

CONNECTING UNITS FOR LIGAND-DRUG CONJUGATES AND METHODS OF MAKING AND USING THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the filing date of CN202410042053.3, filed January 10, 2024, and CN202510022398.7, filed January 07, 2025, the entire disclosures of which are incorporated by reference herein.

TECHNICAL FIELD

10 The disclosure relates to the field of pharmaceutical technology, in particular to a self-stabilizing bridged ring connecting unit and an application thereof in an antibody-drug conjugate, and specifically, to small molecule linkers of ligand-drug conjugates, such as antibody-drug conjugates, and methods for preparing and using ADCs. The disclosure also relates to linker-drug compounds and antibody-linker-drug conjugates in treatment of oncological, infectious and immune diseases.

BACKGROUND

Unless otherwise indicated herein, the materials described in this section are not prior art to the claims in this application and are not admitted being prior art by inclusion in this section.

20 Ligand-drug conjugates are a class of biopharmaceuticals that connect ligands with targeted delivery function to biologically active drugs through stable connecting units, making full use of the high specificity of ligand binding to the target of interest and the high lethality of the drug, and at the same time effectively circumventing the drawbacks such as the drug's indiscriminate attack on normal tissues. A ligand may be a small molecule agonist or antagonist or an antibody or its binding fragment that binds to a receptor on the surface of cells.

30 Antibody-Drug Conjugates (ADCs) are a new type of targeted therapy that combine the advantages of high selectivity of antibodies and high activity of cytotoxic drugs. With their "high efficiency and low toxicity" characteristics, ADCs have become a research hotspot in the field of targeted cancer therapy. In recent years, the rapid development has led to the three generations of ADCs. Currently, 14 ADC drugs have been approved for marketing, including Gemtuzumab ozogamicin (Mylotarg®, Pfizer), Brentuximab vedotin (Adcetris®, Seagen and Millennium), Trastuzumab emtansine (Kadcyla®, Genetech), Inotuzumab ozogamicin (Besponsa®, Pfizer), Moxetumomab pasudotox (Lumoxiti®, AstraZeneca AB), Polatuzumab vedotin (Polivy®, Genetech), Trastuzumab deruxtecan (Enhertu®, AstraZeneca and Daiichi Sankyo), Enfortumab vedotin (Padcev®, Astellas), Sacituzumab govitecan (Trodelvy®, Gilead), Belantamab mafodotin (Blenrep®, GSK), Loncastuximab tesirine (Zynlonta®, ADC Therapeutics SA), Disitamab vedotin (Aidixi®, RemeGen), Tisotumab vedotin (Tivdak®, Genmab), and Mirvetuximab soravtansine (Elahere®, AbbVie). More than 200 ADC drug candidates have entered clinical trials. While ADCs are playing an increasingly important role in targeted cancer therapy, the development of ADC drugs still faces many challenges, including but not limited to safety and stability in the blood (i.e., off-target killing of normal cells); reproducibility of homogeneously coupling linkers and drugs to the antibody in each batch; availability of one or more specifically effective tumor-

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specific antigens (e.g., antigens expressed only in tumor cells, not in normal cells); ability to prevent ADC drugs from hydrophobic aggregation that may reduce the efficacy of binding antigen; and ability of selecting a proper preclinical animal model for recapturing the human indications and reducing the risk of development failure.

In particular, the off-target effect is a key factor affecting the safety of ligand-drug conjugates such as ADC (Regul. Toxicol. Pharmacol., 2013, 67, 382-391). The off-target effect is caused by the instability of the linker that links the ligand (e.g., antibody) and the toxin (also referred to as drug unit, drug load, warhead), which could lead to premature release of the active payload in circulation. Therefore, the development of specific and stable linkers is important to improve the half-life of circulating ADCs, reduce the off-target toxicity of ADC drugs, and ensure better pharmacokinetic (PK) properties *in vivo*.

For ADCs, the toxin is often attached to the lysine residues of the antibody or the cysteine residues reduced by interchain disulfide bonds via a difunctionalized linker. However, a large number (>80) of lysine residues are distributed on the surface of the antibody, resulting in poor selectivity during the coupling process and poor uniformity between different batches of ADCs. For example, by reducing the interchain disulfide bond, eight sulfhydryl groups may be available for coupling, resulting in non-homogeneously coupled product with a drug-antibody-ratio (DAR) varying from 0, 2, 4, to 8. To improve targeted coupling, the antibody can be generated and modified using genetic engineering and recombination techniques. But the challenges in synthesis, preparation and process of ADCs remain. Third generation of coupling techniques focus on specific sites of amino acids and utilize specific molecules and reagents, such as mono- and bis-sulfone reagents, 3, 4-disubstituted maleimides, divinyl pyrimidines, dibromo-pyridazine diones, specific functional structural domains or reactive motifs, such as glycosylated coupling site N297, glycan analogs with bioorthogonal motifs. With targeted couplings via proximity effects to improve values of homogeneous DAR, there comes new challenges and shortcomings, such as effective differentiation between disulfides and thiols of selected reagents in the coupling reaction, for example, water solubility of the selected reagents, stability of the coupled compounds, the precise control of reaction conditions, amplification and quality control of the reaction, immune reaction to genetically modified and/or glycosylated antibodies, and the stability of such antibodies *in vivo*, etc. (*RSC Adv.*, **2017**, 7, 24828-24832; *Bioconjugate. Chem.*, **2021**, 32(9), 1947-1959; *Nat Commun.*, **2015**, 6, 6645- 6653).

Maleimide can react with the thiol group of the antibody under mild conditions to produce a thioether product by Michael's addition reaction. Studies have shown that the addition process is reversible in plasma, and the addition product exchanges with the free sulfhydryl groups of the plasma protein, resulting in drug shedding, toxic side effects, and reduced efficacy of the antibody-drug conjugate (*Nature. Biotech.* **2012**, 30, 184-189; *Bioconj. Chem.* **2011**, 22, 1946-953; *Bioconjugate*, 1946-953; *Bioconjugate. Chem.*, **2008**, 19, 759-765). To solve this problem, WO2016025752 discloses a method of introducing a strong electron-withdrawing group outside the succinimide ring. This method uses the electron-withdrawing effect to reduce the reactivity of the thioether addition product with albumin, and yet the entire succinimide and drug linker are

difficult to preserve under natural conditions due to the presence of the electron-withdrawing group. As the succinimide with a strong electron-withdrawing group is easily hydrolyzed to open the ring, the hydrolysis product can no longer further be hydrolyzed and unable to add the thiol group.

WO2018185526 discloses a coupling method using a pair of thiol groups generated by reducing the interchain disulfide bonds of natural antibodies to bridge the bismaleimide linker. The bridging method can achieve the site-specific coupling of the interchain disulfide bonds of natural antibodies after reduction, solve the problem of uniformity in the preparation of ligand-drug conjugates, and alleviate the off-target toxicity of ligand-drug conjugates to a certain extent. However, it is difficult to avoid the off-target toxicity caused by the reverse Michael's addition reaction of the maleimide linker with the free thiol groups of proteins in plasma during the circulation of the ligand-drug conjugate in the body, and its safety is difficult to be guaranteed. In addition, the simple introduction of double linkers has not effectively solved the hydrophobic aggregation problem of the ligand-drug conjugates present in the current technology.

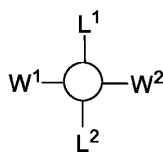
Thus, there is a need for stable linker structures for use in generating linker-drug compounds and ligand-drug conjugates that would overcome significant technical problems including those noted above. The technical solutions provided in this disclosure provides significant advantages over the existing technology and addressed this need.

SUMMARY

The following summary is illustrative only and is not intended to be in any way limiting. In addition to the illustrative aspects, embodiments, and features described above, further aspects, embodiments, and features will become apparent by reference to the drawings and the following detailed description.

To overcome the above-mentioned defects in the preparation of ligand-drug conjugates in the prior art, the application discloses, among others, compositions and methods for the site-specific coupling between ligand and drug that reduce hydrophobic aggregation and improve the uniformity of the ligand-drug conjugate, which in turn, provides significant advantages such as, without limitation, stabilizing conjugates' circulation *in vivo*, reduce off-target toxicity, and ensure better pharmacokinetics.

In one aspect, the application discloses a linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvate thereof, said linker-drug compound being formed by linking together a connector, a linker and a drug unit including a drug and/or a drug precursor. In one embodiment, said linker-drug compound is formed by linking together a connector, a linker and a drug. In one embodiment, said linker-drug compound is formed by linking together a connector, linker and drug precursor. In one embodiment, said connector may be a compound as shown in Formula I, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvate thereof, t



Formula I

wherein,

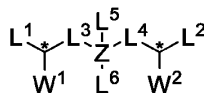
L^1 and L^2 are the same or different, each independently selected from, without limitation, hydrogen, deuterium, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10 heterocyclic, carboxylic acid, amide, ester, sulfite, sulfonate, phosphoric acid, pyrophosphoric acid, natural or unnatural amino acid residues, polyethylene glycol, or a combination thereof;

W^1 and W^2 are the same or different, each independently selected from, without limitation, a connecting group. In one embodiment, at least one of which is a group comprising a maleimide structure;

L^1 and L^2 and/or W^1 and W^2 are covalently linked to the circle, and the circle represents a structure selected from scaffolds comprising basic groups.

The disclosure further provides a double joint structure with unique structural characteristics. Specifically, in the double linker of the present disclosure, the existence of a basic group (e.g., Z in Formula II) can promote the self-opening of succinimide structure (also called maleimide structure) in the linking group (e.g., W^1 or W^2 in Formula II), thereby avoiding the occurrence of reverse-Michael addition reaction, and further inhibiting the shedding of Linker-Drug in the prepared ligand-drug conjugate, thereby the overall stability and hydrophilicity of the prepared ligand-drug conjugate are enhanced..

In some embodiments, said connector is shown in Formula II:



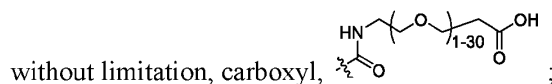
Formula II

wherein,

the carbon atom at the position shown* is a chiral carbon, selected from the *R* absolute configuration or the *S* absolute configuration;

L^1 and L^2 are the same or different, each independently selected from, without limitation, hydrogen, deuterium, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10 heterocyclic, carboxylic acid, amide, ester, sulfite, sulfonate, phosphoric acid, pyrophosphoric acid, natural or unnatural amino acid residues, polyethylene glycol, or a combination thereof;

In one embodiment, L^1 and L^2 are the same or different, each independently selected from,



without limitation, carboxyl, ;

In one embodiment, L^1 and L^2 are the same or different, each independently selected from,

without limitation, the carboxyl group,  ;

L³ and L⁴ are the same or different, each independently selected from, without limitation, the combination of one or more of substituted or unsubstituted C₁-C₆ alkylene, substituted or unsubstituted C₂-C₆ alkenyl, substituted or unsubstituted C₂-C₆ alkynyl, aryl, heteroaryl, C₃-C₁₀ cycloalkyl, 3-10-membered heterocyclic, polyethylene glycol;

In one embodiment, L³ and L⁴ are the same or different, each independently selected from, without limitation, substituted or unsubstituted C₁-C₆ alkylene;

In one embodiment, L³ and L⁴ are the same or different, each independently selected from, 10 without limitation, C₁-C₆ alkylene;

In one embodiment, L³ and L⁴ are the same or different, each independently selected from, without limitation, methylene, ethylene;

L⁵ and L⁶ are each independently present or absent, identical or different when present, selected from, without limitation, hydrogen, deuterium, oxygen, sulfur, hydroxyl, carbonyl, amide, ester, tert-butoxycarbonyl (Boc), benzyloxycarbonyl (Cbz), allyloxycarbonyl (Alloc), benzyl, substituted or unsubstituted C₁-C₆ alkyl, substituted or unsubstituted C₂-C₆ alkenyl, substituted or unsubstituted C₂-C₆ alkynyl, aryl, heteroaryl, C₃-C₁₀ cycloalkyl, 3-10-membered heterocyclic, polyethylene glycol in one or more combinations;

In one embodiment, L⁶ is absent, L⁵ is present, and L⁵ is selected from, without limitation, 20 hydrogen, substituted or unsubstituted C₁-C₆ alkyl;

In one embodiment, L⁶ is absent, L⁵ is present, and L⁵ is selected from, without limitation, hydrogen, C₁-C₆ alkyl;

In one embodiment, L⁶ is absent, L⁵ is present, and L⁵ is hydrogen;

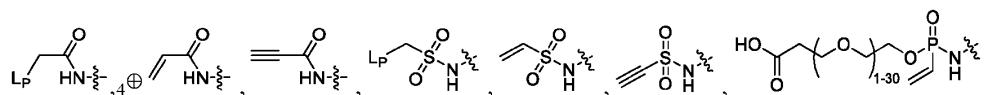
W¹ and W² are the same or different, each independently selected from, without limitation, a connecting group, at least one of which is selected from a connecting group comprising a maleimide structure;

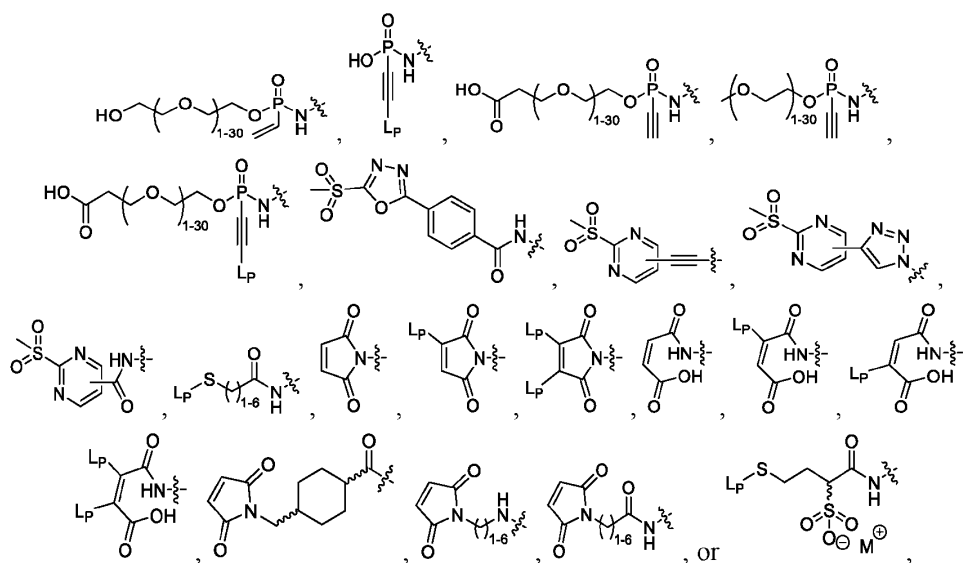
Z is N, O, S or quaternary amines;

In one embodiment, Z is N.

In some embodiments, W^1 and W^2 are the same or different, each independently selected from, without limitation, a group linked to a carboxyl group, an amino group, a carbonyl group, a sulfhydryl group, an azide group, an alkenyl group, a conjugated dienyl group, an alkynyl group, a tetrazine group, and in one embodiment, from a group linked to a sulfhydryl group, wherein at least one of W^1 or W^2 is selected from a connecting group comprising the structure of a maleimide.

In some embodiments, W¹ and W² are the same or different, each independently selected from, without limitation, the following structures or its racemates, enantiomers, diastereomers, pharmaceutically acceptable salts, or solvates thereof:





wherein $M^{\oplus}$ is selected from $H^{\oplus}$, $Li^{\oplus}$, $Na^{\oplus}$, $K^{\oplus}$ or $NH_4^{\oplus}$.

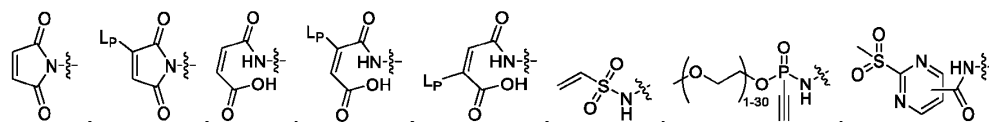
wherein,

the position shown by the wavy line at the end of the structure is the attachment site, and the wavy line in the middle of the structure indicates either of the two chiralities, the *R*-absolute or *S*-absolute configuration;

- 10 In one embodiment, at least one of W^1 or W^2 is selected from a connecting group comprising a maleimide structure;

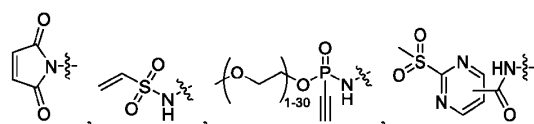
each L_p is selected from the same or different groups including, but not limited to, substituted or unsubstituted C_1 - C_6 alkyl groups, chlorine, bromine, iodine, OMs, OTs;

In one embodiment, W^1 and W^2 are the same or different, each independently selected from, without limitation, the following structures, or its racemates, enantiomers, diastereomers, pharmaceutically acceptable salts or solvates thereof:



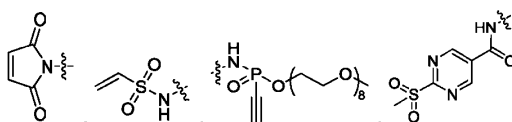
- In one embodiment, at least one of W^1 or W^2 is selected from a connecting group comprising the maleimide structure. In one embodiment, each L_p is selected from the same or a different moiety including, but not limited to, C_1 - C_6 alkyls, chlorine, bromine, iodine, OMs, or OTs;
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In one embodiment, W^1 and W^2 are the same or different, each independently selected from, without limitation, the following structures or its racemates, enantiomers, diastereomers, pharmaceutically acceptable salts or solvates thereof:



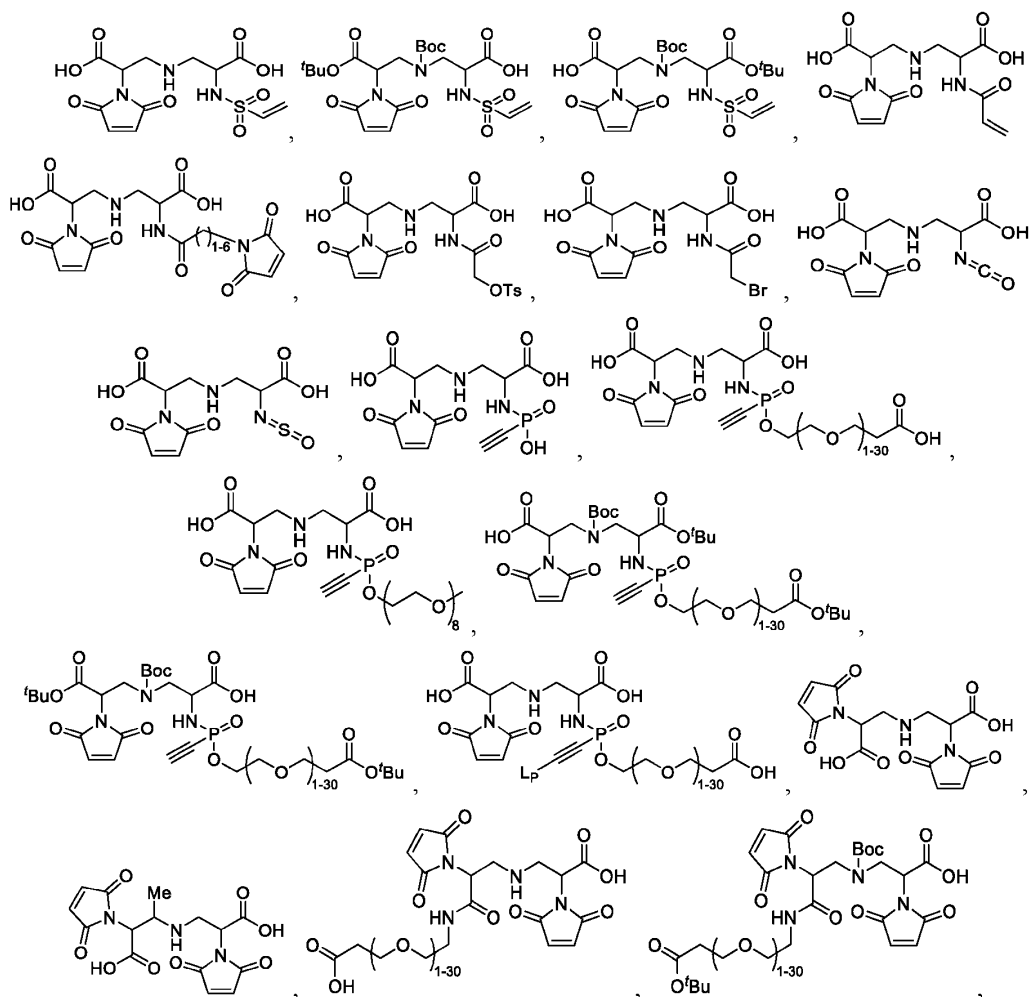
In one embodiment, at least one of W^1 and W^2 is selected from a connecting group comprising a maleimide structure.

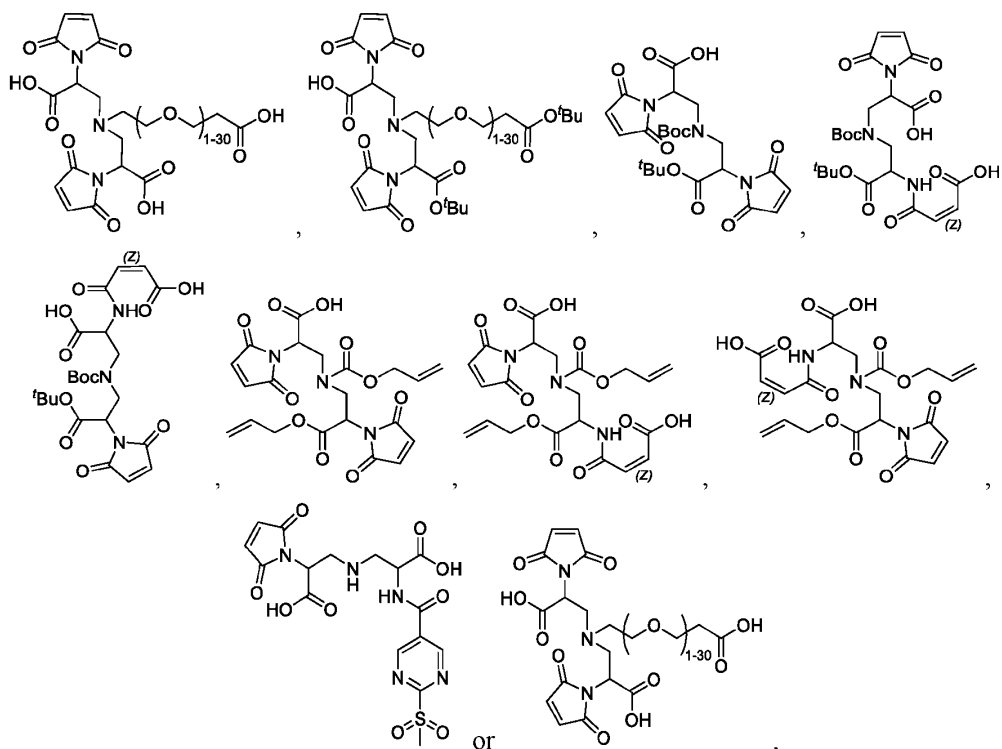
In one embodiment, W^1 and W^2 are the same or different, each independently selected from, without limitation, the following structures or racemates, enantiomers, diastereomers, pharmaceutically acceptable salts or solvates thereof:



In one embodiment, at least one of W^1 or W^2 is selected from a connecting group comprising a maleimide structure.

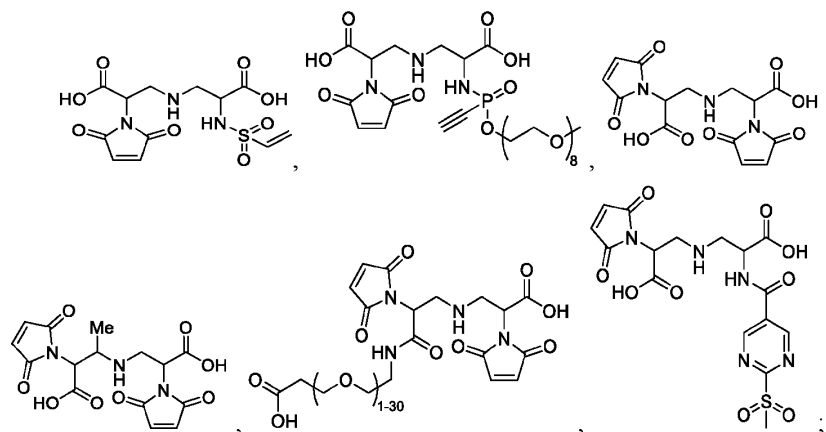
10 In some embodiments, said connector group is derived from, without limitation, the following structures:



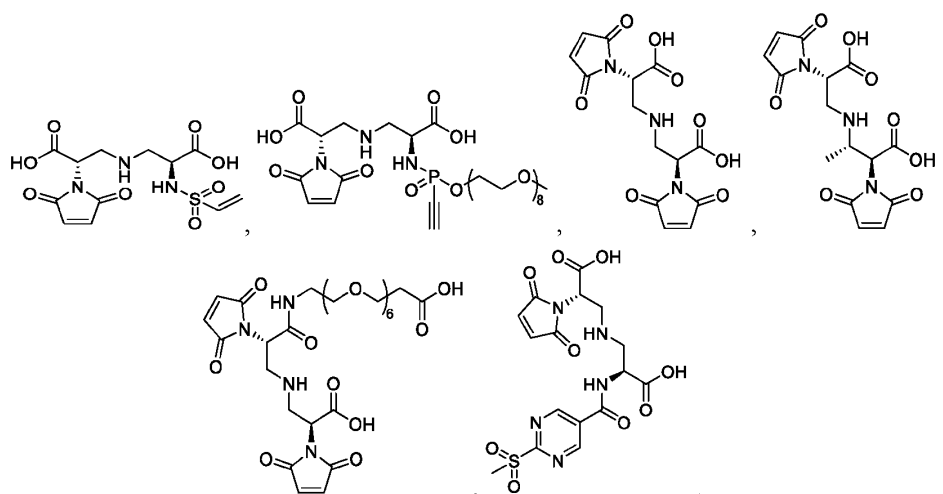


wherein: L_P is selected from the same or different groups, including but not limited to deuterium, chlorine, bromine, iodine, OMs, OTs;

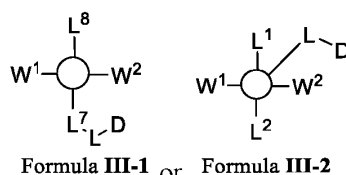
In one embodiment, said connector group may be derived from, without limitation, the following structures:



10 In one embodiment, said connector group may derived from, without limitation, the following structures:



In some embodiments, said linker-drug compound has a structure as shown in Formula III-1 or Formula III-2, in one embodiment, having a structure as shown in Formula III-1,



wherein,

L^1 and L^2 are the same or different, each independently selected from, without limitation, hydrogen, deuterium, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10-membered heterocyclic, carboxylic acid, amide, ester, sulfite, sulfonate, phosphoric acid, pyrophosphoric acid, natural or unnatural amino acid residues polyethylene glycol, or a combination thereof;

L^8 is selected from the group consisting of hydrogen, deuterium, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10-membered heterocyclic, carboxylic acid, amide, ester, sulfinyl, sulfonate, phosphoric acid, pyrophosphoric acid, natural or unnatural amino acid residues, polyethylene glycol, or a combination thereof;

L^7 is selected from the group consisting of one or more combinations of a substituted or unsubstituted C_1 - C_6 alkylene group, a substituted or unsubstituted C_2 - C_6 alkenyl group, a substituted or unsubstituted C_2 - C_6 alkynyl group, an aryl group, a heteroaryl group, a C_3 - C_{10} cycloalkyl group, a 3-10-membered heterocycloalkyl group, a carbonyl group, an amide group, an ester group, a sulfinyl group, a sulfonate group, a phosphoric acid group, a pyrophosphoric acid group, a natural or unnatural amino acid residue, or a polyethylene glycol;

W^1 and W^2 are the same or different, each independently selected from, without limitation, a

connecting group. In one embodiment, at least one of which is selected from a group comprising a maleimide structure;

L^1 , L^2 , L^8 , L^7 and/or W^1 , W^2 are covalently linked to the circle;

Circles are selected from scaffolds containing basic groups;

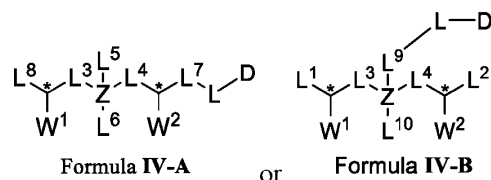
L is a linking moiety;

D is a drug unit. In one embodiment, D may be a drug and/or a drug precursor. In one embodiment, the drug unit is linked to L by a covalent bond;

In one embodiment, D is a drug and said drug is linked to L by a covalent bond;

In one embodiment, D is selected from a drug precursor and said drug precursor is linked to L by a covalent bond.

In some embodiments, said linker-drug compound has a structure such as Formula IV-A or IV-B, in one embodiment, having a structure such as Formula IV-A,

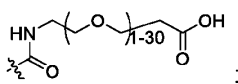


wherein,

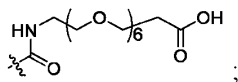
*the carbon atom at the position shown is a chiral carbon, selected from the *R* absolute configuration or the *S* absolute configuration;

L^1 and L^2 are the same or different, each independently selected from, without limitation, hydrogen, deuterium, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10-membered heterocyclic, carboxylic acid, amide, ester, sulfite, sulfonate, phosphoric acid, pyrophosphoric acid, natural or unnatural amino acid residues polyethylene glycol, or a combination thereof;

In one embodiment, L^1 and L^2 are the same or different, each independently selected from, without limitation, carboxyl,



In one embodiment, L^1 and L^2 are the same or different, each independently selected from, without limitation, the carboxyl group,



L^3 and L^4 are the same or different, each independently selected from, without limitation, the combination of one or more of substituted or unsubstituted C_1 - C_6 alkylene, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10-membered heterocyclic, polyethylene glycol;

In one embodiment, L^3 and L^4 are the same or different, each independently selected from,

without limitation, substituted or unsubstituted C₁-C₆ alkylene;

In one embodiment, L³ and L⁴ are the same or different, each independently selected from, without limitation, C₁-C₆ alkylene;

In one embodiment, L³ and L⁴ are the same or different, each independently selected from, without limitation, methylene, ethylene;

L⁵ and L⁶ are each independently present or absent, and when present are identical or different, and are selected from, without limitation, hydrogen, deuterium, oxygen, sulfur, hydroxyl, carbonyl, amide, ester, tert-butoxycarbonyl (Boc), benzyloxycarbonyl (Cbz), allyloxycarbonyl (Alloc), benzyl, substituted or unsubstituted C₁-C₆ alkylidene, substituted or unsubstituted C₂-C₆ alkenyl, substituted or unsubstituted C₂-C₆ alkynyl, aryl, heteroaryl, C₃-C₁₀ cycloalkyl, 3-10-membered heterocyclic, polyethylene glycol, or a combination thereof;

In one embodiment, L⁶ is absent, L⁵ is present, and L⁵ is selected from, without limitation, hydrogen, substituted or unsubstituted C₁-C₆ alkyl;

In one embodiment, L⁶ is absent, L⁵ is present, and L⁵ is selected from, without limitation, hydrogen, C₁-C₆ alkyl;

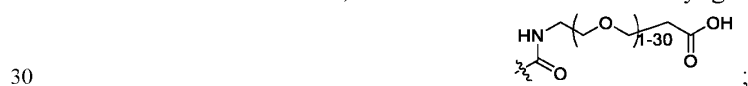
In one embodiment, L⁶ is absent, L⁵ is present, and L⁵ is hydrogen;

L⁷ is selected from the group consisting of one or more combinations of a substituted or unsubstituted C₁-C₆ alkylene group, a substituted or unsubstituted C₂-C₆ alkenyl group, a substituted or unsubstituted C₂-C₆ alkynyl group, an aryl group, a heteroaryl group, a C₃-C₁₀ cycloalkyl group, a 3-10-membered heterocycloalkyl group, a carbonyl group, an amide group, an ester group, a sulfinyl group, a sulfonate group, a phosphoric acid group, a pyrophosphoric acid group, a natural or unnatural amino acid residue, or a polyethylene glycol;

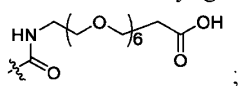
In one embodiment, L⁷ is carbonyl;

L⁸ is selected from the group consisting of hydrogen, deuterium, substituted or unsubstituted C₁-C₆ alkyl, substituted or unsubstituted C₂-C₆ alkenyl, substituted or unsubstituted C₂-C₆ alkynyl, aryl, heteroaryl, C₃-C₁₀ cycloalkyl, 3-10-membered heterocyclic, carboxylic acid, amide, ester, sulfinyl, sulfonate, phosphoric acid, pyrophosphoric acid, natural or unnatural amino acid residues, polyethylene glycol, or a combination thereof;

In one embodiment, L⁸ is selected from the carboxyl group,



In one embodiment, L⁸ is selected from the carboxyl group,



L⁹ is selected from the group consisting of one or more combinations selected from the group consisting of oxygen, sulfur, carbonyl, amide, ester, substituted or unsubstituted C₁-C₆ alkylene, substituted or unsubstituted C₂-C₆ alkenyl, substituted or unsubstituted C₂-C₆ alkynyl, aryl, heteroaryl, C₃-C₁₀ cycloalkyl, 3-10-membered heterocycloalkyl, or polyethylene glycol;

L¹⁰ present or absent, when present is selected from hydrogen, deuterium, oxygen, hydroxyl, carbonyl, amide, ester, tert-butoxycarbonyl (Boc), benzyloxycarbonyl (Cbz), allyloxycarbonyl

(Alloc), benzyl, substituted or unsubstituted C₁-C₆ alkylene, substituted or unsubstituted C₂-C₆ alkenyl, substituted or unsubstituted C₂-C₆ alkynyl, aryl, heteroaryl, C₃-C₁₀ cycloalkyl, 3-10-membered heterocyclic, polyethylene glycol, or a combination thereof;

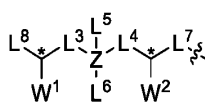
In one embodiment, L¹⁰ is not present;

W1 and W2 are the same or different, each independently selected from, without limitation, a connecting group. In one embodiment, at least one of W1 and W2 is selected from a group comprising a maleimide structure;

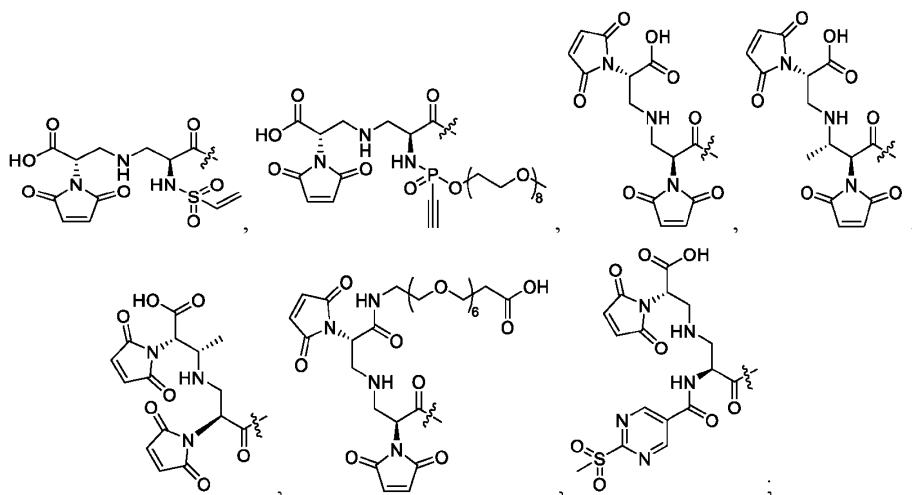
In one embodiment, W¹ and W² are as defined above;

Z is N, O, S or quaternary amines;

10 In one embodiment, Z is N;



In one embodiment, in Formula IV-A, $\begin{array}{c} L^8 \quad L^3 \quad L^5 \quad L^4 \quad L^7 \\ | \quad | \quad | \quad | \quad | \\ W^1 \quad L^6 \quad W^2 \end{array}$, as a whole is selected from the following structures:



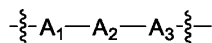
L is a linking moiety;

D is a drug unit. In one embodiment, D may be a drug and/or a drug precursor. In one embodiment, the drug unit D is linked to L by a covalent bond;

In one embodiment, D is a drug and said drug is linked to L by a covalent bond;

20 In one embodiment, D is a drug precursor and said drug precursor is linked to L by a covalent bond.

In some embodiments, said linking moiety L has the structure shown in Formula V below,



Formula V

wherein,

A₁, A₂, and A₃ each absent or present, independently;

when A₁ is present, A₁ is a modifying unit, in one embodiment, from a branchable unit and/or

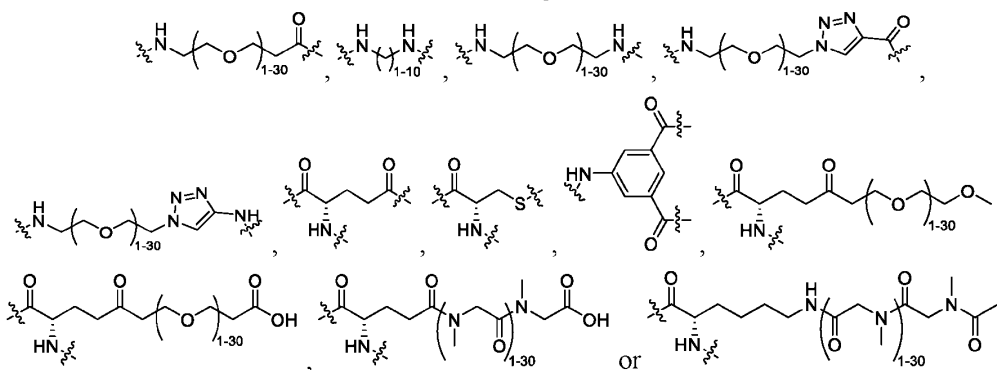
a hydrophilic unit;

when A₂ is present, A₂ is a breakable or a non-breakable unit;

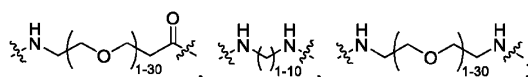
when A₃ is present, A₃ is an elimination unit and/or a chelation unit;

the position shown by the wavy line is the connection site.

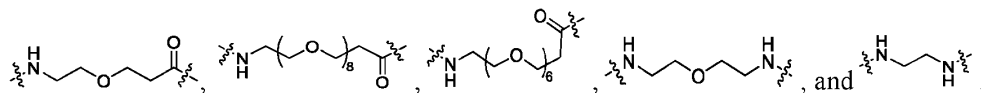
In some embodiments, A₁ may be present or absent. In one embodiment, when A₁ is present, A₁ is selected from, without limitation, the following structures, or combinations thereof:



10 In one embodiment, when A₁ is present, A₁ is selected from, without limitation, the following structures, or combinations thereof:



In one embodiment, when A₁ is present, A₁ is selected from, without limitation, the following structures or combinations thereof:



wherein the positions shown by the wavy lines are connection sites.

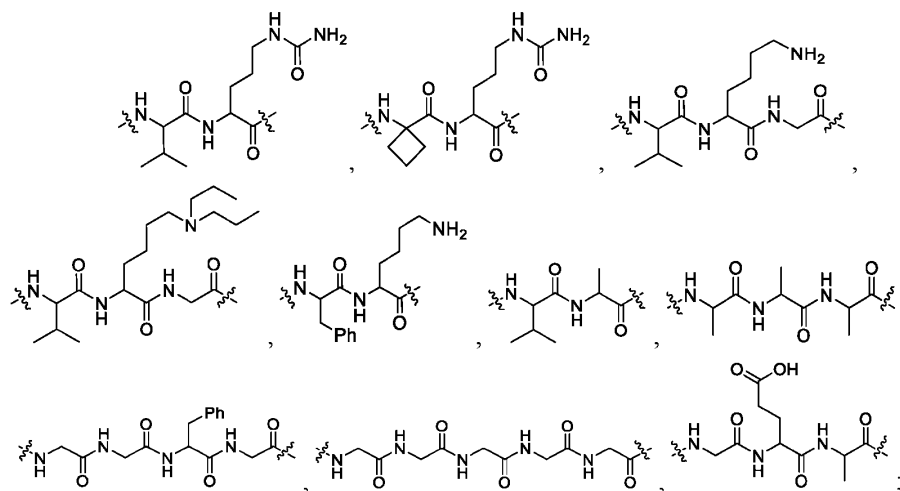
In some embodiments, A₂ may be present or absent. In one embodiment, when A₂ is present, said A₂ is selected from, without limitation, a breakable or unbreakable unit. In one embodiment, the unbreakable unit is selected from, without limitation, a substituted or unsubstituted C₁-C₆ alkyl, a substituted or unsubstituted C₂-C₆ alkenyl, a substituted or unsubstituted C₂-C₆ alkynyl, an aryl, a heteroaryl, a C₃-C₁₀ cycloalkyl, a 3-10-membered heterocyclic, an amide, an ester, polyethylene glycol, or a combination thereof.

In some embodiments, the breakable unit is selected from, without limitation, a non-enzymatically sensitive connection unit, an enzymatically sensitive connection unit, or a combination thereof. In one embodiment, said non-enzymatically sensitive connection unit is selected from, without limitation, glutathione-sensitive disulfide bond linker unit, pH-sensitive hydrazone linker unit. In one embodiment, said enzymatically sensitive connection unit is selected from, without limitation, a histone protease breakable connection unit, a phosphodiesterase and/or pyrophosphate esterase breakable connection unit, a β -glucuronidase breakable connection unit, a β -galactosidase breakable connection unit, a sulfate esterase breakable connection unit or a

combination thereof.

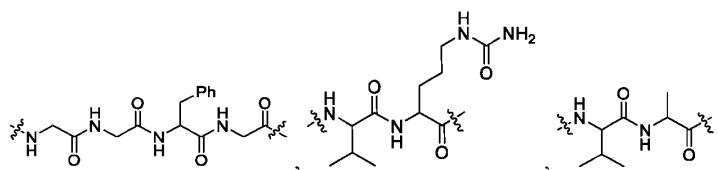
In some embodiments, when A₂ is present, said A₂ is a breakable unit, wherein said breakable unit is selected from, without limitation, a peptide residue comprising 2-10 natural or unnatural amino acid residues. In one embodiment, said natural or unnatural amino acids may be further optionally substituted with one or more substituents.

In one embodiment, said breakable unit is selected from, without limitation:

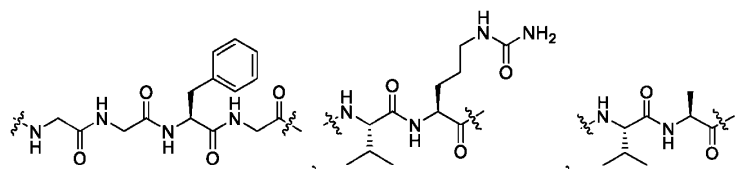


10

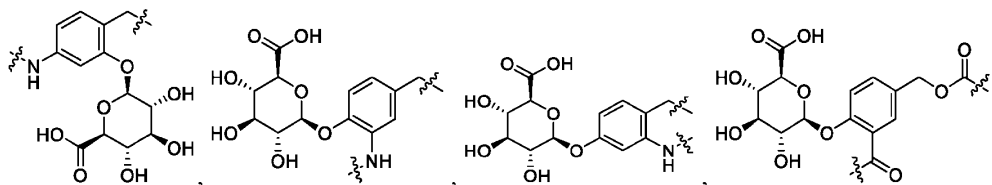
In one embodiment, said breakable unit is selected from:

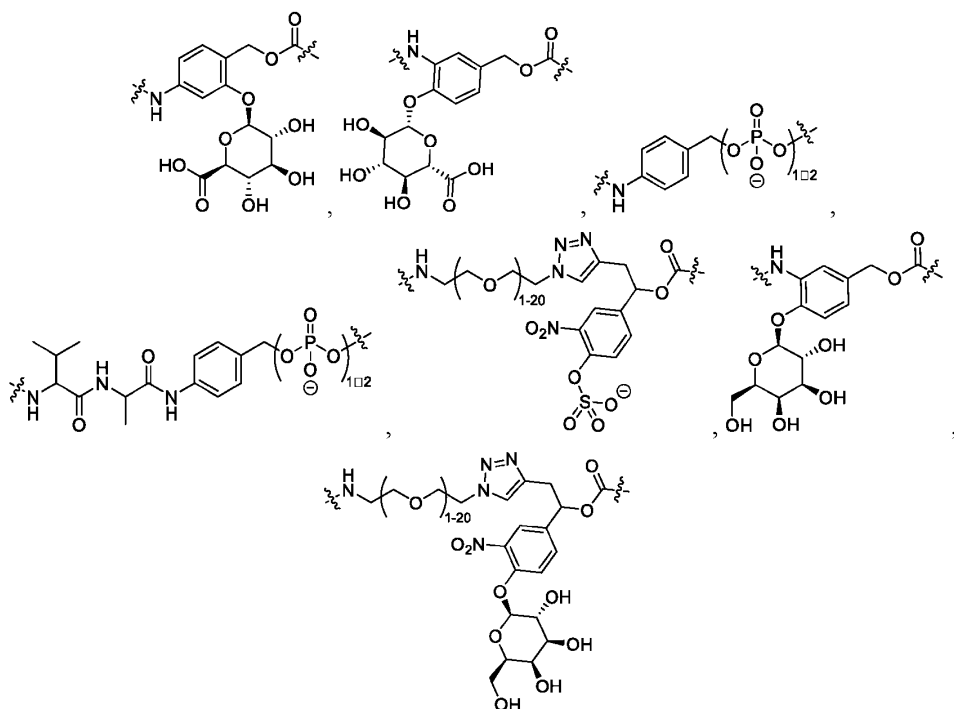


In one embodiment, said breakable unit is selected from:

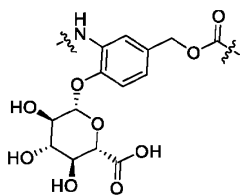


Alternatively, said breakable unit is selected from, without limitation, the following structures or isomers thereof:



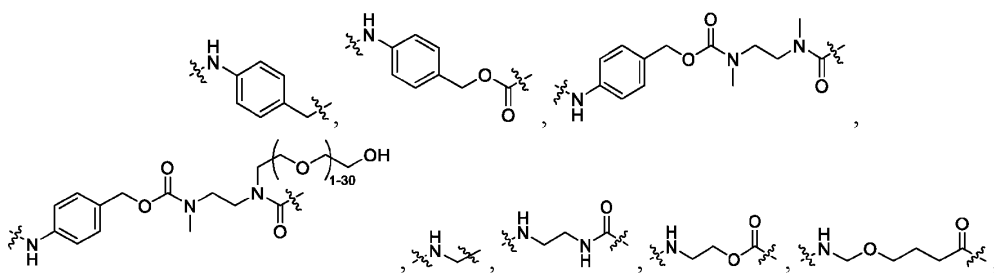


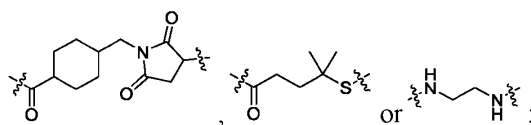
In one embodiment, said breakable unit is selected from the following structures or isomers thereof:



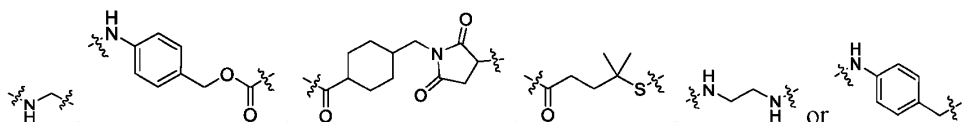
wherein the position shown by the wavy line is the connection site.

In some embodiments, when A₃ is present, A₃ is selected from, without limitation, the following structures:





In one embodiment, when A₃ is present, A₃ is selected from, without limitation, the following structures:



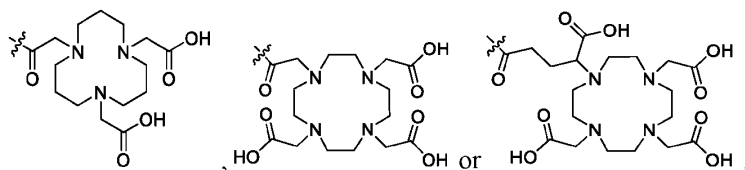
wherein the position shown by the wavy line is the connecting site.

In some embodiments, said drug is selected from, without limitation, a cytotoxic drug, a radioisotope, a detection and/or diagnostic reagent, an enzyme inhibitor, a protein degrading agent, an immunomodulator, a peptide, or a nucleotide.

10 In some embodiments, said drug is selected from, without limitation, cytotoxic drugs.

In some embodiments, said drug is obtained by chelating a drug precursor with a metal ion, such as a radioisotope.

In some embodiments, said drug precursor is selected from, without limitation,



In one embodiment, said drug precursor is obtained by chelating the drug with a metal ion, such as a radioisotope.

In some embodiments, said cytotoxic agent is selected from, without limitation, a DNA damaging agent, a DNA topoisomerase inhibitor, a microtubule and/or microtubule protein inhibitor, an RNA polymerase inhibitor, or a combination thereof.

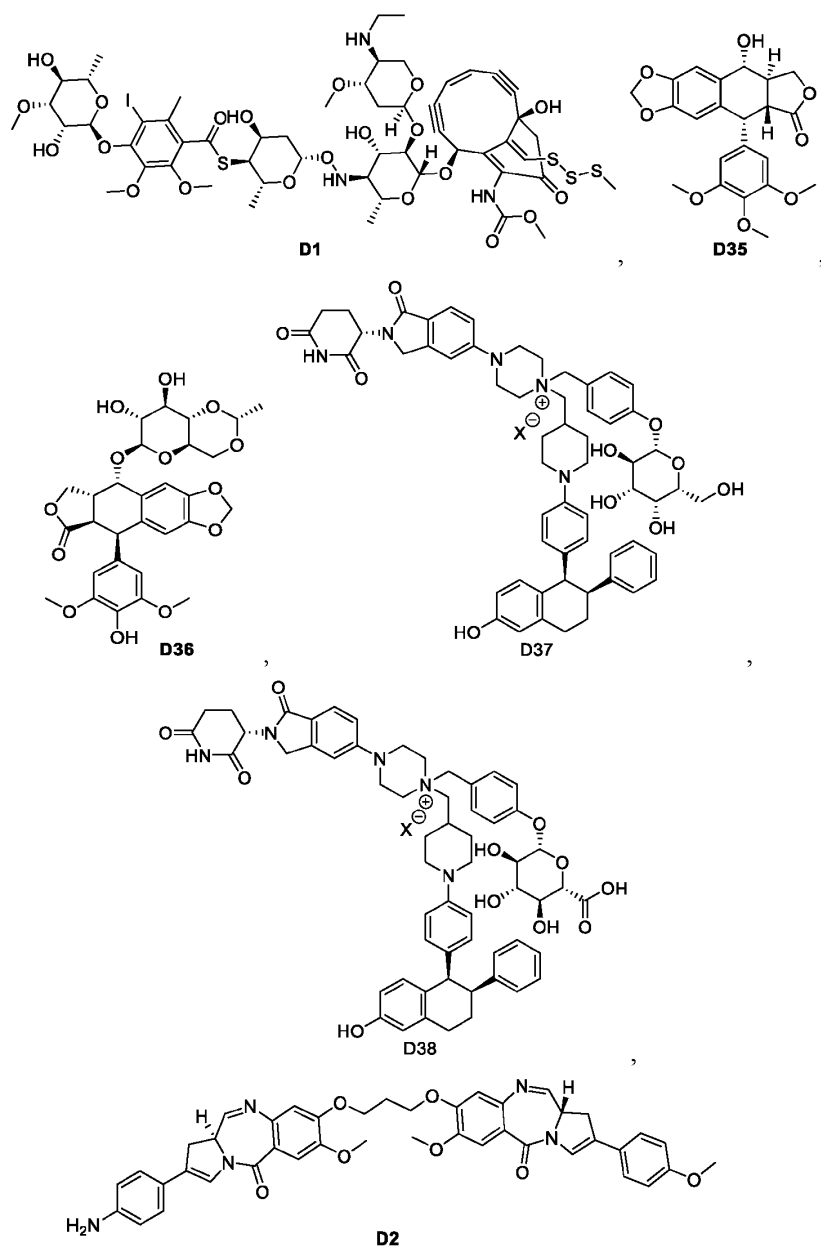
20 In one embodiment, said DNA damaging agents include, but are not limited to, derivatives of calicheamicin, anthramycin, ecteinascidins toxin, adriamycin, mitomycin, duocarmycin, or a combination thereof.

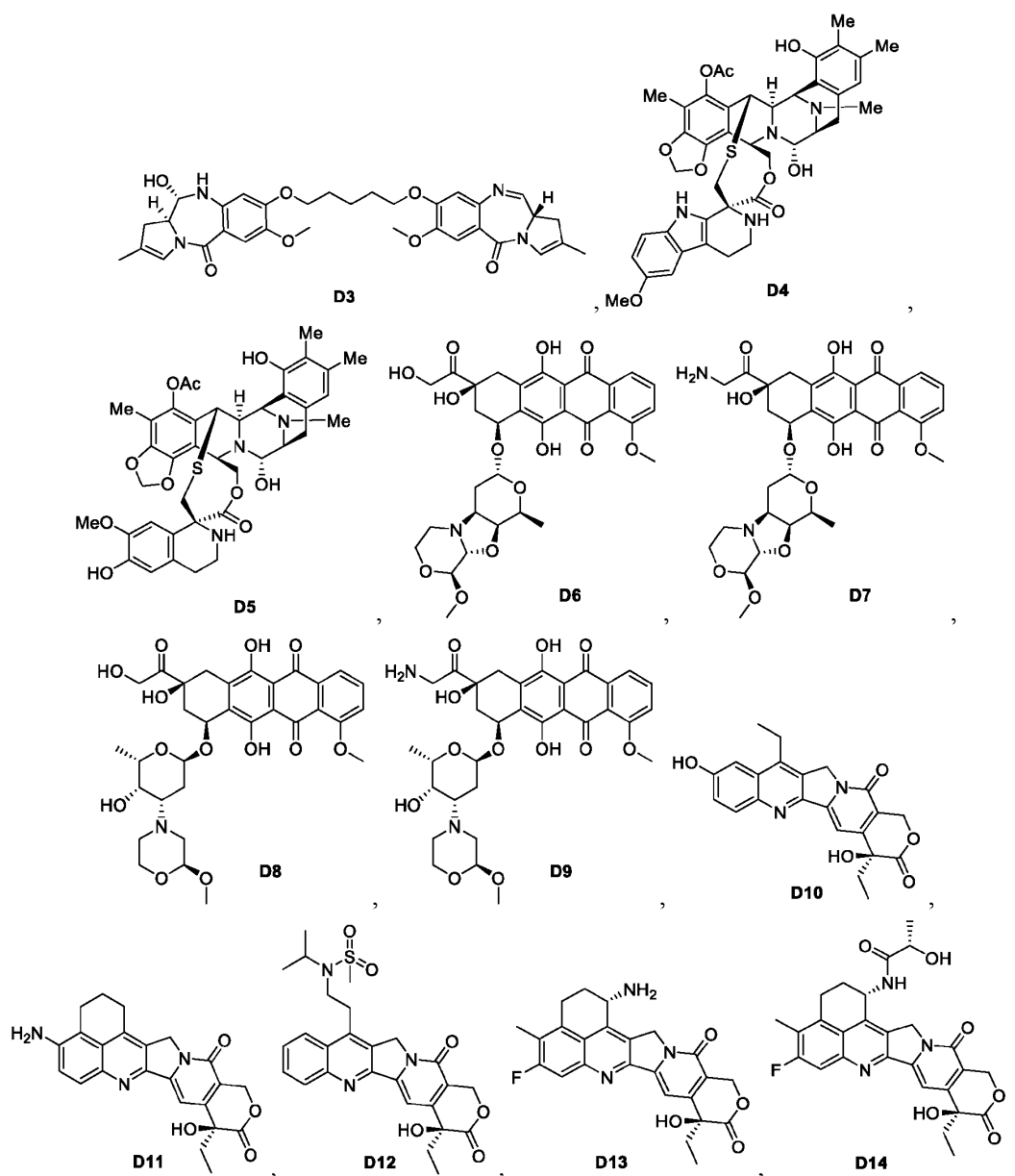
In one embodiment, said DNA topoisomerase inhibitors include, but are not limited to, derivatives of camptothecin, podophyllotoxin, etoposide, or a combination thereof.

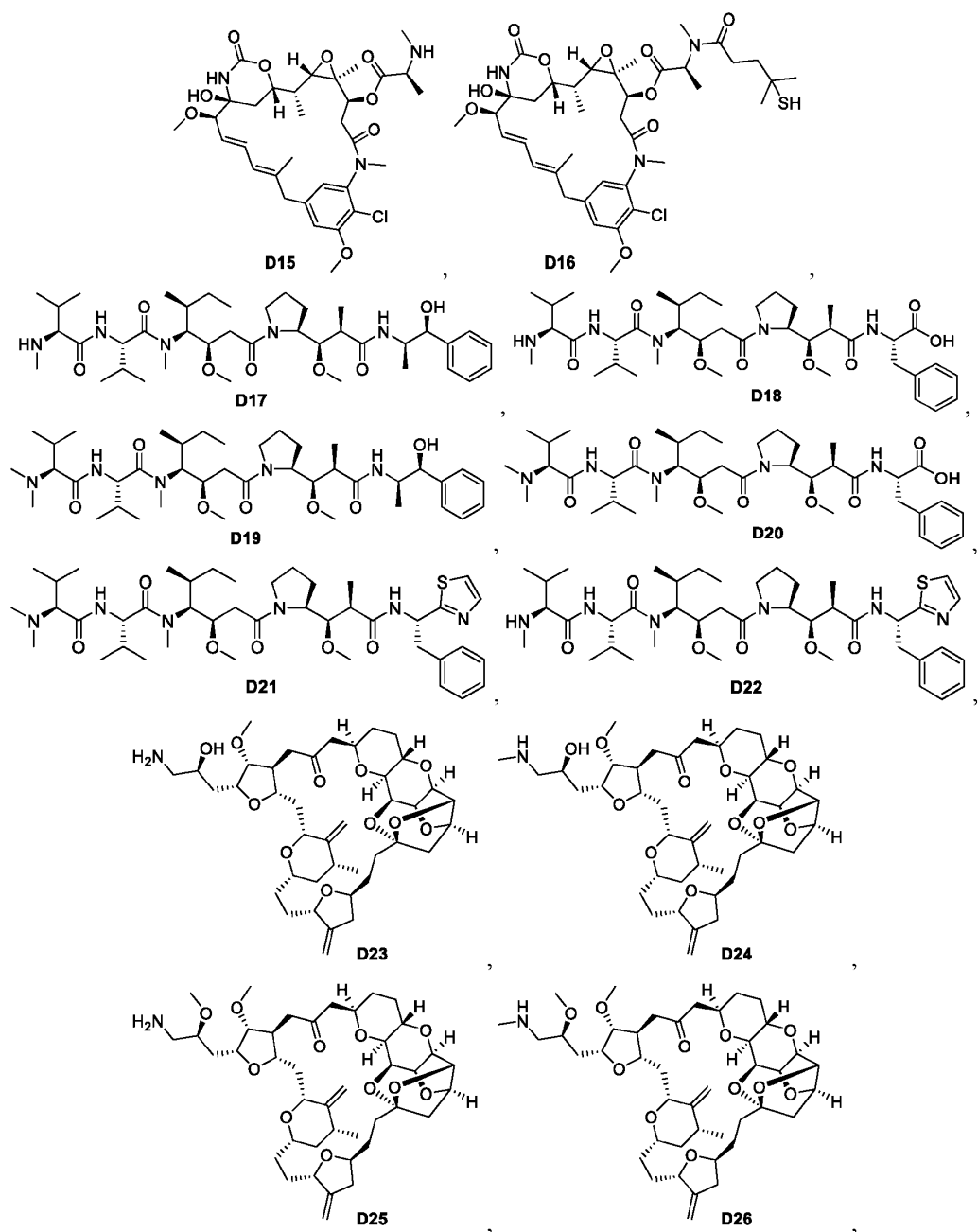
In one embodiment, said microtubule and/or microtubule protein inhibitors include, but are not limited to, derivatives of maytansine, auristatin, eribulin, tubulysin, or a combination thereof.

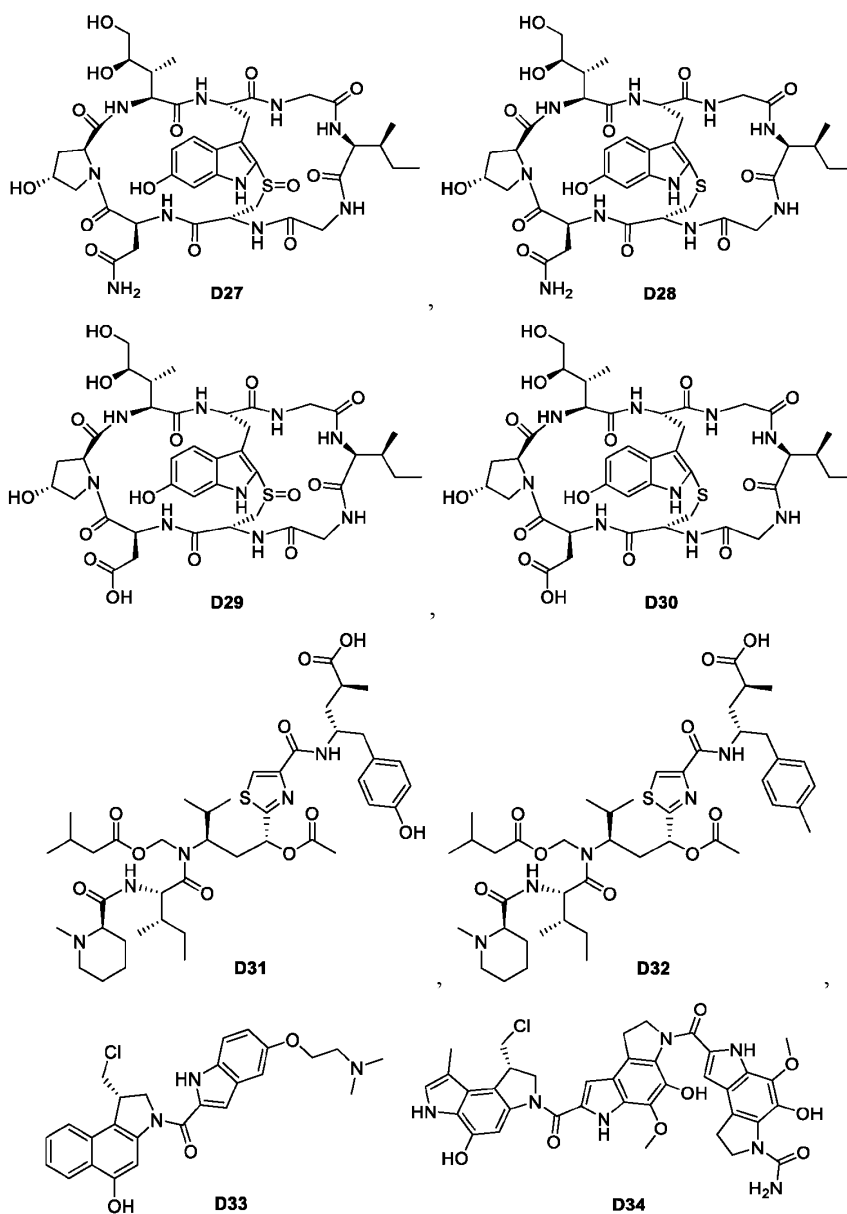
In one embodiment, said RNA polymerase inhibitors include, but are not limited to, amanitin peptide derivatives.

In some embodiments, said drug is selected from, without limitation, the following structures:



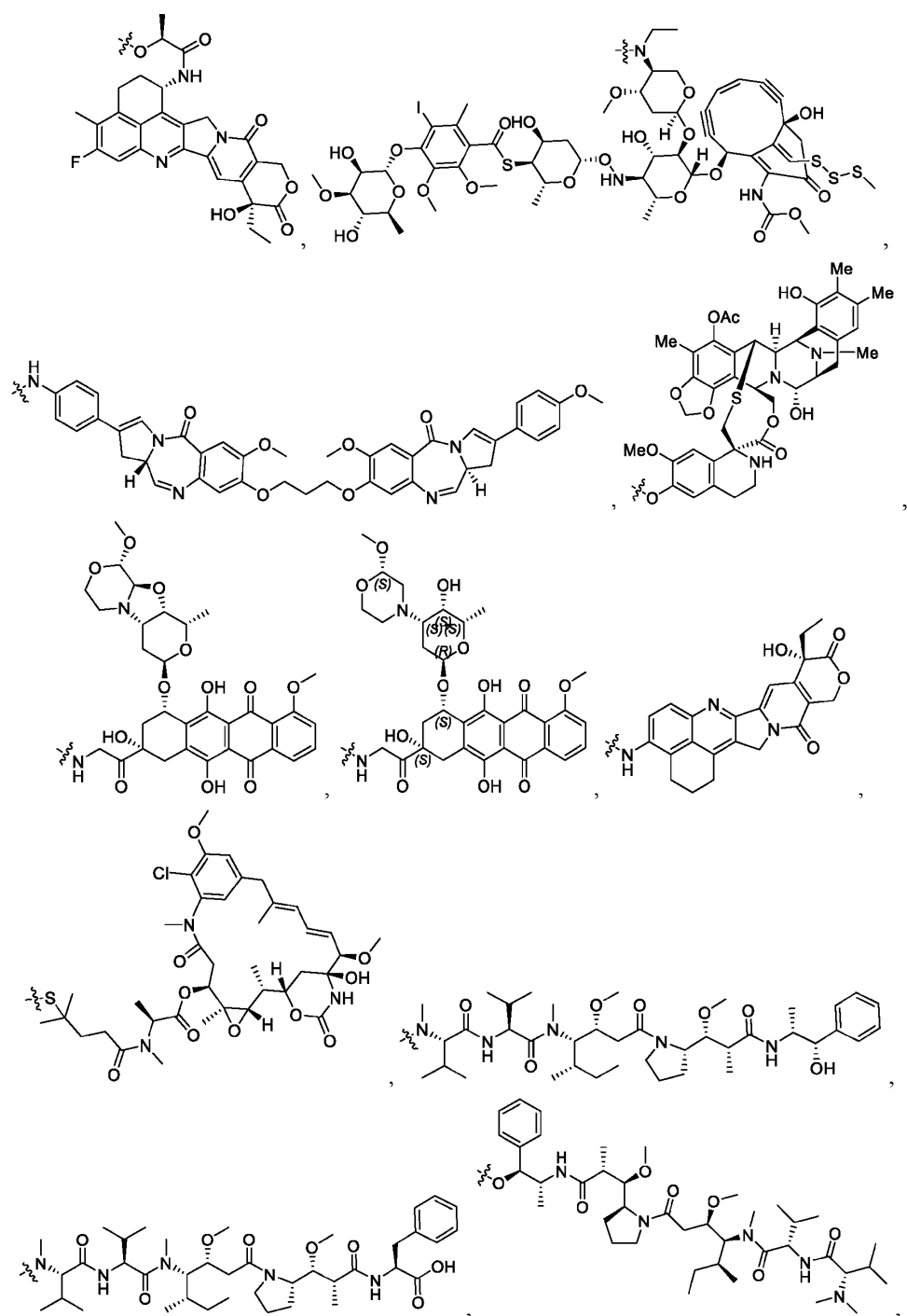


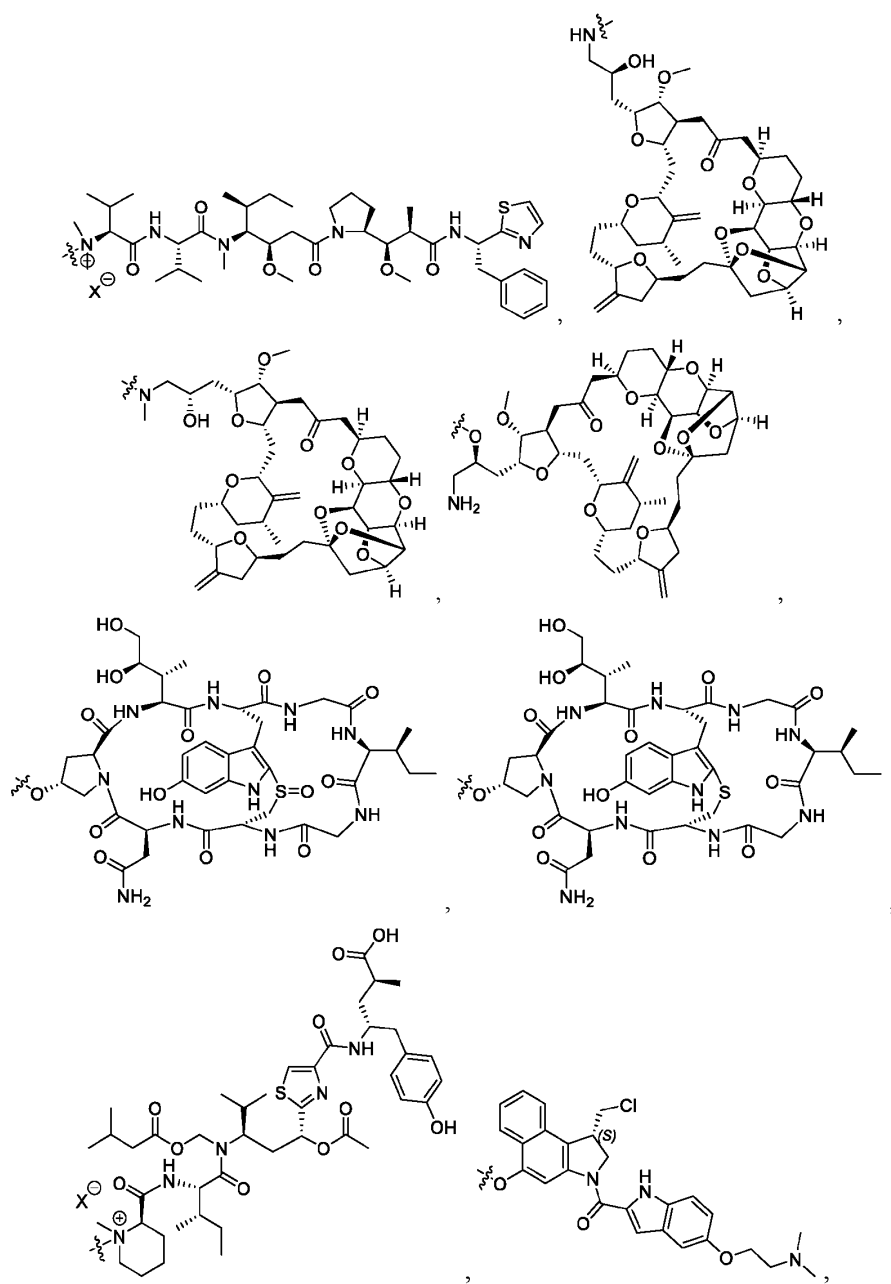


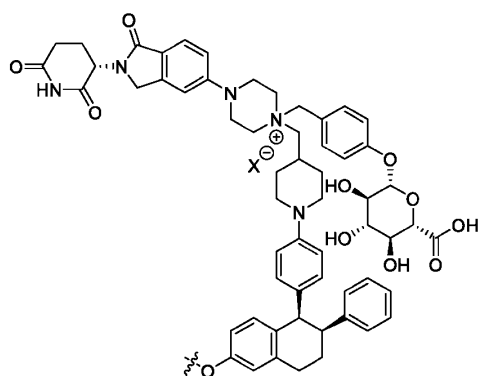


wherein each X^- is independently selected from Cl^- , Br^- , I^- , $MeSO_3^-$, CF_3COO^- . In one embodiment, X^- is CF_3COO^- ;

In one embodiment, D is selected from the following structures:



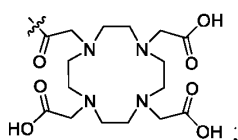




wherein each X^- is independently selected from Cl^- , Br^- , I^- , $MeSO_3^-$, CF_3COO^- . in one embodiment, X^- is CF_3COO^- .

In some embodiments, said drug is obtained by chelation of a drug precursor with a metal ion, such as a radioisotope.

In some embodiments, said drug precursor includes, but is not limited to, the following structure:

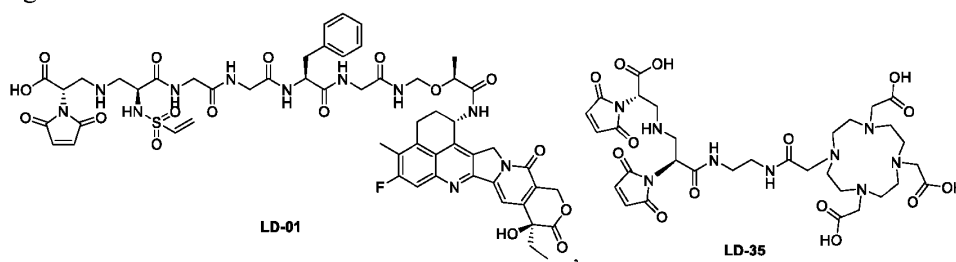


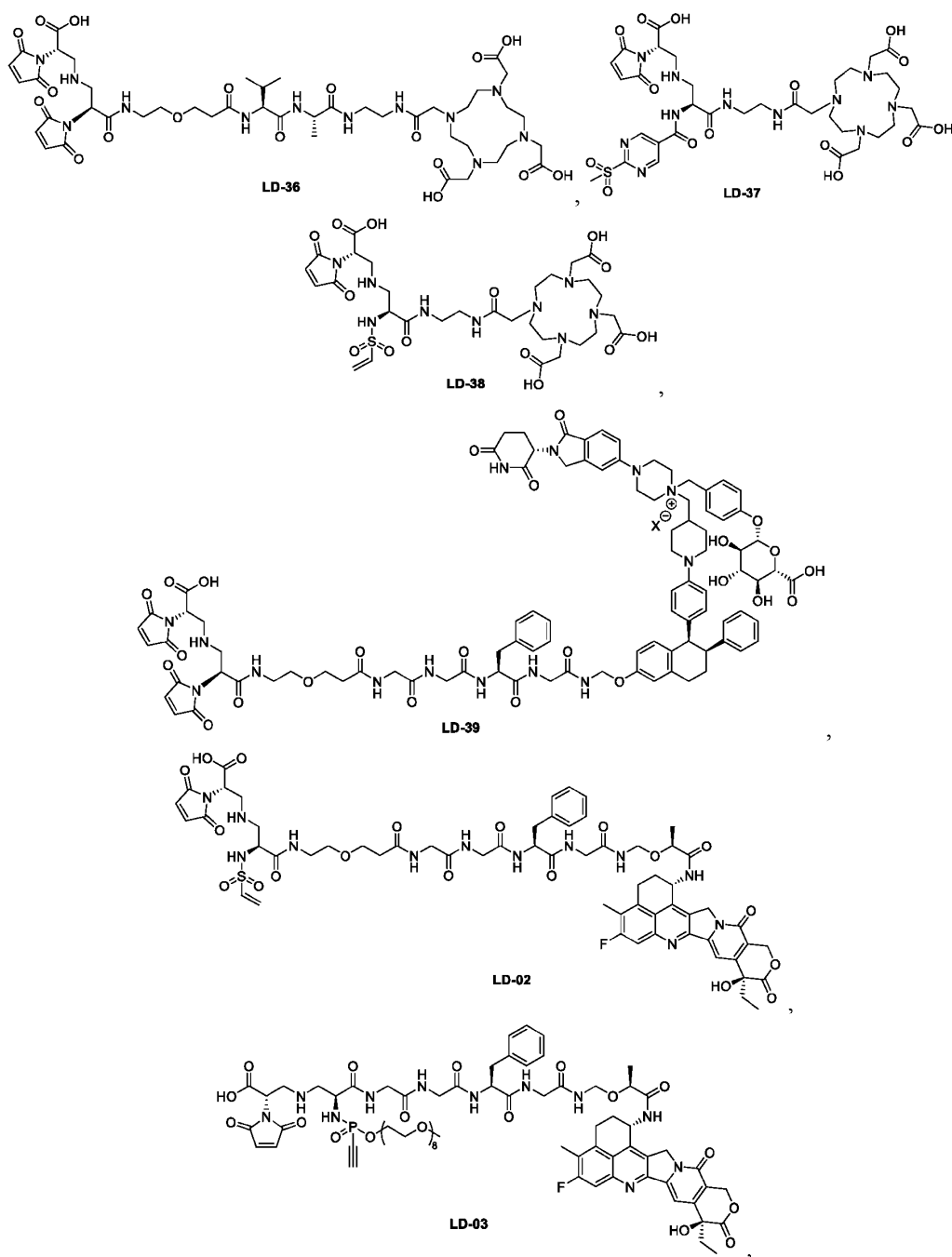
In one embodiment, said drug precursor is obtained by chelating the drug with a metal ion, such as a radioisotope.

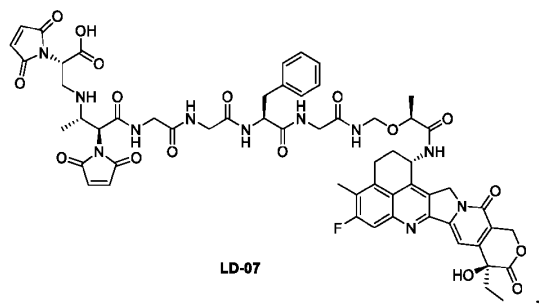
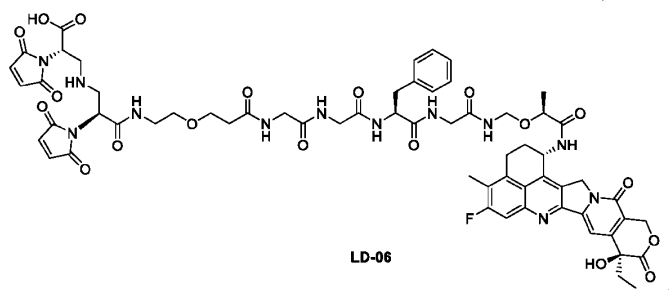
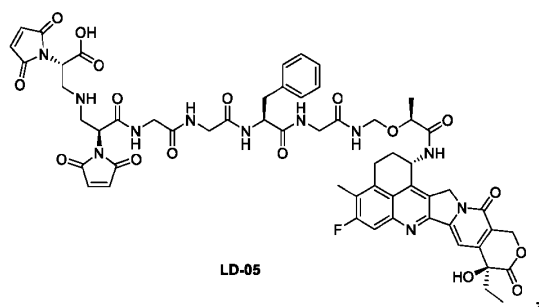
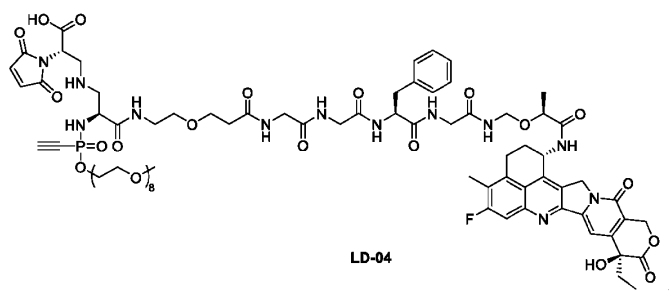
In some embodiments, said radioisotope is selected from, without limitation, ^{18}F , ^{67}Ga , ^{68}Ga , ^{99m}Tc , ^{123}I , ^{125}I , ^{131}I , ^{90}Y , ^{177}Lu , ^{47}Sc , ^{64}Cu , ^{67}Cu , ^{32}P , ^{186}Re , ^{188}Re , ^{89}Sr , ^{153}Sm , ^{198}Au , ^{166}Ho , ^{165}Dy , ^{169}Er , ^{149}Tb , ^{161}Tb , ^{211}At , ^{212}Bi , ^{213}Bi , ^{212}Pb , ^{223}Ra , ^{225}Ac , or a combination thereof.

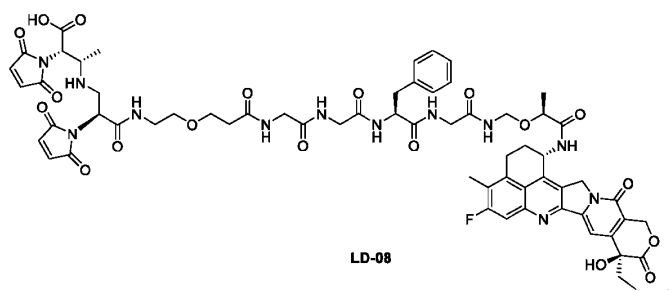
In one embodiment, said radioisotope is selected from, without limitation, ^{68}Ga , ^{99m}Tc , ^{131}I , ^{90}Y , ^{177}Lu , ^{64}Cu , ^{211}At , ^{213}Bi , ^{212}Pb , ^{225}Ac , or a combination thereof

In some embodiments, said connecting unit-drug is selected from, without limitation, the following structures:

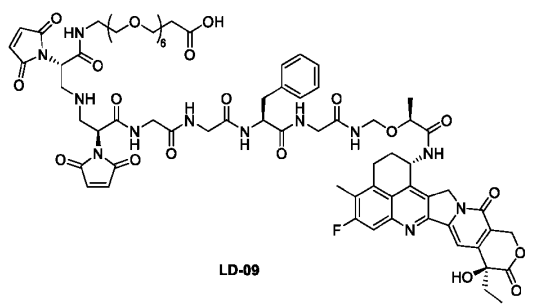




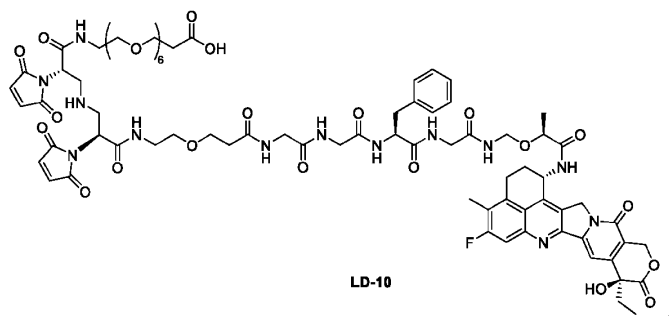




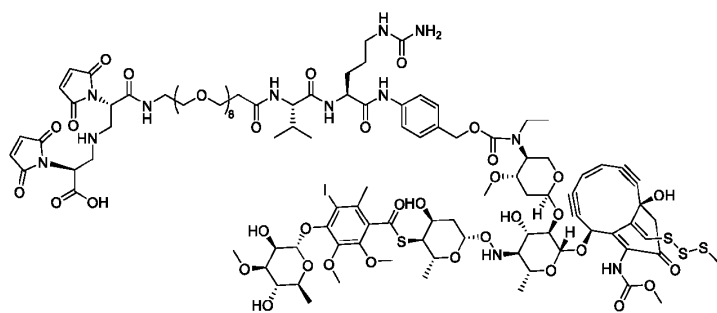
LD-08



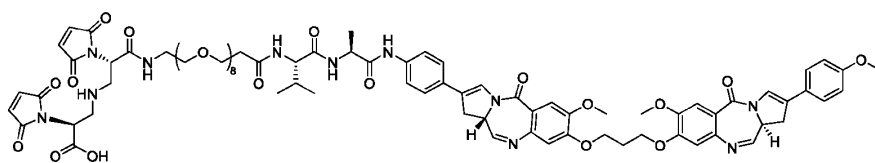
LD-09



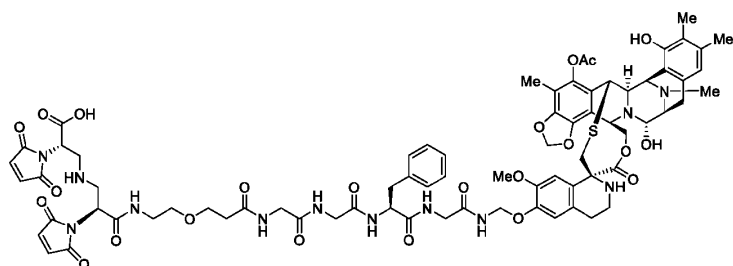
LD-10



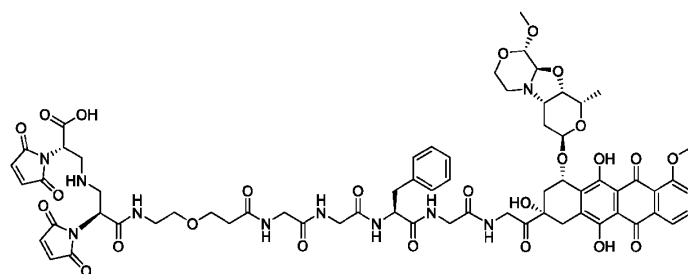
LD-11



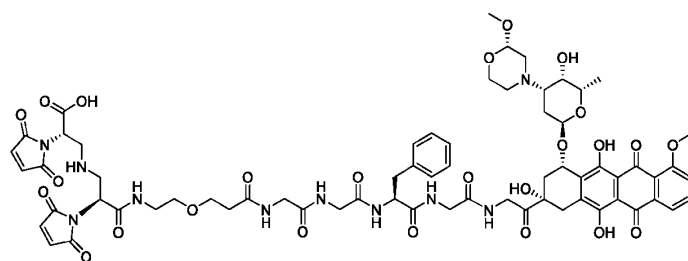
LD-12



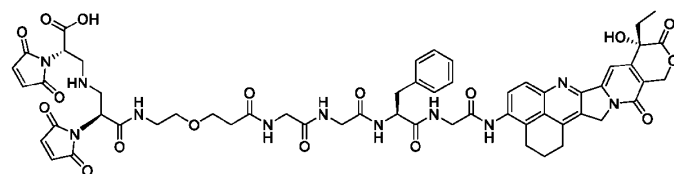
LD-13



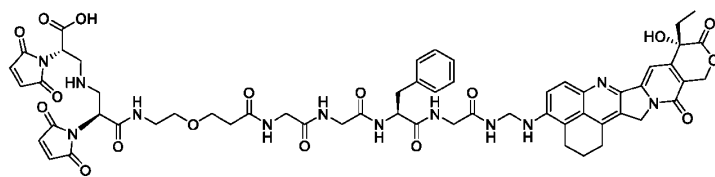
LD-15



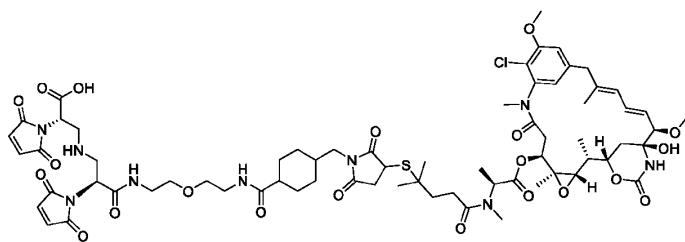
LD-17



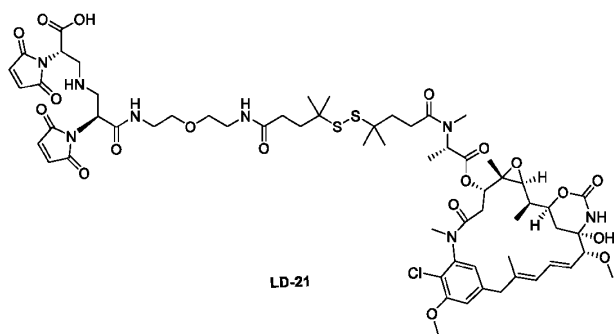
LD-18



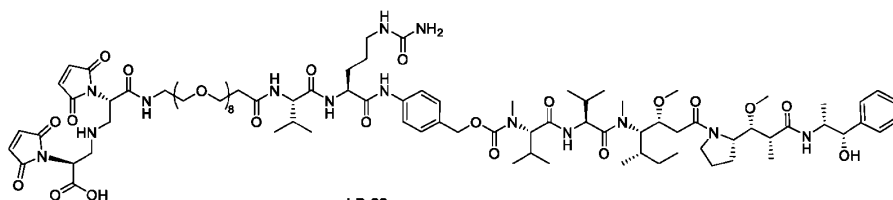
LD-19



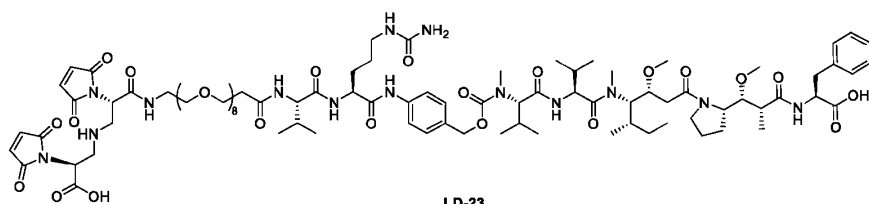
LD-20



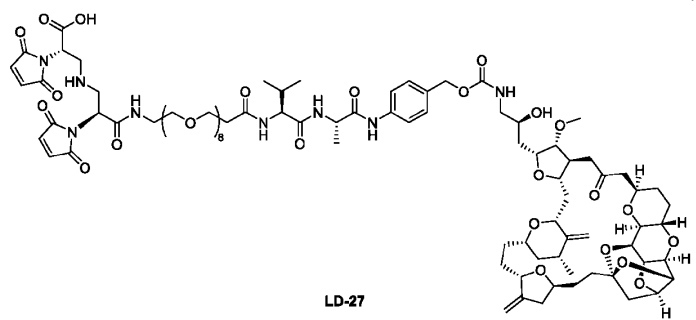
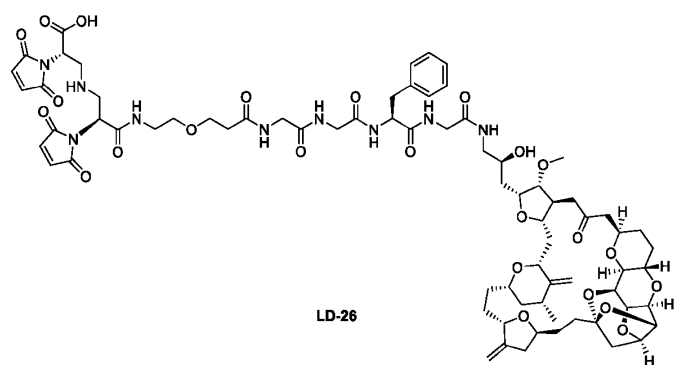
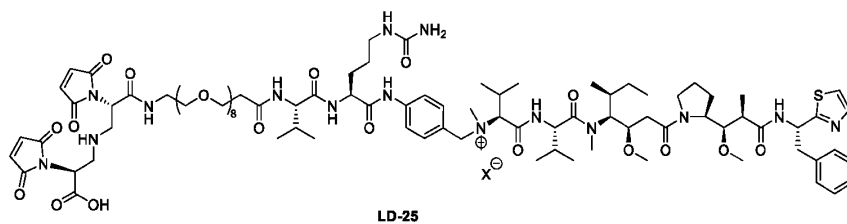
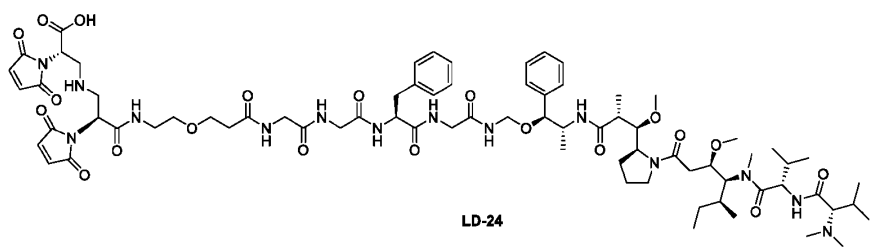
LD-21

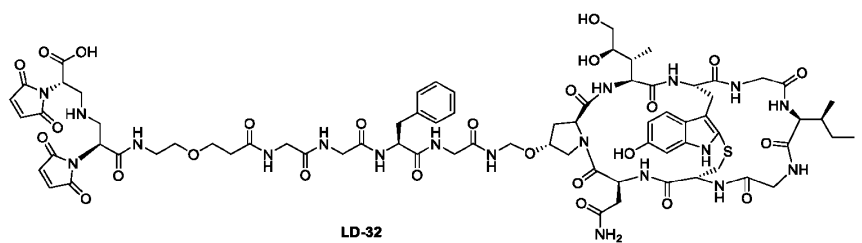
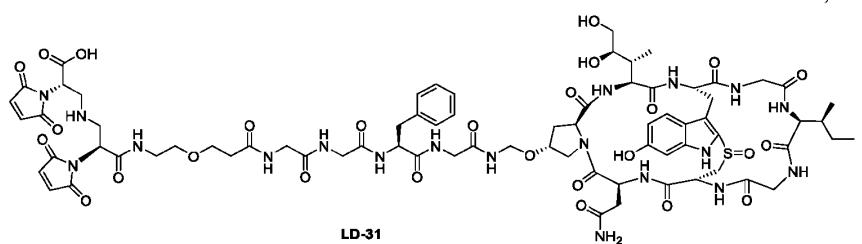
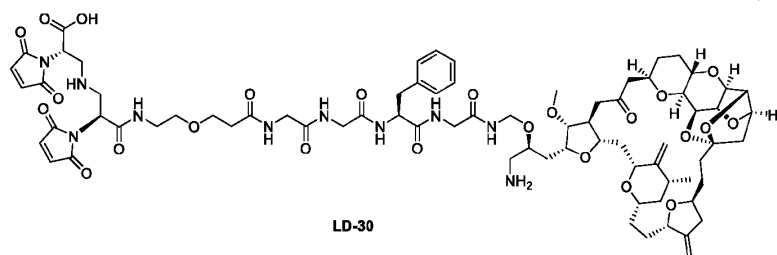
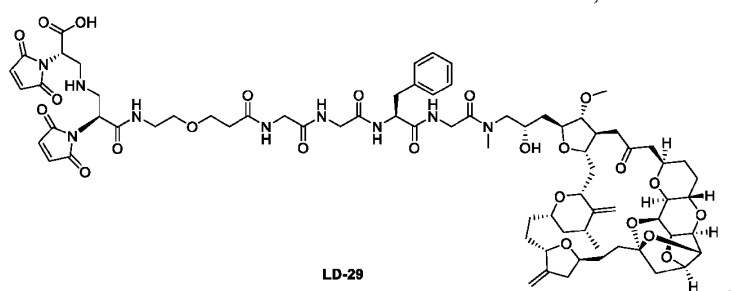
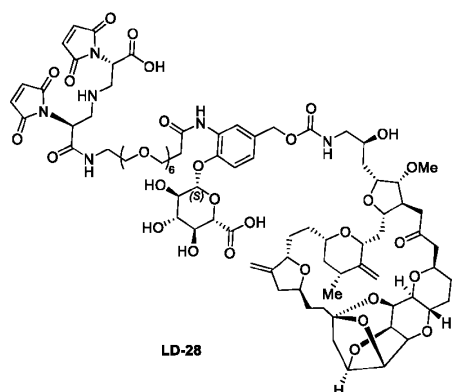


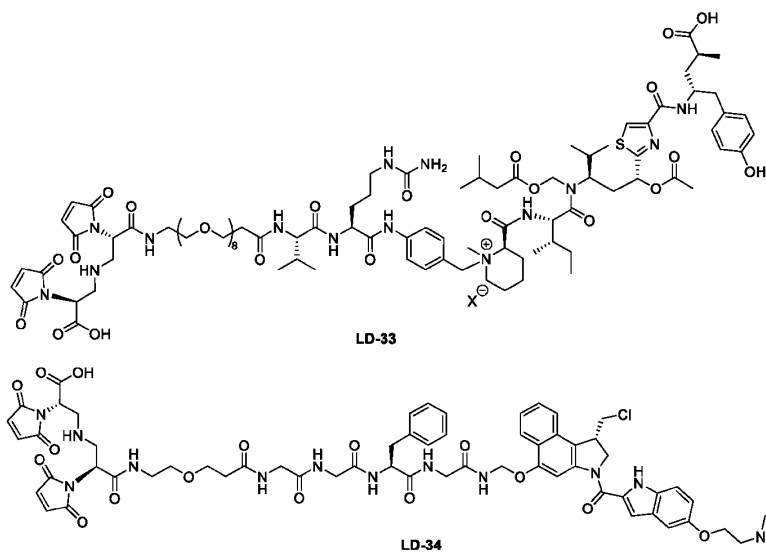
LD-22



LD-23

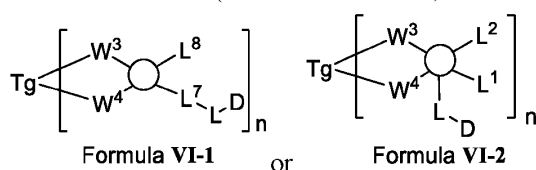






wherein each X^- is independently selected from Cl^- , Br^- , I^- , CF_3COO^- , $MeSO_3^-$. In one embodiment, X^- is CF_3COO^- .

In a further aspect, the application provides a ligand-drug conjugate or its racemate, enantiomer, diastereomer, ring-opening form, pharmaceutically acceptable salt or solvate thereof as shown in Formula VI-1 or Formula VI-2 (in one embodiment, Formula VI-1).



wherein,

10 Tg is selected from the ligand or targeting moiety that binds to the target;

L^1 and L^2 are the same or different, each independently selected from, without limitation, hydrogen, deuterium, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10-membered heterocyclic, carboxylic acid, amide, ester, sulfite, sulfonate, phosphoric acid, pyrophosphoric acid, natural or unnatural amino acid residues polyethylene glycol, or a combination thereof;

20 L^8 is selected from the group consisting of hydrogen, deuterium, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10-membered heterocyclic, carboxylic acid, amide, ester, sulfinyl, sulfonate, phosphoric acid, pyrophosphoric acid, natural or unnatural amino acid residues, polyethylene glycol, or a combination thereof;

L^7 is selected from the group consisting of one or more combinations of a substituted or unsubstituted C_1 - C_6 alkylene group, a substituted or unsubstituted C_2 - C_6 alkenyl group, a

substituted or unsubstituted C₂-C₆ alkynyl group, an aryl group, a heteroaryl group, a C₃-C₁₀ cycloalkyl group, a 3-10-membered heterocycloalkyl group, a carbonyl group, an amide group, an ester group, a sulfinyl group, a sulfonate group, a phosphoric acid group, a pyrophosphoric acid group, a natural or unnatural amino acid residue, or a polyethylene glycol;

W³ and W⁴ are the same or different, each independently and non-limitingly selected from a connecting group. In one embodiment, at least one of W³ and W⁴ is selected from a group comprising a succinimide structure; in one embodiment, the succinimide structure is a closed-ring form, an open-ring form or any combination thereof; in one embodiment, the succinimide structure is in an open-ring form or any combination of closed-ring form and open-ring form; in one embodiment, the succinimide structure is in a ring-opening form;

L¹, L², L⁸, L⁷ and/or W³, W⁴ are covalently linked to the circle. In one embodiment, the circles are selected from scaffolds containing basic groups;

L is a linking moiety;

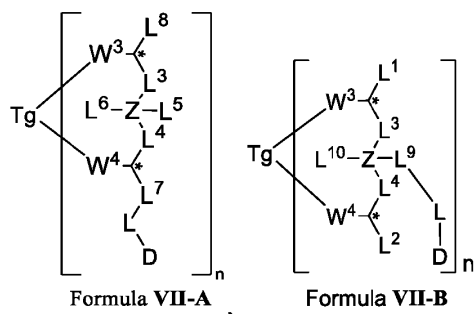
n is an integer or a decimal from 1 to 10. In some embodiments, n may be an integer or a decimal between 1-1.5, 1.5-2, 2-2.5, 2.5-3, 3-3.5, 3.5-4, 4-4.5, 4.5-5, 5-5.5, 5.5-6, 6-6.5, 6.5-7, 7-7.5, 7.5-8, 8-8.5, 8.5-9, 9-9.5, or 9.5-10;

D is a drug unit. In one embodiment, D may be a drug and/or a drug precursor. In one embodiment, the drug unit D is linked to L by a covalent bond;

In one embodiment, D is a drug and said drug is linked to L by a covalent bond;

In one embodiment, D is a drug precursor and said drug precursor is linked to L by a covalent bond.

