

ANTIBODY-AURISTATIN DRUG CONJUGATES AND METHODS OF MAKING AND USING THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the filing date of CN 202410042571.5, filed January 11, 2024, and CN202510016135.5, filed January 06, 2025, the disclosures of which are herein incorporated by reference in its entirety.

TECHNICAL FIELD

10 The application relates to the field of biopharmaceutical technology, and specifically, to ligand-drug conjugates, linker-drug compounds, methods of preparation and uses thereof.

BACKGROUND

Antibody-Drug Conjugates (ADCs) are a new type of targeted therapy that combine the advantages of high selectivity of antibodies and high activity of cytotoxic drugs. With their "high efficiency and low toxicity" characteristics, ADCs have become a research hotspot in the field of targeted cancer therapy. In recent years, the rapid development has led to the three generations of ADCs. At present, 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®, 20 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 trials. ADCs are playing an increasingly important role in the field of targeted tumor therapy.

Antibody-drug conjugates (ADCs) are composed of three components: a high specificity and affinity antibody (mAb), a stable linker, and a small-molecule cytotoxic drug (payload/warhead, warhead). The antibody in ADCs is most commonly immunoglobulin G1 or IgG1, and the warhead part is generally a cytotoxic drug that acts on microtubules, DNA or RNA, such as 30 maytansinoids, auristatins, calicheamicins, camptothecins, pyrrolbenzodiazepines and amatoxins. There are two types of linkers: one is a cleavable linker and the other is a non-cleavable linker.

Auristatins are a class of microtubule inhibitors that block microtubule from binding to GTP and block microtubule binding to the vincristine binding site, thereby inducing cell apoptosis and inhibiting tumor growth. MMAE and MMAF are currently used as ADC warheads (see US6884869 and US7498298, respectively), both of which are pentapeptides derived from Dolastatin 10. Both MMAE and MMAF show good anti-tumor activity, however, due to the lack of selectivity and low therapeutic index, they cannot be used clinically as a single drug. Five ADCs on the market use MMAE as their warhead and one ADC drug uses MMAF as its warhead, namely, Adcetris, Polivy, Padcev, Edexil, Tivdak and Blenrep. These ADCs with auristatins as payloads 40 all have some shortcomings. For example, ADCs with MMAE have adverse effects including

neurotoxicity (peripheral neuropathy) and hematotoxicity (thrombocytopenia and neutropenia), and ADCs with MMAF could develop ophthalmotoxicity. In addition, the linkers often used in these ADCs are VCLinker, and the highest DAR is 4 because VCLinkers are highly hydrophobic. High DAR may lead to the precipitation and aggregation of ADCs, therefore increasing DAR may not enhance its efficacy.

Furthermore, most of the current ADCs that use MMAE as their warhead introduce the linker from the secondary amine at the N-terminus of MMAE. There are few examples of introducing the linker from the hydroxyl group of MMAE, which have only been reported by Seagen, the Technical University of Denmark, Mersana, and Shanghai New Concept Company.

10 Seagen introduced a linker with a hydrazone bond after esterification of the hydroxyl group of the MMAE analog Auristatin E (see US2005009751A1). Such linkers with ester and hydrazone bonds are unstable in human plasma and will undergo a certain degree of hydrolysis, resulting in partial off-target toxicity (see Nat Biotechnol. 2003 Jul;21(7):778-84). Seagen also introduced a self-eliminating group, methylene alkoxy carbamate (MAC), into the hydroxyl group of MMAE and then introduced a β -glucuronic acid glycoside linker (see CN105813653). Although the ADC with such a linker has good stability in human plasma, the release of its warhead involves the catalysis of β -glucuronidase and the subsequent self-elimination process, which is relatively complicated.

20 The Technical University of Denmark introduced linker from the hydroxyl group of MMAE analogs (see WO2020260597A1). In a way like the esterification reaction for introducing linker by Seagen, the ester bond was more difficult to be hydrolyzed by enzyme after the introduction of linker, and it was not easy to release the warhead. Mersana's method for introducing the linker from the hydroxyl group of MMAE analogs (see CN110234357A) is like that of Seagen. By introducing ester bond or carbonate structure, the ester bond is more difficult to be hydrolyzed by enzyme, and the carbonate plasma stability is poorer, which leads to the reduction of the drug's efficacy. Shanghai New Idea Company disclosed the method of introducing a carbamate to the hydroxyl group of MMAE analog to introduce a Mc-Vc-PAB linker (see CN106279352 A), which again is less stable in plasma and has a potential off-target risk.

30 Thus, it is of great clinical importance to develop new strategies of introducing linkers to obtain Auristatin-based ADCs with higher safety and efficacy.

SUMMARY

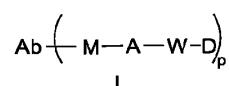
The following summary is illustrative only and is not intended to be in any way limiting. In addition to the illustrative aspects, embodiments, and features described above, further aspects, embodiments, and features will become apparent by reference to the drawings and the following detailed description.

40 The application provides, among others, better anti-tumor auristatin ADCs with higher safety and efficacy to meet the clinical needs, linker-drug compounds, and its use to generate ADCs, methods for making the linker-drug compounds and ADCs, pharmaceutical compositions or formulations comprising ADCs or the linker-drug compounds, methods for treating or preventing diseases such as cancer using the ADCs, the linker-drug compounds, or pharmaceutical

compositions or formulations as disclosed herein.

For example, the application provides, among others, compositions and methods for connecting auristatin or its derivatives at its hydroxyl group to an enzymatically cleavable peptide unit through an amino-methylene structure to form corresponding linker-payload compound. The method shows the significant advantage over the existing ADCs with increased the DAR value of ADC to as much as 8. In addition, the resulting ADCs have better hydrophilicity at high loading, better plasma stability at high drug loading values, and significantly more advantages in terms of drug efficacy. Compared with existing drugs, such as ADCs with Vc-MMAE as the linker-payload, the ADCs disclosed herein show superiority over the existing drugs by achieving similar tumor inhibitory effect with much lower doses, with significantly improved MTD while with significantly reduced neurotoxicity and hepatotoxicity.

In one aspect, the application provides antibody-drug conjugates (ADCs) as shown in General Formula I, or pharmaceutically acceptable salts or solvates thereof:



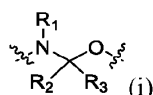
wherein,

Ab is an antibody or its antigen-binding fragment that binds specifically to HER2; In one embodiment, said antibody or its antigen-binding fragment comprises a light chain and a heavy chain; in one embodiment, the light chain comprises complementarity-determining regions (CDRs), CDR-L1, CDR-L2, and CDR-L3 with the amino acid sequences shown in SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3, respectively; and, in certain embodiments, said heavy chain comprises CDR-H1, CDR-H2, and CDR-H3, the amino acid sequences thereof are shown in SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, respectively;

M is a linker unit connected to Ab;

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

W represents an amino methylene oxide structural unit as shown in formula (i):



wherein,

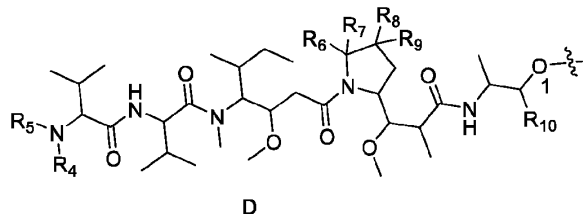
the wavy line on the left side represents the site of attachment between the nitrogen atom and A in formula (i), the wavy line on the right side represents the site of attachment between the oxygen atom and the drug D in formula (i), and the oxygen atom is a common group of the drug D and W;

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

p is an integer or decimal between 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

D is an auristatin having the structure shown in Formula D, its isomers, endo-, racemates,

enantiomers, or mixtures thereof, or a pharmaceutically acceptable salt thereof;



wherein,

R₄, R₅ are each independently hydrogen, deuterium, alkyl and a deuterated alkyl group; or, in one embodiment, R₄, R₅ may be 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, and a deuterioalkyl group; B is O, NR₁₅, or CR₁₆R₁₇, wherein R₁₅, R₁₆, R₁₇ are independently hydrogen, deuterium, and alkyl; n and m are independently an integer number from 0 to 8 (e.g. 0, 1, 2, 3, 4, 5, 6, 7, 8); in one embodiment, the nitrogen atom bonded to R₄ and R₅ may form 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 azide group, alkyl, or NR₁₈R₁₉, or, in one embodiment, 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 azide group, alkyl, and NR₁₈R₁₉, wherein R₁₈ and R₁₉ are independently hydrogen, or alkyl;

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

The wavy line in formula D represents the site of attachment between the oxygen atom at position 1 in the structure of drug D to W. Said oxygen atom is a group commonly shared between drug D and W.

In certain embodiments, said light chain comprises a light chain variable region having an amino acid sequence of SEQ ID NO: 7.

In certain embodiments, said light chain further comprises a light chain constant region of amino acid sequence SEQ ID NO: 8.

In certain embodiments, the amino acid sequence of said light chain is SEQ ID NO: 9.

In certain embodiments, said heavy chain comprises a heavy chain variable region having an amino acid sequence of SEQ ID NO: 10.

In certain embodiments, said heavy chain further comprises a heavy chain constant region of amino acid sequence SEQ ID NO: 11.

In certain embodiments, the amino acid sequence of said heavy chain is SEQ ID NO: 12.

In certain embodiments, said light chain comprises CDR-L1, CDR-L2, and CDR-L3, the nucleotide sequences of which are shown in SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, respectively.

In certain embodiments, said heavy chain comprises CDR-H1, CDR-H2, CDR-H3, the

nucleotide sequences of which are shown in SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, respectively.

In certain embodiments, said light chain comprises a light chain variable region having a nucleotide sequence of SEQ ID NO: 19.

In certain embodiments, said light chain further comprises a light chain constant region having a nucleotide sequence of SEQ ID NO: 20.

In certain embodiments, the nucleotide sequence of said light chain is SEQ ID NO: 21.

In certain embodiments, said heavy chain comprises a heavy chain variable region having a nucleotide sequence of SEQ ID NO: 22.

10 In certain embodiments, said heavy chain further comprises a heavy chain constant region having a nucleotide sequence of SEQ ID NO: 23.

In certain embodiments, the nucleotide sequence of said heavy chain is SEQ ID NO: 24.

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

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

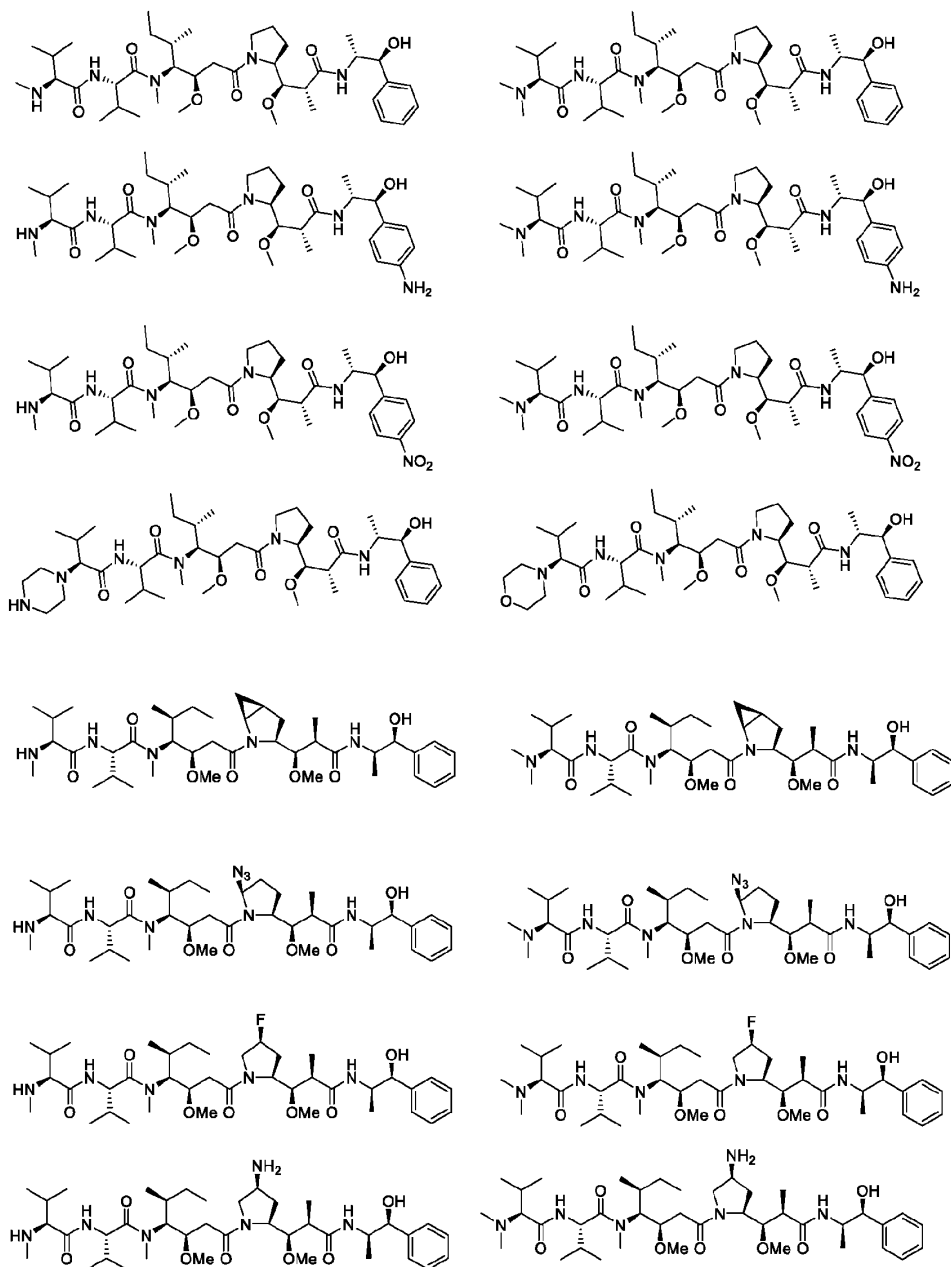
In some embodiments, R_6 , R_7 , R_8 , R_9 in Formula D are each hydrogen atoms.

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

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

In certain embodiments, R_{10} in formula D is a phenyl group, and, in some embodiment, optionally, said phenyl may be substituted with one or more of substituents. In certain embodiments, said substituent may be amino or nitro.

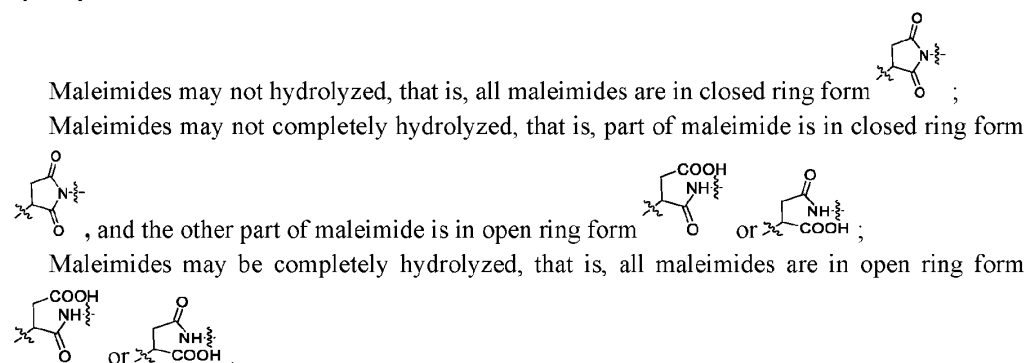
Example compounds for drug unit D may be, without limitation, the following:



In the above embodiments, drug 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. In some embodiments, the site of hydrolysis may be the maleimide portion of the linker unit. When the antibody contains

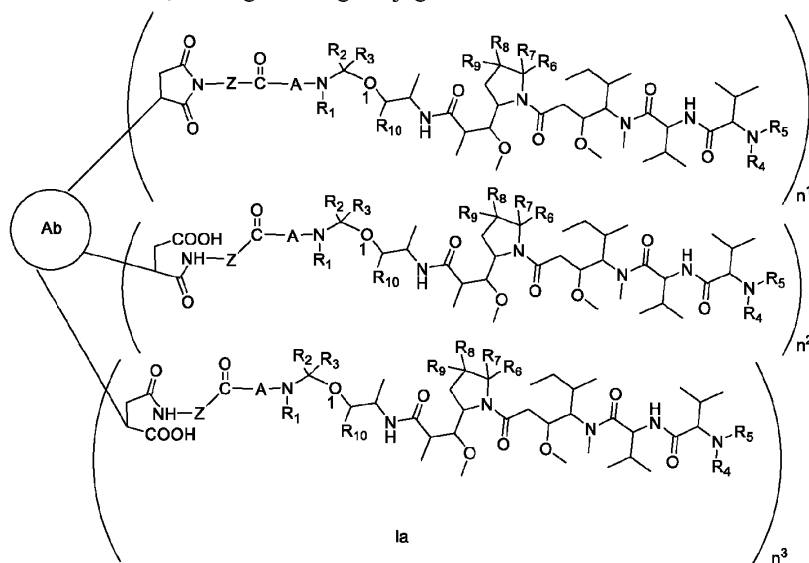
multiple linker-drugs, the following scenarios may occur depending on with the degree of hydrolysis:



Therefore, 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 may all be in closed-ring form, some in an open-ring form, or all in open-ring form.

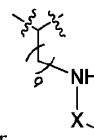
In the above mentioned maleimide structural formulas, the left wavy line represents the linkage site to Ab, and the right wavy line represents other structures in M.

In certain embodiments, said ligand-drug conjugate has the structure shown in formula Ia:

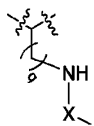


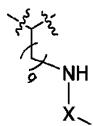
wherein:

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 alkylene-, 3-8-membered heterocyclic-, -C₁-C₁₀-alkylene (3-8-membered heterocyclic-), -(3-8-membered



heterocyclic)-C₁-C₁₀-alkylene-, -(CH₂CH₂O)_r-, -(CH₂CH₂O)_r-CH₂-, or independently -C₁-C₁₀ alkylene-, -C₃-C₈ carbocyclic-, -arylidene-, -C₁-C₁₀ alkylene-arylene-, -arylene-C₁₀-alkylene-, -C₁-C₁₀ alkylene-(C₃-C₈ carbocyclic)-, -(C₃-C₈ carbocyclic)-C₁-C₁₀ alkylene-, -(3-8-membered heterocyclic)-, -C₁-C₁₀ alkylene-(3-8-membered heterocyclic)-, -(3-8-membered heterocyclic)-C₁-C₁₀ alkylene-, -(CH₂CH₂O)_r-, -(CH₂CH₂O)_r-CH₂; Y is a hydrophilic structure such as a group selected from carboxyl group, phosphoric acid group, polyphosphoric acid group, phosphite group, sulphonic acid group, sulfurous acid group, sulfinic acid group, or poly(ethylene glycol) (PEG) group; in one embodiment, the heterocyclic rings may independently comprise 1 to 3 atoms independently selected from N, O, and S; in one embodiment, said -C₁-C₁₀ alkylene-, -C₃-C₈ carbocyclic-, and heterocyclic rings may be each independently substituted with one or more substituents; in one embodiment, the substituents may be independently deuterium, halogen, hydroxyl, a cyano group, a nitro group, an amino group, alkyl, heteroalkyl, substituted alkyl, alkoxy group, carboxylic group, or cycloalkyl;



the left wavy line in  represents the linkage site to the N on the maleimide, the right wavy line represents the linkage site to the carbonyl group;

r is independently the integer numbers between 1 and 10 including, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10; q is independently an integer between 1 and 8, such as 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.

In some embodiments, n¹, n², n³ are independently 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20. For example, n¹, n², n³ may be 0, 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, 9.9 or 10.

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

In certain embodiments, A is a peptide residue formed from 2 to 4 amino acids independently phenylalanine and glycine. In certain embodiments, 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 some embodiments, q may be 1, 2, 3, 4, 5, 6, 7, 8; in some embodiment, q is 1.



In certain embodiments, Z is $\text{X}-\text{Y}$, wherein X is a methylene group, the methylene group is optionally substituted with a carboxyl group, and Y is selected from a carboxyl group, a

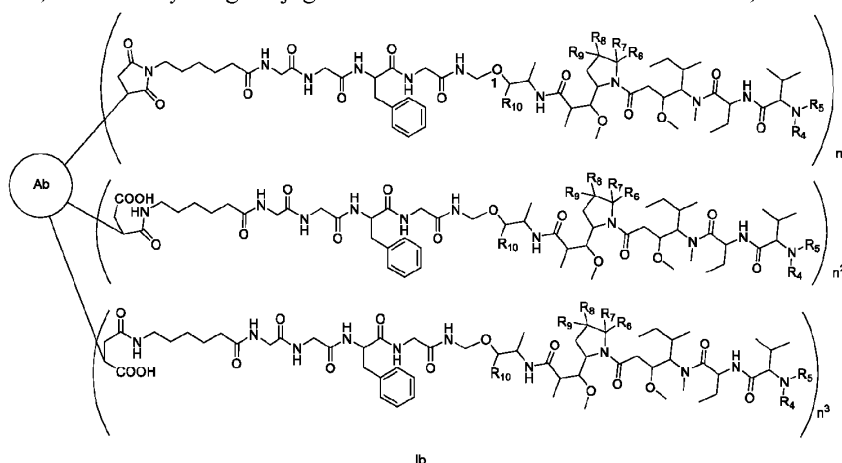


phosphate group; $\text{X}-\text{Y}$ the left wavy line in represents the attachment site to the N on the maleimide, and the right wavy line represents the attachment site to the carbonyl group.

In certain embodiments, R_1 , R_2 and R_3 are each independently hydrogen, deuterium, alkyl group, alkyl halide group, alkyl deuteride group, and hydroxyalkyl group.

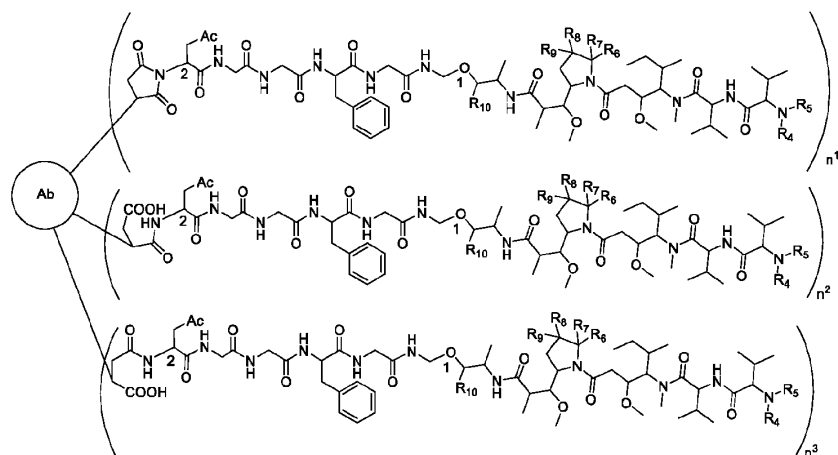
10 In some embodiments, R_1 , R_2 , and R_3 may be simultaneously hydrogen or deuterium.

In some embodiments, R_1 , R_2 , and R_3 may be simultaneously hydrogen atoms. In some embodiments, the antibody-drug conjugate has a structure as shown in formula 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.

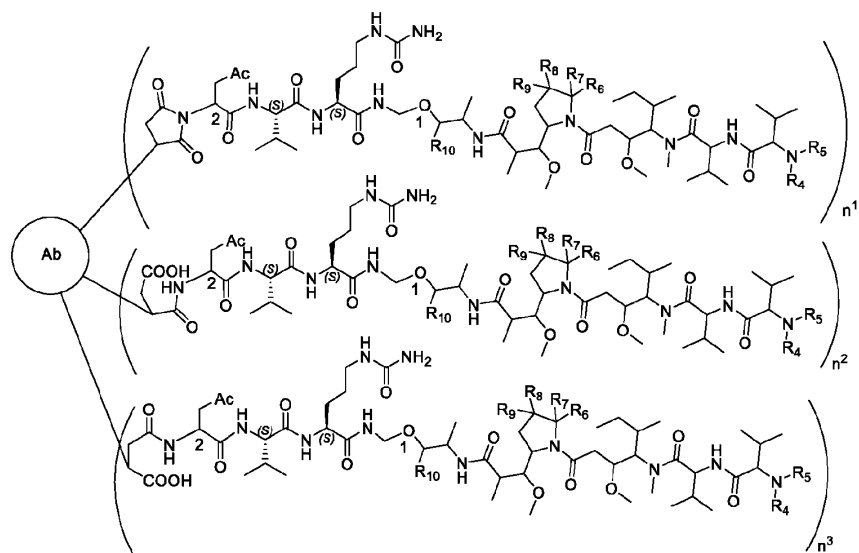
In certain embodiments, said antibody-drug conjugate has a structure as shown in formula Ic,



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.

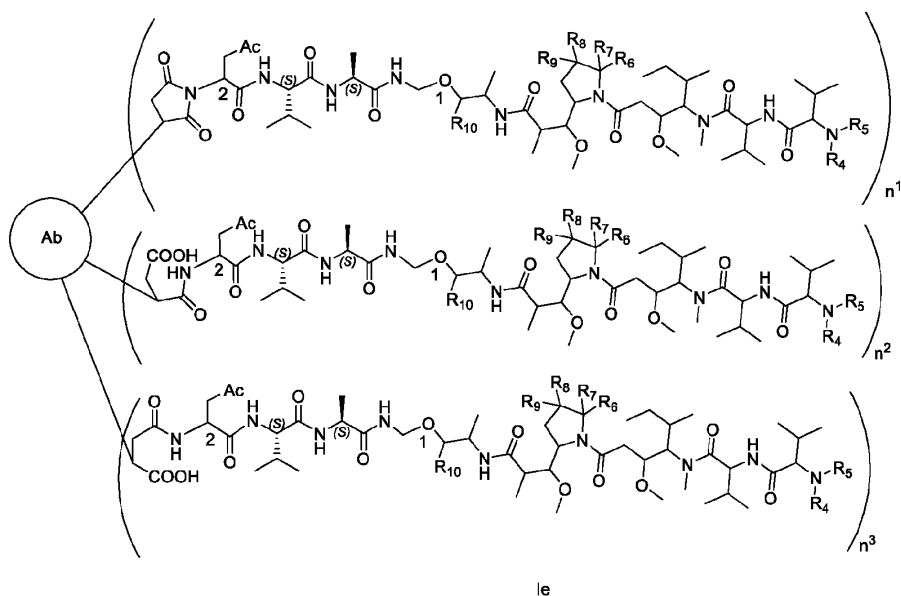
In one embodiment, said antibody-drug conjugate has a structure as shown in Formula Id,



Id

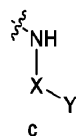
wherein R_4 , R_5 , R_6 , R_7 , R_8 , R_9 , R_{10} , n^1 , n^2 , n^3 are as defined above.

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



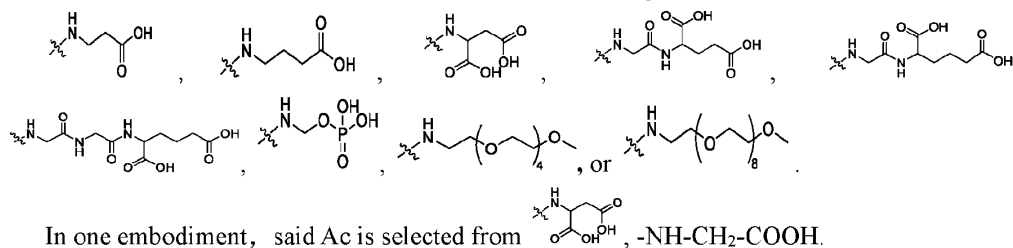
wherein R_4 , R_5 , R_6 , R_7 , R_8 , R_9 , R_{10} , n^1 , n^2 , n^3 are as defined above.

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



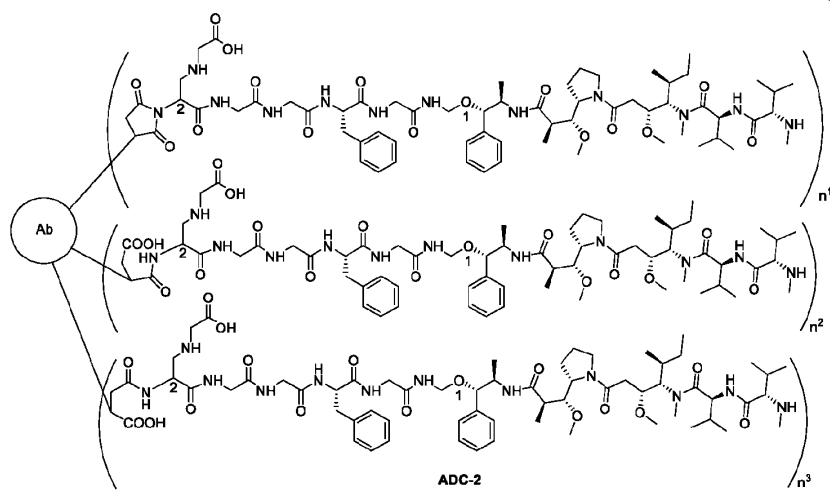
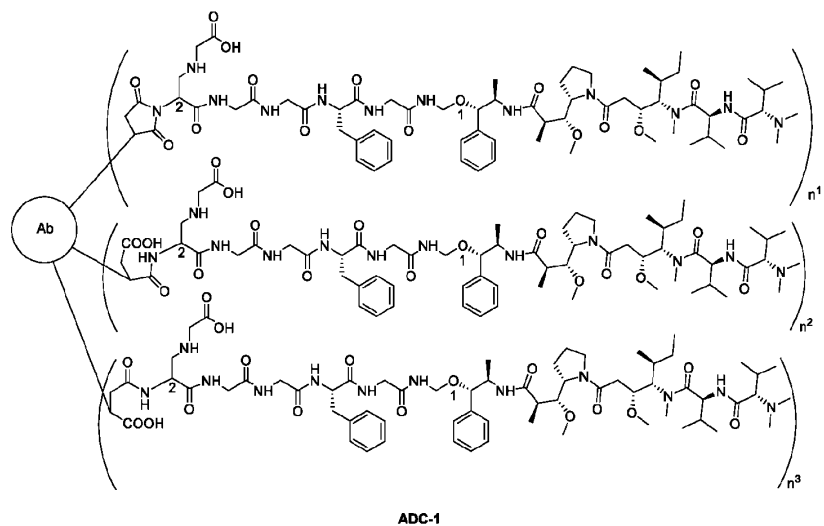
Ac is linked to methylene carbon at the position-2 as labeled in Formula Ic, Id or Ie via an amino functional group, X, Y, R_4 , R_5 , R_6 , R_7 , R_8 , R_9 , R_{10} , n^1 , n^2 , n^3 are as defined above.

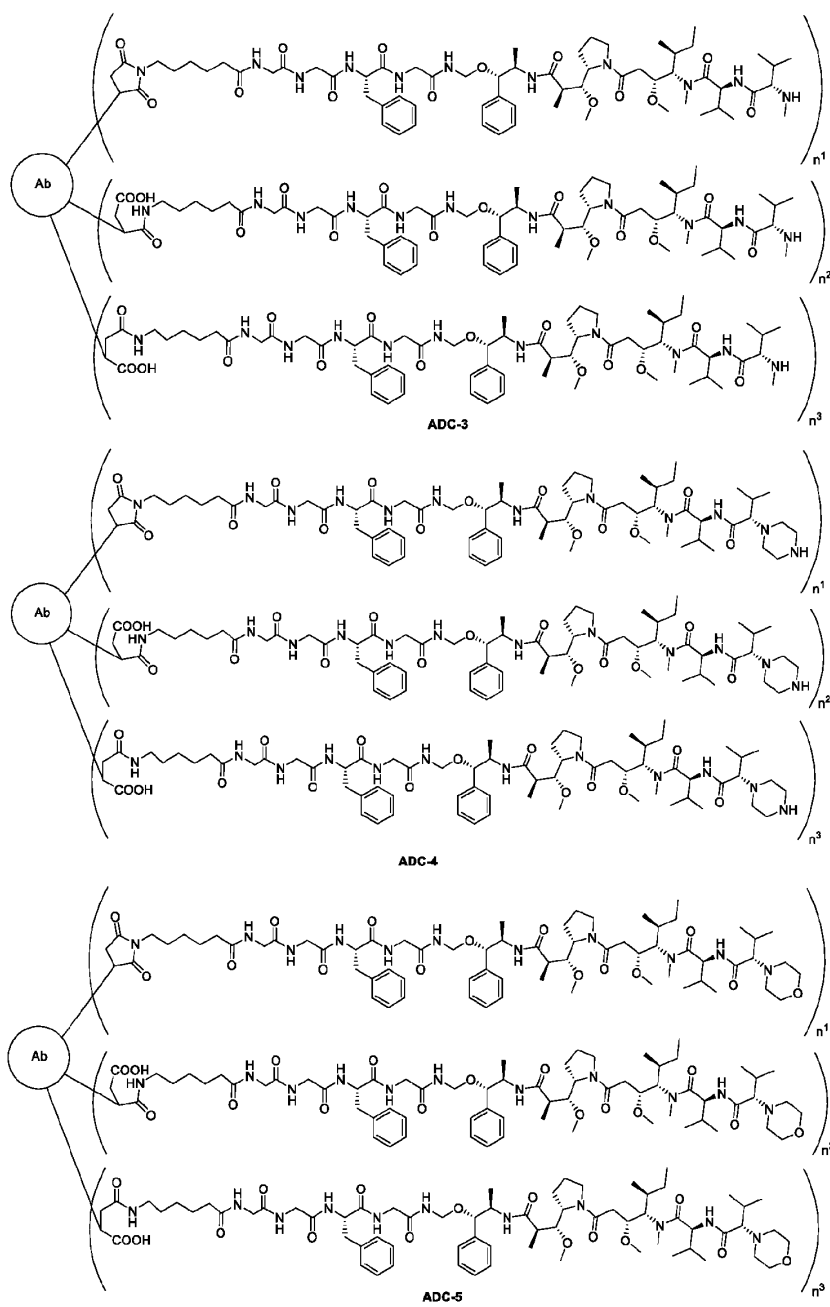
- In certain embodiments, said Ac may be derived from glycine, (D/L) alanine, (D/L) leucine, (D/L) isoleucine, (D/L) valine, (D/L) phenylalanine, (D/L) proline, (D/L) tryptophan, (D/L) serine, (D/L) tyrosine, (D/L) cysteine, (D/L) arginine, (D/L) histidine, (D/L) methionine, (D/L) asparagine, (D/L) glutamine, (D/L) threonine, (D/L) aspartic acid, (D/L) glutamic acid, natural or non-natural amino acid derivatives, or the following structures:

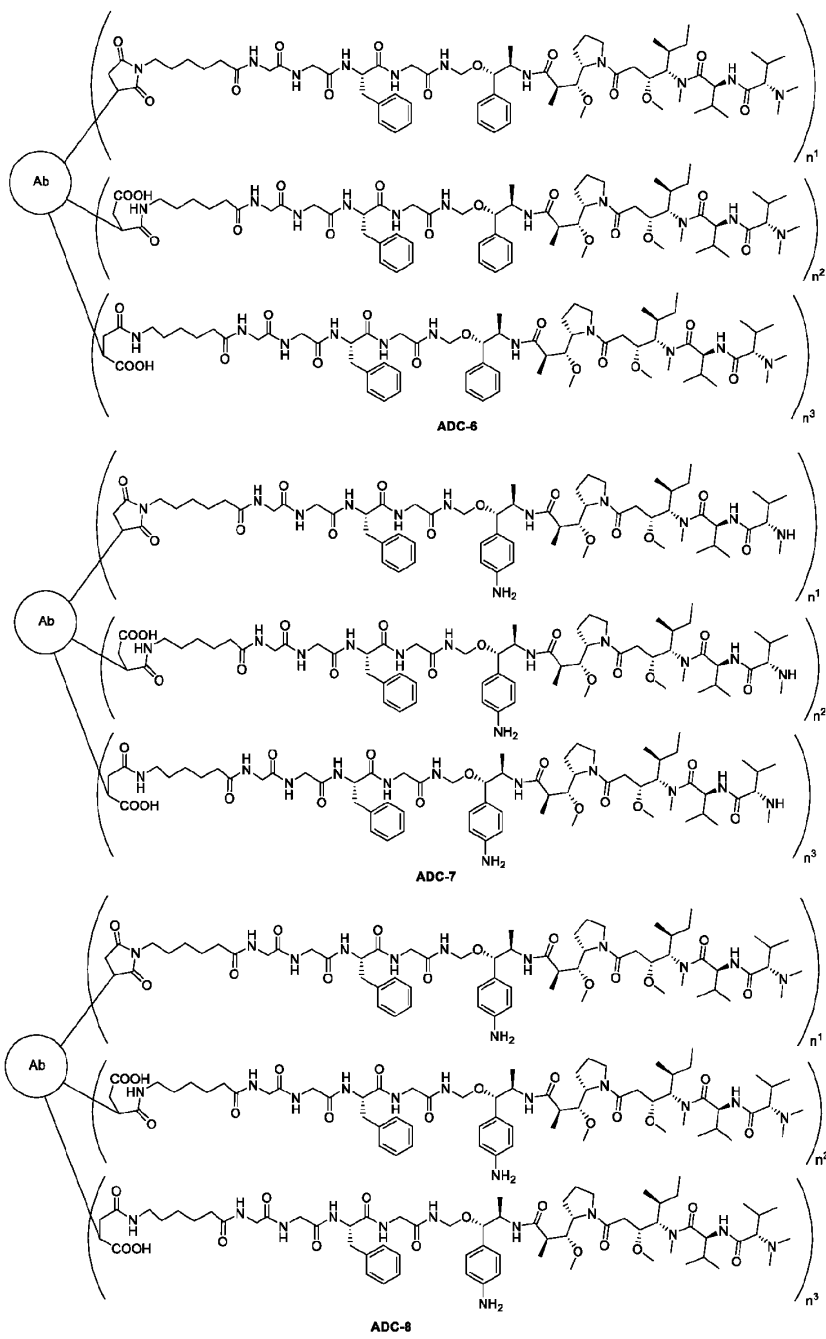


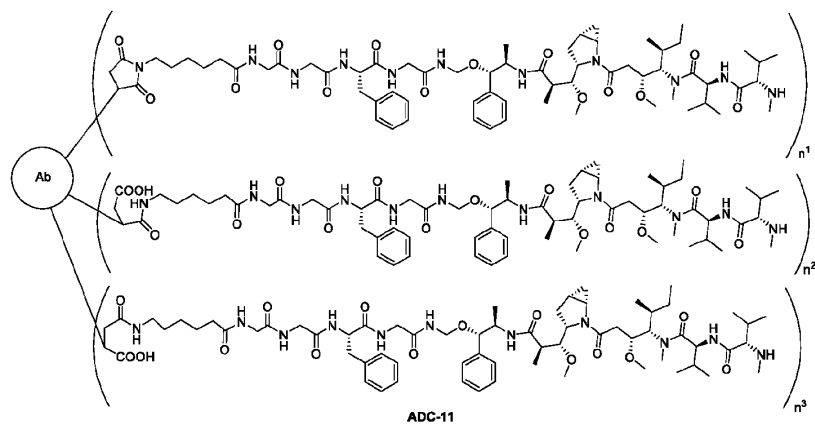
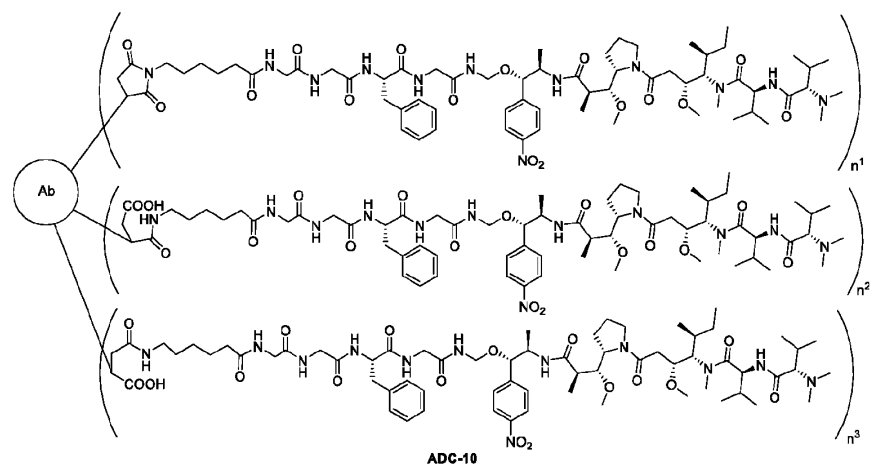
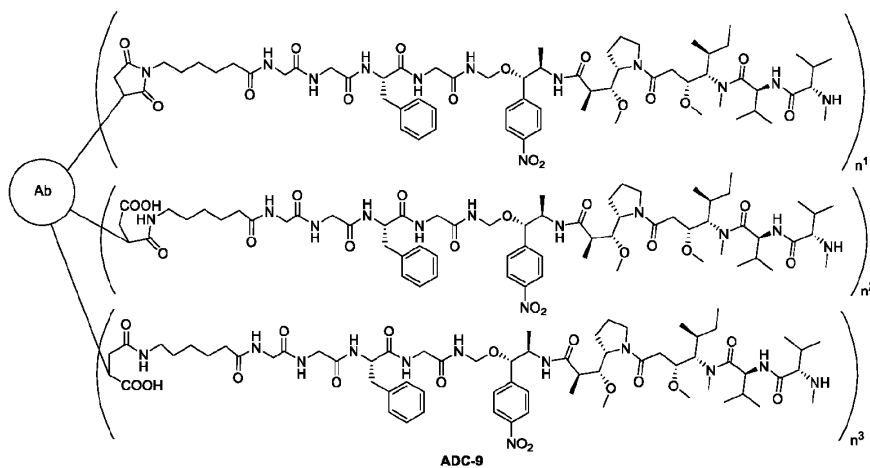
In one embodiment, said Ac is selected from $\text{-NH-CH}_2\text{-COOH}$.

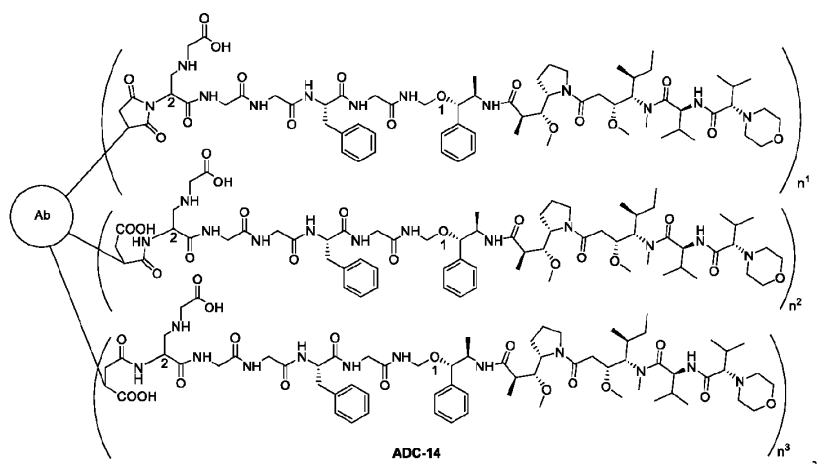
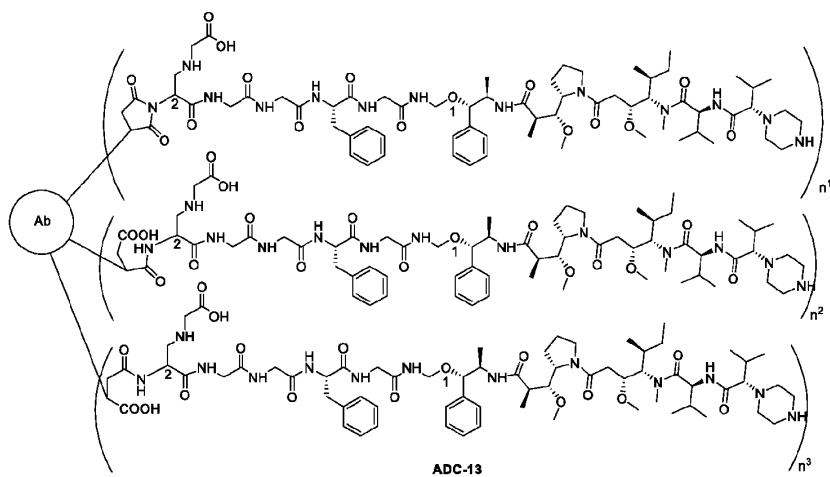
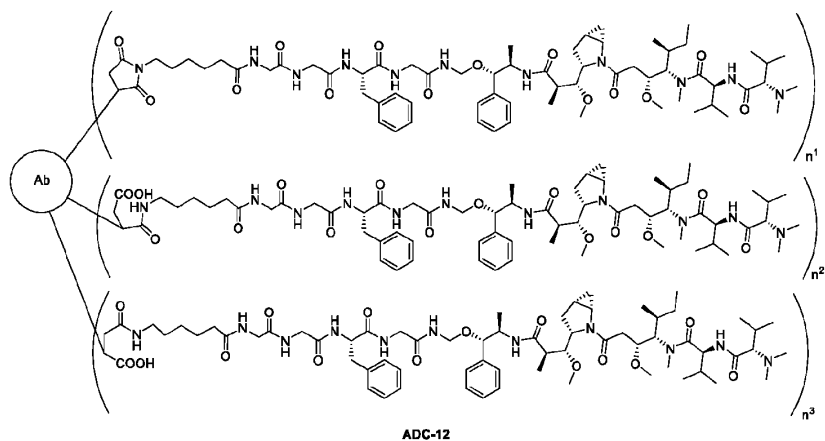
The antibody-drug conjugate of the present disclosure may be non-limitingly selected from the following structures:

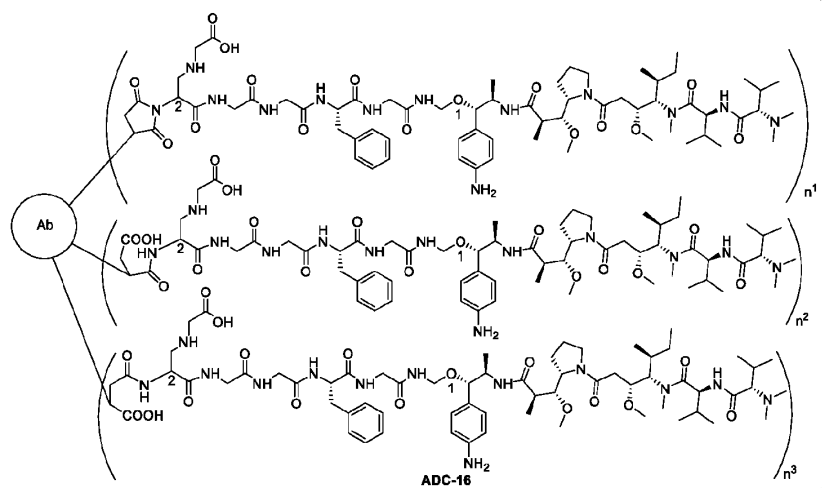
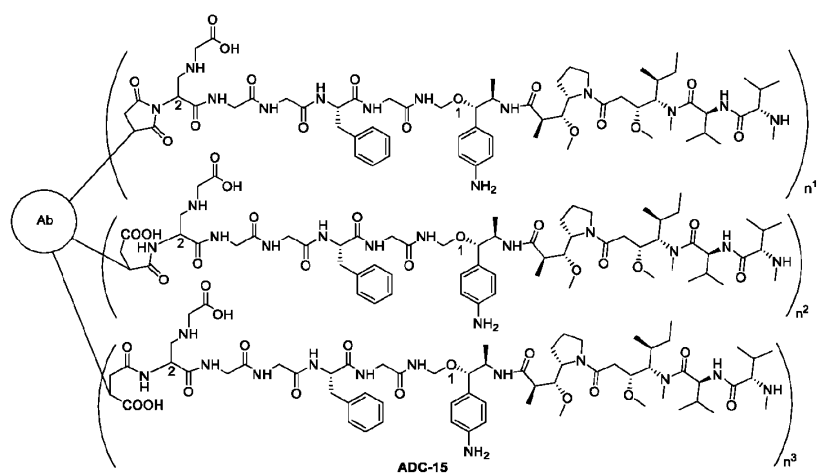


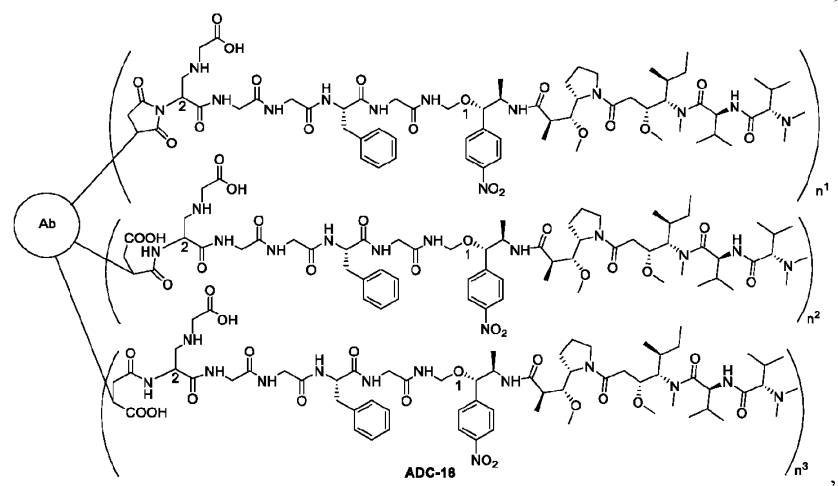
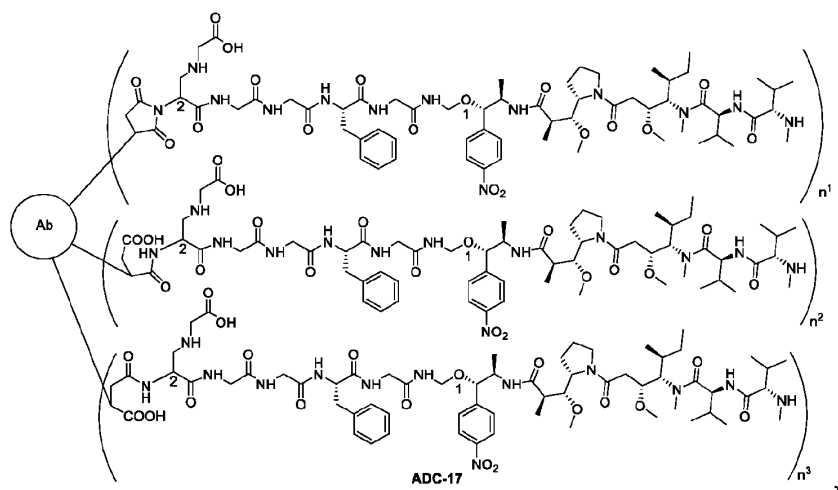


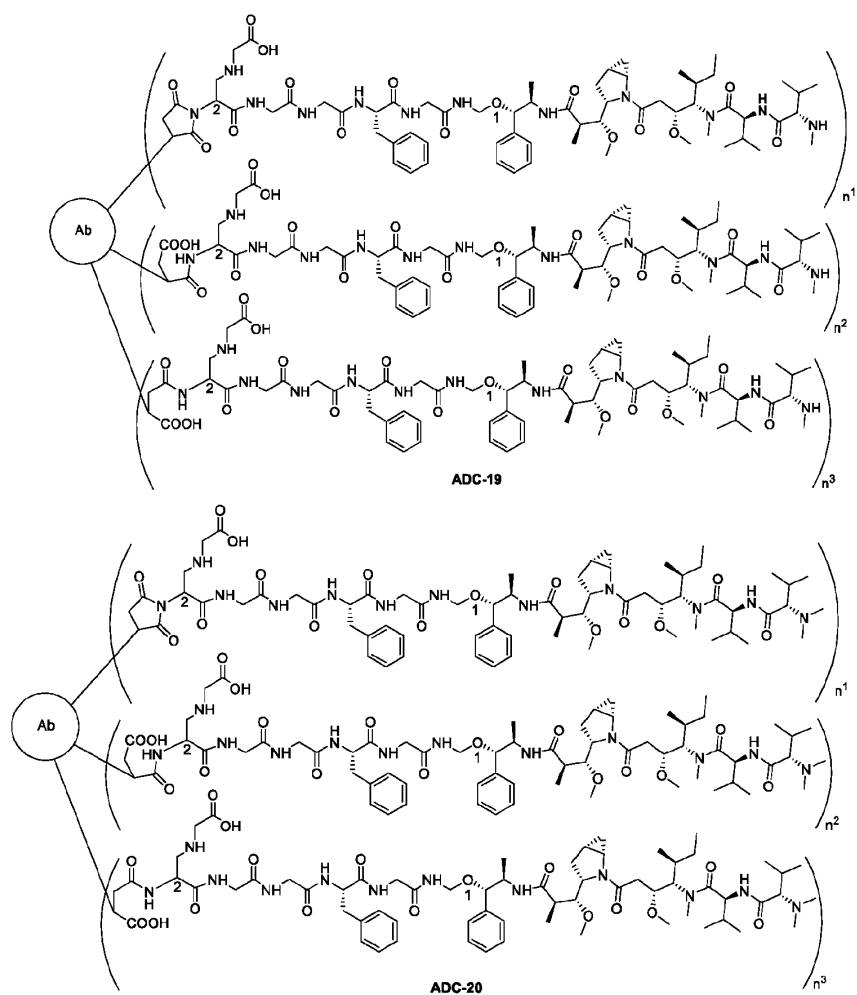


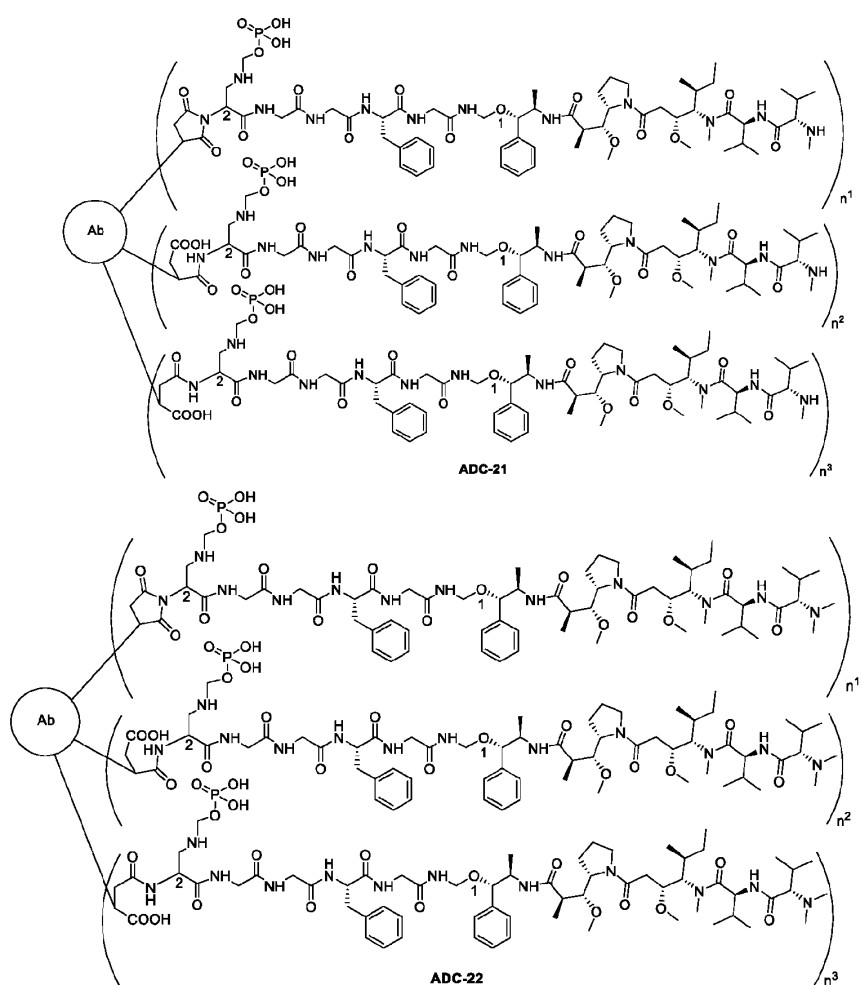


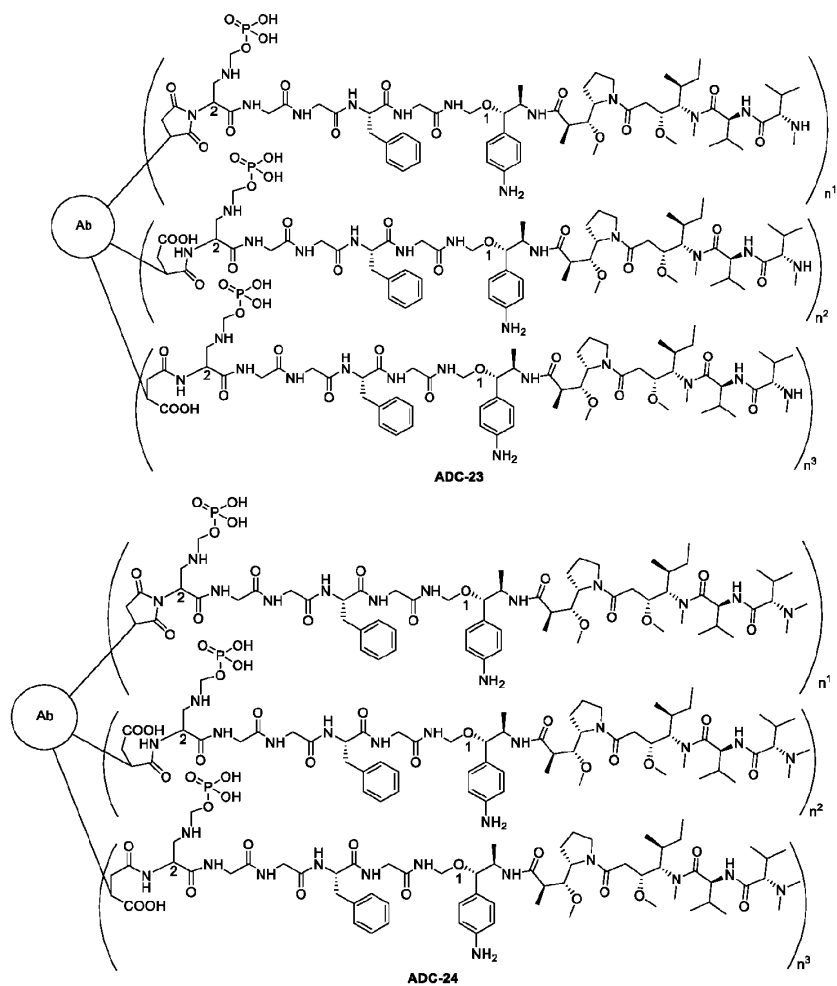


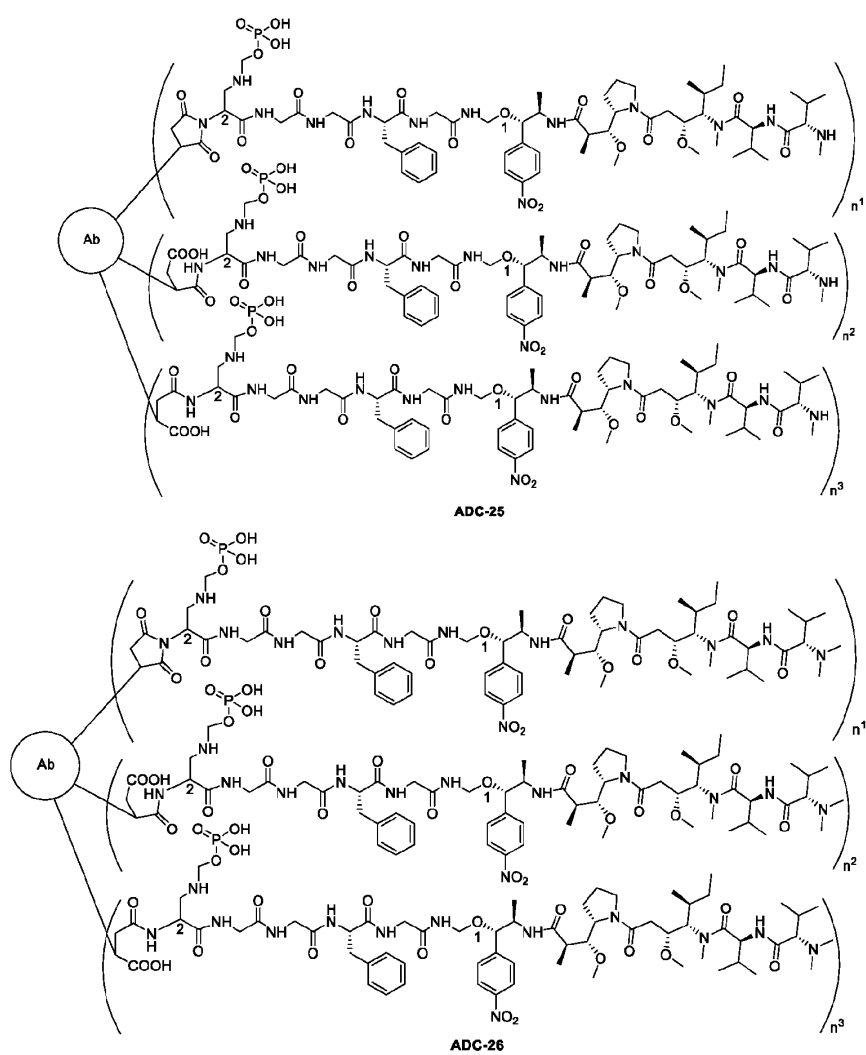


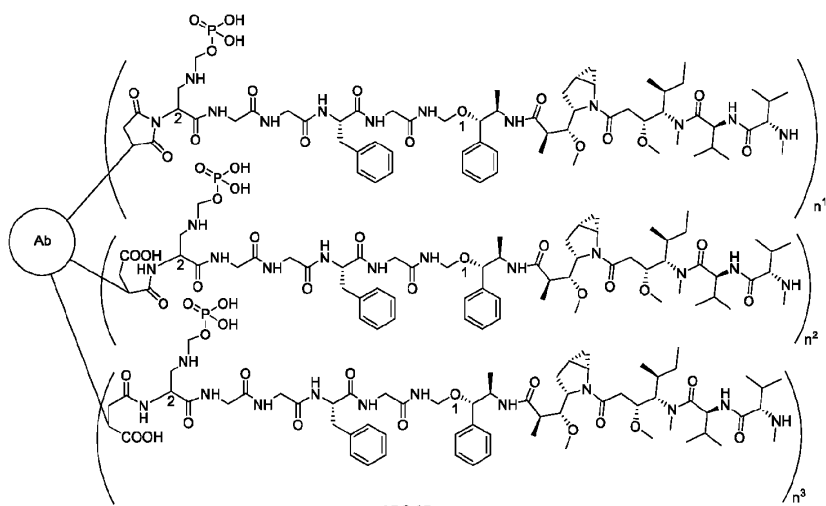




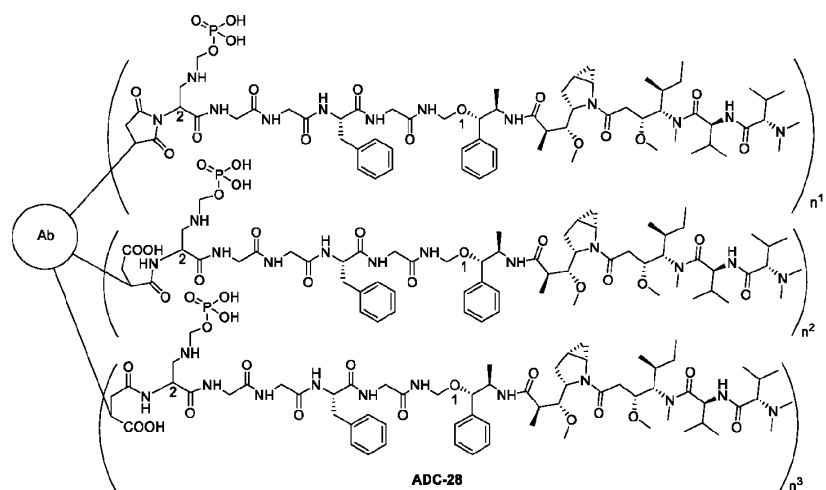




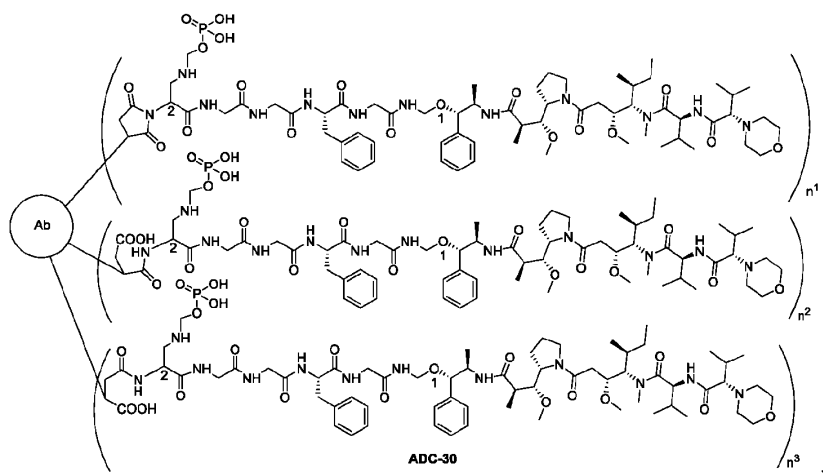
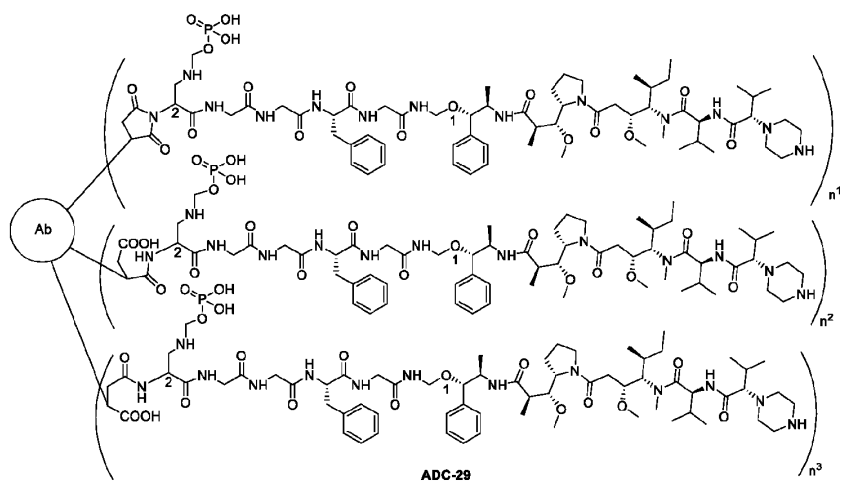


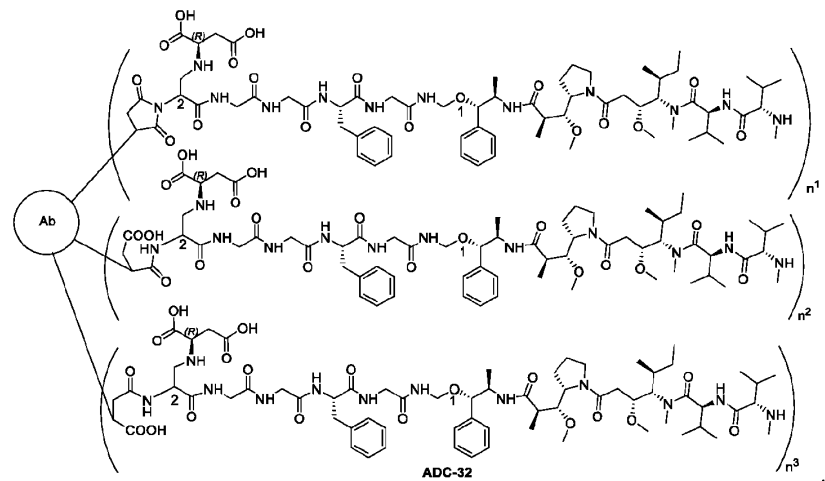
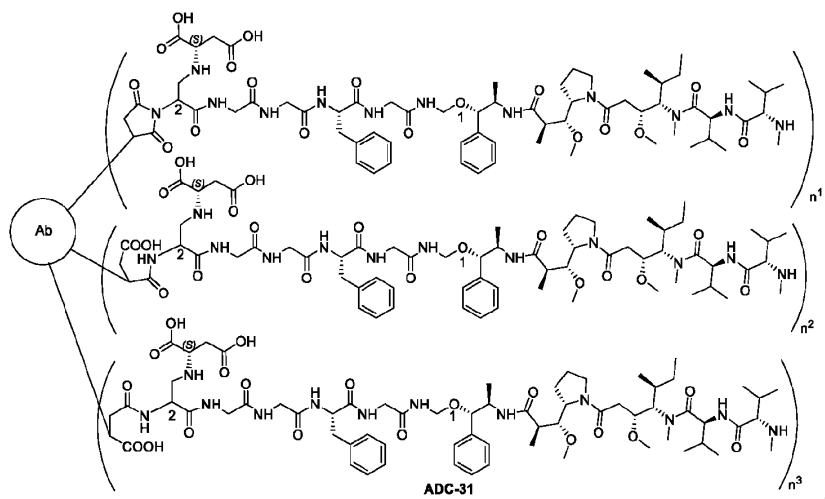


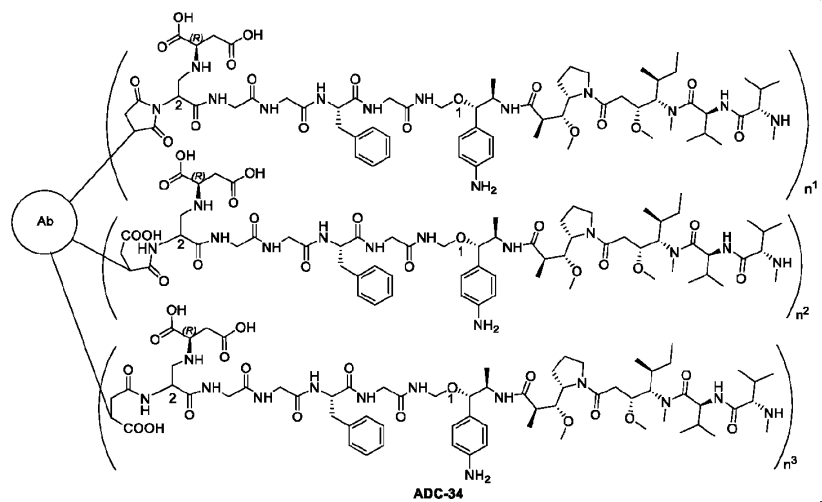
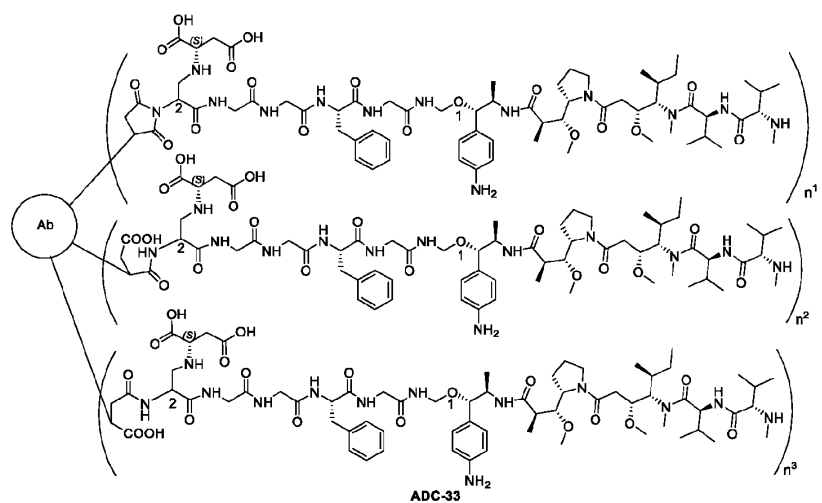
ADC-27

