

CONJUGATE OF ERIBULIN DRUG

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present disclosure is based on and claims priority to CN patent application No. 202310082754.5, filed on January 17, 2023, CN patent application No. 202310563948.7, filed on May 18, 2023, and CN patent application No. 2024100454262, filed on January 11, 2024. The entire content of each of these applications is hereby incorporated by reference.

TECHNICAL FIELD

[0002] The present invention relates to the technical field of medicine, in particular to a ligand-drug conjugate, a linker-drug compound, and preparation methods and uses thereof.

BACKGROUND

[0003] As a specific treatment that can target cancer cells, the development of ADCs (antibody-drug conjugates) has made significant progress since 2000. Currently, there are 15 ADC drugs on the market around the world, namely Mylotarg, Besponsa, Kadcyla, Polivy, Lumoxiti, Enhertu, Adcetris, Padcev, Tivdak, Blenrep, Trodelvy, Akalux, Zynlonta, Disitamab Vedotin and Elahere. The treatment fields are mainly focused on hematological tumors and solid tumors. An ADC consists of three key components: a monoclonal antibody or an antibody fragment that selectively binds to an antigen on tumor cell surface, a cleavable and non-cleavable linker, and a small molecule cytotoxin. The ADC makes full use of the specificity of the antibody binding to the tumor cell antigen and the high efficiency of small molecule drug.

[0004] Microtubules are hollow fiber filaments composed of α -tubulin and β -tubulin. They are one of the components of the cytoskeleton and play an important role in cell signal transduction, intracellular migration and transport, and cell shape maintenance. Moreover, during mitosis, microtubule rearranges to form spindles, which are crucial for the movement and separation of sister chromatids. Most of the biological functions of microtubule in cells are regulated by polymerization kinetics, and interfering with the dynamic behavior of microtubule can have a significant impact on spindle formation and cell division. Because cancer cells divide rapidly, they are more sensitive than normal cells to drugs that affect the normal function of tubulin. Currently, important microtubule inhibitors used as ADC payloads include Auristatin and Eribulin, which are derived from marine species, and Maytansinoids derived from plants.

[0005] Eribulin is a simplified synthetic derivative of macrocyclic compound Halichondrin B. In

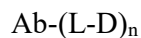
November 2010, Eribulin (methanesulfonate) was first approved by the US FDA for clinical treatment of metastatic breast cancer patients who have received at least two chemotherapy regimens (including anthracycline and taxane chemotherapy drugs). Its trade name is Halaven™, developed and marketed by Eisai. Eribulin can selectively bind to the β -tubulin (+) end to inhibit the elongation of microtubules. In addition to directly killing cancer cells, it can also transform the tumor microenvironment and serve as a small molecule toxin to enhance the “bystander effect” of the ADC. The most common adverse reactions of Eribulin are fatigue, neutropenia, hair loss, peripheral neuropathy, nausea, constipation, etc. At present, there are few studies on ADCs with Eribulin as the warhead, and all of them have the linker introduced on the amino group at C35 of Eribulin. There are no instances of the linker being introduced on the hydroxyl group at C34. Moreover, there is no research on using the precursor of Eribulin having dihydroxyl groups as the warhead.

[0006] To sum up, although Eribulin can be used clinically as a single drug, designing a stable ADC molecule containing Eribulin or its derivatives for targeted delivery to tumor cells can make the treatment safer and more effective and better meet clinical needs.

SUMMARY OF THE INVENTION

[0007] Based on a comprehensive understanding of ADC drugs, the inventors of the application creatively designed and synthesized a series of ADC molecules containing Eribulin or its derivatives. Through experiments, it was found that the designed ADC molecules exhibited good anti-tumor activity.

[0008] In a first aspect, the present application relates to a ligand-drug conjugate of formula I or a pharmaceutically acceptable salt or solvate thereof,



Formula I

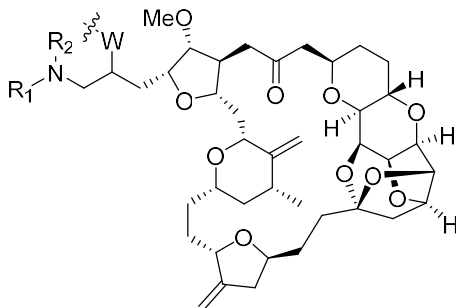
[0009] wherein,

[0010] Ab is a ligand unit;

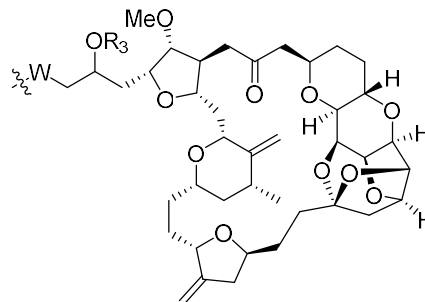
[0011] L is a linker for covalently connecting Ab to D;

[0012] n is selected from integers or decimals between 1 and 40;

[0013] -D is as shown in formula II or formula III:



Formula II



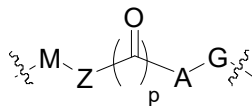
Formula III

[0014] wherein W is an oxygen atom or a sulfur atom,

[0015] R₁ and R₂ are the same or different, and are each independently selected from: hydrogen atom, alkyl, alkoxy, alkenyl, cycloalkyl, aryl, heteroaryl, heterocyclyl, -C(O)-Q₁-Q₂ and -SO₂-Q₁-Q₂, wherein Q₁ is selected from the group consisting of O, N, S atoms and a chemical bond, Q₂ is selected from the group consisting of alkyl, cycloalkyl, heterocyclyl, spirocyclyl, bridged ring, alkenyl, aryl and heteroaryl; optionally, the alkyl, alkoxy, alkenyl, cycloalkyl, heterocyclyl, spirocyclyl, bridged ring, aryl and heteroaryl are each independently

[0016] substituted by one or more substituents selected from the group consisting of hydrogen atom, alkyl, alkoxy, halogen, deuterium, amino, cyano, hydroxy, mercapto, azido, nitro, carboxyl, acyl, carbonyl, hydroxyalkyl, cycloalkyl, heterocyclyl, aryl and heteroaryl; or R₁ and R₂, together with a nitrogen atom connected thereto, form a 3- to 8-membered heterocyclyl; optionally, the heterocyclyl is substituted by one or more substituents selected from the group consisting of hydrogen atom, alkyl, alkoxy, halogen, deuterium, amino, cyano, hydroxy, mercapto, azido, nitro, carboxyl, acyl, carbonyl, hydroxyalkyl, cycloalkyl, heterocyclyl, aryl and heteroaryl; R₃ is selected from the group consisting of hydrogen atom, alkyl, acyl, sulfonyl, cycloalkyl, heterocyclyl, aryl and heteroaryl; optionally, the alkyl, acyl, sulfonyl, cycloalkyl, heterocyclyl, aryl and heteroaryl are each independently substituted by one or more substituents selected from the group consisting of hydrogen atom, alkyl, alkoxy, halogen, deuterium, amino, cyano, hydroxy, mercapto, azido, nitro, carboxyl, acyl, carbonyl, hydroxyalkyl, cycloalkyl, heterocyclyl, aryl and heteroaryl.

[0017] In some embodiments, L has a structure shown in formula IV:



Formula IV

[0018] wherein,

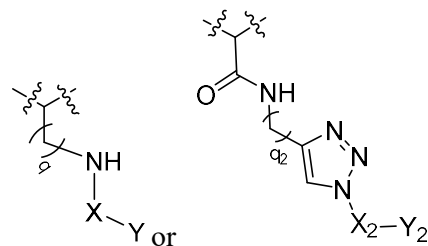
[0019] M is a linking unit covalently connected to Ab;

[0020] Z is selected from the group consisting of -C1-C10 alkylene-, -C3-C8 carbocycle-, -

[illegible]

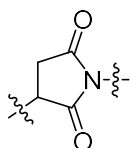
[0021] A is a peptide residue consisting of 2 to 7 amino acids;

[0023] p is 0 or 1.

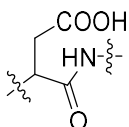


[0024] In some embodiments, a wavy line on the left in indicates connecting to a linking site on M, and a wavy line on the right indicates connecting to a carbonyl.

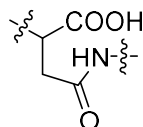
[0025] In some embodiments, the linking unit M has a succinimide structure as represented by formula a, or a structure as represented by formula b or formula c in which the ring in succinimide group is in open form:



Formula a



Formula b

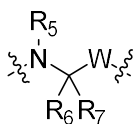


Formula c

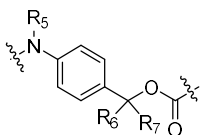
[0026] In formula a, formula b or formula c, a wavy line on the left indicates connecting to a linking site on Ab, and a wavy line on the right indicates connecting to a linking site on Z.

[0027] In some embodiments, A is a polypeptide residue composed of 2 to 7 amino acids selected from the group consisting of phenylalanine, glycine, valine, lysine, citrulline, serine, glutamic acid, aspartic acid, and cysteic acid.

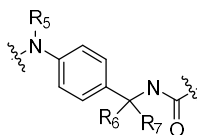
[0028] In some embodiments, the spacer unit G is selected from the group consisting of structures represented by formulas Va, Vb, Vc and Vd and any combinations thereof:



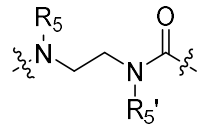
Formula Va



Formula Vb



Formula Vc



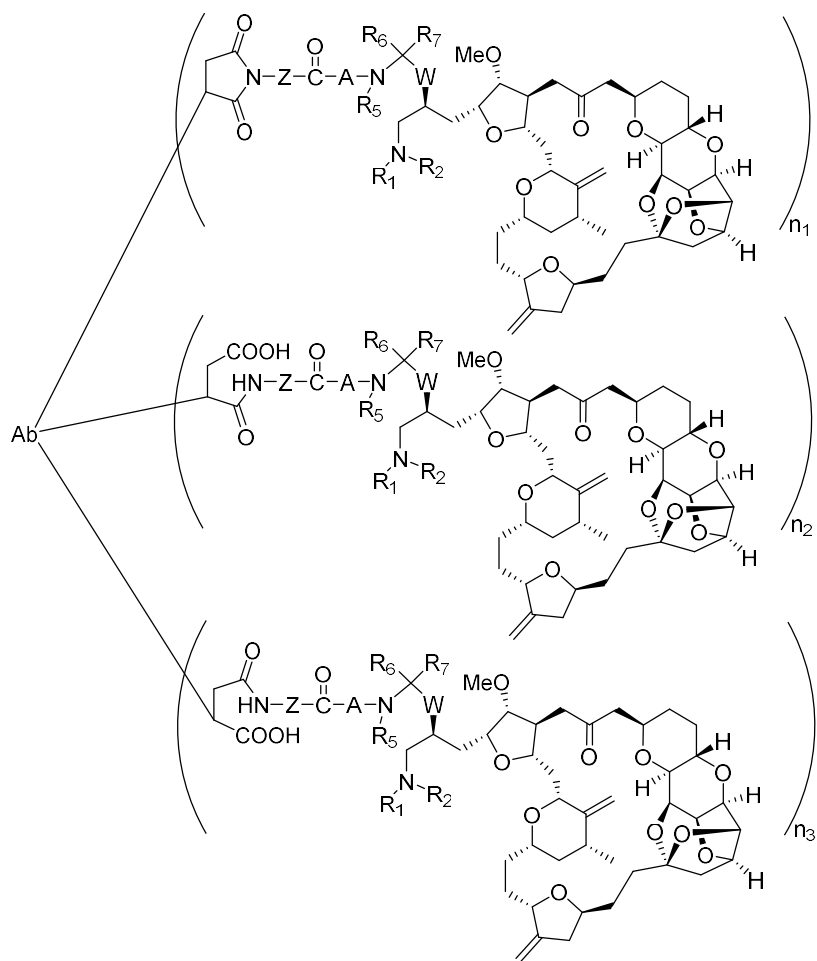
Formula Vd

[0029] wherein a wavy line on the left represents a linking site between a nitrogen atom and peptide residue A, a wavy line on the right represents a linking site between an oxygen atom and drug D, and W is an oxygen atom or a sulfur atom and is a joint group between the drug D and the spacer unit G; and

