

ANTIBODY-AURISTATIN DRUG CONJUGATE AND PHARMACEUTICAL USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the filing date of CN202410048317.6, filed January 11, 2024, and CN202510015917.8, filed January 6, 2025, the entire disclosures of which are incorporated by reference herein.

TECHNICAL FIELD

10 The disclosure relates to the field of pharmaceutical technology, and specifically, to antibody-drug conjugates, linker-Auristatin-like drug compounds, and methods for preparing and using antibody-Auristatin-like drug conjugates.

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.

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
20 targeted cancer therapy. In recent years, the rapid development has led to the three generations of ADCs. Currently, 14 ADCs 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), and more than 200 ADC drug candidates have entered clinical
30 trials. ADCs are playing an increasingly important role in the field of targeted tumor therapy.

ADCs consist of three components: a monoclonal antibody with high binding specificity, a linker, and a small molecule cytotoxic drug (also known as payload or warhead). The antibody of an ADC is often an IgG1, and the payload is generally a cytotoxic drug such as maytansinoid, auristatins, calicheamicins, camptothecin, amatoxins, pyrrolbenzodiazepines, and others that may act on microtubules, DNA, or RNA, and the linker could be either a cleavable linker or a non-cleavable linker.

Auristatins are cytotoxic analogs of Dolastatin 10, a natural antimitotic agent found in the sea hare *Dolabella auricularia*. They are 100 to 1000 times more toxic than doxorubicin, a
40 conventional cancer chemotherapy drug. Auristatins bind to microtubules at a site very close to the vincristine binding site, which is located on the β -tubulin subunit near the GTP-binding pocket.

While the primary interaction of Auristatins is with amino acid residues at the vinca site, their structure may also allow for some additional contact with the GTP-binding pocket on β -tubulin, further contributing to microtubule destabilization. By binding to this site, Auristatins prevent the conformational changes required for tubulin dimers to properly assemble into microtubules, leading to microtubule depolymerization and ultimately cell death. Thus, by blocking microtubule binding to the vincristine binding site and the GTP-binding pocket, Auristatins inhibit microtubule formation, thereby stops cell division and leads to cell death.

Two Auristatins, MMAE and MMAF (US Patent 6,884,869, US Patent 7,498,298), are commonly used as ADC warheads. Both MMAE and MMAF are pentapeptides derived from the modification of Dolastatin 10 and exhibit excellent antitumor activity. While the lack of selectivity and low therapeutic index prevent them from being used as a single therapeutic agent in the clinic, the high cytotoxicity makes them ideal warheads for ADCs. MMAE has been used as the warhead for at least five approved ADCs (Adcetris, Polivy, Padcev, Aidixi, and Tivdak), and MMAF has been used for at least one ADC (Blenrep). However, these ADCs with Auristatins as their warhead have many shortcomings with significant adverse effects such as neurotoxicity (e.g., peripheral neuropathy) and hematotoxicity (e.g., thrombocytopenia and neutropenia) when MMAE is used, and ophthalmotoxicity when MMAF is used. To improve the efficacy and safety, various chemical modifications have been tested, including ways to modify linkers since most of the linkers used in these ADCs are VC linkers with a drug-antibody ratio (DAR) of up to 4. However, VC linker's high hydrophobicity leads to the problem that a high DAR tends to cause precipitation and aggregation of ADC, therefore one could not increase its DAR to enhance the efficacy. Several attempts of chemical modifications target the hydroxyl group of MMAE (see US2005009751A1, Nat. Biotechnol. 2003 Jul;21(7):778-84, CN105813653, WO2020260597A1, and CN106279352A); however significant reduction in adverse effects has yet to be seen with these attempts.

Along with chemical modifications, ADCs have undergone three generations, marked by improvements in antibodies. The first-generation ADCs use murine or chimeric antibodies, combined with unstable linkers and low-potency cytotoxic drugs, and employed random coupling. For example, Mylotarg is a first-generation of ADCs with relatively low efficacy and substantial toxic side effects. The second-generation ADCs use humanized antibodies combined with more stable linkers. For example, Adcetris and Kadcyra contain humanized antibodies, but still produced off-target toxicity. The third-generation ADCs use fully humanized antibodies combined with more potent toxins and site-directed techniques to achieve better efficacy; however, "off-target" toxic side effects still exist.

All 14 ADCs currently on the market have antibodies that are monospecific monoclonal antibodies targeting a single target. In contrast, bispecific monoclonal antibodies can be configured to reduce "off-target" binding by improving binding specificity, thereby reducing adverse reactions. Such bispecific antibodies may have binding specificity for two tumor-associated antigens (TAA) or two epitopes of the same TAA. The anti-EGFR $\times$ HER3 bispecific antibody-camptothecin drug conjugate was one of the first bispecific antibody-drug conjugates

(BsADCs) to be tested in clinical trials (WO2023083381A1, the entire reference is incorporated by reference herein). This bispecific antibody-drug conjugate is stable in treatment and has excellent uniformity, with a drug-to-antibody ratio (DAR) of 6.0-8.0. Optimizing the antibody, linker and toxin of this new class of ADCs is of great clinical significance.

SUMMARY

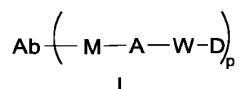
The following summary is illustrative only and is not intended to be in any way limiting. In addition to the illustrative aspects, embodiments, and features described above, further aspects, embodiments, and features will become apparent by reference to the drawings and the following detailed description.

Herein one of the technical problems to be solved lies in exploring and discovering better anti-tumor Auristatin based bispecific antibody-drug conjugates (BsADCs) with higher safety and efficacy to meet the clinical needs.

The application discloses, among others, ADCs comprising a ligand unit and a drug unit, antibodies including without limitation bispecific antibodies or monoclonal antibodies as the ligand unit, linker-drug compounds including without limitation linker-auristatin (and its pharmaceutically or chemically applicable derivatives), ADCs comprising bispecific antibody as ligand units and auristatin derivatives as payload (i.e., drug units), linkers connecting the ligand unit with the drug unit, methods of making the ADCs, pharmaceutical compositions comprising the ADCs and methods of making thereof, and method of treating or preventing diseases such as cancers using ADCs.

In one embodiment, the application provides methods for connecting a drug payload, auristatin or its derivatives, to the enzymatically cleavable peptide unit through the amino-methylene structure from the hydroxyl site to form the corresponding linker-payload compound, which can increase the DAR value of ADC to 8, with better hydrophilicity at high loading, better plasma stability at high drug loading values, and significant advantages in terms of drug efficacy over convention ADCs. Compared with convention ADCs, such as ADCs with Vc-MMAE as the linker-payload, the same tumor inhibitory effect can be achieved with a lower dose, and, with improved MTD, the neurotoxicity and hematotoxicity are significantly reduced.

In one aspect, the disclosure provides antibody-drug conjugates as shown in the Formula I or pharmaceutically acceptable salts or solvates thereof:



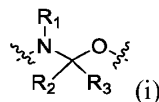
wherein,

Ab is a bispecific antibody or its antigen-binding fragment targeting EGFR and HER3;

M is a linker unit connected to Ab;

A is independently a peptide consisting of 2 to 7 amino acids, wherein, optionally, each of said amino acids is independently substituted by one or more substituents selected from a group comprising deuterium, halogen, hydroxyl, cyano group, amino group, nitro group, alkyl, substituted alkyl, alkoxy, cycloalkyl, and substituted cycloalkyl;

W denotes an amino methylene oxide structural unit as shown in Formula (i):



wherein:

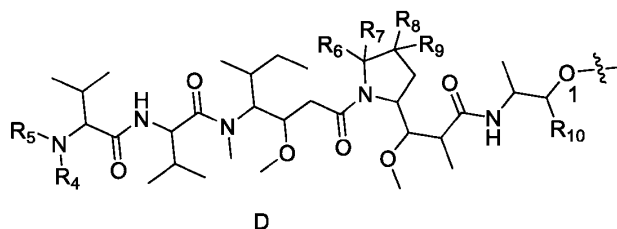
the wavy line on the left side indicates the site of attachment of the nitrogen atom to A in Formula (i), the wavy line on the right side indicates the site of attachment of the oxygen atom to the drug D in Formula (i), the oxygen atom is a common group of the drug unit D and W;

the wavy line on the left side denotes the site of attachment between the nitrogen atom and A in Formula (i), the wavy line on the right side denotes the site of attachment between the oxygen atom and the drug unit D in Formula (i), and the oxygen atom is a common group of the drug D and W;

R₁, R₂ and R₃ are each independently hydrogen, deuterium, an alkyl group and substituted alkyl group;

p is an integer or decimal independently 1 to 20 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20); and

drug unit D is auristatin having the structure shown in Formula D, or in the form of its isomers, endo-, racemates, enantiomers, or mixtures thereof, or a pharmaceutically acceptable salt thereof.



D

wherein,

R₄, R₅ are each independently hydrogen, deuterium, alkyl, or deuterated alkyl, or R₄, R₅ are linked to form the following structure: $-(\text{CR}_{11}\text{R}_{12})_n-\text{B}-(\text{CR}_{13}\text{R}_{14})_m-$, wherein R₁₁, R₁₂, R₁₃, and R₁₄ are independently hydrogen, deuterium, alkyl, or deuterated alkyl; B is independently O, NR₁₅, CR₁₆R₁₇, wherein R₁₅, R₁₆, R₁₇ are independently hydrogen, deuterium, or alkyl; n and m are independently an integer number from 0 to 8 respectively (e.g. 0, 1, 2, 3, 4, 5, 6, 7, 8); the nitrogen atom bonded to R₄ and R₅ may form a ring with $-(\text{CR}_{11}\text{R}_{12})_n-\text{B}-(\text{CR}_{13}\text{R}_{14})_m-$;

R₆, R₇, R₈, R₉ are each independently hydrogen, deuterium, halogen, an azido group, alkyl and NR₁₈R₁₉; alternatively, any two of R₆, R₇, R₈, R₉ together with the atom to which they are bonded form a cycloalkyl group, and the remaining two groups are each independently hydrogen, halogen, an azido group, alkyl, and NR₁₈R₁₉, wherein R₁₈ and R₁₉ are independently hydrogen, or alkyl;

R₁₀ is aryl or heteroaryl, and said aryl or heteroaryl is optionally substituted with one or more substituents comprising hydrogen, halogen, alkyl, an alkoxy group, an amino group, or a nitro group;

the wavy line in Formula D denotes the site of attachment between the oxygen atom at position

l in the structure of drug unit D to W; said oxygen atom is commonly shared between D and W.

In one embodiment, the antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof, as described in Formula I, is characterized in that the Ab comprises an IgG1 heavy chain, a κ light chain, and a single-chain Fv (scFv) domain. In one embodiment, the scFv domain forms a construct with the IgG1 heavy chain or the κ light chain. In one embodiment, said IgG1 heavy chain and κ light chain form an IgG having a binding specificity for EGFR. In one embodiment, said scFv domain has binding specificity for HER3. In one embodiment, said scFv domain is attached to the C-terminus or the N-terminus of said IgG1 heavy chain via a linker. In one embodiment, said scFv domain is attached to the C-terminus or N-terminus of the κ light chain via a linker. In one embodiment, the linker comprises an amino acid sequence having the amino acid sequence (gly-gly-gly-gly-gly-ser)_n, wherein n is an integer of at least 1, for example, n may be an integer of 1 to 10, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In one embodiment, the scFv domain has a structural order of N-terminus-heavy chain variable region-connector-light chain variable region-C-terminus. In one embodiment, the scFv domain has a structural order of N-terminus-light chain variable region-connector-heavy chain variable region-C-terminus. In one embodiment, said connector comprises an amino acid sequence of (gly-gly-gly-gly-gly-ser)_m, wherein m is an integer of at least 3, for example, m may be 3, 4, 5, or 6.

In one embodiment, the antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof is characterized in that the κ light chain of the antibody Ab comprises CDRs having at least 98%, 99%, or 100% sequence identity to SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11. In one embodiment, the IgG1 heavy chain comprises CDRs having at least 98%, 99%, or 100% sequence identity to SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14. In one embodiment, the scFv domain comprises heavy chain variable region CDRs having at least 98%, 99%, or 100% sequence identity to SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, and light chain variable region CDRs having at least 98%, 99%, or 100% sequence identity to SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20.

In one embodiment, the antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof, as described in Formula I, is characterized in that the light chain of the antibody Ab comprises a variable region having at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 5. In one embodiment, the IgG1 heavy chain comprises a variable region having at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 6. In one embodiment, the scFv domain comprises a variable region of the heavy chain having at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 7 and a light chain variable region having at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 8.

In one embodiment, Ab may have a light chain amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 2, and the construct of the heavy chain with the scFv domain may have an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 4.

In one embodiment, Ab may have a κ light chain with CDRs having at least 98%, 99%, or 100%

sequence identity to SEQ ID NO: 29, SEQ ID NO: 30, and SEQ ID NO: 31, an IgG1 heavy chain with CDRs having at least 98%, 99%, or 100% sequence identity to SEQ ID NO: 32, SEQ ID NO: 33, and SEQ ID NO: 34, and a scFv domain comprising heavy chain variable region CDRs having at least 98%, 99%, or 100% sequence identity to SEQ ID NO: 35, SEQ ID NO: 36, and SEQ ID NO: 37, and light chain variable region CDRs having at least 98%, 99%, or 100% sequence identity to SEQ ID NO: 38, SEQ ID NO: 39, and SEQ ID NO: 40.

10 In certain embodiments, Ab may have a light chain with a variable region having at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 25, an IgG1 heavy chain having at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 26. In one embodiment, Ab may have a scFv domain comprising a heavy chain variable region having at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 27 and a light chain variable region having at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 28.

In certain embodiments, Ab may have a light chain having at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 22, and a construct comprising a heavy chain and the single-chain Fv (scFv) domain, and the construct has at least 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 24.

20 In one embodiment, Ab may have a light chain nucleic acid coding sequence having at least 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 1, and the construct of the heavy chain with the scFv domain may have a nucleic acid coding sequence having at least 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 3.

In one embodiment, the antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof as described in Formula I is characterized in that Ab comprises: two IgG1 heavy chains; two κ light chains; and two scFv domains. In one embodiment, each of the scFv domains is linked to each of the IgG1 heavy chain at the C-terminus.

In some embodiments, R_4 and R_5 are each independently hydrogen, or C_1 - C_4 alkyl.

In some embodiments, R_4 and R_5 are linked to form the following structure: $-(CH_2)_2-B-(CH_2)_2-$, B is O, or NH, and said nitrogen atom bonded to R_4 and R_5 form a ring with $-(CH)_2-B-(CH_2)_2-$.

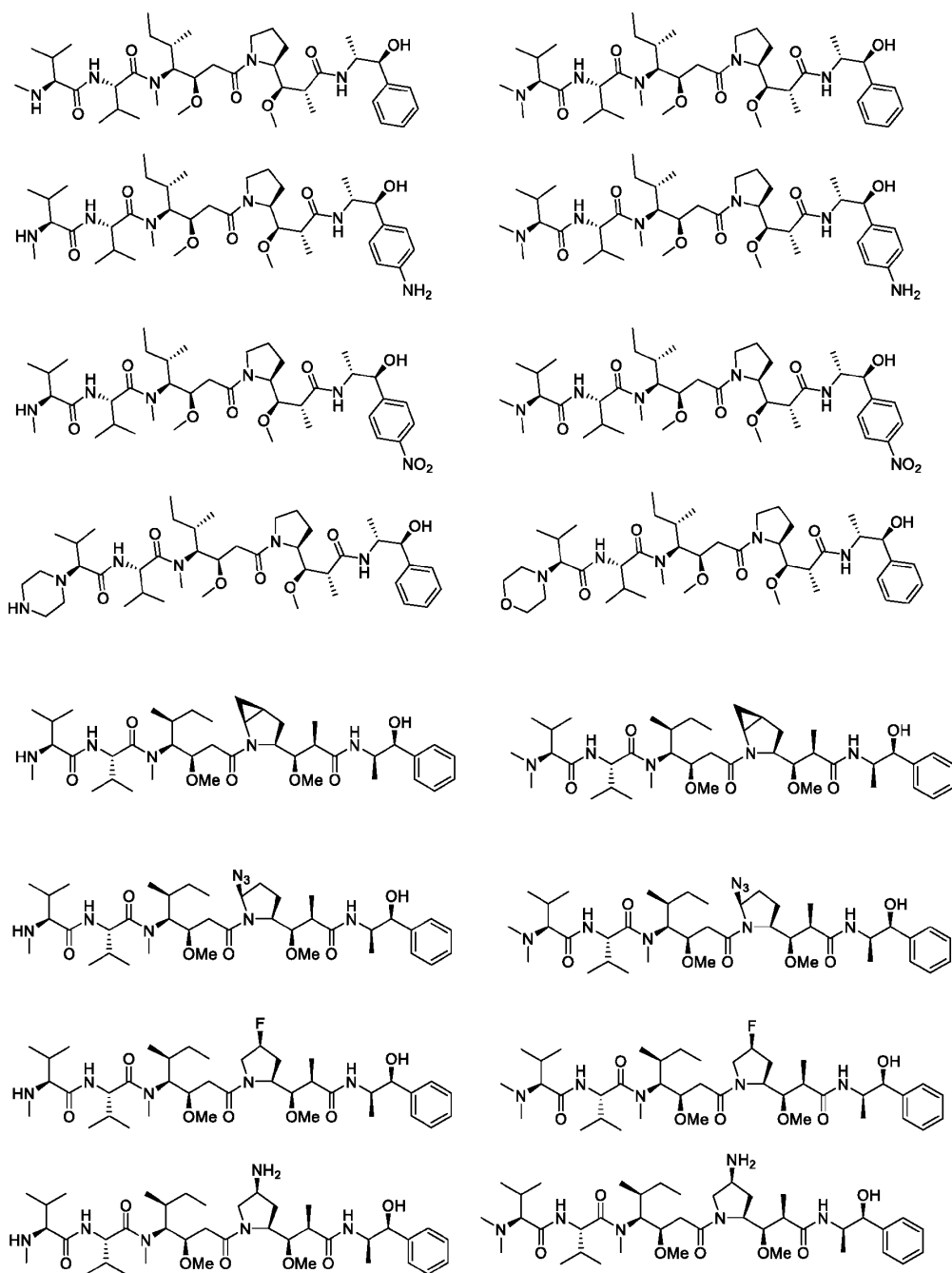
In some embodiments, R_6 , R_7 , R_8 , R_9 in Formula D are each hydrogen.

30 In one embodiment, one of R_6 , R_7 , R_8 , R_9 in Formula D is independently halogen, an azido group, amino, and the remaining three are each hydrogen.

In some embodiments, any two groups of R_6 , R_7 , R_8 , R_9 in Formula D form a cyclopropyl group together with the atom to which it is bonded, the remaining two groups being each independently hydrogen.

In one embodiment, R_{10} in Formula D is substituted or unsubstituted phenyl. In one embodiment, the substituted phenyl is substituted with one or more of substituents. In one embodiment, said substituent is amino or nitro.

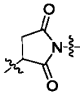
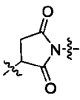
In one embodiment, D is independently, without limitation, the following compounds:



In the above embodiments, the D may be linked to W via a hydroxyl group.

In some embodiments, the linker unit M comprises maleimide. In these embodiments, said antibody-drug conjugate can be hydrolyzed under hydrolysis conditions, the site of hydrolysis is the maleimide portion of the linker unit. When the ligand contains multiple linker-drugs, the

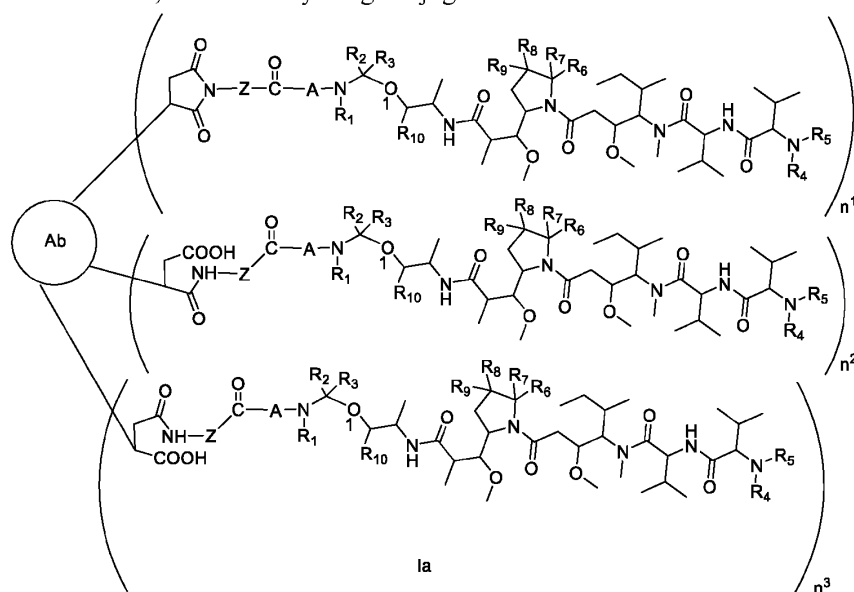
following scenarios may occur depending on with the degree of hydrolysis:

- (1) maleimides are not hydrolyzed, that is, all maleimides are in closed loop form  ;
- (2) maleimides are not completely hydrolyzed, that is, part of the maleimide is in closed ring form , and the other part of maleimide is in open ring form  or  ;
- (3) maleimides are completely hydrolyzed, that is, all maleimides are in open ring form  or .

In some embodiments, when there are multiple maleimide group-containing linker units M in the ADC (i.e., Ab is linked to multiple maleimide group-containing drug-linkers), these maleimide groups can all be in closed-loop form, some in an open-loop form, or all in open-loop form.

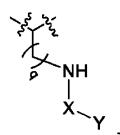
10 In the above mentioned maleimide structural formulas, the left wavy line denotes the linkage site to Ab, and the right wavy line denotes other structures in M.

In one embodiment, said antibody-drug conjugate has the structure shown in Formula Ia:



wherein,

Z is independently -C₁-C₁₀ alkylene-, -C₃-C₈ cycloalkyl-, -aryl-, -C₁-C₁₀ alkylene-aryl-, -aryl-C₁-C₁₀ alkylene-, -C₁-C₁₀ alkylene-(C₃-C₈ cycloalkyl)-, -(C₃-C₈ cycloalkyl)-C₁-C₁₀ alkyl-, 3-8-membered heterocycle-, (3-8-heterocyclic)-C₁-C₁₀-alkyl-, -(CH₂CH₂O)_i-, -(CH₂CH₂O)_i-CH₂-, or

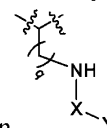


wherein X is independently -C₁-C₁₀ alkylene-, -C₃-C₈ carbocyclic-, -arylidene-, -C₁-C₁₀ alkylene-, -C₁₀-alkylene-, -C₁-C₁₀ alkylene-(C₃-C₈ carbocyclic)-, -(C₃-C₈ carbocyclic)-, -(C₃-C₈ cycloalkyl)-C₁-C₁₀ alkyl-, (3-8-membered heterocyclic)-, -(3-8-membered heterocyclic)-C₁-C₁₀ alkylene-, -(CH₂CH₂O)_r-, -(CH₂CH₂O)_r-CH₂-;

Y is a hydrophilic structure derived from carboxyl group, phosphoric acid, polyphosphoric acid, phosphite, sulphonic acid, sulfurous acid, sulfinic acid, or poly(ethylene glycol) (PEG);

said heterocyclic rings each independently comprise 1 to 3 atoms independently selected from N, O, S;

- 10 said -C₁-C₁₀ alkylene-, -C₃-C₈ carbocyclic-, and heterocyclic rings are each independently substituted with one or more substituents, said substituents being independently selected from deuterium, halogen, hydroxyl, cyano group, nitro group, amino group, alkyl, heteroalkyl,



substituted alkyl, alkoxy group, carboxylic group, or cycloalkyl; the left wavy line in denotes the linkage site to the N on the maleimide, the right wavy line denotes the linkage site to the carbonyl group;

r is an integer between 1 and 10; in one embodiment, r may be 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

q is an integer between 1 and 8; in one embodiment, q may be 1, 2, 3, 4, 5, 6, 7, 8;

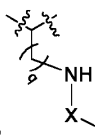
n¹, n², n³ are independently integers or decimals between 0 and 20, n¹, n², n³ are not simultaneously 0, and n¹ + n² + n³ ≤ 20, e.g. 1 ≤ n¹ + n² + n³ ≤ 2, or 7 ≤ n¹ + n² + n³ ≤ 8.

- 20 In some embodiments, n¹, n², n³ are independently integers such as 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20. In some embodiments, n¹, n², n³ are independently decimals such as 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, or 9.9.

In one embodiment, A in Formula I or Formula Ia is a peptide residue formed from 2 to 7 amino acids. The amino acids may be from phenylalanine (F), glycine (G), valine (V), lysine (K), alanine (A), citrulline, serine (S), glutamate (E), or aspartic acid (D).

- 30 In one embodiment, A is a peptide residue formed from 2 to 4 amino acids. In one embodiment, the amino acids may be independently selected from phenylalanine and glycine. In one embodiment, A is a tetrapeptide residue formed from glycine-glycine-phenylalanine-glycine.

In some embodiments, Z is -C₁-C₁₀ alkylene-, such as -C₄-C₆ alkylene-, or -C₅ alkylene-.



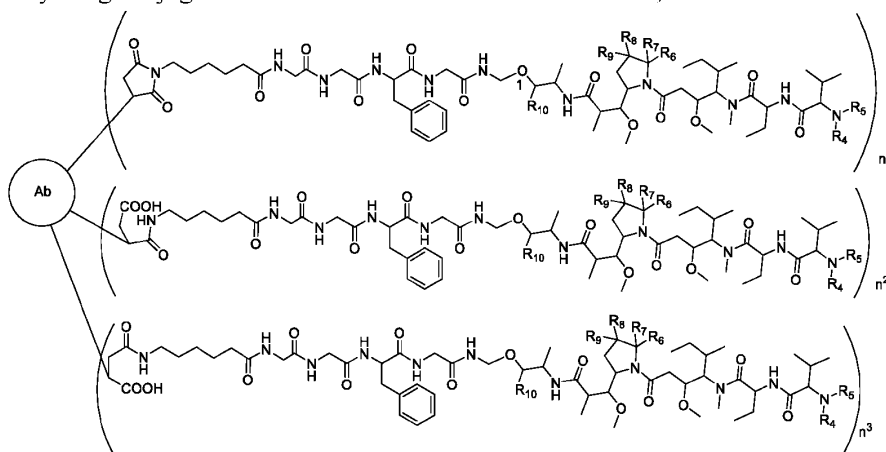
In some embodiments, Z is $\text{X}-\text{Y}$, wherein q is independently an integer between 1 and 8; in one embodiment, q may be 1, 2, 3, 4, 5, 6, 7, or 8. In one embodiment, q is 1.

In one embodiment, X is a methylene group, which may be optionally substituted with a carboxyl group. In one embodiment, Y is a carboxyl group or a phosphate group. In one embodiment, the wavy line on the left indicates the connection point to the nitrogen atom on the maleimide, while the wavy line on the right indicates the connection point to the carbonyl group.

In one embodiment, R_1 , R_2 and R_3 are each independently hydrogen, deuterium, alkyl, alkyl halide, deuterated alkyl, and hydroxyalkyl group.

In some embodiments, R_1 , R_2 , and R_3 are simultaneously hydrogen or deuterium.

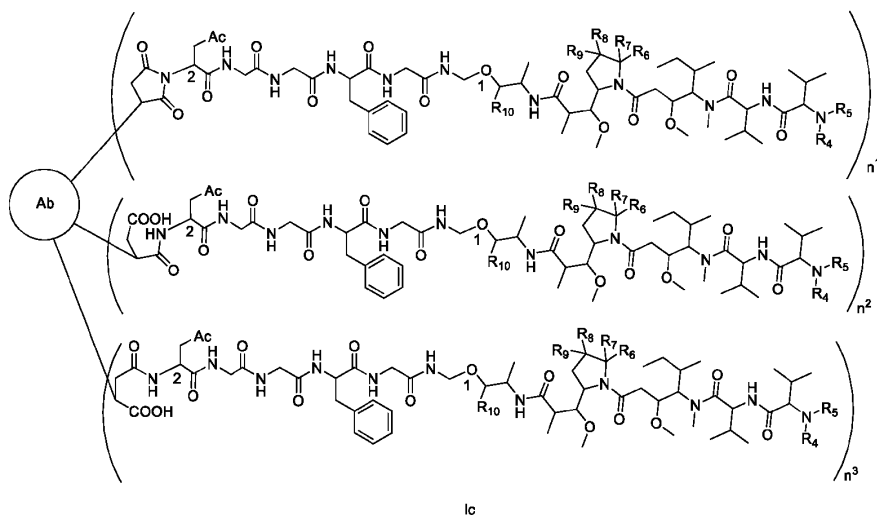
10 In some embodiments, R_1 , R_2 , and R_3 are simultaneously hydrogen. In some embodiments, the antibody-drug conjugate has a structure as shown in Formula Ib,



Ib

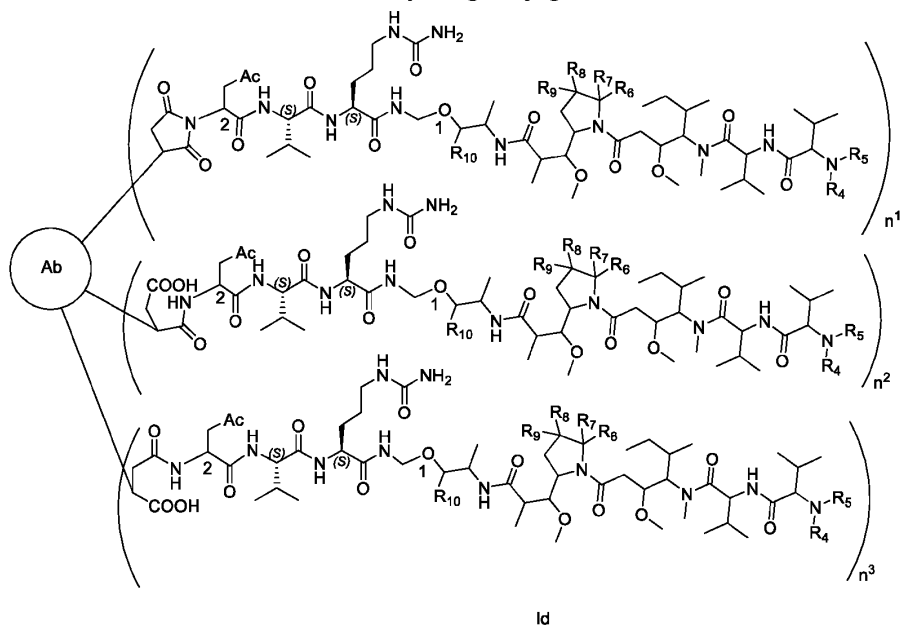
wherein R_4 , R_5 , R_6 , R_7 , R_8 , R_9 , R_{10} , n^1 , n^2 , n^3 are as defined above.

In one embodiment, said antibody-drug conjugate has a structure as shown in Formula Ic,



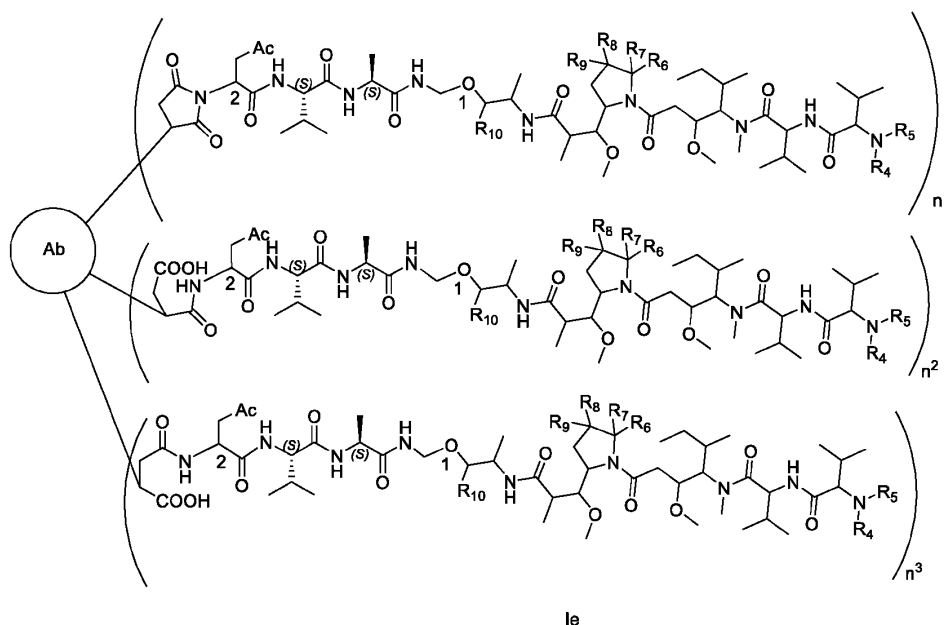
wherein R_4 , R_5 , R_6 , R_7 , R_8 , R_9 , R_{10} , n^1 , n^2 , n^3 are as defined above.

In one embodiment, said antibody-drug conjugate has a structure as shown in Formula Id,



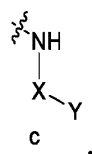
wherein R_4 , R_5 , R_6 , R_7 , R_8 , R_9 , R_{10} , n^1 , n^2 , n^3 are as defined above.

In one embodiment, said antibody-drug conjugate has a structure as shown in Formula Ie,



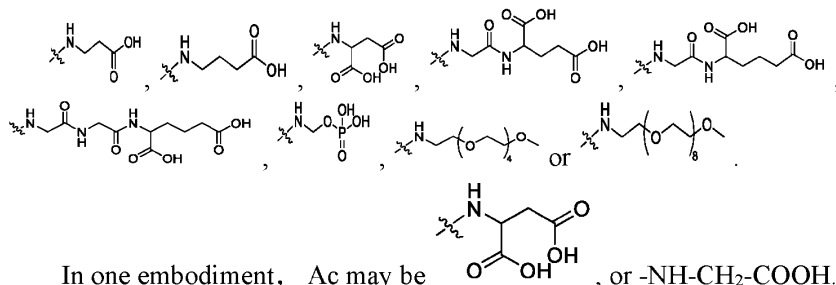
wherein R_4 , R_5 , R_6 , R_7 , R_8 , R_9 , R_{10} , n^1 , n^2 , n^3 are as defined above.

In some embodiments, in Formula Ic, Id, or Ie, Ac is a hydrophilic structural unit having the structure shown in Formula c:

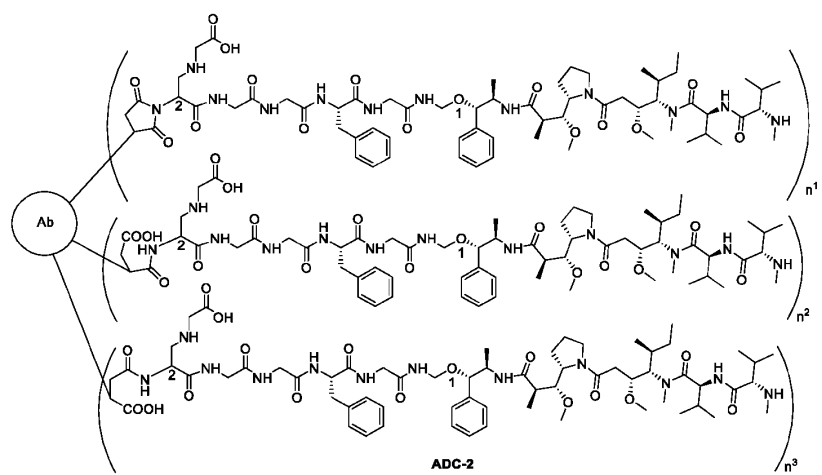
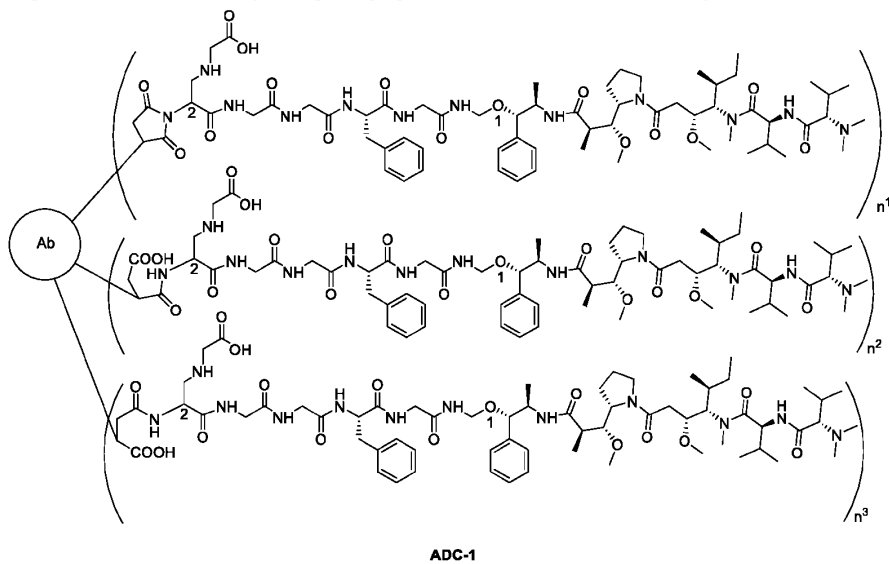


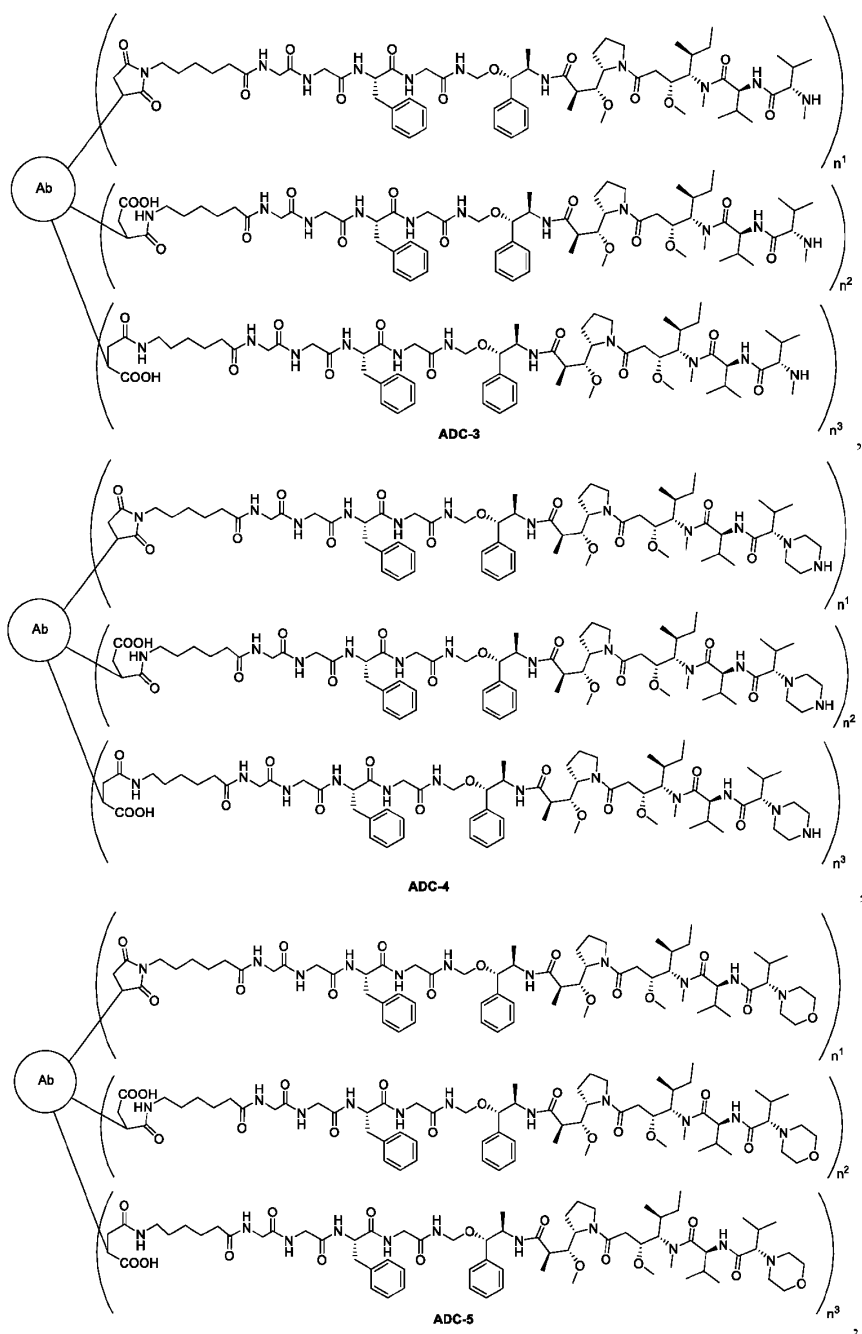
Ac is linked to methylene carbon at the position-2 labeled in Formula Ic, Id or Ie via, in one embodiment, an amino functional group; X, Y, R_4 , R_5 , R_6 , R_7 , R_8 , R_9 , R_{10} , n^1 , n^2 , n^3 are as defined above.

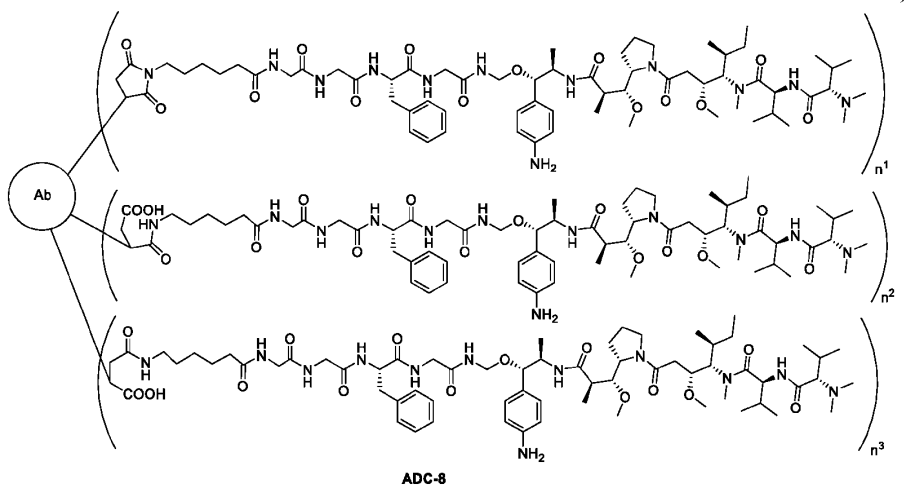
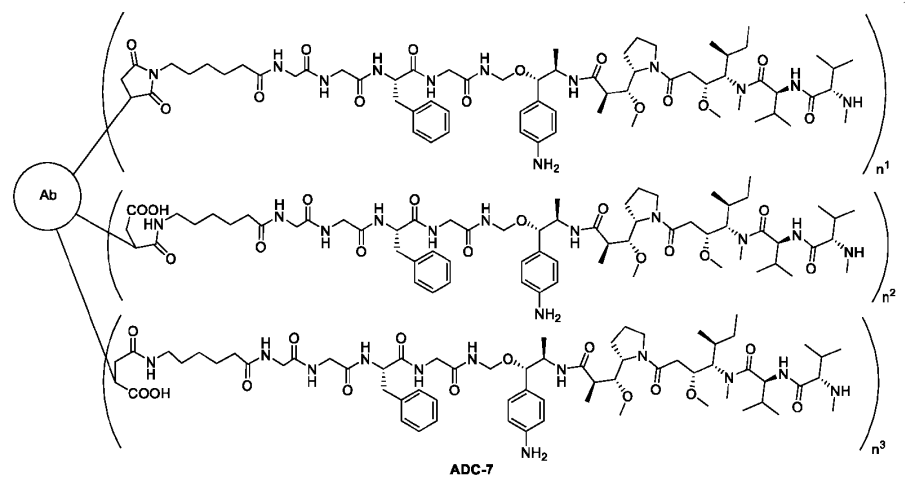
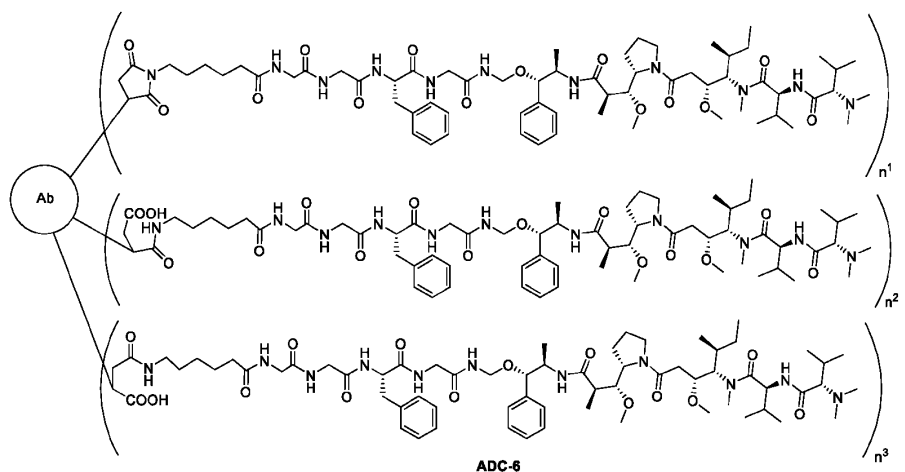
In one embodiment, said Ac comprises a group derived from glycine, (D/L) alanine, (D/L) leucine, (D/L) isoleucine, (D/L) valine, (D/L) phenylalanine, (D/L) proline, (D/L) tryptophan, (D/L) serine, (D/L) tyrosine, (D/L) cysteine, (D/L) cystine, (D/L) arginine, (D/L) histidine, (D/L) methionine, (D/L) asparagine, (D/L) glutamine, (D/L) threonine, (D/L) aspartic acid, (D/L) glutamic acid, natural or non-natural amino acid derivatives, or the following structures.

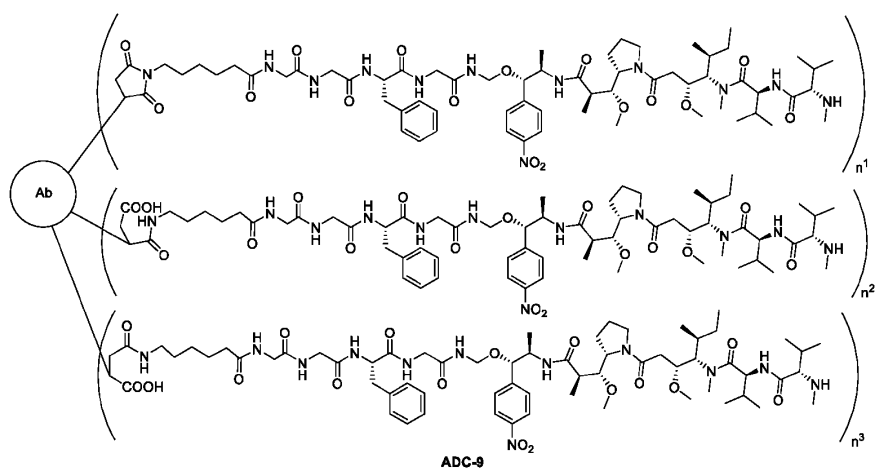


The representative antibody-drug conjugates as disclosed herein may include the following:

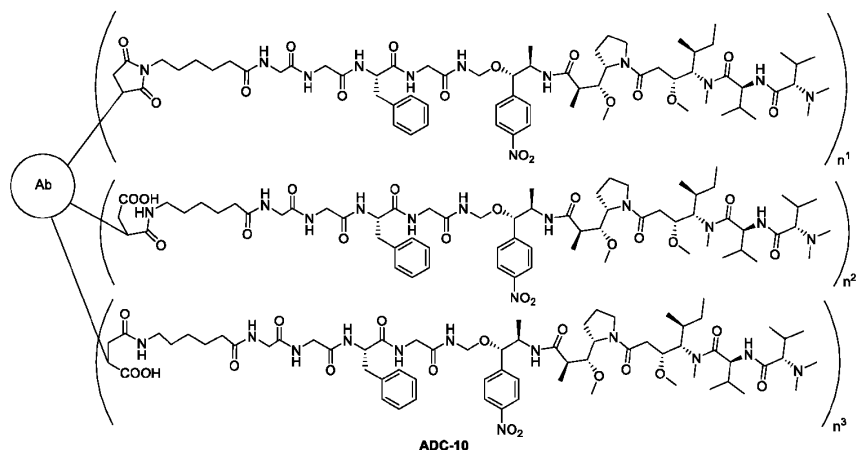




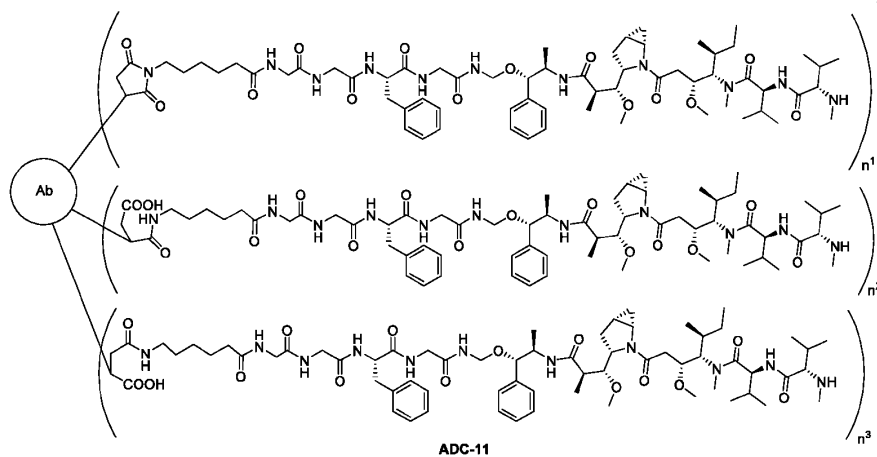




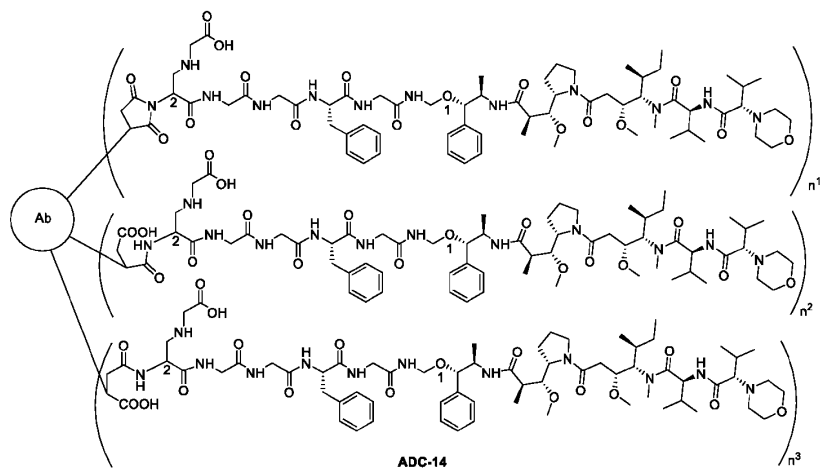
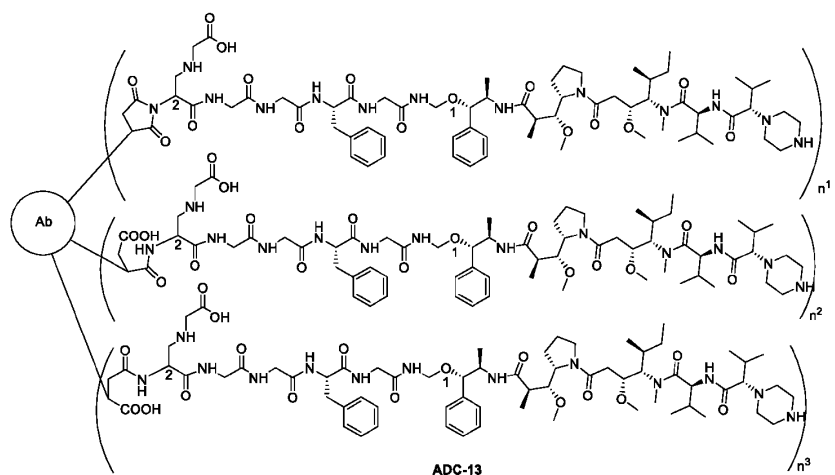
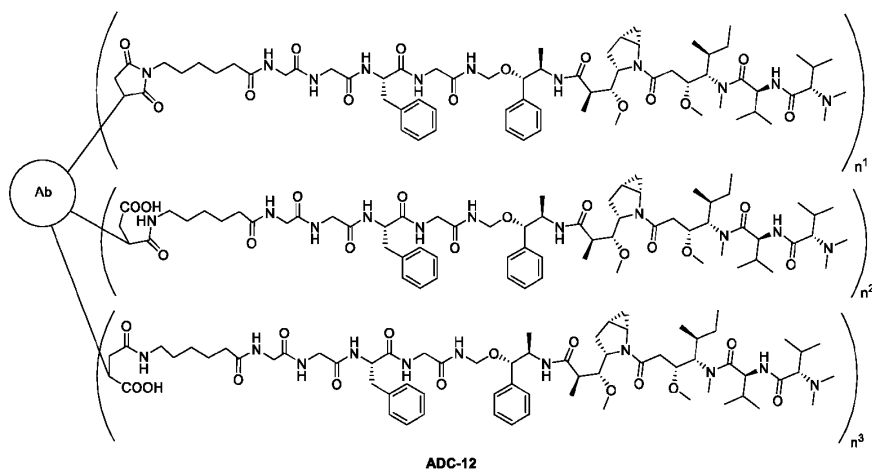
ADC-9

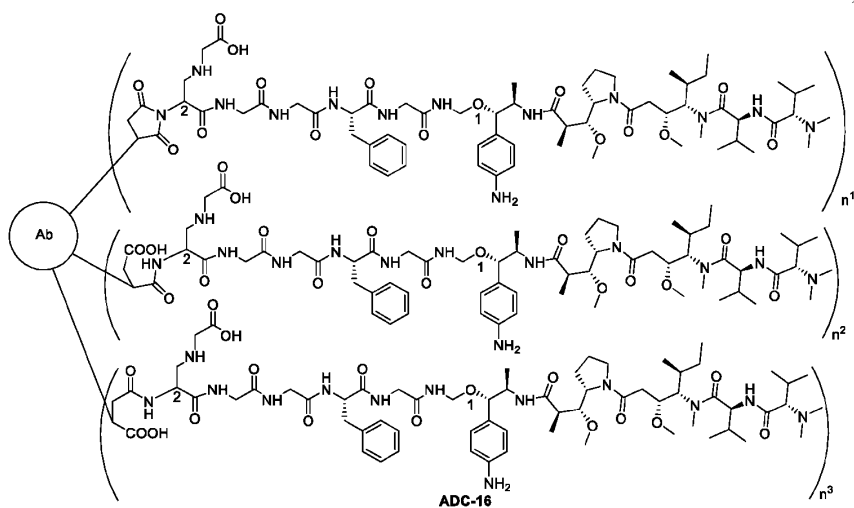
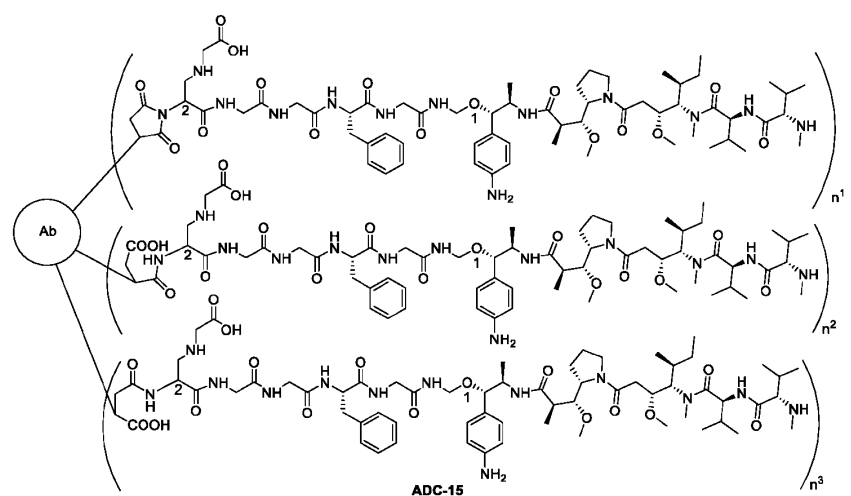


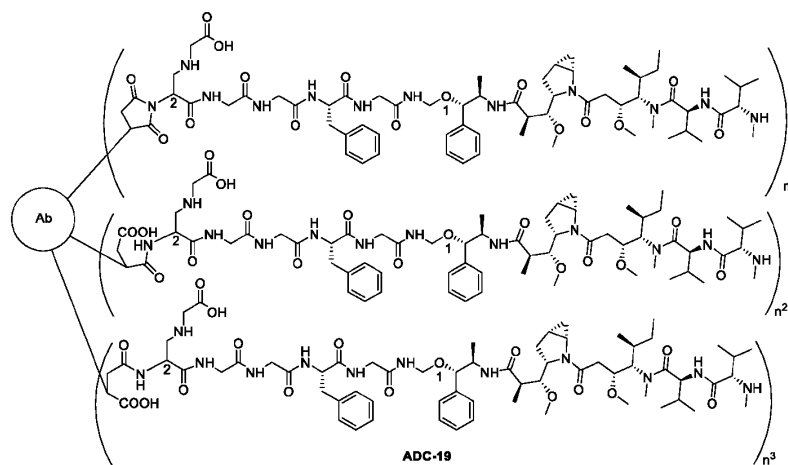
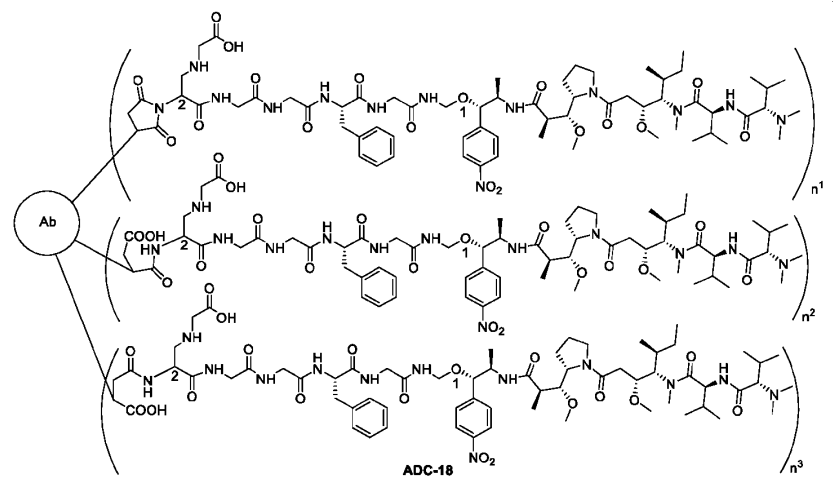
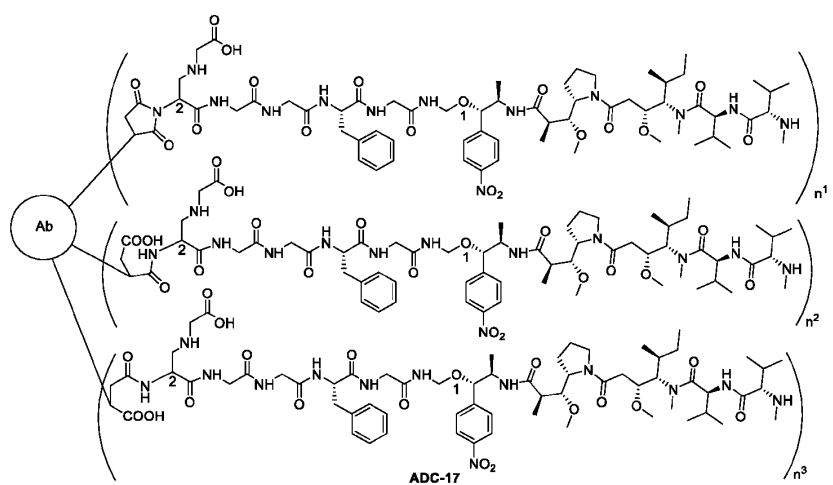
ADC-10

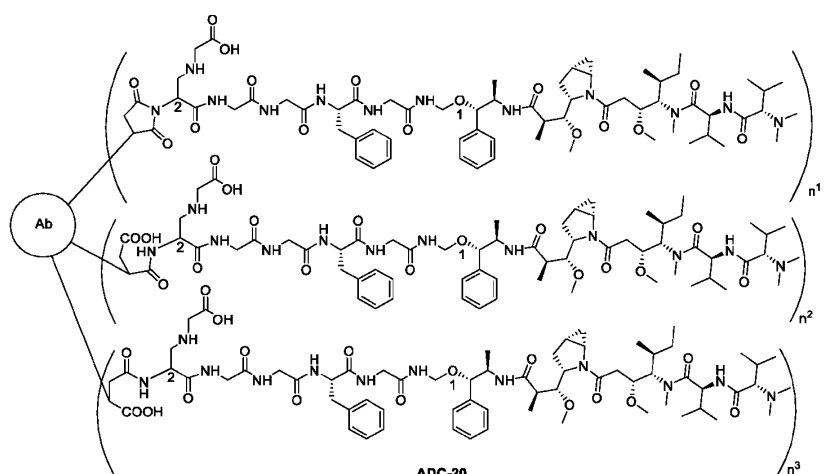


ADC-11

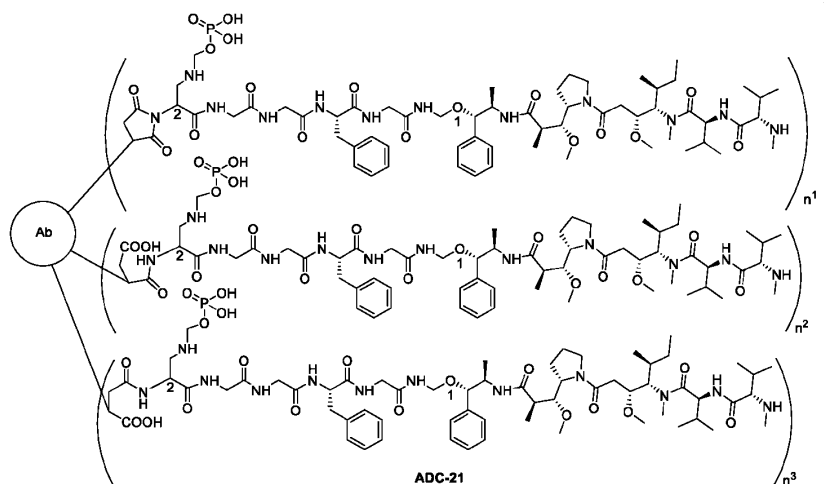




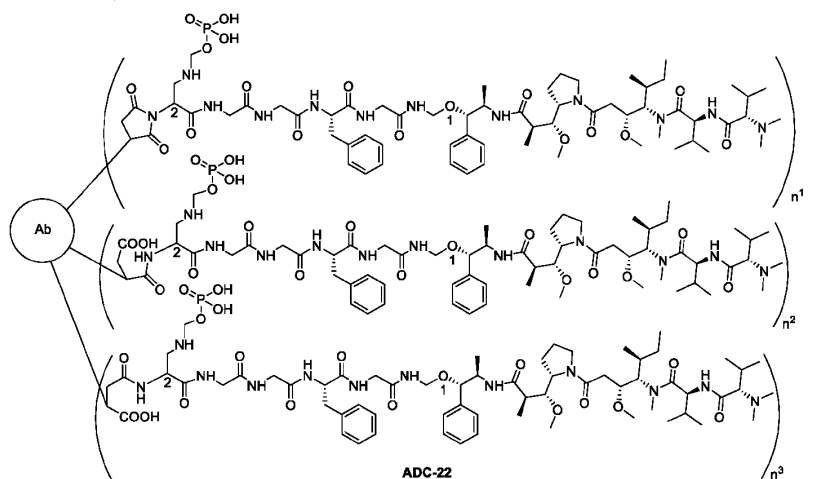




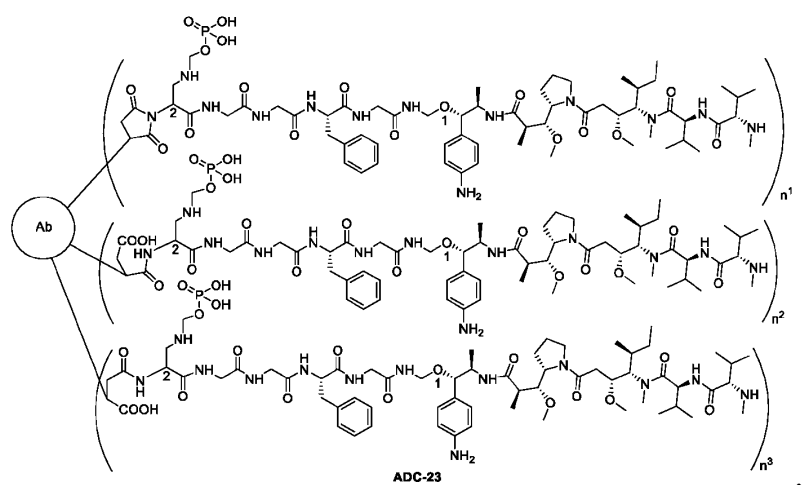
ADC-20



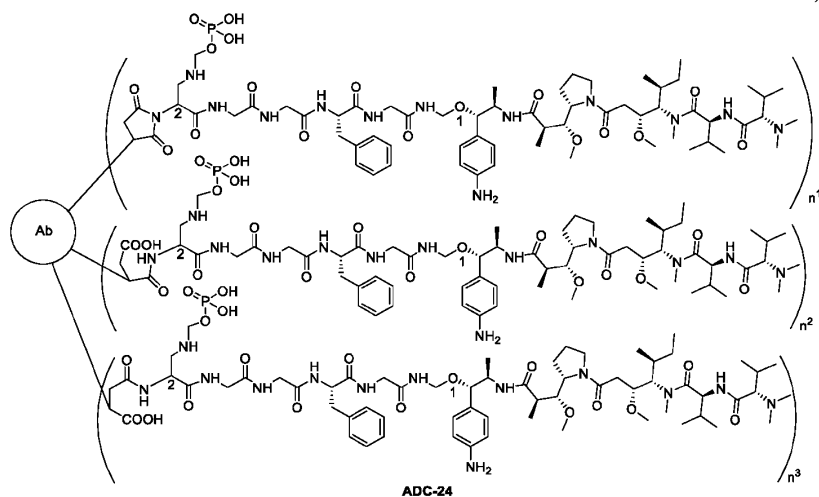
ADC-21



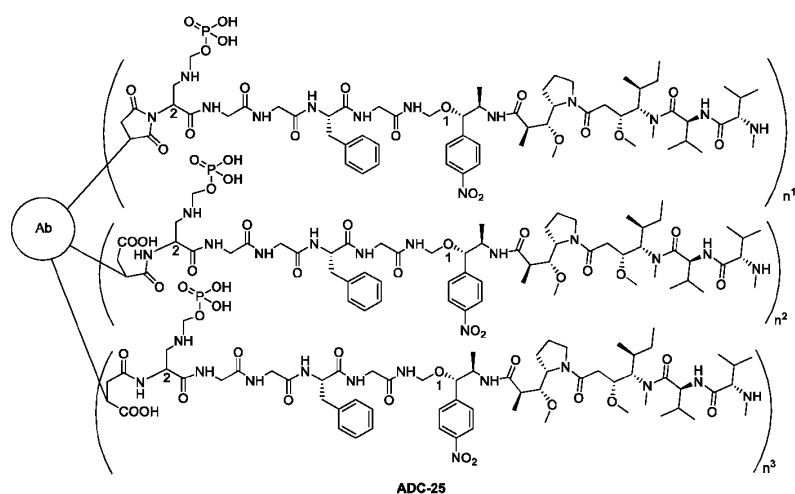
ADC-22



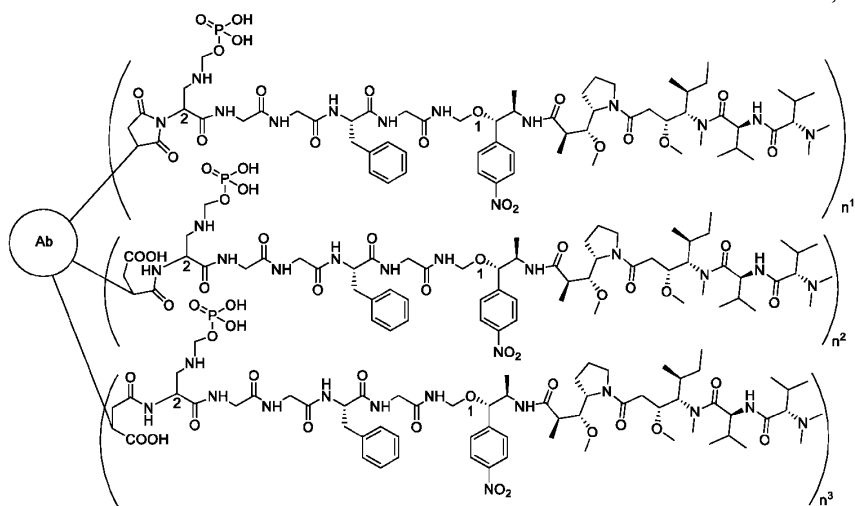
ADC-23



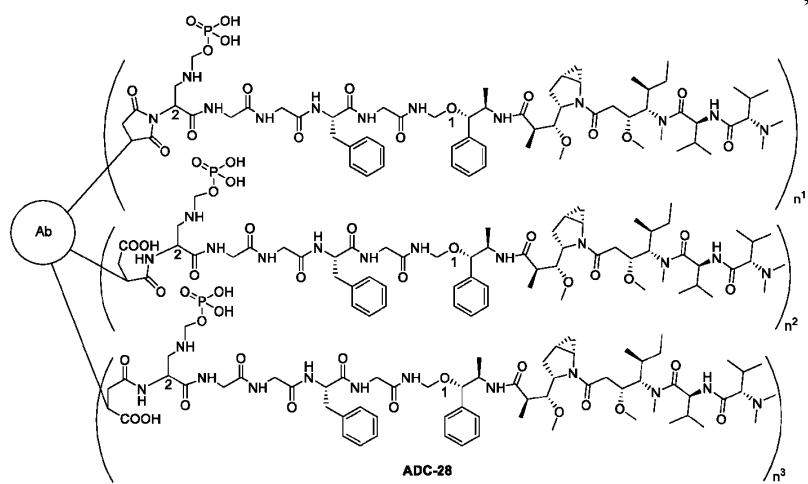
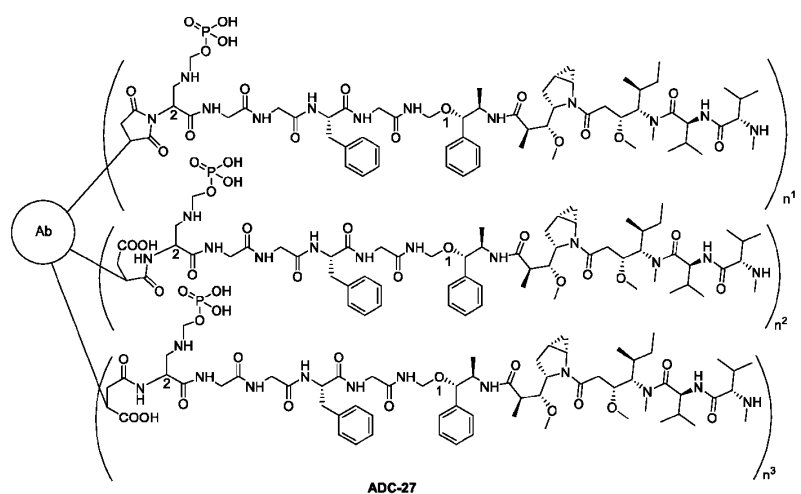
ADC-24

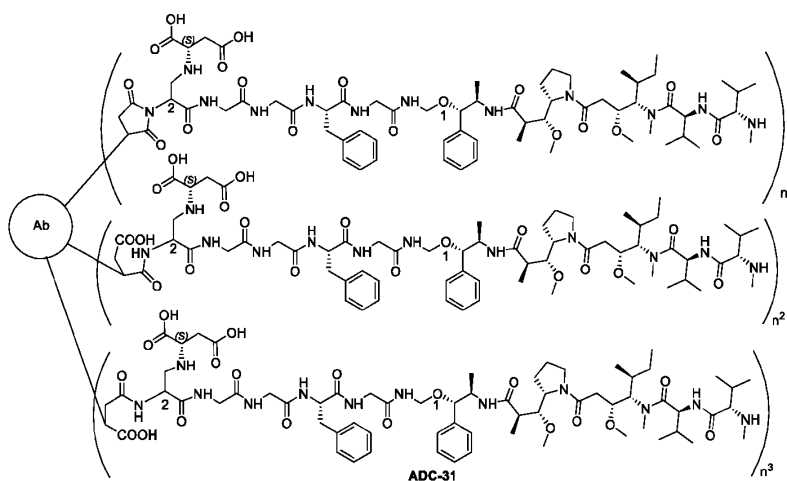
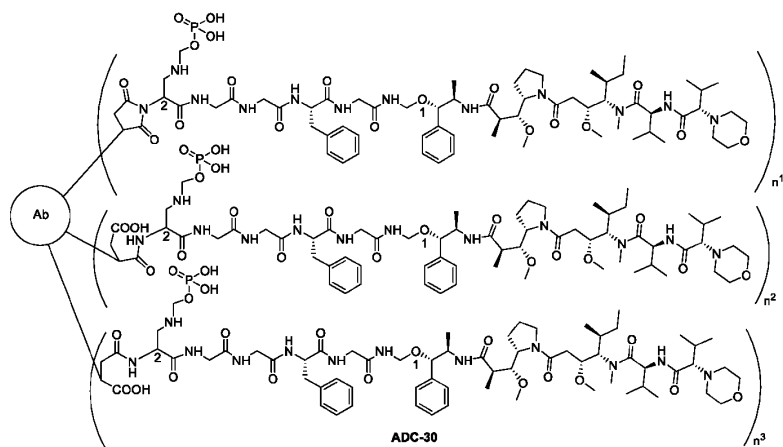
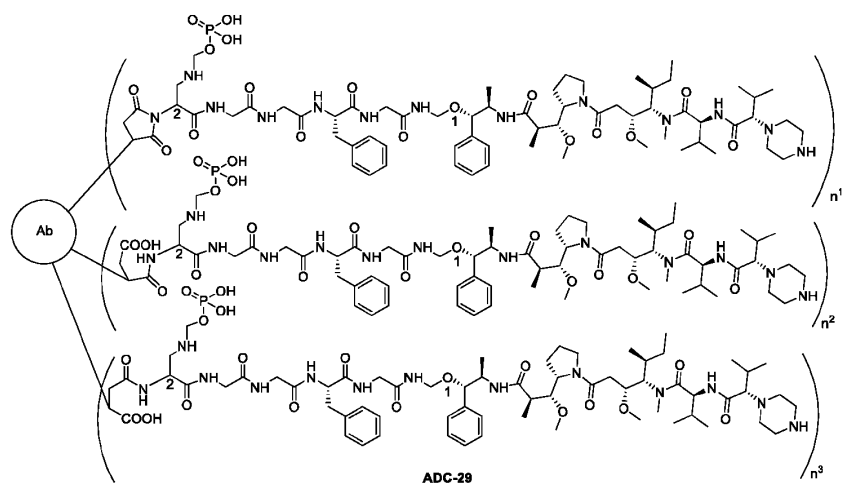