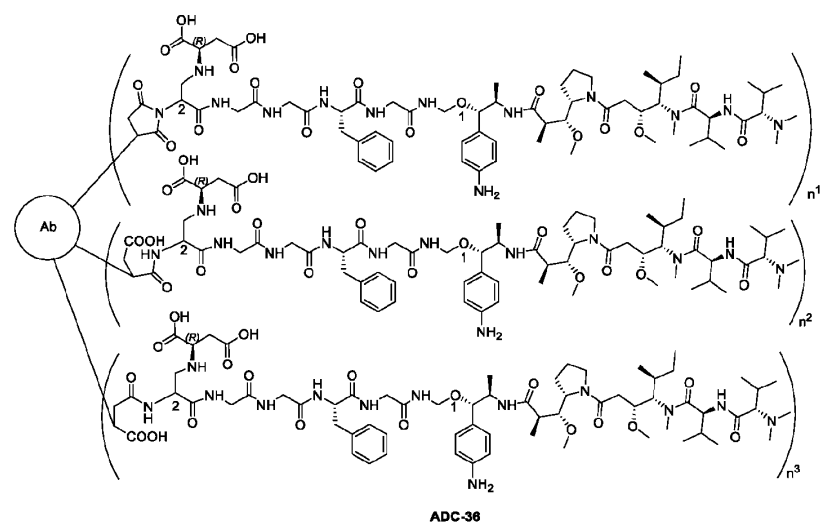
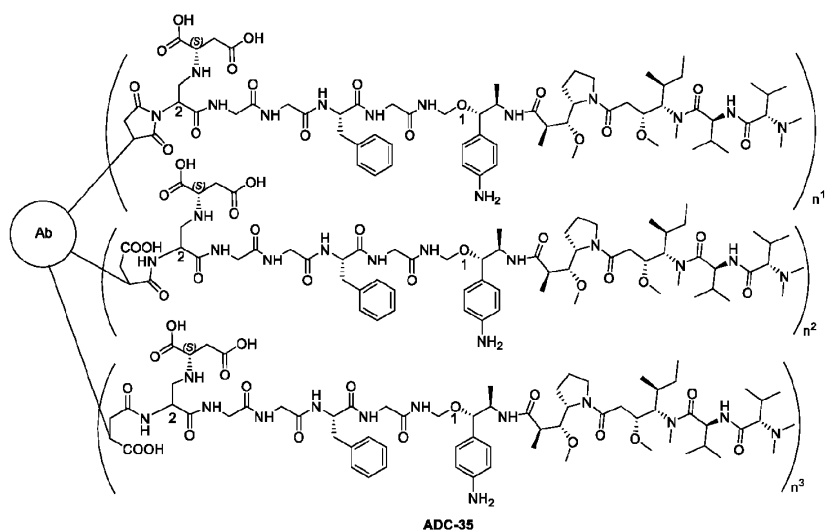


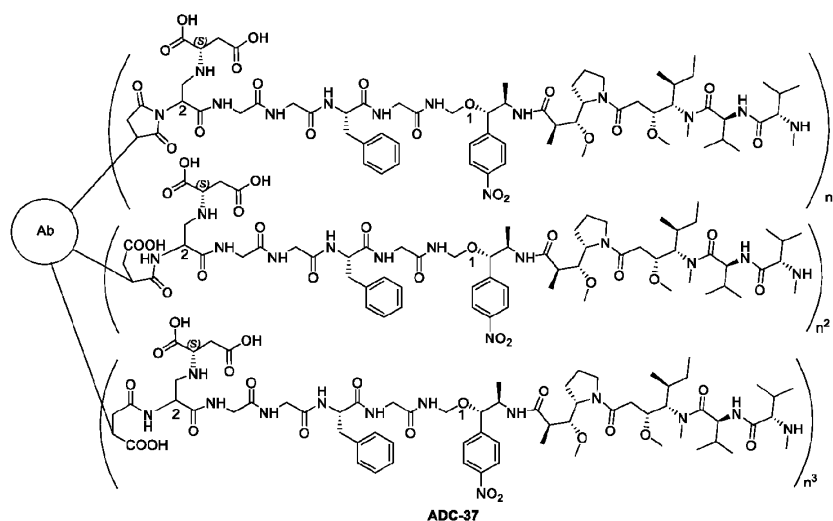
ADC-28



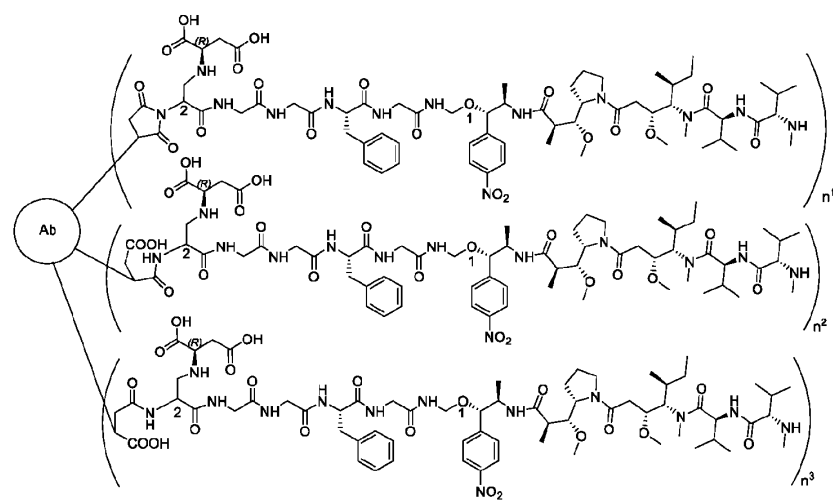




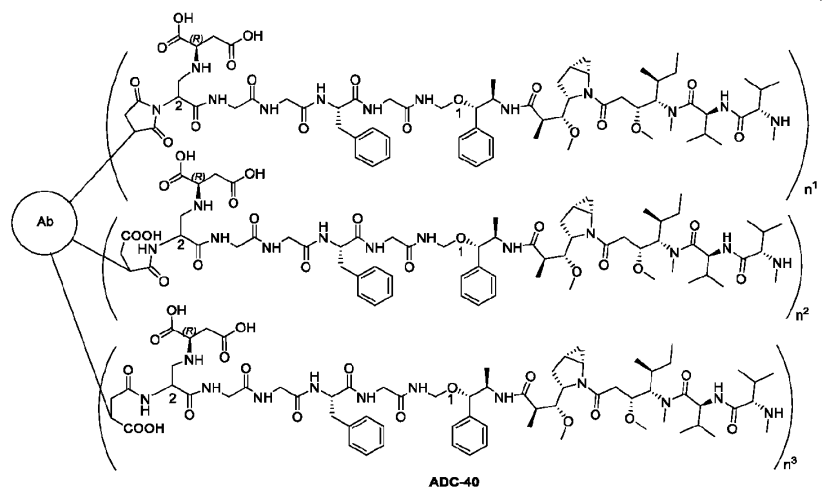
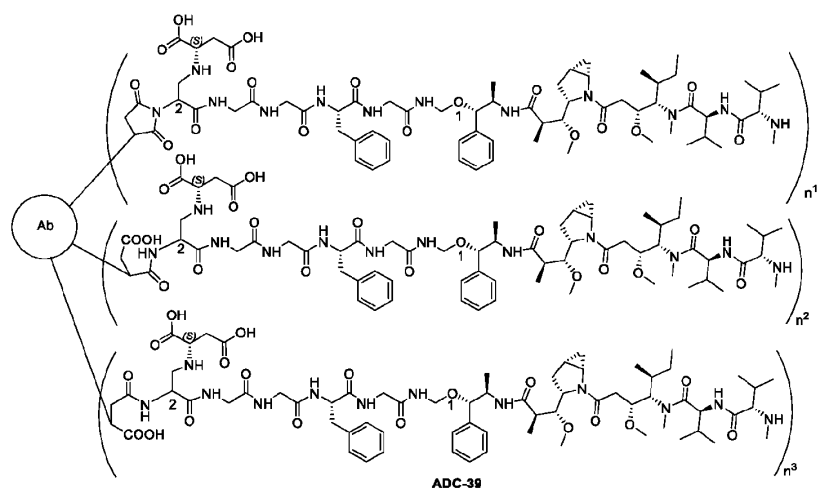


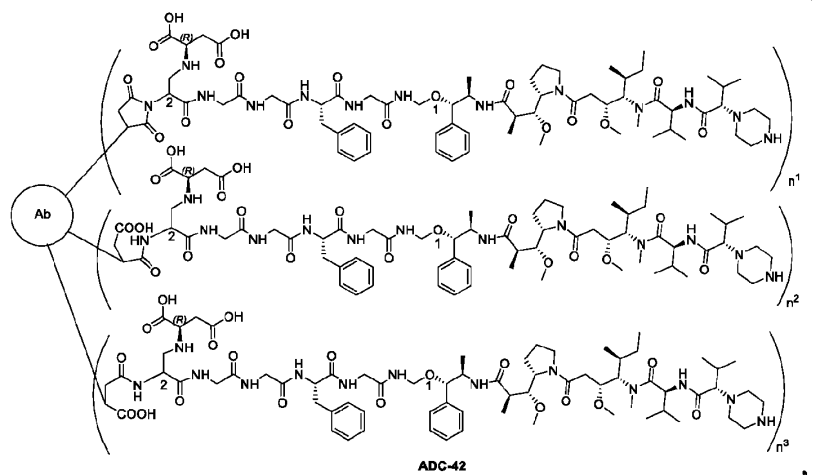
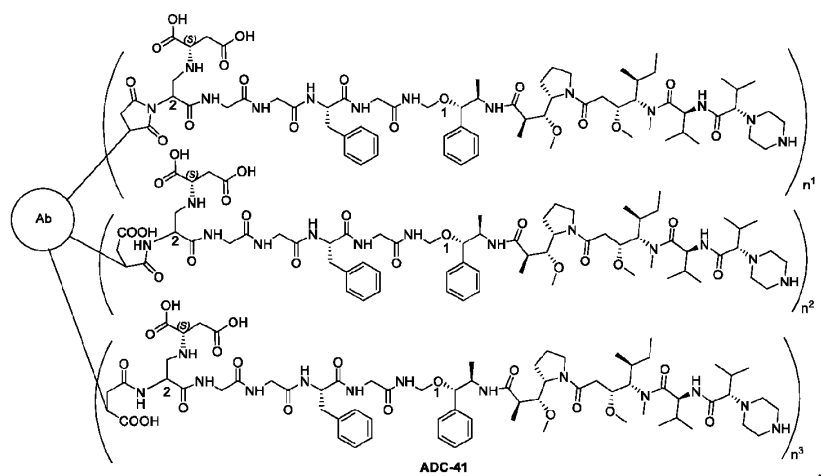


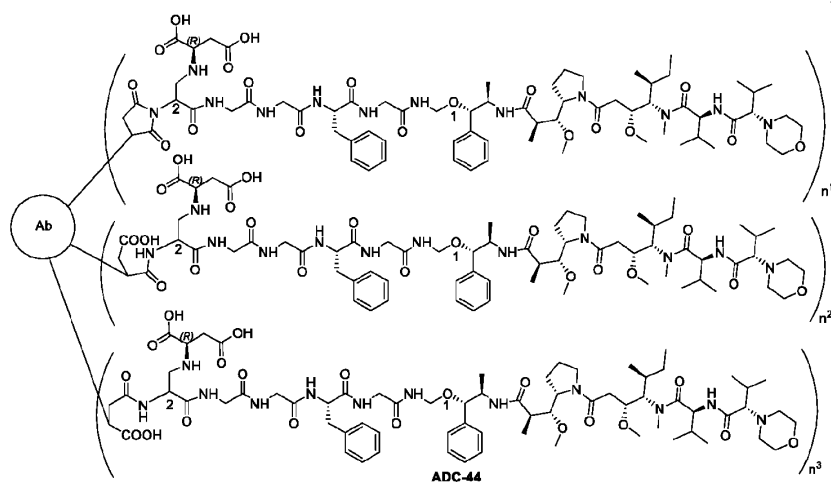
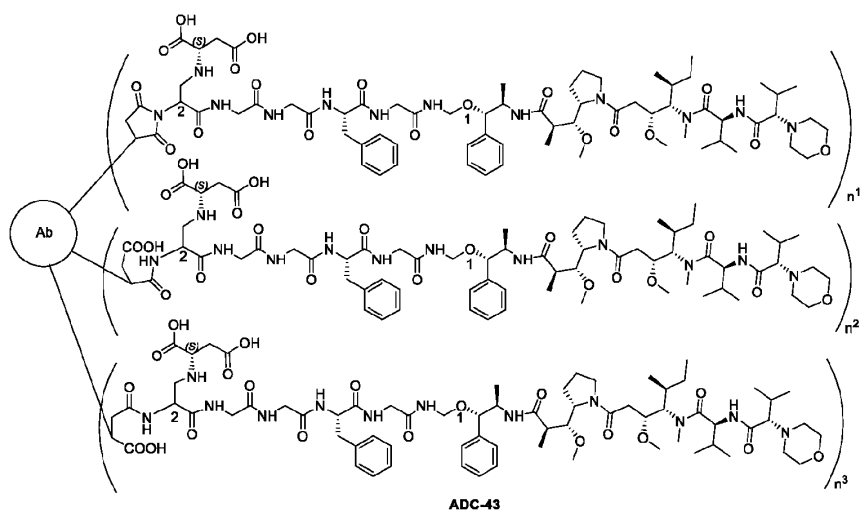
ADC-37

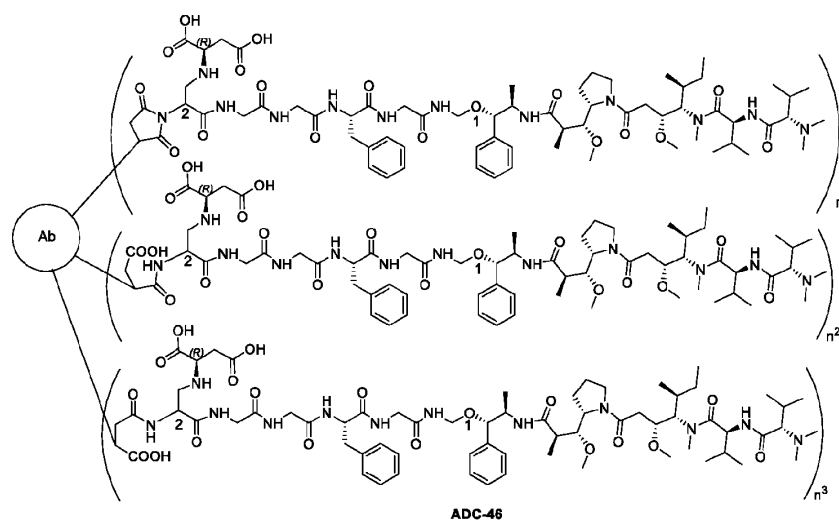
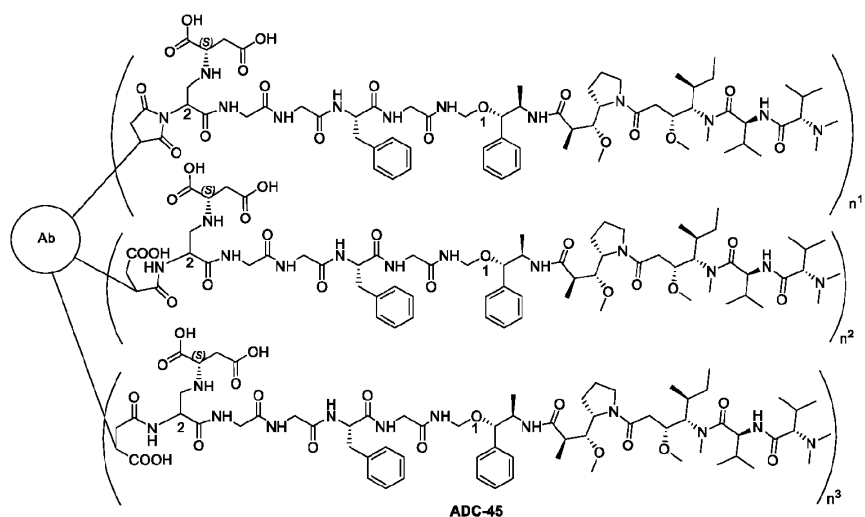


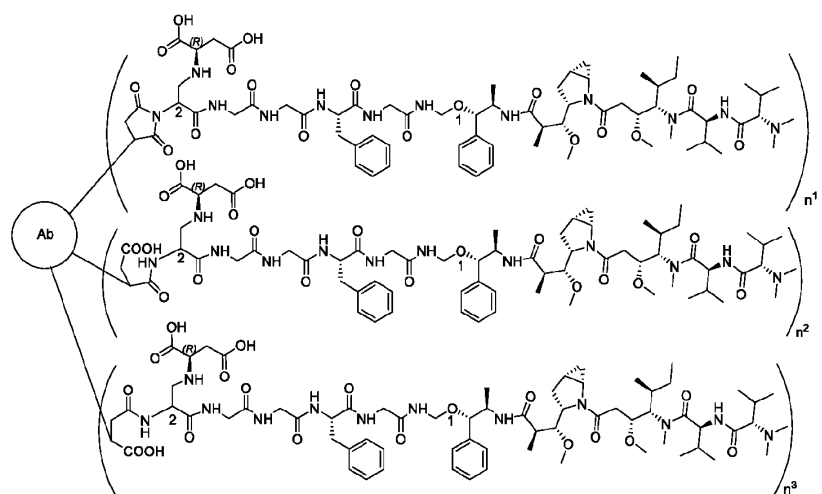
ADC-38



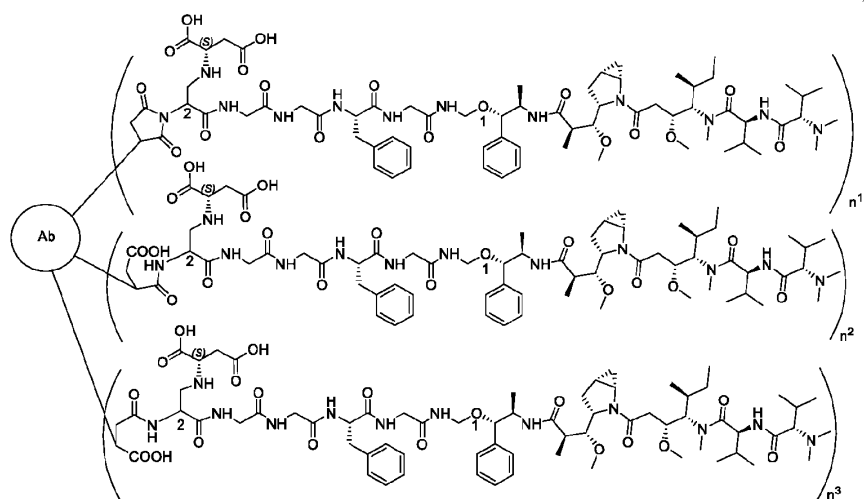




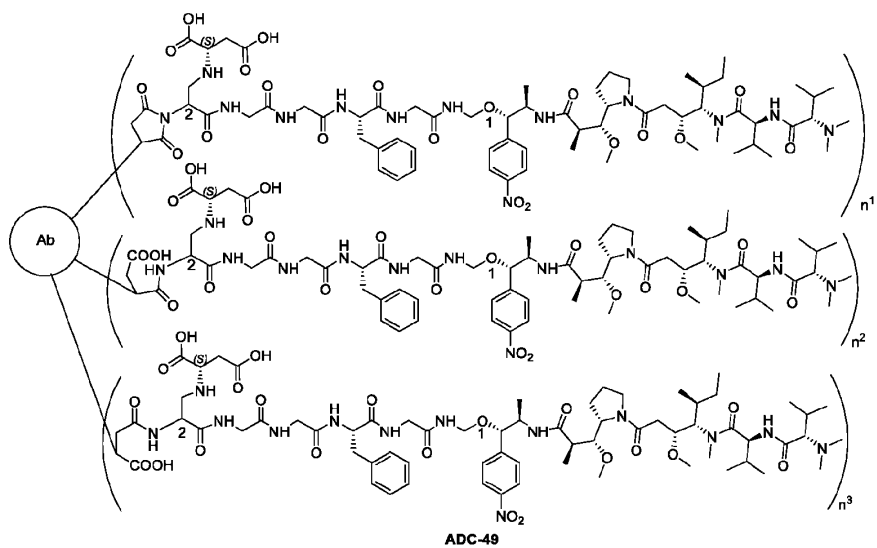




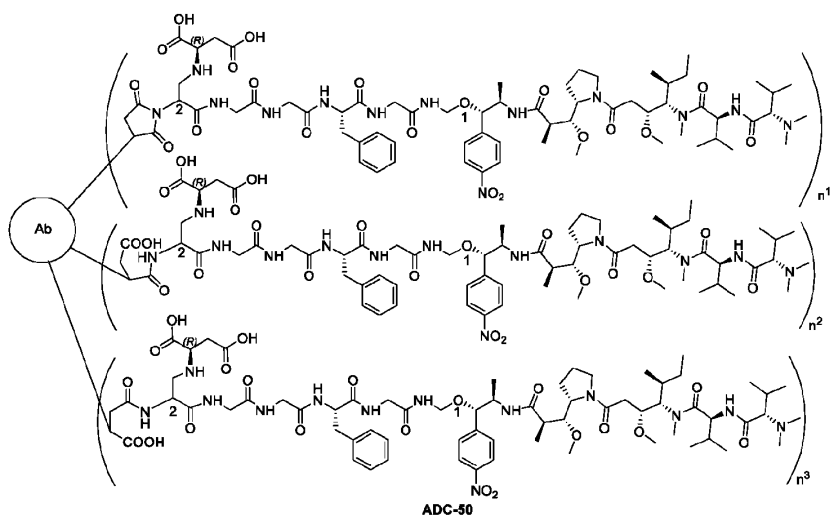
ADC-47

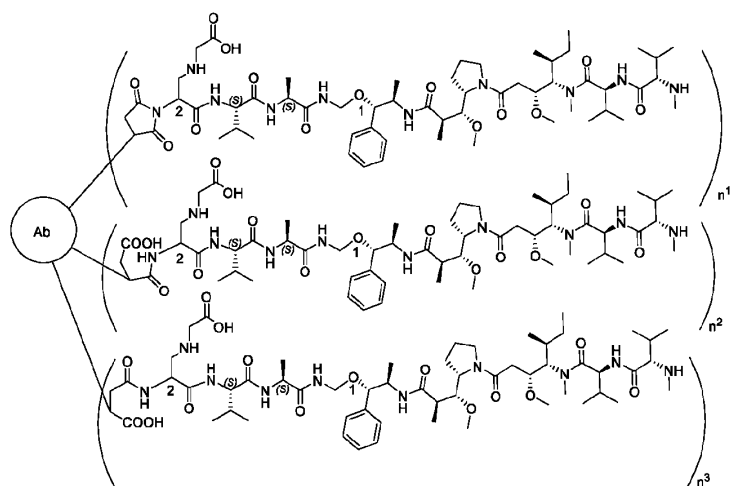


ADC-48

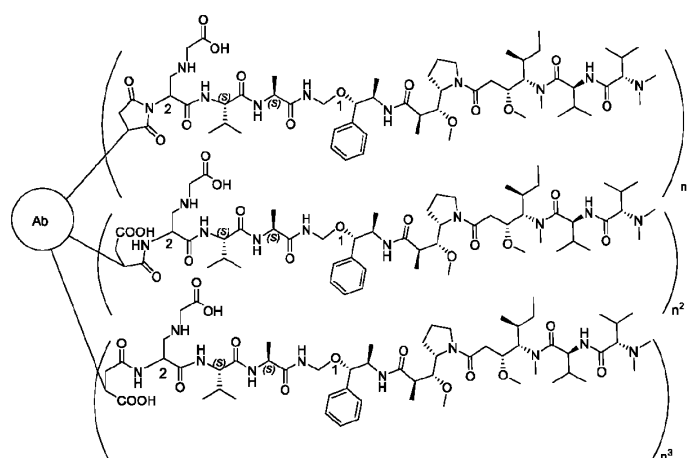


or

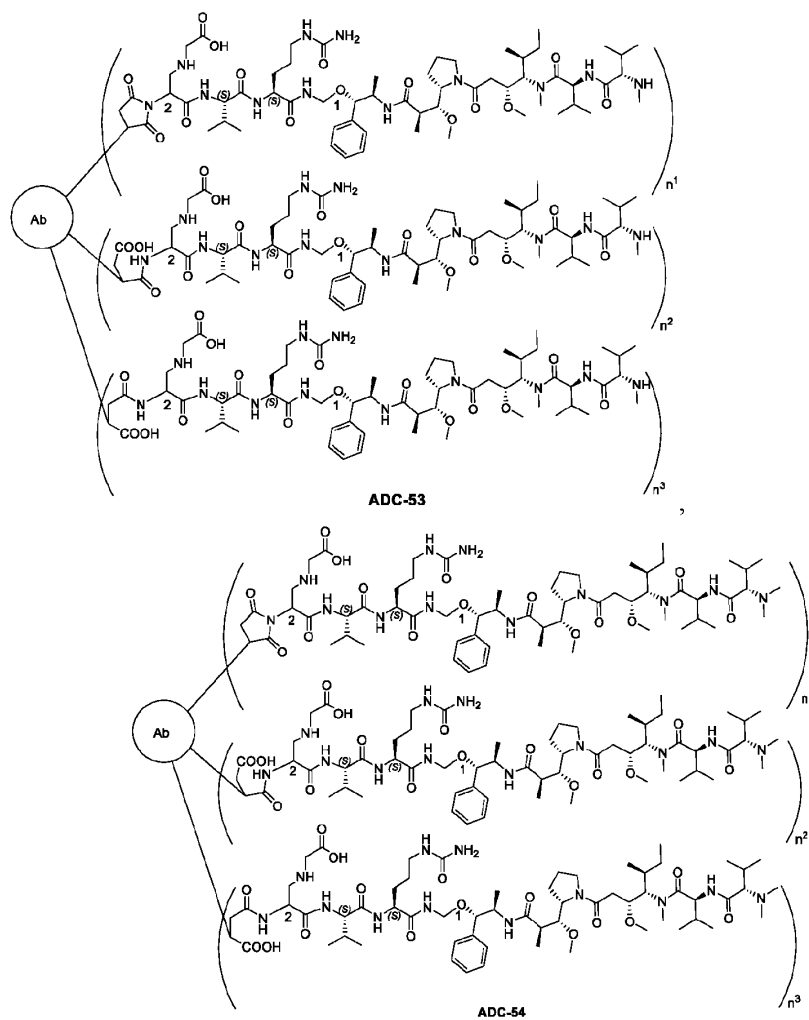




ADC-51

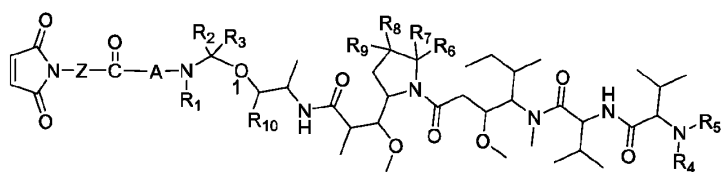


ADC-52



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

In another aspect, the application provides a linker-drug compound. In some embodiment, the linker-drug compound is as shown in formula II, or a pharmaceutically acceptable salt or solvate thereof,

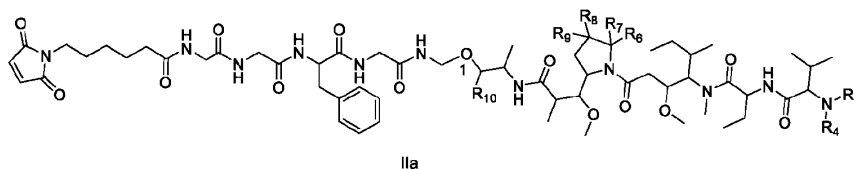


II

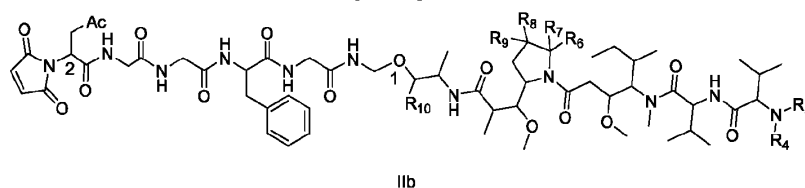
wherein,

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

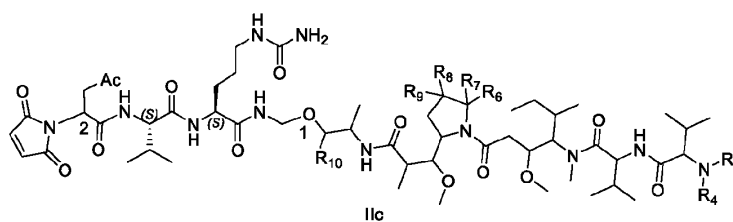
In certain embodiments, said linker-drug compound has the structure shown in formula IIa.



In certain embodiments, said linker-drug compound has the structure shown in formula IIb,

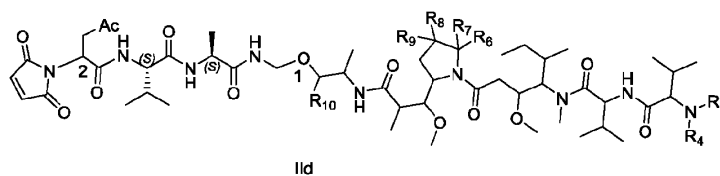


In certain embodiment, said linker-drug compound has the structure shown in Formula IIc,

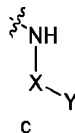


10

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

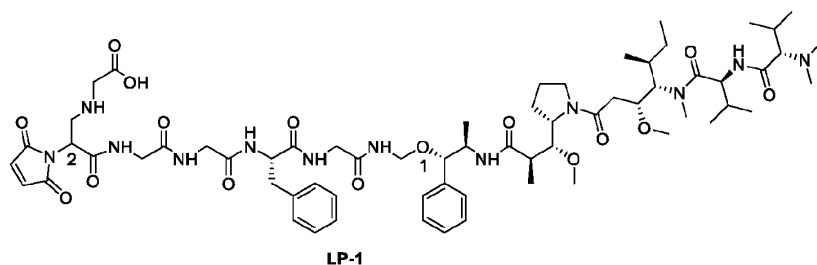


wherein Ac is a hydrophilic structural unit having the structure shown in formula c:

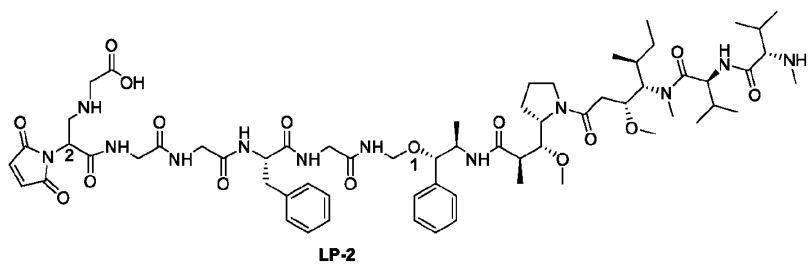


wherein X, Y are as defined above, and Ac is linked to methylene carbon at the position-2 already labeled in formula IIb, Formula IIc or Formula IId via -NH-.

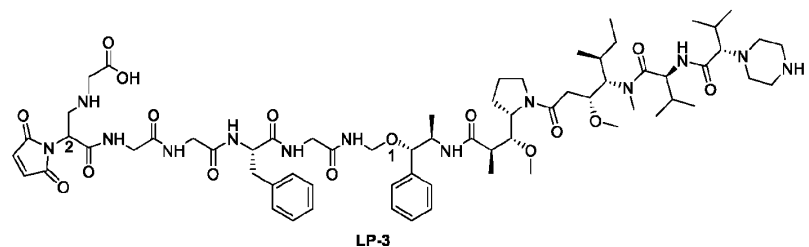
Example linker-drug compounds may include the following structures:



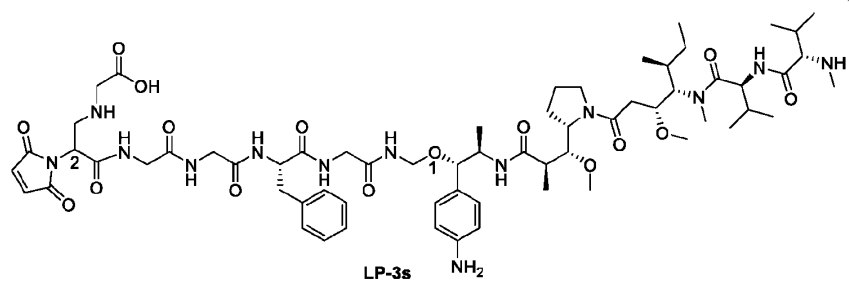
LP-1



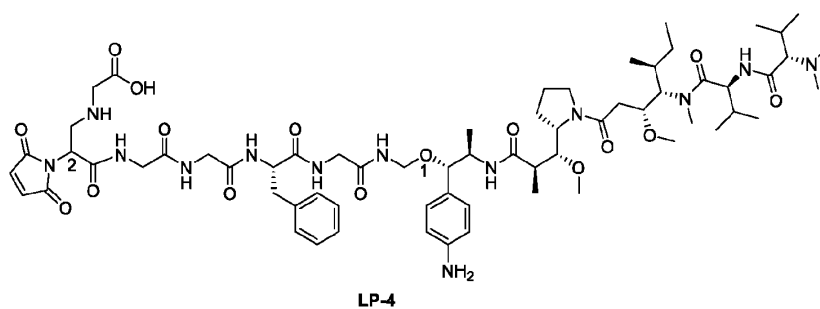
LP-2



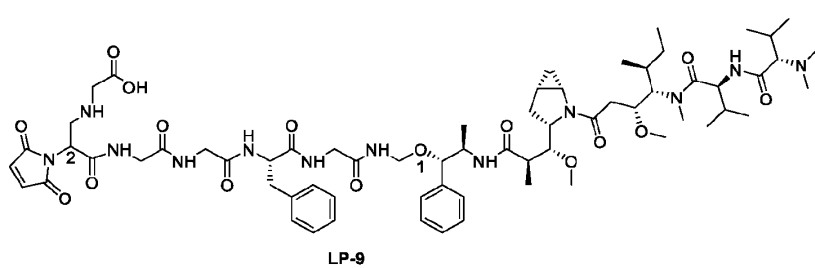
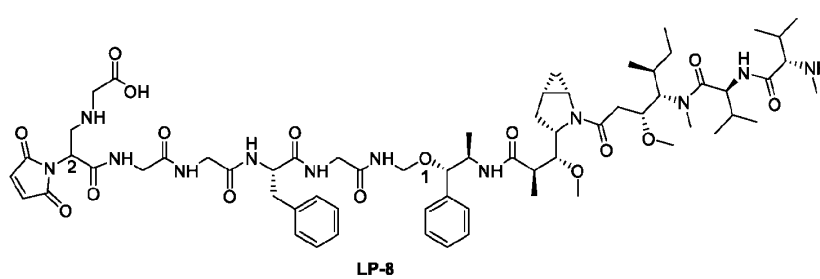
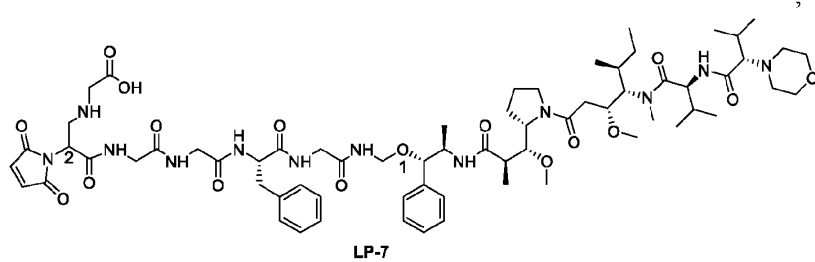
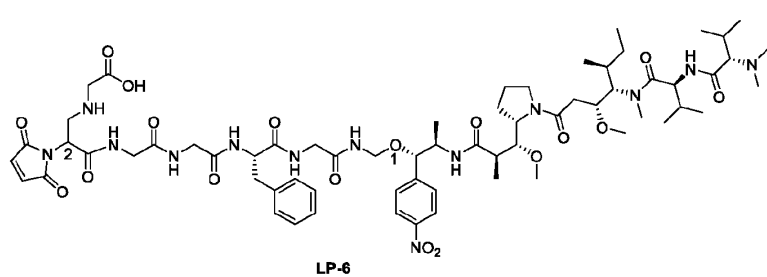
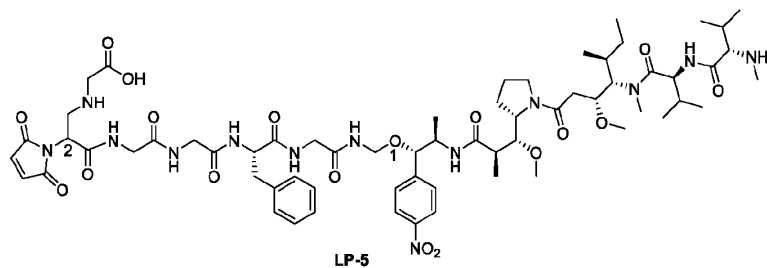
LP-3

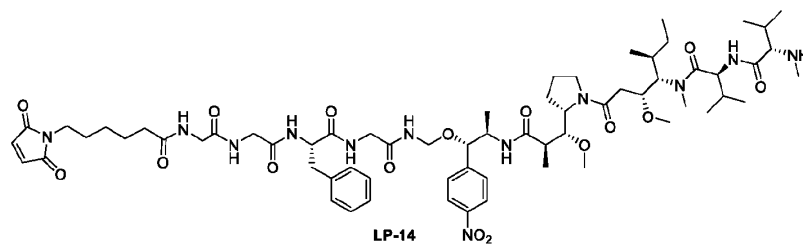
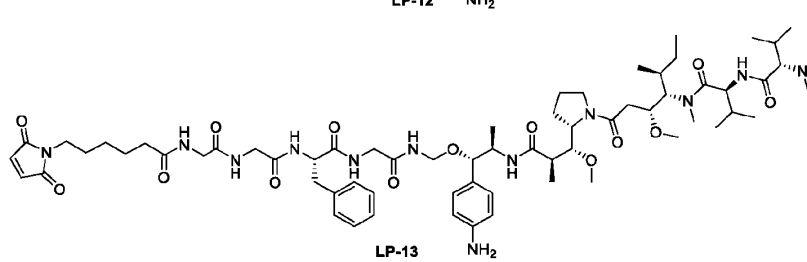
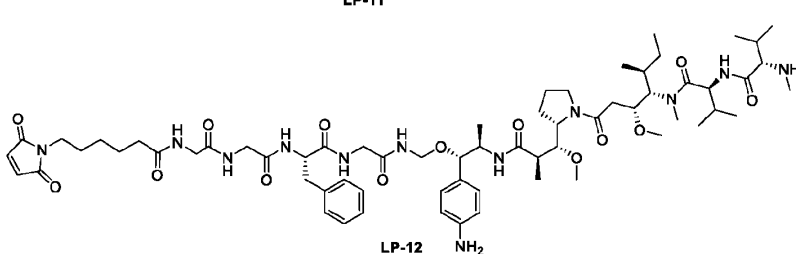
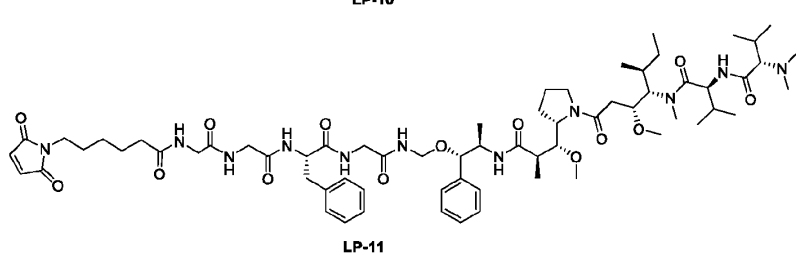
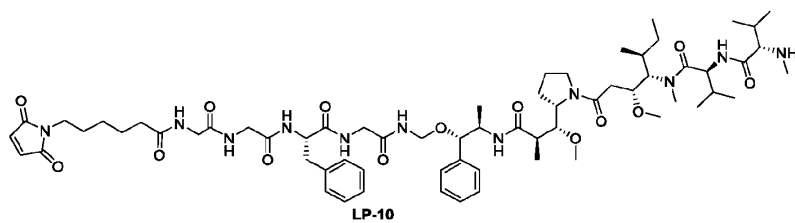


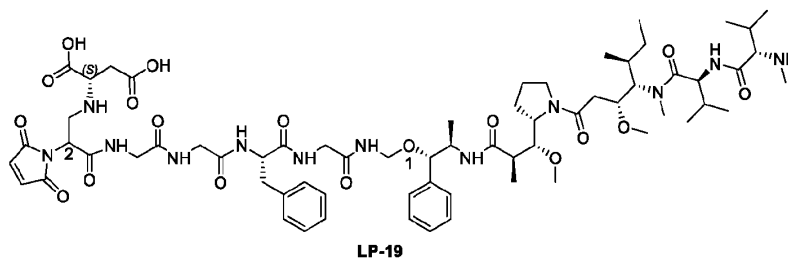
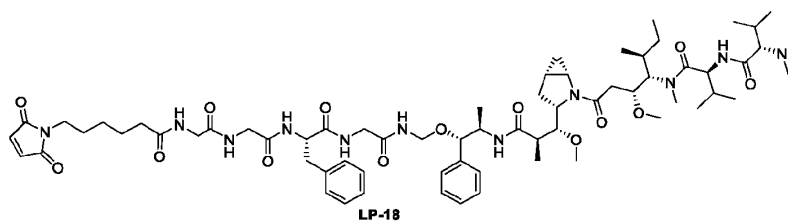
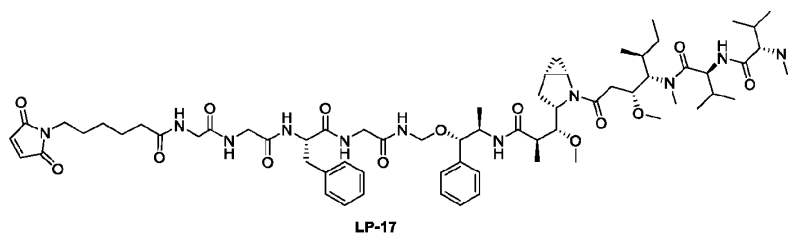
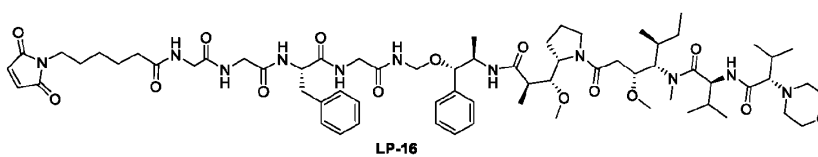
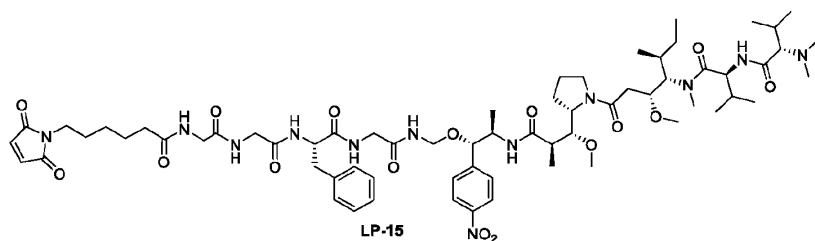
LP-3s

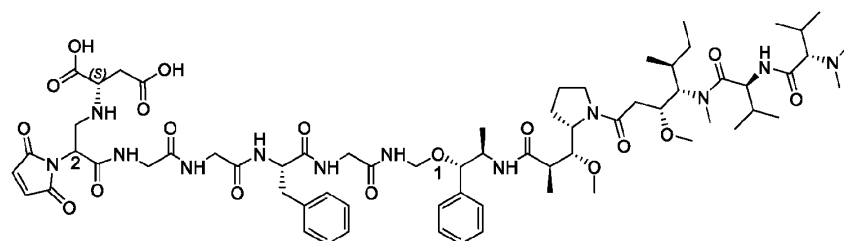


LP-4

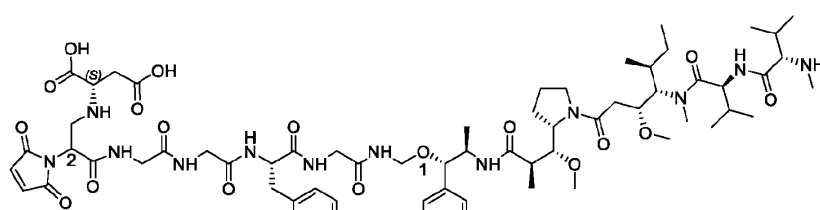




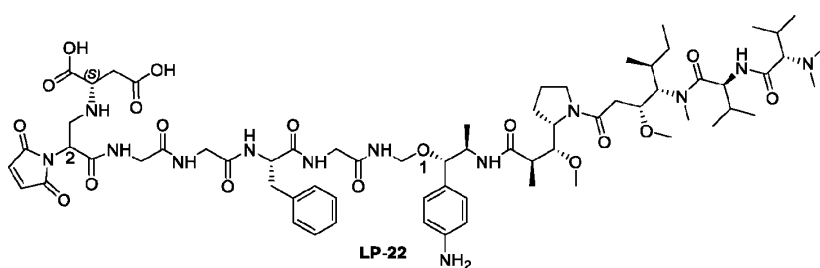




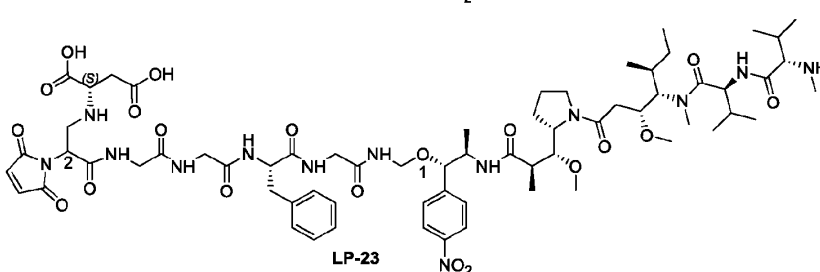
LP-20



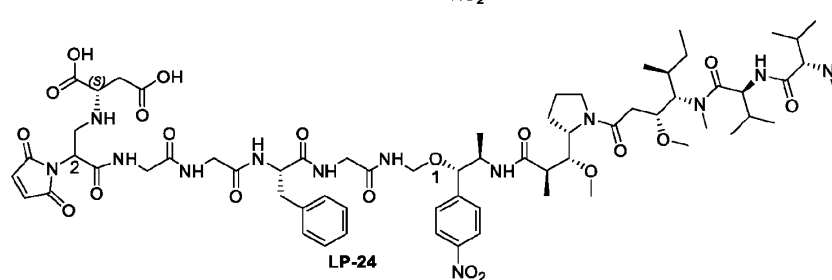
LP-21



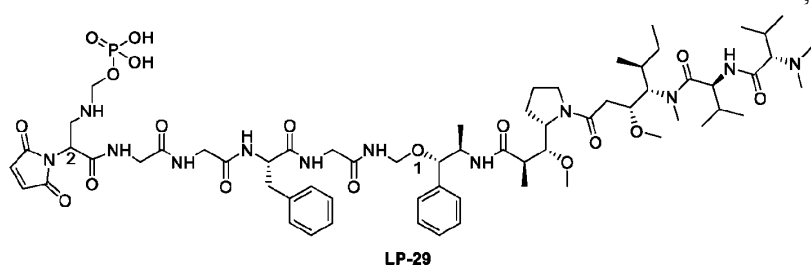
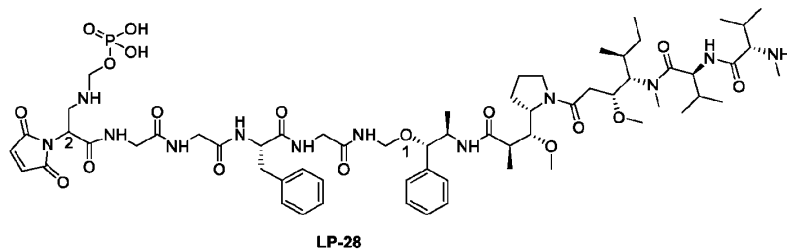
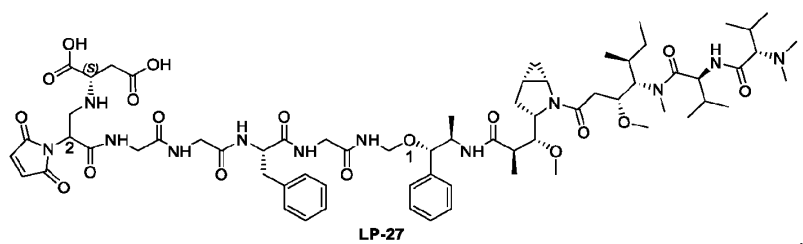
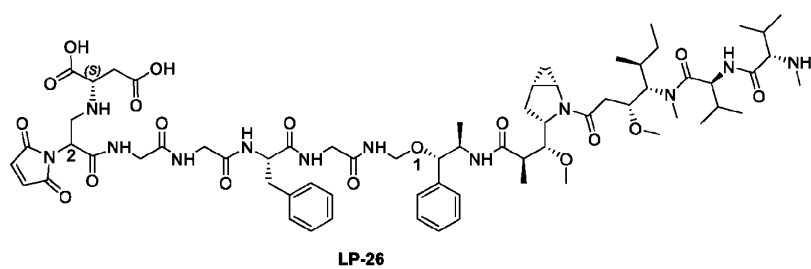
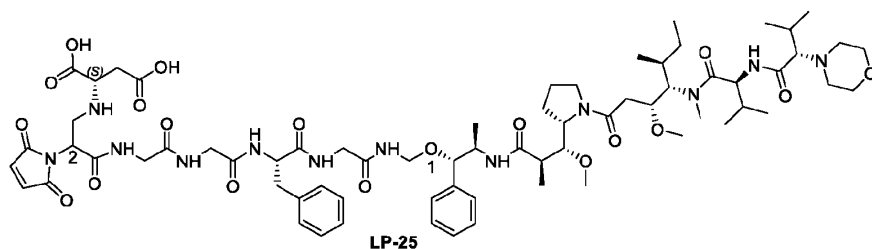
LP-22

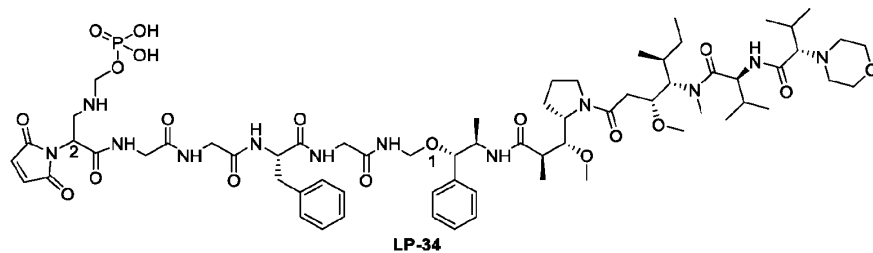
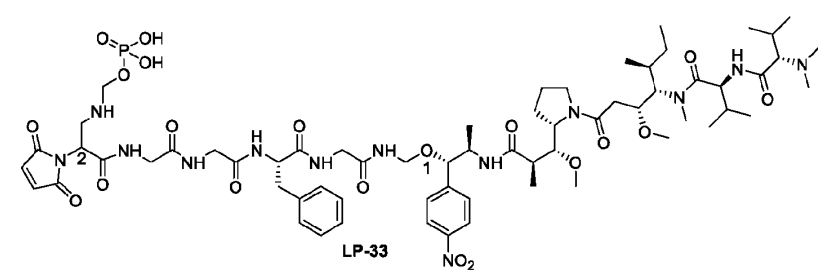
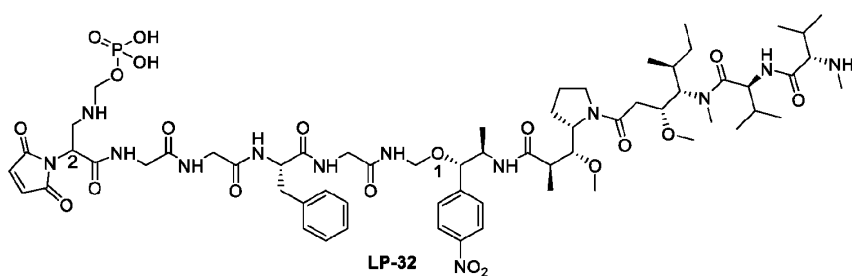
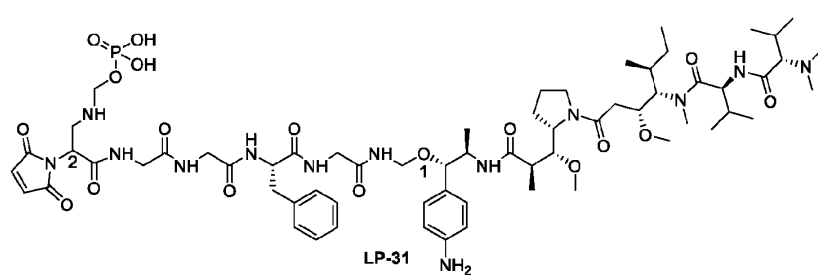
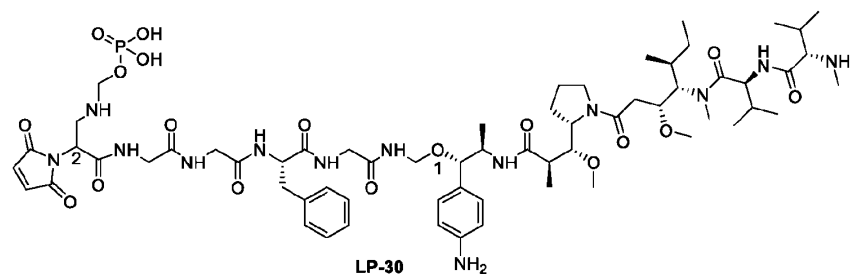


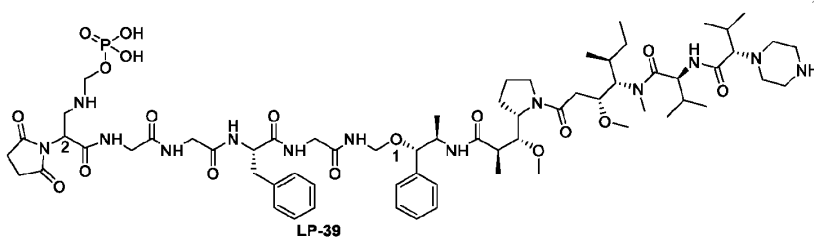
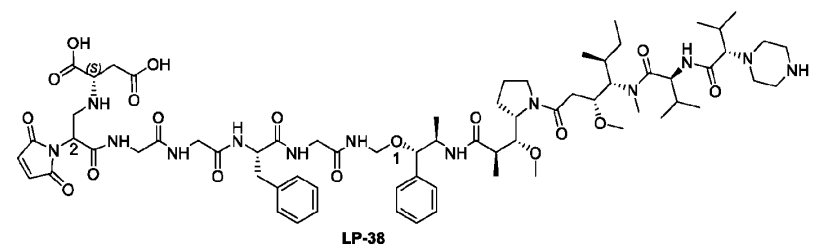
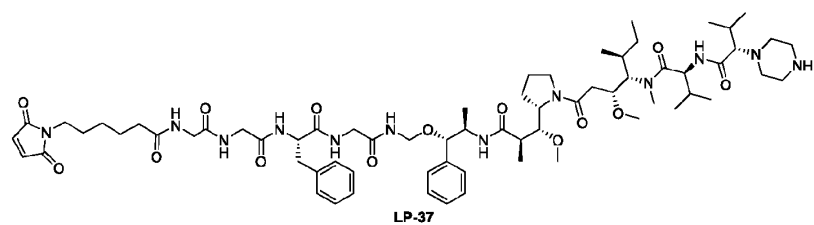
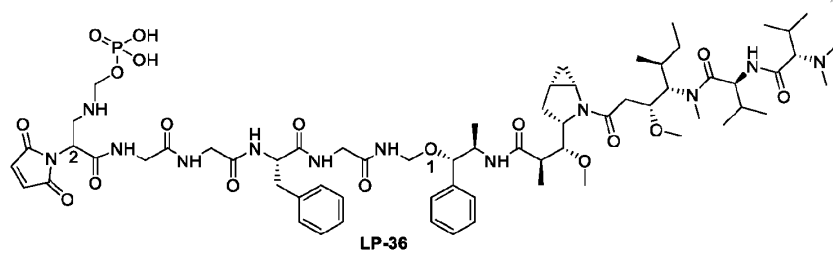
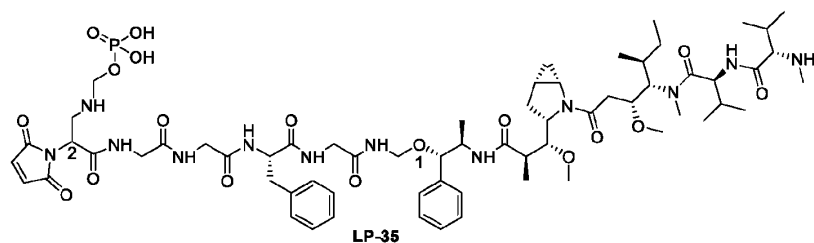
LP-23

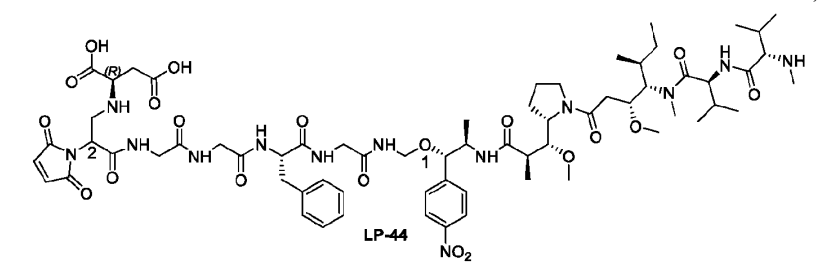
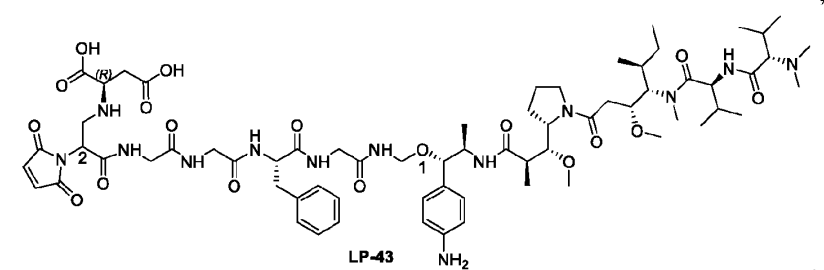
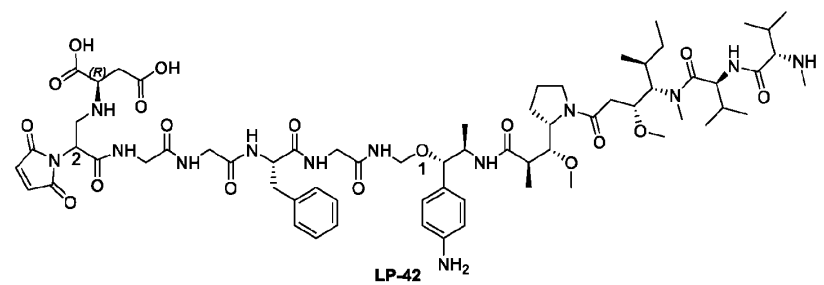
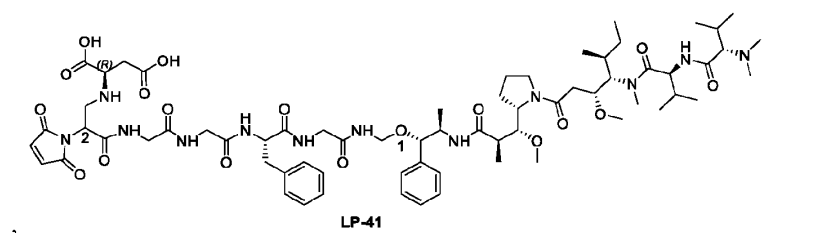
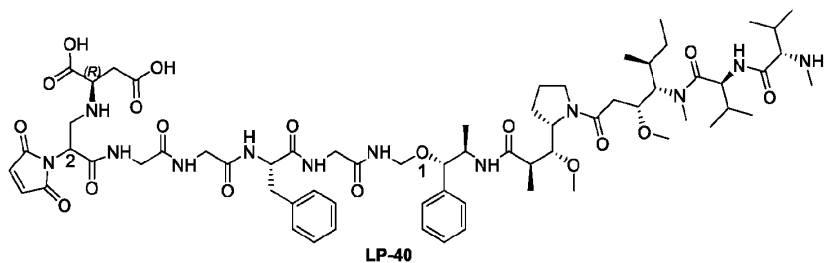


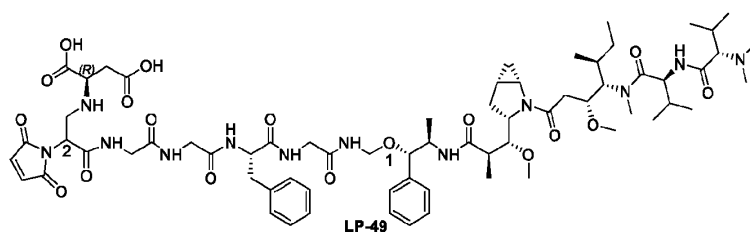
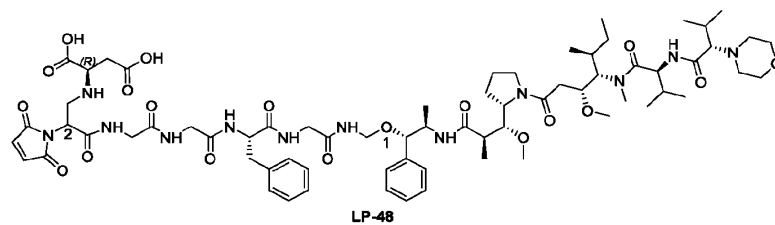
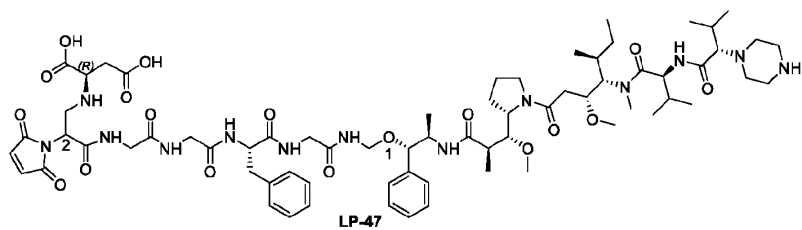
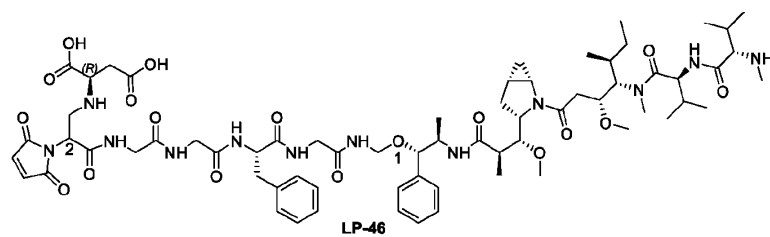
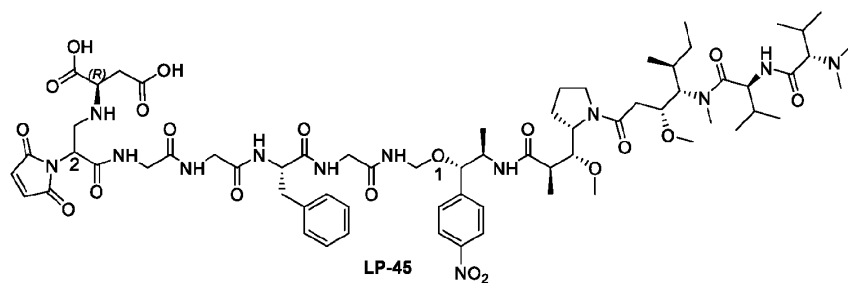
LP-24

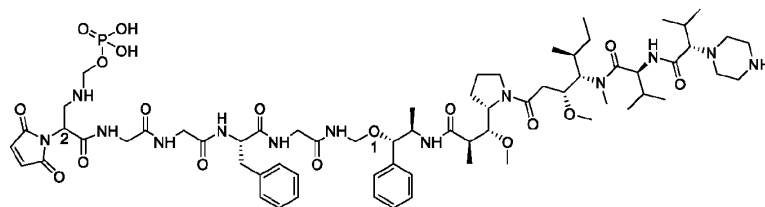




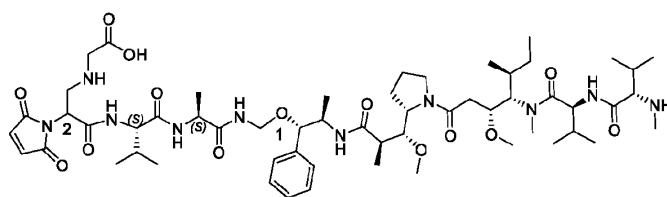




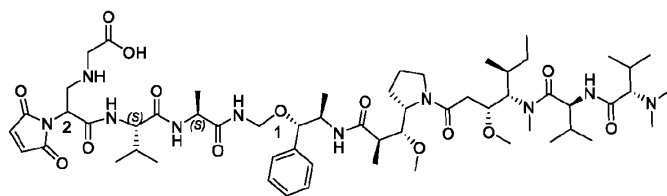




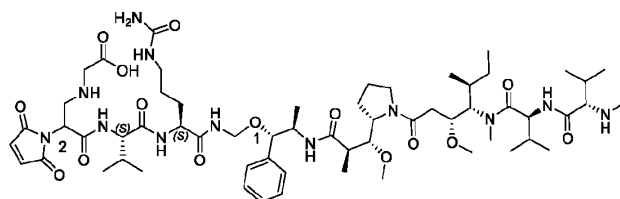
LP-50



LP-51

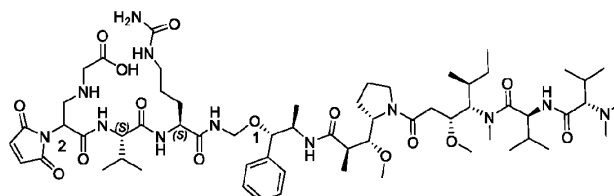


LP-52



LP-53

or



LP-54

;

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

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

10

In certain embodiments, said tumor expresses HER2.

In certain embodiments, said tumor is cancer.

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

In a further aspect, the application provides a pharmaceutical composition comprising an effective amount of an antibody-drug conjugate of the present disclosure, a pharmaceutically acceptable salt or solvate thereof, a linker-drug compound of the present disclosure or its isomer, mesomorph, racemate, enantiomer or a mixture thereof, a pharmaceutically acceptable salt or solvate thereof, or a pharmaceutically acceptable carriers, diluents or excipients.

In a further aspect, the application provides a pharmaceutical formulation comprising an effective amount of a ligand-drug conjugants or a pharmaceutically acceptable salt or solvate thereof, or a linker-drug compound, its isomer, mesomorph, racemate, enantiomer or a mixture thereof, or a pharmaceutically acceptable salt or solvate thereof.

In a further aspect, the application provides methods of treating or preventing a tumor. In one embodiment, the method comprises administering to a subject in need thereof an effective amount of an antibody-drug conjugate of the present disclosure, or a pharmaceutically acceptable salt or solvate thereof, or a linker-drug compound of the present disclosure, or its isomer, mesomorph, racemate, enantiomer or a mixture thereof, or a pharmaceutically acceptable salts or solvates. In certain embodiments, said subject is a mammal, such as a human.

In a further aspect, the application provides the use of said linker drug compounds, their isomers, meso-, endo-, racemates, enantiomers or mixture thereof, or its pharmaceutically acceptable salt or solvate, for preparing an antibody-drug conjugate or its pharmaceutically acceptable salt or solvate. In certain embodiments, the antibody-drug conjugate may be the auristatin ligand-drug compound as disclosed herein.

In an further aspect, the application provides methods for preparing an antibody-drug conjugate compound or a pharmaceutically acceptable salt or solvate thereof, said method comprising: coupling a reduced antibody or an antigen-binding fragment thereof with a linker-drug compound as disclosed herein, its an isomer, meso-, endo-, racemate, enantiomer or a mixture thereof, or a pharmaceutically acceptable salt or solvate thereof, to obtain the ligand-drug conjugate. In one embodiment, the antibody or its antigen-binding fragment can be reduced by a thiol reducing agent 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 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-C-1a (**3B**), FITC-IgG1-LP-1a (**3C**), ADC-Dxd (**3D**), ADC-1-1b (**3E**), ADC-1-1c (**3F**), ADC-1-1d (**3G**), ADC-1-1e (**3H**), and RC-48 (**3I**);

Figure 4 shows the SEC-HPLC results of ADC-1-1a (**4A**), ADC-C-1a (**4B**), and RC-48 (**4C**), ADC-1-1b (**4D**), ADC-1-1c (**4E**), ADC-1-1d (**4F**), ADC-1-1e (**4G**);

Figure 5 shows the *in vitro* efficacy of HER2-ADC in NCI-N87 cells (**5A**), HER2-ADC in SK-OV-3 cells (**5B**), HER2-ADC in Calu-3 cells (**5C**), HER2-ADC in JIMT-1 cells (**5D**), HER2-ADC in A431 cells (**5E**), HER2-ADC in MDA-MB-468 cells (**5F**), and HER2-ADC in a cell model of mixed tumor cells, JIMT-1+MDA-MB-468 (**5G**);

Figure 6 shows the *in vivo* efficacy of HER2-ADC in JIMT-1;

20 **Figure 7** shows the *in vivo* efficacy of HER2-ADC in mixed tumors (JIMT-1+MDA-MB-468);

Figure 8 shows the *in vitro* efficacy of ADC-1-1a and RC-48 in NCI-N87 cells (**8A**), ADC-1-1a and RC-48 in SK-OV-3 cells (**8B**), ADC-1-1a and RC-48 in Calu-3 cells (**8C**), ADC-1-1a and RC-48 in JIMT-1 cells (**8D**), ADC-1-1a and RC-48 in NCI-H1975 cells (**8E**), ADC-1-1a and RC-48 in A431 cells (**8F**), ADC-1-1a and RC-48 in MDA-MB-468 cells (**8G**), and ADC-1-1a and RC-48 in the mixed tumor cell model JIMT-1+MDA-MB-468 (**8H**);

Figure 9 shows the *in vivo* efficacy of ADC-1-1a and RC-48 in JIMT-1;

Figure 10 shows the *in vivo* efficacy of ADC-1-1a and RC-48 in mixed tumors (JIMT-1+MDA-MB-468);

Figure 11 shows the *in vivo* efficacy of ADC-1-1a and RC-48 in NCI-N87; and

30 **Figure 12** shows the *in vivo* efficacy of ADC-1-1a and RC-48 in NCI-H1975.