In some embodiments, said ligand-drug conjugate or its racemate, enantiomer, diastereoisomer, ring-opening form, pharmaceutically acceptable salt or solvate thereof, is characterized in that said ligand-drug conjugate has a structure as shown below in Formula VII-A or VII-B, in one embodiment, having a structure as shown in Formula VII-A;



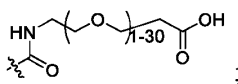
wherein,

Tg is selected from the ligand or its targeting moiety that has the binding specificity to a target; the carbon atom at the position shown* is a chiral carbon, selected from the *R* absolute configuration or the *S* absolute configuration;

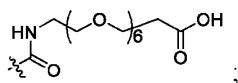
L¹ and L² are the same or different, each independently selected from, without limitation, hydrogen, deuterium, substituted or unsubstituted C₁-C₆ alkyl, substituted or unsubstituted C₂-C₆ alkenyl, substituted or unsubstituted C₂-C₆ alkynyl, aryl, heteroaryl, C₃-C₁₀ cycloalkyl, 3-10-

membered heterocyclic, carboxylic acid, amide, ester, sulfite, sulfonate, phosphoric acid, pyrophosphoric acid, natural or unnatural amino acid residues polyethylene glycol, or a combination thereof;

In one embodiment, L^1 and L^2 are the same or different, each independently selected from, without limitation, carboxyl,



In one embodiment, L^1 and L^2 are the same or different, each independently selected from, without limitation, the carboxyl group,



- 10 L^3 and L^4 are the same or different, each independently selected from, without limitation, the combination of one or more of substituted or unsubstituted C_1 - C_6 alkylene, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10-membered heterocyclic, or polyethylene glycol;

In one embodiment, L^3 and L^4 are the same or different, each independently selected from, without limitation, substituted or unsubstituted C_1 - C_6 alkylene;

In one embodiment, L^3 and L^4 are the same or different, each independently selected from, without limitation, C_1 - C_6 alkylene;

In one embodiment, L^3 and L^4 are the same or different, each independently selected from, without limitation, methylene, ethylene;

- 20 L^5 and L^6 are each independently present or absent, and when present are identical or different, and are selected from, without limitation, hydrogen, deuterium, oxygen, sulfur, hydroxyl, carbonyl, amide, ester, tert-butoxycarbonyl (Boc), benzyloxycarbonyl (Cbz), allyloxycarbonyl (Alloc), benzyl, substituted or unsubstituted C_1 - C_6 alkylidene, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10-membered heterocyclic, polyethylene glycol, or a combination thereof;

In one embodiment, L^6 is absent, L^5 is present, and L^5 is selected from, without limitation, hydrogen, substituted or unsubstituted C_1 - C_6 alkyl;

In one embodiment, L^6 is absent, L^5 is present, and L^5 is selected, without limitation from hydrogen, C_1 - C_6 alkyl;

- 30 In one embodiment, L^6 is absent, L^5 is present, and L^5 is hydrogen;

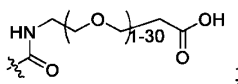
L^7 is selected from the group consisting of one or more combinations of a substituted or unsubstituted C_1 - C_6 alkylene group, a substituted or unsubstituted C_2 - C_6 alkenyl group, a substituted or unsubstituted C_2 - C_6 alkynyl group, an aryl group, a heteroaryl group, a C_3 - C_{10} cycloalkyl group, a 3-10-membered heterocycloalkyl group, a carbonyl group, an amide group, an ester group, a sulfinyl group, a sulfonate group, a phosphoric acid group, a pyrophosphoric acid group, a natural or unnatural amino acid residue, or a polyethylene glycol;

In one embodiment, L^7 is carbonyl;

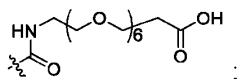
L^8 is selected from the group consisting of hydrogen, deuterium, substituted or unsubstituted

C₁-C₆ alkyl, substituted or unsubstituted C₂-C₆ alkenyl, substituted or unsubstituted C₂-C₆ alkynyl, aryl, heteroaryl, C₃-C₁₀ cycloalkyl, 3-10-membered heterocyclic, carboxylic acid, amide, ester, sulfinyl, sulfonate, phosphoric acid, pyrophosphoric acid, natural or unnatural amino acid residues, polyethylene glycol, or a combination thereof;

In one embodiment, L⁸ is selected from the carboxyl group,



In one embodiment, L⁸ is selected from the carboxyl group,



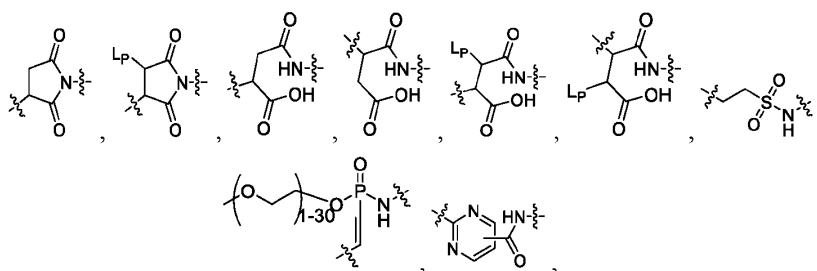
L⁹ is selected from the group consisting of one or more combinations selected from the group consisting of oxygen, sulfur, carbonyl, amide, ester, substituted or unsubstituted C₁-C₆ alkylene, substituted or unsubstituted C₂-C₆ alkenyl, substituted or unsubstituted C₂-C₆ alkynyl, aryl, heteroaryl, C₃-C₁₀ cycloalkyl, 3-10-membered heterocycloalkyl, or polyethylene glycol;

L¹⁰ present or absent, when present is selected from hydrogen, deuterium, oxygen, hydroxyl, carbonyl, amide, ester, tert-butoxycarbonyl (Boc), benzyloxycarbonyl (Cbz), allyloxycarbonyl (Alloc), benzyl, substituted or unsubstituted C₁-C₆ alkylene, substituted or unsubstituted C₂-C₆ alkenyl, substituted or unsubstituted C₂-C₆ alkynyl, aryl, heteroaryl, C₃-C₁₀ cycloalkyl, 3-10-membered heterocyclic, polyethylene glycol, or a combination thereof;

In one embodiment, L¹⁰ is absent;

W³ and W⁴ are the same or different, each independently selected from, without limitation, a connecting group, in one embodiment, at least one of which is selected from a group comprising a succinimide structure; in one embodiment, the succinimide structure is a closed-ring form, an open-ring form or any combination thereof; in one embodiment, the succinimide structure is in an open-ring form or any combination of closed-ring form and open-ring form; in one embodiment, the succinimide structure is in a ring-opening form;

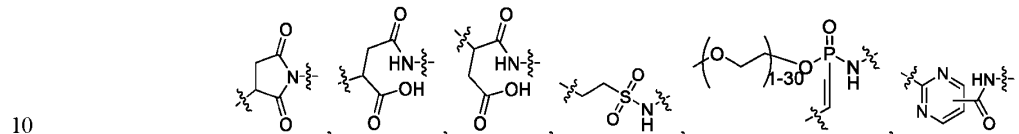
In one embodiment, W³ and W⁴ are the same or different, each independently selected from, without limitation, the following structures or any combination of the following racemates, enantiomers, diastereomers, pharmaceutically acceptable salts or solvates thereof, or the following structures:



In one embodiment, at least one of W³ and W⁴ is selected from groups comprising the structure of the butyrylimine; in one embodiment, each L_P is selected from the same or a different

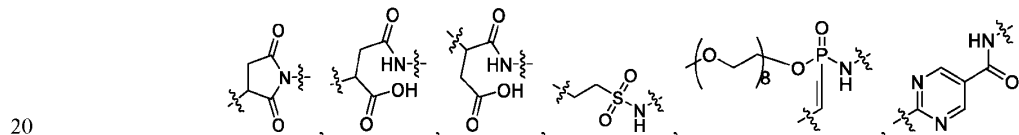
group, including, but not limited to C1- C6 alkyl groups, chlorine, bromine, iodine, OMs, OTs; in one embodiment, the succinimide structure is a closed-ring form, an open-ring form or any combination thereof; in one embodiment, the succinimide structure is in an open-ring form or any combination of closed-ring form and open-ring form; in one embodiment, the succinimide structure is in a ring-opening form;

In one embodiment, W^3 and W^4 are the same or different, each independently selected from, without limitation, the following structures or any combination of the following structures or its racemates thereof, enantiomers, diastereomers, pharmaceutically acceptable salts or solvates or the following structures:



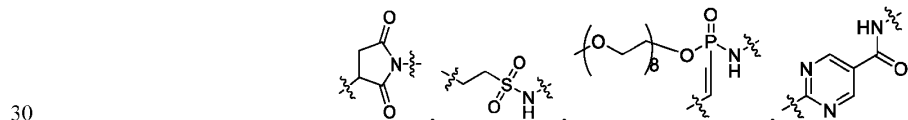
In one embodiment, at least one of W^3 and W^4 is selected from a group comprising the structure of the succinimide; in one embodiment, the succinimide structure is a closed-ring form, an open-ring form or any combination thereof; in one embodiment, the succinimide structure is in an open-ring form or any combination of closed-ring form and open-ring form; in one embodiment, the succinimide structure is in a ring-opening form;

In one embodiment, W^3 and W^4 are the same or different, each independently selected from, without limitation, the following structures or any combination of the following structures or racemates thereof, enantiomers, diastereomers, pharmaceutically acceptable salts or solvates or the following structures:



In one embodiment, at least one of W^3 and W^4 is selected from a group comprising the structure of the succinimide; in one embodiment, the succinimide structure is a closed-ring form, an open-ring form or any combination thereof; in one embodiment, the succinimide structure is in an open-ring form or any combination of closed-ring form and open-ring form; in one embodiment, the succinimide structure is in a ring-opening form;

In one embodiment, W^3 and W^4 are the same or different, each independently selected from, without limitation, the following structures or any combination of the following structures or its racemates, enantiomers, diastereomers, pharmaceutically acceptable salts or solvates thereof, or any combination of the following structures:



In one embodiment, at least one of W^3 and W^4 is selected from a group comprising the structure of the succinimide; in one embodiment, the succinimide structure is a closed-ring form, an open-

ring form or any combination thereof; in one embodiment, the succinimide structure is in an open-ring form or any combination of closed-ring form and open-ring form; in one embodiment, the succinimide structure is in a ring-opening form;

Z is N, O, S or quaternary amines;

In one embodiment, Z is N;

L is a linking moiety;

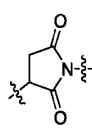
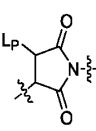
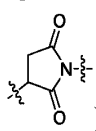
n is an integer or a decimal from 1 to 10 (e.g., 1-1.5, 1.5-2, 2-2.5, 2.5-3, 3-3.5, 3.5-4, 4-4.5, 4.5-5, 5-5.5, 5.5-6, 6-6.5, 6.5-7, 7-7.5, 7.5-8, 8-8.5, 8.5-9, 9-9.5, or 9.5-10);

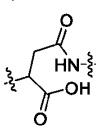
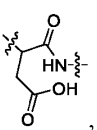
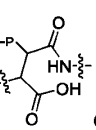
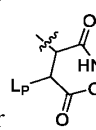
D is a drug unit. In one embodiment, D may be a drug and/or a drug precursor. In one embodiment, the drug unit D is linked to L by a covalent bond;

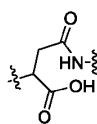
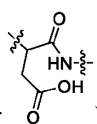
In one embodiment, D is a drug and said drug is linked to L by a covalent bond;

In one embodiment, D is a drug precursor and said drug precursor is linked to L by a covalent bond.

In the ligand-drug conjugate of the present disclosure, although the succinimide structure in the chemical structural formula shows a closed-ring form, it actually covers three situations: full closed ring, partial ring opening and full ring opening of succinimide structure. Specifically, in the ligand-drug conjugate of the disclosure, the succinimide structure comprises a closed-ring form, an open-ring form or any combination thereof, wherein the closed-ring form of the succinimide

structure is, for example,  or  (in one embodiment, ), the ring-opening

form of succinimide structure is, for example, , ,  or  (in one

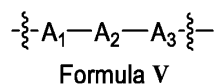
embodiment  or ), where Lp is as described above. In some embodiments, the succinimide structure is an open-ring form of succinimide structure, or any combination of closed-ring form and open-ring form. In some more embodiments, the succinimide structure is a ring-opening form of succinimide structure.

In some embodiments, Tg is a ligand or the targeting moiety thereof that binds to a target; In one embodiment, Tg is selected from an antibody, an antigen-binding protein, or a peptide; In one embodiment, Tg is an antibody.

In some embodiments, Tg that has the binding affinity to a target is selected from a monospecific antibody, a bispecific antibody, or a multispecific antibody, an anti-EGFRvIII antibody, an anti-DLL-3 antibody, an anti-PSMA antibody, an anti-CD70 antibody, an anti-MUC16 antibody, an anti-ENPP3 antibody, an anti-TDGF1 antibody, an anti-ETBR antibody, an anti-MSLN antibody, an anti-TIM-1 antibody, an anti LRRC15 antibody, anti-LIV-1 antibody, anti-CanAg/AFP antibody, anti-Claudin 18.2 antibody, anti-PLAUR antibody, anti-Mesothelin

antibody, anti-HER2(ErbB2) antibody, anti-EGFR antibody, anti-c-MET antibody, anti-SLITRK6 antibody, anti-KIT/CD117 antibody, anti-STEAP1 antibody, anti-STEAP1 antibody, and anti-SLITRK6 antibody. STEAP1 antibody, anti-SLAMF7/CS1 antibody, anti-NaPi2B/SLC34A2 antibody, anti-GPNMB antibody, anti-HER3(ErbB3) antibody, anti-HLA-G antibody, anti-MUC1/CD227 antibody, anti-EGFR/HER3 antibody, anti-AXL antibody, anti-GPC3 antibody, anti-CD166 antibody, anti-B7- H3(CD276) antibody, anti-PTK7/CCK4 antibody, anti-PRLR antibody, anti-EFNA4 antibody, anti-5T4 antibody, anti-NOTCH3 antibody, anti-Nectin 4 antibody, anti-TROP-2 antibody, anti-NKG2D antibody, anti-CD142 antibody, anti-CA6 antibody, anti-GPR20 antibody, anti-CD174 antibody, anti-CD71 antibody, anti-EphA2 antibody, anti-CD71 antibody, anti-EphA2 antibody, anti-LYPD3 antibody, anti-TF antibody, anti-FGFR2 antibody, anti-FGFR3 antibody, anti-FR α antibody, anti-CEACAMs antibody, anti-GCC antibody, anti-CXCR4 antibody, anti-GPRC5D antibody, anti-CCR5 antibody, anti-CCR3 antibody, anti-LGR4 antibody, anti-SSTR2 antibody, anti-integrin Av antibody, anti-CAIX antibody, anti-P-cadherin antibody, anti-GD3 antibody, anti-Cadherin 6 antibody, anti-LAMP1 antibody, anti-FLT3 antibody, anti-BCMA antibody, anti-CD79b antibody, anti-CD19 antibody, anti-ROR1 antibody, anti-CD33 antibody, anti-CD45 antibody, anti-CD56 antibody, anti-CD74 antibody, anti-CD22 antibody, anti-CD30 antibody, anti-CD37 antibody, anti-CD138 antibody, anti-Nectin-4 antibody, anti-CD352 antibody, anti-CD25 antibody, or anti-CD123 antibody and combinations thereof to form a double or multiple antibody, e.g., an anti-TROP-2 antibody.

In some embodiments, the linking moiety L has the structure shown in Formula V below,



wherein,

A₁, A₂, and A₃ each are present or absent, independently;

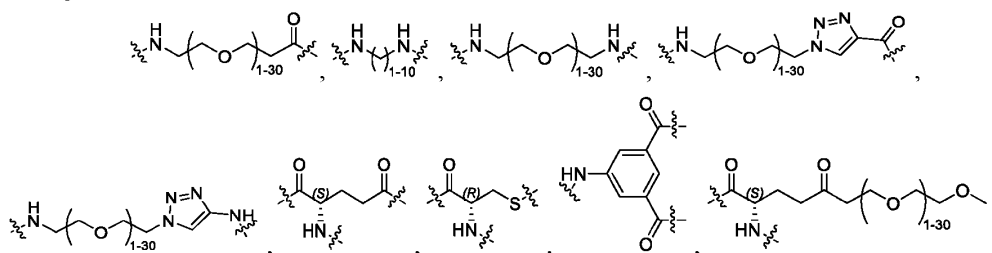
when A₁ is present, A₁ is selected from a modifying unit, in one embodiment, from a branchable unit and/or a hydrophilic unit;

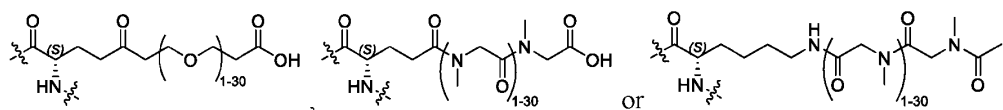
when A₂ is present, A₂ is selected from a breakable unit or a non-breakable unit;

when A₃ is present, A₃ is selected from an elimination unit and/or a chelation unit;

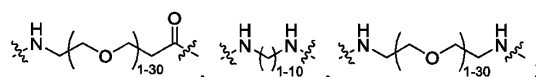
the position shown by the wavy line is the connection site.

In some embodiments, when A₁ is present, A₁ is selected from, without limitation, the following structures or combinations thereof:

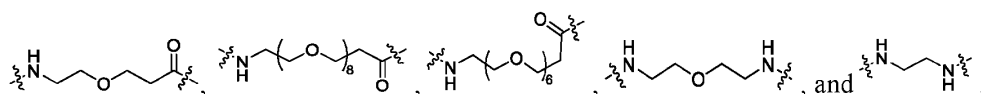




In one embodiment, when A₁ is present, A₁ is selected from, without limitation, the following structures or combinations thereof:



In one embodiment, when A₁ is present, A₁ is selected from, without limitation, the following structures or combinations thereof:



wherein the positions shown by the wavy lines are connection sites.

- In some embodiments, when A₂ is present, said A₂ is selected from, without limitation, a breakable or unbreakable unit, wherein the unbreakable unit is non-limitingly selected from a substituted or unsubstituted C₁-C₆ alkyl group, a substituted or unsubstituted C₂-C₆ alkenyl group, a substituted or unsubstituted C₂-C₆ alkynyl group, an aryl, a heteroaryl group, a C₃-C₁₀ cycloalkyl group, a 3-10-membered heterocycloalkyl group, an amide, an ester group, or a polyethylene glycol, or a combination thereof.

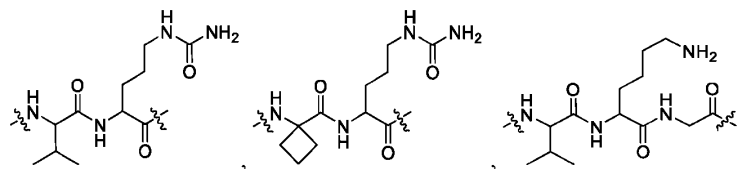
In some embodiments, when A₂ is present, said A₂ is selected from, without limitation, a breakable or non-breakable unit, wherein the breakable unit is selected from, without limitation, a non-enzymatically sensitive connection unit, an enzymatically sensitive connection unit, or a combination thereof;

- In one embodiment, said non-enzymatically sensitive linker unit is selected from, without limitation, glutathione-sensitive disulfide bond linker unit, pH-sensitive hydrazone linker unit;

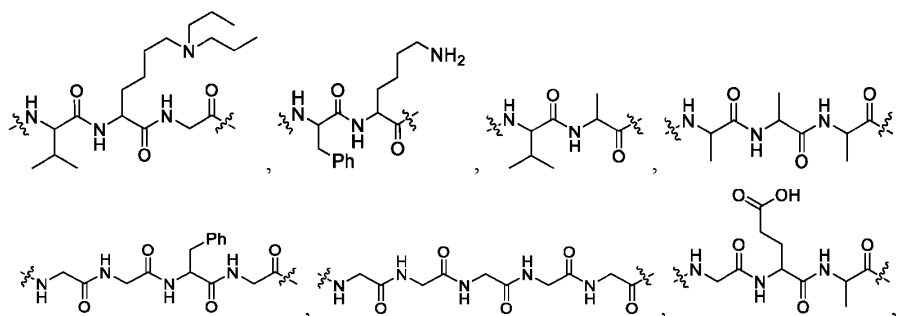
In one embodiment, said enzymatically sensitive connection unit is selected from, without limitation, a histone protease breakable connection unit, a phosphodiesterase and/or pyrophosphate esterase breakable connection unit, a β -glucuronidase breakable connection unit, a β -galactosidase breakable connection unit, a sulfate esterase breakable connection unit or a combination thereof.

In some embodiments, when A₂ is present, said A₂ is a breakable unit, wherein said breakable unit is selected from, without limitation, a peptide residue comprising 2-10 natural or unnatural amino acid residues, said natural or unnatural amino acids optionally further substituted with one or more substituents.

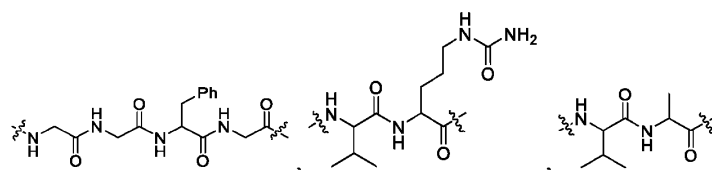
In one embodiment, said breakable unit is selected from, without limitation:



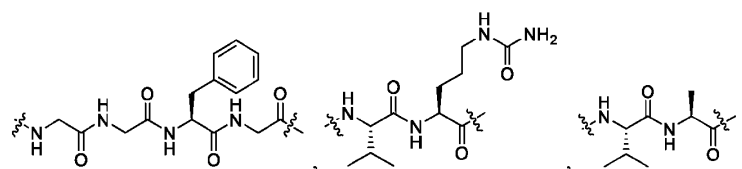
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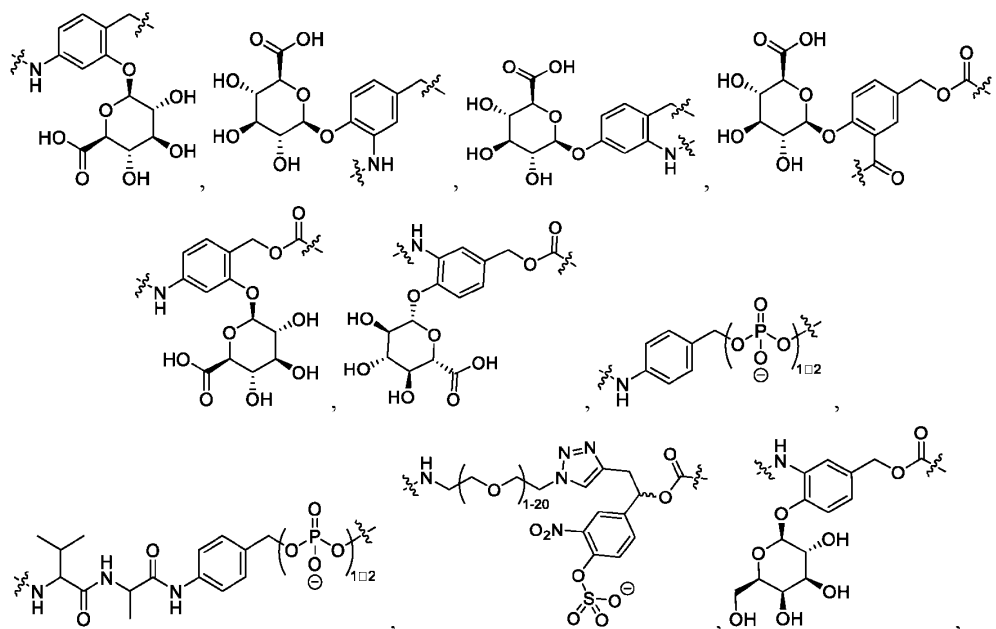
in one embodiment, said breakable unit is selected from:

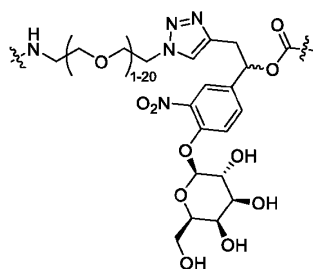


in one embodiment, said breakable unit selected from:

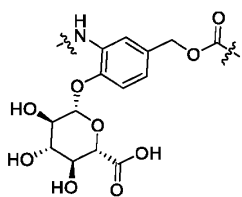


alternatively, said breakable unit is selected from, without limitation, the following structures or isomers thereof:



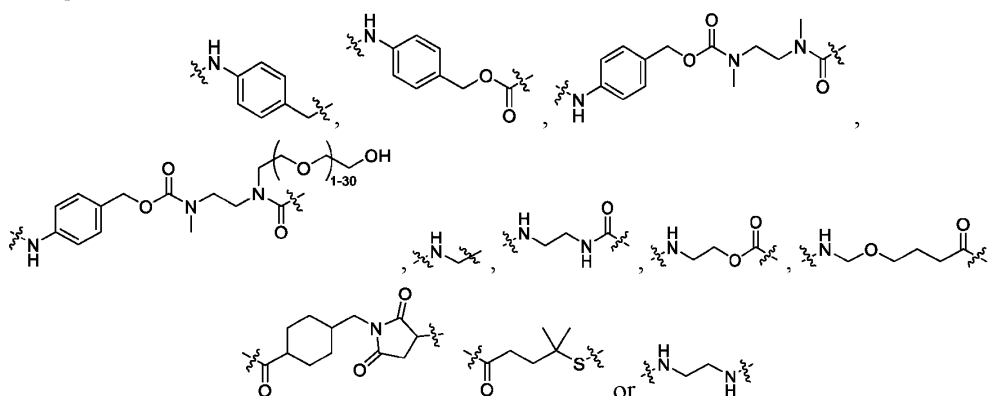


in one embodiment, said breakable unit is selected from the following structures or isomers thereof:

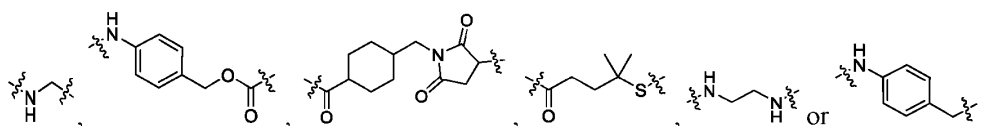


wherein the position shown by the wavy line is the connection site.

In some embodiments, when A₃ is present, A₃ is selected from, without limitation, the following structures:



10 In one embodiment, when A₃ is present, A₃ is selected from, without limitation, the following structures:



wherein the position shown by the wavy line is the connection site.

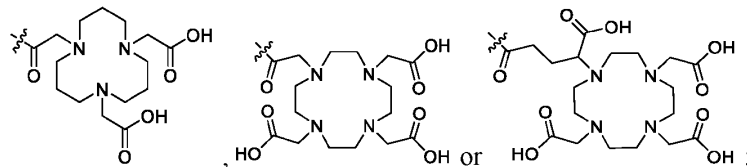
In some embodiments, said drug is selected from, without limitation, a cytotoxic drug, a radioisotope, a detection and/or diagnostic reagent, an enzyme inhibitor, a protein degrading agent, an immunomodulator, a peptide, or a nucleotide.

In some embodiments, said drug is a cytotoxic drug.

In some embodiments, said drug is obtained by chelation of a drug precursor with a metal ion,

such as a radioisotope.

In some embodiments, said drug precursor is selected from, without limitation,



In one embodiment, said drug precursor is obtained by chelating the drug with a metal ion, such as a radioisotope.

In some embodiments, said cytotoxic agent is selected from, without limitation, a DNA damaging agent, a DNA topoisomerase inhibitor, a microtubule and/or microtubule protein inhibitor, an RNA polymerase inhibitor, or a combination thereof.

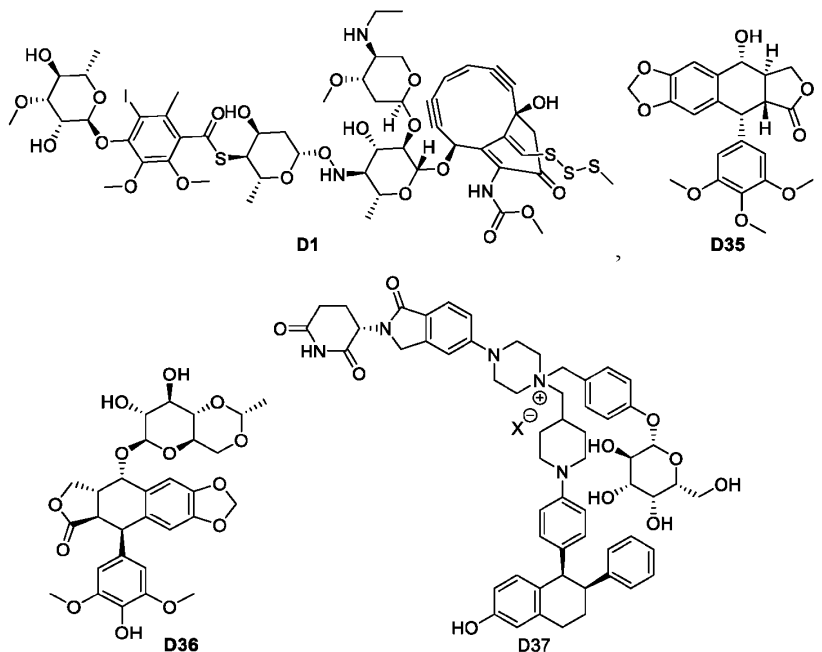
In one embodiment, said DNA damaging agents include, but are not limited to, calicheamicin, anthramycin, ecteinascidin toxin, adriamycin, mitomycin, duocarmycin, or a combination thereof.

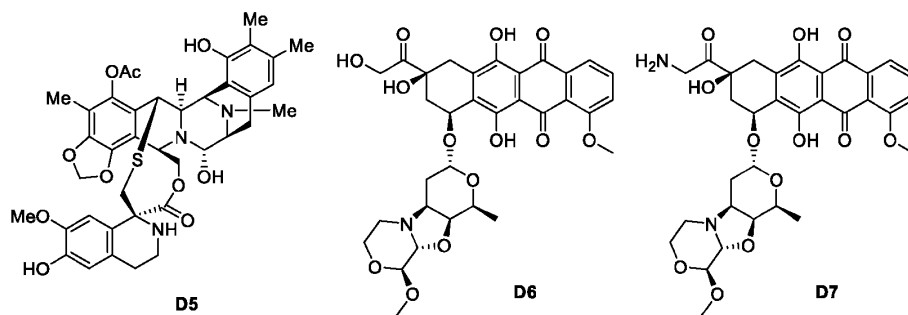
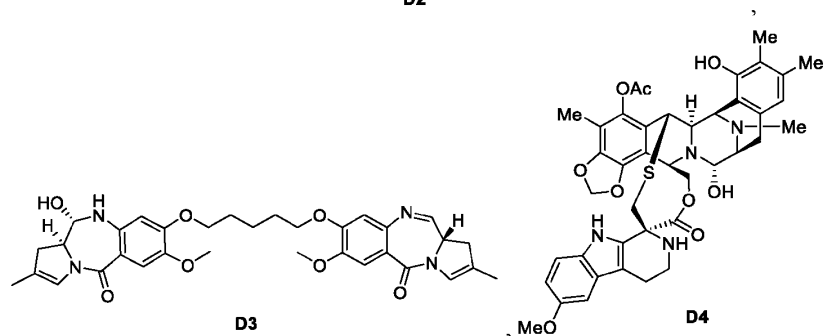
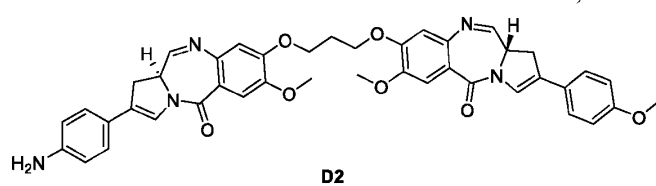
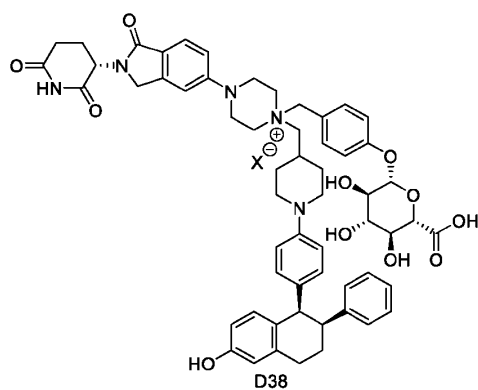
In one embodiment, said DNA topoisomerase inhibitors include, but are not limited to, camptothecin derivatives, podophyllotoxin derivatives, etoposide derivatives, or a combination thereof.

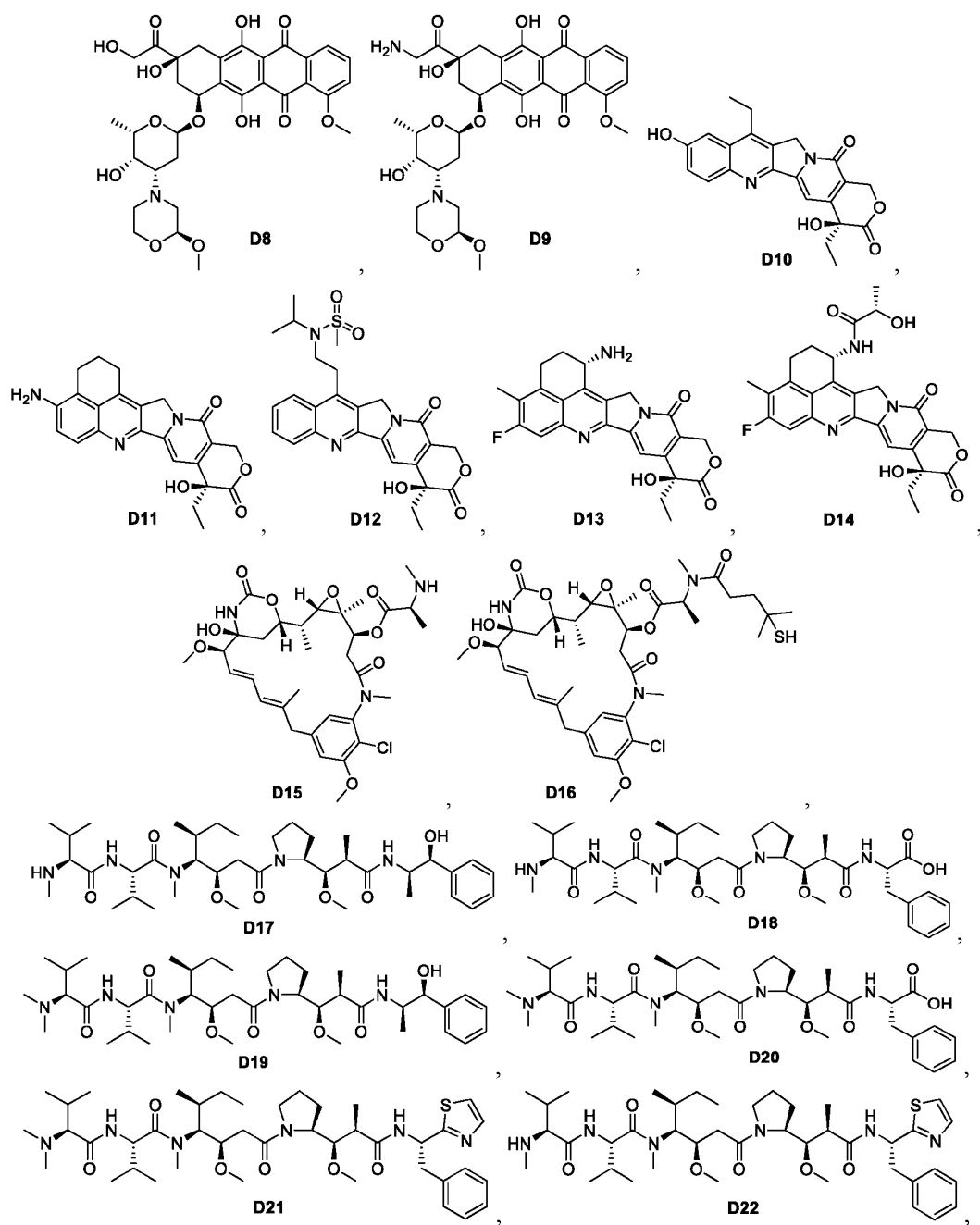
In one embodiment, said microtubule and/or microtubule protein inhibitors include, but are not limited to, maytansine, auristatin-like compounds, eribulin, tubulysin, or a combination thereof.

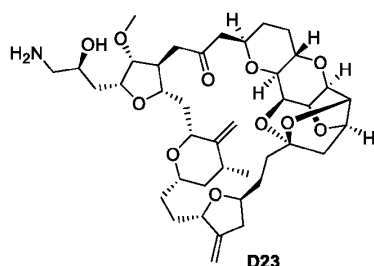
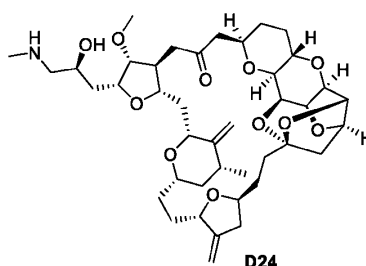
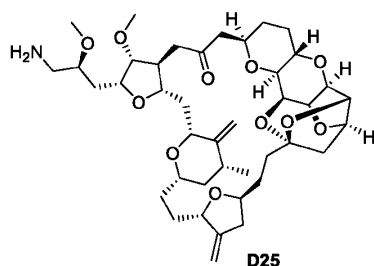
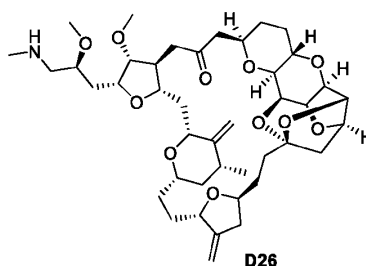
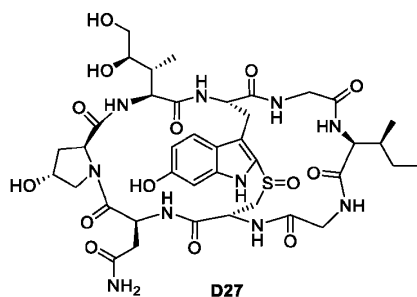
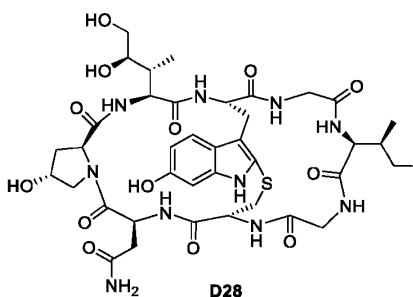
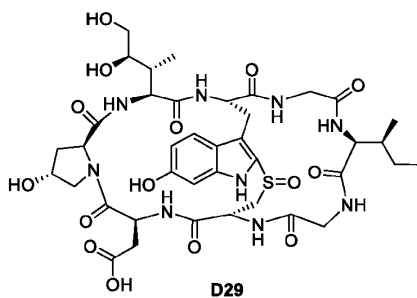
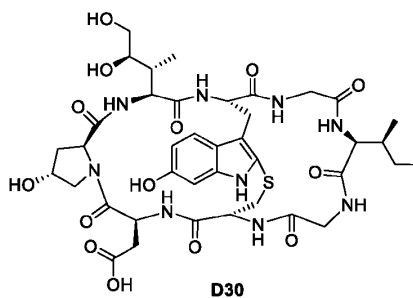
In one embodiment, said RNA polymerase inhibitors include, but are not limited to, amanitin peptide derivatives, or a combination thereof.

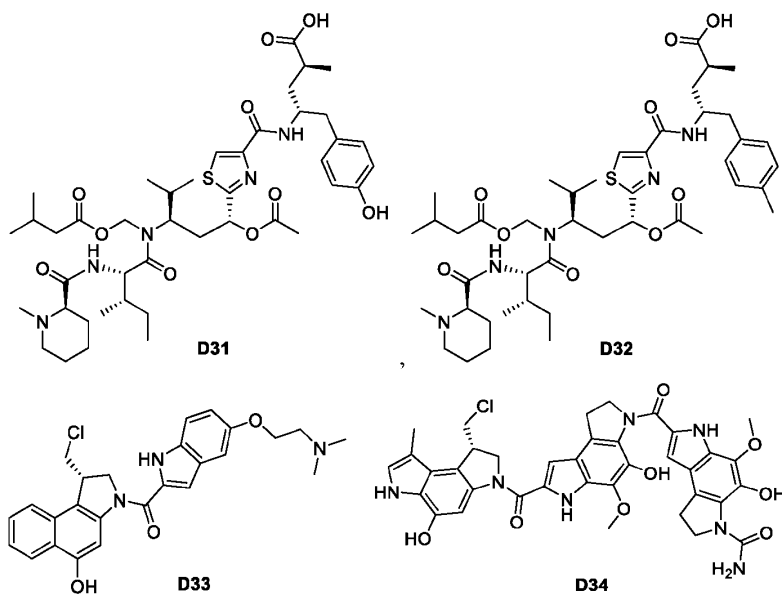
In some embodiments, said drug is selected from, without limitation, the following structures:





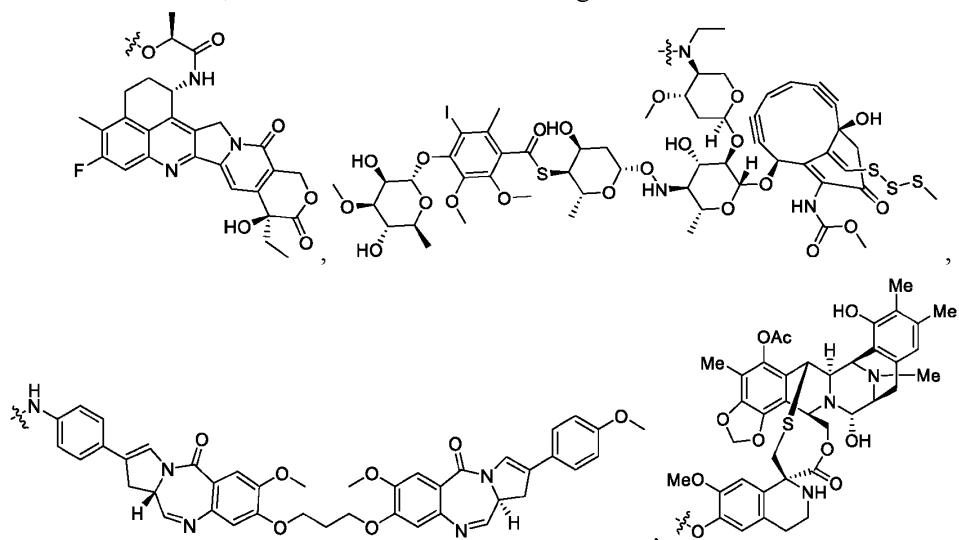


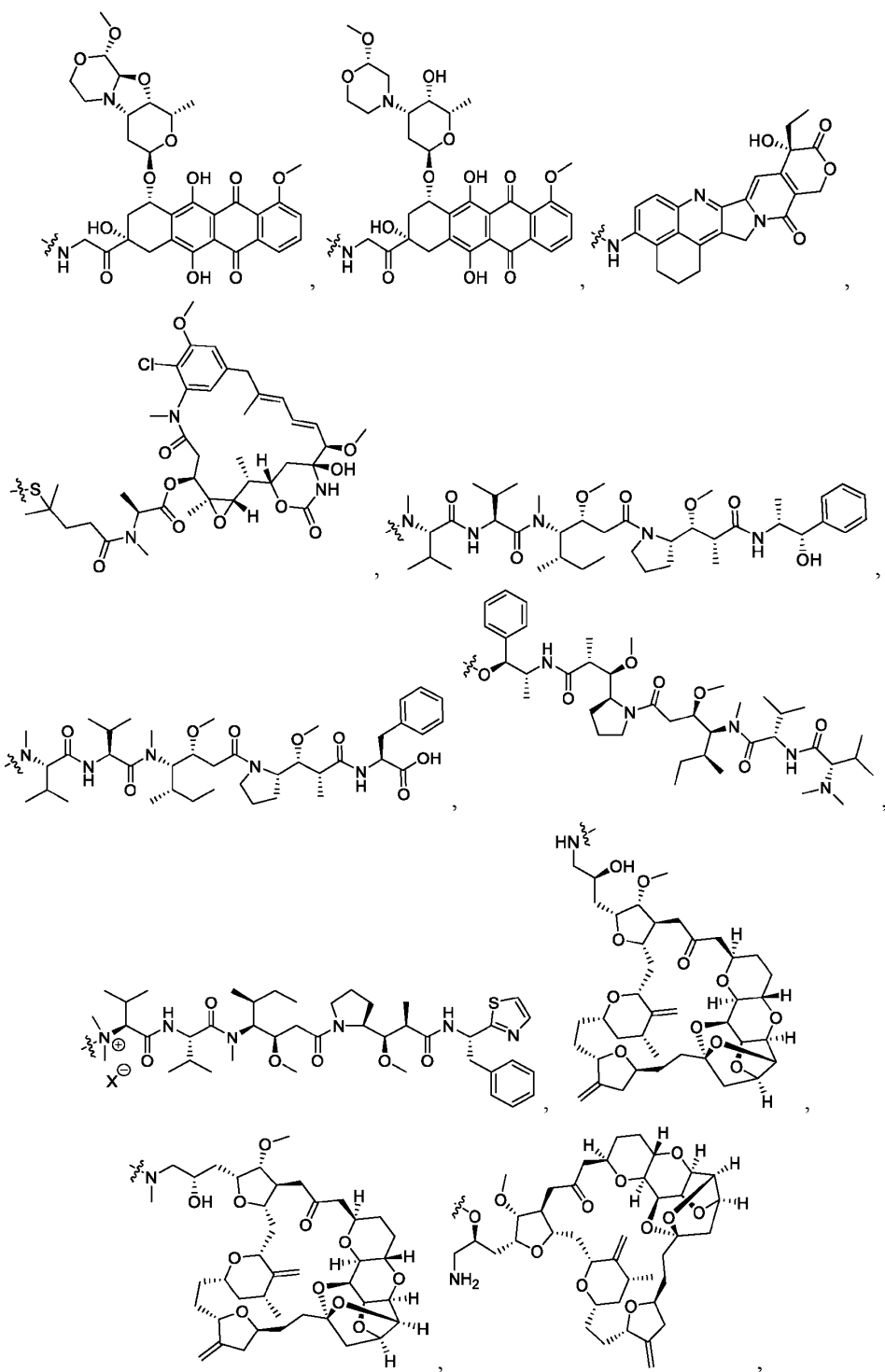
**D23****D24****D25****D26****D27****D28****D29****D30**

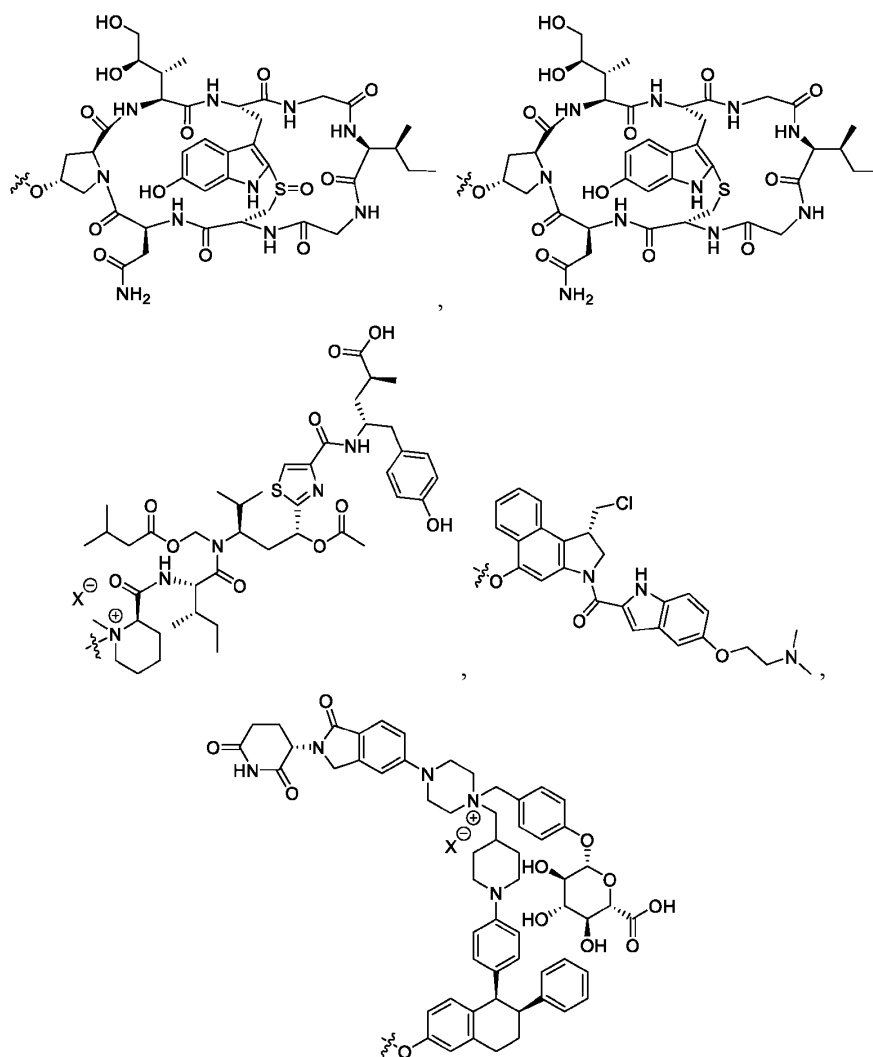


wherein each X^- is independently selected from Cl^- , Br^- , I^- , $MeSO_3^-$, CF_3COO^- , in one embodiment, CF_3COO^- ;

In one embodiment, D is selected from the following structures:



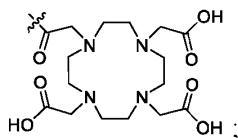




wherein each X^- is independently selected from Cl^- , Br^- , I^- , $MeSO_3^-$, CF_3COO^- , in one embodiment, CF_3COO^- .

In some embodiments, said drug is obtained by chelation of a drug precursor with a metal ion, such as a radioisotope.

In some embodiments, said drug precursor includes, but is not limited to, the following structure,



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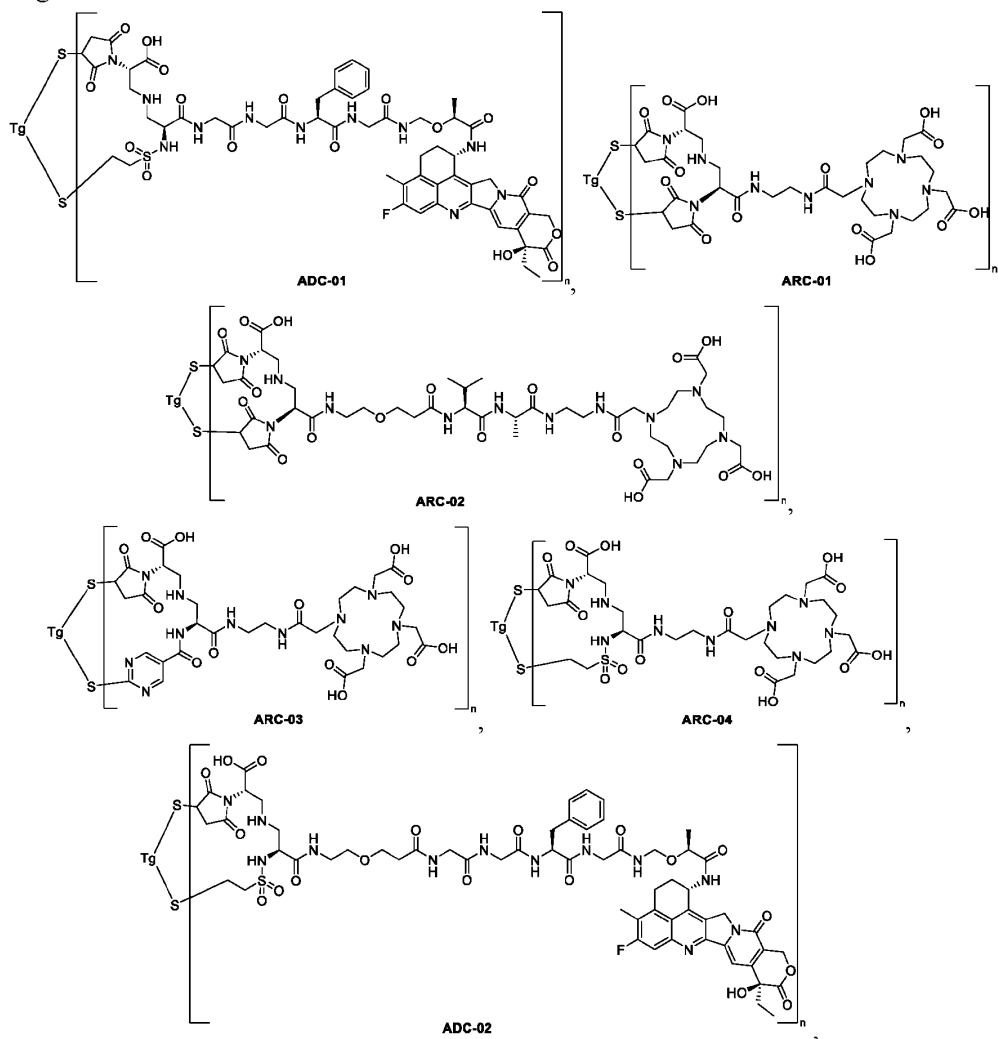
In one embodiment, said drug precursor is obtained by chelating the drug with a metal ion,

such as a radioisotope.

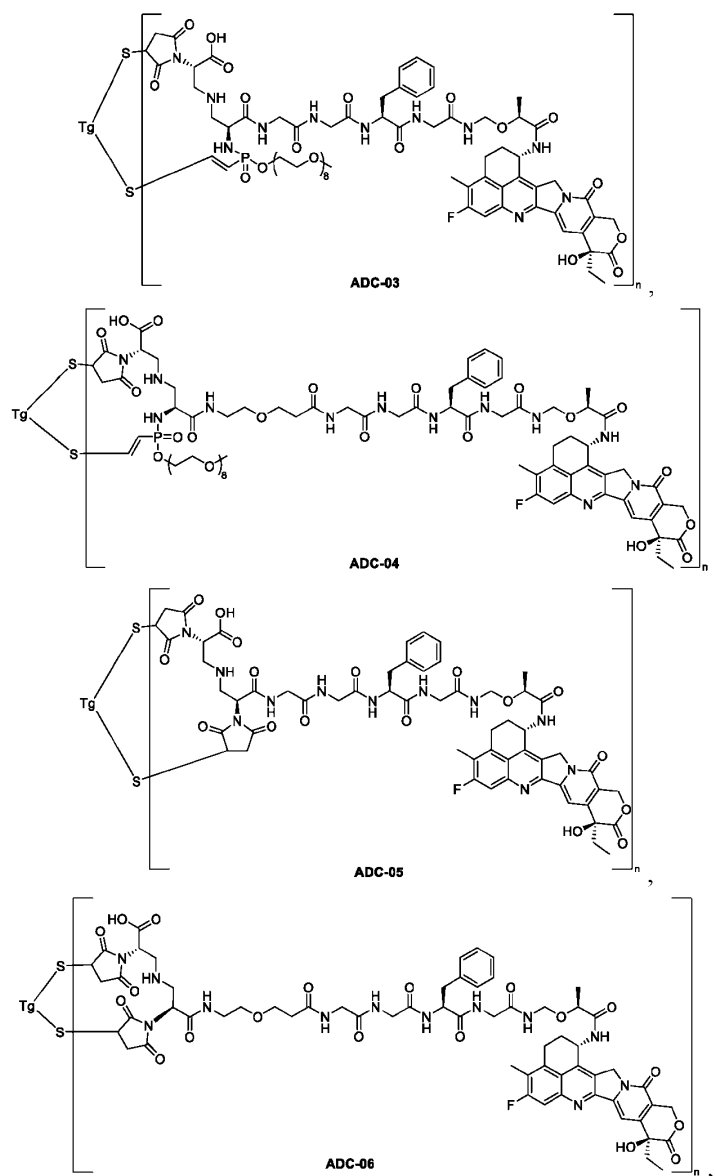
In some embodiments, said radioisotope is selected from, without limitation, ^{18}F , ^{67}Ga , ^{68}Ga , $^{99\text{m}}\text{Tc}$, ^{123}I , ^{125}I , ^{131}I , ^{90}Y , ^{177}Lu , ^{47}Sc , ^{64}Cu , ^{67}Cu , ^{32}P , ^{186}Re , ^{188}Re , ^{89}Sr , ^{153}Sm , ^{198}Au , ^{166}Ho , ^{165}Dy , ^{169}Er , ^{149}Tb , ^{161}Tb , ^{211}At , ^{212}Bi , ^{213}Bi , ^{212}Pb , ^{223}Ra , ^{225}Ac , or a combination thereof.

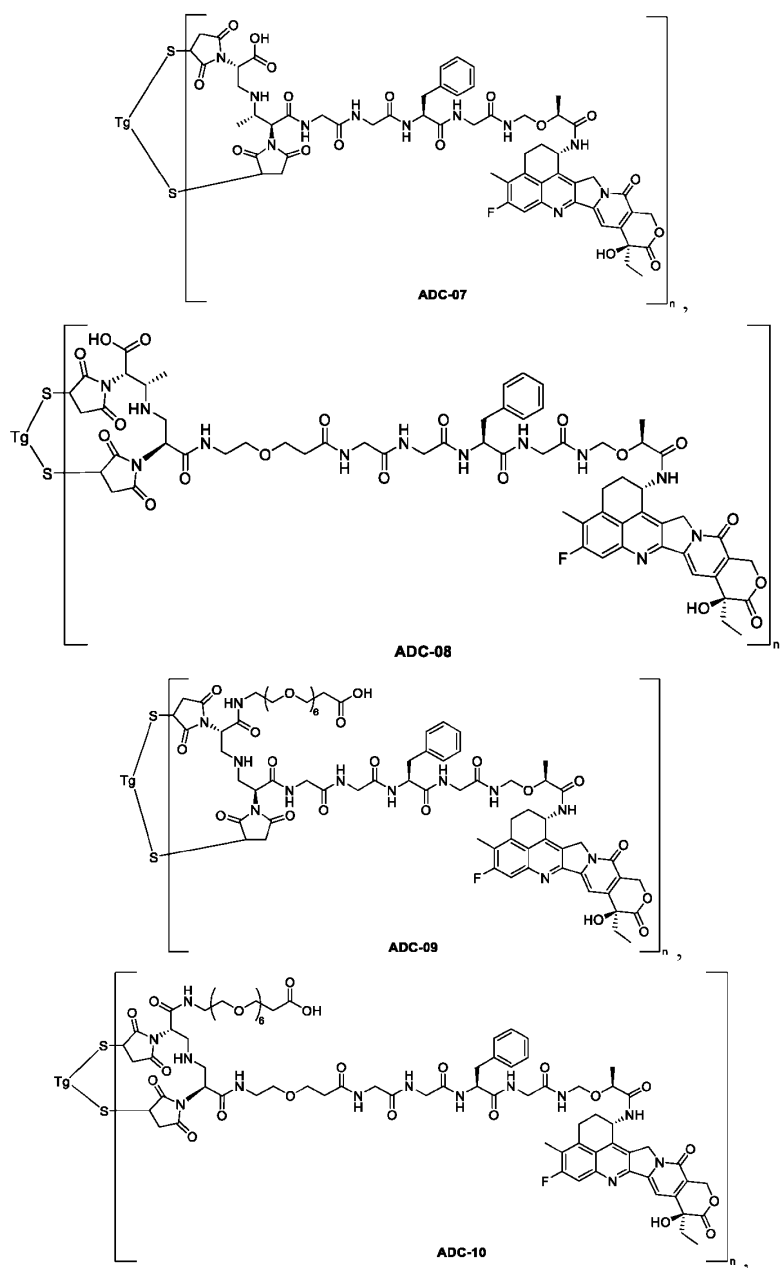
In one embodiment, said radioisotope is selected from, without limitation, ^{68}Ga , $^{99\text{m}}\text{Tc}$, ^{131}I , ^{90}Y , ^{177}Lu , ^{64}Cu , ^{211}At , ^{213}Bi , ^{212}Pb , ^{225}Ac , or a combination thereof.

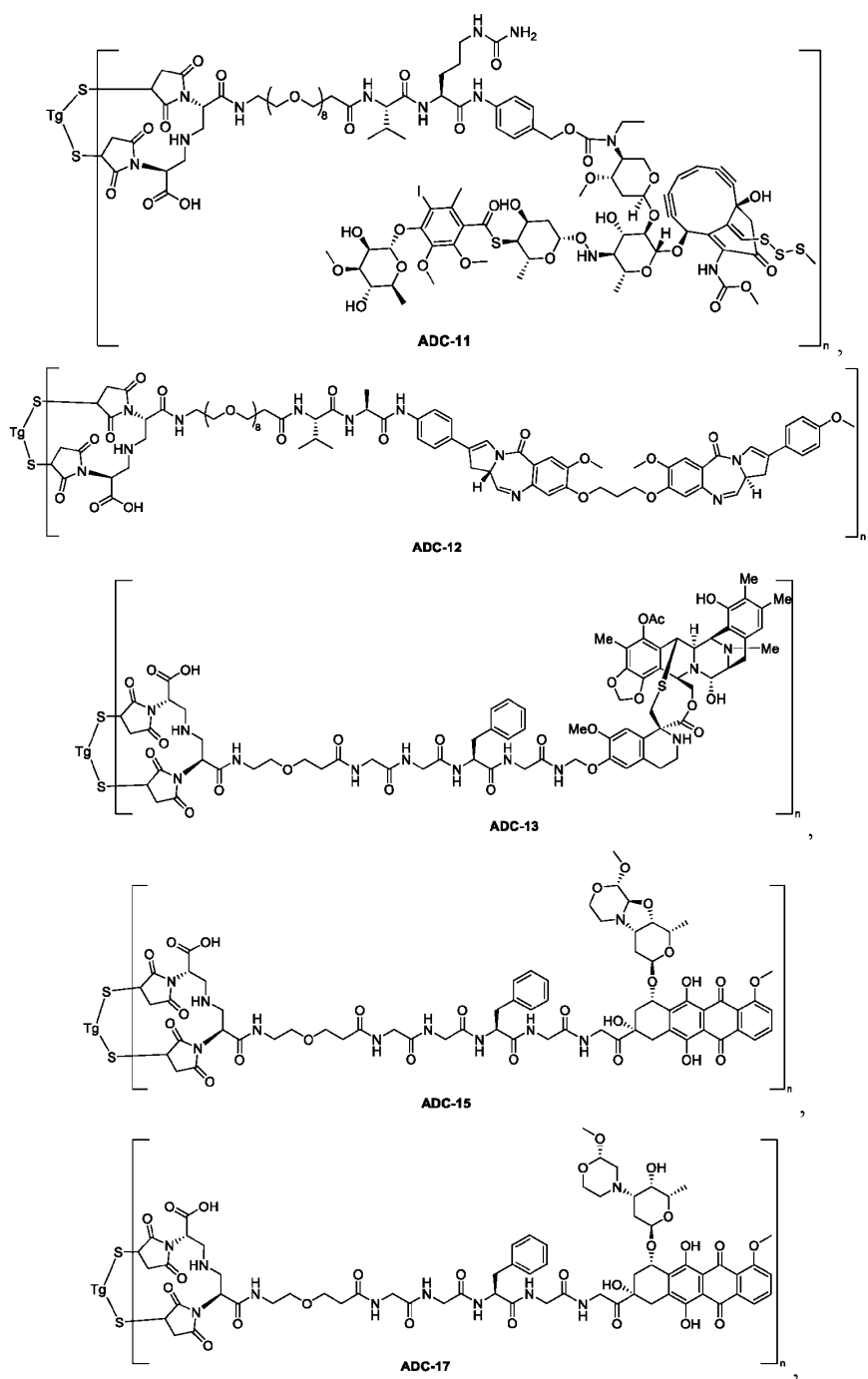
In some embodiments, said ligand-drug conjugate is selected from, without limitation, the following structures:

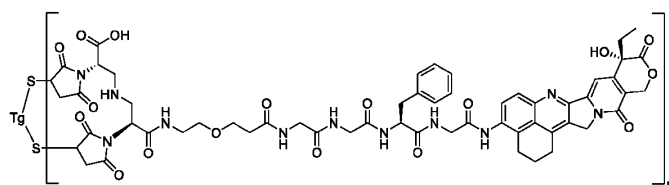


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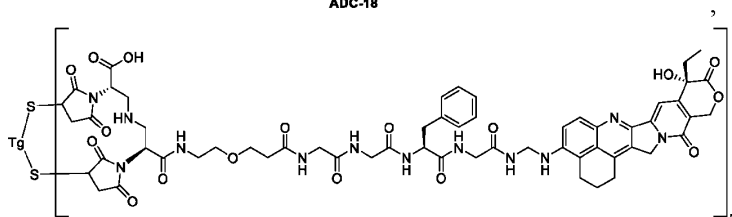




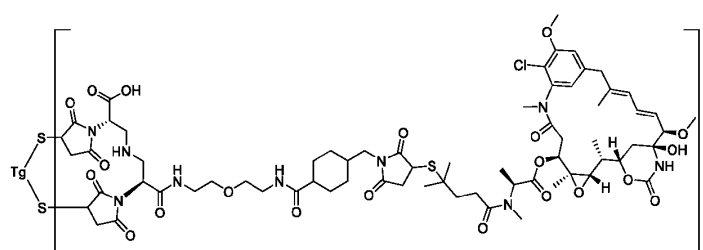




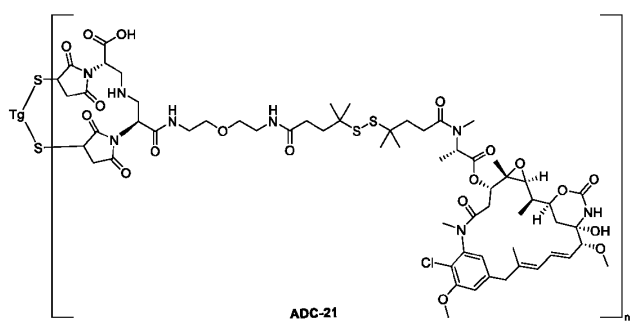
ADC-18



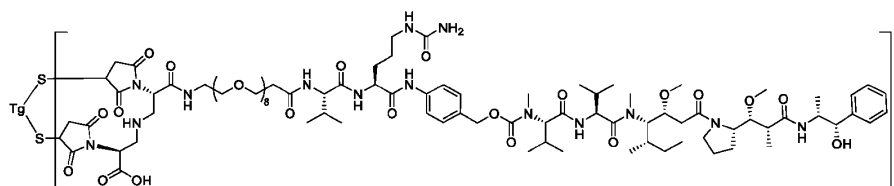
ADC-19



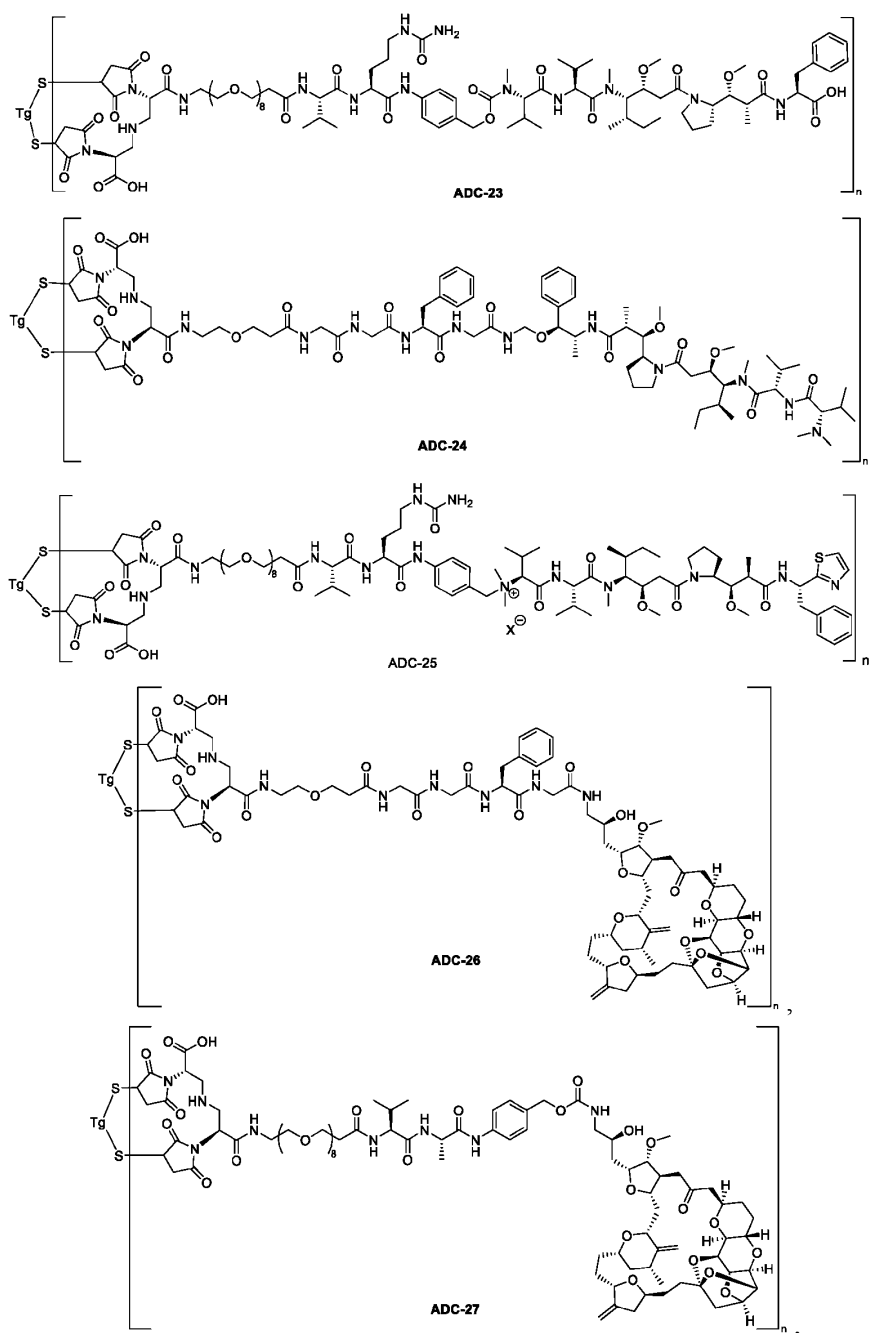
ADC-20

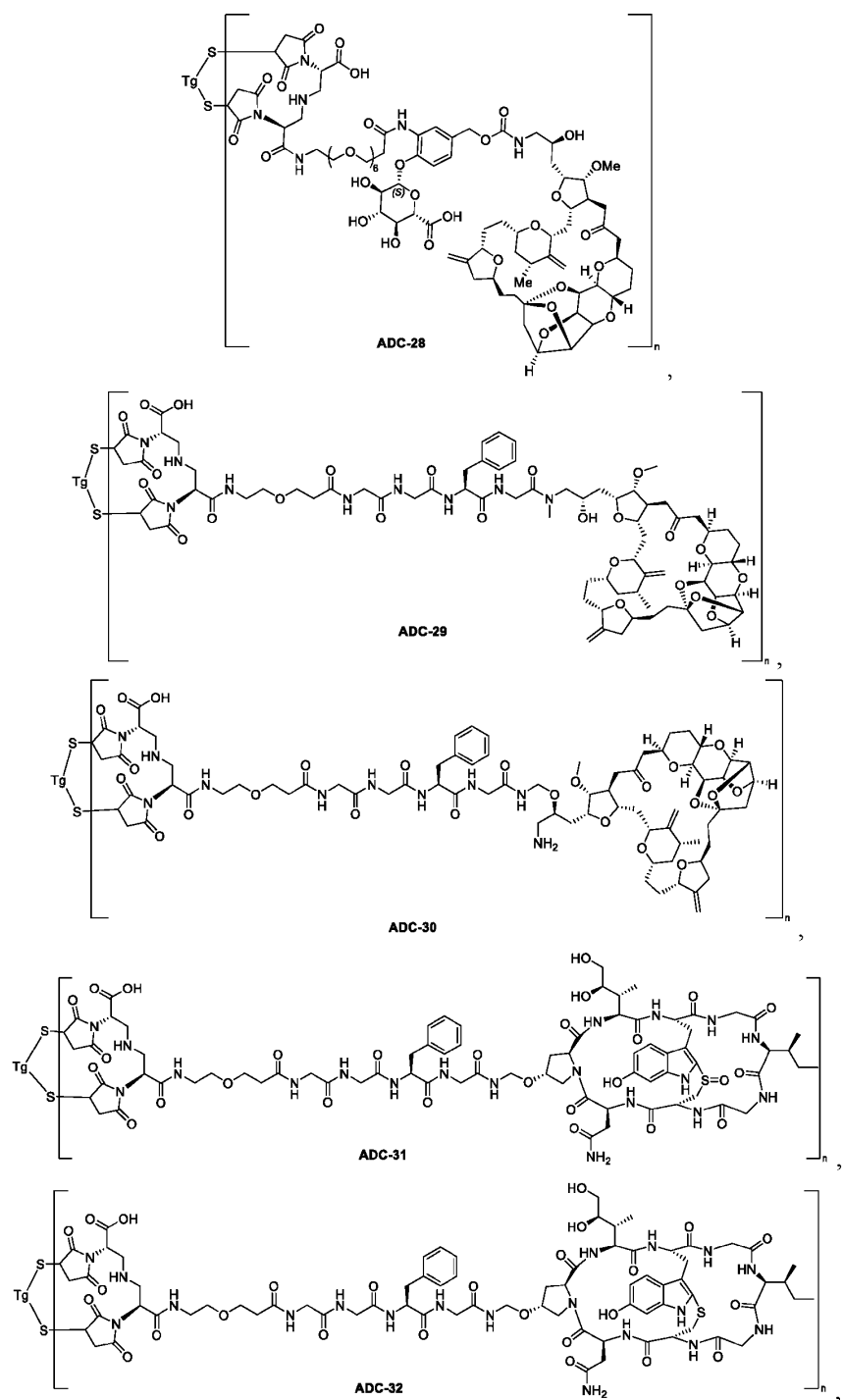


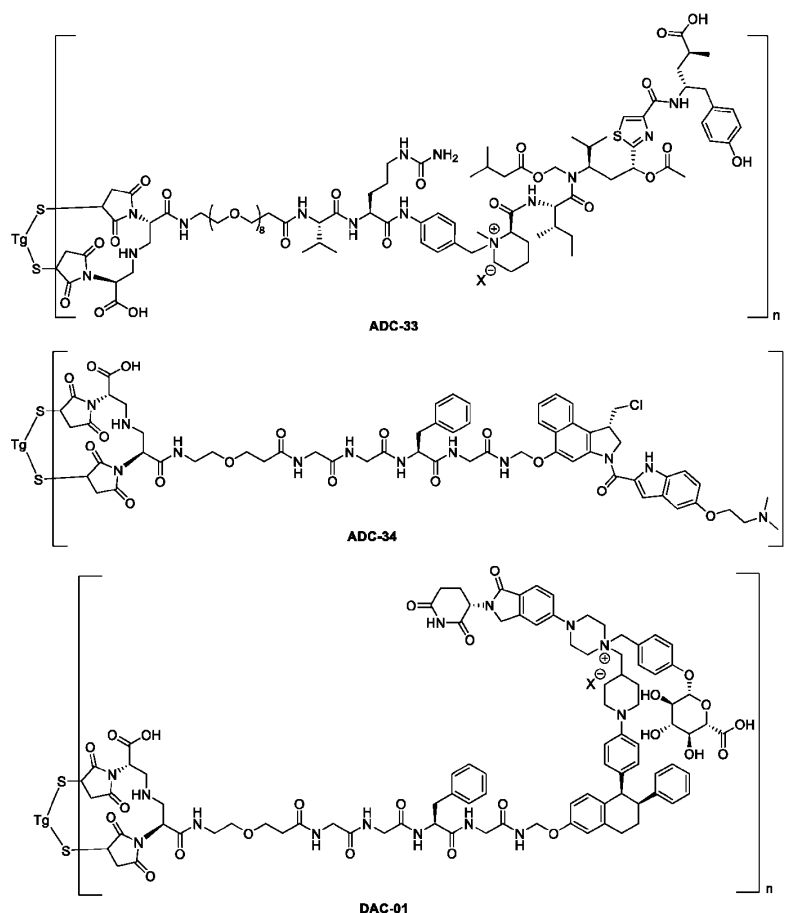
ADC-21



ADC-22







wherein,

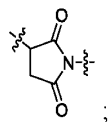
each X^- is independently selected from Cl^- , Br^- , I^- , $MeSO_3^-$, CF_3COO^- , in one embodiment, CF_3COO^- ;

n is an integer or a decimal from 1 to 10. In one embodiment, n is an integer or a decimal from 1-1.5, 1.5-2, 2-2.5, 2.5-3, 3-3.5, 3.5-4, 4-4.5, 4.5-5, 5-5.5, 5.5-6, 6-6.5, 6.5-7, 7-7.5, 7.5-8, 8-8.5, 8.5-9, 9-9.5, or 9.5-10.

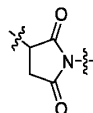
10 Tg is selected from ligands, in one embodiment, from antibodies;

In one embodiment, when the ligand-drug conjugate contains a succinimide group, the ligand-drug conjugate can be hydrolyzed under hydrolysis conditions, and with different degrees of hydrolysis, the following scenarios can occur:

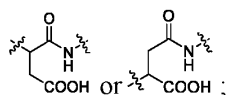
(1) succinimide moieties are not hydrolyzed at all, i.e., the succinimide moieties are all in the closed ring form



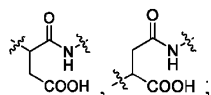
- (2) incomplete hydrolysis of the succinimide moiety, i.e., part of the succinimide moiety is in the closed-ring form,



and the other part of the succinimide moiety is in the open-ring form



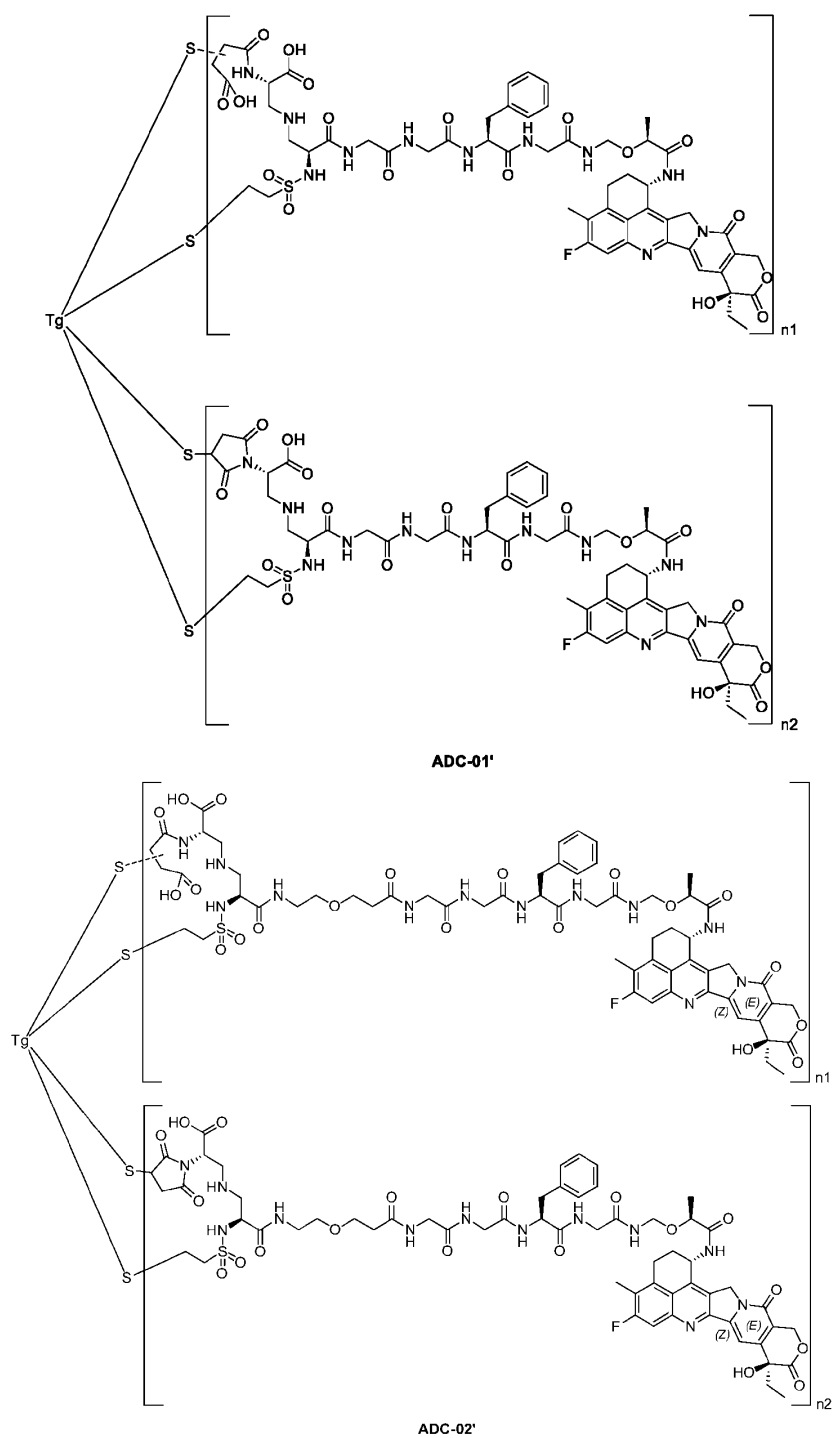
- (3) the succinimide moieties are completely hydrolyzed, i.e., the succinimide moieties are all in the ring-opened form

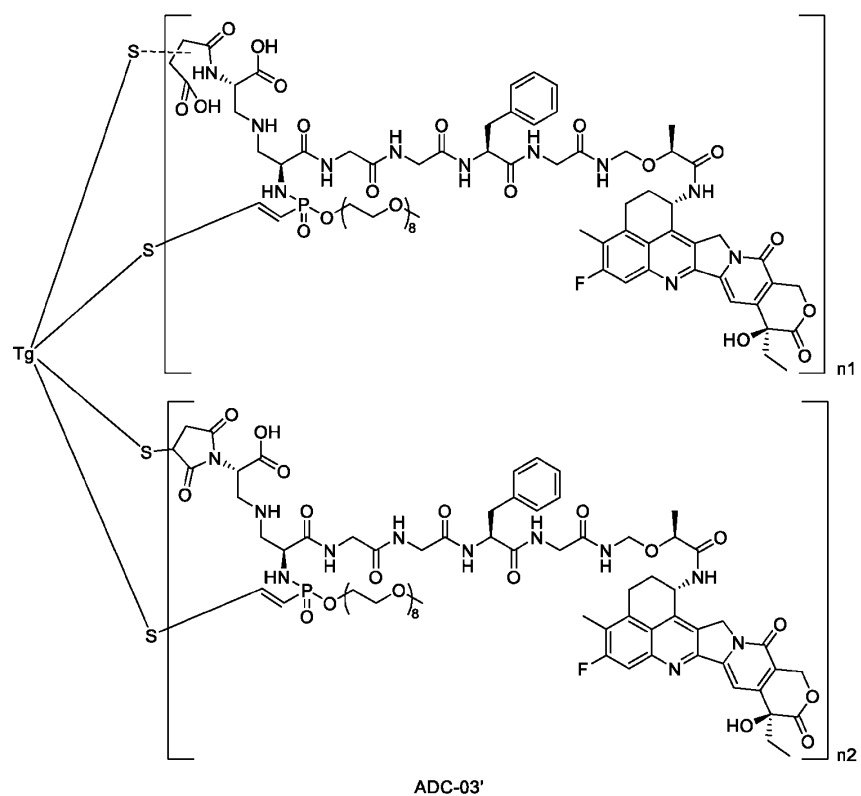


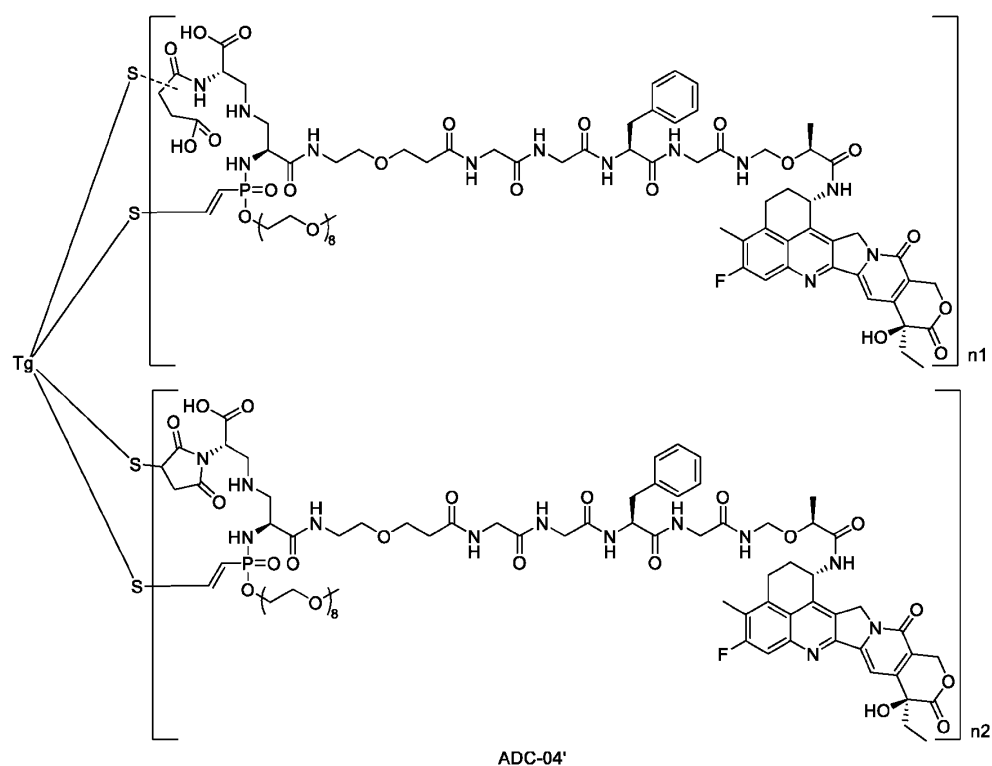
positions shown by wavy lines are connection sites;

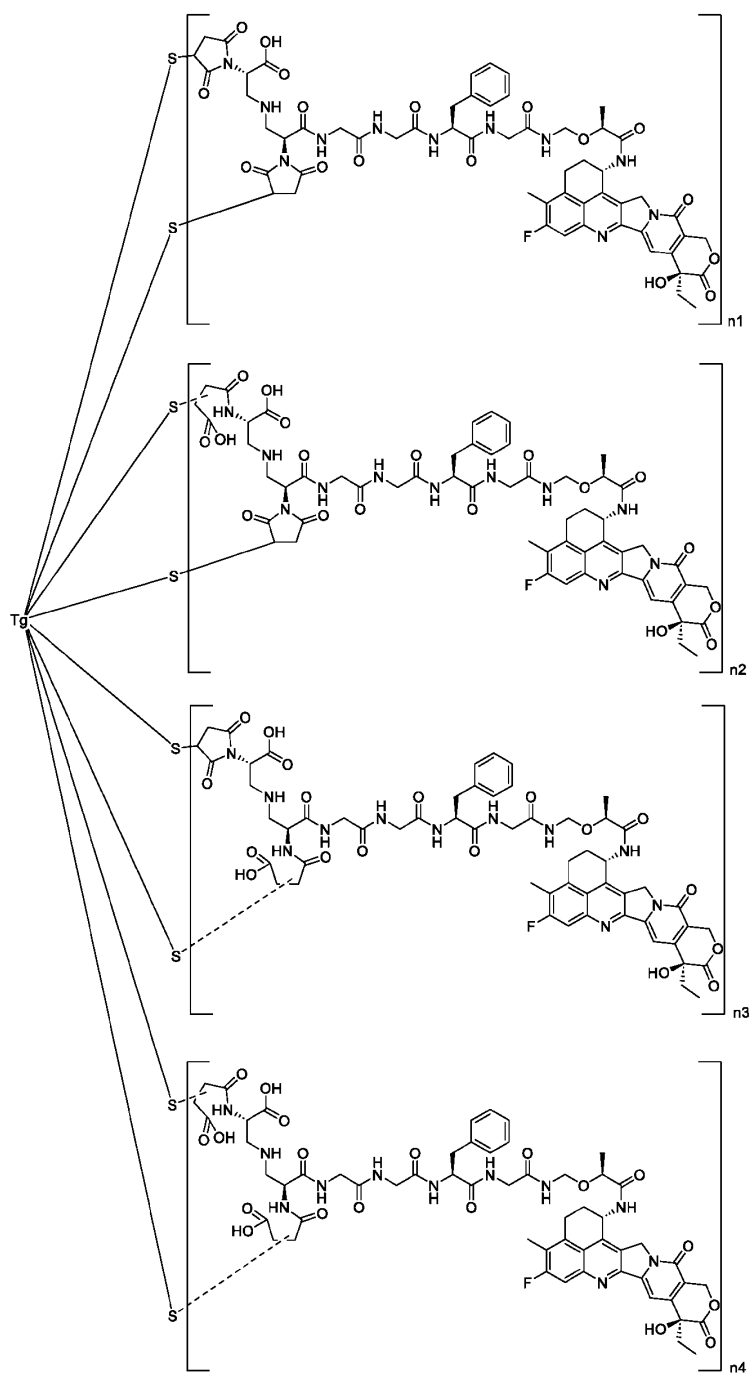
- 10 When multiple succinimide moieties are present in a ligand-drug conjugate, these succinimide moieties may be in the closed ring form, partially open ring form, or fully open ring form, and although the succinimide moieties appearing in the chemical formula of the ligand-drug conjugate are in the closed ring form, they actually cover the three scenarios where the succinimide is fully closed, partially open, and fully open ring, in one embodiment, covering both partial ring-opening and full ring-opening of succinimide, and, in one embodiment, covering the full ring-opening of succinimide.

In some embodiments, the ligand-drug conjugate is selected from the following structures without limitation:

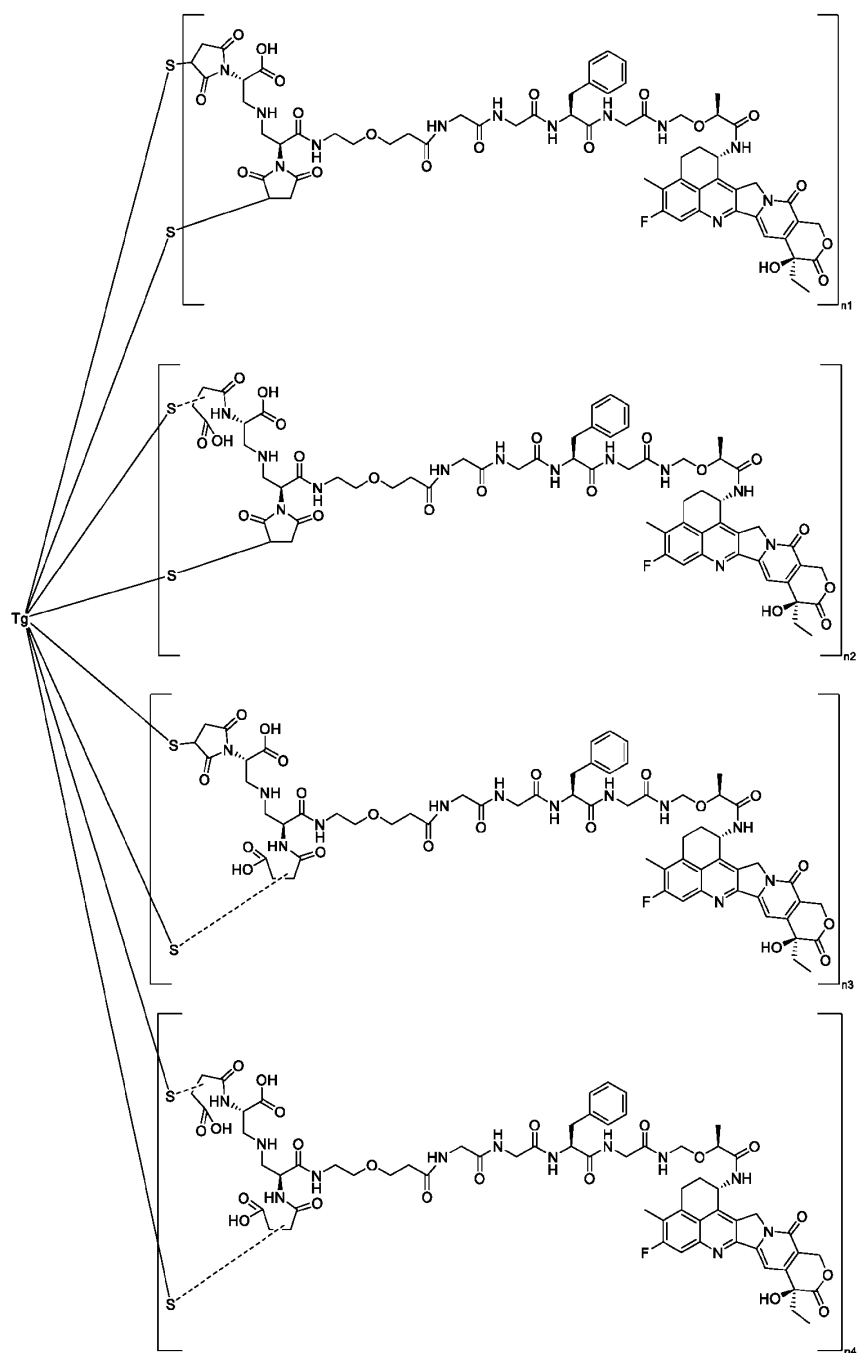






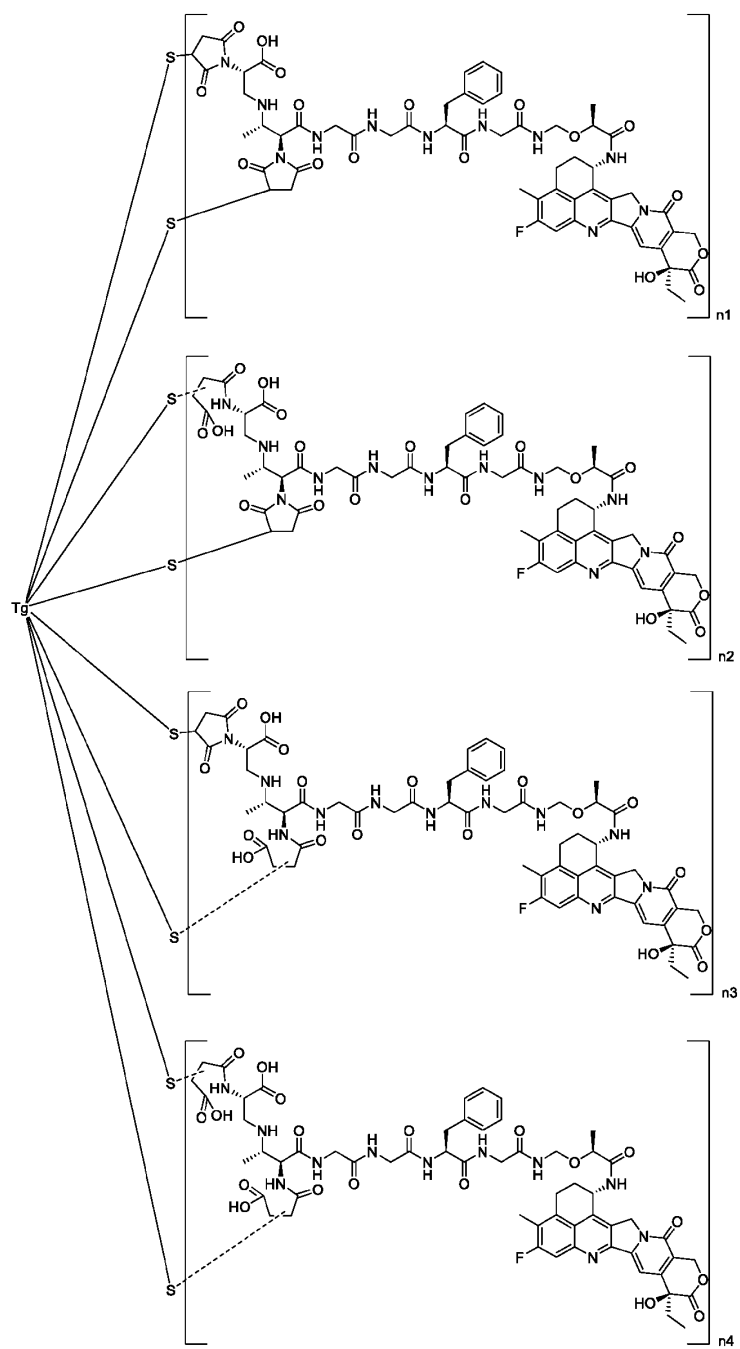


ADC-05'

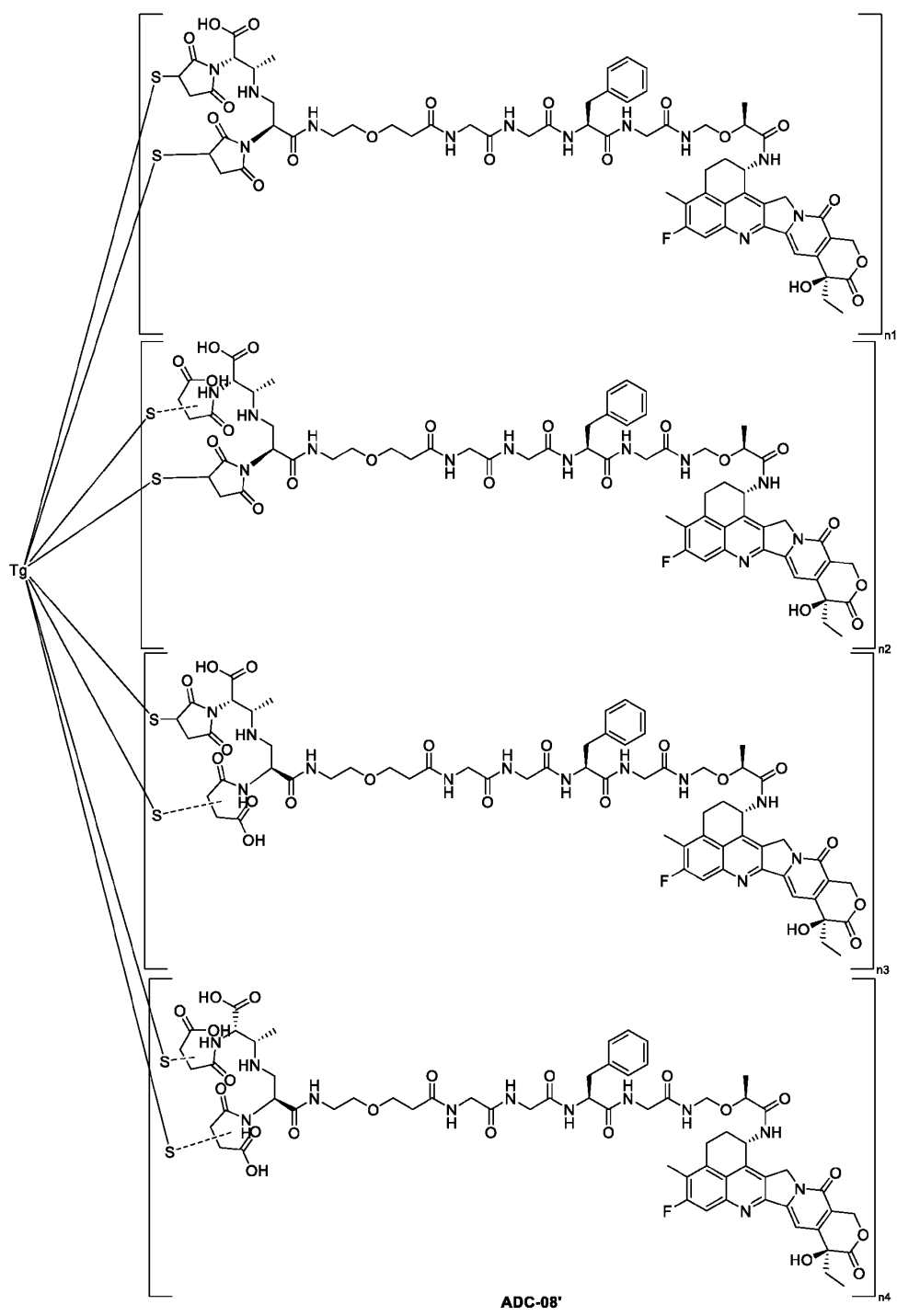


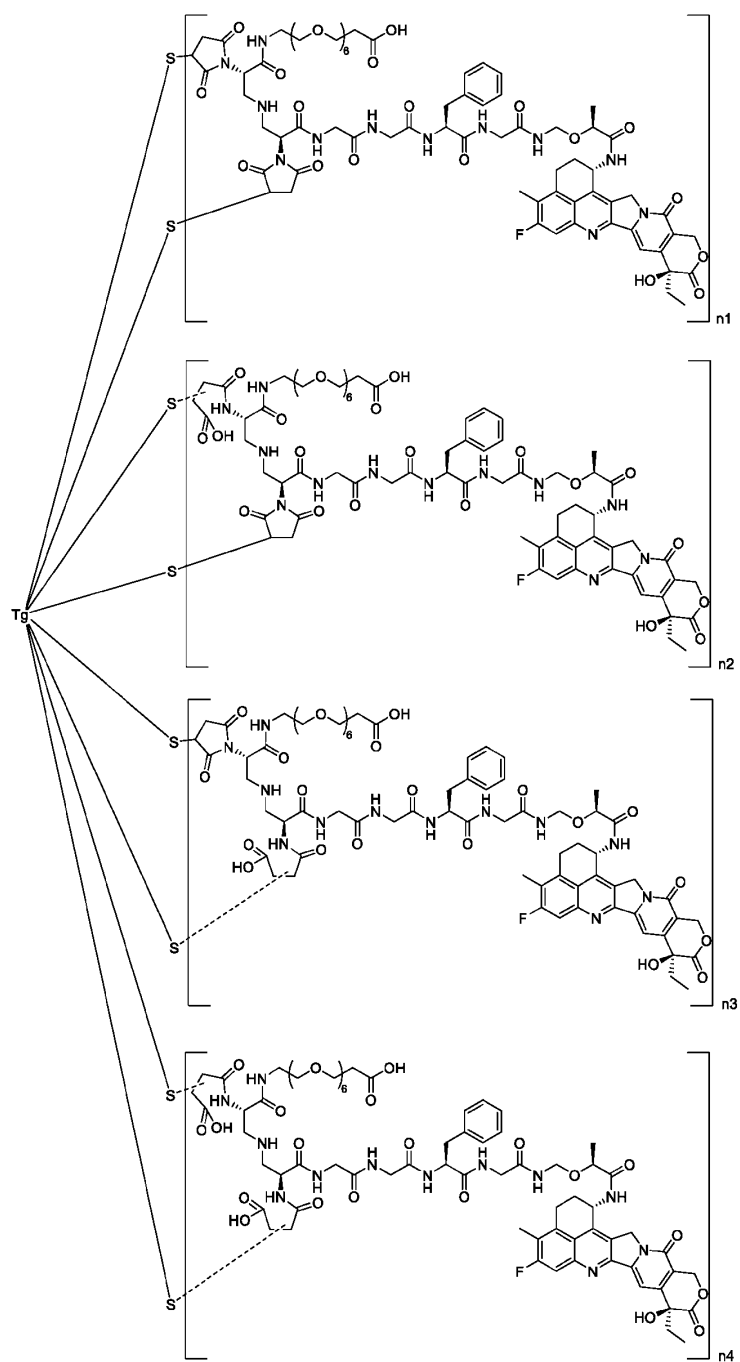
ADC-06*

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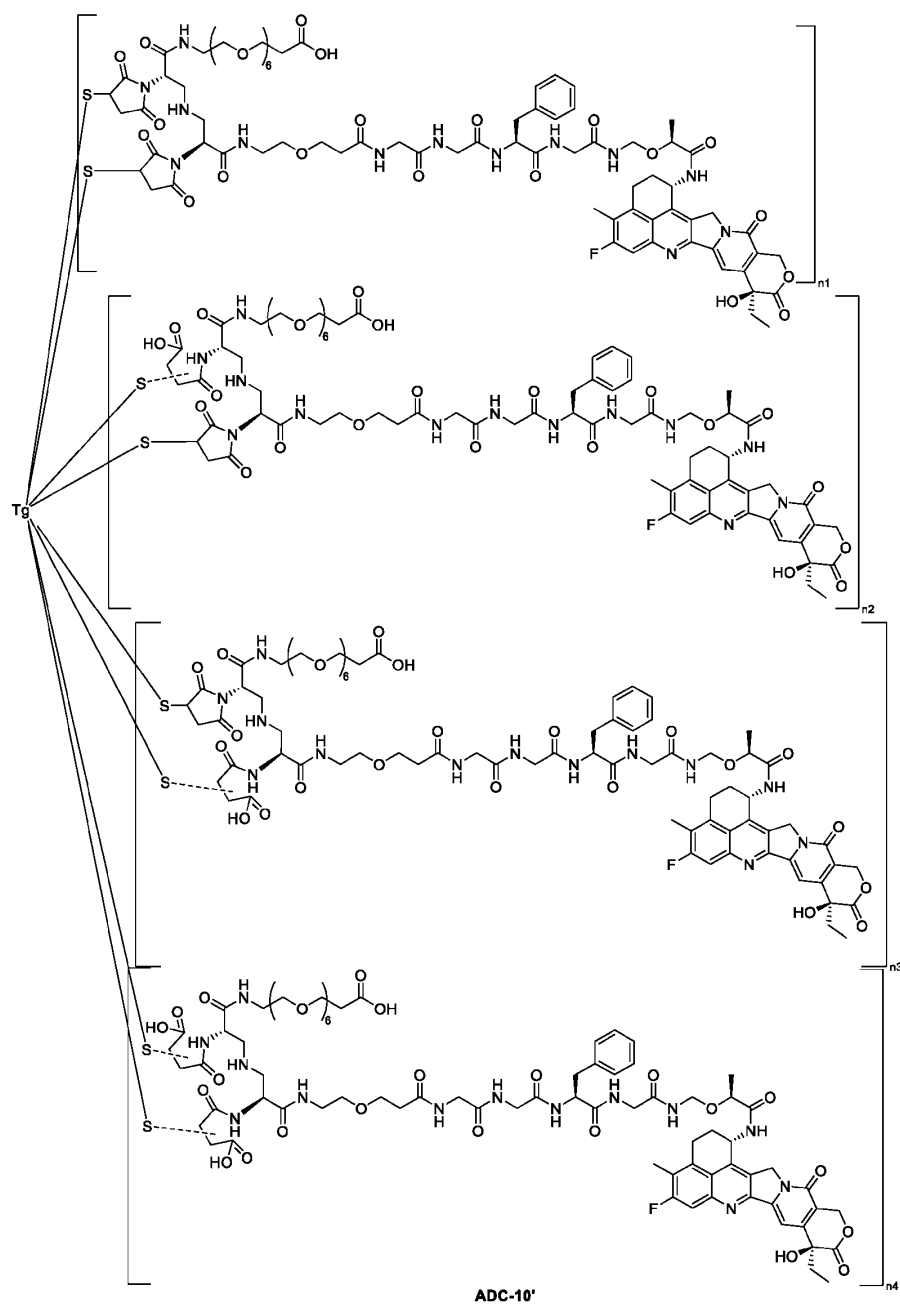