ADC-25

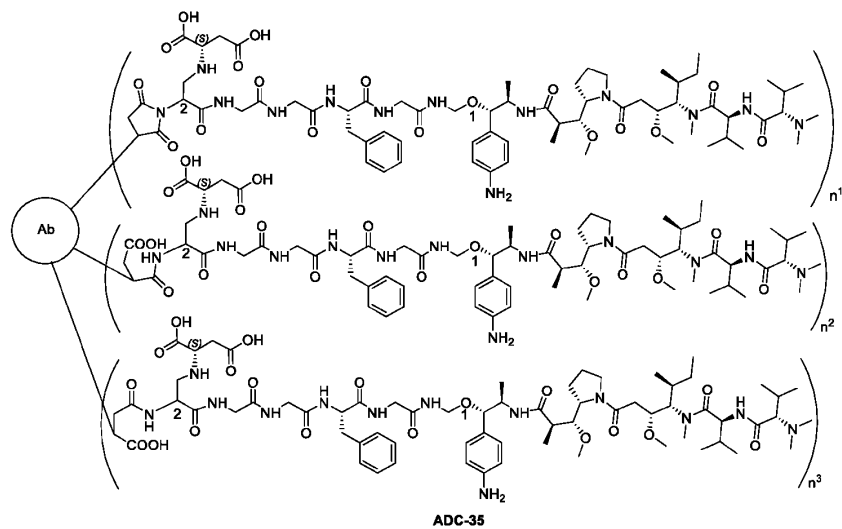
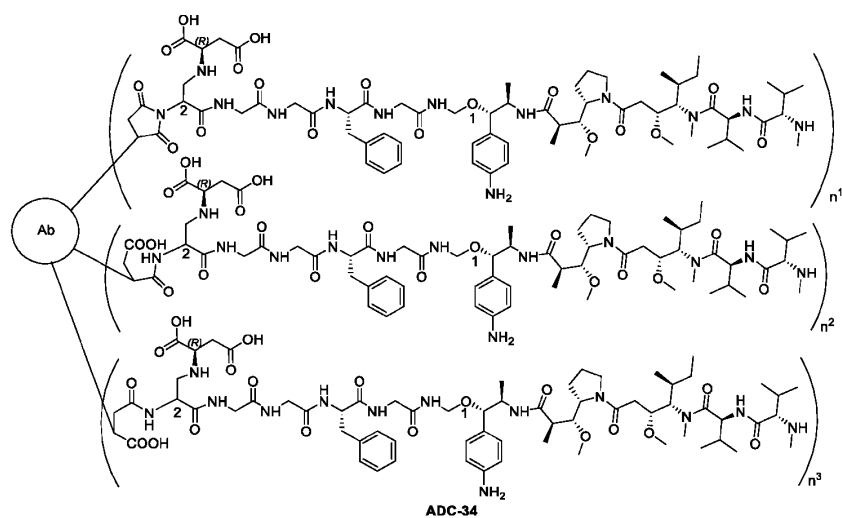


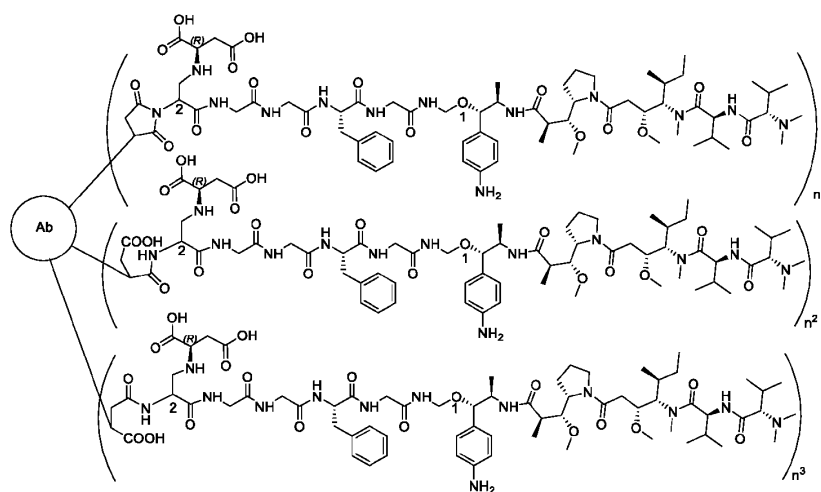
ADC-26



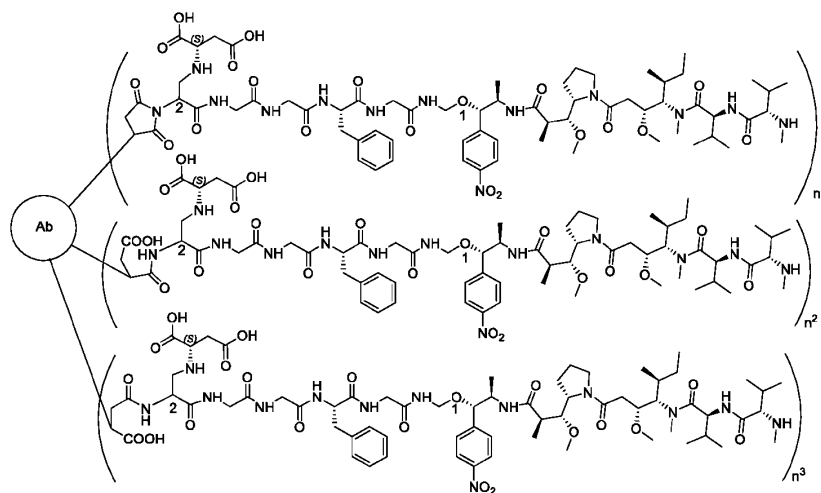




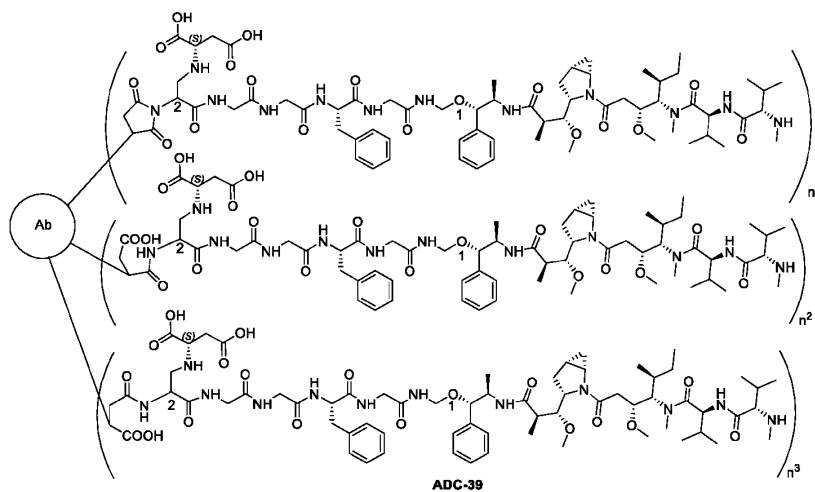
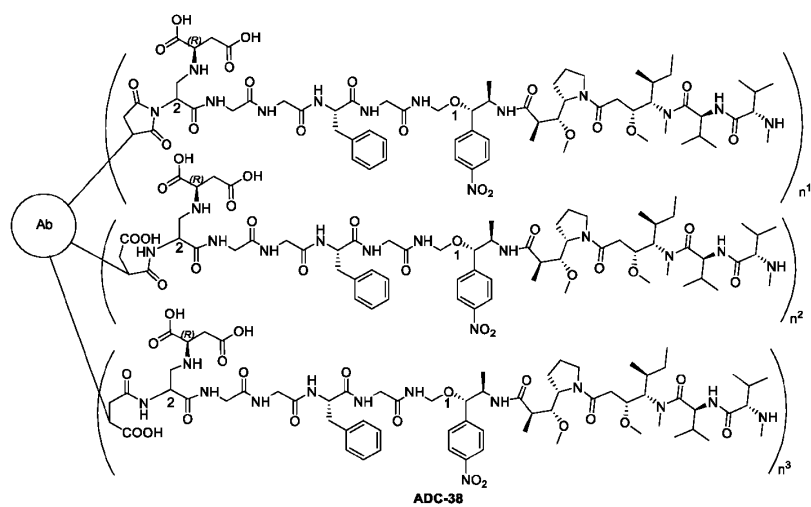


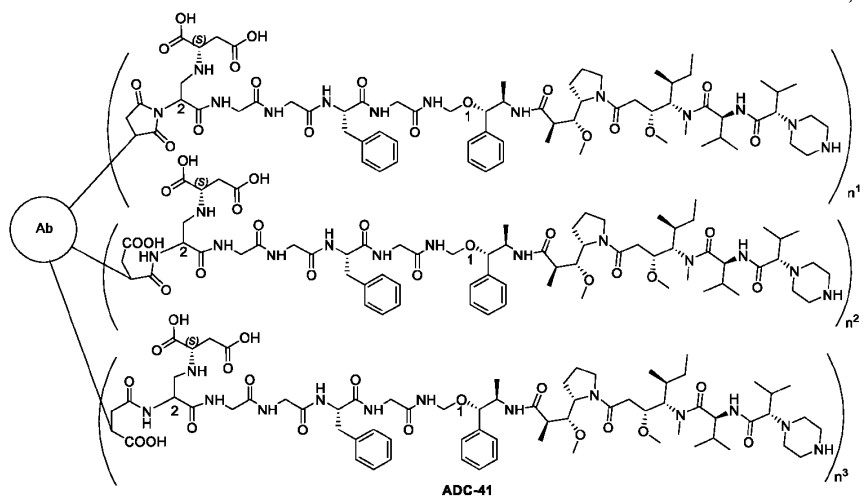
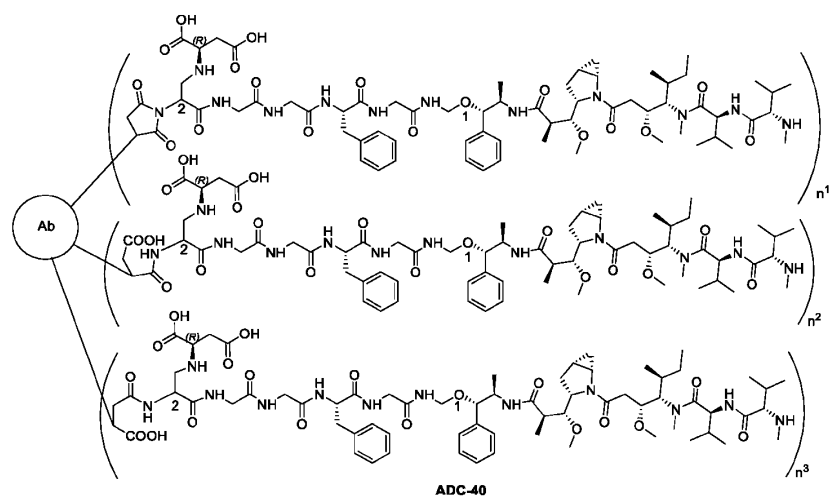


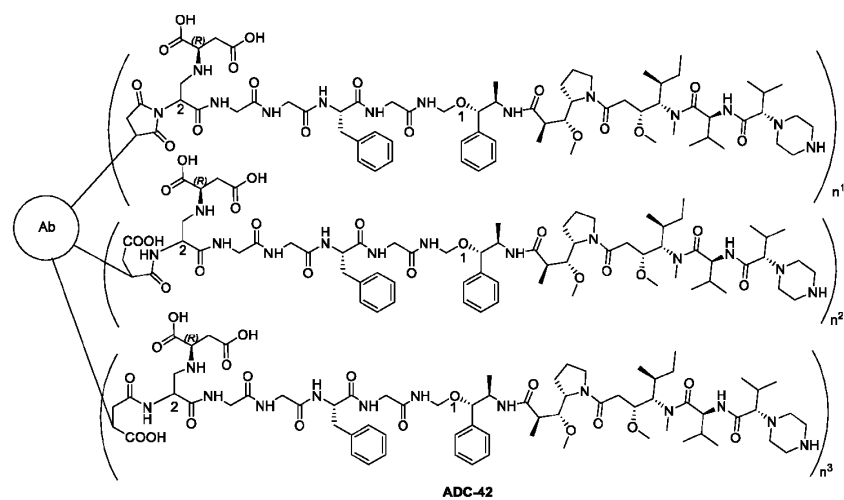
ADC-36



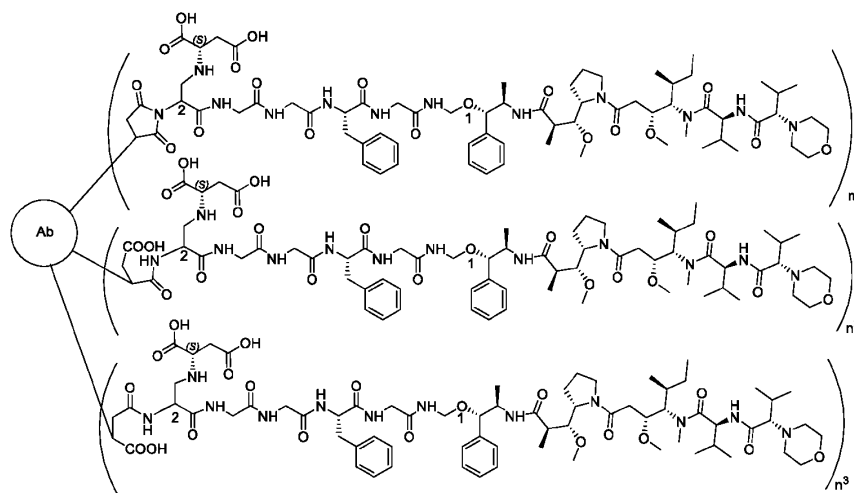
ADC-37



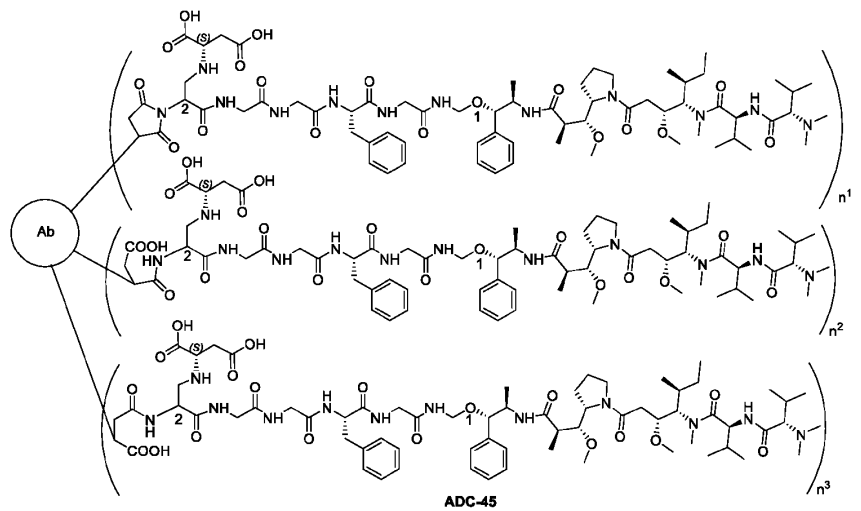
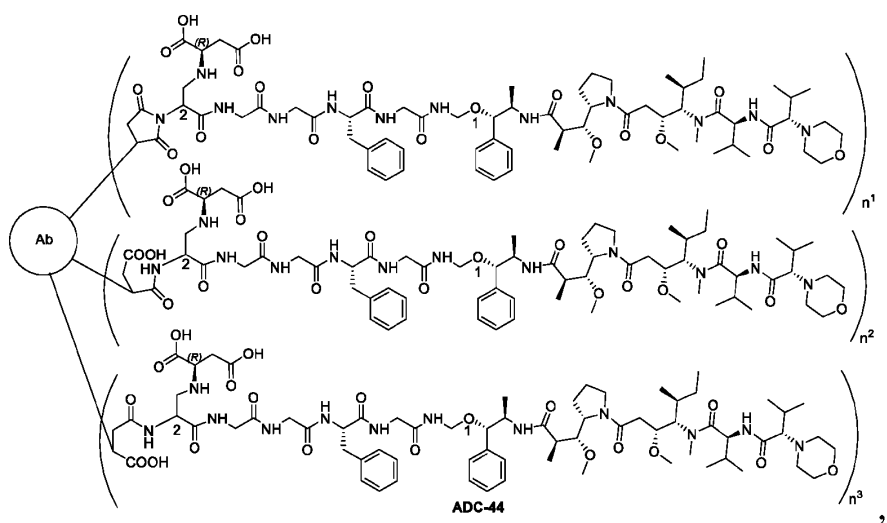


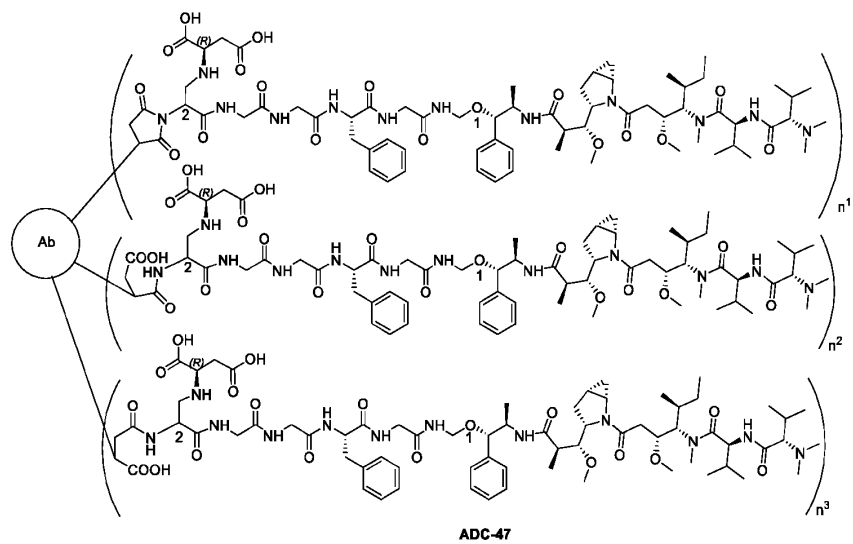
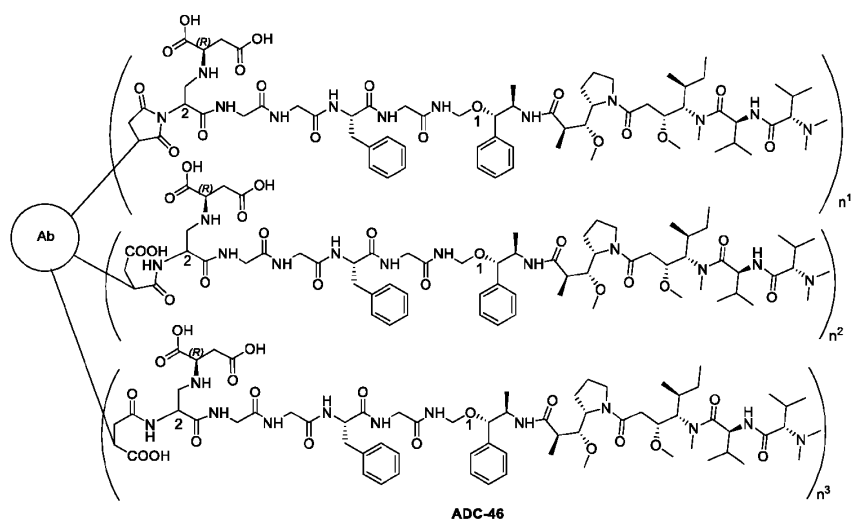


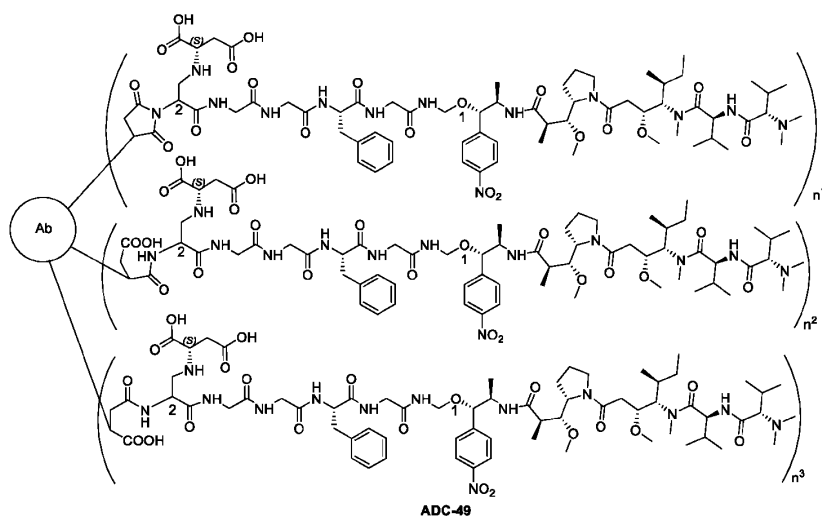
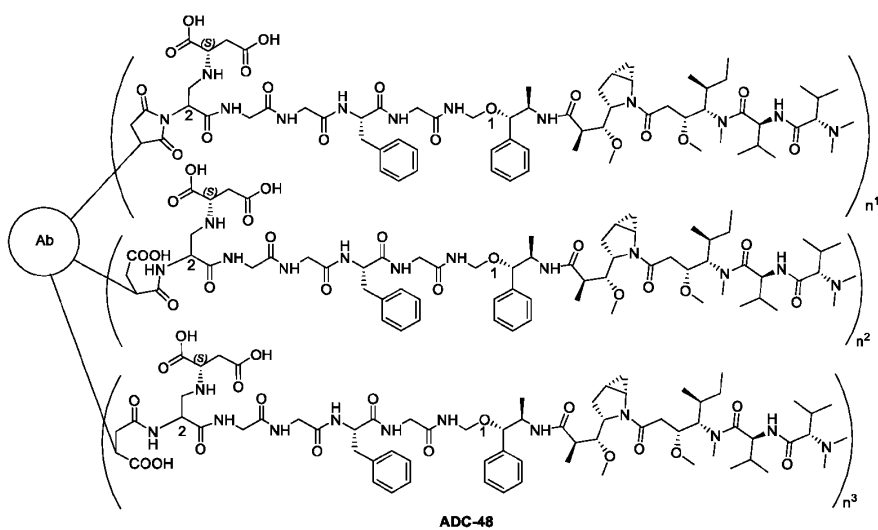
ADC-42



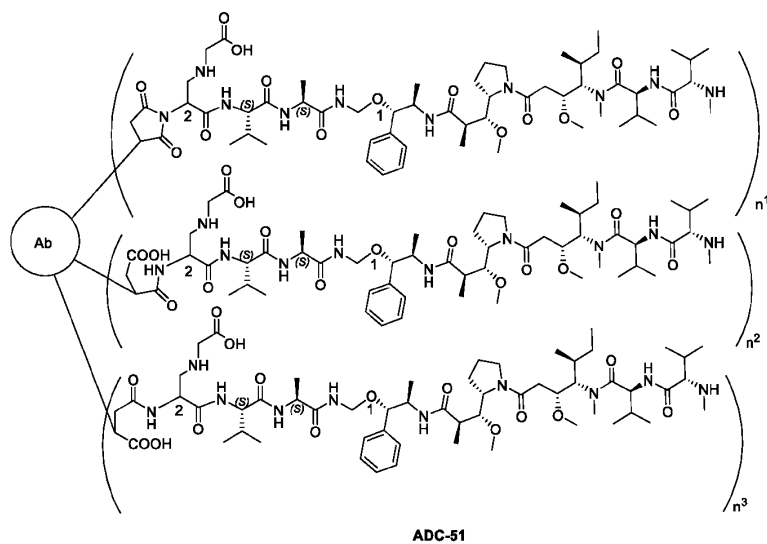
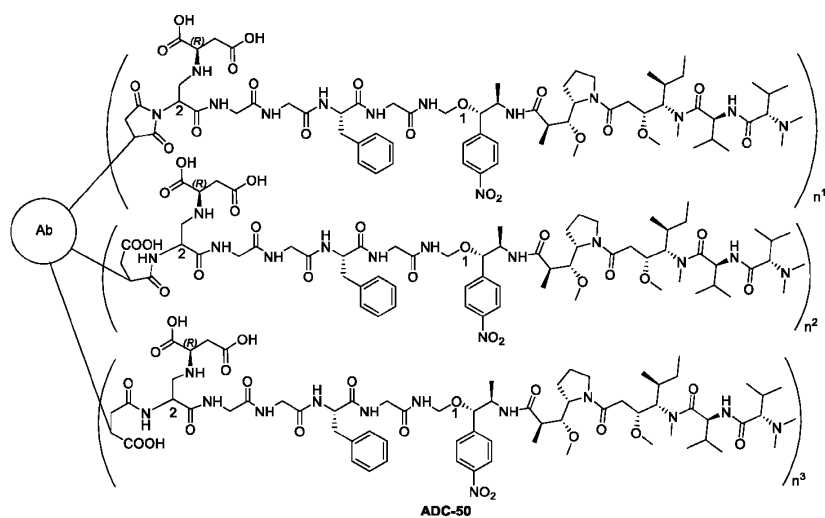
ADC-43

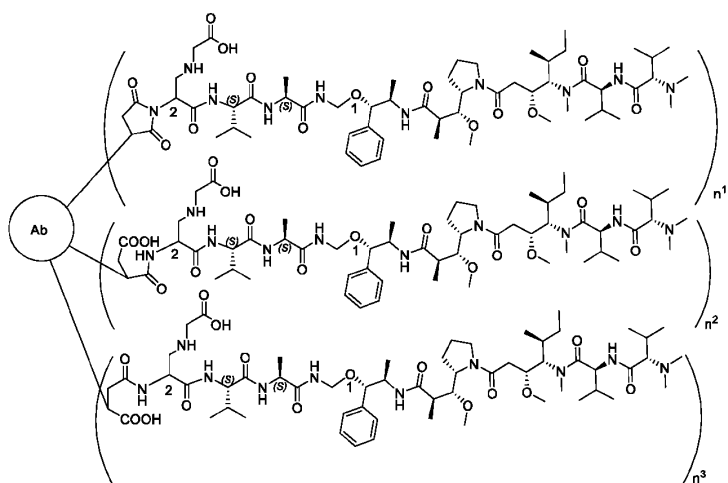




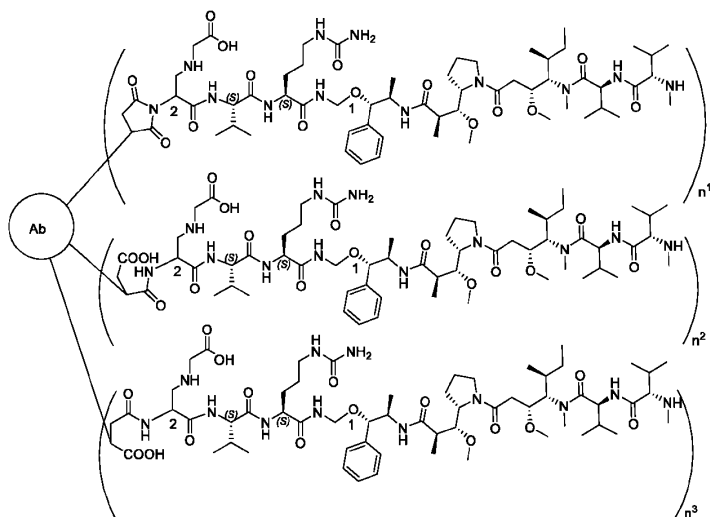


or

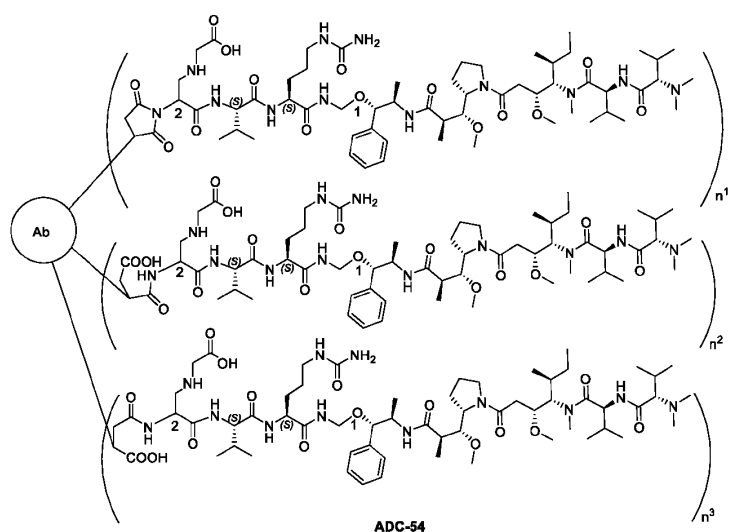




ADC-52

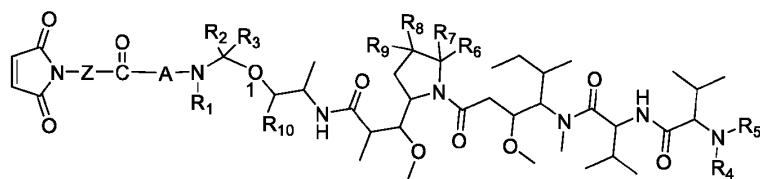


ADC-53



wherein the configuration of the chiral carbon at the position-2 is of either R-type or S-type.

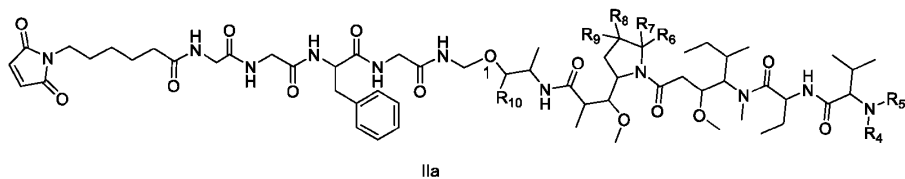
In a further aspect, the disclosure provides a linker-drug compounds. In one embodiment, the linker-drug compound has a structure as shown in Formula II, or a pharmaceutically acceptable salt or solvate thereof,



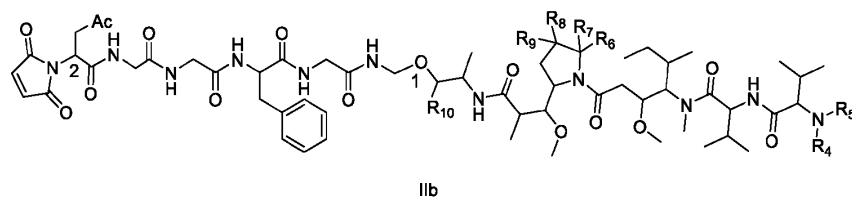
wherein,

Z, A, R₁, R₂, R₃, R₄, R₅, R₁₁, R₁₂, R₁₃, R₁₄, B, R₁₅, R₁₆, R₁₇, n, m, R₆, R₇, R₈, R₉, R₁₈, R₁₉, R₁₀ are as defined in any of the above.

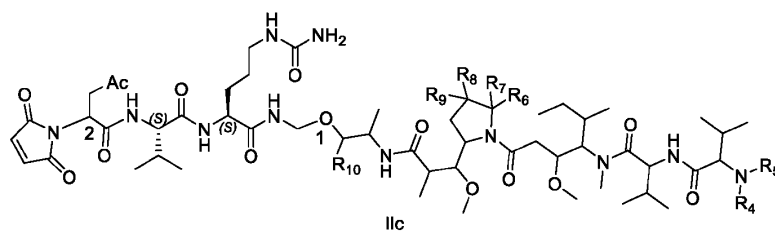
10 In one embodiment, said linker-drug compound may have the structure shown in Formula IIa.



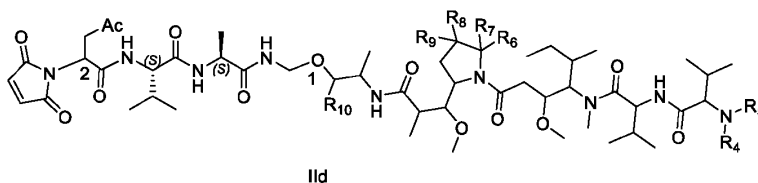
In one embodiment, said linker-drug compound has the structure shown in Formula IIb,



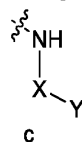
In one embodiment, said linker-drug compound has the structure shown in Formula IIc,



In one embodiment, said linker-drug compound has the structure shown in Formula IId,

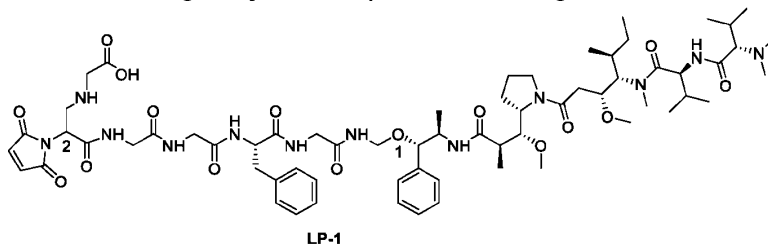


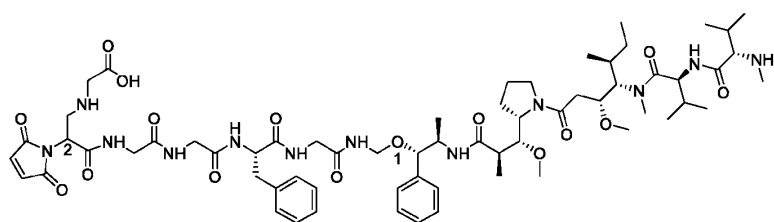
wherein Ac is a hydrophilic structural unit having the structure shown in Formula c:



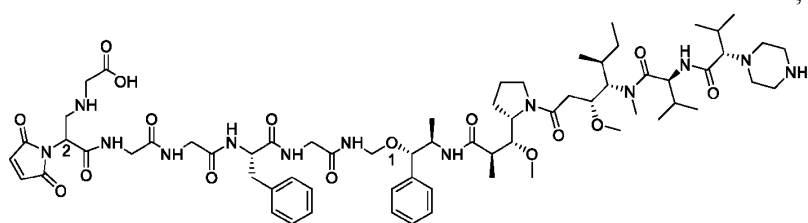
- 10 wherein X, Y are as defined above, and Ac is linked to methylene carbon at the position-2 already labeled in Formula IIb, Formula IIc or Formula IId via -NH-.

Representative linker-drug compounds may be the following structures:

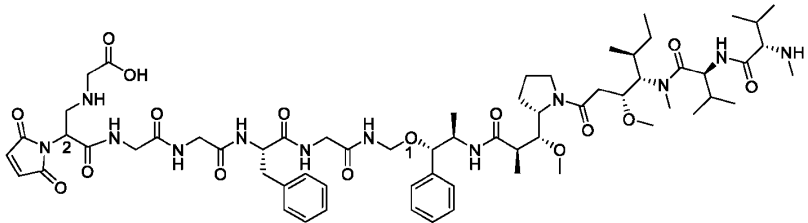




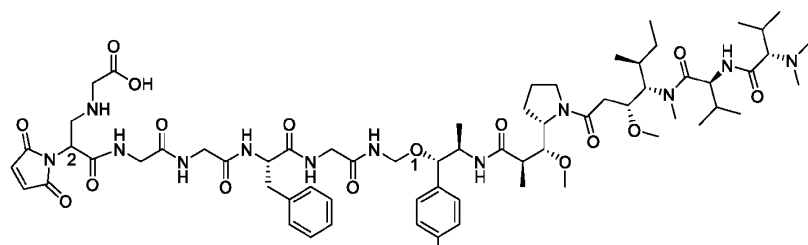
LP-2



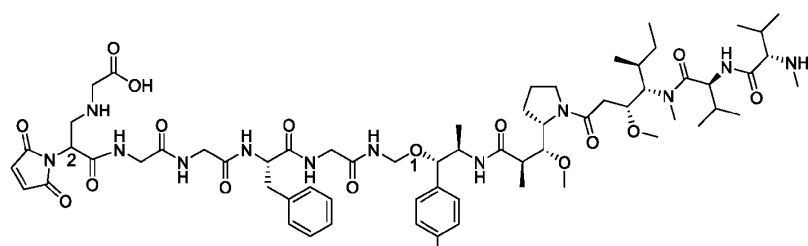
LP-3



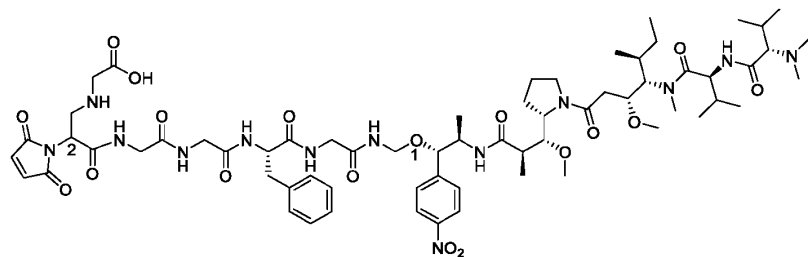
LP-3s



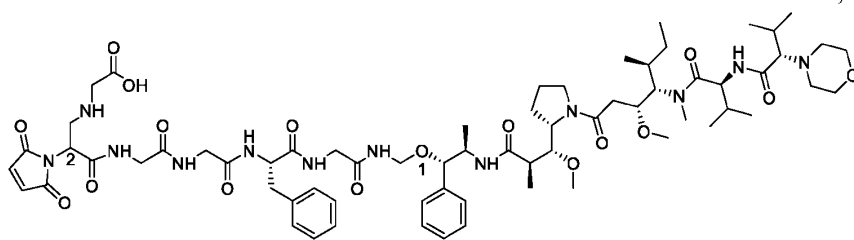
LP-4



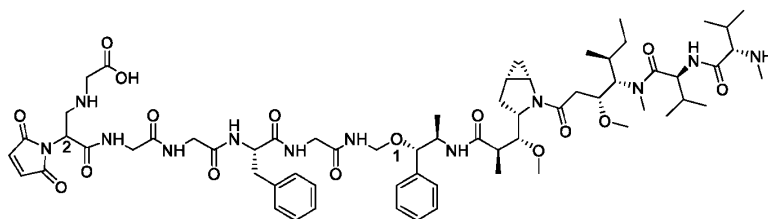
LP-5



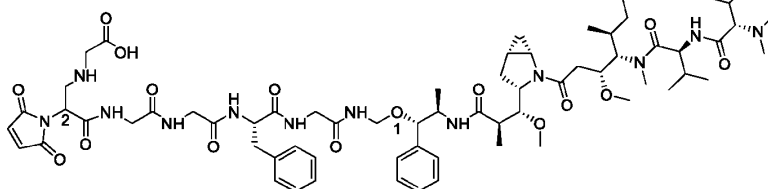
LP-6



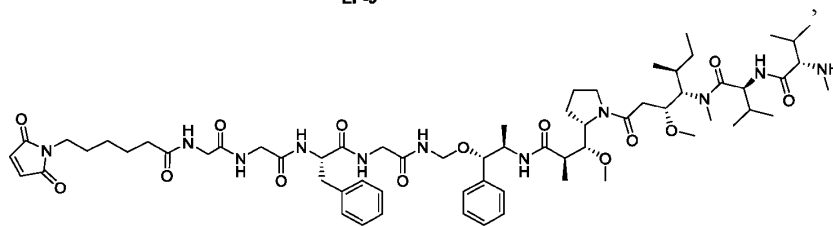
LP-7



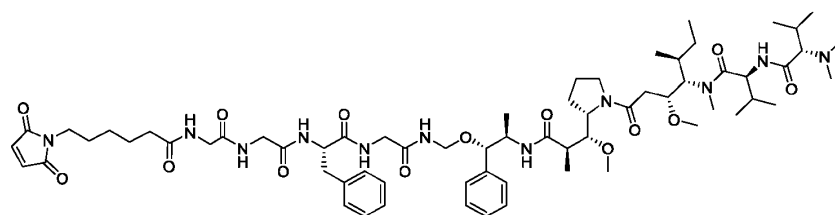
LP-8



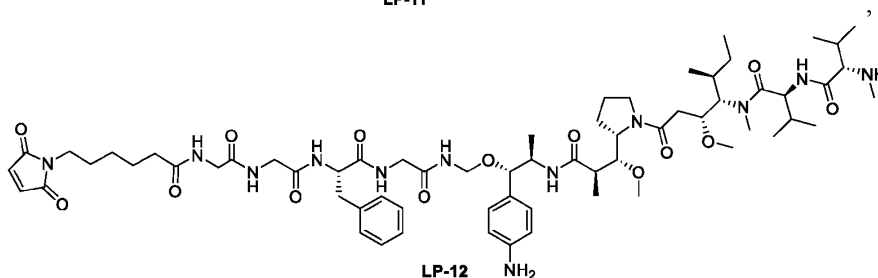
LP-9



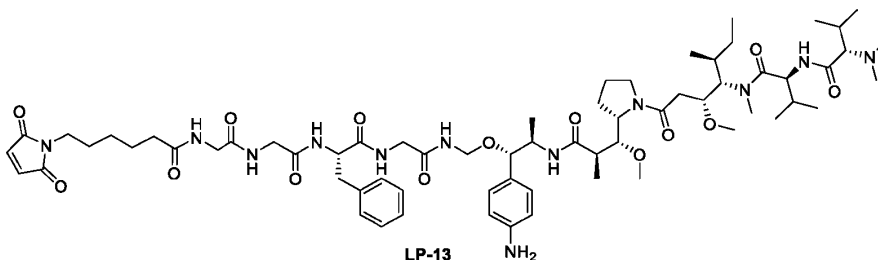
LP-10



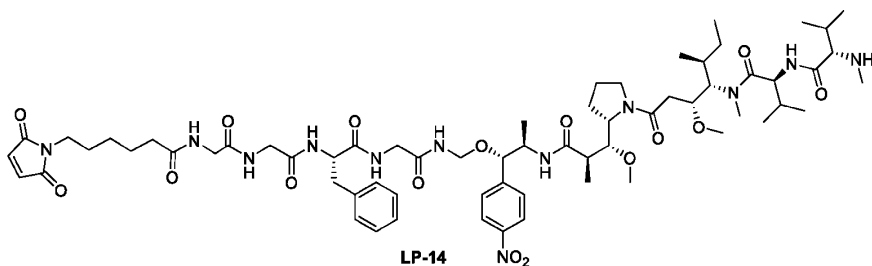
LP-11



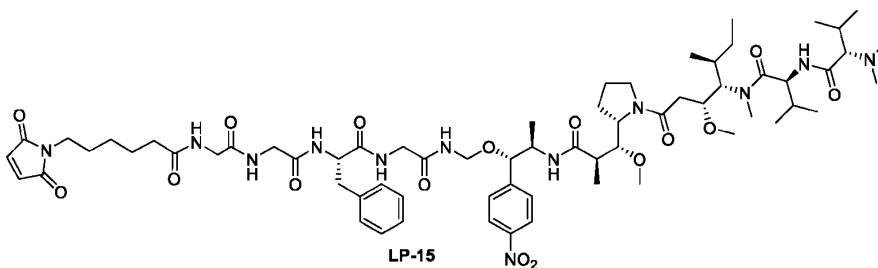
LP-12



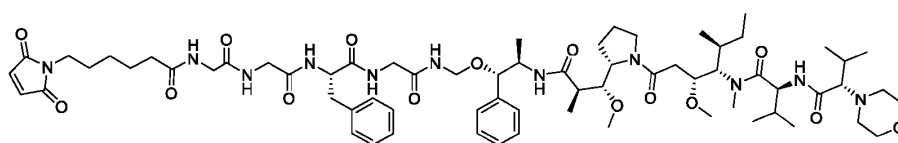
LP-13



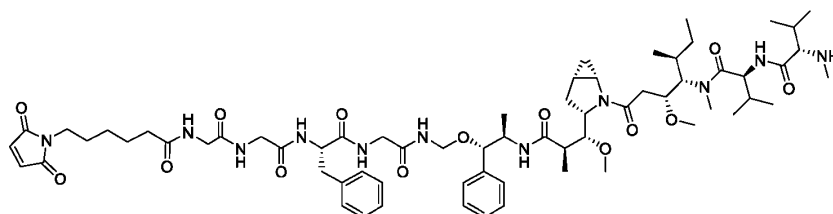
LP-14



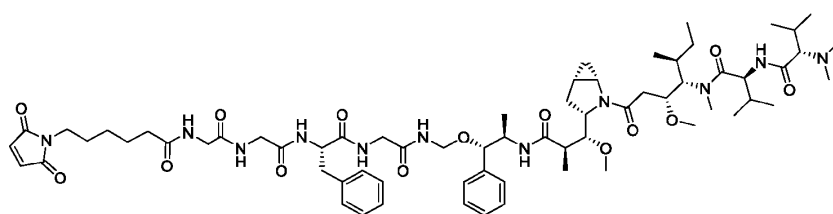
LP-15



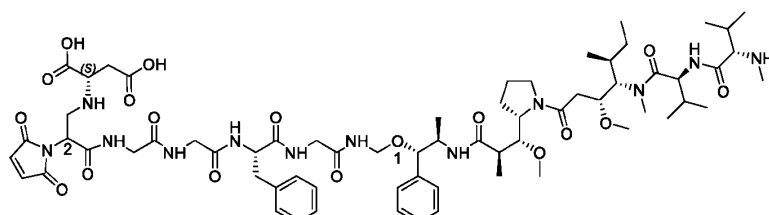
LP-16



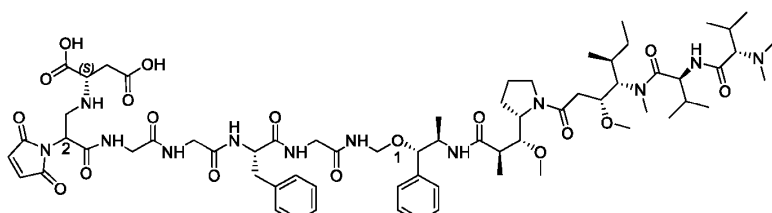
LP-17



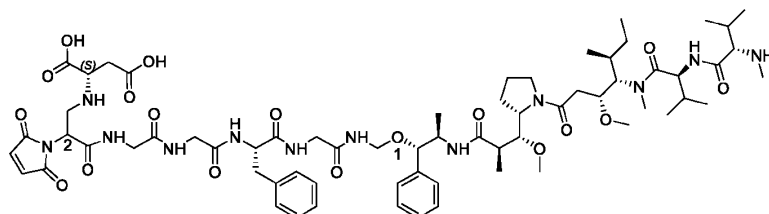
LP-18



LP-19

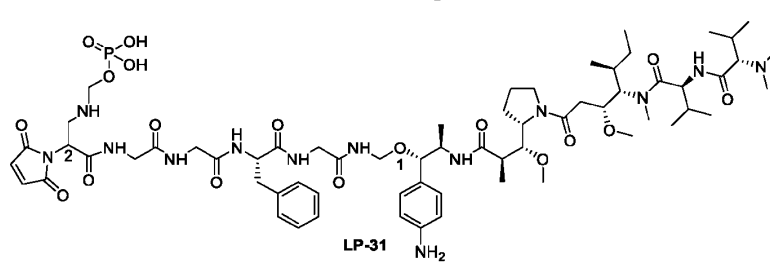
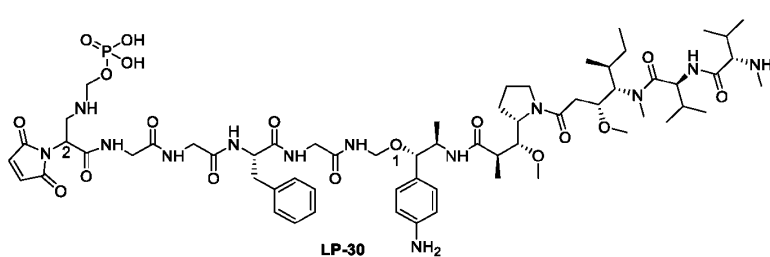
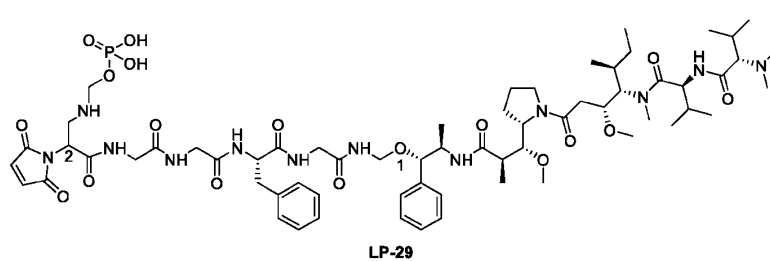
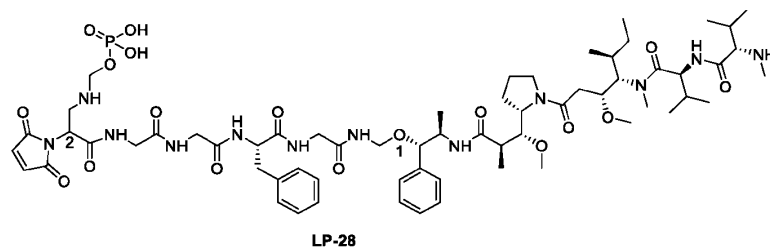
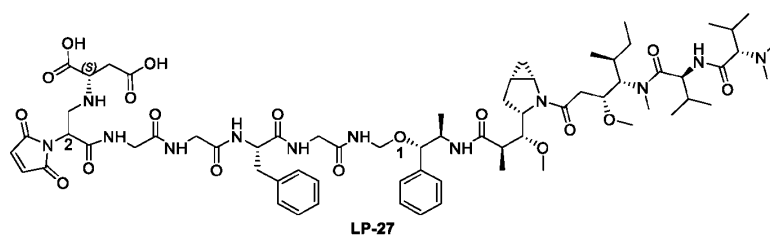


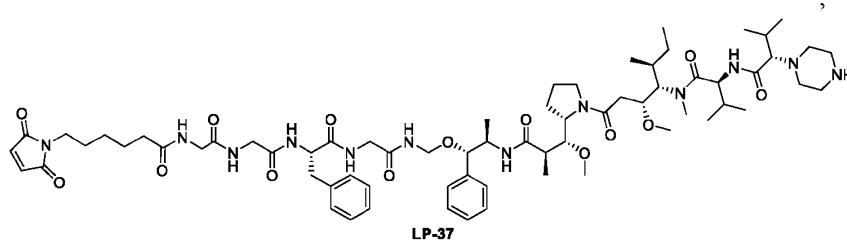
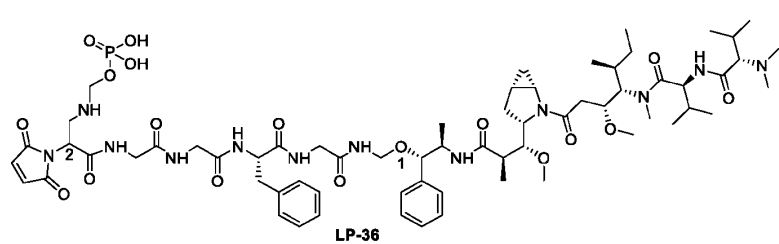
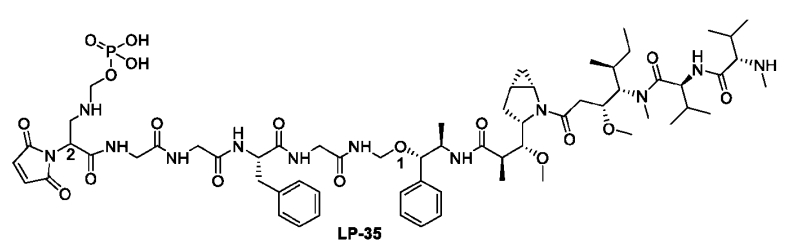
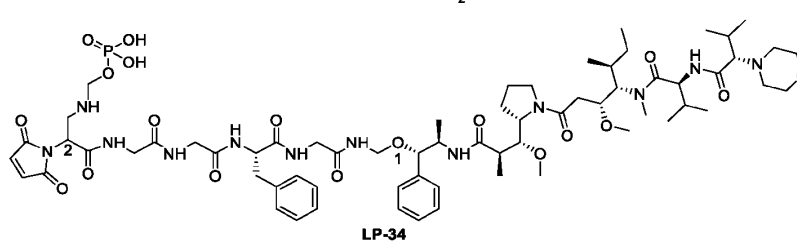
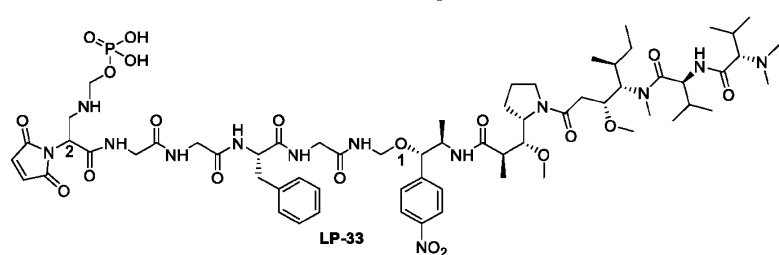
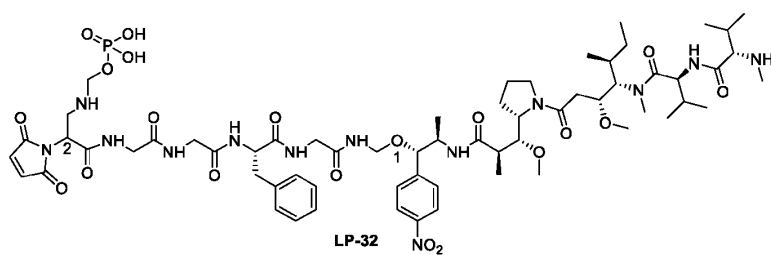
LP-20

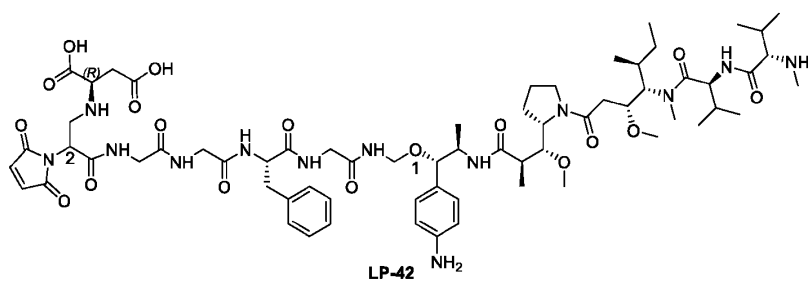
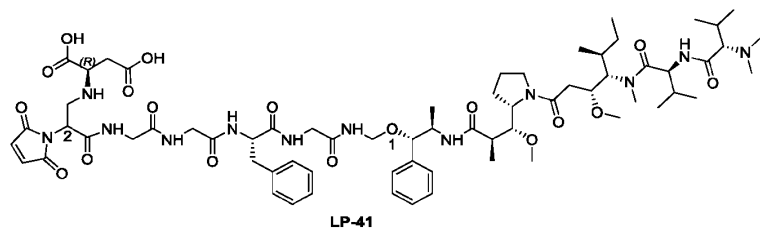
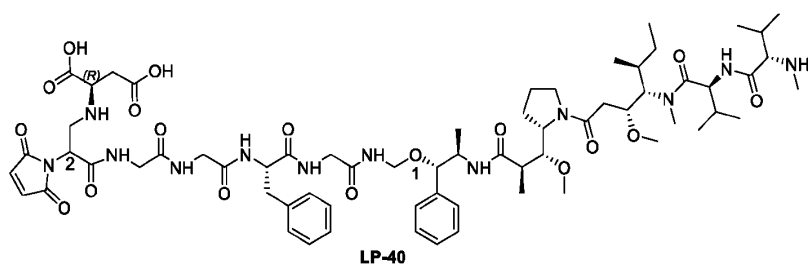
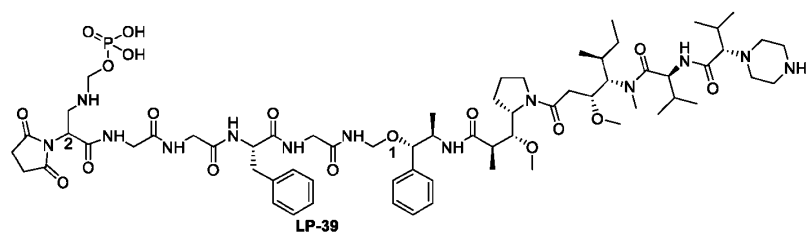
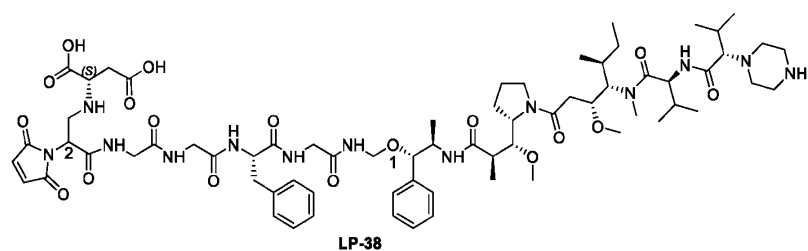


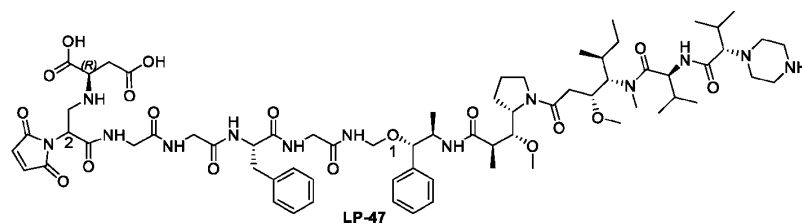
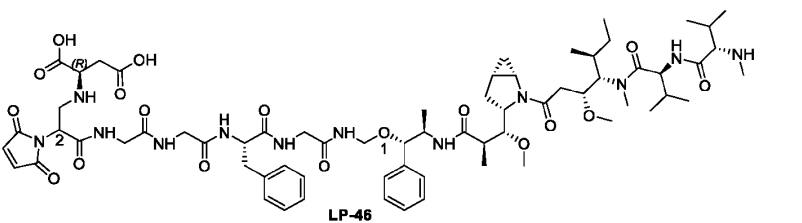
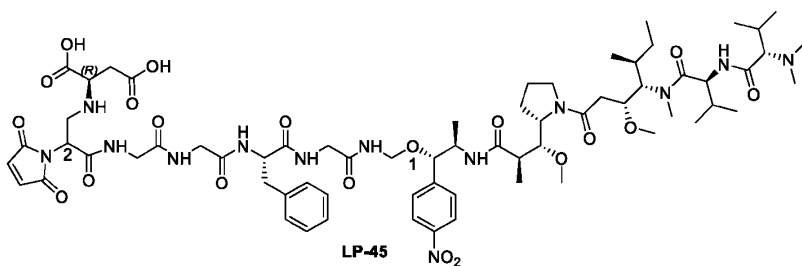
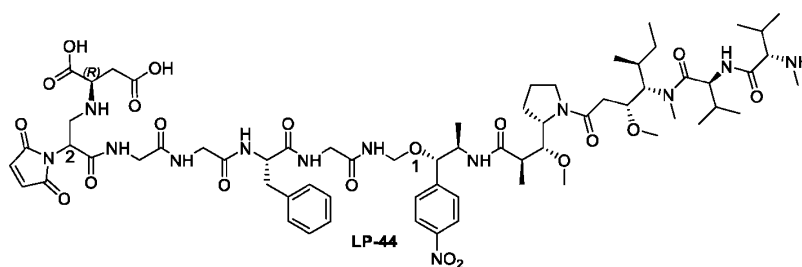
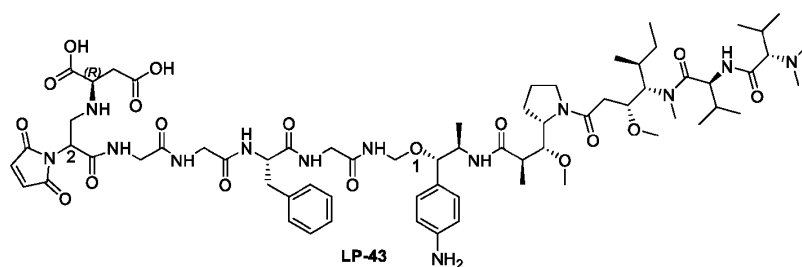
LP-21

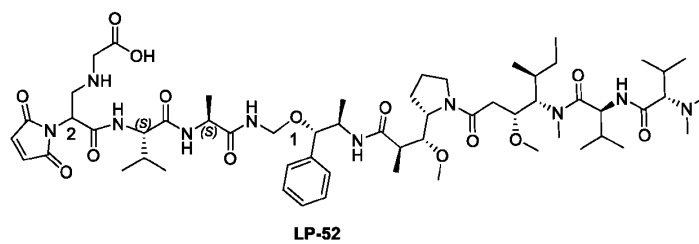
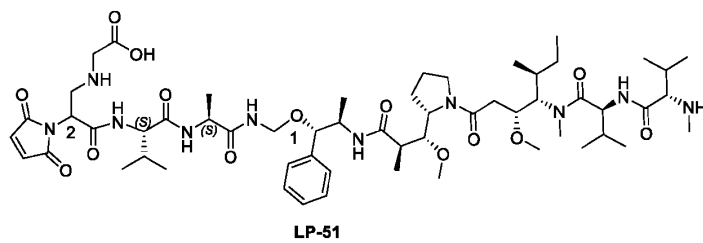
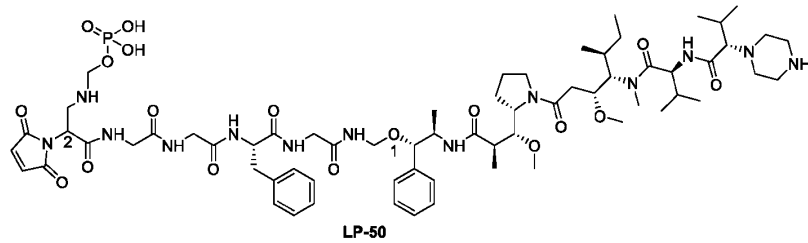
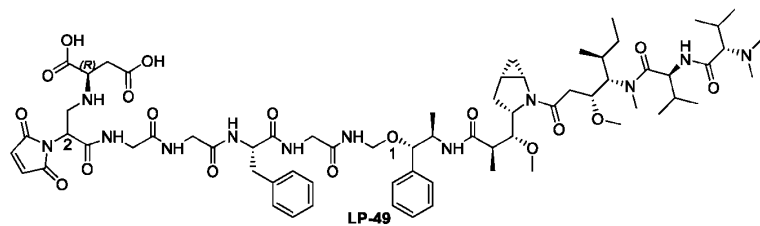
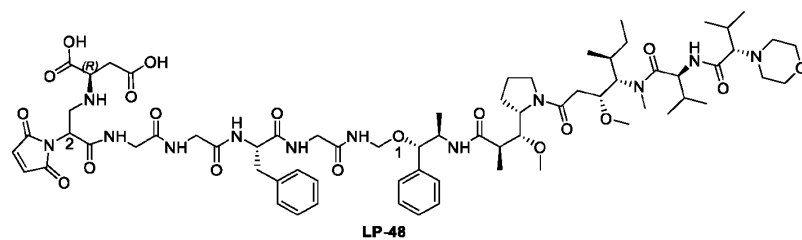


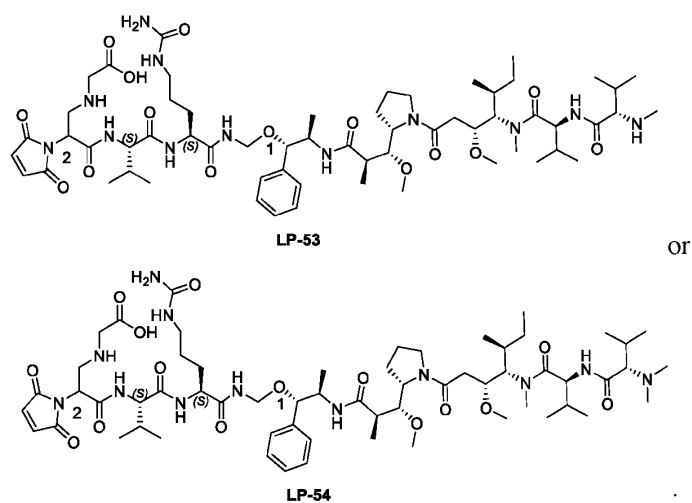












wherein the configuration of the chiral carbon at the position-2 is of either R or S.

In a further aspect, the disclosure provides a pharmaceutical composition comprising an effective amount of the antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof, a linker-drug compound, its isomer, meso-, endo-, racemic, enantiomeric, or a mixture thereof, or a pharmaceutically acceptable salt or solvate thereof, or a pharmaceutically acceptable carriers, diluents or excipients.

10 In a further aspect, the disclosure provides a pharmaceutical formulation or preparation comprising an effective amount of the antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof, a linker-drug compound, its isomer, meso, racemate, enantiomer or a mixture thereof, or a pharmaceutically acceptable salt or solvate thereof.

In a further aspect, the disclosure provides the use of the antibody-drug conjugates or pharmaceutically acceptable salts or solvates thereof, the linker-drug compounds, its isomer, mesomer, racemate, enantiomer or a mixture thereof, or a pharmaceutically acceptable salt or solvate thereof for preparing a drug for treating or preventing tumors.

In some embodiments, said tumor expresses EGFR, HER3, or both.

In one embodiment, said tumor is cancer.

20 Representative tumor may be solid or non-solid tumors, such as breast cancer, 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, rectal cancer, colorectal cancer, bone cancer, skin cancer, thyroid cancer, pancreatic cancer, melanoma, glioma, neuroblastoma, glioblastoma multiforme, sarcoma, lymphoma, and leukemia.

In a further aspect, the disclosure provides a method of treating or preventing a tumor comprising administering to a subject in need thereof an effective amount of the antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof, a linker-drug compound, or its isomer, mesomer, racemate, enantiomer or a mixture thereof, or a pharmaceutically acceptable

salts or solvates. In one embodiment, said subject is a mammal, such as a human.

In a further aspect, the disclosure provides the use of the linker-drug compound, its isomer, mesomorph, racemate, enantiomer or a mixture thereof, or a pharmaceutically acceptable salt or solvate thereof for preparing the antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof. In one embodiment, said antibody-drug conjugates are the antibody-drug conjugates as disclosed herein.

10 In a further aspect, the disclosure provides a method for preparing the antibody-drug conjugate compound or a pharmaceutically acceptable salt or solvent thereof, said method comprising: synthesizing a antibody-drug conjugate, or a pharmaceutically acceptable salt or solvent compound thereof, by coupling the reduced antibody or antigen-binding fragment thereof with a linker-drug compound, its structural or chiral isomer including, without limitation, endo-, enantio-isomer, or a mixture thereof, or a pharmaceutically acceptable salts or solvates, to obtain said antibody-drug conjugates. In some embodiments, antibodies or antigen-binding fragments thereof can be reduced by thiol-type reducing agents such as tris(2-carboxyethyl)phosphine (TCEP).

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 shows the LC-MS spectrum of the compound LP-1a;

Figure 2 shows the LC-MS spectrum of VcMMAE;

10 **Figure 3** shows the RP-HPLC results of ADC-1-1a (**3A**); ADC-2-1a (**3B**); ADC-C-1a (**3C**); ADC-1-1b (**3D**); Parental anti-EGFR mAb-LP-1a (**3E**); and Parental anti-HER3 mAb-LP-1a (**3F**);

Figure 4 shows the SEC-HPLC results of ADC-1-1a (**4A**); ADC-2-1a (**4B**); and ADC-C-1a (**4C**); ADC-1-1b (**4D**); Parental anti-EGFR mAb-LP-1a (**4E**); and Parental anti-HER3 mAb-LP-1a (**4F**);

Figure 5 shows the *in vitro* efficacy of BsADCs and the parental ADCs in human skin cancer (epidermoid carcinoma) cells, A431 (**5A**); human lung cancer (non-small cell lung carcinoma) cells, HCC827 (**5B**); human hypopharyngeal tumor (squamous cell carcinoma) cells, Fadu (**5C**); human lung cancer (non-small cell lung carcinoma) cells, NCI-H1975 (**5D**); human B-cell lymphom, Raji-Luc (**5E**); and mixed cancer cells of A431+Raji-Luc (**5F**);

20 **Figure 6** shows the *in vivo* efficacy of BsADCs and the parental ADCs in two mixed tumors (A431+SW620);

Figure 7 shows the *in vitro* efficacy of ADC-1-1a, ADC-2-1a, ADC-C-1a and the corresponding toxins in human skin cancer (epidermoid carcinoma) cells, A431 (**7A**); human lung cancer (non-small cell lung carcinoma) cells, HCC827 (**7B**); human hypopharyngeal tumor (squamous cell carcinoma) cells, Fadu (**7C**); human lung cancer (non-small cell lung carcinoma) cells, NCI-H1975 (**7D**); and human colorectal cancer cells, SW620 (**7E**);

Figure 8 shows the *in vivo* efficacy of ADC-2-1a and ADC-C-1a in mixed tumors of A431+SW620;

Figure 9 shows the *in vivo* efficacy of ADC-1-1a and ADC-2-1a in mixed tumors of A431+SW620 (**9A**); A431 tumor alone (**9B**), and SW620 tumor alone (**9C**);

30 **Figure 10** shows the *in vivo* efficacy of ADC-1-1a in mixed tumors of A431+SW620 (**10A**, **10B**); A431 tumor alone (**10C** and **10D**); SW620 tumor alone (**10E** and **10F**); and NCI-H1975 tumor alone (**10G** and **10H**).

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

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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, antibody-drug conjugates, antibody-drug conjugates, antibody-drug conjugates and/or immuno-conjugates derived from such antibodies, linkers, toxins, pharmaceutical compositions containing the antibodies, monoclonal and/or recombinant bispecific antibodies, antibody-drug conjugates and/or immuno-conjugates, the methods for making the antibodies, linkers, toxins, antibody-drug conjugates, and compositions, and the methods for treating cancer using the antibody-drug conjugates and/or immuno-conjugates and compositions.

10 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.

Unless stated to the contrary, terms used in the claims and specification herein have the meanings set forth below.

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 aspects, the ligand serves to deliver the drug unit to a specific target cell population with which the ligand unit interacts.

20 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 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 aspects, 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
30 into a cysteine residue of the ligand unit by, for example, targeted mutagenesis or chemical reaction.

The term "drug" refers to cytotoxic drugs, i.e., molecules that have a strong ability to disrupt the normal growth of tumor or cancer cells within the cell. Cytotoxic drugs can, in principle, kill tumor cells at sufficiently high concentrations, but because of their lack of specificity, they kill tumor or cancer cells while also causing apoptosis of normal cells, which can lead to serious side effects.

The term "antibody-drug conjugate" refers to the attachment of a monoclonal antibody or a functional antibody fragment or a targeted protein, etc., to a cytotoxic drug by means of a stabilized
40 linkage unit.

As used herein, "antibody" or "antibody unit" includes, to the extent thereof, any part of an antibody structure. This unit may bind, reactively associate, or complex with a receptor, antigen, or other receptor unit possessed by the target cell population. The antibody may be any protein or protein-like molecule that binds, complexes, or reacts with a portion of the cell population to be treated or biologically modified. The antibodies comprising the antibody-drug coupling compounds of the disclosure maintain their original antigen-binding capacity in the wild. Thus, the antibodies of the disclosure are capable of binding exclusively to antigens. 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 associated with tissue growth and differentiation (such as are known or foreseen to have functionality), lymphokines, cytokines, molecules involved in the regulation of cellular cycling, molecules involved in angiogenic molecules (if known or predicted to be functional). Tumor-associated factors may be cluster differentiation factors (e.g., CD proteins).

Antibodies applied in antibody-drug conjugates include, but are not limited to, antibodies directed against cell surface receptors and tumor-associated antigens. Such tumor-associated antigens are well known in the industry and can be prepared by methods and information for antibody preparation well known in the industry. To develop effective cellular level targets that can be used in cancer diagnosis and therapy, researchers seek to find transmembrane or other tumor-associated peptides. These targets can be specifically expressed on the surface of one or more cancer cells with little or no expression on the surface of one or more non-cancer cells. Typically, such tumor-associated polypeptides are more overexpressed on the surface of cancer cells relative to the surface of non-cancer cells. Identification of such tumor-associated factors can greatly enhance the specific targeting properties of antibody-based cancer therapies. For convenience, information related to antigens known to the industry is labeled below, including name, other names, and genebank accession number. Nucleic acid and protein sequences corresponding to the tumor-associated antigens can be found in publicly available databases, such as Genbank, and the antibody-targeted tumor-associated antigens include all amino acid sequence variants and isoforms having at least 70%, 80%, 85%, 90%, or 95% homology to the sequences identified in the references, or possessing biological properties and characteristics that are identical to the tumor-associated antigen sequences of the cited literature. biological properties and characteristics of the tumor-associated antigen.

Antibodies of the disclosure include, but are not limited to, murine antibodies, chimeric antibodies, humanized antibodies and fully human antibodies, for example humanized antibodies and fully human antibodies.

The term "inhibition" or "inhibition of" means that a detectable amount is reduced or completely prevented.

The term "cancer" refers to a physiological condition or disease characterized by dysregulated cell growth. "Tumor" includes cancer cells.

The term "linker" or "linker fragment" or "linker unit" refers to a fragment or bond of a chemical structure that is attached to a ligand at one end and to the drug at the other end or may be

attached to other connectors and then to the drug. or to other connectors and then to the drug.