DETAILED DESCRIPTION

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

40

The disclosure provides, among others, antibodies including for example bispecific

olated antibodies, methods of making such antibodies, monoclonal and/or recombinant antibodies, linkers, payloads, toxins, ligand-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, 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.

20 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. 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 30 another aspect, the sulfur atom is a sulfur atom that has been introduced into a cysteine residue of the ligand unit by, for example, targeted mutagenesis or chemical reaction.

The term "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.

40 The term "ligand-drug conjugate" refers to a molecule in which a ligand is attached to a drug by means of a stabilizing linker unit. In the present invention, "ligand-drug coupling" is preferably an antibody drug conjugate (ADC), which refers to the attachment of a monoclonal antibody or a functional antibody fragment or a targeted protein, etc., to a cytotoxic payload, toxin, or drug by means of a stabilized 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

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 present application maintain their original antigen-binding capacity in the wild. Thus, the antibodies of the present invention 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 be functional), 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 present invention include, but are not limited to, murine antibodies, chimeric antibodies, humanized antibodies and fully human antibodies, preferably 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

27-131, 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 into 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 "payload" refers to the pharmaceutically active component of ADC that delivers a toxic cargo to a specific target and elicits the desired therapeutic response. Many of the payloads for oncology ADCs are natural product based with some making covalent interactions with their target. Payloads include the microtubulin inhibitors monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF) and mertansine, DNA binder calicheamicin and topoisomerase 1 inhibitors SN-38 and exatecan resulting in a renaissance for natural product total synthesis. Glucocorticoid receptor modulators (GRMs) represent to most active payload class for iADCs. Approaches releasing marketed GRM molecules such as dexamethasone and

ve been developed. Modified GRM molecules have also been developed that enable the attachment of the linker with the term ADCidified describing the medicinal chemistry process of payload optimization to facilitate linker attachment. Alternatives to small molecule payloads have also been investigated, for example, siRNA. The term "antibody-drug conjugate" refers to the attachment of an antibody to a biologically active drug by means of a stabilized linkage unit. In the present invention, "ligand-drug conjugate" is preferably an antibody drug conjugate (ADC), which 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 present invention 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.

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, preferably an alkyl group comprising from 1 to 12 carbon atoms, more preferably an alkyl group comprising from 1 to 10 carbon atoms, and most preferably an alkyl group 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-ethylpentyl, 4-methylpentyl, 4-methylpentyl, n-nonyl, 2, 2-methylpentyl, 4-methylpentyl, n-nonyl ethylpentyl, n-nonyl, 2-methyl-2-ethylhexyl, 2-methyl-3-ethylhexyl, 2, 2-diethylpentyl, n-decyl, 3, 3-diethylhexyl, 2, 2-diethylhexyl, and their various branched isomers, and the like. More preferred 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 preferably being one or more of the following groups independently selected from alkyl, alkenyl, alkynyl, alkoxy, alkylthio, alkylamino, halogen, mercapto, hydroxy, nitro, cyano, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, cycloalkoxy, heterocycloalkoxy, cycloalkane thio, heterocycloalkylthio, oxo.

"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 selected from the group consisting of: - halogen, -OR', -NR'R'', -SR', -SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -CONR'R'', -OC(O)NR'R''', -NR'C(O)R'', -NR'-C(O)NR''R''', -NR''C(O)₂R'', -NH-C(NH₂)=NH, -NR'C(NH₂)=NH, -NH-C(NH₂)=NR', -S(O)R', -S(O)₂R', -S(O)₂NR'R'', -NR'S(O)₂R'', -CN and -NO₂, the number of substituents is from 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.

The term "heteroalkyl" means a group formed by substitution of one or more carbons on an alkyl group 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, preferably from 3 to 12 carbon atoms, more preferably from 3 to 10 carbon atoms, and most preferably 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.

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 preferably one or more of the following groups independently selected from alkyl, alkenyl, alkynyl, alkoxy, alkylthio, alkylamino, halogen, mercapto, hydroxy, nitro, cyano, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, cycloalkoxy, heterocycloalkoxy, cycloalkylthio, heterocycloalkylthio.

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 selected from nitrogen, oxygen, or S(O)_m (wherein m is 0, 1, or 2), and the remaining ring atoms are carbon. Preferably, it contains from 3 to 12 ring atoms, 1 to 4 of which are heteroatoms; more preferably, 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 ring heterocyclic groups.

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, preferably 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 selected from alkyl, alkenyl, alkynyl, alkoxy, alkylthio, alkylamino, halogen, deuterium atom, mercapto, hydroxyl, nitro, cyano, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, cycloalkoxy, heterocycloalkoxy, cycloalkylthio or heterocycloalkylthio.

n "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 1 or 2 other heteroatoms (e.g. oxygen and/or sulfur atoms), or 2 nitrogen atoms and 1 or 2 other heteroatoms (e.g. oxygen and/or sulfur atoms). "5-8-membered monocyclic heteroaryl" include but are not limited to furyl, 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-oxadiazole base, 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, benzofuranyl, benzisofuranyl, benzothiophene, indolyl, isoindolyl, benzoxazolyl, benzimidazolyl, indazolyl, benzotriazolyl, quinolyl, 2-quinolinone, 4-quinolinone, 1-isoquinolinone, isoquinolyl, acridine, phenanthridine, benzo pyridazine, phthalazine, quinazoline, quinoxaline, quinazolidine, quinazolines, and so on; specific examples include but not limited to quinazoliny, quinoxaliny, puriny, naphthyridiny, etc.

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

The term "deuteroalkyl" means an alkyl group 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 -NH₂.

The term "nitro" refers to -NO₂.

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.

'derivative" can be produced from the parent compound in one or more chemical reaction steps.

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.

The term "solvent compounds" refers to the linker-drug compounds or ligand-drug couplings of the present invention 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 present invention 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 the organism, to facilitate the absorption of the active ingredient and thus the biological activity.

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 backups in semi-solid ointments and creams; preservatives, antioxidants, flavor enhancers, aromatics, co-solvents, emulsifiers, permeability enhancers, osmolality 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.

The present application provides an Auristatin drug conjugate with a high-stability hydrophilic linking unit. The conjugate is introduced into the linker by forming an ether bond between an aminomethyl group with a high-stability hydrophilic linking unit and a hydroxyl group. The conjugate can carry multiple toxins, has good plasma stability, good water solubility, uniformity and safety, can specifically bind to receptors highly expressed in tumor cells, and release toxins in tumor cells. It has good anti-tumor activity and can be used to prevent or treat diseases such as tumors.

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

th conditions recommended by the manufacturer. All percentages, proportions, ratios, or portions are by weight unless otherwise indicated. Unless otherwise defined, all professional and scientific used in the text are used in the same sense as those familiar to those skilled in the art. In addition, any methods and materials similar or equivalent to those documented may be applied to the methods of the present invention. The preferred embodiments and materials described herein are for exemplary purposes only. The generic steps employed in the following embodiments of the present disclosure are:

Generic Step A: ADC Preparation

- 10 After cellular expression and purification by Protein A affinity chromatography with molecular sieve chromatography, the antibody was replaced in 20 mM acetate, pH 6.0 buffer, and the antibody was concentrated or diluted to a protein concentration of 3 mg/mL. The linker-payload was dissolved to 20 mg/mL using 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, the corresponding ADC was obtained by ultrafiltration using a 30 KDa ultrafiltration tube, and the linker-payload that was not coupled to the antibody was removed. ADC samples were obtained.

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

- 20 Place the vials filled with samples on the sample plate and set the corresponding position, injection volume, number of injection needles and injection method for each sample according to the Standard Operating Procedures for the Use of UPLC. The column model was Proteomix RP-1000 (4.6*100 mm, 5 μ m, 1000Å), Sepax, Item No. 465950-4610.

The method parameters are as follows:

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	Room temperature			
Injection Volume	25 μ g			
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

2: SEC detects the monomer rate of antibodies or ADCs

Place the sample bottle on the sample plate and set the corresponding position, injection volume, number of injection needles and injection method for each sample according to the Standard Operating Procedures for the Use of UPLC.

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

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

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

The parameters:

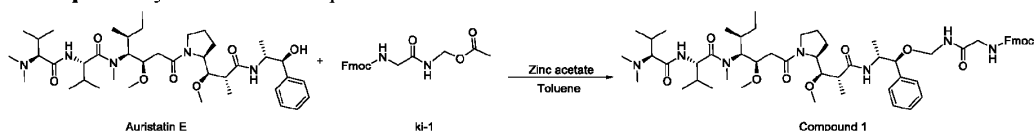
Parameters	Set up
Flow Rates	0.3 mL/min
Wavelength	280 nm
Column Temperature	30 °C
Sample Plate Temperature	Room temperature
Injection Volume	20 μ g
Maximum Pressure	150 bar/15 MPa/2175 PSI
Gradient	Equal degree
Running Time	20 minutes.

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.

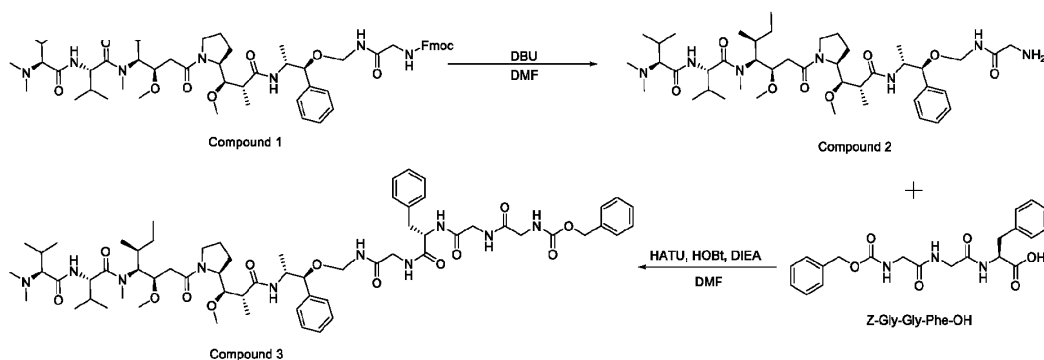
EXAMPLES

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 synthesis method refers to the synthesis of compound **1** in CN111686259A), zinc acetate (414.2 mg, 5.64 mmol, 2.0 eq) and toluene (15 mL) were added to a 50 mL single-mouth round-bottom flask in sequence, and nitrogen was replaced 3 times. The reaction was refluxed at 115 °C for 4 h, and the reaction was stopped. The mixture was cooled to room temperature, filtered, and the filtrate was concentrated under reduced pressure. The mixture was 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.

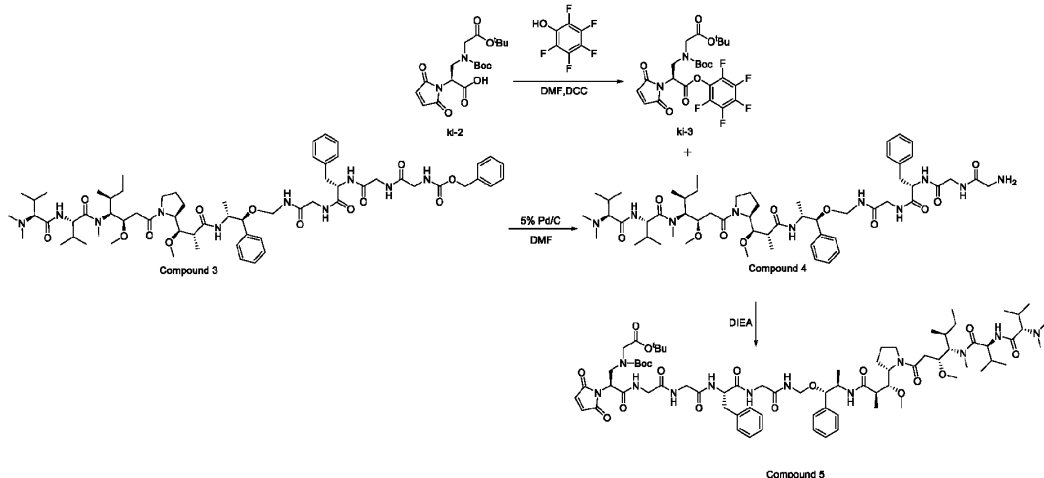
Example 2. Synthesis of compound 3



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 added to a 10 mL EP tube in sequence and stirred at room temperature for later use.

Compound 1 (605 mg, 0.581 mmol, 1.0 eq) and DMF (8 mL) were added to another 25 mL single-necked round-bottom flask in sequence. After stirring at room temperature and dissolving, DBU (95.6 μ L, 0.64 mmol, 1.1 eq) was added. The reaction was carried out at room temperature for 0.5 h. The raw material disappeared under TLC monitoring to produce compound 2. The above mixture was then added to this flask, 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. After the reaction was completed, the product was purified by reverse phase preparative column to obtain a white solid product (487 mg, 69.08%). LC-MS m/z (ES⁺): [M+H]⁺: 1213.6

Example 3. Synthesis of Compound 5



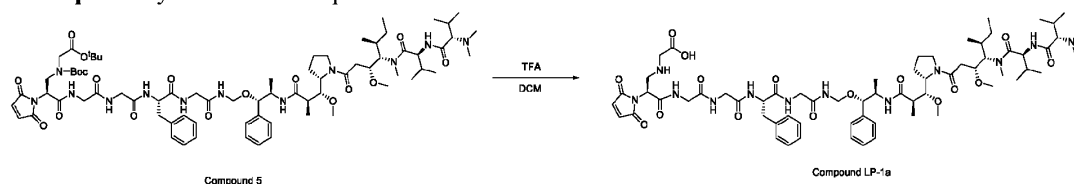
Compound 3 (487 mg, 0.402 mmol, 1.0 eq), 5% Pd/C (48.7 mg) and DMF (5 mL) were added to a 25 mL single-mouth round-bottom flask in sequence. After hydrogen replacement 3 times, the mixture was reacted at room temperature for 1 h. The reaction was monitored by HPLC. The raw material disappeared and a new peak was generated, which was compound 4, recorded as reaction solution ①.

In another 25 mL single-mouth bottle, ki-2 (176 mg, 0.442 mmol, 1.1 eq (synthesis method 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) were added. The mixture was reacted at room

r 30 min. The reaction was monitored by TLC. The reaction was completed to obtain ki-3, recorded as reaction solution ②.

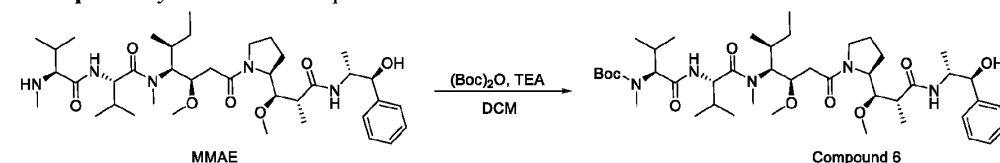
Then the reaction solution ① was filtered into a new 25 mL single-mouth round-bottom flask, and DIEA (73 μ L, 0.442 mmol, 1.1 eq) was added under ice-water bath. The reaction solution ② was filtrated, and the temperature was raised to room temperature for 1 hour after the addition. The reaction was monitored by HPLC. The reaction solution was filtered, and the filtrate was added to the reaction solution containing compound 4 under ice-bath stirring, and then added, the ice bath was removed, and the reaction was carried out at room temperature for 1 hour, and HPLC was monitored. The reaction solution was directly purified by reverse phase preparation, and the preparation 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



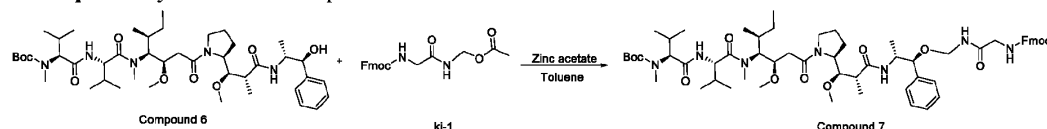
Compound 5 (100 mg, 0.0685 mmol, 1.0 eq) was dissolved in 10 mL dry dichloromethane and 4 mL TFA, and reacted at room temperature for 3 h, monitored by HPLC. After the reaction, the solvent was removed by concentration under reduced pressure, purified by reverse phase preparative column, and the preparative solution was 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) were added to a 100 mL single-necked bottle, and DCM (20 mL) was used to dissolve the mixture. TEA (563 mg, 5.57 mmol, 2.0eq) was added under an ice-water bath. After the addition, the mixture was heated to room temperature and reacted for 72 h. The reaction of the raw material MMAE was completed by TLC monitoring. Post-treatment: The reaction solution was concentrated under reduced pressure and purified by column chromatography (eluent: DCM/MeOH=20/1) to obtain a white solid (2.28g, 100%). LC-MSm/z (ES⁺): [M+H]⁺: 818.4.

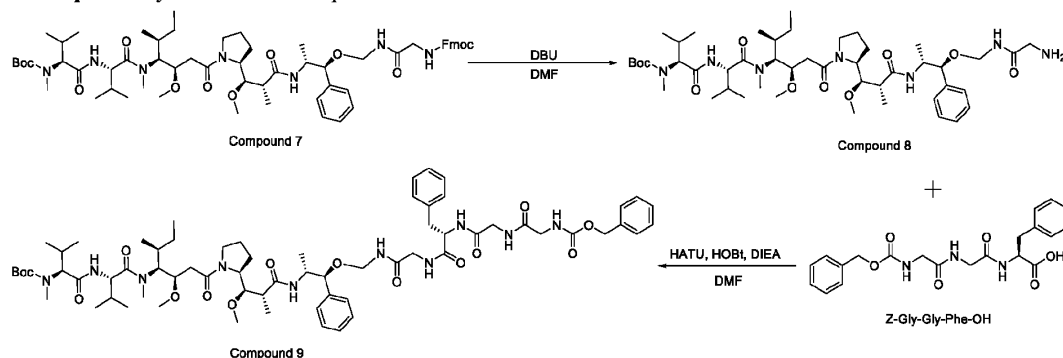
Example 6. Synthesis of compound 7



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), and toluene (30 mL) were added to a 100 mL single-mouth bottle in sequence. After N₂ replacement 3 times, the temperature was raised to 115°C under N₂

4 h, the reaction was stopped, cooled to room temperature, 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 (1.071 g, 34%). LC-MSm/z (ES+): [M+H]⁺: 1126.4.

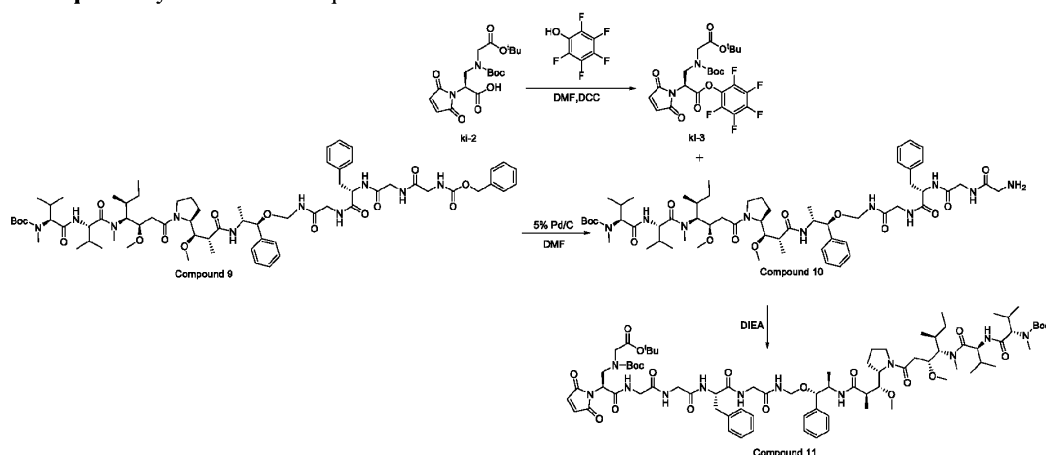
Example 7. Synthesis of Compound 9



Compound 7 (900 mg, 0.8mmol, 1.0eq) and DMF (9 mL) were added to a 50 mL single-necked bottle. After stirring and dissolving, DBU (134 mg, 0.88mmol, 1.1eq) was added dropwise under an ice-water bath. After addition, the temperature was raised to room temperature for reaction for 30 min. The reaction was completed by TLC monitoring and recorded as reaction solution ①.

In another 50 mL single-necked bottle, Z-Gly-Gly-Phe-OH (364 mg, 0.88mmol, 1.1eq), HATU (365 mg, 0.96mmol, 1.2eq), HOBT (129.7 mg, 0.96mmol, 1.2eq) and DMF (7 mL) were added dropwise under an ice-water bath. After dissolving, reaction solution ① and DIEA (103.4 mg, 0.8mmol, 1.0eq) were added dropwise in turn under an ice-water bath. After addition, the temperature was raised to room temperature for reaction for 1 h. The reaction was monitored by HPLC. The reaction solution was purified by reverse phase preparative method, and the preparative solution was lyophilized to obtain a white solid (960 mg, 92.4%). LC-MS m/z (ES+): [M+H]⁺: 1299.6.

Example 8. Synthesis of Compound 11



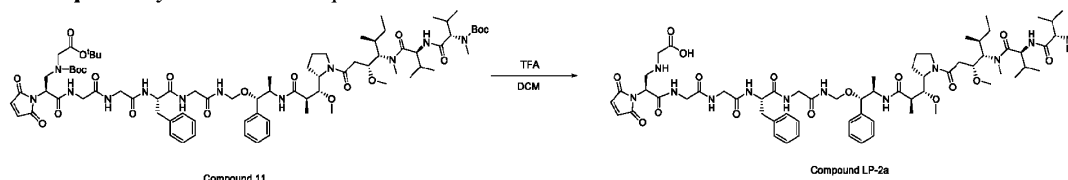
Compound 10 (960 mg, 0.74mmol, 1.0eq), 5% Pd/C (960mg) and DMF (10mL) were added to a 50 mL single-mouth bottle. After H₂ replacement 3 times, the mixture was reacted

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

In another 25 mL single-mouth bottle, ki-2 (322.7mg, 0.81mmol, 1.1eq), DCC (167mg, 0.81mmol, 1.1eq) and DMF (5 mL) were added to dissolve the mixture. Pentafluorophenol (149mg, 0.81mmol, 1.1eq) was added under ice-water bath. After the addition was completed, the mixture was heated to room temperature and reacted for 30min. The reaction was monitored by TLC. After the reaction was completed, ki-3 was obtained and recorded as reaction solution ②.

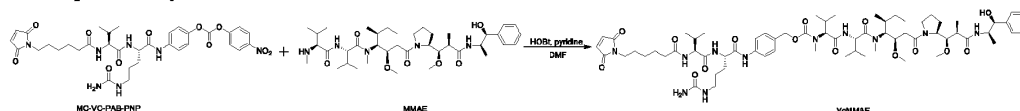
The reaction solution ① was filtered into a 50 mL single-mouth bottle, and DIEA (105 mg, 0.81 mmol, 1.1 eq) was added under an ice-water bath. The reaction solution filtrate of ② was heated to room temperature for 1 h after addition. The reaction was monitored by HPLC. The reaction solution was purified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

Example 9. Synthesis of Compound LP-2a



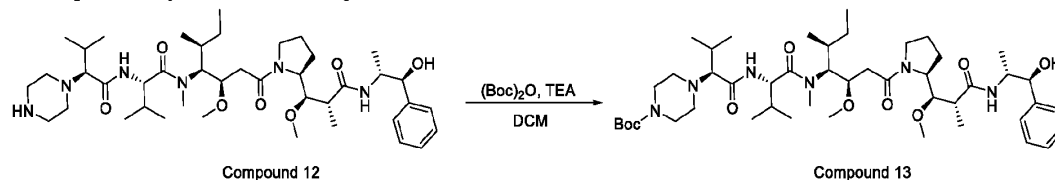
Compound 12 (400 mg, 0.259 mmol, 1.0 eq) was dissolved in 20 mL dry dichloromethane and 8 mL TFA, and reacted at room temperature for 3 h, monitored by HPLC. After the reaction was completed, the solvent was removed by concentration under reduced pressure, and the crude product was purified by reverse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

Example 10. Synthesis of VcMMAE



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

Example 11. Synthesis of Compound 13

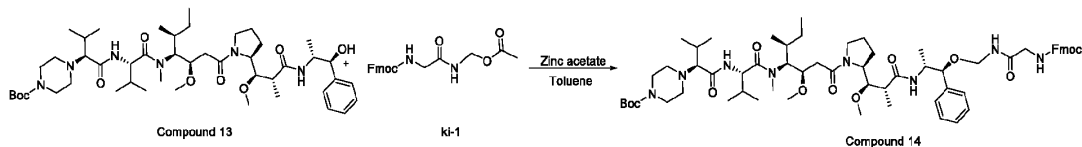


Referring to the synthesis method of Example 5, compound 13 was synthesized using

(refer to the synthesis of compound 17 in patent CN106279352) as a raw material.

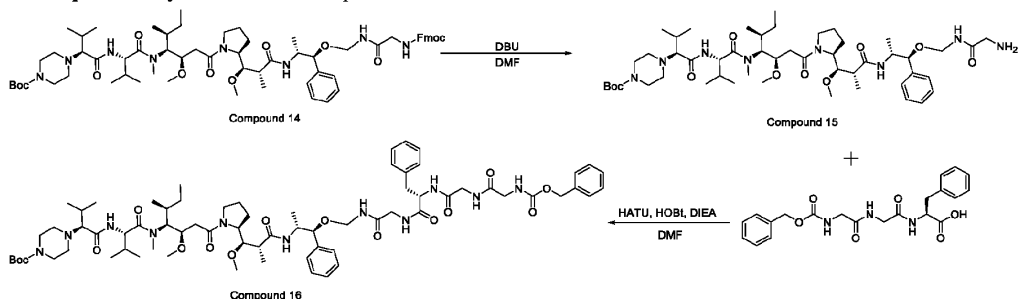
LC-MSm/z (ES⁺): [M+H]⁺: 873.6

Example 12. Synthesis of Compound 14



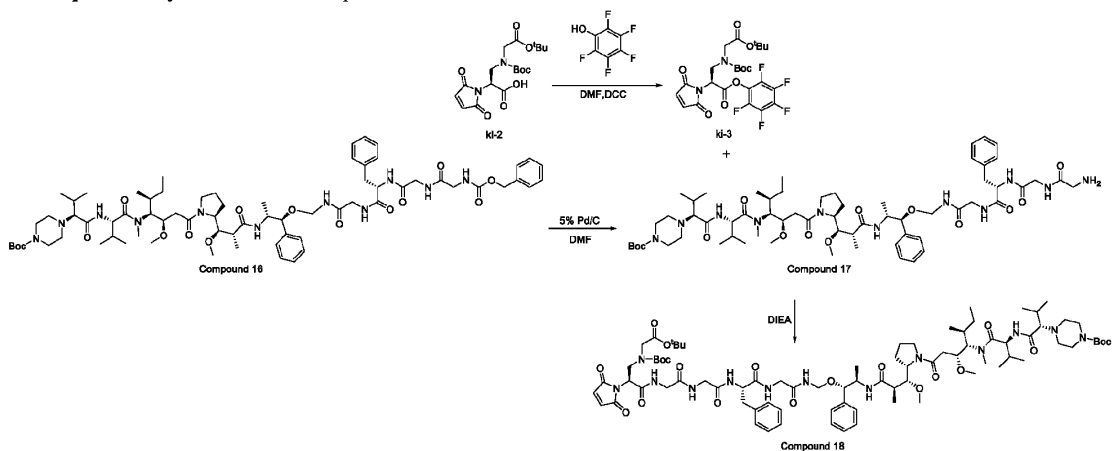
Referring to the synthesis method of **Example 6**, compound 14 was synthesized using compound 13 and ki-1 as raw materials. LC-MS m/z (ES⁺): [M+H]⁺: 1180.7.

Example 13. Synthesis of Compound 16



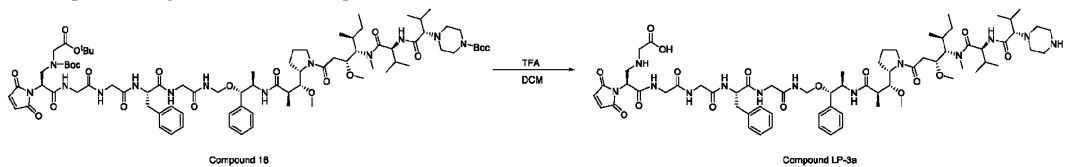
Compound 16 was synthesized from compound 14 with reference to the synthetic method of **Example 7**. LC-MSm/z (ES⁺): [M+H]⁺: 1353.9.

Example 14. Synthesis of Compound 18



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

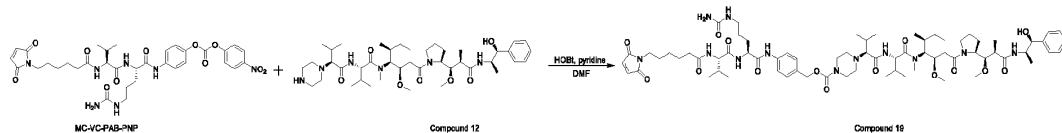
Example 15. Synthesis of Compound LP-3a



Compound LP-3a was synthesized from compound 18 with reference to the synthetic

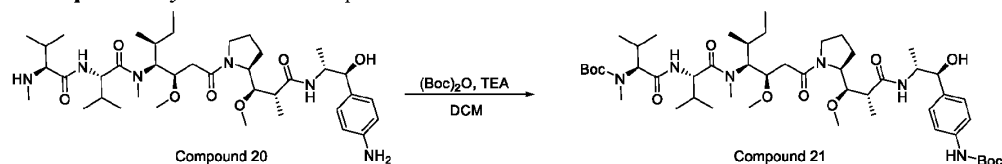
Example 9. LC-MSm/z (ES^+): $[M+H]^+$: 1344.7.

Example 16. Synthesis of Compound 23



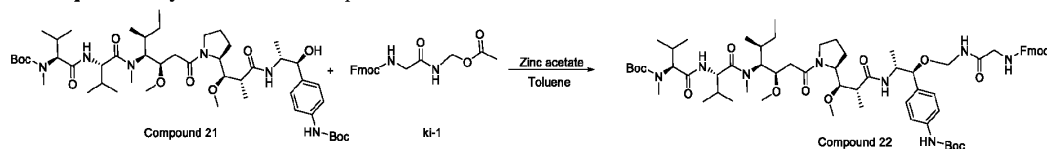
Compound 19 was synthesized from compound 12 and MC-VC-PAB-PNP with reference to the synthesis of **Example 10**. LC-MS m/z (ES^+): $[M+H]^+$: 1371.8.

Example 17. Synthesis of Compound 21



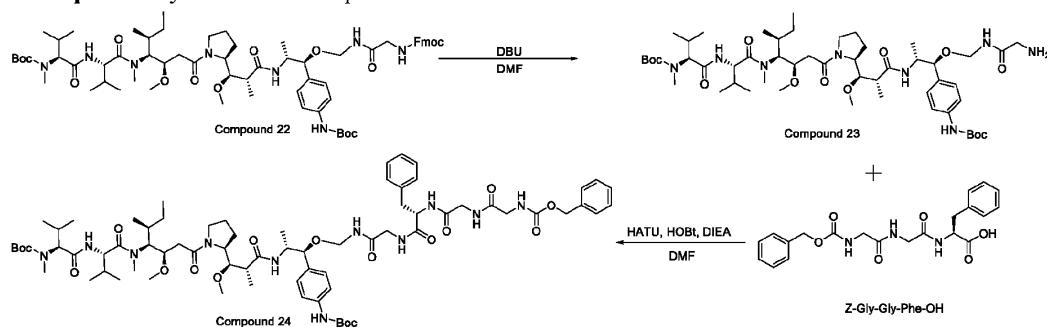
Compound 21 was synthesized from compound 20 (synthesized with reference to US2017014524A1) with reference to the synthesis method of **Example 5**. LC-MSm/z (ES^+): $[M+H]^+$: 933.6.

Example 18. Synthesis of Compound 22



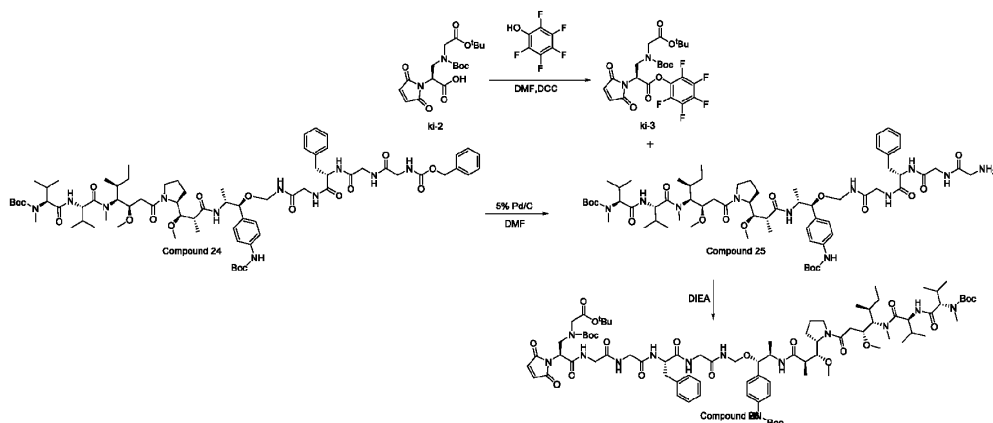
Compound 22 was synthesized from compound 21 and ki-1 with reference to the synthetic method of **Example 6**. LC-MSm/z (ES^+): $[M+H]^+$: 1241.7.

Example 19. Synthesis of Compound 24



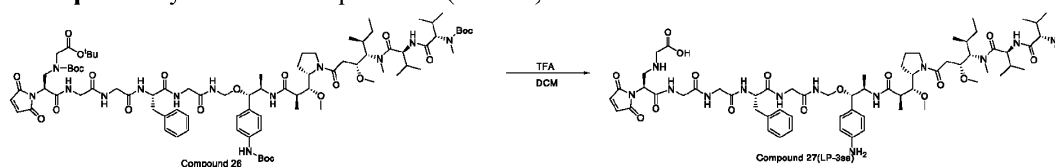
Compound 24 was synthesized from compound 22 with reference to the synthetic method of **Example 7**. LC-MSm/z (ES^+): $[M+H]^+$: 1414.8.

Example 20. Synthesis of Compound 26



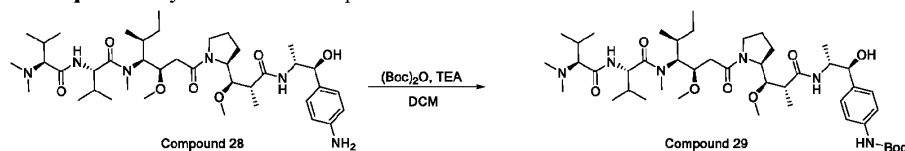
Compound 26 was synthesized from compound 24 with reference to the synthetic method of **Example 8**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1660.9.

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



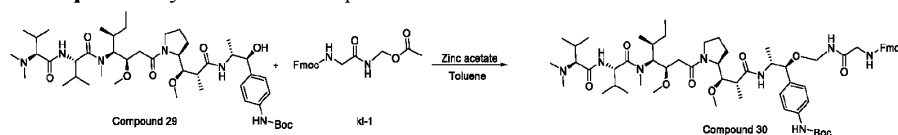
Compound 27 (LP-3sa, whose structure is shown in the structural formula of LP-3s, where the configuration of the chiral carbon at the position-2 is of the S-type) was synthesized from compound 26 with reference to the synthetic method of **Example 9**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1304.7.

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



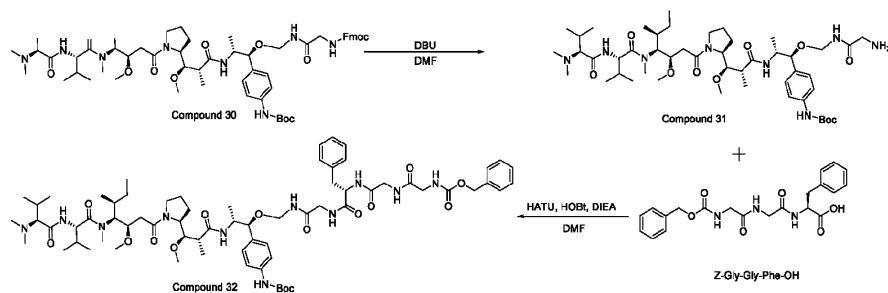
Compound 29 was synthesized from compound 28 (synthesized with reference to US2017014524A1) with reference to the synthesis method of **Example 5**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 847.6.

Example 23. Synthesis of Compound 30



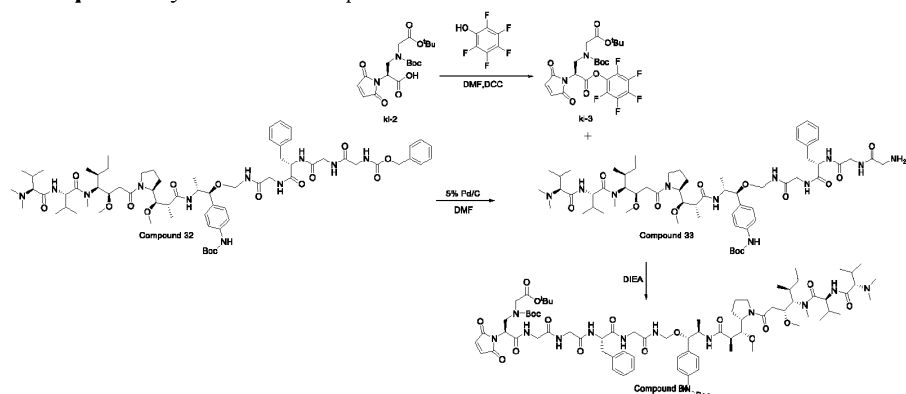
Compound 30 was synthesized from compound 29 and ki-1 with reference to the synthetic method of **Example 6**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1155.7.

Example 24. Synthesis of Compound 32



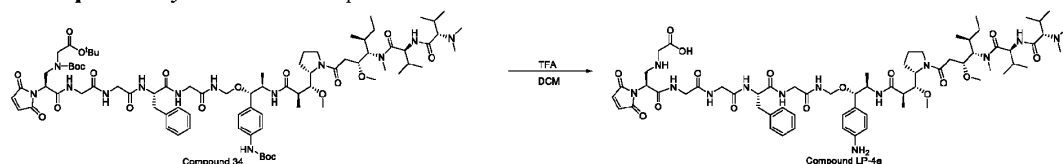
Compound **32** was synthesized from compound **30** with reference to the synthetic method of **Example 7**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1328.8.

Example 25. Synthesis of Compound 34



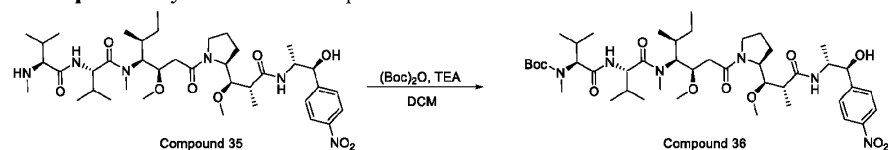
Compound **34** was synthesized from compound **32** with reference to the synthetic method of **Example 8**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1574.9.

Example 26. Synthesis of Compound LP-4a



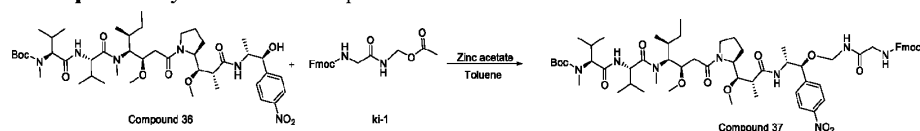
Compound **LP-4a** was synthesized from compound **34** with reference to the synthetic method of **Example 9**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1318.7.

Example 27. Synthesis of Compound 36



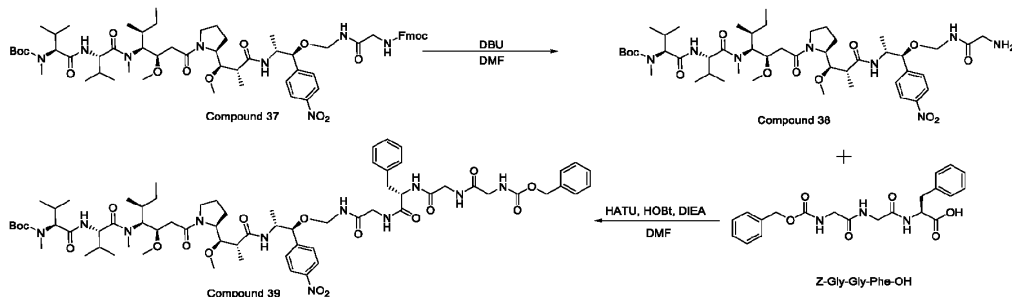
Compound **36** was synthesized from compound 35 (synthesized with reference to US20210346523A1) by the synthetic method of **Example 5**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 863.5.

Example 28. Synthesis of Compound 37



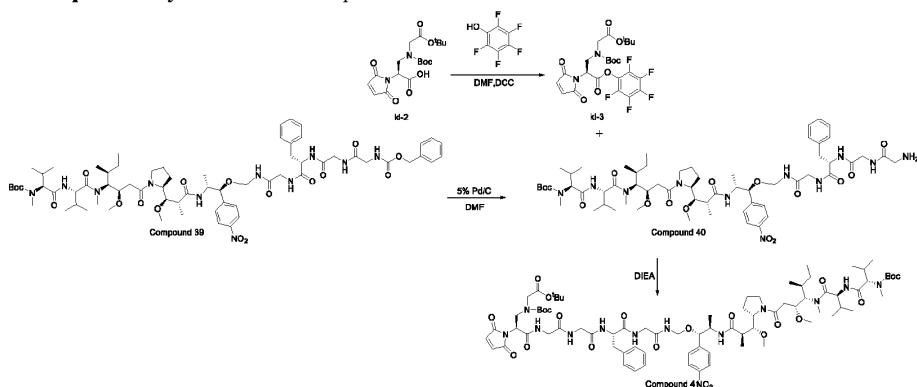
nd 37 was synthesized from compound 36 and ki-1 with reference to the synthetic method of **Example 6**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1171.7.

Example 29. Synthesis of Compound 39



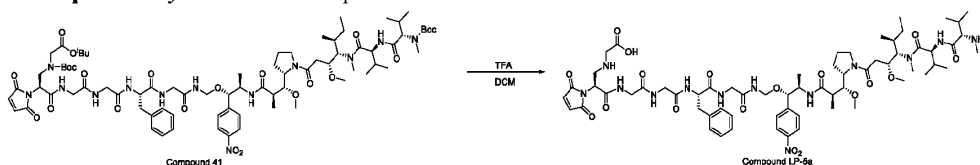
Compound 39 was synthesized from compound 37 with reference to the synthetic method of **Example 7**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1344.7.

Example 30. Synthesis of Compound 41



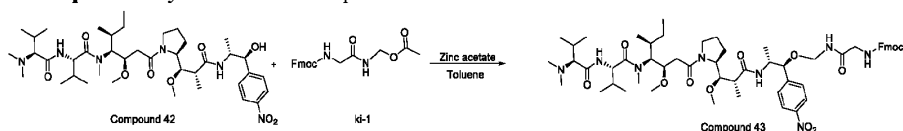
Compound 41 was synthesized from compound 39 with reference to the synthetic method of **Example 8**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1590.9.

Example 31. Synthesis of Compound LP-5a



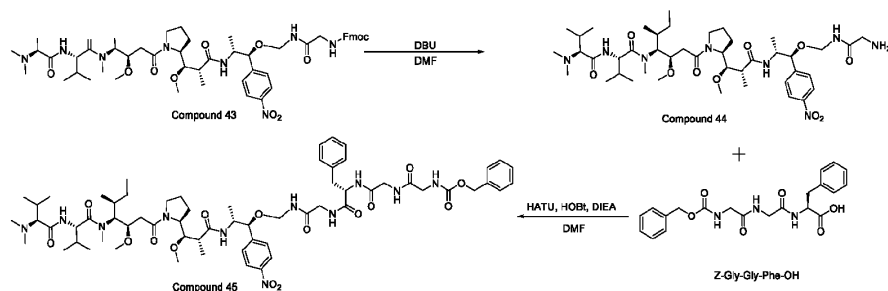
Compound LP-5a was synthesized from compound 41 with reference to the synthetic method of **Example 9**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1334.7.

Example 32. Synthesis of Compound 43



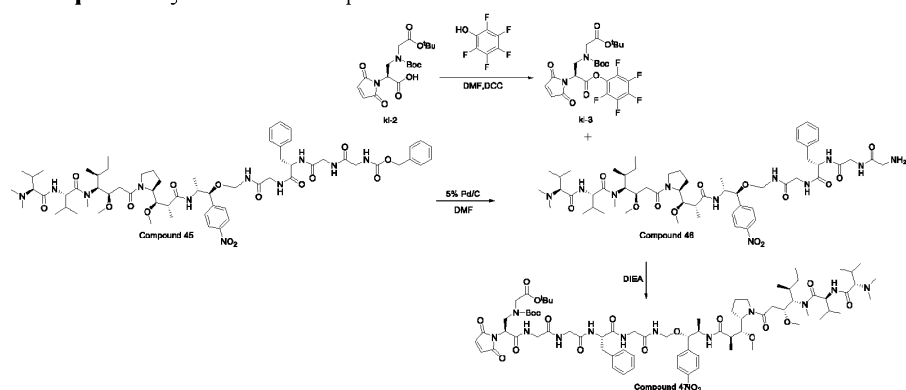
Compound 43 was synthesized with reference to the synthesis method of **Example 6**, using compound 42 (with reference to the synthesis of compound 17 in US20210346523A1) and ki-1 as raw material. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1085.6.

Example 33. Synthesis of Compound 45



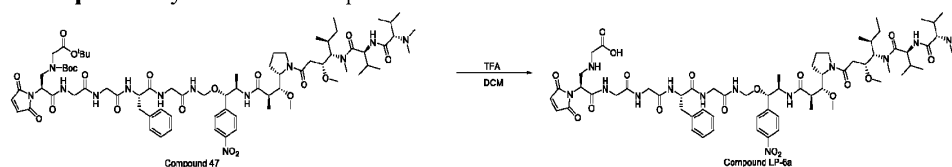
Compound **45** was synthesized from compound **43** with reference to the synthetic method of **Example 7**. LC-MSm/z (ES^+): $[M+H]^+$: 1258.7.

Example 34. Synthesis of Compound **47**



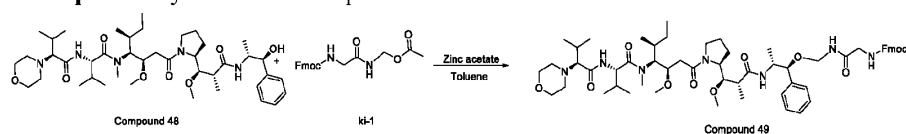
Compound **47** was synthesized from compound **45** with reference to the synthetic method of **Example 8**. LC-MSm/z (ES^+): $[M+H]^+$: 1504.8.

Example 35. Synthesis of Compound **LP-6a**



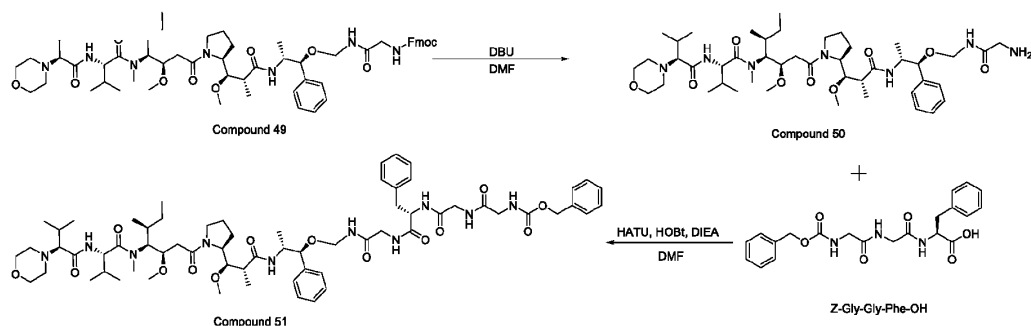
Compound **LP-6a** was synthesized from compound **47** with reference to the synthetic method of **Example 9**. LC-MSm/z (ES^+): $[M+H]^+$: 1348.7.

Example 36. Synthesis of Compound **49**



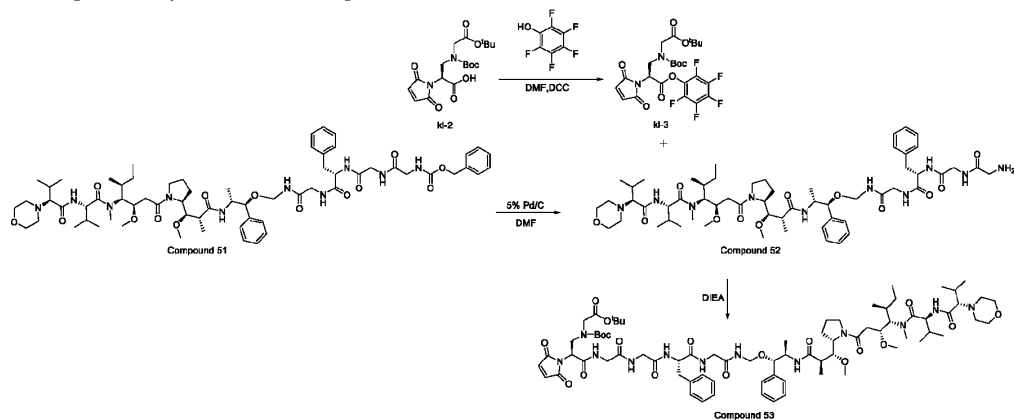
Referring to the synthesis method of **Example 6**, compound **49** was synthesized from compound **48** (referring to the synthesis of compound 6 in CN106279352) and ki-1. LC-MSm/z (ES^+): $[M+H]^+$: 1082.6.

Example 37. Synthesis of Compound **51**



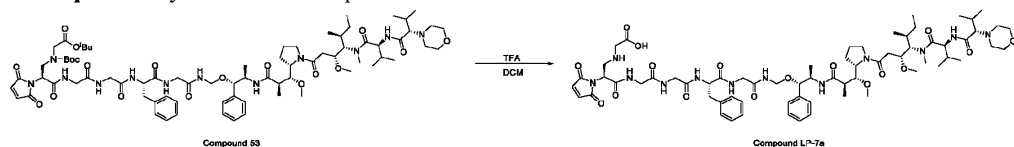
Compound **51** was synthesized from compound **49** with reference to the synthetic method of **Example 7**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$:1255.7.

Example 38. Synthesis of Compound 53



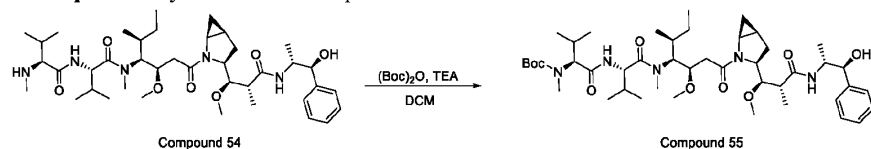
Compound **53** was synthesized from compound **51** with reference to the synthetic method of **Example 8**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$:1501.8.

Example 39. Synthesis of Compound LP-7a



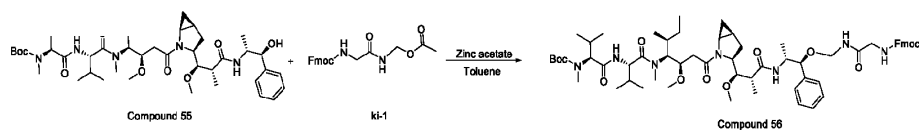
Compound **LP-7a** was synthesized from compound **53** with reference to the synthetic method of **Example 9**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$:1345.7.

Example 40. Synthesis of Compound 55



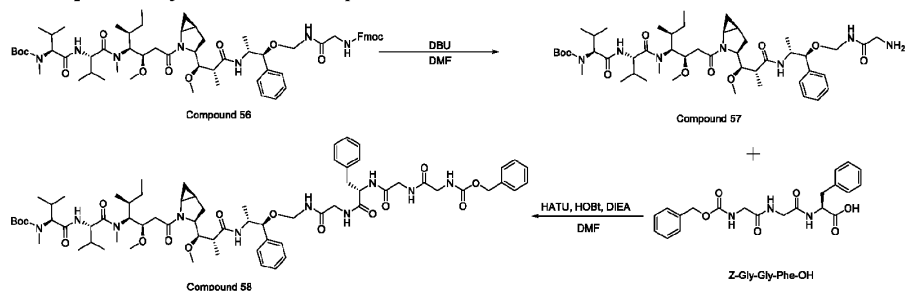
Referring to the synthesis method of **Example 5**, Compound **55** was synthesized from Compound **54** (referring to the synthesis of Compound 1 in CN113121639A). LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$:830.6.

Example 41. Synthesis of Compound 56



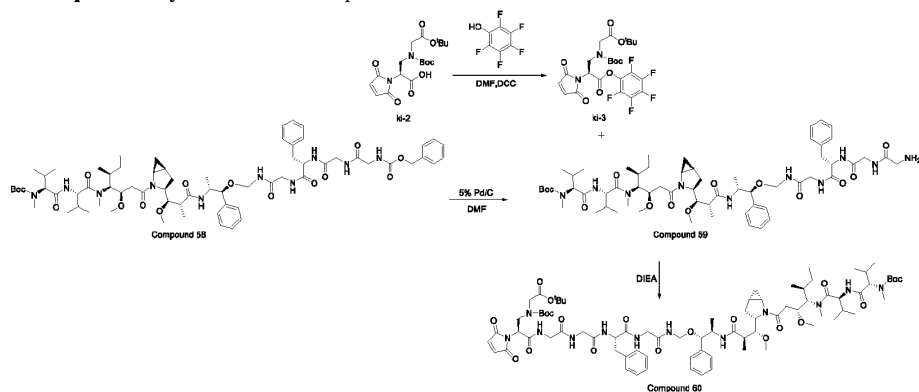
Compound **56** was synthesized from compound **55** and ki-1 with reference to the synthetic method of **Example 6**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$:1138.7.

Example 42. Synthesis of Compound 58



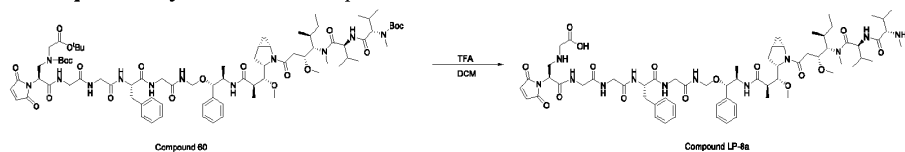
Compound **58** was synthesized from compound **56** with reference to the synthetic method of **Example 7**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$:1311.8.

Example 43. Synthesis of Compound 60



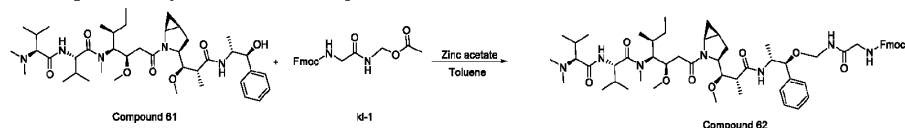
Compound **60** was synthesized from compound **58** with reference to the synthetic method of **Example 8**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$:1557.9.

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.

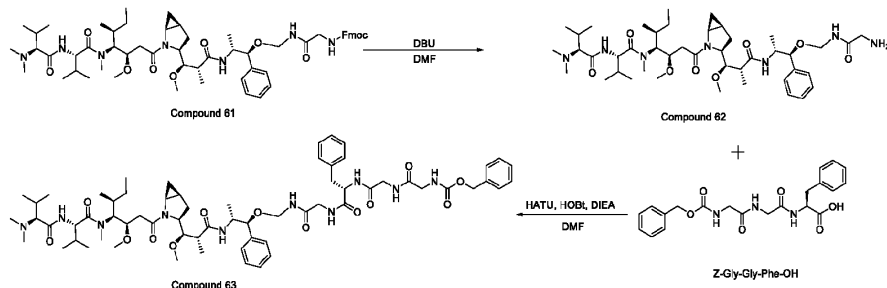
Example 45. Synthesis of Compound 62



Referring to the synthesis method of **Example 6**, compound **62** was synthesized from

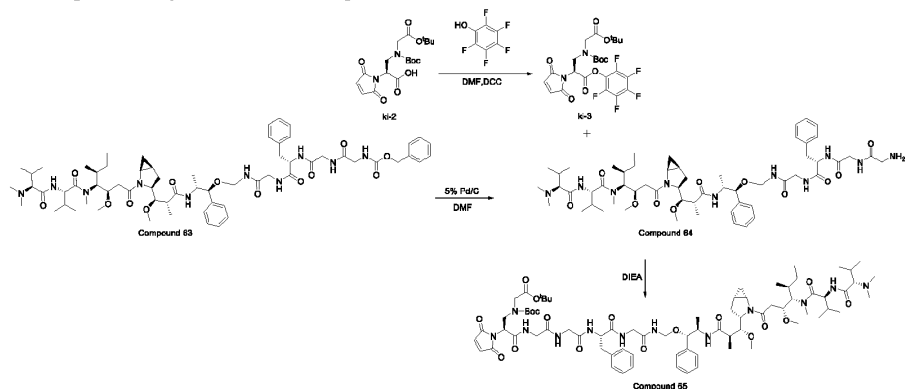
(referring to the synthesis of Compound 1 in CN113121639A) and ki-1. LC-MSm/z (ES^+): $[M+H]^+$:1052.6.

Example 46. Synthesis of Compound 63



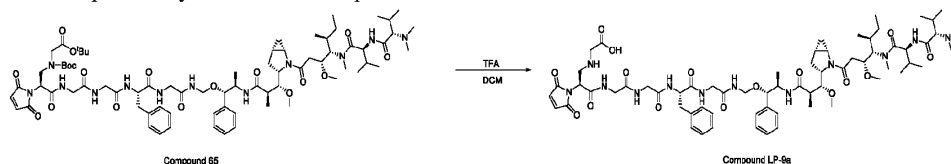
Compound 63 was synthesized from compound 61 with reference to the synthetic method of **Example 7**. LC-MSm/z (ES^+): $[M+H]^+$:1225.7.

Example 47. Synthesis of Compound 65



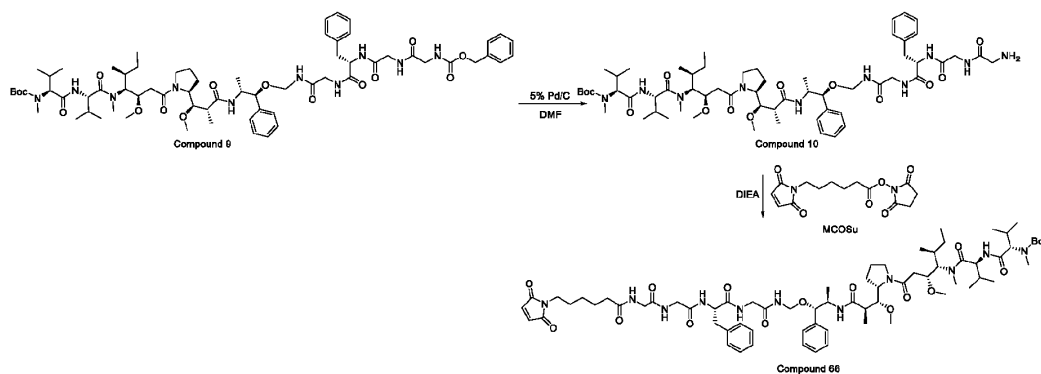
Compound 65 was synthesized from compound 63 with reference to the synthetic method of **Example 8**. LC-MSm/z (ES^+): $[M+H]^+$:1471.8.

Example 48 Synthesis of Compound LP-9a



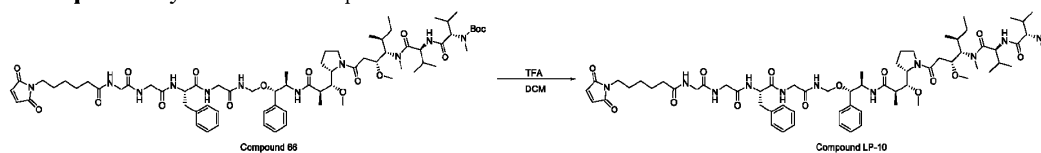
Compound LP-9a was synthesized from compound 65 with reference to the synthetic method of **Example 9**. LC-MSm/z (ES^+): $[M+H]^+$:1315.7.

Example 49. Synthesis of Compound 66



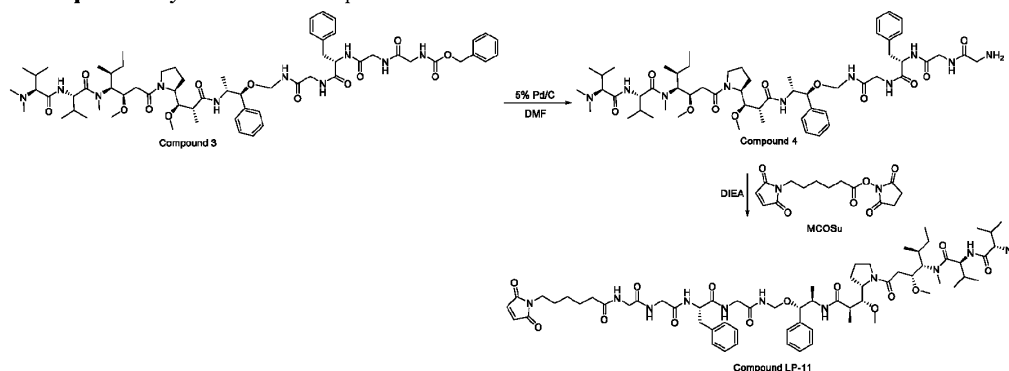
Compound **9** (960 mg, 0.74 mmol, 1.0 eq), 5% Pd/C (960 mg) and DMF (10 mL) were added to a 50 mL single-mouth bottle. After H₂ replacement 3 times, the reaction was carried out at room temperature for 1 h. The reaction was monitored by HPLC. The reaction was completed and recorded as reaction solution ①. The reaction solution ① was filtered into a 50 mL single-mouth bottle. MCOSu (274 mg, 0.89 mmol, 1.2 eq) and DIEA (105 mg, 0.81 mmol, 1.1 eq) were added in turn under ice-water bath. After the addition, the temperature was raised to room temperature for 1 h. The reaction was monitored by HPLC. The reaction solution was purified by reverse phase preparation and the preparation solution was lyophilized to obtain a white solid (847.5 mg, 84.3%). LC-MSm/z (ES⁺): [M+H]⁺: 1358.2.

Example 50. Synthesis of Compound LP-10



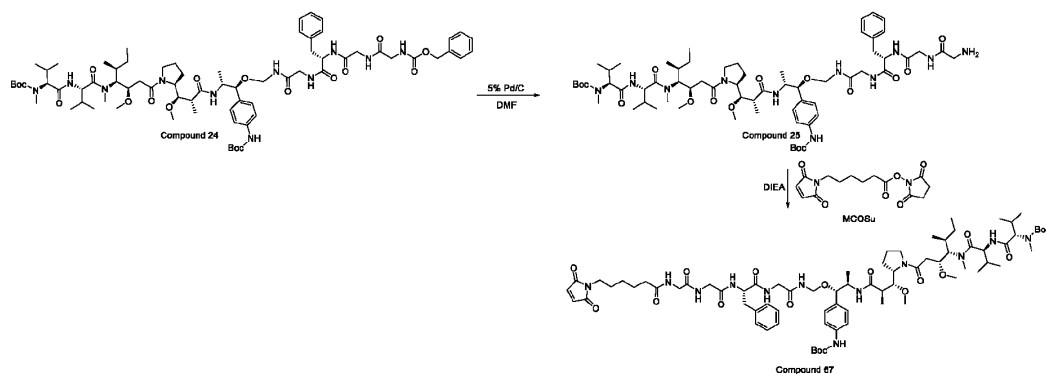
Compound **LP-10** was synthesized from compound **66** with reference to the synthetic method of **Example 9**. LC-MSm/z (ES⁺): [M/2+H]⁺: 630.0.

Example 51. Synthesis of Compound LP-11



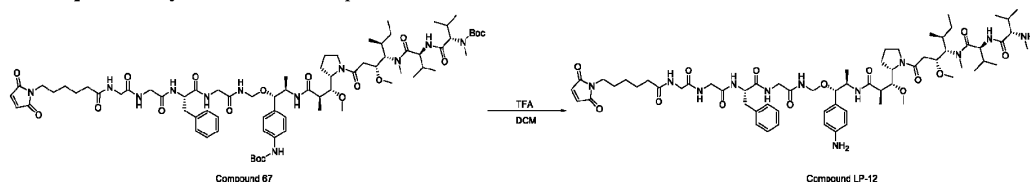
Compound **LP-11** was synthesized from compound **3** with reference to the synthetic method of **Example 49**. LC-MSm/z (ES⁺): [M+H]⁺: 1272.8.

Example 52. Synthesis of Compound 67



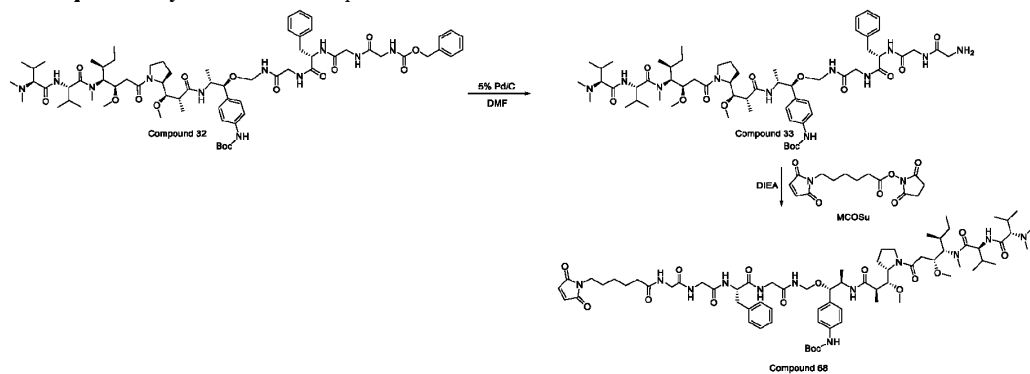
Compound **67** was synthesized from compound **24** with reference to the synthetic method of **Example 49**. LC-MSm/z (ES⁺):[M+H]⁺: 1473.9.

Example 53. Synthesis of Compound LP-12



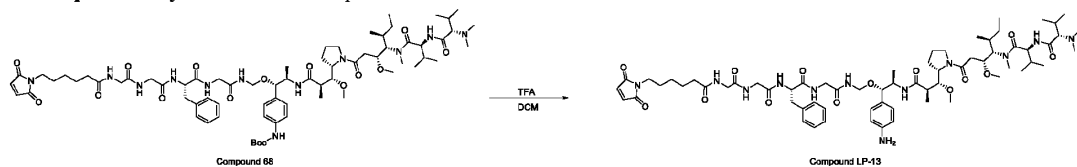
Compound **LP-12** was synthesized from compound **67** with reference to the synthetic method of **Example 9**. LC-MSm/z (ES⁺):[M+H]⁺: 1273.8.

Example 54. Synthesis of Compound 68



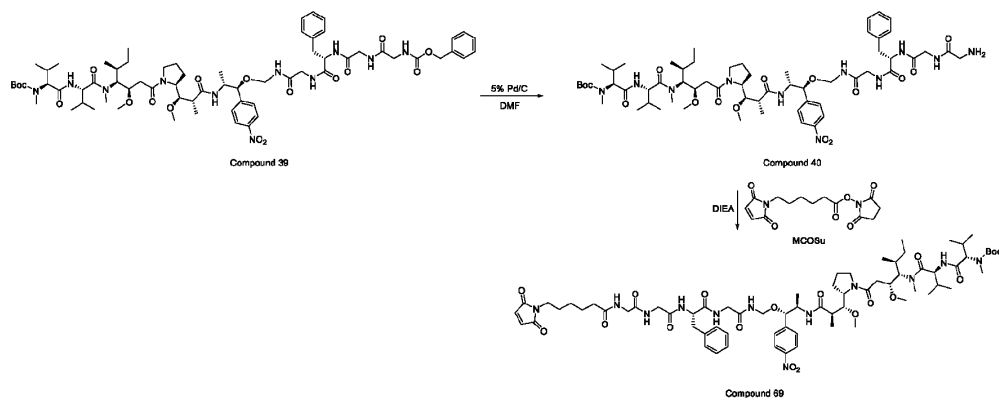
10 Compound **68** was synthesized from compound **32** with reference to the synthetic method of **Example 49**. LC-MSm/z (ES⁺):[M+H]⁺: 1387.8.

Example 55. Synthesis of Compound LP-13



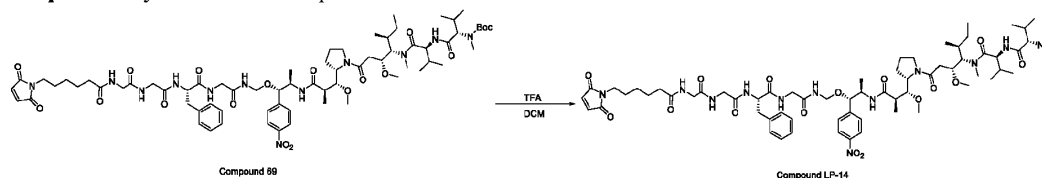
Compound **LP-13** was synthesized from compound **68** with reference to the synthetic method of **Example 9**. LC-MSm/z (ES⁺):[M+H]⁺: 1287.8.

Example 56. Synthesis of Compound 69



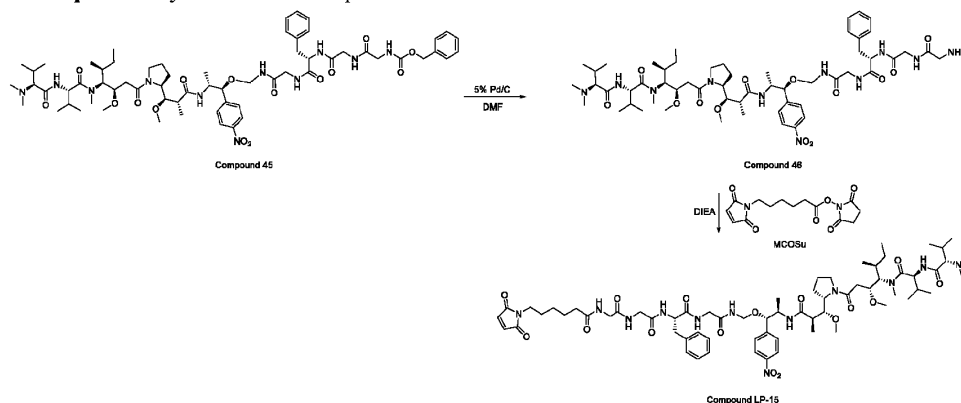
Compound **69** was synthesized from compound **39** with reference to the synthetic method of **Example 49**. LC-MSm/z (ES^+): $[M+H]^+$: 1403.8.