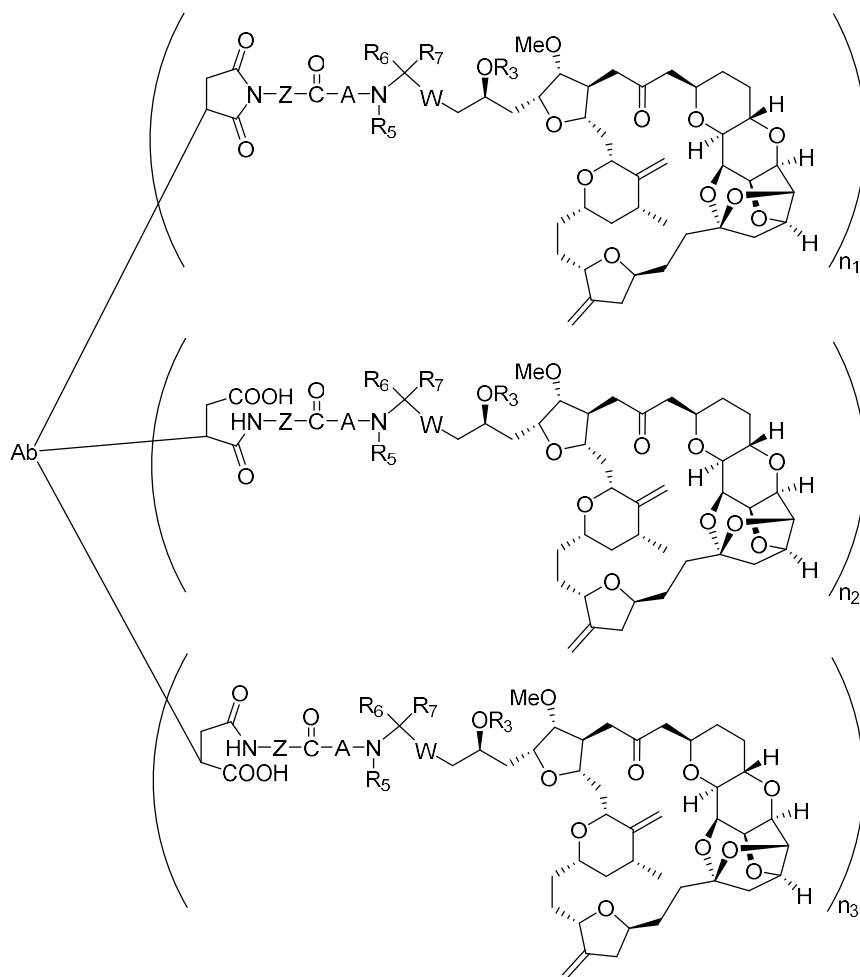
[0030] R_5 , R_5' , R_6 and R_7 are each independently selected from the group consisting of hydrogen atom, deuterium atom, alkyl and substituted alkyl.

[0031] In some embodiments, the ligand-drug conjugate or the pharmaceutically acceptable salt

or solvate thereof of the present application has a structure represented by formula VI or formula VII:



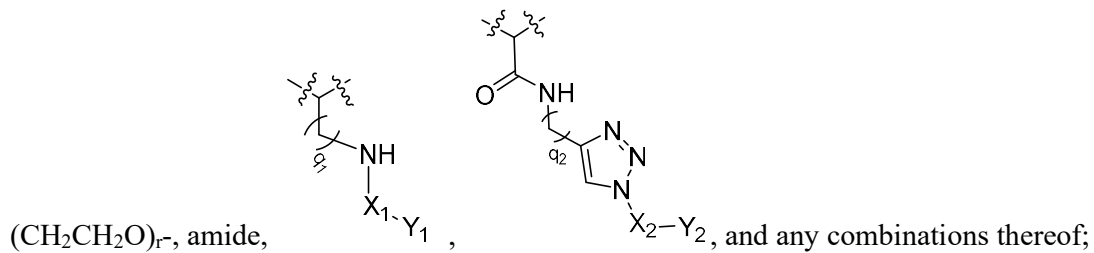
Formula VI



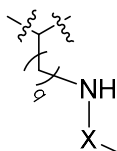
Formula VII

[0032] wherein, the Ab, Z, A, R₅, R₆, R₇, R₃ and W are as defined above; n₁, n₂ and n₃ are independently selected from integers or decimals between 0 and 40 (e.g. 0-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35 or 35-40), n₁, n₂ and n₃ are not 0 at the same time, and n₁+n₂+n₃≤40.

[0033] In some embodiments, Z is selected from the group consisting of -C1-C10 alkylene-, -



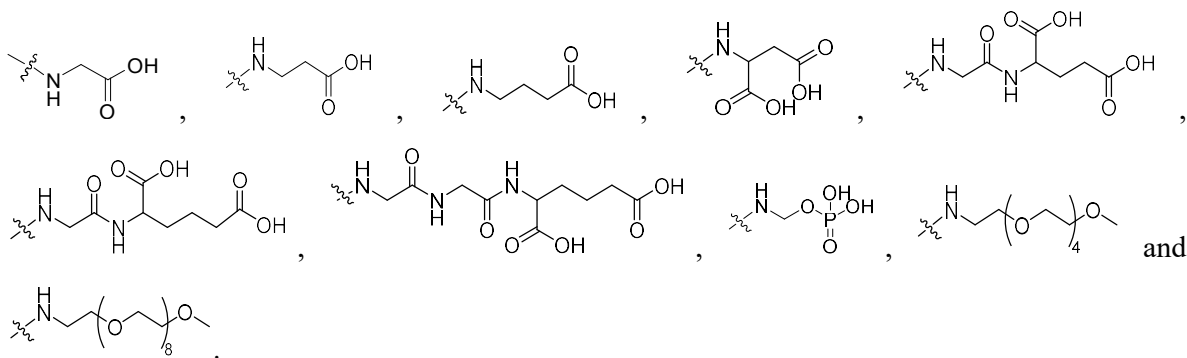
[0034] In some embodiments, Z is -C2-alkylene or -C5-alkylene;



[0035] In some embodiments, Z is -NH-X-Y , wherein, q_1 is selected from integers between 1 and 8 (e.g. 1, 2, 3, 4, 5, 6, 7 and 8).

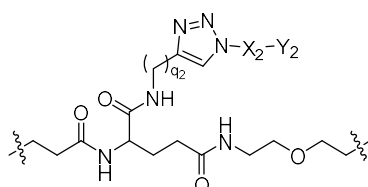
[0036] In some embodiments, q_1 is 1.

[0037] In some embodiments, q_1 is 1, and $\text{-NH-X}_1\text{-Y}_1$ composes the hydrophilic structural unit Ac_1 , the Ac_1 is selected, without limitation, from the group consisting of (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 unnatural amino acid derivatives and the following structures:

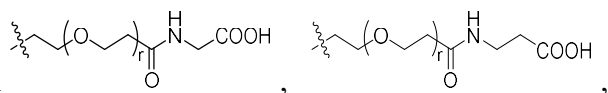


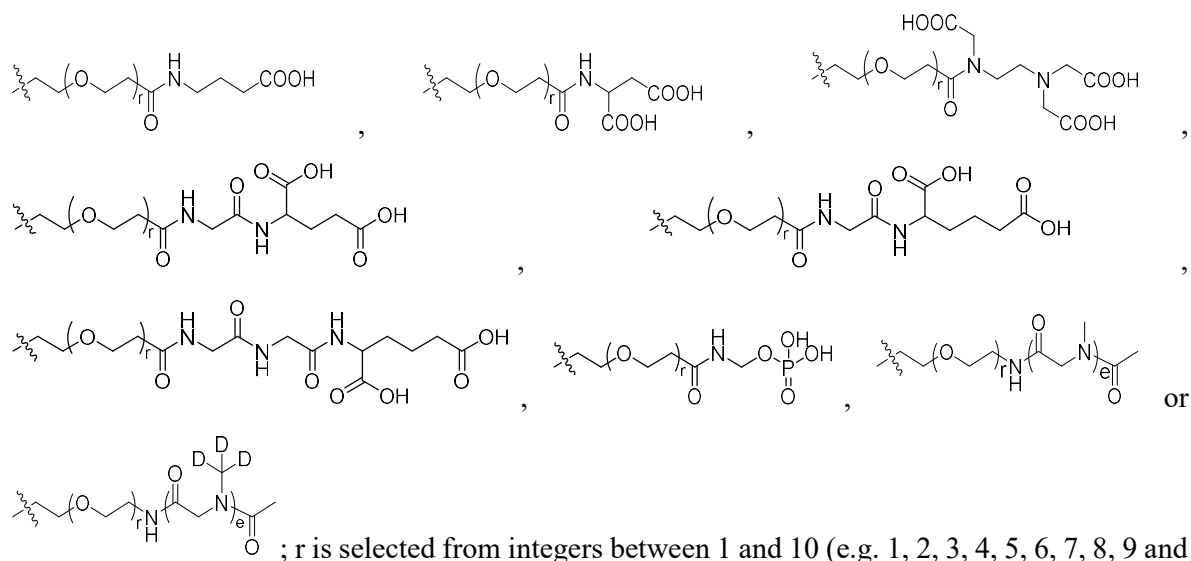
[0038] In some embodiments, q_1 is 1, and $\text{-NH-X}_1\text{-Y}_1$ composes the hydrophilic structural unit

Ac_1 , the Ac_1 is selected from the following structures:

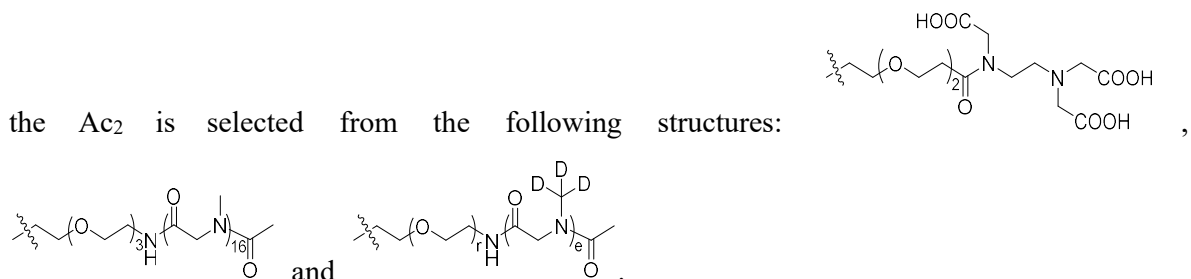
[0039] In some embodiments, Z is , wherein, q_2 is selected from integers between 1 and 8 (e.g. 1, 2, 3, 4, 5, 6, 7 and 8); preferably, q_2 is 1; preferably, X_2 is $\text{-(C1-C10 alkylene)-(CH}_2\text{CH}_2\text{O)}_r\text{-(C=O)-}$ or $\text{-(C1-C10 alkylene)-(CH}_2\text{CH}_2\text{O)}_r\text{-NR}_4\text{-}$.