Linkers, including extensions, spacers and amino acid units, can be synthesized by methods known in the art, such as those described in US2005-0238649A1. The linkers can be "cleavable linkers" that facilitate the release of the drug in the cell. For example, acid-unstable linkers (e.g., hydrazone), protease-sensitive (e.g., peptidase-sensitive) linkers, photo-unstable linkers, dimethyl linkers, or disulfide-containing linkers can be used (Chari et al. Cancer Research 52:127-131, 1992; U.S. Patent No. 5, 208, 020).

According to the mechanism of intracellular drug release, as used herein, "linkers" or "linkers of antibody-drug conjugates" can be categorized into two types: non-cleavable linkers and cleavable linkers. For antibody-drug conjugates containing a non-cleavable linker, the mechanism of drug release is as follows: after the conjugate binds to the antigen and is endocytosed by the cell, the antibody is enzymatically cleaved in the lysosome, releasing an active molecule consisting of the small molecule drug, the linker, and the amino acid residues of the antibody. 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. Therefore, 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). For antibody-drug conjugates containing cleavable linkers, the mechanism of drug release is that the conjugate binds to the antigen and is endocytosed by the cell, then breaks and releases the active ingredient (the small-molecule drug itself) in the target cell. Cleavable linkers are mainly categorized into chemical-sensitive linkers and enzyme-sensitive linkers. Chemically sensitive linkers can be selectively broken due to differences in the nature of the plasma and cytoplasm or tumor microenvironment. Such properties include pH, glutathione concentration, etc. pH-sensitive linkers, which are relatively stable in the neutral or weakly alkaline environment of blood (pH 7.3-7.5), will be hydrolyzed within the weakly acidic tumor microenvironment (pH 5.0-6.5) and lysosomes (pH 4.5-5.0), e.g., hydrazones, carbonates, acetals, and ketals. Antibody-drug conjugates based on such linkers typically have a short half-life (2-3 days) due to the limited plasma stability of acid-cleavable linkers. This short half-life has somewhat limited the use of pH-sensitive linkers in the new generation of antibody-drug conjugates. For glutathione-sensitive linkers, also known as disulfide-bonded linkers. Drug release is based on the difference between the high intracellular glutathione concentration (millimolar range) and the relatively low glutathione concentration in the blood (micromolar range). This is particularly true for tumor cells, whose low oxygen content leads to enhanced reductase activity and thus to higher glutathione concentrations. Disulfide bonds are thermodynamically stable and therefore have better stability in plasma. Enzyme-cleavable linkers, such as peptide linkers, provide better control of drug release. Lysosomal-cleavable peptide linkers can be efficiently cleaved by lysosomal proteases such as Cathepsin (Cathepsin B). This peptide linker is thought to be very stable in the plasma circulation due to the unfavorable extracellular pH and serum protease inhibitors resulting in proteases that are normally inactive outside the cell. Given the high plasma stability and good intracellular break selectivity and potency, enzyme-cleavable linkers are widely used as cleavable

linkers for antibody-drug conjugates.

The term "antibody drug conjugate" refers to the attachment of a monoclonal antibody or antibody fragment to a biologically active toxic drug through a stabilized linkage unit.

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.

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

10 The term "alkyl" refers to a saturated aliphatic hydrocarbon group, which is a straight or branched chain group comprising from 1 to 20 carbon atoms, for example alkyl comprising from 1 to 12 carbon atoms, for example, alkyl comprising from 1 to 10 carbon atoms, and most for example alkyl comprising from 1 to 6 or 1 to 4 carbon atoms. 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-dimethylpentyl, n-nonyl, 2, 2-dimethylpentyl, 2, 2-methylpentyl, 2, 2-methylpentyl 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. Examples are lower alkyl groups containing 1 to 6 (e.g., 1 to 4) carbon atoms, and non-limiting embodiments include methyl, ethyl, n-propyl, isopropyl, n-butyl, iso-butyl, 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 for example being one or more of the following groups independently alkyl, alkenyl, alkynyl, alkoxy, alkylthio, alkylamino, halogen, mercapto, hydroxy, nitro, cyano, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, cycloalkoxy, heterocycloalkoxy, cycloalkane thio, heterocycloalkylthio, oxo.

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The term "substituted alkyl" means that the hydrogen in the alkyl group is replaced by a substituent group which, unless otherwise indicated in the text, may be one or more groups independently the group consisting of: - halogen, -OR', -NR'R' ', -SR', -SiR'R''R''', -OC(O)R ' , -

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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 1 to (2m'+1), wherein m' is the total number of carbon atoms in the group, e.g., 1, 2, 3, 4, 5, or 6. r', R'' and R''' each designate hydrogen, C₁₋₈ alkyl, aryl, aryl substituted by 1-3 halogens, C₁₋₈ alkyl substituted by 1-3 halogens, C₁₋₈ alkoxy or C₁₋₈ thioalkoxy, or unsubstituted aryl-C₁₋₄alkyl. When R' and R'' are attached to the same nitrogen atom, they may form a 3-, 4-, 5-, 6- or 7- meta-ring with that nitrogen atom. For example, -NR'R'' includes 1-pyrrolidinyl and 4-morpholinyl.

10 The term "heteroalkyl" refers to a group formed by substitution of one or more carbons on alkyl by N, O or S.

The term "cycloalkyl" refers to a saturated or partially unsaturated monocyclic or polycyclic cyclic hydrocarbon group, the ring of the cycloalkyl group containing from 3 to 20 carbon atoms, for example from 3 to 12 carbon atoms, for example, from 3 to 10 carbon atoms, and most for example from 3 to 8 carbon atoms. Non-limiting examples of monocyclic cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cyclohexadienyl, cycloheptenyl, cycloheptatrienyl, cyclooctyl, and the like; multicyclic cycloalkyl groups include cycloalkyl groups of spirocyclic, thick, and bridged rings.

20 The term "alkoxy" refers to -O-(alkyl) and -O-(cycloalkyl), wherein alkyl or cycloalkyl is defined above. Non-limiting examples of alkoxy include: methoxy, ethoxy, propoxy, butoxy, cyclopropoxy, cyclobutoxy, cyclopentyloxy, cyclohexyloxy. The alkoxy group may be optionally substituted or non-substituted, and when substituted, the substituent is for example one or more of the following groups independently alkyl, alkenyl, alkynyl, alkoxy, alkylthio, alkylamino, halogen, mercapto, hydroxy, nitro, cyano, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, cycloalkoxy, heterocycloalkoxy, cycloalkylthio, and heterocycloalkylthio.

30 The term "heterocyclic" refers to saturated or partially unsaturated monocyclic or polycyclic cyclic hydrocarbons comprising from 3 to 20 ring atoms, wherein one or more of the ring atoms (e.g., 1, 2, 3, or 4) is a heteroatom independently nitrogen, oxygen, or S(O)_m (wherein m is 0, 1, or 2), and the remaining ring atoms are carbon. For example, it contains from 3 to 12 ring atoms, 1 to 4 of which are heteroatoms; for example, it contains from 3 to 10 or 3 to 8 ring atoms. Non-limiting examples of monocyclic heterocyclic groups include pyrrolidinyl, piperidinyl, piperazinyl, morpholinyl, thiomorpholinyl, homopiperazinyl, and the like. Polycyclic heterocyclic groups include spiro, thick and bridged heterocyclic groups.

40 The term "aryl" refers to a 6- to 14-membered all-carbon monocyclic or densely packed polycyclic (i.e., a ring sharing adjacent pairs of carbon atoms) group having a conjugated π -electronic system, for example from 6 to 10 members, e.g., phenyl. The aryl group may be substituted or non-substituted, and when substituted, the substituent may be one or more of the following groups, non-limitingly independently alkyl, alkenyl, alkynyl, alkoxy, alkylthio, alkylamino, halogen, deuterium atom, mercapto, hydroxy, nitro, cyano, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, cycloalkoxy, heterocycloalkoxy, cycloalkanethio, or heterocycloalkanethio.

The term "heteroaryl" includes 5-8-membered monocyclic heteroaryl and 8-12-membered thick heteroaryl.

The term "5-8-membered monocyclic heteroaryl" refers to an aromatic monocyclic cyclic group containing 5-8 ring atoms, at least one of which is a heteroatom, such as a nitrogen atom, an oxygen atom or a sulfur atom. Optionally, the ring atoms (e.g., carbon atoms, nitrogen atoms, or sulfur atoms) in the cyclic structure may be substituted with oxygen. "5-8-membered monocyclic heteroaryl" includes, for example, "5-7-membered monocyclic heteroaryl", "5-6-membered monocyclic heteroaryl", "5-6-membered monocyclic nitrogen-containing heteroaryl", nitrogen-containing heteroaryl", "6-membered monocyclic nitrogen-containing heteroaryl", etc. Said "nitrogen-containing heteroaryl" contains heteroatoms which contain at least one nitrogen atom, e.g. only one or two nitrogen atoms, or one nitrogen atom and one or two other nitrogen atoms. and one or two other heteroatoms (e.g. oxygen and/or sulfur atoms), or two nitrogen atoms and one or two other heteroatoms (e.g. oxygen and/or sulfur atoms). Specific examples of "5-8-membered monocyclic heteroaryl" include, but are not limited to, furanyl, thienyl, pyrrolyl, thiazolyl, isothiazolyl, thiadiazolyl, oxazolyl, isoxazolyl, oxadiazolyl, imidazolyl, pyrazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,4-oxadiazoleyl, 1,2,4-oxadiazoleyl, 1,2,4-oxadiazoleyl, 1,2,4-oxadiazoleyl, 1,2,4-oxadiazoleyl, and 1,2,4-oxadiazoleyl. 4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, pyridinyl, 2-pyridinone, 4-pyridinone, pyrimidinyl, pyridazinyl, pyrazinyl, 1,2,3-triazinyl, 1,3,5-triazinyl, 1,2,4,5-tetrazinyl, azacycloheptatriene, 1,3-diazacycloheptatriene, azacyclooctatetraenyl, and others.

The term "8-12-membered heteroaryl" refers to an unsaturated aromatic ring structure containing 8-12 ring atoms (at least one of which is a heteroatom, e.g., a nitrogen atom, an oxygen atom, or a sulfur atom) formed by two or more ring structures sharing two adjacent atoms with each other. Optionally, the ring atoms (e.g., carbon atoms, nitrogen atoms, or sulfur atoms) in the ring structure may be oxidized. "8-12-membered thickened heteroaryl" includes "8-10-membered thickened heteroaryl", "8-9-membered thickened heteroaryl", and the like; specific examples include, but are not limited to: pyrrolo[pyrrole], pyrrolo[pyrrole], pyrrolo[pyrrole] and pyrrolo-pyrrole, pyrrolo-furan, pyrazolopyrrole, pyrazolothiophene, furothiophene, pyrazolino-oxazole, benzene and furo furan, benzisofuranyl, benzothienyl, indolyl, isoindolyl, benzoxazolyl, benzimidazolyl, indazolyl, benzotriazolyl, quinolyl, 2-quinolynyl, 4-quinolinolone, 1-isoquinolinolone, isoquinolynyl, acridyl, phenanthridyl, pyridazine, phthalazine, quinazolinyl, quinazoline, quinazoline, quinazolidine, quinazoline, quinazolidine, quinazoline, quinoxaline, quinazoline, quinazolidine, quinazolinyl, quinazolinyl, quinazolinyl, quinazoline, quinazolinyl, quinazolyl, quinazolinyl, quinazolinyl quinazolinyl, quinoxalinyl, purinyl, naphthyridinyl, etc.

The term "haloalkyl" means alkyl substituted with one or more halogens, wherein alkyl is as defined above.

The term "deuteroalkyl" means alkyl substituted with one or more deuterium atoms, wherein the alkyl group is as defined above.

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

The term "halogen" means fluorine, chlorine, bromine or iodine.

The term "amino" refers to $-NH_2$.

The term "nitro" refers to $-NO_2$.

The term "derivative" refers to a substance that has a chemical structure similar to that of a compound but also contains a chemical group that is not present in at least one of the compounds and/or lacks a chemical group that is present in at least one of the compounds. The compound to which the derivative is compared is referred to as the "parent" compound. Typically, the "derivative" can be produced from the parent compound in one or more chemical reaction steps.

10 The term "pharmaceutically acceptable salt" refers to a pharmaceutically acceptable organic or inorganic salt of a compound (e.g., a drug, a linker-drug compound or a ligand-drug coupling). The compound or coupling may contain at least one amino or carboxyl group and may therefore form an addition salt with a corresponding acid or base. Exemplary salts include, but are not limited to sulfate, trifluoroacetate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, acid citrate, tartrate, oleate, tannate, pantothenate, hydrotartrate, ascorbate, salicylate, formate, benzoate Glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, potassium and sodium salts.

20 The term "solvent compounds" refers to the linker-drug compounds or ligand-drug couplings of the disclosure formed with one or more solvent molecules including, but not limited to, water, ethanol, acetonitrile, isopropanol, DMSO, ethyl acetate, and the like.

The term "pharmaceutical composition" refers to a mixture containing one or more of the compounds described in the disclosure or their physiologically/medicinally useful salts or precursor drugs together with other chemical components, as well as other components such as physiologically/medicinally useful carriers and/or excipients. The pharmaceutical compositions are intended to facilitate the administration of the drug to an organism, to facilitate the absorption of the active ingredient and thus the biological activity.

30 Pharmaceutical compositions containing the active ingredients may also contain one or more inactive pharmaceutical ingredients such as diluents, solubilizers, alcohols, binders, controlled release polymers, enteric polymers, disintegrants, excipients, colorants, flavorants, sweeteners, antioxidants, preservatives, pigments, additives, fillers, suspension agents, surfactants (for example, anionic, cationic, amphoteric and nonionic), and the like. Various FDA-approved topical inactive ingredients are found at the FDA's "The Inactive Ingredients Database" that contains inactive ingredients specifically intended as such by the manufacturer, whereby inactive ingredients can also be considered active ingredients under certain circumstances, according to the definition of an active ingredient given in 21 CFR 210.3(b)(7). Alcohol is a good example of an ingredient that may be considered either active or inactive depending on the product Formulation.

40 The term "carrier" refers to a system that alters the way a drug enters and distributes itself in the body, controls the rate of release, and delivers the drug to its target. Drug carrier release and targeting systems can reduce drug degradation and loss, decrease side effects and increase bioavailability.

The term "excipient" refers to an additive or supplement other than the main drug in a pharmaceutical preparation. For example, binders, fillers, disintegrants, lubricants in tablets; matrix backup in semi-solid ointments and creams; preservatives, antioxidants, flavoring agents, aromatics, co-solvents, emulsifiers, permeability enhancers, osmolarity regulators, coloring agents and so on in liquid preparations can be called excipients.

The term "diluent" or "filler" is mainly used to increase the weight and/or volume of a preparation. The addition of diluents not only ensures a certain volume size, but also reduces the dosage deviation of the main ingredients, improves the compression molding of the drug, etc.

10 The phrase "therapeutically effective" is intended to qualify the amount that will achieve the goal of improvement in disease severity and/or the frequency of incidence over non-treatment, while limiting, reducing, or avoiding adverse side effects typically associated with disease therapies. A "therapeutic effect" relieves to some extent one or more of the symptoms of a cancer disease or disorder. In reference to the treatment of a cancer, a therapeutic effect refers to one or more of the following: 1) reduction in the number of cancer cells by, for example, killing the cancer cells; 2) reduction in tumor size; 3) inhibition (i.e., slowing to some extent, preferably stopping) of cancer cell infiltration into peripheral organs; 4) inhibition (i.e., slowing to some extent, preferably stopping) of tumor metastasis; 5) inhibition, to some extent, of tumor growth; 6) relieving or reducing to some extent one or more of the symptoms associated with the disorder; and/or 7) relieving or reducing the side effects associated with the administration of anticancer
20 agents. "Therapeutic effective amount" is intended to qualify the amount required to achieve a therapeutic effect.

The disclosure discloses that an anti-EGFRxHER3 bispecific ADC, of which a highly stable hydrophilic linking unit formed through an ether bond between an aminomethyl group and a hydroxyl group can carry multiple toxins, simultaneously targets both EGFR and HER3 with good molecular stability and good preclinical efficacy, which may potentially exert excellent therapeutic effect.

The disclosure may be understood more readily by reference to the following detailed description of specific embodiments and examples included herein. Although the disclosure has been described with reference to specific details of certain embodiments thereof, it is not intended
30 that such details should be regarded as limitations upon the scope of the disclosure.

EXAMPLES

The experimental methods that do not specify particular conditions in the following examples are generally consistent with normal conditions or conditions recommended by the manufacturer. Unless otherwise stated, all percentages, proportions, ratios, or parts are expressed by weight. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as known to those skilled in the art. In addition, any methods and materials similar to or equivalent to those described herein may be applied to the methods disclosed herein. The preferred embodiments and materials described herein are for exemplary purposes only.

10 The general steps used in the following embodiments:

Generic Step A: Preparing ADCs by coupling

The monoclonal antibody was cloned and expressed by a proper gene expression system and purified by Protein-A affinity chromatography with molecular sieve chromatography. The purified antibody was placed in 20 mM acetate, pH 6.0 buffer, which was concentrated or diluted to the antibody concentration at 3 mg/mL. The linker-payload was dissolved to 20 mg/mL DMA and set aside. To open the interchain disulfide bonds of the antibody, 20-fold TECP was added according to the molecular ratio and the reaction was carried out at room temperature for 3 h. Then 20-fold linker-payload was added according to the molecular ratio and the reaction was carried out at room temperature for 1 h. After the reaction was completed, 20 the resulting ADC was obtained by ultrafiltration using a 30 KDa ultrafiltration tube, and the free linker-payload was removed.

Generic Step B: Measuring DAR by reversed-phase high-performance liquid chromatography (RP-HPLC)

Following the Standard Operating Procedures for the Use of Ultra Performance Liquid Chromatography (UPLC), the sample vial filled with the ADC sample was placed on the sample plate to set up the conditions of injection volume, number of injection needles, and injection method for each sample (see the list of parameters below). The chromatographic column model is Proteomix RP-1000 (4.6*100mm, 5 μ m, 1000Å), Sepax, Item No. 465950-4610.

30

Parameters and Set-Up:

Parameters	Set-up			
Mobile phase	A: 0.1% TFA aqueous solution; B: 0.1% TFA acetonitrile solution			
Flow rates	0.5 mL/min			
Wavelength	214 nm and 280 nm			
Column temperature	65 °C			
Sample plate temperature	indoor temperature			
Injection volume	25 μg			
Maximum pressure	100bar/10MPa/1450PSI			
Gradient	Time (min)	Flow rate (mL/min)	Mobile phase A (%)	Mobile phase B (%)
	0.0	0.5	75	25
	3	0.5	75	25

	28	0.5	50	50
	30	0.5	5	95
	32	0.5	5	95
	33	0.5	75	25
	40	0.5	75	25

Generic Step C: Detecting the monomer rate of antibodies or ADCs by SEC

Following the Standard Operating Procedures for the Use of UPLC, the sample vials on the sample plate were set for conditions, including injection volume, number of injection needles and injection method for each sample, including:

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

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

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

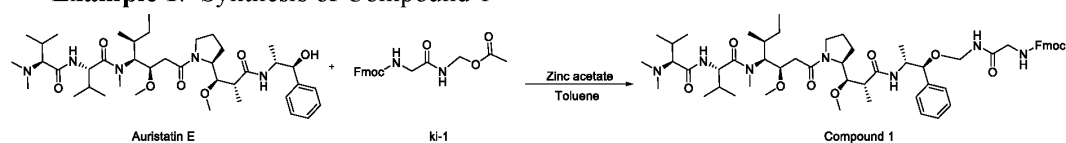
Parameters and values

Parameters	Set up
Flow rates	0.3 mL/min
Wavelength	280 nm
Column temperature	30 °C
Sample plate temperature	Indoor temperature
Injection volume	20 μ g
Maximum pressure	150 bar/15 MPa/2175 PSI
Gradient	Equal degree
Running time	20 minutes.

Generic Step D: Studying plasma stability

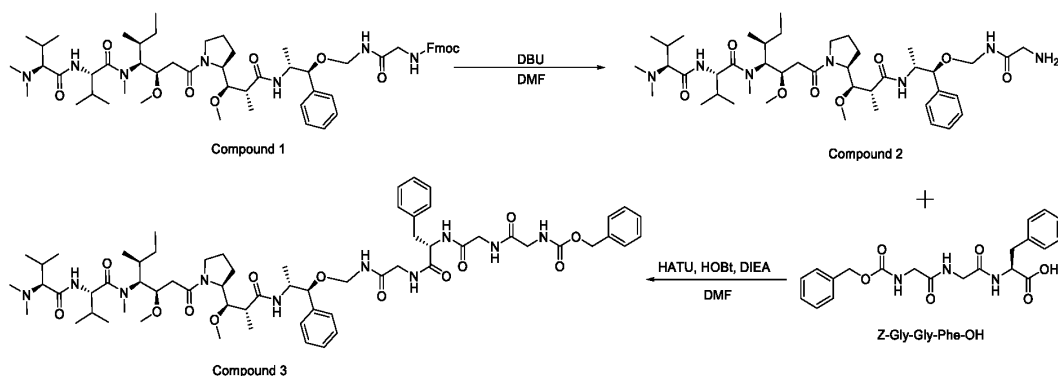
- 10 The ADC sample was mixed with IgG-free plasma, the final concentration of ADC was set at 0.6 mg/mL, and the reaction was placed in a water bath box in a 37 °C thermostat for incubation with the incubation time set for 0, 3, and 7 days using unincubated plasma as a control. After incubation, the sample ADC was purified and extracted for measuring the drug-antibody ratio (DAR), which reflects the stability of the sample ADC in plasma.

Example 1. Synthesis of Compound 1



- 20 Auristatin E (826 mg, 1.128 mmol, 1.0 eq), ki-1 (831.4 mg, 2.256 mmol, 2.0 eq, the method of synthesis refers to the synthesis of compound 1 in CN111686259A), zinc acetate (414.2 mg, 5.64 mmol, 2.0 eq), zinc acetate (414.2 mg, 5.64 mmol, 2.0 eq) and toluene (15 mL), were added to a 50 mL flask in sequence, and nitrogen was replaced 3 times. The reaction was refluxed at 115 °C for 4 h. The reaction was stopped, cooled to room temperature, filtered, and the filtrate was concentrated under reduced pressure, purified by reverse phase preparative column, and freeze-dried to obtain a white solid (605 mg, 51.55%). LC-MS m/z (ES⁺): [M+H]⁺: 1041.3.

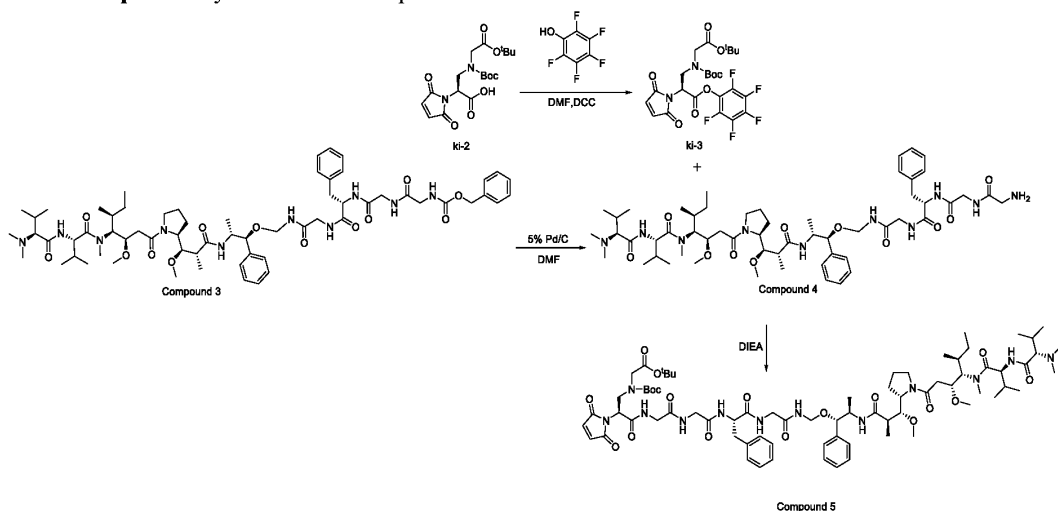
Example 2. Synthesis of Compound 3



Z-Gly-Gly-Phe-OH (240 mg, 0.581 mmol, 1.0 eq), HATU (264 mg, 0.697 mmol, 1.2 eq), HOBt (94 mg, 0.697 mmol, 1.2 eq), and DMF (2 mL) were sequentially added to a 10 mL EP tube and kept aside with stirring at room temperature.

Compound 1 (605 mg, 0.581 mmol, 1.0 eq) and DMF (8 mL) were sequentially added to a 25 mL flask, dissolved by stirring at room temperature. Then, DBU (95.6 μ L, 0.64 mmol, 1.1 eq) was added and the reaction was carried out at room temperature for 0.5 h. The raw material disappeared as monitored by TLC and compound 2 was produced. Then the mixture mentioned above was added to this vial and DIEA (96 μ L, 0.581 mmol, 1.0 eq) was added. The reaction was carried out at room temperature for 1 h and monitored by HPLC. At the end of the reaction, the white solid product (487 mg, 69.08%) was purified by using a reversed-phase preparative column. LC-MS m/z (ES^+): $[M+H]^+$: 1213.6.

Example 3. Synthesis of Compound 5



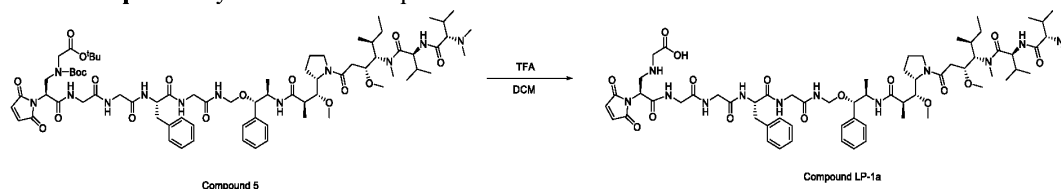
Compound 3 (487 mg, 0.402 mmol, 1.0 eq), 5% Pd/C (48.7 mg) and DMF (5 mL) were added sequentially to a 25 mL flask, and the reaction was carried out at room temperature for 1 h after three times of hydrogen substitution. The reaction was monitored by using HPLC, and the raw materials disappeared to produce a new peak for compound 4, which was recorded as the reaction solution ①.

In a 25 mL flask filled with ki-2 (176 mg, 0.442 mmol, 1.1 eq, the method of synthesis refers to CN108452321A), Pentafluorophenol (81 mg, 0.442 mmol, 1.1 eq), DCC (91 mg, 0.442 mmol, 1.1 eq), and DMF (3 mL), the reaction was carried out at room temperature for

30 min, monitored by using TLC, and recorded as reaction solution (2). The reaction was monitored by TLC, and ki-3 was obtained at the end of the reaction, which was recorded as reaction solution (2).

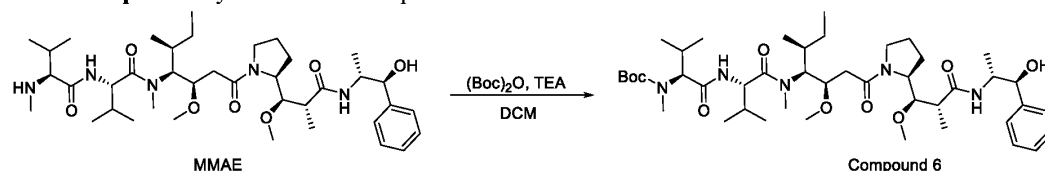
The reaction solution ① was then filtered into a new 25 mL flask, and in an ice-water bath, the filtrate of the reaction solution of DIEA (73 μ L, 0.442 mmol, 1.1 eq) and ② was added, and the reaction solution was added and brought to room temperature for 1 h, which was monitored by HPLC. The reaction solution was filtered, and the filtrate was added to the reaction solution containing compound 4 while stirred in an ice bath, and then the ice bath was withdrawn, and the reaction was carried out at room temperature for 1 h, while monitored. By using HPLC. The reaction solution was purified directly by reversed-phase preparation column, and the preparative solution was lyophilized to obtain a white solid product (320 mg, 54.53%). LC-MS m/z (ES^+): $[M/2+H]^+$: 730.4.

Example 4. Synthesis of Compound LP-1a



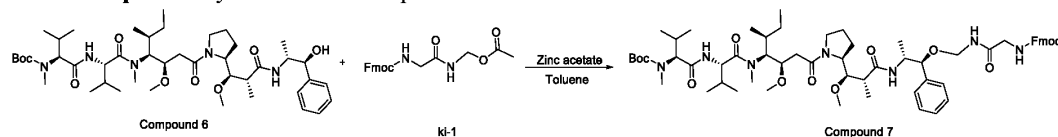
Compound 5 (100 mg, 0.0685 mmol, 1.0 eq) was dissolved in 10 mL of dry dichloromethane and 4 mL of TFA, and reacted at room temperature for 3 h, monitored by HPLC. At the completion of the reaction, the solvent was removed by vacuum concentration, purified by reversed-phase preparative column, and lyophilized to obtain a white solid product (21 mg, 29.78%). LC-MS m/z (ES^+): $[M/2+H]^+$: 652.4. The LC-MS spectrum is shown in **Figure 1**.

Example 5. Synthesis of Compound 6



MMAE (2.0 g, 2.79 mmol, 1.0eq), (Boc)₂O (1.21g, 5.57 mmol, 2.0eq), DCM (20 mL) was added dropwise to a 100 mL flask, and under an ice-water bath, TEA (563 mg, 5.57 mmol, 2.0 eq) was added. After the addition was complete, the reaction was allowed to stand at room temperature for 72 h, monitored by TLC until the raw material MMAE was completely reacted. Post-treatment: after vacuum concentration, the reaction solution was purified by column chromatography (eluent: DCM/MeOH=20/1) to obtain a white solid (2.28 g, 100%).

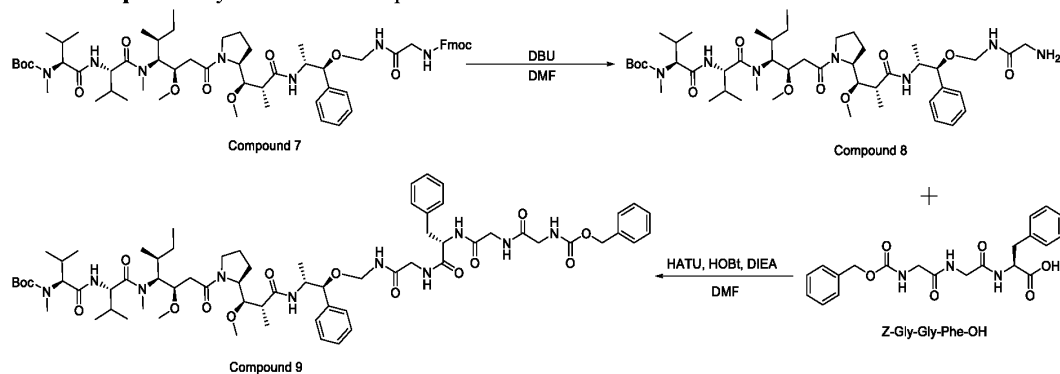
Example 6. Synthesis of Compound 7



In a 100 mL flask, compound 6 (2.28 g, 2.79 mmol, 1.0 eq), ki-1 (2.05 g, 5.57 mmol, 2.0 eq), zinc acetate (1.02 g, 5.57 mmol, 2.0 eq) were added sequentially and dissolved in toluene (30 mL), and the reaction was stopped by replacing nitrogen 3 times, and then ramping up to

115°C for 4 h under the protection of nitrogen. The reaction was cooled to room temperature, filtered, and the filtrate was vacuum concentrated. The reaction was stopped, cooled down to room temperature, filtered, and the filtrate was concentrated under reduced pressure, purified by reversed-phase preparative column, and the preparative solution was lyophilized to obtain a white solid product (1.071 g, 34%). LC-MSm/z (ES⁺): [M+H]⁺: 1126.4.

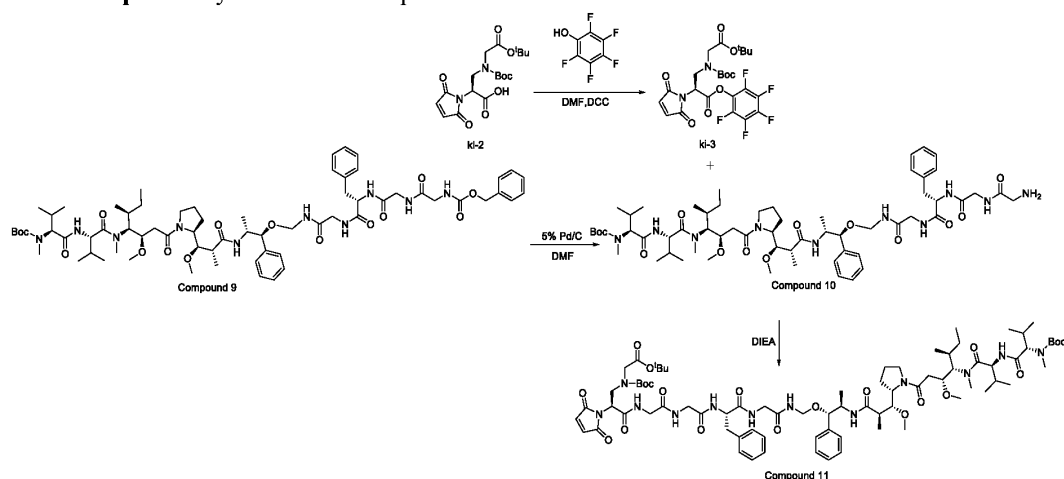
Example 7. Synthesis of compound 9



Compound 7 (900 mg, 0.8 mmol, 1.0 eq) and DMF (9 mL) were added to a 50 mL flask, stirred to dissolve until the clearing, and under an ice-water bath, added dropwise DBU (134 mg, 0.88 mmol, 1.1 eq), after addition, the reaction was brought to room temperature for 30 min. The reaction was monitored by TLC and the end of the reaction was recorded as reaction solution ①.

In another 50 mL flask, Z-Gly-Gly-Phe-OH (364 mg, 0.88 mmol, 1.1 eq), HATU (365 mg, 0.96 mmol, 1.2 eq), HOBT (129.7 mg, 0.96 mmol, 1.2 eq) and DMF (7 mL) were added to dissolve until the clearing, and under an ice-water bath, the reaction solution ① and DIEA (103.4 mg, 0.8 mmol, 1.0 eq) was sequentially added dropwise and the reaction was brought to room temperature for 1 h. The reaction was monitored by HPLC. The reaction solution was purified by reversed-phase preparation column and lyophilized to obtain a white solid (960 mg, 92.4%). LC-MSm/z (ES⁺): [M+H]⁺: 1299.6.

Example 8. Synthesis of Compound 11



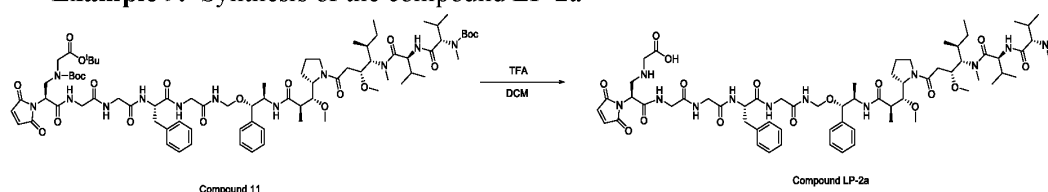
Compound 10 (960 mg, 0.74 mmol, 1.0 eq), 5% Pd/C (960 mg) and DMF (10 mL) were added to a 50 mL flask, H₂ was replaced 3 times, and then reacted at room temperature for 1 h.

The reaction was monitored by HPLC and was recorded as reaction solution ①.

In another 25 mL flask, add ki-2 (322.7 mg, 0.81 mmol, 1.1 eq), DCC (167 mg, 0.81 mmol, 1.1 eq) and DMF (5 mL), added pentafluorophenol (149 mg, 0.81 mmol, 1.1 eq) in an ice-water bath, and the reaction was allowed to proceed for 30 min at room temperature. The reaction was monitored by TLC, and after completion of the reaction, ki-3 was obtained, which was recorded as reaction solution ②.

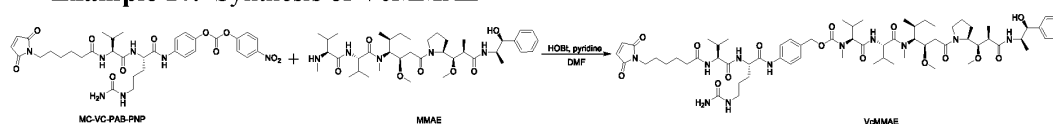
The reaction solution ① was filtered into a 50 mL flask, and in an ice-water bath, DIEA (105 mg, 0.81 mmol, 1.1 eq) and the filtrate of the reaction solution ② were added, and the reaction solution was raised to room temperature for 1 h, and the reaction was monitored by HPLC. The reaction solution was purified by reversed-phase preparation column and lyophilized to obtain a white solid (895 mg, 78.5%). LC-MSm/z (ES⁺): [M/2+H]⁺: 773.4.

Example 9. Synthesis of the compound LP-2a



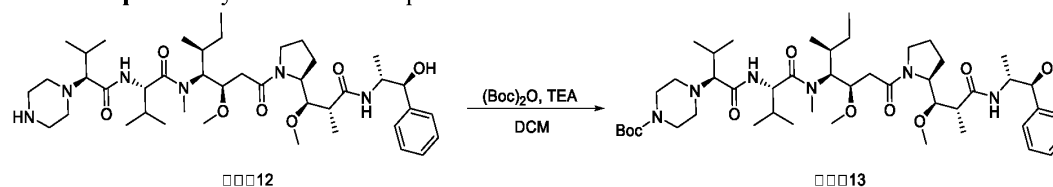
Compound 12 (400 mg, 0.259 mmol, 1.0 eq) was dissolved in 20 mL of dry dichloromethane, 8 mL of TFA, and reacted at room temperature for 3 h. The reaction was monitored by HPLC. At the end of the reaction, the solvent was removed by vacuum concentrator, and the crude product was purified by reversed-phase preparative column, and the preparative solution was lyophilized to produce a white solid (248 mg, 74%). LC-MSm/z (ES⁺): [M/2+H]⁺: 645.3.

Example 10. Synthesis of VcMMAE

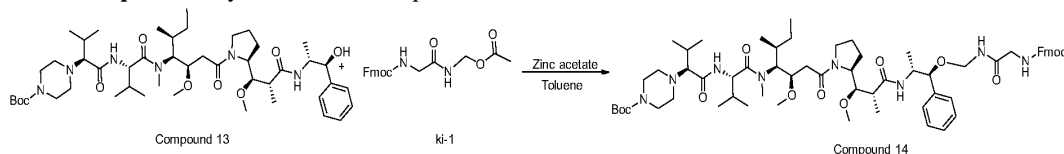


In a 25 mL beaker, MMAE (120 mg, 0.167 mmol, 1.0 eq) and MC-VC-PAB-PNP (186 mg, 0.25 mmol, 1.5 eq) were added, solubilized with DMF (5 mL), and then HOBT (27.1 mg, 0.20 mmol, 1.2 eq) and pyridine (1 mL) were added sequentially. The reaction was stirred overnight at room temperature and monitored by HPLC. After the reaction, the crude product was purified by a reversed-phase preparative column, and the preparative solution was lyophilized to obtain a white solid (158.3 mg, 72%) with LC-MSm/z (ES⁺): [M/2+H]⁺: 659.0. The LC-MS spectrum is shown in Figure 2.

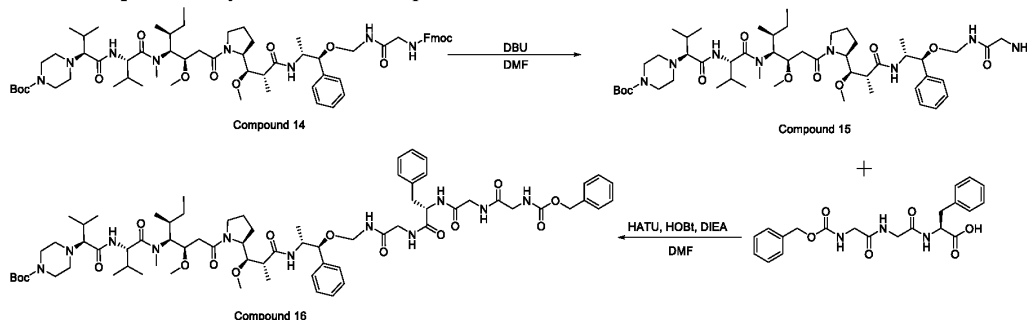
Example 11. Synthesis of Compound 13



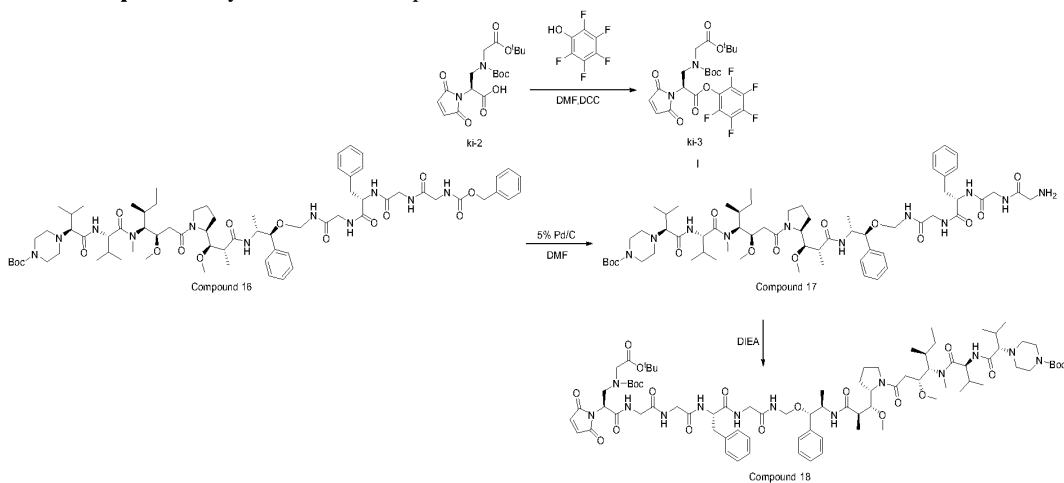
Referring to the synthesis method of Example 5, compound 13 was synthesized from compound 12 (referring to the synthesis of compound 17 in patent CN106279352). LC-MSm/z (ES⁺): [M+H]⁺: 873.6

Example 12. Synthesis of Compound 14

Compound 14 was synthesized from compound 13 and ki-1 with reference to the synthetic method of Example 6. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1180.7.

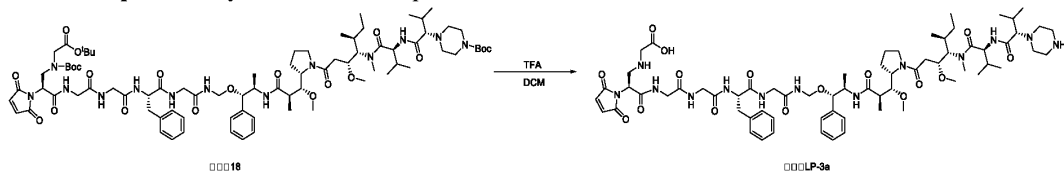
Example 13. Synthesis of Compound 16

Compound 16 was synthesized from compound 14 with reference to the synthetic method of Example 7. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1353.9.

Example 14. Synthesis of Compound 18

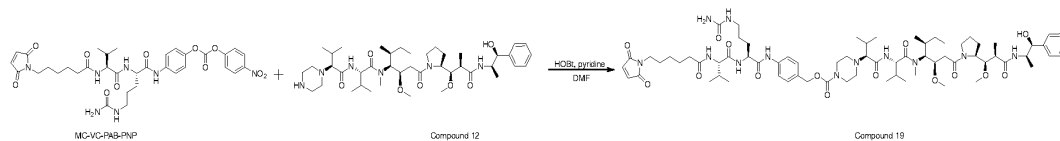
10

Compound 18 was synthesized from compound 16 with reference to the synthetic method of Example 8. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1600.9.

Example 15. Synthesis of Compound LP-3a

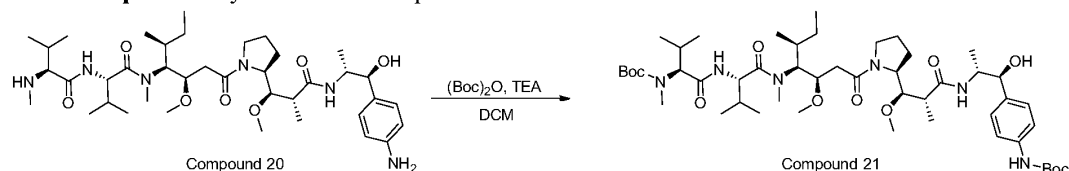
Compound LP-3a was synthesized from compound 18 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1344.7.

Example 16. Synthesis of Compound 23



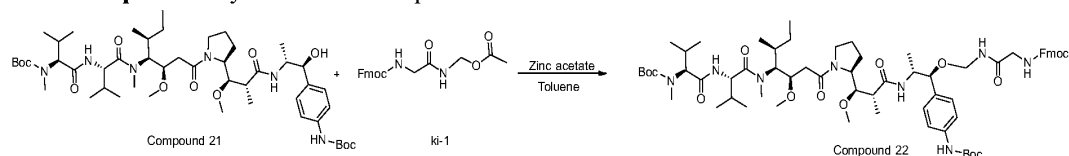
Compound 19 was synthesized from compound 12 and MC-VC-PAB-PNP with reference to the synthesis of Example 10. LC-MS m/z (ES^+): $[\text{M}+\text{H}]^+$: 1371.8.

Example 17. Synthesis of Compound 21



Compound 21 was synthesized from compound 20 (synthesized with reference to US2017014524A1) with reference to the synthesis method of Example 5. LC-MS m/z (ES^+): $[\text{M}+\text{H}]^+$: 933.6.

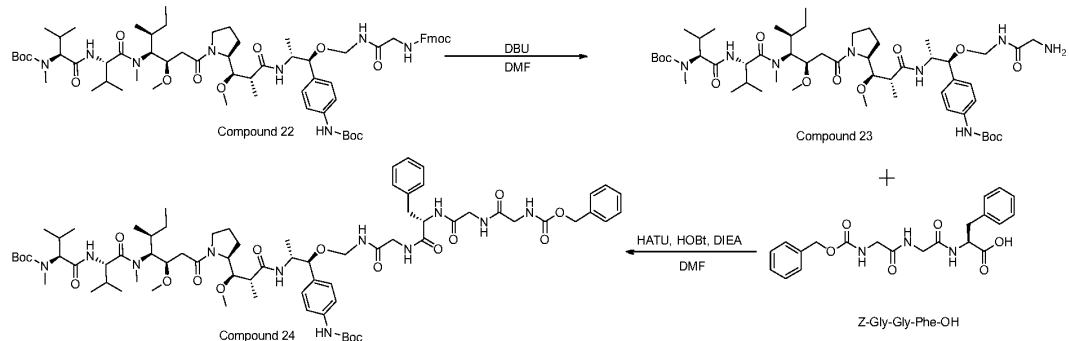
Example 18. Synthesis of Compound 22



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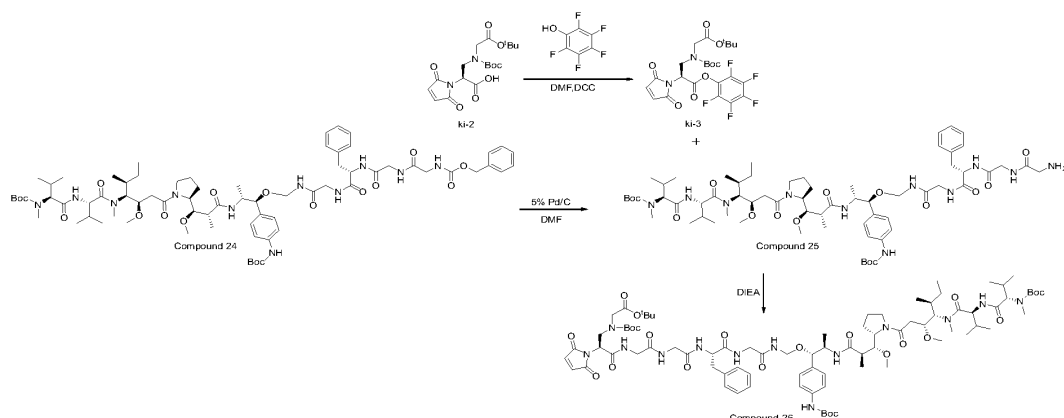
Compound 22 was synthesized from compound 21 and ki-1 with reference to the synthetic method of Example 6. LC-MS m/z (ES^+): $[\text{M}+\text{H}]^+$: 1241.7.

Example 19. Synthesis of Compound 24



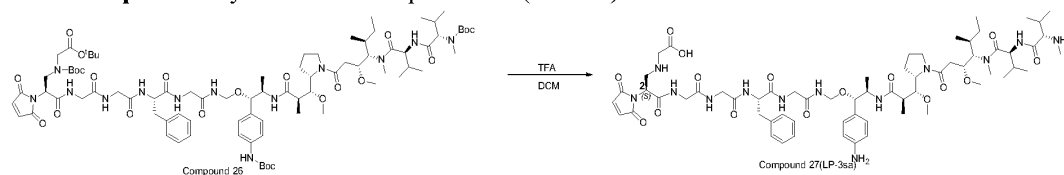
Compound 24 was synthesized from compound 22 with reference to the synthetic method of Example 7. LC-MS m/z (ES^+): $[\text{M}+\text{H}]^+$: 1414.8.

Example 20. Synthesis of Compound 26



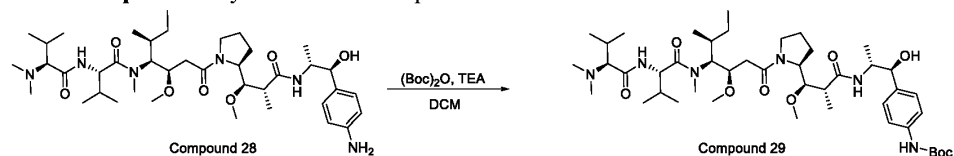
Compound 26 was synthesized from compound 24 with reference to the synthetic method of Example 8. LC-MSm/z (ES^+): $[M+H]^+$:1660.9.

Example 21. Synthesis of compound 27 (LP-3sa)



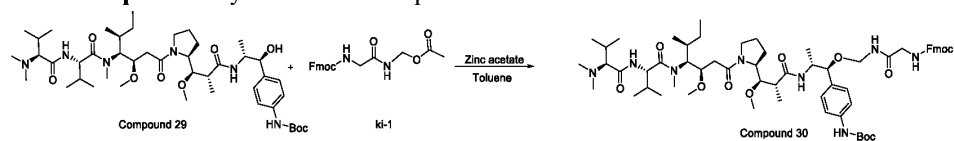
Compound 27 (LP-3sa, whose structure is shown in the structural formula of LP-3s, where the configuration of the chiral carbon at the position-2 is S-type) was synthesized from compound 26 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[M+H]^+$:1304.7.

10 **Example 22.** Synthesis of Compound 29



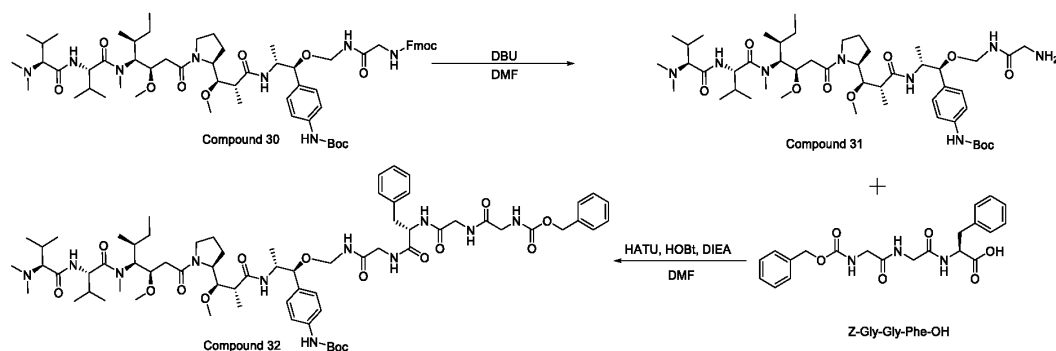
Compound 29 was synthesized from compound 28 (synthesized with reference to patent US2017014524A1) with reference to the synthesis method of Example 5. LC-MSm/z (ES^+): $[M+H]^+$:847.6.

Example 23. Synthesis of Compound 30



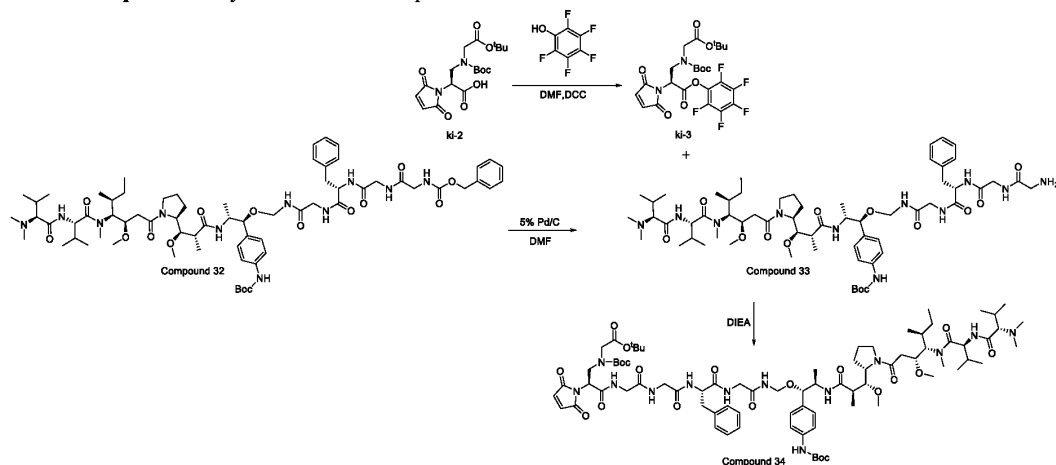
Compound 30 was synthesized from compound 29 and ki-1 with reference to the synthetic method of Example 6. LC-MSm/z (ES^+): $[M+H]^+$:1155.7.

Example 24. Synthesis of Compound 32



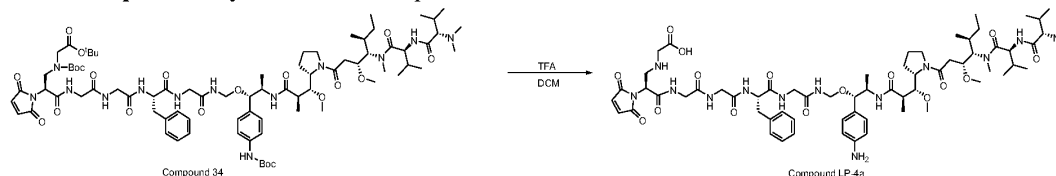
Compound 32 was synthesized from compound 30 with reference to the synthetic method of Example 7. LC-MSm/z (ES^+): $[M+H]^+$:1328.8.

Example 25. Synthesis of Compound 34



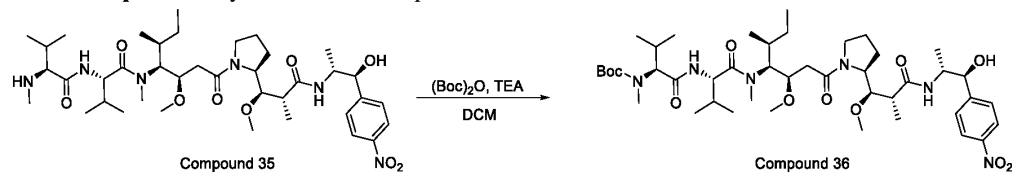
Compound 34 was synthesized from compound 32 with reference to the synthetic method of Example 8. LC-MSm/z (ES^+): $[M+H]^+$:1574.9.

Example 26. Synthesis of Compound LP-4a



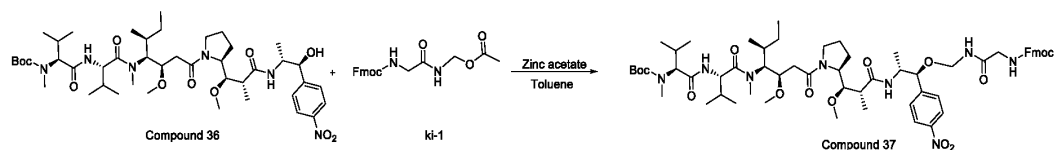
10 Compound LP-4a was synthesized from compound 34 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[M+H]^+$:1318.7.

Example 27. Synthesis of Compound 36



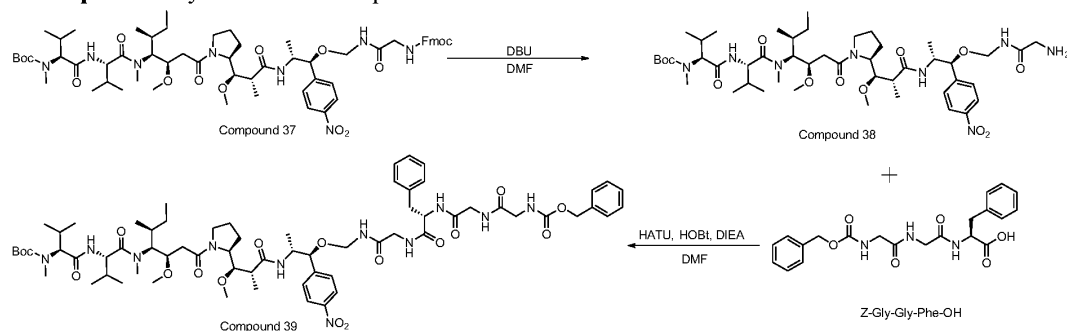
Compound 36 was synthesized from compound 35 (synthesized with reference to US20210346523A1) by the synthetic method of Example 5. LC-MSm/z (ES^+): $[M+H]^+$:863.5.

Example 28. Synthesis of Compound 37



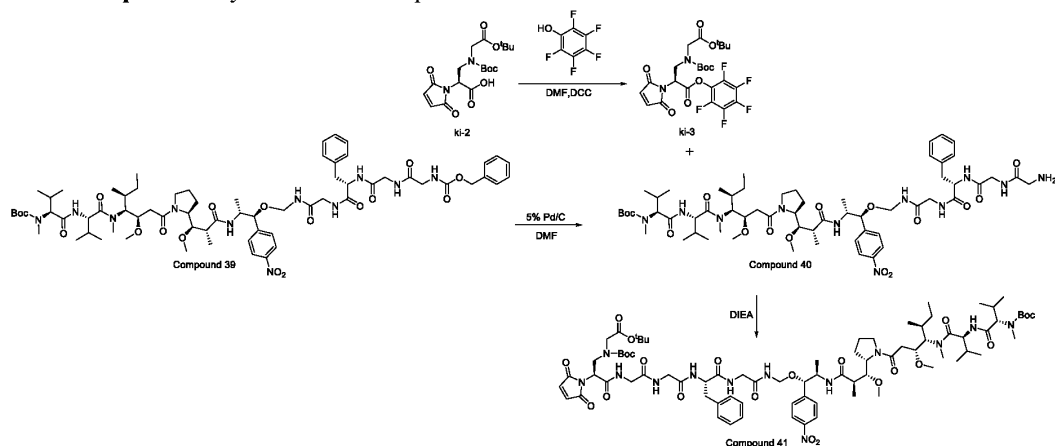
Compound 37 was synthesized from compound 36 and ki-1 with reference to the synthetic method of Example 6. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$:1171.7.

Example 29. Synthesis of Compound 39



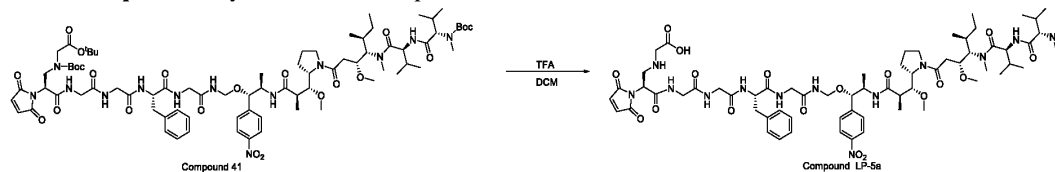
Compound 39 was synthesized from compound 37 with reference to the synthetic method of Example 7. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$:1344.7.

Example 30. Synthesis of Compound 41



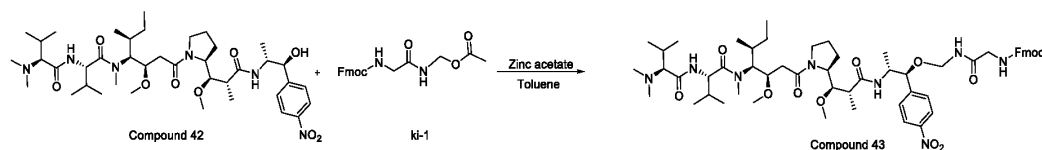
10 Compound 41 was synthesized from compound 39 with reference to the synthetic method of Example 8. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$:1590.9.

Example 31. Synthesis of Compound LP-5a



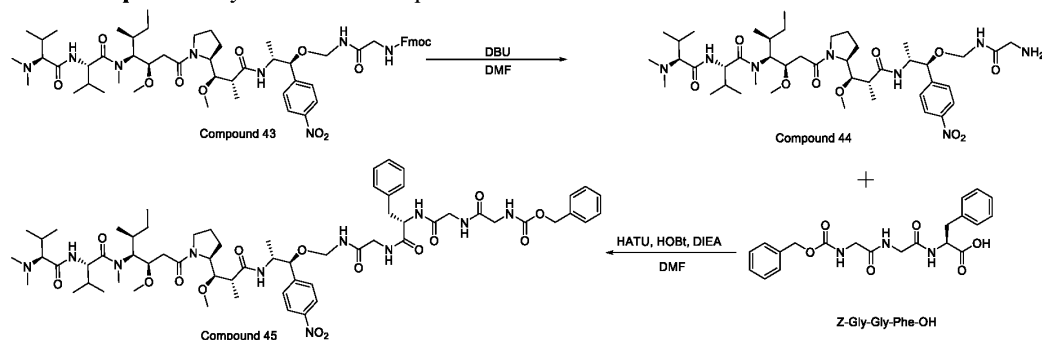
Compound LP-5a was synthesized from compound 41 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$:1334.7.

Example 32. Synthesis of Compound 43



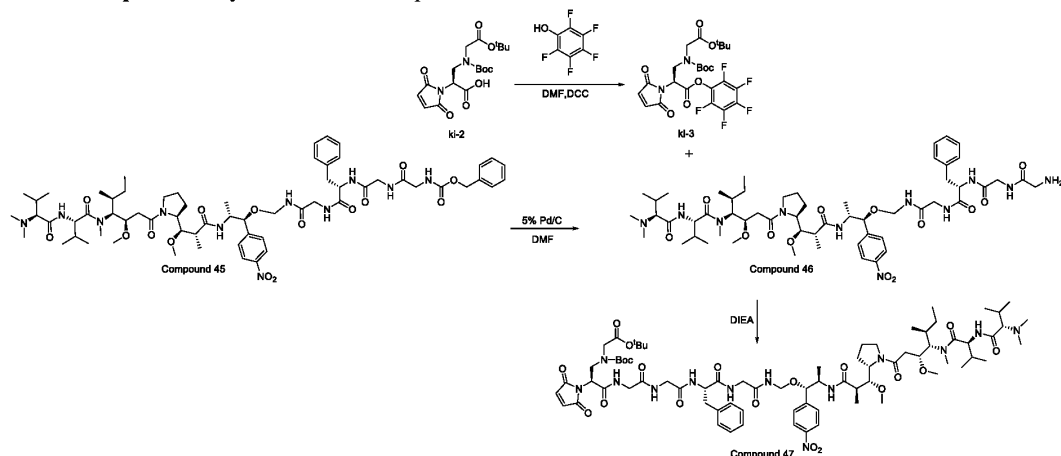
Compound 43 was synthesized with reference to the synthesis method of Example 6, using compound 42 (with reference to the synthesis of compound 17 in US20210346523A1) and ki-1 as raw material. LC-MSm/z (ES⁺):[M+H]⁺:1085.6.

Example 33. Synthesis of Compound 45



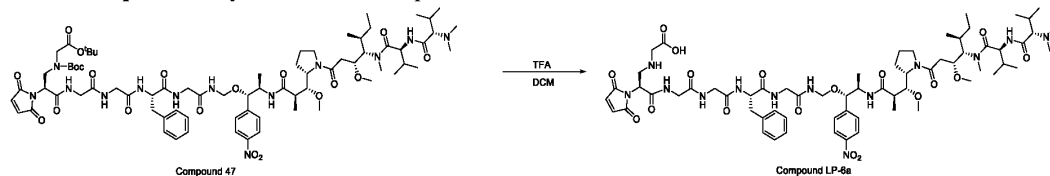
Compound 45 was synthesized from compound 43 with reference to the synthetic method of Example 7. LC-MSm/z (ES⁺):[M+H]⁺:1258.7.

Example 34. Synthesis of Compound 47



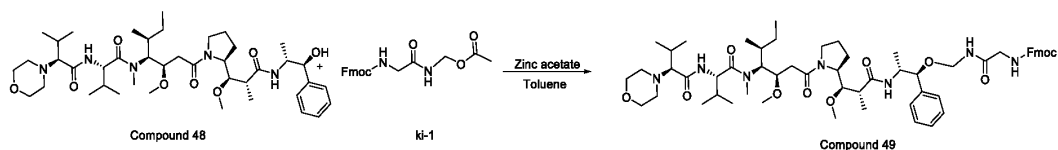
Compound 47 was synthesized from compound 45 with reference to the synthetic method of Example 8. LC-MSm/z (ES⁺):[M+H]⁺:1504.8.

Example 35. Synthesis of Compound LP-6a



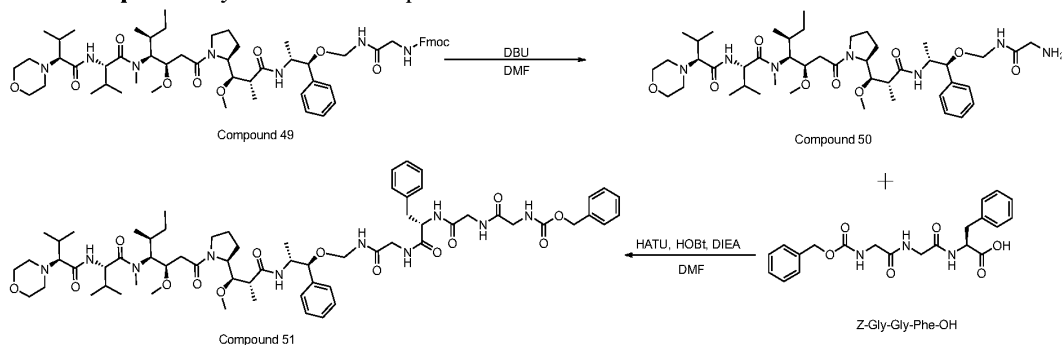
Compound LP-6a was synthesized from compound 47 with reference to the synthetic method of Example 9. LC-MSm/z (ES⁺):[M+H]⁺:1348.7.

Example 36. Synthesis of Compound 49



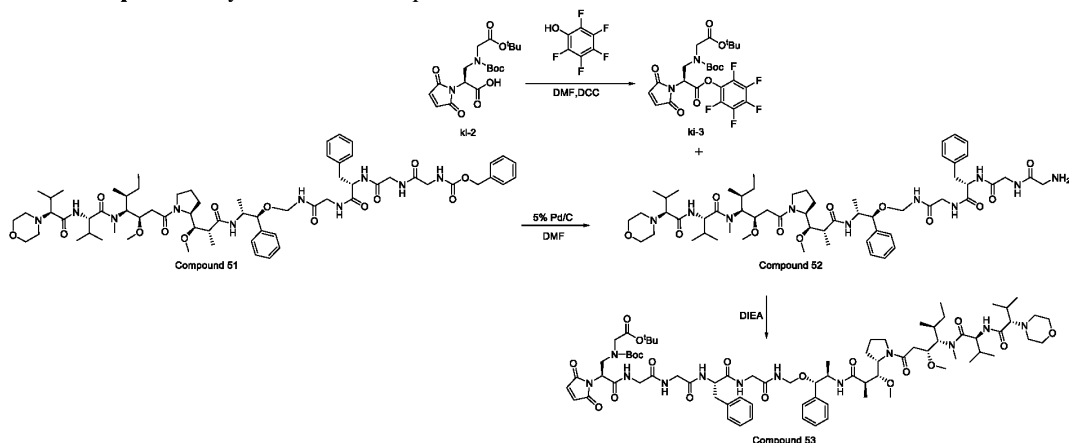
Referring to the synthesis method of Example 6, compound 49 was synthesized from compound 48 (referring to the synthesis of compound 6 in CN106279352) and ki-1. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$:1082.6.

Example 37. Synthesis of Compound 51



Compound 51 was synthesized from compound 49 with reference to the synthetic method of Example 7. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$:1255.7.

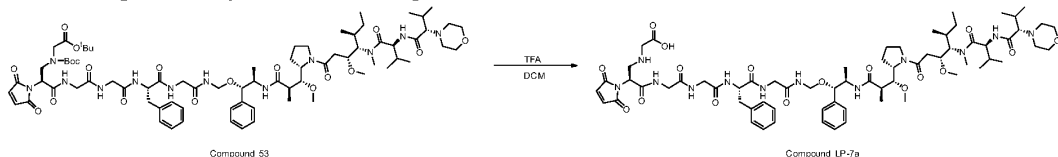
Example 38. Synthesis of Compound 53



10

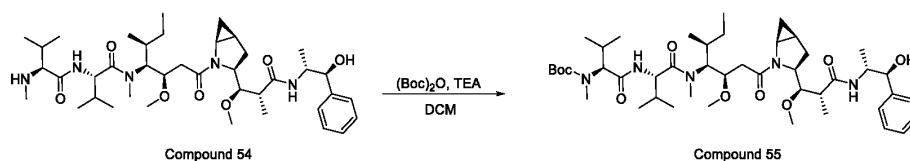
Compound 53 was synthesized from compound 51 with reference to the synthetic method of Example 8. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$:1501.8.

Example 39. Synthesis of Compound LP-7a



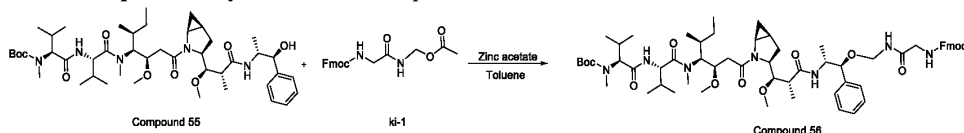
Compound LP-7a was synthesized from compound 53 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$:1345.7.

Example 40. Synthesis of Compound 63 55



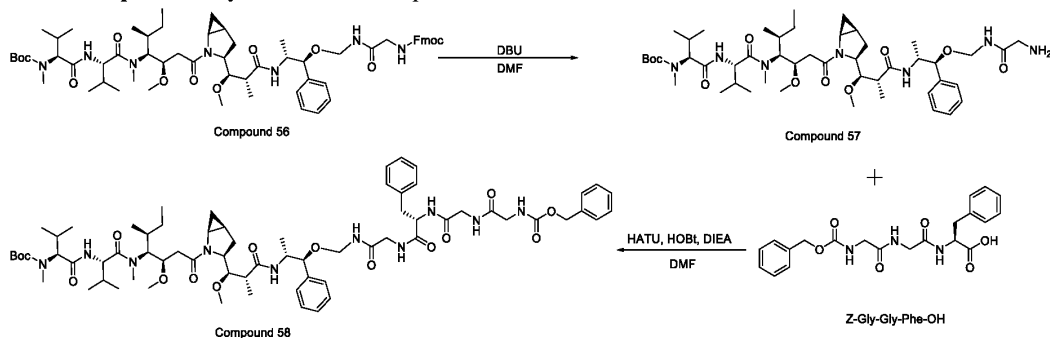
Referring to the synthesis method of Example 5, compound 55 was synthesized from compound 54 (referring to the synthesis of compound 1 in CN113121639A). LC-MSm/z (ES^+): $[M+H]^+$:830.6.

Example 41. Synthesis of Compound 56



Compound 56 was synthesized from compound 55 and ki-1 with reference to the synthetic method of Example 6. LC-MSm/z (ES^+): $[M+H]^+$:1138.7.

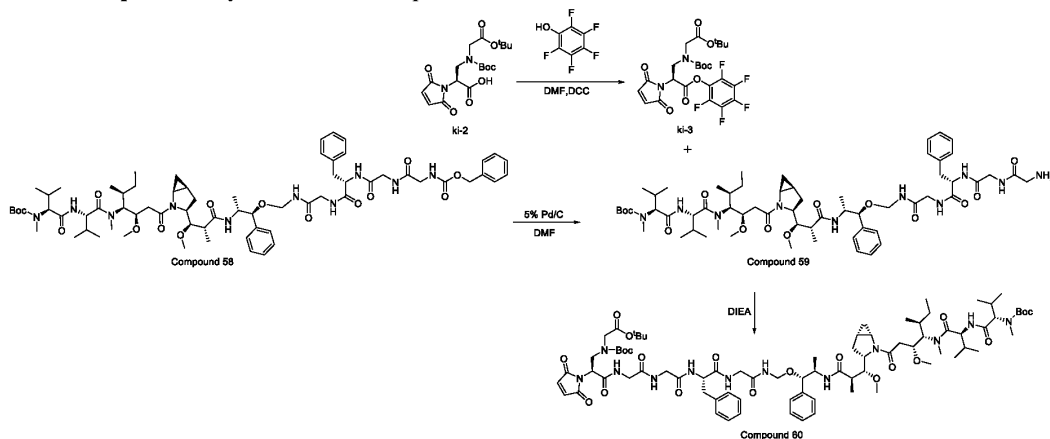
Example 42. Synthesis of Compound 58



10

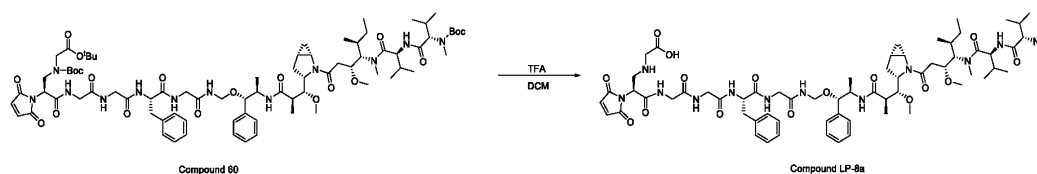
Referring to the synthetic method of Example 7, compound 58 was synthesized from compound 56. LC-MSm/z (ES^+): $[M+H]^+$:1311.8.