Example 57. Synthesis of Compound LP-14



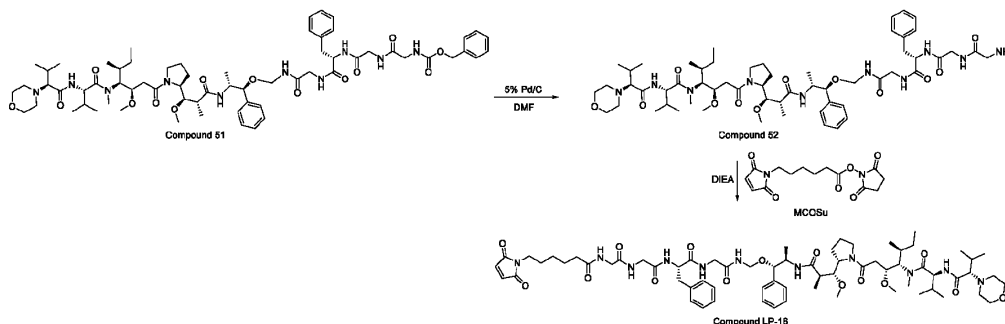
Compound **LP-14** was synthesized from compound **69** with reference to the synthetic method of **Example 9**. LC-MSm/z (ES^+): $[M+H]^+$: 1303.7.

Example 58. Synthesis of Compound LP-15



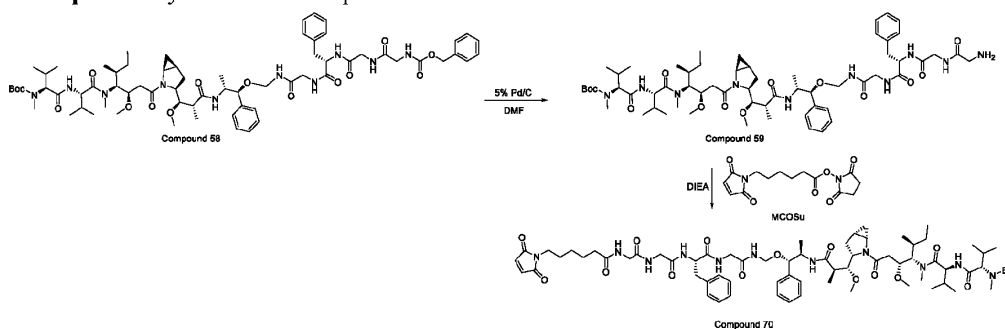
10 Compound **LP-15** was synthesized from compound **45** with reference to the synthetic method of **Example 49**. LC-MSm/z (ES^+): $[M+H]^+$: 1403.8.

Example 59. Synthesis of Compound LP-16



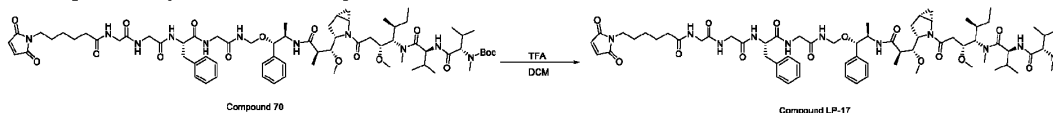
Compound **LP-16** was synthesized from compound **51** with reference to the synthetic method of **Example 49**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1314.8.

Example 60. Synthesis of Compound **70**



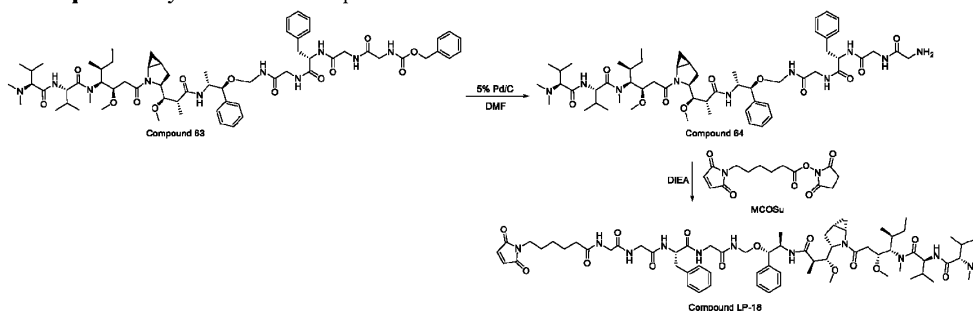
Compound **70** was synthesized from compound **58** with reference to the synthetic method of **Example 49**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1370.8.

Example 61. Synthesis of Compound **LP-17**



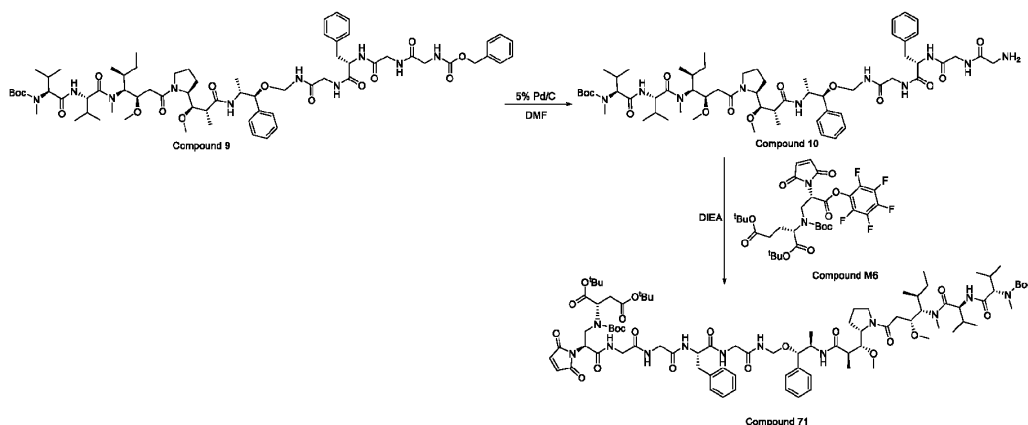
Compound **LP-17** was synthesized from compound **70** with reference to the synthetic method of **Example 9**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1270.8.

Example 62. Synthesis of Compound **LP-18**



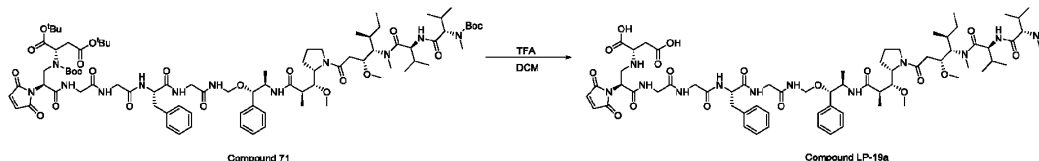
Compound **LP-18** was synthesized from compound **63** with reference to the synthetic method of **Example 49**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1284.8.

Example 63. Synthesis of Compound **71**



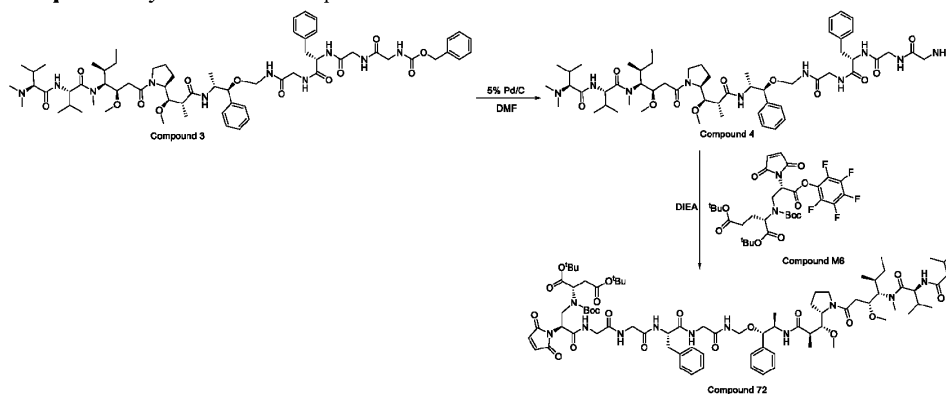
Compound 9 (960 mg, 0.74 mmol, 1.0 eq), 5% Pd/C (960 mg) and DMF (10 mL) were added to a 50 mL single-mouth bottle. After H₂ replacement 3 times, the reaction was carried out at room temperature for 1 h. The reaction was monitored by HPLC. The reaction was completed and recorded as reaction solution ①. The reaction solution ① was filtered into a 50 mL single-mouth bottle. Compound M6 (512.5 mg, 0.74 mmol, 1.0 eq, compound M6 refers to the synthesis of compound M6 in CN113827736) and DIEA (105 mg, 0.81 mmol, 1.1 eq) were added in turn under ice-water bath. After the addition, the temperature was raised to room temperature for 1 h. The reaction was monitored by HPLC. The reaction solution was purified by reverse phase preparation and the preparation 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

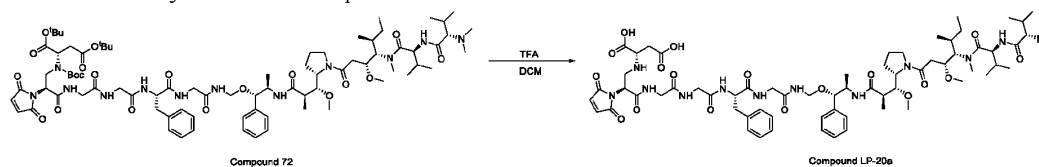


Compound LP-19a was synthesized from compound 71 with reference to the synthetic method of **Example 9**. LC-MSm/z (ES⁺):[M/2+H]⁺: 674.4.

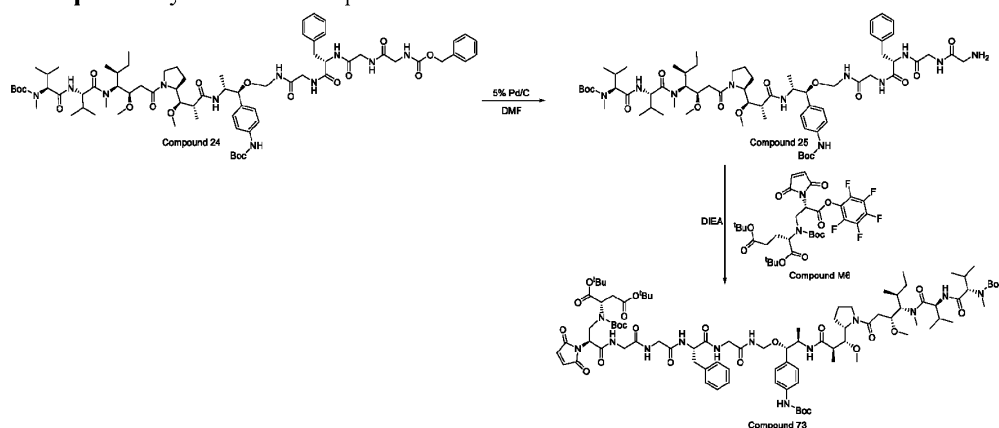
Example 65. Synthesis of Compound 72



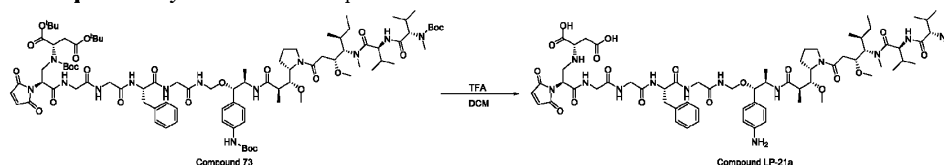
Compound 72 was synthesized from compound 3 with reference to the synthetic method of **Example 63**. LC-MSm/z (ES⁺):[M/2+H]⁺: 783.5.

Synthesis of Compound **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.

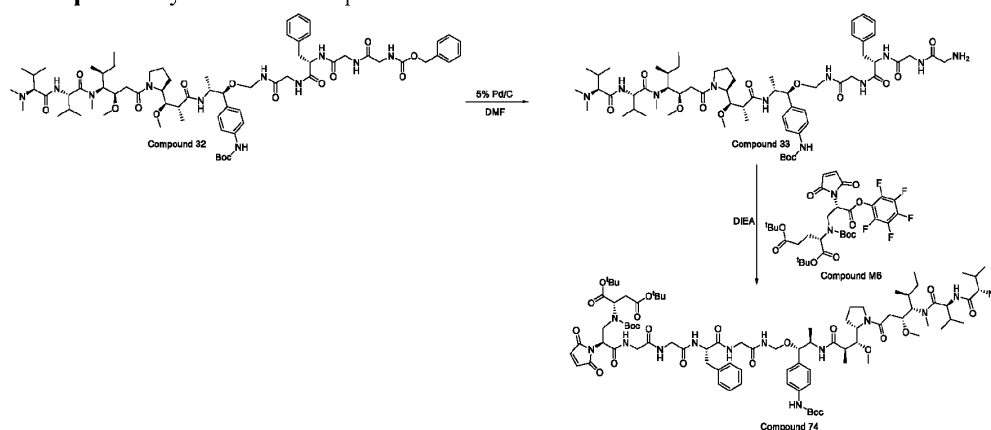
Example 67. Synthesis of Compound **73**

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**

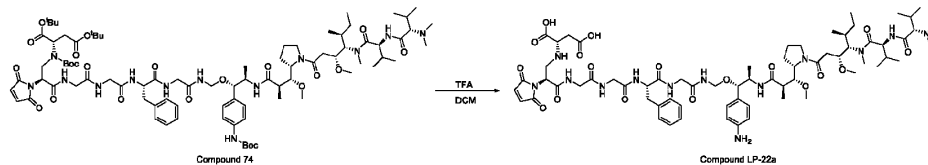
10

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

Example 69. Synthesis of Compound **74**

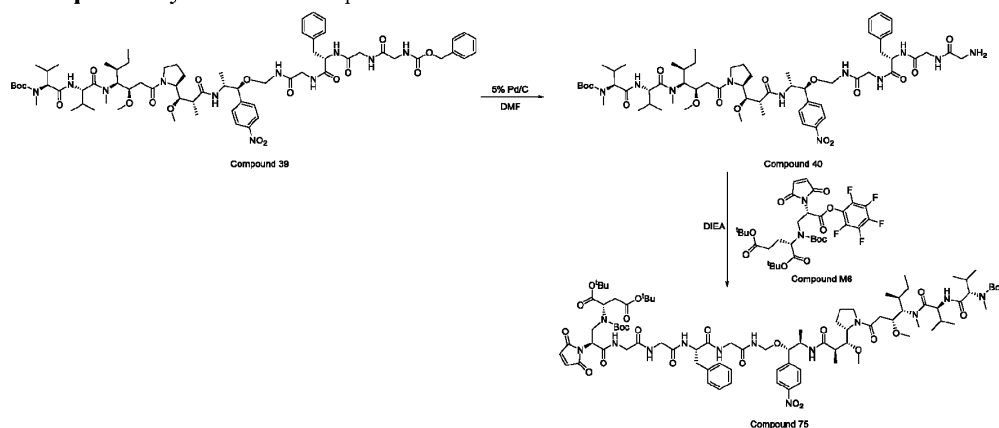
nd **74** was synthesized from compound **32** with reference to the synthetic method of **Example 63**. LC-MSm/z (ES⁺):[M/2+H]⁺: 845.1.

Example 70. Synthesis of Compound LP-22a



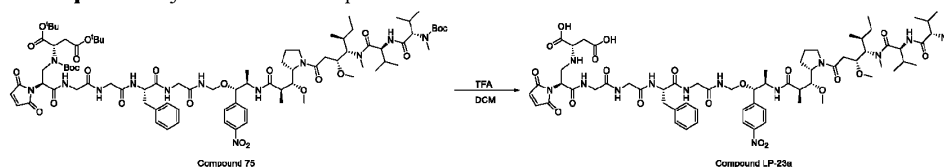
Compound **LP-22a** was synthesized from compound **74** with reference to the synthetic method of **Example 9**. LC-MSm/z (ES⁺):[M+H]⁺: 1376.8.

Example 71. Synthesis of Compound 75



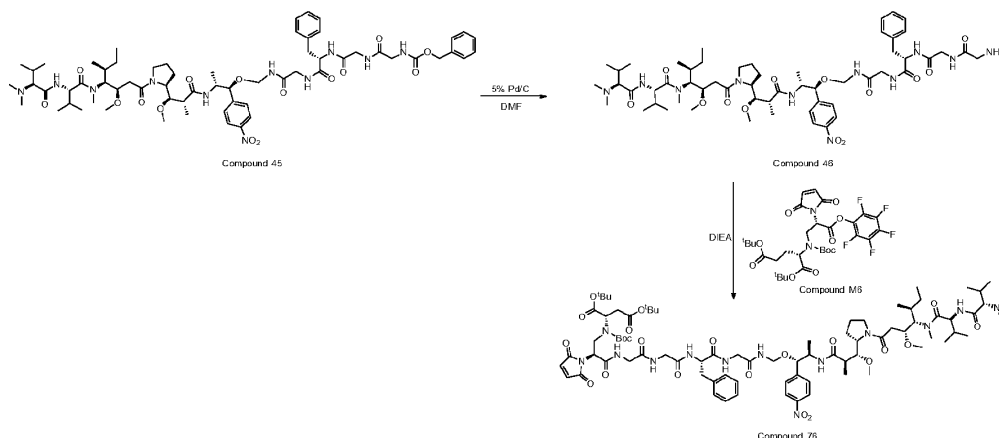
Compound **75** was synthesized from compound **39** with reference to the synthetic method of **Example 63**. LC-MSm/z (ES⁺):[M/2+H]⁺: 853.1.

Example 72. Synthesis of Compound LP-23a



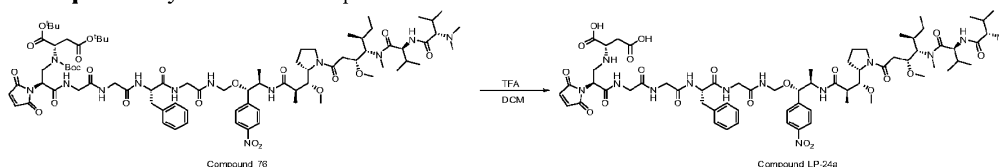
Compound **LP-23a** was synthesized from compound **75** with reference to the synthetic method of **Example 9**. LC-MSm/z (ES⁺):[M+H]⁺: 1392.7.

Example 73. Synthesis of Compound 76



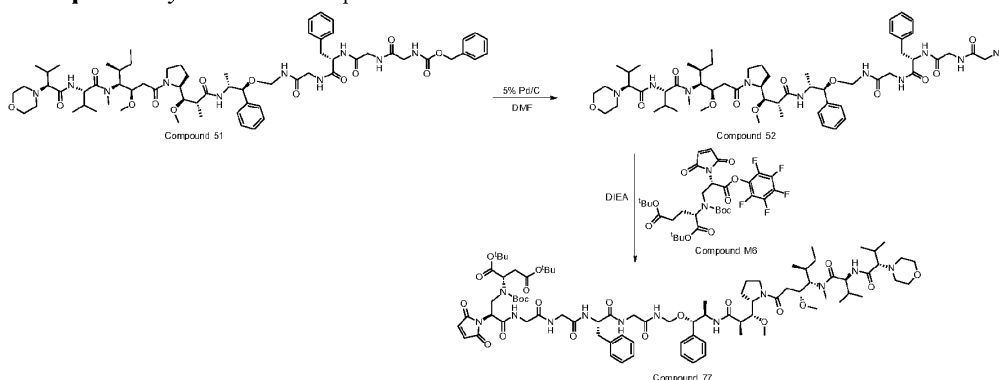
Compound 76 was synthesized from compound 45 with reference to the synthetic method of **Example 63**. LC-MSm/z (ES^+): $[\text{M}/2+\text{H}]^+$: 810.1.

Example 74. Synthesis of Compound LP-24a



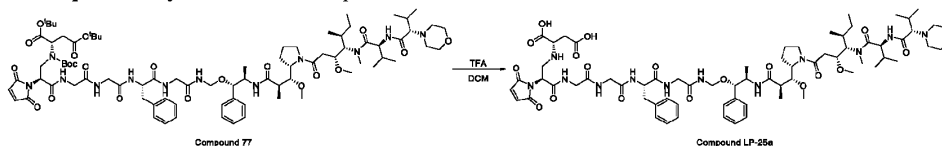
Compound LP-24a was synthesized from compound 76 with reference to the synthetic method of **Example 9**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1406.8.

Example 75. Synthesis of Compound 77



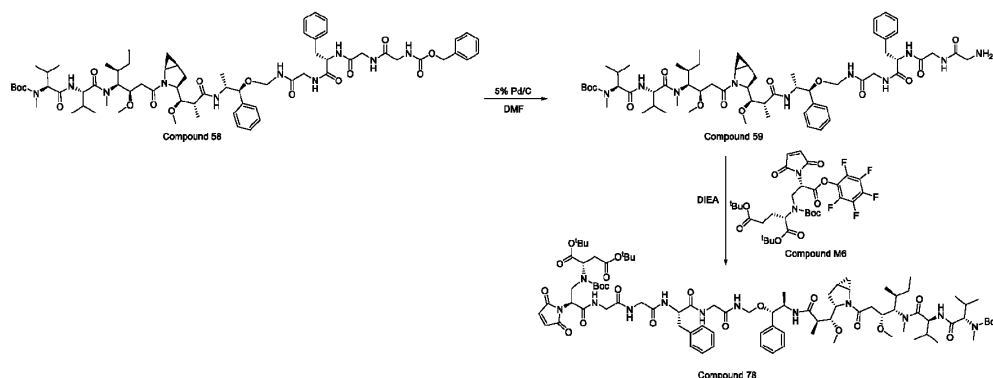
Compound 77 was synthesized from compound 51 with reference to the synthetic method of **Example 63**. LC-MSm/z (ES^+): $[\text{M}/2+\text{H}]^+$: 808.5.

Example 76. Synthesis of Compound LP-25a



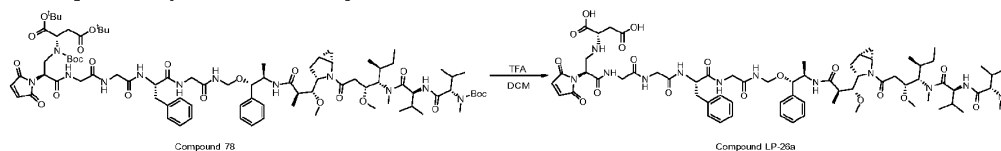
Compound LP-25a was synthesized from compound 77 with reference to the synthetic method of **Example 9**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1403.8.

Example 77. Synthesis of Compound 78



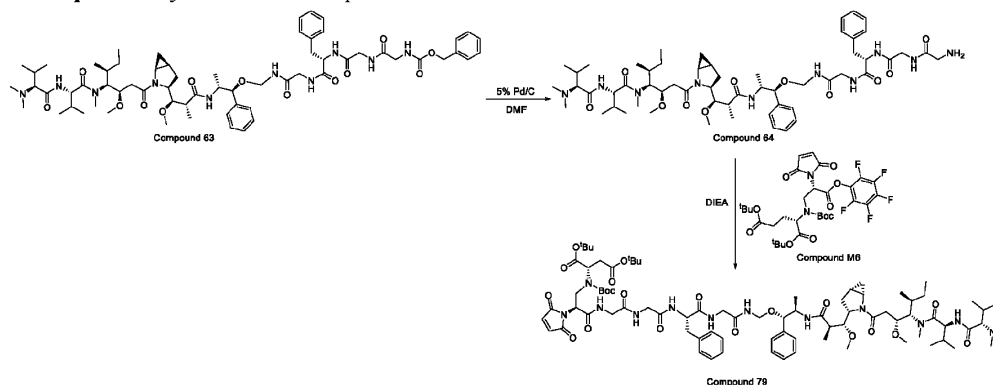
Compound **78** was synthesized from compound **58** with reference to the synthetic method of **Example 63**. LC-MSm/z (ES^+): $[\text{M}/2+\text{H}]^+$: 836.5.

Example 78. Synthesis of Compound LP-26a



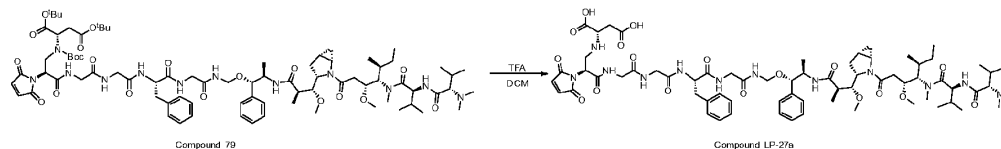
Compound **LP-26a** was synthesized from compound **78** with reference to the synthetic method of **Example 9**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1359.7.

Example 79. Synthesis of Compound 79



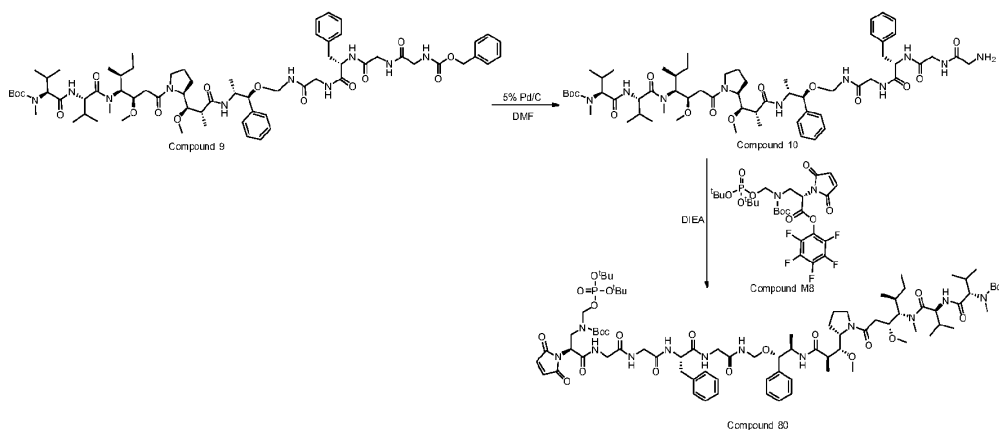
Compound **79** was synthesized from compound **63** with reference to the synthetic method of **Example 63**. LC-MSm/z (ES^+): $[\text{M}/2+\text{H}]^+$: 793.5.

Example 80. Synthesis of Compound LP-27a



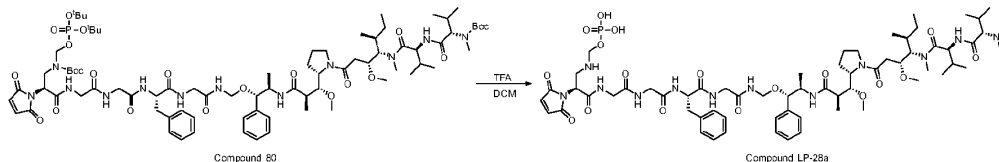
Compound **LP-27a** was synthesized from compound **79** with reference to the synthetic method of **Example 9**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1373.8.

Example 81. Synthesis of Compound 80



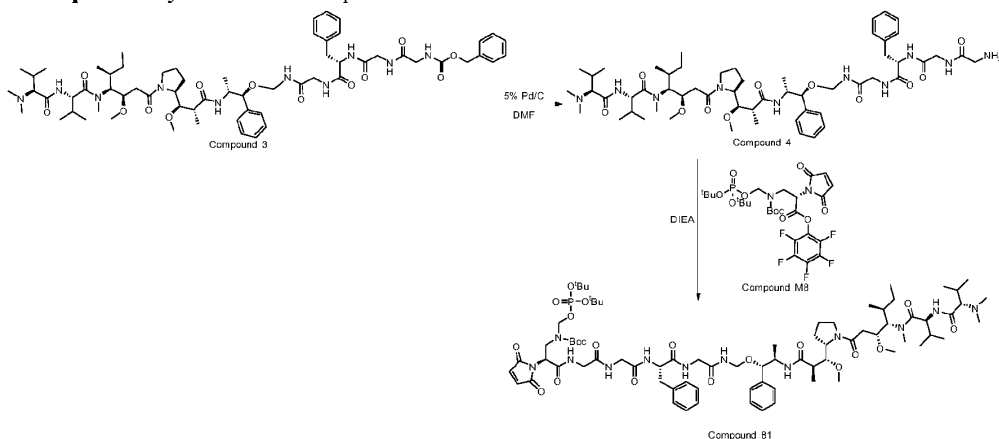
Compound 9 (960 mg, 0.74 mmol, 1.0 eq), 5% Pd/C (960 mg) and DMF (10 mL) were added to a 50 mL single-mouth bottle. After H₂ replacement 3 times, the reaction was carried out at room temperature for 1 h. The reaction was monitored by HPLC. The reaction was completed and recorded as reaction solution ①. The reaction solution ① was filtered into a 50 mL single-mouth bottle. Compound M8 (497.7 mg, 0.74 mmol, 1.0 eq, compound M8 refers to the synthesis of compound M8 in CN13827736) and DIEA (105 mg, 0.81 mmol, 1.1 eq) were added in turn under ice-water bath. After the addition, the temperature was raised to room temperature for 1 h. The reaction was monitored by HPLC. The reaction solution was purified by reverse phase preparation and the preparation 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



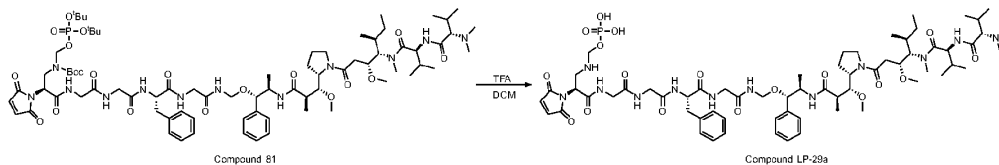
Compound LP-28a was synthesized from compound 80 with reference to the synthetic method of **Example 9**. LC-MSm/z (ES⁺): [M/2+H]⁺: 671.4.

Example 83. Synthesis of Compound 81



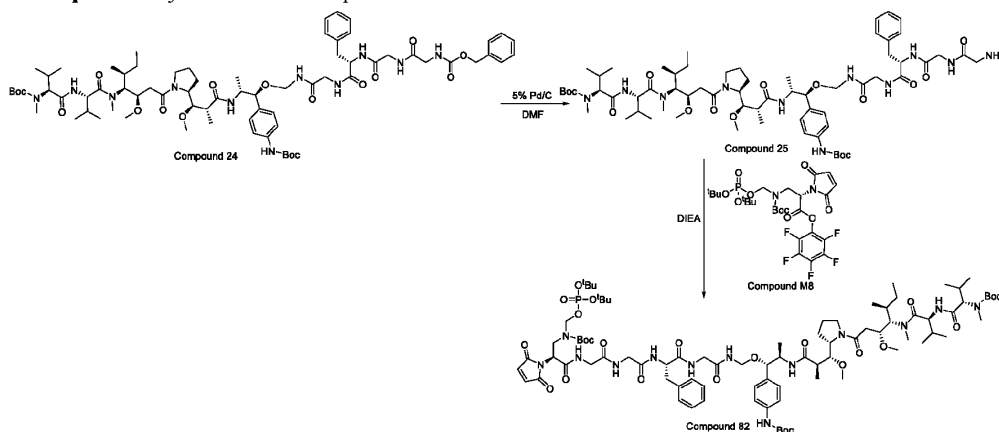
nd **81** was synthesized from Compound **3** with reference to the synthesis of **Example 81**. LC-MSm/z (ES^+): $[M/2+H]^+$: 784.5.

Example 84. Synthesis of Compound LP-29a



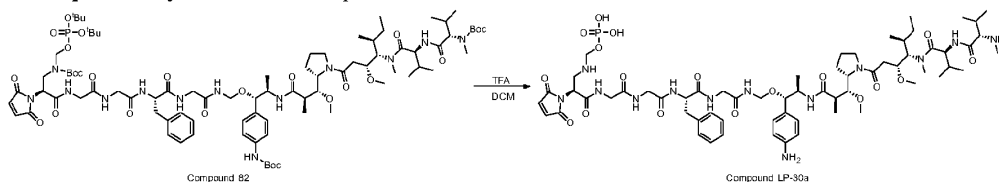
Compound **LP-29a** was synthesized from compound **81** with reference to the synthetic method of **Example 9**. LC-MSm/z (ES^+): $[M/2+H]^+$: 678.4.

Example 85. Synthesis of Compound 82



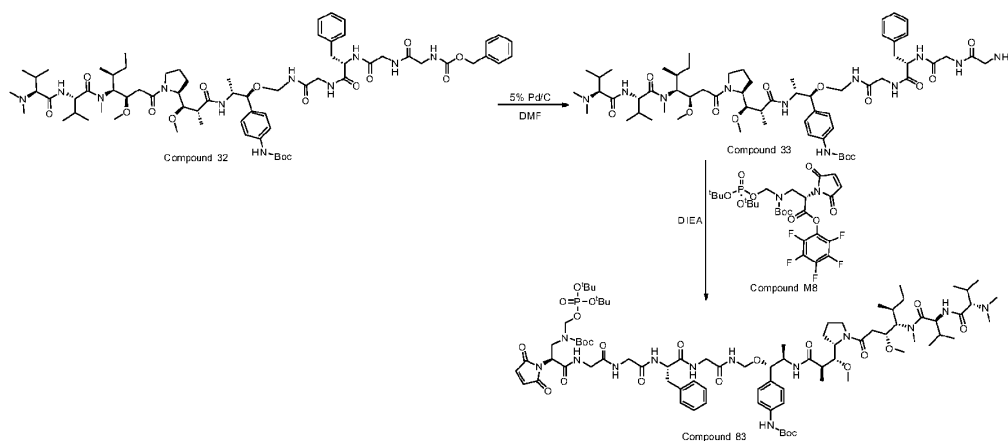
Compound **82** was synthesized from compound **24** with reference to the synthetic method of **Example 81**. LC-MSm/z (ES^+): $[M/2+H]^+$: 885.0.

Example 86. Synthesis of Compound LP-30a



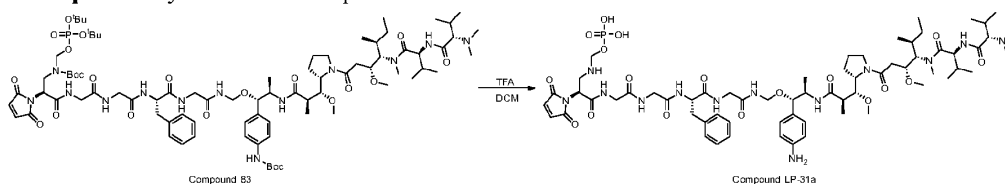
Compound **LP-30a** was synthesized from compound **82** with reference to the synthetic method of **Example 9**. LC-MSm/z (ES^+): $[M/2+H]^+$: 678.9.

Example 87. Synthesis of Compound 83



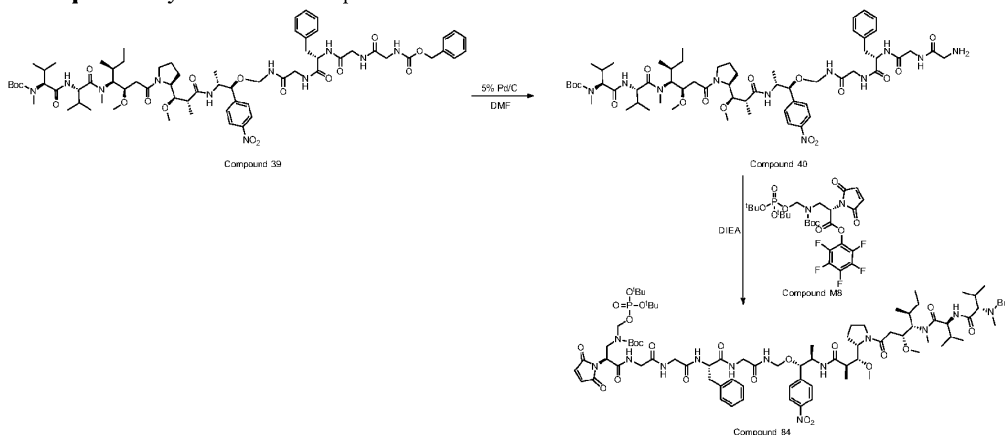
Compound **83** was synthesized from compound **32** with reference to the synthetic method of **Example 81**. LC-MSm/z (ES^+): $[\text{M}/2+\text{H}]^+$: 842.0.

Example 88. Synthesis of Compound LP-31a



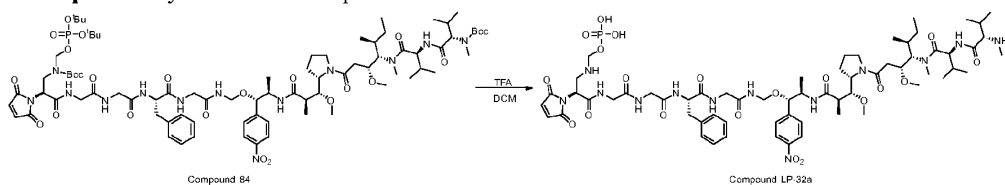
Compound **LP-31a** was synthesized from compound **83** with reference to the synthetic method of **Example 9**. LC-MSm/z (ES^+): $[\text{M}/2+\text{H}]^+$: 685.9.

Example 89. Synthesis of Compound 84



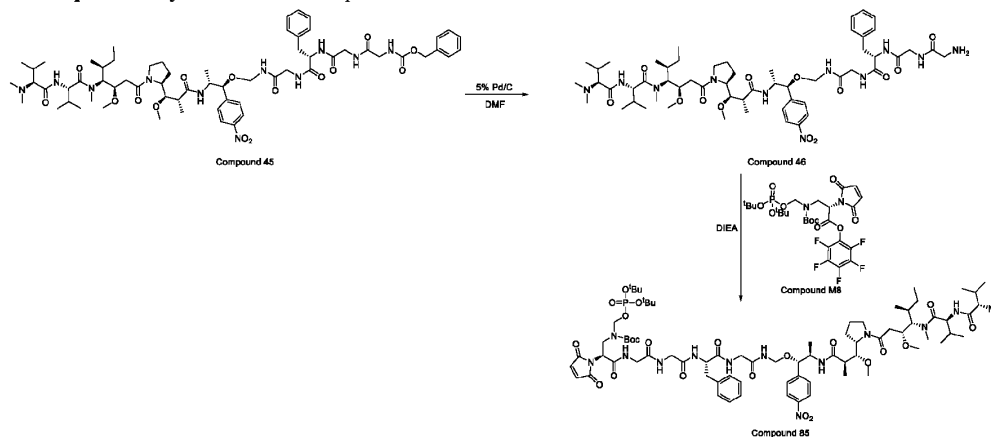
Compound **84** was synthesized from compound **39** with reference to the synthetic method of **Example 81**. LC-MSm/z (ES^+): $[\text{M}/2+\text{H}]^+$: 850.1.

Example 90. Synthesis of Compound LP-32a



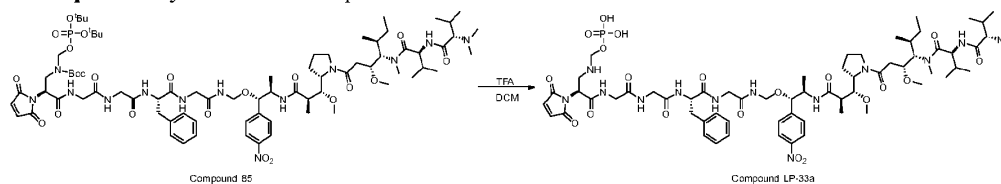
nd **LP-32a** was synthesized from compound **84** with reference to the synthetic method of **Example 9**. LC-MSm/z (ES⁺):[M/2+H]⁺ : 693.9.

Example 91. Synthesis of Compound **85**



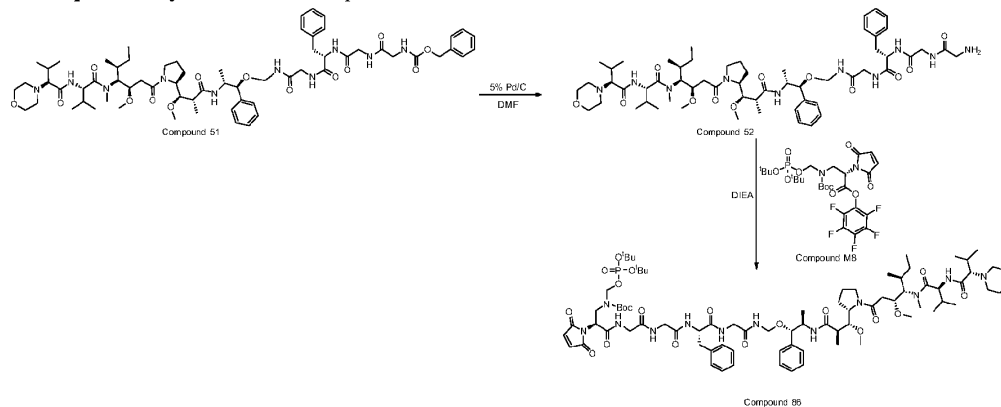
Compound **85** was synthesized from compound **45** with reference to the synthetic method of **Example 81**. LC-MSm/z (ES⁺):[M/2+H]⁺ : 806.9.

Example 92. Synthesis of Compound **LP-33a**



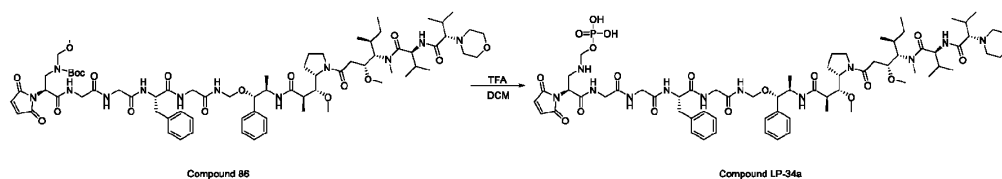
Compound **LP-33a** was synthesized from compound **85** with reference to the synthetic method of **Example 9**. LC-MSm/z (ES⁺):[M/2+H]⁺ : 700.9.

Example 93. Synthesis of Compound **86**



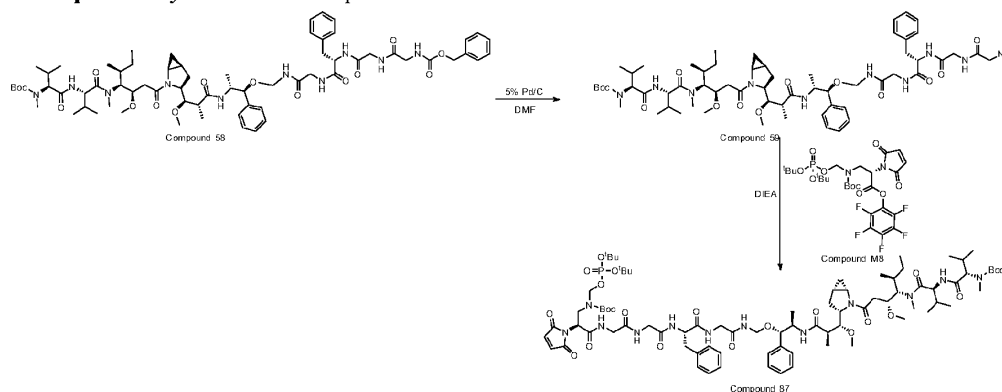
Compound **86** was synthesized from compound **51** with reference to the synthetic method of **Example 81**. LC-MSm/z (ES⁺):[M/2+H]⁺ : 805.5.

Example 94. Synthesis of Compound **LP-34a**



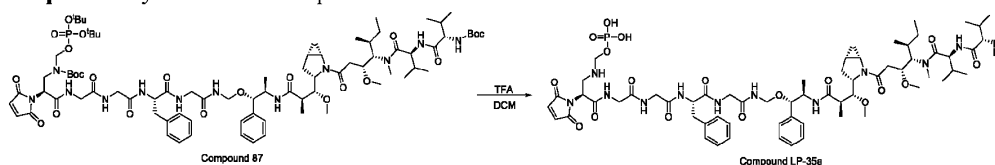
Compound **LP-34a** was synthesized from compound **86** with reference to the synthetic method of **Example 9**. LC-MSm/z (ES^+): $[\text{M}/2+\text{H}]^+$: 699.4.

Example 95. Synthesis of Compound 87



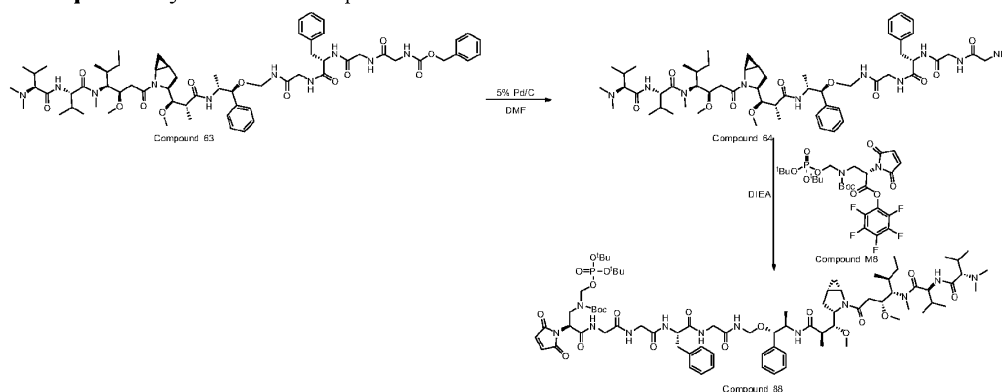
Compound **87** was synthesized from compound **58** with reference to the synthetic method of **Example 81**. LC-MSm/z (ES^+): $[\text{M}/2+\text{H}]^+$: 833.5.

Example 96. Synthesis of Compound LP-35a



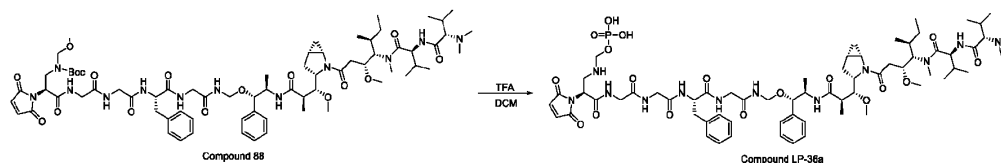
Compound **LP-35a** was synthesized from compound **87** with reference to the synthetic method of **Example 9**. LC-MSm/z (ES^+): $[\text{M}/2+\text{H}]^+$: 677.4.

Example 97. Synthesis of Compound 88



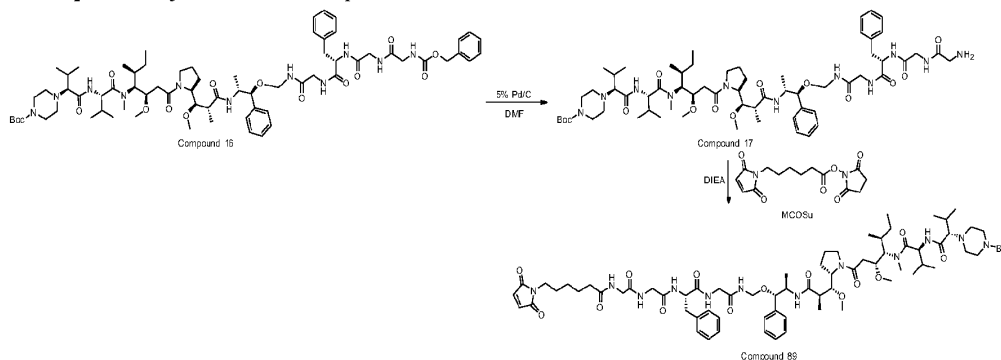
Compound **88** was synthesized from compound **63** with reference to the synthetic method of **Example 81**. LC-MSm/z (ES^+): $[\text{M}/2+\text{H}]^+$: 790.5.

Example 98. Synthesis of Compound LP-36a



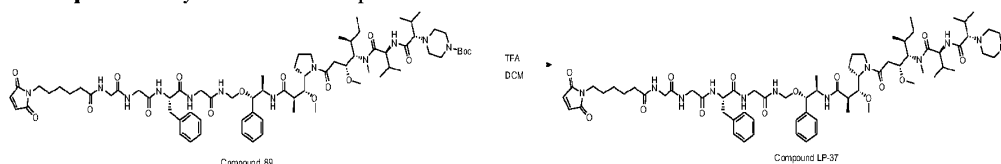
Compound **LP-36a** was synthesized from compound **88** with reference to the synthetic method of **Example 9**. LC-MSm/z (ES^+): $[\text{M}/2+\text{H}]^+$: 670.4.

Example 99. Synthesis of Compound 89



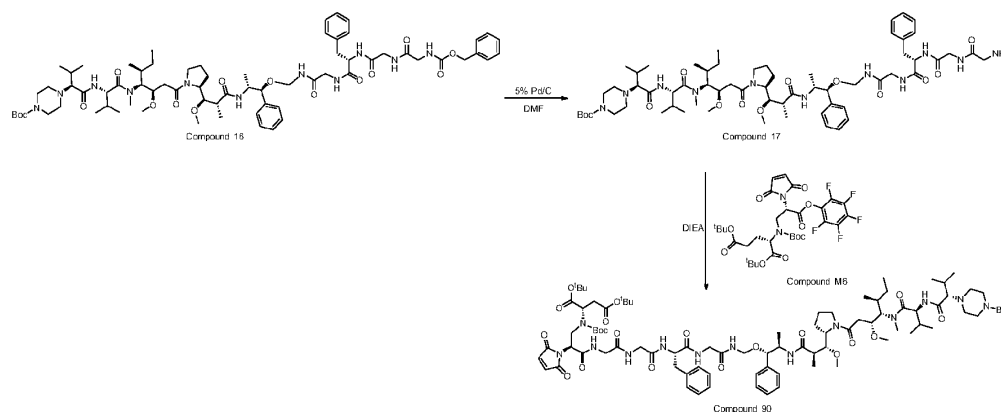
Compound **89** was synthesized from compound **16** with reference to the synthetic method of **Example 49**. LC-MSm/z (ES^+): $[\text{M}/2+\text{H}]^+$: 707.4.

Example 100. Synthesis of Compound LP-37



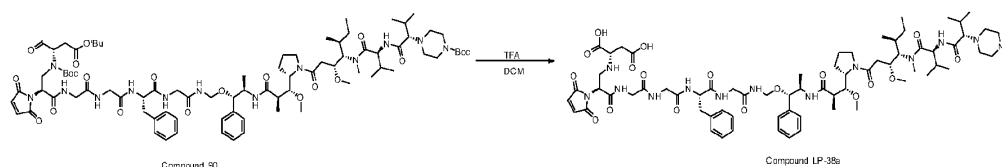
10 Compound **LP-37** was synthesized from compound **89** with reference to the synthetic method of **Example 9**. LC-MSm/z (ES^+): $[\text{M}+\text{H}]^+$: 1313.8.

Example 101. Synthesis of Compound 90



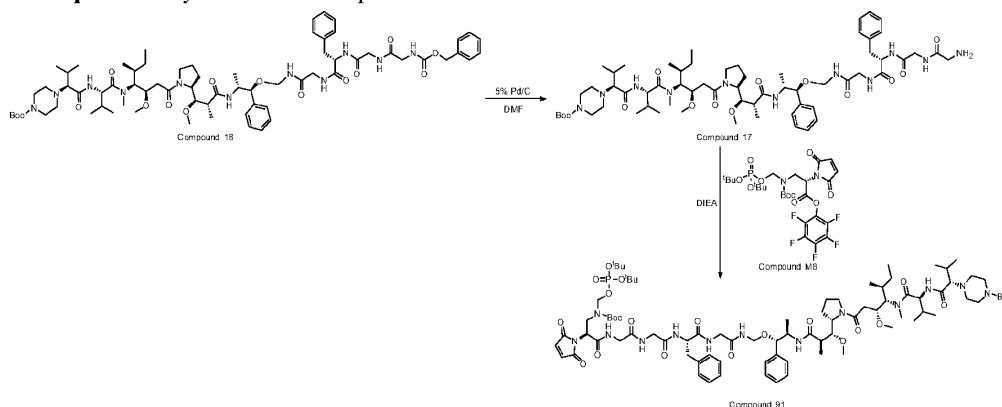
Compound **90** was synthesized from compound **16** with reference to the synthetic method of **Example 63**. LC-MSm/z (ES^+): $[\text{M}/2+\text{H}]^+$: 858.0.

Example 102. Synthesis of Compound LP-38a



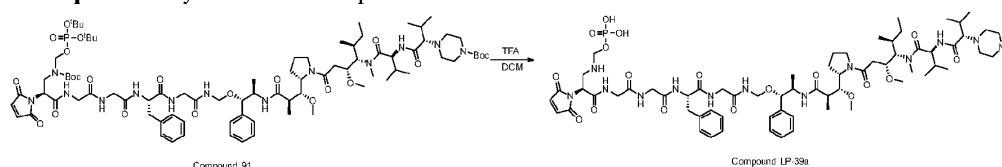
Compound **LP-38a** was synthesized from compound **90** with reference to the synthetic method of **Example 9**. LC-MSm/z (ES^+): $[M/2+H]^+$: 701.9.

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.

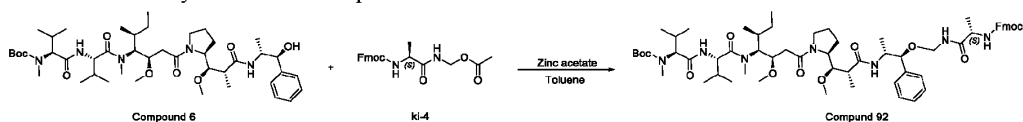
Example 105. Expression and purification of TA001 antibody:

Expi293 suspension cells (Shanghai Aopuma Biotech Co., Ltd.) were cultured. One day before transfection, cells were inoculated at a certain density in OPM-293 CD05 Medium (Shanghai Aopuma Biotech Co., Ltd.) and cultured overnight in a cell culture shaker at 37 °C, 5% CO₂, and 120 rpm. The next day, PEI-MAX was used to transfect the antibody expression plasmid. OPM-293 ProFeed (Shanghai Aopuma Biotech Co., Ltd.) was added as feed on the first and third days after transfection, and the supernatant was collected by centrifugation on the sixth day after transfection.

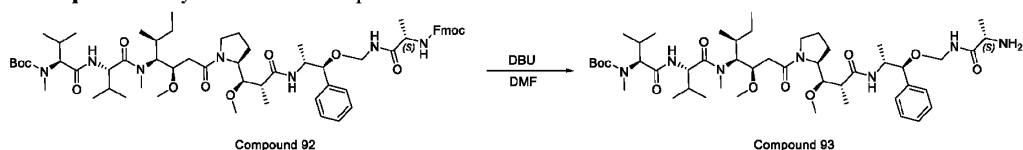
The supernatant was initially purified using a Protein A affinity chromatography column, and then finely purified by SEC or CHT to remove impurities such as polymers.

The amino acid sequences of Antibody TA001 are listed as SEQ ID NO: 9 for its light chain; SEQ ID NO: 7 for its light chain variable region; SEQ ID NO: 12 for its heavy chain; and SEQ ID NO: 10 for its heavy chain variable region. In addition, the amino acid sequences of CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, CDR-H3, as well as the light chain constant region and the heavy chain constant region are listed.

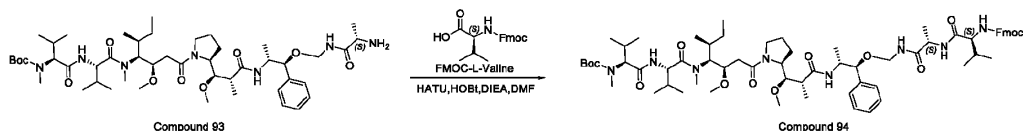
Synthesis of Compound 92



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, 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.

10 **Example 107.** Synthesis of Compound 93

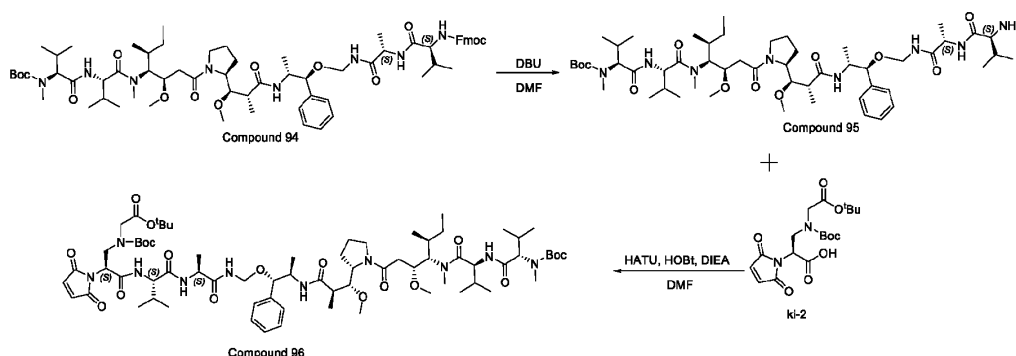
Compound **92** (1.0 g, 0.877 mmol, 1.0 eq) and DMF (10 mL) were added to a 50 mL single-necked round-bottom flask in sequence. After stirring at room temperature and dissolving, DBU (144.1 μL, 0.985 mmol, 1.1 eq) was added and reacted at room temperature for 0.5 h. The starting material disappeared 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 108. Synthesis of Compound 94

20

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 μL, 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.

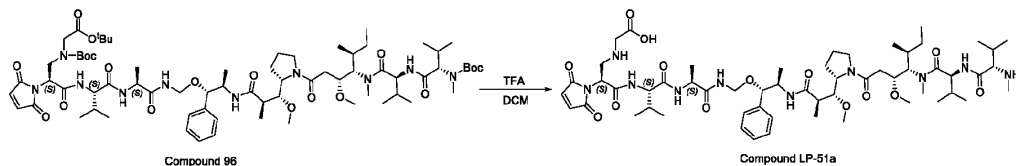
Example 109. Synthesis of Compound 96



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.

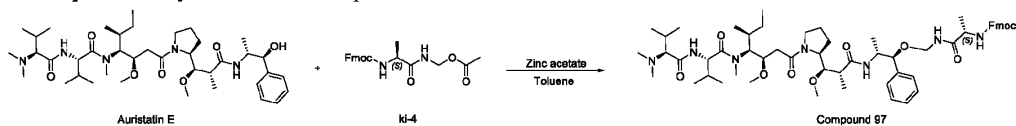
In another 10 mL single-necked round-bottom flask, compound **94** (360.1 mg, 0.291 mmol, 1.0 eq) and DMF (2 mL) were added in sequence. After stirring at room temperature and dissolving, DBU (47.8 μ L, 0.32 mmol, 1.1eq) was added and reacted at room temperature for 0.5 h. The reaction was monitored 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 110. Synthesis of Compound LP-51a



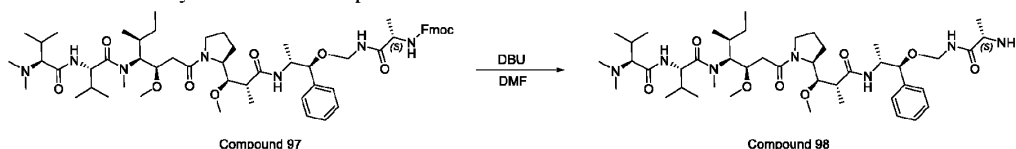
Compound **96** (200 mg, 0.143 mmol, 1.0 eq) was dissolved in 10 mL dry dichloromethane and 4 mL TFA, and reacted at room temperature for 3 h, monitored by HPLC. After the reaction was completed, the solvent was removed by concentration under reduced pressure, and the crude product was purified by reverse 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 111. Synthesis of Compound 97

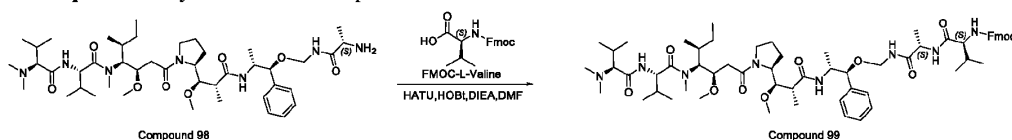


Auristatin E (2.04 g, 2.79 mmol, 1.0eq), ki-4 (2.13 g, 5.57 mmol, 2.0eq, the synthesis method refers to the synthesis of compound 4b in WO2020146541), zinc acetate (1.02 g, 5.57 mmol, 2.0eq), and toluene (30 mL) were added in sequence. After N₂ replacement 3 times, the temperature was raised to 115 °C under N₂ protection for 4h, the reaction was stopped, cooled to room temperature, 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.

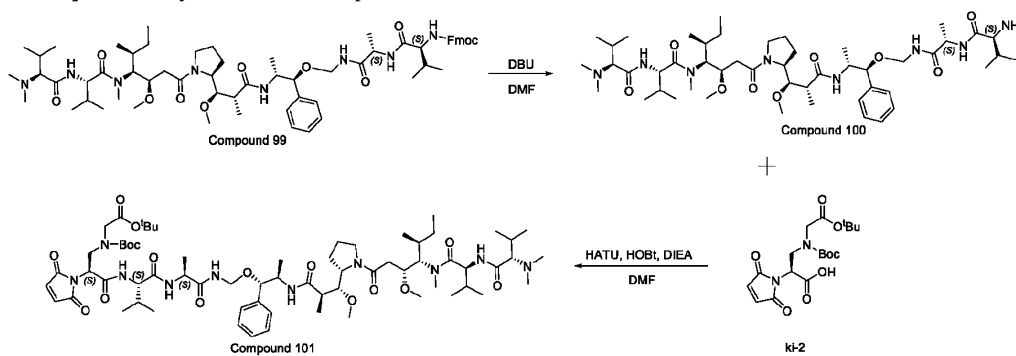
Synthesis of Compound 98



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.

10 **Example 113.** Synthesis of Compound 99

Compound 98 (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 114. Synthesis of Compound 101

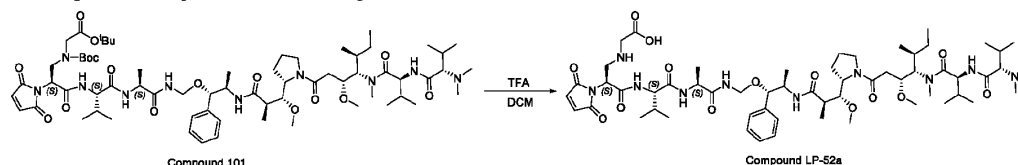
20

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

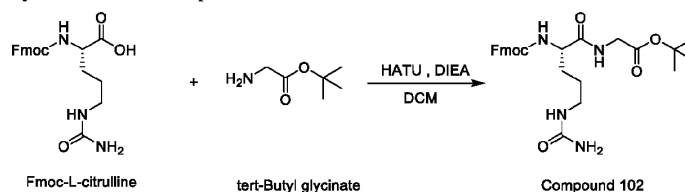
HPLC. After the reaction was completed, the product was purified by reverse phase preparative column to obtain a white solid product (299.1 mg, 78.5%). LC-MS m/z (ES⁺): [M+2H]²⁺: 656.4.

Example 115. Synthesis of Compound LP-52a



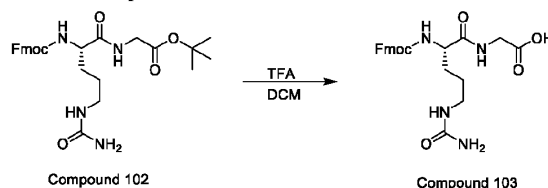
Compound **101** (200 mg, 0.152 mmol, 1.0 eq) was dissolved in 10 mL dry dichloromethane and 4 mL TFA, and reacted at room temperature for 3 h, monitored by HPLC. After the reaction was completed, the solvent was removed by concentration under reduced pressure, and the crude product was purified by reverse 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.

Example 116. Synthesis of Compound 102



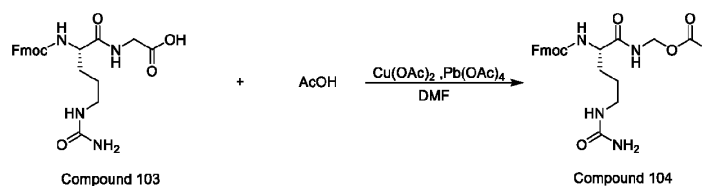
Compound DIEA (4.88 g, 37.74 mmol, 3 eq) and HATU (5.74 g, 15.09 mmol, 1.2 eq) were added to a solution of compound Fmoc-L-citrulline (5 g, 12.58 mmol, 1 eq) and glycine tert-butyl ester (2.48 g, 18.87 mmol, 1.5 eq) in DCM (10 mL), and the reaction solution was stirred at room temperature overnight. 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 117. Synthesis of Compound 103



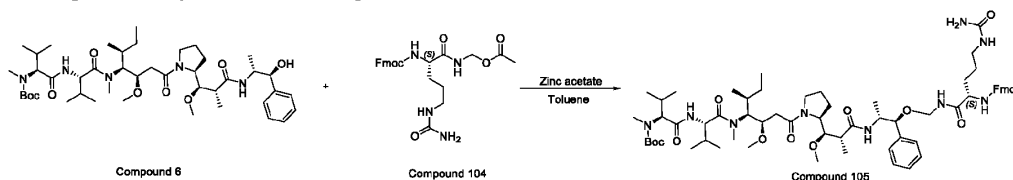
TFA (2 mL) was added to a solution of compound **102** (1.60 g, 3.13 mmol, 1 eq) in DCM (10 mL) at 0 °C, and the reaction was stirred overnight at room temperature. LCMS showed that the reaction was complete. The reaction solution was evaporated under reduced pressure, and the residue was purified by 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 118. Synthesis of Compound 104



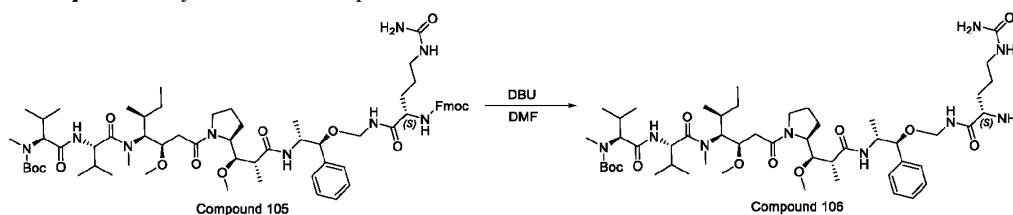
To a solution of compound **103** (1.01 g, 2.22 mmol, 1 eq) in DMF (20 mL) were added acetic acid (400.35 mg, 382.02 μ L, 6.66 mmol, 3 eq), copper acetate (121.09 mg, 113.38 μ L, 0.67 mmol, 0.3 eq) and lead acetate (1.97 g, 0.89 mL, 4.45 mmol, 2 eq), and the reaction was stirred at 60°C for 1 hour. LCMS 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 119. Synthesis of Compound 105



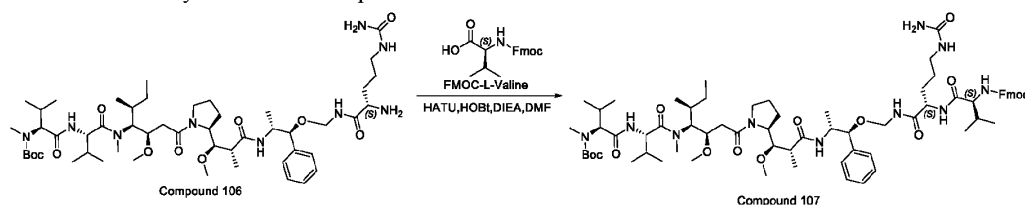
Compound **6** (2.12 g, 2.59 mmol, 1.0eq), compound **104** (2.77 g, 5.18 mmol, 2.0eq), zinc acetate (1.08 g, 5.18 mmol, 2.0eq) were added to a 100 mL single-mouth bottle in sequence, dissolved in toluene (30 mL), replaced with N₂ three times, heated to 115°C under N₂ protection for 4 hours, stopped 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 120. Synthesis of Compound 106

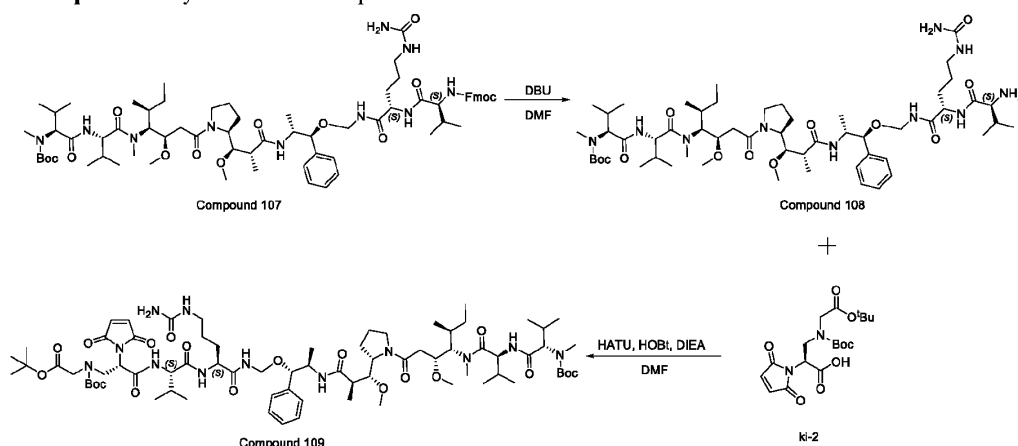


Compound **105** (1.02 g, 0.832 mmol, 1.0 eq) and DMF (9 mL) were added to a 50 mL single-necked round-bottom flask in sequence. After stirring at room temperature and dissolving, DBU (136.7 μ L, 0.915 mmol, 1.1 eq) was added and reacted at room temperature for 0.5 h. The starting material disappeared 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.

Synthesis of Compound 107



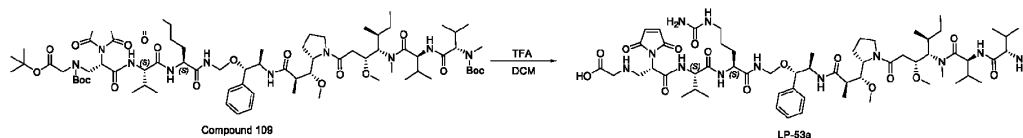
Compound **106** (496.5 mg, 0.494 mmol, 1.0 eq) and Fmoc-L-valine (186.3 mg, 0.494 mmol, 1.0 eq) were added to a 25 mL single-necked round-bottom flask, and DMF (5 mL) was added to dissolve. Then, HATU (225.6 mg, 0.593 mmol, 1.2 eq), HOBT (80 mg, 0.593 mmol, 1.2 eq), and DIEA (191.6 μ L, 1.483 mmol, 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.

10 **Example 122. 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.