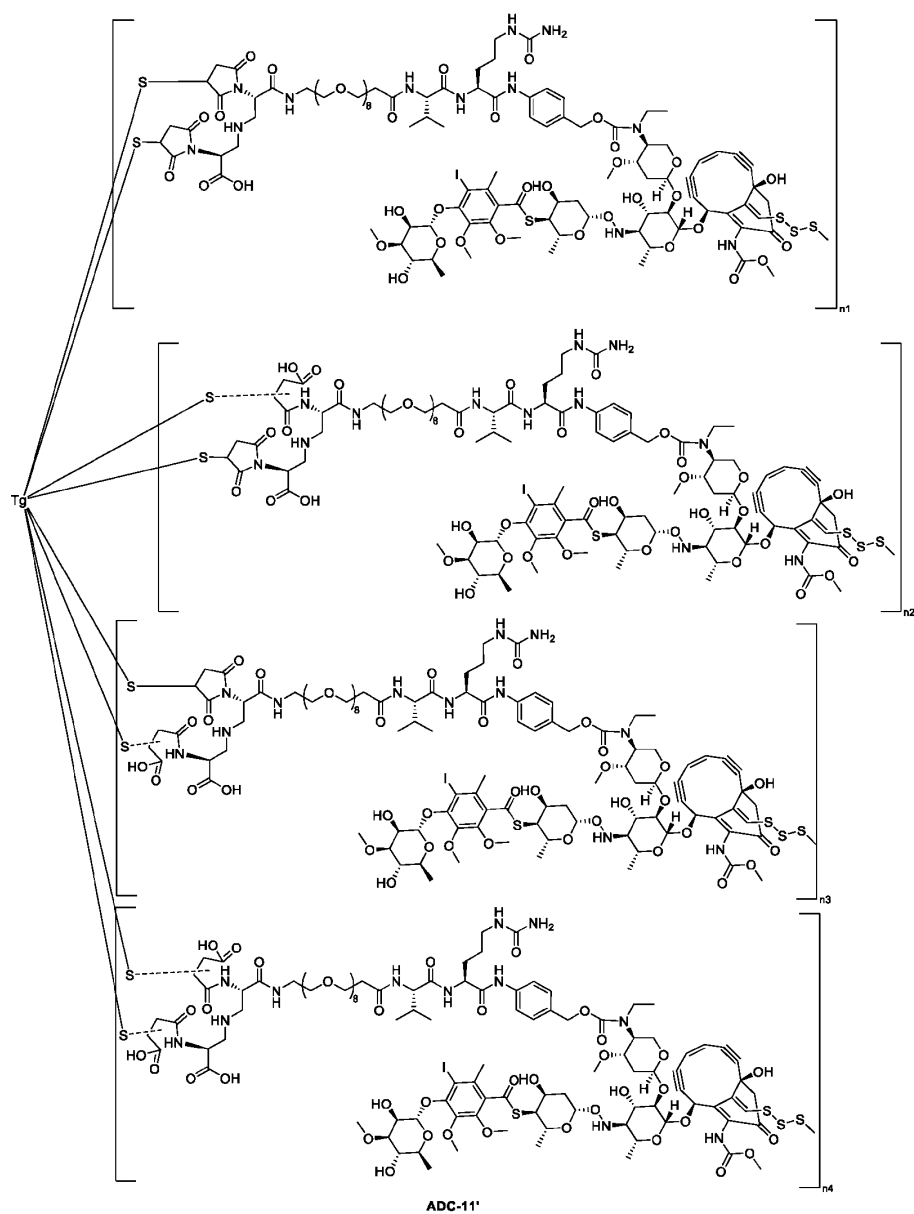
ADC-07'

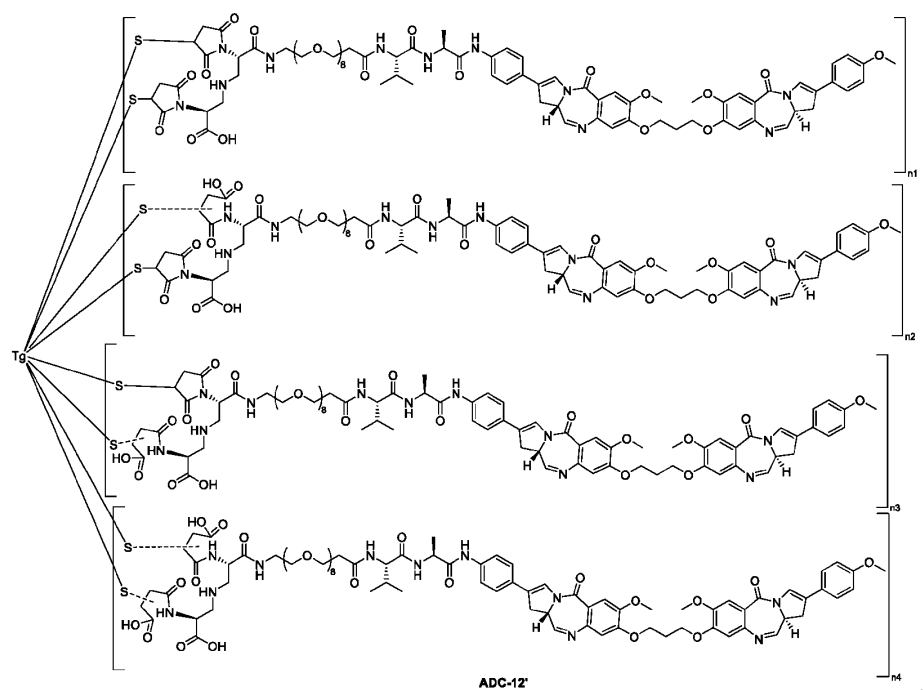


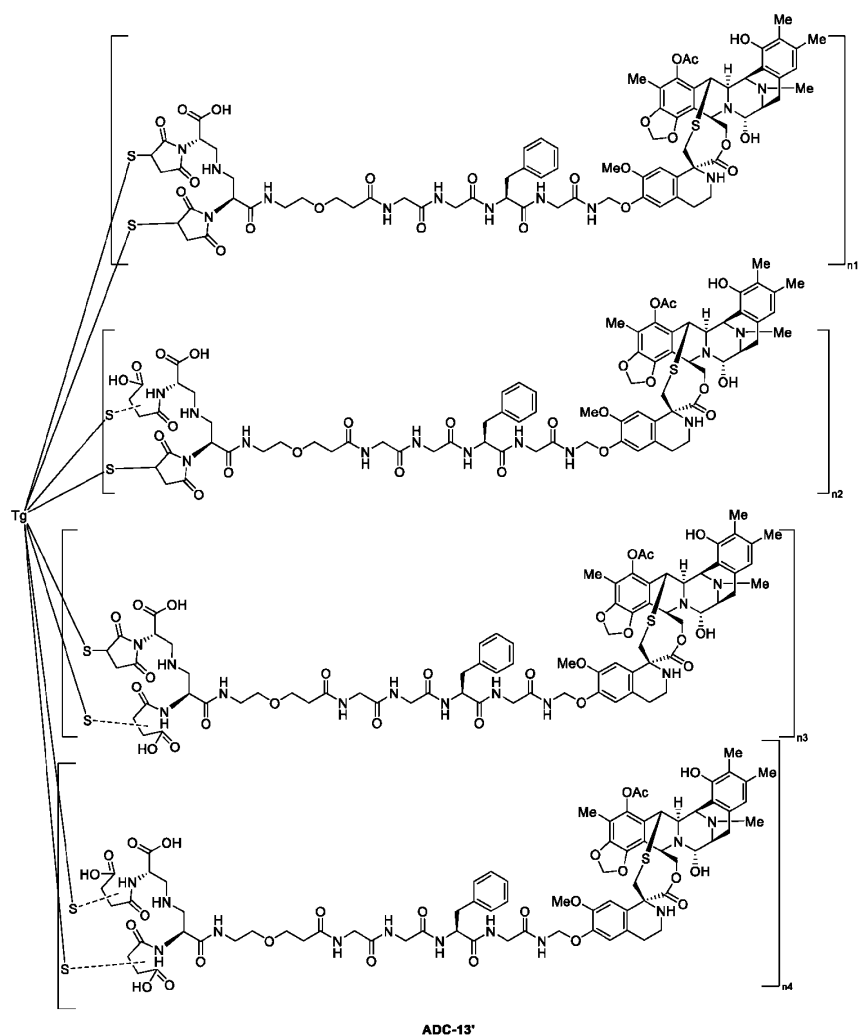


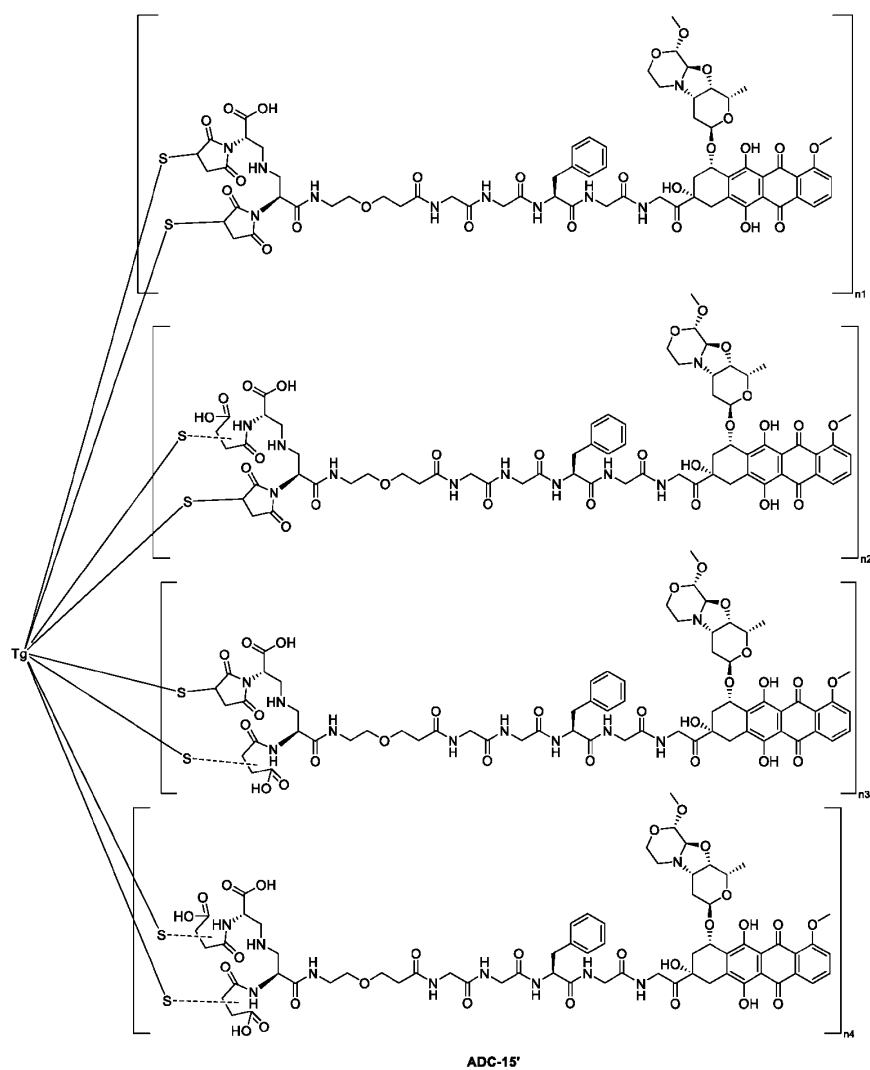
ADC-09'

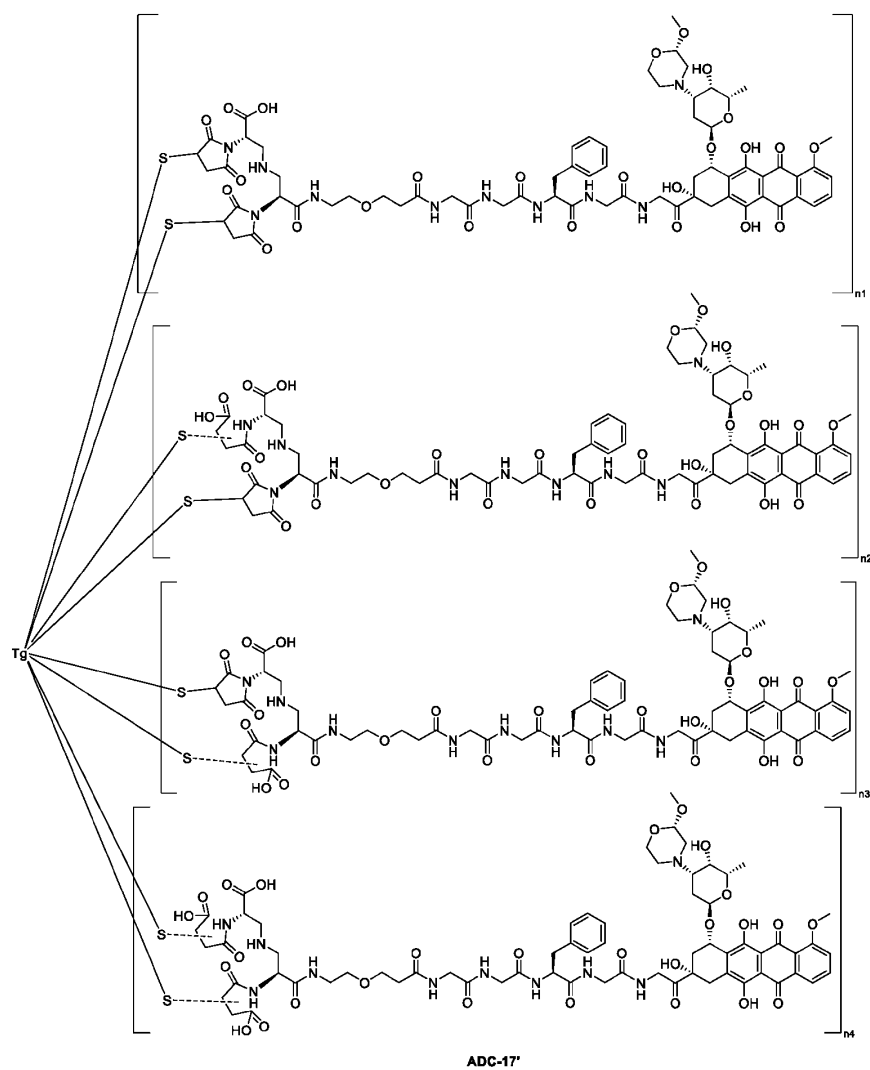


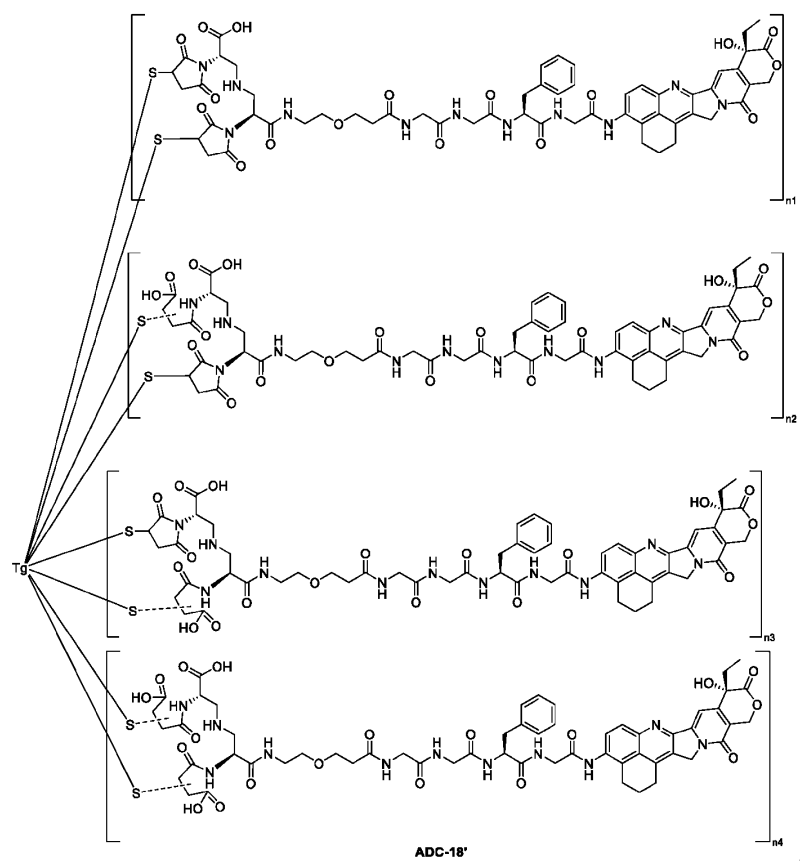


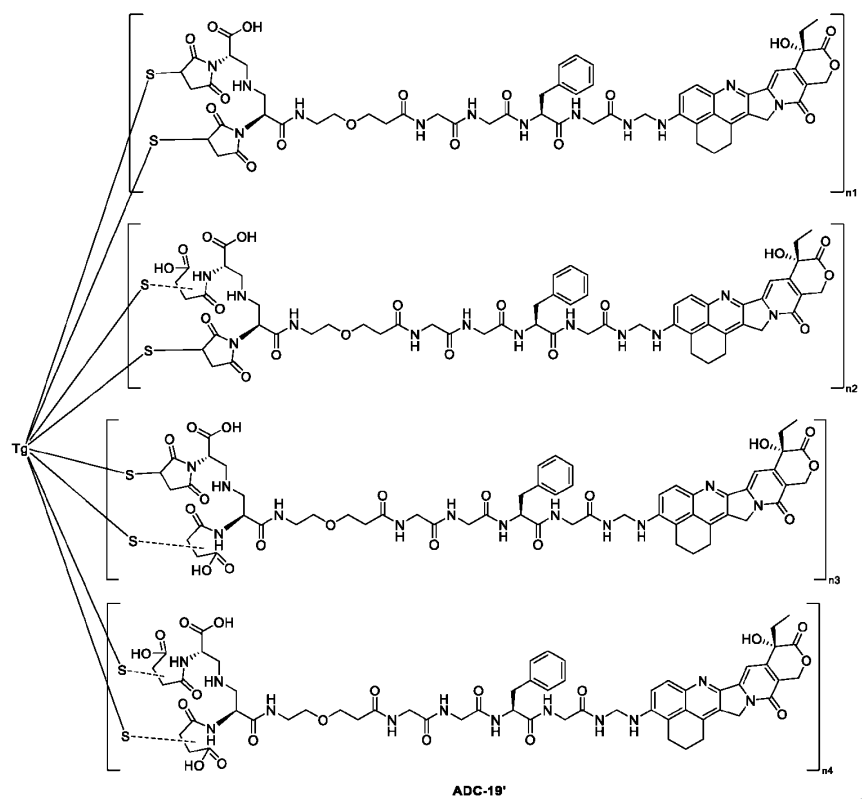


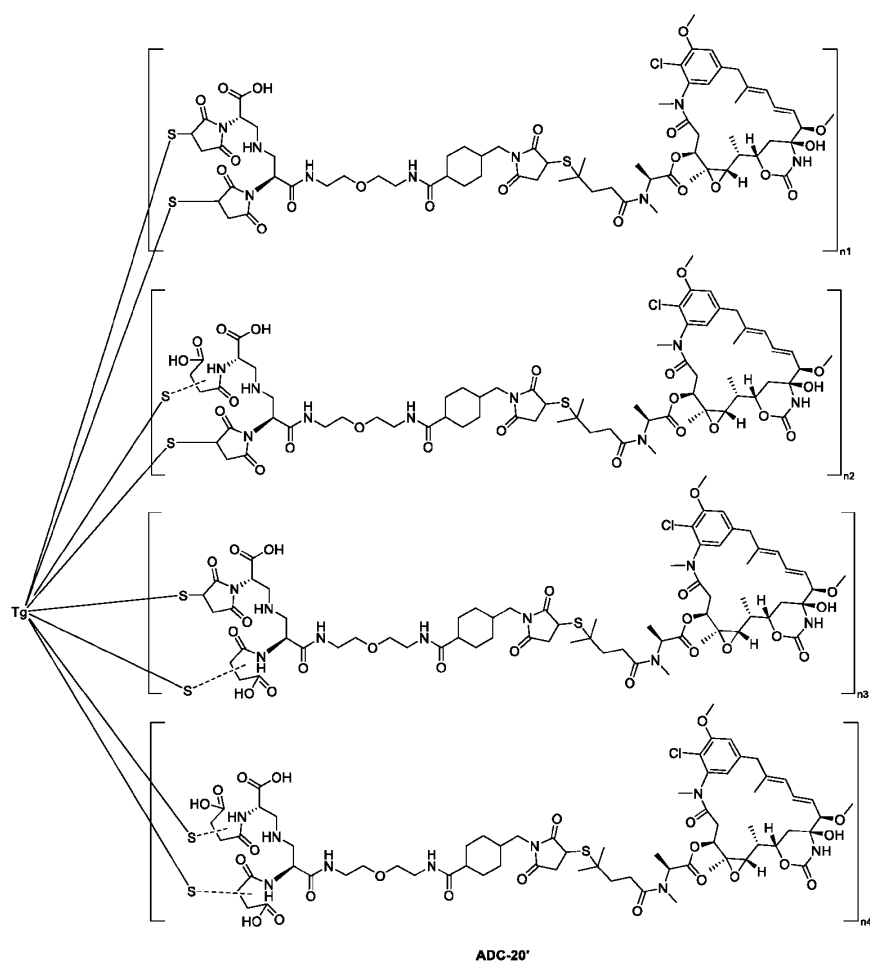


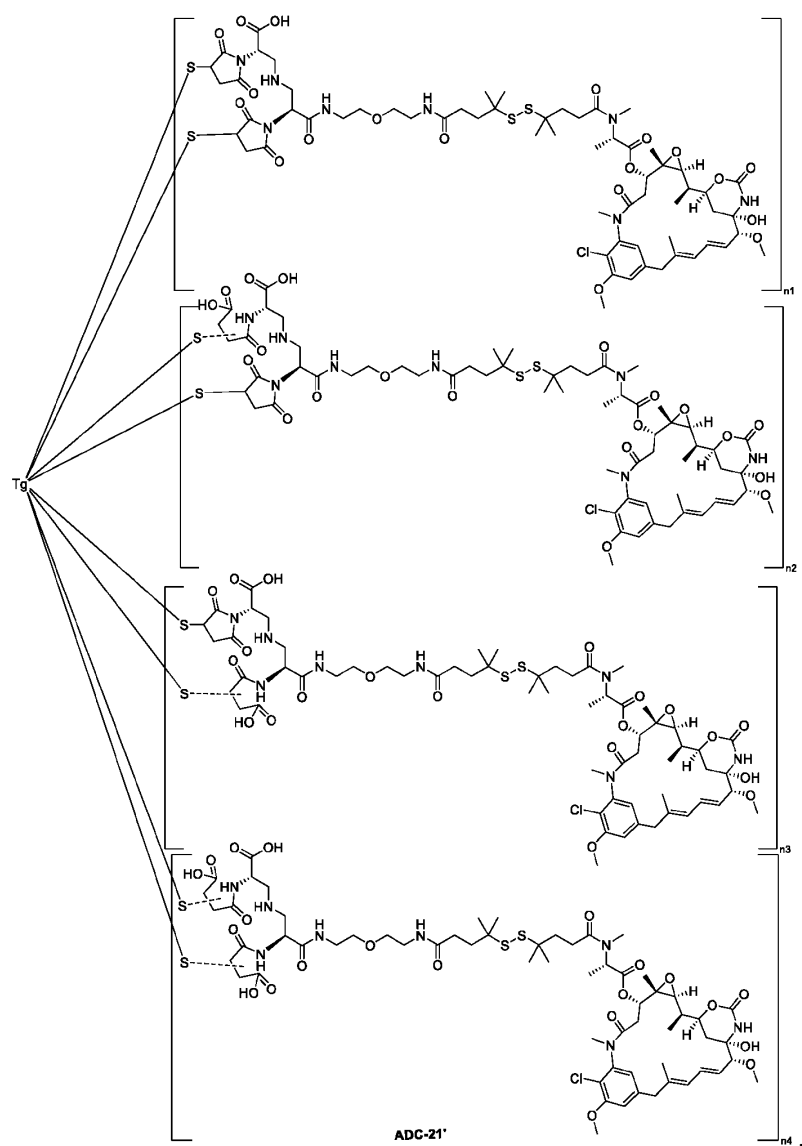


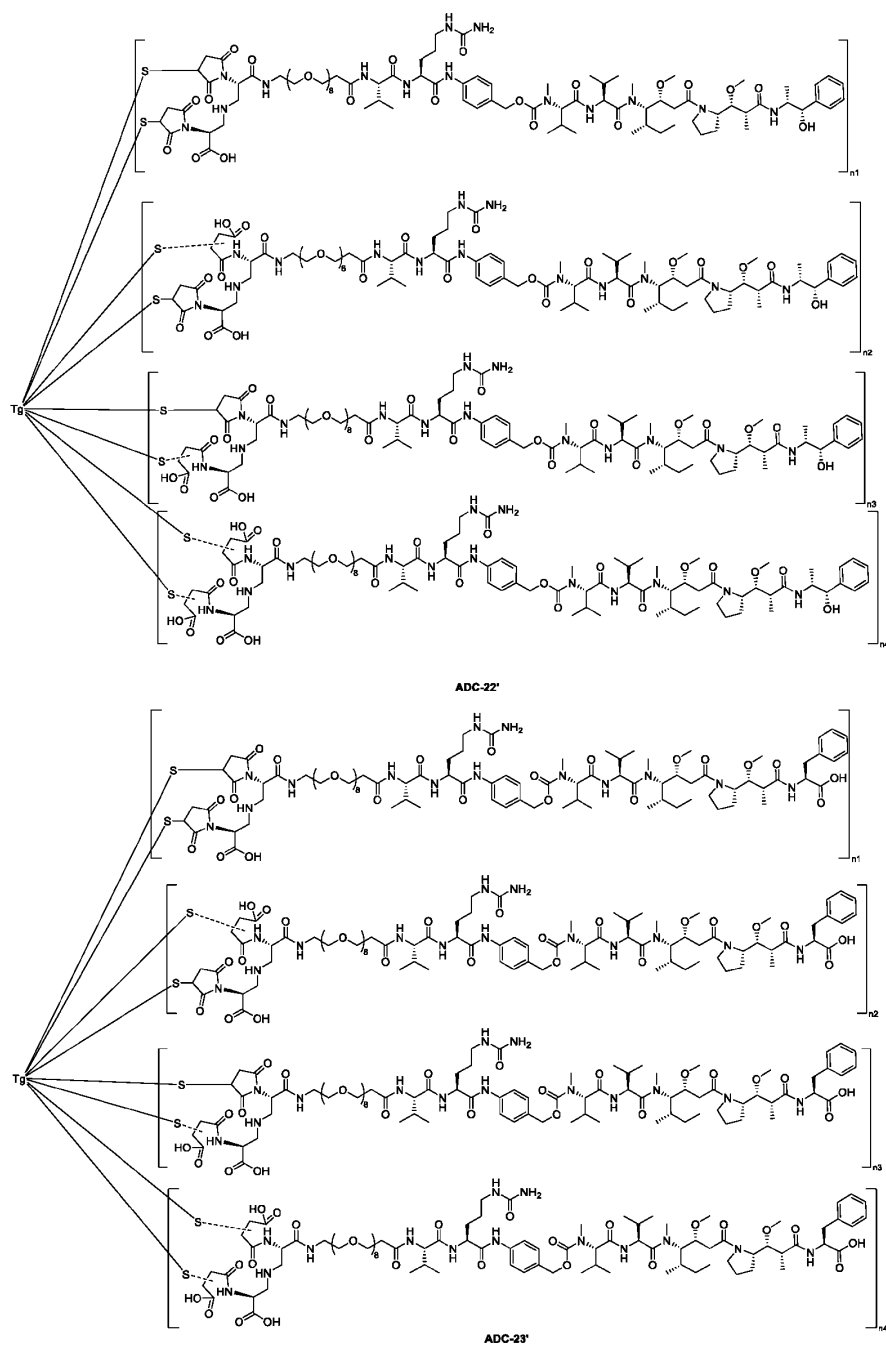


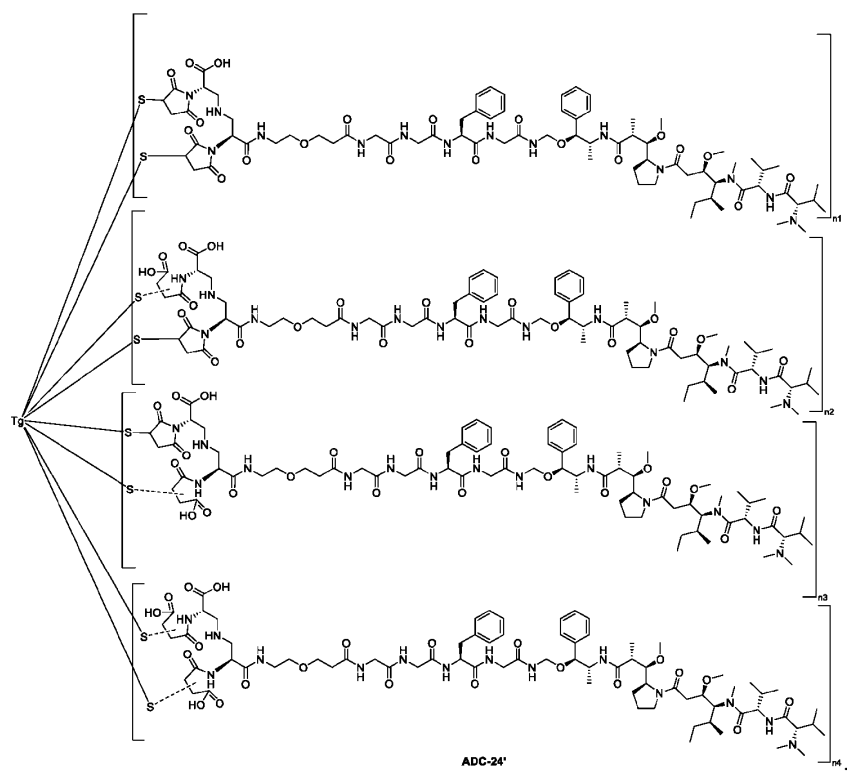


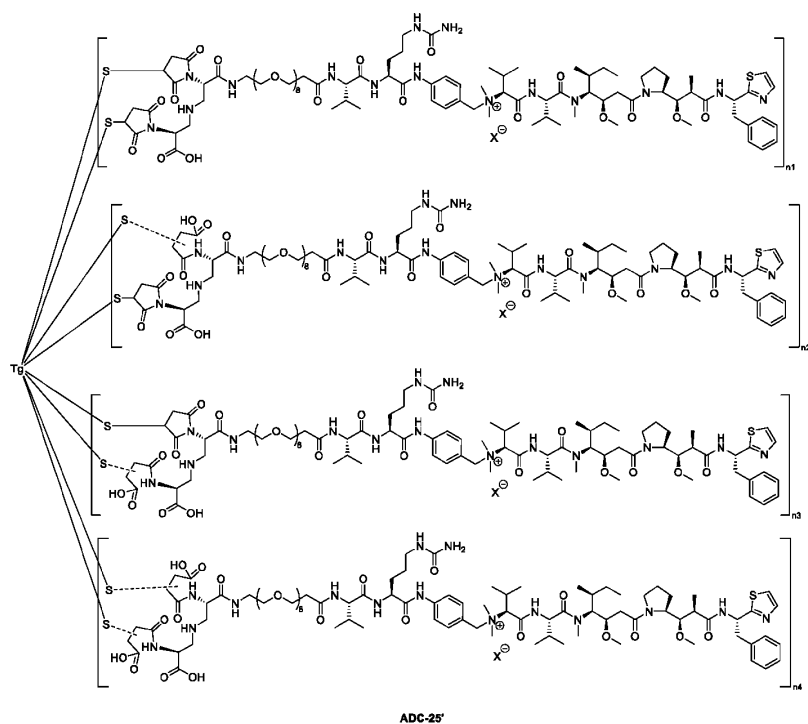


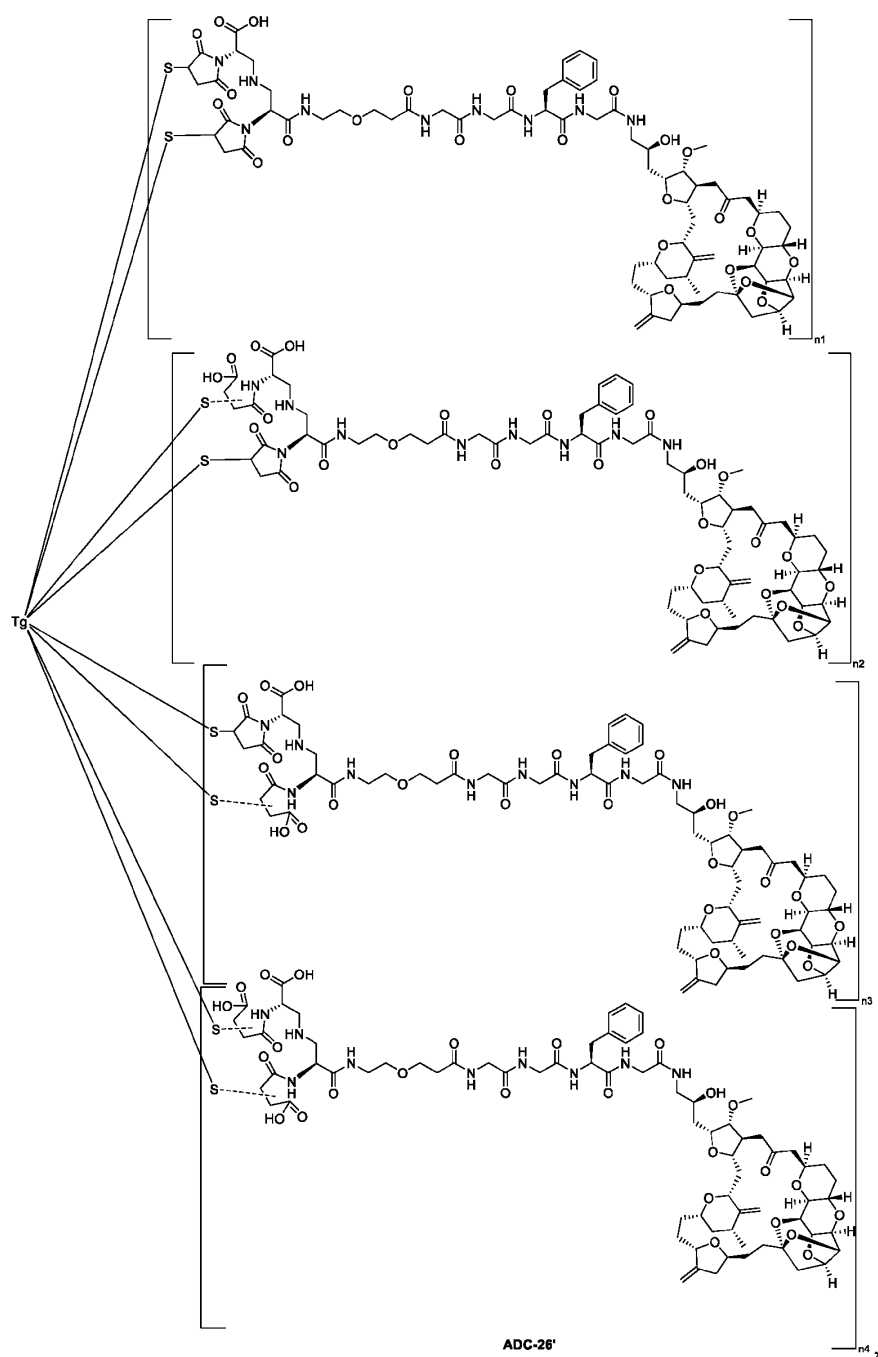


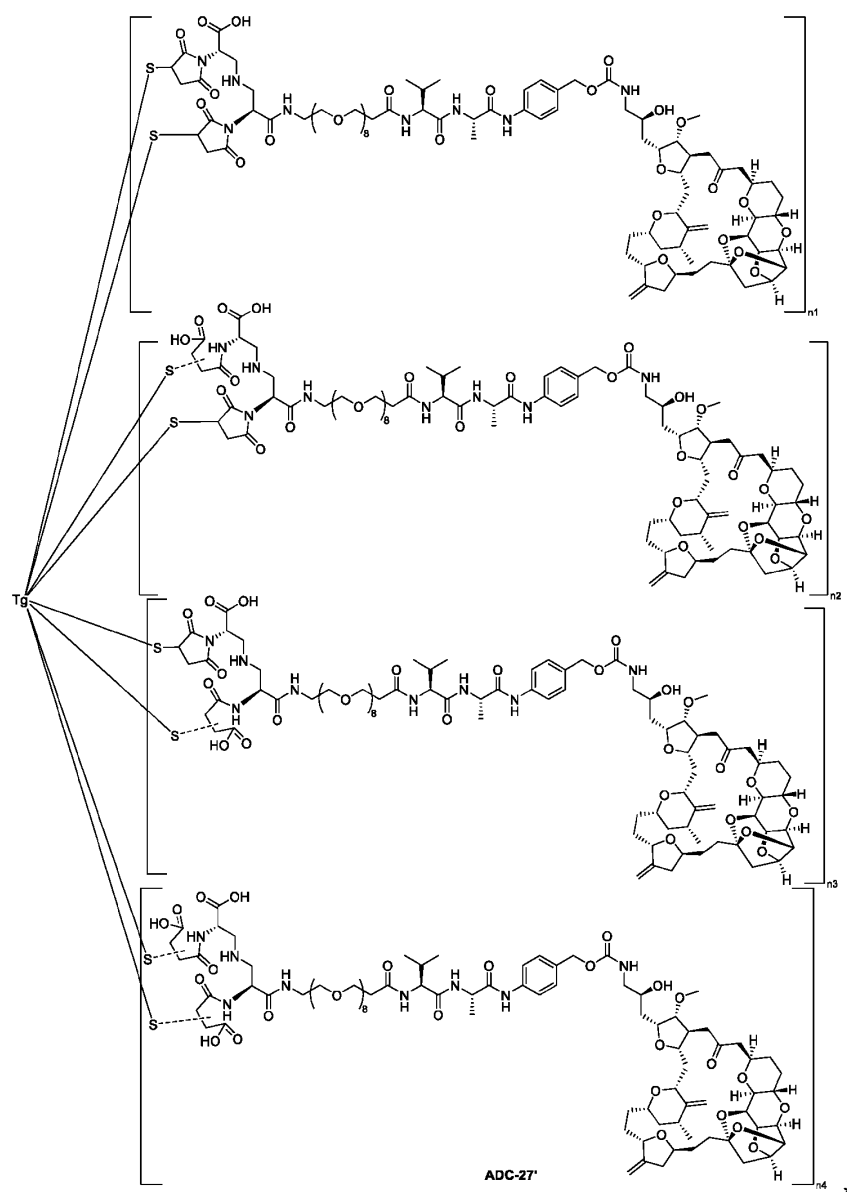


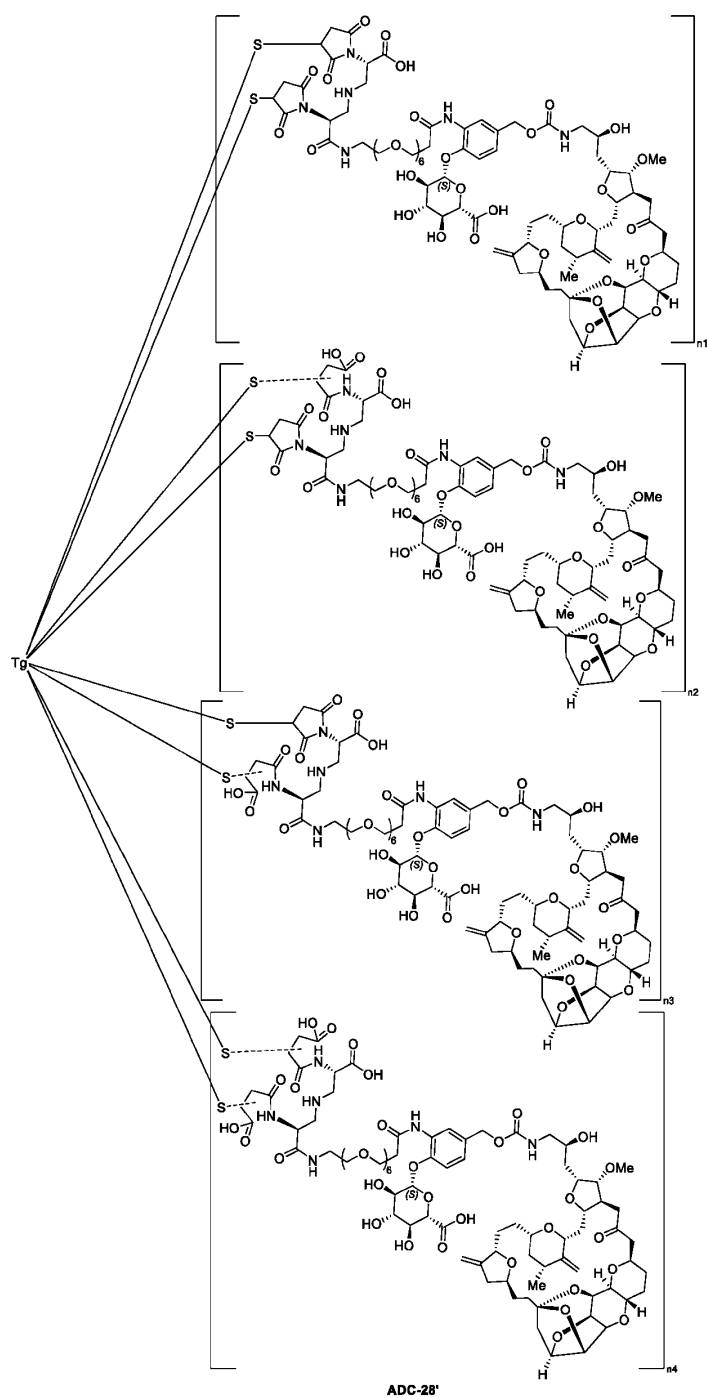


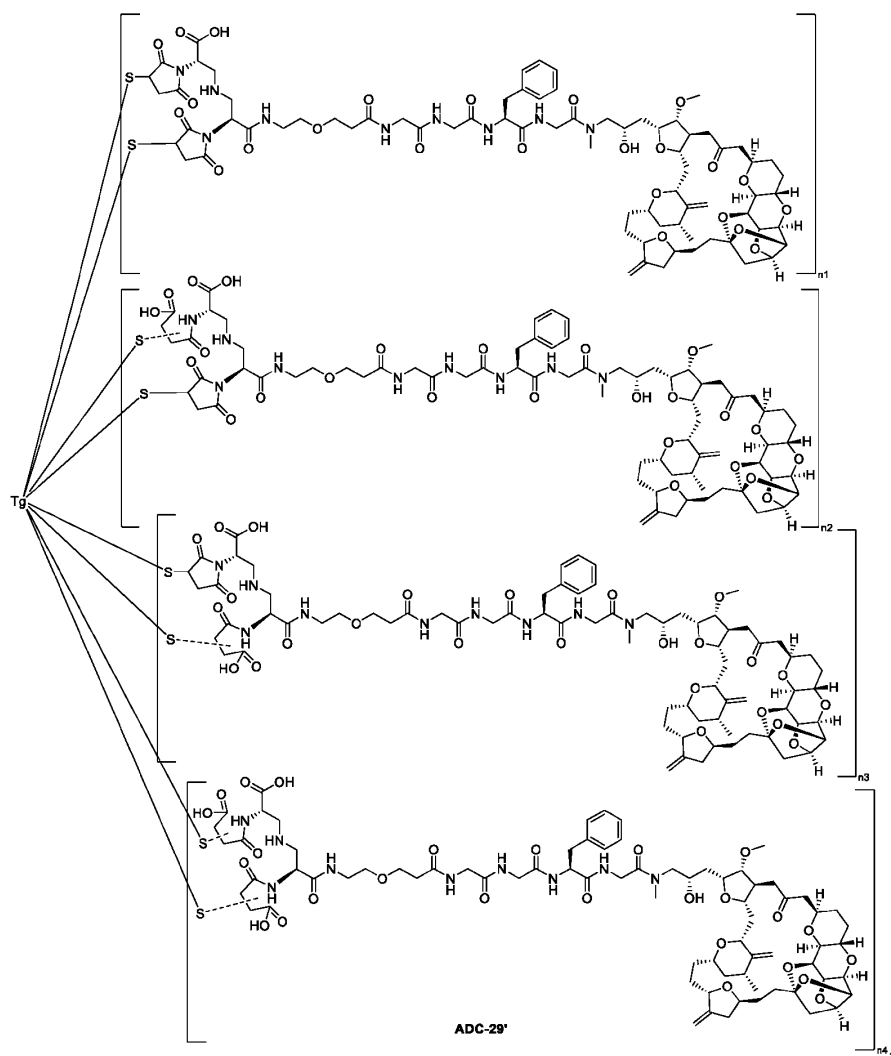


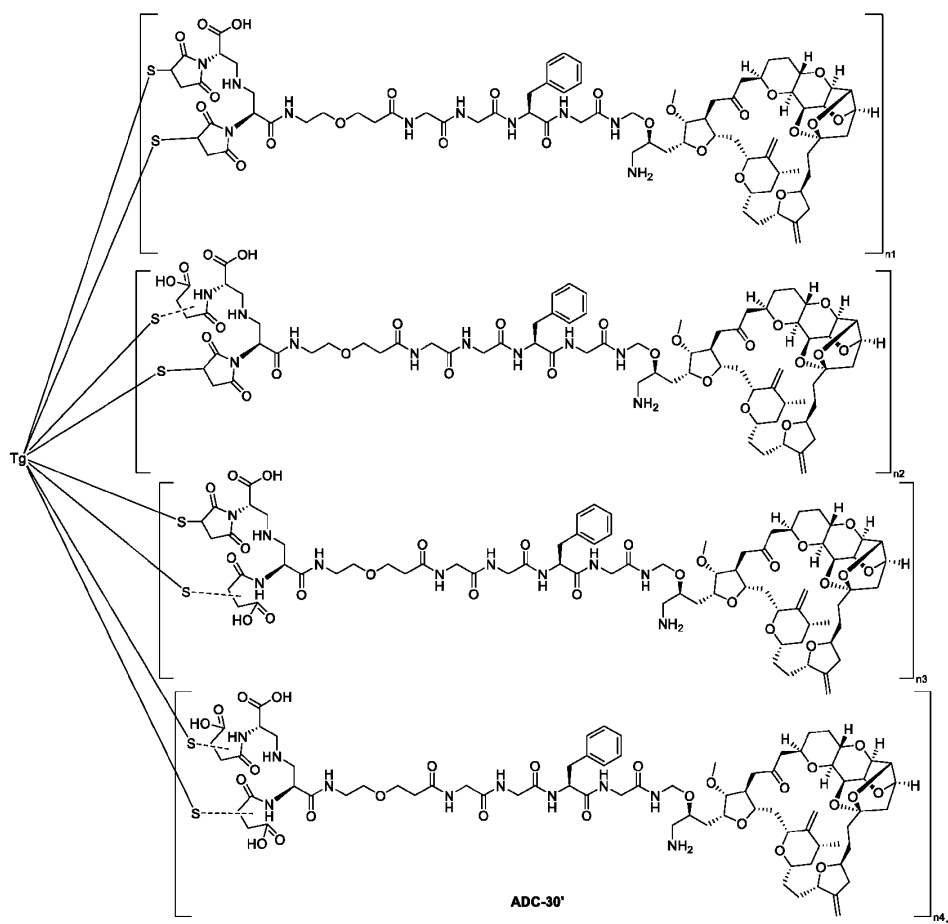


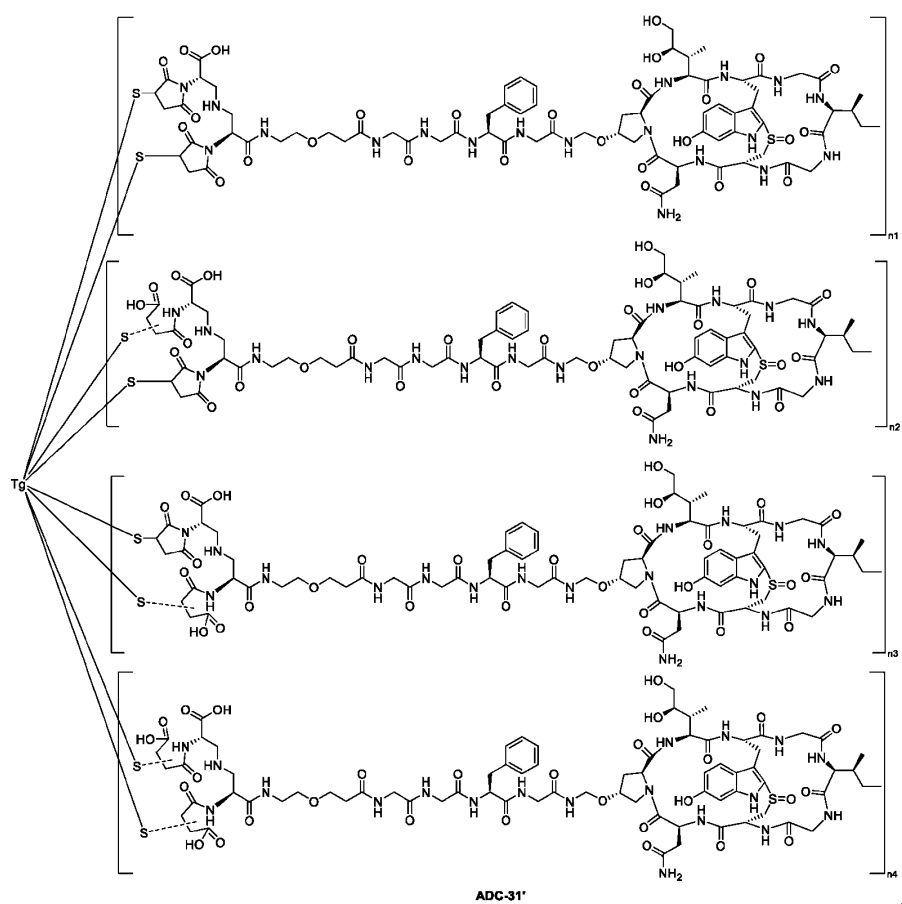


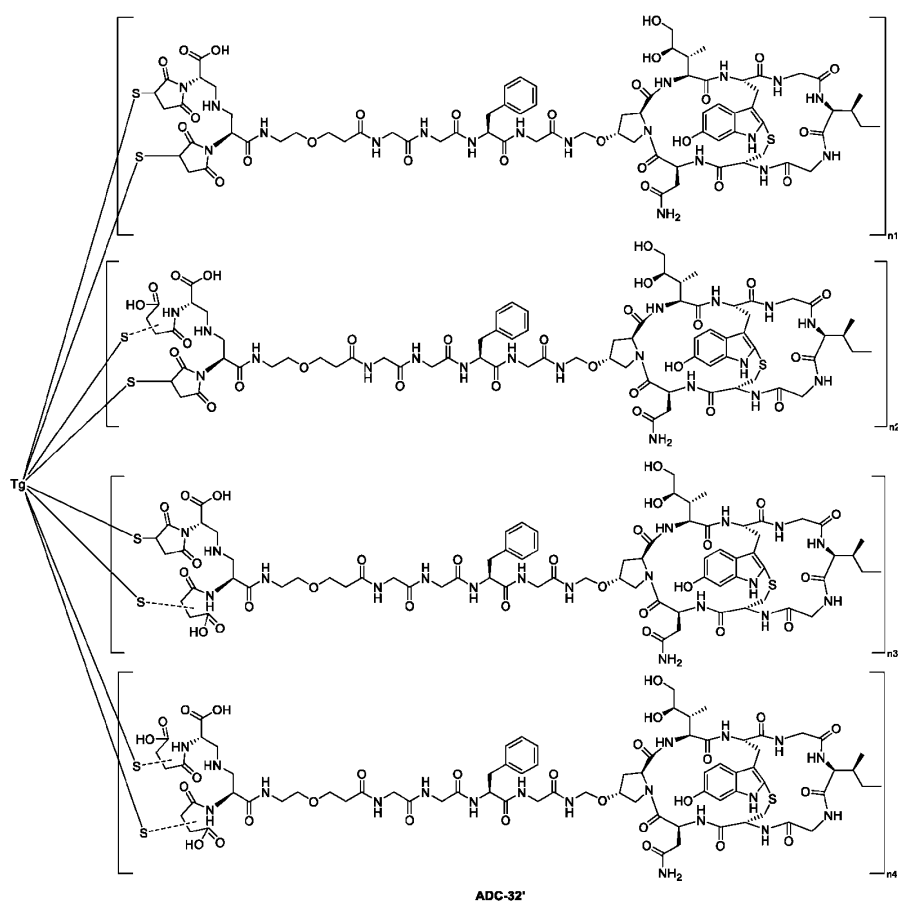


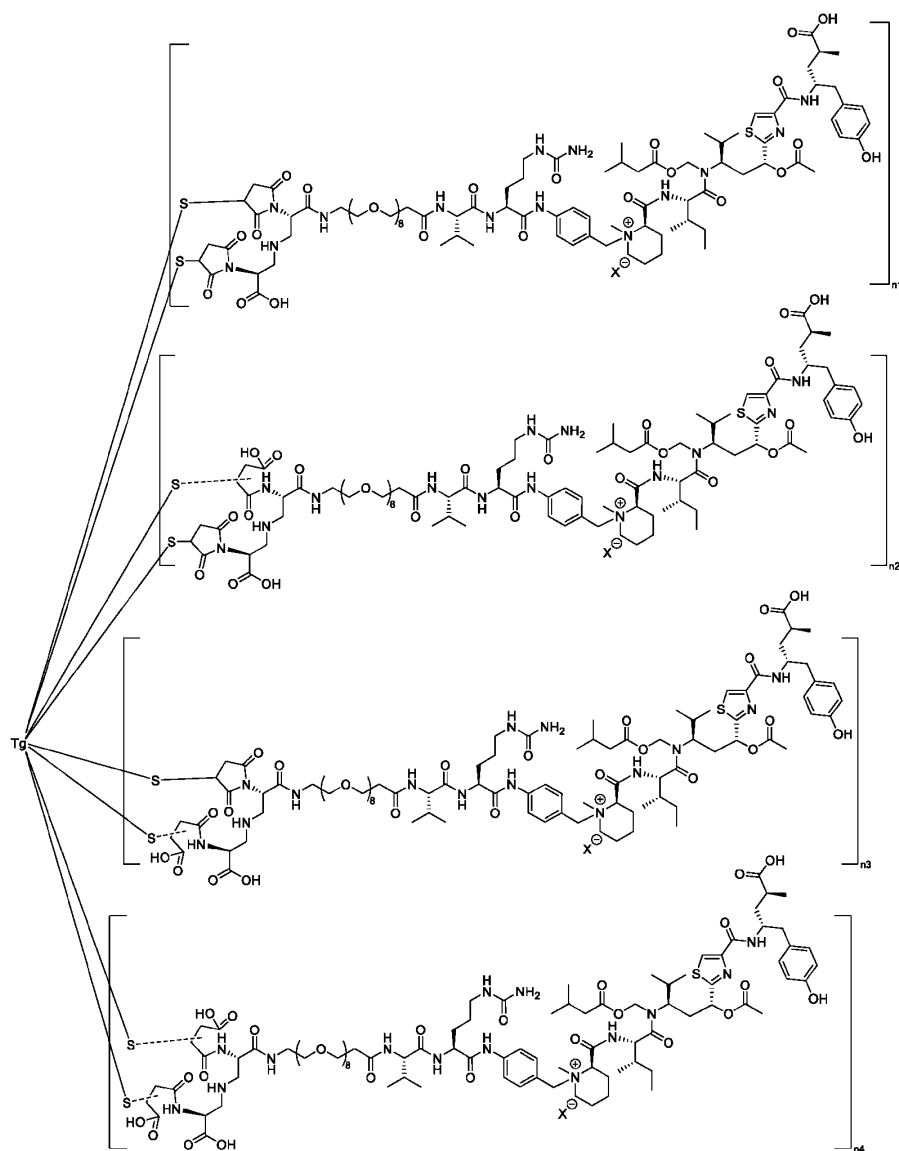




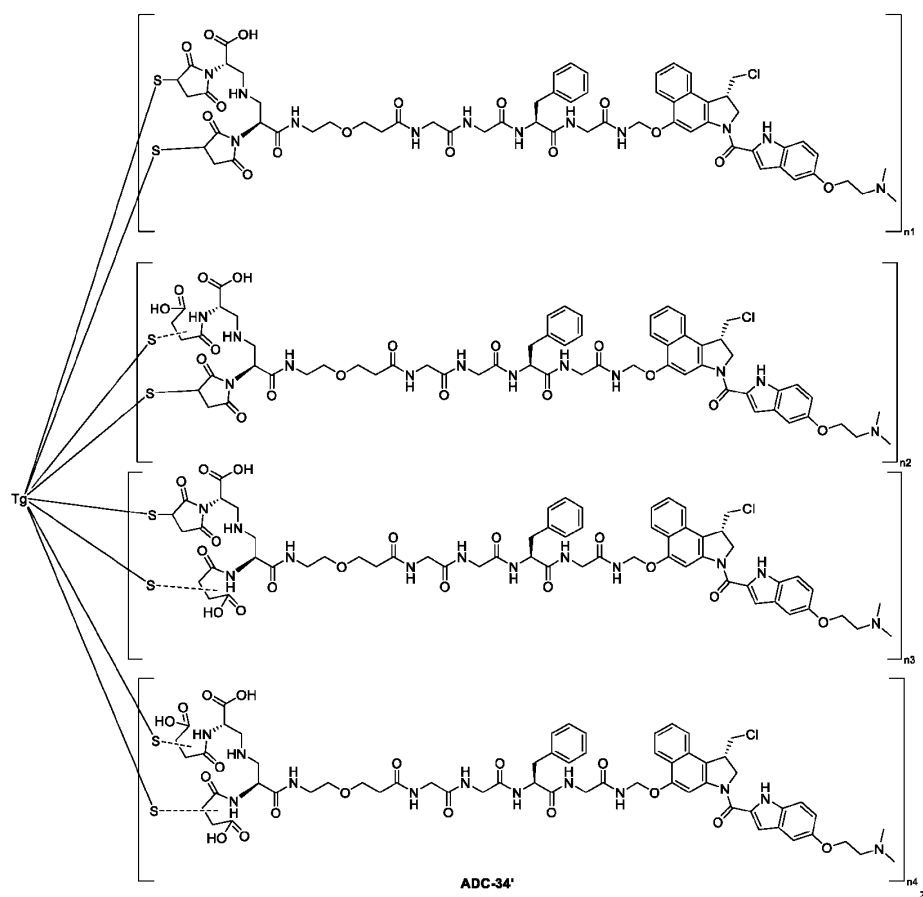


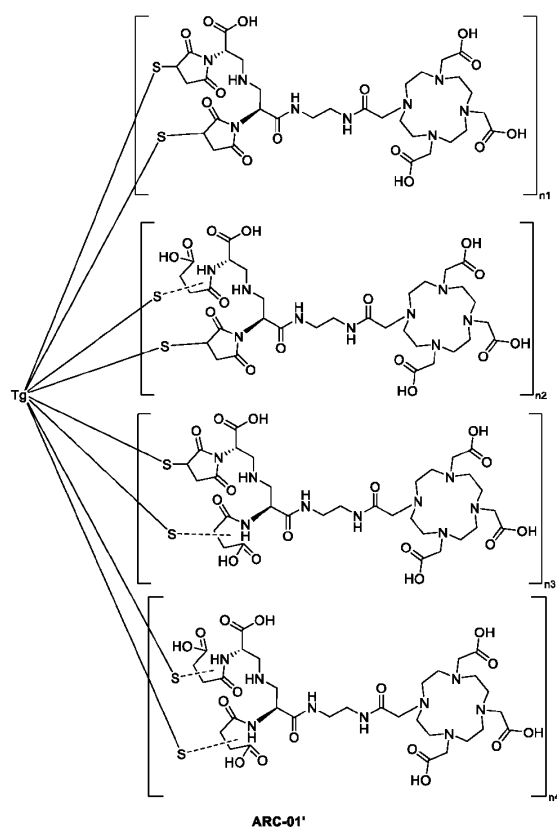


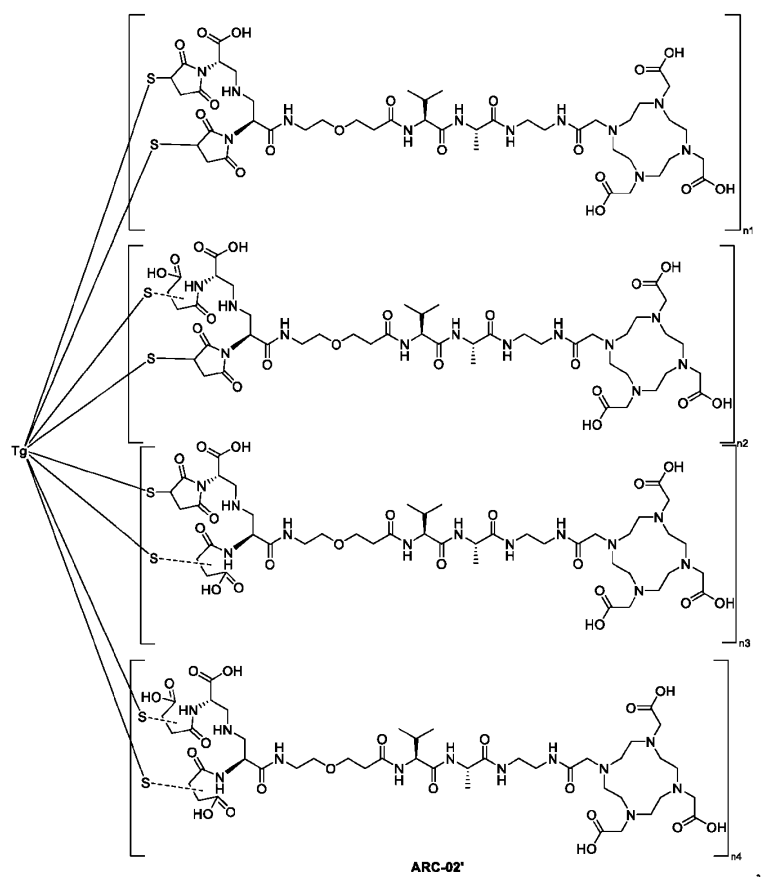


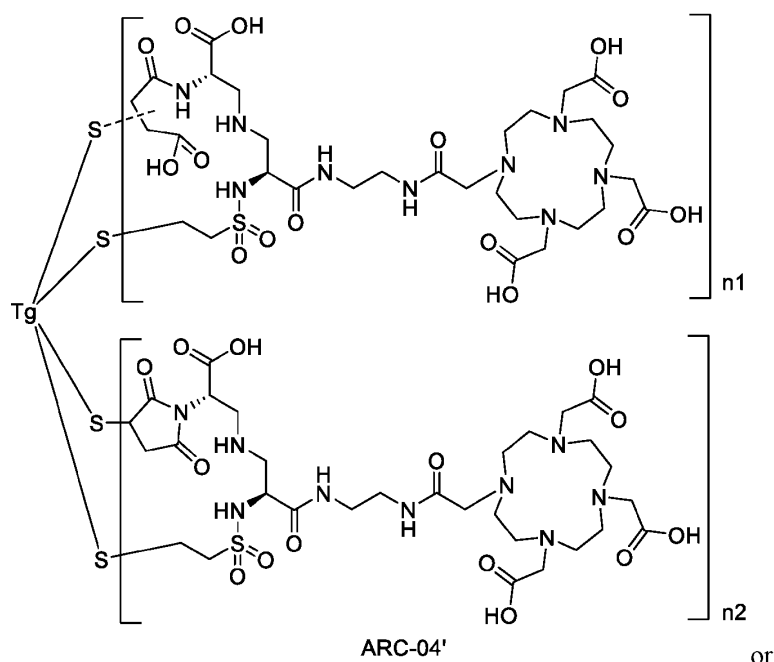
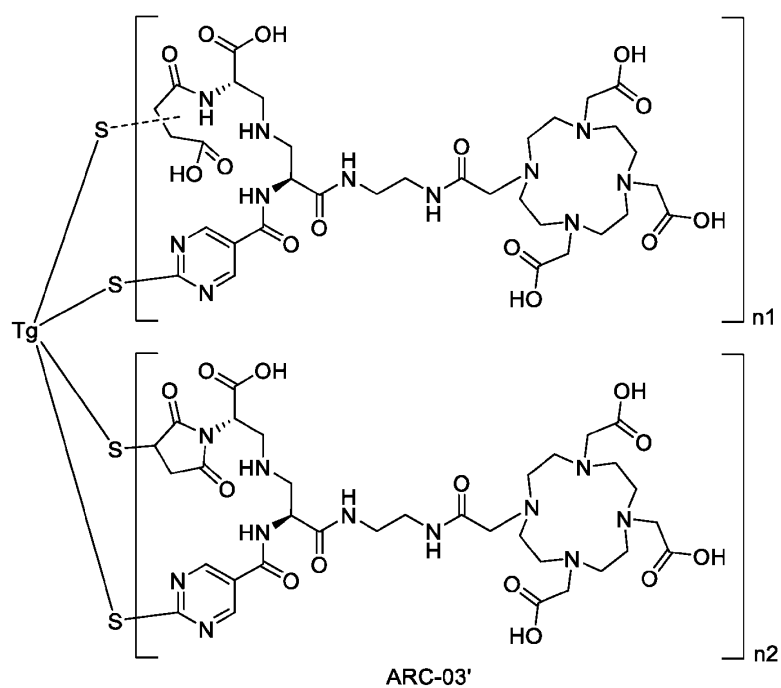


ADC-33'

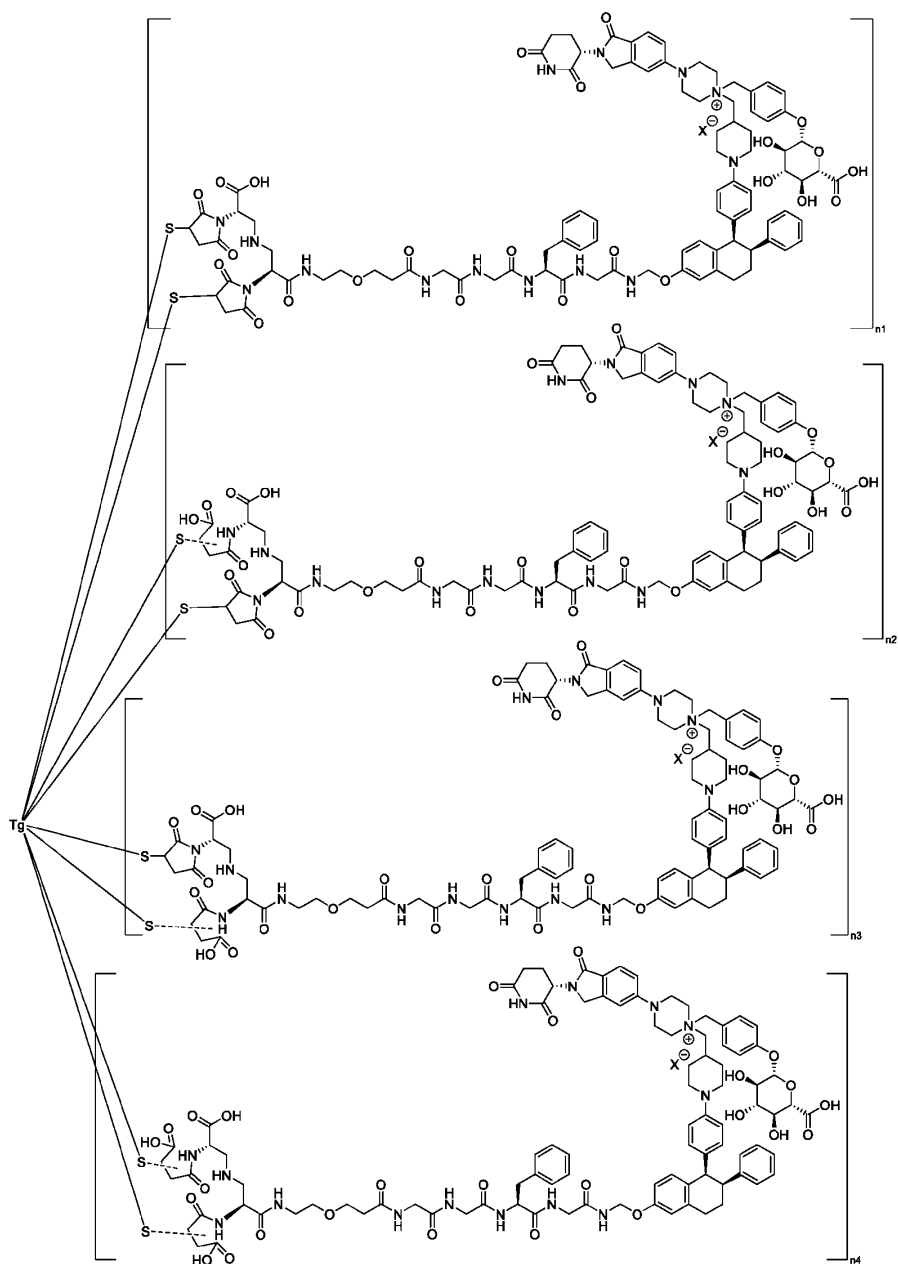








or



DAC-01'

wherein
 the dotted line indicates the bond connected with the succinimide ring-opening structure at any position;
 when n3 and n4 exist:

n_1 , n_2 , n_3 and n_4 are independently selected from integers or decimals of 0-10, and n_1 , n_2 , n_3 and n_4 are not 0 at the same time; in one embodiment, at least one of n_2 , n_3 and n_4 is not 0; In one embodiment, n_1 , n_2 and n_3 are 0, and n_4 is not 0;

and the sum of $n_1+n_2+n_3+n_4$ is an integer or decimal from 1 to 10, for example, 1-1.5, 1.5-2, 2-2.5, 2.5-3, 3-3.5, 3.5-4, 4-4.5, 4.5-5, 5-5.5, 5.5-6, 6-6.5, 6.5-7, 7-7.5, 7.5-8, 8-8.5, 8.5-9, 9-9.5 or 9.5-10, in one embodiment, an integer or decimal selected from 2-8, and in one embodiment an integer or decimal selected from 3-6;

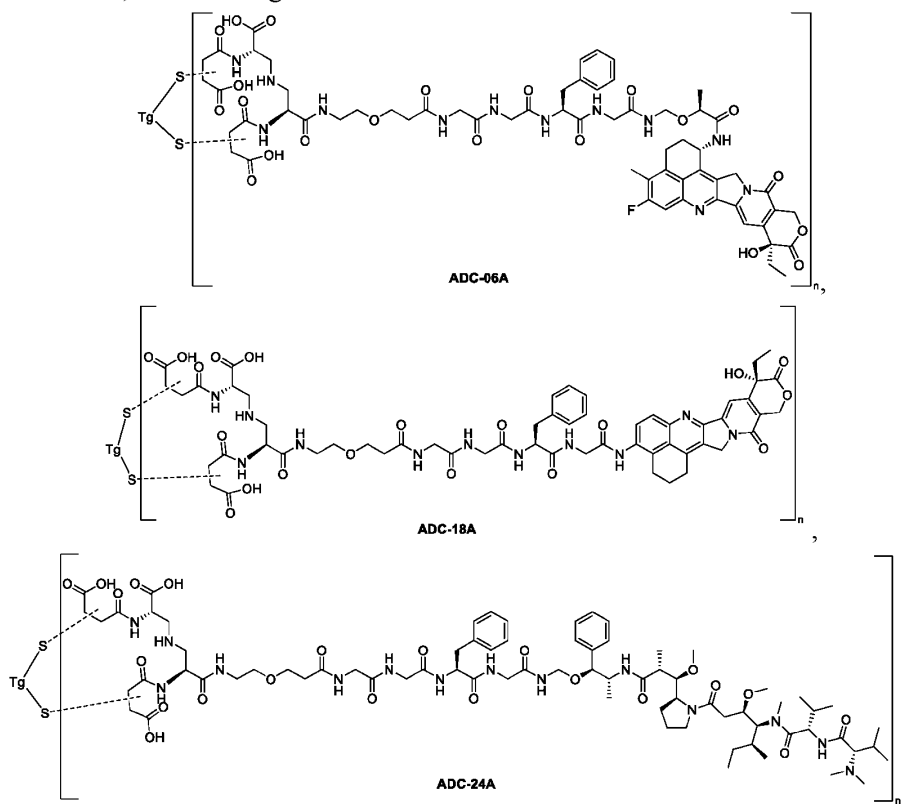
when n_3 and n_4 do not exist:

n_1 and n_2 are independently selected from integers or decimals of 0-10, and n_1 and n_2 are not simultaneously 0; in one embodiment, n_1 is not 0; in one embodiment, n_2 is 0;

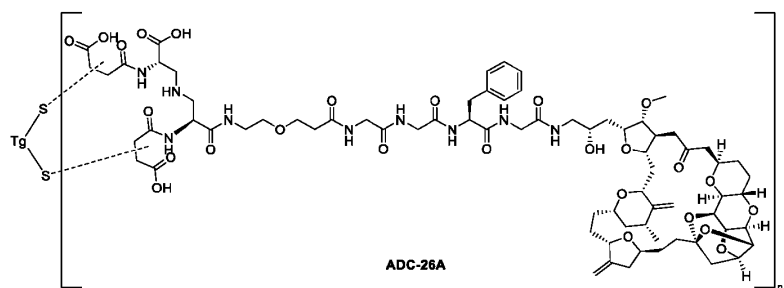
and the sum of n_1+n_2 is an integer or decimal from 1 to 10, such as 1-1.5, 1.5-2, 2-2.5, 2.5-3, 3-3.5, 3.5-4, 4-4.5, 4.5-5, 5-5.5, 5.5-6, 6-6.5, 6.5-7, 7-7.5, 7.5-8, 8-8.5, 8.5-9, 9-9.5 or 9.5-10, in one embodiment, an integer or decimal selected from 2-8, and in one embodiment an integer or decimal selected from 3-6;

Tg is selected from ligands, in one embodiment, from antibodies.

In some embodiments, the ring-opening form of said ligand-drug conjugate is selected from, without limitation, the following structures:



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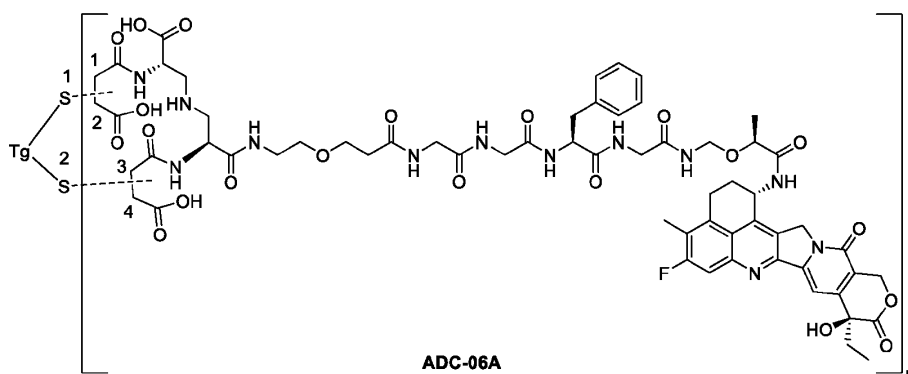
wherein,

dashed lines indicate bonds connected to the open ring structure of the succinimide at arbitrary sites;

n is an integer or decimal number selected from 1-10; for example, n may be an integer or decimal number between 1-1.5, 1.5-2, 2-2.5, 2.5-3, 3-3.5, 3.5-4, 4-4.5, 4.5-5, 5-5.5, 5.5-6, 6-6.5, 6.5-7, 7-7.5, 7.5-8, 8-8.5, 8.5-9, 9-9.5 or 9.5-10); in one embodiment, n may be an integer or decimal number from 2-8; in one embodiment, n may be an integer or decimal number from 3-6;

T_g is selected from ligands, in one embodiment, from antibodies.

- 10 In the ligand-drug conjugate disclosed herein, the dotted line represents the bond connected with the succinimide ring-opening structure at any site that can be connected and does not exceed the conventional valence state of each atom. For example, those skilled in the art can understand that in ADC-06A, the dotted line connected to No.1 S atom means that it is connected to succinimide ring-opening structure at No.1 C atom or No.2 C atom, and the dotted line connected to No.2 S atom means that it is connected to succinimide ring-opening structure at No.3 C atom or No.4 C atom.



- 20 The dotted line in other ligand-drug conjugates disclosed herein is understood with reference to the foregoing contents, especially with reference to the explanation of the dotted line in ADC-06A.

In some embodiments, said pharmaceutically acceptable salt comprises a salt of sodium, potassium, calcium, or magnesium derived from an acidic functional group in the ligand-drug conjugate or linker-drug compound. In one embodiment, the pharmaceutically acceptable salt may be a salt derived from a basic functional group in the ligand-drug conjugate or linker-drug

compound including, for example, an acetate, trifluoroacetate, citrate, oxalate, tartrate, malate, nitrate, chloride, bromide, iodide, sulfate, bisulfate, phosphate, lactate, oleate, ascorbate salicylate, formate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate or p-toluenesulfonate.

In a further aspect, the application provides methods of preparing a ligand-drug conjugate or its racemate, enantiomer, diastereoisomer, ring-opening form, pharmaceutically acceptable salt or solvate thereof. In one embodiment, the method includes the step of reacting a reduced Tg with a linker-drug compound or its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvates in a coupling reaction under suitable conditions to provide the desired conjugate. In one embodiment, said method may further comprise the step of purifying the conjugate.

10 In a further aspect, the application provides pharmaceutical compositions comprising a linker-drug compound, its racemate thereof, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvate, or a ligand-drug conjugate or its racemate thereof, enantiomer, diastereoisomer, ring-opening form, pharmaceutically acceptable salt or solvate. In one embodiment, the pharmaceutical composition may optionally further comprise a pharmaceutically acceptable carrier.

In a further aspect, the application provides pharmaceutical formulations or preparations comprising a linker-drug compound, its racemate thereof, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvate, or a ligand-drug conjugate or its racemate thereof, enantiomer, diastereoisomer, ring-opening form, pharmaceutically acceptable salt or solvates.

20 In a further aspect, the application provides uses for the linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvate thereof, the ligand-drug conjugate or its racemate, enantiomer, diastereoisomer, ring-opening form, pharmaceutically acceptable salt or solvate thereof, the pharmaceutical compositions and/or pharmaceutical formulations or preparations as described herein. In one embodiment, the application provides such uses in the preparation of a drug for the treatment and/or prevention a disease. In one embodiment, the application provides such uses for the treatment and/or prevention a disease. In one embodiment, the disease may be cancer or tumors.

30 In one embodiment, said cancer or tumor may be selected from adenocarcinoma, ovarian cancer, cervical cancer, uterine cancer, prostate cancer, renal cancer, urothelial cancer, bladder cancer, hepatocellular carcinoma, gastric cancer, endometrial cancer, salivary gland cancer, esophageal cancer, lung cancer, colon cancer, breast cancer (e.g. triple-negative breast cancer), rectal cancer, colorectal cancer, bone cancer, skin cancer, thyroid cancer, pancreatic cancer, melanoma, glioma, neuroblastoma, solid or hematologic tumors such as glioblastoma multiforme, sarcoma, lymphoma, and leukemia.

In a further aspect, the application provides methods of treating and/or preventing cancer or tumors. In one embodiment, the method include the step of administering to a subject in need of such treatment a prophylactically or therapeutically effective amount of the linker-drug compound or its racemate thereof, enantiomeric isomer, diastereomeric isomer, pharmaceutically acceptable salt or solvate, the ligand-drug conjugate or its racemate enantiomer, diastereoisomer, ring-opening
40 form, pharmaceutically acceptable salt or solvate, the pharmaceutical composition, or the

pharmaceutical formulation or preparation as described herein.

In one embodiment, said cancer or tumor may be adenocarcinoma, ovarian cancer, cervical cancer, uterine cancer, prostate cancer, renal cancer, urothelial cancer, bladder cancer, hepatocellular carcinoma, gastric cancer, endometrial cancer, salivary gland cancer, esophageal cancer, lung cancer, colon cancer, breast cancer, rectal cancer, colorectal cancer, bone cancer, skin cancer, thyroid cancer, pancreatic cancer, melanoma, glioma, neuroblastoma, solid or hematologic tumors such as glioblastoma multiforme, sarcoma, lymphoma, or leukemia. In one embodiment, the breast cancer may be triple-negative breast cancer. In one embodiment, the solid or hematologic tumors may be glioblastoma multiforme.

10 **BENEFICIAL EFFECTS**

1. The linker-drug compound and ligand-drug conjugate described in the application offer significant technical advantages over existing drugs of the same type. They exhibit superior homogeneity, uniformity (for example, the ratio of DAR = 4 is significantly higher), and stability, while effectively enhancing the hydrophilicity of the ligand-drug conjugate and minimizing aggregation. These attributes position them as highly promising candidates with excellent tumor inhibiting effect in vivo and in vitro, exceptional therapeutic potential, great development value, and the capacity to deliver excellent clinical outcomes.

20 2. The disclosure additionally provides a double linker structure with unique structural characteristics. Specifically, compared with the common double-linking structure in the prior art, the existence of basic groups (such as Z in formula II) can promote the self-ring opening of succinimide structure (also called maleimide structure) in linking groups (such as W1 or W2 in formula II) in the double linker disclosed in the disclosure, thereby avoiding the occurrence of the reverse-Michael addition reaction and further inhibiting the shedding of Linker-Drug from the prepared ligand-drug conjugate (see, for example, Figure 1), therefore, the overall stability and hydrophilicity of the prepared ligand-drug conjugate are significantly enhanced.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other features of this disclosure may become more fully apparent from the following description and appended claims, taken in conjunction with the accompanying drawings. Understanding that these drawings depict only several embodiments arranged in accordance with the disclosure and are, therefore, not to be considered limiting of its scope, the disclosure may be described with additional specificity and detail through use of the accompanying drawings, in which:

Figure 1 schematically shows the mechanism of the preferred double linker structure;

Figure 2 shows the *in vitro* efficacy of TROP2-ADC-06 in human cancer cell lines of A431 (2A), BxPC3 (2B), Fadu (2C), HCC827 (2D), and A431+SW620 (2E);

Figure 3 shows the *in vitro* efficacy of TROP2-ADC-24 in human cancer cell lines of A431 (3A), BxPC3 (3B), Fadu (3C), SW620 (3D), and A431+SW620 (3E);

Figure 4 shows the *in vivo* tumor suppressing effects of TROP2-ADC-06 and control ADCs (4A) and TROP2-ADC-24 and control ADCs (4B) at high and low doses in a subcutaneous xenograft model of human epidermal carcinoma cells A431 in BALB/c-nu mice; and

Figure 5 shows the *in vivo* tumor suppressing effects of TROP2-ADC-06 and control ADCs at high and low doses in a subcutaneous xenograft model of mixed human epidermal carcinoma cells A431 and human colon cancer cells SW620 in BALB/c-nu mice.

DETAILED DESCRIPTION

In the following detailed description, reference is made to the accompanying drawings, which form a part hereof. In the drawings, similar symbols typically identify similar components, unless context dictates otherwise. The illustrative embodiments described in the detailed description, drawings, and claims are not meant to be limiting. Other embodiments may be utilized, and other changes may be made, without departing from the spirit or scope of the subject matter presented herein. It will be readily understood that the aspects of the disclosure, as generally described herein, and illustrated in the Figures, can be arranged, substituted, combined, separated, and designed in a wide variety of different configurations, all of which are explicitly contemplated herein.

The disclosure provides, among others, antibodies including for example bispecific antibodies, isolated antibodies, methods of making such antibodies, monoclonal and/or recombinant antibodies, linkers, toxins, linker-drug compounds, ligand-drug conjugates, antibody-drug conjugates, antibody-drug conjugates and/or immuno-conjugates derived from such antibodies, linkers, toxins, pharmaceutical compositions, formulations or preparations containing the antibodies, monoclonal and/or recombinant bispecific antibodies, linker-drug compounds, antibody-drug conjugates and/or immuno-conjugates, the methods for making the antibodies, linkers, toxins, linker-drug compounds, antibody-drug conjugates, and compositions, and the methods for treating cancer using the linker-drug compounds, ligand-drug conjugates, antibody-drug conjugates and/or immuno-conjugates and compositions.

Unless otherwise indicated, the following terms and phrases, as used herein, are intended to have the meanings set forth below. When a trade name is used herein, unless otherwise indicated in the context, the trade name includes the product formulation, generic drug and active ingredient of said trade name product.

The term "ligand" is a targeting agent that binds specifically to a target component. Said ligand is capable of binding specifically to the cellular component or to other target molecules of interest. The target portion or target is typically on the surface of the cell. In some embodiments, the ligand serves to deliver the drug unit to a specific target cell population with which the ligand unit interacts. Ligands include, but are not limited to, proteins, polypeptides and peptides, and non-proteins such as sugars. Suitable ligand units include, for example, antibodies, such as full-length (intact) antibodies and antigen-binding fragments thereof. In some embodiments where the ligand unit is a non-antibody targeting reagent, it may be a peptide or polypeptide, or a non-protein molecule. Examples of such targeting reagents include interferons, lymphokines, hormones, growth and colony stimulating factors, vitamins, nutrient transporter molecules, or any other cell-binding molecule or substance. In some embodiments, the linker is covalently attached to the sulfur atom of the ligand. In some embodiments, the sulfur atom is a sulfur atom of a cysteine residue that forms an interchain disulfide bond of the antibody. In another aspect, the sulfur atom is a sulfur atom of a cysteine residue that has been imported into the ligand unit, which forms the interchain disulfide bond of the antibody. In another aspect, the sulfur atom is a sulfur atom that has been introduced into a cysteine residue of the ligand unit by, for example, targeted mutagenesis or chemical reaction.

The term "basic" or "basic group" refers to the basic structure in the acid-base ionization theory, acid-base proton theory and acid-base electron theory. Acid-base ionization theory (Arrhenius): any substance that can ionize to produce H^+ in aqueous solution is called an acid, and any substance that can ionize to produce OH^- is called a base, and the essence of the acid-base reaction is that H^+ interacts with OH^- to produce water. Acid-base proton theory (Bronsted-Laurie Acid-Base Theory): any molecule or ion that can release a proton (a hydrogen ion, H^+) is an acid (Bronsted Acid), and any molecule or ion that can accept a hydrogen ion is a base (Bronsted Base Theory). Molecules or ions are bases (Bronsted bases); acid-base electron theory (Lewis's acid-base theory): any substance (group, molecule, ion, or group of atoms) that can accept pairs of electrons is called an acid, and any substance (group, molecule, ion, or group of atoms) that can give pairs of electrons is called a base.

The term "connector" refers to a chemical structural fragment or bond that is connected to a ligand at one end and to the drug at the other end by other connecting units, or directly to the drug at the other end.

The term "racemate" refers to a mixture or molecular complex formed by mixing enantiomorphous levamers and dextrorotamers in equal quantities to form a mixture or molecular complex that is not cyclotropic.

The term "enantiomer" refers to stereoisomers that are objects and mirror images of each other. All enantiomers have rotational properties, one of which is left-handed and the other right-handed, therefore, enantiomers are also called optical isomers. The relationship between the two isomers is the same as the stereo structure of an object in a mirror, the stereo structure and its image in the mirror are enantiomers of each other.

The term "diastereoisomer" refers to a conformational isomer that is not enantiomorphous, or diastereomer for short. Diastereoisomerism exists when there are two or more chiral

centers in a molecule. Diastereomers have different physical properties, different specific optical rotation, similar chemical properties, but different reaction rates.

The term "chiral carbon" refers to a carbon atom to which four different groups are attached.

The term "absolute configuration" refers to the true spatial arrangement of the groups in a chiral molecule, i.e. the absolute spatial relationship. When the stereo structure of a compound, as expressed in its structural formula, agrees with the true stereo structure of the compound molecule, the configuration of this stereo structure is the absolute configuration.

The term "non-limiting" means that the event or circumstance subsequently described may or may not occur, and the description includes occasions when the event or circumstance does or does not occur.

The term "linking site" means a chemical bond or unit of attachment to a ligand, wherein the linking site is selected, without limitation, from a carboxyl, amino, carbonyl, mercapto, azido, alkenyl, conjugated dienyl, alkynyl, tetrazinyl linkage, and in one embodiment from a linkage to a sulfhydryl group.

The term "linker unit" (or "linker") includes an extension, a spacer, or an amino acid unit, which can be synthesized by methods known in the art, such as those described in US20050238649A1.

According to the mechanism of intracellular drug release, as used herein, "linker units" can be categorized into two types: unbreakable linker units and breakable linker units. In the case of ligand-drug conjugates containing an unbreakable linker, the mechanism of drug release is as follows: upon binding of the conjugate to the antigen, an active molecule consisting of the small molecule drug, the linker unit, and the amino acid residues of the antibody is released. The resulting change in the structure of the drug molecule does not diminish its cytotoxicity, but because the active molecule is electrically charged (amino acid residues), it cannot penetrate neighboring cells. Thus, such active drugs cannot kill neighboring tumor cells that do not express the targeted antigen (antigen-negative cells) (bystander effect) (Ducry et al., 2010, Bioconjugate Chem. 21: 5-13). The linker unit may also be a "breakable linker" that facilitates the release of the drug in the cell. For example, acid-unstable linkers (e.g., hydrazone), protease-sensitive (e.g., peptidase-sensitive) linkers, light-unstable linkers, or disulfide-containing linkers can be used (Chari et al. Cancer Research 52:127-131, 1992; U.S. Patent No. 5, 208, 020).

The term "amino acid residue" refers to the incomplete amino acid structure remaining after the amino group of an amino acid has lost a hydrogen and the carboxyl group has lost a hydroxyl group, having an amino end and a carbonyl end. For example, A₂ is a peptide residue comprising 2-10 natural or non-natural amino acid residues, wherein the 2-10 amino acid species may be the same or different from each other.

The term "modifying unit" means a chemical bond or extension, a spacer, an amino acid unit with a branching structure, a sugar unit, a polyethylene glycol unit, a poly sarcosine unit, a carbonyl group, an amide group, a carboxylic acid group, a phosphoric acid group, a sulfonic acid group, a sulfinyl group, an alkyl group, a cycloalkyl group, a heterocyclic group, an aryl group, a heteroaryl group, or a combination thereof, which is linked to a linker at one end and to another linker at the other end. The role of the modifying unit is to enhance the

hydrophilicity of the antibody-drug conjugate or to have a branching structure to increase drug loading.

The term "elimination unit" refers to a chemical structure that possesses the ability to release an active drug by self-elimination.

The term "chelating unit" refers to a chemical structure that complexes with a metal ion through two or more chelating ligands. There are typically two types of coordination bonds between a chelating unit and a metal ion: 1) the chelating unit contains an acidic group that dissociates to H^+ and then coordinates with the metal ion; and 2) a neutral group containing a lone electron pair on the ligand coordinates with the metal ion.

10 The term "protein degraders" refers to structures that degrade target proteins through mediating the recognition of the target protein by ubiquitination ligases. There are two main classes, PROTACs, which specifically degrade target proteins via the ubiquitin-proteasome pathway, and molecular gels, which also bind ubiquitination ligases, thereby recognizing and degrading entirely new substrates.

The term "peptide" refers to a fragment of a compound between an amino acid and a protein, consisting of two or more amino acid molecules connected to each other by a peptide bond, and is a structural and functional fragment of a protein, and non-restricted examples include hormones, enzymes, and so on.

20 The term "enzyme inhibitor" refers to a substance that inhibits the activity of a specific enzyme in an organism that is associated with a disease to achieve a therapeutic effect.

The term "nucleotide" refers to a class of compounds consisting of three substances, namely, purine or pyrimidine bases, ribose or deoxyribose, and phosphate, which have a variety of important biological functions.

The term "radioisotope" refers to an element that is radioactive, and non-limiting examples of radioisotopes include ^{68}Ga , ^{99m}Tc , ^{131}I , ^{90}Y , ^{177}Lu , ^{64}Cu , ^{211}At , ^{213}Bi , ^{212}Pb , ^{225}Ac .

30 The term "drug load" or "drug-antibody ratio (DAR)" refers to the average amount of cytotoxic drug loaded on each ligand or antibody, which can also be expressed as the ratio of drug to antibody amount and can be an integer or a decimal. The amount of drug loaded may range from an average of 1-10 ligations per antibody, for example, it may be any integer selected from between 1-10 including endpoints 1 and 10, or any decimal number between 1-10.

The term "derivative" means a product resulting from the substitution of atoms or groups in the molecule of a compound by other atoms or groups.

40 The term "alkyl" refers to a saturated aliphatic hydrocarbon group, which is a straight or branched chain group containing 1 to 20 carbon atoms (i.e., " C_1 - C_{20} alkyl"), in one embodiment, an alkyl group containing 1 to 12 carbon atoms (i.e., " C_1 - C_{12} alkyl"), in one embodiment, an alkyl group containing 1 to 10 carbon atoms (i.e., " C_1 - C_{10} alkyl"), and in a further embodiment, an alkyl group containing 1 to 6 carbon atoms (i.e., " C_1 - C_6 alkyl"). Non-limiting examples include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, tert-butyl, sec-butyl, n-pentyl, 1,1-dimethylpropyl, 1,2-dimethylpropyl, 2,2-dimethylpropyl, 1-ethylpropyl, 2-methylbutyl, 3-methylbutyl, n-hexyl, 1-ethyl-2-methylpropyl, 1,1,2-trimethylpropyl, 1,1-dimethylbutyl, 1,2-dimethylbutyl, 2,2-dimethylbutyl, 1,3-dimethylbutyl, 2-ethylbutyl, 2-methylpentyl, 3-

methylpentyl, 4-methylpentyl, 2,3-dimethylbutyl, n-heptyl, 2-methylhexyl, 3-methylhexyl, 4-methylhexyl, 5-methylhexyl, 2,3-dimethylpentyl, 2,4-dimethylpentyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, 3,3-dimethylpentyl, 2-ethylpentyl, 3-ethylpentyl, n-octyl, 2,3-dimethylhexyl, 2,4-dimethylhexyl, 2,5-dimethylhexyl, 2,2-dimethylhexyl, 3,3-dimethylhexyl, 4,4-dimethylhexyl, 2-ethylhexyl, 3-ethylhexyl, 4-ethylhexyl, 2-methyl-2-ethylpentyl, 2-methyl-3-ethylpentyl, n-nonyl, n-ethylpentyl, 2,2-ethylpentyl, 4-methylpentyl, 4-methylpentyl, n-nonyl, 2,2-methylpentyl, 4-methylpentyl, n-nonyl ethylpentyl, n-nonyl, 2-methyl-2-ethylhexyl, 2-methyl-3-ethylhexyl, 2,2-diethylpentyl, n-decyl, 3,3-diethylhexyl, 2,2-diethylhexyl, and their various branched isomers, and the like. Additional examples are lower alkyl groups

10 containing from 1 to 6 carbon atoms, and non-limiting embodiments include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, tert-butyl, sec-butyl, n-pentyl, 1,1-dimethylpropyl, 1,2-dimethylpropyl, 2,2-dimethylpropyl, 1-ethylpropyl, 2-methylbutyl, 3-methylbutyl, n-hexyl, 1-ethyl-2-methylpropyl, 1,1,2-trimethylpropyl, 1,1-dimethylbutyl, 1,2-dimethylbutyl, 2,2-dimethylbutyl, 1,3-dimethylbutyl, 2-ethylbutyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 2,3-dimethylbutyl, and the like. The alkyl group may be substituted or non-substituted, and when substituted, the substituent group may be substituted at any available point of attachment, said substituent group in one embodiment, being one or more of the following groups independently selected from alkyl, alkenyl, alkynyl, alkoxy, alkylthio, alkylamino, halogen, mercapto, hydroxy, nitro, cyano, cycloalkyl, heterocycloalkyl, aryl,

20 heteroaryl, cycloalkoxy, heterocycloalkoxy, cycloalkane thio, heterocycloalkylthio, or oxo.

The term "substituted alkyl" means that the hydrogen in said alkyl group is replaced by a substituent group which, unless otherwise indicated in the text, may be a variety of groups selected from, without limitation, for example, the following group: -halogen, -OR', -NR'R'', -SR', -SiR'R''R''', -OC(O)R', -C(O)R', -CO₂ R', -CONR'R'', -OC(O)NR'R'', -NR'C(O)R', -NR'C(O)NR''R''', -NR'C(O)₂ R'', -NH-C(NH₂)=NH, -NR'C(NH₂)=NH, -NH-C(NH₂)=NR', -S(O)R', -S(O)₂ R', -S(O)₂ NR'R'', -NR'S(O)₂R'', -CN and -NO₂, the number of substituents is from 0 to (2m'+1), where m' is the total number of carbon atoms in the group. R', R'' and R''' each independently refer to hydrogen, unsubstituted C₁₋₈ alkyl, unsubstituted C₆₋₁₂ aryl (or C₆₋₁₀ aryl), C₆₋₁₂ aryl (or C₆₋₁₀ aryl) substituted by 1-3 halogens, unsubstituted C₁₋₈ alkyl,

30 C₁₋₈ alkoxy or C₁₋₈ thioalkoxy, or unsubstituted C₆₋₁₂ aryl (or C₆₋₁₀ aryl)-C₁₋₄ alkyl. When R' and R'' are attached to the same nitrogen atom, they may form a 3-, 4-, 5-, 6-, or 7-membered ring together with that nitrogen atom. For example, -NR'R'' includes 1-pyrrolidinyl and 4-morpholinyl.

The term "alkylene" means a saturated straight or branched aliphatic hydrocarbon group having two residues derived from the removal of two hydrogen atoms from the same carbon atom or from two different carbon atoms of the parent alkane, which is a straight or branched group comprising from 1 to 20 carbon atoms, in one embodiment, from 1 to 12 carbon atoms, in one embodiment, from 1 to 6 carbon atoms. alkyl or alkylene groups containing from 1 to 12 carbon atoms, in one embodiment, from 1 to 6 carbon atoms. Non-limiting examples of

40 alkyl or alkylene groups include, but are not limited to, methylene (-CH₂-), 1,1-ethylidene (-CH(CH₃)-), 1,2-ethylidene (-CH₂CH₂-), 1,1-propylidene (-CH(CH₂CH₃)-), 1,2-propylidene (-CH₂CH(CH₃)-), 1,3-propylidene (-CH₂CH₂CH₂-), 1,4-butylene (-CH₂CH₂CH₂CH₂-), and 1,5-

butylidene ($-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$), among others. The alkyl or alkylene group may be substituted or non-substituted, and when substituted, the substituent may be substituted at any available linkage, said substituent in one embodiment, being independently selected from alkyl, alkenyl, alkynyl, alkoxy, alkylthio, alkylamino, halogen, mercapto, hydroxy, nitro, cyano, cycloalkyl, heterocyclo, aryl, heteroaryl, cycloalkoxy, heterocycloalkoxy, cycloalkanethoxy, cycloalkanethiol, heterocycloalkanethiol and one or more substituents in the oxo group.

The term "substituted alkylidene" means that the hydrogen in said alkylidene is replaced by a substituent group which, unless otherwise indicated, may be a variety of groups selected from, without limitation, the following examples: -halogen, -OR', -NR''R''', -SR', -SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -CONR''R''', -OC(O)NR'R''', -NR''C(O)R'', -NR'-C(O)NR''R''', -NR''C(O)₂R'', -NH-C(NH₂)=NH, -NR'C(NH₂)=NH, -NH-C(NH₂)=NR', -S(O)R', -S(O)₂R', -S(O)₂NR'R'', -NR'S(O)₂R'', -CN and -NO₂, the number of substituents is from 0 to (2m'+1), where m' is the total number of carbon atoms in the group. r', R'' and R''' each independently refer to hydrogen, unsubstituted C₁₋₈ alkyl, unsubstituted C₆₋₁₂ aryl (or C₆₋₁₀ aryl), C₆₋₁₂ aryl (or C₆₋₁₀ aryl) substituted by 1-3 halogens, unsubstituted C₁₋₈ alkyl, C₁₋₈ alkoxy or C₁₋₈ thioalkoxy, or unsubstituted C₆₋₁₂ aryl (or C₆₋₁₀ aryl)-C₁₋₄ alkyl. When R' and R'' are attached to the same nitrogen atom, they may form a 3-, 4-, 5-, 6-, or 7-membered ring together with that nitrogen atom. For example, -NR'R'' includes 1-pyrrolidinyl and 4-morpholinyl.

The terms "alkenyl" or "alkynyl" refer to unsaturated straight or branched aliphatic hydrocarbon groups.

The term "substituted alkenyl" or "substituted alkynyl" means that the hydrogen in the alkenyl or alkynyl group is replaced by a substituent group.