[0040] In some embodiments, q_2 is 1, and $\text{-X}_2\text{-Y}_2$ composes the hydrophilic structural unit Ac_2 ,

the Ac_2 is selected, without limitation, from .



[0041] In some embodiments, q_2 is 1, and $-X_2-Y_2$ composes the hydrophilic structural unit Ac_2 ,



[0042] In some embodiments, A is a polypeptide residue composed of 2 to 5 amino acids selected from the group consisting of phenylalanine, glycine, valine, lysine, citrulline, serine, glutamic acid, aspartic acid, and cysteine acid.

[0043] In some embodiments, A is a peptide residue formed by 2 to 4 amino acids selected from phenylalanine and glycine.

[0044] In some embodiments, A is a tetrapeptide residue composed of glycine (G), glycine (G), phenylalanine (F), and glycine (G).

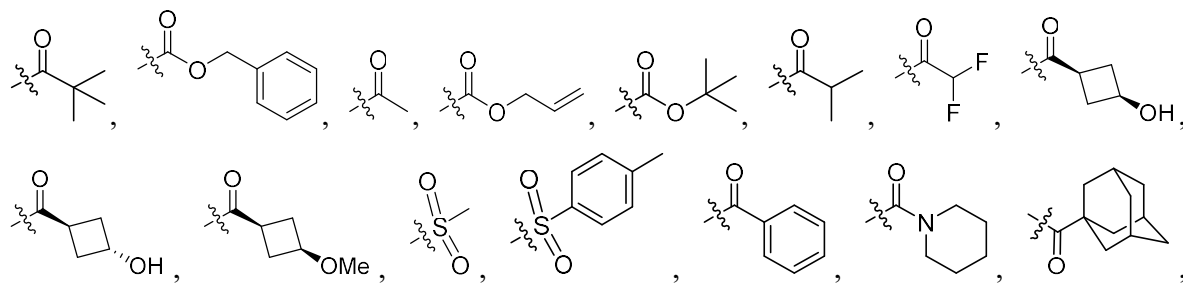
[0045] In some embodiments, A is -GGFG-.

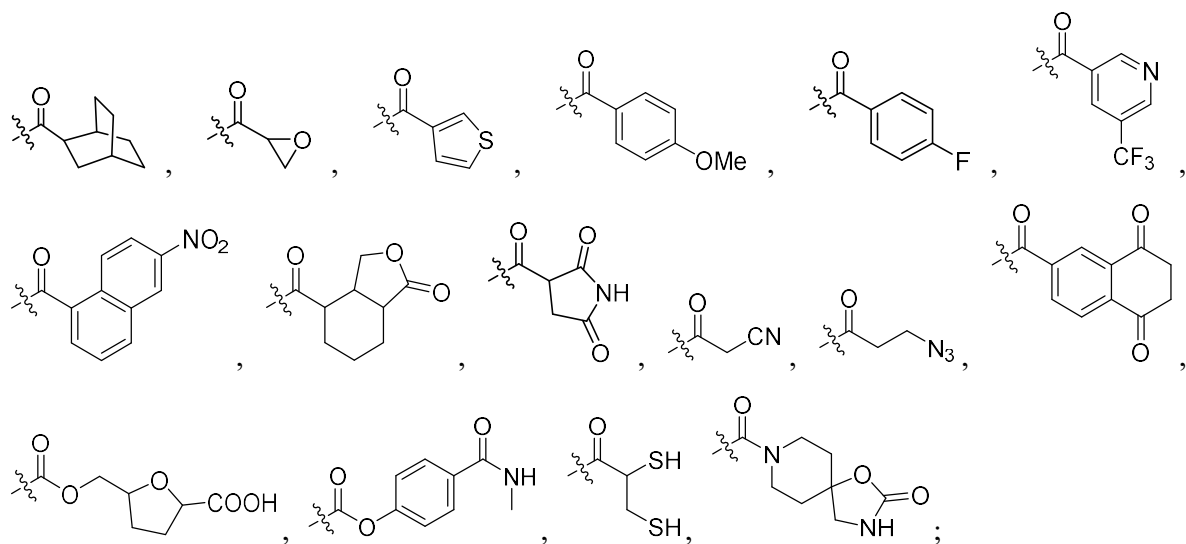
[0046] In some embodiments, W is an oxygen atom or a sulfur atom, R_1 and R_2 are the same or different, and are each independently selected from the group consisting of: hydrogen atom, alkyl, alkoxy, alkenyl, cycloalkyl, $-C(O)-Q_1-Q_2$ and $-SO_2-Q_1-Q_2$, wherein Q_1 is selected from the group consisting of O and a chemical bond, and Q_2 is selected from the group consisting of alkyl, heterocyclyl, alkenyl, aryl, heteroaryl and cycloalkyl; optionally, the alkyl, heterocyclyl, alkenyl, aryl, heteroaryl and cycloalkyl are each independently substituted with one or more substituents

selected from the group consisting of hydrogen atom, alkyl, hydroxyalkyl, halogen, deuterium, mercapto, amino, hydroxy, cycloalkyl and heterocyclyl; or R₁ and R₂ together with a nitrogen atom connected thereto, form a 3- to 6-membered heterocyclyl, and optionally the heterocyclyl is substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, alkoxy, halogen, deuterium, amino, aryl, and hydroxyl; R₃ is selected from the group consisting of hydrogen atom and alkyl, and optionally the alkyl is substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, alkoxy, halogen, deuterium, amino, hydroxyl, cycloalkyl and heterocyclyl.

[0047] In some embodiments, W is an oxygen atom, R₁ and R₂ are the same or different, and are each independently selected from the group consisting of: hydrogen atom, alkyl, alkenyl, haloalkyl, -C(O)-Q₁-Q₂ and -SO₂-Q₁-Q₂, wherein Q₁ is selected from the group consisting of O and a chemical bond, Q₂ is selected from the group consisting of alkyl, alkenyl, aryl and 3- to 8-membered cycloalkyl, and optionally, the alkyl, alkenyl, aryl and cycloalkyl are each independently substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, hydroxyalkyl, halogen, aryl, and hydroxyl; or R₁ and R₂ together with a nitrogen atom connected thereto, form a 3- to 6-membered heterocyclyl containing one to two nitrogen atoms and optionally one oxygen atom; optionally, the 3- to 6-membered heterocyclyl is substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, and haloalkyl; R₃ is selected from the group consisting of hydrogen atom and alkyl, and the alkyl is substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, halogen, deuterium, amino and hydroxyl.

[0048] In some embodiments, W is an oxygen atom, R₁ and R₂ are the same or different, and are each independently selected from the group consisting of: hydrogen atom, C₁-C₄ alkyl (such as methyl, ethyl), C₂-C₄ alkenyl (such as allyl), C₁-C₄ haloalkyl (such as difluoroethyl) and the following structures:



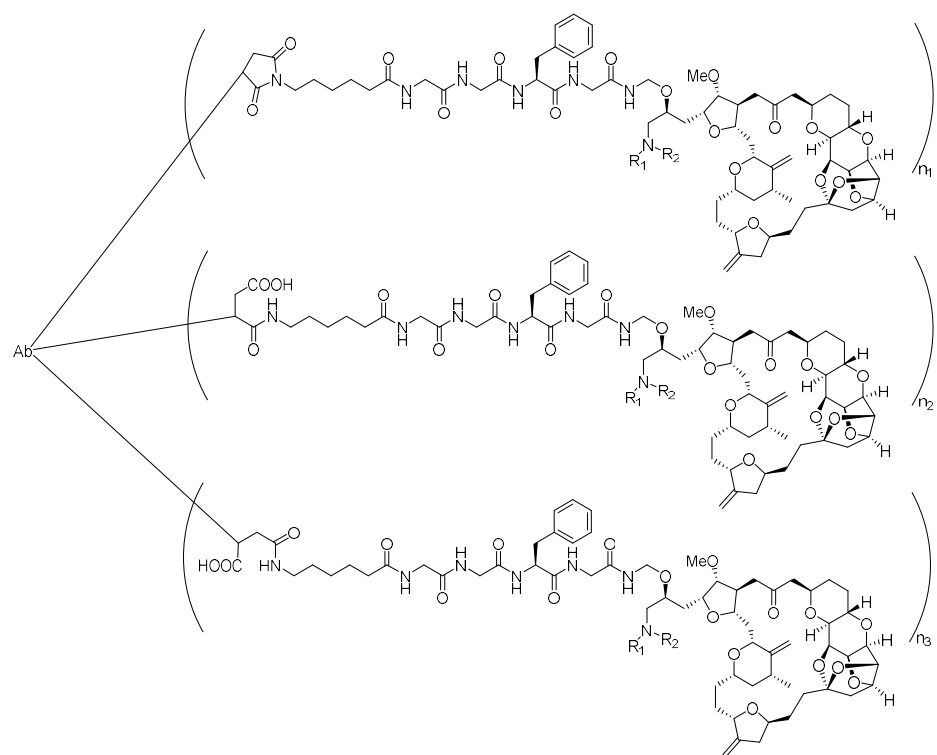


[0049] or R₁ and R₂, together with a nitrogen atom connected thereto, form a 3- to 6-membered heterocyclyl containing one to two nitrogen atoms and optionally one oxygen atom (such as azyclopropyl, piperidinyl, piperazinyl, morpholinyl); optionally, the 3- to 6-membered heterocyclyl is substituted with one or more substituents selected from the group consisting of hydrogen atom, C₁-C₄ alkyl (e.g., methyl), and C₁-C₄ haloalkyl (e.g., trifluoromethyl); and R₃ is selected from the group consisting of hydrogen atom and methyl.

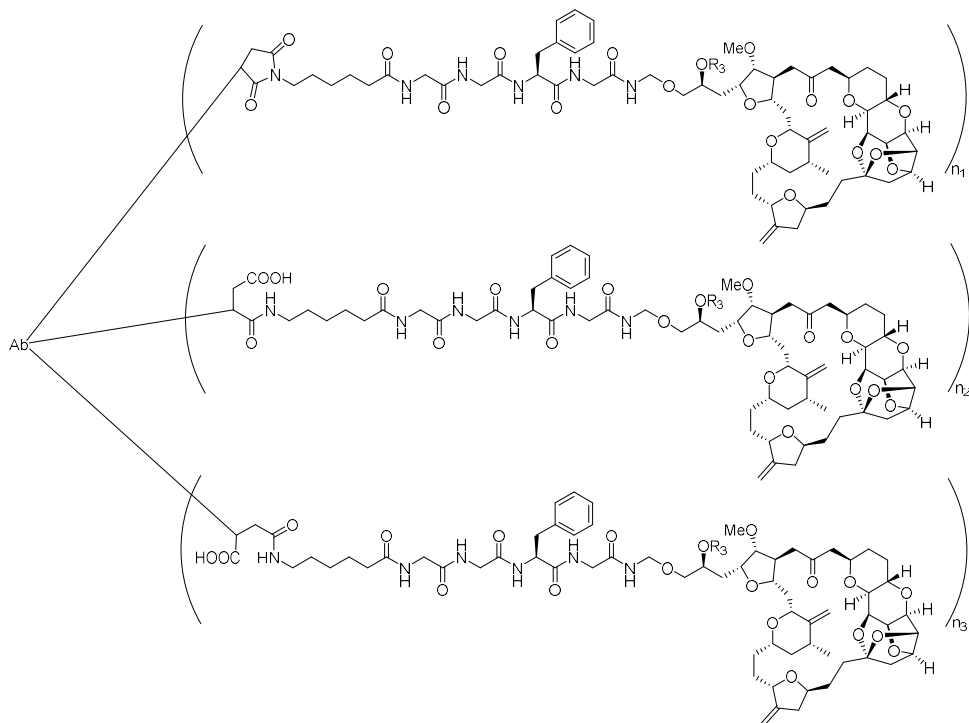
[0050] In some embodiments, R₅, R₆ and R₇ are each independently selected from the group consisting of hydrogen atom, deuterium atom, alkyl, haloalkyl, deuterated alkyl and hydroxyalkyl.