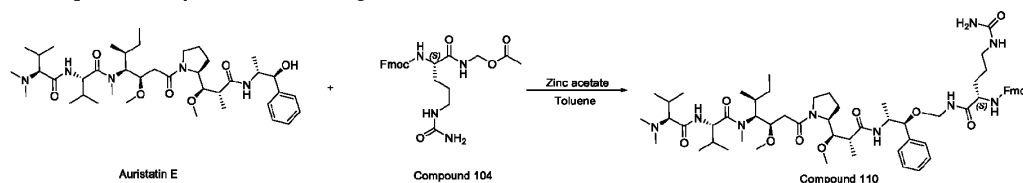
In another 10 mL single-necked round-bottom flask, compound **107** (385.8 mg, 0.291 mmol, 1.0 eq) and DMF (2 mL) were added in sequence. After stirring and dissolving at room temperature, DBU (47.8 μ L, 0.32 mmol, 1.1 eq) was added and reacted at room temperature for 0.5 h. The reaction was monitored 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 123. Synthesis of Compound LP-53a



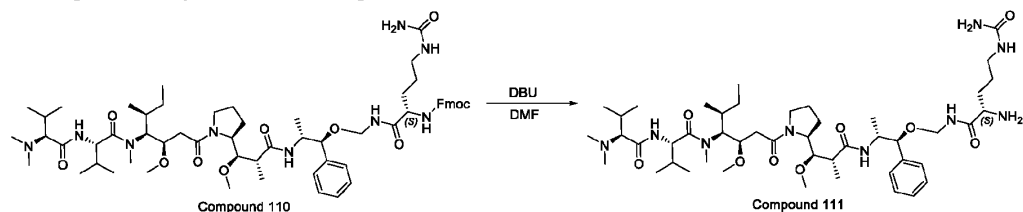
Compound **109** (200 mg, 0.135 mmol, 1.0 eq) was dissolved in 10 mL dry dichloromethane and 4 mL TFA, and reacted at room temperature for 3 h, monitored by HPLC. After the reaction was completed, the solvent was removed by concentration under reduced pressure, and the crude product was purified by reverse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

Example 124. Synthesis of Compound 110



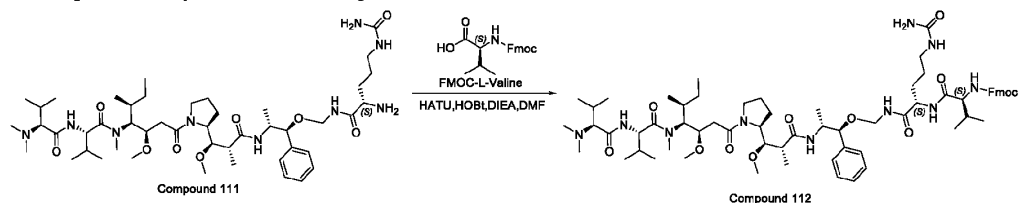
10 Auristatin E (2.3 g, 3.14 mmol, 1.0 eq), compound **104** (2.94 g, 6.28 mmol, 2.0 eq), zinc acetate (1.15 g, 6.28 mmol, 2.0 eq) and toluene (30 mL) were added to a 100 mL single-necked bottle in sequence. After N₂ replacement for 3 times, the temperature was raised to 115°C under N₂ protection for 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 125. Synthesis of Compound 111



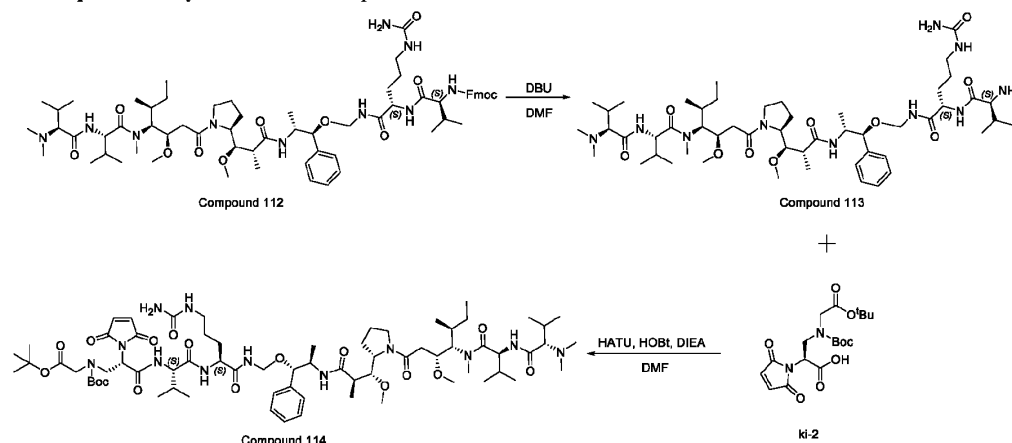
20 Compound **110** (918.7 mg, 0.805 mmol, 1.0 eq) and DMF (9 mL) were added to a 50 mL single-necked round-bottom flask in sequence. After stirring at room temperature and dissolving, DBU (132.4 μL, 0.886 mmol, 1.1 eq) was added and reacted at room temperature for 0.5 h. The starting material disappeared 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 126. Synthesis of Compound 112



id **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]⁺: 1239.7.

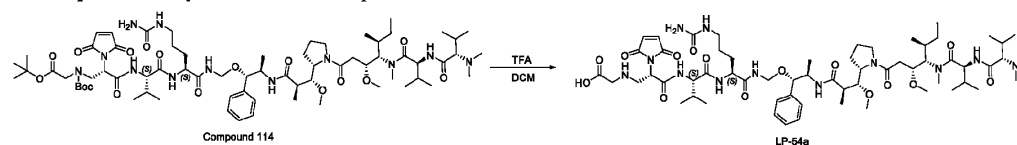
Example 127. Synthesis of Compound **114**



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 **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.

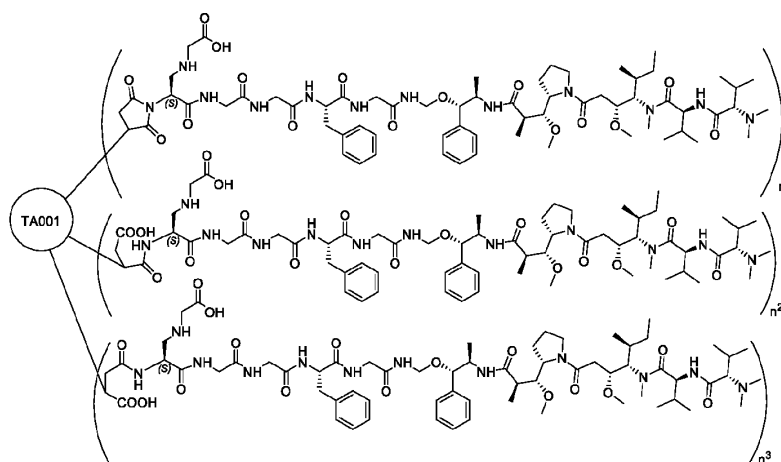
Example 128. Synthesis of Compound **LP-54a**



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.

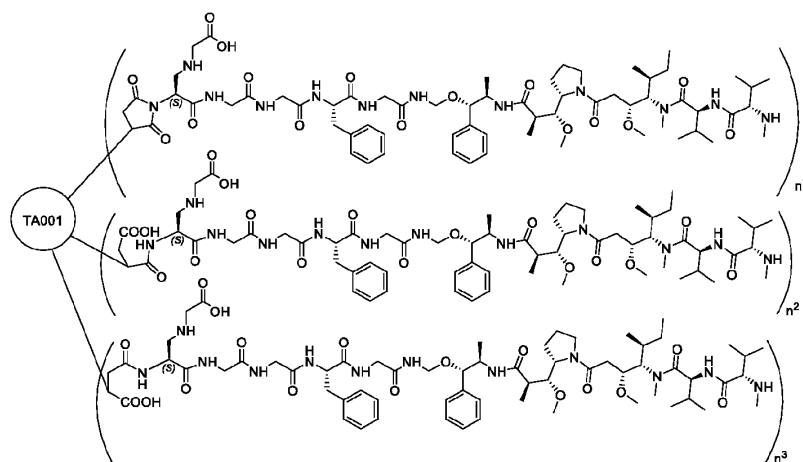
ADC-1-1a

ADC-1-1a was prepared by combining 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 S-type) with antibody TA001 according to the method of generic Step A. The sequence information of TA001 is shown in **Example 105**. The RP-HPLC detection results of **ADC-1-1a** are shown in **Figure 3A**, and the SEC-HPLC detection results are shown in **Figure 4A**.

**ADC-1-1a****Example 130. ADC-2-1a**

ADC-2-1a was prepared as the corresponding linker-payload (**LP-2a**, the structure of which is shown in the structural formula of **LP-2**, where the configuration of the chiral carbon at position 2 is of the S-type) was prepared in accordance with the method of generic Step A. was prepared with the antibody TA001 to obtain, and the sequence information of TA001 is shown in **Example 105**.

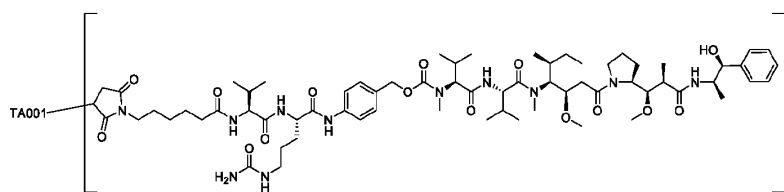
ADC-2-1a was prepared by combining the corresponding linker-payload (**LP-2a**, whose structure is shown in the structural formula of **LP-2**, wherein the configuration of the chiral carbon at position-2 is S-type) with antibody TA001 according to the method of generic Step A. The sequence information of TA001 is shown in **Example 105**.



ADC-2-1a

Control Example 1. ADC- C-1a

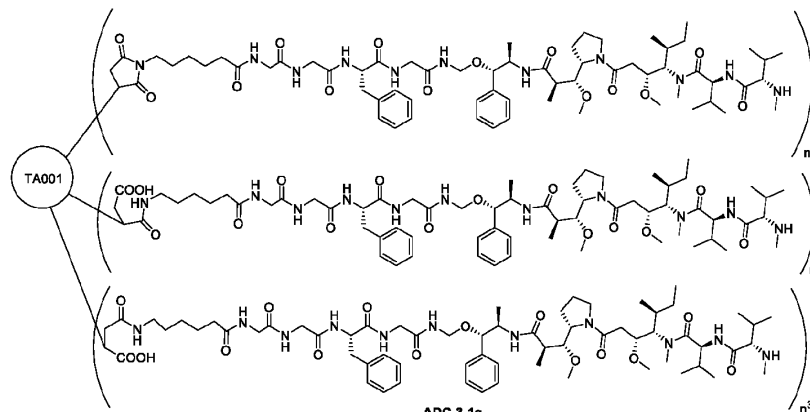
ADC-C-1a was prepared by combining compound **VcMMAE** with antibody TA001 according to the method of generic Step A. The RP-HPLC detection results of **ADC-C-1a** are shown in **Figure 3B**, and the SEC-HPLC detection results are shown in **Figure 4B**.



ADC-C-1a

Example 131. ADC-3-1a

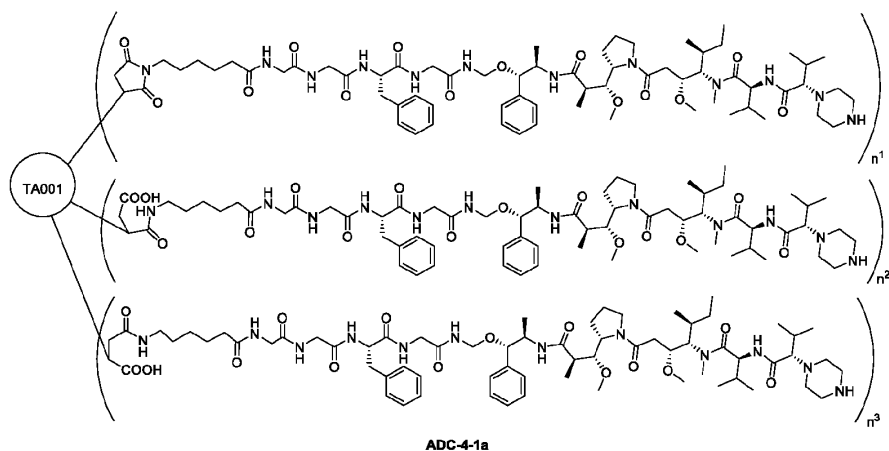
ADC-3-1a was prepared as the corresponding linker-payload (**LP-10**) was prepared with antibody TA001 according to the method of generic Step A.



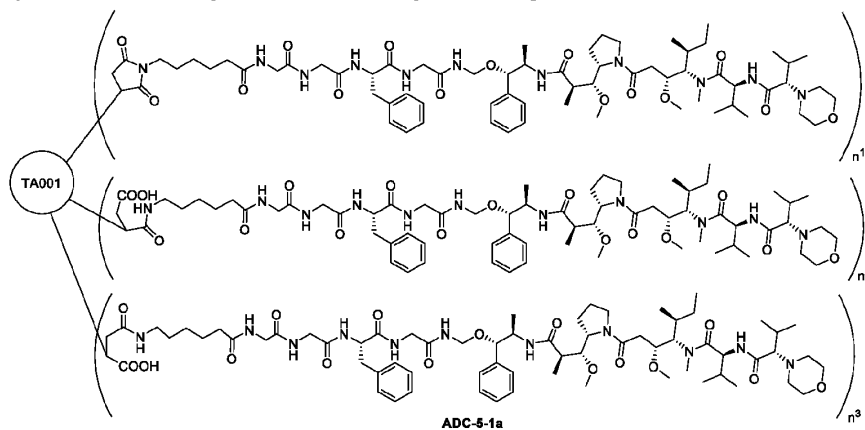
ADC-3-1a

Example 132. ADC-4-1a

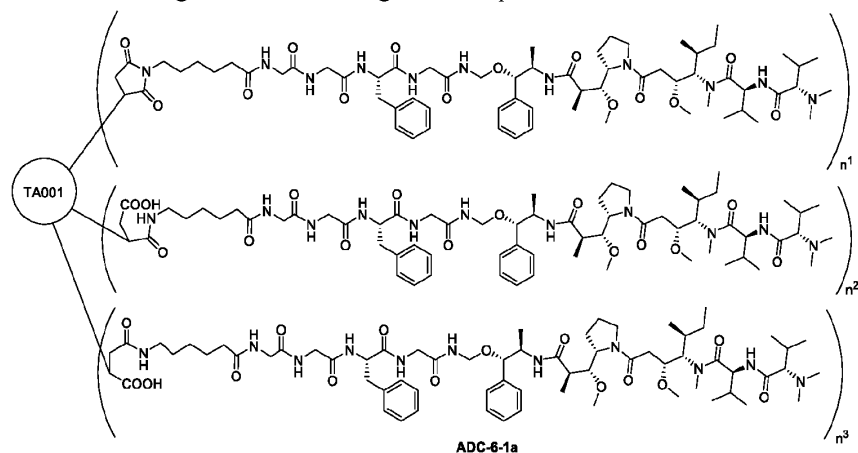
ADC-4-1a was prepared by combining the corresponding linker-payload (**LP-37**) with antibody TA001 according to the method of generic Step A.

**Example 133. ADC-5-1a**

ADC-5-1a was prepared by combining the corresponding linker-payload (**LP-16**) with antibody TA001 according to the method of generic Step A.

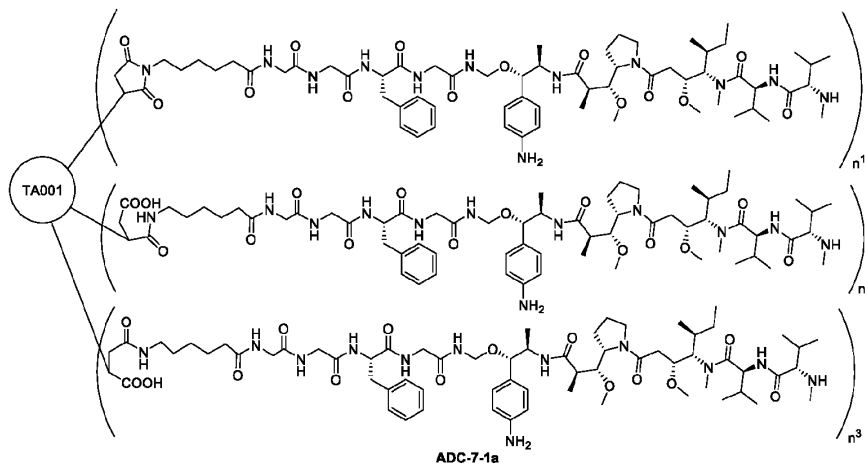
**Example 134. ADC-6-1a**

ADC-6-1a was prepared by combining the corresponding linker-payload (**LP-11**) with antibody TA001 according to the method of generic Step A.

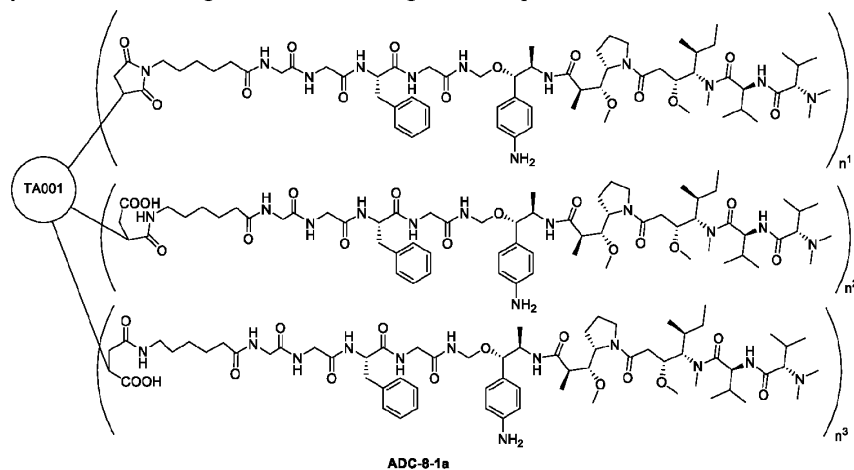


ADC-7-1a

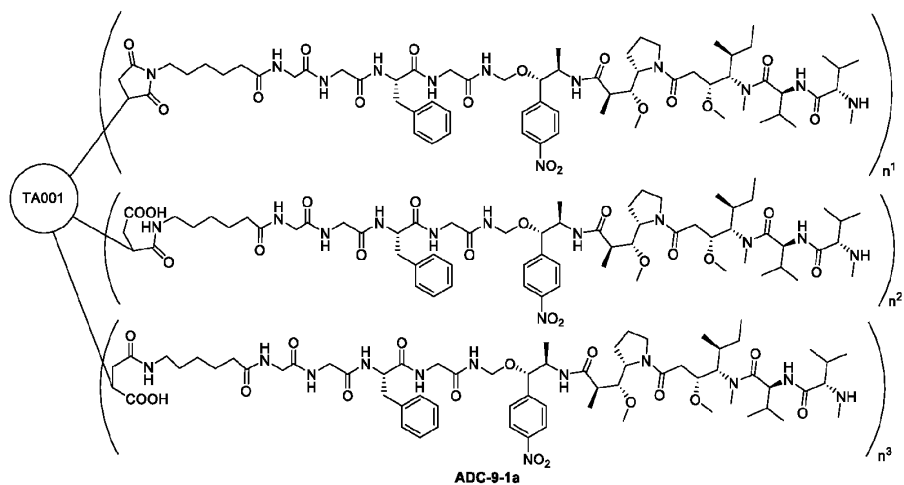
ADC-7-1a was prepared by combining the corresponding linker-payload (**LP-12**) with antibody TA001 according to the method of generic Step A.

**Example 136. ADC-8-1a**

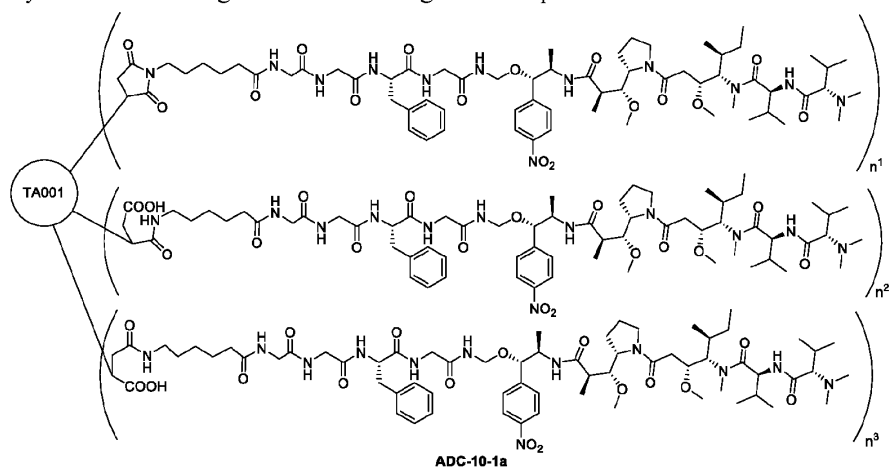
ADC-8-1a was prepared by combining the corresponding linker-payload (**LP-13**) with antibody TA001 according to the method of generic step A.

**Example 137. ADC-9-1a**

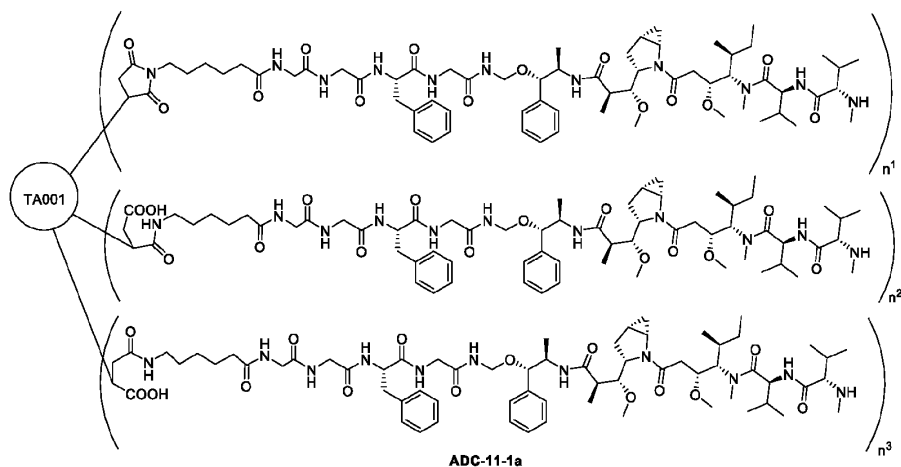
10 **ADC-9-1a** was prepared by combining the corresponding linker-payload (**LP-14**) with antibody TA001 according to the method of generic Step A.

**Example 138. ADC-10-1a**

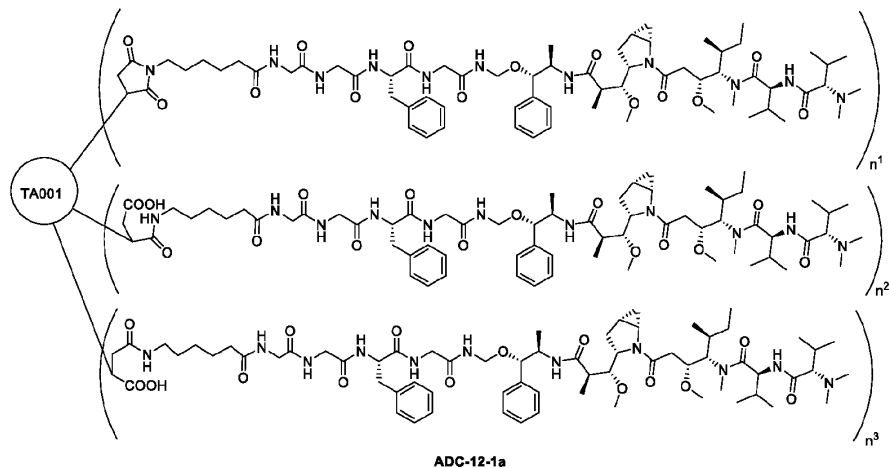
ADC-10-1a was prepared by combining the corresponding linker-payload (**LP-15**) with antibody TA001 according to the method of generic Step A.

**Example 139. ADC-11-1a**

ADC-11-1a was prepared by combining the corresponding linker-payload (**LP-17**) with antibody TA001 according to the method of generic Step A.

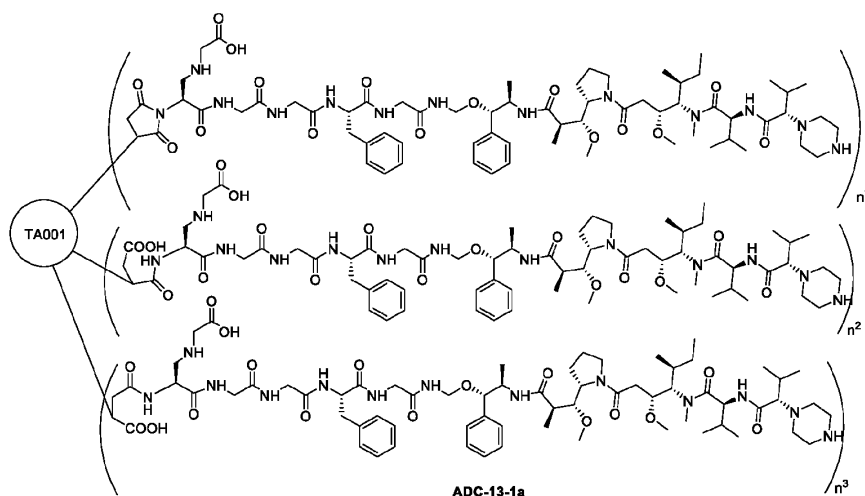
**Example 140. ADC-12-1a**

ADC-12-1a was prepared by combining the corresponding linker-payload (**LP-18**) with antibody TA001 according to the method of generic Step A.

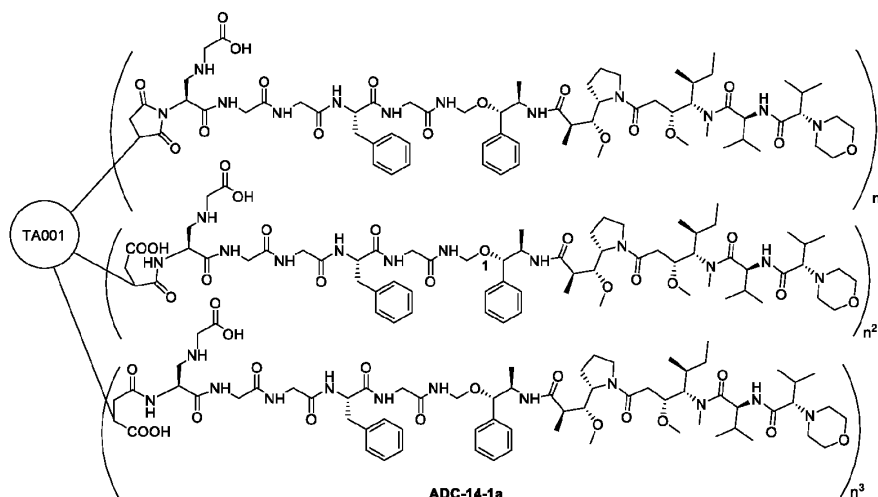
**Example 141. ADC-13-1a**

ADC-13-1a was prepared by combining the corresponding linker-payload (**LP-3a**, whose structure is shown in the structural formula of **LP-3**, wherein the configuration of the chiral carbon at position-2 is S-type) with the antibody TA001 according to the method of generic

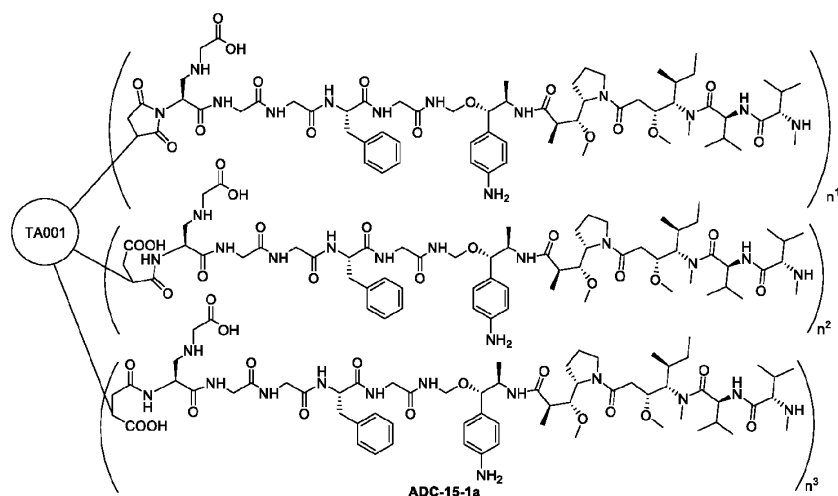
10 Step A.

**Example 142. ADC-14-1a**

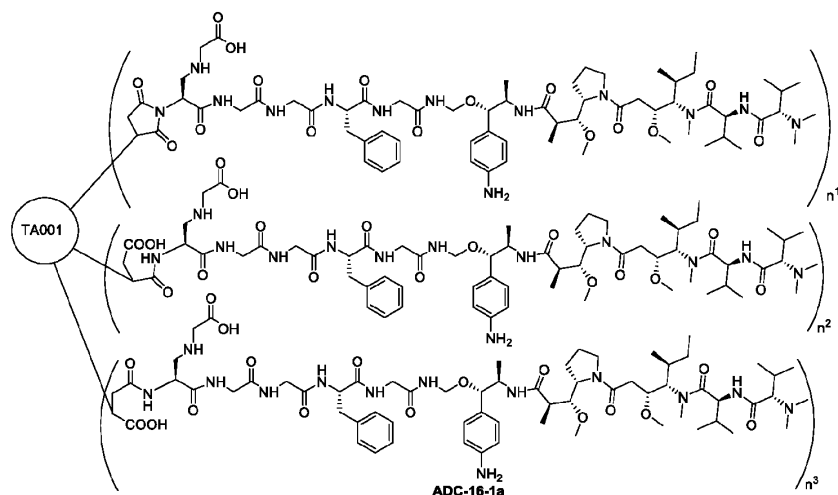
ADC-14-1a was prepared by reacting the corresponding linker-payload (**LP-7a**, whose structure is shown in the structural formula of **LP-7**, wherein the configuration of the chiral carbon at position-2 is S-type) with antibody TA001 according to the method of generic Step A.

**Example 143. ADC-15-1a**

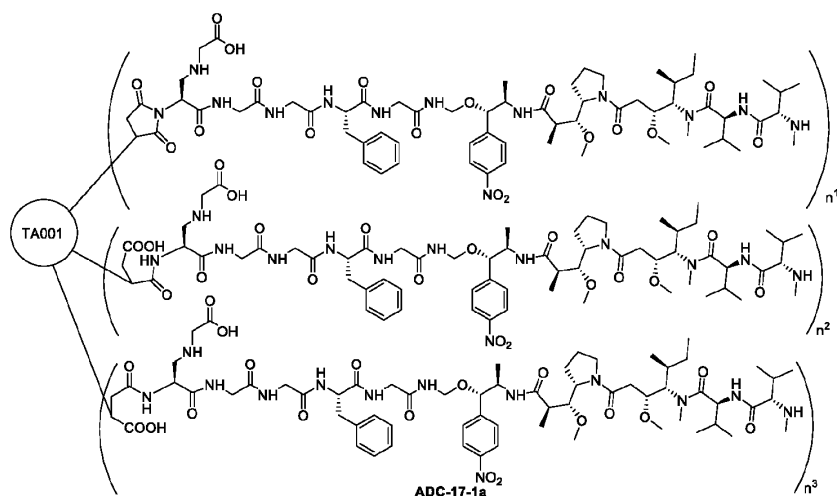
ADC-15-1a was prepared by combining the corresponding linker-payload (**LP-3sa**, whose structure is shown in the structural formula of **LP-3s**, wherein the configuration of the chiral carbon at position-2 is S-type) with the antibody TA001 according to the method of generic Step A.

**Example 144. ADC-16-1a**

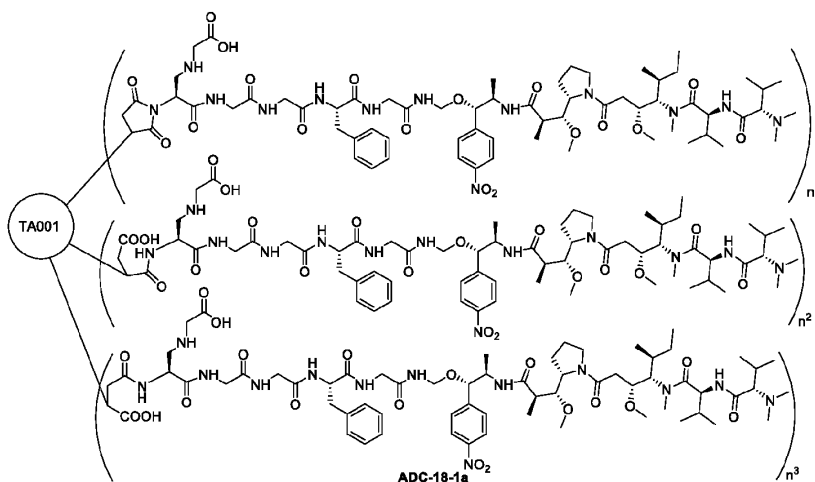
ADC-16-1a was prepared by combining the corresponding linker-payload (**LP-4a**, whose structure is shown in the structural formula of **LP-4**, wherein the configuration of the chiral carbon at position-2 is S-type) with the antibody TA001 according to the method of generic Step A.

**Example 145. ADC-17-1a**

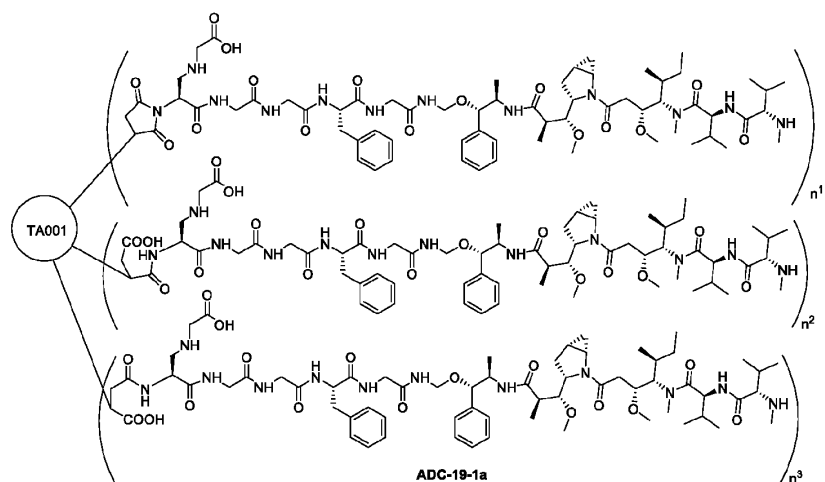
ADC-16-1a was prepared by combining the corresponding linker-payload (**LP-4a**, whose structure is shown in the structural formula of **LP-4**, wherein the configuration of the chiral carbon at position-2 is S-type) with the antibody TA001 according to the method of generic Step A.

**Example 146. ADC-18-1a**

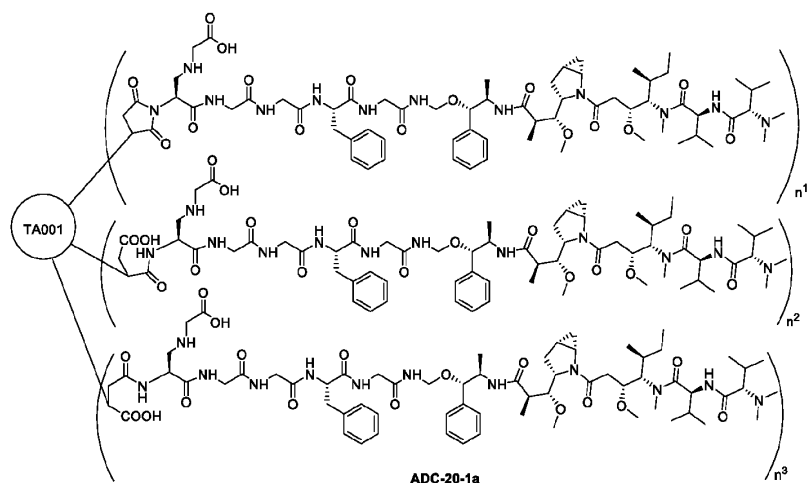
ADC-18-1a was prepared by combining the corresponding linker-payload (**LP-6a**, whose structure is shown in the structural formula of **LP-6**, wherein the configuration of the chiral carbon at position-2 is S-type) with the antibody TA001 according to the method of generic Step A.

**Example 147. ADC-19-1a**

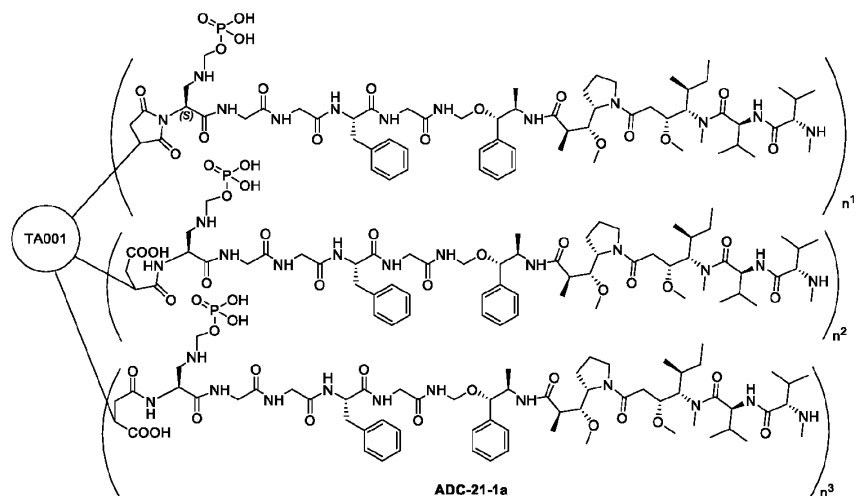
ADC-19-1a was prepared by reacting the corresponding linker-payload (**LP-8a**, whose structure is shown in the structural formula of **LP-8**, wherein the configuration of the chiral carbon at position-2 is S-type) with the antibody TA001 according to the method of generic Step A.

**Example 148. ADC-20-1a**

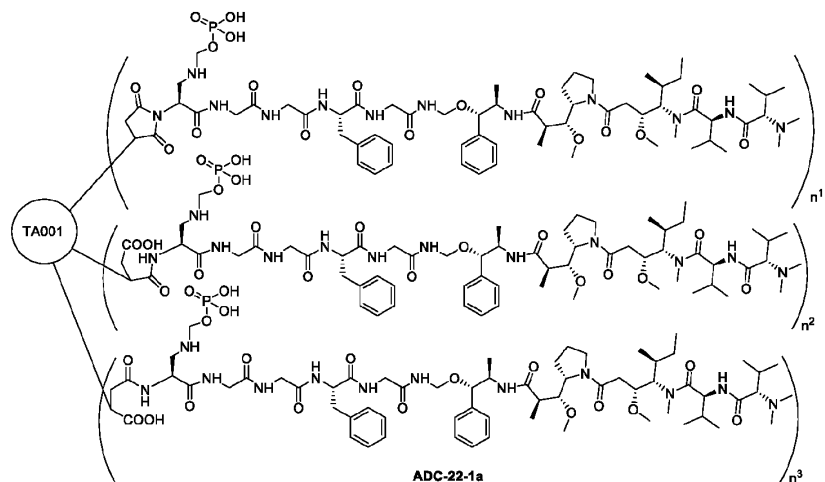
ADC-20-1a was prepared by combining the corresponding linker-payload (LP-9a, whose structure is shown in the structural formula of LP-9, wherein the configuration of the chiral carbon at position-2 is S-type) with the antibody TA001 according to the method of generic Step A.

**Example 149. ADC-21-1a**

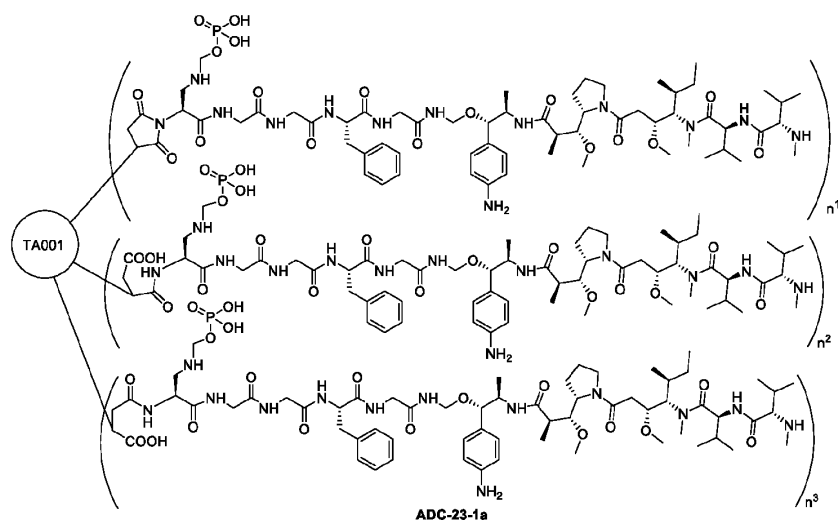
ADC-21-1a was prepared by combining the corresponding linker-payload (LP-28a, whose structure is shown in the structural formula of LP-28, wherein the configuration of the chiral carbon at position-2 is S-type) with the antibody TA001 according to the method of generic Step A.

**Example 150. ADC-22-1a**

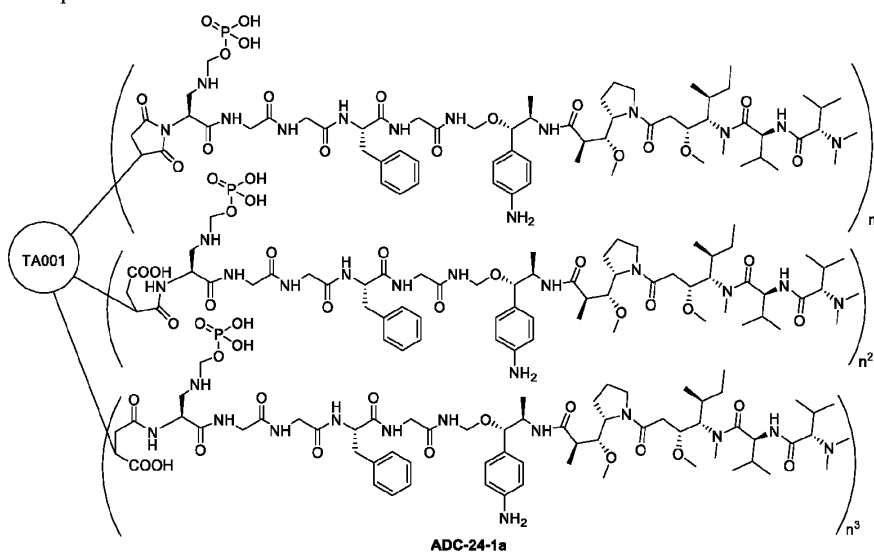
ADC-22-1a was prepared by combining the corresponding linker-payload (LP-29a, whose structure is shown in the structural formula of LP-29, wherein the configuration of the chiral carbon at position-2 is S-type) with the antibody TA001 according to the method of generic Step A.

**Example 151. ADC-23-1a**

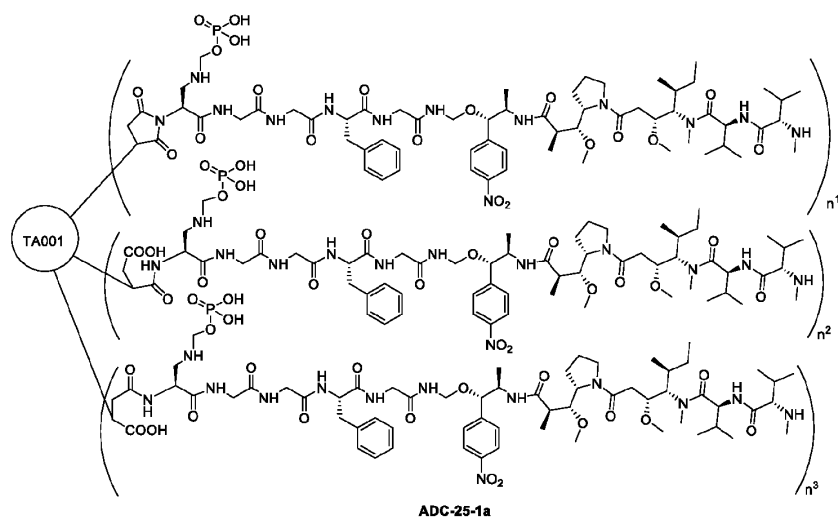
ADC-23-1a was prepared by combining the corresponding linker-payload (LP-30a, whose structure is shown in the structural formula of LP-30, wherein the configuration of the chiral carbon at position-2 is S-type) with the antibody TA001 according to the method of generic Step A.

**Example 152. ADC-24-1a**

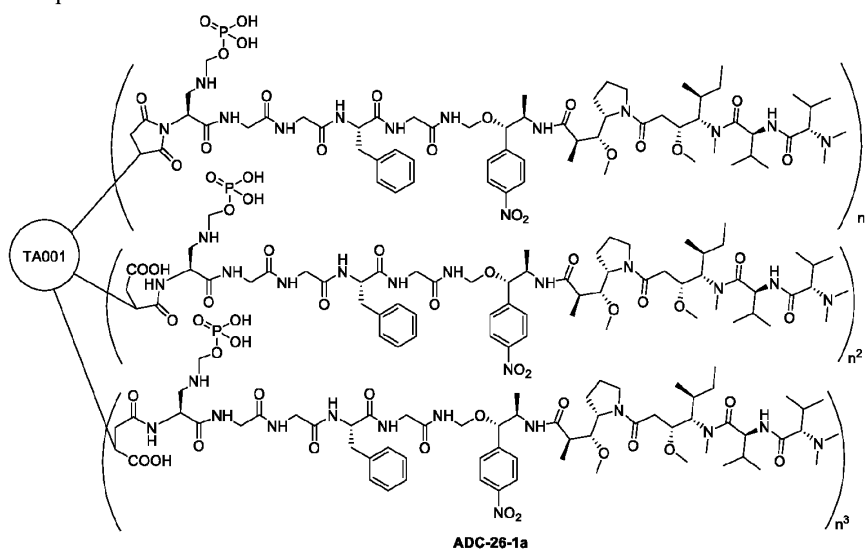
ADC-24-1a was prepared by combining the corresponding linker-payload (LP-31a, whose structure is shown in the structural formula of LP-31, wherein the configuration of the chiral carbon at position-2 is S-type) with the antibody TA001 according to the method of generic Step A.

**Example 153. ADC-25-1a**

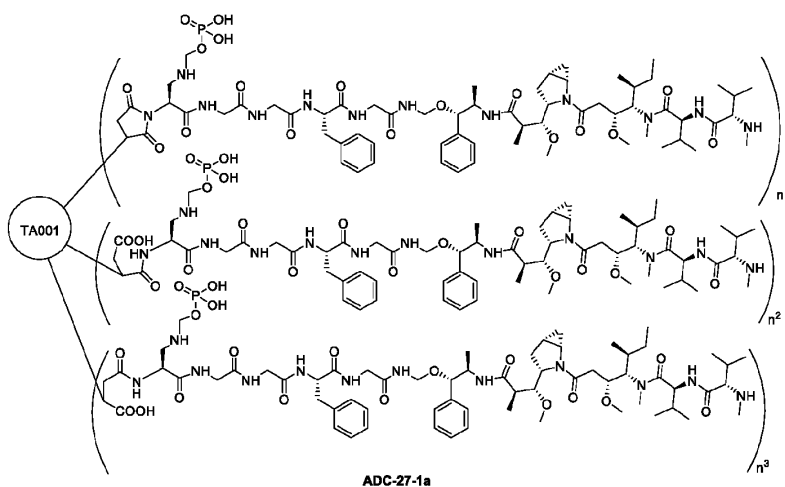
ADC-25-1a was prepared by combining the corresponding linker-payload (LP-32a, whose structure is shown in the structural formula of LP-32, wherein the configuration of the chiral carbon at position-2 is S-type) with the antibody TA001 according to the method of generic Step A.

**Example 154. ADC-26-1a**

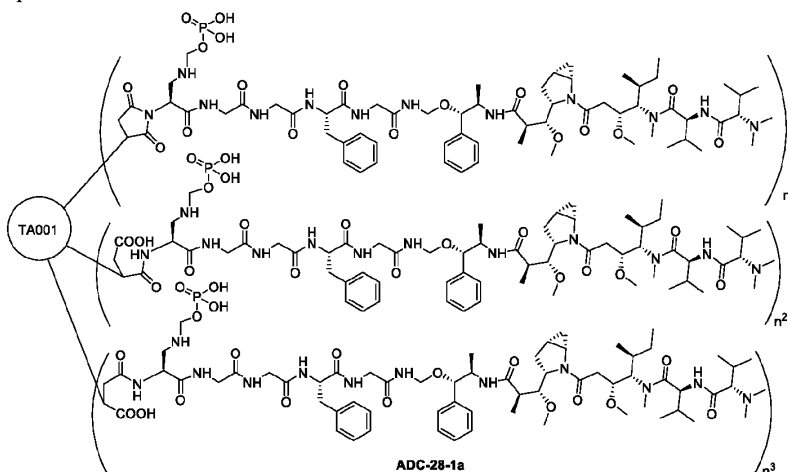
ADC-26-1a was prepared by combining the corresponding linker-payload (**LP-33a**, whose structure is shown in the structural formula of **LP-33**, wherein the configuration of the chiral carbon at position-2 is S-type) with the antibody TA001 according to the method of generic Step A.

**Example 155. ADC-27-1a**

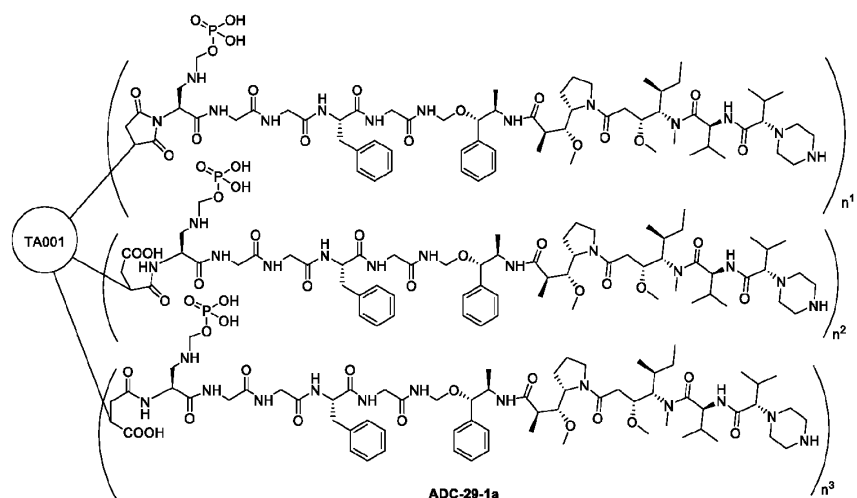
ADC-27-1a was prepared by reacting the corresponding linker-payload (**LP-35a**, whose structure is shown in the structural formula of **LP-35**, wherein the configuration of the chiral carbon at position-2 is S-type) with antibody TA001 according to the method of generic Step A.

**Example 156. ADC-28-1a**

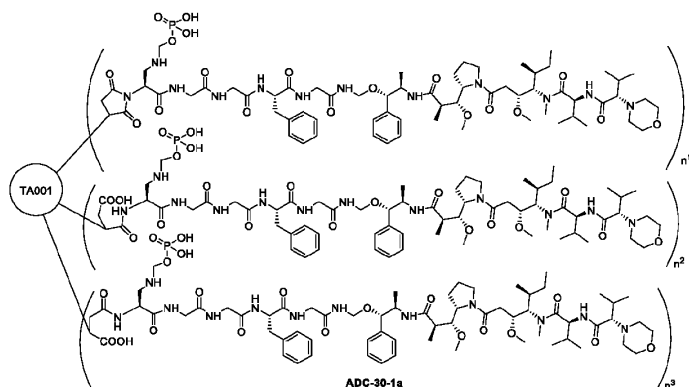
ADC-28-1a was prepared by combining the corresponding linker-payload (LP-36a, whose structure is shown in the structural formula of LP-36, wherein the configuration of the chiral carbon at position-2 is S-type) with the antibody TA001 according to the method of generic Step A.

**Example 157. ADC-29-1a**

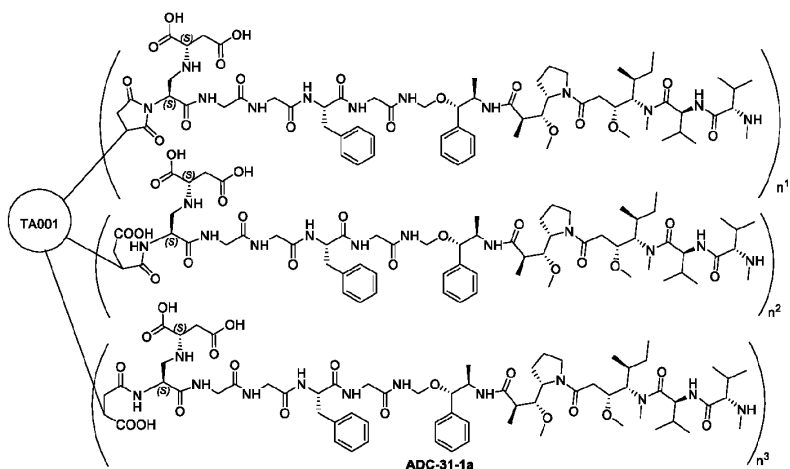
ADC-29-1a was prepared by combining the corresponding linker-payload (LP-39a, whose structure is shown in the structural formula of LP-39, wherein the configuration of the chiral carbon at position-2 is S-type) with the antibody TA001 according to the method of generic Step A.

**Example 158. ADC-30-1a**

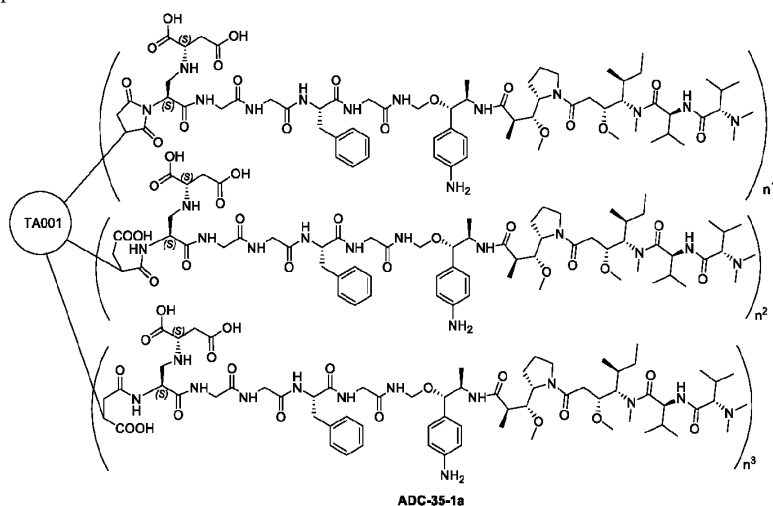
ADC-30-1a was prepared by combining the corresponding linker-payload (LP-34a, whose structure is shown in the structural formula of LP-34, wherein the configuration of the chiral carbon at position-2 is S-type) with the antibody TA001 according to the method of generic Step A.

**Example 159. ADC-31-1a**

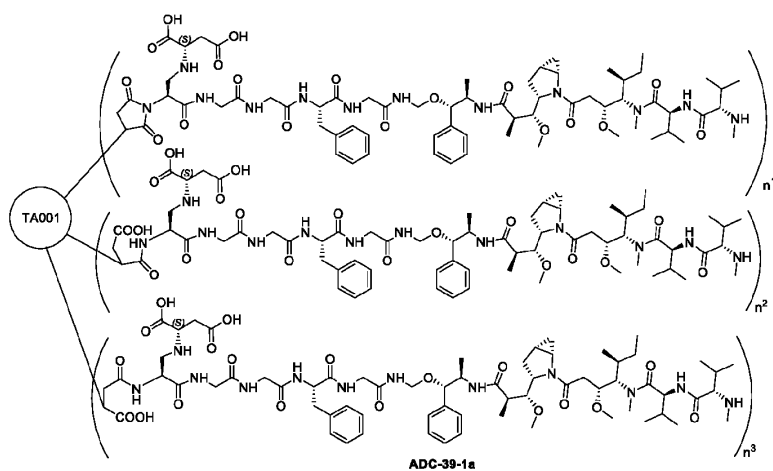
ADC-31-1a was prepared by combining the corresponding linker-payload (LP-19a, whose structure is shown in the structural formula of LP-19, wherein the configuration of the chiral carbon at position-2 is S-type) with the antibody TA001 according to the method of generic Step A.

**Example 160. ADC-35-1a**

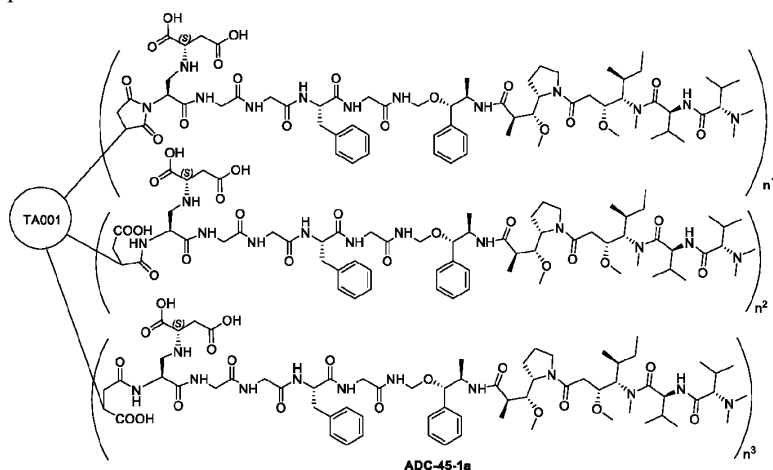
ADC-35-1a was prepared by combining the corresponding linker-payload (**LP-22a**, whose structure is shown in the structural formula of **LP-22**, wherein the configuration of the chiral carbon at position-2 is S-type) with the antibody TA001 according to the method of genericStep A.

**Example 161. ADC-39-1a**

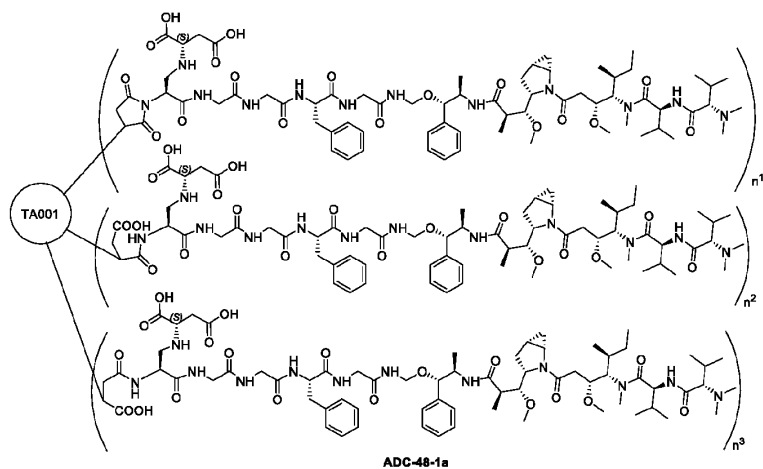
ADC-39-1a was prepared by combining the corresponding linker-payload (**LP-26a**, whose structure is shown in the structural formula of **LP-26**, wherein the configuration of the chiral carbon at position-2 is S-type) with the antibody TA001 according to the method of generic Step A.

**Example 162. ADC-45-1a**

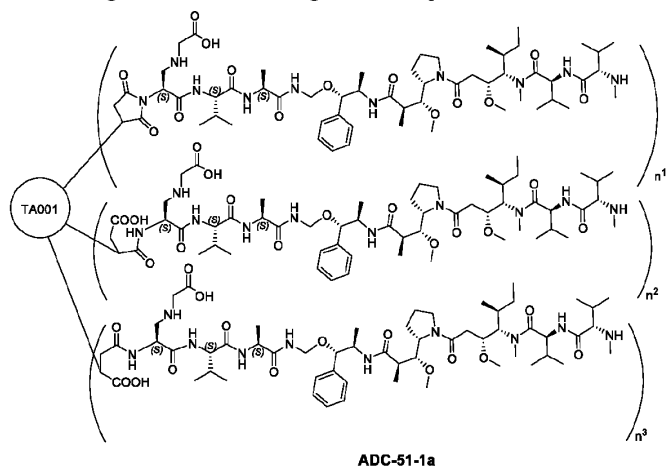
ADC-45-1a was prepared by combining the corresponding linker-payload (**LP-20a**, whose structure is shown in the structural formula of **LP-20**, wherein the configuration of the chiral carbon at position-2 is S-type) with the antibody TA001 according to the method of generic Step A.

**Example 163. ADC-48-1a**

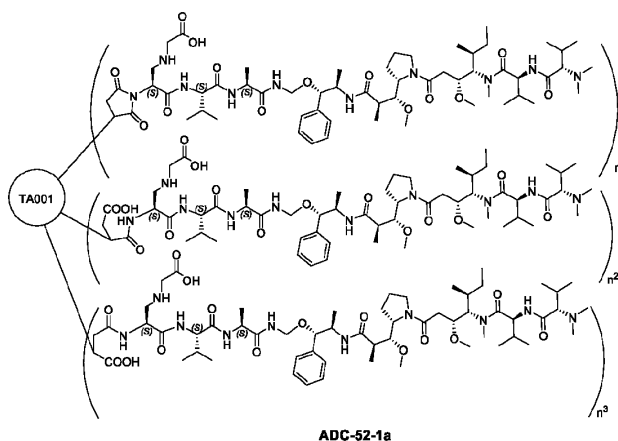
ADC-48-1a was prepared by combining the corresponding linker-payload (**LP-27a**, whose structure is shown in the structural formula of **LP-27**, wherein the configuration of the chiral carbon at position-2 is S-type) with the antibody TA001 according to the method of generic Step A.

**Example 164.** Preparation of ADC-51-1a

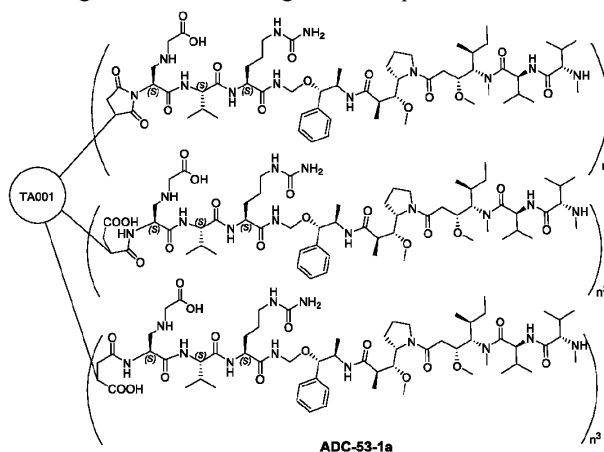
ADC-51-1a was prepared by combining the corresponding linker-payload (LP-51a) with antibody TA001 according to the method of generic Step A.

**Example 165.** Preparation of ADC-52-1a

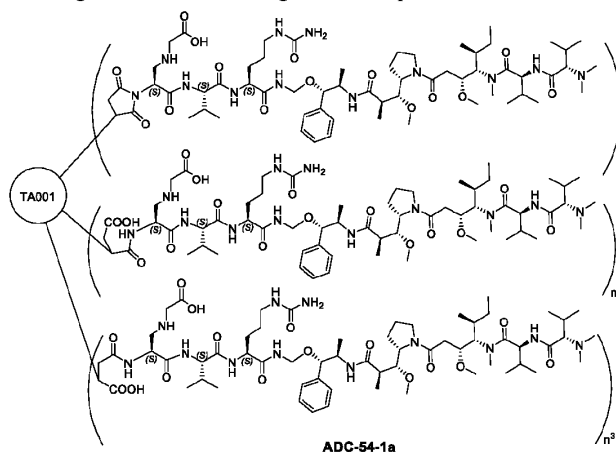
ADC-52-1a was prepared by combining the corresponding linker-payload (LP-52a) with antibody TA001 according to the method of generic Step A.

**Example 166.** Preparation of ADC-53-1a

ADC-53-1a was prepared by combining the corresponding linker-payload (**LP-53a**) with antibody TA001 according to the method of generic Step A.

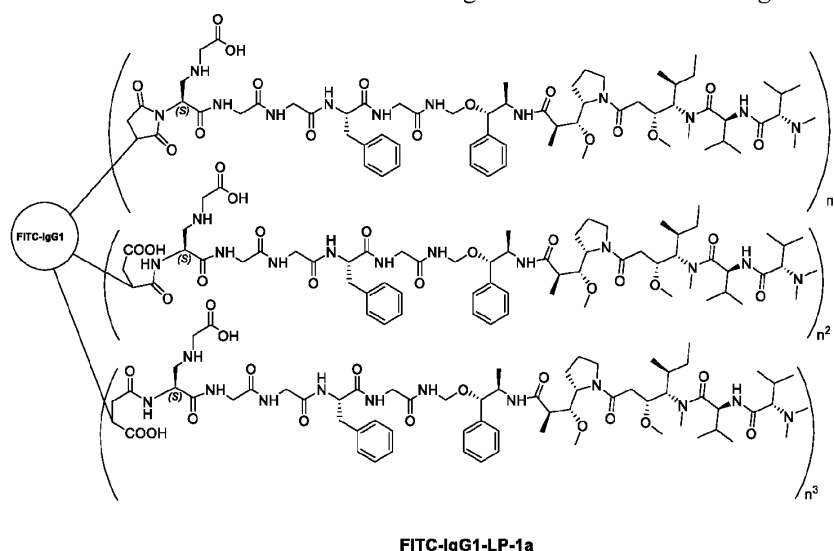
**Example 167.** Preparation of ADC-54-1a

ADC-54-1a was prepared by combining the corresponding linker-payload (**LP-54a**) with antibody TA001 according to the method of generic Step A.



Example 2. FITC-IgG1-LP-1a

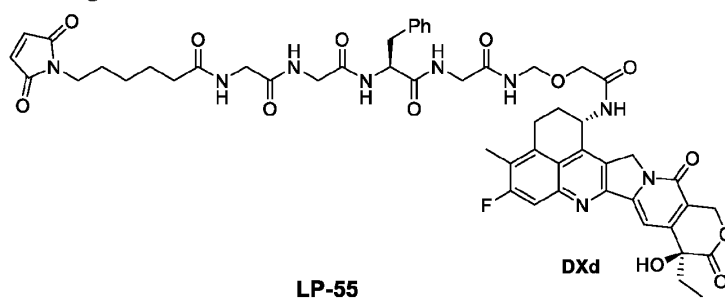
FITC-IgG1-LP-1a was prepared by combining the corresponding linker-payload (**LP-1**, wherein the configuration of the chiral carbon at position-2 is S-type) with an irrelevant antibody FITC-IgG1 (an IgG antibody of the same type that does not target any antigen) according to the method of generic Step A. The RP-HPLC detection results of FITC-IgG1-LP-1a are shown in **Figure 3C**.

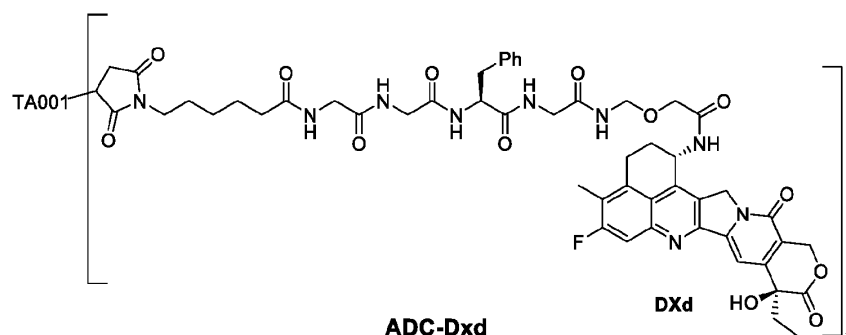


The amino acid sequences of the unrelated antibody FITC-IgG1 are listed as SEQ ID NO: 25 for the light chain; SEQ ID NO: 26 for the light chain variable region; SEQ ID NO: 27 for the heavy chain; and SEQ ID NO: 28 for the heavy chain variable region.

Control Example 3. Preparation of ADC-Dxd

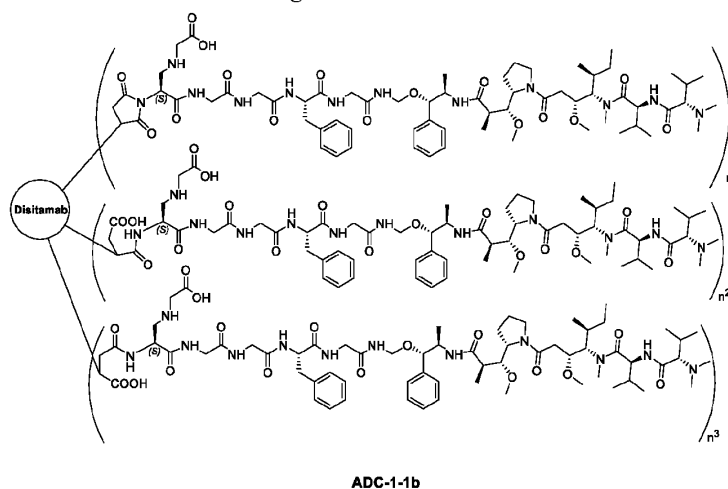
ADC-Dxd was prepared by combining the corresponding linker-payload (**LP-55**, synthesized according to the method described in Example 58 of CN104755494A) with antibody TA001 according to the method of generic Step A. The RP-HPLC test results of ADC-Dxd are shown in **Figure 3D**.





Control Example 4. Preparation of ADC-1-1b

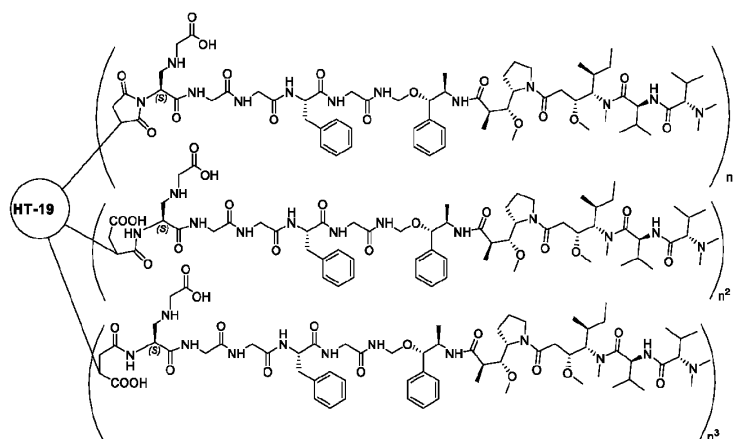
ADC-1-1b was prepared by combining the corresponding linker-payload (**LP-1a**, whose structure is shown in the structural formula of **LP-1**, wherein the configuration of the chiral carbon at position-2 is S-type) with the anti-HER2 antibody Disitamab according to the method of generic Step A. The RP-HPLC test results of **ADC-1-1b** are shown in **Figure 3E**, and the SEC-HPLC test results are shown in **Figure 4D**.