The term "cycloalkyl" refers to a saturated or partially unsaturated monocyclic or polycyclic cyclic hydrocarbon substituent, wherein the cycloalkyl ring comprises from 3 to 20 carbon atoms (i.e. "C₃-C₂₀ cycloalkyl"), in one embodiment, from 3 to 12 carbon atoms (i.e. "C₃-C₁₂ cycloalkyl"), in one embodiment, comprising 3 to 10 carbon atoms (i.e., "C₃-C₁₀ cycloalkyl"), in a further embodiment, comprising 3 to 8 carbon atoms (i.e., "C₃-C₈ cycloalkyl"). Non-limiting examples of monocyclic cycloalkyl groups (e.g., "C₃-C₈ cycloalkyl") include cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cyclohexadienyl, cycloheptenyl, cycloheptatrienyl, cyclooctyl, and the like; and multi-cyclic cycloalkyl groups include cycloalkyl groups of spirocycles, thickened rings, and bridged rings.

The term "heterocyclyl" or "heterocyclic" means a saturated or partially unsaturated monocyclic or polycyclic cyclic hydrocarbon substituent comprising from 3 to 20 ring atoms (i.e., a "3-20-membered heterocyclyl", "3-20-atom heterocyclyl", "3-20-element heterocyclyl", or "3-20 heterocyclyl"), wherein one or more of the ring atoms is a heteroatom selected from the group consisting of nitrogen, oxygen, or S(O)_m (wherein m is an integer 0 to 2) of a heteroatom, but excluding the ring portion of -O-O-, -O-S- or -S-S-, with the remaining ring atom(s) being carbon. In one embodiment, it contains 3 to 12 ring atoms (i.e., a "3-12 heterocyclic group"), 1 to 4 of which are heteroatoms; in one embodiment, the cycloalkyl ring contains 3 to 10 ring atoms (i.e., a "3-10 heterocyclic group"). Non-limiting examples of monocyclic heterocyclic groups (e.g., 3-7-membered heterocyclic groups) include pyrrolidinyl, piperidinyl, piperazinyl, morpholinyl, thiomorpholinyl, homopiperazinyl, and the like.

Polycyclic heterocyclic groups include spiro, thick and bridged ring heterocyclic groups.

The term "cycloalkylalkyl" means that the alkyl group is substituted with one or more cycloalkyl groups, in one embodiment, with a cycloalkyl group, wherein alkyl is as defined above and wherein cycloalkyl is as defined above. For example, C₃-C₈ cycloalkyl C₁-C₆ alkyl.

The term "haloalkyl" means that the above alkyl group is substituted with one or more halogens, wherein the alkyl group is as defined above, e.g., halo-C₁-C₆ alkyl. Similarly, the term "deuteroalkyl" refers to the above alkyl group substituted with one or more deuteriums, wherein the alkyl group is as defined above, e.g., deutero-C₁-C₆ alkyl.

The term "aryl" refers to a group of a carbocyclic aromatic system. For example, "C₆-C₁₀ aryl" refers to a group of a carbocyclic aromatic system having 6-10 carbon atoms, and non-limiting examples include, but are not limited to, phenyl, naphthalenyl, and the like.

The term "heteroaryl" refers to an aromatic heterocyclic ring, typically a heterocyclic ring having from 1 to 3 heteroatoms selected from N, O or S; the heteroaryl ring may optionally be further thickened or attached to aromatic and non-aromatic carbon and heterocyclic rings. Non-limiting examples of said heteroaryl groups are, for example, pyridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, indolyl, imidazolyl, thiazolyl, isothiazolyl, thiadiazolyl, pyrrolyl, phenyl-pyrrolyl, furanyl, phenyl-furanyl, oxazolyl, isoxazolyl, pyrazolyl, thiophenyl, benzofuranyl, benzothiophenyl, benzo-1,3-dioxolane (benzodiazem), isodihydroindolyl, benzimidazolyl, indazolyl, quinolinyl, isoquinolinyl, 1,2,3-triazolyl, 1-phenyl-1,2,3-triazolyl, 2,3-dihydroindolyl, 2,3-dihydrobenzofuranyl, 2,3-dihydrobenzothiophene, benzopyranyl, 2,3-dihydrobenzoxazinyl, 2,3-dihydroquinoxalinyl, and others.

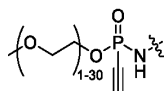
The term "substituted aryl" or "substituted heteroaryl" or "substituted heterocyclyl" means that the hydrogen in the aryl or heteroaryl or heterocyclyl group is replaced by a substituent group, unless the context indicates otherwise. Unless otherwise specified, the substituent of the aryl or heteroaryl or heterocyclyl group may be the following examples: -halogen, -OR', -NR'R'', -SR', -SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -CONR'R'', -OC(O)NR'R'', -NR''C(O)R'', -NR'-C(O)NR''R''', -NR''C(O)₂R'', -NH-C(NH₂)=NH, -NR'C(NH₂)=NH, -NH-C(NH₂)=NR', -S(O)R', -S(O)R'', -S(O)₂R', -S(O)₂NR'R'', -NR'S(O)₂R'', -CN and -NO₂, with substituent numbers from 0 to (2m'+1), where m' is the total number of carbon atoms in the group. R', R'' and R''' each independently refer to hydrogen, unsubstituted C₁₋₈ alkyl, unsubstituted C₆-C₁₂ aryl (or C₆-C₁₀ aryl), C₆-C₁₂ aryl (or C₆-C₁₀ aryl) substituted by 1-3 halogens, unsubstituted C₁₋₈ alkyl, C₁₋₈ alkoxy or C₁₋₈ thioalkoxy, or unsubstituted C₆-C₁₂ aryl (or C₆-C₁₀ aryl)-C₁₋₄ alkyl. When R' and R'' are attached to the same nitrogen atom, they may form a 3-, 4-, 5-, 6-, or 7-membered ring with that nitrogen atom. For example, -NR'R'' includes 1-pyrrolidinyl and 4-morpholinyl.

The term "alkoxy" refers to -O-(alkyl) and -O-(cycloalkyl), wherein the definition of alkyl or cycloalkyl is as described above. Non-limiting examples of C₁-C₆ alkoxy include: methoxy, ethoxy, propoxy, butoxy, cyclopropyloxy, cyclobutyloxy, cyclopentyloxy, cyclohexyloxy. Alkoxy can be optionally substituted or unsubstituted, and when substituted, substituents are in one embodiment, one or more of the following groups, which are independently selected from alkyl, alkenyl, alkynyl, alkoxy, alkylthio, alkylamino, halogen, sulfhydryl, hydroxyl, nitro, cyano, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, cycloalkyloxy, heterocycloalkyloxy,

cycloalkylthio, heterocycloalkylthio.

Further in the application, it is understood by those skilled in the art that any group which is not further specified or limited in front of or behind the group means that the group is an unsubstituted group. For example, "C₁-C₆ alkyl" has the same meaning as "unsubstituted C₁-C₆ alkyl". The remaining similar groups are explained and understood with reference to the foregoing.

In addition, any group in the present application containing a numerical value in the lower right corner of the bracket to represent the number of repetitions of the repeating unit, if the numerical value is a range value, it means that the number of repetitions of the repeating unit is selected from any integer point value within the range including the end point of the range value or any smaller range value. For example, in the formula,



the number of repetitions of the repeating unit -O-CH₂-CH₂- is 1-30, which means that the number of repetitions of the repeating unit is selected from any integer point value within the range including the two endpoints of 1 and 30, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30, or the number of repetitions of the repeating unit is selected from any smaller range value within the range including the two endpoints of 1 and 30, such as 1-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8, 8-9, 9-10, 10-11, 11-12, 12-13, 13-14, 14-15, 15-16, 16-17, 17-18, 18-19, 19-20, 20-21, 21-22, 22-23, 23-24, 24-25, 25-26, 26-27, 27-28, 28-29, 29-30, 1-5, 5-10, 10-15, 15-20, 20-25 or 25-30, etc. The remaining similar groups are explained and understood with reference to the foregoing.

The term "hydroxyl" refers to the -OH group.

The term "halogen" means halogen atoms or its ionic forms, including for example fluorine/fluoride, chlorine/chloride, bromine/bromide, or iodine/iodide.

The term "amino" refers to substituted or unsubstituted amino group (i.e., primary, secondary, or tertiary amino group) including for example -NH₂ or its ionic form.

The term "nitro" refers to -NO₂.

The term "amide group" means -C(O)N(alkyl) or (cycloalkyl), wherein alkyl, cycloalkyl is as defined above.

The term "carboxyl" means -C(O)OH or its ionic form.

The term "sulfinyl" means -S(O)OH or its ionic form.

The term "sulfonic acid group" means -S(O)₂ OH or its ionic form.

The term "phosphate group" refers to -P(O)₂ OH or its ionic form.

The term "pyrophosphate group" means -P(O)₂ OP(O)₂ OH or its ionic form.

The term "ester group" means -C(O)O(alkyl) or (cycloalkyl), wherein alkyl, cycloalkyl is as defined above.

The term "polyethylene glycol" means an oligomer or polymer of ethylene oxide.

The term "solvent compound" or "solvent compound" refers to a linker, linker-drug or ligand-drug conjugate forming a pharmaceutically usable solvent compound with one or more solvent molecules, non-limiting examples of solvent molecules include water, ethanol, acetonitrile, isopropanol, DMSO, ethyl acetate, ethanol, acetonitrile, isopropanol, DMSO,

ethyl acetate.

The term "pharmaceutically acceptable salt" or "pharmaceutically acceptable salt" refers to a salt of the linker-drug or ligand-drug conjugate, or a salt of any compound described herein. Such salts are safe and effective when used in mammals and have the desired biological activity. In some embodiments, the ligand-drug conjugate compound contains at least one carboxyl group and thus can form salts with bases. Non-limiting examples of pharmaceutically acceptable salts include sodium salts, potassium salts, calcium salts or magnesium salts. In some embodiments, the ligand-drug conjugate compound contains at least one amino group and thus can form salts with acids. Non-limiting examples of pharmaceutically acceptable salts include hydrochloride, hydrobromide, hydroiodide, sulfate, hydrogen sulfate, citrate, acetate, succinate, ascorbate, oxalate, nitrate, sorbate, hydrogen phosphate, dihydrogen phosphate, salicylate, hydrogen citrate, tartrate, maleate, fumarate, formate, benzoate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate.

The term "ligand" or "targeting moiety" is a targeting agent such as a macromolecular compound, that can recognize and specifically binds to a target moiety such as an antigen or receptor associated with a target cell. The target moiety or target is usually on the cell surface. The role of the ligand is to present the drug to the target cell population bound to the ligand, and these ligands include but are not limited to protein hormones, lectins, growth factors, antibodies or other molecules that can bind to cells. In one embodiment, the ligand is represented by Tg, and the ligand can form a connecting bond with the connecting unit through a heteroatom on the ligand, such as an antibody or an antigen-binding fragment thereof, wherein the antibody may be a chimeric antibody, a humanized antibody, a fully human antibody or a mouse antibody, in one embodiment, a monoclonal antibody.

In some embodiments, the role of the ligand unit is to deliver the drug unit to a specific target cell population with which the ligand unit interacts. Ligands include, but are not limited to, proteins, polypeptides and peptides, and non-proteins such as sugars. Suitable ligand units include, for example, antibodies, such as full-length (complete) antibodies and antigen-binding fragments thereof. In embodiments where the ligand unit is a non-antibody targeting agent, it can be a peptide or polypeptide, or a non-protein molecule. Examples of such targeting agents include interferons, lymphokines, hormones, growth factors and colony stimulating factors, vitamins, nutrient transport molecules, or any other cell binding molecules or substances. In some embodiments, the linker is covalently attached to the sulfur atom of the ligand. In some embodiment, the sulfur atom is the sulfur atom of a cysteine residue, which forms an interchain disulfide bond of the antibody. In another aspect, the sulfur atom is a sulfur atom of a cysteine residue that has been introduced into the ligand unit, which forms an interchain disulfide bond of the antibody. In another aspect, the sulfur atom is a sulfur atom of a cysteine residue that has been introduced into the ligand unit (e.g., by site-directed mutagenesis or chemical reaction). In other aspects, the sulfur atom to which the linker is bound is selected from a cysteine residue that forms an interchain disulfide bond of the antibody or a cysteine residue that has been introduced into the ligand unit (e.g., by site-directed mutagenesis or chemical reaction). In some embodiments, the EU index numbering system is according to Kabat {[Kabat E.A et al., (1991)] Sequences of proteins of Immunological

Interest, Fifth Edition, NIH Publication 91-3242}.

As used herein, "antibody" or "antibody unit" includes any part of the antibody structure within the scope to which it belongs. This unit can bind, reactively associate, or complex a receptor, antigen or other receptor unit possessed by the targeted cell population. The antibody can be any protein or protein-like molecule that can bind, complex or react with a part of the cell population to be treated or biomodified. In some embodiment, the antibodies constituting the antibody-drug conjugates retain their antigen binding ability in their original wild state. Therefore, the antibody components can bind specifically to the antigen. The antigens involved include, for example, tumor-associated antigens (TAA), cell surface receptor proteins and other cell surface molecules, cell survival regulators, cell proliferation regulators, molecules related to tissue growth and differentiation (such as known or predicted functional), lymphokines, cytokines, molecules involved in cell cycle regulation, molecules involved in angiogenesis, and molecules related to angiogenesis (such as known or predicted functional). Tumor-related factors can be cluster differentiation factors (such as CD proteins).

Antibodies used in antibody-drug conjugates include, but are not limited to, antibodies against cell surface receptors and tumor-associated antigens (TAAs). Such tumor-associated antigens are well known in the industry and can be prepared by antibody preparation methods and information well known in the industry. To develop effective cellular level targets that can be used for cancer diagnosis and treatment, researchers strive to find transmembrane or other tumor-associated polypeptides. These targets can be specifically expressed on the surface of one or more cancer cells, and rarely or not expressed on the surface of one or more non-cancerous cells. Generally, such tumor-associated polypeptides are more overexpressed on the surface of cancer cells relative to the surface of non-cancerous cells. Confirming such tumor-associated factors can greatly improve the specific targeting characteristics of antibody-based cancer treatment. For convenience, antigen-related information well known in the industry is marked as follows, including name, other names, and gene bank accession number. Nucleic acid and amino acid sequences corresponding to tumor-associated antigens can be found in public databases, such as Genbank. The antibody targets the corresponding tumor-associated antigen, including all amino acid sequence variants and isotypes, and has at least 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% homology with the sequence confirmed in the reference, or has biological properties and characteristics that are completely consistent with the tumor-associated antigen sequence in the cited reference.

The antibodies described herein are, without limitation, mono-, di- or multi-specific antibodies, or the following antibodies: anti-EGFRvIII antibody, anti-DLL-3 antibody, anti-PSMA antibody, anti-CD70 antibody, anti-MUC16 antibody, anti-ENPP3 antibody, anti-TDGF1 antibody, anti-ETBR antibody, anti-MSLN antibody, anti-TIM-1 antibody, anti-LRRC15 antibody, Anti-LIV-1 antibody, anti-CanAg/AFP antibody, anti-Claudin18.2 antibody, anti-Mesothelin antibody, anti-HER2(ErbB2) antibody, anti-EGFR antibody, anti-c-MET antibody, anti-SLITRK6 antibody, anti-KIT/CD117 antibody, anti-STEAP1 antibody, anti-SLAMF7/CS1 antibody, anti-NaPi2B/SLC34A2 antibody, anti-GPNMB antibody, anti-HER3(ErbB3) antibody, anti-MUC1/CD227 antibody, anti-AXL antibody, anti-CD166 antibody, anti-B7-H3(CD276) antibody, anti-PTK7/CCK4 antibody, anti-PRLR antibody, anti-

EFNA4 antibody, anti-5T4 antibody, anti NOTCH3 antibody, anti-Nectin 4 antibody, anti-TROP2 antibody, anti-CD142 antibody, anti-CA6 antibody, anti-GPR20 antibody, anti-CD174 antibody, anti-CD71 antibody, anti-EphA2 antibody, anti-LYPD3 antibody, anti-FGFR2 antibody, anti-FGFR3 antibody, anti-FR α antibody, anti-CEACAMs antibody, anti-GCC antibodies, anti-Integrin Av antibodies, anti-CAIX Antibodies, anti-P-cadherin antibodies, anti-GD3 antibodies, anti-Cadherin 6 antibodies, anti-LAMP1 antibodies, anti-FLT3 antibodies, anti-BCMA antibodies, anti-CD79b antibodies, anti-CD19 antibodies, anti-CD33 antibodies, anti-CD56 antibodies, anti-CD74 antibodies, anti-CD22 antibodies, anti-CD30 antibodies, anti-CD30 antibodies, anti-CD22 antibodies, anti-CD30 antibody, anti-CD37 antibody, anti-
 10 CD138 antibody, anti-CD352 antibody, anti-CD25 antibody, anti-CD123 antibody, or combination thereof.

Antibodies of the application include, but are not limited to, murine antibodies, chimeric antibodies, humanized antibodies and fully human antibodies, in one embodiment, humanized antibodies and fully human antibodies.

The three-letter codes and single-letter codes for amino acids used in the disclosure are as described in *J. boil. chem.* 1968, 243, 3558.

The term "natural amino acids" refers to amino acids that can be synthesized biologically. Natural amino acids are generally of the L-type, with a few exceptions, such as glycine, and include both natural and biologically synthesized ones.

20 The term "unnatural amino acids" refers to amino acids that can only be synthesized artificially.

The term "drug" refers to atoms, ions, small molecules, peptides, nucleotides, etc. with biological activity. Drugs are represented by D, which are chemical molecules that can strongly disrupt the normal growth of tumor cells. In principle, drugs can kill tumor cells at sufficiently high concentrations, but due to the lack of specificity, while killing tumor cells, they can also cause apoptosis of normal cells, leading to serious side effects. The term includes toxins, such as small molecule toxins or enzyme-active toxins from bacteria, fungi, plants or animals, radioactive isotopes (e.g. ^{68}Ga , $^{99\text{m}}\text{Tc}$, ^{131}I , ^{90}Y , ^{177}Lu , ^{64}Cu , ^{211}At , ^{213}Bi , ^{212}Pb , ^{225}Ac), toxic drugs, chemotherapeutic drugs, antibiotics and nucleolytic enzymes, and protein degraders.
 30 Sometimes, drug or toxin may be used interchangeably.

The term "ligand-drug conjugate" refers to a ligand connected to a biologically active drug (or toxin) through a stable linking unit. In this application, "ligand-drug conjugate" is in one embodiment, an antibody-drug conjugate (ADC), which refers to a monoclonal antibody or antibody fragment connected to a biologically active toxic drug through a stable linking unit.

In one embodiment, the cytotoxic drug is coupled to the thiol-SH of the opened cysteine interchain and/or the thiol-SH of the site-directed mutated cysteine residue through a linking unit, an optional modification unit and a linker unit. In some embodiments, the number of drug molecules that can be coupled to the antibody in the coupling reaction may be less than or equal to the theoretical maximum value.

40 In some embodiments and without being bound by the theory, the loading of the ligand-cytotoxic drug conjugate can be controlled by the following non-limiting methods, including:

1) controlling the molar ratio of the linking agent and the monoclonal antibody,

- 2) controlling the reaction time and temperature, or
- 3) selecting different reaction reagents.

The disclosure also includes various deuterated forms of drugs, linkers, linker units-drugs containing linkers, and ligand-drug conjugates containing linkers. Each available hydrogen atom connected to a carbon atom can be independently replaced by a deuterium atom. Those skilled in the art can synthesize deuterated forms of drugs, linkers, linker units-drugs containing linkers, and ligand-drug conjugates containing linkers with reference to relevant literature. Commercially available deuterated starting materials can be used to prepare deuterated forms of drugs, linkers, linker-drugs containing linkers, and ligand-drug conjugates containing linkers, or they can be synthesized using conventional techniques using deuterated reagents, non-limiting examples of which include deuterated borane, trideuterated borane tetrahydrofuran solution, deuterated lithium aluminum hydride, deuterated ethyl iodide, and deuterated methyl iodide, etc.

The term "pharmaceutical composition" "pharmaceutical formulation" or "pharmaceutical preparation" refers to a mixture containing one or more antibody-drug conjugates or their stereoisomers, pharmaceutically acceptable salts or solvates, or linker drugs or their stereoisomers, pharmaceutically acceptable salts or solvates, and other chemical components, as well as other components such as physiologically or pharmaceutically acceptable carriers and excipients. The purpose of a pharmaceutical composition is to promote administration to an organism, facilitate the absorption of active ingredients, and thus exert biological activity. The preparation of conventional pharmaceutical compositions can be found in various pharmacopieas including for example the Chinese Pharmacopoeia or USP.

The term "carrier" refers to a system that can change the way a drug enters the human body and its distribution in the body, control the release rate of the drug, and deliver the drug to the targeted organ. The drug carrier release and targeting system can reduce drug degradation and loss, reduce side effects, and improve bioavailability. For example, polymer surfactants that can be used as carriers can self-assemble to form various forms of aggregates due to their unique amphiphilic structure. Examples include micelles, microemulsions, gels, liquid crystals, vesicles, etc. These aggregates can encapsulate drug molecules and have good permeability to membranes and can be used as excellent drug carriers.

The term "treatment" generally refers to obtaining a desired pharmacological and/or physiological effect. The effect may be preventive, in terms of completely or partially preventing, modulating, or alleviating a disease or its symptoms; and/or therapeutic, in terms of partially or completely stabilizing or curing a disease and/or side effects due to a disease. As used herein, "treatment" encompasses any treatment of a patient's disease, including: (a) preventing a disease or symptom from occurring in a patient who is susceptible to the disease or symptom but has not yet been diagnosed with the disease; (b) inhibiting the symptoms of the disease, i.e., preventing its development; or (c) alleviating the symptoms of the disease, i.e., causing the disease or symptom to regress.

The term "subject" includes a human or non-human animal. Exemplary human subjects include humans (referred to as patients, for example) or normal individuals suffering from a disease (e.g., a disease described herein). The term "non-human animal" includes all

vertebrates, such as non-mammals (e.g., birds, amphibians, reptiles) and mammals, such as non-human primates, livestock and/or domesticated animals (e.g., sheep, dogs, cats, cows, pigs, etc.).

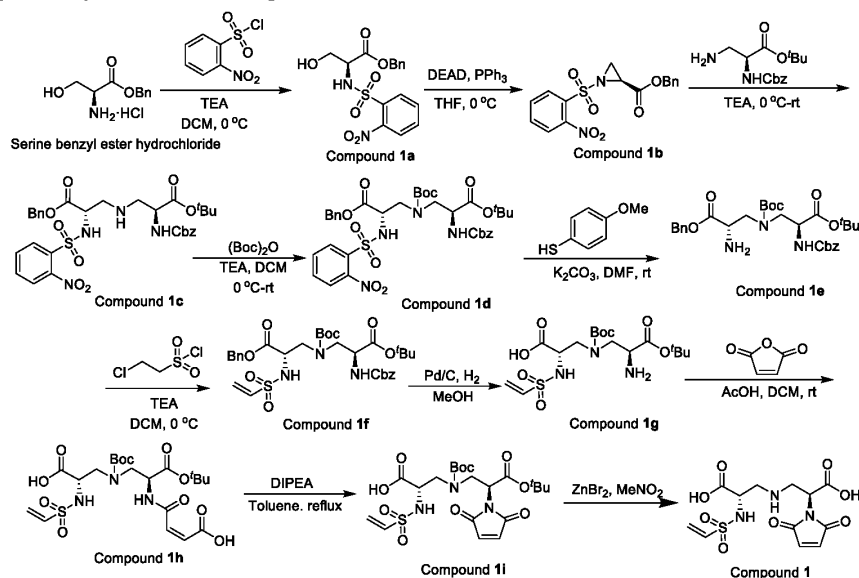
The term "effective amount" refers to the amount of a drug that, after being administered, will alleviate one or more symptoms of the condition being treated to some extent.

The terms "a", "an" and "the" as used herein are defined to mean "one or more" and include the plural unless the context is inappropriate.

EXAMPLES

The application is further elaborated below in connection with specific embodiments which, it should be understood, are intended only to illustrate the invention and are not intended to limit the scope of the invention. Test methods for which specific conditions are not indicated in the following embodiments are generally in accordance with conventional conditions or in accordance with conditions recommended by the manufacturer.

Example 1: Synthesis of Compound 1



Step 1: Compound 1a

Serine benzyl ester hydrochloride (43.16 mmol, 40 g) was weighed and dissolved in DCM (200 mL). The system was cooled to 0 °C, and TEA (90.46 mmol, 12.6 mL) was added dropwise. After the addition was completed, the mixture was stirred for 20 min. At the same temperature, a solution of o-nitrobenzenesulfonyl chloride (45.32 mmol, 10.04 g) in DCM (30 mL) was added dropwise to the system. After the reaction was completed by TLC monitoring, saturated NaHCO₃ aqueous solution (100 mL) was added to the reaction system to quench the reaction. The reaction was extracted with DCM (100 mL×2 times). The organic phases were combined and dried over anhydrous Na₂SO₄. The system was filtered, concentrated, and purified by column chromatography (PE/EA = 1/1). After the solvent was concentrated, an oily liquid compound 1a was obtained with an equivalent yield of LC-MS [M+H]⁺: m/z 381.1.

Step 2: Compound 1b

At room temperature, PPh₃ (27.6 mmol, 7.2 g) was weighed into a three-necked flask, the system was replaced with nitrogen atmosphere, and ultra-dry THF (80 mL) was added to dissolve. The system was cooled to 0 °C, and a solution of compound **1a** (26.3 mmol, 10.0 g) in ultra-dry THF (24 mL) was added dropwise. After 10 min, the addition was completed, and then a solution of DEAD (27.6 mmol, 4.3 mmol) in ultra-dry THF (30 mL) was added dropwise to the system. The reaction was allowed to proceed at this temperature for 1 h, and the reaction was complete when monitored by TLC. Saturated NaHCO₃ aqueous solution (120 mL) was added to the reaction system for quenching, and EA (120 mL×2 times) was added for extraction. The organic phases were combined, dried over anhydrous Na₂SO₄, concentrated, and purified by column chromatography (PE/EA = 7/1). After the solvent was concentrated, 8.6 g of yellow solid product compound **1b** was obtained with a yield of 91%. LC-MS [M+H]⁺: m/z 363.5.

Step 3: Compound **1c**

At room temperature, compound **1b** (21.0 mmol, 7.6 g) was dissolved in THF (200 mL). The system was cooled to 0 °C and (*S*)-3-amino-2-carboxylaminopropionic acid tert-butyl ester (25.3 mmol, 7.44 g) and Et₃N (4.2 mmol, 0.584 mL) were added. The reaction was allowed to warm naturally to room temperature and react overnight. The reaction was monitored for completion by TLC. The reaction system was directly concentrated and purified by column chromatography (PE/EA = 10/1-2/1). Concentration gave 9.8 g of yellow oily liquid compound **1c** with a yield of 71.1%. LC-MS [M+H]⁺: m/z 657.2.

Step 4: Compound **1d**:

Compound **1c** (14.8 mmol, 9.72 g) was dissolved in DCM (330 mL) at room temperature, cooled to 0 °C, Et₃N (44.7 mmol, 6.21 mL) was added dropwise to the reaction system, stirred for 20 min after the completion of addition, and then (Boc)₂O (61.0 mmol, 14 mL) was added, and the reaction was heated up to 35 °C for 5.5 h. The direct concentration and purification followed by column chromatography (PE/EA = 10/1-2/1) to obtain yielded 6.4 g of product compound **1d** with a yield of 57.2%, LC-MS [M+H]⁺: m/z 757.6.

Step 5: Compound **1e**

Compound **1d** (8.5 mmol, 6.4 g) was dissolved in DMF (120 mL) at room temperature, K₂CO₃ (170 mmol, 23.4 g), 4-methoxybenzenethiol (85 mmol, 10.5 mL) were added in sequence. The reaction was allowed to proceed for 30 min at room temperature and the reaction was monitored by TLC for completion. Water (300 mL) and EA (500 mL) were added and extracted three times. The organic phase was dried over anhydrous Na₂SO₄, concentrated and purified by column chromatography (PE/EA = 10/1-5/1-2/1) to obtain 4.0 g of oily compound **1e** with a yield of 82%, LC-MS [M+H]⁺: m/z 572.0.

Step 6: Compound **1f**

Compound **1e** (3.96 mmol, 3.0 g) was weighed and dissolved in dichloromethane (30 mL) at room temperature, TEA (4.0 mmol, 0.56 mL) was added, and the system was kept at 0 °C, and chloroethylsulfonyl chloride (0.67 g, 4 mmol) was added dropwise. The reaction was allowed to proceed at room temperature for 5 h, and the reaction was complete after TLC monitoring. After concentration, 2.3 g of oily product compound **1f** was obtained, with a yield of 85%, and LC-MS [M+H]⁺: m/z 662.8.

Step 7: Compound **1g**

Compound **1f** (2.3 g, 3.5 mmol) was weighed and dissolved in methanol (30 mL) at room temperature, 5% Pd/C (0.23 g) was added, and the system was replaced with H₂ atmosphere. The reaction was continued for 5 h, and the reaction was completed after TLC monitoring. Pd/C was removed by filtration, and **1g** of oily product was obtained after concentrated, with an equivalent yield, which was used directly in the next step without purification.

Step 8: Compound **1h**

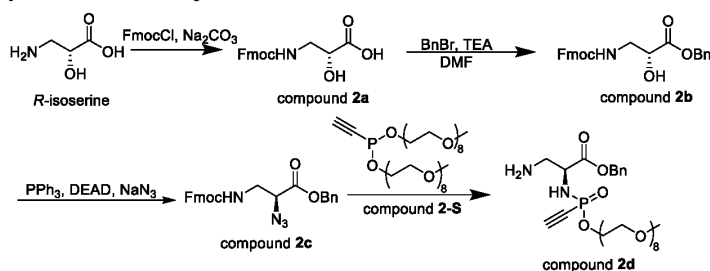
Compound **1g** (2.96 mmol, 1.4 g) was weighed at room temperature and dissolved in DCM (65 mL), and then AcOH (28.64 mmol, 1.64 mL) and maleic anhydride (8.87 mmol, 0.87 g) were added. After reacting at room temperature for 2 h, the reaction was complete as monitored by HPLC, and the system was directly concentrated, purified and lyophilized to obtain 1.2 g of white solid product **1h**, with a yield of 75.3%, LC-MS [M+H]⁺: m/z 536.7.

Step 9: Compound **1i**

Compound **1h** (2.3 mmol, 1.2 g) was weighed at room temperature and dissolved in toluene (25 mL), and DIPEA (3.46 mmol, 0.572 mL) was added. The temperature was raised to 125 °C and refluxed for 2 h. The reaction was completed by HPLC monitoring. After lyophilization, 0.65 g of white solid product **1i** was obtained with a yield of 54.4%, LC-MS [M+H]⁺: m/z 518.3.

Step 10: Compound **1**

Compound **1i** (0.97 mmol, 0.5 g) was weighed at room temperature and dissolved in dry nitromethane (10 mL), anhydrous zinc bromide (19.4 mmol, 4.55 g) was added, and the temperature was raised to 35 °C for 2 h. The reaction was monitored by HPLC to be complete. The nitromethane was removed after water pump decompression and rotary evaporation at 40 °C. The residue was purified by preparative purification to obtain 150 mL of preparative solution. After lyophilization, 0.32 g of white solid product compound **1** was obtained, with a yield of 91%. LC-MS [M-H]⁻: m/z 359.9.

Example 2: Synthesis of Compound **2**Step 1: Compound **2a**

At room temperature, R-isoserine (113.2 mmol, 12.0 g) was weighed and dissolved in THF (500 mL). The system was cooled to 0 °C, and an aqueous solution (250 mL) of Na₂CO₃ (138.7 mmol, 15.0 g) was added, followed by a THF solution (200 mL) of Fmoc-Cl (138.7 mmol, 36.6 g). The reaction was allowed to rise to room temperature for 6 h, and the reaction was complete as determined by TLC. The product was concentrated to remove most of the THF, and EA (200 mL × 3 times) was added for extraction. The product was washed with a saturated aqueous NaCl solution, and the organic phase was dried over anhydrous Na₂SO₄.

The organic phase was filtered and concentrated and dried at 50°C for 2 h to obtain 36.1 g of a white solid product **2a** with a yield of 97%. LC-MS [M-H]⁻: m/z 326.3.

Step 2: Compound **2b**

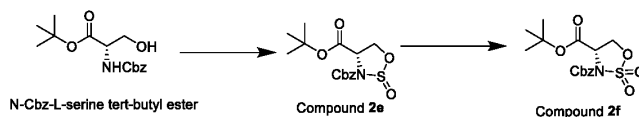
At room temperature, DMF (100 mL) was added to a clean and dry three-necked flask, the reaction system was cooled to 0 °C, compound **2a** (61 mmol, 20.0 g) was weighed and added to the reaction flask, TEA (62 mmol, 8.8 mL) was added to the reaction system, and a solution of BnBr (62 mmol, 7.5 mL) in DMF (50 mL) was added dropwise. The temperature was naturally raised to room temperature for 2 h. After TLC monitoring, the reaction was complete. The reaction solution was placed under ice water and quenched with saturated aqueous sodium bicarbonate solution. EA (100 mL×3 times) was added for extraction. The organic phase was dried over anhydrous Na₂SO₄, filtered and concentrated to obtain a dark brown oily liquid. 23.9 g of the product compound **2b** was obtained by column chromatography (PE/EA = 10/1, 5/1, 2/1) with a yield of 95%. LC-MS [M+H]⁺: m/z 418.5.

Step 3: Compound **2c**

Compound **2b** (52.7 mmol, 22.0 g) was weighed and dissolved in anhydrous THF (100 mL). The atmosphere in the system was replaced with N₂ three times. PPh₃ (79 mmol, 20.9 g) and DEAD (79 mmol, 12.56 mL) were added in turn under ice-water cooling. The reaction was maintained at this temperature for 0.5 h, and NaN₃ (53 mmol, 3.51 g) was added. The temperature was naturally raised to room temperature and the reaction was carried out for 2.5 h. The reaction was completed by TLC monitoring. The reaction solution was placed under ice water and quenched by adding saturated sodium bicarbonate aqueous solution. EA (100 mL×3 times) was added to the filtrate for extraction, and the organic phase was dried over anhydrous Na₂SO₄. The organic phase was filtered and concentrated and purified by column chromatography (PE/EA = 10/1, 5/1, 1/1) to obtain 18.6 g of slightly yellow oily product compound **2c** with a yield of 80%. LC-MS [M+H]⁺: m/z 443.0.

Step 4: Compound **2d**

Compound **2c** (34 mmol, 14.9 g) was weighed and dissolved in anhydrous DMF (100 mL). The atmosphere in the system was replaced with N₂ three times. Compound **2-S** (prepared by the method of reference DOI: 10.1002/anie.201904193, 170 mmol, 140 g) was added at room temperature. The reaction was maintained at this temperature for 16 h. The reaction was complete when monitored by HPLC. Piperidine (20 mL) was added to the reaction mixture and the reaction was maintained at room temperature for 6 h. The reaction was complete when detected by HPLC. The solution was concentrated under reduced pressure by an oil pump to remove most of the DMF. The solution was prepared and purified to obtain 2.7 L of the preparation solution, which was lyophilized to obtain 18.9 g of a slightly yellow oily product, compound **2d**, with a yield of 86%. LC-MS [M+H]⁺: m/z 649.3.



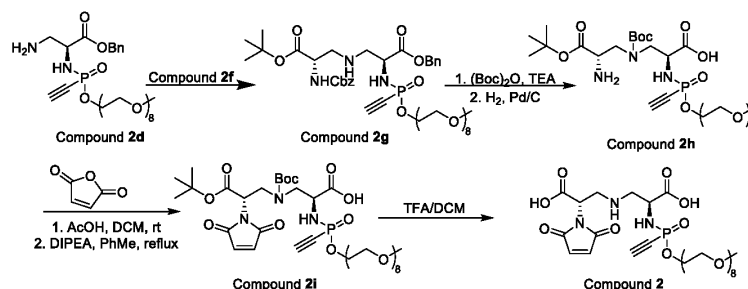
Step 5: Compound **2e**

Ultra-dry CH₃CN (50 mL) was added to a clean and dry three-necked flask at room temperature, the reaction system was cooled to -40 °C. SOCl₂ (38.0 mmol, 2.76 mL) was

added to the reaction system, and the of CH₃CN (100 mL) solution of the compound N-Cbz-L-serine tert-butyl ester (34 mmol, 10.0 g) was added dropwise. The reaction was proceeded at this temperature for 2 h, the CH₃CN (50 mL) solution of Pyridine (76 mmol, 6.1 mL) was added dropwise, and the reaction was naturally warm to room temperature for 7 h. The reaction was complete as monitored by TLC. The reaction solution was quenched in iced water and extracted with EA (100 mL x 3 times). The organic phase was dried over anhydrous Na₂SO₄, filtered and concentrated to obtain a dark brown oily liquid. 5.9 g of product compound **2e** was purified by column chromatography (PE/EA = 10/1, 5/1, 2/1) with a yield of 51%. LC-MS [M+H]⁺: m/z 342.1.

10 Step 6: Compound **2f**

Compound **2e** (17.3 mmol, 5.9 g) was weighed and dissolved in CH₃CN (30 mL). RuO₂·XH₂O (1.73 mmol, 2.3 g) was added at room temperature. The system was cooled to 0 °C and an aqueous solution of NaIO₄ (20 mmol, 4.27 g) (30 mL) was added. The reaction was continued for 2.5 h. The reaction was monitored for completion by TLC. The insoluble solid was removed by filtration. EA (50 mL x 3 times) was added to the filtrate for extraction. The organic phase was dried over anhydrous Na₂SO₄ and filtered and concentrated to obtain a black oily material. The product was purified by column chromatography (PE/EA = 1/1) to obtain 4.76 g of a colorless oily product, compound **2f** with a yield of 77%. LC-MS [M+H]⁺ : m/z 358.2.



Step 7: Compound **2g**

At room temperature, compound **2f** (13.3 mmol, 4.76 g) was weighed and dissolved in THF (120 mL), compound **2d** (14 mmol, 9.1 g) and Et₃N (16 mmol, 2.27 mL) were added. The reaction was raised to 60 °C for 4 h. After the reaction was complete as monitored by TLC, the solvent was concentrated and purified by column chromatography (PE/EA = 10/1-1/1-1-EA) to obtain 6.5 g of product compound **2g** with a yield of 53%. LC-MS [M+H]⁺ : m/z 926.6.

Step 8: Compound **2h**

At room temperature, compound **2g** (6.5 mmol, 6 g) was weighed and dissolved in DCM (65 mL), Et₃N (13 mmol, 1.84 mL) was added, the system was cooled to 0°C, (Boc)₂O (13 mmol, 3 mL) was added, and the reaction was warm naturally to room temperature and react overnight. The next day, water was added to the reaction system, extracted with DCM (50 mL×2 times), washed with saturated NaCl aqueous solution, dried with anhydrous Na₂SO₄, filtered and concentrated to obtain an oily compound, which was directly used in the next reaction without purification.

At room temperature, the above crude compound was weighed and dissolved in methanol

(100 mL), 5% Pd/C (6 g) was added, the system was replaced with H₂ atmosphere, the reaction was continued overnight and monitored for completion by TLC. Pd/C was removed by filtration and concentrated to obtain an oily product. The product was purified to obtain 1.3 L of eluate. After lyophilization, 3.44 g of the compound **2h** was obtained and the two-step yield was 66%. LC-MS [M-Boc-H]⁺ : m/z 700.3.

Step 9: Compound **2i**

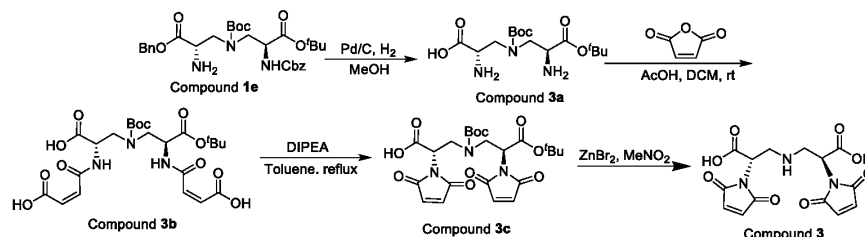
At room temperature, compound **2h** (4.24 mmol, 3.4 g) was dissolved in DCM (40 mL) and AcOH (0.32 mL) and maleic anhydride (8.48 mmol, 0.84 g) were added. After 2 h of reaction at room temperature, the reaction was complete as monitored by HPLC, and the system was directly concentrated and used for the next step of hardening without purification.

At room temperature, the above crude compound was dissolved in toluene (25 mL), DIPEA (8.48 mmol, 1.4 mL) was added, and the temperature was raised to 125°C and refluxed for 2 h. The reaction was complete as monitored by TLC and HPLC. After lyophilization, 2.34 g of white solid product **2i** was obtained, with a two-step yield of 63%, LC-MS [M-H]⁺ : m/z 880.2.

Step 10: Compound **2**

Compound **2i** (1.13 mmol, 1 g) was weighed and dissolved in dichloromethane (10 mL) at room temperature, TFA (5 mL) was added, and the reaction was carried out at room temperature for 2 h. The reaction was monitored by HPLC. The solvent was removed by rotary evaporation at 40 °C under reduced pressure with a water pump, and the residue was purified by preparative purification to obtain about 900 mL of preparative solution. After lyophilization, 0.39 g of the white solid product, compound **2** was obtained with 47% yield, LC-MS [M-H]⁺ : m/z 724.8.

Example 3: Synthesis of Compound **3**



Step 1: Compound **3a**

Compound **1e** (3 g, 5.2 mmol) was weighed and dissolved in methanol (50 mL) at room temperature, 10% Pd/C (0.52 g) was added, and the system was replaced with H₂ atmosphere, and the reaction was carried out for 15 h. The complete reaction was monitored by TLC. The Pd/C was removed by filtration and concentrated to obtain the oily product **3a**, which was used directly in the next step without purification.

Step 2: Compound **3b**

At room temperature, the crude compound **3a** (5.2 mmol) was dissolved in DCM (55 mL), and AcOH (26 mmol, 1.49 mL) and maleic anhydride (10.4 mmol, 1.02 g) were added. After reacting for 2 h at room temperature, the reaction was complete as monitored by HPLC, and the system was directly concentrated and purified by lyophilizing to obtain 2.12 g of white solid product **3b**, with a yield of 75%. LC-MS [M-H]⁺ : m/z 542.3.

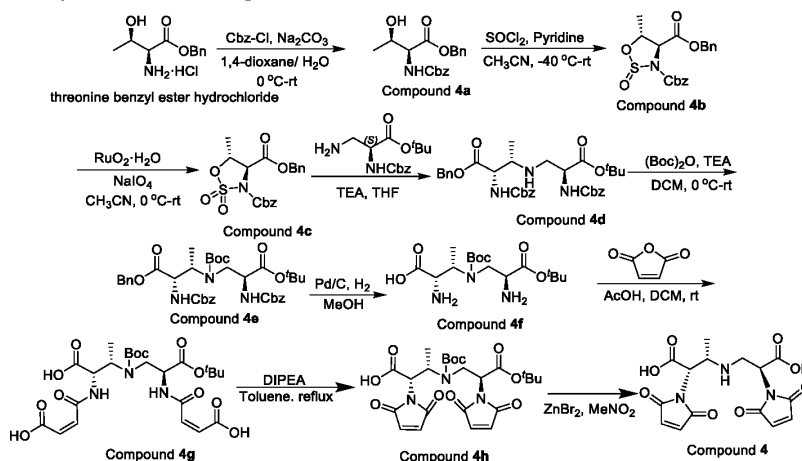
Step 3: Compound **3c**

At room temperature, compound **3b** (3.68 mmol, 2 g) was weighed and dissolved in toluene (25 mL), and DIPEA (14.72 mmol, 2.4 mL) was added. The temperature was raised to 125 °C and refluxed for 4 h. The reaction was monitored by HPLC to be complete. After lyophilized, 1.16 g of white solid product **DM** was obtained with a yield of 61%, LC-MS [M-H]⁻: m/z 506.0.

Step 4: Compound **3**

At room temperature, compound **3c** (1.97 mmol, 1 g) was weighed and dissolved in dry nitromethane (20 mL), anhydrous zinc bromide (39.4 mmol, 9.25 g) was added, the reaction was heated up to 35 °C for 2 h, and the complete reaction was monitored by HPLC. The water pump was decompressed, and nitromethane was removed by rotary evaporation at 40 °C. The residue was prepared and purified to obtain 350 mL of preparative solution, lyophilized to obtain 0.49 g of white solid product compound **3** with a yield of 70%. LC-MS [M-H]⁻: m/z 350.1.

Example 4: Synthesis of Compound **4**



Step 1: Compound **4a**

At room temperature, threonine benzyl ester hydrochloride (69.80 mmol, 17.17 g) was weighed and dissolved in THF (250 mL), the system was cooled to 0 °C, an aqueous solution of Na₂ CO₃ (139.60 mmol, 14.80 g) (250 mL) was added and alkalized for 20 min, and then a THF solution (200 mL) of Cbz-Cl (90.73 mmol, 15.28 g) (200 mL) was dropwise added. The reaction was warmed up to room temperature for 5 h, and the reaction was complete when detected by TLC. Concentrate to remove most of the THF, add EA (200 mL × 3 times) to extract, washed with saturated NaCl aqueous solution, the organic phase was dried with anhydrous Na₂SO₄, filtered and concentrated, and dried at 50 °C for 2 h to obtain 22 g of white solid product **4a** with a yield of 92%, LC-MS [M+H]⁺: m/z 344.4.

Step 2: Compound **4b**

Ultra-dry CH₃CN (50 mL) was added to a clean and dry three-necked flask at room temperature, the reaction system was cooled down to -40 °C, SOCl₂ (38.0 mmol, 2.76 mL) was added to the reaction system, and a solution of CH₃CN (100 mL) of compound **4a** (14.6 mmol, 5.0 g) was added dropwise (1 h dropwise completion). The reaction was carried out at this temperature for 1 h. A solution of CH₃CN (50 mL) of Pyridine (91.2 mmol, 7.3 mL) was

added dropwise and the reaction was naturally brought to room temperature overnight. The reaction was monitored for completeness by TLC on the following day, and the reaction solution was quenched in ice water, extracted by adding EA (100 mL x 3 times), and the organic phase was dried by anhydrous Na₂SO₄, the organic phase was filtered and concentrated to give a dark brown oily liquid, which was purified by column chromatography (PE/EA = 50/1, 40/1, 10/1, 5/1, 2/1) to give 3.2 g of the product, compound **4b**, in a yield of 56%, and LC-MS [M+H]⁺ : m/z 390.6.

Step 3: Compound **4c**

Compound **4b** (8.2 mmol, 3.2 g) was weighed and dissolved in CH₃CN (30 mL) and RuO₂-2H₂O (0.85 mmol, 0.113 g) was added at room temperature. The system was cooled down to 0 °C and an aqueous solution of NaIO₄ (16.94 mmol, 3.62 g) was added (30 mL). The reaction was monitored by TLC for 2.5 h. The reaction was complete, the insoluble solids were removed by filtration, the filtrate was extracted by adding EA (50 mL x 3 times), anhydrous Na₂SO₄ was dried, and the organic phase was filtered and concentrated to give a black oily substance, which was purified by column chromatography (PE/EA = 1/1) to give 2.76 g of the colorless oily product, compound **4c**, in 83% yield, and LC-MS [M+H]⁺ : m/z 406.2.

Step 4: Compound **4d**

At room temperature, compound **4c** (6.8 mmol, 2.76 g) was weighed and dissolved in THF (40 mL), tert-butyl (*S*)-3-amino-2-carboxylaminopropionate (7.44 mmol, 2.19 g) was added and Et₃N (2.13 mmol, 0.295 mL) was added. The reaction was warmed to 60 °C for 3 h. The solvent was concentrated and removed, and the product was purified by column chromatography (PE/EA = 10/1-1/1-EA) to give 1.98 g of product compound **4d** in 47% yield, LC-MS [M+H]⁺ : m/z 620.7.

Step 5: Compound **4e**

At room temperature, compound **4d** (3.07 mmol, 1.9 g) was weighed and dissolved in DCM (50 mL), Et₃N (11.50 mmol, 1.60 mL) was added, the system was cooled down to 0°C, (Boc)₂O (11.50 mmol, 2.64 mL) was added, and the reaction was carried out naturally after warming to room temperature overnight. On the next day, water was added to the reaction system, extracted by DCM (50 mL x 2 times), washed by saturated aqueous NaCl, dried by anhydrous Na₂SO₄, filtered and concentrated to give an oily compound, purified by column chromatography (PE/EA = 15/1-5/1) to give 1 g of solid product **4e** in 45% yield, LC-MS [M+H]⁺ : m/z 720.7.

Step 6: Compound **4f**

At room temperature, compound **4e** (1.26 mmol, 0.9 g) was weighed and dissolved in methanol (25 mL), 5% Pd/C (1.26 g) was added, the system was replaced with H₂ atmosphere, the reaction was carried out overnight, and the complete reaction was monitored by TLC. The Pd/C was removed by filtration and concentrated to give an oily product that was used directly in the next step without purification.

Step 7: Compound **4g**

At room temperature, the crude compound **4f** (1.26 mmol) from step 6 was dissolved in DCM (30 mL), then AcOH (2.86 mmol, 0.16 mL), maleic anhydride (4.44 mmol, 0.44 g) was added. After 2 h at room temperature, the reaction was monitored for completeness by HPLC,

and the system was concentrated directly to prepare a purified lyophilized 0.53 g white solid product compound **4g** in 75% yield, LC-MS $[M-H]^-$: m/z 556.5.

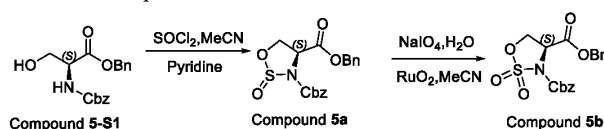
Step 8: Compound **4h**

At room temperature, the compound **4g** (0.84 mmol, 0.47 g) was weighed and dissolved in toluene (25 mL), DIPEA (3.46 mmol, 0.572 mL) was added, and refluxed at 125 °C for 2 h. The reaction was monitored for completeness by TLC and HPLC. Preparation was lyophilized to give 0.24 g of white solid product **4h** in 54% yield, LC-MS $[M-H]^-$: m/z 520.4.

Step 9: Compound **4**

- At room temperature, compound **4h** (0.44 mmol, 0.23 g) was weighed and dissolved in dry
 10 nitromethane (10 mL), anhydrous zinc bromide (8.8 mmol, 1.95 g) was added, the reaction was heated up to 35 °C for 2 h, and the reaction was monitored by HPLC. Water pump decompression, 40 °C rotary evaporation to remove nitromethane, the residue was prepared and purified to obtain 200 mL of preparative solution, lyophilized to obtain 0.088 g of white solid product compound **4**, the yield was 55%, LC-MS $[M-H]^-$: m/z 364.3.

Example 5: Synthesis of Compound 5

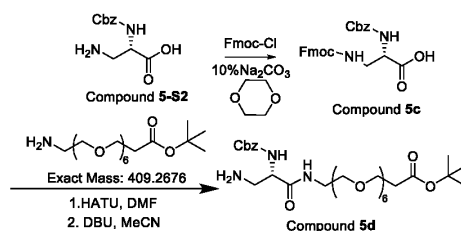


Step 1: Compound **5a**

- MeCN (60 mL) and SOCl₂ (5.06 g, 42.56 mmol) were added to a 500 mL three-necked
 20 flask, cooled to -40 °C, and a solution of compound **5-S1** (5.0 g, 15.2 mmol) in MeCN (150 mL) was added dropwise to the reaction solution. After the addition was complete, the reaction was continued at low temperature for 1.5 h. A solution of Pyridine (8.05 g, 101.87 mmol) in MeCN (30 mL) was added dropwise to the reaction solution at low temperature, and the mixture was heated to room temperature for 3 h. The completion of the raw materials in the reaction was monitored by TLC. H₂O was added to quench the reaction at low temperature, and EA (300 mL) was added to extract once. The EA phase was washed with saturated NaCl (100 mL*2 times), dried with anhydrous Na₂SO₄, filtered and dried to obtain 6.3 g of brown oil, which was directly used for the next step without purification; LC-MS $[M+18]^+$: m/z 393.1.

Step 2: Compound **5b**

- MeCN (60 mL) was added to a 250 mL single-mouth bottle. After compound **5a** (15.2
 30 mmol) was dissolved, RuO₂ (200 mg, 1.52 mmol) was added under an ice-water bath, and then a solution of NaIO₄ (6.5 g, 30.4 mmol) in H₂O (60 mL) was added dropwise. After the addition was complete, the reaction was continued at low temperature for 3 h. The reaction was monitored by TLC. The filter cake was filtered and washed with EA twice. EA was added to the filtrate, and the phases were separated by H₂O extraction. The EA phase was washed with saturated NaCl, dried over anhydrous Na₂SO₄, filtered and dried to obtain 6.28 g of brown oil. The crude product was purified by column chromatography (eluent: 100% PE-15% EA-20% EA-25% EA) to obtain a colorless oil (4.16 g), with a two-step yield of 70%; LC-MS $[M+23]^+$: m/z 414.1.



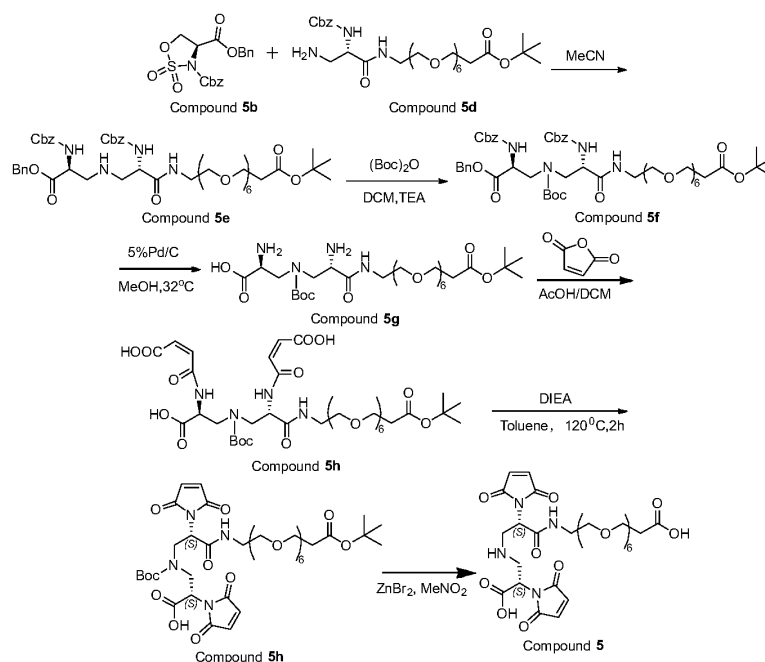
Step 3: Compound 5c

1,4-dioxane (10 mL), compound 5-S2 (2.38 g, 10 mmol) were added to a 150 mL single-mouth bottle, 10% Na₂CO₃ (22 mL) was added under ice-water bath, and then Fmoc-Cl (3.1 g, 12 mmol) in 1,4-dioxane (15 mL) solution was added dropwise. After the addition, the reaction was continued in ice-water bath for 1 hour, and then the temperature was raised to room temperature for 1 hour. The reaction of the raw materials was completed by TLC monitoring. The reaction solution was poured into 120 mL of ice water, and EA was added for extraction twice. The H₂O phase was adjusted to pH = 5-6 with HCl, and EA was added for extraction twice. The EA phase was washed with saturated NaCl in turn, dried over anhydrous Na₂SO₄, filtered and dried to obtain 4.38 g of white foamy solid, which was directly reacted in the next step without purification. LC-MS [M-1]⁺: m/z 459.1.

Step 4: Compound 5d

Compound 5c (4.19 g, 9.1 mmol), amino-hexaethylene glycol tert-butyl ester (4.09 g, 10 mmol), HATU (4.14 g, 10.9 mmol), HOBT (1.47 g, 10.9 mmol) and DMF (30 mL) were added to a 100 mL single-mouth bottle and dissolved. DIEA (1.75 g, 13.6 mmol) was added dropwise under an ice-water bath. After the addition was complete, the temperature was raised to room temperature and reacted for 1 h. The reaction was monitored by HPLC. The reaction solution was directly purified by HPLC and the prepared solution was lyophilized to obtain a white solid (5.04 g) with a yield of 65%. LC-MS [M+23]⁺: m/z 874.4.

The above white solid (2.64 g, 3.07 mmol) and MeCN (15 mL) were added to a 100 mL single-necked bottle. After stirring to dissolve, DBU (561 mg, 3.68 mmol) was added dropwise under an ice-water bath. After the addition, the temperature was raised to room temperature and reacted for 20 min. TLC monitoring showed that the raw materials had reacted. The reaction solution was directly wet-loaded and passed through a rapid column chromatography (eluent: 100% DCM-3% MeOH-5% MeOH-9% MeOH) to obtain a colorless oil (1.95 g) with a yield of 100%. LC-MS [M+23]⁺: m/z 652.4.



Step 5: Compound 5e

Compound **5b** (1.63 g, 4.17 mmol) and MeCN (10 mL) were added to a 50 mL single-necked bottle. After stirring, a solution of compound **5d** (1.75 g, 2.78 mmol) in MeCN (8 mL) was added. After addition, the mixture was reacted at room temperature for 30 min, then heated to 70 °C for 1 h. The reaction was monitored by TLC. The reaction solution was directly wet-loaded and flash column chromatographed (eluent: 100% DCM-3% MeOH-4% MeOH-5% MeOH) to obtain a colorless oil (1.64 g) with a yield of 63%; LC-MS [M+1]⁺: m/z 941.2.

10 Step 6: Compound 5f

Compound **5e** (1.64 g, 1.74 mmol), (Boc)₂O (1.14 g, 5.22 mmol) and DCM (17 mL) were added to a 100 mL single-necked bottle. After stirring, TEA (528 mg, 5.22 mmol) was added. After the addition, the mixture was reacted at room temperature for 30 min. The reaction was monitored by TLC. The reaction solution was directly wet-loaded and passed through a rapid column chromatography (eluent: 100% DCM-3% MeOH- 4% MeOH) to obtain a colorless oil (1.34 g) with a yield of 100%; LC-MS [M+H]⁺: m/z 1041.2.

Step 7: Compound 5g

Compound **5f** (1.33 g, 1.28 mmol), 5% Pd/C (1.33 g) and MeOH (20 mL) were added to a 100 mL single-mouth bottle. After H₂ replacement 3 times, the temperature was raised to 32 °C for 2.5 h. The reaction was monitored by HPLC and the raw materials were completely reacted. The reaction solution was directly filtered, the filter cake was washed twice with MeOH, and the filtrate was dried to obtain 701.1 mg of foamy solid, with a yield of 80%, LC-MS [M+H]⁺: m/z 683.3.

Step 8: Compound 5h

Compound **5g** (700 mg, 1.03 mmol) and DCM (7 mL) were added to a 100 mL single-mouth bottle. After stirring, maleic anhydride (226 mg, 2.3 mmol) and AcOH (433 mg, 7.21

mmol) were added. After the addition was complete, the reaction was allowed to proceed at room temperature for 2 h. TLC monitoring indicated that the reaction of the raw materials was complete. The reaction solution was directly wet loaded and passed through a rapid column chromatography (eluent: 100% DCM-5% MeOH-10% MeOH) to obtain a white foamy solid (730.2 mg) with a yield of 83%. LC-MS [M-H]⁻: m/z 877.2.

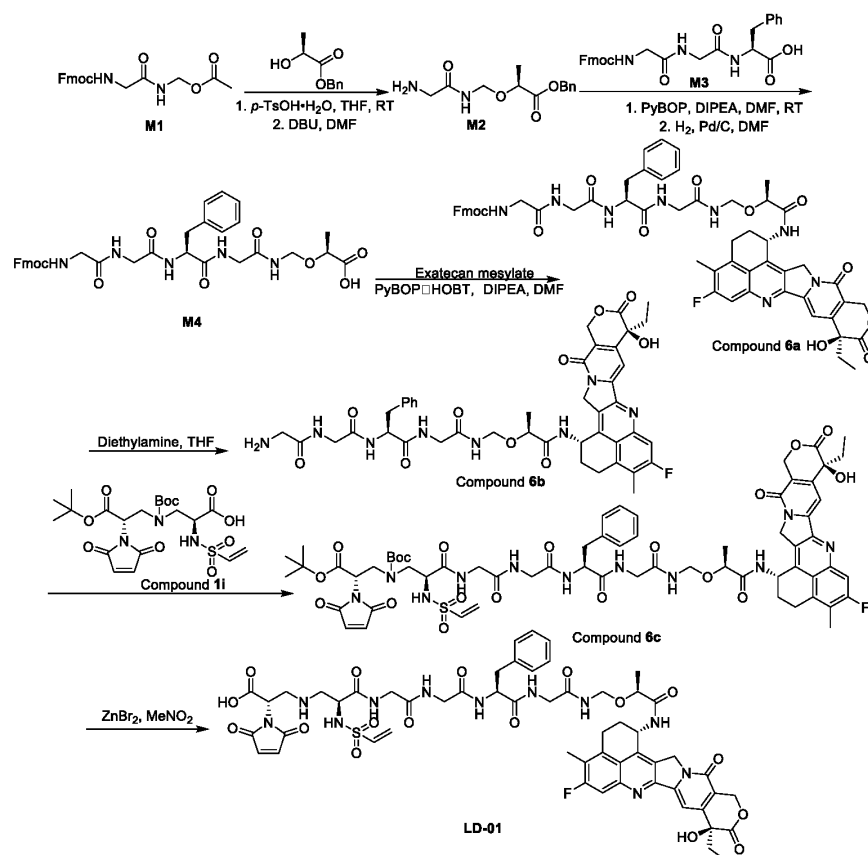
Step 9: Compound **5i**

Compound **5h** (90 mg, 0.102 mmol), DIEA (53 mg, 0.41 mmol) and toluene (9 mL) were added to a 100 mL single-necked bottle, stirred and dissolved, replaced with N₂ three times, and then heated to 120 °C for 2 h. The reaction was completed by HPLC monitoring. Post-treatment: the reaction solution was directly dried under reduced pressure at 45 °C to obtain a crude oil, which was directly reacted in the next step without purification; LC-MS: [M-H]⁻=841.2.

Step 10: Compound **5**

Compound **5i** (50 mg, 0.06 mmol) and nitromethane (5 mL) were added to a 25 mL single-necked bottle. After stirring to dissolve, zinc bromide (276 mg, 1.2 mmol) was weighed. After N₂ replacement three times, the temperature was raised to 35 °C for reaction. The reaction of the raw materials was monitored by HPLC. The reaction solution was directly dried under reduced pressure at 45 °C to obtain a crude product. The product was prepared and purified to obtain a white foamy solid (29 mg) with a yield of 70%. LC-MS [M-H]⁻: m/z 685.2.

Example 6: Synthesis of LD-01



Step 1: Compound **M2**

M1 (7.45 g, 20.3 mmol) and 120 mL THF were added to a 250 mL round-bottom flask and stirred in an ice-water bath for 15 min. p-Toluenesulfonic acid monohydrate (385 mg, 2.03 mmol) was added and stirred for 10 min. S-hydroxybenzyl acetate (6.0 mL, 41.5 mmol) was added dropwise. After the addition was completed, stirring was continued in an ice-water bath. After about 3 h, the raw material disappeared by TLC detection. The reaction was quenched with saturated sodium bicarbonate. The mixed solution was extracted with ethyl acetate (100 mL*3 times), dried over anhydrous sodium sulfate, and concentrated and used directly in the next reaction. The above crude compound and 100 mL of DMF were added to a 250 mL round-bottom flask, followed by DBU (21 mmol, 3.2 mL). The reaction was stirred at room temperature for about 0.5 h. After TLC detection, the reaction was completed, and the mixture was concentrated under reduced pressure by a water pump. The crude product was purified by flash column chromatography (eluent: DCM/MeOH=20/1-10/1) to obtain 3.2 g of white foamy solid **M2**. The yield was 59%, LC-MS [M+H]⁺: m/z 267.3.

Step 2: Compound **M4**

M2 (3.2 g, 12 mmol), **M3** (6 g, 12 mmol) and DMF (20 mL) were added to a 100 mL single-mouth bottle, cooled in an ice-water bath, and PyBOP (9.5 g, 18 mmol) and DIEA (3 mL, 18 mmol) were added to the reaction bottle in turn. After the addition, the temperature was raised to room temperature for 4 hours. The reaction was monitored by HPLC to be complete. The

reaction liquid was directly concentrated under reduced pressure by an oil pump. The obtained crude product was directly used for the next reaction without purification.

The above crude compound, 5% Pd/C (3.2 g) and MeOH (30 mL) were added to a 100 mL single-mouth bottle. After H₂ replacement 3 times, the reaction was carried out at room temperature for 2.5 hours. The reaction was monitored by HPLC and the raw materials were reacted. The reaction solution was directly filtered, and the filter cake was washed twice with MeOH. The filtrate was concentrated under reduced pressure by a water pump and then purified by preparative liquid phase. The preparative liquid was lyophilized to obtain 6.4 g of light-yellow compound **M4** with a yield of 80%. LC-MS [M-H]⁻: m/z 658.6.

10 Step 3: Compound **6a**

Compound **M4** (6.4 g, 9.6 mmol), exitecan mesylate (5.4 g, 9.6 mmol) and DMF (20 mL) were added to a 100 mL single-necked bottle and cooled in an ice-water bath. PyBOP (10.6 g, 20 mmol), HOBt (2.73 g, 20 mmol) and DIEA (3.3 mL, 20 mmol) were added to the reaction bottle in sequence. After the addition, the temperature was raised to room temperature and the reaction was allowed to react for 4 h. The reaction was completed under HPLC monitoring. The product was purified by preparative liquid phase and the preparative solution was lyophilized to obtain 9.5 g of light-yellow compound **6a** with a yield of 92%. LC-MS [M+H]⁺: m/z 1077.6.

Step 4: Compound **6b**

20 Compound **6a** (9.5 g) and 100 mL THF were added to a 250 mL round-bottom flask, followed by diethylamine (20 mL). The mixture was stirred at room temperature for 3 h. After the reaction was completed as detected by HPLC, the mixture was concentrated under reduced pressure by a water pump. The crude product was purified by preparative liquid phase, and the preparative liquid was lyophilized to obtain 7.54 g of light-yellow compound **6b** with a yield of 100%. LC-MS [M+H]⁺: m/z 855.3.

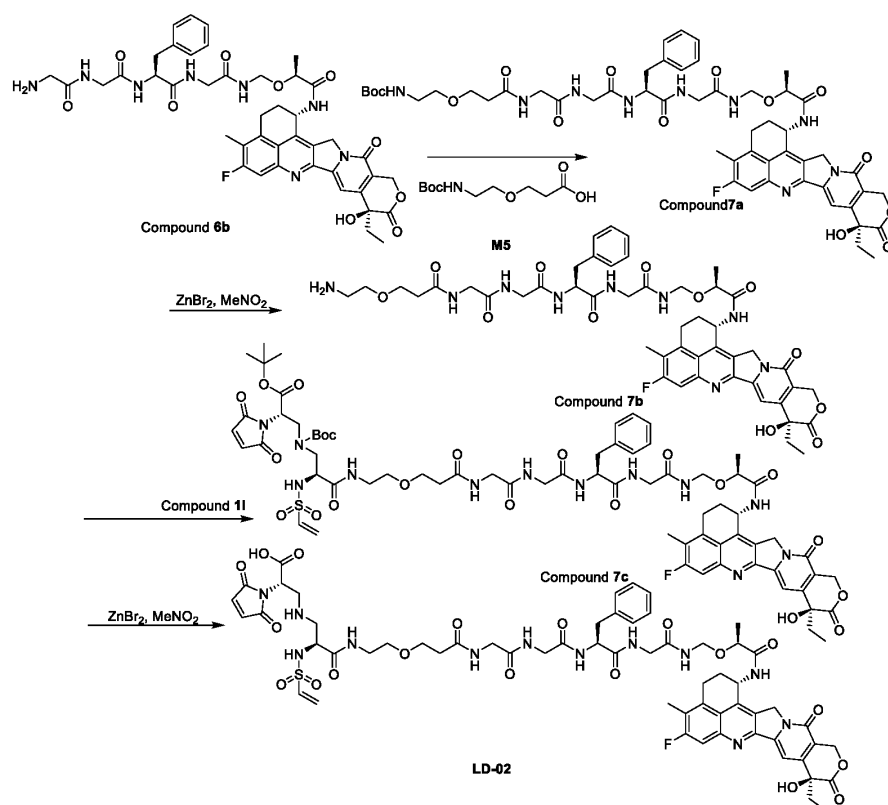
Step 5: Compound **6c**

30 Compound **6b** (60 mg, 0.07 mmol), compound **1i** (38 mg, 0.07 mmol) and 5 mL DMF were added to a 50 mL round-bottom flask and cooled in an ice-water bath. PyBop (58.4 mg, 0.11 mmol) and HOBt (15 mg, 0.11 mmol) were added in sequence. DIEA (58 μ L, 0.35 mmol) was added dropwise under stirring in an ice bath. The reaction was stirred at room temperature for 2 h. The reaction of compound **6b** was completed by HPLC monitoring. The product was purified by preparative liquid phase and lyophilized to obtain 64 mg of compound **6c** with a yield of 67%. LC-MS [M+H]⁺: m/z 1354.8.

Step 6: **LD-01**

40 Compound **6c** (60 mg, 0.044 mmol) and nitromethane (5 mL) were added to a 25 mL single-necked bottle. After stirring to dissolve, zinc bromide (200 mg, 0.88 mmol) was weighed. After N₂ replacement three times, the temperature was raised to 35 °C for reaction. The reaction of the raw materials was monitored by HPLC. The reaction solution was directly dried under reduced pressure at 45 °C to obtain a crude product. The product was prepared and purified to obtain 33 mg of white solid **LD-01** with a yield of 62%. LC-MS [M+H]⁺: m/z 1198.7.

Example 7: LD-02



Step 1: Compound 7a

Compound **6b** (60 mg, 0.07 mmol), compound **M5** (24 mg, 0.1 mmol) and 5 mL DMF were added to a 25 mL round-bottom flask and cooled in an ice-water bath. PyBop (58.6 mg, 0.11 mmol) and HOBt (15.3 mg, 0.11 mmol) were added in sequence. DIPEA (58 μL , 0.35 mmol) was added dropwise under stirring in an ice bath. The reaction was stirred at room temperature for 1 h. The reaction was completed after HPLC monitoring. The product was purified by preparative liquid phase and the preparative solution was lyophilized to obtain 66 mg of compound **7a** with a yield of 88%. LC-MS $[\text{M-Boc}+\text{H}]^+$: m/z 970.7.

10 Step 2: Compound 7b

Compound **7a** (66 mg, 0.062 mmol) and nitromethane (5 mL) were added to a 25 mL single-necked bottle. After stirring to dissolve, zinc bromide (213 mg, 0.93 mmol) was weighed. After N_2 replacement three times, the temperature was raised to 40 $^\circ\text{C}$ for reaction for 0.5 h. The reaction of the raw materials was monitored by HPLC. The reaction solution was directly dried under reduced pressure at 40 $^\circ\text{C}$ and then prepared for purification. The prepared solution was lyophilized to obtain 49 mg of white solid compound **7b** with a yield of 80%. LC-MS $[\text{M}+\text{H}]^+$: m/z 970.5.

Step 3: Compound 7c

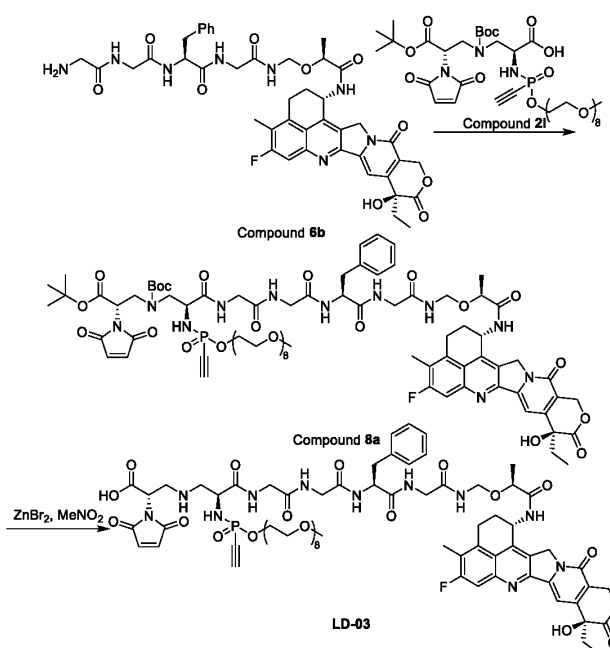
20 Compound **7b** (49 mg, 0.05 mmol), compound **1i** (15 mg, 0.06 mmol) and 5 mL DMF were added to a 25 mL round-bottom flask and cooled in an ice-water bath. PyBop (41 mg, 0.075 mmol) and HOBt (10.3 mg, 0.075 mmol) were added in sequence. DIPEA (42 μL , 0.25 mmol) was added dropwise with stirring in an ice bath. The reaction was stirred at room temperature

overnight. The reaction was completed by HPLC monitoring. The product was purified by preparative liquid phase and the preparative liquid was lyophilized to obtain 51 mg of compound **7c** with a yield of 56%. LC-MS $[M/2+H]^+$: m/z 735.3.

Step 4: **LD-02**

Compound **7c** (51 mg, 0.035 mmol) and nitromethane (5 mL) were added to a 25 mL single-necked bottle. After stirring to dissolve, zinc bromide (159 mg, 0.69 mmol) was weighed. After N₂ replacement three times, the temperature was raised to 40 °C for reaction for 0.5 h. The reaction of the raw materials was monitored by HPLC. The reaction solution was directly dried under reduced pressure at 40 °C and then prepared for purification. The prepared solution was lyophilized to obtain 27 mg of white solid compound **LD-02** with a yield of 59%. LC-MS $[M+H]^+$: m/z 1313.7.

Example 8: **LD-03**



Step 1: Compound **8a**

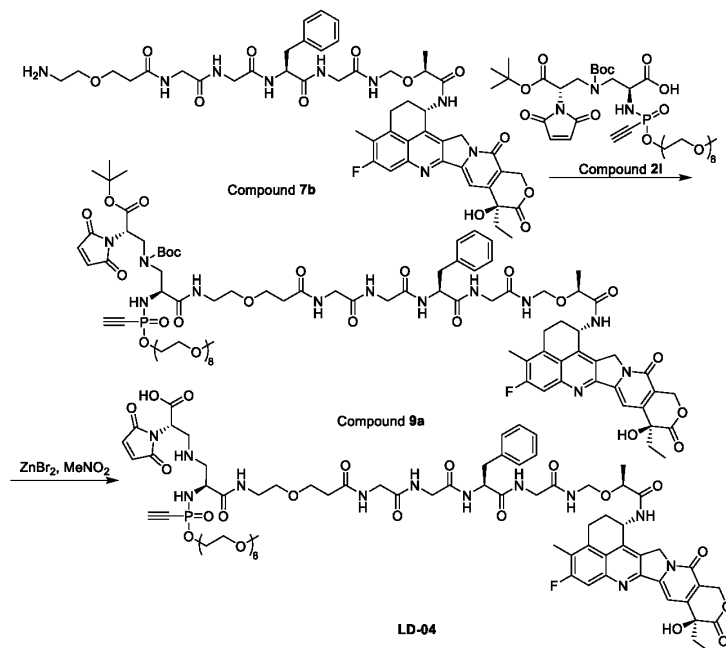
Compound **6b** (50 mg, 0.058 mmol), compound **2i** (53 mg, 0.06 mmol) and 5 mL of DMF were added to a 25 mL round-bottom flask. PyBop (51 mg, 0.09 mmol) and HOBt (12.9 mg, 0.09 mmol) were added in sequence under ice-water cooling. DIPEA (49 μ L, 0.29 mmol) was added dropwise under ice-bath stirring. The reaction was stirred at room temperature overnight. The reaction was completed by HPLC monitoring. The product was purified by preparative liquid phase and the preparative liquid was lyophilized to obtain 60 mg of white solid compound **8a** in a yield of 60%. LC-MS $[(M-Boc)/2+H]^+$: m/z 810.6.

Step 2: **LD-03**

Compound **8a** (60 mg, 0.035 mmol) and nitromethane (5 mL) were added to a 25 mL single-necked bottle. After stirring to dissolve, zinc bromide (161 mg, 0.69 mmol) was weighed. After N₂ replacement three times, the temperature was raised to 40 °C for reaction for 0.5 h. The reaction of the raw materials was monitored by HPLC. The reaction solution

was directly dried under reduced pressure at 40 °C and then prepared for purification. The prepared solution was lyophilized to obtain 23 mg of white solid compound **LD-03** with a yield of 42%. LC-MS $[M/2+H]^+$: m/z 782.3.

Example 9: LD-04



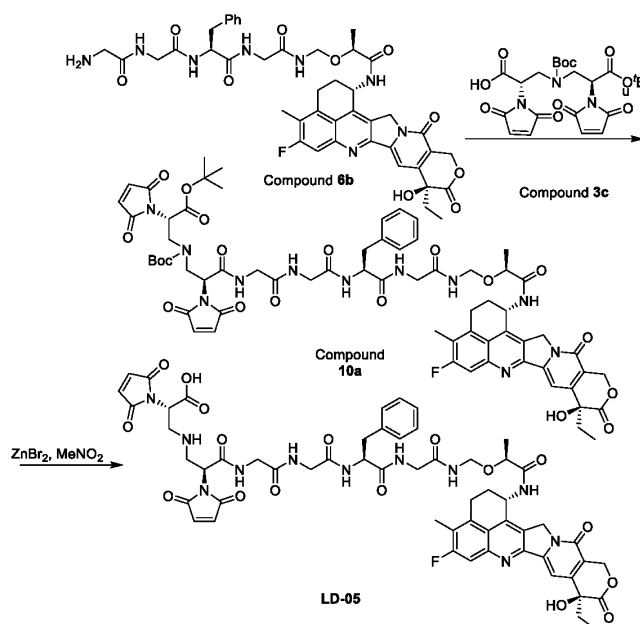
Step 1: Compound 9a

Compound **7b** (100 mg, 0.103 mmol), compound **2i** (109 mg, 0.123 mmol) and 5 mL DMF were added to a 25 mL round-bottom flask. PyBop (122 mg, 0.21 mmol) and HOBt (33 mg, 0.21 mmol) were added in sequence under ice-water cooling. DIPEA (86 μ L, 0.52 mmol) was added dropwise under ice-bath stirring. The reaction was stirred at room temperature for 4 h. The reaction was completed after HPLC monitoring. The product was purified by preparative liquid phase and the preparative solution was lyophilized to obtain 130 mg of white solid compound **9a** with a yield of 72%. LC-MS $[M/2+H]^+$: m/z 874.5.

Step 2: LD-04

Compound **9a** (130 mg, 0.074 mmol) and nitromethane (5 mL) were added to a 25 mL single-necked bottle. After stirring to dissolve, zinc bromide (256 mg, 1.12 mmol) was weighed. After N_2 replacement three times, the temperature was raised to 40 °C for reaction for 0.5 h. The reaction of the raw materials was monitored by HPLC. The reaction solution was directly dried under reduced pressure at 40 °C and then prepared for purification. The prepared solution was freeze-dried to obtain 71 mg of white solid compound **LD-04** with a yield of 57%. LC-MS $[M/2+H]^+$: m/z 839.5.

Example 10: LD-05



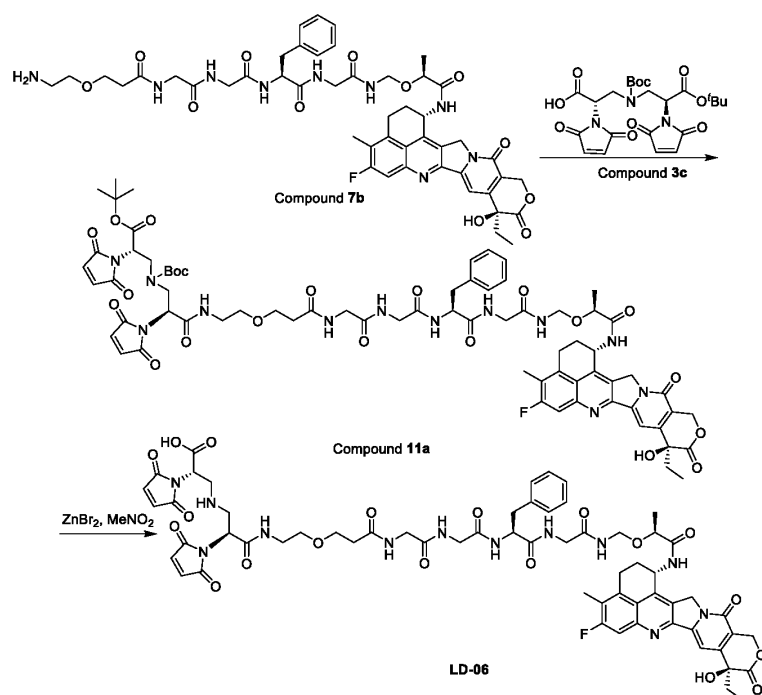
Step 1: Compound **10a**

Compound **6b** (100.5 mg, 0.117 mmol), compound **3c** (61.2 mg, 0.117 mmol) and 5 mL DMF were added to a 25 mL round-bottom flask. PyBop (104.3 mg, 0.176 mmol) and HOBt (29.6 mg, 0.176 mmol) were added in sequence under ice-water cooling. DIPEA (99 μL , 0.6 mmol) was added dropwise under ice-bath stirring. The reaction was stirred at room temperature for 14 h. The reaction was completed after HPLC monitoring. The product was purified by preparative liquid phase and the preparative solution was lyophilized to obtain 98.3 mg of white solid compound **10a** with a yield of 61%. LC-MS $[\text{M}+\text{H}]^+$: m/z 1344.7.

10 Step 2: **LD-05**

Compound **10a** (98.3 mg, 0.073 mmol) and nitromethane (5 mL) were added to a 25 mL single-necked bottle. After stirring to dissolve, zinc bromide (329.7 mg, 1.46 mmol) was weighed. After N_2 replacement three times, the temperature was raised to 40 $^\circ\text{C}$ for reaction for 0.5 h. The reaction of the raw materials was monitored by HPLC. The reaction solution was directly dried under reduced pressure at 40 $^\circ\text{C}$ and then prepared for purification. The prepared solution was lyophilized to obtain 55.0 mg of white solid compound **LD-05** with a yield of 63%. LC-MS $[\text{M}+\text{H}]^+$: m/z 1188.6.

Example 11: **LD-06**



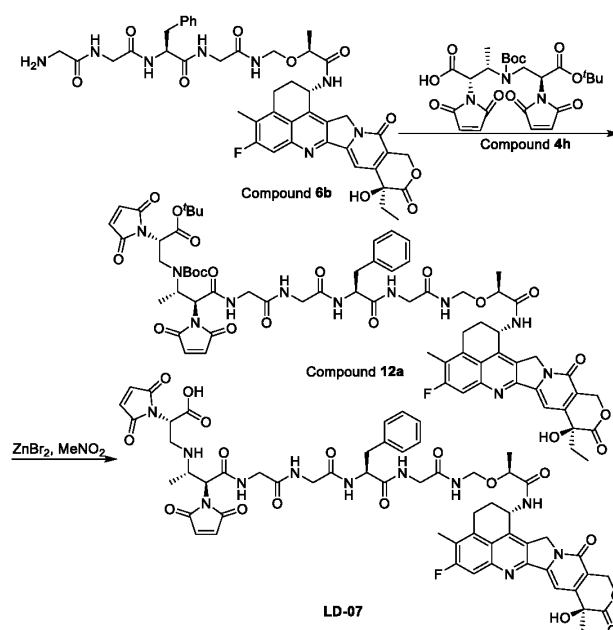
Step 1: Compound **11a**

Compound **7b** (200.5 mg, 0.206 mmol), compound **3c** (108.9 mg, 0.206 mmol) and 5 mL DMF were added to a 25 mL round-bottom flask. PyBop (237 mg, 0.4 mmol) and HOBt (69.7 mg, 0.4 mmol) were added in sequence under ice-water cooling. DIPEA (171 μL , 1.03 mmol) was added dropwise under ice-bath stirring. The reaction was stirred at room temperature for 5 h. The reaction was completed under HPLC monitoring. The product was purified by preparative liquid phase and the preparative liquid was lyophilized to obtain 177.1 mg of white solid compound **11a** with a yield of 59%. LC-MS [(M-Boc)/2+H]⁺: m/z 680.3.

10 Step 2: **LD-06**

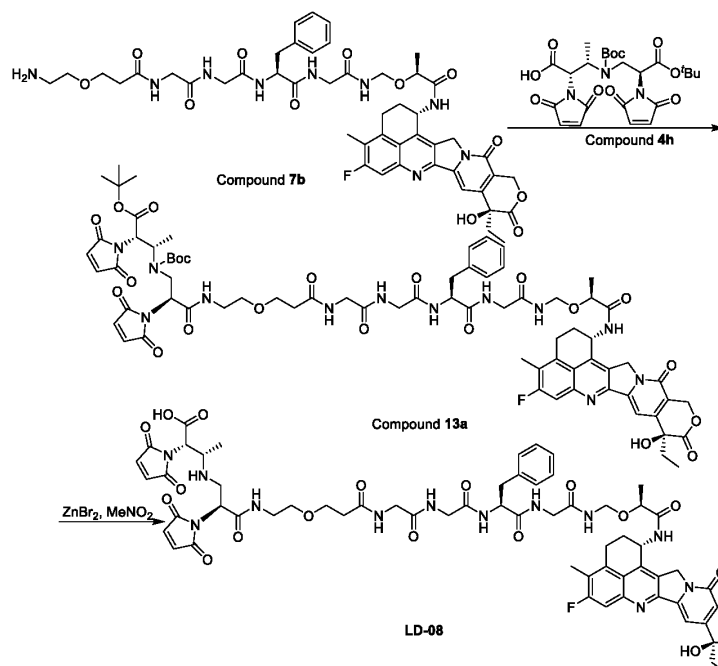
Compound **11a** (177.1 mg, 0.12 mmol) and nitromethane (15 mL) were added to a 25 mL single-necked bottle. After stirring to dissolve, zinc bromide (568.8 mg, 2.4 mmol) was weighed. After N₂ replacement three times, the temperature was raised to 40 °C for reaction for 0.5 h. The reaction of the raw materials was monitored by HPLC. The reaction solution was directly dried under reduced pressure at 40 °C and then prepared for purification. The prepared solution was freeze-dried to obtain 105.6 mg of white solid compound **LD-06** with a yield of 67%. LC-MS [M+H]⁺: m/z 1303.6.

Example 12: **LD-07**



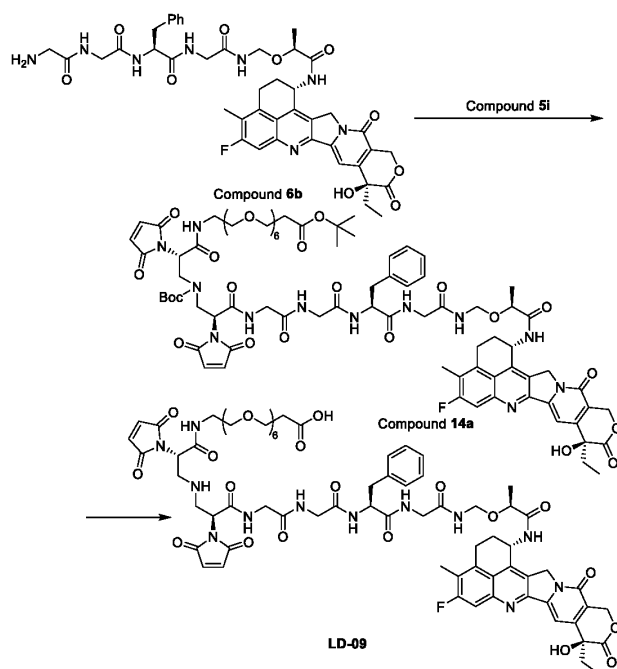
Referring to the synthesis method of **Example 10**, 34.4 mg of white solid **LD-07** was prepared, LC-MS $[\text{M}+\text{H}]^+$: m/z 1202.3.

Example 13: LD-08



Referring to the synthesis method of **Example 11**, 29.5 mg of white solid **LD-08** was prepared, LC-MS $[\text{M}+\text{H}]^+$: m/z 1317.8.

Example 14: LD-09



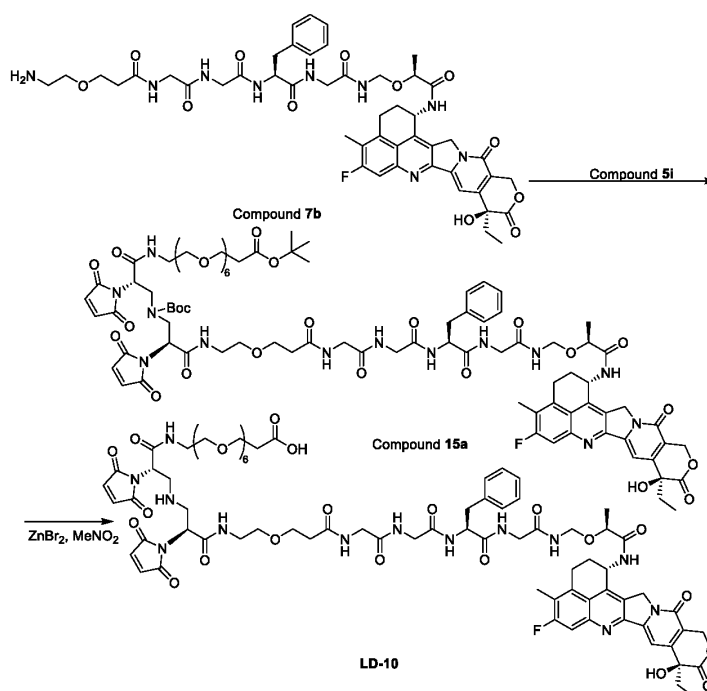
Step 1: Compound 14a

Compound 5i (40.3 mg, 0.047 mmol), compound 6b (42.9 mg, 0.047 mmol), HOPO (31.1 mg, 0.24 mmol) and DCM (5 mL) were added to a 25 mL single-necked bottle. DIEA (17 μ L, 0.1 mmol) and DCC (50.5 mg, 0.24 mmol) were added under an ice-water bath. After the addition, the temperature was raised to room temperature and reacted for 4 h. After the reaction was completed under HPLC monitoring, the reaction solution was directly purified by HPLC preparation. The prepared solution was lyophilized to obtain 31.8 mg of light yellow solid compound 14a, with a yield of 41%; LC-MS $[M/2+H]^+$: m/z 840.4.

10 Step 2: LD-09

Compound 14a (31.8 mg, 0.019 mmol) and CH_3NO_2 (3 mL) were added to a 5 mL single-necked bottle to dissolve the turbidity. DMF (0.2 mL) was then added to assist dissolution. $ZnBr_2$ (90.4 mg, 0.5 mmol) was then added. After N_2 replacement, the temperature was raised to 40 $^{\circ}C$ for reaction for 1 h. The solvent was dried under reduced pressure at 40 $^{\circ}C$ using an oil pump to obtain a crude product. After the reaction was complete under HPLC monitoring, the product was directly prepared and purified by HPLC. The prepared solution was lyophilized to obtain 12.8 mg of a light yellow solid with a yield of 44%; LC-MS $[M/2+H]^+$: m/z 762.3.

Example 15: LD-10



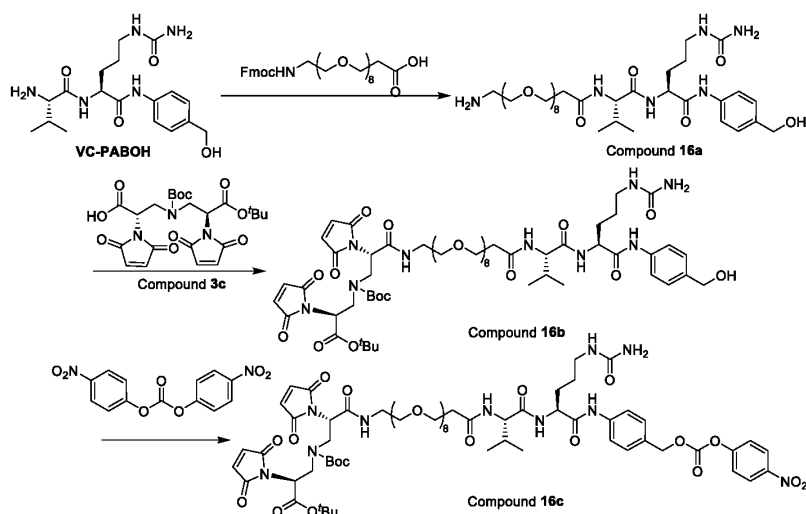
Step 1: Compound **15a**

Compound **5i** (28.7 mg, 0.034 mmol), compound **7b** (33.2 mg, 0.034 mmol), HOPO (31.6 mg, 0.27 mmol) and DCM (3 mL) were added to a 25 mL single-necked bottle. DIEA (17 uL, 0.1 mmol) and DCC (56.8 mg, 0.27 mmol) were added under an ice-water bath. After the addition, the temperature was raised to room temperature and reacted for 4 h. After the reaction was completed under HPLC monitoring, the reaction solution was directly purified by HPLC preparation. The prepared solution was lyophilized to obtain 30.2 mg of light yellow solid compound **15a**, with a yield of 49%; LC-MS [M/2+H]⁺: m/z 897.8.

10 Step 2: **LD-10**

Compound **15a** (30.2 mg, 16.7 umol) and CH₃NO₂ (3 mL) were added to a 25 mL single-necked bottle to dissolve the turbid solution. DMF (0.2 mL) was then added to assist dissolution. ZnBr₂ (104.7 mg, 0.5 mmol) was then added. After N₂ replacement, the temperature was raised to 40 °C for reaction for 1 h. The solvent was dried under reduced pressure at 40 °C using an oil pump to obtain a crude product. After the reaction was complete under HPLC monitoring, the product was directly prepared and purified by HPLC. The prepared solution was freeze-dried to obtain 8.2 mg of a light yellow solid with a yield of 30%; LC-MS [M/2+H]⁺: m/z 820.0.

Example 16: **LD-11**



Step 1: Compound 16a

Compound VC-PABOH (20.3 g, 52.7 mmol) and DMF (100 mL) were added to a 250 mL single-necked bottle, cooled in an ice-water bath, and compound 5,8,11,14,17,20,23,26-octaoxa-2-azanonaconic acid 1-(9H-fluoren-9-ylmethyl) ester (3.55 g, 53 mmol), EDCI·HCl (15.5 g, 79.1 mmol) and DMAP (650.6 mg, 5.27 mmol) were added in sequence. After the addition, the temperature was raised to room temperature and reacted for 16 h. After TLC monitoring, 50 mL of water was added, and the reaction was complete. Dichloromethane (100 mL×three times) was used for extraction, and the organic solutions were combined, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure.

The crude compound and THF (100 mL) were added to a 250 mL single-necked bottle, diethylamine (25 mL) was added at room temperature, and the reaction was maintained at room temperature for 2 h. After the reaction was completed under TLC monitoring, the product was concentrated under reduced pressure by a water pump. The residue was purified by flash column chromatography (eluent DCM/MeOH = 50/1 – 10/1) to obtain 30.9 g of yellow oily compound 16a with a yield of 73%; LC-MS [M+H]⁺: m/z 803.8.

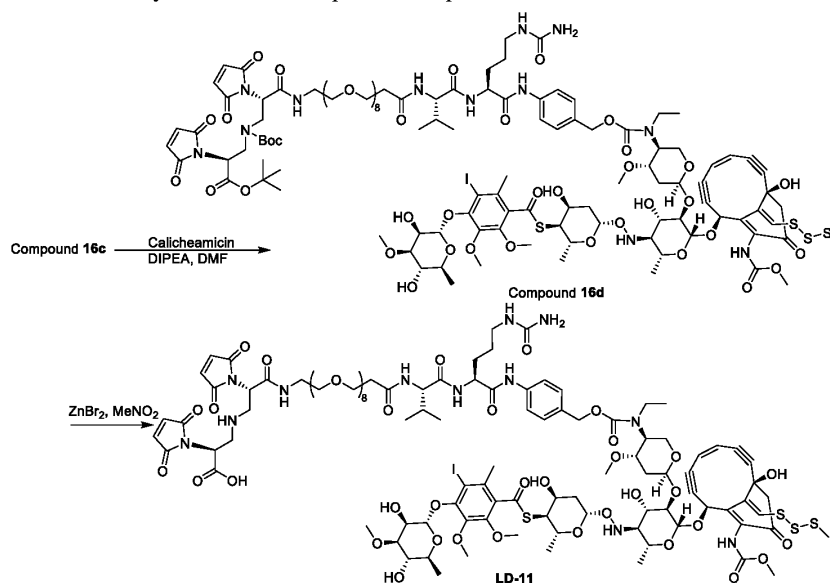
Step 2: Compound 16b

Compound 3c (199.7 mg, 0.394 mmol) and DCM (10 mL) were added to a 25 mL single-mouth bottle, cooled in an ice-water bath, and HOSu (50.7 mg, 0.433 mmol) and DIPEA (72 μL, 0.433 mmol) were added in sequence. After addition, the reaction was allowed to proceed for 2 h. The reaction was completely monitored by TLC, and the insoluble matter was removed by filtration. The product was concentrated under reduced pressure and used directly in the next step without purification.

The above crude compound and DMF (10 mL) were added to a 25 mL single-mouth bottle, cooled in an ice-water bath, and compound 16a (349.1 mg, 0.433 mmol) and DIPEA (72 μL, 0.433 mmol) were added in sequence. After addition, the temperature was raised to room temperature and the reaction was allowed to proceed for about 4 h. The product was directly purified by HPLC, and the prepared solution was lyophilized to obtain 400.8 mg of slightly yellow solid compound 16b with a yield of 78%; LC-MS [M+H]⁺: m/z 1292.6.

Step 3: Compound 16c

Compound **16b** (400.8 mg, 0.31 mmol) and DCM (10 mL) were added to a 25 mL single-necked bottle, and di(p-nitrobenzene) carbonate (102.3 mg, 0.32 mmol) and DIPEA (33 μ L, 0.32 mmol) were added in sequence at room temperature. The mixture was reacted for 2 h after the addition was completed. After the reaction was completed under TLC monitoring, the insoluble matter was removed by filtration, and the mixture was concentrated under reduced pressure and used directly in the next step without purification.



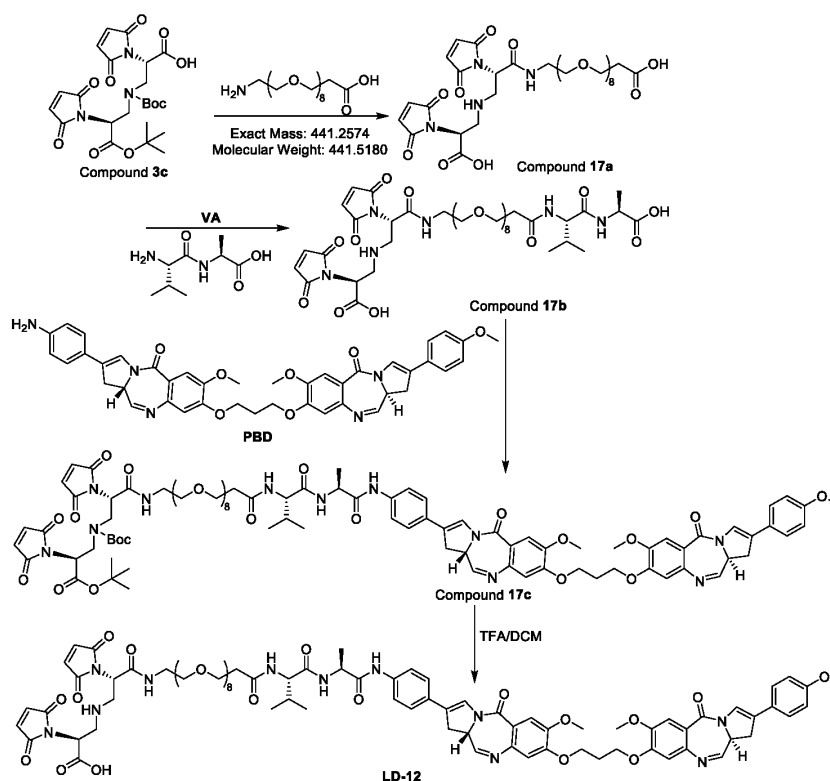
Step 4: Compound **16d**

Compound **16c** (0.01 mmol) and DMF (5 mL) were added to a 25 mL single-necked bottle and cooled in an ice-water bath. Compound calicinomycin (123.2 mg, 0.09 mmol) and DIPEA (2 μ L, 0.01 mmol) were added in sequence. After the addition, the temperature was raised to room temperature and the reaction was carried out for about 5 h. The complete reaction of calicinomycin was monitored by HPLC. HPLC preparation and purification were performed directly. The preparation solution was lyophilized to obtain 75.9 mg of white solid compound **16d** with a yield of 63%; LC-MS $[\text{M}/2+\text{H}]^+$: m/z 1336.5.

Step 5: LD-11

Compound **16d** (50.4 mg, 18.9 μ mol) and CH_3NO_2 (3 mL) were added to a 25 mL single-necked bottle to dissolve the turbid solution. DMF (0.2 mL) was then added to assist dissolution. ZnBr_2 (86.8 mg, 0.374 mmol) was then added. After N_2 replacement, the temperature was raised to 40 $^\circ\text{C}$ for reaction for 1 h. After HPLC monitoring, the reaction was complete, and the solvent was dried under reduced pressure at 40 $^\circ\text{C}$ by an oil pump to obtain a crude product. The product was directly prepared and purified by HPLC. The prepared solution was lyophilized to obtain 22.8 mg of light yellow solid LD-11 with a yield of 47%; LC-MS $[\text{M}/2+\text{H}]^+$: m/z 1258.7.

Example 17: LD-12



Step 1: Compound 17a

Compound 3c (200.0 mg, 0.394 mmol) and DCM (10 mL) were added to a 25 mL single-necked bottle, cooled in an ice-water bath, and HOSu (50.4 mg, 0.433 mmol) and DIPEA (72 μL , 0.433 mmol) were added in sequence. The mixture was reacted for 2 h after addition. After TLC monitoring, the insoluble matter was removed by filtration. The mixture was concentrated under reduced pressure and used directly in the next step. DMF (10 mL) was added to the above crude compound, cooled in an ice-water bath, and amino-PEG8-carboxylic acid (182.3 mg, 0.394 mmol) and DIPEA (67 μL , 0.4 mmol) were added in sequence. The mixture was heated to room temperature and reacted for about 3 h after addition. The mixture was directly purified by HPLC. The prepared solution was lyophilized to obtain 267.2 mg of white solid compound 17a with a yield of 88%; LC-MS $[\text{M}-\text{H}]^-$: m/z 773.7.