[0051] In some embodiments, R₅, R₆ and R₇ are simultaneously hydrogen atoms.

[0052] In some embodiments, the ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof of the present application has a structure represented by formula VIa or formula VIIa:



Formula VIa

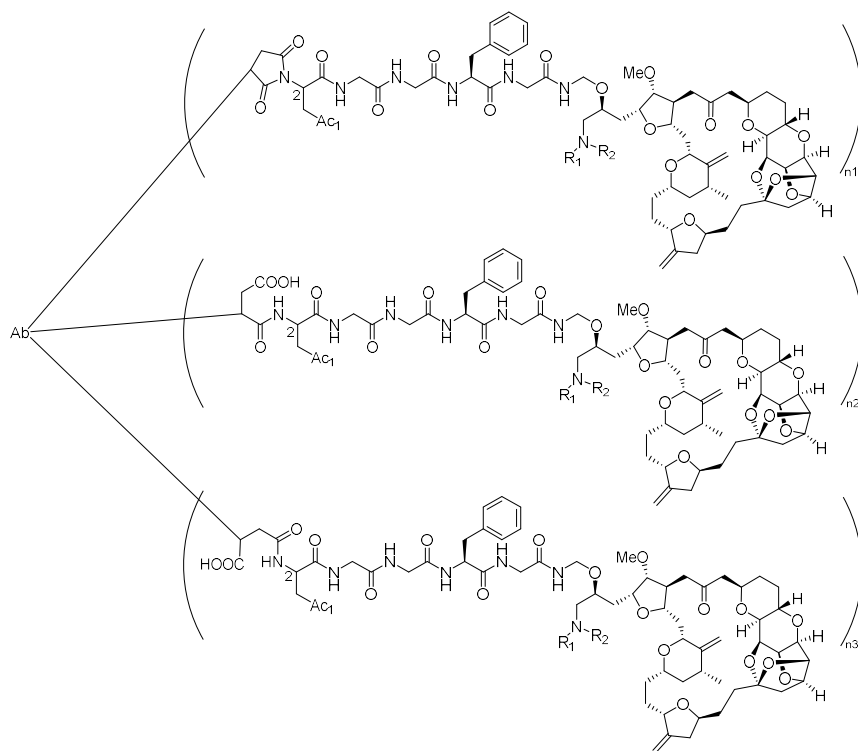


Formula VIIa

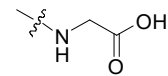
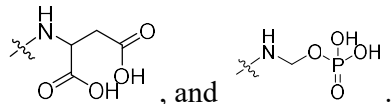
[0053] wherein, the R_1 , R_2 , R_3 , n_1 , n_2 and n_3 are as defined above.

[0054] In some embodiments, the ligand-drug conjugate or the pharmaceutically acceptable salt

or solvate thereof of the present application has a structure represented by formula VIb or formula VIIb:



Formula VIb

*NC(=O)O

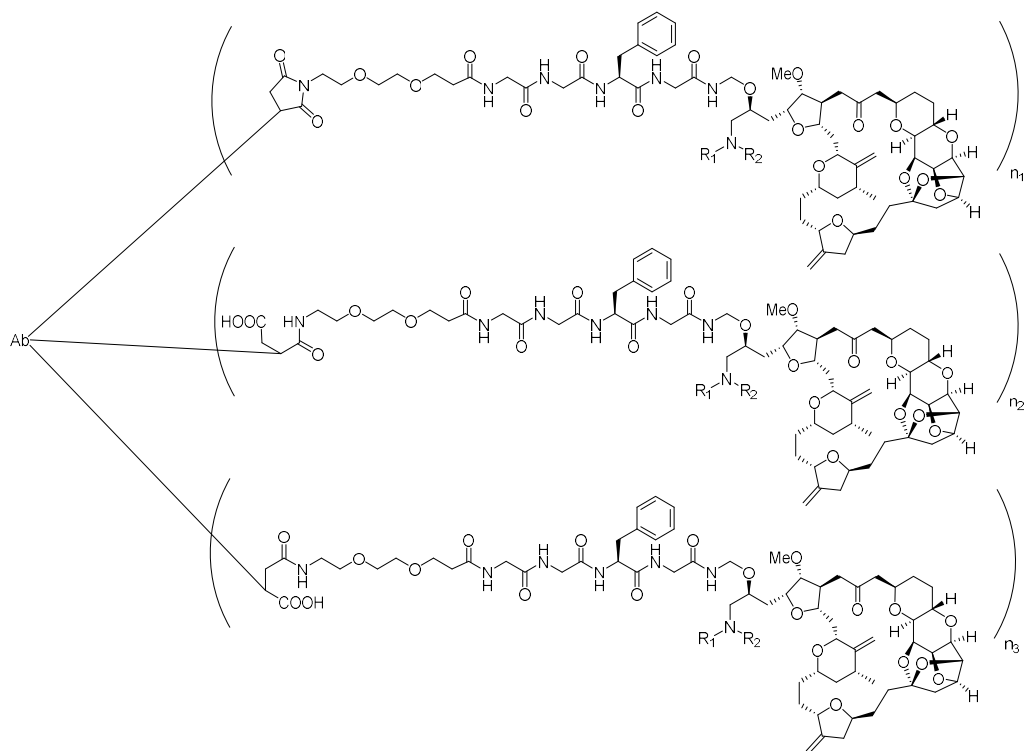
The figure displays three chemical structures of antibodies (Ab) conjugated to a dendritic molecule, labeled Ab1, Ab2, and Ab3. Each structure consists of an antibody (Ab) linked to a dendritic molecule via a linker. The linker is a common structure for all three, featuring a succinyl group, a carboxymethyl group, and a carboxylic acid group. The dendritic molecule is a complex, branched structure with multiple hydroxyl groups and a terminal carboxylic acid group. The structures are shown in a schematic representation, with the antibody part enclosed in a box and the dendritic part shown as a separate structure.

The figure displays three chemical structures of antibodies (Ab) conjugated to a dendritic molecule, labeled Ab₁, Ab₂, and Ab₃. The antibodies are represented by a Y-shaped structure with a central 'Ab' label. The dendritic molecule is a complex, branched structure with multiple hydroxyl groups and a central core. The conjugation is achieved through a linker that includes a benzamide group and a chiral auxiliary (R₁, R₂).

- Ab₁**: The antibody is linked to the dendritic molecule via a succinyl group (a five-membered ring with two carbonyl groups).
- Ab₂**: The antibody is linked to the dendritic molecule via a carboxylic acid group (HOOC).
- Ab₃**: The antibody is linked to the dendritic molecule via a succinyl group (a five-membered ring with two carbonyl groups).

The linker structure is common to all three conjugates and includes a benzamide group (a benzene ring attached to an amide group) and a chiral auxiliary (R₁, R₂) which is a 1,2-diol derivative.

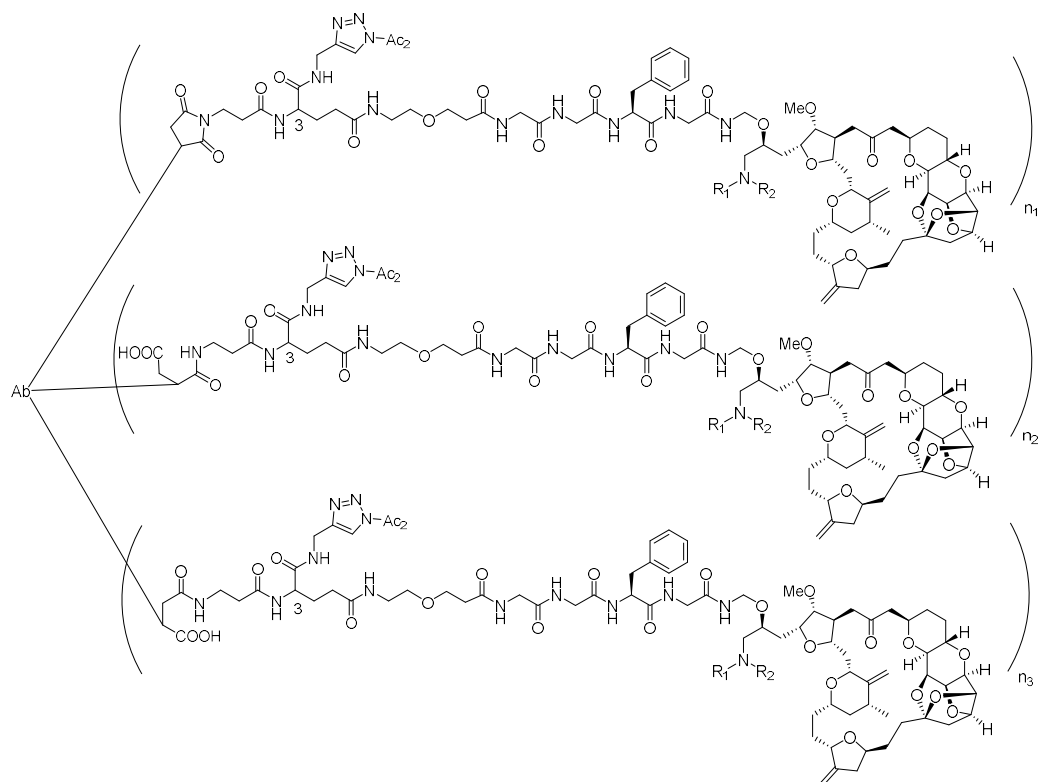
The figure displays three chemical structures of antibodies (Ab) conjugated to a dendritic molecule, labeled Ab₁, Ab₂, and Ab₃. The antibodies are linked to the dendritic structure via a common linker. The linker consists of a benzyl group connected to a carbonyl group, which is further connected to a chain of amide bonds. The dendritic structure is a complex, branched molecule with multiple hydroxyl groups and a terminal amine group (R₁NR₂). The antibodies are shown in their natural conformation, with the antigen-binding site (Ab) highlighted in red. The structures are labeled Ab₁, Ab₂, and Ab₃ from top to bottom, indicating different conjugation sites or conditions.



Formula VIId

[0063] wherein the R₁, R₂, n₁, n₂ and n₃ are as defined above.