Example 43. Synthesis of Compound 60



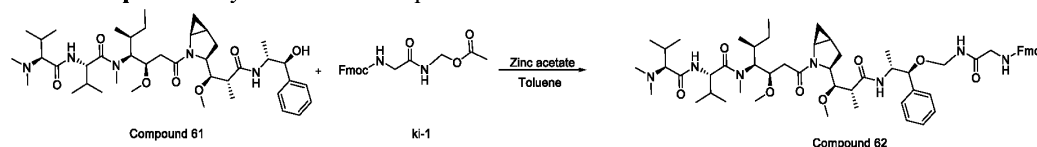
Compound 60 was synthesized from compound 58 with reference to the synthetic method of Example 8. LC-MSm/z (ES^+): $[M+H]^+$:1557.9.

Example 44. Synthesis of compound LP-8a



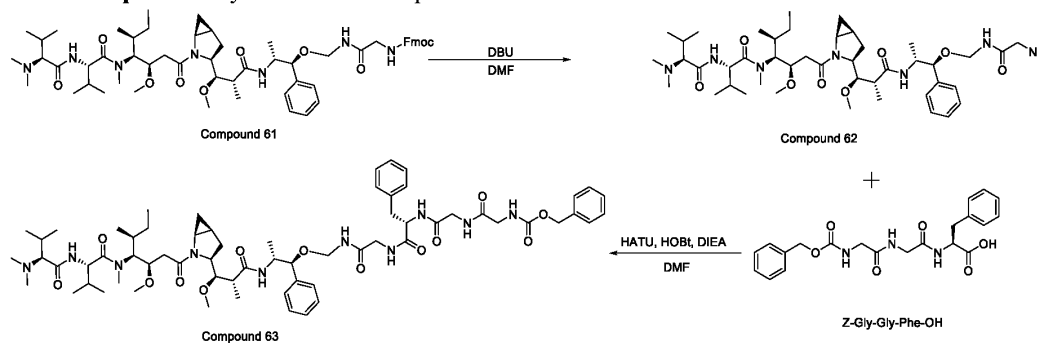
Compound LP-8a was synthesized from compound 60 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1301.7.

Example 45. Synthesis of Compound 62



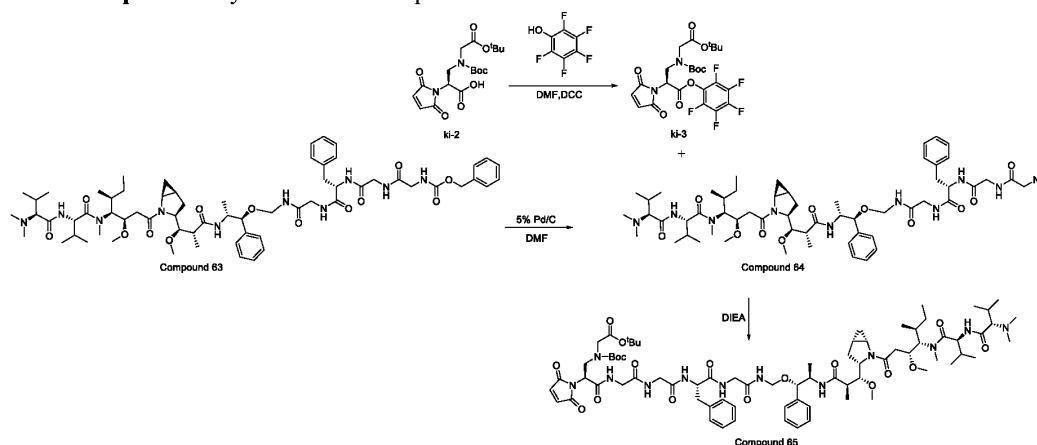
Referring to the synthesis method of Example 6, Compound 62 was synthesized from Compound 61 (referring to the synthesis of Compound 1 in CN113121639A) and ki-1. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1052.6.

Example 46. Synthesis of Compound 63



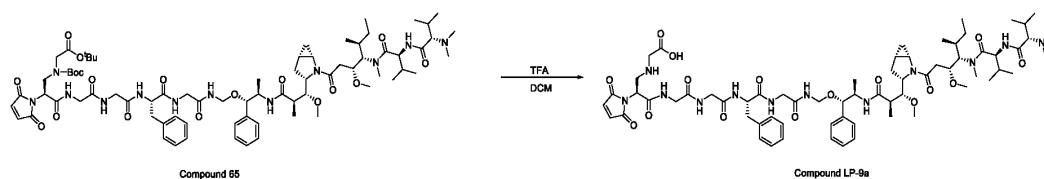
Compound 63 was synthesized from compound 61 with reference to the synthetic method of Example 7. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1225.7.

Example 47. Synthesis of Compound 65



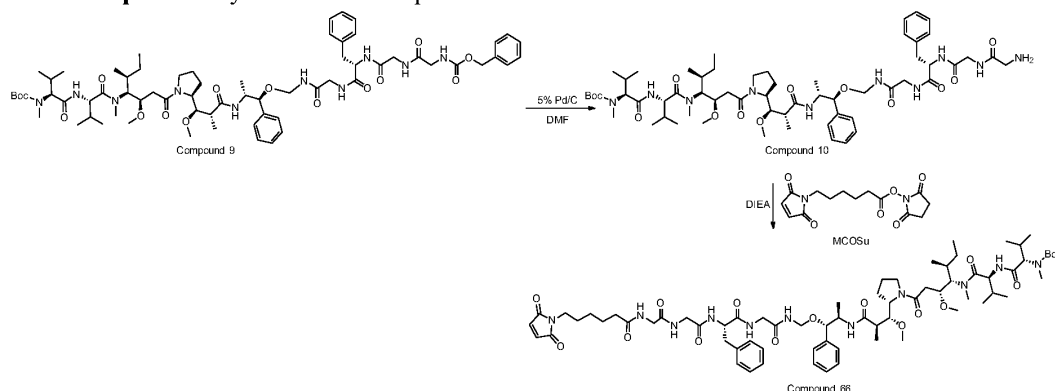
Compound 65 was synthesized from compound 63 with reference to the synthetic method of Example 8. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1471.8.

Example 48. Synthesis of Compound LP-9a



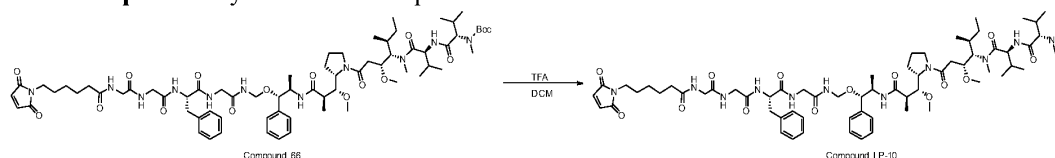
Compound LP-9a was synthesized from compound 65 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1315.7.

Example 49. Synthesis of Compound 66



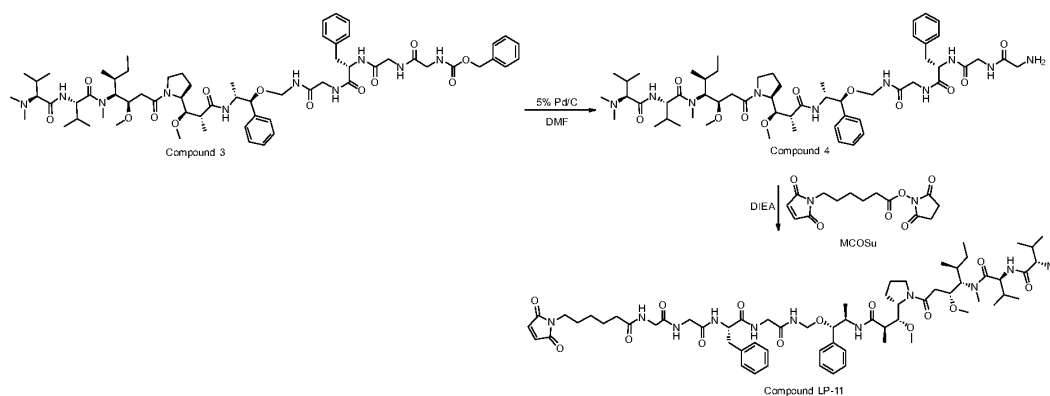
Compound 9 (960mg, 0.74mmol, 1.0eq), 5% Pd/C (960mg) and DMF (10mL) were added to a 50 mL flask, H_2 was replaced 3 times, and then the reaction was carried out at room temperature for 1h. The reaction was monitored by HPLC. When the reaction was completed, it was recorded as reaction solution ①. Reaction solution ① was filtered to a 50 mL flask, and MCOSU was added sequentially in an ice water bath (274 mg, 0.89 mmol, 1.2eq) and DIEA (105 mg, 0.81 mmol, 1.1eq) were sequentially added in an ice-water bath, and the reaction was brought to room temperature for 1 h. The reaction was monitored by HPLC. The reaction solution was purified by reversed-phase preparation, and the preparative solution was lyophilized to give a white solid (847.5 mg, 84.3%). LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1358.2.

Example 50. Synthesis of Compound LP-10



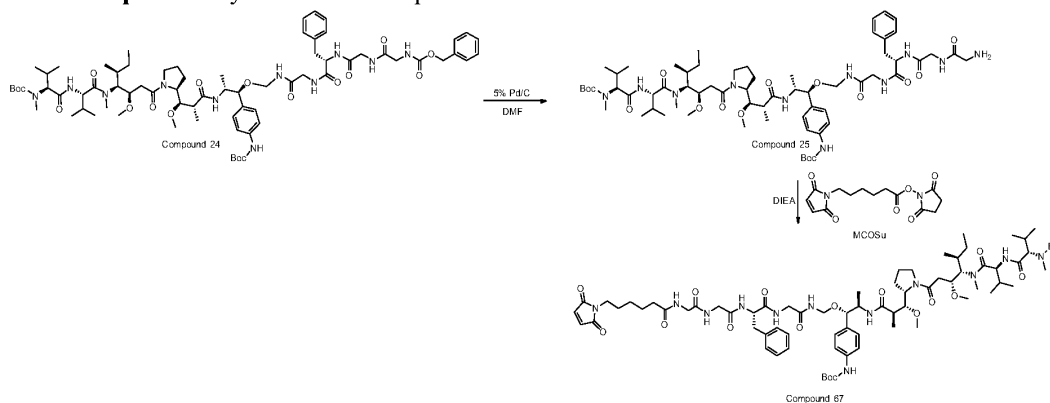
Compound LP-10 was synthesized from compound 66 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[\text{M}/2+\text{H}]^+$: 630.0.

Example 51. Synthesis of Compound LP-11



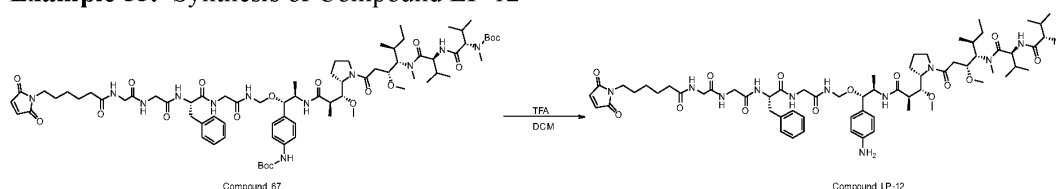
Compound LP-11 was synthesized from compound 3 with reference to the synthetic method of Example 49. LC-MSm/z (ES^+): $[M+H]^+$: 1272.8.

Example 52. Synthesis of Compound 67



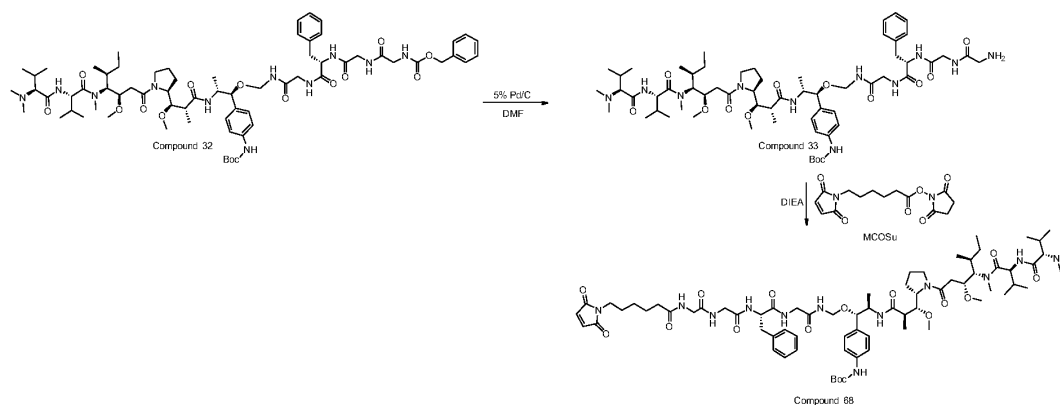
Compound 67 was synthesized from compound 28 with reference to the synthetic method of Example 49. LC-MSm/z (ES^+): $[M+H]^+$: 1473.9.

Example 53. Synthesis of Compound LP-12



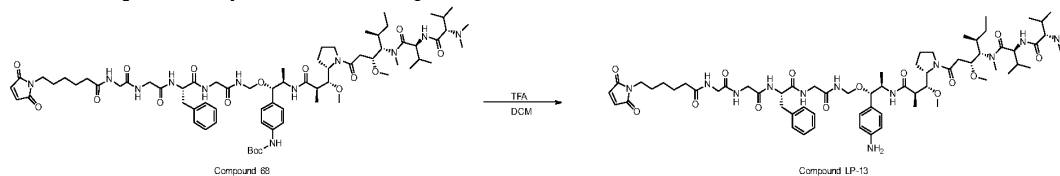
Compound LP-12 was synthesized from compound 67 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[M+H]^+$: 1273.8.

Example 54. Synthesis of Compound 68



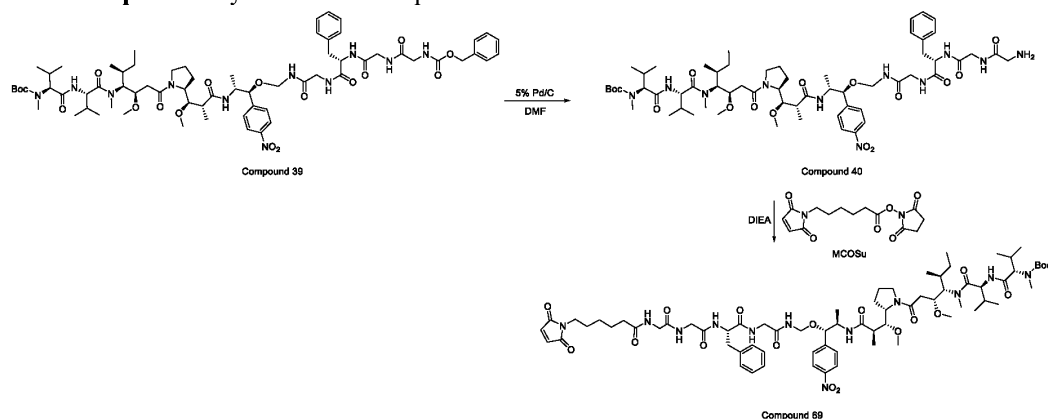
Compound 68 was synthesized from compound 36 with reference to the synthetic method of Example 49. LC-MSm/z (ES^+): $[M+H]^+$: 1387.8.

Example 55. Synthesis of Compound LP-13



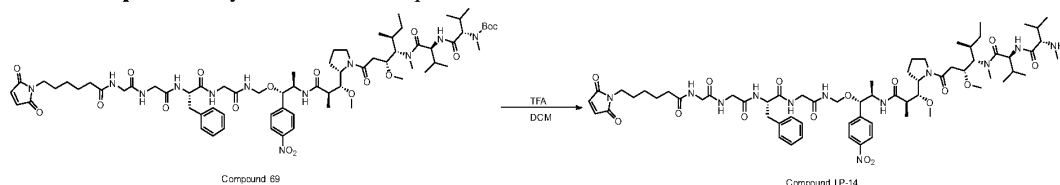
Compound LP-13 was synthesized from compound 68 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[M+H]^+$: 1287.8.

Example 56. Synthesis of Compound 69



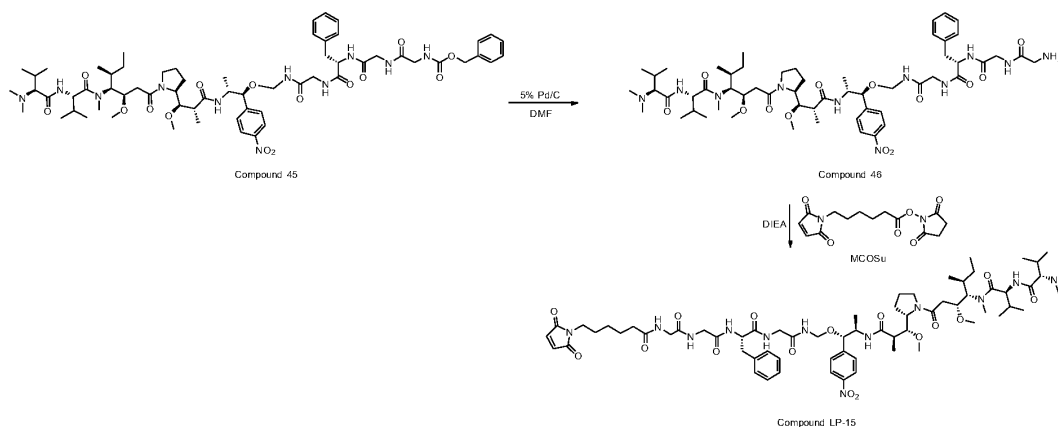
10 Compound 69 was synthesized from compound 39 with reference to the synthetic method of Example 49. LC-MSm/z (ES^+): $[M+H]^+$: 1403.8.

Example 57. Synthesis of Compound LP-14



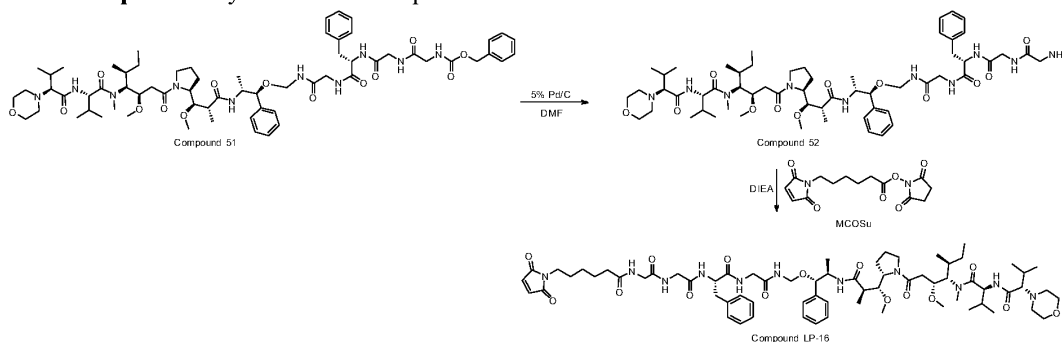
Compound LP-14 was synthesized from compound 69 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[M+H]^+$: 1303.7.

Example 58. Synthesis of Compound LP-15



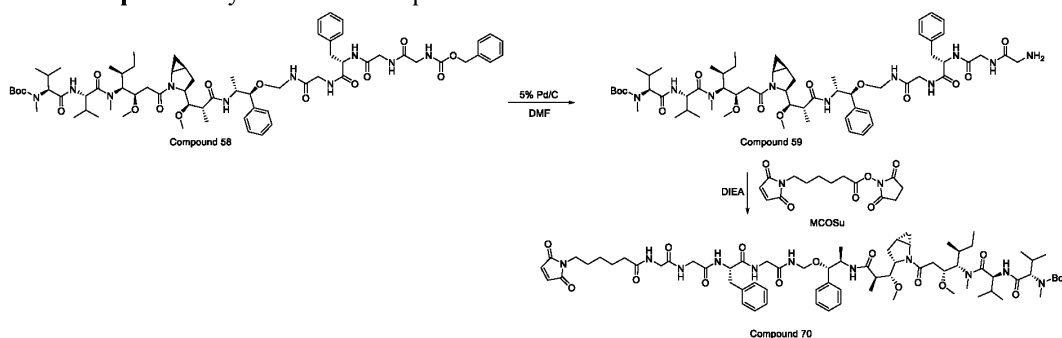
Compound LP-15 was synthesized from compound 45 with reference to the synthetic method of Example 49. LC-MSm/z (ES^+): $[M+H]^+$:1403.8.

Example 59. Synthesis of Compound LP-16



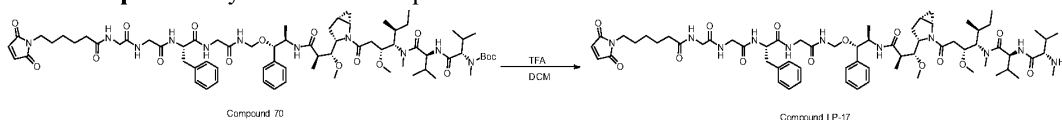
Compound LP-16 was synthesized from compound 51 with reference to the synthetic method of Example 49. LC-MSm/z (ES^+): $[M+H]^+$:1314.8.

Example 60. Synthesis of Compound 70

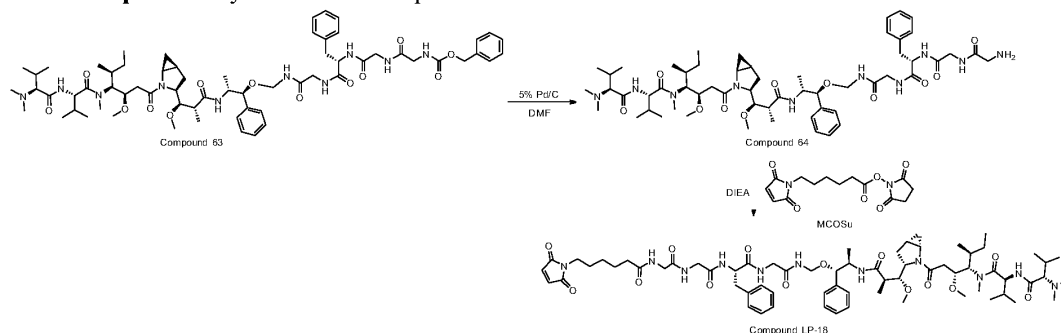


10 Compound 70 was synthesized from compound 58 with reference to the synthetic method of Example 49. LC-MSm/z (ES^+): $[M+H]^+$:1370.8.

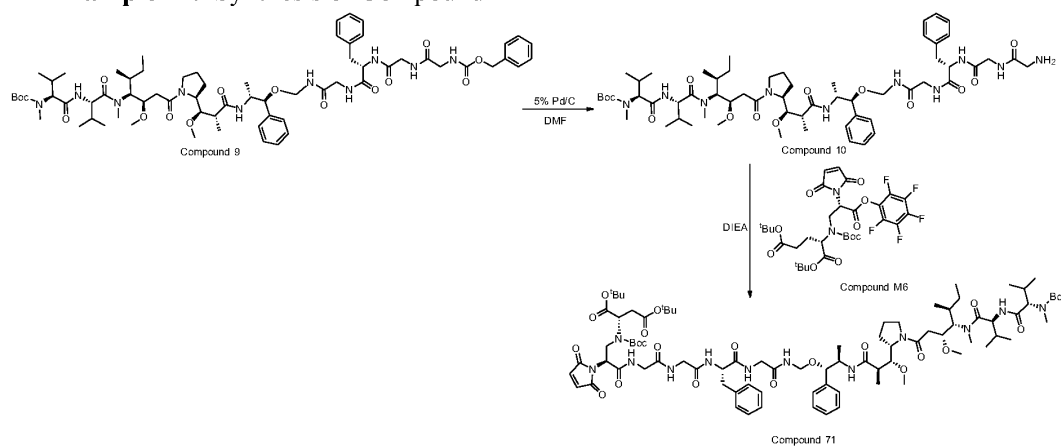
Example 61. Synthesis of Compound LP-17



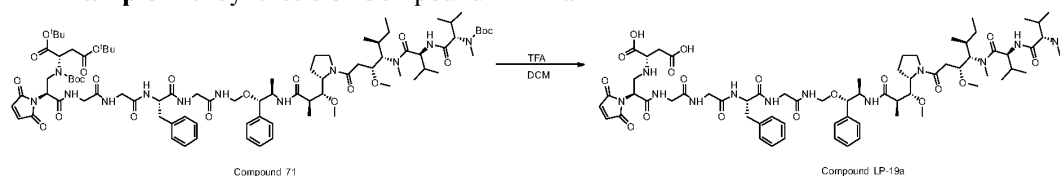
Compound LP-17 was synthesized from compound 70 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[M+H]^+$:1270.8.

Example 62. Synthesis of Compound LP-18

Compound LP-18 was synthesized from compound 63 with reference to the synthetic method of Example 49. LC-MSm/z (ES^+): $[M+H]^+$: 1284.8.

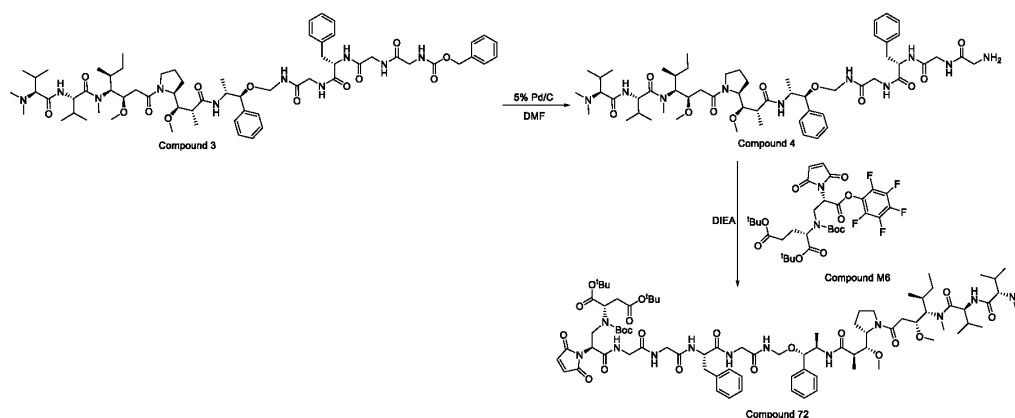
Example 63. Synthesis of Compound 71

In a 50 mL flask, compound 9 (960mg, 0.74mmol, 1.0eq), 5% Pd/C (960mg), and DMF (10mL) were added. After 3 times of H_2 replacement, the reaction was kept at room temperature for 1h. The reaction was monitored by HPLC. The reaction was completed and recorded as reaction solution ①. The reaction solution ① was filtered into a 50mL flask, and compound M6 (512.5mg, 0.74mmol, 1.0eq, compound M6 refer to the synthesis of compound M6 in CN113827736) and DIEA (105mg, 0.74mmol, 1.0eq) were added sequentially in the ice water bath, and the reaction was brought to room temperature for 1 h. The reaction was monitored by HPLC. The reaction solution was purified by reversed-phase preparation and the preparative solution was lyophilized to obtain a white solid (860 mg, 70%). LC-MSm/z (ES^+): $[M+H]^+$: 1660.1.

Example 64. Synthesis of Compound LP-19a

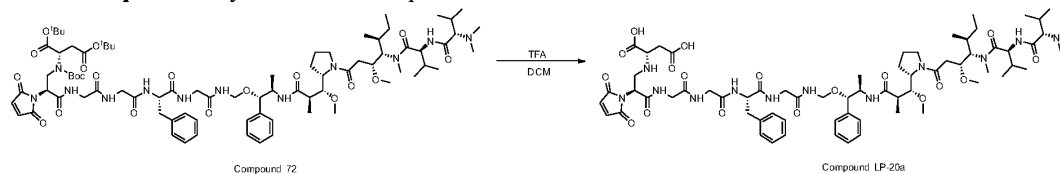
Compound LP-19a was synthesized from compound 71 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[M/2+H]^+$: 674.4.

Example 65. Synthesis of Compound 72



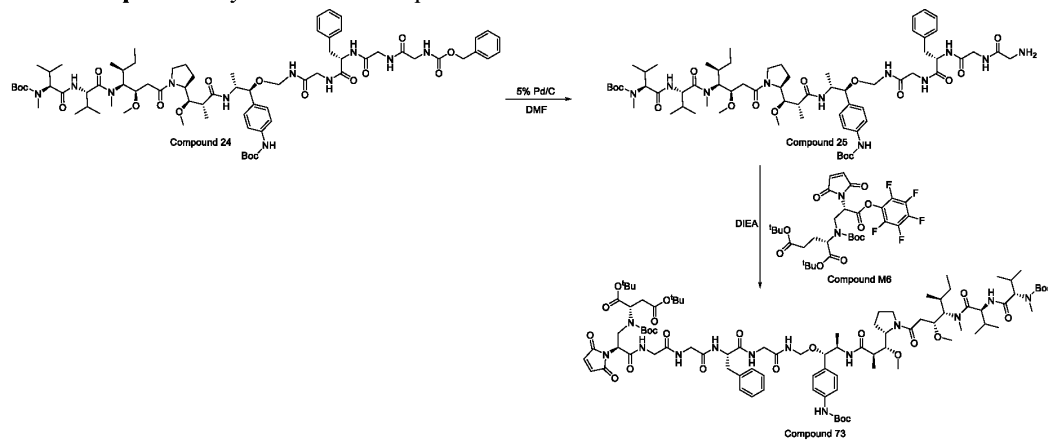
Compound 72 was synthesized from compound 3 with reference to the synthetic method of Example 63. LC-MSm/z (ES^+): $[\text{M}/2+\text{H}]^+$: 783.5.

Example 66. Synthesis of compounds LP- 20a



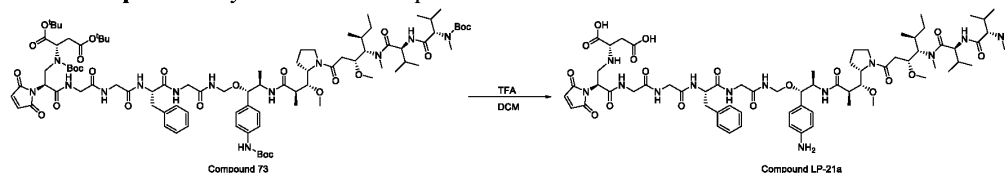
Compound LP-20a was synthesized from compound 72 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[\text{M}/2+\text{H}]^+$: 681.4.

Example 67. Synthesis of Compound 73

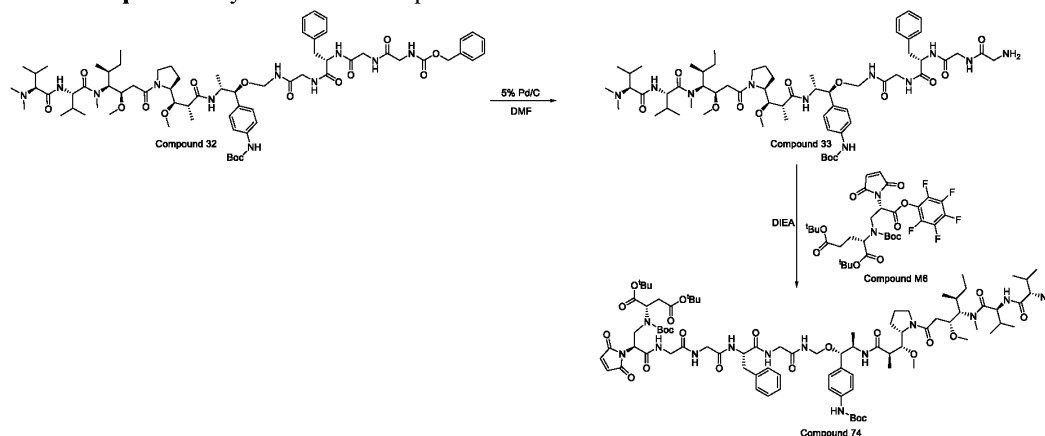


Compound 73 was synthesized from compound 24 with reference to the synthetic method of Example 63. LC-MSm/z (ES^+): $[\text{M}/2+\text{H}]^+$: 888.5.

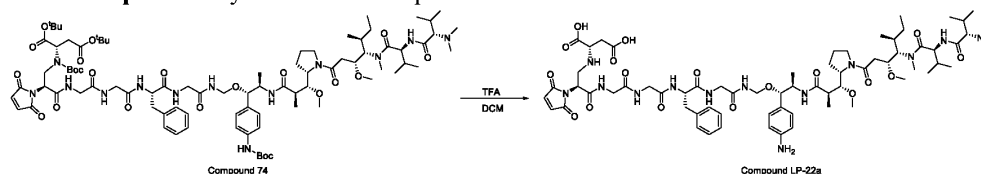
Example 68. Synthesis of Compound LP-21a



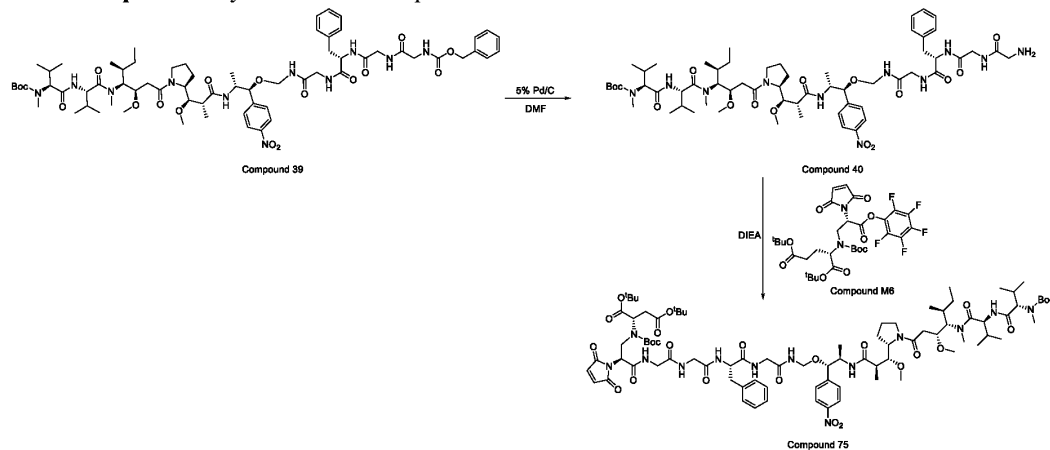
Compound LP-21a was synthesized using compound 73 as the raw material with reference to the synthesis method of Example 9. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1362.7.

Example 69. Synthesis of Compound 74

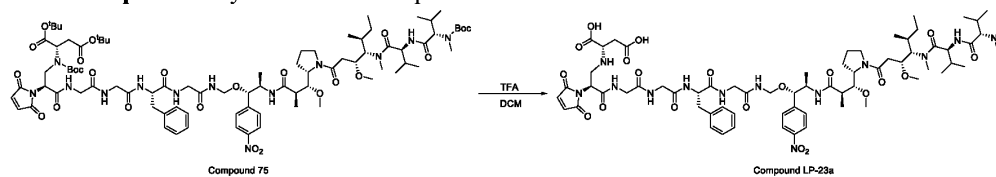
Compound 74 was synthesized from compound 32 with reference to the synthetic method of Example 63. LC-MSm/z (ES^+): $[M/2+H]^+$: 845.1.

Example 70. Synthesis of Compound LP-22a

Compound LP-22a was synthesized from compound 74 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[M+H]^+$: 1376.8.

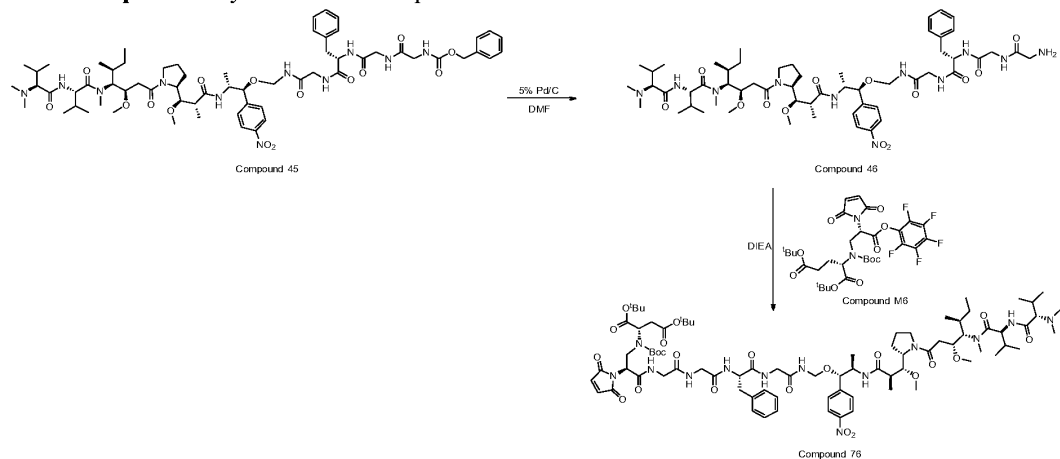
Example 71. Synthesis of Compound 75

Compound 75 was synthesized from compound 39 with reference to the synthetic method of Example 63. LC-MSm/z (ES^+): $[M/2+H]^+$: 853.1.

Example 72. Synthesis of Compound LP-23a

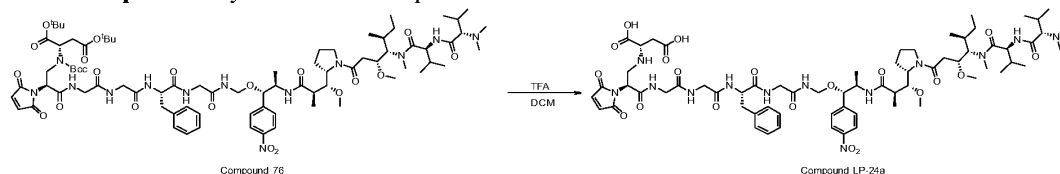
Compound LP-23a was synthesized from compound 75 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[M+H]^+$: 1392.7.

Example 73. Synthesis of Compound 76



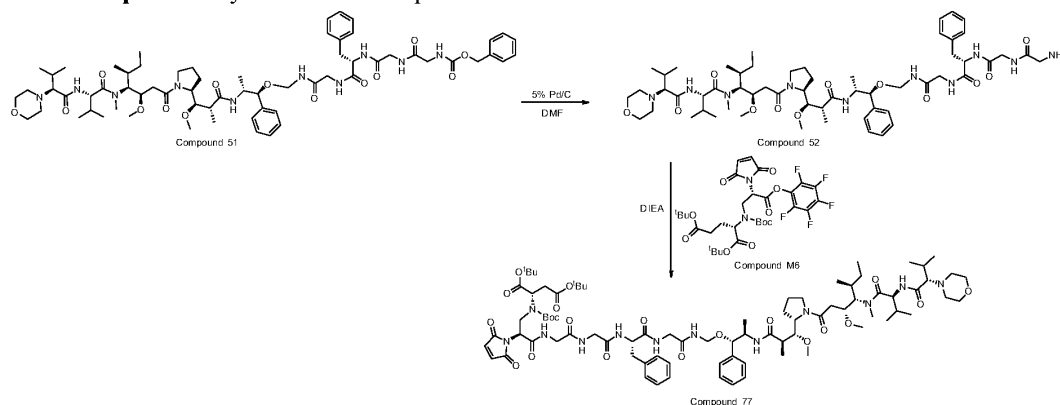
Compound 76 was synthesized from compound 45 with reference to the synthetic method of Example 63. LC-MSm/z (ES^+): $[M/2+H]^+$: 810.1.

Example 74. Synthesis of Compound LP-24a



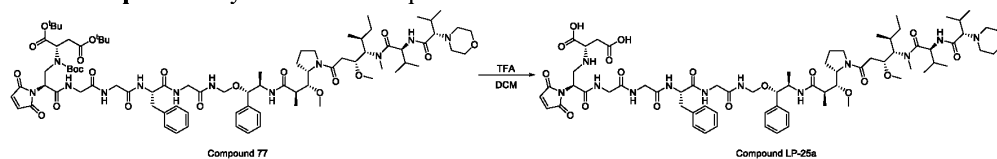
Compound LP-24a was synthesized from compound 76 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[M+H]^+$: 1406.8.

Example 75. Synthesis of Compound 77



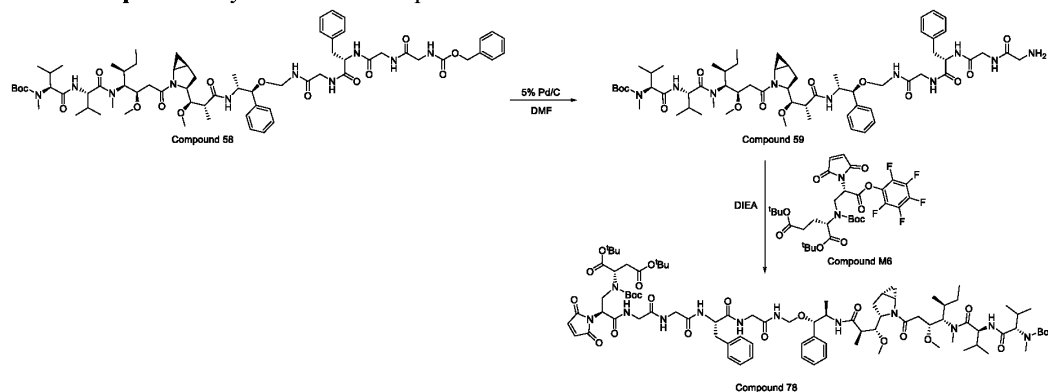
Compound 77 was synthesized from compound 51 with reference to the synthetic method of Example 63. LC-MSm/z (ES^+): $[M/2+H]^+$: 808.5.

Example 76. Synthesis of compound LP-25a



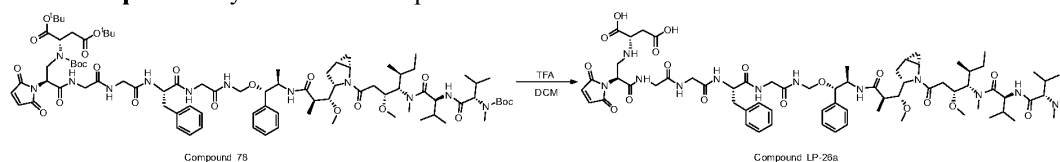
Compound LP-25a was synthesized from compound 77 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[M+H]^+$: 1403.8.

Example 77. Synthesis of Compound 78



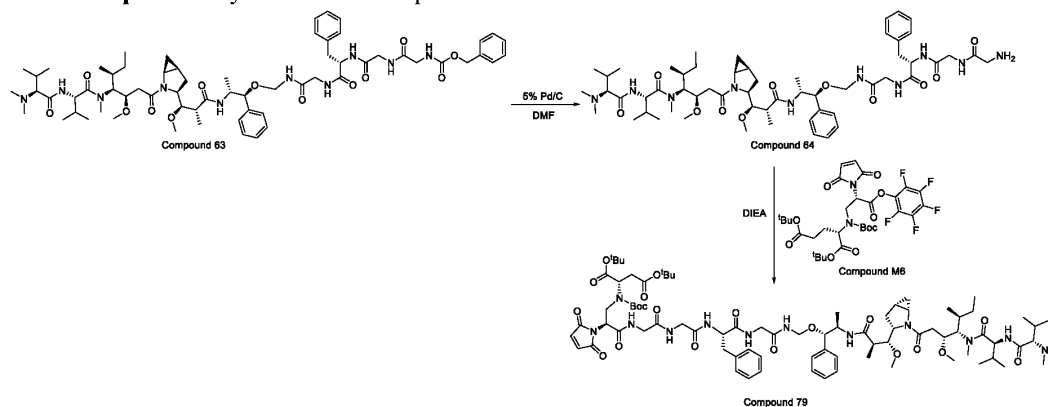
Compound 78 was synthesized from compound 58 with reference to the synthetic method of Example 63. LC-MSm/z (ES^+): $[M/2+H]^+$: 836.5.

Example 78. Synthesis of Compound LP-26a



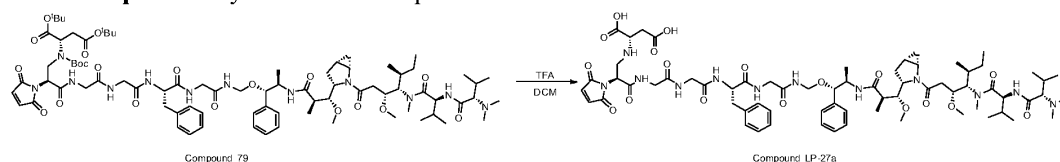
Compound LP-26a was synthesized from compound 78 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[M+H]^+$: 1359.7.

Example 79. Synthesis of Compound 79

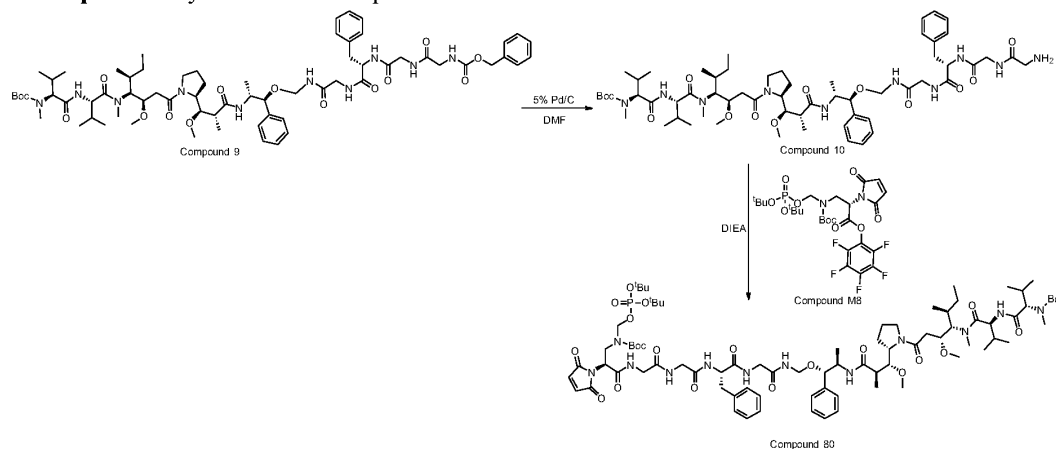


Compound 79 was synthesized from compound 63 with reference to the synthetic method of Example 63. LC-MSm/z (ES^+): $[M/2+H]^+$: 793.5.

Example 80. Synthesis of Compound LP-27a

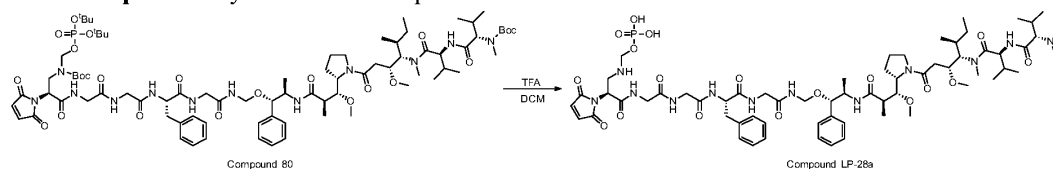


Compound LP-27a was synthesized from compound 79 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[M+H]^+$: 1373.8.

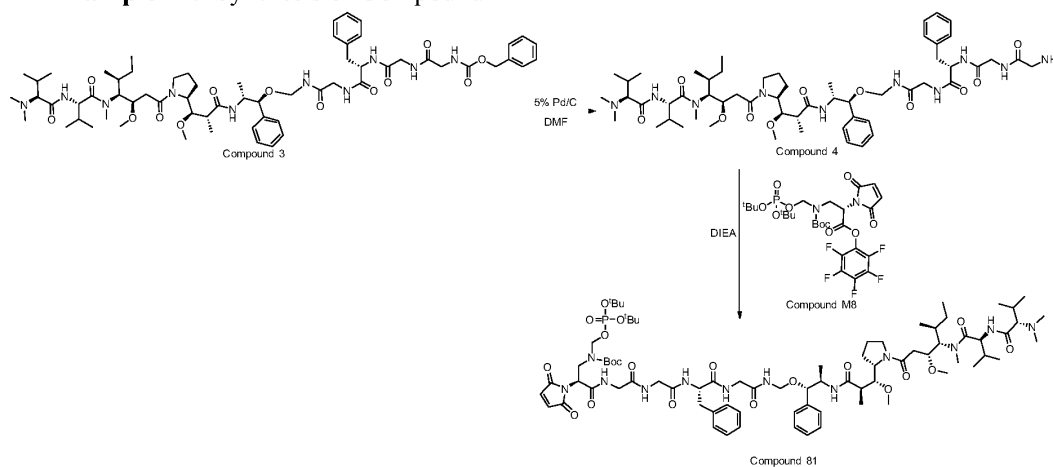
Example 81. Synthesis of Compound 80

Compound 9 (960mg, 0.74mmol, 1.0eq), 5% Pd/C (960mg) and DMF (10mL) were added in a 50 mL flask, H₂ was displaced 3 times, and the reaction was kept at room temperature for 1h. The reaction was monitored by HPLC. The reaction was completed and recorded as reaction solution ①. The reaction solution ① was filtered into a 50mL flask, and then compound M8 (497.7mg, 0.74mmol, 1.0eq, compound M8 refer to the synthesis of compound M8 in CN113827736) and DIEA (105mg, 0.81mmol, 1.0eq) were added sequentially under ice-water bath, and after the addition, the reaction was brought to room temperature for 1 h.

10 The reaction was monitored by HPLC. The reaction solution was purified by reversed-phase preparation and the preparative solution was lyophilized to obtain a white solid (918 mg, 75%). LC-MSm/z (ES⁺): [M/2+H]⁺: 827.5.

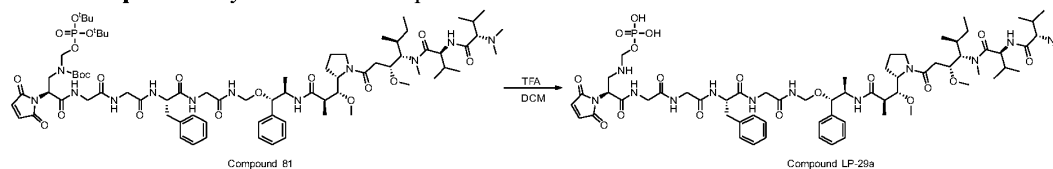
Example 82. Synthesis of Compound LP-28a

Compound LP-28a was synthesized from compound 80 with reference to the synthetic method of Example 9. LC-MSm/z (ES⁺):[M/2+H]⁺: 671.4.

Example 83. Synthesis of Compound 81

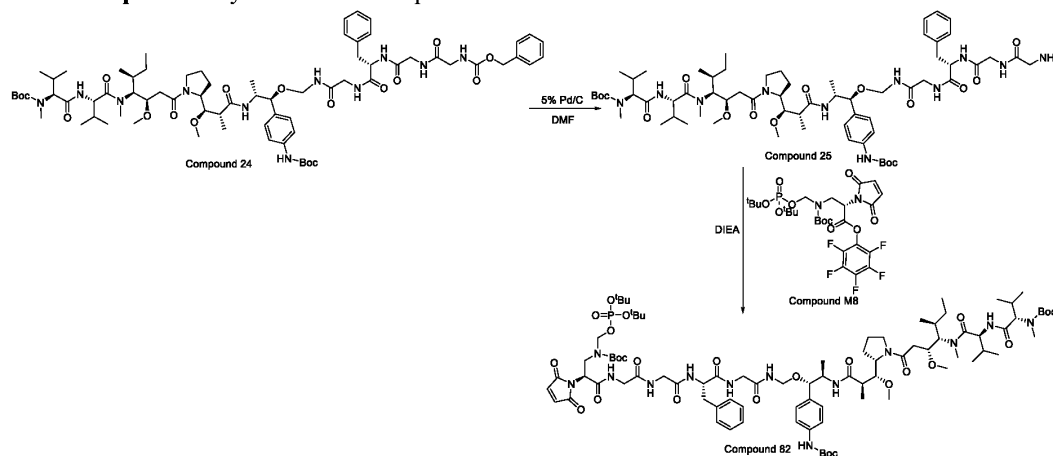
Compound 81 was synthesized from Compound 3 with reference to the synthesis of Example 81. LC-MSm/z (ES^+): $[M/2+H]^+$: 784.5.

Example 84. Synthesis of Compound LP-29a



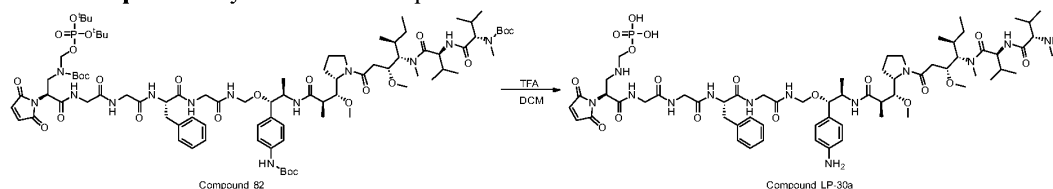
Compound LP-29a was synthesized from compound 81 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[M/2+H]^+$: 678.4.

Example 85. Synthesis of Compound 82



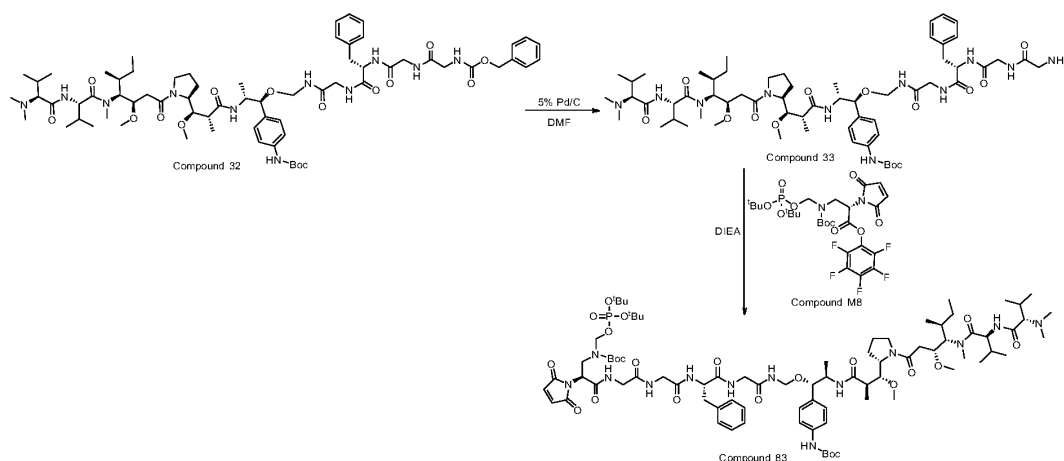
Compound 82 was synthesized from compound 24 with reference to the synthetic method of Example 81. LC-MSm/z (ES^+): $[M/2+H]^+$: 885.0.

Example 86. Synthesis of Compound LP-30a



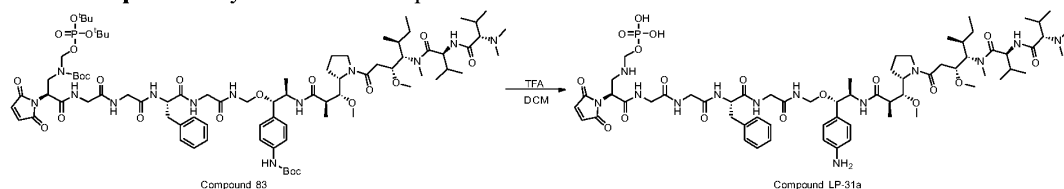
Compound LP-30a was synthesized from compound 82 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[M/2+H]^+$: 678.9.

Example 87. Synthesis of Compound 83



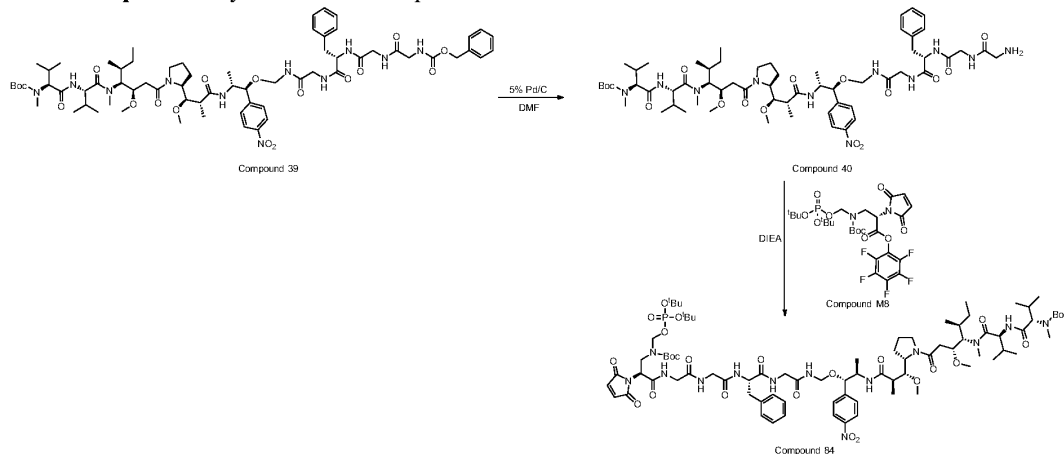
Compound 83 was synthesized from compound 32 with reference to the synthetic method of Example 81. LC-MSm/z (ES^+): $[M/2+H]^+$: 842.0.

Example 88. Synthesis of Compound LP-31a



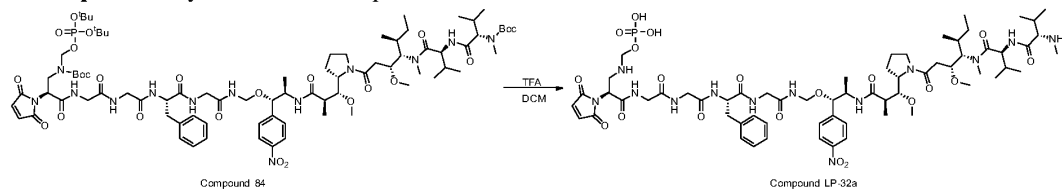
Compound LP-31a was synthesized from compound 83 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[M/2+H]^+$: 685.9.

Example 89. Synthesis of Compound 84



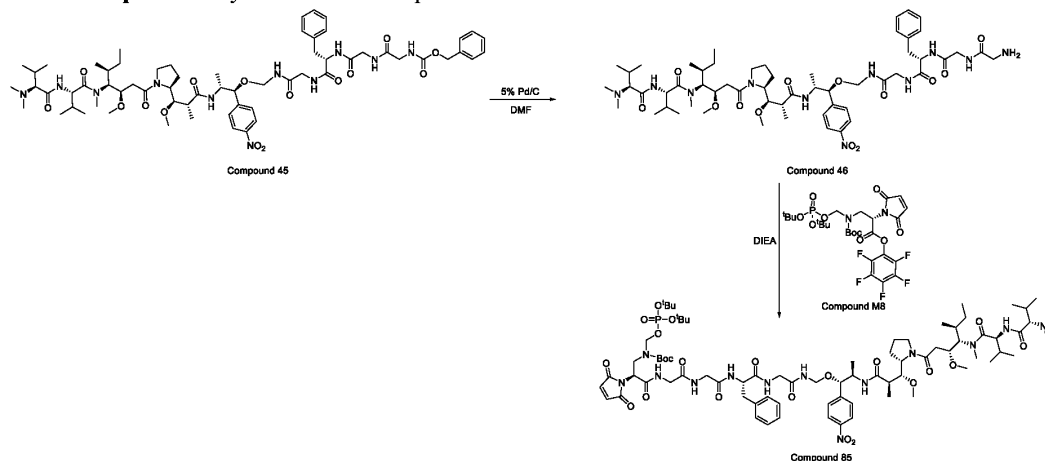
10 Compound 84 was synthesized from compound 39 with reference to the synthetic method of Example 81. LC-MSm/z (ES^+): $[M/2+H]^+$: 850.1.

Example 90. Synthesis of Compound LP-32a



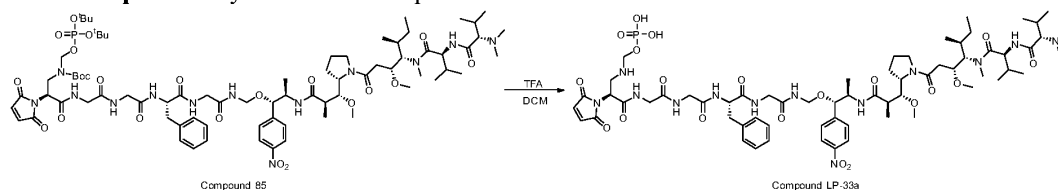
Compound LP-32a was synthesized from compound 84 with reference to the synthetic method of Example 9. LC-MSm/z (ES⁺): [M/2+H]⁺: 693.9.

Example 91. Synthesis of Compound 85



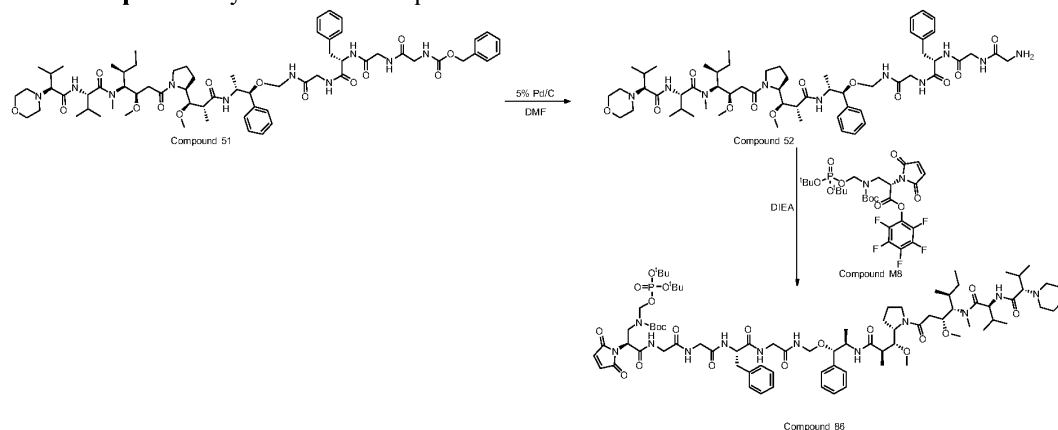
Compound 85 was synthesized from compound 45 with reference to the synthetic method of Example 81. LC-MSm/z (ES⁺): [M/2+H]⁺: 806.9.

Example 92. Synthesis of Compound LP-33a



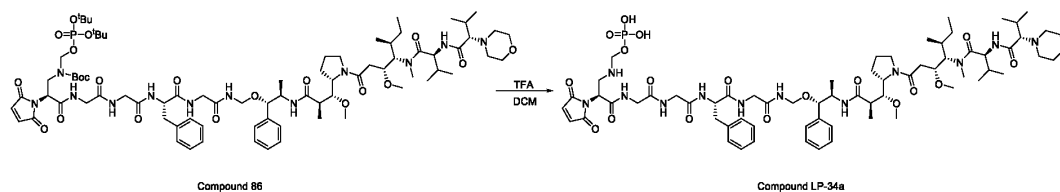
Compound LP-33a was synthesized from compound 85 with reference to the synthetic method of Example 9. LC-MSm/z (ES⁺): [M/2+H]⁺: 700.9.

Example 93. Synthesis of Compound 86



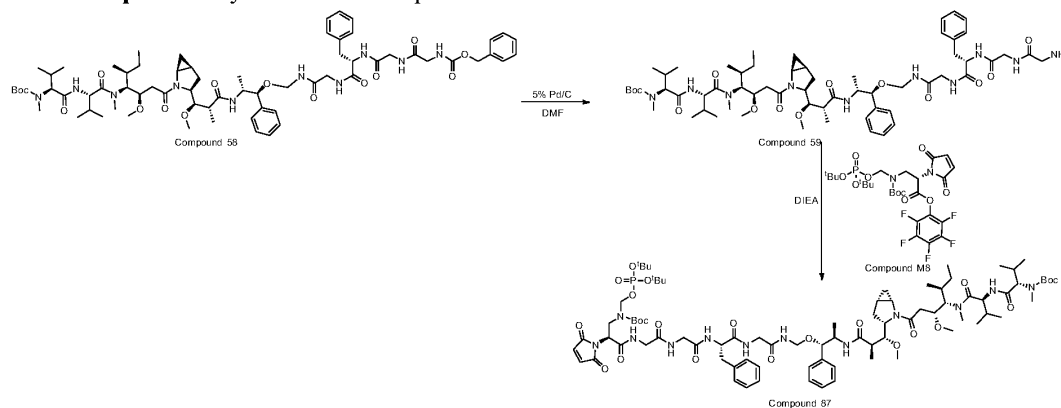
Compound 86 was synthesized from compound 51 with reference to the synthetic method of Example 81. LC-MSm/z (ES⁺): [M/2+H]⁺: 805.5.

Example 94. Synthesis of Compound LP-34a



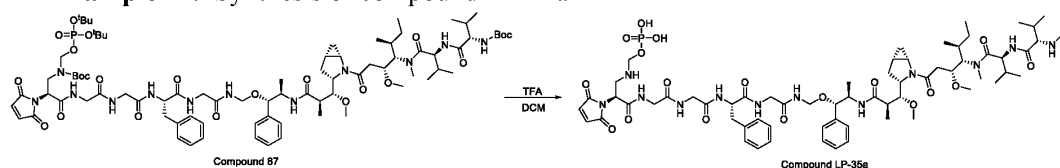
Compound LP-34a was synthesized from compound 86 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[M/2+H]^+$: 699.4.

Example 95. Synthesis of Compound 87



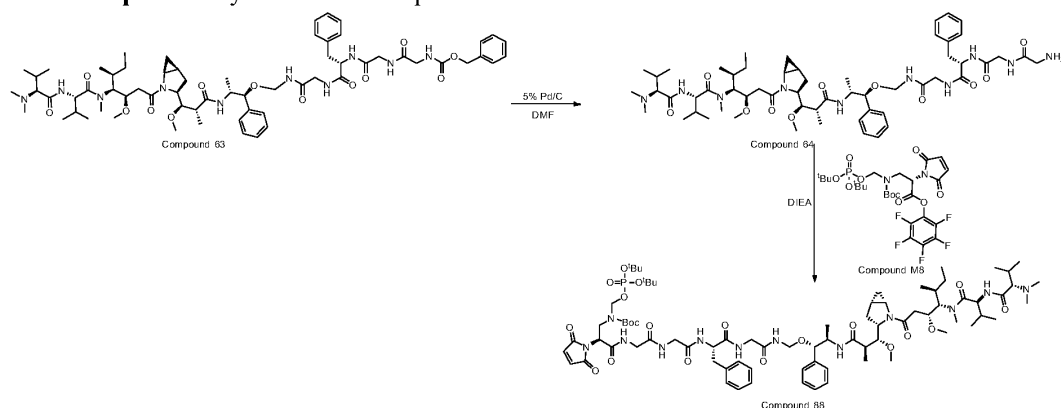
Compound 87 was synthesized from compound 58 with reference to the synthetic method of Example 81. LC-MSm/z (ES^+): $[M/2+H]^+$: 833.5.

Example 96. Synthesis of compound LP-35a



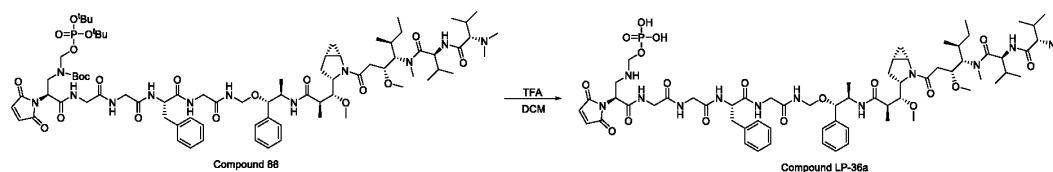
Compound LP-35a was synthesized from compound 87 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[M/2+H]^+$: 677.4.

Example 97. Synthesis of Compound 88



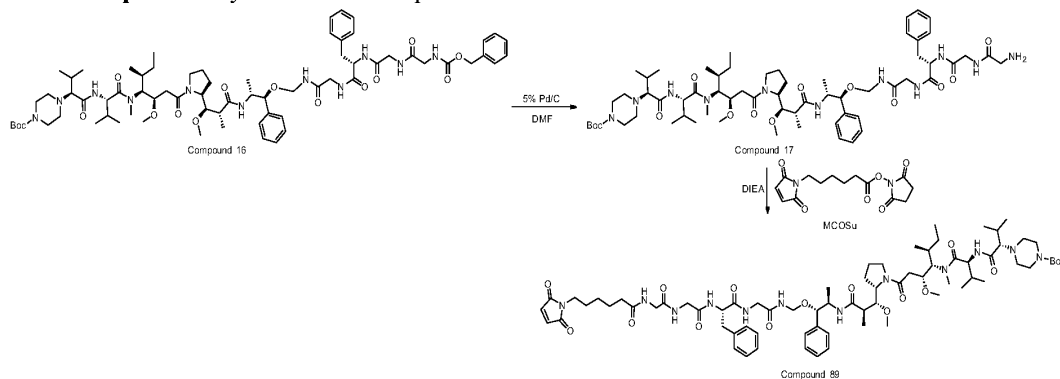
Compound 88 was synthesized from compound 63 with reference to the synthetic method of Example 81. LC-MSm/z (ES^+): $[M/2+H]^+$: 790.5.

Example 98. Synthesis of Compound LP-36a



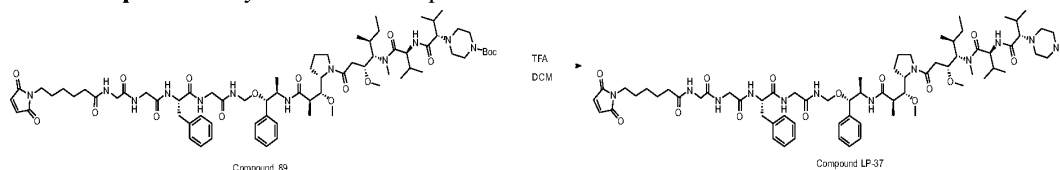
Compound LP-36a was synthesized from compound 88 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[M/2+H]^+$: 670.4.

Example 99. Synthesis of Compound 89



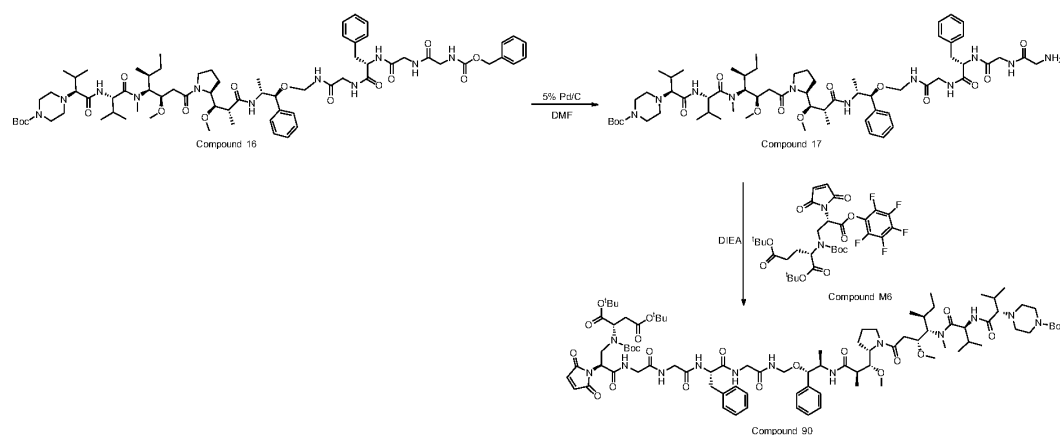
Referring to the synthesis of Example 49, Compound 89 was synthesized from Compound 16. LC-MSm/z (ES^+): $[M/2+H]^+$: 707.4.

Example 100. Synthesis of Compound LP-37



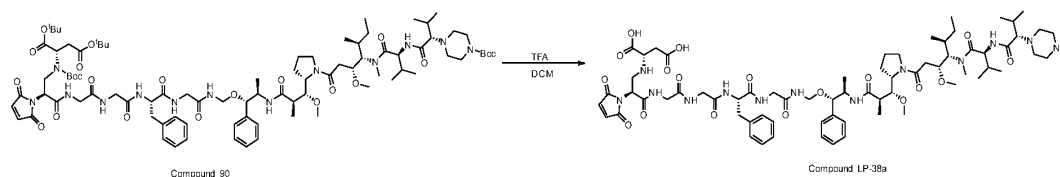
Compound LP-37 was synthesized from compound 89 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[M+H]^+$: 1313.8.

Example 101. Synthesis of Compound 90



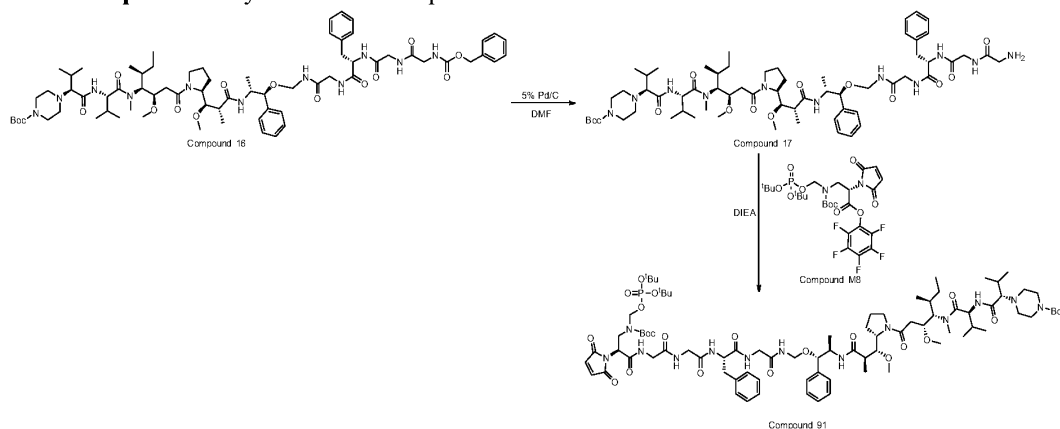
Compound 90 was synthesized from compound 16 with reference to the synthetic method of Example 63. LC-MSm/z (ES^+): $[M/2+H]^+$: 858.0.

Example 102. Synthesis of Compound LP-38a



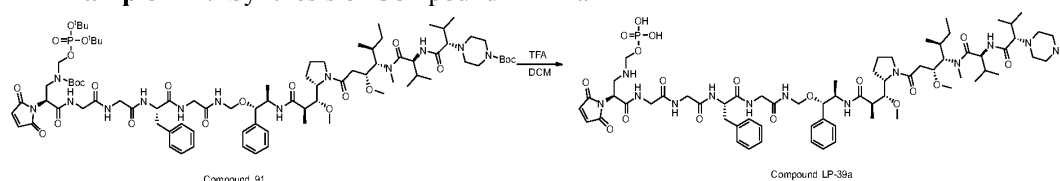
Compound LP-38a was synthesized from compound 90 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[M/2+H]^+$: 701.9.

