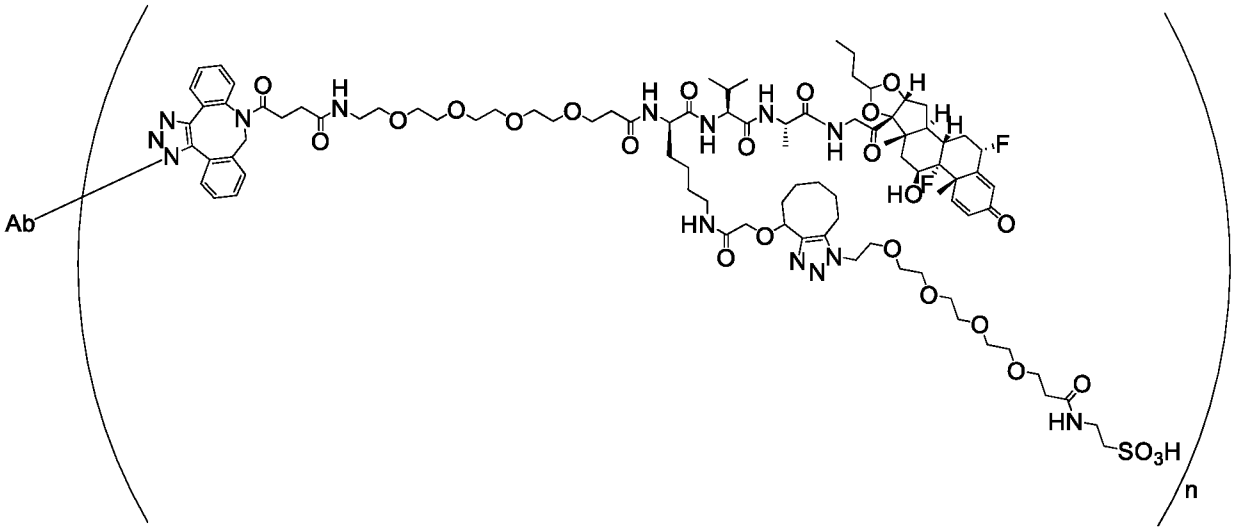




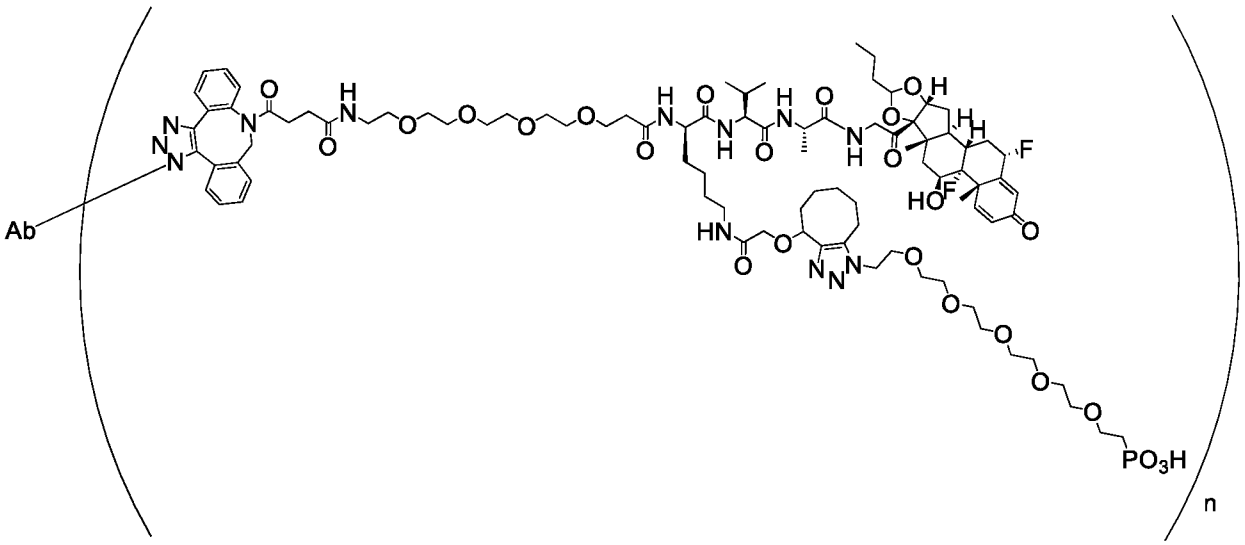
,



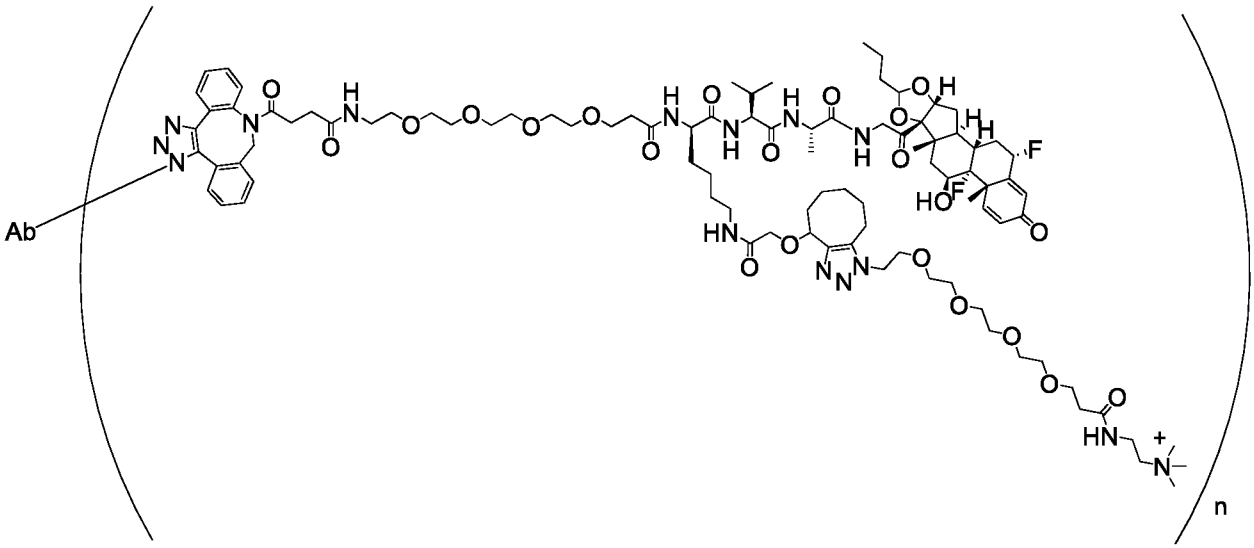
,



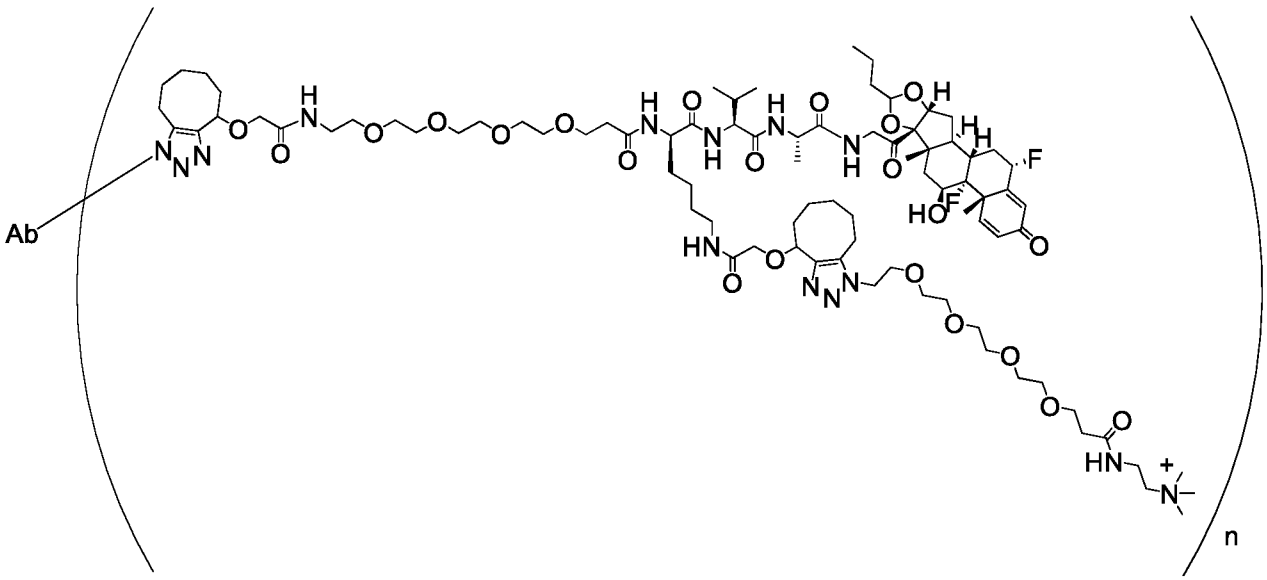
,


Ab
PO3H
n

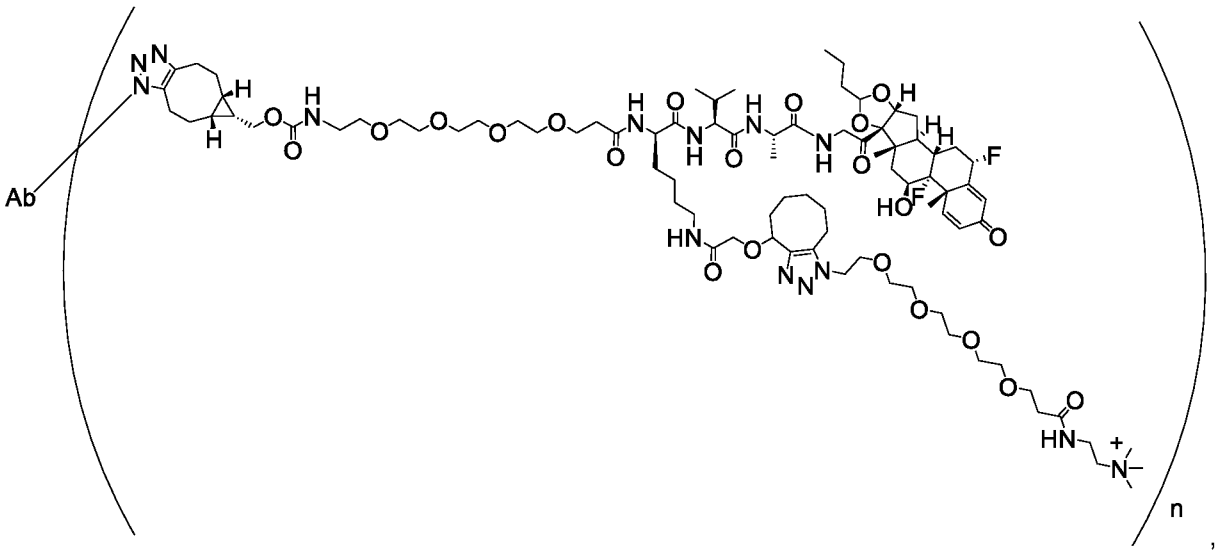
,

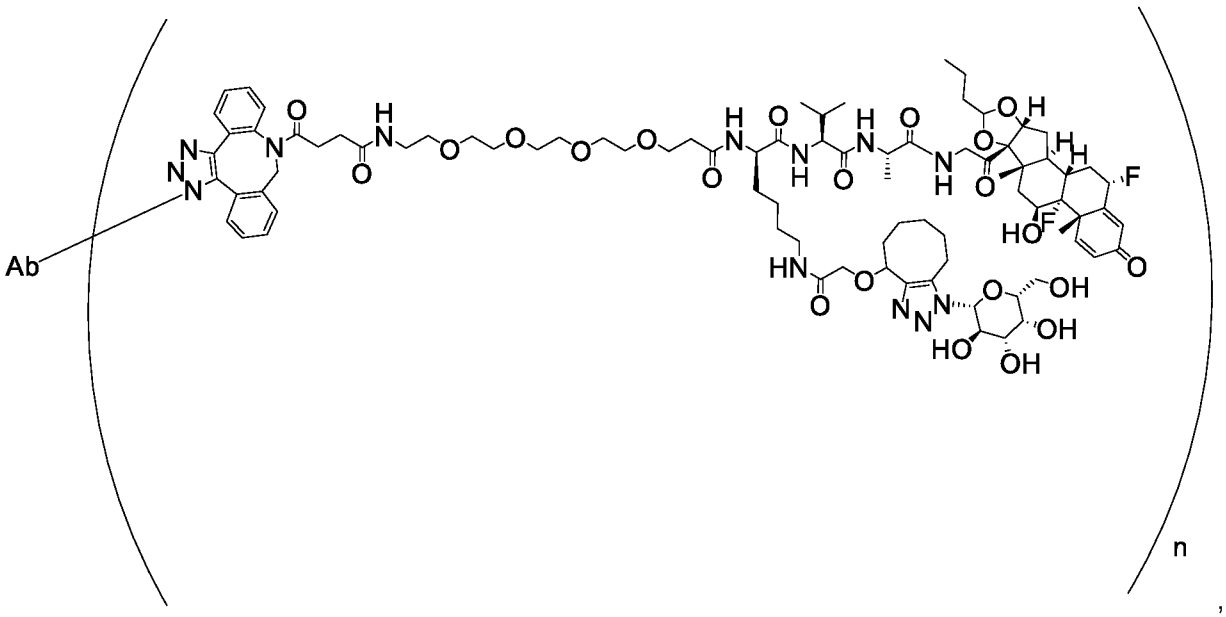

Ab
n

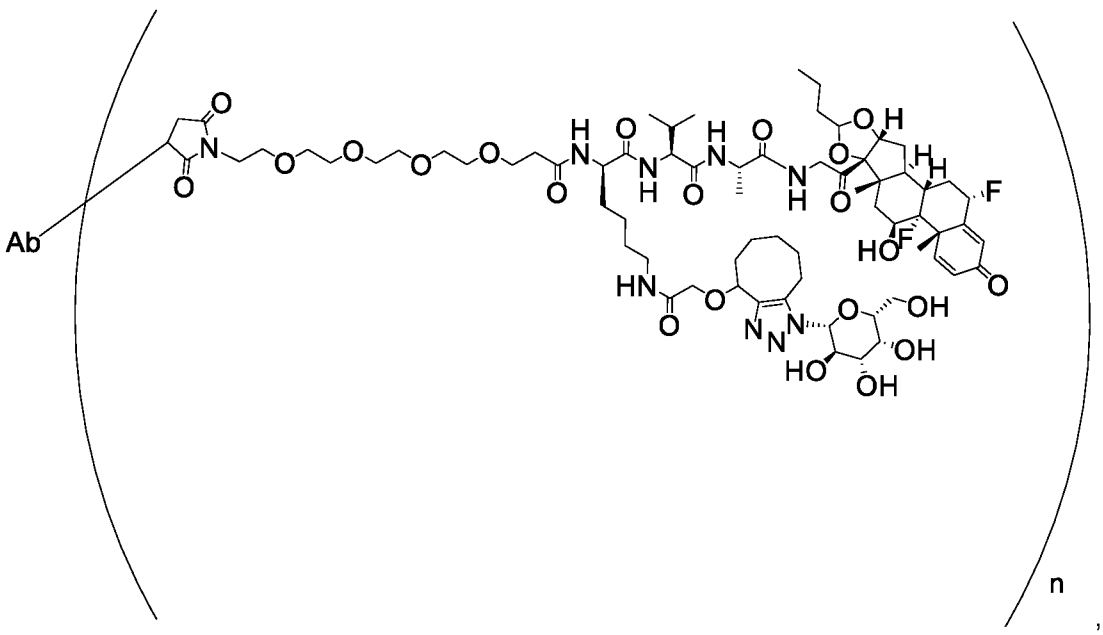
,

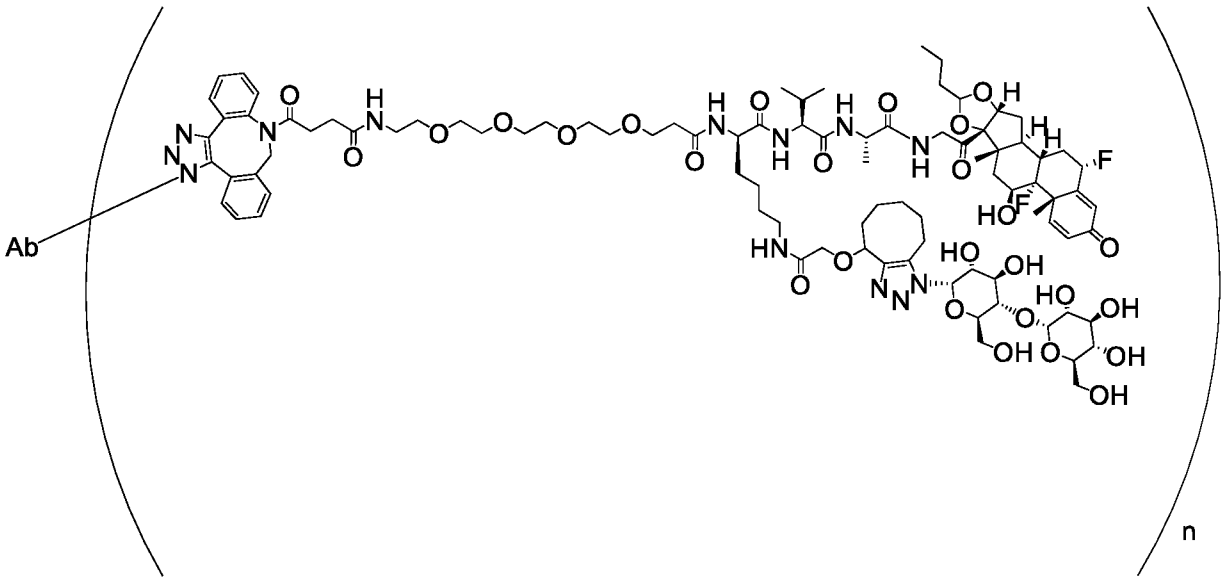

Ab
n

,

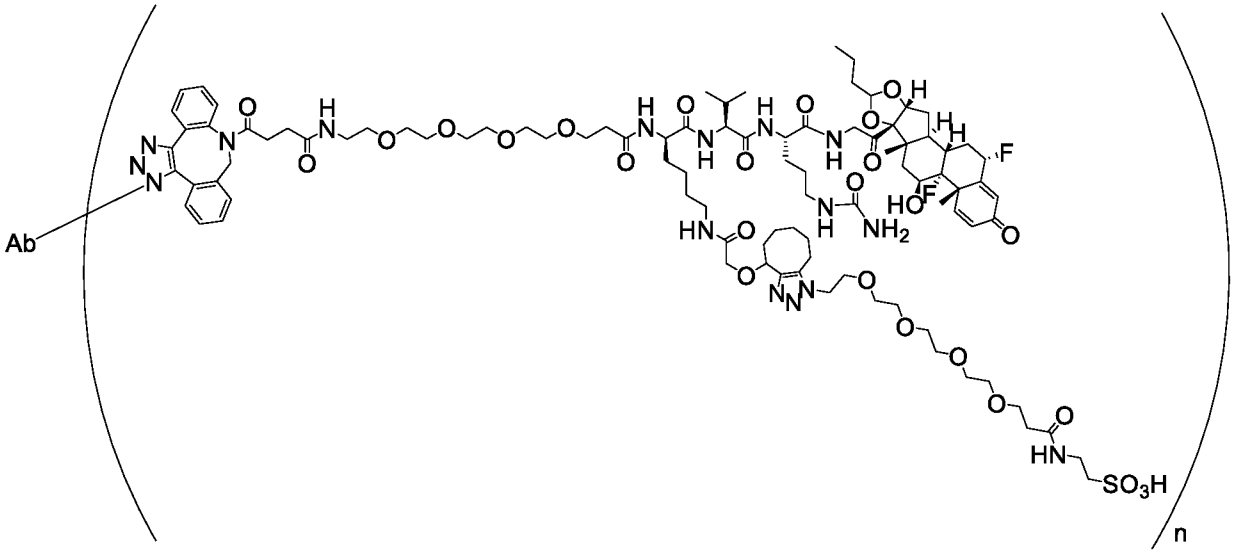




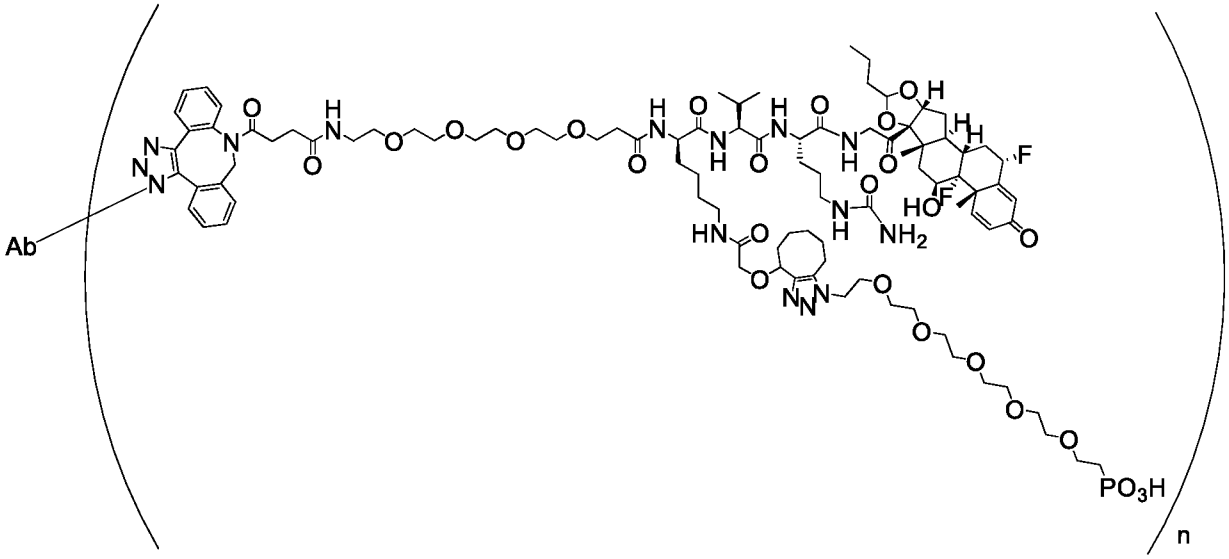



Ab
n

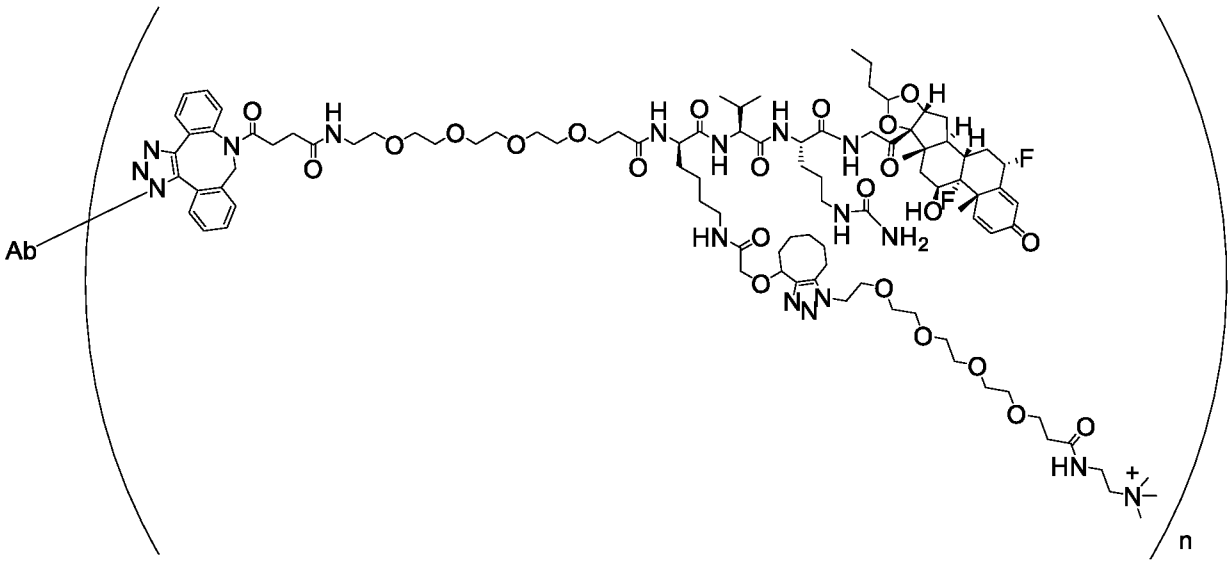
,


Ab
SO3H
n

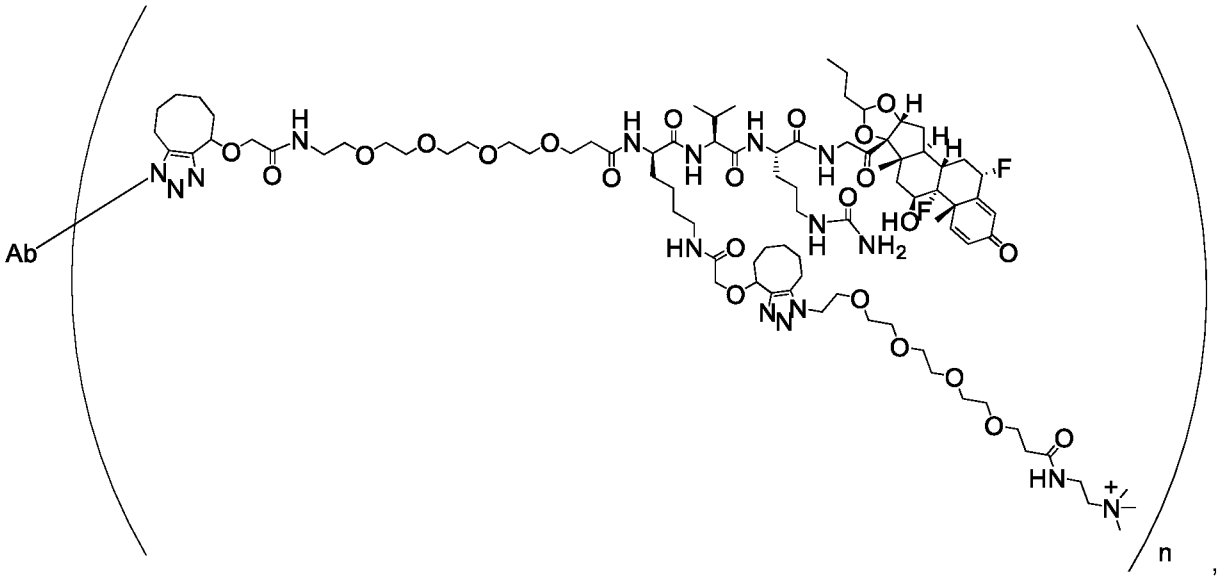
,


Ab
PO3H
n

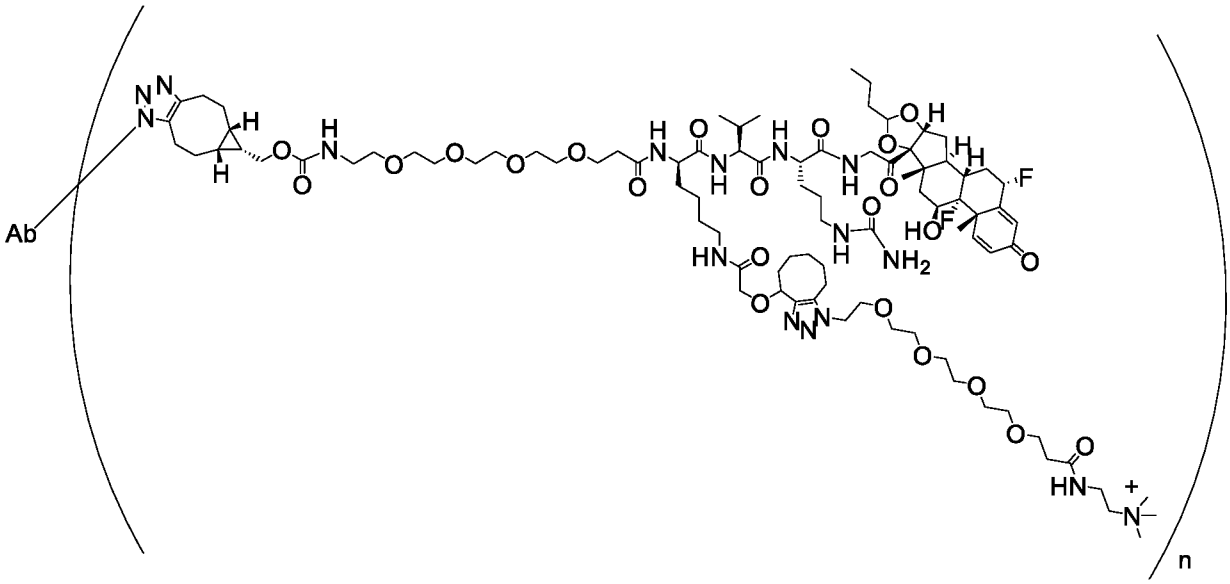
,



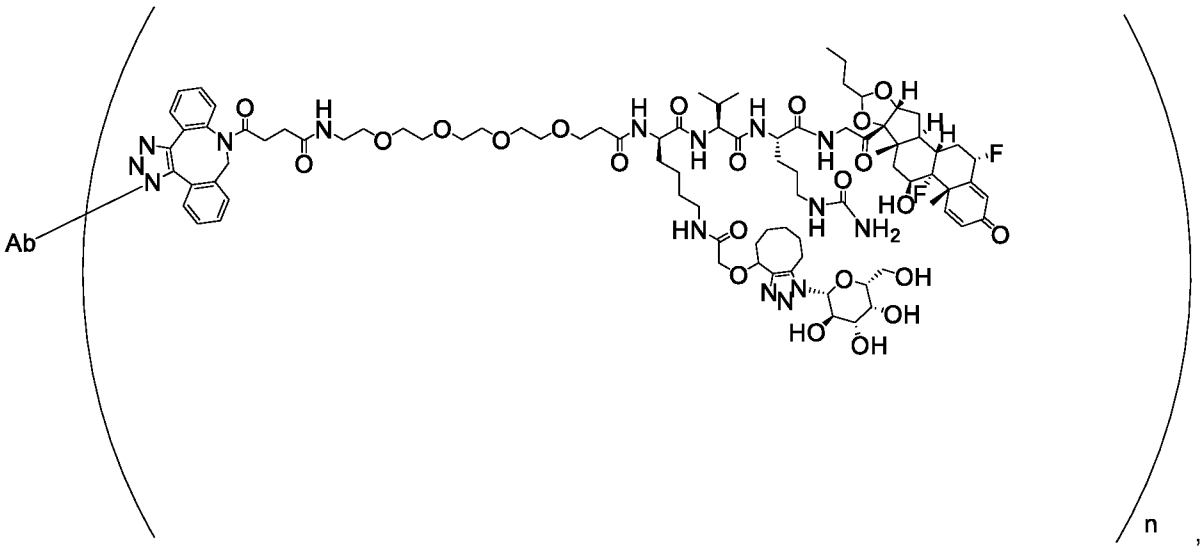
,

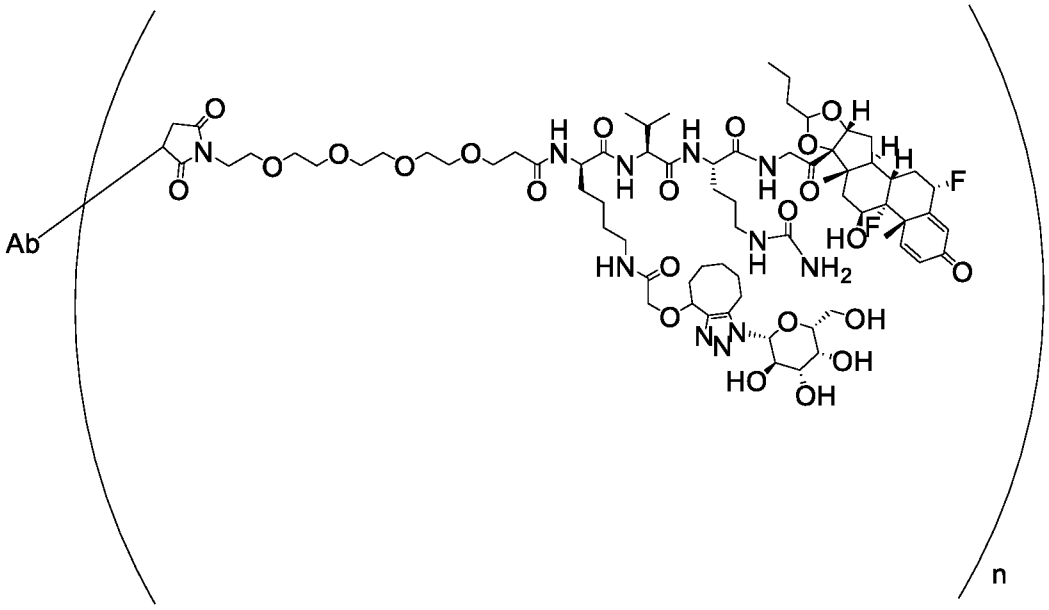


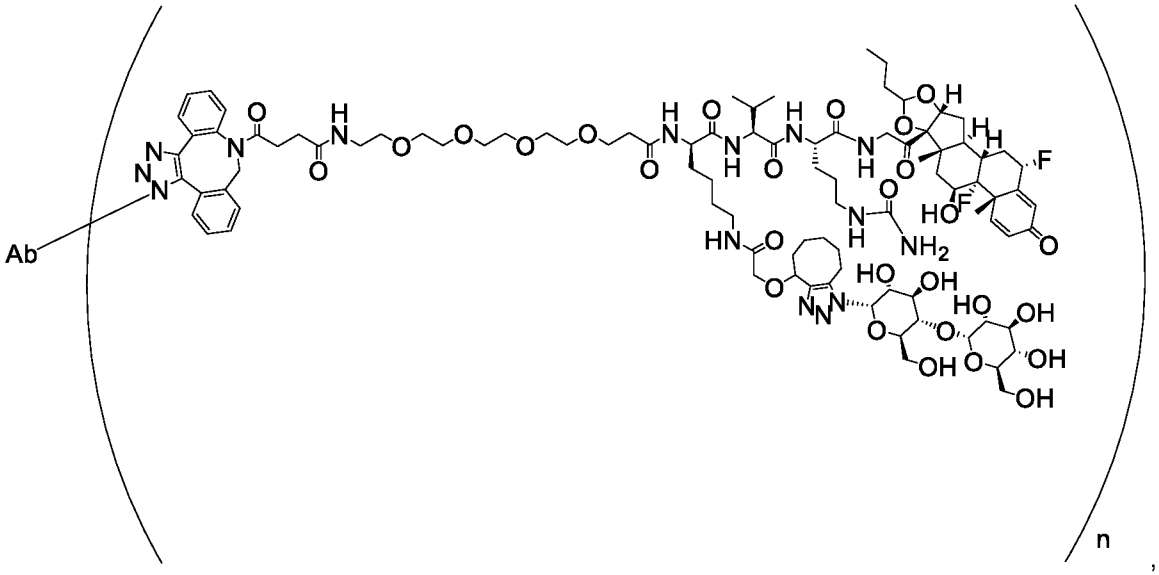
,

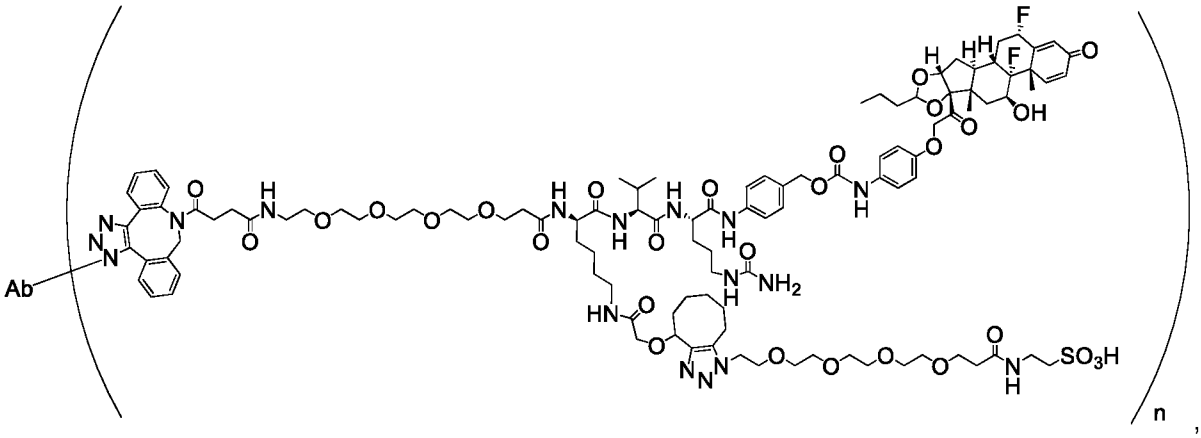


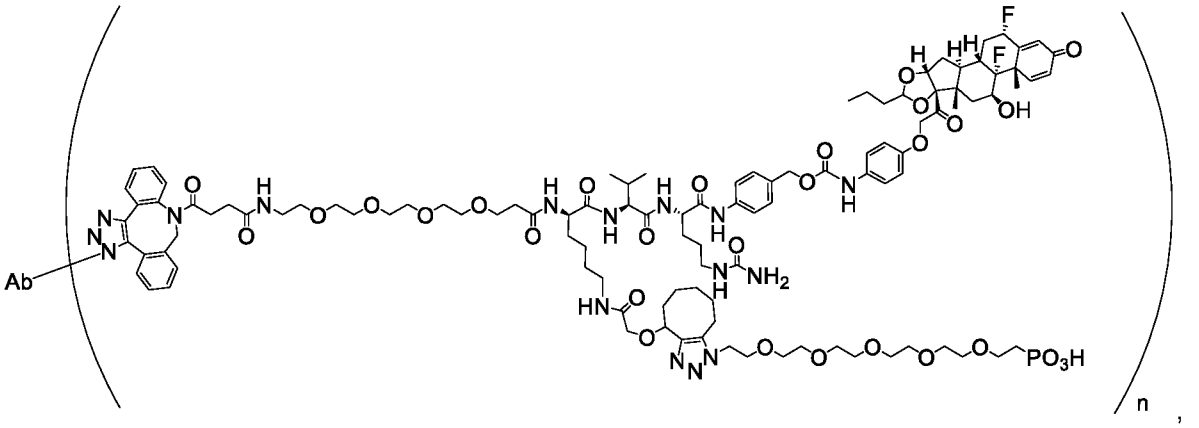
,

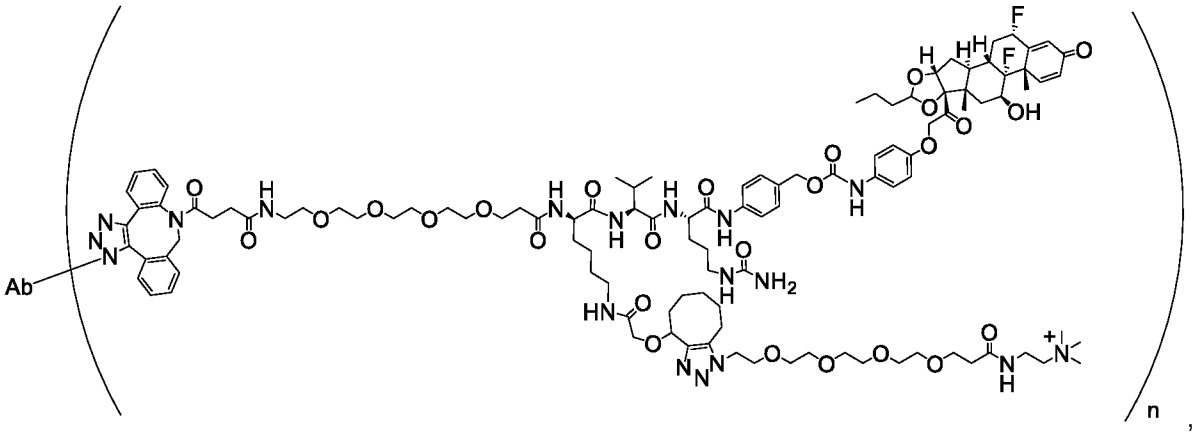


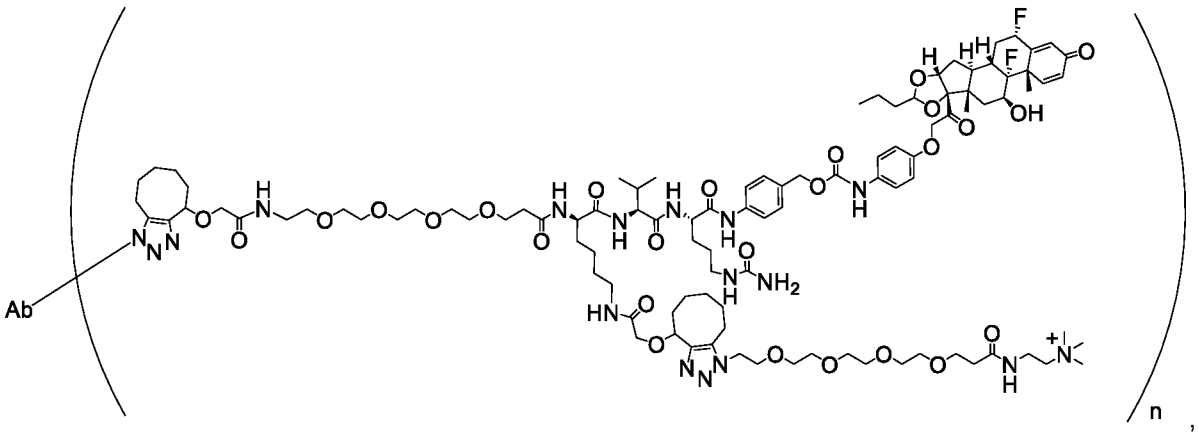


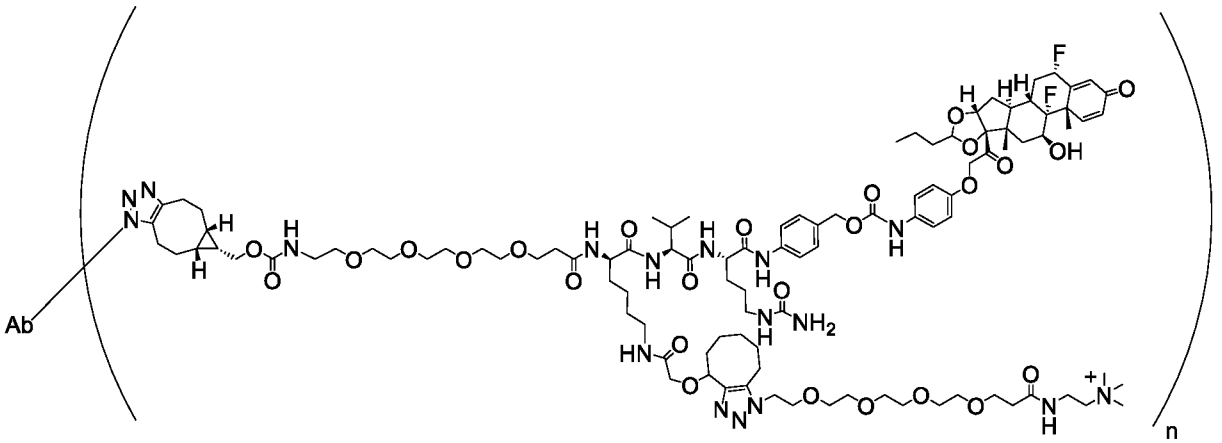


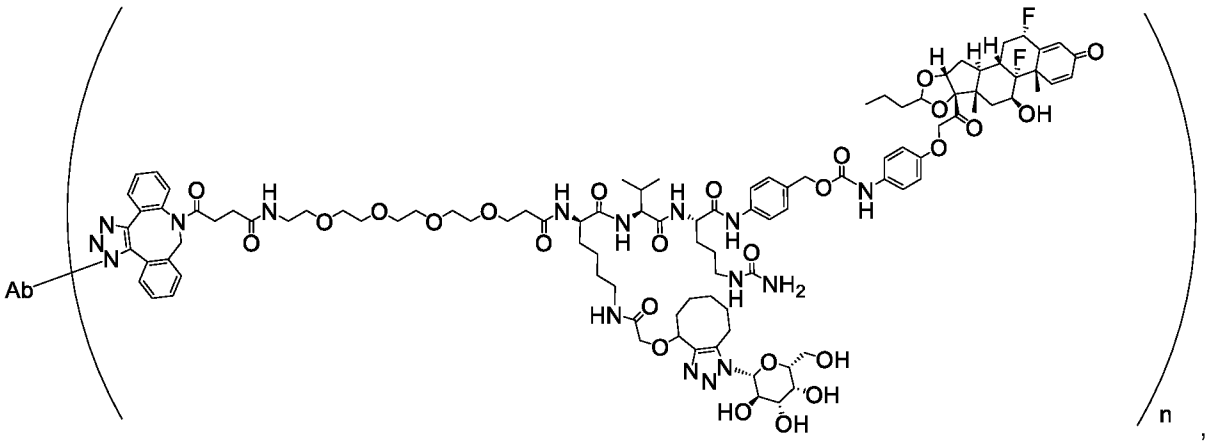


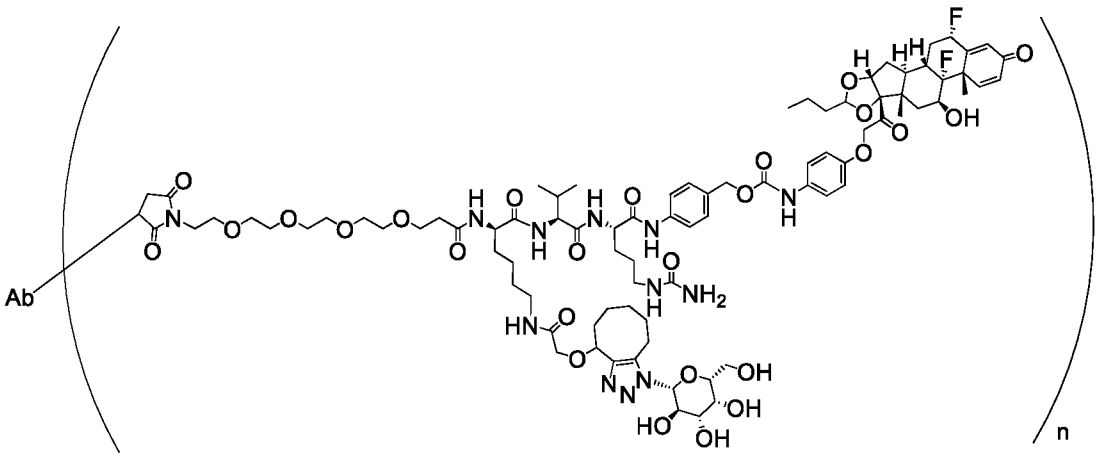


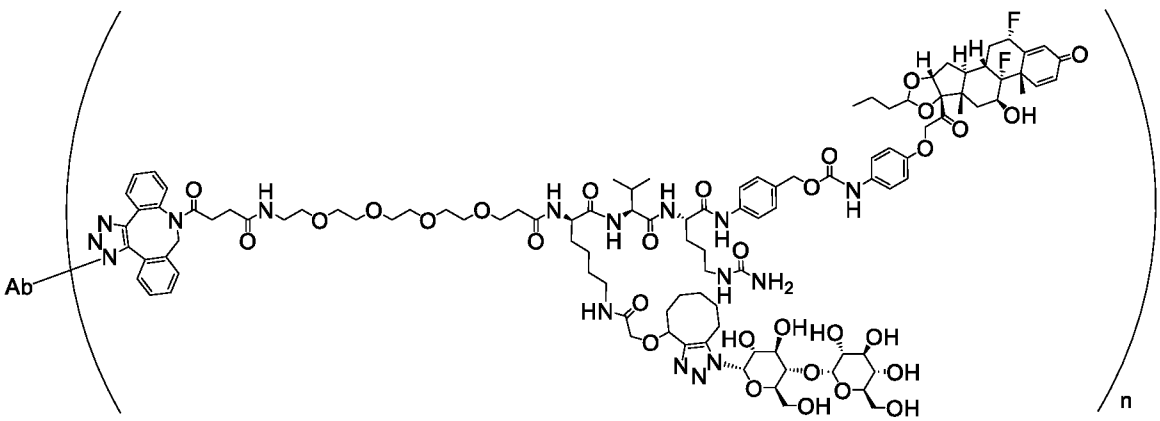


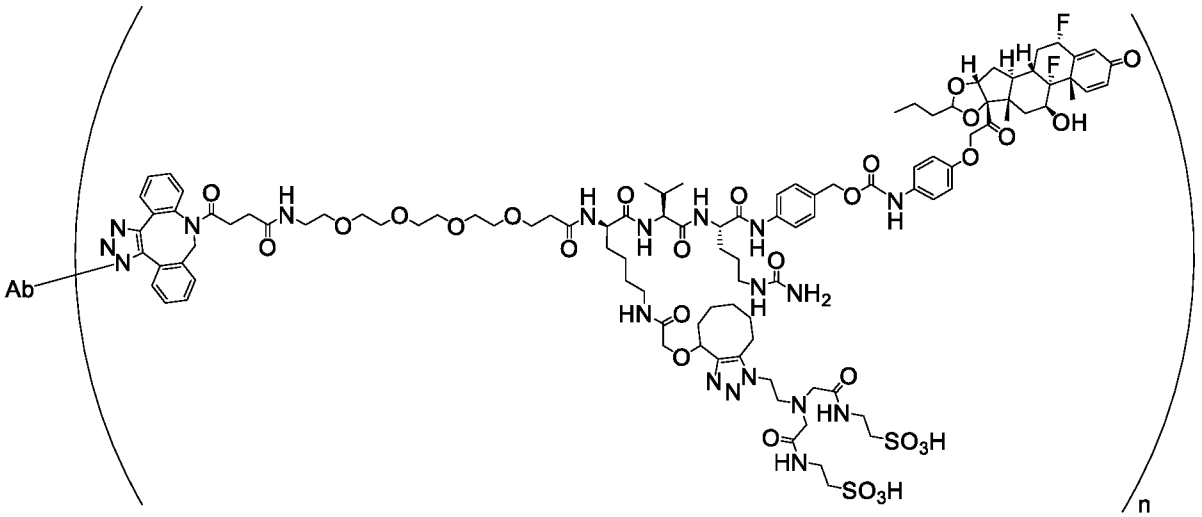


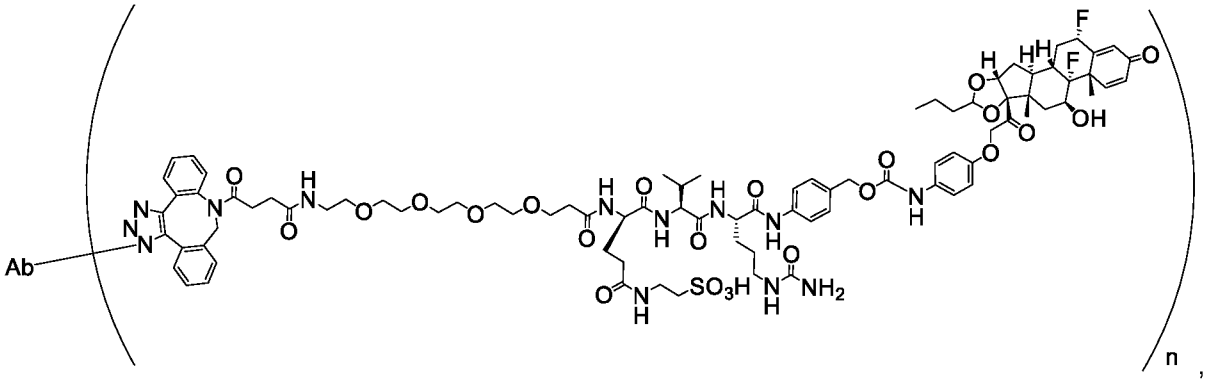


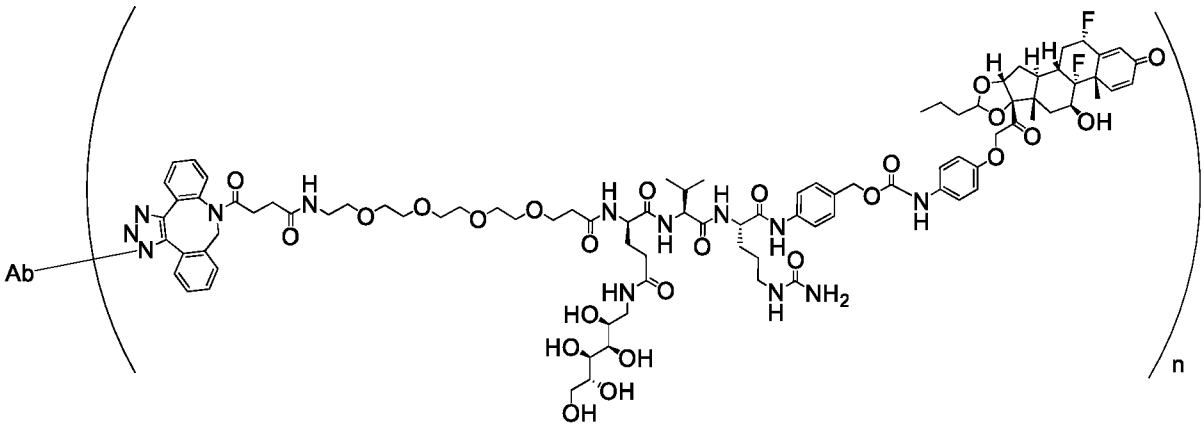


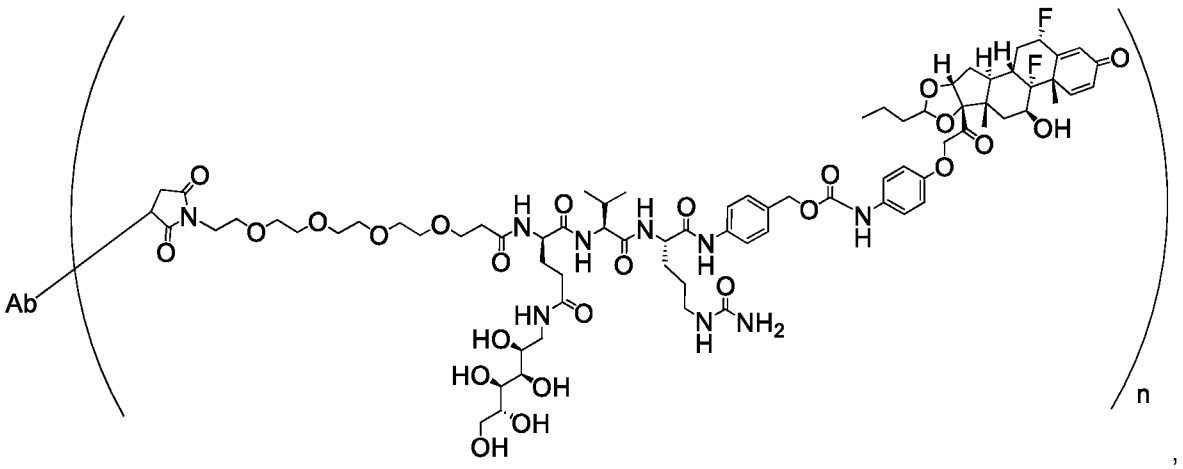


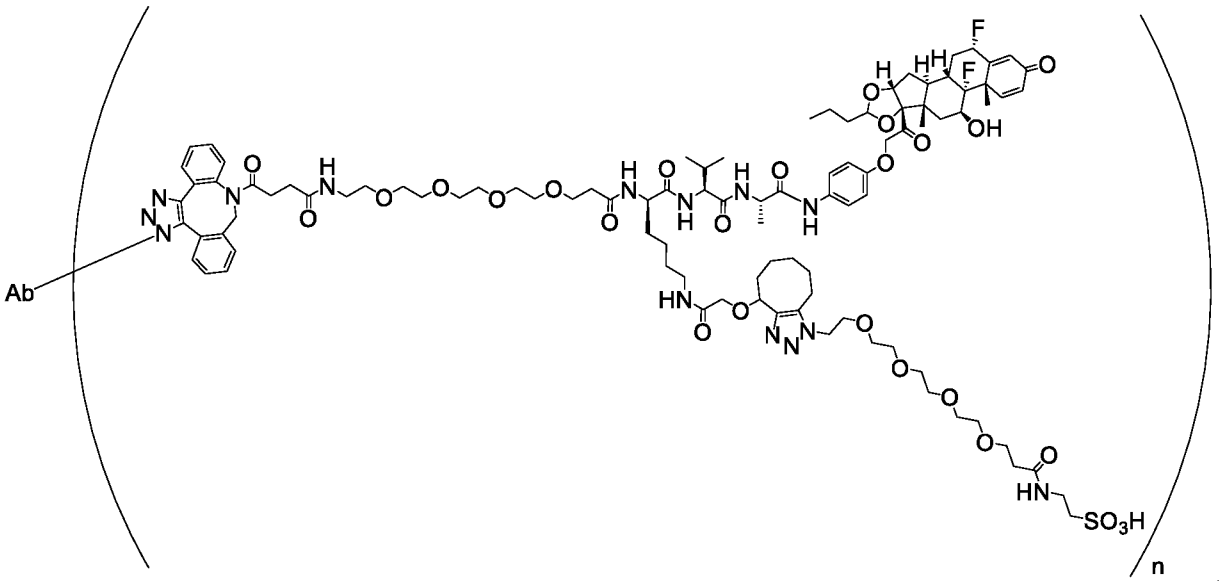




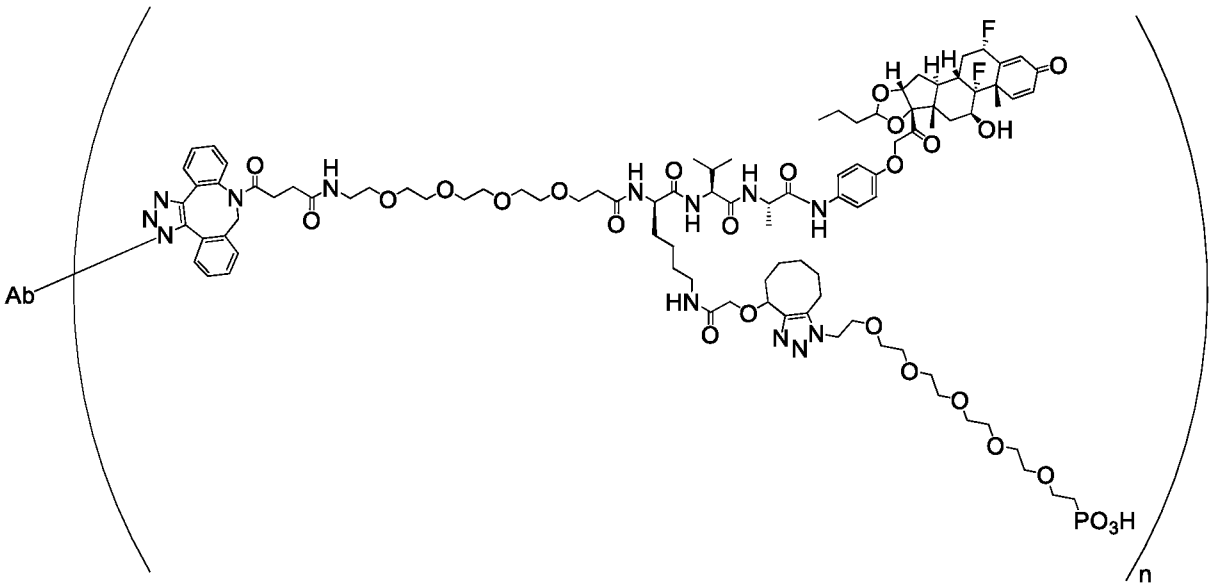




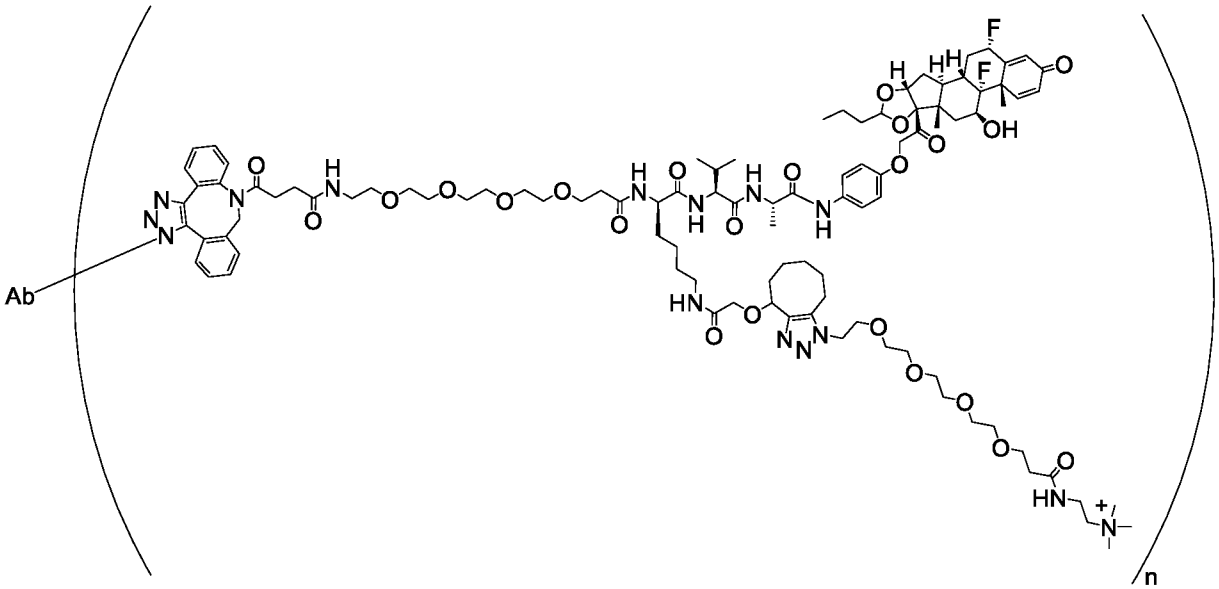




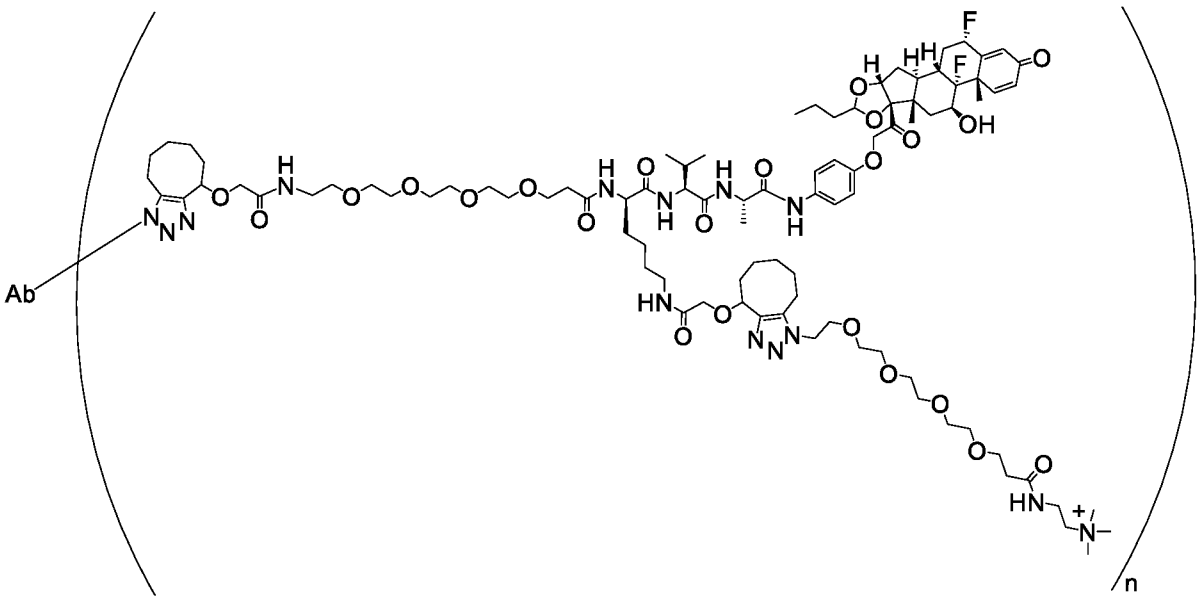
,

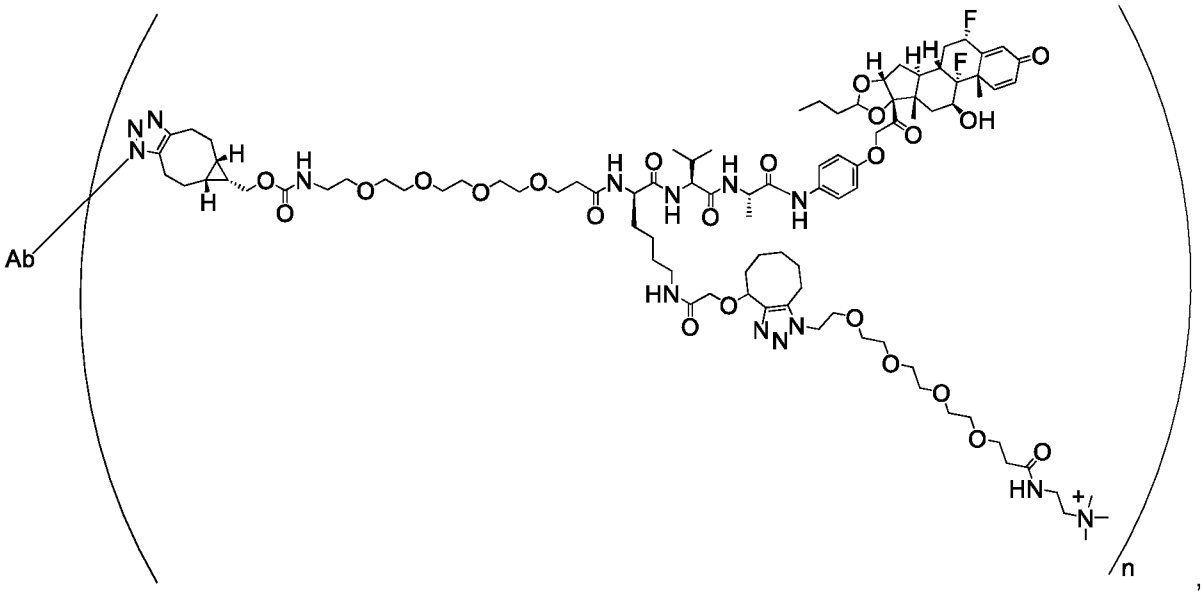


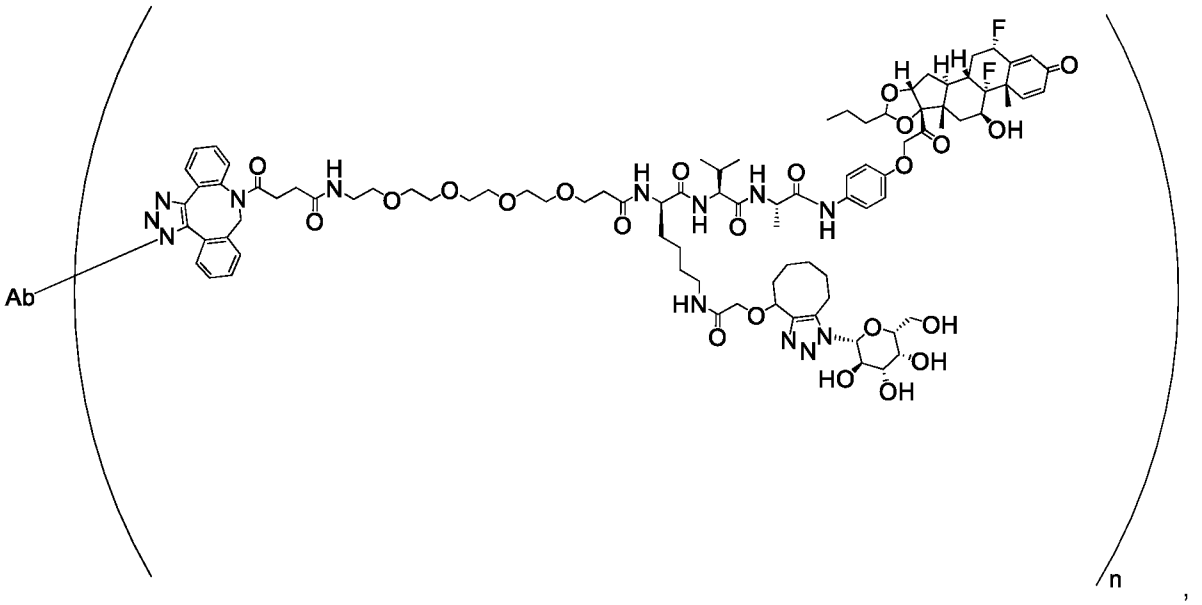
,

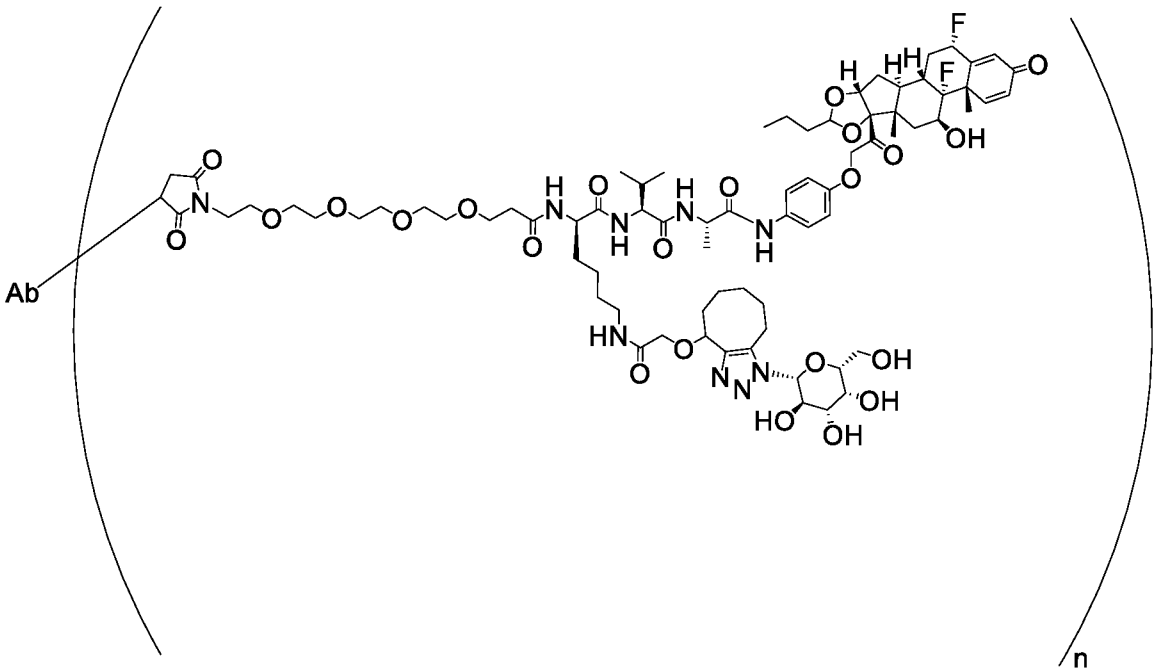


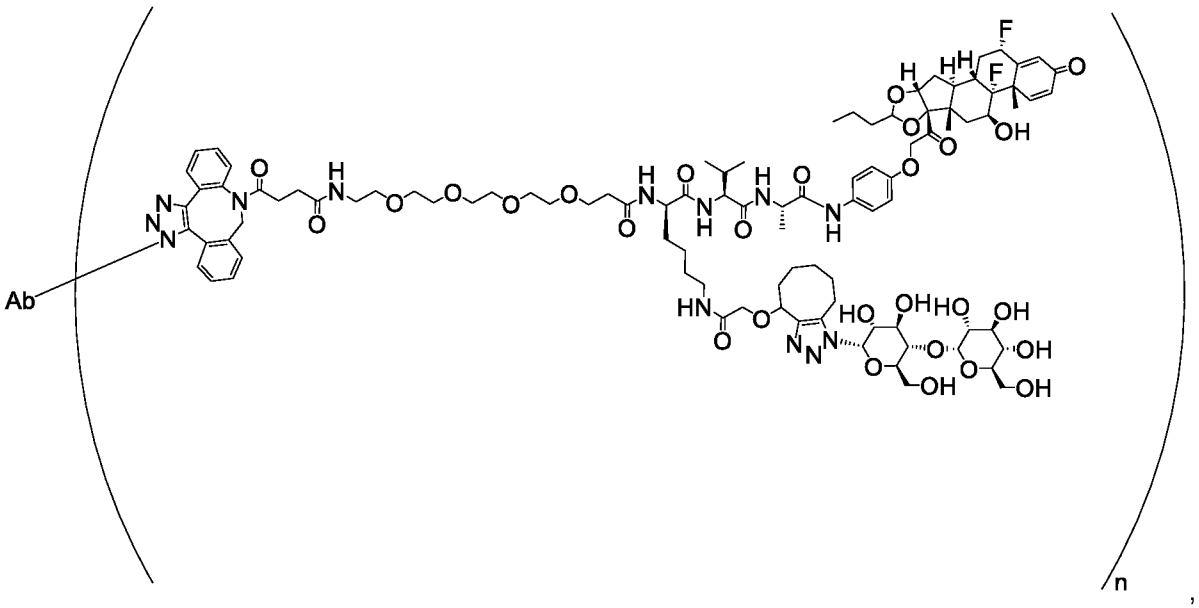
,

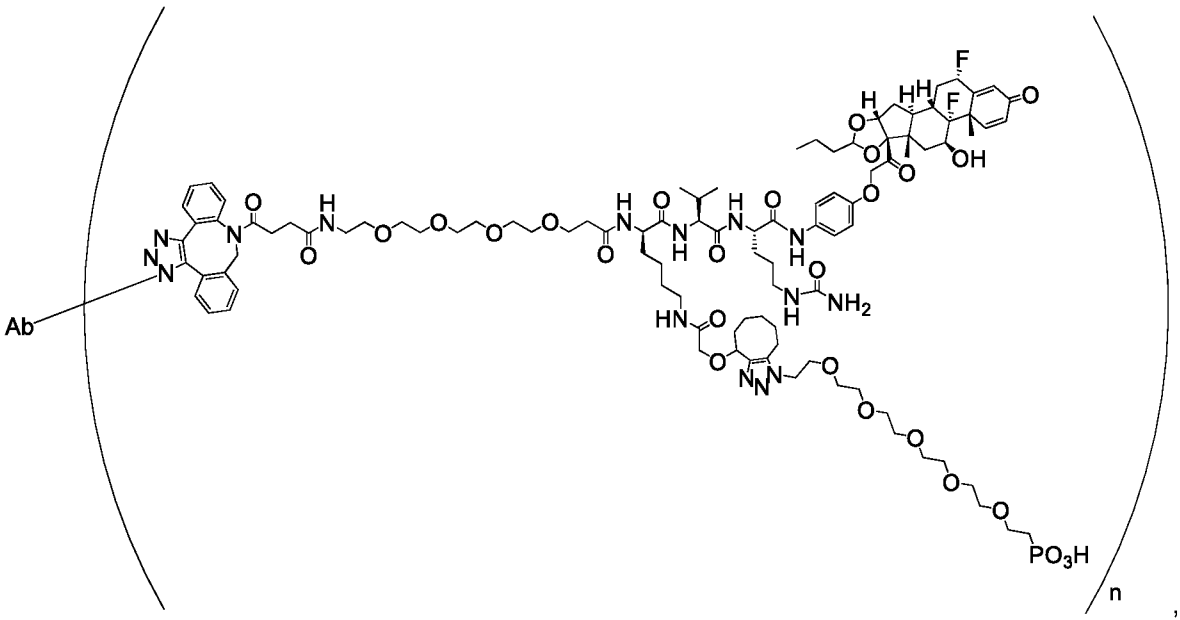




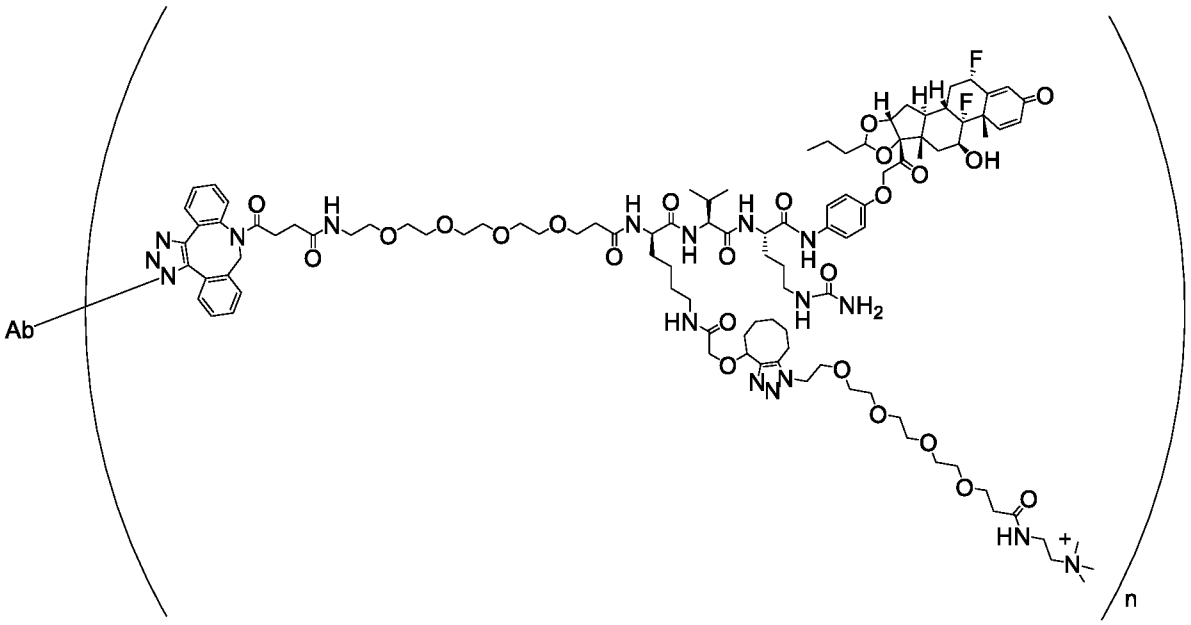




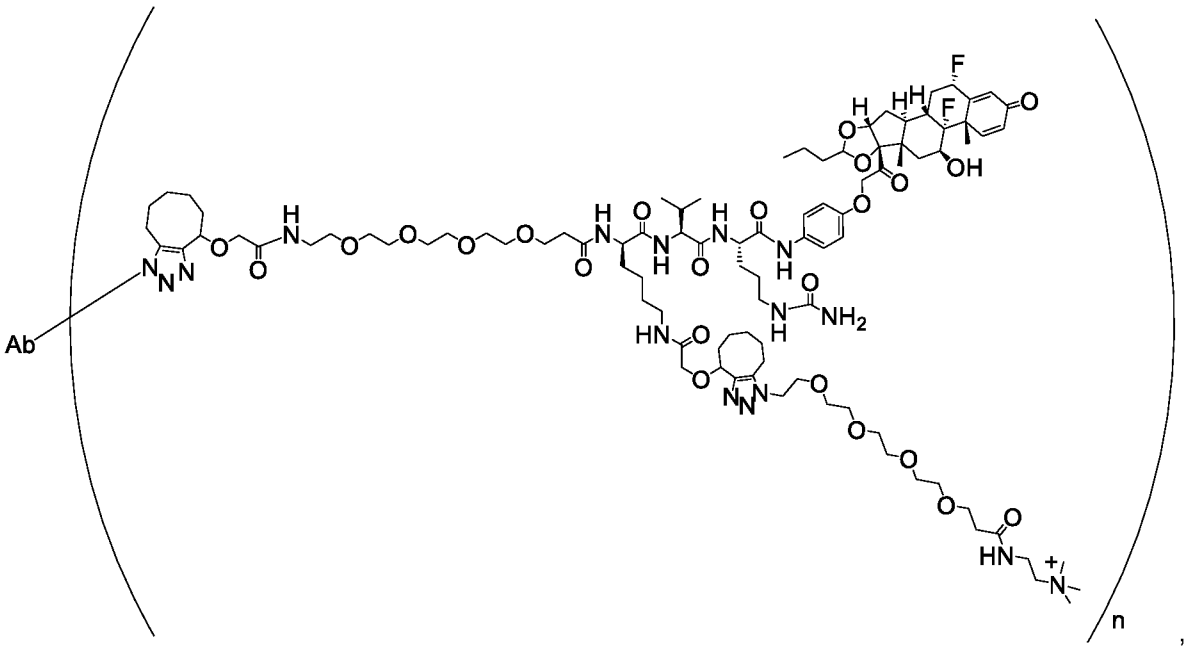


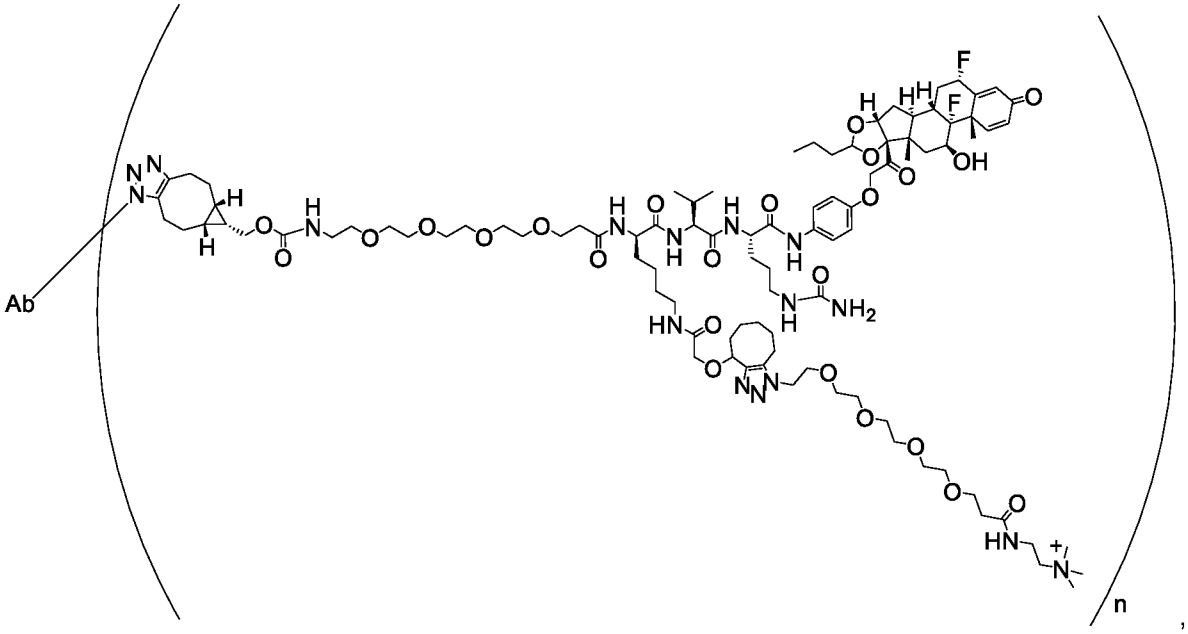

Ab
F
H
H
F
OH
O
N
H
NH2
HN
PO3H
n

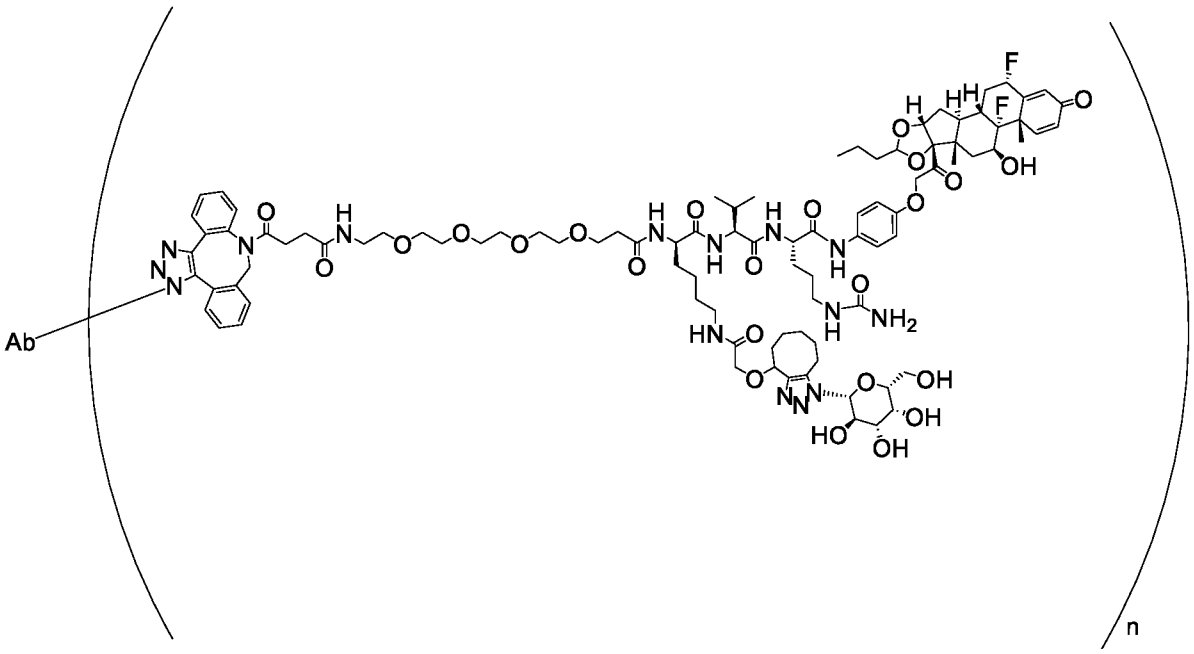
,

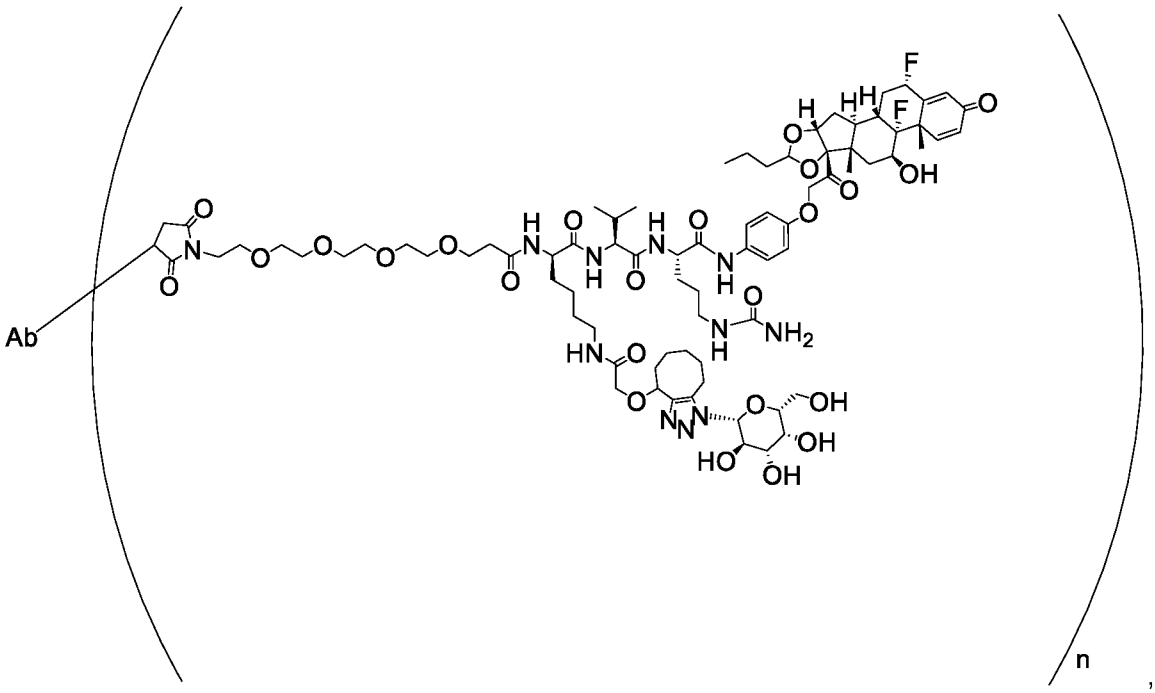

Ab
F
H
H
F
OH
O
N
H
NH2
HN
HN
+
N
n

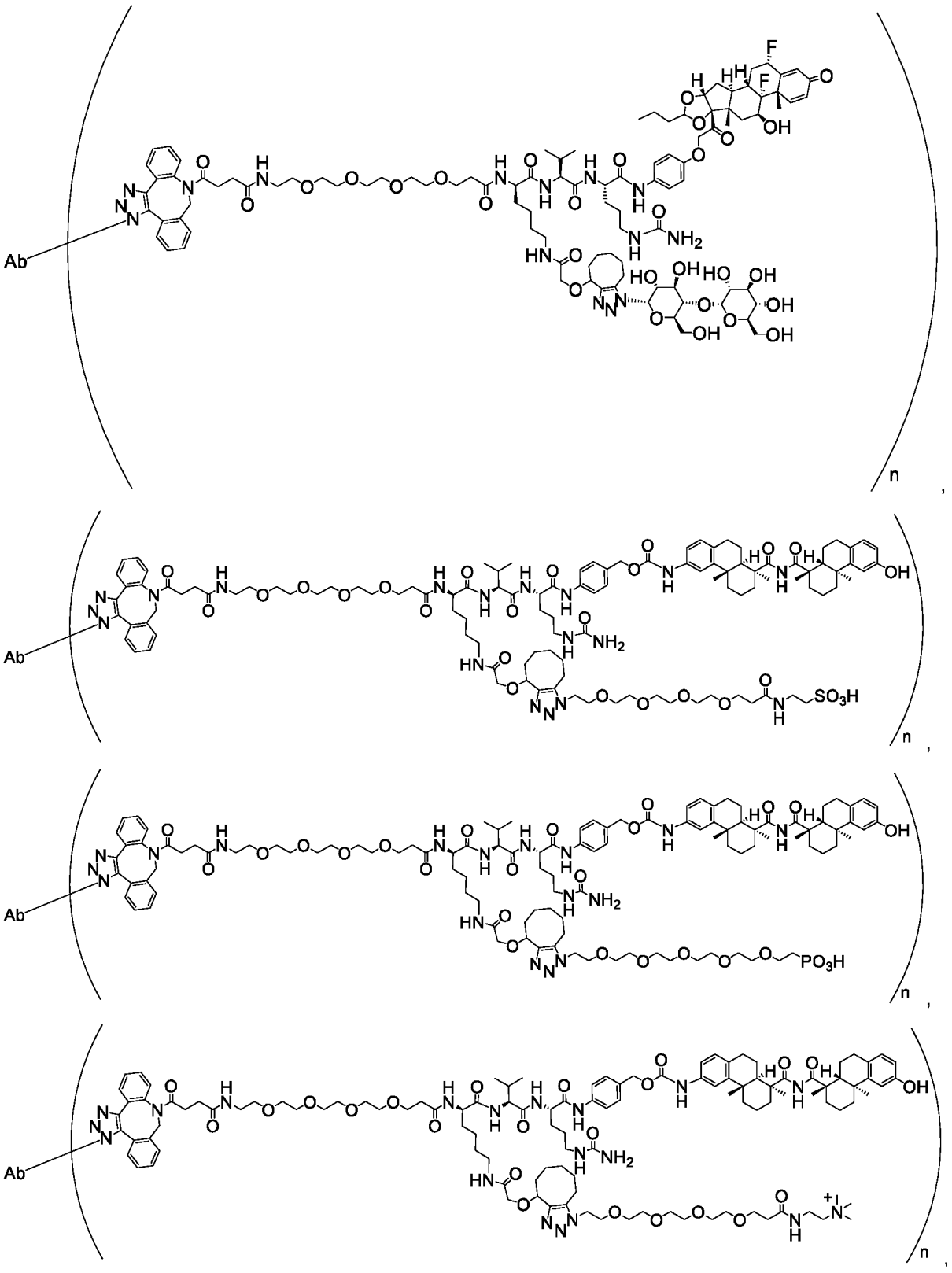
,

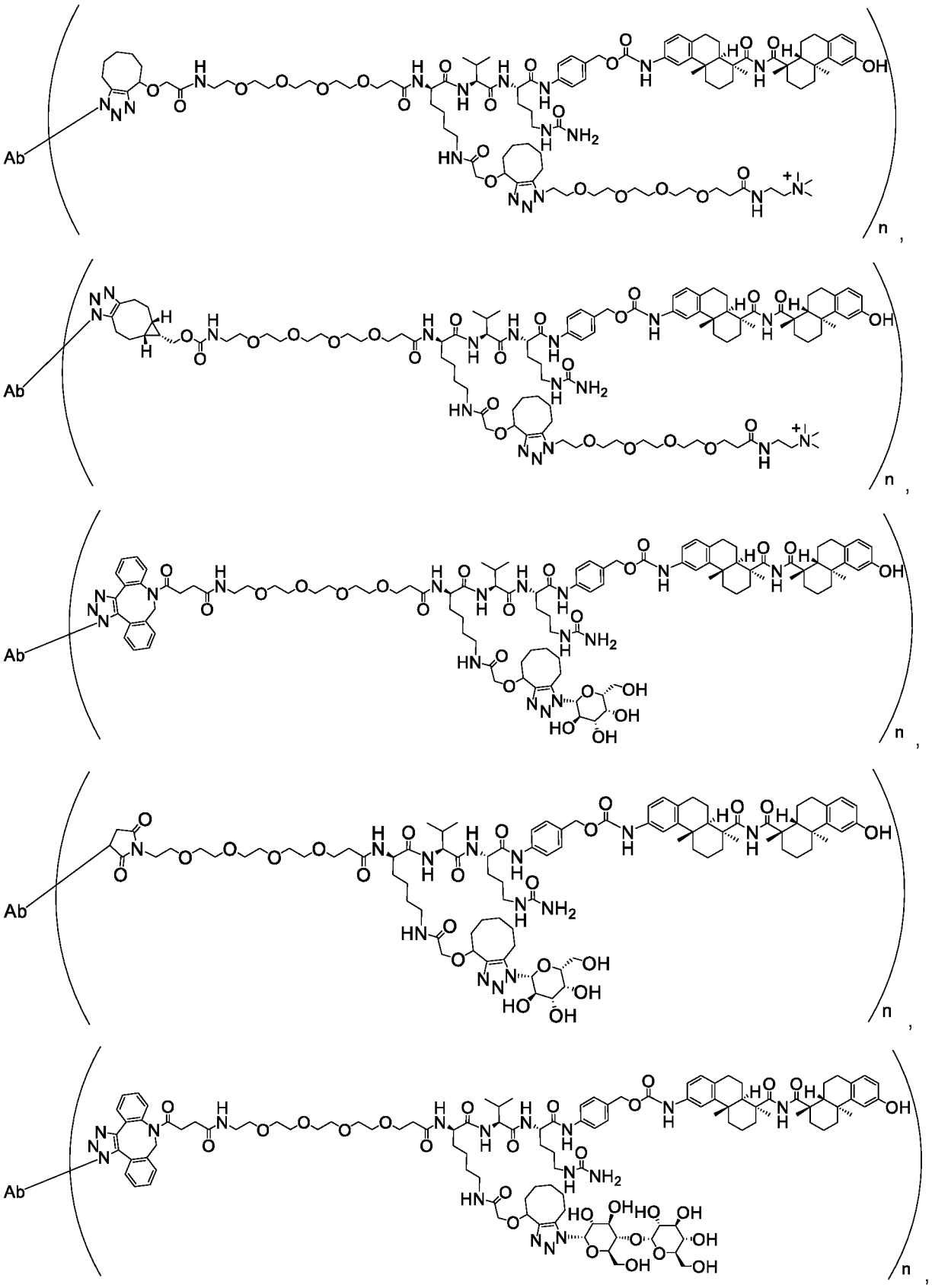


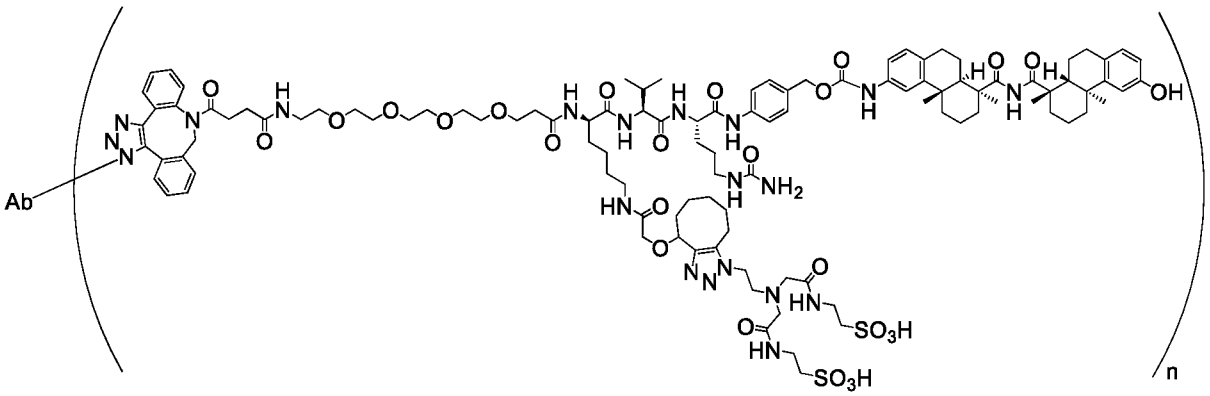




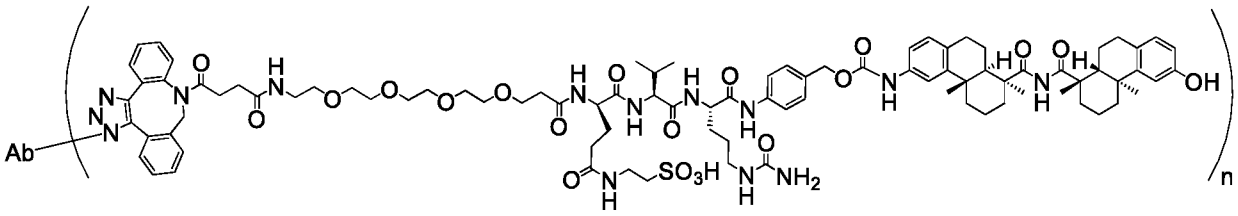




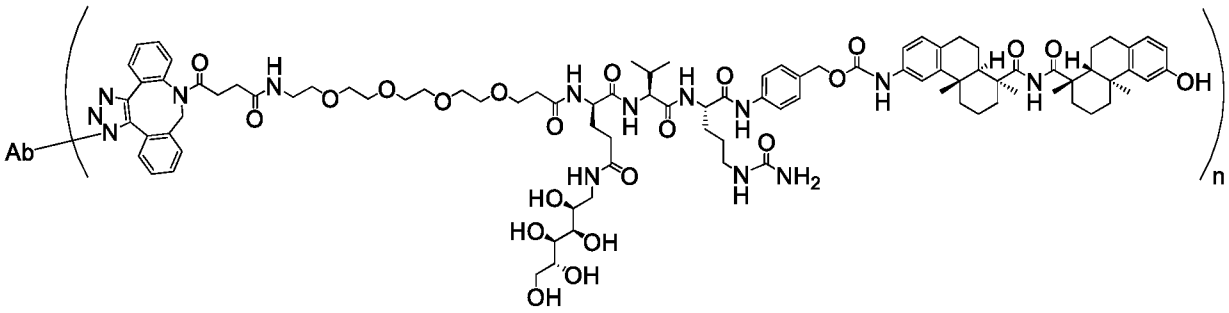




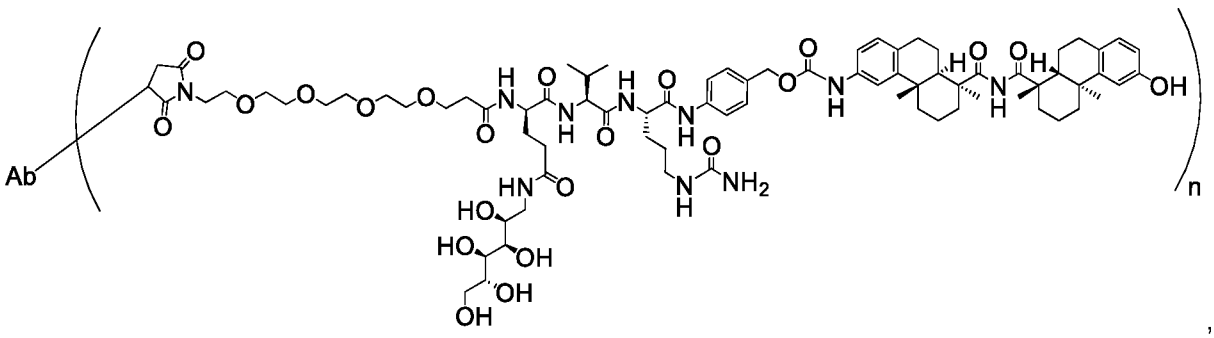
,



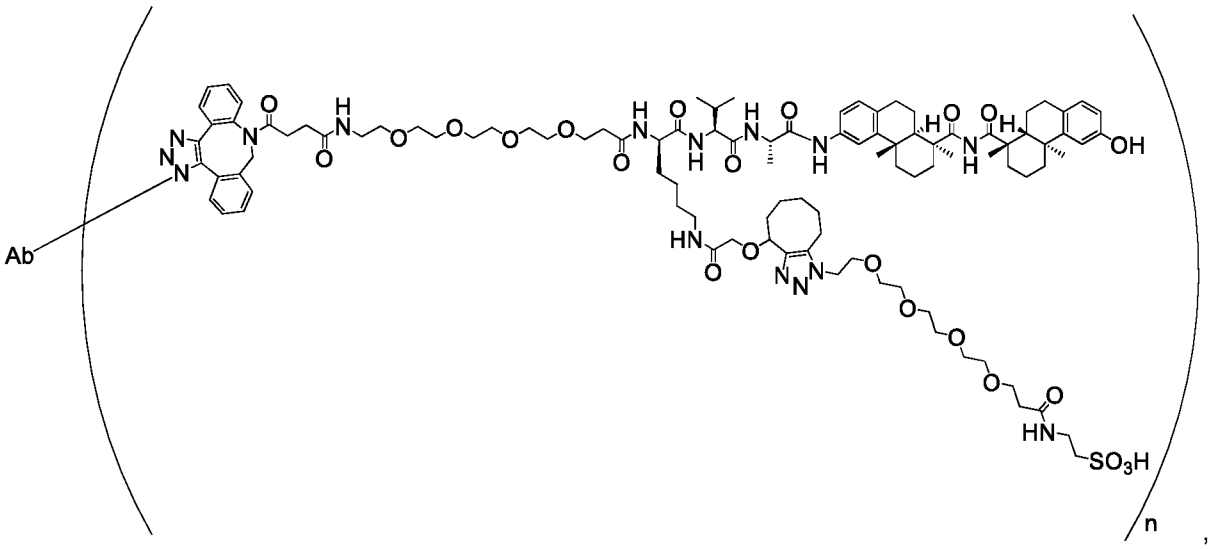
,



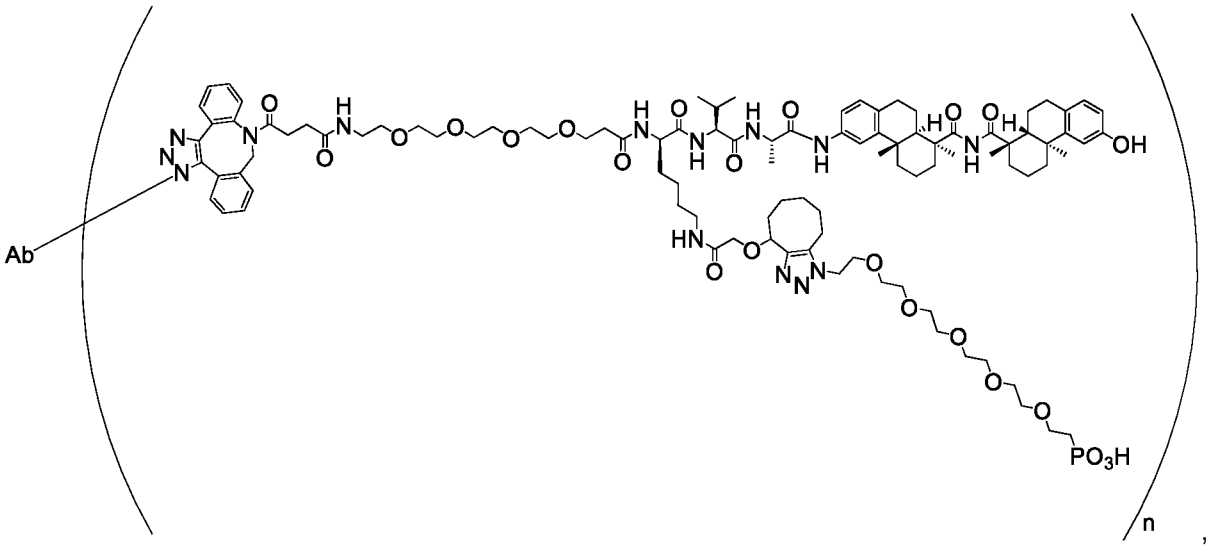
,



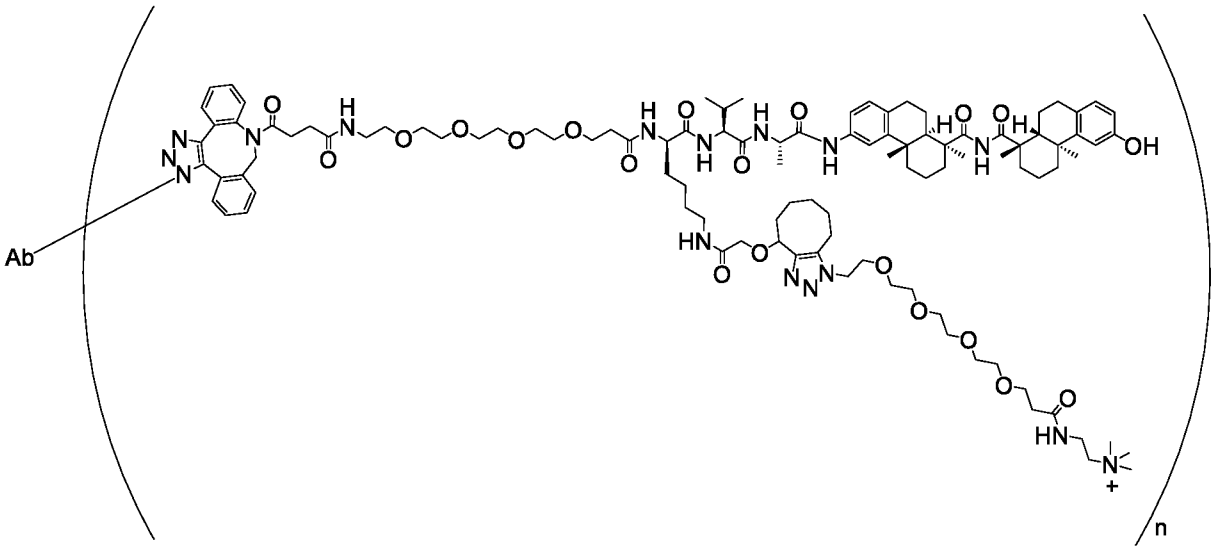
,


Ab
OH
HN
O
N
N
O
HN
SO3H
n

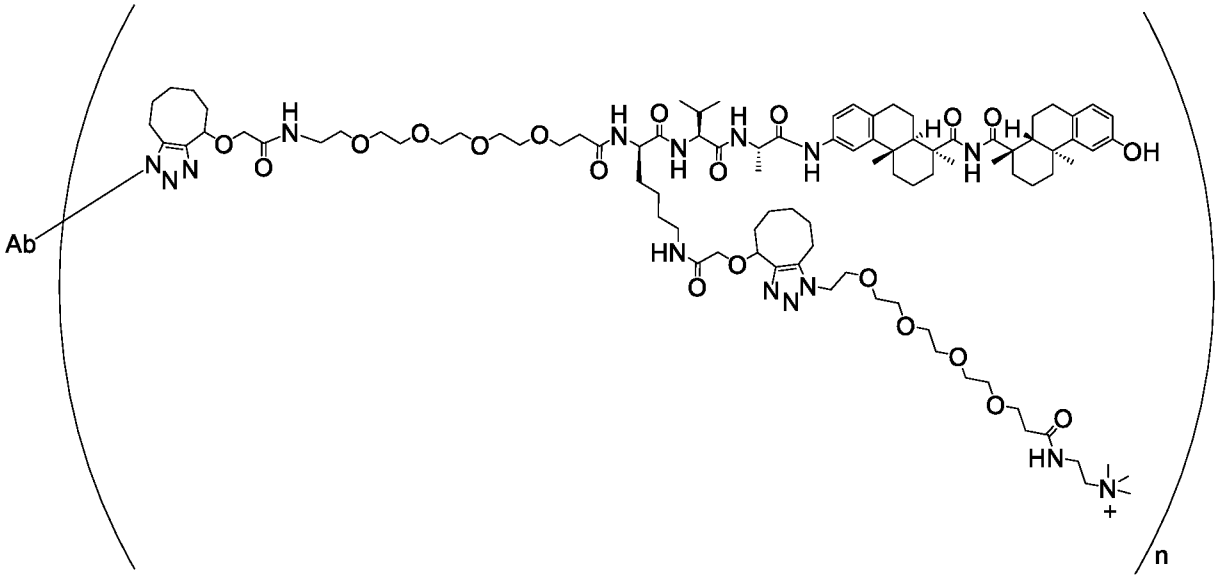
,


Ab
OH
HN
O
N
N
O
PO3H
n

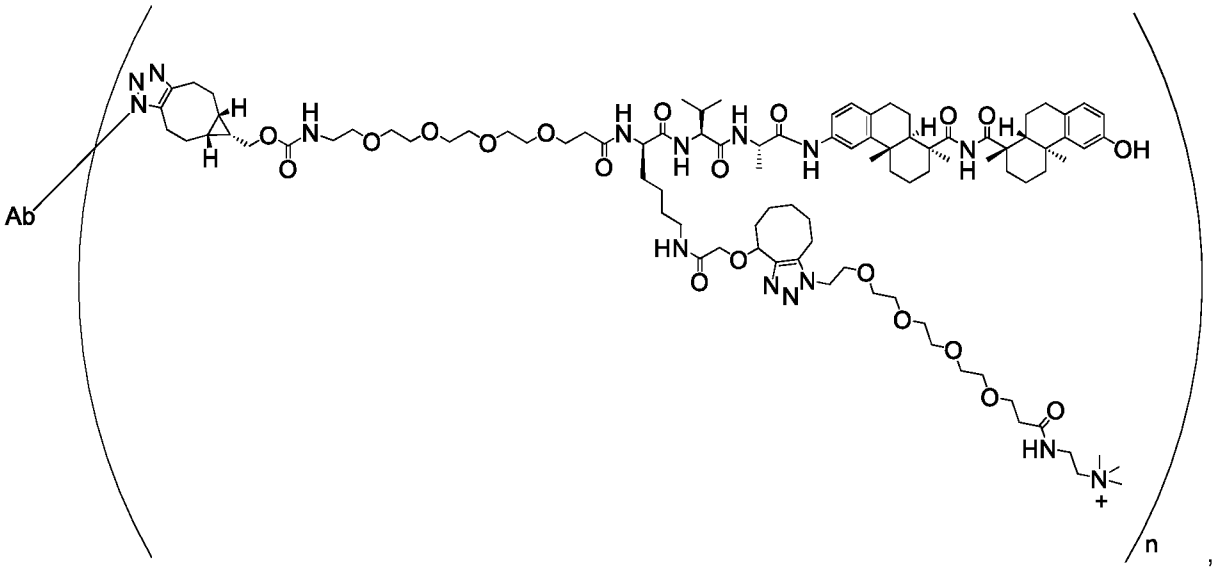
,


Ab
OH
HN
O
N
N
O
HN
N+
n

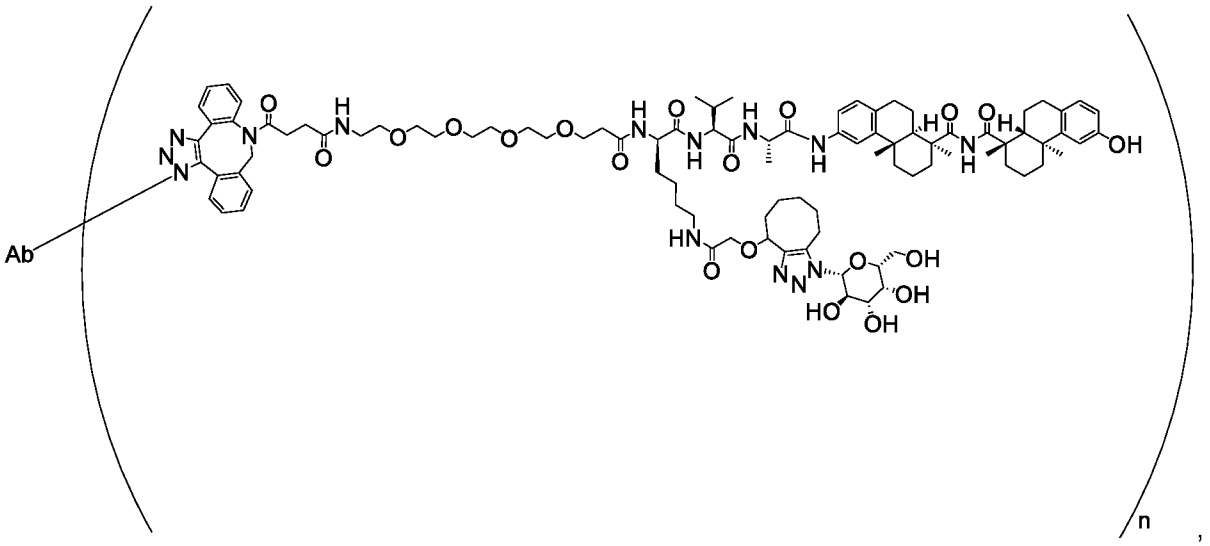
,



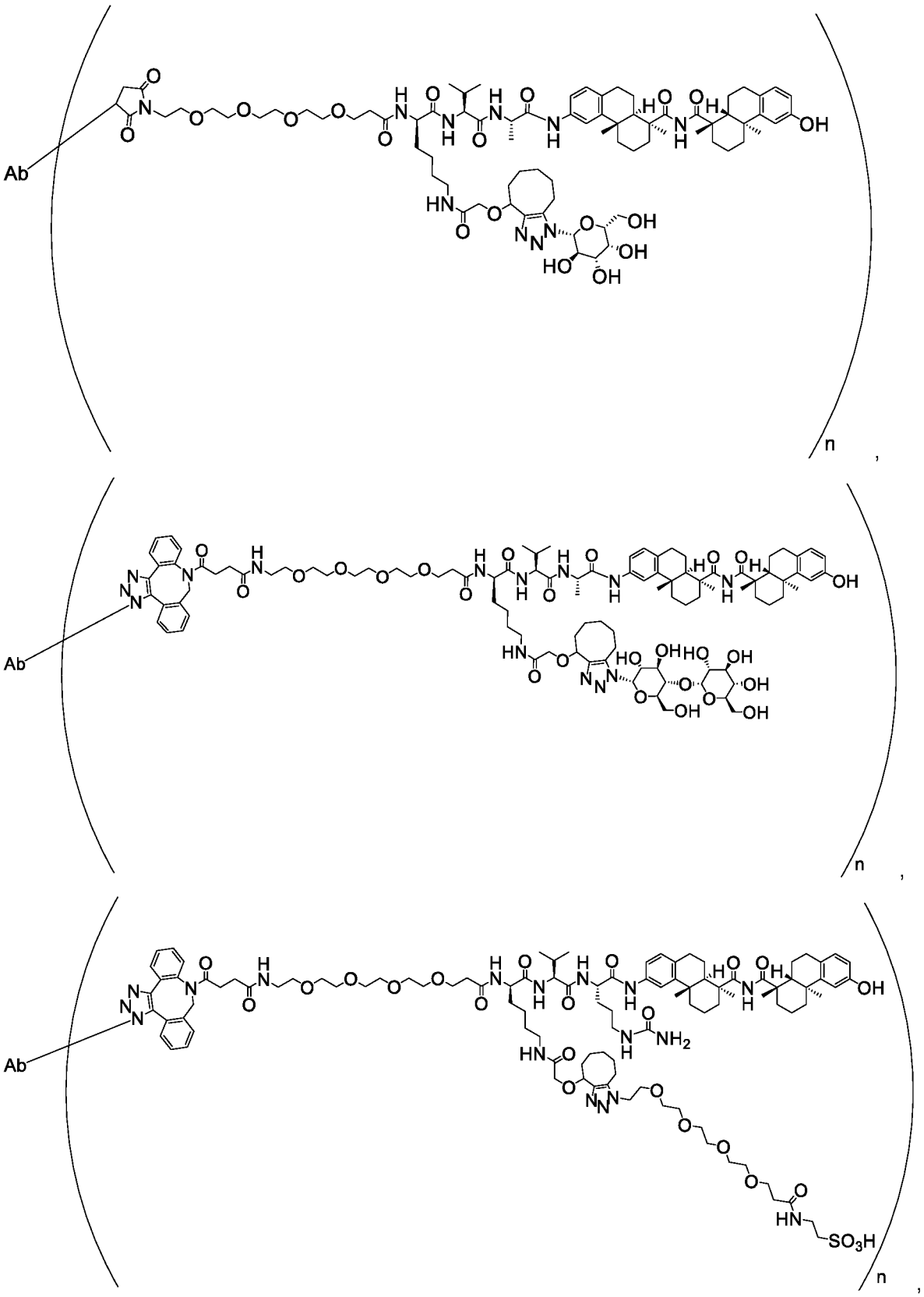
,

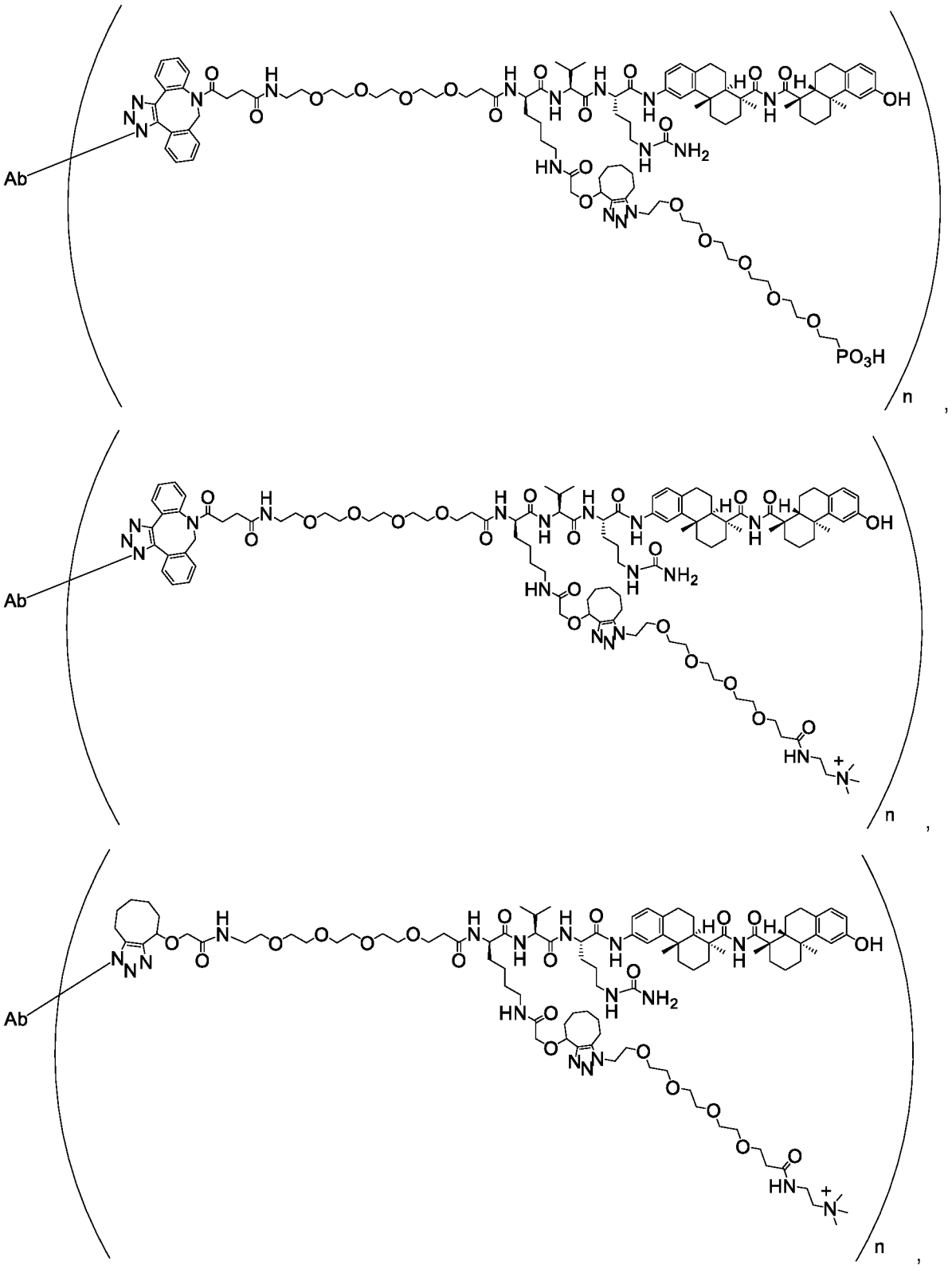


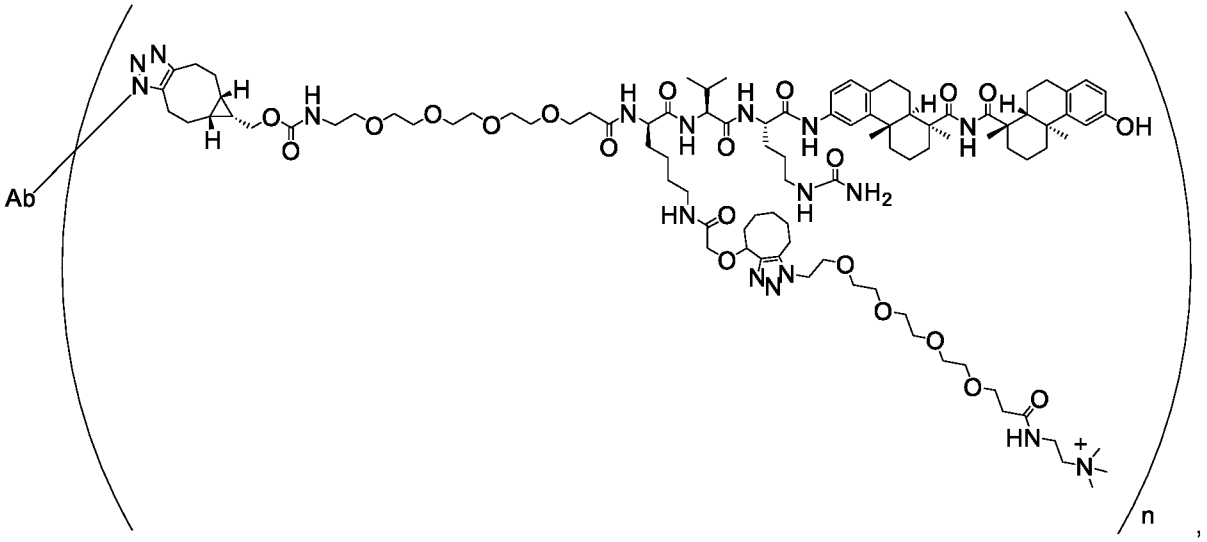
,

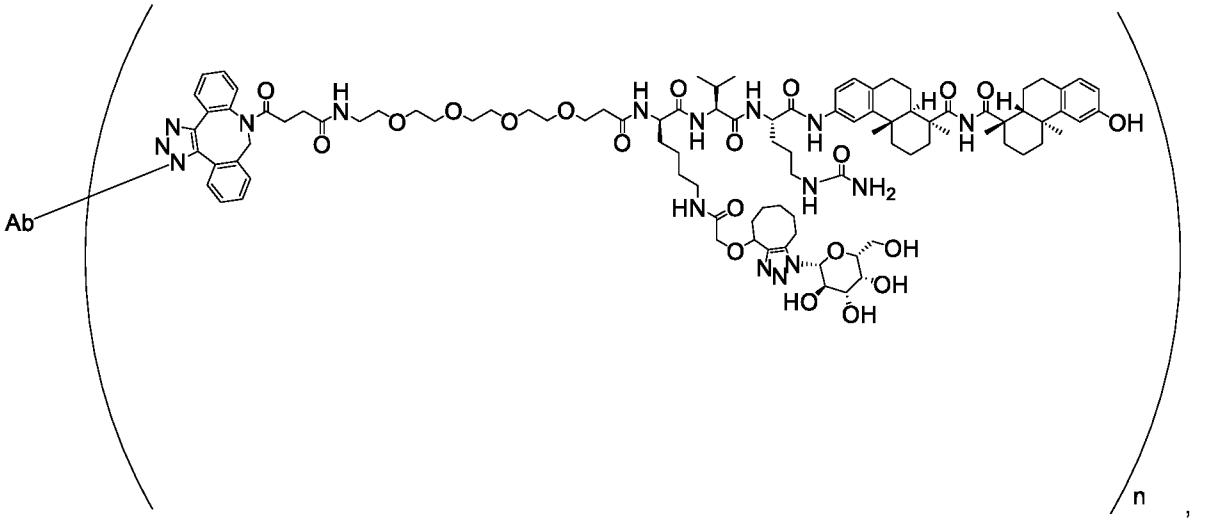


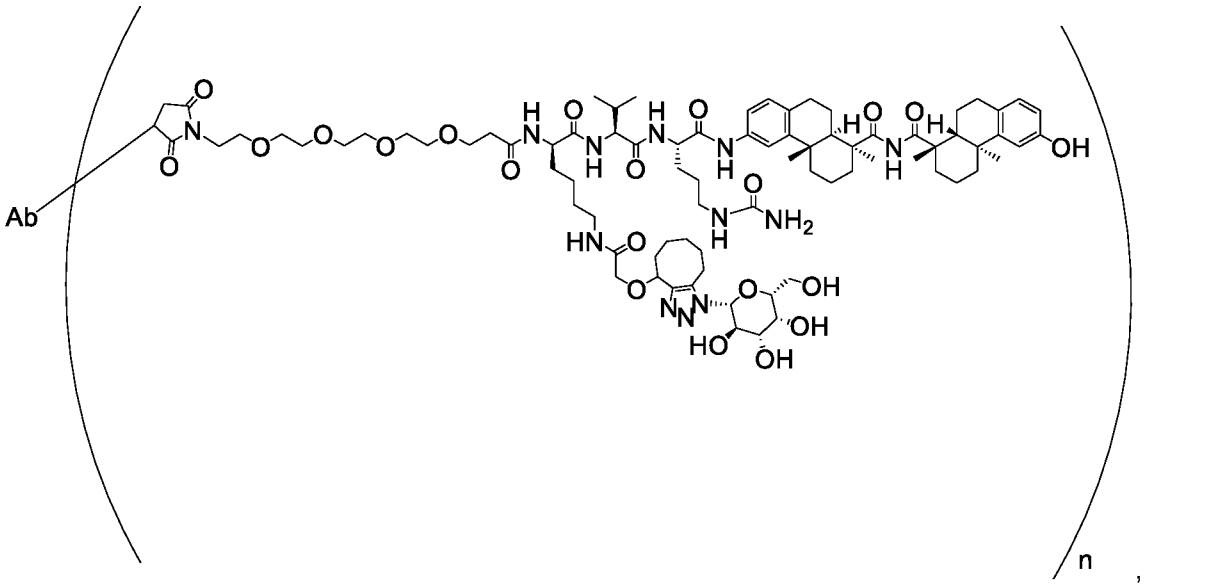
,

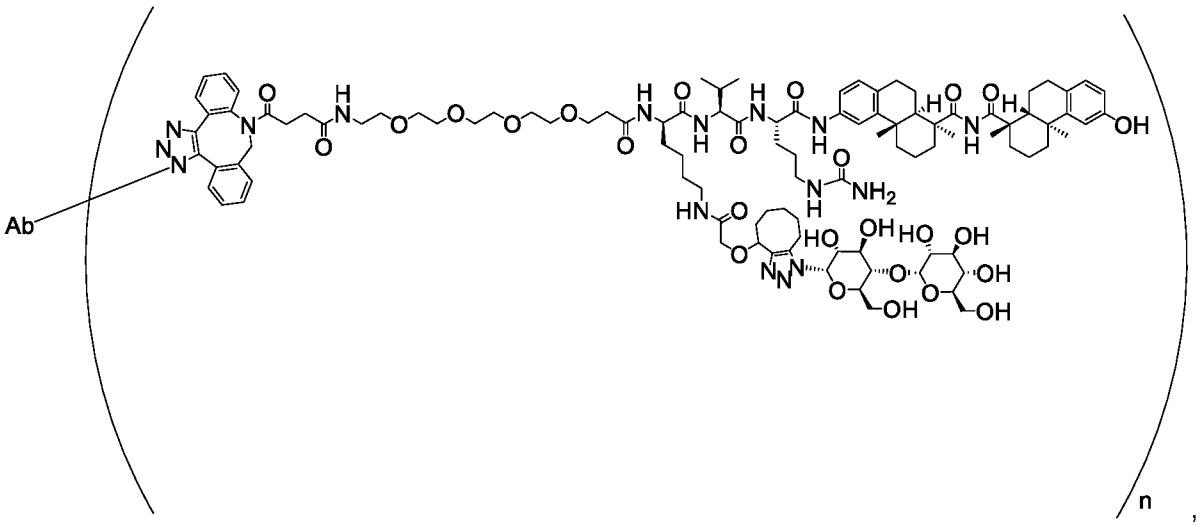




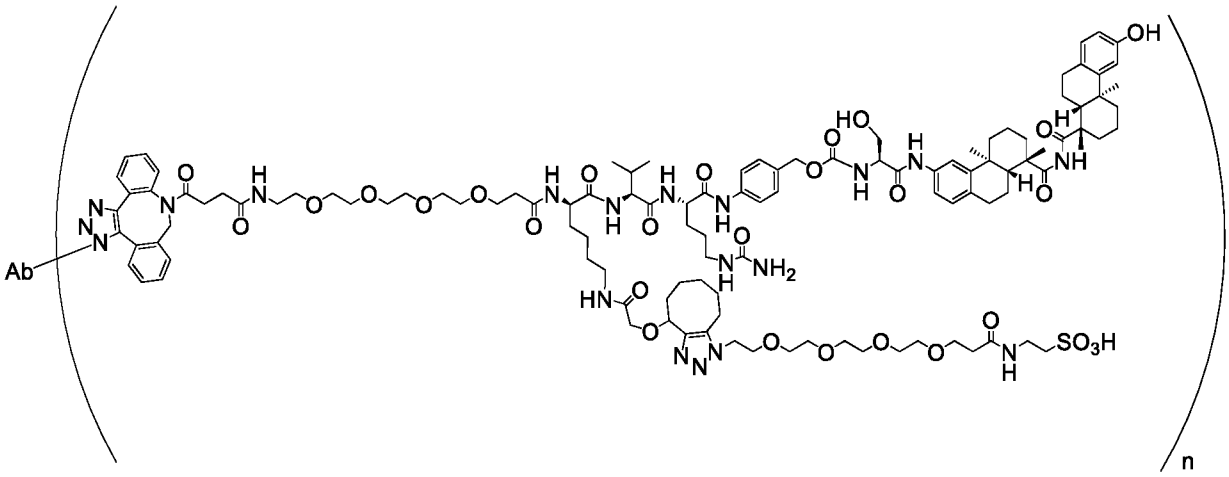




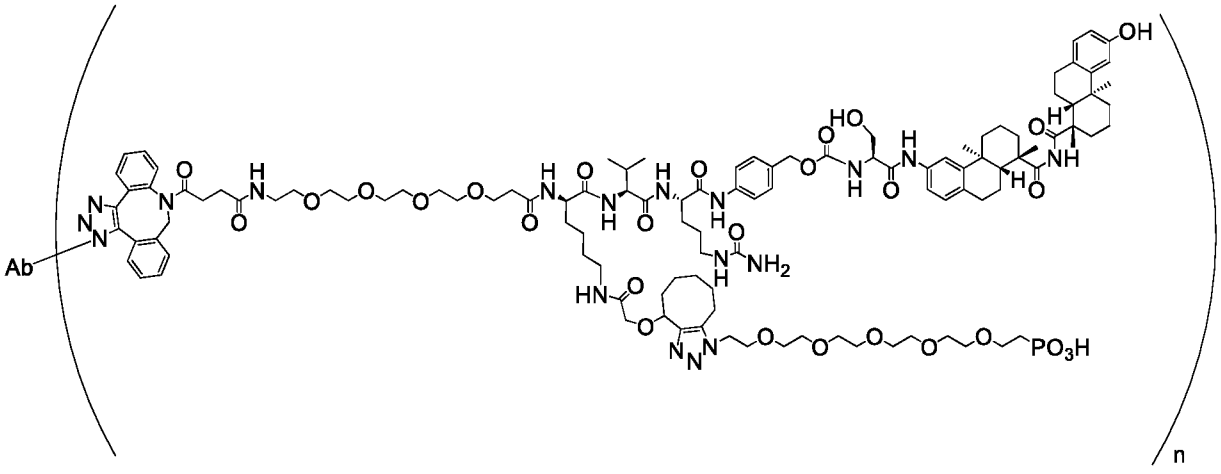




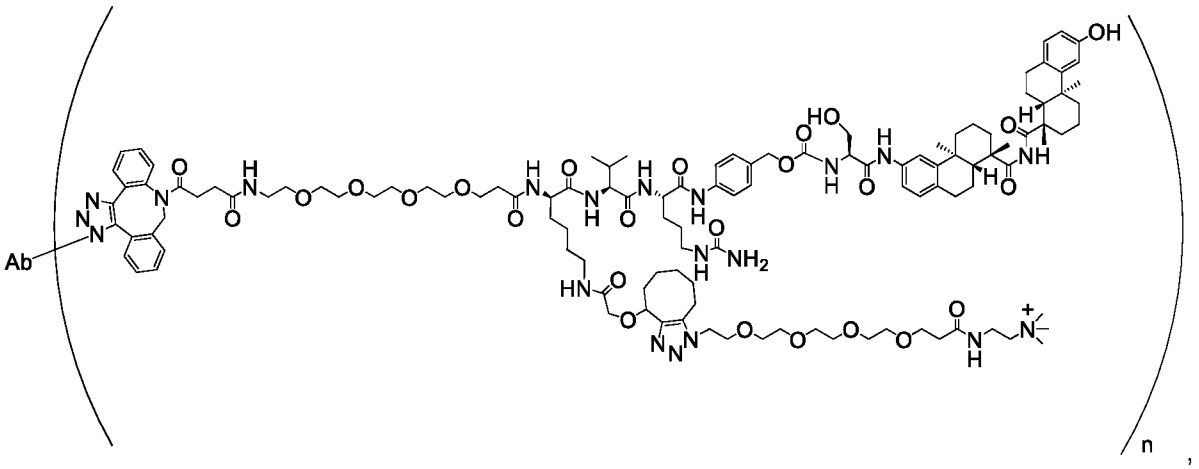
,

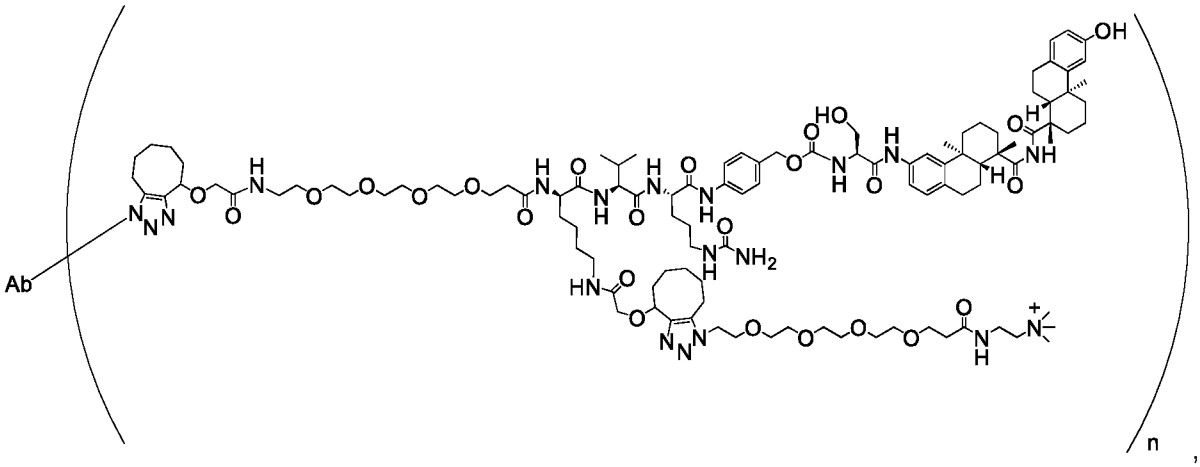


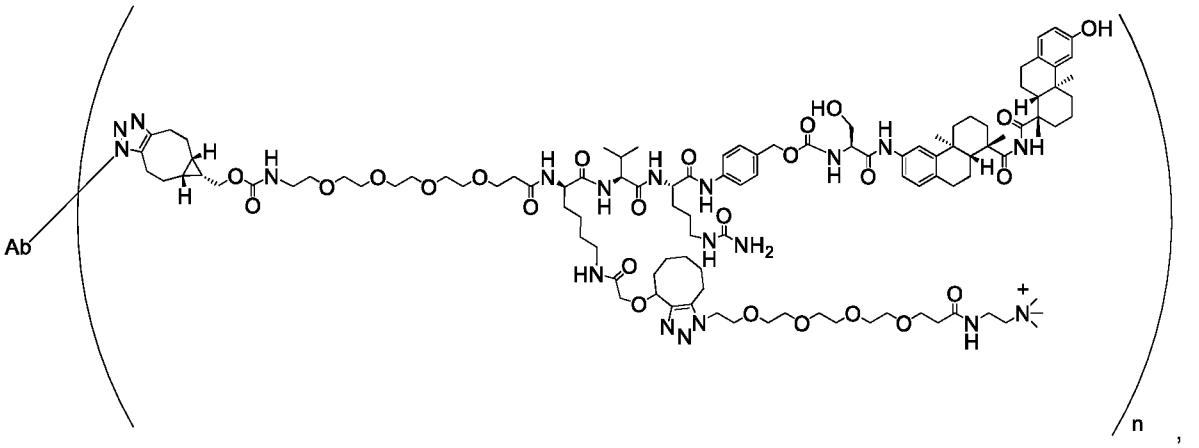
,

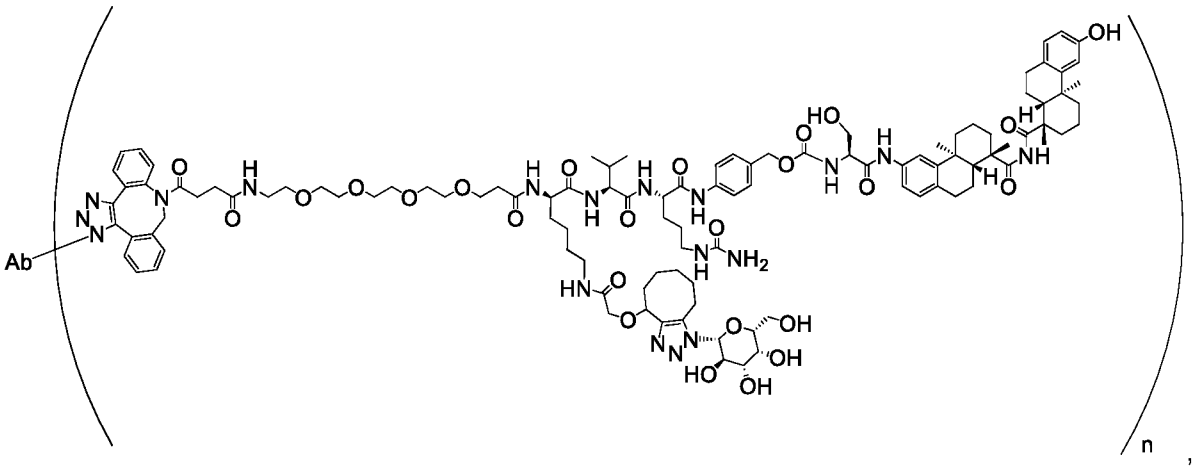


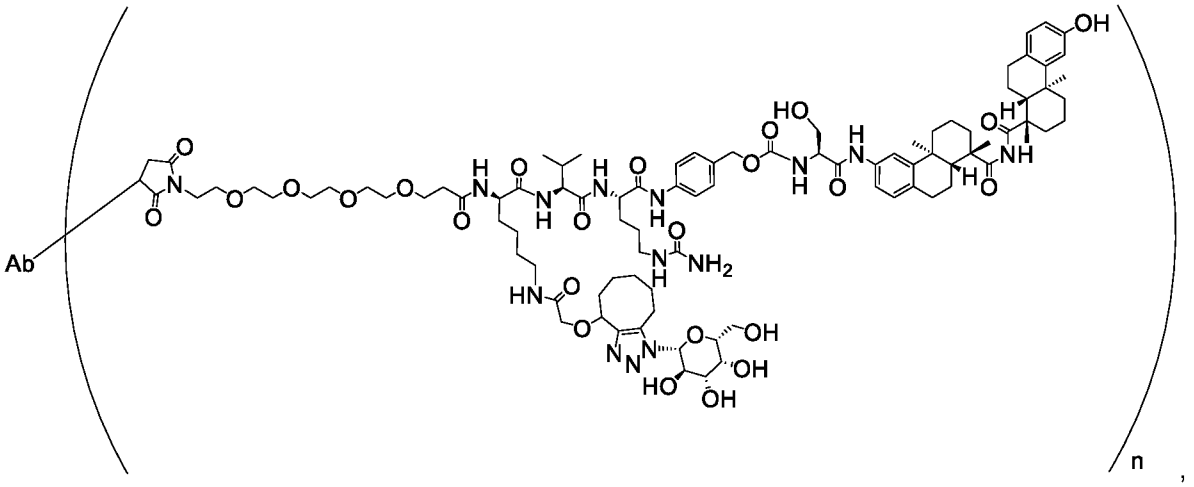
,

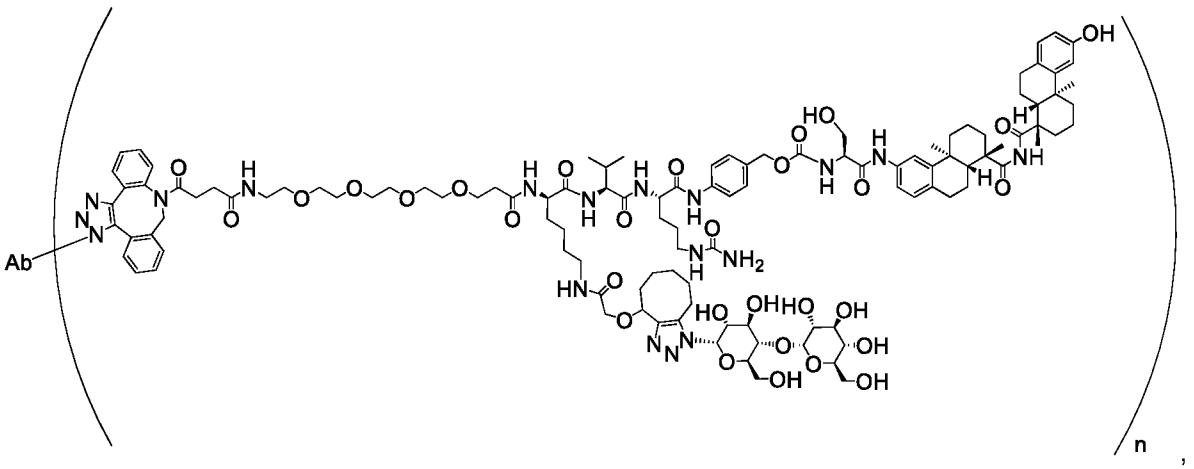


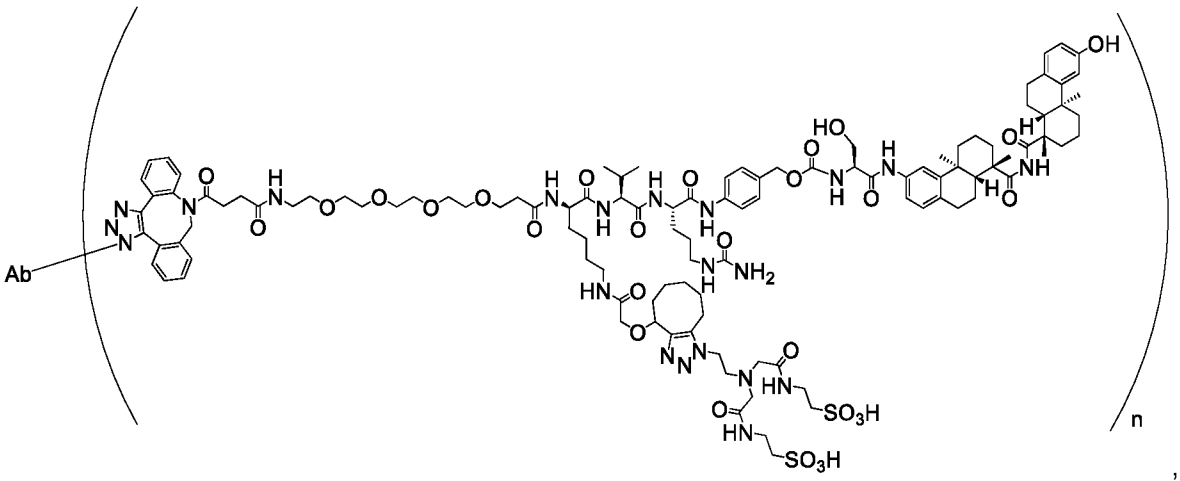




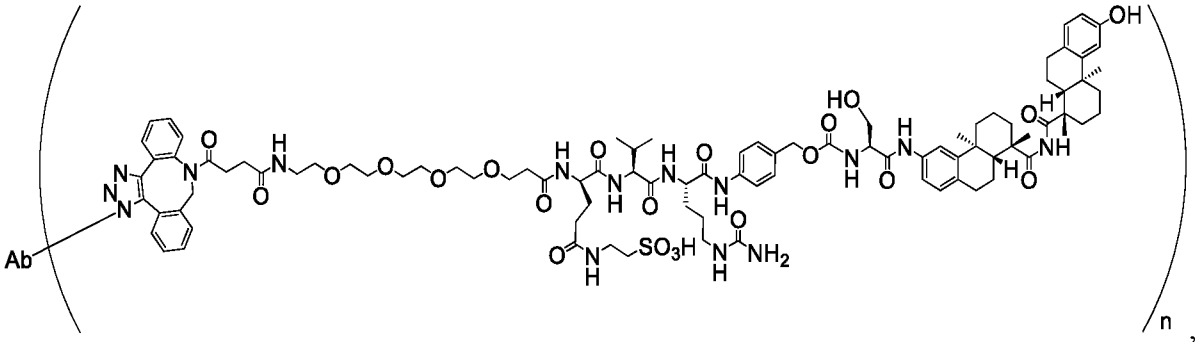




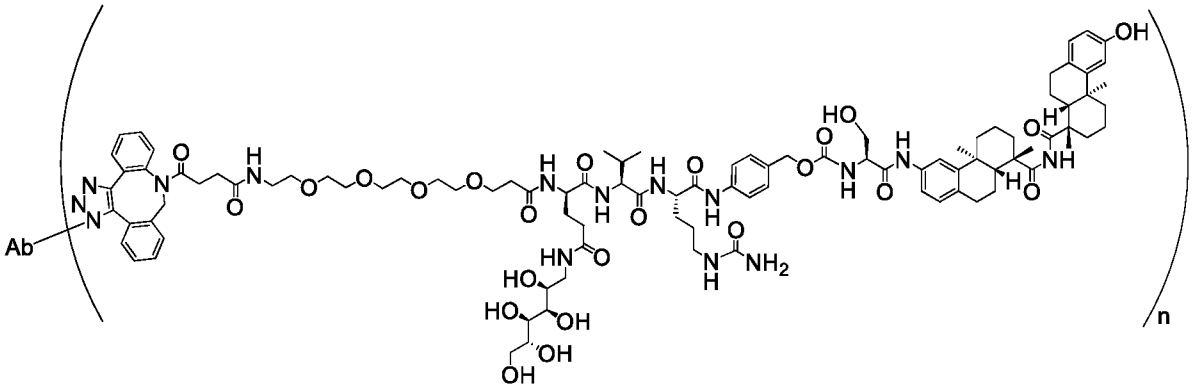




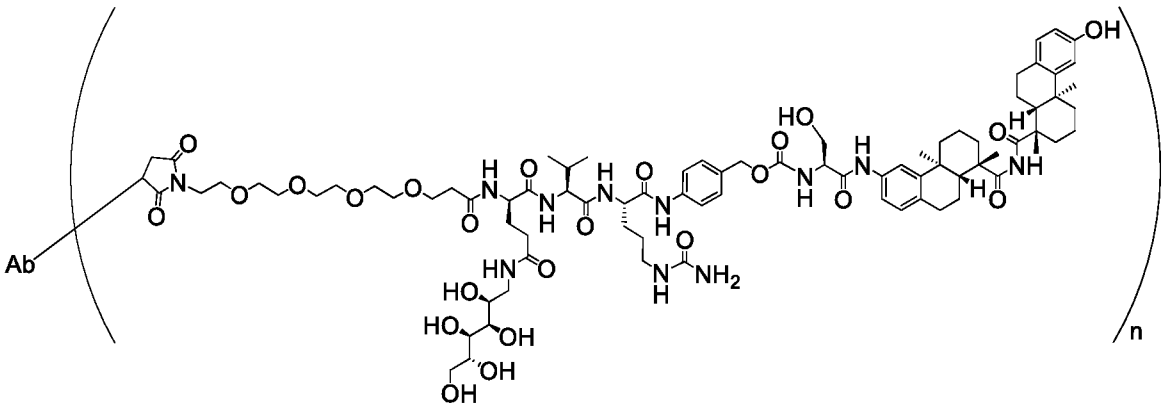
,



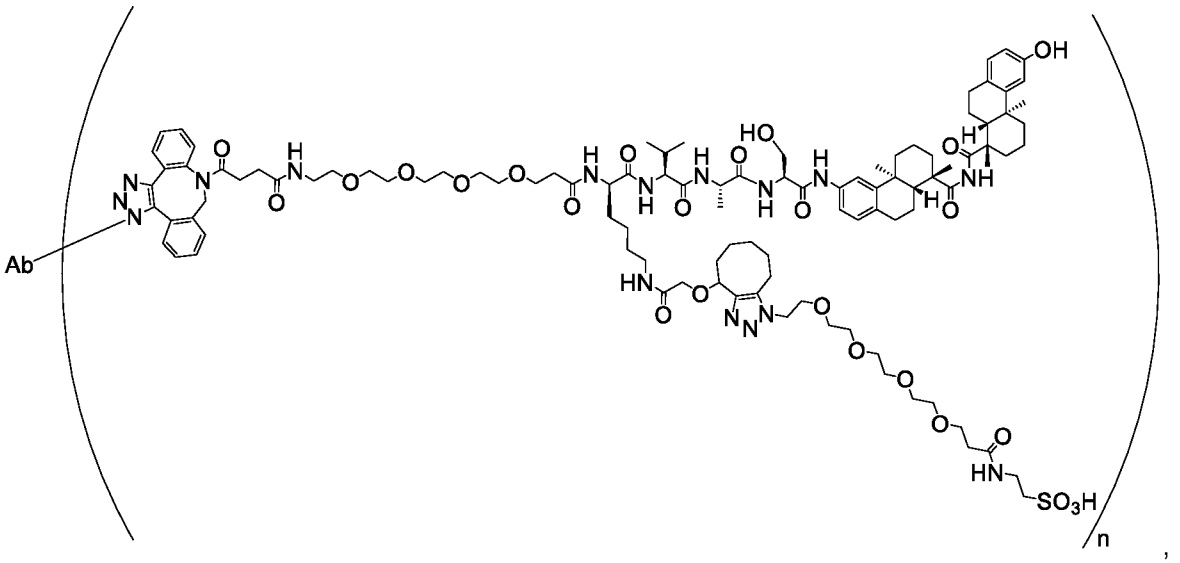
,



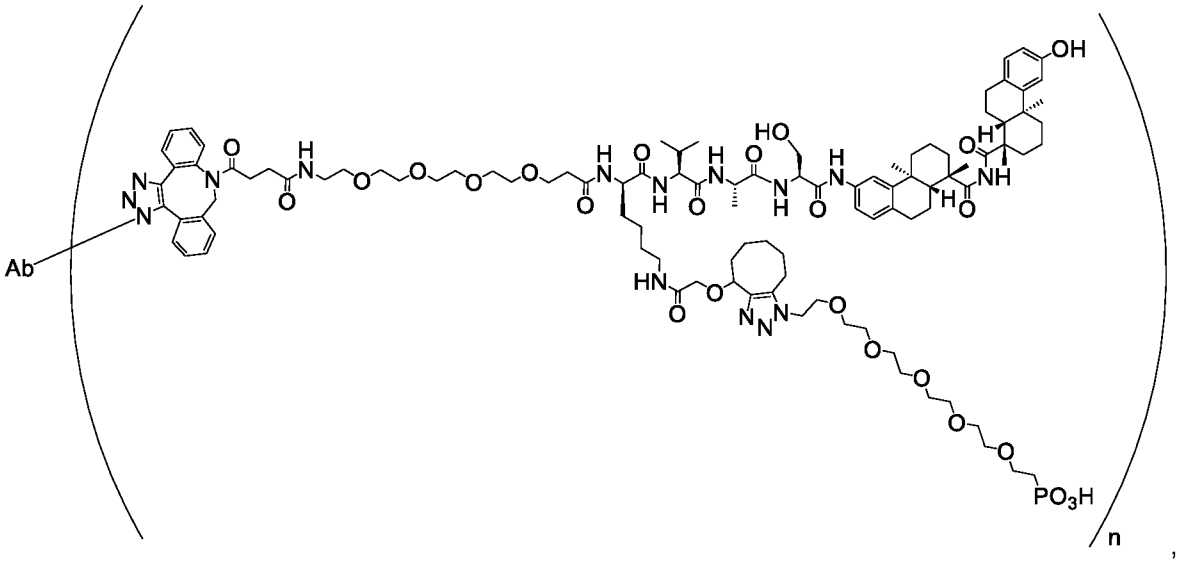
,



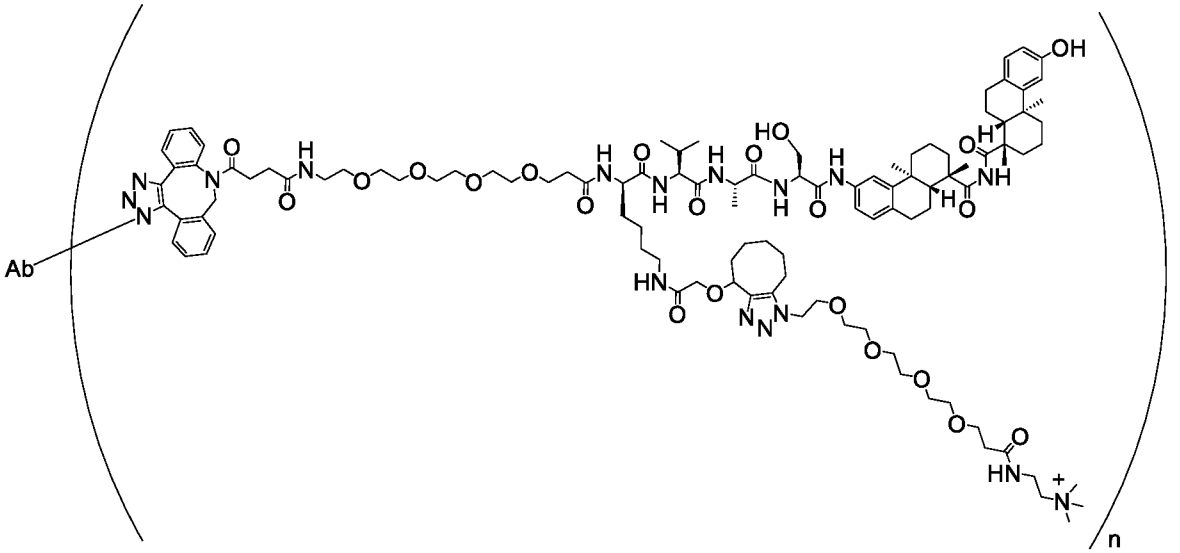
,


Ab
OH
HO
HN
O
N
H
NH
HN
SO3H
n

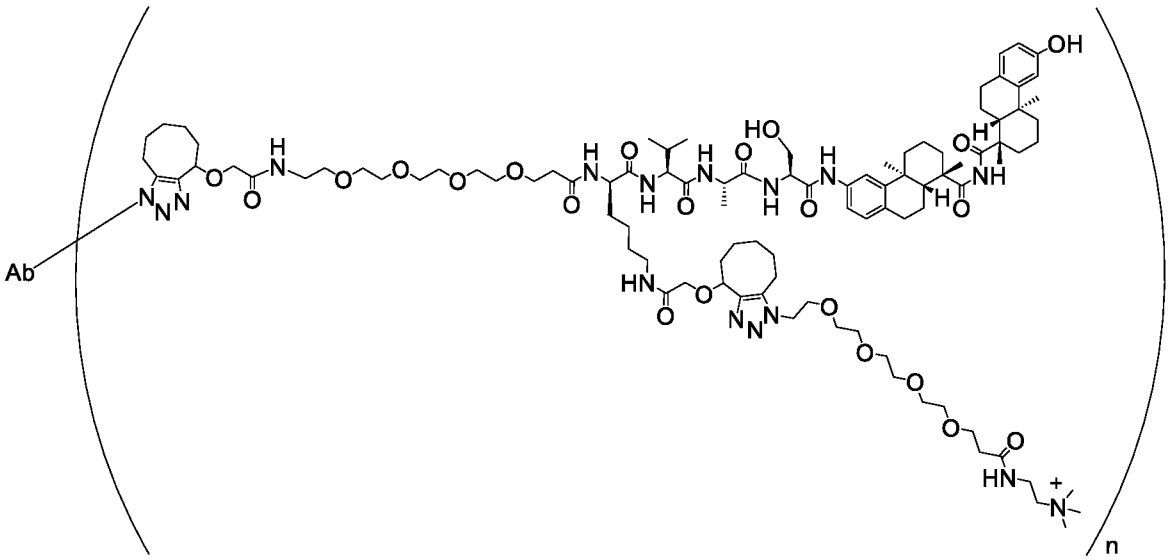
,

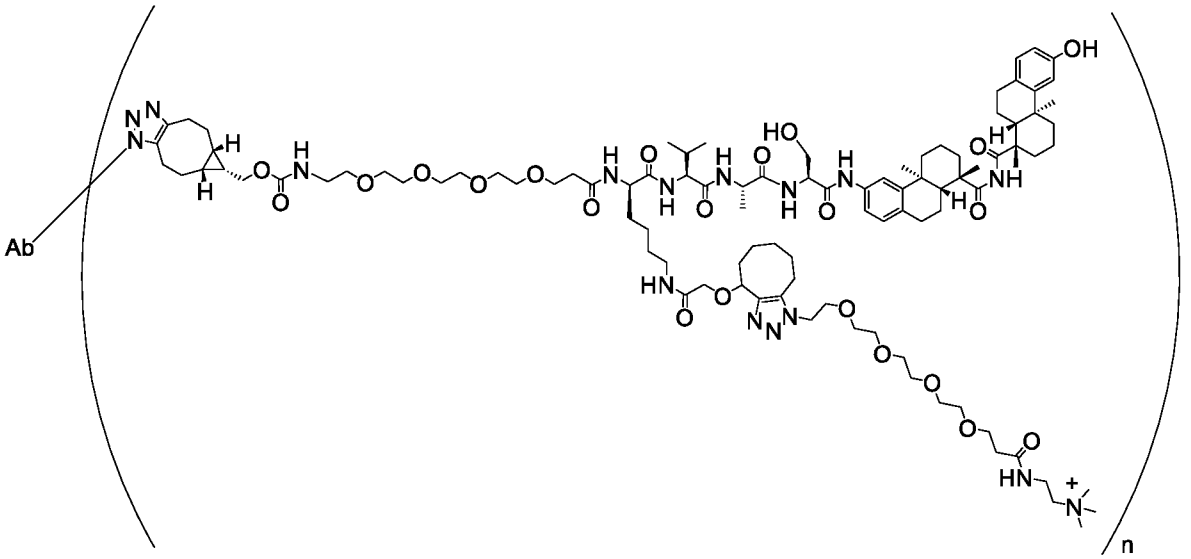

Ab
OH
HO
HN
O
N
H
NH
PO3H
n

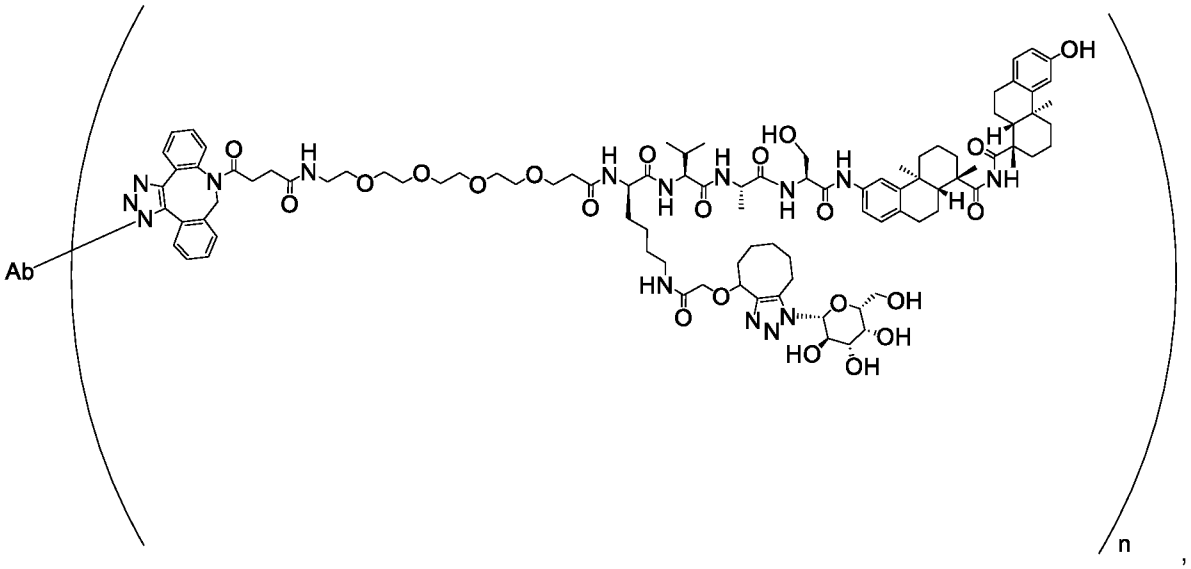
,

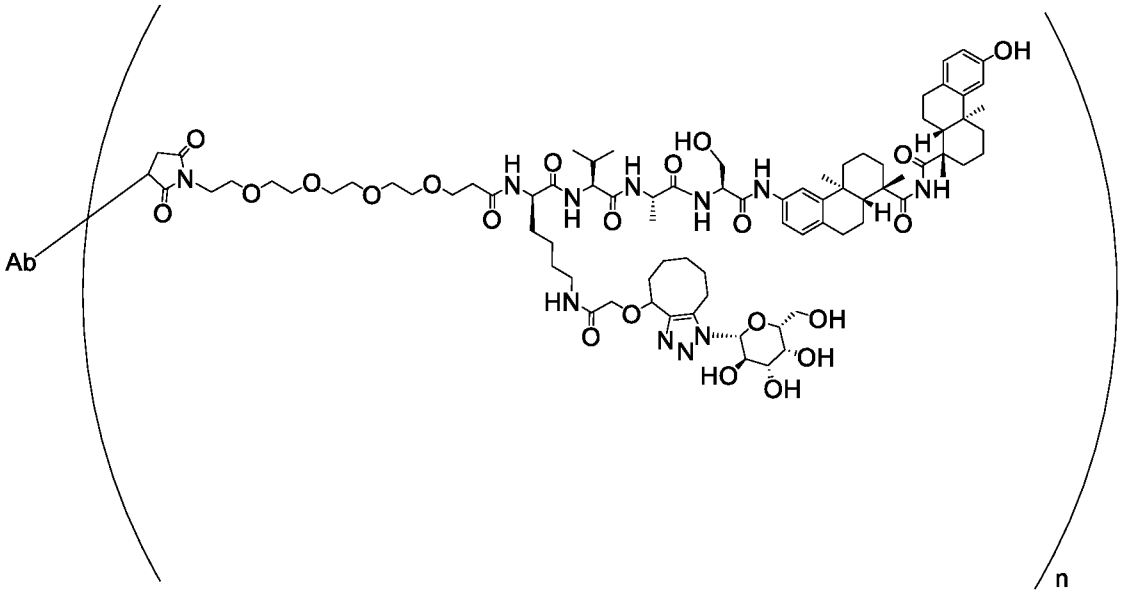

Ab
OH
HO
HN
O
N
H
NH
+
N
n

,

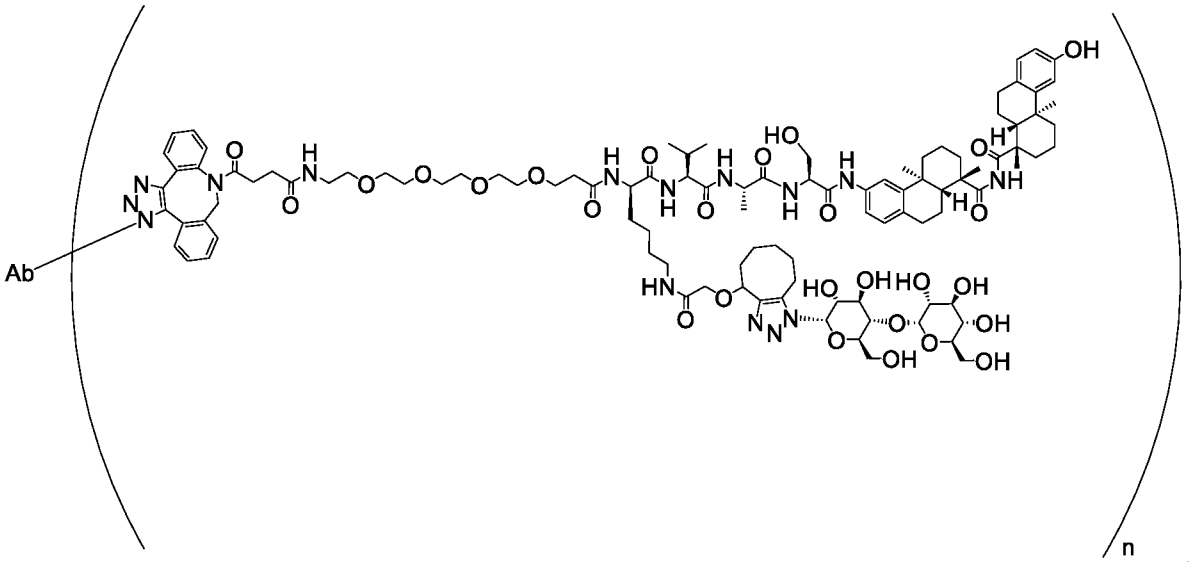




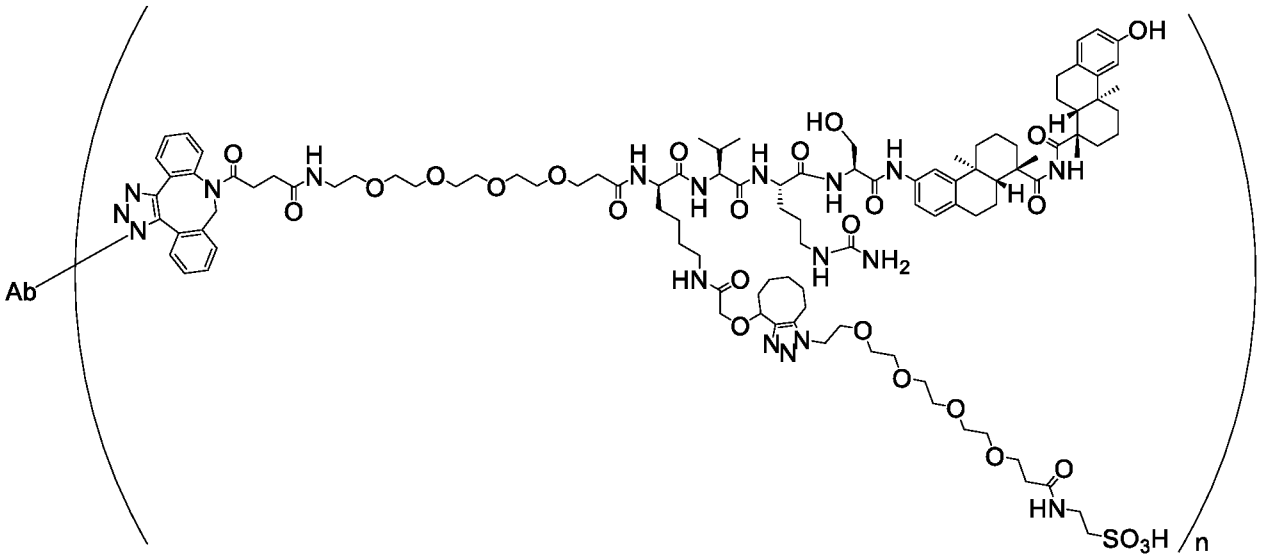




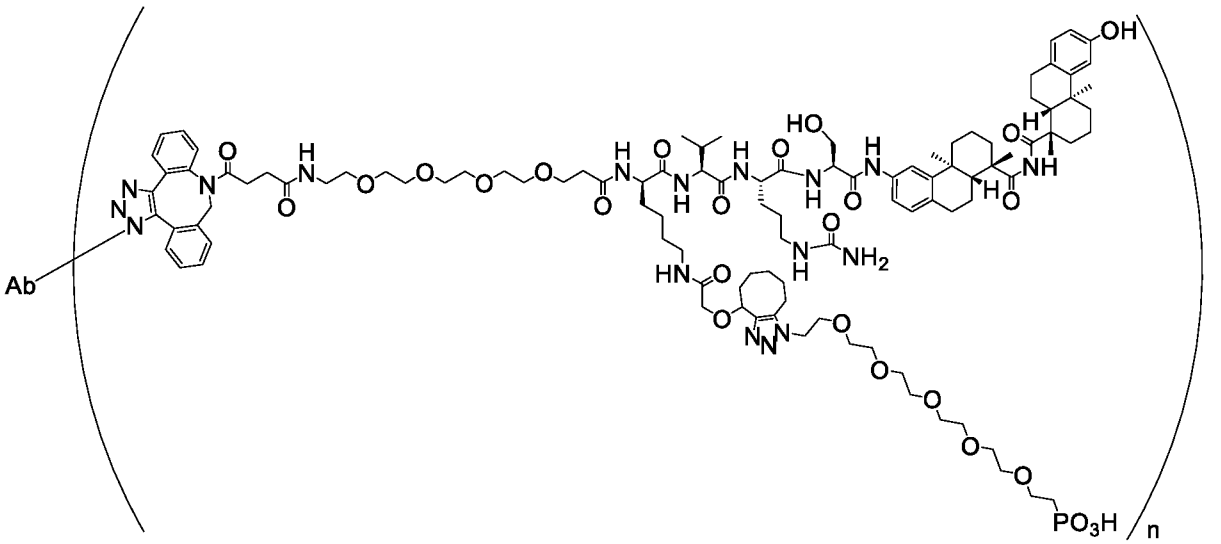
,

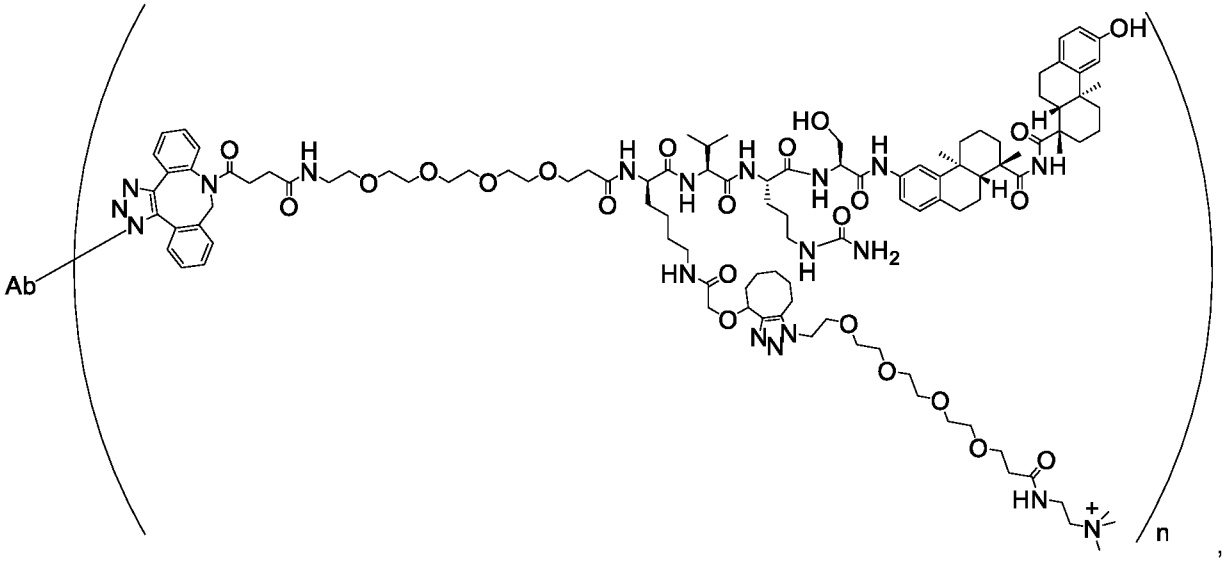


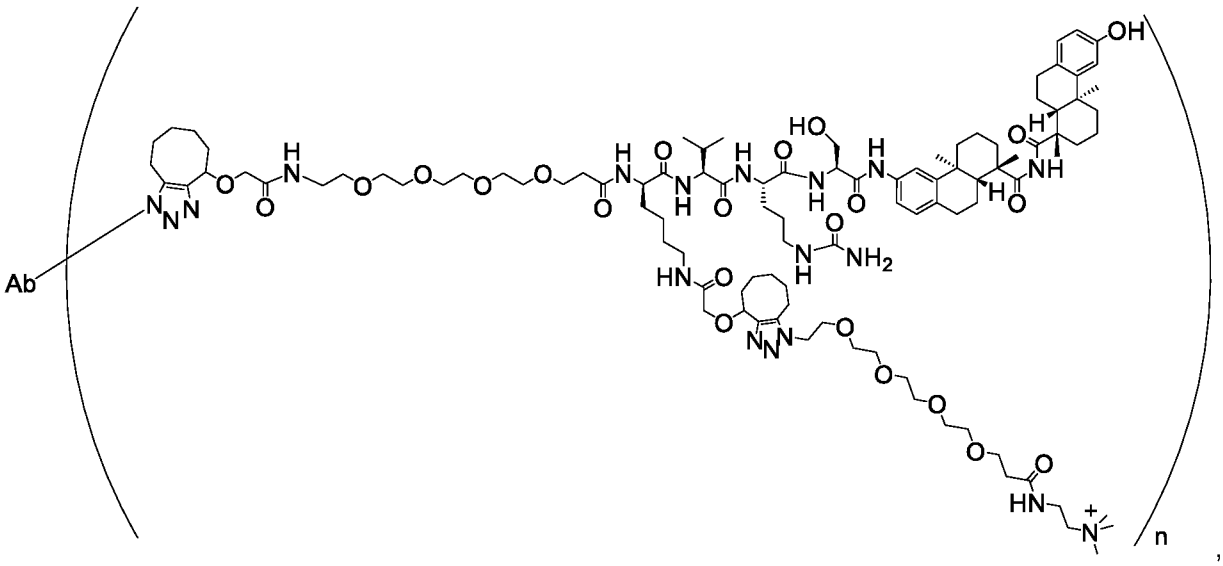
,

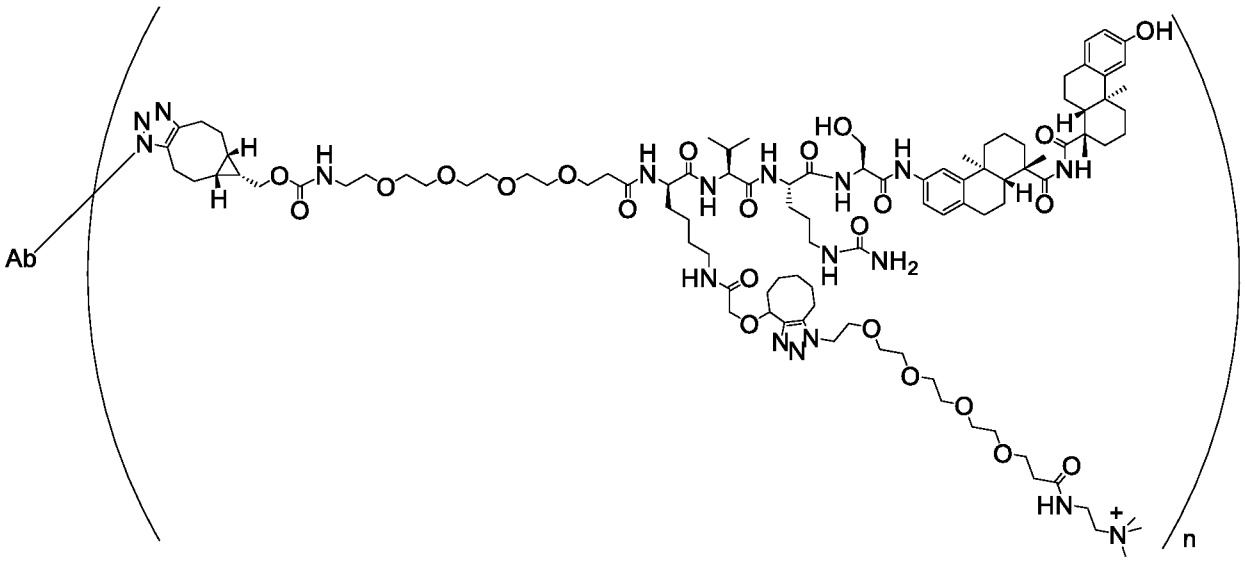


,

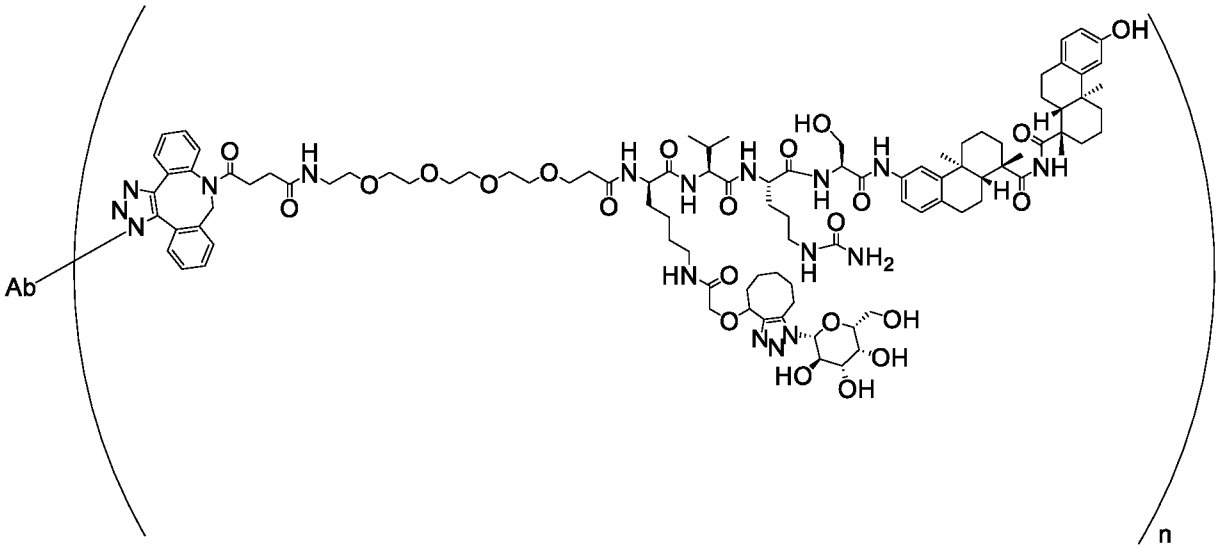




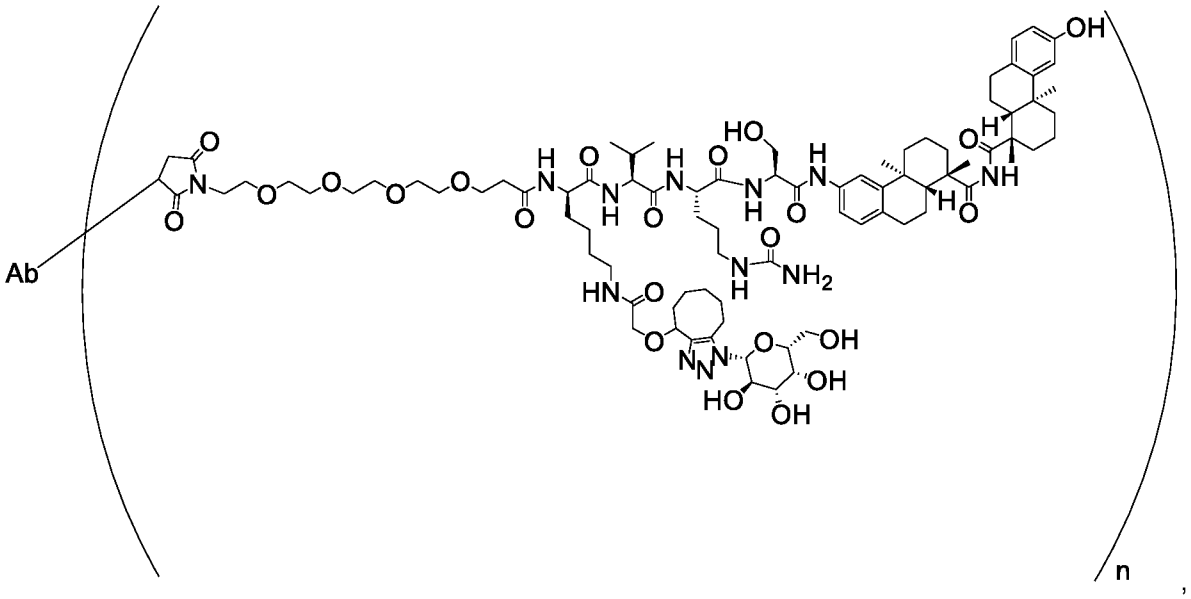




,

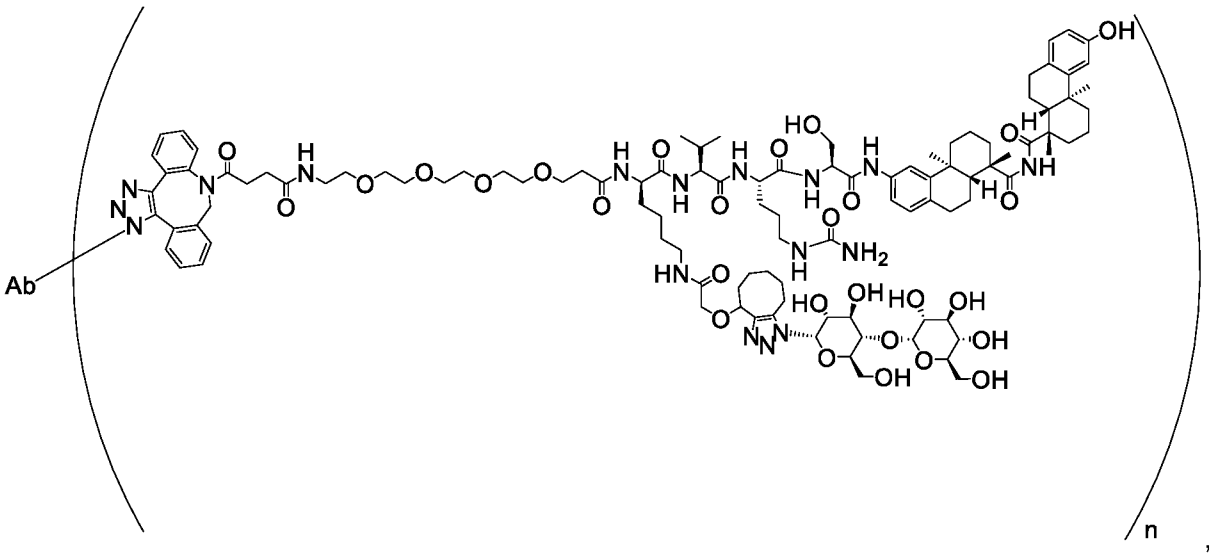


,



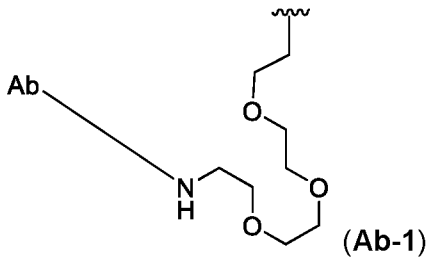
,

and



or a stereoisomeric form thereof, or a regioisomer thereof, or a mixture of regioisomers thereof, wherein each **Ab** is an antibody, or an antigen-binding fragment thereof, and subscript **n** is an integer from 1 to 30. In some embodiments, **n** is an integer from 1 to 4. In some embodiments **n** is 2. In some embodiments, **n** is 4.

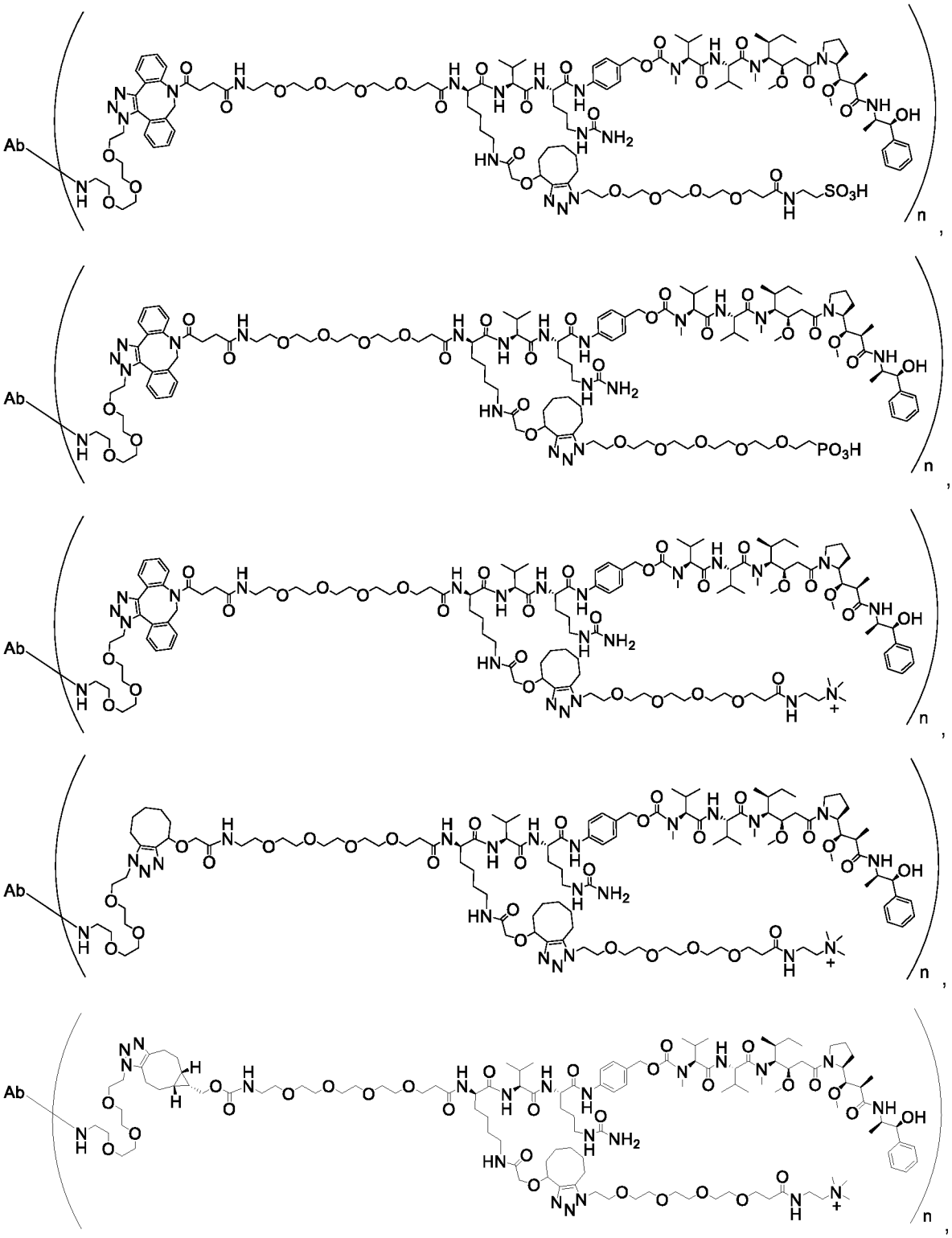
**[0145]** In some instances, **BA** is a modified antibody of formula (**Ab-1**)

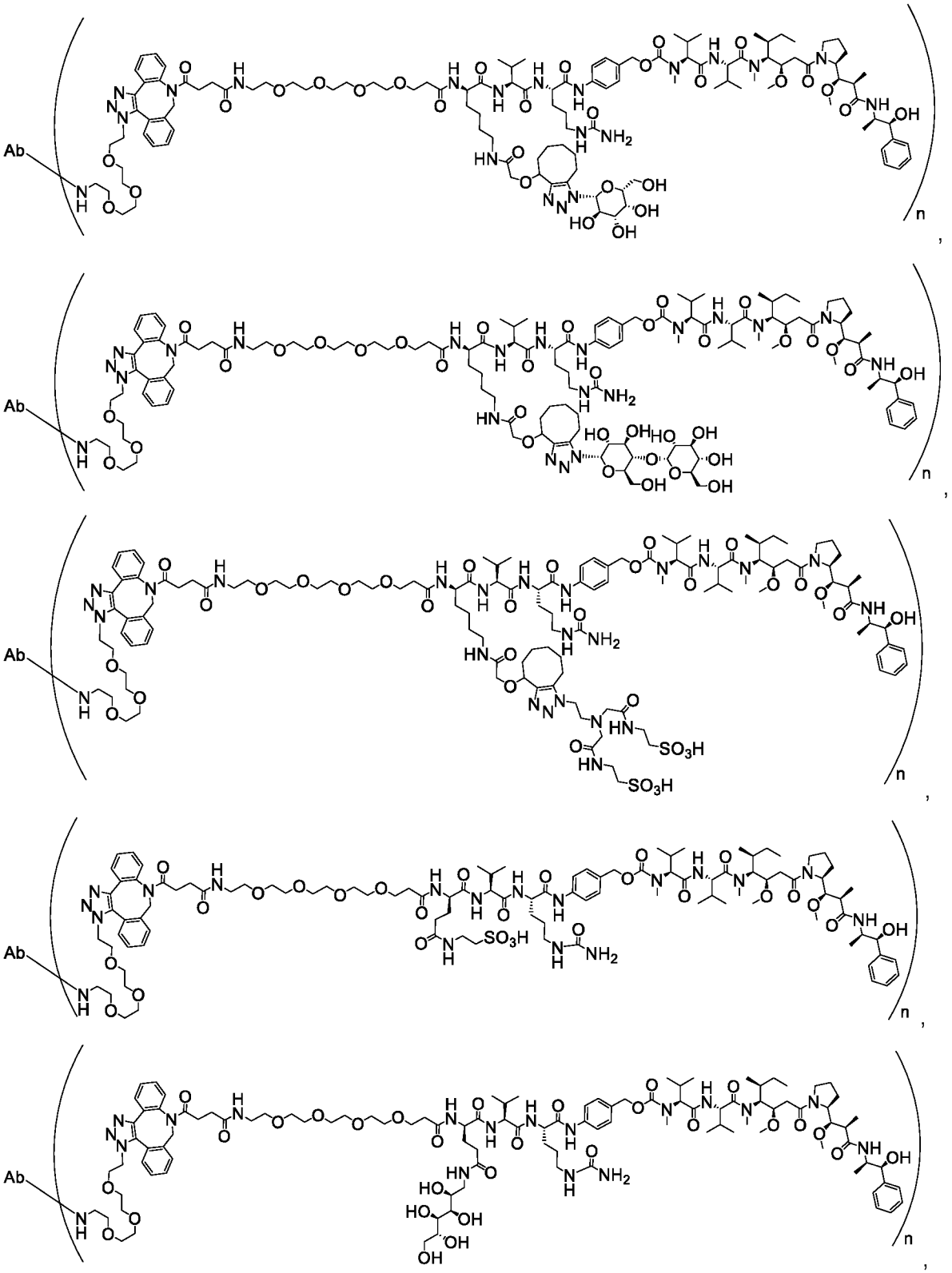


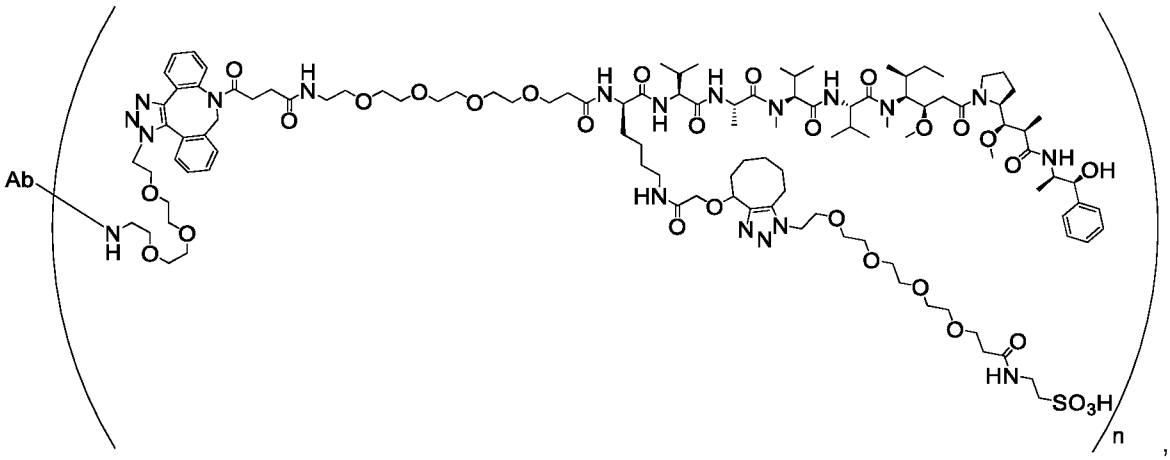
(**Ab-1**)

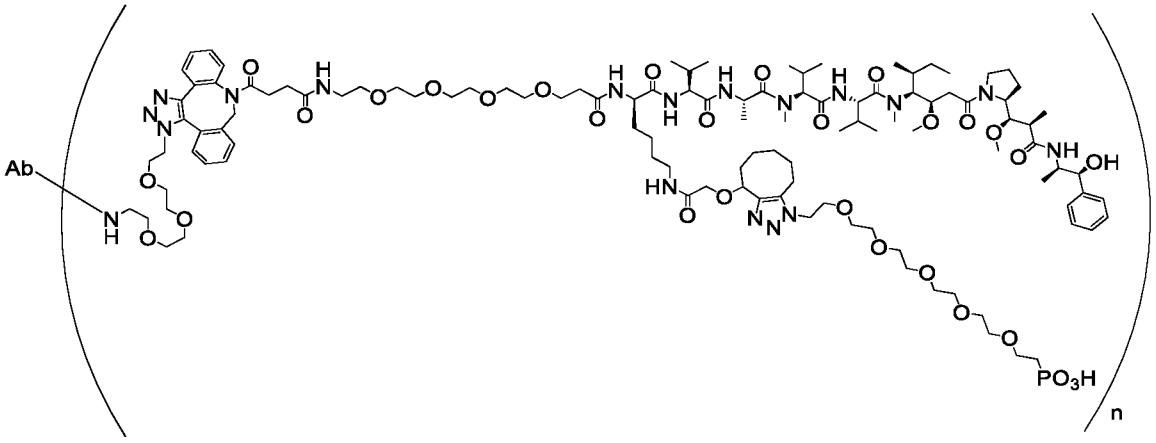
wherein the  indicates the atom through which **Ab-1** is bonded to the adjacent groups in the formula.

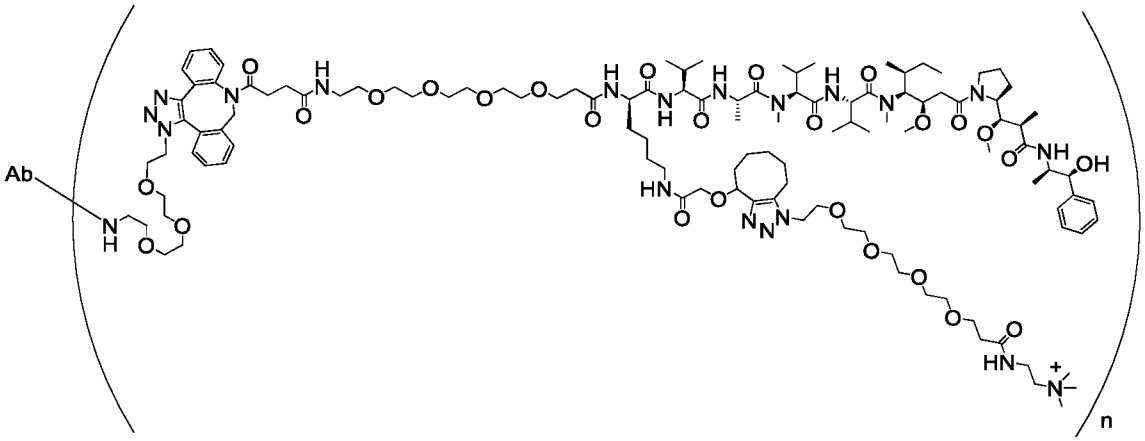
**[0146]** In some instances, a compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), is an antibody drug conjugate (ADC), wherein BA is Ab-1 as defined herein, where Ab is an antigen or antigen-binding fragment thereof, selected from the group consisting of:

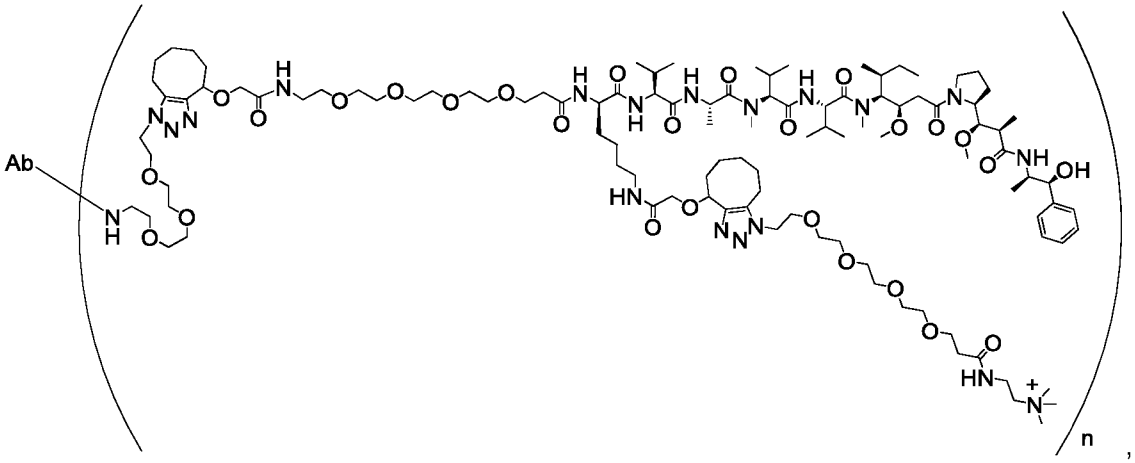


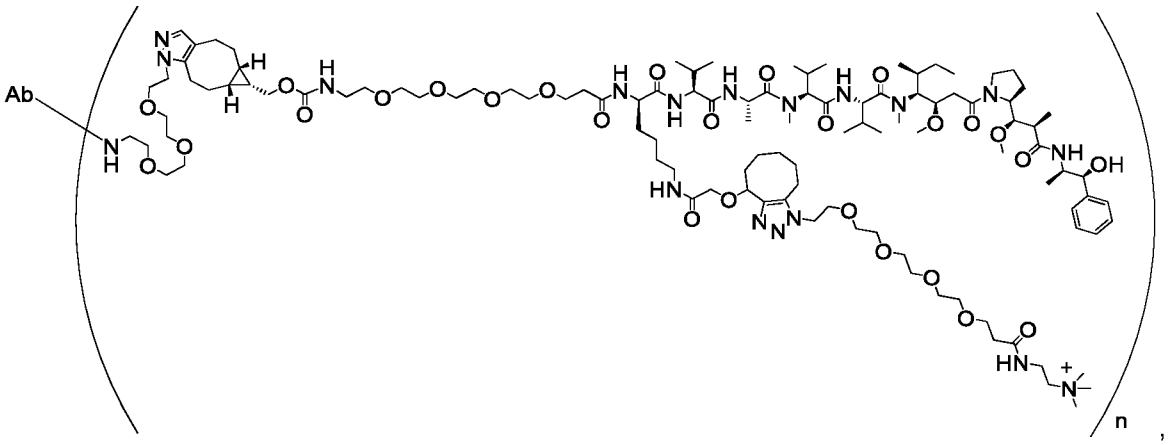


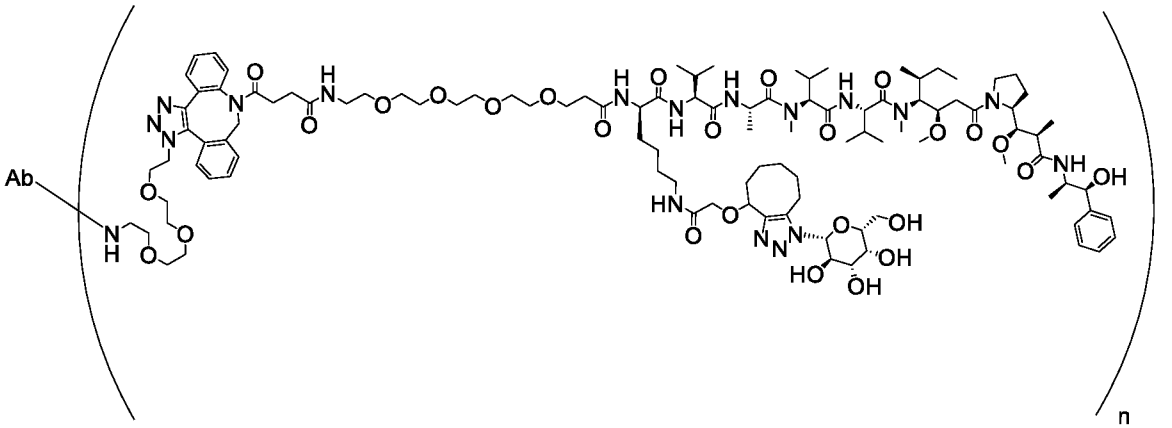


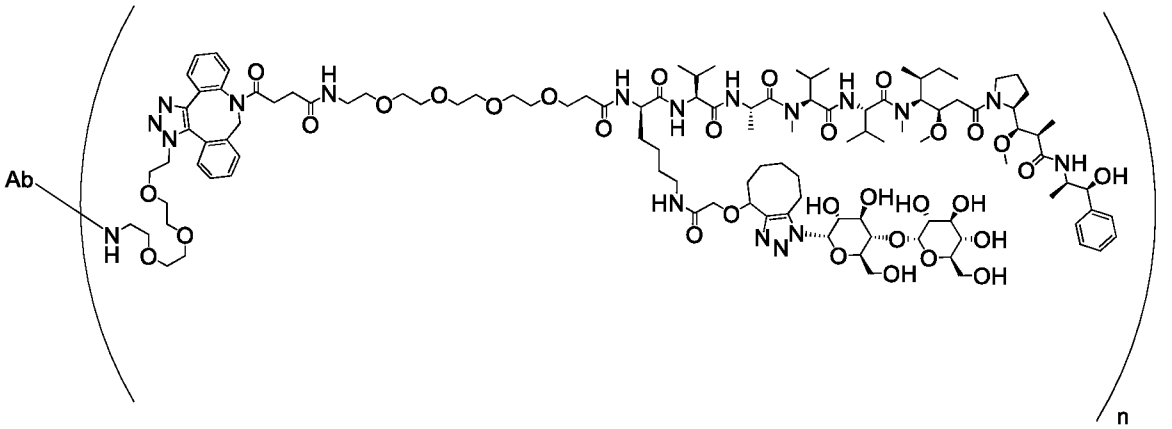


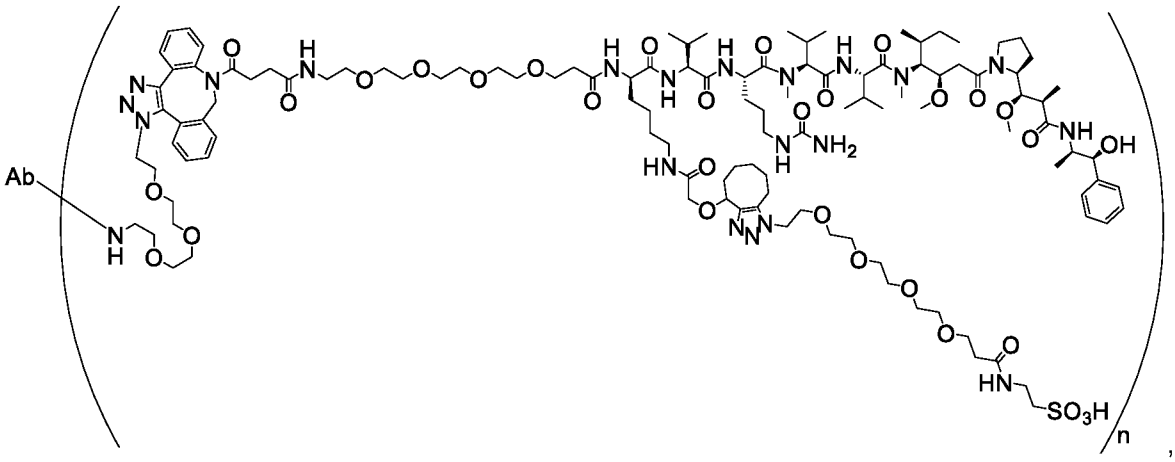


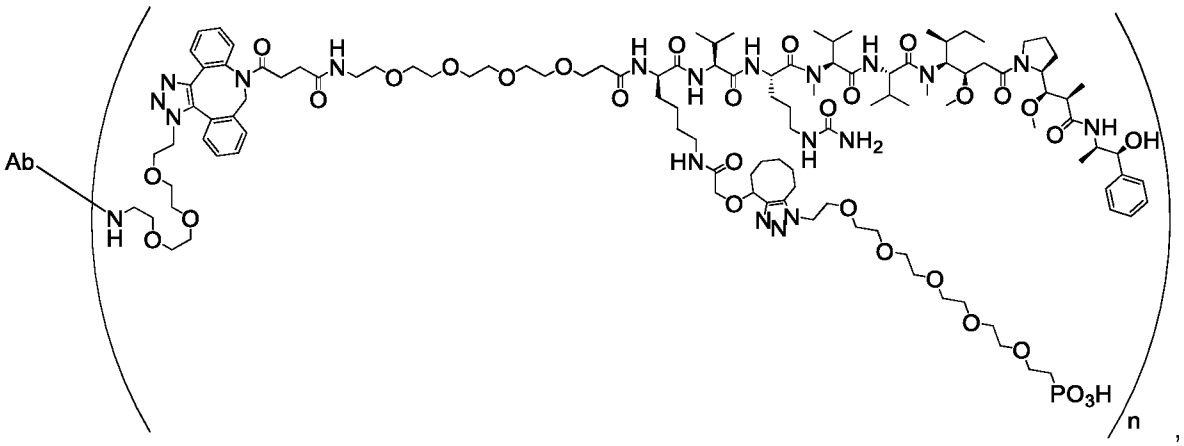


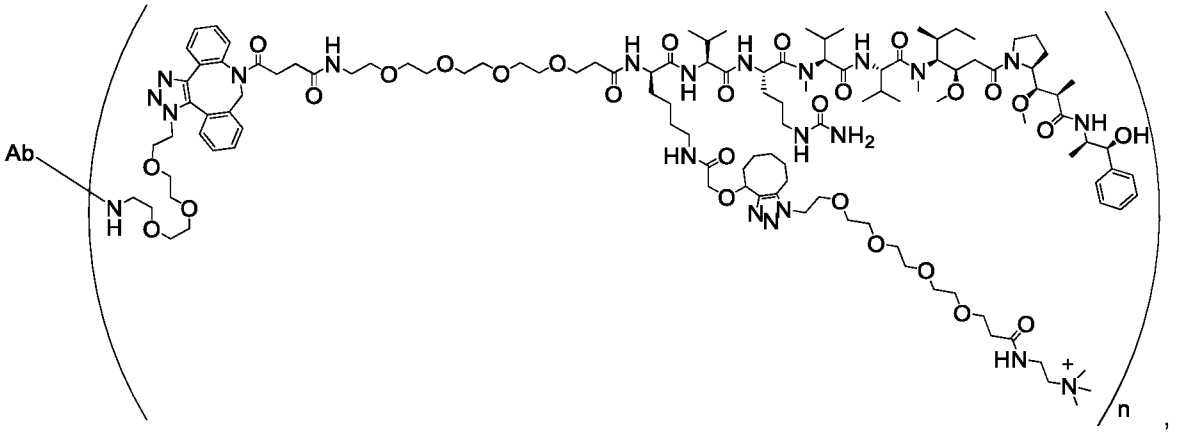


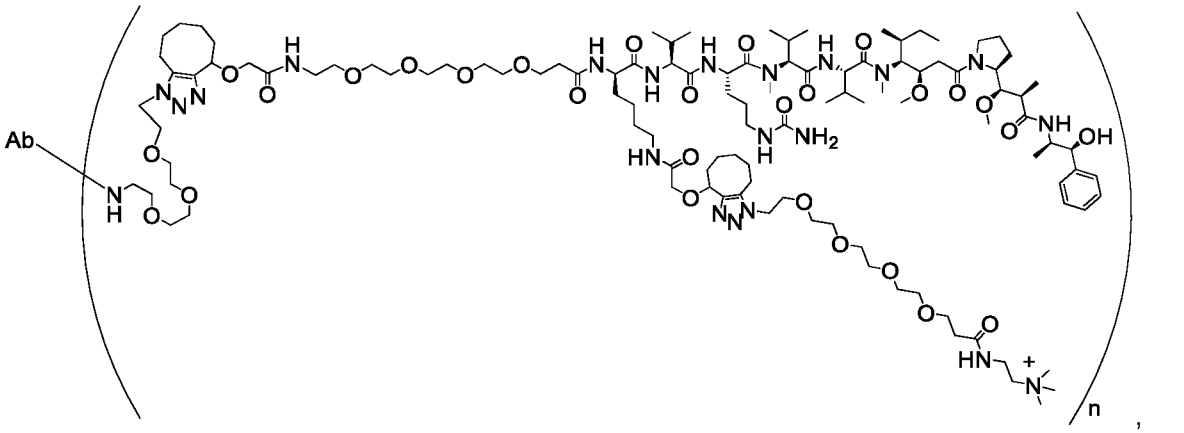


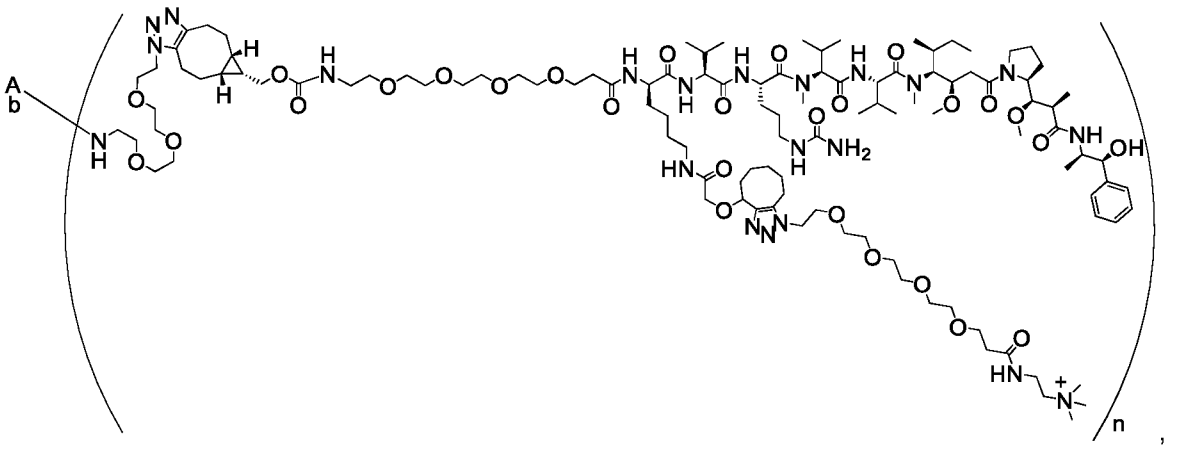


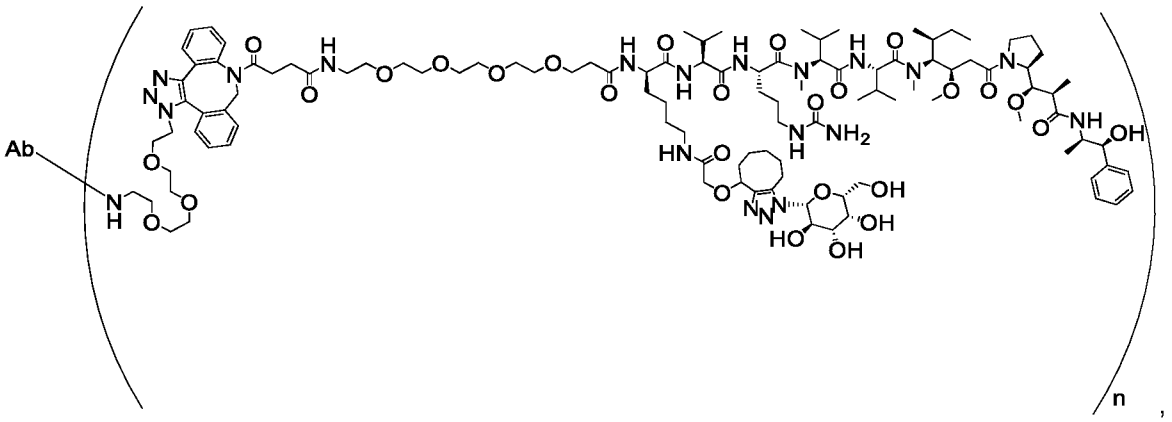


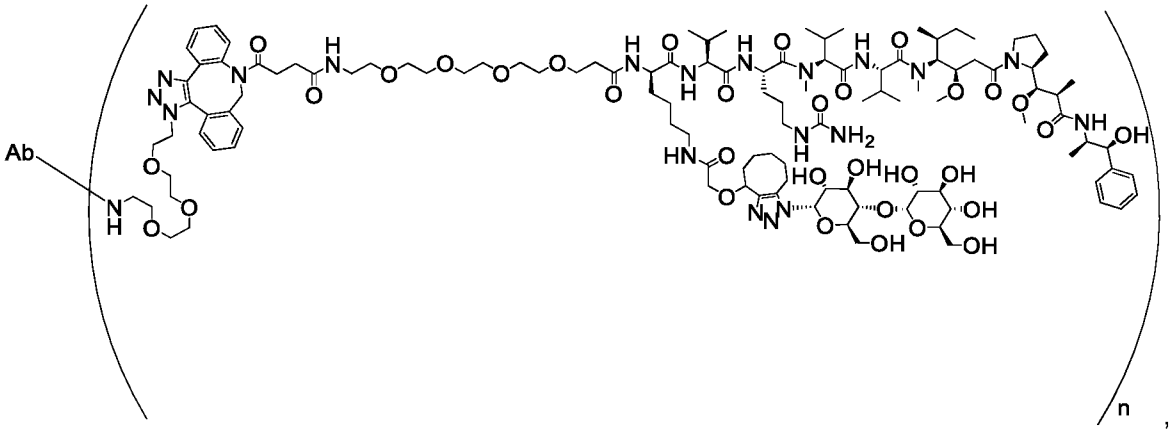


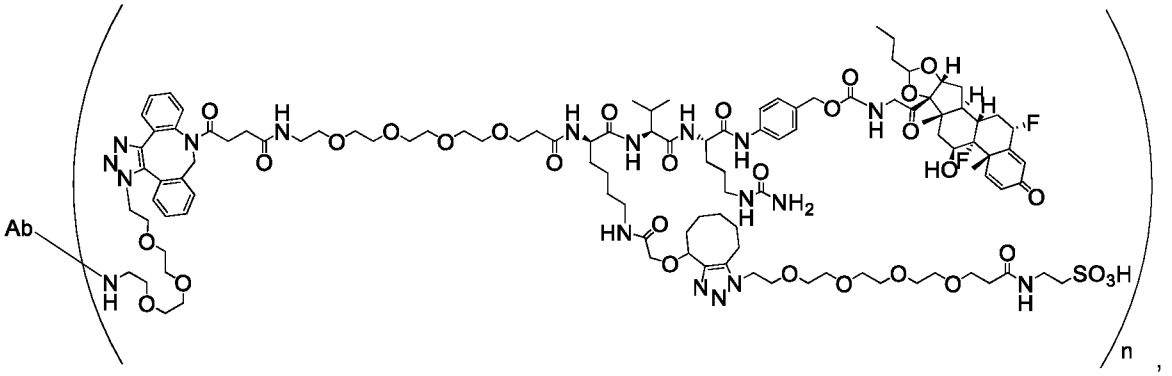


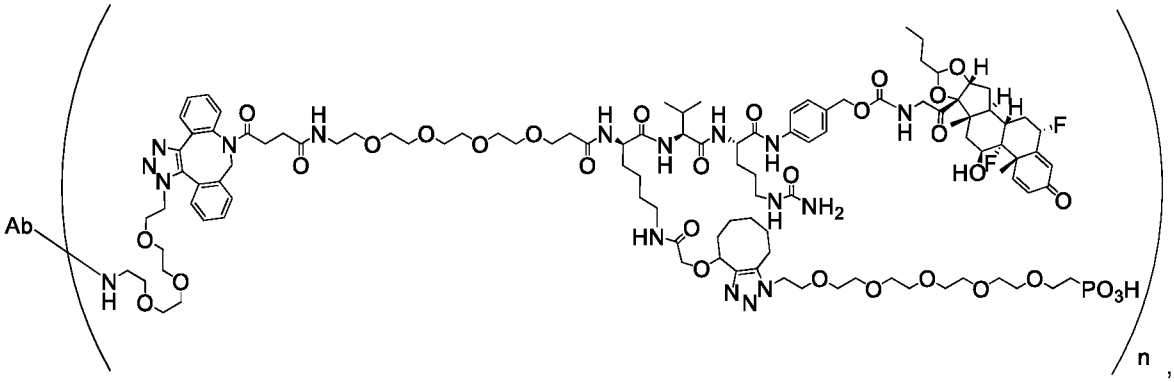


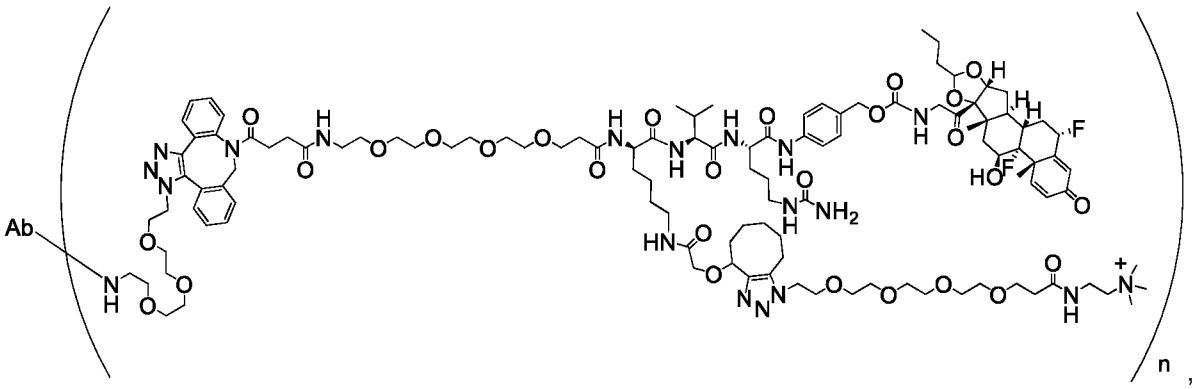


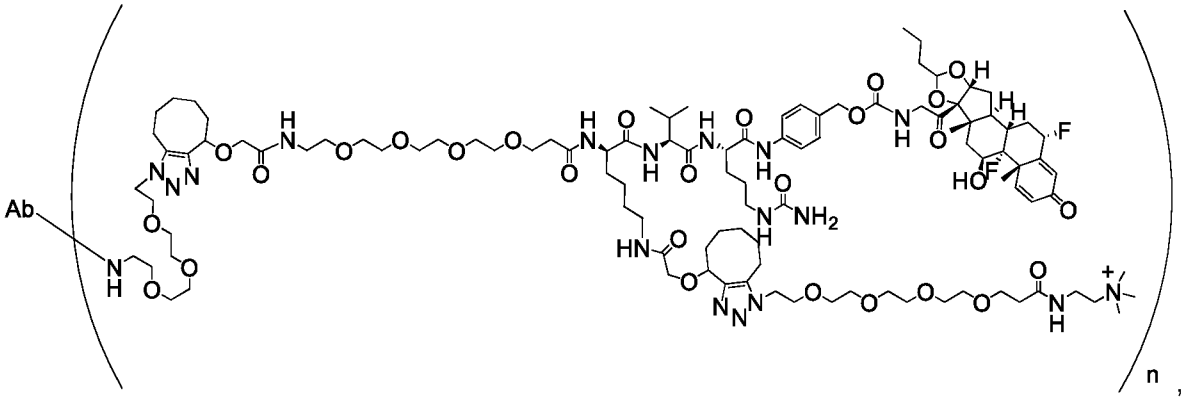


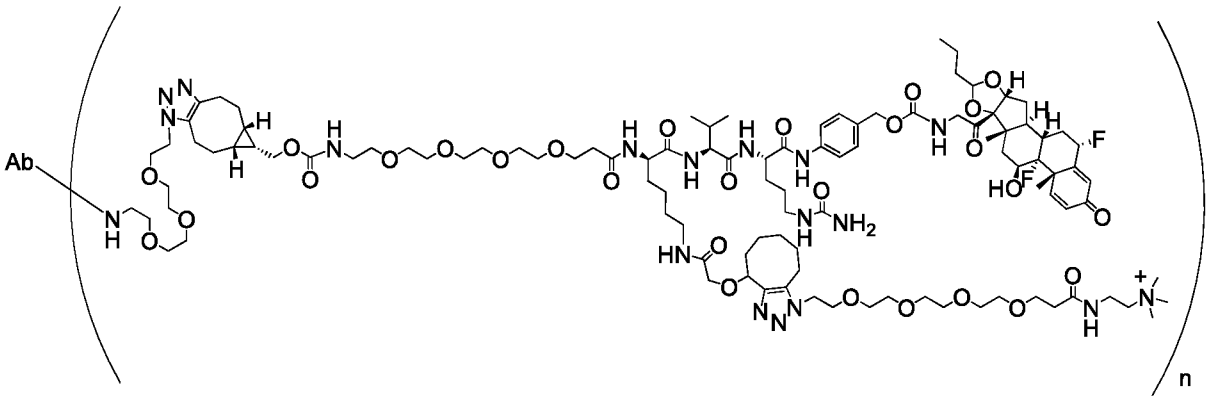


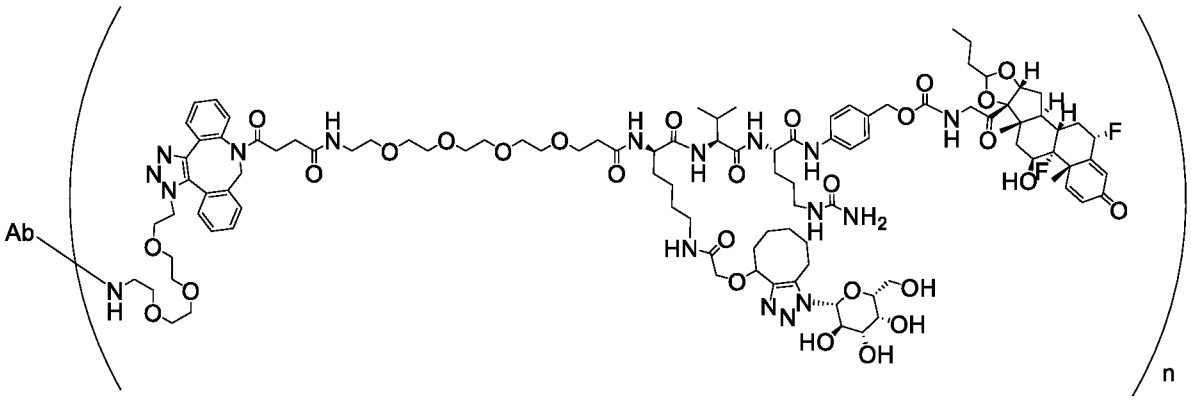


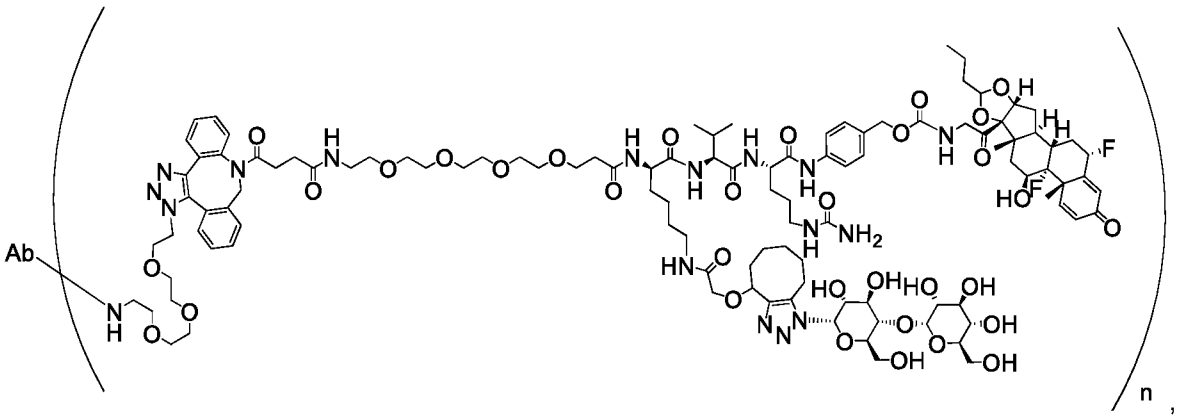




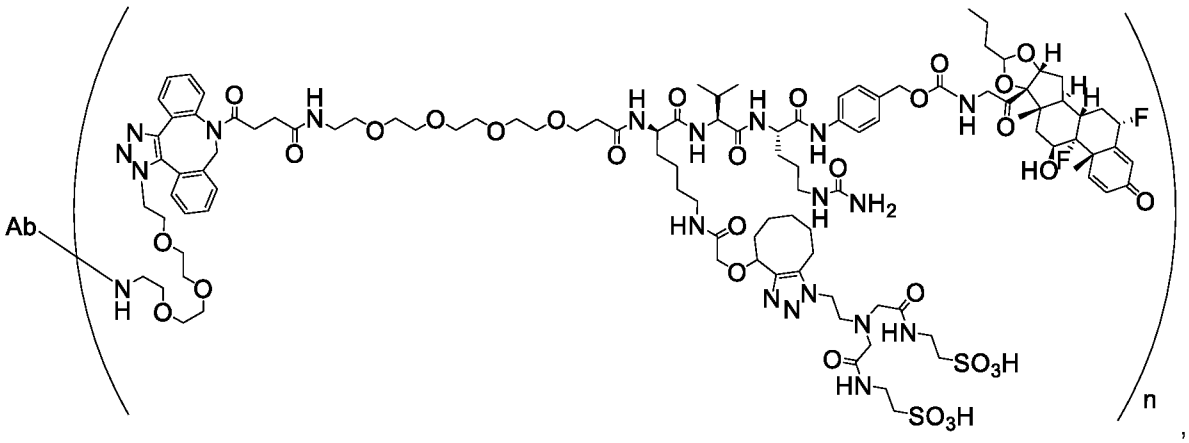




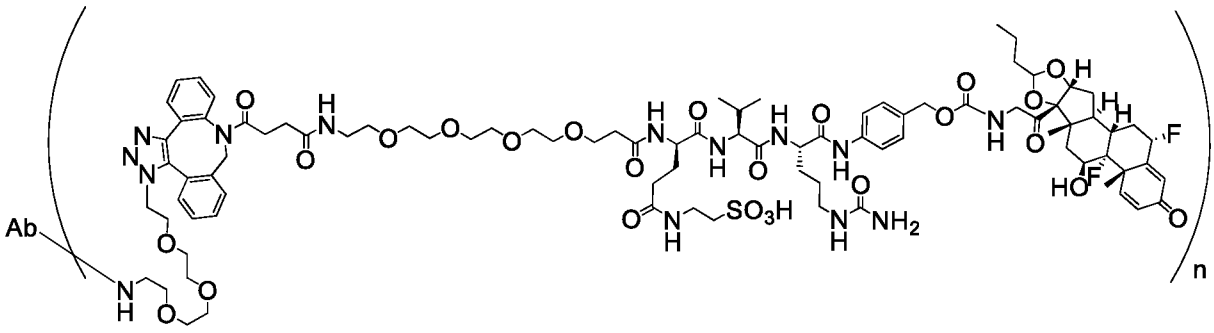




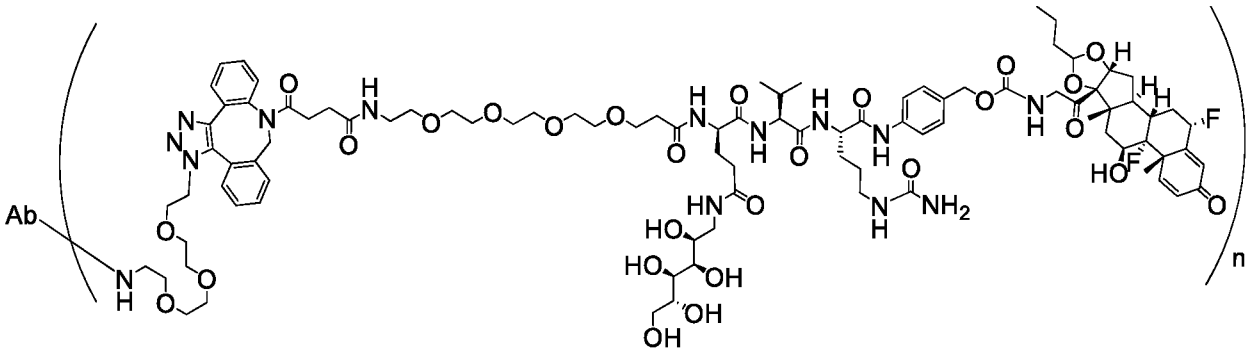
,



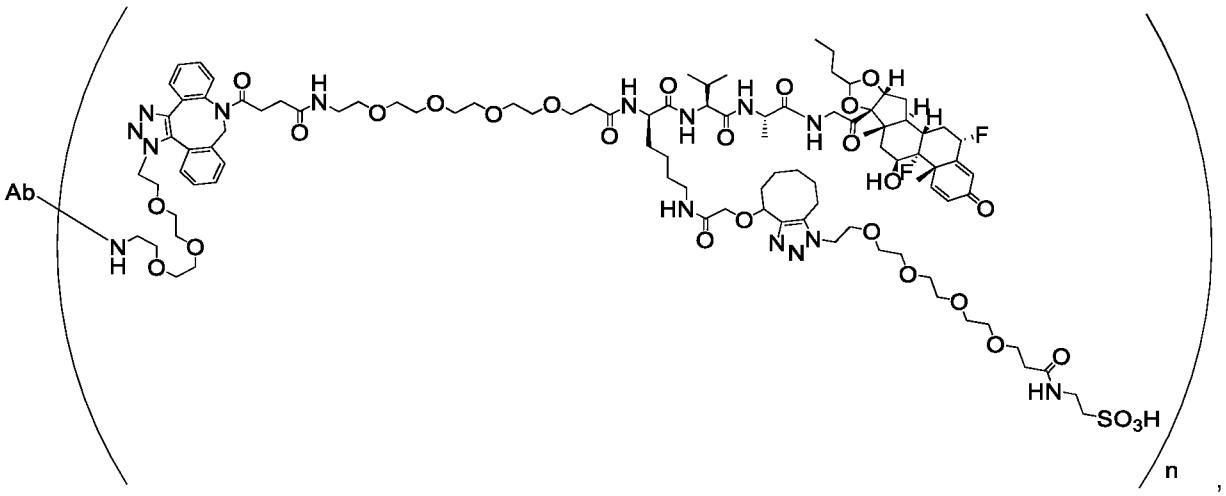
,



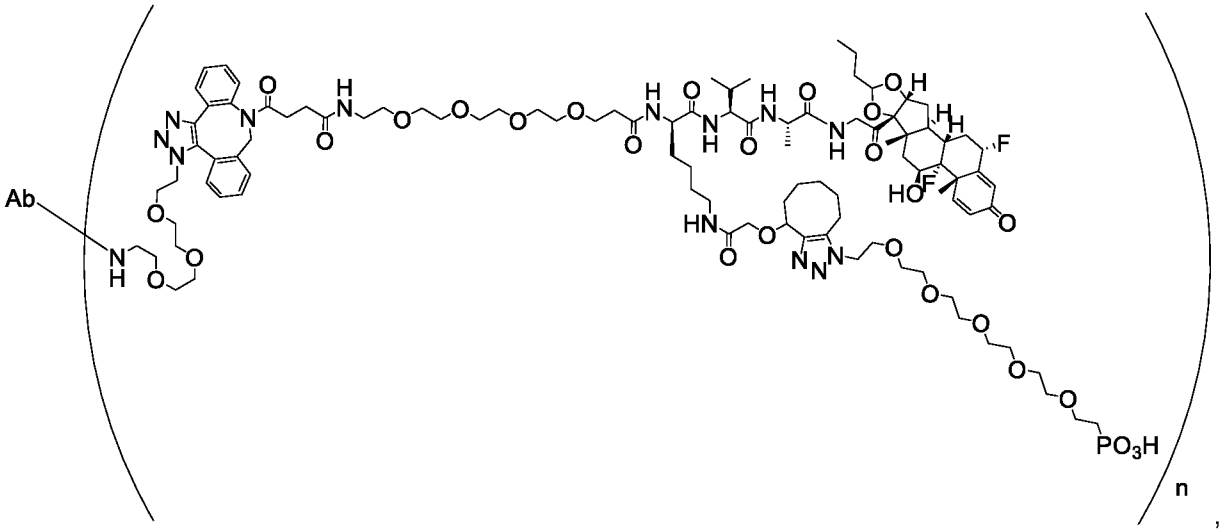
,



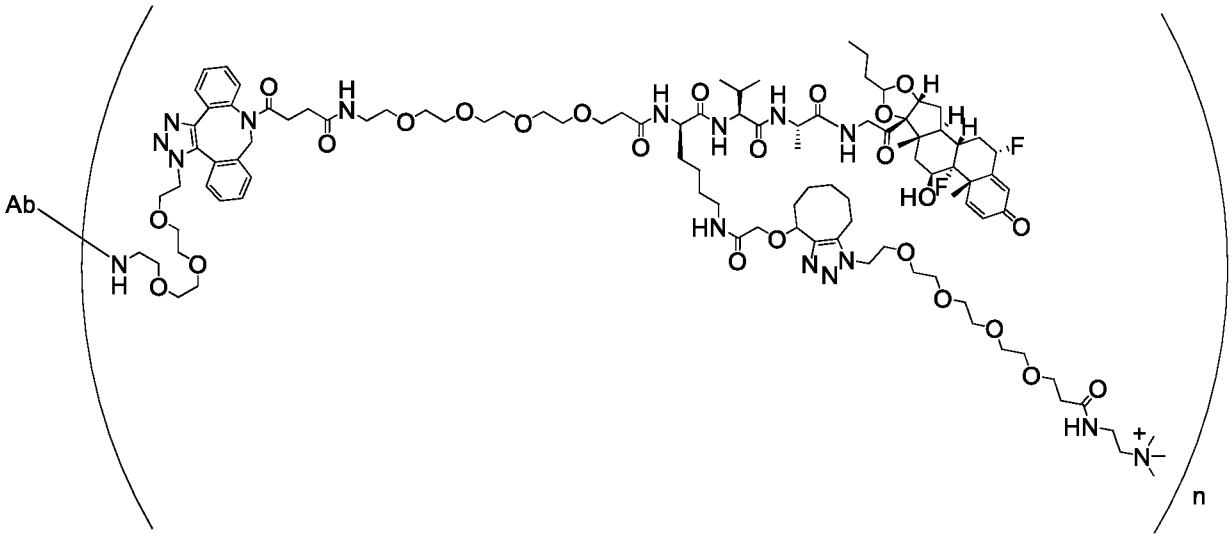
,


Ab
N
H
O
HN
O
N
N
HO
F
F
O
HN
O
N
N
O
O
O
O
O
HN
O
SO3H
n

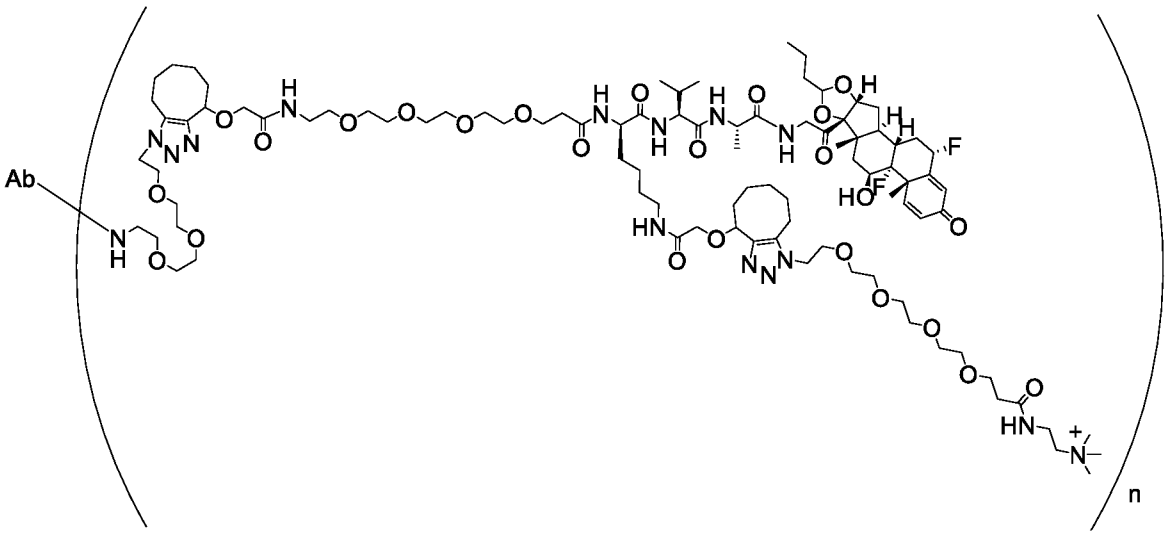
,

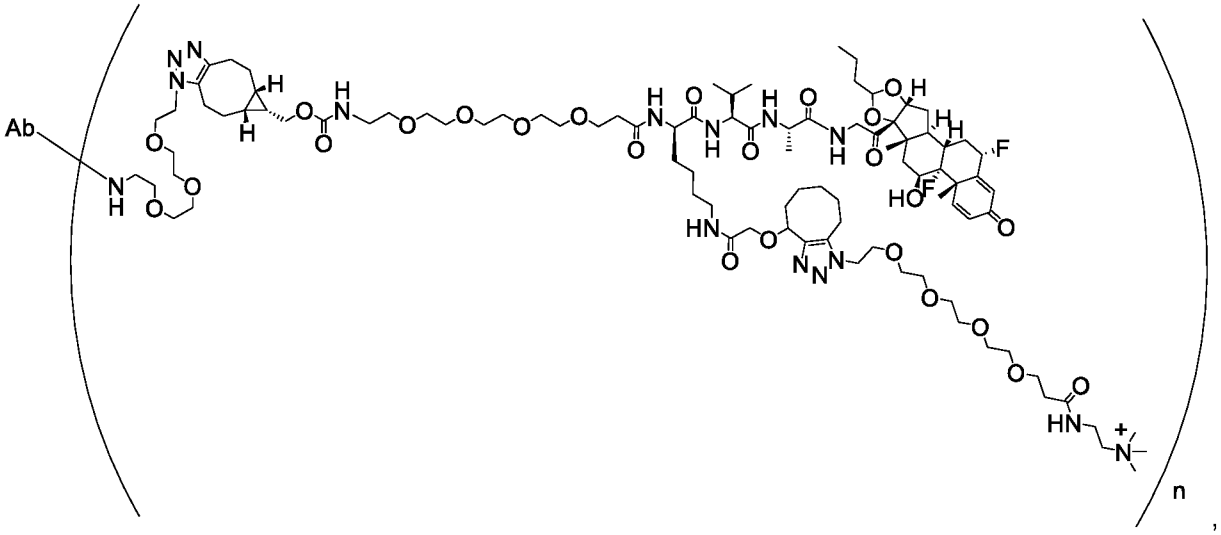

Ab
N
H
O
HN
O
N
N
HO
F
F
O
HN
O
N
N
O
O
O
O
O
PO3H
n

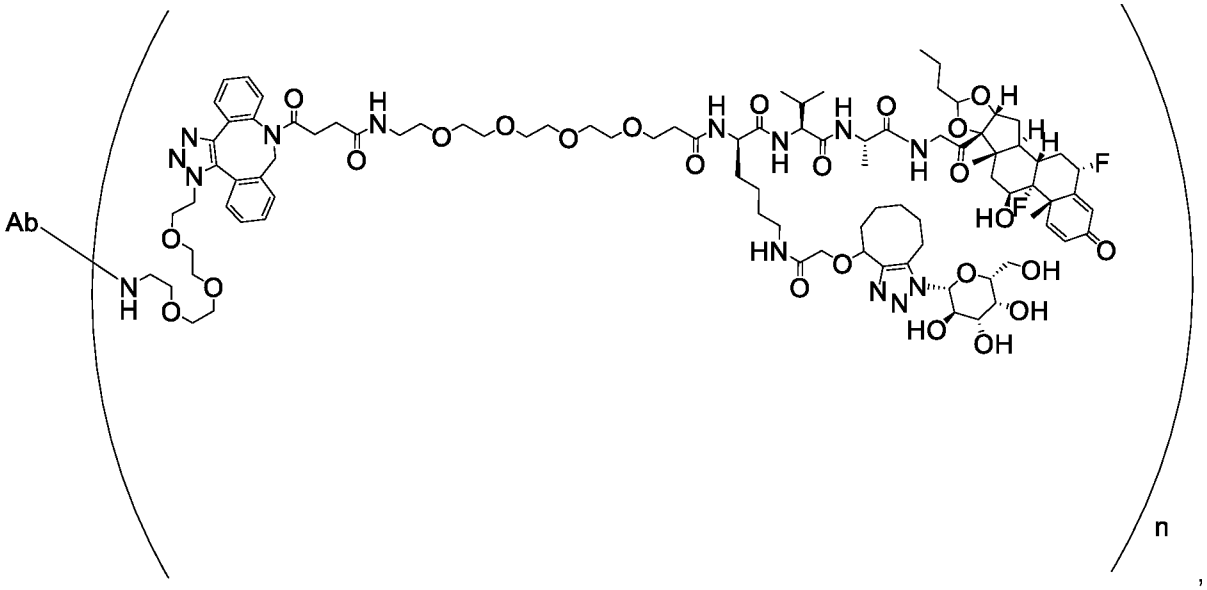
,

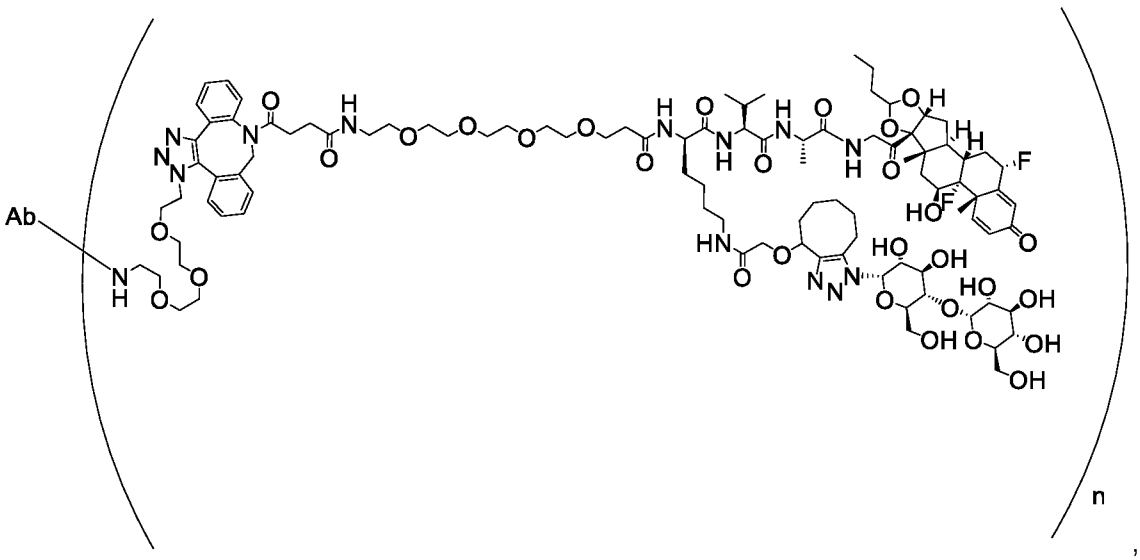

Ab
N
H
O
HN
O
N
N
HO
F
F
O
HN
O
N
N
O
O
O
O
O
HN
+
N
n

,

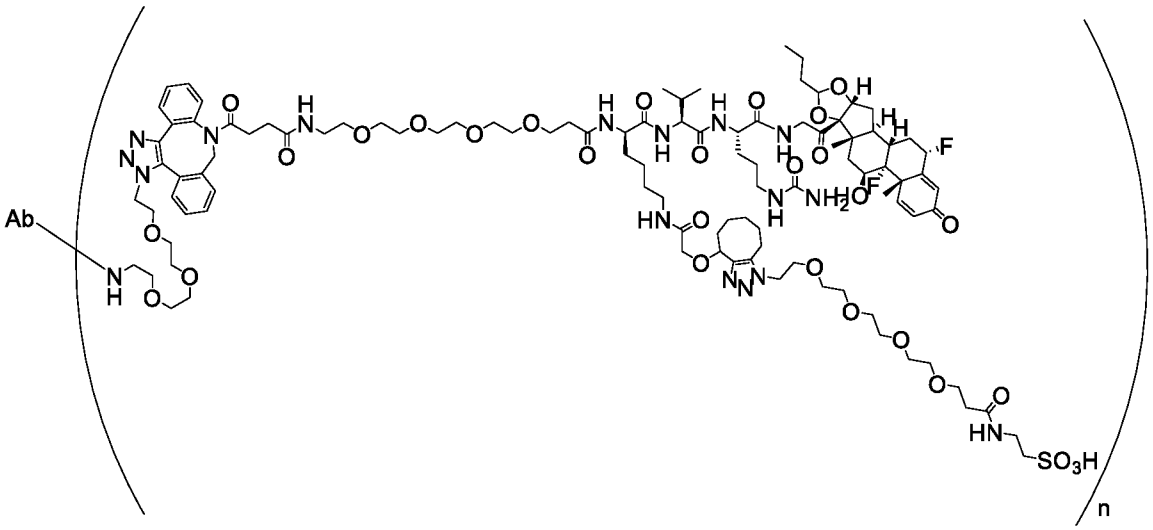




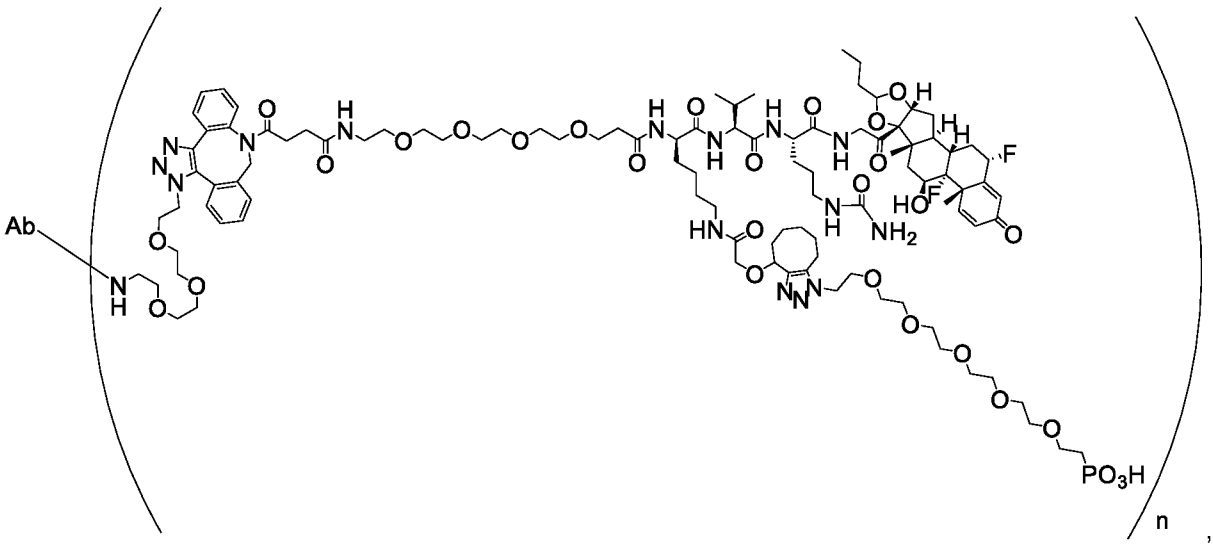




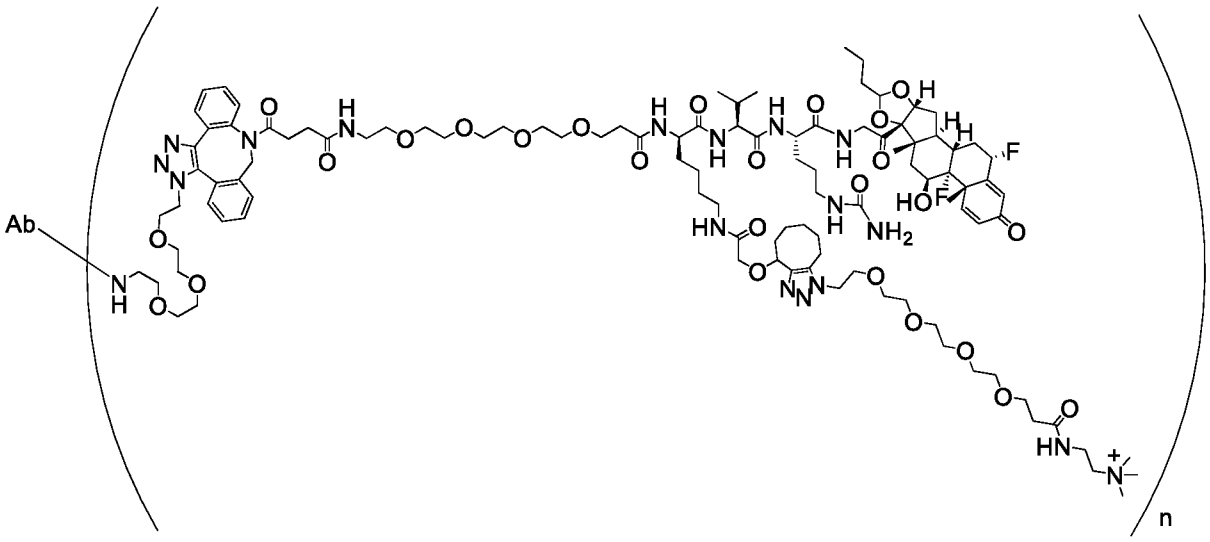
,

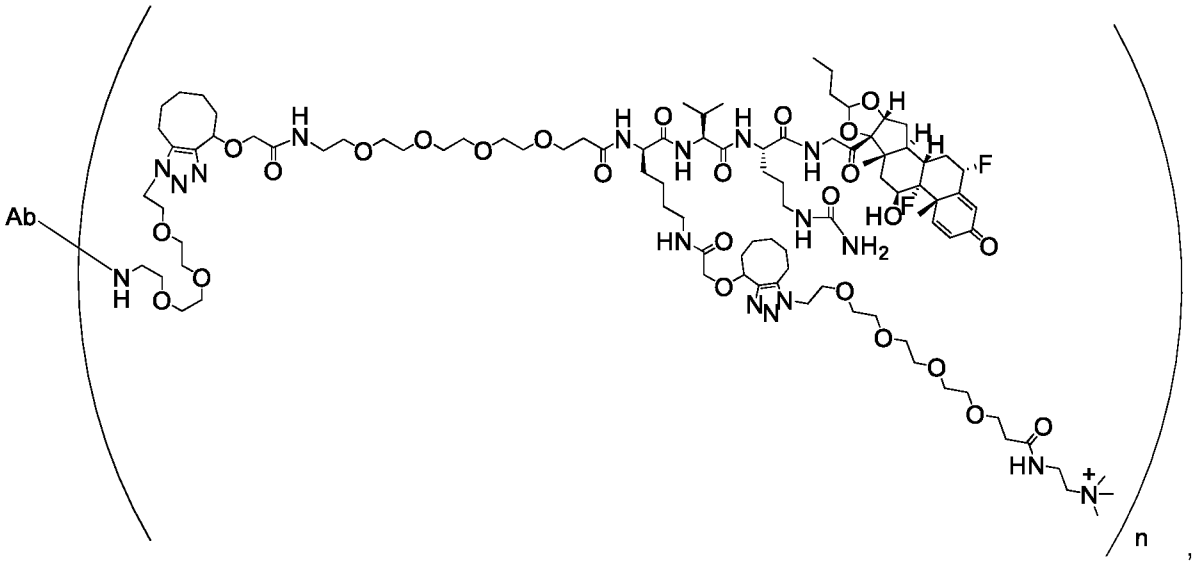


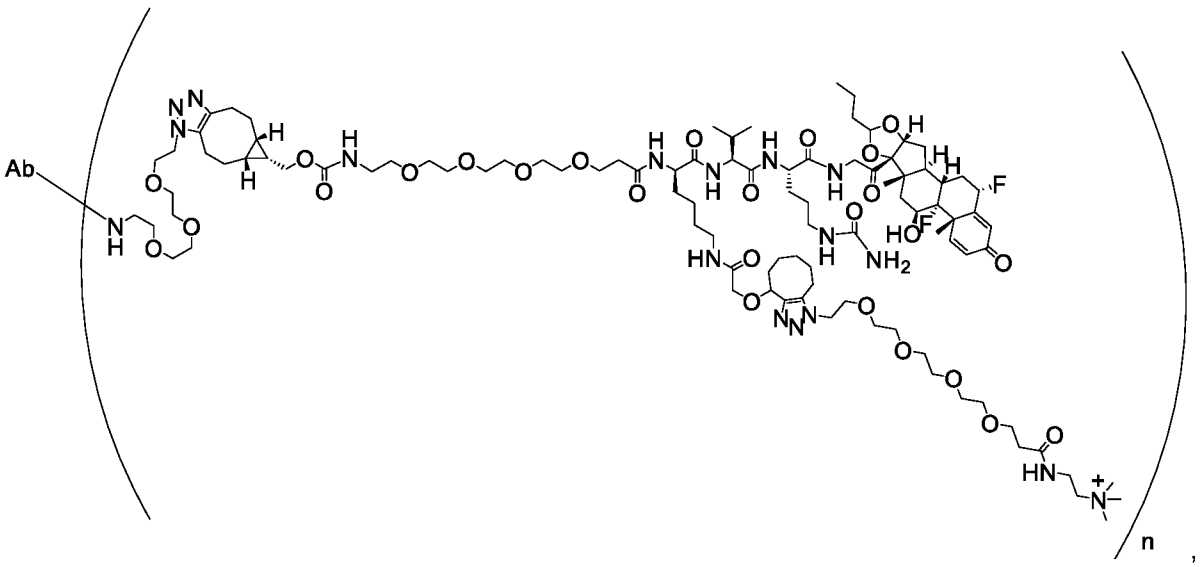
,

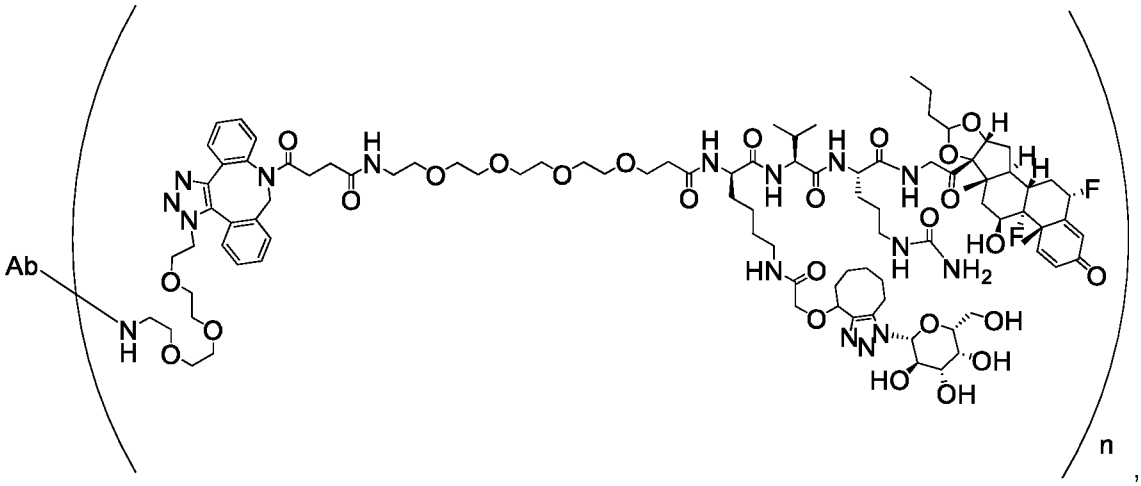


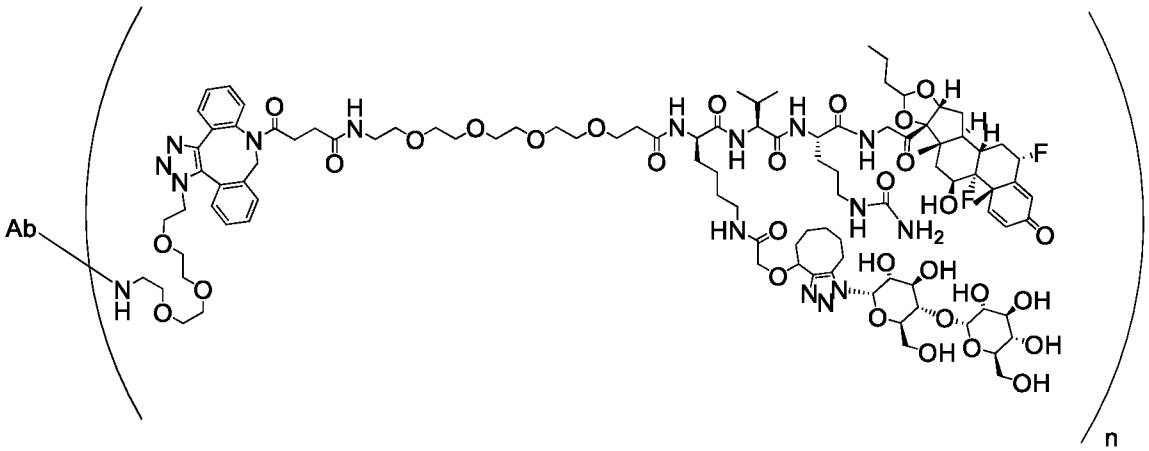
,

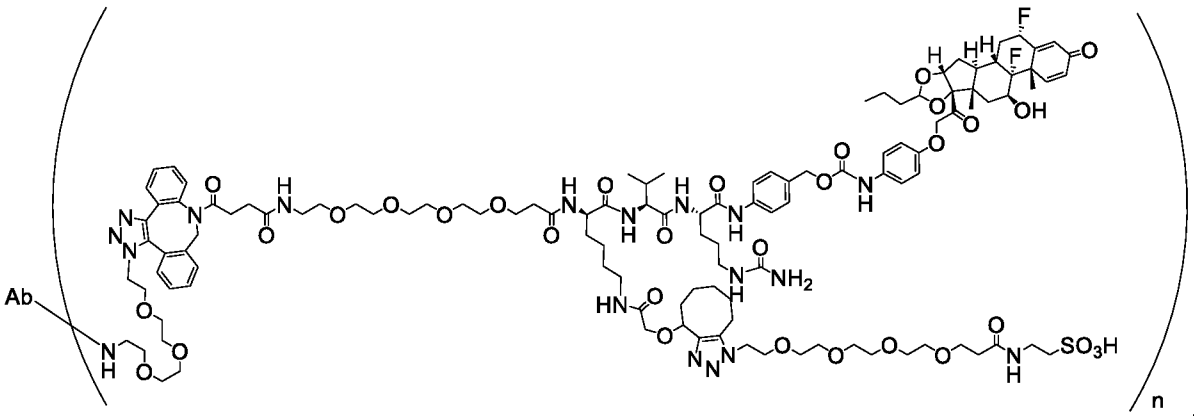


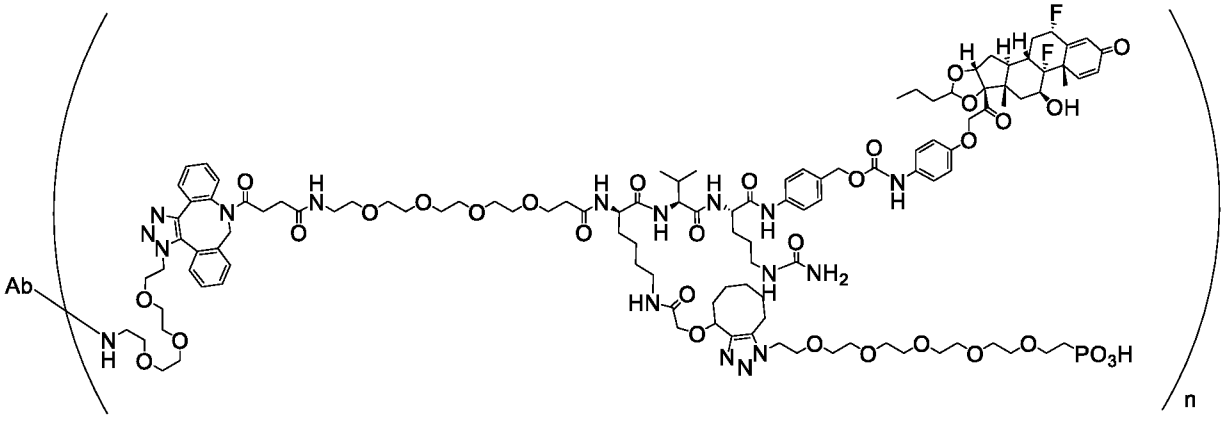




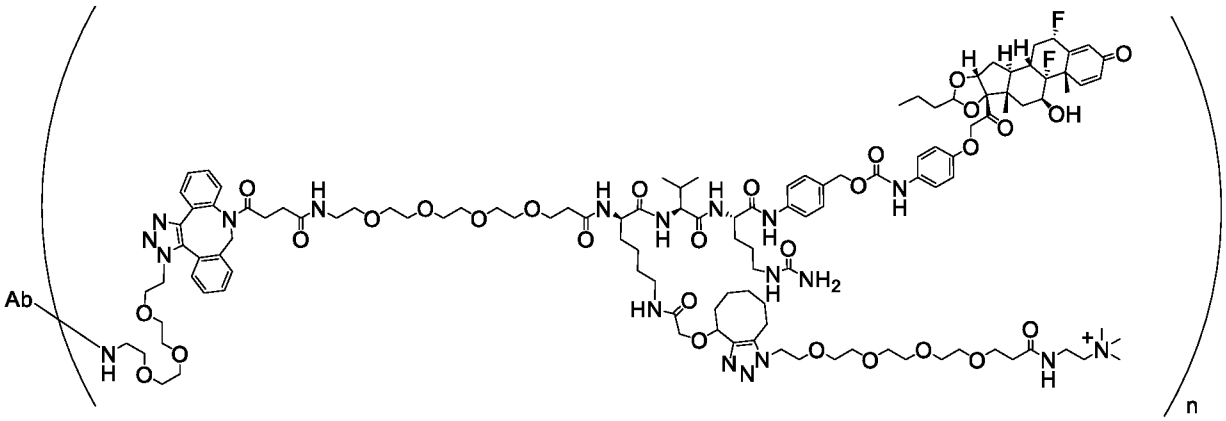

Ab
n


Ab
n

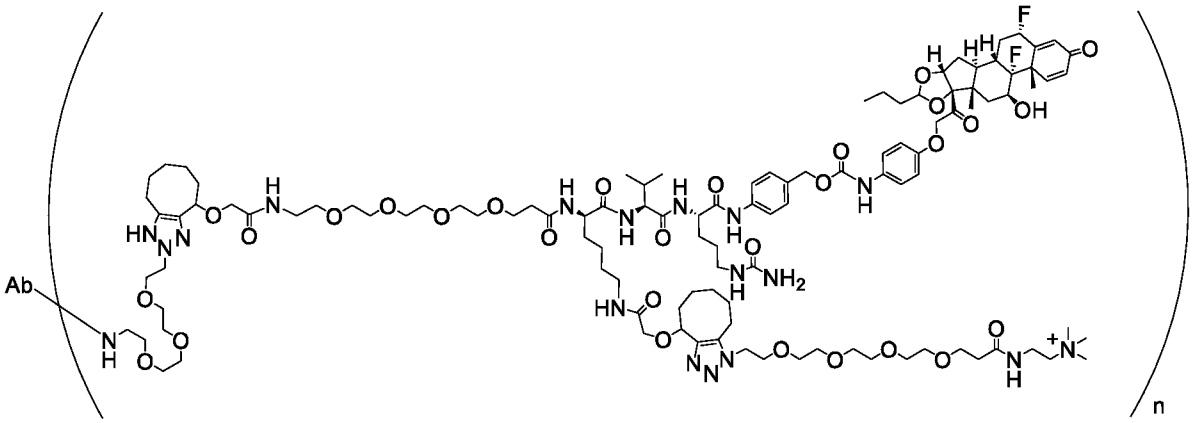

Ab
n


Ab
n

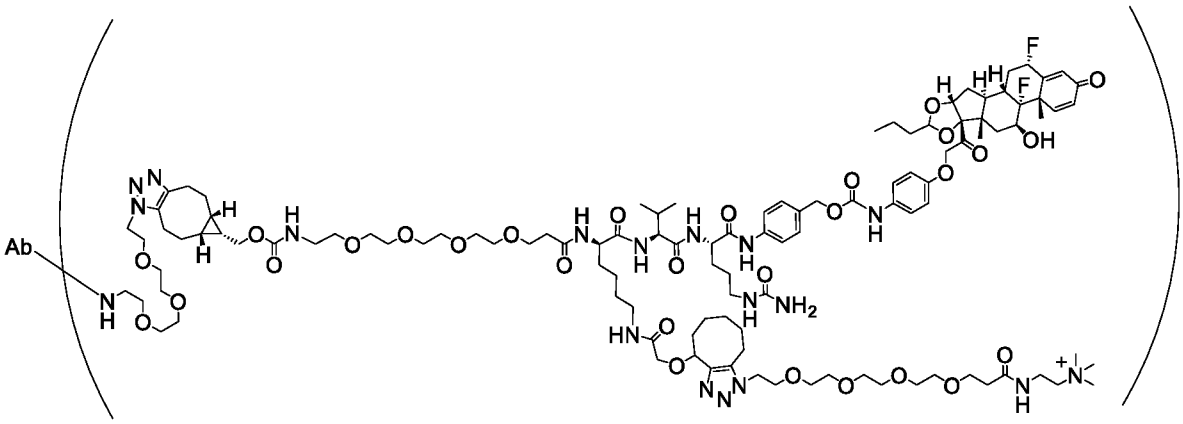
,



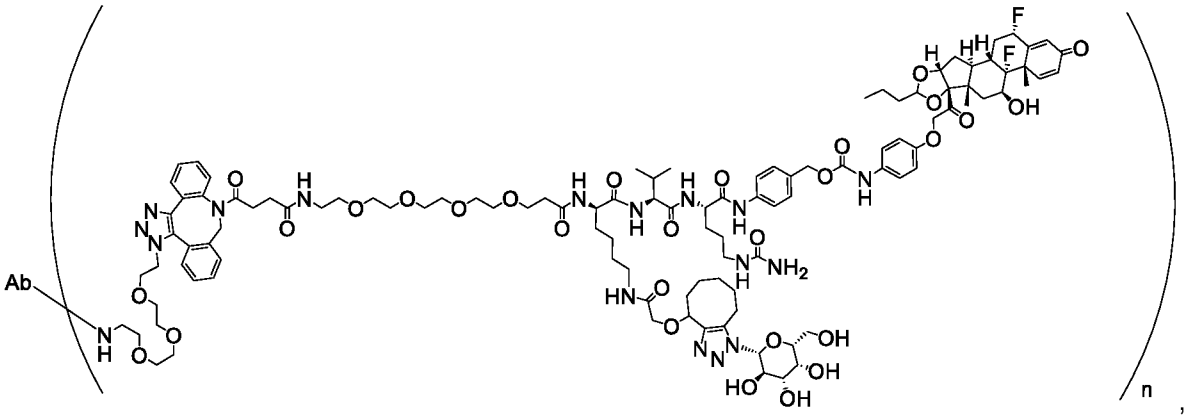
,



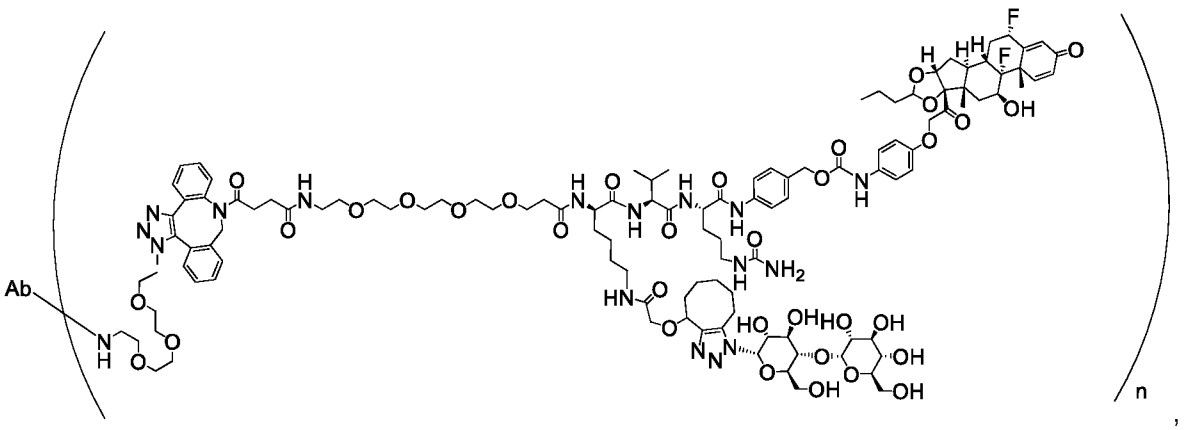
,



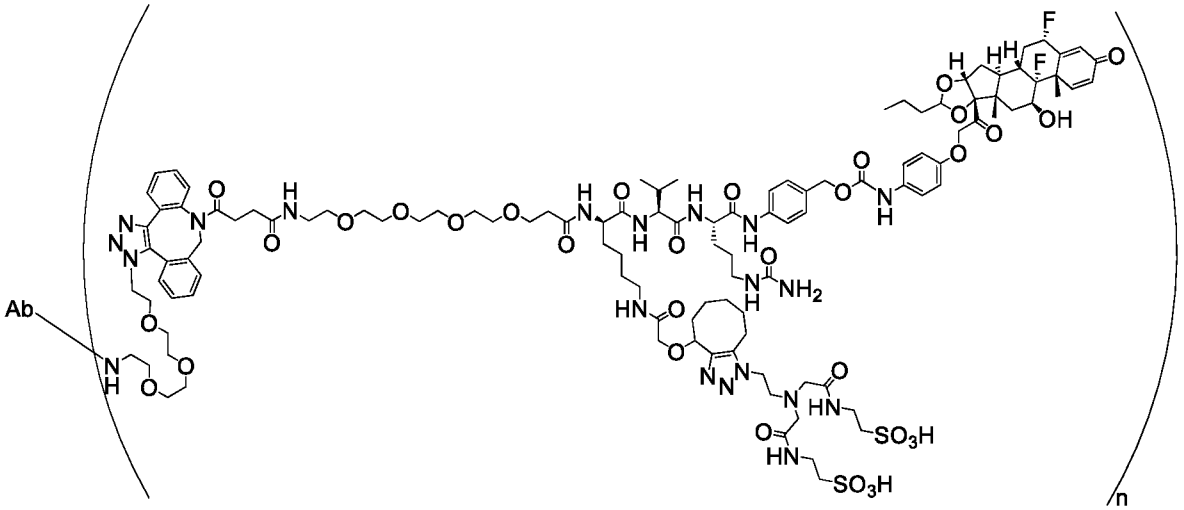
,



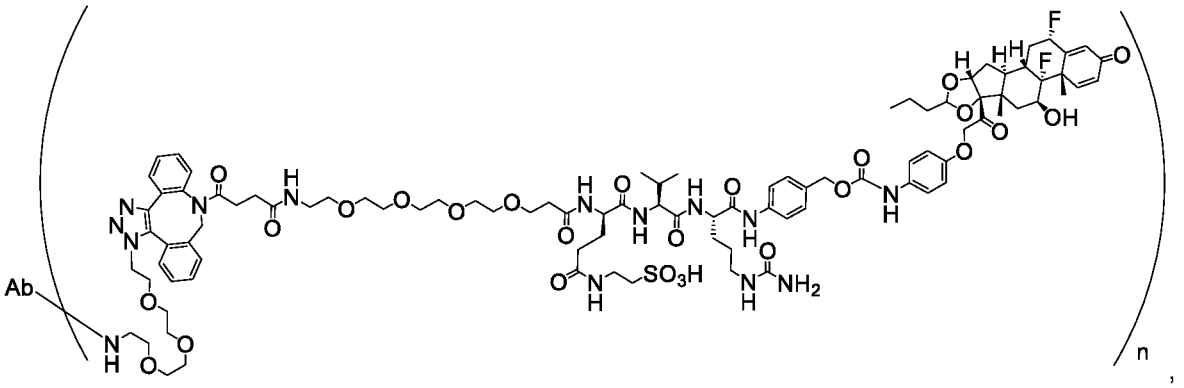
,



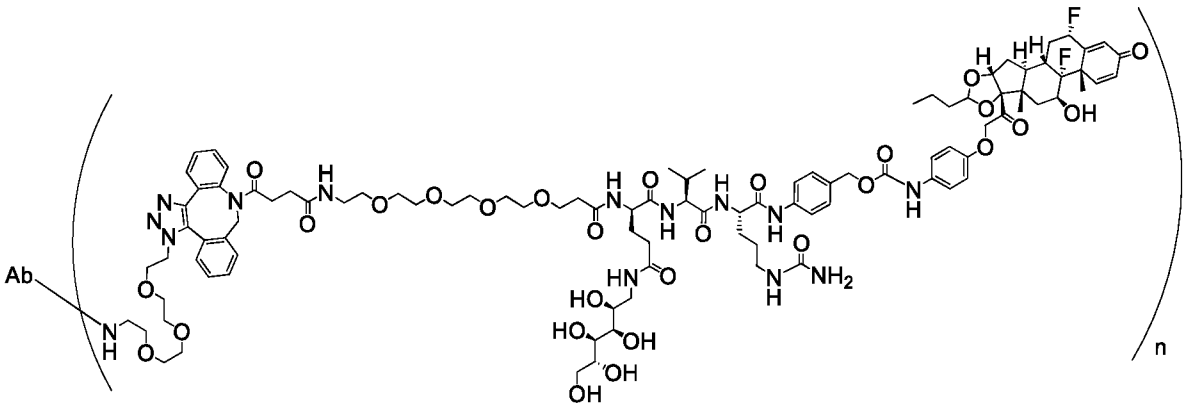
,



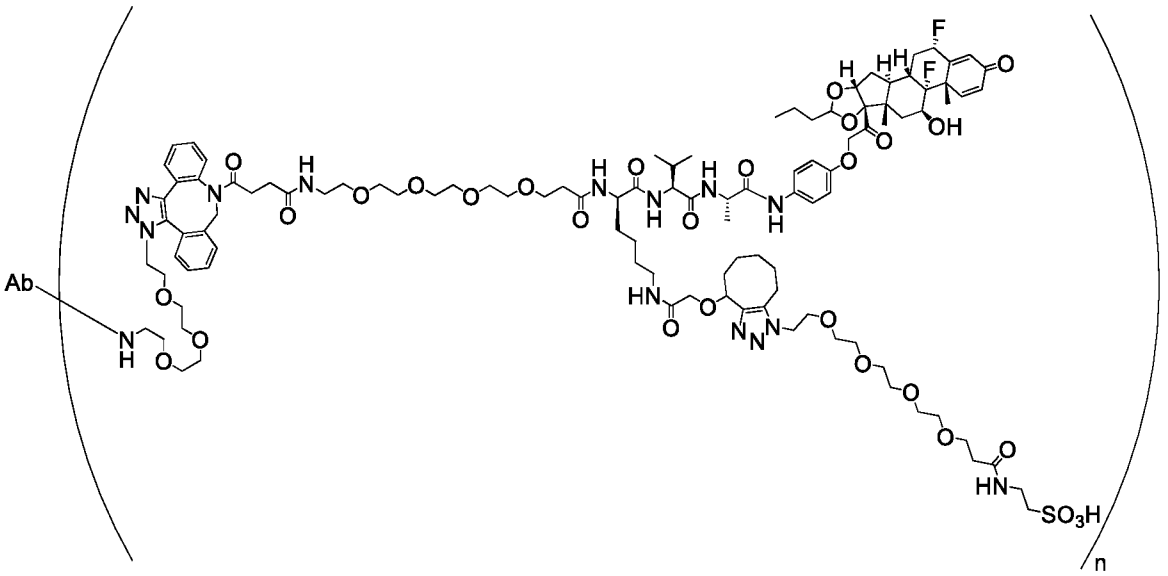
,



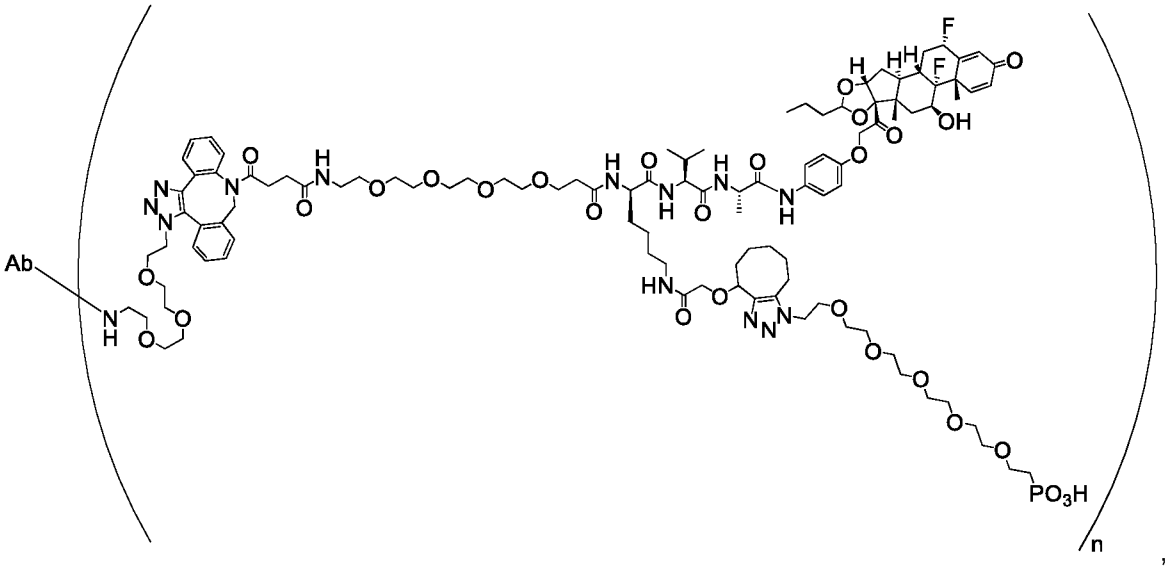
,



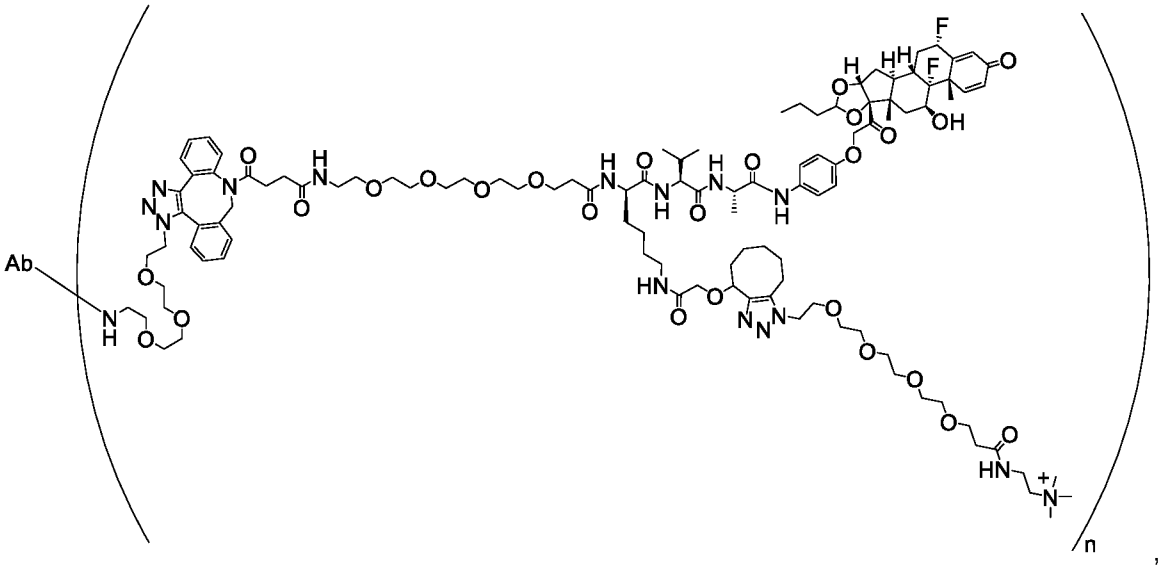
,


Ab
F
OH
HN
SO3H
n

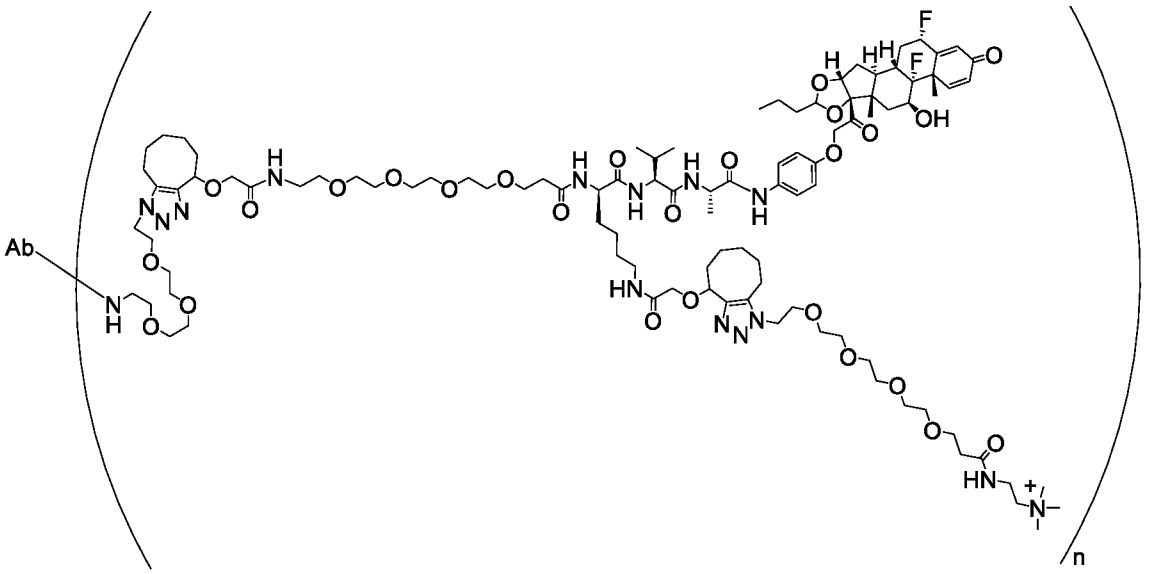
,

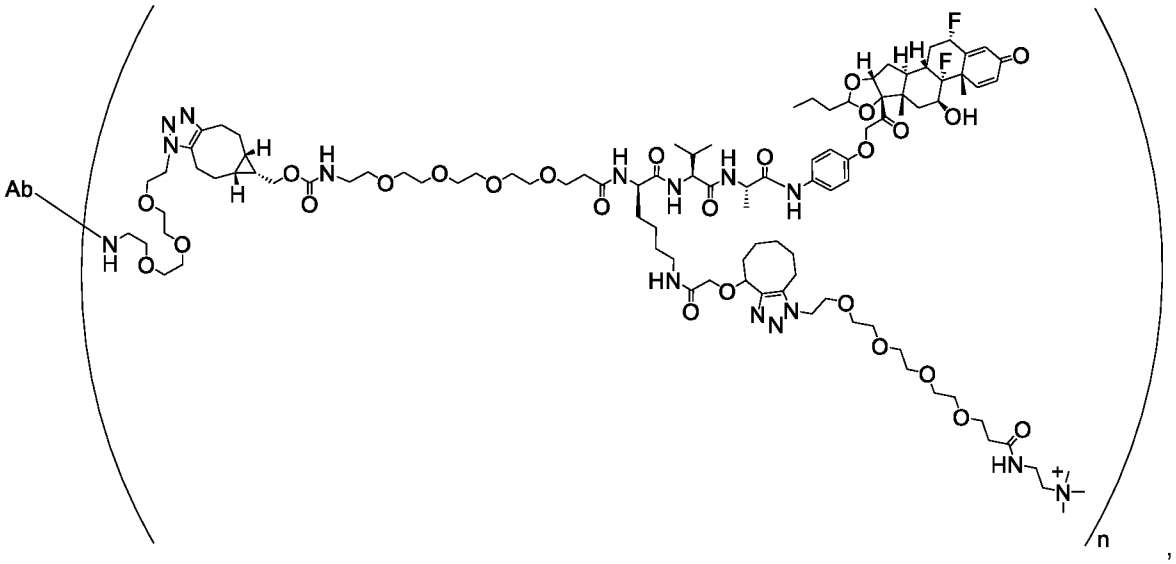

Ab
F
OH
HN
PO3H
n

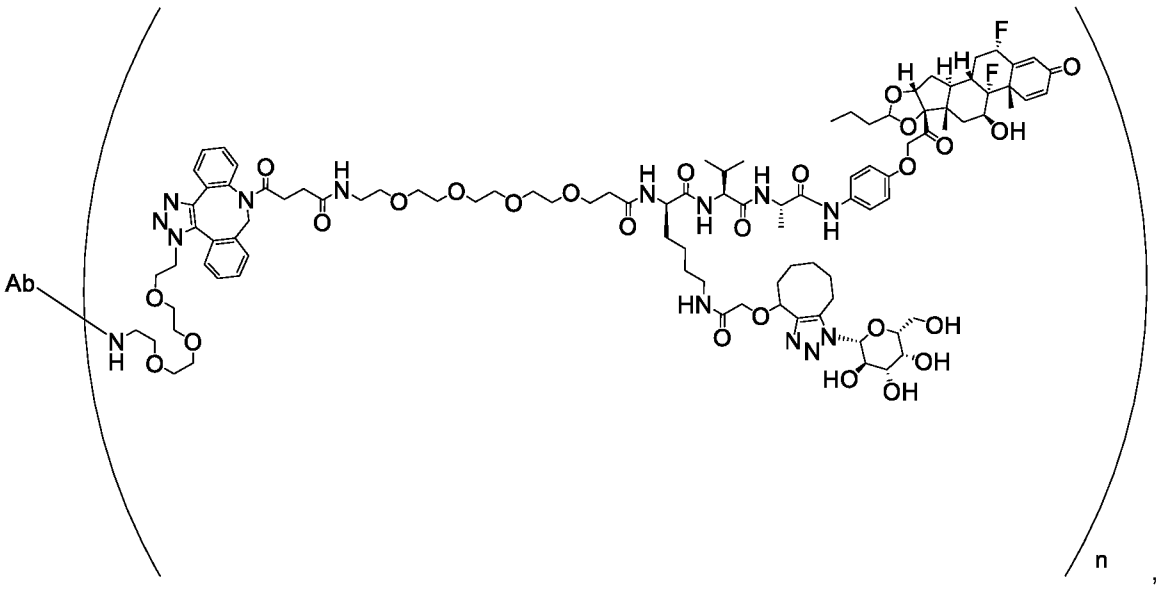
,

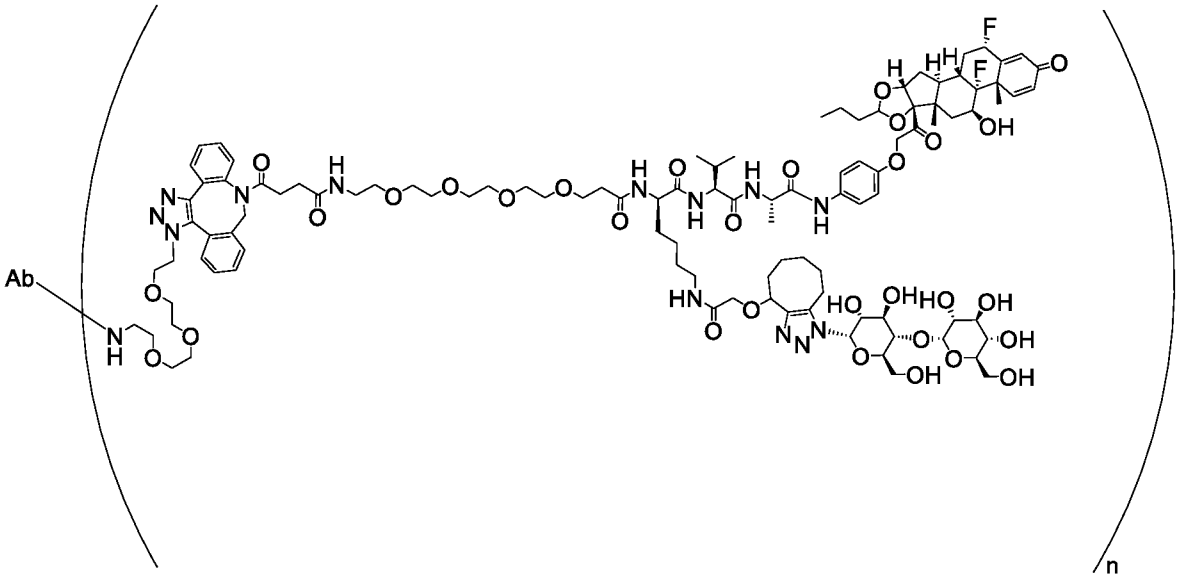

Ab
F
OH
HN
n

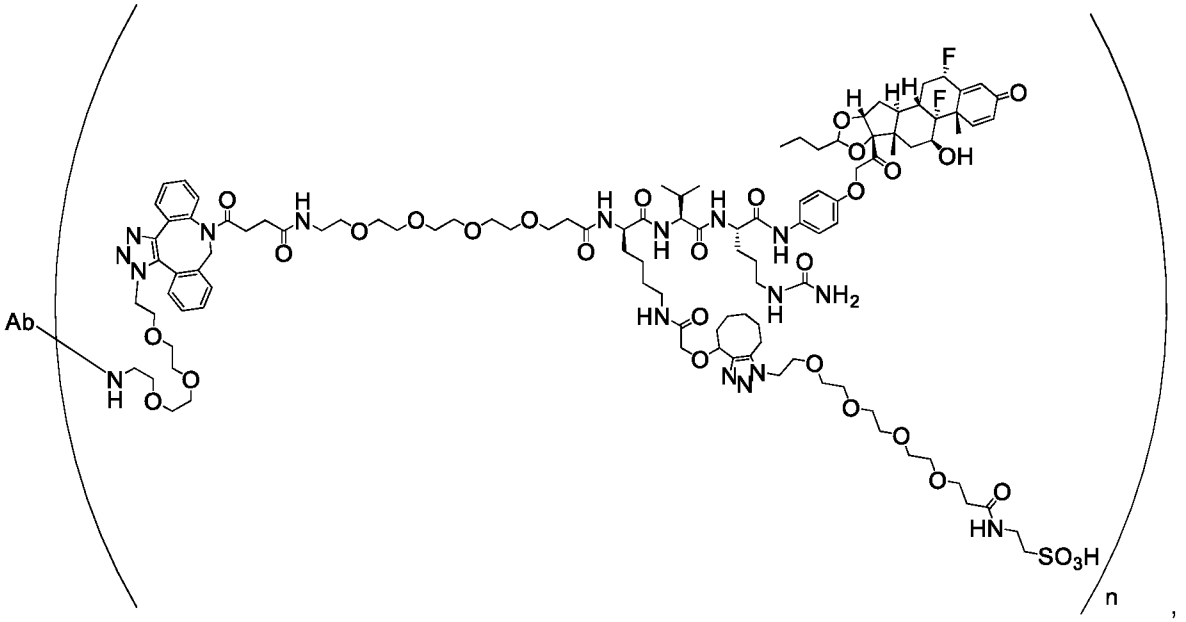
,

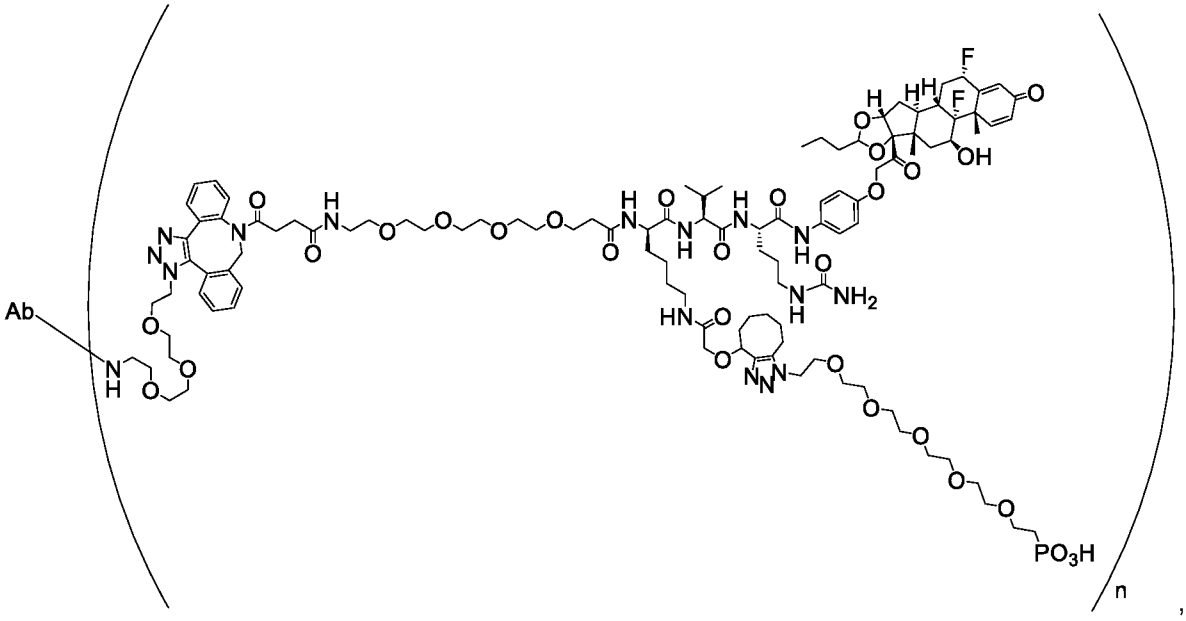


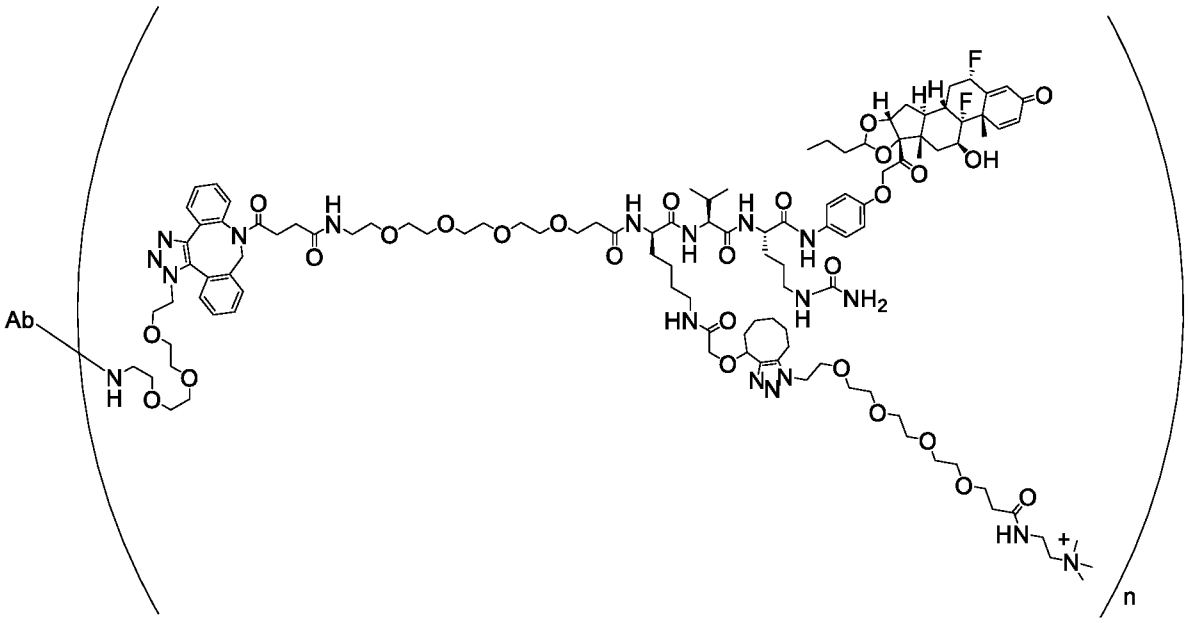


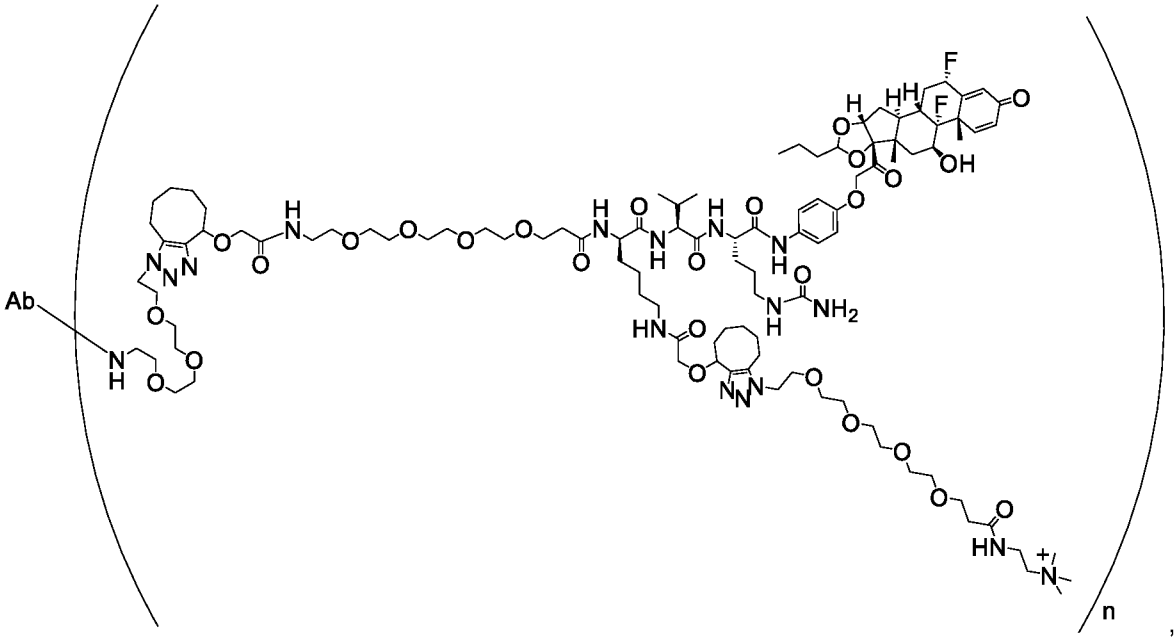


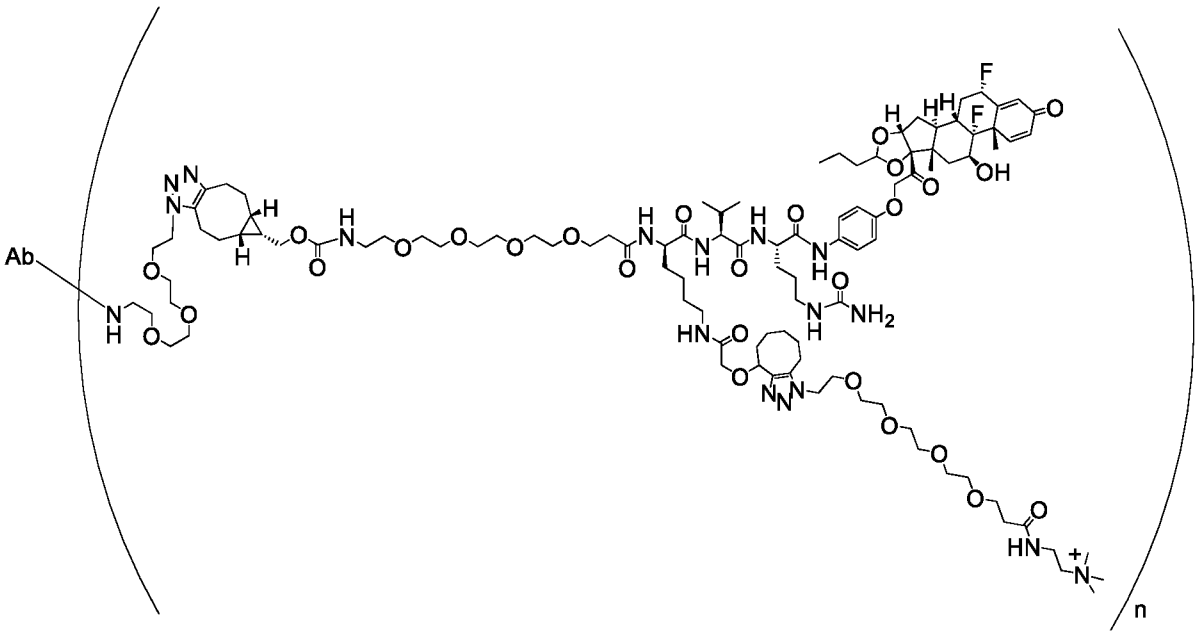


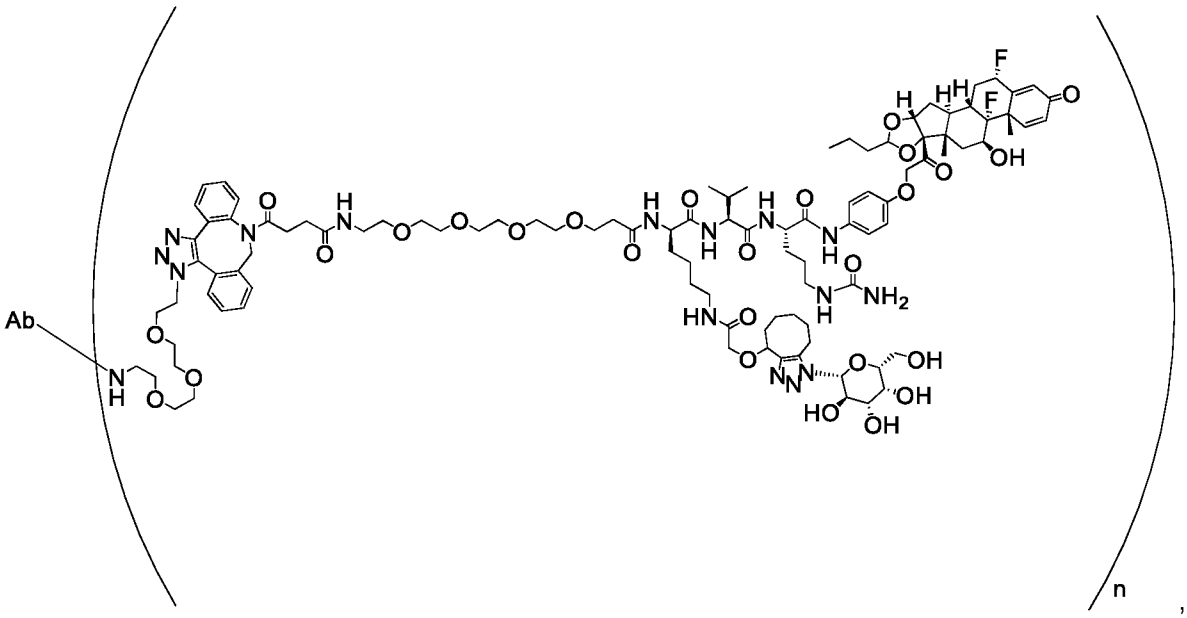


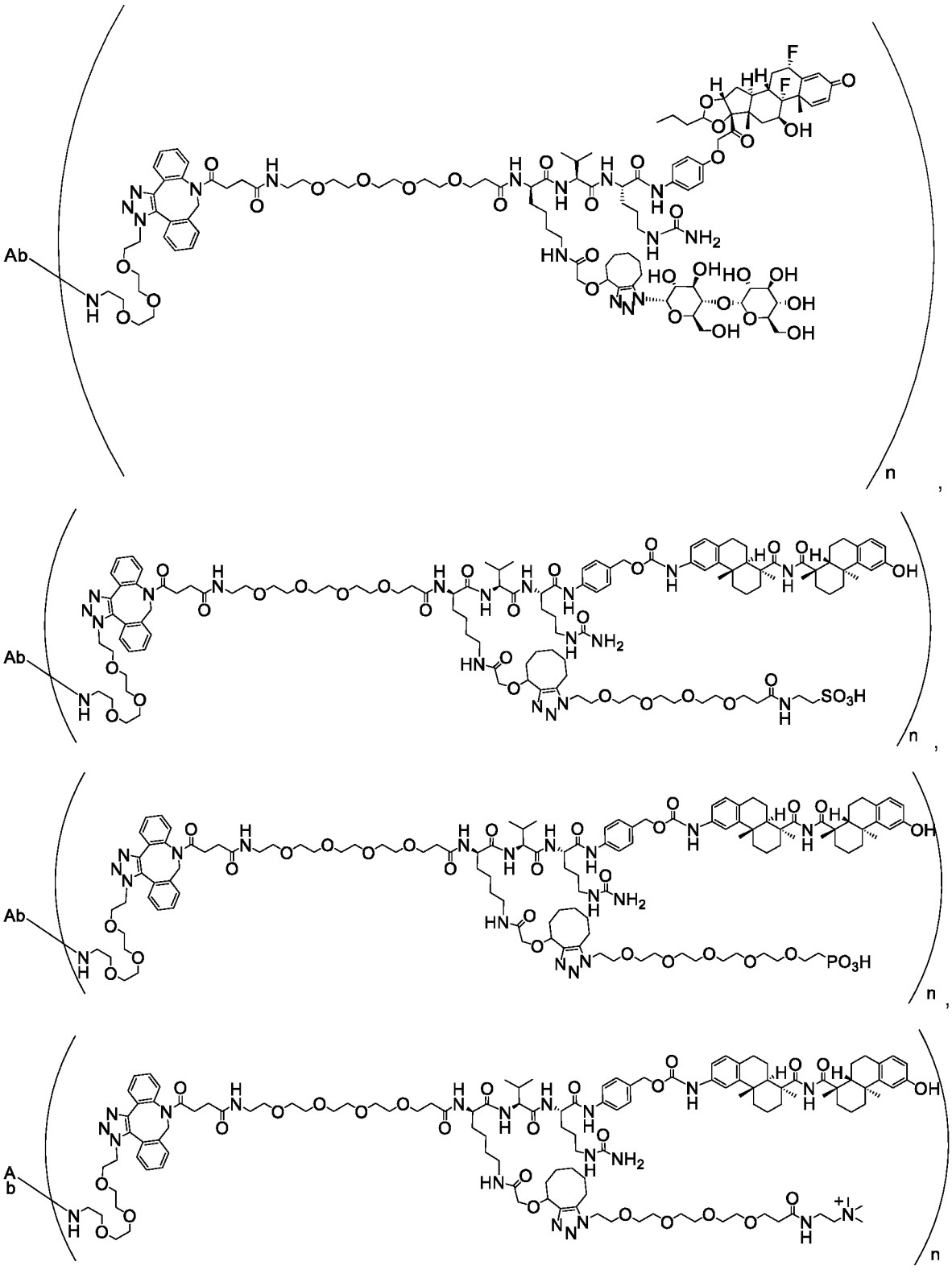


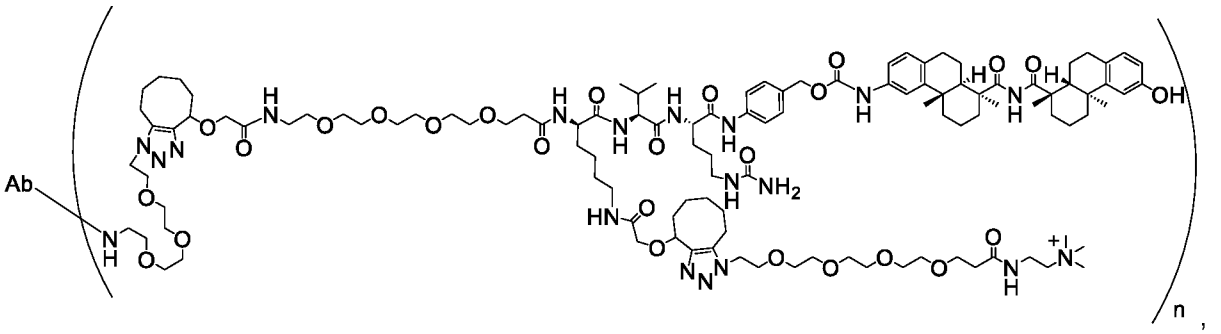


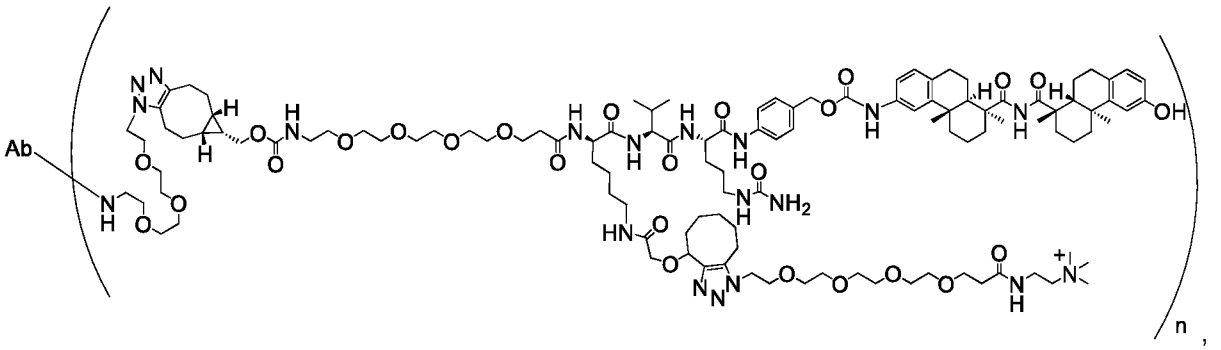


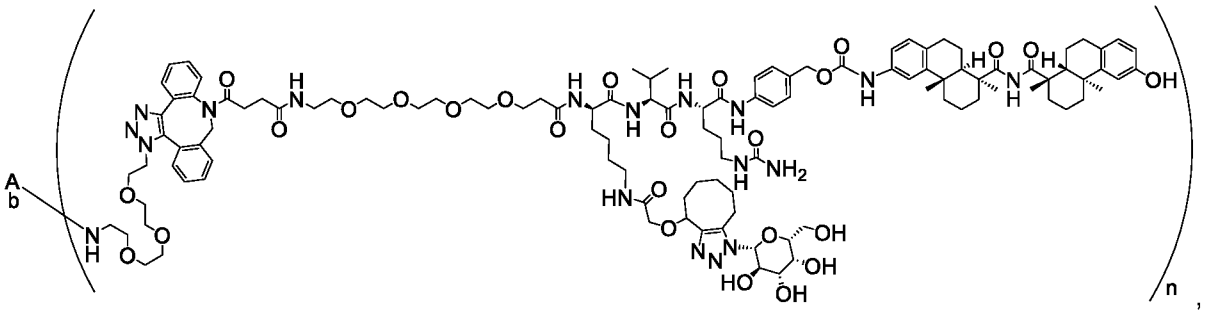


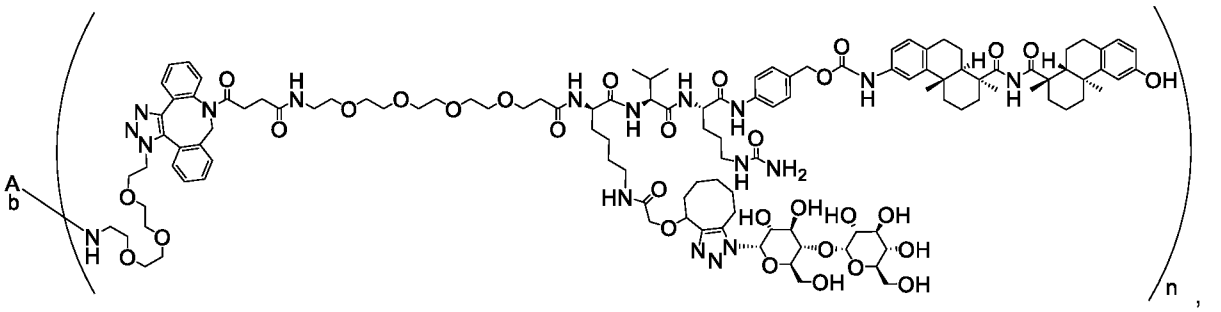


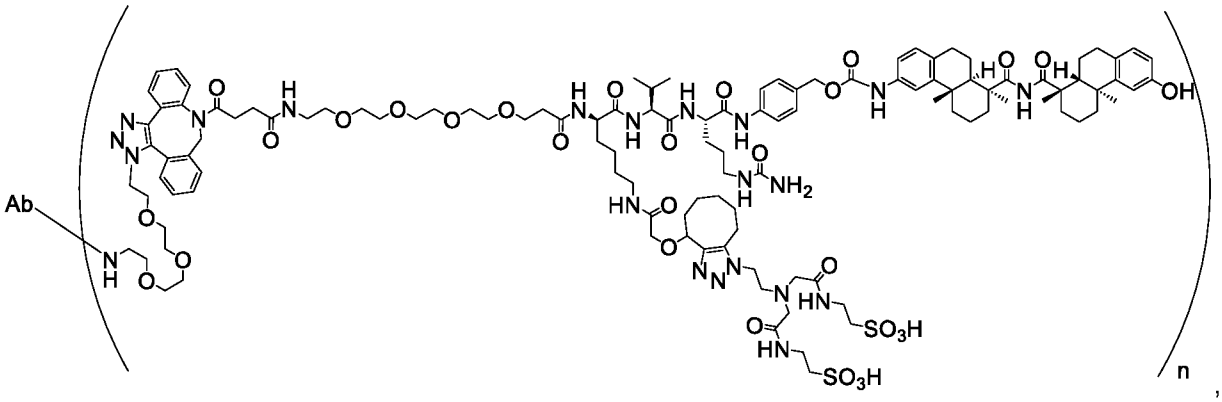


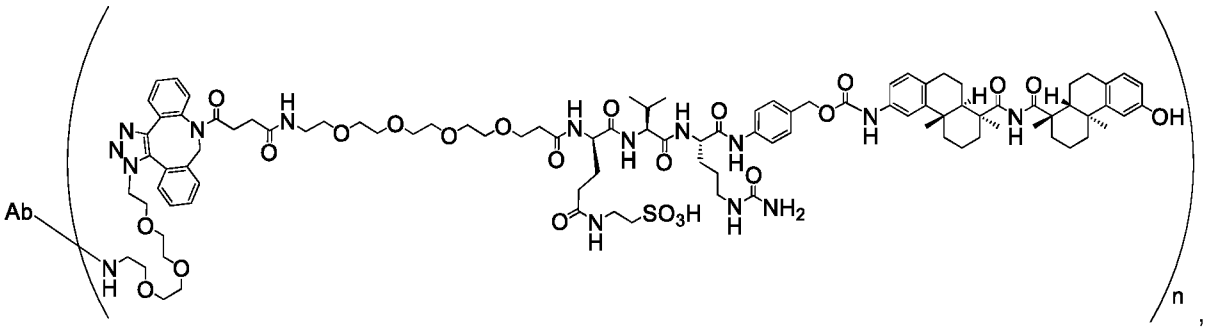


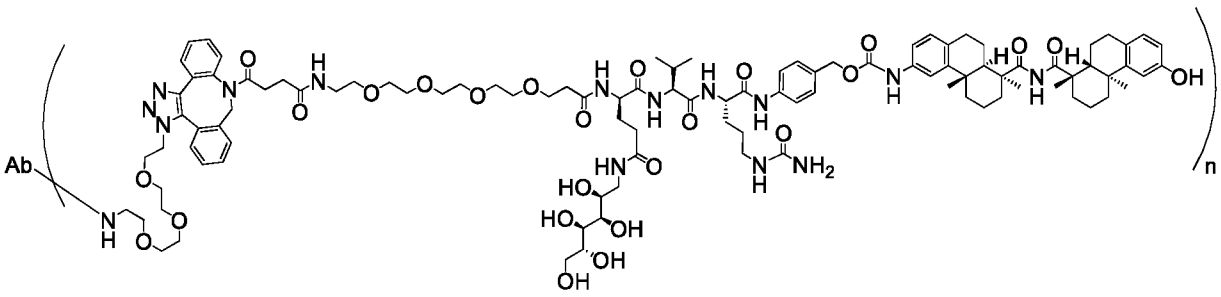


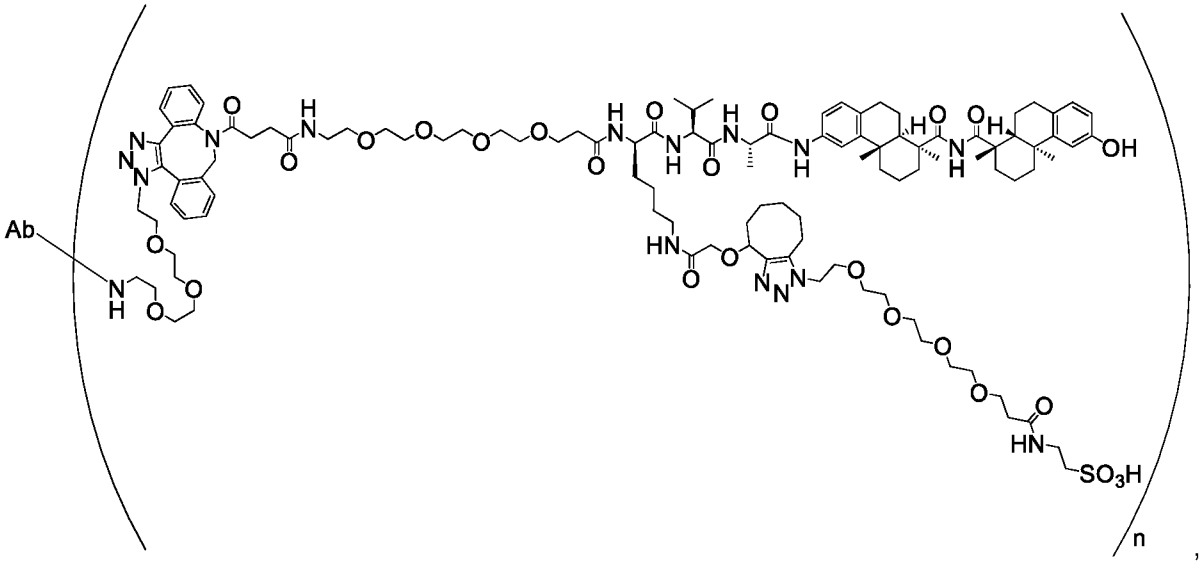


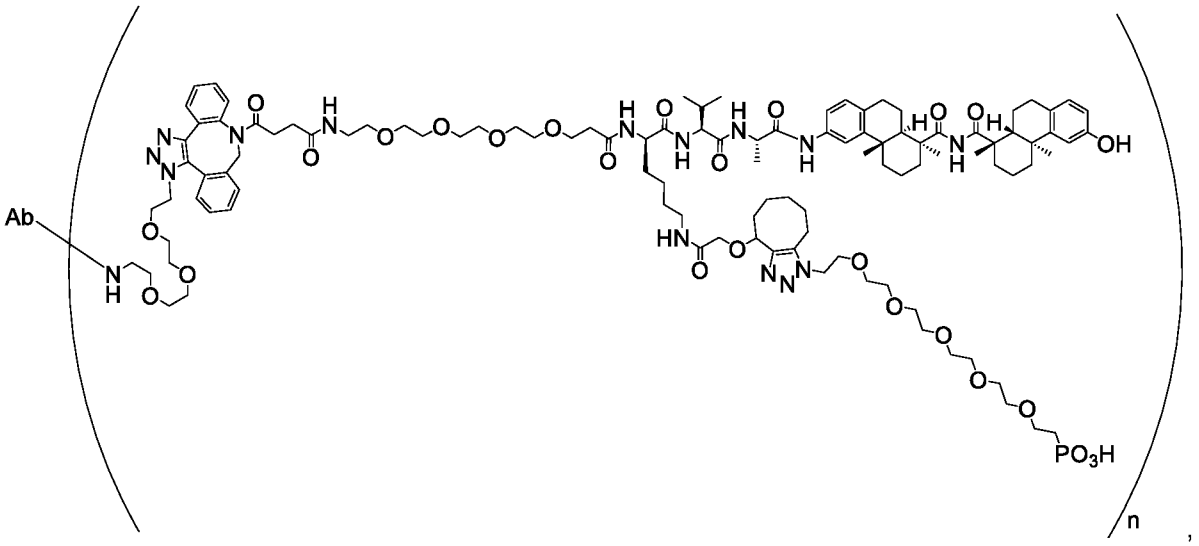


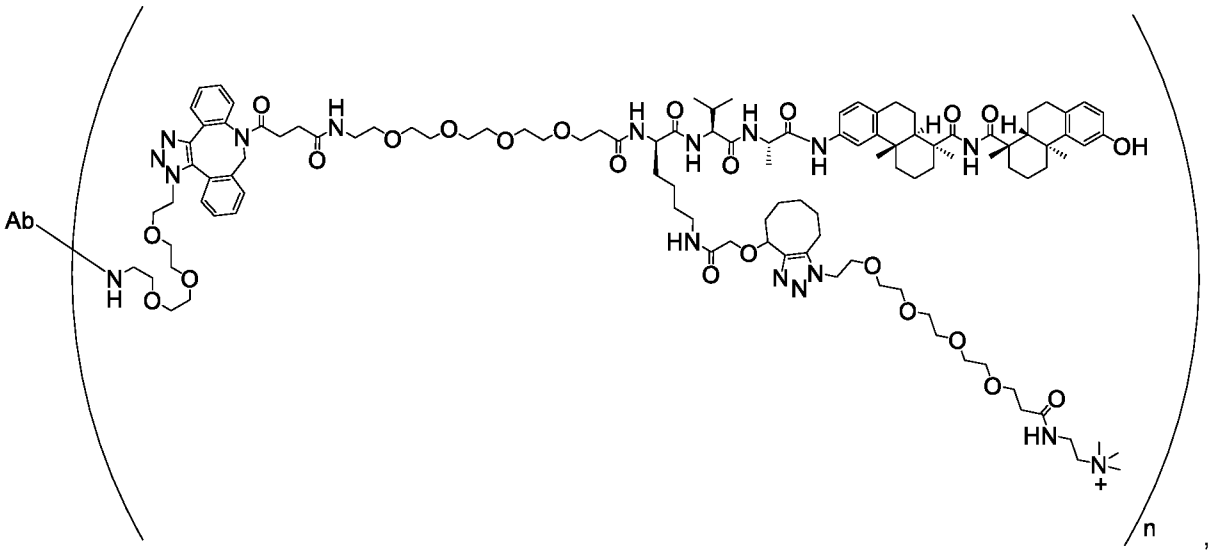


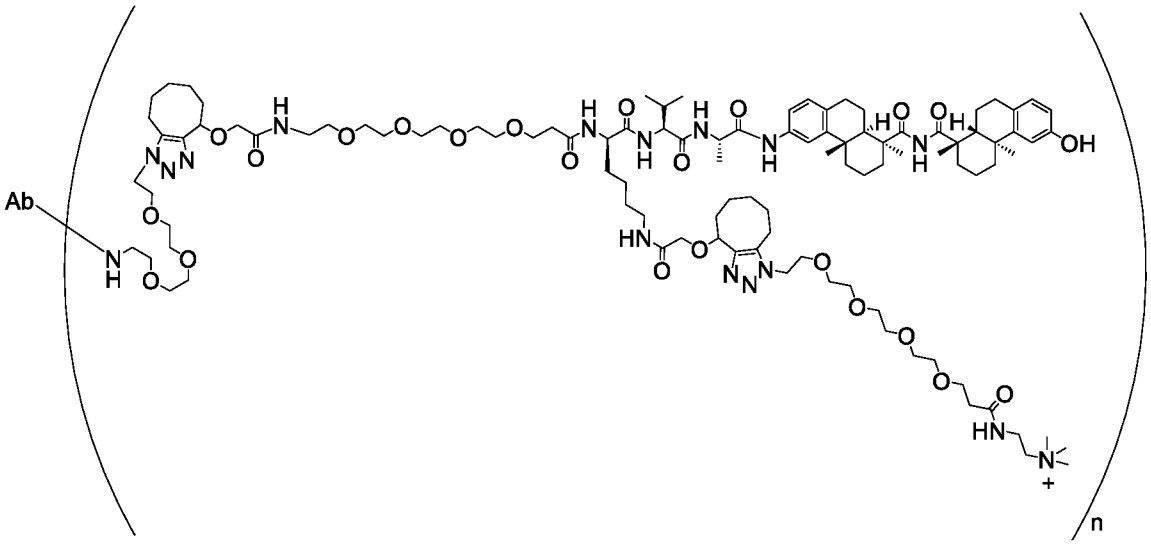


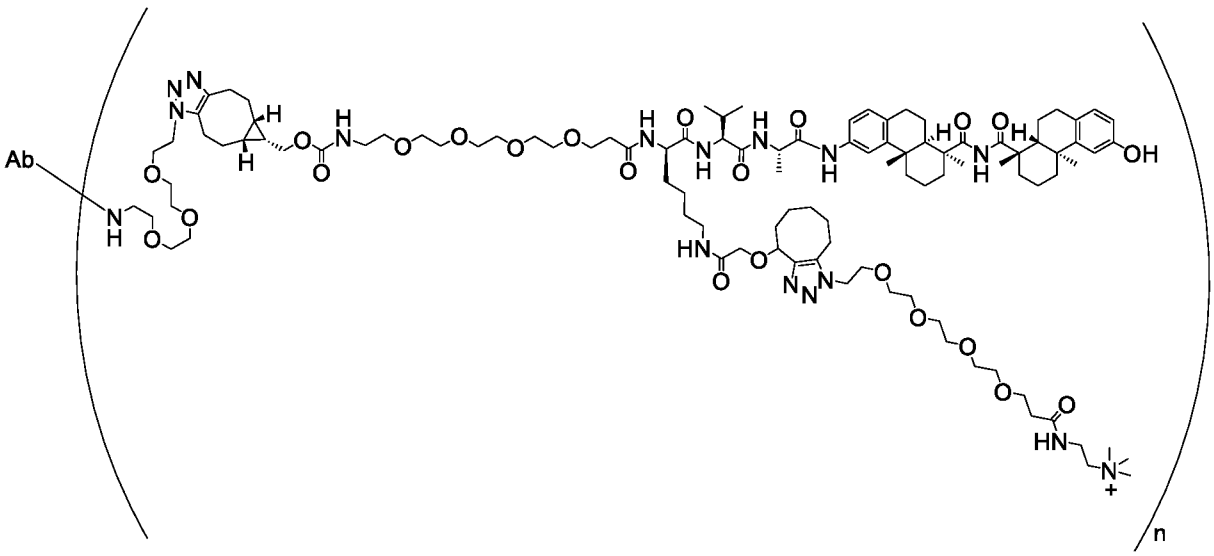


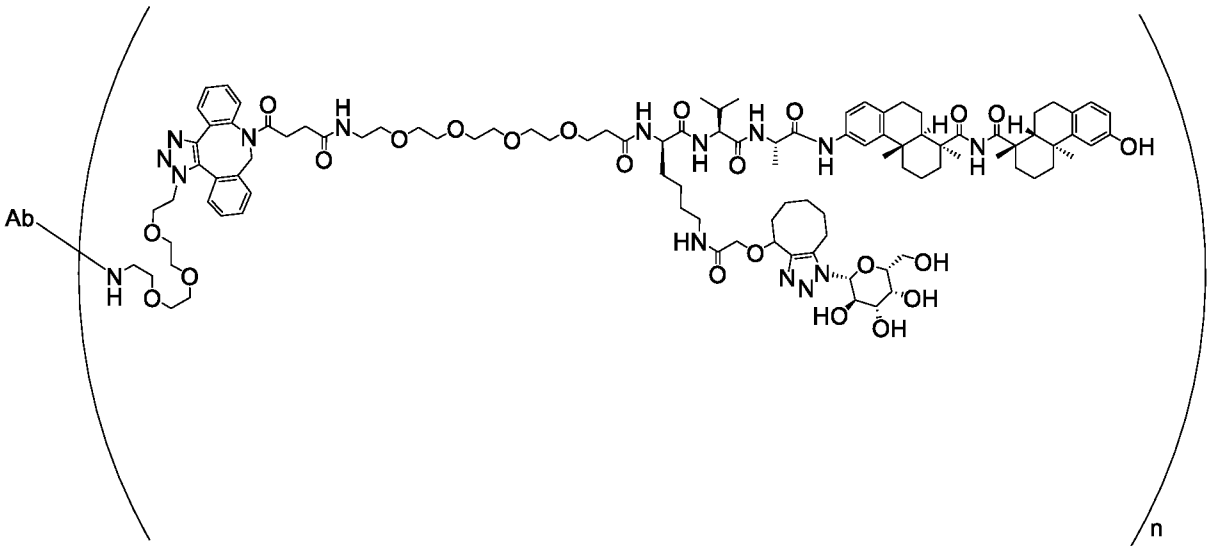




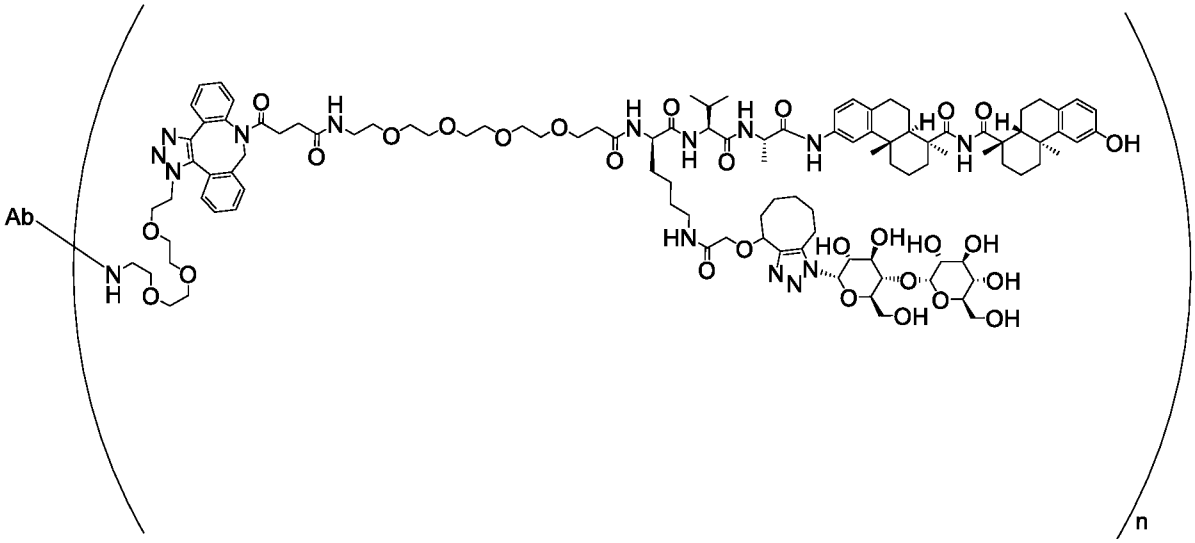




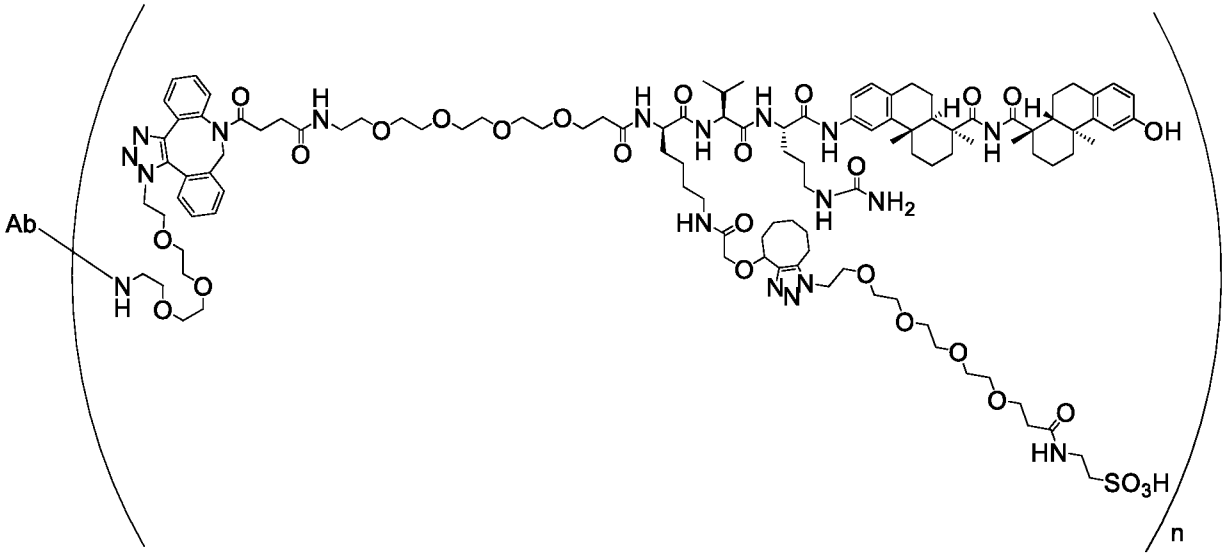




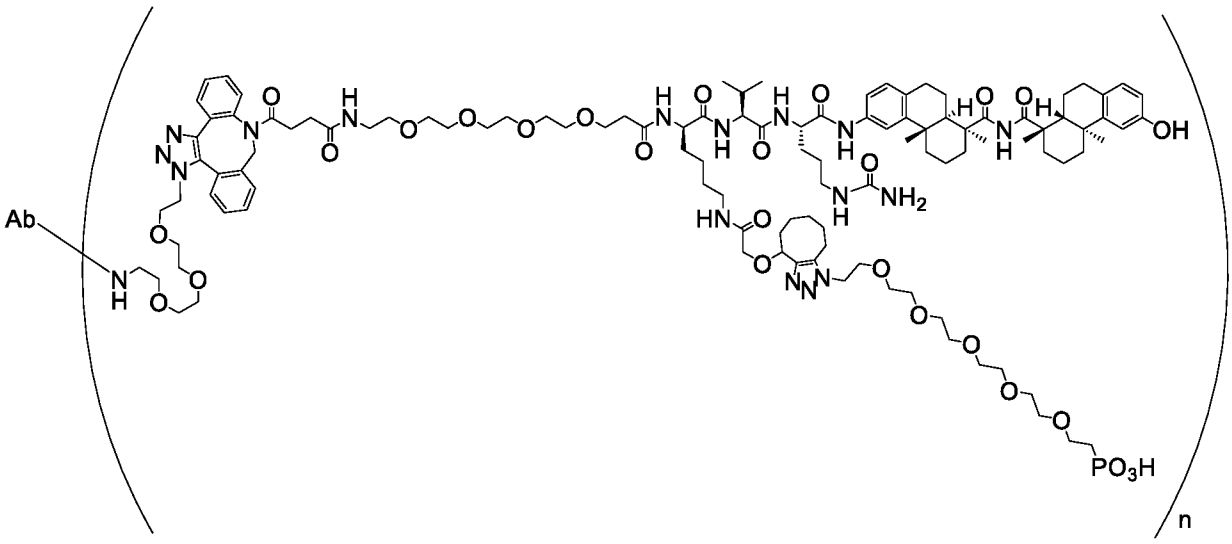
,



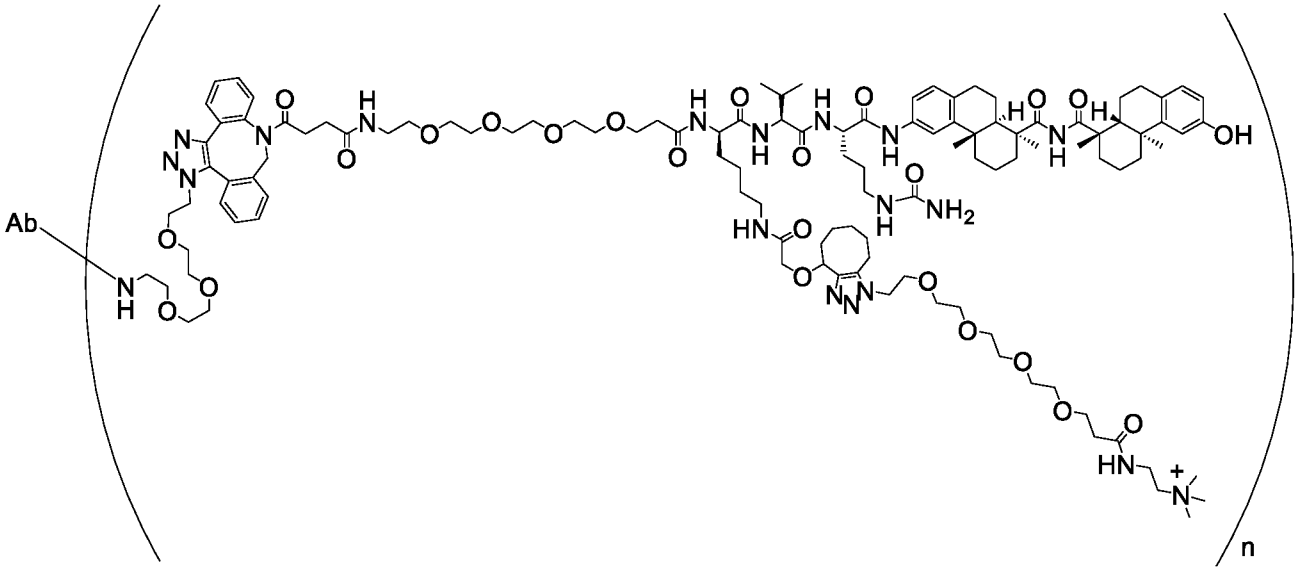
,



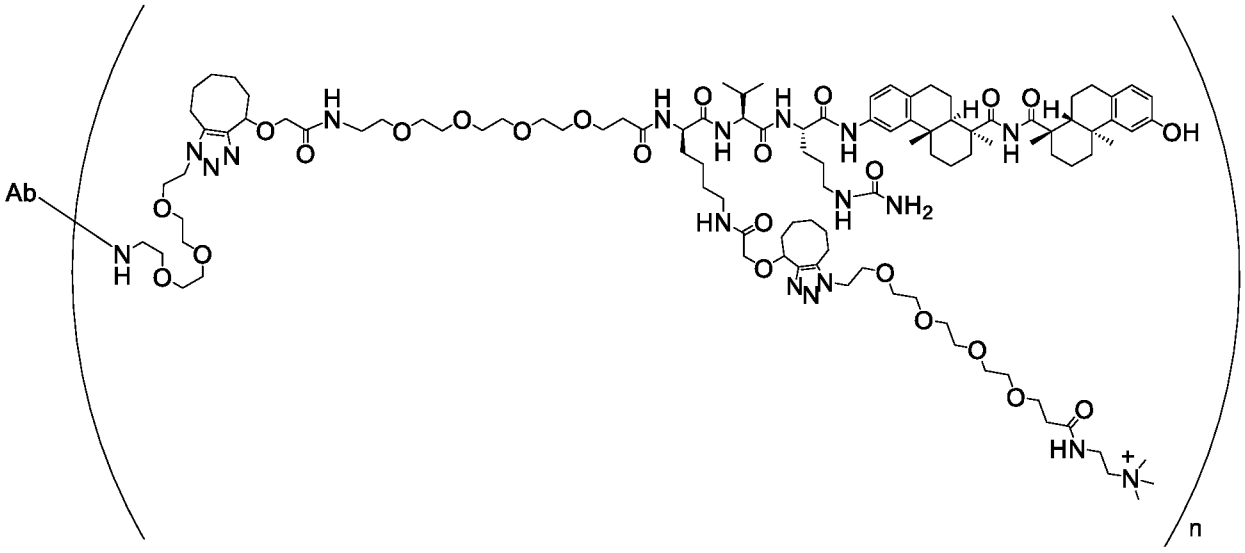
,


Ab
OH
PO3H
n

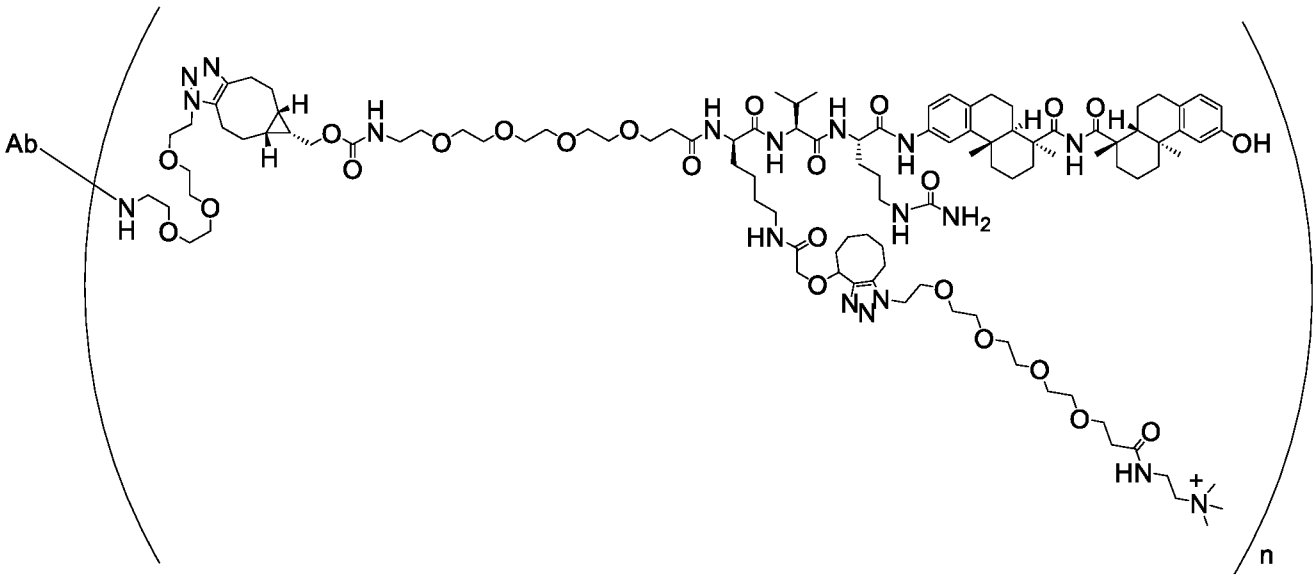
,


Ab
OH
n

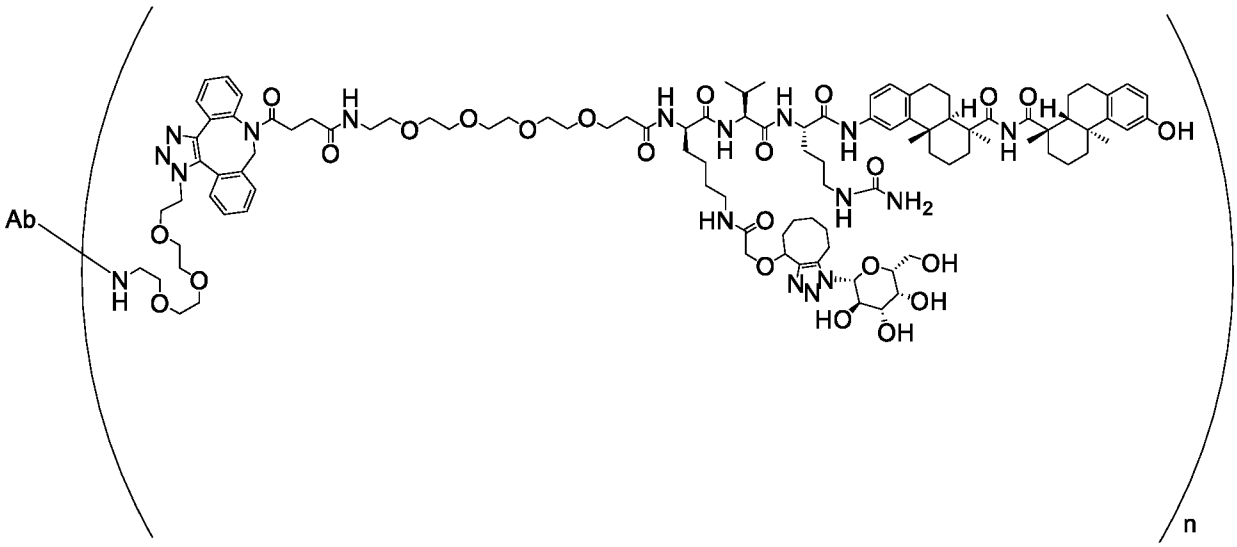
,


Ab
OH
n

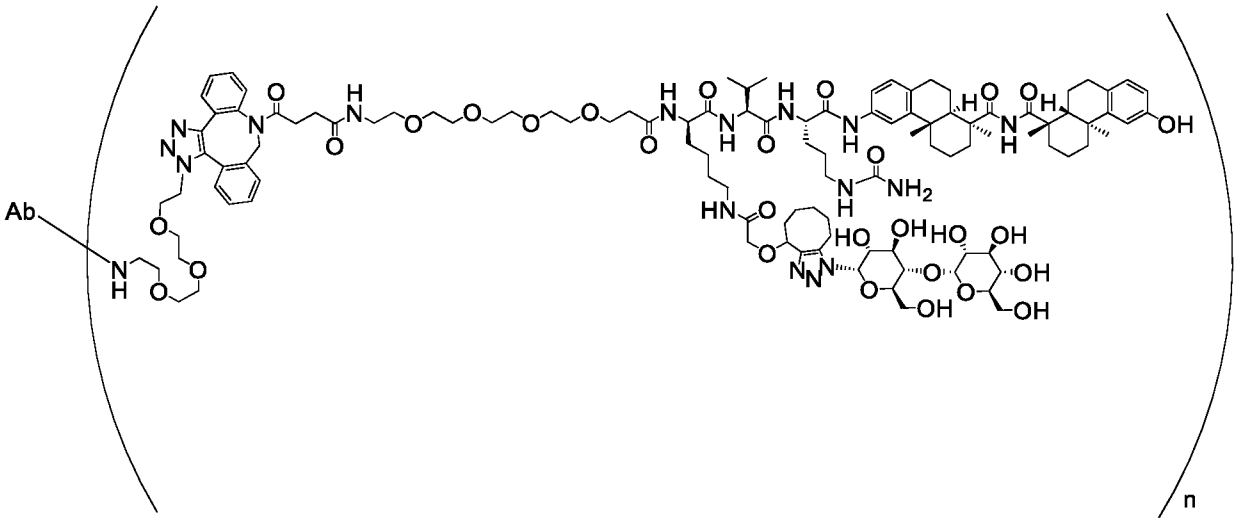
,


Ab
n

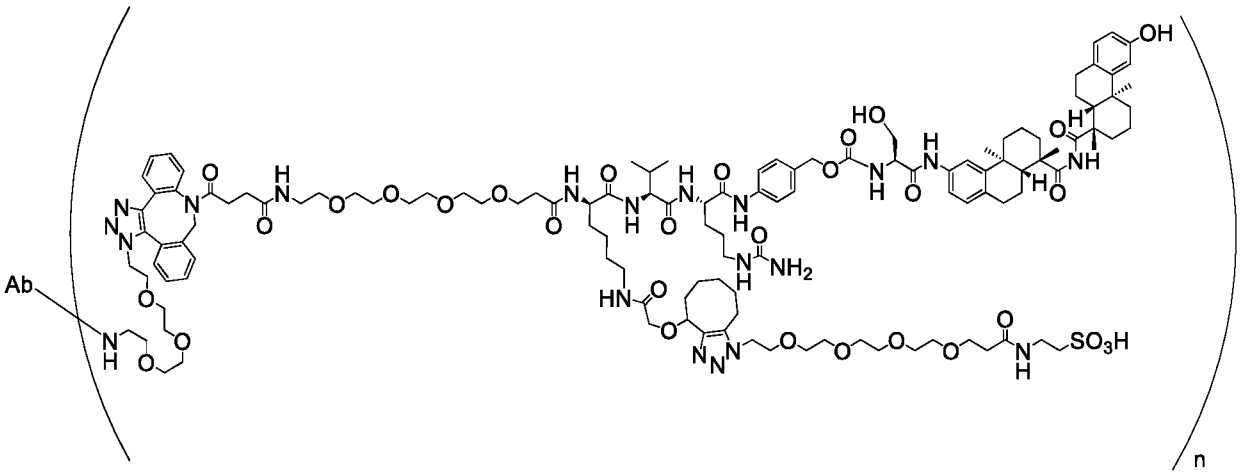
,


Ab
n

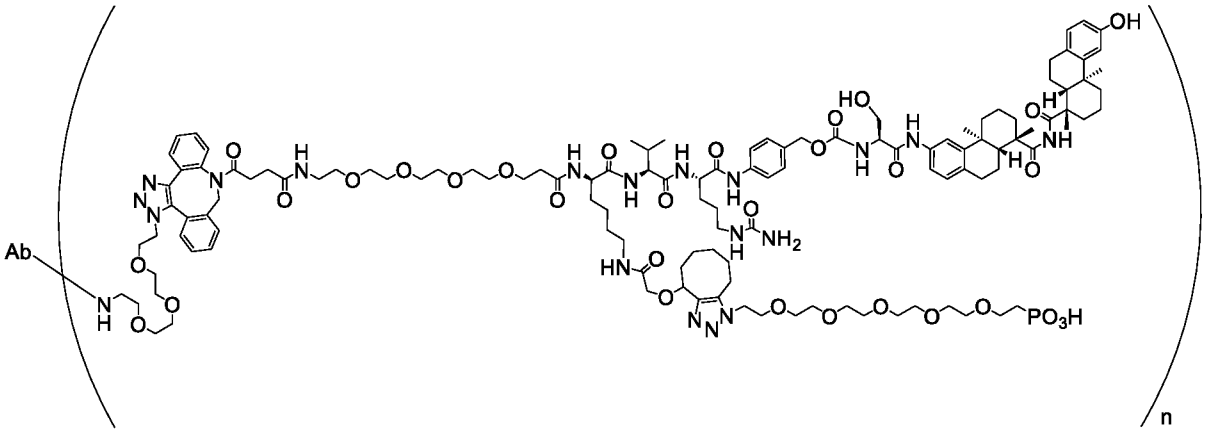
,


Ab
n

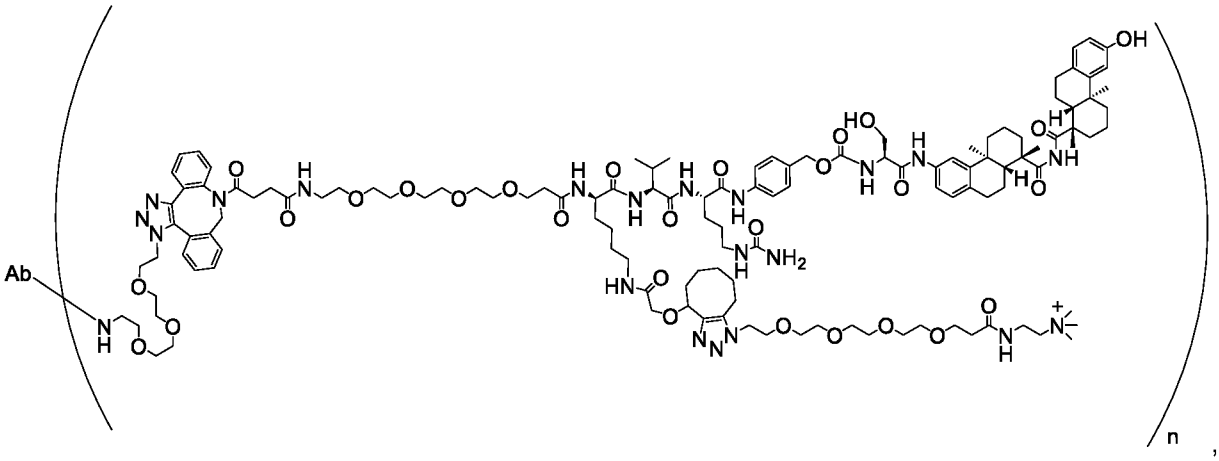
,



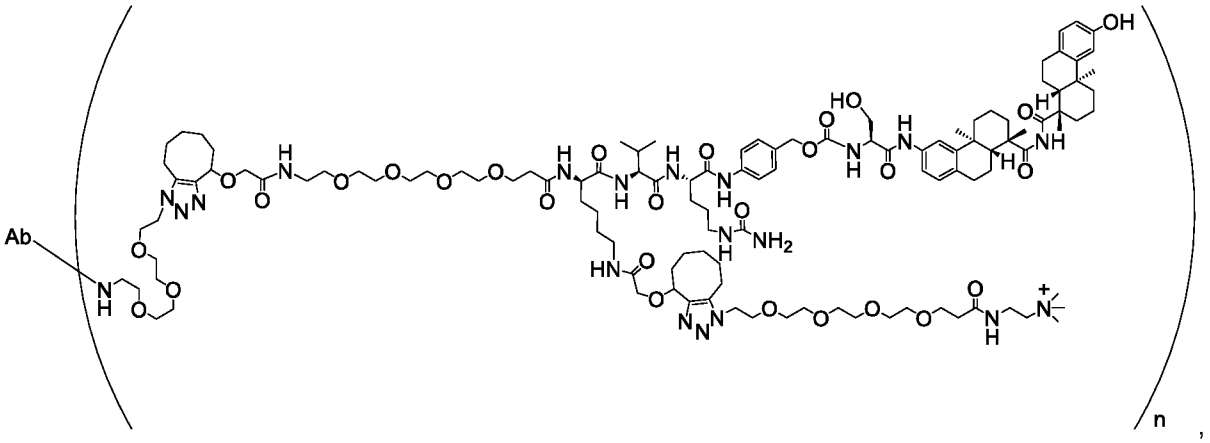
,

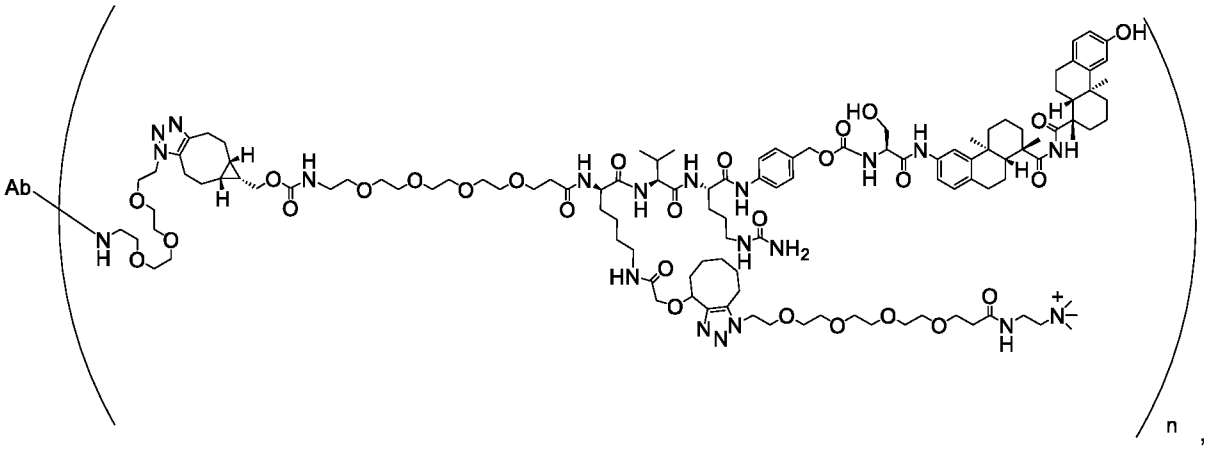


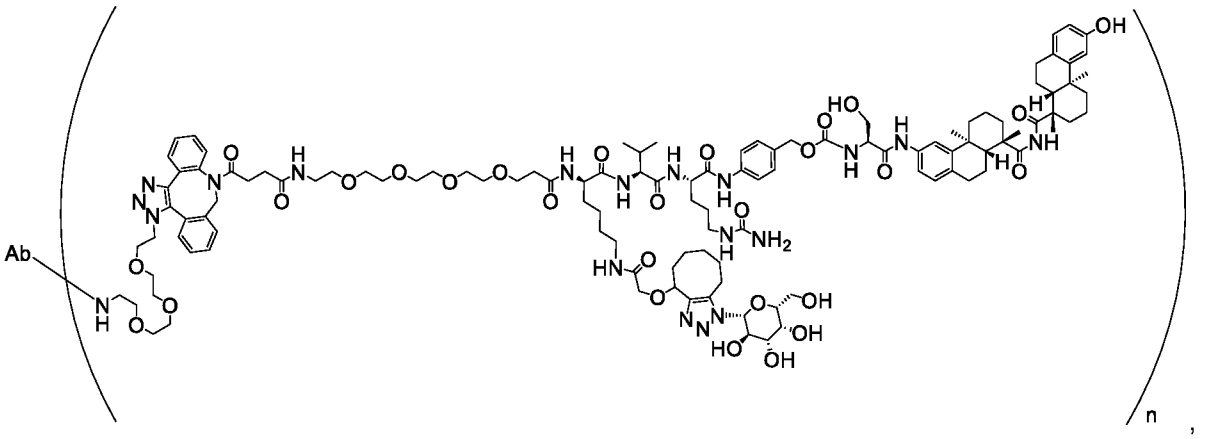
,

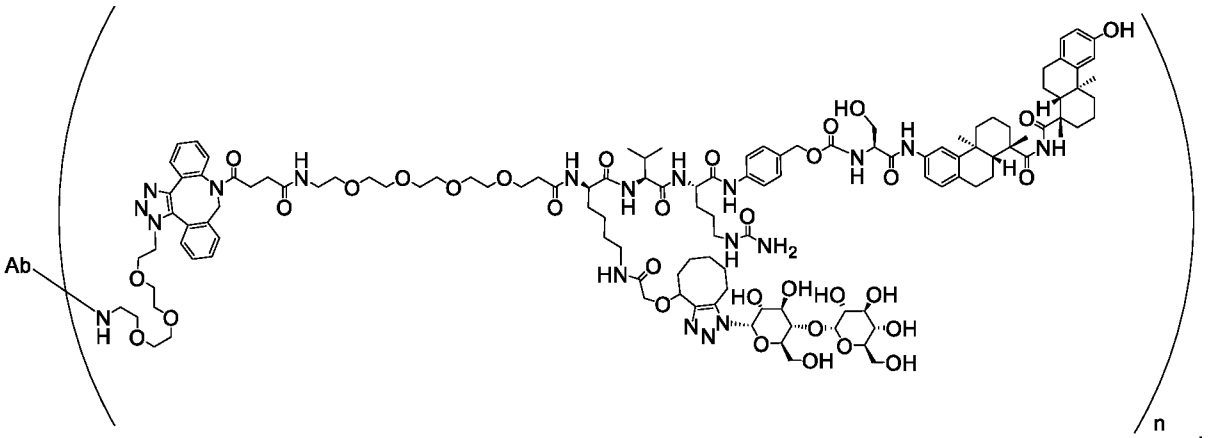


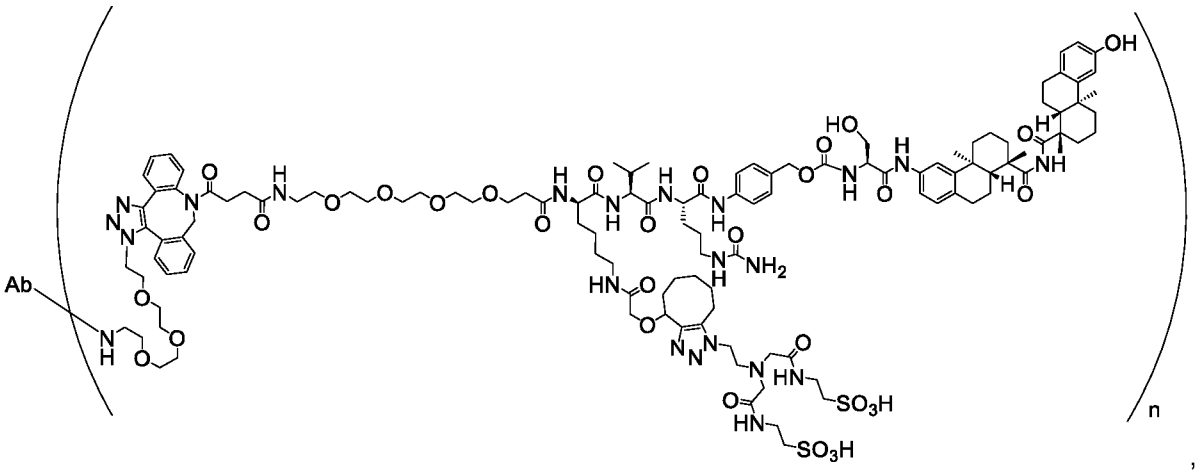
,



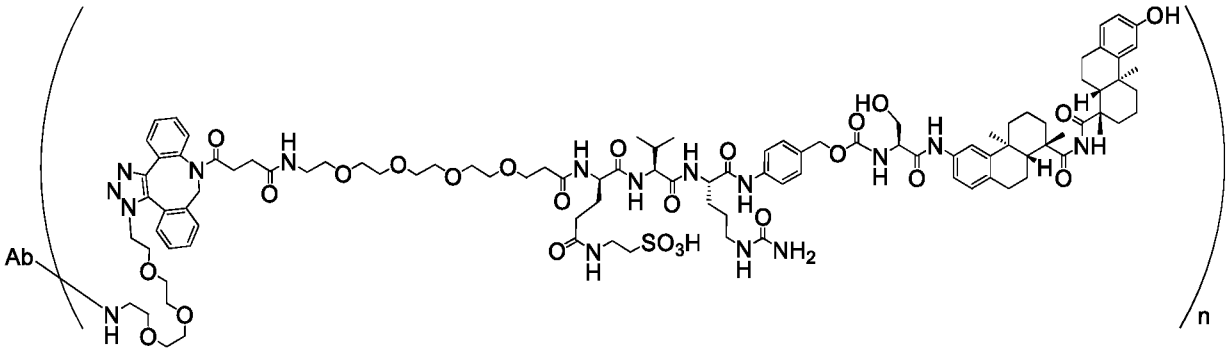




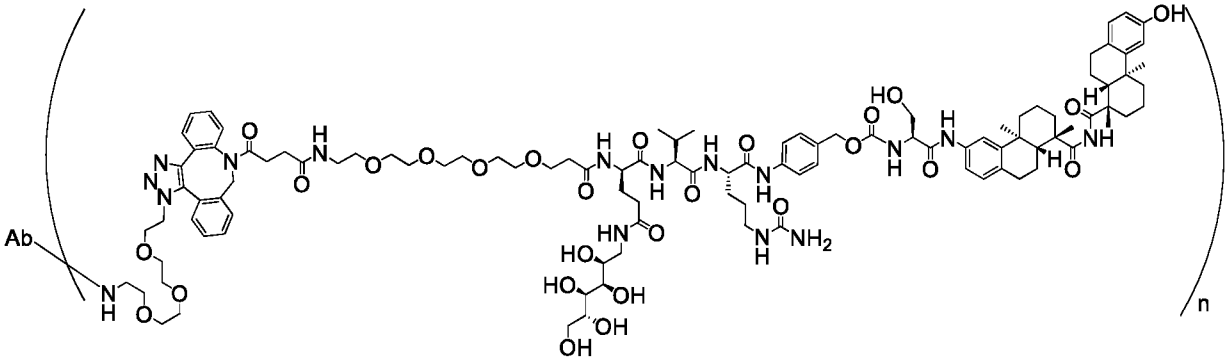




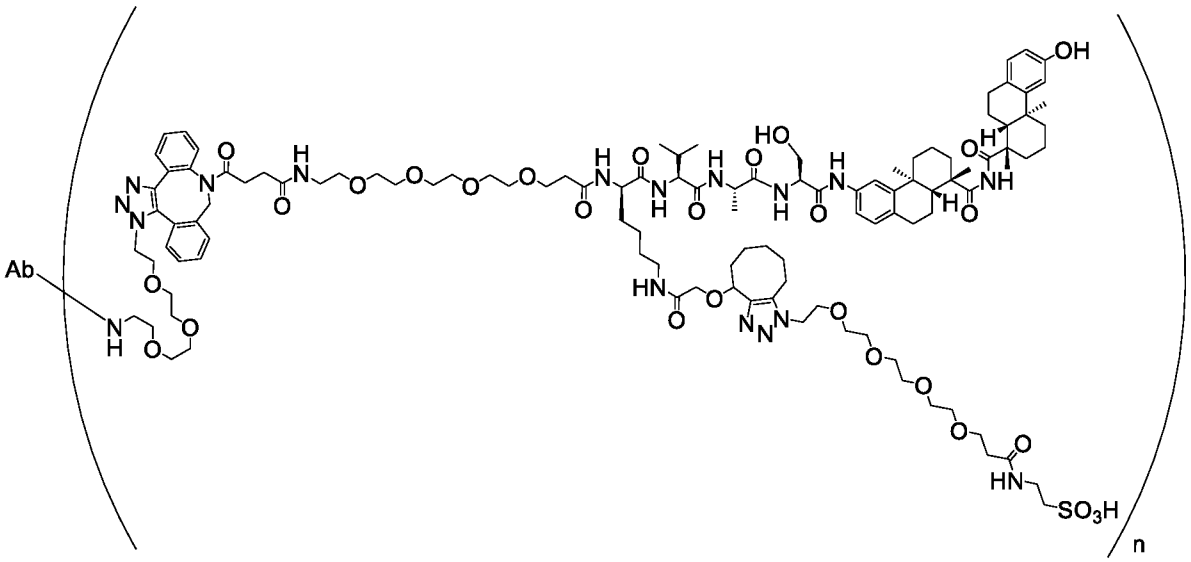
,



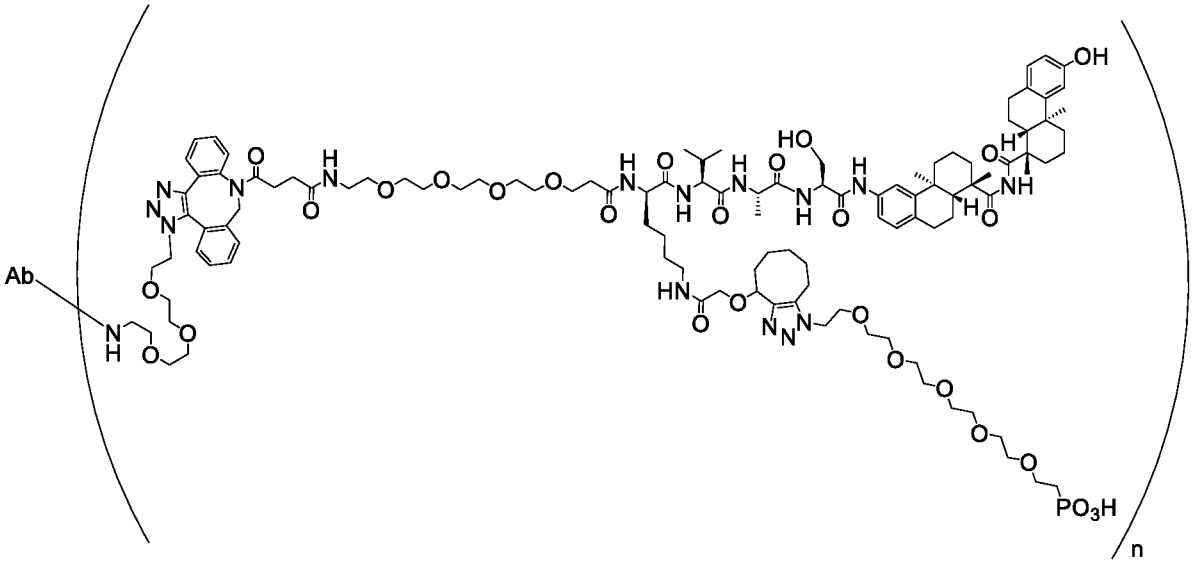
,



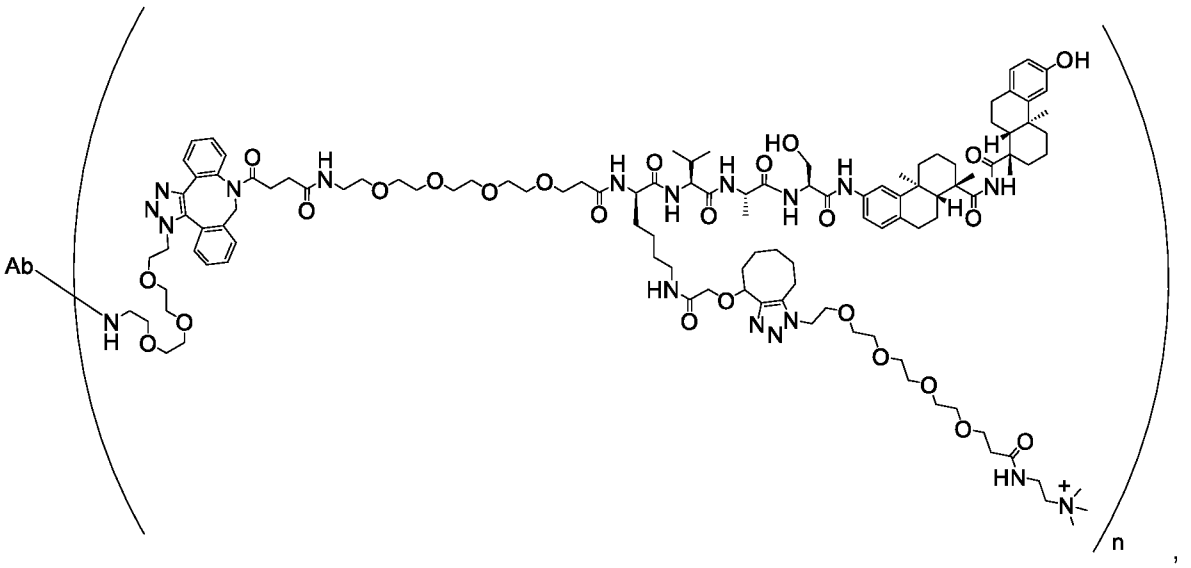
,


Ab
OH
HO
HN
$SO_3H$
n

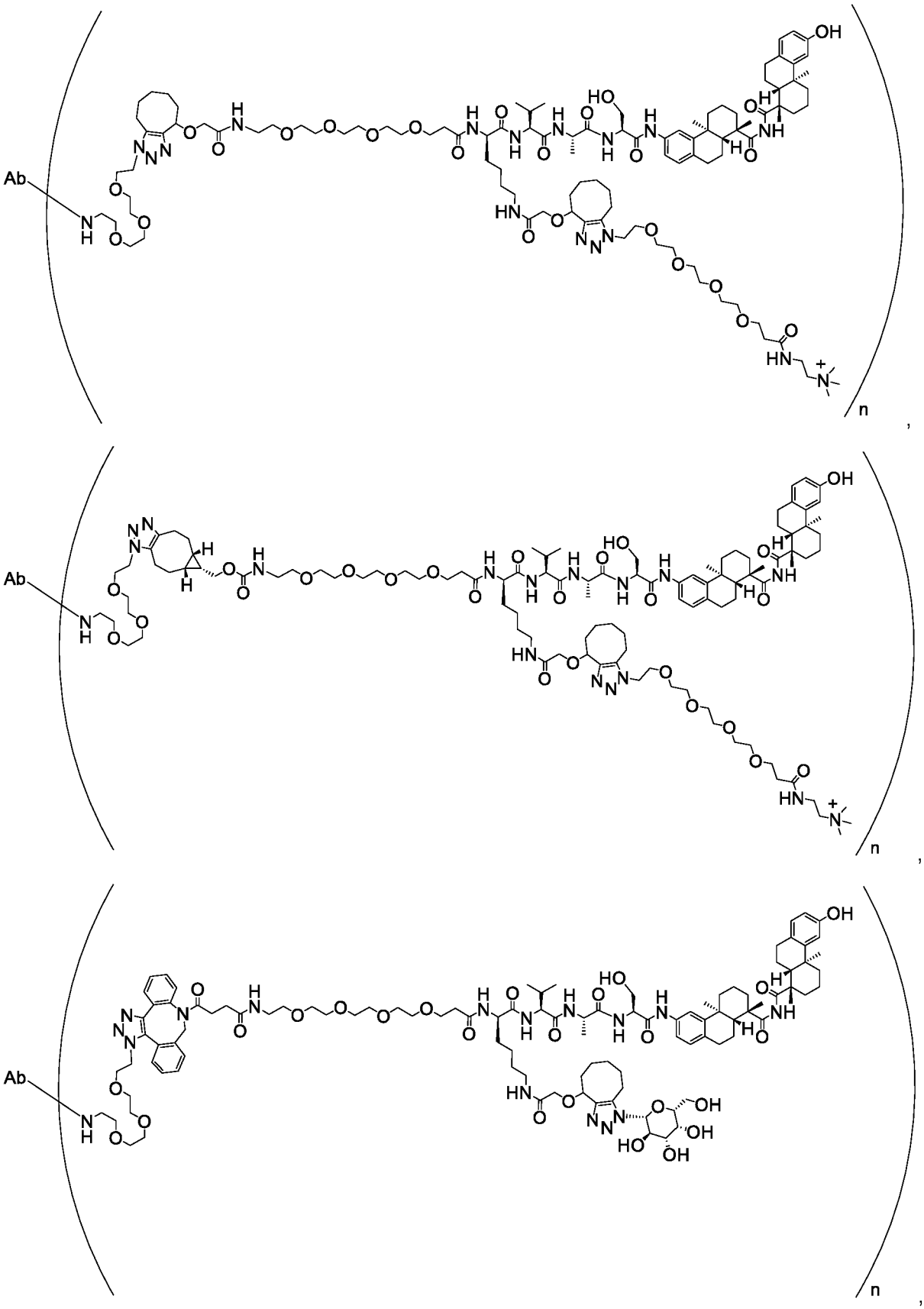
,

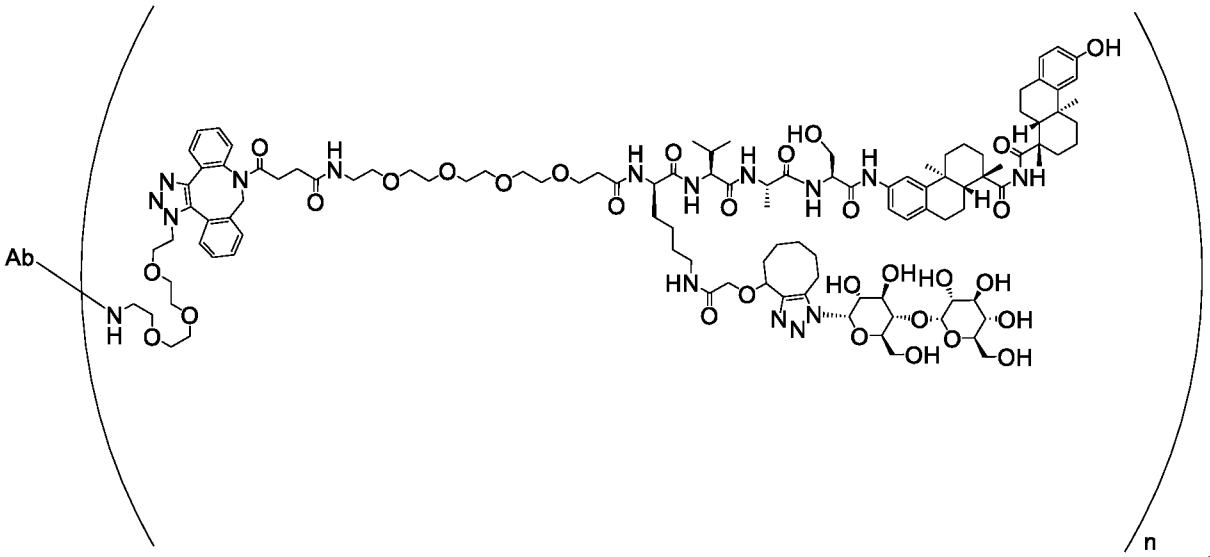

Ab
OH
HO
HN
$PO_3H$
n

,

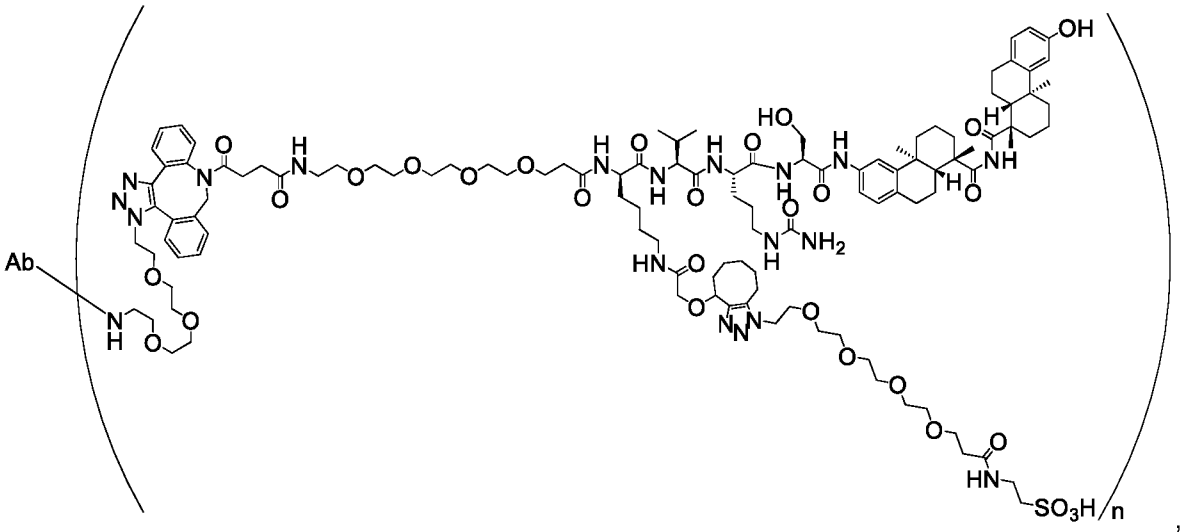

Ab
OH
HO
HN
n

,

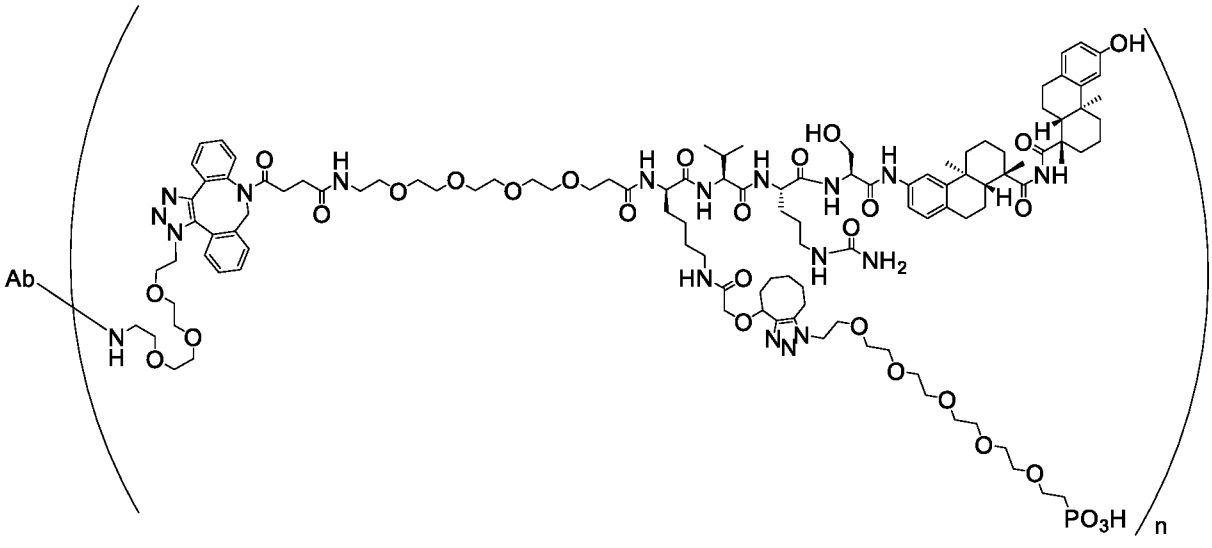




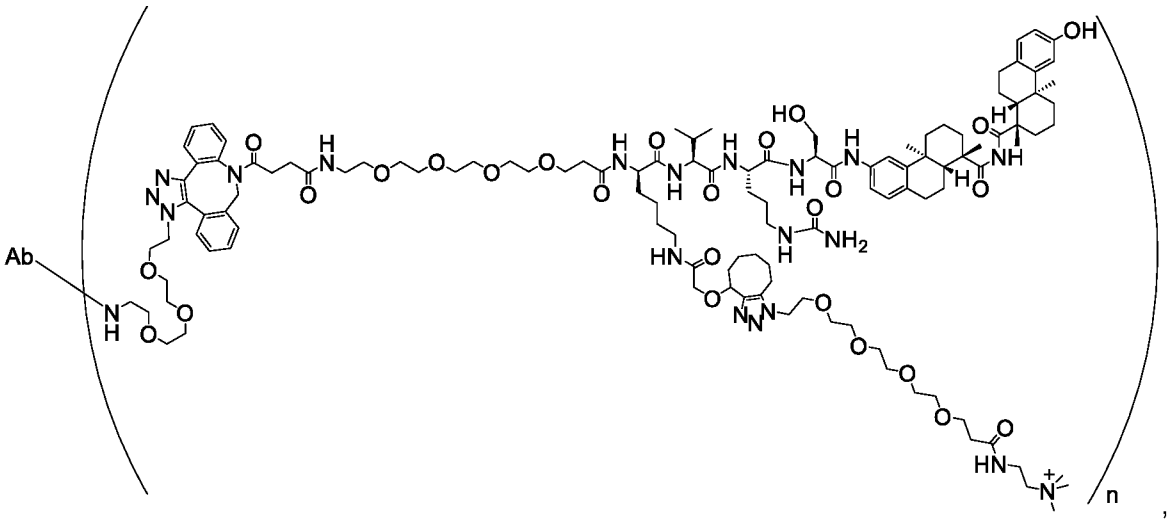
,



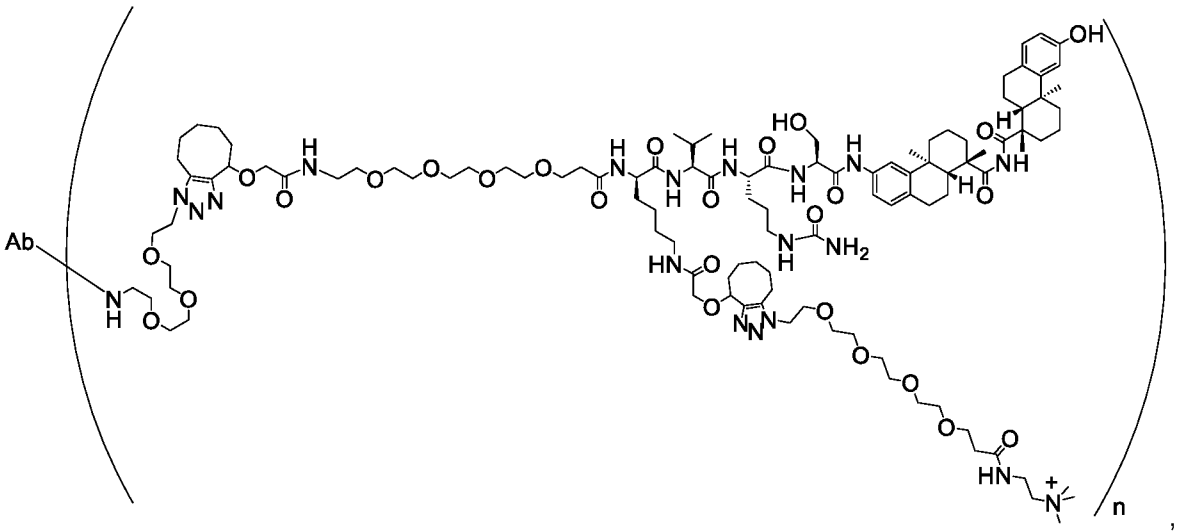
,



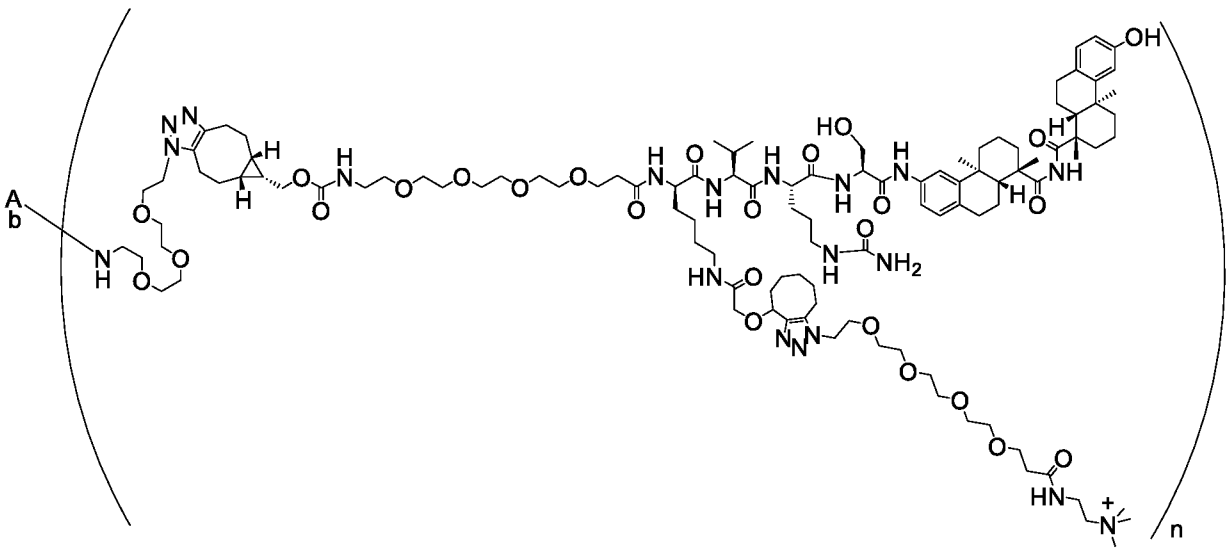
,



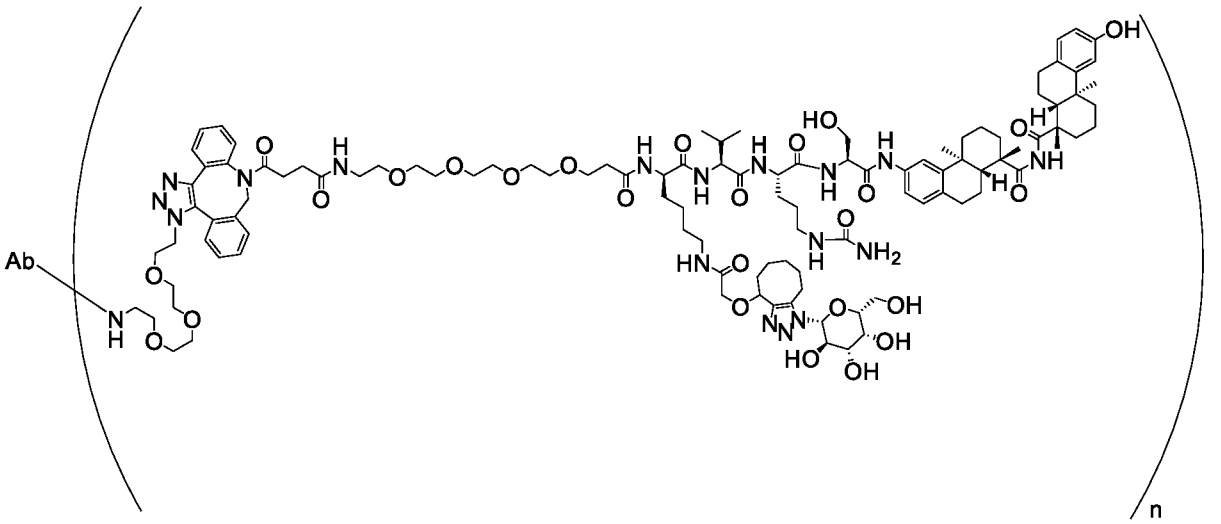
,

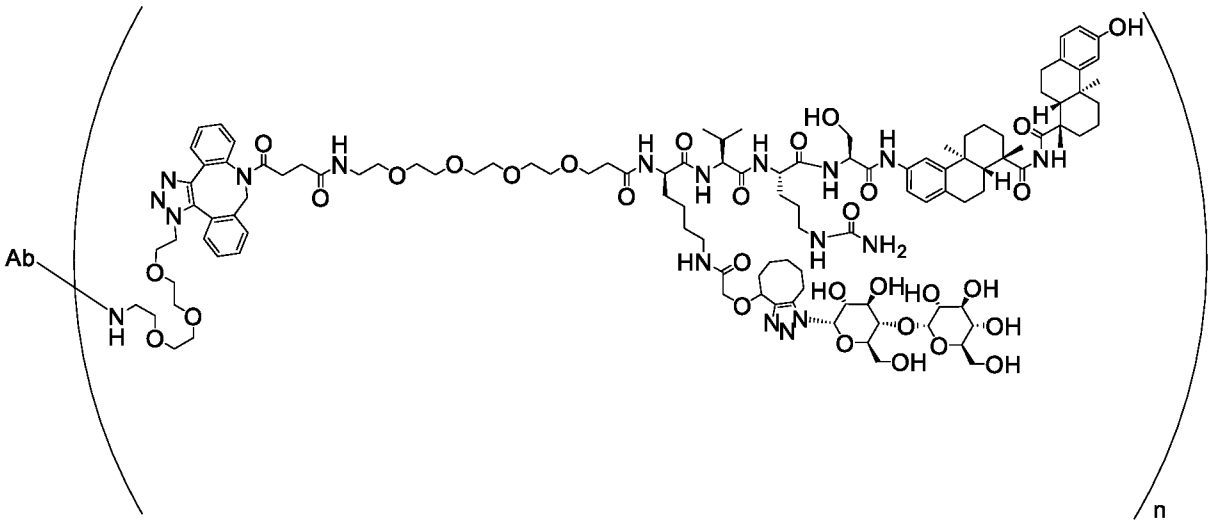


,



,





or a stereoisomeric form thereof, or a regioisomer thereof, or a mixture of regioisomers thereof, wherein each **Ab** is an antibody, or an antigen-binding fragment thereof, and subscript **n** is an integer from 1 to 30. In some embodiments, **n** is an integer from 1 to 4. In some embodiments, **n** is 2. In some embodiments, **n** is 4.

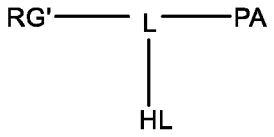
**[0147]** Provided herein is a pharmaceutical composition comprising a compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), or pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

**[0148]** Also provided herein is a method of treating a disease or disorder in a patient in need thereof comprising administering to the patient a compound of any one of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), or a composition comprising a

compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**).

**[0149]** In another aspect is provided a compound comprising a reactive linker bonded to at least one payload moiety and bonded to at least one hydrophilic residue via a covalent linker, wherein said covalent linker is bonded directly or indirectly to each of the reactive linker, the payload moiety, and the hydrophilic residue.

**[0150]** In a further aspect is provided a compound according to Formula (**VI**):




(**VI**)

or a pharmaceutically acceptable salt, solvate, stereoisomer, or derivative thereof,

wherein:

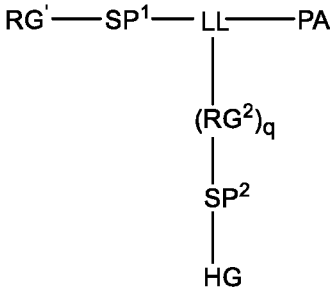
**RG'** is a reactive group;

**L** is a trivalent linker;

**HL** is a hydrophilic residue;and

**PA** is a payload residue.

**[0151]** In one instance, a compound of Formula (**VI**) is according to Formula (**VII**):




(**VII**)

wherein:

**LL** is a trivalent linker;

**RG'** is a reactive group;

**$RG^2$** is a reactive group residue;

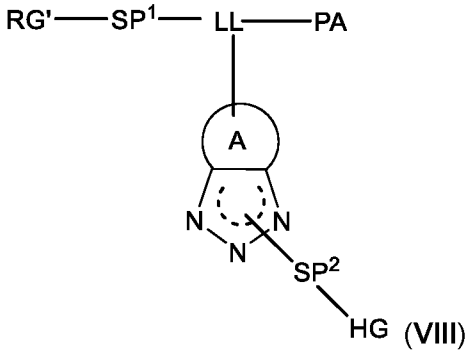
**$SP^1$** and **$SP^2$** are independently, in each instance, absent, or a spacer group residue;

**HG** is a hydrophilic residue;

**PA** is a payload residue; and

subscript **q** is 0 or 1.

**[0152]** In one instance, a compound of Formula (**VII**) is according to Formula (**VIII**):



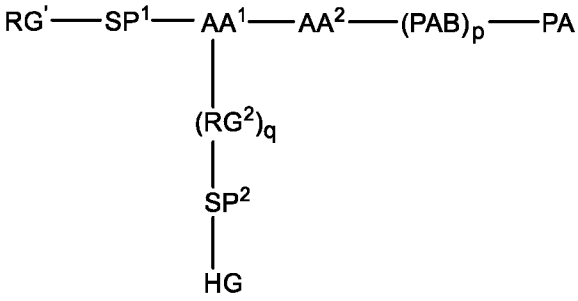

(**VIII**)

wherein

ring **A** is fused to the triazole and is selected from the group consisting of cycloalkyl, cycloalkenyl, heterocycloalkyl, and heterocycloalkenyl;

wherein cycloalkyl, cycloalkenyl, heterocycloalkyl, and heterocycloalkenyl are optionally substituted with alkyl, -OH, or -N$R^aR^b$, where each of $R^a$ and $R^b$ is alkyl or -H.

**[0153]** In another instance, a compound of Formula (**VII**) is according to Formula (**IX**):

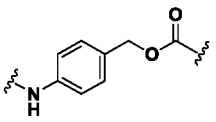



(**IX**)

wherein:

$AA^1$ is a trivalent linker comprising an amino acid residue;

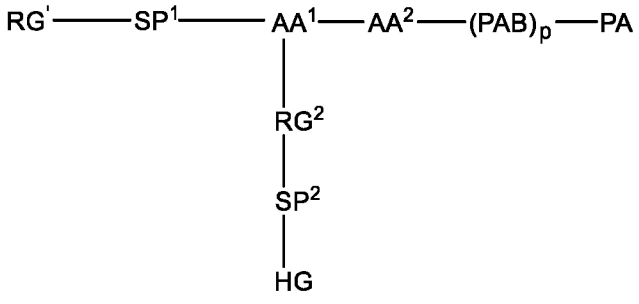
$AA^2$ is a dipeptide, tripeptide or tetrapeptide residue; and

**PAB** is  , wherein the  indicates the atom through which the PAB is bonded to the adjacent groups in the formula;

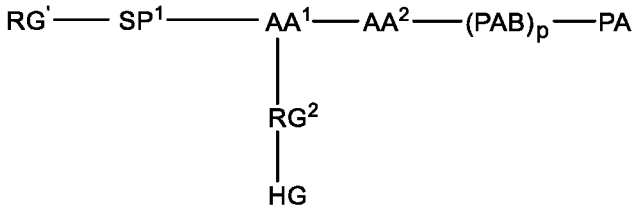
subscript **p** is 0 or 1; and

subscript **q** is 0 or 1.

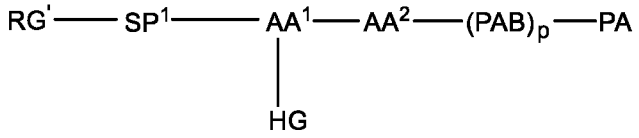
**[0154]** In certain instances, a compound of Formula (**IX**) is according to Formula (**IX**):according to Formula (**IXa**), Formula (**IXb**) or Formula (**IXc**):




(**IXa**),




(**IXb**),

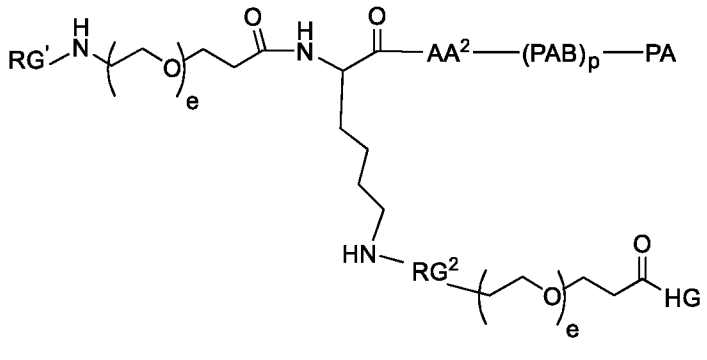



(**IXc**).

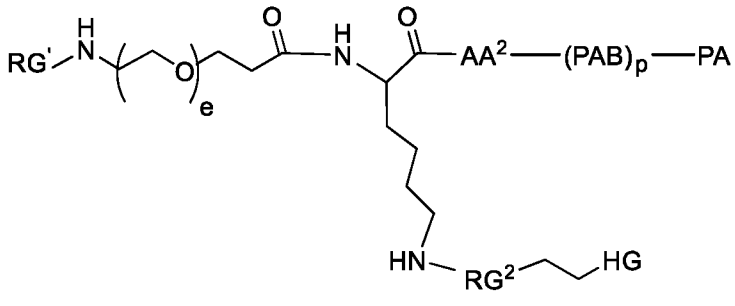
**[0155]** $AA^1$ is a trivalent linker comprising an amino acid residue and is directly or indirectly linked to an antibody, a payload and a hydrophilic group. In some examples, any one of $AA^1$ or $AA^2$ comprises, independently in each instance, an amino acid selected from alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, or citrulline, a derivative thereof, or a combination thereof. In certain embodiments, $AA^1$ is an amino acid selected from alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, or citrulline, a derivative thereof, or a combination thereof. In certain embodiments, $AA^1$ is an amino acid with three functional groups to link to a payload, to a binding agent (e.g., antibody or antigen binding fragment thereof), and to a linker comprising a hydrophilic group, e.g., lysine, aspargine, glutamic acid, aspartic acid, glutamine, cysteine, threonine, serine, or tyrosine. In certain embodiments, $AA^1$ is lysine. In certain embodiments $AA^1$ is glutamine. In certain embodiments, $AA^1$ is lysine or a derivative of lysine. In certain embodiments, $AA^1$ is L-lysine.

In certain embodiments, the $AA^1$ is D-lysine. In certain embodiments, $AA^1$ is glutamic acid. In certain embodiments, $AA^1$ is aspartic acid. In certain embodiments, the $AA^2$ is valine-citrulline. In some embodiments, the $AA^2$ is citrulline-valine. In some embodiments, the $AA^2$ is valine-alanine. In some embodiments, the $AA^2$ is alanine-valine. In some embodiments, the $AA^2$ is valine-glycine. In some embodiments, the $AA^2$ is glycine-valine. In some embodiments, the $AA^1$-$AA^2$ is glutamine-valine-citrulline. In some embodiments, the $AA^1$-$AA^2$ is lysine-valine-citrulline. In some embodiments, the $AA^1$-$AA^2$ is lysine-valine-alanine. In some embodiments, the $AA^1$-$AA^2$ is glutamine-valine-alanine.

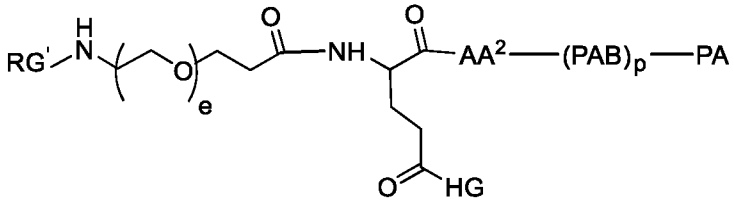
**[0156]** In certain instances, a compound of Formula (**IXa**), Formula (**IXb**) or Formula (**IXc**) is according to according to Formula (**Xa**), (**Xb**), (**Xc**) or (**Xd**):



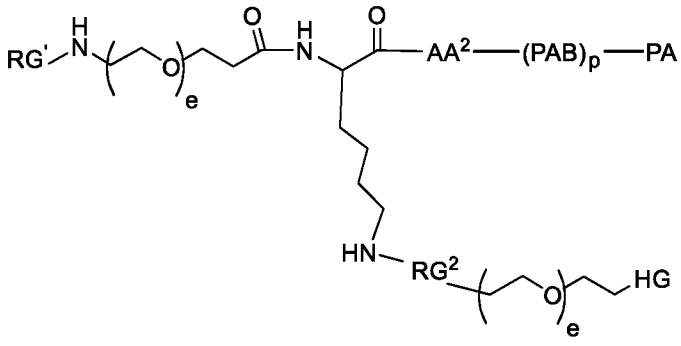

(**Xa**)




(**Xb**)



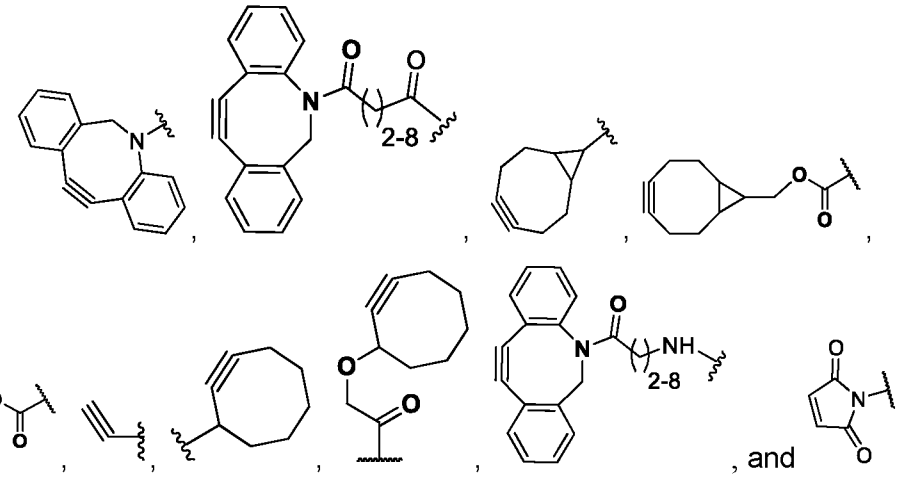
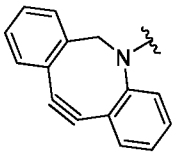
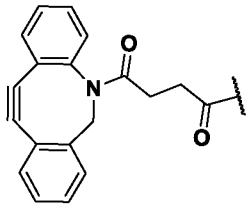

(**Xc**), or

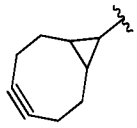
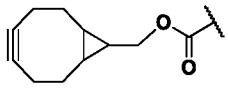
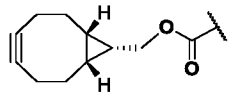
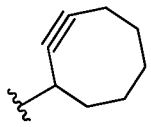
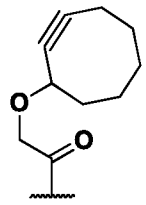
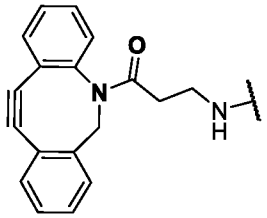
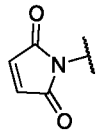


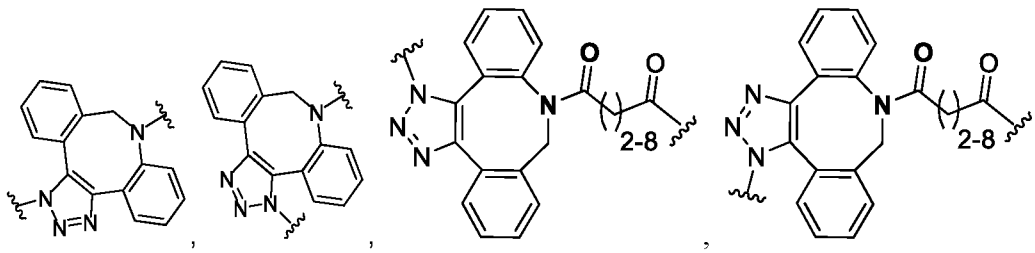
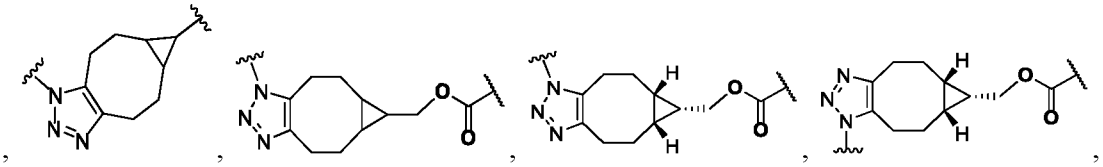
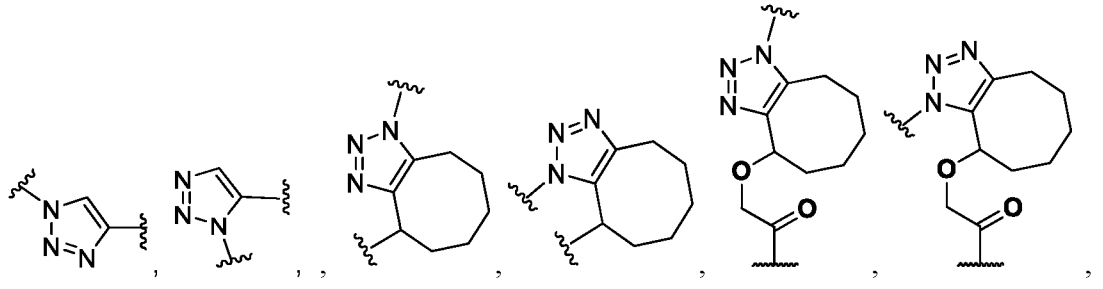
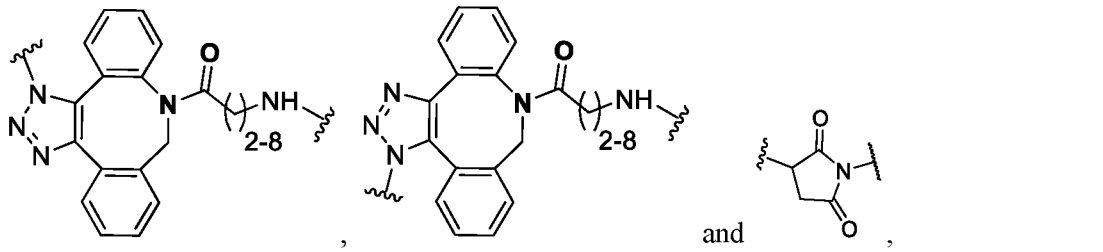
(**Xd**),

wherein:

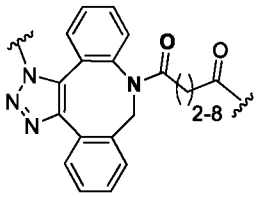
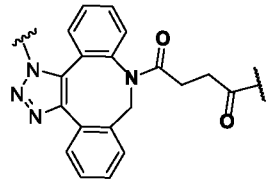
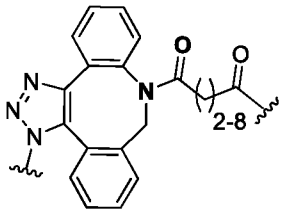
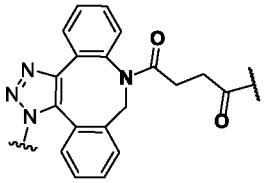
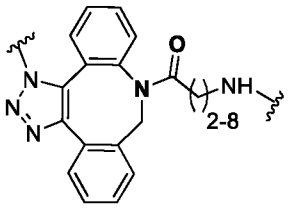
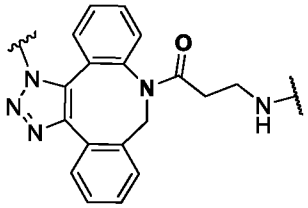
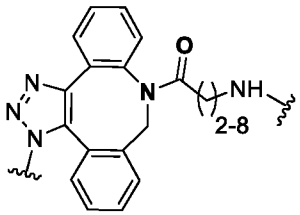
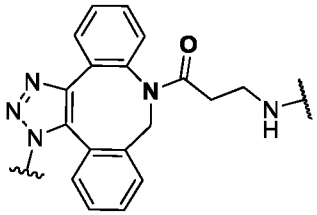
subscript **e** is independently, in each instance, an integer from 0 to 6, or an integer from 0 to 5. In some instances, subscript **p** is 0. In some instances, subscript **p** is 1. In any of these examples, subscript **e** is 1, 2, 3, or 4. In some examples, subscript **e** is 1. In some examples, subscript **e** is 2. In some examples, subscript **e** is 3. In some examples, subscript **e** is 4. In some examples, subscript **e** is 5. In some examples, subscript **e** is 6. In certain embodiments, the $AA^2$ is valine-citrulline. In some embodiments, the $AA^2$ is citrulline-valine. In some embodiments, the $AA^2$ is valine-alanine. In some embodiments, the $AA^2$ is alanine-valine. In some embodiments, the $AA^2$ is valine-glycine. In some embodiments, the $AA^2$ is glycine-valine. In some embodiments, the $AA^1$-$AA^2$ is glutamine-valine-citrulline. In certain embodiments, the lysine is L-lysine. In certain embodiments, the lysine is D-lysine.

**[0157]** In certain embdiments, **RG′** is, independently in each instance, a click chemistry residue. In some instances, **RG′** is, independently in each instance, selected from the group consisting of  , and , wherein the  indicates the atom through which the **RG′** is bonded to the adjacent groups in the formula. In one case, **RG′** is  . In another case, **RG′** is  . In

some instances, **RG′** is  . In some instances, **RG′** is  . In some instances, **RG′** is  . In some instances, **RG′** is  . In some instances, **RG′** is  . In some instances, **RG′** is  . In some instances, **RG′** is  . In some instances, **RG′** is  .

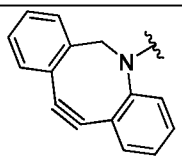
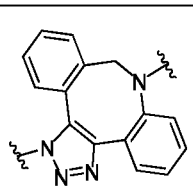
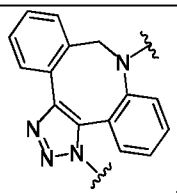
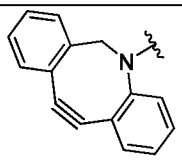
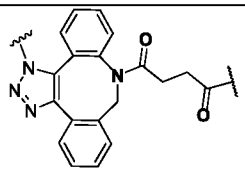
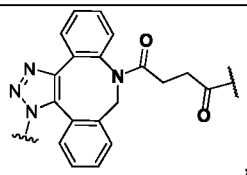
**[0158]** In some instances, $RG^2$ is independently, in each instance, selected from the group consisting of  ,  ,  ,  , and .

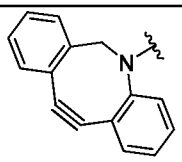
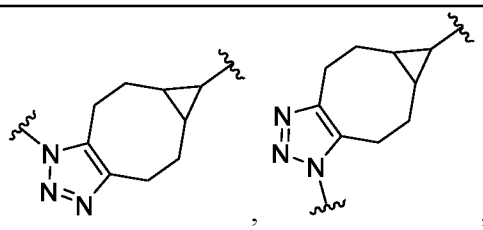
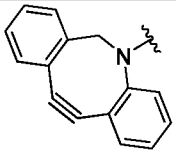
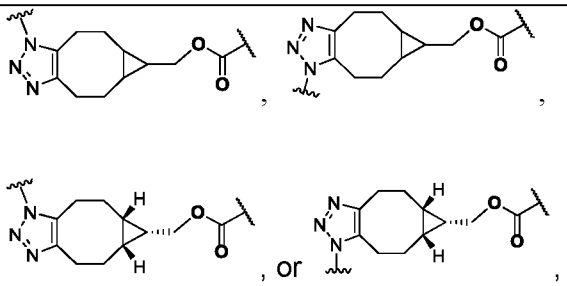
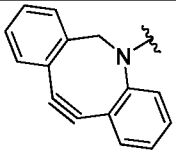
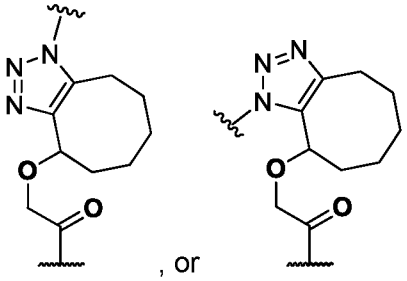
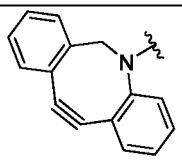
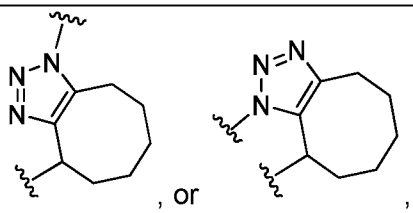
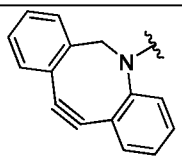
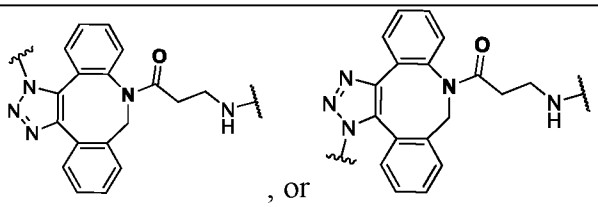
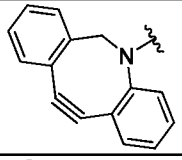
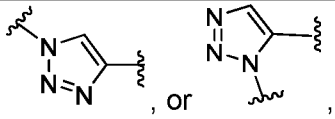
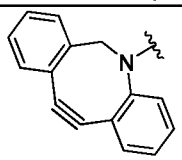
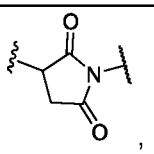
wherein the  indicates the atom through which the $RG^2$ is bonded to the adjacent groups in the formula.

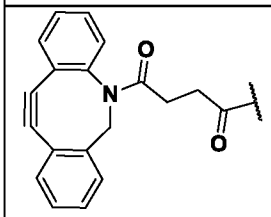
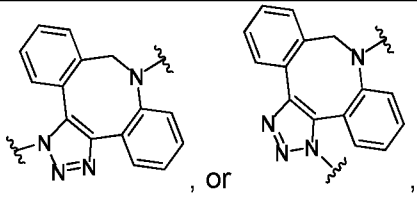
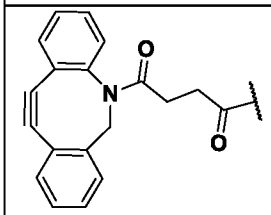
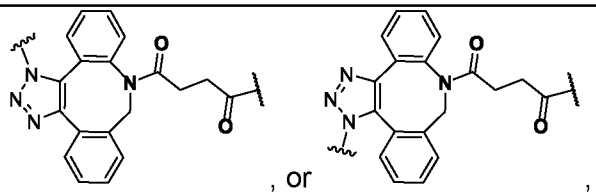
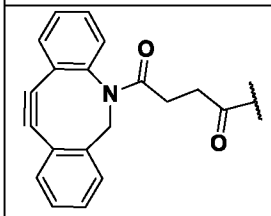
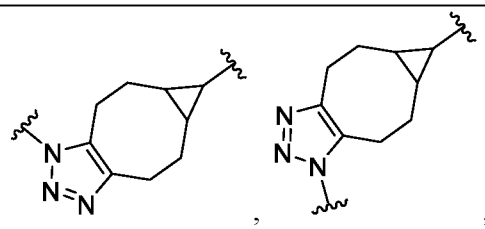
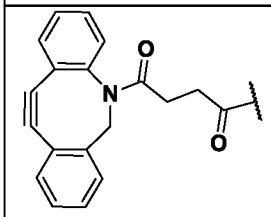
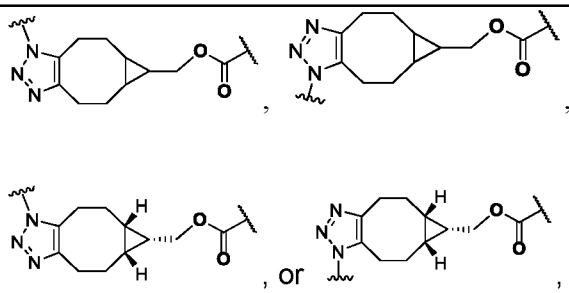
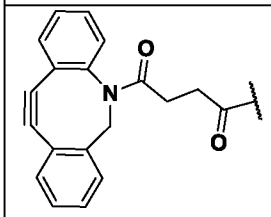
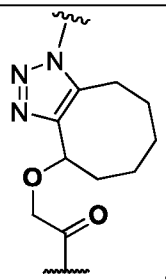
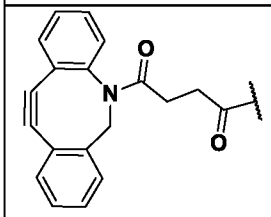
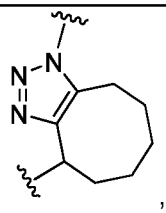
**[0159]** In certain embodiments,  is  . In certain embodiments,  is  . In certain embodiments,  is  . In some embodiments,  is  .

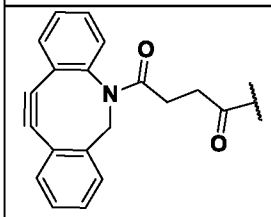
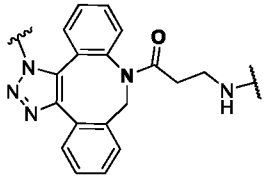
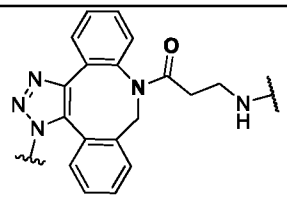
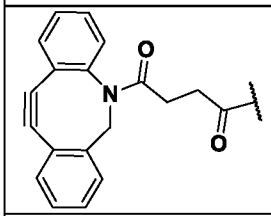
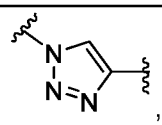
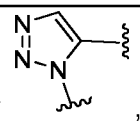
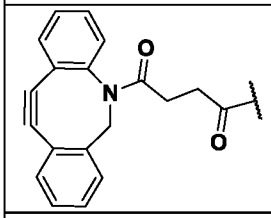
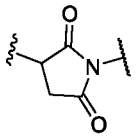
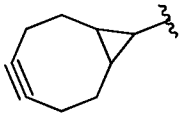
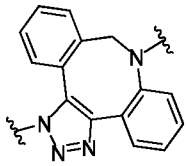
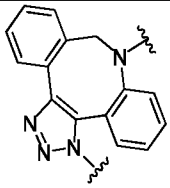
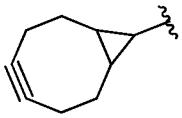
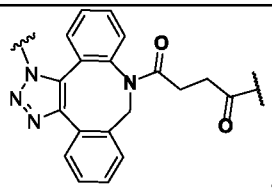
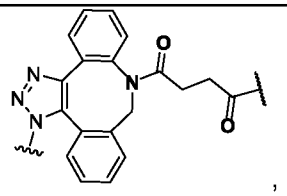
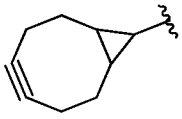
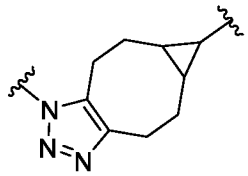
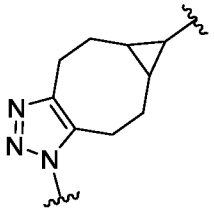
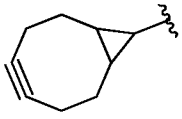
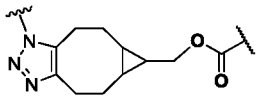
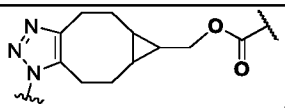
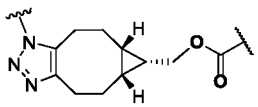
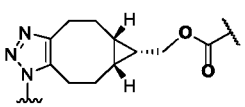
**[0160]** In certain instances, **RG'** and $RG^2$ are independently, in each instance, as shown in Table R'.

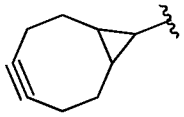
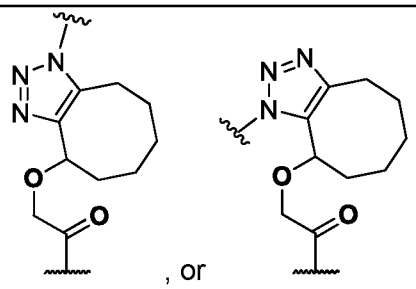
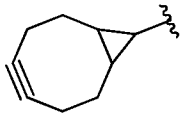
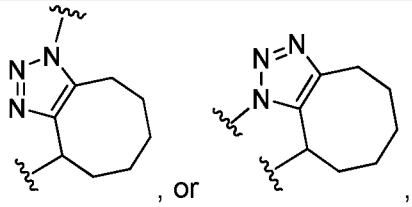
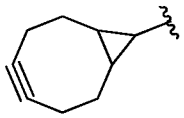
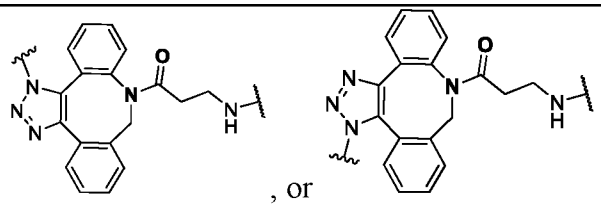
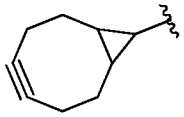
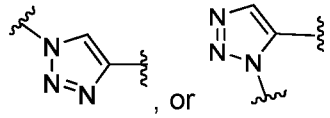
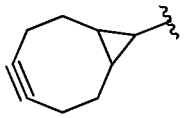
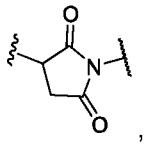
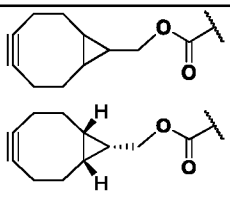
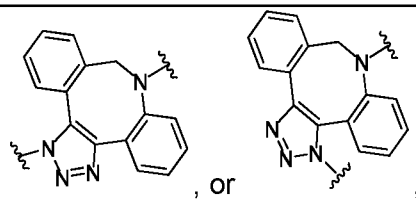
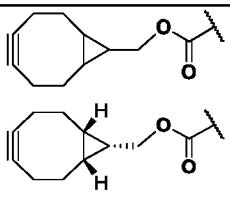
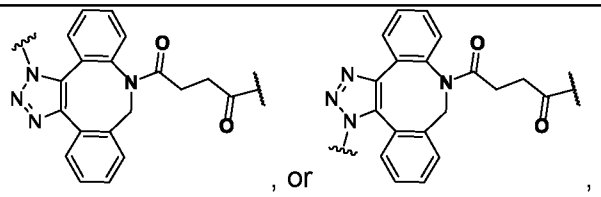
**Table R'**

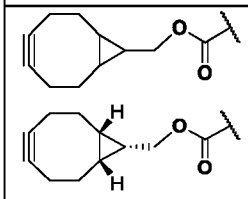
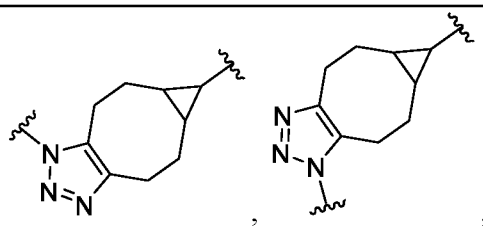
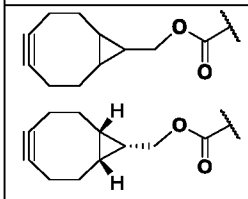
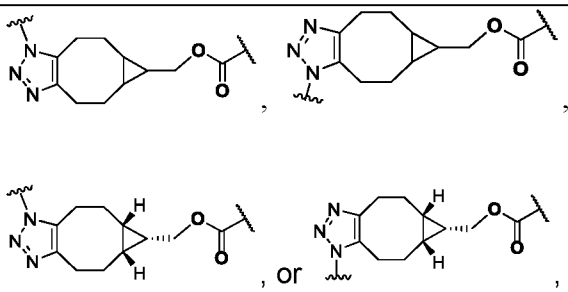
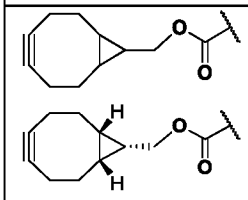
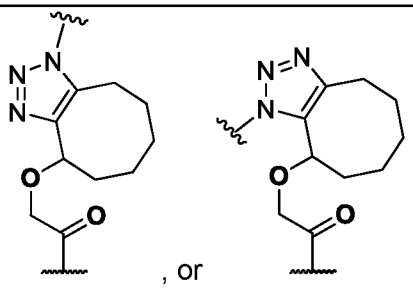
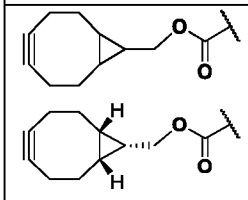
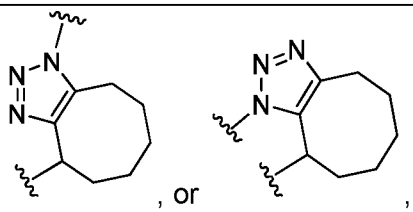
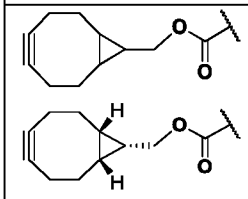
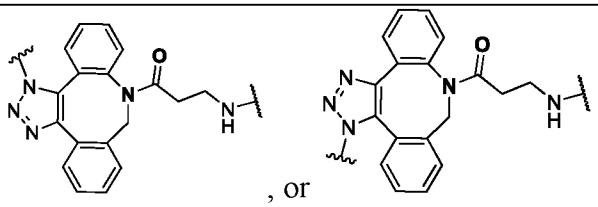
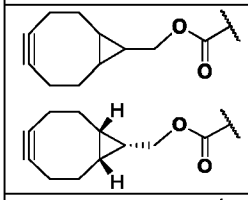
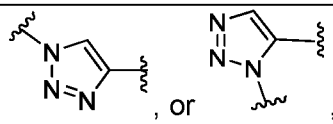
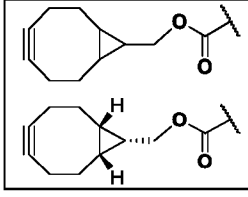
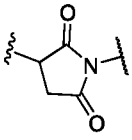
| RG' | $RG^2$ |
|---|---|
|  |  , or  , |