Step 2: Compound 17b

Compound 17a (267.9 mg, 0.345 mmol) and DMF (10 mL) were added to a 25 mL single-necked bottle, cooled in an ice-water bath, and HOSu (43.7 mg, 0.35 mmol) and DIPEA (60 μL , 0.35 mmol) were added in sequence. The mixture was reacted for 2 h after addition. After TLC monitoring, the insoluble matter was removed by filtration. The mixture was concentrated under reduced pressure by an oil pump and used directly in the next step. DMF (10 mL) was added to the above crude compound, cooled in an ice-water bath, and VA (65.6 mg, 0.345 mmol) and DIPEA (58 μL , 0.345 mmol) were added in sequence. The mixture was heated to room temperature and reacted for about 4 h after addition. The mixture was directly prepared and purified by HPLC. The prepared solution was lyophilized to obtain 199.2 mg

of white solid compound **17b** with a yield of 61%; LC-MS $[M-H]^-$: m/z 943.6.

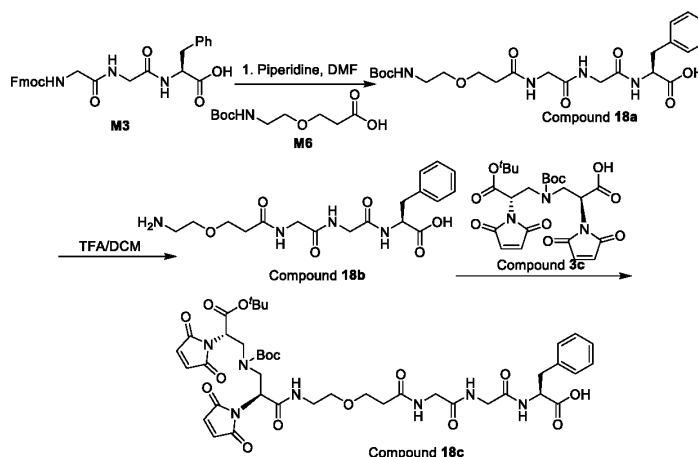
Step 3: Compound **17c**

Compound **17b** (50.6 mg, 0.053 mmol) and DMF (5 mL) were added to a 25 mL single-necked bottle and cooled in an ice-water bath. PBD (40.1 mg, 0.053 mmol), EDCI·HCl (16.8 mg, 0.08 mmol) and DIPEA (14 μ L, 0.08 mmol) were added in sequence. After the addition, the temperature was raised to room temperature and the reaction was carried out for about 7 h. The reaction was monitored by HPLC. The product was directly prepared and purified by HPLC. The prepared solution was lyophilized to obtain 52.3 mg of white solid compound **17c** with a yield of 54%; LC-MS $[M/2+H]^+$: m/z 905.1.

10 Step 4: **LD-12**

Compound **17c** (30.4 mg, 16.6 μ mol) and CH_3NO_2 (3 mL) were added to a 25 mL single-necked bottle to dissolve the turbid solution. DMF (0.2 mL) was then added to assist dissolution. $ZnBr_2$ (77.4 mg, 0.332 mmol) was then added. After N_2 replacement, the temperature was raised to 40 °C for reaction for 1 h. After HPLC monitoring, the reaction was completed, and the solvent was dried under reduced pressure at 40 °C by an oil pump to obtain a crude product. The product was directly prepared and purified by HPLC. The prepared solution was lyophilized to obtain 12.9 mg of light yellow solid **LD-12** with a yield of 47%; LC-MS $[M/2+H]^+$: m/z 827.0.

Example 18: **LD-13**



20

Step 1: Compound **18a**

Compound **M3** (7.8 g, 15.5 mmol) and DMF (100 mL) were added to a 250 mL single-mouth bottle, and Piperidine (200 mL) was added at room temperature. After addition, the reaction was allowed to proceed for 2 h. After TLC monitoring, the insoluble matter was removed by filtration, and the mixture was concentrated under reduced pressure for later use.

Compound **M6** (3.6 g, 15.5 mmol) and DMF (100 mL) were added to a 250 mL single-mouth bottle, and the mixture was cooled in an ice-water bath. HOSu (2 g, 17 mmol) and DIPEA (2.83 mL, 17 mmol) were added in sequence. After addition, the reaction was allowed to proceed for 3 h. 20 mL of DMF solution of the above crude compound was added. After HPLC monitoring, the reaction was completed, and the mixture was directly purified by HPLC. The prepared solution was freeze-dried to obtain 7 g of white solid compound **18a**, with a yield

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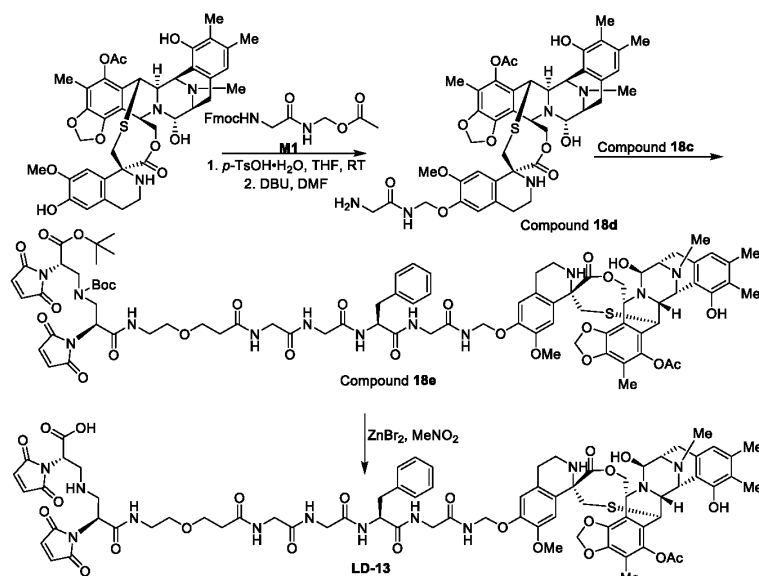
of 92%; LC-MS [M-H]⁻: m/z 493.4.

Step 2: Compound **18b**

Compound **18a** and DCM (100 mL) were added to a 250 mL single-necked bottle and cooled in an ice-water bath. TFA (30 mL) was slowly added dropwise. After the addition was complete, the temperature was slowly raised to room temperature and the reaction was carried out for 2 h. After the reaction was completed as monitored by HPLC, the insoluble matter was removed by filtration and the mixture was concentrated under reduced pressure by a water pump. The obtained yellow oily crude product was directly purified by HPLC. The prepared solution was lyophilized to obtain 4.9 g of white solid compound **18b** with a yield of 87%; LC-MS [M-H]⁻: m/z 393.8.

Step 3: Compound **18c**

Compound **18b** (4.7 g, 12 mmol) and DMF (50 mL) were added to a 100 mL single-necked bottle and cooled in an ice-water bath. Compound **3c** (6.1 g, 12 mmol), EDCI·HCl (2.82 g, 14.4 mmol) and DIPEA (2.4 mL, 14.4 mmol) were added in sequence. After the addition was complete, the temperature was raised to room temperature and the reaction was carried out for about 17 h. The reaction was monitored by HPLC. The product was directly prepared and purified by HPLC. The prepared solution was lyophilized to obtain 8.1 g of white solid compound **18c** with a yield of 77%; LC-MS [M+H]⁺: m/z 882.5.



Step 4: Compound **18d**

M1 (24.1 mg, 0.064 mmol) and 10 mL THF were added to a 25 mL round-bottom flask and stirred in an ice-water bath for 15 min. p-Toluenesulfonic acid monohydrate (1.4 mg, 0.0064 mmol) was added and stirred for 10 min. Trabectedin (49.5 mg, 0.064 mmol) was added and stirred in an ice-water bath after the addition was complete. The raw material disappeared after TLC detection about 3 h. The reaction was quenched with saturated sodium bicarbonate. The mixed solution was extracted with ethyl acetate (100 mL*3 times), dried over anhydrous sodium sulfate, concentrated and used directly in the next reaction.

The above crude compound and 10 mL of DMF were added to a 25 mL round-bottom flask, followed by DBU (2 mL). The reaction was stirred at room temperature for about 0.5 h. After TLC detection, the reaction was completed, and the mixture was concentrated under reduced pressure by a water pump. The crude product was purified by flash column chromatography (eluent: DCM/MeOH=20/1-10/1-1/1) to obtain 38.3 mg of white foamy solid **18d**. The yield was 71%. LC-MS [M+H]⁺: m/z 832.3.

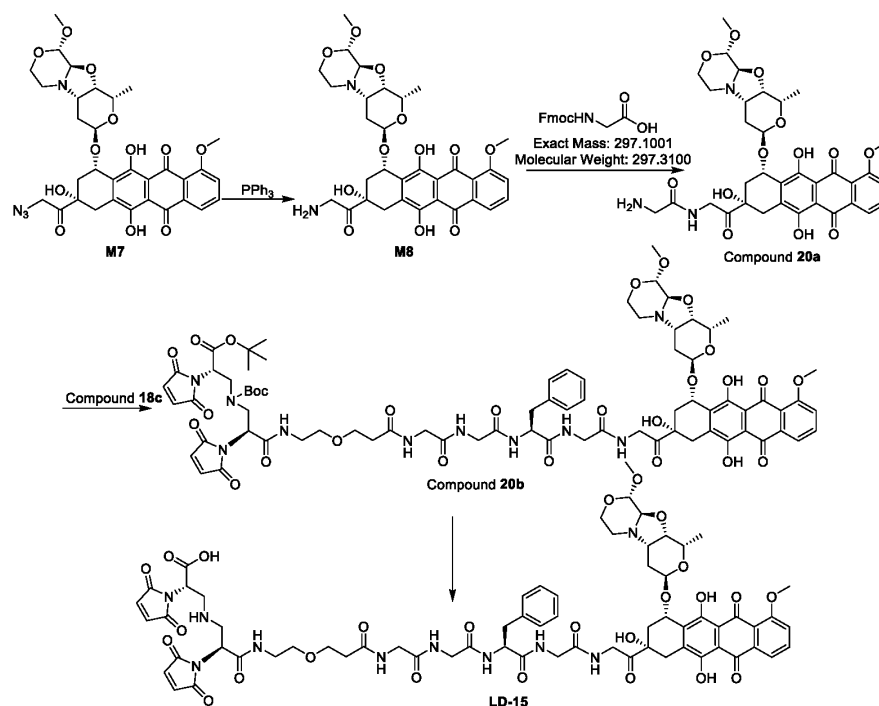
Step 5: Compound **18e**

Compound **18c** (40.9 mg, 0.046 mmol), compound **18d** (38.2 mg, 0.046 mmol) and 5 mL DMF were added to a 25 mL round-bottom flask and cooled in an ice-water bath. PyBop (55.7 mg, 0.1 mmol) and HOBt (13.6 mg, 0.1 mmol) were added in sequence. DIEA (31 μ L, 0.184 mmol) was added dropwise under stirring in an ice bath. The reaction was stirred at room temperature for 2 h. The reaction was completed after HPLC monitoring. The product was purified by preparative liquid phase and the preparative solution was lyophilized to obtain 43.7 mg of compound **18e** with a yield of 55%. LC-MS [M/2+H]⁺: m/z 849.8.

Step 6: **LD-13**

Compound **18e** (43.7 mg, 0.026 mmol) and nitromethane (5 mL) were added to a 25 mL single-necked bottle. After stirring to dissolve, zinc bromide (125.0 mg, 0.52 mmol) was weighed. After N₂ replacement three times, the temperature was raised to 35 °C for reaction. The reaction of the raw materials was monitored by HPLC. The reaction solution was directly dried under reduced pressure at 45 °C to obtain a crude product. The product was prepared and purified to obtain 16.1 mg of white solid **LD-13** with a yield of 40%. LC-MS [M+H]⁺: m/z 1541.7.

Example 19: LD-15



Step 1: Compound **M8**

M7 (45.0 mg, 0.068 mmol, prepared according to the method of WO2018103739A1) and 10 mL THF were added to a 25 mL round-bottom flask and stirred in an ice-water bath for 15 min. PPh₃ (37.2 mg, 0.14 mmol) was added and continued to stir for about 3 h. The starting material disappeared by TLC detection. The reaction was quenched with saturated sodium bicarbonate. The mixed solution was extracted with ethyl acetate (100 mL*3 times), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure with a water pump. The crude product was purified by flash column chromatography (eluent: DCM/MeOH=50/1-20/1) to obtain 36.1 mg of white foamy solid **M8** with a yield of 83%. LC-MS [M+H]⁺: m/z 641.6.

Step 2: Compound **20a**

Compound **M8** (36.1 mg, 0.056 mmol), Fmoc-glycine (16.9 mg, 0.056 mmol) and 5 mL DMF were added to a 25 mL round-bottom flask, cooled in an ice-water bath. PyBop (63.4 mg, 0.112 mmol) and HOBt (15.4 mg, 0.112 mmol) were added in sequence. DIEA (37 μ L, 0.224 mmol) was added dropwise under stirring in an ice-water bath, stirred at room temperature for 2 h. The reaction solution was concentrated under reduced pressure with an oil pump to remove the solvent, and the obtained residue was directly used for the next step reaction.

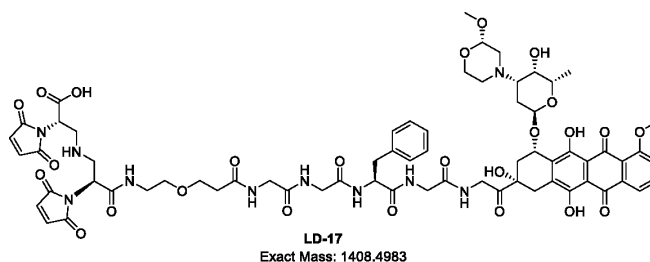
DMF (10 mL) was added to the above crude compound, diethylamine (2.5 mL) was added at room temperature, and the reaction was stirred at room temperature for 3 h. The reaction was monitored by HPLC until completion, and the preparative liquid phase was purified. The preparative liquid was lyophilized to obtain 30.6 mg of compound **20a**, with a yield of 77%, LC-MS [M+H]⁺: m/z 698.8.

Step 3: Compound **20b**

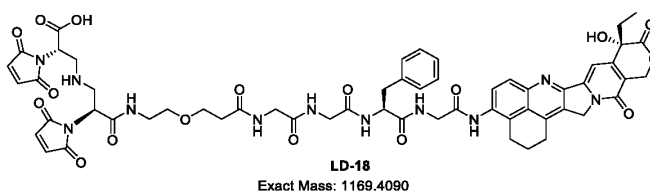
Compound **20a** (30.3 mg, 0.043 mmol), compound **18c** (38.9 mg, 0.043 mmol) and 5 mL DMF were added to a 25 mL round-bottom flask and cooled in an ice-water bath. PyBop (47.3 mg, 0.086 mmol) and HOBt (11.9 mg, 0.086 mmol) were added in sequence. DIEA (35 μ L, 0.22 mmol) was added dropwise under stirring in an ice bath. The reaction was stirred at room temperature for 6 h. The reaction was completed under HPLC monitoring. The reaction solution was concentrated under reduced pressure with an oil pump to remove the solvent and purified by preparative liquid phase. The preparative solution was lyophilized to obtain 41.8 mg of compound **20b** with a yield of 62%. LC-MS $[M/2+H]^+$: m/z 781.6.

10 Step 6: **LD-15**

Compound **20b** (20.4 mg, 0.013 mmol) and nitromethane (5 mL) were added to a 25 mL single-necked bottle. After stirring to dissolve, zinc bromide (62.9 mg, 0.26 mmol) was weighed. After N₂ replacement three times, the temperature was raised to 35 °C for reaction. The reaction of the raw materials was monitored by HPLC. The reaction solution was directly dried under reduced pressure at 45 °C to obtain a crude product. The product was prepared and purified to obtain 7.3 mg of white solid **LD-15** with a yield of 38%. LC-MS $[M+H]^+$: m/z 1407.6.

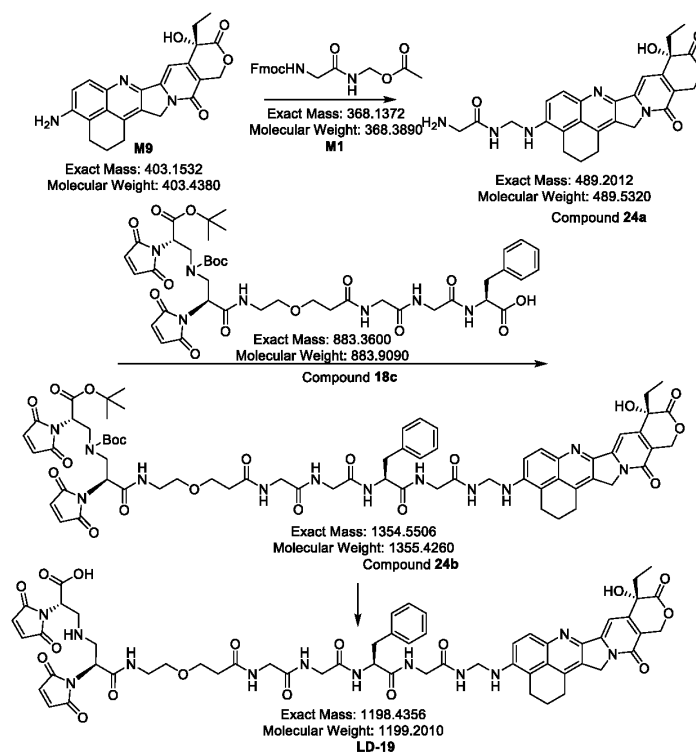
Example 20: LD-17

20 Referring to the synthetic route of **Example 19**, 15.8 mg of white solid compound **LD-17** was prepared; LC-MS $[M/2+H]^+$: m/z 704.5.

Example 21: LD-18

Referring to the synthetic route of **Example 19**, 20.0 mg of white solid compound **LD-18** was prepared; LC-MS $[M+H]^+$: m/z 1170.8.

Example 22: LD-19



Step 1: Compound **18d**

M1 (56 mg, 0.15 mmol) and 15 mL THF were added to a 25 mL round-bottom flask and stirred in an ice-water bath for 15 min. Toluenesulfonic acid monohydrate (2.9 mg, 0.015 mmol) was added and stirred for 10 min. **M9** (60 mg, 0.149 mmol) was added and stirred in an ice-water bath after the addition was complete. The raw material disappeared after TLC detection about 1 h. The reaction was quenched with saturated sodium bicarbonate. The mixed solution was extracted with ethyl acetate (100 mL*3 times), dried over anhydrous sodium sulfate, concentrated and used directly in the next reaction.

10 The above crude compound and 15 mL of DMF were added to a 25 mL round-bottom flask, followed by DBU (3 mL). The reaction was stirred at room temperature for about 0.5 h. After the reaction was completed as detected by TLC, the mixture was concentrated under reduced pressure by a water pump. The crude product was purified by flash column chromatography (eluent: DCM/MeOH=20/1-10/1-1/1) to give 41 mg of white solid **24a** in a yield of 56%. LC-MS [M+H]⁺: m/z 490.3.

Step 2: Compound **24b**

20 Compound **24a** (41 mg, 0.083 mmol), compound **18c** (73.6 mg, 0.083 mmol) and 5 mL DMF were added to a 25 mL single-necked bottle and cooled in an ice-water bath. PyBop (96.5 mg, 0.17 mmol) and HOBt (25.4 mg, 0.17 mmol) were added in sequence. DIEA (75 μ L, 0.45 mmol) was added dropwise under stirring in an ice bath. The reaction was stirred at room temperature for 5 h. The reaction was completed under HPLC monitoring. The reaction solution was concentrated under reduced pressure with an oil pump to remove the solvent and purified by preparative liquid phase. The preparative solution was lyophilized to

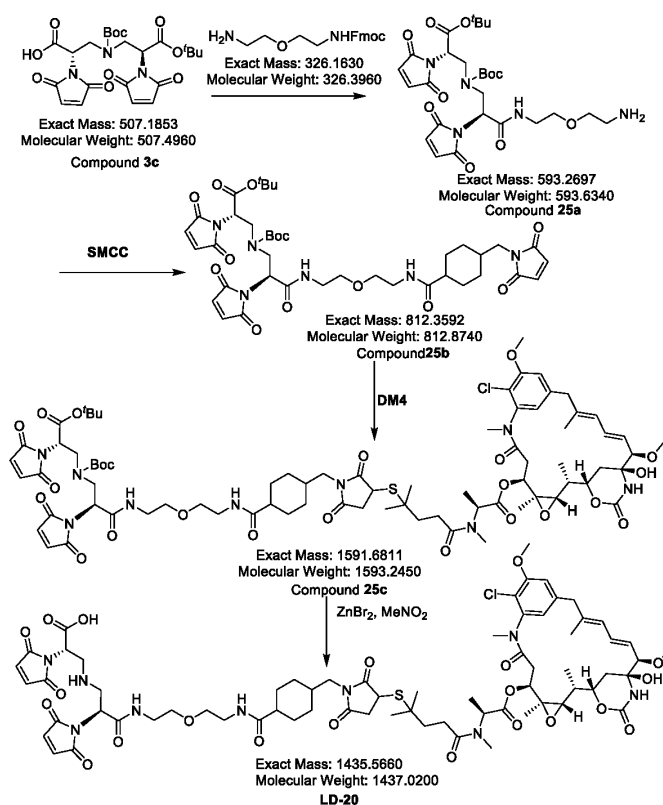
obtain 52 mg of compound **24b** with a yield of 46%. LC-MS $[M+H]^+$: m/z 1355.5.

Step 3: LD-19

Compound **24b** (30 mg, 0.022 mmol) and nitromethane (5 mL) were added to a 25 mL single-necked bottle. After stirring to dissolve, zinc bromide (102.7 mg, 0.45 mmol) was weighed. After N₂ replacement three times, the temperature was raised to 35 °C for reaction. The reaction of the raw materials was monitored by HPLC. The reaction solution was directly dried under reduced pressure at 45 °C to obtain a crude product. The product was prepared and purified to obtain 9 mg of white solid **LD-19** with a yield of 34%. LC-MS $[M+H]^+$: m/z 1199.3.

10

Example 23: LD-20



Step 1: Compound 25a

Compound **3c** (200 mg, 0.394 mmol) and DCM (10 mL) were added to a 25 mL single-mouth bottle, cooled in an ice-water bath. HOSu (50 mg, 0.433 mmol) and DIPEA (72 μ L, 0.433 mmol) were added in sequence. After the addition, the reaction was allowed to proceed for 2 h. After TLC monitoring, the insoluble matter was removed by filtration, and the mixture was concentrated under reduced pressure and used directly in the next step. DMF (10 mL) was added to the above crude compound, cooled in an ice-water bath, and 2-[2-(Fmoc-amino)ethoxy]ethylamine hydrochloride (148.8 mg, 0.394 mmol) and DIPEA (101 μ L, 0.6 mmol) were added in sequence. The mixture was naturally heated to room temperature and reacted for about 2 h. The mixture was concentrated under reduced pressure by an oil pump and used directly in the next step.

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DMF (10 mL) and diethylamine (2 mL) were added to the above crude compound, and the reaction was allowed to proceed for about 0.5 h. After the reaction was completed as monitored by HPLC, the DMF was removed by oil pump concentration, and the product was directly prepared and purified, and freeze-dried to obtain 135 mg of white solid compound **25a** with a yield of 58%; LC-MS [M+H]⁺: m/z 594.6.

Step 2: Compound **25b**

Compound **25a** (130 mg, 0.22 mmol) and DMF (10 mL) were added to a 25 mL single-necked bottle and cooled in an ice-water bath. SMCC (85.3 mg, 0.25 mmol) and DIPEA (42 uL, 0.25 mmol) were added in sequence. The mixture was reacted for 1 h after the addition was completed. After the reaction was completed under TLC monitoring, the insoluble matter was removed by filtration, the mixture was concentrated under reduced pressure with a water pump, and purified by flash column chromatography with an eluent of (PE/EA = 50/1-20/1-5/1) to obtain 189 mg of compound **25b** with a yield of 100%; LC-MS [M+H]⁺: m/z 813.7.

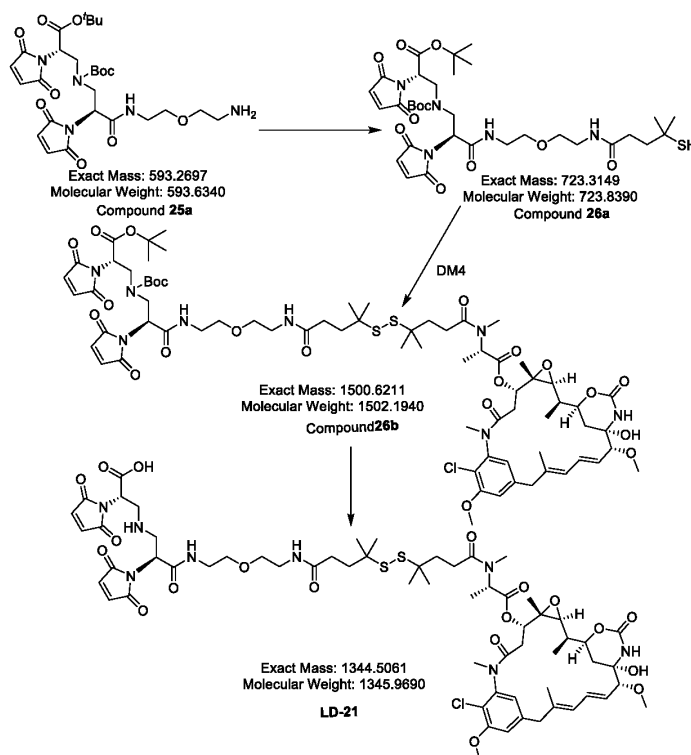
Step 3: Compound **25c**

Compound **25b** (150 mg, 0.185 mmol) and DMF (5 mL) were added to a 25 mL single-necked bottle, and DM4 (150 mg, 0.18 mmol) and DIPEA (34 uL, 0.2 mmol) were added in sequence at room temperature. The reaction was carried out at room temperature for about 13 h. The reaction was monitored by HPLC. The product was directly prepared and purified by HPLC. The prepared solution was lyophilized to obtain 124 mg of white solid compound **25c** with a yield of 42%; LC-MS [M+H]⁺: m/z 1592.5.

Step 4: **LD-12**

Compound **25c** (40 mg, 25 umol) and CH₃NO₂ (3 mL) were added to a 25 mL single-necked bottle to dissolve the turbid solution. DMF (0.2 mL) was then added to assist dissolution. ZnBr₂ (116.9 mg, 0.5 mmol) was then added. After N₂ replacement, the temperature was raised to 40 °C for reaction for 1 h. After HPLC monitoring, the reaction was completed and the solvent was dried under reduced pressure at 40 °C by an oil pump to obtain a crude product. The crude product was directly prepared and purified by HPLC. The prepared solution was lyophilized to obtain 18 mg of light yellow solid **LD-20** with a yield of 50%; LC-MS [M+H]⁺: m/z 1436.6.

Example 24: LD-21



Step 1: Compound 26a

4-Mercapto-4-methylpentanoic acid (50 mg, 0.337 mmol) and DCM (10 mL) were added to a 25 mL single-necked bottle, cooled in an ice-water bath. HOSu (39.4 mg, 0.337 mmol) and DIPEA (58 μ L, 0.35 mmol) were added in sequence. After the addition was complete, the reaction was allowed to react for 2 h. The insoluble matter was removed by filtration, and DMF (10 mL) was added to the above crude compound after reduced pressure concentration. The mixture was cooled in an ice-water bath, and compound 25a (208.6 mg, 0.35 mmol) and DIPEA (58 μ L, 0.35 mmol) were added in sequence. The temperature was naturally raised to room temperature and the reaction was allowed to react for about 2 h. The reaction was monitored by HPLC. DMF was removed by oil pump concentration, and the mixture was directly prepared and purified. 196 mg of compound 26a was obtained by freeze-drying with a yield of 80%; LC-MS $[M+H]^+$: m/z 724.4.

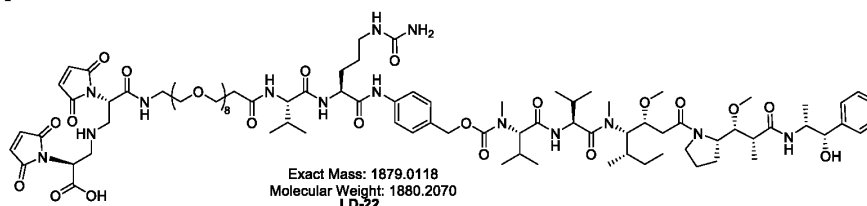
Step 2: Compound 26b

Compound 26a (196 mg, 0.27 mmol) and DCM (10 mL) were added to a 25 mL single-necked bottle and cooled in an ice-water bath. DM4 (115 mg, 0.14 mmol) and I_2 (138 mg, 0.54 mmol) were added in sequence. The reaction was allowed to react for 4 h after the addition was complete. The reaction was monitored by HPLC. Vitamin C solution in DCM was added under ice-water cooling until the reaction solution was colorless. The DCM was removed by concentration and the solution was directly prepared and purified. After freeze-drying, 108 mg of compound 26b was obtained with a yield of 51%; LC-MS $[M/2+H]^+$: m/z 751.5.

Step 3: LD-21

Compound **26b** (48 mg, 32 μmol) and CH_3NO_2 (3 mL) were added to a 25 mL single-necked bottle to dissolve the turbid solution. DMF (0.2 mL) was then added to assist dissolution. ZnBr_2 (176.5 mg, 0.64 mmol) was then added. After N_2 replacement, the temperature was raised to 40°C for reaction for 1 h. After HPLC monitoring, the reaction was completed, and the solvent was dried under reduced pressure at 40°C by an oil pump to obtain a crude product. The product was directly prepared and purified by HPLC. The prepared solution was lyophilized to obtain 22 mg of white solid **LD-21** with a yield of 51%; LC-MS $[\text{M}+\text{H}]^+$: m/z 1345.5.

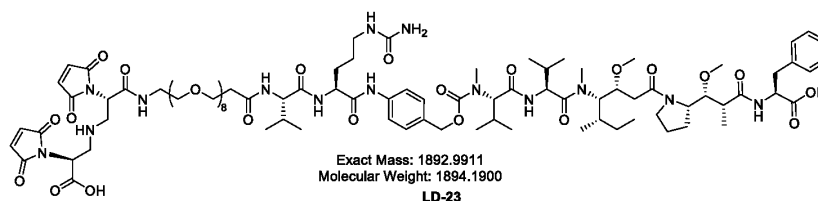
Example 25: LD-22



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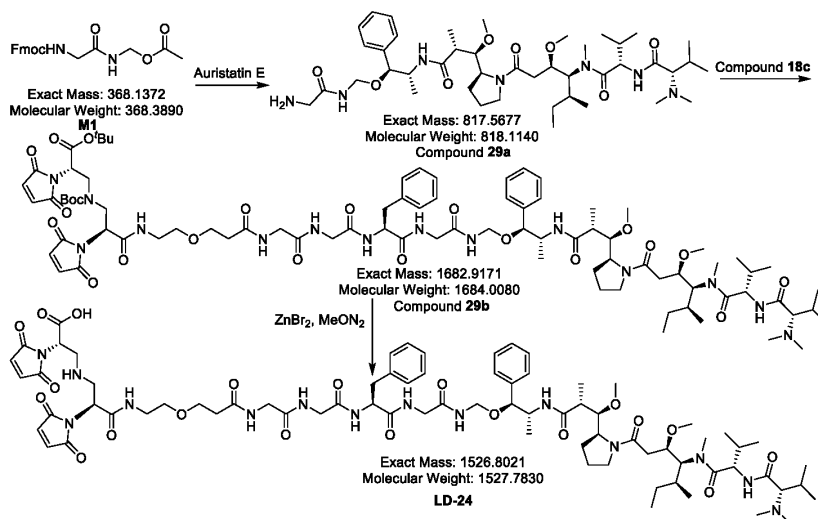
Referring to the synthetic route of **Example 16**, 48 mg of white solid compound **LD-22** was prepared; LC-MS $[\text{M}/2+\text{Na}]^+$: m/z 962.4.

Example 26: LD-23



Referring to the synthetic route of **Example 16**, 39 mg of white solid compound **LD-23** was prepared; LC-MS $[\text{M}/2+\text{H}]^+$: m/z 947.8.

Example 27: LD-24



Step 1: Compound 29a

M1 (50 mg, 0.136 mmol) and 15 mL THF were added to a 25 mL round-bottom flask and

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stirred in an ice-water bath for 15 min. Toluenesulfonic acid monohydrate (2.6 mg, 0.0136 mmol) was added and stirred for 10 min. **Auristatin E** (88.2 mg, 0.12 mmol) was added and stirred in an ice-water bath after the addition was complete. The starting material disappeared after TLC detection for about 1 h. The reaction was quenched with saturated sodium bicarbonate. The mixed solution was extracted with ethyl acetate (50 mL * 3 times), dried over anhydrous sodium sulfate, and concentrated for the next step.

The above crude compound and 10 mL DMF were added to a 25 mL round-bottom flask. DBU (2 mL) was added for deprotection. The reaction was stirred at room temperature for about 0.5 h. After the reaction was completed by HPLC monitoring, it was directly prepared and purified. The prepared solution was lyophilized to obtain 53 mg of white solid **29a**, with a two-step yield of 54%; LC-MS $[M+H]^+$: m/z 818.6.

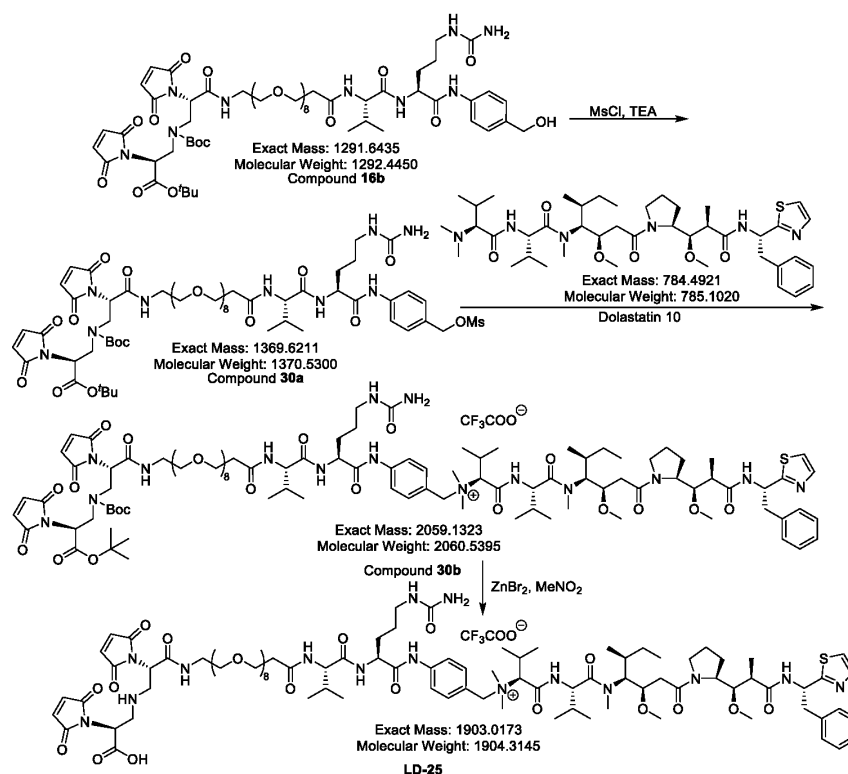
Step 2: Compound **29b**

Compound **29a** (43 mg, 52.6 μ mol), compound **18c** (54.5 mg, 60 μ mol) and 5 mL DMF were added to a 25 mL single-necked bottle and cooled in an ice-water bath. EDCI·HCl (27.1 mg, 0.12 mmol) and DIEA (20 μ L, 0.12 mmol) were added in sequence. The mixture was stirred at room temperature for 4 h. The reaction was completed after HPLC monitoring. The solvent DMF was removed by vacuum concentration using an oil pump, and the mixture was purified by preparative liquid phase and freeze-dried to obtain 58 mg of compound **29b** with a yield of 57%. LC-MS $[(M-Boc)/2+H]^+$: m/z 792.7.

Step 3: **LD-24**

Compound **29b** (20 mg, 11.9 μ mol) and nitromethane (5 mL) were added to a 25 mL single-necked bottle. After stirring to dissolve, zinc bromide (55.1 mg, 0.24 mmol) was weighed. After N₂ replacement three times, the temperature was raised to 35 °C for reaction. The reaction of the raw materials was monitored by HPLC. The reaction solution was directly dried under reduced pressure at 40 °C to obtain a crude product. The product was prepared and purified to obtain 11 mg of white solid **LD-24** with a yield of 61%. LC-MS $[M+H]^+$: m/z 764.6.

Example 28: LD-25



Step 1: Compound **30a**

Compound **16b** (101.4 mg, 0.078 mmol) and 15 mL of anhydrous dichloromethane were added to a 25 mL round-bottom flask, and the mixture was stirred and cooled in an ice-water bath for 15 min. TEA (17 μ L, 0.117 mmol) and MsCl (13.7 mg, 0.117 mmol) were added. After the addition was complete, stirring was continued in an ice-water bath. After about 1 h, TLC detection showed that the raw material disappeared. After dilution with 10 mL of dichloromethane, the reaction was quenched with saturated sodium bicarbonate, and the mixed solution was extracted with ethyl acetate (50 mL * 3 times), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. After preparation and purification, 75 mg of solid compound **30a** was obtained, with a yield of 70%; LC-MS [M+H]⁺: m/z 1370.5.

Step 2: Compound **30b**

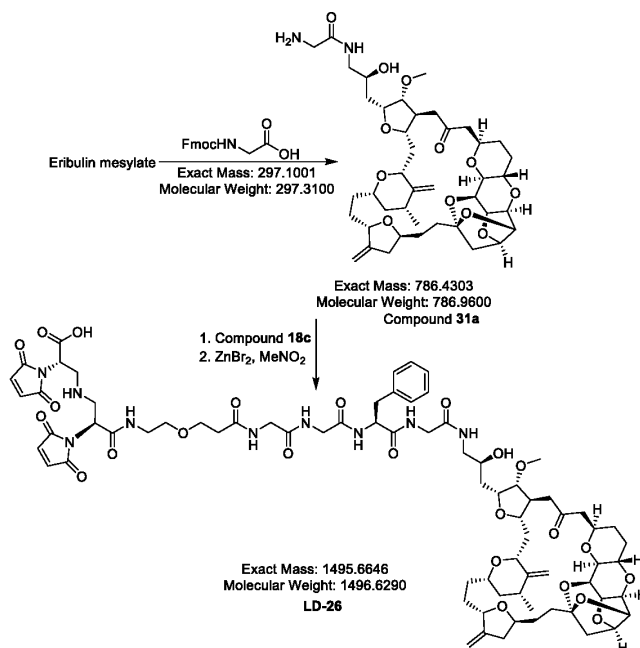
Compound **30a** (48 mg, 35 μ mol), Dolastatin 10 (24.5 mg, 30 μ mol) and 2-butanone (10 mL) were added to a 25 mL single-necked bottle and the temperature was raised to reflux for reaction for 23 h. The reaction was completed after HPLC monitoring. The solvent was removed by vacuum concentration using an oil pump, and the product was purified by preparative liquid phase and lyophilized to obtain 15.6 mg of trifluoroacetate salt of compound **30b** with a yield of 24%. LC-MS [M/2]⁺: m/z 1030.3.

Step 3: **LD-25**

Compound **30b** (15.6 mg, 7.2 μ mol) and nitromethane (5 mL) were added to a 25 mL single-necked bottle. After stirring to dissolve, zinc bromide (37.6 mg, 0.144 mmol) was weighed. After N₂ replacement three times, the temperature was raised to 40 °C for reaction. The reaction of the raw materials was monitored by HPLC. The reaction solution was directly

dried under reduced pressure at 40 °C to obtain a crude product. The product was prepared and purified to obtain 5.1 mg of trifluoroacetate salt of white solid **LD-25** with a yield of 35%. LC-MS [M/2]⁺: m/z 951.6.

Example 29: LD-26



Step 1: Compound **31a**

Compound **Eribulin mesylate** (20 mg, 24.2 μ mol), **Fmoc-glycine** (9.5 mg, 30 μ mol) and 5 mL **DMF** were added to a 25 mL round-bottom flask, cooled in an ice-water bath. **PyBop** (26.7 mg, 0.048 mmol) and **HOBt** (7.5 mg, 0.048 mmol) were added in sequence. **DIEA** (20 μ L, 0.121 mmol) was added dropwise under stirring in an ice-water bath, stirred at room temperature for 17 h. The reaction solution was concentrated under reduced pressure with an oil pump to remove the solvent, and the obtained residue was directly used for the next step reaction.

DMF (5 mL) was added to the above crude compound, **diethylamine** (1 mL) was added at room temperature, and the reaction was stirred at room temperature for 1 h. The reaction was monitored by **HPLC** until complete, and the preparative liquid phase was purified. The preparative liquid was lyophilized to obtain 14.1 mg of compound **31a**, with a yield of 73%, LC-MS [M+H]⁺: m/z 788.1.

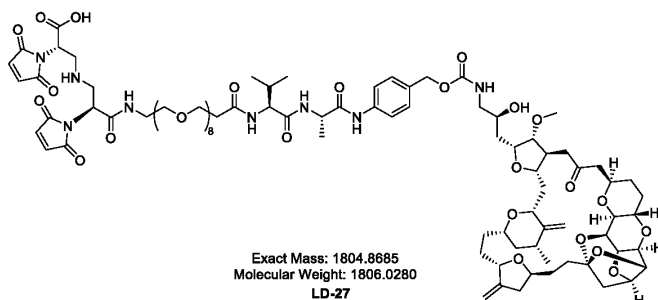
Step 2: **LD-26**

Compound **31a** (14.1 mg, 17.9 μ mol), compound **18c** (21.9 mg, 24 μ mol) and 5 mL **DMF** were added to a 25 mL round-bottom flask, cooled in an ice-water bath. **PyBop** (27.1 mg, 0.048 mmol) and **HOBt** (7.9 mg, 0.048 mmol) were added in sequence. **DIEA** (12 μ L, 0.072 mmol) was added dropwise under ice-water stirring, and the reaction was stirred at room temperature for 12 h. The reaction was monitored by **HPLC** to be complete. The reaction solution was concentrated under reduced pressure with an oil pump to remove the solvent, and the preparative liquid was purified. The preparative liquid was freeze-dried and directly used

for the next step reaction.

The above crude compound and nitromethane (5 mL) were added to a 25 mL single-mouth bottle, and DMF (0.2 mL) was added to stir and dissolve. Zinc bromide (85.0 mg, 0.36 mmol) was weighed, and N₂ was replaced 3 times and then raised to 40 °C for reaction. HPLC monitored the reaction of the raw materials. The reaction was completed, and the reaction solution was directly 40 °C and dried under reduced pressure to obtain a crude product, which was purified to obtain 6.7 mg of white solid **LD-26** with a two-step yield of 25%. LC-MS [M+H]⁺: m/z 1497.0.

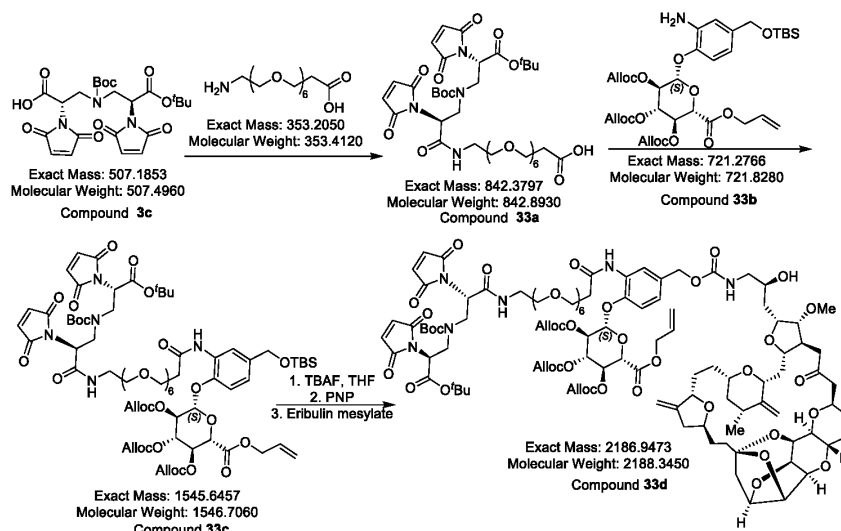
Example 30: LD-27



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Referring to the synthetic route of **Example 16**, 9.3 mg of white solid compound **LD-27** was prepared; LC-MS [M/2+H]⁺: m/z 903.5.

Example 31: LD-28



Step 1: Compound **33a**

Compound **3c** (152.2 mg, 0.30 mmol) and DMF (10 mL) were added to a 25 mL single-necked bottle and cooled in an ice-water bath. HOSu (43.7 mg, 0.36 mmol) and DIPEA (60 μL, 0.36 mmol) were added in sequence. The mixture was reacted for 2 h after the addition was completed. The insoluble matter was removed by filtration and concentrated under reduced pressure. DMF (10 mL) was added to the crude compound. Amino-6-polyethylene glycol-carboxylic acid (117.8 mg, 0.33 mmol) and DIPEA (55 μL, 0.33 mmol) were added in sequence at room temperature. The reaction was allowed to react for about 2 h. The reaction

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was completed under HPLC monitoring. DMF was removed by oil pump concentration. The mixture was directly prepared and purified. 204.5 mg of compound **33a** was obtained by freeze-drying. The yield was 79%; LC-MS [M-H]⁻: m/z 841.6.

Step 2: Compound **33c**

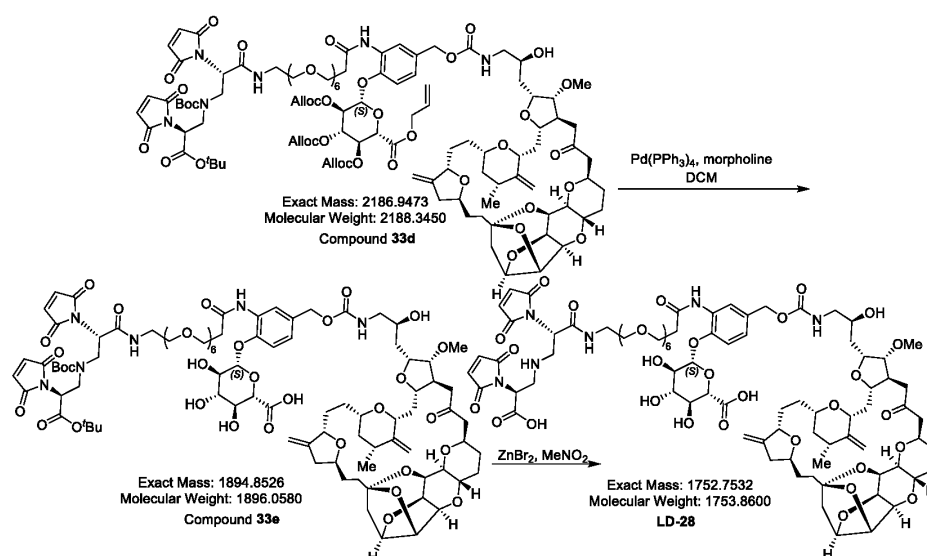
Compound **33a** (204.5 mg, 0.243 mmol) and DMF (10 mL) were added to a 25 mL single-necked bottle and cooled in an ice-water bath. Compound **33b** (178.0 mg, 0.243 mmol, prepared by the method of reference: 10.1002/chem.202003471). PyBOP (268.8 mg, 0.5 mmol), HOBt (70.3 mg, 0.5 mmol) and DIPEA (83 μ L, 0.5 mmol) were added in sequence. The reaction was allowed to proceed for 4 h. The reaction was completed under HPLC monitoring. The product was directly prepared and purified, and lyophilized to obtain 221.9 mg of compound **33c** with a yield of 59%; LC-MS [M/2+H]⁺: m/z 774.2.

Step 3: Compound **33d**

Compound **33c** (221.9 mg, 144 μ mol) and THF (5 mL) were added to a 25 mL single-mouth bottle, and then TBAF (288 μ L, 1 mmol/L) was added. The reaction was maintained at room temperature for 4 h. After the reaction was completed by HPLC monitoring, the mixture was filtered through silica gel pads, and the filter cake was rinsed with EA three times. The filtrate was concentrated and directly used for the next reaction.

Anhydrous dichloromethane (5 mL) was added to a 25 mL single-mouth bottle of the above crude compound, and di(p-nitrobenzene) carbonate (90.4 mg, 288 μ mol) and DIPEA (48 μ L, 288 μ mol) were added in sequence at room temperature. After reacting at room temperature for 2 h, the insoluble matter was removed by filtration, and the filtrate was concentrated and directly used for the next reaction.

Anhydrous DMF (5 mL) was added to a 25 mL single-mouth bottle of the above crude compound, followed by Eribulin mesylate (33.0 mg, 40 μ mol) and DIPEA (37 μ L, 220 μ mol) in sequence. The reaction was maintained at room temperature for 8 h after the addition. The reaction was completed under HPLC monitoring, and the product was directly prepared and purified. After freeze-drying, 33.4 mg of compound **33d** was obtained with a yield of 38%; LC-MS [M/2+H]⁺: m/z 1093.6.



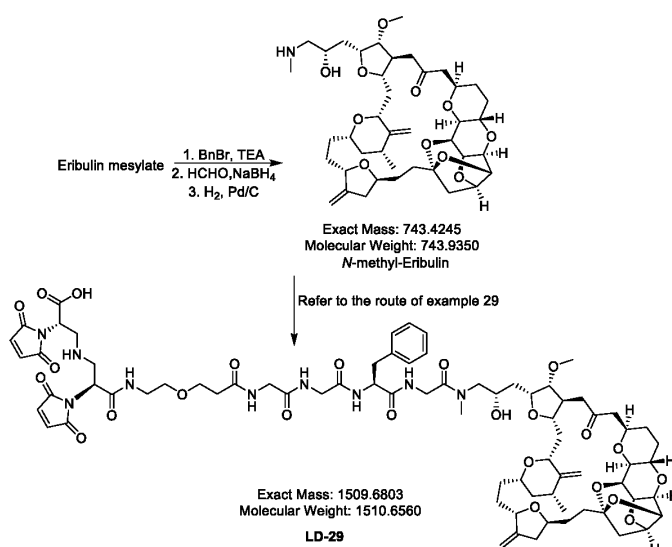
Step 4: Compound **33e**

Compound **33d** (33.4 mg, 15.3 μmol) and DCM (10 mL) were added to a 25 mL single-mouth bottle, and the system was replaced with N_2 three times. Tetrakis(triphenyl)phosphine palladium (2.3 mg, 1.53 μmol) and Morpholine (3 μL , 32 μmol) were added in sequence. The reaction was maintained at room temperature for 1.5 h. The reaction of the raw materials was monitored by HPLC. The reaction solution was filtered through diatomaceous earth to remove insoluble matter. The filter cake was rinsed three times with dichloromethane. The filtrate was concentrated under reduced pressure to obtain a crude product. The product was prepared and purified to obtain 10.4 mg of solid compound **33e** with a yield of 36%. LC-MS $[\text{M}/2+\text{H}]^+$: m/z 948.5.

Step 5: **LD-28**

Compound **33e** (10.4 mg, 5.5 μmol) and nitromethane (4 mL) were added to a 25 mL single-necked bottle, and DMF (0.2 mL) was added and stirred to dissolve. Zinc bromide (28.5 mg, 0.11 mmol) was weighed, and the temperature was raised to 40 $^\circ\text{C}$ after N_2 replacement three times. The reaction of the raw materials was monitored by HPLC. The reaction solution was directly dried under reduced pressure at 40 $^\circ\text{C}$ to obtain a crude product. The product was prepared and purified to obtain 5.7 mg of solid compound **LD-28** with a yield of 60%. LC-MS $[\text{M}/2+\text{H}]^+$: m/z 870.7.

Example 32: **LD-29**



Step 1: N-methyl-eribulin

Eribulin mesylate (20.3 mg, 24.6 μmol) and 10 mL of anhydrous dichloromethane were added to a 25 mL round-bottom flask, cooled in an ice-water bath. TEA (4 μL , 29.5 μmol) and BnBr (4 μL , 0.117 mmol) were added, and the reaction was continued at this temperature for 1 h. After TLC monitoring, the raw material disappeared, 10 mL of dichloromethane was added to dilute, and the reaction was quenched with saturated sodium bicarbonate. The mixed solution was extracted with ethyl acetate (50 mL * 3 times), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure and directly used for the next reaction.

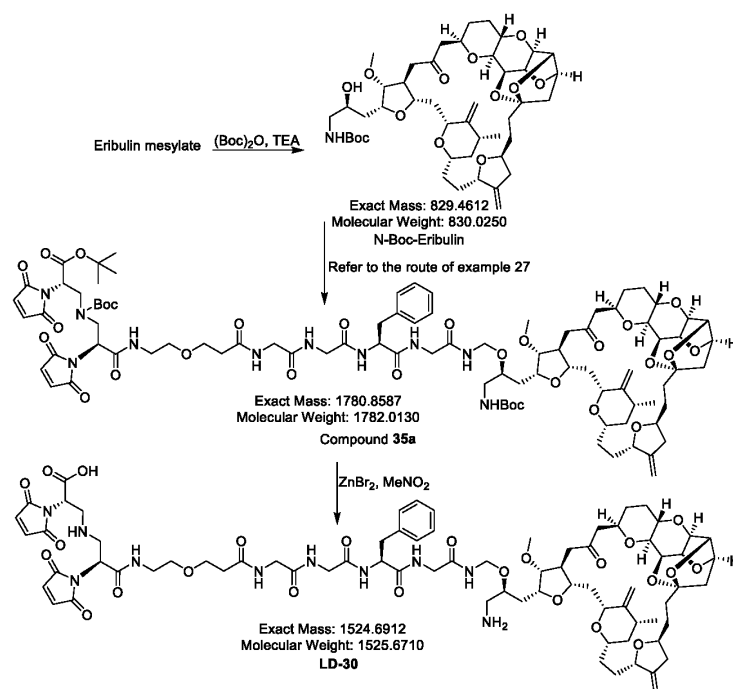
- 10 The above compound and 5 mL of formaldehyde aqueous solution were added to a 25 mL single-mouth bottle, cooled and stirred in an ice-water bath for 1 h. Sodium borohydride (4 mg) was added, and the reaction was maintained at this temperature for 0.5 h, and sodium borohydride (4 mg) was added. After 0.5 h, TLC monitoring showed that the reaction was complete. Acetone was added for 2 mL quenched the reaction, filtered on silica gel. The filter cake was rinsed with EA 3 times, and the filtrate was concentrated under reduced pressure and directly used for the next step reaction.

- 20 The above compound and methanol (5 mL) were added to a 25 mL single-mouth bottle, and 20 mg Pd/C was added. After the atmosphere in the system was replaced by a hydrogen balloon three times, the reaction was maintained at room temperature. HPLC monitored the reaction of the raw materials, filtered to remove Pd/C, and the filter cake was rinsed with methanol three times, concentrated, prepared, purified, and freeze-dried to obtain 11.3 mg **N-methyl-eribulin**, with a three-step yield of 61%; LC-MS $[\text{M}+\text{H}]^+$: m/z 744.8.

Step 2: LD-29

Referring to the synthetic route of **Example 31**, 4.8 mg of white solid **LD-29** was prepared; LC-MS $[\text{M}/2+\text{H}]^+$: m/z 755.9.

Example 33: LD-30



Step 1: N-Boc-Azhibulin

Eribulin mesylate (20.5 mg, 24.8 μ mol) and 10 mL of anhydrous dichloromethane were added to a 25 mL round-bottom flask, cooled in an ice-water bath. TEA (7 μ L, 49.6 μ mol) and (Boc)₂O (6 μ L, 24.8 μ mol) were added, and the temperature was naturally raised to room temperature for 4 h. The starting material disappeared after TLC monitoring. Saturated sodium bicarbonate aqueous solution was added to quench the reaction. The mixed solution was extracted with dichloromethane (50 mL * 3 times), dried over anhydrous sodium sulfate, filtered, concentrated under reduced pressure, prepared and purified, and freeze-dried to obtain 19.9 mg of white solid N-Boc-Eribulin, with a yield of 92%; LC-MS [M-Boc+H]⁺: m/z 730.6.

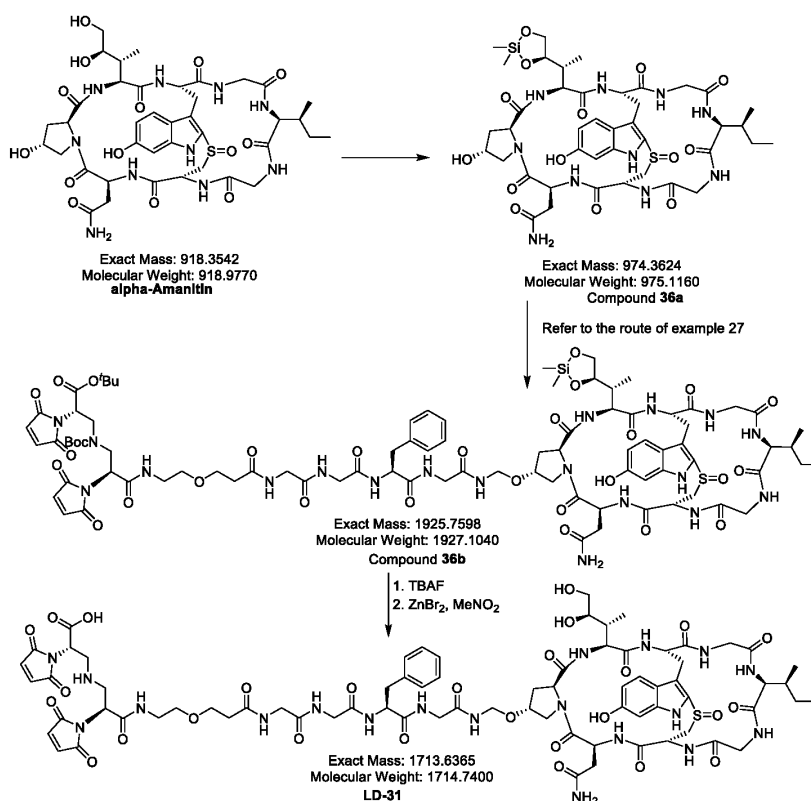
Step 2: Compound 35a

Referring to the synthetic route of **Example 29**, 6.8 mg of compound 35a was prepared; LC-MS [M/2+H]⁺: m/z 892.0.

Step 3: LD-30

Compound 35a (6.8 mg, 0.0038 mmol) and nitromethane (5 mL) were added to a 25 mL single-necked bottle, and DMF (0.2 mL) was added and stirred to dissolve. Zinc bromide (26.8 mg, 0.115 mmol) was added, and the temperature was raised to 40 °C after N₂ replacement three times. The reaction of the raw material was monitored by HPLC. The reaction solution was directly dried under reduced pressure at 40 °C to obtain a crude product. The product was prepared and purified to obtain 3.3 mg of white solid LD-30 with a yield of 57%. LC-MS [M/2+H]⁺: m/z 763.6.

Example 34: LD-31



Step 1: Compound **36a**

α -Amanitin (23.4 mg, 25.5 μ mol) and 10 mL of anhydrous dichloromethane were added to a 25 mL round-bottom flask, and TEA (7 μ L, 51 μ mol) and dichlorodimethylsilane (5 μ L, 24.8 μ mol) were added in sequence at room temperature. The temperature was naturally raised to room temperature for reaction for 3 h. The disappearance of the raw material was monitored by HPLC. The reaction solution was directly concentrated under reduced pressure and purified, and then lyophilized to obtain 23.1 mg of compound **36a** with a yield of 93%; LC-MS $[M+H]^+$: m/z 975.6.

10 Step 2: Compound **36b**

Referring to the synthetic route of **Example 29**, 12.8 mg of compound **36b** was prepared; LC-MS $[M/2+H]^+$: m/z 964.4.

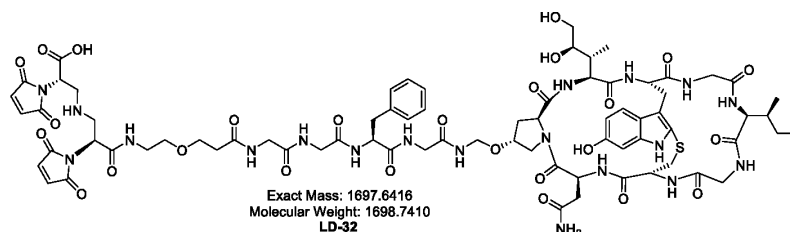
Step 3: **LD-31**

Compound **36b** (12.8 mg, 6.6 μ mol) and THF (5 mL) were added to a 25 mL single-mouth bottle, and then TBAF (14 μ L, 1 mmol/L) was added. The reaction was maintained at room temperature for 1 h. After the reaction was completed by HPLC monitoring, the mixture was filtered through silica gel pads. The filter cake was rinsed with methanol three times. The filtrate was concentrated and directly used for the next step reaction.

The above crude compound and nitromethane (5 mL) were added to a 25 mL single-mouth bottle. DMF (0.2 mL) was added and stirred to dissolve. Zinc bromide (32.7 mg, 0.133 mmol) was added. After N₂ replacement three times, the temperature was raised to 40 °C for reaction. After the reaction of the raw materials was completed by HPLC monitoring, the reaction

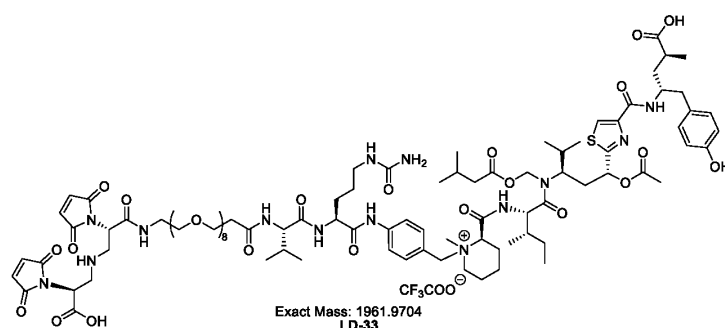
solution was directly dried under reduced pressure at 40 °C to obtain the crude product. After preparation and purification, 3.1 mg of white solid **LD-31** was obtained, with a two-step yield of 28%. LC-MS $[M/2+H]^+$: m/z 858.5.

Example 35: LD-32



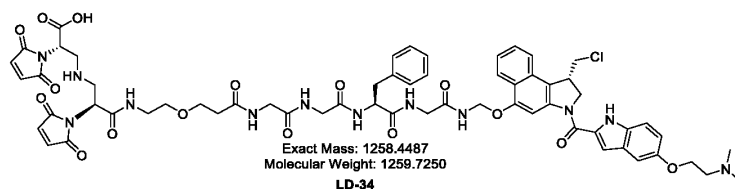
Referring to the synthetic route of **Example 34**, 8.3 mg of the white solid compound **LD-32** was prepared; LC-MS $[M/2+H]^+$: m/z 850.4.

Example 36: LD-33



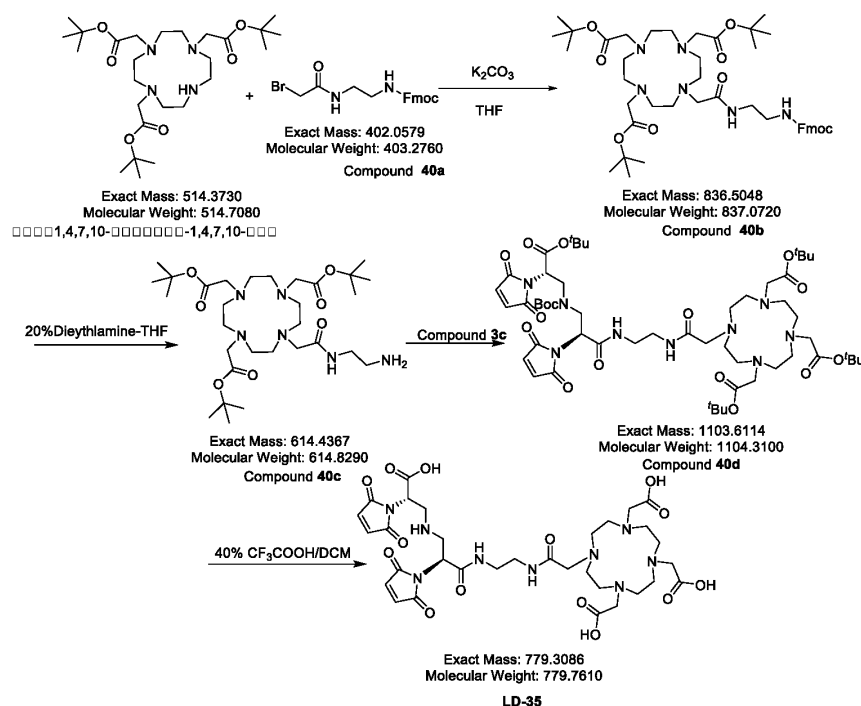
10 Referring to the synthetic route of **Example 28**, 6.2 mg of white solid compound **LD-33** was prepared; LC-MS $[M/2]^+$: m/z 981.8.

Example 37: LD-34



Referring to the synthetic route of **Example 11**, 17.3 mg of the white solid compound **LD-34** was prepared; LC-MS $[M+Na]^+$: m/z 1281.7.

Example 38: LD-35



Step 1: Compound 40b

Tri-tert-butyl 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (8.62 g, 16.7 mmol) and 100 mL of anhydrous THF were added to a 250 mL round-bottom flask, and compound **40a** (6.81 g, 16.8 mmol) and potassium carbonate (2.37 g, 16.8 mmol) were added in sequence at room temperature. After maintaining the reaction at room temperature for 2.5 h. TLC monitoring showed that the starting material disappeared. Water (100 mL) was added to the reaction solution, and the solution was extracted with ethyl acetate (100 mL $\times$ three times), dried over anhydrous sodium sulfate, filtered, and the filtrate was concentrated under reduced pressure to obtain 14.9 g of compound **40b**, which was directly used in the next step without purification; LC-MS $[M-Boc+H]^+$: m/z 737.7.

Step 2: Compound 40c

The above crude compound and THF (100 mL) were added to a 250 mL single-necked bottle, and diethylamine (20 mL) was added at room temperature and stirred to dissolve. The mixture was reacted at room temperature for 6 h. The reaction was monitored by HPLC until the raw material reaction was complete. The reaction solution was directly dried under reduced pressure at 40 °C to obtain a crude product. The crude product was prepared and purified to obtain 7.65 g of solid compound **40c** with a yield of 74.5%. LC-MS $[M-Boc+H]^+$: m/z 515.8.

Step 3: Compound 40d

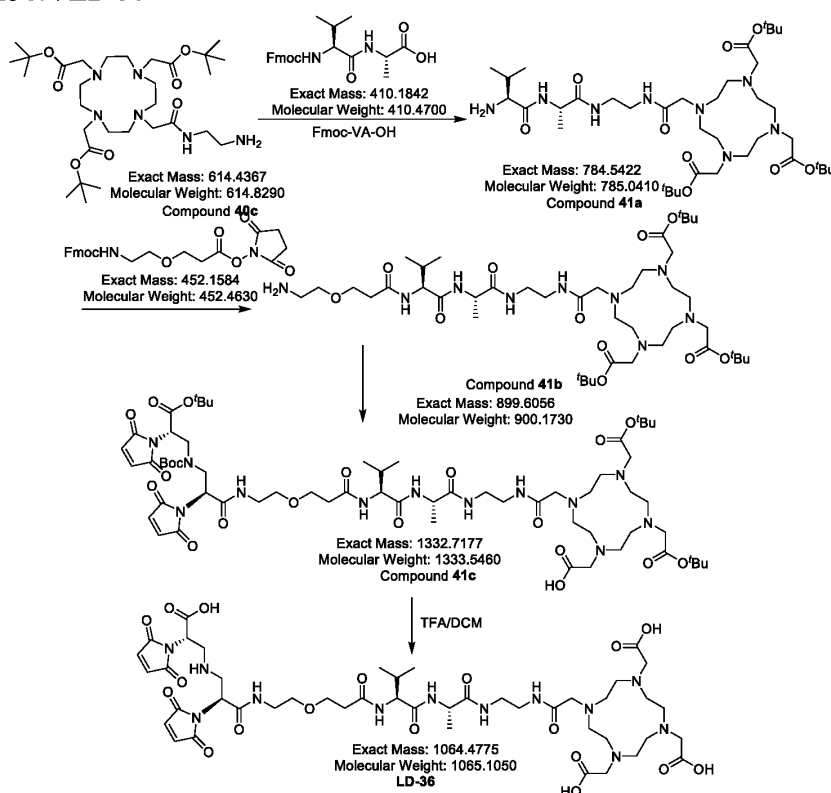
Compound **40c** (152.2 mg, 0.248 mmol) and DMF (5 mL) were added to a 25 mL single-necked bottle, and then compound **3c** (126.3 mg, 0.248 mmol) was added. Under ice-water cooling, EDCI·HCl (100.9 mg, 0.496 mmol) and DIPEA (84 μ L) were added in sequence. The temperature was raised to room temperature and the reaction was reacted for 18 h. After

the reaction was completed under HPLC monitoring, the crude product was concentrated under reduced pressure by an oil pump to obtain a crude product. The product was prepared and purified to obtain 227.1 mg of solid compound **40d** with a yield of 82.9%. LC-MS $[M+H]^+$: m/z 1104.7.

Step 4: **LD-35**

Compound **40c** (202.3 mg, 0.183 mmol) and dichloromethane (10 mL) were added to a 25 mL single-necked bottle, and TFA (2 mL) was added at room temperature. The reaction of the raw materials was monitored by HPLC. The reaction solution was directly concentrated under reduced pressure at 40 °C to obtain a crude product. The crude product was prepared and purified to obtain 95.9 mg of a white hygroscopic solid **LD-35** with a yield of 67%. LC-MS $[M-H]^-$: m/z 778.7.

Example 39: **LD-36**



Step 1: Compound **41a**

Compound Fmoc-VA-OH (201.6 mg, 0.49 mmol) and DCM (15 mL) were added to a 25 mL single-mouth bottle, and HOSu (60.5 mg, 0.52 mmol) and DIPEA (87 μ L) were added sequentially at room temperature. After reacting at room temperature for 2 h, the insoluble matter was removed by filtration, and the filtrate was concentrated to obtain a crude product. Compound **40c** (296.4 mg, 0.49 mmol) and DMF (10 mL) were added to a 25 mL single-mouth bottle of the above crude compound, and DIPEA (87 μ L) was added. The reaction was carried out at room temperature for 2 h. After the reaction was completed by HPLC, the crude product was concentrated under reduced pressure by an oil pump to obtain a crude product.

DMF (6 mL) and diethylamine (1.5 mL) were added to a 25 mL single-mouth bottle of the above crude compound, and the reaction was carried out at room temperature for 4 h. After the reaction was completed by HPLC, the crude product was concentrated under reduced pressure by an oil pump to obtain a crude product. The product was purified and 308.2 mg solid compound **41a**, three-step reaction yield 80.1%, LC-MS [M+H]⁺: m/z 785.7.

Step 2: Compound **41b**

Compound **41a** (308.2 mg, 0.39 mmol) and DMF (10 mL) were added to a 25 mL single-necked bottle, and then N-fluorenylmethoxycarbonyl-monoethylene glycol-succinimide ester (179.5 mg, 0.39 mmol) and DIPEA (66 μ L) were added. The reaction was allowed to react at room temperature for 5 h. After the reaction was completed as monitored by HPLC, the mixture was concentrated under reduced pressure by an oil pump to obtain a crude product. DMF (6 mL) and diethylamine (2 mL) were added to a 25 mL single-necked bottle of the above crude compound. The reaction was allowed to react at room temperature for 4 h. After the reaction was completed as monitored by HPLC, the mixture was concentrated under reduced pressure by an oil pump to obtain a crude product. The product was prepared and purified to obtain 287.2 mg of solid compound **41b** with a yield of 82.3%. LC-MS [M+H]⁺: m/z 901.1.

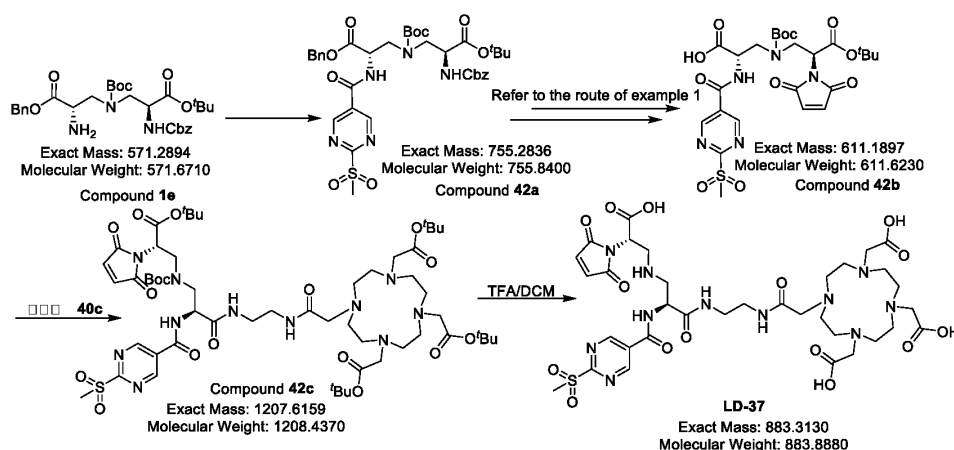
Step 3: Compound **41c**

Compound **41b** (120.6 mg, 0.134 mmol) and DMF (5 mL) were added to a 25 mL single-necked bottle, and then compound **3c** (69.8 mg, 0.134 mmol) was added. Under ice-water cooling, EDCI·HCl (31.7 mg, 0.268 mmol) and DIPEA (67 μ L) were added in sequence. The temperature was raised to room temperature and the reaction was reacted for 13 h. After the reaction was completed under HPLC monitoring, the crude product was concentrated under reduced pressure by an oil pump to obtain a crude product. The product was purified to obtain 124.6 mg of solid compound **41c** with a yield of 69.8%. LC-MS [M-Boc+H]⁺: m/z 1234.2.

Step 4: **LD-36**

Compound **41c** (124.6 mg, 0.093 mmol) and dichloromethane (5 mL) were added to a 25 mL single-necked bottle, and TFA (1.5 mL) was added at room temperature. The reaction of the raw materials was monitored by HPLC. The reaction solution was directly concentrated under reduced pressure at 40 °C to obtain a crude product. The crude product was prepared and purified to obtain 55.6 mg of a white hygroscopic solid **LD-36** with a yield of 56.1%. LC-MS [M-H]⁻: m/z 1063.5.

Example 40: LD-37



Step 1: Compound **42a**

Compound **1e** (250.4 mg, 0.44 mmol) and DMF (10 mL) were added to a 25 mL single-necked bottle and cooled in an ice-water bath. 2-(Methylsulfonyl)pyrimidine-5-carboxylic acid (90.7 mg, 0.44 mmol), PyBOP (268.8 mg, 0.5 mmol), HOBt (70.3 mg, 0.5 mmol) and DIPEA (83 μ L, 0.5 mmol) were added in sequence. The reaction was allowed to proceed for 4 h after the addition was completed. The reaction was monitored to be complete by HPLC. The reaction solution was concentrated by oil pump and then directly prepared and purified. After freeze-drying, 211.9 mg of compound **42a** was obtained with a yield of 63.7%; LC-MS [M+H]⁺: m/z 756.8.

Step 2: Compound **42b**

Referring to the synthetic route of **Example 1**, 64.3 mg of solid compound **42b** was prepared; LC-MS [M-Boc+H]⁺: m/z 512.9.

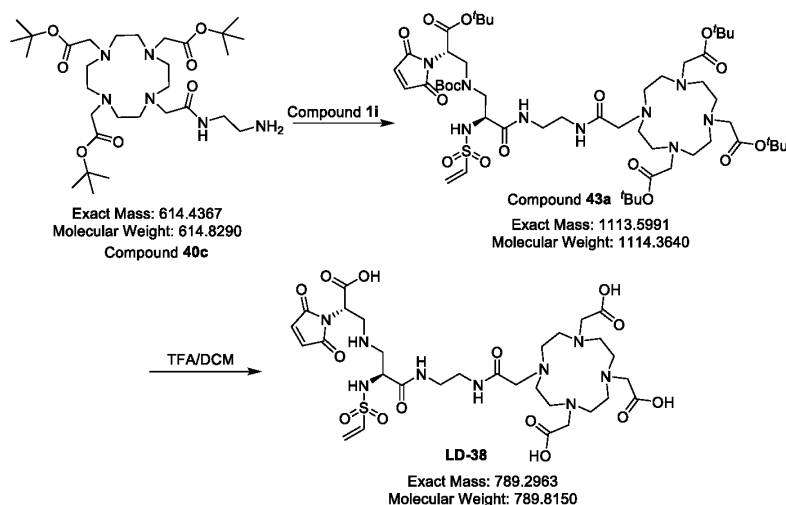
Step 3: Compound **42c**

Compound **42b** (64.3 mg, 0.105 mmol) and DMF (5 mL) were added to a 25 mL single-necked bottle, and then compound **3c** (55.6 mg, 0.105 mmol) was added. Under ice-water cooling, EDCI·HCl (41.8 mg, 0.21 mmol) and DIPEA (54 μ L) were added in sequence. The temperature was raised to room temperature and the reaction was reacted for 25 h. After the reaction was completed under HPLC monitoring, the crude product was concentrated under reduced pressure by an oil pump to obtain a crude product. The product was purified to obtain 62.7 mg of solid compound **42c** with a yield of 49.4%. LC-MS [M-Boc+H]⁺: m/z 1109.1.

Step 4: **LD-37**

Compound **42c** (62.7 mg, 0.052 mmol) and dichloromethane (5 mL) were added to a 25 mL single-necked bottle, and TFA (1.5 mL) was added at room temperature. The reaction of the raw materials was monitored by HPLC. The reaction solution was directly concentrated under reduced pressure at 40 °C to obtain a crude product. The crude product was prepared and purified to obtain 30.5 mg of a white hygroscopic solid **LD-37** with a yield of 67%. LC-MS [M-H]⁻: m/z 882.5.

Example 41: LD-38



Step 1: Compound 43a

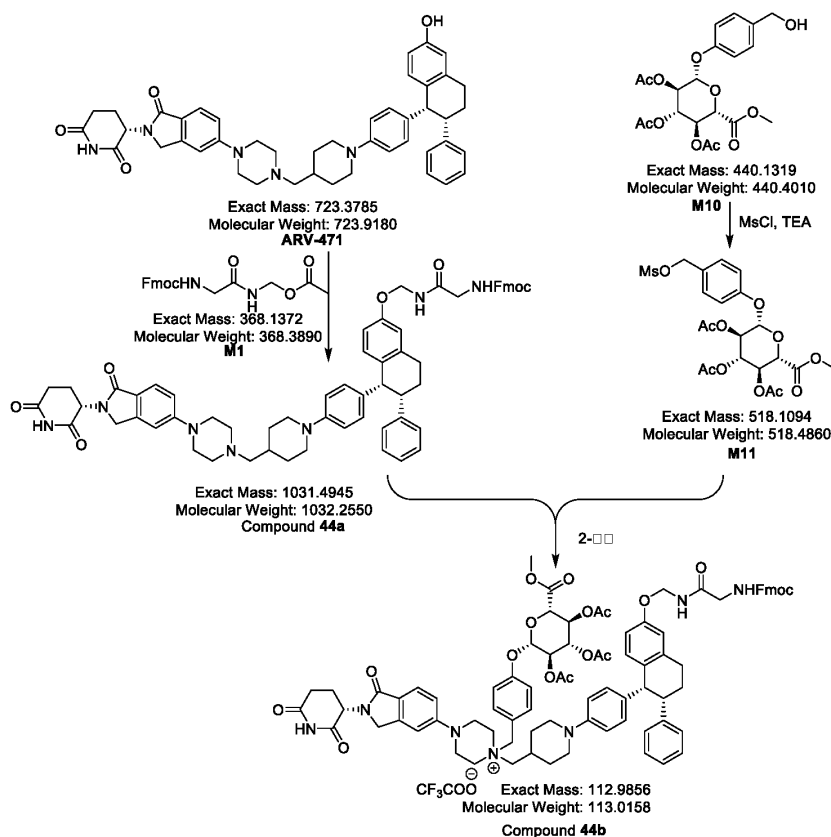
Compound **1i** (145.5 mg, 0.28 mmol) and DCM (15 mL) were added to a 25 mL single-necked bottle, and HOSu (32.7 mg, 0.28 mmol) and DIPEA (48 μ L) were added in sequence at room temperature. After reacting at room temperature for 2 h, the insoluble matter was removed by filtration, and the filtrate was concentrated to obtain a crude product.

Compound **40c** (170.7 mg, 0.28 mmol) and DMF (10 mL) were added to a 25 mL single-necked bottle containing the above crude compound, and DIPEA (50 μ L) was added. The reaction was carried out at room temperature for 2 h. After the reaction was completed under HPLC monitoring, the crude product was concentrated under reduced pressure by an oil pump to obtain a crude product, which was prepared and purified to obtain 199.7 mg of solid compound **43a** with a yield of 64.0%. LC-MS [M-Boc+H]⁺: m/z 1014.9.

Step 2: LD-38

Compound **43a** (60.5 mg, 0.054 mmol) and dichloromethane (5 mL) were added to a 25 mL single-necked bottle, and TFA (1.5 mL) was added at room temperature. The reaction of the raw materials was monitored by HPLC. The reaction solution was directly concentrated under reduced pressure at 40 °C to obtain a crude product. The crude product was prepared and purified to obtain 23.4 mg of a white hygroscopic solid **LD-38** with a yield of 54.9%. LC-MS [M-H]⁻: m/z 788.5.

Example 42: LD-39



Step 1: Compound 44a

M1 (50.3 mg, 0.136 mmol) and 15 mL THF were added to a 25 mL round-bottom flask and stirred in an ice-water bath for 15 min. Toluenesulfonic acid monohydrate (2.5 mg, 0.0136 mmol) was added and stirring was continued for 10 min. ARV-471 (72.9 mg, 0.10 mmol) was added and stirring was continued in an ice-water bath after the addition was complete. After about 1 h, the raw material disappeared by HPLC detection. The product was directly prepared and purified. The prepared solution was lyophilized to obtain 53.5 mg of white solid compound **44a** with a yield of 51.8%; LC-MS $[M+H]^+$: m/z 1032.5.

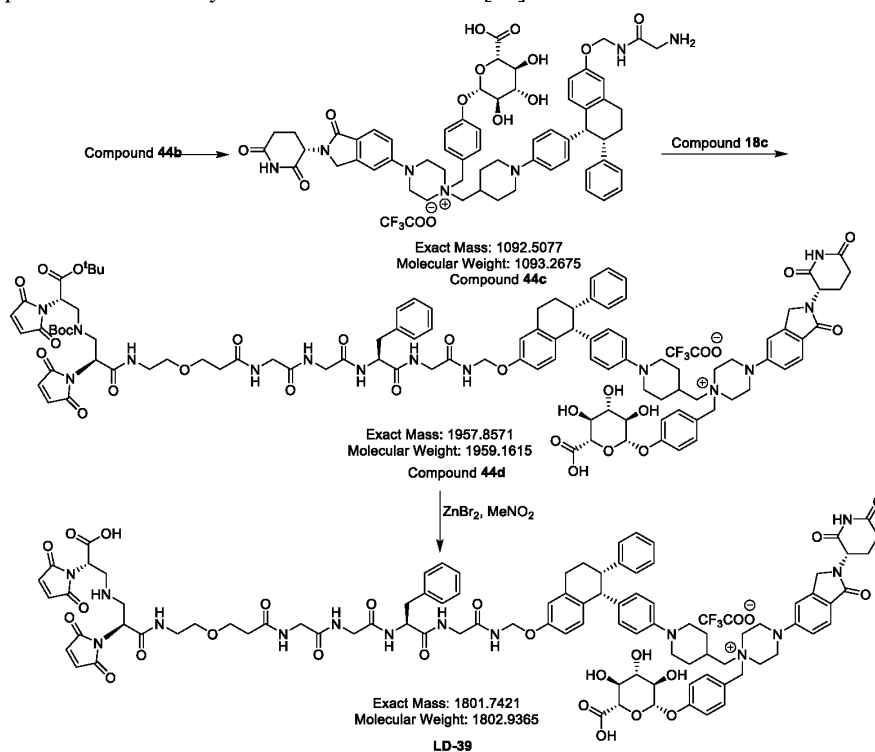
10 Step 2: Compound M11

Compound **M10** (300.2 mg, 682 μ mol, prepared by the method of reference DOI: 10.1021/jm990124q) and 15 mL of anhydrous dichloromethane were added to a 25 mL single-necked bottle and cooled in an ice-water bath. TEA (190 μ L, 1.36 mmol) and MsCl (125 μ L) were added in sequence. The reaction was stirred at room temperature for 1 h. The reaction was completed under HPLC monitoring. The solvent was removed by concentration under reduced pressure, purified by preparative liquid phase, and lyophilized to obtain 142.1 mg of oily compound **M11** with a yield of 40.2%. LC-MS $[M-OMs+H]^+$: m/z 423.4.

Step 3: Compound 44b

20 Compound **44a** (53.5 mg, 51.8 μ mol), compound **M11** (27.2 mg, 51.8 μ mol) and butanone (10 mL) were added to a 25 mL single-necked bottle. After stirring to dissolve, the temperature was raised to 60 °C for 13 h. The reaction of the raw materials was monitored

by HPLC. The reaction solution was directly dried under reduced pressure at 40 °C to obtain a crude product. The crude product was prepared and purified to obtain 37.6 mg of white solid compound **44b** with a yield of 49.9%. LC-MS [M]⁺: m/z 1455.0.



Step 4: Compound **44c**

Compound **44b** (37.6 mg, 25.8 μ mol) and DMF (4 mL) were added to a 25 mL single-necked bottle, and diethylamine (1 mL) was added at room temperature. The reaction was maintained at room temperature for 1 h. After the reaction was completed under HPLC monitoring, the crude product was concentrated under reduced pressure by an oil pump and prepared and purified to obtain 18.6 mg of solid compound **44c** with a yield of 65.9%. LC-MS [M]⁺: m/z 1092.6.

Step 5: Compound **44d**

Compound **44c** (18.6 mg, 17 μ mol) and DMF (5 mL) were added to a 25 mL single-necked bottle, and then compound **18c** (16.0 mg, 17 μ mol) was added. PyBOP (18.4 mg, 35 μ mol), HOBt (5.3 mg, 35 μ mol) and DIPEA (12 μ L) were added in sequence at room temperature. The reaction was allowed to proceed for 6 h at room temperature. After the reaction was completed under HPLC monitoring, the crude product was concentrated under reduced pressure by an oil pump and prepared and purified to obtain 16.4 mg of solid compound **44d** with a yield of 49.2%. LC-MS [M/2]⁺: m/z 979.3.

Step 6: **LD-39**

Compound **44d** (16.4 mg, 8.4 μ mol) and dichloromethane (3 mL) were added to a 10 mL single-necked bottle, and TFA (1 mL) was added at room temperature. The reaction of the raw materials was monitored by HPLC. The reaction solution was directly concentrated

under reduced pressure at 40 °C to obtain a crude product. The crude product was prepared and purified to obtain 5.9 mg of a white hygroscopic solid **LD-39** with a yield of 39%. LC-MS [M/2]⁺: m/z 901.2.

Example 43: Expression and purification of anti-TROP2 antibody:

Expi293 (Shanghai OPM Biotech Co., Ltd.) suspension cells were used to express anti-TROP2 antibodies (also known as TROP2 antibodies herein). One day before transfection, cells were inoculated at a density of 0.9×10^6 cells/mL in a 1 L shake flask containing 300 mL OPM-293 CD05 Medium (81075-001, Shanghai OPM Biotech Co., Ltd.) and cultured overnight at 37°C, 5% CO₂, and 120 rpm in a cell culture shaker. The next day, PEI-MAX
10 was used to transfect the antibody expression plasmid, where the mass ratio of plasmid: PEI-MAX was 1:3. OPM-293 ProFeed was added at 5% (v/v) on the first day after transfection, and OPM-293 ProFeed was added again at 5% (v/v) on the third day after transfection. The supernatant was collected by centrifugation on the sixth day after transfection.

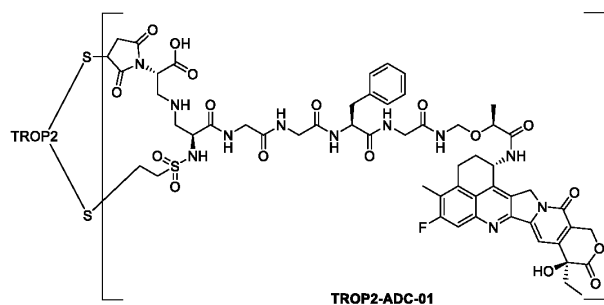
The cell expression supernatant was collected and passed through a Protein A affinity chromatography column (UniMab 50, Suzhou Nanovita Technology Co., Ltd.), eluted with 0.05 M sodium acetate (pH 3.6), and the captured antibody was adjusted to pH 7.0 with 1 M Tris-HCl (pH 8.8).

The amino acid sequences of the heavy chain (HC, SEQ ID NO: 17) and the light chain (LC, SEQ ID NO: 18), HC variable region (VH, SEQ ID NO: 13), and LC variable region (VL, SEQ ID NO: 14) of the anti-TROP2 antibody are listed in Sequence Listing. The amino acid
20 sequences of complementarity determining regions (CDRs) were underlined in SEQ ID NO: 13, 14 and listed as SEQ ID NO: 1-6 for CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDRL2, and CDRL3, respectively.

Example 44: Preparation of ligand-drug conjugates

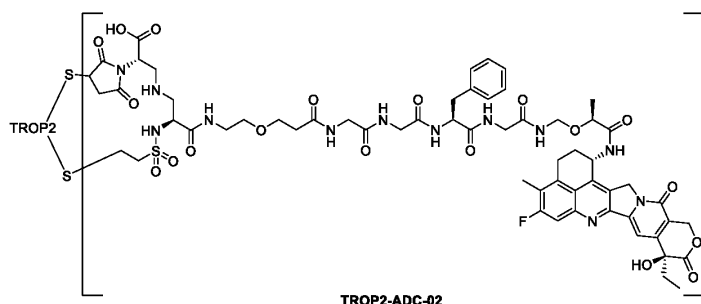
The anti-TROP2 antibody-drug conjugates were prepared by coupling anti-TROP2 antibody with linker-drug compounds. The anti-TROP2 antibody was replaced in 20 mM NaAc-Hac (pH 6.0) buffer and the protein concentration was adjusted to 5 mg/mL. A 25-fold equivalent of TCEP was added and the reaction was carried out at room temperature for 2 h to open the interchain disulfide bonds of the antibody. Then, a 20-fold equivalent of linker-drug
30 compound was added and reacted for 2 h at room temperature to couple the linker-drug compound with the antibody. Finally, ultrafiltration was performed using an ultrafiltration centrifuge tube to remove the linker-drug compound that was not coupled to the antibody. The samples of antibody-drug conjugate between anti-TROP2 antibody and linker-drug compound (TROP2-ADC) were obtained.

Example 45:



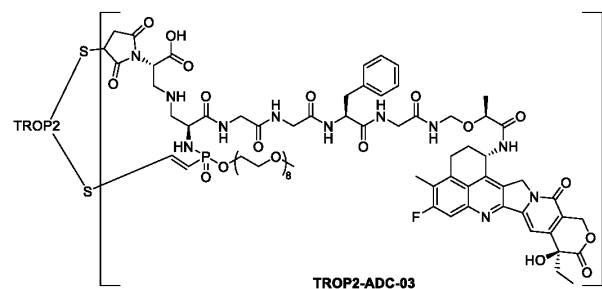
The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 46:



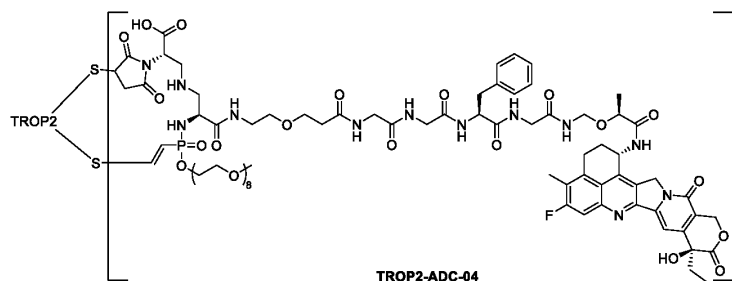
The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 47:

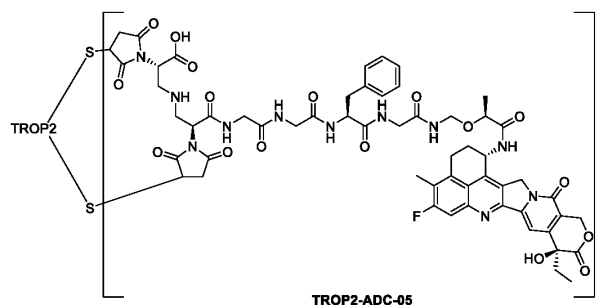


10 The ADC of this example was prepared according to the universal preparation method of
ligand-drug conjugate in Example 44.