Example 103. Synthesis of Compound 91



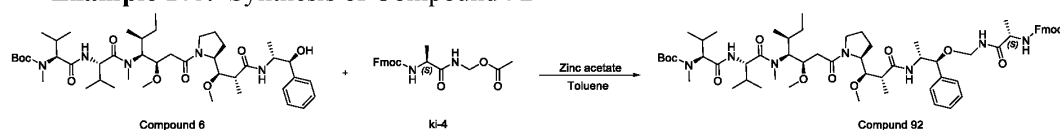
Compound 91 was synthesized from compound 16 with reference to the synthesis of Example 81. LC-MSm/z (ES^+): $[M/2+H]^+$: 855.0.

Example 104. Synthesis of Compound LP-39a



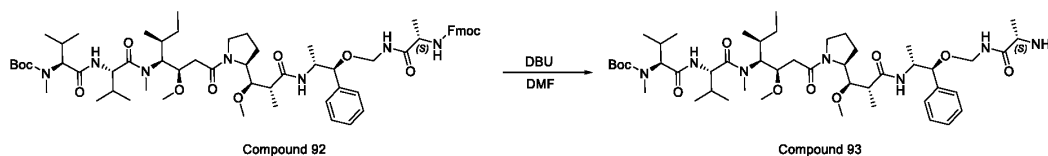
Compound LP-39a was synthesized from compound 91 with reference to the synthetic method of Example 9. LC-MSm/z (ES^+): $[M/2+H]^+$: 698.9.

Example 105. Synthesis of Compound 92



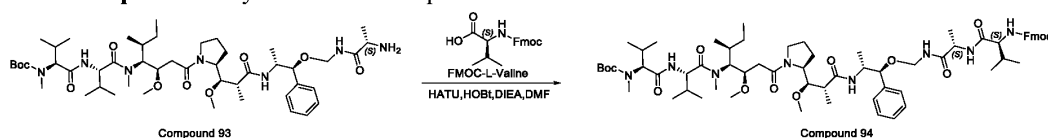
Compound 6 (2.28 g, 2.79 mmol, 1.0 eq), ki-4 (2.13 g, 5.57 mmol, 2.0 eq, the synthesis method refers to the synthesis of compound 4b in WO2020146541), zinc acetate (1.02 g, 5.57 mmol, 2.0 eq), and toluene (30 mL) were added in sequence. After N₂ replacement 3 times, the temperature was raised to 115 °C under N₂ protection for 4 hours, the reaction was stopped, cooled to room temperature, and filtered. The filtrate was concentrated under reduced pressure, purified by reverse phase preparative column, and the preparative solution was lyophilized to obtain a white solid product (1.2 g, 37.8%). LC-MSm/z (ES^+): $[M+H]^+$: 1140.5.

Example 106. Synthesis of Compound 93



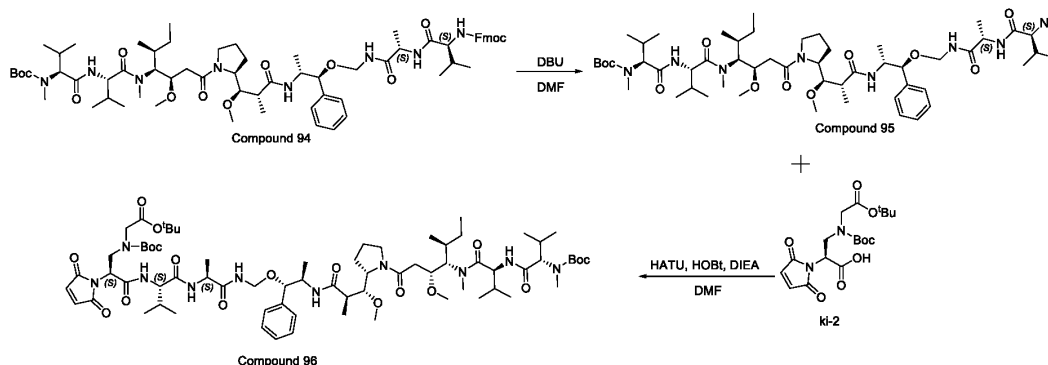
Compound 92 (1.0 g, 0.877 mmol, 1.0 eq) and DMF (10 mL) were added to a 50 mL single-necked round-bottom flask in sequence. After stirring at room temperature and dissolving, DBU (144.1 μ L, 0.985 mmol, 1.1 eq) was added and reacted at room temperature for 0.5 h. The starting material disappeared under TLC monitoring, and compound 93 was produced. The reaction solution was directly purified by reverse phase preparation, and the preparation solution was lyophilized to obtain a white solid product (661.0 mg, 82.1%). LC-MS m/z (ES⁺): [M+H]⁺: 918.6.

Example 107. Synthesis of Compound 94



Compound 93 (0.53 g, 0.581 mmol, 1.0 eq) and Fmoc-L-valine (197.2 mg, 0.581 mmol, 1.0 eq) were added to a 25 mL single-necked round-bottom flask, and DMF (5 mL) was added to dissolve. Then, HATU (264 mg, 0.697 mmol, 1.2 eq), HOBt (94 mg, 0.697 mmol, 1.2 eq), and DIEA (288 μ L, 1.743 mmol, 3.0 eq) were added in an ice bath. After the addition, the mixture was heated to room temperature and stirred for 1 h and then monitored by HPLC. After the reaction was completed, the product was purified by reverse phase preparative column to obtain a white solid product (540.2 mg, 75.0%). LC-MS m/z (ES⁺): [M+H]⁺: 1239.8.25 mL.

Example 108. Synthesis of Compound 96

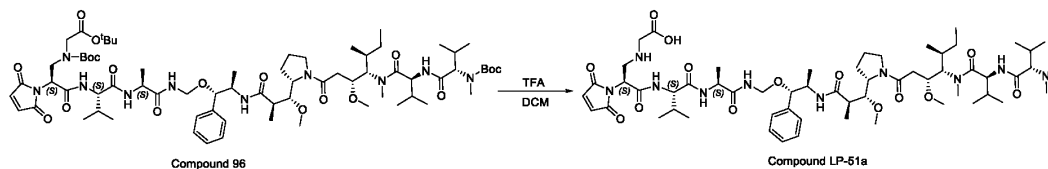


In a 10 mL EP tube, ki-2 (115.9 mg, 0.291 mmol, 1.0 eq), HATU (132 mg, 0.349 mmol, 1.2 eq), HOBt (47 mg, 0.349 mmol, 1.2 eq), and DMF (2 mL) were added in sequence and stirred at room temperature for later use.

In another 10 mL single-necked round-bottom flask, compound 94 (360.1 mg, 0.291 mmol, 1.0 eq) and DMF (2 mL) were added in sequence. After stirring at room temperature and dissolving, DBU (47.8 μ L, 0.32 mmol, 1.1eq) was added and reacted at room temperature for 0.5 h. The reaction was monitored by HPLC. The starting material disappeared and compound 95 was produced. Then the above mixture was added to this bottle, and DIEA (48 μ L, 0.291 mmol, 1.0 eq) was added. The reaction was carried out at room temperature for 1 h and monitored by HPLC. After the reaction was completed, the product was purified by reverse

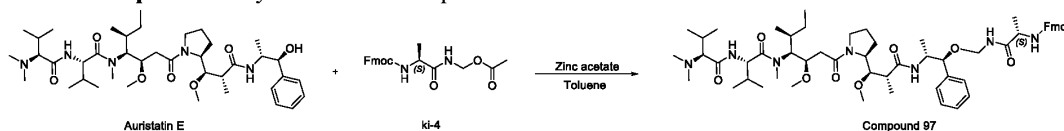
phase preparative column to obtain a white solid product (308.6 mg, 76%). LC-MS m/z (ES⁺): [M+2H]²⁺: 699.4.

Example 109. Synthesis of LP-51a



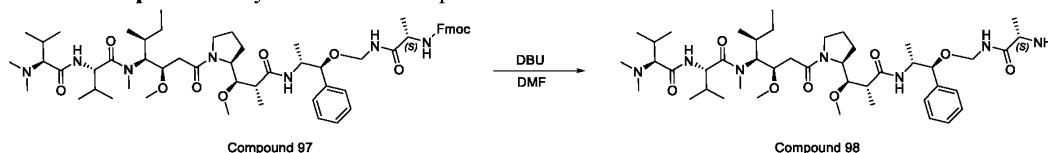
Compound 96 (200 mg, 0.143 mmol, 1.0 eq) was dissolved in 10 mL dry dichloromethane and 4 mL TFA, and reacted at room temperature for 3 h, monitored by HPLC. After the reaction was completed, the solvent was removed by concentration under reduced pressure, and the crude product was purified by reverse phase preparative column, and the preparative solution was lyophilized to obtain a white solid (127.4 mg, 78%), LC-MS m/z (ES⁺): [M+2H]²⁺: 571.3.

Example 110. Synthesis of Compound 97



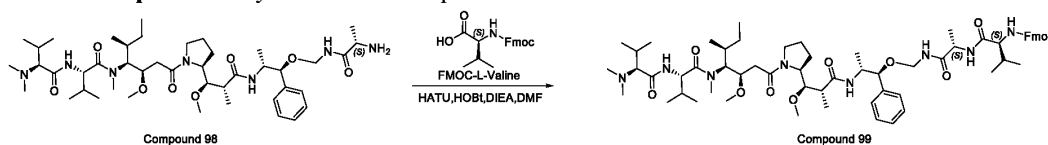
Auristatin E (2.04 g, 2.79 mmol, 1.0eq), ki-4 (2.13 g, 5.57 mmol, 2.0eq, the synthesis method refers to the synthesis of compound 4b in WO2020146541), zinc acetate (1.02 g, 5.57 mmol, 2.0eq), and toluene (30 mL) were added in sequence. After N₂ replacement 3 times, the temperature was raised to 115 °C under N₂ protection for 4h, the reaction was stopped, cooled to room temperature, and filtered. The filtrate was concentrated under reduced pressure, purified by reverse phase preparative column, and the preparative solution was lyophilized to obtain a white solid product (1.3 g, 44.2%). LC-MS m/z (ES⁺): [M+H]⁺: 1053.7.

Example 111. Synthesis of Compound 98



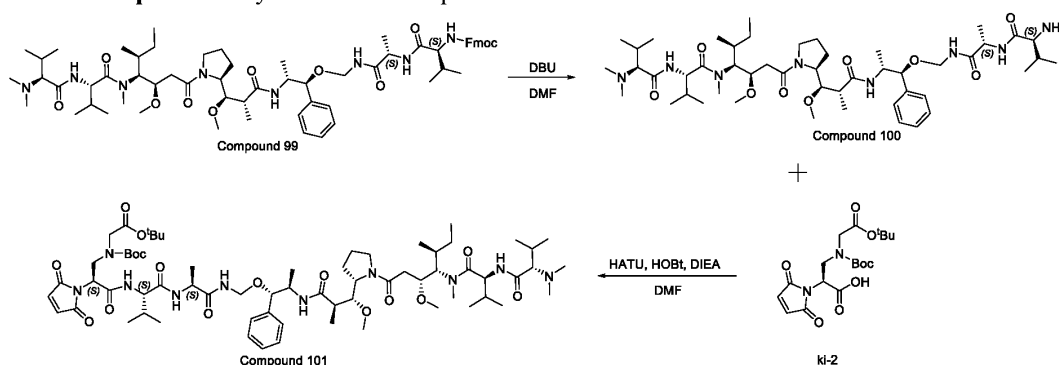
Compound 97 (924.7 mg, 0.877 mmol, 1.0 eq) and DMF (9 mL) were added to a 50 mL single-necked round-bottom flask in sequence. After stirring at room temperature and dissolving, DBU (144.1 μ L, 0.985 mmol, 1.1 eq) was added and reacted at room temperature for 0.5 h. The starting material disappeared under TLC monitoring, and compound 98 was produced. The reaction solution was directly purified by reverse phase preparation, and the preparation solution was lyophilized to obtain a white solid product (622.5 mg, 85.3%). LC-MS m/z (ES⁺): [M+H]⁺: 832.6.

Example 112. Synthesis of Compound 99



Compound 93 (483.5 mg, 0.581 mmol, 1.0 eq) and Fmoc-L-valine (197.2 mg, 0.581 mmol, 1.0 eq) were added to a 25 mL single-necked round-bottom flask, and DMF (5 mL) was added to dissolve. Then, HATU (264 mg, 0.697 mmol, 1.2 eq), HOBt (94 mg, 0.697 mmol, 1.2 eq), and DIEA (288 μ L, 1.743 mmol, 3.0 eq) were added in an ice bath. After the addition, the mixture was heated to room temperature and stirred for 1 h and then monitored by HPLC. After the reaction was completed, the product was purified by reverse phase preparative column to obtain a white solid product (517.4 mg, 77.2%). LC-MS m/z (ES⁺): [M+H]⁺: 1153.7.

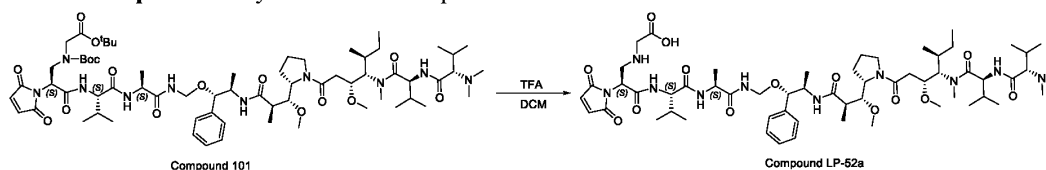
Example 113. Synthesis of Compound 101



10 In a 10 mL EP tube, ki-2 (115.9 mg, 0.291 mmol, 1.0 eq), HATU (132 mg, 0.349 mmol, 1.2 eq), HOBt (47 mg, 0.349 mmol, 1.2 eq), and DMF (2 mL) were added in sequence and stirred at room temperature for later use.

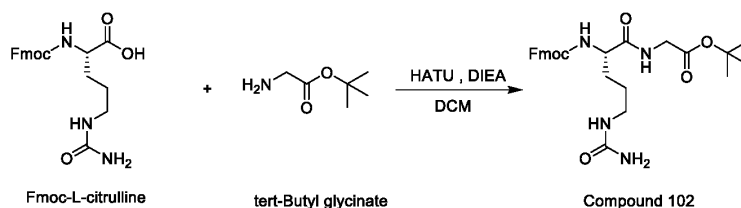
In another 10 mL single-necked round-bottom flask, compound 99 (335.1 mg, 0.291 mmol, 1.0 eq) and DMF (2 mL) were added in sequence. After stirring at room temperature and dissolving, DBU (47.8 μ L, 0.32 mmol, 1.1eq) was added and reacted at room temperature for 0.5 h. The reaction was monitored by HPLC. The starting material disappeared and compound 100 was produced. Then the above mixture was added to this bottle, and DIEA (48 μ L, 0.291 mmol, 1.0 eq) was added. The reaction was carried out at room temperature for 1 h and monitored by HPLC. After the reaction was completed, the product was purified by reverse phase preparative column to obtain a white solid product (299.1 mg, 78.5%). LC-MS m/z (ES⁺): [M+2H]²⁺: 656.4.

Example 114. Synthesis of Compound LP-52a



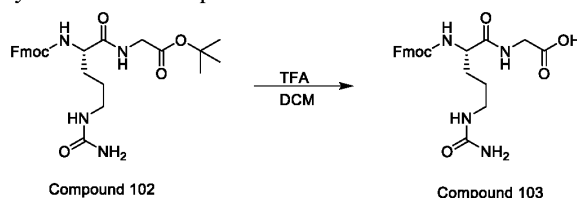
Compound 101 (200 mg, 0.152 mmol, 1.0 eq) was dissolved in 10 mL dry dichloromethane and 4 mL TFA, and reacted at room temperature for 3 h, monitored by HPLC. After the reaction was completed, the solvent was removed by concentration under reduced pressure, and the crude product was purified by reverse phase preparative column, and the preparative solution was lyophilized to obtain a white solid (122.3 mg, 69.4%), LC-MS m/z (ES⁺): [M+2H]²⁺: 578.3.

30 **Example 115.** Synthesis of Compound 102



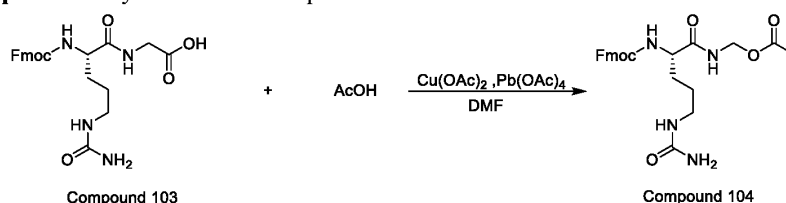
Compound DIEA (4.88 g, 37.74 mmol, 3 eq) and HATU (5.74 g, 15.09 mmol, 1.2 eq) were added to a solution of compound Fmoc-L-citrulline (5 g, 12.58 mmol, 1 eq) and glycine tert-butyl ester (2.48 g, 18.87 mmol, 1.5 eq) in DCM (10 mL), and the reaction solution was stirred at room temperature overnight. LCMS showed that the reaction was complete. Dichloromethane/methanol (10:1, 150 mL) and water (50 mL) were added to the reaction solution, and the mixture was washed twice with dilute hydrochloric acid (0.5 M), 50 mL each time, and twice with saturated brine, 50 mL each time, dried over anhydrous sodium sulfate, filtered, and dried under reduced pressure. The residue was purified by silica gel column chromatography (eluent: methanol/dichloromethane 0: 1 to 1:9) to obtain a white solid product (5.7 g, 88.73%). LC-MS m/z (ES⁺): [M+H]⁺: 511.3.

Example 116. Synthesis of Compound 103



TFA (2 mL) was added to a solution of compound 102 (1.60 g, 3.13 mmol, 1 eq) in DCM (10 mL) at 0 °C, and the reaction was stirred overnight at room temperature. LCMS showed that the reaction was complete. The reaction solution was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography to obtain a white solid product (1.82 g, 63.83%). LC-MS m/z (ES⁺): [M+H]⁺: 455.2.

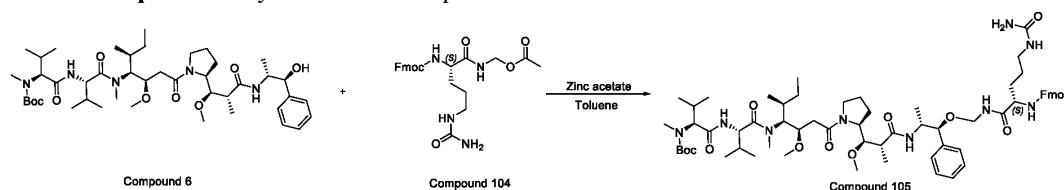
Example 117. Synthesis of Compound 104



To a solution of compound 103 (1.01 g, 2.22 mmol, 1 eq) in DMF (20 mL) were added acetic acid (400.35 mg, 382.02 μ L, 6.66 mmol, 3 eq), copper acetate (121.09 mg, 113.38 μ L, 0.67 mmol, 0.3 eq) and lead acetate (1.97 g, 0.89 mL, 4.45 mmol, 2 eq), and the reaction was stirred at 60°C for 1 hour. LCMS showed that the reaction was complete. The reaction solution was dried under reduced pressure, saturated NaHCO₃ solution was added to the residue to adjust pH = 7, DCM/MeOH (10:1, 1L) was added, the mixed solution was extracted 3 times with DCM/MeOH (10:1), 500mL each time, the organic phases were combined, washed with saturated brine (100mL), the organic phases were dried over anhydrous Na₂SO₄, filtered and dried under reduced pressure. The residue was purified by silica gel column chromatography (eluent: methanol/dichloromethane 0:1 to 10:1) to obtain a white solid product (256.5mg,

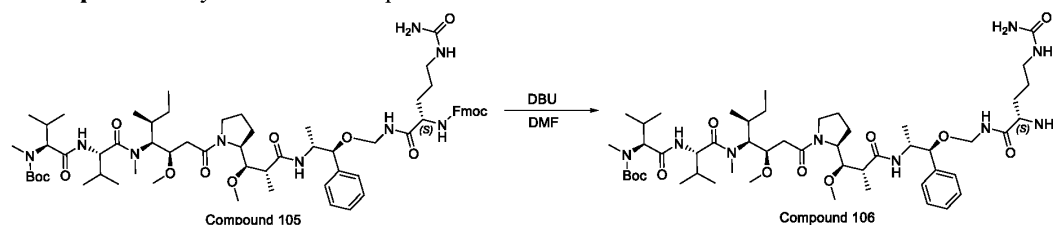
24.63%). LC-MS m/z (ES⁺): [M+Na]⁺: 491.3.

Example 118. Synthesis of Compound 105



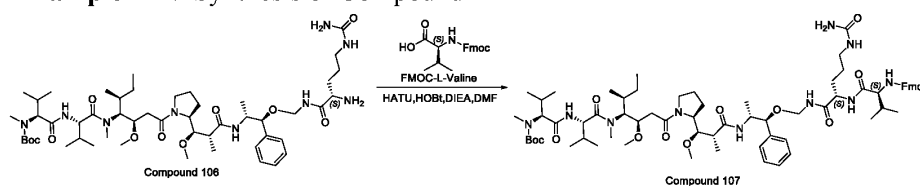
Compound 6 (2.12 g, 2.59 mmol, 1.0eq), compound 104 (2.77 g, 5.18 mmol, 2.0eq), zinc acetate (1.08 g, 5.18 mmol, 2.0eq) were added to a 100 mL single-mouth bottle in sequence, dissolved in toluene (30 mL), replaced with N₂ three times, heated to 115°C under N₂ protection for 4 hours, stopped the reaction, cooled to room temperature, and filtered. The filtrate was concentrated under reduced pressure, purified by reverse phase preparative column, and the preparative solution was lyophilized to obtain a white solid product (1.35 g, 42.4%). LC-MS m/z (ES⁺): [M+H]⁺: 1226.7.

Example 119. Synthesis of Compound 106



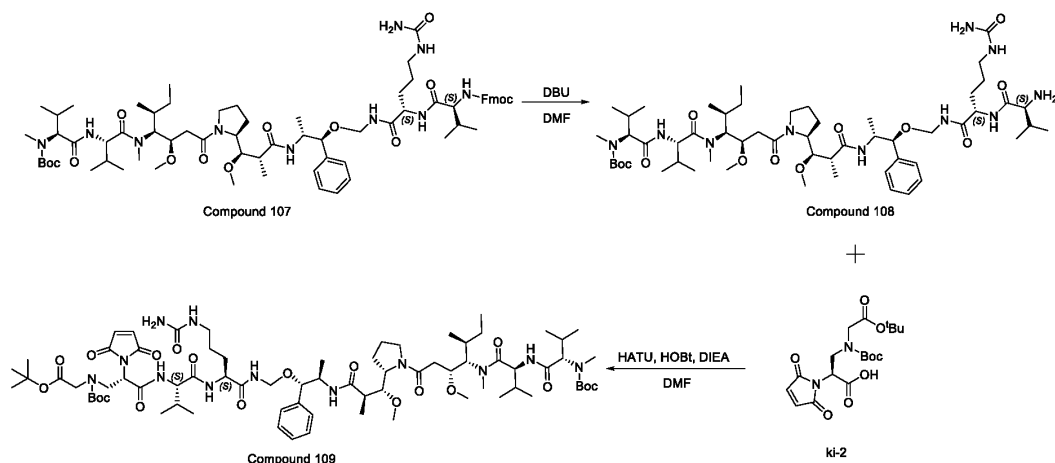
Compound 105 (1.02 g, 0.832 mmol, 1.0 eq) and DMF (9 mL) were added to a 50 mL single-necked round-bottom flask in sequence. After stirring at room temperature and dissolving, DBU (136.7 μ L, 0.915 mmol, 1.1 eq) was added and reacted at room temperature for 0.5 h. The starting material disappeared under TLC monitoring, and compound 98 was produced. The reaction solution was directly purified by reverse phase preparation, and the preparation solution was lyophilized to obtain a white solid product (726.6 mg, 87%). LC-MS m/z (ES⁺): [M+H]⁺: 1004.6.

Example 120. Synthesis of Compound 107



Compound 106 (496.5 mg, 0.494 mmol, 1.0 eq) and Fmoc-L-valine (186.3 mg, 0.494 mmol, 1.0 eq) were added to a 25 mL single-necked round-bottom flask, and DMF (5 mL) was added to dissolve. Then, HATU (225.6 mg, 0.593 mmol, 1.2 eq), HOBt (80 mg, 0.593 mmol, 1.2 eq), and DIEA (191.6 μ L, 1.483 mmol, 3.0 eq) were added in an ice bath. After the addition, the mixture was heated to room temperature and stirred for 1 h and then monitored by HPLC. After the reaction was completed, the product was purified by reverse phase preparative column to obtain a white solid product (496.8 mg, 75.8%). LC-MS m/z (ES⁺): [M+H]⁺: 1325.8.

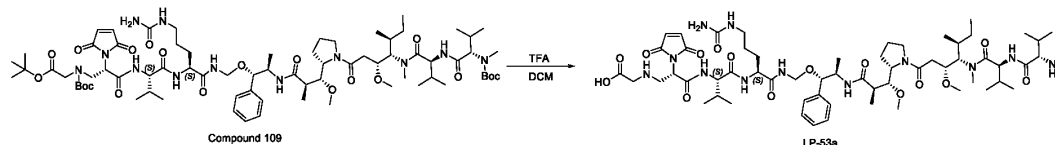
Example 121. Synthesis of Compound 109



In a 10 mL EP tube, ki-2 (115.9 mg, 0.291 mmol, 1.0 eq), HATU (132 mg, 0.349 mmol, 1.2 eq), HOBT (47 mg, 0.349 mmol, 1.2 eq), and DMF (2 mL) were added in sequence and stirred at room temperature for later use.

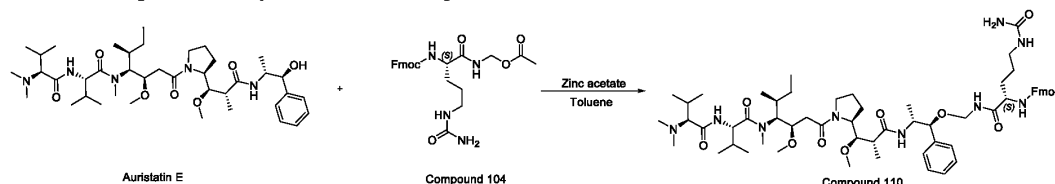
In another 10 mL single-necked round-bottom flask, compound 107 (385.8 mg, 0.291 mmol, 1.0 eq) and DMF (2 mL) were added in sequence. After stirring and dissolving at room temperature, DBU (47.8 μ L, 0.32 mmol, 1.1 eq) was added and reacted at room temperature for 0.5 h. The reaction was monitored by HPLC. The starting material disappeared and compound 108 was produced. Then the above mixture was added to this bottle, and DIEA (48 μ L, 0.291 mmol, 1.0 eq) was added. The reaction was carried out at room temperature for 1 h and monitored by HPLC. After the reaction was completed, the product was purified by reverse phase preparative column to obtain a white solid product (331.6 mg, 76.8%). LC-MS m/z (ES⁺): [M+2H]²⁺: 742.4.

Example 122. Synthesis of Compound LP-53a



Compound 109 (200 mg, 0.135 mmol, 1.0 eq) was dissolved in 10 mL dry dichloromethane and 4 mL TFA, and reacted at room temperature for 3 h, monitored by HPLC. After the reaction was completed, the solvent was removed by concentration under reduced pressure. The crude product was purified by reverse phase preparative column, and the preparative solution was lyophilized to obtain a white solid (107.9 mg, 65.2%), LC-MS m/z (ES⁺): [M+2H]²⁺: 614.3.

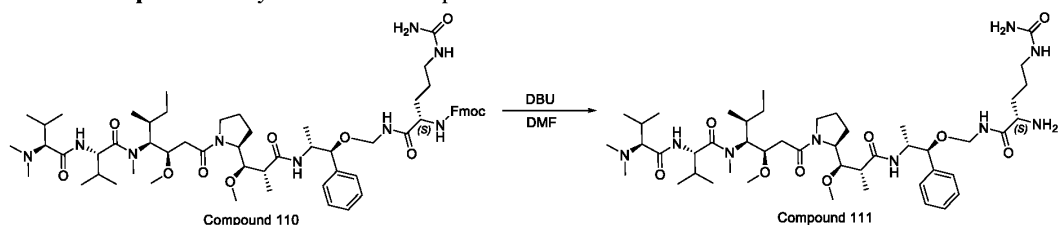
Example 123. Synthesis of Compound 110



Auristatin E (2.3 g, 3.14 mmol, 1.0 eq), compound 104 (2.94 g, 6.28 mmol, 2.0 eq), zinc acetate (1.15 g, 6.28 mmol, 2.0 eq) and toluene (30 mL) were added to a 100 mL single-necked

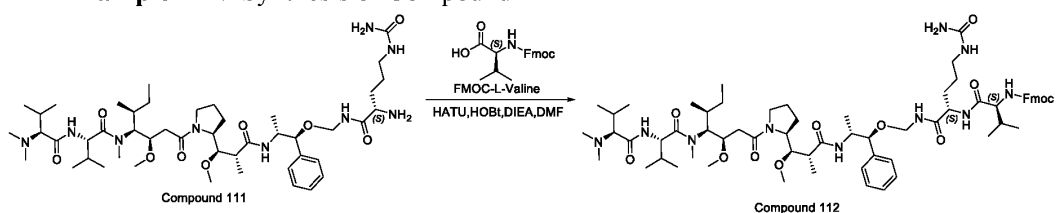
bottle in sequence. After N₂ replacement for 3 times, the temperature was raised to 115°C under N₂ protection for 4 h, the reaction was stopped, cooled to room temperature, and filtered,. The filtrate was concentrated under reduced pressure, purified by reverse phase preparative column, and the preparative solution was lyophilized to obtain a white solid product (1.5 g, 43.2%). LC-MSm/z (ES⁺): [M+H]⁺: 1140.7.

Example 124. Synthesis of Compound 111



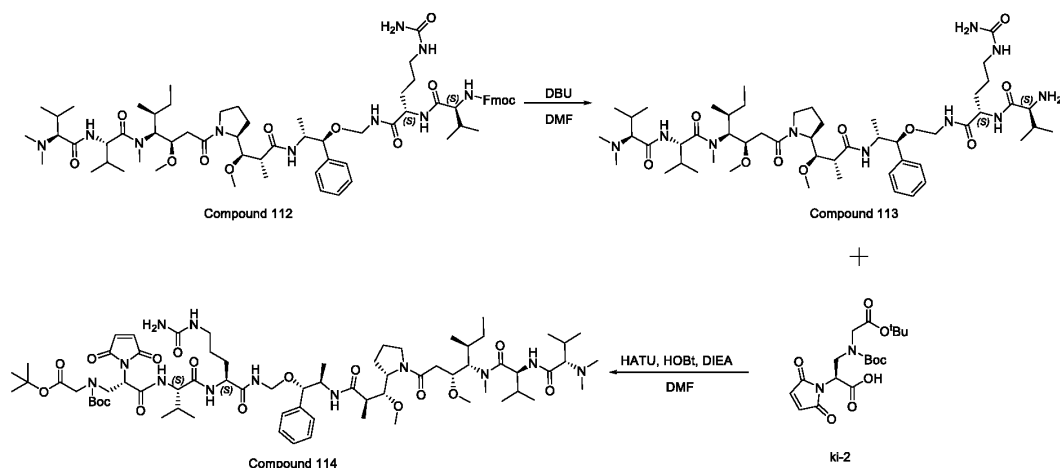
Compound 110 (918.7 mg, 0.805 mmol, 1.0 eq) and DMF (9 mL) were added to a 50 mL single-necked round-bottom flask in sequence. After stirring at room temperature and dissolving, DBU (132.4 μ L, 0.886 mmol, 1.1 eq) was added and reacted at room temperature for 0.5 h. The starting material disappeared under TLC monitoring to produce compound 98. The reaction solution was directly purified by reverse phase preparation, and the preparation solution was lyophilized to obtain a white solid product (623.5 mg, 84.3%). LC-MS m/z (ES⁺): [M+H]⁺: 918.6.

Example 125. Synthesis of Compound 112



Compound 111 (481.3 mg, 0.524 mmol, 1.0 eq) and Fmoc-L-valine (177.9 mg, 0.524 mmol, 1.0 eq) were added to a 25 mL single-necked round-bottom flask, and DMF (5 mL) was added to dissolve. Then, HATU (239 mg, 0.629 mmol, 1.2 eq), HOBt (85 mg, 0.629 mmol, 1.2 eq), and DIEA (203 μ L, 1.572 mmol, 3.0 eq) were added in an ice bath. After the addition, the mixture was heated to room temperature and stirred for 1 h and then monitored by HPLC. After the reaction was completed, the product was purified by reverse phase preparative column to obtain a white solid product (495.7 mg, 76.3%). LC-MS m/z (ES⁺): [M+H]⁺: 1239.

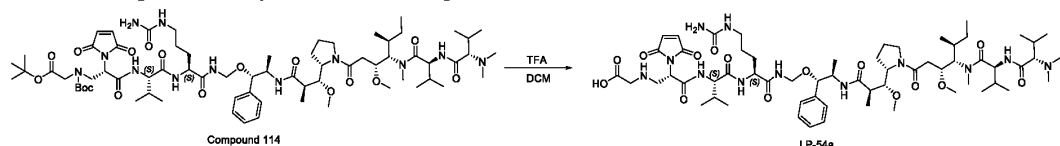
Example 126. Synthesis of Compound 114



In a 10 mL EP tube, ki-2 (115.9 mg, 0.291 mmol, 1.0 eq), HATU (132 mg, 0.349 mmol, 1.2 eq), HOBT (47 mg, 0.349 mmol, 1.2 eq), and DMF (2 mL) were added in sequence and stirred at room temperature for later use.

In another 10 mL single-necked round-bottom flask, compound 112 (360.7 mg, 0.291 mmol, 1.0 eq) and DMF (2 mL) were added in sequence. After stirring at room temperature and dissolving, DBU (47.8 μ L, 0.32 mmol, 1.1eq) was added and reacted at room temperature for 0.5 h. The reaction was monitored by HPLC. The starting material disappeared and compound 113 was produced. Then the above mixture was added to this bottle, and DIEA (48 μ L, 0.291 mmol, 1.0 eq) was added. The reaction was carried out at room temperature for 1 h and monitored by HPLC. After the reaction was completed, the product was purified by reverse phase preparative column to obtain a white solid product (314 mg, 77.2%). LC-MS m/z (ES⁺): [M+2H]²⁺: 699.4.

Example 127. Synthesis of Compound LP-54a



Compound 114 (200 mg, 0.143 mmol, 1.0 eq) was dissolved in 10 mL dry dichloromethane and 4 mL TFA, and reacted at room temperature for 3 h, monitored by HPLC. After the reaction was completed, the solvent was removed by concentration under reduced pressure, and the crude product was purified by reverse phase preparative column, and the preparative solution was lyophilized to obtain a white solid (120.6 mg, 67.9%), LC-MS m/z (ES⁺): [M+2H]²⁺: 621.3.

Example 128. Expression and purification of Antibody SI000

Expi293 (Shanghai Aopumai Biotech Co., Ltd.) suspension cells were used to express the SI000 antibody. One day before transfection, Expi293 cells were inoculated in culture medium at a density of 0.9×10^6 cells/mL and cultured overnight in a carbon dioxide shaking incubator at 37°C, 5% CO₂, and 120rpm; on the day of transfection, PEI-MAX was used to transfect the expression plasmid; on the first day after transfection, 5% (volume ratio) of feed was added; on the third day after transfection, 5% (volume ratio) of feed was added again; on the sixth day after transfection, expression was terminated and the supernatant was collected by

centrifugation.

The harvested supernatant was purified by ProteinA affinity chromatography, eluted with 0.05M sodium acetate (pH3.6), adjusted to pH7.0 with 1M Tris-HCl (pH8.8), and then impurities such as polymers were removed by gel filtration chromatography.

The amino acid sequences of Antibody SI000 are listed as SEQ ID NO: 2 and 4 for its light chain and the heavy chain with a single chain Fv (scFv) structural domain, respectively; and SEQ ID NO: 5 and 6 for its light chain variable region and heavy chain variable region, respectively; and SEQ ID NO: 9, 10, 11, 12, 13, and 14 for CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3, respectively. The amino acid sequences of the scFv domain are listed as SEQ ID NO: 7 and 8 for the heavy and light chain variable regions, respectively; and SEQ ID NO: 15, 16, 17, 18, 19, and 20 for CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3, respectively.

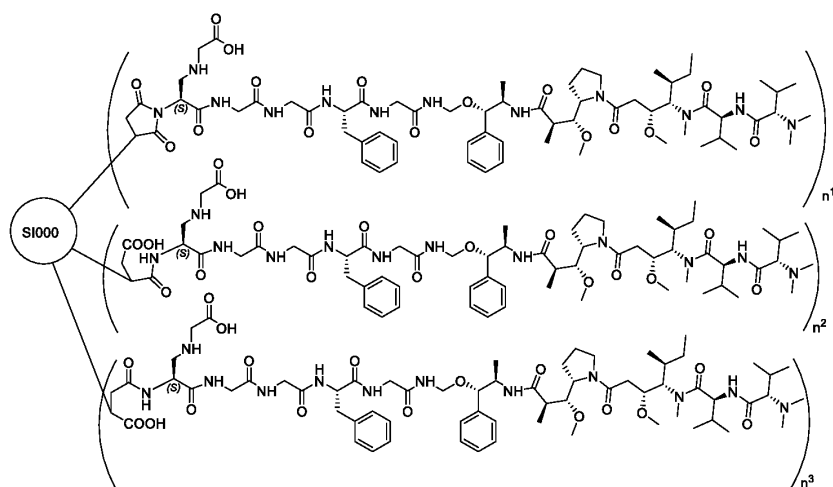
Example 129. Expression and purification of Antibody SI001

The methods for expressing and purifying antibody SI001 were the same as described in Example 128.

The amino acid sequences of Antibody SI001 are listed as SEQ ID NO: 22 and 23 for its light chain and the heavy chain with a single chain Fv (scFv) structural domain, respectively; and SEQ ID NO: 25 and 26 for its light chain variable region and heavy chain variable region, respectively; and SEQ ID NO: 29, 30, 31, 32, 33, and 34 for CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3, respectively. The amino acid sequences of the scFv domain are listed as SEQ ID NO: 27 and 28 for the heavy and light chain variable regions, respectively; and SEQ ID NO: 35, 36, 37, 38, 39, and 40 for CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3, respectively.

Example 130. Preparation of ADC-1-1a

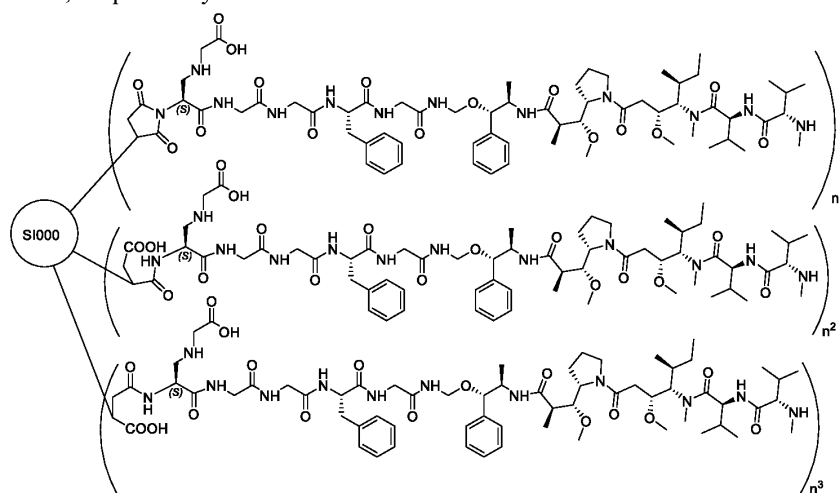
ADC-1-1a was prepared by conjugating the corresponding linker-payload (LP-1a, whose structure is shown in the structural formula of LP-1, wherein the configuration of the chiral carbon at position 2 is the S-type) with the bispecific antibody, SI000, according to Generic Step A. The sequence information of SI000 is shown in Example 105. The RP-HPLC assay and SEC-HPLC assay of ADC-1-1a were performed and the results are shown in **Figure 3A** and **Figure 4A**, respectively.



ADC-1-1a

Example 131. Preparation of ADC-2-1a

ADC-2-1a was prepared by conjugating the corresponding linker-payload (LP-2a, whose structure is shown in the structural formula of LP-2, wherein the configuration of the chiral carbon at position 2 is the S-type) with the bispecific antibody, SI000, according to Generic Step A. The sequence information of SI000 is shown in Example 105. The RP-HPLC assay and SEC-HPLC assay of ADC-2-1a were performed and the results are shown in **Figure 3B** and **Figure 4B**, respectively.

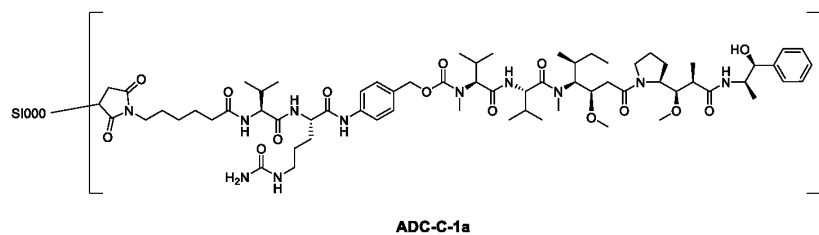


ADC-2-1a

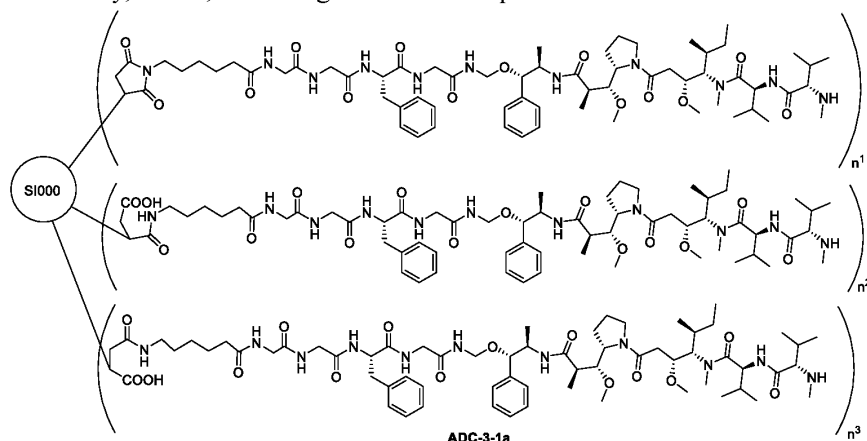
10

Example 132. Preparation of ADC-C-1a

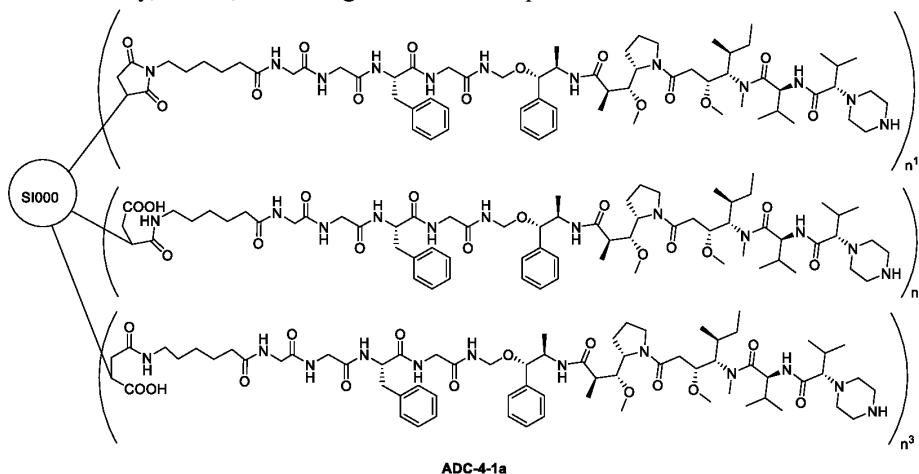
ADC-C-1a was prepared by conjugating VcMMAE with bispecific antibody, SI000, according to Generic Step A. The RP-HPLC assay and SEC-HPLC assay of ADC-C-1a were performed and the results are shown in **Figure 3C** and **Figure 4C**, respectively.

**Example 133.** Preparation of ADC-3-1a

ADC-3-1a was prepared by conjugating the corresponding linker-payload (LP-10) with the bispecific antibody, SI000, according to General Step A.

**Example 134.** Preparation of ADC-4-1a

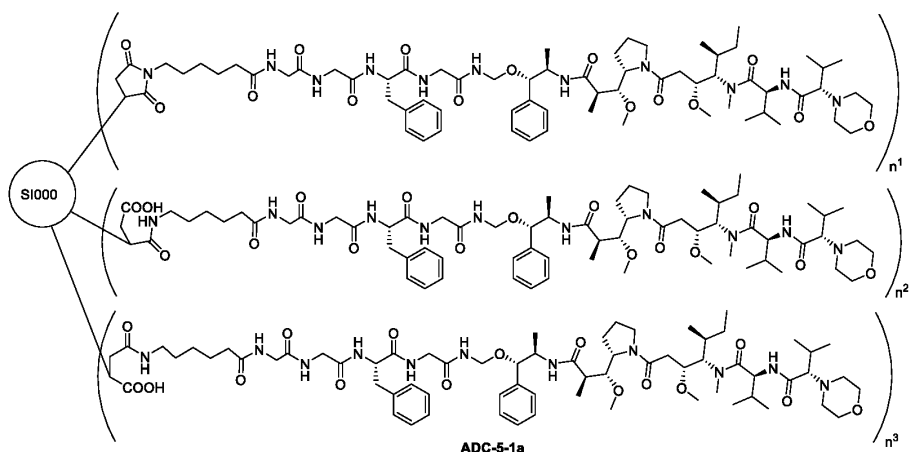
ADC-4-1a was prepared by conjugating the corresponding linker-payload (LP-37) with the bispecific antibody, SI000, according to Generic Step A.



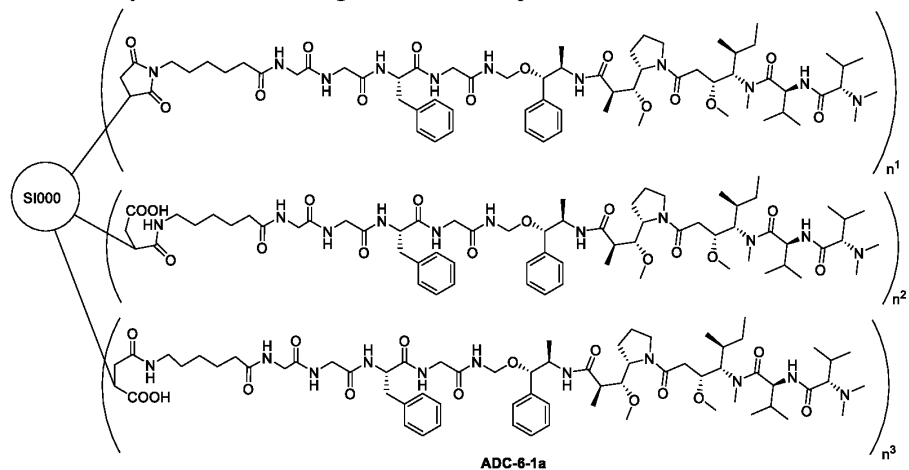
10

Example 135. Preparation of ADC-5-1a

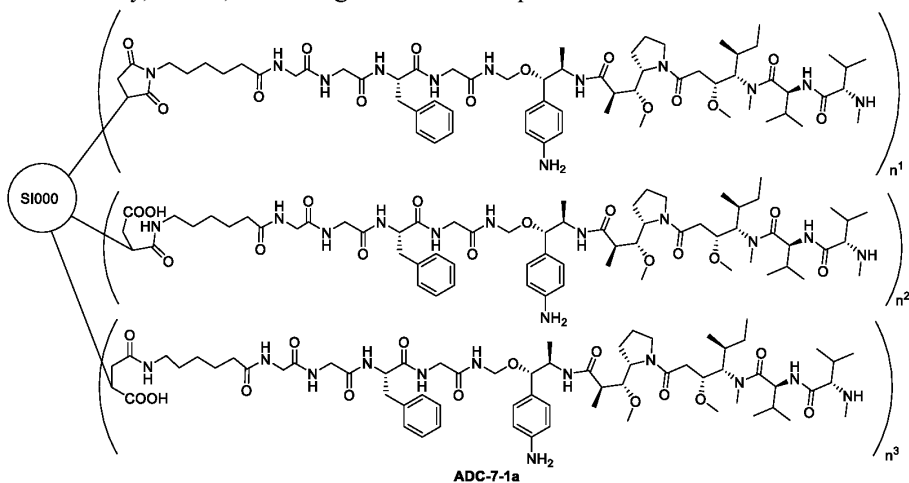
ADC-5-1a was prepared by conjugating the corresponding linker-payload (LP-16) with the bispecific antibody, SI000, according to Generic Step A.

**Example 136.** Preparation of ADC-6-1a

ADC-6-1a was prepared by conjugating the corresponding linker-payload (LP-11) with the bispecific antibody, SI000, according to Generic Step A.

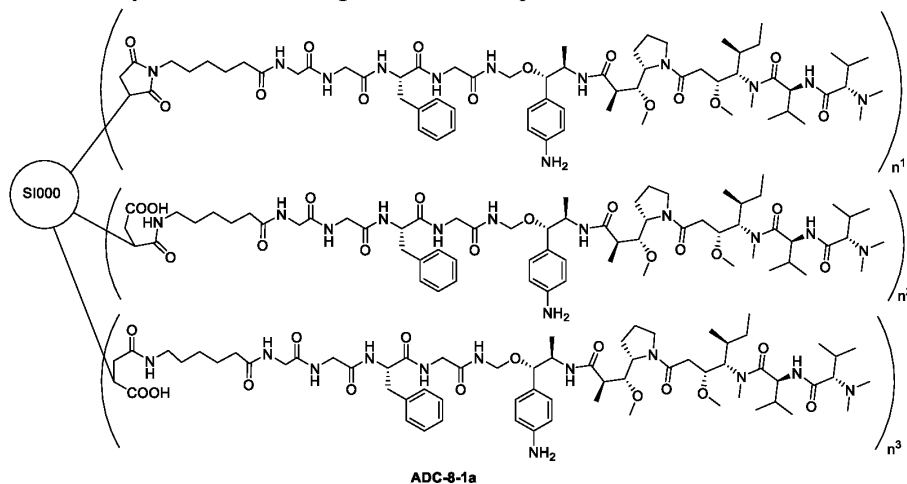
**Example 137.** Preparation of ADC-7-1a

ADC-7-1a was prepared by conjugating the corresponding linker-payload (LP-12) with the bispecific antibody, SI000, according to General Step A.

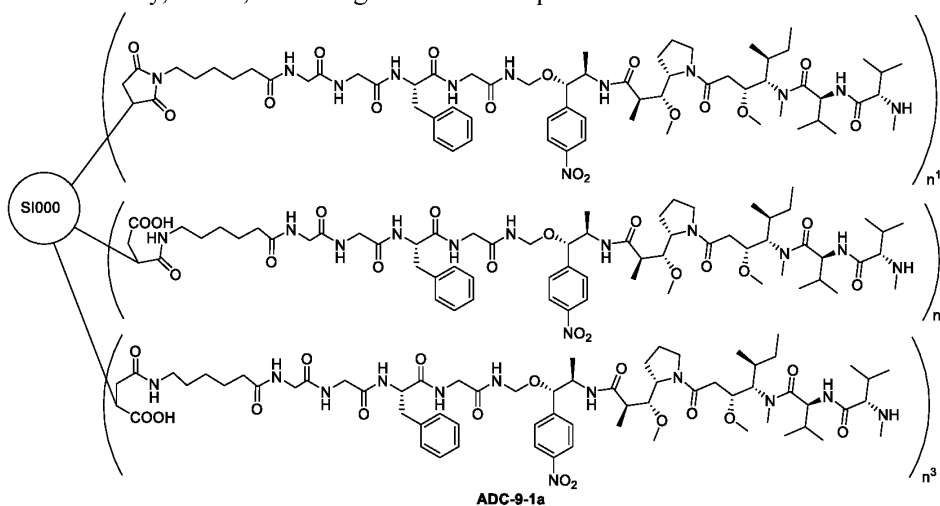


Example 138. Preparation of ADC-8-1a

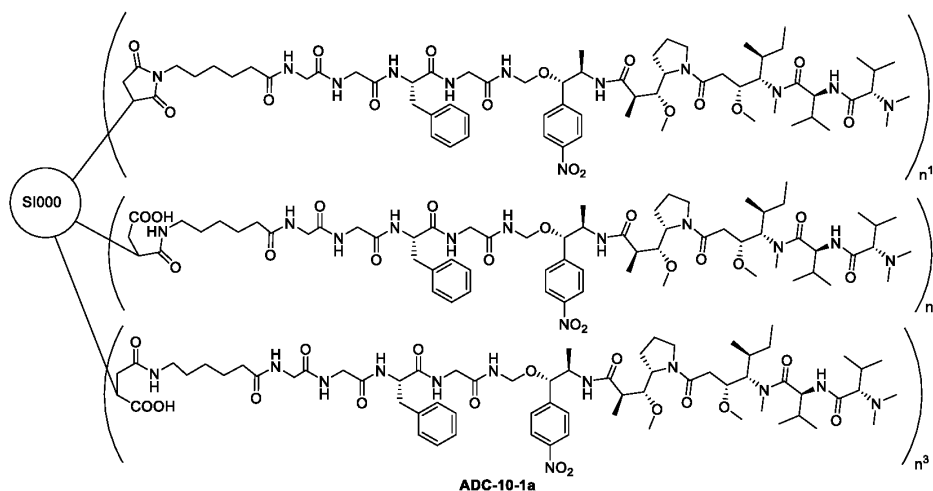
ADC-8-1a was prepared by conjugating the corresponding linker-payload (LP-13) with the bispecific antibody, SI000, according to Generic Step A.

**Example 139.** Preparation of ADC-9-1a

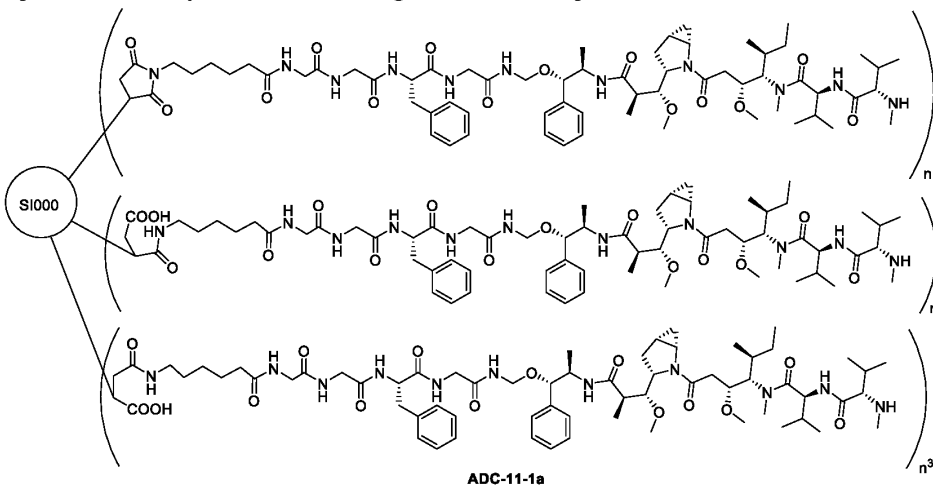
ADC-9-1a was prepared by conjugating the corresponding linker-payload (LP-14) with the bispecific antibody, SI000, according to Generic Step A.

**Example 140.** Preparation of ADC-10-1a

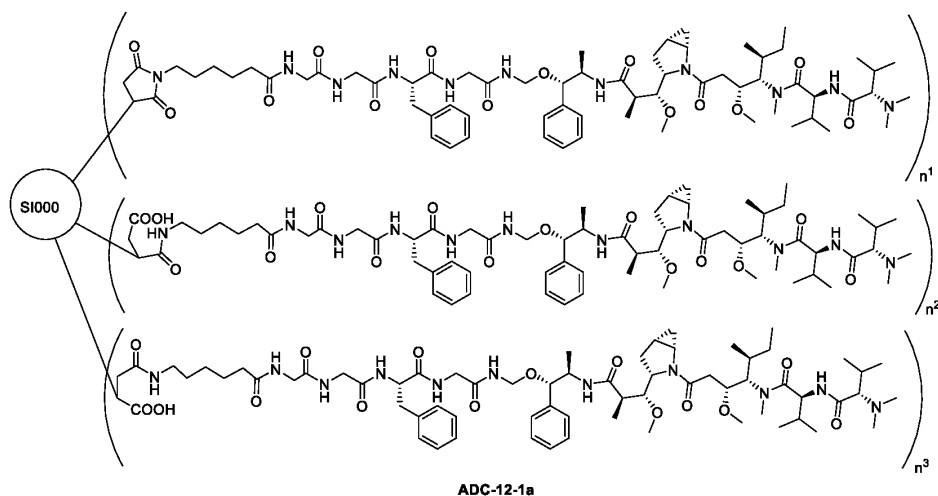
ADC-10-1a was prepared by conjugating the corresponding linker-payload (LP-15) with the bispecific antibody, SI000, according to General Step A.

**Example 141. Preparation of ADC-11-1a**

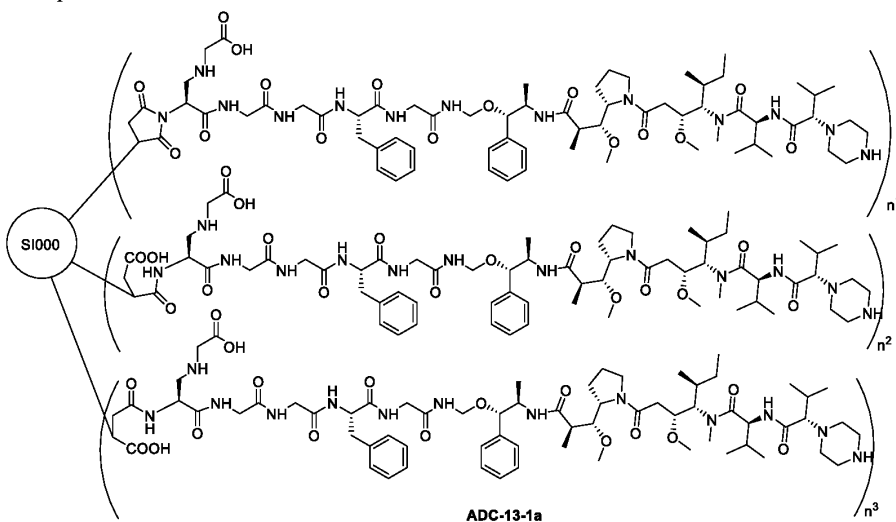
ADC-11-1a was prepared by conjugating the corresponding linker-payload (LP-17) with the bispecific antibody, SI000, according to Generic Step A.

**Example 142. Preparation of ADC-12-1a**

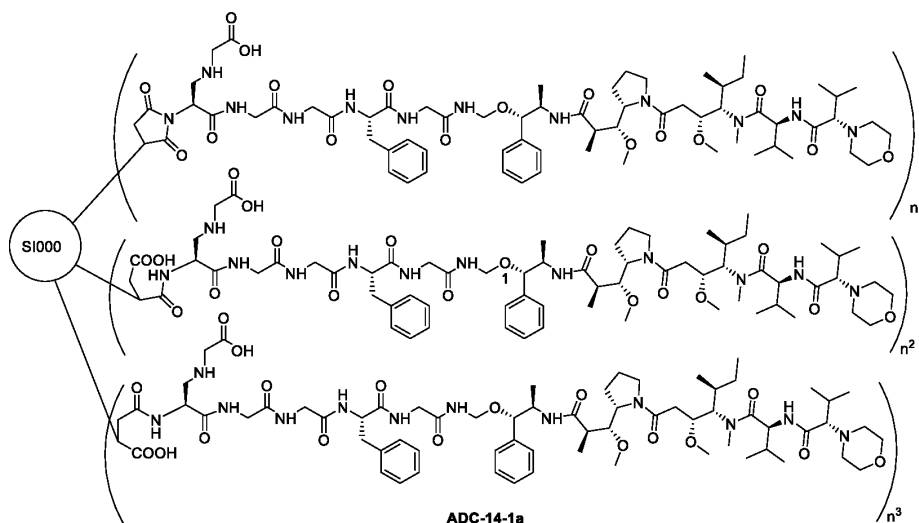
ADC-12-1a was prepared by conjugating the corresponding linker-payload (LP-18) with the bispecific antibody, SI000, according to Generic Step A.

**Example 143.** Preparation of ADC-13-1a

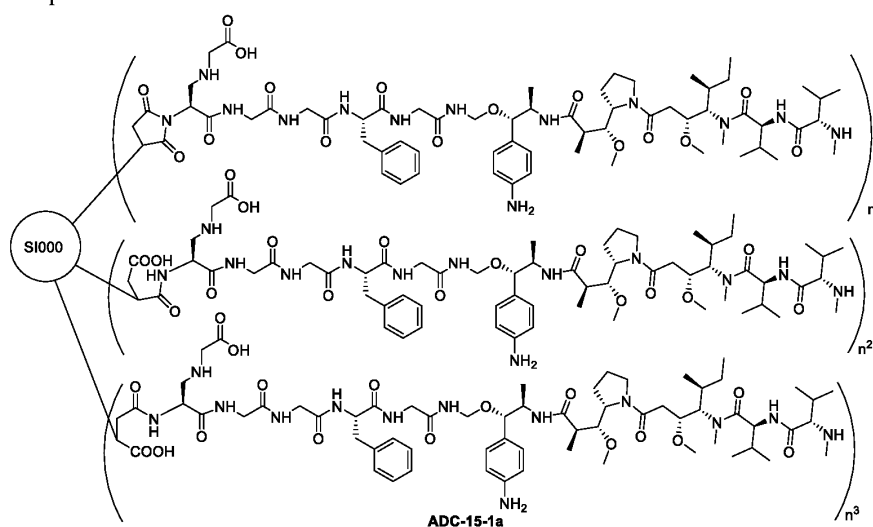
ADC-13-1a was prepared by conjugating the corresponding linker-payload (LP-3a, whose structure is shown in the structural formula of LP-3, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.

**Example 144.** Preparation of ADC-14-1a

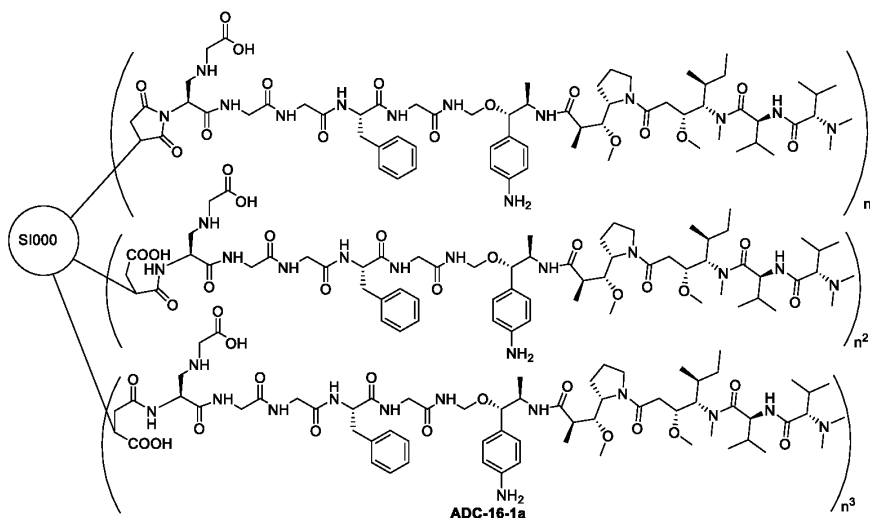
ADC-14-1a was prepared by conjugating the corresponding linker-payload (LP-7a, whose structure is shown in the structural formula of LP-7, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.

**Example 145.** Preparation of ADC-15-1a

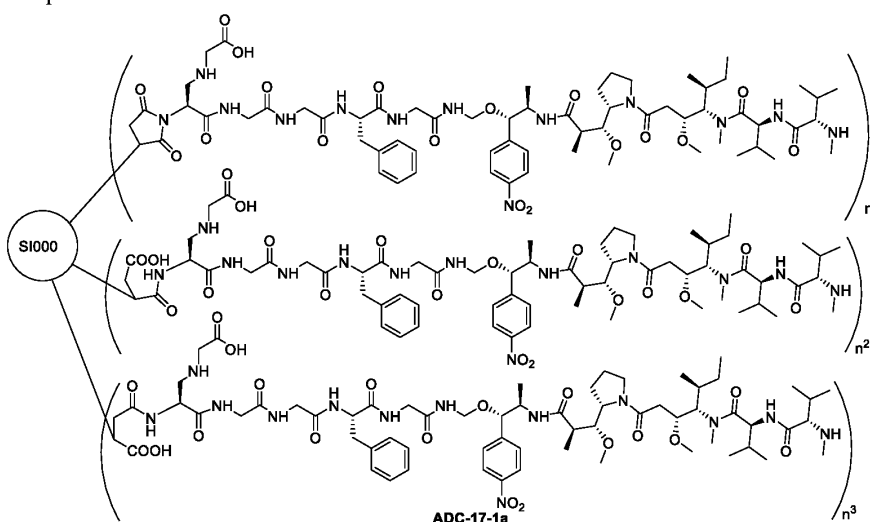
ADC-15-1a was prepared by conjugating the corresponding linker-payload (LP-3sa, whose structure is shown in the structural formula of LP-3s, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.

**Example 146.** Preparation of ADC-16-1a

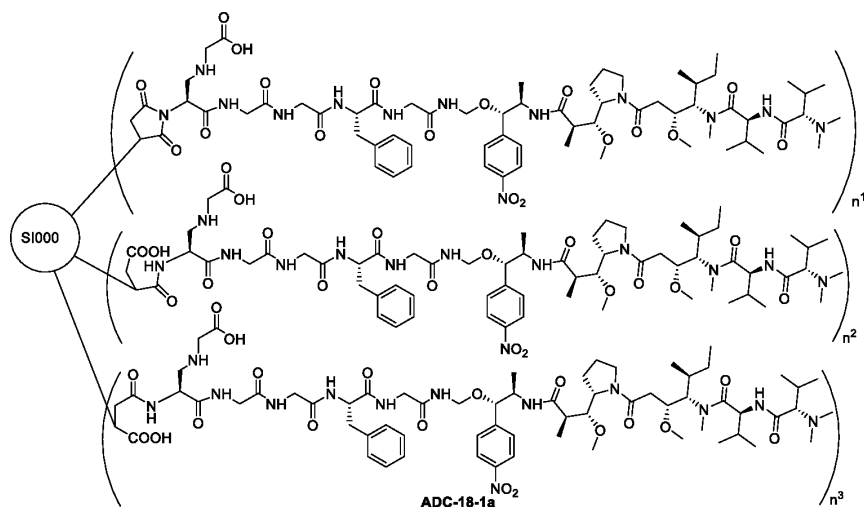
ADC-16-1a was prepared by conjugating the corresponding linker-payload (LP-4a, whose structure is shown in the structural formula of LP-4, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.

**Example 147.** Preparation of ADC-17-1a

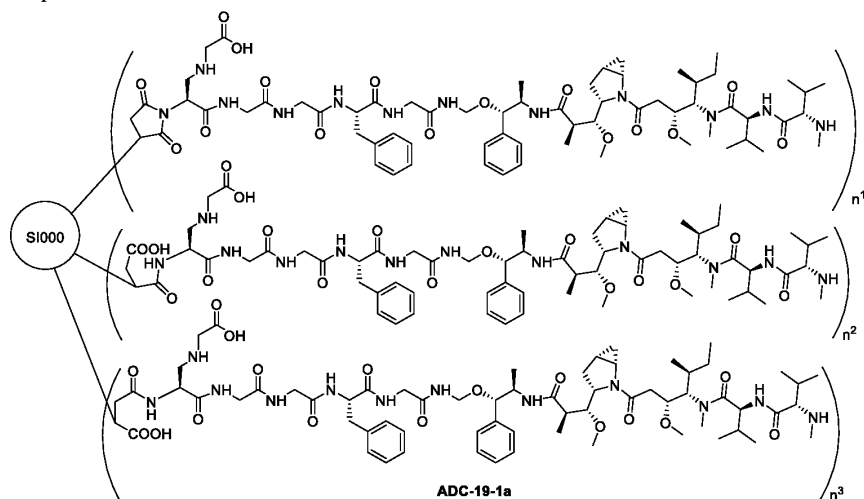
ADC-17-1a was prepared by conjugating the corresponding linker-payload (LP-5a, whose structure is shown in the structural formula of LP-5, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.

**Example 148.** Preparation of ADC-18-1a

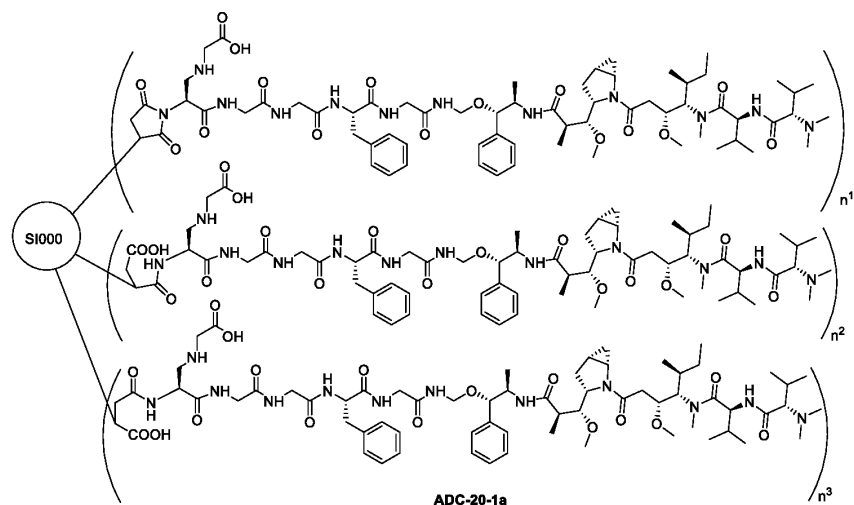
ADC-18-1a was prepared by conjugating the corresponding linker-payload (LP-6a, whose structure is shown in the structural formula of LP-6, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.

**Example 149.** Preparation of ADC-19-1a

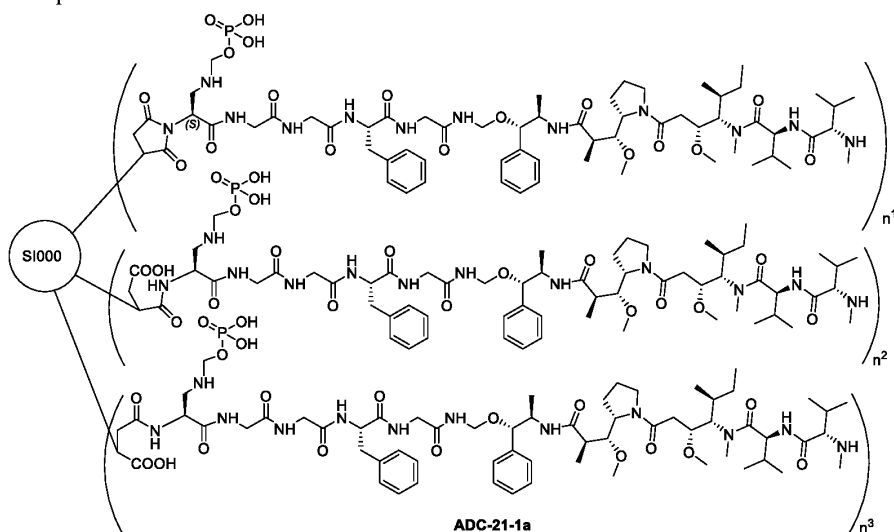
ADC-19-1a was prepared by conjugating the corresponding linker-payload (LP-8a, whose structure is shown in the structural formula of LP-8, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.

**Example 150.** Preparation of ADC-20-1a

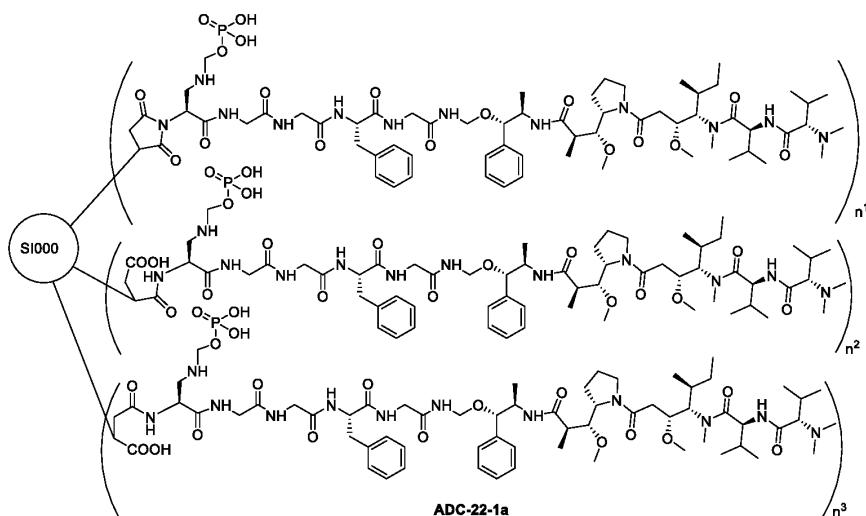
ADC-20-1a was prepared by conjugating the corresponding linker-payload (LP-9a, whose structure is shown in the structural formula of LP-9, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.