|  |  , or  , |

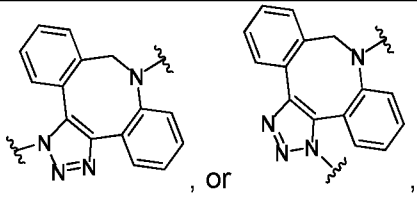
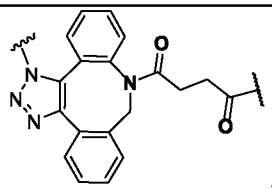
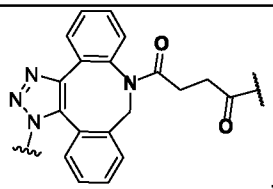
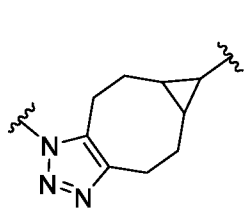
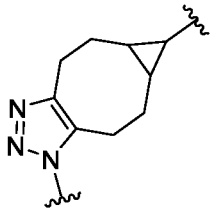
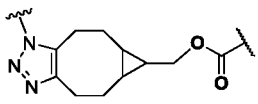
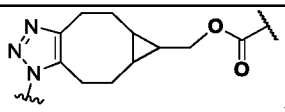
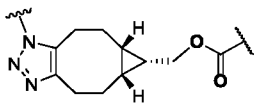
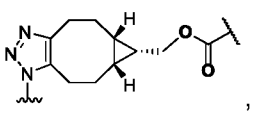
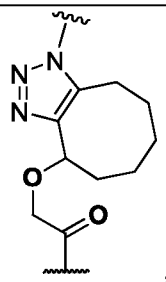
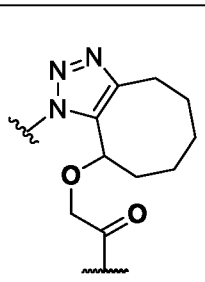
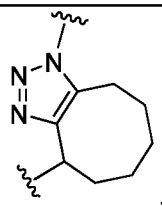
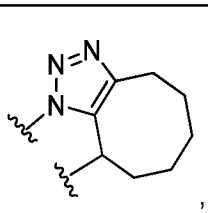
| RG' | $RG^2$ |
|---|---|
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |

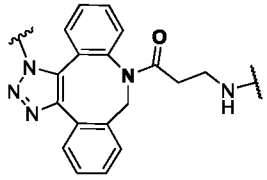
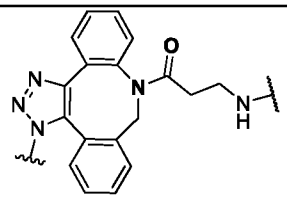
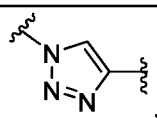
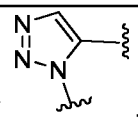
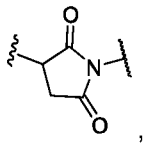
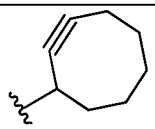
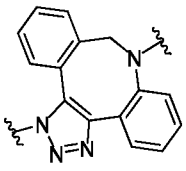
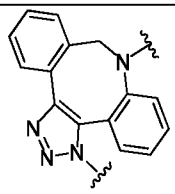
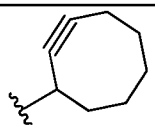
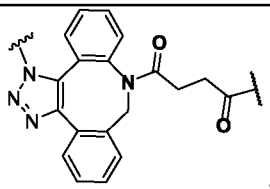
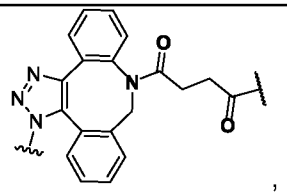
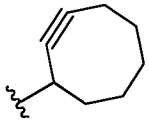
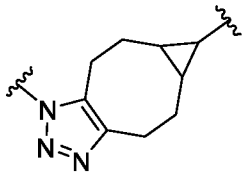
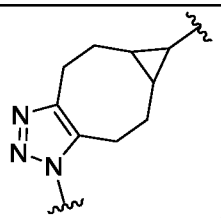
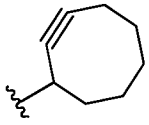
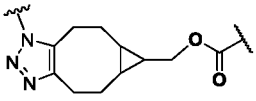
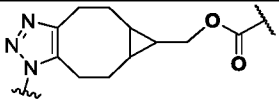
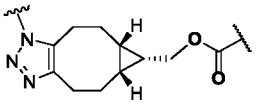
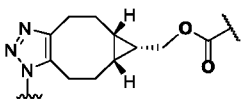
| RG' | $RG^2$ |
|---|---|
|  |  , or , |
|  |  , or , |
|  |  , , |
|  |  , , , or , |
|  |  , or |
|  |  , or , |

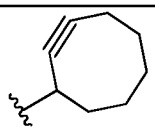
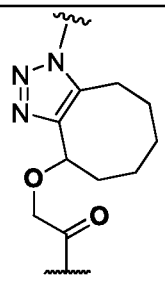
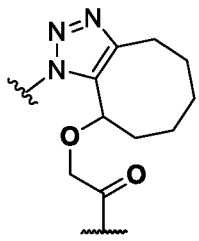
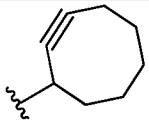
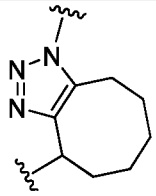
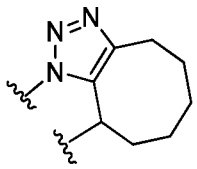
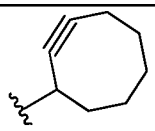
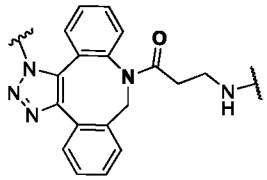
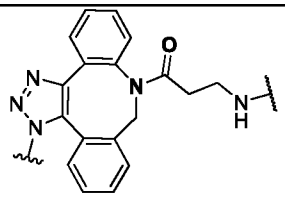
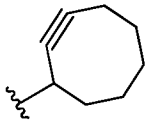
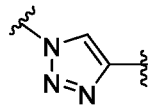
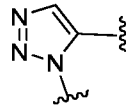
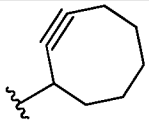
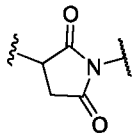
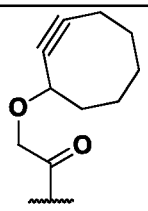
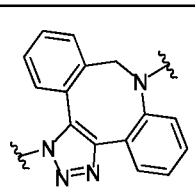
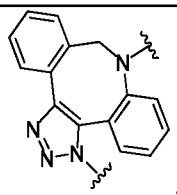
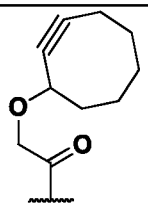
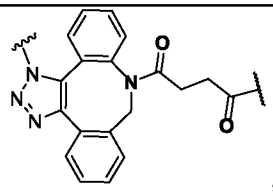
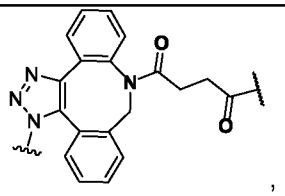
| RG' | $RG^2$ |
|---|---|
|  |  , or  |
|  |  , or  , |
|  |  , |
|  |  , or  , |
|  |  , or  , |
|  |  ,  , |
|  |  ,  ,  , or  , |

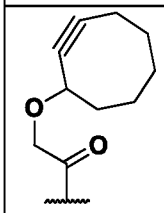
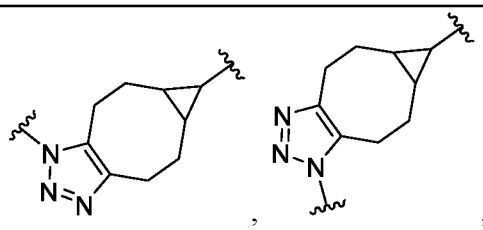
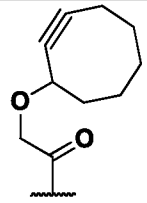
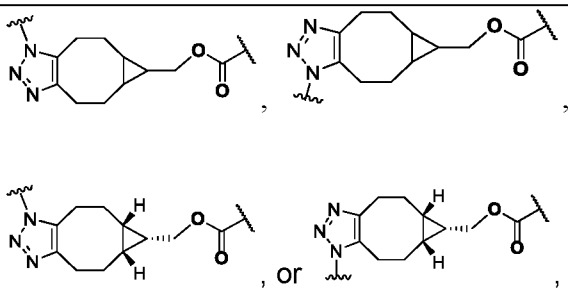
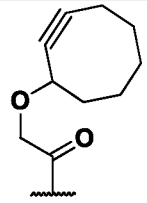
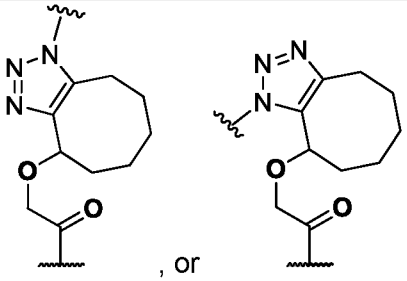
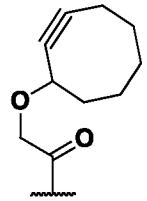
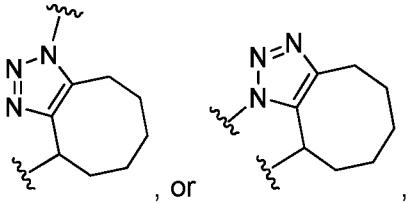
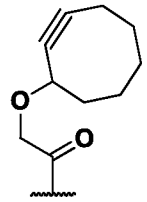
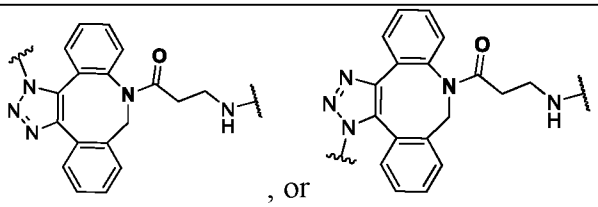
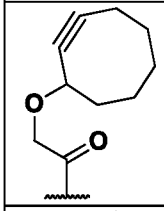
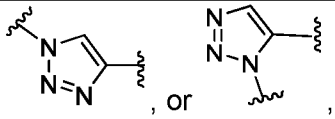
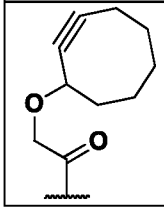
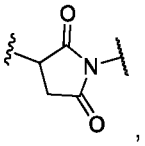
| RG' | $RG^2$ |
|---|---|
|  |  , or |
|  |  , or , |
|  |  , or |
|  |  , or , |
|  |  , |
|  , or |  , or , |
|  , or |  , or , |

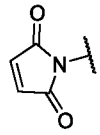
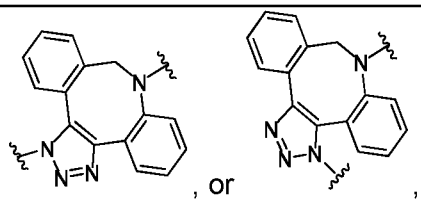
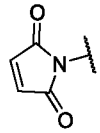
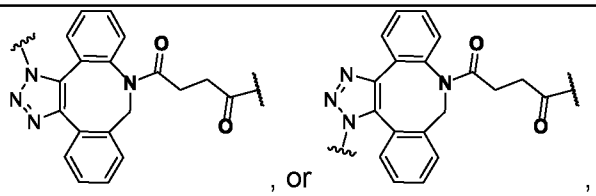
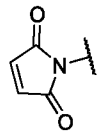
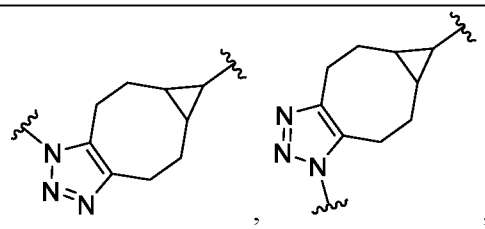
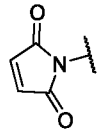
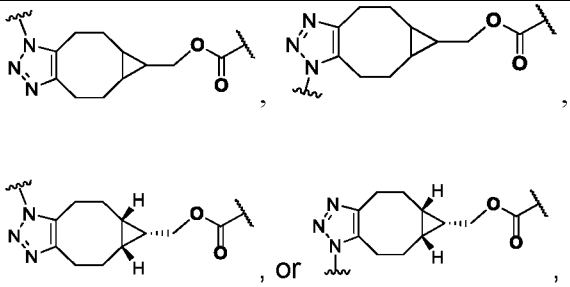
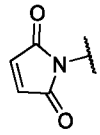
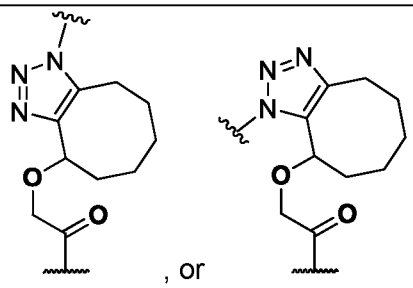
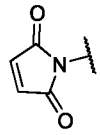
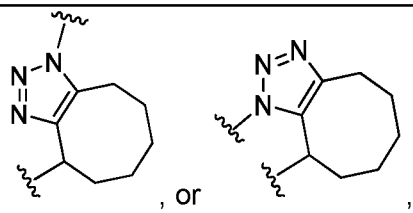
| RG' | $RG^2$ |
|---|---|
|  , or |  , , |
|  , or |  , , , or , |
|  , or |  , or |
|  , or |  , or , |
|  , or |  , or |
|  , or |  , or , |
|  , or |  , |

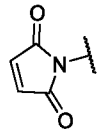
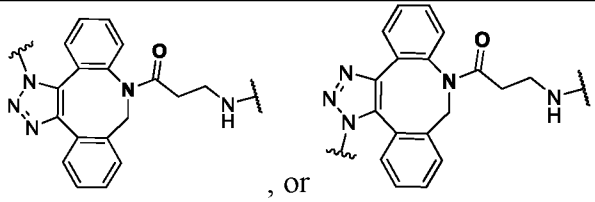
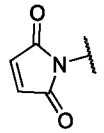
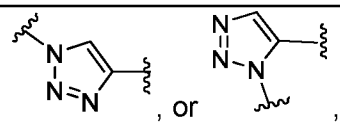
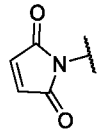
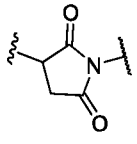
| RG' | $RG^2$ |
|---|---|
|  |  , or  , |
|  |  , or  , |
|  |  ,  , |
|  |  ,  ,  , or  , |
|  |  , or  |
|  |  , or  , |

| RG' | $RG^2$ |
|---|---|
|  |  , or  |
|  |  , or  , |
|  |  , |
|  |  , or  , |
|  |  , or  , |
|  |  ,  , |
|  |  ,  ,  , or  , |

| RG' | $RG^2$ |
|---|---|
|  |  , or  |
|  |  , or  , |
|  |  , or  |
|  |  , or  , |
|  |  , |
|  |  , or  , |
|  |  , or  , |

| RG' | $RG^2$ |
|---|---|
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |

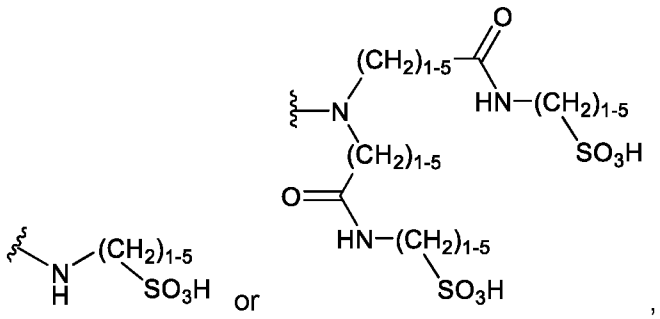
| RG′ | $RG^2$ |
|---|---|
|  |  , or , |
|  |  , or , |
|  |  , , |
|  |  , or , |
|  |  , or |
|  |  , or , |

| RG′ | $RG^2$ |
|---|---|
|  |  , or |
|  |  , or  , |
|  |  , |

wherein the  indicates the atom through which the **RG′** or $RG^2$ is bonded to the adjacent groups in the formula.

**[0161]** Any combination of a row from Table R′ and a spacer $SP^2$ as described herein may be present in a compound of Formula (**VI**) described herein.

**[0162]** In certain instances, for any compound of Formula (**VI**), Formula (**VII**), Formula (**VIII**), Formula (**IX**), Formula (**IXa**), Formula (**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**), Formula (**Xc**), or Formula (**Xd**), **HG** is

 or  ,

wherein

the  indicates the atom through which the **HG** is bonded to the adjacent groups in the formula.

**[0163]** In some instances, for any compound of Formula (**VI**), Formula (**VII**), Formula (**VIII**), Formula (**IX**), Formula (**IXa**), Formula (**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**), Formula (**Xc**) or Formula (**Xd**), **HG** is $–(CH_2)_{1\text{-}5}SO_3H$, $–(CH_2)_{n''}–NH\text{-}(CH_2)_{1\text{-}5}SO_3H$, $–(CH_2)_{n''}–C(O)NH\text{-}(CH_2)_{1\text{-}5}SO_3H$,

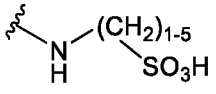
$-(CH_2CH_2O)_{m''}-C(O)NH-(CH_2)_{1-5}SO_3H$, $-(CH_2)_{n''}-N((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}SO_3H)_2$, $-(CH_2)_{n''}-C(O)N((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}SO_3H)_2$, or $-(CH_2CH_2O)_{m''}-C(O)N((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}SO_3H)_2$, **wherein subscript n″ is 1, 2, 3, 4, or 5, and subscript m″ is 1, 2, 3, 4, or 5.** In one embodiment, **HG** is $-(CH_2)_{1-5}SO_3H$. In another embodiment, **HG** is $-(CH_2)_{n''}-NH-(CH_2)_{1-5}SO_3H$, wherein subscript n″ is 1, 2, 3, 4, or 5. In another embodiment, **HG** is $-(CH_2)_{n''}-C(O)NH-(CH_2)_{1-5}SO_3H$, wherein n″ is 1, 2, 3, 4, or 5. In another embodiment, **HG** is $-(CH_2CH_2O)_{m''}-C(O)NH-(CH_2)_{1-5}SO_3H$, wherein subscript m″ is 1, 2, 3, 4, or 5. In another embodiment, **HG** is $-(CH_2)_{n''}-N((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}SO_3H)_2$, wherein subscript n″ is 1, 2, 3, 4, or 5. In another embodiment, **HG** is $-(CH_2)_{n''}-C(O)N((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}SO_3H)_2$, wherein subscript n″ is 1, 2, 3, 4, or 5. In another embodiment, **HG** is $-(CH_2CH_2O)_{m''}-C(O)N((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}SO_3H)_2$, wherein subscript m″ is 1, 2, 3, 4, or 5.

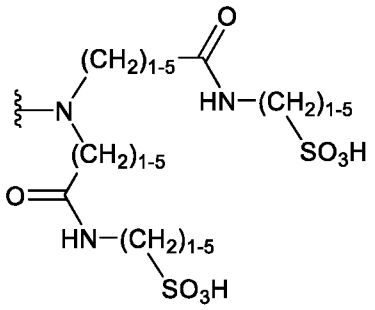
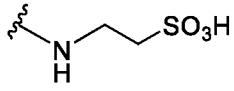
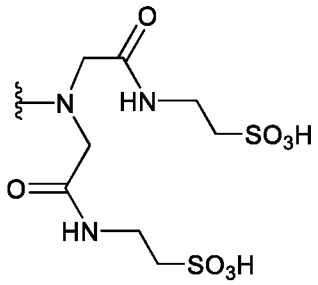
**[0164]** In some instances, for any compound of Formula (**VI**), Formula (**VII**), Formula (**VIII**), Formula (**IX**), Formula (**IXa**), Formula (**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**), Formula (**Xc**), or Formula (**Xd**), **HG** is is $-(CH_2)_{1-5}PO_3H$, $-(CH_2)_n-NH-(CH_2)_{1-5}PO_3H$, $-(CH_2)_n-C(O)NH-(CH_2)_{1-5}PO_3H$, $-(CH_2CH_2O)_m-C(O)NH-(CH_2)_{1-5}PO_3H$, $-(CH_2)_n-N((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}PO_3H)_2$, $-(CH_2)_n-C(O)N((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}PO_3H)_2$, or $-(CH_2CH_2O)_m-C(O)N((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}PO_3H)_2$, wherein n is 1, 2, 3, 4, or 5, and m is 1, 2, 3, 4, or 5. In one embodiment, **HG** is $-(CH_2)_{1-5}PO_3H$. In another embodiment, **HG** is $-(CH_2)_n-NH-(CH_2)_{1-5}PO_3H$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is $-(CH_2)_n-C(O)NH-(CH_2)_{1-5}PO_3H$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is $-(CH_2CH_2O)_m-C(O)NH-(CH_2)_{1-5}PO_3H$, wherein m is 1, 2, 3, 4, or 5. In another embodiment, **HG** is $-(CH_2)_n-N((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}PO_3H)_2$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is $-(CH_2)_n-C(O)N((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}PO_3H)_2$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is $-(CH_2CH_2O)_m-C(O)N((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}PO_3H)_2$, wherein m is 1, 2, 3, 4, or 5.

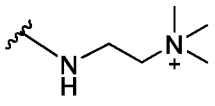
**[0165]** In some instances, for any compound of Formula (**VI**), Formula (**VII**), Formula (**VIII**), Formula (**IX**), Formula (**IXa**), Formula (**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**), Formula (**Xc**) or Formula (**Xd**), **HG** is is $-(CH_2)_{1-5}N^+(\mathbf{R^M})_3$, $-(CH_2)_n-NH-(CH_2)_{1-5}N^+(\mathbf{R^M})_3$, $-(CH_2)_n-C(O)NH-(CH_2)_{1-5}N^+(\mathbf{R^M})_3$, $-(CH_2CH_2O)_m-C(O)NH-(CH_2)_{1-5}N^+(\mathbf{R^M})_3$, $-(CH_2)_n-N((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+(\mathbf{R^M})_3)_2$, $-(CH_2)_n-C(O)N((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+(\mathbf{R^M})_3)_2$, or $-(CH_2CH_2O)_m-C(O)N((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+(\mathbf{R^M})_3)_2$, wherein n is 1, 2, 3, 4, or 5, m is 1, 2, 3, 4, or 5, and $\mathbf{R^M}$, at each occurrence, is independently H, $C_{1-6}$alkyl, $C_{3-7}$cycloalkyl or $C_{1-6}$alkyl-$C_{3-7}$cycloalkyl, or, two $\mathbf{R^M}$ together with the nitrogen atom to which they are attached, form a 3-7-membered heterocycloalkyl ring. In one embodiment, **HG** is $-(CH_2)_{1-5}N^+(\mathbf{R^M})_3$. In another

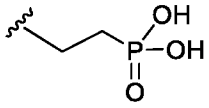
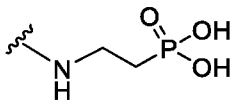
embodiment, **HG** is -$(CH_2)_n$–NH-$(CH_2)_{1-5}N^+(\mathbf{R^M})_3$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is -$(CH_2)_n$–C(O)NH-$(CH_2)_{1-5}N^+(\mathbf{R^M})_3$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is $-(CH_2CH_2O)_m$–C(O)NH-$(CH_2)_{1-5}N^+(\mathbf{R^M})_3$, wherein m is 1, 2, 3, 4, or 5. In another embodiment, **HG** is –$(CH_2)_n$–N$((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+(\mathbf{R^M})_3)_2$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is –$(CH_2)_n$–C(O)N$((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+(\mathbf{R^M})_3)_2$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is $-(CH_2CH_2O)_m$–C(O)N$((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+(\mathbf{R^M})_3)_2$, wherein m is 1, 2, 3, 4, or 5.

**[0166]** In some instances, for any compound of Formula (**VI**), Formula (**VII**), Formula (**VIII**), Formula (**IX**), Formula (**IXa**), Formula (**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**), Formula (**Xc**), or Formula (**Xd**), **HG** is is –$(CH_2)_{1-5}N^+Me_3$, –$(CH_2)_n$–NH-$(CH_2)_{1-5}N^+Me_3$, –$(CH_2)_n$–C(O)NH-$(CH_2)_{1-5}N^+Me_3$, $-(CH_2CH_2O)_m$–C(O)NH-$(CH_2)_{1-5}N^+Me_3$, –$(CH_2)_n$–N$((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+Me_3)_2$, –$(CH_2)_n$–C(O)N$((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+Me_3)_2$, or $-(CH_2CH_2O)_m$–C(O)N$((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+Me_3)_2$, wherein n is 1, 2, 3, 4, or 5, and m is 1, 2, 3, 4, or 5. In one embodiment, **HG** is –$(CH_2)_{1-5}N^+Me_3$. In another embodiment, **HG** is -$(CH_2)_n$–NH-$(CH_2)_{1-5}N^+Me_3$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is -$(CH_2)_n$–C(O)NH-$(CH_2)_{1-5}N^+Me_3$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is $-(CH_2CH_2O)_m$–C(O)NH-$(CH_2)_{1-5}N^+Me_3$, wherein m is 1, 2, 3, 4, or 5. In another embodiment, **HG** is –$(CH_2)_n$–N$((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+Me_3)_2$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is –$(CH_2)_n$–C(O)N$((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+Me_3)_2$, wherein n is 1, 2, 3, 4, or 5. In another embodiment, **HG** is $-(CH_2CH_2O)_m$–C(O)N$((CH_2)_{1-5}C(O)NH(CH_2)_{1-5}N^+Me_3)_2$, wherein m is 1, 2, 3, 4, or 5.

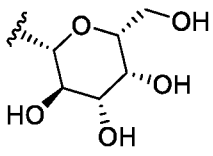
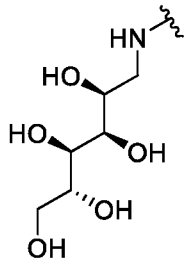
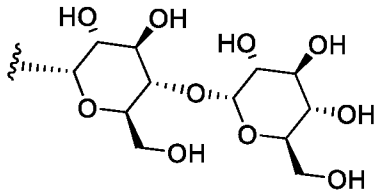
**[0167]** In certain instances, for any compound of Formula (**VI**), Formula (**VII**), Formula (**VIII**), Formula (**IX**), Formula (**IXa**), Formula (**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**), Formula (**Xc**), or Formula (**Xd**), **HG** is  , or salts thereof. In certain instances, for any compound of Formula (**VI**), Formula (**VII**), Formula (**VIII**), Formula (**IX**), Formula (**IXa**), Formula (**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**), Formula (**Xc**), or Formula (**Xd**),

**HG** is  , or salts thereof. In one instance **HG** is  , or salts thereof. In another instance, **HG** is  , or salts thereof.

**[0168]** In some examples, for any compound of Formula (**VI**), Formula (**VII**), Formula (**VIII**), Formula (**IX**), Formula (**IXa**), Formula (**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**), Formula (**Xc**), or Formula (**Xd**), **HG** is an amine, or salts thereof, for instance a quarternary amine, e.g.,  , wherein the  indicates the atom through which the **HG** is bonded to the adjacent groups in the formula.

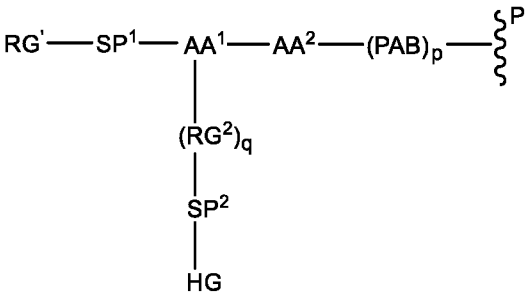
**[0169]** In other examples, for any compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), **HG** is a phosphonic acid, or salts thereof, e.g.,  wherein the  indicates the atom through which the **HG** is bonded to the adjacent groups in the formula. In other examples, for any compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), **HG** is a phosphonic acid, or salts thereof, e.g.,  wherein the  indicates the atom through which the **HG** is bonded to the adjacent groups in the formula.

**[0170]** In yet other examples, for any compound of Formula (**VI**), Formula (**VII**), Formula (**VIII**), Formula (**IX**), Formula (**IXa**), Formula (**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**),

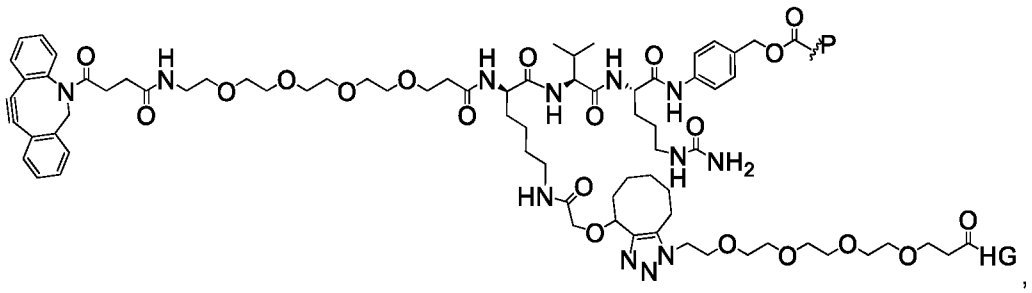
Formula (**Xc**), or Formula (**Xd**), **HG** is a sugar residue, e.g.,  (galactose),  (glucamine), or  (maltose), wherein the  indicates the atom through which the **HG** is bonded to the adjacent groups in the formula.

**[0171]** In certain instances, for any compound of Formula (**VI**), Formula (**VII**), Formula (**VIII**), Formula (**IX**), Formula (**IXa**), Formula (**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**), Formula (**Xc**), or Formula (**Xd**), $SP^1$ and $SP^2$ are independently, in each instance, absent, or selected from the group consisting of $C_{1-6}$ alkylene, -NH-, -C(O)-, $(-CH_2-CH_2-O)_e$, -NH-$CH_2$-$CH_2$-$(-O-CH_2-CH_2)_e$-C(O)-, -C(O)-$(CH_2)_u$-C(O)-, -C(O)-NH-$(CH_2)_v$-, polyglycine (e.g., $((glycine)_4\text{-serine})_f$ wherein subscript **f** is an integer from 1 to 6), and combinations thereof, wherein subscript **e** is an integer from 0 to 4, subscript **u** is an integer from 1 to 8, and subscript **v** is an integer from 1 to 8. In certain instances, for any compound of Formula (**VI**), Formula (**VII**), Formula (**VIII**), Formula (**IX**), Formula (**IXa**), Formula (**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**), Formula (**Xc**), or Formula (**Xd**), $SP^1$ and $SP^2$ are independently, in each instance, as shown in Table S. In certain embodiments, $((glycine)_4\text{-serine})_f$ is $(glycine)_4$-serine.

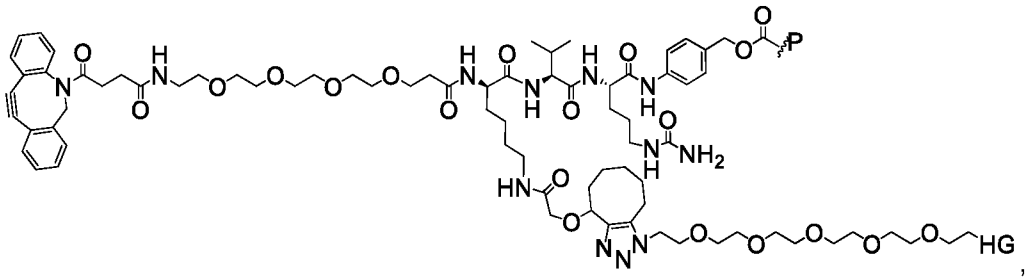
**[0172]** In some instances, for any compound of Formula (**VI**), Formula (**VII**), Formula (**VIII**), Formula (**IX**), Formula (**IXa**), Formula (**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**), Formula (**Xc**), or Formula (**Xd**),



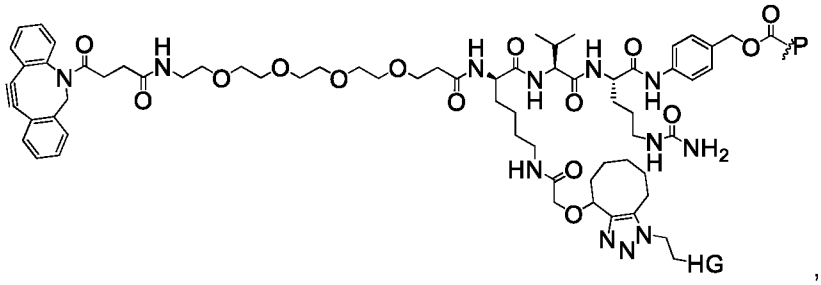

is selected from the group consisting of:



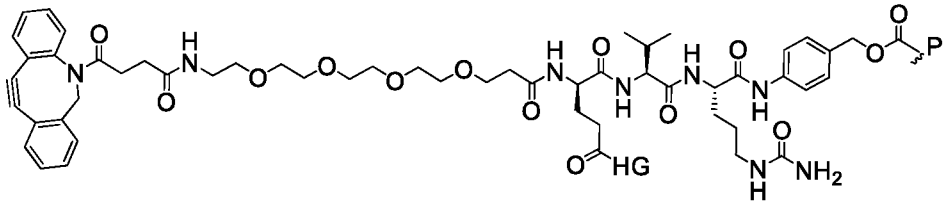
,



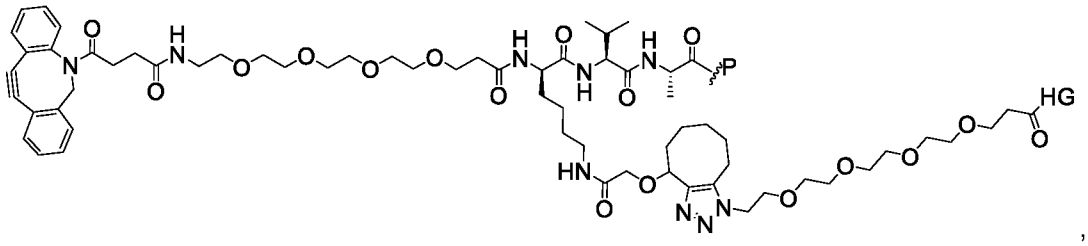
,



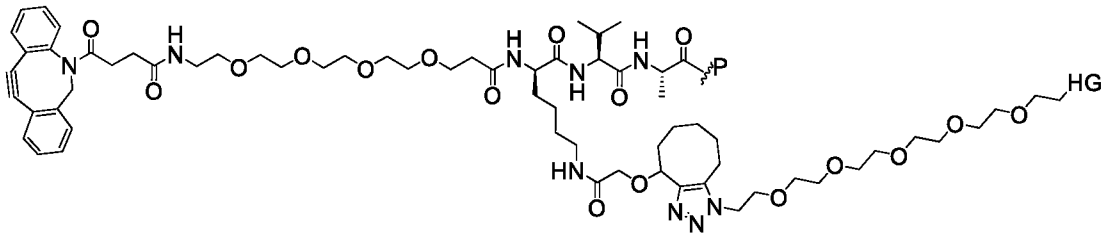
,



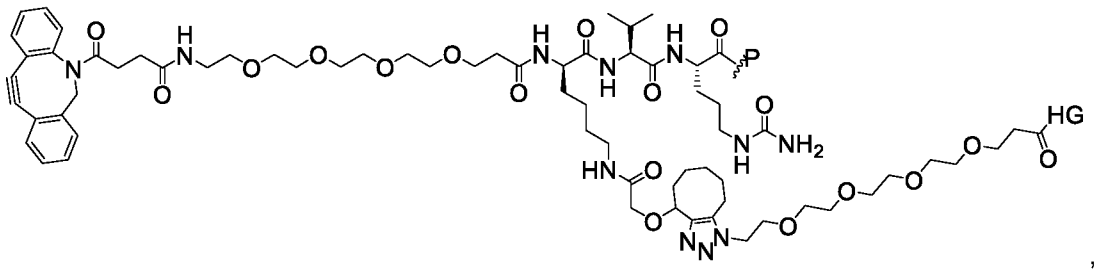
,



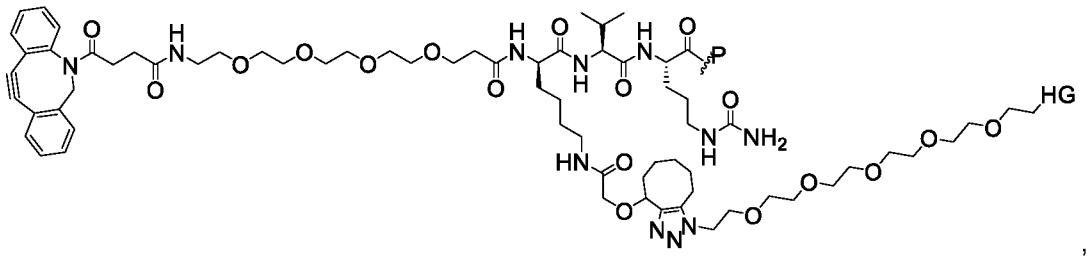
,



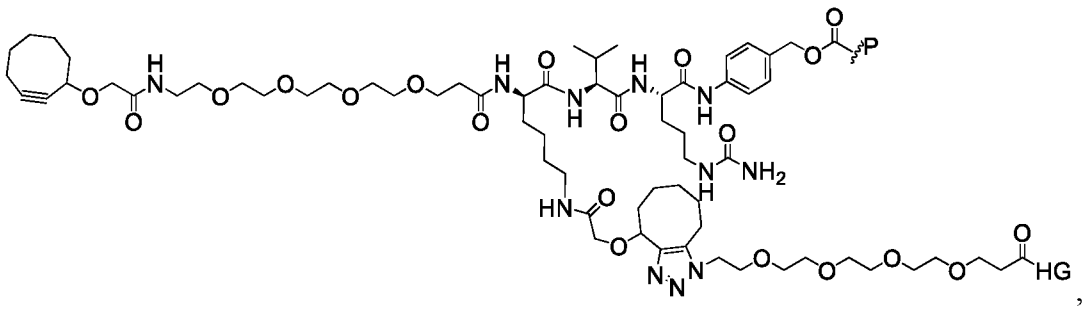
,



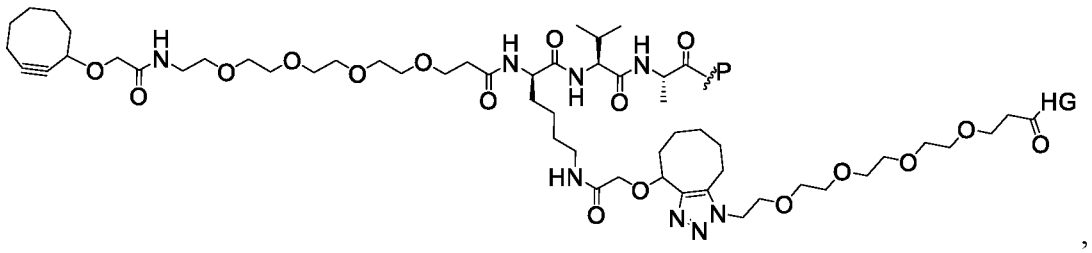
,



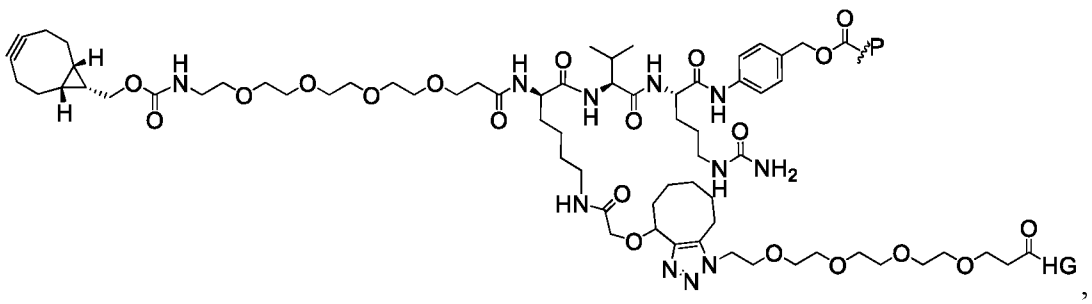
,



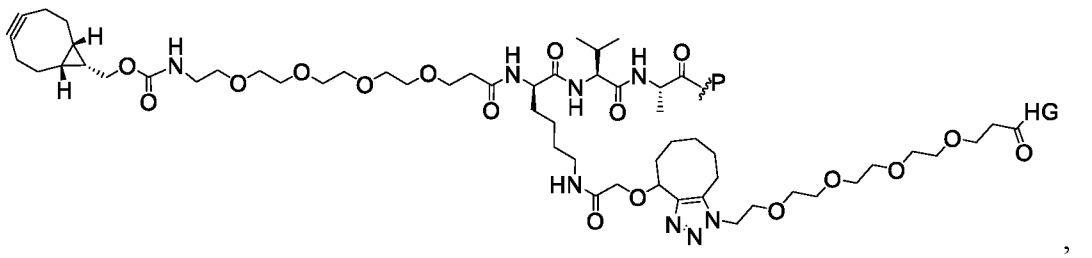
,



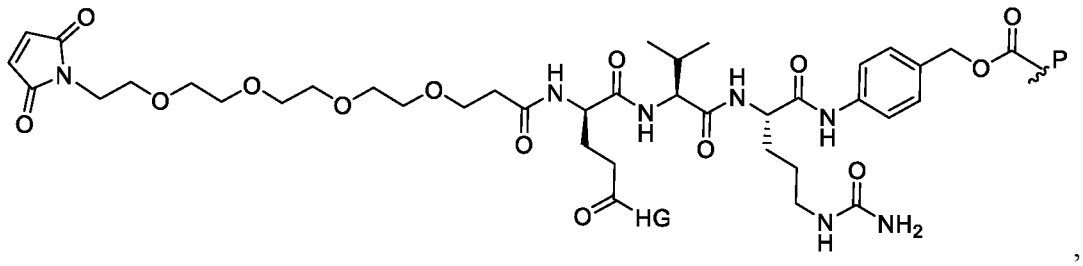
,



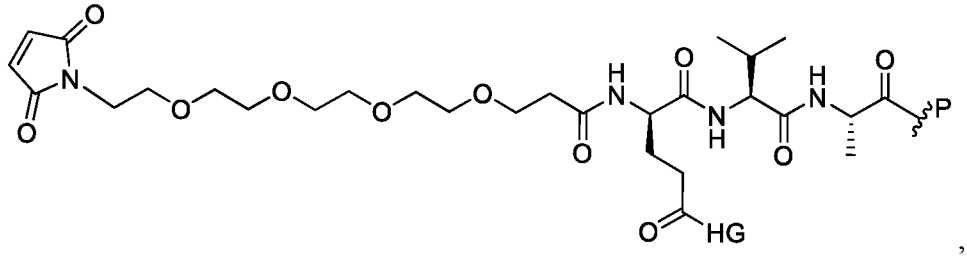
,



,



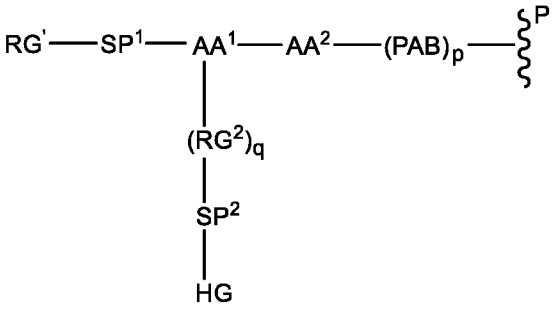
and



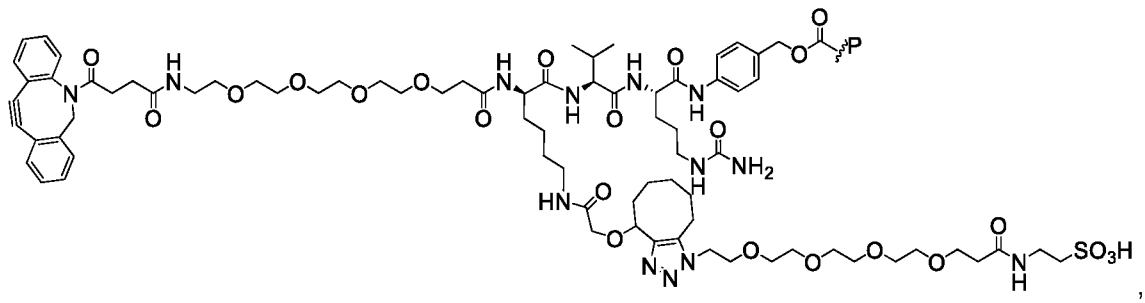
or a stereoisomeric form thereof, or a regioisomer thereof, or a mixture of regioisomers thereof, wherein

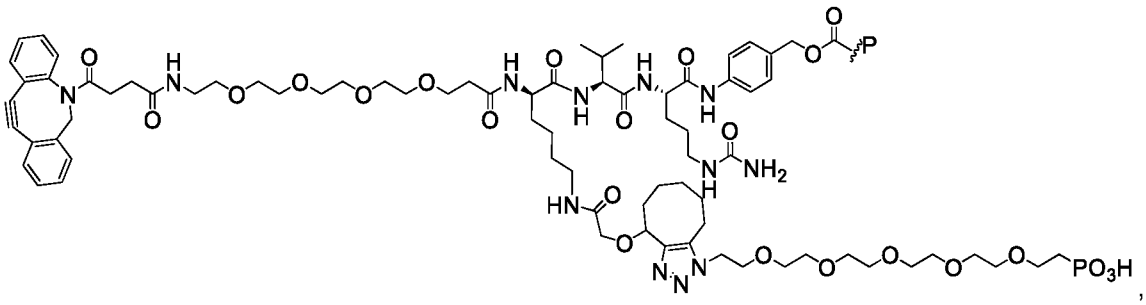
each  is a bond to the payload residue.