- The amino acid sequences of Disitamab are listed as SEQ ID NO: 29 for the light chain; SEQ ID NO: 30 for the light chain variable region; SEQ ID NO: 31 for the heavy chain; and SEQ ID NO: 32 for the heavy chain variable region.

Control Example 5. Preparation of ADC-1-1c

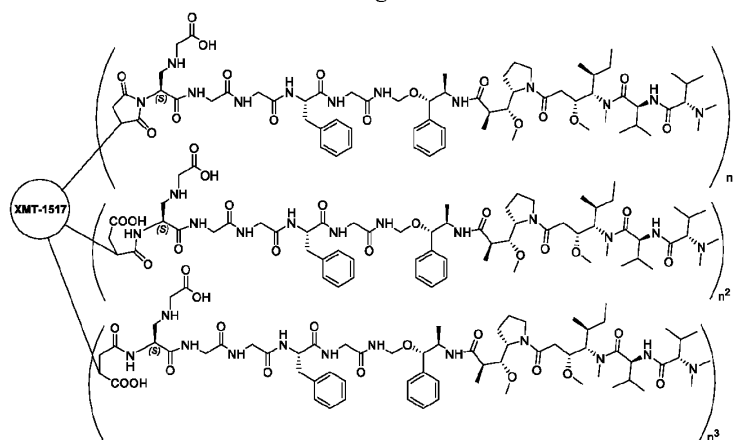
ADC-1-1c was prepared by combining the corresponding linker-payload (**LP-1a**, whose structure is shown in the structural formula of **LP-1**, wherein the configuration of the chiral carbon at position-2 is S-type) with the anti-HER2 antibody HT-19 according to the method of generic Step A. The RP-HPLC test results of **ADC-1-1c** are shown in **Figure 3F**, and the SEC-HPLC test results are shown in **Figure 4E**.

**ADC-1-1e**

The amino acid sequences of Disitamab are listed as SEQ ID NO: 33 for the light chain; SEQ ID NO: 34 for the light chain variable region; SEQ ID NO: 35 for the heavy chain; and SEQ ID NO: 36 for the heavy chain variable region.

Control Example 6. Preparation of ADC-1-1d

ADC-1-1d was prepared by combining 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 S-type) with the anti-HER2 antibody XMT-1517 according to the method of generic Step A. The RP-HPLC test results of **ADC-1-1d** are shown in **Figure 3G**, and the SEC-HPLC test results are shown in **Figure 4F**.

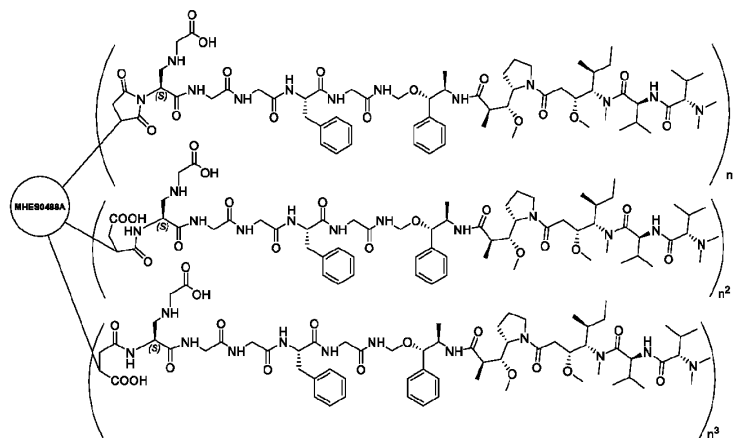
**ADC-1-1d**

The amino acid sequences of XMT-1517 are listed as SEQ ID NO: 37 for the light chain; SEQ ID NO: 38 for the light chain variable region; SEQ ID NO: 39 for the heavy chain; and SEQ ID NO: 40 for the heavy chain variable region.

Control Example 7. Preparation of ADC-1-1e

ADC-1-1e was prepared by combining 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 S-type) with the anti-HER2 antibody MHES0488A according to the

eric Step A. The RP-HPLC test results of **ADC-1-1e** are shown in **Figure 3H**, and the SEC-HPLC test results are shown in **Figure 4G**.



ADC-1-1e

The amino acid sequences of MHES0488A are listed as SEQ ID NO: 41 for the light chain; SEQ ID NO: 42 for the light chain variable region; SEQ ID NO: 43 for the heavy chain; and SEQ ID NO: 44 for the heavy chain variable region.

Example 168. Detection of DAR of ADC by RP-HPLC

As determined by RP-HPLC in generic Step B, the average drug/antibody ratio, DAR, of the corresponding ADCs is shown in **Table 1**. RC-48 was purchased from Remegen Biosciences (batch no.: RC48-X₁-202302003, the results of the RP-HPLC assay are shown in **Figure 3I**, SEC-HPLC assay are shown in **Figure 4C**). For **ADC-C-1a** and **ADC-Dxd**, its DAR = n, and for the other ADCs, DAR = n₁ + n₂ + n₃. As can be seen from **Table 1**, the ADCs disclosed in the present application have the excellent property of high DAR value and can significantly increase the drug concentration at the target site at the same dose of ADC drug.

Example 169. SEC Detection of ADC Monomer Rates

The percentage of aggregation of each ADC was assessed by generic Step C, and the results are shown in **Figure 4** and **Table 2**. Using the classic MC linker VcMMAE RC-48 has a DAR of 4.19, a monomer rate of more than 99%, and a relatively low percentage of aggregation and degradation. In contrast, **ADC-C-1a** that uses VcMMAE as its linker-payload had a DAR of 7.63, a monomer rate of 17.59%, but showed a high percentage of aggregation, indicating that ADCs using the classic MC linker VcMMAE may be prone to aggregation when made into high DAR.

The percentage of aggregation of ADCs of coupling different HER2 antibodies with LP-1a may be different (**Table 2**). When coupling TA001 with LP-1a, the monomer rate of ADC-1-1a was high, indicating that antibody TA001 contributed to the stability of ADC as compared to other antibodies. The ADCs with antibody TA001 also displayed reduced levels of aggregation and degradation.

ADC-1-1b, which was made of an acidic stable linker on the hydroxyl group of the toxin, had a DAR of 7.48, which was nearly twice of the DAR of RC-48. However, its monomer rate was above 99% which was like that of RC-48, and the percentages of aggregation and degradation degree were low. When compared with **ADC-C-1a**, **ADC-1-1a** had a similar DAR

by a much higher monomer rate than **ADC-C-1a**. This indicates that the high-DAR ADC obtained by coupling an acidic stable linker on the hydroxyl group of the toxin possesses the characteristics of low levels of degradation and aggregation while having the excellent property of high monomer rate.

Example 170. *In vitro* plasma stability of ADC

The plasma stability study of ADC was carried out according to generic Step D. As shown in **Table 3**, ADCs of coupling acidic stable linkers on the hydroxyl groups of toxins have less loss of drug during plasma incubation. Specifically, when antibody TA001 was used as the antibody, ADC-1-1a was the most stable, while ADC-C-1a with the classic MC-linked showed a significant DAR reduction after 7 days of incubation. Thus, the ADC drug having an acidic stable linker into the hydroxyl group of the toxin shows good plasma stability and no major changes in the DAR value.

Example 171. Anti-tumor cell activity of ADC

To evaluate the *in vitro* tumor cell killing activity of ADC, human tumor cell lines of different cancer types (**Table 4**) and mixed tumor cells (JIMT-1 + MDA-MB-468) were used as experimental models. A certain number of tumor cells were seeded in a 96-well plate through a gradient of dilutions of the test antibody or ADC drug. After ADC treatment for 5 days, the cell viability was assessed by using CellTiter-Glo® Luminescent Cell Viability Assay, and the killing effect of the test antibody and ADC drug on the tumor cell line was evaluated by calculating the half-maximal inhibitory concentration (IC₅₀).

The starting dose of antibody drug or ADC drug was 100 nM, the dilution multiple was 10 times, a total of 8 detection concentration points, and the treatment lasted for 5 days. Cell survival rate = (experimental group-blank)/(control group-blank group) × 100%.

JIMT-1 is a breast cancer cell line expressing moderate level of HER2, whereas MDA-MB-468 is a HER2-negative triple-negative breast cancer cell line (**Table 4**). The *in vitro* mixed tumor model of JIMT-1 + MDA-MB-468 (1:2.5) cells was set up as follows: take the test cells in the logarithmic growth phase, discard the culture medium and rinse the cells with sterilized 1×PBS, and add an appropriate amount of 0.25% Trypsin-EDTA to digest the cells according to the size of the culture flask; seed JIMT-1 cells into a 96-well plate at a density of 2000 cells/well, and MDA-MB-468 cells into a 96-well plate at a density of 5000 cells/well with the seeding ratio of the two lines of cells per well at 1:2.5, add 300 μL of sterilized 1×PBS or sterilized ddH₂O to the edge of the 96-well plate without adding cell suspension to seal the edge, and place it in a CO₂ incubator for overnight culture.

With the counts of viable cells after ADC treatment for 5 days, the percentages of cell viability were plotted as the survival curves shown in **Figures 5A, 5B, 5C, 5D, 5E, 5F, and 5G**, and the IC₅₀ values were calculated and listed in **Table 5**. The *in vitro* data showed that in the cancer cell models of high-, medium-, and low-HER2 expression (**Table 4**), ADC-1-1a with antibody TA001 consistently exerted higher tumor cell killing activity than that ADCs coupling other anti-HER2 antibodies with LP-1a. In the mixed tumor cell model (JIMT-1 + MDA-MB-468), ADC-1-1a led to higher tumor cell killing activity than the anti-HER2 antibodies (i.e., Disitamab, HT-19, XMT1517, and MHES0488A) of other ADCs (i.e., ADC-1-1b, ADC-1-1c, ADC-1-1d, and ADC-1-1e, respectively) when coupled with LP-1a. It remains possible that the treatment by ADC-1-1a induced more bystander effects.

In addition, the tumor cell killing activity of ADC-1-1a is better than that of ADC-Dxd,

is that, compared with the ADC with Dxd as a toxin, the ADC with Auristatins as a toxin has an advantage in *in vitro* anti-tumor activity.

Example 172. *In vivo* efficacy of anti-HER2-ADCs in JIMT-1-derived tumor in mice

To evaluate the *in vivo* efficacy of anti-HER2-ADC, a xenograft tumor model was established by transplanting JIMT-1 breast cancer cells into *BALB/c-mu* mice. JIMT-1 (5×10^6 /mouse) cell suspension (0.1 mL/mouse) was subcutaneously injected into the right scapula of 6~7-week-old *BALB/c-mu* mice. The mice were randomly divided into 13 groups, including vehicle control group (Vehicle), six treatment groups for ADC-1-1a, ADC-1-1b, ADC-1-1c, ADC-1-1d, ADC-1-1e, and ADC-Dxd with each treatment group divided by two dose groups (0.25 mg/kg, 0.5 mg/kg) and with 6 mice per dose group. When the average tumor volume of each mice grew to about 166 mm³, drug administration began (D0). Mice of each dose group were administered with the same type of ADC through a tail vein injection of 10 mL/kg body weight, with a dosing cycle of 7 days (QW), and 4 consecutive cycles of administration. All dose groups were observed 28 days (D28) for evaluating tumor growth inhibition by ADC.

The average of tumor volumes was recorded through the cycle and calculated on D28 (Table 6 and 7). In JIMT-1-derived single tumor model, ADC-1-1a treatment from both 0.25mg/kg and 5mg/kg groups led to smaller average tumor volume than the treatment by ADCs of anti-HER2 antibodies other than antibody TA001 coupled with LP-1a in a pairwise comparison of tumor growth and inhibition curves (Figure 6).

In addition, ADC-1-1a exerted stronger tumor inhibition effect than ADC-Dxd, indicating that Auristatins are more efficacious toxins than Dxd.

Example 173. *In vivo* efficacy of anti-HER2-ADC in mixed tumors (JIMT-1 + MDA-MB-468)

JIMT-1 is a breast cancer cell line expressing moderate level of HER2, whereas MDA-MB-468 is a HER2-negative triple-negative breast cancer cell line (Table 4). The *in vivo* mixed tumor model of JIMT-1 + MDA-MB-468 was established in a subcutaneous mixed transplant tumor model in *BALB/c-mu* mice. A mixture of JIMT-1 (1×10^6) and MDA-MB-468 (2.5×10^6) cells (0.1 mL/mouse) was subcutaneously injected into the right scapula of 6~7-week-old *BALB/c-mu* mice. The mice were randomly divided into 13 groups, including vehicle control group (Vehicle), six treatment groups for ADC-1-1a, ADC-1-1b, ADC-1-1c, ADC-1-1d, ADC-1-1e, and ADC-Dxd with each treatment group divided by two dose groups (0.25 mg/kg, 0.5 mg/kg) and with 6 mice per dose group. When the average tumor volume of each mice grew to about 172 mm³, drug administration began (D0). Mice of each dose group were administered with the same type of ADC through a tail vein injection of 10 mL/kg body weight, with a dosing cycle of 7 days (QW), and 4 consecutive cycles of administration. All dose groups were observed 28 days (D28) for evaluating tumor growth inhibition by ADC.

The average of tumor volumes was recorded through the cycle and calculated on D28 (Table 7). In the (JIMT-1 + MDA-MB-468)-derived mixed tumor model, ADC-1-1a treated mice in both 0.25mg/kg and 5mg/kg groups showed smaller average tumor volume than the mice treated by ADCs of LP-1a coupled with anti-HER2 antibodies other than antibody TA001 in a pairwise comparison of tumor growth and inhibition curves (Figure 7). This shows that antibody TA001 disclosed in the present application made the differences when compared with ADCs with other anti-HER2 antibodies for treating mixed tumors.

In addition, the tumor inhibition effect of ADC-1-1a is also stronger than that of ADC-

icates that the ADC with Auristatins as toxins has advantages in the treatment of mixed tumors compared with the ADC with Dxd as toxins.

Example 174. Anti-Tumor Cell Activity of ADC

To evaluate ADC drug candidates for their activity of killing tumor cells *in vitro*, JIMT-1+MDA-MB-468 and other human tumor cell lines of different cancer types (1:1) were used to assemble the experimental cell models of heterogeneous tumor cells (cell lines are shown in **Table 8**). A certain number of tumor cells were seeded in a 96-well plate, and gradient dilutions of ADC and controls were added to the cells for 5-day treatment. Cell viability was monitored using CellTiter-Glo® Luminescent Cell Viability Assay, and the killing effect of ADC on the tumor cell line was evaluated by calculating the half inhibitory concentration (IC₅₀).

The starting dose of ADC was 100 nM, the dilution multiple was 10-fold with a total of 8 detection concentration points. Cell survival rate = (experimental group-blank)/(control group-blank group)×100%.

The JIMT-1+MDA-MB-468 (1:1) *in vitro* heterogeneous tumor cell model was constructed as follows: take the test cells in the logarithmic growth phase, discard the culture medium and rinse the cells with sterilized 1×PBS, and add an appropriate amount of 0.25% Trpsin-EDTA to digest the cells according to the size of the culture flask. After the cells are digested, resuspended and counted, the HER2-moderately expressed breast cancer cell line JIMT-1 is first seeded into a 96-well plate at a density of 3500 cells/well, and then the HER2-negative triple-negative breast cancer cell line MDA-MB-468 is seeded into a 96-well plate at a density of 3500 cells/well. The seeding ratio of the two cells per well is 1:1. Finally, add 300μL of sterilized 1×PBS or sterilized H₂O to the edge of the 96-well plate without adding cell suspension to seal the edge, and place it in a CO₂ incubator for overnight culture.

The corresponding *in vitro* data are shown in **Table 9**, as well as **Figures 8A, 8B, 8C, 8D, 8E, 8F, 8G, and 8H**. The results showed that in HER2 high-expression, medium-expression, and low-expression cell models, **ADC-1-1a** showed stronger tumor cell killing activity than the commercial drug, RC-48. In addition, in the heterogeneous tumor cell model of JIMT-1+MDA-MB-468, **ADC-1-1a** showed a stronger bystander effect and stronger killing activity than RC-48.

Example 175. *In Vivo* Efficacy of ADC-1-1a to Single Tumor (JIMT-1)

BALB/c-Nude subcutaneous xenograft of a human-derived tumor cell line (JIMT-1) was utilized as an experimental model to evaluate the *in vivo* efficacy of ADC-1-1a. A certain number of tumor cell suspensions were subcutaneously injected into *BALB/c-Nude*. When the tumor volume grew to about 170 mm³, the tail vein was injected with a lysate (Vehicle), a naked antibody control (TA001), an unrelated antibody ADC control (FITC-IgG1-LP-1a), a payload control (Auristatin E), a positive control (RC-48), and a positive control (RC-48). RC-48 and the corresponding ADC-1-1a were administered once a week for four times for continuous observation, and the tumor volume and body weight of the mice were measured twice a week to evaluate the inhibitory effect of injected ADC on tumor growth. The results, as shown in **Table 10, 11, and Figure 9**, showed that, in HER2-expressing JIMT-1 tumors, 1 mg/kg of ADC-1-1a exhibited stronger tumor suppression than 1 mg/kg of naked antibody control (TA001), 1 mg/kg of unrelated antibody ADC control (FITC-IgG1-LP-1a), 0.039 mg/kg of payload control (Auristatin E) at equimolar doses of payload, 0.25 mg/kg, 0.5 mg/kg; 1 mg/kg of ADC-1-1a exhibited stronger tumor suppression than 0.5 mg/kg, 1 mg/kg, and 2 mg/kg of the positive

8); and mice were well tolerated by ADC-1-1a. This suggests that ADC-1-1a possesses significant *in vivo* antitumor activity and safety.

Example 176. *In vivo* Efficacy of ADC-1-1a to mixed tumors (JIMT-1 + MDA-MB-468)

BALB/c-Nude subcutaneous xenograft of mixed human tumor cell lines, such as JIMT-1 + MDA-MB-468, was utilized as an experimental model to evaluate the *in vivo* efficacy of ADC-1-1a. A certain number of mixed suspensions of tumor cells were seeded subcutaneously in *BALB/c-Nude* mice. When the tumor volume grew to about 160 mm³, the tail vein was injected with lysate (Vehicle), naked anti-control (TA001), unrelated antibody ADC control (FITC-IgG1-LP-1a), payload control (Auristatin E), and positive control (RC-48) and the corresponding ADC drug (ADC-1-1a) were administered four times once a week for continuous observation. Tumor volume and body weight of the mice were measured twice every week to evaluate the inhibitory effect of the ADC drug candidate on tumor growth. The results are shown in **Table 12, 13** and **Figure 10**. In the mixed tumors derived from the mixture of HER2-positive JIMT-1 cells and HER2-negative MDA-MB-468 cells, 1 mg/kg of ADC-1-1a showed stronger tumor inhibition than 1 mg/kg of naked anti-control (TA001), 1 mg/kg of irrelevant antibody ADC control (FITC-IgG1-LP-1a), and 0.039 mg/kg of payload control (Auristatin E); at the equivalent molar dose of payload, 0.25 mg/kg, 0.5 mg/kg and 1 mg/kg of ADC-1-1a showed stronger tumor inhibition than 0.5 mg/kg, 1 mg/kg and 2 mg/kg of positive control (RC-48); and mice tolerated ADC-1-1a well. This shows that ADC-1-1a has significant *in vivo* anti-tumor activity and safety.

Example 177. *In vivo* Efficacy of ADC-1-1a in single form of tumor derived from NCI-N87

BALB/c-Nude subcutaneous xenograft model of a human tumor cell line (NCI-N87) was established as an experimental model to evaluate the *in vivo* efficacy of ADC-1-1a. A certain number of tumor cell suspensions were subcutaneously injected in *BALB/c-Nude*. When the tumor volume grew to about 180 mm³, the tail vein was injected with a lysate (Vehicle), a naked antibody control (TA001), an unrelated antibody ADC control (FITC-IgG1-LP-1a), a payload control (Auristatin E), a positive control (RC-48), and a positive control (RC-48). (RC-48) and the ADC drug candidate (e.g., ADC-1-1a) were administered once a week for four times for continuous observation, and the tumor volume and body weight of the mice were measured twice a week to evaluate the inhibitory effect of the ADC drug tested for tumor growth. The results are shown in **Table 14, 15** and **Figure 11**. In HER2-expressing NCI-N87 cell-derived tumors, 1 mg/kg ADC-1-1a showed a stronger tumor inhibition effect than 1 mg/kg naked anti-control (TA001), 1 mg/kg irrelevant antibody ADC control (FITC-IgG1-LP-1a), and 0.039 mg/kg payload control (Auristatin E); at equimolar doses of payload, 0.25 mg/kg, 0.5 mg/kg and 1 mg/kg ADC-1-1a showed a stronger tumor inhibition effect than 0.5 mg/kg, 1 mg/kg and 2 mg/kg positive control (RC-48); and mice tolerated ADC-1-1a well. These data established that ADC-1-1a has significant *in vivo* anti-tumor activity and safety.

Example 178. *In Vivo* Efficacy of ADC-1-1a in NCI-H1975 cell-derived tumors

BALB/c-Nude subcutaneous xenograft of a human tumor cell line (NCI-H1975) was utilized as an experimental model in this invention to evaluate the *in vivo* efficacy of ADC-1-1a. A certain number of tumor cell suspensions were inoculated subcutaneously in *BALB/c-Nude*. When the tumor volume grew to about 180 mm³, the tail vein was injected with a lysate (Vehicle), a naked antibody control (TA001), an unrelated antibody ADC control (FITC-IgG1-LP-1a), a payload control (Auristatin E), a positive control (RC-48), and a positive control

h were used to evaluate the efficacy of ADC-1-1a *in vivo*. RC-48 and the corresponding ADC drug (ADC-1-1a) were administered once a week for four times for continuous observation, and the tumor volume and body weight of the mice were measured twice a week to evaluate the inhibitory effect of the tested ADC drugs on tumor growth.

The results are shown in **Table 16, 17** and **Figure 12**. In HER2-expressing NCI-H1975-derived tumors, 1 mg/kg ADC-1-1a showed stronger anti-tumor effects when compared to 1 mg/kg naked antibody control substance (TA001), 1 mg /kg irrelevant antibody ADC reference substance (FITC-IgG1-LP-1a) and 0.039 mg/kg payload reference substance (Auristatin E); at equimolar doses of payload, 0.25 mg/kg, ADC-1-1a at 0.5 mg/kg and 1 mg/kg showed stronger anti-tumor effects than the positive control (RC-48) at 0.5 mg/kg, 1 mg/kg and 2 mg/kg; and mice have a stronger anti-tumor effect on ADC -1-1a is well tolerated. This indicates that ADC-1-1a has significant anti-tumor activity and safety *in vivo*.

It can be understood that the above specific description of the present disclosure is only used to illustrate the present application and is not limited to the technical solutions described in the embodiments of the present application. Those skilled in the art should understand that the present application can still be modified or replaced by equivalents to achieve the same technical effects; the above modifications or equivalent replacements are all within the protection scope of the present application.

ANTIBODY-AURISTATIN DRUG CONJUGATES AND METHODS OF MAKING AND USING THEREOF

TABLES

Table 1. The effect of linker-payload and antibody on the value of DAR

Antibody	Linker-Payload	ADC	DAR
TA001	LP-1a	ADC-1-1a	7.52
	LP-1a	FITC-IgG1-LP-1a	7.04
	LP-2a	ADC-2-1a	7.49
	VcMMAE	ADC-C-1a	7.63
	LP-10	ADC-3-1a	7.18
	LP-11	ADC-6-1a	7.23
	LP-17	ADC-11-1a	7.22
	LP-18	ADC-12-1a	7.30
	LP-8a	ADC-19-1a	7.32
	LP-9a	ADC-20-1a	7.39
	LP-28a	ADC-21-1a	7.29
	LP-29a	ADC-22-1a	7.32
	LP-35a	ADC-27-1a	7.45
	LP-36a	ADC-28-1a	7.47
	LP-21a	ADC-31-1a	7.38
	LP-22a	ADC-35-1a	7.39
	LP-26a	ADC-39-1a	7.38
	LP-20a	ADC-45-1a	7.42
	LP-27a	ADC-48-1a	7.37
	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
	LP-55	ADC-Dxd	7.16
Disitamab	VcMMAE	RC-48	4.19
	LP-1a	ADC-1-1b	7.48
HT-19	LP-1a	ADC-1-1c	7.66
XMT-1517	LP-1a	ADC-1-1d	7.69
MHES0488A	LP-1a	ADC-1-1e	7.62

Table 2. The effect of the acidic connector on stability of ADC

ADC	Antibody	DAR	Aggregates (100%)	Monomer rate (100%)	Degradation (100%)
ADC-1-1a	TA001	7.52	0.36	99.05	0.59
ADC-C-1a	TA001	7.63	81.24	17.59	1.17
RC-48	Disitamab	4.19	0.15	99.85	0.00
ADC-1-1b	Disitamab	7.48	0.81	98.54	0.65
ADC-1-1c	HT-19	7.66	1.71	97.53	0.76
ADC-1-1d	XMT-1517	7.69	5.67	93.82	0.51
ADC-1-1e	MHES0488A	7.62	1.47	97.86	0.67

Table 3. In vitro plasma stability of ADC

ADC	Antibody	Linker-payload	Incubate 0 day to extract	Incubate 3 days to extract	Incubate 7 days to extract
ADC-1-1a	TA001	LP-1a	7.54	7.50	7.44
ADC-2-1a	TA001	LP-2a	7.51	7.41	7.40
ADC-C-1a	TA001	VcMMAE	7.23	6.69	6.39
ADC-1-1b	Disitamab	LP-1a	7.58	7.52	7.46
ADC-1-1c	HT-19	LP-1a	7.70	7.62	7.52
ADC-1-1d	XMT-1517	LP-1a	7.72	7.70	7.55
ADC-1-1e	MHES0488A	LP-1a	7.59	7.56	7.46

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.

Table 4. Human cancer cell lines

Cell line	Cancer of origin	Level of HER2 expression	Source
NCI-N87	Gastric carcinoma of human stomach	High	Shanghai Cell Bank
SK-OV-3	Adenocarcinoma of human ovary	High	Shanghai Cell Bank
Calu-3	Human non-small-cell lung cancer	Intermediate to High	Shanghai Tongpai
JIMT-1	Human breast cancer	Positive	Shanghai Tongpai
A431	Epidermoid carcinoma of human skin	Low	Pricella
MDA-MB-468	Triple-negative human breast cancer	Negative	Shanghai Tongpai

Table 5. IC50 (nM) of ADC mediated cytotoxicity

Cell Line	NCI-N87	SK-OV-3	Calu-3	JIMT-1	A431	MDA-MB-468	JIMT-1 + MDA-MB-468
Antibody, drug, ADC	Cell model of single tumor						Cell model of mixed tumors
TA001	>100	>100	>100	>100	>100	>100	>100
ADC-1-1a	0.02	0.1	0.08	0.02	17.51	86.48	7.86
ADC-Dxd	0.44	>100	0.32	>100	>100	26.43	91.49
Auristatin E	0.06	0.13	0.83	0.05	0.03	0.07	0.05
Dxd	1	31.75	2.43	113.3	3.53	0.87	6
Disitamab	>100	>100	>100	>100	—	>100	>100
ADC-1-1b	0.03	0.16	0.1	0.04	—	>100	9.11
HT-19	>100	>100	>100	>100	>100	>100	>100
ADC-1-1c	0.03	0.41	0.42	0.99	25.09	101.55	17.93
XMT-1517	>100	>100	>100	>100	>100	>100	>100
ADC-1-1d	0.04	1.47	0.87	23.85	61.49	103.34	55.27
MHES0488A	>100	>100	>100	>100	>100	>100	>100
ADC-1-1e	0.02	0.13	0.16	0.08	19.8	87.75	13.97

Table 6. Average tumor volume in each group

Group	Average tumor volume (mm ³)								
	D0	D3	D7	D11	D14	D18	D21	D25	D28
Vehicle	165. 72	328.9 7	368.7 5	515.4 9	532.7 1	577.2 3	725.9 6	812.5 0	959.86
ADC-1-1a (0.25mg/kg)	166. 16	261.5 3	311.6 9	341.5 5	325.7 7	317.0 9	374.2 6	358.7 1	338.29
ADC-1-1a (0.5mg/kg)	166. 58	258.5 9	255.1 4	240.7 7	179.1 5	119.2 9	98.62	52.42	36.53
ADC-1-1b (0.25mg/kg)	166. 02	280.2 9	351.2 4	426.3 7	443.4 8	447.8 5	481.9 9	445.3 3	496.47
ADC-1-1b (0.5mg/kg)	165. 36	290.6 4	313.7 9	287.0 5	227.8 1	161.5 8	121.5 6	71.07	57.06
ADC-1-1c (0.25mg/kg)	167. 77	263.6 2	312.1 7	349.3 7	390.4 8	425.7 6	474.3 9	453.8 6	459.80
ADC-1-1c (0.5mg/kg)	164. 68	298.2 2	333.3 8	364.7 5	266.3 1	199.4 4	178.3 4	114.1 0	86.49
ADC-1-1d (0.25mg/kg)	167. 08	280.8 5	363.2 8	450.4 3	440.2 9	472.0 9	498.2 5	491.9 3	600.14
ADC-1-1d (0.5mg/kg)	165. 92	267.7 7	325.6 0	397.7 3	363.4 0	380.7 8	362.1 3	332.5 2	316.09
ADC-1-1e (0.25mg/kg)	166. 39	277.7 3	337.6 2	370.4 2	342.4 0	331.8 9	340.0 3	312.1 3	312.34
ADC-1-1e (0.5mg/kg)	164. 49	260.3 2	315.8 1	308.2 0	262.6 0	223.4 3	160.3 1	104.5 3	99.13
ADC-Dxd (0.25mg/kg)	167. 49	340.1 1	401.7 5	428.9 4	499.5 9	535.8 1	625.5 3	763.6 9	856.29
ADC-Dxd (0.5mg/kg)	165. 96	337.1 2	488.2 8	614.0 8	620.6 3	690.0 6	883.8 0	932.1 2	1041.4 7

Table 6. Average tumor volume in each group

Group	Average tumor volume (mm ³)								
	D0	D4	D7	D10	D14	D17	D21	D24	D28
Vehicle	175.21	248.90	390.60	452.21	581.21	662.58	758.88	814.06	1049.56
ADC-1-1a (0.25mg/kg)	171.11	257.97	321.89	335.03	367.44	379.28	326.05	282.33	256.05
ADC-1-1a (0.5mg/kg)	173.63	213.46	252.54	269.39	175.16	147.97	93.35	72.77	60.01
ADC-1-1b (0.25mg/kg)	170.92	221.80	305.58	361.70	348.82	389.33	379.73	381.00	311.02
ADC-1-1b (0.5mg/kg)	172.20	200.51	233.91	257.81	224.01	175.91	156.47	110.88	83.75
ADC-1-1c (0.25mg/kg)	174.41	237.33	313.45	377.63	412.17	414.80	414.22	404.33	422.13
ADC-1-1c (0.5mg/kg)	173.74	202.09	244.33	266.61	230.63	162.96	140.02	121.46	104.26
ADC-1-1d (0.25mg/kg)	173.08	263.56	311.24	384.37	406.44	412.27	480.58	449.30	474.65
ADC-1-1d (0.5mg/kg)	173.82	231.85	271.14	311.92	322.38	291.00	285.89	250.87	259.97
ADC-1-1e (0.25mg/kg)	171.73	267.28	285.94	364.61	266.41	348.09	351.23	310.38	299.67
ADC-1-1e (0.5mg/kg)	174.24	222.59	232.34	259.16	220.43	219.18	169.49	128.40	116.60
ADC-Dxd (0.25mg/kg)	170.95	259.30	334.26	385.15	457.89	529.19	566.52	626.42	700.24
ADC-Dxd (0.5mg/kg)	172.88	229.73	364.42	443.99	527.51	555.62	650.28	686.09	791.80

Table 8. Human cancer cell lines

Cell line	Cancer of origin	Level of HER2 expression	Source
NCI-N87	Gastric carcinoma of human stomach	High	Shanghai Cell Bank
SK-OV-3	Adenocarcinoma of human ovary	High	Shanghai Cell Bank
Calu-3	Human non-small-cell lung cancer	Intermediate to High	Shanghai Tongpai
JIMT-1	Human breast cancer	Positive	Shanghai Tongpai
NCI-H1975	Human non-small-cell lung cancer	Low	ATCC
A431	Epidermoid carcinoma of human skin	Low	Pricella
MDA-MB-468	Triple-negative human breast cancer	Negative	Shanghai Tongpai

Table 9. IC₅₀ (nM) of ADCs

Cancer cell line	Cell Model	TA001	ADC-1-1a	RC-48	FITC-IgG1	FITC-IgG1-LP-1a
NCI-N87	Single tumor	> 100	0.02	0.08	> 100	86.83
SK-OV-3		> 100	0.10	3.18	> 100	> 100
Calu-3		> 100	0.07	0.24	> 100	> 100
JIMT-1		> 100	0.22	> 100	> 100	> 100
NCI-H1975		> 100	27.68	52.31	> 100	29.76
A431		> 100	19.79	49.18	> 100	72.03
MDA-MB-468		> 100	27.96	81.77	> 100	31.58
JIMT-1 + MDA-MB-468 (1:1)	Mixed breast cancer	> 100	1.42	20.92	> 100	> 100

Table 10. Tumor growth in the xenograft model of human JIMT-1 cells in *BALB/c-Nude* mice

ADC or parts (dosage)	Mean tumor volume (mm ³)								
	D0	D4	D7	D11	D14	D18	D21	D25	D28
Vehicle	170.51	308.18	395.94	512.03	611.45	737.96	833.94	1020.15	1191.96
TA001 (1mg/kg)	169.07	262.89	351.59	420.24	500.40	625.02	747.09	929.02	1045.52
FITC-IgG1- LP-1a (1mg/kg)	169.51	299.11	403.92	498.71	568.33	679.53	829.11	933.58	970.27
Auristatin E (0.039mg/kg)	169.70	278.91	393.33	503.84	602.47	715.34	814.71	922.74	1080.01
RC-48 (0.5mg/kg)	169.44	285.93	375.20	440.92	529.56	616.65	690.75	775.61	843.14
RC-48 (1mg/kg)	168.56	254.30	282.93	342.31	425.27	479.29	520.40	563.43	595.87
RC-48 (2mg/kg)	169.21	225.40	242.72	259.41	323.35	355.89	371.32	370.96	418.45
ADC-1-1a (0.25 mg/kg)	168.46	250.20	296.14	327.53	380.08	372.76	382.74	379.99	392.53
ADC-1-1a (0.5 mg/kg)	166.96	216.19	213.41	147.33	120.74	85.21	58.52	37.56	32.24
ADC-1-1a (1mg/kg)	169.55	225.27	197.17	106.31	64.31	29.03	19.39	6.04	3.79

Table 11. Changes in body weight of *BALB/c-Nude* mice

ADC dose-escalation	Average body weight of mice (g)								
	D0	D4	D7	D11	D14	D18	D21	D25	D28
Vehicle	22.05	22.67	23.01	23.36	23.61	23.91	24.60	25.29	25.49
TA001 (1mg/kg)	20.91	21.67	22.35	22.84	23.34	23.65	23.98	24.91	25.02
FITC- IgG1-LP-1a (1mg/kg)	22.71	23.24	23.49	24.13	24.35	25.42	25.79	26.46	26.84
Auristatin E (0.039mg/kg)	22.52	23.27	23.67	23.97	24.45	25.35	25.58	26.08	26.33
RC-48 (0.5mg/kg)	23.10	23.51	23.55	23.83	24.38	24.77	25.53	25.87	25.92
RC-48 (1mg/kg)	20.29	21.38	21.95	22.29	22.60	23.06	23.94	24.43	24.64
RC-48 (2mg/kg)	21.98	22.43	22.94	23.15	23.50	23.93	23.95	24.23	24.61
ADC-1-1a (0.25 mg/kg)	20.63	21.33	21.64	22.24	22.63	22.54	23.48	23.58	23.05
ADC-1-1a (0.5 mg/kg)	22.38	23.02	23.16	23.16	23.82	24.24	24.88	25.23	25.14
ADC-1-1a (1mg/kg)	21.58	22.49	22.27	22.86	23.00	23.74	24.37	24.71	24.46

Table 12. Tumor growth in the xenograft model of mixed human cancer cells,
JIMT-1 + MDA-MB-468, in *BALB/c-Nude* mice

ADC dose-escalation	Mean tumor volume (mm ³)								
	D0	D4	D7	D11	D14	D18	D21	D25	D28
Vehicle	163.31	245.62	323.80	475.57	583.46	688.02	739.60	863.79	940.83
TA001 (1mg/kg)	160.04	226.20	290.88	385.75	432.65	522.48	602.56	713.43	702.35
FITC-IgG1-LP-1 (1mg/kg)	165.04	239.50	316.49	427.54	470.82	548.11	609.41	668.68	625.63
Auristatin E (0.039mg/kg)	162.77	243.99	319.25	426.61	459.63	592.66	657.86	751.11	827.54
RC-48 (0.5mg/kg)	162.08	240.67	289.35	389.83	452.63	549.55	571.84	726.30	830.61
RC-48 (1mg/kg)	160.50	211.47	263.34	349.93	411.45	457.96	509.56	567.71	626.94
RC-48 (2mg/kg)	159.36	218.36	245.20	290.89	299.57	336.40	326.06	334.69	323.66
ADC-1-1a (0.25 mg/kg)	163.01	232.18	263.24	318.37	314.29	325.70	300.44	275.61	272.20
ADC-1-1a (0.5 mg/kg)	160.71	203.18	218.40	240.70	209.92	164.94	125.08	114.98	105.67
ADC-1-1a (1mg/kg)	161.10	201.06	206.97	178.17	122.04	58.04	35.77	16.81	11.19

Table 13. Changes in body weight of *BALB/c-Nude* mice

ADC dose escalation	Average body weight of mice (g)								
	D0	D4	D7	D11	D14	D18	D21	D25	D28
Vehicle	21.92	22.34	22.82	23.67	23.94	24.53	24.93	25.17	25.44
TA001 (1mg/kg)	20.93	20.86	21.16	21.74	21.88	22.37	22.64	22.66	22.79
FITC-IgG1-LP-1a (1mg/kg)	22.16	22.05	22.57	23.11	23.90	23.90	24.27	24.80	24.87
Auristatin E (0.039mg/kg)	22.59	23.03	23.55	23.98	24.36	24.08	24.87	25.84	25.23
RC-48 (0.5mg/kg)	22.20	22.40	22.94	23.20	23.64	23.77	24.39	25.15	25.04
RC-48 (1mg/kg)	21.50	21.41	21.65	22.68	22.99	22.87	23.33	23.62	23.91
RC-48 (2mg/kg)	22.33	22.72	22.98	23.41	23.27	23.33	23.97	24.77	24.60
ADC-1-1a (0.25 mg/kg)	21.09	21.28	21.40	22.01	22.60	22.12	22.52	23.29	23.20
ADC-1-1a (0.5 mg/kg)	21.76	21.87	22.09	22.81	23.07	22.91	23.38	23.91	23.79
ADC-1-1a (1mg/kg)	21.30	21.44	21.99	22.23	22.53	22.43	22.74	23.27	23.54

Table 14. Tumor growth in the xenograft model of human NCI-N87 cells in *BALB/c-Nude* mice

ADC dose-escalation	Mean tumor volume (mm ³)								
	D0	D4	D7	D11	D14	D18	D21	D25	D28
Vehicle	178.44	305.86	446.38	632.97	814.42	953.51	1282.90	1254.71	1504.86
TA001 (1mg/kg)	180.51	274.08	385.38	539.95	707.68	931.55	1162.69	1323.24	1742.58
FITC- IgG1-LP-1a (1mg/kg)	178.82	309.64	444.48	606.08	719.33	875.64	936.01	919.83	1115.46
Auristatin E (0.039mg/kg)	178.67	279.83	423.84	531.86	720.56	933.02	949.94	1189.56	1407.85
RC-48 (0.5mg/kg)	178.03	291.27	447.85	540.51	704.27	766.51	939.50	1034.78	1197.28
RC-48 (1mg/kg)	179.45	228.65	334.34	330.91	393.75	441.03	430.54	423.93	438.27
RC-48 (2mg/kg)	178.71	215.17	234.02	246.66	262.66	188.90	189.30	183.21	163.03
ADC-1-1a (0.25 mg/kg)	179.23	227.72	323.97	296.66	285.62	261.81	222.55	207.63	189.54
ADC-1-1a (0.5 mg/kg)	180.08	224.11	246.78	161.95	161.66	140.39	140.82	105.73	76.24
ADC-1-1a (1mg/kg)	176.94	195.74	234.50	142.41	112.90	122.16	123.58	110.40	73.15

Table 15. Changes in body weight of *BALB/c-Nude* mice

ADC dose-escalation	Average body weight of mice (g)								
	D0	D4	D7	D11	D14	D18	D21	D25	D28
Vehicle	21.56	22.33	22.23	22.32	22.68	23.43	23.85	24.48	24.47
TA001 (1mg/kg)	21.73	23.06	23.00	23.70	24.00	24.58	24.84	25.54	25.45
FITC- IgG1-LP-1a (1mg/kg)	22.78	22.91	23.61	24.03	24.27	24.92	25.00	26.16	25.58
Auristatin E (0.039mg/kg)	22.59	23.40	23.43	23.56	24.05	24.90	25.12	25.76	25.77
RC-48 (0.5mg/kg)	22.39	23.56	23.45	23.71	24.09	24.78	25.36	25.76	25.40
RC-48 (1mg/kg)	22.51	23.62	23.35	23.43	24.06	24.88	24.97	25.51	25.23
RC-48 (2mg/kg)	22.45	23.30	23.48	23.58	24.21	24.85	24.97	25.46	25.56
ADC-1-1a (0.25 mg/kg)	22.20	23.10	22.65	23.07	23.25	24.03	24.32	25.01	23.81
ADC-1-1a (0.5 mg/kg)	21.32	22.86	22.77	23.39	23.04	23.56	23.78	24.42	24.47
ADC-1-1a (1mg/kg)	22.31	23.20	23.35	23.54	23.56	24.26	24.70	24.93	25.01

Table 16. Tumor growth in the xenograft model of human NCI-H1975 cells in *BALB/c-Nude* mice

ADC dose- escalation	Mean tumor volume (mm ³)								
	D0	D4	D7	D11	D14	D18	D21	D25	D28
Vehicle	185.25	430.55	927.01	1575.49	2548.40	3237.64	3748.82	-	-
TA001 (1mg/kg)	183.50	378.96	897.38	1402.59	2235.08	2464.19	3281.38	3340.45	-
FITC- IgG1-LP-1a (1mg/kg)	182.87	433.25	930.44	1338.53	1702.10	1905.93	2048.41	1748.48	1812.88
Auristatin E (0.039mg/kg)	184.46	419.59	966.38	1705.10	2977.08	3388.75	4265.69	-	-
RC-48 (0.5mg/kg)	182.69	357.32	770.91	1411.08	2316.22	3236.15	4186.43	-	-
RC-48 (1mg/kg)	184.26	337.07	653.58	964.69	1285.60	1517.80	1895.17	1409.44	1338.06
RC-48 (2mg/kg)	184.17	331.57	503.20	630.03	763.95	805.70	721.09	599.11	361.17
ADC-1-1a (0.25 mg/kg)	184.18	382.15	560.40	574.60	409.15	291.92	169.47	110.12	57.36
ADC-1-1a (0.5 mg/kg)	183.91	335.96	422.33	350.87	183.11	107.94	54.24	34.86	35.38
ADC-1-1a (1mg/kg)	182.52	217.98	310.25	231.26	111.76	63.03	35.91	23.53	15.49

Note: "-" means animal died, no data available

Table 17. Changes in body weight of *BALB/c-Nude* mice

form	Average body weight of mice (g)								
	D0	D4	D7	D11	D14	D18	D21	D25	D28
Vehicle	21.52	21.82	23.12	23.64	24.90	25.70	28.32	-	-
TA001 (1mg/kg)	23.00	23.10	24.27	24.84	25.55	25.87	27.28	29.45	-
FITC- IgG1-LP-1a (1mg/kg)	22.68	22.78	23.68	23.84	24.31	23.89	24.08	24.35	24.44
Auristatin E (0.039mg/kg)	23.09	23.37	24.76	25.24	26.57	27.19	27.90	-	-
RC-48 (0.5mg/kg)	22.15	22.08	22.95	23.61	24.68	25.35	25.97	-	-
RC-48 (1mg/kg)	21.68	21.83	22.41	22.66	23.02	23.38	24.11	23.56	23.87
RC-48 (2mg/kg)	21.66	21.45	22.03	22.35	23.01	23.37	23.45	23.46	23.57
ADC-1-1a (0.25 mg/kg)	22.12	21.55	22.17	22.54	22.92	22.71	23.09	23.29	23.53
ADC-1-1a (0.5 mg/kg)	22.69	22.57	23.26	23.55	24.15	24.36	24.77	24.52	24.63
ADC-1-1a (1mg/kg)	22.47	22.52	22.93	23.27	23.71	23.57	24.40	24.28	24.06

Note: "-" means animal died, no data available

**ANTIBODY-AURISTATIN DRUG CONJUGATES AND METHODS OF MAKING AND
USING THEREOF****SEQUENCE LISTING**

>SEQ ID NO.1: TA001 CDR-L1
RASQDVNTAVA

>SEQ ID NO.2: TA001 CDR-L2
SASFLYS

>SEQ ID NO.3: TA001 CDR-L3
QQHYTTPPT

>SEQ ID NO.4: TA001 CDR-H1
DTYIH

>SEQ ID NO.5: TA001 CDR-H2
RIYPTNGYTRYADSVKG

>SEQ ID NO.6: TA001 CDR-H3
WGGDGFYAMDY

>SEQ ID NO.7: TA001 light chain (LC) variable region (VL) amino
acid sequence
DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSG
SRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIK

>SEQ ID NO.8: TA001 LC constant region amino acid sequence
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS
TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

>SEQ ID NO.9: TA001 LC amino acid sequence
DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSG
SRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG
TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC

>SEQ ID NO.10: TA001 heavy chain (HC) variable region (VH) amino
acid sequence
EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIIYPTNGYTRYADSV
KGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSS

>SEQ ID NO.11: TA001 HC constant region amino acid sequence
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS
LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP
KPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL
HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMEALHNHYT
QKSLSLSPG

>SEQ ID NO.12: TA001 HC amino acid sequence
EVQLVESGGGLVQPGGSLRLSCAASGFINIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSV
KGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI
SRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSP
G

>SEQ ID NO.13: TA001 CDR-L1 nucleic acid sequence
CGTGCCAGTCAGGATGTGAATACTGCTGTAGCC

>SEQ ID NO.14: TA001 CDR-L2 nucleic acid sequence
TCGGCATCCTTCCTCTACTCT

>SEQ ID NO.15: TA001 CDR-L3 nucleic acid sequence
CAGCAACATTATACTACTCCTCCACG

>SEQ ID NO.16: TA001 CDR-H1 nucleic acid sequence
GACACCTATATACAC

>SEQ ID NO.17: TA001 CDR-H2 nucleic acid sequence
AGGATTTATCCTACGAATGGTTATACTAGATATGCCGATAGCGTCAAGGGC

>SEQ ID NO.18: TA001 CDR-H3 nucleic acid sequence
TGGGGAGGGGACGGCTTCTATGCTATGGACTAC

>SEQ ID NO.19: TA001 VL nucleic acid sequence
GATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGGTCACCATCA
CCTGCCGTGCCAGTCAGGATGTGAATACTGCTGTAGCCTGGTATCAACAGAAACCAGGAAAAGC
TCCGAAACTACTGATTTACTCGGCATCCTTCCTCTACTCTGGAGTCCCTTCTCGCTTCTCTGGC
TCCAGATCTGGGACGGATTTCACTCTGACCATCAGCAGTCTGCAGCCGGAAGACTTCGCAACTT
ATTACTGTCAGCAACATTATACTACTCCTCCACGTTTCGGACAGGGTACCAAGGTGGAGATCAA

A

>SEQ ID NO.20: TA001 LC constant region nucleic acid sequence
CGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAA
CTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGT
GGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACACAGAGCAGGACAGCAAGGACAGC
ACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACG
CCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTG
TTAG

>SEQ ID NO.21: TA001 LC nucleic acid sequence
GATATCCAGATGACCCAGTCCCCGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGGTCACCATCA
CCTGCCGTGCCAGTCAGGATGTGAATACTGCTGTAGCCTGGTATCAACAGAAACCAGGAAAAGC
TCCGAAACTACTGATTTACTCGGCATCCTTCCCTCTACTCTGGAGTCCCTTCTCGCTTCTCTGGC
TCCAGATCTGGGACGGATTTCACTCTGACCATCAGCAGTCTGCAGCCGGAAGACTTCGCAACTT
ATTACTGTCAGCAACATTATACTACTCCTCCACGTTCCGACAGGGTACCAAGGTGGAGATCAA
ACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGA
ACTGCCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGG
TGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACACAGAGCAGGACAGCAAGGACAG
CACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTAC
GCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGT
GTTAG

>SEQ ID NO.22: TA001 VH nucleic acid sequence
GAGGTTTCAGCTGGTGGAGTCTGGCGGTGGCCTGGTGCAGCCAGGGGGCTCACTCCGTTTGTCTT
GTGCAGCTTCTGGCTTCAACATTAAAGACACCTATATACACTGGGTGCGTCAGGCCCCGGGTAA
GGGCTTGGGAATGGGTTGCAAGGATTTATCCTACGAATGGTTATACTAGATATGCCGATAGCGTC
AAGGGCCGTTTCACTATAAGCGCAGACACATCCAAAACACAGCCTACCTGCAGATGAACAGCC
TGCGTGCTGAGGACACTGCCGTCTATTATTGTTCTAGATGGGGAGGGGACGGCTTCTATGCTAT
GGACTACTGGGGTCAAGGAACCTGGTCACCGTCTCCTCG

>SEQ ID NO.23: TA001 HC constant region nucleic acid sequence
GCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCTCCTCCAAGAGCACCTCTGGGGGCA
CAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTC
AGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGTGTCTACAGTCCTCAGGACTCTACTCC
CTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGA
ATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCA
CACATGCCACCGTGCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCA
AAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGA
GCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAA
GACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCTCTG
CACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCC
CCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCTGCC
CCCATCCCGGGAGGAGATGACCAAGAACCAGGTGACCTGCCTGGTCAAAGGCTTCTAT
CCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGC
CTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTATAGCAAGCTCACCGTGGACAAGAGCAG
GTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACG

CAGAAGAGCCTCTCCCTGTCTCCGGGTTGA

>SEQ ID NO.24: TA001 HC nucleic acid sequence

GAGGTTTCAGCTGGTGGAGTCTGGCGGTGGCCTGGTGCAGCCAGGGGGCTCACTCCGTTTGTCTCT
GTGCAGCTTCTGGCTTCAACATTAAAGACACCTATATACACTGGGTGCGTCAGGCCCGGGTAA
GGGCCTGGAATGGGTTGCAAGGATTTATCCTACGAATGGTTATACTAGATATGCCGATAGCGTC
AAGGGCCGTTTCACTATAAGCGCAGACACATCCAAAAACACAGCCTACCTGCAGATGAACAGCC
TGCCTGCTGAGGACACTGCCGTCTATTATTGTTCTAGATGGGGAGGGGACGGCTTCTATGCTAT
GGACTACTGGGGTCAAGGAACCCCTGGTCACCGTCTCCTCGGCTAGCACCAAGGGCCCATCGGTC
TTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCA
AGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCA
CACCTTCTCCGGGTCTCTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCC
TCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGG
TGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCACCGTGCCACGACC
TGAACCTCTGGGGGACCGTCAGTCTTCTCTTCCCCCAAACCCAAGGACACCCCTCATGATC
TCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGT
TCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTA
CAACAGCACGTACCGTGTGGTCAGCGTCTCACCCTCCTGCACCAGGACTGGCTGAATGGCAAG
GAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAAG
CCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAA
GAACCAGGTGACCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGG
GAGAGCAATGGGCAGCCGGAGAACAACATAAGACCACGCCTCCCGTGCTGGACTCCGACGGCT
CCTTCTTCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTC
ATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCG
GGTGA

>SEQ ID NO.25: FITC-IgG1 LC amino acid sequence

DVVMQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLRWYLQKPGQSPKVLIIYKVSNRFSQV
DRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPWTFGGGTKLEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE
KHKVYACEVTHQGLSSPVTKSFNRGEC

>SEQ ID NO.26: FITC-IgG1 LC VL amino acid sequence

DVVMQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLRWYLQKPGQSPKVLIIYKVSNRFSQV
DRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPWTFGGGTKLEIK

>SEQ ID NO.27: FITC-IgG1 HC amino acid sequence

EVKLDDETGGGLVQGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYYEYSD
SVKGRFTISRDDSKSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSTVTVSSASTKGPSVFP
LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS
SLGTQTYICNVNHKPSNTKVDKRVPEPKSCDKHTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
KCAVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCVMHEALHNHYTQKSLSLSPG

>SEQ ID NO.28: FITC-IgG1 HC VH amino acid sequence

EVKLDDETGGGLVQGRPMKLSCVASGFTFSDYWMNWVRQSPEKGLEWVAQIRNKPYNYYEYSD

SVKGRFTISRDDSKSSVYLQMNLRVEDMGIYCTGSYYGMDYWGQGTSVTVSS

>SEQ ID NO.29: Disitamab LC amino acid sequence

DIQMTQSPSSVSASVGDRVITITCKASQDVGTAVAWYQQKPGKAPKLLIYWASIRHTGVPSRFSG
SGSGTDFTLTISSSLQPEDFATYYCHQFATYTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYAC
EVTHQGLSSPVTKSFNRGEC

>SEQ ID NO.30: Disitamab LC VL amino acid sequence

DIQMTQSPSSVSASVGDRVITITCKASQDVGTAVAWYQQKPGKAPKLLIYWASIRHTGVPSRFSG
SGSGTDFTLTISSSLQPEDFATYYCHQFATYTFGGGTKEIK

>SEQ ID NO.31: Disitamab HC amino acid sequence

EVQLVQSGAEVKKPGATVKISCKVSGYTFTDYYIHWVQQAPGKGLEWMGRVNPDHGDSYYNQKF
KDKATITADKSTDTAYMELSSLRSEDTAVYFCARNYLFDPHWGQGTTLVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG
TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE
VTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK
VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPG

>SEQ ID NO.32: Disitamab HC VH Amino acid sequence

EVQLVQSGAEVKKPGATVKISCKVSGYTFTDYYIHWVQQAPGKGLEWMGRVNPDHGDSYYNQKF
KDKATITADKSTDTAYMELSSLRSEDTAVYFCARNYLFDPHWGQGTTLVTVSS

>SEQ ID NO.33: HT-19 LC amino acid sequence

EIVLTQSPGTLSSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFS
GSGSGTDFTLTISRLEPEDFAVYYCQQYHHSPLTFGGGTKEIKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKV
YACEVTHQGLSSPVTKSFNRGEC

>SEQ ID NO.34: HT-19 LC VL amino acid sequence

EIVLTQSPGTLSSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFS
GSGSGTDFTLTISRLEPEDFAVYYCQQYHHSPLTFGGGTKEIK

>SEQ ID NO.35: HT-19 HC amino acid sequence

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSYISSSSSSTIYYADSV
KGRFTISRDNANKNSLYLQMNLSRAEDTAVYYCARGGHGYFDLWGRGTLVTVSSASTKGPSVFPL
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSL
LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPG

>SEQ ID NO.36: HT-19 HC VH Amino acid sequence

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSYISSSSSSTIYYADSV
KGRFTISRDNANKNSLYLQMNLSRAEDTAVYYCARGGHGYFDLWGRGTLVTVSS

>SEQ ID NO.37: XMT-1517 LC amino acid sequence
EIVLTQSPGTLSSLSPGERATLSCRASQSVSSDYLAWYQQKPGQAPRLLIYGASSRATGIPDRFS
GSGSGTDFTLTISRLEPEDFAVYYCQQYVSYWTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSG
TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC

>SEQ ID NO.38: XMT-1517 LC VL amino acid sequence
EIVLTQSPGTLSSLSPGERATLSCRASQSVSSDYLAWYQQKPGQAPRLLIYGASSRATGIPDRFS
GSGSGTDFTLTISRLEPEDFAVYYCQQYVSYWTFGGGTKVEIK

>SEQ ID NO.39: XMT-1517 HC amino acid sequence
QVQLVESGGGVVQPGRSRLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWIYDGSNKYYADSV
KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKEAPYYAKDYMDVWGKGTTVTVSSASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPETVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSP
G

>SEQ ID NO.40: XMT-1517 HC VH amino acid sequence
QVQLVESGGGVVQPGRSRLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVIWIYDGSNKYYADSV
KGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKEAPYYAKDYMDVWGKGTTVTVSS

>SEQ ID NO.41: MHES0488A LC amino acid sequence
DIVMTQSPDSLAVSLGERATINCRASQSVSGSRFTYMHWYQQKPGQPPKLLIKYASILESGVPD
RFSGSGSGTDFTLTITSSSLQAEDVAVYYCQHSWEIPWTFGQGTKVEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYE
KHKVYACEVTHQGLSSPVTKSFNRGEC

>SEQ ID NO.42: MHES0488A LC VL amino acid sequence
DIVMTQSPDSLAVSLGERATINCRASQSVSGSRFTYMHWYQQKPGQPPKLLIKYASILESGVPD
RFSGSGSGTDFTLTITSSSLQAEDVAVYYCQHSWEIPWTFGQGTKVEIK

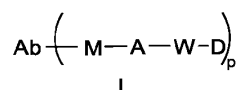
>SEQ ID NO.43: MHES0488A HC amino acid sequence
EVQLVQSGAEVKKPGASVKVSCKASGYSFTGYWMNWVRQAPGQGLEWIGMIHPLDAEIRANQKF
RDRVTITVDTSTSTAYLELSSLRSEDTAVYYCARGTYDGGFEYWGQGLTVTVSSASTKGPSVFP
LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSS
SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR
TPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPG

>SEQ ID NO.44: MHES0488A HC VH amino acid sequence
EVQLVQSGAEVKKPGASVKVSCKASGYSFTGYWMNWVRQAPGQGLEWIGMIHPLDAEIRANQKF
RDRVTITVDTSTSTAYLELSSLRSEDTAVYYCARGTYDGGFEYWGQGLTVTVSS

ANTIBODY-AURISTATIN DRUG CONJUGATES AND METHODS OF MAKING AND USING THEREOF

CLAIMS

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



wherein,

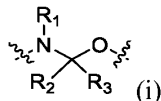
Ab is an antibody or its antigen-binding fragment having a binding affinity to HER2;

wherein said antibody comprises a light chain and a heavy chain, said light chain comprising CDR-L1, CDR-L2 and CDR-L3 each having an amino acid sequence as shown in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, respectively; said heavy chain comprising CDR-H1, CDR-H2, CDR-H3, each having an amino acid sequence as shown in SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, respectively;

M is a linker connected to Ab;

A is a peptide residue comprising 2-7 amino acids, wherein, optionally, said amino acids are each independently substituted with one or more substituents, said substituents being selected from: deuterium, halogen, hydroxyl, a cyano group, an amino group, a nitro group, alkyl, substituting alkyl, alkoxy, cycloalkyl, and substituted cycloalkyl;

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



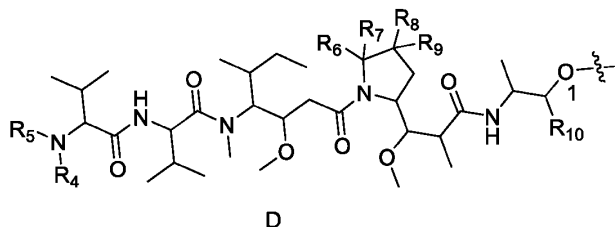
wherein,

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 the D in Formula (i), where the oxygen atom is the common group of D and W;

R₁, R₂ and R₃ are each hydrogen, deuterium, alkyl, or substituted alkyl;

p is an integer or decimal from 1-20; and

D is an auristatin having the structure shown in Formula D, its isomeric, endo-, racemic, enantiomeric, or mixtures thereof, or pharmaceutically acceptable salts thereof.



wherein,

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

R₆, R₇, R₈, R₉ are each independently hydrogen, deuterium, a halogen, an azido group, alkyl, or 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 selected from hydrogen, a halogen, an azido group, alkyl, and NR₁₈R₁₉, wherein R₁₈, R₁₉ are independently hydrogen or alkyl;

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

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 said oxygen atom is shared by D and W.

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

- a light chain variable region having the amino acid sequence SEQ ID NO: 7,
- a light chain constant region having an amino acid sequence SEQ ID NO: 8,
- an amino acid sequence of SEQ ID NO: 9,

wherein said heavy chain comprises:

- a heavy chain variable region having an amino acid sequence SEQ ID NO: 10,
- a heavy chain constant region having an amino acid sequence SEQ ID NO: 11, or
- an amino acid sequence of SEQ ID NO: 12.

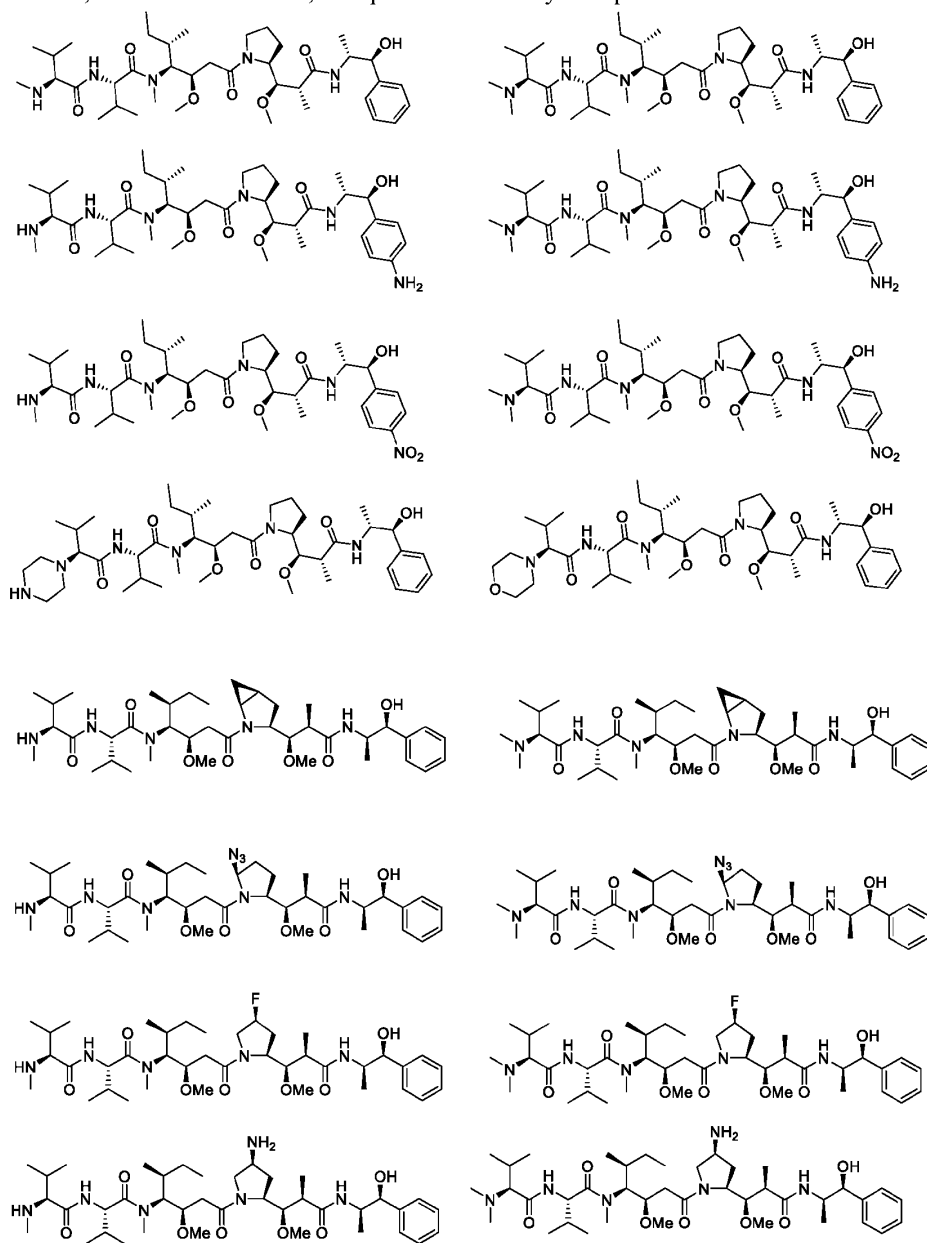
3. The antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof according to Claim 1 or 2, said antibody or antigen-binding fragment thereof comprising a murine antibody, a rabbit antibody, a phage display source antibody, a yeast display source antibody, a chimeric antibody, a humanized antibody, or a fully human antibody.

4. The antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-3, wherein R₄, R₅ are each independently hydrogen, C₁-C₄ alkyl, or R₄, R₅ are linked to form the following structure: $-(\text{CH}_2)_2-\text{B}-(\text{CH}_2)_2-$, B is O, NH, and nitrogen atoms bonded to R₄ and R₅ form a ring with $-(\text{CH}_2)_2-\text{B}-(\text{CH}_2)_2-$.

5. The antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-4, wherein R₆, R₇, R₈, R₉ in Formula D are each hydrogen; alternatively, one of R₆, R₇, R₈, R₉ in Formula D is halogen, an azido group, amino, and the remaining three are each hydrogen; alternatively, any two of R₆, R₇, R₈, R₉ 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.

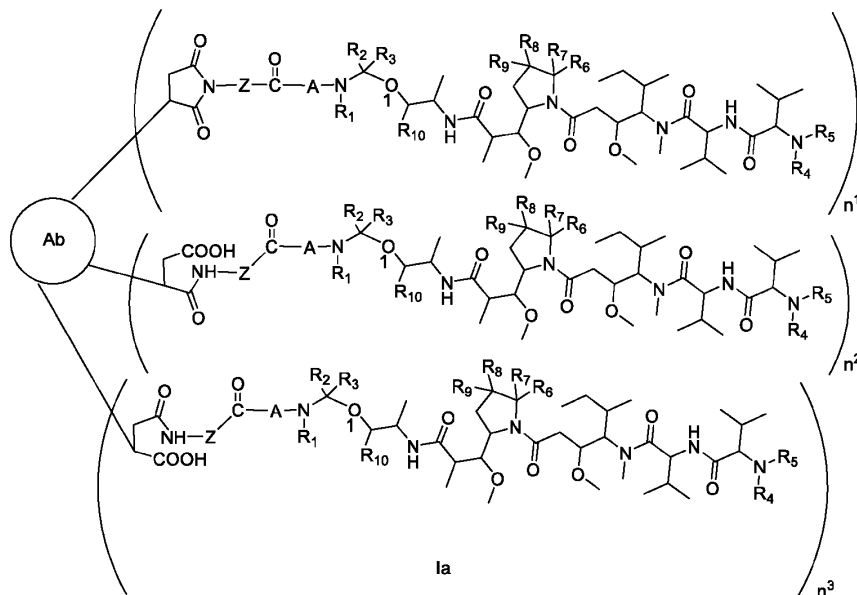
6. The antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof

according to any one of Claims 1-5, wherein R₁₀ in Formula D is phenyl, and wherein optionally said phenyl is substituted with one or more of substituents comprising amino, or nitro, or
 wherein D is selected from the following compounds, its isomers, endo-, racemates, enantiomers, or mixtures thereof, or a pharmaceutically acceptable salt thereof:



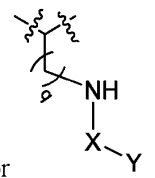
7. The antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-6, said antibody-drug conjugate having the structure shown in

Formula Ia:



wherein,

Z is $-\text{C}_1\text{-C}_{10}$ alkylene-, $-\text{C}_3\text{-C}_8$ carbocyclic-, -arylidene-, $-\text{C}_1\text{-C}_{10}$ alkylene-arylidene-, -arylidene- $-\text{C}_1\text{-C}_{10}$ -alkylene-, $-\text{C}_1\text{-C}_{10}$ alkylene- $(\text{C}_3\text{-C}_8$ carbocyclic)-, $-(\text{C}_3\text{-C}_8$ carbocyclic)- $-\text{C}_1\text{-C}_{10}$ alkylene-, -3-8 membered heterocyclic-, $-\text{C}_1\text{-C}_{10}$ alkylene-(3-8 membered heterocyclic)-, -(3-8



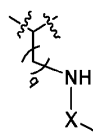
membered heterocyclic)- $-\text{C}_1\text{-C}_{10}$ alkylene-, $-(\text{CH}_2\text{CH}_2\text{O})_r$ -, $-(\text{CH}_2\text{CH}_2\text{O})_r\text{-CH}_2$ - or

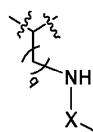
wherein X is $-\text{C}_1\text{-C}_{10}$ alkylene-, $-\text{C}_3\text{-C}_8$ carbocyclic-, -aryl-, $-\text{C}_1\text{-C}_{10}$ alkyl-arylene-, -aryl- $-\text{C}_1\text{-C}_{10}$ alkyl-, $-\text{C}_1\text{-C}_{10}$ alkylene- $(\text{C}_3\text{-C}_8$ carbocyclic)-, $-(\text{C}_3\text{-C}_8$ carbocyclic)- $-\text{C}_1\text{-C}_{10}$ alkylene-, -3-8 membered heterocyclic-, $-\text{C}_1\text{-C}_{10}$ alkyl-(3-8 membered heterocyclic)-, -(3-8 membered heterocyclic)- $-\text{C}_1\text{-C}_{10}$ alkylene-, $-(\text{CH}_2\text{CH}_2\text{O})_r$ -, or $-(\text{CH}_2\text{CH}_2\text{O})_r\text{-CH}_2$ -;

Y is a hydrophilic structure derived from carboxylic acid, phosphoric acid, polyphosphoric acid, phosphite, sulphonic acid, sulfenic acid, or poly(ethylene glycol) (PEG);

said 3-8 membered heterocyclic each independently comprise 1-3 atoms selected from N, O, and S;

said $-\text{C}_1\text{-C}_{10}$ alkylene-, $-\text{C}_3\text{-C}_8$ carbocyclic -, and 3-8 membered heterocyclic are each independently substituted with one or more deuterium, halogen, hydroxyl, cyano, nitro, amino, alkyl, heteroalkyl, substituted alkyl, substituted 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;

n^1 , n^2 , n^3 are independently chosen from an integer or a decimal between 0 and 20, wherein n^1 , n^2 , n^3 are not simultaneously 0 and $n^1 + n^2 + n^3 \leq 20$.

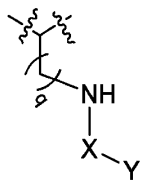
8. The antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-7, wherein A comprises:

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

2-4 amino acids selected from phenylalanine and glycine, or

a tetrapeptide consisting of glycine-glycine-phenylalanine-glycine.