**Example 151. Preparation of ADC-21-1a**

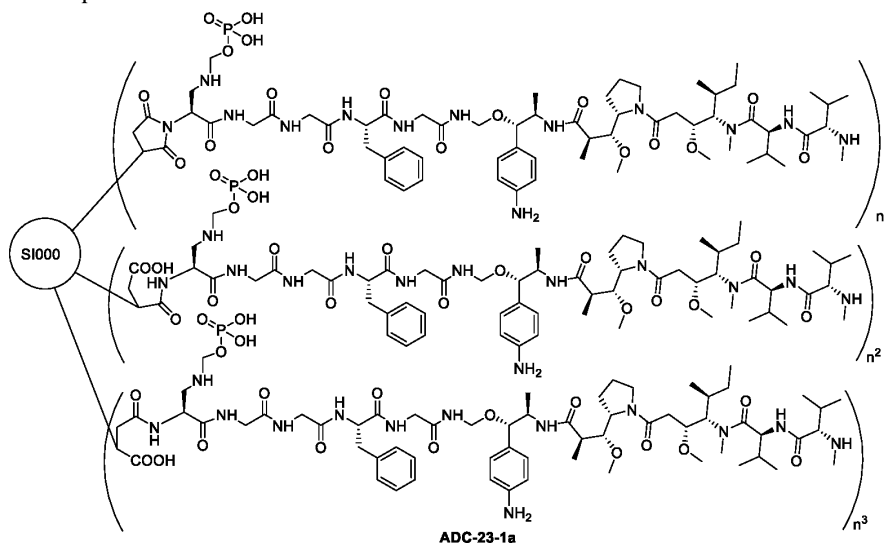
ADC-21-1a was prepared by conjugating the corresponding linker-payload (LP-28a, whose structure is shown in the structural formula of LP-28, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.

**Example 152. Preparation of ADC-22-1a**

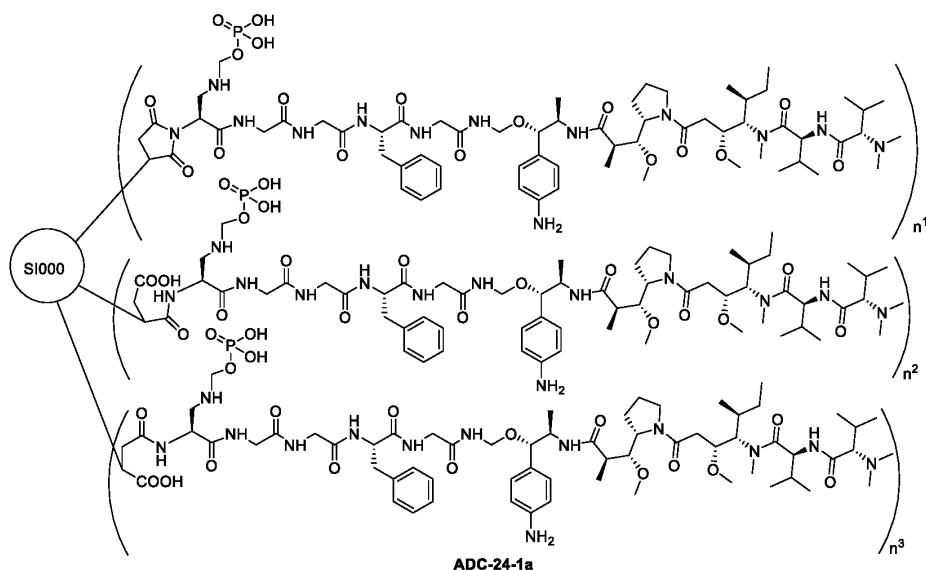
ADC-22-1a was prepared by conjugating the corresponding linker-payload (LP-29a, whose structure is shown in the structural formula of LP-29, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.

**Example 153.** Preparation of ADC-23-1a

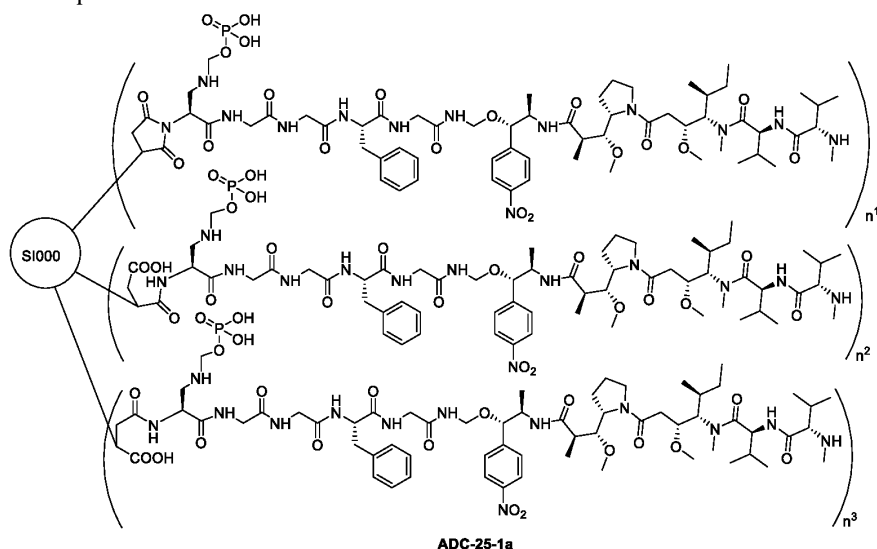
ADC-23-1a was prepared by conjugating the corresponding linker-payload (LP-30a, whose structure is shown in the structural formula of LP-30, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.

**Example 154.** Preparation of ADC-24-1a

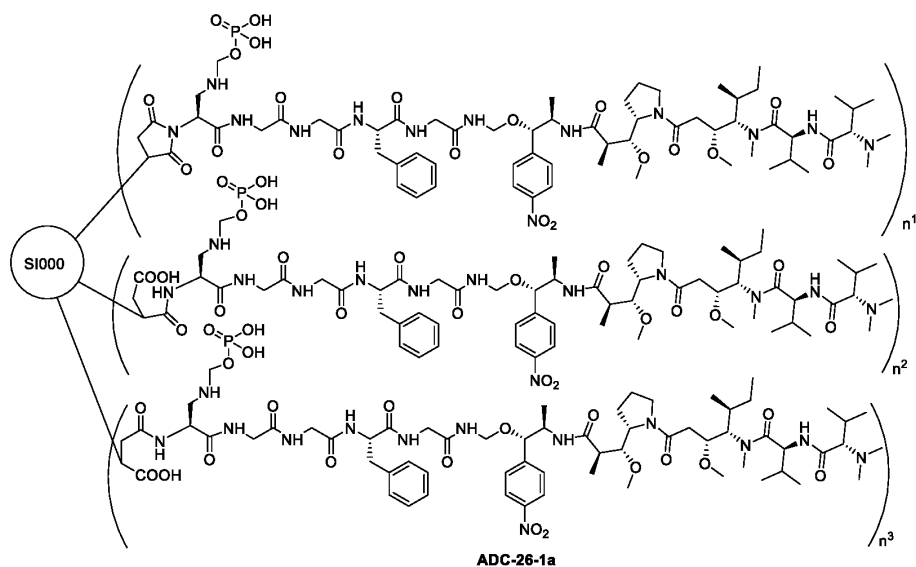
ADC-24-1a was prepared by conjugating the corresponding linker-payload (LP-31a, whose structure is shown in the structural formula of LP-31, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.

**Example 155. Preparation of ADC-25-1a**

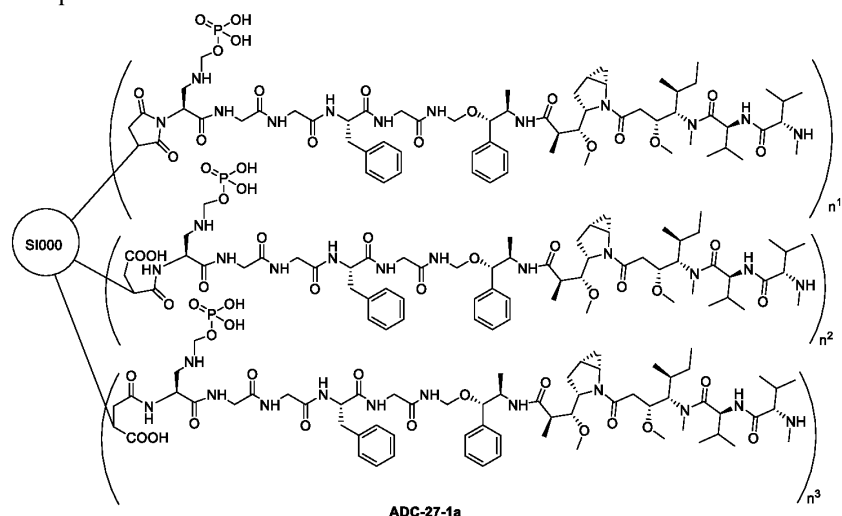
ADC-25-1a was prepared by conjugating the corresponding linker-payload (LP-32a, whose structure of which is shown in the structural formula of LP-32, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.

**Example 156. Preparation of ADC-26-1a**

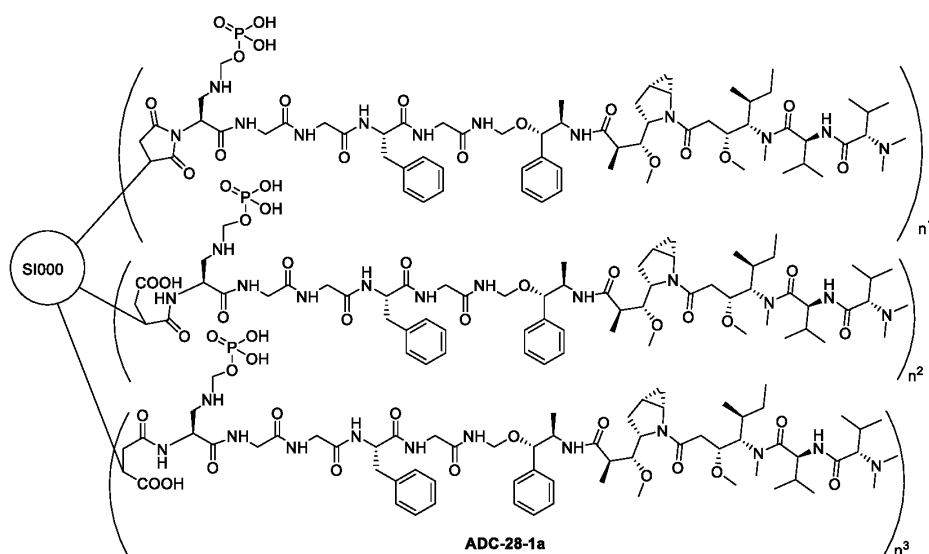
ADC-26-1a was prepared by conjugating the corresponding linker-payload (LP-33a, whose structure is shown in the structural formula of LP-33, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.

**Example 157.** Preparation of ADC-27-1a

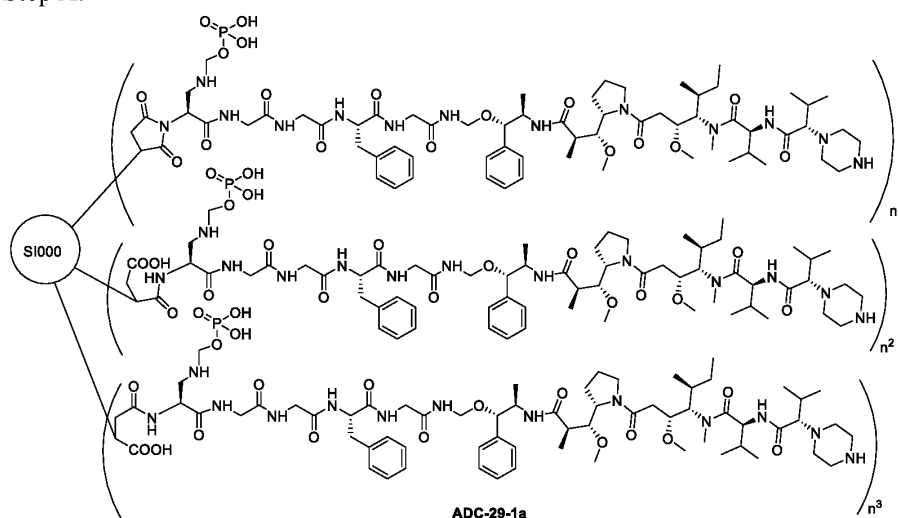
ADC-27-1a was prepared by conjugating the corresponding linker-payload (LP-35a, whose structure is shown in the structural formula of LP-35, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.

**Example 158.** Preparation of ADC-28-1a

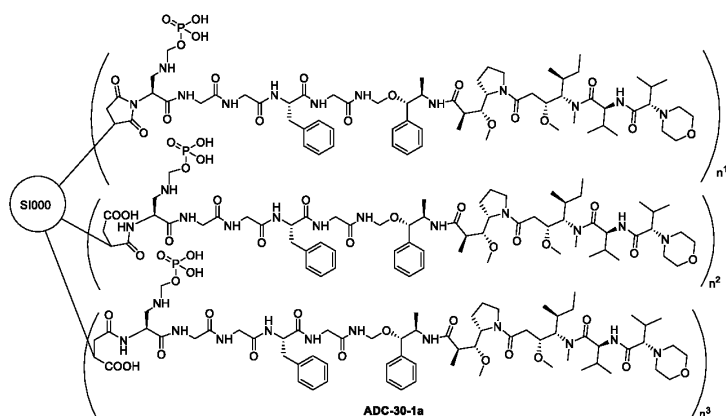
ADC-28-1a was prepared by conjugating the corresponding linker-payload (LP-36a, whose structure is shown in the structural formula of LP-36, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.

**Example 159. Preparation of ADC-29-1a**

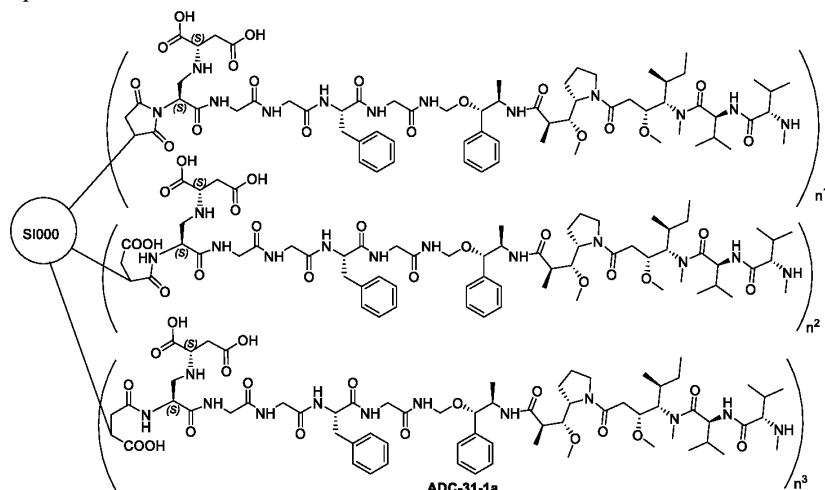
ADC-29-1a was prepared by conjugating the corresponding linker-payload (LP-39a, whose structure is shown in the structural formula of LP-39, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.

**Example 160. Preparation of ADC-30-1a**

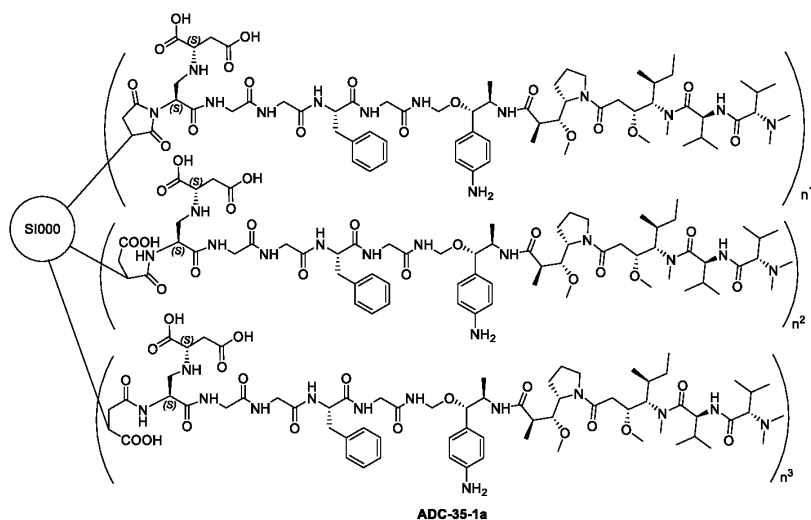
ADC-30-1a was prepared by conjugating the corresponding linker-payload (LP-34a, whose structure of which is shown in the structural formula of LP-34, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.

**Example 161.** Preparation of ADC-31-1a

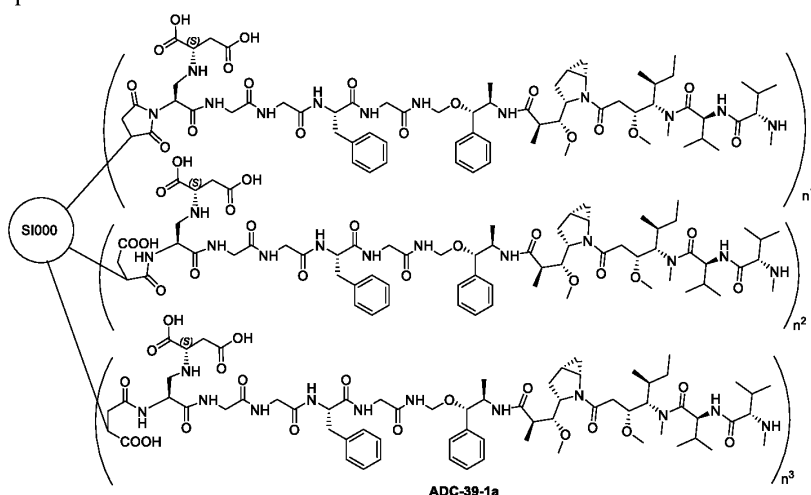
ADC-31-1a was prepared by conjugating the corresponding linker-payload (LP-19a, whose structure is shown in the structural formula of LP-19, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.

**Example 162.** Preparation of ADC-35-1a

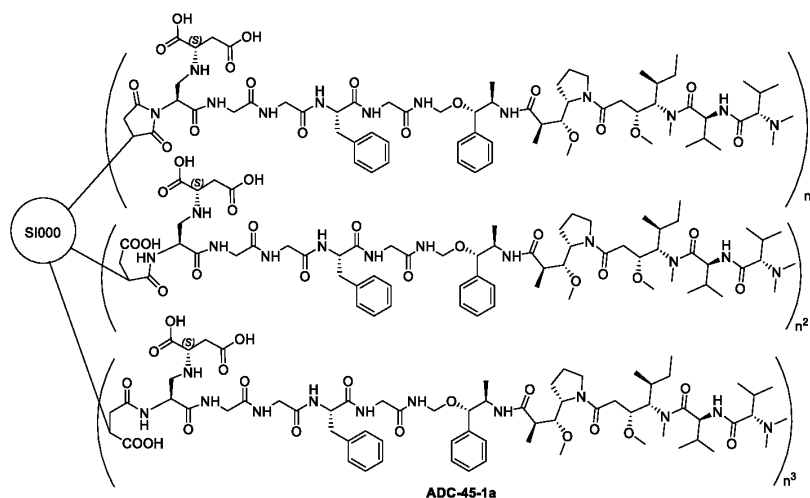
ADC-35-1a was prepared by conjugating the corresponding linker-payload (LP-22a, whose structure is shown in the structural formula of LP-22, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.

**Example 163.** Preparation of ADC-39-1a

ADC-39-1a was prepared by conjugating the corresponding linker-payload (LP-26a, whose structure is shown in the structural formula of LP-26, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.

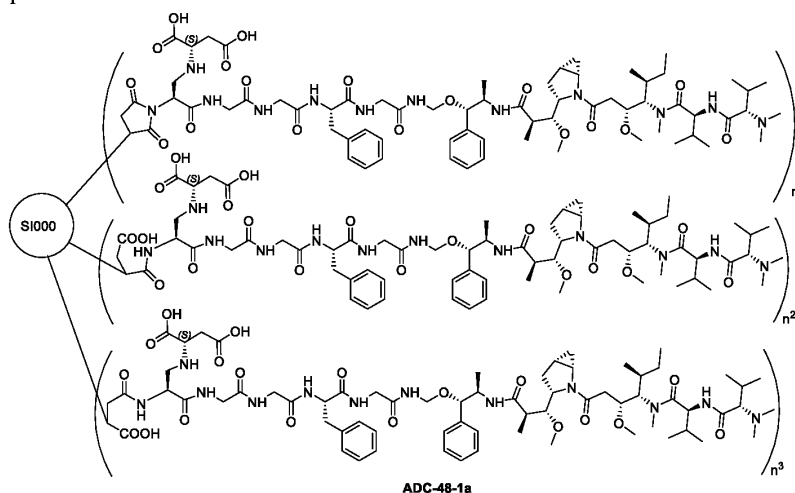
**Example 164.** Preparation of ADC-45-1a

ADC-45-1a was prepared by conjugating the corresponding linker-payload (LP-20a, whose structure is shown in the structural formula of LP-20, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.



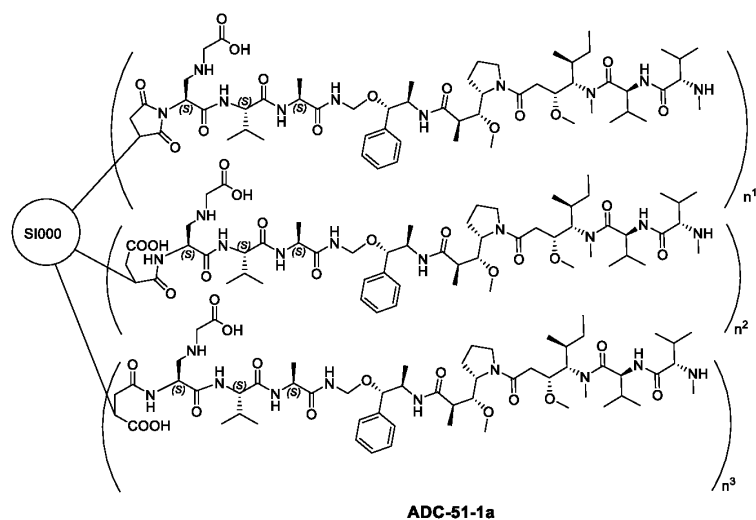
Example 165. Preparation of ADC-48-1a

ADC-48-1a was prepared by conjugating the corresponding linker-payload (LP-27a, whose structure is shown in the structural formula of LP-27, where the configuration of the chiral carbon at the position-2 is of the S-type) with the bispecific antibody, SI000, according to Generic Step A.

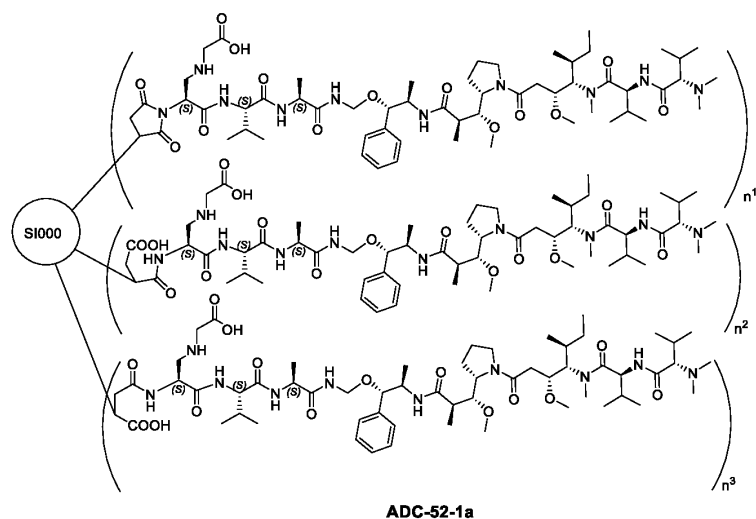


Example 166. Preparation of ADC-51-1a

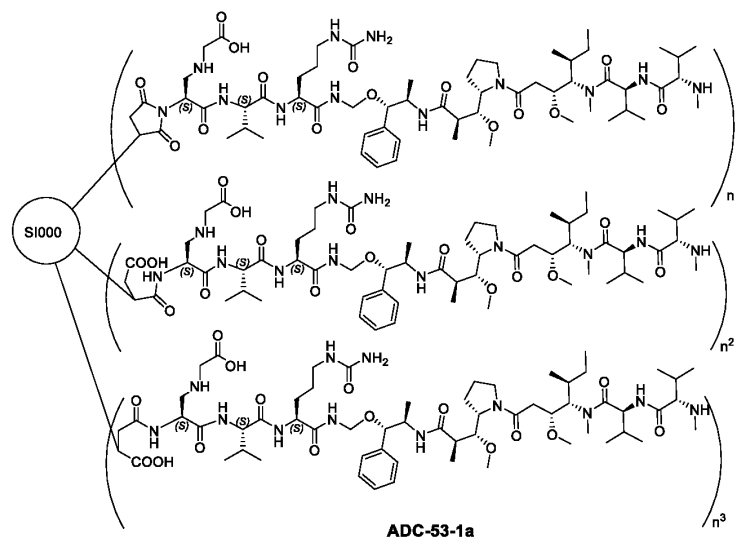
ADC-51-1a was prepared by conjugating the corresponding linker-payload (LP-51a) with the bispecific antibody, SI000, according to Generic Step A.

**Example 167.** Preparation of ADC-52-1a

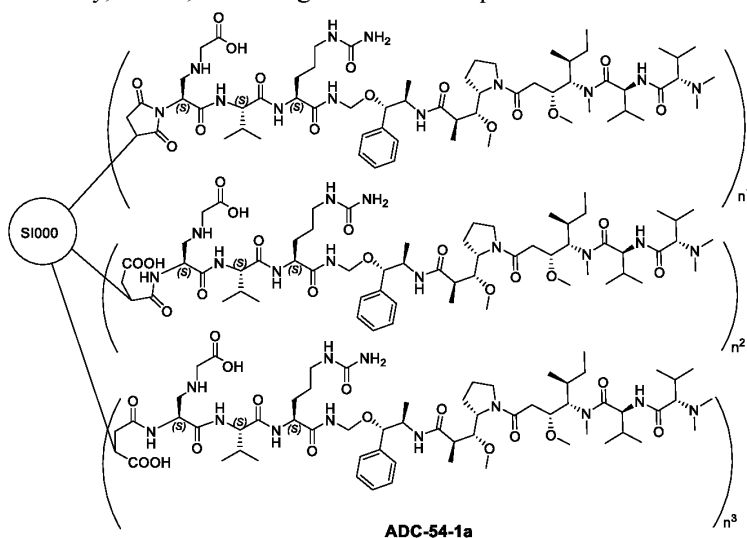
ADC-52-1a was prepared by conjugating the corresponding linker-payload (LP-52a) with the bispecific antibody, SI000, according to Generic Step A.

**Example 168.** Preparation of ADC-53-1a

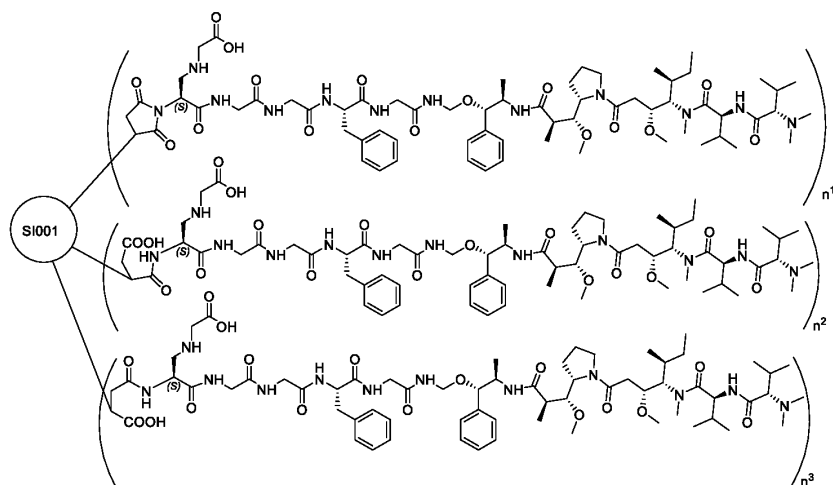
ADC-53-1a was prepared by conjugating the corresponding linker-payload (LP-53a) with the bispecific antibody, SI000, according to Generic Step A.

**Example 169.** Preparation of ADC-54-1a

ADC-54-1a was prepared by conjugating the corresponding linker-payload (LP-54a) with the bispecific antibody, SI000, according to Generic Step A.

**Example 170.** Preparation of ADC-1-1b

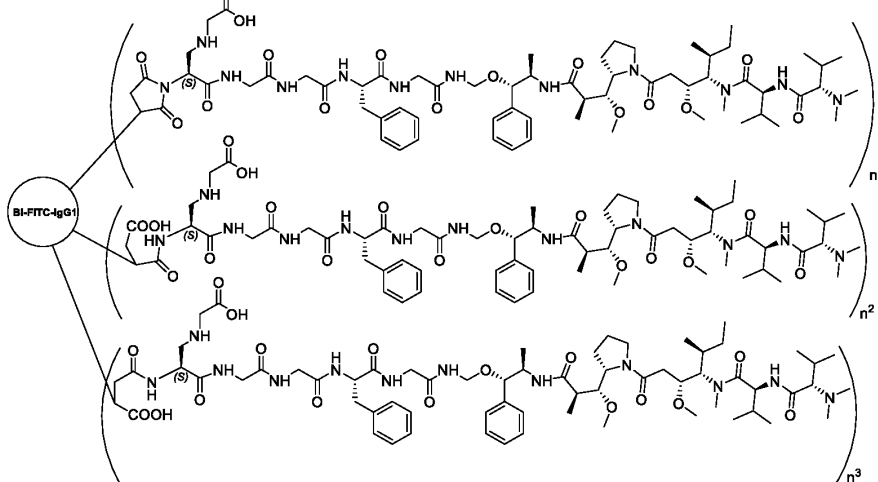
ADC-1-1b was prepared by conjugating the corresponding linker-payload (LP-1a, whose structure is shown in the structural formula of LP-1, wherein the configuration of the chiral carbon at position-2 is the S-type) with the bispecific antibody, SI000, according to Generic Step A. The sequence information of SI001 is shown in Example 129. The RP-HPLC detection results of ADC-1-1b are shown in **Figure 3D**, and the SEC-HPLC detection results are shown in **Figure 4D**.



ADC-1-1b

Control Example 1. Preparation of Bi-FITC-IgG1-LP-1a

Bi-FITC-IgG1-LP-1a was prepared by conjugating the corresponding linker-payload (LP-1a, whose structure is shown in the structural formula of LP-1, wherein the configuration of the chiral carbon at the position-2 is of S-type) with an unrelated antibody, Bi-FITC-IgG1, according to Generic Step A.

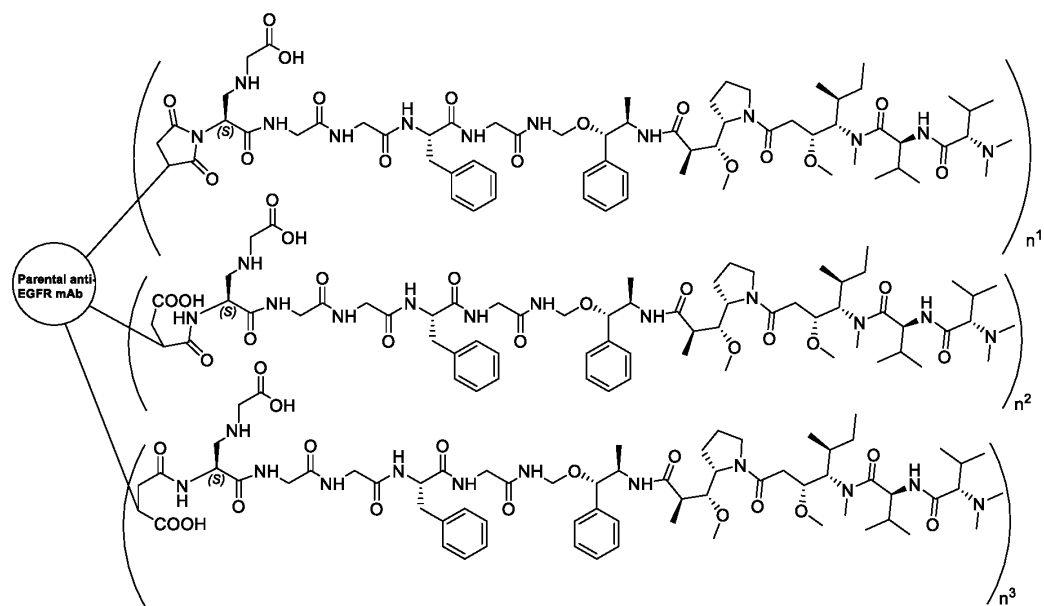


Bi-FITC-IgG1-LP-1a

The unrelated antibody, Bi-FITC-IgG1, is an isotype IgG antibody that does not target any antigen, and the amino acid sequence of its light chain and heavy chain with a scFv domain are listed as SEQ ID NO: 41 and SEQ ID NO: 42, respectively.

Control Example 2. Preparation of Parental anti-EGFR mAb-LP-1a

Parental anti-EGFR mAb-LP-1a was prepared by conjugating the corresponding linker-payload (LP-1a, whose structure is shown in the structural formula of LP-1, in which the configuration of the chiral carbon at the position-2 is of S-type) with the parental antibody, Parental anti-EGFR mAb, according to Generic Step A.

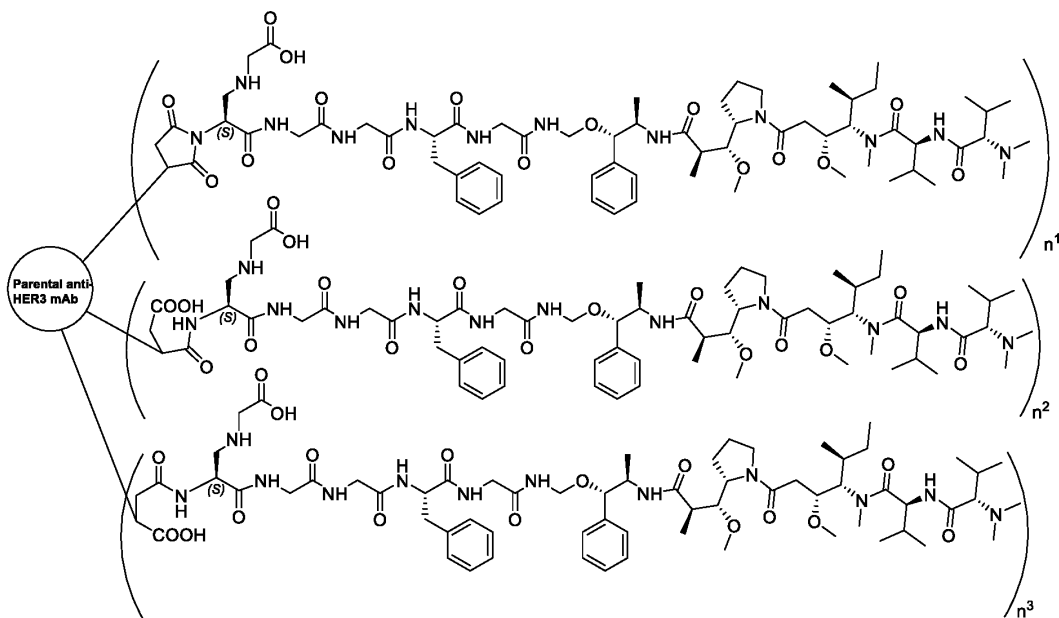


Parental anti-EGFR mAb-LP-1a

Parental anti-EGFR mAb is an antibody that targets EGFR only and the amino acid sequence of its light chain and heavy chain are listed as SEQ ID NO: 43 and 44, respectively.

Control Example 3. Preparation of Parental anti-HER3 mAb-LP-1a

Parental anti-HER3 mAb-LP-1a was prepared by conjugating the corresponding linker-payload (LP-1a, whose structure is shown in the structural formula of LP-1, in which the configuration of the chiral carbon at the position-2 is of S-type) with the parental antibody, Parental anti-HER3 mAb, according to Generic Step A.



Parental anti-HER3 mAb-LP-1a

Parental anti-HER3 mAb is an antibody that targets HER3 only and the amino acid sequence of its light chain and heavy chain are listed as SEQ ID NO: 45 and 46, respectively.

Example 171. DAR of ADC by Reversed Phase High Performance Liquid Chromatography (RP-HPLC)

The average values of Drug/Antibody Ratio or DAR of ADCs were determined by RP-HPLC according to Generic Step B (see **Table 1** below). Except for ADC-C-1a, whose DAR=n; for the rest of ADCs, DAR=n1+n2+n3. The ADCs disclosed here have the comparative advantageous property of higher DAR values than that of control ADC, indicating that at the same dosage, the administered ADC drug may significantly increase its location concentration at the target site.

Table 1. DAR of ADCs

Antibodies	Linker-payload number	ADC number	DAR
SI000	LP-1a	ADC-1-1a	7.34
	LP-1a	Bi-FITC-IgG1-LP-1a	7.38
	LP-1a	Parental anti-EGFR mAb-LP-1a	7.47
	LP-1a	Parental anti-HER3 mAb-LP-1a	7.51
	LP-2a	ADC-2-1a	7.48
	VcMMAE	ADC-C-1a	7.08
	LP-10	ADC-3-1a	7.19
	LP-11	ADC-6-1a	7.25
	LP-17	ADC-11-1a	7.23
	LP-18	ADC-12-1a	7.31
	LP-8a	ADC-19-1a	7.36
	LP-9a	ADC-20-1a	7.41
	LP-28a	ADC-21-1a	7.32
	LP-29a	ADC-22-1a	7.36
	LP-35a	ADC-27-1a	7.42
	LP-36a	ADC-28-1a	7.46
	LP-21a	ADC-31-1a	7.36
	LP-22a	ADC-35-1a	7.44
	LP-26a	ADC-39-1a	7.36
	LP-20a	ADC-45-1a	7.44
	LP-27a	ADC-48-1a	7.42
	LP-51a	ADC-51-1a	7.29
	LP-52a	ADC-52-1a	7.31
	LP-53a	ADC-53-1a	7.38
	LP-54a	ADC-54-1a	7.31
SI001	LP-1a	ADC-1-1b	7.39

Example 172. SEC Detection of ADC Monomer Rates

The degree of ADC aggregation was measured, according to Generic Step C, and the SEC-HPLC peaks were shown in **Figure 4**, and summarized in **Table 2**, from which it can be seen that the monomerization rate of ADC-1-1a, ADC-1-1b, Parental anti-EGFR mAb-LP-1a, Parental anti-HER3 mAb-LP-1a and ADC-2-1a, which introduced an acid-stable linker on the

hydroxyl group of the payload, was greater than 95%, and the degree of aggregation and degradation was low, whereas the ADC-C-1a, which used the classical MC linker, had a higher degree of aggregation, but low degradation.

Table 2. The degree of ADC aggregation

Target conjugates	Antibodies	Aggregates (100%)	Mono rate (100%)	Degradation (100%)
ADC-1-1a	SI000	1.27	98.61	0.12
ADC-2-1a	SI000	2.73	97.11	0.16
ADC-C-1a	SI000	17.591	81.632	0.777
ADC-1-1b	SI001	0.99	98.91	0.1
Parental anti-EGFR mAb-LP-1a	Parental anti-EGFR mAb	1.5	95.52	2.98
Parental anti-HER3 mAb-LP-1a	Parental anti-HER3 mAb	4.2	95.53	0.27

Example 173. The *in vitro* plasma stability of ADC

The plasma stability study of ADCs was carried out according to Generic Step D, and the results are shown in **Table 3**. The experimental results show that the introduction of an acid-stable linker on the hydroxyl group of the payload can reduce the loss of ADC samples during plasma culture, while the ADC with a classical MC junction (ADC-C-1a) has a significant decrease in DAR after 7 days of culture. In other words, introducing an acid-stable linker on the hydroxyl group of the payload can significantly improve the plasma stability of the ADC drug while leaving its DAR value almost unchanged.

Table 3. The *in vitro* plasma stability of ADC

Sample Name	Antibodies	Linker-payload	Incubate 0 day to extract	Incubate 3 days to extract	Incubate 7 days to extract
ADC-1-1a	SI000	LP-1a	7.72	7.68	7.68
ADC-2-1a	SI000	LP-2a	7.68	7.63	7.59
ADC-C-1a	SI000	VcMMAE	7.51	7.3	6.7
ADC-1-1b	SI001	LP-1a	7.69	7.66	7.61
Parental anti-EGFR mAb-LP-1a	Parental anti-EGFR mAb	LP-1a	7.66	7.61	7.52
Parental anti-HER3 mAb-LP-1a	Parental anti-HER3 mAb	LP-1a	7.61	7.51	7.33

Remarks: Incubated for 0 days means the DAR value measured after adding the ADC to the plasma and starting the purification and extraction step; Incubated for 3 days means the DAR value measured after the ADC is added to the plasma and left at 37°C for 3 DAR values were measured after the purification and extraction steps were started after 3 days of incubation; 7 days of incubation were measured after the purification and extraction steps were started after ADC was added to plasma and left at 37°C for 7 days.

Example 174. The Relative Affinity of Antibody and that of ADC

Comparison of the relative affinity of antibody SI000 to ADC-1-1a, ADC-2-1a, ADC-C-1a

for EGFR and HER3 by double antigen sandwich ELISA. Specific steps are described below:

Recombinant EGFR-His*6 antigen-coated plate, after 1% bovine serum protein closure; respectively, diluted SI000, ADC-1-1a, ADC-2-1a and ADC-C-1a, respectively, and then at a starting concentration of 5000ng/mL, successive 3-fold gradient dilution, a total of 11 concentrations; the sample was incubated on the coated enzyme plate for a certain period of time, and then incubated with biotin-labeled HER3-Fc antigen for a period of time, followed by incubation with streptavidin HRP labeling; finally, TMB color development was carried out, terminated by sulfuric acid solution, and the absorbance value at 450 nm was detected on the enzyme marker, and the corresponding ELISA data are shown in the following table.

10 CONCLUSION: The P-values of ADC-1-1a, ADC-2-1a and ADC-C-1a after coupling were in the range of 50%-150% (**Table 4**), indicating that all three ADCs maintained a similar affinity for SI000, which suggests that the coupling of antibody SI000 to linker-payload does not affect its affinity for the antigen.

Table 4. The Relative Affinity of Antibody and that of ADC

Sample Name	P-value
ADC-1-1a	68.2%
ADC-2-1a	69.9%
ADC-C-1a	68.7%

$$\text{P-value} = (\text{EC50 of Bare Anti (SI000)} / \text{EC50 of ADC}) * 100\%$$

Example 175. Test 1 for assessing the anti-tumor cell activity of ADCs

To evaluate the *in vitro* efficacy ADCs, several human cancer cell lines were used as cell models of human cancer, including human epidermal cancer cell line, A431; human non-small cell lung cancer cell line, HCC827; human non-small cell lung cancer cell line, NCI-H1975; 20 human pharyngeal squamous cell carcinoma cell line, FaDu; and human B cell lymphoma cells, cells that stably express luciferase reporter gene, Raji-Luc, coexpressing EGFR and HER3 (**Table 5**).

Table 5. Cancer cell line related information

Cell line name	Origin of Human Cancer	Source	EGFR Status	HER3 Status
A431	Human epidermoid carcinoma	ATCC	Positive	Positive
HCC827	Human non-small cell lung cancer	Shanghai cell bank	Positive	Positive
Fadu	Squamous carcinoma of the human pharynx	Shanghai cell bank	Positive	Positive
NCI-H1975	Human non-small cell lung cancer	Shanghai cell bank	Positive	Positive
Raji_Luc	Human B-cell Lymphoma	In-House	Negative	Negative

1) Experimental method:

An appropriate number of tumor cells were uniformly inoculated into a 96-well transparent-bottomed, white-walled plate and incubated in a carbon dioxide incubator. After 24 hours, the cells were confirmed to be in normal condition under a microscope and then treated with drugs. The test antibodies and ADC drugs were diluted with detection medium. The starting concentration of the drugs was 100 nM, the dilution multiple was 10 times, and

there was a total of 8 detection concentration points. After mixing, the corresponding cell wells were added, and the incubation volume was 100 μ L/well, set the last two columns as the control group (i.e., cells + medium, no drug treatment) and the blank group (i.e., no cells, only medium, used to deduct the background), and incubate in a carbon dioxide incubator at 37°C for 5 days.

For the monoclonal tumor cell models, including A431, HCC827, Fadu, and H1975, the CTG high sensitivity chemiluminescence detection technology was used to quantify adenosine triphosphate (ATP) in living cells to calculate cell viability. After the drug incubation was complete, 100 μ L of CTG (Promega, G7571) was added to each well and the reaction was protected from light for 10 minutes to fully lyse the target cells. After the reaction was completed, the relative luminescence (RLU) in each experimental group was detected using a Molecular Devices i3X multifunctional enzyme-linked instrument. The cell survival rate was calculated according to the formula: survival rate (%) = (experimental group - blank group)/(control group - blank group) $\times$ 100%. Then, Graphpad Prism was used to fit the four-parameter curve and calculate the IC50 to evaluate the killing effect of the test antibody-conjugated drug on tumor cells.

For the cell model of mixed tumors, EGFR/HER3-positive cancer cells, A431, and EGFR/HER3-negative cancer cells, Raji-Luc were seeded in a 1:2 ratio in 96-well plates with transparent bottom and white wall plates for drug treatment. In addition, a negative cell control was set up to receive the same drug treatment (the only negative cells, Raji-Luc cells, were seeded in 96-well plates with transparent bottom and white wall plates). Since EGFR/HER3-negative cells Raji stably express the luciferase reporter gene, the Bio-Lite Luciferase Assay System can be used to quantitatively detect luciferase in living cells to calculate the survival rate of EGFR/HER3-negative cells, Raji-Luc. After the drug incubation is complete, add 100 μ L of Bio-Lite (Vazyme, DD1201-02) to each well and incubate for 10 minutes to allow the cells to lyse and release luciferase, which reacts with the luciferin in the detection reagent to emit a stable light signal. The relative luminescence (RLU) of each experimental group was detected under dark conditions using a Molecular Devices i3X multifunctional enzyme labeling instrument. The survival rate (%) of the negative tumor cell line Raji-Luc in the mixed tumor model was calculated according to the formula: survival rate (%) = (experimental group - blank group)/(control group - blank group) $\times$ 100%, the survival rate of Raji-Luc in the cell model of mixed tumors was calculated, and then the IC50 was calculated using Graphpad Prism four-parameter fitting curve to evaluate the killing effect of ADC drugs on negative tumor cells through bystander effect.

2) Experimental results:

The corresponding *in vitro* data are shown in **Table 6**, as well as in **Figures 5A, 5B, 5C, 5D, 5E, and 5F**. ADCs are obtained by conjugating LP-1a with different antibodies (**Table 6**). Compared with EGFR and HER3 double-negative cells, Raji-Luc, ADC-1-1a, ADC-1-1b, and Parental anti-EGFR mAb-LP-1a all showed good performance in EGFR and HER3 double-positive cells. The selective killing effect shows that the anti-EGFR $\times$ HER3 bispecific antibodies, SI000 and SI001, have good specificity. In addition, in the HER3-positive cells with medium and low expression of EGFR (i.e., Fadu and NCI-H1975), ADC-1-1a is slightly more effective than parental anti-EGFR Ab-LP-1a and significantly better than parental anti-HER3 Ab-LP-1a, which shows that the ADC obtained by combining the bispecific antibody SI000 with the linker-payload has more advantages in *in vitro* anti-tumor activity than the

single-target antibody ADC. In the cell model of mixed tumors, A431+Raji-Luc, with Raji-Luc as the negative cell control, the ADC obtained by combining each antibody with the linker-payload can kill the negative cells through the bystander effect. And in these experiments, ADC-1-1a exhibits the strongest killing activity, which shows that the ADC obtained by combining SI000 with the linker-payload has more advantages in *in vitro* anti-tumor activity than a single target.

Table 6. IC50 (nM) of ADCs

Cell model of single tumor	ADC-1-1a	ADC-1-1b	Parental anti-EGFR mAb-LP-1a	Parental anti-HER3 mAb-LP-1a	SI000	SI001	Parental anti-EGFR mAb	Parental anti-HER3 mAb
A431	0.01	0.06	0.01	11.89	>100	>100	>100	>100
HCC827	0.04	0.08	0.03	102.03	0.76	>100	0.63	>100
Fadu	0.01	0.26	0.01	12.71	0.16	>100	>100	>100
NCI-H1975	0.01	0.62	0.02	88.25	>100	>100	>100	>100
Raji-Luc	>100	>100	92.81	72.23	>100	>100	>100	>100
Cell model of mixed tumors								
A431+Raji-Luc (1:2)	4.94	53.75	6.15	28.52	>100	>100	>100	>100

Example 176. Evaluation of *in vivo* efficacy of ADC

The present application established a subcutaneous heterogeneous tumor model of EGFR/HER3-positive human epidermal cancer cells, A431, and EGFR/HER3-positive human colon cancer cells, SW620, in BALB/c-Nude mice. To evaluate the *in vivo* efficacy of ADC-1-1a, ADC-1-1b, Parental anti-EGFR mAb-LP-1a and Parental anti-HER3 mAb-LP-1a, A431 (0.5×10⁶) and SW620 (1×10⁶) cell mixture (0.1 mL/mouse) was subcutaneously injected into the right scapula of 6~7-week-old *BALB/c-mu* mice. When the average tumor volume of the mice grew to about 188 mm³, they were randomly divided into 9 groups, namely vehicle control group (Vehicle), ADC-1-1a (3 mg/kg, 6 mg/kg) treatment group, ADC-1-1b (3 mg/kg, 6 mg/kg) treatment group, Parental anti-EGFR Ab-LP-1a (2.25 mg/kg, 4.5 mg/kg) treatment group, and Parental anti-HER3 Ab-LP-1a (2.25 mg/kg, 4.5 mg/kg) treatment group, with 6 mice in each group, and drug administration began (D0). Each treatment group was injected with 10 mL/kg body weight via tail vein, with one dosing cycle (QW) of 7 days. The drug was administered for 4 consecutive cycles, and all groups were observed 28 days after group administration (D28) to evaluate the inhibitory effect of the tested ADC drug on tumor growth.

After the dosing cycle, the average tumor volume of each treatment group at 28 days was statistically analyzed. As shown in **Figure 6**, at equimolar doses, in the A431/SW620 mixed tumor model, 3 mg/kg and 6 mg/kg of bispecific antibody-drug conjugates (ADC-1-1a and ADC-1-1b) showed stronger tumor inhibition than single-target parental ADCs (Parental anti-EGFR Ab-LP-1a, Parental anti-HER3 Ab-LP-1a). This indicates that bispecific antibody-ADCs obtained by combining either antibody SI000 or SI001with the linker-payload have more advantages in the treatment of mixed tumors than single-target parental antibodies.

Example 177. ADC anti-tumor cell activity test 2

A variety of human-derived tumor cell lines (human epidermoid carcinoma cell A431, human non-small cell lung cancer cell HCC827, human non-small cell lung cancer cell NCI-

H1975, human pharyngeal squamous carcinoma cell FaDu, human lung squamous carcinoma cell line HARA-B, and human colon cancer cell SW620, with expression of the corresponding antigens EGFR and HER3 as shown in **Table 7**) were utilized as experimental models in the present invention to evaluate the in vitro efficacy of the corresponding ADC drugs.

(1) Experimental method: Uniformly inoculate the appropriate number of tumor cell lines in 96-well plates and incubate them in a carbon dioxide incubator. 24 hours later, after confirming the normal cell status under the microscope, carry out the drug dosing treatment. The drugs were diluted with assay medium (ADC-1-1a The starting concentration of the drug was 100 nM, and the dilution was 10-fold, with a total of 8 concentration points; the starting concentration of ADC-2-1a and ADC-C-1a was set at 500 nM, and the dilution was 7-fold, with a total of 8 concentration points), and the theoretical coupling ratio of the payload to the antibody (DAR) was 8:1, so that the starting concentration of Auristatin was 0.8 nM. The starting concentration of Auristatin was 0.8 μM, with a 10-fold concentration gradient dilution and 8 concentration points; the starting concentration of MMAE was set to 4.0 μM, with a 7-fold concentration gradient dilution and 8 concentration points, which was mixed and added into the corresponding cell wells, where the last two columns were the control (i.e., cells + medium, no drug treatment) and the blank (i.e., without cells, containing only medium, for deduction of the background) and put into the carbon dioxide incubator at 37°C for 5 days. After the drug incubation was completed, 20 μL of MTS (Promega, G3581) was added to each well to react for 2-3 hours, which can be bioreduced by living cells to become a colored methyl dirty product, and the absorbance value readings at a wavelength of 490 nm were read using an enzyme labeling instrument (Molecular Device, model: SpectraMAX190), according to the formula: survival rate (%)=(experimental group-blank group)/(control group-blank group)*100%, the cell survival rate was calculated, followed by Graphpad Prism four-parameter fitting curve, calculation of IC₅₀ as well as Efficacy (%), to evaluate the killing effect of ADC drugs on tumor cells.

(2) Experimental results: the corresponding in vitro data are shown in **Tables 8, 9, 10, 11 and 12**, as well as in **Figures 7A, 7B, 7C, 7D and 7E**. In **Table 8**, the payload of ADC-1-1a is Auristatin E, the payload of ADC-2-1a and ADC-C-1a is MMAE, and IC₅₀: ADC-1-1a = ADC-C-1a < Auristatin E < ADC-2-1a < MMAE. This suggests that ADC-1-1a, ADC-2-1a and ADC-C-1a all have good selective killing effects on EGFR and HER3 double-positive cells A431. In addition, based on the results of **Tables 9, 10, 11 and 12**, it can be concluded that ADC-1-1a and ADC-2-1a exhibit cytotoxic effects on EGFR and HER3 double-positive cell lines, and no significant cytotoxic effect was observed in EGFR negative cell line (SW620) except at the highest concentration (100nM), indicating that ADC-1-1a and ADC-2-1a have good selectivity for EGFR-positive cell lines.

Table 7 Cell line related information

Cell line	Type of Cancer	Source	EGFR Status	HER3 Status
A431	Human epidermoid carcinoma	ATCC	Positive	Positive
HCC827	Human non-small cell lung cancer	Shanghai cell bank	Positive	Positive
Fadu	Squamous carcinoma of the human pharynx	Shanghai Cell Bank	Positive	Positive

NCI-H1975	Human non-small cell lung cancer	Shanghai cell bank	Positive	Positive
SW620	Human colorectal cancer	Shanghai cell bank	Negative	Positive

Table 8: *In vitro* efficacy of 3 ADCs (ADC-1-1a, ADC-2-1a and ADC-C-1a) and 2 payloads (Auristatin E and MMAE) in A431

A431	
Group	IC ₅₀ (nM)
ADC-1-1a	0.001
ADC-2-1a	0.017
ADC-C-1a	0.001
Auristatin E	0.009
MMAE	0.040

Table 9: *In vitro* efficacy of 2 ADCs (ADC-1-1a and ADC-2-1a) and 2 payloads (Auristatin E and MMAE) in HCC827

HCC827	
Group	IC ₅₀ (nM)
ADC-1-1a	0.020
ADC-2-1a	0.017
Auristatin E	1.103
MMAE	0.223

Table 10: *In vitro* efficacy of 2 ADCs (ADC-1-1a and ADC-2-1a) and 2 payloads (Auristatin E and MMAE) in Fadu

Fadu	
Group	IC ₅₀ (nM)
ADC-1-1a	0.001
ADC-2-1a	0.024
Auristatin E	0.031
MMAE	0.054

Table 11: *In vitro* efficacy of 2 ADCs (ADC-1-1a and ADC-2-1a) and 2 payloads (Auristatin E and MMAE) in NCI-H1975

NCI-H1975	
Group	IC ₅₀ (nM)
ADC-1-1a	0.0005
ADC-2-1a	0.073
Auristatin E	0.045
MMAE	0.368

Table 12: *In vitro* efficacy of 2 ADCs (ADC-1-1a and ADC-2-1a) and two payloads (Auristatin E and MMAE) in SW620

SW620	
Group	IC ₅₀ (nM)
ADC-1-1a	115.333

ADC-2-1a	488.103
Auristatin E	0.067
MMAE	4.091

Example 178. ADC-2-1a of *in vivo* potency assay

BALB/c-mu subcutaneous inoculation of a human tumor cell line (A431+SW620) was utilized as an experimental model in the present invention to evaluate the *in vivo* efficacy of ADC-2-1a and ADC-C-1a. A certain number of mixed suspensions of tumor cells were inoculated subcutaneously in *BALB/c-mu*, and when the tumor volume grew to about 180 mm³, the lysate and the corresponding ADC drugs (ADC-2-1a and ADC-C-1a) were injected into the tail vein, and the drugs were administered once a week for four times, under continuous observation, and the tumor volume was measured twice a week, to evaluate the inhibitory effect of the subjected ADC drugs on the growth of the tumors. As shown in **Figure 8**, in EGFR-positive HER3-positive A431 and EGFR-negative HER3-positive SW620 mixed tumors, ADC-2-1a at 5 mg/kg and 10 mg/kg exhibited a stronger tumor-suppressing effect relative to ADC-C-1a at 5 mg/kg and 10 mg/kg. This suggests that using the same payload and bispecific antibody, ADCs with acidic stabilizing linkers introduced from the hydroxyl group have better *in vivo* antitumor activity compared to ADCs with MC-VC-PAB linkers, and ADC-2-1a can exhibit a cytotoxic effect on EGFR negative tumors through bystander effects.

Example 179. *In vivo* potency assay of ADC-1-1a and ADC-2-1a

BALB/c-mu subcutaneous inoculation of human-derived tumor cell lines (A431+SW620, A431, SW620) was utilized as an experimental model in the present invention to evaluate the *in vivo* efficacy of ADC-1-1a and ADC-2-1a. A certain number of mixed suspensions of tumor cells were inoculated subcutaneously in *BALB/c-mu*, and when the tumor volume grew to about 150-200 mm³, the lysate and the corresponding ADC drugs were injected intravenously in the tail (ADC-1-1a, ADC-2-1a, Bi-FITC-IgG1-LP-1a, parental anti-EGFR Ab-LP-1a and parental anti-HER3 Ab-LP-1a) were administered four times once a week for continuous observation, and tumor volume was measured twice a week to evaluate the inhibitory effect of the tested ADC drugs on tumor growth.

The results are shown in **Figure 9**. As shown in Figure 9A, in the mixed tumors of EGFR-positive HER3-positive A431 and EGFR-negative HER3-positive SW620 (A431+SW620), both ADC-1-1a and ADC-2-1a showed tumor inhibition.

As shown in Figure 9B, in the EGFR-positive HER3-positive A431 monomer tumor (A431), both ADC-1-1a and ADC-2-1a showed tumor inhibition.

As shown in Figure 9C, in the EGFR-negative HER3-positive SW620 monomer tumor (SW620), both ADC-1-1a and ADC-2-1a showed tumor inhibition.

Example 180. *In vivo* potency assay of ADC-1-1a

In the present disclosure, *BALB/c-mu* subcutaneous inoculation of human-derived tumor cell lines (A431+SW620, A431, SW620, NCI-H1975) was used as an experimental model to evaluate the *in vivo* efficacy of ADC-1-1a. A certain number of mixed suspensions of tumor cells were inoculated in *BALB/c-mu* subcutaneously, and when the tumor volume grew to about 150-200 mm³, lysate, antibody, payload and the corresponding ADC drugs were injected into the tail vein, and the drugs were administered once a week for four times, under continuous observation, and the volume of the tumors was measured twice a week, and the inhibitory effect

of the subjected ADC drugs on the growth of the tumors was evaluated.

The results are shown in **Figure 10**. From **Figure 10A**, it can be seen that, in EGFR-positive HER3-positive A431 and EGFR-negative HER3-positive SW620 mixed tumors (A431+SW620), 2.5 mg/kg, 5mg/kg, and 10mg/kg ADC-1-1a produced significant tumor suppression effects, and the level of tumor suppression showed a certain quantitative relationship with the dose of the drug administered; from **Figure 10A**, the tumor-suppressive effect of 10mg/kg ADC-1-1a was superior to that of equimolar doses of naked antibody SI000, Bi-FITC-IgG1-LP-1a, and payload Auristatin E. From **Figure 10B**, the tumor-suppressive effect of 2.5mg/kg, 5mg/kg, and 10mg/kg ADC-1-1a was superior to that of equimolar doses of parental anti-EGFR mAb-LP-1a and parental anti-HER3 mAb-LP-1a.

As can be seen from **Figure 10C**, in EGFR-positive HER3-positive A431 monosomy (A431), 1, 2, and 4 mg/kg of ADC-1-1a produced significant tumor suppressive effects, and the level of tumor suppression showed a quantitative relationship with the dose of the drug administered; the tumor suppressive effect of 4 mg/kg of ADC-1-1a was superior to that of equimolar doses of the nude anti-SI000, Bi-FITC-IgG1 -LP-1a, and payload Auristatin E; as shown in **Figure 10D**, the tumor suppressive effect of 1 mg/kg of ADC-1-1a was superior to that of equimolar doses of parental anti-EGFR mAb-LP-1a, and the tumor suppressive effect of 1, 2, and 4 mg/kg of ADC-1-1a was superior to that of equimolar doses of parental anti-HER3 mAb-LP-1a.

As shown in **Figure 10E**, produced significant tumor suppressive effects of ADC-1-1a at 2.5 mg/kg, 5 mg/kg, and 10 mg/kg in EGFR-negative HER3-positive SW620 monosomy (SW620), and the level of tumor suppression showed a quantitative relationship with the dose of the drug administered; the tumor suppressive effect of ADC-1-1a at 10 mg/kg was superior to that of equimolar doses of the nude anti- SI000, Bi-FITC-IgG1-LP-1a, and payload Auristatin E. As shown in **Figure 10F**, the tumor-suppressive effect of ADC-1-1a at 2.5 mg/kg, 5 mg/kg, and 10 mg/kg was superior to that of the equimolar doses of parental anti-EGFR mAb-LP-1a and parental anti -HER3 mAb-LP-1a, indicating ADC-1-1a can exhibit a cytotoxic effect on EGFR negative tumors through bystander effects.

As can be known from **Figure 10G**, in EGFR-positive HER3-positive NCI-H1975 monosomy (NCI-H1975), 1mg/kg, 2mg/kg, and 4mg/kg of ADC-1-1a yielded a significant tumor-suppressing effect; the tumor-suppressing effect of 4mg/kg of ADC-1-1a was superior to that of equimolar doses of the naked anti-SI000, irrelevant antibody ADC Bi -FITC-IgG1-LP-1a, and payload Auristatin E; as shown in **Figure 10H**, the tumor-suppressive effect of 1 mg/kg, 2 mg/kg, and 4 mg/kg of ADC-1-1a was superior to that of equimolar doses of parental anti-EGFR mAb-LP-1a, and comparable to the equimolar dose of Parental anti-HER3 mAb-LP -1a.

It is understood that the above specific description of the present invention is only used to illustrate the present invention and is not limited to the technical solutions described in the embodiments of the present invention, and the person of ordinary skill in the art should be able to understand that modifications or equivalent replacements can still be made to the present invention to achieve the same technical effect; the above modifications or equivalent replacements are within the scope of protection of the present application.

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Each of the references is incorporated herein by reference in its entirety.

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WO2020260597A1

CN106279352A

10 WO2023083381A1

**ANTIBODY-AURISTATIN DRUG CONJUGATES AND PHARMACEUTICAL USE
THEREOF****SEQUENCE LISTING**

>SEQ ID NO.1: SI000 κ light chain (κ LC) nucleic acid sequence
GACATCTTGCTGACTCAGTCTCCAGTCATCCTGTCTGTGAGTCCAGGAGAAAGAGTCAGTTTCT
CCTGCAGGGCCAGTCAGAGTATTGGCACAAACATACACTGGTATCAGCAAAGAACAAATGGTTC
TCCAAGGCTTCTCATAAAGTATGCTTCTGAGTCTATCTCTGGGATTCCTTCCAGGTTTAGTGGC
AGTGGATCAGGGACAGATTTTACTCTTAGCATCAACAGTGTGGAGTCTGAAGATATTGCAGATT
ATTACTGTCAACAAAATAATAACTGGCCAACCACGTTTCGGTGCTGGGACCAAGCTGGAGCTGAA
ACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGA
ACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGG
TGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAG
CACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTAC
GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGT
GTTAG

>SEQ ID NO.2: SI000 κ LC amino acid sequence
DILLTQSPVILSVSPGERVVSFSCRASQSIGTNIHWYQQRNGSPRLLIKYASESISGIPSRFSG
SGSGTDFTLINSVESEDIADYYCQNNNWPPTTFGAGTKLELKRTVAAPSVFI FPPSDEQLKSG
TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC

>SEQ ID NO.3: SI000 heavy chain (HC) with scFv nucleic acid
sequence
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GGGTCTGGAGTGGCTGGGAGTGATATGGAGTGGTGGAAACACAGACTATAATACACCTTTTACA
TCCAGACTGAGCATCAACAAGGACAATTCCAAGAGCCAAGTTTTCTTTAAATGAACAGTCTGC
AATCTAATGACACAGCCATATATTACTGTGCCAGAGCCCTCACCTACTATGATTACGAGTTTGC
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CCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGG
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CTTCCCGGCTGTCTTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC
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ACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAA
CAGCACGTACCGTGTGGTCAGCGTCTCACCGTCTGCACCAGGACTGGCTGAATGGCAAGGAG

TACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCA
 AAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAA
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 TCTTCCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATG
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 GATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTGGCCAACATAAACCGCGAT
 GGAAGTGCAGTACTATGTGGACTCTGTGAAGGGCCGATTACCATCTCCAGAGACGACGCCA
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 GAGAGATCGTGGGGTGGGCTACTTCGATCTCTGGGGCCGTGGCACCCCTGGTCACCGTCTCGAGC
 GGTGGAGGCGGTTTCAGGCGGAGGTGGTTCGGGCGGTGGCGGCTCCAGTCTGCCCTGACTCAGC
 CTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATCTCCTGCACTGGAACCAGCAGTGA
 CGTTGGTGGTTATAACTTTGTCTCCTGGTACCAACAACACCCAGGCAAAGCCCCCAAACATCATG
 ATCTATGATGTGAGTATCGGCCCTCAGGGGTGTCTGATCGCTTCTCCGGCTCCAAGTCTGGCA
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 ATATGGGAGCAGCAGCACTCATGTGATTTTCGGCGGAGGGACCAAGGTGACCGTCCTATAA

>SEQ ID NO.4: SI000 HC with scFv amino acid sequence

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 PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS
 SSLGTQTYICNVNHKPSNTKVDKRVPEPKSCDKHTCTPPCPAPELLGGPSVFLFPPKPKDTLMIS
 RTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
 YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE
 SNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSHEALHNHYTQKSLSLSPG
 GGGSGGGGSQVQLQESGGGLVKPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANINRD
GSASYYVDSVKGRFTISRDDAKNSLYLQMNSLRAEDTAVYYCARDRGVGYFDLWGRGTLTVSS
 GGGSGGGSGGGGSQSALTQPASVSGSPGQSITISCTGTSSDVGGYNFVSWYQQHPGKAPKLM
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 SGSGTDFTLSINSVESEDIADYYCQNNNNWPTTFGAGTKLELK

>SEQ ID NO.6: SI000 HC variable region (VH) amino acid sequence
QVQLKQSGPGLVQPSSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSGNTDYNTPFT
SRLSINKDNSKSVFFKMNLSQNDTAIYYCARALTYDYEFAYWGQGLVTVSS

>SEQ ID NO.7: SI000 scFv VH amino acid sequence

QVQLQESGGGLVKPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANINRDGSASYVDSV
KGRFTISRDDAKNSLYLQMNSLRAEDTAVYYCARDRGVGYFDLWGRGTLVTVSS

>SEQ ID NO.8: SI000 scFv VL amino acid sequence
QSALTQPASVSGSPGQSITISCTGTSSDVGGYNFVSWYQQHPGKAPKLMYDVSDRPSGVSDRF
SGSKSGNTASLIISGLQADDEADYYCSSYGSSSTHVIFGGGTKVTVL

>SEQ ID NO.9: SI000 VL CDR-L1
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>SEQ ID NO.10: SI000 VL CDR-L2
YASESIS

>SEQ ID NO.11: SI000 VL CDR-L3
QQNNNWPTT

>SEQ ID NO.12: SI000 VH CDR-H1
NYGVH

>SEQ ID NO.13: SI000 VH CDR-H2
VIWSGGNTDYNTPFST

>SEQ ID NO.14: SI000 VH CDR-H3
ALTYDYEFAY

>SEQ ID NO.15: SI000 scFv VH CDR-H1
SYWMS

>SEQ ID NO.16: SI000 scFv VH CDR-H2
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>SEQ ID NO.17: SI000 scFv VH CDR-H3
DRGVGYFDL

>SEQ ID NO.18: SI000 scFv VL CDR-L1
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>SEQ ID NO.19: SI000 scFv VL CDR-L2
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>SEQ ID NO.20: SI000 scFv VL CDR-L3

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>SEQ ID NO.21: SI001 κLC nucleic acid sequence

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AACTCCTGGTAAAGCTCCTAAACTTCTTATTTATAAAGTTTCTAATCGTTTTTCTGGTGTTCCT
TCTCGTTTTTCTGGTTCTGGTTCTGGTACTGATTTTACTTTTACTATTTCTTCTCTTCAACCTG
AAGATATTGCTACTTATTATTGTTTTCAATATTCTCATGTTTCCTTGGACTTTTGGTCAAGGTAC
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CAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAAATTCTATCCCAGAGAGGCCA
AAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCA
GGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAG
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SRFSGSGSGTDFTFTISSLPEDIATYYCFQYSHVPWTFGQGTKLQITRTVAAPSVFI FPPSDE
QLKSGTASVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSLTLSKADYE
KHKVYACEVTHQGLSSPVTKSFNRGEC

>SEQ ID NO.23: SI001 HC with scFv nucleic acid sequence

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GTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCTGGACT

CCGACGGCTCCTTCTTCCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAA
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CCCAAATCATGATCTATGATGTCAGTGATCGGCCCTCAGGGGTGTCTGATCGCTTCTCCGGCT
CCAAGTCTGGCAACACGGCCTCCCTGATCATCTCTGGCCTCCAGGCTGACGACGAGGCTGATTA
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GTCCTATAA

>SEQ ID NO.24: SI001HC with scFv amino acid sequence

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PSVFP LAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
TVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKHTCTPPCPAPELLGGPSVFLFPPKPKDT
LMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL
NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLS
LSPGGGGSGGGGSQVQLQESGGGLVKPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVAN
INRDGSASYVDSVKGRFTISRDDAKNSLYLQMNSLRAEDTAVYYCARDRGVGYFDLWGRGTLV
TVSSGGGGSGGGGSQALTPASVSGSPGQSITISCTGTSSDVGGYNFVSWYQQHPGKA
PKLMIYDVSDRPSGVSDRFGSGKSGNTASLIISGLQADDEADYYCSSYGSSTHVI FGGGTKVT
VL

>SEQ ID NO.25: SI001 VL amino acid sequence

DIQMTQSPSSLSASVGDRVTITCRSSQNI VHSNGNTYLDWYQQTPGKAPKLLIYKVS NRFSGVP
SRFSGSGSGTDFTFTISSLQPEDATYYCFQYSHVPWTFGQGTKLQIT

>SEQ ID NO.26: SI001 IgG1 VH amino acid sequence

QVQLQQSGAEVKKPGSSVKVSKASGYTFTNYYIYWVRQAPGQGLEWIGGINPTSGGSNFNEKF
KTRVTITADESSTTAYMELSSLRSEDTAFYFCTRQGLWFDS DGRGDFWVGQTTVTVSS

>SEQ ID NO.27: SI001 scFv VH amino acid sequence

QVQLQESGGGLVKPGGSLRLSCAASGFTFSSYWMSWVRQAPGKGLEWVANINRDGSASYVDSV

KGRFTISRDDAKNSLYLQMNSLRAEDTAVYYCARDRGVGYFDLWGRGTLVTVSS

>SEQ ID NO.28: SI001 scFv VL amino acid sequence
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SGSKSGNTASLIISGLQADDEADYYCSSYGSSSTHVIFGGGTKVTVL

>SEQ ID NO.29: SI001 κLC CDR-L1
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>SEQ ID NO.30: SI001 κLC CDR-L2
KVSNRFS

>SEQ ID NO.31: SI001 κLC CDR-L3
FQYSHVPWT

>SEQ ID NO.32: SI001 IgG1 VH CDR-H1
NYIY

>SEQ ID NO.33: SI001 IgG1 VH CDR-H2
GINPTSGGSNFNEKFKT

>SEQ ID NO.34: SI001 IgG1 VH CDR-H3
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>SEQ ID NO.35: SI001 scFv VH CDR-H1
SYWMS

>SEQ ID NO.36: SI001 scFv VH CDR-H2
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>SEQ ID NO.37: SI001 scFv VH CDR-H3
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>SEQ ID NO.38: SI001 scFv VL CDR-L1
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>SEQ ID NO.39: SI001 scFv VL CDR-L2
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>SEQ ID NO.40: SI001 scFv VL CDR-L3
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>SEQ ID NO.41: Bi-FITC-IgG1 LC amino acid sequence
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QLKSGTASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYLSSTLTLSKADYE
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>SEQ ID NO.42: Bi-FITC-IgG1 HC with scFv amino acid sequence
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LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS
SLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISR
TPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
KCAVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGG
GGGSGGGGSEVKLDDETGGGLVQGRPMKLSCVASGFTFSDYWMNWVRQSPKGLGWVAQIRNKP
YNITYYSDSVKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSTVTVSSG
GGGSGGGGSGGGGSDVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLRWYLQK
PGQSPKVLIIKVSNRFSQVDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPWTFGGGTKL
EIK.