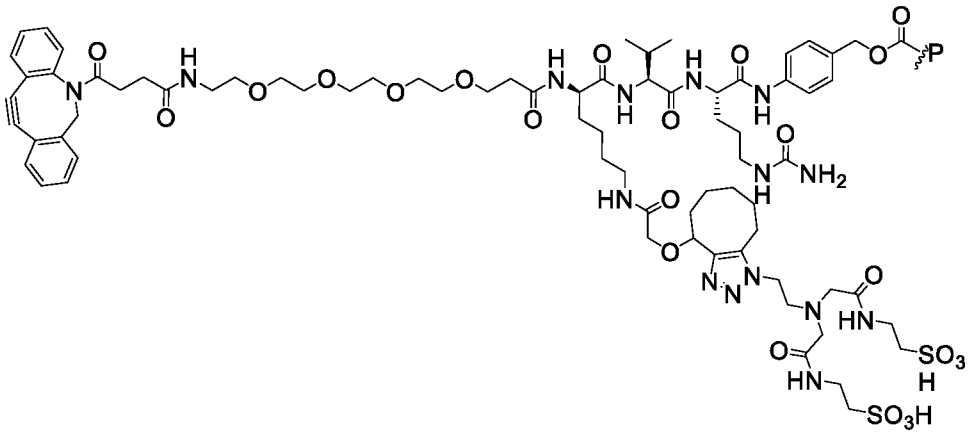
**[0173]** In some instances, for any compound of Formula (**VI**), Formula (**VII**), Formula (**VIII**), Formula (**IX**), Formula (**IXa**), Formula (**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**), Formula (**Xc**), or Formula (**Xd**),

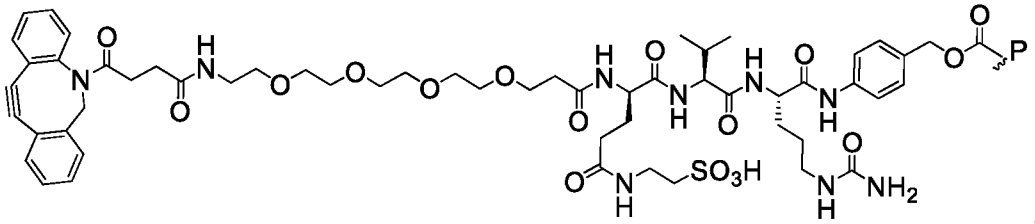


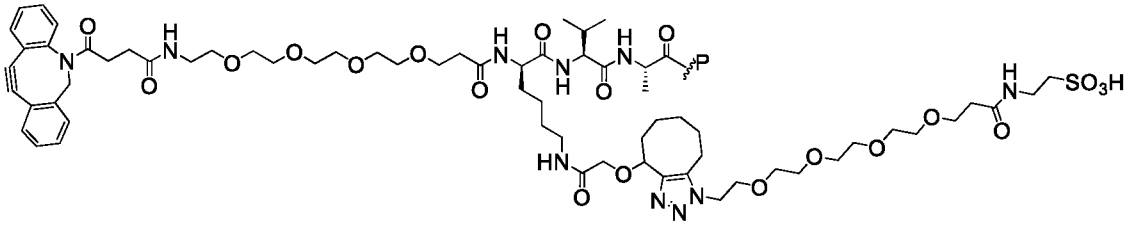
is selected from the group consisting of:

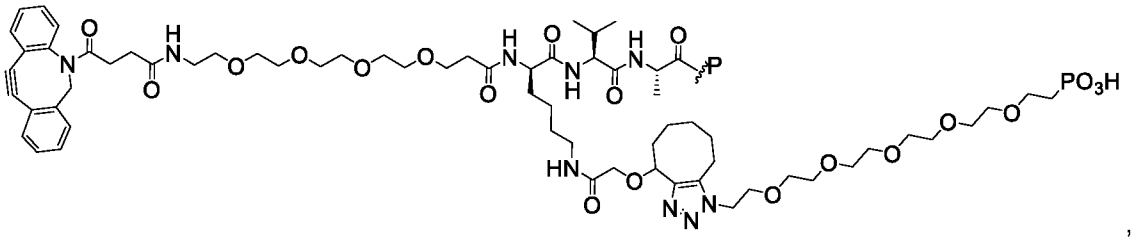


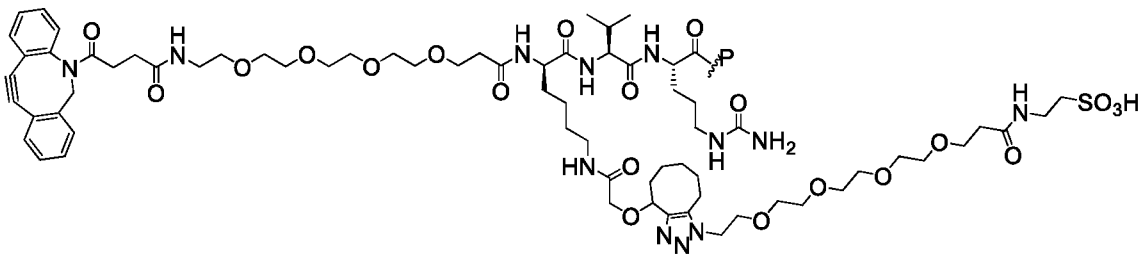


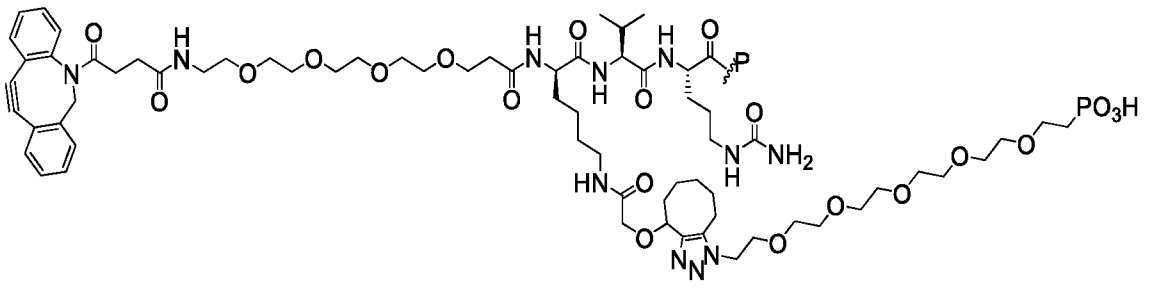




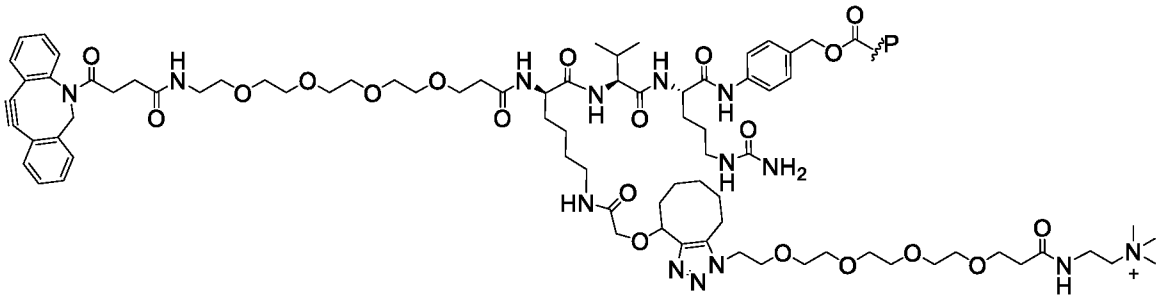




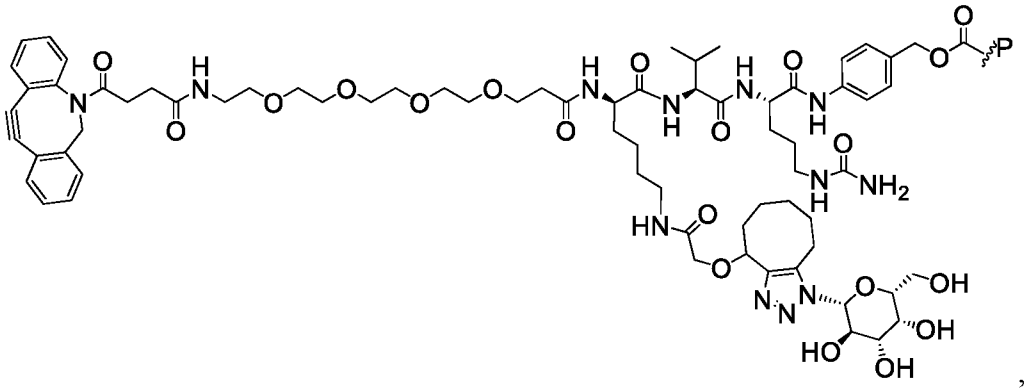




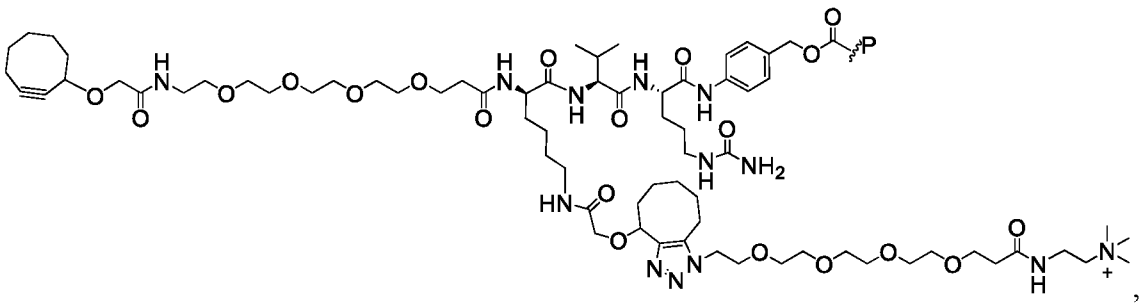
,



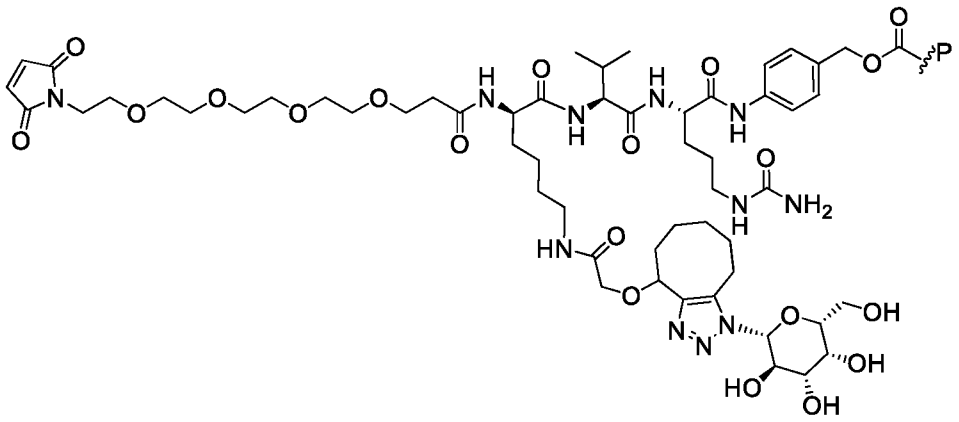
,



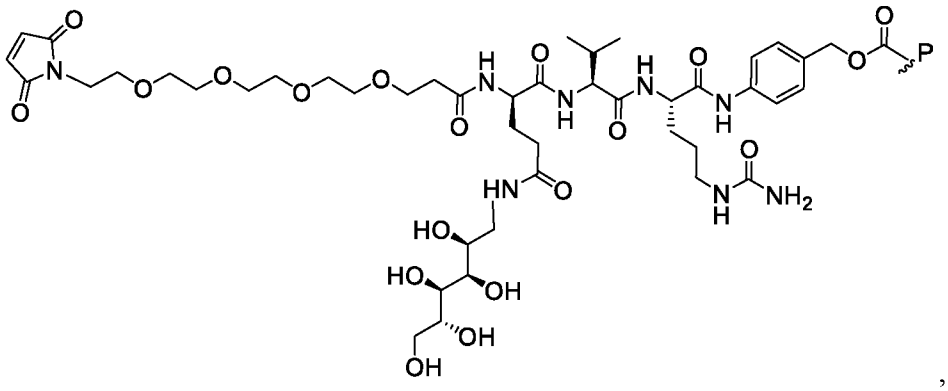
,



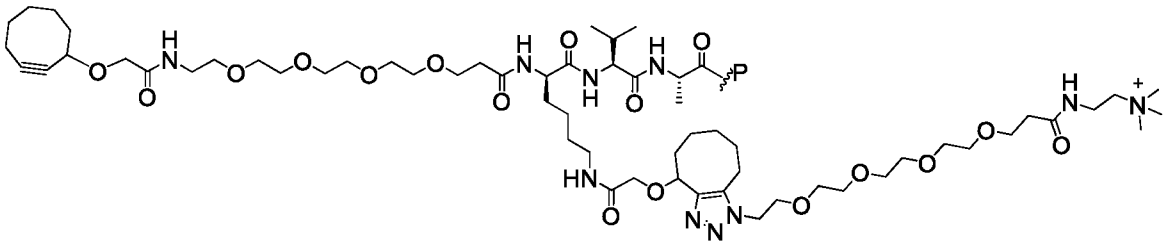
,



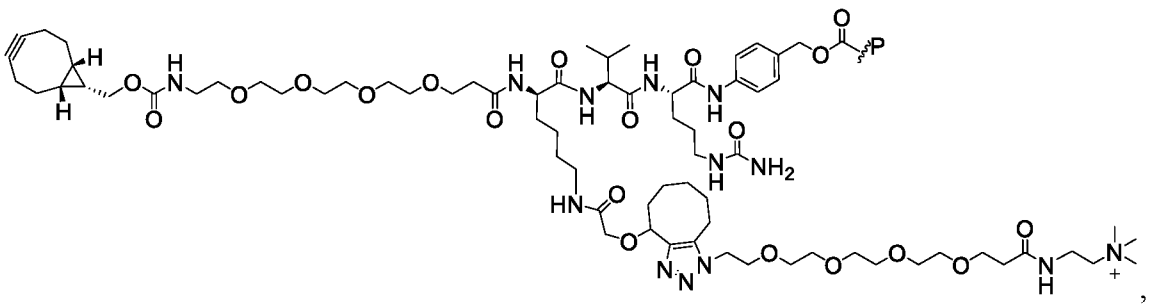
,



,

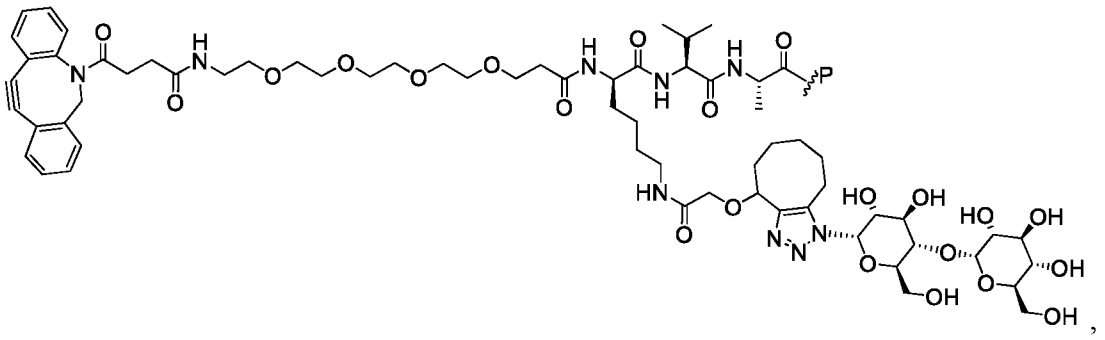


,



,

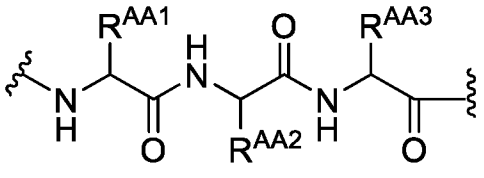
and



or a stereoisomeric form thereof, or a regioisomer thereof, or a mixture of regioisomers thereof, wherein

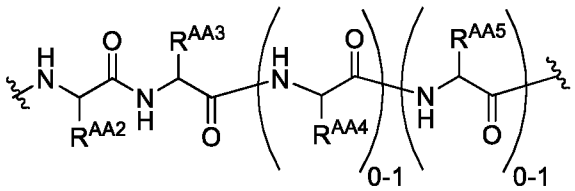
each  is a bond to the payload residue.

**[0174]** In some instances, for any compound of Formula (**VI**), Formula (**VII**), Formula (**VIII**), Formula (**IX**), Formula (**IXa**), Formula (**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**), Formula (**Xc**), or Formula (**Xd**), **LL** is according to Formula (**LL1**):


(LL1)

**[0175]** wherein $R^{AA1}$, $R^{AA2}$, and $R^{AA3}$ are each, independently, amino acid side chains, at least one of which is bonded directly or indirectly to $-(RG^2)_q-SP^2-HG$. In some cases, $R^{AA1}$ is a lysine, glutamine, glutamic acid or aspartic acid side chain bonded directly or indirectly to **HG**, and $R^{AA2}$ and $R^{AA3}$ are either valine and alanine or valine and citrulline sidechains respectively.

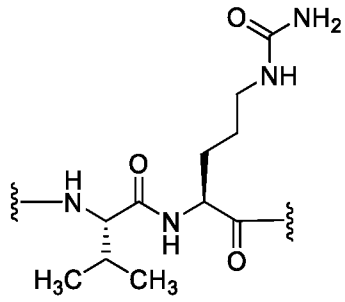
**[0176]** In some instances, for any compound of Formula (**IX**), Formula (**IXa**), Formula (**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**), Formula (**Xc**), or Formula (**Xd**), $AA^2$ is



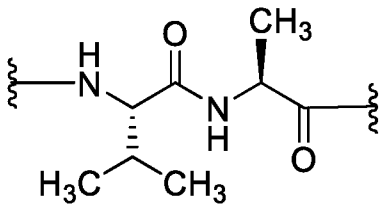
wherein $R^{AA2}$, $R^{AA3}$, $R^{AA4}$, and $R^{AA5}$ are each, independently, amino acid side chains, at least one of which is bonded directly or indirectly to $-(RG^2)_q-SP^2-HG$, wherein the  indicates

the atom through which $AA^2$ is bonded to the adjacent groups in the formula. In some examples, $R^{AA2}$, $R^{AA3}$, $R^{AA4}$, and $R^{AA5}$, are independently in each instance, an amino acid side chain selected from the side chains of alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, or citrulline, a derivative thereof, or a combination thereof.

**[0177]** In some instances, for any compound of Formula (**IX**), Formula (**IXa**), Formula (**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**), Formula (**Xc**), or Formula (**Xd**), $AA^2$ is



or



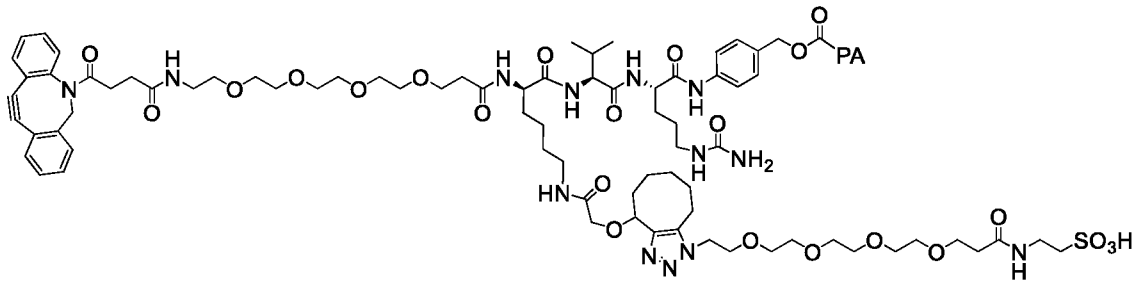
,

wherein the  indicates the atom through which $AA^2$ is bonded to the adjacent groups in the formula.

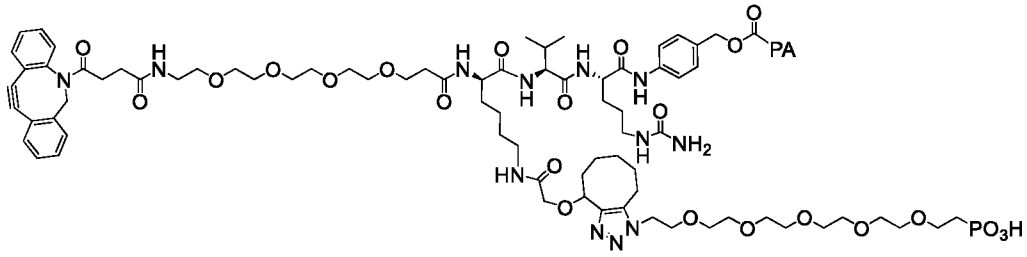
**[0178]** In some instances, for any compound of Formula (**Xa**), Formula (**Xb**), Formula (**Xc**), or Formula (**Xd**), subscript **e** is 4. In some instances, for any compound of Formula (**Xa**), Formula (**Xb**), Formula (**Xc**), or Formula (**Xd**), subscript **e** is 5.

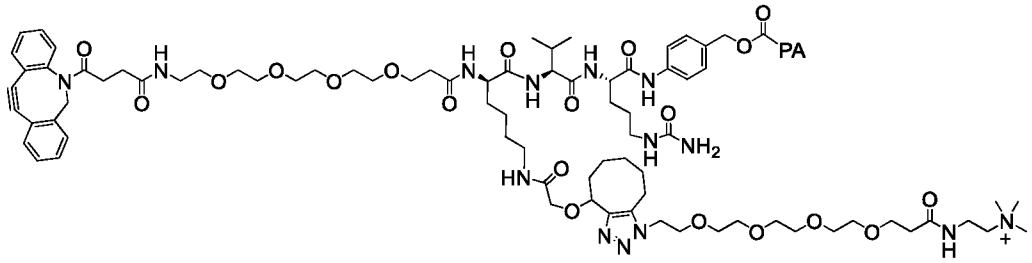
**[0179]** In some instances, for any compound of Formula (**IX**), Formula (**IXa**), Formula (**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**), Formula (**Xc**), or Formula (**Xd**), **PA** is the residue of a group selected from the group consisting of a dolastatin, an auristatin, a maytansinoid, a plant alkaloid, a taxane, a vinca alkaloid, a steroid, and a liver X receptor (LXR) modulator. In some cases, **PA** is a dolastatin. In some cases, **PA** is a dolastatin. In some cases, **PA** isn auristatin. In some cases, **PA** is a maytansinoid. In some cases, **PA** is a doplant alkaloid. In some cases, **PA** is a taxane. In some cases, **PA** is a vinca alkaloid. In some cases, **PA** is a steroid. In some cases, **PA** is a LXR modulator. In some cases, a LXR modulator is a LXR agonist. In some cases, a LXR modulator is a LXR antagonist.

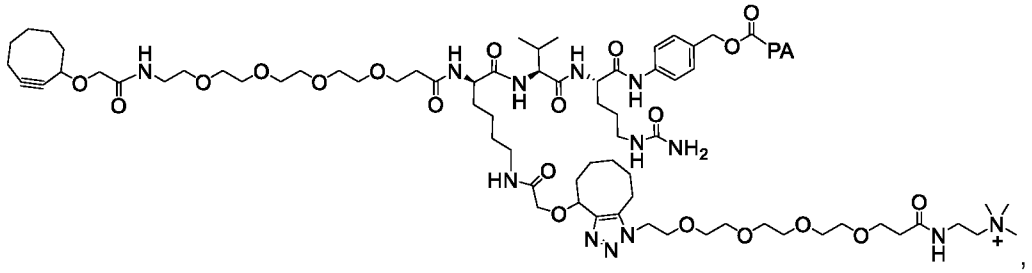
**[0180]** In certain instances, any compound of Formula (**IX**), Formula (**IXa**), Formula (**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**), Formula (**Xc**), or Formula (**Xd**), is selected from the group consisting of:



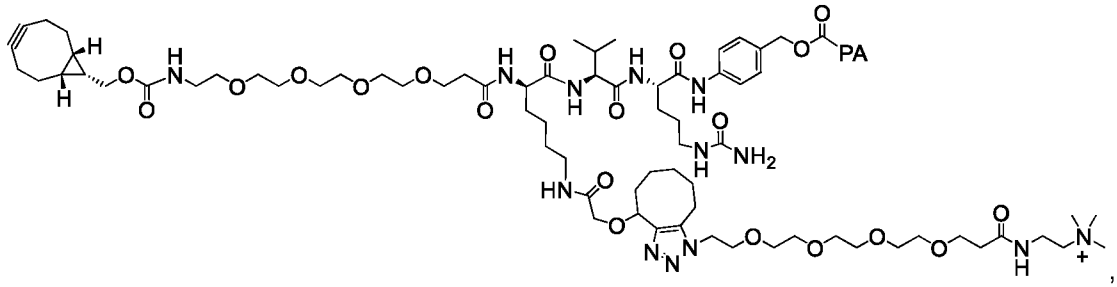
,



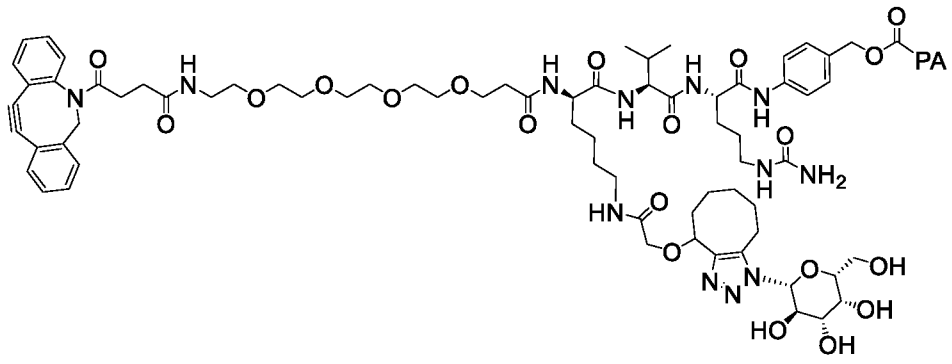




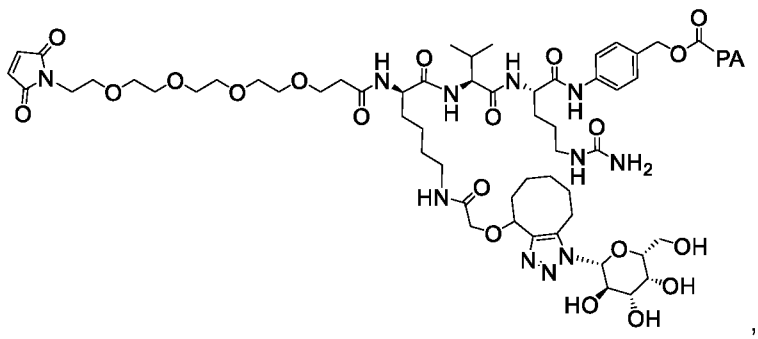
,

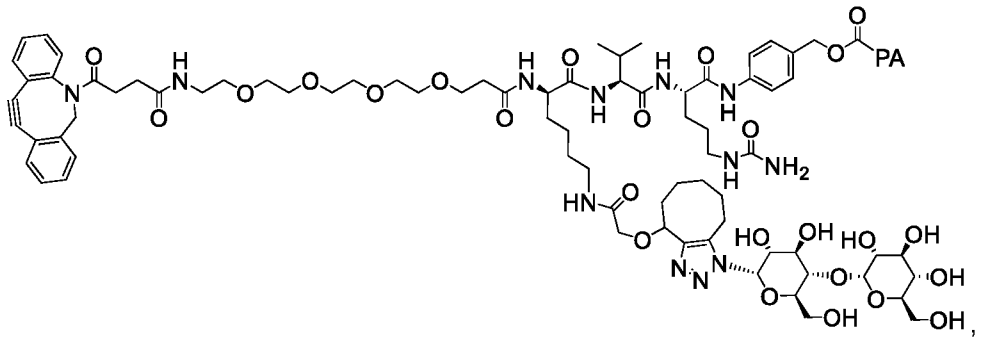


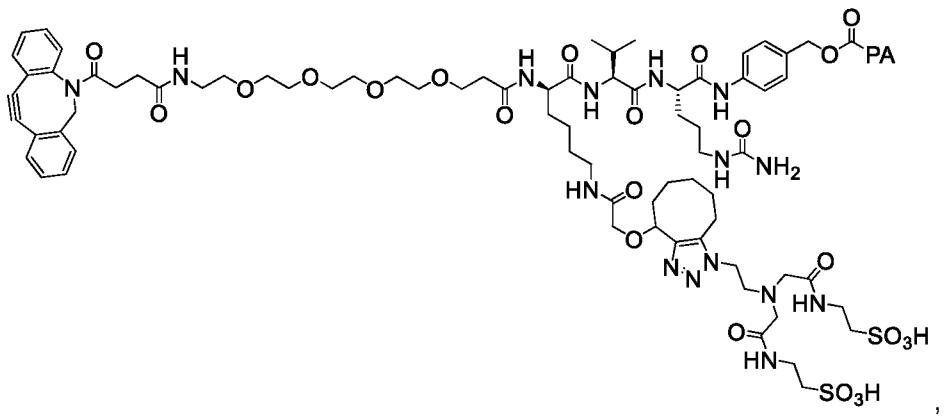
,

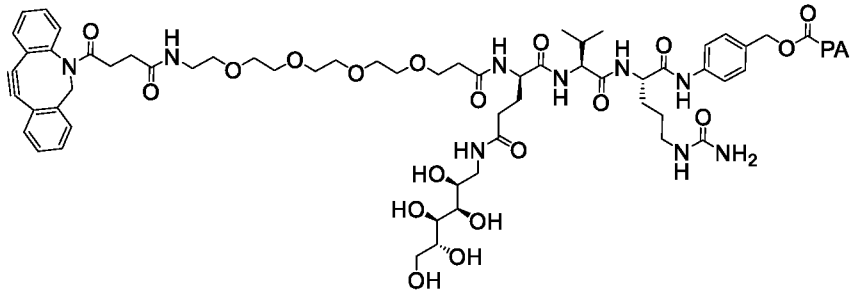


,

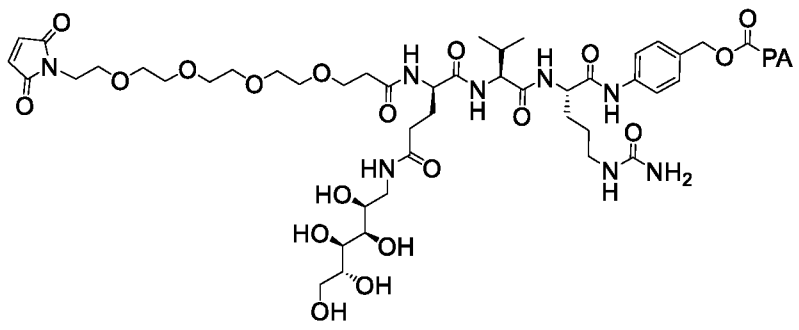


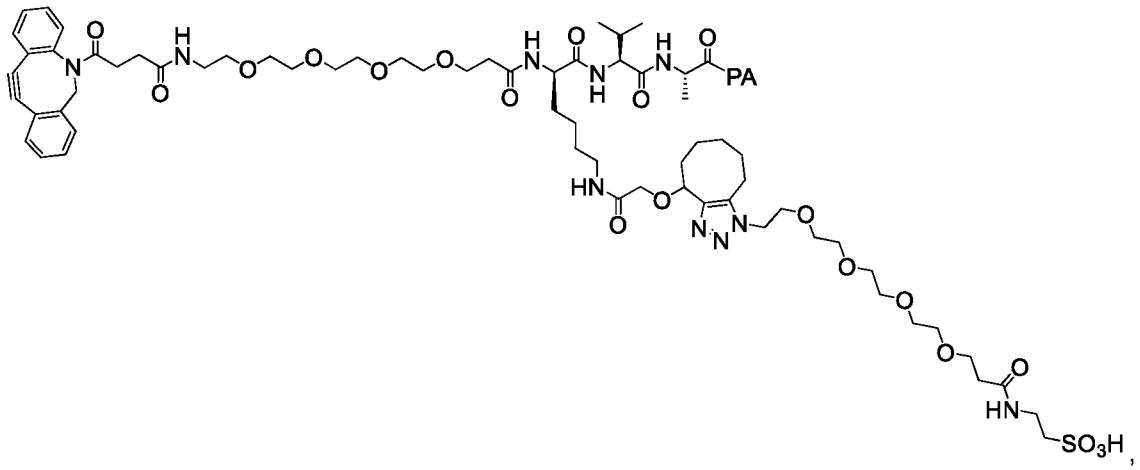


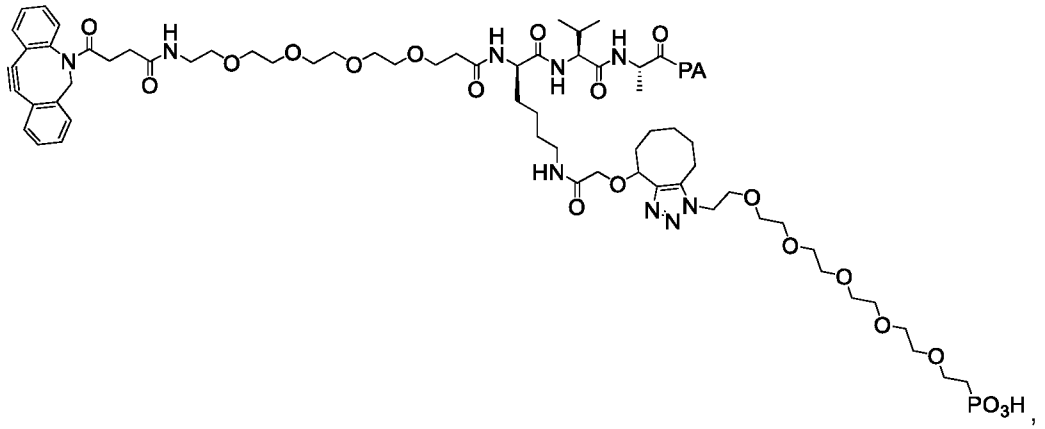


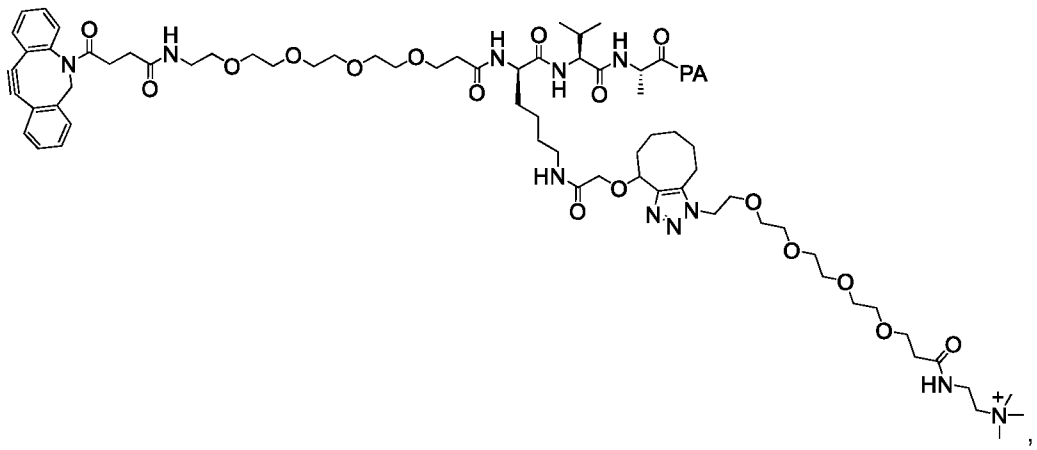


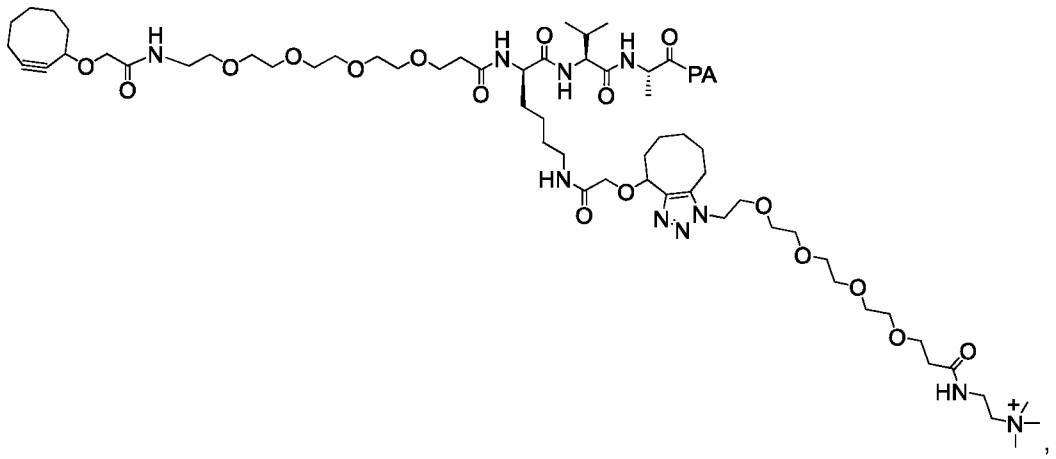
,Z

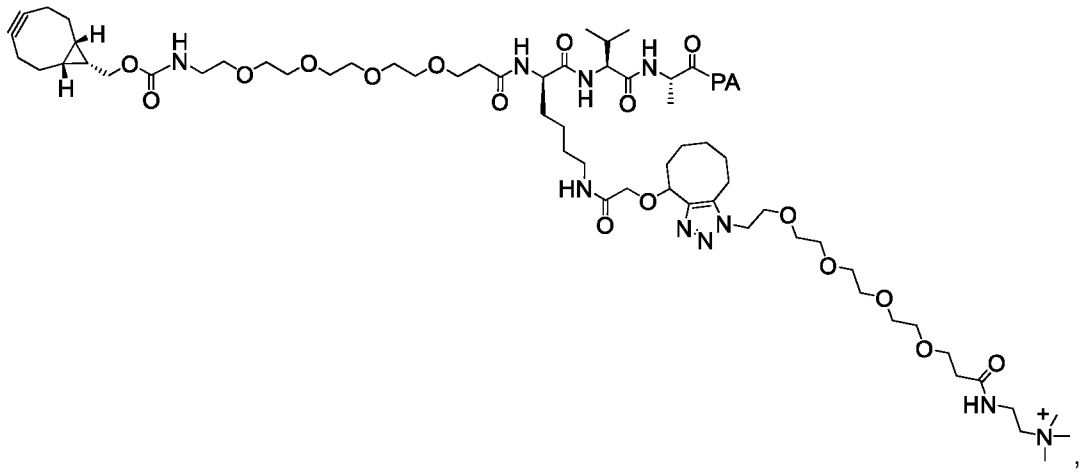


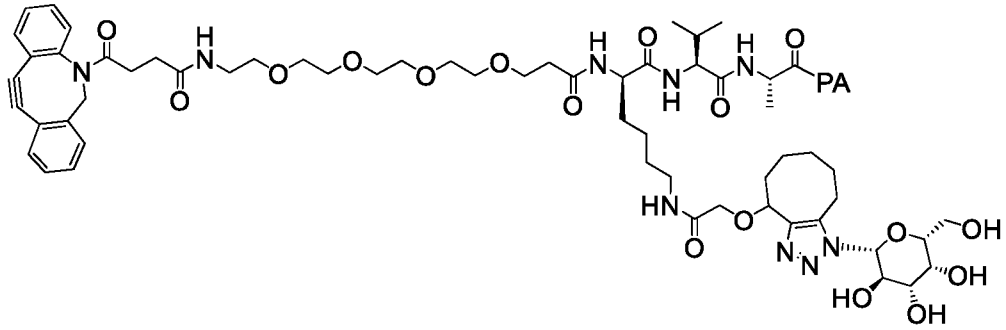

PA
HN
SO3H

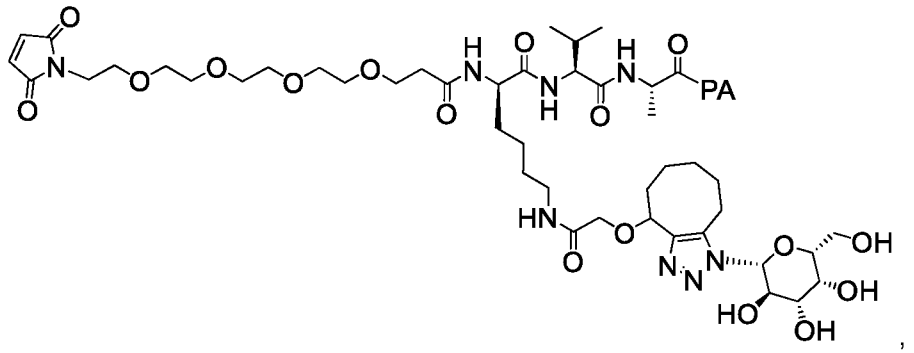

PA
HN
PO3H

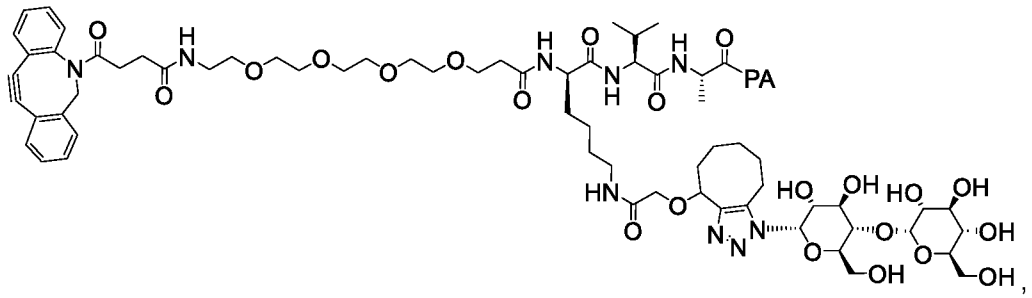

PA
HN

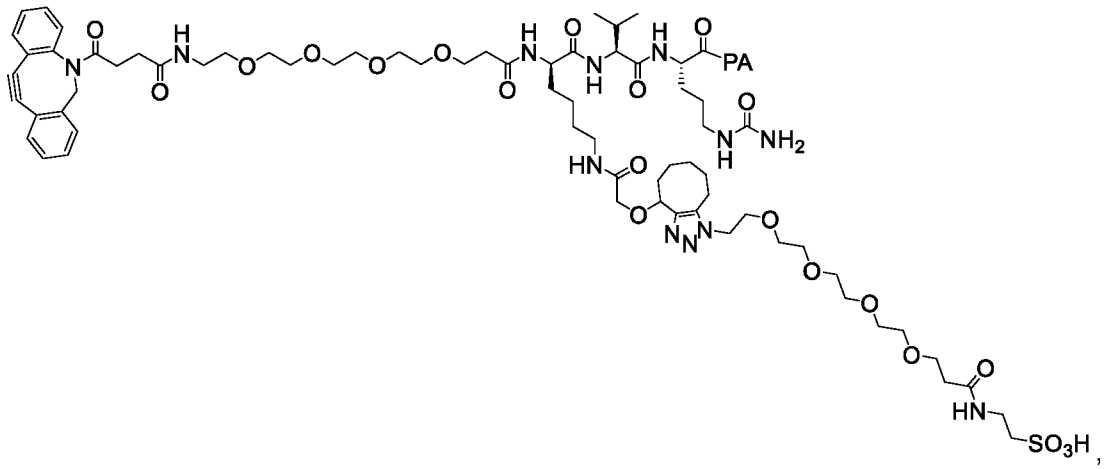

PA
HN

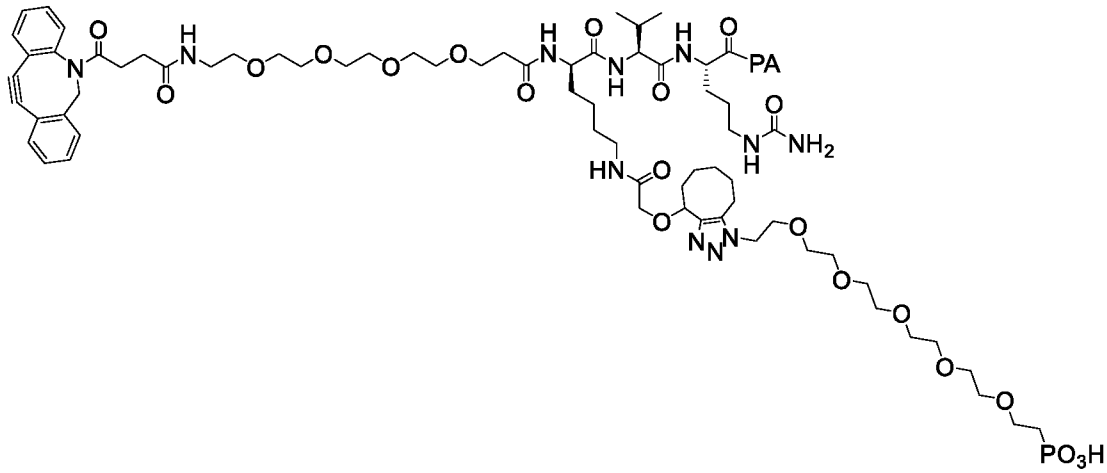


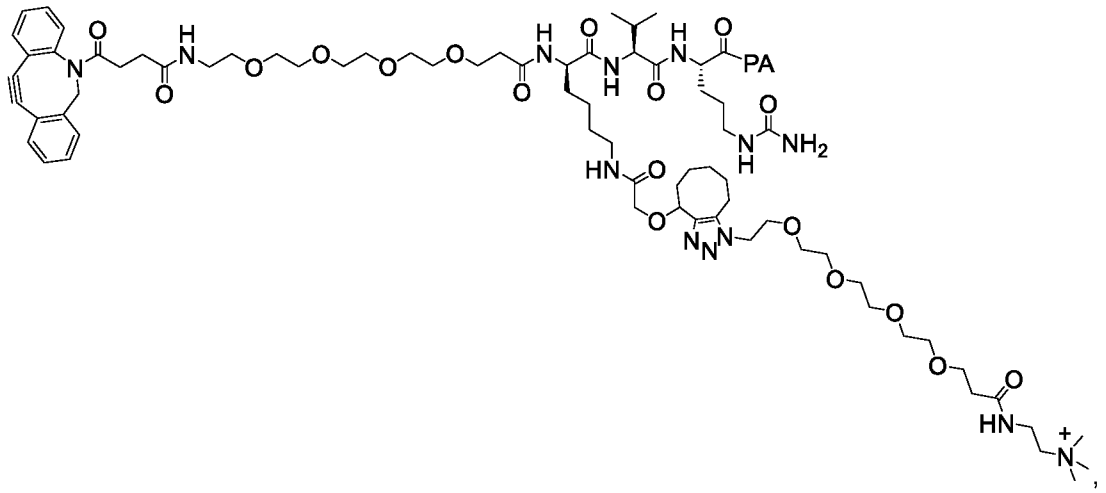


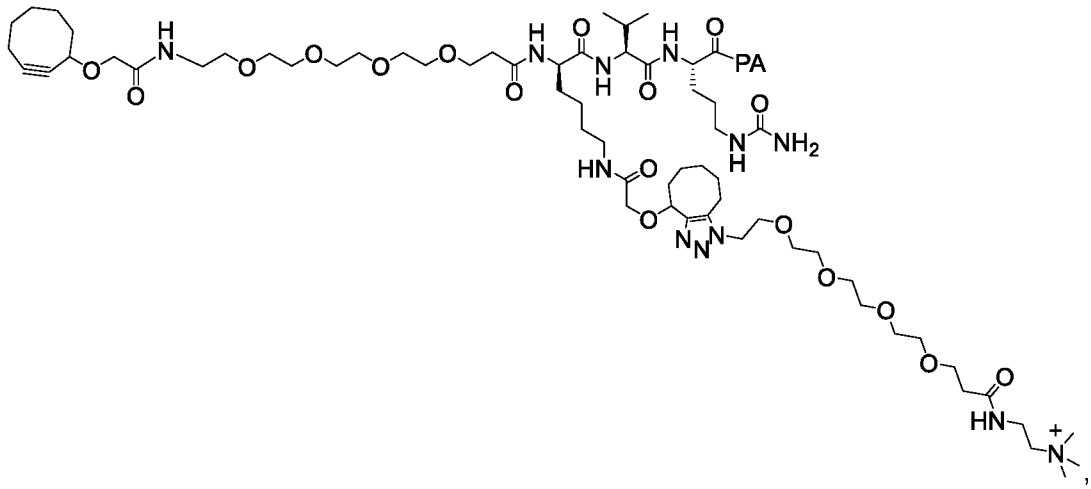


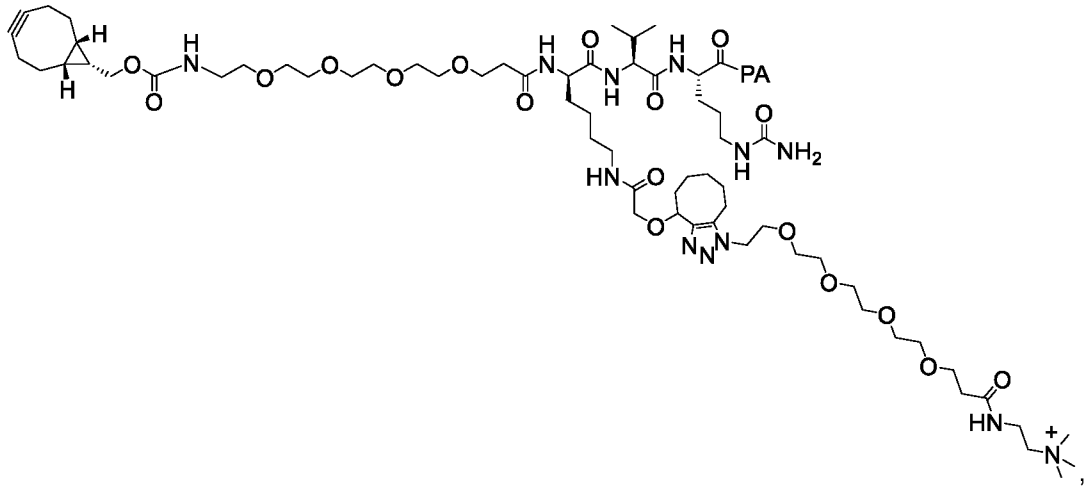


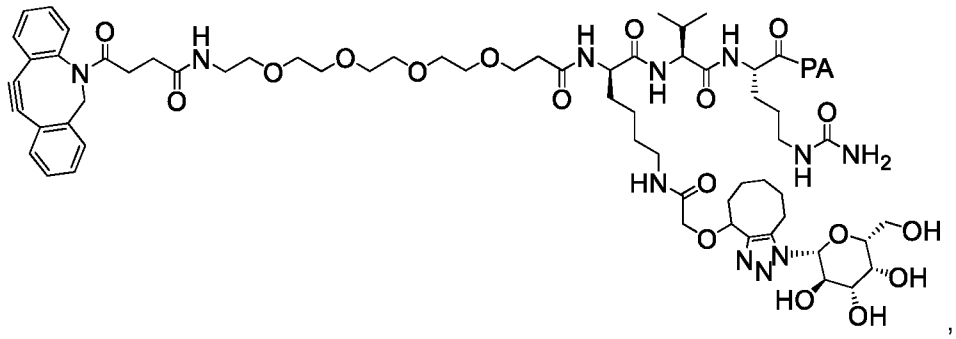


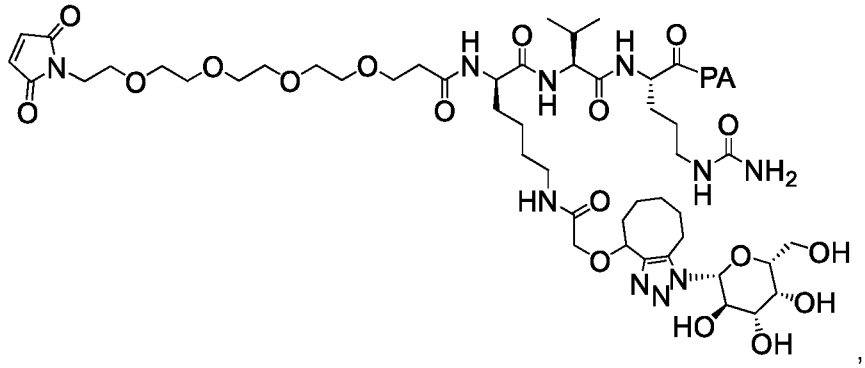




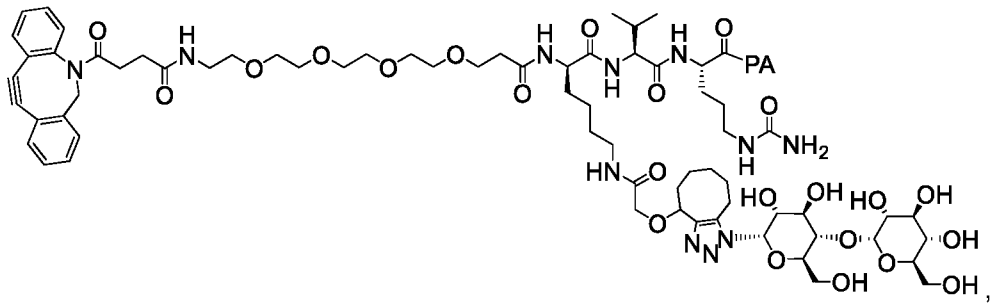






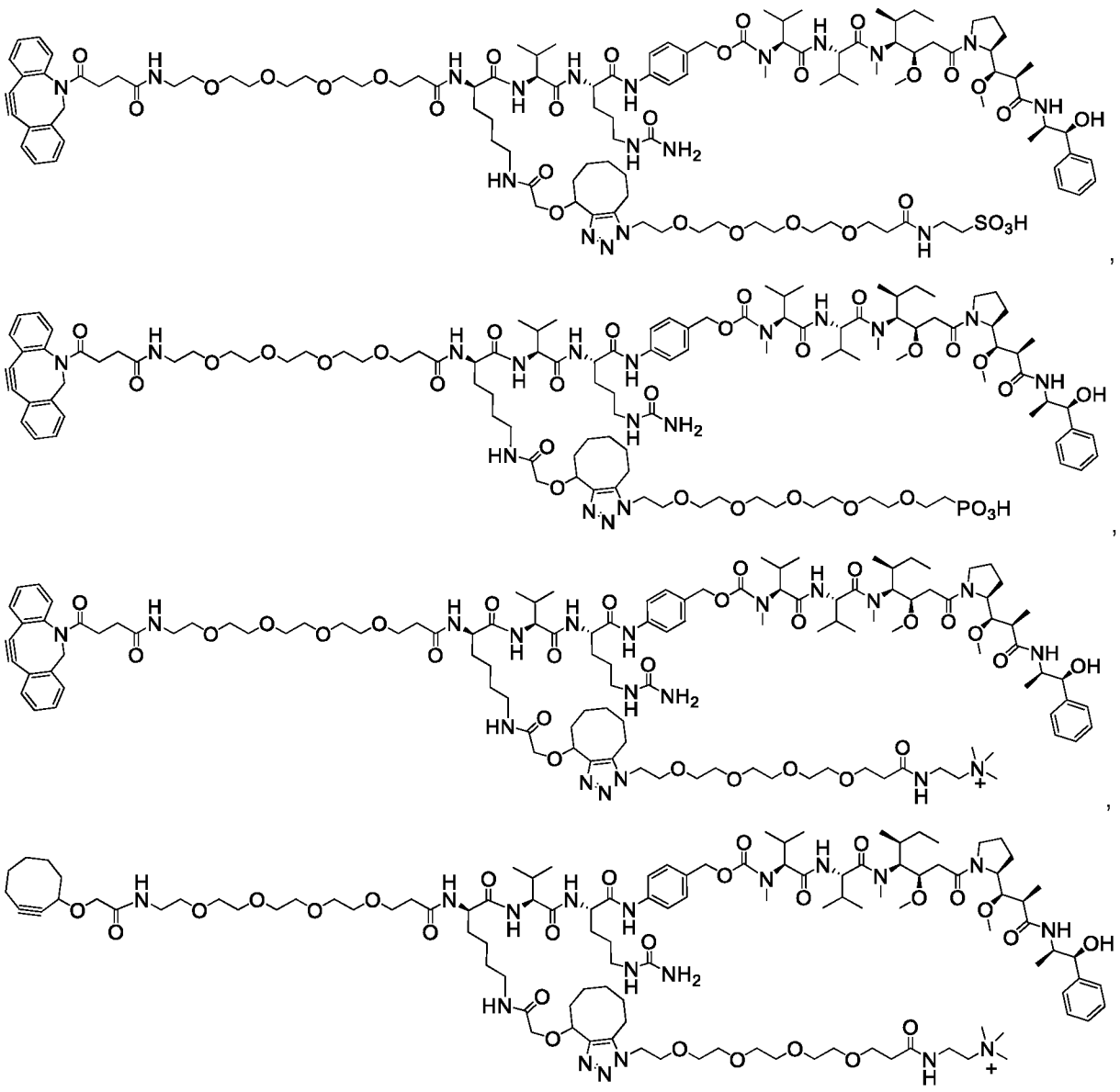


and

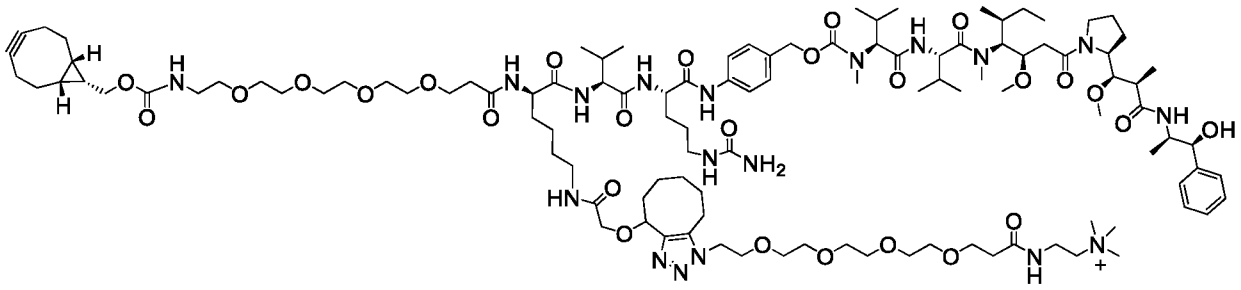


or a stereoisomeric form thereof, or a regioisomer thereof, or a mixture of regioisomers thereof, wherein **PA** is a payload residue.

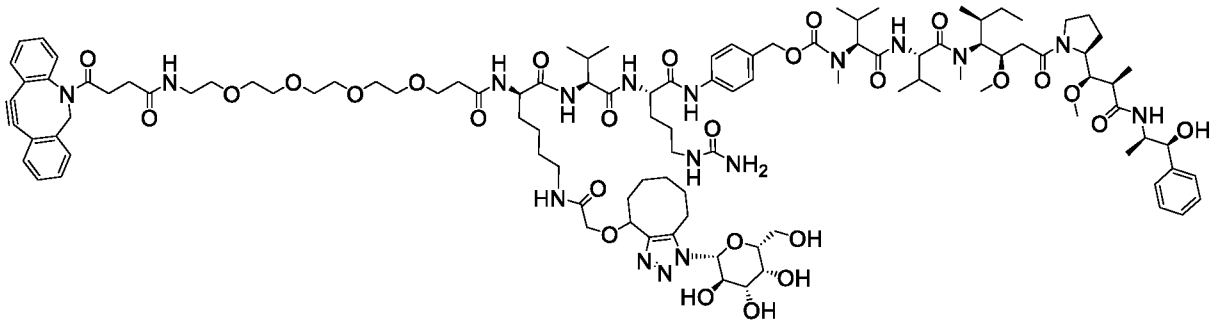
**[0181]** In some instances, any compound of Formula (**IX**), Formula (**IXa**), Formula (**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**), Formula (**Xc**), or Formula (**Xd**), is selected from the group consisting of:



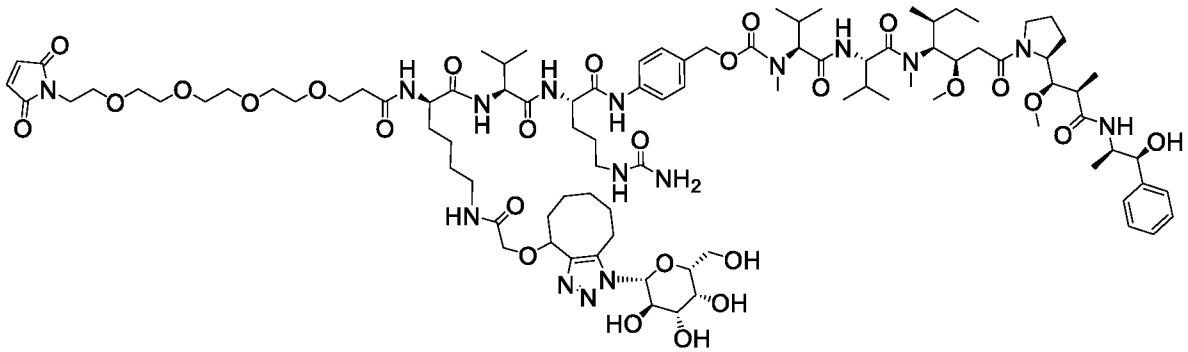
,



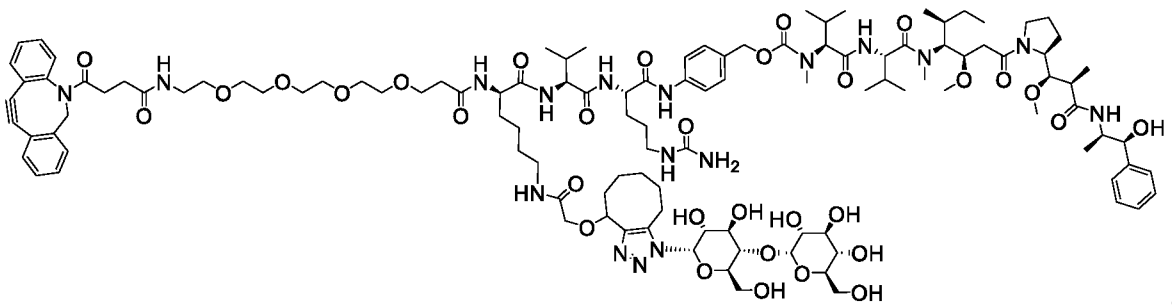
,



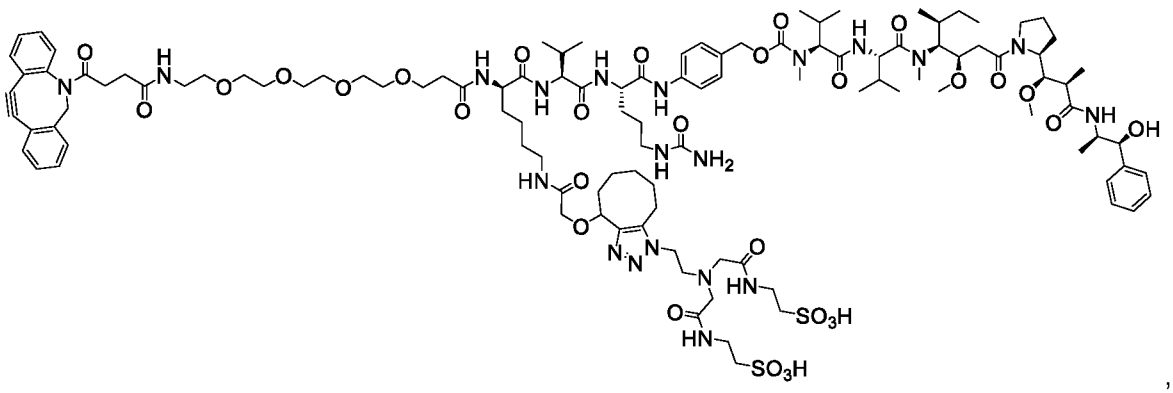
,



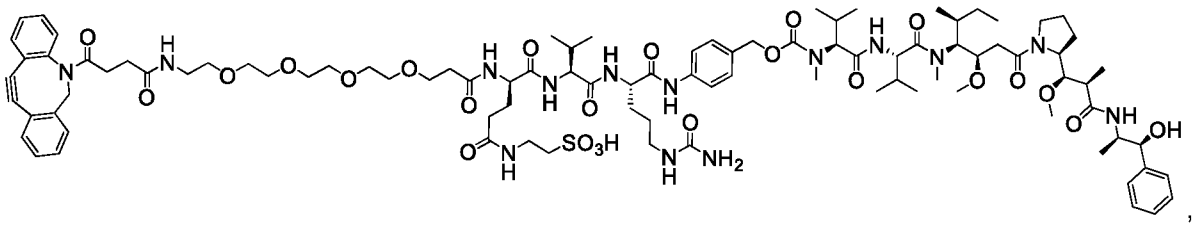
,



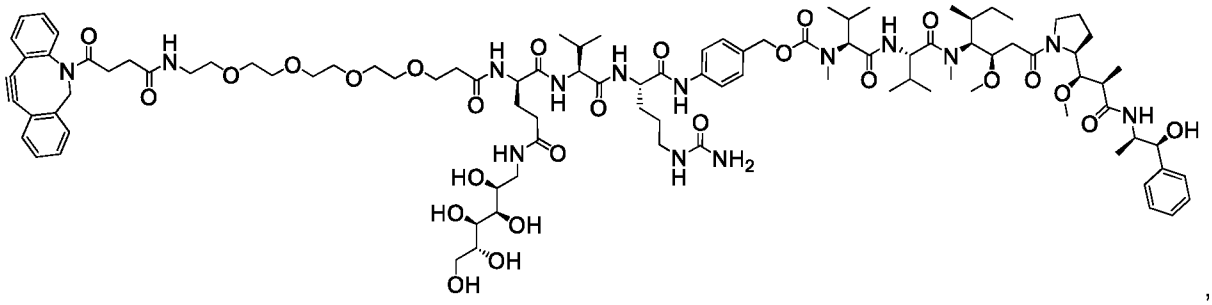
,



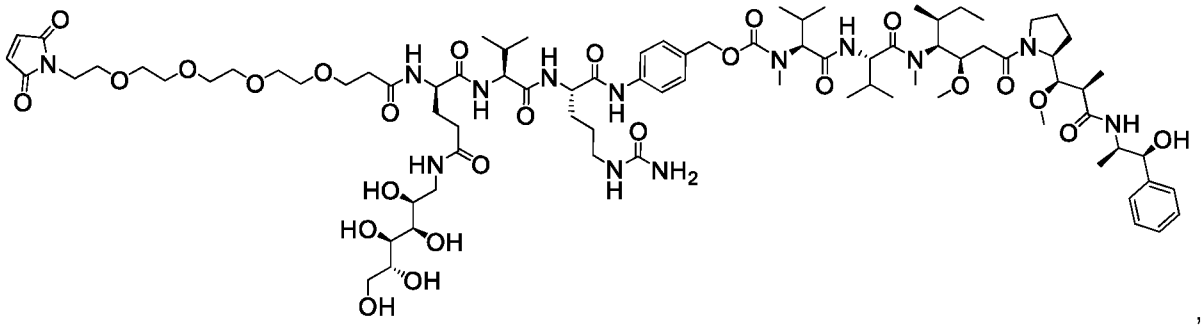
,



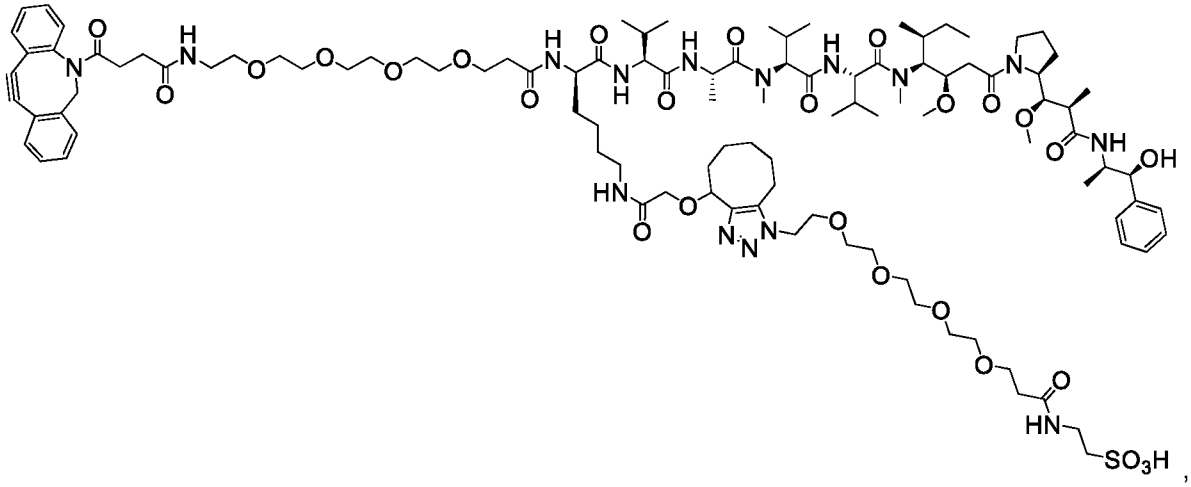
,



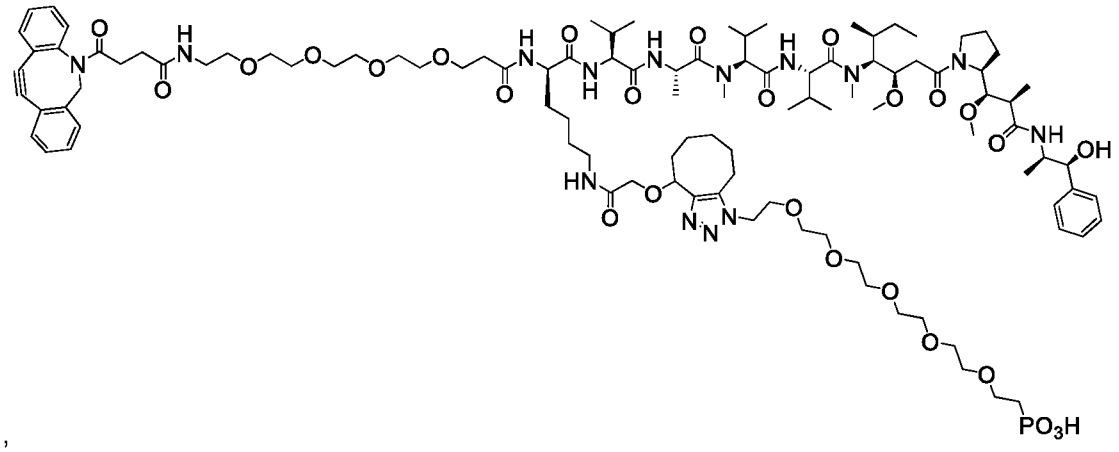
,



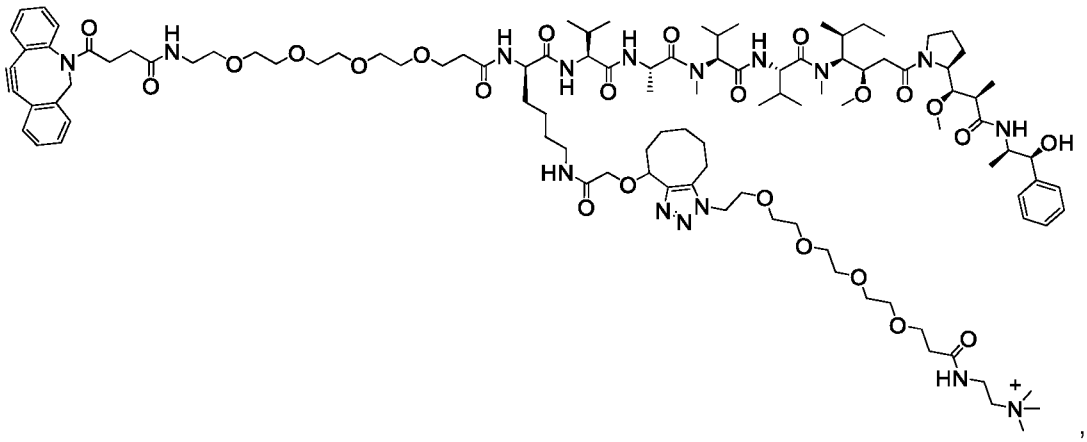
,



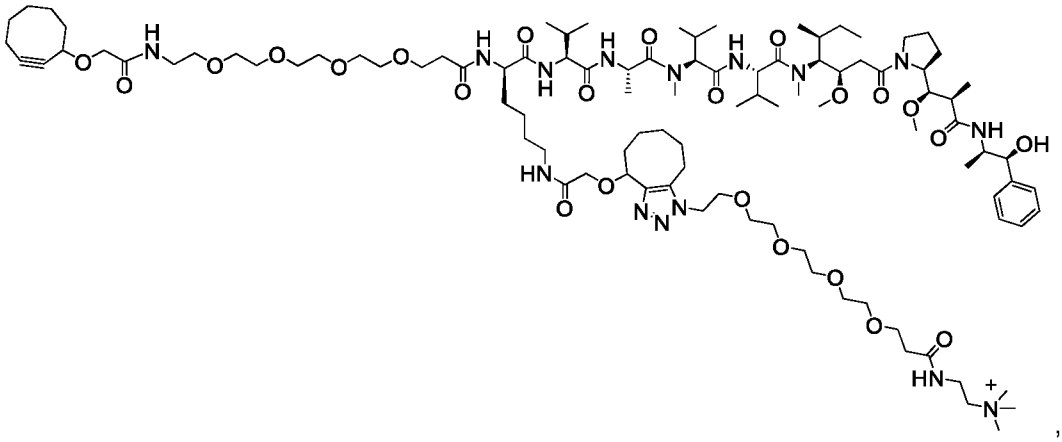
,



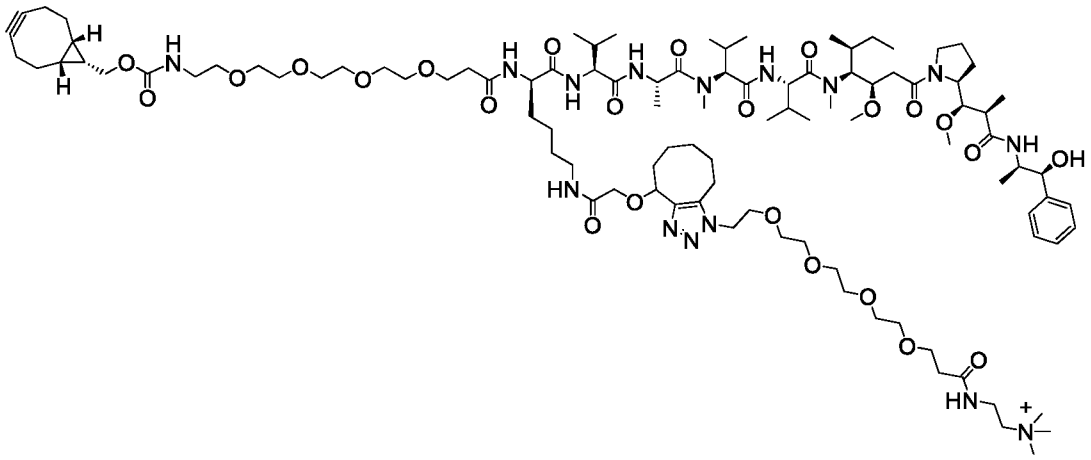
,



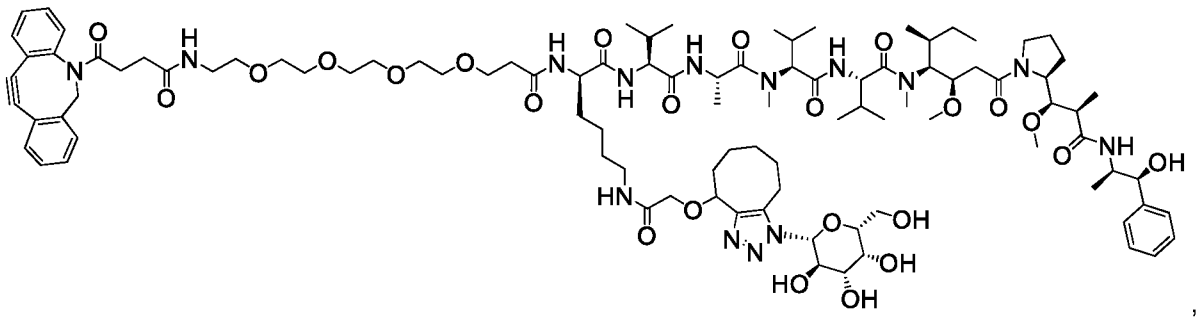
,



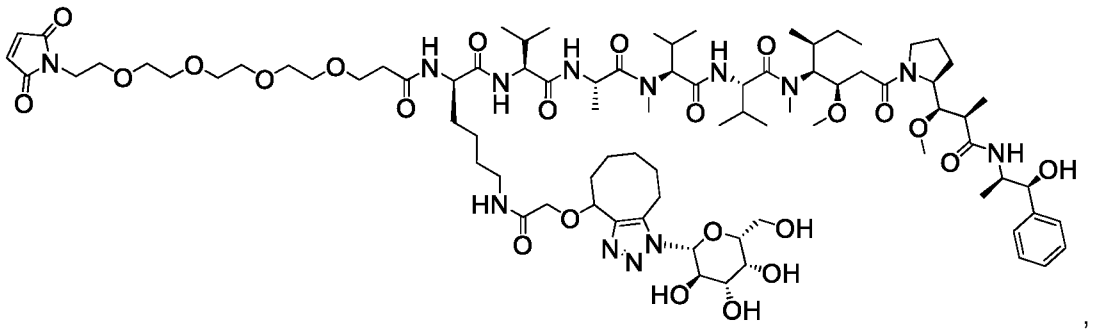
,



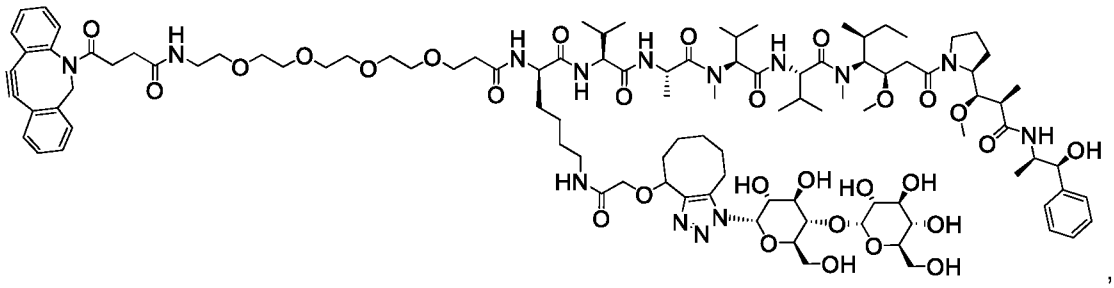
,



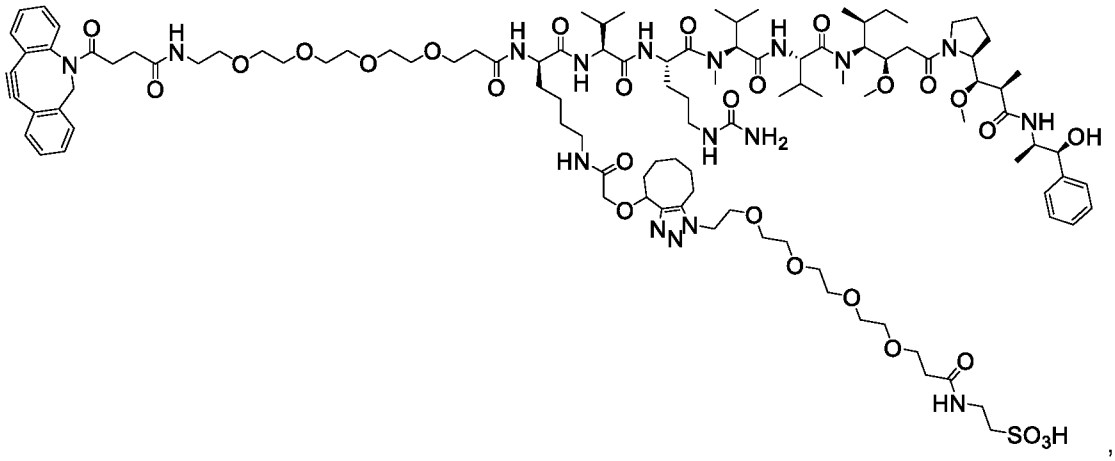
,



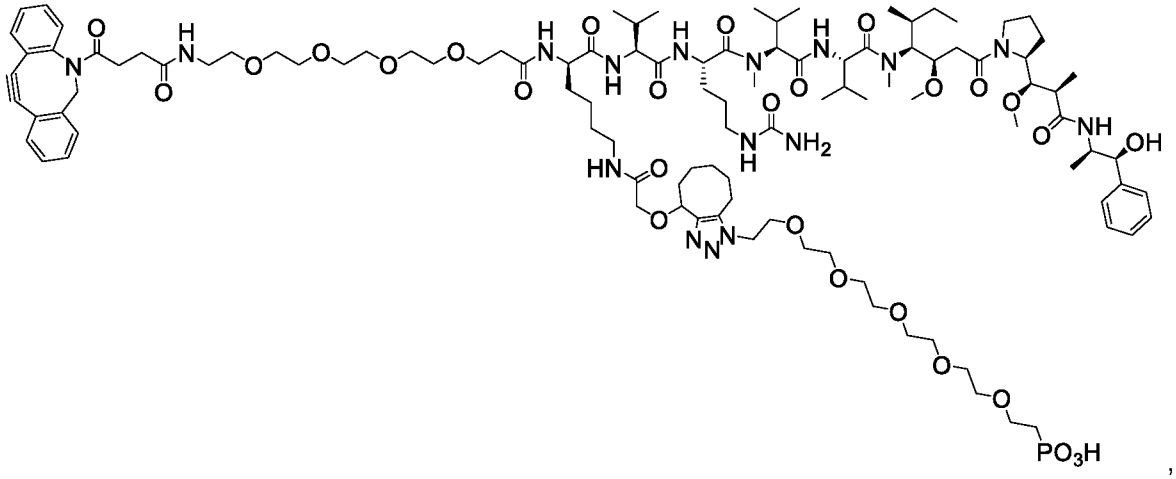
,



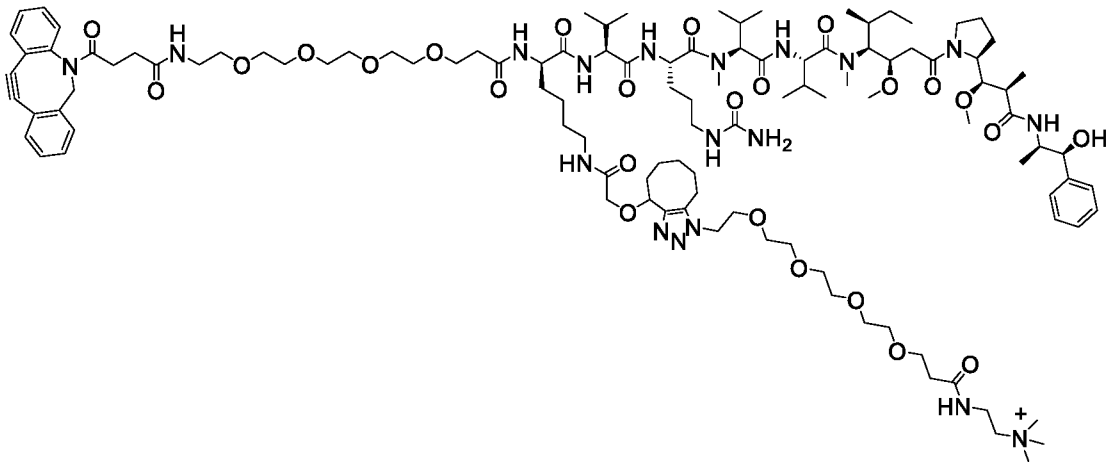
,

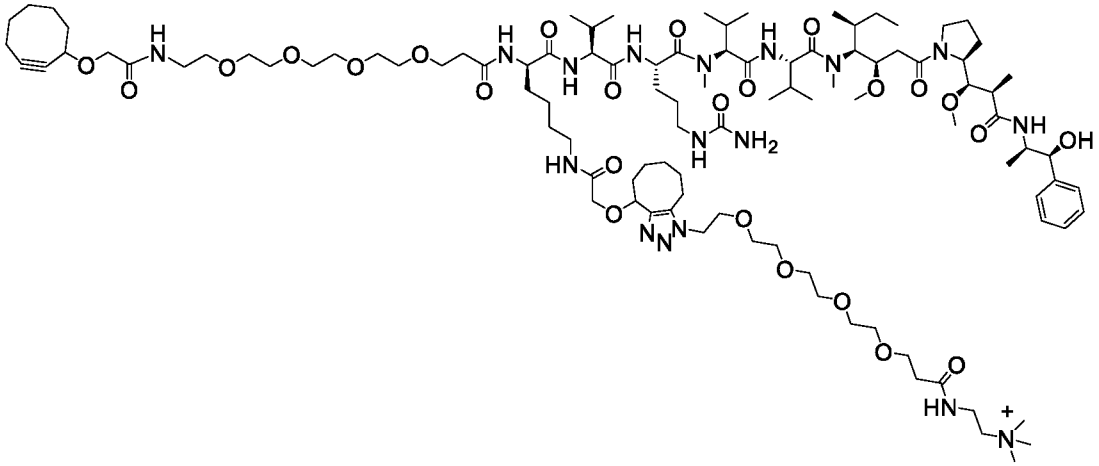


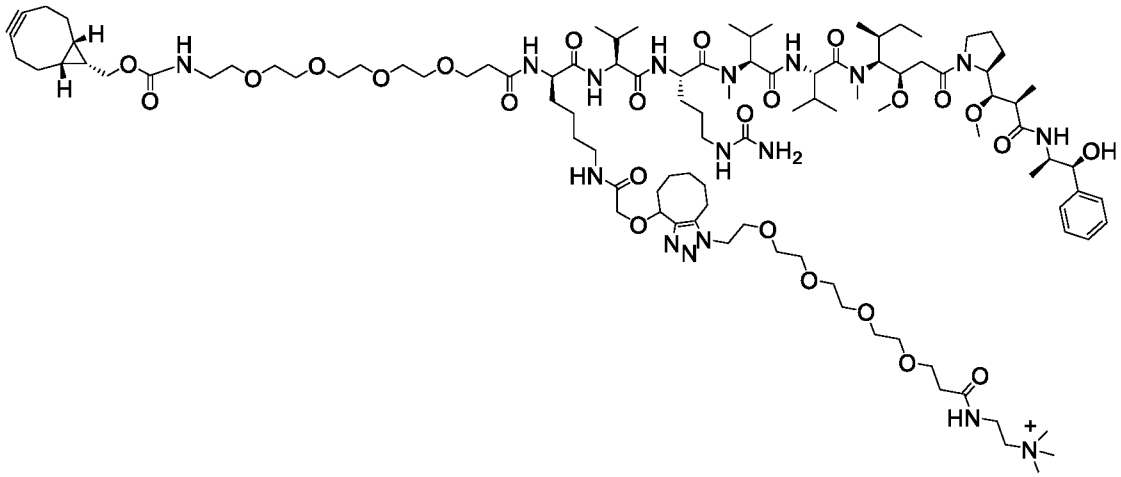
,

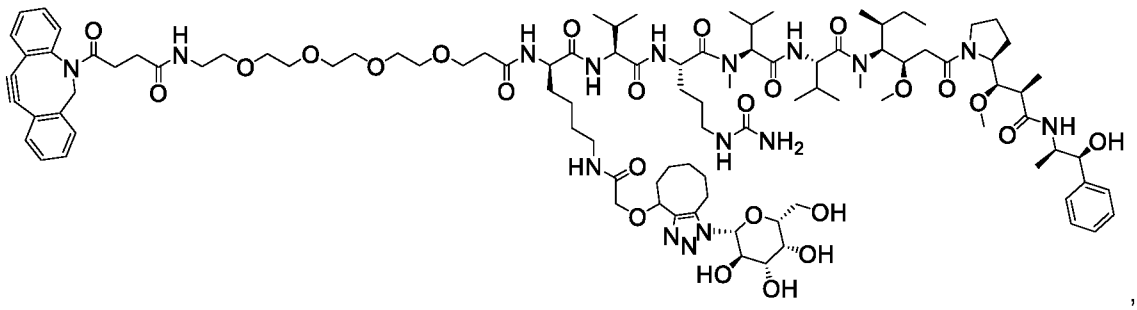


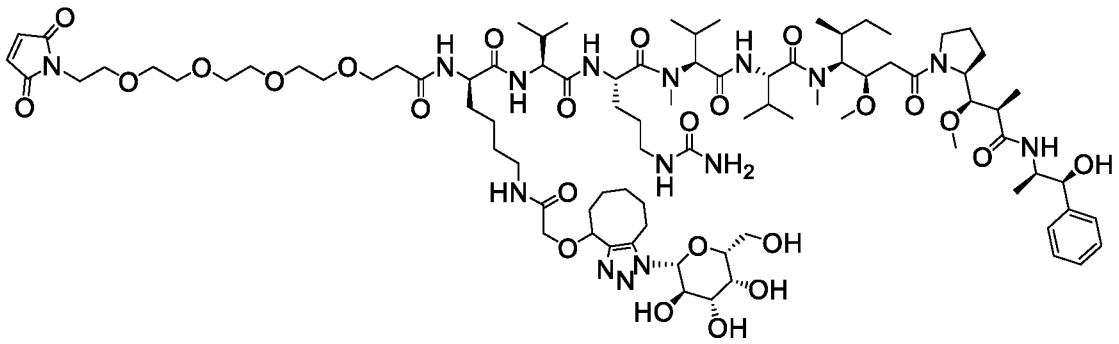
,

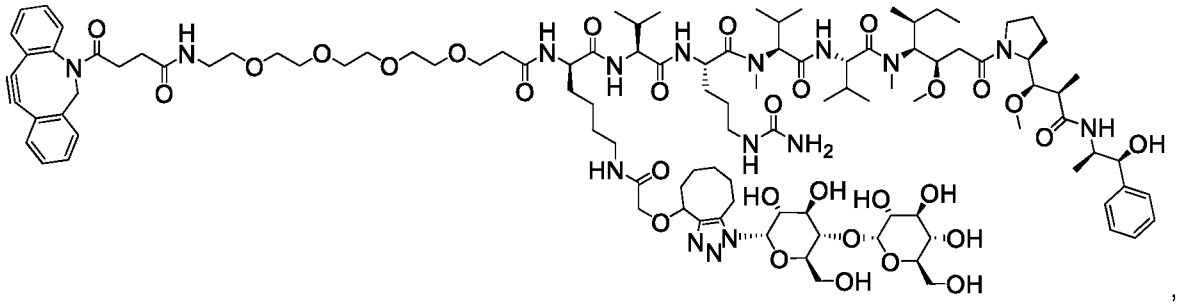


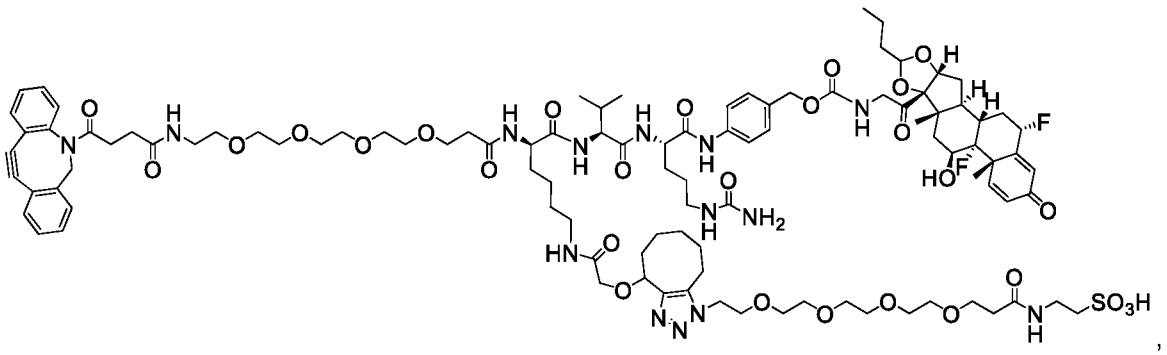


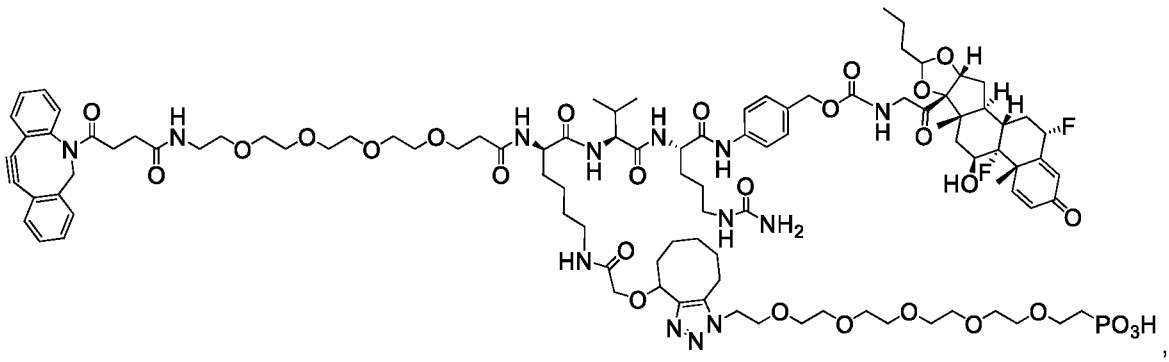


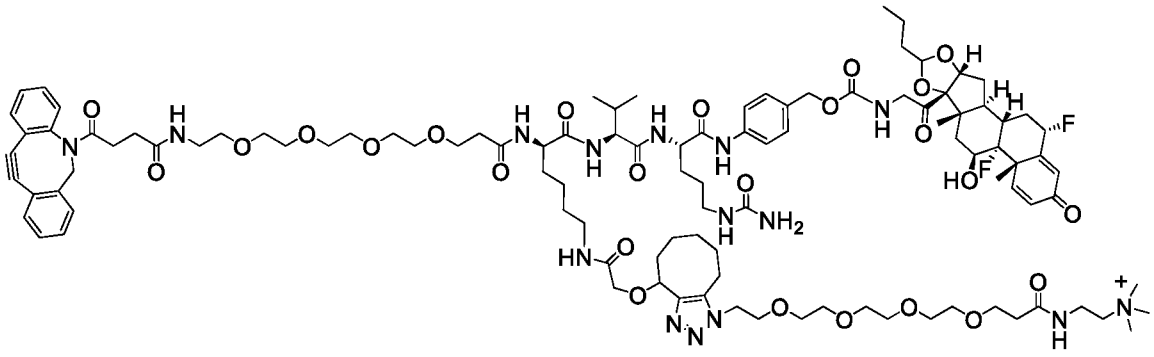


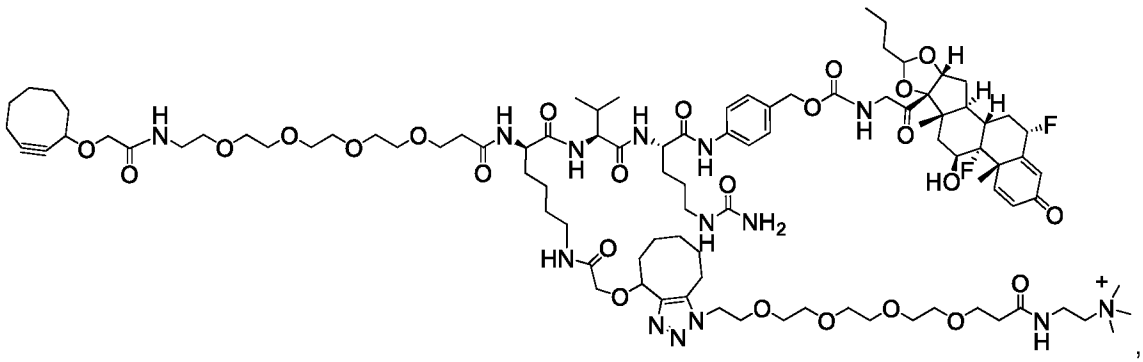




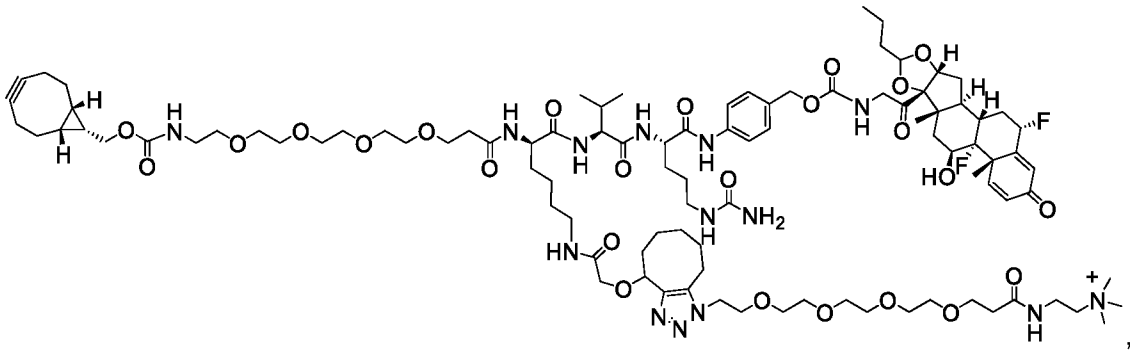




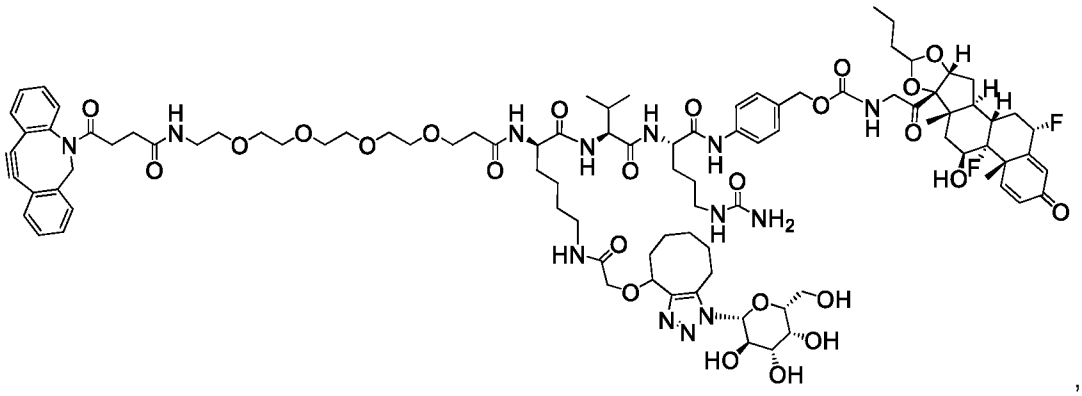




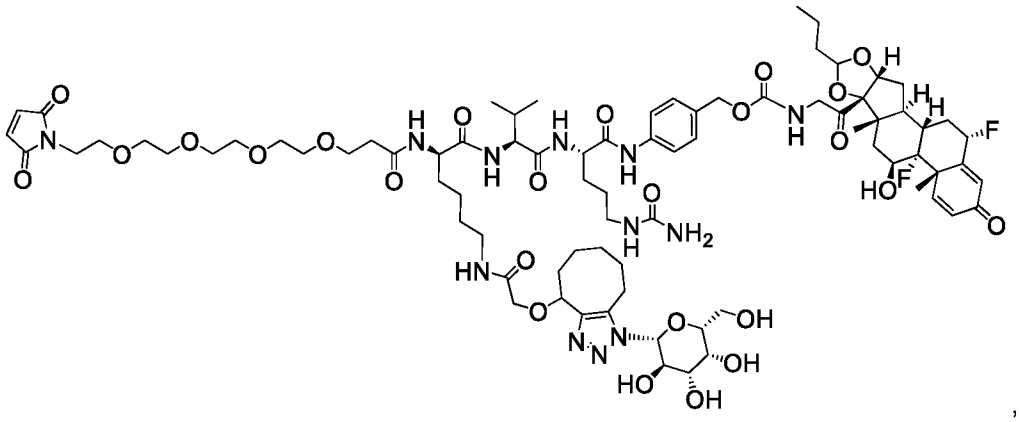
,



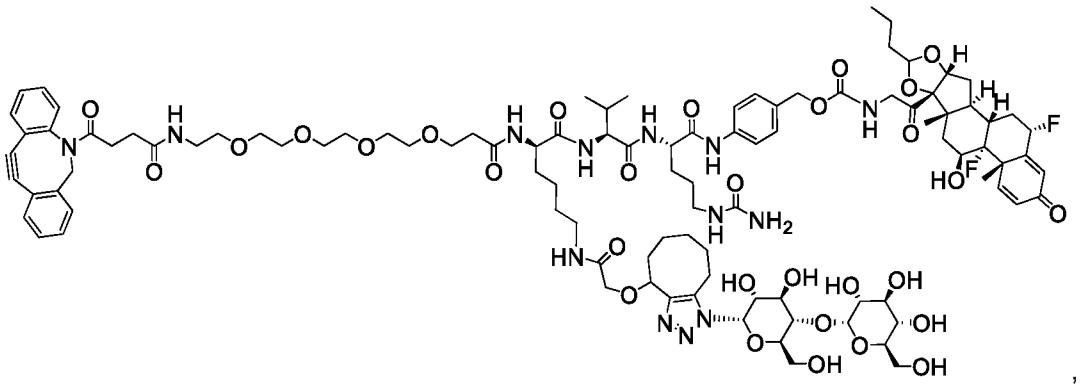
,



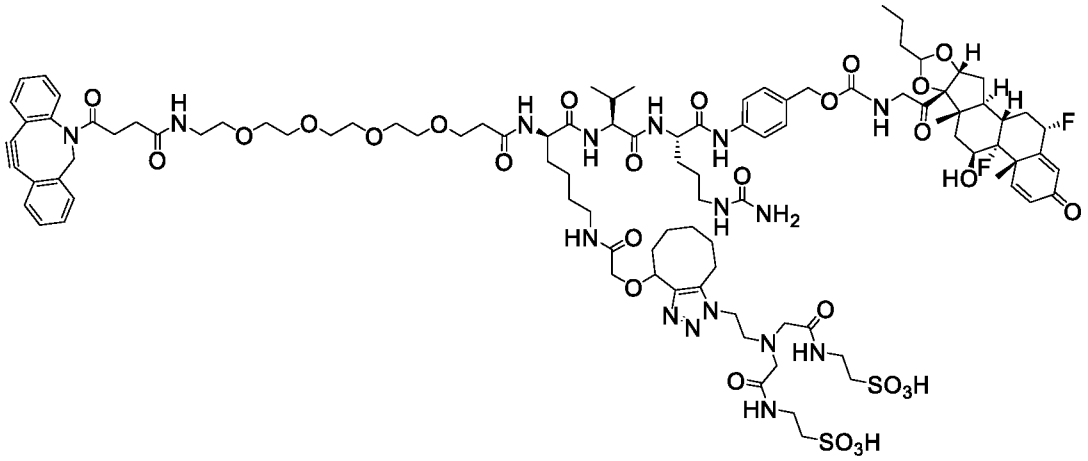
,



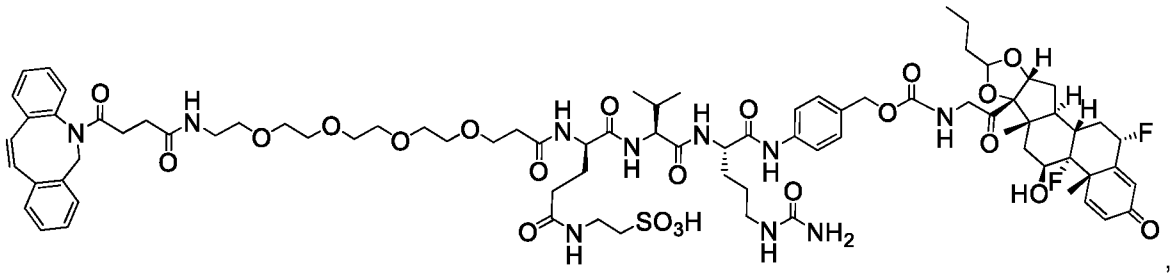
,



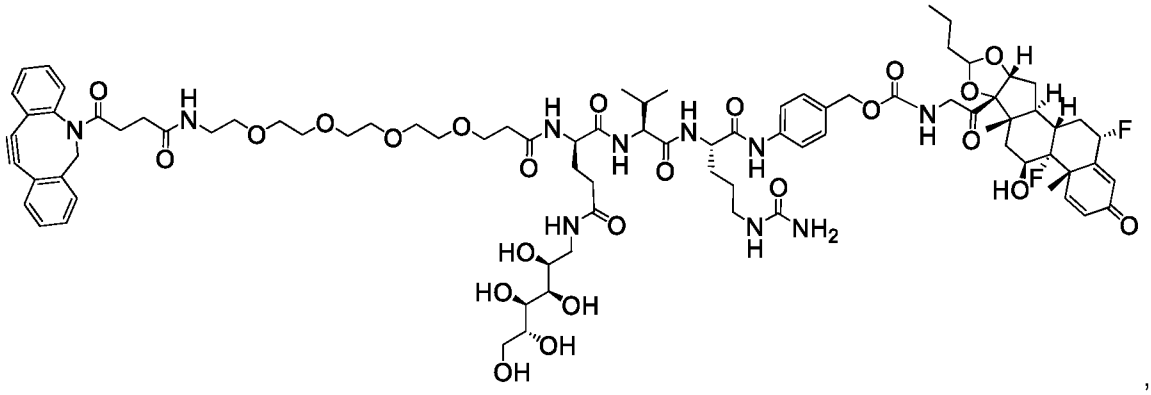
,



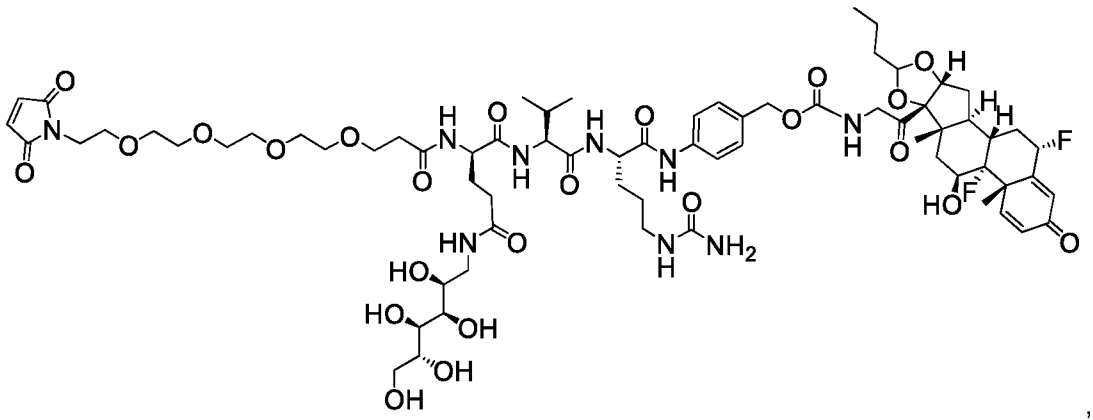
,



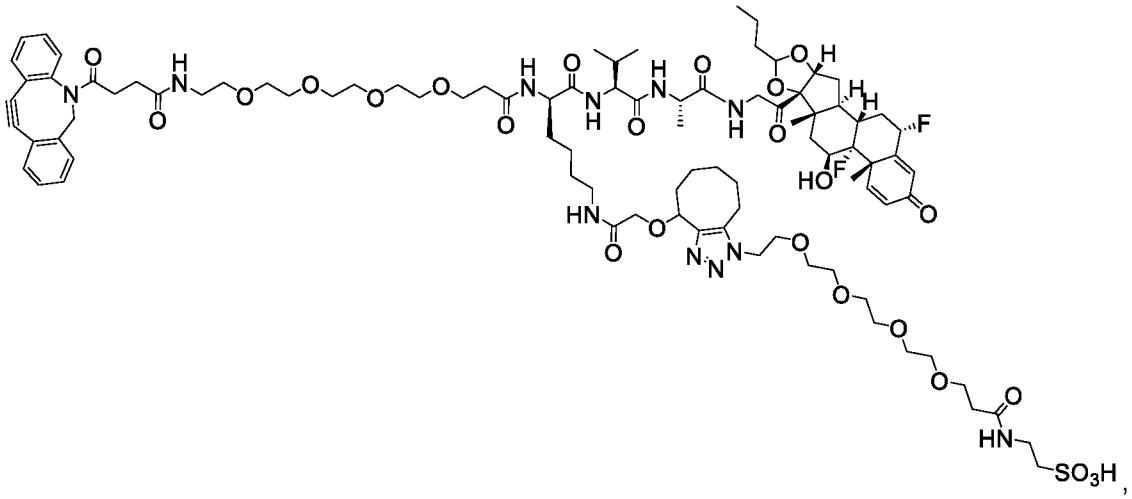
,



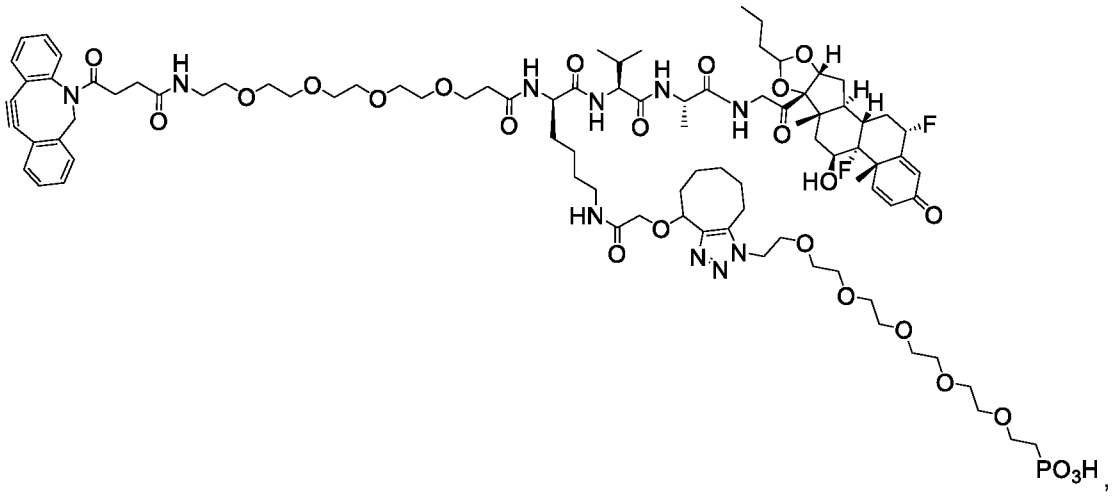
,



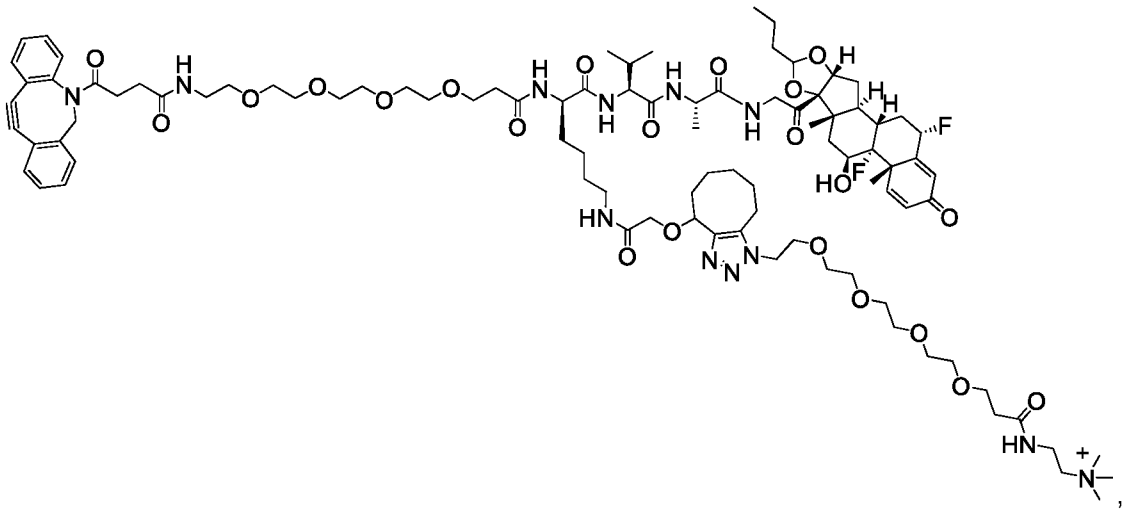
,

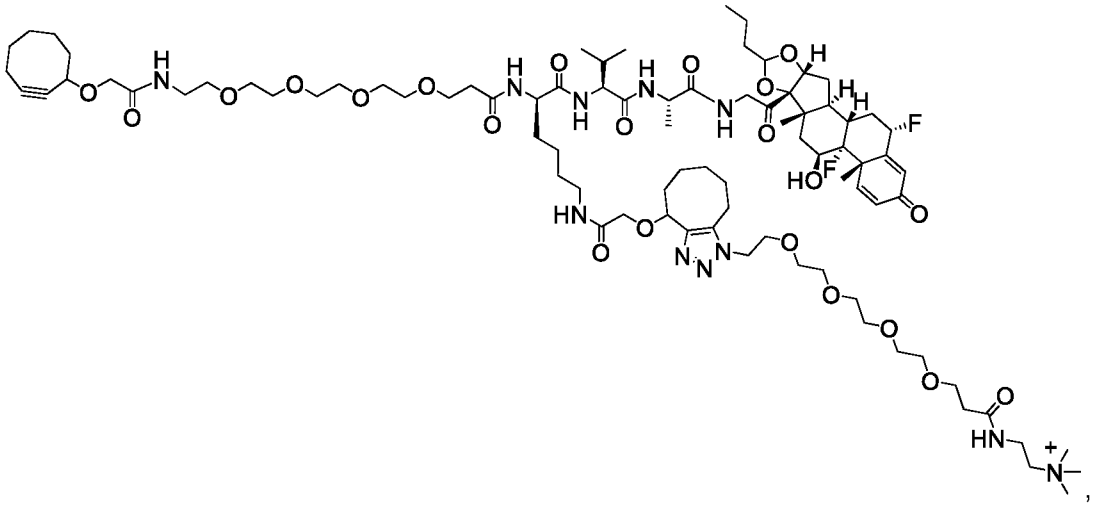


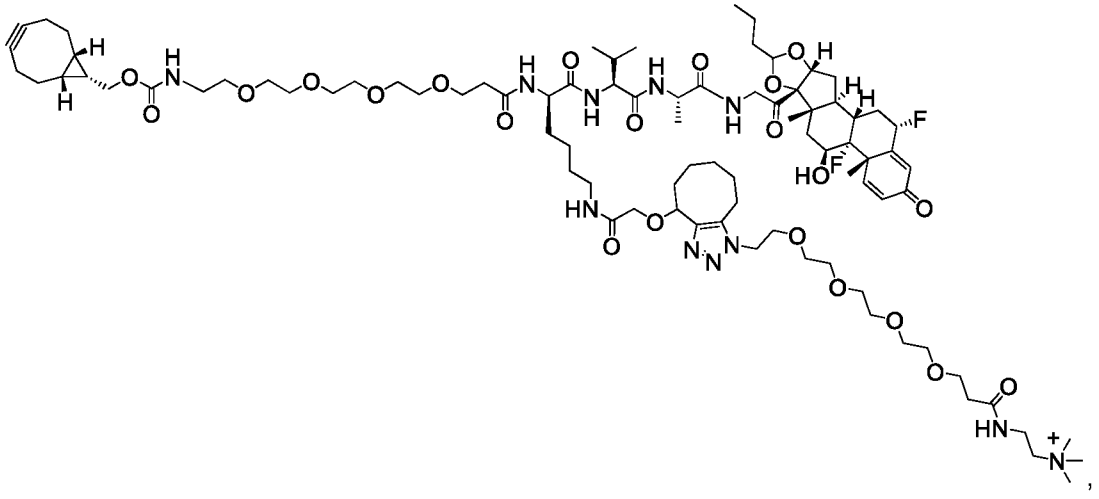
,

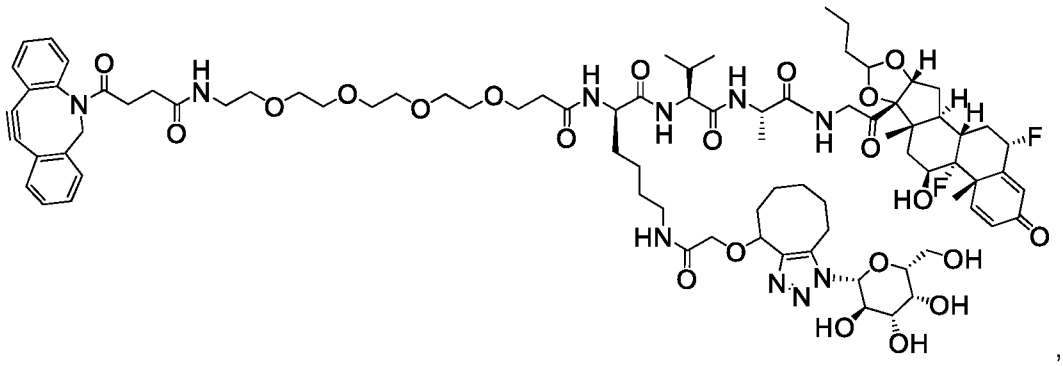


,

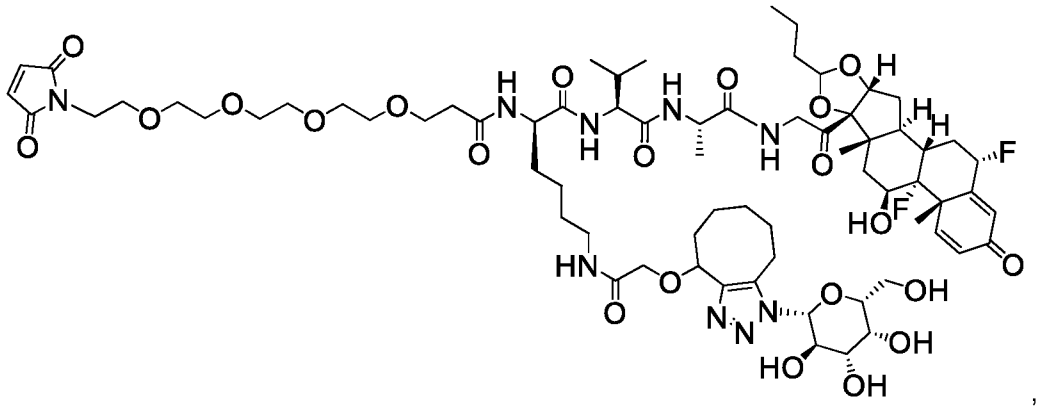




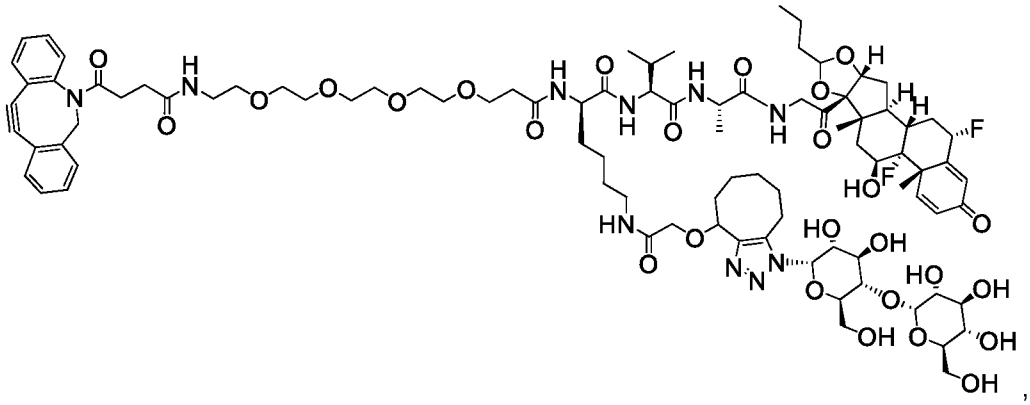




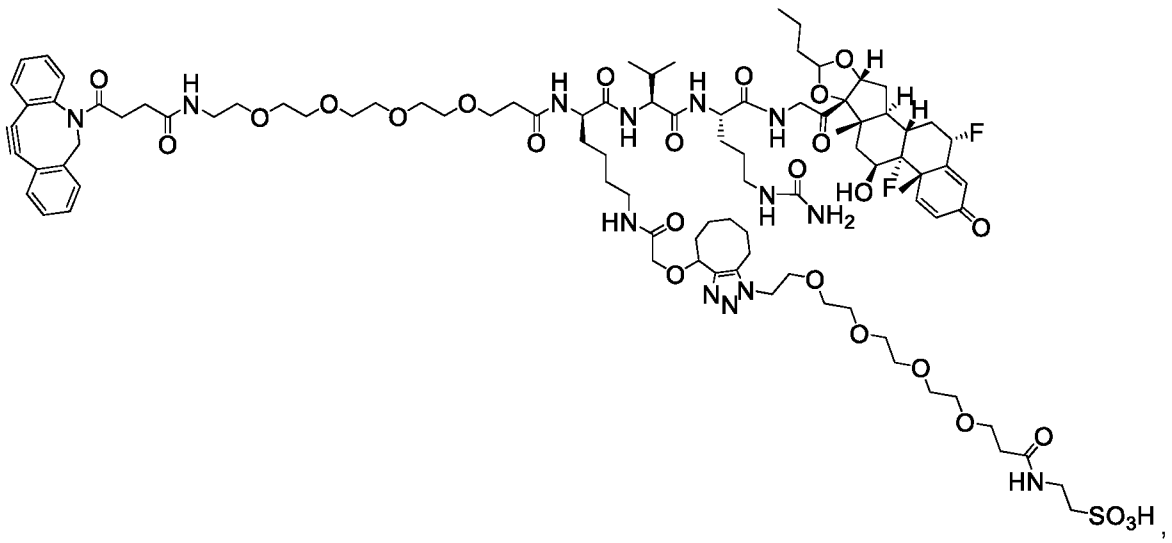
,



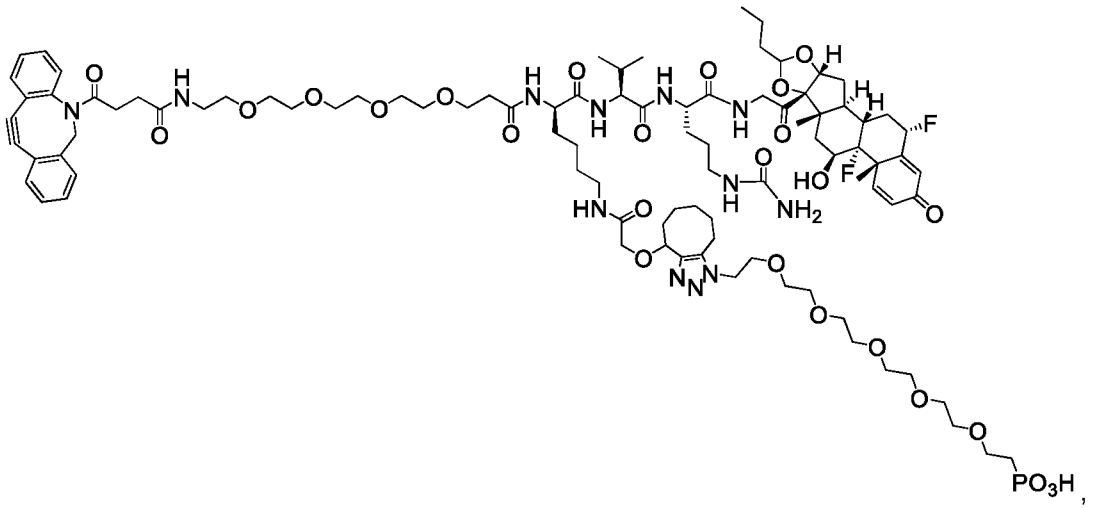
,

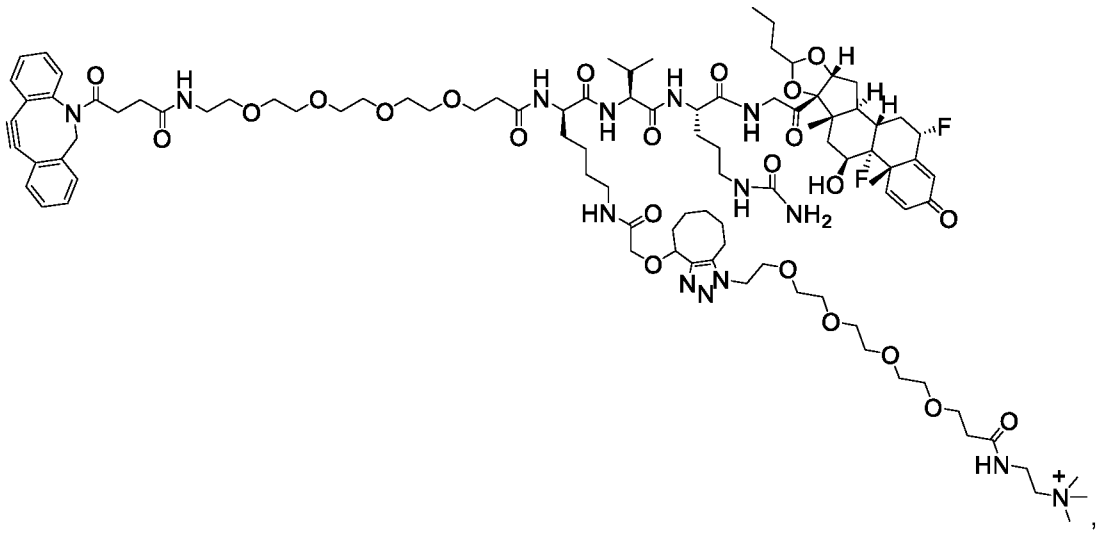


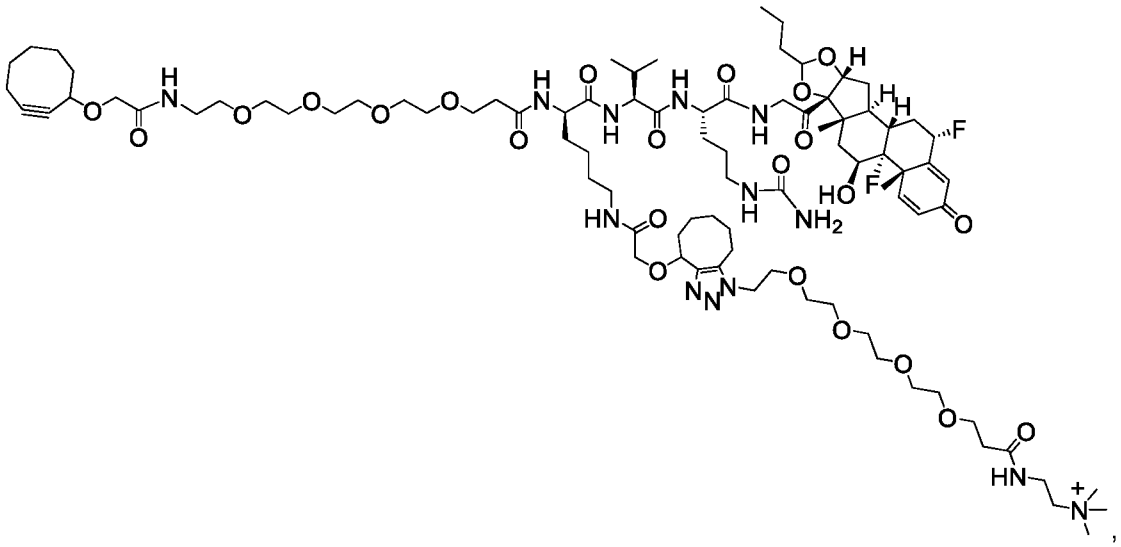
,

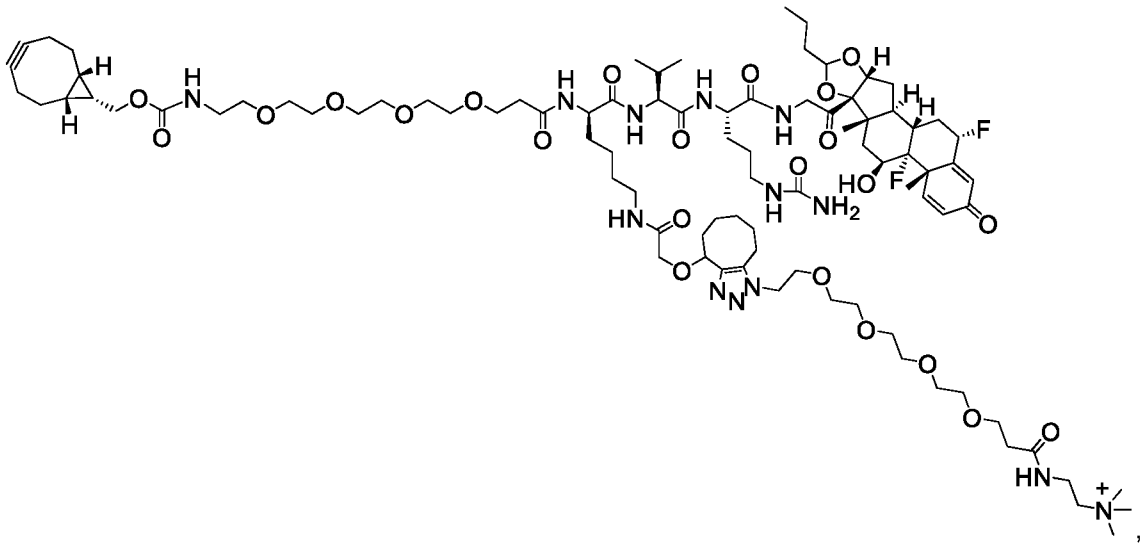


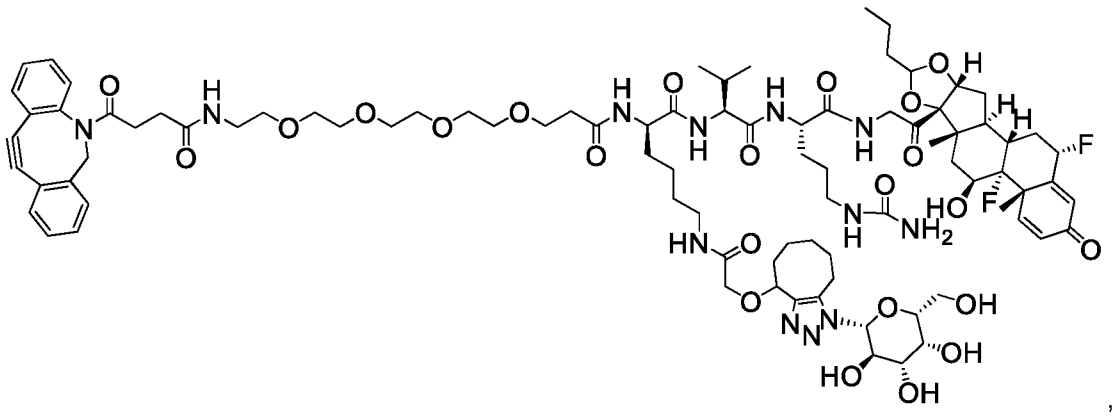
,

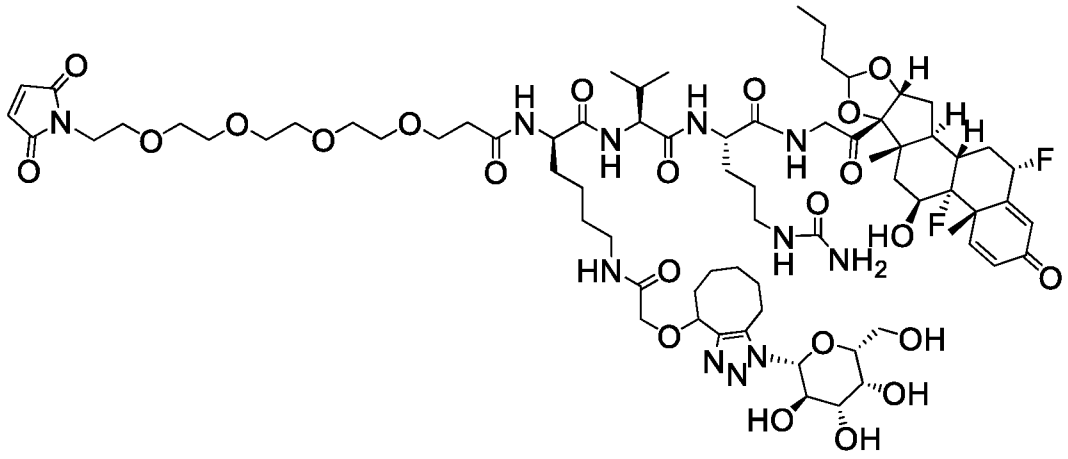


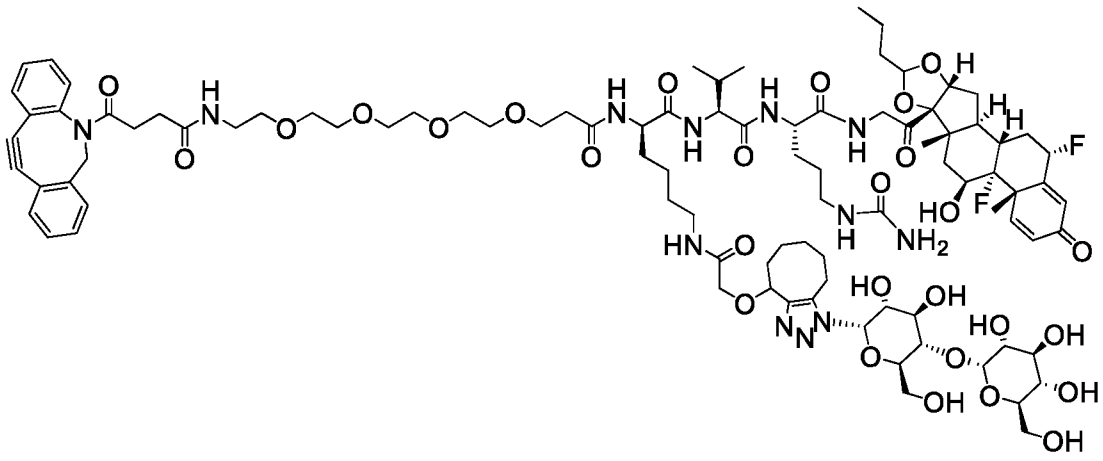


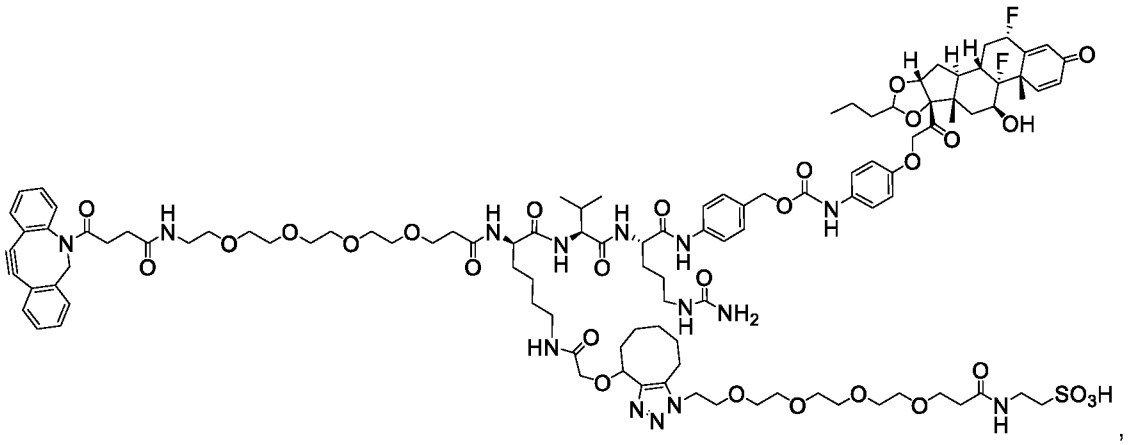


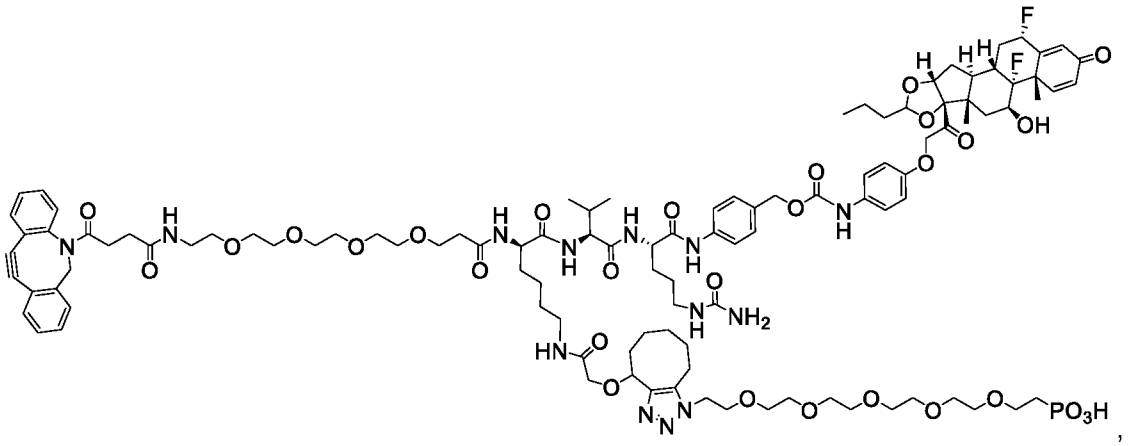


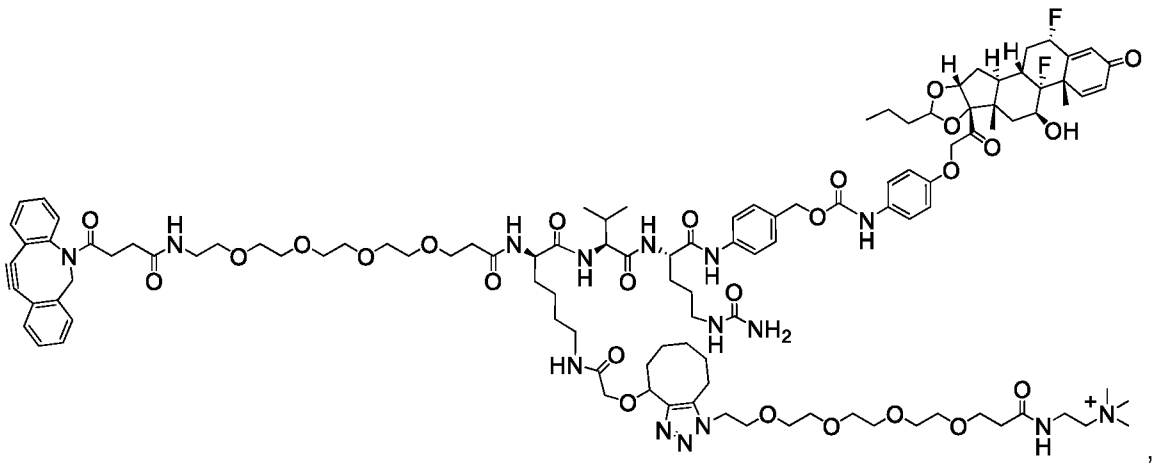


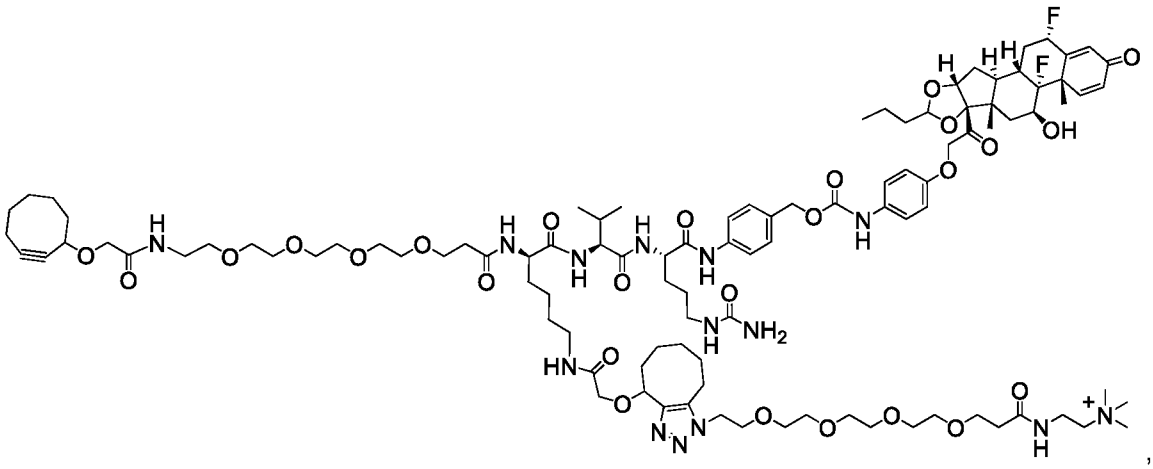




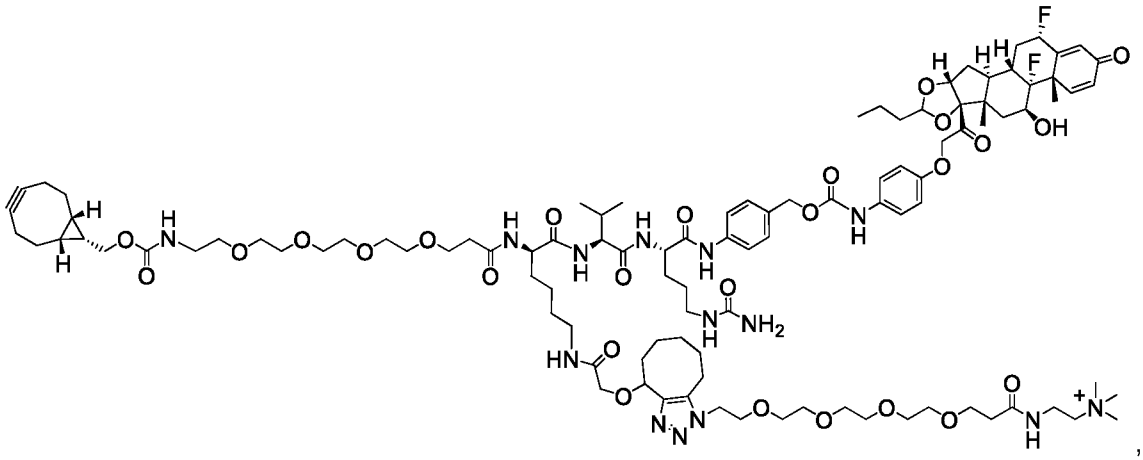




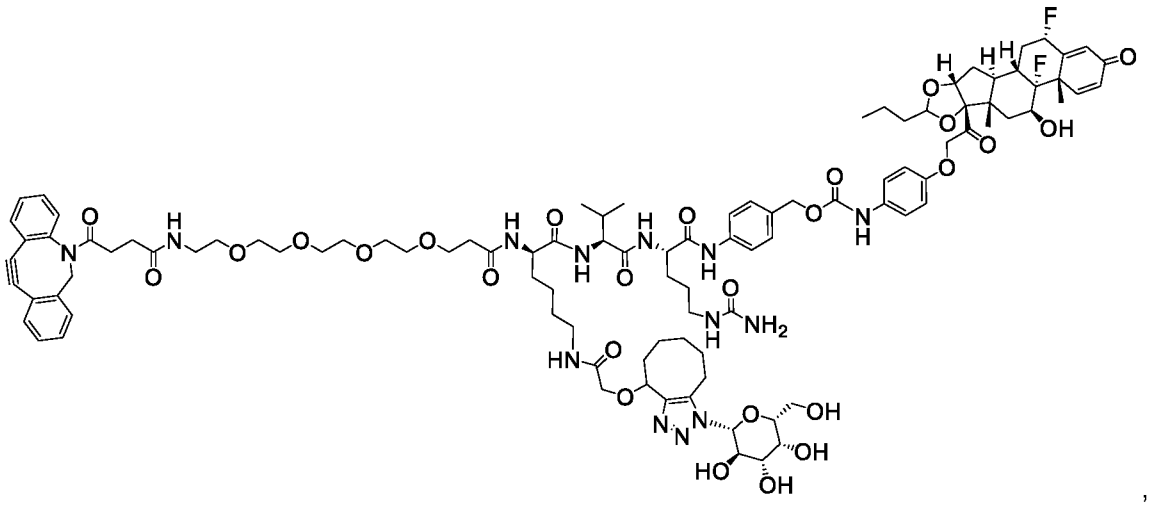




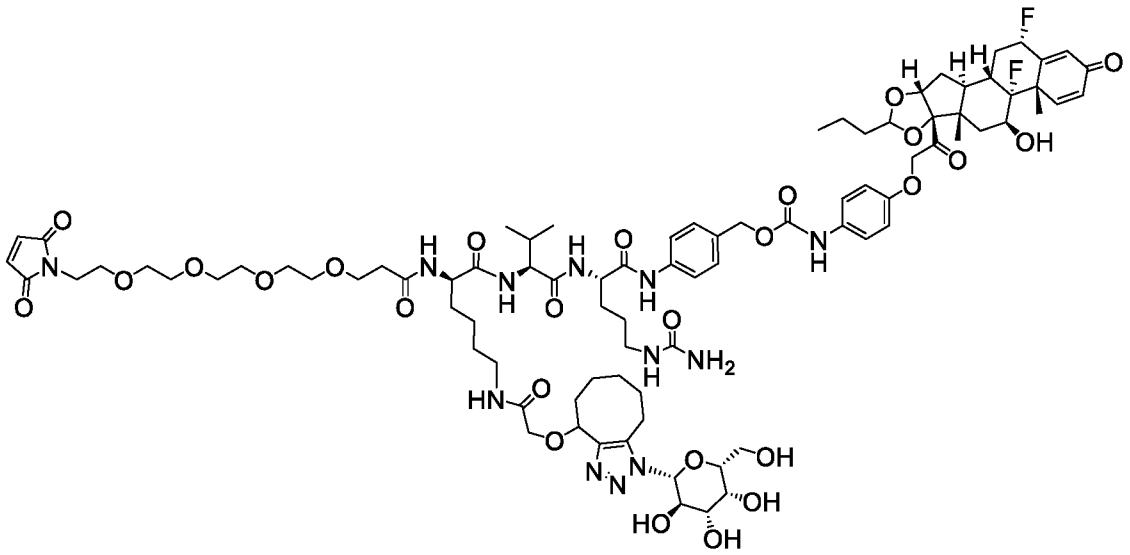
,



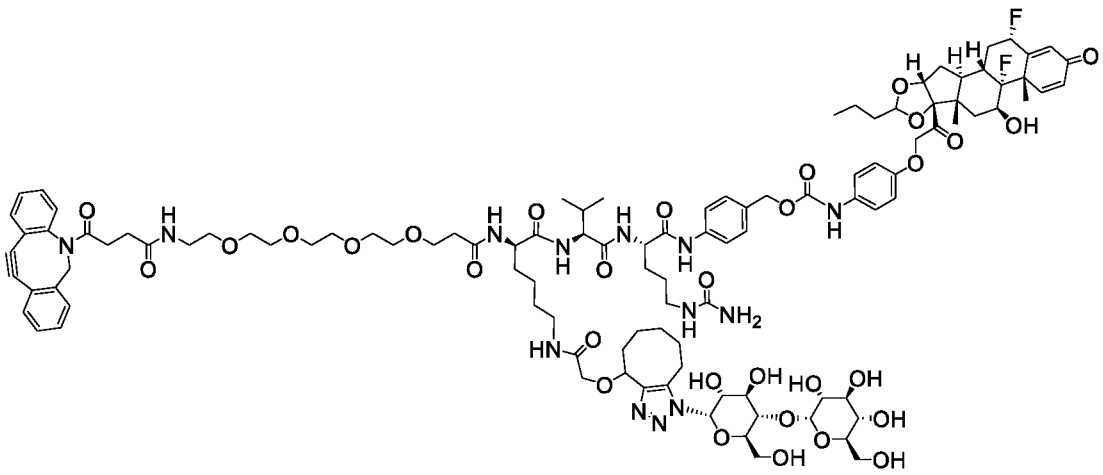
,



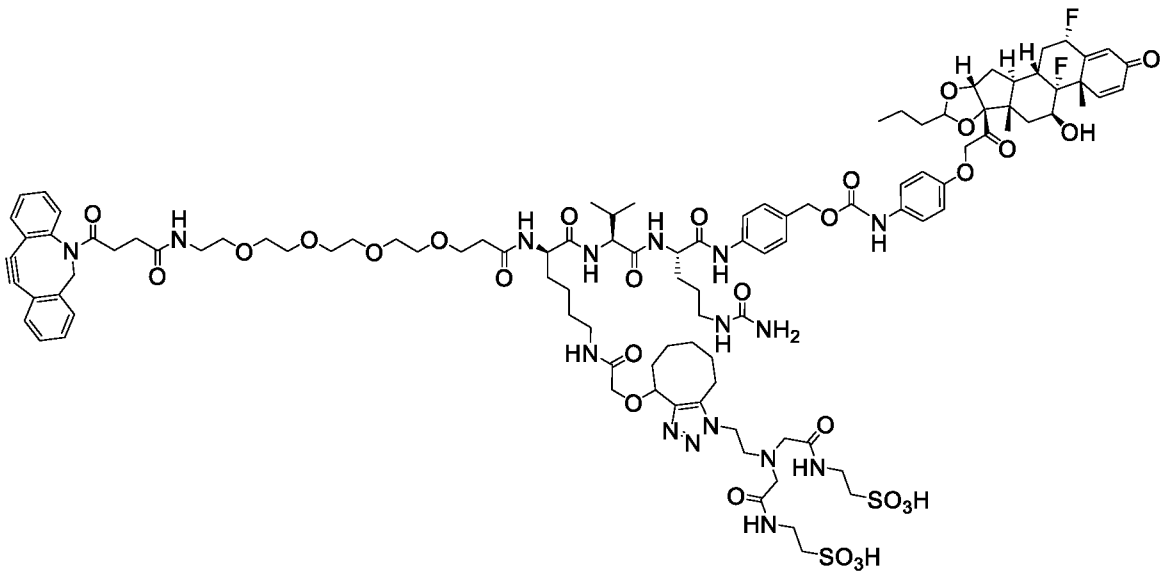
,



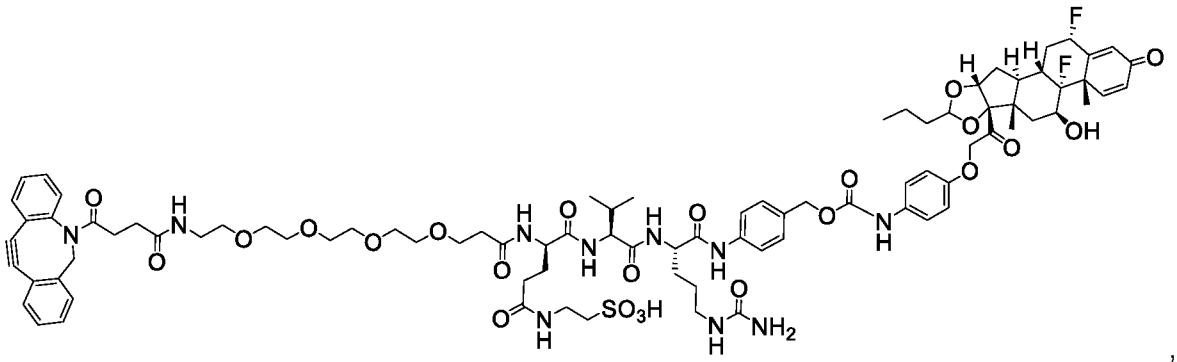
,



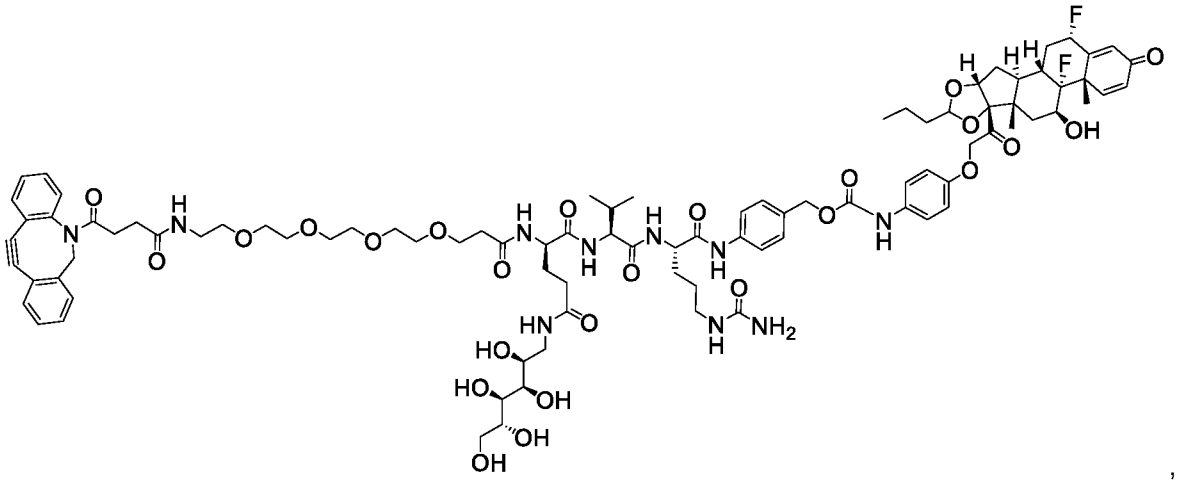
,



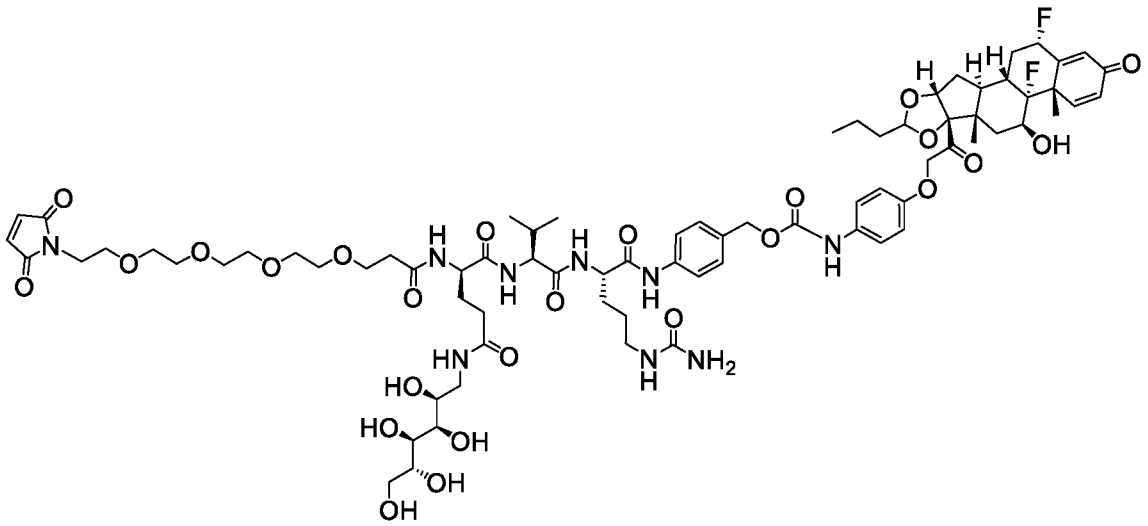
,



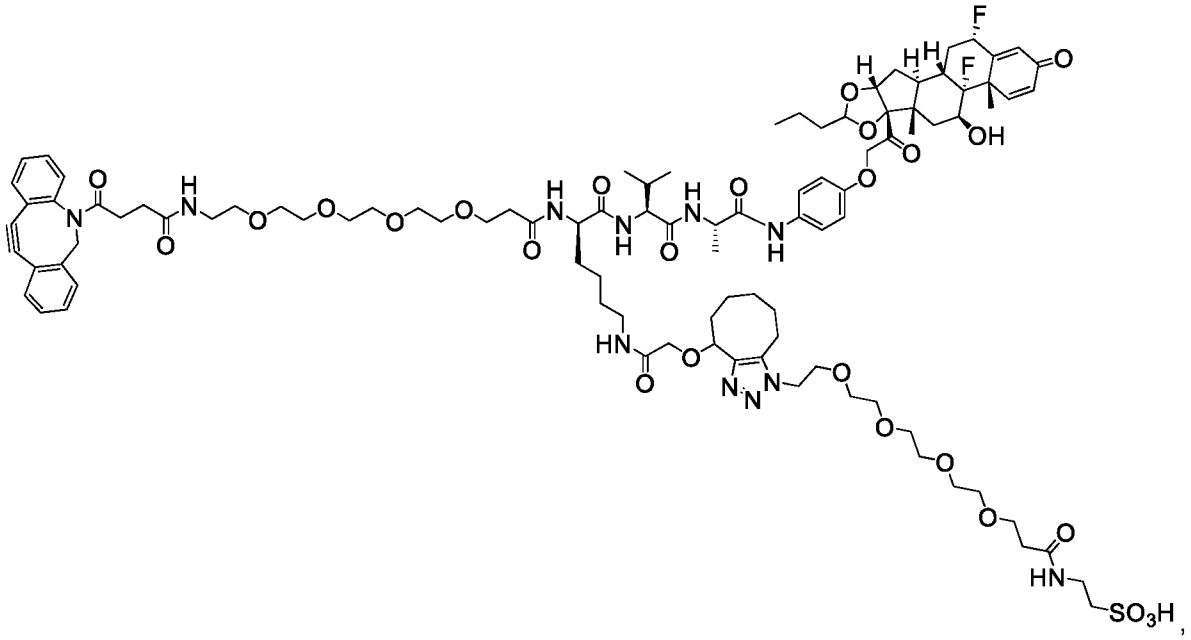
,

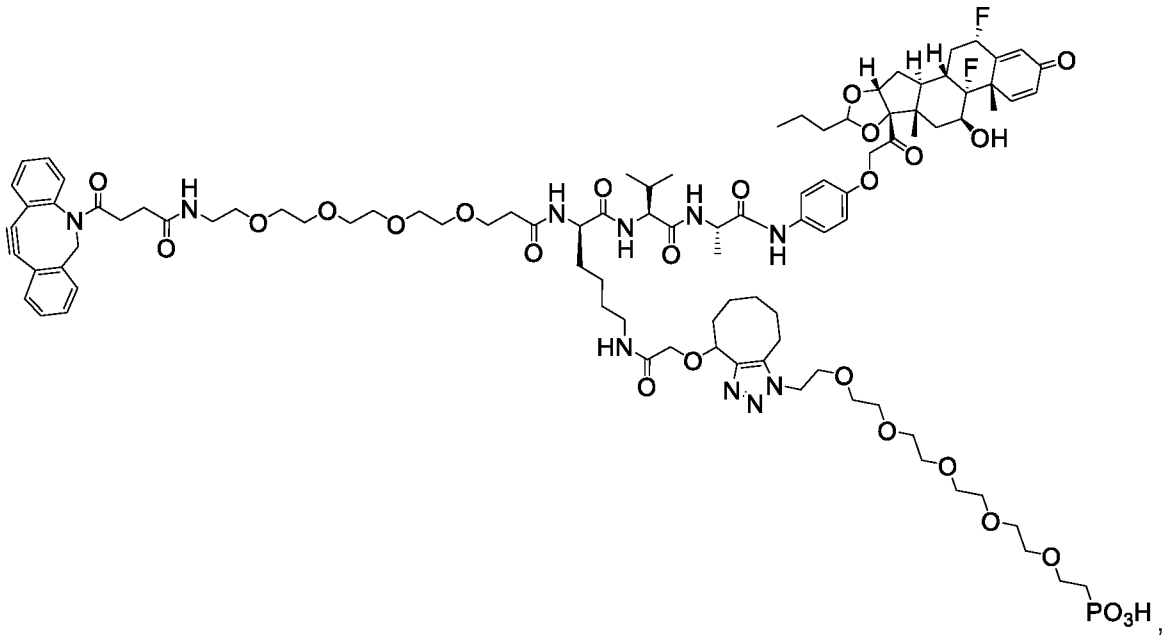


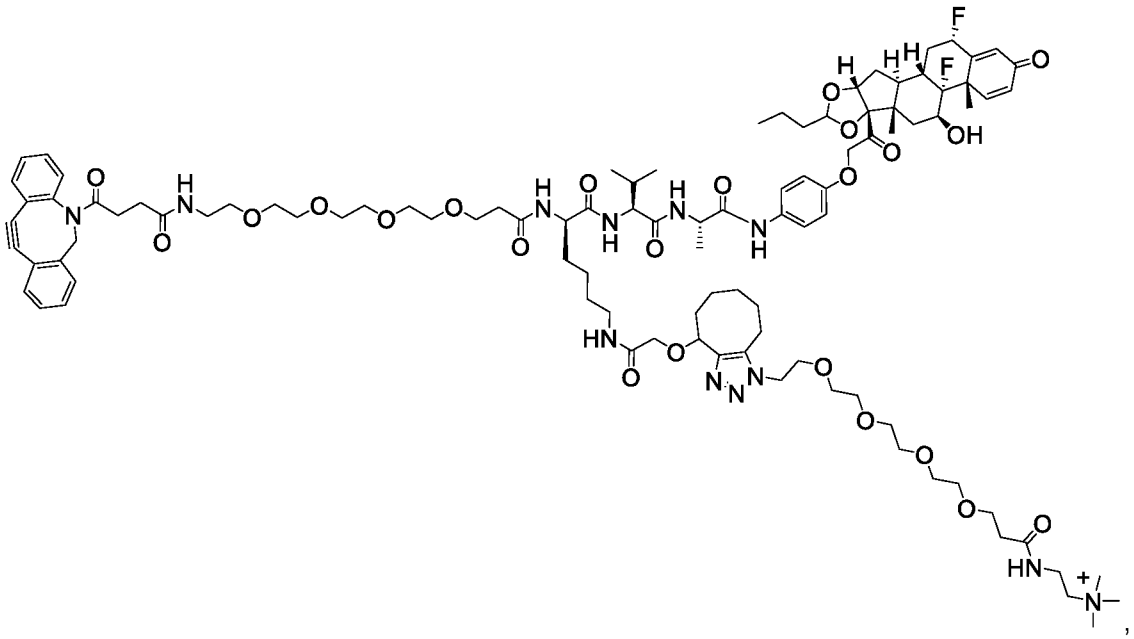
,

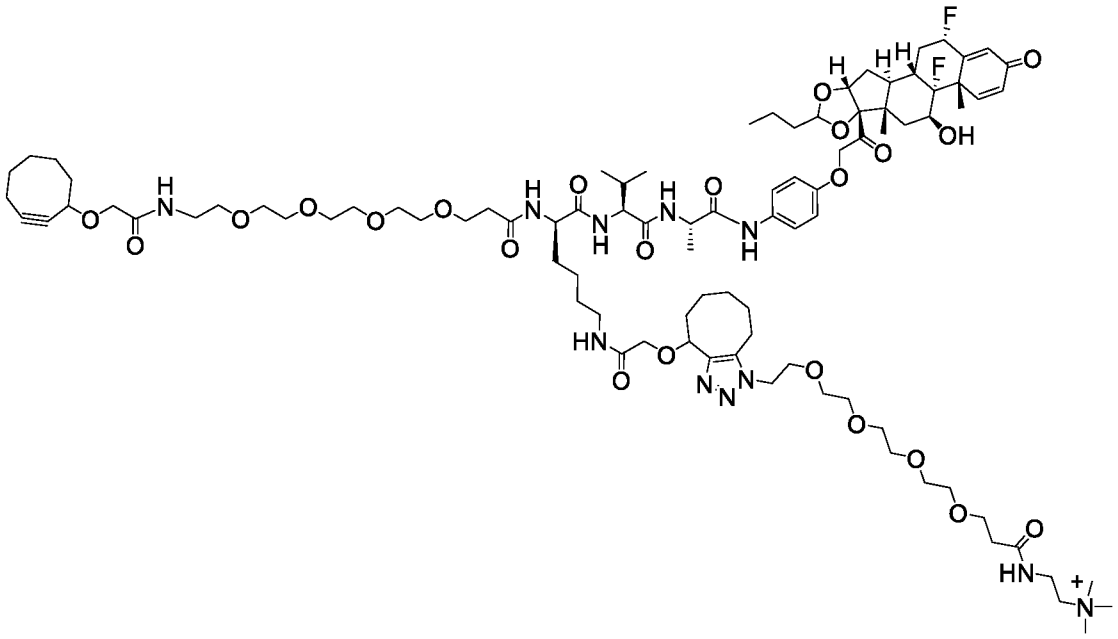


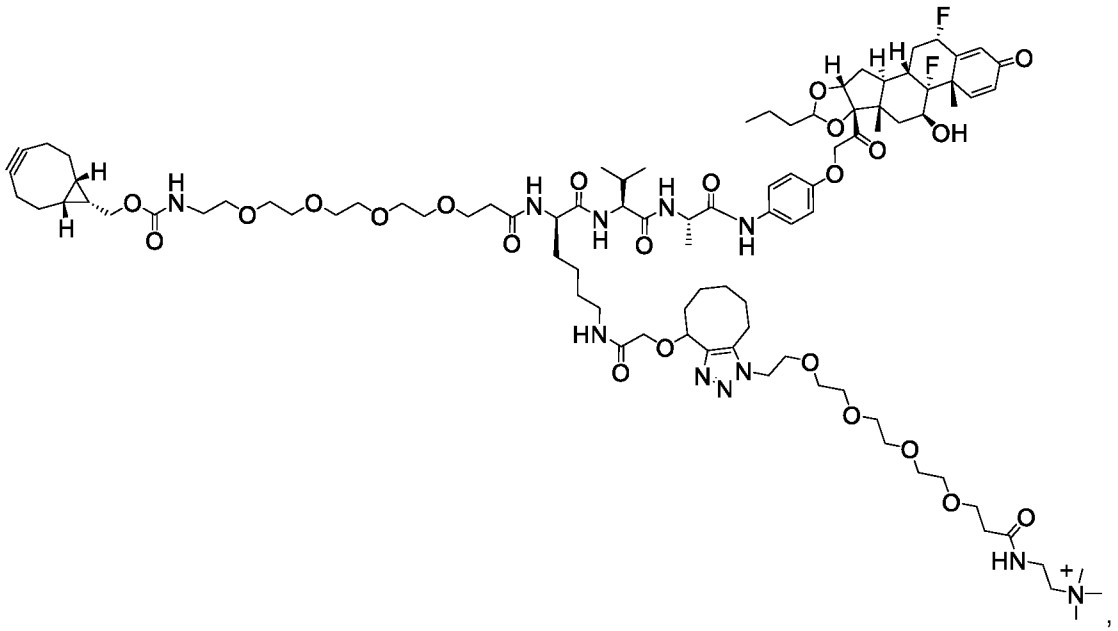
,



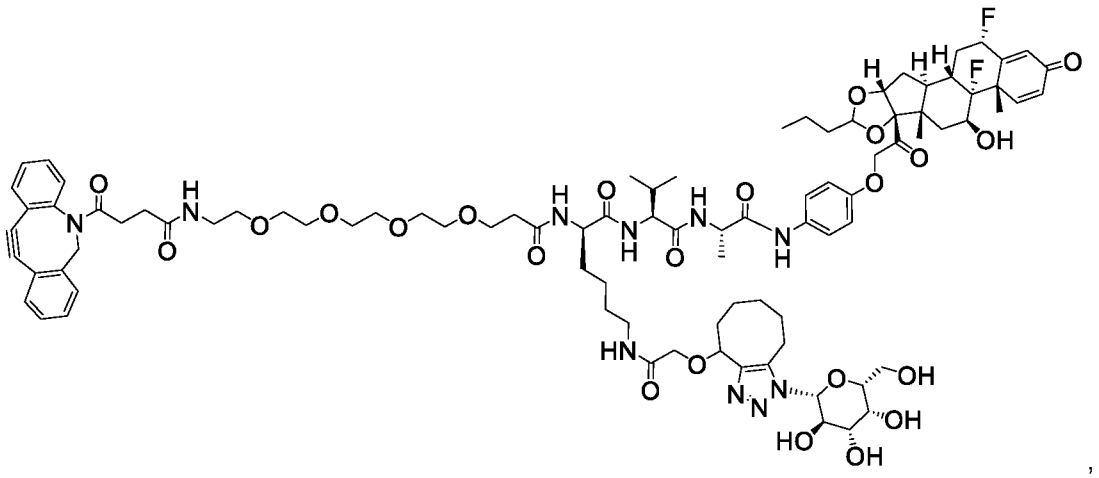




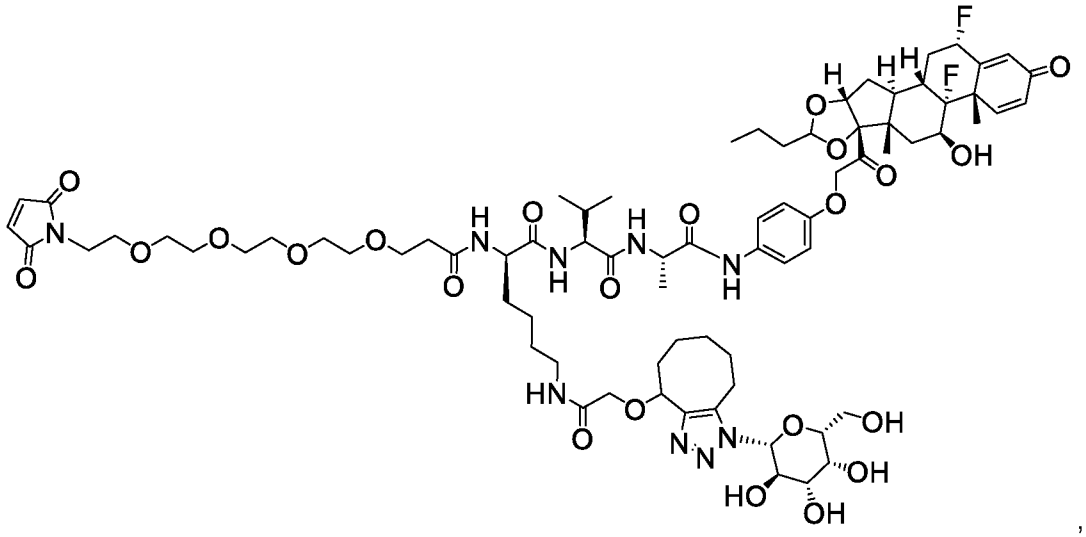




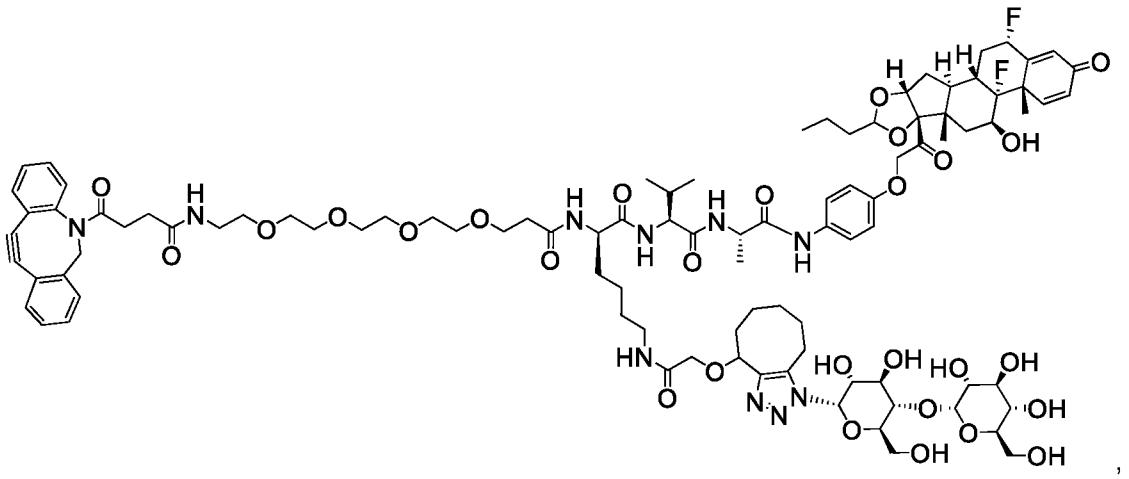
,



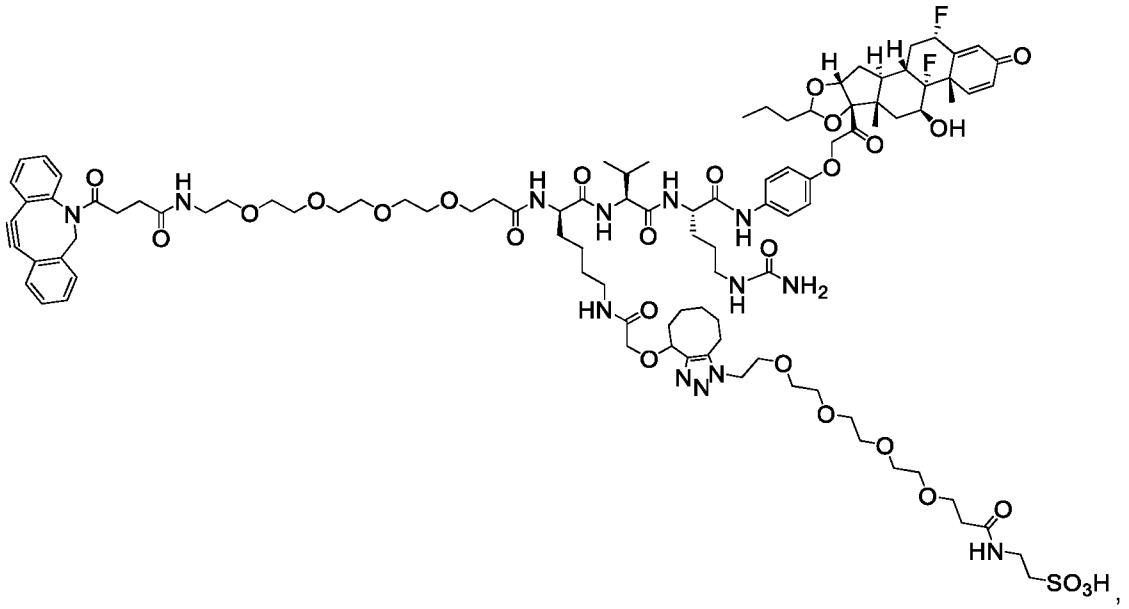
,



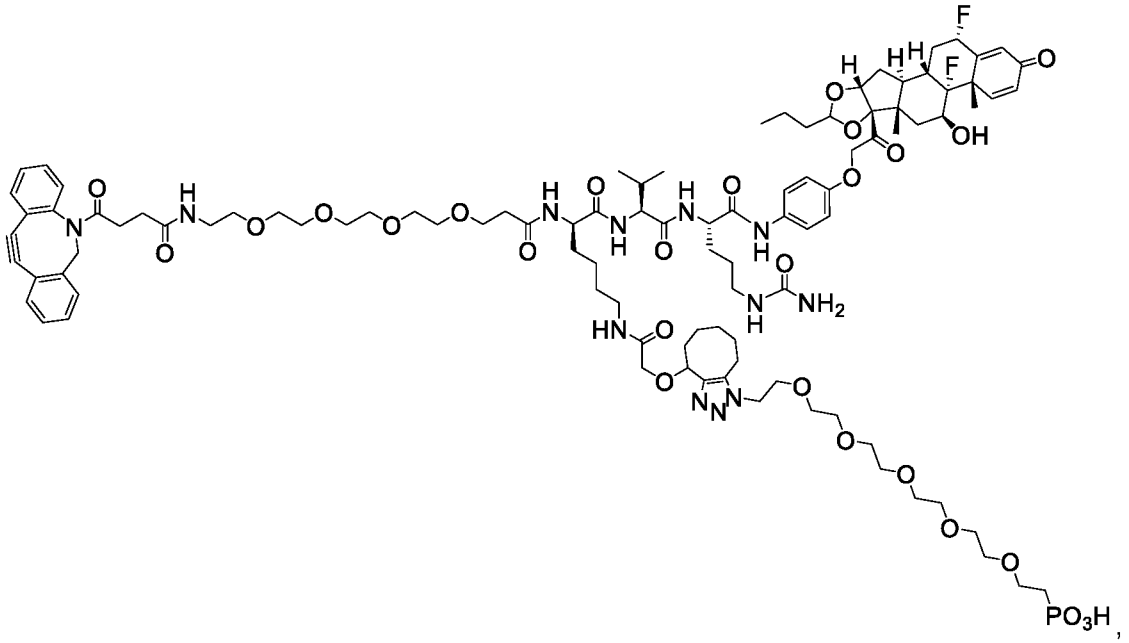
,



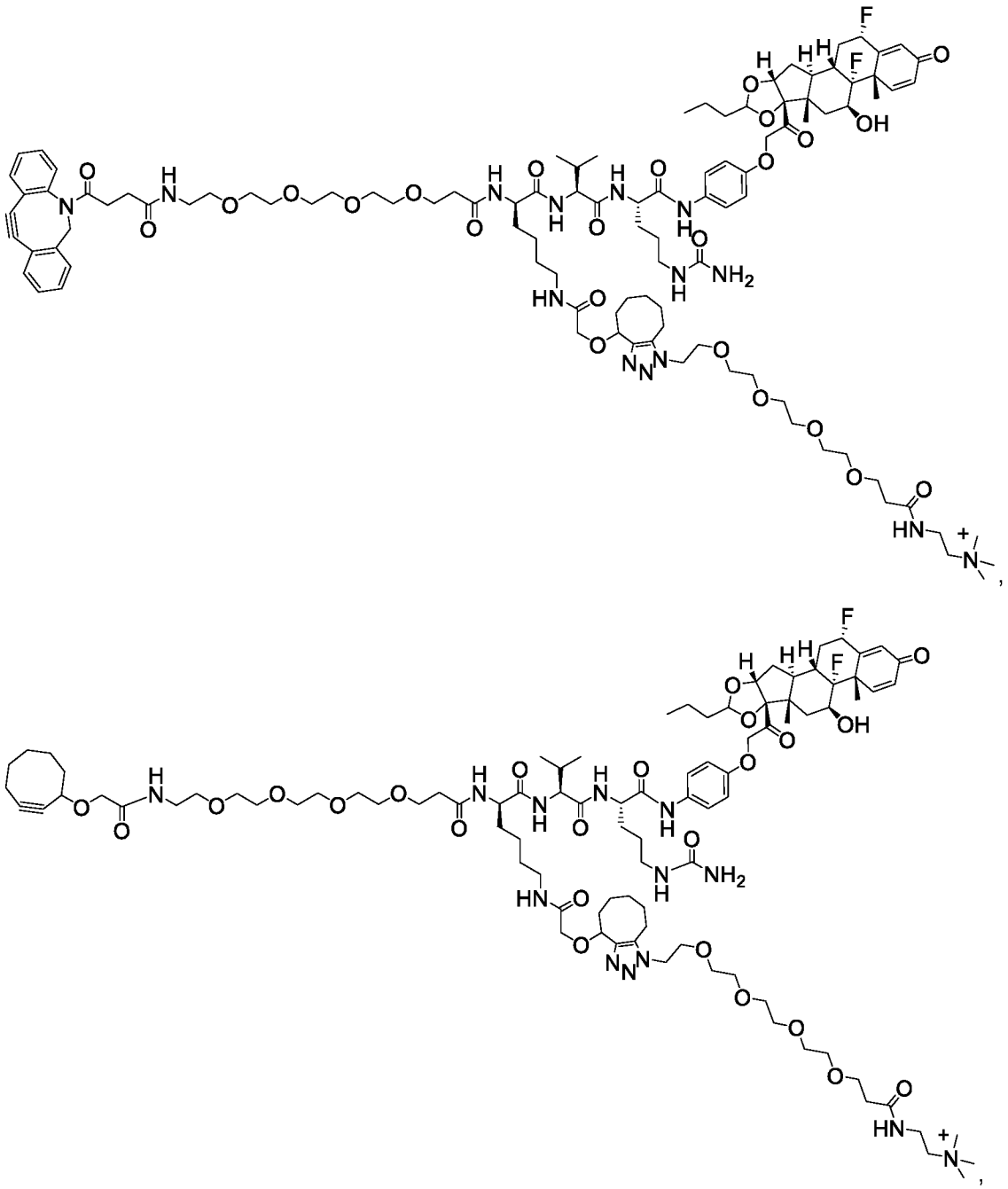
,

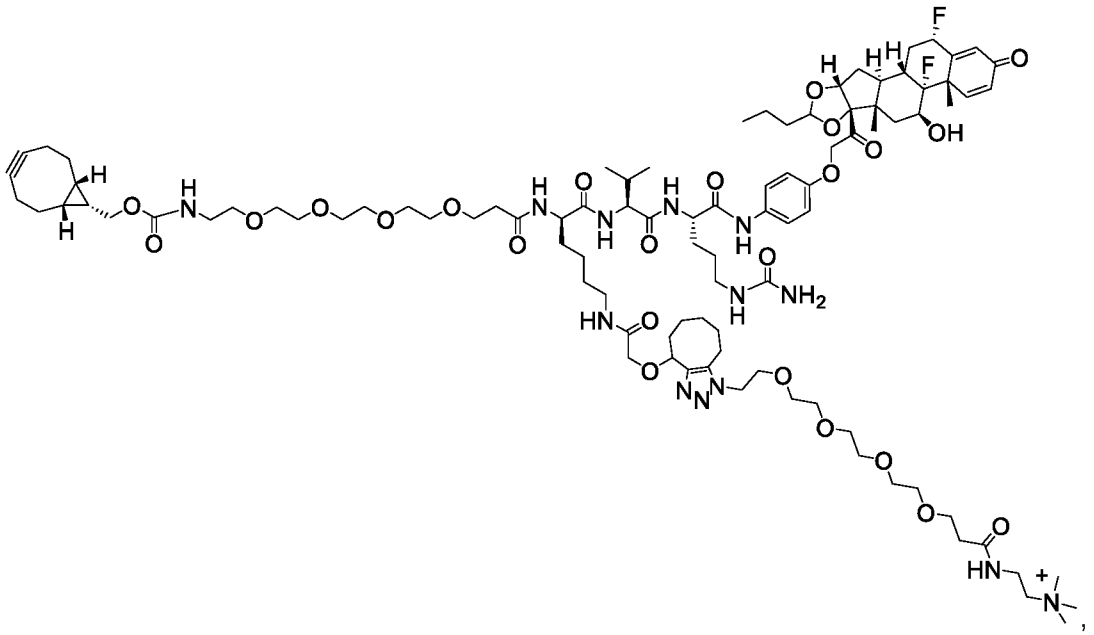


,

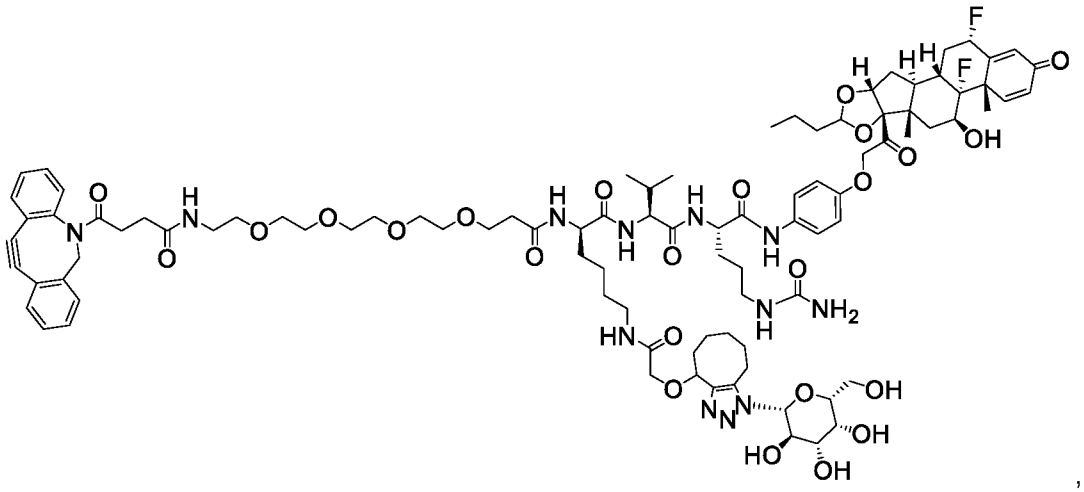


,

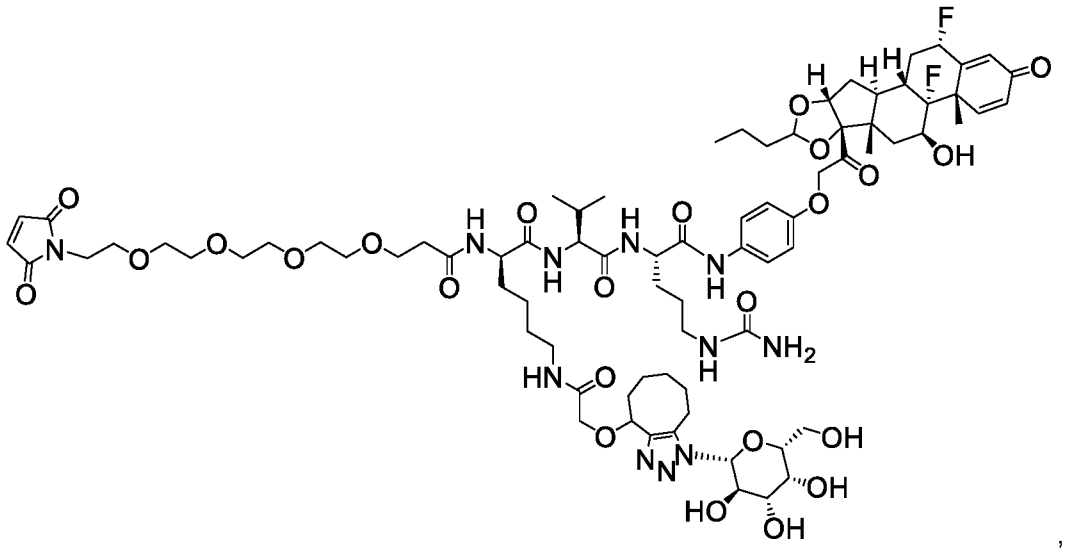




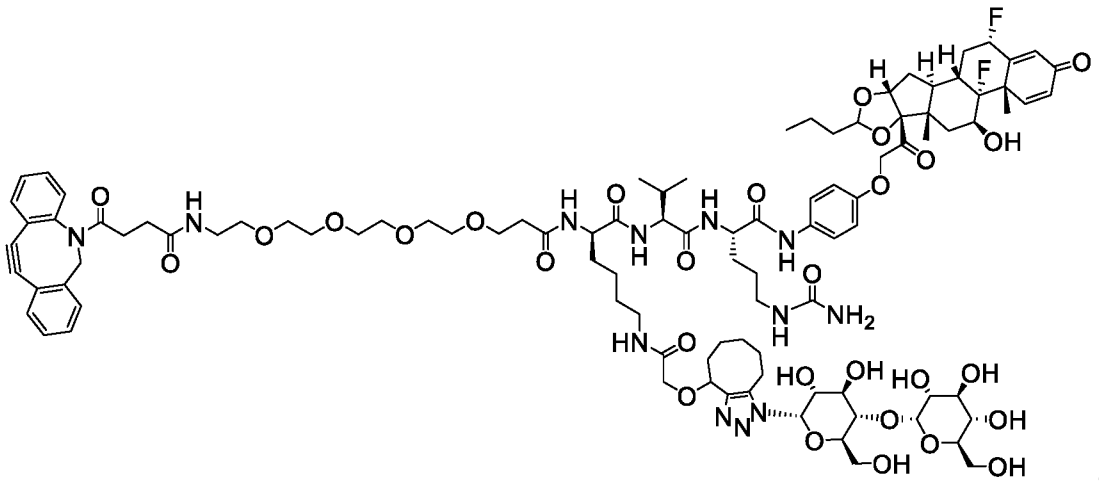
,



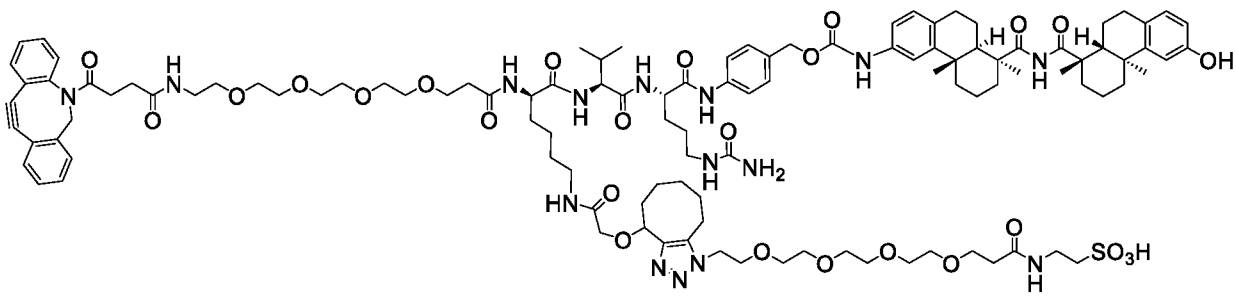
,



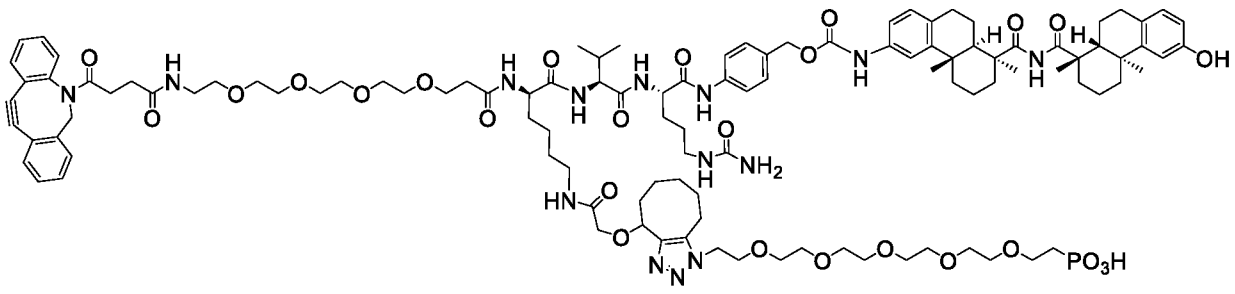
,



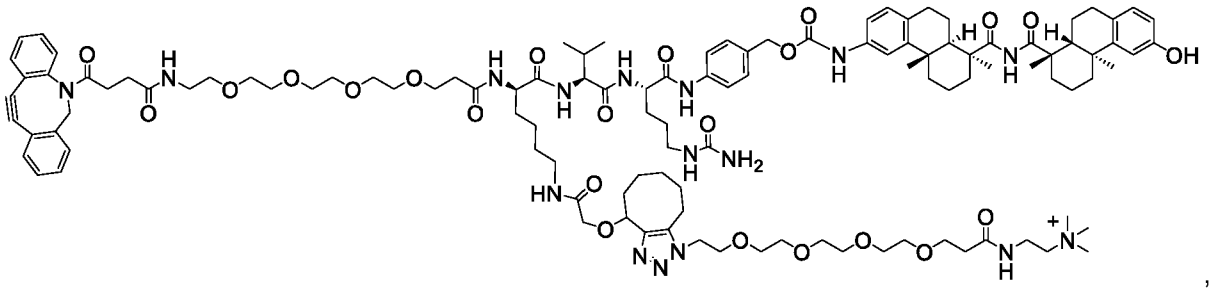
,



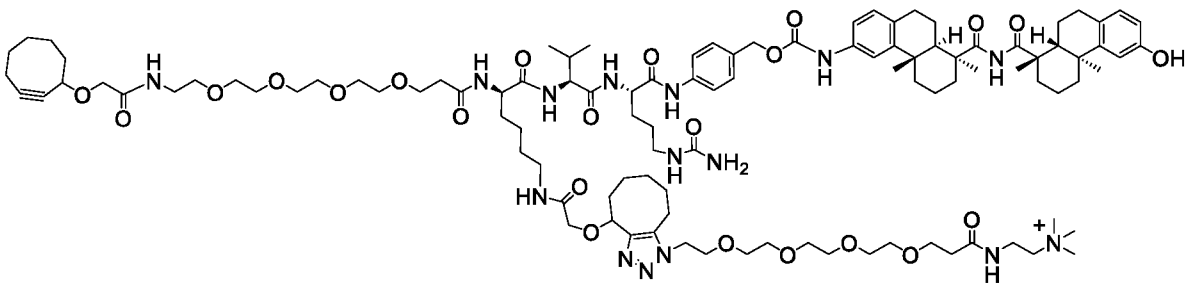
,



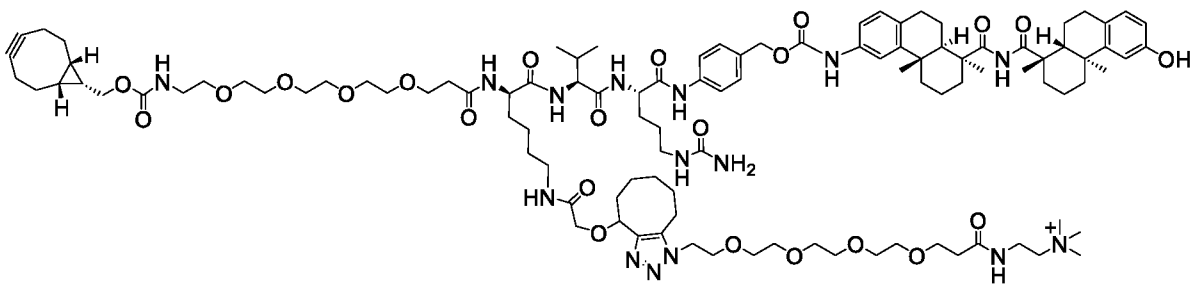
,



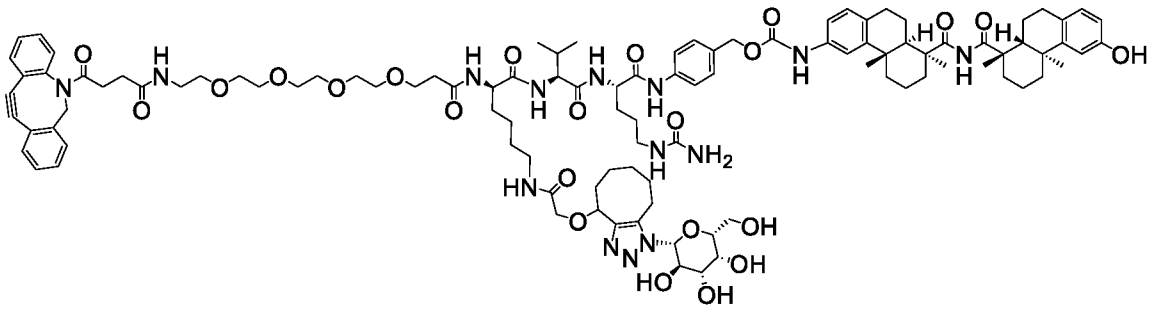
,



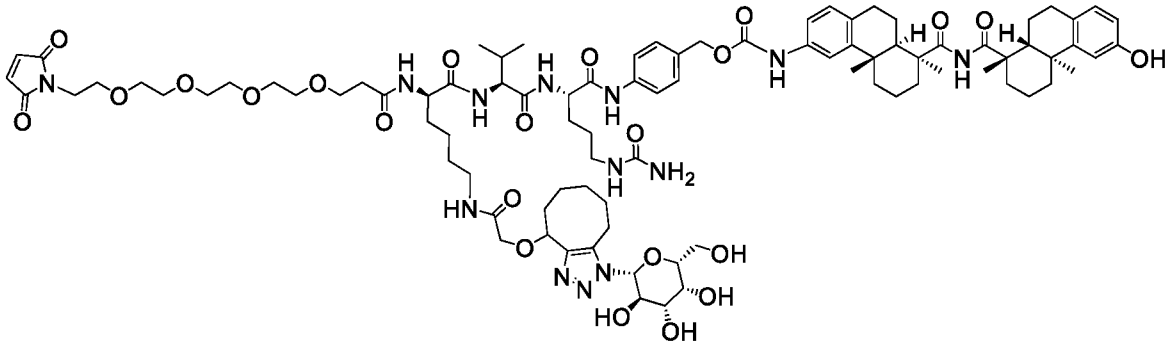
,



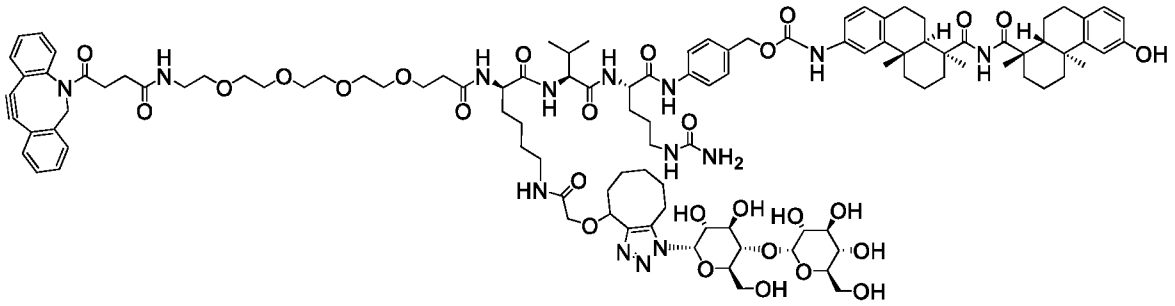
,



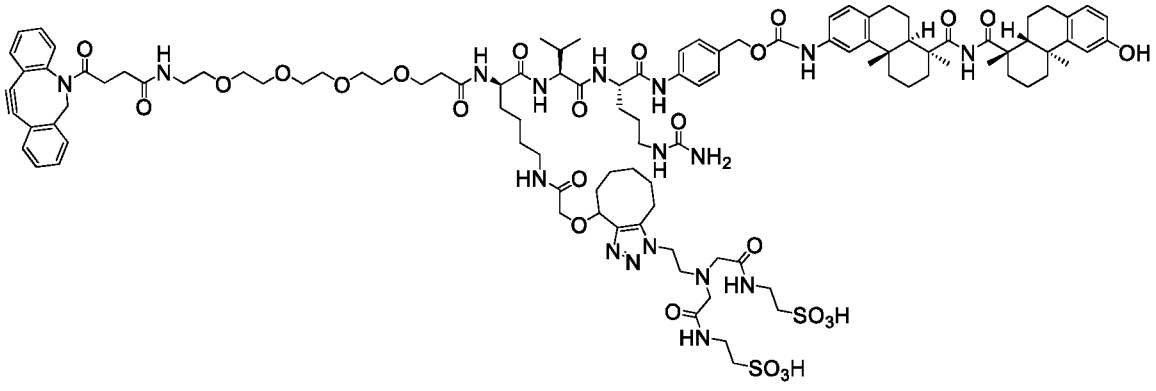
,



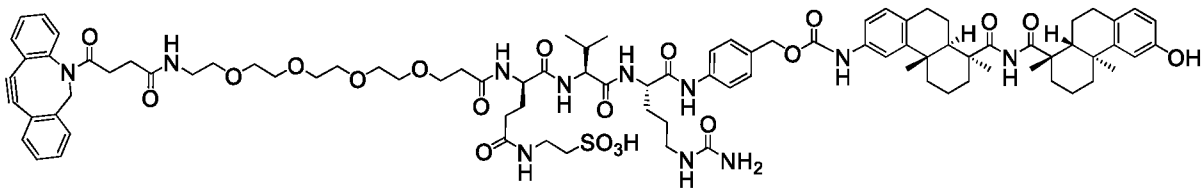
,



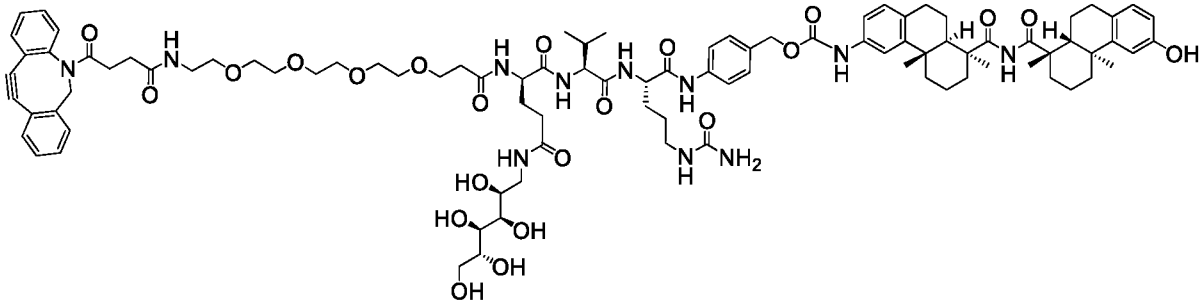
,



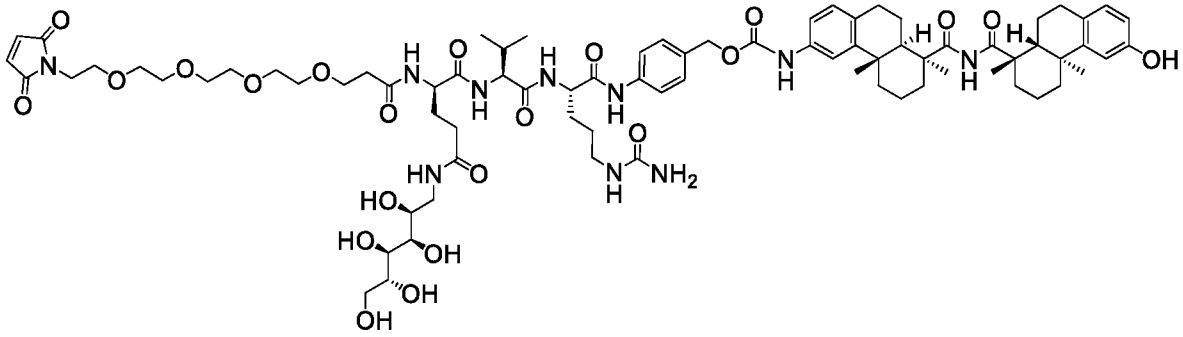
,



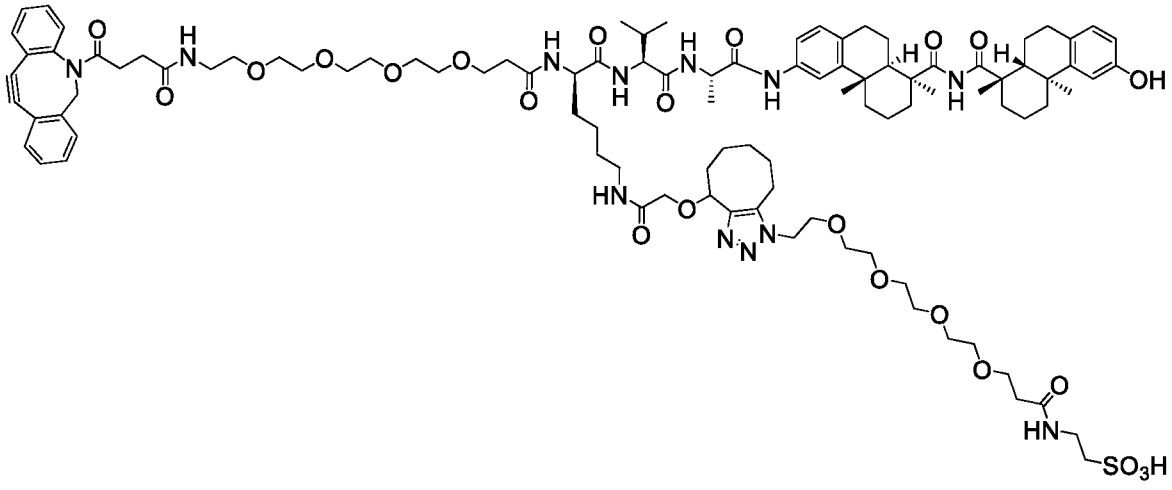
,



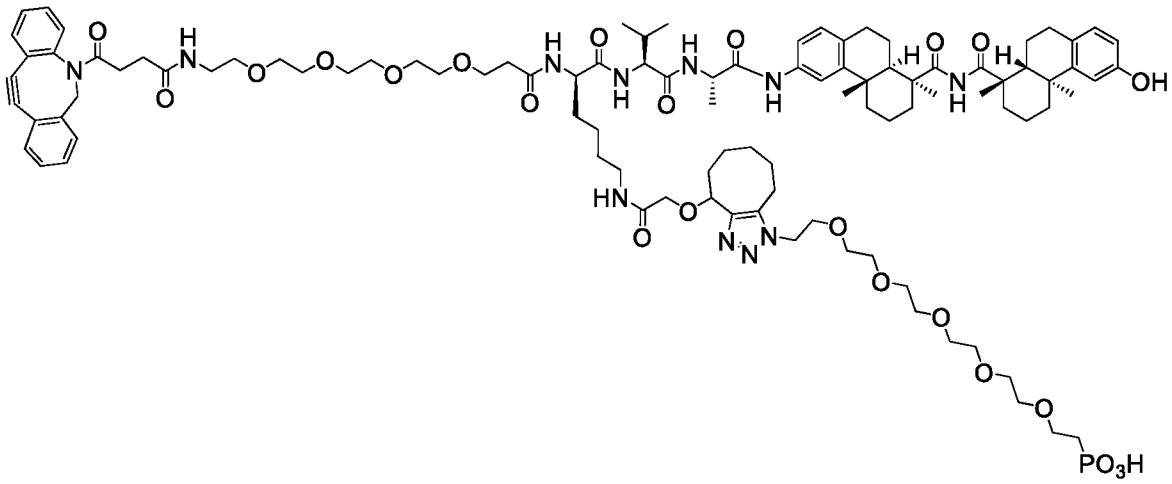
,

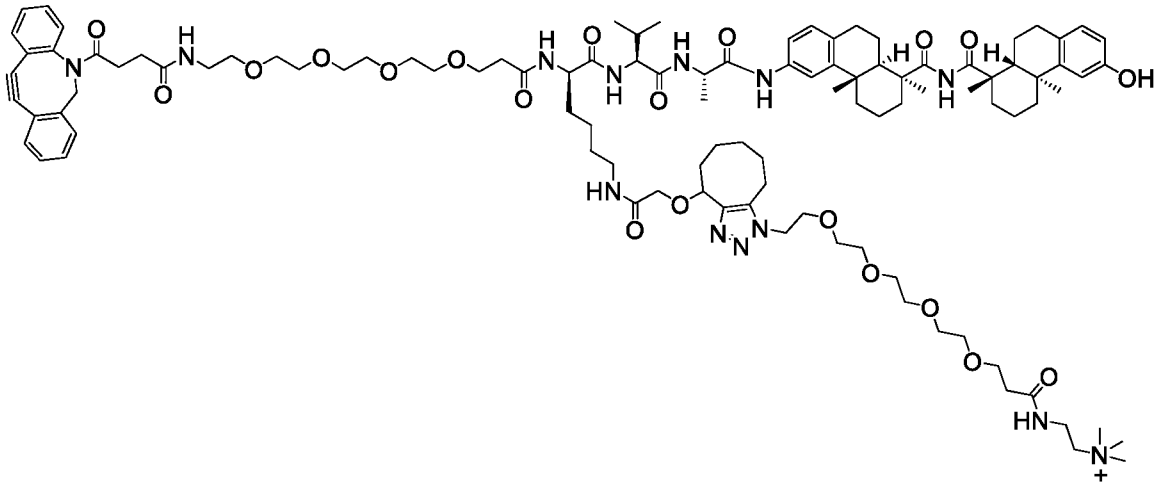


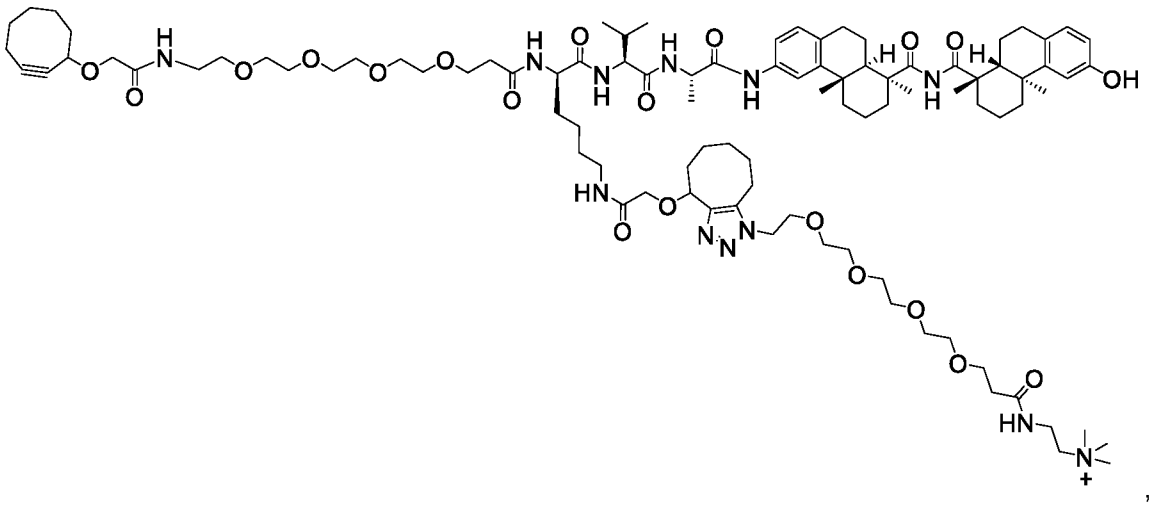
,

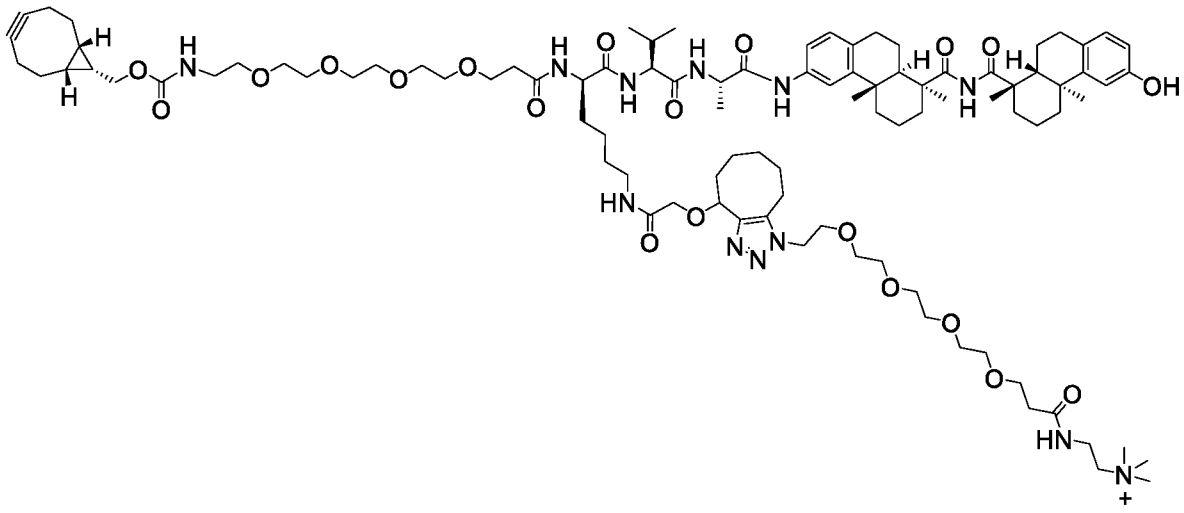


,

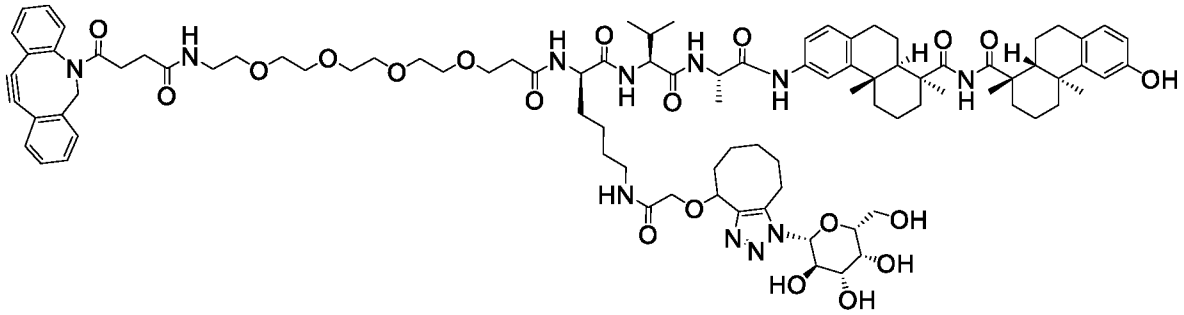




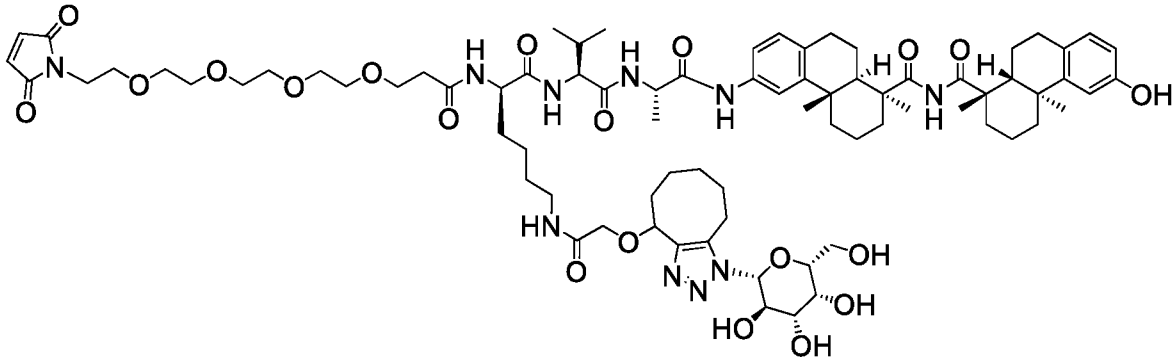




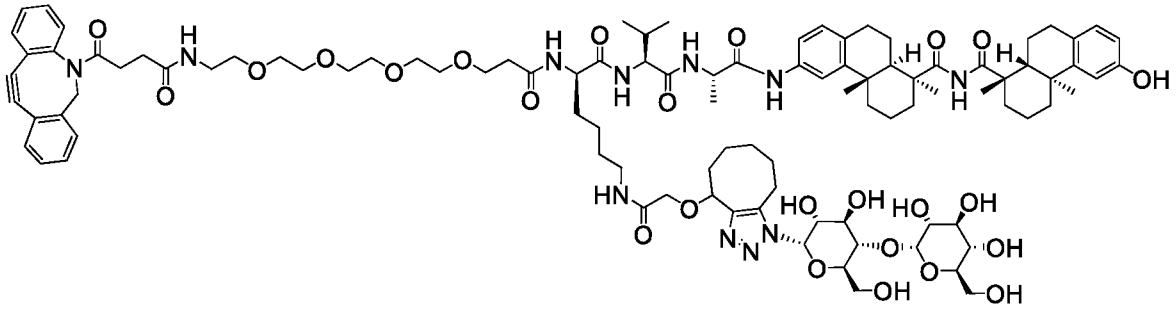
,



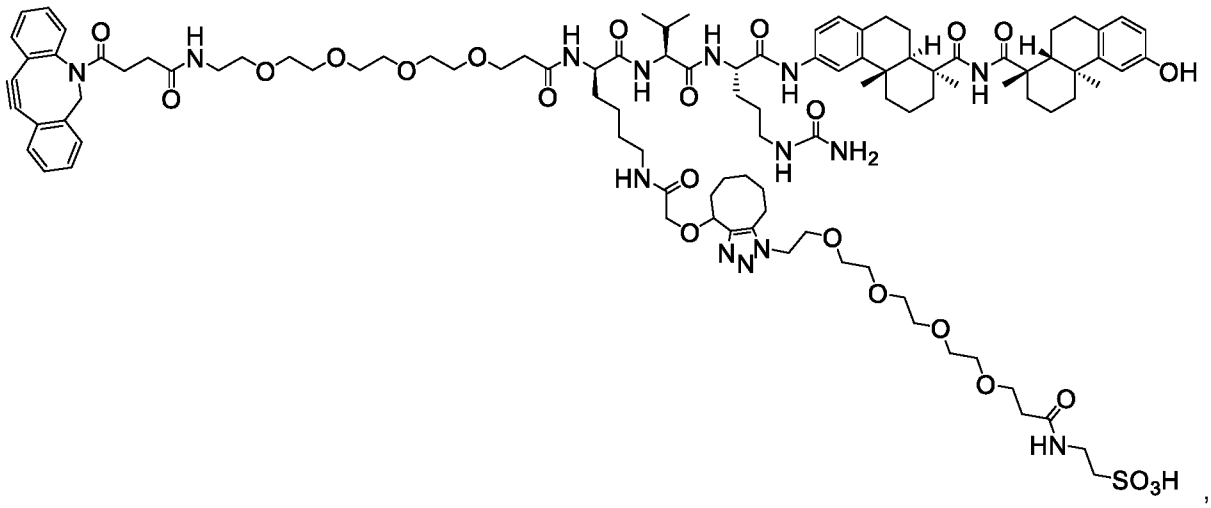
,

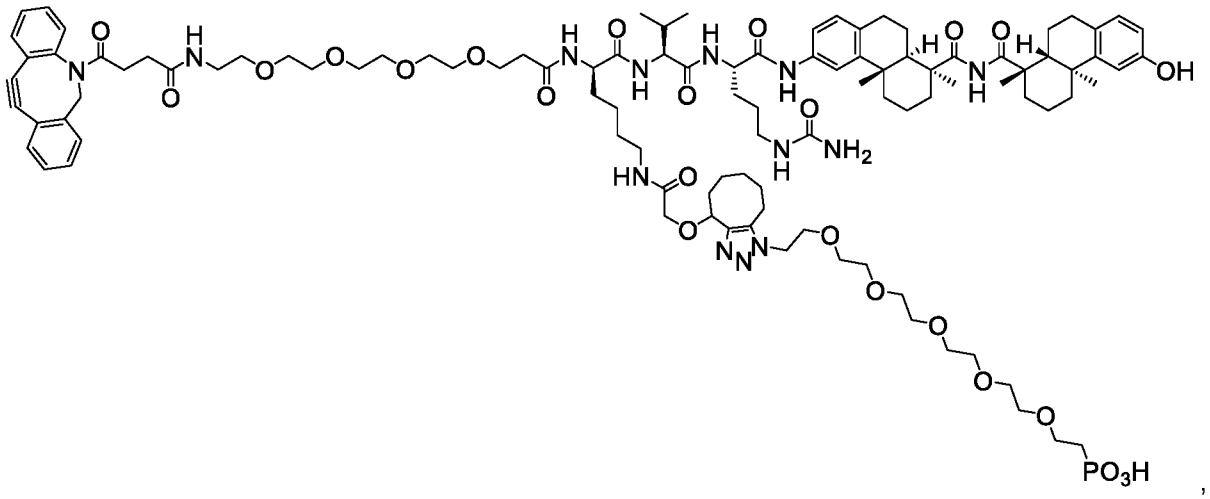


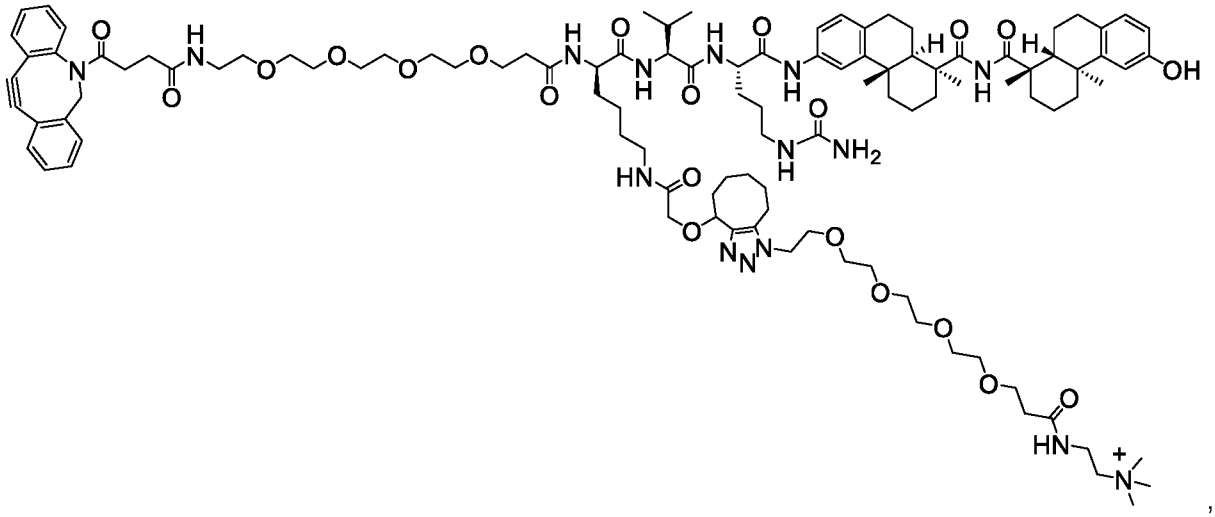
,

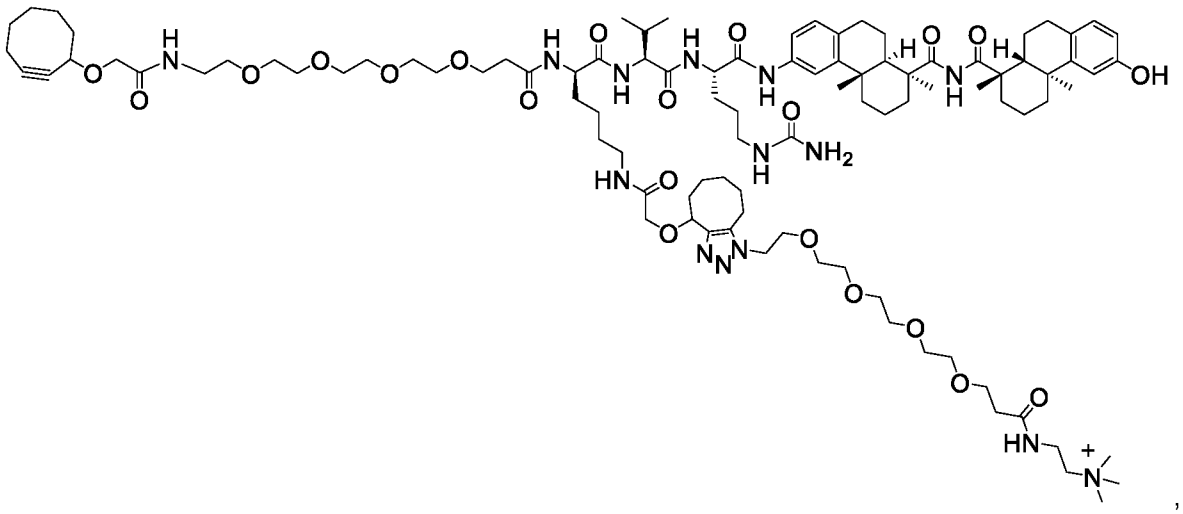


,

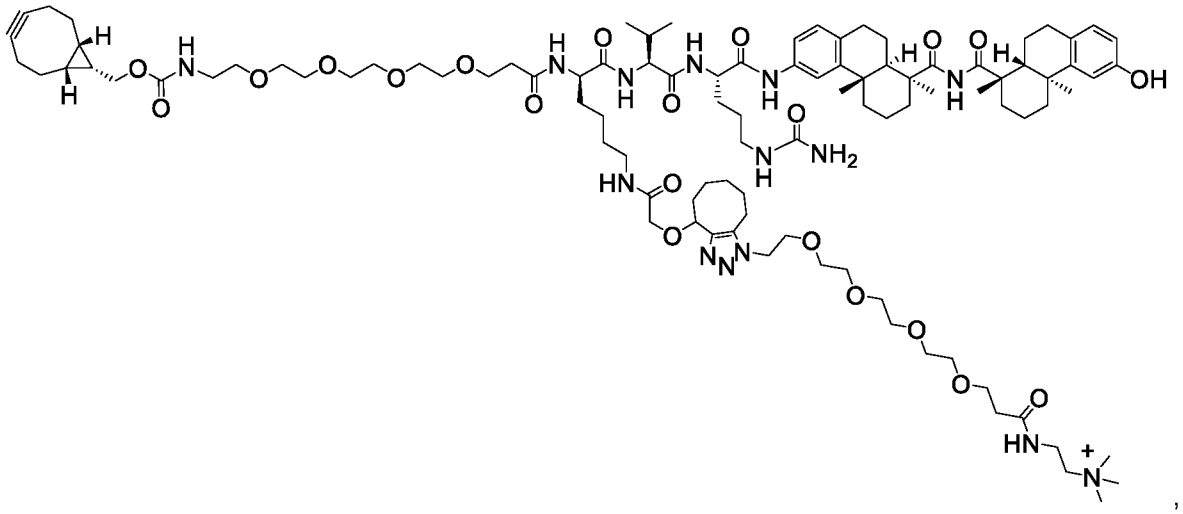




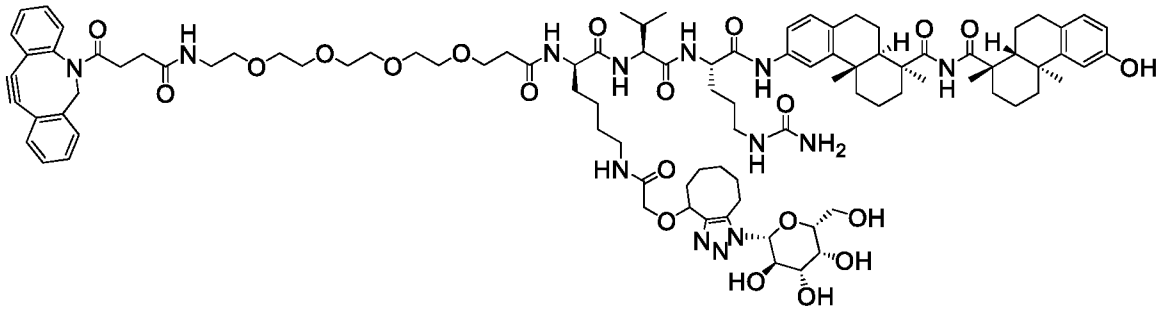




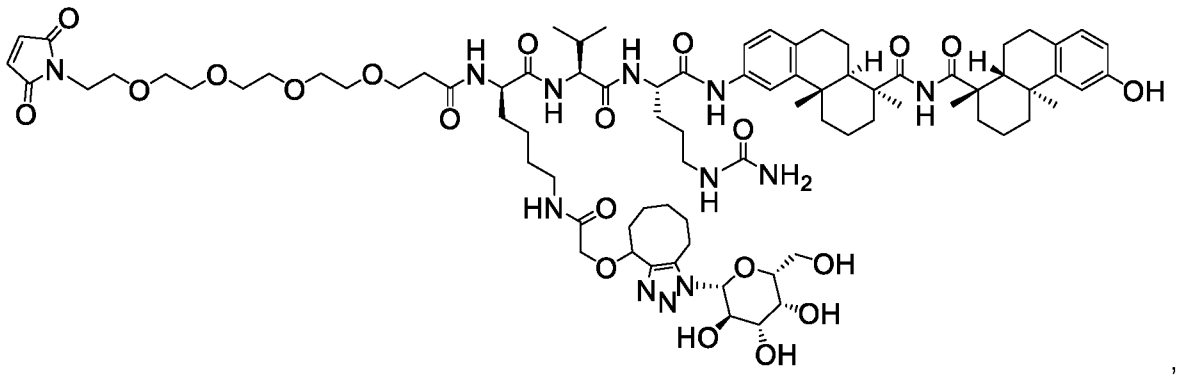
,



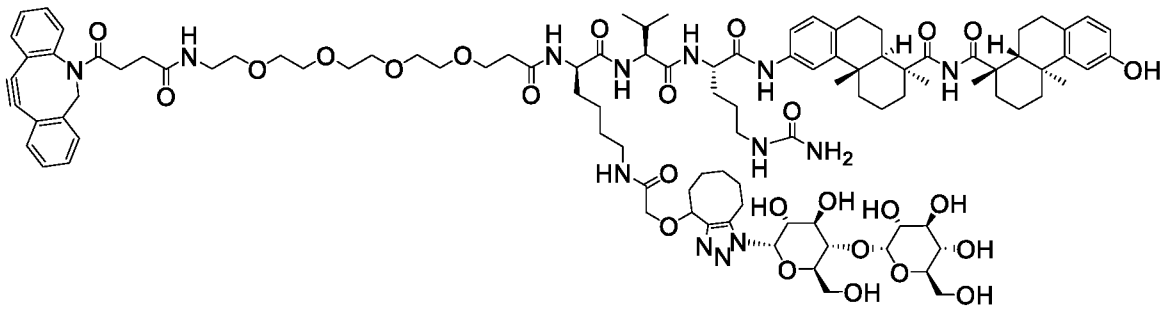
,



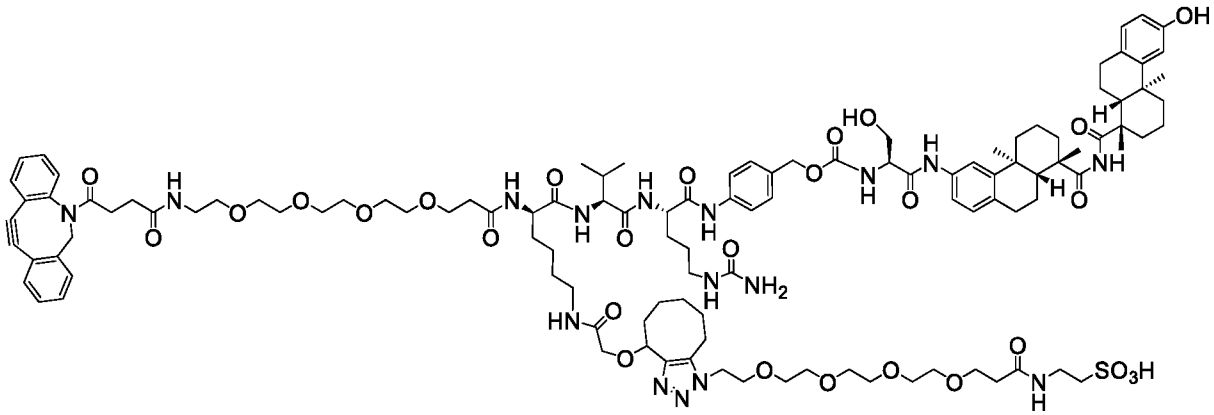
,



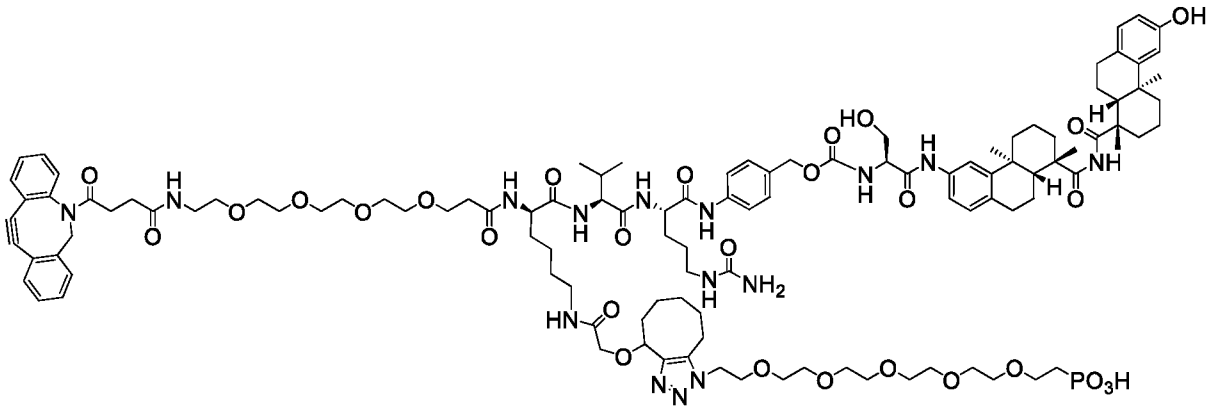
,



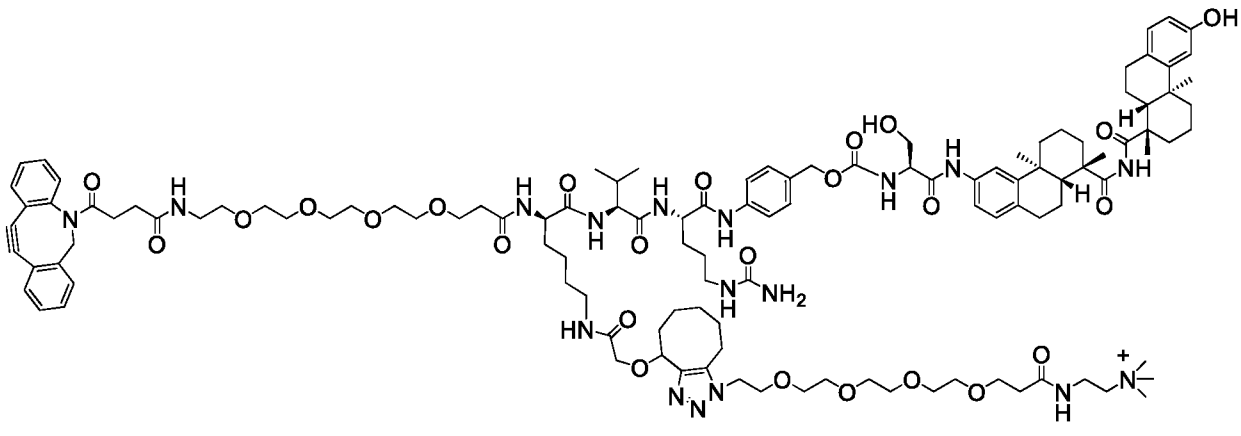
n ,

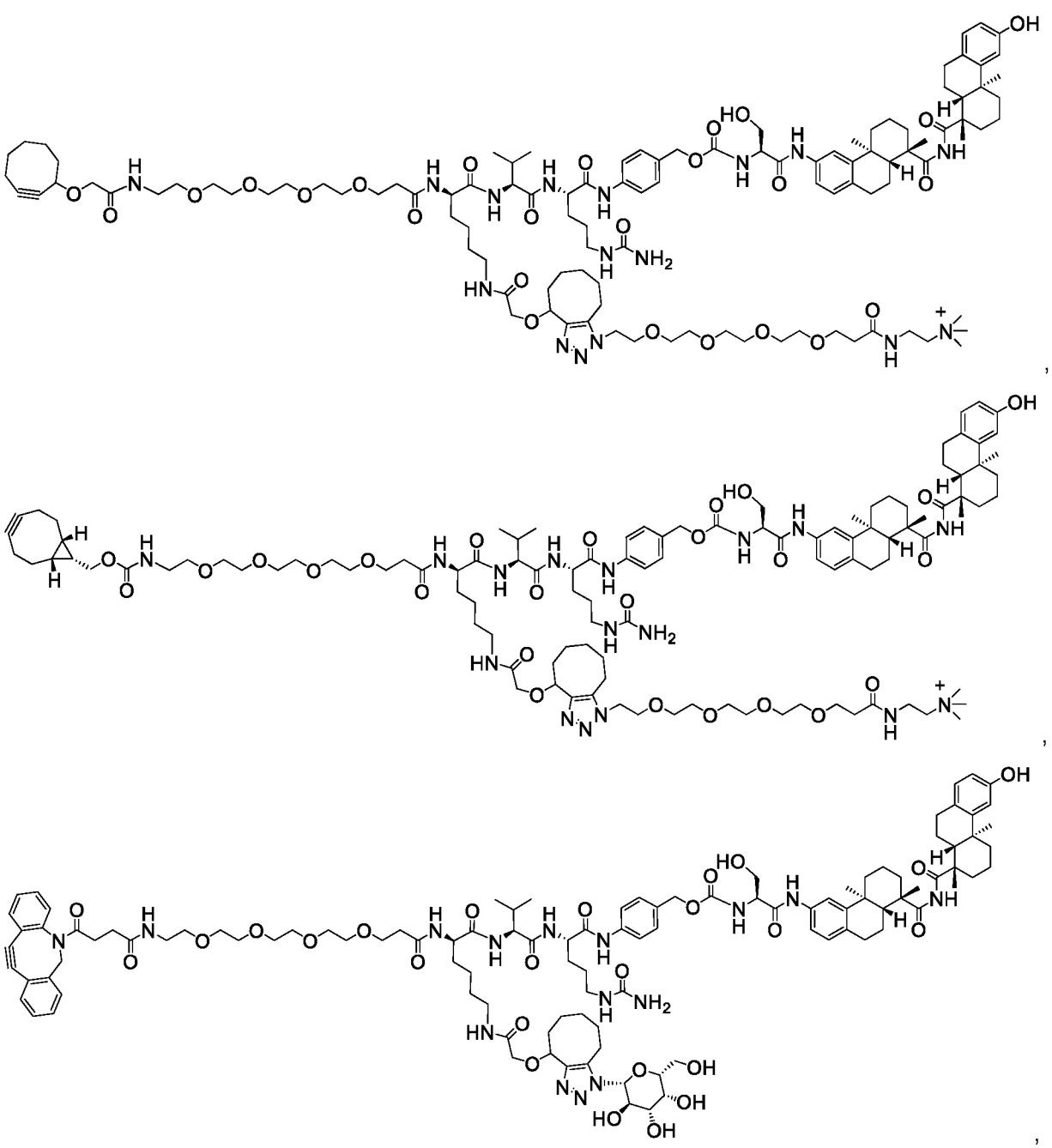


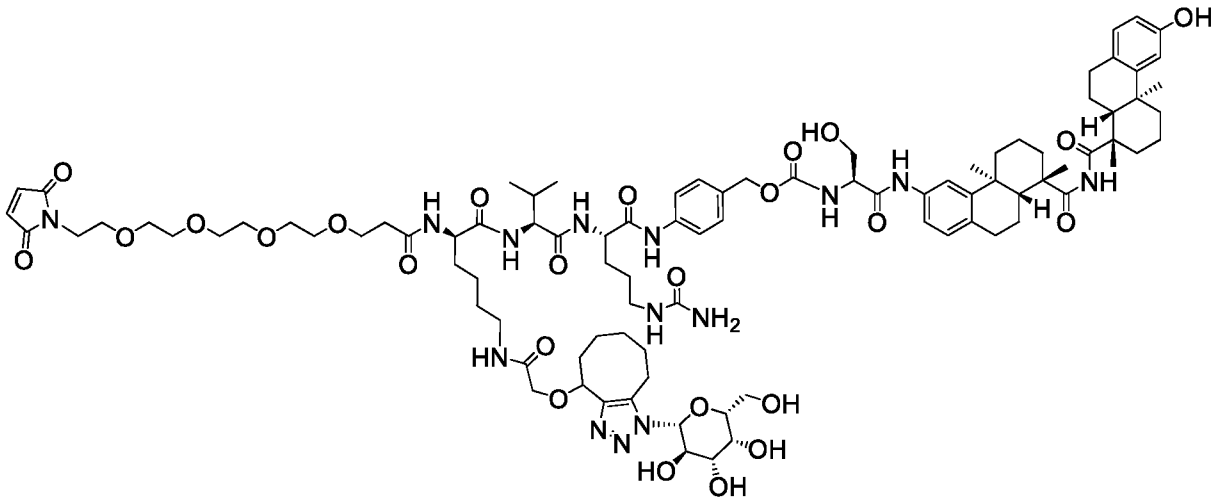
,



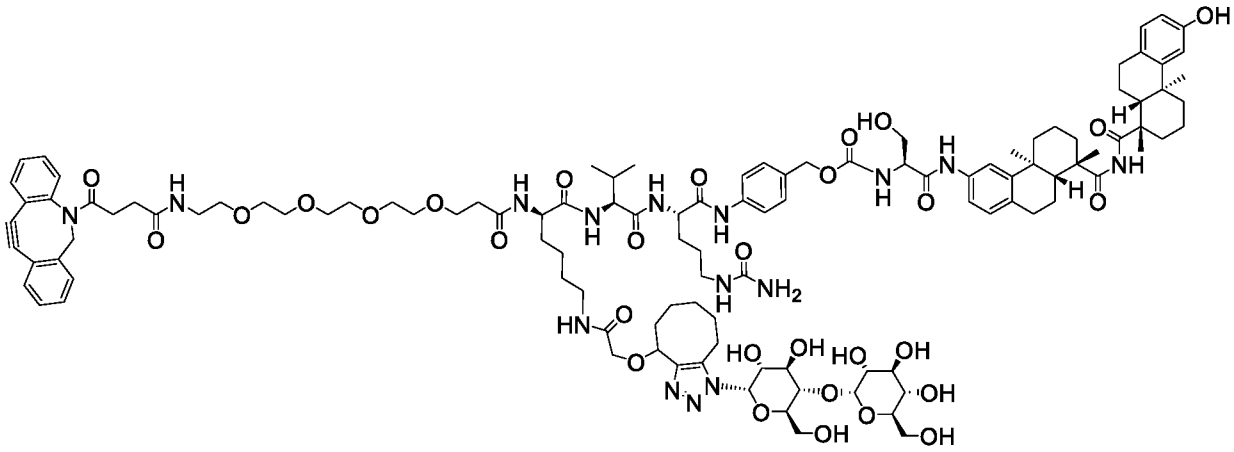
,



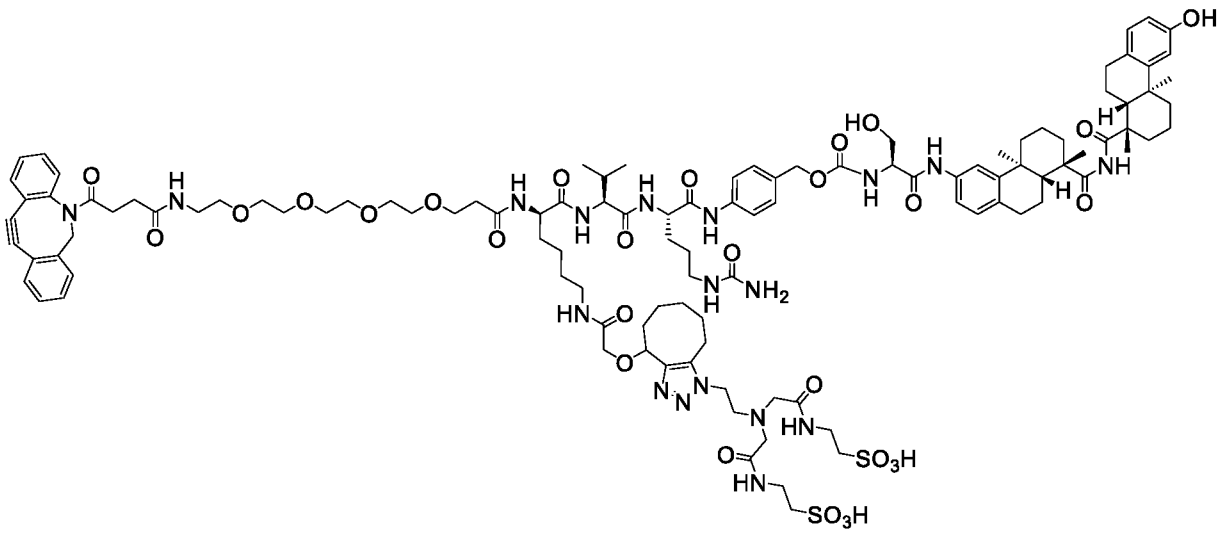




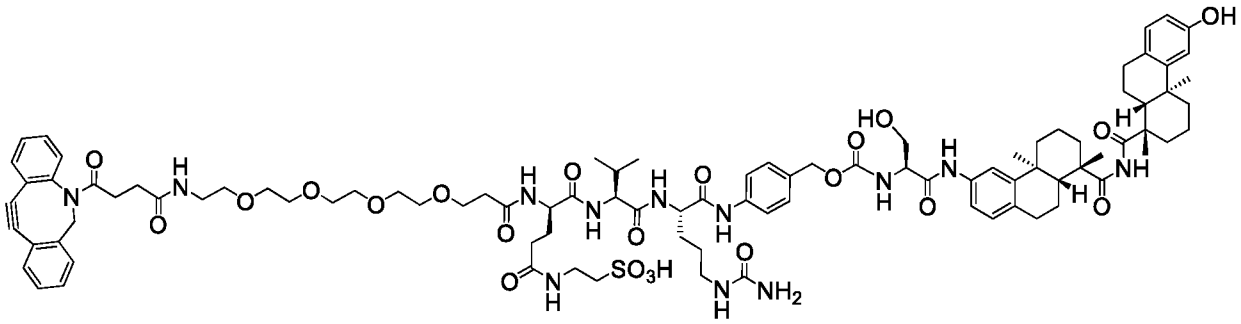
,



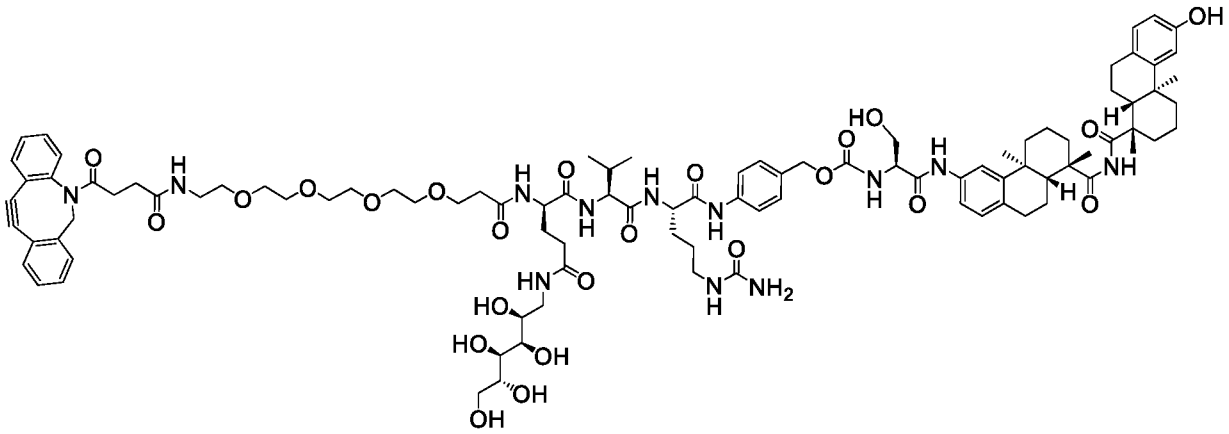
,



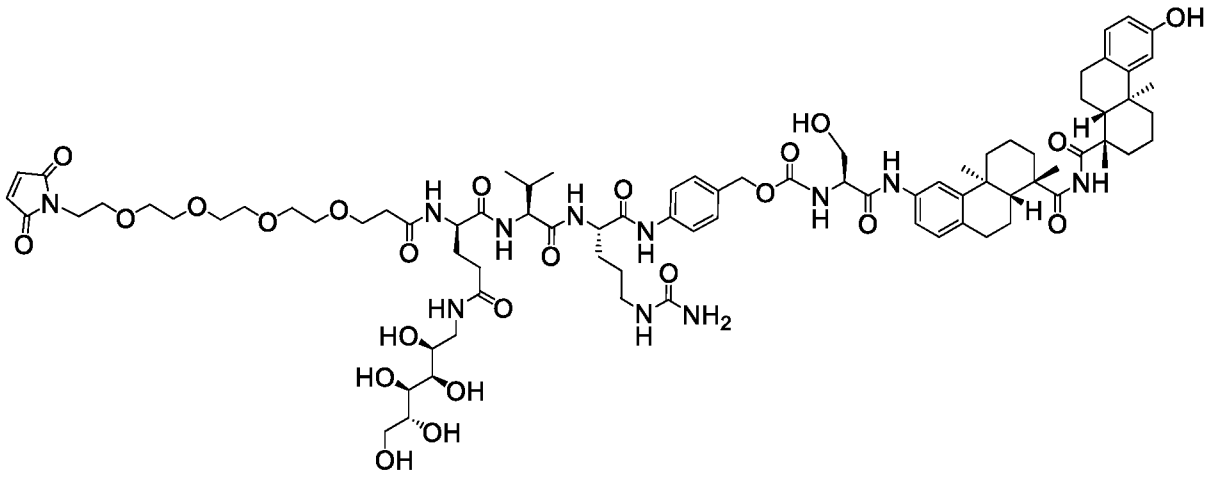
,



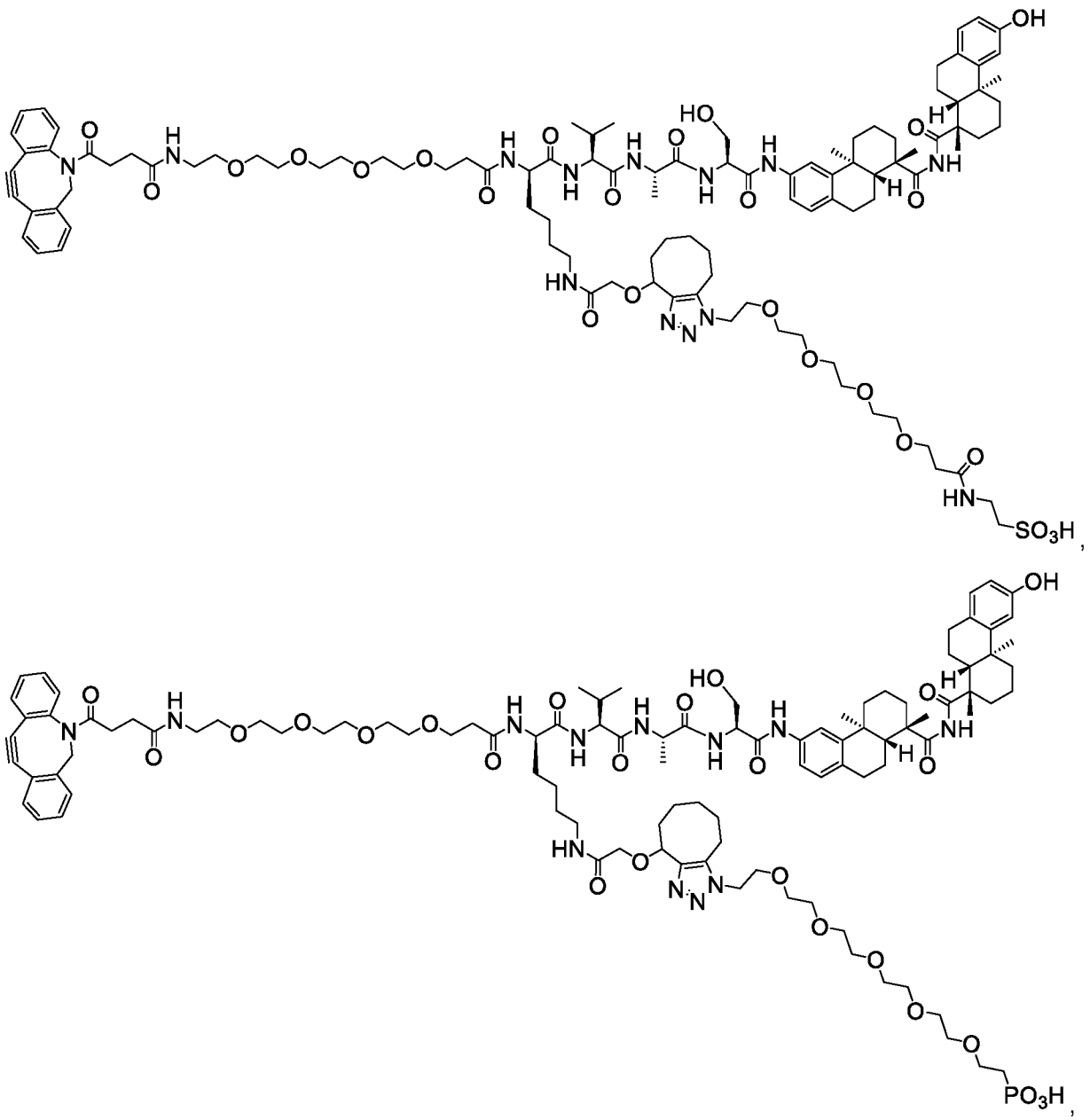
,

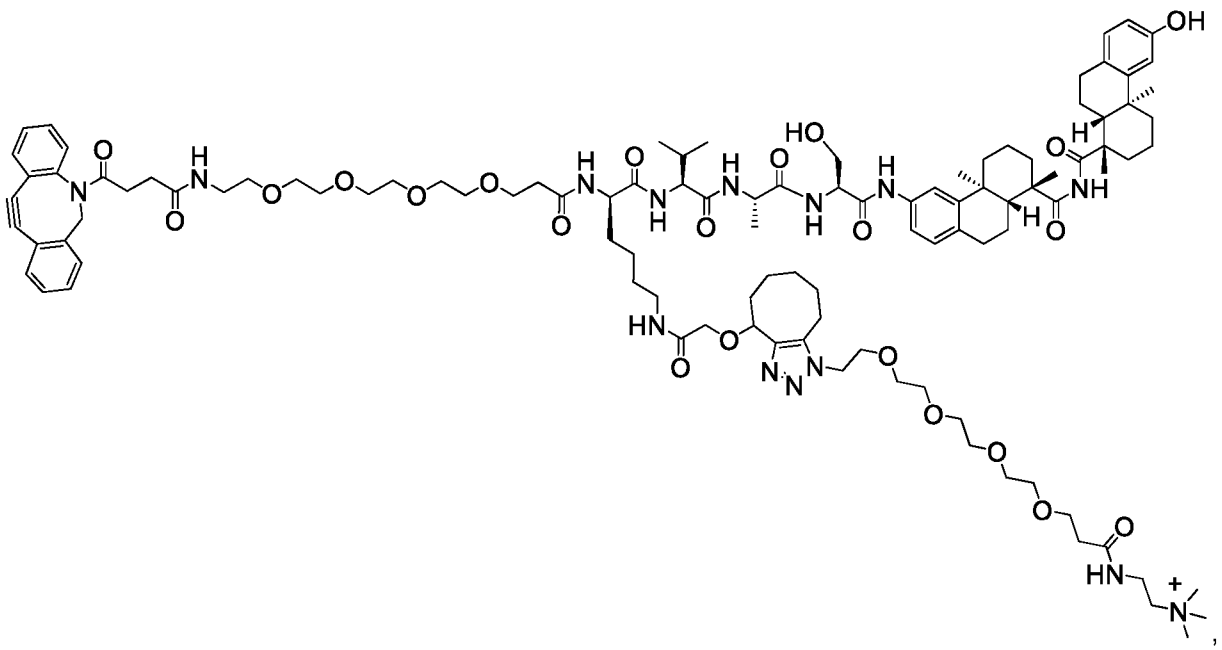


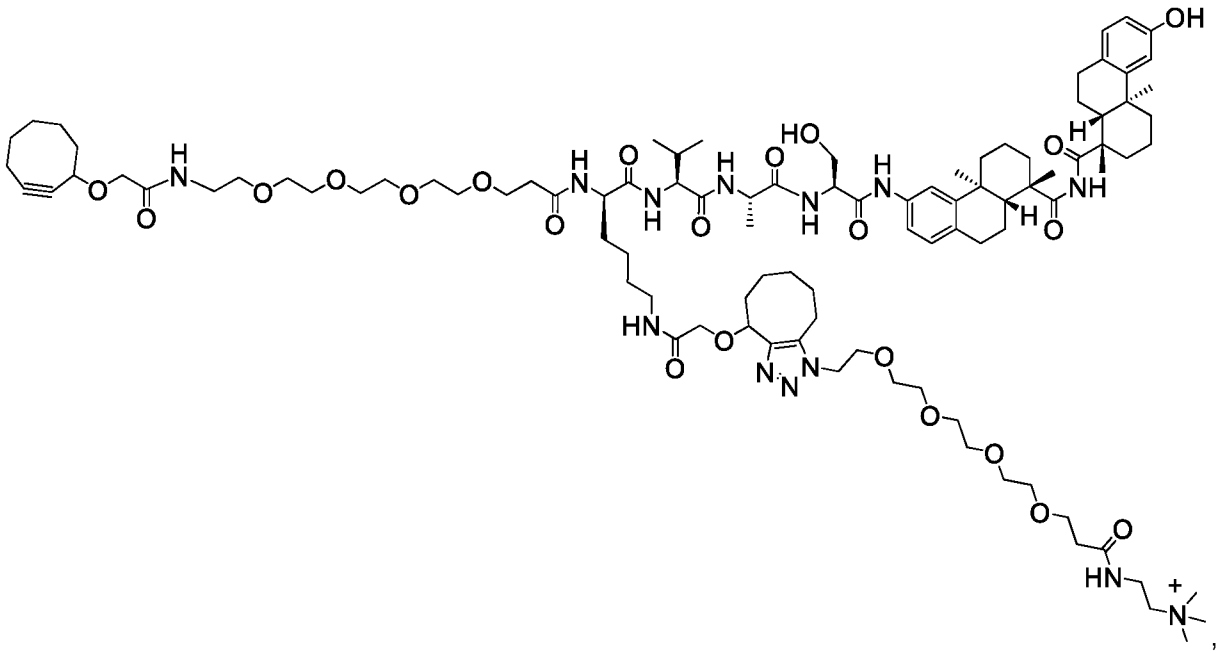
,

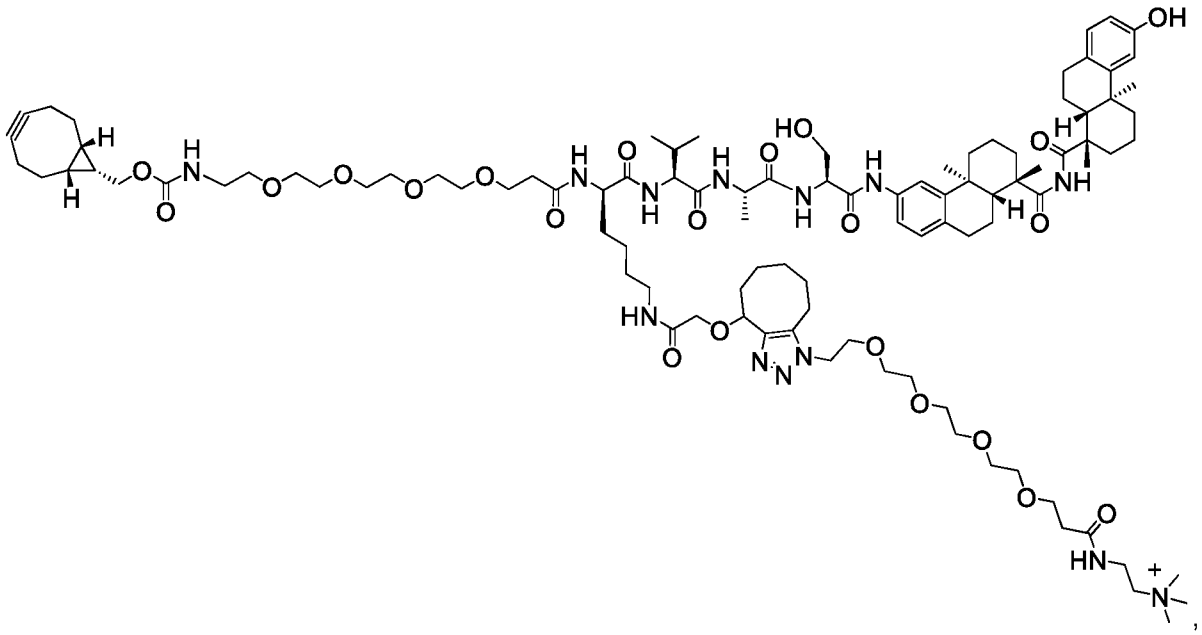


,

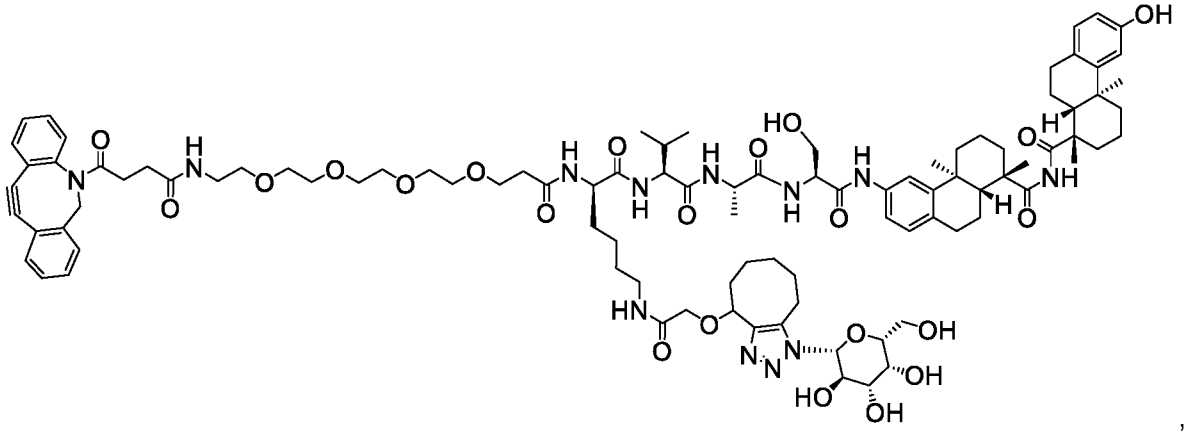




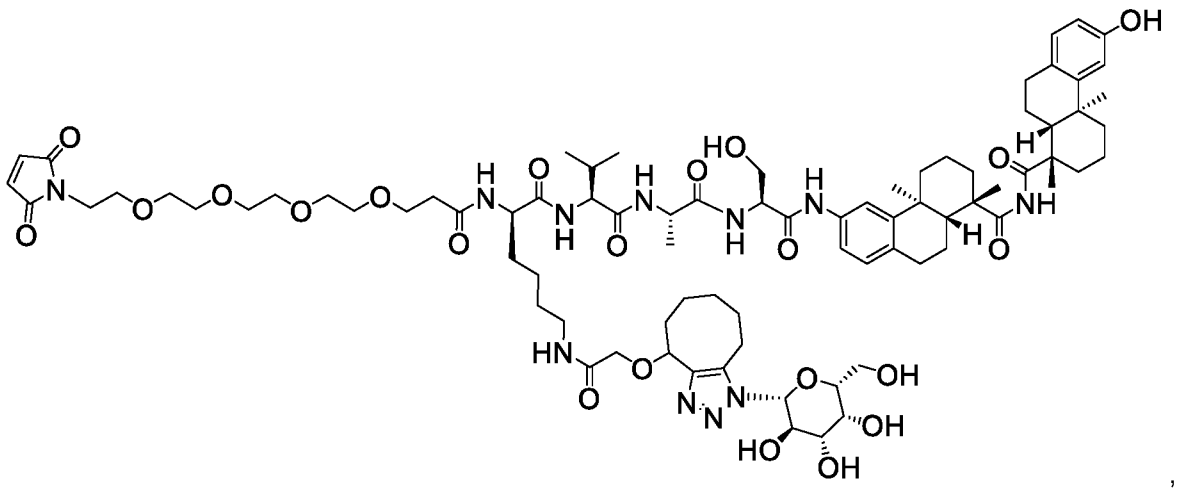




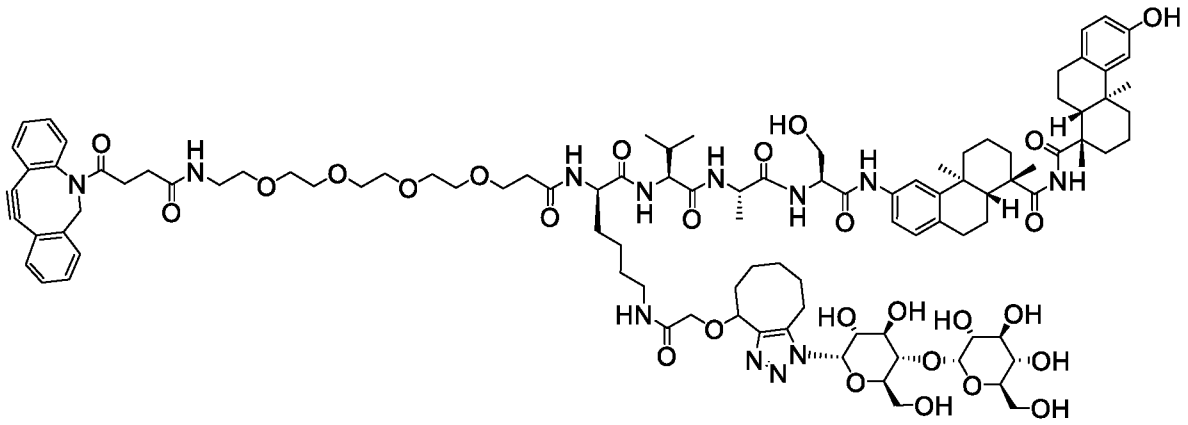
,



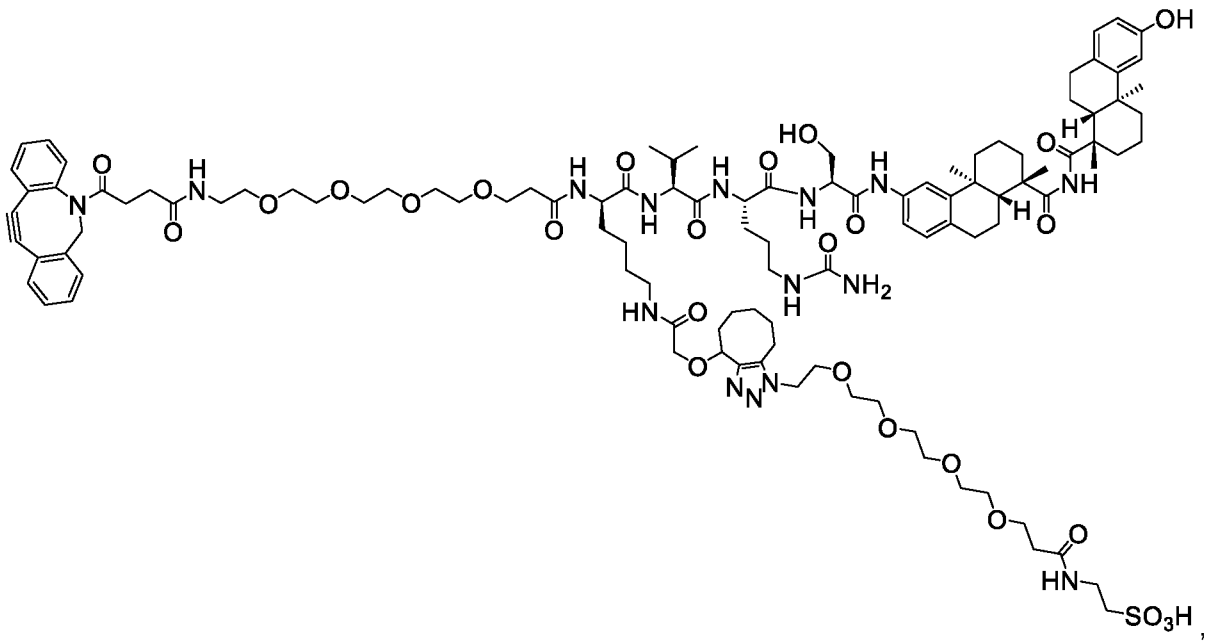
,



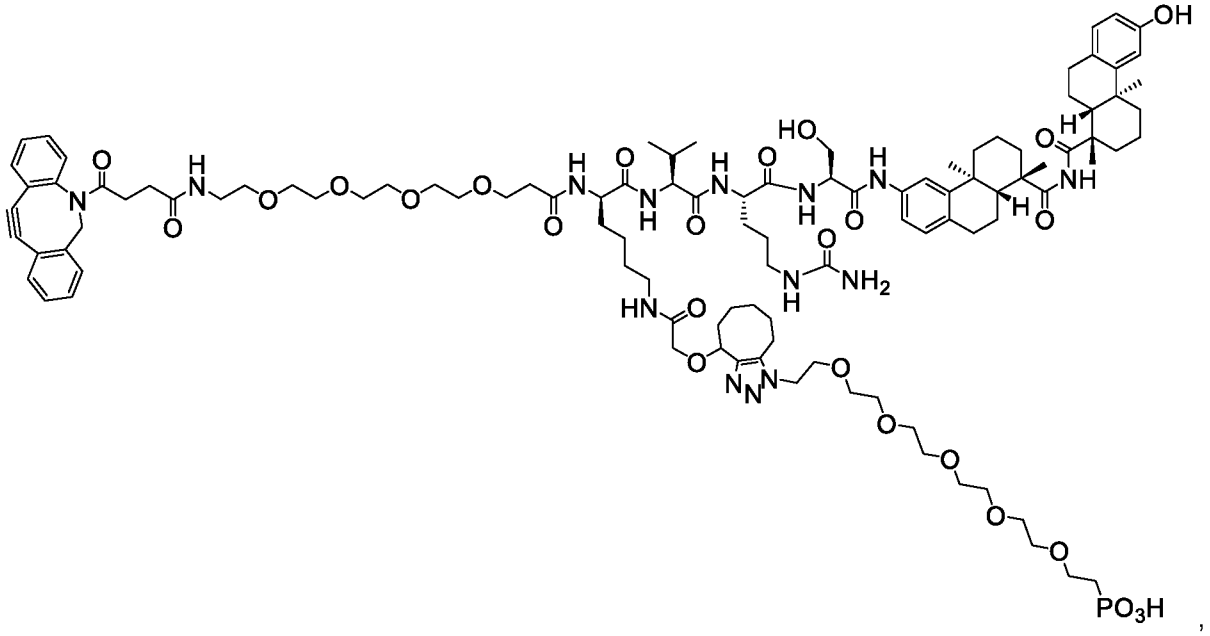
,



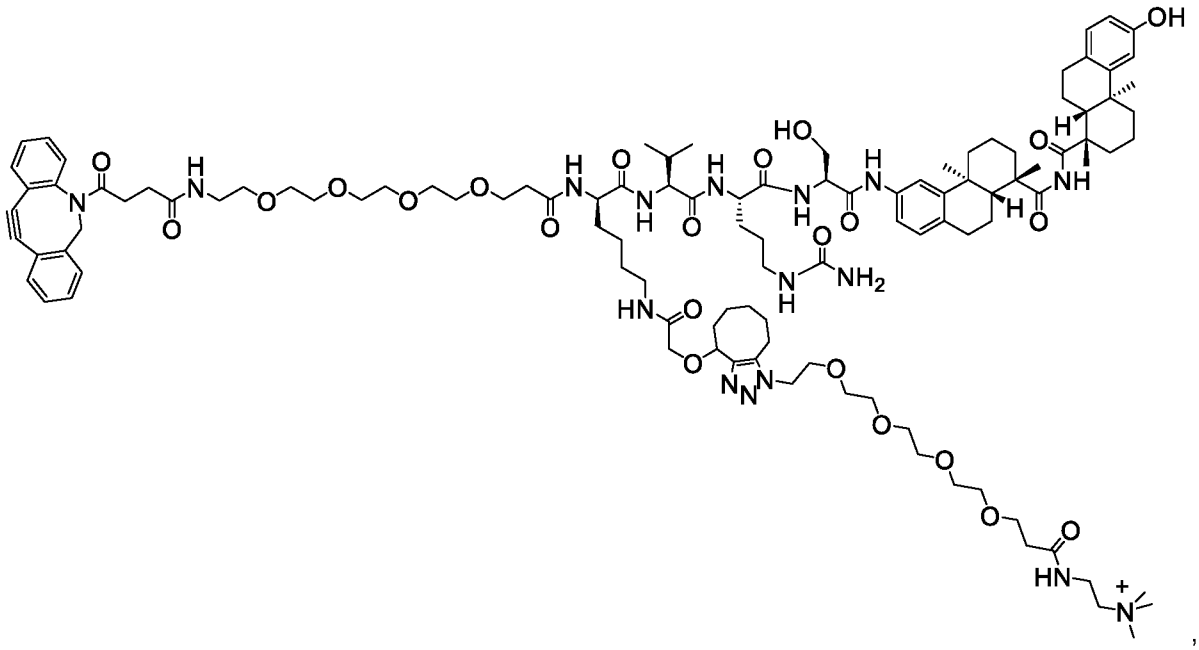
,



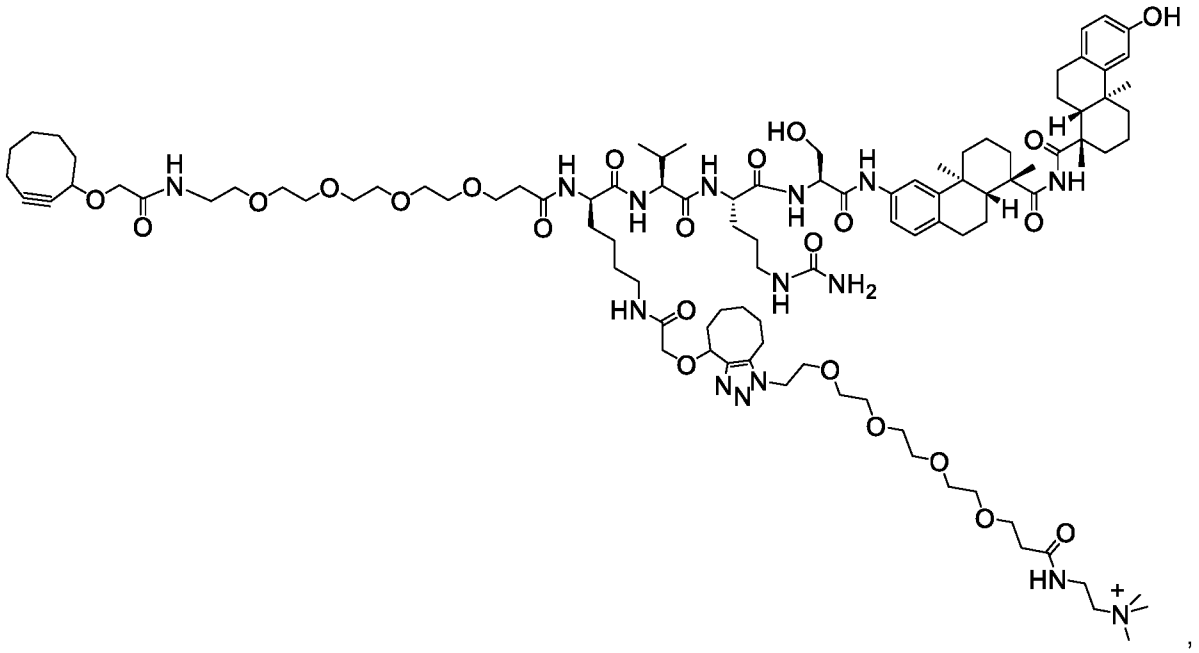
,



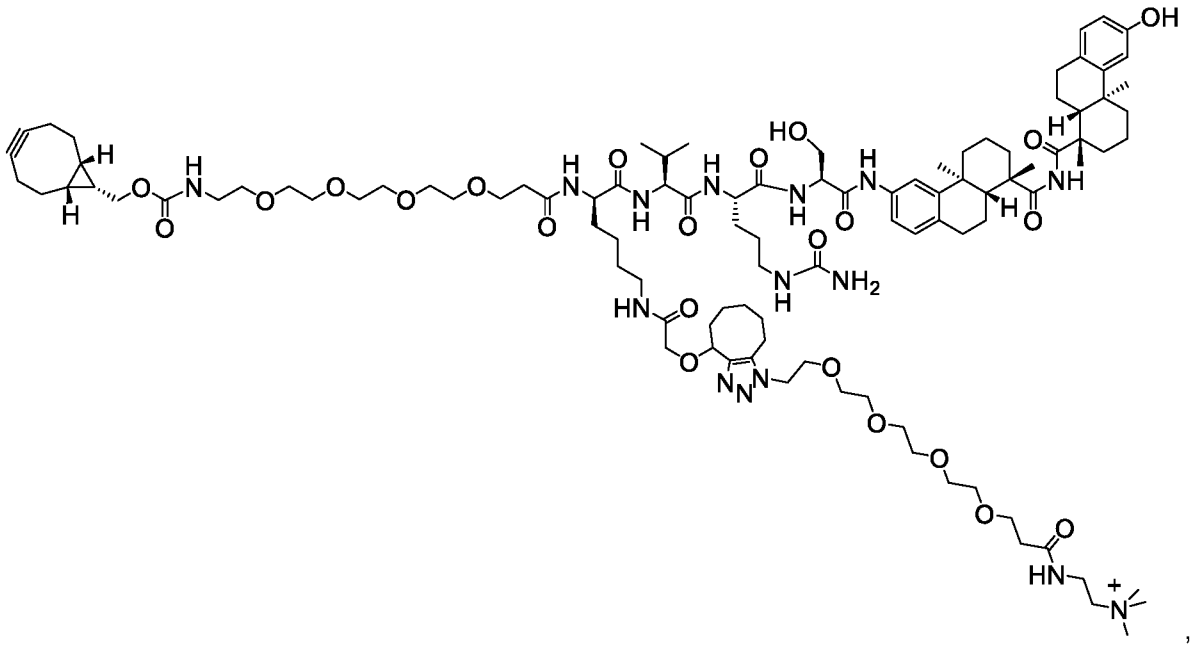
,



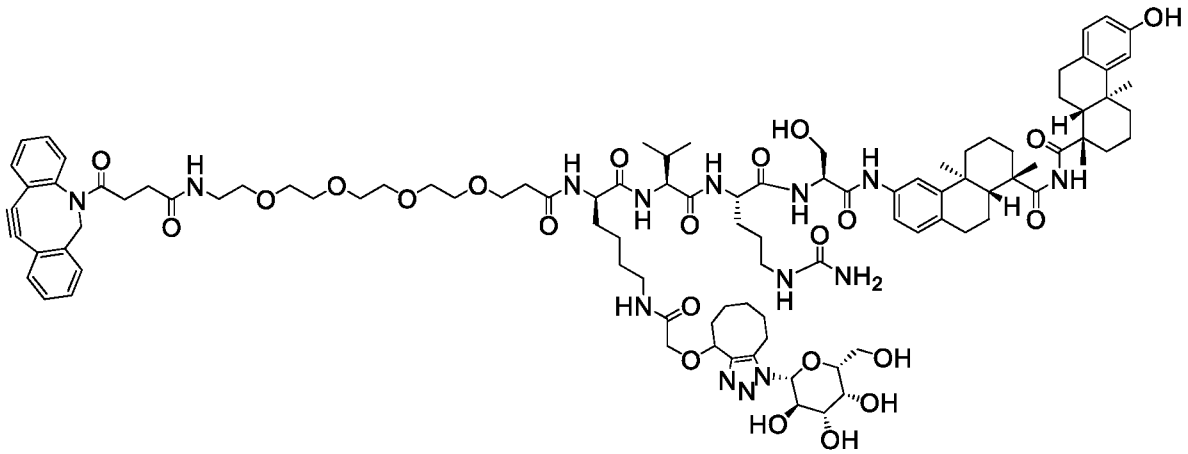
,



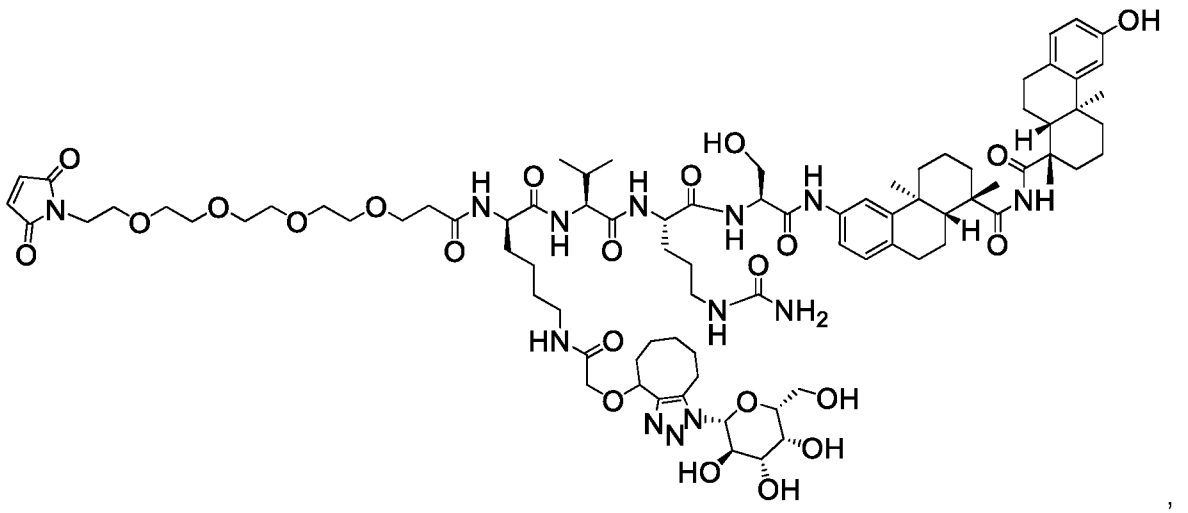
,



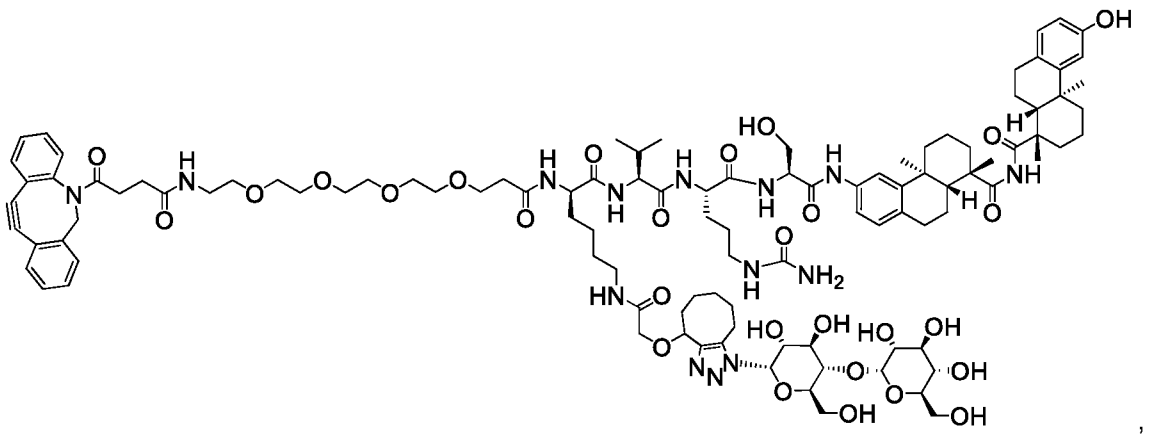
,



,



,



or a stereoisomeric form thereof, or a regioisomer thereof, or a mixture of regioisomers thereof.

**[0182]** Also provided is a method of preparing a compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), comprising the step of contacting a binding agent (**BA**) with a compound according to Formula (**VI**), Formula (**VII**), Formula (**VIII**), Formula (**IX**), Formula (**IXa**), Formula (**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**), Formula (**Xc**), or Formula (**Xd**), under conditions suitable for forming a bond between the binding agent and the compound. In one instance, the binding agent is a modified binding agent comprising an azido group ($\overset{\S}{\S}\!\!-N_3$) wherein the $\overset{\S}{\S}\!\!-$ indicates the atom through which the azido group is bonded to the adjacent groups in the formula.

**[0183]** Provided herein is a linker-payload comprising the compound of any of Formula (**VI**), Formula (**VII**), Formula (**VIII**), Formula (**IX**), Formula (**IXa**), Formula (**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**), Formula (**Xc**) or Formula (**Xd**), bonded to a linker.

**[0184]** Provided is a linker-payload comprising the compound of any of Formula (**VI**), Formula (**VII**), Formula (**VIII**), Formula (**IX**), Formula (**IXa**), Formula (**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**), Formula (**Xc**), or Formula (**Xd**), bonded to an oxygen or a primary or secondary nitrogen of the payload.

**[0185]** Further provided herein is an antibody-drug-conjugate comprising a compound or linker-payload described above bonded to an antibody, or an antigen binding fragment thereof.

**[0186]** In one aspect is provided a method of treating a proliferative disease, a metabolic disease, inflammation, or a neurodegenerative disease in a subject comprising administering to the subject an effective treatment amount of a compound according Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**),

Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), or a pharmaceutical composition comprising a compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**) or Formula (**Vd**).

**[0187]** In one aspect is provided a method for the treatment of a disease, disorder, or condition in a subject comprising administering to the subject an effective treatment amount of a compound according Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), or a pharmaceutical composition comprising a compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**).

**[0188]** In one aspect is provided a method for the treatment of a proliferatice disease in a subject comprising administering to the subject an effective treatment amount of a compound according Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), or a pharmaceutical composition comprising a compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**).

**[0189]** In one aspect is provided a method for the treatment of a metabolic disease in a subject comprising administering to the subject an effective treatment amount of a compound according Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), or a pharmaceutical composition comprising a compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**).

**[0190]** In one aspect is provided a method for the treatment of inflammation in a subject comprising administering to the subject an effective treatment amount of a compound according Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), or a pharmaceutical composition comprising a compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**) or Formula (**Vd**).

**[0191]** In one aspect is provided a method for the treatment of a neurodegenerative disease in a subject comprising administering to the subject an effective treatment amount of a compound according Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**),

or a pharmaceutical composition comprising a compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**).

**[0192]** In some examples, set forth herein is a pharmaceutical composition comprising a compound set forth herein, including any of the foregoing compounds, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

**[0193]** Those of skill in the art will recognize that the amino acid residue may be achiral or chiral, for example, an L-amino acid or D-amino acid. The amino acids generally include an amino acid side chain. The side chain can be the side chain of any amino acids known to those of skill. In certain embodiments, the side chain is the side chain of histidine, alanine, isoleucine, arginine, leucine, asparagine, lysine, aspartic acid, methionine, cysteine, phenylalanine, glutamic acid, threonine, glutamine, tryptophan, valine, ornithine, selenocysteine, serine, glycine, homoglycine (*e.g.*, β-homoglycine), or tyrosine. Those of skill in the art will recognize that the peptide may be achiral or chiral, for example, including racemic DL-amino acids or non-racemic D- or L-amino acids and diastereomeric mixtures thereof. The side chains of the peptides are as described in the context of amino acids, above. Those of skill in the art will recognize that the N-alkyl amino acid residue includes an alkyl substituent, as defined herein, at the terminal amino group of the amino acid or the terminal amino group of the peptide.

**[0194]** In some examples, including any of the foregoing, subscript n is an integer from 1 to 30. In some examples, including any of the foregoing, subscript n is 1. In some examples, including any of the foregoing, subscript n is 2. In some examples, including any of the foregoing, subscript n is 3. In some examples, including any of the foregoing, subscript n is 4. In some examples, including any of the foregoing, subscript n is 5. In some examples, including any of the foregoing, subscript n is 6. In some examples, including any of the foregoing, subscript n is 7. In some examples, including any of the foregoing, subscript n is 8. In some examples, including any of the foregoing, subscript n is 9. In some examples, including any of the foregoing, subscript n is 10. In some examples, including any of the foregoing, subscript n is 11. In some examples, including any of the foregoing, subscript n is 12. In some examples, including any of the foregoing, subscript n is 13. In some examples, including any of the foregoing, subscript n is 14. In some examples, including any of the foregoing, subscript n is 15. In some examples, including any of the foregoing, subscript n is 16. In some examples, including any of the foregoing, subscript n is 17. In some examples, including any of the foregoing, subscript n is 18. In some examples, including any of the foregoing, subscript n is 19. In some examples, including any of the foregoing, subscript n is 20. In some examples, including any of the foregoing, subscript n is 21. In some examples,

including any of the foregoing, subscript n is 22. In some examples, including any of the foregoing, subscript n is 23. In some examples, including any of the foregoing, subscript n is 24. In some examples, including any of the foregoing, subscript n is 25. In some examples, including any of the foregoing, subscript n is 26. In some examples, including any of the foregoing, subscript n is 27. In some examples, including any of the foregoing, subscript n is 28. In some examples, including any of the foregoing, subscript n is 29. In some examples, including any of the foregoing, subscript n is 30.

**Binding Agents (BA)**

**[0195]** Suitable binding agents for any of the conjugates provided in the instant disclosure include, but are not limited to, antibodies, lymphokines, hormones, growth factors, viral receptors, interleukins, or any other cell binding or peptide binding molecules or substances. Suitable binding agents also include polypeptides.

**[0196]** In some examples, including any of the foregoing, the binding agent (**BA**) is selected from any polypeptide. Example polypeptides include, but are not limited to, natural polypeptides and unnatural polypeptides. Example polypeptides include, but are not limited to, those produced from genetically modified organisms.

**[0197]** In some examples, including any of the foregoing, the **BA** is selected from receptors, cytokines, proteins, enzymes, binding agents, milk peptides, ribosomal peptides, nonribosomal peptides, peptones, and peptide fragments. In some examples, including any of the foregoing, the **BA** is selected selected from antimicrobial peptides, tachykinin peptides, vasoactive intestinal peptides, pancreatic polypeptide-related peptides, opiod peptides, and calcitonin peptides. In some examples, including any of the foregoing, the **BA** is selected B-type natriuretic peptide (BNP), lactotripeptides, neuropeptides, lipopeptides, proteoses, or hormones.

**[0198]** In some examples, including any of the foregoing, the **BA** is selected from short amino acid chains comprising two or more amino acids bonded together. In some examples, including any of the foregoing, the **BA** is selected from dipeptides (Val-Cit), tripeptides, and tetrapeptides (*e.g.*, Val-Gly-Ser-Ala) having two, three, or four amino acids bonded together, respectively. In some examples, including any of the foregoing, the **BA** is selected from dipeptides, tripeptides, tetrapeptides, pentapeptides, hexapeptides, heptapeptides, octapeptides, nonapeptides, decapeptides, undecapeptides, and icosapeptides.

**[0199]** In some examples, including any of the foregoing, the **BA** is selected from any proteins. In some examples, the proteins include only natural amino acids. In some examples, the proteins further include non-natural amino acids.In some emodiments, the binding agent is an antibody of an antigen-binding fragment thereof. The antibody can be in any form known

to those of skill in the art. The term "antibody", as used herein, means any antigen-binding molecule or molecular complex comprising at least one complementarity determining region (CDR) that specifically binds to or interacts with a particular antigen. The term "antibody" includes immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (*e.g.*, IgM). Each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or $V_H$) and a heavy chain constant region. The heavy chain constant region comprises three domains, $C_H1$, $C_H2$ and $C_H3$. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or $V_L$) and a light chain constant region. The light chain constant region comprises one domain ($C_L1$). The $V_H$ and $V_L$ regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each $V_H$ and $V_L$ is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In different embodiments of the invention, the FRs of the antiboides suitable for the compounds herein (or antigen-binding portion thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs. The term "antibody", as used herein, also includes antigen-binding fragments of full antibody molecules. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc. Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')2 fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies,

chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.* monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein. An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a $V_H$ domain associated with a $V_L$ domain, the $V_H$ and $V_L$ domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain $V_H$-$V_H$, $V_H$-$V_L$ or $V_L$-$V_L$ dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric $V_H$ or $V_L$ domain. In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) $V_H$-$C_H1$; (ii) $V_H$-$C_H2$; (iii) $V_H$-$C_H3$; (iv) $V_H$-$C_H1$-$C_H2$; (v) $V_H$-$C_H1$-$C_H2$-$C_H3$; (vi) $V_H$-$C_H2$-$C_H3$; (vii) $V_H$-$C_L$; (viii) $V_L$-$C_H1$; (ix) $V_L$-$C_H2$; (x) $V_L$-$C_H3$; (xi) $V_L$-$C_H1$-$C_H2$; (xii) $V_L$-$C_H1$-$C_H2$-$C_H3$; (xiii) $V_L$-$C_H2$-$C_H3$; and (xiv) $V_L$-$C_L$. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. As with full antibody molecules, antigen-binding fragments may be monospecific or multispecific (*e.g.*, bispecific). A multispecific antigen-binding fragment of an antibody will typically 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 on the same antigen. Any multispecific antibody format, including the exemplary bispecific antibody formats disclosed herein, may be adapted for use in the context of an antigen-binding fragment of an antibody of the present invention using routine techniques available in the art.

In certain embodiments of the invention, antibodies of the invention are human antibodies. The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian

species, such as a mouse, have been grafted onto human framework sequences. The term "human antibody" does not include naturally occurring molecules that normally exist without modification or human intervention/manipulation, in a naturally occurring, unmodified living organism. The antibodies of the invention may, in some embodiments, be recombinant human antibodies. The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further below), antibodies isolated from a recombinant, combinatorial human antibody library (described further below), antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic for human immunoglobulin genes (see *e.g.*, Taylor *et al.* (1992) Nucl. Acids Res. 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the $V_H$ and $V_L$ regions of the recombinant antibodies are sequences that, while derived from and related to human germline $V_H$ and $V_L$ sequences, may not naturally exist within the human antibody germline repertoire *in vivo*. Human antibodies can exist in two forms that are associated with hinge heterogeneity. In one form, an immunoglobulin molecule comprises a stable four chain construct of approximately 150-160 kDa in which the dimers are held together by an interchain heavy chain disulfide bond. In a second form, the dimers are not linked via inter-chain disulfide bonds and a molecule of about 75-80 kDa is formed composed of a covalently coupled light and heavy chain (half-antibody). These forms have been extremely difficult to separate, even after affinity purification. The frequency of appearance of the second form in various intact IgG isotypes is due to, but not limited to, structural differences associated with the hinge region isotype of the antibody. A single amino acid substitution in the hinge region of the human IgG4 hinge can significantly reduce the appearance of the second form (Angal *et al.* (1993) Molecular Immunology 30:105) to levels typically observed using a human IgG1 hinge. The instant invention encompasses antibodies having one or more mutations in the hinge, $C_H2$ or $C_H3$ region which may be desirable, for example, in production, to improve the yield of the desired antibody form. The antibodies of the invention may be isolated antibodies. An "isolated antibody," as used herein, means an antibody that has been identified and separated and/or recovered from at least one component of its natural environment. For example, an antibody that has been separated or removed from at least one component of an organism, or from a tissue or cell in which the antibody naturally exists or is naturally produced, is an "isolated antibody" for purposes of the

present invention. An isolated antibody also includes an antibody *in situ* within a recombinant cell. Isolated antibodies are antibodies that have been subjected to at least one purification or isolation step. According to certain embodiments, an isolated antibody may be substantially free of other cellular material and/or chemicals. The antibodies used herein can comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences from which the antibodies were derived. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present invention includes antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the $V_H$ and/or $V_L$ domains are mutated back to the residues found in the original germline sequence from which the antibody was derived. In other embodiments, only certain residues are mutated back to the original germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.*, a germline sequence that is different from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies of the present invention may contain any combination of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. Antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present invention. Antibodies useful for the compounds

herein also include antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. The term "epitope" refers to an antigenic determinant that interacts with a specific antigen-binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. In certain circumstances, an epitope may include moieties of saccharides, phosphoryl groups, or sulfonyl groups on the antigen.

**[0200]** In certain embodiments, the antibody comprises a light chain. In certain embodiments, the light chain is a kappa light chain. In certain embodiments, the light chain is a lambda light chain. In certain embodiments, the antibody comprises a heavy chain. In some aspects, the heavy chain is an IgA. In some aspects, the heavy chain is an IgD. In some aspects, the heavy chain is an IgE. In some aspects, the heavy chain is an IgG. In some aspects, the heavy chain is an IgM. In some aspects, the heavy chain is an IgG1. In some aspects, the heavy chain is an IgG2. In some aspects, the heavy chain is an IgG3. In some aspects, the heavy chain is an IgG4. In some aspects, the heavy chain is an IgA1. In some aspects, the heavy chain is an IgA2.

**[0201]** In some embodiments, the antibody is an antibody fragment. In some aspects, the antibody fragment is an Fv fragment. In some aspects, the antibody fragment is a Fab fragment. In some aspects, the antibody fragment is a $F(ab')_2$ fragment. In some aspects, the antibody fragment is a Fab' fragment. In some aspects, the antibody fragment is an scFv (sFv) fragment. In some aspects, the antibody fragment is an scFv-Fc fragment.

**[0202]** In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a polyclonal antibody.

**[0203]** In some embodiments, the antibody is a chimeric antibody. In some embodiments, the antibody is a humanized antibody. In some embodiments, the antibody is a human antibody.

**[0204]** The antibody can have binding specificity for any antigen deemed suitable to those of skill in the art. In certain embodiments, the antigen is a transmembrane molecule (*e.g.*, receptor) or a growth factor. Exemplary antigens include, but are not limited to, molecules such as renin; a growth hormone, including human growth hormone and bovine growth hormone; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; lipoproteins; alpha1-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; clotting factors such as factor

vmc, factor IX, tissue factor (TF), and von Willebrands factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, such as urokinase or human urine or tissue-type plasminogen activator (t-PA); bombesin; thrombin; hemopoietic growth factor; tumor necrosis factor-alpha and -beta; enkephalinase; RANTES (regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein (MIP-I-alpha); a serum albumin, such as human serum albumin; Muellerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; a microbial protein, such as betalactamase; DNase; 19E; a cytotoxic T-lymphocyte associated antigen (CTLA), such as CTLA-4; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT4, NT-5, or NT-6), or a nerve growth factor such as NGF-β; platelet-derived growth factor (PDGF); fibroblast growth factor such as aFGF and bFGF; fibroblast growth factor receptor 2 (FGFR2), epidermal growth factor (EGF); transforming growth factor (TGF) such as TGF-alpha and TGF-beta, including TGF-β1, TGF-β2, TGF- β3, TGF-β4, or TGF- β5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(I-3)-IGF-I (brain IGF-I), insulin-like growth factor binding proteins, EpCAM, GD3, FLT3, PSMA, PSCA, MUCI, MUCI6, STEAP, CEA, TENB2, EphA receptors, EphB receptors, folate receptor, FOLRI, mesothelin, cripto, alphavbeta6, integrins, VEGF, VEGFR, EGFR, transferrin receptor, IRTA1, IRTA2, IRTA3, IRTA4, IRTA5; CD proteins such as CD2, CD3, CD4, CD5, CD6, CD8, CD11, CD14, CD19, CD20, CD21, CD22, CD25, CD26, CD28, CD30, CD33, CD36, CD37, CD38, CD40, CD44, CD52, CD55, CD56, CD59, CD70, CD79, CD80. CD81, CD103, CD105, CD134, CD137, CD138, CDI52, or an antibody which binds to one or more tumor-associated antigens or cell-surface receptors disclosed in US Publication No. 2008/0171040 or US Publication No. 2008/0305044 and incorporated in their entirety by reference; erythropoietin; osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon, such as interferon-alpha, -beta, and -gamma; colony stimulating factors (CSFs), *e.g.,* M-CSF, GM-CSF, and G-CSF; interleukins (ILs), *e.g.,* IL-1 to IL-10; superoxide dismutase; T-cell receptors; surface membrane proteins; decay accelerating factor; viral antigen such as, for example, a portion of the HIV envelope; transport proteins; homing receptors; addressins; regulatory proteins; integrins, such as CDIIa, CDIIb, CDIIc, CDI8, an ICAM, VLA-4 and VCAM; a tumor associated antigen such as AFP, ALK, B7H4, BAGE proteins, β-catenin, brc-abl, BRCA1, BORIS, CA9 (carbonic anhydrase IX), caspase-8, CD20, CD40, CD123, CDK4, CEA, CLEC12A, c-kit, cMET, CTLA4, cyclin-B1, CYP1B1, EGFR, EGFRvIII, endoglin, Epcam, EphA2, ErbB2/Her2, ErbB3/Her3, ErbB4/Her4, ETV6-AML, Fra-1, FOLR1, GAGE proteins (*e.g.*, GAGE-1, -2), GD2, GD3, GloboH, glypican-3, GM3, gp100, Her2, HLA/B-raf, HLA/EBNA1, HLA/k-ras, HLA/MAGE-A3, hTERT, IGF1R, LGR5, LMP2, MAGE proteins (*e.g.*,

MAGE-1, -2, -3, -4, -6, and -12), MART-1, mesothelin, ML-IAP, Muc1, Muc16 (CA-125), MUM1, NA17, NGEP, NY-BR1, NY-BR62, NY-BR85, NY-ESO1, OX40, p15, p53, PAP, PAX3, PAX5, PCTA-1, PDGFR-α, PDGFR-β, PDGF-A, PDGF-B, PDGF-C, PDGF-D, PLAC1, PRLR, PRAME, PSCA, PSGR, PSMA (FOLH1), RAGE proteins, Ras, RGS5, Rho, SART-1, SART-3, Steap-1, Steap-2, STn, survivin, TAG-72, TGF-β, TMPRSS2, Tn, TNFRSF17, TRP-1, TRP-2, tyrosinase, and uroplakin-3, and fragments of any of the above-listed polypeptides. In some embodiments, the antigen is a tumor antigen, including antigens specific for a type of tumor or antigens that are shared, overexpressed or modified on a particular type of tumor. Examples include, but are not limited to: alpha-actinin-4 with lung cancer, ARTC1 with melanoma, BCR-ABL fusion protein with chronic myeloid leukemia, B-RAF, CLPP or Cdc27 with melanoma, CASP-8 with squamous cell carcinoma, and hsp70-2 with renal cell carcinoma as well as the following shared tumor-specific antigens, for example: BAGE-1, GAGE, GnTV, KK-LC-1, MAGE-A2, NA88-A, TRP2-INT2. In some embodiments, the antigen is PRLR or HER2. In some embodiments, the antibody is an anti-PRLR or anti HER2 antibody.

**[0205]** The binding agent linkers can be bonded to the binding agent, *e.g.*, antibody or antigen-binding molecule, through an attachment at a particular amino acid within the antibody or antigen-binding molecule. Exemplary amino acid attachments that can be used in the context of this aspect of the disclosure include, *e.g.*, lysine (*see*, e.g., US 5,208,020; US 2010/0129314; Hollander *et al.*, *Bioconjugate Chem.*, 2008, 19:358-361; WO 2005/089808; US 5,714,586; US 2013/0101546; and US 2012/0585592), cysteine (*see*, e.g., US 2007/0258987; WO 2013/055993; WO 2013/055990; WO 2013/053873; WO 2013/053872; WO 2011/130598; US 2013/0101546; and US 7,750,116), selenocysteine (*see*, e.g., WO 2008/122039; and Hofer *et al.*, *Proc. Natl. Acad. Sci., USA, 2008, 105*:12451-12456), formyl glycine (*see*, e.g., Carrico *et al.*, *Nat. Chem. Biol.*, 2007, 3:321-322; Agarwal *et al.*, *Proc. Natl. Acad. Sci., USA, 2013, 110*:46-51, and Rabuka *et al.*, *Nat. Protocols*, 2012, *10*:1052-1067), non-natural amino acids (*see*, *e.g.*, WO 2013/068874, and WO 2012/166559), and acidic amino acids (*see*, *e.g.*, WO 2012/05982). Linkers can also be conjugated to an antigen-binding protein via attachment to carbohydrates (*see*, *e.g.*, US 2008/0305497, WO 2014/065661, and Ryan *et al.*, *Food & Agriculture Immunol.*, 2001, *13*:127-130).

**[0206]** In some examples, the binding agent is an antibody or antigen binding molecule, and the antibody is bonded to the linker through a lysine residue. In some embodiments, the antibody or antigen binding molecule is bonded to the linker through a cysteine residue.

**[0207]** Linkers can also be conjugated to one or more glutamine residues via transglutaminase-based chemo-enzymatic conjugation (*see, e.g.,* Dennler *et al., Bioconjugate Chem.* 2014, 25, 569-578). For example, in the presence of transglutaminase, one or more glutamine residues of an antibody can coupled to a primary amine compound. Primary amine

compounds include payloads or linker-payloads, which directly provide antibody drug conjugates via transglutaminase-mediated coupling. Primary amine compounds also include linkers and spacers that are functionalized with reactive groups that can be subsequently reacted with further compounds towards the synthesis of antibody drug conjugates.

Antibodies comprising glutamine residues can be isolated from natural sources or engineered to comprise one or more glutamine residues. Techniques for engineering glutamine residues into an antibody polypeptide chain (glutaminyl-modified antibodies or antigen binding molecules) are within the skill of the practitioners in the art. In certain embodiments, the antibody is aglycosylated.

**[0208]** In certain embodiments, the antibody or a glutaminyl-modified antibody or antigen binding molecule comprises at least one glutamine residue in at least one polypeptide chain sequence. Site specific conjugation techniques can also be employed to direct conjugation to particular residues of the antibody or antigen binding protein (see, e.g., Schumacher et al. *J Clin Immunol* (2016) 36(Suppl 1): 100). Site specific conjugation techniques, include, but are not limited to glutamine conjugation via transglutaminase (see e.g., Schibli, *Angew Chemie Inter Ed.* 2010, 49 ,9995). In certain embodiments, the antibody or a glutaminyl-modified antibody or antigen binding molecule comprises two heavy chain polypeptides, each with one Gln295 residue. In further embodiments, the antibody or a glutaminyl-modified antibody or antigen binding molecule comprises one or more glutamine residues at a site other than a heavy chain 295, for instance at Gln55 (Q55). Included herein are antibodies of this section bearing N297Q mutation(s) described herein. Briefly, in some embodiments, an antibody including a glutamine residue is treated with a primary amine compound, described in more detail below, in the presence of the enzyme transglutaminase. An exemplary N297 mutant is Asn297Gln (N297Q) mutant. For example, in some embodiments, such an antibody can be prepared by site-directed mutagenesis to remove or disable a sequence or to insert a glutamine residue at a site apart from any interfering structure. Such an antibody can also be isolated from natural or artificial sources. The amide groups in the inserted glutamine residues can be linked to azido groups which are suitable for the click chemistry reactions described herein. Advantageously, the number of inserted glutamine residues can be controlled and consequently, the number of azido groups in the antibody or a glutaminyl-modified antibody or antigen binding molecule can also be controlled, thereby allowing for improved control of Antibody-Drug ratios (ADR), see, for instance, Example 29 and Table 5.

**[0209]** In a specific instance, for a compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), described herein, the Drug Antibody Ratio (DAR) in any antibody drug conjugate sample described herein is about 2 (i.e., **n** is 2). In such instances,

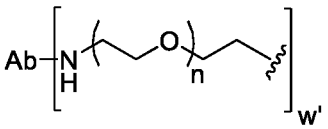
the antibody or antigen binding molecule comprises two heavy chain polypeptides, each with one Gln295 residue, and each Gln295 residue is linked to an azido group which is suitable for click chemistry reactions described herein, allowing a DAR of about 2.

**[0210]** In another specific instance, for a compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**) or Formula (**Vd**), described herein, the Drug Antibody Ratio in any antibody drug conjugate sample described herein is about 4 (i.e., **n** is 4). In such instances, the antibody or antigen binding molecule comprises two heavy chain polypeptides, each with one Gln295 residue, and each Gln295 residue is linked to an azido group which is suitable for click chemistry reactions described herein. In addition, the antibody or antigen binding molecule is further modified prior to conjugation by site-directed mutagenesis to remove or disable a sequence or to insert a glutamine residue at a site apart from any interfering structure. For example, an N297 mutant, Asn297Gln (N297Q) mutant, in each heavy chain is linked to an azido group. Thus the glutaminyl-modified antibody or antigen binding molecule comprises 4 azido groups suitable for click chemistry reactions described herein, allowing a DAR of about 4. See Fig. 19.

**[0211]** In another specific instance, for a compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**) or Formula (**Vd**), described herein, the Drug Antibody Ratio in any antibody drug conjugate sample described herein is about 5 or about 6 (i.e., **n** is 5 or 6). In such instances, the antibody or antigen binding molecule comprises two heavy chain polypeptides, each with one Gln295 residue, and each Gln295 residue is linked to an azido group which is suitable for click chemistry reactions described herein. In addition, the antibody or antigen binding molecule is further modified prior to conjugation by site-directed mutagenesis to remove or disable a sequence or to insert a glutamine residue at a site apart from any interfering structure. For example, an N297 mutant, Asn297Gln (N297Q) mutant, in each heavy chain is linked to an azido group. In certain instances, antibodies to be conjugated contain one or more additional naturally occurring glutamine residues in their variable regions, which can be accessible to transglutaminase and therefore are able to be conjugated, for instance Gln55 (Q55). In these instances, the antibodies after conjugation via transglutaminase will have a DAR value higher than 4. Thus, the glutaminyl-modified antibody or antigen binding molecule may comprise 5 or 6 azido groups suitable for click chemistry reactions described herein, allowing a DAR of about 5 or about 6.

**[0212]** In some embodiments, **BA** of the conjugates described herein is an antibody or an antigen-binding fragment thereof. In some examples, the binding agent that reacts with a compound of Formula (**VI**), Formula (**VII**), Formula (**VIII**), Formula (**IX**), Formula (**IXa**), Formula

(**IXb**), Formula (**IXc**), Formula (**Xa**), Formula (**Xb**), Formula (**Xc**), or Formula (**Xd**), as described herein, is an antibody or an antigen binding fragment thereof. In such instances, the binding agent is an antibody or an antigen-binding fragment thereof, is optionally modified with azido groups, or the binding agent is a modified antibody or antigen-binding fragment thereof of Formula **Ab-1** described herein, or a combination thereof. In some embodiments, the conjugates described herein are derived from azido-functionalized antibodies or antigen binding fragments thereof. In some embodiments, the conjugates described herein are derived from PEG-functionalized antibodies or antigen-binding fragements thereof. In some embodiments, the conjugates described herein are derived from azido and PEG-functionalized antibodies or antigen binding fragments thereof where the PEG is attached on a first end to the antibody or antigen binding fragments thereof and the PEG is attached on the second end to an azido group which is suitable for click chemistry reactions described herein. In certain embodiments, BA of the conjugates described herein is:




where w' is an integer from 1 to 10.

**[0213]** In some examples, herein, the d-Lys can be replaced with a reagent set forth in FIG. 20.

**Primary Amine Compounds**

**[0214]** The primary amine compound useful for the transglutaminase mediated coupling of an antibody (or antigen binding compound) comprising a glutamine can be any primary amine compound deemed useful by the practitioner of ordinary skill. Generally, the primary amine compound has the formula $H_2N$-R, where R can be any group compatible with the antibody and reaction conditions. In certain embodiments, R is alkyl, substituted alkyl, heteroalkyl, or substituted heteroalkyl.

**[0215]** In some embodiments, the primary amine compound comprises a reactive group or protected reactive group. Useful reactive groups include azides, alkynes, cycloalkynes, thiols, alcohols, ketones, aldehydes, acids, esters, hydrozides, analines, and amines. In certain embodiments, the reactive group is selected from the group consisting of azide, alkyne, sulfhydryl, cycloalkyne, aldehyde, and carboxyl.

**[0216]** In certain embodiments, the primary amine compound is according to the formula $H_2N$-**LL**-**X**, where **LL** is a divalent spacer and **X** is a reactive group or protected reactive group. In particular embodiments, **LL** is a divalent polyethylene glycol (PEG) group. In certain embodiments, **X** is selected from the group consisting of –SH, $–N_3$, alkyne, aldehyde, and tetrazole. In particular embodiments, **X** is $–N_3$.