9. The antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof according to Claim 7 or 8, wherein Z is $-C_1-C_{10}$ alkylene-, $-C_4-C_6$ alkylene-, $-C_5$ alkylene-, or



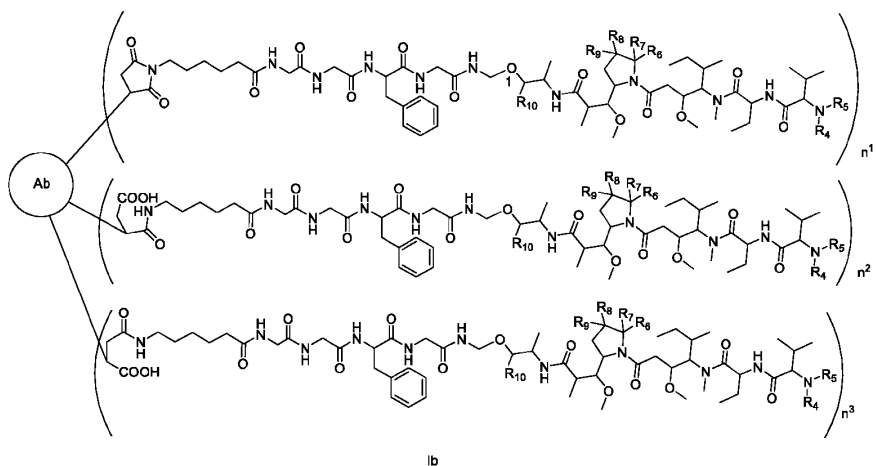
$X-Y$, wherein q is an integer between 1 and 8.

10. The antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 7-9, 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

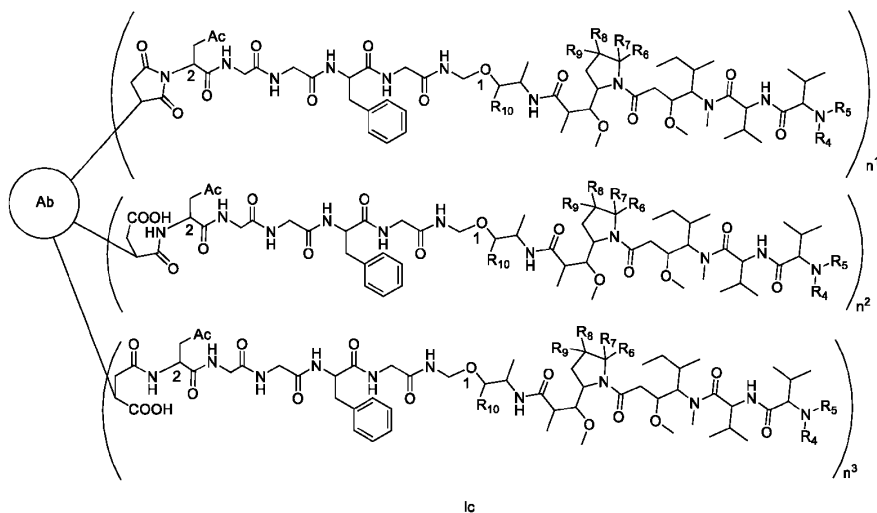
R_1 , R_2 and R_3 are all hydrogen atom.

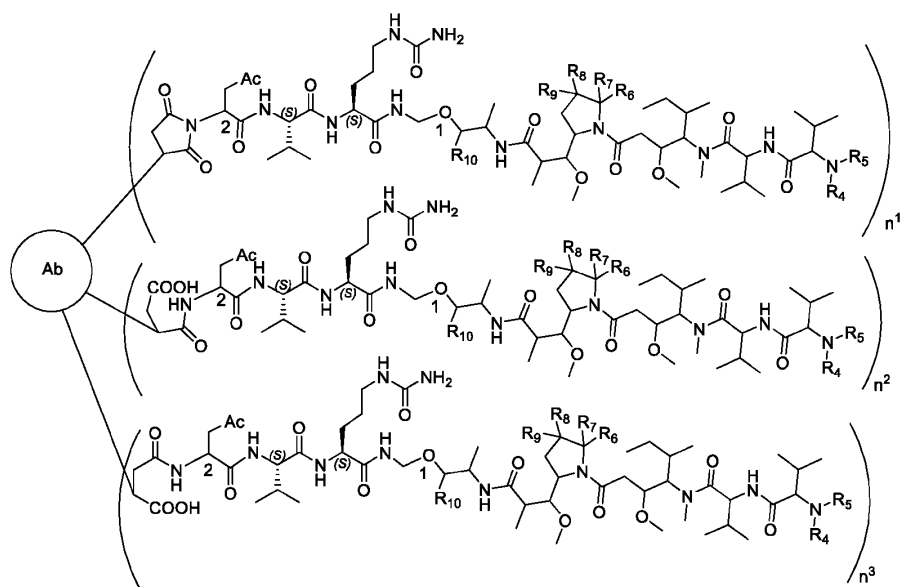
11. The antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-10, said antibody-drug conjugate having a structure as shown in Formula Ib.



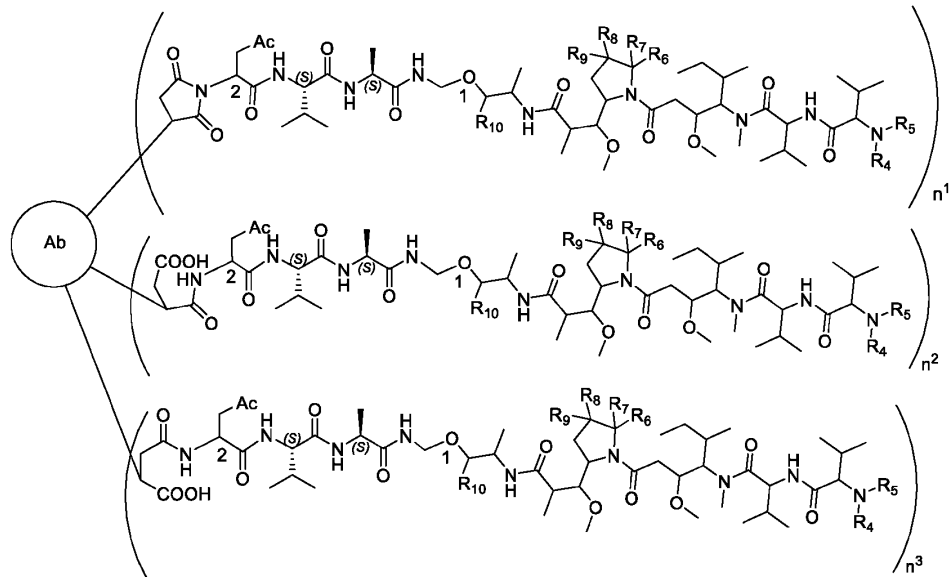
wherein R_4 , R_5 , R_6 , R_7 , R_8 , R_9 , R_{10} , n^1 , n^2 , n^3 are as defined in any one of Claims 1-7.

12. The antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-10, said antibody-drug conjugate having a structure as shown in Formula Ic, Formula Id or Formula Ie.



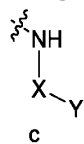


Id



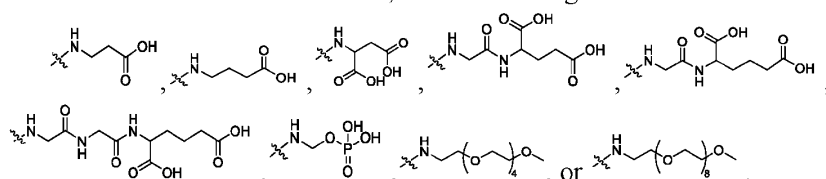
Ie

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

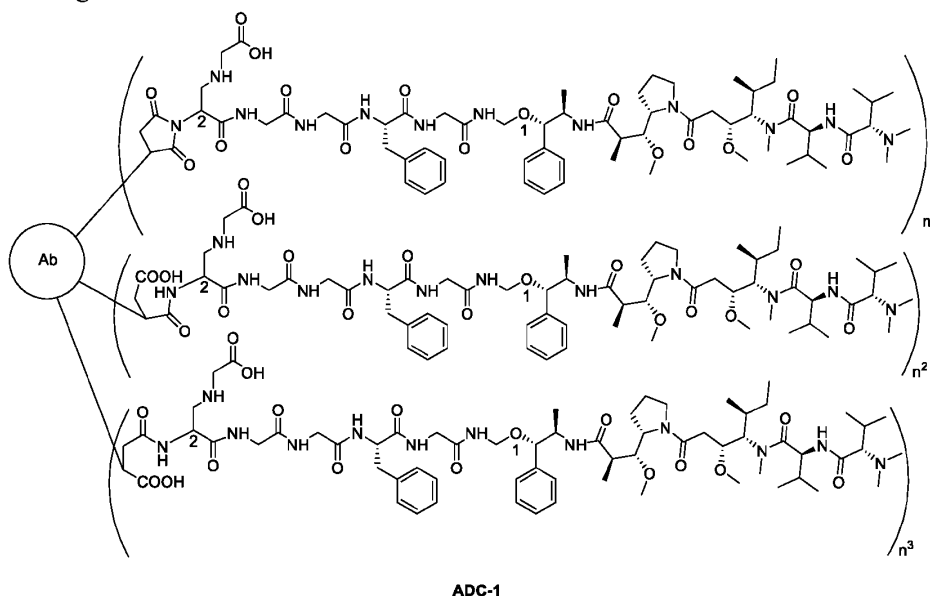


Ac is linked to methylene carbon at the position-2 as labeled via an amino functional group, X, Y are as defined in claim 7; R₄, R₅, R₆, R₇, R₈, R₉, R₁₀, n¹, n², n³ are as defined in any one of Claims 1-7,

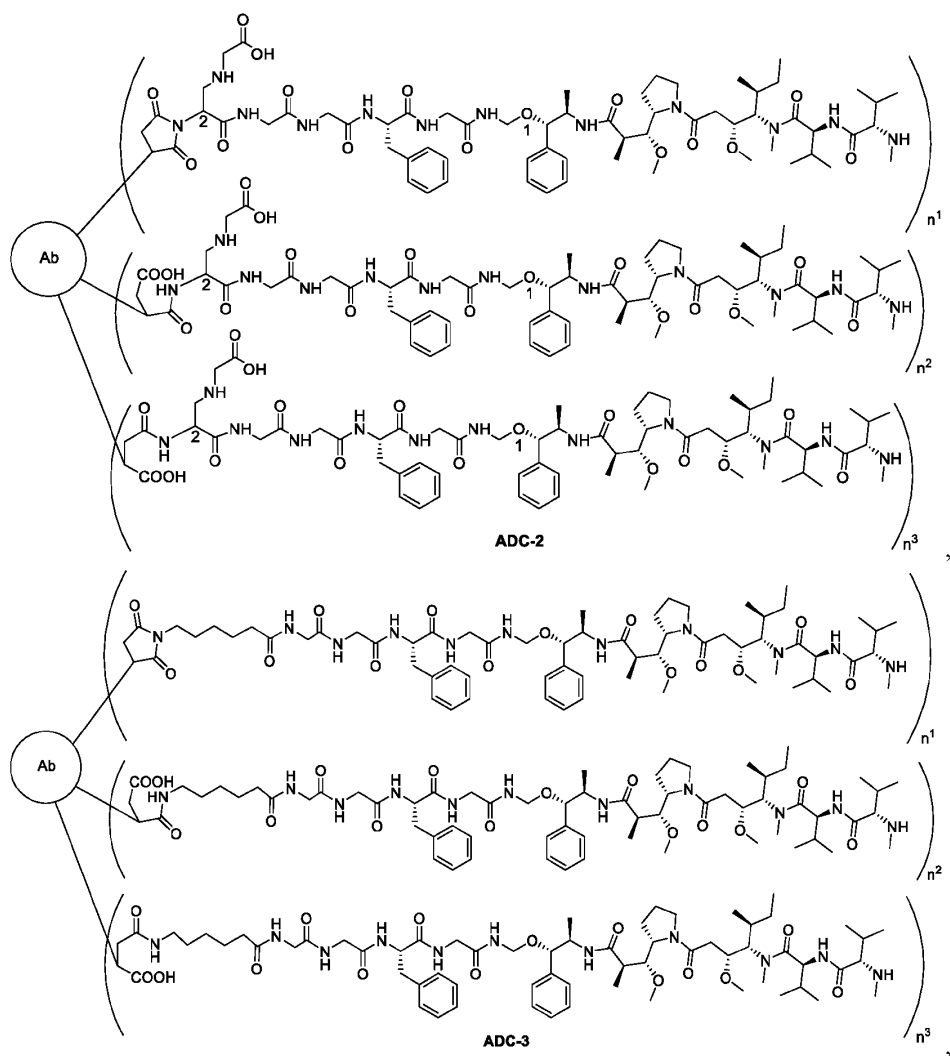
preferably, said Ac is selected from glycine, (D/L) alanine, (D/L) leucine, (D/L) isoleucine, (D/L) valine, (D/L) phenylalanine, (D/L) proline, (D/L) tryptophan, (D/L) serine, (D/L) tyrosine, (D/L) cysteine, (D/L) cystine, (D/L) arginine, (D/L) histidine, (D/L) methionine, (D/L) asparagine, (D/L) glutamine,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

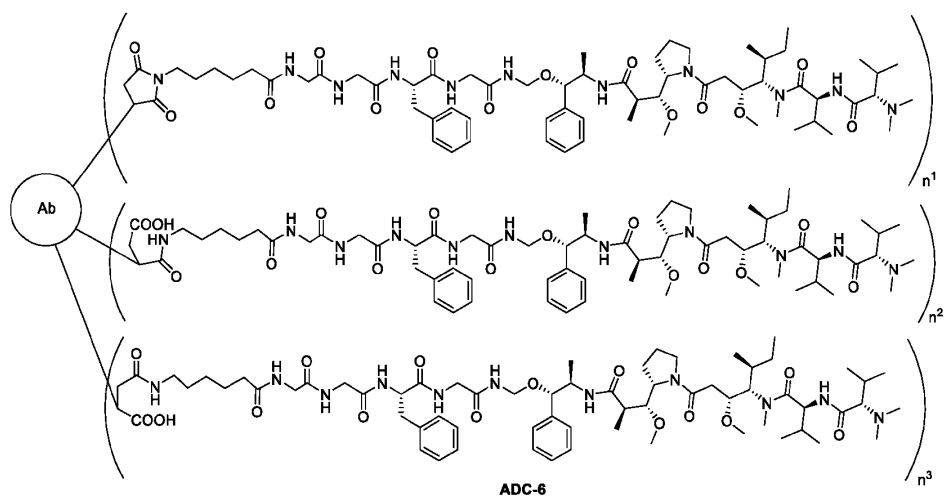
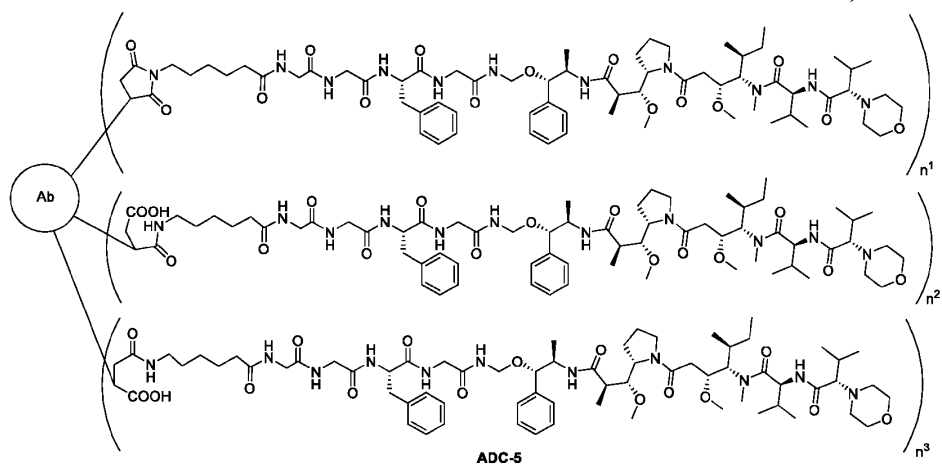
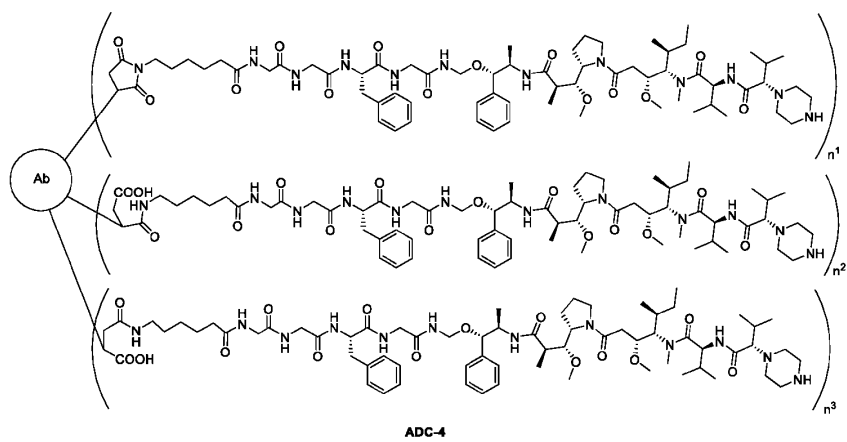


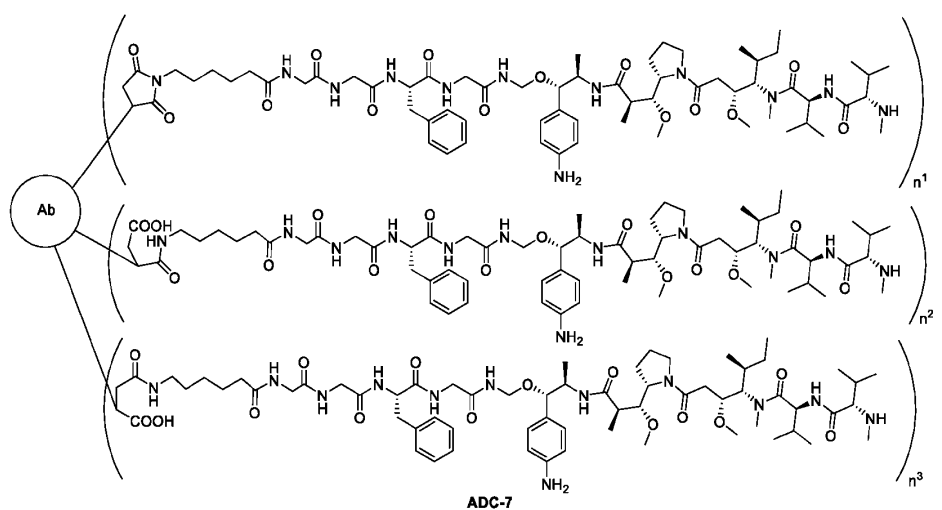
13. The antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-12, said antibody-drug coupling compound being selected from the following structures:



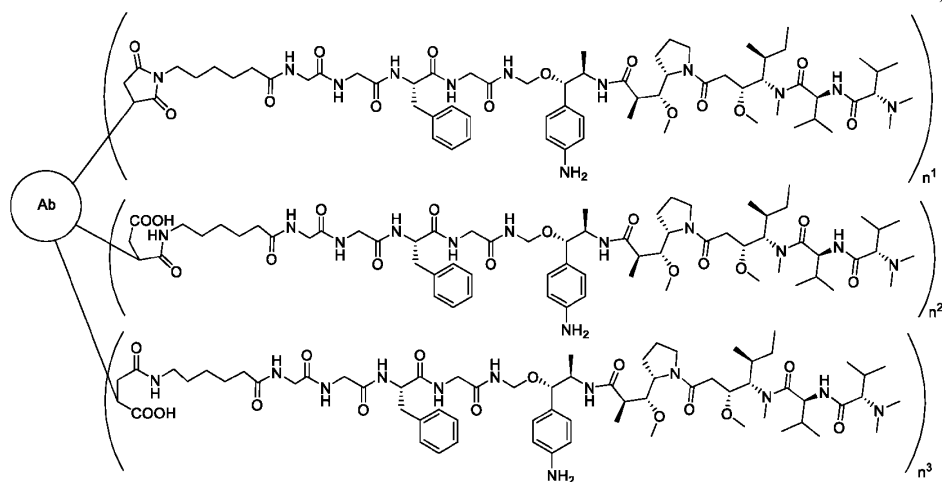
ADC-1



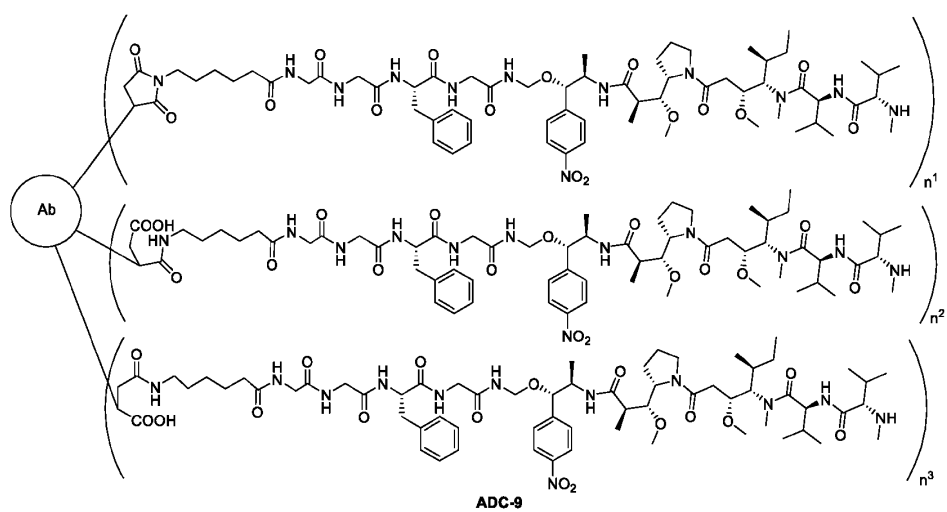




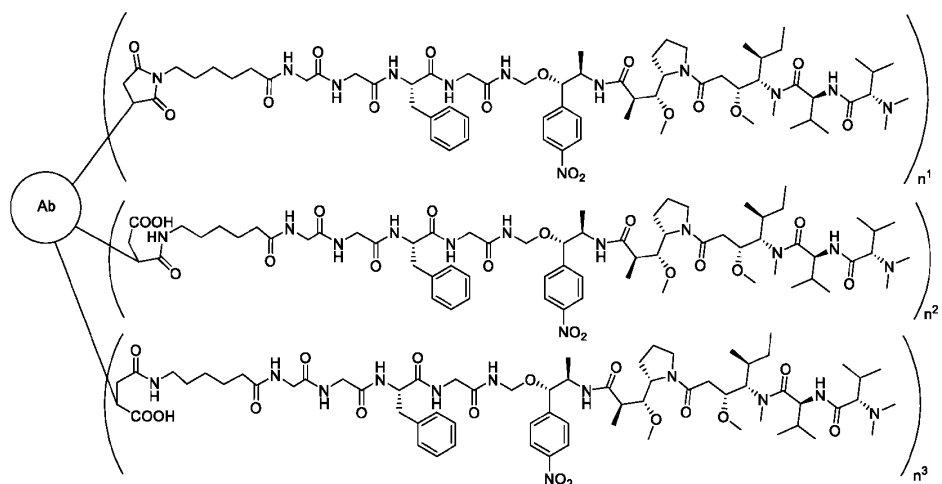
ADC-7



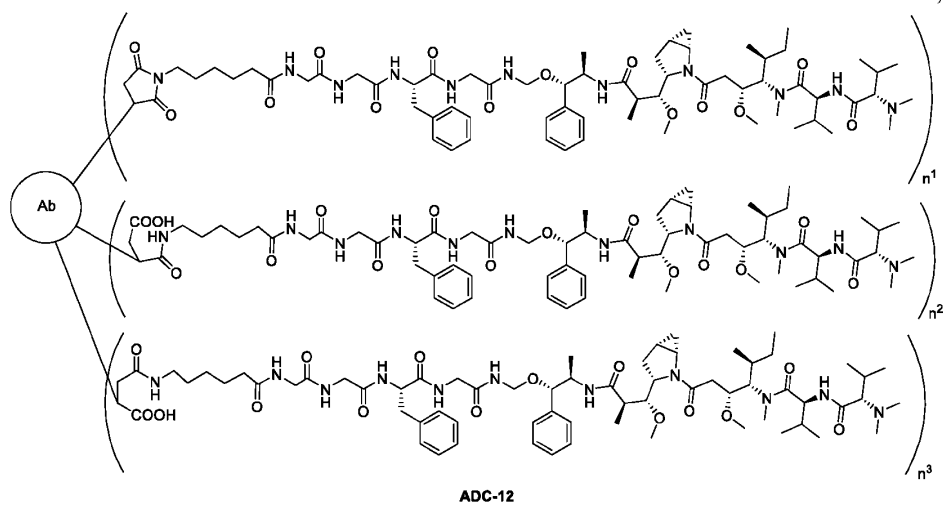
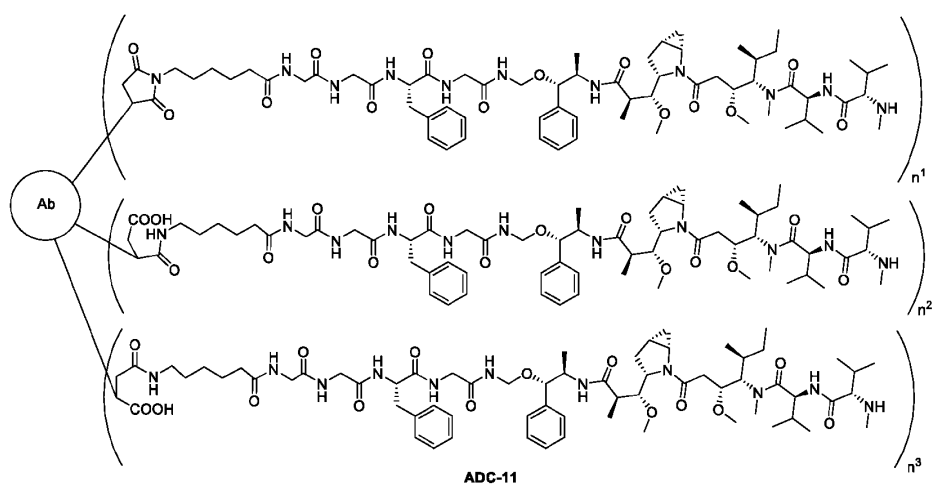
ADC-8

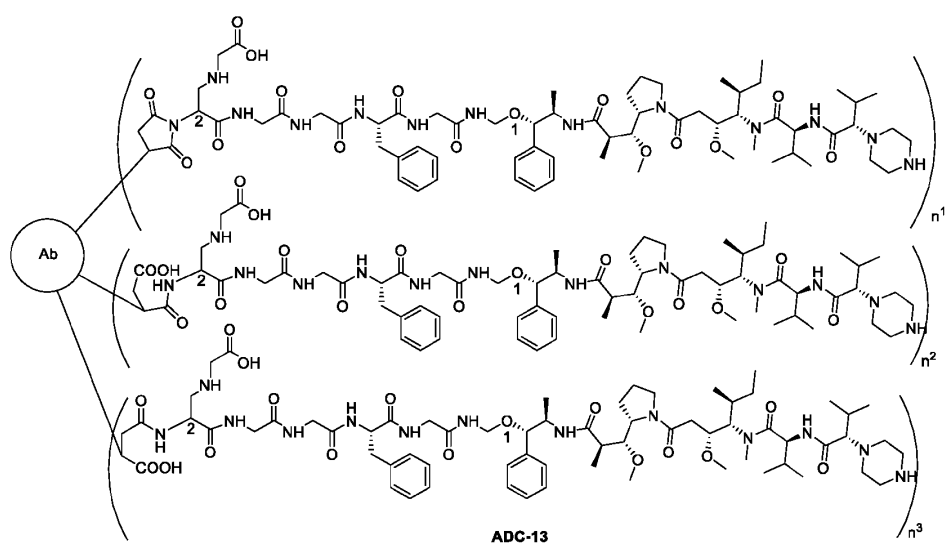


ADC-9

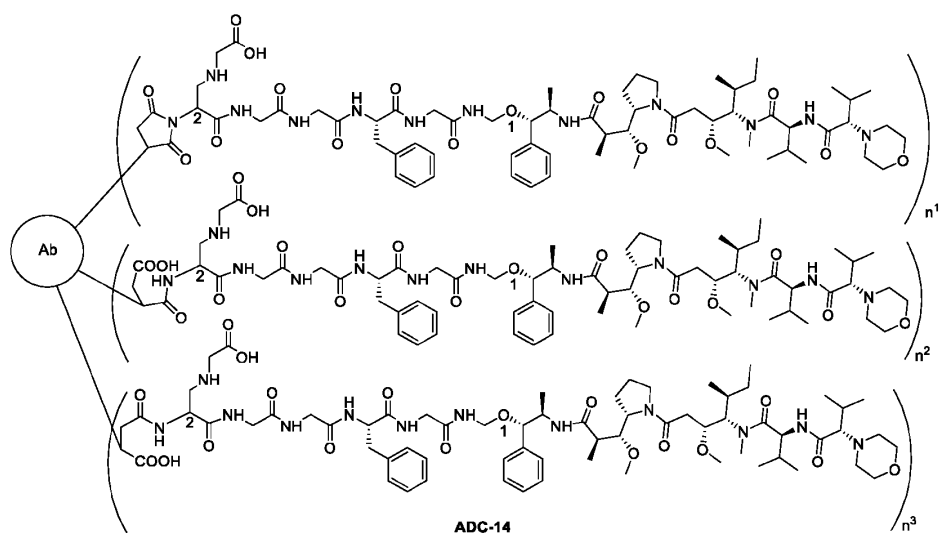


ADC-10

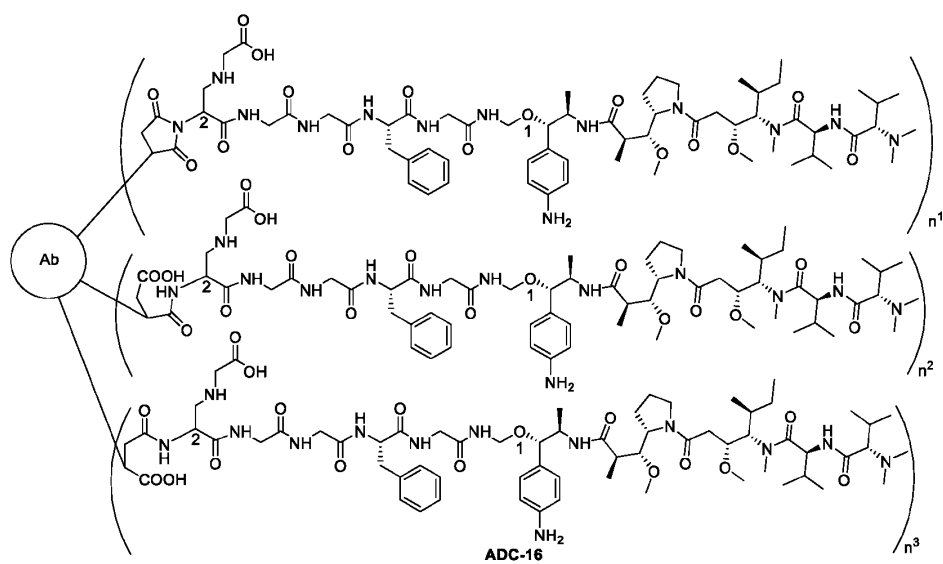
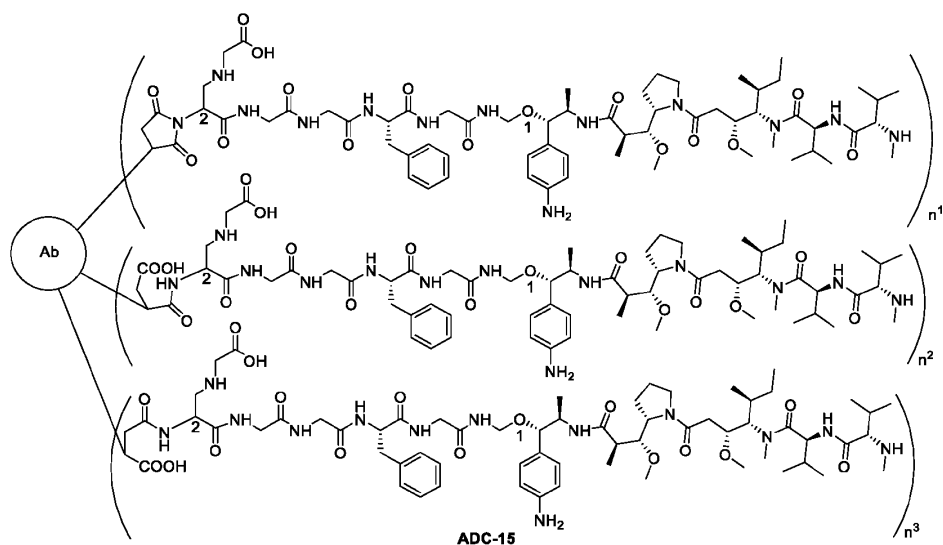


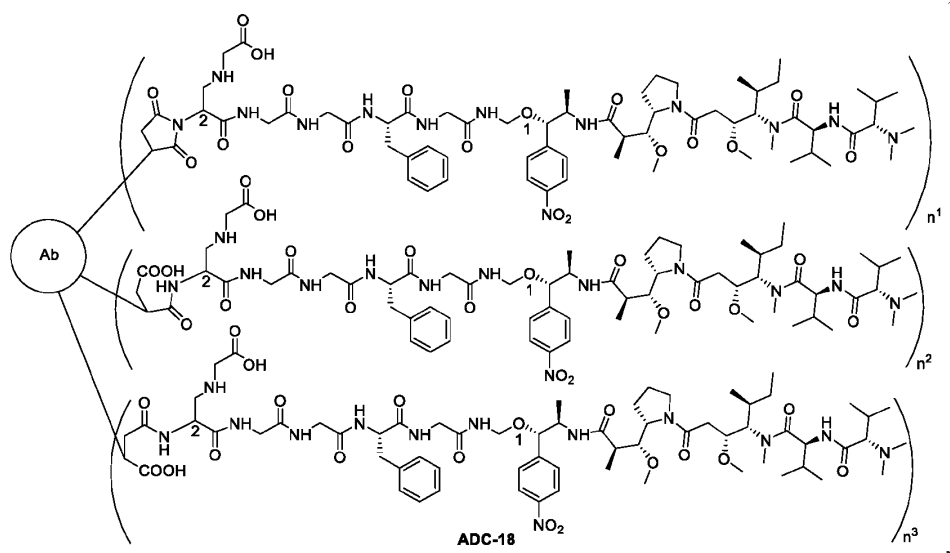
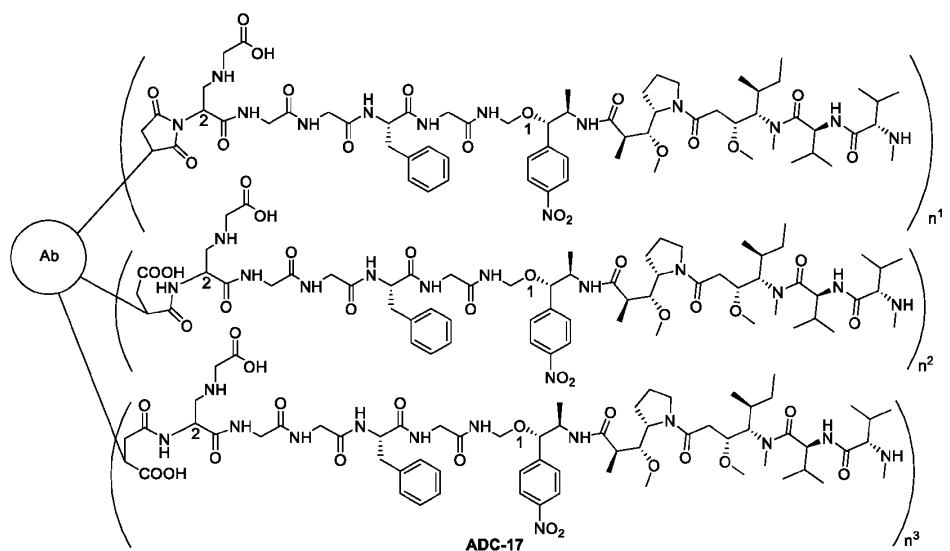


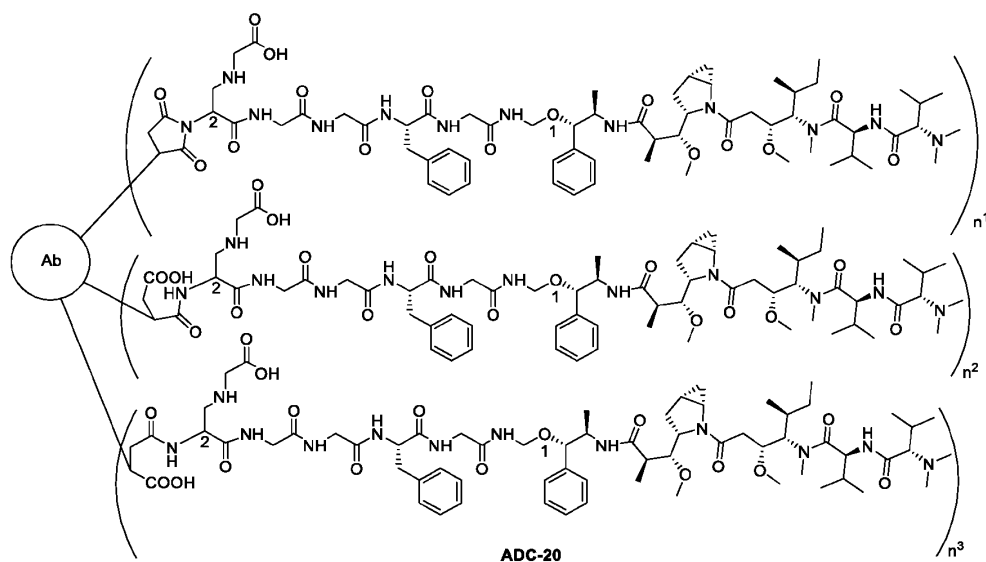
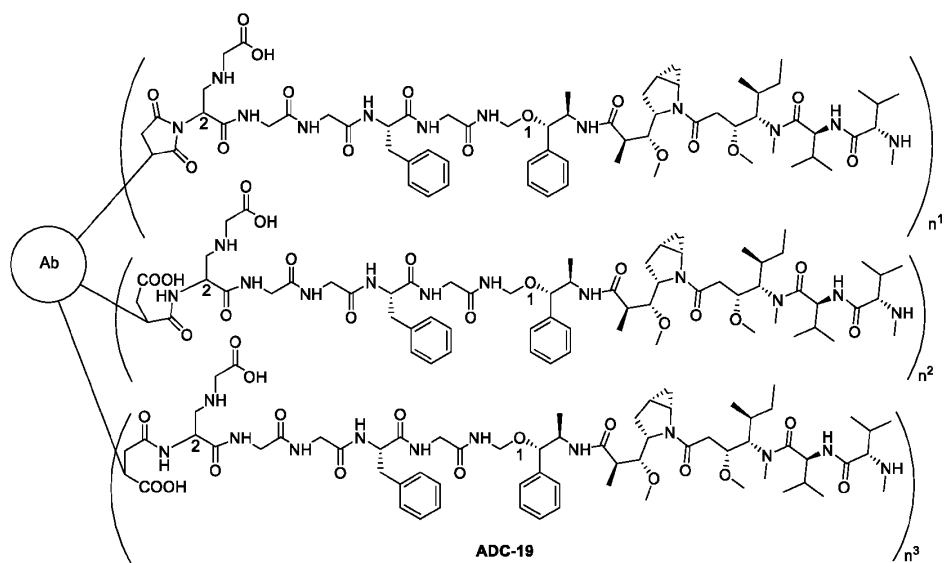
ADC-13

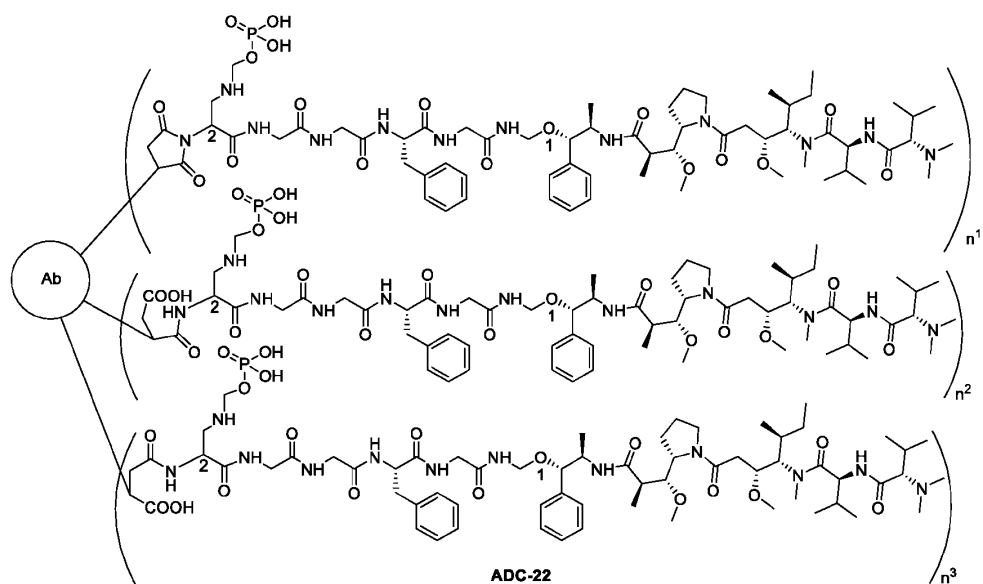
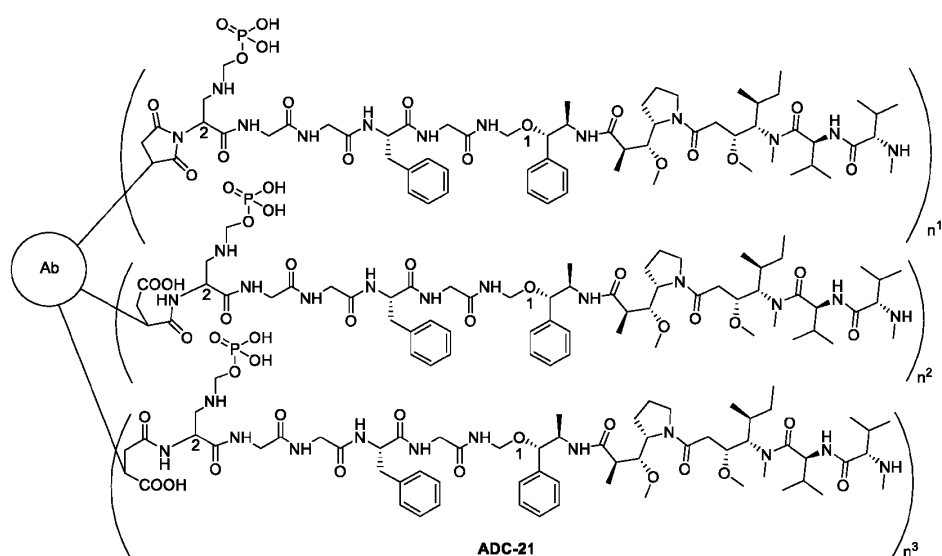


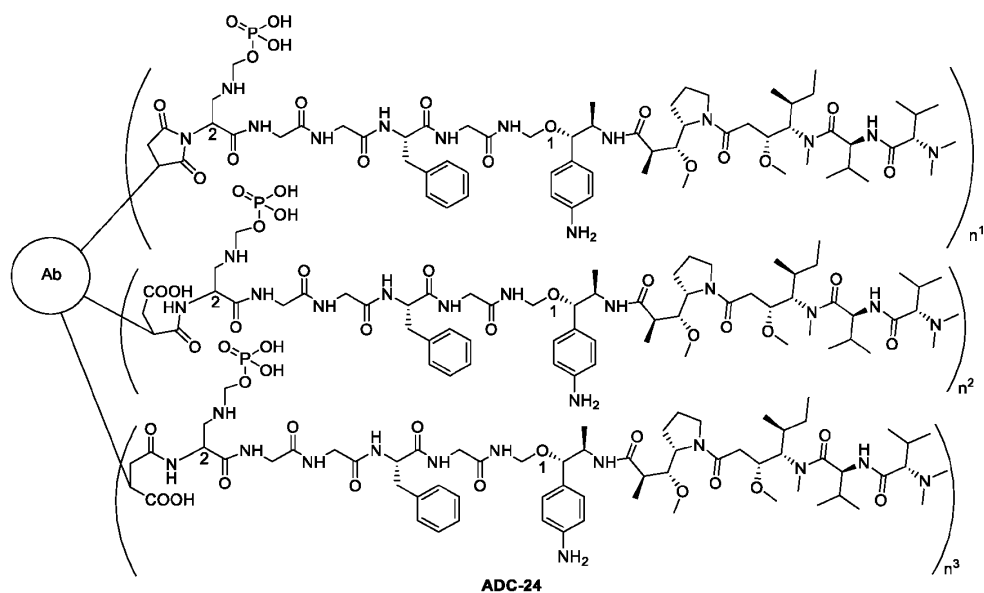
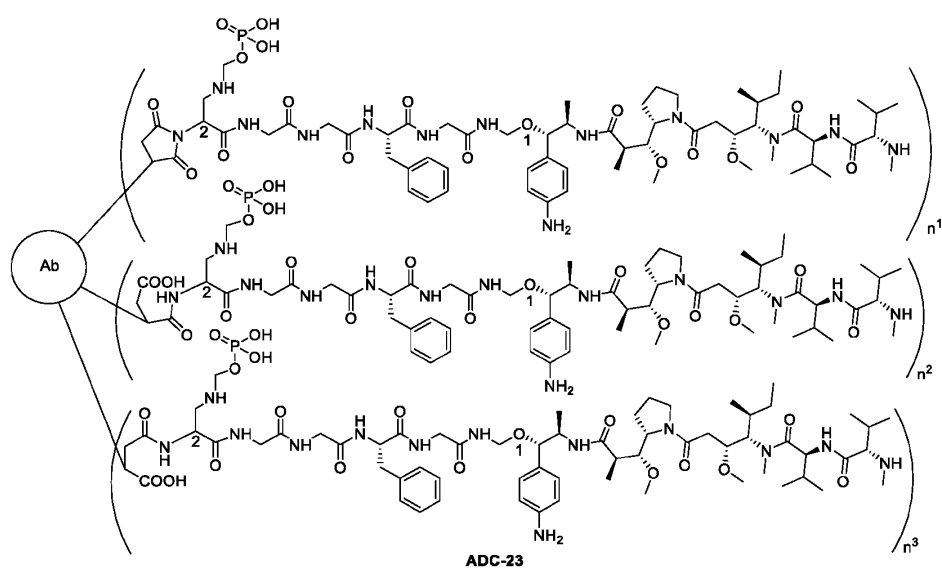
ADC-14

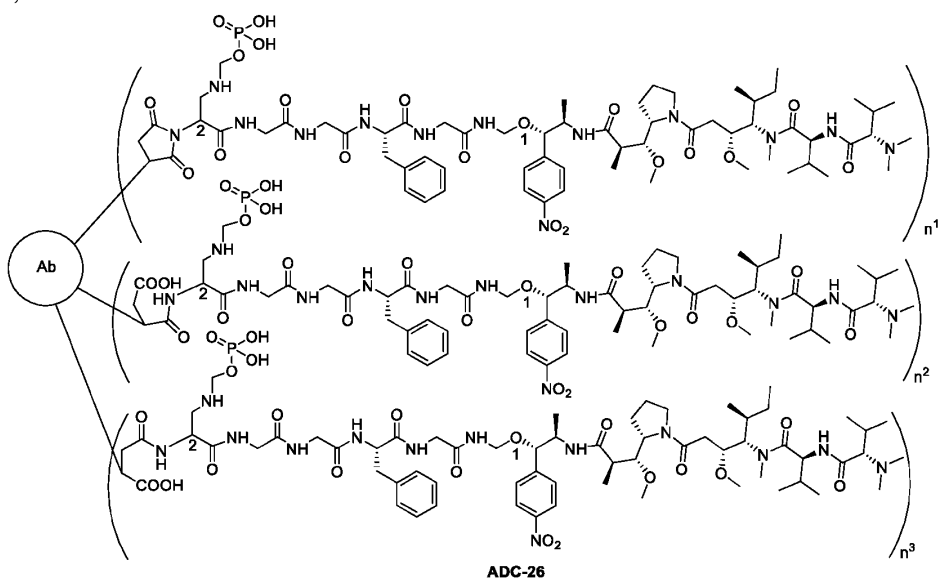
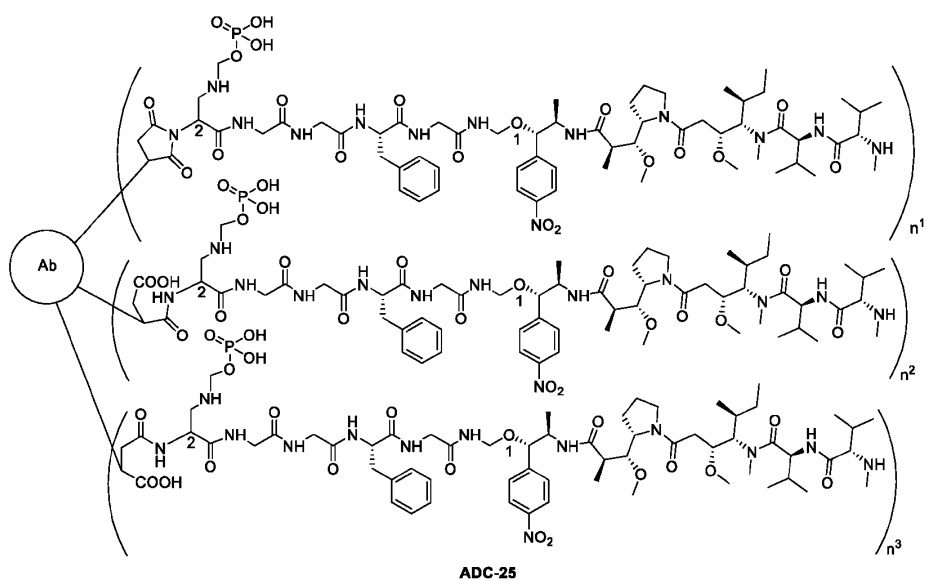


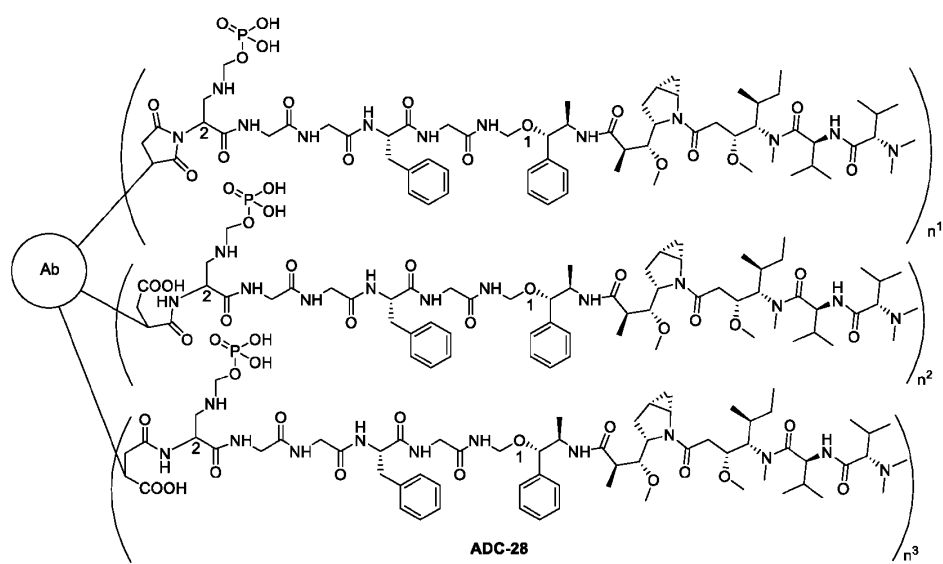
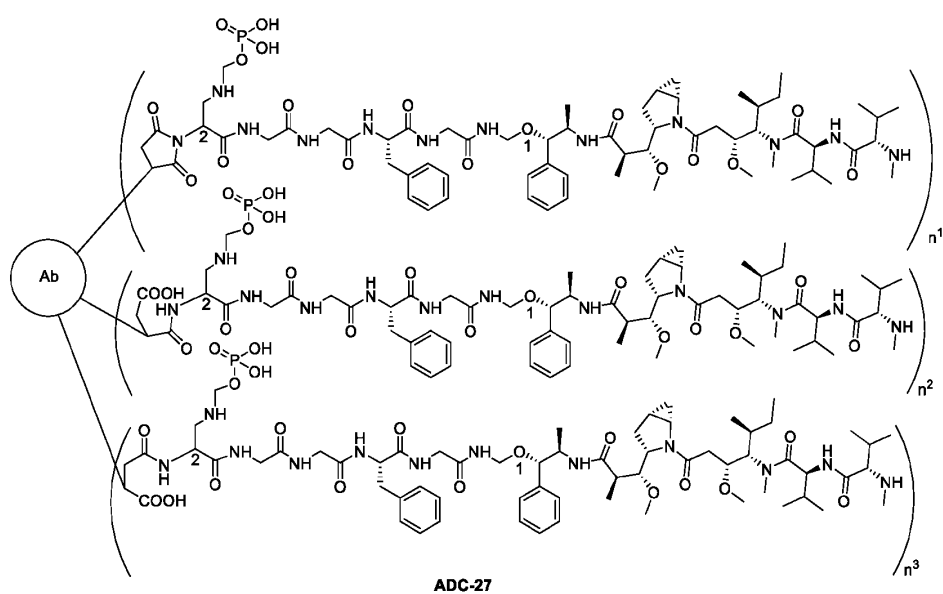


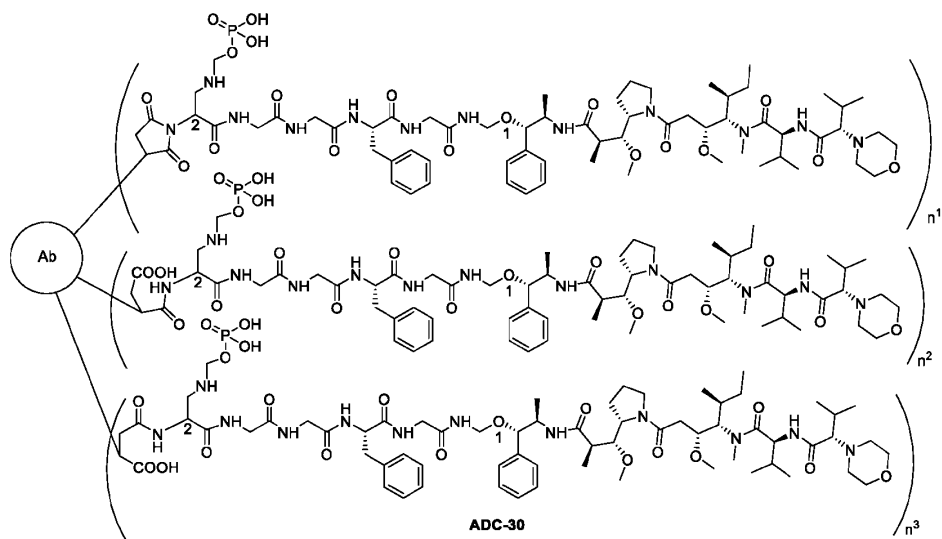
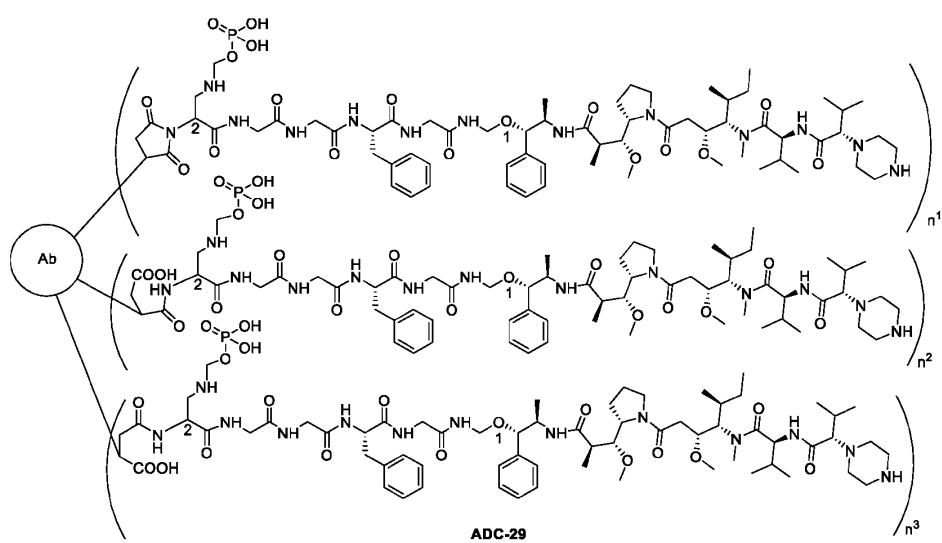


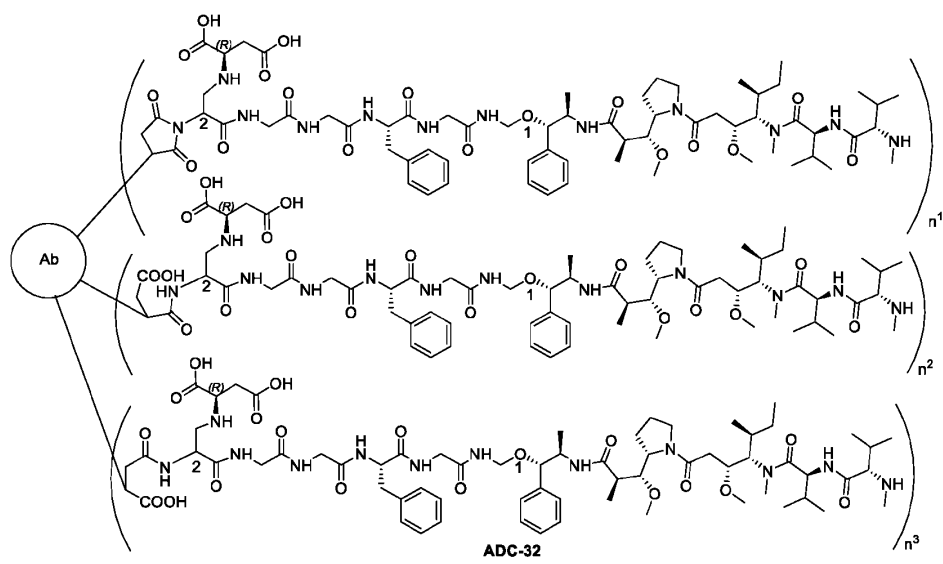
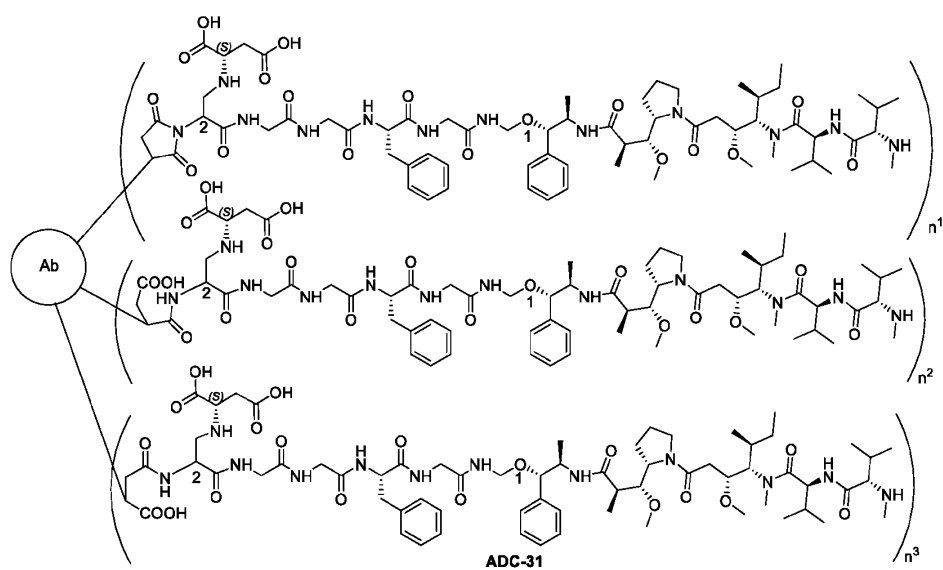


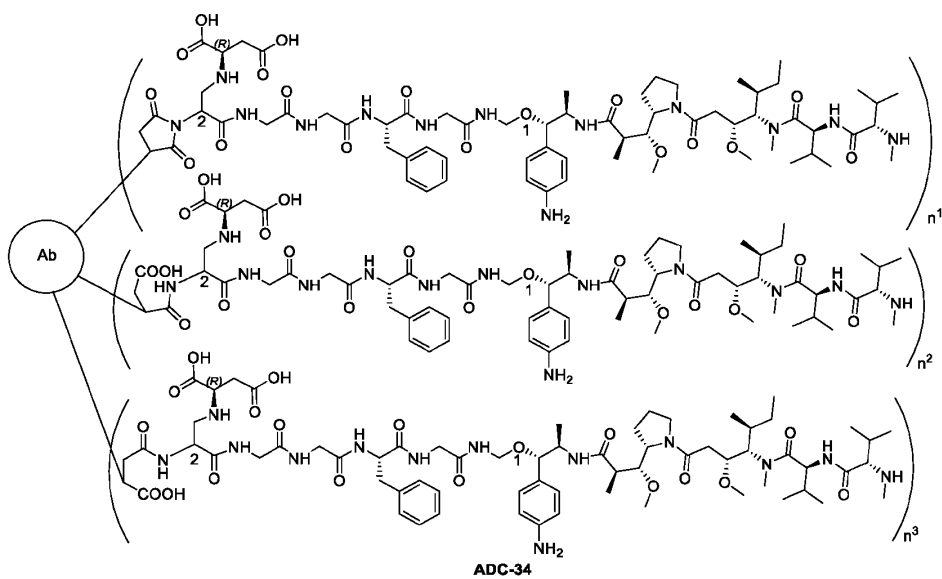
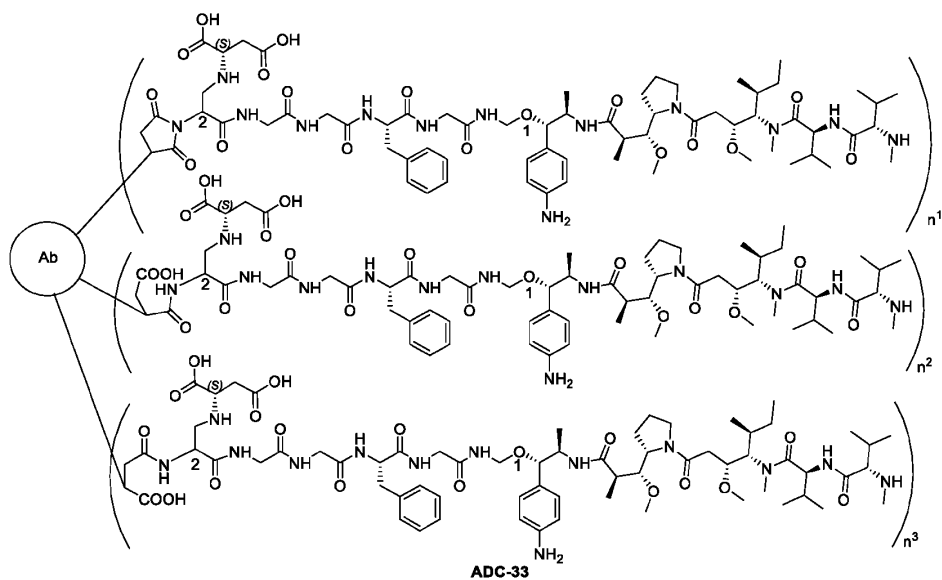


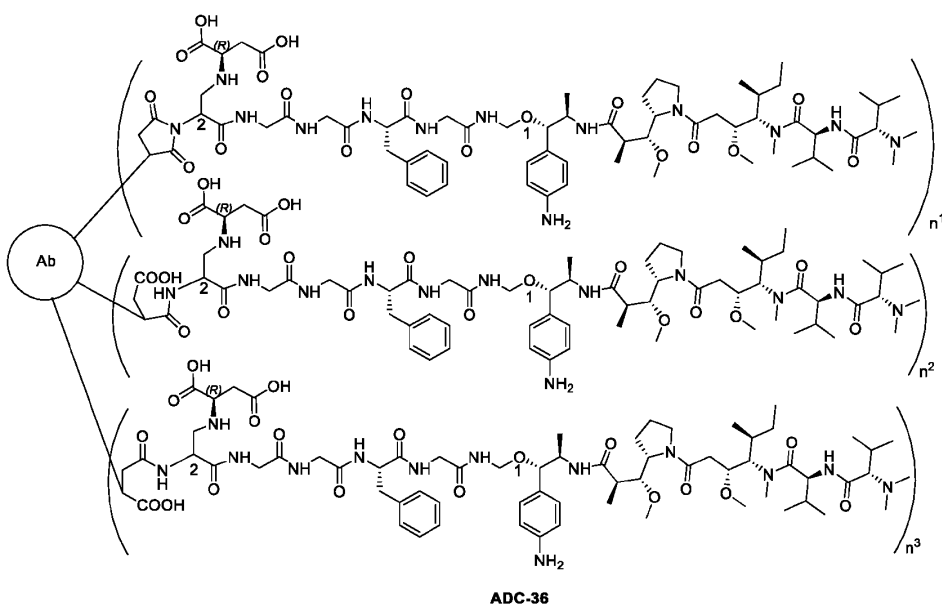
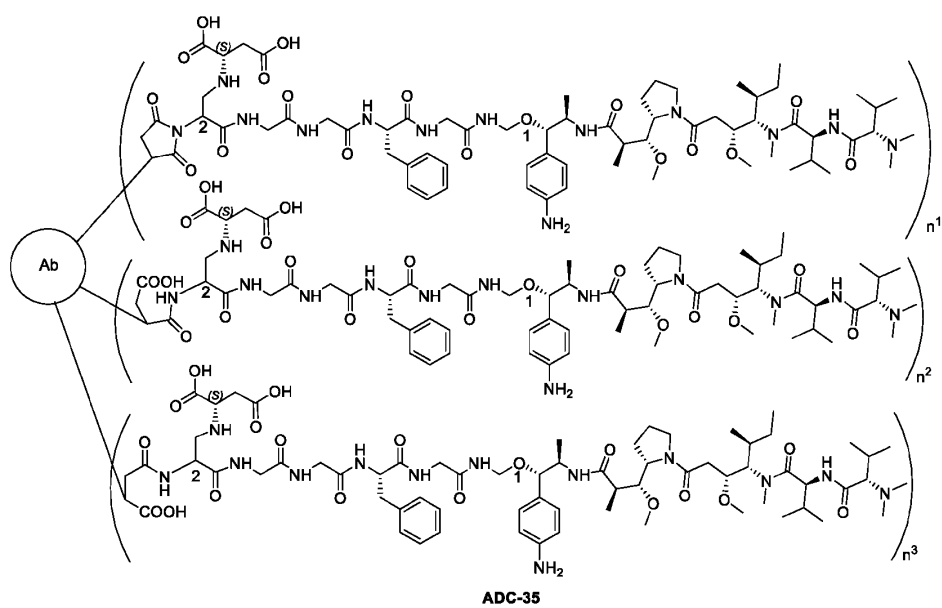


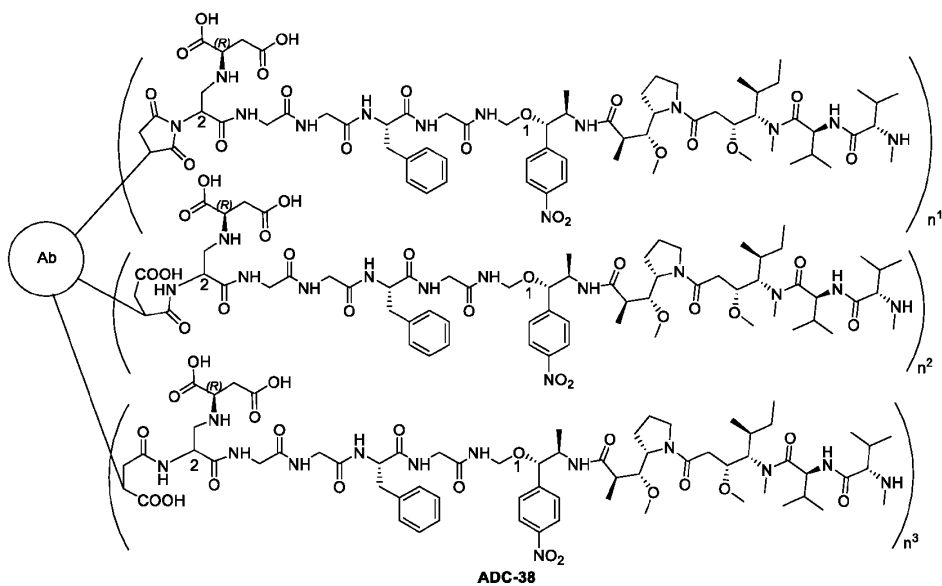
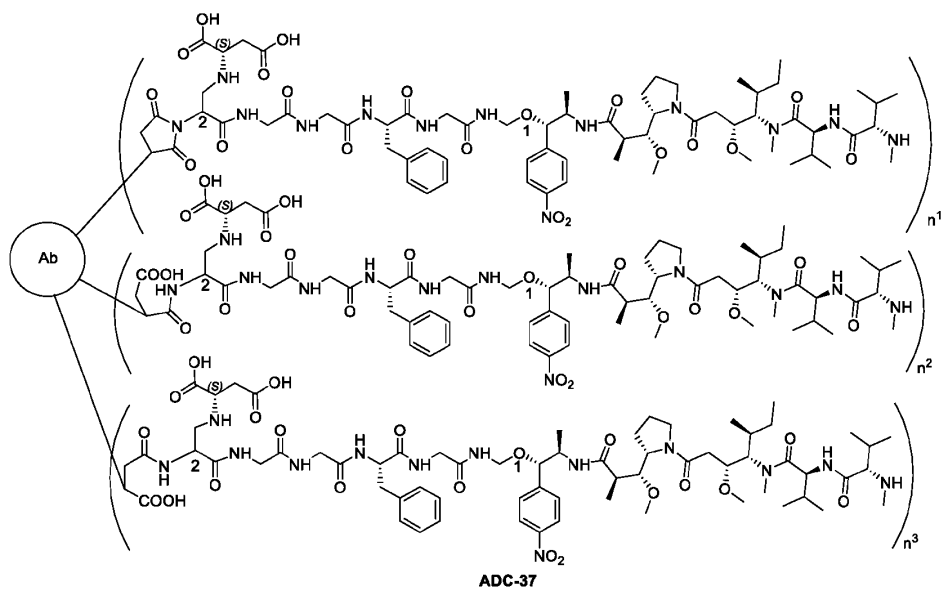