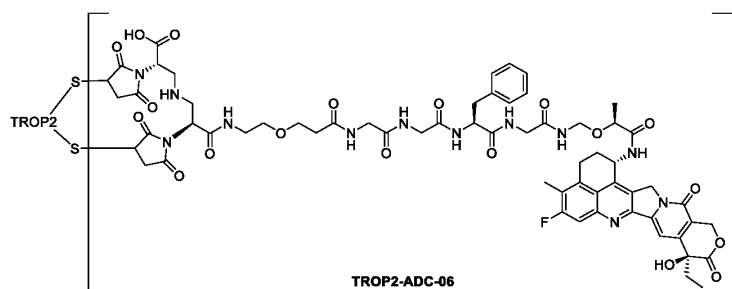
Example 48:



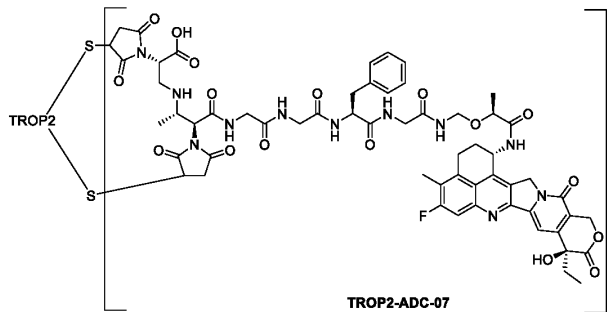
The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 49:

The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

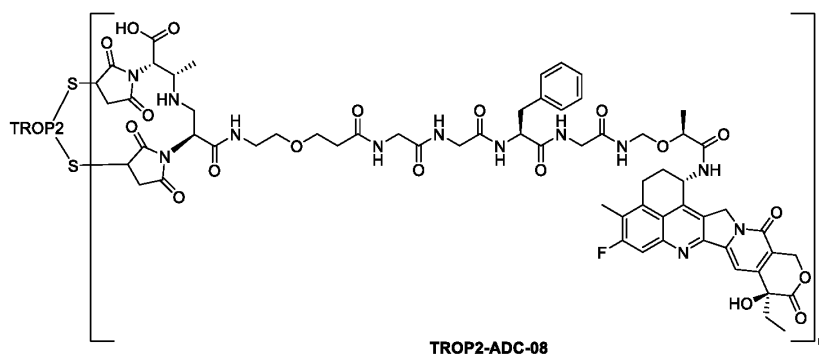
Example 50:

The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 51:

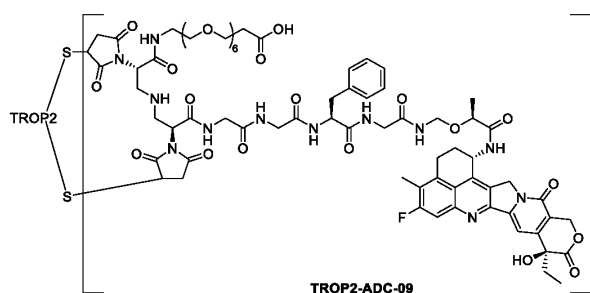
The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 52:



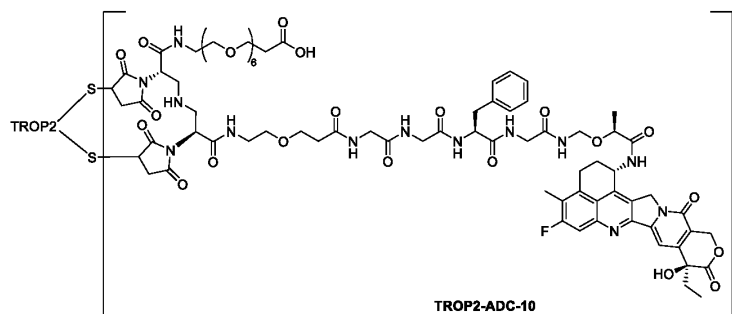
The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 53:



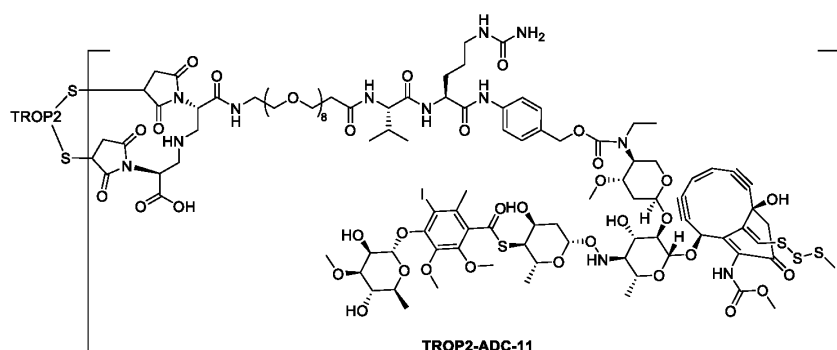
The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 54:



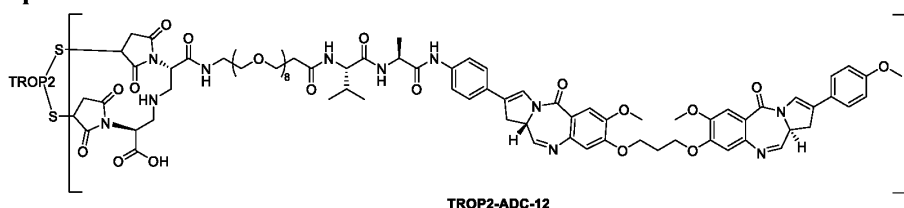
10 The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 55:



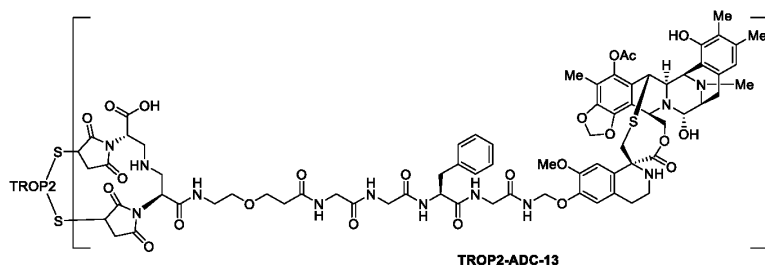
The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 56:



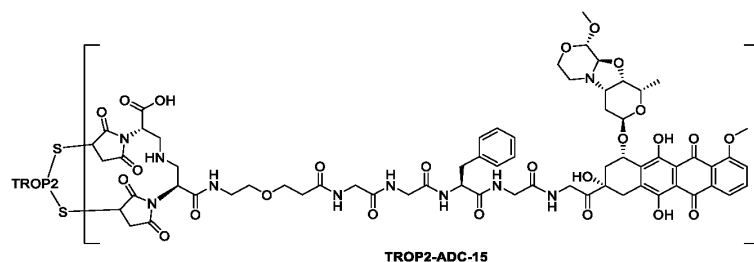
The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 57:



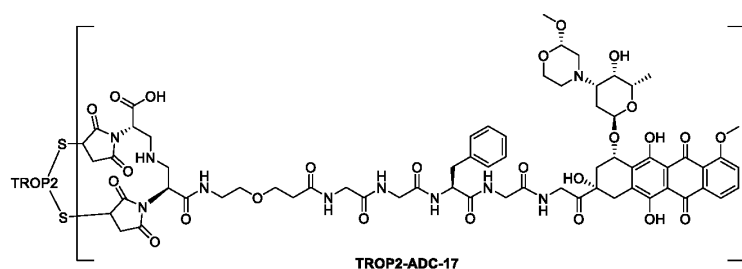
10 The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 58:



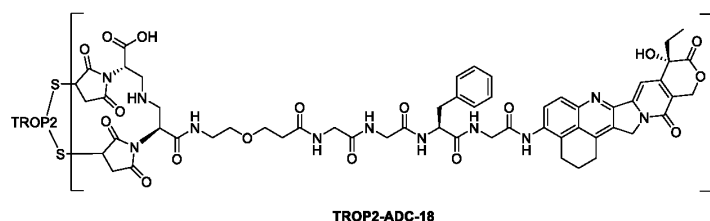
The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 59:



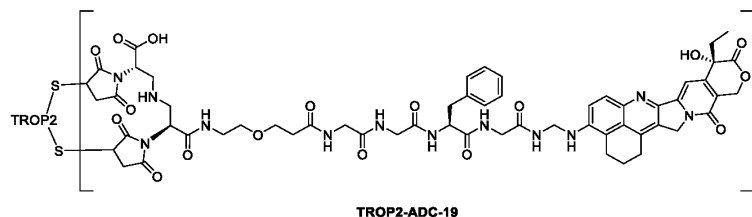
The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 60:



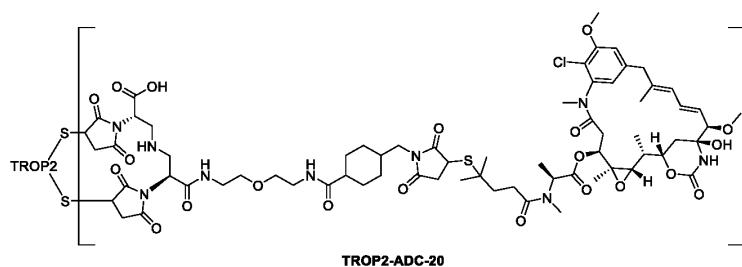
The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 61:



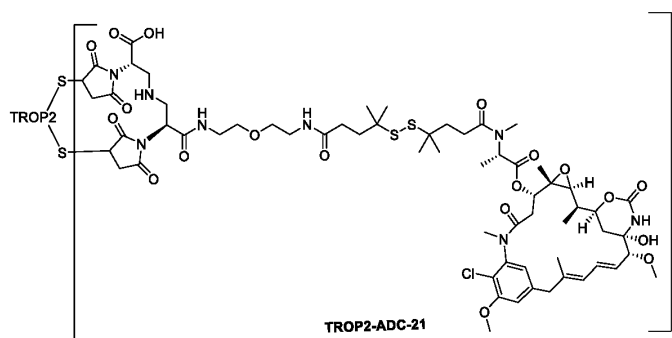
10 The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 62:



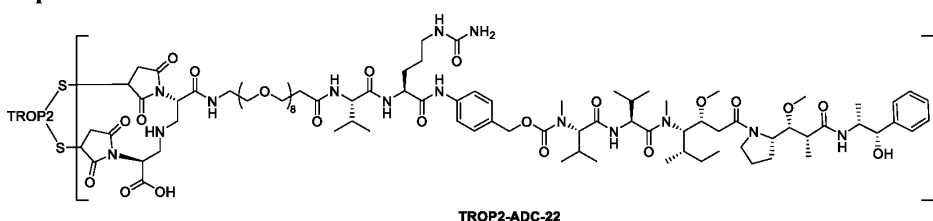
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Example 63:



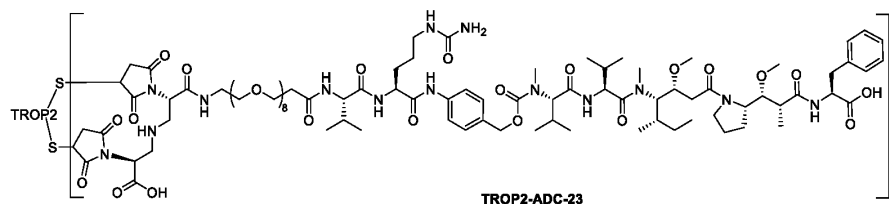
The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 64:



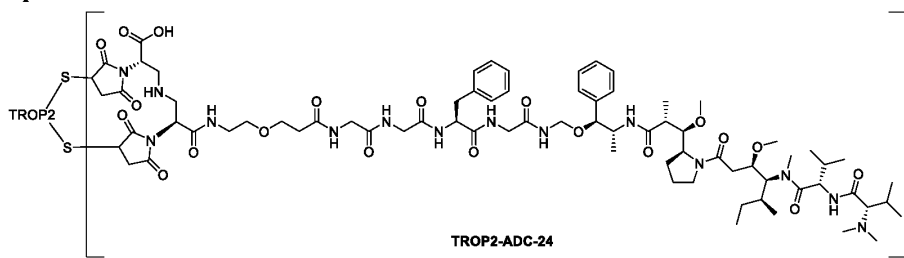
The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 65:



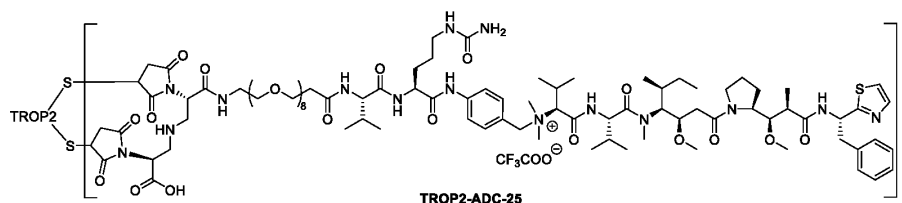
10 The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 66:



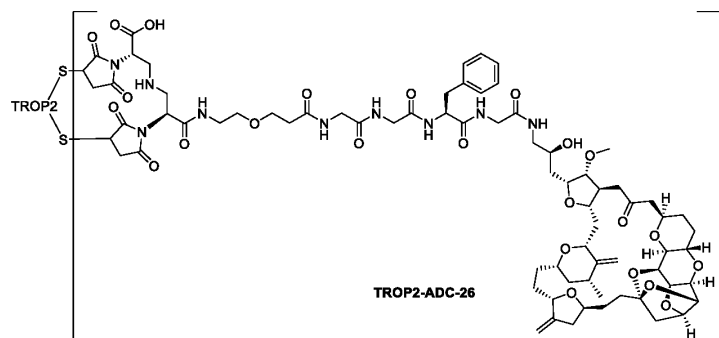
The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 67:



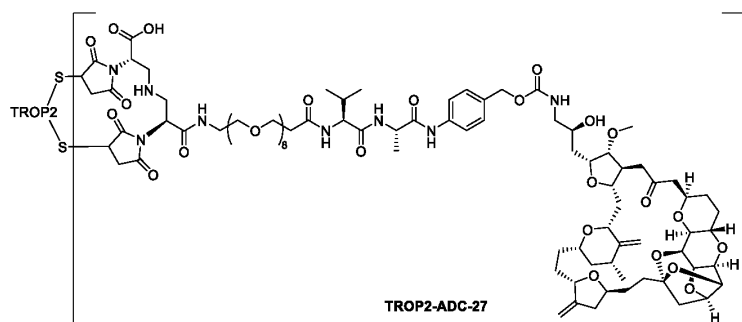
The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 68:



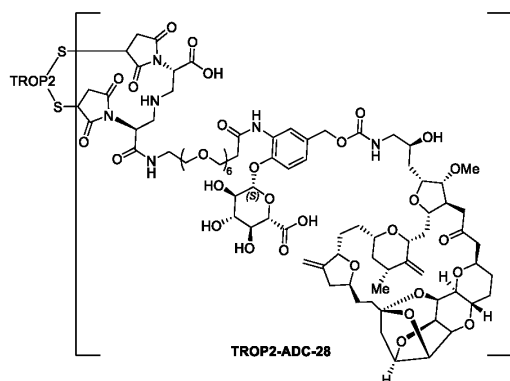
The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 69:



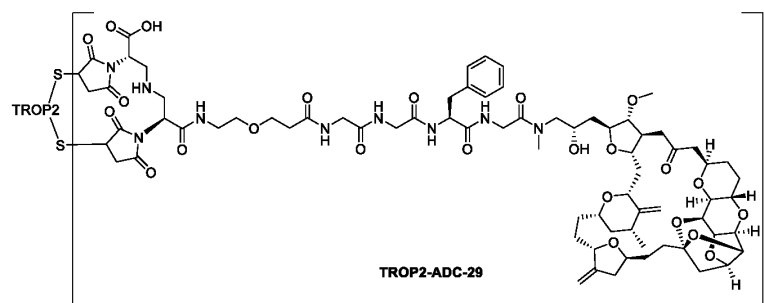
10 The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 70:



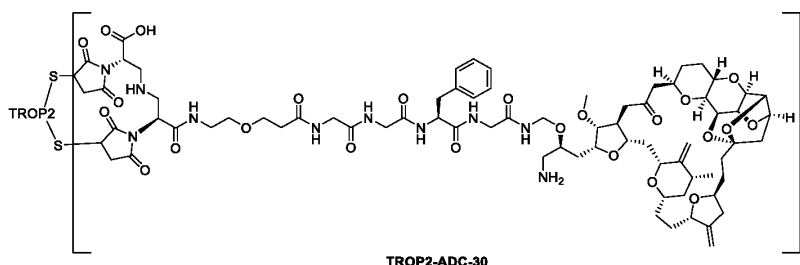
The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 71:



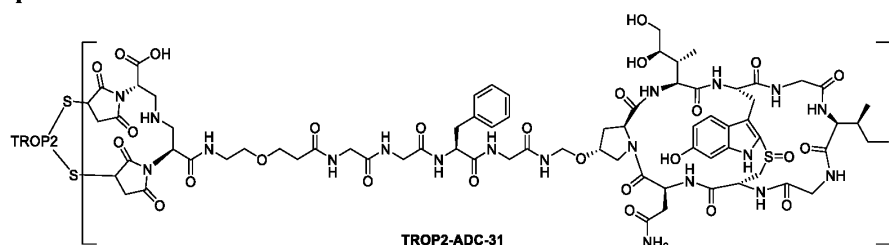
The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 72:



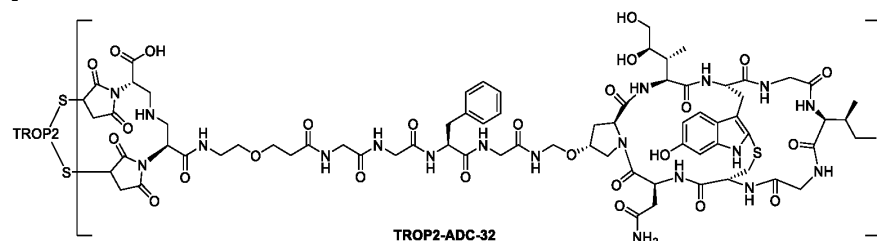
The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 73:

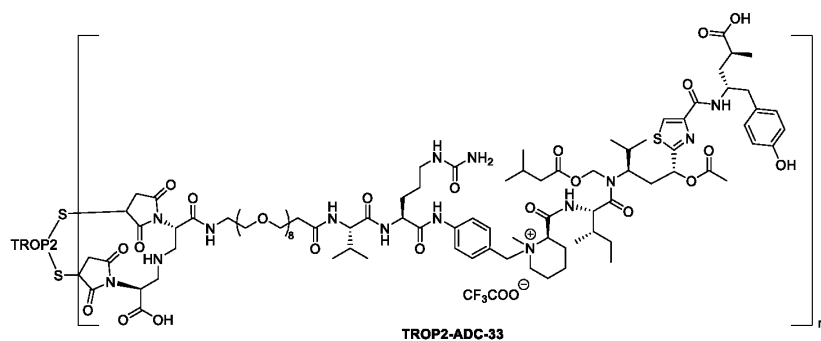


The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

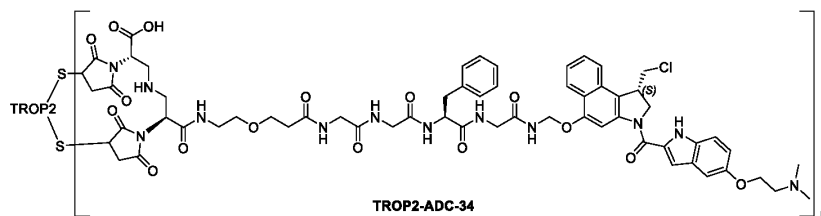
Example 74:



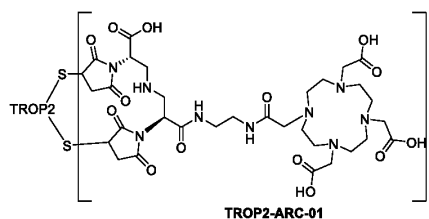
The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 75:

The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

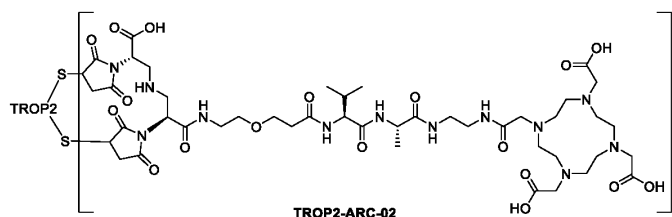
Example 76:

The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 77:

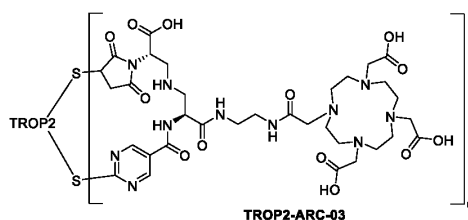
10

The ARC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 78:

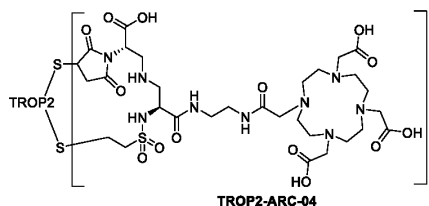
The ARC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 79:



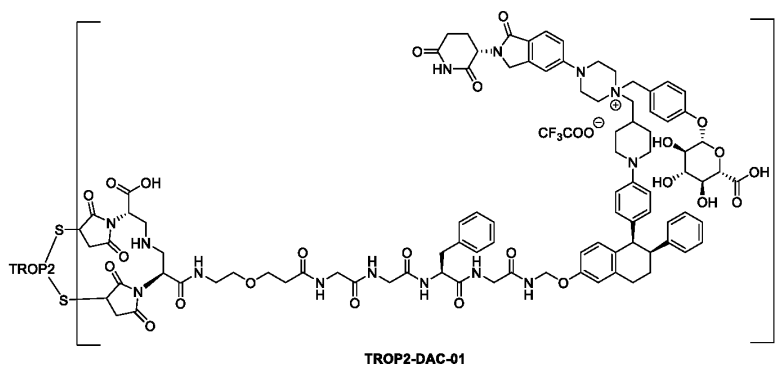
The ARC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 80:



The ARC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 81:

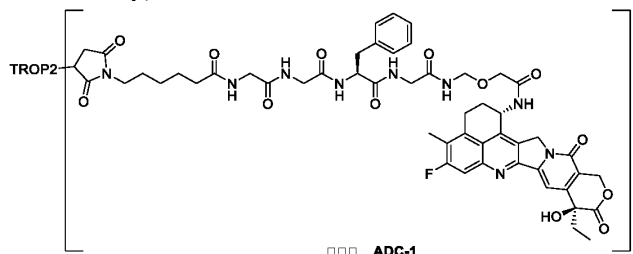


10 wherein TROP2 is an antibody targeting TROP2.

The ADC of this example was prepared according to the universal preparation method of ligand-drug conjugate in Example 44.

Example 82: Control ADC-1

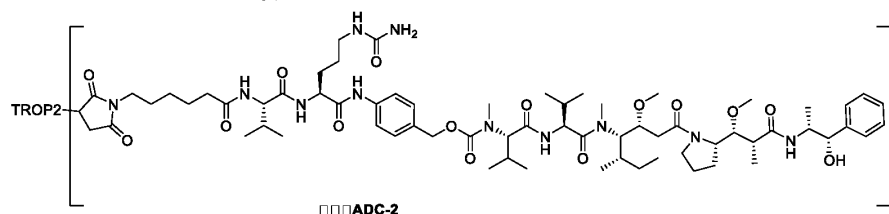
Control **ADC-1** was prepared according to the method of WO2015098099A1 (incorporated herein by reference in its entirety).



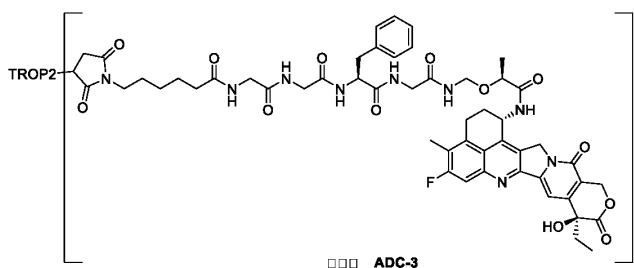
wherein TROP2 is an antibody targeting TROP2.

Example 83: Control ADC-2

Control **ADC-2** was prepared according to the method of WO2015074528A1 (incorporated herein by reference in its entirety).

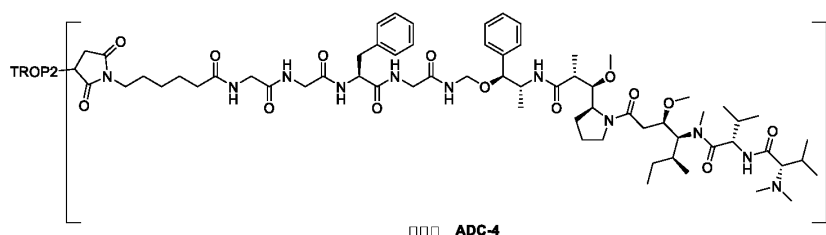


Example 84: control ADC-3



Compound **6b** was condensed with the MC linker to prepare the Linker-Drug of the control ADC, ADC-3 (see **Example 82** for the method of preparation).

Example 85: Control ADC-4



10 Compound **29a** was condensed with MC-Gly-Gly-Phe linker to prepare Linker-Drug of the control ADC, **ADC-4** (see **Example 83** for the method of preparation).

Example 86: DAR values

To determine DAR values, HIC-HPLC method was used:

Column name: Sepax ME-HIC

Manufacturer: Sepax Item No.: 535335-4603

Table 1. Methodological parameters

Parameters	Set Up
Flow Rates	0.8 mL/Min
Wavelength	214 nm and 280 nm
Column Temperature	25 °C
Sample Plate Temperature	Indoor Temperature
Mobile Phase	A: 1.5 M (NH ₄) ₂ SO ₄ + 0.025 M Na ₃ PO ₄ Ph 7.0 B: 0.025 M Na ₃ PO ₄ + 25% IPA Ph 7.0

Injection Volume	25 µg					
Gradient	T (min)	Flow rates (mL/min)	A (%)	B (%)	C (%)	D (%)
	0	0.8	100	0	0	0
	2	0.8	100	0	0	0
	15	0.8	0	100	0	0
	20	0.8	0	100	0	0
	21	0.8	100	0	0	0
	30	0.8	100	0	0	0

The result shows the distribution of DAR values of ADC groups (**Table 2**).

Table 2. DAR distribution of antibody-drug conjugates

ADC	mAb-0	mAb-1	mAb-2	mAb-3	mAb-4	mAb-5	mAb-6	mAb-7	mAb-8	mAb-9	mAb-10	Average DAR
TROP2-ADC-06	4.05	0.00	5.71	0.00	88.22	0.00	2.02	0.00	0.00	0.00	0.00	3.76
ADC-1	21.14	0.00	22.11	0.00	22.02	0.00	20.56	0.00	14.17	0.00	0.00	3.69
ADC-3	11.28	0.00	19.14	0.00	39.96	0.00	22.04	0.00	7.58	0.00	0.00	3.91
TROP2-ADC-24	0.42	0.00	10.19	0.00	85.45	0.00	2.11	0.00	1.83	0.00	0.00	3.89
ADC-2	5.57	0.00	23.82	0.00	36.05	0.00	23.49	0.00	11.07	0.00	0.00	4.21
ADC-4	6.22	0.00	37.56	0.00	30.56	0.00	18.73	0.00	6.93	0.00	0.00	3.65

Conclusion: the distribution of DAR values of the antibody-drug conjugates disclosed in the present application is more concentrated than that of control ADCs, and the proportion of ADC with DAR value 4 is higher than that of control ADCs. This result unveils the excellent property of uniform DAR values of the ADCs.

Example 87: Hydrolysis of ADCs

The antibody-drug conjugates were analyzed by mass spectrometry to determine the hydrolysis of the antibody-drug conjugate linker. The succinimide linker in the antibody-drug conjugate of this application exists in the form of partial ring opening or even complete ring opening.

As shown in **Table 3**, the actual molecular weight of the antibody-drug conjugates was 147.02 Da for TROP2-ADC-06 and 145.11 Da for TROP2-ADC-24, which are higher than the theoretical molecular weight, indicating that the succinimidyl linker is in the form of a fully open ring.

Table 3. Molecular weight of ADCs before and after hydrolysis

ADC	Theoretical molecular weight (Da)	Actual molecular weight (Da)
Antibody	145175.23	/
TROP2-ADC-06	150385.07	150532.09
TROP2-ADC-24	151282.43	151427.54

Example 88. Monomer rate of ADCs by SEC-HPLC

Column: Biocore SEC-300 5 μ m, 4.6 $\times$ 300 mm;

Manufacturer: NanoChrom, Item No.: B213-050030-04630S;

Mobile phase: 50 mM PB+300 mM NaCl+200 mM Arg+5%IPA, pH =6.5.

Table 4. Methodological parameters

Parameters	Set up
Flow Rates	0.3 mL/min
Wavelength	214 nm and 280 nm
Column Temperature	30°C
Sample Plate Temperature	Indoor temperature
Injection Volume	20 μ g
Maximum Pressure	150 bar/15MPa/2175PSI
Gradient	Equal degree
Running Time	20 minutes.

The result shows the stability of ADCs as measured by their monomer rates (**Table 5**)

Table 5. Antibody-drug coupler monomer rate data

ADC	ADC Lot Release					
	BCA concentration	Nanodrop concentration	SEC %			Free Drug
	mg/ml	mg/ml	HMW	POI	LMW	% mol/mol
TROP2-ADC-06	4.50	4.08	1.47	97.46	1.07	Less than 0.074
TROP2-ADC-24	4.10	4.08	0.76	98.12	1.12	Less than 0.092

Therefore, the antibody-drug conjugates as disclosed herein have excellent properties of low aggregation rate, low degradation rate and high monomer rate. In the meantime, the antibody-drug conjugates have low content of small molecule toxins, indicating that the antibody-drug conjugates have high stability.

Example 89: Plasma stability of ADCs

Each ADC sample was aseptically diluted to a final drug concentration of 0.6 mg/mL and incubated in a 37°C water bath for 0, 3, and 7 days, respectively. The sample and control ADCs were purified and extracted, and their DAR values were determined.

Results: As compared to their controls, TROP2-ADC-06 and TROP2-ADC-24 showed no significant decrease in DAR value after incubation in plasma for 7 days (**Table 6**),

Table 6. Plasma stability of antibody-drug conjugates

ADC Sample	DAR value		
	37°C 0d	37°C 3d	37°C 7d
TROP2-ADC-06	3.75	3.73	3.72
ADC-3 (control)	3.97	3.24	2.86
ADC-1 (control)	4.20	3.47	3.00
TROP2-ADC-24	3.77	3.74	3.72

ADC-4 (control)	3.60	2.90	2.45
ADC-2 (control)	3.91	3.52	3.03

Therefore, the antibody-drug conjugates disclosed herein have excellent stability in plasma.

Example 90: *In vitro* pharmacodynamics of ADCs

1. Experimental materials: see **Table 7**.

Table 7: Cell lines and the level of TROP2 expression

Cell Line	Cancer Type	Source	TROP2 Expression per Cell
A431	Human squamous skin cancer	ATCC	988,598
Bxpc-3	Human pancreatic cancer	ATCC	812,566
Fadu	Squamous carcinoma of human pharynx	Shanghai Cell Bank, CAS	712,558
Hcc827	Human non-small cell lung cancer	Shanghai Cell Bank, CAS	334,314
Sw620-Luc #B8c	Monoclonal stable transducer of human colorectal cancer overexpressing luciferase	In-house	Undetectable

2. Preparation of culture media: Assay medium: RPMI-1640 medium + 10% FBS + 100 U/mL Penicillin/Streptomycin; Basal medium: Genpex Bio; FBS: ExCell Bio;

3. Testing reagents: Cell Titer-Glo Luminescent Cell Viability Assay (CTG): Promega, Item G7571; Bio-Lite Luciferase Assay System (Bio-Lite): Vazyme, Catalog No. DD1201-02

4. Experimental methodology

10 An appropriate number of tumor cells were evenly seeded into a 96-well plate. After 24 hours of incubation, the cells were treated with ADC sample drugs in the detection medium. The starting concentration of each ADC was 500 nM, and the dilution factor was 7 times for a total of 8 concentration points. After mixing, the sample drug was added to the corresponding cell wells at 100 μ L/well. The last two wells were designated for having the control group, i.e., cells plus culture medium but no drug treatment, and the blank group, i.e., only culture medium but no cells. The plate was placed for incubation at 37°C for 5 days.

20 For the monomeric tumor cell model, CTG high sensitivity chemiluminescence detection technology was used to quantify adenosine triphosphate (ATP) in living cells for calculating the cell survival rate. At the completion of cell incubation, 100 μ L CTG (Promega, G7571) was added to each well and kept in the dark for 10 minutes of the reaction that fully lyses the test cells. After the reaction was completed, the chemiluminescence intensity (Relative Luminescence, RLU) was measured according to the formula: survival rate (%) = (experimental group - blank group) / (control group - blank group) $\times$ 100%, and the cell survival rate was calculated, and then Graphpad Prism four-parameter fitting curve was applied to

calculate IC₅₀ that evaluates the killing effect of the test antibody-drug conjugate on tumor cells.

For the heterogeneous tumor cell model, since the TROP2-negative cell SW620 stably expresses the luciferase (Luciferase) reporter gene, the highly sensitive Bio-Lite Luciferase Assay System can be used to quantitatively detect Luciferase in living cells to calculate the survival rate of TROP2-negative cell SW620. After the drug incubation is completed, 100 μ L Bio-Lite (Vazyme, DD1201-02) was added to each well and incubated for 10 minutes to cause cell lysis to release luciferase and fully react with Luciferin in the detection reagent to emit a stable light signal. The chemiluminescence intensity (Relative Luminescence, RLU), and the survival rate of TROP2-negative SW620 cells in the heterogeneous tumor model was calculated according to the formula: survival rate (%) = (experimental group-blank group)/(control group-blank group) $\times$ 100%. Then, Graphpad Prism four-parameter fitting curve was used to calculate IC₅₀ for evaluating the killing effect of ADC drugs on negative tumor cells through bystander effect.

5. The results of TROP2-ADC-06 treatment are shown in **Table 8**.

Table 8. Killing effect of antibody-drug conjugate on tumor cells

TROP2-ADC-06 Group	A431		BxPC-3		Fadu		HCC827		A431+SW620- Luc #B8C	
	IC50 (nM)	Efficacy (%)	IC50 (nM)	Efficacy (%)	IC50 (nM)	Efficacy (%)	IC50 (nM)	Efficacy (%)	IC50 (nM)	Efficacy (%)
TROP2-ADC-06	8.76	69.64	9.38	74.14	4.55	97.00	7.64	67.78	9.38	74.14
Control ADC-3	24.60	70.35	29.22	75.16	11.15	97.56	19.42	68.46	29.22	75.16
Control ADC-1	279.34	66.02	176.53	64.45	13.84	96.84	56.76	68.28	176.53	64.45

As shown in **Table 8**, and **Figure 2**, the *in vitro* killing effect of TROP2-ADC-06, as measured by IC₅₀, was superior to that of the control ADC-1 and control ADC-3 on TROP2-expressing cancer cells, including the monomeric cell model of A431, BxPC-3, Fadu, and HCC827, as well as the heterogeneous tumor model of A431 plus SW620-Luc #B8C. Since the TROP2 expression is undetectable in SW620-Luc #B8C cells, the killing effect in this heterogeneous tumor model is a bystander effect of paracrine signals. In this context, the bystander effect of TROP2-ADC-06 is superior to control ADC-1 and control ADC-3.

6. The results of TROP2-ADC-24 treatment are shown in **Table 9**.

Table 9: Killing effect of antibody-coupled drugs and toxins on each tumor cell

TROP2-ADC-24 Group	A431		BxPC-3		Fadu		SW620-Luc #B8C		A431+SW620- Luc #B8C	
	IC50 (nM)	Efficacy (%)	IC50 (nM)	Efficacy (%)	IC50 (nM)	Efficacy (%)	IC50 (nM)	Efficacy (%)	IC50 (nM)	Efficacy (%)
TROP2-ADC-24	0.12	90.96	0.16	91.74	0.87	86.87	>100	8.98	3.56	68.56

As shown in **Table 9**, and **Figure 3**, under the experimental conditions, TROP2-ADC-24 shows excellent killing activity *in vitro* among the TROP2 positive cell models (A431, BxPC-3 and Fadu); in the TROP2-negative cell model (SW620-Luc #B8C), TROP2-ADC-24 has no obvious killing effect in the dose range, and the ADC drug disclosed in this application has excellent safety; in the heterogeneous tumor model (A431+SW620-Luc #B8C), TROP2-ADC-24 can kill negative cells by bystander effect, and the ADC disclosed in this application has excellent killing activity.

Example 91: *In vivo* efficacy of ADCs in a mouse model of human A431 cancer cells

To evaluate the *in vivo* efficacy of antibody-drug conjugates, a subcutaneous xenograft model of human epidermal carcinoma cells A431 was established in *BALB/c-mu* mice. The A431 cell suspension (1.3×10^6 cells in 0.1 mL) were injected subcutaneously into the right scapula of 6~7-week-old *BALB/c-mu* mice. As the tumor grew, the mice were randomly divided into 13 groups, namely, a lysate control group (Vehicle) and 12 dosing groups, and each group requires 6 mice. The 12 dosing groups were designated for administering TROP2-ADC-06 and its controls (i.e., control ADC-3 and control ADC-1), and TROP2-ADC-24 and its controls (i.e., control ADC-4 and control ADC-2) at a low and high dose. When the average tumor volume reached about 250 mm³, D0 administration started. Each group of mice was administered by tail vein injection at 10 mL/kg body weight every 7 days (QW) for 3 consecutive cycles, and all groups were observed up to 21 days. Among them, TROP2-ADC-06, control ADC-3 and control ADC-1 were 3 mg/Kg in the low-dose group and 6 mg/kg in the high-dose group; TROP2-ADC-24, control ADC-4 and control ADC-2 were 1.5 mg/Kg in the low dose group and 3 mg/Kg in the high dose group.

At the end of the dosing cycle, the inhibitory effect of ADC drugs on tumor growth was evaluated by a two-factor statistical analysis of the mean tumor volume in each treatment group over 21 days (**Figure 4A**). The results showed that, the anti-tumor effect of the TROP2-ADC-06 (low and high doses) treatment group was excellent, and the anti-tumor effect was significantly better than that of the control ADC-1 (low and high doses) treatment group at the same dose ($P < 0.0001$, $P < 0.0001$).

At the end of the dosing cycle, a one-way statistical analysis of the mean tumor volume on day 21 (D21) for each treatment group (see **Table 10**). The results showed that the average tumor volume of TROP2-ADC-06 (low dose, high dose) treatment group was significantly lower than that of Vehicle control group. The mean tumor volume was significantly reduced in the TROP2-ADC-06 (low dose) treatment group compared to the control ADC-3 (low dose) treatment group at the same dose ($P = 0.0217$), and in the TROP2-ADC-06 (high dose) treatment group compared to the control ADC-3 (high dose) treatment group at the same dose (see **Table 10**). Meanwhile, the mean tumor volume was significantly reduced in the TROP2-ADC-06 (low or high dose) treatment group as compared to the control ADC-1 (low or high dose) treatment group ($P = 0.0009$, $P = 0.0012$).

At the end of the dosing cycle, the inhibitory effect of ADC drugs on tumor growth was evaluated by a two-factor statistical analysis of the mean tumor volume in each treatment group over 21 days. At the same doses, the tumor suppressive effect was significantly better in the TROP2-ADC-24 (low and high doses) treatment group than in the control ADC-4 (low and high doses) treatment group ($P<0.0001$, $P=0.0042$), and the tumor suppressive effect was significantly better in the control ADC-2 (low and high doses) treatment group ($P<0.0001$, $P<0.0001$) (see **Figure 4B**).

At the end of the dosing cycle, a one-way statistical analysis of the mean tumor volume on day 21 (D21) for each treatment group showed (see **Table 10**). At the same dose, the mean tumor volume was reduced in the TROP2-ADC-24 (low and high doses) treatment group compared to the control ADC-4 (low and high dose) treatment group, while the mean tumor volume was significantly reduced in the TROP2-ADC-24 (low and high doses) treatment group compared to the control ADC-2 (low and high dose) treatment group ($P<0.0001$, $P=0.0056$).

Table 10. Pharmacodynamic analysis of ADCs in the subcutaneous xenograft model of human epidermal cancer cell, A431 in *BALB/c-mu* mice

Experimental Group		D21			
		Mean tumor volume mm ³	P-value	T/C (%)	TGI (%)
G1	Vehicle	1726.35±198.79	-	-	-
G2	TROP2-ADC-06 (low dose, QW*3, i.v.)	777.15±102.20	0.0217 (G2 vs. G4) 0.0009 (G2 vs. G6)	45.71	54.29
G3	TROP2-ADC-06 (high dose, QW*3, i.v.)	287.38±41.51	0.5343 (G3 vs. G5) 0.0012 (G3 vs. G7)	16.84	83.16
G4	Control ADC-3 (low dose, QW*3, i.v.)	1475.05±146.06	-	86.17	13.83
G5	Control ADC-3 (high dose, QW*3, i.v.)	661.00±63.65	-	38.60	61.40
G6	Control ADC-1 (low dose, QW*3, i.v.)	1701.14±287.49	-	99.19	0.81
G7	Control ADC-1 (high dose, QW*3, i.v.)	1193.59±99.77	-	69.12	30.88
G8	TROP2-ADC-24 (low dose, QW*3, i.v.)	284.56±41.12	0.9688 (G8 vs. G10) <0.0001 (G8 vs. G12)	16.54	83.46
G9	TROP-ADC-24 (high dose, QW*3, i.v.)	278.82±18.58	0.9969 (G9 vs. G11) 0.0056 (G9 vs. G13)	16.49	83.51
G10	Control ADC-4 (low dose, QW*3, i.v.)	450.88±83.50	-	26.17	73.83
G11	Control ADC-4 (high dose, QW*3, i.v.)	385.66±41.56	-	22.73	77.27
G12	Control ADC-2	1718.97±256.80	-	100.58	-0.58

	(low dose, QW*3, i.v.)				
G13	Control ADC-2 (high dose, QW*3, i.v.)	995.54±192.34	-	57.65	42.35

Note:

1. Mean tumor volume MTV is expressed as "mean± standard error;"
2. Relative tumor suppression rate $TGI\% = (1-T/C) \times 100\%$, $T/C\% = T_{RTV} / C_{RTV} \times 100\%$;
3. *P* value was obtained by comparing tumor volume at D21 between the combination therapy group and the monotherapy group, and was considered statistically significant at $P < 0.05$,

* denotes $P < 0.05$, ** denotes $P < 0.01$, and *** denotes $P < 0.001$;

Therefore, based on the statistical analyses of pharmacodynamic data from the subcutaneous graft tumor model of human epidermal carcinoma cell A431 in *BALB/c-mu* mice, TROP2-ADC-06 showed significantly better efficacy relative to both control ADC-3 and control ADC-1; in comparison, TROP2-ADC-24 showed significant better efficacy relative to control ADC-2 and control ADC-4.

Example 92: *In vivo* efficacy of ADC in a mouse model of mixed human cancer cells

To evaluate the *in vivo* efficacy of ADC drug candidates killing heterogeneous tumors, a subcutaneous xenograft model of TROP2-positive human epidermoid carcinoma cells A431 and TROP2-negative human colon carcinoma cells SW620 was established in *BALB/c-mu* mice. A mixture of A431 (0.5×10^6) and SW620 (1×10^6) cells in 0.1 mL was injected subcutaneously into the right scapula of *BALB/c-mu* mice at 6~7 weeks of mouse age. As the tumor grew, the mice were randomly divided into 7 groups, namely, a lysate control group (Vehicle) and 6 dosing groups, and each group requires 6 mice. The 6 dosing groups were designated for administering low dose and high dose TROP2-ADC-06 (3 mg/Kg in low dose group and 6 mg/Kg in high dose group) and its controls (i.e., control ADC-3 and control ADC-1). When the average tumor volume reached about 169 mm³, D0 administration started. Each group of mice was administered by tail vein injection at 10 mL/kg body weight every 7 days (QW) for 4 consecutive cycles, and all groups were observed up to 28 days to evaluate the inhibitory effect of ADC drugs on tumor growth.

At the end of the dosing cycle, a two-way statistical analysis of the mean tumor volume in each treatment group over 28 days (**Figure 5**). The TROP2-ADC-06 (low dose) treatment group showed excellent anti-tumor effect, and was significantly better than that of control ADC-3 (low dose) treatment group at the same dose ($P < 0.0001$); and the tumor suppression effect of TROP2-ADC-06 (high dose) treatment group was significantly better than that of control ADC-3 (high dose) and control ADC-1 (high dose) treatment groups at the same dose ($P = 0.0002$, $P < 0.0001$).

At the end of the dosing cycle, a one-way statistical analysis of the mean tumor volume at day 28 (D28) for each treatment group showed (see **Table 11**). The mean tumor volume was significantly reduced in the TROP2-ADC-06 (low dose) treatment group compared to the control ADC-3 (low dose) treatment group ($P=0.0155$), and compared to the control ADC-1 (low dose) treatment group at the same dose; the mean tumor volume of the TROP2-ADC-06 (high dose) treatment group was significantly reduced compared to control ADC-3 (high dose) at the same dose ($P=0.4467$), and the mean tumor volume was significantly reduced than that of control ADC-1 (high dose) treatment group at the same dose ($P=0.006$).

Table 11. Pharmacodynamic analysis of heterogeneous tumors of A431 and SW620 in a mouse xenograft model of human cancer cells

Experimental Group		D28			
		Mean tumor volume mm ³	P-value	T/C (%)	TGI (%)
G1	Vehicle	1450.70±111.69	-	-	-
G2	TROP2-ADC-06 (low dose, QW*4, i.v.)	422.26±118.47	0.0155 (G2 vs. G4) 0.7353 (G2 vs. G6)	28.81	71.19
G3	TROP2-ADC-06 (high dose, QW*4, i.v.)	85.13±28.89	0.4467 (G3 vs. G5) 0.006 (G3 vs. G7)	5.82	94.18
G4	Control ADC-3 (low dose, QW*4, i.v.)	1192.95±260.01	-	82.67	17.33
G5	Control ADC-3 (high dose, QW*4, i.v.)	508.56±67.67	-	35.29	64.71
G6	Control ADC-1 (low dose, QW*4, i.v.)	746.39±72.39	-	51.69	48.31
G7	Control ADC-1 (high dose, QW*4, i.v.)	932.37±170.55	-	64.98	35.02

Note:

1. Mean tumor volume MTV is expressed as "mean± standard error";
2. Relative tumor suppression rate $TGI\% = (1 - T/C) \times 100\%$, $T/C\% = T_{RTV} / C_{RTV} \times 100\%$;
3. P value was obtained by comparing tumor volume at D28 between the combination therapy group and the monotherapy group and was considered statistically significant at $P < 0.05$,

* indicates $P < 0.05$, ** indicates $P < 0.01$, and *** indicates $P < 0.001$;

Therefore, TROP2-ADC-06 showed stronger antitumor effect than control ADC-3 and control ADC-1 in the subcutaneous heterogeneous transplanted tumor model of TROP2-positive human epidermal cancer cell A431 and Trop2-negative human colon cancer cell SW620 BALB/c-nu mice.

BACN2541PCT

CONNECTING UNITS FOR LIGAND-DRUG CONJUGATES AND METHODS OF MAKING AND USING THEREOF

SEQUENCE LISTING

>SEQ ID NO.1: Anti-TROP2 antibody CDR-H1
SFDIN

>SEQ ID NO.2: Anti-TROP2 antibody CDR-H2
WIFPGDGNTKYSQKFQG

>SEQ ID NO.3: Anti-TROP2 antibody CDR-H3
GEALYYFDY

>SEQ ID NO.7: Anti-TROP2 antibody CDR-L1
RASQDINKYLA

>SEQ ID NO.8: Anti-TROP2 antibody CDR-L2
STSTLQS

>SEQ ID NO.9: Anti-TROP2 antibody CDR-L3
LQYDDLFT

>SEQ ID NO.13: Anti-TROP2 antibody heavy chain (HC) variable region (VL) amino acid sequence
QVQLVQSGAEVKKPGASVKLSCKASGYTFTSFDINWVRQAPQRLEWMGWIFPGDGNTKYSQKF
QGRATITRDTSASTAYMELSSLRSEDTAVYYCVRGEALYYFDYWGQGTLVTVSS

>SEQ ID NO.14: Anti-TROP2 antibody light chain (LC) variable region (VL) amino acid sequence
DIQMTQSPSSLSASVGDRVTITCRASQDINKYLAWYQQKPGKVPKLLIYSTSTLQSGVPSRFSG
SGSGTDFLTITSSLPEDVATYYCLQYDDLFTFGQGTKLEIK

>SEQ ID NO.17: Anti-TROP2 antibody HC amino acid sequence
QVQLVQSGAEVKKPGASVKLSCKASGYTFTSFDINWVRQAPQRLEWMGWIFPGDGNTKYSQKF
QGRATITRDTSASTAYMELSSLRSEDTAVYYCVRGEALYYFDYWGQGTLVTVSSASTKGPSVFP
LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSS
SLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR
TPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES

BACN2541PCT

NGQPENNYKTTTPVLDSGSEFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPG

>SEQ ID NO.18: Anti-TROP2 antibody LC amino acid sequence
DIQMTQSPSSLSASVGDRVTITCRASQDINKYLAWYQQKPGKVPKLLIYSTSTLQSGVPSRFSG
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CEVTHQGLSSPVTKSFNRGEC

>SEQ ID No.21: Anti-TROP2 antibody CDR-H1 nucleic acid sequence
TCCTTCGACATTAAC

>SEQ ID No.22: Anti-TROP2 antibody CDR-H2 nucleic acid sequence
TGGATCTTCCCCGCGCAGCGCAACACCAAGTACTCCCAGAAGTTCCAGGGA

>SEQ ID No.23: Anti-TROP2 antibody CDR-H3 nucleic acid sequence
GGAGAGGCTCTGTACTATTTTGATTAT

>SEQ ID No.27: Anti-TROP2 antibody CDR-L1 nucleic acid sequence
AGAGCTTCTCAGGATATCAATAAGTATCTGGCT

>SEQ ID No.28: Anti-TROP2 antibody CDR-L2 nucleic acid sequence
TCTACATCTACCCTGCAGTCT

>SEQ ID No.29: Anti-TROP2 antibody CDR-L3 nucleic acid sequence
CTGCAGTATGATGATCTGTTACAC

>SEQ ID NO.33: Anti-TROP2 antibody VH nucleic acid sequence
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GCGCCTGGAGTGGATGGGCTGGATCTTCCCCGCGCAGCGCAACACCAAGTACTCCCAGAAGTTC
CAGGGAAGAGCTACCATCACCAGAGATACATCCGCTTCTACAGCTTACATGGAGCTGTCTAGCC
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>SEQ ID NO.34: Anti-TROP2 antibody VL nucleic acid sequence
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GCCTAAGCTGCTGATCTACTCTACATCTACCCTGCAGTCTGGAGTGCCTTCTAGATTTTCTGGA
TCTGGCTCTGGCACCGATTTTACACTGACAATCTCTTCTCTGCAGCCTGAGGATGTGGCTACAT
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BACN2541PCT

>SEQ ID NO.37: Anti-TROP2 antibody HC nucleic acid sequence
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GCGCCTGGAGTGGATGGGCTGGATCTTCCCCGGCGACGGCAACACCAAGTACTCCCAGAAGTTC
CAGGGAAGAGCTACCATCACCAGAGATACATCCGCTTCTACAGCTTACATGGAGCTGTCTAGCC
TGAGATCTGAGGATACAGCTGTGTATTACTGTGTGAGAGGAGAGGCTCTGTACTATTTTGATTA
TTGGGGCCAGGGCACCCTGGTGACAGTGTCTTCTGCTAGCACCAGGGGCCATCGGTCTTCCCC
CTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACT
ACTTCCCCGAACCGGTGACGGTGTCTGGAAGTCAAGCGCCCTGACCAGCGGCGTGCACACCTT
CCCGGCTGTCTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGC
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CCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAACCCAAAGGACACCCTCATGATCTCCCGG
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>SEQ ID NO.38: Anti-TROP2 antibody LC nucleic acid sequence
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TCTGGCTCTGGCACCGATTTTACACTGACAATCTCTTCTCTGCAGCCTGAGGATGTGGCTACAT
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AG

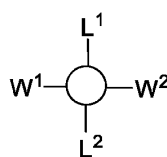
CONNECTING UNITS FOR LIGAND-DRUG CONJUGATES AND METHODS OF MAKING AND USING THEREOF

CLAIMS

1. A linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvent compound thereof, said linker-drug is formed from a connector linked to a drug unit through a linker, wherein,

the drug unit is a drug or a drug precursor,

the connector is a compound as shown in Formula I, or its racemate, enantiomer, diastereomer, or pharmaceutically acceptable salt or solvate thereof,



Formula I

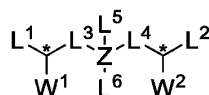
wherein,

L^1 , L^2 are identical or different, and each independently comprises hydrogen, deuterium, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10 membered heterocyclic, carboxylic acid, amide, ester, sulfite, sulfonate, phosphoric acid, pyrophosphoric acid, natural or unnatural amino acid residues, polyethylene glycol, or a combination thereof;

W^1 , W^2 are identical or different, and each comprises a connecting group, wherein at least one of W^1 , W^2 comprises a maleimide structure;

L^1 , L^2 , W^1 , or W^2 are covalently linked to the circle, wherein the circle represents a scaffold comprising a basic group.

2. The linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvent compound thereof, according to Claim 1, wherein said connector comprises a structure as shown in Formula II:



Formula II

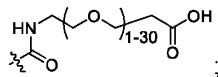
wherein,

the carbon atom at the position shown * is a chiral carbon having an absolute configuration of R or S;

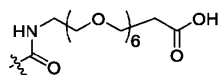
L^1 , L^2 are identical or different, and each independently comprises hydrogen, deuterium, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10 membered heterocyclic, carboxylic acid, amide, ester, sulfite, sulfonate, phosphoric acid, pyrophosphoric acid, natural or

unnatural amino acid residues, polyethylene glycol, or a combination thereof;

preferably, L^1 , L^2 are identical or different, and each independently comprises carboxyl, or



preferably, L^1 , L^2 are identical or different, and each independently comprises carboxyl, or



L^3 , L^4 are identical or different, and each independently comprises substituted or unsubstituted C_1 - C_6 alkylene, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10-membered heterocyclic, polyethylene glycol, or a combination thereof;

preferably, L^3 , L^4 are identical or different, and each independently comprises substituted or unsubstituted C_1 - C_6 alkylene;

preferably, L^3 , L^4 are identical or different, and each independently comprises C_1 - C_6 alkylene;

preferably, L^3 , L^4 are identical or different, and each independently comprises methylene, or ethylene;

L^5 , L^6 are present or absent, identical or different, and when present, each independently comprises hydrogen, deuterium, oxygen, sulfur, hydroxyl, carbonyl, amide, ester, tert-butoxycarbonyl (Boc), benzyloxycarbonyl (Cbz), allyloxycarbonyl (Alloc), benzyl, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10-membered heterocyclic, polyethylene glycol, or a combination thereof;

preferably, L^6 is absent, L^5 is present, and L^5 is hydrogen, or substituted or unsubstituted C_1 - C_6 alkyl;

preferably, L^6 is absent, L^5 is present, and L^5 is hydrogen, or C_1 - C_6 alkyl;

preferably, L^6 is absent, L^5 is present, and L^5 is hydrogen;

W^1 , W^2 are identical or different, and each independently comprises a connecting group, wherein at least one of W^1 and W^2 comprises a maleimide structure;

Z is N, O, S or a quaternary amine;

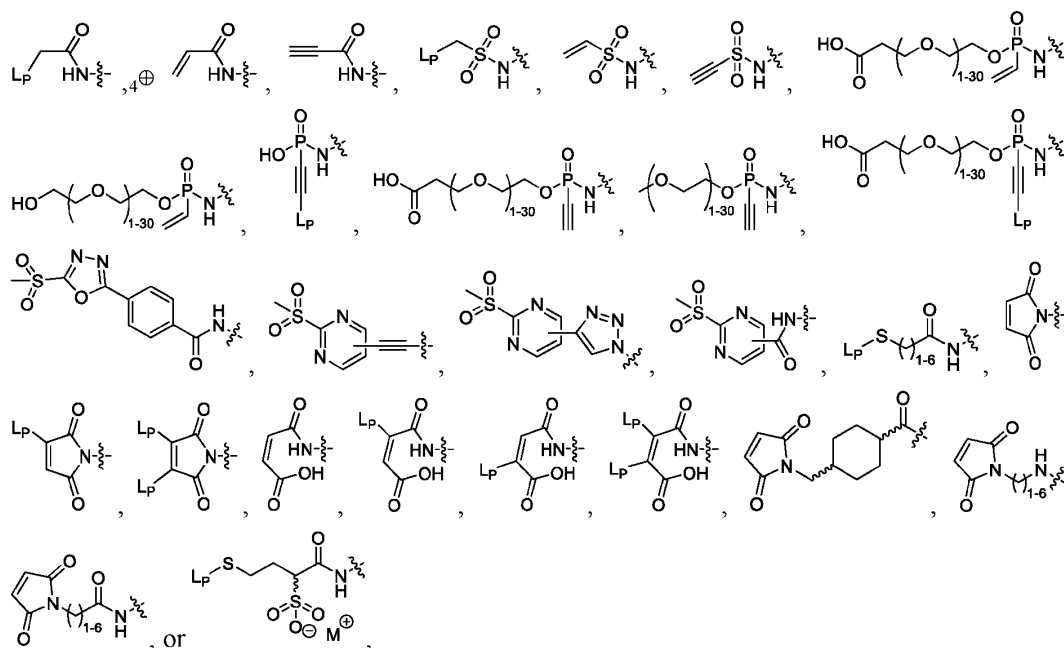
preferably, Z is N.

3. The linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvent compound thereof, according to Claims 1 or 2, wherein W^1 , W^2 are identical or different, and each independently comprises a connecting group connected to carboxyl, amino, carbonyl, thiol, azido, alkenyl, a conjugated dienophile, alkenyl, tetrazinyl,

preferably the connecting group is connected to thiol,

wherein at least one of W^1 or W^2 comprises a maleimide structure.

4. The linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvent compound thereof, according to Claims 1-3, wherein W^1 , W^2 are identical or different, and each independently comprises the following, its racemate, enantiomer, diastereomer, or pharmaceutically acceptable salts or solvates thereof:



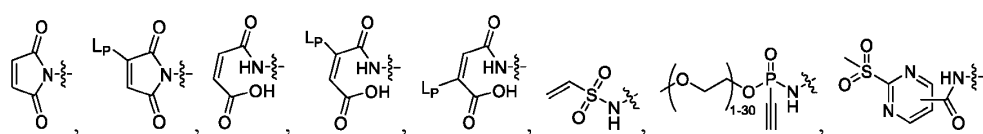
wherein M^+ is H^+ , Li^+ , Na^+ , K^+ or NH_4^+ ,

wherein the position shown by the wavy line at the end of the structure represents the attachment site, and the wavy line in the middle of the structure indicates either of the two chiral configurations: R- or S-;

and at least one of W^1 or W^2 comprises a maleimide structure;

each L_P is identical or different, and comprises substituted or unsubstituted C1-C6 alkyl, chloride, bromide, iodide, OMs, or OTs;

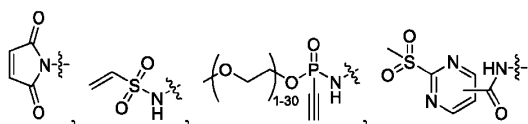
preferably, W^1 , W^2 are identical or different, and each independently comprises the following, their racemates, enantiomers, diastereomers, or pharmaceutically acceptable salts or solvates thereof:



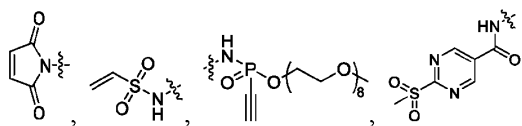
and at least one of W^1 or W^2 comprises a maleimide structure, and

each L_P is identical or a different moiety, and comprises C1-C6 alkyl, chloride, bromide, iodide, OMs, or OTs;

preferably, W^1 , W^2 are identical or different, and each independently comprises the following, their racemates, enantiomers, diastereomers, pharmaceutically acceptable salts or solvates thereof:

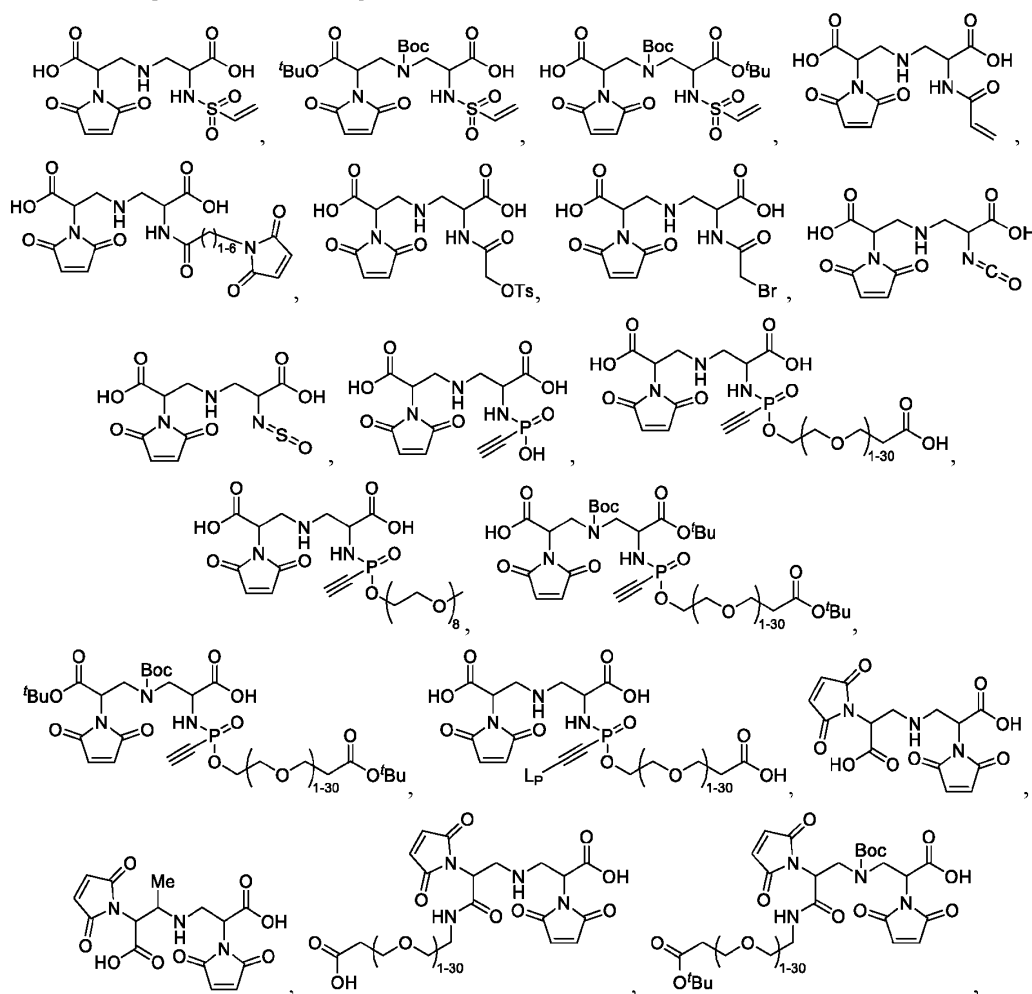


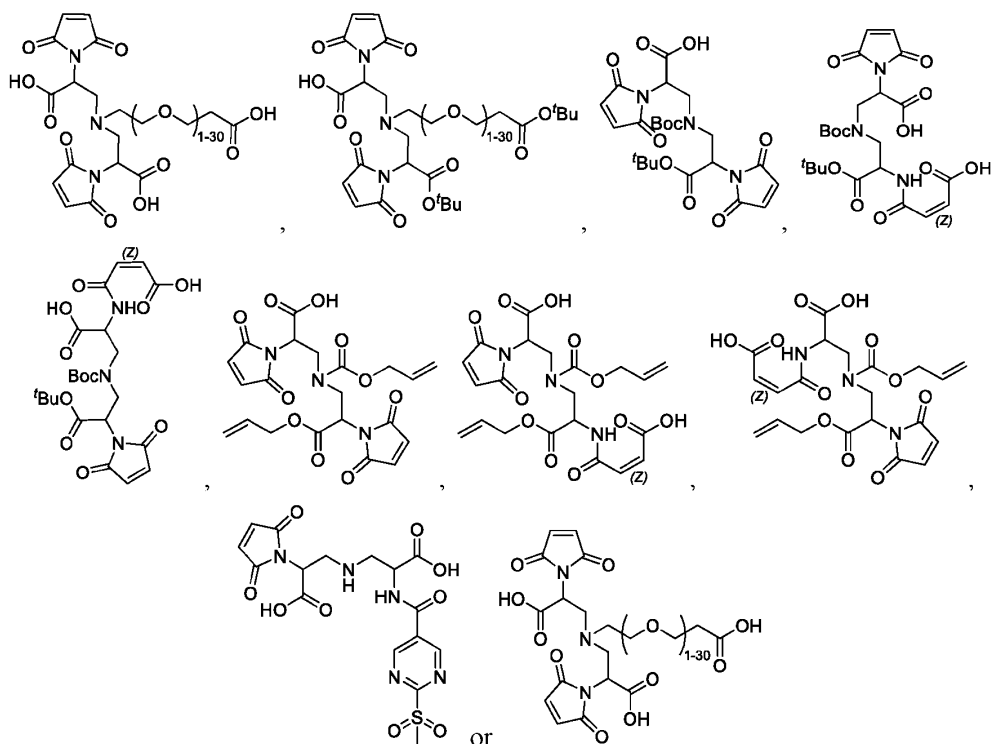
and at least one of W^1 or W^2 comprises a maleimide structure;
preferably, W^1 , W^2 are identical or different, and each independently comprises the following,
their racemates, enantiomers, diastereomers, pharmaceutically acceptable salts or solvates thereof:



and at least one of W^1 or W^2 comprises a maleimide structure.

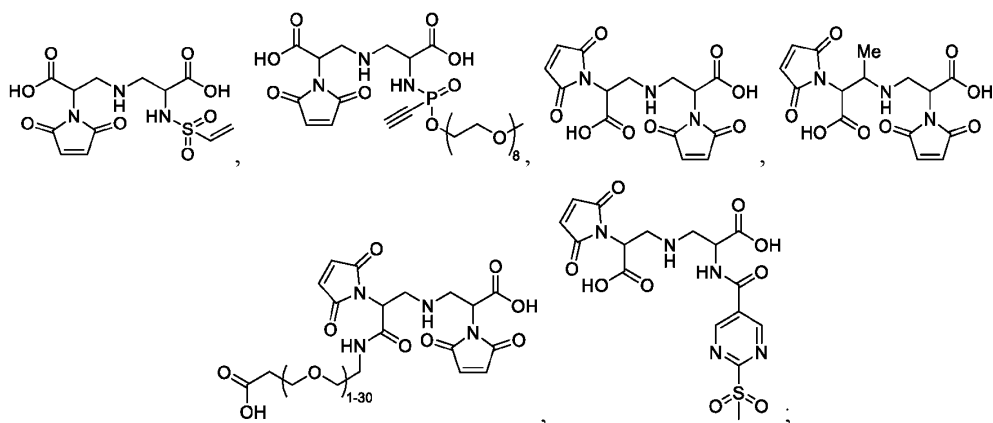
5. The linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 1-4, wherein said connector comprises the following:



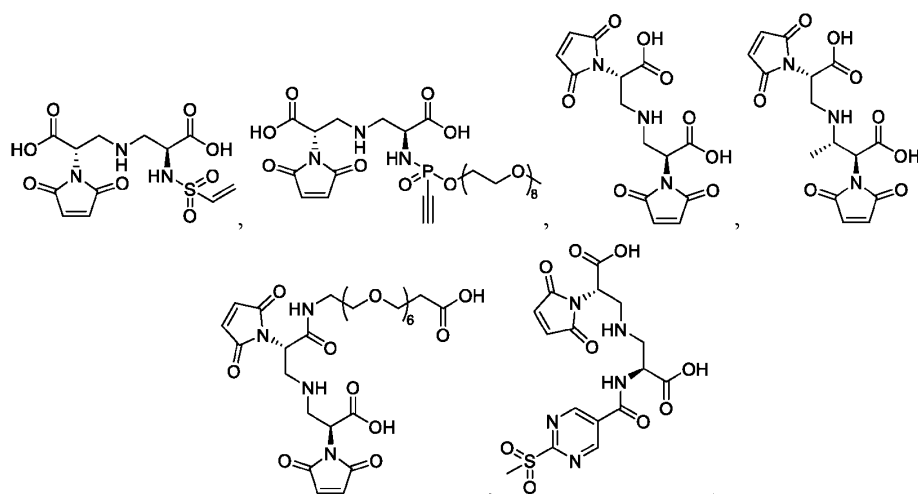


wherein L_P is identical or different, and comprises deuterium, chloride, bromide, iodide, OM_s, or OT_s;

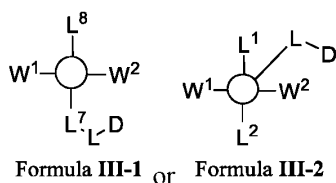
preferably, said connector comprises the following:



preferably, said connector comprises the following:



6. The linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 1-5, wherein said linker-drug compound has a structure as shown in Formula III-1 or Formula III-2, preferably the structure shown in Formula III-1.



wherein,

L^1 , L^2 are identical or different, and each independently comprises hydrogen, deuterium, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10 membered heterocyclic, carboxylic acid, amide, ester, sulfinyl, sulfonate, phosphoric acid, pyrophosphoric acid, natural or unnatural amino acid residues, polyethylene glycol, or a combination thereof;

L^8 comprises hydrogen, deuterium, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10-membered heterocyclic, carboxylic acid, amide, ester, sulfinyl, sulfonate, phosphoric acid, pyrophosphoric acid, natural or unnatural amino acid residues, polyethylene glycol, or a combination thereof;

L^7 comprises substituted or unsubstituted C_1 - C_6 alkylene, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10-membered heterocyclic, carbonyl, amide, ester, sulfinyl, sulfonate, phosphoric acid, pyrophosphoric acid, natural or unnatural amino acid residue, polyethylene glycol, or a combination thereof;

W^1 , W^2 are identical or different, and each independently comprises a connecting group,

wherein at least one of W^1 , W^2 comprises a maleimide structure;

L^1 , L^2 , L^8 , L^7 , W^1 , or W^2 are covalently linked to the circle, wherein the circle represents a scaffold containing a basic group;

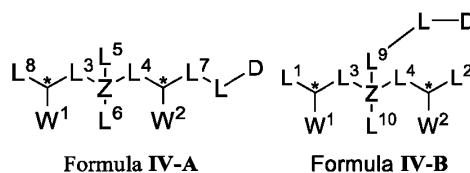
L is the linker;

D is the drug unit comprising a drug or a drug precursor, wherein said drug unit is linked to L covalently;

preferably, D is a drug linked to L covalently;

alternatively and preferably, D is a drug precursor linked to L covalently.

7. The linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 1-6, wherein said linker-drug compound has a structure as shown in Formula **IV-A** or **IV-B**, preferably a structure as shown in Formula **IV-A**.

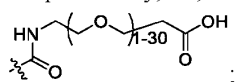


wherein,

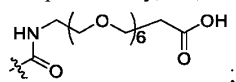
the carbon atom at the position shown * is a chiral carbon, having an absolute configuration of R or S;

L^1 , L^2 are identical or different, and each independently comprises hydrogen, deuterium, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10 membered heterocyclic, carboxylic acid, amide, ester, sulfinyl, sulfonate, phosphoric acid, pyrophosphoric acid, natural or unnatural amino acid residues, polyethylene glycol, or a combination thereof;

preferably, L^1 , L^2 are identical or different, and each independently comprises a carboxyl, or



preferably, L^1 , L^2 are identical or different, and each independently comprises a carboxyl, or



L^3 , L^4 are identical or different, and each independently comprises substituted or unsubstituted C_1 - C_6 alkylene, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10-membered heterocyclic, polyethylene glycol, or a combination thereof;

preferably, L^3 , L^4 are identical or different, and each independently comprises substituted or unsubstituted C_1 - C_6 alkylene;

preferably, L^3 , L^4 are identical or different, and each independently comprises C_1 - C_6 alkylene;

preferably, L^3 , L^4 are identical or different, and each independently comprises methylene, or ethylene;

L^5 , L^6 are present or absent, identical or different, and, when present, each independently comprises hydrogen, deuterium, oxygen, sulfur, hydroxyl, carbonyl, amide, ester, tert-butoxycarbonyl (Boc), benzyloxycarbonyl (Cbz), allyloxycarbonyl (Alloc), benzyl, substituted or unsubstituted C_1 - C_6 alkylidene, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10-membered heterocyclic, polyethylene glycol, or a combination thereof;

preferably, L^6 is absent, L^5 is present, and L^5 is hydrogen, or substituted or unsubstituted C_1 - C_6 alkyl;

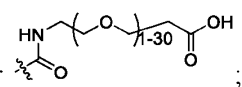
preferably, L^6 is absent, L^5 is present, and L^5 is hydrogen, or C_1 - C_6 alkyl;

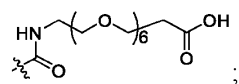
preferably, L^6 is absent, L^5 is present, and L^5 is hydrogen;

L^7 comprises substituted or unsubstituted C_1 - C_6 alkylene, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10-membered heterocyclic, carbonyl, amide, ester, sulfinyl, sulfonate, phosphoric acid, pyrophosphoric acid, natural or unnatural amino acid residue, polyethylene glycol, or a combination thereof;

preferably, L^7 is carbonyl;

L^8 comprises hydrogen, deuterium, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10-membered heterocyclic, carboxyl, amide, ester, sulfinyl, sulfonate, phosphoric acid, pyrophosphoric acid, natural or unnatural amino acid residues, or polyethylene glycol, or a combination thereof;

preferably, L^8 is a carboxyl, or  ;

preferably, L^8 is carboxyl, or  ;

L^9 comprises oxygen, sulfur, carbonyl, amide, ester, substituted or unsubstituted C_1 - C_6 alkylene, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10-membered heterocyclic, polyethylene glycol, or a combination thereof;

L^{10} is present or absent, and when present, comprises hydrogen, deuterium, oxygen, hydroxyl, carbonyl, amide, ester, tert-butoxycarbonyl (Boc), benzyloxycarbonyl (Cbz), allyloxycarbonyl (Alloc), benzyl, substituted or unsubstituted C_1 - C_6 alkylene, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10-membered heterocyclic, polyethylene glycol, or a combination thereof;

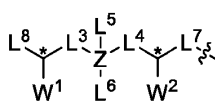
preferably, L^{10} is absent;

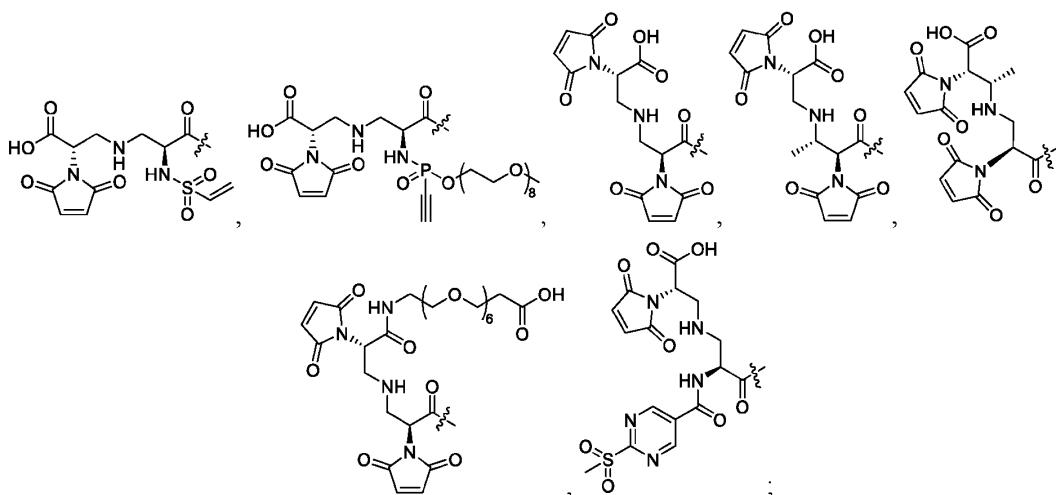
W^1 , W^2 are identical or different, and each independently comprises a linking, wherein at least one of W^1 , W^2 comprises a maleimide structure;

preferably, W^1 , W^2 are as defined according to any one of Claims 3-4;

Z is selected from N, O, S or a quaternary amine;

preferably, Z is N;

preferably, in Formula IV-A,  as a whole is selected from the following:



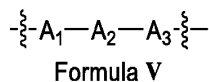
L is the linker;

D is the drug unit comprising a drug or a drug precursor, wherein said drug unit is linked to L covalently;

preferably, D is a drug linked to L covalently;

alternatively and preferably, D is a drug precursor linked to L covalently.

8. The linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 1-7, wherein said linker L has the structure shown in Formula V:



wherein,

A₁, A₂, and A₃ each is present or absent independently;

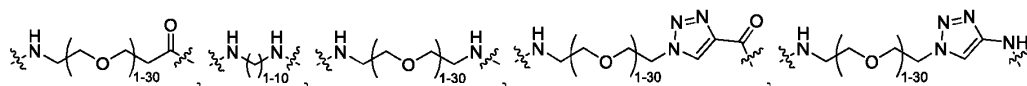
when A₁ is present, A₁ is a modifying unit, preferably selected from a branchable unit or a hydrophilic unit;

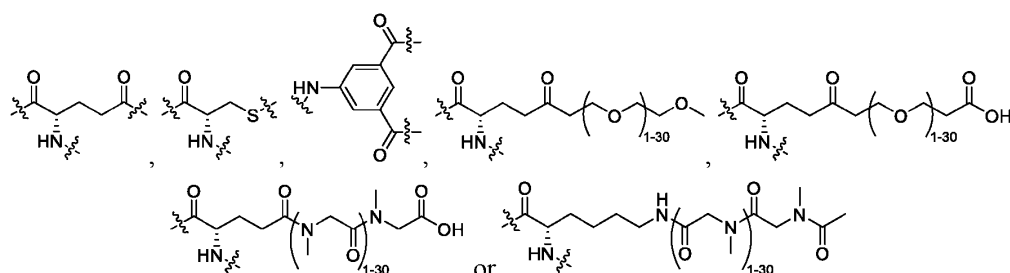
when A₂ is present, A₂ is selected from a breakable unit or a non-breakable unit;

when A₃ is present, A₃ is selected from an elimination unit or a chelation unit;

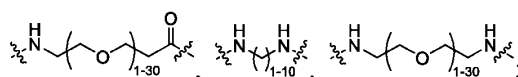
the position shown by the wavy line represents the connection site.

9. The linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvent compound thereof, according to Claim 8, wherein A₁ is present or absent, and when A₁ is present, A₁ comprises the following or their combinations thereof:

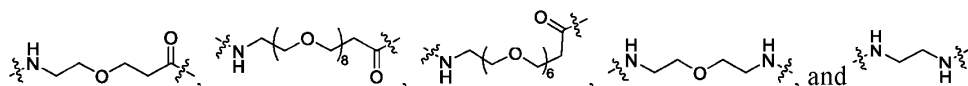




preferably, when A₁ is present, A₁ is the following or combinations thereof:



preferably, when A₁ is present, A₁ is selected from the following or combinations thereof:



where the positions shown by the wavy lines represent connection sites.

10. The linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvent compound thereof, according to Claims 8-9, wherein A₂ is present or absent, and when A₂ is present, said A₂ comprises a breakable or a unbreakable unit, wherein the unbreakable unit comprises substituted or unsubstituted C₁-C₆ alkyl, substituted or unsubstituted or unsubstituted C₂-C₆ alkenyl, substituted or unsubstituted C₂-C₆ alkynyl, aryl, heteroaryl, C₃-C₁₀ cycloalkyl, 3-10-membered heterocyclic, amide, ester, polyethylene glycol, or a combination thereof.

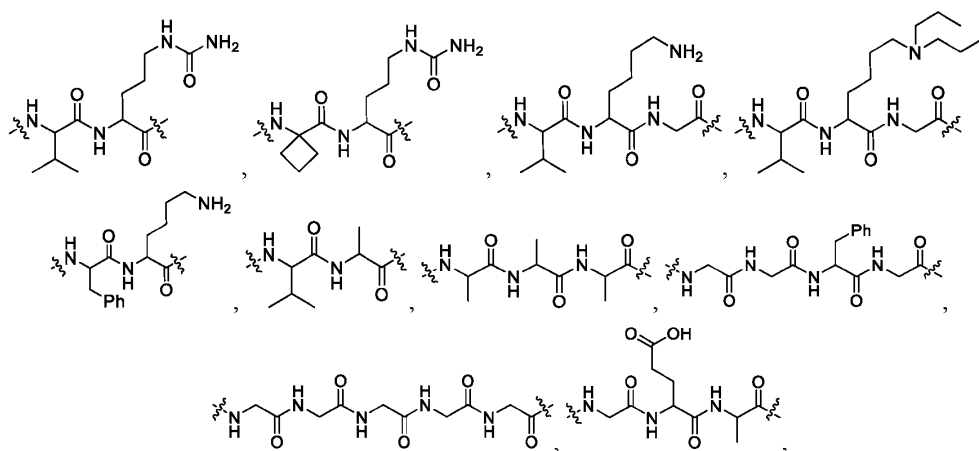
11. The linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 8-10, wherein A₂ is present or absent, and when A₂ is present, said A₂ comprises a breakable unit or a non-breakable unit, wherein the breakable unit comprises a non-enzymically sensitive linker unit, an enzymatically sensitive linker unit, or a combination thereof;

preferably, said non-enzyme-sensitive linker unit comprises a glutathione-sensitive disulfide bond linker unit, or a pH-sensitive hydrazone linker unit;

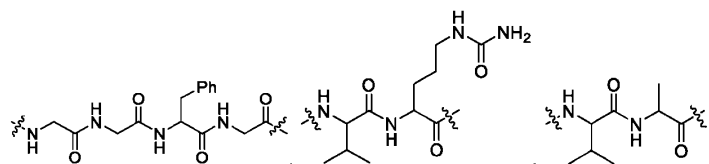
preferably, said enzyme-sensitive linker unit comprises a histone protease breakable ligating unit, a phosphodiesterase or pyrophosphate esterase breakable linker unit, a β -glucuronidase breakable linker unit, a β -galactosidase breakable linker unit, a sulfate esterase breakable linker unit, or a combination thereof.

12. The linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 8-11, wherein A₂ is present or absent, and when A₂ is present, said A₂ is a breakable unit, wherein said breakable unit comprises peptide residues comprising 2-10 natural or non-natural amino acid residues, said natural or unnatural amino acids optionally substituted with one or more substituents,

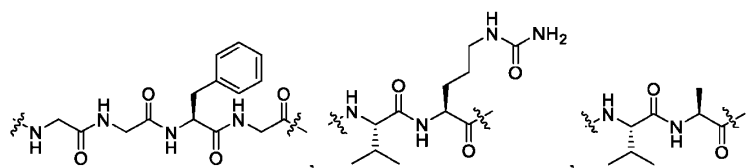
preferably, said breakable unit comprises:



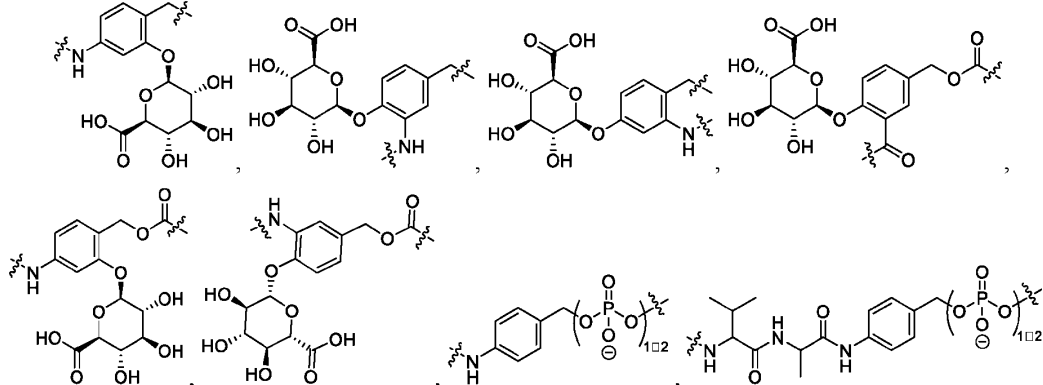
preferably selected from:

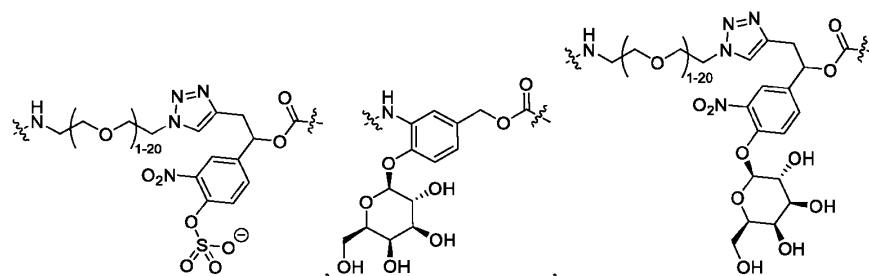


preferably selected from:

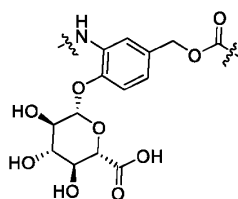


alternatively, said breakable unit comprises the following or isomers thereof:



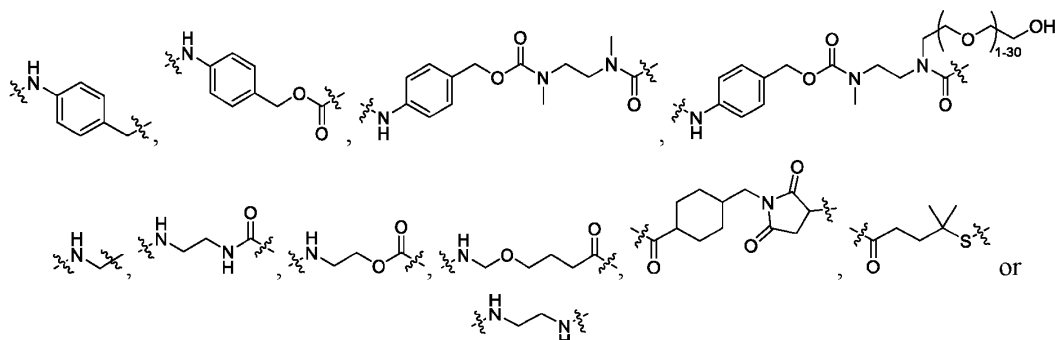


preferably selected from the following or isomers thereof;

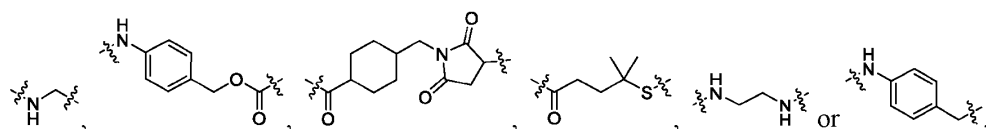


wherein the position shown by the wavy line is the connection site.

13. The linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 8-12, wherein A_3 is present or absent, and when A_3 is present, A_3 comprises the following:



preferably, when A_3 is present, A_3 comprises the following:



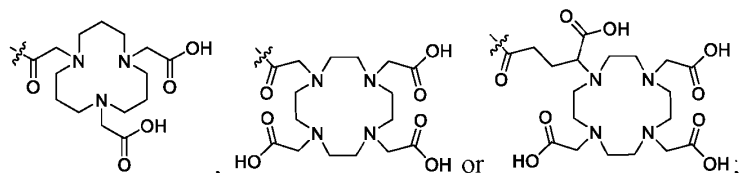
wherein the position shown by the wavy line is the connection site.

14. The linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 1-13, wherein: said linker-drug compound comprises one or more of the following (i)-(iii):

- (i) said drug unit comprises cytotoxic drugs, radioisotopes, detection or diagnostic reagents, enzyme inhibitors, protein degraders, immunomodulators, peptides or nucleotides; preferably, said drug comprises cytotoxic drugs;

(ii) said drug is obtained by chelating a drug precursor with a metal ion, preferably a radioisotope;

(iii) said drug precursor comprises



preferably, said drug precursor is obtained by chelating the drug unit with a metal ion, preferably a radioisotope.

15. The linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claim 14, wherein said cytotoxic drug comprises DNA damaging agents, DNA topoisomerase inhibitors, microtubule or microtubule protein inhibitors, or RNA polymerase inhibitors;

preferably, said DNA damaging agents comprises derivatives of calicheamicin, anthramycin, eteinasidins, adriamycin, mitomycin, or duocarmycin;

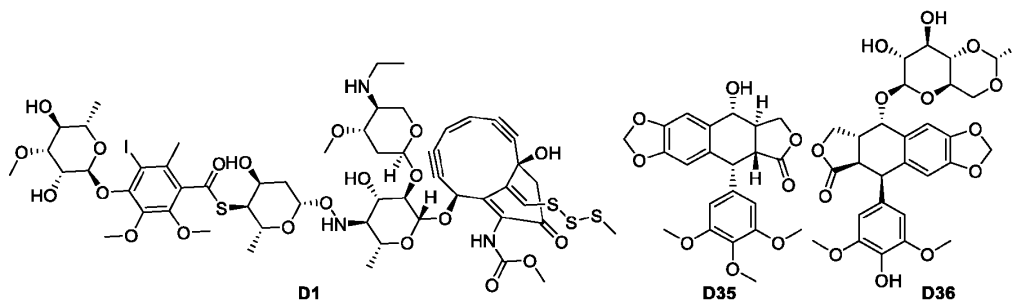
preferably, said DNA topoisomerase inhibitors comprises derivatives of camptothecin, podophyllotoxin, or etoposide;

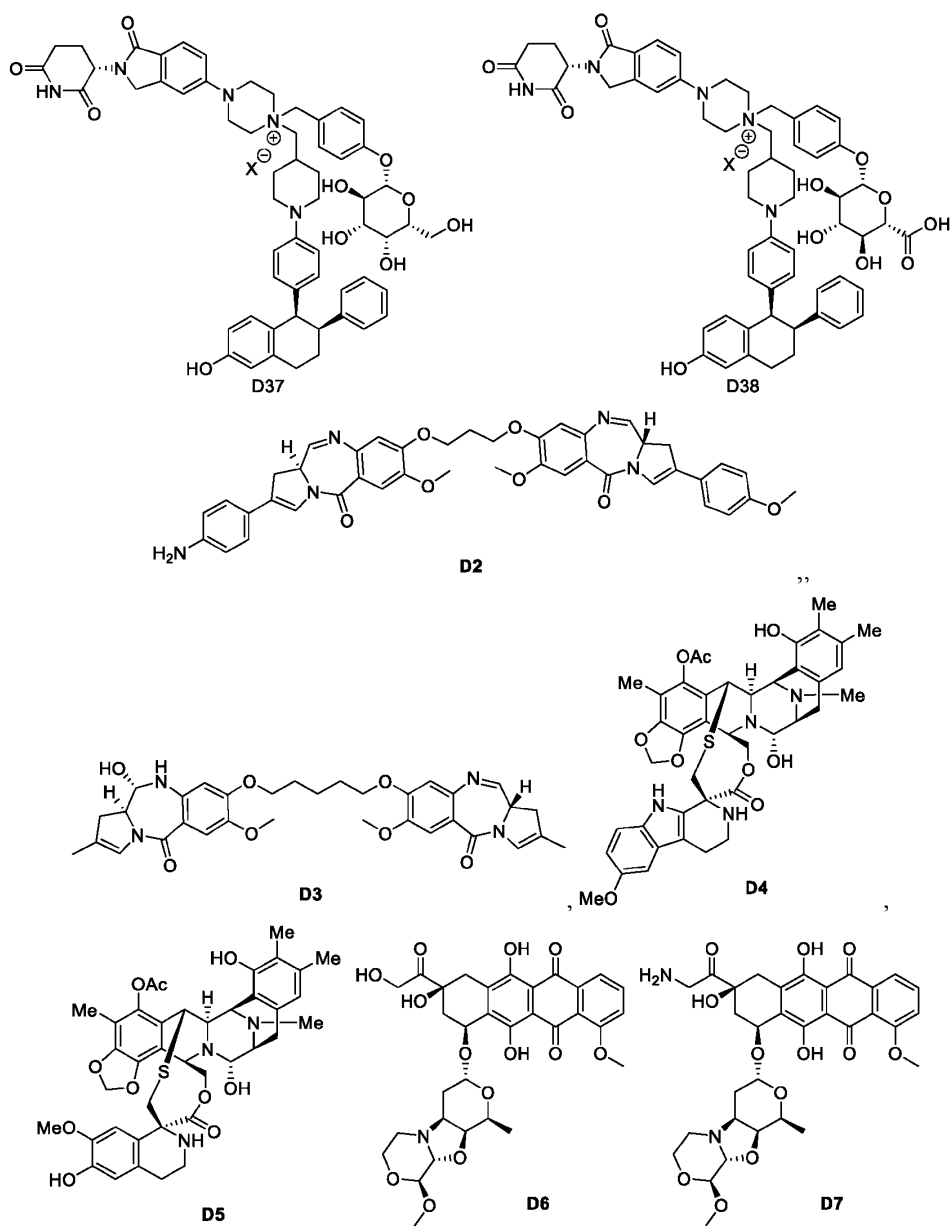
preferably, said microtubule or microtubule protein inhibitors comprises derivatives of maytansine, auristatin, eribulin, or tubulysin;

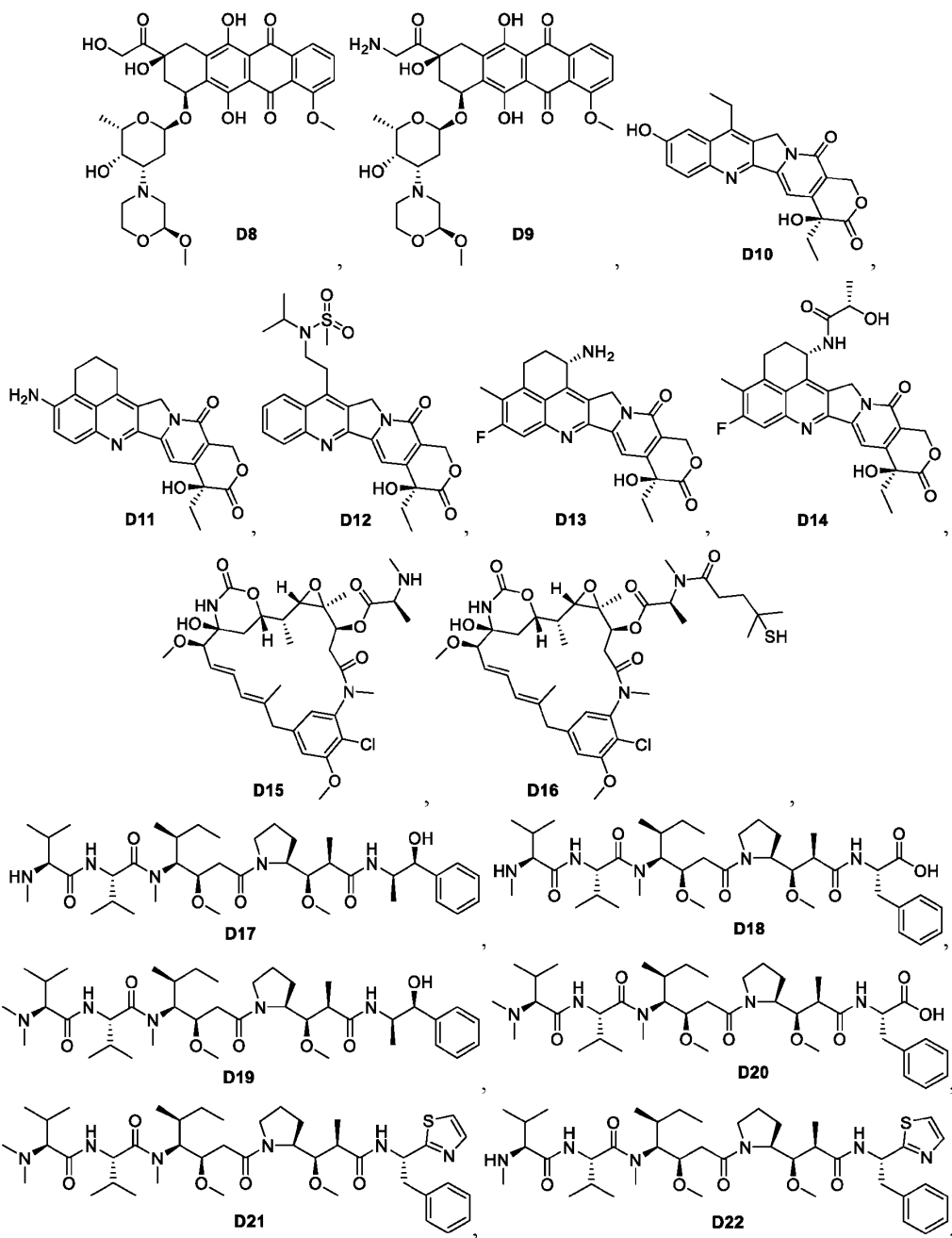
preferably, said RNA polymerase inhibitors comprises derivatives of amanitin.

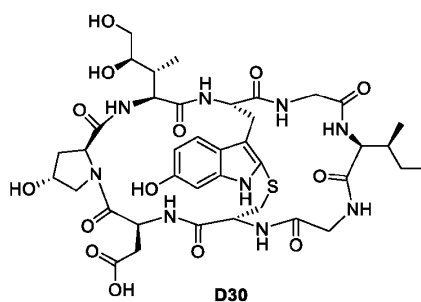
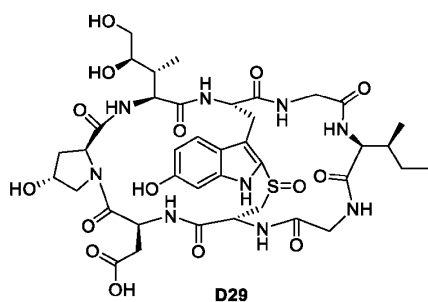
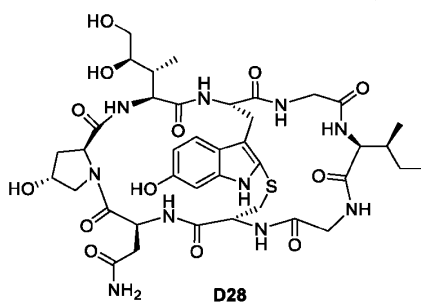
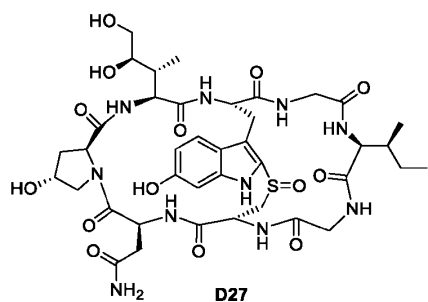
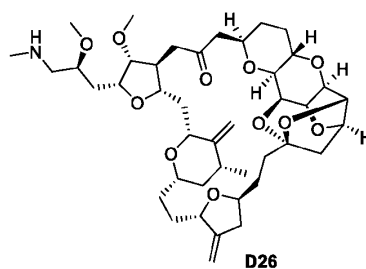
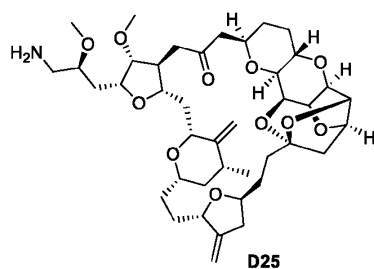
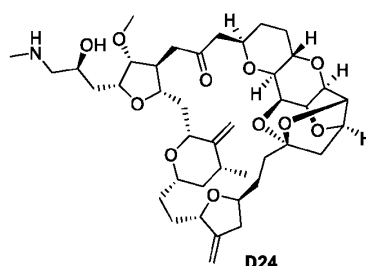
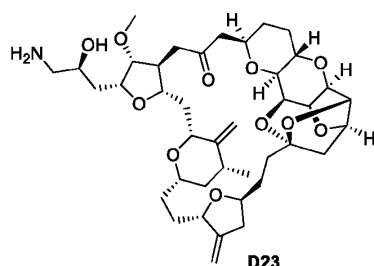
16. The linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 1-15, wherein said linker-drug has one or more technical features selected from one or more of (i)-(iii) below:

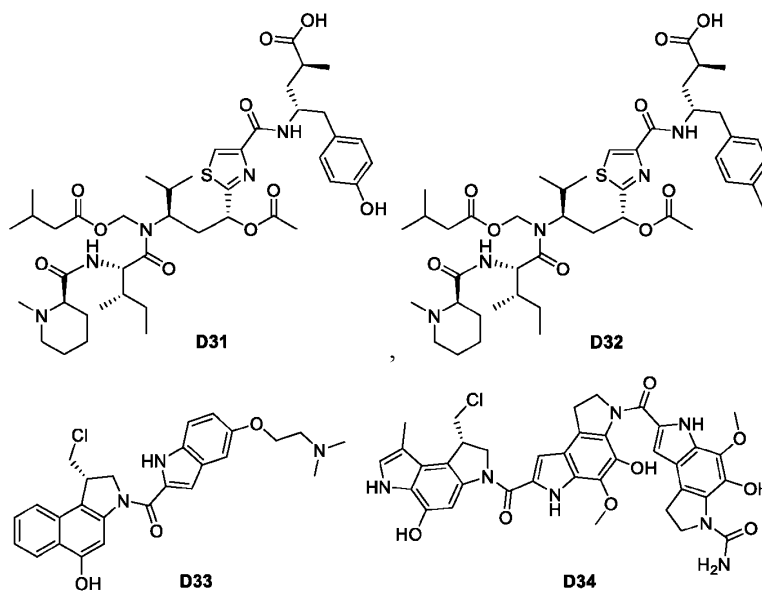
(i) said drug comprises the following:





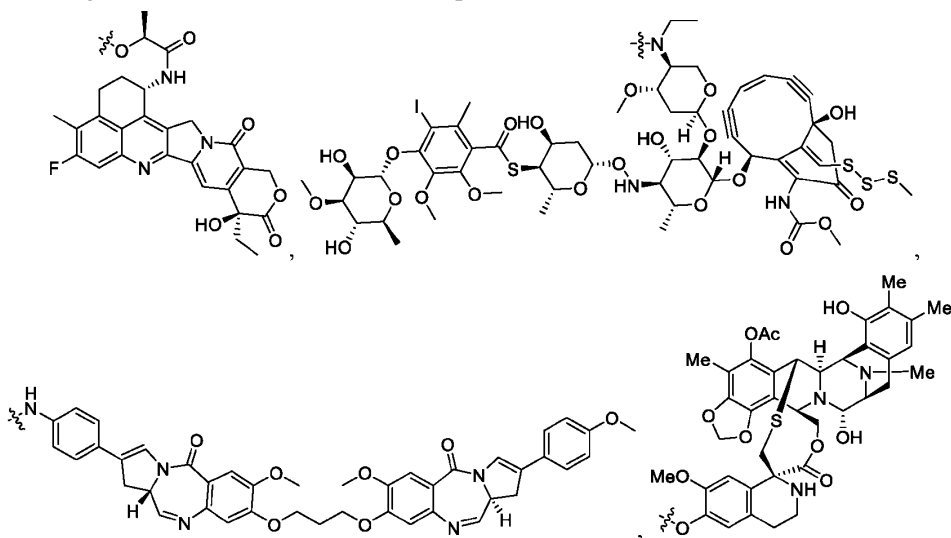


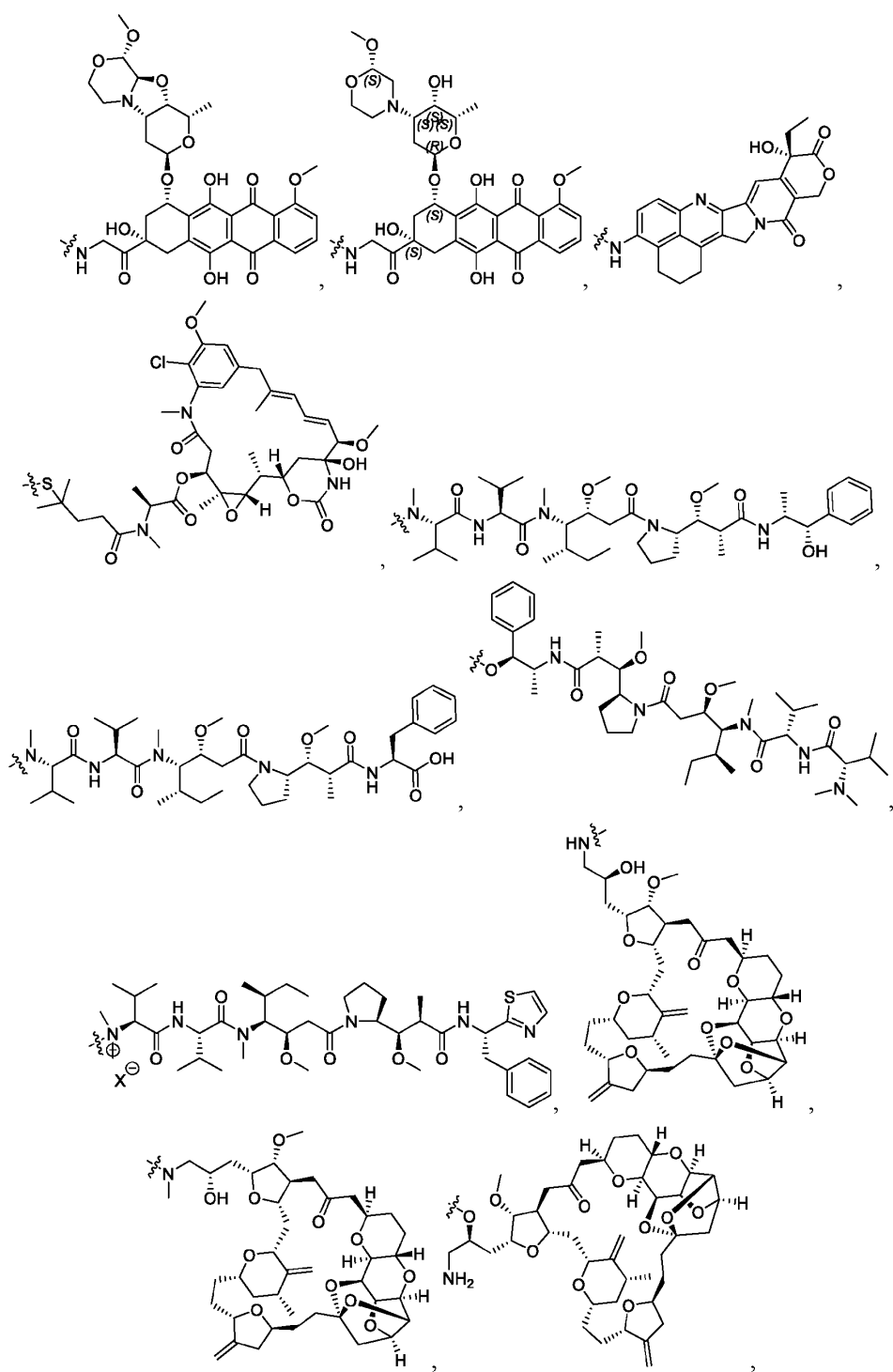


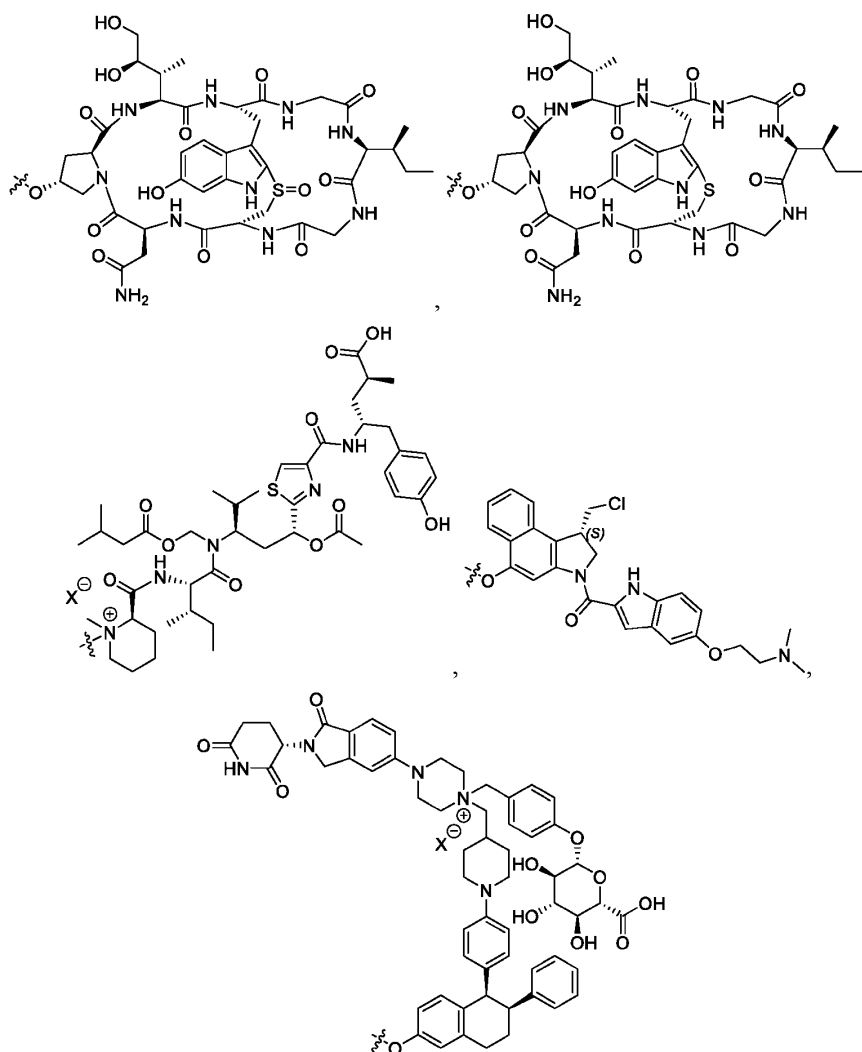


wherein each X^- is independently selected from Cl^- , Br^- , I^- , $MeSO_3^-$, CF_3COO^- , preferably CF_3COO^- ;

preferably, D is selected from the following:

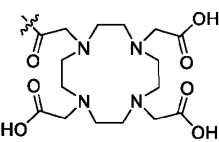






wherein each X^- is independently selected from Cl^- , Br^- , I^- , $MeSO_3^-$, CF_3COO^- , preferably CF_3COO^- ;

(ii) said drug is obtained by chelating a drug precursor with a metal ion, preferably a radioisotope;



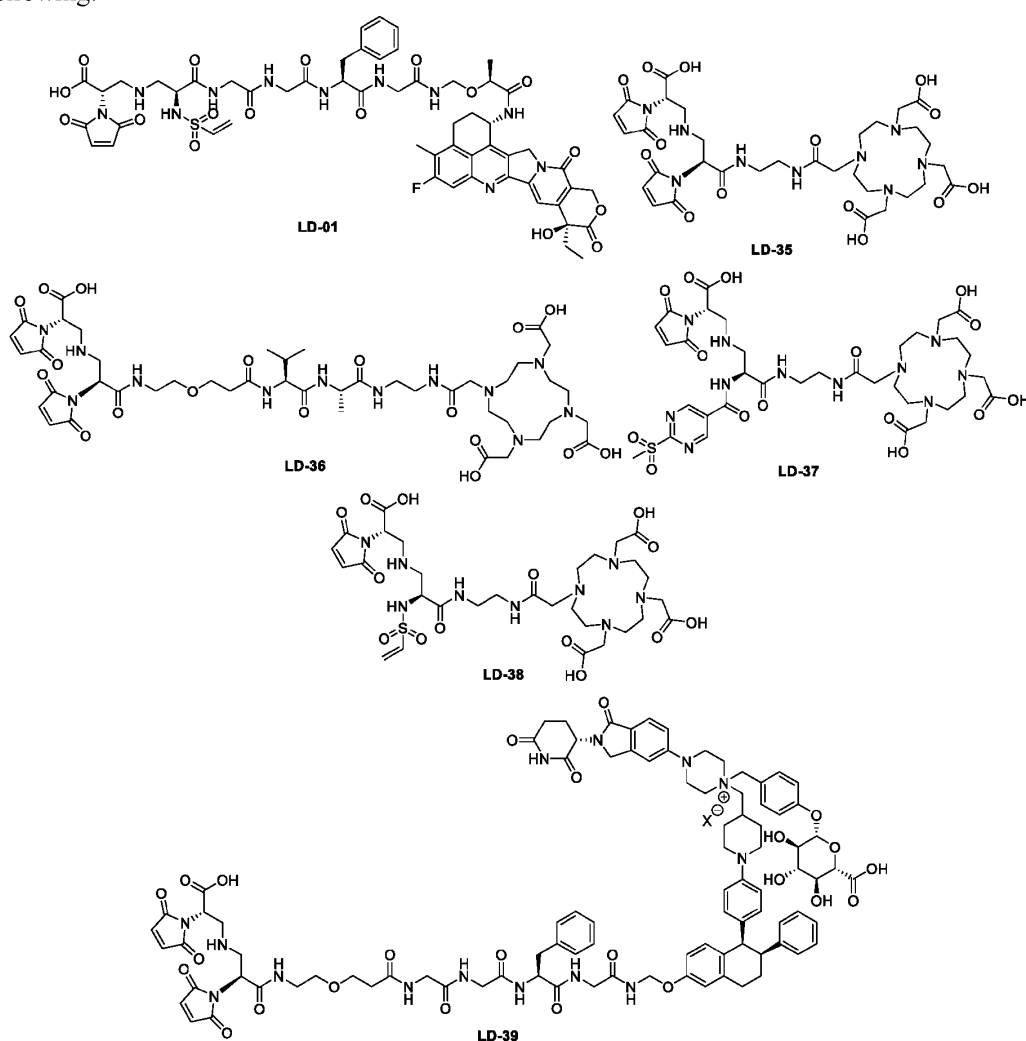
(iii) said drug precursor comprises the following structure:

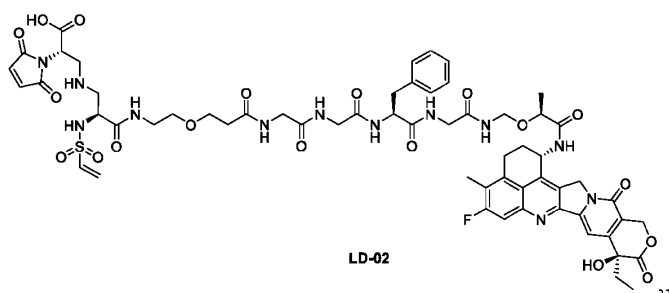
preferably, said drug precursor is obtained by chelating the drug with a metal ion, preferably a radioisotope.