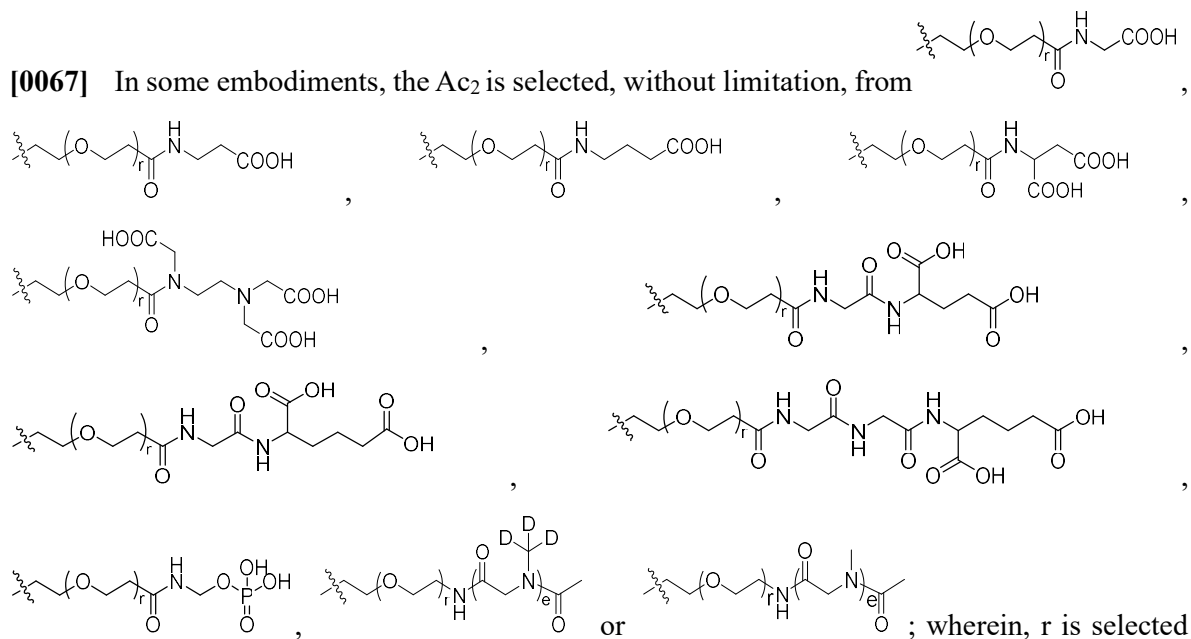
[0064] In some embodiments, the ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof of the present application has a structure represented by formula VIe:



Formula VIe

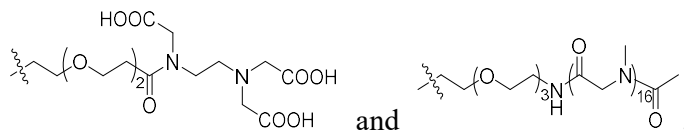
[0065] wherein the R_1 , R_2 , n_1 , n_2 and n_3 are as defined above;

[0066] Ac_2 is a hydrophilic structural unit composed of $-X_2-Y_2$, and the X_2 and Y_2 are as defined above.

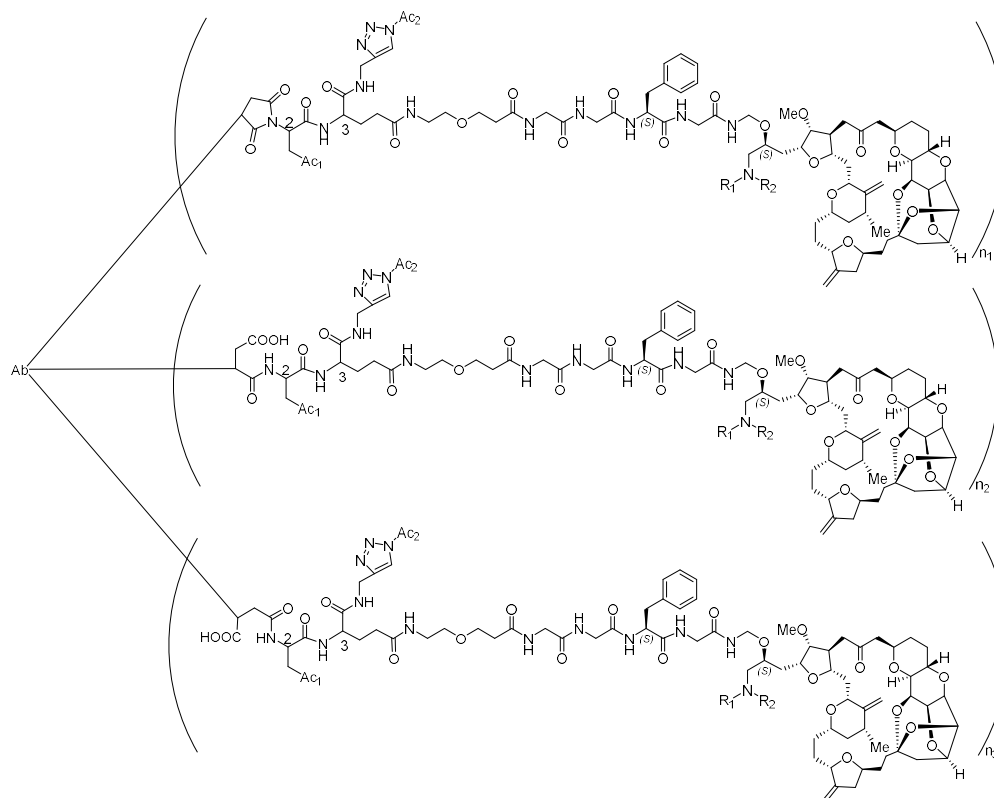


from integers between 1 and 10; e is selected from integers between 1 and 20.

[0068] In some embodiments, Ac₂ is selected from the following structures:



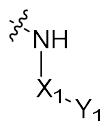
[0069] In some embodiments, the ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof of the present application has a structure represented by formula VI:



Formula VI

[0070] wherein the R₁, R₂, n₁, n₂ and n₃ are as defined above;

[0071] Ac₁ is a hydrophilic structural unit having the structure shown in formula d:

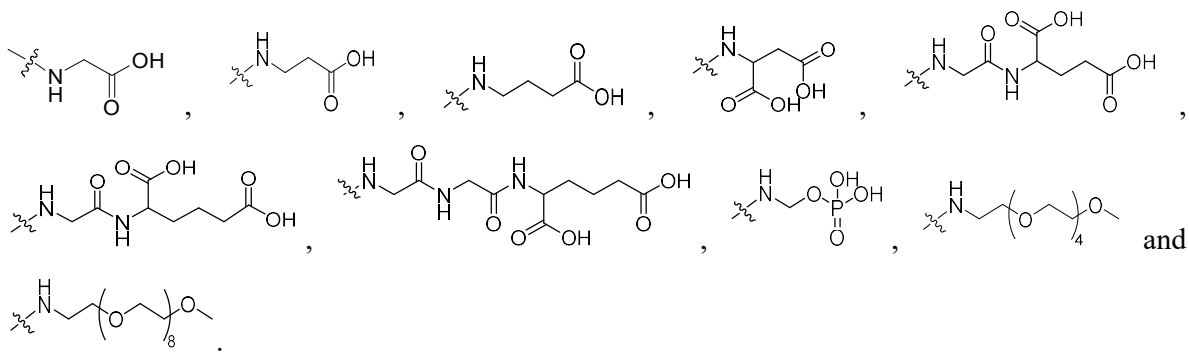


Formula d

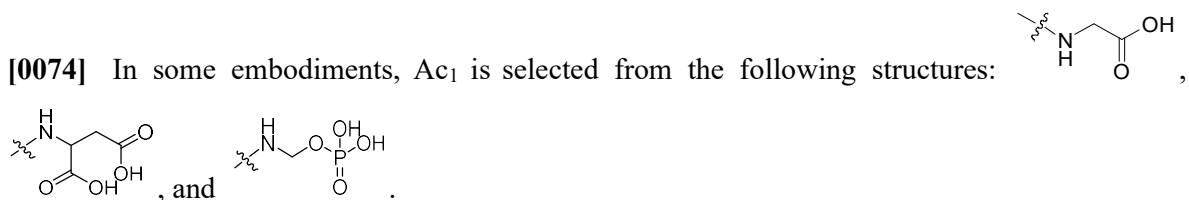
[0072] the X₁ and Y₁ are as defined above;

[0073] In some embodiments, the Ac₁ is selected, without limitation, from the group consisting

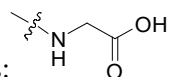
of (D/L) alanine, (D/L) leucine, (D/L) isoleucine, (D/L) valine, (D/L) phenylalanine, (D/L) proline, (D/L) tryptophan, (D/L) serine, (D/L) tyrosine, (D/L) cysteine, (D/L) cystine, (D/L) arginine, (D/L) histidine, (D/L) methionine, (D/L) asparagine, (D/L) glutamine, (D/L) threonine, (D/L) aspartic acid, (D/L) glutamic acid, natural or unnatural amino acid derivatives and the following structures:



[0074] In some embodiments, Ac₁ is selected from the following structures:

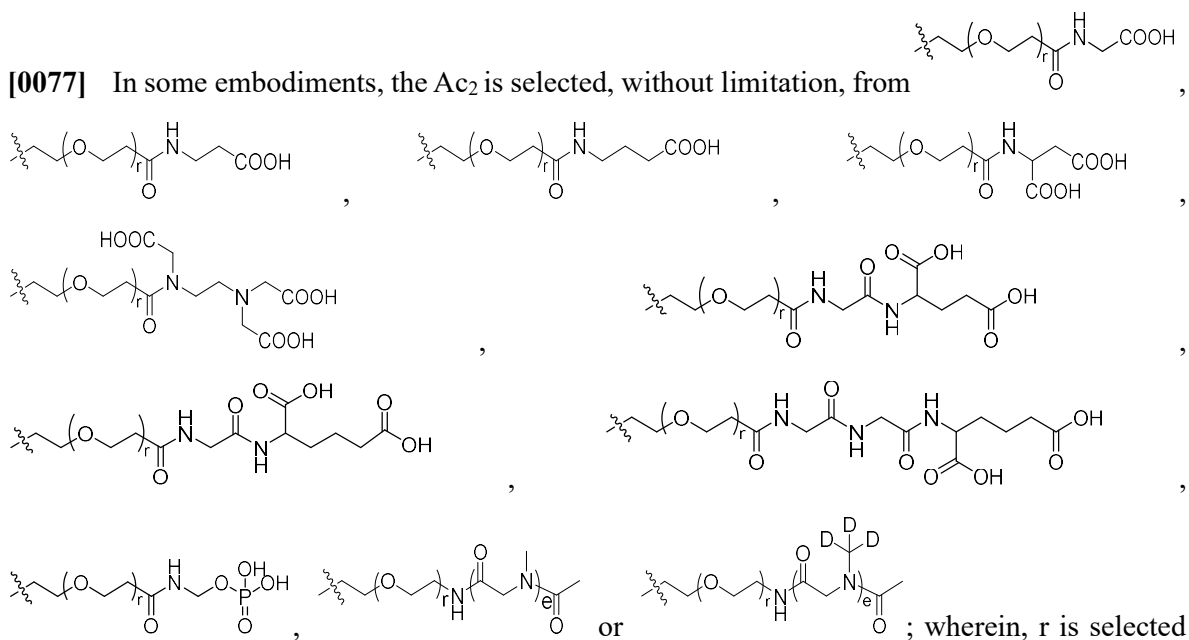


[0075] In some embodiments, Ac₁ is:



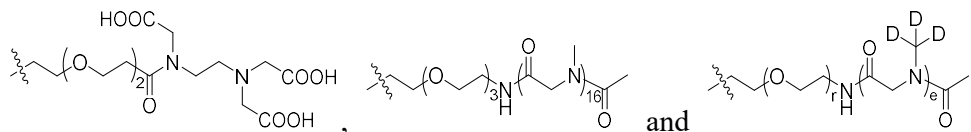
[0076] Ac₂ is a hydrophilic structural unit composed of -X₂-Y₂, and the X₂ and Y₂ are as defined above.