**[0217]** In certain embodiments, the primary amine compound is according to one of the following formulas:

$H_2N$-$(CH_2)_{n'}$-X;

$H_2N$-$(CH_2CH_2O)_{n'}$-$(CH_2)_{p'}$-X;

$H_2N$-$(CH_2)_{n'}$-N(H)C(O)-$(CH_2)_{m'}$-X;

$H_2N$-$(CH_2CH_2O)_{n'}$-N(H)C(O)-$(CH_2CH_2O)_{m'}$-$(CH_2)_{p'}$-X;

$H_2N$-$(CH_2)_{n'}$-C(O)N(H)-$(CH_2)_{m'}$-X;

$H_2N$-$(CH_2CH_2O)_{n'}$-C(O)N(H)-$(CH_2CH_2O)_{m'}$-$(CH_2)_{p'}$-X;

$H_2N$-$(CH_2)_{n'}$-N(H)C(O)-$(CH_2CH_2O)_{m'}$-$(CH_2)_{p'}$-X;

$H_2N$-$(CH_2CH_2O)_{n'}$-N(H)C(O)-$(CH_2)_{m'}$-X;

$H_2N$-$(CH_2)_{n'}$-C(O)N(H)-$(CH_2CH_2O)_{m'}$-$(CH_2)_{p'}$-X; and

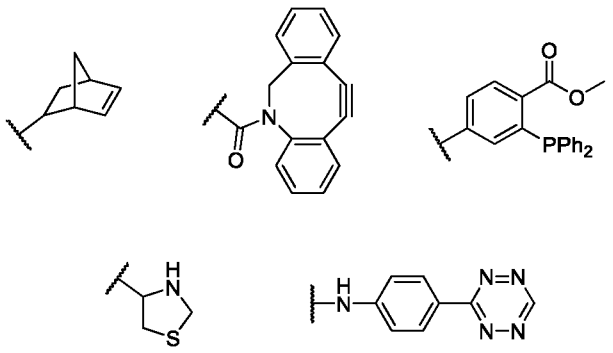
$H_2N$-$(CH_2CH_2O)_{n'}$-C(O)N(H)-$(CH_2)_{m'}$-X;

where subscript **n'** is an integer selected from 1 to 12;

subscript **m'** is an integer selected from 0 to 12;

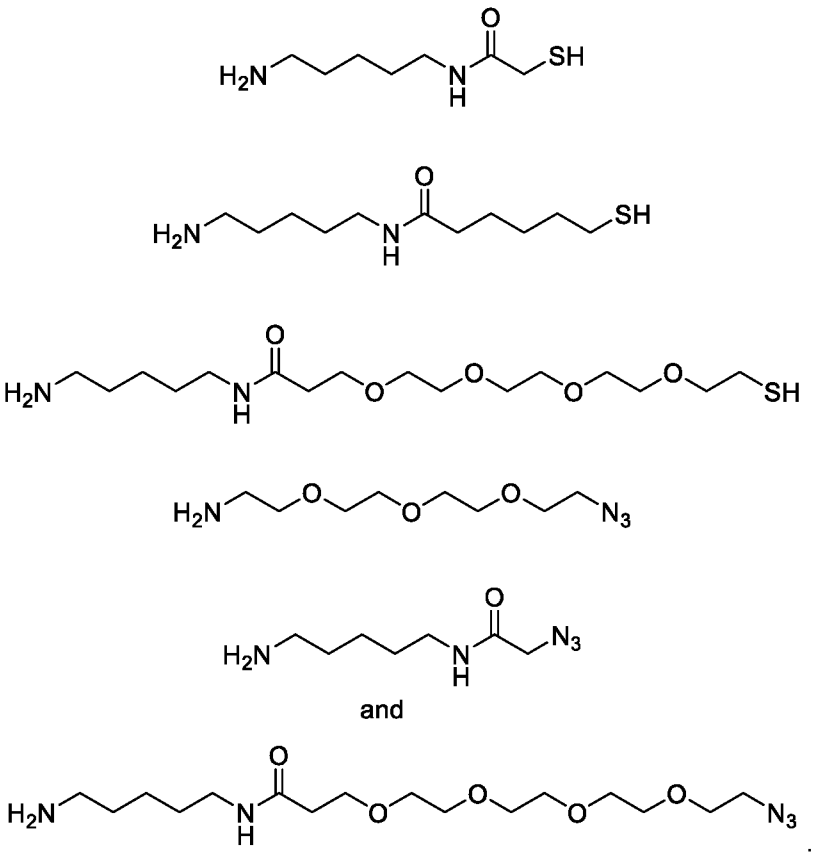
subscript **p'** is an integer selected from 0 to 2;

and X is selected from the group consisting of –SH, $–N_3$, **–C≡CH,** –C(O)H, tetrazole, and any of




**[0218]** In the above, any of the alkyl or alkylene (*i.e.*, $-CH_2-$) groups can optionally be substituted, for example with $C_{1\text{-}8}$alkyl, methylformyl, or $–SO_3H$. In certain embodiments, the alkyl groups are unsubstituted.

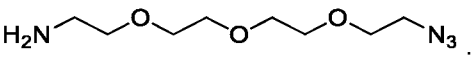
**[0219]** In certain embodiments, the primary amine compound is selected from the group consisting of:



and



[0001] In particular embodiments, the primary amine compound is



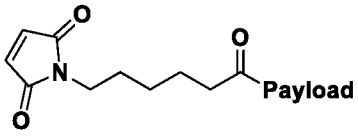
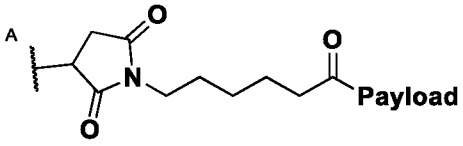
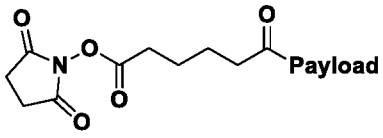
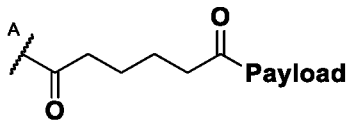
Exemplary conditions for the above reactions are provided in the Examples below.

**Linkers**

**[0220]** The linker **LL** portion of the conjugates described herein is a moiety, for instance a divalent moiety, that covalently links a binding agent to a payload compound described herein. In other instances, the linker **LL** is a trivalent or multivalent moiety that covalently links a binding agent to a payload compound described herein. Suitable linkers may be found, for example, in *Antibody-Drug Conjugates and Immunotoxins*; Phillips, G. L., Ed.; Springer Verlag: New York, 2013; *Antibody-Drug Conjugates*; Ducry, L., Ed.; Humana Press, 2013; *Antibody-Drug Conjugates*; Wang, J., Shen, W.-C., and Zaro, J. L., Eds.; Springer International Publishing, 2015, the contents of each incorporated herein in their entirety by reference. Payload compounds include compounds of FIG. 1, and their residues following bonding or incorporation with linker **LL**. Those of skill in the art will recognize that certain functional groups of the payload moieties are convenient for linking or bonding to linkers and/or binding agents. Those groups include amines, hydroxyls, phosphates, and sugars.

**[0221]** In certain embodiments, the linkers are stable in physiological conditions. In certain embodiments, the linkers are cleavable, for instance, able to release at least the payload

portion in the presence of an enzyme or at a particular pH range or value. In some embodiments, a linker comprises an enzyme-cleavable moiety. Illustrative enzyme-cleavable moieties include, but are not limited to, peptide bonds, ester linkages, hydrazones, and disulfide linkages. In some embodiments, the linker comprises a cathepsin-cleavable linker.

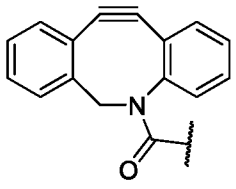
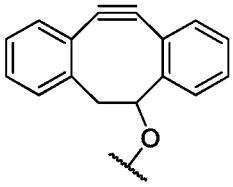
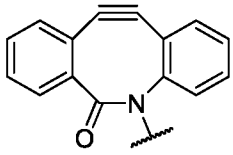
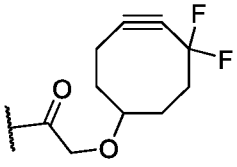
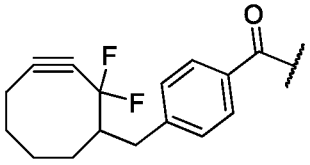
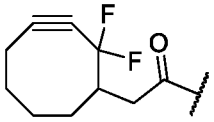
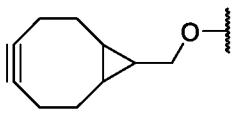
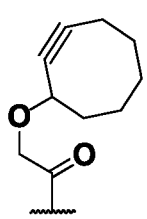
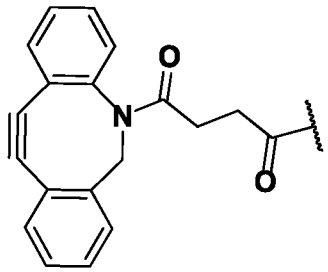
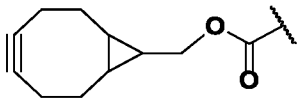
**[0222]** In some embodiments, the linker comprises a non-cleavable moiety. In some embodiments, the non-cleavable linker is derived from  or a residue thereof. In some embodiments, the non-cleavable linker-payload is  , or a regioisomer thereof. In some embodiments, the non-cleavable linker is derived from  or a residue thereof. In some embodiments, the non-cleavable linker-payload is  , or a regioisomer thereof. In one embodiment, the linker is maleimide cyclohexane carboxylate or 4-(*N*-maleimidomethyl)cyclohexanecarboxylic acid (MCC). In the structures,  indicates a bond to a binding agent. In the structures, in some examples,  indicates a click chemistry residue which results from the reaction of, for example, a binding agent and a linker payload.

**[0223]** In some embodiments, suitable linkers include, but are not limited to, those that are chemically bonded to two cysteine residues of a single binding agent, *e.g.*, antibody. Such linkers can serve to mimic the antibody's disulfide bonds that are disrupted as a result of the conjugation process.

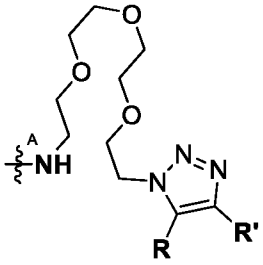
**[0224]** In some embodiments, the linker comprises one or more amino acids. Suitable amino acids include natural, non-natural, standard, non-standard, proteinogenic, non-proteinogenic, and L-, or D- α-amino acids. In some embodiments, the linker comprises alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, or citrulline, a derivative thereof, or combination thereof. In certain embodiments, one or more side chains of the amino acids is linked to a side chain group, described below. In some embodiments, the linker comprises valine and citrulline. In some embodiments, the

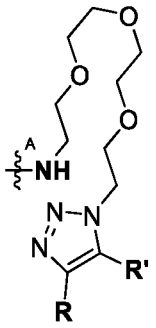
linker comprises lysine, valine, and citrulline. In some embodiments, the linker comprises lysine, valine, and alanine. In some embodiments, the linker comprises valine and alanine.

**[0225]** In some embodiments, the linker comprises a self-immolative group. The self-immolative group can be any such group known to those of skill. In particular embodiments, the self-immolative group is *p*-aminobenzyl (**PAB**), or a derivative thereof. Useful derivatives include *p*-aminobenzyloxycarbonyl (**PABC**). Those of skill will recognize that a self-immolative group is capable of carrying out a chemical reaction which releases the remaining atoms of a linker from a payload.

**[0226]** In certain embodiments, reactive groups include, but are not limited to, alkynes. In certain embodiments, the alkynes are alkynes capable of undergoing 1,3-cycloaddition reactions with alkynes in the absence of copper catalysts such as strained alkynes. Strained alkynes are suitable for strain-promoted alkyne-azide cycloadditions (SPAAC), cycloalkynes, *e.g.*, cyclooctynes, ane benzannulated alkynes. Suitable alkynes include, but are not limited to, dibenzoazacyclooctyne or  (DIBAC), dibenzocyclooctyne or  (DIBO), biarylazacyclooctynone or  (BARAC), difluorinated cyclooctyne or  , or  , or  (DIFO), substituted, *e.g.*, fluorinated alkynes, aza-cycloalkynes, bicycle[6.1.0]nonyne or  (BCN), and derivatives thereof. Particularly useful alkynes include  ,  , and  .

**[0227]** In certain embodiments, the binding agent is bonded directly to a reactive group **RG**. In certain embodiments, the binding agent is bonded to a reactive group **RG** via a spacer. In particular embodiments, the binding agent is bonded to a reactive group.via a PEG spacer. In certain embodiments, the binding agent is prepared by functionalizing with one or more azido groups. Each azido group is capable of reacting with an alkyne in **RG** to form $RG^1$ or **RG'**. In particular embodiments, the binding agent is derivatized with –PEG-$N_3$ linked to a glutamine residue. Exemplary –$N_3$ derivatized binding agents, methods for their preparation, and methods for their use in reacting with **RG** are provided herein. In certain embodiments, **RG** is an alkyne suitable for participation in 1,3-cycloadditions, and $RG^1$ or **RG'** is a 1,2,3-triazolyl moiety formed from the reaction of **RG** with an azido-functionalized binding agent. By way of further example, in certain embodiments, **RG** is linked to the binding agent as shown in



or


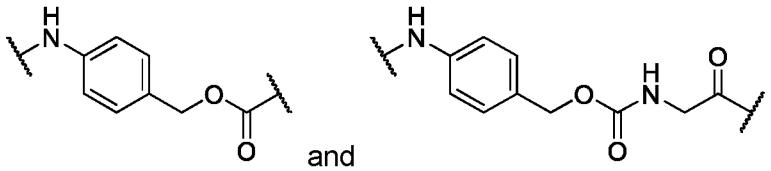
, or a mixture of each regioisomer. Each **R** and **R'** is as described herein.

**[0228]** In an analogous manner, each **HG** described herein may be an azido-functionalized hydrophilic group which reacts with an alkyne in **RG** to form $RG^2$.

**[0229]** In certain embodiments, $RG^1$, or **RG'** is derived from the reaction of a reactive group with a cysteine or lysine residue of an antibody or antigen–binding fragment thereof. In certain embodiments, $RG^1$, or **RG'** is derived from a click chemistry reaction. In some embodiments of said click chemistry reaction, $RG^1$, or **RG'** is derived from a 1,3 cycloaddition reaction between an alkyne and an azide. Non–limiting examples of such $RG^1$, or **RG'** include those derived from strained alkynes, *e.g.*, those suitable for strain–promoted alkyne–azide cycloadditions (SPAAC), cycloalkynes, *e.g.*, cyclooctynes, benzannulated alkynes, and alkynes capable of undergoing 1,3 cycloaddition reactions with azides in the absence of copper catalysts. Suitable $RG^1$, or **RG'** also include, but are not limited to those derived from DIBAC, DIBO, BARAC, substituted, *e.g.*, fluorinated alkynes, aza–cycloalkynes, BCN, and derivatives thereof. Conjugates containing such $RG^1$, or **RG'** groups can be derived from antibodies that have been functionalized with azido groups. Such functionalized antibodies include antibodies functionalized with azido–polyethylene glycol groups. In certain embodiments, such functionalized antibody is derived by reacting an antibody comprising at least one glutamine residue with a compound according to the formula $H_2N$– **LL'**–$N_3$, wherein

LL' is a divalent polyethylene glycol group, in the presence of the enzyme transglutaminase, *e.g.*, microbial transglutaminase. Suitable glutamine residues of an antibody include Q295 or Q55, or those derived by insertion or mutation, *e.g.*, N297Q mutation.

**[0230]** Those of skill will recognize **PAB** and **PABC** as residues of *p*-aminobenzyloxycarbonyl and *p*-aminobenzylcarbamate with the following structures respectively:



and

The **PAB** and **PABC** residues have been shown to facilitate cleavage of certain linkers *in vitro* and *in vivo*.

**[0231]** In some embodiments, the linker is DIBAC-PEG$_e$-dLys(COT-PEG$_6$)-VC-PAB. Subscript **e** is an integer from 0 to 4. In some examples subscript **e** is 0. In some examples subscript **e** is 1. In some examples subscript **e** is 2. In some examples subscript **e** is 3. In some examples subscript **e** is 4.

**[0232]** In some embodiments, the linker is DIBAC-PEG$_e$-dLys(COT-PEG$_e$-taurine)-VC-PAB. Subscript **e** is an integer from 0 to 4. In some examples subscript **e** is 0. In some examples subscript **e** is 1. In some examples subscript **e** is 2. In some examples subscript **e** is 3. In some examples subscript **e** is 4.

**[0233]** In some embodiments, the linker is DIBAC-PEG$_e$-dLys(COT-PEG$_e$-taurine)-VC-PAB. Subscript **e** is an integer from 0 to 4. In some examples subscript **e** is 0. In some examples subscript **e** is 1. In some examples subscript **e** is 2. In some examples subscript **e** is 3. In some examples subscript **e** is 4.

**[0234]** In some embodiments, the linker is DIBAC-PEG$_4$-dLys(COT-dualtaurine)-VC-PAB. Subscript **e** is an integer from 0 to 4. In some examples subscript **e** is 0. In some examples subscript **e** is 1. In some examples subscript **e** is 2. In some examples subscript **e** is 3. In some examples subscript **e** is 4.

**[0235]** In some embodiments, the linker is DIBAC-PEG$_e$-dGlu(taurine)-VC-PAB. Subscript **e** is an integer from 0 to 4. In some examples subscript **e** is 0. In some examples subscript **e** is 1. In some examples subscript **e** is 2. In some examples subscript **e** is 3. In some examples subscript **e** is 4.

**[0236]** In some embodiments, the linker is DIBAC-PEG$_e$-dLys(COT-PEG$_4$-taurine)-VC-PAB. Subscript **e** is an integer from 0 to 4. In some examples subscript **e** is 0. In some examples subscript **e** is 1. In some examples subscript **e** is 2. In some examples subscript **e** is 3. In some examples subscript **e** is 4.

**[0237]** In some embodiments, the linker is DIBAC-$PEG_e$-dLys(COT-$PEG_4$-taurine)-VA. Subscript **e** is an integer from 0 to 4. In some examples subscript **e** is 0. In some examples subscript **e** is 1. In some examples subscript **e** is 2. In some examples subscript **e** is 3. In some examples subscript **e** is 4.

**[0238]** In some embodiments, the linker is DIBAC-$PEG_e$-dLys(COT-$PEG_4$-taurine)-VC. Subscript **e** is an integer from 0 to 4. In some examples subscript **e** is 0. In some examples subscript **e** is 1. In some examples subscript **e** is 2. In some examples subscript **e** is 3. In some examples subscript **e** is 4.

**[0239]** In some embodiments, the linker is DIBAC-$PEG_e$-dGlu(taurine)-VC. Subscript **e** is an integer from 0 to 4. In some examples subscript **e** is 0. In some examples subscript **e** is 1. In some examples subscript **e** is 2. In some examples subscript **e** is 3. In some examples subscript **e** is 4.

**[0240]** In some embodiments, the linker is DIBAC-$PEG_e$-dLys(COT-$PEG_e$-taurine)-VA. Subscript **e** is an integer from 0 to 4. In some examples subscript **e** is 0. In some examples subscript **e** is 1. In some examples subscript **e** is 2. In some examples subscript **e** is 3. In some examples subscript **e** is 4.

**[0241]** In some embodiments, the linker is DIBAC-$PEG_e$-dLys(COT-$PEG_e$-taurine)-VC. Subscript **e** is an integer from 0 to 4. In some examples subscript **e** is 0. In some examples subscript **e** is 1. In some examples subscript **e** is 2. In some examples subscript **e** is 3. In some examples subscript **e** is 4.

**[0242]** In some embodiments, the linker is DIBAC-$PEG_e$-dLys(COT-$PEG_e$-$N^+Me_3$)-vcPAB. Subscript **e** is an integer from 0 to 4. In some examples subscript **e** is 0. In some examples subscript **e** is 1. In some examples subscript **e** is 2. In some examples subscript **e** is 3. In some examples subscript **e** is 4.

**[0243]** In some embodiments, the linker is DIBAC-$PEG_e$-dLys(COT-$PEG_e$-phosphate)-vcPAB. Subscript **e** is an integer from 0 to 4. In some examples subscript **e** is 0. In some examples subscript **e** is 1. In some examples subscript **e** is 2. In some examples subscript **e** is 3. In some examples subscript **e** is 4. In some examples subscript **e** is 3. In some examples subscript **e** is 5.

**[0244]** In some embodiments, the linker is DIBAC-$PEG_e$-dLys(COT-galactose)-vcPAB. Subscript **e** is an integer from 0 to 4. In some examples subscript **e** is 0. In some examples subscript **e** is 1. In some examples subscript **e** is 2. In some examples subscript **e** is 3. In some examples subscript **e** is 4.

**[0245]** In some embodiments, the linker is MAL-$PEG_e$-dLys(COT-galactose)-vcPAB. Subscript **e** is an integer from 0 to 4. In some examples subscript **e** is 0. In some examples subscript **e** is 1. In some examples subscript **e** is 2. In some examples subscript **e** is 3. In some examples subscript **e** is 4.

**[0246]** In some embodiments, the linker is DIBAC-$PEG_e$-dGlu(glucamide)-vcPAB. Subscript **e** is an integer from 0 to 4. In some examples subscript **e** is 0. In some examples subscript **e** is 1. In some examples subscript **e** is 2. In some examples subscript **e** is 3. In some examples subscript **e** is 4.

**[0247]** In some embodiments, the linker is MAL-$PEG_e$-dGlu(glucamide)-vcPAB. Subscript **e** is an integer from 0 to 4. In some examples subscript **e** is 0. In some examples subscript **e** is 1. In some examples subscript **e** is 2. In some examples subscript **e** is 3. In some examples subscript **e** is 4.

**[0248]** In some embodiments, the linker is DIBAC-$PEG_e$-dLys(COT-$PEG_e$-$N^+Me_3$)-vcPAB. Subscript **e** is an integer from 0 to 4. In some examples subscript **e** is 0. In some examples subscript **e** is 1. In some examples subscript **e** is 2. In some examples subscript **e** is 3. In some examples subscript **e** is 4.

**[0249]** In some embodiments, the linker is COT-$PEG_e$-dLys(COT-$PEG_e$-$N^+Me_3$)-vcPAB. Subscript **e** is an integer from 0 to 4. In some examples subscript **e** is 0. In some examples subscript **e** is 1. In some examples subscript **e** is 2. In some examples subscript **e** is 3. In some examples subscript **e** is 4.

**[0250]** In some embodiments, the linker is BCN-$PEG_e$-dLys(COT-$PEG_e$-$N^+Me_3$)-vcPAB. Subscript **e** is an integer from 0 to 5. In some examples subscript **e** is 0. In some examples subscript **e** is 1. In some examples subscript **e** is 2. In some examples subscript **e** is 3. In some examples subscript **e** is 4. In some examples subscript **e** is 5.

**[0251]** In some embodiments, the linker is DIBAC-$PEG_e$-dLys(COT-$PEG_e$-$N^+Me_3$)-VA . Subscript **e** is an integer from 0 to 4. In some examples subscript **e** is 0. In some examples subscript **e** is 1. In some examples subscript **e** is 2. In some examples subscript **e** is 3. In some examples subscript **e** is 4.

**[0252]** In some embodiments, the linker is DIBAC-$PEG_4$-dLys(COT-$PEG_6$)-VC-PAB.

**[0253]** In some embodiments, the linker is DIBAC-$PEG_4$-dLys(COT-$PEG_4$-taurine)-VC-PAB.

**[0254]** In some embodiments, the linker is DIBAC-$PEG_4$-dLys(COT-$PEG_4$-taurine)-VC-PAB.

**[0255]** In some embodiments, the linker is DIBAC-$PEG_4$-dLys(COT-dualtaurine)-VC-PAB.

**[0256]** In some embodiments, the linker is DIBAC-$PEG_4$-dGlu(taurine)-VC-PAB.

**[0257]** In some embodiments, the linker is DIBAC-$PEG_4$-dLys(COT-$PEG_4$-taurine)-VC-PAB.

**[0258]** In some embodiments, the linker is DIBAC-$PEG_4$-dLys(COT-$PEG_4$-taurine)-VA.

**[0259]** In some embodiments, the linker is DIBAC-$PEG_4$-dLys(COT-$PEG_4$-taurine)-VC.

**[0260]** In some embodiments, the linker is DIBAC-$PEG_4$-dGlu(taurine)-VC.

**[0261]** In some embodiments, the linker is DIBAC-$PEG_4$-dLys(COT-$PEG_4$-taurine)-VA.

**[0262]** In some embodiments, the linker is DIBAC-$PEG_4$-dLys(COT-$PEG_4$-taurine)-VC.

**[0263]** In some embodiments, the linker is DIBAC-$PEG_4$-dLys(COT-$PEG_4$-$N^+Me_3$)-vcPAB

**[0264]** In some embodiments, the linker is DIBAC-$PEG_4$-dLys(COT-$PEG_5$-phosphate)-vcPAB

**[0265]** In some embodiments, the linker is DIBAC-$PEG_4$-dLys(COT-galactose)-vcPAB

**[0266]** In some embodiments, the linker is MAL-$PEG_4$-dLys(COT-galactose)-vcPAB

**[0267]** In some embodiments, the linker is DIBAC-$PEG_4$-dGlu(glucamide)-vcPAB

**[0268]** In some embodiments, the linker is MAL-$PEG_4$-dGlu(glucamide)-vcPAB

**[0269]** In some embodiments, the linker is DIBAC-PEG4-dLys(COT-PEG4-N+$Me_3$)-vcPAB

**[0270]** In some embodiments, the linker is COT-$PEG_4$-dLys(COT-$PEG_4$-$N^+Me_3$)-vcPAB

**[0271]** In some embodiments, the linker is BCN-$PEG_4$-dLys(COT-$PEG_4$-$N^+Me_3$)-vcPAB

**[0272]** In some embodiments, the linker is DIBAC-$PEG_4$-dLys(COT-$PEG_4$-$N^+Me_3$)-VA

**Reactive Linker-Payloads**

**[0273]** Conjugates provided herein can be prepared from reactive linker-payloads comprising reactive groups **$RG^1$** or **RG'**, or **$RG^2$**, as described above. The reactive linker payloads can be linked to hydrophilic groups, as described herein, and/or binding agents according to the methods described below.

**[0274]** In some embodiments, set forth herein is a reactive linker-payload which comprises a reactive group linked to at least one payload moiety and linked to at least one hydrophilic moiety via a covalent linker, wherein said covalent linker is bonded directly or indirectly to each of the reactive group, the payload moiety, the hydrophilic moiety.

**[0275]** As illustrated herein, in some examples, the reactive group is bonded directly to a covalent linker, such as a lysine amino acid. This means that the reactive group is one bond position away from the covalent linker. In some of these examples, the covalent linker is also bonded directly to a payload moiety. This means that the covalent linker is one bond position away from a payload such as, but not limited to, MMAE, a steroid, an LXR modulator, or any payload set forth herein. In some of these examples, the covalent linker is also bonded

directly to a hydrophilic moiety. This means that the covalent linker is one bond position away from a hydrophilic residue, such as the hydrophilic residues set forth herein. In some of these examples, the covalent linker is a lysine amino acid or a derivative thereof.

**[0276]** In other examples, the reactive group is bonded indirectly to a covalent linker. This means that the reactive group is more than one bond position away from the covalent linker. This also means that the reactive group is bonded through another moiety to the covalent linker. For example, the reactive group may be bonded to a polyethylene glycol group which is bonded to the covalent linker. In some of these examples, the covalent linker is also bonded indirectly to a payload moiety. This means that the covalent linker is more than one bond position away from a payload such as, but not limited to, MMAE, or a steroid, or any payload set forth herein. This also means that the covalent linker is bonded through another moiety to the payload. For example, the covalent linker may be bonded to a dipeptide, such as but not limited to Val-Ala or Val-Cit, which may be bonded to PAB which may be bonded to the payload. In some of these examples, the covalent linker is also bonded indirectly to a hydrophilic moiety. This means that the covalent linker is more than one bond position away from a hydrophilic moiety, such as the hydrophilic residues set forth herein. This also means that the covalent linker is bonded through another moiety to the hydrophilic residue. For example, the covalent linker may be bonded to a polyethylene glycol group which may be bonded to a reactive group which may be bonded to the hydrophilic residue. In some of these examples, the covalent linker is a lysine amino acid or a derivative thereof.

**[0277]** In the formulas, herein, each $AA^1$ is an amino acid. In some examples in the formulas, herein, each $AA^2$ is an amino acid. In some examples in the formulas, herein, each $AA^2$ is a di-peptide. In some examples in the formulas, herein, each $AA^2$ is a tri-peptide. Suitable amino acids for each $AA^1$ or $AA^2$ include natural, non-natural, standard, non-standard, proteinogenic, non-proteinogenic, and L-, or D-amino acids. In some embodiments, the linker comprises alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, or citrulline, a derivative thereof, or a combination thereof. In certain embodiments, one or more side chains of the amino acids are linked to a side chain group, described below. In certain embodiments, $AA^1$ is an amino acid selected from alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, or citrulline, a derivative thereof, or a combination thereof. In certain embodiments, $AA^1$ is an amino acid with three functional groups to link to a payload, to a binding agent (e.g., antibody or antigen binding fragment thereof), and to a linker comprising a hydrophilic group, e.g., lysine, aspargine, glutamic acid, aspartic acid, glutamine, cysteine,

threonine, serine, or tyrosine. In certain embodiments, $AA^1$ is lysine. In certain embodiments, $AA^1$ is glutamine. In certain embodiments, $AA^1$ is lysine or a derivative of lysine. In certain embodiments, $AA^1$ is L-lysine. In certain embodiments, the $AA^1$ is D-lysine. In certain embodiments, $AA^1$ is glutamic acid. In certain embodiments, the $AA^2$ is valine-citrulline. In some embodiments, the $AA^2$ is citrulline-valine. In some embodiments, the $AA^2$ is valine-alanine. In some embodiments, the $AA^2$ is alanine-valine. In some embodiments, the $AA^2$ is valine-glycine. In some embodiments, the $AA^2$ is glycine-valine. In some embodiments, the $AA^1$-$AA^2$ is glutamine-valine-citrulline. In some embodiments, the $AA^1$-$AA^2$ is lysine-valine-citrulline. In some embodiments, the $AA^1$-$AA^2$ is lysine-valine-alanine. In some embodiments, the $AA^1$-$AA^2$ is glutamine-valine-alanine.

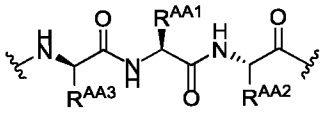
**[0278]** In some embodiments, $AA^2$ is a dipeptide selected from valine–citrulline, citrulline–valine, lysine–phenylalanine, phenylalanine–lysine, valine–asparagine, asparagine–valine, threonine–asparagine, asparagine–threonine, serine–asparagine, asparagine–serine, phenylalanine–asparagine, asparagine–phenylalanine, leucine–asparagine, asparagine–leucine, isoleucine–asparagine, asparagine–isoleucine, glycine–asparagine, asparagine–glycine, glutamic acid–asparagine, asparagine–glutamic acid, citrulline–asparagine, asparagine–citrulline, alanine–asparagine, or asparagine–alanine.

**[0279]** In some examples, $AA^1$-$AA^2$ is lysine-valine–alanine or alanine–valine-lysine.

**[0280]** In some examples, $AA^1$-$AA^2$ is lysine-valine–citrulline or citrulline–valine-lysine.

**[0281]** In some examples, $AA^2$ is valine–citrulline or citrulline–valine.

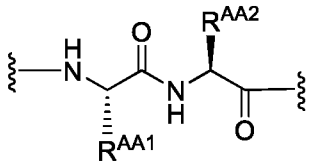
**[0282]** In some examples, $AA^2$ is valine–alanine or alanine–valine.

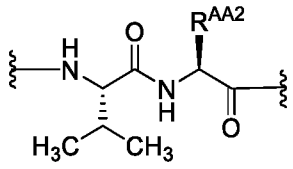
**[0283]** In some examples, $AA^1$-$AA^2$ is  . In some of these examples, $R^{AA1}$ is an amino acid side chain, $R^{AA2}$ is an amino acid side chain, and $R^{AA3}$ is an amino acid side chain that is bonded directly or indirectly to a hydrophilic moiety.

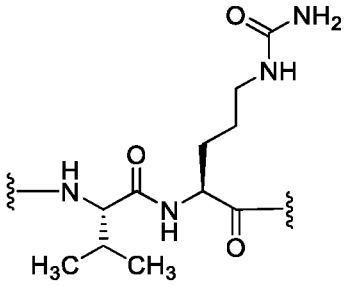
**[0284]** In some examples, $R^{AA1}$ is a valine sidechain.

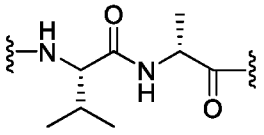
**[0285]** In some examples $R^{AA2}$ is an alanine sidechain.

**[0286]** In some examples, $R^{AA2}$ is a citrulline sidechain.

**[0287]** In some examples, $AA^1$-$AA^2$ is  . In some of these examples, $R^{AA1}$ is an amino acid side chain, and wherein $R^{AA2}$ is an amino acid side chain.

**[0288]** In some examples, $AA^2$ is  .

**[0289]** In some examples, $AA^2$ is  .

**[0290]** In some examples, $AA^2$ is  .

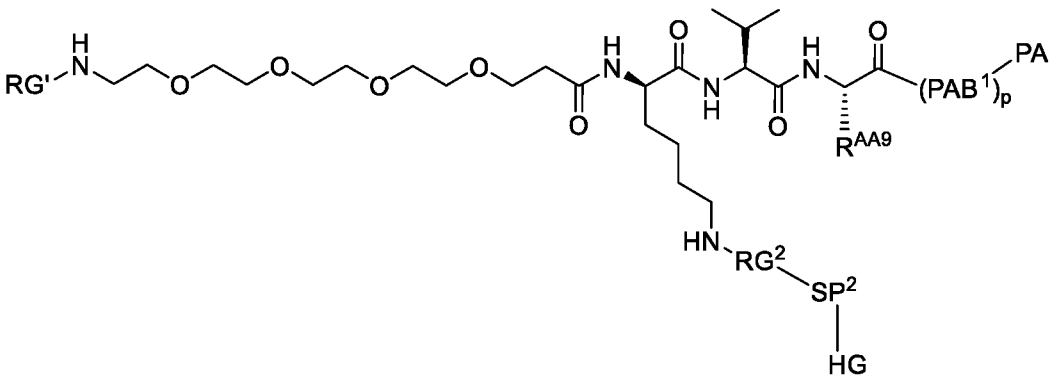
**[0291]** In some examples, set forth herein is a linker-payload comprising a compound set forth herein.

**[0292]** In some examples, set forth herein is a linker-payload comprising a compound set forth herein bonded to an oxygen or a primary or secondary nitrogen of any compound described herein.

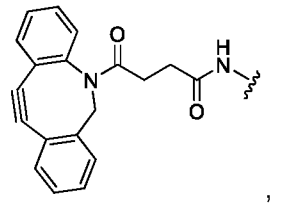
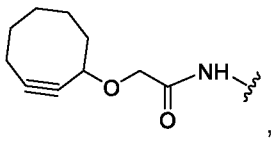
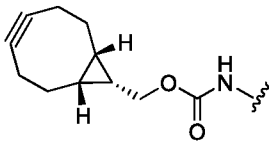
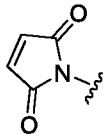
**[0293]** In some examples, set forth herein is an antibody-drug-conjugate comprising the compound or linker-payload set forth herein bonded to an antibody, or an antigen binding fragment thereof.

**[0294]** Conjugates provided here can be prepared from reactive linker-paylaods with reactive groups **RG** as described above and in the Examples section and figures. The reactive linker payloads can be linked to hydrophilic groups and/or binding agents according to the methods described herein, e.g., in the Examples section and figures.

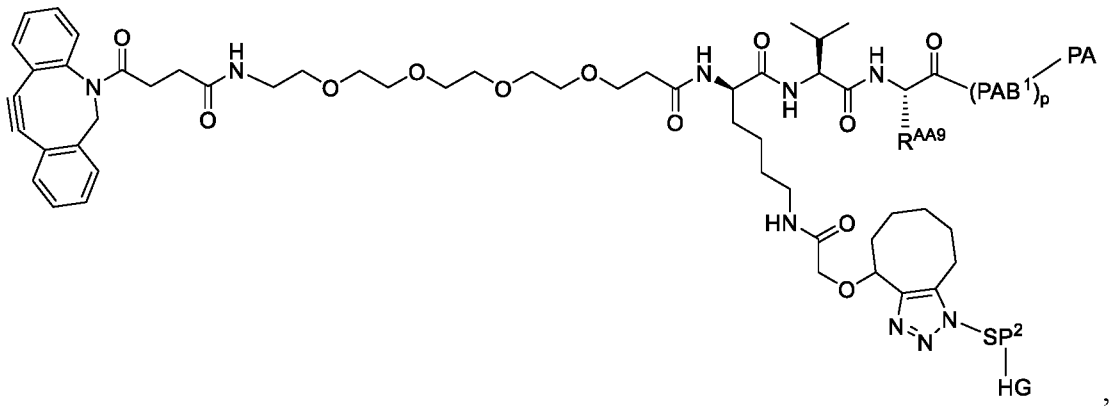
**[0295]** In some embodiments, the reactive linker-payload is:



or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof, or a regioisomer thereof, wherein **PAB**[1] is **PAB** or **PABC** as described herein; **p** is 0 or 1; $R^{AA9}$ is methyl or -$(CH_2)_3$-NH-C(=O)-$NH_2$; and **RG'**, $RG^2$, **PA**, $SP^2$ and **HG** are as defined herein.

**[0296]** In some embodiments, **RG'** is  ,  ,  , or  , where each  indicates the atom through which the group is attached to the rest of the molecule.

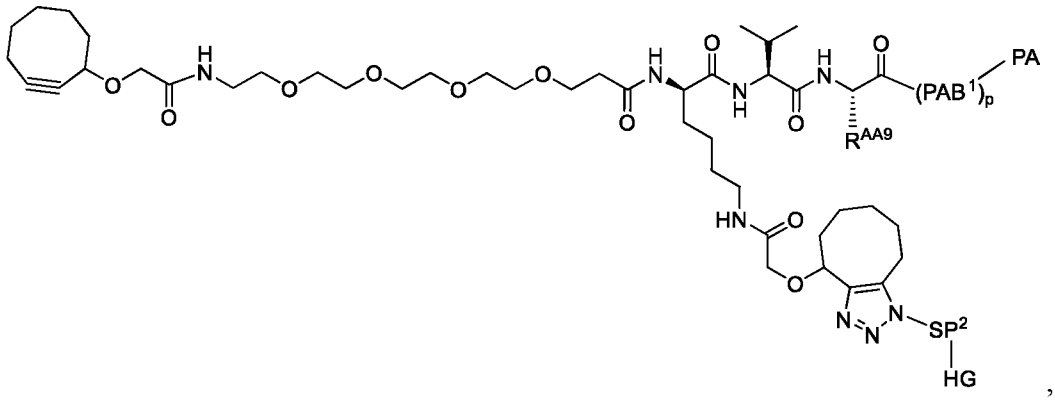
**[0297]** In some embodiments, a reactive linker payload is:

 ,

or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof, or a regioisomer thereof, wherein **PAB**[1] is **PAB** or **PABC** as described herein; **p** is 0 or 1; $R^{AA9}$ is methyl or -$(CH_2)_3$-NH-C(=O)-$NH_2$; and **PA**, $SP^2$ and **HG** are as defined herein. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload A of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload B of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload C of FIG. 1. In some of these embodiments, **HG** is a taurine

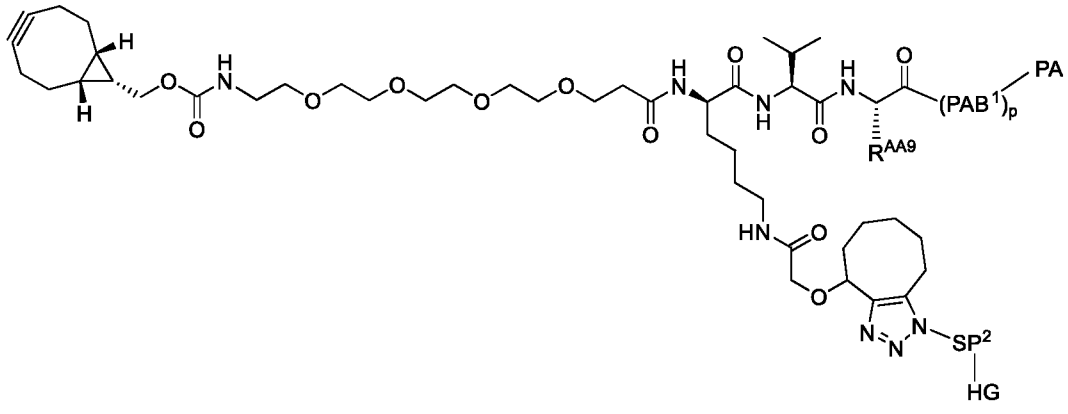
or dualtaurine as defined herein and **PA** is payload D of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload E of FIG. 1.

**[0298]** In some embodiments, a reactive linker payload is:



or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof, or a regioisomer thereof, wherein **PAB**$^1$ is **PAB** or **PABC** as described herein; **p** is 0 or 1; $R^{AA9}$ is methyl or -$(CH_2)_3$-NH-C(=O)-$NH_2$; and **PA**, **SP**$^2$ and **HG** are as defined herein. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload A of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload B of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload C of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload D of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload E of FIG. 1.

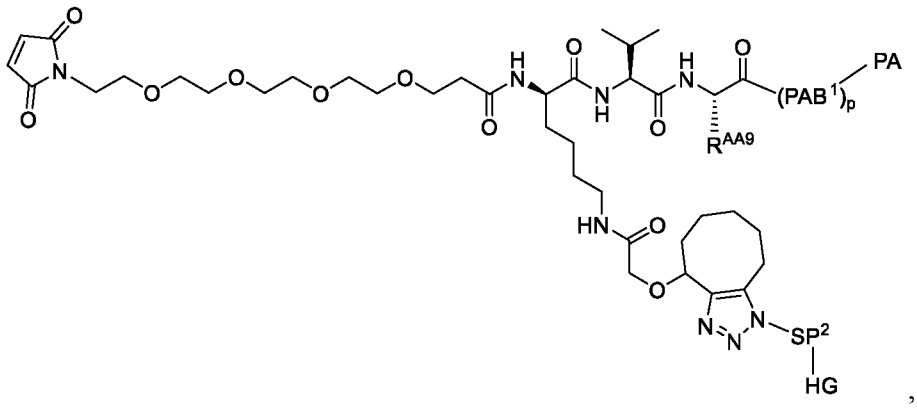
**[0299]** In some embodiments, a reactive linker payload is:



or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof, or a regioisomer thereof, wherein **PAB**$^1$ is **PAB** or **PABC** as described herein; **p** is 0 or 1; $R^{AA9}$ is methyl or -$(CH_2)_3$-NH-C(=O)-$NH_2$; and **PA**, **SP**$^2$ and **HG** are as defined herein. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload A of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and

**PA** is payload B of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload C of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload D of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload E of FIG. 1.

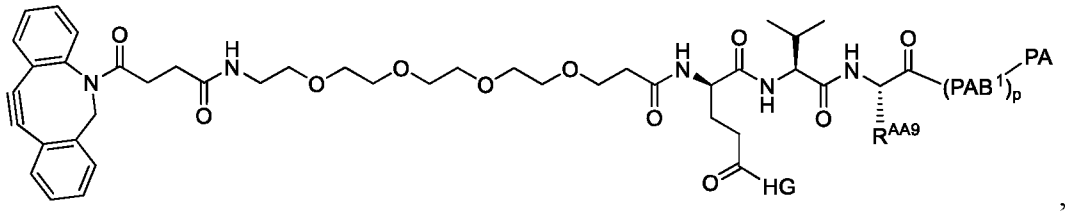
**[0300]** In some embodiments, a reactive linker payload is:



,

or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof, or a regioisomer thereof, wherein **PAB$^1$** is **PAB** or **PABC** as described herein; **p** is 0 or 1; **R$^{AA9}$** is methyl or -$(CH_2)_3$-NH-C(=O)-$NH_2$; and **PA**, **SP$^2$** and **HG** are as defined herein. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload A of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload B of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload C of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload D of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload E of FIG. 1.

**[0301]** In some embodiments, a reactive linker payload is:

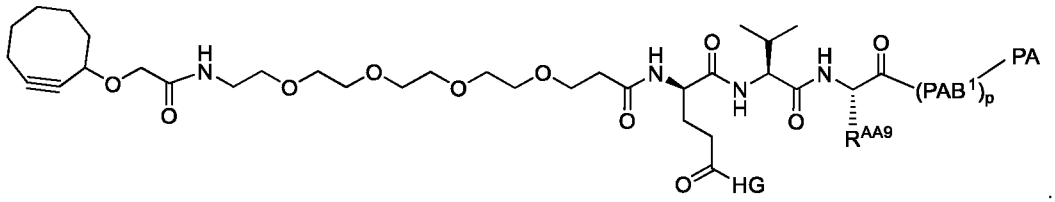


,

or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof, or a regioisomer thereof, wherein **PAB$^1$** is **PAB** or **PABC** as described herein; **p** is 0 or 1; **R$^{AA9}$** is methyl or -$(CH_2)_3$-NH-C(=O)-$NH_2$; and **PA**, and **HG** are as defined herein. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload A of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload B of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload C of FIG. 1. In some of these embodiments, **HG** is a taurine or

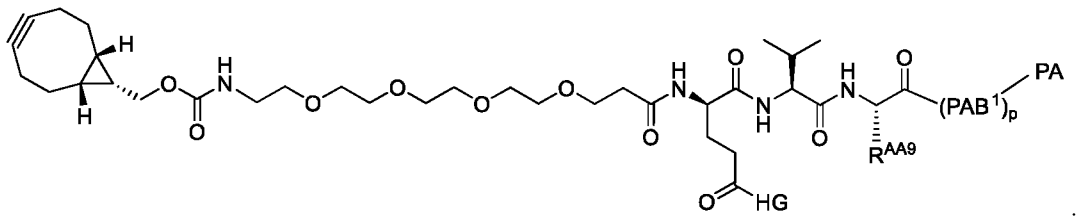
dualtaurine as defined herein and **PA** is payload D of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload E of FIG. 1.

**[0302]** In some embodiments, a reactive linker payload is:



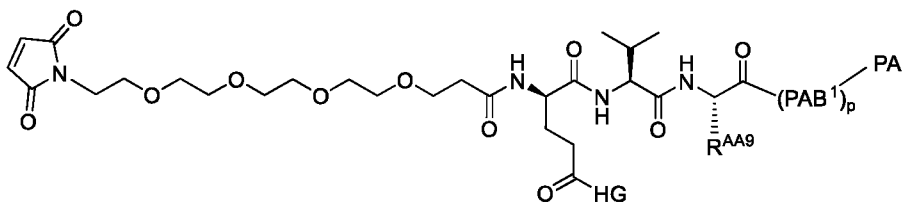
or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof, or a regioisomer thereof, wherein **PAB$^1$** is **PAB** or **PABC** as described herein; **p** is 0 or 1; **R$^{AA9}$** is methyl or -$(CH_2)_3$-NH-C(=O)-$NH_2$; and **PA**, and **HG** are as defined herein. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload A of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload B of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload C of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload D of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload E of FIG. 1.

**[0303]** In some embodiments, a reactive linker payload is:



or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof, or a regioisomer thereof, wherein **PAB$^1$** is **PAB** or **PABC** as described herein; **p** is 0 or 1; **R$^{AA9}$** is methyl or -$(CH_2)_3$-NH-C(=O)-$NH_2$; and **PA**, and **HG** are as defined herein. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload A of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload B of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload C of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload D of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload E of FIG. 1.

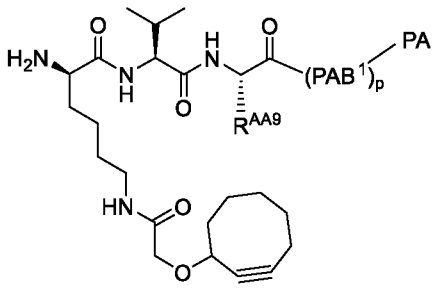
**[0304]** In some embodiments, a reactive linker payload is:



or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof, or a regioisomer thereof, wherein **PAB**$^{1}$ is **PAB** or **PABC** as described herein; **p** is 0 or 1; $R^{AA9}$ is methyl or -$(CH_2)_3$-NH-C(=O)-$NH_2$; and **PA**, and **HG** are as defined herein. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload A of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload B of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload C of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload D of FIG. 1. In some of these embodiments, **HG** is a taurine or dualtaurine as defined herein and **PA** is payload E of FIG. 1.

**[0305]** In any instance of the reactive linker payloads described herein, the reactive linker payload is linked to a binding agent (e.g., **Ab** or **Ab-1** as defined herein) via reaction of an azide (e.g., by click chemistry) with a strained alkyne. In other embodiments, the reactive linker payload is linked to a binding agent (e.g., **Ab** or **Ab-1** as defined herein) via a nucleophilic attack on the succinimide moiety in the reactive linker payload.

**[0306]** In some embodiments, a reactive linker payload is:



or a pharmaceutically acceptable salt, solvate, or stereoisomeric form thereof, or a regioisomer thereof, wherein **PAB**$^{1}$ is **PAB** or **PABC** as described herein; **p** is 0 or 1; $R^{AA9}$ is methyl or -$(CH_2)_3$-NH-C(=O)-$NH_2$; and **PA** is as defined herein. In such instances, a hydrophilic linker comprising an azide is linked to the reactive linker payload via reaction of an azide (e.g., by click chemistry) with a strained alkyne.

**Methods of preparing compounds**

**[0307]** The compounds provided herein can be prepared, isolated, or obtained by any method apparent to those of skill in the art. Exemplary methods of preparation are described in detail

in the examples below. In certain embodiments, compounds provided herein can be prepared according to methods described in the Examples and in Figures 1-19.

**[0308]** The conjugates described herein can be synthesized by coupling the linker-payloads described herein with a binding agent, for example, an antibody under standard conjugation conditions (see, *e.g.*, Doronina *et al. Nature Biotechnology* 2003, 21, 7, 778, which is incorporated herein by reference in its entirety). When the binding agent is an antibody, the antibody may be coupled to a linker-payload via one or more cysteine or lysine residues of the antibody. Linker-payloads can be coupled to cysteine residues, for example, by subjecting the antibody to a reducing agent, for example, dithiotheritol, to cleave the disulfide bonds of the antibody, purifying the reduced antibody, for example, by gel filtration, and subsequently treating the antibody with a linker-payload containing a suitable reactive moiety, for example, a maleimido group. Suitable solvents include, but are not limited to water, DMA, DMF, and DMSO. Linker-payloads containing a reactive group, for example, an activated ester or acid halide group, can be coupled to lysine residues of the antibody. Suitable solvents include, but are not limited to water, DMA, DMF, and DMSO. Conjugates can be purified using known protein techniques, including, for example, size exclusion chromatography, dialysis, and ultrafiltration/diafiltration.

**[0309]** Binding agents, for example antibodies, can also be conjugated via click chemistry reactions. In some embodiments of said click chemistry reactions, the linker-payload includes a reactive group, for example an alkyne, that is capable of undergoing a 1,3-cycloaddition reaction with an azide. Such suitable reactive groups are described above. The antibody includes one includes one or more azide groups. Such antibodies include antibodies functionalized with, for example, azido-polyethylene glycol groups. In certain embodiments, such functionalized antibody is derived by treating an antibody having at least one glutamine residue, for example, heavy chain Gln295, with a primary amine compound in the presence of the enzyme transglutaminase. Such antibodies may also include Asn297Gln (N297Q) mutants. In certain embodiments, such functionalized antibody is derived by treating an antibody having at least two glutamine residues, for example, heavy chain Gln295 and heavy chain Gln297, with a primary amine compound in the presence of the enzyme transglutaminase. Such antibodies include Asn297Gln (N297Q) mutants. In certain embodiments, the antibody has two heavy chains as described in this paragraph for a total of two or a total of four glutamine residues. In certain instances, antibodies to be conjugated contain one or more additional naturally occurring Glutamine residues in their variable regions, which can be accessible to transglutaminase and therefore are able to be conjugated, for instance Gln55 (Q55). In these instances, the antibodies after conjugation via transglutaminase may have a DAR value higher than 4. Such antibodies include Asn297Gln

(N297Q) mutants. In some embodiments, the antibody has two heavy chains as described in this paragraph for a total of two or a total of four or a total of five or six glutamine residues.

**[0310]** In certain embodiments, the antibody comprises a glutamine residue at one or more heavy chain positions numbered 295 in the EU numbering system. In the present disclosure, this position is referred to as glutamine 295, or as Gln295, or as Q295. Those of skill will recognize that this is a conserved glutamine residue in the wild type sequence of many antibodies. In other useful embodiments, the antibody can be engineered to comprise a glutamine residue. Techniques for modifying an antibody sequence to include a glutamine residue are within the skill of those in the art (see, e.g., Ausubel *et al. Current Protoc. Mol. Biol.*).

**[0311]** In certain embodiments, the antibody comprises two glutamine residues, one in each heavy chain. In particular embodiments, the antibody comprises a Q295 residue in each heavy chain. In further embodiments, the antibody comprises one, two, three, four, five, six, seven, eight, or more glutamine residues. These glutamine residues can be in heavy chains, light chains, or in both heavy chains and light chains. These glutamine residues can be wild-type residues, or engineered residues. The antibodies can be prepared according to standard techniques. In certain embodiments, the antibodies comprise a Q55 residue.

**[0312]** Those of skill will recognize that antibodies are often glycosylated at residue N297, near residue Q295 in a heavy chain sequence. Glycosylation at residue N297 can interfere with a transglutaminase at residue Q295 (Dennler *et al.*, *supra*). Accordingly, in advantageous embodiments, the antibody is not glycosylated. In certain embodiments, the antibody is deglycoslated or aglycosylated. In particular embodiments, an antibody heavy chain has an N297 mutation. Alternatively stated, the antibody is mutated to no longer have an asparagine residue at position 297. In particular embodiments, an antibody heavy chain has an N297Q mutation. Such an antibody can be prepared by site-directed mutagenesis to remove or disable a glycosylation sequence or by site-directed mutagenesis to insert a glutamine residue at a site apart from any interfering glycosylation site or any other interfering structure. Such an antibody also can be isolated from natural or artificial sources.

**[0313]** The antibody without interfering glycosylation is then treated with a primary amine compound. In certain embodiments, an aglycosylated antibody is treated with a primary amine compound to produce a glutaminyl-modified antibody. In certain embodiments, a deglycosylated antibody is reacted with a primary amine compound to produce a glutaminyl-modified antibody.

**[0314]** The primary amine can be any primary amine that is capable of forming a covalent bond with a glutamine residue in the presence of a transglutaminase. Useful primary amines

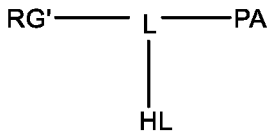
are described herein. The transglutaminase can be any transglutaminase deemed suitable by those of skill in the art. In certain embodiments, the transglutaminase is an enzyme that catalyzes the formation of an isopeptide bond between a free amine group on the primary amine compound and the acyl group on the side chain of a glutamine residue. Transglutaminase is also known as protein-glutamine-γ-glutamyltransferase. In particular embodiments, the transglutaminase is classified as EC 2.3.2.13. The transglutaminase can be from any source deemed suitable. In certain embodiments, the transglutaminase is microbial. Useful transglutaminases have been isolated from *Streptomyces mobaraense, Streptomyces cinnamoneum*, *Streptomyces griseo-carneum*, *Streptomyces lavendulae*, and *Bacillus subtilis.* Non-microbial transglutaminases, including mammalian transglutaminases, can also be used. In certain embodiments, the transglutaminase can be produced by any technique or obtained from any source deemed suitable by the practitioner of skill. In particular embodiments, the transglutaminase is obtained from a commercial source.

**[0315]** In particular embodiments, the primary amine compound comprises a reactive group capable of further reaction after transglutamination. In these embodiments, the glutaminyl-modified antibody can be reacted or treated with a reactive payload compound or a reactive linker-payload compound to form an antibody-payload conjugate. In certain embodiments, the primary amine compound comprises an azide as described herein.

**[0316]** In certain embodiments, the glutaminyl-modified antibody is reacted or treated with a reactive linker-payload to form an antibody-payload conjugate. The reaction can proceed under conditions deemed suitable by those of skill in the art. In certain embodiments, the glutaminyl-modified antibody is contacted with the reactive linker-payload compound under conditions suitable for forming a bond between the glutaminyl-modified antibody and the linker-payload compound. Suitable reaction conditions are well known to those in the art.

**[0317]** Examples of such reactions are provided in the Examples below.

**[0318]** In some examples, set forth herein is a method of making a conjugate comprising treating or contacting a compound with a binding agent under coupling conditions, wherein the compound comprises a reactive linker bonded to at least one payload moiety and linked to at least one cyclodextrin moiety via a covalent linker, wherein said covalent linker is bonded directly or indirectly to each of the reactive linker, the payload moiety, the cyclodextrin moiety. In some examples, the compound which reacts with a binding agent is a compound according to Formula (**VI**):



(VI)

or a pharmaceutically acceptable salt, solvate, stereoisomer, or derivative thereof,

wherein **RG'** is a reactive group; **L** is a trivalent linker; **HL** is a hydrophilic residue;and **PA** is a payload residue.

**[0319]** In some examples, the binding agent that reacts with a compound of Formula (**II**) is an antibody or an antigen binding fragment thereof.

**[0320]** In some examples, herein, the D-Lys can be replaced with a reagent set forth in FIG. 20.

**[0321]** In some examples, the cyclodextrin-containing moieties may react in a bioorthogonal reaction selected from a [3+2] click reaction, a Diels-Alder reaction, a reductive-amination, a photoclick reaction, or other reactions.

**[0322]** Other useful bioorthogonal reactions suitable for use with the methods herein include, but are not limited to, the Staudinger ligation, a click reaction, a tetrazine ligation, and a photo-click reaction.

**[0323]** The following reference shows example reactions and reagents that may be used with the bioorthogonal reactions set forth herein: Zheng, Mengmeng, *et al.*, *Molecules* 2015, *20*, 3190-3205, the contents of which are herein incorporated by reference in their entirety for all purposes.

**Pharmaceutical Compositions and Methods of Treatment**

**[0324]** Provided herein are methods of treating and preventing diseases, conditions, or disorders comprising administering a therapeutically or prophylactically effective amount of one or more of the compounds disclosed herein, for example, one or more of the compounds of a formula provided herein. A person of skill will appreciate that the diseases, disorders, and/or conditions include, but are not limited to, those associated with the antigens listed herein.

**[0325]** The compounds described herein can be administered alone or together with one or more additional therapeutic agents. The one or more additional therapeutic agents can be administered just prior to, concurrent with, or shortly after the administration of the compounds described herein. The present disclosure also includes pharmaceutical compositions comprising any of the compounds described herein in combination with one or more additional therapeutic agents, and methods of treatment comprising administering such combinations to subjects in need thereof.

**[0326]** Suitable additional therapeutic agents include, but are not limited to: a second glucocorticoid, an autoimmune therapeutic agent, a hormone, a biologic, or a monoclonal

antibody. Suitable therapeutic agents also include, but are not limited to any pharmaceutically acceptable salts, acids, or derivatives of a compound set forth herein.

**[0327]** In some embodiments of the methods described herein, multiple doses of a compound described herein (or a pharmaceutical composition comprising a combination of a compound described herein and any of the additional therapeutic agents mentioned herein) may be administered to a subject over a defined time course. The methods according to this aspect of the disclosure comprise sequentially administering to a subject multiple doses of a compound described herein. As used herein, "sequentially administering" means that each dose of the compound is administered to the subject at a different point in time, *e.g.*, on different days separated by a predetermined interval (*e.g.*, hours, days, weeks or months). The present disclosure includes methods which comprise sequentially administering to the patient a single initial dose of a compound described herein, followed by one or more secondary doses of the compound, and optionally followed by one or more tertiary doses of the compound.

**[0328]** The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the compounds described herein. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses can all contain the same amount the compound described herein, but generally can differ from one another in terms of frequency of administration. In certain embodiments, the amount of the compound contained in the initial, secondary and/or tertiary doses varies from one another (*e.g.*, adjusted up or down as appropriate) during the course of treatment. In certain embodiments, two or more (*e.g.*, 2, 3, 4, or 5) doses are administered at the beginning of the treatment regimen as "loading doses" followed by subsequent doses that are administered on a less frequent basis (*e.g.*, "maintenance doses").

**[0329]** In certain exemplary embodiments of the present disclosure, each secondary and/or tertiary dose is administered 1 to 26 (*e.g.*, 1, 1½, 2, 2½, 3, 3½, 4, 4½, 5, 5½, 6, 6½, 7, 7½, 8, 8½, 9, 9½, 10, 10½, 11, 11½, 12, 12½, 13, 13½, 14, 14½, 15, 15½, 16, 16½, 17, 17½, 18, 18½, 19, 19½, 20, 20½, 21, 21½, 22, 22½, 23, 23½, 24, 24½, 25, 25½, 26, 26½, or more) weeks after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose the compound which is administered to a patient prior to the administration of the very next dose in the sequence with no intervening doses.

**[0330]** The methods according to this aspect of the disclosure may comprise administering to a patient any number of secondary and/or tertiary doses of the compound. For example, in certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain embodiments, only a single tertiary dose is administered to the patient. In other embodiments, two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient. The administration regimen may be carried out indefinitely over the lifetime of a particular subject, or until such treatment is no longer therapeutically needed or advantageous.

**[0331]** In embodiments involving multiple secondary doses, each secondary dose may be administered at the same frequency as the other secondary doses. For example, each secondary dose may be administered to the patient 1 to 2 weeks or 1 to 2 months after the immediately preceding dose. Similarly, in embodiments involving multiple tertiary doses, each tertiary dose may be administered at the same frequency as the other tertiary doses. For example, each tertiary dose may be administered to the patient 2 to 12 weeks after the immediately preceding dose. In certain embodiments of the disclosure, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

**[0332]** The present disclosure includes administration regimens in which 2 to 6 loading doses are administered to a patient at a first frequency (*e.g.*, once a week, once every two weeks, once every three weeks, once a month, once every two months, etc.), followed by administration of two or more maintenance doses to the patient on a less frequent basis. For example, according to this aspect of the disclosure, if the loading doses are administered at a frequency of once a month, then the maintenance doses may be administered to the patient once every six weeks, once every two months, once every three months, etc.

**[0333]** The present disclosure includes pharmaceutical compositions of the compounds and/or conjugates described herein, *e.g.*, the compounds of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), *e.g.*, compositions comprising a compound described herein, a salt, stereoisomer, polymorph thereof, and a pharmaceutically acceptable carrier, diluent, and/or excipient. Examples of suitable carriers, diluents and excipients include, but are not limited to, buffers for maintenance of proper composition pH (*e.g.*, citrate buffers, succinate buffers, acetate buffers, phosphate buffers, lactate buffers, oxalate buffers, and the like), carrier proteins (*e.g.*, human serum albumin), saline, polyols (*e.g.*, trehalose, sucrose,

xylitol, sorbitol, and the like), surfactants (*e.g.*, polysorbate 20, polysorbate 80, polyoxolate, and the like), antimicrobials, and antioxidants.

**[0334]** In some examples, set forth herein is a method of treating a disease, disorder or condition comprising administering to a patient having said disorder a therapeutically effective amount of a compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), or a pharmaceutical composition thereof.

**[0335]** In some examples, set forth herein is a method of preventing a disease, disorder or condition comprising administering to a patient having said disorder a prophylactically effective amount of a compound of Formula (**I**), Formula (**II**), Formula (**III**), Formula (**IV**), Formula (**IVa**), Formula (**IVb**), Formula (**IVc**), Formula (**Va**), Formula (**Vb**), Formula (**Vc**), or Formula (**Vd**), or a pharmaceutical composition thereof.

**[0336]** In some examples, set forth herein is a method of treating or preventing a disease, disorder, or condition selected from the group consisting of a proliferative disorder, a neurodegenerative disorder, an immunological disorder, an autoimmune disease, an inflammatory disorder, a dermatological disease, a metabolic disease, cardiovascular disease, and a gastrointestinal disease.

**[0337]** Provided herein are methods for modulating LDLR (low-density lipoprotein receptor) protein expression or cholesterol efflux in a cell comprising contacting said cell with an antibody drug conjugate (ADC), wherein the ADC comprises an antibody targeting said cell, hydrophilic residue, and LXR agonist.

**[0338]** The proliferative disorder can be any proliferative disorder known to those of skill. In certain embodiments, proliferative disorders include, without limitation, oncology disorders, where the oncology disorder can be any cancer disorder known to those of skill. In certain embodiments, provided herein are methods of treating or preventing a melanoma. In certain embodiments, provided herein are methods of treating or preventing metastatic melanoma. In certain embodiments, provided herein are methods of treating or preventing lung cancer. In certain embodiments, provided herein are methods of treating or preventing EGFR-tyrosine kinase inhibitor resistant lung cancer. In certain embodiments, provided herein are methods of treating or preventing oral cancer. In certain embodiments, provided herein are methods of treating or preventing oral squamous cell carcinoma. In certain embodiments, provided herein are methods of treating or preventing prostate cancer. In certain embodiments, provided herein are methods of treating or preventing Hodgkin's lymphoma. In certain embodiments, provided herein are methods of treating or preventing breast cancer.

**[0339]** The neurodegenerative disorder can be any neurodegenerative disorder known to those of skill. In certain embodiments, provided herein are methods of treating or preventing Alzheimer's disease. In certain embodiments, provided herein are methods of treating or preventing Parkinson's disease. In certain embodiments, provided herein are methods of treating or preventing Huntington's disease. In certain embodiments, provided herein are methods of treating or preventing amyotrophic lateral sclerosis. In certain embodiments, provided herein are methods of treating or preventing myelin gene expression. In certain embodiments, provided herein are methods of treating or preventing myelination and remyelination conditions, diseases, or disorders.

**[0340]** The immunological disorder can be any immunological disorder known to those of skill. In certain embodiments, provided herein are methods of treating or preventing inflammatory bowel disease. In certain embodiments, provided herein are methods of treating or preventing ulcerative colitis. In certain embodiments, provided herein are methods of treating or preventing Crohn's disease.

**[0341]** The inflammatory disorder can be any inflammatory disorder known to those of skill. In certain embodiments, provided herein are methods of treating or preventing arthritis. In certain embodiments, provided herein are methods of treating or preventing rheumatoid arthritis.

**[0342]** The metabolic disease can be any metabolic disease known to those of skill. In certain embodiments, dyslipidemia is selected from the group consisting of hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, hyperlipoproteinemia, HDL deficiency, ApoA-I deficiency, and cardiovascular disease such as coronary artery disease (including, for example, treatment and prevention of angina, myocardial infarction and sudden cardiac death); atherosclerosis (including, for example, treatment and prevention of atherosclerosis); and restenosis (including, for example, preventing or treating atherosclerotic plaques which develop as a consequence of medical procedures such as balloon angioplasty). In certain embodiments, provided herein are methods of treating or preventing diabetes.

**[0343]** The cardiovascular disease can be any cardiovascular disease known to those of skill. In certain embodiments, provided herein are methods of treating or preventing atherosclerosis. In certain embodiments, provided herein are methods of treating or preventing atherosclerosis derived from abnormal macrophage processing. In certain embodiments, provided herein are methods of treating or preventing atherosclerosis derived from the formation of oxidized low-density lipoproteins (oxLDLs), where marcrophages fail to process oxLDLs. In certain embodiments, provided herein are methods of treating or preventing ischemic heart disease. In certain embodiments, provided herein are methods of treating or preventing stroke. In certain embodiments, provided herein are methods of treating or preventing hypertensive heart disease. In certain embodiments, provided herein are

methods of treating or preventing aortic aneurysm. In certain embodiments, provided herein are methods of treating or preventing endocarditis. In certain embodiments, provided herein are methods of treating or preventing peripheral artery disease. In certain embodiments, provided herein are methods of treating or preventing combinations of any of the diseases provided in this paragraph.

**[0344]** In some examples, set forth herein is a method for modulating the function of a nuclear receptor. By way of non-limiting example, the function may be selected from expression/secretion of inflammatory mediators (*e.g.* cytokines, chemokines), cholesterol regulation, cholesterol intake, cholesterol efflux, cholesterol oxidation, migration, chemotaxis, apoptosis and necrosis, an inflammatory activity, lipid regulation, apoptosis, migration, chemotaxis, gene transcription, and protein expression.

**[0345]** In some examples, set forth herein is a method of treating a disease or disorder in a patient in need thereof comprising administering to the patient a compound or pharmaceutical composition set forth herein. In some examples, the administered compound is an antibody-drug conjugate set forth herein.

**[0346]** In some examples, set forth herein is a method of an antibody-drug conjugate comprising the step of contacting a binding agent with a linker-payload compound under conditions suitable for forming a bond between the binding agent and the compound.

**[0347]** In some examples, set forth herein is a method of treating a proliferative disease, a metabolic disease, inflammation, or a neurodegenerative disease in a subject comprising administering to the subject of an effective treatment amount of a compound or pharmaceutical composition set forth herein. In some examples, the administered compound is an antibody-drug conjugate set forth herein.

**[0348]** In some examples, set forth herein is a method of treating a disease, disorder, or condition in a subject comprising administering to the subject of an effective treatment amount of a compound or pharmaceutical composition set forth herein. In some examples, the administered compound is an antibody-drug conjugate set forth herein.

**[0349]** In some examples, set forth herein is a method of treating a proliferative disease in a subject comprising administering to the subject of an effective treatment amount of a compound or pharmaceutical composition set forth herein. In some examples, the administered compound is an antibody-drug conjugate set forth herein.

**[0350]** In some examples, set forth herein is a method of treating a metabolic disease in a subject comprising administering to the subject of an effective treatment amount of a compound or pharmaceutical composition set forth herein. In some examples, the administered compound is an antibody-drug conjugate set forth herein.

**[0351]** In some examples, set forth herein is a method of treating inflammation in a subject comprising administering to the subject of an effective treatment amount of a compound or pharmaceutical composition of set forth herein. In some examples, the administered compound is an antibody-drug conjugate set forth herein.

**[0352]** In some examples, set forth herein is a method of treating a neurodegenerative disease in a subject comprising administering to the subject of an effective treatment amount of a compound or pharmaceutical composition set forth herein. In some examples, the administered compound is an antibody-drug conjugate set forth herein.

## EXAMPLES

**[0353]** Reagents and solvents were obtained from commercial sources such as Sinopharm Chemical Reagent Co. (SCRC), Sigma-Aldrich, Alfa, or other vendors, unless explicitly stated otherwise.

**[0354]** 1H NMR and other NMR spectra were recorded on a Bruker AVIII 400 or Bruker AVIII 500. The data were processed with Nuts software or MestReNova software, measuring proton shifts in parts per million (ppm) downfield from an internal standard tetramethylsilane (TMS).

**[0355]** HPLC-MS measurements were run on an Agilent 1200 HPLC/6100 SQ System using the follow conditions:

**[0356]** Method A for HPLC-MS measurements included, as the Mobile Phase: A: Water (0.01% trifluoroacetic acid (TFA)), B: acetonitrile (0.01% TFA); Gradient Phase: 5% of B increased to 95% of B within 15 minutes (min); Flow Rate: 1.0 mL/min; Column: SunFire C18, 4.6x50 mm, 3.5 µm; Column Temperature: 50 ºC. Detectors: Analog to Digital Converter (ADC) Evaporative Light-scattering Detector (ELSD), Diode array detector (DAD) (214 nm and 254 nm), electrospray ionization-atmospheric ionization (ES-API).

**[0357]** Method B for HPLC-MS measurements included, as the Mobile Phase: A: Water (10 mM $NH_4HCO_3$), B: acetonitrile; Gradient Phase: 5% to 95% of B within 15 min; Flow Rate: 1.0 mL/min; Column: XBridge C18, 4.6x50 mm, 3.5 µm; Column Temperature: 50 ºC. Detectors: ADC ELSD, DAD (214 nm and 254 nm), mass selective detector (MSD) (ES–API).

**[0358]** LC-MS measurements were run on an Agilent 1200 HPLC/6100 SQ System using the following conditions:

**[0359]** Method A for LC-MS measurements included, as the Instrument: WATERS 2767; column: Shimadzu Shim–Pack, PRC–ODS, 20x250mm, 15 µm, two connected in series; Mobile Phase: A: Water (0.01% TFA), B: acetonitrile (0.01% TFA); Gradient Phase: 5% of B increased to 95% of B within 3 min; Flow Rate: 1.8 – 2.3 mL/min; Column: SunFire C18,

4.6x50 mm, 3.5 µm; Column Temperature: 50 ºC. Detectors: ADC ELSD, DAD (214 nm and 254 nm), ES–API.

**[0360]** Method B for LC-MS measurement included, as the Instrument: Gilson GX–281; column: Xbridge Prep C18 10 µm OBD, 19x250 mm; Mobile Phase: A: Water (10 mM NH4HCO3), B: Acetonitrile; Gradient Phase: 5% to 95% of B within 3 min; Flow Rate: 1.8 – 2.3 mL/min; Column: XBridge C18, 4.6x50 mm, 3.5 µm; Column Temperature: 50 ºC. Detectors: ADC ELSD, DAD (214 nm and 254 nm), MSD (ES–API).

**[0361]** Preparative high-pressure liquid chromatography (Prep-HPLC) in an acidic (Method A) or basic (Method B) solvent system was utilized on a Gilson GX–281 instrument. The acidic solvent system used a Waters SunFire 10 µm C18 column (100Å, 250x19 mm), and solvent A for prep-HPLC was water/0.05% TFA and solvent B was acetonitrile. The elution conditions were a linear gradient increase of solvent B from 5% to 100% over a time period of 20 min at a flow rate of 30 mL/min. The basic solvent system included a Waters Xbridge 10 µm C18 column (100 Å, 250x19 mm), and solvent A used for prep-HPLC was water/10 mM ammonium bicarbonate ($NH_4HCO_3$) and solvent B was acetonitrile. The elution conditions were a linear gradient increase of solvent B from 5% to 100% over a time period of 20 min at a flow rate of 30 mL/min.

**[0362]** Flash chromatography was performed on a Biotage instrument, with Agela Flash Column silica–CS cartridges; Reversed phase flash chromatography was performed on Biotage instrument, with Boston ODS or Agela C18 cartridges.

**[0363]** Analytical chiral HPLC method - SFC conditions

**[0364]** Instrument: SFC Method Station (Thar, Waters)

**[0365]** Column: CHIRALPAK AD-H/AS-H/OJ-H/OD-H 4.6×100mm, 5 µm (Daicel)

**[0366]** Column temperature: 40 ºC

**[0367]** Mobile phase: CO2/ IPA(0.1% DEA)= 55/45

**[0368]** Flow: 4.0 ml/min

**[0369]** Back Pressure: 120 Bar

**[0370]** Injection volume: 2 µL

**[0371]** Preparative chiral HPLC method - SFC conditions

**[0372]** Instrument: SFC-80 (Thar, Waters)

**[0373]** Column: CHIRALPAK AD-H/AS-H/OJ-H/OD-H 20×250mm, 10 µm (Daicel)

**[0374]** Column temperature: 35 ºC

**[0375]** Mobile phase: CO2/ IPA(0.2% Methanol Ammonia)= 30/70

**[0376]** Flow rate: 80 g/min

**[0377]** Back pressure: 100 bar

**[0378]** Detection wavelength: 214 nm

**[0379]** Cycle time: 6.0 min

**[0380]** Sample solution: 1500 mg dissolved in 70 mL Methanol

**[0381]** Injection volume: 2 mL (loading: 42.86 mg/injection)

**[0382]** As used herein, the symbols and conventions used in these processes, schemes, and examples, regardless of whether a particular abbreviation is specifically defined, are consistent with those used in the contemporary scientific literature, for example, the Journal of the American Chemical Society or the Journal of Biological Chemistry. Specifically, but without limitation, the following abbreviations may be used in the Examples and throughout the specification:

**[0383]** Specifically, but without limitation, the following abbreviations may be used in the Examples and throughout the specification:

| **Abbreviation** | **Term** |
|---|---|
| ADC | Antibody-drug conjugate |
| Aglycosylated antibody | Antibody does not have any glycan |
| aq | Aqueous |
| Boc | N-tert-butoxycarbonyl |
| BupH™ | Thermo Scientific Prod# 28372, containing 100 mM sodium phosphate and 150 mM sodium chloride, potassium free, pH was adjusted from 7.2 to 7.6-7.8 MQ, unless otherwise noted. |
| COT | Cyclooctynol |
| Da | Dalton |
| DAR | Drug to antibody ratio. |
| DCM | Dichloromethane |
| DIBAC | Dibenz[*b*,*f*]azocine, 11,12-didehydro-5,6-dihydro- or Dibenzocyclooctyne or Dibenz[b,f]azocine-5(6H)-butanoic acid, 11,12-didehydro |
| DIBAC-Suc | Dibenz[b,f]azocine-5(6H)-butanoic acid, 11,12-didehydro |
| BCN | Bicyclo[6.1.0]nonyne |
| MAL | Maleimide |

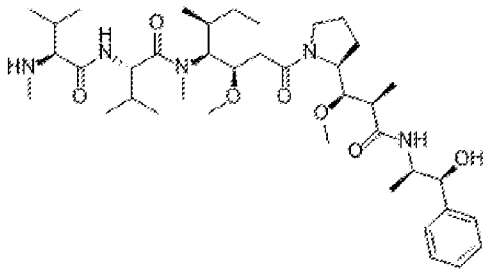
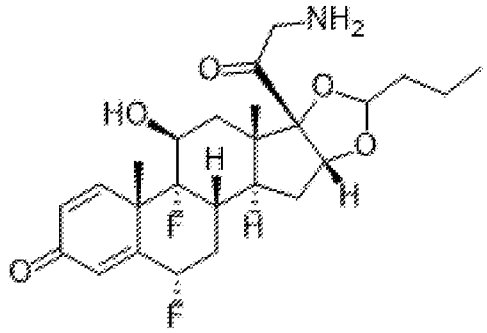
| | |
|---|---|
| COT | Cyclooctynol |
| DIBACT | 3*H*-Benzo[*c*]-1,2,3-triazolo[4,5-*e*][1]benzazocine, 8,9-dihydro- |
| DIPEA | Diisopropylethylamine |
| DMF | N,N-dimethylformamide |
| DMSO | Dimethylsulfoxide |
| ESI | Electrospray ionization |
| Fmoc | Fluorenylmethyloxycarbonyl |
| Fmoc–vcPAB–PNP | N–Fmoc–L–valine–L–citrulline–p–aminobenzyl alcohol p–nitrophenyl carbonate |
| g | Gram |
| HATU | 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate |
| HC | Heavy chain of immunoglobulin |
| HEK | Human embryonic kidney (cells) |
| HPLC | High performance liquid chromatography |
| hr or hrs | Hours |
| LC | Light chain of immunoglobulin |
| LC | Liquid chromatography |
| MC | Maleimidocaproyl |
| mg | milligrams |
| min | minutes |
| mL | milliliters |
| mM | millimolar |
| MMAE | Monomethyl auristatin E |
| MS | Mass spectrometry |
| MSD | Mass-selective detector |
| MTG | Microbial transglutaminase (MTG EC 2.3.2.13, Zedira, Darmstadt, Germany) |
| MW | Molecular weight |
| ncADC | Non-Cytotoxic antibody drug conjugation |
| NHS | N-hydroxy succinimide |
| nM | nanomolar |
| NMR | Nuclear magnetic resonance |
| NOESY | Nuclear Overhauser effect spectroscopy |
| PAB | Para-aminobezyloxy(carbonyl) |
| PBS | 10 mM sodium phosphate buffer and 150 mM sodium chloride |

| | |
|---|---|
| PBSg | 10 mM phosphate, 150 mM sodium chloride, 5% glycerol |
| PEG | Polyethyleneglycol |
| ppm | Parts per million (chemical shift) |
| RP | Reversed phase |
| RT | Room temperature |
| SDS-PAGE | Sodium dodecylsulfate polyacrylamide gel electrophoresis |
| SEC | Size exclusion chromatography |
| Suc | Succinic acid |
| TCEP | Tris(2-carboxyethyl)phosphine hydrochloride |
| TEA | Triethylamine |
| TFA | Trifluoroacetic acid |
| TG | Transglutaminase |
| THF | Tetrahydrofuran |
| TOF | Time-of-flight |
| UPLC | Ultra Performance Liquid Chromatography |
| UV | Ultraviolet |
| VA | Valine-Aniline |
| VC | Valine-citrulline |
| µL | microliters |
| µM | micromolar |

## PREPARATION METHODS

TABLE 1

List of payloads

| # | Structure | HPLC purity | cLogP | MF | MW (Cal.) | MS (M+H) | Synthesis |
|---|---|---|---|---|---|---|---|
| A |  | >95 | 3.51 | $C_{39}H_{67}N_5O_7$ | 717.98 | N.D. | Commercial |
| B |  | 98 | 2.33 | $C_{25}H_{33}F_2NO_5$ | 465.54 | 466.2 | FIG. 4 |



SUBSTITUTE SHEET (RULE 26)

| # | Structure | HPLC purity | cLogP | MF | MW (Cal.) | MS (M+H) | Synthesis |
|---|---|---|---|---|---|---|---|
| C |  | 97 | 3.94 | $C_{31}H_{37}F_2NO_6$ | 557.63 | 558.2 | FIG. 4 |
| D |  | >95 | 7.28 | $C_{34}H_{44}N_2O_3$ | 528.72 | 529.3 | FIG. 5 |
| E |  | 92 | 5.94 | $C_{37}H_{49}N_3O_4$ | 615.8 | 616.3 | FIG. 5 |

TABLE 2

List of charged linker payloads

| LP | Ex | Payload | Linker name | Structure |
|---|---|---|---|---|
| 1a | 17 | MMAE | DIBAC-PEG$_4$-dLys(COT-PEG$_6$)-vcPAB |  |
| Ib | 17A | MMAE | DIBAC-PEG$_4$-dLys(COT-PEG$_4$-N$^+$Me$_3$)-vcPAB |  |
| Ic | 18 | MMAE | DIBAC-PEG$_4$-dLys(COT-PEG$_4$-taurine)-vcPAB |  |
| Id | 18A | MMAE | DIBAC-PEG$_4$-dLys(COT-PEG$_5$-phosphate)-vcPAB |  |

| | | | |
|---|---|---|---|
| Ie-1 | 18B | MMAE | DIBAC-PEG$_4$-dLys(COT-galactose)-vcPAB |
| If-1 | 18C | MMAE | MAL-PEG$_4$-dLys(COT-galactose)-vcPAB |
| Ig | 18D | MMAE | DIBAC-PEG$_4$-dGlu(glucamide)-vcPAB |
| Ih-1 | 18E | MMAE | MAL-PEG$_4$-dGlu(glucamide)-vcPAB |

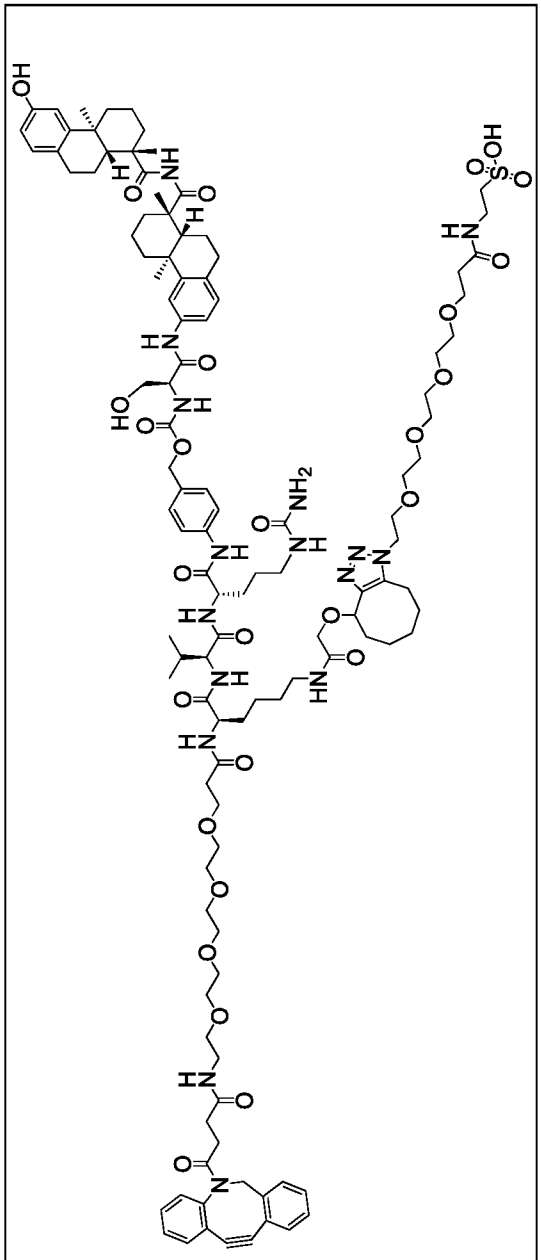
| | | | |
|---|---|---|---|
| 1e | 19 | B | DIBAC-PEG4-dLys(COT-PEG4-taurine)-vcPAB |
| IIb | 19A | B | DIBAC-PEG4-dLys(COT-PEG4-N+Me3)-vcPAB |
| 1f | 20 | B | DIBAC-PEG4-dLys(COT-dualtaurine)-vcPAB |

| | | | |
|---|---|---|---|
| 1h | 21 | B | DIBAC-PEG4-dGlu(taurine)-vcPAB |
| 1i | 22 | C | DIBAC-PEG4-dLys(COT-PEG4-taurine)-vcPAB |
| 1j | 23 | C | DIBAC-PEG4-dLys(COT-PEG4-taurine)-VA |

| | | | |
|---|---|---|---|
| 1k | 24 | C | DIBAC-PEG$_4$-dLys(COT-PEG$_4$-taurine)-vc |
| IIId | 24A | C | COT-PEG$_4$-dLys(COT-PEG$_4$-N$^+$Me$_3$)-vcPAB |
| IIIe | 24B | C | BCN-PEG$_4$-dLys(COT-PEG$_4$-N$^+$Me$_3$)-vcPAB |

| | | | |
|---|---|---|---|
| 1l | 25 | C | DIBAC-PEG$_4$-dGlu(taurine)-vc |
| 1m | 26 | D | DIBAC-PEG$_4$-dLys(COT-PEG$_4$-taurine)-VA |
| IVb | 26A | D | DIBAC-PEG$_4$-dLys(COT-PEG$_4$-N$^+$Me$_3$)-VA |

| | | | |
|---|---|---|---|
| 1q | 27 | E | DIBAC-PEG$_4$-dLys(COT-PEG$_4$-taurine)-vc |



## EXAMPLE 1

**General preparation of linker payloads** (See FIG. 2)

**[0384]** Synthesis of Linker-Payloads **1** (except **1h** and **1l**) started from Fmoc-vcPAB-PNP (**2a**), Fmoc-Val-Ala-OH (**2b**) and Fmoc-Val-Cit-OH (**2c**) with payloads **A-D** gave the carbamates (**3a-g**). Amides (**5a-h**) were synthesized from **3a-f** with Fmoc-*D*-Lys-COT (**4a**). **5a-f** were cyclized with azides (**6a-d**) to afford **7**. Finally **7** reacted with acid **8a** or its active ester **8b** to give Linker-Payloads **1** (except **1h** and **1l**).

**[0385]** Synthesis of Linker-Payloads **I, II, III and IV** (except **Ig-1, Ih-1, 1h** and **1l**) started from Fmoc-vcPAB-PNP (**2a**), Fmoc-Val-Ala-OH (**2b**) and Fmoc-Val-Cit-OH (**2c**) with payloads **A-E**, gave the carbamates (**3a-g**). Amides (**5a-g**) were synthesized from **3a-g** with Fmoc-*D*-Lys-COT (**4a**). **5a-g** were cyclized with azides (**6a-g**) to afford **7**. Finally **7** reacted with acid **8a** or active esters **8b-e** to give Linker-Payloads **I, II, III and IV** (except **Ig-1, Ih-1**).

## EXAMPLE 2

**Preparation of payloads 1h and 1l** (See FIG. 3)

**[0386]** Synthesis of linker-payloads **1h** and **1l** started from **3b** and **3e** with Fmoc-dGlu-taurine (**4b**) under the condensation condition of EDCI followed by the de-Fmoc reaction and finally amidation with active ester **8b**. Synthesis of linker-payloads **1h** and **1l** started from condensation of **3b** and **3e** with Fmoc-dGlu-taurine (**4b**), separately, followed by a reaction removing Fmoc and finally amidation with active ester **8b**..

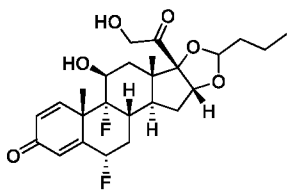
**[0387]** Synthesis of linker-payloads **Ig-1** and **Ih-1** was started from amide coupling reactions of **3a** with Fmoc-dGlu-acetal glucamide (**4c**), followed by the condensation with **8e,** and finally de-protection by TFA and then condensation with **8b.**

## EXAMPLE 3

**Preparation of linker payloads B and C** (See FIG. 4)

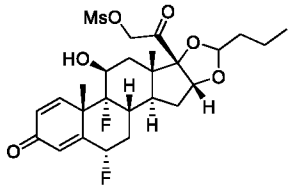
**[0388]** MMAE (**A**) was commercially available with CAS 474645-27-7. Steroidal payloads **B** and **C** were prepared according to Fig. 4 starting from commercial fluocinolone acetonide **9** (CAS: 67-73-2). Compound **10**, obtained from **9** by ketal-exchange with butyraldehyde in the presence of perchloric acid, was converted to mesylate **11** followed by replacement of the mesylate group with azide moiety to form **12** that were further reduced to amine **B**. Otherwise, the mesylate moiety in **11** was also replaced by 4-amino-phenol to afford aniline **C**.

**[0389] (1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-8-(2-hydroxyacetyl)-9,13-dimethyl-6-propyl-5,7-dioxapentacyclo[10.8.0.$0^{2,9}$.$0^{4,8}$.$0^{13,18}$]icosa-14,17-dien-16-one (10)**



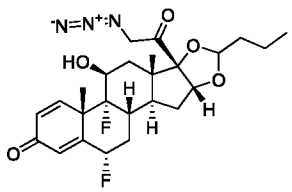
**[0390]** To a mixture of fluocinolone acetonide (**9**, 0.90 g, 2.0 mmol) and silica gel (18 g) in heptanes (90 mL) was added butyraldehyde (0.27 mL, 3.0 mmol) at 10 °C and the suspension was stirred at 10-20 °C for 10 minutes. To the mixture was added perchloric acid (70%, 0.68 mL, 8.3 mmol) dropwise at 0 °C. The reaction mixture was then stirred at 10-20 °C overnight. Most of fluocinolone acetonide **9** was consumed according to TLC and LCMS. The reaction mixture was diluted with petroleum ether and quenched with *sat. aq.* $Na_2CO_3$. The suspension was filtered and the solid was washed with DCM/methanol (v/v = 1). The combined filtrate was concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (0-100% ethyl acetate in petroleum ether) to give compound **10** (0.15 g, 16% yield) as a white solid. ESI m/z: 467.1 $(M + H)^+$.

**[0391] 2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-Difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethyl methanesulfonate (11)**



**[0392]** To a solution of compound **10** (0.28 g, 0.65 mmol)) and triethylamine (0.13 g, 1.3 mmol) in DCM (3 mL) was added methanesulfonyl chloride (89 mg, 0.78 mmol) at 0 °C. After stirred at 0 °C for 0.5 h, the reaction mixture was diluted with DCM (20 mL). The mixture was washed with $H_2O$ (20 mL×2), dried over anhydrous $Na_2SO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography on silica gel (0-50% ethyl acetate in petroleum ether) to give compound **11** (0.26 g, >99% yield) as a white solid. ESI m/z: 545 $(M + H)^+$.

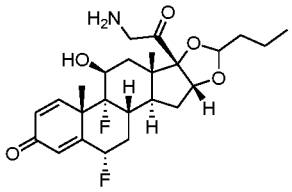
**[0393] (1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-8-(2-Azidoacetyl)-12,19-difluoro-11-hydroxy-9,13-dimethyl-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-16-one (12)**



**[0394]** A suspension of compound **11** (1.0 g, 1.8 mmol) and sodium azide (1.2 g, 18 mmol) in acetone (15 mL) was stirred at 50 °C overnight. The mixture was cooled to RT and poured into water (80 mL). The aqueous mixture was extracted with ethyl acetate (50 mL x 3). The combined organic layer was washed with brine (30 mL), dried over anhydrous $Na_2SO_4$, filtered and concentrated *in vacuo* to afford crude compound **12** (0.90 g, > 99% yield) as a yellow solid, which was used for the next step without further purification. ESI m/z: 492 $(M + H)^+$.

**Payload B**

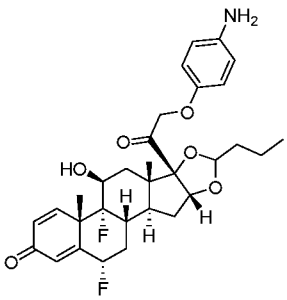
**[0395] (1*S*,2*S*,4*R*,6*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-8-(2-Aminoacetyl)-12,19-difluoro-11-hydroxy-9,13-dimethyl-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-16-one; trifluoroacetic acid salt (compound B)**



**[0396]** To a solution of compound **12** (0.85 g, 1.7 mmol) in THF (20 mL) was added *aq.* hydrochloride (1 N, 10 mL). The mixture was stirred at 28-32 °C until it turned clear, to the mixture was then added triphenylphosphine (0.68 g, 2.6 mmol). The resulting yellow clear solution was stirred at RT for 18 h. The mixture was concentrated *in vacuo* and the residue was purified by reversed phase flash chromatography (0-50% acetonitrile in *aq.* TFA (0.05%)) to give compound **B** (0.56 g, 57% yield, TFA salt) as an off-white solid. ESI m/z: 466 $(M + H)^+$. $^1$H NMR (400 MHz, $MeOD_{d4}$) **δ** 7.33 (d, *J* = 9.9 Hz, 1H), 6.40-6.29 (m, 2H), 5.69-5.45 (m, 1H), 4.93-4.92 (m, 1H), 4.71 (t, *J* = 4.3 Hz, 1H), 4.35-4.27 (m, 2H), 3.90-3.84 (m, 1H), 2.81-2.54 (m, 1H), 2.42-2.06 (m, 3H), 1.82-1.32 (m, 11H), 1.09-0.87 (m, 6H) ppm. $^{19}$F NMR (376 MHz, $CD_3OD$) **δ** -77.01, -166.24, -166.92, -188.81, -188.83 ppm. *Anal.* HPLC: 100%, Retention time: 6.86 min (method A).

**Payload C**

**[0397]** (1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-8-[2-(4-Aminophenoxy)acetyl]-12,19-difluoro-11-hydroxy-9,13-dimethyl-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-16-one (C)

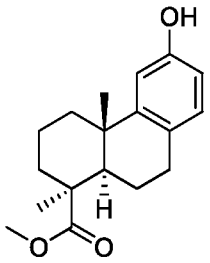


**[0398]** A mixture of compound **11** (93 mg, 0.17 mmol), 4-aminophenol (37 mg, 0.34 mmol) and cesium carbonate (0.11 g, 0.34 mmol) in acetone (0.5 mL) was refluxed for 2 hours. The mixture was cooled to RT and diluted with $H_2O$ (10 mL). The mixture was extracted with ethyl acetate (10 mL×3). The combined organic layer was washed with water (20 mL) and brine (20 mL), dried over $Na_2SO_4$, filtered and concentrated *in vacuo*. The residue was purified by prep-HPLC to give payload **C** (6.0 mg, 6.3% yield) as a white solid. ESI m/z: 298 $(M/2 + H)^+$, 558 $(M + H)^+$ (10%). $^1H$ NMR (500 MHz, $MeOD_{d4}$) $\delta$ 7.34 (d, $J$ = 10.0 Hz, 1H), 6.78-6.71 (m, 4H), 6.37-6.33 (m, 2H), 5.63-5.49 (m, 1H), 5.10-4.99 (m, 1H), 4.77-4.63 (m, 2H), 4.33 (d, $J$ = 9.1 Hz, 1H), 2.74-2.57 (m, 1H), 2.39-2.13 (m, 3H), 1.98-1.31 (m, 12H), 1.03-0.93 (m, 6H) ppm. *Anal.* HPLC: purity 97.4%, Retention time: 7.55 min (method B).

## EXAMPLE 4

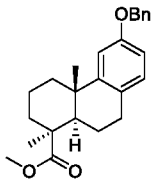
**Preparation of payload D** (See FIG. 5)

**[0399] Methyl (1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate (14)**



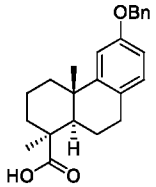
**[0400]** To a solution of podocarpic acid (**13**, 90 g, 0.33 mol) in methanol (200 mL) and toluene (600 mL) was added with (trimethylsilyl)diazomethane (2 M in hexane, 200 mL). The reaction mixture was stirred at room temperature for 2 hours. The podocarpic acid was then totally consumed according to LCMS. The volatiles were removed *in vacuo*, and the residue was triturated from petroleum ether (2 L) to give compound **14** (91 g, 96% yield) as a white solid. ESI m/z: 289 $(M + H)^+$. $^1H$ NMR (400 MHz, $DMSO_{d6}$) $\delta$ 8.95 (s, 1H), 6.79 (d, $J$ = 8.2 Hz, 1H), 6.63 (d, $J$ = 2.4 Hz, 1H), 6.48 (dd, $J$ = 8.2, 2.4 Hz, 1H), 3.58 (s, 3H), 2.80-2.55 (m, 2H), 2.20-2.02 (m, 3H), 1.96-1.71 (m, 2H), 1.56-1.45 (m, 2H), 1.27 (t, $J$ = 13.5 Hz, 1H), 1.21 (s, 3H), 1.09 (td, $J$ = 13.5, 4.1 Hz, 1H), 0.91 (s, 3H) ppm.

**[0401] Methyl (1*S*,4a*S*,10a*R*)-6-(benzyloxy)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate (20)**



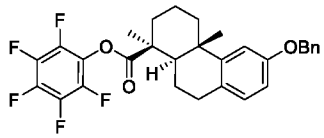

**[0402]** A mixture of compound **14** (12 g, 40 mmol) and cesium carbonate (14 g, 44 mmol) in DMF (100 mL) was stirred at 20-25 °C for 15 minutes. To the mixture was added benzyl bromide (7.1 mL, 60 mmol) at room temperature. After stirred at room temperature for 4 hours, the resulting mixture was poured into cold water and extracted with ethyl acetate. The combined organic solution was washed with water and brine, dried over sodium sulfate and concentrated in vacuo. The crude product was purified by flash chromatography (0-10% ethyl acetate in petroleum ether) to give the title compound **20** (13 g, 89% yield) as a white solid. ESI m/z: 379 $(M + H)^+$. $^1H$ NMR (500 MHz, $MeOD_{d4}$) $\delta$ 7.60-7.20 (m, 5H), 7.00-6.82 (m, 2H), 6.73 (d, $J$ = 7.1 Hz, 1H), 5.03 (s, 2H), 3.66 (s, 3H), 2.95-2.58 (m, 2H), 2.36-2.10 (m, 3H), 2.10-1.85 (m, 2H), 1.70-1.48 (m, 2H), 1.44-1.21 (m, 4H), 1.15 (t, $J$ = 17.2 Hz, 1H), 1.01 (s, 3H) ppm.

**[0403] (1*S*,4a*S*,10a*R*)-6-(Benzyloxy)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylic acid (21)**



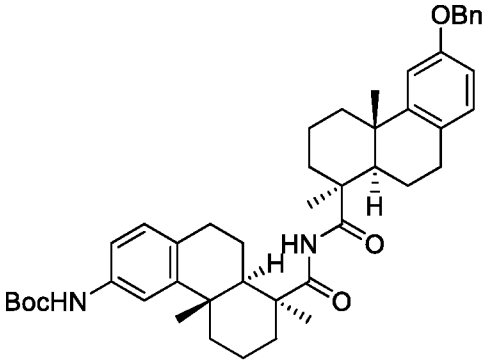

**[0404]** A mixture of compound **20** (11 g, 29 mmol) and potassium *tert*-butoxide (33 g, 0.29 mol) in DMSO (0.19 L) was stirred at 100 °C for an hour until the methyl group was totally removed, which was monitored by LCMS and TLC. After cooled to 25 °C, the mixture was quenched with aqueous hydrochloride (1 N) and extracted with ethyl acetate. The combined organic solution was washed with brine, dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (0-24% ethyl acetate in petroleum ether) to give compound **21** (7.5 g, 71% yield) as a white solid. ESI m/z: 365 $(M + H)^+$. $^1H$ NMR (500 MHz, $MeOD_{d4}$) $\delta$ 7.42 (d, $J$ = 7.4 Hz, 2H), 7.36 (t, $J$ = 7.5 Hz, 2H), 7.30 (t, $J$ = 7.3 Hz, 1H), 6.92 (d, $J$ = 8.4 Hz, 1H), 6.87 (d, $J$ = 2.5 Hz, 1H), 6.72 (dd, $J$ = 8.4, 2.5 Hz, 1H), 5.02 (s, 2H), 2.82 (dd, $J$ = 16.3, 4.4 Hz, 1H), 2.77-2.65 (m, 1H), 2.24 (d, $J$ = 13.2 Hz, 2H), 2.19 (dd, $J$ = 13.8, 6.0 Hz, 1H), 2.11-1.96 (m, 2H), 1.64-1.56 (m, 1H), 1.53 (d, $J$ = 11.0 Hz, 1H), 1.35 (td, $J$ = 13.3, 3.7 Hz, 1H), 1.30 (s, 3H), 1.13 (s, 3H), 1.11-1.05 (m, 1H) ppm.

**[0405] Pentafluorophenyl (1*S*,4a*S*,10a*R*)-6-(benzyloxy)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate (22)**



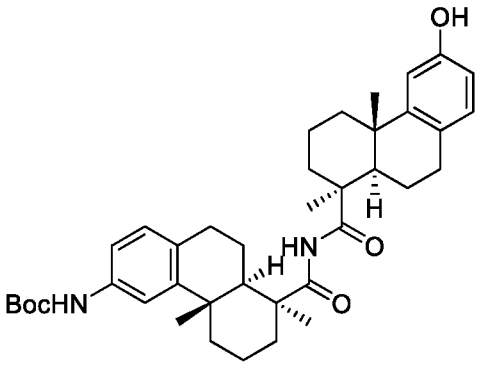
**[0406]** To a solution of **21** (9.6 g, 26 mmol) in DMF (100 mL) was added DIPEA (14 mL, 79 mmol), and perfluorophenyl 2,2,2-trifluoroacetate (15 g, 53 mmol). This mixture was stirred at room temperature overnight, which was monitored by LCMS. The reaction mixture was then diluted with ether (200 mL) and washed with water (300 mL) and brine (200 mL). The organic solution was dried over sodium sulfate, and concentrated *in vacuo*. The residue was purified by flash chromatography (0-10% ethyl acetate in petroleum ether) to give compound **22** (12 g, 88% yield) as a white solid. ESI m/z: 531 $(M + H)^+$. $^1H$ NMR (500 MHz, $DMSO_{d6}$) $\delta$ 7.43 (d, $J$ = 7.1 Hz, 2H), 7.38 (t, $J$ = 7.4 Hz, 2H), 7.31 (t, $J$ = 7.2 Hz, 1H), 6.93 (dd, $J$ = 10.2, 5.5 Hz, 2H), 6.76 (dd, $J$ = 8.4, 2.5 Hz, 1H), 5.05 (s, 2H), 2.81 (dd, $J$ = 16.3, 4.5 Hz, 1H), 2.77-2.68 (m, 1H), 2.28-2.19 (m, 2H), 2.18 (dd, $J$ = 13.4, 5.6 Hz, 1H), 2.00-1.83 (m, 2H), 1.74 (d, $J$ = 11.8 Hz, 1H), 1.65 (d, $J$ = 14.1 Hz, 1H), 1.47 (s, 3H), 1.38-1.27 (m, 2H), 1.08 (s, 3H) ppm.

**[0407] *tert*-Butyl *N*-[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-(benzyloxy)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamate (23)**



**[0408]** To a solution of compound **18** (2.3 g, 6.2 mmol) in THF (20 mL) was added dropwise *n*-BuLi (2.5 M in hexane, 5.5 mL, 14 mmol) at -78 °C. The reaction was stirred at this temperature for 1 hour. To the mixture was added a solution of **22** (3.0 g, 5.6 mmol) in THF (20 mL), and the resulting mixture was then stirred at 10-20 °C overnight until compound **22** was consumed, which was monitored by LCMS. The reaction was quenched with *sat. aq.* ammonium chloride and extracted with ethyl acetate. The combined organic solution was washed with water and brine, dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by flash chromatography (0-30% ethyl acetate in petroleum ether) to give compound **23** (1.59 g, 51% yield) as a white solid. ESI m/z: 719 $(M + 1)^+$.

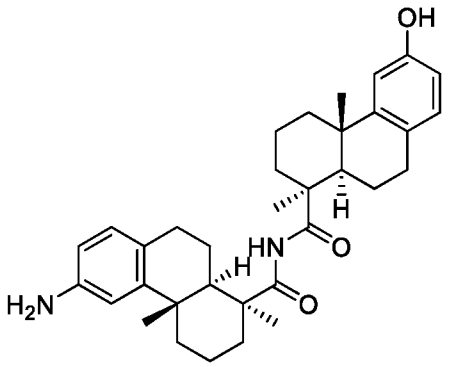
**[0409]** ***tert*-Butyl *N*-[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamate (24)**




**[0410]** To a solution of **23** (2.0 g, 2.78 mmol) in ethyl acetate (40 mL) was added wet palladium on carbon (10% Pd, 0.9 g) under nitrogen protection. The mixture was degassed and fulfilled with hydrogen and stirred at room temperature under hydrogen balloon overnight until **23** was totally consumed, which was monitored by LCMS. The mixture was filtered through Celite and the filtration was concentrated in vacuo. The residue was purified by silica gel column chromatography (0-55% ethyl acetate in petroleum ether) to give **24** (1.06 g, 61% yield) as a white solid. ESI m/z: 629 $(M + H)^+$. $^1H$ NMR (500 MHz, $DMSO_{d6}$) $\delta$ 9.10 (s, 1H), 8.98 (s, 1H), 8.11 (s, 1H), 7.40 (s, 1H), 7.15 (d, $J$ = 7.5 Hz, 1H), 6.90 (d, $J$ = 8.4 Hz, 1H), 6.81 (d, $J$ = 8.3 Hz, 1H), 6.63 (d, $J$ = 2.3 Hz, 1H), 6.50 (dd, $J$ = 8.2, 2.4 Hz, 1H), 2.84 (td, $J$ = 16.3, 3.8 Hz, 2H), 2.77-2.64 (m, 2H), 2.30-2.22 (m, 2H), 2.14 (t, $J$ = 10.9 Hz, 4H), 2.00-1.80 (m, 4H), 1.65-1.54 (m, 4H), 1.45 (s, 9H), 1.34-1.28 (m, 2H), 1.27 (d, $J$ = 2.5 Hz, 6H), 1.15-1.08 (m, 2H), 0.99 (s, 6H) ppm.

**Payload D**

**[0411] (1*S*,4a*S*,10a*R*)-*N*-[(1*S*,4a*S*,10a*R*)-6-Amino-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl]-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide (D)**



**[0412]** To the solution of compound **24** (0.17 g, 0.27 mmol) in DCM (10 mL) was added dropwise TFA (3 mL) at room temperature. The reaction mixture was stirred at room temperature for an hour until Boc was removed according to LCMS. The volatiles were removed *in vacuo* and the residue was purified by prep-HPLC (method B) to give **D** (0.10 g, 70% yield) as a white solid.

**[0413]** ESI m/z: 529.3 $(M + 1)^+$.

**[0414]** $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.14 (s, 1H), 6.92 (d, $J$ = 8.3 Hz, 1H), 6.86 (d, $J$ = 8.1 Hz, 1H), 6.73 (d, $J$ = 2.5 Hz, 1H), 6.65-6.57 (m, 2H), 6.50 (dd, $J$ = 8.1, 2.3 Hz, 1H), 4.75 (s, 1H), 3.49 (s, 1H), 2.99-2.85 (m, 2H), 2.79 (tt, $J$ = 11.6, 5.8 Hz, 2H), 2.34-2.14 (m, 6H), 2.15-1.95 (m, 4H), 1.74-1.51 (m, 5H), 1.46-1.34 (m, 2H), 1.30 (s, 6H), 1.21-1.06 (m, 8H) ppm.

**[0415]** $^1$H NMR (400 MHz, $DMSO_{d6}$) $\delta$ 8.99 (s, 1H), 8.09 (s, 1H), 6.81 (d, $J$ = 8.0 Hz, 1H), 6.68 (d, $J$ = 8.0 Hz, 1H), 6.63 (d, $J$ = 2.5 Hz, 1H), 6.50 (dd, $J$ = 8.0, 2.5 Hz, 1H), 6.48 (d, $J$ = 2.5 Hz, 1H), 6.34 (dd, $J$ = 8.0, 2.5 Hz, 1H), 4.69 (s, 2H), 2.86-2.60 (m, 4H), 2.28-2.10 (m, 6H), 1.94-1.75 (m, 4H), 1.65-1.53 (m, 4H), 1.35-1.20 (m, 8H), 1.20-1.06 (m, 2H), 0.98 (s, 6H) ppm.

**[0416]** $^{13}$C NMR (100 MHz, $DMSO_{d6}$) $\delta$ 174.03, 173.92, 155.34, 148.39, 147.63, 146.43, 129.56, 129.09, 124.60, 121.65, 113.23, 112.58, 111.81, 110.77, 52.32, 52.09, 45.56, 45.52, 39.20, 39.36, 38.23, 38.17, 37.18, 37.12, 31.08, 31.00, 27.65, 27.64, 23.08, 23.03, 21.43, 21.27, 19.64, 19.61 ppm.

**[0417]** HPLC (method B): Retention time: 8.92 min, purity: 99.4%. chiral HPLC: >99.9% (in column AD, AS, OD and OJ).

**[0418]** Optical rotation ($\alpha$): +2.53° (1.7 g/100 mL THF, 25 °C).

**Payload E**

**[0419] (1*S*,4a*S*,10a*R*)-6-((*S*)-2-Amino-3-hydroxypropanamido)-*N*-((1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide (E)**

**[0420]** To a solution of Fmoc-Ser-OH (30 mg, 0.1 mmol) in DMF (1 mL) were added HATU (38 mg, 0.1 mmol), and DIPEA (39 mg, 0.3 mmol) at 25 °C. The resulting mixture was stirred at this temperature for an hour. To the mixture was then added **D** (30 mg, 0.06 mmol), and the reaction mixture was stirred at 25 °C for 16 h. To the mixture was added piperidine (0.2 mL), and the resulting mixture was stirred for additional 30 min at rt. The volatiles were removed in vacuo and the residue was directly purified by prep-HPLC (method B) to give the desired product (18 mg, 51% yield) as a white solid. ESI m/z: 616 $(M + 1)^+$. $^1$H NMR (500 MHz, DMSO-$_{d6}$) $\delta$ 9.74 (br s, 1H, CON*H*-Ph), 9.00 (s, 1H, O*H*), 8.11 (s, 1H, N*H* of imidine), 7.58 (s, 1H), 7.41 (dd, $J$ = 8.2, 2.0 Hz, 1H), 6.96 (d, $J$ = 8.4 Hz, 1H), 6.82 (d, $J$ = 8.3 Hz, 1H), 6.63 (d, $J$

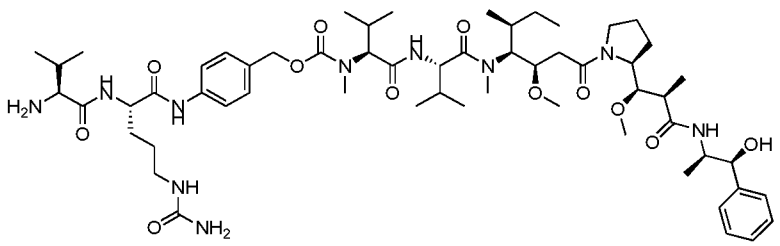
= 2.3 Hz, 1H), 6.50 (dd, *J* = 8.2, 2.4 Hz, 1H), 4.82 (t, *J* = 5.5 Hz, 1H, O*H* on Ser), 3.62-3.45 (m, 3H), 2.97-2.61 (m, 4H), 2.33-2.21 (m, 2H), 2.21-2.03 (m, 4H), 1.96-1.77 (m, 4H), 1.70-1.50 (m, 4H), 1.36-1.20 (m, 8H), 1.23-1.06 (m, 2H), 1.06-0.93 (m, 6H) ppm.

## EXAMPLE 5

**Preparation of Intermediates 3a-c, 3g** (See FIG. 6)

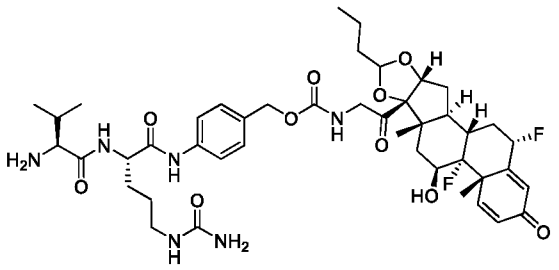
**[0421]** General Procedure A: To a solution of payload (**A**, **B**, **C**, or **E** 1.0 eq.) in DMF (0.3 mL per 10 mg of payload) were added Fmoc-vcPAB-PNP **2a** (1.1 eq.), HOBt (1.5 eq.) and DIPEA (2.0 eq.) at RT. The mixture was stirred at RT for 3 hours until payload was totally consumed, which was monitored by LCMS. To the reaction mixture was added diethyl amine (0.03 mL per 10 mg of payload) and the mixture was stirred at RT (18-30 °C) for one hour until Fmoc was removed. The mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by reversed phase flash chromatography or prep-HPLC to titled compound (**3a**, **3b, 3c or 3g**).

**[0422] 4-((*S*)-2-((*S*)-2-Amino-3-methylbutanamido)-5-ureidopentanamido)benzyl (*S*)-1-((*S*)-1-(((3*R*,4*S*,5*S*)-1-((*S*)-2-((1*R*,2*R*)-3-((1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-ylamino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-ylamino)-3-methyl-1-oxobutan-2-yl(methyl)carbamate (3a) (vcPAB-MMAE)**



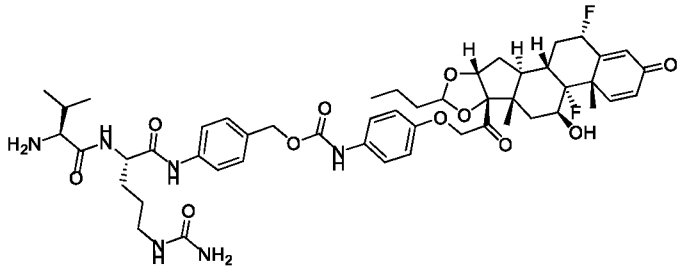
**[0423]** [Ref: WO2012/166560] Following the general procedure A from compound **2a** (90 mg, purity 75%, 88 µmol) with payload **A** (45 mg, 63 µmol), compound **3a** (28 mg, 40% yield) as a white powder was obtained. ESI m/z: 1123.5 $(M + H)^+$. $^1H$ NMR ($DMSO_{d6}$, 500 MHz): $\delta$ 10.12 (br s, 1H), 8.25-8.05 (m, 2H), 7.89-7.58 (m, 3H), 7.34-7.16 (m, 7H), 5.98 (t, *J* = 5.5 Hz, 1H), 5.42-5.34 (m, 3H), 5.06-4.95 (m, 2H), 4.78-4.57 (m, 1H), 4.51-4.26 (m, 3H), 3.80-3.40 (m, 2H), 3.28-3.18 (m, 7H), 3.12-2.84 (m, 10H), 2.43-2.40 (m, 1H), 2.30-2.27 (m, 1H), 2.15-1.91 (m, 5H), 1.96-1.68 (m, 4H), 1.59-1.34 (m, 6H), 1.06-0.93 (m, 6H), 0.90-0.70 (m, 26H) ppm.

**[0424] {4-[(2*S*)-2-[(2*S*)-2-Amino-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethyl}carbamate (3b)**



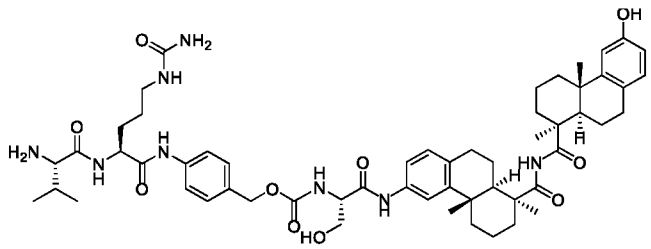
**[0425]** Following the general procedure A from compound **2a** (93 mg, 0.20 mmol) with payload **B**, compound **3b** was obtained after purification by reversed phase flash chromatography (50-80% acetonitrile in *aq.* ammonium bicarbonate (10 mM)) as a white solid. ESI m/z: 871 $(M + H)^+$.

**[0426] {4-[(2*S*)-2-[(2*S*)-2-Amino-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-(4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethoxy}phenyl)carbamate (3c)**



**[0427]** Following the general procedure A from compound **2a** (0.10 g, 0.22 mmol) with payload **C**, compound **3c** (160 mg, 76% yield) was obtained after purification by reversed phase flash chromatography (50-80% acetonitrile in *aq.* ammonium bicarbonate (10 mM)) as a white solid. ESI m/z: 963.4 $(M + H)^+$.

**[0428] {4-[(2*S*)-2-[(2*S*)-2-Amino-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}-2-hydroxyethyl]carbamate (3g)**



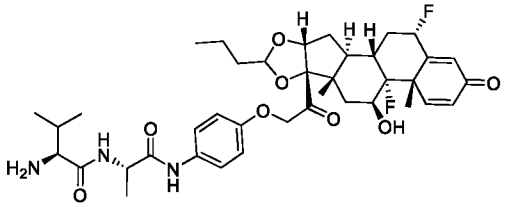
**[0429]** To a solution of Fmoc-vc-PAB-PNP (58 mg, 76 μmol) and **E** (36 mg, 58 μmol) in DMF (3 mL) were added HOBt (7.9 mg, 58 μmol) and DIPEA (15 mg, 0.12 mmol), and the mixture was stirred at 30 °C for 16 hours. Compound **E** was then totally consumed according to LCMS. To the resulting mixture was added diethylamine (0.1 mL) and it was stirred at RT for an hour until Fmoc was removed, which was monitored by LCMS. After filtered, the filtrate was directly purified by prep-HPLC (method B) to give compound **3g** (36 mg, 48% yield) as a light yellow solid. ESI m/z: 1021 $(M + 1)^+$. $^1H$ NMR (400 MHz, $DMSO_{d6}$) $\delta$ 10.02 (s, 1H), 9.82 (s, 1H), 9.00 (s, 1H), 8.69-8.65 (m, 1H), 8.11-8.00 (m, 4H), 7.65-7.53 (m, 3H), 7.40-7.30 (m, 3H), 7.30-7.20 (m, 1H), 6.96 (d, $J$ = 8.0 Hz, 1H), 6.81 (d, $J$ = 8.0 Hz, 1H), 6.65-6.61 (m, 1H), 6.50 (dd, $J$ = 8.0 Hz, 2.0 Hz, 1H), 6.00-5.95 (m, 1H), 5.48 (s, 2H), 5.00-4.95 (m, 3H), 4.60-4.40 (m, 1H), 4.25-4.20 (m, 1H), 3.65-3.55 (m, 4H), 3.15-2.55 (m, 10H), 2.40-2.20 (m, 3H), 2.20-2.00 (m, 5H), 2.00-1.80 (m, 4H), 1.86-1.55 (m, 6H), 1.27 (d, $J$ = 4.8 Hz, 9H), 1.20-1.10 (m, 2H), 0.97-0.90 (m, 6H) ppm.

## EXAMPLE 6

**Preparation of Intermediates 3d and 3f** (See FIG. 7)

**[0430]** General Procedure B: To a solution of Fmoc-Val-Ala-OH (**2b**, 1.2 eq.) in DMF (25 mL per gram of payload) were added HATU (1.5 eq.) and DIPEA (3.0 eq.) at RT. The mixture was stirred at RT for 5 minutes followed by addition of payload (**C** or **D**, 1.0 eq.). The mixture was stirred for additional 2 hours and LCMS showed the completion of reaction. To the reaction mixture was added diethyl amine (5 eq.). The mixture was stirred at RT for 2 hours, until the Fmoc was totally removed according to LCMS. The mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by reversed phase flash chromatography (0-100% acetonitrile in *aq.* ammonium bicarbonate (10 mM)) or prep-HPLC (Method B) to give the desired product (**3d** or **3f**, 64-72% yield from payload).

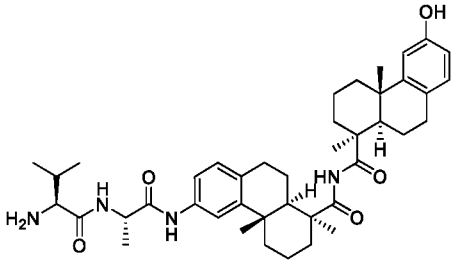
**(2*S*)-2-Amino-*N*-[(1*S*)-1-[(4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethoxy}phenyl)carbamoyl]ethyl]-3-methylbutanamide (3d)**



**[0431]** Following the general procedure B from compound **2b** (0.50 g, 0.90 mmol) with payload **C**, compound **3d** (0.69 g, 72% yield) was obtained after purification by reversed

phase flash chromatography (0-100% acetonitrile in *aq.* ammonium bicarbonate (10 mM)) as viscous yellow oil. ESI m/z: 728 $(M + H)^+$.

**(1*S*,4a*S*,10a*R*)-6-((*S*)-2-((*S*)-2-Amino-3-methylbutanamido)propanamido)-*N*-((1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide (3f)**

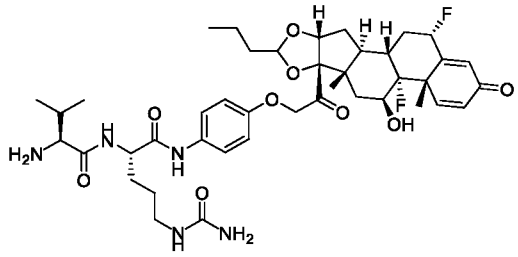


**[0432]** Following the general procedure B from compound **2b** with payload **D** (53 mg, 0.10 mmol), compound **3f** (45 mg, 64% yield) was obtained after purification by prep-HPLC (method B) as a white solid. ESI m/z: 699 $(M + 1)^+$. $^1$H NMR (500 MHz, $MeOD_{d4}$) $\delta$ 8.40 (s, 1H), 7.47 (s, 1H), 7.32 (d, $J$ = 8.0 Hz, 1H), 7.03 (d, $J$ = 8.3 Hz, 1H), 6.88 (d, $J$ = 8.2 Hz, 1H), 6.72 (d, $J$ = 2.4 Hz, 1H), 6.56 (dd, $J$ = 8.3, 2.4 Hz, 1H), 4.60-4.48 (m, 1H), 3.22-3.11 (m, 1H), 3.02-2.93 (m, 1H), 2.92-2.76 (m, 3H), 2.74-2.70 (m, 1H), 2.43-2.31 (m, 3H), 2.28 (d, $J$ = 14.1 Hz, 3H), 2.16-1.96 (m, 3H), 1.81 (s, 1H), 1.78-1.65 (m, 4H), 1.53-1.42 (m, 4H), 1.38 (d, $J$ = 5.3 Hz, 6H), 1.33-1.22 (m, 2H), 1.14 (d, $J$ = 6.6 Hz, 6H), 1.09 (d, $J$ = 18.6 Hz, 6H) ppm.

## EXAMPLE 7

**Preparation of Intermediate 3e** (See FIG. 8)

**(2*S*)-2-[(2*S*)-2-Amino-3-methylbutanamido]-5-(carbamoylamino)-*N*-(4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethoxy}phenyl)pentanamide (3e)**



**[0433]** To a solution of Fmoc-Val-Cit-OH (0.23 g, 0.43 mmol) in DMF (5 mL) were added HATU (0.38 g, 0.43 mmol) and DIPEA (93 mg, 0.72 mmol) at RT. The mixture was stirred at RT for 5 minutes followed by addition of payload **C** (0.20 g, 0.36 mmol). The mixture was stirred for additional 2 hours and LCMS showed the completion of reaction. To the reaction mixture was added diethylamine (0.5 mL). The mixture was stirred at RT for an hour, until the

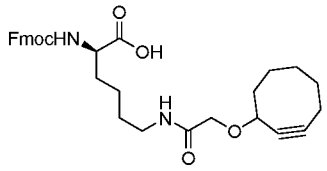
Fmoc was totally removed according to LCMS. The mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was purified by reversed phase flash chromatography (0-100% acetonitrile in *aq.* ammonium bicarbonate (10 mM)) and then methanol) to give compound **3e** (38% yield from payload **C**). ESI m/z: 814 $(M + 1)^+$.

## EXAMPLE 8

**Preparation of Intermediate 4** (See FIG. 9)

**[0434]** Intermediate **4a** was synthesized by the amidation from Fmoc-(D)-Lys-OH with commercial activated ester **25**.

**(2R)-6-[2-(Cyclooct-2-yn-1-yloxy)acetamido]-2-{[(9H-fluoren-9-ylmethoxy)carbonyl]amino}hexanoic acid (4a)**



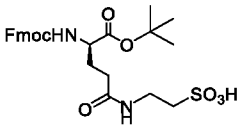

**[0435]** To a mixture of compound **25** (65 mg, 0.23 mmol, CAS: 1425803-45-7) in DMF (2 mL) were added Fmoc-D-Lys-OH (85 mg, 0.23 mmol) and triethylamine (52 mg, 0.51 mmol). The reaction mixture was stirred at room temperature for 30 minutes. The mixture was directly separated by reversed phase flash chromatography (0-100% acetonitrile in water (0.05% TFA)) to give intermediate 4a (85 mg, yield 70%) as a white solid. ESI m/z: 533 (M + H)+. 1H NMR (MeODd4, 500 MHz): δ 7.70 (d, J = 7.5 Hz, 2H), 7.59 (t, J = 8.0 Hz, 2H), 7.30 (t, J = 7.5 Hz, 2H), 7.22 (t, J = 7.4 Hz, 2H), 4.35-4.22 (m, 2H), 4.22-4.09 (m, 2H), 4.09-3.99 (m, 1H), 3.94-3.81 (m, 1H), 3.79-3.67 (m, 1H), 3.15 (t, J = 6.9 Hz, 2H), 2.17-1.96 (m, 3H), 1.96-1.86 (m, 1H), 1.85-1.66 (m, 4H), 1.66-1.41 (m, 5H), 1.41-1.25 (m, 3H) ppm.

## EXAMPLE 9

**Preparation of Intermediate 4b** (See FIG. 10)

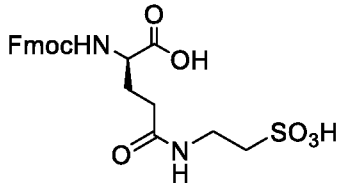
**[0436]** Intermediate **4b** was synthesized from Fmoc-(*D*)-Glu-O$^t$Bu (**26**). Compound **26** was activated with HOSu and then amidated by taurine to provide compound **27**, which was hydrolyzed by TFA to give intermediate **4b**.

**[0437] 2-[(4*R*)-5-(*tert*-Butoxy)-4-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-5-oxopentanamido]ethane-1-sulfonic acid (27)**



**[0438]** To a solution of Fmoc-(*D*)-Glu-O$^t$Bu (**26**) (2.0 g, 4.7 mmol) in DCM (20 mL) were added HOSu (1.1 g, 9.6 mmol) and EDCI (1.8 g, 9.4 mmol) at RT. The mixture was stirred at RT overnight, which was monitored by LCMS. The reaction mixture was diluted with DCM (100 mL), washed with water (50 mL x 2) and brine (50 mL), dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was dissolved in DMF (2 mL) and to the solution were added taurine (1.2 g, 9.6 mmol) and DIPEA (1.4 g, 14 mmol). The resulting mixture was stirred at RT overnight until the reaction was completed according to LCMS. The reaction mixture was directly purified by prep-HPLC (method B) to give compound **27** (2.0 g, 81% yield) as a white solid. ESI m/z: 477 $(M - 55 + H)^+$.

**(2*R*)-2-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-4-[(2-sulfoethyl)carbamoyl]butanoic acid (4b)**




**[0439]** To a solution of compound **27** (0.52 g, 0.98 mmol) in DCM (2 mL) was added TFA (2 mL). The reaction mixture was stirred at RT for 3 hours until hydrolysis was completed according to LCMS. The volatiles were removed *in vacuo* and the residue was purified by reversed phase flash chromatography (0-100% acetonitrile in *aq.* ammonium bicarbonate (10 mM)) to give intermediate **4b** (0.45 g, 97% yield) as a white solid. ESI m/z: 477 $(M + H)^+$. $^1$H NMR ($DMSO_{d6}$, 500 MHz) $\delta$ 7.89 (d, $J$ = 7.5 Hz, 2H), 7.72 (d, $J$ = 7.5 Hz, 2H), 7.42 (t, $J$ = 7.5 Hz, 2H), 7.34 (t, $J$ = 7.5 Hz, 2H), 7.21-7.17 (br s, 1H), 4.27-4.23 (m, 2H), 3.82-3.79 (m, 1H), 3.27-3.19 (m, 1H), 2.71-2.67 (m, 1H), 2.54 (t, $J$ = 7.5 Hz, 2H), 2.13-1.89 (m, 2H), 1.80-1.75 (m, 1H), 1.07-1.06 (m, 4H) ppm (the proton COO*H* was not revealed).

**[0440]** Compound **4c** was prepared by following the synthetic procedures outlined in *J. Org. Chem.* 2010, 75, 3685-3691, the compound **4c** was obtained with 25% total yield. ESI m/z: 613.3 (M + H)+.

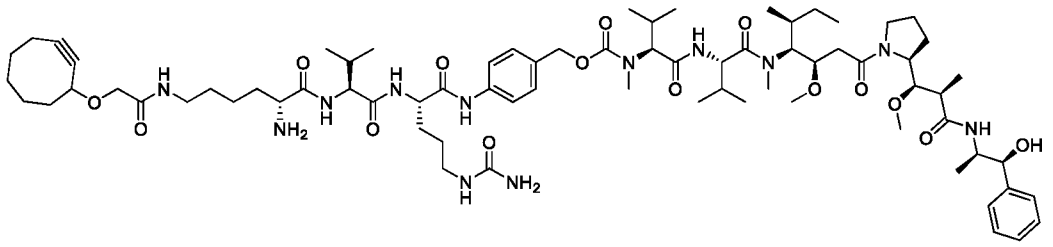
## EXAMPLE 10

**Preparation of Intermediates 5a-g** (See FIG. 11)

**[0441]** <u>General Procedure D:</u> To a solution of compound **4a** (1.2 eq.) in DMF (0.2 mL per 10 mg of **4a**) were added HATU (1.4 eq.) and DIPEA (3 eq.) at RT. The mixture was stirred at RT for 5 minutes before the addition of compound **3** (1.0 eq.). The reaction mixture was then stirred at RT for 2 hours until compound **3a-g** was totally consumed, which was monitored by LCMS. To the reaction mixture was added diethyl amine (5.0 eq.). The mixture was stirred at RT for 2 hours. The mixture was filtered and the filtrate was concentrated. The residue was

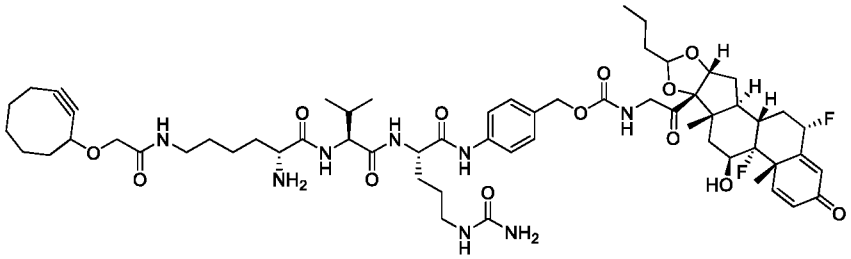
purified by reversed phase flash chromatography (0-100% acetonitrile in *aq.* ammonium bicarbonate (10 mM)) or prep-HPLC (Method B) to give compound **5a-g**.

**4-((2*S*)-2-((2*S*)-2-((2*R*)-2-Amino-6-(2-(cyclooct-2-ynyloxy)acetamido)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl (*S*)-1-((*S*)-1-(((3*R*,4*S*,5*S*)-1-((*S*)-2-((1*R*,2*R*)-3-((1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-ylamino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-ylamino)-3-methyl-1-oxobutan-2-yl(methyl)carbamate (5a)**



**[0442]** Following the general procedure D from compound **3a** (38 mg, 34 μmol) with compound **4** (34 mg, 64 μmol), compound **5a** (17 mg, 35% yield) was obtained as a white solid. ESI m/z: 1415 $(M + 1)^+$. $^1H$ NMR ($DMSO_{d6}$, 400 MHz) $\delta$ 10.09-10.02 (m, 1H), 8.54 (d, $J$ = 8.4 Hz, 1H), 8.38 (d, $J$ = 7.5 Hz, 1H), 8.35-8.26 (m, 0.5H), 8.12-8.02 (m, 3H), 7.94-7.85 (m, 0.5H), 7.66-7.54 (m, 3H), 7.34-7.23 (m, 6H), 7.20-7.13 (m, 1H), 6.08-5.97 (m, 1H), 5.54-5.37 (m, 3H), 5.13-4.94 (m, 2H), 4.52-4.21 (m, 6H), 4.03-3.70 (m, 4H), 3.63-3.51 (m, 1H), 3.25-3.17 (m, 8H), 3.13-2.82 (m, 10H), 2.31-1.91 (m, 10H), 1.85-1.64 (m, 9H), 1.64-1.25 (m, 15H), 1.07-0.96 (m, 6H), 0.90-0.74 (m, 26H) ppm.

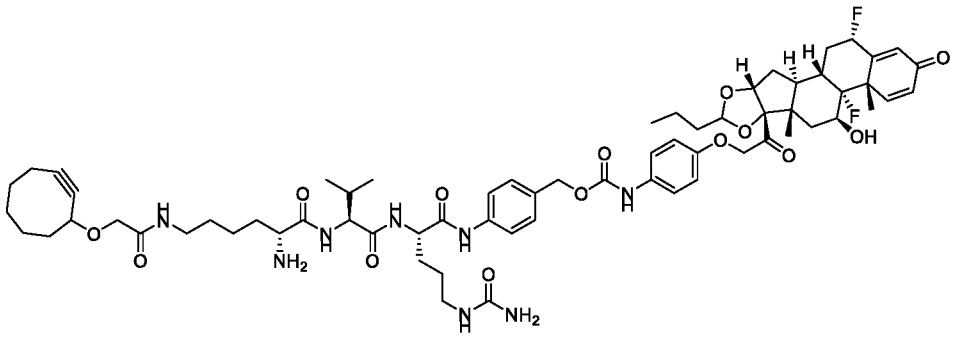
**{4-[(2*S*)-2-[(2*S*)-2-[(2*S*)-2-Amino-6-[2-(cyclooct-2-yn-1-yloxy)acetamido]hexanamido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethyl}carbamate (5b)**



**[0443]** Following the general procedure D from **3b** (0.20 g, 0.23 mmol), the compound **5b** (0.12 g, 45% yield) was obtained as a white solid after prep-HPLC (method B). ESI m/z: 1385 $(M + 1)^+$. $^1H$ NMR (400 MHz, $MeOD_{d4}$) $\delta$ 7.65-7.55 (m, 2H), 7.40-7.26 (m, 3H), 6.39-6.27 (m, 2H), 5.65-5.45 (m, 1H), 5.13-5.01 (m, 2H), 4.71-4.50 (m, 2H), 4.40-4.14 (m, 4H), 4.11-3.82

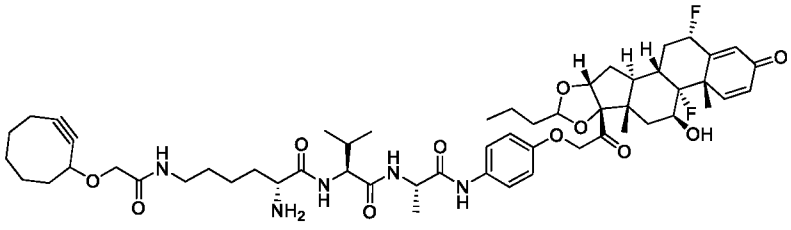
(m, 3H), 3.46-3.39 (m, 1H), 3.29-3.09 (m, 4H), 2.76-2.54 (m, 1H), 2.41-2.10 (m, 7H), 2.09-1.99 (m, 1H), 1.96-1.80 (m, 5H), 1.78-1.21 (m, 23H), 1.06-0.82 (m, 12H) ppm.

**{4-[(2*S*)-2-[(2*S*)-2-[(2*R*)-2-Amino-6-[2-(cyclooct-2-yn-1-yloxy)acetamido]hexanamido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-(4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethoxy}phenyl)carbamate (5c)**



**[0444]** Following the general procedure D from **3c (55 mg, 54 μmol) with 4a**, compound **5c** (0.10 g, yield 57%) was obtained as a white solid. ESI m/z: 1255.5 (M + 1)$^+$. $^1$H NMR (400 MHz, MeOD$_{d4}$) $\delta$ 7.61 (d, $J$ = 8.4 Hz, 2H), 7.32-7.39 (m, 5H), 6.84-6.88 (m, 2H), 6.31-6.36 (m, 2H), 5.05-5.16 (m, 3H), 4.71-4.83 (m ,1H), 4.50-4.54 (m,1H), 4.18-4.33 (m 3H), 3.00-2.85 (m, 2H), 3.40-3.51 (m, 1H), 3.00-3.29 (m, 6H), 1.31-2.35 (m, 34 H), 1.29 (t, $J$ =7.2 Hz, 2H), 0.93-1.02 (m ,12H) ppm.

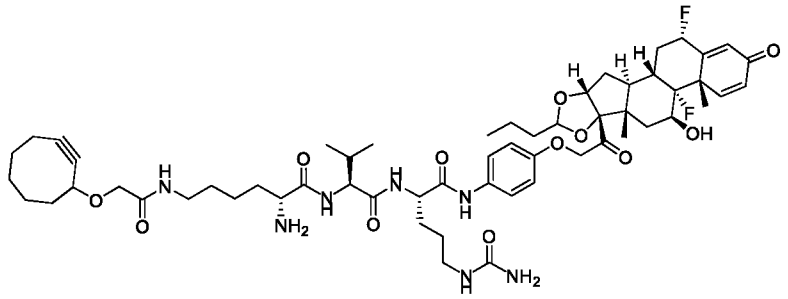
**(2*R*)-2-Amino-6-[2-(cyclooct-2-yn-1-yloxy)acetamido]-*N*-[(1*S*)-1-{[(1*S*)-1-[(4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethoxy}phenyl)carbamoyl]ethyl]carbamoyl}-2-methylpropyl]hexanamide (5d)**



**[0445]** Following the general procedure D from **3d** (0.28 g, 0.38 mmol), compound **5d** (0.21 g, 46% yield) was obtained as a white solid after prep-HPLC (method B). ESI m/z: 1021.5 (M + 1)$^+$. $^1$H NMR (400 MHz, MeOD$_{d4}$) $\delta$ 7.33-7.60 (m, 3H), 6.87-6.91 (m ,2H), 6.32-6.37 (m, 2H), 5.47-5.65 (m, 1H), 5.07-5.30 (m, 1H), 4.72-4.86 (m, 3H), 4.34-4.51 (m, 3H), 3.83-4.20 (m,

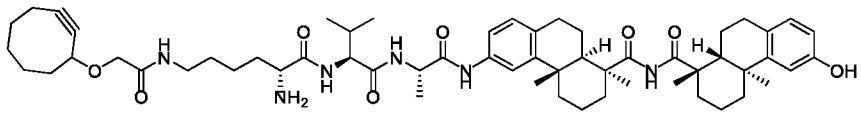
3H), 3.33-3.49 (m, 1H), 3.14-3.27 (m, 3H), 2.59-2.75 (m, 1H), 1.31-2.39 (m, 33 H), 0.93-1.05 (m, 12H) ppm.

**(2*R*)-2-Amino-*N*-[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethoxy}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]-6-[2-(cyclooct-2-yn-1-yloxy)acetamido]hexanamide (5e)**



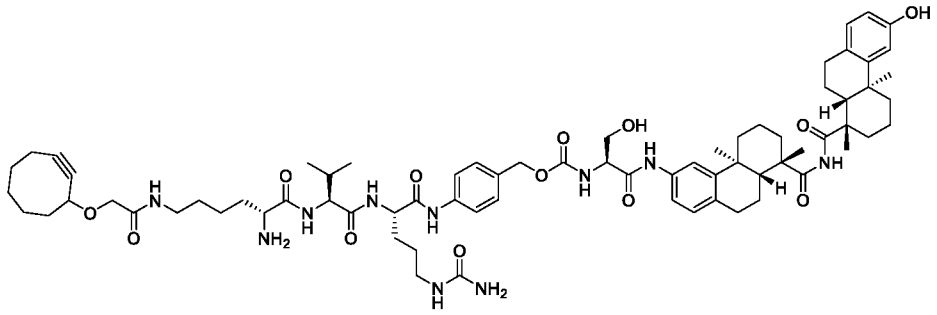
**[0446]** Following the general procedure D from **3e** (0.10 g, 0.12 mmol), compound **5d** (0.12 g, 88% yield) was obtained as a white solid after purification by reversed phase flash chromatography (0-100% acetonitrile in *aq.* ammonium bicarbonate (10 mM)). ESI m/z: 553.7 $(M/2 + 1)^+$.

**(1*S*,4a*S*,10a*R*)-6-((2*S*)-2-((2*S*)-2-((2*R*)-2-Amino-6-(2-(cyclooct-2-ynyloxy)acetamido)hexanamido)-3-methylbutanamido)propanamido)-*N*-((1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl)-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxamide (5f)**



**[0447]** Following the general procedure D from **3f** (80 mg, 0.11 mmol), compound **5f** (48 mg, 84% yield) was obtained as a white solid after prep-HPLC (method B). ESI m/z: 991.5 $(M + 1)^+$.

**{4-[(2*S*)-2-[(2*S*)-2-[(2*R*)-2-Amino-6-[2-(cyclooct-2-yn-1-yloxy)acetamido]hexanamido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}-2-hydroxyethyl]carbamate (5g)**



**[0448]** To a solution of compound **4a** (24 mg, 44 μmol) in DMF (2 mL) were added HATU (17 mg, 44 μmol) and compound **3g** (35 mg, 34 μmol) subsequently at RT. The mixture was stirred for a few minutes at RT until the mixture was homogenous. To this mixture was added DIPEA (8.8 mg, 68 μmol) at RT by syringe. The resulting mixture was stirred at RT for 2 hours until the **3g** was mostly consumed according to LCMS. To this reaction mixture was then added diethylamine or piperidine (0.1 mL, excess)[1] dropwise at RT and the mixture was stirred for an hour until Fmoc group was removed, which was monitored by LCMS. The reaction mixture was directly purified by prep-HPLC (method B) to give compound **5g** (15 mg, 33% yield) as a white solid. ESI m/z: 1313.6 $(M + H)^+$. $^1H$ NMR (500 MHz, $MeOD_{d4}$) $\delta$ 7.59 (d, $J$ = 8.5 Hz, 2H), 7.51 (s, 1H), 7.36-7.26 (m, 3H), 7.01 (d, $J$ = 8.5 Hz, 1H), 6.88 (d, $J$ = 8.0 Hz, 1H), 6.72-6.71 (m, 1H), 6.57-6.54 (m, 1H), 5.09 (s, 2H), 4.64-4.52 (m, 1H), 4.35-4.28 (m, 2H), 4.21 (d, $J$ = 7.0 Hz, 1H), 4.01-3.98 (m, 1H), 3.88-3.84 (m, 3H), 3.43 (t, $J$ = 6.5 Hz, 1H), 3.26-3.10 (m, 4H), 3.00-2.76 (m, 3H), 2.38-2.24 (m, 7H), 2.19-2.02 (m, 9H), 1.98-1.78 (m, 4H), 1.74-1.54 (m, 12H), 1.45-1.26 (m, 14H), 1.13 (s, 6H), 1.00 (t, $J$ = 7.5 Hz, 6H) ppm.

## EXAMPLE 10-1

**[0449] Preparation of Compounds 10a-d** (FIG. 11C)

**[0450]** Compounds 1**0a-d** were prepared by an amide coupling procedure according to FIG. 11C.

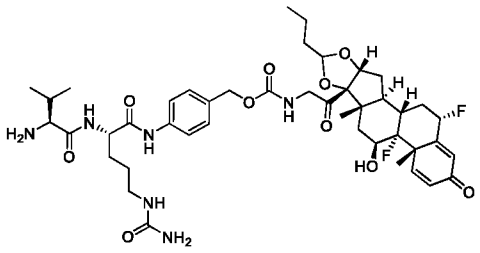
**[0451] General Procedure D2 for compounds 10a-d:** To a solution of DIBAC-suc-$PEG_4$-acid **8a** (1.1-1.3 eq.) in DMF (1 mL per 5-10 mg of **8a**) were added HATU (1.5 eq.) and DIPEA (5.0 eq.) at RT. The mixture was stirred at RT for half an hour followed by addition of compound **3** (1.0 eq.). The resulting mixture was stirred at RT until compound **3** was consumed, which was monitored by LCMS. After filtration, the filtrate was directly purified by prep-HPLC to give compound 1**0a-d**.

## EXAMPLE 10A

**Preparation of Compound 10a** (See FIG. 11A)

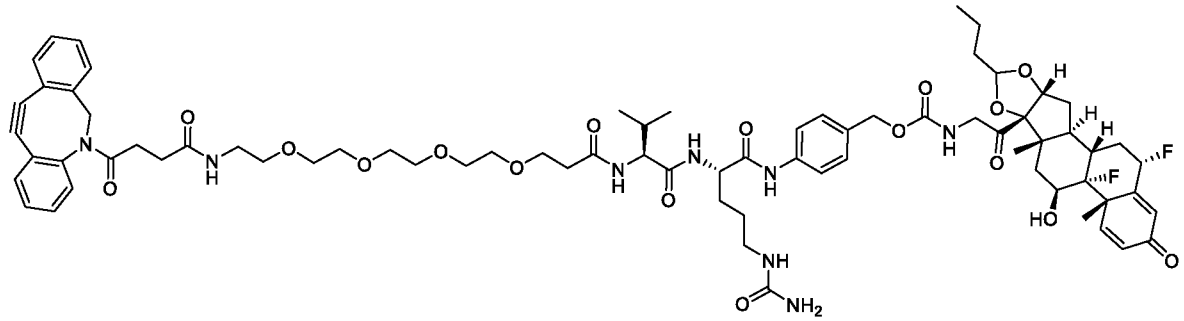
**{4-[(2*S*)-2-[(2*S*)-2-Amino-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-**

**12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethyl}carbamate 10a**



**[0452]** To a solution of Fmoc-VC-PAB-PNP (**10-1**, 0.17 g, 0.22 mmol) and compound **B,** (93 mg, 0.20 mmol) in DMF (3 mL) was added with DIPEA (51 mg, 0.40 mmol) at RT by syringe. The mixture was stirred at RT for 3 hours and most of materials were consumed according to LCMS. To the resulting mixture was added piperidine (0.3 mL, excess) and it was stirred at RT for an hour until Fmoc was totally removed, which was monitored by LCMS. After filtering through a membrane, the filtrate was directly purified by prep-HPLC (method B) to give compound **3b** (0.13 g, 73% yield) as a white solid. ESI m/z: 871 $(M + 1)^+$.

**{4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-Azatricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethyl}carbamate 10a**

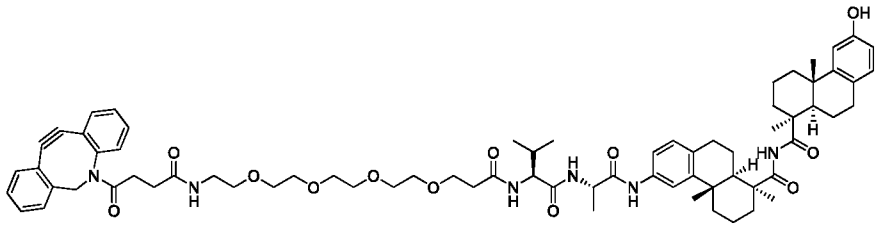


**[0453]** To a solution of acid **8a** (30 mg, 54 μmol) in DMF (5 mL) were added DIPEA (13 mg, 0.10 mmol) and HATU (31 mg, 81 μmol) at RT successively. The resulting mixture was stirred at this temperature for 0.5 hour before the amine **3b** (43 mg, 50 μmol) was added. The reaction mixture was stirred at RT for 3 hours until the amine was totally consumed, which was monitored by LCMS. The reaction mixture was filtered through membrane and the filtrate was then separated by prep-HPLC (method B) to give compound **10a** (16 mg, 23% yield) as a white solid. ESI m/z: 1406 $(M + H)^+$. $^1$H NMR (500 MHz, $DMSO_{d6}$) $\delta$ 9.99 (s, 1H), 8.11 (d, $J$ =

7.5 Hz, 1H), 7.88 (d, *J* = 8.5 Hz, 1H), 7.80-7.75 (m, 1H), 7.70-7.66 (m, 1H), 7.65-7.60 (m, 3H), 7.53-7.33 (m, 6H), 7.33-7.28 (m, 3H), 6.30 (dd, *J* = 10.0 Hz, 1.5 Hz, 1H), 6.11 (s, 1H),6.10-6.00 (m, 1H), 5.72-5.55 (m, 2H), 5.41 (s, 2H), 5.05-5.01 (m, 1H), 4.97 (s, 2H), 4.80-4.72 (m, 1H), 4.60-4.58 (m, 1H), 4.43-4.33 (m, 1H), 4.25-4.10 (m,3H), 3.88-3.80 (m,1H), 3.65-3.55 (m, 3H), 3.50-3.40 (m, 12H), 3.30-3.25 (m, 2H), 3.12-2.90 (m, 4H), 2.70-2.55 (m, 2H), 2.48-2.35 (m, 2H), 2.30-2.20 (m, 2H),2.15-1.95 (m, 4H), 1.86-1.65 (m, 3H), 1.64-1.54 (m, 5H), 1.49 (s, 4H), 1.46-1.34 (m, 5H), 0.90-0.80 (m, 12H) ppm. *Anal. HPLC*: 100%, Retention time: 7.40 min (method B). Solubility: 0.02 mg/mL water.

## EXAMPLE 10B

**Preparation of 1-(4-{2-Azatricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-*N*-[(1*S*)-1-{[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-{[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl]carbamoyl}-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}ethyl]carbamoyl}-2-methylpropyl]-3,6,9,12-tetraoxapentadecan-15-amide 10b** (See FIG. 11B)




**[0454]** Prepared by an amide coupling procedure according to FIG. 11B. Following general procedure D2, amine **3f** (20 mg, 29 μmol), acid **8a** (18 mg, 33 μmol), HATU (33 mg, 87 μmol), and DIPEA (33 mg, 87 μmol), were stirred in 1 mL of DMF at 15-20 °C for 16 hours, purified by prep-HPLC (method B). Yield of **10b**:10 mg, 28%. ESI m/z: 1234 $(M + H)^+$.

**[0455]** $^1$H NMR (500 MHz, methanol$_{d4}$) $\delta$ 7.65 (d, *J* = 7.4 Hz, 1H), 7.62-7.51 (m, 2H), 7.48-7.28 (m, 6H), 7.27-7.21 (m, 1H), 7.00 (d, *J* = 8.5 Hz, 1H), 6.88 (d, *J* = 8.0 Hz, 1H), 6.72 (d, *J* = 2.4 Hz, 1H), 6.56 (dd, *J* = 8.3, 2.4 Hz, 1H), 5.15-5.10 (m, 1H), 4.52-4.43 (m, 1H), 4.20 (d, *J* = 6.5 Hz, 0.5H), 4.04 (d, *J* = 7.9 Hz, 0.5H), 3.77-3.64 (m, 3H), 3.63-3.49 (m, 12H), 3.47-3.39 (m, 2H), 3.24 (t, *J* = 5.5 Hz, 2H), 2.99-2.66 (m, 5H), 2.57-2.42 (m, 2H), 2.42-1.94 (m, 14H), 1.76-1.63 (m, 4H), 1.48-1.21 (m, 13H), 1.14-1.10 (m, 6H), 1.05-0.97 (m, 6H) ppm.

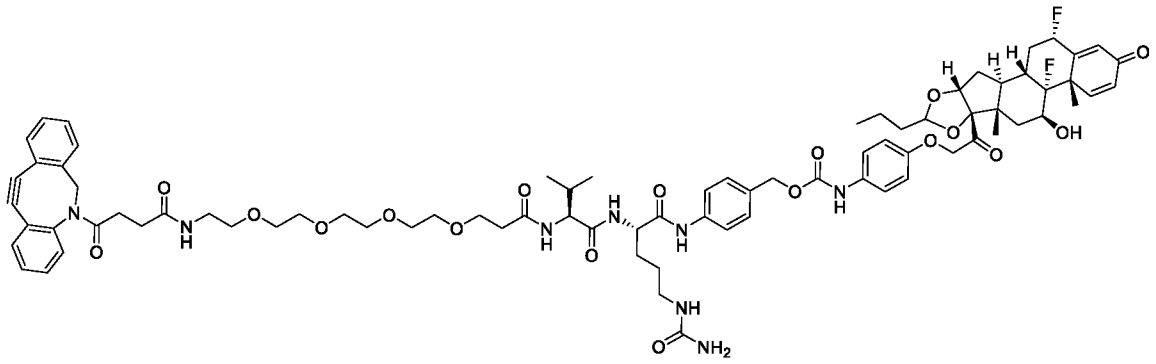
**[0456]** Anal. HPLC: >99%, Retention time: 9.21 min (method B).

**[0457]** Solubility: <0.1 mg/mL water.

## EXAMPLE 10C

**[0458] {4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-Azatricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-**

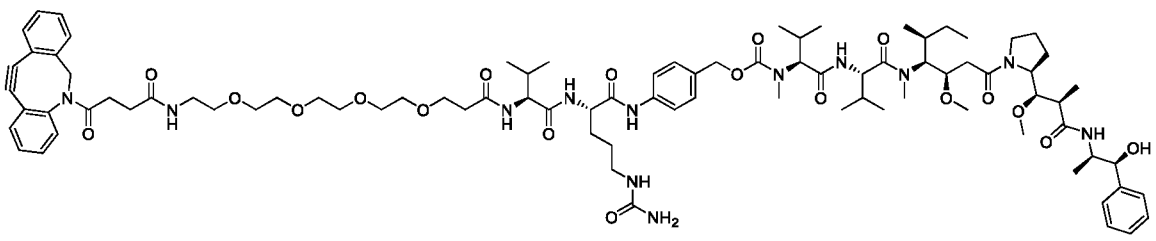
**methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-(4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethoxy}phenyl)carbamate (10c)**



**[0459]** Following the general procedure D2 from compound **3c** (58 mg, 60 μmol) with compound **8a**, compound **10c** (20 mg, 22% yield) was obtained as a white solid. ESI m/z: 1499 (M + H)$^+$. $^1$H NMR (400 MHz, DMSO$_{d6}$) $\delta$ 10.02 (s, 1H), 9.59 (s, 1H), 8.14 (d, *J* = 7.6 Hz, 1H), 7.88 (d, *J* = 8.8 Hz, 1H), 7.80-7.75 (m, 1H), 7.70-7.66 (m, 1H), 7.65-7.60 (m, 3H), 7.53-7.45 (m, 3H), 7.40-7.28 (m, 7H), 6.84 (d, *J* = 9.2 Hz, 2H), 6.30 (dd, *J* = 10.4 Hz, 1.6 Hz, 1H), 6.11 (s, 1H), 6.10-6.00 (m, 1H), 5.72-5.55 (m,1H), 5.52 (s, 1H), 5.43 (s, 2H), 5.16-5.05 (m, 4H), 4.88-4.70 (m, 3H), 4.43-4.33 (m, 1H), 4.25-4.20 (m, 2H), 3.65-3.55 (m, 3H), 3.50-3.40 (m, 12H), 3.30-3.25 (m, 2H), 3.12-2.90 (m, 4H), 2.70-2.55 (m, 2H), 2.48-2.43 (m, 1H), 2.40-2.35 (m, 1H), 2.30-2.20 (m, 2H), 2.15-1.95 (m, 4H), 1.86-1.75 (m, 2H), 1.64-1.54 (m, 5H), 1.49 (s, 4H), 1.46-1.34 (m, 4H), 1.23 (s, 2H), 0.90-0.80 (m, 12H) ppm. Solubility: <0.01 mg/mL water.