17. The linker unit-drug or racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvate thereof as claimed in any one of claims 14-16, wherein: said radioisotope comprises ^{18}F , ^{67}Ga , ^{68}Ga , $^{99\text{m}}\text{Tc}$, ^{123}I , ^{125}I , ^{131}I , ^{90}Y , ^{177}Lu , ^{47}Sc , ^{64}Cu , ^{67}Cu , ^{32}P , ^{186}Re , ^{188}Re , ^{89}Sr , ^{153}Sm , ^{198}Au , ^{166}Ho , ^{165}Dy , ^{169}Er , ^{149}Tb , ^{161}Tb , ^{211}At , ^{212}Bi , ^{213}Bi , ^{212}Pb , ^{223}Ra , ^{225}Ac , ^{226}Th , ^{227}Th , or ^{230}U ;

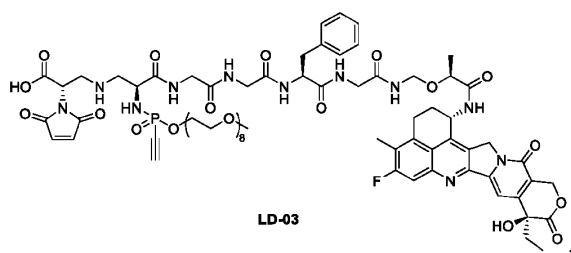
preferably, said radioisotope comprises ^{68}Ga , $^{99\text{m}}\text{Tc}$, ^{131}I , ^{90}Y , ^{177}Lu , ^{64}Cu , ^{211}At , ^{213}Bi , ^{212}Pb , or ^{225}Ac .

18. The linker-drug or racemate, enantiomer, diastereomer, pharmaceutically acceptable salt or solvate thereof as claimed in any one of claims 1-17, wherein said linker-drug comprises the following:

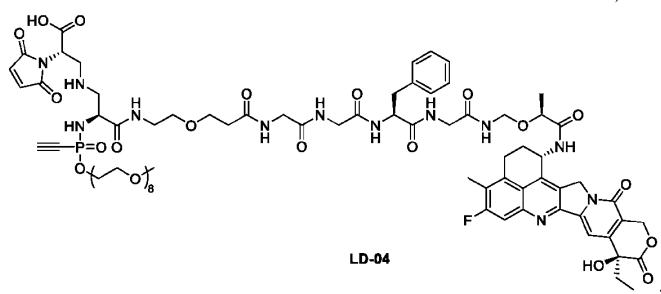




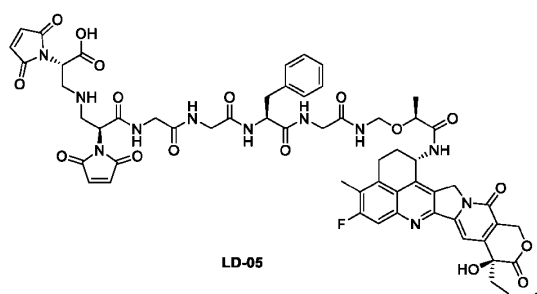
LD-02



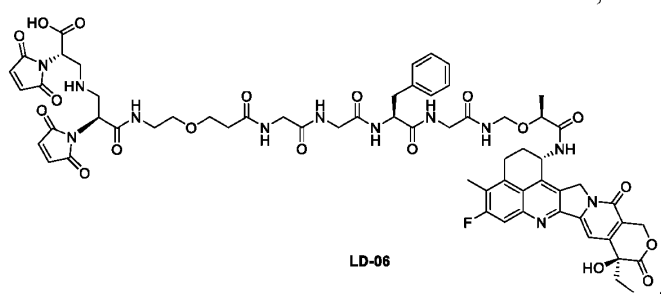
LD-03



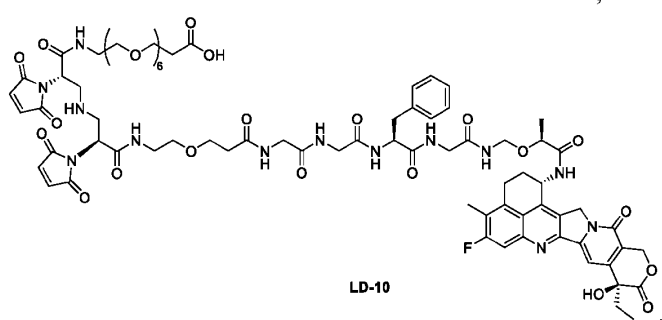
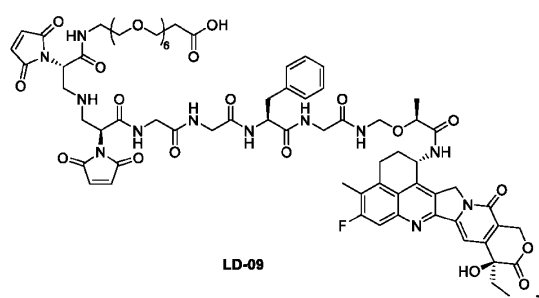
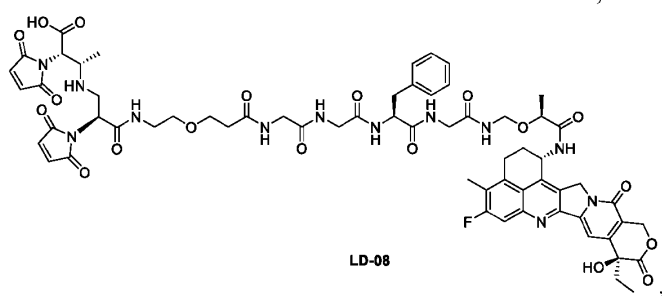
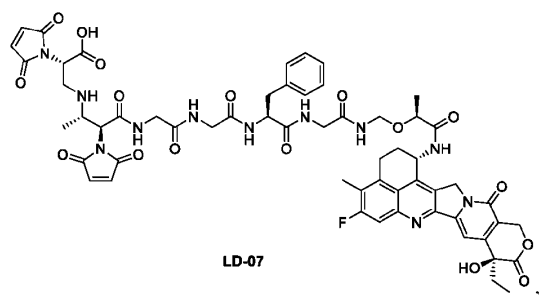
LD-04

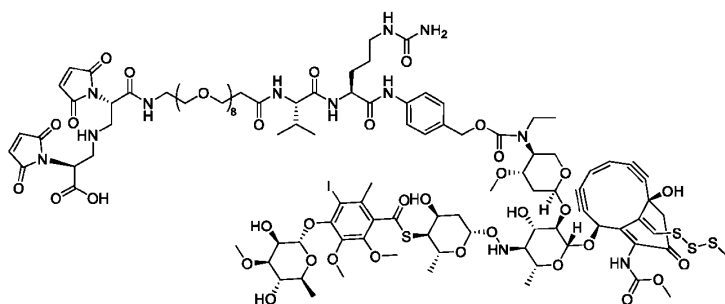


LD-05

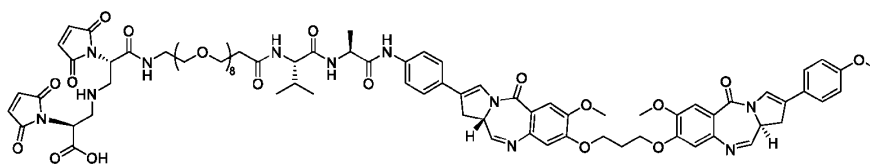


LD-06

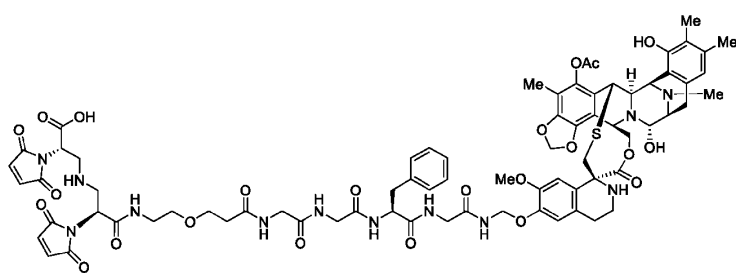




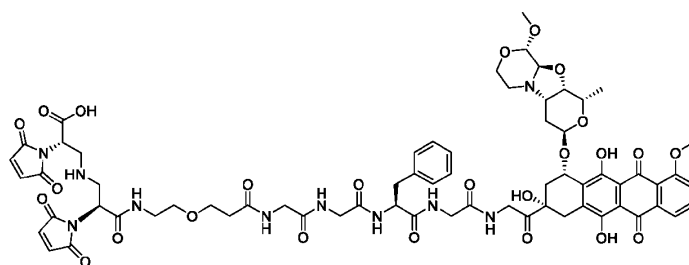
LD-11



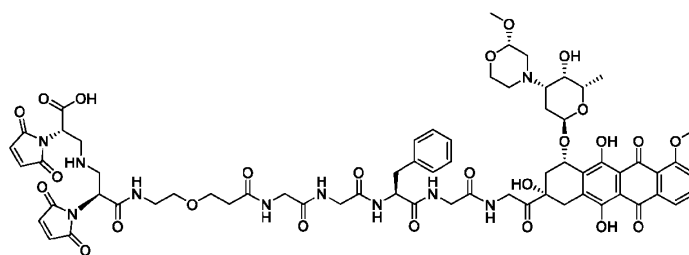
LD-12



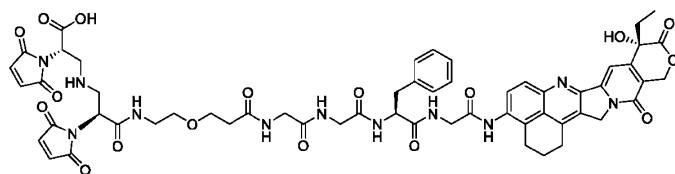
LD-13



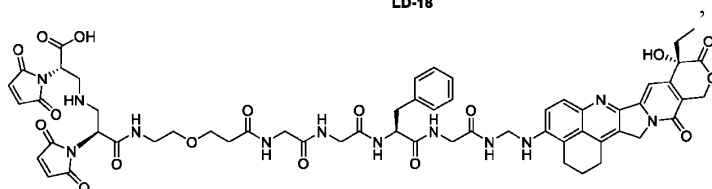
LD-15



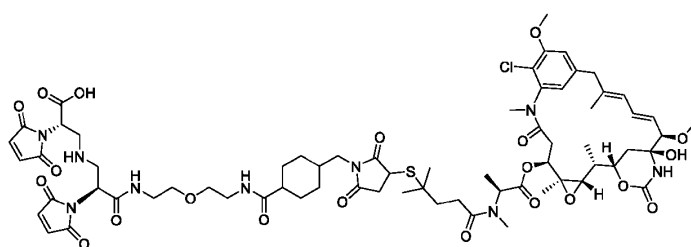
LD-17



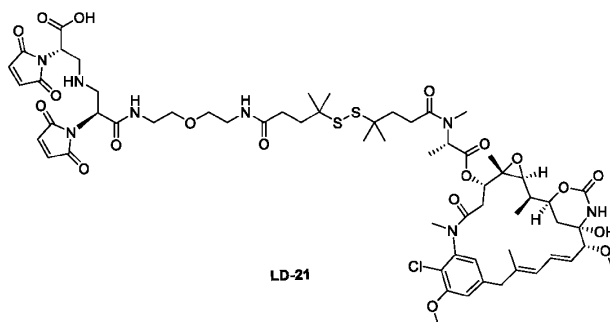
LD-18



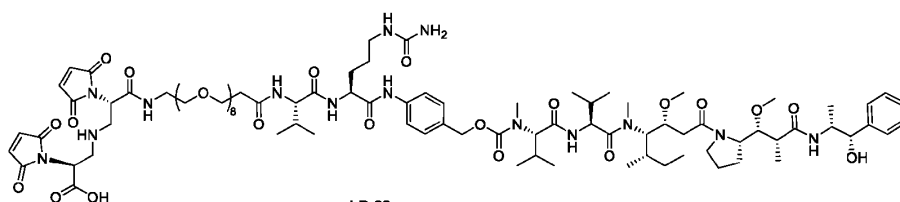
LD-19



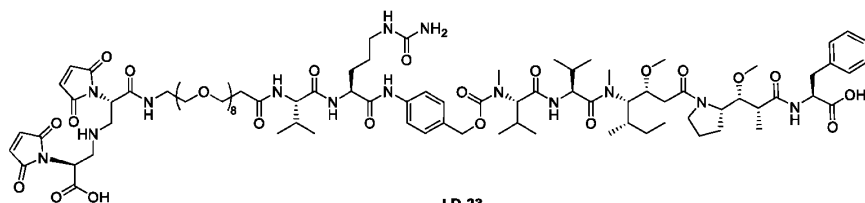
LD-20



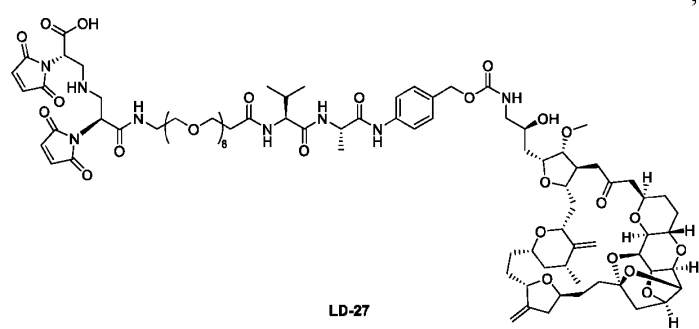
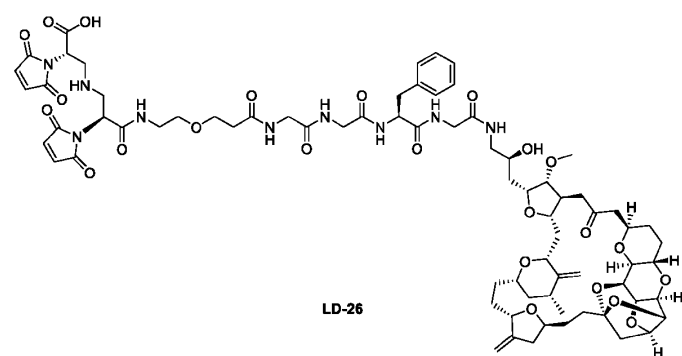
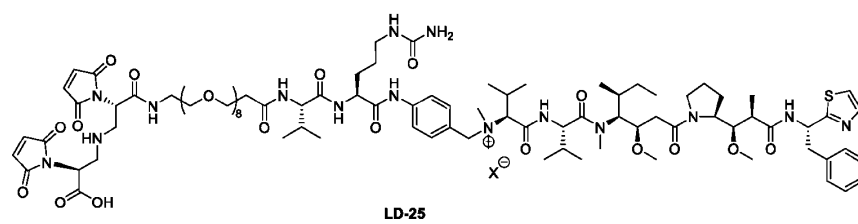
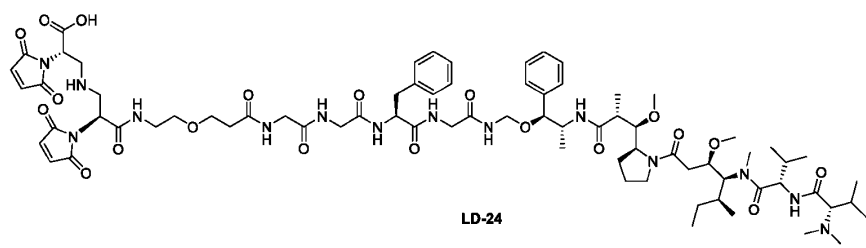
LD-21

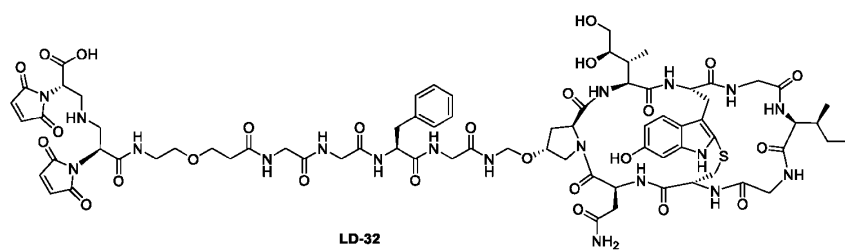
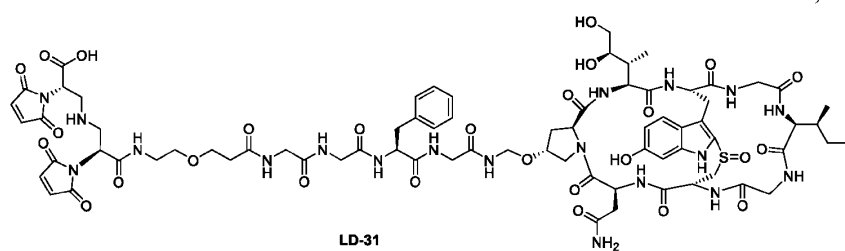
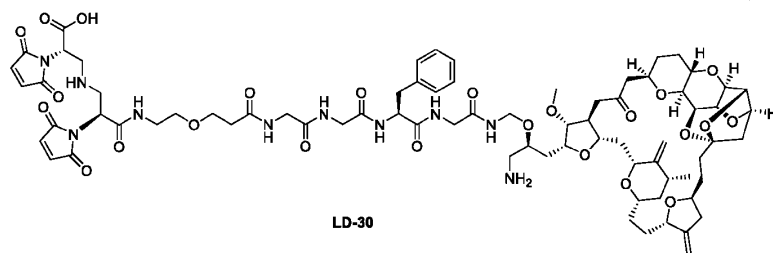
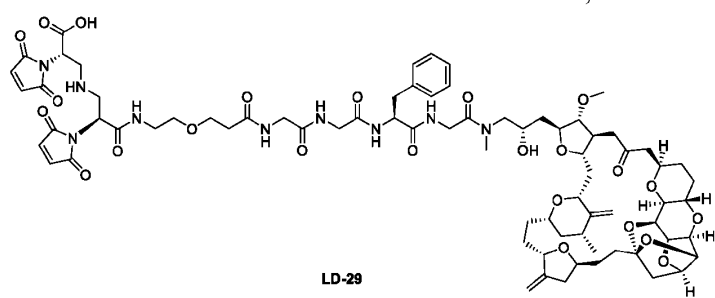
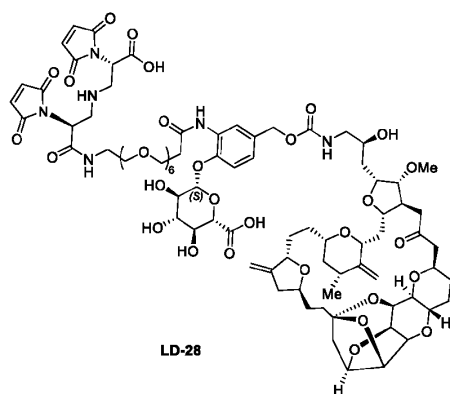


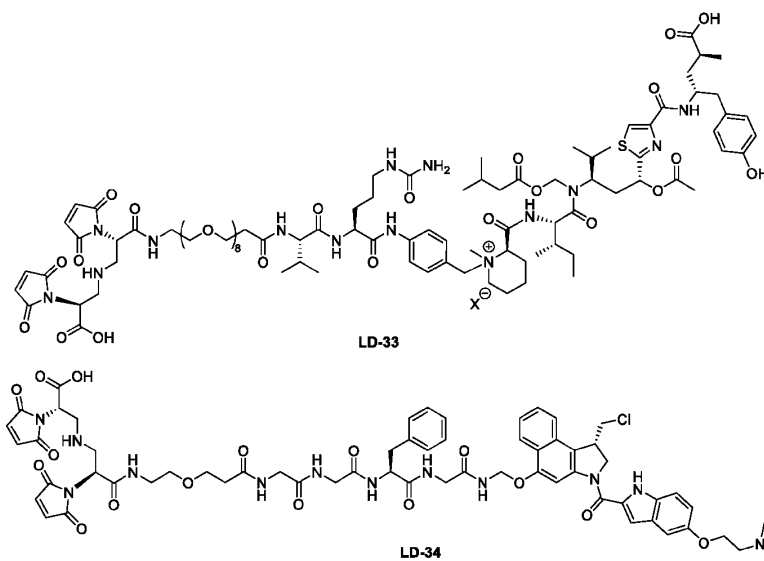
LD-22



LD-23

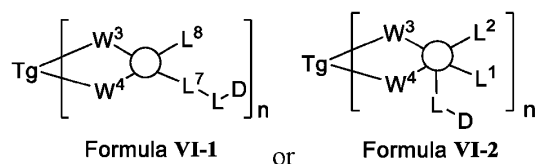






wherein each X^- is independently selected from Cl^- , Br^- , I^- , CF_3COO^- , $MeSO_3^-$, preferably CF_3COO^- .

19. A ligand-drug conjugate as shown in Formula **VI-1** or Formula **VI-2**, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof,



wherein,

Tg is selected from the ligand or targeting portion that binds to the target;

L^1 , L^2 are identical or different, and each independently comprises hydrogen, deuterium, substituted or unsubstituted C_1-C_6 alkyl, substituted or unsubstituted C_2-C_6 alkenyl, substituted or unsubstituted C_2-C_6 alkynyl, aryl, heteroaryl, C_3-C_{10} cycloalkyl, 3-10 membered heterocyclic, carboxylic acid, amide, ester, sulfite, sulfonate, phosphoric acid, pyrophosphoric acid, natural or unnatural amino acid residues, polyethylene glycol, or a combination thereof;

L^8 comprises hydrogen, deuterium, substituted or unsubstituted C_1-C_6 alkyl, substituted or unsubstituted C_2-C_6 alkenyl, substituted or unsubstituted C_2-C_6 alkynyl, aryl, heteroaryl, C_3-C_{10} cycloalkyl, 3-10-membered heterocyclic, carboxylic acid, amide, ester, sulfinyl, sulfonate, phosphoric acid, pyrophosphoric acid, natural or unnatural amino acid residues, or polyethylene glycol, or a combination thereof;

L^7 comprises one or more combinations of substituted or unsubstituted C_1-C_6 alkylene, substituted or unsubstituted C_2-C_6 alkenyl, substituted or unsubstituted C_2-C_6 alkynyl, aryl,

heteroaryl, C₃-C₁₀ cycloalkyl, 3-10-membered heterocyclic, carbonyl, amide, ester, sulfinyl, sulfonate, phosphoric acid group, pyrophosphoric acid group, natural or unnatural amino acid residue, or polyethylene glycol;

W³, W⁴ are identical or different, and each independently comprises a connecting group, wherein at least one of W³, W⁴ comprises a succinimide structure; preferably, the succinimide structure is a closed-ring form, an open-ring form or any combination thereof; preferably, the succinimide structure is in an open-ring form or any combination of closed-ring form and open-ring form; preferably, the succinimide structure is in an open-ring form;

L¹, L², L⁸, L⁷ or W³, W⁴ are covalently linked to the circle, wherein the circle represents a scaffold containing a basic group;

L is a linker;

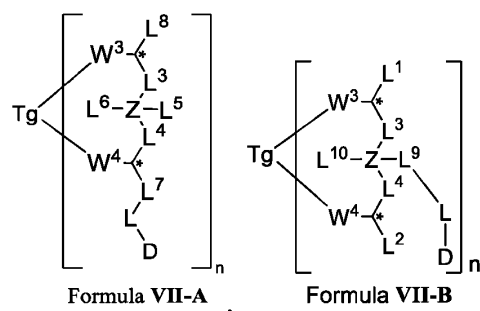
n is an integer or decimal from 1 to 10;

D is the drug unit derived from a drug or a drug precursor, and said drug unit is linked to L covalently;

preferably, D is a drug linked to L covalently;

alternatively preferably, D is a drug precursor is linked to L covalently.

20. The ligand-drug conjugate as shown in Formula **VI-1** or Formula **VI-2**, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof, according to Claim 19, wherein said ligand-drug conjugate has a structure as shown in the following Formula **VII-A** or **VII-B**, preferably having a structure as shown in Formula **VII-A**;

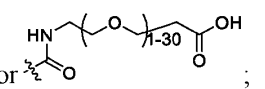


wherein,

Tg is the ligand or the targeting portion thereof having a binding affinity to a target;

The carbon atom at the position shown * is a chiral carbon having an absolute configuration of R or S;

L¹, L² are identical or different, and each independently comprises hydrogen, deuterium, substituted or unsubstituted C₁-C₆ alkyl, substituted or unsubstituted C₂-C₆ alkenyl, substituted or unsubstituted C₂-C₆ alkynyl, aryl, heteroaryl, C₃-C₁₀ cycloalkyl, 3-10 membered heterocyclic, carboxyl, amide, ester, sulfite, sulfonate, phosphoric acid, pyrophosphoric acid, natural or unnatural amino acid residues, polyethylene glycol, or a combination thereof;

preferably, L^1 , L^2 each independently comprises carboxyl, or  ;

preferably, L^1 , L^2 each independently comprises the carboxyl,  ;

L^3 , L^4 are identical or different, and each independently comprises substituted or unsubstituted C_1 - C_6 alkylene, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10-membered heterocyclic, polyethylene glycol, or a combination thereof;

preferably, L^3 , L^4 each independently comprises substituted or unsubstituted C_1 - C_6 alkylene;

preferably, L^3 , L^4 each independently comprises C_1 - C_6 alkylene;

preferably, L^3 , L^4 each independently comprises methylene, ethylene;

L^5 , L^6 are present or absent, identical or different, and when present each independently comprises hydrogen, deuterium, oxygen, sulfur, hydroxyl, carbonyl, amide, ester, tert-butoxycarbonyl (Boc), benzyloxycarbonyl (Cbz), allyloxycarbonyl (Alloc), benzyl, substituted or unsubstituted C_1 - C_6 alkylidene, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10-membered heterocyclic, polyethylene glycol, or a combination thereof;

preferably, L^6 is absent, L^5 is present, and L^5 comprises hydrogen, substituted or unsubstituted C_1 - C_6 alkyl;

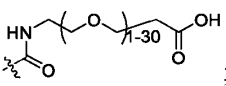
preferably, L^6 is absent, L^5 is present, and L^5 comprises hydrogen, C_1 - C_6 alkyl;

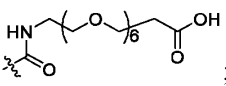
preferably, L^6 is absent, L^5 is present, and L^5 is hydrogen;

L^7 comprises substituted or unsubstituted C_1 - C_6 alkylene, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, a 3-10-membered heterocyclic, carbonyl, amide, ester, sulfinyl, sulfonate, phosphoric acid, pyrophosphoric acid, natural or unnatural amino acid residue, polyethylene glycol, or a combination thereof;

preferably, L^7 is carbonyl;

L^8 comprises hydrogen, deuterium, substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-10-membered heterocyclic, carboxylic acid, amide, ester, sulfinyl, sulfonate, phosphoric acid, pyrophosphoric acid, natural or unnatural amino acid residues, or polyethylene glycol, or a combination thereof;

preferably, L^8 is selected from the carboxyl,  ;

preferably, L^8 is selected from the carboxyl,  ;

L^9 comprises one or more combinations selected from the group consisting of oxygen, sulfur, carbonyl, amide, ester, substituted or unsubstituted C_1 - C_6 alkylene, substituted or unsubstituted C_2 - C_6 alkenyl, substituted or unsubstituted C_2 - C_6 alkynyl, aryl, heteroaryl, C_3 - C_{10} cycloalkyl, 3-

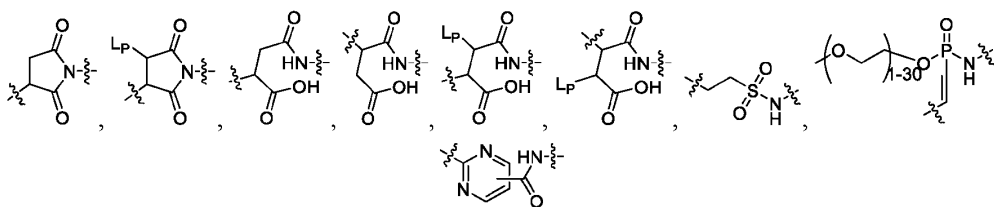
10-membered heterocyclic, and polyethylene glycol;

L¹⁰ present or absent, when present is selected from hydrogen, deuterium, oxygen, hydroxyl, carbonyl, amide, ester, tert-butoxycarbonyl (Boc), benzyloxycarbonyl (Cbz), allyloxycarbonyl (Alloc), benzyl, substituted or unsubstituted C₁-C₆ alkylene, substituted or unsubstituted C₂-C₆ alkenyl, substituted or unsubstituted C₂-C₆ alkynyl, aryl, heteroaryl, C₃-C₁₀ cycloalkyl, 3- 10-membered heterocyclic, a combination of one or more of polyethylene glycol;

preferably, L^{10} is not present;

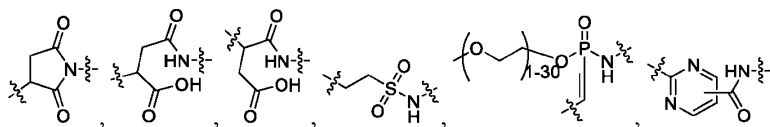
W³, W⁴ are identical or different, and each independently comprises a connecting group, wherein at least one of W³, W⁴ comprises a succinimide structure; preferably, the succinimide structure is a closed-ring form, an open-ring form or any combination thereof; preferably, the succinimide structure is in an open-ring form or any combination of closed-ring form and open-ring form; preferably, the succinimide structure is in an open-ring form;

preferably, W³, W⁴ are identical or different, and each independently comprises the following, their racemates, enantiomers, diastereomers, pharmaceutically acceptable salts or solvates thereof, or the combination thereof:



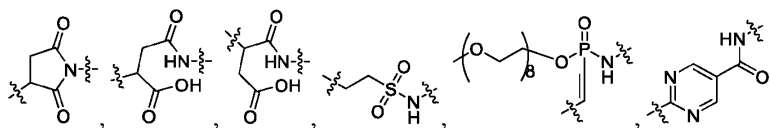
and at least one of W^3 , W^4 is selected from groups comprising succinimide structure, and each L_P is selected from the same or a different, including, but not limited to C1- C6 alkyl groups, chloride, bromide, iodide, OMs, OTs; preferably, the succinimide structure is a closed-ring form, an open-ring form or any combination thereof; preferably, the succinimide structure is in an open-ring form or any combination of closed-ring form and open-ring form; preferably, the succinimide structure is in an open-ring form;

preferably, W³, W⁴ are identical or different, and each independently comprises the following or any combination of the following or racemates thereof, enantiomers, diastereomers, pharmaceutically acceptable salts or solvates or the following:



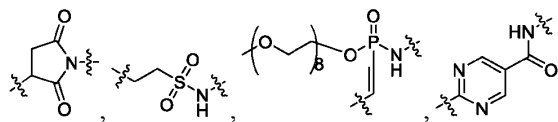
and at least one of W³, W⁴ is selected from a group comprising the succinimide structure; preferably, the succinimide structure is a closed-ring form, an open-ring form or any combination thereof; preferably, the succinimide structure is in an open-ring form or any combination of closed-ring form and open-ring form; preferably, the succinimide structure is in an open-ring form;

preferably, W³, W⁴ are identical or different, and each independently comprises the following or any combination of the following or racemates thereof, enantiomers, diastereomers, pharmaceutically acceptable salts or solvates or the following:



and at least one of W^3 , W^4 is selected from a group comprising the succinimide structure; preferably, the succinimide structure is in a closed-ring form, an open-ring form, or any combination thereof; preferably, the succinimide structure is in an open-ring form or any combination of closed-ring form and open-ring form; preferably, the succinimide structure is in an open-ring form;

preferably, W^3 , W^4 are identical or different, and each independently comprises the following or any combination of the following or racemates, enantiomers, diastereomers, pharmaceutically acceptable salts or solvates thereof, or any combination of the following:



and at least one of W^3 , W^4 is selected from a group comprising the succinimide structure; preferably, the succinimide structure is a closed-ring form, an open-ring form or any combination thereof; preferably, the succinimide structure is in an open-ring form or any combination of closed-ring form and open-ring form; preferably, the succinimide structure is in an open-ring form;

Z is selected from N, O, S or quaternary amines;

preferably, Z is N;

L is a linker;

n is chosen from whole numbers or decimals from 1 to 10;

D is selected from drug/drug precursors and said drug/drug precursor is linked to L covalently;

preferably, D is a drug and said drug is linked to L covalently; or

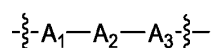
preferably, D is a drug precursor and said drug precursor is linked to L covalently.

21. The ligand-drug conjugate, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 19 or 20, wherein the Tg is selected from a ligand or targeting portion thereof having a binding affinity to the target; preferably, the Tg is selected from an antibody, a protein or a polypeptide that binds to an antigen; or preferably, the Tg is selected from an antibody.

22. The ligand-drug conjugate, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 19-21, wherein the Tg bound to the target is selected from a mono-specific antibody, a bispecific antibody, or a multispecific antibody, including, but not limited to, an anti-EGFRvIII antibody, an anti-DLL-3 antibody, anti-PSMA antibody, anti-CD70 antibody, anti-MUC16 antibody, anti-ENPP3 antibody, anti-TDGF1 antibody, anti-ETBR antibody, anti-MSLN antibody, anti-TIM-1 antibody, anti-LRRC15 antibody, anti-LIV-1 antibody, anti-CanAg/AFP antibody, anti-Claudin 18.2 antibody, anti-PLAUR antibody, anti-Mesothelin antibody, anti-HER2(ErbB2) antibody, anti-EGFR antibody, anti-c-MET antibody, anti-SLITRK6 antibody, anti-KIT/CD117 antibody, anti-

STEAP1 antibody, anti-SLAMF7/CS1 antibody, anti-NaPi2B/SLC34A2 antibody, anti-GPNMB antibody, anti-HER3 antibody, anti HLA-G antibody, anti-MUC1/CD227 antibody, anti-EGFR/HER3 antibody, anti-AXL antibody, anti-GPC3 antibody, anti-CD166 antibody, anti-B7-H3(CD276) antibody, anti-PTK7/CKK4 antibody, anti-PRLR antibody, anti-EFNA4 antibody, anti-5T4 antibody, anti-NOTCH3 antibody, anti-Nectin 4 Antibodies, Anti-TROP-2 Antibodies, Anti-NKG2D Antibodies, Anti-CD142 Antibodies, Anti-CA6 Antibodies, Anti-GPR20 Antibodies, Anti-CD174 Antibodies, Anti-CD71 Antibodies, Anti-EphA2 Antibodies, Anti-LYPD3 Antibodies, Anti-TF Antibodies, Anti-FGFR2 Antibodies, Anti-FGFR3 Antibodies, Anti-FR α Antibodies, Anti-CEACAMs Antibodies, Anti-GCC Antibodies anti-CXCR4 antibody, anti-GPRC5D antibody, anti-CCR5 antibody, anti-CCR3 antibody, anti-LGR4 antibody, anti-SSTR2 antibody, anti-Integrin Av antibody, anti-CAIX antibody, anti-cadherin antibody, anti-GD3 antibody, anti-cadherin 6 antibody, anti-LAMP1 antibody, anti-FLT3 antibody, anti-BCMA antibody, anti-CD79 antibody, anti-CD79D3 antibody, anti-TF antibody, anti-FGFR2 antibody, anti-FGFR3 antibody, anti-CEACAMs antibody, anti-GCC antibody, anti-FGFR2 antibody, anti-FR α antibody, anti-CEACAMs antibody, anti-GCC antibody BCMA antibody, anti-CD79b antibody, anti-CD19 antibody, anti-ROR1 antibody, anti-CD33 antibody, anti-CD45 antibody, anti-CD56 antibody, anti-CD74 antibody, anti-CD22 antibody, anti-CD30 antibody, anti-CD37 antibody, anti-CD138 antibody, anti-Nectin-4 antibody, anti-CD352 antibody, anti-CD25 antibody, or anti-CD123 antibody. CD123 antibody and combinations thereof to form a double or multiple antibody, preferably an anti-TROP-2 antibody.

23. The ligand-drug conjugate, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 19-22, wherein said linker L has the structure shown in the Formula V below.



Formula V

wherein,

A₁, A₂, and A₃ each exist or do not exist independently;

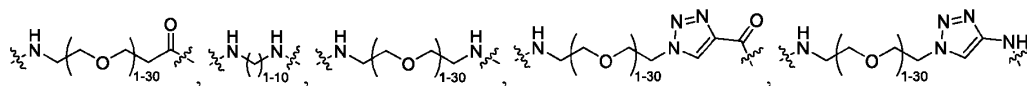
when A₁ is present, A₁ is selected from a modifying unit, preferably from a branchable unit or a hydrophilic unit;

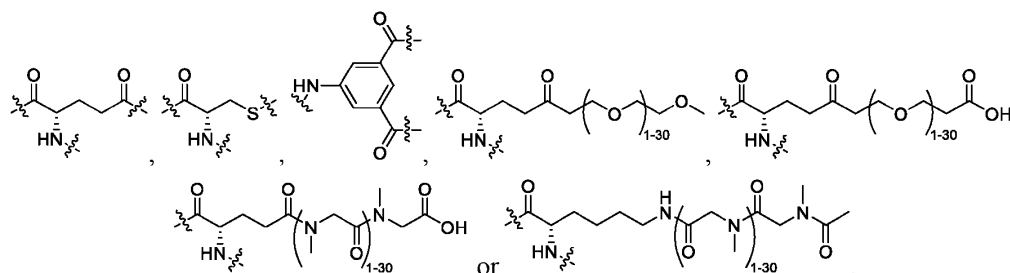
when A₂ is present, A₂ is selected from breakable or non-breakable units;

when A₃ is present, A₃ is selected from the elimination unit or chelation unit;

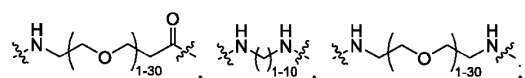
the position shown by the wavy line is the connection site.

24. The ligand-drug conjugate, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof, according to Claim 23, wherein A₁ is present or absent, and when A₁ is present, A₁ comprises the following, or combinations thereof:

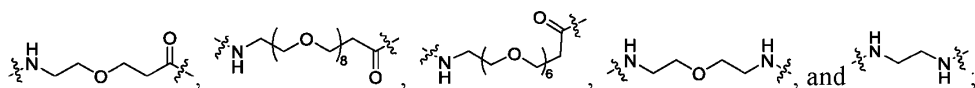




preferably, A₁ is present or absent, and when A₁ is present, A₁ comprises the following or combinations thereof:



preferably, A₁ is present or absent, and when A₁ is present, A₁ comprises the following or combinations thereof:



where the positions shown by the wavy lines are connection sites.

25. The ligand-drug conjugate, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 23-24, wherein A₂ is present or absent, and when A₂ is present, said A₂ comprises a breakable or unbreakable unit, wherein the unbreakable unit comprises substituted or unsubstituted C₁-C₆ alkyl, substituted or unsubstituted C₂-C₆ alkenyl, substituted or unsubstituted C₂-C₆ alkynyl, aryl, heteroaryl, C₃-C₁₀ cycloalkyl, 3-10-membered heterocyclic, amide, ester, or polyethylene glycol or a combination thereof.

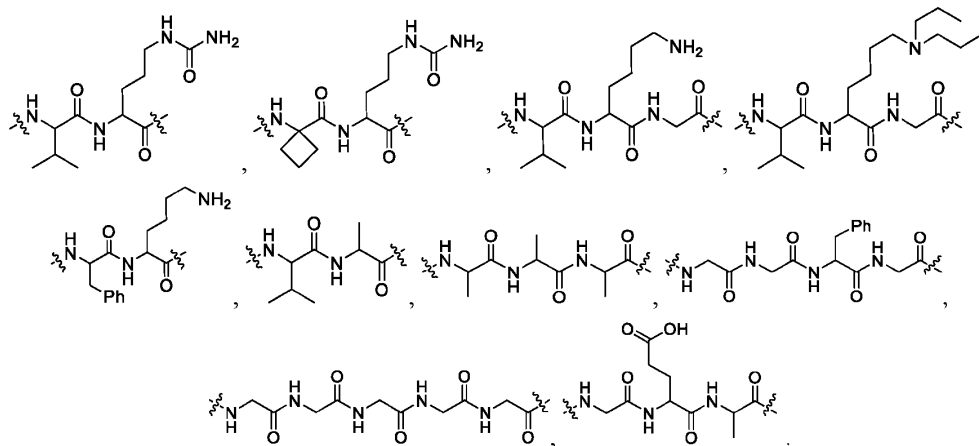
26. The ligand-drug conjugate, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 23-25, wherein A₂ is present or absent, and when A₂ is present said A₂ comprises a breakable or non-breakable unit, wherein the breakable unit comprises a non-enzyme-sensitive ligating units, enzyme-sensitive ligation units, or a combination thereof;

preferably, said non-enzyme-sensitive linker unit comprises glutathione-sensitive disulfide bond linker unit, pH-sensitive hydrazone linker unit;

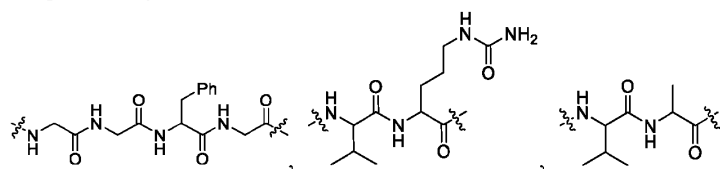
preferably, said enzyme-sensitive ligating unit comprises a histone protease breakable ligating unit, a phosphodiesterase or pyrophosphate esterase breakable ligating unit, a β -glucuronidase breakable ligating unit, a β -galactosidase breakable ligating unit, a sulfate esterase breakable ligating unit or a combination thereof.

27. The ligand-drug conjugate, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 23-26, wherein A₂ is present or absent, and when A₂ is present, said A₂ is a breakable unit, wherein said breakable unit comprises a peptide residue comprising from 2 to 10 natural or non-natural amino acid residues, wherein said natural or unnatural amino acid is optionally substituted with

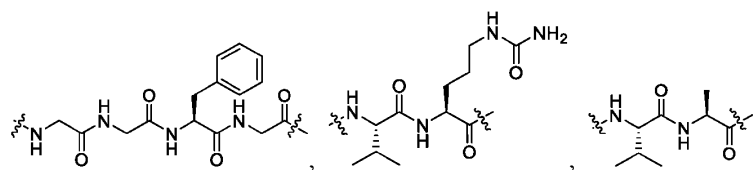
one or more substituents,
preferably, said breakable unit comprises:



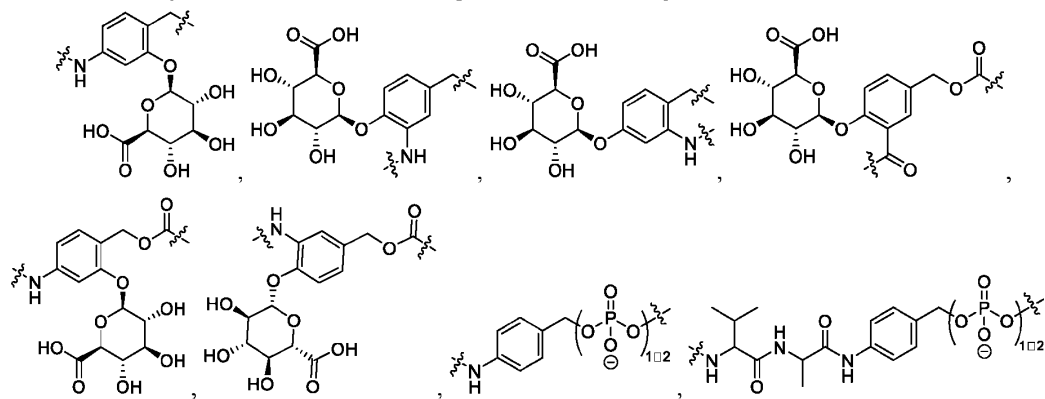
preferably:

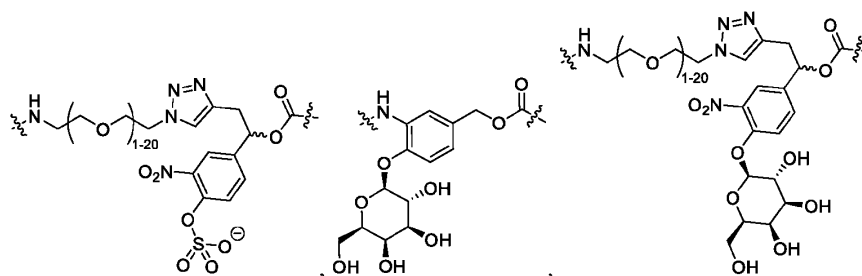


Preferably:

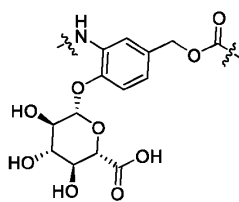


Alternatively, said breakable unit comprises the following or isomers thereof:



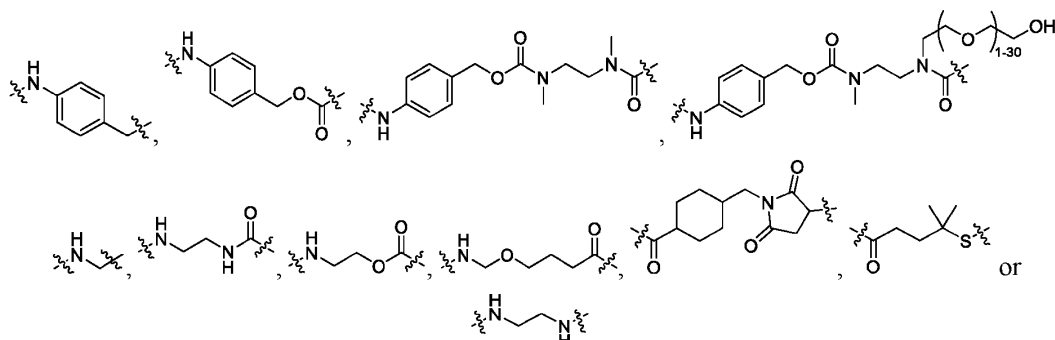


preferably the following or isomers thereof:

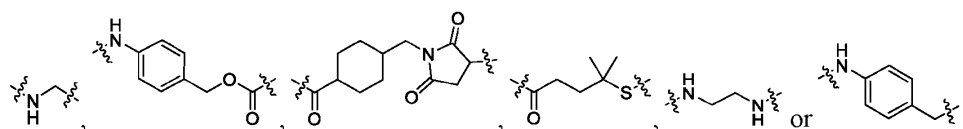


wherein the position shown by the wavy line is the connection site.

28. The ligand-drug conjugate, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 23-27, wherein A₃ is present or absent, and when A₃ is present, A₃ comprises the following:



preferably, A₃ is present or absent, and when A₃ is present, A₃ comprises the following:



wherein the position shown by the wavy line is the connection site.

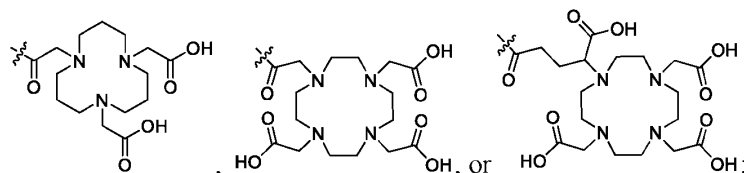
29. The ligand-drug conjugate, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 19-28, wherein: said ligand-drug conjugate has one or more technical features selected from one or more of the following (i)-(iii):

(i) said drug comprises cytotoxic drugs, radioisotopes, detection or diagnostic reagents, enzyme inhibitors, protein degraders, immunomodulators, peptides or nucleotides;

preferably, said drug comprises cytotoxic drugs;

(ii) said drug is obtained by chelating a drug precursor with a metal ion, preferably a radioisotope;

(iii) said drug precursor comprises



preferably, said drug precursor is obtained by chelating the drug with a metal ion, preferably a radioisotope.

30. The ligand-drug conjugate, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof, according to Claim 29, wherein: said cytotoxic drug comprises DNA damaging agents, DNA topoisomerase inhibitors, microtubule or microtubule protein inhibitors, RNA polymerase inhibitors;

preferably, said DNA damaging agents comprise derivatives of calicheamicin, anthramycin, ecteinascidins toxin, adriamycin, mitomycin, Duocarmycin;

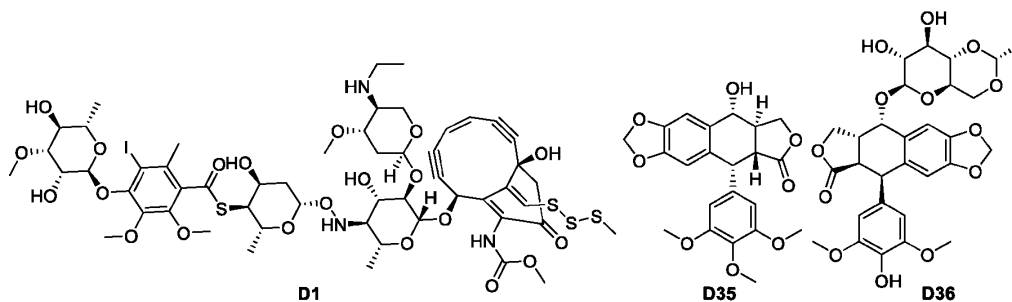
preferably, said DNA topoisomerase inhibitors comprise derivatives of camptothecin, podophyllotoxin, or etoposide;

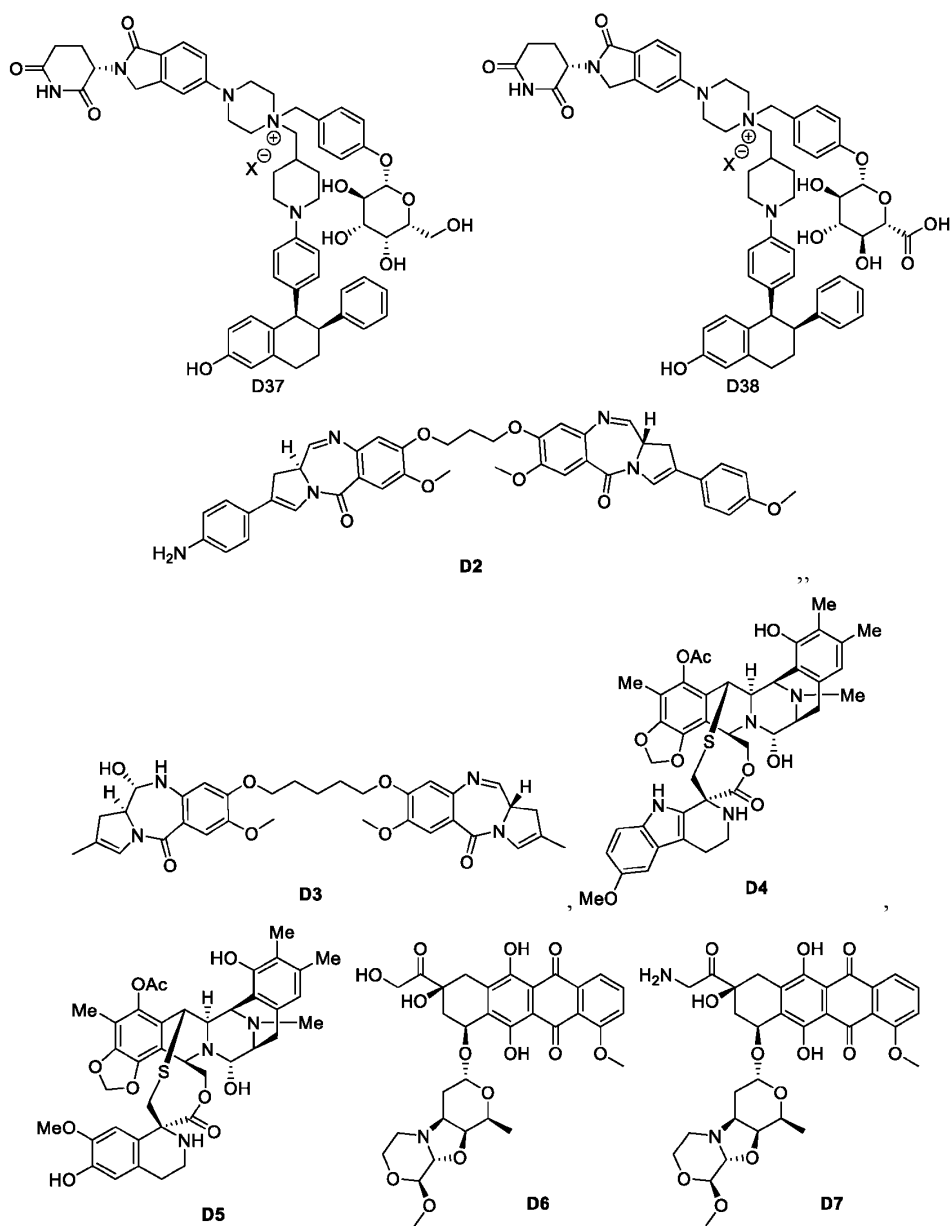
preferably, said microtubule or microtubule protein inhibitors comprise derivatives of maytansine, auristatin, eribulin, or tubulysin;

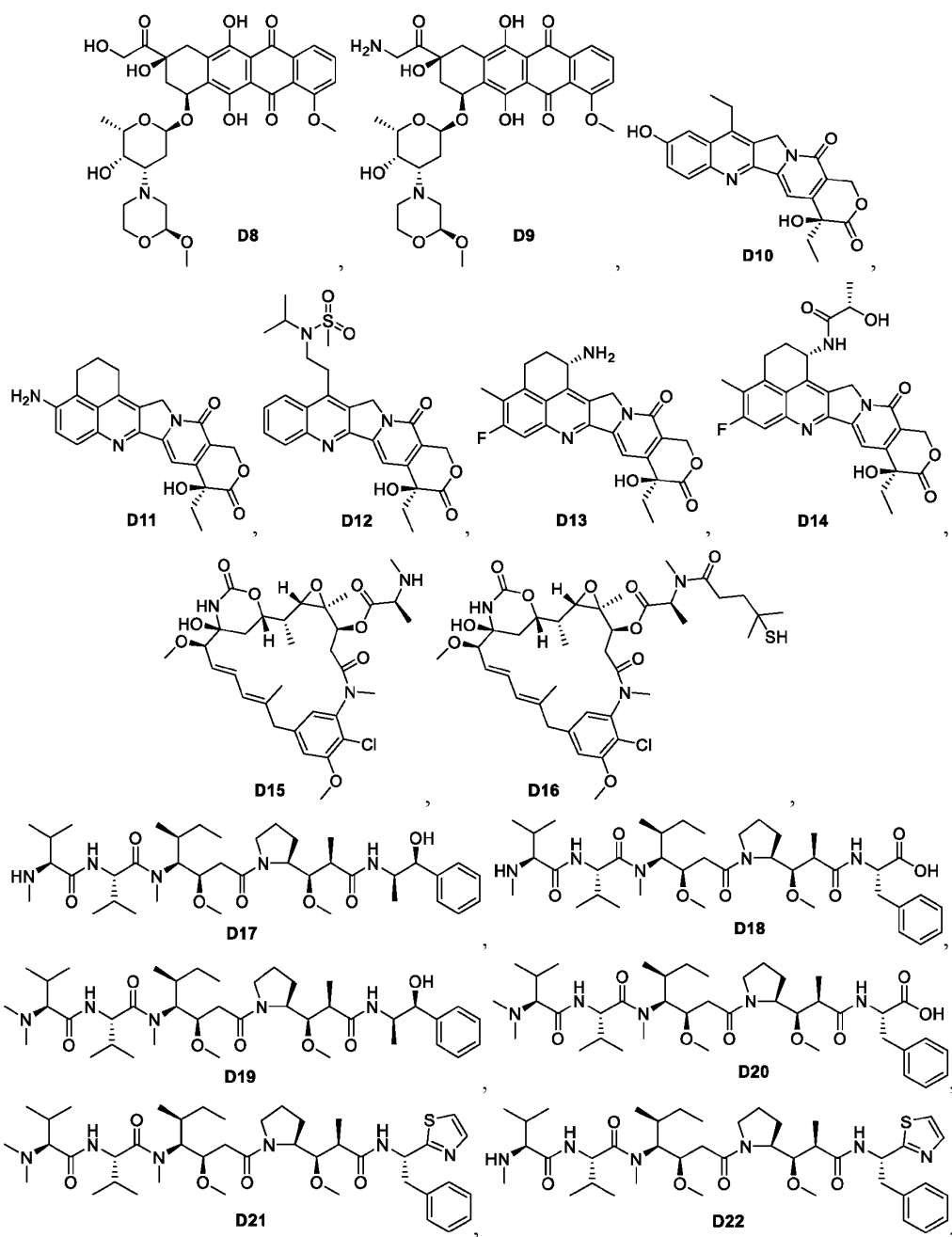
preferably, said RNA polymerase inhibitors comprise derivatives of amanitin.

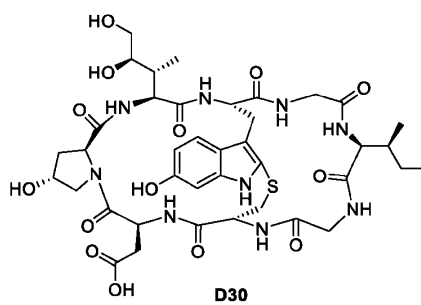
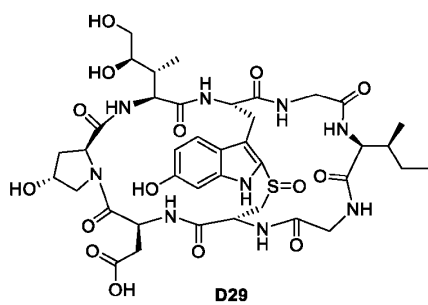
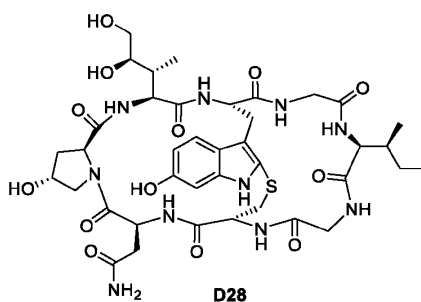
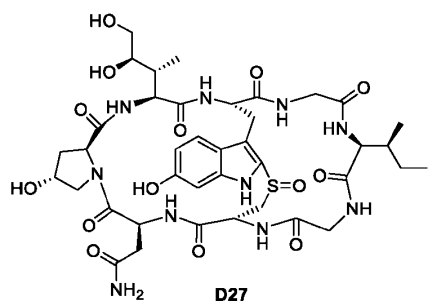
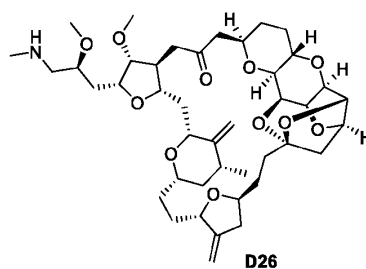
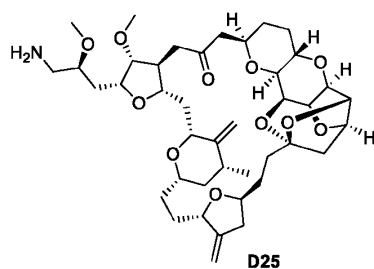
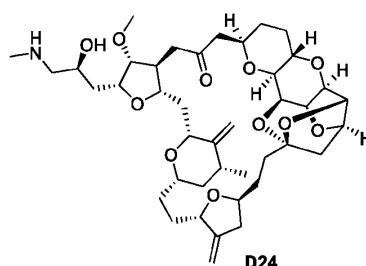
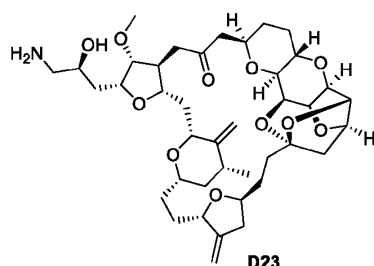
31. The ligand-drug conjugate, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 19-30, wherein said ligand-drug conjugate has one or more technical features selected from one or more of the following (i)-(iii):

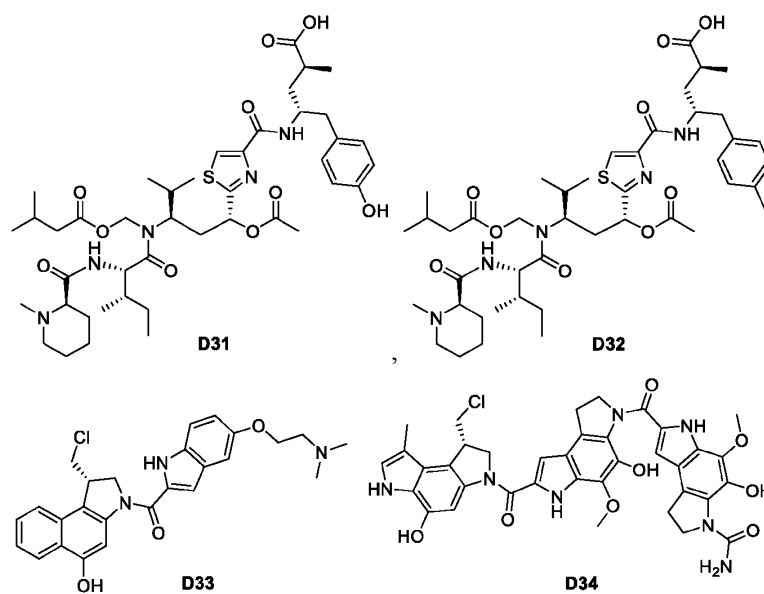
(i) said drug comprises the following:





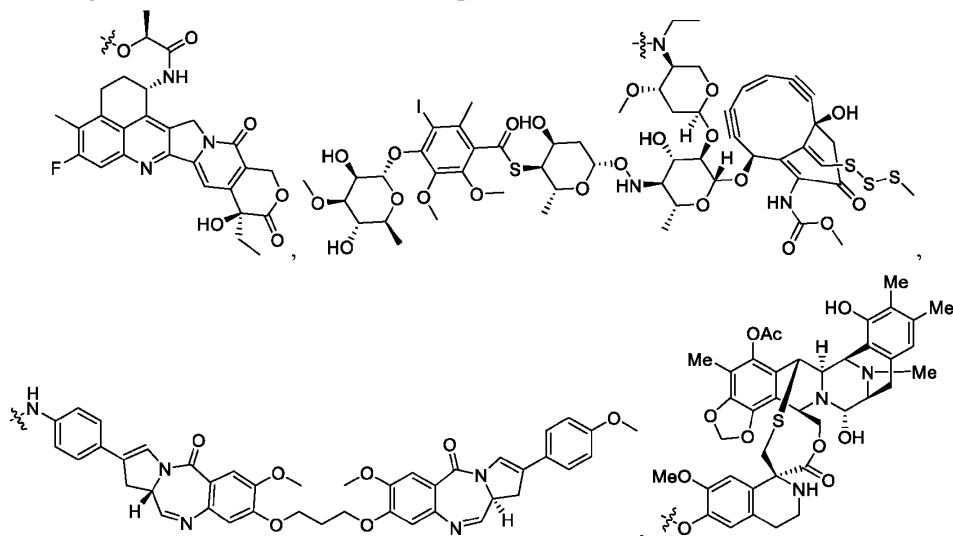


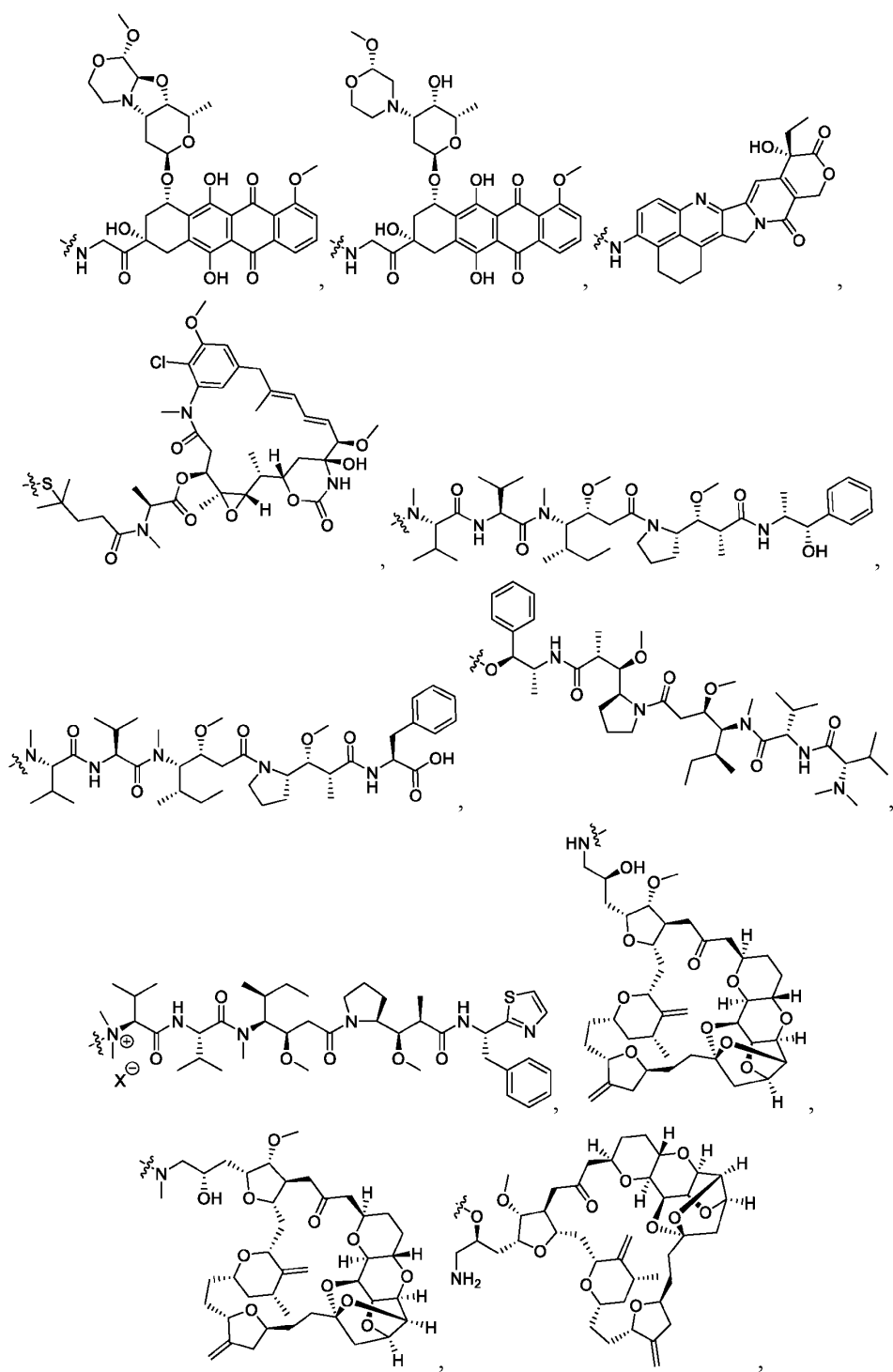


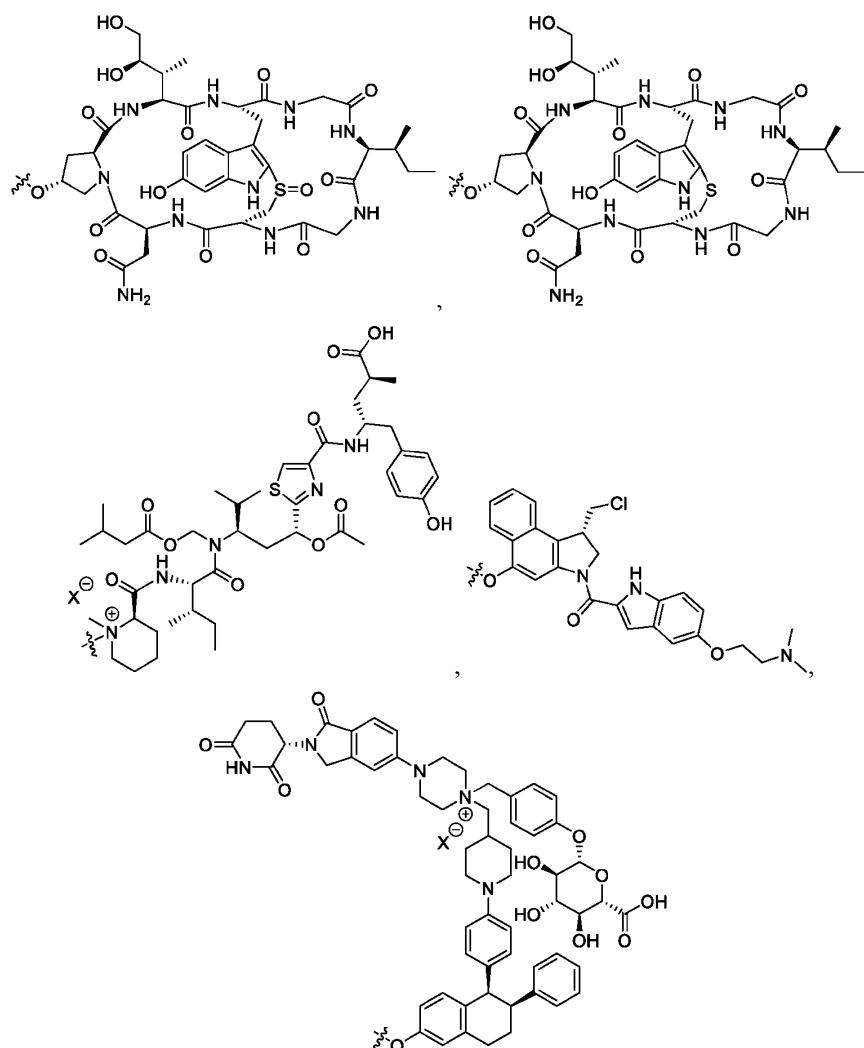


wherein each X^- is independently selected from Cl^- , Br^- , I^- , $MeSO_3^-$, CF_3COO^- , preferably CF_3COO^- ;

preferably, D is selected from the following:

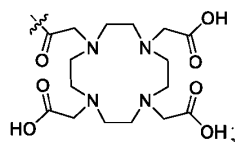






wherein each X^- is independently selected from Cl^- , Br^- , I^- , $MeSO_3^-$, CF_3COO^- , preferably CF_3COO^- ;

- (ii) said drug is obtained by chelating a drug precursor with a metal ion (e.g. a radioisotope);
- (iii) said drug precursor includes, but is not limited to, the following structure:



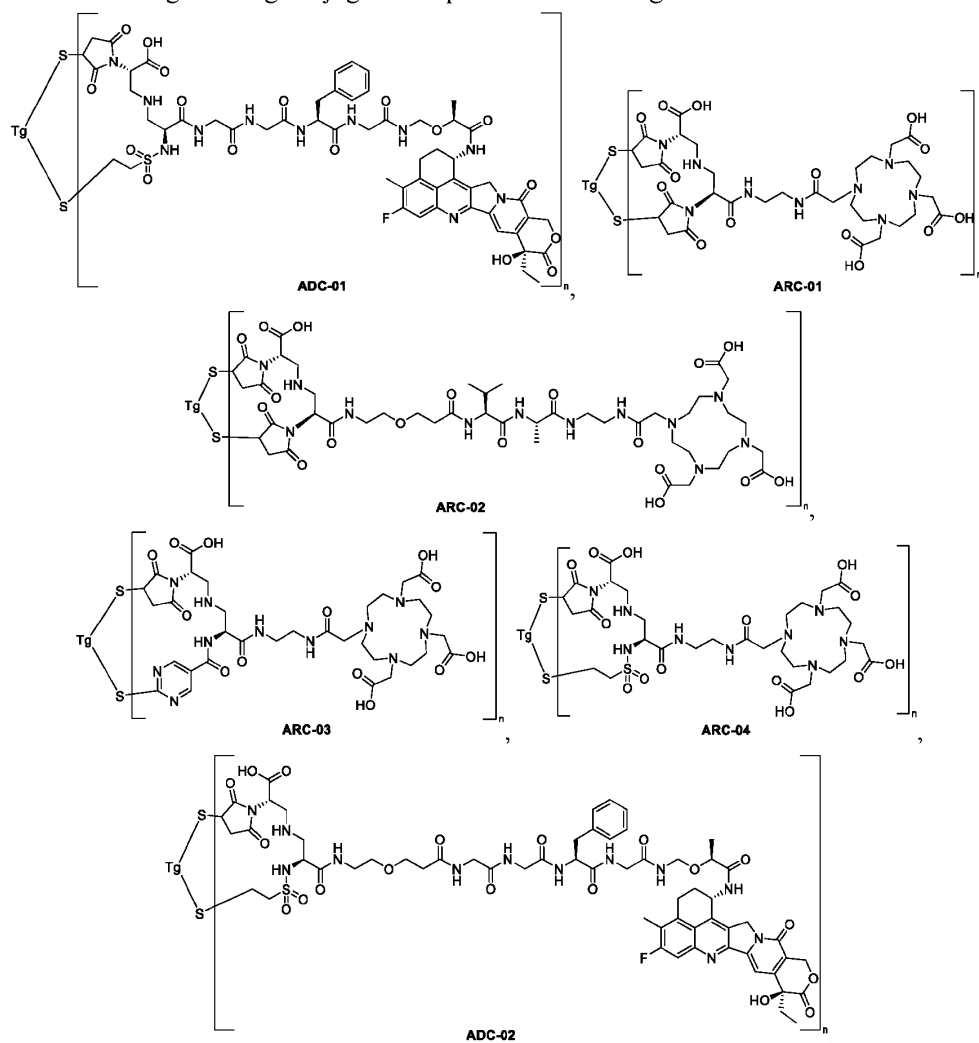
preferably, said drug precursor is obtained by chelating the drug with a metal ion comprising a radioisotope.

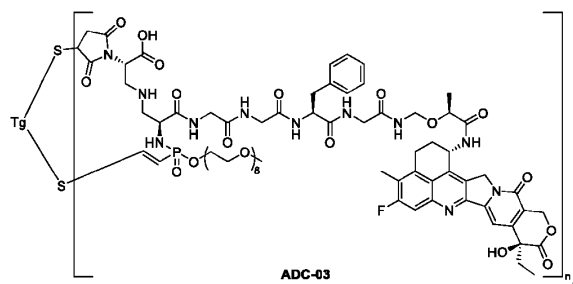
32. The ligand-drug conjugate, its racemate, enantiomer, diastereomer, open-ring form, or

pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 29-31, wherein: said radioisotope comprises ^{18}F , ^{67}Ga , ^{68}Ga , $^{99\text{m}}\text{Tc}$, ^{123}I , ^{125}I , ^{131}I , ^{90}Y , ^{177}Lu , ^{47}Sc , ^{64}Cu , ^{67}Cu , ^{32}P , ^{186}Re , ^{188}Re , ^{89}Sr , ^{153}Sm , ^{198}Au , ^{166}Ho , ^{165}Dy , ^{169}Er , ^{149}Tb , ^{161}Tb , ^{211}At , ^{212}Bi , ^{213}Bi , ^{212}Pb , ^{223}Ra , ^{225}Ac , ^{226}Th , ^{227}Th , or ^{230}U ;

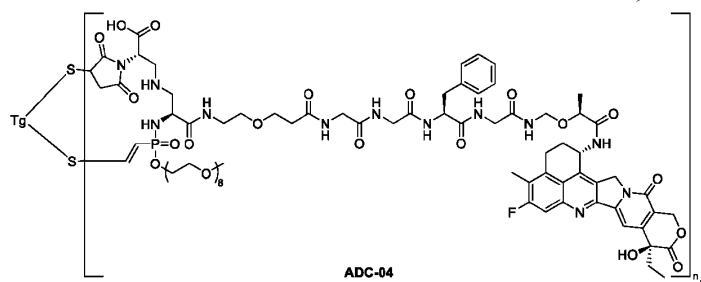
preferably, said radioisotope comprises ^{68}Ga , $^{99\text{m}}\text{Tc}$, ^{131}I , ^{90}Y , ^{177}Lu , ^{64}Cu , ^{211}At , ^{213}Bi , ^{212}Pb , or ^{225}Ac .

33. The ligand-drug conjugate, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 19-32, wherein said ligand-drug conjugate comprises the following:

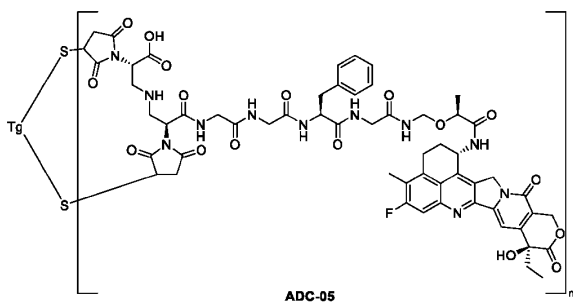




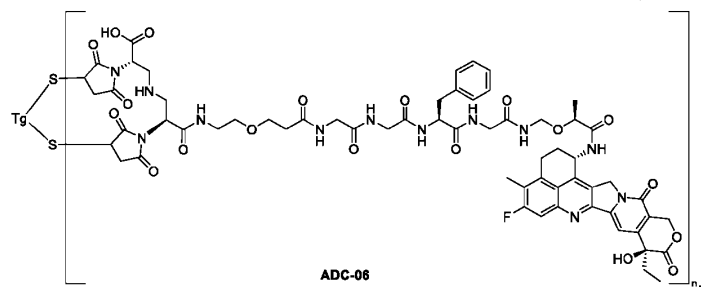
ADC-03



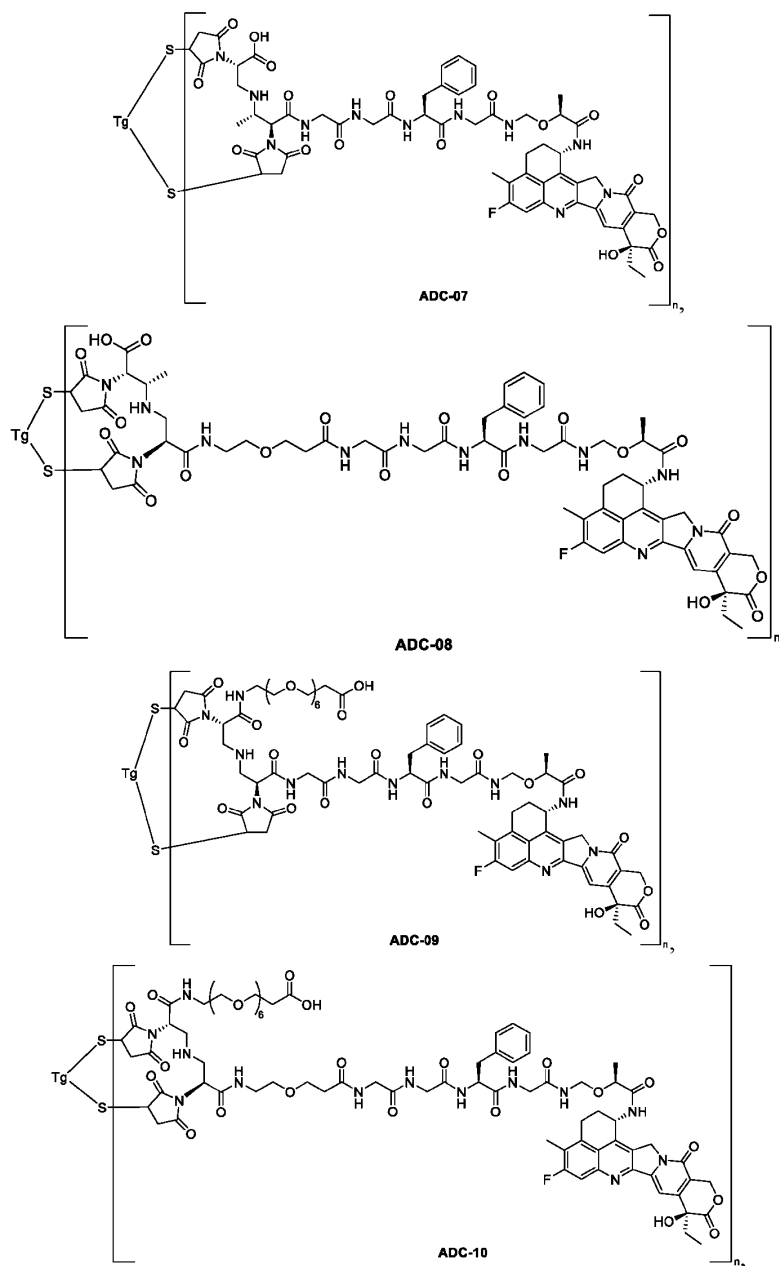
ADC-04

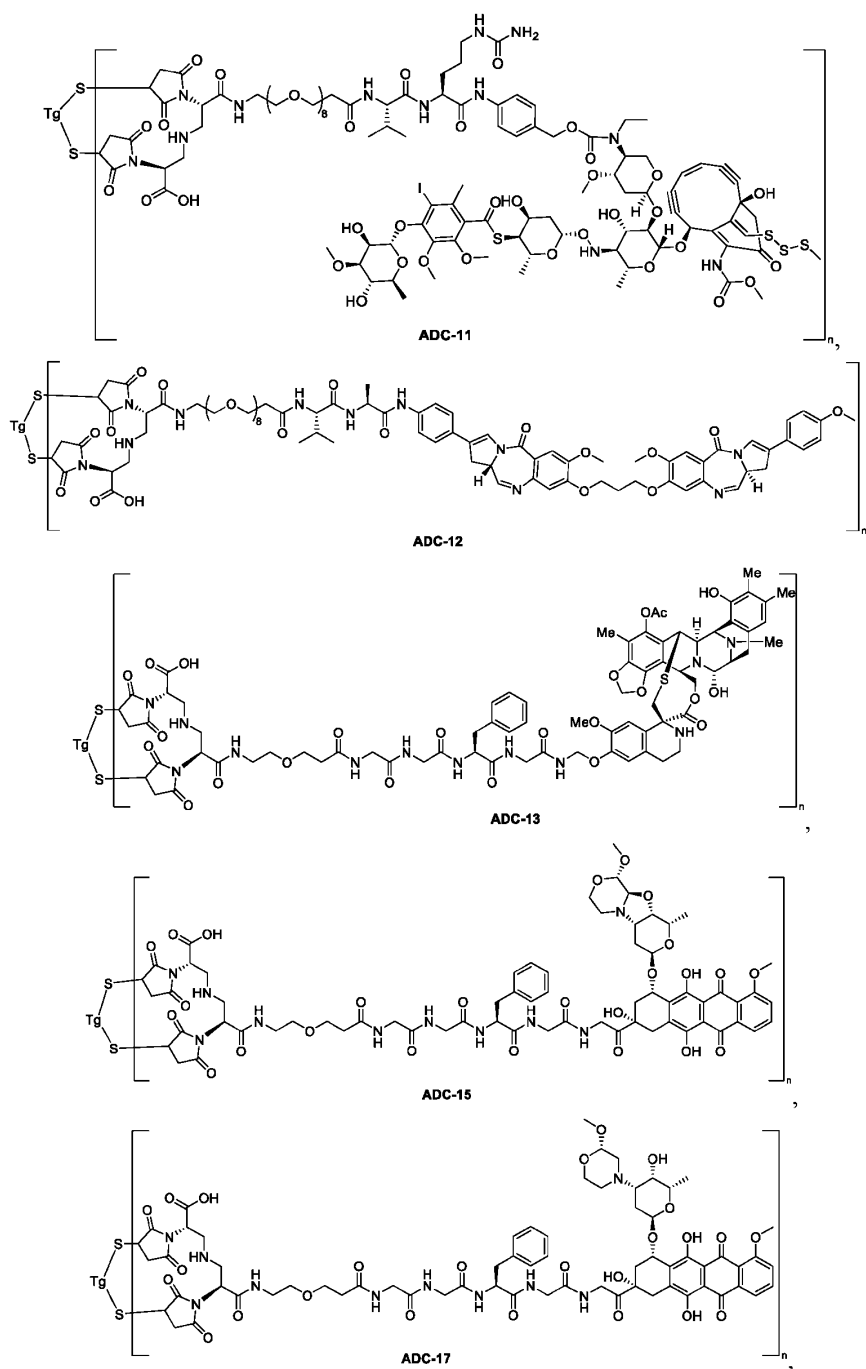


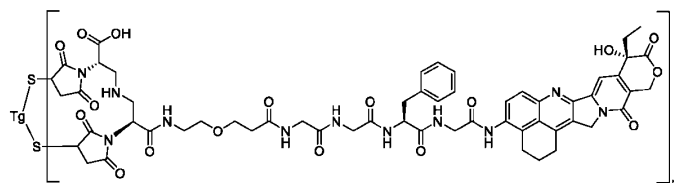
ADC-05



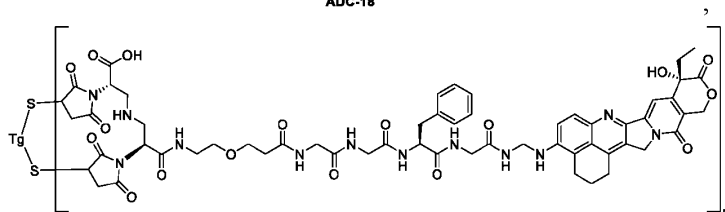
ADC-06



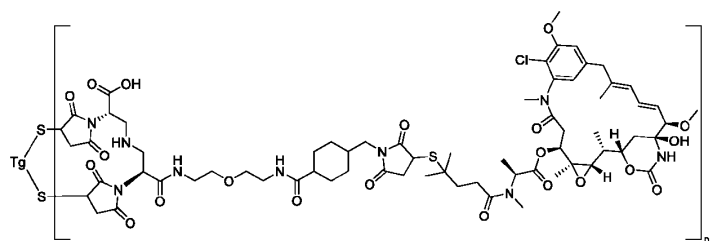




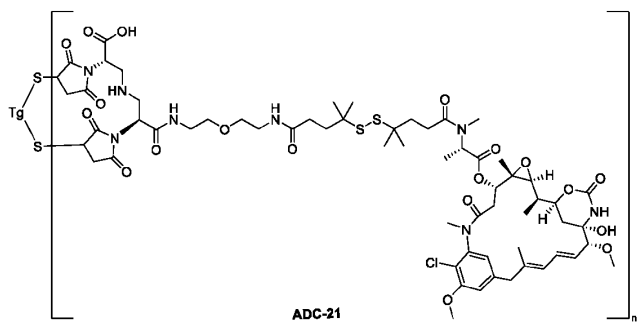
ADC-18



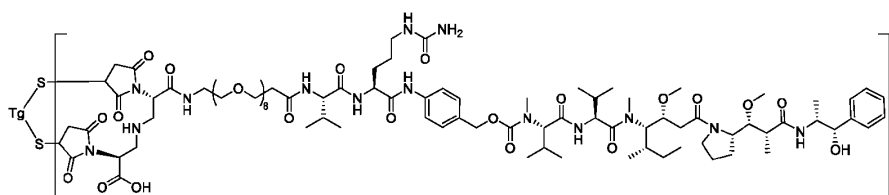
ADC-19



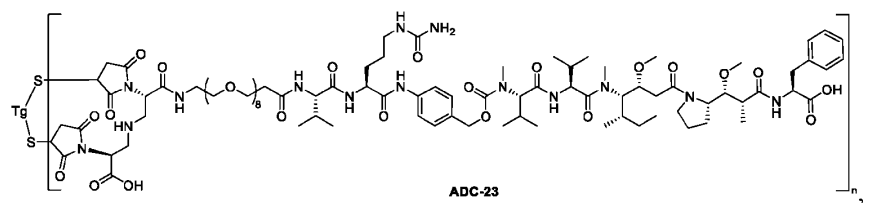
ADC-20



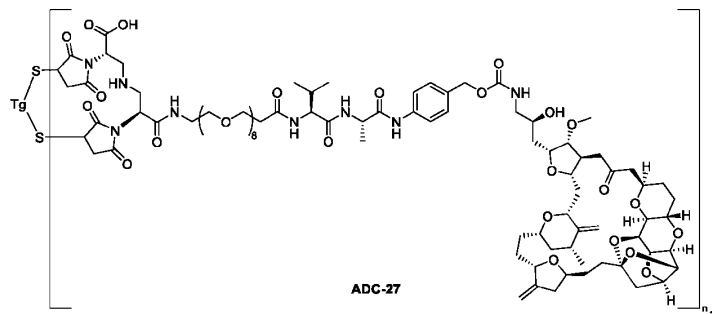
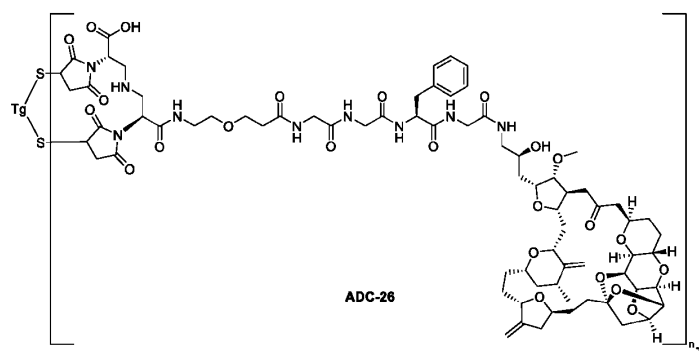
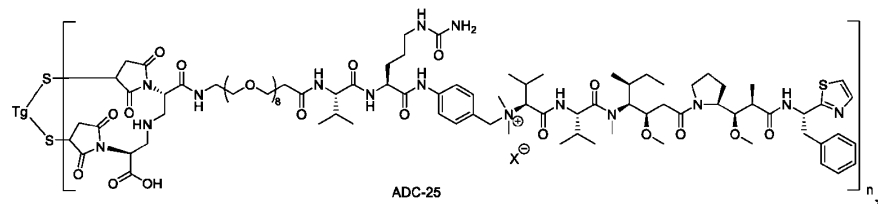
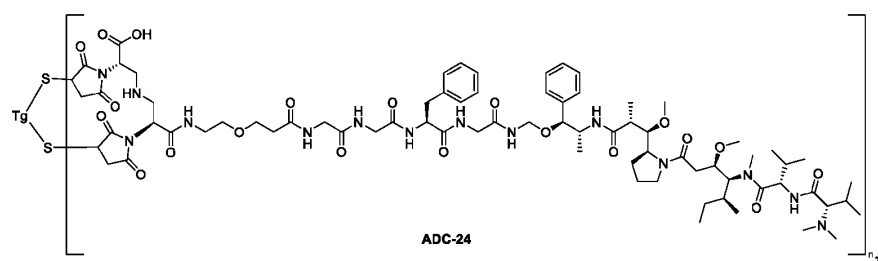
ADC-21

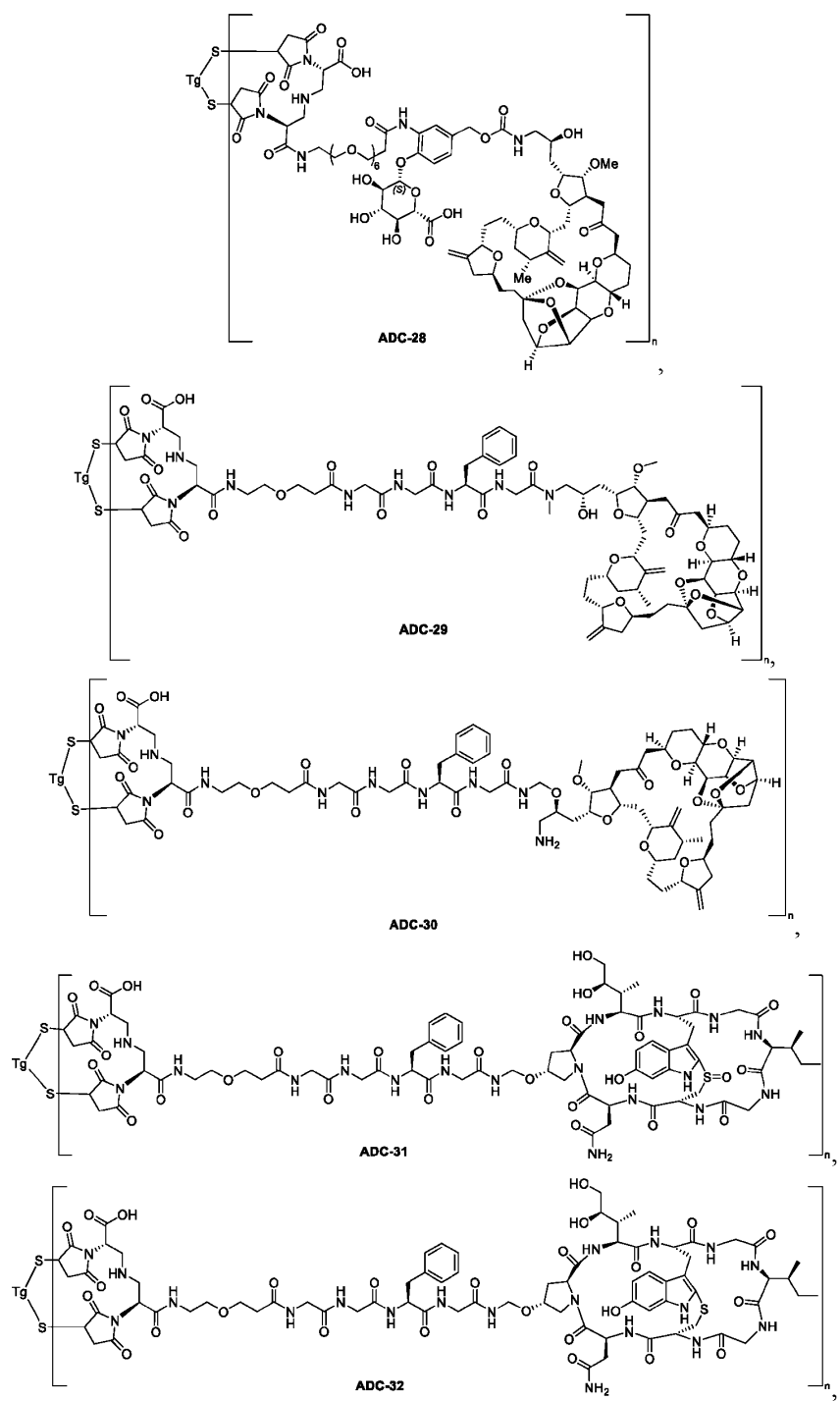


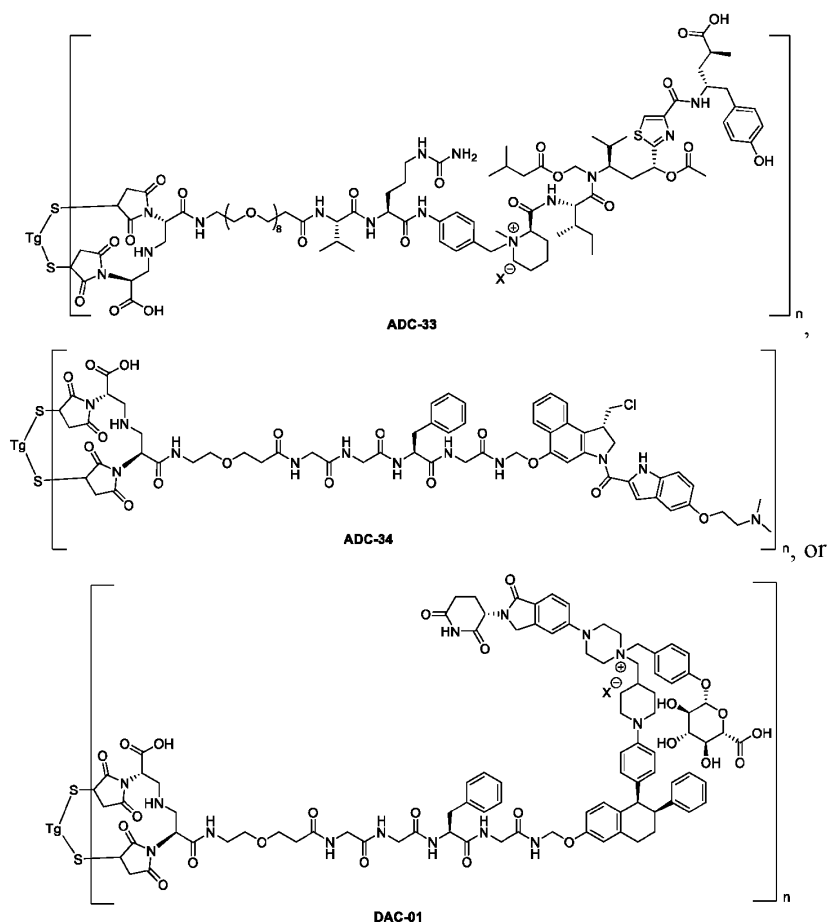
ADC-22



ADC-23







wherein,

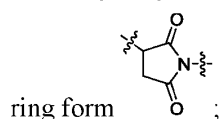
each X^- is Cl^- , Br^- , I^- , $MeSO_3^-$, CF_3COO^- , preferably CF_3COO^- ;

n is an integer or decimal from 1 to 10;

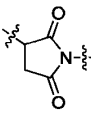
Tg is a ligand, preferably an antibody;

wherein, when the ligand-drug conjugate comprises one or more succinimide structures, the ligand-drug conjugate is configured to be hydrolyzed under readily hydrolyzable conditions, and wherein different degrees of hydrolysis is configured to result in one or more of the following:

no hydrolysis of the succinimide structures, and all the succinimide structures are in the closed



incomplete hydrolysis of the succinimide structures, and therefore some of the succinimide

structures are in the closed-ring form  and some of the succinimide structures are in the

open-ring form  or ;

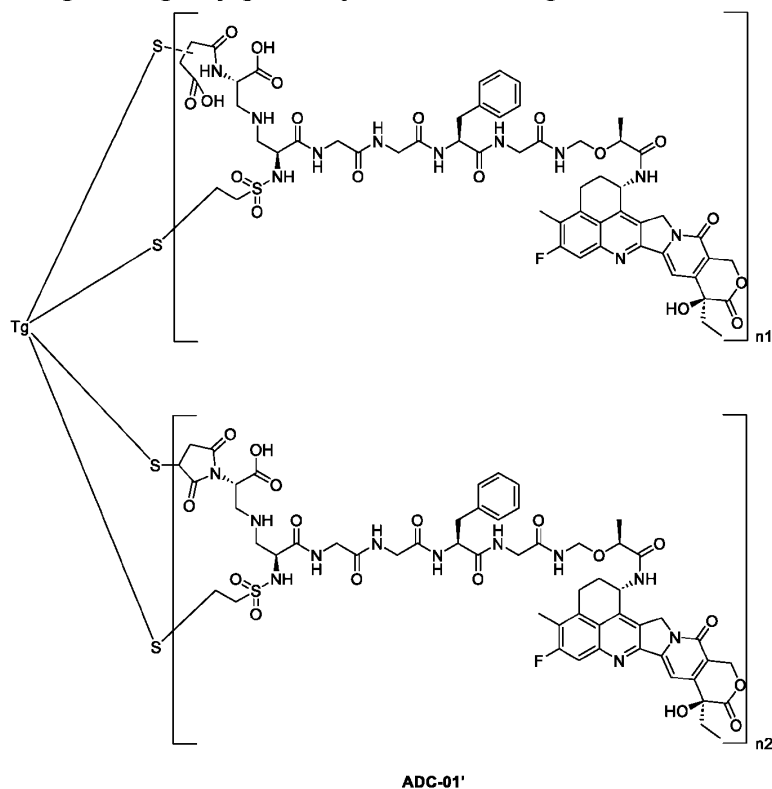
complete hydrolysis of the succinimide structures, and all the succinimide structures are in the

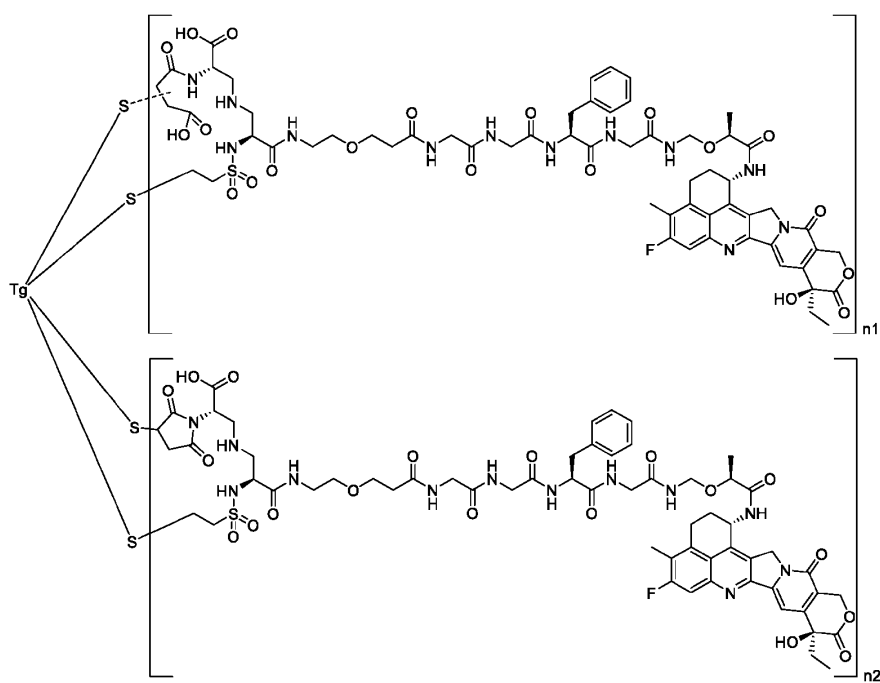
ring-opened form ;

the position shown by the wavy line is the connection site;

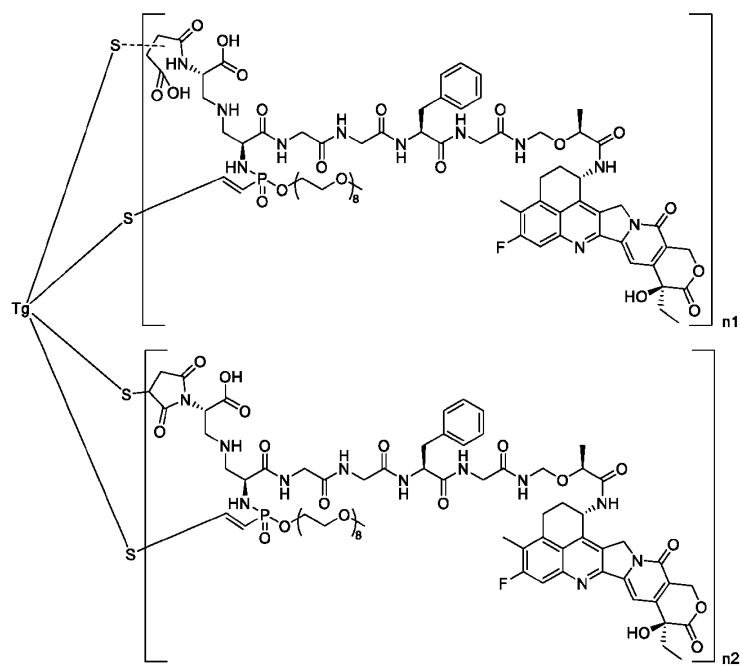
when multiple succinimide structures are present in a ligand-drug conjugate, these succinimide structures are in the closed-ring form, partially open-ring form, or fully open-ring form.