>SEQ ID NO.43: Parental anti-EGFR mAb LC amino acid sequence
DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQRTNGSPRLLIKYASESISGIPSRFSG
SGSGTDFTLSINSVEEDIADYYCQQNNNWPTTFGAGTKLELKRVAAPSVFIFPPSDEQLKSG
TASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC

>SEQ ID NO.44: Parental anti-EGFR mAb HC amino acid sequence
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PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS
SSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS
RTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE
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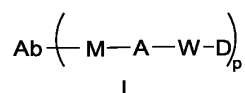
>SEQ ID NO.45: Parental anti-HER3 mAb LC amino acid sequence
QSALTQPASVSGSPGQSITISCTGTSSDVGGYNFVSWYQQHPGKAPKLMYDVS DRPSGVSDRF

SGSKSGNTASLIISGLQADDEADYYCSSYGSSSTHVI FGGGTKVTVLRTVAAPSVFI FPPSDEQ
LKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSTLTLSKADYEK
HKVYACEVTHQGLSSPVTKSFNRGEC

>SEQ ID NO.46: Parental anti-HER3 mAb HC amino acid sequence
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KGRFTISRDDAKNSLYLQMNSLRAEDTAVYYCARDRGVGYFDLWGRGTLVTVSSASTKGPSVFP
LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS
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KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

CLAIMS

1. An antibody-drug conjugate as shown in general Formula I or a pharmaceutically acceptable salt or solvate thereof,



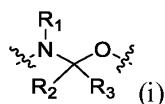
wherein:

Ab is a bispecific antibody having a binding affinity to EGFR and HER3 or an antigen-binding fragment thereof;

M is a linker unit attached to Ab;

A is a peptide residue having from 2 to 7 amino acids, wherein, optionally, each amino acid is independently substituted with one or more substituents comprising deuterium, halogens, hydroxyl group, cyano group, amino group, nitro group, alkyl, substituted alkyls, alkoxy, cycloalkyl, or substituting cycloalkyl;

W is an amino methylene oxide structural unit as shown in Formula (i):



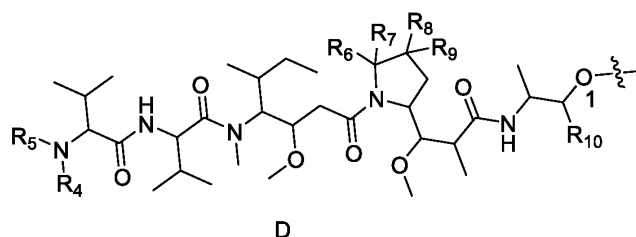
wherein:

the left wavy line indicates the site of attachment of the nitrogen atom to A in Formula (i), and the right wavy line indicates the site of attachment of the oxygen atom to D in Formula (i), where the oxygen atom is a common group shared by D and W;

R₁, R₂ and R₃ are each independently selected from hydrogen, deuterium, alkyl and a substituted alkyl;

p is an integer or a decimal between 1-20; and

D is a drug unit, wherein D is auristatin, its isomeric, endo-, racemic, enantiomeric, or mixtures thereof, or pharmaceutically acceptable salts or solvates thereof, having the structure shown in Formula D.



wherein:

R₄, R₅ are each independently hydrogen, deuterium, alkyl, and a deuterated alkyl group, or R₄, R₅ are linked to form the following structure: $-(\text{CR}_{11}\text{R}_{12})_n\text{-B-(CR}_{13}\text{R}_{14})_m-$, wherein R₁₁, R₁₂, R₁₃, and R₁₄ are independently hydrogen, deuterium, alkyl, or a deuterated alkyl group; B is O, NR₁₅, or CR₁₆R₁₇, wherein R₁₅, R₁₆, and R₁₇ are independently hydrogen, deuterium, or alkyl; n and m is independently an integer from 0-8; and R₄ and R₅ bonded nitrogen atom forms a ring with $-(\text{CR}_{11}\text{R}_{12})_n\text{-B-(CR}_{13}\text{R}_{14})_m-$,

R₆, R₇, R₈, R₉ are each independently hydrogen, deuterium, halogen, an azido group, alkyl and NR₁₈R₁₉, or any two of R₆, R₇, R₈, R₉ form a cycloalkyl group together with the atom to which they are bonded, and the remaining two are each independently hydrogen, halogen, an azido group, alkyl, or NR₁₈R₁₉, wherein R₁₈, R₁₉ are independently hydrogen, or alkyl;

R₁₀ is aryl or heteroaryl, each being optionally substituted with one or more substituents comprising hydrogen, halogen, alkyl, an alkoxy group, an amino group, or a nitro group,

the wavy line in Formula D indicates the site of attachment of the oxygen atom at position 1 in the structure of D to W, wherein the oxygen atom is a group shared by D and W.

2. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to Claim 1, wherein Ab comprises:

an IgG1 heavy chain,

a κ light chain, and

a single-chain Fv (scFv) domain, wherein the scFv domain is attached via a linker to the C-terminus or N-terminus of the IgG1 heavy chain, or the C-terminus or N-terminus of the κ light chain,

wherein Ab has a binding specificity to EGFR and HER3.

3. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to Claim 2, wherein the IgG1 heavy chain and the κ light chain form an IgG having a binding specificity for EGFR, and wherein the scFv domain has binding specificity for HER3.

4. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to Claim 2, wherein the scFv domain forms a construct with the IgG1 heavy chain or the κ light chain.

5. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to Claim 2, wherein the scFv domain has a structural order of N-terminal-heavy chain variable-region-connector-light chain variable-region-C-terminal or N-terminal-light chain variable-region-connector-heavy chain variable region-C-terminal.

6. The antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof according to Claim 2, wherein the linker comprises an amino acid sequence (gly-gly-gly-gly-gly-ser)_n, wherein n is an integer of at least 1, preferably n is an integer between 1 to 10, and wherein the connector comprises an amino acid sequence of (gly-gly-gly-gly-ser)_m, wherein m is an integer of at least 3, preferably m is 3, 4, 5, or 6.

7. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-6,

wherein Ab comprises a κ light chain having CDRs as shown in SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11, an IgG1 heavy chain comprising a CDR as shown in SEQ ID NO: 12, SEQ ID NO: 13, and SEQ ID NO: 14, and the scFv domain comprising heavy chain variable region CDRs as shown in SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 17, and light chain variable region CDRs as shown in SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20, or

wherein Ab comprises a κ light chain having CDRs as shown in SEQ ID NO:29, SEQ ID NO:30, and SEQ ID NO:31; an IgG1 heavy chain contains CDRs as shown in SEQ ID NO:32, SEQ ID NO:33, and SEQ ID NO:34; and an scFv domain comprising heavy chain variable region CDRs as shown in SEQ ID NO:35, SEQ ID NO:36, and SEQ ID NO:37, and light chain variable region CDRs as shown in SEQ ID NO:38, SEQ ID NO:39, and SEQ ID

NO:40.

8. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-7,

wherein Ab comprises a light chain having a variable region as shown in SEQ ID NO: 5, an IgG1 heavy chain having a variable region as shown in SEQ ID NO: 6, and a scFv domain having a heavy chain variable region as shown in SEQ ID NO: 7 and a light chain variable region as shown in SEQ ID NO: 8, or

wherein Ab comprises a light chain having a variable region as shown in SEQ ID NO:25; an IgG1 heavy chain having a variable region as shown in SEQ ID NO:26; and a scFv domain having a heavy chain variable region as shown in SEQ ID NO:27 and a light chain variable region as shown in SEQ ID NO:28.

9. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-8,

wherein Ab comprises a light chain having an amino acid sequence of SEQ ID NO: 2, and a construct comprising a heavy chain and a scFv domain, wherein the construct has an amino acid sequence of SEQ ID NO: 4, or

wherein Ab comprises a light chain having an amino acid sequence of SEQ ID NO:22, and a construct comprising a heavy chain and a scFv domain, wherein the construct has an amino acid sequence of SEQ ID NO:24.

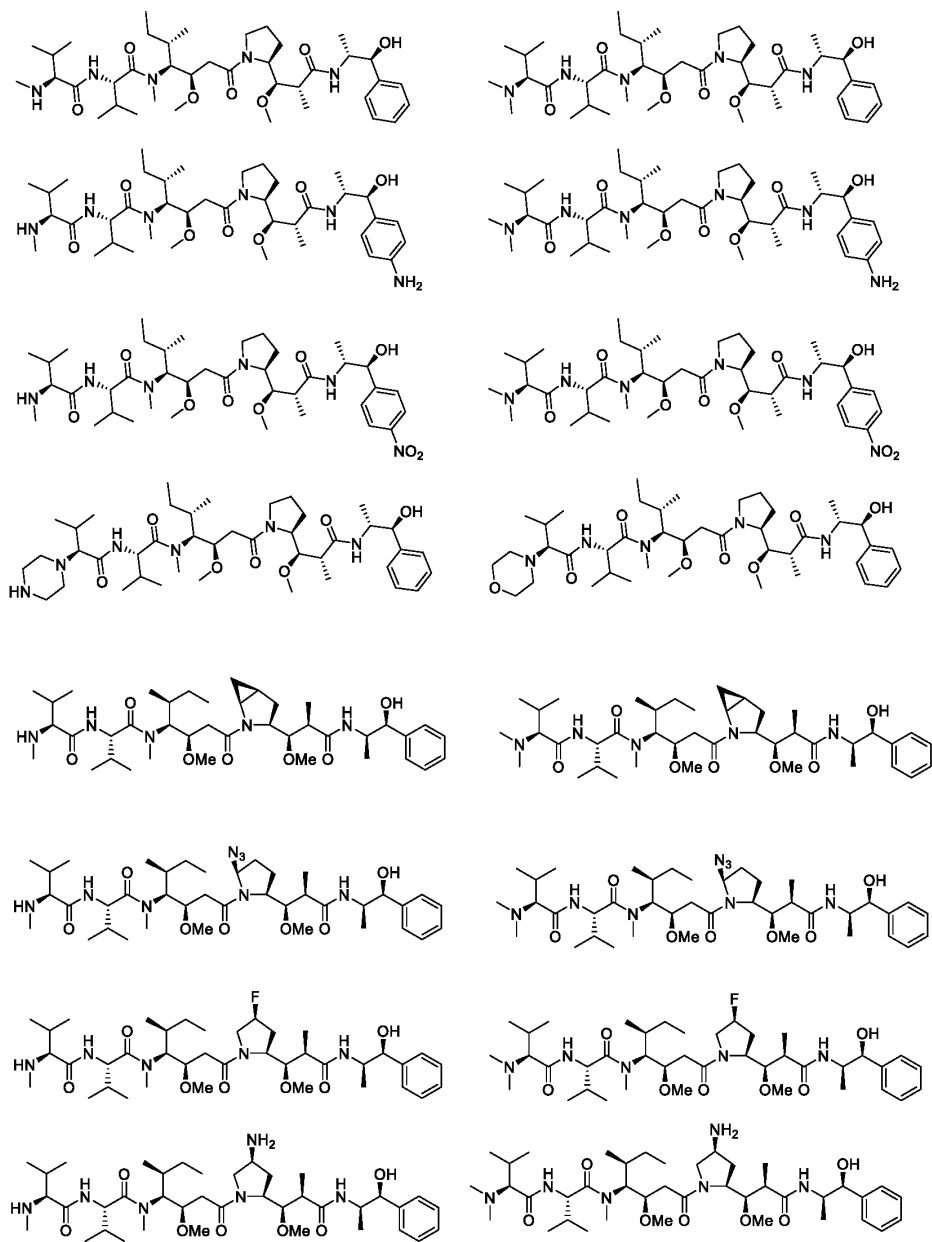
10. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-9, wherein Ab comprises: two IgG1 heavy chains; two κ light chains; and two scFv domains, wherein each of the scFv domains is linked to each of the IgG heavy chain at its C-terminus.

11. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-10, wherein R_4 , R_5 are each independently hydrogen, $C_1 - C_4$ alkyl, or R_4 , R_5 are linked to form $-(CH_2)_2-B-(CH_2)_2-$, B is O or NH, wherein the nitrogen atom bonded to R_4 and R_5 form a ring with $-(CH_2)_2-B-(CH_2)_2-$.

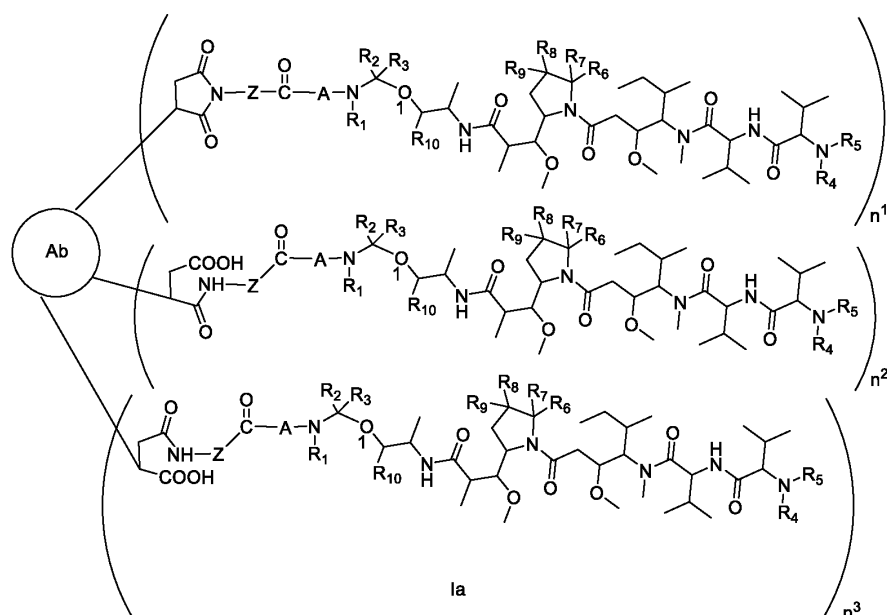
12. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-11, wherein R_6 , R_7 , R_8 , R_9 in Formula D are each hydrogen; alternatively, one of R_6 , R_7 , R_8 , R_9 in Formula D is halogen, an azido group, or an amino group, and the remaining three are each hydrogen; alternatively, any two of R_6 , R_7 , R_8 , R_9 in Formula D form a cyclopropyl group together with the atoms to which they are bonded, and each of the remaining two groups is independently hydrogen.

13. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-12, wherein R_{10} in Formula D is phenyl, wherein, optionally the phenyl is substituted with one or more of an amino group or a nitro group, or

wherein D is selected from the following compounds, or its isomeric, endo-, racemic, enantiomeric, or mixtures thereof, or pharmaceutically acceptable salts or solvates thereof:



14. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-13, wherein the antibody-drug conjugate having the structure shown in Formula Ia:



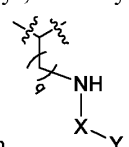
wherein:

Z is -C₁-C₁₀ alkylene-, -C₃-C₈ carbocyclic-, -arylidene-, -C₁-C₁₀ alkylene-arylidene-, -arylidene-C₁-C₁₀-alkylene-, -C₁-C₁₀ alkylene-(C₃-C₈ carbocyclic)-, -(C₃-C₈ carbocyclic)-C₁-C₁₀ alkylene-, -3-8 heterocyclic-, -C₁-C₁₀ alkylene- (3-8 heterocyclic)-, -(3-8 heterocyclic)-

C₁-C₁₀ alkylene-, -(CH₂CH₂O)_r-, -(CH₂CH₂O)_r-CH₂- or

X is -C₁-C₁₀ alkyl-, -C₃-C₈ carbocyclic-, -aryl-, -C₁-C₁₀ alkyl-aryl-, -aryl-C₁-C₁₀-alkyl-, -C₁-C₁₀ alkyl-(C₃-C₈ carbocyclic)-, -(C₃-C₈ carbocyclic)-C₁-C₁₀ alkyl-, -3-8- heterocyclic-, -C₁-C₁₀ alkyl-(3-8 heterocyclic)-, -(3-8- heterocyclic)-C₁-C₁₀ alkylene-, -(CH₂CH₂O)_r-, -(CH₂CH₂O)_r-CH₂-;

Y is a hydrophilic structure comprising a carboxylic acid group, a phosphoric acid group, a polyphosphoric acid group, a phosphite group, a sulphonic acid group, a sulfenic acid group, or poly(ethylene glycol) (PEG); the heterocyclic ring each independently comprise 1-3 atoms selected from N, O, and S; said -C₁-C₁₀ alkylene-, -C₃-C₈ carbocyclic -, and heterocyclic are each independently substituted with one or more substituents comprising deuterium, halogen, hydroxyl, cyano group, nitro group, amine, alkyl, heteroalkyl, substituted alkyl, alkoxy, carboxyl, or cycloalkyl;

the left wavy line in  denotes the attachment site to N on the maleimide, and the right wavy line denotes the attachment site to the carbonyl group; r is an integer from 1-10; q is an integer from 1-8;

n¹, n², n³ are independently an integer or decimal between 0 and 20, n¹, n², n³ are not simultaneously 0 and n¹ + n² + n³ ≤ 20.

15. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof

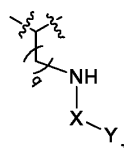
according to any one of Claims 1-14, wherein A comprises,

2-7 amino acids selected from phenylalanine (F), glycine (G), valine (V), lysine (K), alanine (A), citrulline, serine (S), glutamic acid (E), and aspartic acid (D),

2-4 amino acids selected from phenylalanine and glycine, or
a tetrapeptide residue glycine-glycine-phenylalanine-glycine.

16. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to Claim 14 or 15, wherein Z is $-C_1-C_{10}$ alkylene-, $-C_4-C_6$ alkylene-, or $-C_5$ alkylene-, or

Z is



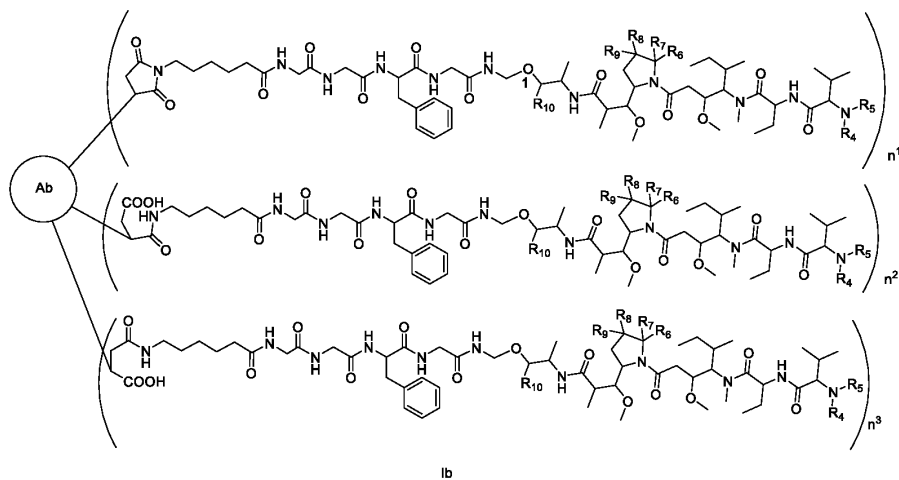
wherein q is an integer between 1 and 8.

17. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of Claims 14-16, wherein R_1 , R_2 and R_3 are each independently hydrogen, deuterium, alkyl, an alkyl halide group, an alkyl deuteride group, or a hydroxyalkyl group,

R_1 , R_2 and R_3 are all hydrogen or deuterium, or

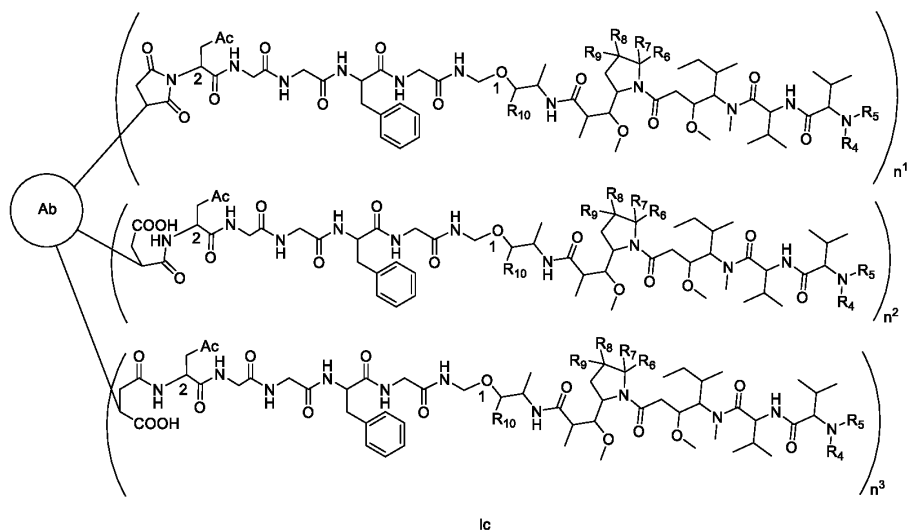
R_1 , R_2 and R_3 are all hydrogen.

18. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-17, wherein the antibody-drug conjugate has a structure as shown in Formula Ib:

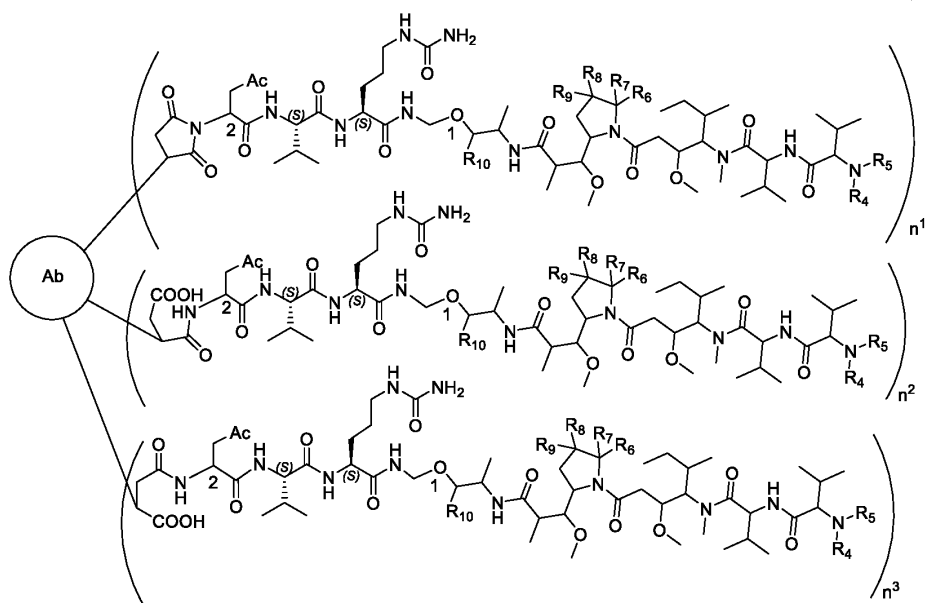


Ib

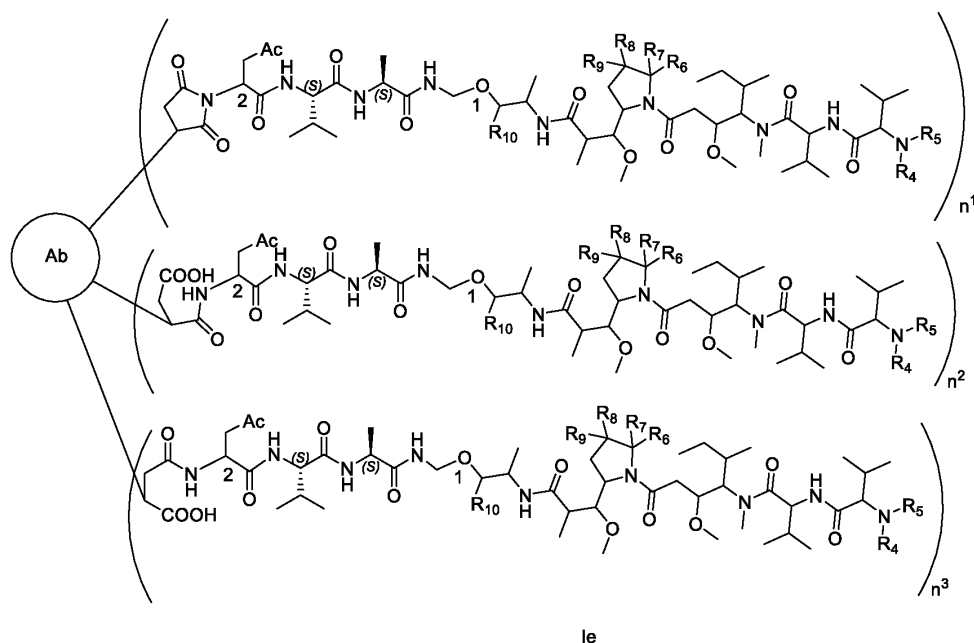
19. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-18, wherein the antibody-drug conjugate comprises a structure as shown in Formula Ic, Formula Id, or Formula Ie:



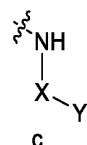
Ic



Id

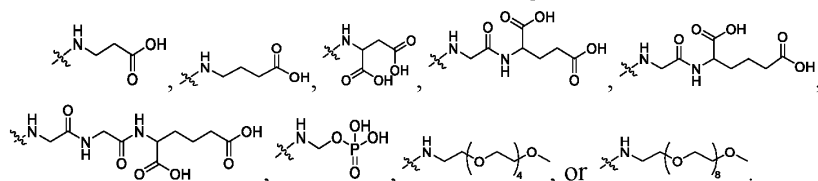


wherein Ac is a hydrophilic structural unit having the structure shown in Formula c:

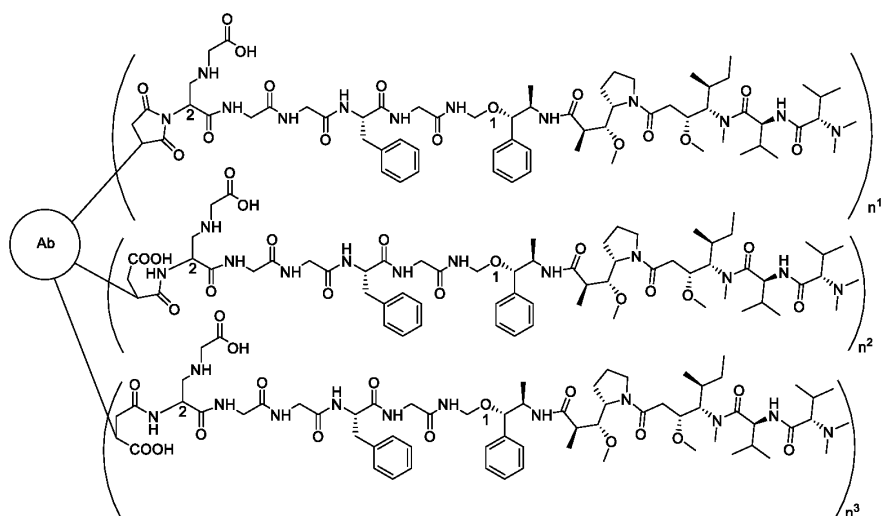


wherein Ac is linked to methylene carbon at the position 2 as labeled via an amino group, and X, Y are as defined in Claim 11, or

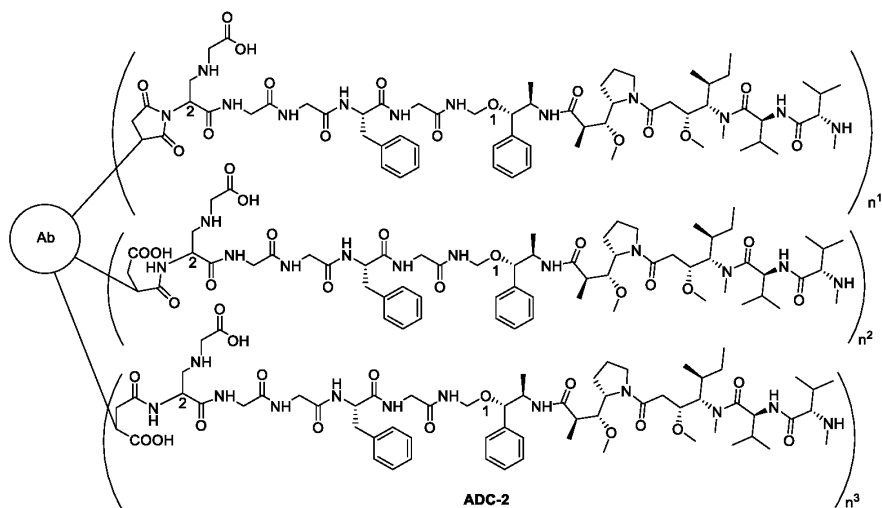
Ac is selected from glycine, (D/L) alanine, (D/L) leucine, (D/L) isoleucine, (D/L) valine, (D/L) phenylalanine, (D/L) proline, (D/L) tryptophan, (D/L) serine, (D/L) tyrosine, (D/L) cysteine, (D/L) cystine, (D/L) arginine, (D/L) histidine, (D/L) methionine, (D/L) asparagine, (D/L) glutamine, (D/L) threonine, (D/L) aspartic acid, (D/L) glutamic acid, natural or non-natural amino acid derivatives, or the following structures:



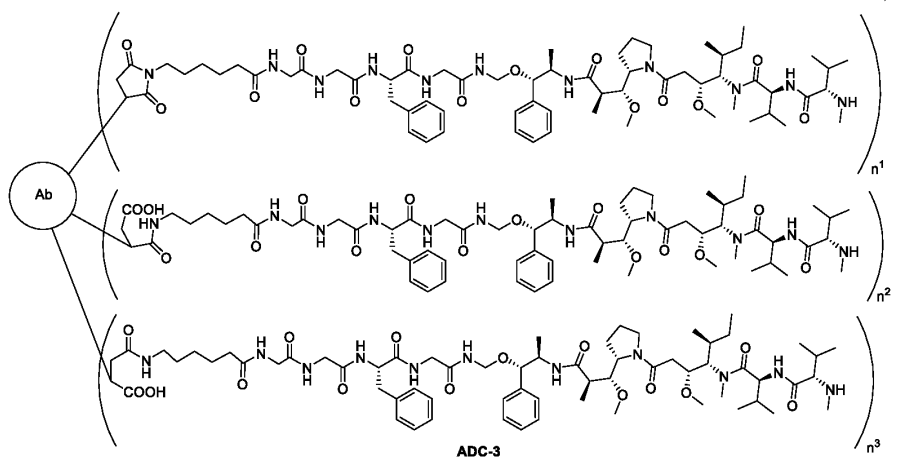
20. The antibody-drug conjugate or pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-19, the antibody-drug conjugate being selected from the following structures:



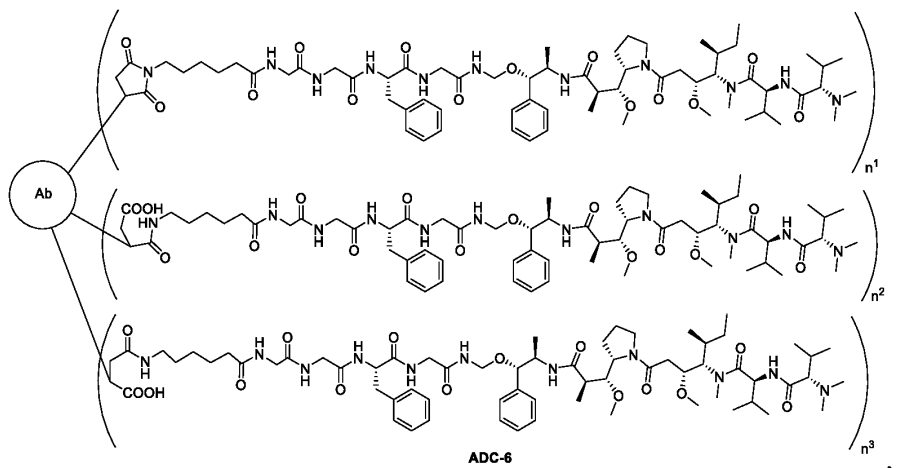
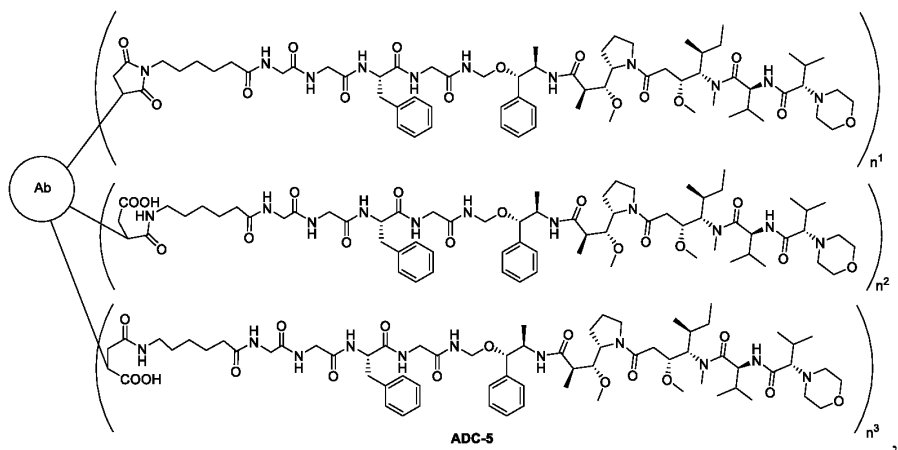
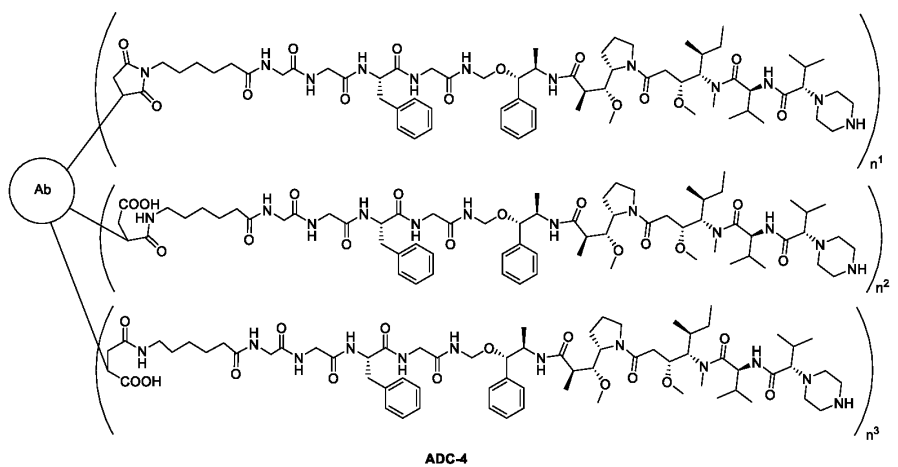
ADC-1

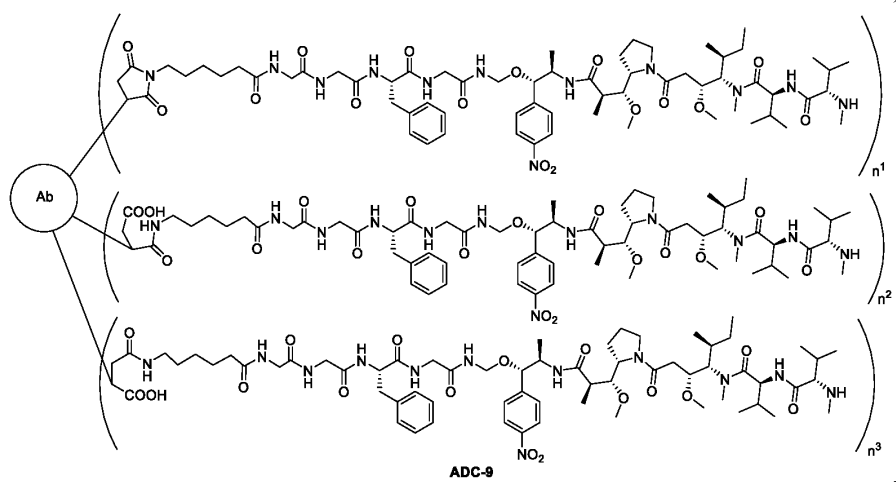
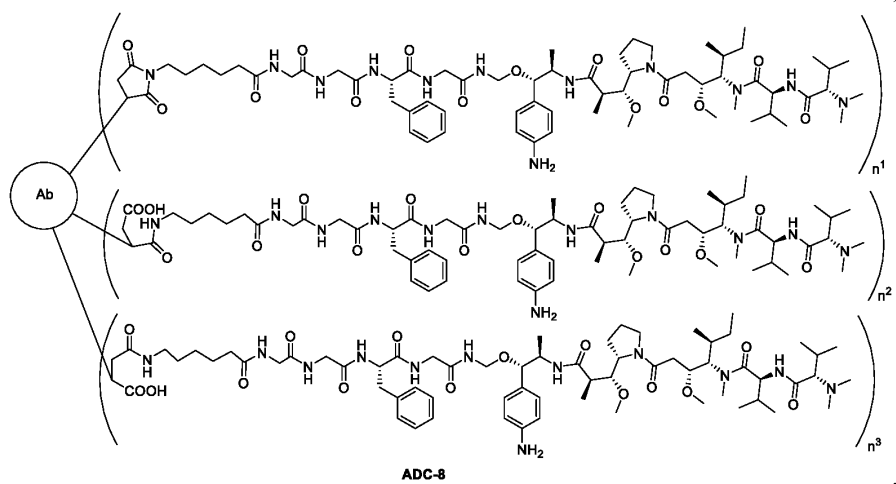
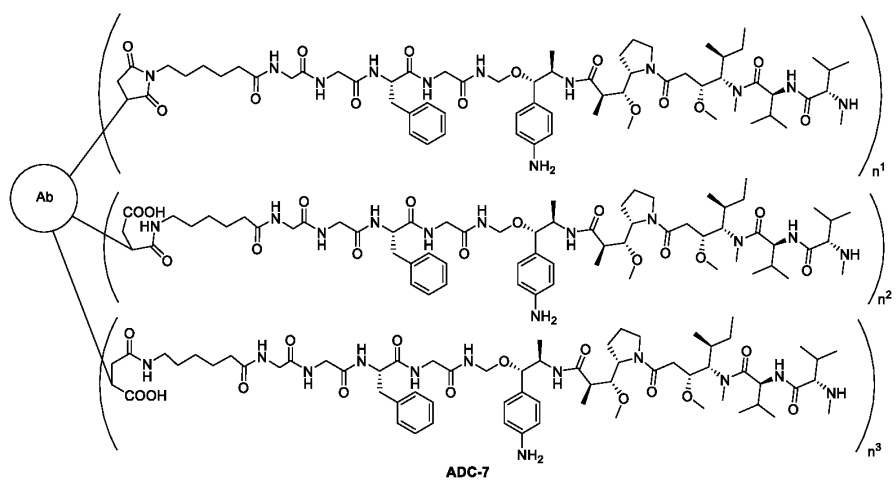


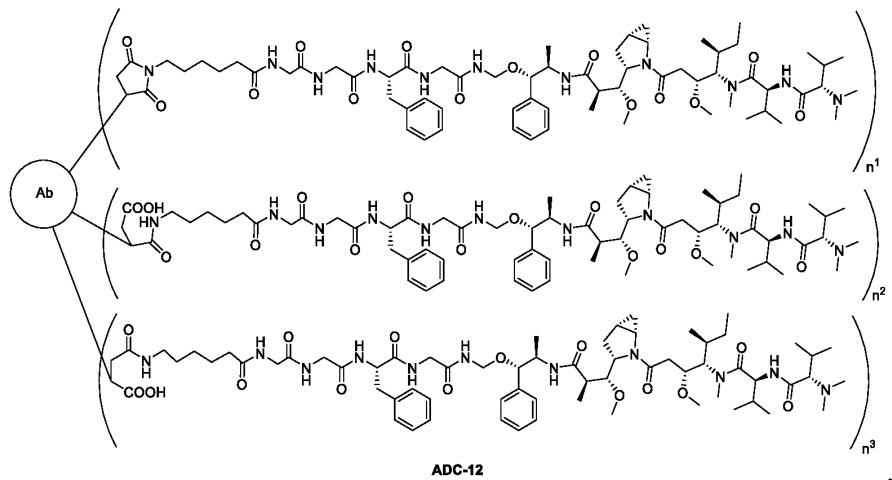
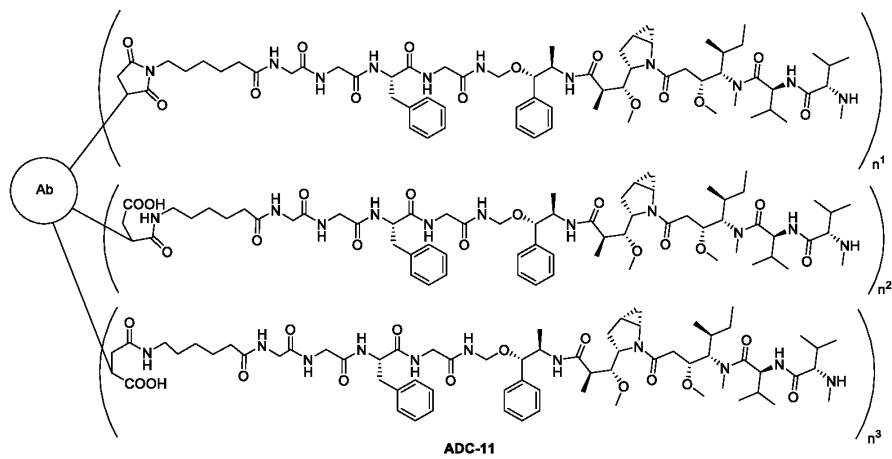
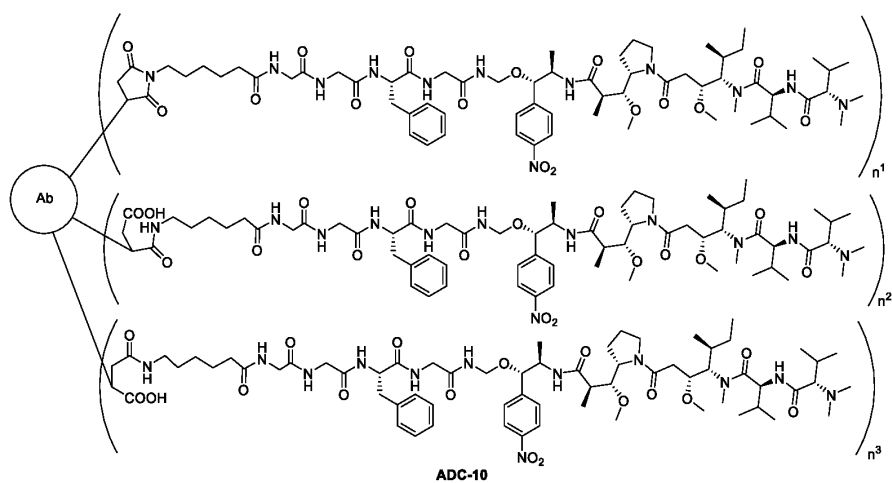
ADC-2

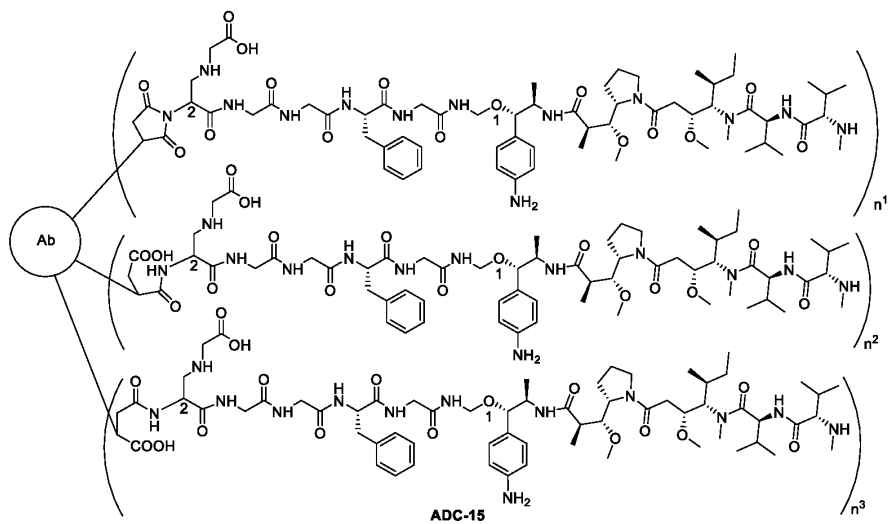
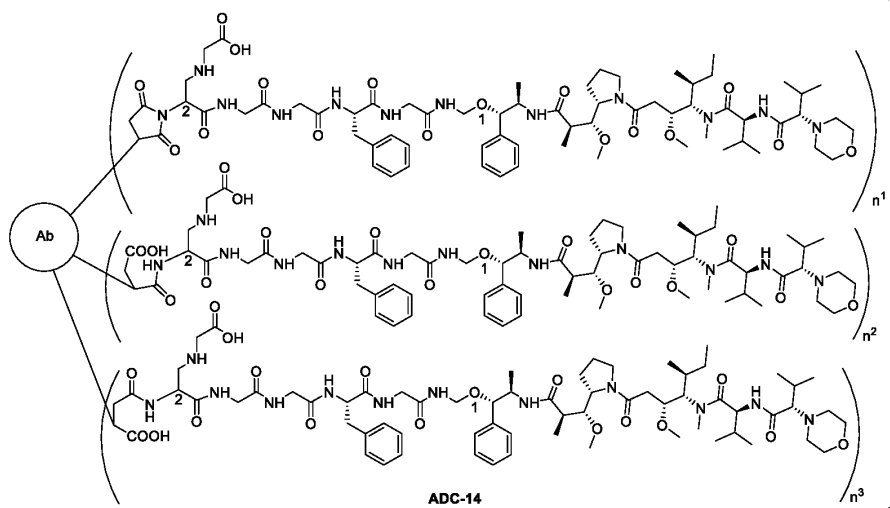
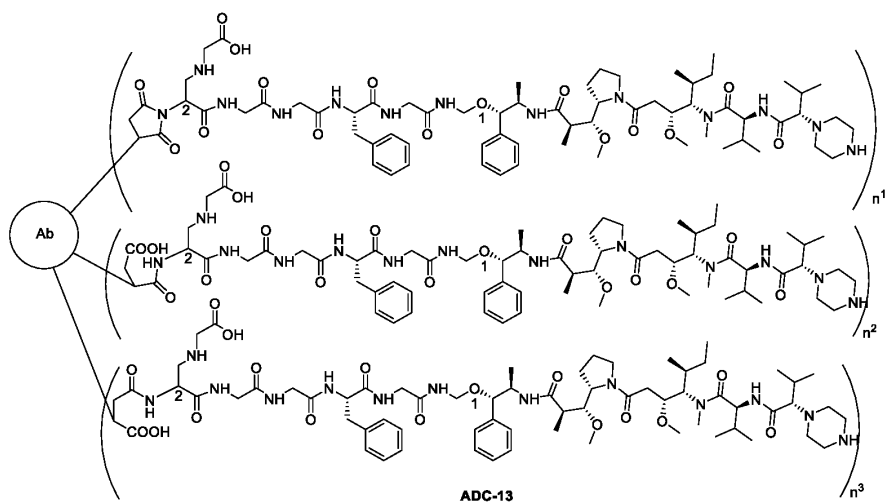


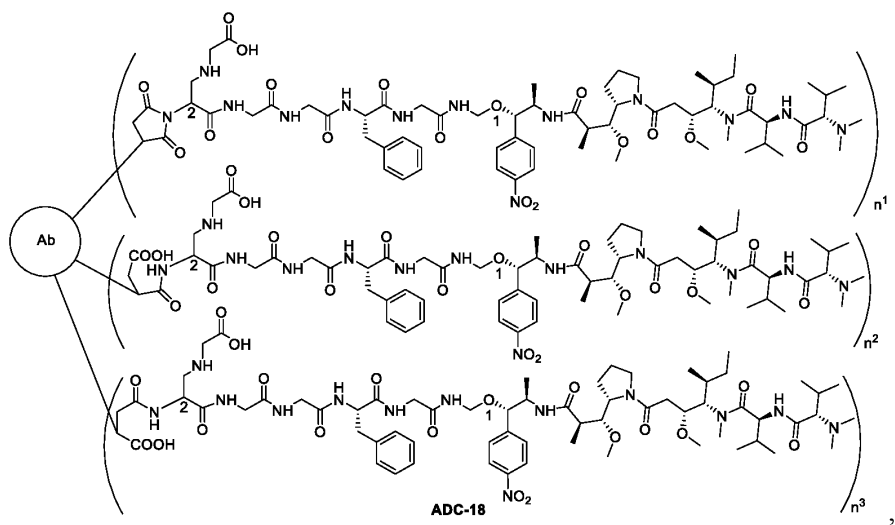
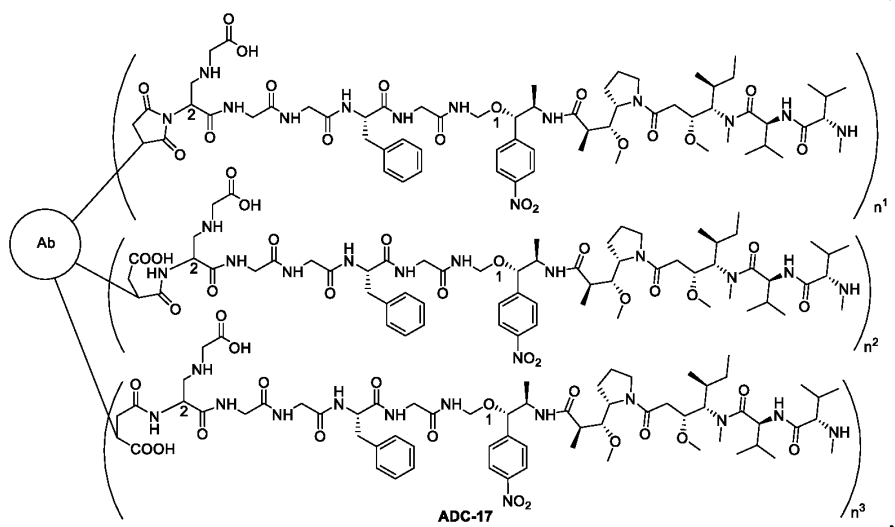
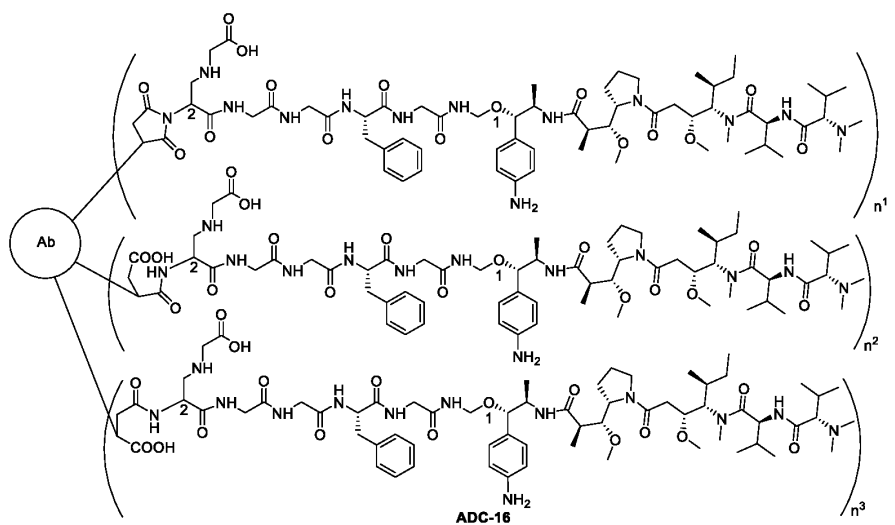
ADC-3

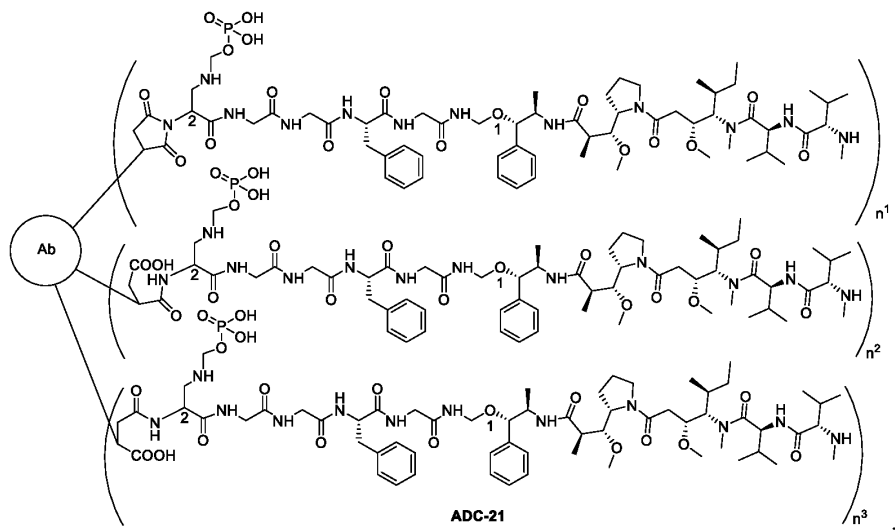
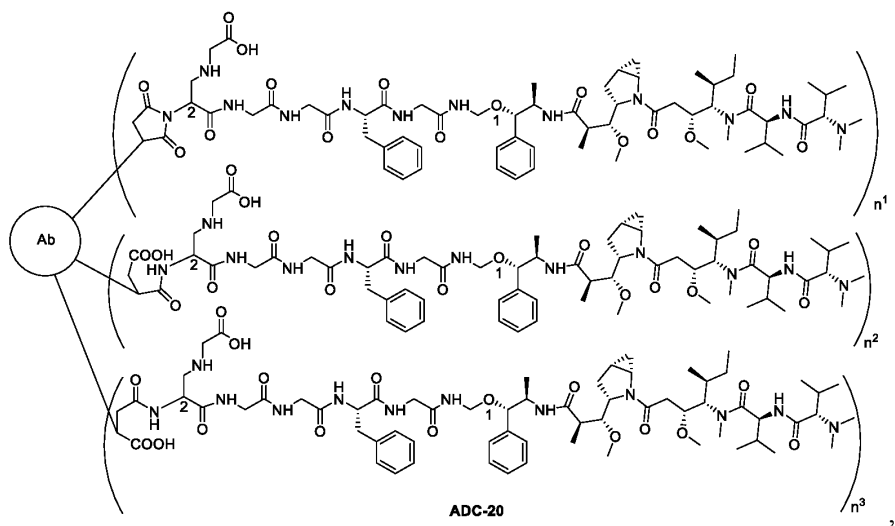
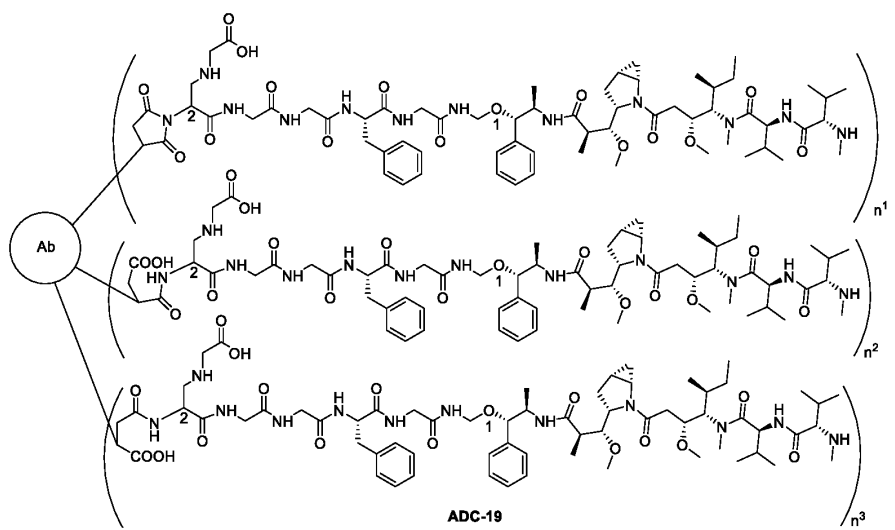


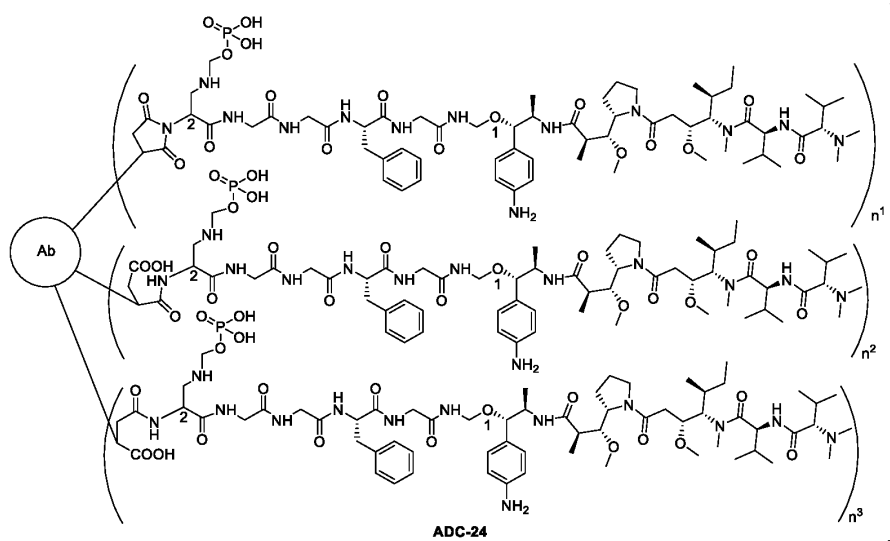
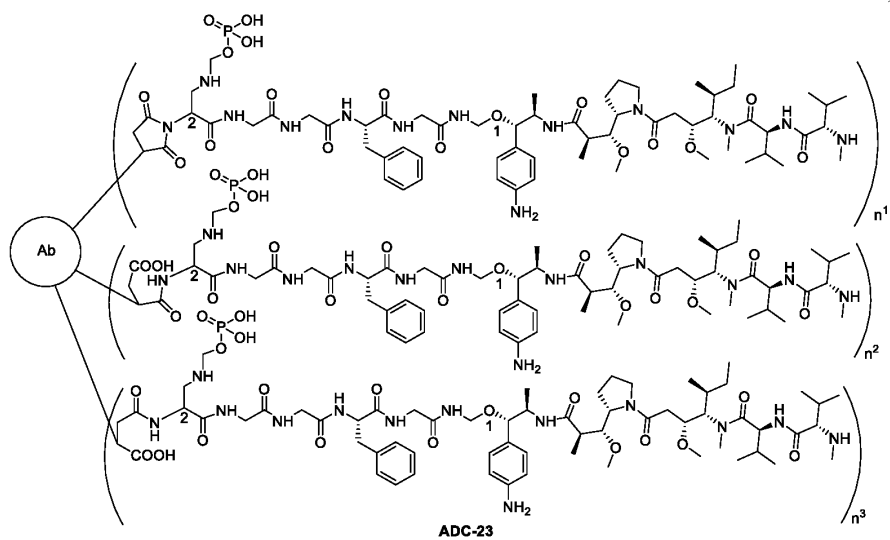
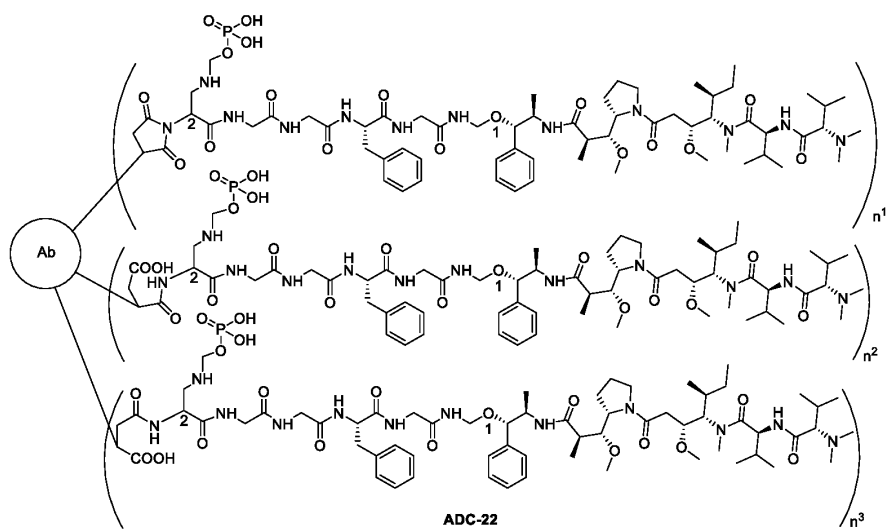


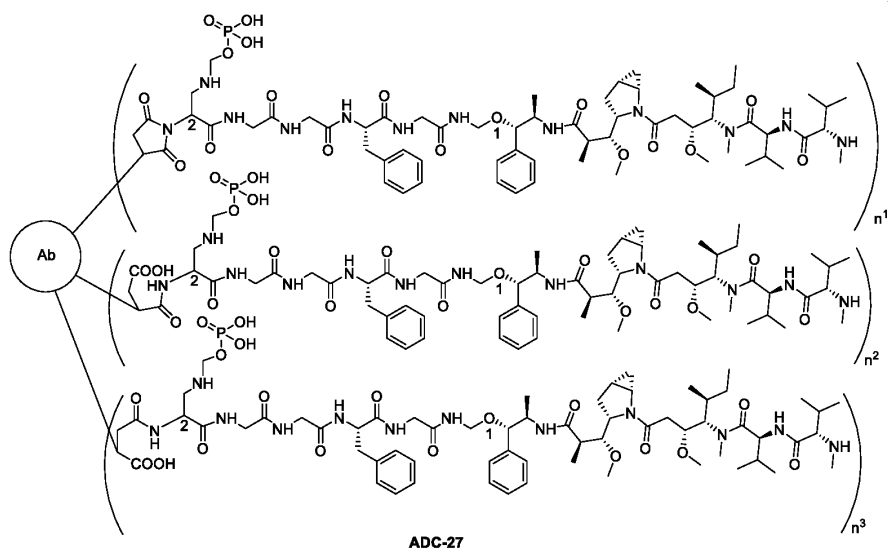
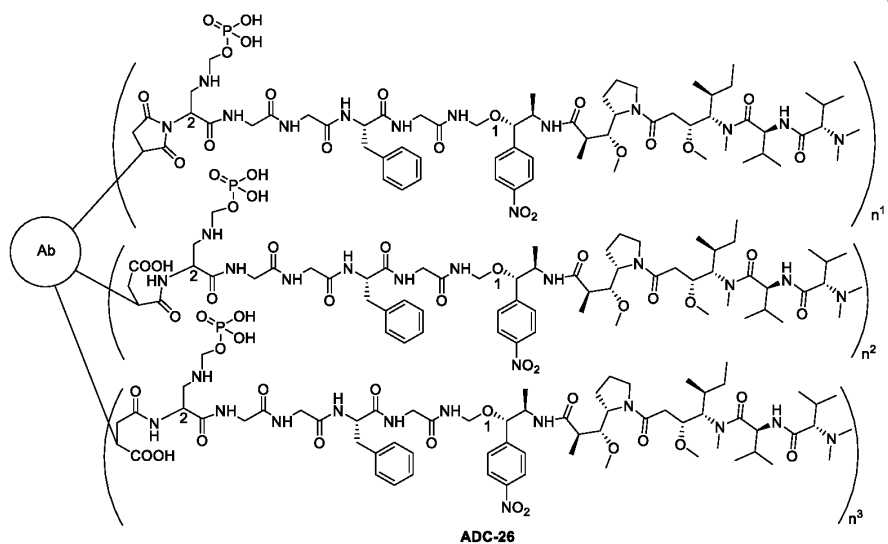
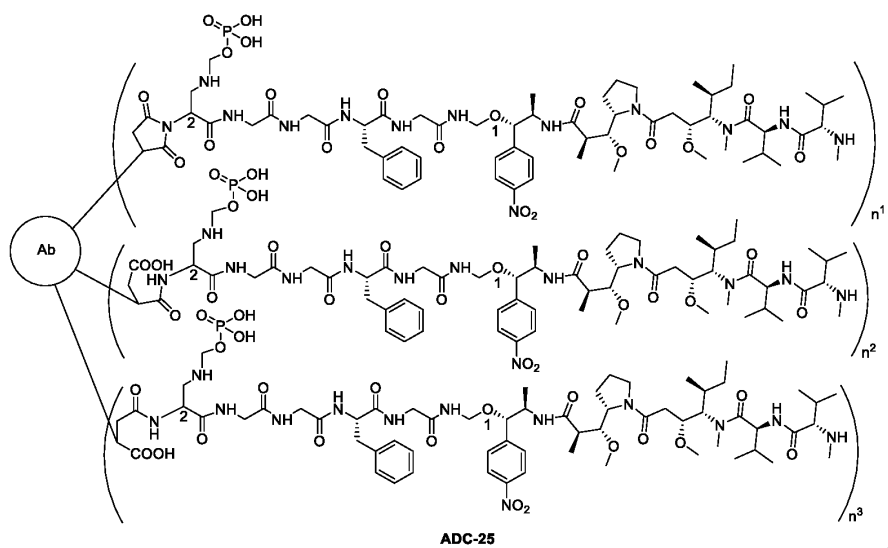


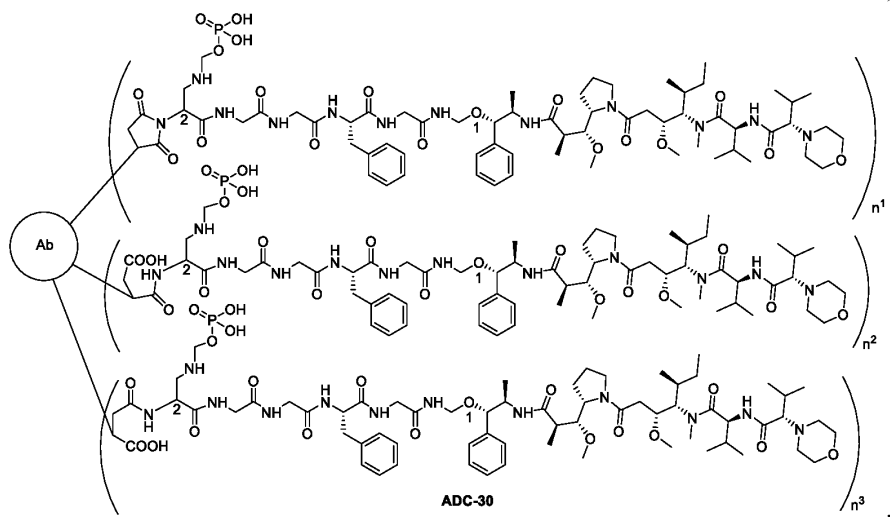
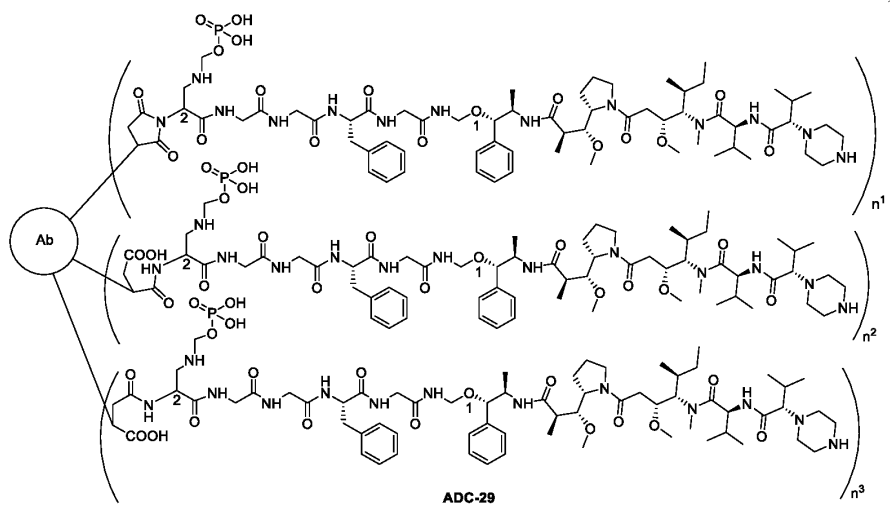
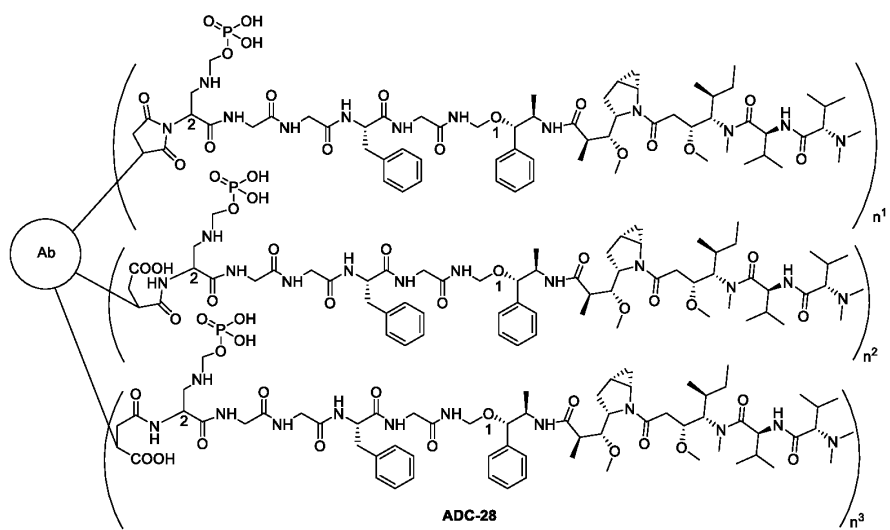


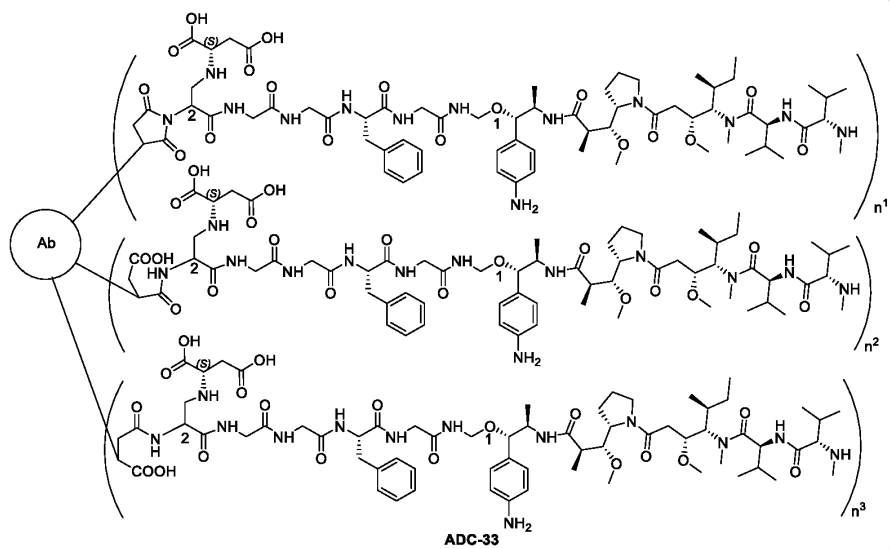
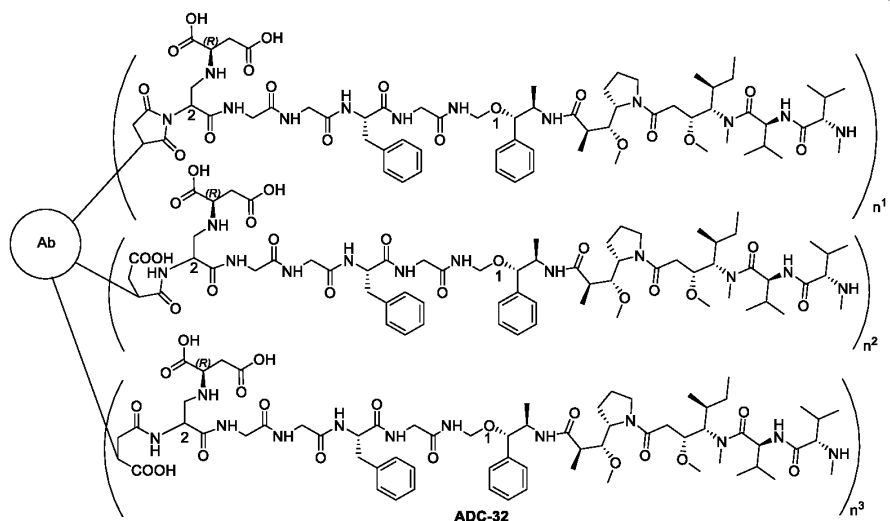
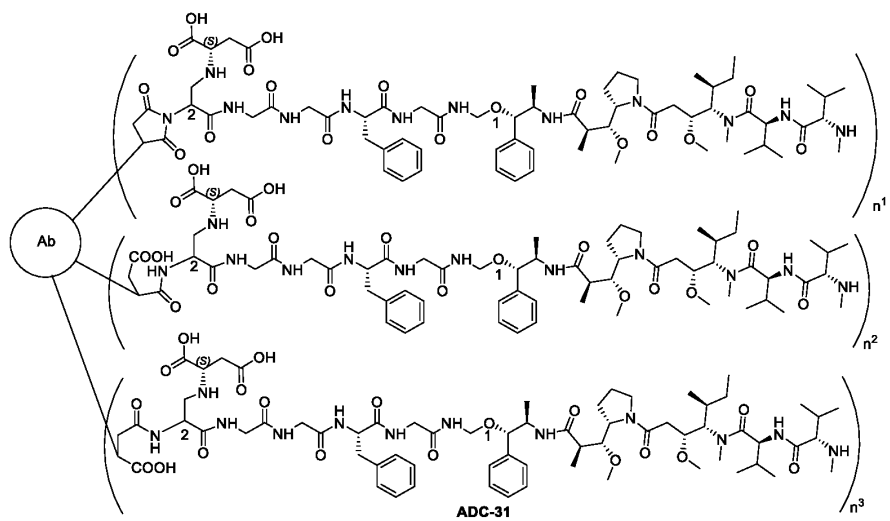


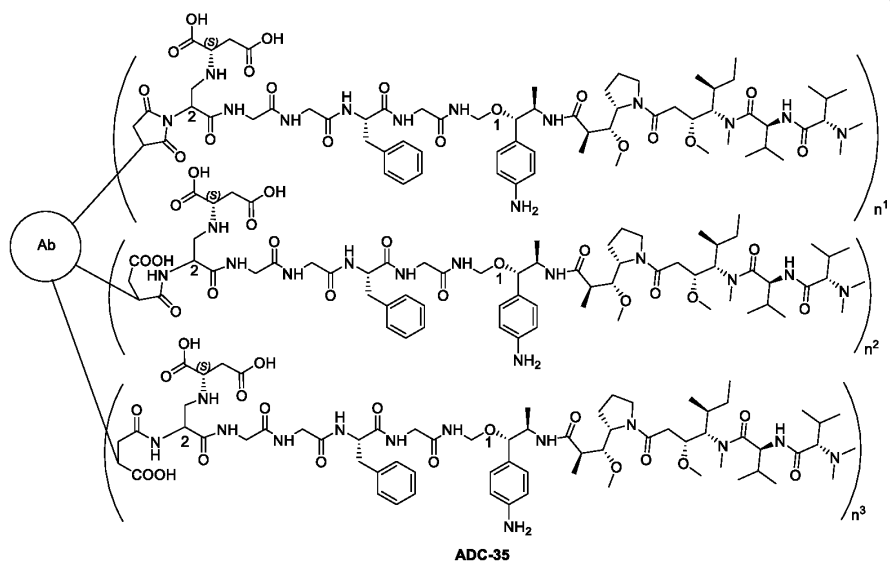
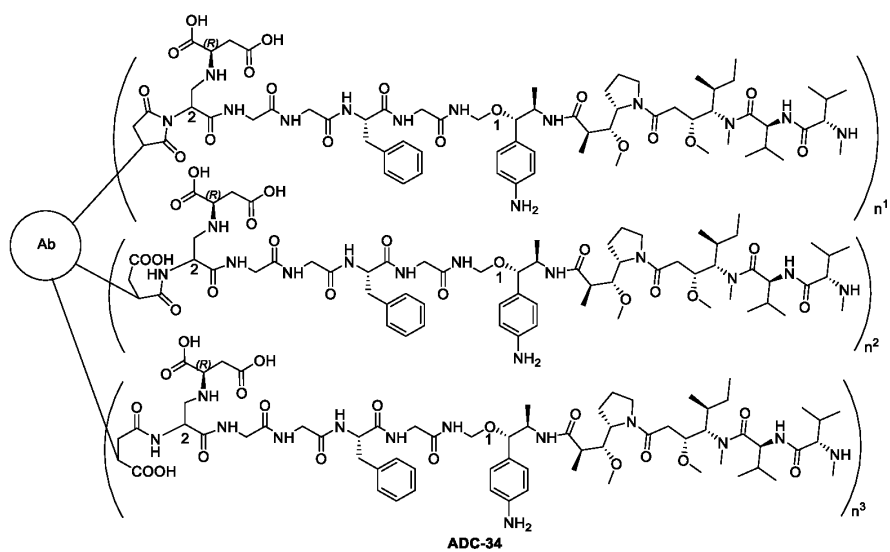