[0077] In some embodiments, the Ac₂ is selected, without limitation, from

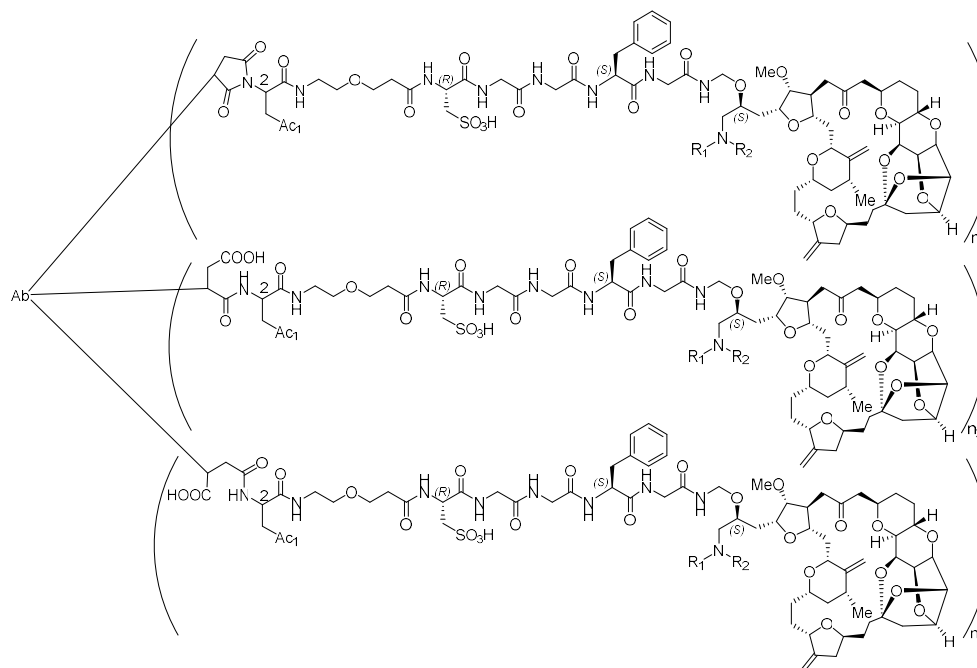


from integers between 1 and 10; e is selected from integers between 1 and 20.

[0078] In some embodiments, Ac₂ is selected from the following structures:



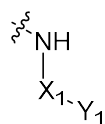
[0079] In some embodiments, the ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof of the present application has a structure represented by formula VIg:



Formula VIg

[0080] wherein the R₁, R₂, n₁, n₂ and n₃ are as defined above;

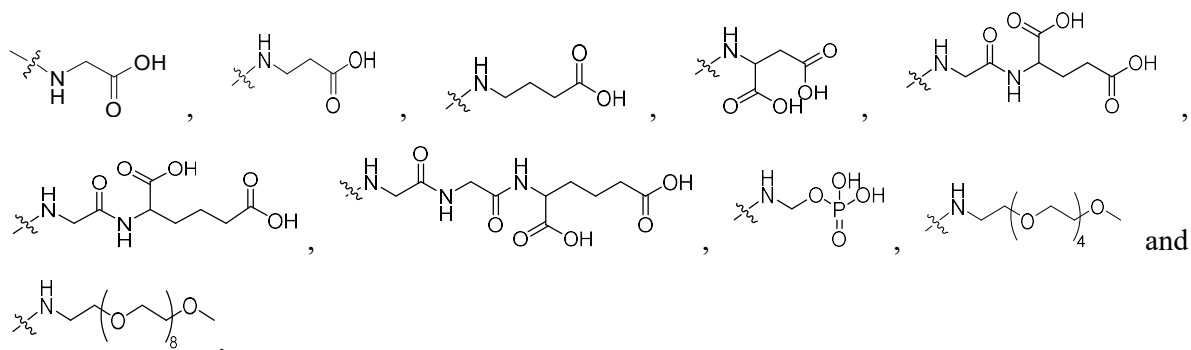
[0081] Ac₁ is a hydrophilic structural unit having the structure shown in formula d:



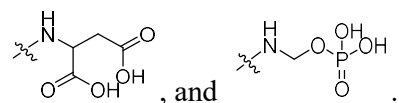
Formula d

[0082] the X₁ and Y₁ are as defined above;

[0083] In some embodiments, the Ac₁ is selected, without limitation, from the group consisting of (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 unnatural amino acid derivatives and the following structures:

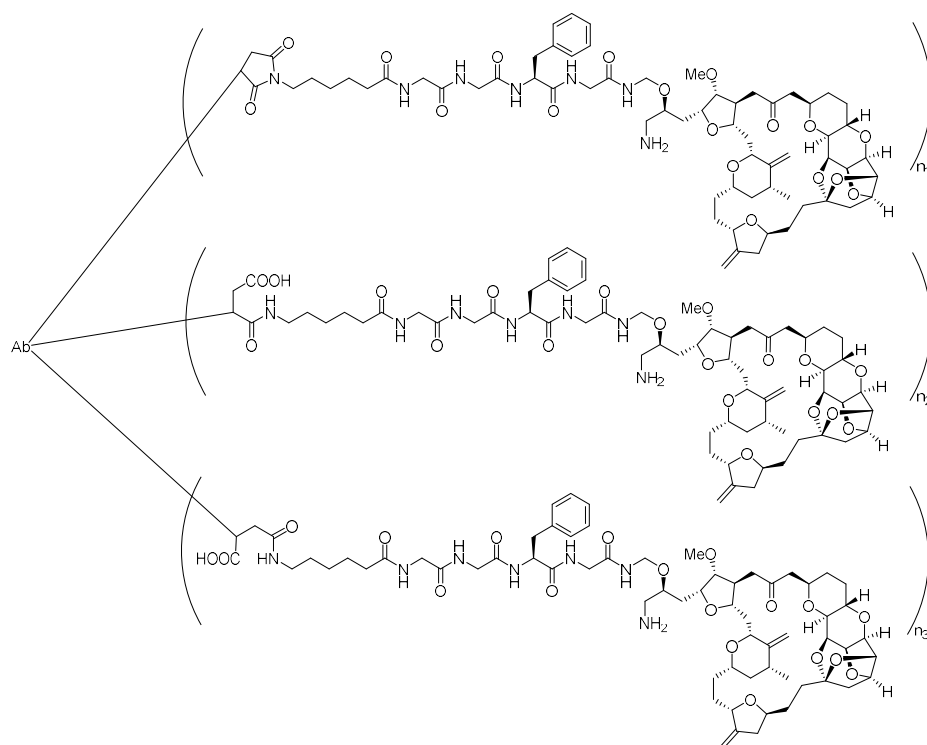


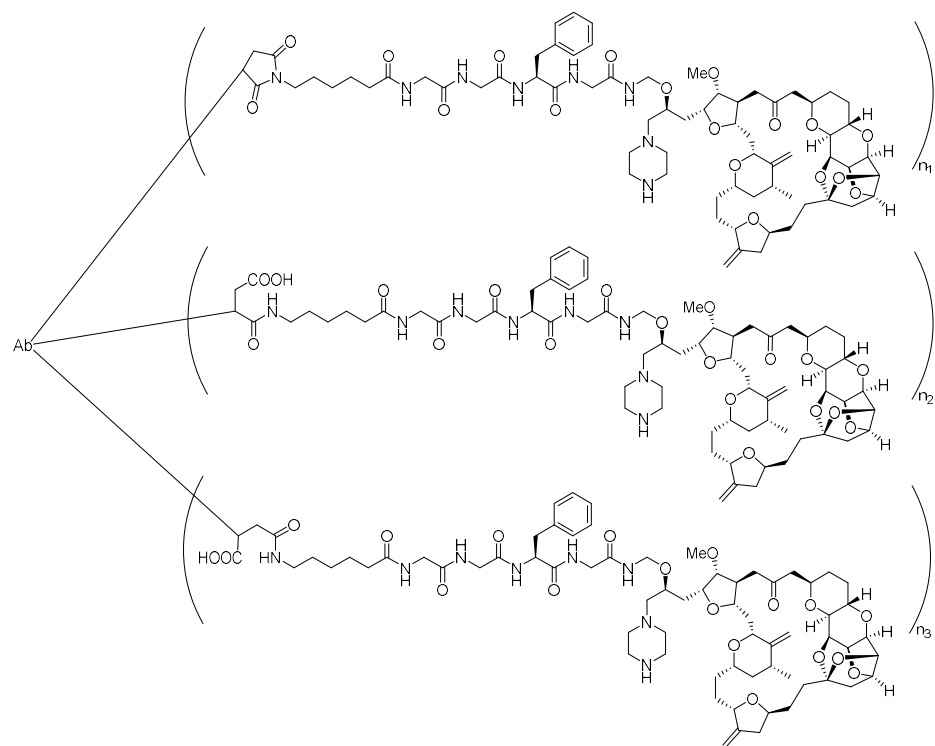
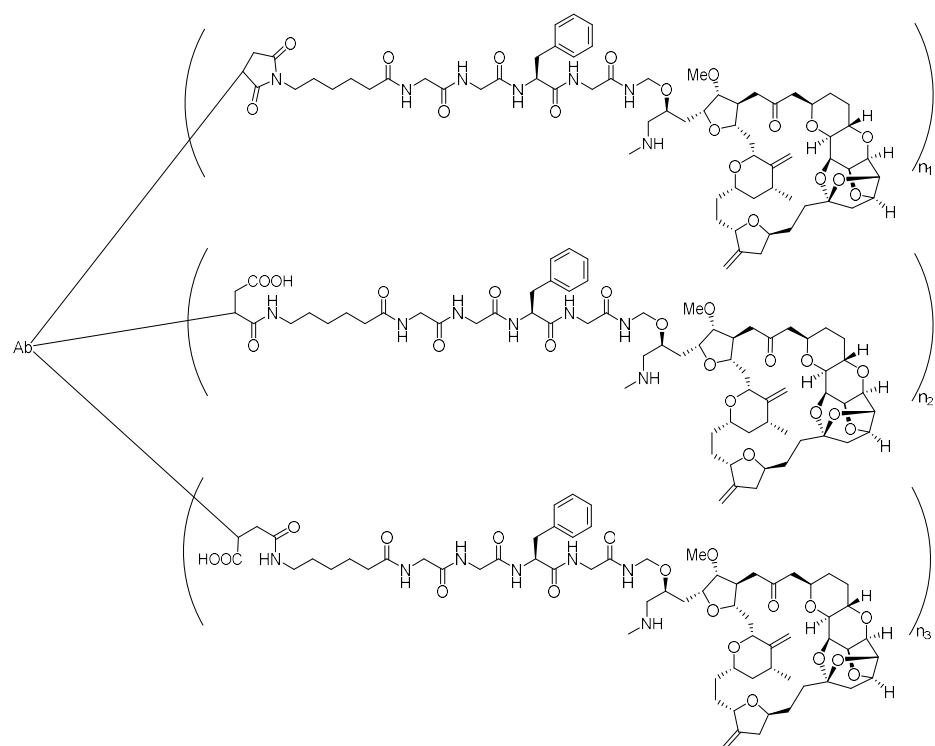
[0084] In some embodiments, Ac₁ is selected from the following structures: ,