34. The ligand-drug conjugate, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 19-33, wherein said ligand-drug conjugate comprises the following:

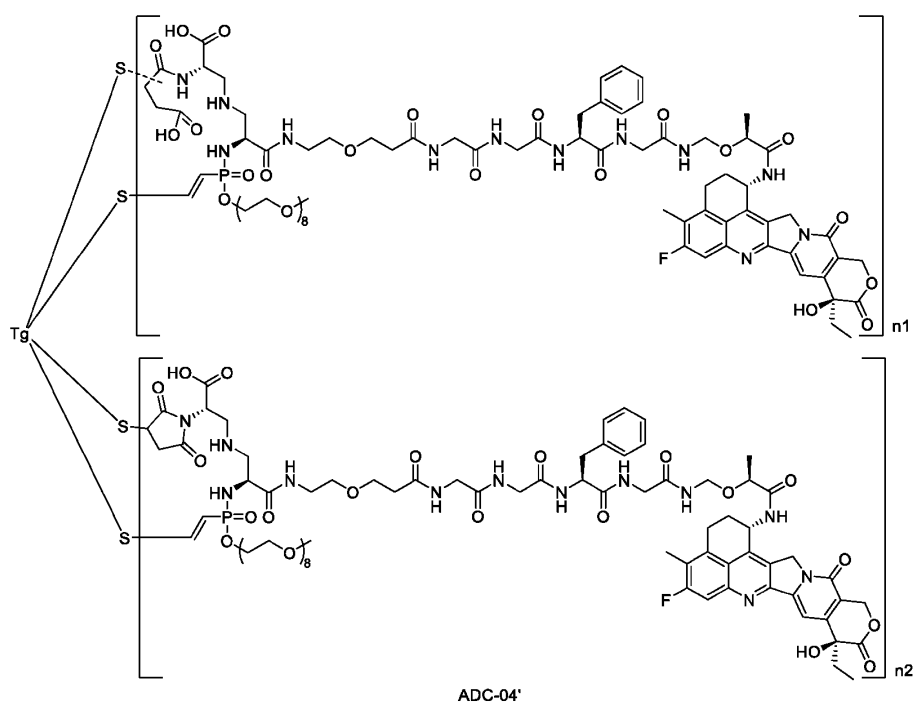


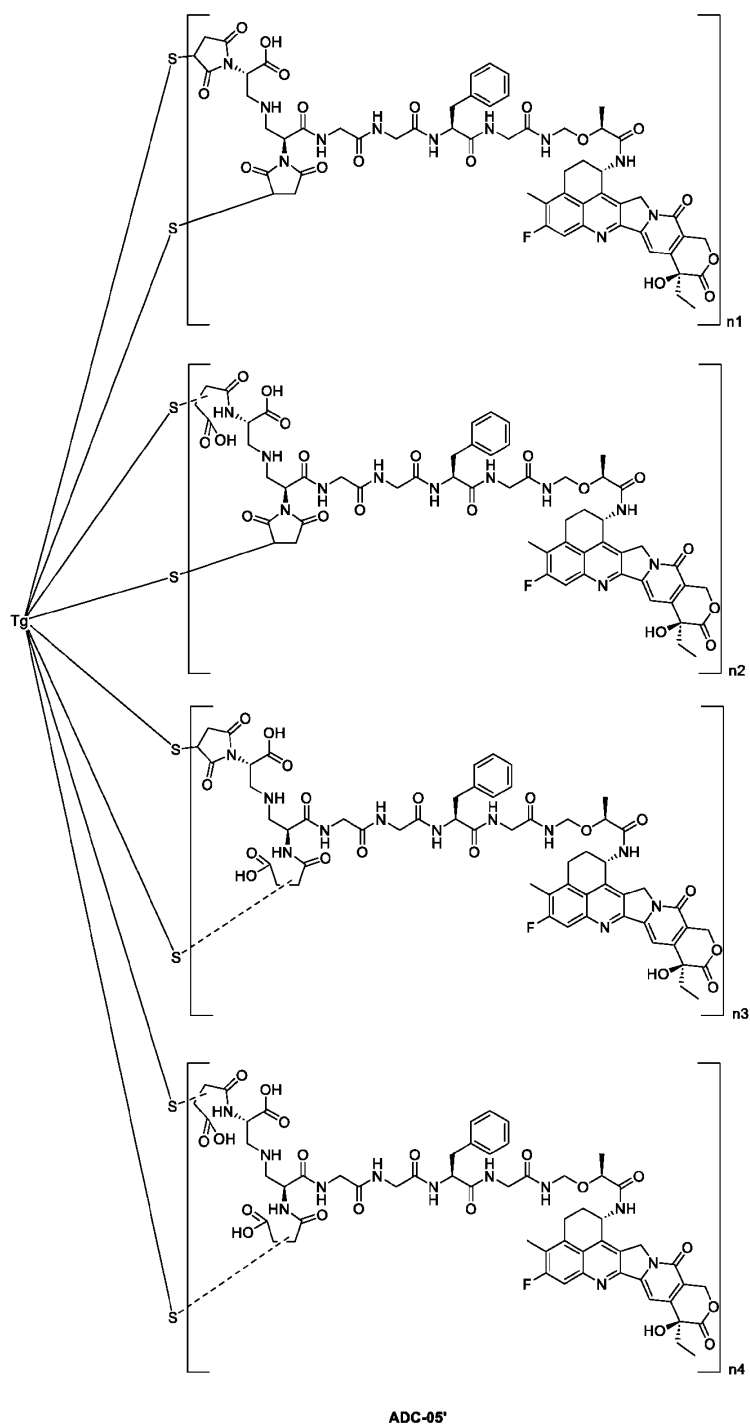


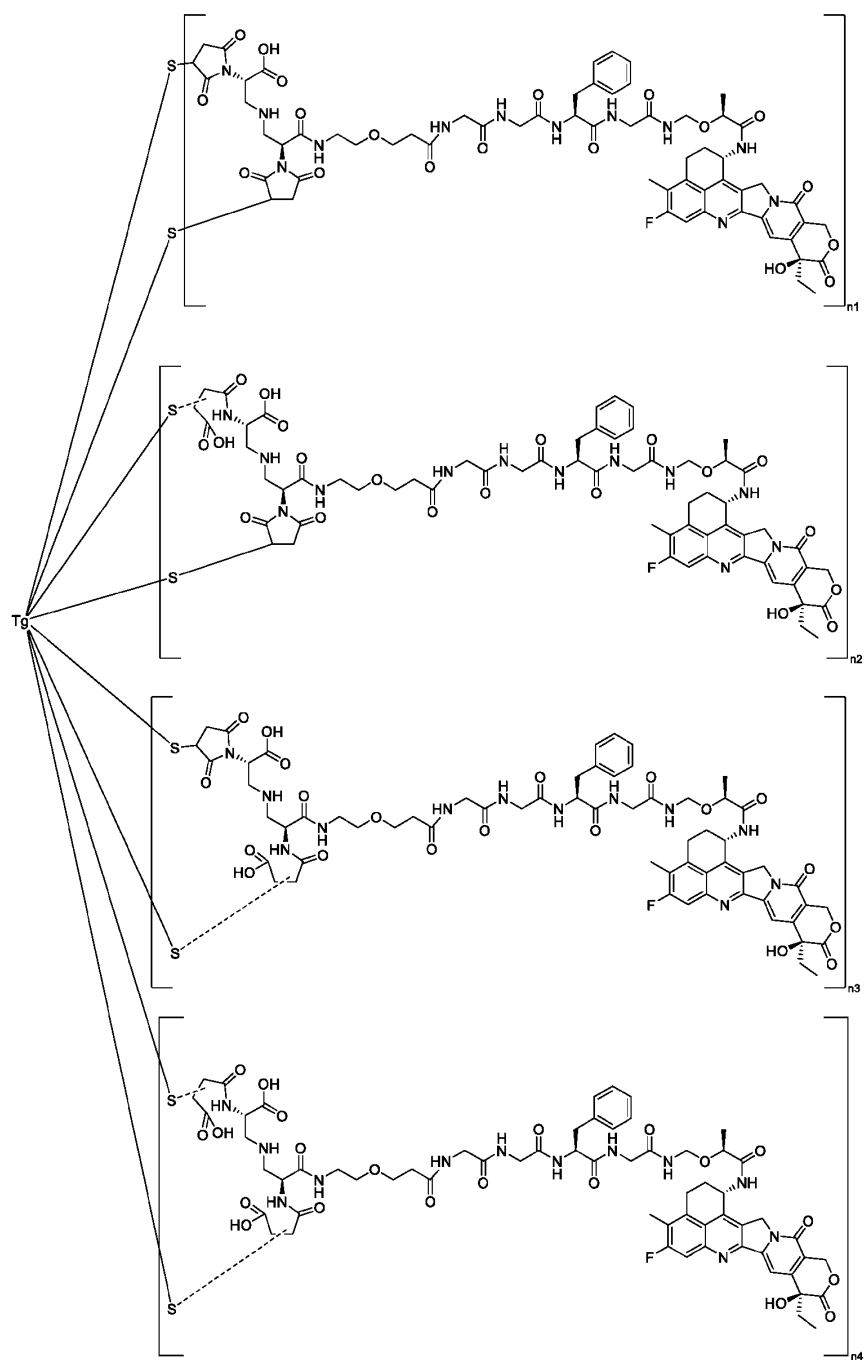
ADC-02'



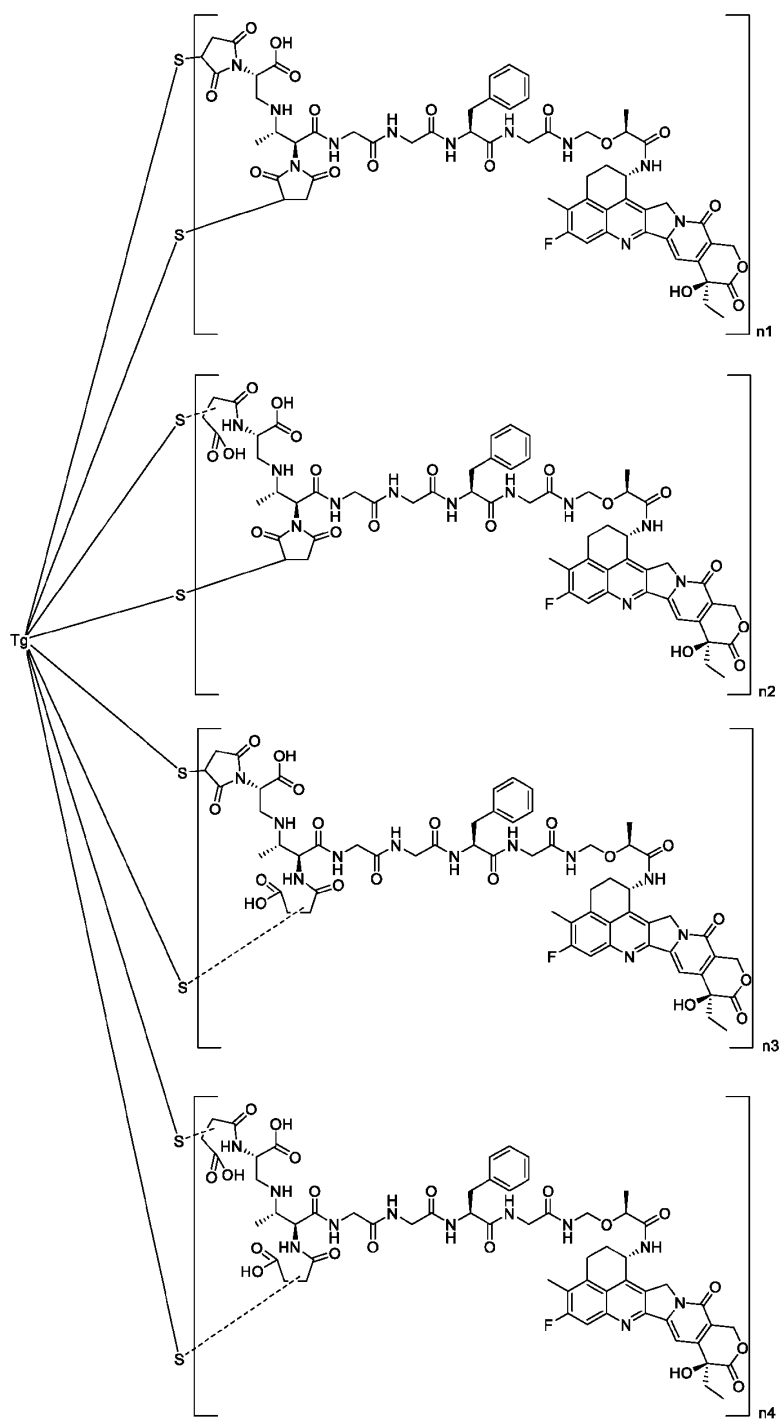
ADC-03'





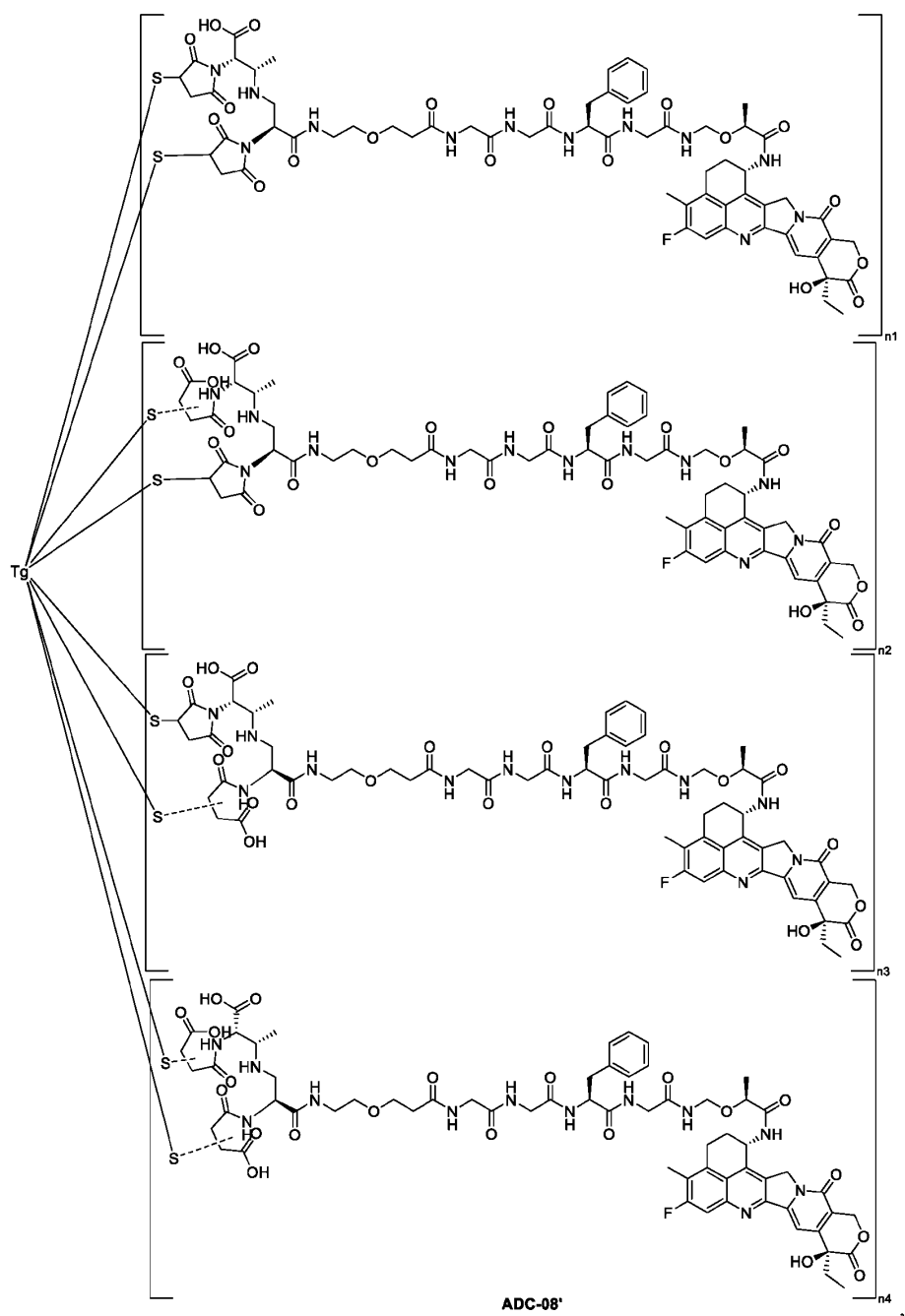


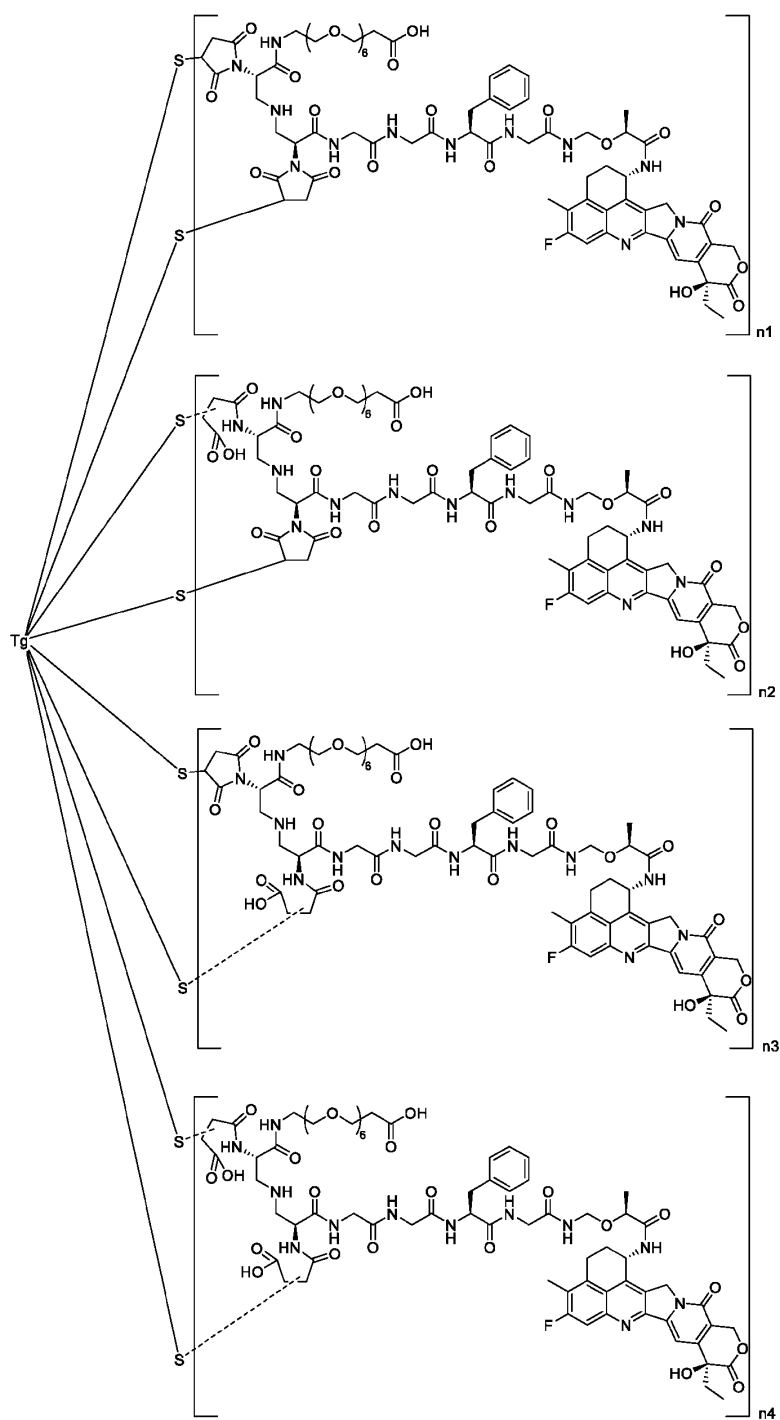
ADC-06*



ADC-07'

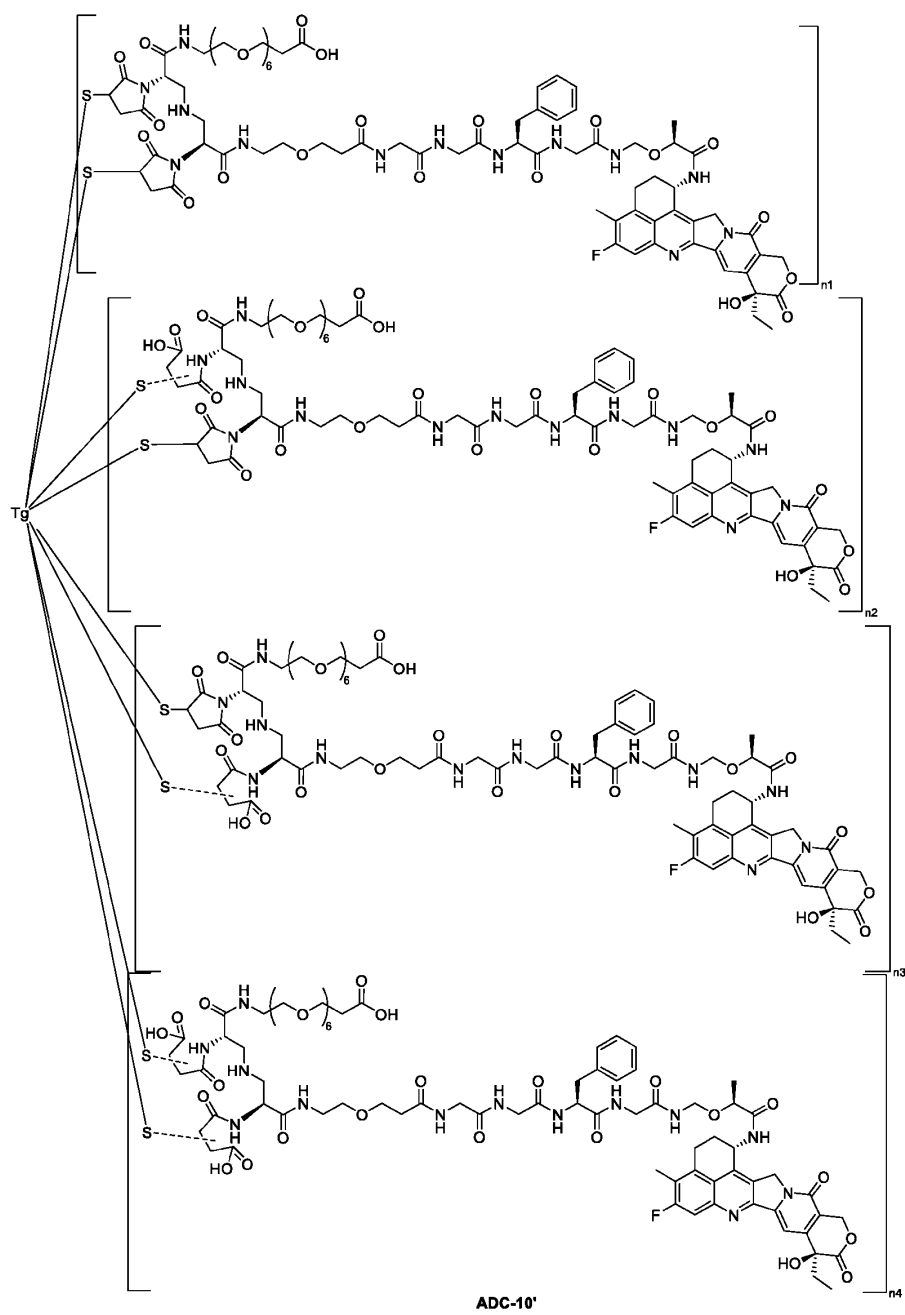
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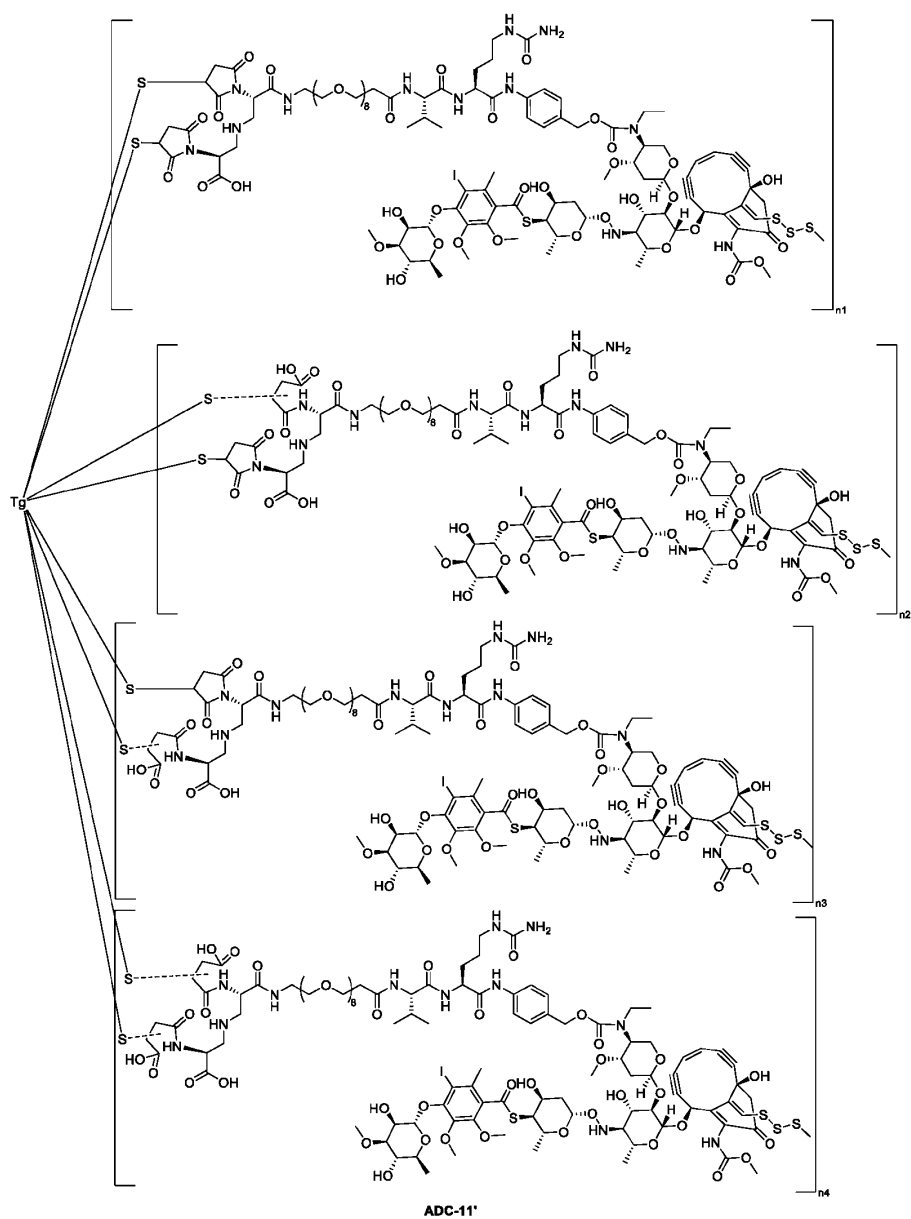


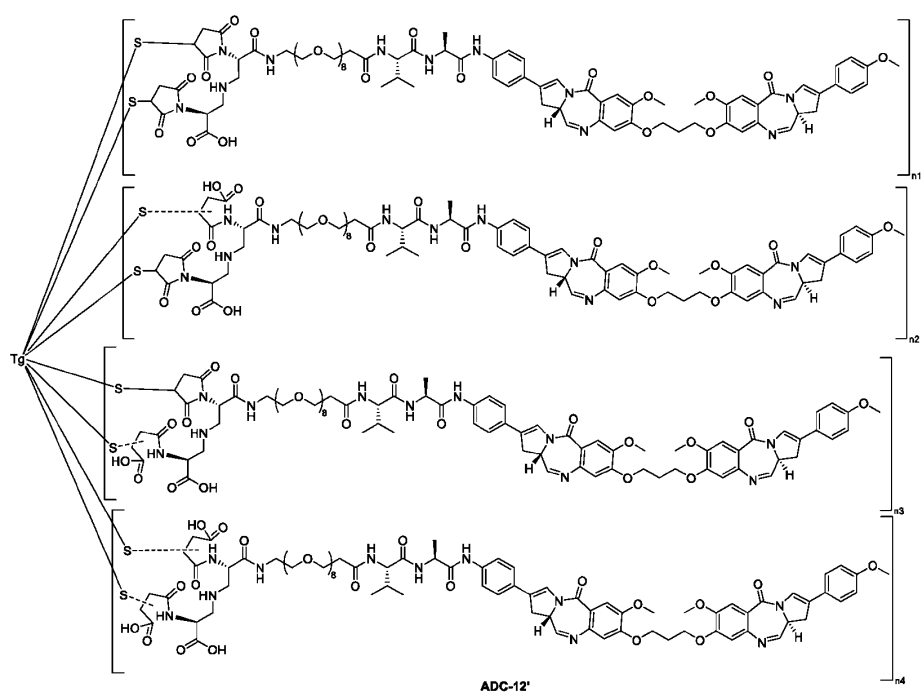


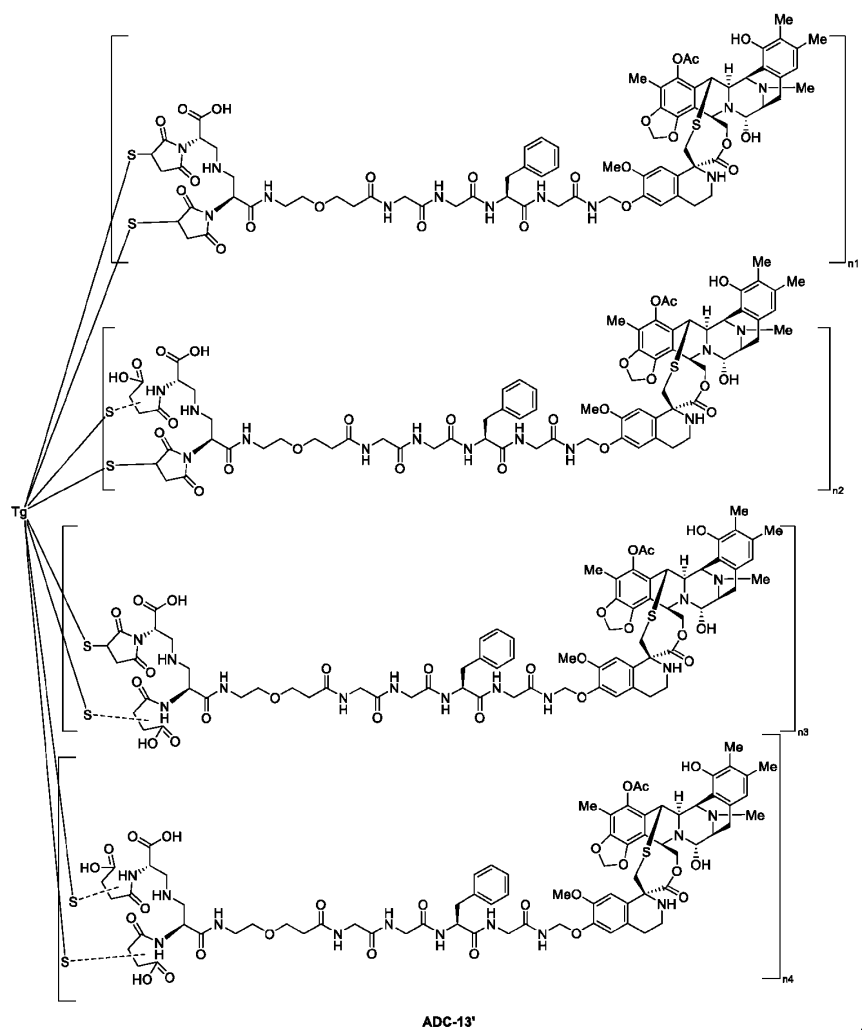
ADC-09'

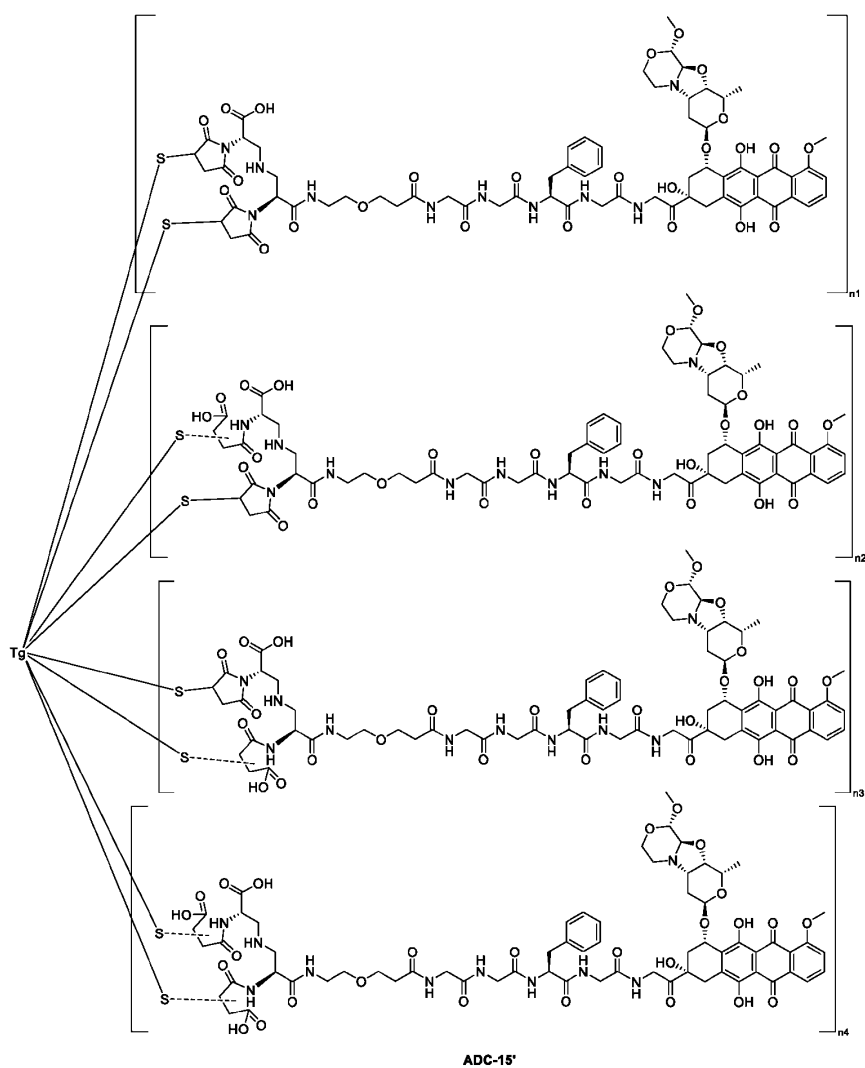
2

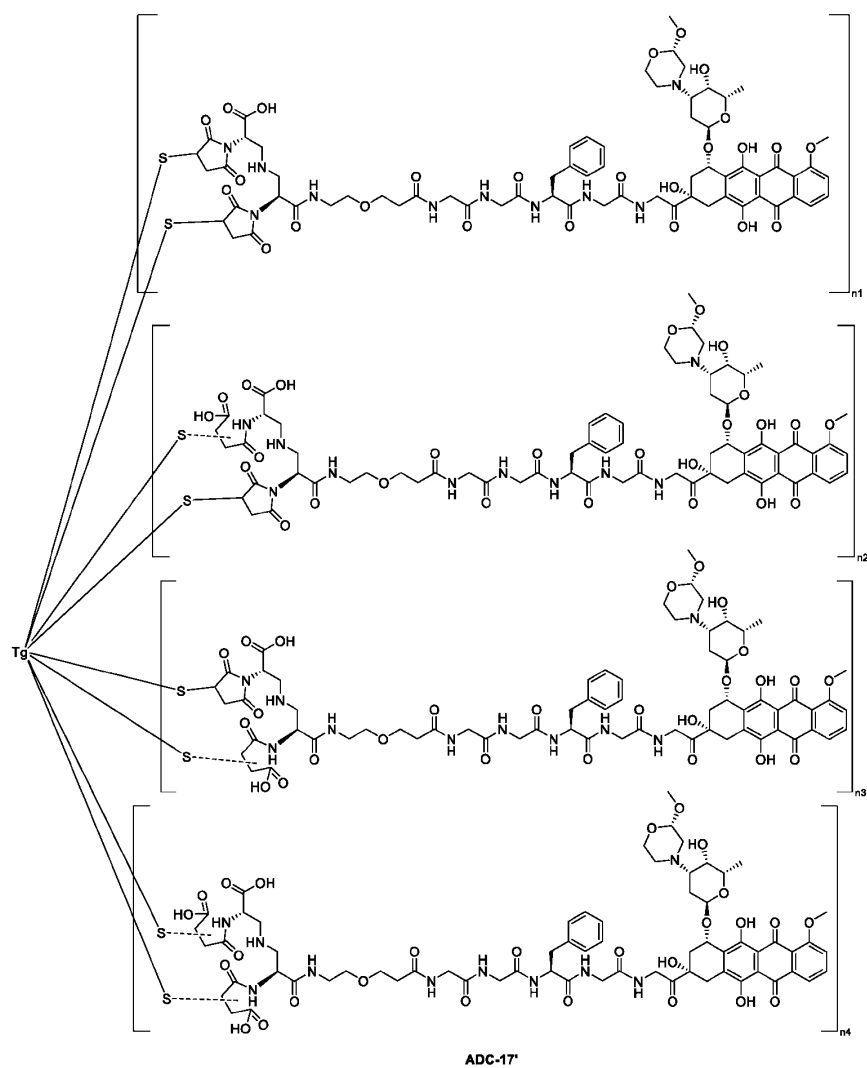


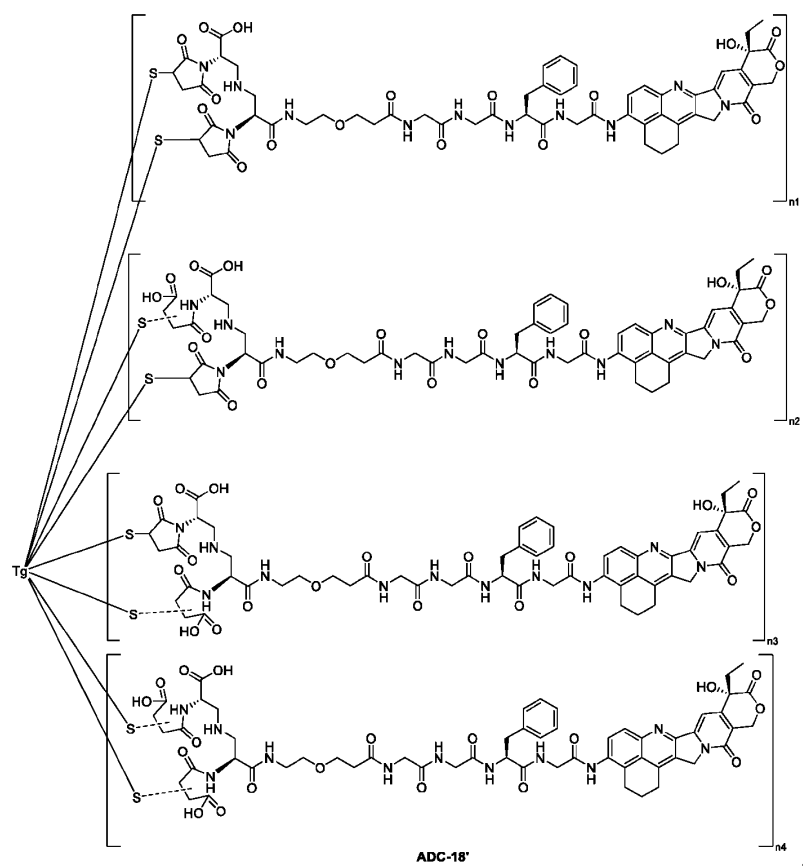


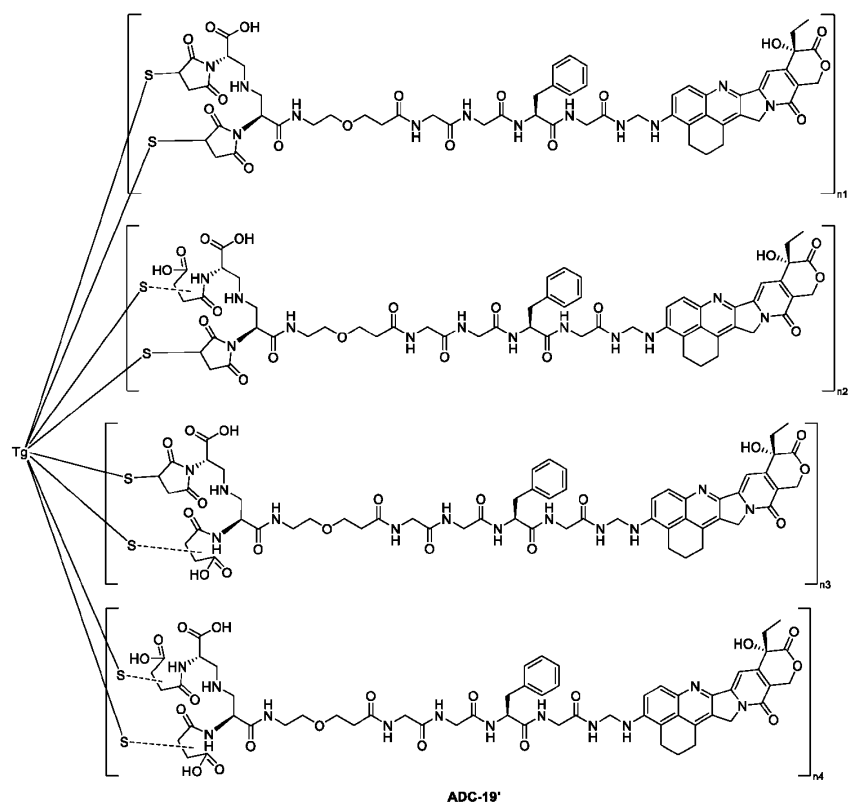


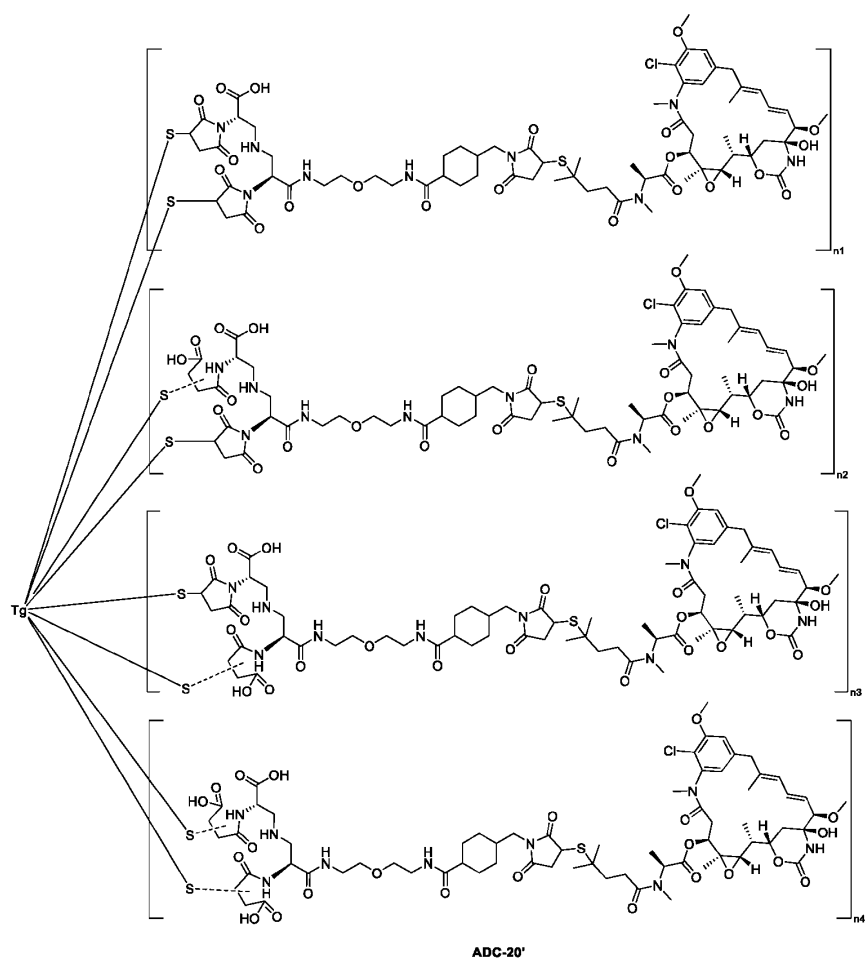


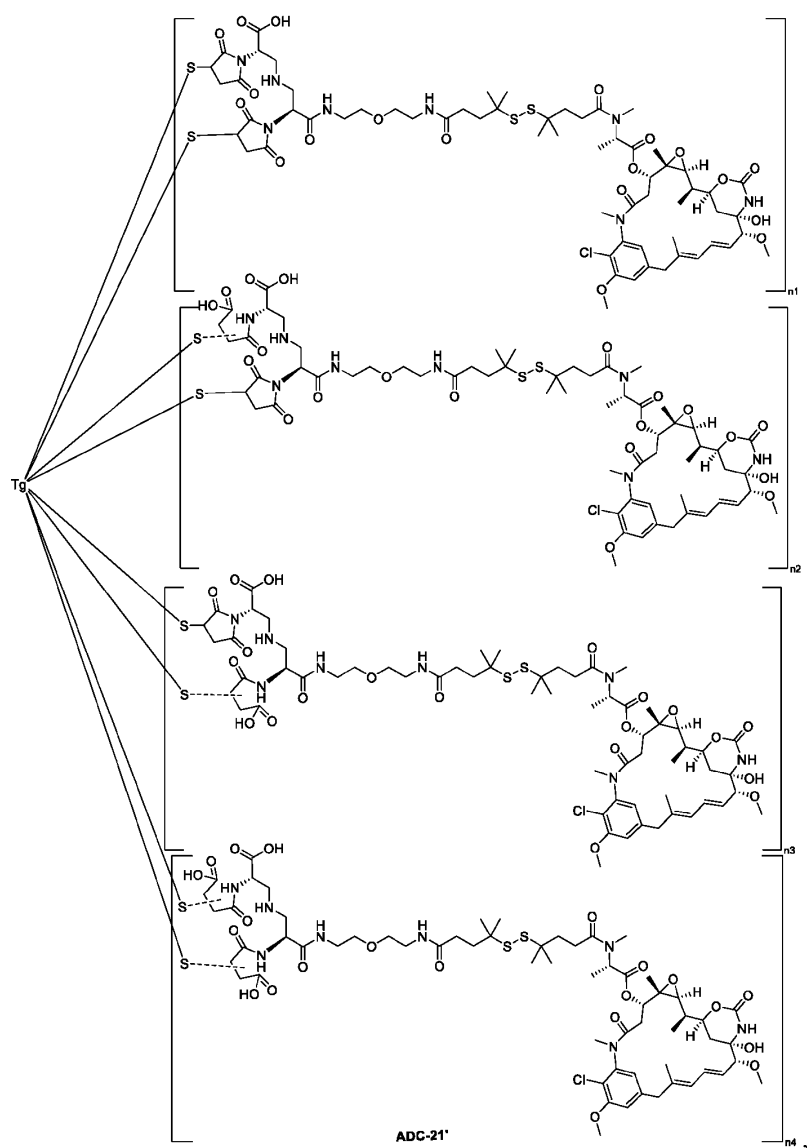


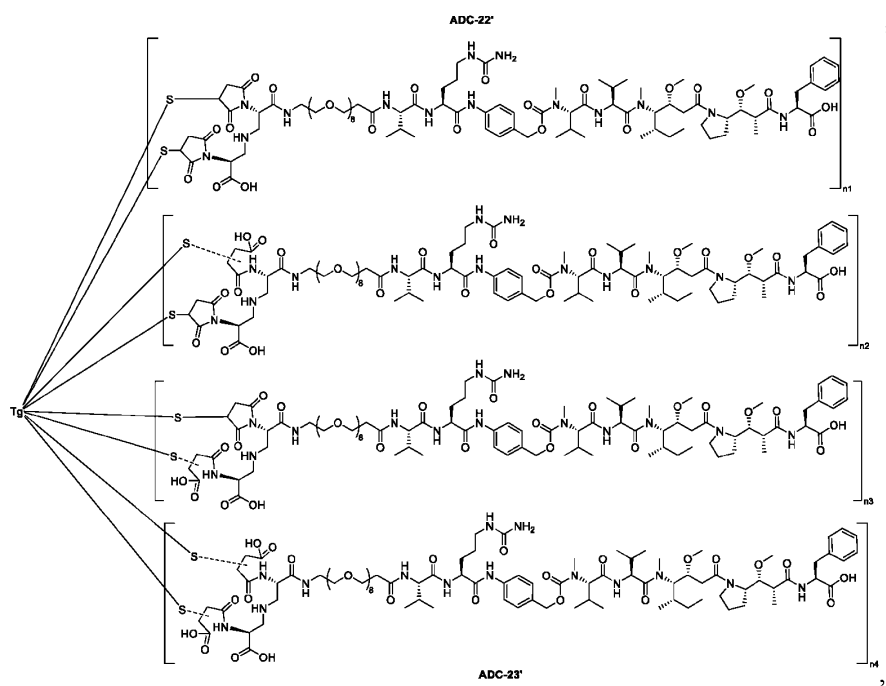
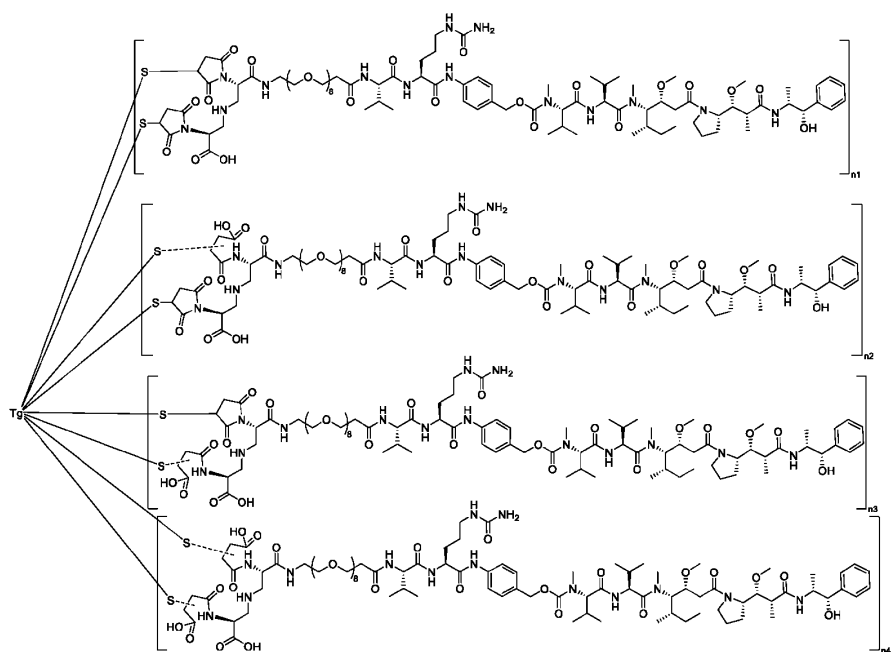


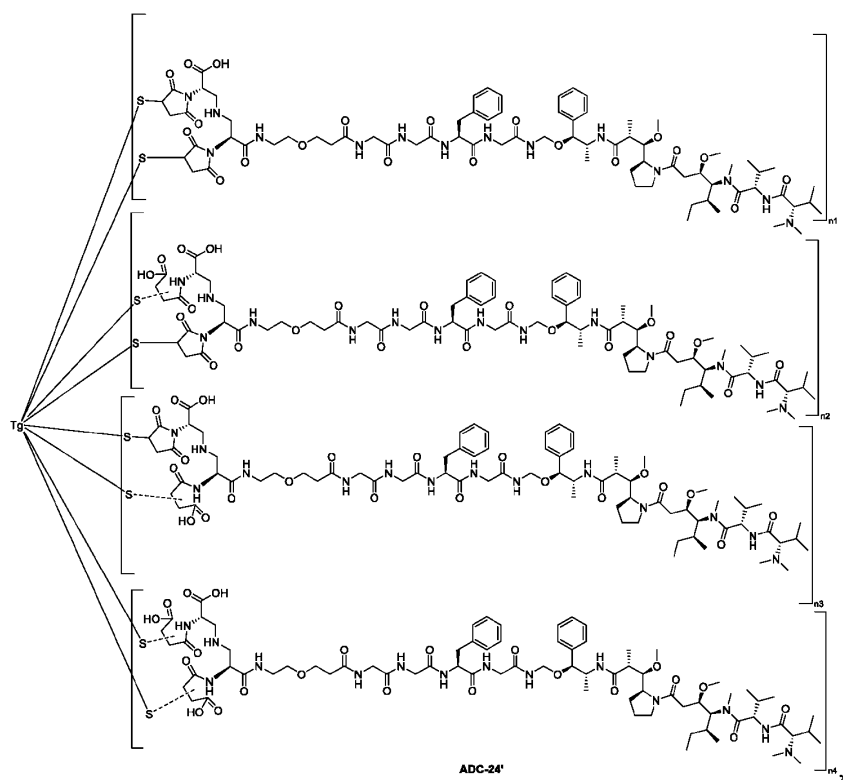


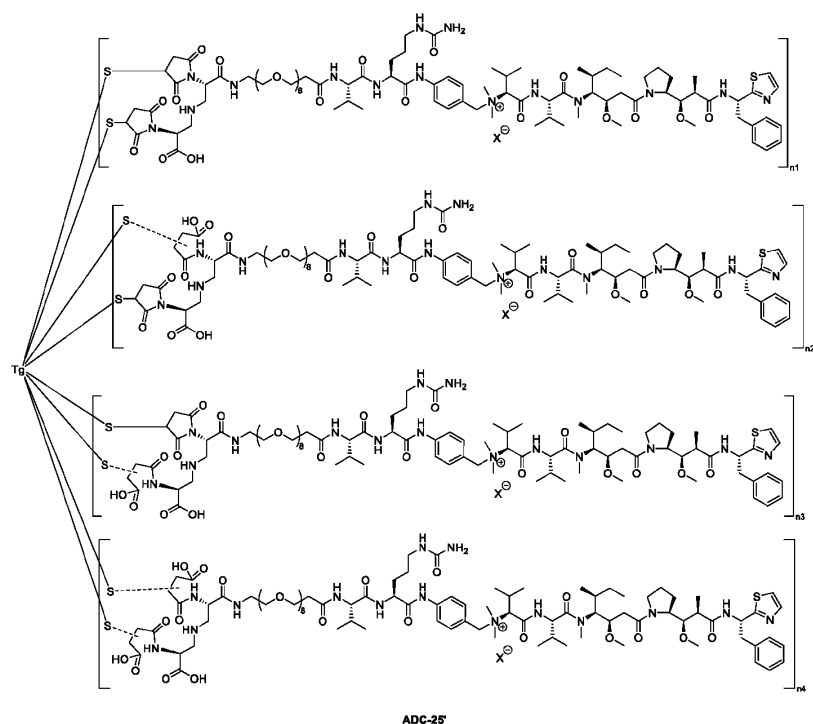


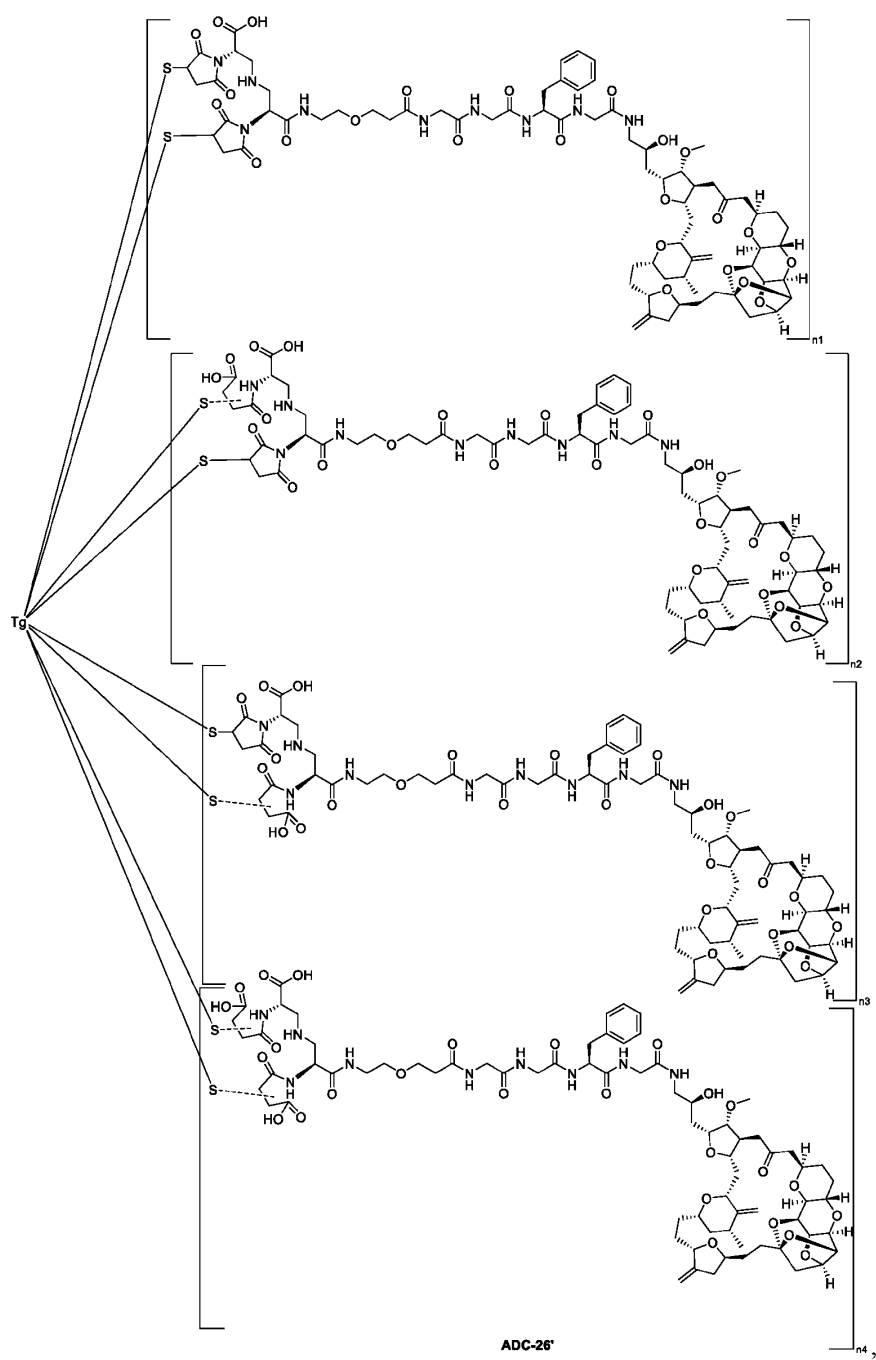


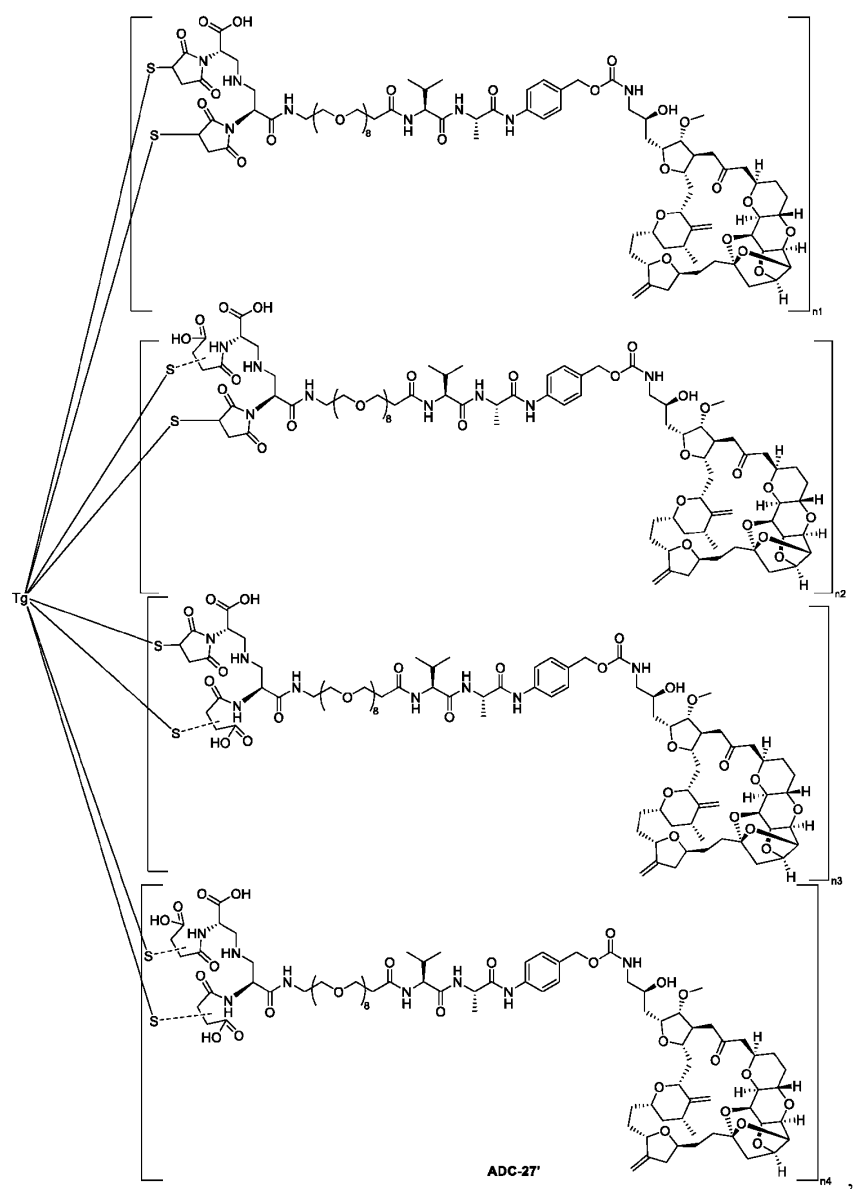


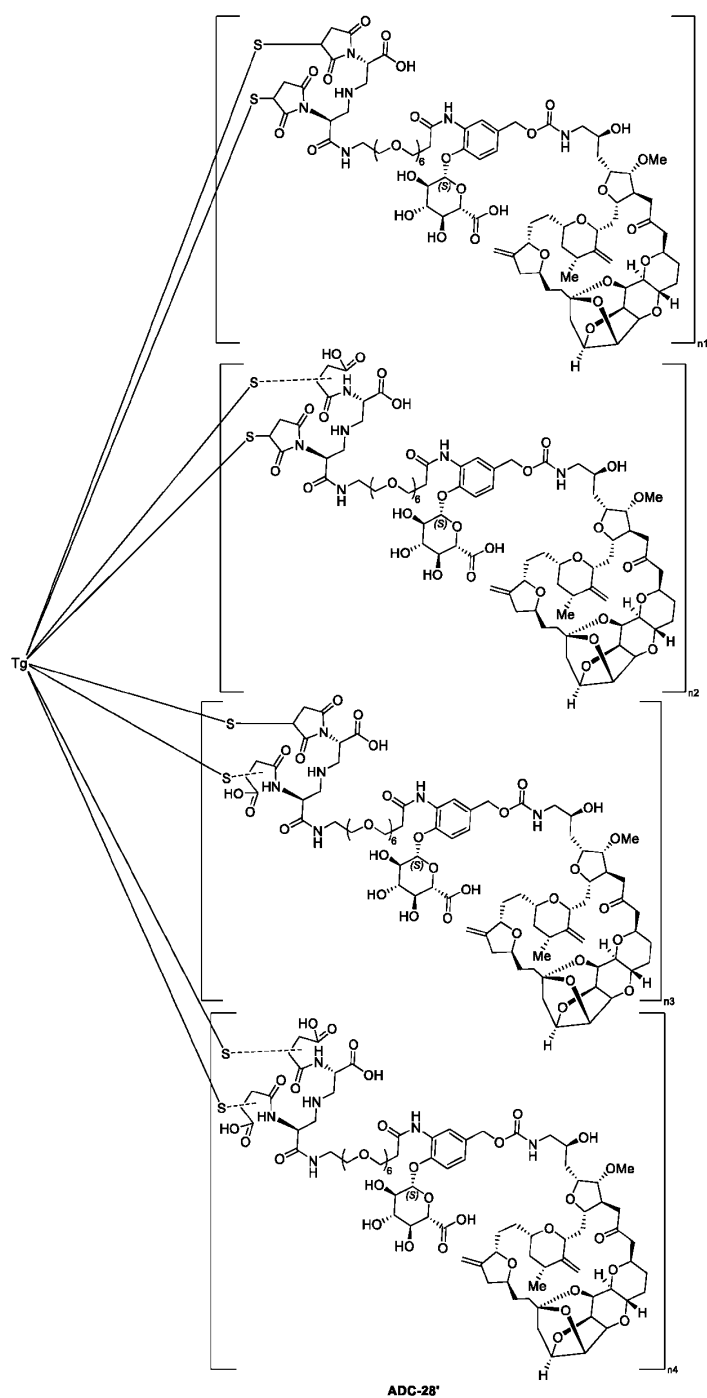


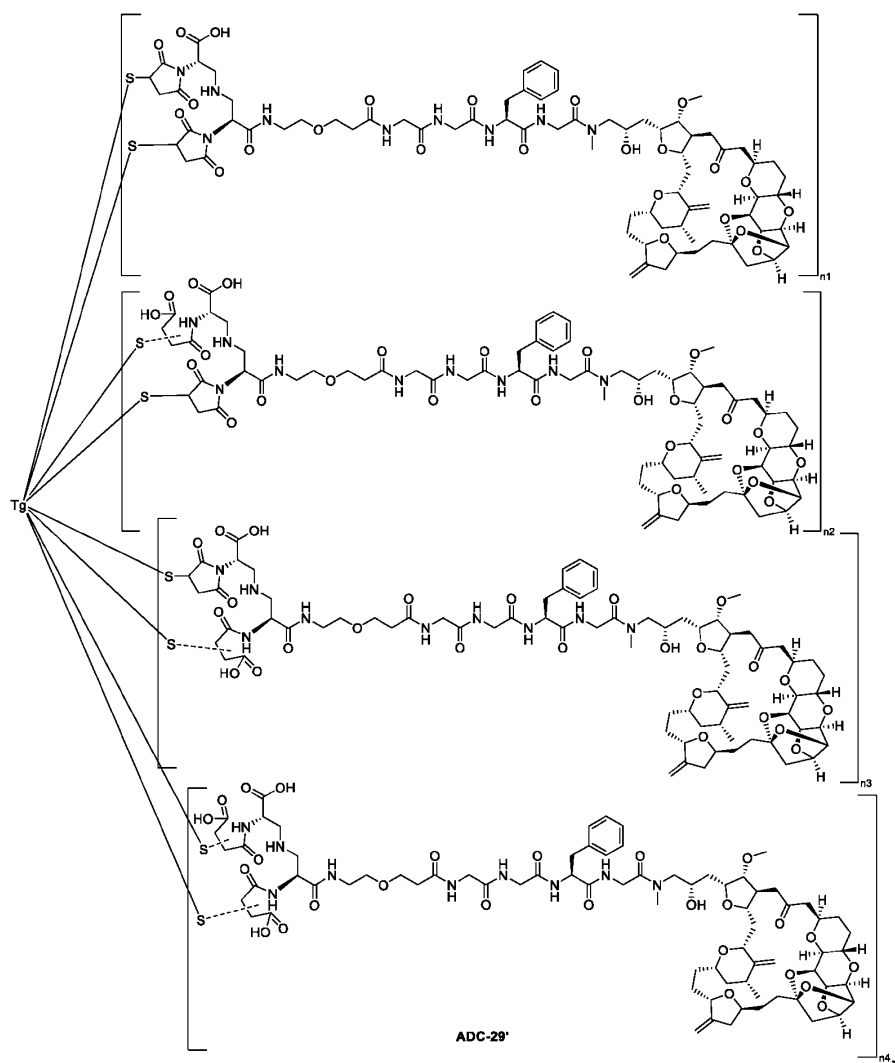


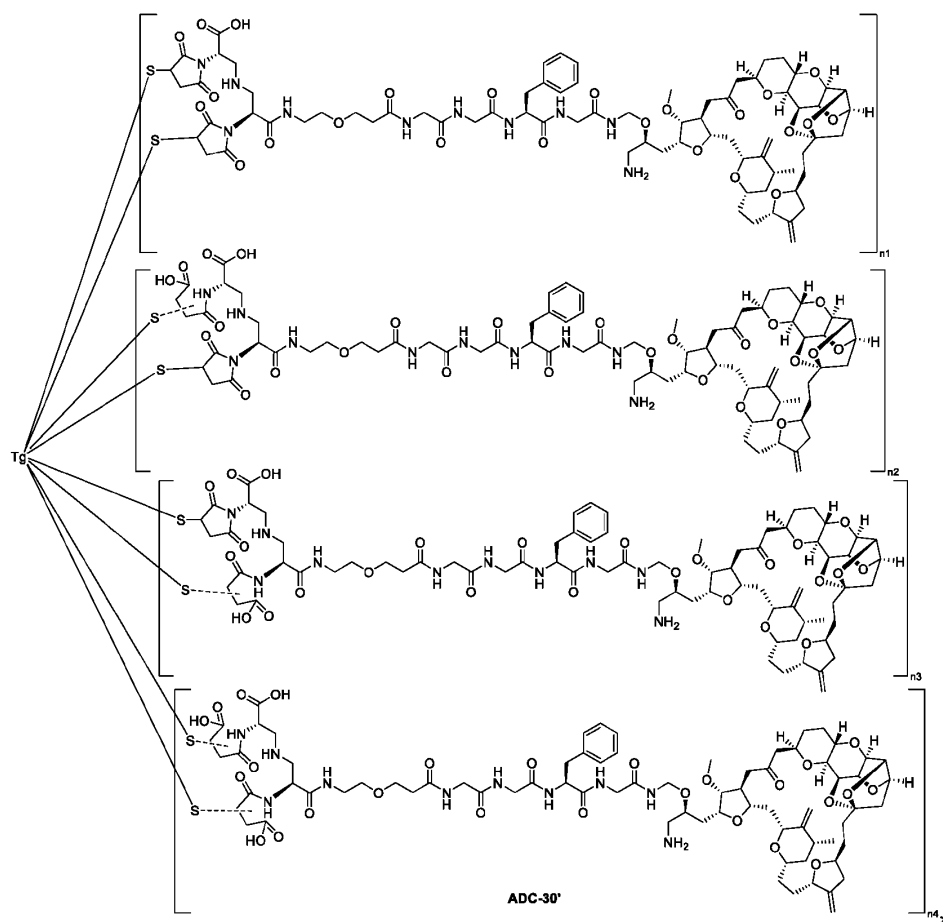


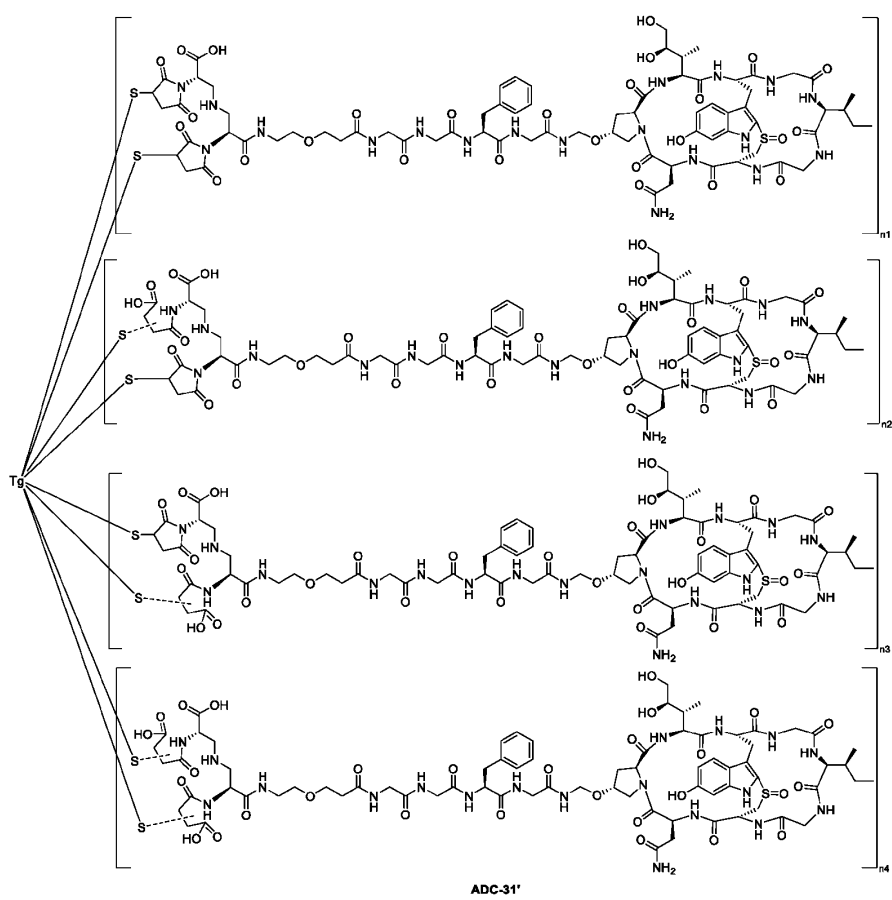


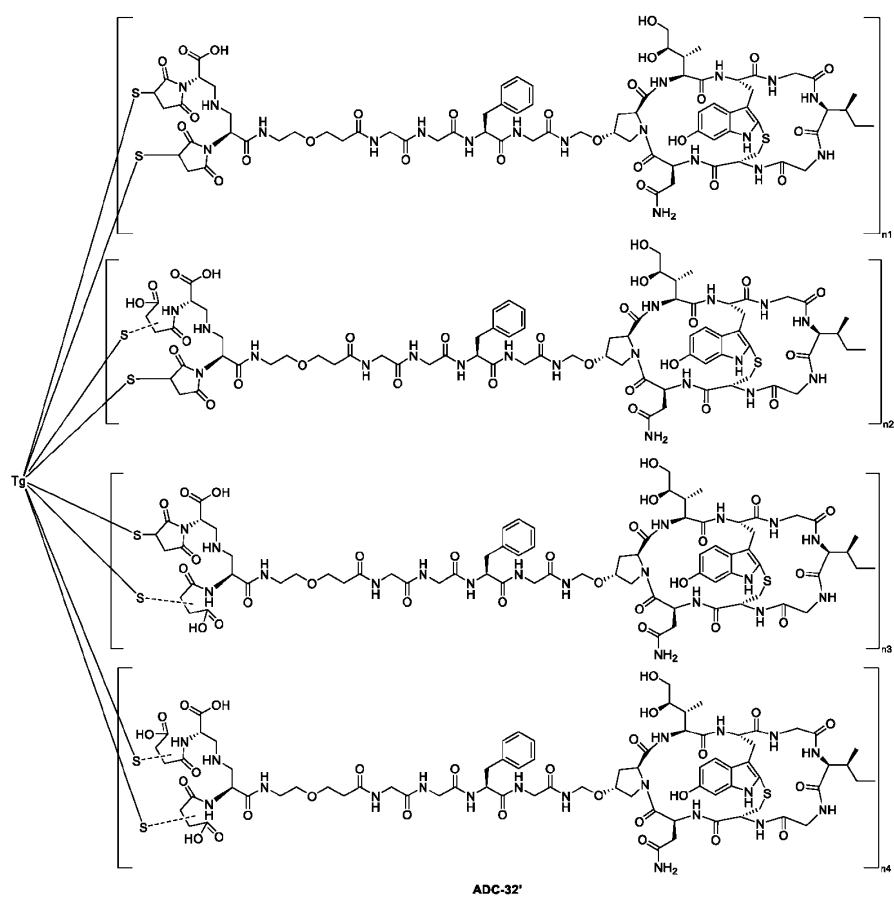


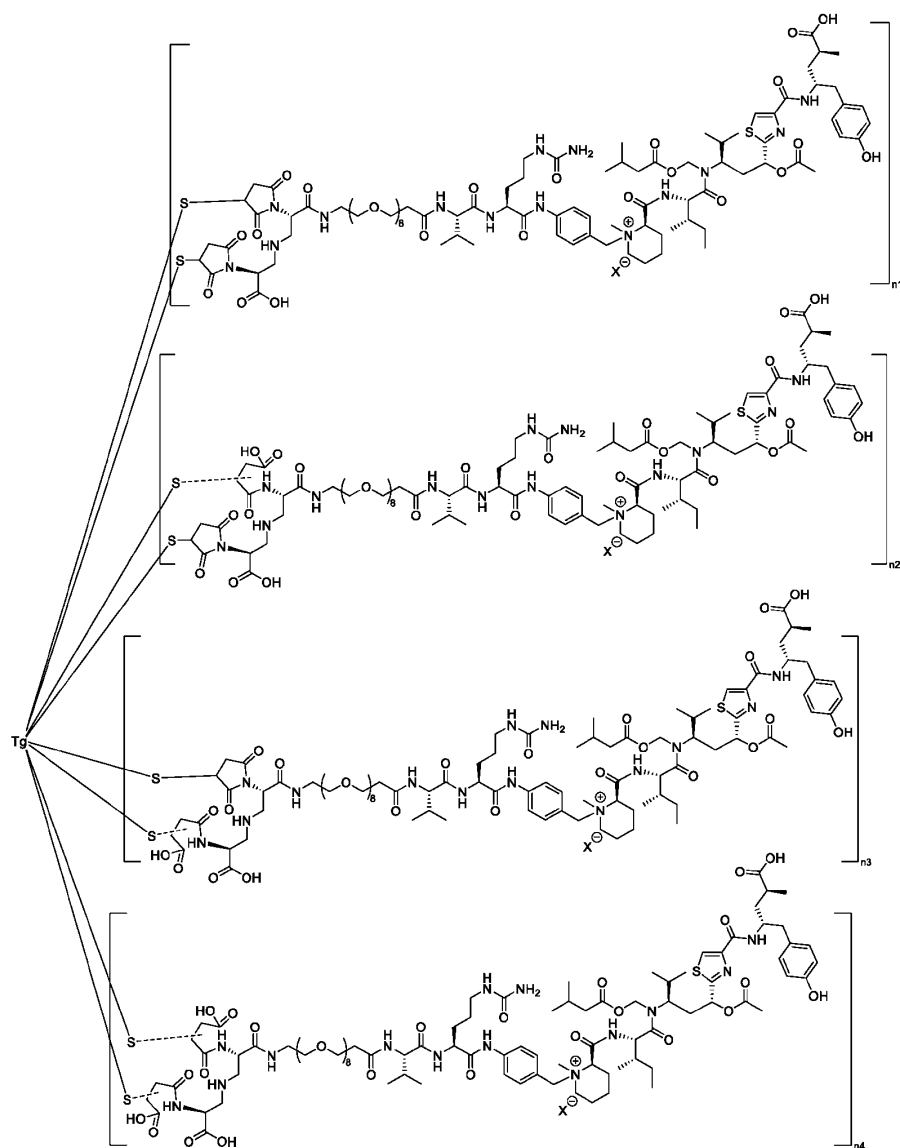




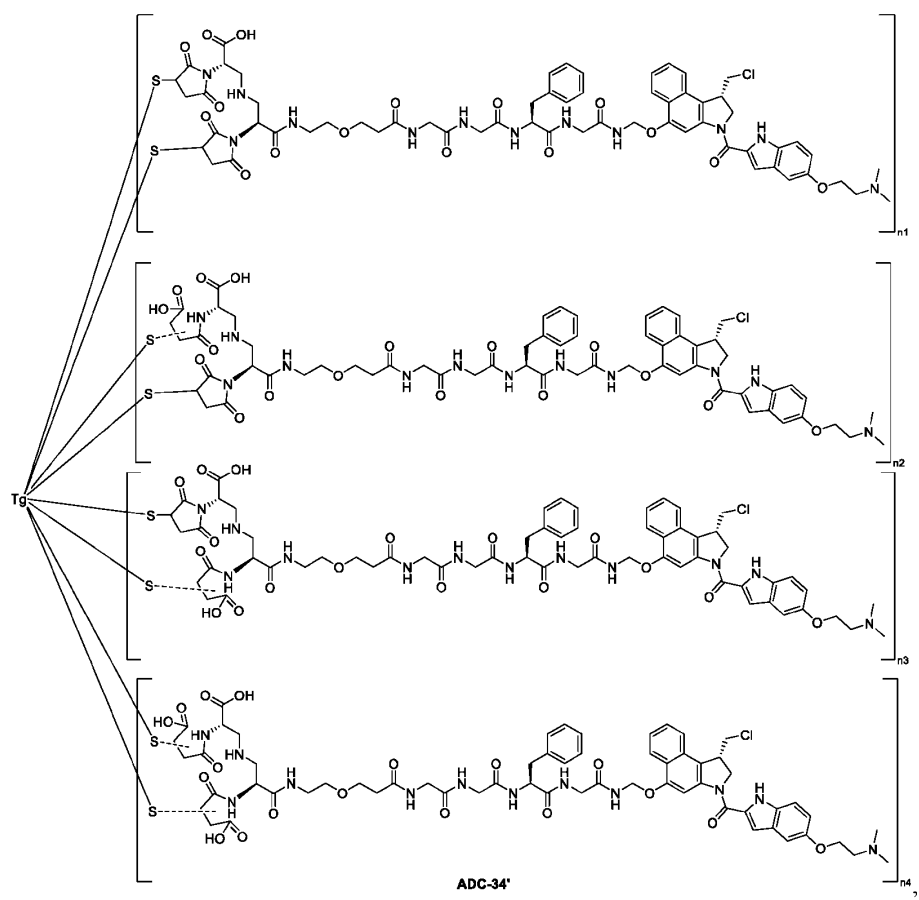


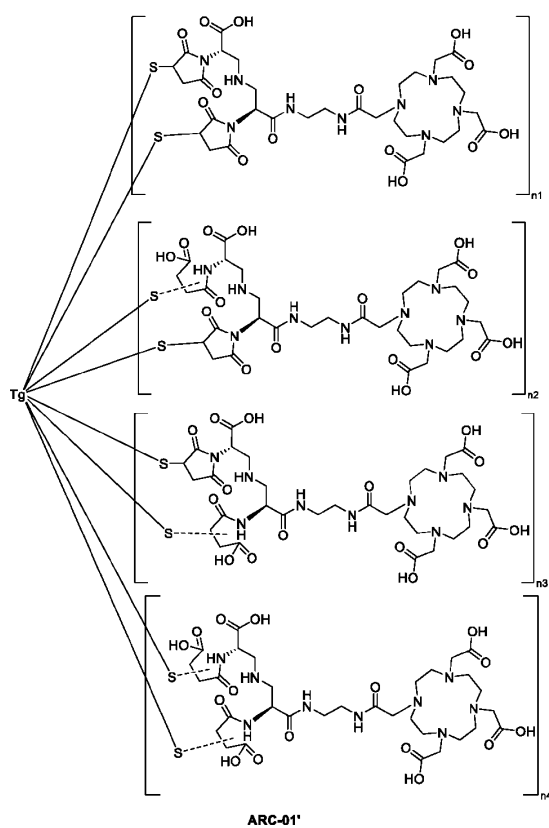






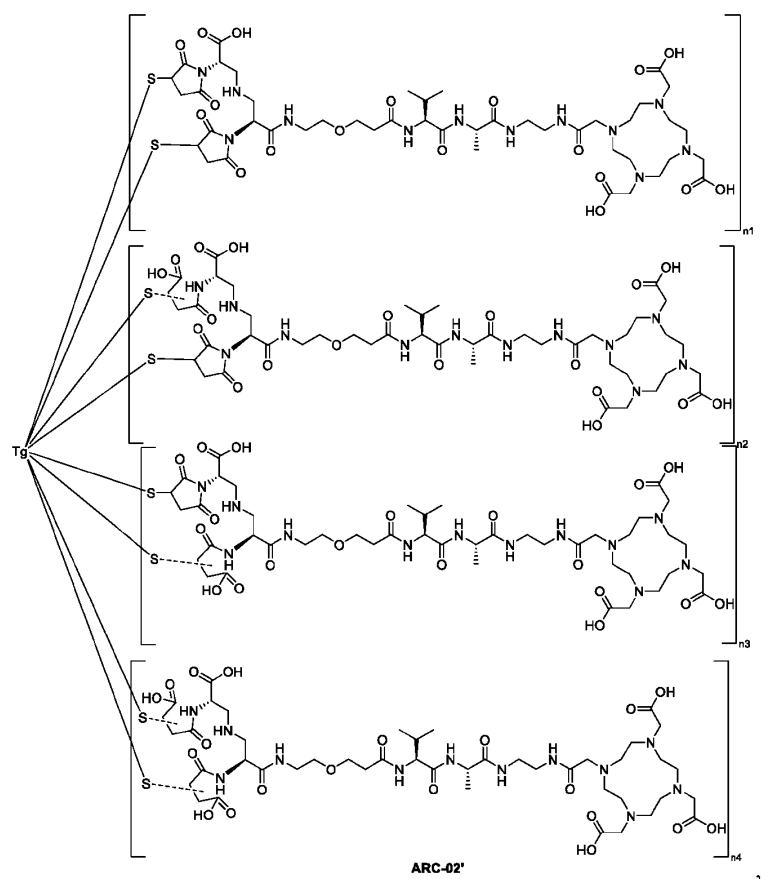
ADC-33'

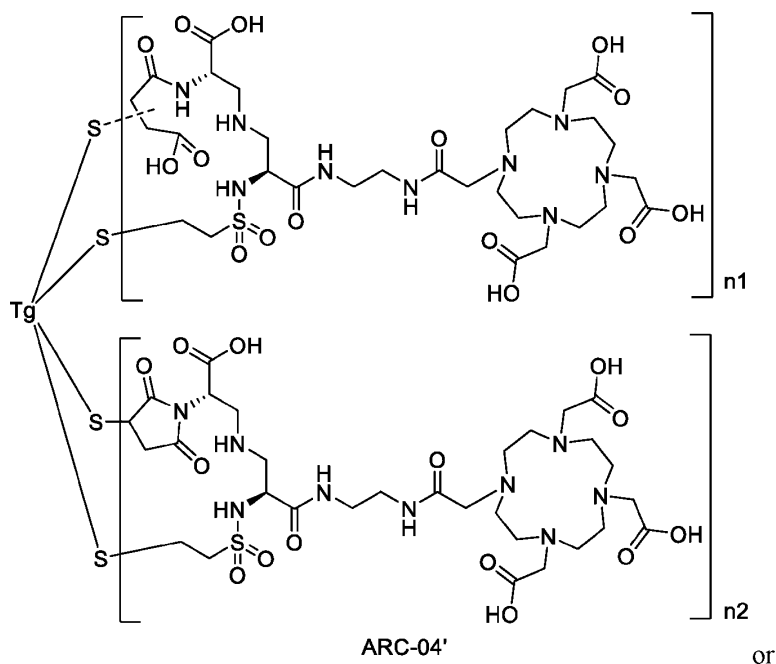
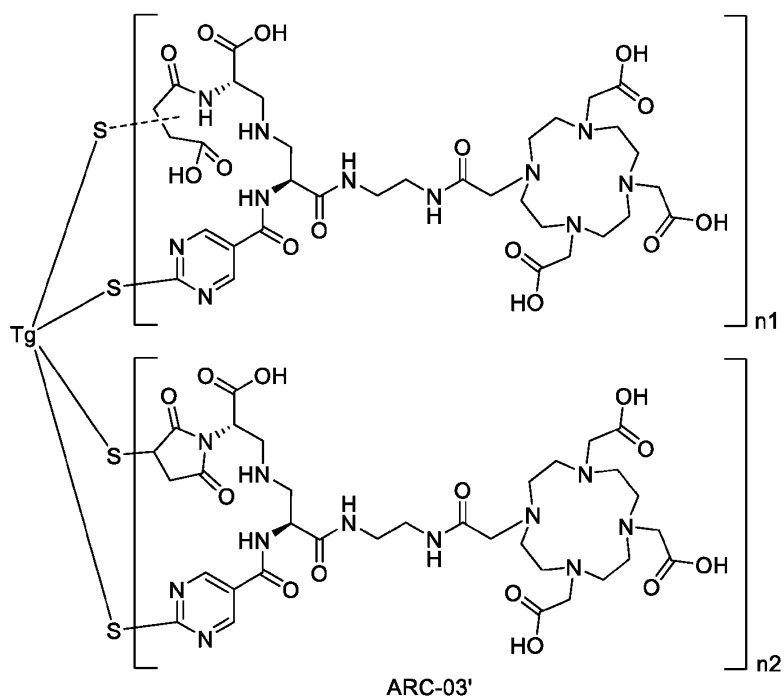


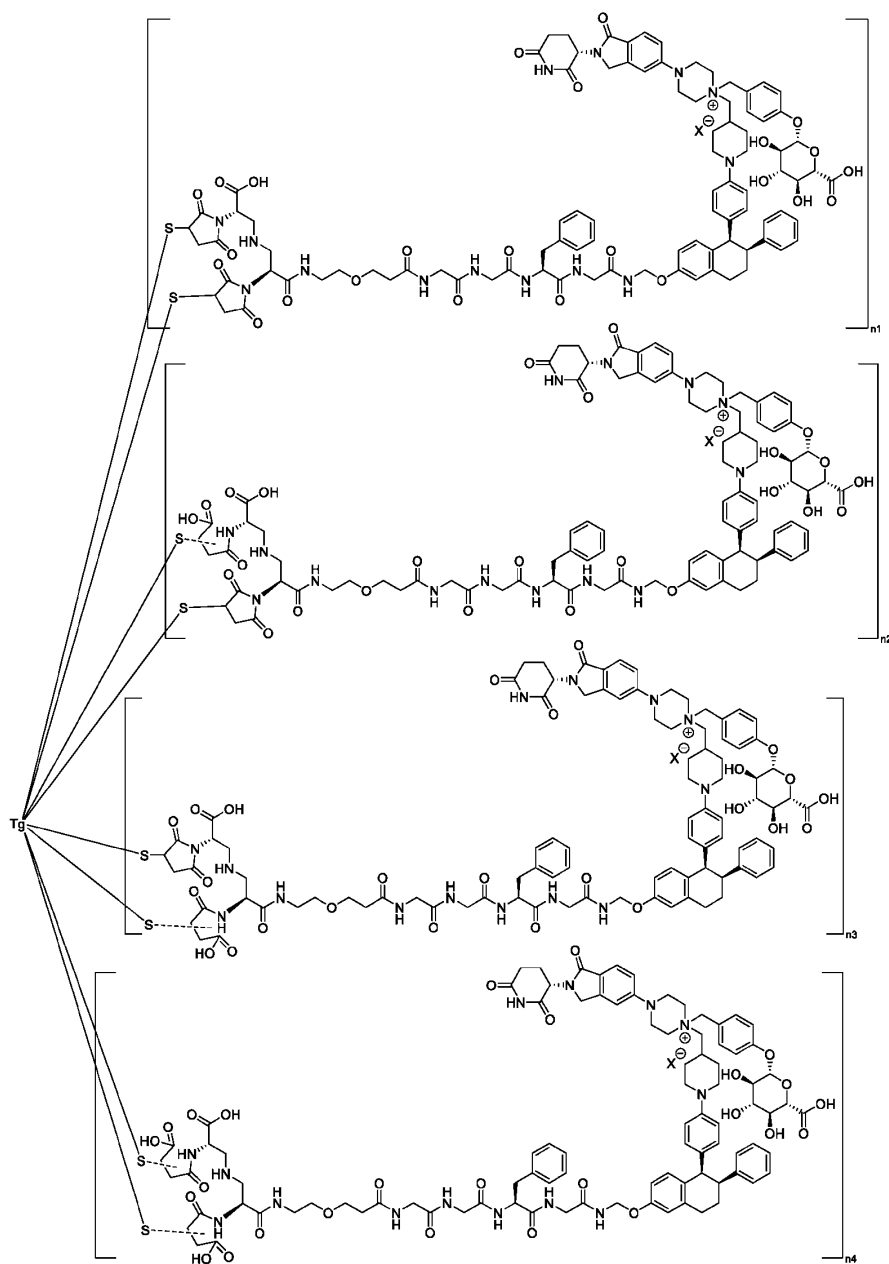


ARC-01'

5







DAC-91'

wherein

the dotted line indicates the bond connected to the succinimide open-ring form at any position;
when n3 and n4 exist:

n1, n2, n3 and n4 are independently selected from integers or decimals from 1 to 10, and

n_1 , n_2 , n_3 and n_4 are not 0 at the same time; preferably, at least one of n_2 , n_3 and n_4 is not 0; Preferably, n_1 , n_2 and n_3 are 0, and n_4 is not 0;

and the sum of $n_1+n_2+n_3+n_4$ is an integer or decimal between 1 to 10, preferably an integer or decimal selected from 2 to 8, or preferably an integer or decimal from 3 to 6;

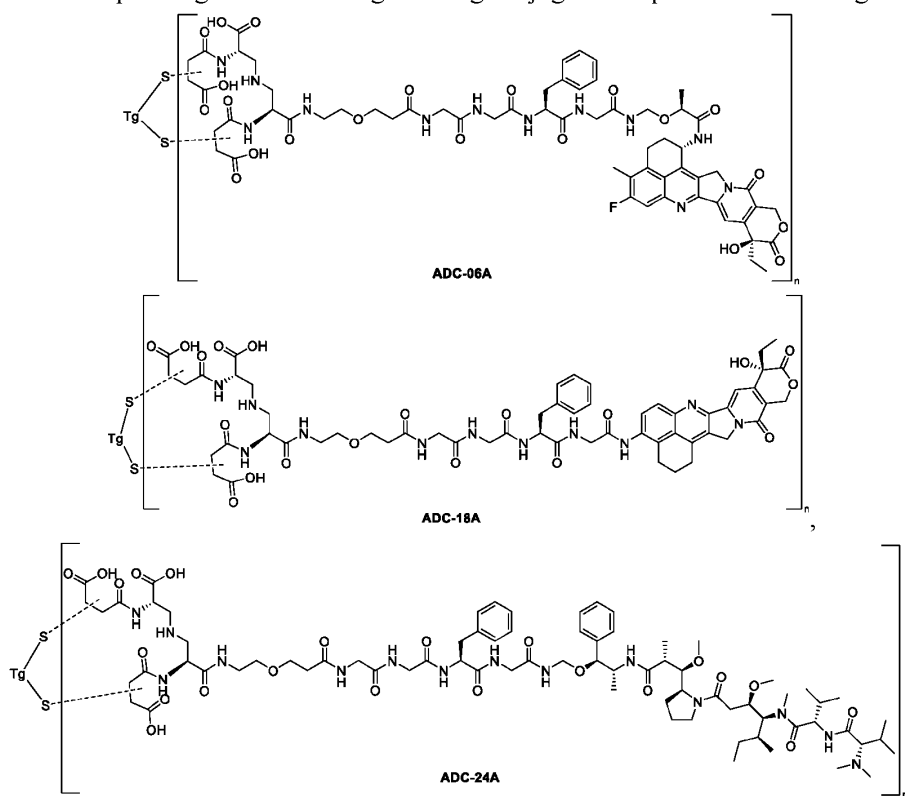
when n_3 and n_4 do not exist:

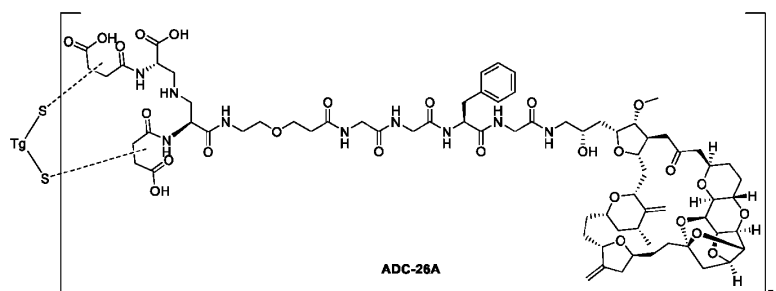
n_1 and n_2 are independently selected from integers or decimals from 1 to 10, and n_1 and n_2 are not simultaneously 0; preferably, n_1 is not 0; preferably, n_2 is 0;

and the sum of n_1+n_2 is an integer or decimal from 1 to 10, preferably an integer or decimal selected from 2 to 8, and preferably an integer or decimal selected from 3 to 6;

Tg is selected from ligands, preferably from antibodies.

35. The ligand-drug conjugate, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 19-34, wherein the open-ring form of said ligand-drug conjugate comprises the following:





wherein,

dashed lines indicate bonds connected to the succinimide ring-open form at any position;

n is an integer or a decimal from 1 to 10, preferably an integer or decimal from 2 to 8, or preferably an integer or a decimal from 3 to 6;

Tg is a ligand, preferably an antibody.

36. The linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 1-18, or the ligand-drug conjugate, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 19-35, wherein said pharmaceutically acceptable salt comprises a salt of sodium, potassium, calcium or magnesium derived from an acidic functional group of the linker-drug compound or the ligand-drug conjugate, or a salt of acetate, trifluoroacetate, citrate, oxalate, tartrate, malate, nitrate, chloride, bromide, iodide, sulfate, bisulfate, phosphate, lactate, oleate, ascorbate, salicylate, formate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, or p-toluenesulfonate derived from a basic functional group of the linker-drug compound or the ligand-drug conjugate.

37. A method of preparing the ligand-drug conjugate, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 19-35, comprising reacting reduced Tg with the linker-drug compound according to any one of Claims 1-18, its a racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvate, through a coupling reaction under suitable conditions; optionally, said method comprising the step of purifying the ligand-drug conjugate.

38. A pharmaceutical composition comprising the linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 1-18, or the ligand-drug conjugate, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 19-35, pharmaceutically acceptable salt or solvates, and, optionally, a pharmaceutically acceptable carrier.

39. A pharmaceutical preparation, comprising the linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 1-18, or the ligand-drug conjugate, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 19-35.

40. The linker unit-drug, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-18, or the ligand-drug conjugate, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 19-35, or the ligand-drug conjugate or open-ring form, its pharmaceutically acceptable salt or solvate thereof according to Claim 38, the use of a pharmaceutical composition or a pharmaceutical preparation according to Claim 39 in the preparation of a drug for the treatment or prevention of cancer or tumors;

alternatively, the linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-18, or the ligand-drug conjugate, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 19-35, the pharmaceutical composition according to Claim 38, the pharmaceutical preparation according to Claim 39, which is used for the treatment or prevention of cancer or tumors;

preferably, said cancer or tumor is adenocarcinoma, ovarian cancer, cervical cancer, uterine cancer, prostate cancer, renal cancer, urothelial cancer, bladder cancer, hepatocellular carcinoma, gastric cancer, endometrial cancer, salivary gland cancer, esophageal cancer, lung cancer, colon cancer, breast cancer comprising triple-negative breast cancer, rectal cancer, colorectal cancer, bone cancer, skin cancer, thyroid cancer, pancreatic cancer, melanoma, glioma, neuroblastoma, solid or hematologic tumors comprising glioblastoma multiforme, sarcoma, lymphoma, and leukemia.

41. A method of treating or preventing cancer or tumors, comprising administering to a subject in need thereof a prophylactically or therapeutically effective amount of the linker-drug compound, its racemate, enantiomer, diastereoisomer, pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-18, the ligand-drug conjugate, its racemate, enantiomer, diastereomer, open-ring form, or pharmaceutically acceptable salt or solvent compound thereof, according to any one of Claims 19-35, or the pharmaceutical composition according to Claim 38 or the pharmaceutical preparation according to Claim 39;

Preferably, said cancer or tumor is adenocarcinoma, ovarian cancer, cervical cancer, uterine cancer, prostate cancer, renal cancer, urothelial cancer, bladder cancer, hepatocellular carcinoma, gastric cancer, endometrial cancer, salivary gland cancer, esophageal cancer, lung cancer, colon cancer, breast cancer comprising triple-negative breast cancer), rectal cancer, colorectal cancer, bone cancer, skin cancer, thyroid cancer, pancreatic cancer, melanoma, glioma, neuroblastoma, solid or hematologic tumors comprising glioblastoma multiforme, sarcoma, lymphoma, and leukemia.