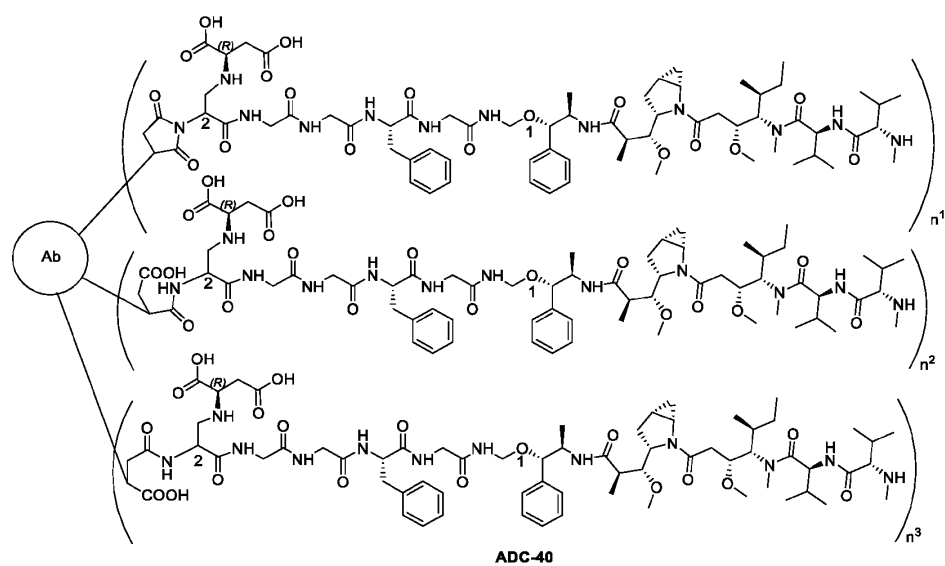
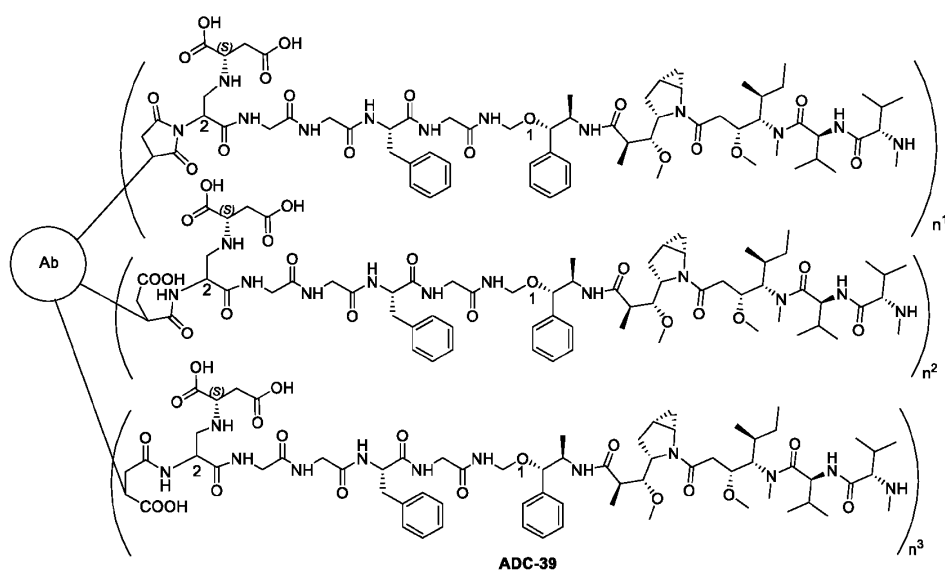


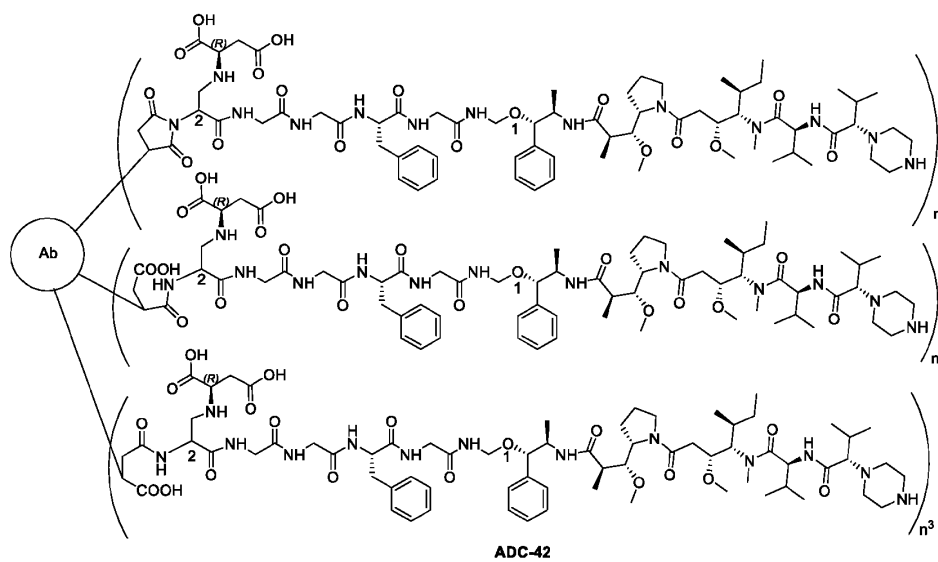
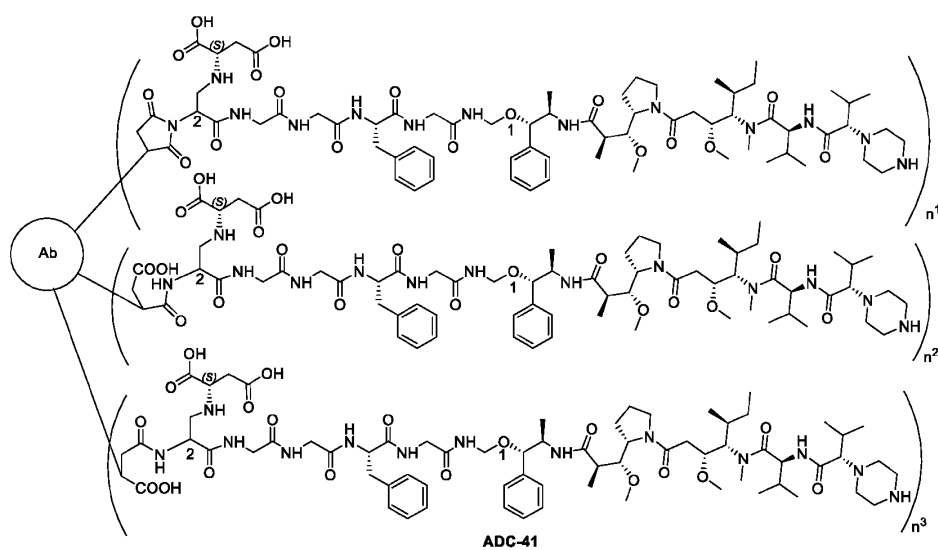


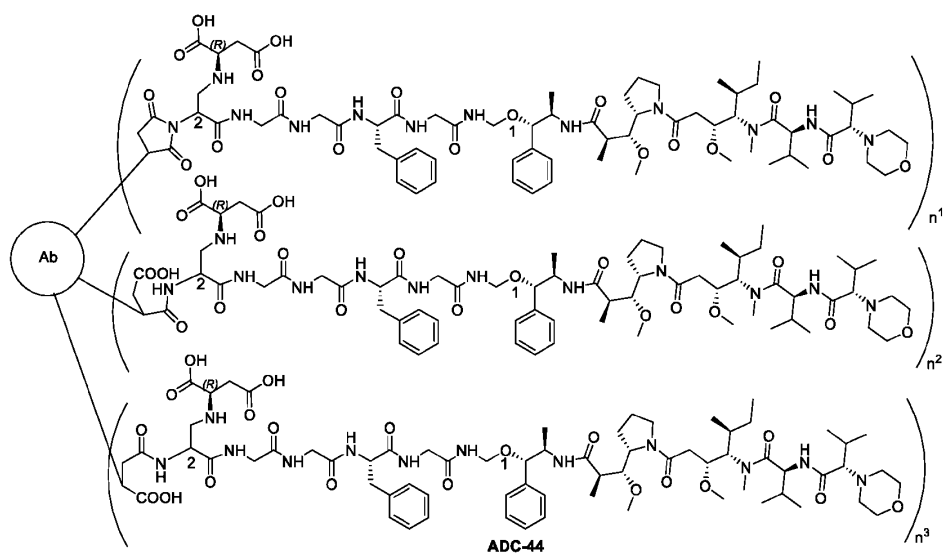
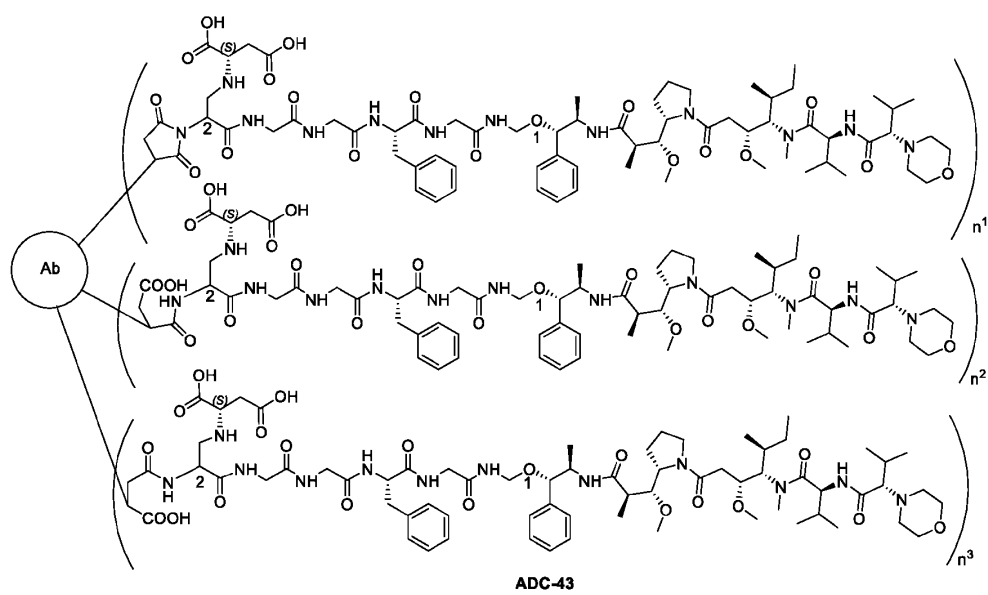


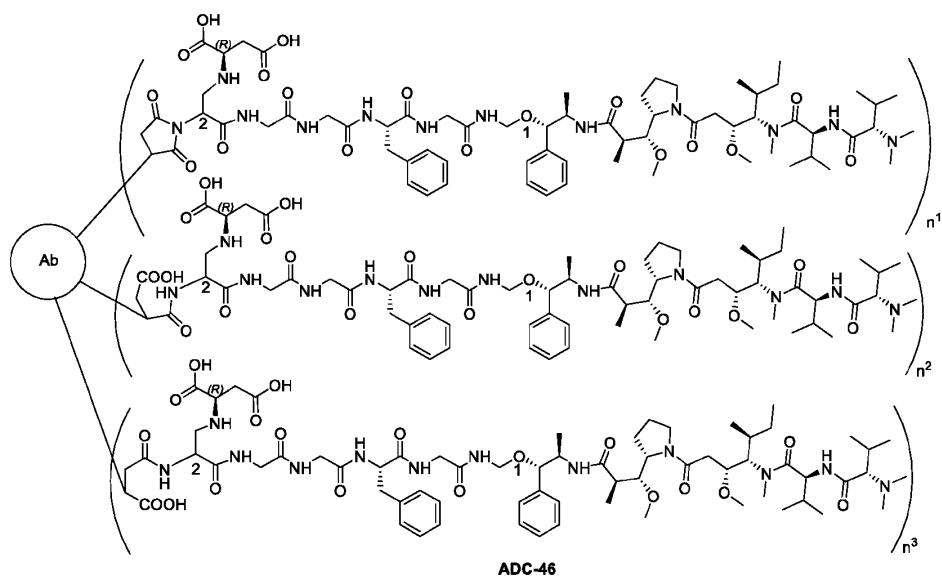
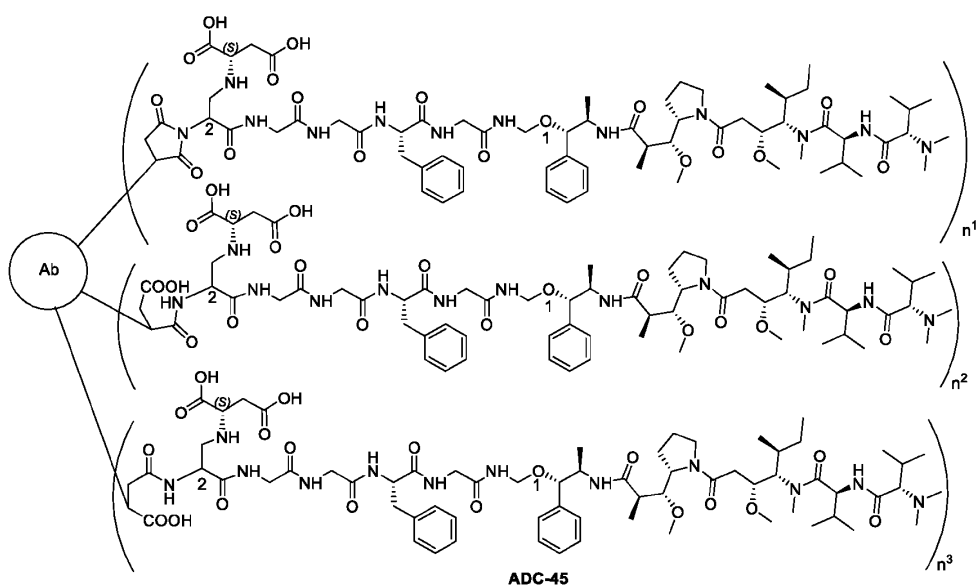


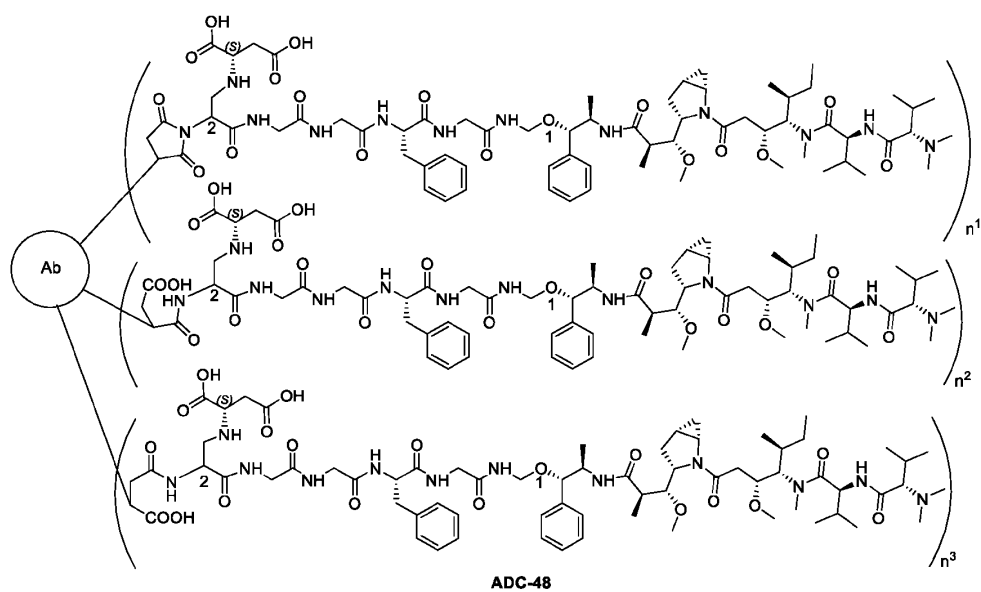
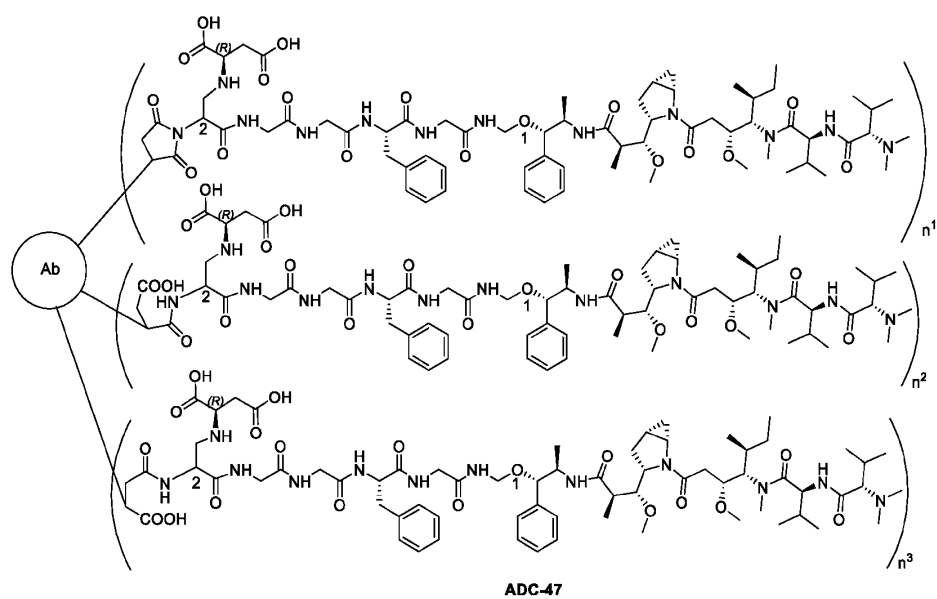


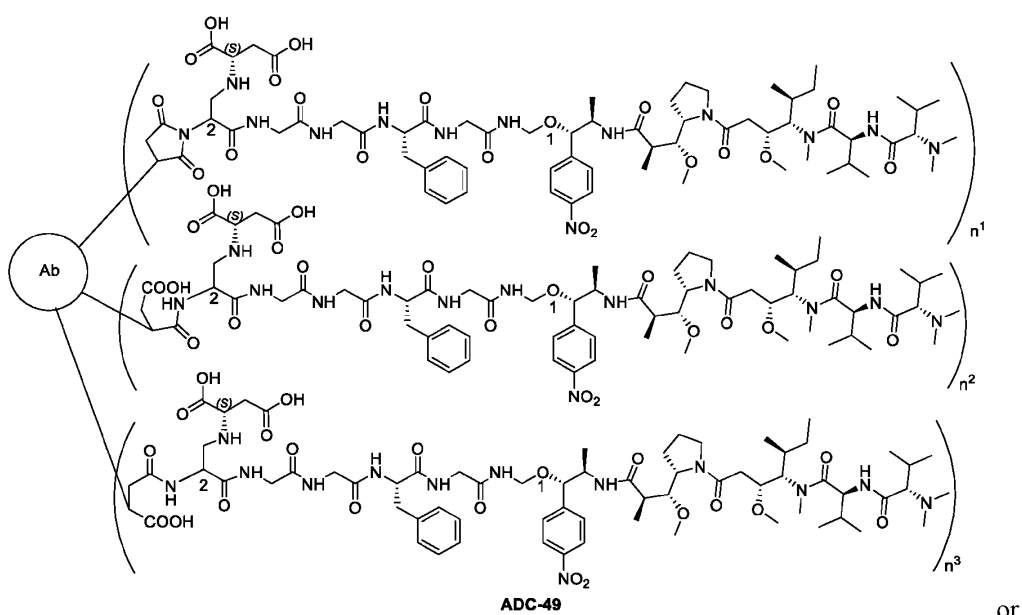




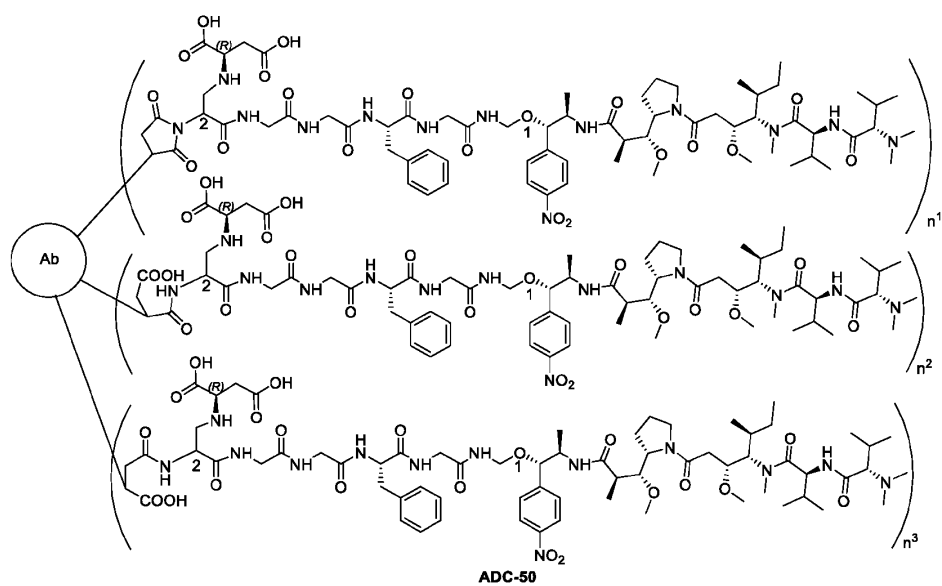


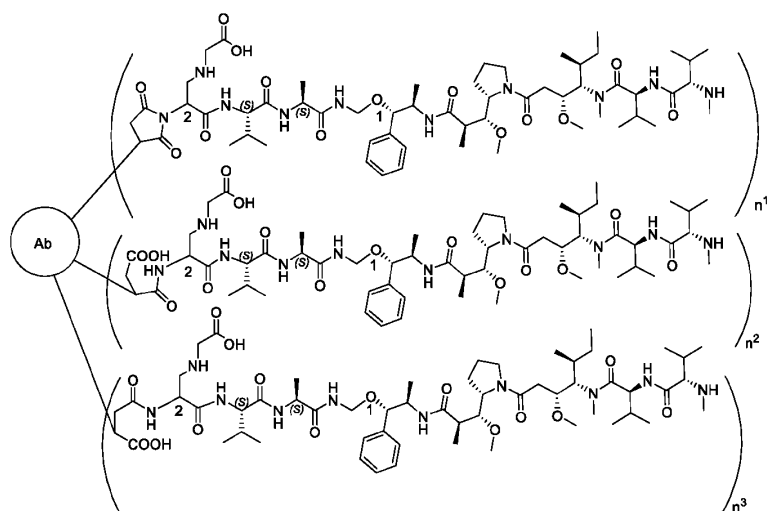




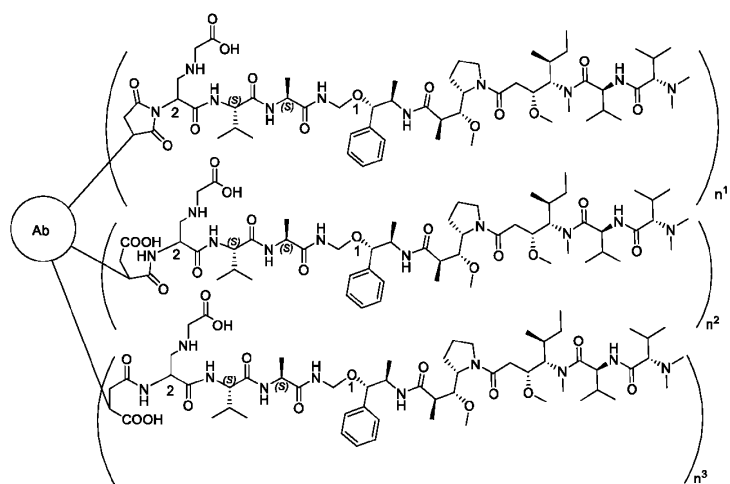


or

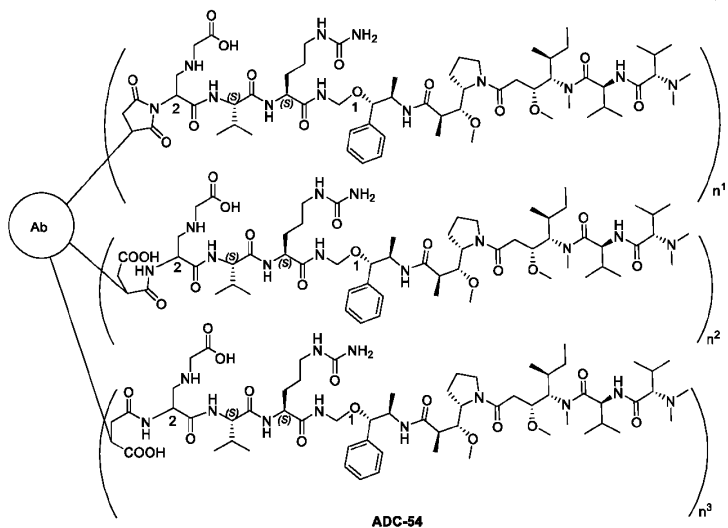
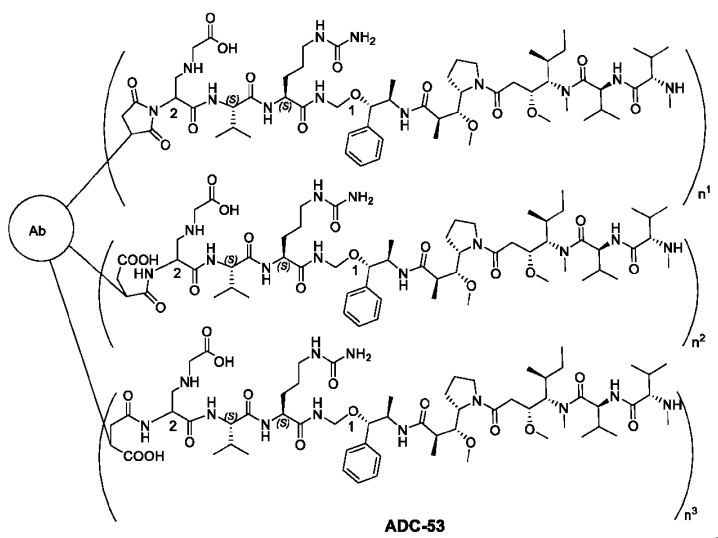




ADC-51

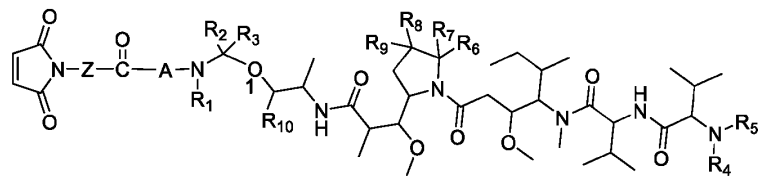


ADC-52



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

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

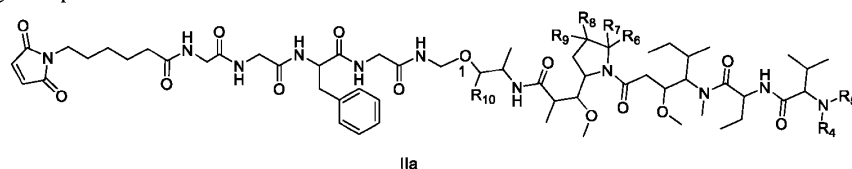


II

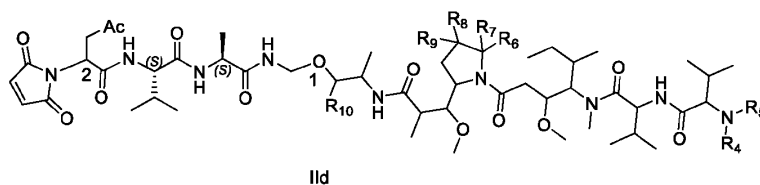
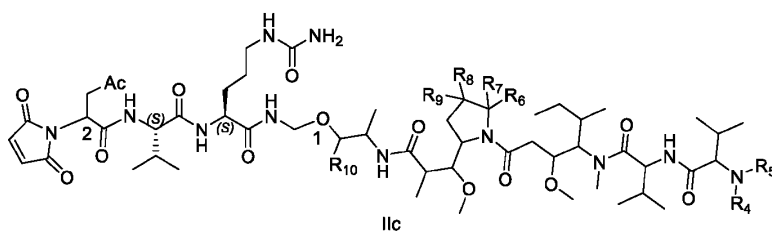
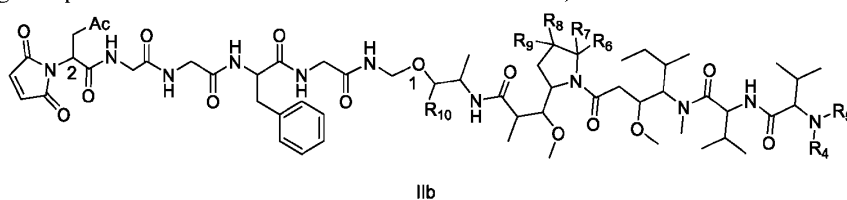
wherein,

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 as defined in any one of Claims 1-10.

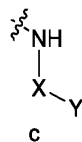
15. The linker- drug compound of Claim 14, its isomeric, endo-, racemic, enantiomeric, or mixture thereof, or pharmaceutically acceptable salts or solvates thereof, wherein said linker- drug compound has the structure shown in Formula IIa:



16. The linker- drug compound of Claim 15-, its isomeric, endo-, racemic, enantiomeric, or mixture thereof, or pharmaceutically acceptable salts or solvates thereof, wherein said linker- drug compound has the structure shown in Formula IIb, Formula IIc or Formula IId:



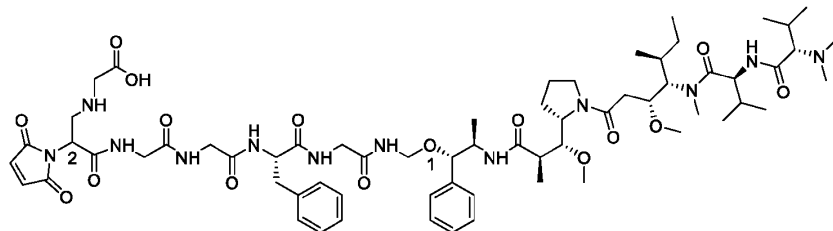
wherein Ac is a hydrophilic structural unit having the structure shown in Formula c:



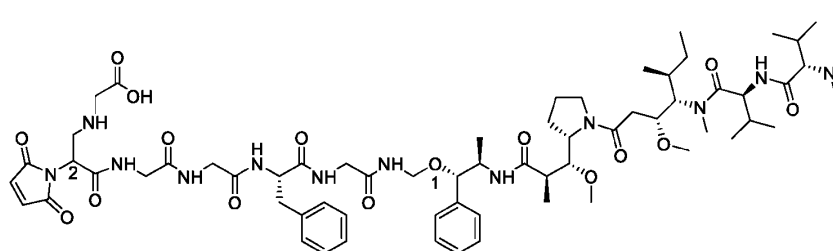
wherein X, Y are as defined in Claim 7, and Ac is linked to methylene carbon at the position-2 as labeled via -NH-.

17. The linker-drug compound according to any one of Claims 14-16, its isomeric, endo-,

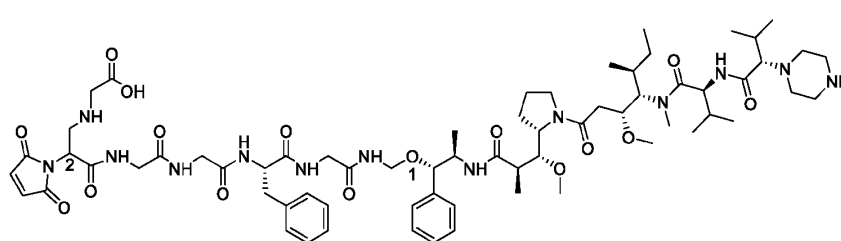
racemic, enantiomeric, or mixture thereof, or pharmaceutically acceptable salts or solvates thereof, said linker-drug compound being selected from the following structures:



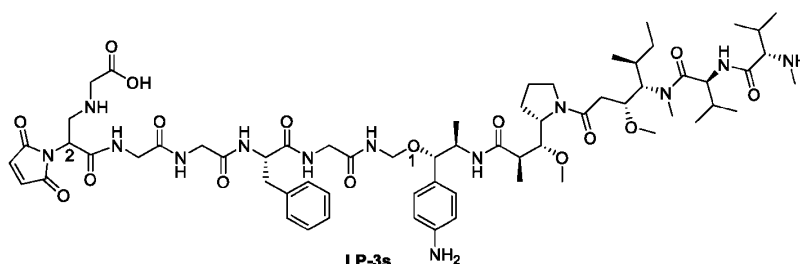
LP-1



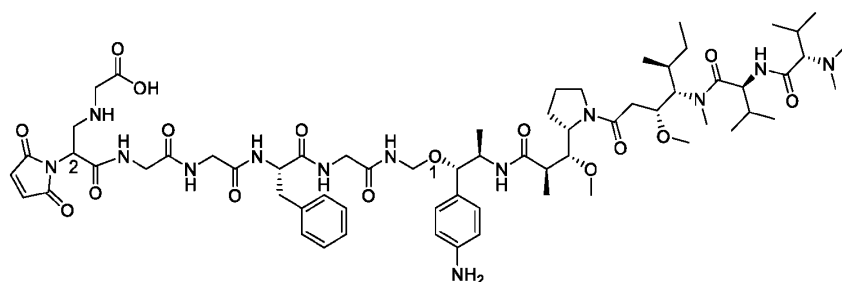
LP-2



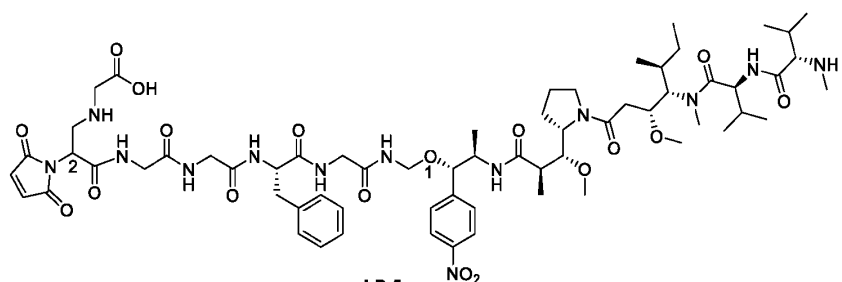
LP-3



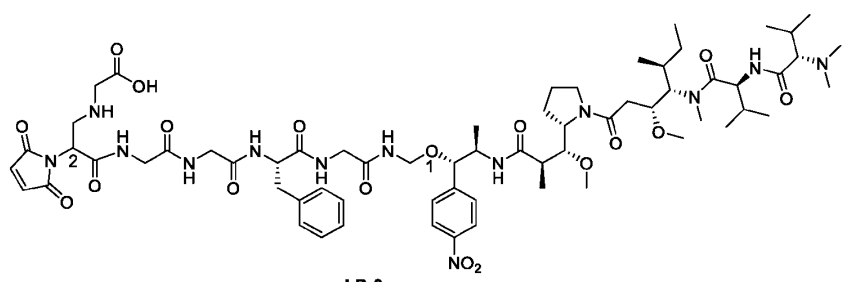
LP-3s



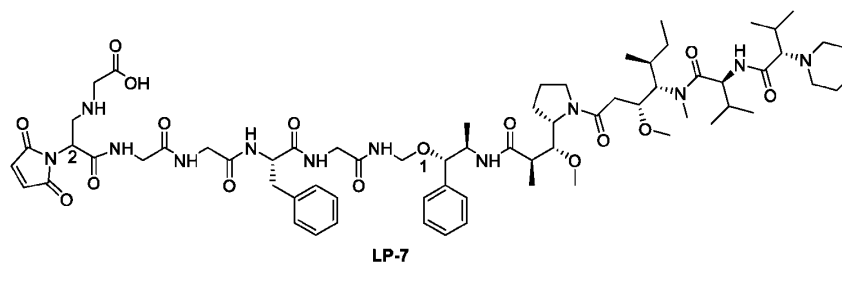
LP-4



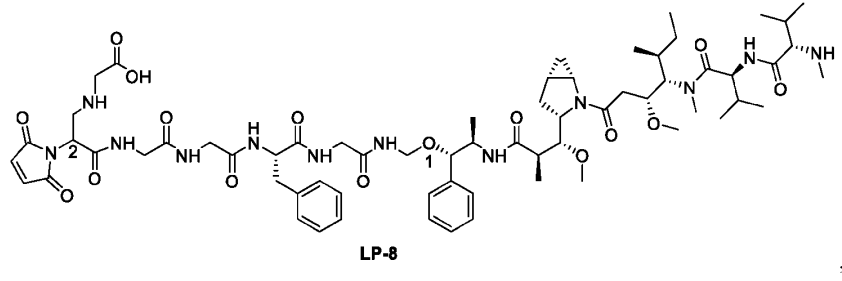
LP-5



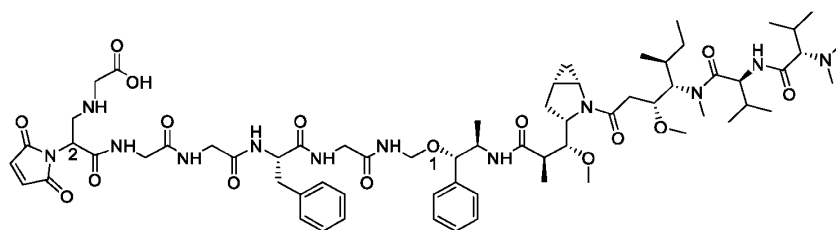
LP-6



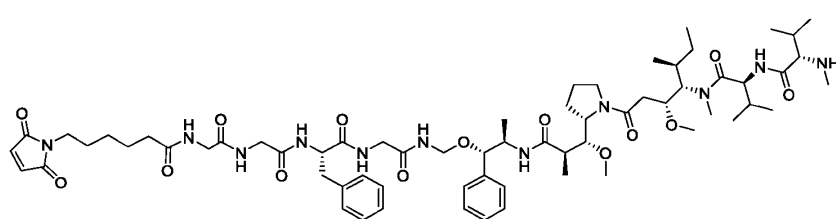
LP-7



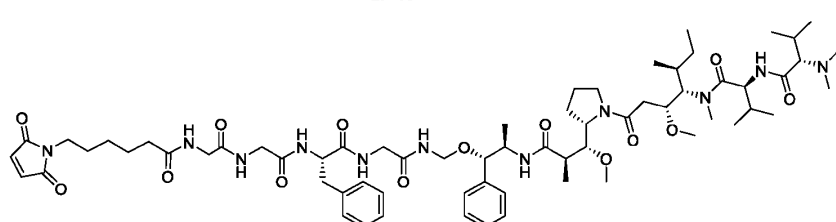
LP-8



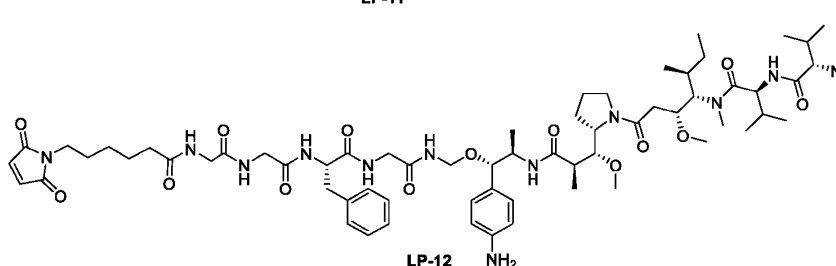
LP-9



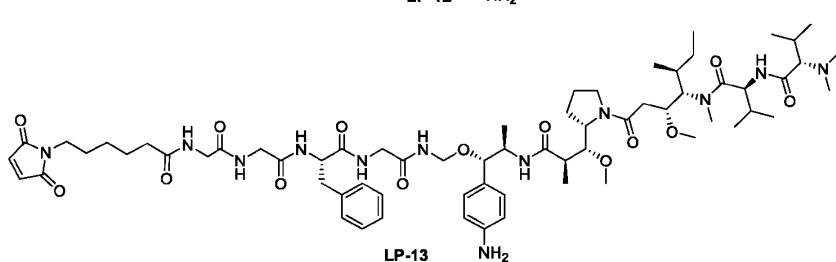
LP-10



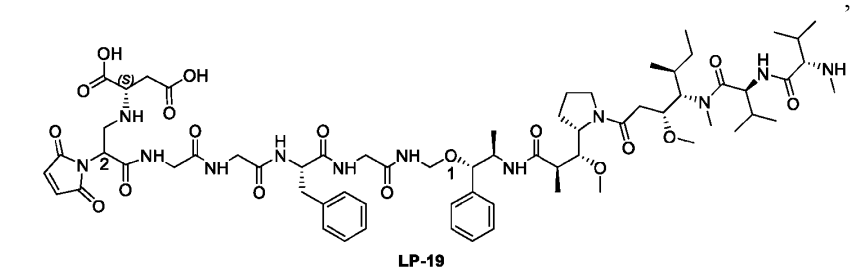
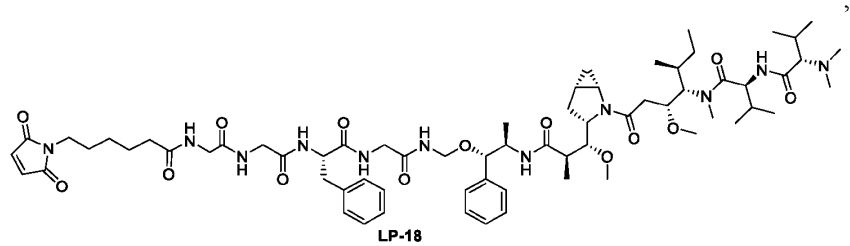
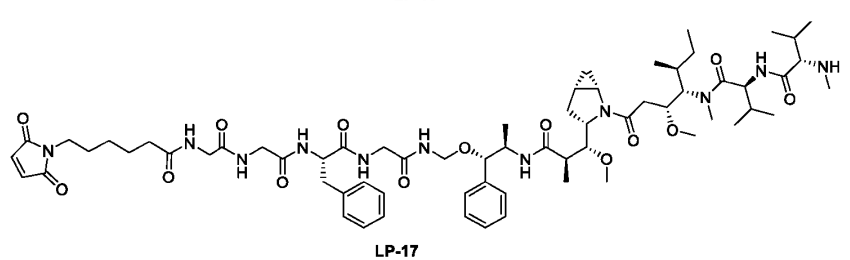
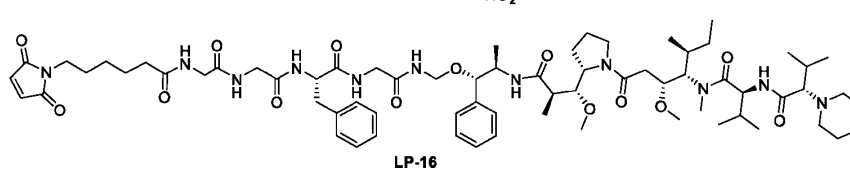
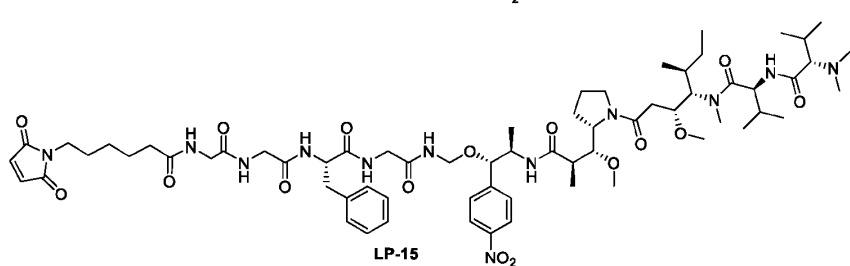
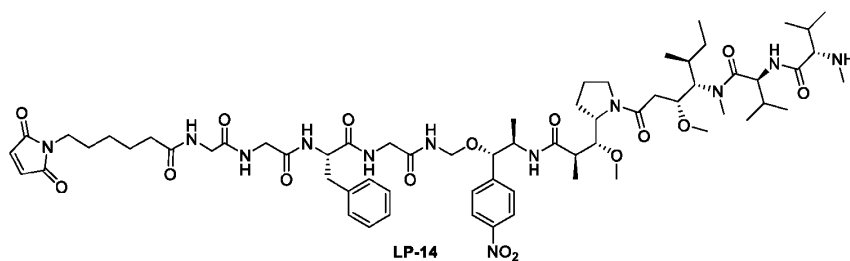
LP-11

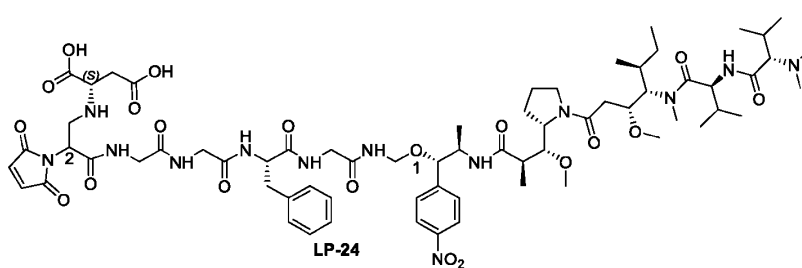
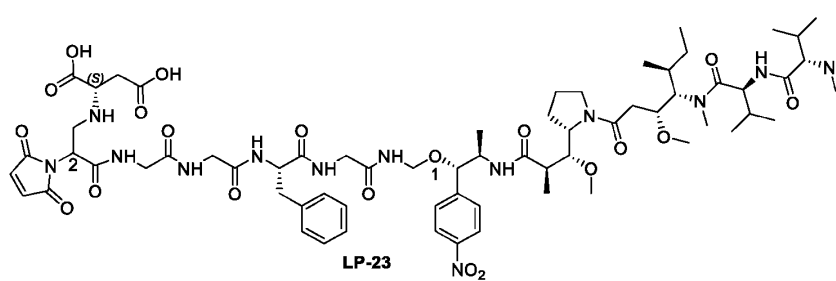
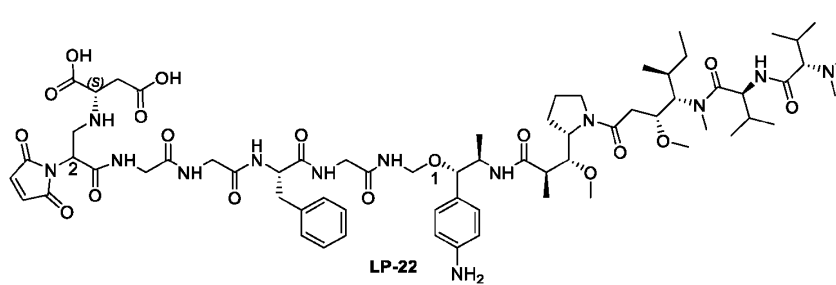
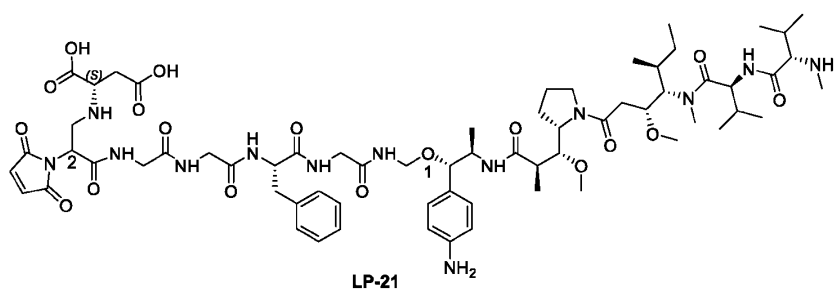
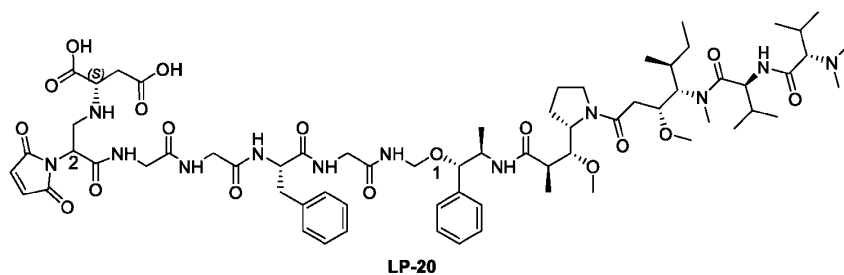


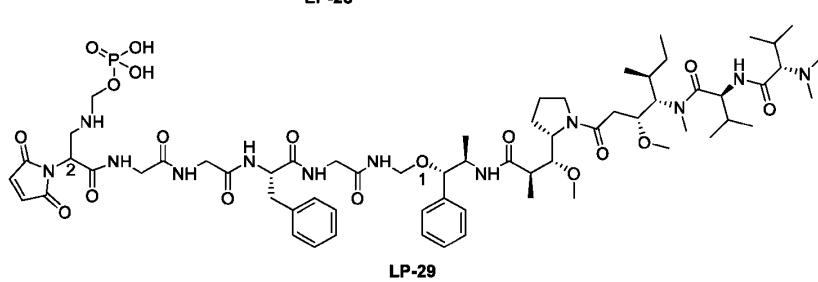
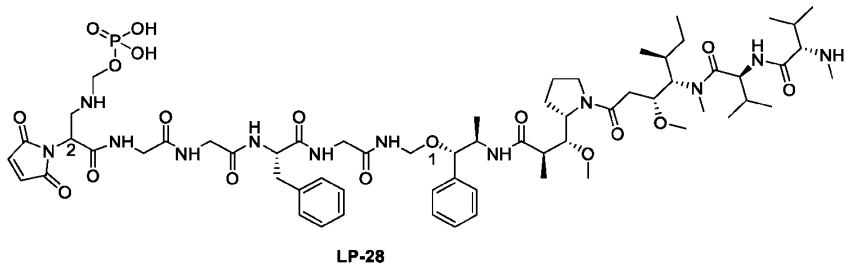
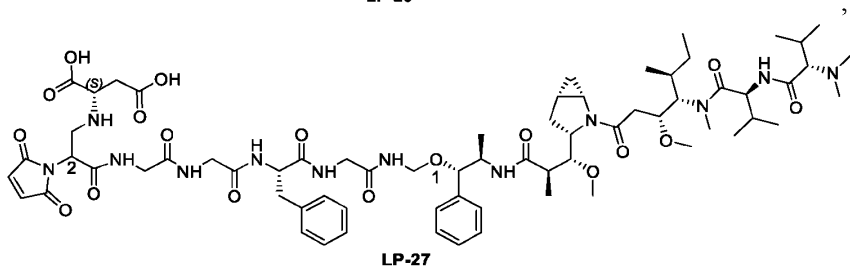
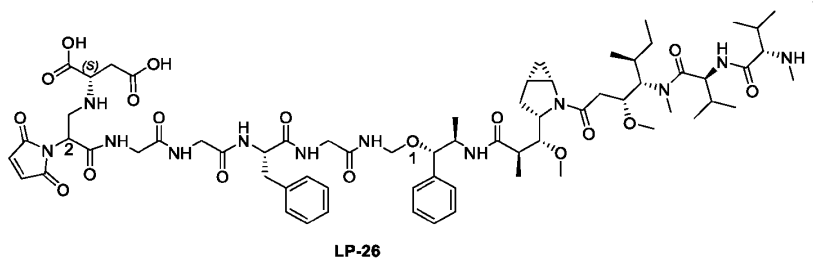
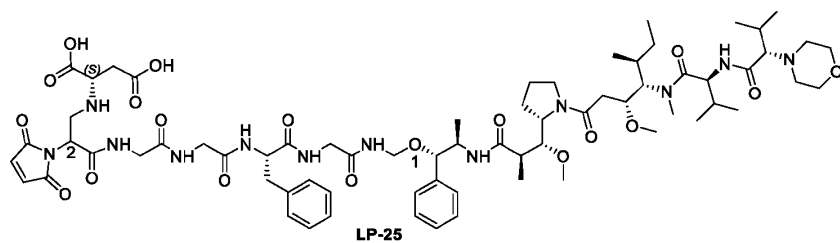
LP-12

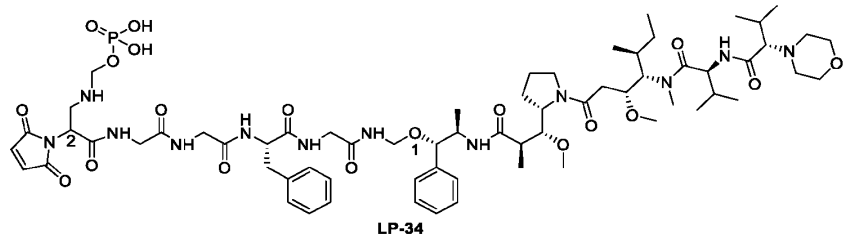
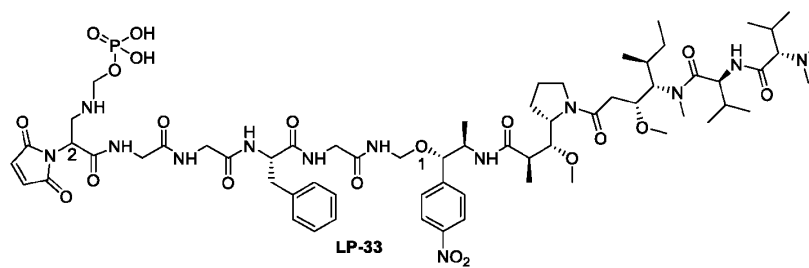
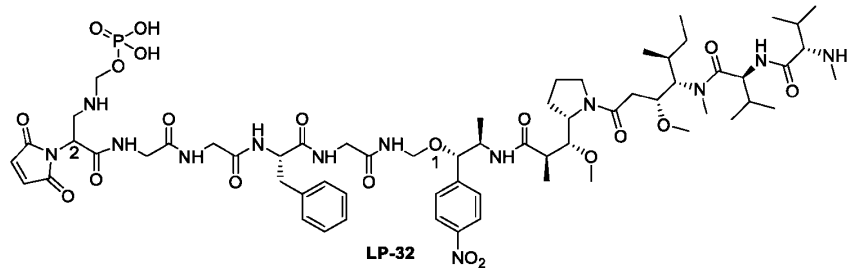
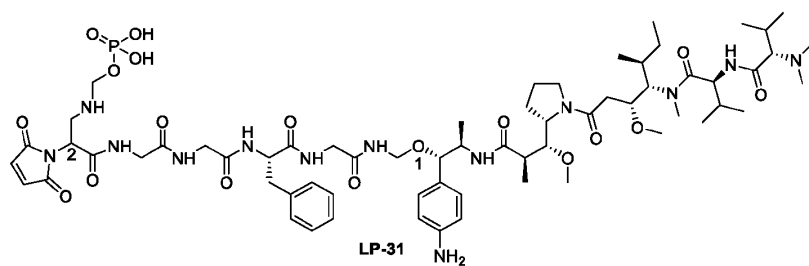
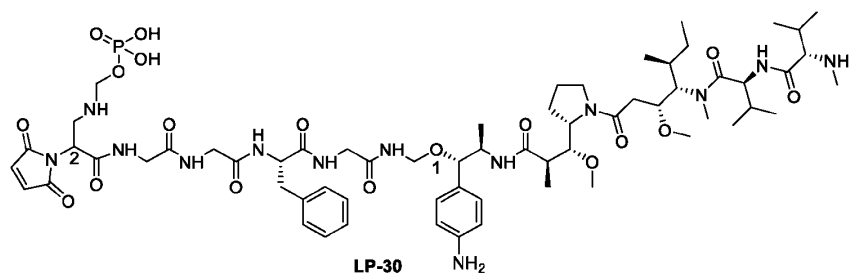


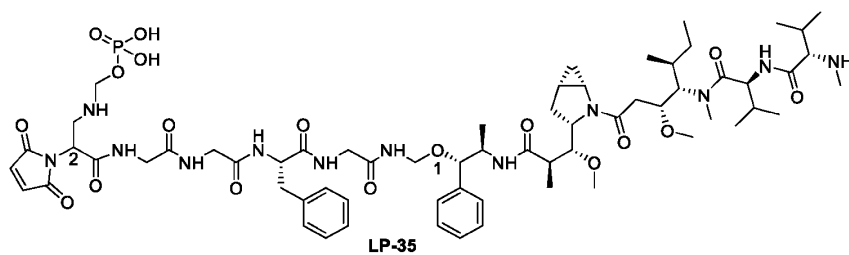
LP-13



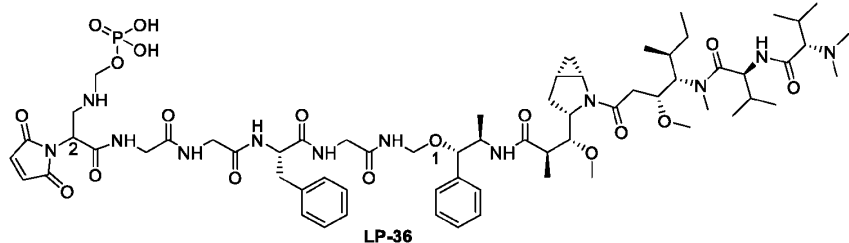




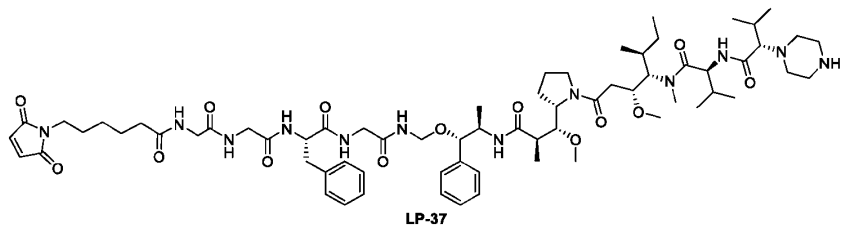




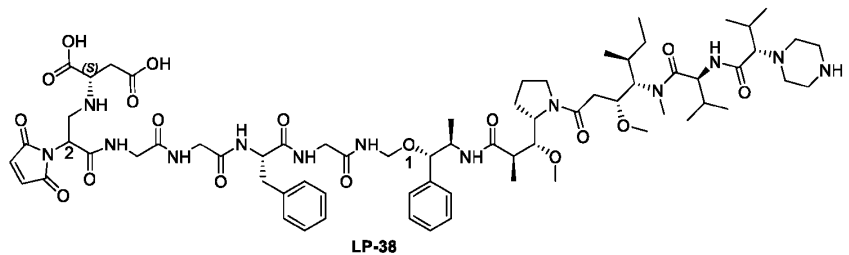
LP-35



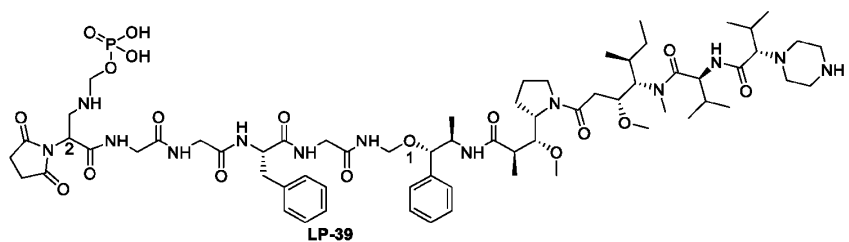
LP-36



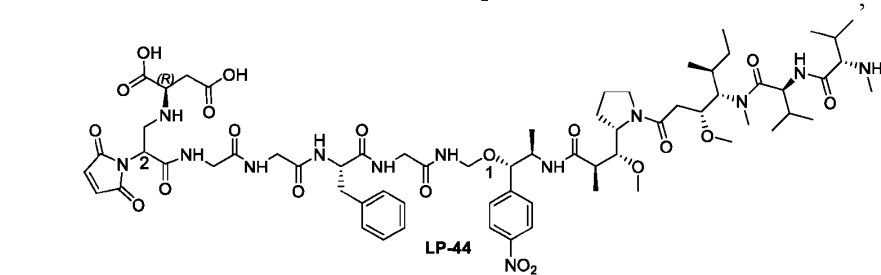
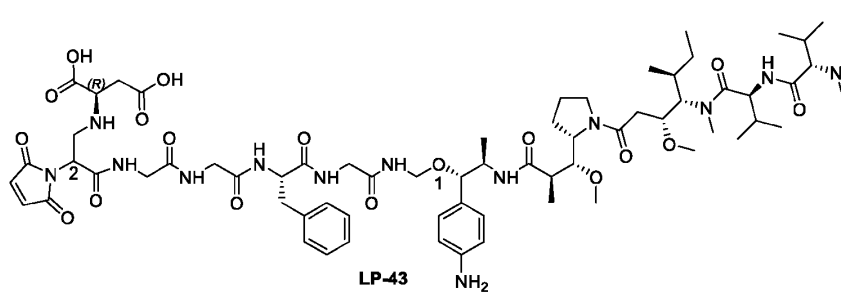
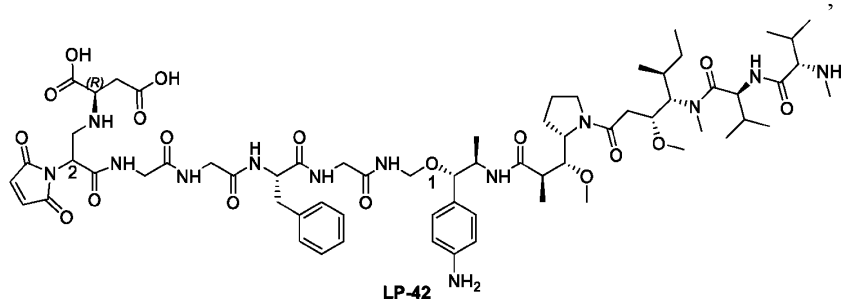
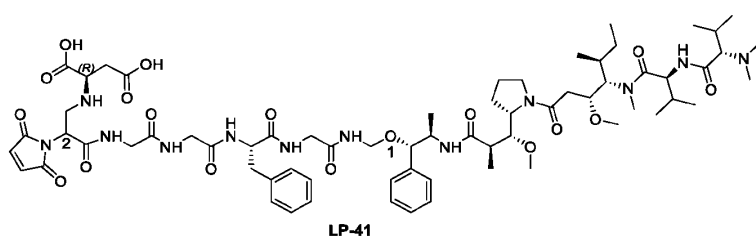
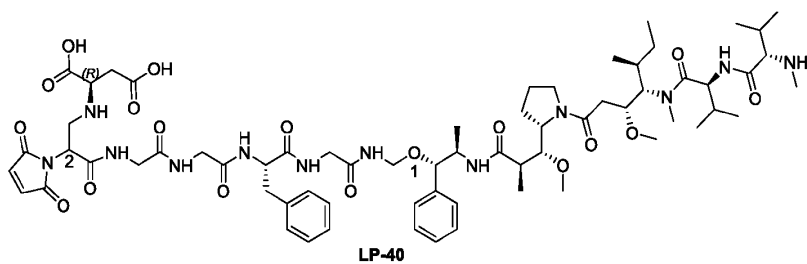
LP-37

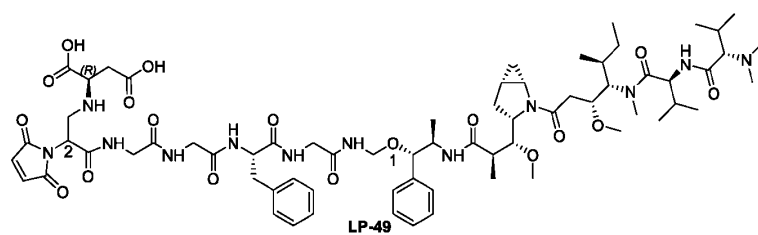
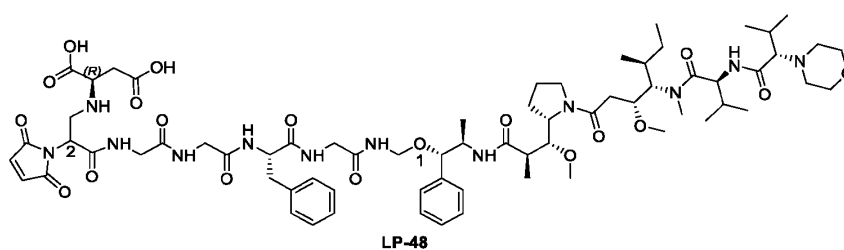
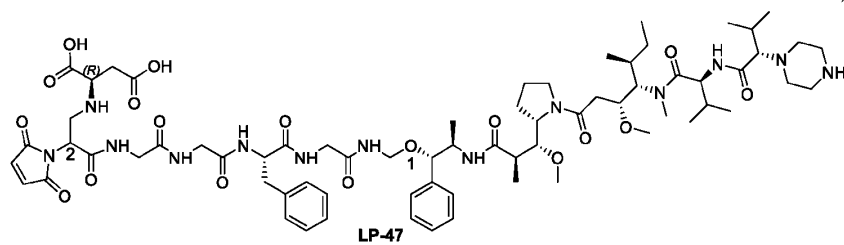
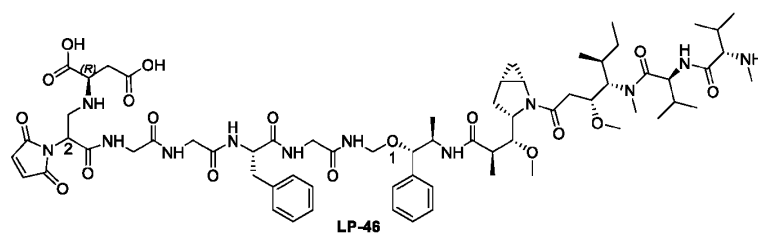
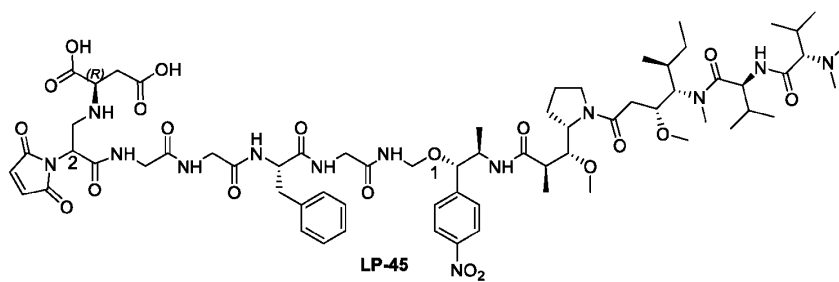


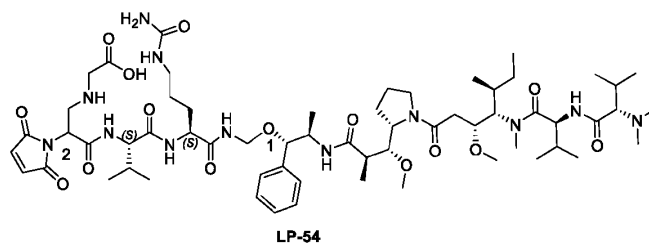
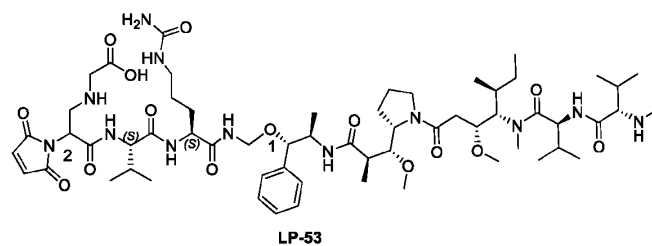
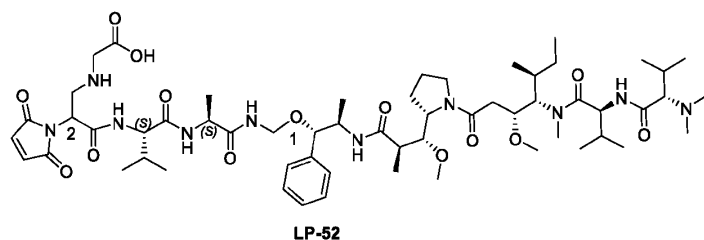
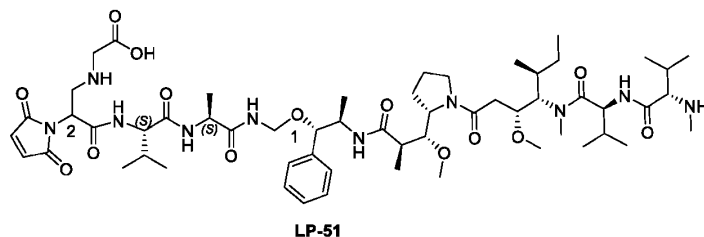
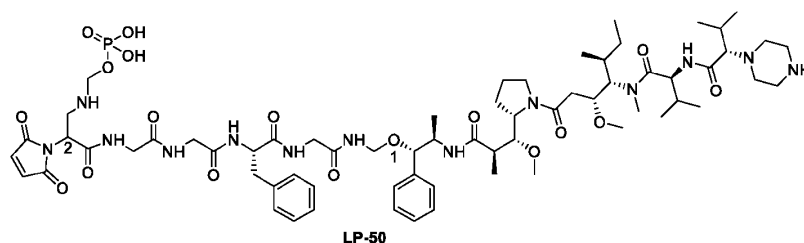
LP-38



LP-39







or

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

18. The use of the antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-13, or the linker- drug compound of any one of Claims 14-17, its isomeric, endo-, racemic, enantiomeric, or mixture thereof, or a pharmaceutically acceptable salt or solvate thereof, for the preparation of a drug, said drug is used for the

treatment or prevention of tumors, wherein:

said tumor expresses HER2,

said tumor is cancer, or

said tumor is breast cancer, ovarian cancer, cervical cancer, uterine cancer, prostate cancer, renal cancer, urethral 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, polymorphous glioblastoma, sarcoma, lymphoma, or Leukemia.

19. A pharmaceutical composition comprising an effective amount of the antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-13, or the linker-drug compound of any one of Claims 14-17, its isomeric, endo-, racemic, enantiomeric, or mixture thereof, or a pharmaceutically acceptable salt or solvates, wherein, optionally, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier, diluent or excipient.

20. A pharmaceutical formulation comprising an effective amount of the antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-13, or the linker-drug compound of any one of Claims 14-17-, its isomeric, endo-, racemic, enantiomeric, or mixture thereof, a pharmaceutically acceptable salt or solvent compounds.

21. The use of the linker-drug compound of any one of Claims 14-17, its isomer, endo-, racemate, enantiomer, or mixtures thereof, or pharmaceutically acceptable salts or solvates thereof, for the preparation of antibody-drug couplings or pharmaceutically acceptable salts or solvates thereof, wherein, optionally, said antibody-drug coupling substance is the antibody-drug conjugate according to any one of Claims 1-13.

22. A method for preparing the antibody-drug conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-13, said method comprising:

reacting a reduced antibody or antigen-binding fragment thereof with the linker-drug compound according to any one of Claims 14-17, its isomers, endo-, racemates, enantiomers, or mixtures thereof, or a pharmaceutically acceptable salt or solvent compound thereof, to obtain said antibody-drug conjugate.