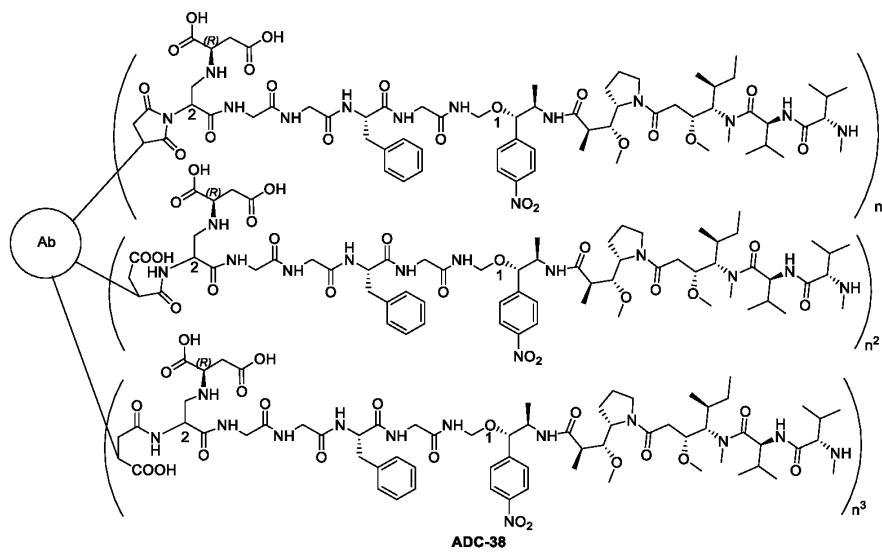
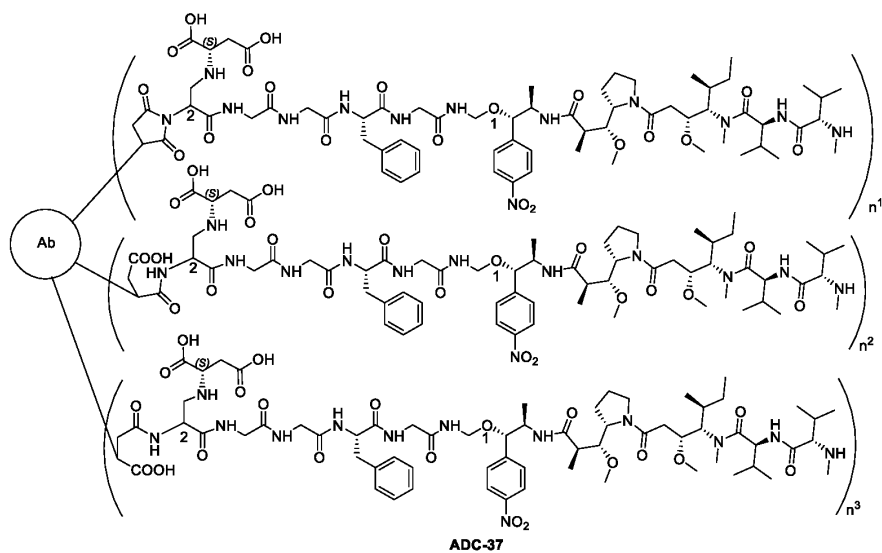
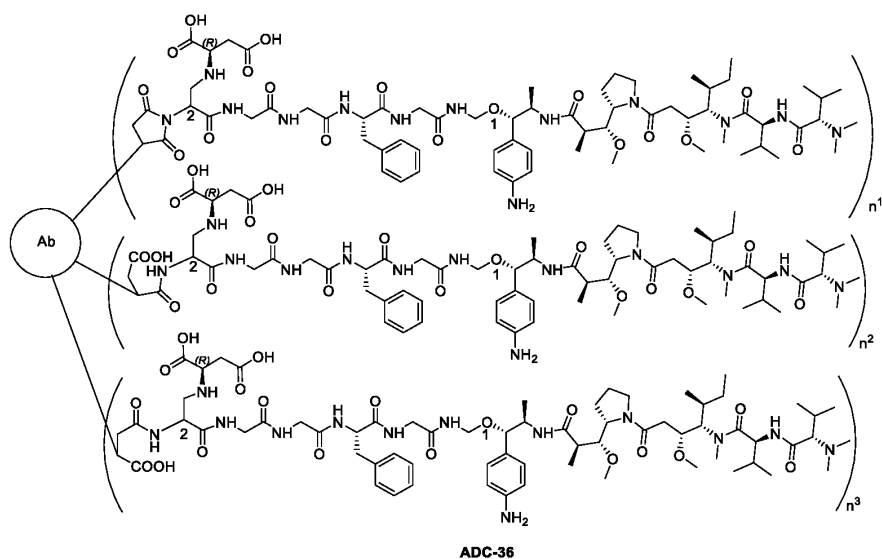


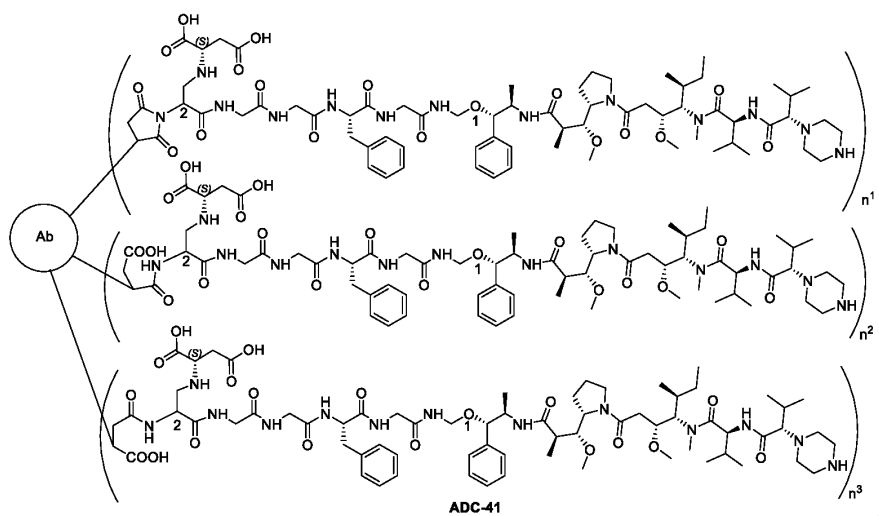
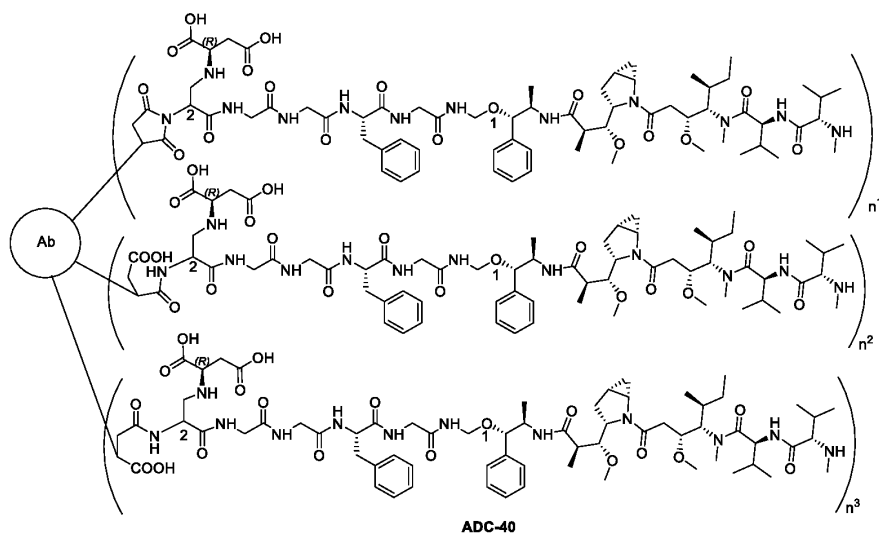
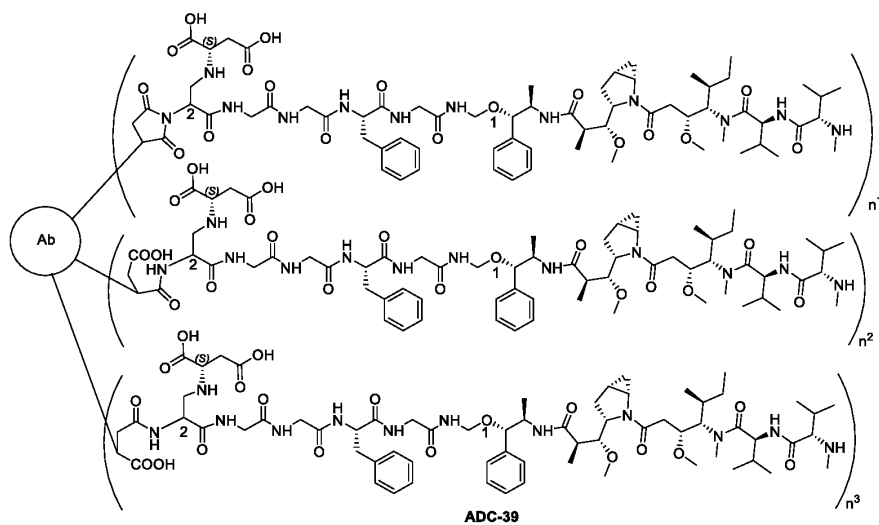


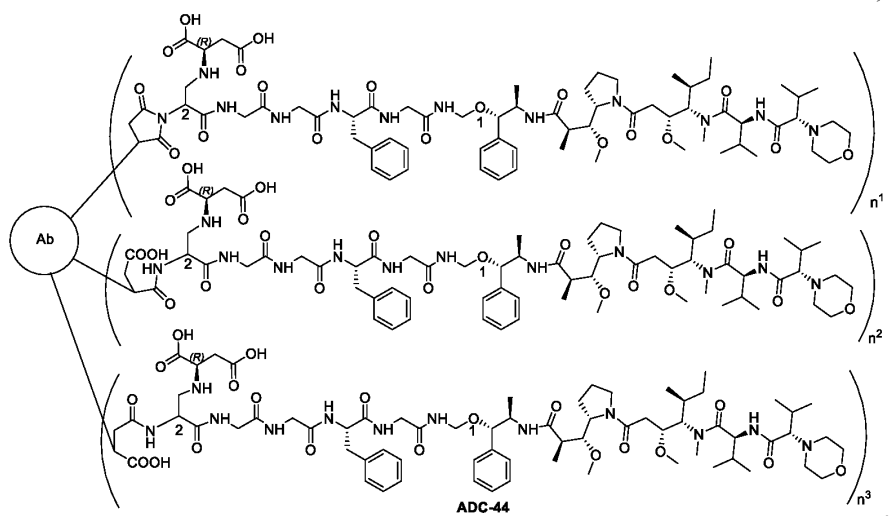
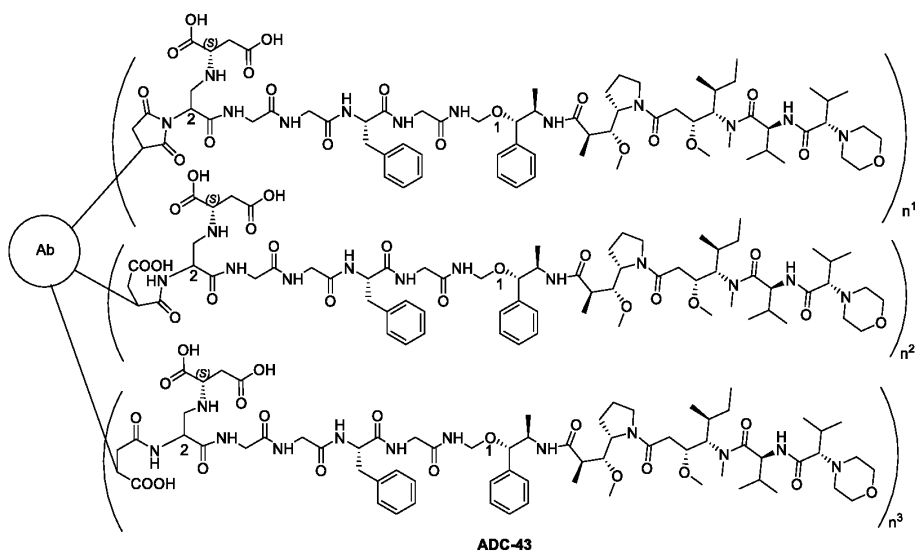
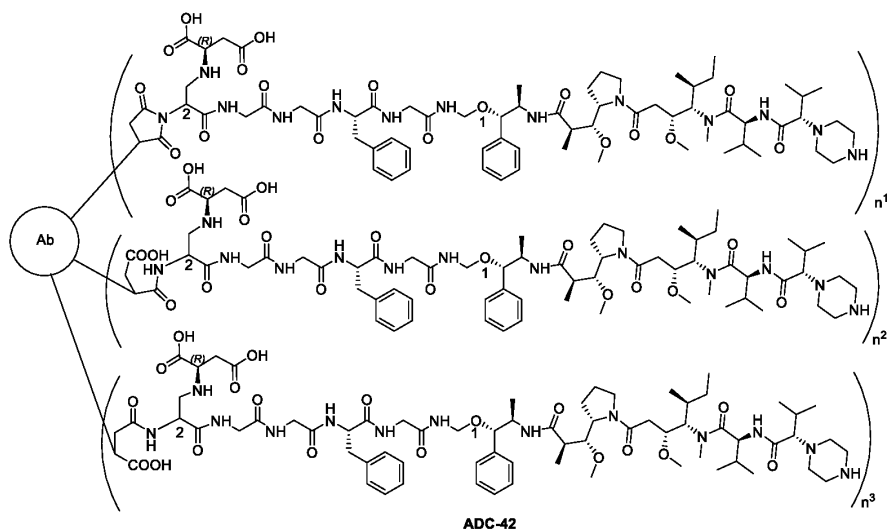


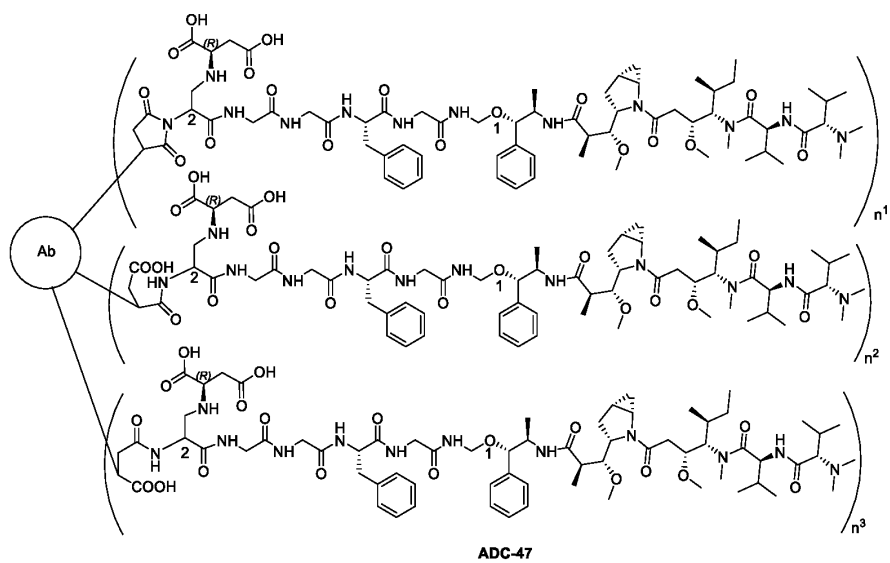
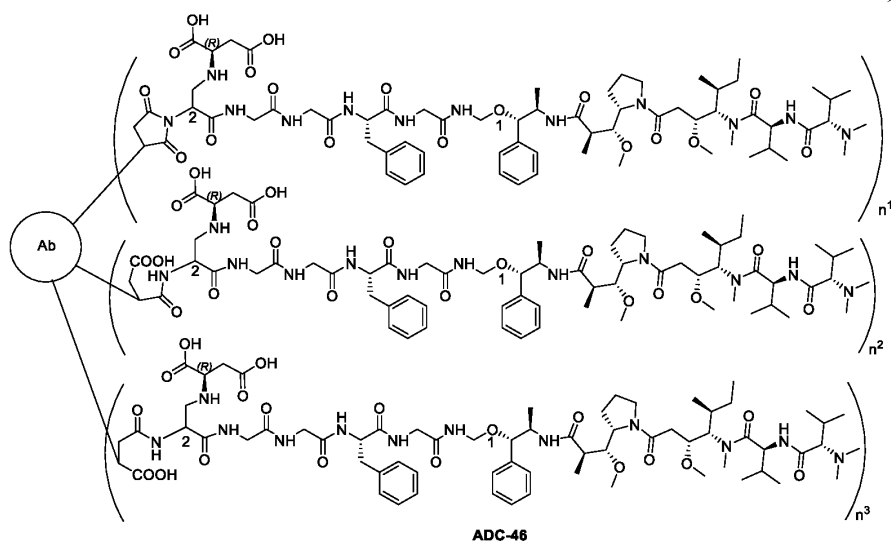
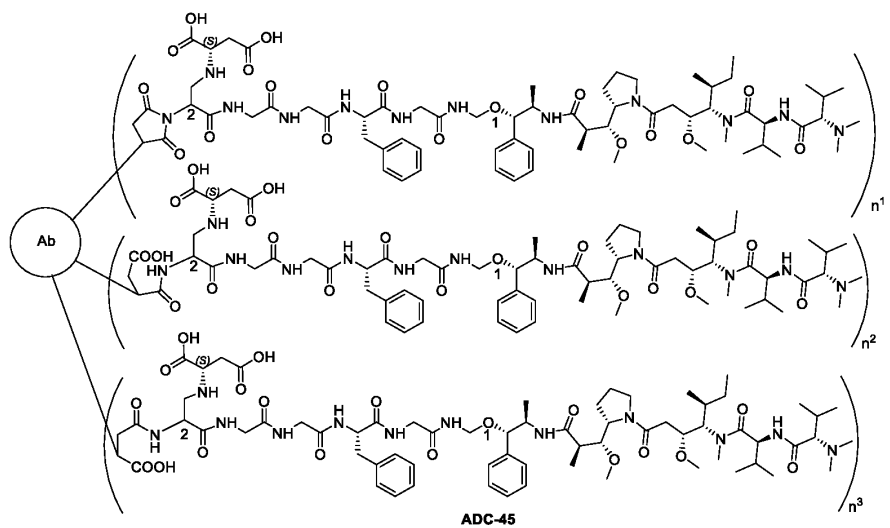


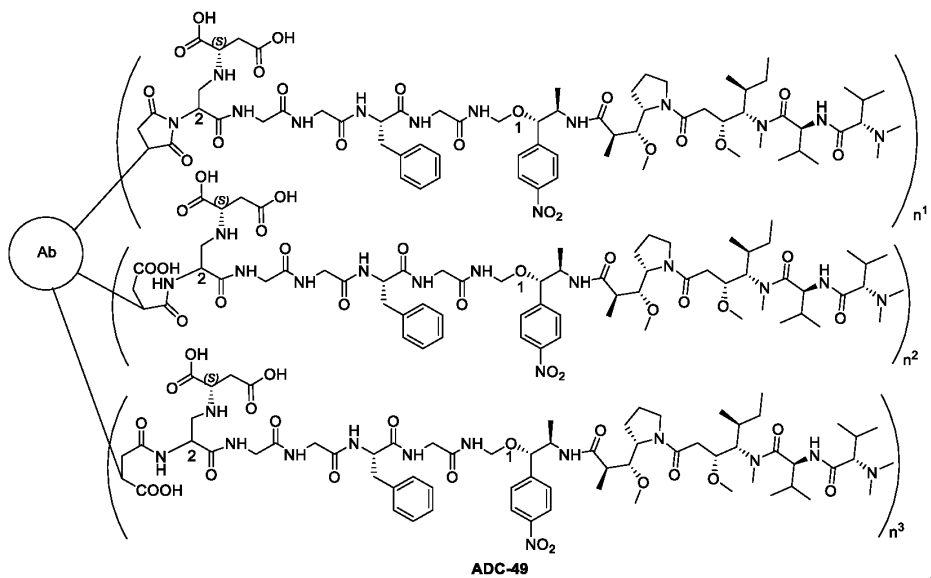
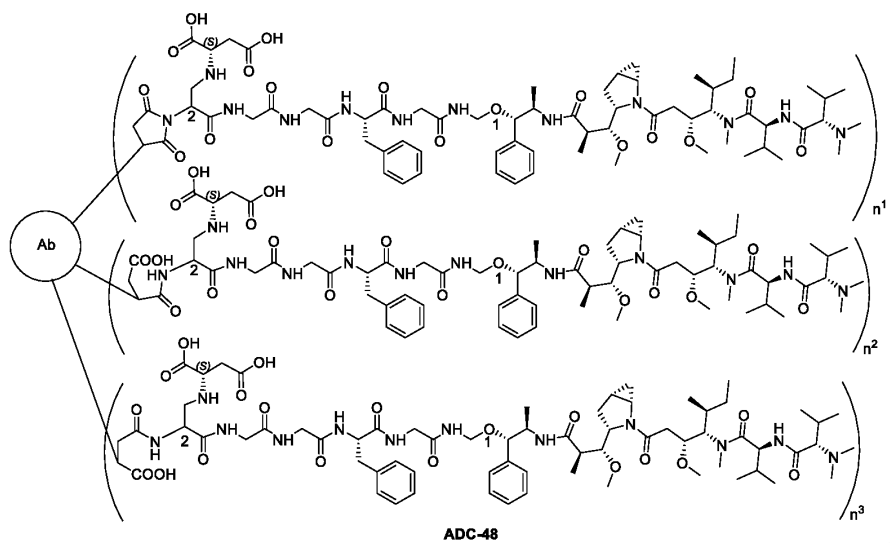




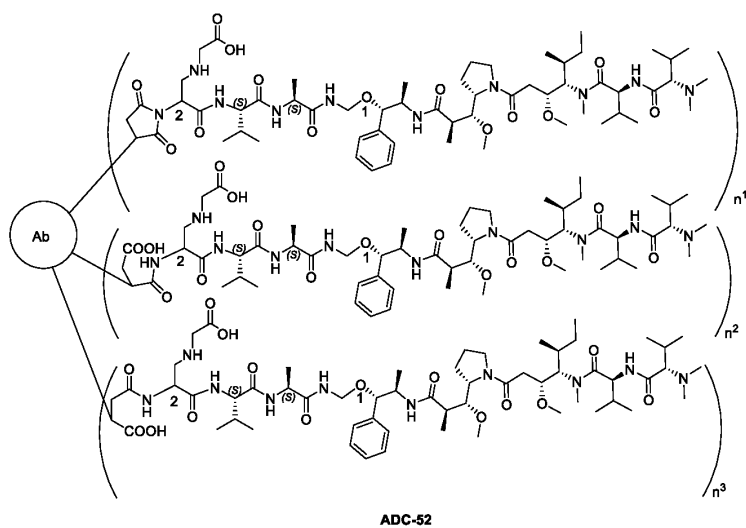
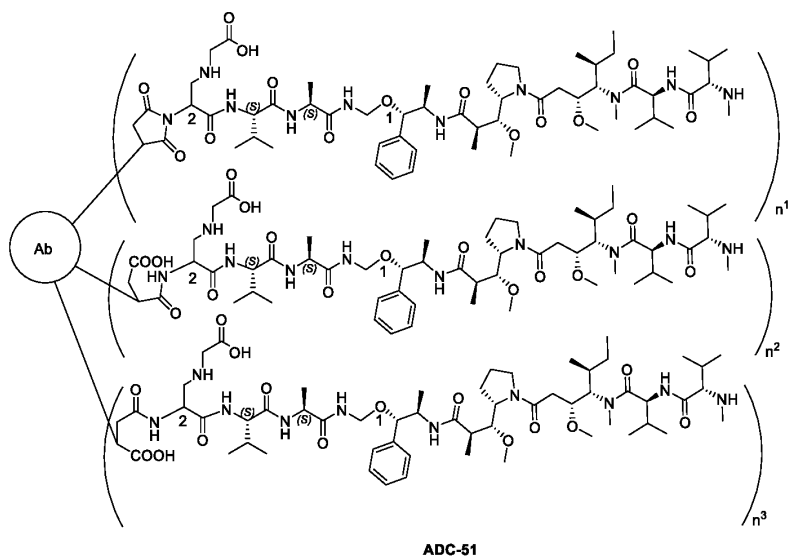
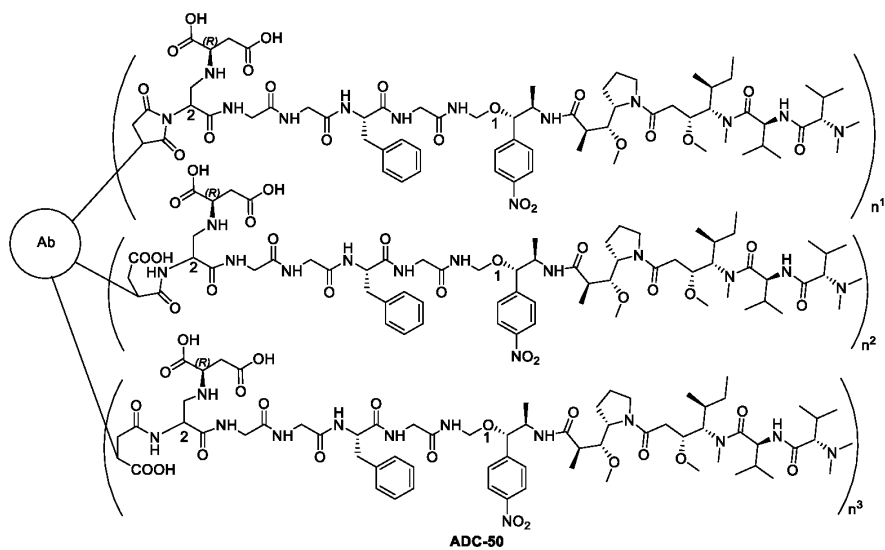


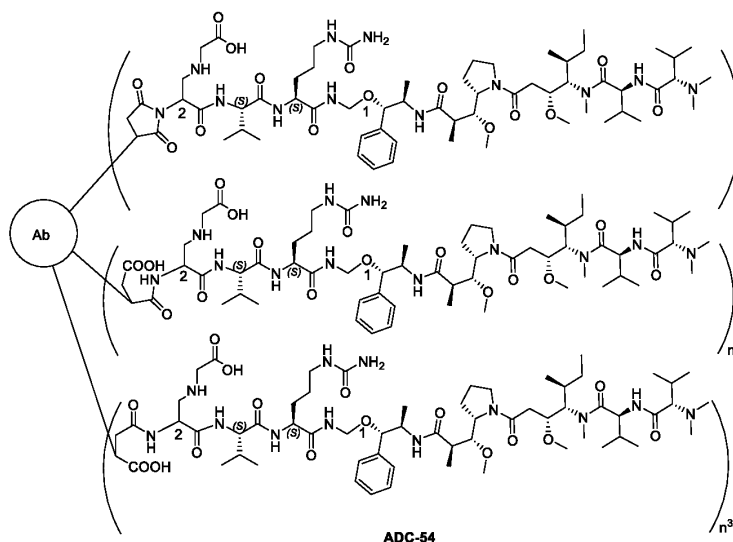
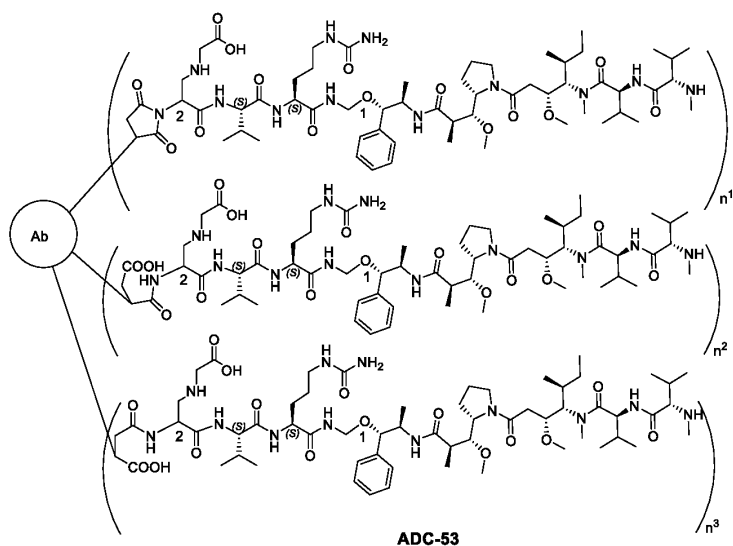






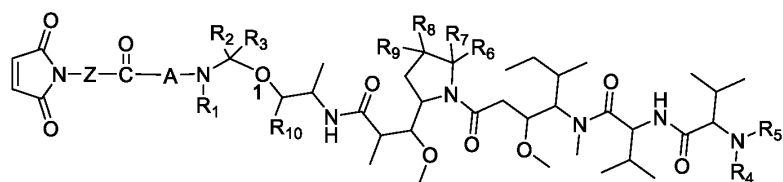
, OR





wherein the configuration of the chiral carbon at the position 2 is R or S.

21. A linker-drug compound as shown in Formula II, its isomeric, endo-, racemic, enantiomeric, or mixtures thereof, or pharmaceutically acceptable salts or solvates thereof,



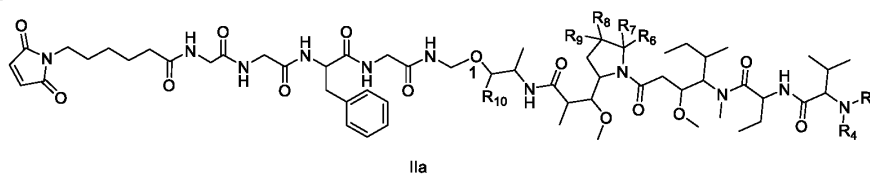
II

wherein:

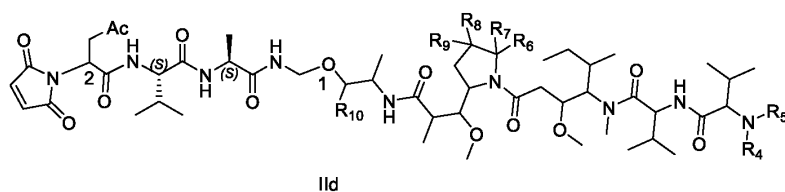
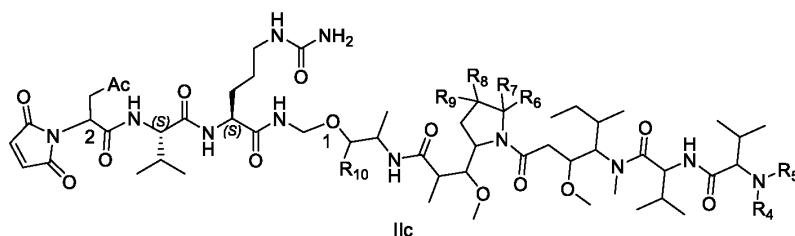
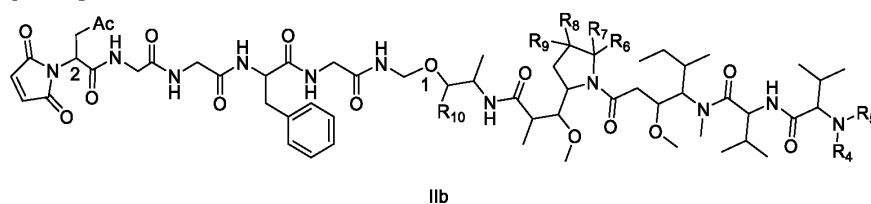
Z, A, R₁, R₂, R₃, R₄, R₅, R₁₁, R₁₂, R₁₃, R₁₄, B, R₁₅, R₁₆, R₁₇, n, m, R₆, R₇, R₈, R₉, R₁₈, R₁₉, R₁₀ are each defined in any one of Claims 1-13.

22. The linker-drug compound of Claim 21, its isomeric, endo-, racemic, enantiomeric, or mixtures thereof, or pharmaceutically acceptable salts or solvates thereof, wherein the linker-

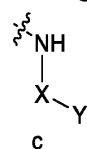
drug compound has the structure shown in Formula IIa:



23. The linker-drug compound of Claim 22, its isomeric, endo-, racemic, enantiomeric, or mixtures thereof, or pharmaceutically acceptable salts or solvates thereof, wherein the linker-drug compound has the structure shown in Formula IIb, Formula IIc or Formula IId:

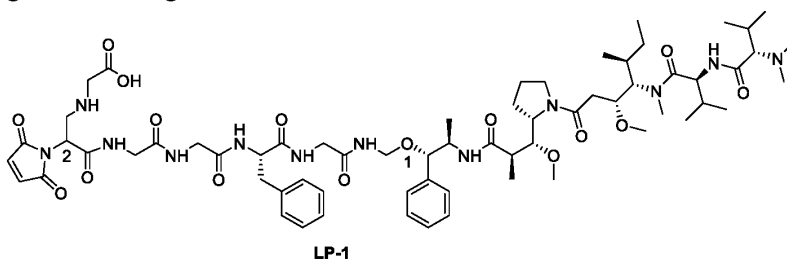


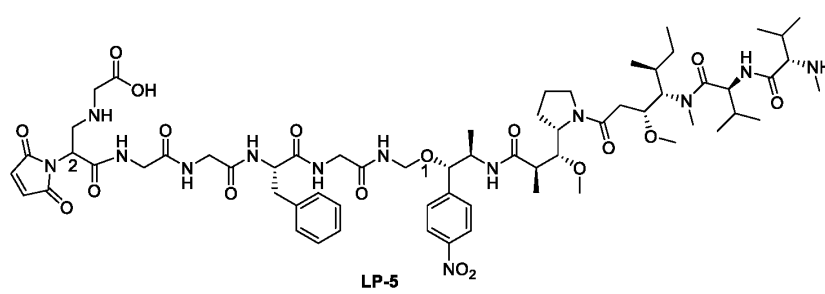
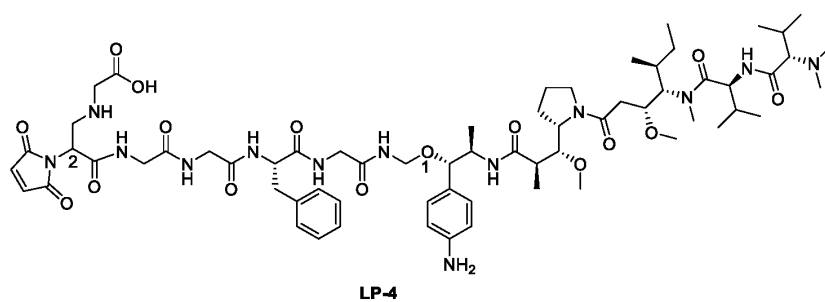
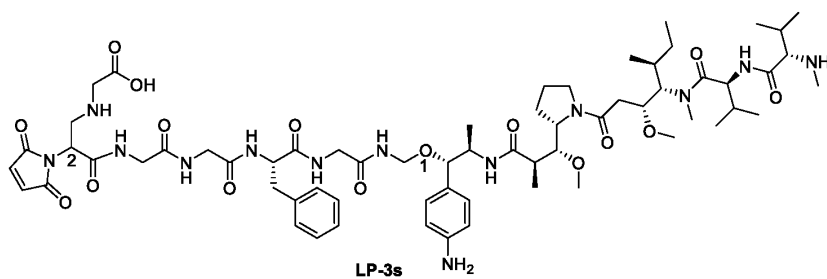
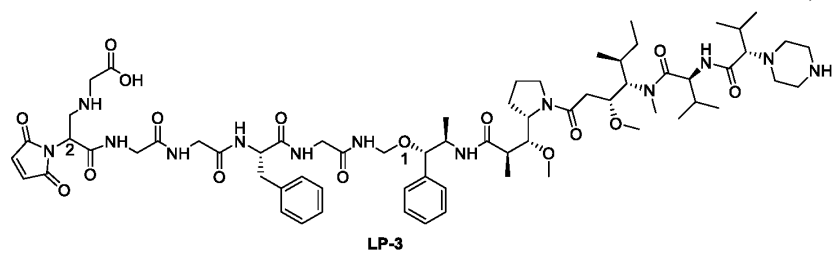
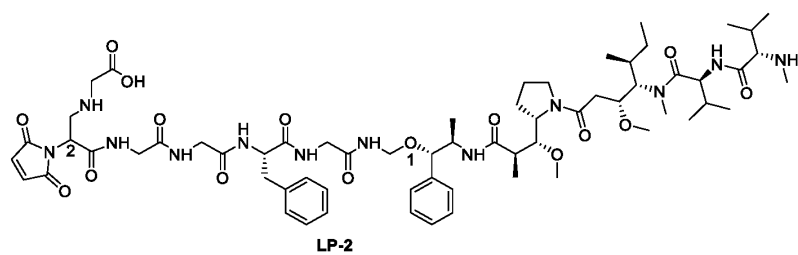
wherein Ac is a hydrophilic structural unit having the structure shown in Formula c:

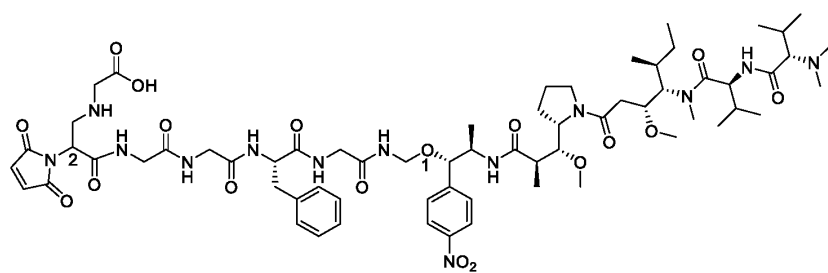


wherein X, Y are as defined in Claim 14 and Ac is linked to methylene carbon at the position 2 as labeled via -NH-.

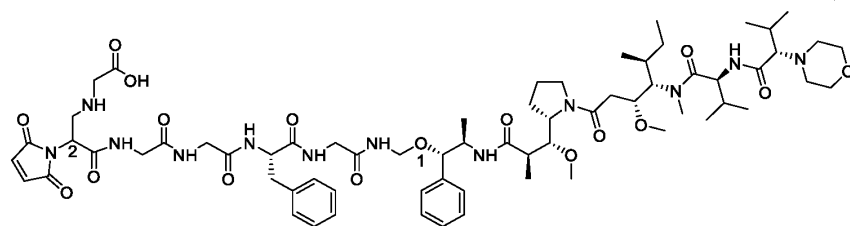
24. The linker-drug compound of any one of Claims 21-23, its isomeric, endo-, racemic, enantiomeric, or mixtures thereof, or pharmaceutically acceptable salts or solvates thereof, having the following structures:



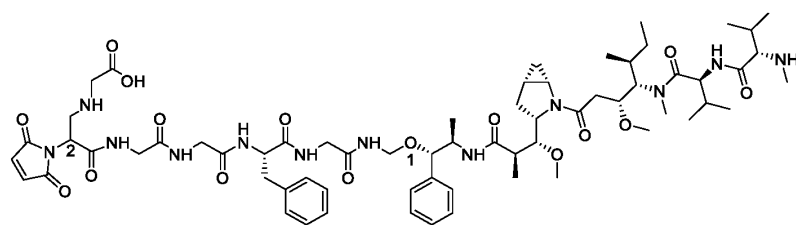




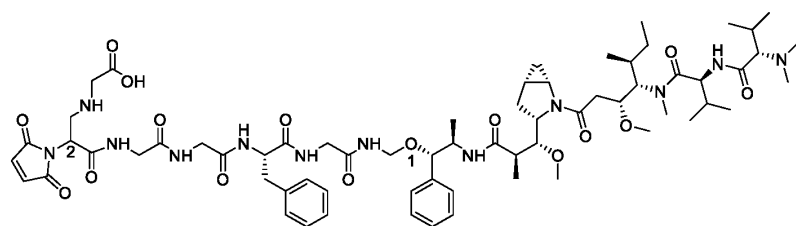
LP-6



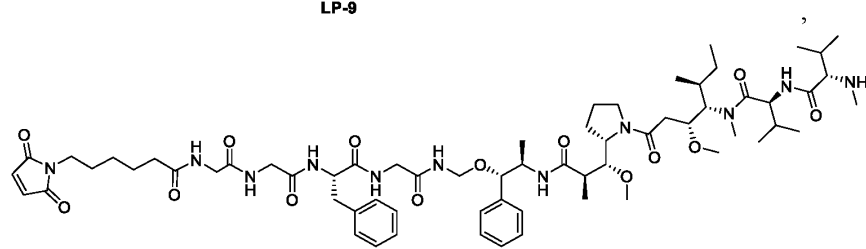
LP-7



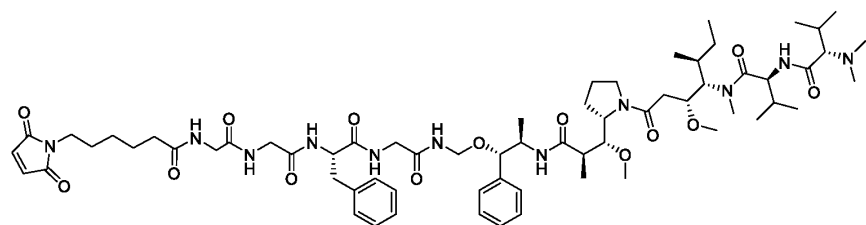
LP-8



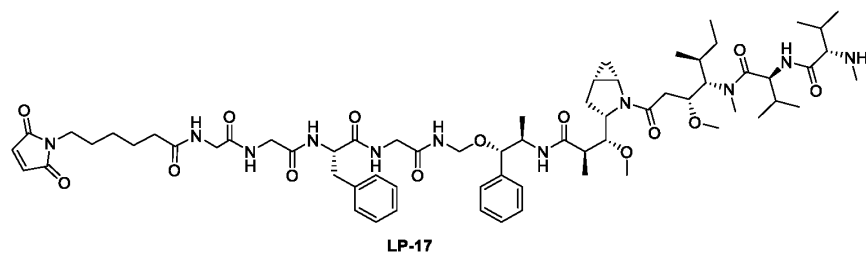
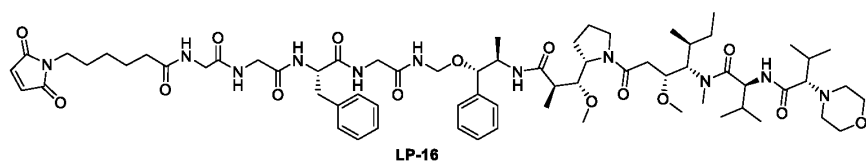
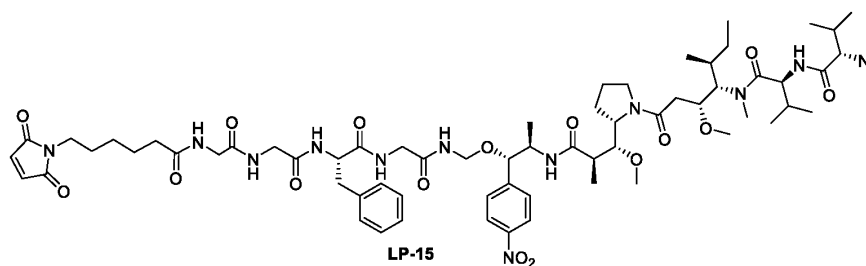
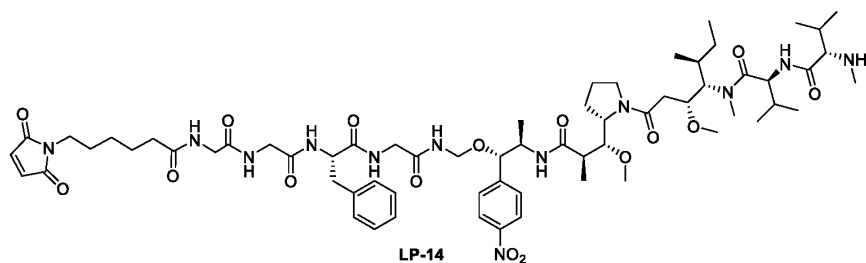
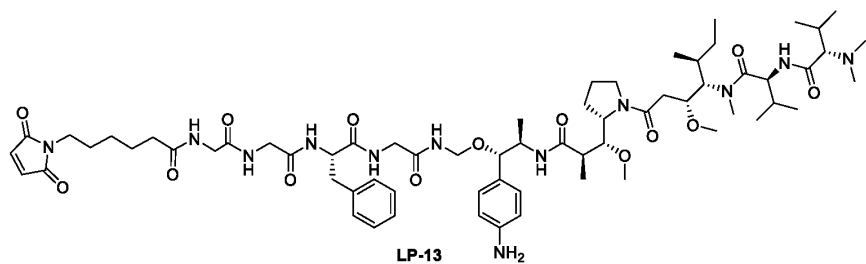
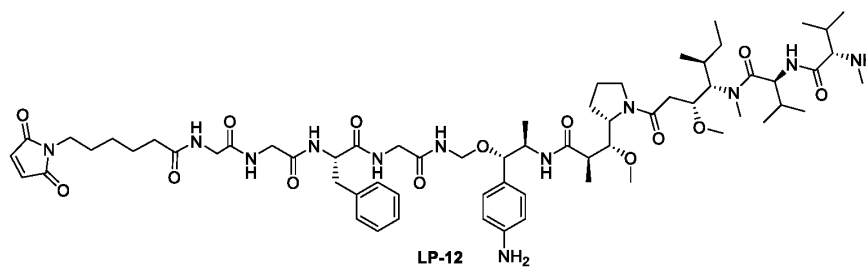
LP-9

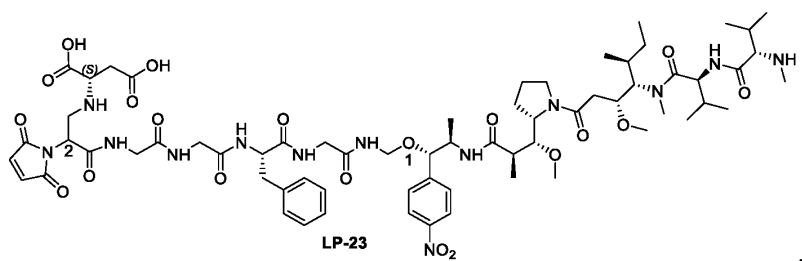
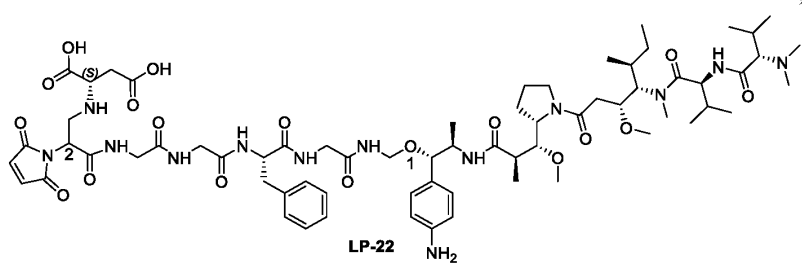
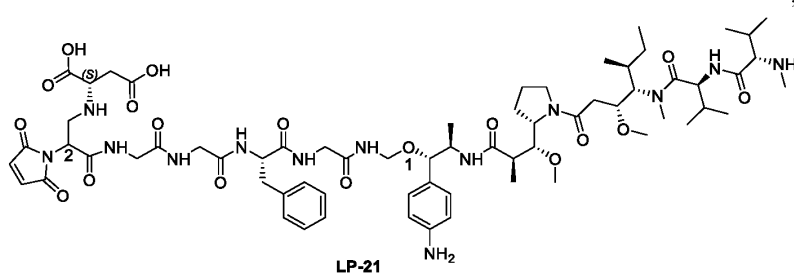
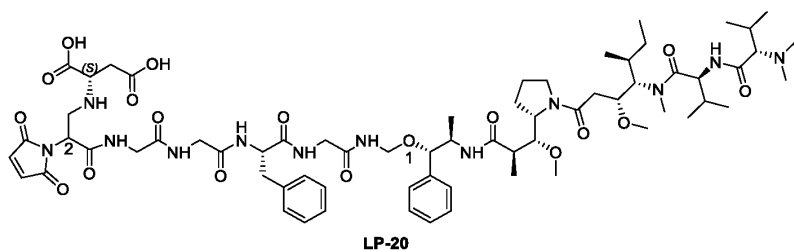
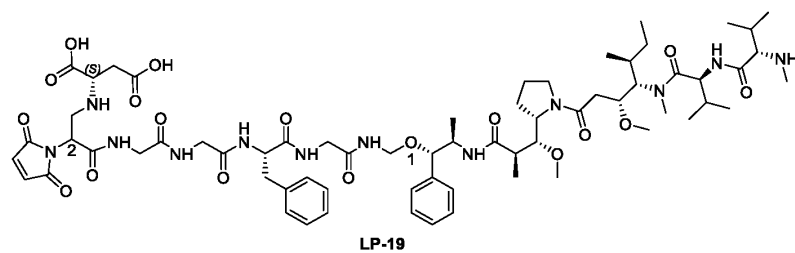
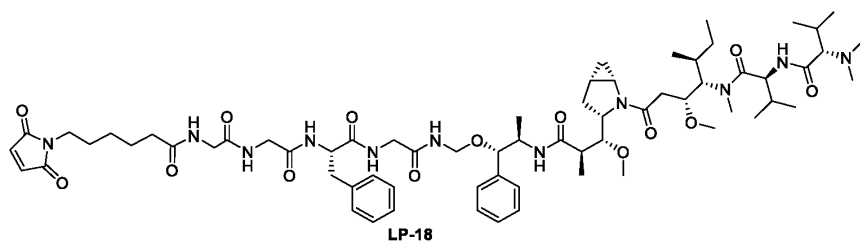


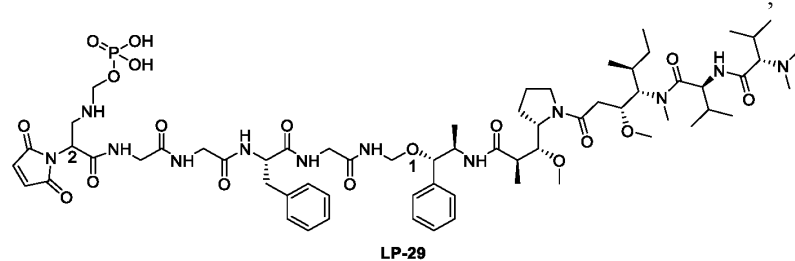
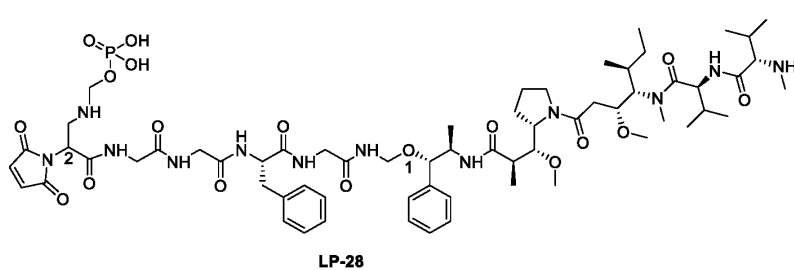
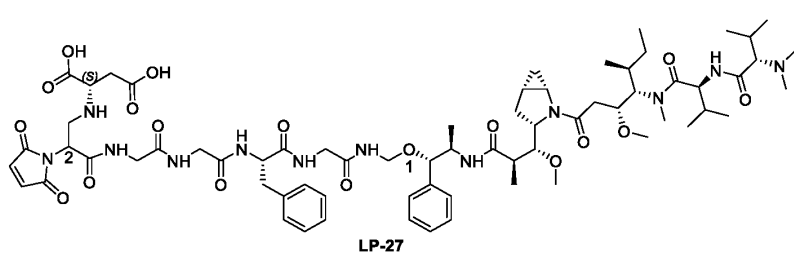
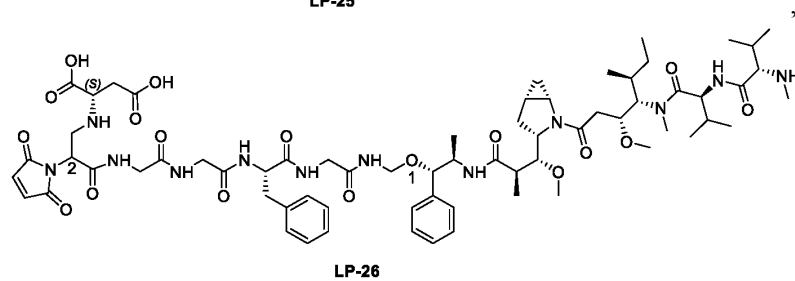
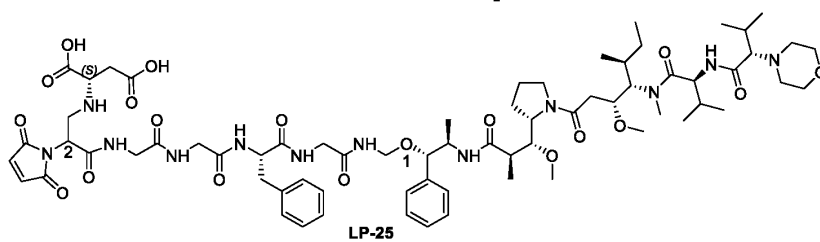
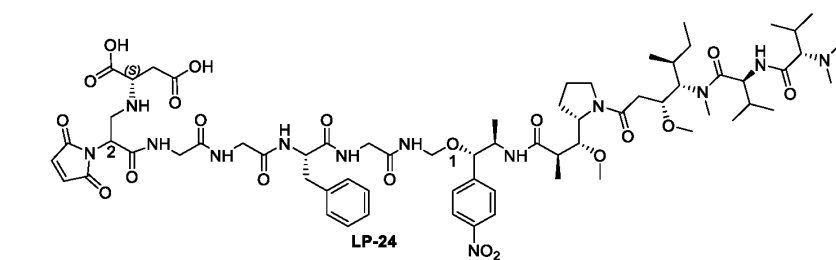
LP-10

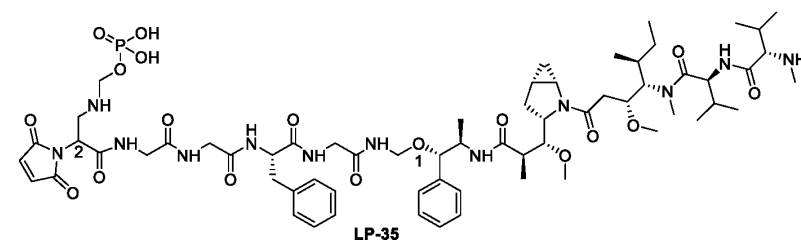
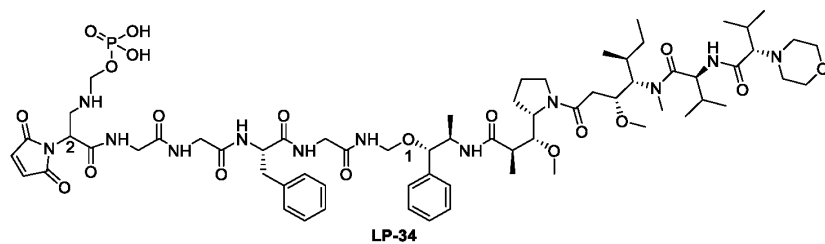
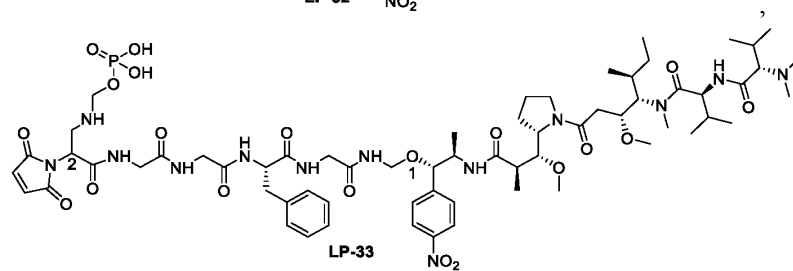
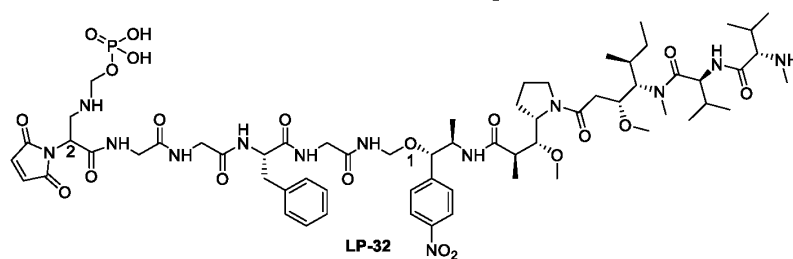
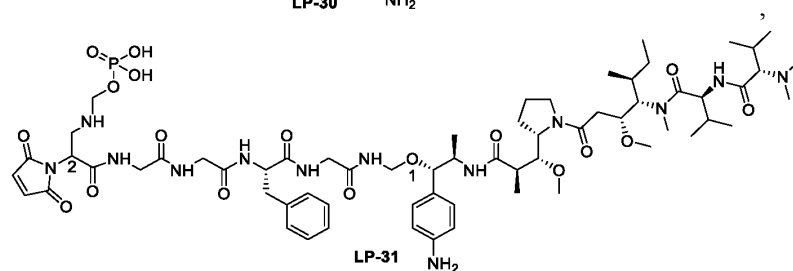
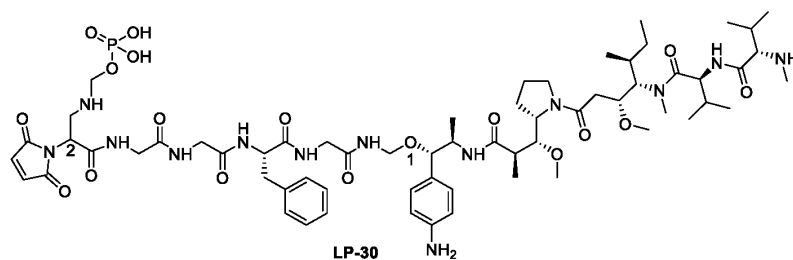


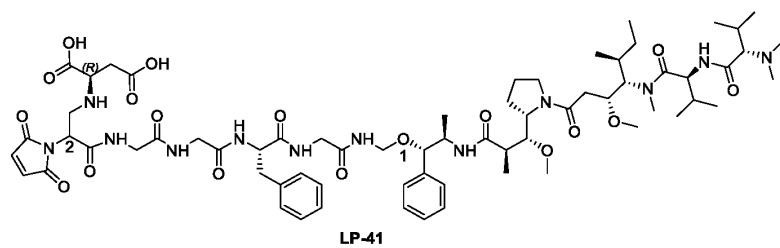
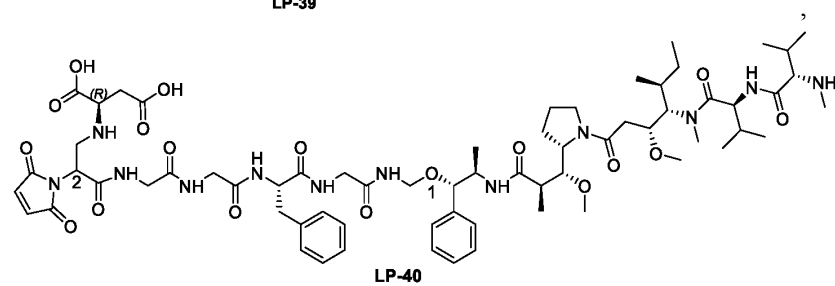
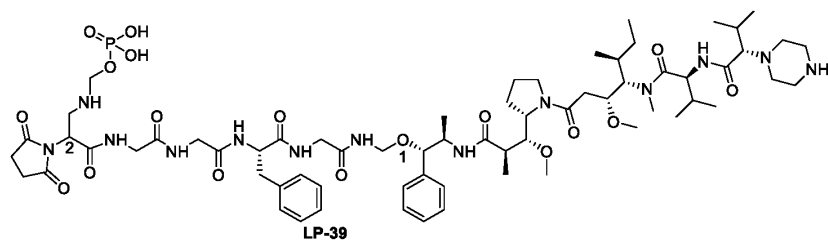
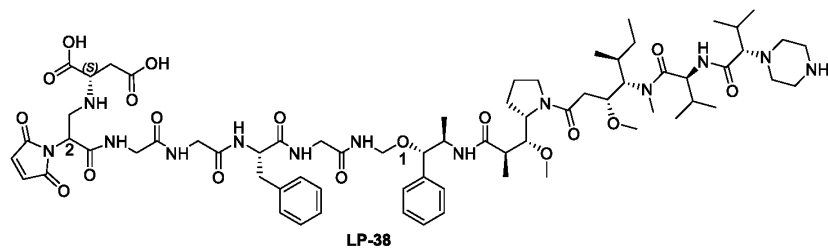
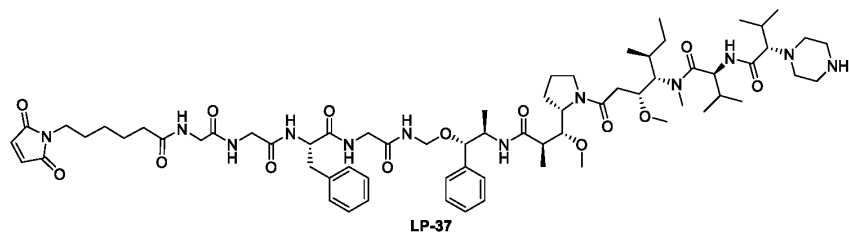
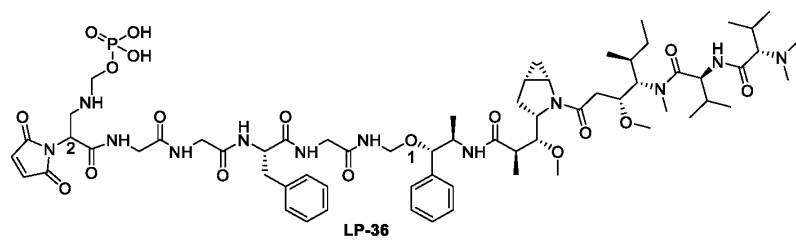
LP-11

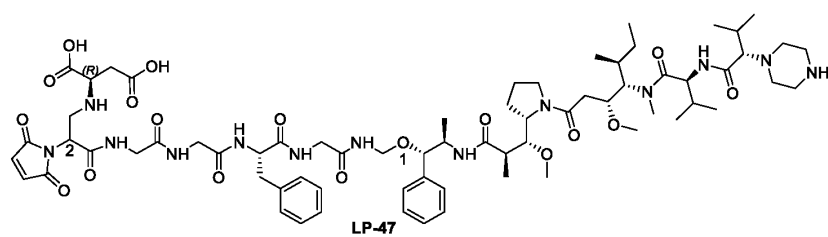
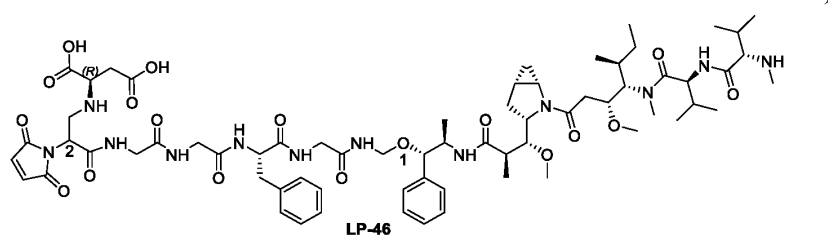
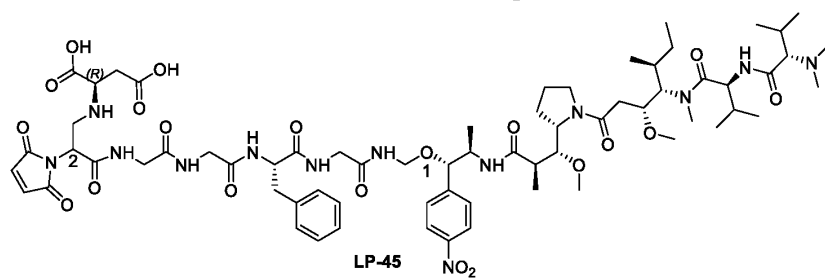
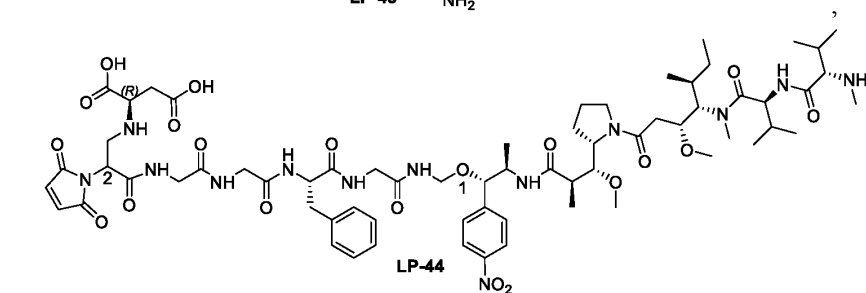
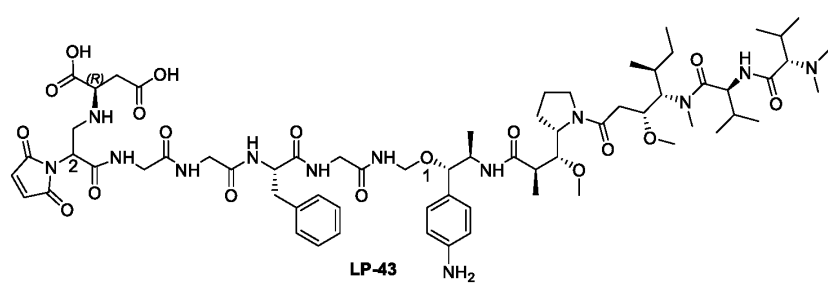
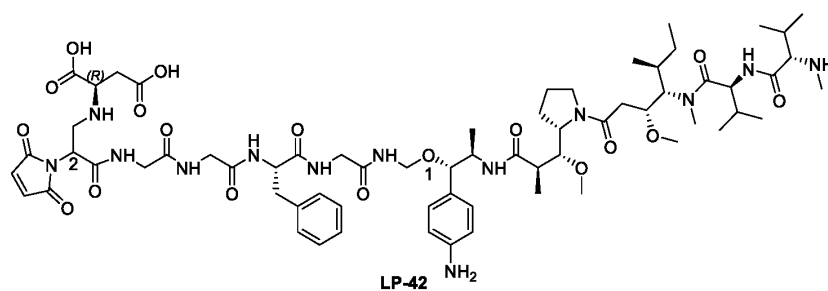


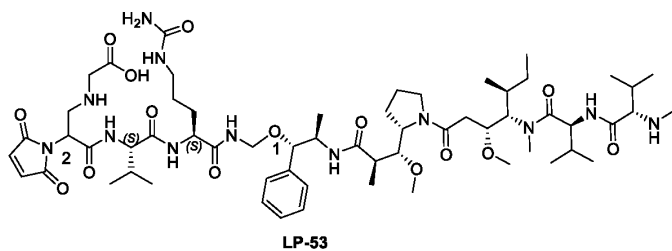
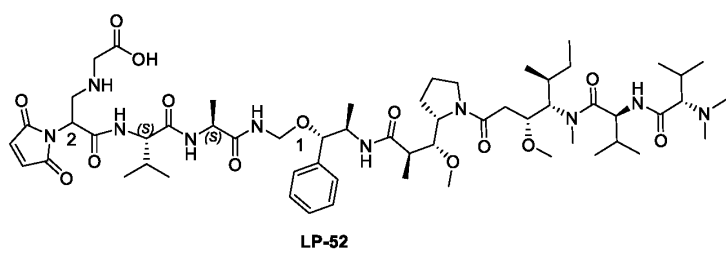
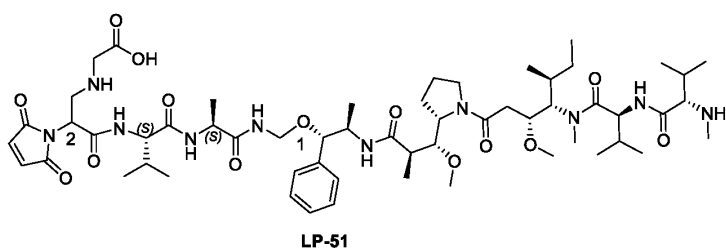
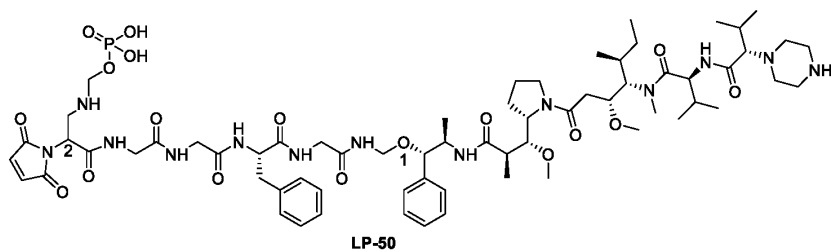
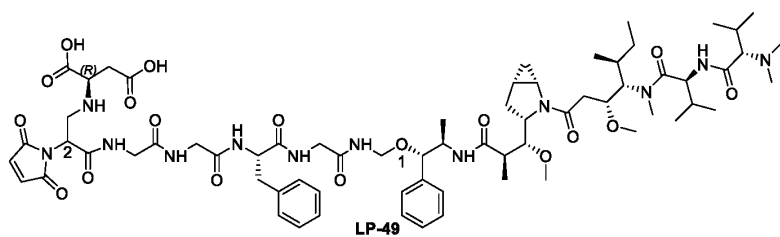
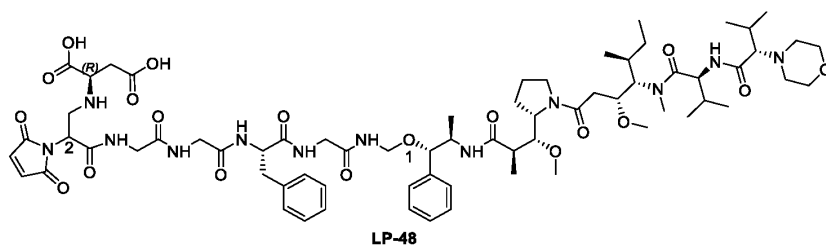




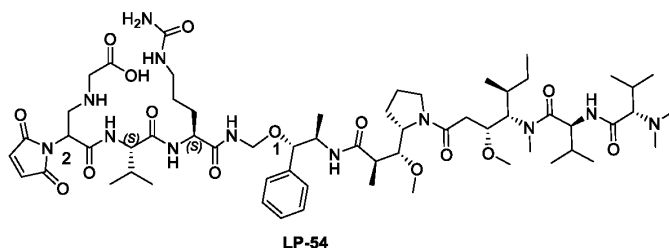








OR



wherein the configuration of the chiral carbon at the position 2 is R or S.

25. A pharmaceutical composition, comprising an effective amount of the antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof according any one of Claims 1-17, or the linker-drug compound of any one of Claims 21-24 its isomeric, endo-, racemic, enantiomeric, or mixtures thereof, or a pharmaceutically acceptable salt or solvates, and optionally a pharmaceutically acceptable carrier, diluent or excipient.

26. A pharmaceutical preparation, comprising the antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-20, or the linker-drug compound of any one of Claims 21-24, its isomeric, endo-, racemic, enantiomeric, or mixtures thereof, or a pharmaceutically acceptable salt or solvate thereof.

27. The antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-20, the linker-drug compound of any one of Claims 21-24, its isomeric, endo-, racemic, enantiomeric, or mixtures thereof, or pharmaceutically acceptable salts or solvates thereof, for the preparation of a drug,

wherein the drug is used for the treatment or prevention of tumors, or

wherein the tumor expresses EGFR, HER3, or both, wherein the tumor is cancer, or wherein the tumor comprises adenocarcinoma, ovarian cancer, cervical cancer, uterine cancer, prostate cancer, renal cancer, urothelial cancer, bladder cancer, liver cancer, gastric cancer, endometrial cancer, salivary gland cancer, esophageal cancer, lung cancer, colon cancer, rectal cancer, colorectal cancer, bone cancer, skin cancer, thyroid cancer, pancreatic cancer, melanoma, glioma, neuroblastoma, glioblastoma multiforme, sarcoma, lymphoma and Leukemia and other solid or hematologic tumors.

28. A method of treating or preventing a tumor, comprising administering to a subject in need thereof a prophylactically or therapeutically effective amount of the antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-20, or the linker-drug compound according to any one of Claims 21-24, its isomeric, endo-, racemic, enantiomeric, or mixtures thereof, or pharmaceutically acceptable salt or solvate thereof,

wherein the tumor expresses EGFR, HER3, or both,

wherein the tumor is cancer, or

wherein tumor comprises 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, rectal cancer, colorectal cancer, bone cancer, skin cancer, thyroid cancer, pancreatic cancer, melanoma, glioma, neuroblastoma, polymorphous glioblastoma, sarcoma, lymphoma, Leukemia, or other solid or hematologic tumors.

29. The linker-drug compound according to any one of Claims 21-24, its isomeric, endo-, racemic, enantiomeric, or mixtures thereof, or pharmaceutically acceptable salts or solvates

thereof, for the preparation of an antibody-drug conjugates or pharmaceutically acceptable salts or solvates thereof, wherein, optionally, the antibody-drug conjugate comprises the antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-20.