## EXAMPLE 10D

**[0460] {4-[(2*S*)-2-[(2*S*)-2-[1-(4-{2-Azatricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-[(1*S*)-1-{[(1*S*)-1-{[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-{[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]carbamoyl}-1-methoxy-2-methylethyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl](methyl)carbamoyl}-2-methylpropyl]carbamoyl}-2-methylpropyl]-*N*-methylcarbamate (10d)**



**[0461]** Following the general procedure D2 from vcPAB-MMAE **3a** (6.9 mg, 6.1 μmol) with compound **8a**, compound **10d** (2.0 mg, 20% yield) was obtained as a white solid. ESI m/z: 830 (M/2 + H)$^+$. $^1$H NMR (500 MHz, DMSO$_{d6}$) $\delta$ 9.98 (s, 1H), 8.29 (s, 1H), 8.11 (d, $J$ = 7.3 Hz, 1H), 8.04 (s, 1H), 7.92-7.82 (m, 2H), 7.75 (t, $J$ = 5.1 Hz, 1H), 7.68 (d, $J$ = 7.2 Hz, 1H), 7.65-7.53 (m, 3H), 7.53-7.42 (m, 3H), 7.41-7.22 (m, 9H), 7.22-7.10 (m, 1H), 6.06-5.91 (m, 1H), 5.75 (s, 1H), 5.40 (s, 2H), 5.33 (d, $J$ = 4.8 Hz, 1H), 5.15-4.91 (m, 3H), 4.79-4.57 (m, 1H), 4.54-4.46 (m, 1H), 4.46-4.32 (m, 2H), 4.32-4.17 (m, 2H), 4.08-3.88 (m, 2H), 3.67-3.53 (m, 4H), 3.50-3.40 (m, 12H), 3.27-3.15 (m, 8H), 3.14-2.91 (m, 8H), 2.91-2.80 (m, 3H), 2.62-2.53 (m, 1H), 2.41-2.33 (m, 2H), 2.32-2.19 (m, 2H), 2.17-2.05 (m, 2H), 2.05-1.90 (m, 4H), 1.86-1.65 (m, 5H), 1.64-1.52 (m, 2H), 1.52-1.40 (m, 2H), 1.39-1.27 (m, 2H), 1.07-0.95 (m, 6H), 0.90-0.67 (m, 26H) ppm.

## EXAMPLE 11

**Preparation of Intermediate 6c** (See FIG. 12)

**[0462]** Azido-intermediate **6c** was synthesized by the amidation from the activated ester **30** with taurine as described in FIG. 12.

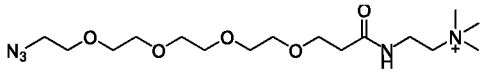
**1-Azido-15-oxo-3,6,9,12-tetraoxa-16-azaoctadecane-18-sulfonic acid (6c)**

**[0463]** To a solution of 2,5-dioxopyrrolidin-1-yl 1-azido-3,6,9,12-tetraoxapentadecan-15-oate **30** (0.10 g, 0.26 mmol) and taurine (39 mg, 0.31 mmol) in anhydrous DMF (4 mL) was added diisopropylethylamine (15 mg, 0.52 mmol). The mixture was stirred at 25 °C overnight. The reaction mixture was filtered and the solution was purified by prep-HPLC (method A) to give intermediate **6c** (0.80 g, yield 78%) as colorless oil. ESI m/z: 399.1 (M + H)$^+$. $^1$H NMR (500 MHz, $D_2O$) $\delta$ 3.69 (t, $J$ = 6.0 Hz, 2H), 3.64-3.59 (m, 14H), 3.49 (t, $J$ = 6.5 Hz, 2H), 3.41 (t, $J$ = 4.5 Hz, 2H), 3.00 (t, $J$ = 7.0 Hz, 2H), 2.45 (t, $J$ = 6.0 Hz, 2H) ppm.

## EXAMPLE 11A

**[0464]** Azido-intermediate **6b** was synthesized by the amidation from the activated ester **30** with compound **6b-1** as described in FIG. 13A.

**[0465] [2-(1-Azido-3,6,9,12-tetraoxapentadecan-15-amido)ethyl]trimethylazanium chloride (6b)**



**[0466]** To a solution of azido-PEG$_4$-NHS **30** (0.19 g, 0.50 mmol) in anhydrous DMF (4 mL) were added compound **6b-1** (83 mg, 0.60 mmol) and DIPEA (19 g, 1.5 mmol). The mixture was stirred at 25 °C overnight. The mixture was filtered and the filtrate was purified by prep-HPLC (method A) to give compound **6b** (0.13 g, 64% yield) as colorless oil. ESI m/z: 376 (M +

H)+. $^1$H NMR (500 MHz, $DMSO_{d6}$) $\delta$ 3.65-3.58 (m, 4H), 3.58-3.45 (m, 16H), 3.45-3.30 (m, 12H), 2.35 (t, $J$ = 6.5 Hz, 2H) ppm.

## EXAMPLE 11B

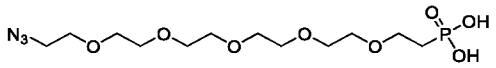
**[0467]** Azido-intermediate **6a** was commercially available with CAS 86770-69-6.

## EXAMPLE 11C

**[0468] [2-(1-Azido-3,6,9,12-tetraoxapentadecan-15**

**[0469]** Azido-intermediate **6f** was synthesized from compound **6d-1** by substitution of the bromine with azide moiety followed by the hydrolysis.

**[0470] 17-Azido-3,6,9,12,15-pentaoxaheptadecylphosphonic acid (6f)** (See FIG. 13B)



**[0471]** To a 10 mL round bottom flask were added **6d-1** (50 mg, 0.11 mmol), sodium azide (28 mg, 0.43 mmol), acetonitrile (2 mL) and water (2 mL). The mixture was stirred at 80 °C for 16 hours. LCMS showed **6d-1** was completely consumed. The reaction was cooled to RT and acetonitrile was removed *in vacuo*. The residue was partitioned between ethyl acetate (20 mL) and $H_2O$ (20 mL). The organic layer was washed with $H_2O$ (15 mL x 2), brine (15 mL), dried over anhydrous $Na_2SO_4$, filtered and concentrated to give **6d-2** (35 mg, yield:76%, ESI m/z: 428.2 (M + H)+) as red oil, which was dissolved in anhydrous DMF (2 mL). To the solution was added bromotrimethylsilane (TMSBr, 0.12 g, 0.32 mmol) at 0 °C. The mixture was stirred at 0 °C for 30 minutes, and then at RT for 16 hours. The volatiles were removed *in vacuo* and the residue was co-evaporated with dry toluene (3 times). The residue was dissolved in water and lyophilized to afford crude **6f** (crude yield >100%) as oil for the next step without further purification.

## EXAMPLE 11E

**[0472]** Azido-intermediate **6e** was commercially available with CAS 35899-89-9.

## EXAMPLE 11F

**[0473]** Azido intermediate maltose-$N_3$ (**6g**) was synthesized according to *Tetrahedron Letters*, **2001**, 42 (7), 1325-1328.

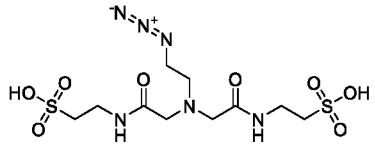
## EXAMPLE 12

**Preparation of Intermediate 6d** (See FIG. 13)

**[0474]** Azido-intermediate **6d** with dual-sulfonate was synthesized as described in FIG. 13. Azidoethanamine **31** reacted with 2 equivalents of bromoacetate **32**, followed by hydrolysis to

give dual acid **33**, which was converted to activated ester **35** with pentafluorophenol **34**. Compound **35** was amidated with taurine to provide the **intermediate 6d.**

**2-{2-[(2-Azidoethyl)({[(2-sulfoethyl)carbamoyl]methyl})amino]acetamido}ethane-1-sulfonic acid (6d)**



**[0475]** To the solution of 2-azidoethanamine **31** (0.52 g, 6.0 mmol) in acetonitrile (50 mL) were added *tert*-butyl 2-bromoacetate **32** (2.6 g, 13 mmol) and sodium carbonate (3.2 g, 30 mmol). The suspension was refluxed for 16 hr. After cooled to RT, the mixture was filtered and the filtrate was concentrated *in vacuo* to give yellow oil (1.6 g, ESI m/z: 315 $(M + H)^+$). 0.62 g of the oily product was dissolved in a solution of hydrochloride in dioxane (4 N, 10 mL). The mixture was stirred at 25 °C overnight and LCMS showed the reaction completion. The volatiles were removed *in vacuo* to give compound **33** (0.39 g, ESI m/z: 203 $(M + H)^+$) as hydrochloride salt, 0.20 g of which was dissolved in DCM (5 mL) for the next step without further purification. To the solution were added DIC (0.38 g, 3.0 mmol), DIPEA (0.77 g, 6.0 mmol) and pentafluorophenol **34** (0.55 g, 3.0 mol). The reaction mixture was stirred at RT overnight. LCMS indicated the reaction was completed. The mixture was concentrated *in vacuo* to give crude **35** (ESI m/z: 289 $(M/2 + Na)^+$), which was dissolved in DMF (5 mL). To the solution were added taurine (0.38 g, 3.0 mmol) and DIPEA (0.52 g, 4.0 mmol). The mixture was stirred at 25 °C overnight and the resulting mixture was directly purified by reversed phase flash chromatography (0-10% acetonitrile in water (with 0.01% TFA)) to give compound **6d** (0.18 g, 34% yield from 2-azidoethanamine) as a white solid. ESI m/z: 417 $(M + H)^+$.

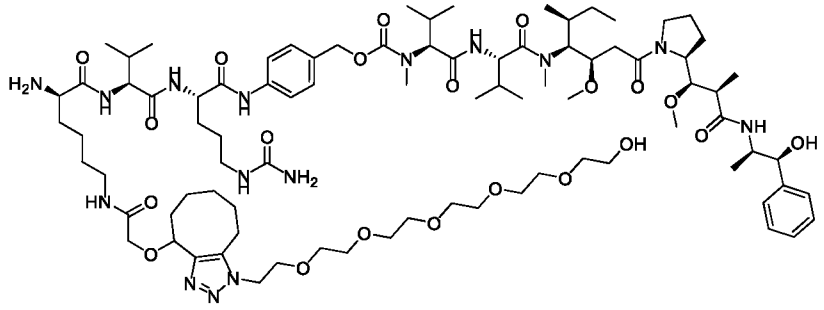
## EXAMPLE 13

**Preparation of Intermediates 7a, 7c, 7e, 7f, 7j, 7k, 7l, 7m, 7q, 7ab, 7ad, 7ae, 7bb, 7cb, 7fb** (See FIG. 14)

**[0476]** <u>General procedure E</u>: To a solution of compound **5** in DMF (0.5 mL per 10 mg of **5**) were added azido intermediate **6** (1.5 eq) and DIPEA (0.1 mL per 10 mg of **5**) at RT. The reaction was stirred at RT for 24 hours, LCMS showed the completion of reaction. The reaction mixture was directly purified by prep-HPLC to give compound **7** as a white solid.

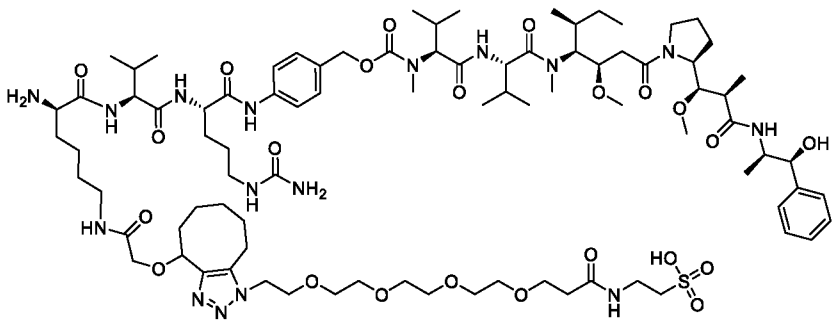
**{4-[(2*S*)-2-[(2*S*)-2-[(2*R*)-2-Amino-6-(2-{[1-(17-hydroxy-3,6,9,12,15-pentaoxaheptadecan-1-yl)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl]oxy}acetamido)hexanamido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-[(1*S*)-1-{[(1*S*)-1-**

**{[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-{[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]carbamoyl}-1-methoxy-2-methylethyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl](methyl)carbamoyl}-2-methylpropyl]carbamoyl}-2-methylpropyl]-*N*-methylcarbamate (7a)**



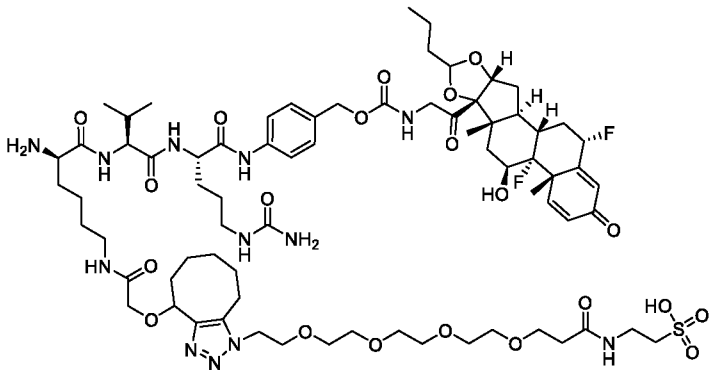
**[0477]** Following the general procedure E from compound **5a** (30 mg, 21 μmol) with compound **6a** (20 mg, 64 μmol), compound **7a** (30 mg, 74% yield) was obtained as a white solid. ESI m/z: 862 (M/2 + H)$^+$. $^1$H NMR (MeOD$_{d4}$, 500MHz): **δ** 7.66-7.57 (m, 2H), 7.44-7.29 (m, 6H), 7.26-7.18 (m, 1H), 5.25-5.04 (m, 2H), 4.88-4.74 (m, 2H), 4.70-4.47 (m, 2H), 4.59-4.48 (m, 4H), 4.29-4.17 (m, 4H), 4.00-3.95 (m, 2H), 3.93-3.88 (m, 2H), 3.76-3.70 (m, 1H), 3.69-3.64 (m, 10H), 3.62-3.59 (m, 2H), 3.59-3.55 (m, 8H), 3.49-3.42 (m, 2H), 3.38-3.36 (m, 4H), 3.31-3.28 (m, 3H), 3.27-3.17 (m, 3H), 3.15-3.06 (m, 3H), 3.00-2.87 (m, 4H), 2.57-2.46 (m, 2H), 2.41-2.18 (m, 2H), 2.17-1.99 (m, 5H), 1.98-1.86 (m, 3H), 1.85-1.68 (m, 6H), 1.67-1.54 (m, 9H), 1.50-1.36 (m, 4H), 1.34-1.25 (m, 1H), 1.22-1.12 (m, 6H), 1.06-0.98 (m, 11H), 0.96-0.93 (m, 3H), 0.92-0.84 (m, 9H), 0.79 (m, 2H). ppm.

**2-{1-[4-({[(5*R*)-5-Amino-5-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-({4-[({[(1*S*)-1-{[(1*S*)-1-{[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-{[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]carbamoyl}-1-methoxy-2-methylethyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl](methyl)carbamoyl}-2-methylpropyl]carbamoyl}-2-methylpropyl](methyl)carbamoyl}oxy)methyl]phenyl}carbamoyl)butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethane-1-sulfonic acid (7c)**



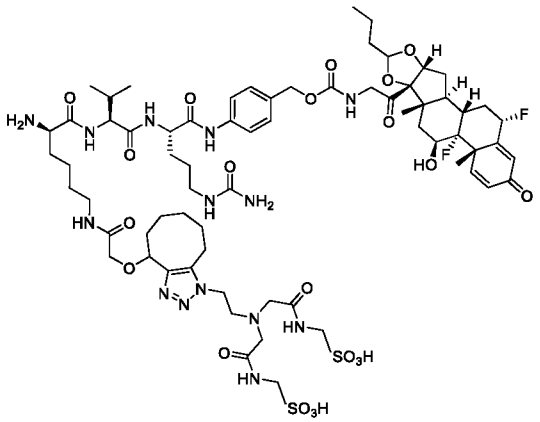
**[0478]** Following the general procedure E from compound **5a** (28 mg, 20 μmol) with compound **6c** (20 mg, 50 μmol), compound **7c** (20 mg, 56% yield) was obtained as a white solid. ESI m/z: 907.3 $(M/2 + H)^+$.

**2-{1-[4-({[(5*R*)-5-Amino-5-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethyl}carbamoyl)oxy]methyl}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethane-1-sulfonic acid (7e)**



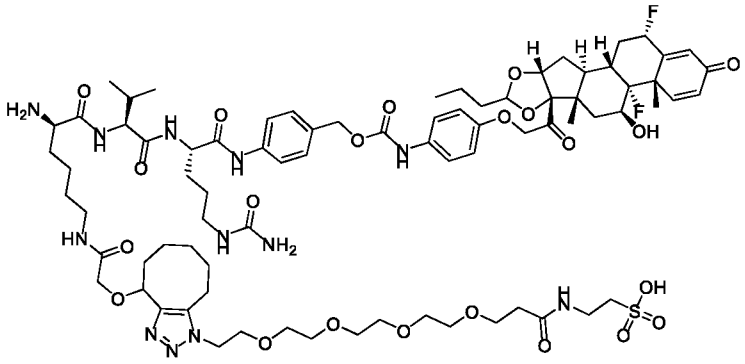
**[0479]** Following the general procedure E from **5b (60 mg, 52 μmol) with 6c**, compound **7e** (60 mg, 74% yield) was obtained as a white solid. ESI m/z: 781 $(M/2 + H)^+$. $^1$H NMR (400 MHz, $MeOD_{d4}$) $\delta$ 7.61 (d, $J$ = 8.5 Hz, 2H), 7.39-7.28 (m, 3H), 6.39-6.30 (m, 2H), 5.66-5.46 (m, 1H), 5.29-5.13 (m, 1H), 5.12-5.04 (m, 3H), 4.72-4.60 (m, 2H), 4.56-4.49 (m, 2H), 4.36-3.84 (m, 8H), 3.76-3.70 (m, 2H), 3.66-3.54 (m, 14H), 3.30-3.23 (m, 2H), 3.21-3.04 (m, 3H), 3.03-2.97 (m, 2H), 2.96-2.84 (m, 1H), 2.75-2.52 (m, 1H), 2.50-2.42 (m, 2H), 2.39-2.01 (m, 6H), 1.99-1.78 (m, 6H), 1.74-1.22(m, 22H), 1.03-0.87 (m, 12H) ppm.

**2-[2-({2-[4-({[(5*R*)-5-Amino-5-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethyl}carbamoyl)oxy]methyl}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]ethyl}({[(2-sulfoethyl)carbamoyl]methyl})amino)acetamido]ethane-1-sulfonic acid (7f)**



**[0480]** Following the general procedure E from **5b** (0.10 g, 86 μmol) with **6d**, compound **7f** (65 mg, 48% yield) was obtained as a white solid. ESI m/z: 790 (M/2 + H)$^+$. $^1$H NMR (500MHz, $DMSO_{d6}$) $\delta$ 10.1-10.0 (m, 1H), 8.60-8.50 (m, 1H), 8.40-8.30 (m, 1H), 8.30-8.20 (m, 2H), 8.15-8.00 (m, 4H), 7.60-7.55 (m, 2H), 7.50-7.40 (m, 1H), 7.30-7.20 (m, 4H), 6.30 (d, $J$ = 10.5 Hz, 1H), 6.15-6.00 (m, 2H), 5.70-5.55 (m, 3H), 4.98 (s, 2H), 4.80-4.70 (m, 1H), 4.59 (t, $J$ = 4.0 Hz, 1H), 4.50-4.45 (m, 1H), 4.40-4.35 (m, 2H), 4.25-4.10 (m, 2H), 3.95-3.80 (m, 4H), 3.20-2.90 (m, 10H), 2.85-2.75 (m, 2H), 2.70-2.60 (m, 4H), 2.31-2.10 (m, 3H), 2.10-1.95 (m, 6H), 1.80-1.65 (m, 6H), 1.65-1.55 (m, 7H), 1.40-1.20 (m, 12H), 1.20-1.10 (m, 1H), 1.06 (t, $J$ = 7.0 Hz, 1H), 1.02-1.00 (m, 1H), 0.90-0.80 (m, 16H) ppm.

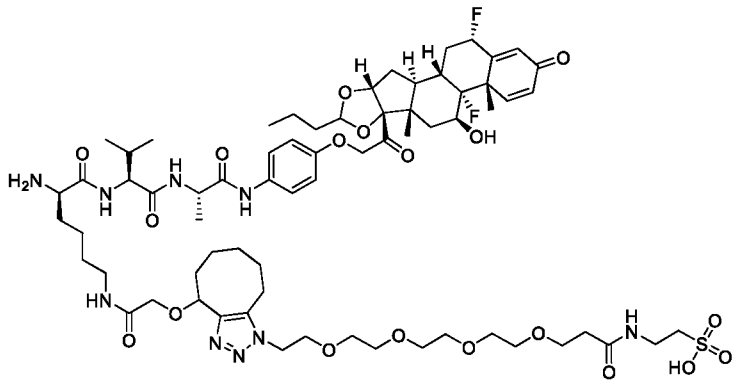
**2-{1-[4-({[(5*R*)-5-Amino-5-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-{[4-({[(4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethoxy}phenyl)carbamoyl]oxy}methyl)phenyl]carbamoyl}butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethane-1-sulfonic acid (7i)**



**[0481]** Following the general procedure E from **5c (55 mg, 54 μmol) with 6c**, compound **7i** (53 mg, yield 43%) was obtained as a white solid. ESI m/z: 827.6 (m/2 + H)$^+$. $^1$HNMR (400 MHz, $MeOD_{d4}$) $\delta$ 7.63-7.61 (m, 2H), 7.40-7.29 (m, 5H), 6.89-6.85 (m, 2H), 6.38-6.33 (m, 2H), 5.65-

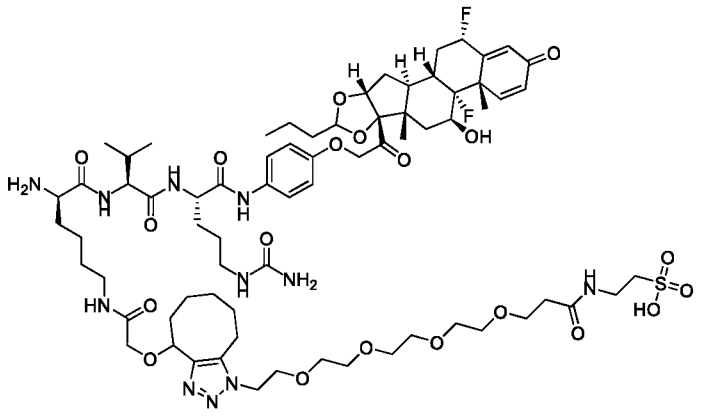
5.48 (m, 1H), 5.31-5.06 (m, 4H), 4.91-4.70 (m, 4H), 4.65-4.22 (m, 5H), 4.07-3.86 (m, 5H), 3.74-3.63 (m, 16H), 3.33-2.82 (m, 4H), 2.76-1.21 (m, 39H), 1.06-0.93 (m ,12H) ppm.

**2-{1-[4-({[(5*R*)-5-Amino-5-{[(1*S*)-1-{[(1*S*)-1-[(4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethoxy}phenyl)carbamoyl]ethyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethane-1-sulfonic acid (7j)**



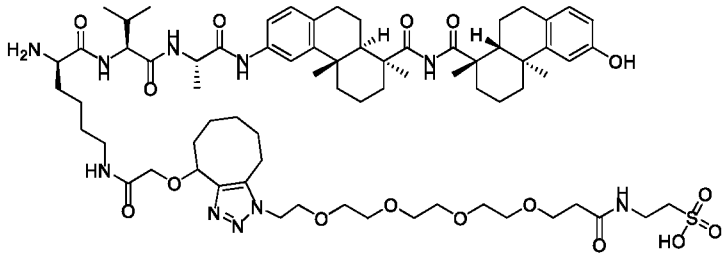
**[0482]** Following the general procedure E from **5d (55 mg, 54 µmol) with 6c**, compound **7j** (70 mg, 67% yield) was obtained as a white solid. ESI m/z: 709.9 (M/2 + H)$^+$. $^1$H NMR (400 MHz, MeOD$_{d4}$) $\delta$ 7.58-7.48 (m, 2H), 7.35 (d, $J$ = 8.8 Hz, 1H), 6.92-6.88 (m, 2H), 6.38-6.34 (m, 1H), 6.33 (s, 1H), 5.65-5.46 (m ,1H), 5.31-5.07 (m, 2H), 4.87-4.44 (m, 7H), 4.36-4.13 (m ,2H), 4.06-3.87 (m, 5H), 3.75-3.55 (m, 16H), 3.33-2.60 (m, 6H), 2.47-1.79 (m, 13H), 1.72-1.43 (m, 21H), 1.03-0.94 (m, 12H) ppm.

**2-{1-[4-({[(5*R*)-5-Amino-5-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethoxy}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethane-1-sulfonic acid (7k)**



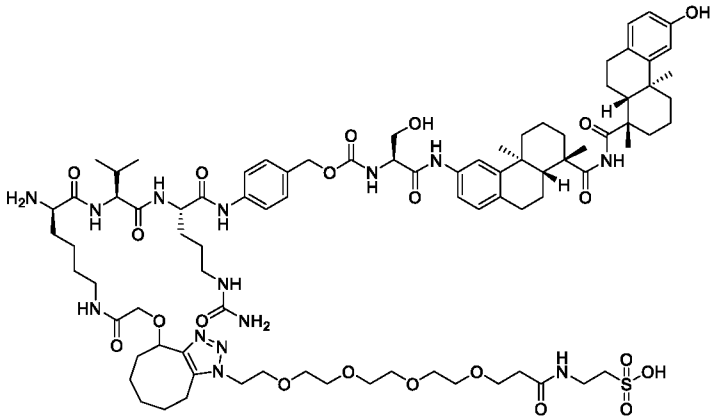
**[0483]** Following the general procedure E from **5e** (60 mg, 54 µmol) with **6c**, compound **7k** (50 mg, 61% yield) was obtained as a white solid. ESI m/z: 753 (M/2 + H)$^+$.

**1-(4-(2-((*R*)-5-Amino-6-((*S*)-1-((*S*)-1-((4b*S*,8*S*,8a*R*)-8-((1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonylcarbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-ylamino)-1-oxopropan-2-ylamino)-3-methyl-1-oxobutan-2-ylamino)-6-oxohexylamino)-2-oxoethoxy)-4,5,6,7,8,9-hexahydro-1*H*-cycloocta[d][1,2,3]triazol-1-yl)-15-oxo-3,6,9,12-tetraoxa-16-azaoctadecane-18-sulfonic acid (7m)**



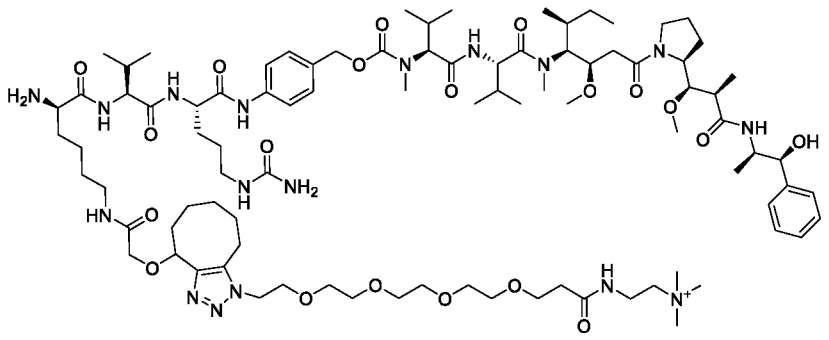
**[0484]** Following the general procedure E from **5f** (40 mg, 40 µmol) with **6c**, compound **7m** (52 mg, 77% yield) was obtained as a white solid. ESI m/z: 695.4 (M/2 + H)$^+$.

**2-{1-[4-({[(5*R*)-5-Amino-5-{[(1*S*)-1-{[(1*S*)-1-({4-[({[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}-2-hydroxyethyl]carbamoyl}oxy)methyl]phenyl}carbamoyl)-4-(carbamoylamino)butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethane-1-sulfonic acid (7q)**



**[0485]** To a solution of compound **6c** (20 mg, 50 μmol) in water (1 mL) was added dropwise *sat. aq.* sodium bicarbonate solution at 0 °C until pH ~ 7. To the stirred solution was then added a solution of compound **5?** (28 mg, 21 μmol) in acetontrile (1 mL) by syringe. The mixture was stirred at 25 °C overnight. The reaction mixture was monitored by LCMS until compound **5?** was totally consumed. The reaction mixture was filtered and purified by prep-HPLC (method A) to give compound **7q** (15 mg, 41% yield) as a white solid. ESI m/z: 856.5 $(M/2 + 1)^+$.

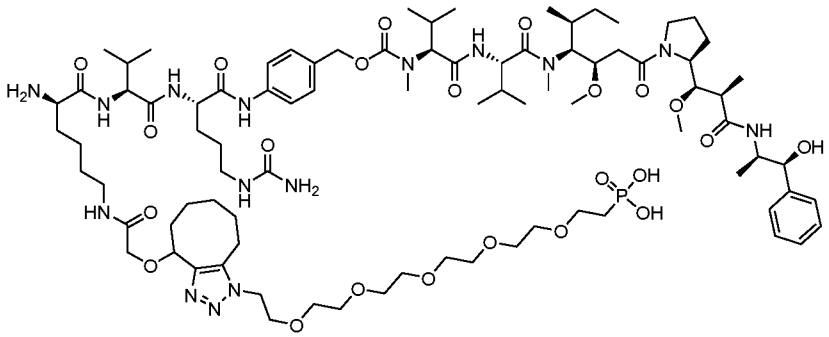
**[0486] (2-{1-[4-({[(5*R*)-5-Amino-5-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-({4-[({[(1*S*)-1-{[(1*S*)-1-{[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-{[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]carbamoyl}-1-methoxy-2-methylethyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl](methyl)carbamoyl}-2-methylpropyl]carbamoyl}-2-methylpropyl](methyl)carbamoyl}oxy)methyl]phenyl}carbamoyl)butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethyl)trimethylazanium chloride (7ab)**



**[0487]** Following the general procedure from **5a (55 mg, 39 μmol)** with **6b** except stirring at 50 °C overnight, compound **7ab** (50 mg, 70% yield) was obtained as a white solid. ESI m/z: 896 $[(M + H)/2]^+$. $^1$H NMR (400 MHz, $DMSO_{d4}$) $\delta$ 9.35 (s, 1H), 8.95 (s, 1H), 8.43-8.34 (m, 1H), 8.07-8.00 (m, 1H), 7.91-7.83 (m, 1H), 7.79-7.60 (m, 4H), 7.33-7.23 (m, 6H), 7.20-7.13 (m, 1H), 6.30-5.80 (m, 1H), 5.49-5.34 (m, 1H), 5.12-4.83 (m, 3H), 4.77-4.71 (m, 1H), 4.54-4.38

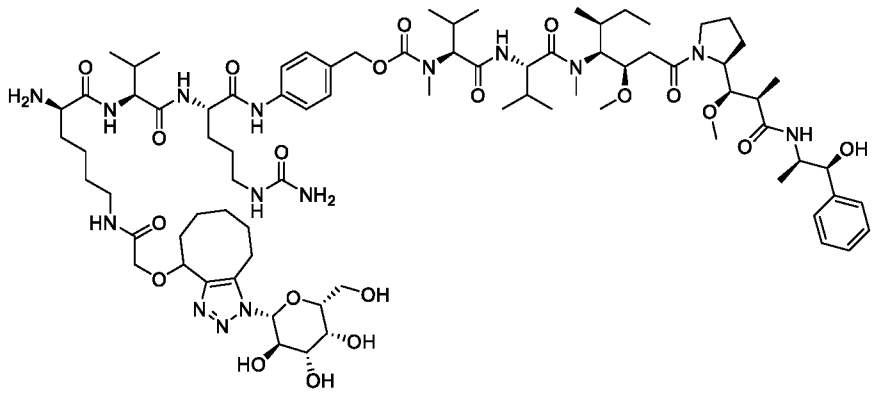
(m, 4H), 4.27 (t, *J* = 11.6 Hz, 1H), 4.03-3.93 (m, 4H), 3.85-3.75 (m, 5H), 3.59 (t, *J* = 6.2 Hz, 3H), 3.50-3.40 (m, 15H), 3.38-3.35 (m, 2H), 3.26-3.16 (m, 7H), 3.13-3.03 (m, 14H), 2.99-2.92 (m, 4H), 2.89-2.78 (m, 4H), 2.44-2.38 (m, 1H), 2.34 (t, *J* = 6.2 Hz, 2H), 2.30-2.22 (m, 2H), 2.15-1.93 (m, 6H), 1.85-1.42 (m, 19H), 1.37-1.23 (m, 3H), 1.06-0.96 (m, 7H), 0.94-0.71 (m, 27H) ppm.

**[0488]** **{17-[4-({[(5*R*)-5-Amino-5-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-({4-[({[(1*S*)-1-{[(1*S*)-1-{[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-{[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]carbamoyl}-1-methoxy-2-methylethyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl](methyl)carbamoyl}-2-methylpropyl]carbamoyl}-2-methylpropyl](methyl)carbamoyl}oxy)methyl]phenyl}carbamoyl)butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12,15-pentaoxaheptadecan-1-yl}phosphonic acid (7ad)**



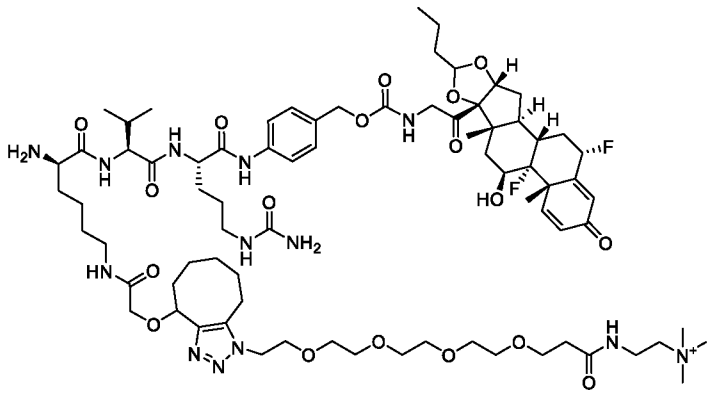
**[0489]** Following the general procedure from compound **5a** (18 mg, 13 μmol) with compound **6f** (14 mg, 38 μmol), compound **7ad** (15 mg, 58% yield) was obtained as a white solid. ESI m/z: 893.9 (M/2 + H)$^+$. $^1$H NMR (MeOD$_{d4}$, 500MHz): $\delta$ 7.98-7.91 (m, 1H), 7.79-7.59 (m, 2H), 7.41-7.20 (m, 6H), 5.51-5.06 (m, 2H), 4.78-4.53 (m, 6H), 4.29-3.88 (m, 9H), 3.78-3.75 (m, 3H), 3.64-3.58 (m, 16H), 3.47-3.36 (m, 6H), 3.29-3.07 (m, 8H), 3.01-2.80 (m, 4H), 2.58-2.05 (m, 10H), 1.96-1.32 (m, 26H), 1.21-1.14 (m, 6H), 1.02-0.71 (m, 25H) ppm.

**[0490]** **{4-[(2*S*)-2-[(2*S*)-2-[(2*R*)-2-Amino-6-[2-({1-[(2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl}oxy)acetamido]hexanamido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-[(1*S*)-1-{[(1*S*)-1-{[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-{[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]carbamoyl}-1-methoxy-2-methylethyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl](methyl)carbamoyl}-2-methylpropyl]carbamoyl}-2-methylpropyl]-*N*-methylcarbamate (7ae)**



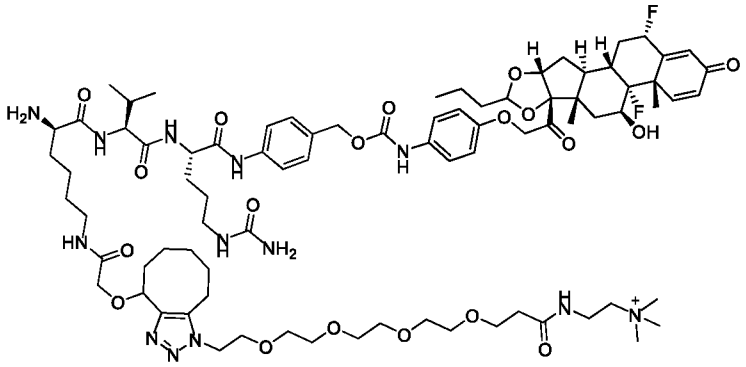
**[0491]** Following the general procedure from compound **5a** (6.0 mg, 4.2 μmol) with compound **6e** (3.0 mg, 15 μmol), the reaction solution of compound **7ae** was obtained and used directly for the next step. ESI m/z: 811 $(M/2 + H)^+$.

**[0492] (2-{1-[4-({[(5*R*)-5-Amino-5-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethyl}carbamoyl)oxy]methyl}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethyl)trimethylazanium chloride (7bb)**



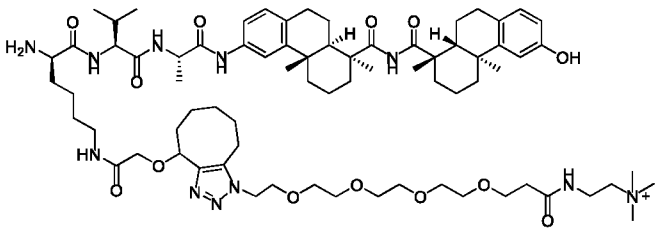
**[0493]** Following the general procedure E from **5b (40 mg, 34 μmol) with 6b** except stirring at 50 °C overnight, compound **7bb** (40 mg, 76% yield) was obtained as a white solid after purified by prep-HPLC (method B). ESI m/z: 770 $[(M + 18)/2]^+$. $^1$H NMR (400 MHz, $DMSO_{d6}$) **δ** 10.13-8.87 (m, 2H), 8.58-8.21 (m, 2H), 8.09-7.85 (m, 2H), 7.82-7.64 (m, 3H), 7.63-7.55 (m, 1H), 7.49-7.37 (m, 1H), 7.34-7.16 (m, 3H), 6.34-6.21 (m, 1H), 6.15-5.99 (m, 2H), 5.78-5.42 (m, 3H), 5.01-4.83 (m, 3H), 4.81-4.69 (m, 2H), 4.63-4.47 (m, 2H), 4.45-4.34 (m, 2H), 4.27-4.07 (m, 3H), 4.04-3.96 (m, 1H), 3.91-3.70 (m, 6H), 3.60 (t, *J* = 6.2 Hz, 2H), 3.53-3.40 (m, 14H), 3.15-3.01 (m, 11H), 3.00-2.88 (m, 3H), 2.83-2.73 (m, 1H), 2.68-2.56 (m, 1H), 2.38-2.19 (m, 4H), 2.13-1.93 (m, 4H), 1.87-1.74 (m, 2H), 1.71-1.64 (m, 2H), 1.62-1.18 (m, 22H), 1.14-1.02 (m, 1H), 0.97-0.70 (m, 12H) ppm.

**[0494]** (2-{1-[4-({[(5*R*)-5-Amino-5-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-{[4-({[(4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethoxy}phenyl)carbamoyl]oxy}methyl)phenyl]carbamoyl}butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethyl)trimethylazanium (7cb)



**[0495]** Following the general procedure from **6b** (44 mg, 35 μmol) stirring at RT for 24 hours, compound **7cb** (44 mg, yield 77%) was obtained as a white solid after purification by reversed phase flash chromatography (0-100% acetonitrile in *aq.* TFA (0.03%)). ESI m/z: 816.0 (m/2 + H)$^+$; 544.5 (M/3 + H)$^+$.

**[0496]** 1-(4-(2-(((*R*)-5-Amino-6-(((*S*)-1-(((*S*)-1-(((4b*S*,8*S*,8a*R*)-8-(((1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carbonyl)carbamoyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-6-oxohexyl)amino)-2-oxoethoxy)-4,5,6,7,8,9-hexahydro-1*H*-cycloocta[d][1,2,3]triazol-1-yl)-*N*,*N*,*N*-trimethyl-15-oxo-3,6,9,12-tetraoxa-16-azaoctadecan-18-aminium (7fb)



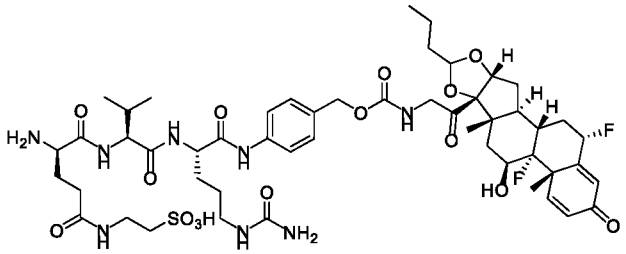
**[0497]** Following the general procedure from **6b** (50 mg, 50 μmol) stirring at RT for 24 hours, compound **7fb** (50 mg, 73% yield) was obtained as a white solid after purification by reversed phase flash chromatography (0-30% acetonitrile in *aq.* TFA (0.03%)). ESI m/z: 684 (M/2 + H)$^+$.

## EXAMPLE 14

**Preparation of Intermediates 7h, 7l** (See FIG. 15)

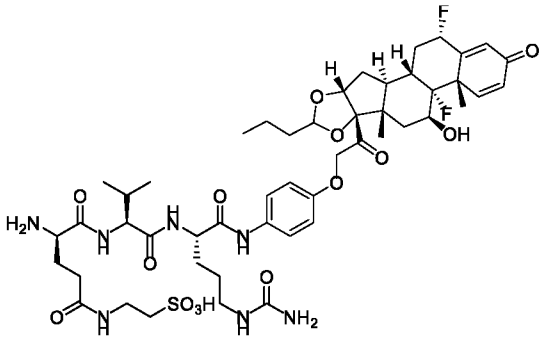
**[0498]** General procedure F: To a solution of compound **4b** (1.2 eq.) in DMF (40 mL per gram of compound **3**) were added EDCI (1.5 eq.), HOBt (2.0 eq.), compound **3** (**3b** or **3e**, 1.0 eq.) and triethylamine (3.0 eq.) successively at RT. The resulting mixture was stirred at RT overnight. LCMS showed compound **4b** was totally consumed (compound **3** was not consumed). To the reaction was added diethylamine (6 mL per gram of compound **3**, excess). The reaction mixture was stirred at RT for 2 hours until Fmoc was removed according to LCMS. The reaction mixture was directly purified by reversed phase flash chromatography (0-100% acetonitrile in *aq.* ammonium bicarbonate (10 mM)) to give compound **7** (**7h** or **7l**, 25-26% yield) as a white solid and unreacted compound **3** could be recovered.

**2-[(4*R*)-4-Amino-4-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethyl}carbamoyl)oxy]methyl}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]carbamoyl}butanamido]ethane-1-sulfonic acid (7h)**



**[0499]** Following the general procedure F from **3b** (50 mg, 57 μmol) with **4b**, compound **7h** (17 mg, 26% yield) was obtained as a white solid after purification by reversed phase flash chromatography (0-100% acetonitrile in water, 17 mg of compound **3b** was recycled (34% recycled yield)). ESI m/z: 1107 (M + H)$^+$. $^1$H NMR (500 MHz, DMSO$_{d6}$) $\delta$ 10.07 (s, 1H), 8.31 (d, $J$ = 7.0 Hz, 2H), 7.81 (t, $J$ = 5.5 Hz, 1H), 7.61-7.60 (m, 2H), 7.45-7.42 (m, 1H), 7.30-7.27 (m, 3H), 6.30 (d, $J$ = 10 Hz, 1H), 6.11-6.03 (m, 2H), 5.86-5.82 (m, 1H), 5.70-5.57 (m, 2H), 5.46 (s, 2H), 4.97 (s, 2H), 4.78-4.76 (m, 1H), 4.59 (t, $J$ = 4.5 Hz, 1H), 4.41-4.12 (m, 4H), 3.86-3.80 (m, 1H), 3.64-3.58 (m, 1H), 3.21-3.16 (m, 1H), 3.01-2.90 (m, 6H), 2.56 (t, $J$ = 7.5 Hz, 2H), 2.35-2.32 (m, 2H), 2.17-2.14 (m, 2H), 2.09-1.28 (m, 18H), 1.17-1.14 (m, 1H), 0.97 (t, $J$ = 6.5 Hz, 2H), 0.89-0.84 (m, 10H) ppm.

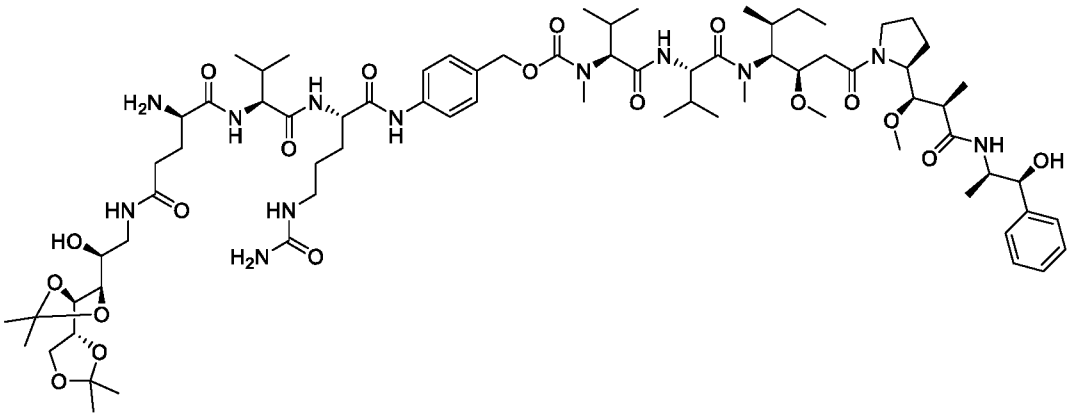
**2-[(4*R*)-4-Amino-4-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethoxy}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]carbamoyl}butanamido]ethane-1-sulfonic acid (7l)**



**[0500]** Following the general procedure F from **3e** (80 mg, 98 μmol) with **4b**, compound **7l** (26 mg, 25% yield) was obtained as a white solid after purification by reversed phase flash chromatography (0-100% acetonitrile in water). ESI m/z: 525.8 $(M/2 + H)^+$.

**Preparation of Intermediate 7ah** (See FIG. 15A)

**[0501] {4-[(2*S*)-2-[(2*S*)-2-[(2*R*)-2-Amino-4-{[(2*S*)-2-[(4*R*,5*R*)-5-[(4*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]-2,2-dimethyl-1,3-dioxolan-4-yl]-2-hydroxyethyl]carbamoyl}butanamido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-[(1*S*)-1-{[(1*S*)-1-{[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-{[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]carbamoyl}-1-methoxy-2-methylethyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl](methyl)carbamoyl}-2-methylpropyl]carbamoyl}-2-methylpropyl]-*N*-methylcarbamate (7ah)**



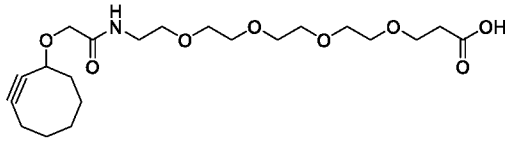
**[0502]** To a solution of intermediate **4c** (25 mg, 41 μmol) in DMF (1 mL) was added HATU (23 mg, 61 μmol) at RT. The solution obtained was stirred at RT for an hour. To this suspension were added a solution of vcPAB-MMAE **3a** (28 mg, 25 μmol) in DMF (1 mL) and subsequently NMM (1 drop, excess). The reaction mixture was stirred at RT for 3 hours and turned clear. The reaction was monitored by LCMS until compound **3a** was totally consumed. To the reaction mixture was then added diethylamine (excess), and the resulting mixture was then stirred at RT overnight. The reaction was completed according to LCMS. The volatiles were removed in vacuo and the residue was purified by reversed phase flash chromatograhy (0-100% acetonitrile in *aq.* TFA (0.5%)) to give compound **7ah** (25 mg, 67% yield) as a white

solid. ESI m/z: 1495 $(M + H)^+$. $^1H$ NMR ($MeOD_{d4}$, 500 MHz): $\delta$ 7.65-7.55 (m, 2H), 7.46-7.28 (m, 6H), 7.23 (t, $J$ = 7.1 Hz, 1H), 5.27-5.02 (m, 2H), 4.72-4.47 (m, 4H), 4.30-4.05 (m, 6H), 4.03-3.78 (m, 4H), 3.78-3.65 (m, 1H), 3.61-3.40 (m, 4H), 3.39-3.27 (m, 5H), 3.26-3.16 (m, 2H), 3.17-3.08 (m, 3H), 3.00-2.91 (m, 3H), 2.58-2.11 (m, 7H), 2.10-1.52 (m, 12H), 1.50-1.25 (m, 15H), 1.24-1.11 (m, 6H), 1.05-0.69 (m, 26H) ppm.

## EXAMPLE 14A

**[0503]** Preparation of intermediate **8c** (See FIG. 15B)

**[0504] 1-[2-(Cyclooct-2-yn-1-yloxy)acetamido]-3,6,9,12-tetraoxapentadecan-15-oic acid (8c)**



**[0505]** To a mixture of compound **36** (0.50 g, 1.8 mmol) and **37** (0.65 g, 1.8 mmol) in DMF (3 mL) was added DIPEA (1.2 g, 9.0 mmol) at RT. The mixture was stirred at RT for 30 minutes. The reaction mixture was directly purified by prep-HPLC (method A) to give OCT-PEG4-acid (**8c**) (0.70 g, 91% yield) as light yellow oil. ESI m/z: 430 $(M + H)^+$.

**[0506]** **8d** and **8e** were prepared using methods similar to the method of preparation of 8c, using suitable starting materials known to one of skill in the art.

## EXAMPLE 15

**Preparation of 1a** (See FIG. 16)

**[0507]** To a solution of DIBAC-suc-$PEG_4$-acid **8a** (1.2-1.3 eq.) in DMF (1 mL per 10 mg of **8a**) were added HATU (1.3 eq.) and DIPEA (5.0 eq.) at RT. The mixture was stirred at RT for half an hour followed by addition of a solution of compound **7a** (1.0 eq.) in DMF (0.6 mg per 10 mg of **7a**). The resulting mixture was stirred at RT until compound **7** was consumed, which was monitored by LCMS. After filtration, the filtrate was directly purified by prep-HPLC to give compound **1a**.

**[0508]** General procedure G (from **8a**, **8c** or **8d**): To a solution of acid **8** (**8a**, **8c** or **8d,** 1.2-1.3 eq.) in DMF (1 mL per 10 mg of **8**) were added HATU (1.3 eq.) and DIPEA (5.0 eq.) at RT. The mixture was stirred at RT for half an hour followed by addition of a solution of compound **7** (1.0 eq.) in DMF (0.6 mg per 10 mg of **7**). The resulting mixture was stirred at RT until compound **7** was consumed, which was monitored by LCMS. After filtration, the filtrate was directly purified by prep-HPLC to give linker-payloads **1**, and **II-V**.

## EXAMPLE 16

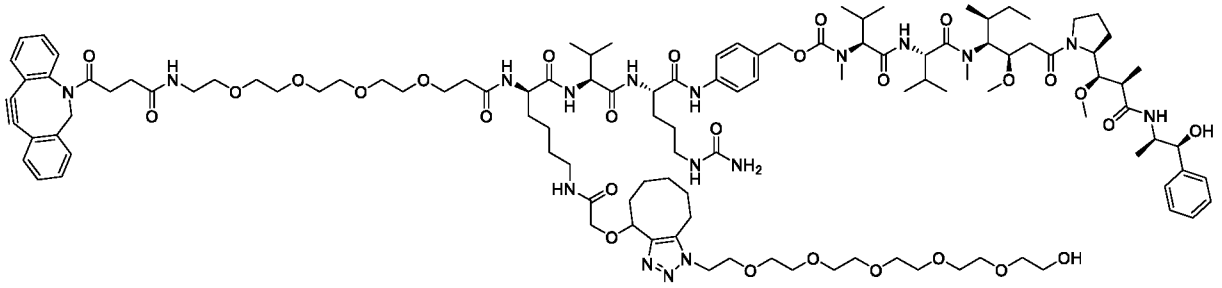
**Preparation of 1a, 1c, 1e, 1f, 1h, 1i, 1j, 1k, 1l, 1m 1q** (See FIG. 17)

**[0509]** General procedure H (from **8b or 8e**): To a solution of compound **7** (1.0 eq.) in DMF (1 mL per 50 mg) were added compound DIBAC-PEG$_4$-NHS **8b** (1.1-1.2 eq.) and DIPEA (5.0 eq.) at RT. The reaction mixture was stirred at RT for 3 hours. The reaction mixture was directly purified by prep-HPLC to give compound **1**.

## EXAMPLE 17

**Preparation of 1a** (See FIG. 17)

**{4-[(2*S*)-2-[(2*S*)-2-[(2*R*)-2-[1-(4-{2-Azatricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-6-(2-{[1-(17-hydroxy-3,6,9,12,15-pentaoxaheptadecan-1-yl)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl]oxy}acetamido)hexanamido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido] phenyl}methyl *N*-[(1*S*)-1-{[(1*S*)-1-{[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-{[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]carbamoyl}-1-methoxy-2-methylethyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl](methyl)carbamoyl}-2-methylpropyl]carbamoyl}-2-methylpropyl]-*N*-methylcarbamate (1a)**

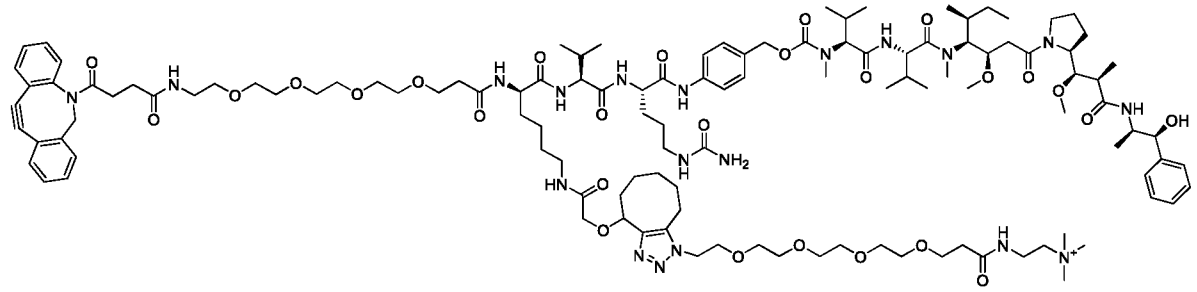


**[0510]** Following the general procedure G from compound **7a** (25 mg, 15 μmol) with compound **8a** (10 mg, 18 μmol), linker-payload **1a** (24 mg, 73% yield) was obtained as a white solid. ESI: 753 (M/3 + H)$^+$. $^1$H NMR (500 MHz, MeOD$_{d4}$) $\delta$ 7.74-7.69 (m, 2H), 7.67-7.64 (m, 1H), 7.63-7.59 (m, 1H), 7.53-7.45 (m, 3H), 7.42-7.29 (m, 9H), 7.25-7.20 (m, 1H), 5.22-5.05 (m, 3H), 4.70-4.49 (m, 4H), 4.47-4.43 (m, 1H), 4.33-4.29 (m, 1H), 4.27-4.16 (m, 4H), 4.00-3.95 (m, 2H), 3.92-3.87 (m, 2H), 3.77-3.69 (m, 2H), 3.68-3.63 (m, 10H), 3.61-3.52 (m, 21H), 3.49-3.41 (m, 5H), 3.38-3.36 (m, 4H), 3.30-3.27 (m, 3H), 3.27-3.22 (m, 3H), 3.20-3.14 (m, 2H), 3.12 (s, 1H), 3.10-3.03 (m, 1H), 2.99-2.86 (m, 4H), 2.82-2.68 (m, 2H), 2.56-2.12 (m, 10H), 2.11-1.97 (m, 5H), 1.91-1.79 (m, 5H), 1.76-1.53 (m, 10H), 1.48-1.37 (m, 3H), 1.34-1.26 (m, 1H), 1.21-1.12 (m, 14H), 1.06-0.93 (m, 14H), 0.92-0.81 (m, 10H) ppm.

## EXAMPLE 17A

**Preparation of Ib** (See FIG. 17)

**[0511]** (2-{1-[4-({[(5*R*)-5-[1-(4-{2-**Azatricyclo[10.4.0.0$^{4,9}$]hexadeca**-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-5-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-({4-[({[(1*S*)-1-{[(1*S*)-1-{[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-{[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]carbamoyl}-1-methoxy-2-methylethyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl](methyl)carbamoyl}-2-methylpropyl]carbamoyl}-2-methylpropyl](methyl)carbamoyl}oxy)methyl]phenyl}carbamoyl)butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethyl)trimethylazanium (Ib)



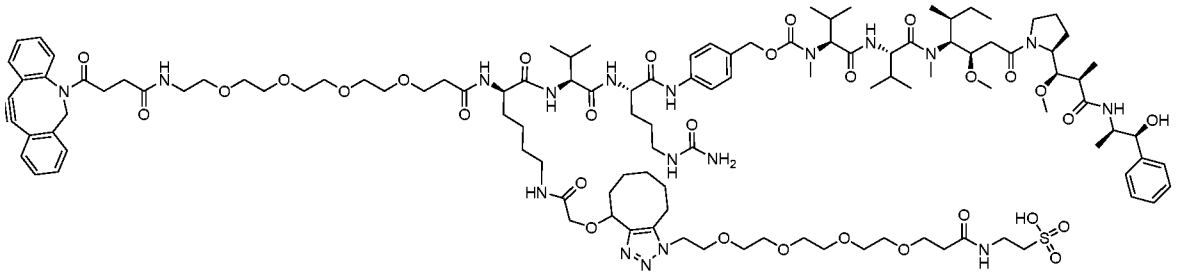
**[0512]** Following the general procedure G from compound **7ab** (45 mg, 25 μmol) with compound **8a** (16 mg, 29 μmol), linker-payload **Ib** (16 mg, 27% yield) was obtained as a white solid. ESI m/z: 766 $[(M+H)/3]^+$. $^1$H NMR (500MHz, $DMSO_{d6}$): $\delta$ 9.78 (s, 1H), 8.34-8.04 (m, 5H), 7.95-7.83 (m, 2H), 7.79-7.74 (m, 1H), 7.70-7.56 (m, 5H), 7.52-7.43 (m, 3H), 7.40-7.11 (m, 12H), 6.08 (s, 1H), 5.43 (s, 2H), 5.06-5.00 (m, 2H), 4.76-4.71 (m, 1H), 4.54-4.39 (m, 4H), 4.35-4.15 (m, 4H), 4.03-3.92 (m, 2H), 3.85-3.73 (m, 5H), 3.64-3.55 (m, 5H), 3.46-3.43 (m, 20H), 3.25-3.16 (m, 11H), 3.13-3.05 (m, 15H), 3.00-2.93 (m, 5H), 2.89-2.82 (m, 4H), 2.80-2.73 (m, 2H), 2.61-2.55 (m, 1H), 2.42-2.32 (m, 5H), 2.31-2.20 (m, 4H), 2.15-2.04 (m, 4H), 2.03-1.95 (m, 4H), 1.83-1.65 (m, 8H), 1.57-1.38 (m, 11H), 1.30-1.19 (m, 10H), 1.06-0.96 (m, 7H), 0.87-0.76 (m, 20H) ppm.

## EXAMPLE 18

**Preparation of 1c** (See FIG. 17)

**[0513]** 2-{1-[4-({[(5*R*)-5-[1-(4-{2-Azatricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-5-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-({4-[({[(1*S*)-1-{[(1*S*)-1-{[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-{[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]carbamoyl}-1-methoxy-2-methylethyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl](methyl)carbamoyl}-2-methylpropyl]carbamoyl}-2-methylpropyl](methyl)carbamoyl}oxy)methyl]phenyl}carbamoyl)butyl]carbamoyl}-2-

**methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethane-1-sulfonic acid (1c)**

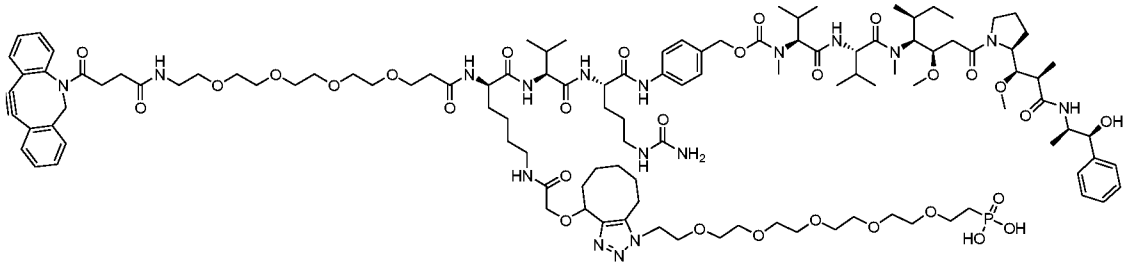


**[0514]** Following the general procedure H from compound **7c** (20 mg, 11 μmol) with compound **8b** (7.1 mg, 11 μmol), compound **1c** (5.0 mg, 19% yield) was obtained as a white solid. ESI m/z: 1174.7 $(M/2 + H)^+$. $^1H$ NMR ($MeOD_{d4}$, 500MHz): $\delta$ 8.38-8.17 (m, 2H), 7.99-7.87 (m, 2H), 7.77-7.56 (m, 4H), 7.49-7.20 (m, 9H), 5.36 (t, $J$ = 4.5 Hz, 1H), 5.21 -5.07 (m, 4H), 4.71-4.18 (m, 9H), 3.98-3.88 (m, 5H), 3.74-3.43 (m, 37H), 3.37-3.36 (m, 6H), 3.29-3.12 (m, 6H), 3.00-2.88 (m, 6H), 2.75 -2.33 (m, 5H), 2.32-1.78 (m, 17H), 1.64-1.34 (m, 15H), 1.20-1.13 (m, 6H), 1.03-0.76 (m, 30H) ppm.

## EXAMPLE 18A

**Preparation of Id** (FIG. 17)

**[0515] {4-[(2*S*)-2-[(2*S*)-2-[(2*R*)-2-[1-(4-{2-Azatricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-6-(2-{[1-(14-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-({4-[({[(1*S*)-1-{[(1*S*)-1-{[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-{[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]carbamoyl}-1-methoxy-2-methylethyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl](methyl)carbamoyl}-2-methylpropyl]carbamoyl}-2-methylpropyl](methyl)carbamoyl}oxy)methyl]phenyl}carbamoyl)butyl]carbamoyl}-2-methylpropyl]carbamoyl}-3,6,9,12-tetraoxatetradecan-1-yl)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl]oxy}acetamido)hexanamido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-[(1*S*)-1-{[(1*S*)-1-{[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-{[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]carbamoyl}-1-methoxy-2-methylethyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl](methyl)carbamoyl}-2-methylpropyl]carbamoyl}-2-methylpropyl]-*N*-methylcarbamate (Id)**

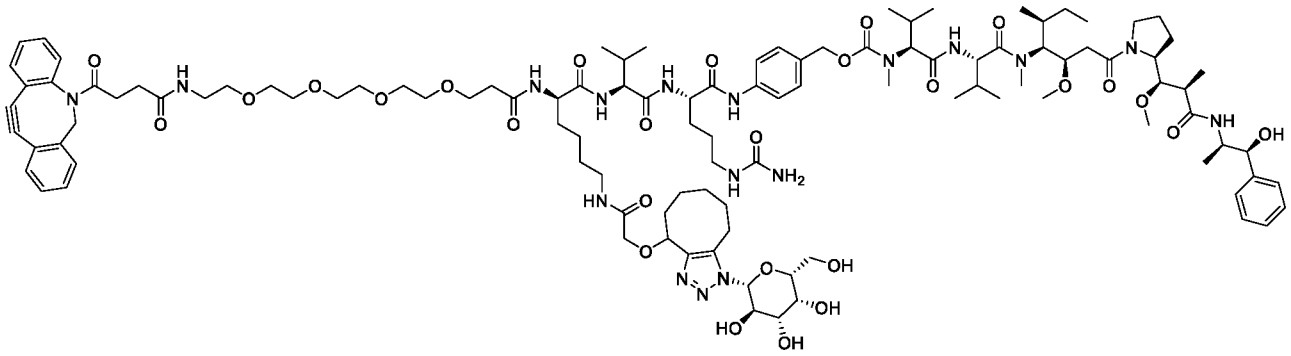


**[0516]** Following the general procedure G from compound **7ad** (12 mg, 6.7 μmol) with compound **8a** (4.4 mg, 8.0 μmol), linker-payload **Id** (3.0 mg, 19% yield) was obtained as a white solid. ESI m/z: 774.7 (M/3 + H)$^+$. $^1$H NMR (MeOD$_{d4}$, 500MHz): $\delta$ 8.00-7.90 (m, 1H), 7.94-7.61 (m, 4H), 7.49-7.47(m, 3H), 7.41-7.14 (m, 9H), 5.37-5.03 (m, 5H), 4.70-4.44 (m, 6H), 4.34-3.90 (m, 7H), 3.77-3.42 (m, 38H), 3.37-3.35 (m, 4H), 3.29 (s, 3H), 3.27-3.01 (m, 9H), 2.97-2.87 (m, 6H), 2.75-2.59 (m, 2H), 2.53-2.16 (m, 9H), 2.06-1.81 (m, 12H), 1.64-1.32 (m, 19H), 1.20-1.13 (m, 6H), 1.03-0.79 (m, 21H) ppm. Anal. HPLC: 95%, Retention time: 5.56 and 6.64 min (method B).

## EXAMPLE 18B

**Preparation of Ie-1** (FIG. 17)

**[0517] {4-[(2*S*)-2-[(2*S*)-2-[(2*R*)-2-[1-(4-{2-Azatricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-6-[2-({1-[(2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl}oxy)acetamido]hexanamido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl}methyl *N*-[(1*S*)-1-{[(1*S*)-1-{[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-{[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]carbamoyl}-1-methoxy-2-methylethyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl](methyl)carbamoyl}-2-methylpropyl]carbamoyl}-2-methylpropyl]-*N*-methylcarbamate (Ie-1) (with triazole isomers)**



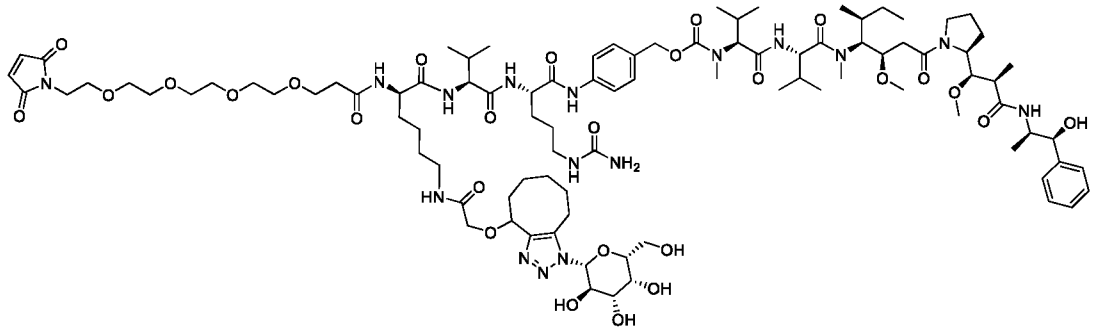
**[0518]** Following the general procedure H from **7ae** (reaction solution) and **8b,** the compound **Ie-1** with triazole isomers (with the ratio 3/2 by HPLC) (13 mg, 30% yield from **5a**) was obtained as a white solid after purification by prep-HPLC (method B). ESI m/z: 1078 (M/2 +

H)$^+$ (100%), 2156.9 (M + H)$^+$ (10%). $^1$H NMR (DMSO$_{d6}$, 500 MHz): $\delta$ 9.69 (m, 1H), 8.08-8.30 (m, 4H), 7.84-7.89 (m, 1H), 7.61-7.75 (m, 6H), 7.45-7.49 (m, 2H), 7.16-7.37 (m, 10H), 5.96 (m, 1H), 5.34-5.51 (m, 4H), 4.97-5.16 (m, 5H), 4.62-4.75 (m, 4H), 3.92-4.49(m, 9H), 3.77-3.80 (m, 3H), 3.40-3.72 (m, 18H), 3.24 (s, 3H), 3.23 (s, 2H), 3.18-3.20 (m, 4H), 2.88-3.17 (m, 17H), 2.54-2.83 (m, 2H), 2.35-2.42 (m, 2H), 2.22-2.29 (m, 3H), 1.96-2.15 (m, 7H), 1.72-1.82 (m, 5H), 1.28-1.65 (m, 16H), 0.87-1.05 (m, 6H), 0.75-0.87 (m, 27H). Anal. HPLC: isomer 1: 60.3%, Retention time: 7.34 min; isomer 2: 39.7%, Retention time: 7.41 min (method B).

## EXAMPLE 18C

**Preparation of If-1** (FIG. 17)

**[0519] {4-[(2*S*)-5-(Carbamoylamino)-2-[(2*S*)-2-[(2*R*)-2-[1-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3,6,9,12-tetraoxapentadecan-15-amido]-6-[2-({1-[(2*R*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-4-yl}oxy)acetamido]hexanamido]-3-methylbutanamido]pentanamido]phenyl}methyl *N*-[(1*S*)-1-{[(1*S*)-1-{[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-{[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]carbamoyl}-1-methoxy-2-methylethyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl](methyl)carbamoyl}-2-methylpropyl]carbamoyl}-2-methylpropyl]-*N*-methylcarbamate (If-1)**

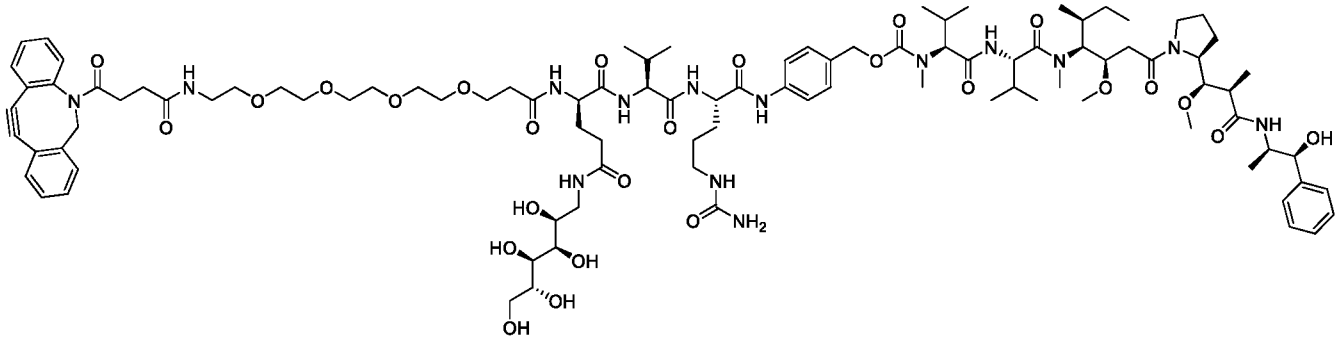


**[0520]** Following the general procedure H from **7ae** (reaction solution) and **8e,** the compound **If-1** with triazole isomers (with the ratio 3/2 by HPLC) (1.5 mg, 18% yield from **5a**) was obtained as a white solid after purification by prep-HPLC (method A). ESI m/z: 974.7 (M/2 + H)$^+$ (100%), 1972 (M + Na)$^+$ (20%). $^1$H NMR (DMSO$_{d6}$, 500 MHz): $\delta$ 9.69 (s, 1H), 8.19-7.97 (m, 4H), 7.92-7.83 (m, 1H), 7.66-7.54 (m, 3H), 7.36-7.23 (m, 6H), 7.22-7.12 (m, 2H), 7.01 (s, 2H), 6.87 (s, 2H), 6.68-6.59 (m, 2H), 6.03-5.93 (m, 1H), 5.47-5.26 (m, 5H), 5.20-5.11 (m, 1H), 5.07-4.95 (m, 2H), 4.80-4.58 (m, 3H), 4.53-4.38 (m, 2H), 4.36-4.12 (m, 5H), 4.04-3.94 (m, 2H), 3.84-3.76 (m, 3H), 3.68-3.37 (m, 21H), 3.26-3.17 (m, 7H), 3.13-2.96 (m, 7H), 2.90-2.81 (m, 3H), 2.44-2.34 (m, 2H), 2.31-2.21 (m, 2H), 2.18 (s, 3H), 2.15-1.94 (m, 9H), 1.77-1.74 (m, 2H), 1.69-1.59 (m, 4H), 1.56-1.42 (m, 9H), 1.32-1.27 (m, 2H), 1.06-0.96 (m, 6H), 0.90-0.72 (m, 26H) ppm. Anal. HPLC: isomer 1: 76.0%, Retention time: 7.19 min; isomer 2: 24.0%, Retention time: 7.28 min (method A).

## EXAMPLE 18D

**Preparation of Ig** (FIG. 18)

**[0521] {4-[(2*S*)-2-[(2*S*)-2-[(2*R*)-2-[1-(4-{2-Azatricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-4-{[(2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxyhexyl]carbamoyl}butanamido]-3-methylbutanamido]-5-(carbamoylamino)pentanamido]phenyl} methyl *N*-[(1*S*)-1-{[(1*S*)-1-{[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-{[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]carbamoyl}-1-methoxy-2-methylethyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl](methyl)carbamoyl}-2-methylpropyl]carbamoyl}-2-methylpropyl]-*N*-methylcarbamate (Ig)**

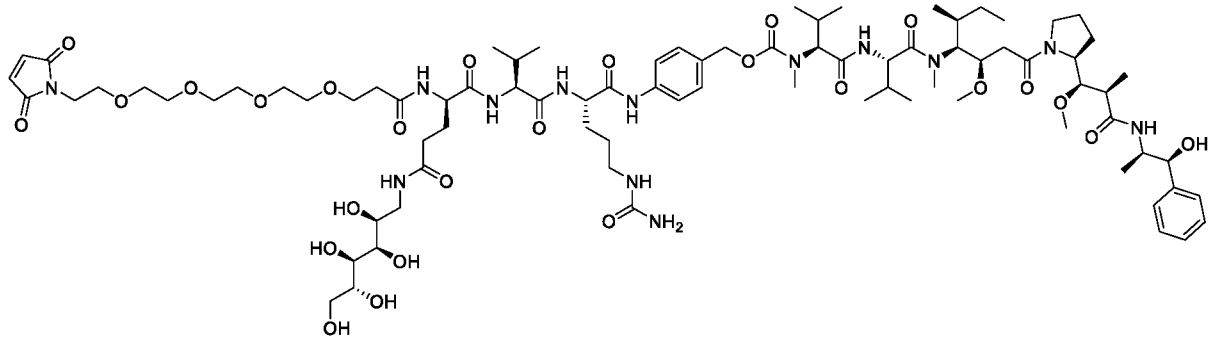


**[0522]** To a mixture of intermediate **7ah** (40 mg, 27 μmol) in DCM (3 mL) was added TFA (0.25 mL) dropwise at 0 °C. The mixture was stirred at RT for an hour until **7ah** was consumed. The de-protection was completed according to LCMS. The volatiles were removed in vacuo and the residue (ESI m/z: 708 (M/2 + H)$^+$) was dissolved in DMF (2 mL). To the solution were added intermediate **8b** (17 mg, 27 μmol) and triethylamine (8.2 mg, 81 μmol). The mixture stirred at RT overnight. The reaction mixture was directly purified by prep-HPLC (method B) to give **Ig** (8.0 mg, 15% yield in two steps from **7ah**) as a white solid. ESI m/z: 650.8 (M/3 + H)$^+$ (100%), 975 (M/2 + H)$^+$ (40%). $^1$H NMR (DMSO$_{d6}$, 500 MHz): $\delta$ 9.75 (s, 1H), 8.31-8.06 (m, 5H), 7.91-7.88 (m, 1H), 7.75-7.74 (m, 2H), 7.68-7.61 (m, 5H), 7.50-7.46 (m, 3H), 7.39-7.24 (m, 11H), 7.17-7.15 (m, 1H), 5.99 (s, 1H), 5.41-5.34 (m, 3H), 5.04-5.01 (m, 2H), 4.77-4.75 (m, 1H), 4.48-4.18 (m, 11H), 4.05-3.95 (m, 3H), 3.62-3.55 (m, 7H), 3.47-3.17 (m, 14H), 3.12-2.97 (m, 10H), 2.88-2.83 (m, 3H), 2.60-2.54 (m, 1H), 2.53-2.51 (m, 2H), 2.42-2.36 (m, 2H), 2.29-2.21 (m, 3H), 2.17-1.99 (m, 8H), 1.79-1.71 (m, 7H), 1.54-1.44 (m, 3H), 1.36-1.28 (m, 2H), 1.05-0.97 (m, 8H), 0.88-0.75 (m, 28H) ppm. Anal. HPLC: 96.9%, Retention time: 7.58 min (method B).

## EXAMPLE 18E

**Preparation of Ih-1** (FIG. 18)

**[0523]** **{4-[(2*S*)-5-(Carbamoylamino)-2-[(2*S*)-2-[(2*R*)-2-[1-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3,6,9,12-tetraoxapentadecan-15-amido]-4-{[(2*R*,3*S*,4*S*,5*S*)-2,3,4,5,6-pentahydroxyhexyl]carbamoyl}butanamido]-3-methylbutanamido]pentanamido]phenyl}methyl *N*-[(1*S*)-1-{[(1*S*)-1-{[(3*R*,4*S*,5*S*)-1-[(2*S*)-2-[(1*R*,2*R*)-2-{[(1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl]carbamoyl}-1-methoxy-2-methylethyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl](methyl)carbamoyl}-2-methylpropyl]carbamoyl}-2-methylpropyl]-*N*-methylcarbamate (Ih-1)**



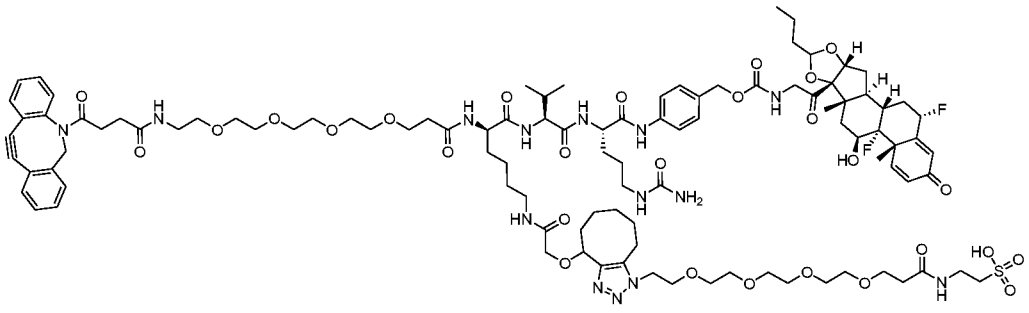
**[0524]** To a solution of intermediate **7ah** (10 mg, 6.7 μmol) in DMF (1 mL) was added intermediate **8e** (5.9 mg, 13 μmol) and DIPEA (1.7 mg, 13 μmol). The mixture was stirred at RT for 24 hours until most of **10a** was consumed according to LCMS. The reaction mixture was filtered through membrane and the filtration was purified by prep-HPLC (Method A) (ESI m/z: 912 $(M/2 + H)^+$). The residue after lyophilization was dissolved in acetonitrile (2 mL) and the solution was added copper(II) chloride dehydrate (19 mg, 0.12 mmol). The mixture was stirred at RT for two days. Desired mass of **Ih** was detected by LCMS as major product. After filtered to remove inorganic salts, the solution was directly purified by prep-HPLC (Method A) to give **Ih-1** (2.0 mg, 17% yield in two steps from **7ah**) as a white solid. ESI m/z: 1742.7 $(M + H)^+$, 1764.8 $(M + Na)^+$. $^1H$ NMR ($DMSO_{d6}$, 500 MHz): $\delta$ 9.75 (s, 1H), 8.42-7.84 (m, 5H), 7.79-7.70 (m, 1H), 7.68-7.52 (m, 3H), 7.44-7.11 (m, 8H), 7.02 (s, 2H), 6.04-5.93 (m, 1H), 5.45-5.31 (m, 3H), 5.15-4.57 (m, 5H), 4.53-3.91 (m, 14H), 3.84-3.74 (m, 1H), 3.63-3.53 (m, 8H), 3.52-3.09 (m, 9H), 3.04-2.92 (m, 4H), 2.90-2.82 (m, 3H), 2.42-2.36 (m, 2H), 2.32-2.21 (m, 2H), 2.19-1.92 (m, 8H), 1.90-1.15 (m, 23H), 1.06-0.97 (m, 6H), 0.90-0.71 (m, 26H) ppm. Anal. HPLC: 99.4%, Retention time: 6.09 min (method A).

## EXAMPLE 19

**Preparation of 1e** (See FIG. 17)

**2-{1-[4-({[(5*R*)-5-[1-(4-{2-Azatricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-5-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-**

**14,17-dien-8-yl]-2-oxoethyl}carbamoyl)oxy]methyl}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethane-1-sulfonic acid (1e)**

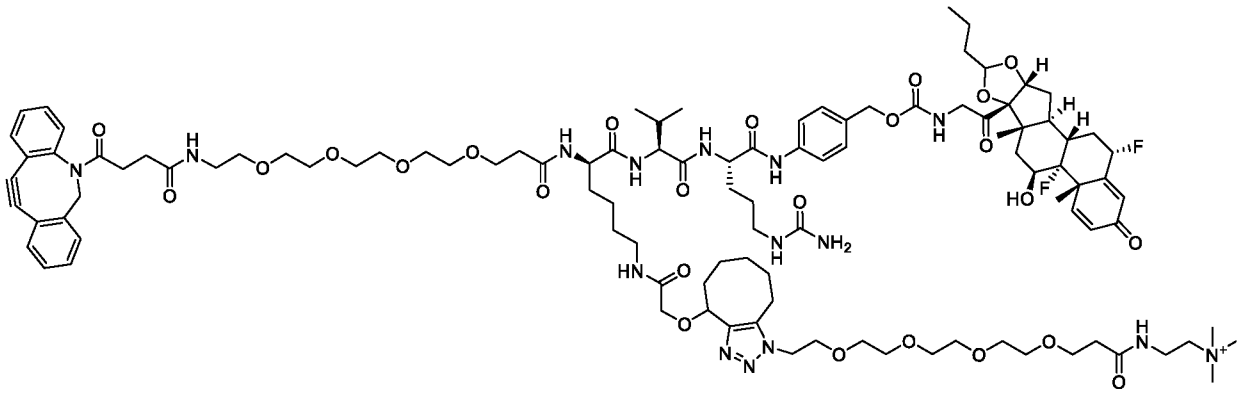


**[0525]** Following the general procedure H from compound **7e** (47 mg, 30 µmol) with **8b**, compound **1e** (28 mg, 45% yield) was obtained as a white solid. ESI m/z: 1049 $(M/2 + H)^+$. 1H NMR (500 MHz, MeODd4) δ 7.75-7.57 (m, 4H), 7.48-7.43 (m, 3H), 7.38-7.23 (m, 6H), 6.37-6.28 (m, 2H), 5.65-5.43 (m, 1H), 5.17-5.03 (m, 3H), 4.69-4.59 (m, 2H), 4.52-4.47 (m, 1H), 4.45-4.41 (m, 1H), 4.34-4.25(m, 2H), 4.23-4.13 (m, 2H), 4.07-3.86 (m, 5H), 3.77-3.68 (m, 4H), 3.64-3.54 (m, 22H), 3.52-3.47 (s, 3H), 3.46-3.40 (m, 4H), 3.29-3.21 (m, 4H), 3.20-3.14 (m, 2H), 3.10-3.01 (m, 1H), 3.00-2.95 (m, 2H), 2.92-2.84 (m, 1H), 2.76-2.60 (m, 2H), 2.47-2.42 (m, 2H), 2.40-2.25 (m, 5H), 2.23-2.13 (m, 3H), 2.07-1.97 (m, 3H), 1.90-1.79 (m, 4H), 1.71-1.55 (m, 14H), 1.52-1.42 (m, 3H), 1.40-1.29 (m, 8H), 1.07-0.85 (m, 12H) ppm. Anal. HPLC: 98%, Retention time: 5.88 min (method B).

## EXAMPLE 19A

**Preparation of IIb** (FIG. 17)

**[0526] (2-{1-[4-({[(5*R*)-5-[1-(4-{2-Azatricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-5-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethyl}carbamoyl)oxy]methyl}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethyl)trimethylazanium chloride (IIb)**

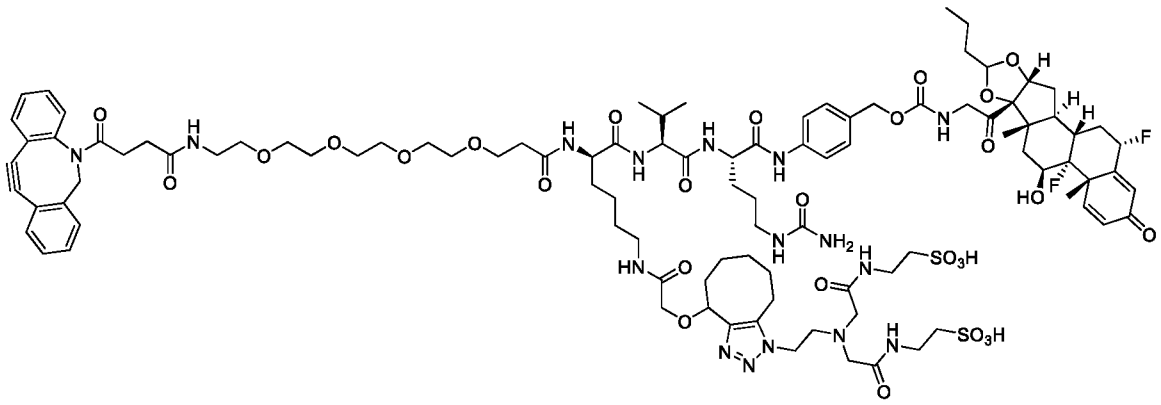


**[0527]** Following the general procedure G from compound **7bb** (35 mg, 23 μmol) with **8a**, compound **IIb** (25 mg, 52% yield) was obtained as a white solid after purified by reversed phase flash chromatography 0-100% acetonitrile in *aq.* ammonium bicarbonate (10 mM) and then 100% methanol). ESI m/z: 1037 $[(M + 18)/2]^+$. $^1$H NMR (400 MHz, $DMSO_{d6}$) $\delta$ 9.82-9.74 (m, 1H), 8.30 (s, 1H), 8.21-8.08 (m, 2H), 7.92-7.82 (m, 1H), 7.76 (t, *J* = 5.5 Hz, 1H), 7.69-7.58 (m, 3H), 7.53-7.18 (m, 9H), 6.34-6.26 (m, 1H), 6.15-6.03 (m, 2H), 5.71-5.53 (m, 1H), 5.43 (s, 2H), 5.31 (t, *J* = 12.4, 7.8 Hz, 1H), 5.10-4.90 (m, 3H), 4.79-4.72 (m, 1H), 4.58 (t, *J* = 4.2 Hz, 1H), 4.52 (t, 1H), 4.44-4.28 (m, 3H), 4.25-4.11 (m, 3H), 3.88-3.74 (m, 4H), 3.64-3.55 (m, 3H), 3.49-3.42 (m, 20H), 3.13-3.02 (m, 12H), 3.00-2.91 (m, 3H), 2.81-2.73 (m, 1H), 2.61-2.54 (m, 1H), 2.41-2.32 (m, 3H), 2.29-2.19 (m, 3H), 2.12-1.93 (m, 7H), 1.83-1.73 (m, 3H), 1.70-1.62 (m, 3H), 1.60-1.31 (m, 20H), 1.31-1.19 (m, 17H), 1.15-1.04 (m, 3H), 0.89-0.79 (m, 12H) ppm.

## EXAMPLE 20

**Preparation of 1f** (See FIG. 17)

**2-[2-({2-[4-({[(5*R*)-5-[1-(4-{2-Azatricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-5-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethyl}carbamoyl)oxy]methyl}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]ethyl}({[(2-sulfoethyl)carbamoyl]methyl})amino)acetamido]ethane-1-sulfonic acid (1f)**

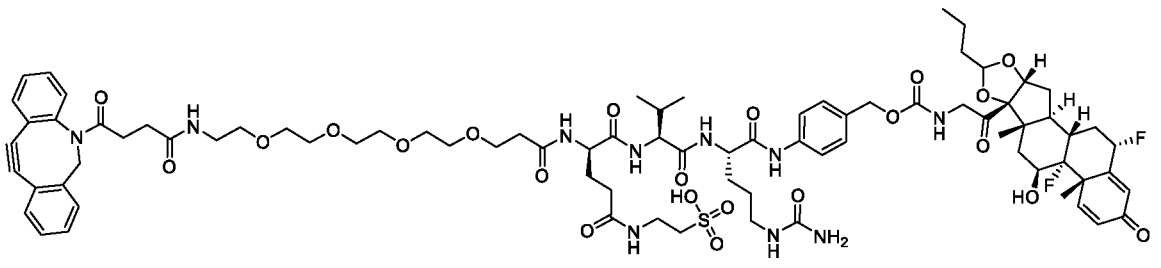


**[0528]** Following the general procedure H from compound **7f** (60 mg, 38 μmol) with **8b**, compound **1f** (25 mg, 31% yield) was obtained as a white solid. ESI m/z: 1058 $(M/2 + H)^+$. $^1H$ NMR (500 MHz, $DMSO_{d6}$) $\delta$ 9.62-9.58 (m, 1H), 8.25-8.00 (m, 6H), 7.90-7.80 (m, 1H), 7.77 (t, $J$ = 5.5 Hz, 1H), 7.70-7.60 (m, 4H),7.55-7.25 (m, 12H), 6.30 (d, $J$ = 10.5 Hz, 1H), 6.10 (s, 1H), 6.05-5.95 (m, 1H), 5.70-5.55 (m, 2H), 5.40 (s, 2H), 5.10-4.90 (m, 4H), 4.80-4.70 (m, 1H), 4.59 (t, $J$ = 4.0 Hz, 1H), 4.50-4.35 (m, 2H), 4.35-4.25 (m, 2H), 4.25-4.10 (m, 3H), 3.90-3.75 (m, 3H), 3.65-3.50 (m, 5H), 3.50-3.40 (m, 16H), 3.20-3.05 (m, 12H), 3.00-2.80 (m, 6H), 2.65-2.55 (m, 6H), 2.40-2.35 (m, 1H), 2.30-2.20 (m, 5H), 2.10-1.95 (m, 5H), 1.85-1.70 (m, 4H), 1.65-1.30 (m, 18H), 0.90-0.80 (m, 12H) ppm. *Anal.* HPLC: 97%, Retention time: 6.82 min (method B).

## EXAMPLE 21

**Preparation of 1h** (See FIG. 17)

**[0529] 2-[(4*R*)-4-[1-(4-{2-Azatricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-4-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{[({2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethyl}carbamoyl)oxy]methyl}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]carbamoyl}butanamido]ethane-1-sulfonic acid (1h)**



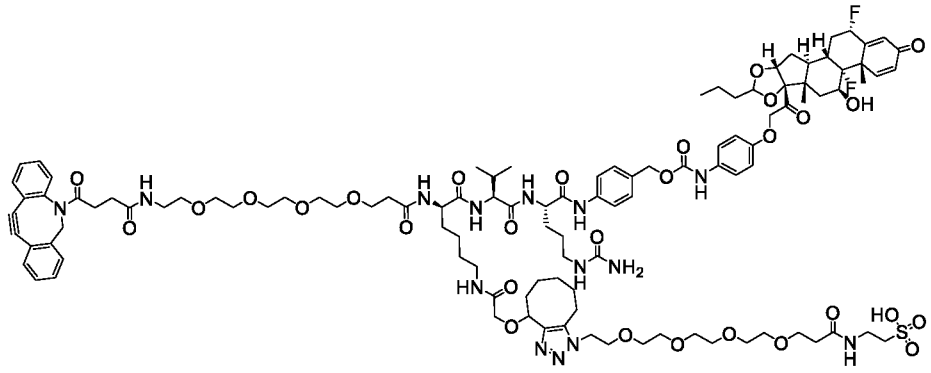
**[0530]** Following the general procedure H from compound **7h** (20 mg, 18 μmol) with **8b**, compound **1h** (11 mg, 37% yield) was obtained as a white solid after purified by reversed phase flash chromatography 0-100% acetonitrile in *aq.* ammonium bicarbonate (10 mM)). ESI

m/z: 821.3 (M/2 + H)$^+$. $^1$H NMR (500 MHz, DMSO$_{d6}$) $\delta$ 9.70 (s, 1H), 8.22 (d, $J$ = 7.0 Hz, 1H), 8.10 (d, $J$ = 7.0 Hz, 2H), 7.95-7.61 (m, 7H), 7.51-7.26 (m, 11H), 6.30 (d, $J$ = 10 Hz, 1H), 6.11 (s, 1H), 6.01-5.99 (m, 1H), 5.69-5.57 (m, 2H), 5.41 (s, 2H), 5.22-4.92 (m, 4H), 4.78-4.58 (m, 1H), 4.35-4.13 (m, 5H), 3.86-3.80 (m, 1H), 3.62-3.40 (m, 14H), 3.30-3.28 (m, 4H), 3.10-2.73 (m, 5H), 2.63-2.53 (m, 2H), 2.41-2.20 (m, 3H), 2.09-1.57 (m, 13H), 1.48-1.12 (m, 15H), 0.90-0.84 (m, 10H) ppm. *Anal.* HPLC: 96%, Retention time: 7.28 min (method B).

## EXAMPLE 22

**Preparation of 1i** (See FIG. 17)

**2-(1-{4-[({5-[1-(4-{2-Azatricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-5-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-{[4-({[(4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethoxy}phenyl)carbamoyl]oxy}methyl)phenyl]carbamoyl}butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl}carbamoyl)methoxy]-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl}-3,6,9,12-tetraoxapentadecan-15-amido)ethane-1-sulfonic acid (1i)**

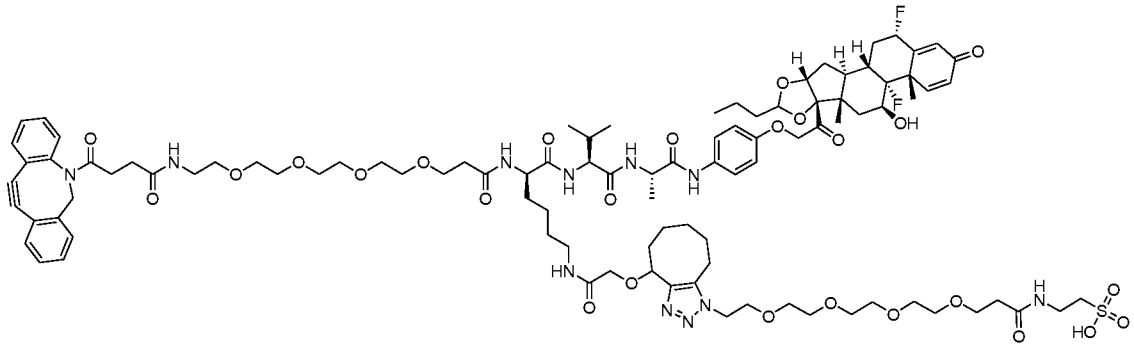


**[0531]** Following the general procedure H from compound **7i** (46 mg, 28 μmol) with compound **8b**, compound **1i** (28 mg, 46% yield) was obtained as a white solid. ESI m/z: 1095.0 (M/2 + H)$^+$. $^1$H NMR (400 MHz, MeOD$_{d4}$) $\delta$ 7.42-7.23 (m, 15H), 6.87-6.83 (m, 2H), 6.36-6.31 (m, 2H), 5.64-5.47 (m, 1H), 5.29-5.05 (m, 5H), 4.94-4.90 (m, 1H), 4.84-4.59 (m, 2H), 4.49-4.41 (m, 2H), 4.34-4.26 (m, 2H), 4.16-4.13 (m ,1H), 4.02-3.86 (m, 4H), 3.72-3.68 (m, 3H), 3.66-3.34 (m, 31H), 3.24-3.14 (m, 5H), 3.08-2.83 (m, 4H), 2.73-2.60 (m, 2H), 2.46-2.36 (m, 9H), 2.12-1.19 (m, 32H), 1.05-0.90 (m ,12H) ppm. *Anal.* HPLC: 100%, Retention time: 7.62 min (method B).

## EXAMPLE 23

**Preparation of 1j** (See FIG. 17)

**[0532]** **2-{1-[4-({[(5*R*)-5-[1-(4-{2-Azatricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-5-{[(1*S*)-1-{[(1*S*)-1-[(4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethoxy}phenyl)carbamoyl]ethyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethane-1-sulfonic acid (1j)**

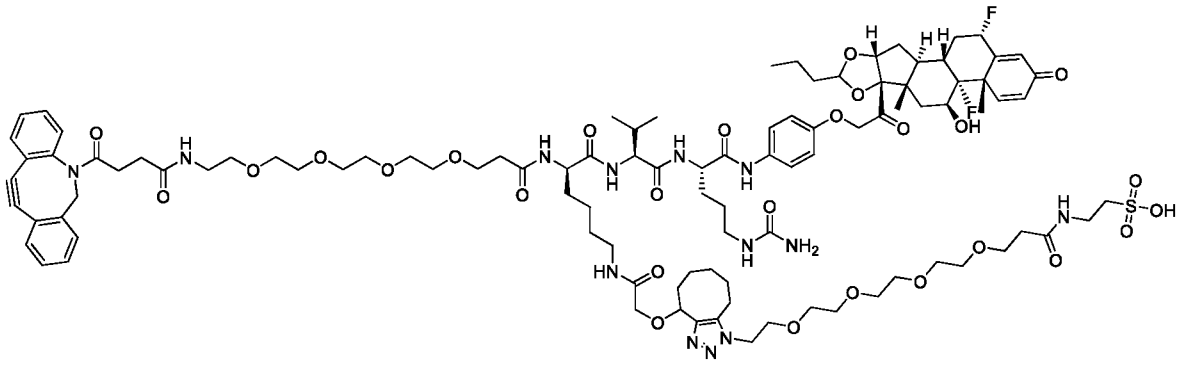


**[0533]** Following the general procedure H from compound **7j** (66 mg, 47 μmol) with compound **8b**, compound **1j** (40 mg, 44% yield) was obtained as a white solid. ESI m/z: 977.5 $(M/2 + H)^+$. $^1$H NMR (400 MHz, $MeOD_{d4}$) $\delta$ 7.66-7.55 (m, 4H), 7.48-7.24 (m, 7H), 6.90-6.85 (m, 2H), 6.35 (d, $J$ = 10.0 Hz, 1H), 6.32 (s, 1H), 5.64-5.47 (m, 1H), 5.28-5.07 (m, 3H), 4.85-4.60 (m, 3H), 4.50-4.44 (m, 2H), 4.34-4.25 (m, 2H), 4.12-3.88 (m, 5H), 3.73-3.49 (m, 29H), 3.45-3.39 (m, 3H), 3.25-3.20 (m, 3H), 3.14-2.86 (m, 5H), 2.74-2.63 (m, 2H), 2.46-1.27 (m, 41H), 1.05-0.92 (m, 12H) ppm. *Anal.* HPLC: 95%, Retention time: 7.55 min (method B).

## EXAMPLE 24

**Preparation of 1k** (See FIG. 17)

**2-{1-[4-({[(5*R*)-5-[1-(4-{2-Azatricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-5-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethoxy}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethane-1-sulfonic acid (1k)**

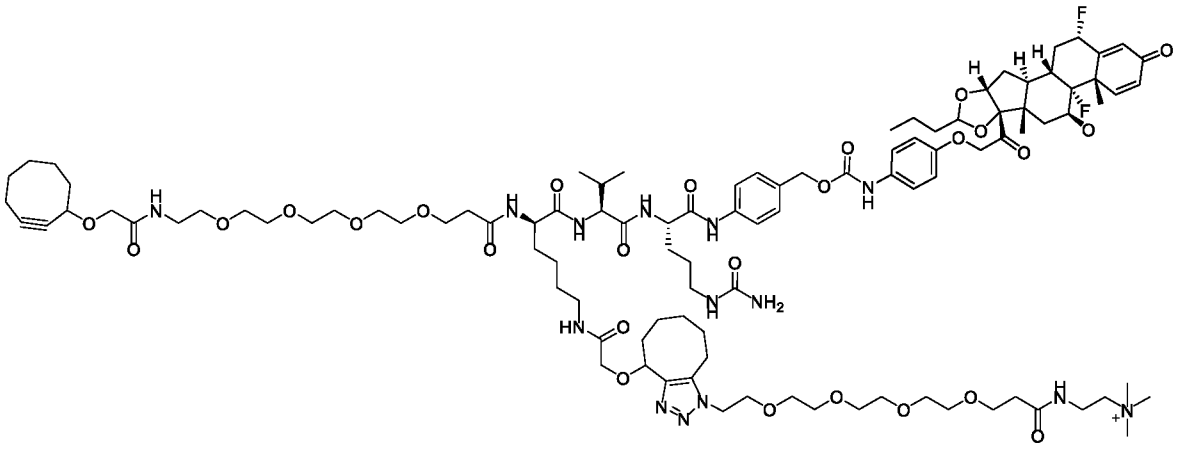


**[0534]** Following the general procedure H from compound **7k** (50 mg, 33 μmol) with compound **8b**, compound **1k** (30 mg, 44% yield) was obtained as a white solid. ESI m/z: 680 $(M/3 + H)^+$. $^1H$ NMR (500 MHz, $DMSO_{d6}$) $\delta$ 9.51 (s, 1H), 8.30-7.97 (m, 3H), 7.92-7.84 (m, 1H), 7.80-7.73 (m, 2H), 7.68 (d, $J$ = 7.5 Hz, 1H), 7.62 (d, $J$ = 7.9 Hz, 1H), 7.50-7.45 (m, 4H), 7.38-7.26 (m, 4H), 7.08 (br s, 3H), 6.90-6.78 (m, 2H), 6.30 (d, $J$ = 10.5 Hz, 1H), 6.11 (s, 1H), 6.01-5.94 (m, 1H), 5.72-5.55 (m, 1H), 5.52 (s, 1H), 5.39 (s, 2H), 5.12 (d, $J$ = 18.5 Hz, 1H), 5.03 (d, $J$ = 14.5 Hz, 1H), 4.84 (d, $J$ = 18.5 Hz, 1H), 4.80-4.71 (m, 2H), 4.55-4.49 (m, 1H), 4.42 (t, $J$ = 5.5 Hz, 1H), 4.38-4.12 (m, 4H), 3.81-3.76 (m, 4H), 3.62-3.53 (m, 4H), 3.46-3.39 (m, 21H), 3.33-3.25 (m, 4H), 3.09-2.93 (m, 7H), 2.81-2.78 (m, 1H), 2.64-2.52 (m, 8H), 2.40-2.36 (m, 2H), 2.27-2.20 (m, 5H), 2.09-1.98 (m, 5H), 1.61-1.35 (m, 29H), 0.89-0.83 (m, 11H) ppm.

## EXAMPLE 24A

**Preparation of IIId** (FIG. 17)

**[0535] (2-{1-[4-({[(5*R*)-5-{[(1*S*)-1-{[(1*S*)-4-(Carbamoylamino)-1-{[4-({[(4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethoxy}phenyl)carbamoyl]oxy}methyl)phenyl] carbamoyl}butyl]carbamoyl}-2-methylpropyl]carbamoyl}-5-{1-[2-(cyclooct-2-yn-1-yloxy)acetamido]-3,6,9,12-tetraoxapentadecan-15-amido}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethyl)trimethylazanium (IIId)**

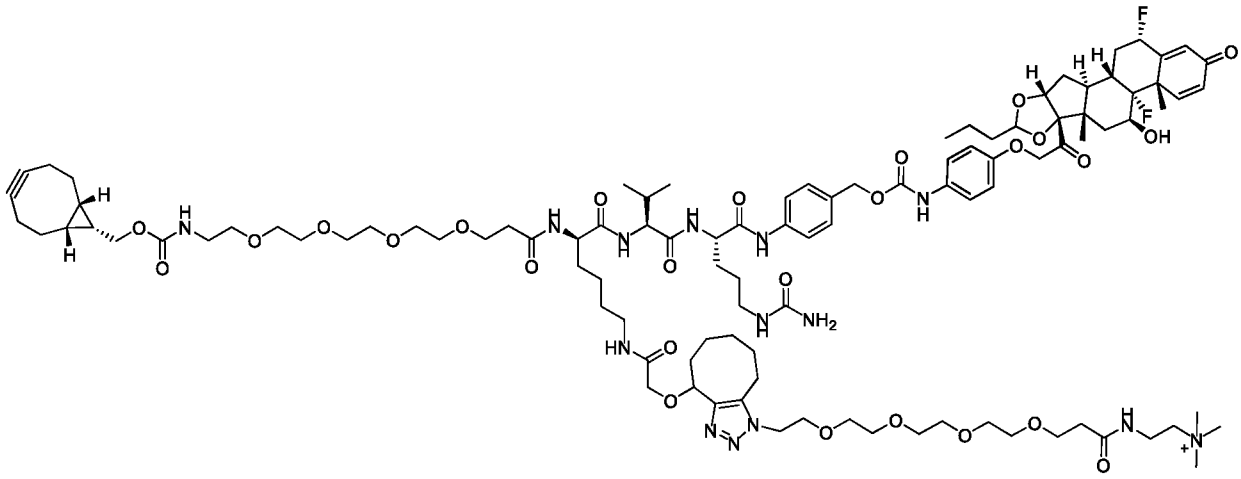


**[0536]** Following the general procedure G from compound **7cb** (20 mg, 12 μmol) with compound **8c** (5.2 mg, 12 μmol), compound **IIId** (8 mg, 32% yield) was obtained as a yellow solid after purification by reversed phase flash chromatography (0-100% acetonitrile in *aq.* TFA (0.01%)). ESI m/z: 681.7 $(M/3 + H)^+$; 1021.7 $(M/2 + H)^+$. $^1H$ NMR (400 MHz, $DMSO_{d6}$) **δ** 9.73-9.67 (m, 1H), 9.61-9.54 (m, 1H), 8.26-8.20 (m, 1H), 8.19-8.04 (m, 2H), 7.91-7.77 (m, 1H), 7.66-7.57 (m, 3H), 7.40-7.26 (m, 4H), 6.84-6.78 (m, 2H), 6.30 (d, *J* = 10 Hz, 1H), 6.12 (s, 1H), 6.01-5.99 (m, 1H), 5.72-5.42 (m, 4H), 5.24-5.06 (m, 3H), 4.91-4.73 (m, 3H), 4.55-4.16 (m, 7H), 3.89-3.73 (m, 5H), 3.58 (t, *J* = 10.4 Hz, 3H), 3.49-3.41 (m, 29H), 3.26-3.22 (m, 4H), 3.08 (s, 9H), 3.04-2.93 (m, 4H), 2.77-2.62 (m, 3H), 2.40-1.98 (m, 13H), 1.89-1.02 (m, 41H), 0.89-0.84 (m, 9H) ppm.

## EXAMPLE 24B

**Preparation of IIIe** (FIG. 17)

**[0537] (2-{1-[4-({[(5*R*)-5-[1-({[(1*R*,8*S*,9*S*)-Bicyclo[6.1.0]non-4-yn-9-ylmethoxy]carbonyl}amino)-3,6,9,12-tetraoxapentadecan-15-amido]-5-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-{[4-({[(4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethoxy}phenyl)carbamoyl]oxy}methyl) phenyl]carbamoyl}butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethyl)trimethylazanium (IIIe)**

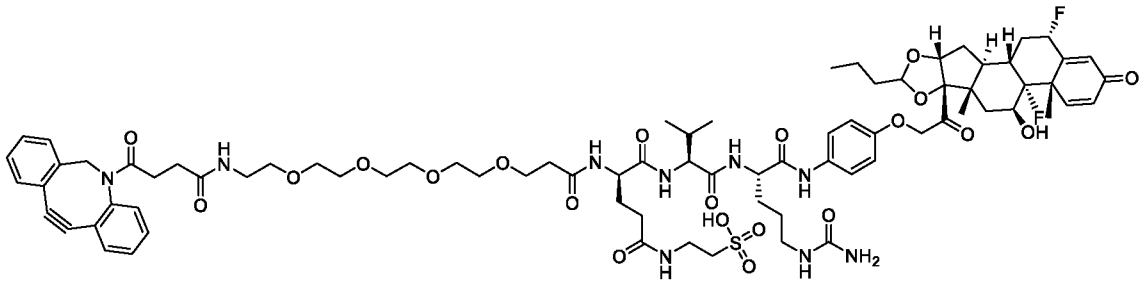


**[0538]** Following the general procedure G from compound **7cb** (25 mg, 15 μmol) with compound **8d** (6.8 mg, 15 μmol), compound **IIIe** (4 mg, 13% yield) was obtained as a yellow solid after purification by reversed phase flash chromatography (0-100% acetonitrile in *aq.* TFA (0.01%)). ESI m/z: 685.6 $(M/3 + H)^+$. $^1H$ NMR (400 MHz, $MeOD_{d4}$) $\delta$ 7.77-7.71 (m, 2H), 7.40-7.34 (m, 5H), 6.90-6.86 (m, 2H), 6.38-6.33 (m, 2H), 5.66-5.47 (m, 1H), 5.38-5.24 (m, 1H), 5.14-5.02 (m, 2H), 4.76-4.58 (m, 4H), 4.52-4.27 (m, 6H), 4.19-4.11 (m, 3H), 3.99-3.86 (m, 4H), 3.75-3.42 (m, 37H), 3.30-3.27 (m, 3H), 3.17 (s, 9H), 3.16-3.00 (m, 2H), 3.0-2.84 (m, 1H), 2.72-2.46 (m, 4H), 2.38-2.04 (m, 15H), 1.83-1.31 (m, 27H), 1.06-0.90 (m, 13H) ppm.

## EXAMPLE 25

**Preparation of 1l** (See FIG. 17)

**2-[(4*R*)-4-[1-(4-{2-Azatricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-4-{[(1*S*)-1-{[(1*S*)-4-(carbamoylamino)-1-[(4-{2-[(1*S*,2*S*,4*R*,8*S*,9*S*,11*S*,12*R*,13*S*,19*S*)-12,19-difluoro-11-hydroxy-9,13-dimethyl-16-oxo-6-propyl-5,7-dioxapentacyclo[10.8.0.0$^{2,9}$.0$^{4,8}$.0$^{13,18}$]icosa-14,17-dien-8-yl]-2-oxoethoxy}phenyl)carbamoyl]butyl]carbamoyl}-2-methylpropyl]carbamoyl}butanamido]ethane-1-sulfonic acid (1l)**



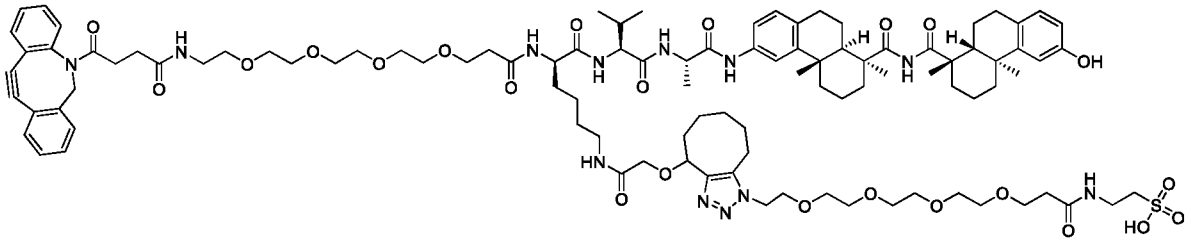
**[0539]** Following the general procedure H from compound **7l** (20 mg, 19 μmol) with compound **8b**, compound **1l** (6.0 mg, 20% yield) was obtained as a white solid. ESI m/z: 793 $(M/2 + H)^+$. $^1H$ NMR (400 MHz, $DMSO_{d6}$) $\delta$ 9.81-9.48 (m, 1H), 8.32-8.18 (m, 1H), 8.12-7.96

(m, 2H), 7.81-7.60 (m, 4H), 7.58-7.43 (m, 5H), 7.41-7.25 (m, 5H), 7.18-6.98 (m, 2H), 6.88-6.81 (m, 2H), 6.30 (d, *J* = 10.3 Hz, 1H), 6.11 (s, 1H), 6.02-5.95 (m, 1H), 5.71-5.52 (m, 2H), 5.40 (s, 2H), 5.15-5.00 (m, 2H), 4.86-4.74 (m, 2H), 4.34-4.13 (m, 4H), 3.63-3.54 (m, 2H), 3.48-3.42 (m, 9H), 3.30-3.28 (m, 2H), 3.12-3.05 (m, 2H), 3.01-2.92 (m, 2H), 2.62-2.56 (m, 1H), 2.42-2.37 (m, 1H), 2.29-2.20 (m, 3H), 2.12-1.95 (m, 7H), 1.86-1.70 (m, 5H), 1.64-1.56 (m, 4H), 1.52-1.22 (m, 14H), 0.92-0.81 (m, 14H) ppm.

## EXAMPLE 26

**Preparation of 1m** (See FIG. 17)

**2-{1-[4-({[(5*R*)-5-[1-(4-{2-Azatricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-5-{[(1*S*)-1-{[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}ethyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethane-1-sulfonic acid (1m)**



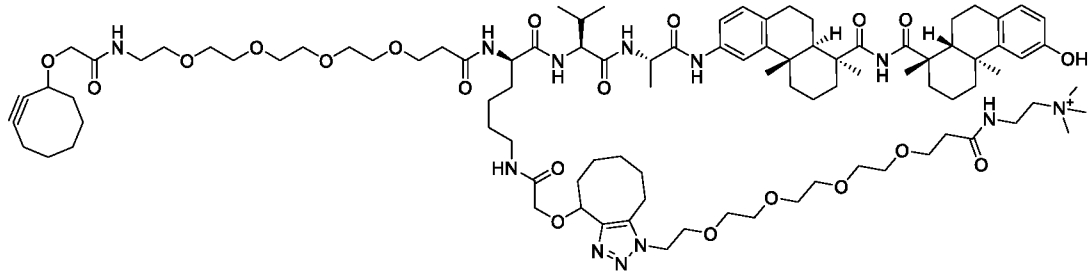
**[0540]** Following the general procedure H from compound **7m** (30 mg, 22 μmol) with compound **8b**, compound **1m** (15 mg, 37% yield) was obtained as a white solid. ESI m/z: 642 (M/3 + H)$^+$. $^1$H NMR (400 MHz, DMSO$_{d6}$) $\delta$ 9.68-9.27 (m, 1H), 8.99 (s, 1H), 8.23-7.85 (m, 4H), 7.79-7.71 (m, 2H), 7.76-7.42 (m ,6H), 7.39-7.28 (m, 3H), 7.21 (s, 1H), 7.09 (s, 1H), 6.96-6.93 (m, 2H), 6.81 (d, *J* = 8.4 Hz, 1H), 6.63 (d, *J* = 2.4 Hz, 1H), 6.50 (dd, *J* = 8.4 Hz, 2.4 Hz, 1H), 5.02 (d, *J* = 14.0 Hz, 1H), 4.93-4.72 (m, 1H), 4.53-4.09 (m, 5H), 3.82-3.75 (m, 4H), 3.62-3.53 (m, 3H), 3.51-3.38 (m, 23H), 3.30-3.27 (m, 6H), 3.12-2.67 (m, 10H), 2.61-2.54 (m, 4H), 2.39-1.52 (m, 31H), 1.45-1.08 (m, 18H), 1.01-0.98 (m, 6H), 0.90-0.82 (m, 6H) ppm.

## EXAMPLE 26A

**Preparation of IVb** (FIG. 17)

**[0541] (2-{1-[4-({[(5*R*)-5-{[(1*S*)-1-{[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}ethyl]carbamoyl}-2-**

**methylpropyl]carbamoyl}-5-{1-[2-(cyclooct-2-yn-1-yloxy)acetamido]-3,6,9,12-tetraoxapentadecan-15-amido}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethyl)trimethylazanium (IVb)**

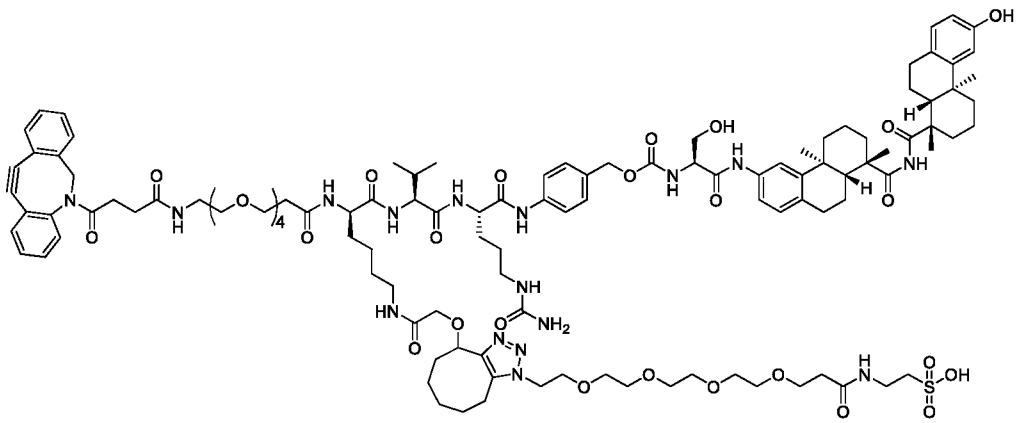


**[0542]** Following the general procedure G from compound **7fb** (20 mg, 15 μmol) with compound **8c**, compound **IVb** (6 mg, 23% yield) was obtained as a yellow solid after purification by prep-HPLC (method A). ESI m/z: 889.8 $(M/2 + H)^+$, 593.5 $(M/3 + H)^+$. $^1H$ NMR (500 MHz, $DMSO_{d6}$) $\delta$ 9.30 (d, $J$ = 8.5 Hz, 1H), 9.02 (s, 1H), 8.76 (d, $J$ = 5.5 Hz, 1H), 8.54-8.48 (m, 1H), 8.32-7.76 (m, 6H), 7.62-7.60 (m, 2H), 7.53-7.51 (m, 2H), 7.44 (d, $J$ = 8.0 Hz, 1H), 6.95 (d, $J$ = 8.5 Hz, 1H), 6.81 (d, $J$ = 8.5 Hz, 1H), 6.63 (s, 1H), 6.50 (d, $J$ = 8.0 Hz, 1H), 5.33-4.71 (m, 2H), 4.55-4.05 (m, 7H), 3.88-3.74 (m, 6H), 3.61-3.58 (m, 4H), 3.50-3.42 (m, 44H), 3.26-3.24 (m, 4H), 3.08 (s, 9H), 2.84-2.65 (m, 3H), 2.44 (t, $J$ = 6.5 Hz, 2H), 2.35-1.26 (m, 35H), 1.16-1.13 (m, 2H), 1.02-0.98 (m, 6H), 0.89-0.84 (m, 6H) ppm.

## EXAMPLE 27

**Preparation of 1q** (See FIG. 17)

**2-{1-[4-({[(5*R*)-5-[1-(4-{2-Azatricyclo[10.4.0.0$^{4,9}$]hexadeca-1(12),4(9),5,7,13,15-hexaen-10-yn-2-yl}-4-oxobutanamido)-3,6,9,12-tetraoxapentadecan-15-amido]-5-{[(1*S*)-1-{[(1*S*)-1-({4-[({[(1*S*)-1-{[(4b*S*,8*S*,8a*R*)-8-({[(1*S*,4a*S*,10a*R*)-6-hydroxy-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl]formamido}carbonyl)-4b,8-dimethyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-3-yl]carbamoyl}-2-hydroxyethyl]carbamoyl}oxy)methyl]phenyl}carbamoyl)-4-(carbamoylamino)butyl]carbamoyl}-2-methylpropyl]carbamoyl}pentyl]carbamoyl}methoxy)-1*H*,4*H*,5*H*,6*H*,7*H*,8*H*,9*H*-cycloocta[d][1,2,3]triazol-1-yl]-3,6,9,12-tetraoxapentadecan-15-amido}ethane-1-sulfonic acid (1q)**



**[0543]** To a solution of compound **7q** (15 mg, 8.8 μmol) and commercial DIBAC-Suc-PEG$_4$-OSu (5.7 mg, 8.8 μmol, CAS 1427004-19-0) in DMF (1 mL) was added DIPEA (2.3 mg, 18 μmol) and the mixture was stirred at RT for 2 hours. Most of The volatiles were removed *in vacuo* and the residue was purified by prep-HPLC (method B) to give **1q** (6.0 mg, 30% yield) as a white solid. ESI m/z: 1123.8 $(M/2 + H)^+$, 749.5 $(M/3 + H)^+$. $^1$H NMR (500 MHz, MeOD$_{d4}$) ***δ*** 7.76-7.16 (m, 14H), 7.06-7.00 (m, 1H), 6.88 (d, *J* = 8.5 Hz, 1H), 6.72-6.71 (m, 1H), 6.56-6.55 (m, 1H), 5.39-5.33 (m, 1H), 5.14-5.09 (m, 5H), 4.61 (s, 18H), 4.50-4.43 (m, 2H), 4.33-4.30 (m, 1H), 3.99 (s, 2H), 3.89-3.85 (m, 3H), 3.73-3.42 (m, 28H), 3.25-2.72 (m, 8H), 2.45 (t, *J* = 7.5 Hz, 2H), 2.36-1.96 (m, 18H), 1.81-1.51 (m, 12H), 1.45-1.32 (m, 15H), 1.12-0.89 (m, 12H) ppm.

**[0544]** Table 2B and Table 2B-1 summarize the results for the Linker-Payloads: HPLC purity and LC retention time on HPLC, M/Z from mess spectra. The analytical HPLC and MS methods are described in General procedures.

**Table 2B. Chemical-physical properties of Linker-Payloads**

| LP | Ex | cLogP | MF | MW | HPLC purity (%) | HPLC RT (min)[1] | MS (m/z) 100% | Highest m/z peak |
|---|---|---|---|---|---|---|---|---|
| **1a** | **17** | 5.07 | $C_{116}H_{177}N_{17}O_{28}$ | 2257.74 | 95 | 6.89 | 753.3 (M/3+H) | 1029.3 (M/2+H) (30%) |
| **1c** | **18** | 2.75 | $C_{117}H_{178}N_{18}O_{30}S$ | 2348.83 | 100 | 7.17 | 783.7 (M/3+H) | 1174.7 (M/2+H) (20%) |
| **1e** | **19** | 2.98 | $C_{103}H_{144}F_2N_{14}O_{28}S$ | 2096.38 | 98 | 5.88 | 699.6 (M/3+H) | 1049.0 (M/2+H) (67%) |
| **1f** | **20** | -1.43 | $C_{100}H_{138}F_2N_{16}O_{28}S_2$ | 2114.38 | 97 | 6.82 | 1057.6 (M/2+H) | 1057.6 (M/2+H) |
| **1h** | **21** | 1.94 | $C_{81}H_{106}F_2N_{10}O_{22}S$ | 1641.82 | 96 | 7.28 | 821.4 (M/2+H) | 821.4 (M/2+H) |
| **1i** | **22** | 3.75 | $C_{109}H_{148}F_2N_{14}O_{29}S$ | 2188.48 | 100 | 7.62 | 1095.0 (M/2+H) | 1095.0 (M/2+H) |

| LP | Ex | cLogP | MF | MW | HPLC purity (%) | HPLC RT (min)[1] | MS (m/z) 100% | Highest m/z peak |
|---|---|---|---|---|---|---|---|---|
| **1j** | **23** | 3.15 | $C_{98}H_{135}F_2N_{11}O_{26}S$ | 1953.24 | 95 | 7.55 | 977.5 (M/2+H) | 977.5 (M/2+H) |
| **1k** | **24** | 2.06 | $C_{101}H_{141}F_2N_{13}O_{27}S$ | 2039.33 | 100 | 5.97 | 1020.3 (M/2+H) | 1020.3 (M/2+H) |
| **1l** | **25** | 2.22 | $C_{79}H_{103}F_2N_9O_{21}S$ | 1584.77 | 100 | 5.89 | 792.8 (M/2+H) | 792.8 (M/2+H) |
| **1m** | **26** | 6.49 | $C_{101}H_{142}N_{12}O_{23}S$ | 1924.34 | 97 | 7.57 | 642.2 (M/3+H) | 962.5 (M/2+H) (70%) |
| **1q** | **27** | 6.59 | $C_{115}H_{160}N_{16}O_{28}S$ | 2245.13 | 99 | 7.24 | 749.5 (M/3+H) | 1123.8 (M/2 + H)$^+$ |

1. There might be COT-isomers.

**Table 2B-1. Chemical-physical properties of Linker-Payloads**

| LP | Ex | cLogP | MF | MW | HPLC purity (%) | HPLC RT (min)[1] | MS (m/z) 100% | Highest m/z peak |
|---|---|---|---|---|---|---|---|---|
| **1a** | **17** | 2.75 | $C_{117}H_{178}N_{18}O_{30}S$ | 2348.83 | 100 | 7.17 | 783.7 (M/3+H) | 1174.7 (M/2+H) (20%) |
| **Ib** | **17A** | 0.79 | $C_{120}H_{186}N_{19}O_{27}^+$ | 2326.87 | 91 | 8.08 | 776.0 (M/3+H) | 776.0 (M/3+H) |
| **1c** | **18** | 5.07 | $C_{116}H_{177}N_{17}O_{28}$ | 2257.74 | 95 | 6.89 | 753.3 (M/3+H) | 1029.3 (M/2+H) (30%) |
| **Id** | **18A** | 3.50 | $C_{116}H_{178}N_{17}O_{30}P$ | 2321.72 | 95 | 5.56, 5.64 | 774.7 (M/3+H) | 774.7 (M/3+H) |
| **Ie-1** | **18B** | 3.58 | $C_{110}H_{163}N_{17}O_{27}$ | 2155.57 | 100 | 7.34 | 1078.3 [M/2+H] | 2156.9 [M+H] (10%) |
| **If-1** | **18C** | 0.98 | $C_{95}H_{150}N_{16}O_{27}$ | 1948.3 | 100 | 7.19 (A) | 974.7 [M/2+H] | 1971.8 [M+Na] (20%) |
| **Ig** | **18D** | 0.68 | $C_{99}H_{148}N_{14}O_{26}$ | 1950.31 | 97 | 7.58 | 650.8 [M/3+H] | 975.8 [M/2+H] (40%) |
| **Ih-1** | **18E** | -1.92 | $C_{84}H_{135}N_{13}O_{26}$ | 1743.04 | >97 | 6.09 (A) | 1742.7 [M+H] | 1764.8 [M+Na] (90%) |
| **1e** | **19** | 2.98 | $C_{103}H_{144}F_2N_{14}O_{28}S$ | 2096.38 | 98 | 5.88 | 699.6 (M/3+H) | 1049.0 (M/2+H) (67%) |
| **IIb** | **19A** | -0.18 | $C_{106}H_{152}F_2N_{15}O_{25}^+$ | 2074.42 | 96 | 8.36 | 1037.6 (M/2+H) | 692.2 (M/3+H) |
| **1f** | **20** | -1.43 | $C_{100}H_{138}F_2N_{16}O_{28}S_2$ | 2114.38 | 97 | 6.82 | 1057.6 (M/2+H) | 1057.6 (M/2+H) |
| **1h** | **21** | 1.94 | $C_{81}H_{106}F_2N_{10}O_{22}S$ | 1641.82 | 96 | 7.28 | 821.4 | 821.4 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | (M/2+H) | (M/2+H) |
| **1i** | **22** | 3.75 | $C_{109}H_{148}F_2N_{14}O_{29}S$ | 2188.48 | 100 | 7.62 | 1095.0 (M/2+H) | 1095.0 (M/2+H) |
| **1j** | **23** | 3.15 | $C_{98}H_{135}F_2N_{11}O_{26}S$ | 1953.24 | 95 | 7.55 | 977.5 (M/2+H) | 977.5 (M/2+H) |
| **1k** | **24** | 2.06 | $C_{101}H_{141}F_2N_{13}O_{27}S$ | 2039.33 | 100 | 5.97 | 1020.3 (M/2+H) | 1020.3 (M/2+H) |
| **IIId** | **24A** | 0.94 | $C_{103}H_{155}F_2N_{14}O_{26}^+$ | 2043.41 | 96 | 6.86 (A) | 681.7 (M/3+H) | 1021.7 (M/2+H) (33%) |
| **IIIe** | **24B** | 1.55 | $C_{104}H_{155}F_2N_{14}O_{26}^+$ | 2055.42 | 100 | 7.15 (A) | 685.7 (M/3+H) | 685.7 (M/3+H) |
| **1l** | **25** | 2.22 | $C_{79}H_{103}F_2N_9O_{21}S$ | 1584.77 | 100 | 5.89 | 792.8 (M/2+H) | 792.8 (M/2+H) |
| **1m** | **26** | 6.49 | $C_{101}H_{142}N_{12}O_{23}S$ | 1924.34 | 97 | 7.57 | 642.2 (M/3+H) | 962.5 (M/2+H) (70%) |
| **IVb** | **26A** | 3.78 | $C_{95}H_{149}N_{12}O_{20}^+$ | 1779.27 | 92 | 6.20 (A) | 593.7 (M/3+H) | 889.8 (M/2+H) |
| **1q** | **27** | 6.59 | $C_{115}H_{160}N_{16}O_{28}S$ | 2245.13 | 99 | 7.24 | 749.5 (M/3+H) | 1123.8 (M/2 + H)$^+$ |
| **10a** | **10A** | 4.39 | $C_{74}H_{94}F_2N_8O_{17}$ | 1405.58 | 100 | 7.40 | 703.5 (M/2+H) | 1405.7 (M+H) (5%) |
| **10b** | **10B** | 9.10 | $C_{72}H_{92}N_6O_{12}$ | 1233.53 | 100 | 9.21 | 617.3 (M/2+H) | 1233.6 (M+H) (80%) |
| **10c** | **10C** | 6.25 | $C_{80}H_{98}F_2N_8O_{18}$ | 1497.67 | 100 | 7.99 | 749.5 (M/2+H) | 1497.7 (M+H) (5%) |
| **10d** | **10D** | 5.37 | $C_{88}H_{128}N_{12}O_{19}$ | 1658.03 | >95 | 8.25 | 829.7 (M/2+H) | 1659.7 (M+H) (20%) |

1. There might be two regioisomers.

## EXAMPLE 28

**[0545]** This Example illustrates the activity of anti-MSR1 antibody steroid non-cytotoxic ADCs comprising an $SO_3H$ moiety, in an *in vitro* lipopolysaccharide (LPS) mediated IL-1β release assay

**[0546]** For the assay, THP-1 cells were seeded onto 96 well plates at 40,000 cells/ well in media containing RPMI supplemented with 10% FBS and pencillin/streptomycinin, and were differentiated with 200 nM Phorbol Myristate Acetate (PMA) for 3 days. After the 3 day differentiation, three-fold serial dilutions of the antibody drug conjugates and unconjugated antibody fresh media were added to the cells at final concentration ranging from 100 nM to 0.01 nM. The last well was left as blank control containing only the media. Seventy-two hours

later, cells were treated with 5 µg/mL of LPS (InVivoGen, Cat# tlrl-eklps) for 5 hours. The cell media was then collected and the IL-1β was measured using a V-PLEX Proinflammatory Panel 1 human kit (Meso Scale Diagnostics, Cat # 15049D-2) as per manufacturer's instructions. Subsequently, the plate was read on a MSD plate reader (Meso Scale Discovery). The $IC_{50}$ values were determined from a four- parameter logistic equation over a 10-point response curve (GraphPad Prism). All $IC_{50}$ values are expressed in molar (M) concentration.

**[0547]** As shown in Table 1A, H1H21234N-N297Q conjugated with the steroid linker payload without $SO_3H$ moiety **(H1H21234N-N297Q- Example 10A)** demonstrated inhibition of LPS mediated IL-1β release from THP1 cells with an $IC_{50}$ value of 1.73 nM and having a reduction of IL-1β released down to 97.2 pg/mL. H1H21234N-N297Q conjugated with the steroid linker payload with a $SO_3H$ moiety **(H1H21234N-N297Q- Example 19)** demonstrated similar inhibition of LPS mediated IL-1β release from THP1 cells with an $IC_{50}$ value of 1.97 nM and having a reduction of IL-1β released down to 97.17 pg/mL. The unconjugated antibody demonstrated inhibition of LPS mediated IL-1β release from THP1 cells with an $IC_{50}$ value of 22.9 nM and having a reduction of IL-1β released down to 343.7 pg/mL showing the lack of efficacy compared to the conjugated antibodies.

**Table 1A**: Activity of anti-MSR1 Ab-steroid ncADCs on LPS-induced IL-1β release from THP1 cells

| Payload Type | Linker Modification | ncADC | 72 hour $IC_{50}$ (M) | IL1beta released at max concentration tested (pg/mL) |
|---|---|---|---|---|
| Steroid | N/A | H1H21234N-N297Q- Example 10A | 1.73E- | 97.24 |
| | $SO_3H$ | H1H21234N-N297Q- Example 19 | 1.97E- | 97.17 |
| N/A | N/A | H1H21234N-N297Q | 2.29E- | 343.7 |

**pH stability**

**[0548]** **pH stability was assessed as follows.** 0.1 mg of sample was dissolved into 0.2-0.3mL of DMSO, and the resulting solution was added dropwise into different pH buffers (1 mL) while maintain clear solutions during testing time period. The samples were collected from several time points (e.g., over 72 hours), and pH stability was determined by LC-MS. pH buffer was prepared as follows: pH 8.0 sodium borate buffer: Add 9.534 g Sodium borate decahydrate, 1.461 g Sodium chloride, and 0.393 g DTPA to 900mL distilled water, make sure all powers dissolve completely, then make final volume to 1L by adding water. pH 7.4 sodium borate

buffer: Add 900 ml Milli-Q water, 9.534 g Sodium borate decahydrate, 1.461 g Sodium chloride, and 0.393 g DTPA to 900mL distilled water, make sure all powers dissolve completely, then make final volume to 1L by adding water. pH 5.0 succinate acid buffer: Add 10 mM succinate acid to 150mL distilled water, and then change the pH to pH5.0 by adding 0.5M NaOH, then make final volume to 250mL by adding water. pH 7.4 PBS buffer: commercially available. Stability was assesed at 72 hours by LCMS.

**Solubility**

**[0549]** Procedure for the solubility test: Dissolve 1mg of testing sample into 1 mL DMSO, and prepare standard solutions A (4.5 mL, 100 µg/mL), B (4.5 mL, 10 µg/mL), C (4.5 mL, 1 µg/mL), D (5 mL, 0.1 µg/mL).

| Standard solution | Sample amount (mg) | DMSO (%) | Total volume (mL) | Conc (mg/mL) |
|---|---|---|---|---|
| DMSO | 1 | 100% | 1 | 1 |
| A | 0.5 | 10% | 5 | 0.1 |
| B | 0.05 | 1% | 5 | 0.01 |
| C | 0.005 | 0.10% | 5 | 0.001 |
| D | 0.0005 | 0.01% | 5 | 0.0001 |

The DMSO solution (1 mg/mL, 0.5 mL) was diluted with water (4.5 mL) to generate solution A (0.1 mg/mL, 5 mL, 0.5 mg). Solution A (0.1 mg/mL, 0.5 mL) was diluted with water (4.5 mL) to generate solution B (0.01 mg/mL, 5 mL, 0.05 mg). Solution B (0.01 mg/mL, 0.5 mL) was diluted with water (4.5 mL) to generate solution C (1 µg/mL, 5 mL, 5 µg). Solution C (1 µg/mL, 0.5 mL) was diluted with water (4.5 mL) to generate solution D (0.1 µg/mL, 5 mL, 0.5 µg). The testing sample (0.05 mg) was suspended in water (1 mL), and was sonicated for 5-30 min to dissolve the sample. If no clear solution was observed, the resulting suspension was centrifuged and the clear supernatant solution was collected for analysis. All the standard solutions A, B, C, D and sample solutions were tested using same method on the same LC-MS instrument. Solubility was assessed by LCMS and calculated based on a standard curve. **Table 3** and **Table 3-1** show data from these experiments.

**Table 3. pH stability, solubility data for linker-payloads**

| LP | Ex | Payload | cLogP | cLogD (pH 5.07) | Solubility (mg/mL) | pH Stability |
|---|---|---|---|---|---|---|
| **1e** | **19** | B | 2.98 | 0.61 | > 10 | Y |

| | | | | | | |
|---|---|---|---|---|---|---|
| **1j** | **23** | C | 3.15 | 1.97 | > 10 | Y |
| **1k** | **24** | C | 2.06 | 0.88 | > 1 | Y |

Y: stable in pH7.4 or 8 buffer at room temperature for more than 3 days;

**Table 3-1. pH stability, solubility data for linker-payloads**

| LP | Ex | Payload | cLogP | cLogD (pH 5.07) | Solubility (mg/mL) | pH Stability |
|---|---|---|---|---|---|---|
| **10d** | **10D** | A | 5.37 | 5.37 | <0.1 | Y |
| **1a** | **17** | A | 2.75 | 1.58 | 0.17 | Y |
| **1c** | **19** | A | 5.07 | 5.07 | 0.16 | |
| **Id** | **18A** | A | 3.50 | 1.23 | 0.25 | Y |
| **Ie-1** | **18B** | A | 3.58 | 3.58 | 0.4 | Y |
| **Ig** | **18D** | A | 0.68 | 0.68 | 0.28 | Y |
| **Ih-1** | **18E** | A | -1.92 | -1.92 | 0.5 | |
| **10a** | **10A** | B | 4.39 | 4.39 | 0.02 | Y |
| **1e** | **19** | B | 2.98 | 0.61 | > 10 | Y |
| **10c** | **10C** | C | 6.25 | 6.25 | < 0.1 | Y |
| **1i** | **22** | C | 3.75 | 2.46 | > 10 | |
| **1j** | **23** | C | 3.15 | 1.97 | > 10 | Y |
| **1k** | **24** | C | 2.06 | 0.88 | > 1 | Y |
| **IIIe** | **24B** | C | 2.22 | -0.16 | > 10 | Y |
| **10d** | **10D** | D | 9.10 | 9.10 | < 0.1 | Y |
| **1q** | **27** | E | 6.59 | 4.22 | 0.17 | Y |

Y: stable in pH7.4 and 8 buffer at RT for more than 3 days

## EXAMPLE 29

**ADC Conjugations** (See FIG. 19)

**[0550]** This example demonstrates a method for site–specific conjugation, generally, of a payload to an antibody or antigen–binding fragment thereof.

**[0551]** The following example demonstrates a method for making the conjugates. Aglycosylated antibody with a human IgG1 isotype in BupH™ (pH 7-8) was mixed with ≥200 molar equivalents of azido–dPEG$_3$–amine (MW. 218.26 g/mol). The resulting solution was mixed with transglutaminase (25 U/mL; 5U MTG per mg of antibody) resulting in a final concentration of the antibody at 0.5–10 mg/mL, and the solution was then incubated at 37 °C for 4–24 hours while gently shaking. The reaction was monitored by SDS–PAGE or ESI–MS. Upon the completion, the excess amine and MTG were removed by Size Exclusion Chromatography (SEC) to generate the azido–functionalized antibody (mAb-$N_3$). This product was analyzed on SDS–PAGE and ESI–MS. The azido–dPEG$_3$–amine added to two sites – Q295 and Q297– of the antibody resulting in an 804 Da increase for the 4DAR aglycosylated antibody–PEG$_3$–azide conjugate. The conjugation sites were identified and confirmed at EEQ$^{Linker}$YQ$^{Linker}$STYR for the 4DAR azido–functionalized antibody via peptide sequence mapping of trypsin digested heavy chains.

**[0552]** The site–specific aglycosylated antibody drug conjugates (ADCs) with a human IgG1 or IgG4 generated against MSR1, HER2 (or PRLR) and containing an N297Q mutation were prepared by a [2+3] click reaction between the azido–functionalized antibody (mAb-$N_3$) with an alkyne containing linker–payload (LP). FeID1 is a non-binding control antibody.

**[0553]** As shown in Table 5, the site–specific aglycosylated antibodies containing an N297Q mutation were conjugated with amine–PEG$_3$–$N_3$ to generate the azido–functionalized antibody conjugates (mAb-$N_3$), including anti MSR1 Ab–PEG$_3$–$N_3$, anti Fel D1 Ab–PEG$_3$–$N_3$, anti HER2 Ab–PEG$_3$–$N_3$, anti PRLR Ab–PEG$_3$–$N_3$.

**[0554]** A site-specific antibody conjugate with linker–payload (**LP**) was prepared by incubating **mAb–PEG$_3$–$N_3$** (1–12 mg / mL) in an aqueous medium (*e.g.*, PBS, PBS containing 5% glycerol, HBS) with ≥6 molar equivalents of an **LP** dissolved in a suitable organic solvent, such as DMSO, DMF or DMA (*i.e.*, the reaction mixture contains 5-20% organic solvent, v/v) at 24 °C to 37 °C for 30 min to 24 hr. The progress of the reaction was monitored by ESI–MS and the absence of **mAb–PEG$_3$–$N_3$** indicated the completion of the conjugation. The excess amount of the **LP** and organic solvent were removed by SEC via elution with PBS, or via protein A column chromatography via elution with acidic buffer followed by neutralization with Tris (pH8.0). The purified conjugates were analyzed by SEC, SDS–PAGE, and ESI–MS. Shown in Table 5 is a list of the MMAE antibody conjugates, steroid antibody conjugates, and LXR agonist antibody conjugates from the corresponding **LP**s, their molecular weights and ESI–DAR values.

**[0555]** In a specific example, the azido-functionalized antibody (1 mg) in 0.800 mL PBSg (PBS, 5% glycerol, pH 7.4) was treated with six molar equivalents of of a linker payload (**LP**) from Table 2 (conc. 10 mg/mL in DMSO) for 2 hours at RT and the excess linker payload (**LP**)

was removed by size exclusion chromatography (SEC, Superdex 200 HR, GE Healthcare). The final product was concentrated by ultra-centrifugation and characterized by UV, SEC, SDS-PAGE and ESI-MS.

**Characterization of ADC by LC–ESI–MS**

**[0556]** Measurement of intact mass for the ADC samples by LC–ESI–MS was performed to determine drug-payload distribution profile and to calculate the average DAR. Each testing sample (20-50 ng, 5uL) was loaded onto an Acquity UPLC Protein BEH C4 column (10K psi, 300 Å, 1.7 µm, 75µm × 100 mm; Cat No. 186003810). After 3 min desalting, the protein was eluted and mass spectra were acquired by a Waters Synapt G2–Si mass spectrometer. The MWs of the naked antibodies, the azido–functionalized antibody conjugates (mAb-$N_3$), the LPs, and the ADCs were listed in Table 5. As summarized in Table 5, most site-specific ADCs have 3.9 - 4DAR for the site specific conjugates.

**Table 5. List of conjugates**

| LP | Ex | MW (LP) | Ab, Ab–$N_3$, or ncADC | Target | MS m/z (ncADC) | DAR (ESI–MS) |
|---|---|---|---|---|---|---|
| 1q | 27 | 2246.7 | H1H21234N-Ex 27 | MSR1 | 155570 | 3.9 |
| 1e | 19 | 2096.4 | H1H21234N-Ex 19 | MSR1 | 154964 | 3.9 |
| 1q | 27 | 2246.2 | H1H1238N-N297Q-Ex 27 | FelD1(isotype control) | 155245 | 4 |
| 1e | 19 | 2096.4 | H1H1238N-N297Q -Ex 19 | FelD1(isotype control) | 154641 | 3.9 |
| 1j | 23 | 1953.2 | H1H6958N2-N297Q –Ex 23 | Anti-PRLRmAb1 | 154070 | 4 |
| 1i | 22 | 2188.5 | H1H6958N2-N297Q –Ex 22 | Anti-PRLRmAb2 | 155011 | 3.9 |
| 1e | 19 | 2096.4 | H1H6958N2-N297Q –Ex 19 | Anti-PRLRmAb3 | 153792 | 4 |
| 1f | 20 | 2114.4 | H1H6958N2-N297Q –Ex 20 | Anti-PRLRmAb4 | 152301 | 3.7 |
| 1j | 23 | 1953.2 | H1H6958N2-N297Q –Ex 23 | Anti-PRLRmAb5 | 153227 | 3.9 |
| 1i | 22 | 2188.5 | H1H6958N2-N297Q –Ex 22 | Anti-PRLRmAb6 | 154168 | 3.9 |
| 1k | 24 | 2039.3 | H1H6958N2-N297Q-Ex 24 | Anti- | 153555 | 3.9 |

| | | | | PRLRmAb7 | | |
|---|---|---|---|---|---|---|
| 1c | 18 | 2348.9 | Anti-HER-N297Q-Ex 18 | HER2 | 155358 | 3.9 |
| 1q | 27 | 2246.7 | Anti-HER-N297Q-Ex 27 | HER2 | 154945 | 3.9 |

## EXAMPLE 30

**[0557]** This example demonstrates the effect on the cleavage efficiency for a steroid or LXR agonist payload when a $SO_3H$ moiety, $N^+Me_3$ moiety, phosphate moiety, or galactose moiety, present on a linker using a Cathepsin B cleavage assay.

**[0558]** For the assay, the linker-payload stock solution (10 mM of linker-payload in DMSO) was added into a solution of 100 mM NaOAc, 10 mM dithiothreitol, at pH5 to obtain a 50 µM substrate solution. Human liver Cathepsin B (Athens Research & Technology, Cat# 16-12-030102) in 50 mM NaOAc, 1 mM EDTA, at pH 5, was added to the substrate solution. The Cathepsin B and substrate solution was mixed with and without 10 mM of a Cathepsin B inhibitor (CA074; APE Bio, Cat# A1926), and incubated at 37 °C for 4 hours. Following the 4 hour incubation, acetic acid and then acetonitrile were added to stop the reaction. The quenched samples then underwent centrifugation at 14,000 rpm. Aliquots of the resultant supernatants were then diluted with equal volume of water and analyzed by LC/MS to determine the amount of payload released. The stability and activity of Cathepsin B was confirmed by incubating with 200 µM fluorogenic substrate for Cathepsin B (Santa Cruz Biotechnology, Cat# 207975) (fluorescence at excitation of 340 nm / emission of 425 nm).

**[0559]** As shown in **Table 1B**, the LXR agonist linker payload without the $SO_3H$ moiety had 13.6% of the free payload release when treated with Cathepsin B for 4 hours, whereas the LXR agonist linker payload with the $SO_3H$ moiety had 44.2% of the free payload release when treated with Cathepsin B for 4 hours. The steroid linker payload without the $SO_3H$ moiety had between 7.5 -17.2% of the free payload releasd when treated with Cathepsin B for 4 hours, whereas the steroid linker payload with $SO_3H$ moiety had 79.6% of the free payload release when treated with Cathepsin B for 4 hours. **Table 1C** and **Table 1D** show additional data for free payload release when linker payloads are treated with Cathepsin B.

**Table 1B**: Amount of payload released from linker-payloads with and without $SO_3H$ moiety in a Cathepsin B assay (See FIG. 18)

| Payload | Payload Type | Linker-Payload (LP) | Linker Modification | Percent of payload released at 4 hrs |
|---|---|---|---|---|
| D | LXR Agonist | **Ex 10B** | N/A | 13.6% |
| | | **Ex 26** | $SO_3H$ | 44.2% |
| B | Steroid | **Ex 10A** | N/A | 7.5 - 17.2% |
| | | **Ex 19** | $SO_3H$ | 79.6% |

**Table 1C**: Amount of payload released from linker-payloads with different $SO_3H$ linkers in a Cathepsin B assay. (See FIG. 18)

| # | Ex | Payload | Linker | | CapB cleavage rate (%) at 4 hrs |
|---|---|---|---|---|---|
| | | | Cleaved piece | linker | |
| **1e** | **19** | B | vcPAB | $PEG_4$-taurine | **79.6** |
| **1f** | **20** | B | vcPAB | Dual taurine | **63.0** |
| **1i** | **22** | C | vcPAB | $PEG_4$-taurine | **57.8** |
| **1j** | **23** | C | VA | $PEG_4$-taurine | **30.8** |
| **1k** | **24** | C | vc | $PEG_4$-taurine | **31.0** |
| **1m** | **26** | D | VA | $PEG_4$-taurine | **44.2** |

**Table 1D**: % of Payload released from linker-payloads (FIG. 19) in a Cathepsin B assay.

| # | Ex | Payload | Linker | | CapB cleavage rate (%) at 4 hrs |
|---|---|---|---|---|---|
| | | | Cleaved piece | linker | |
| **10b** | **10B** | A | vcPAB | - | **63** |
| **Ib** | **17A** | A | vcPAB | $PEG_4$-$N^+Me_3$ | **65** |
| **Id** | **18A** | A | vcPAB | $PEG_5$-phosphate | **50** |
| **Ie-1** | **18B** | A | vcPAB | galactose | **72** |
| **Ig** | **18D** | A | vcPAB | glucamide | **70** |

| | | | | | |
|---|---|---|---|---|---|
| **Ih-1** | **18E** | A | vcPAB | glucamide | **42** |
| **10a** | **10A** | B | vcPAB | - | **18.6** |
| **1e** | **19** | B | vcPAB | $PEG_4$-taurine | **79.6** |
| **1f** | **20** | B | vcPAB | Dual taurine | **63.0** |
| **10c** | **10C** | C | vcPAB | - | **<5** |
| **1i** | **22** | C | vcPAB | $PEG_4$-taurine | **57.8** |
| **1j** | **23** | C | VA | $PEG_4$-taurine | **30.8** |
| **1k** | **24** | C | vc | $PEG_4$-taurine | **31.0** |
| **10b** | **10B** | D | VA | - | **13.6** |
| **1m** | **26** | D | VA | $PEG_4$-taurine | **44.2** |
| **1q** | **27** | E | vcPAB | $PEG_4$-taurine | **30.4** |

## EXAMPLE 31

**[0560]** This experiment demonstrates the effect on the cleavage efficiency of a steroid payload when a hydrophilic linker is present in an anti-MSR1 antibody steroid non-cytotoxic ADC (ncADC), by measuring lysosomal cleavage over time.

**[0561]** For the assay, The ncADCs were added to a freshly prepared lysosome working solution containing 1x catabolic buffer (Xenotech Cat#K5200) and 0.125 mg/mL human liver lysosome proteins (Xenotech Cat#H0610.L). 200 µL of the resulting mixture, which contains 0.25 µM ncADC, was incubated at 37°C with gentle shaking over a 24-hour period. 20 µL aliquots were taken at 0, 0.5, 1.0, 2.0, 4.0, 8.0 and 24 hours and then transferred to a plate containing 80 µL cold acetonitrile to deactivate the lysosome and precipitate the proteins. After the centrifugation at 2,000 rpm for 5 minutes, aliquots of the supernatant were diluted with an equal volume of water before subjecting to analysis by LC-MS to determine the amount of payload release.

**[0562]** As shown in **Table 1E**, ncADC steroid conjugate without the hydrophilic linker **(H1H21234N-N297Q-Example 10A)** released 548 nM steroid at the 24-hour time point. The ncADC steroid conjugate with the hydrophilic linker (**H1H21234N-N297Q-Example 19**) released 760 nM at 24-hour time point.

**Table 1E: Amount of steroid released with and without hydrophilic linker**

| ncADC | Pay-load | Pay-load Type | Linker Modifi-cation | Released Payload Concentration (nM) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0 hour | 0.5 hour | 1.0 hour | 2.0 hour | 4.0 hour | 8.0 hour | 24 hour |
| H1H21234 N-N297Q-**Example 10A** | B | Steroid | N/A | 0 | 43.1 | 105 | 265 | 628 | 719 | 548 |
| H1H21234 N-N297Q-**Example 19** | B | Steroid | $SO_3H$ | 12.6 | 13.8 | 40.4 | 175 | 680 | 961 | 760 |

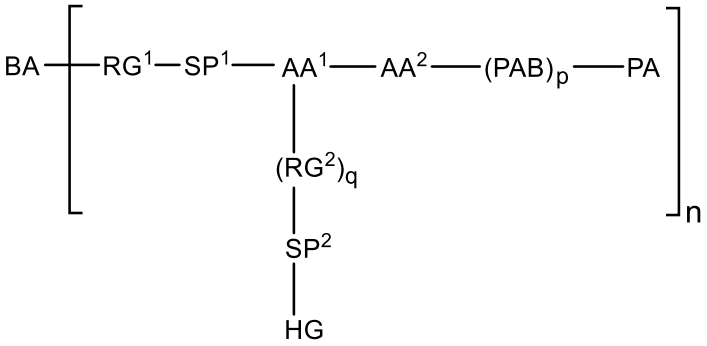
**[0563]** The embodiments and examples described above are intended to be merely illustrative and non–limiting. Those skilled in the art will recognize or will be able to ascertain using no more than routine experimentation, numerous equivalents of specific compounds, materials and procedures. All such equivalents are considered to be within the scope and are encompassed by the appended claims.

## CLAIMS

What is claimed is:

1. A compound, or pharmaceutically acceptable salt, solvate, triazole linkage regioisomer, mixture of triazole linkage regioisomers, or stereoisomer thereof,

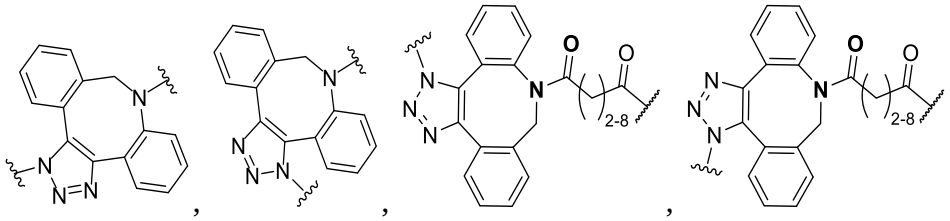
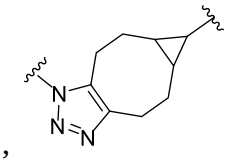
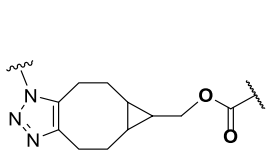
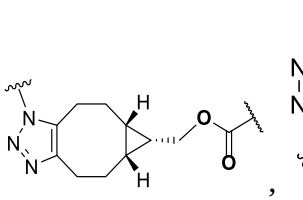
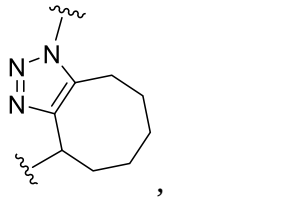
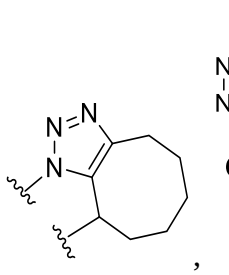
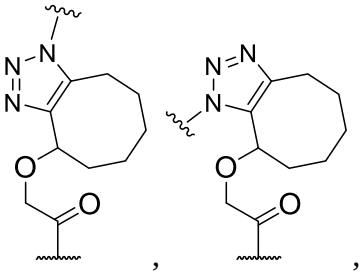
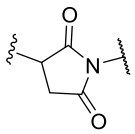
wherein the compound has a structure according to Formula (**IV**):




(**IV**)

wherein:

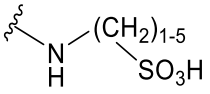
**BA** is an antibody or antigen binding fragment thereof;

**RG$^1$** and **RG$^2$** are reactive group residues independently selected from the group consisting of  ,  ,  ,  ,  , , , , , ,  ,  , , and  , wherein the  indicates

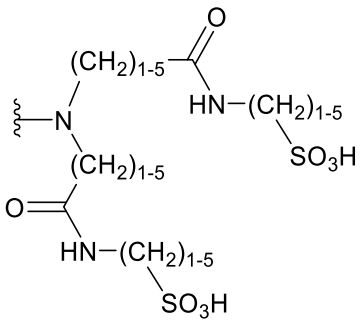
the atom through which the $RG^1$ or $RG^2$ is bonded to the adjacent groups in the formula;

**$SP^1$** and **$SP^2$** are independently, in each instance, absent, or a spacer group residue selected from the group consisting of $C_{1-6}$alkylene, -NH-, -C(O)-, -$(CH_2$-$CH_2$-$O)_e$, -NH-$(CH_2$-$CH_2$-$O)_e$-$CH_2$-$CH_2$-C(O)-, -C(O)-$(CH_2)_u$-C(O)-, -C(O)-NH-$(CH_2)_v$-, and combinations thereof wherein subscript e is an integer from 0 to 6, subscript u is an integer from 1 to 8, and subscript v is an integer from 1 to 8;

**HG** is a hydrophilic residue selected from



and

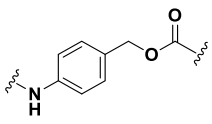


wherein the  indicates the atom through which the **HG** is bonded to the adjacent groups in the formula;

**PA** is a payload residue;

**$AA^1$** is an amino acid residue selected from tryptophan, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, and citrulline;

**$AA^2$** is a dipeptide, tripeptide, or tetrapeptide residue comprising amino acid residues selected from alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, and citrulline;

**PAB** is  , wherein the  indicates the atom through which the **PAB** is bonded to the adjacent groups in the formula;

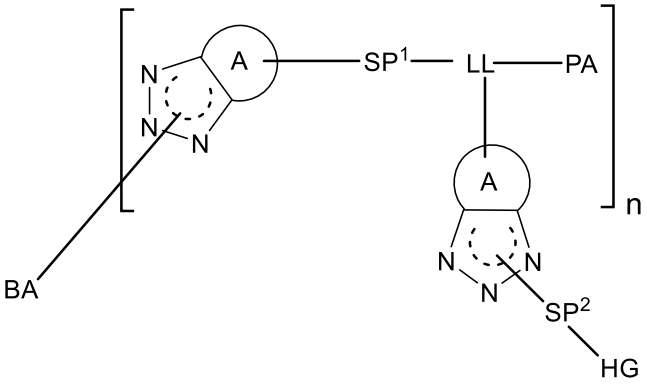
subscript **n** is an integer from 1 to 30;

subscript **p** is 0 or 1; and

subscript **q** is 1.

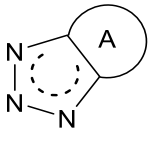
2. The compound of claim 1, wherein subscript **n** is 1, 2, 3 or 4.

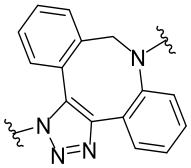
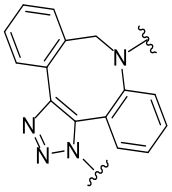
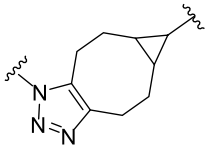
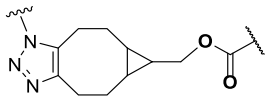
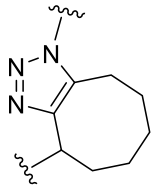
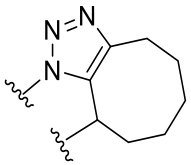
3. The compound of claim 1, according to Formula (**III**):




(**III**)

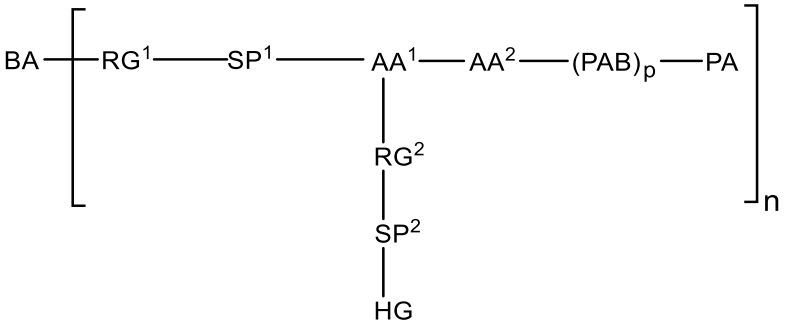
wherein



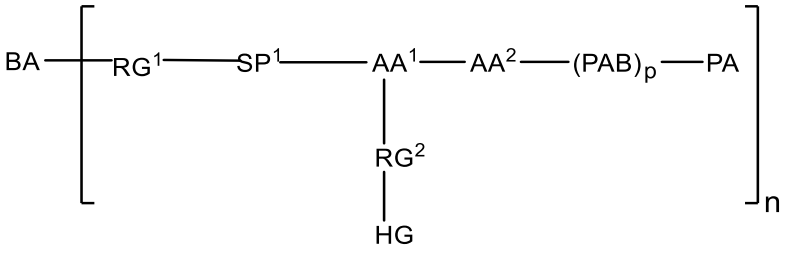

is selected from the group consisting of  ,  ,  ,  ,  , and  ; and

LL is –**AA$^1$-AA$^2$-(PAB)$_p$-**.

4. The compound of claim 1, according to Formula (**IVa**), or Formula (**IVb**):



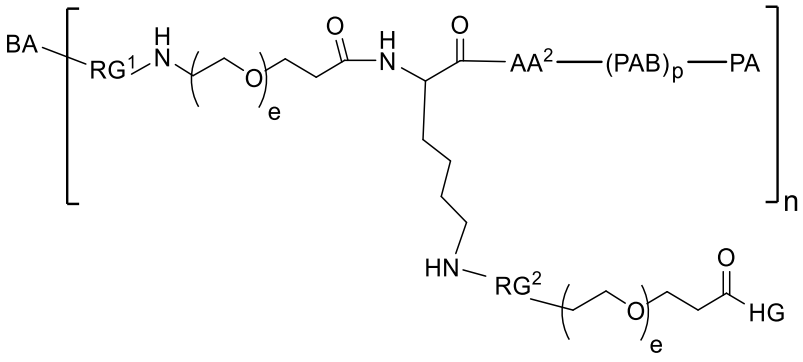

(**IVa**),



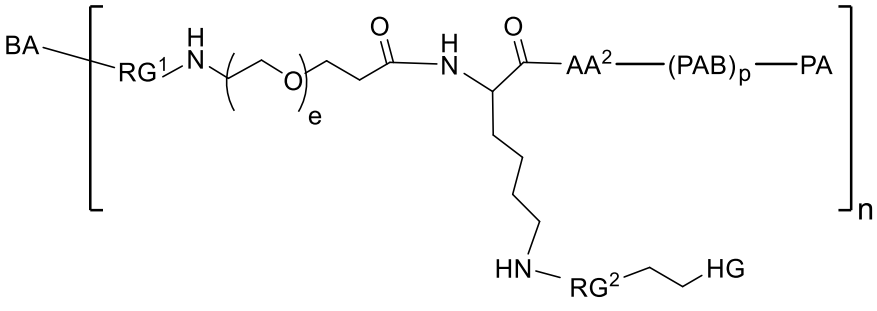

(**IVb**).

5. The compound of claim 4, wherein **AA$^1$** is a lysine, glutamine, glutamic acid, or aspartic acid residue.

6. The compound of claim 4, according to Formula (**Va**), (**Vb**), or (**Vd**) respectively:

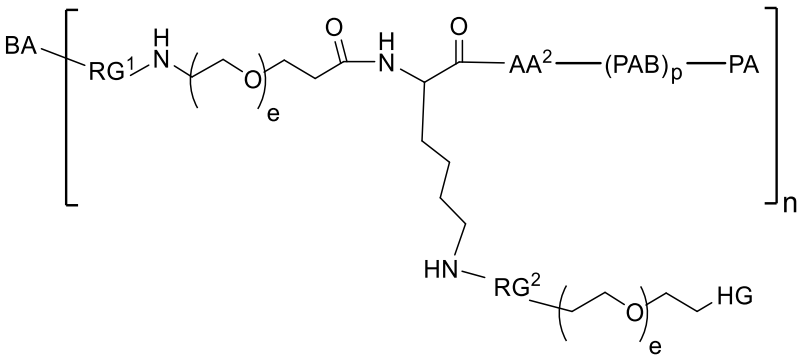



(**Va**),




(**Vb),**

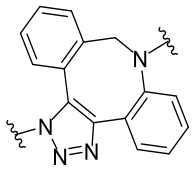
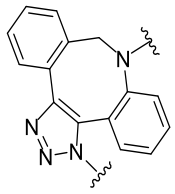
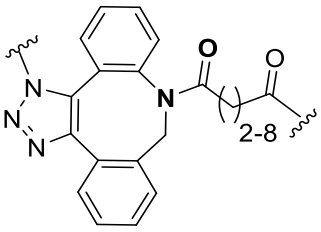
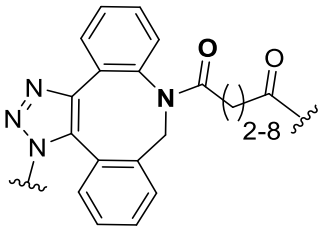
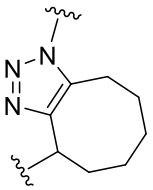
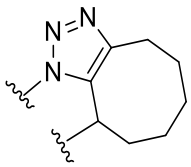
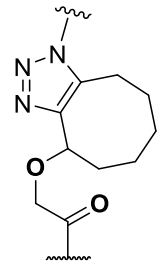
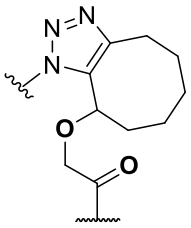
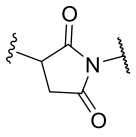
or



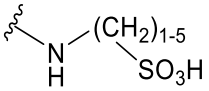

(**Vd**),

wherein:

subscript **e** is independently, in each instance, an integer from 0 to 6.

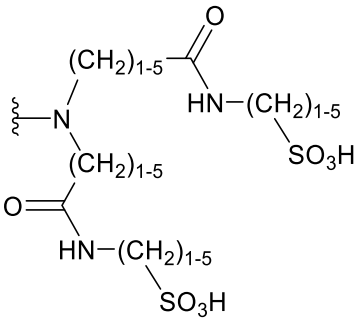
7. The compound of any one of claims 1 or 4-6, wherein **RG[1]** and **RG[2]** are independently, in each instance, selected from the group consisting of  ,  ,  ,  ,  ,  ,  ,  , and  , wherein the  indicates the atom through which the **RG[1]** or **RG[2]** is bonded to the adjacent groups in the formula.

8. The compound of any one of claims 1 or 4-7, wherein **HG** is



wherein the  indicates the atom through which the **HG** is bonded to the adjacent groups in the formula.

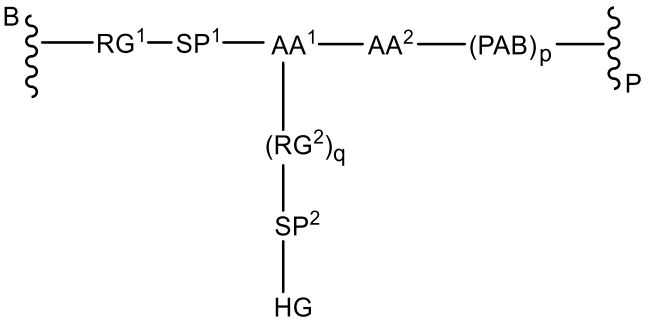
9. The compound of any one of claims 1 or 4-7, wherein **HG** is



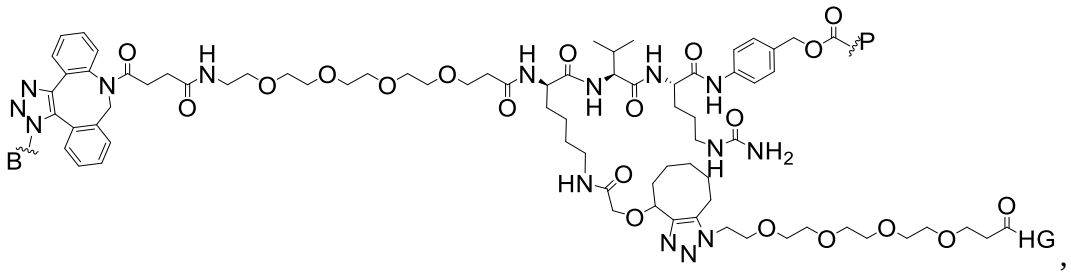
, wherein the  indicates the atom through which the **HG** is bonded to the adjacent groups in the formula.

10. The compound of any one of claims 1, 4-5, and 7-9, wherein **SP[1]** and **SP[2]** are independently, in each instance, absent, or selected from the group consisting of (-$CH_2$-$CH_2$-O)$_e$ and -NH-$CH_2$-$CH_2$-(-O-$CH_2$-$CH_2$)$_e$-C(O)-, wherein subscript **e** is an integer from 0 to 4.

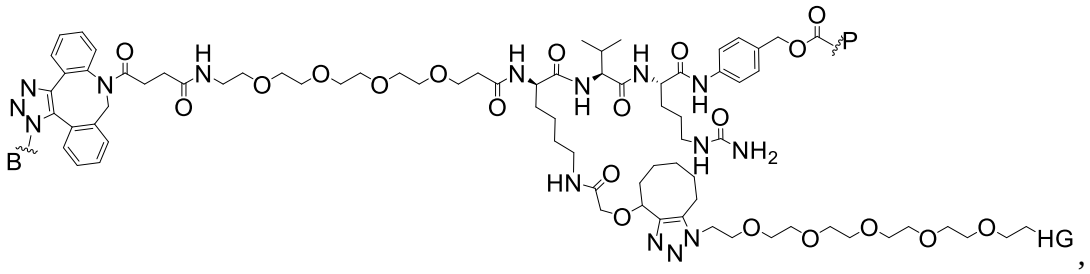
11. The compound claim 1, wherein



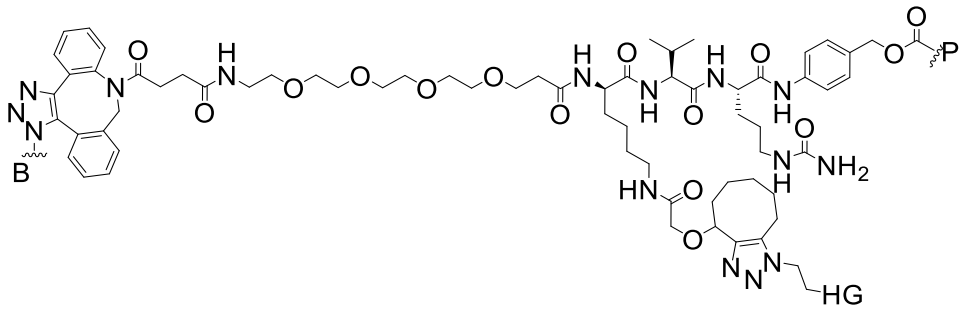
is selected from the group consisting of:

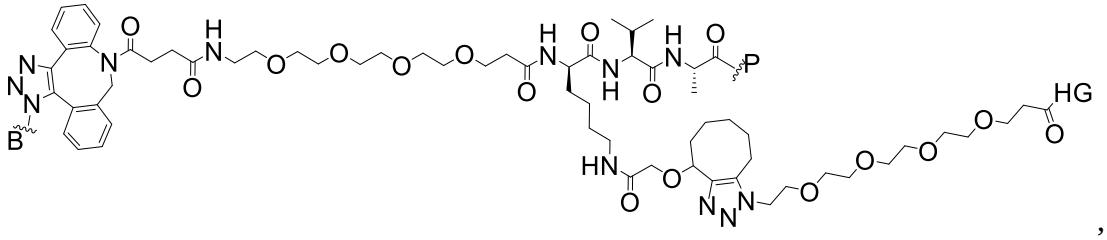


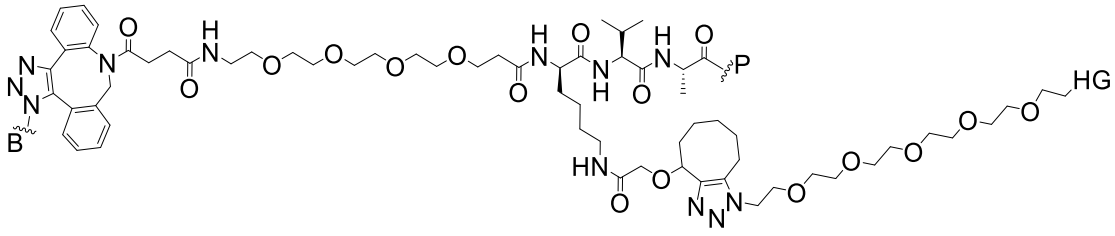
,

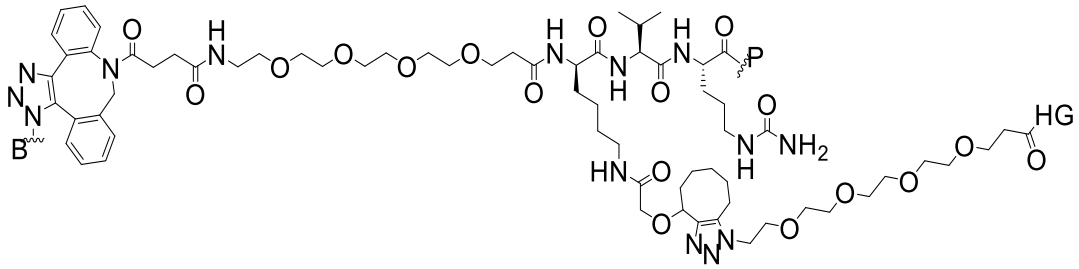


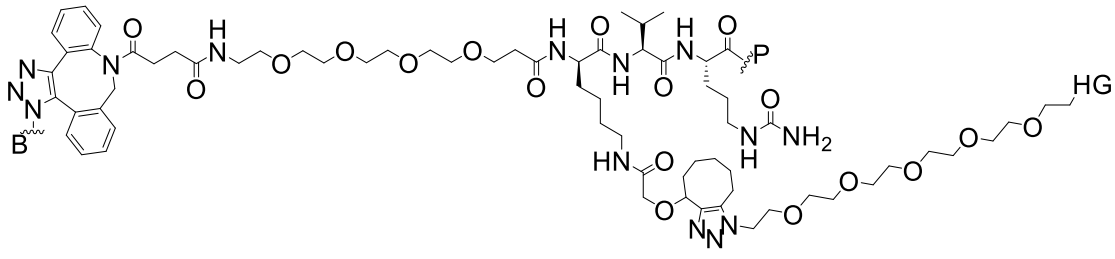
,

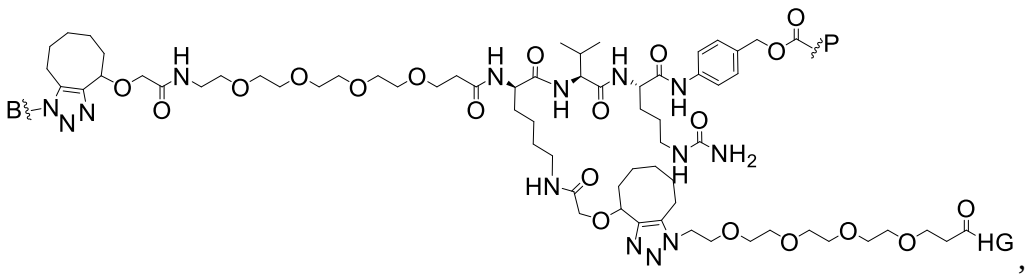

,

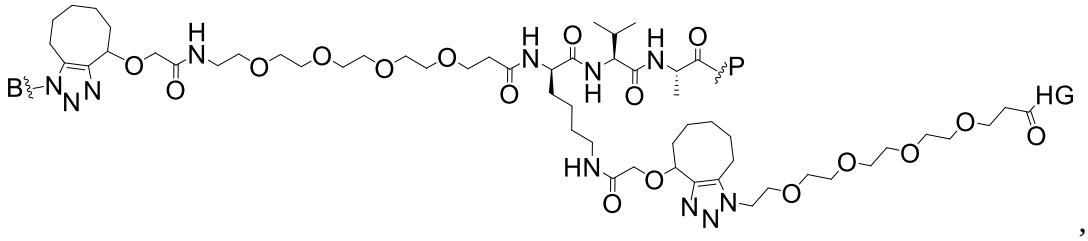

,

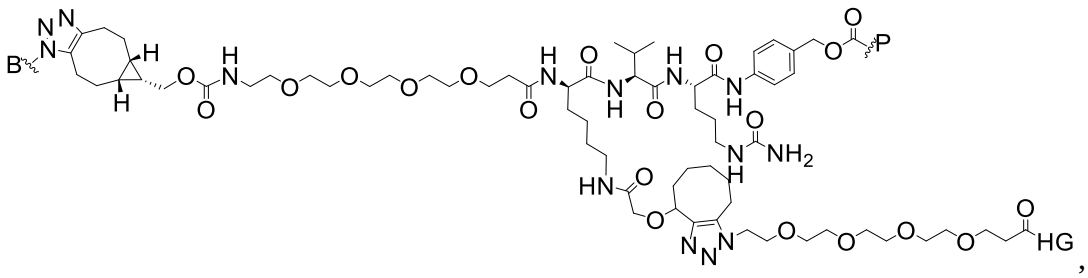

,


,

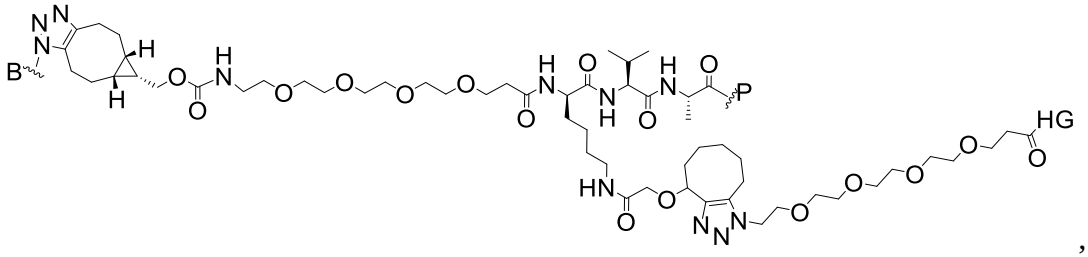

,


,


,


,

and

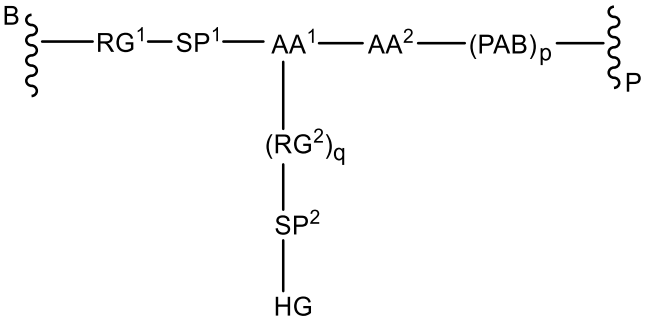

,

or a stereoisomeric form thereof, or a triazole linkage regioisomer thereof, or a mixture of triazole linkage regioisomers thereof, wherein

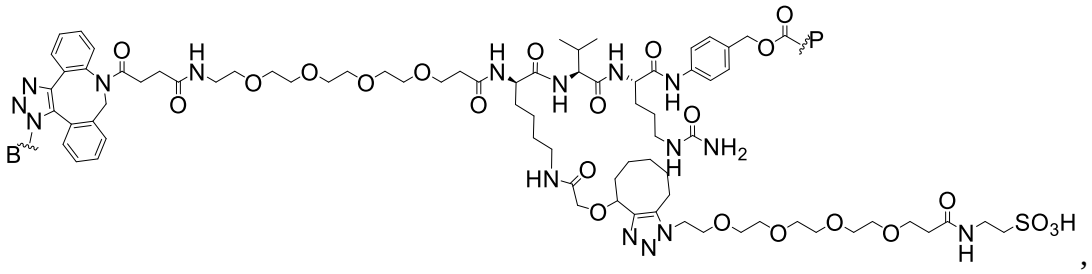
each  is a bond to the antibody or antigen binding fragment thereof; and

each  is a bond to the payload residue.

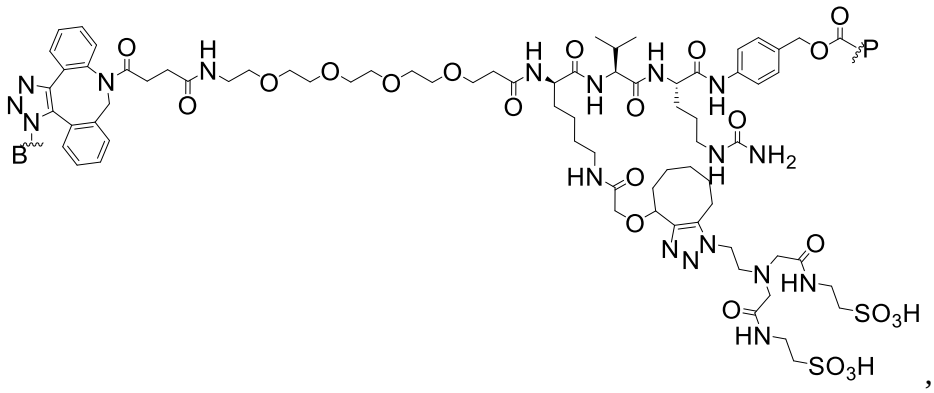
12. The compound of claim 1, wherein



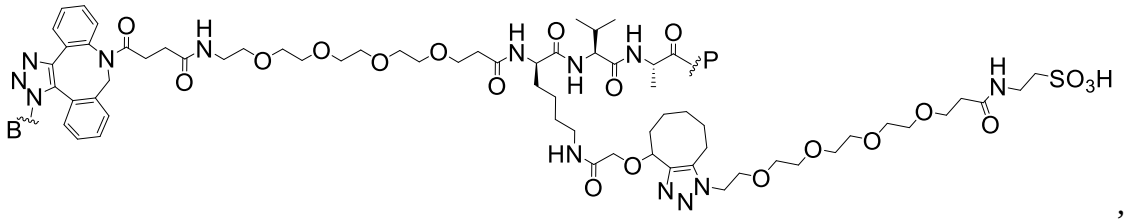
is selected from the group consisting of:



,

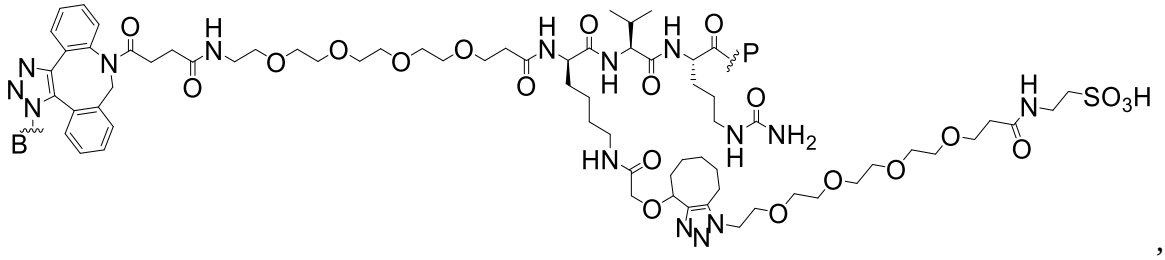


,



,

and



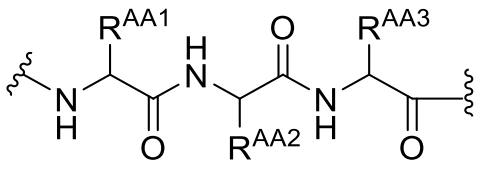
,

or a stereoisomeric form thereof, or a triazole linkage regioisomer thereof, or a mixture of triazole linkage regioisomers thereof, wherein

each  is a bond to the antibody or antigen binding fragment thereof; and

each  is a bond to the payload residue.

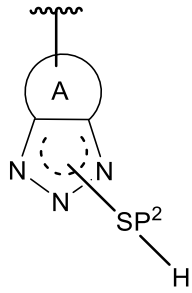
13. The compound of claim 3, wherein **LL** is according to Formula (**LL1**):



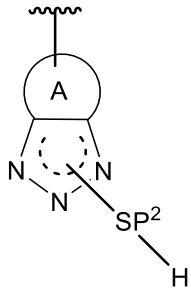
(**LL1**)

wherein $\mathbf{R^{AA1}}$ is an amino acid sidechain residue selected from tryptophan, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, and citrulline,

$\mathbf{R^{AA2}}$, and $\mathbf{R^{AA3}}$ are each, independently, amino acid side chain residues selected from alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, and citrulline; and

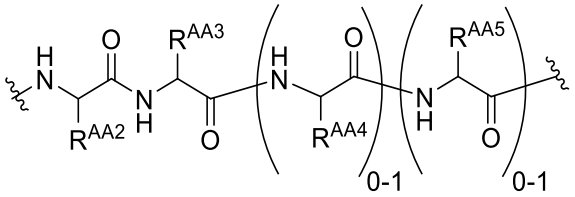
$\mathbf{R^{AA1}}$ is bonded directly to 

.

14. The compound of claim 13, wherein $\mathbf{R^{AA1}}$ is a lysine, glutamine, glutamic acid or aspartic acid side chain residue bonded directly to 

, and $\mathbf{R^{AA2}}$ and $\mathbf{R^{AA3}}$ are either valine and alanine or valine and citrulline sidechain residues respectively.

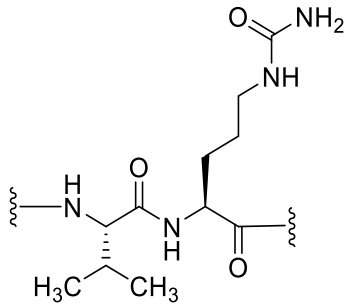
15. The compound of any one of claims 1 or 4-6, wherein $\mathbf{AA^2}$ is

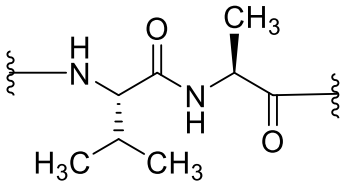



wherein $\mathbf{R^{AA2}}$, $\mathbf{R^{AA3}}$, $\mathbf{R^{AA4}}$, and $\mathbf{R^{AA5}}$ are each, independently, amino acid side chains selected from alanine, valine, leucine, isoleucine, methionine, tryptophan,

phenylalanine, proline, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, and citrulline, wherein the  indicates the atom through which **AA$^2$** is bonded to the adjacent groups in the formula.

16. The compound of any one of claims 1 or 4-6, wherein **AA$^2$** is




or 

,

wherein the  indicates the atom through which **AA$^2$** is bonded to the adjacent groups in the formula.

17. The compound of any one of claims 6-10, wherein subscript e is 4.

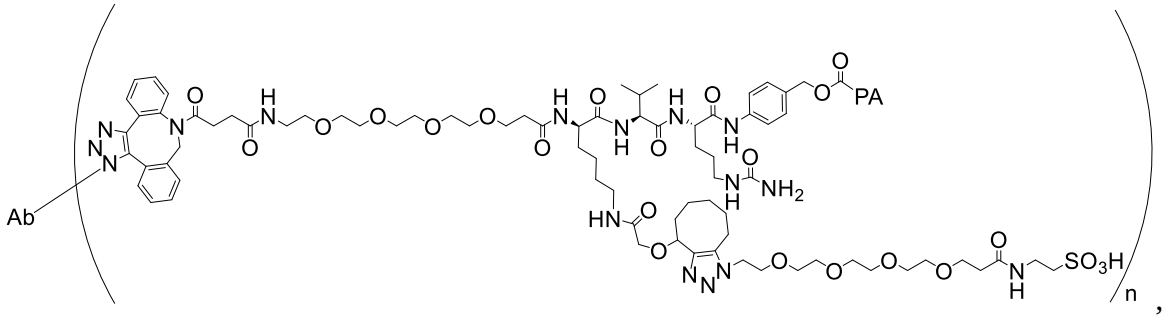
18. The compound of any one of claims 1-17, wherein **BA** is an antibody.

19. The compound of any one of claims 1-17, wherein **BA** is an antibody, or an antigen-binding fragment thereof, selective for an antigen selected from the group consisting of AXL, BAFFR, BCMA, BCR-list components, BDCA2, BDCA4, BTLA, BTNL2, BTNL3, BTNL8, BTNL9, C10 or f54, CCR1, CCR3, CCR4, CCR5, CCR6, CCR7, CCR9, CCR10, CD11c, CD137, CD138, CD14, CD168, CD177, CD19, CD20, CD209, CD209L, CD22, CD226, CD248, CD25, CD27, CD274, CD276, CD28, CD30, CD300A, CD33, CD37, CD38, CD4, CD40, CD44, CD45, CD46, CD48, CD5, CD52, CD55, CD56, CD59, CD62E, CD68, CD69, CD70, CD74, CD79a, CD79b, CD8, CD80, CD86, CD90.2, CD96, CLEC12A, CLEC12B, CLEC7A, CLEC9A, CR1, CR3, CRTAM, CSF1R, CTLA4, CXCR1/2, CXCR4, CXCR5, DDR1, DDR2, DEC-205, DLL4, DR6, FAP, FCamR, FCMR, FcR's, Fire, GITR, HHLA2, HLA class II, HVEM, ICOSLG, IFNLR1, IL10R1, IL10R2, IL12R, IL13RA1, IL13RA2, IL15R, IL17RA, IL17RB, IL17RC, IL17RE, IL20R1, IL20R2, IL21R, IL22R1, IL22RA, IL23R, IL27R, IL29R, IL2Rg, IL31R, IL36R, IL3RA, IL4R, IL6R, IL5R, IL7R, IL9R, Integrins, LAG3, LIFR, MAG/Siglec-4, MMR, MSR1, NCR3LG1, NKG2D, NKp30, NKp46, PDCD1, PROKR1, PVR, PVRIG, PVRL2, PVRL3, RELT, SIGIRR, Siglec-1, Siglec-

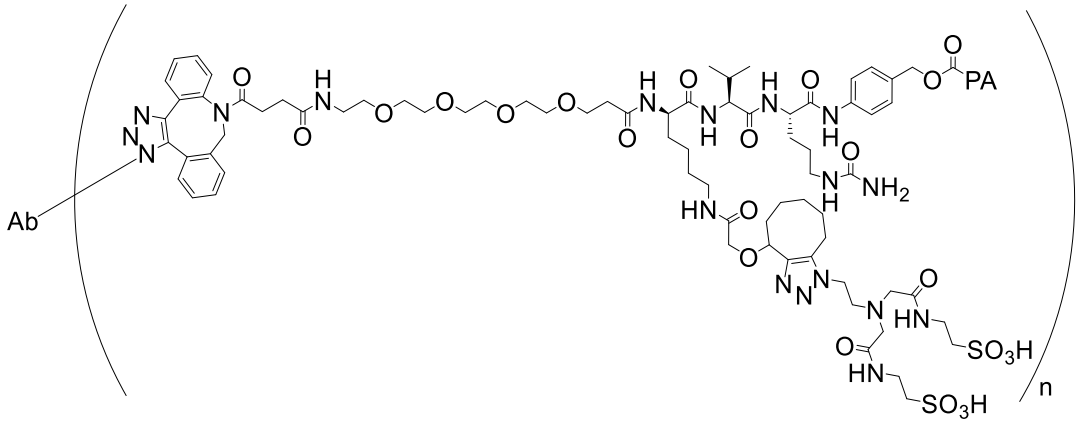
10, Siglec-5, Siglec-6, Siglec-7, Siglec-8, Siglec-9, SIRPA, SLAMF7, TACI, TCR-list components/assoc, PTCRA, TCRb, CD3z, CD3, TEK, TGFBR1, TGFBR2, TGFBR3, TIGIT, TLR2, TLR4, TROY, TSLPR, TYRO, VLDLR, VSIG4, and VTCN1.

20. The compound of any one of claims 1-19, wherein **PA** is the residue of a group selected from the group consisting of a dolastatin, an auristatin, a maytansinoid, a plant alkaloid, a taxane, a vinca alkaloid, a steroid, and a liver X receptor (LXR) modulator.

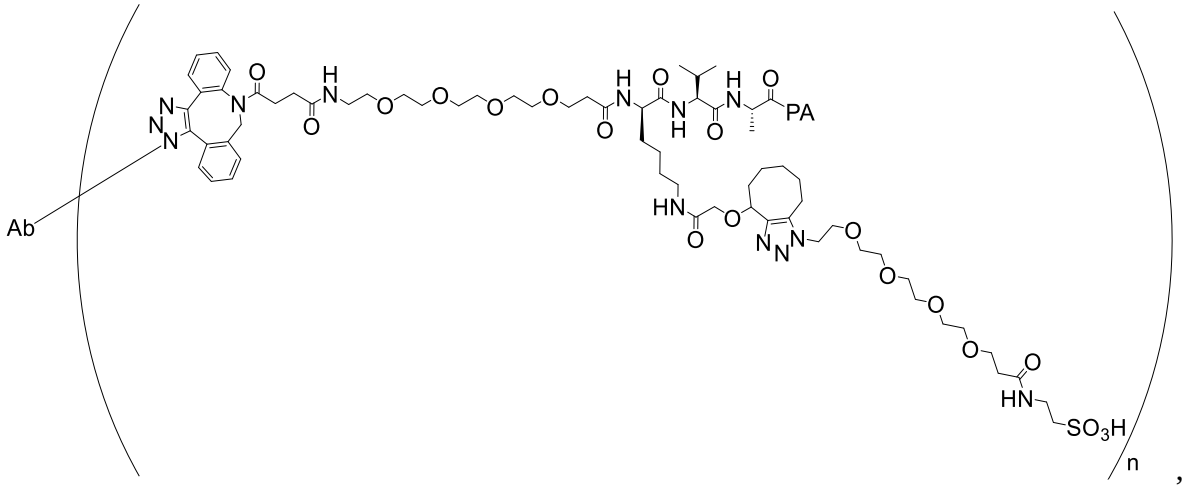
21. The compound of any one of claims 1 and 18-20, selected from the group consisting of:



,

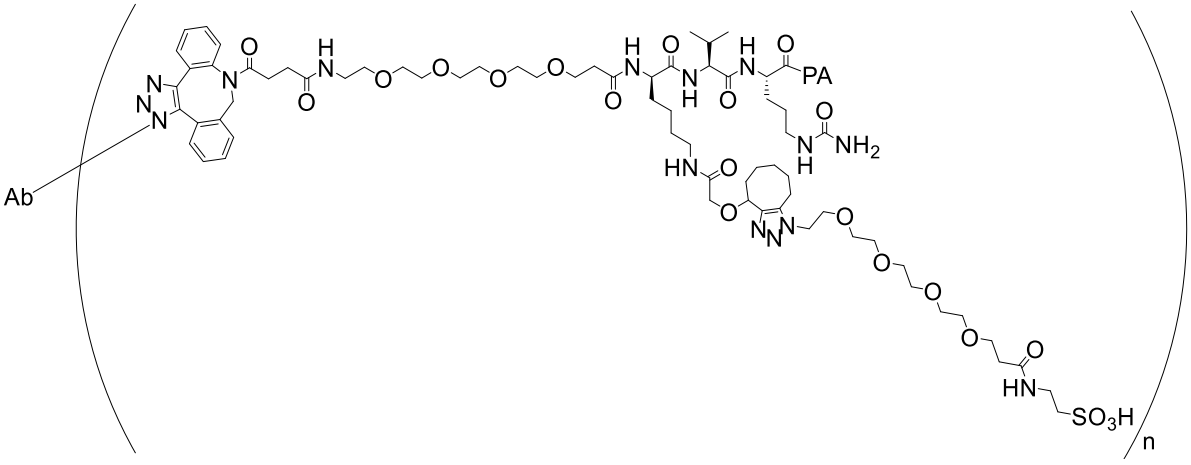


,



,

and




,

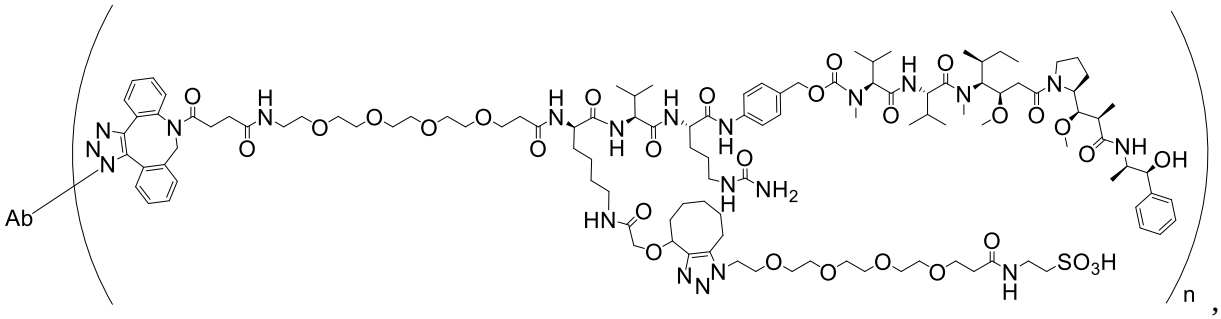
or a stereoisomeric form thereof, or a triazole linkage regioisomer thereof, or a mixture of triazole linkage regioisomers thereof, wherein

each **Ab** is an antibody, or an antigen-binding fragment thereof;

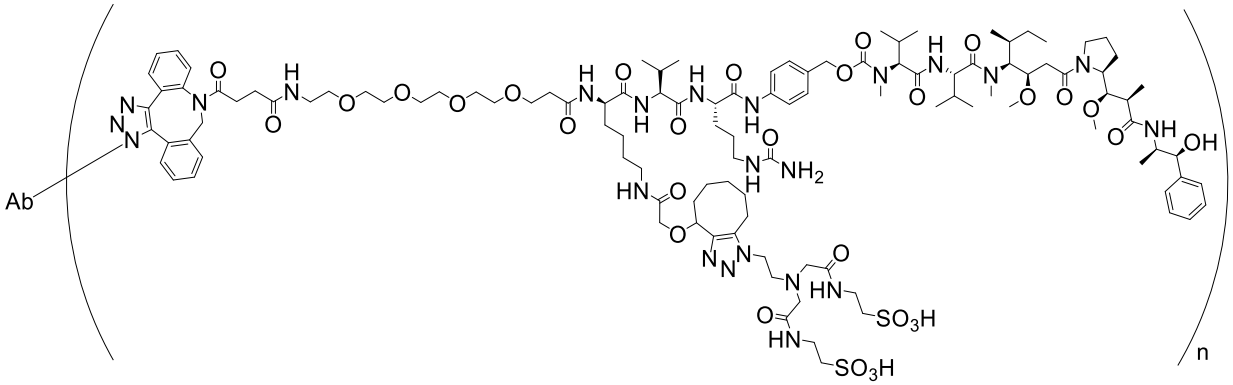
**PA** is a payload residue; and

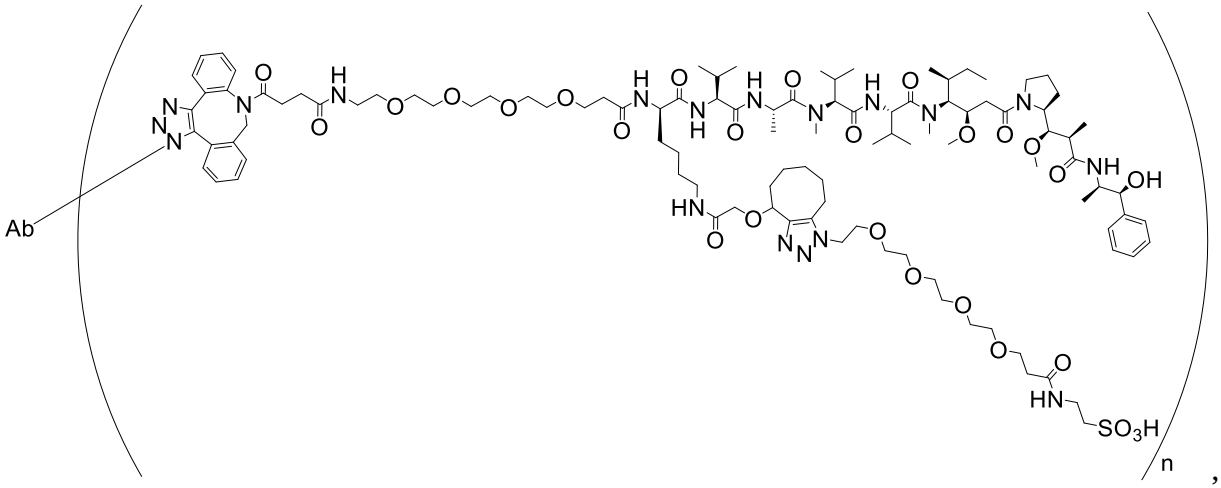
subscript **n** is an integer from 1 to 30.

22. The compound of any one of claims 1 and 18-20, selected from the group consisting of:

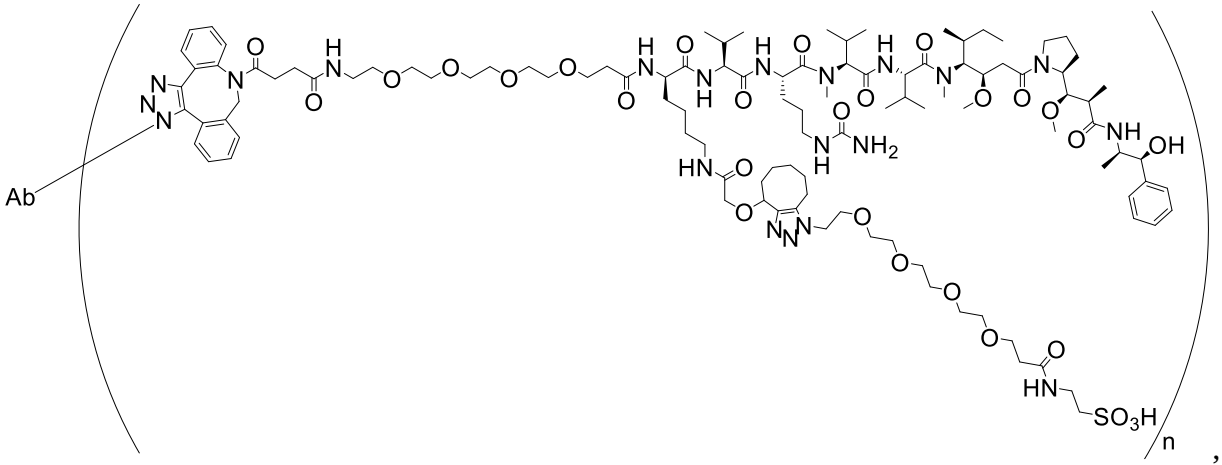



,

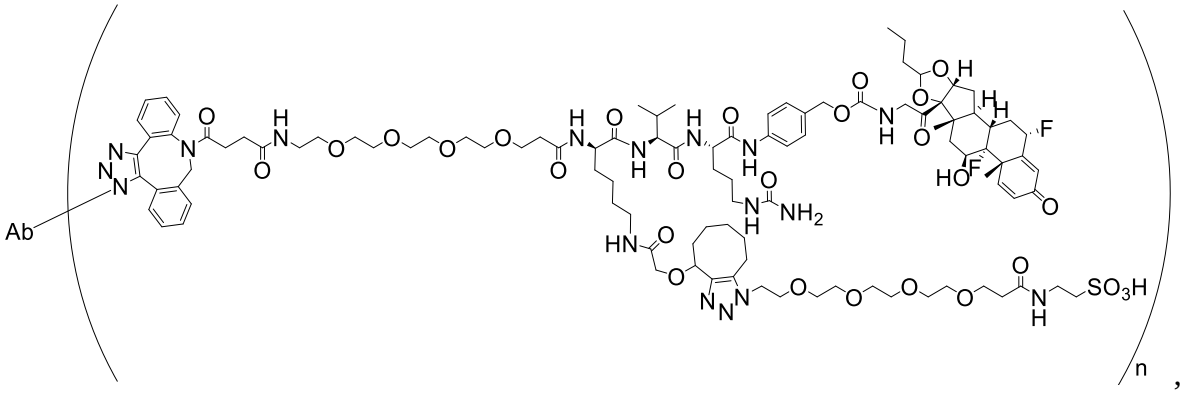




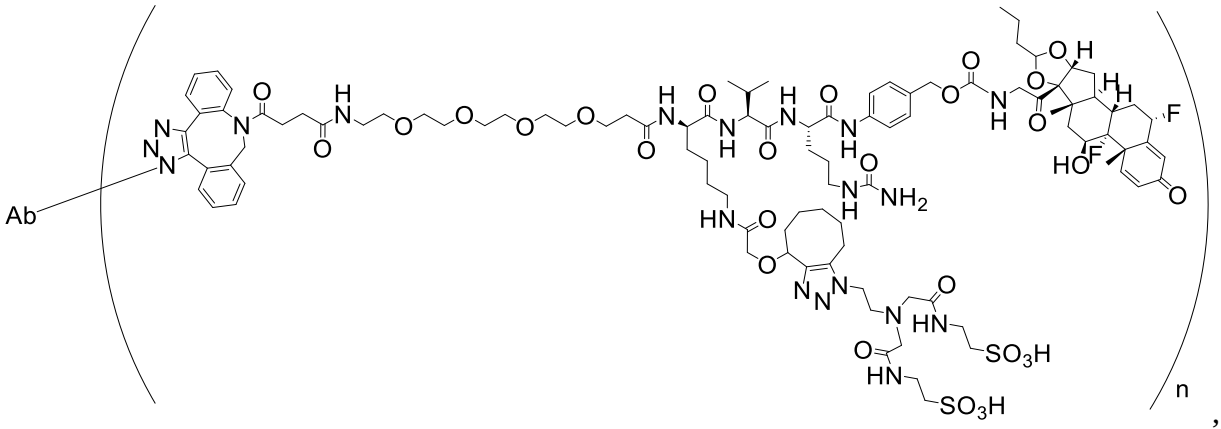
,



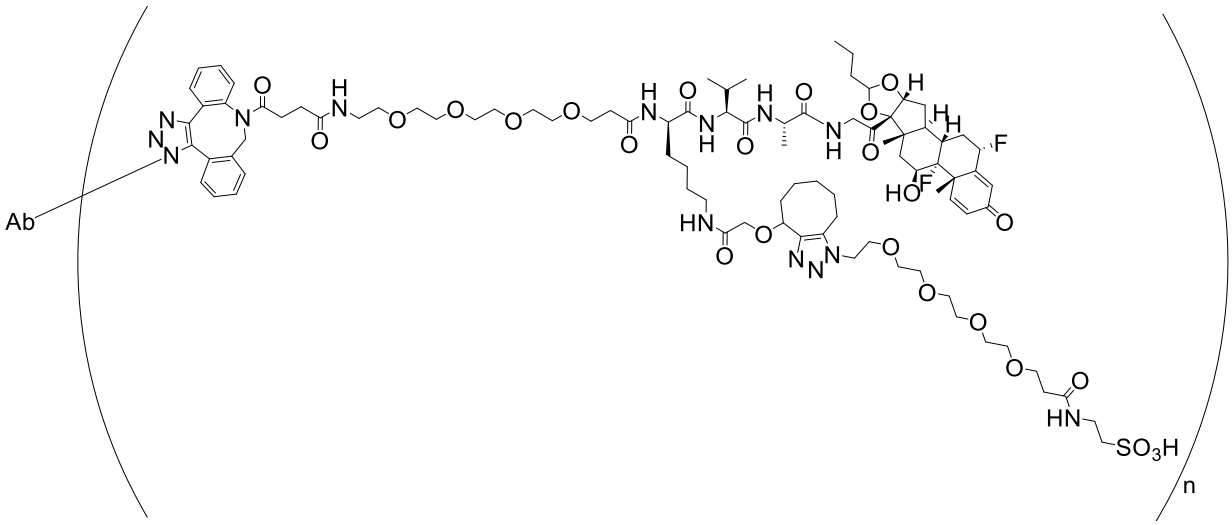
,

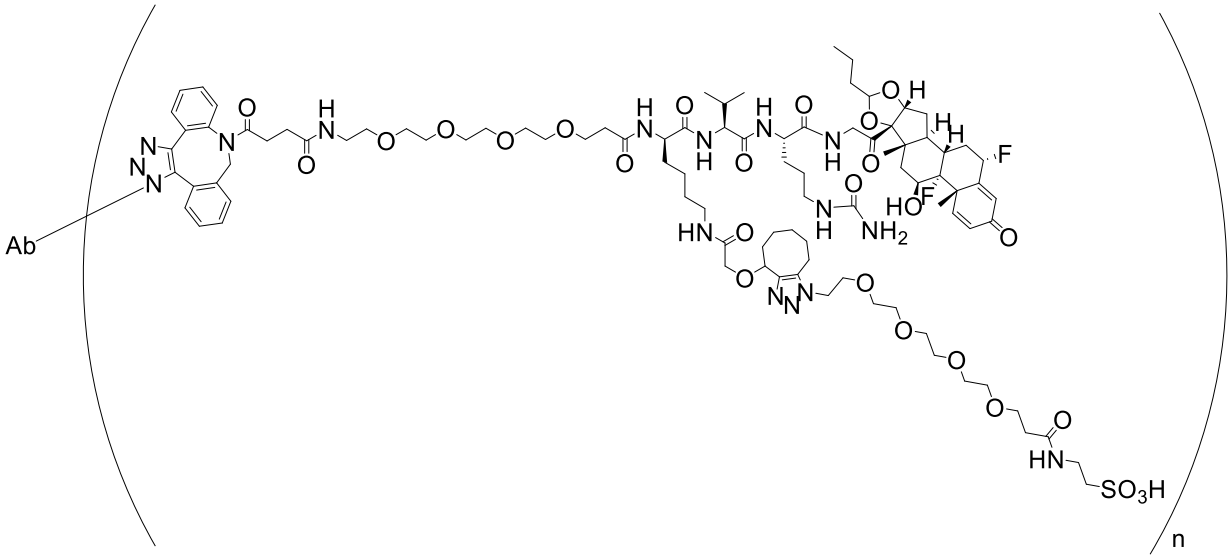


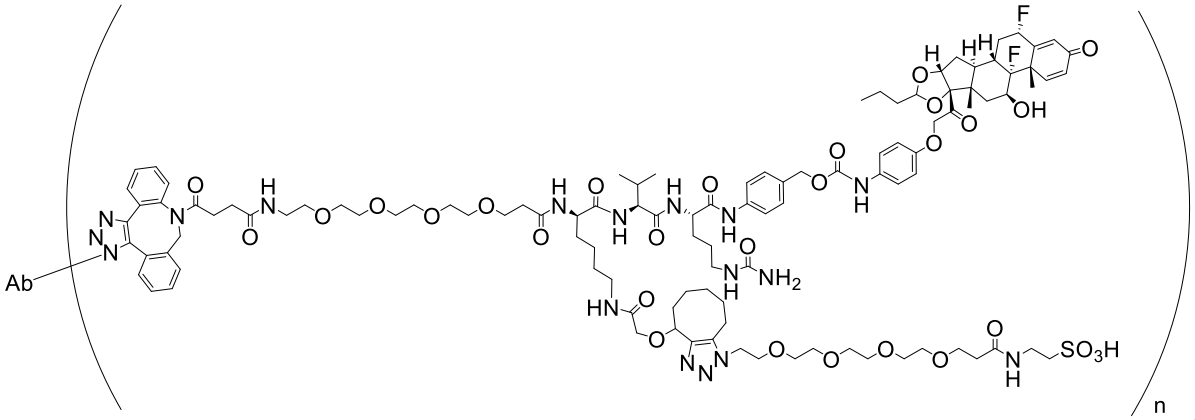
,


Ab
n

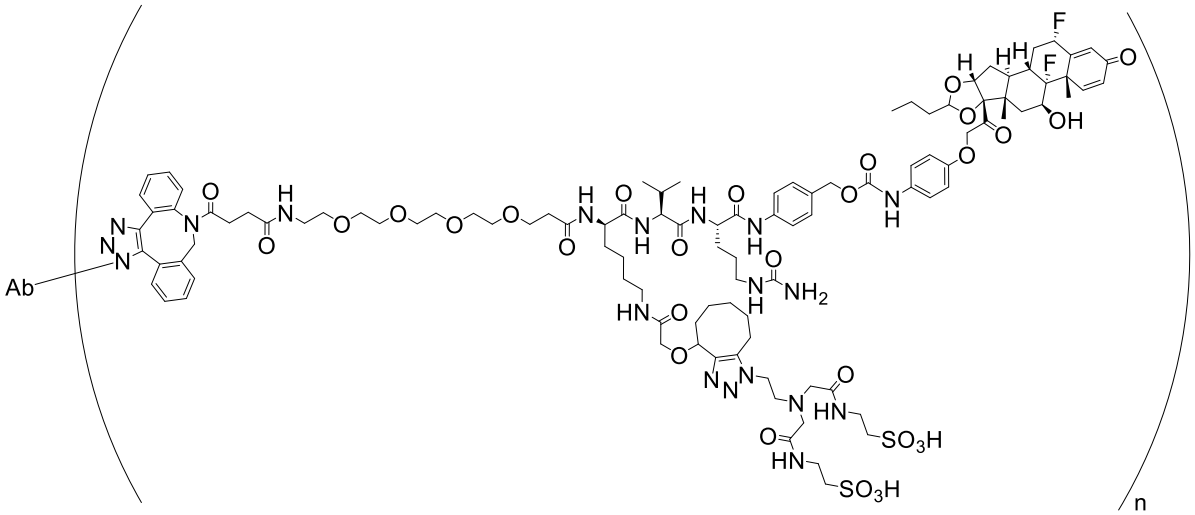
,


Ab
n

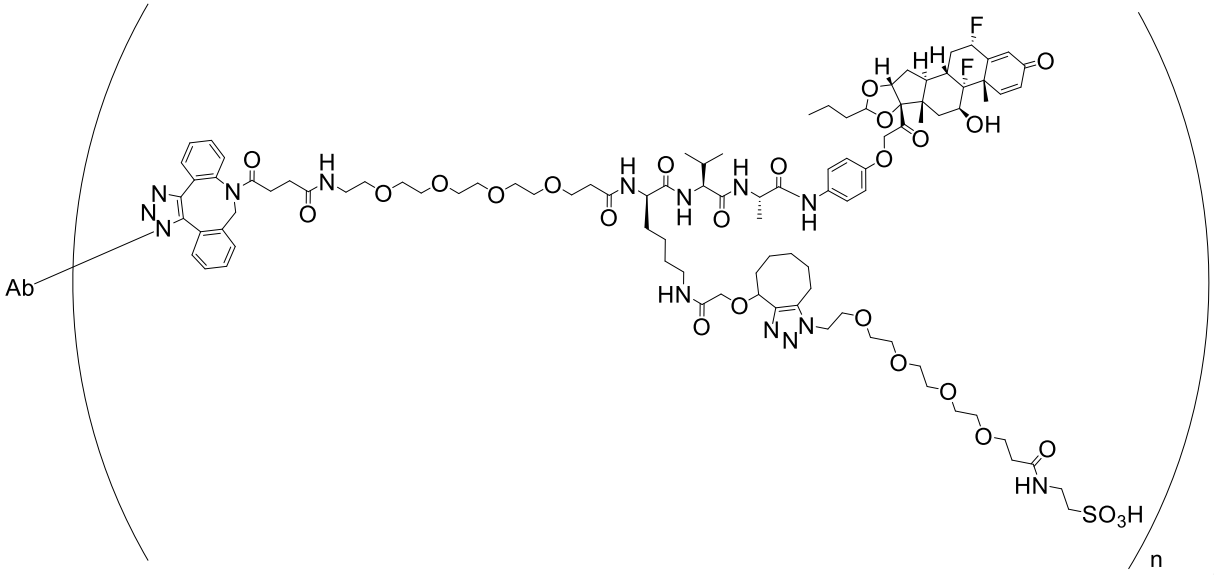

Ab
n



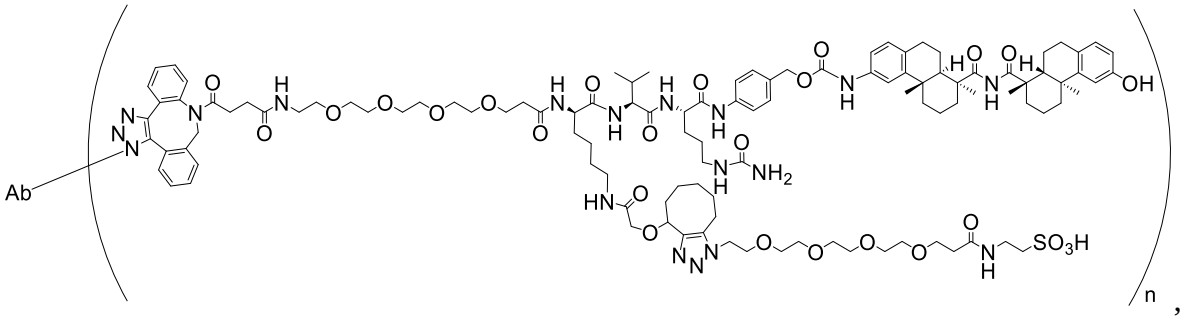
,

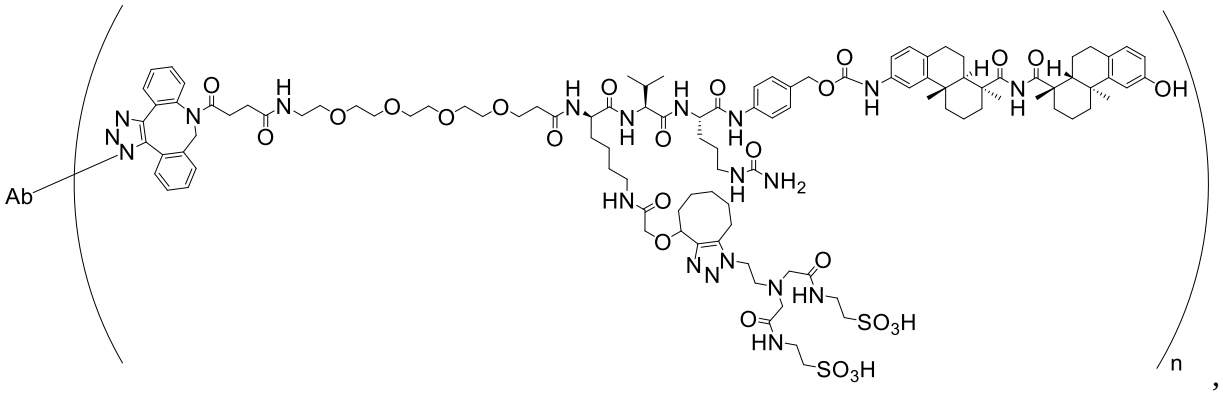


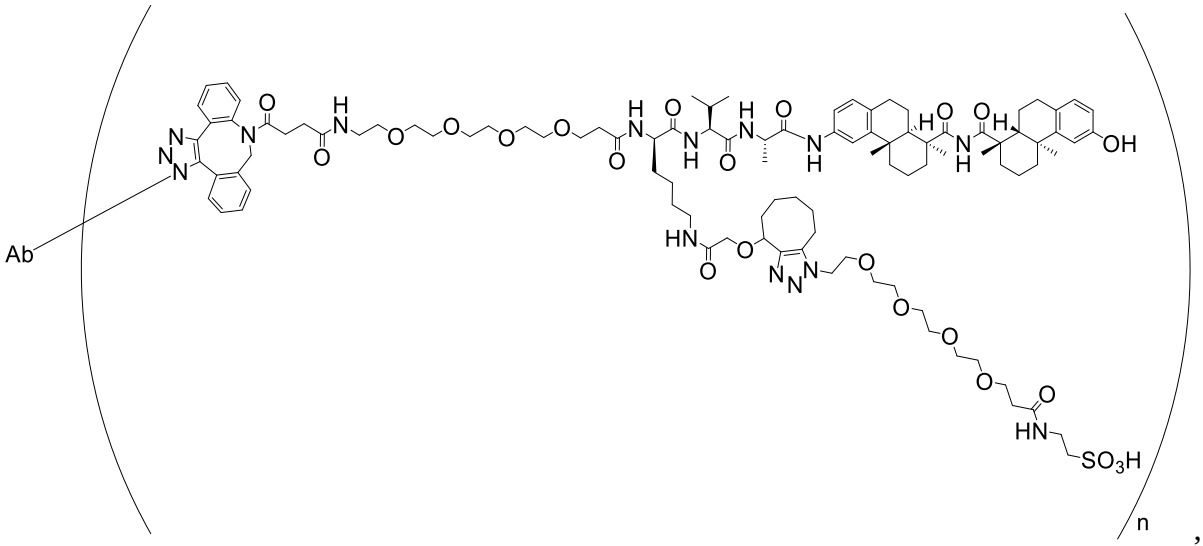
,

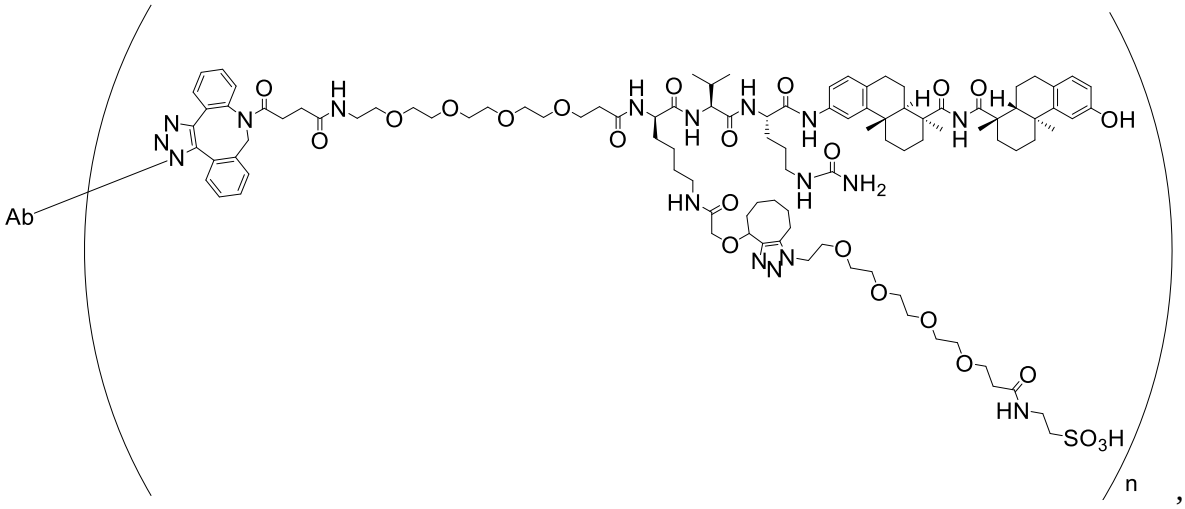


,

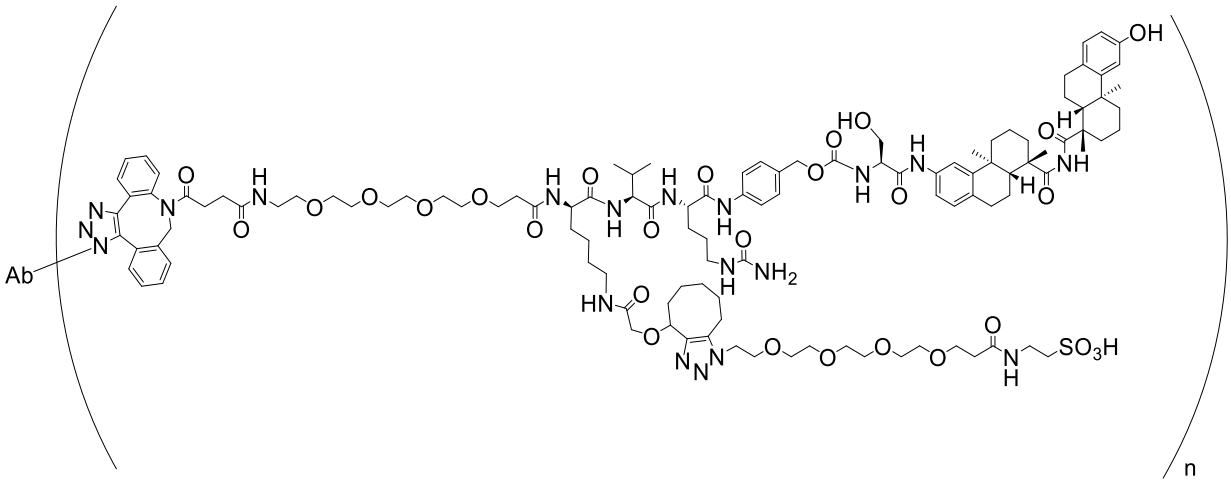


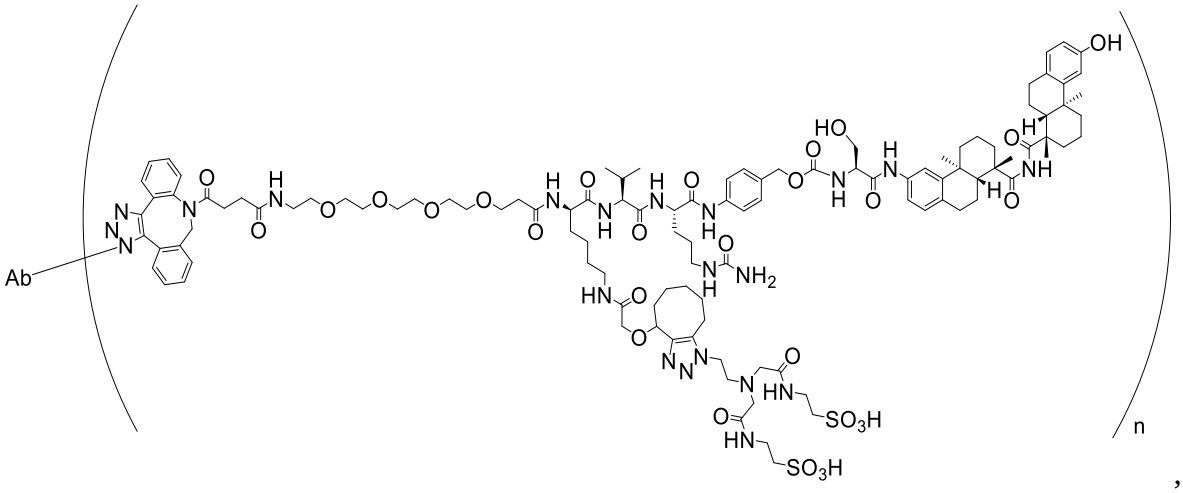




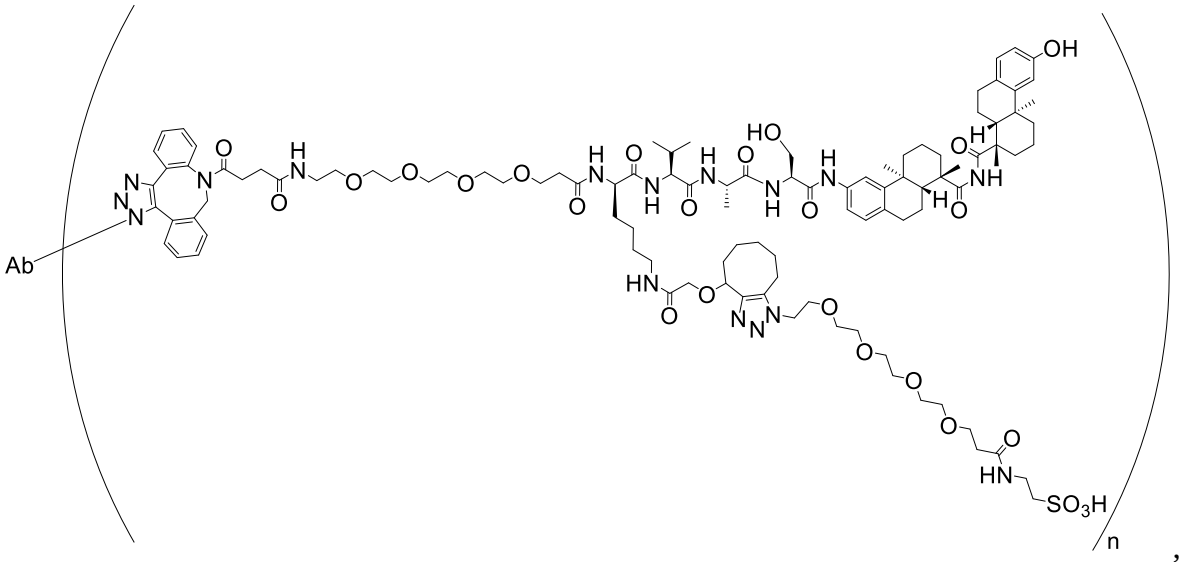


,



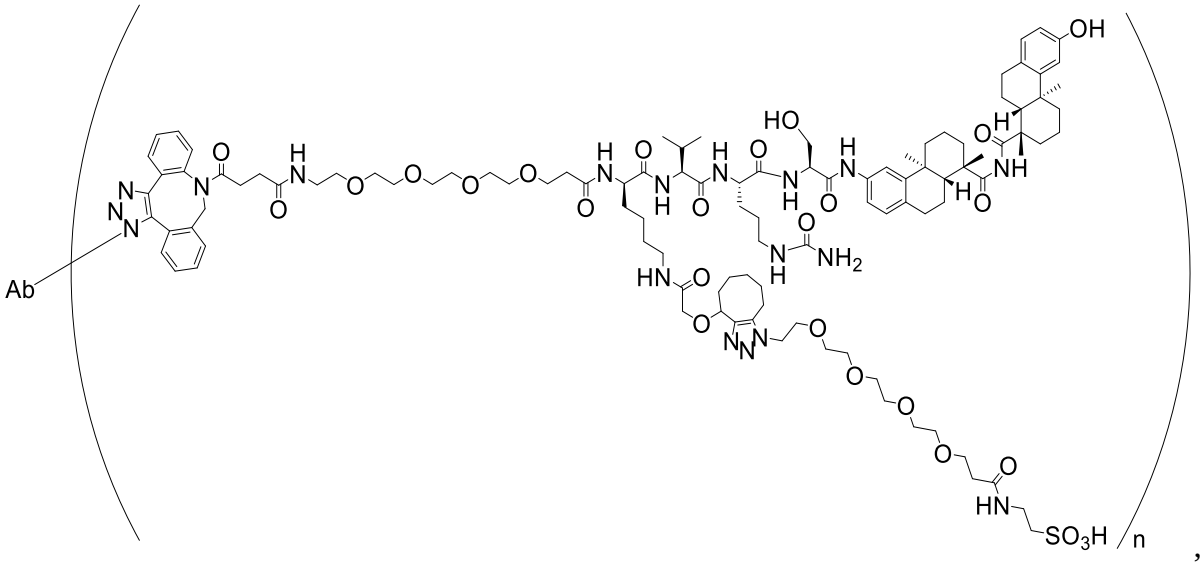


,




,

and



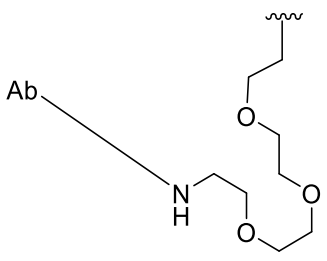

,

or a stereoisomeric form thereof, or a triazole linkage regioisomer thereof, or a mixture of triazole linkage regioisomers thereof, wherein

each **Ab** is the antibody, or an antigen-binding fragment thereof; and

subscript **n** is an integer from 1 to 30.

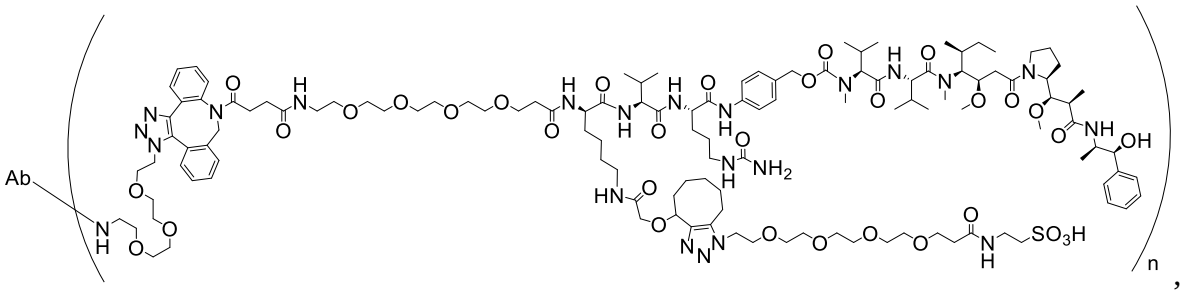
23. The compound of any one of claims 1-22, wherein **BA** is a modified antibody of formula (**Ab-1**)



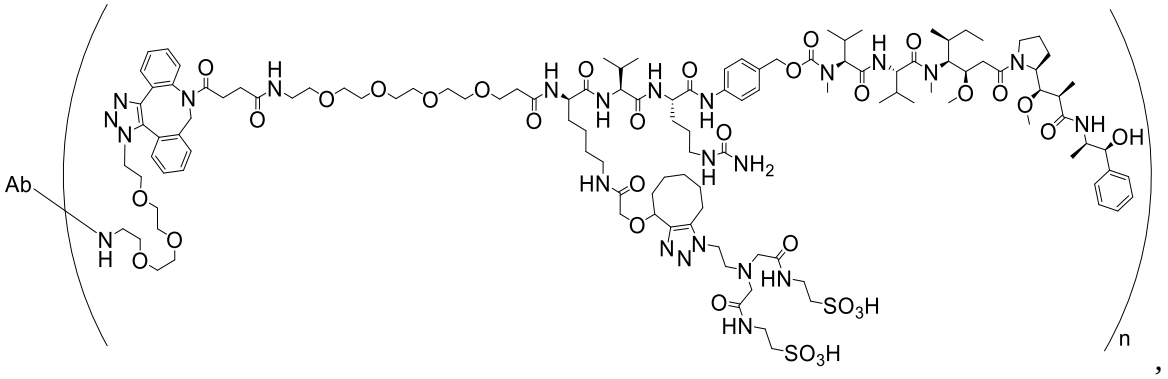

(**Ab-1**)

wherein the  indicates the atom through which **Ab-1** is bonded to the adjacent groups in the formula.

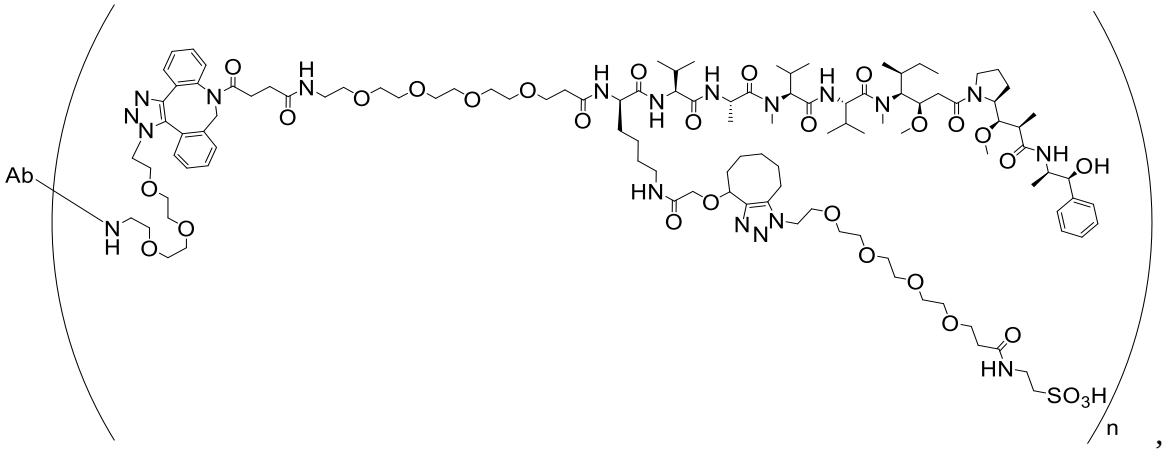
24. The compound of claim 1, selected from the group consisting of:



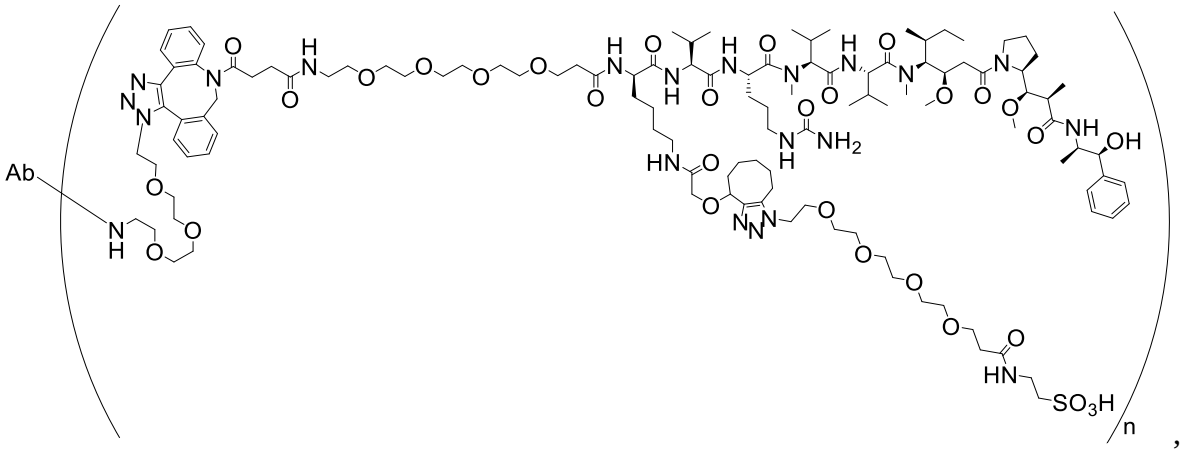
,



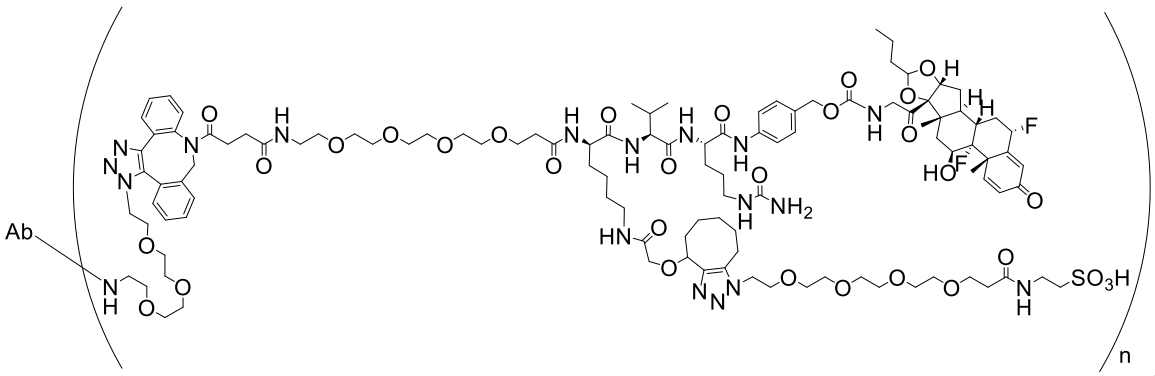
,



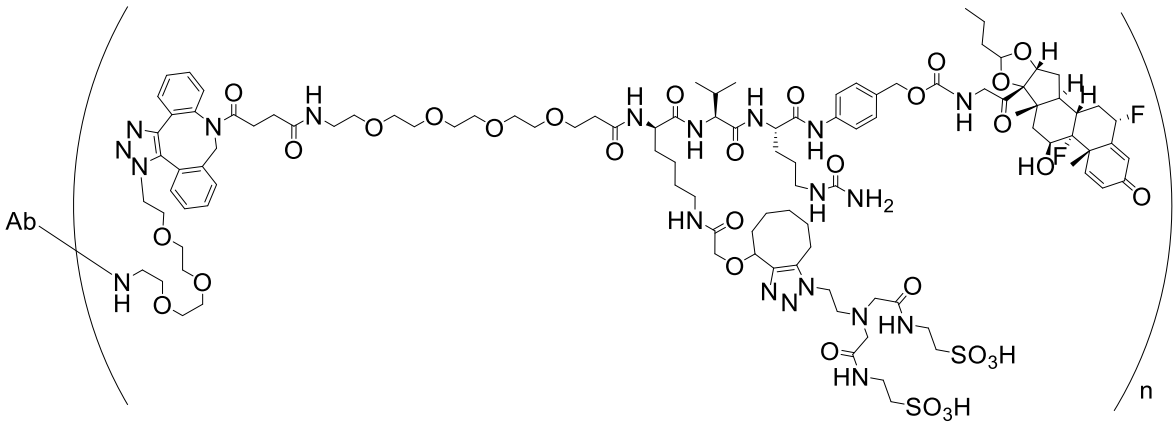
,


Ab
SO3H
n

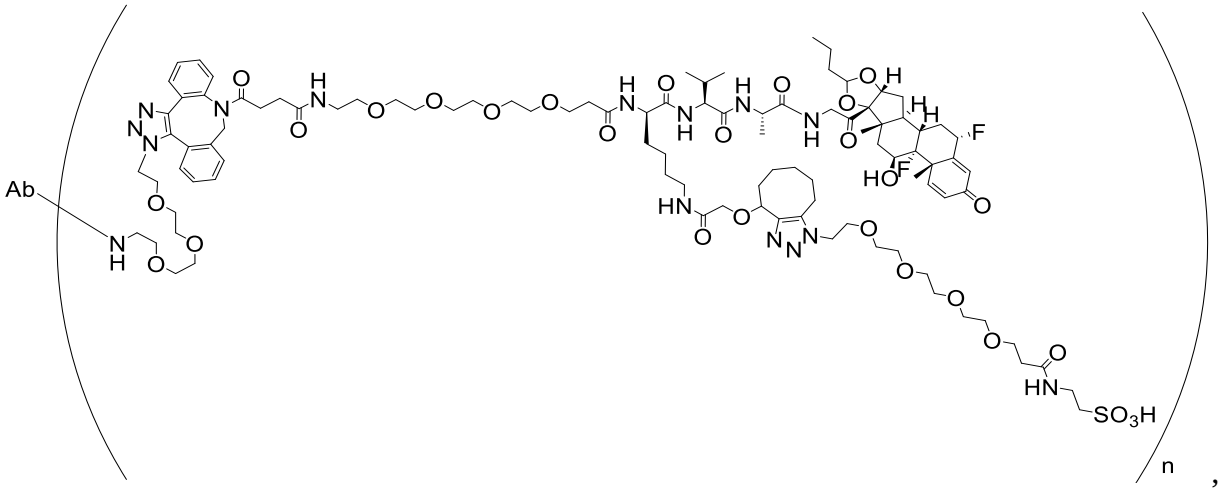
,

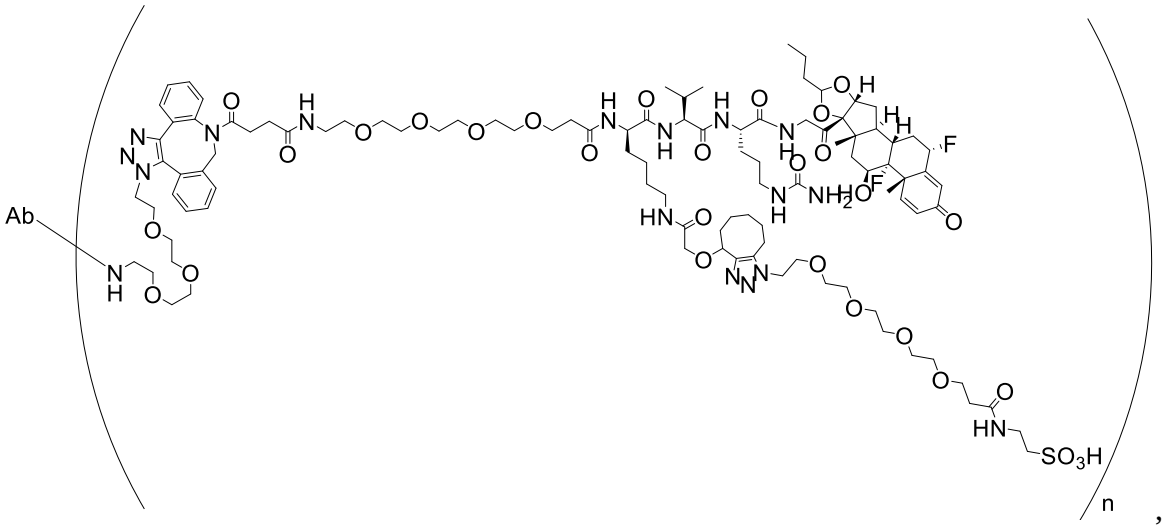

Ab
SO3H
n

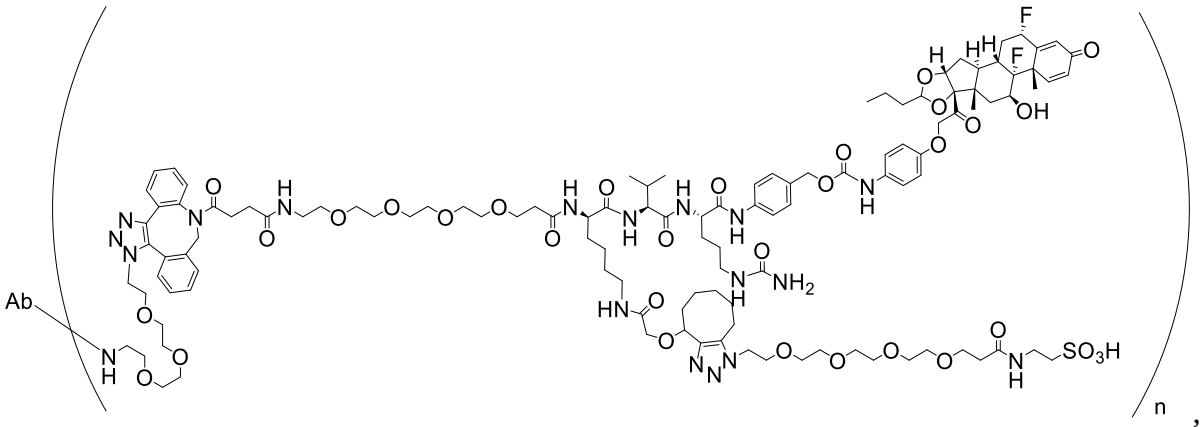
,

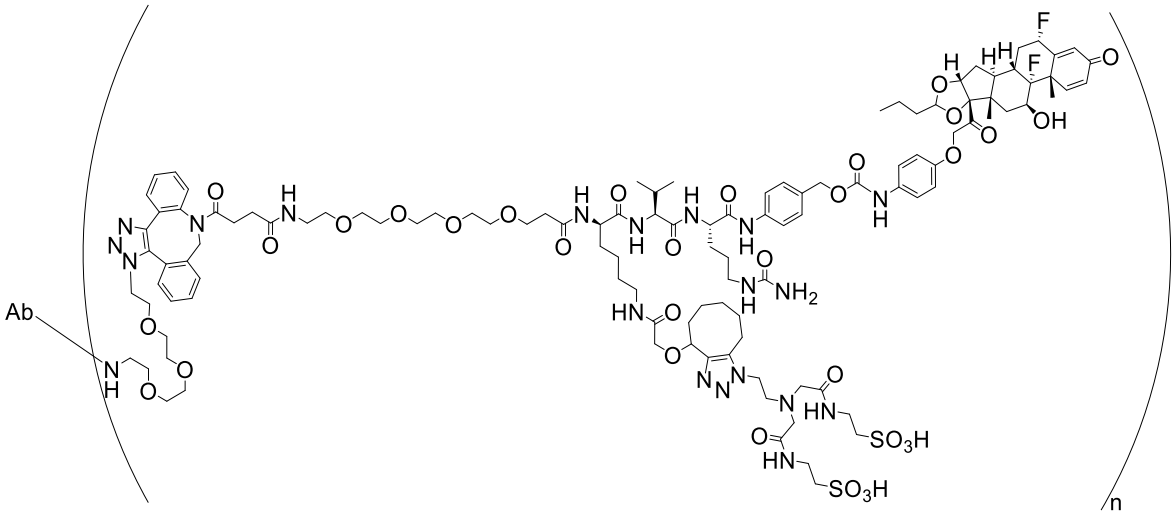

Ab
SO3H
SO3H
n

,

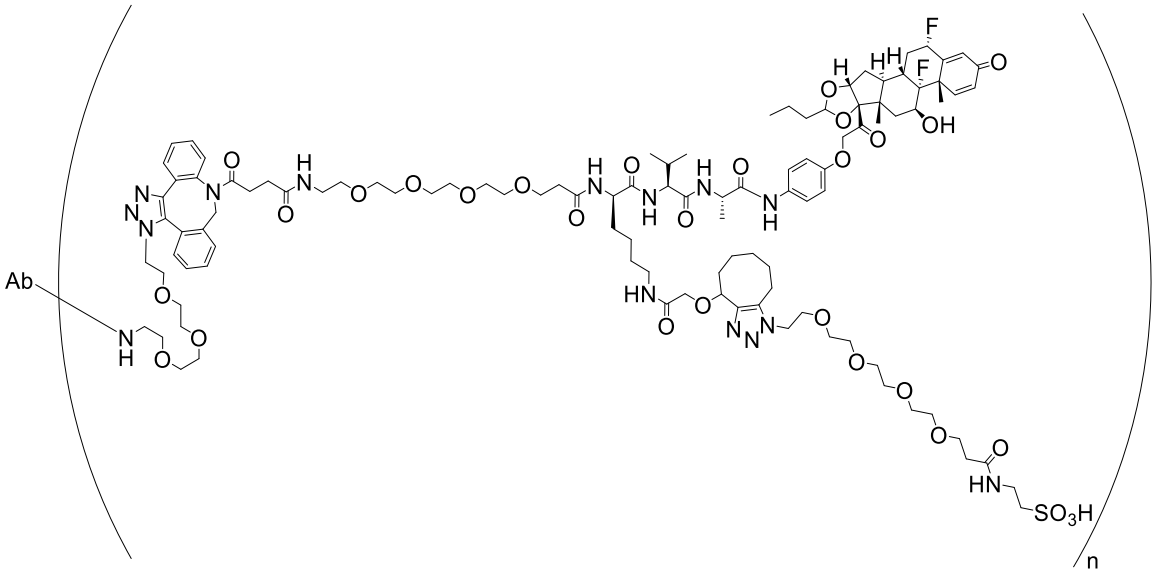




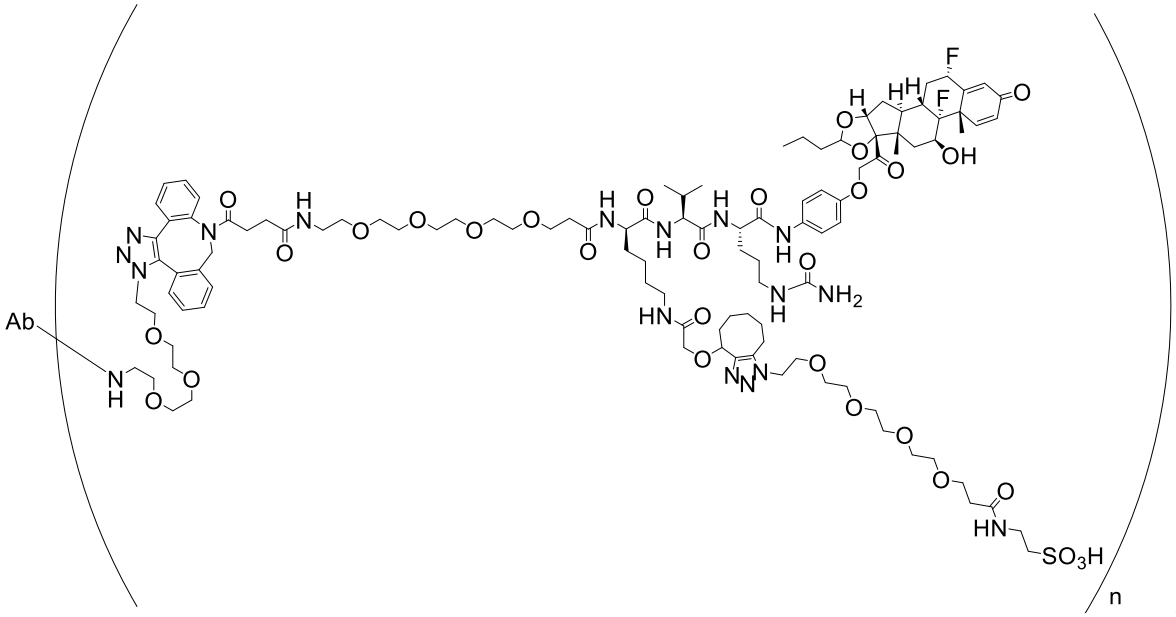





,




, and




,

or a stereoisomeric form thereof, or a triazole linkage regioisomer thereof, or a mixture of triazole linkage regioisomers thereof, wherein

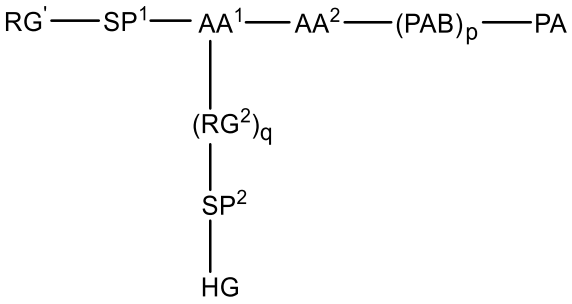
each **Ab** is the antibody, or an antigen-binding fragment thereof; and

subscript **n** is an integer from 1 to 30.

25. A pharmaceutical composition comprising a compound of any one of claims 1-24, or pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

26. A compound of any one of claims 1-24, or a composition of claim 25 for use in treating a disease or disorder in a patient in need thereof.

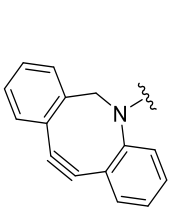
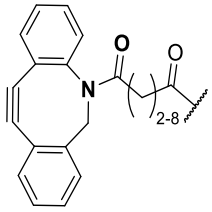
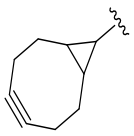
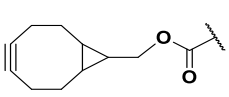
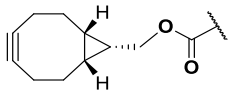
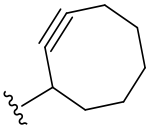
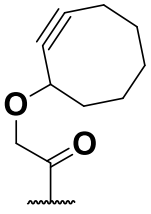
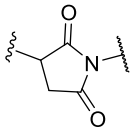
27. A compound or stereoisomeric form, a triazole linkage regioisomer, or mixture of triazole linkage regioisomers thereof, according to Formula (**IX**):

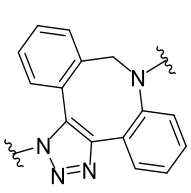
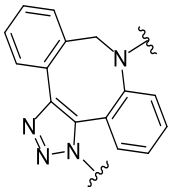
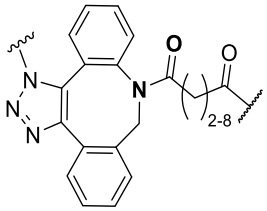
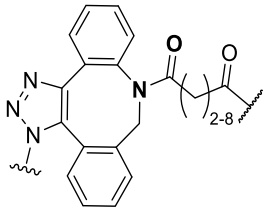
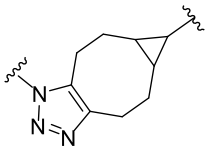
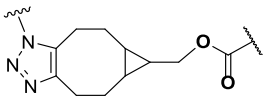
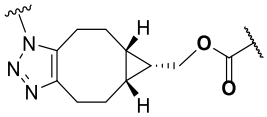
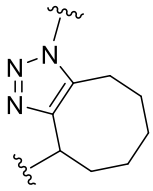
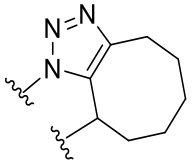
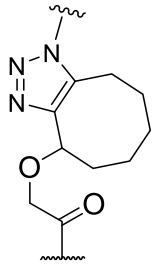
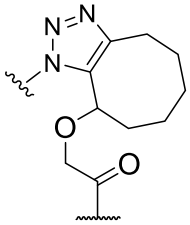
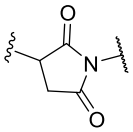



(**IX**)

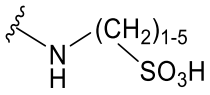
**AA$^1$** is an amino acid residue selected from tryptophan, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, and citrulline;

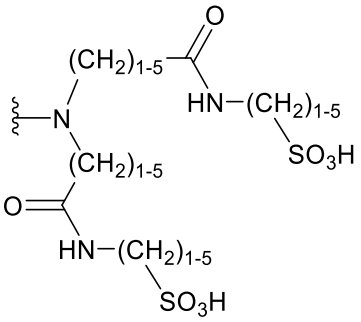
**AA$^2$** is a dipeptide, tripeptide or tetrapeptide residue comprising amino acid residues selected from alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, and citrulline;

**RG′** is a reactive group selected from  ,  ,  ,  ,  ,  ,  , and  , wherein the  indicates the atom through which the **RG′** is bonded to the adjacent groups in the formula;

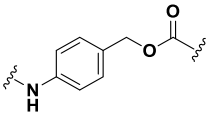
**RG²** is a reactive group residue selected from  ,  ,  ,  ,  ,  ,  ,  ,  ,  ,  , and  , wherein the  indicates the atom through which the $RG^1$ or $RG^2$ is bonded to the adjacent groups in the formula;

**$SP^1$** and **$SP^2$** are independently, in each instance, absent, or a spacer group residue selected from the group consisting of $C_{1\text{-}6}$alkylene, -NH-, -C(O)-, -$(CH_2$-$CH_2$-$O)_e$, -NH-$(CH_2$-$CH_2$-$O)_e$-$CH_2$-$CH_2$-C(O)-, -C(O)-$(CH_2)_u$-C(O)-, -C(O)-NH-$(CH_2)_v$-, and combinations thereof wherein subscript e is an integer from 0 to 6, subscript u is an integer from 1 to 8, and subscript v is an integer from 1 to 8;

**HG** is a hydrophilic residue selected from  and



;

**PAB** is  , wherein the  indicates the atom through which the PAB is bonded to the adjacent groups in the formula;

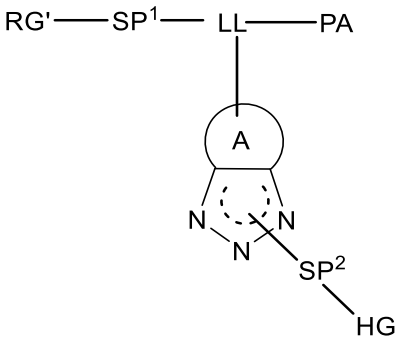
subscript **p** is 0 or 1;

**PA** is a payload residue;

subscript **q** is 1; and

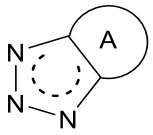
wherein the  indicates the atom through which the **HG** is bonded to the adjacent groups in the formula.

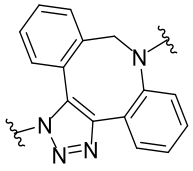
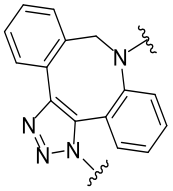
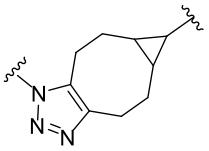
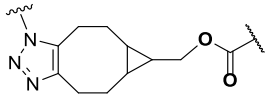
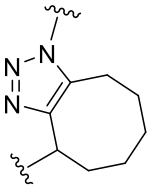
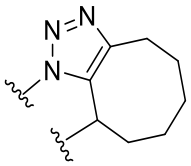
28. The compound of claim 27, according to Formula (**VIII**):



(**VIII**)

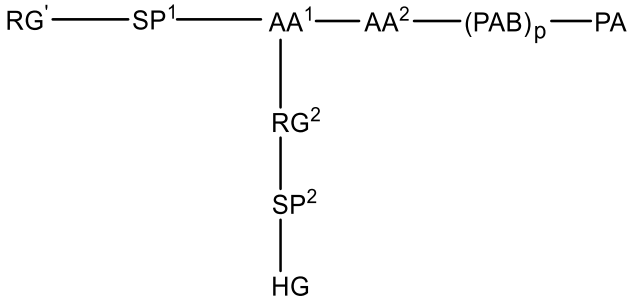
wherein



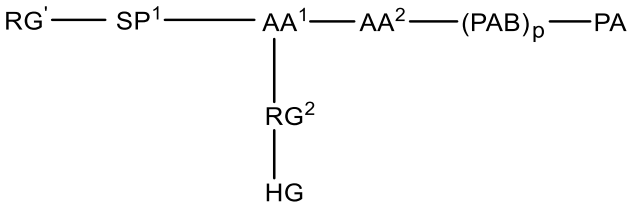
is selected from the groups consisting of  ,  ,  ,  ,  , and  ;

LL is –**AA$^1$-AA$^2$-(PAB)$_p$**.

29. The compound of claim 27, according to Formula (**IXa**) or Formula (**IXb**):



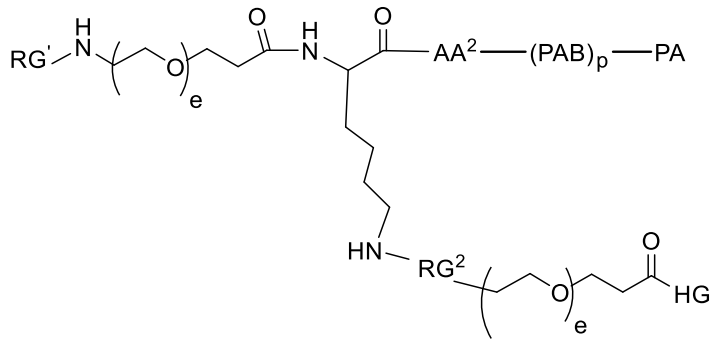
(**IXa**),



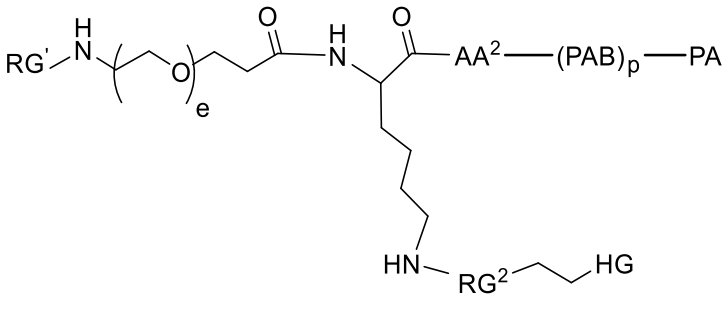
(**IXb**).

30. The compound according to claim 29, wherein **AA$^1$** is a lysine, glutamine, glutamic acid, or aspartic acid residue.

31. The compound of claim 29, according to Formula (**Xa**), (**Xb**) or (**Xd**):

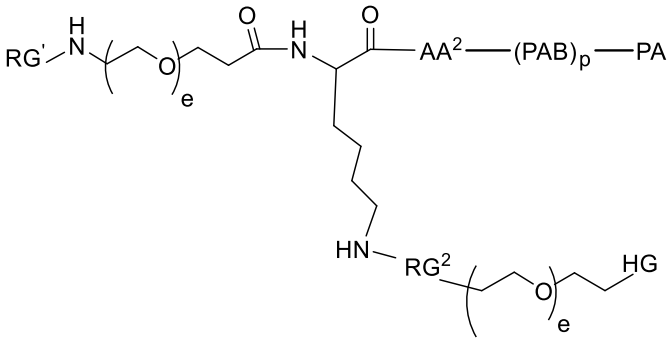


(**Xa**)



(**Xb**)

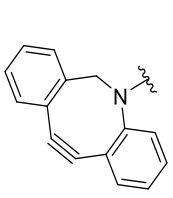
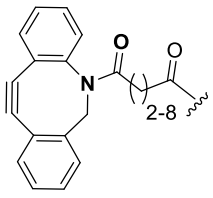
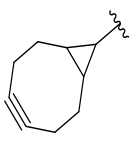
or



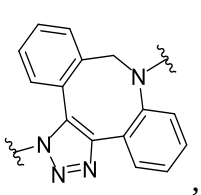
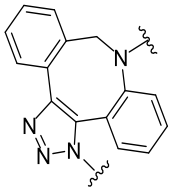
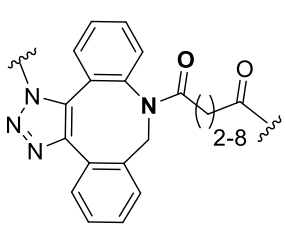
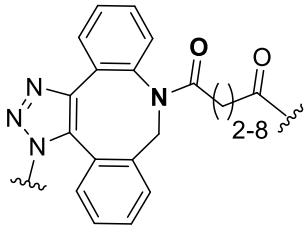
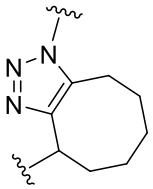
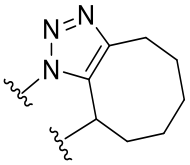
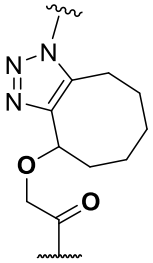
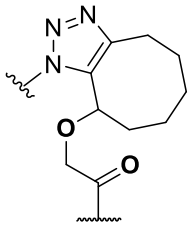
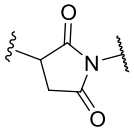
(**Xd**),

wherein:

subscript **e** is independently, in each instance, an integer from 0 to 6.

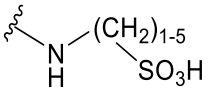
32. The compound of any one of claims 27-31, wherein **RG′** is, independently in each instance, selected from the group consisting of  ,  , and  ,

wherein the  indicates the atom through which the **RG′** is bonded to the adjacent groups in the formula.

33. The compound of any one of claims 27-32, wherein **RG²** is independently, in each instance, selected from the group consisting of  ,  ,  ,  ,  ,  ,  ,  , and  ,

wherein the  indicates the atom through which the **RG²** is bonded to the adjacent groups in the formula.

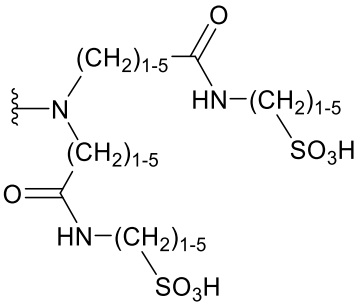
34. The compound of any one of claims 27-33, wherein **HG** is



wherein

the  indicates the atom through which the **HG** is bonded to the adjacent groups in the formula.

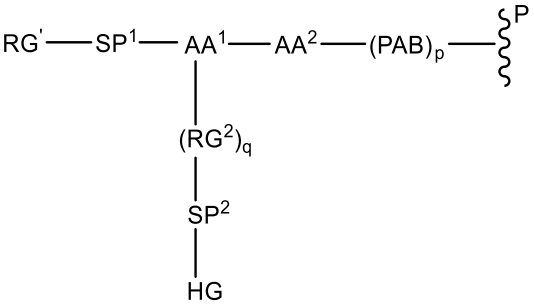
35. The compound of any one of claims 27-34, wherein **HG** is



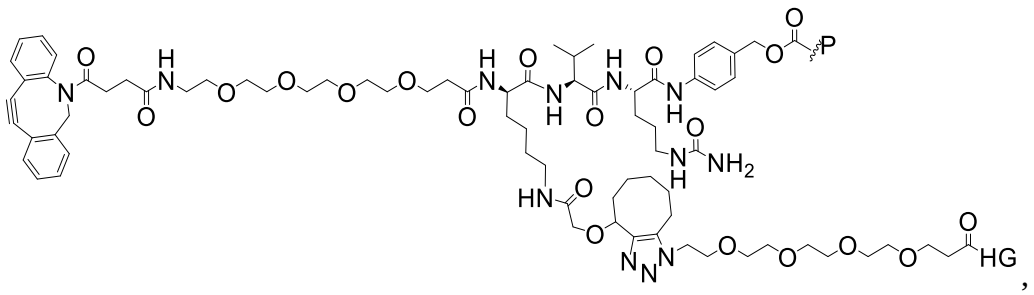
, wherein the  indicates the atom through which the **HG** is bonded to the adjacent groups in the formula.

36. The compound of any one of claims 27-30 and 32-35, wherein **SP[1]** and **SP[2]** are independently, in each instance, absent, or selected from the group consisting of -$(CH_2$-$CH_2$-$O)_e$ and -NH-$CH_2$-$CH_2$-(-O-$CH_2$-$CH_2)_e$-C(O)-.

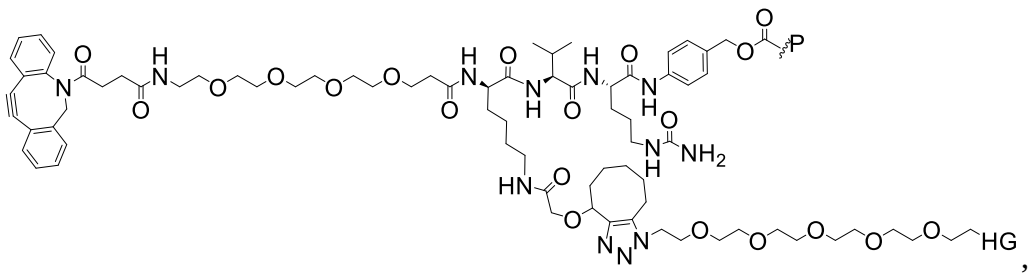
37. The compound of claim 27, wherein



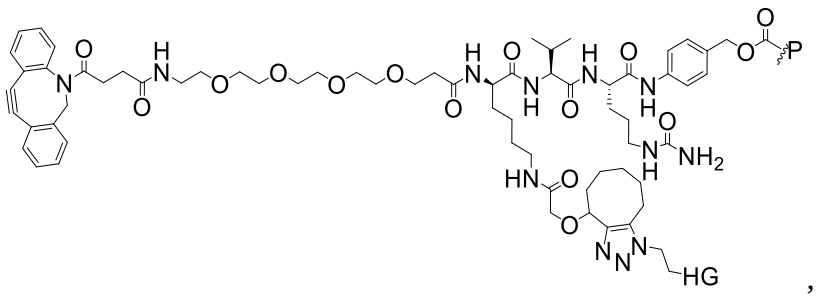
is selected from the group consisting of:

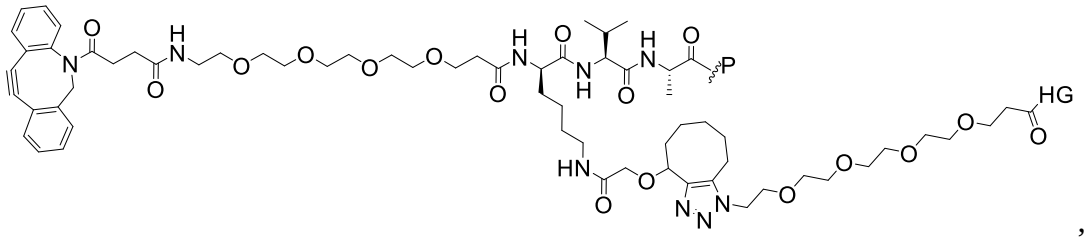


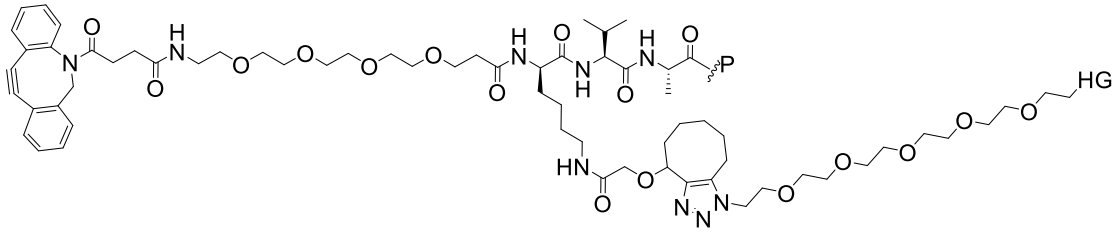
,

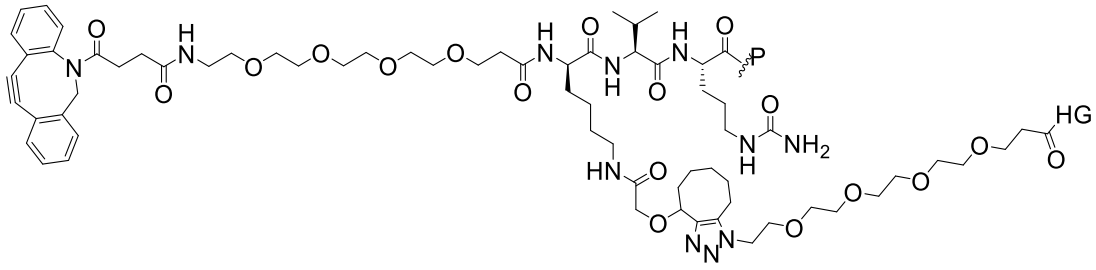


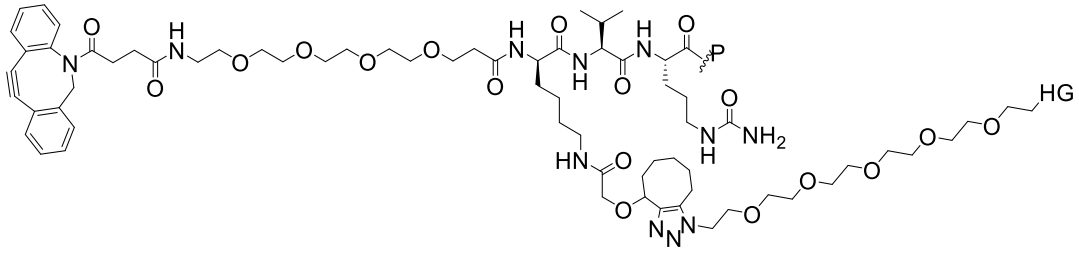
,

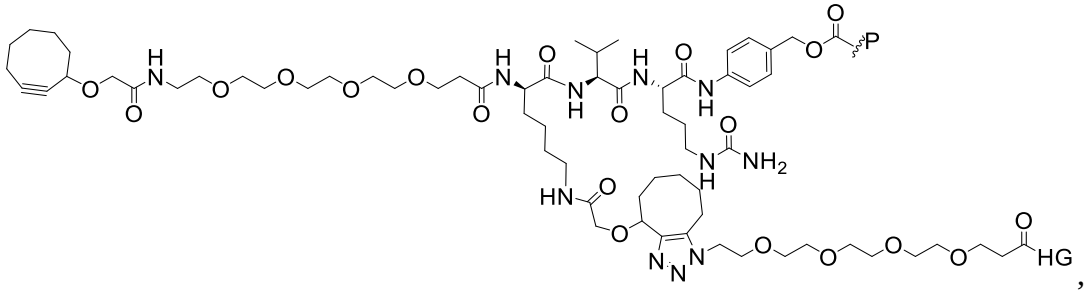

,

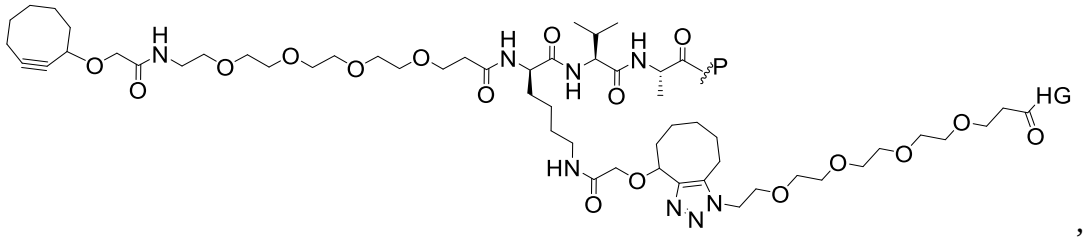

,


,

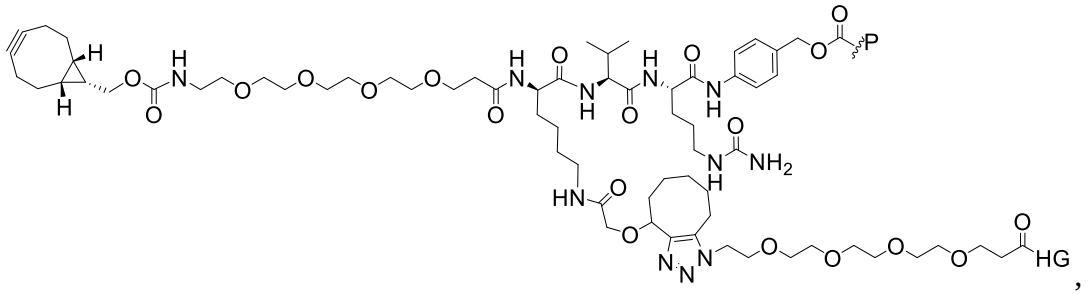

,


,


,

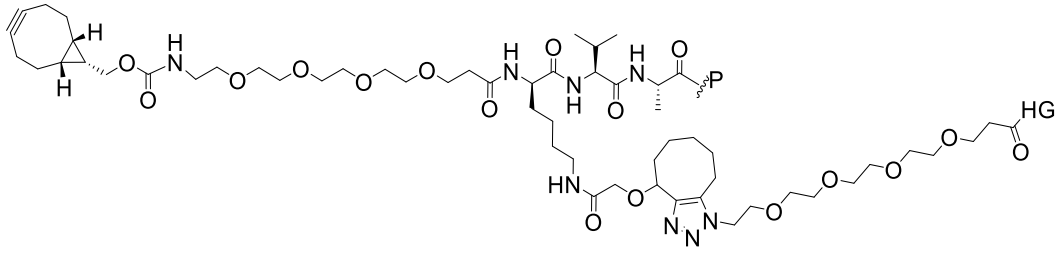


,



,

and

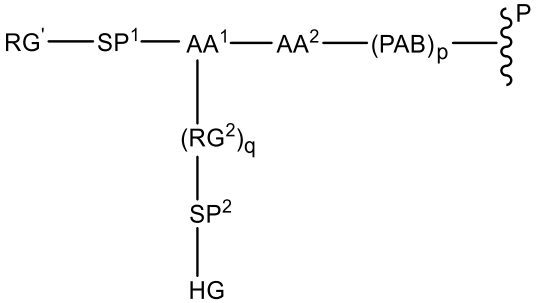


,

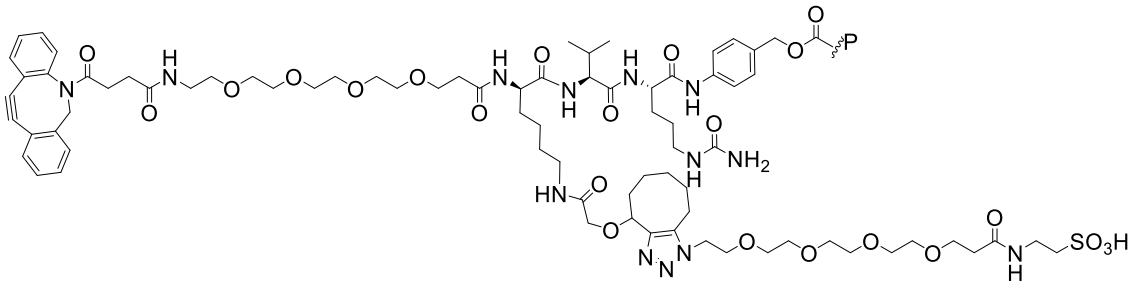
or a stereoisomeric form thereof, or a triazole linkage regioisomer thereof, or a mixture of triazole linkage regioisomers thereof, wherein

each  is a bond to the payload residue.

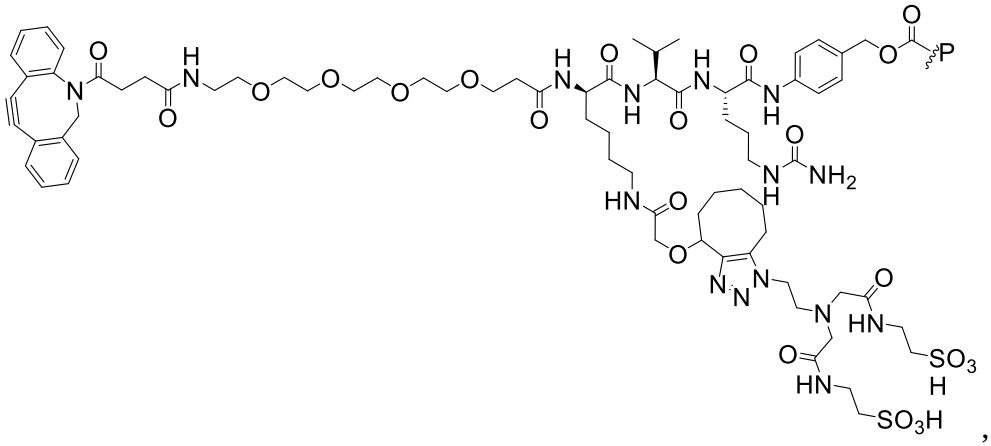
38. The compound of claim 27, wherein



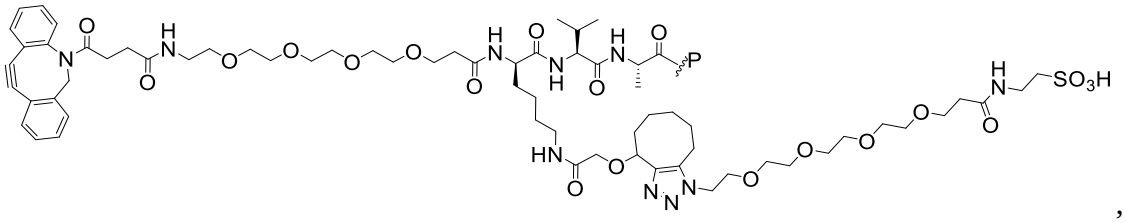
is selected from the group consisting of:



,

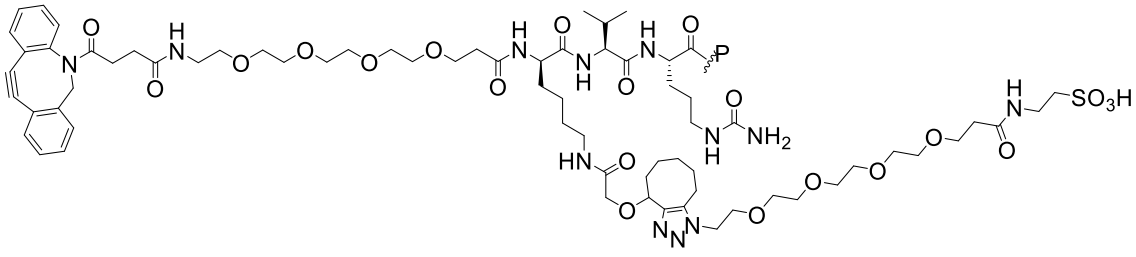


,



,

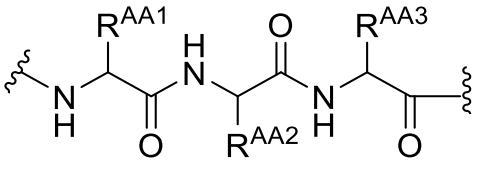
and



or a stereoisomeric form thereof, or a triazole linkage regioisomer thereof, or a mixture of triazole linkage regioisomers thereof, wherein

each  is a bond to the payload residue.

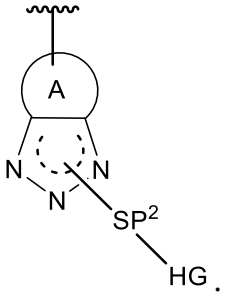
39. The compound of claim 28, wherein **LL** is according to Formula (**LL1**):

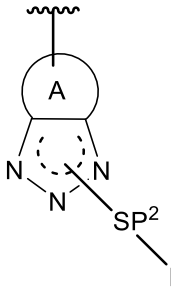


(**LL1**)

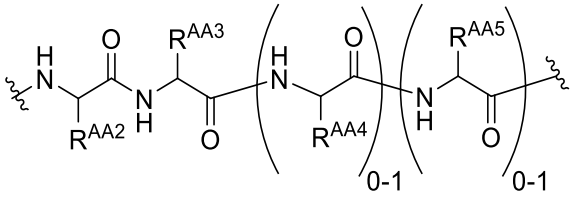
wherein $\mathbf{R^{AA1}}$ is an amino acid sidechain residue selected from tryptophan, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, and citrulline,

$\mathbf{R^{AA2}}$, and $\mathbf{R^{AA3}}$ are each, independently, amino acid side chain residues selected from alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, and citrulline; and

$\mathbf{R^{AA1}}$ is bonded directly to  .

40. The compound of claim 39, wherein $\mathbf{R^{AA1}}$ is a lysine, glutamine, glutamic acid or aspartic acid side chain residue bonded directly to  , and $\mathbf{R^{AA2}}$ and $\mathbf{R^{AA3}}$ are either valine and alanine or valine and citrulline sidechain residues respectively.

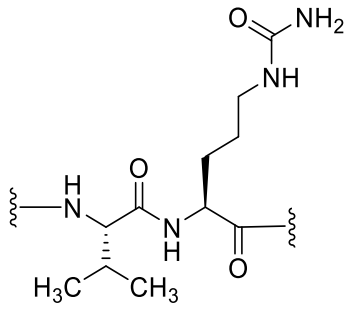
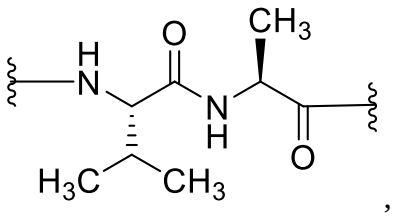
41. The compound of any one of claims 27 and 29-36, wherein $\mathbf{AA^2}$ is



wherein $\mathbf{R^{AA2}}$, $\mathbf{R^{AA3}}$, $\mathbf{R^{AA4}}$, and $\mathbf{R^{AA5}}$ are each, independently, amino acid side chain residues selected from alanine, valine, leucine, isoleucine, methionine, tryptophan, phenylalanine, proline, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, and citrulline,

wherein the  indicates the atom through which $\mathbf{AA^2}$ is bonded to the adjacent groups in the formula.

42. The compound of any one of claims 27 and 29-36, wherein $\mathbf{AA^2}$ is

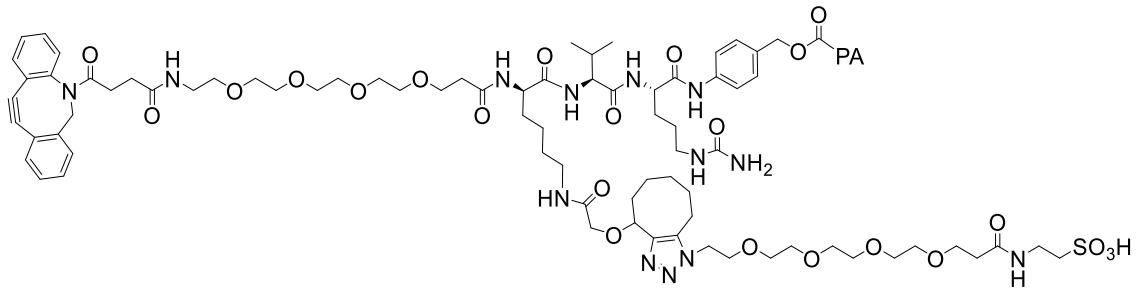
 or  ,

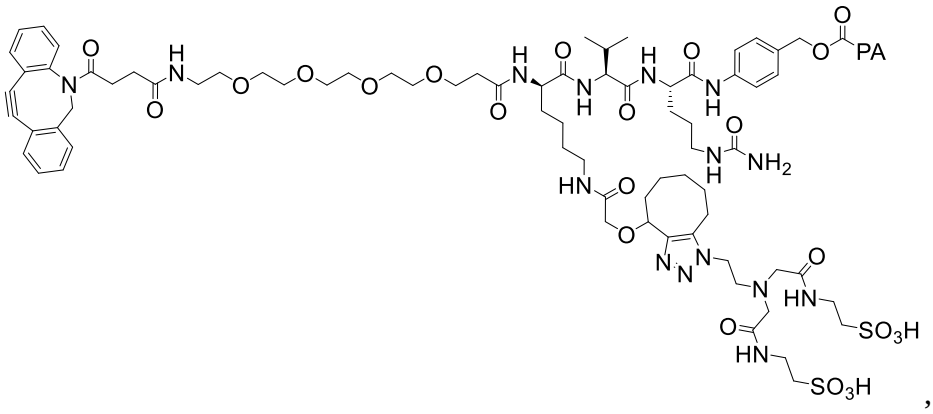
wherein the  indicates the atom through which $\mathbf{AA^2}$ is bonded to the adjacent groups in the formula.

43. The compound of any one of claims 31-36, wherein subscript **e** is 4.

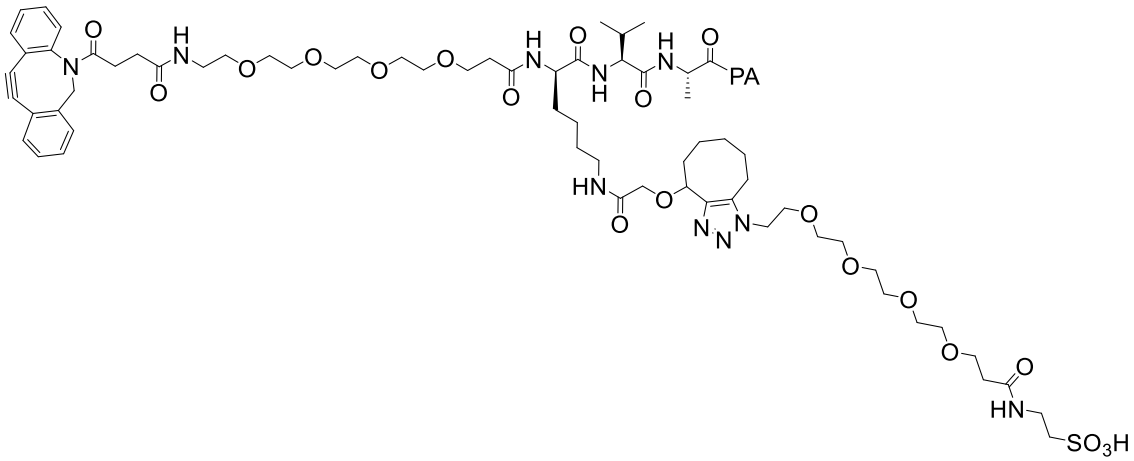
44. The compound of any one of claims 27-43, wherein **PA** is the residue of a group selected from the group consisting of a dolastatin, an auristatin, a maytansinoid, a plant alkaloid, a taxane, a vinca alkaloid, a steroid, and a liver X receptor (LXR) modulator.

45. The compound of claim 27, selected from the group consisting of:

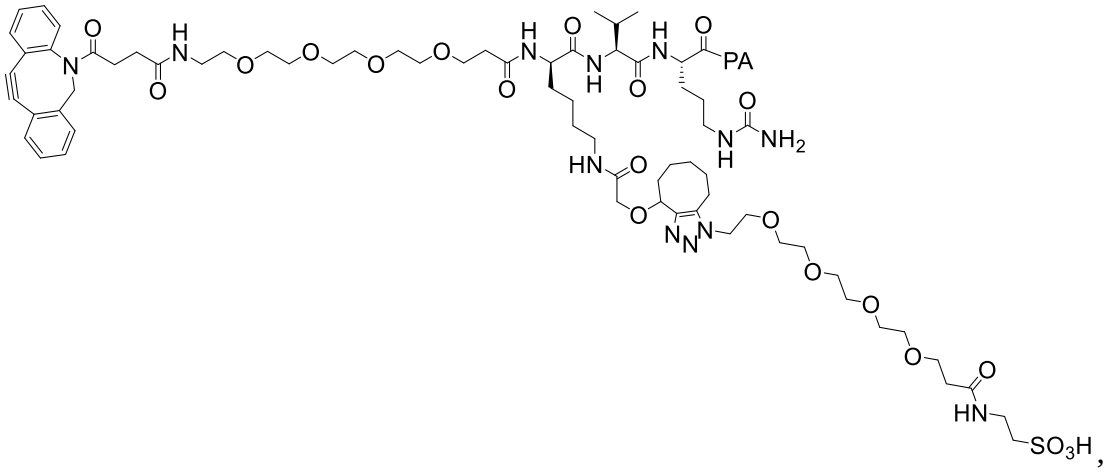




,



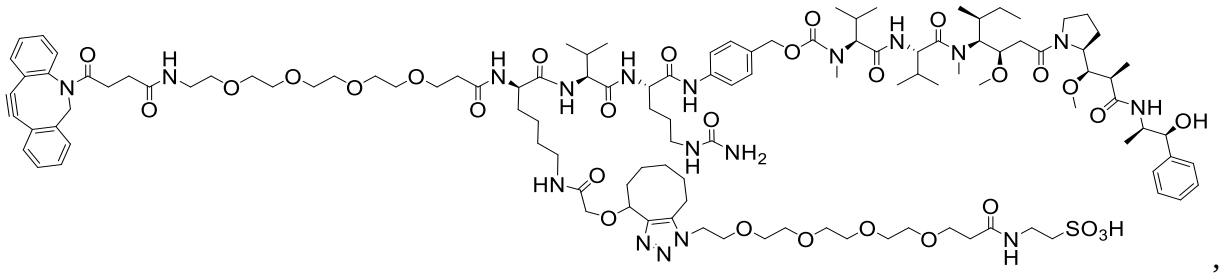
and



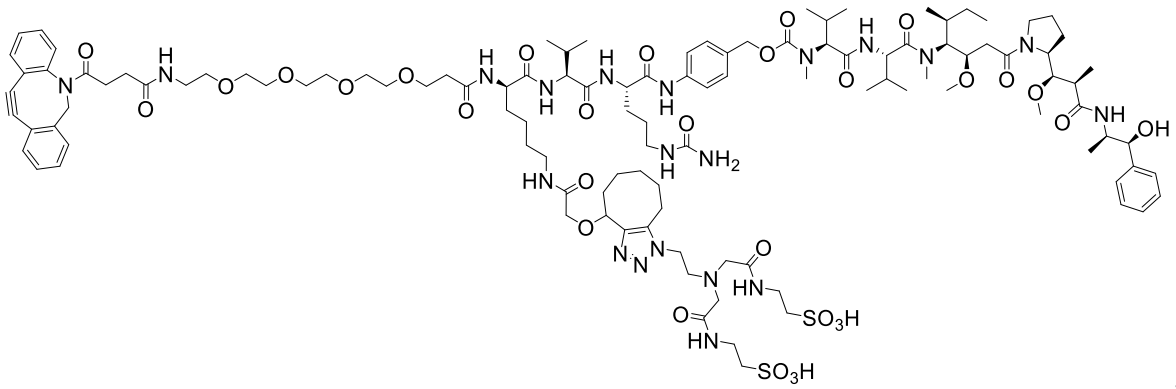
,

or a stereoisomeric form thereof, or a triazole linkage regioisomer thereof, or a mixture of triazole linkage regioisomers thereof, wherein **PA** is a payload residue.

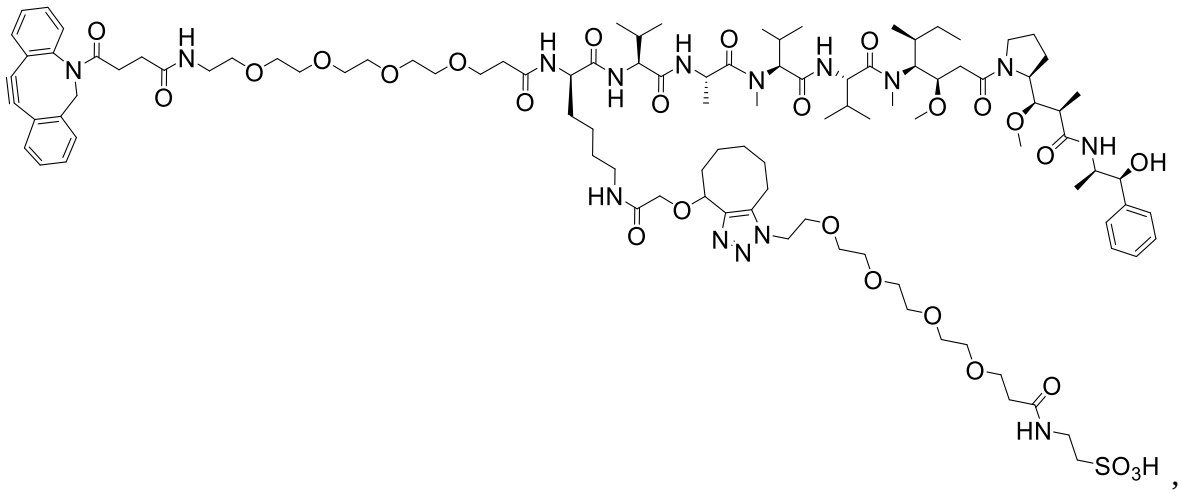
46. The compound of claim 27, selected from the group consisting of:



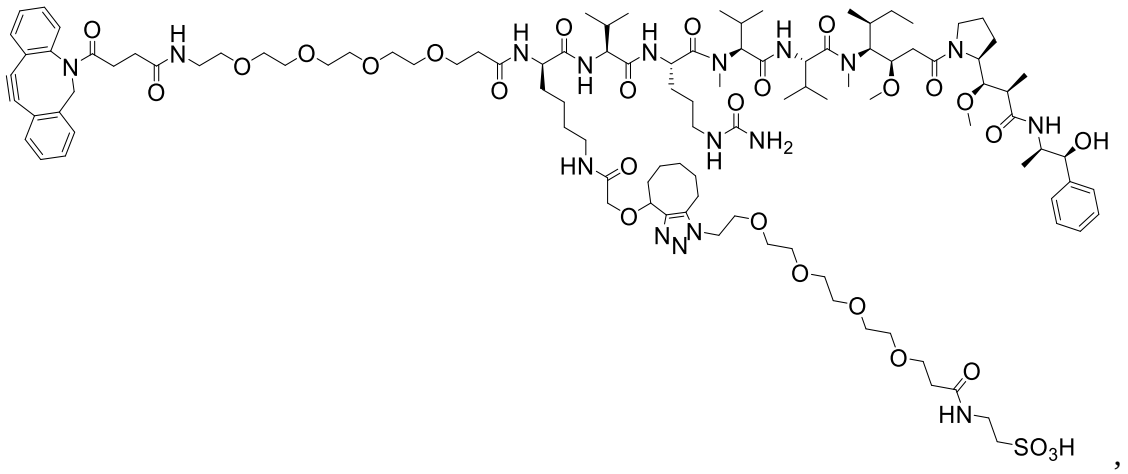
,



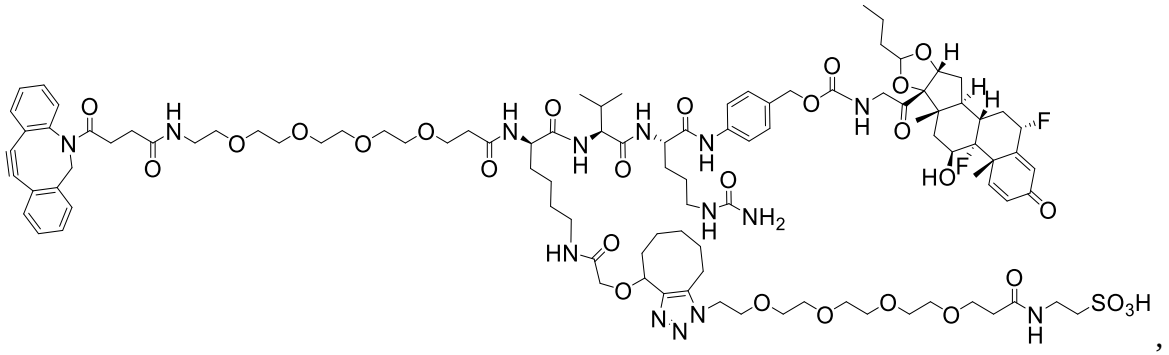
,



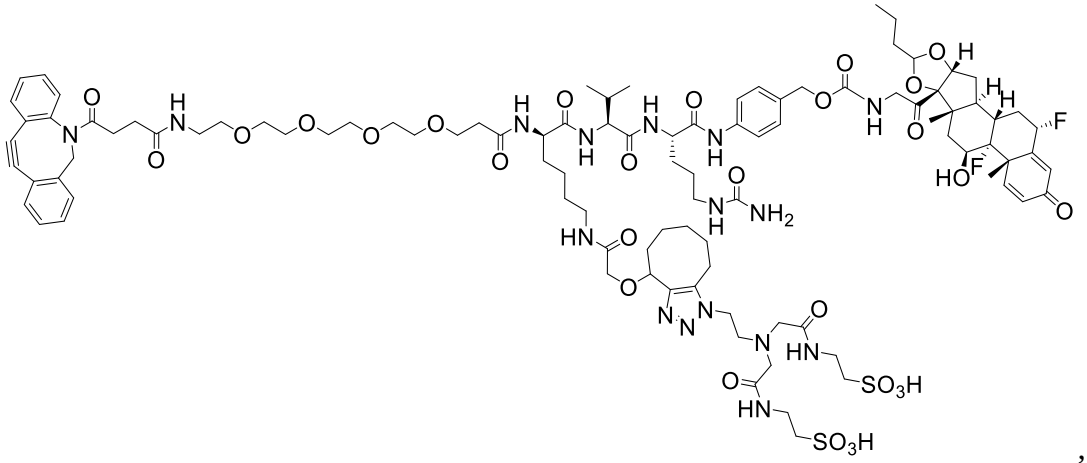
,



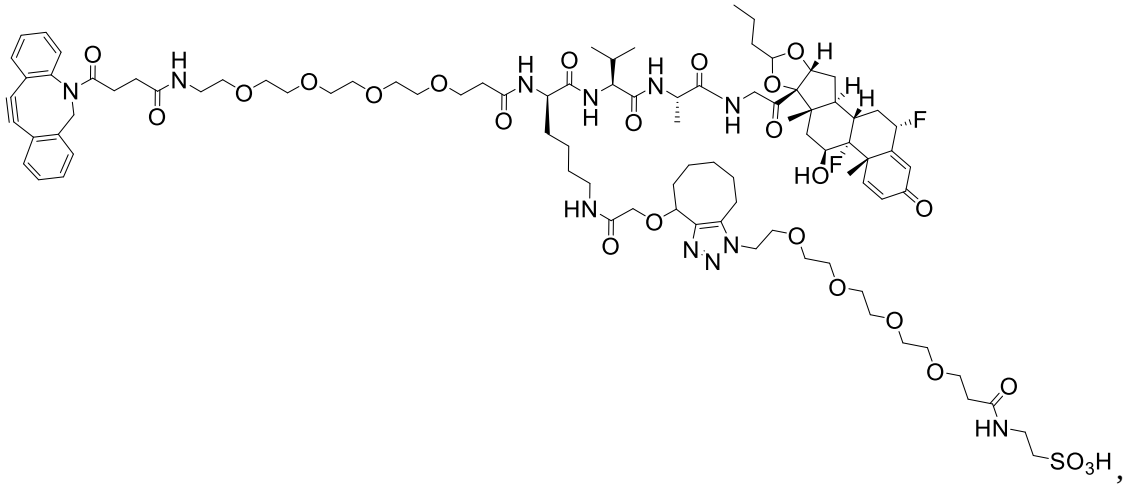
,



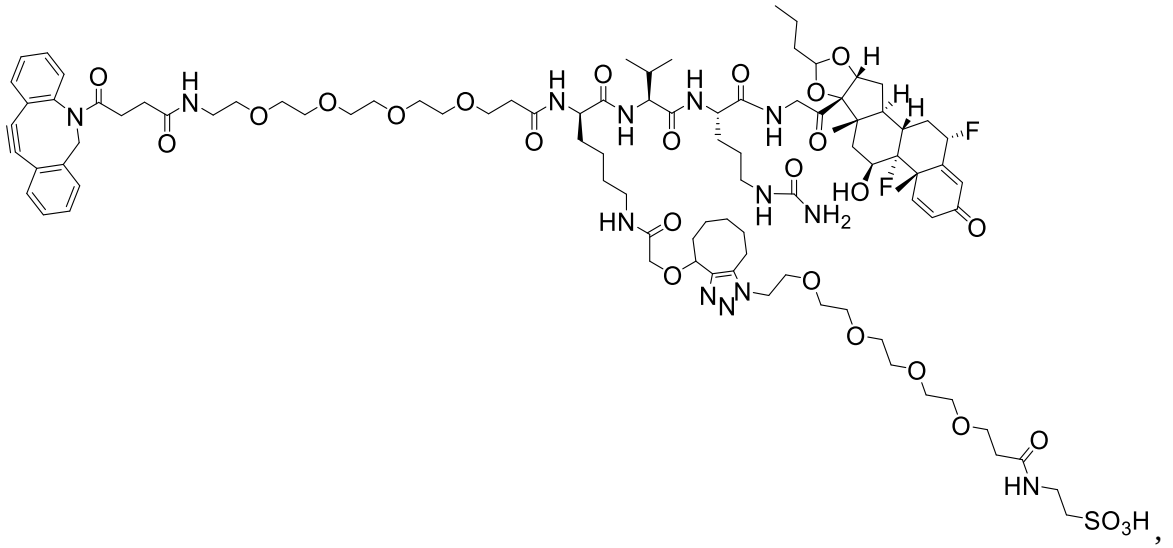
,



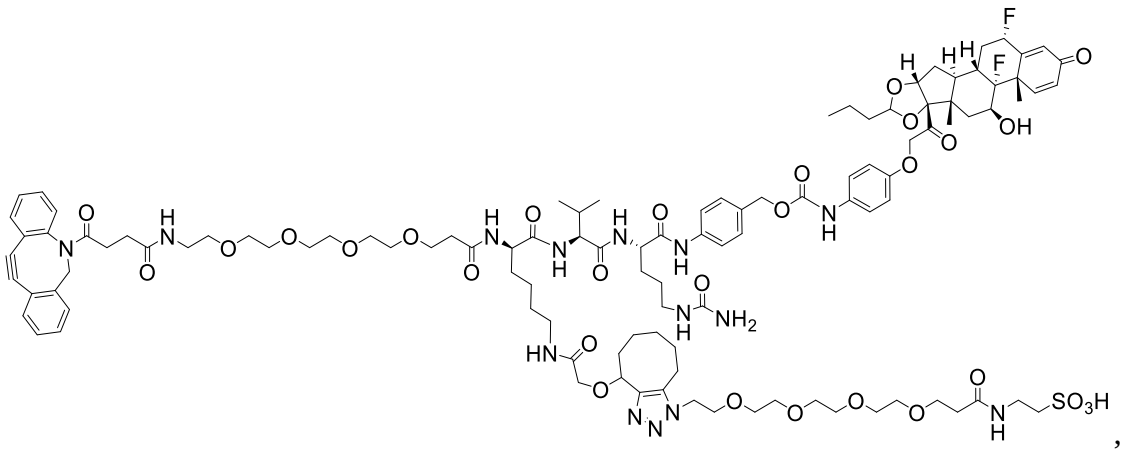
,



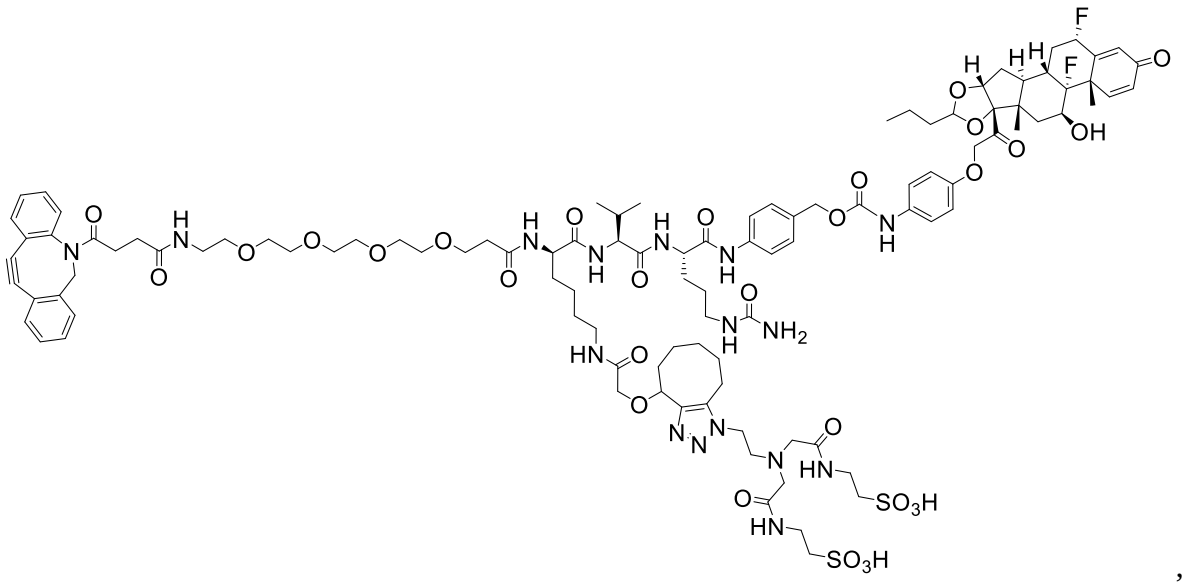
,



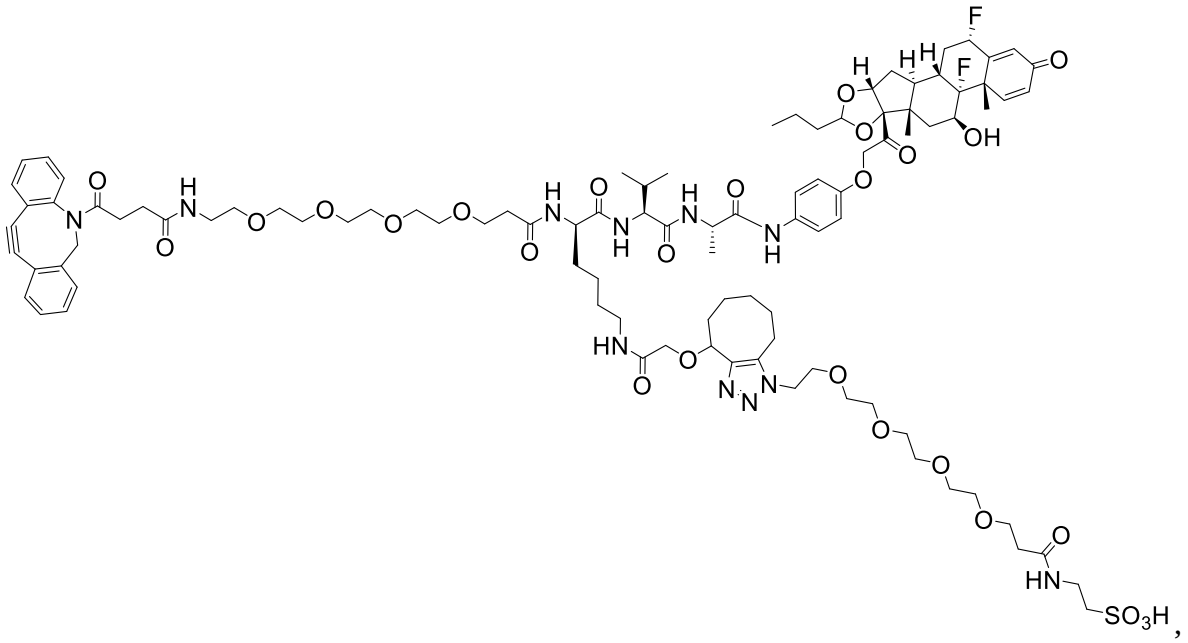
,



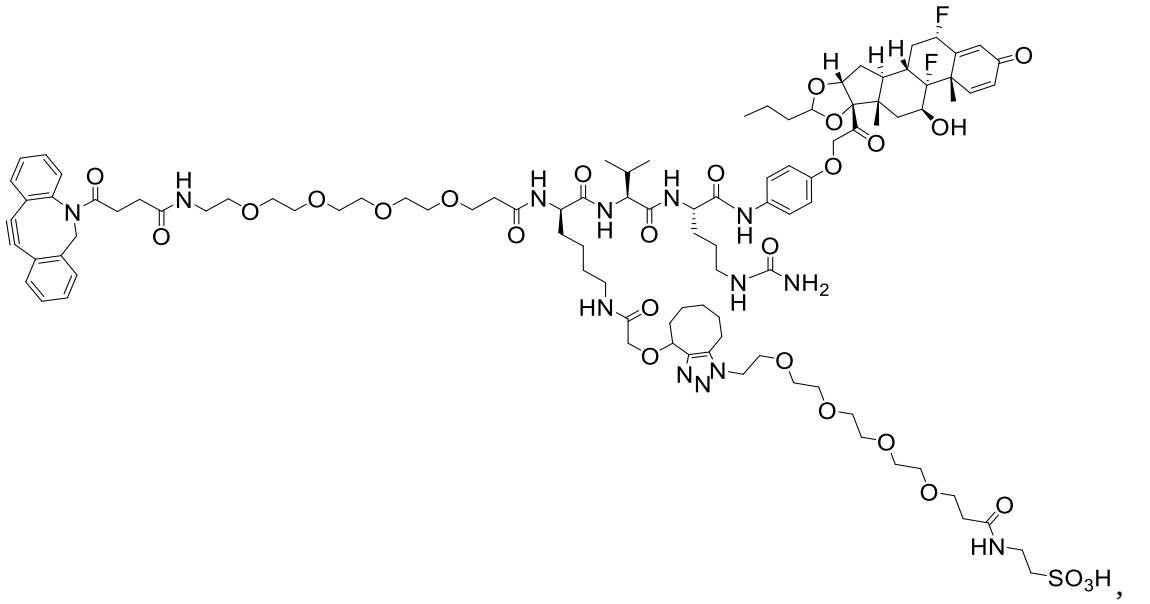
,



,



and



or a stereoisomeric form thereof, or a triazole linkage regioisomer thereof, or a mixture of triazole linkage regioisomers thereof.

47. A method of preparing a compound of claim 1 comprising the step of contacting an antibody or antigen binding fragment thereof with a compound according to any of claims 27-46, under conditions suitable for forming a bond between the antibody or antigen binding fragment thereof and the compound.

48. The method of claim 47, wherein the antibody or antigen binding fragment thereof is a modified antibody or antigen binding fragment thereof comprising an azido group ($\sim N_3$)

wherein the  indicates the atom through which the azido group is bonded to the adjacent groups in the formula.

49. The compound of claim 28 wherein LL is bonded to an oxygen or a primary or secondary nitrogen of the payload.

50. An antibody-drug-conjugate comprising the compound of any of claims 27-46 bonded to an antibody, or an antigen binding fragment thereof.

51. A compound of any one of claims 1-24 or a pharmaceutical composition of claim 25 for use in treating a proliferative disease, a metabolic disease, inflammation, or a neurodegenerative disease in a subject.

52. A compound of any one of claims 1-24 or a pharmaceutical composition of claim 25 for use in the treatment of a proliferative disease in a subject.

53. A compound of any one of claims 1-24 or a pharmaceutical composition of claim 25 for use in the treatment of a metabolic disease in a subject.

54. A compound of any one of claims 1-24 or a pharmaceutical composition of claim 25 for use in the treatment of inflammation in a subject.

55. A compound of any one of claims 1-24 or a pharmaceutical composition of claim 25 for use in the treatment of a neurodegenerative disease in a subject.

56. Use of a compound of any one of claims 1-24 or a pharmaceutical composition of claim 25 in the manufacture of a medicament for treating a proliferative disease, a metabolic disease, inflammation, or a neurodegenerative disease in a subject.