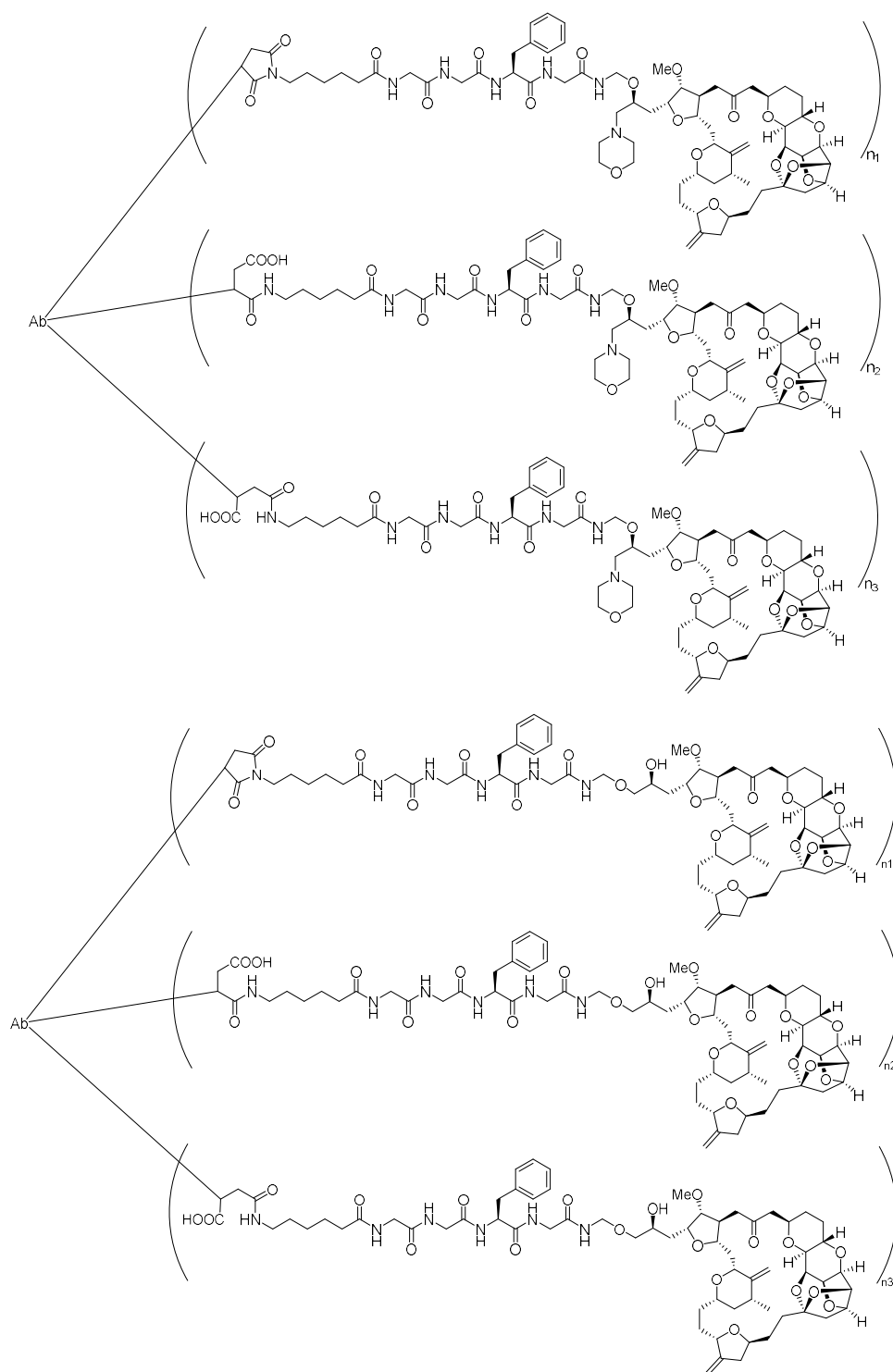


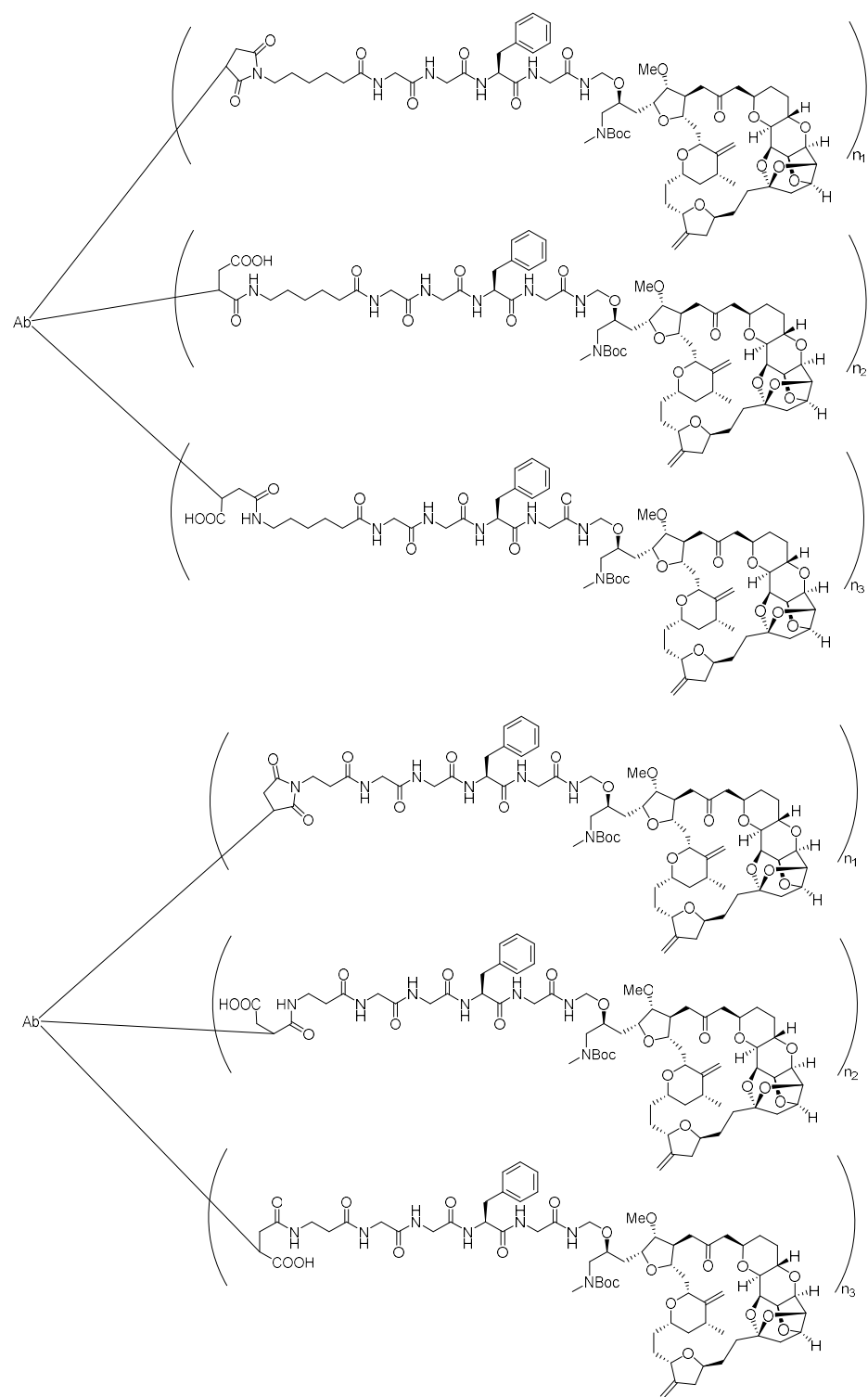
[0085] In some embodiments, Ac₁ is: .

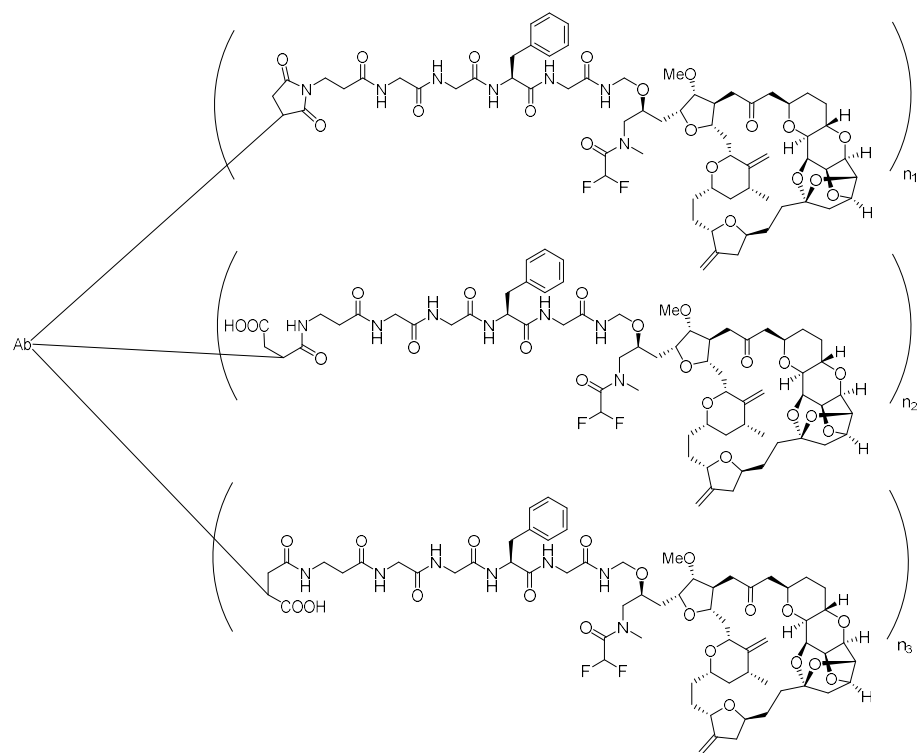
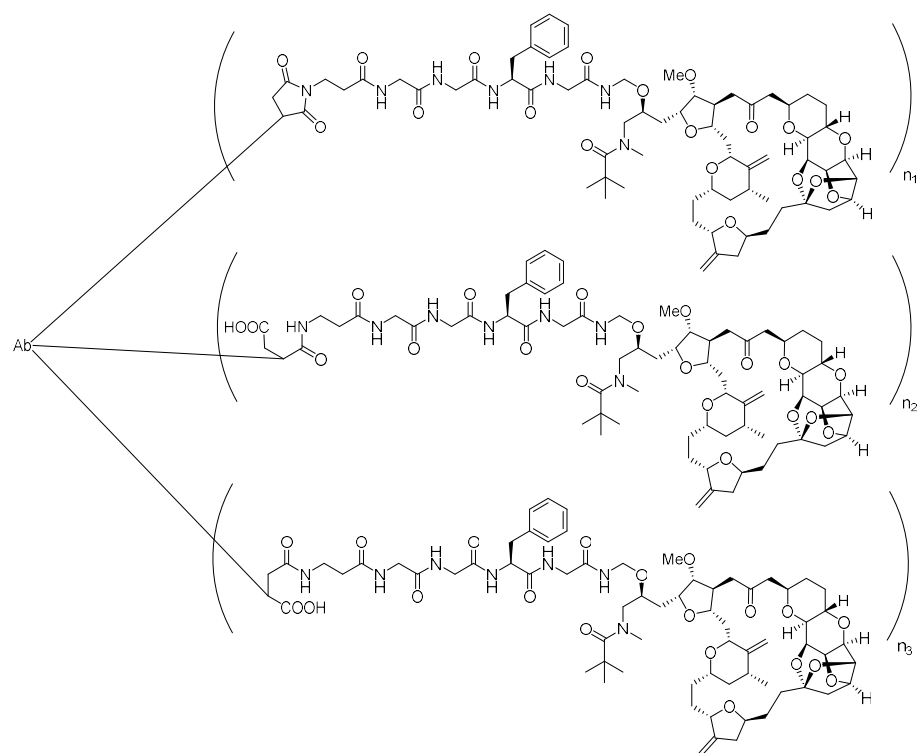
[0086] In some embodiments, the ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof of the present application is selected from the following structures:

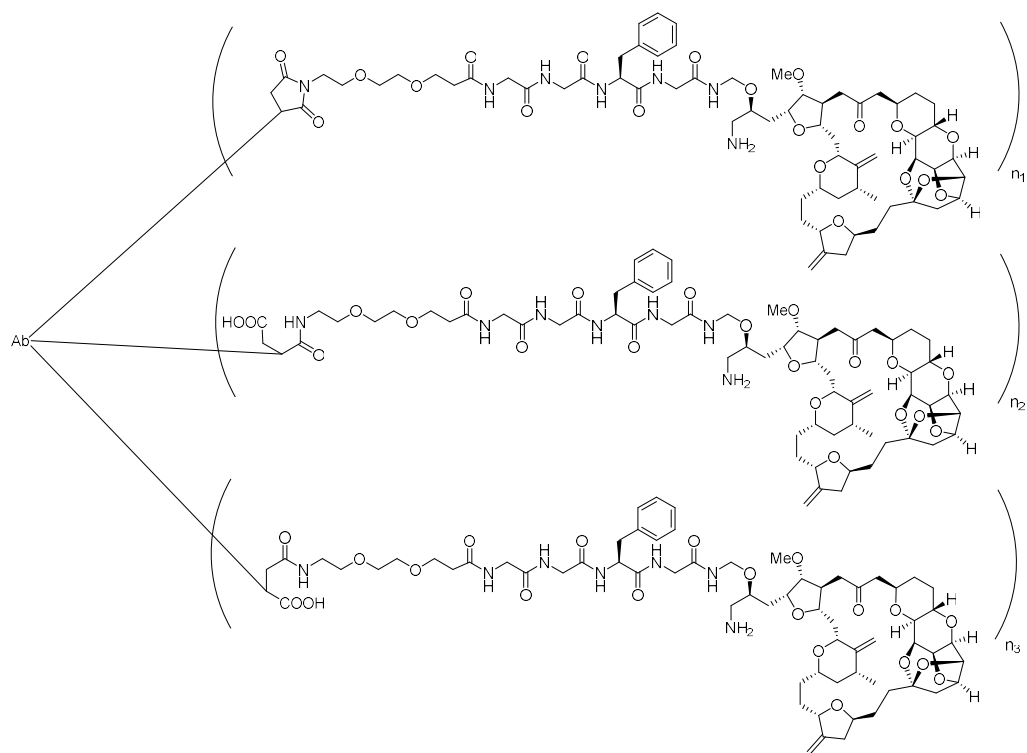
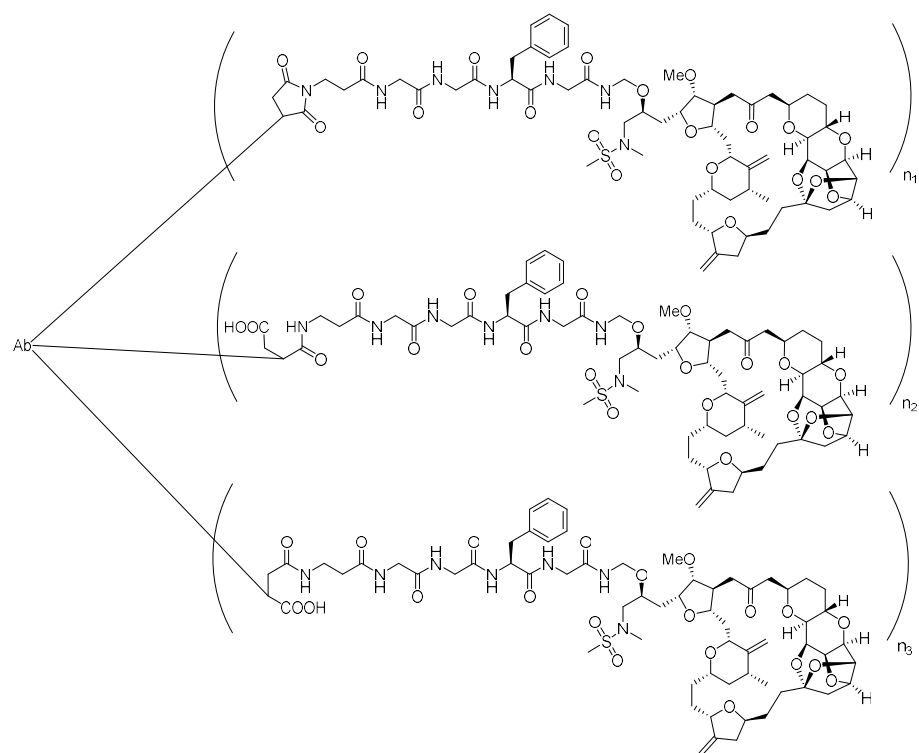


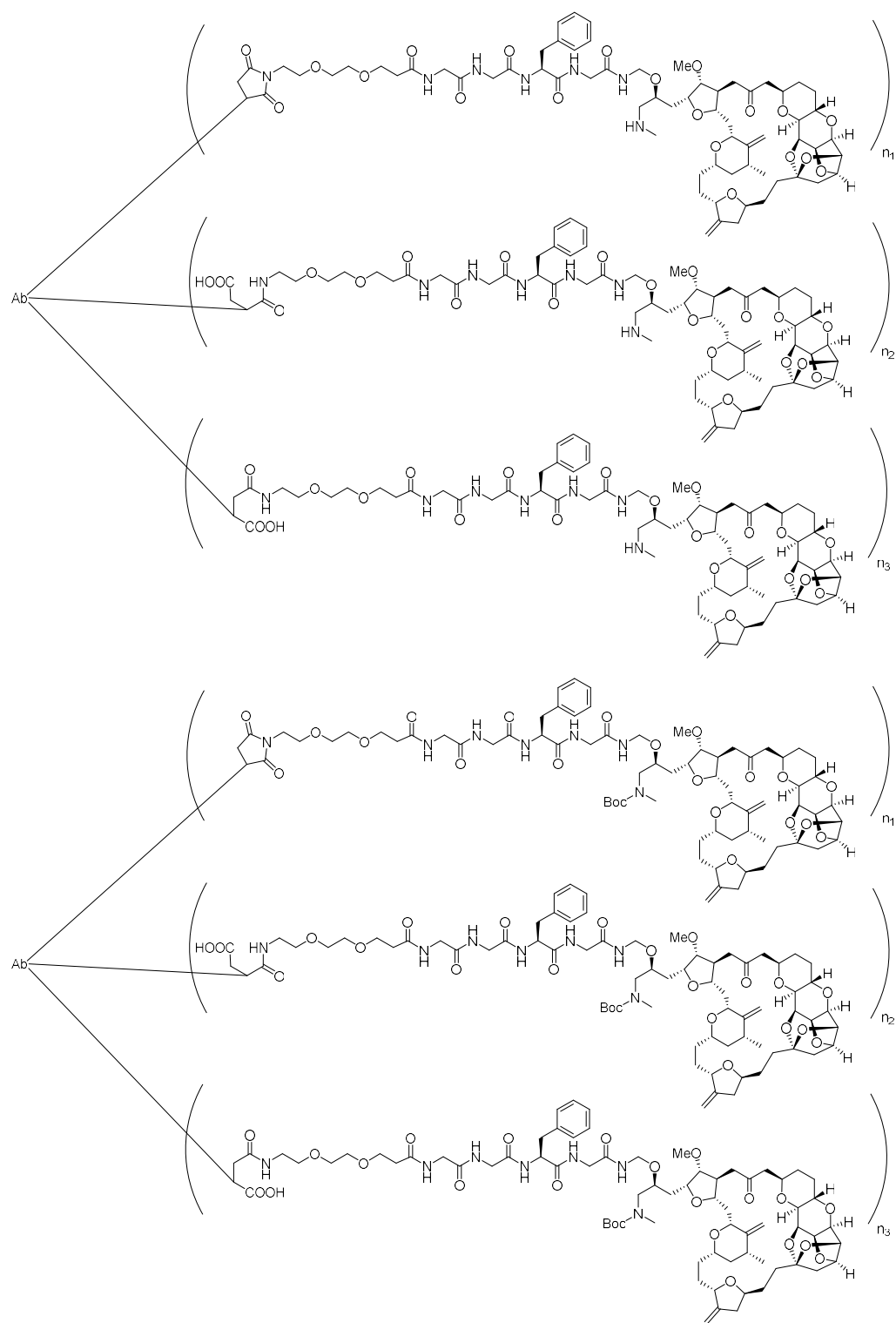


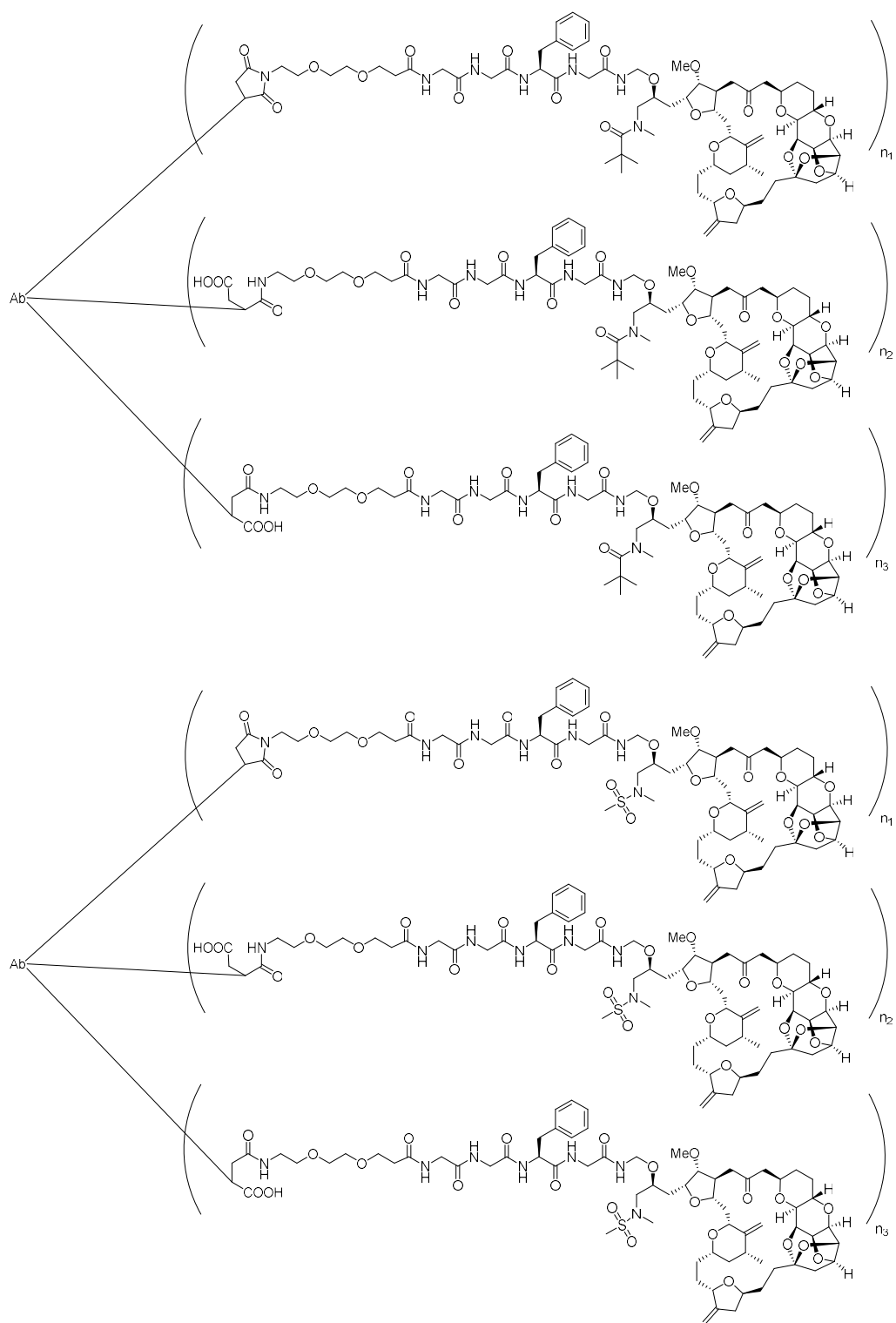


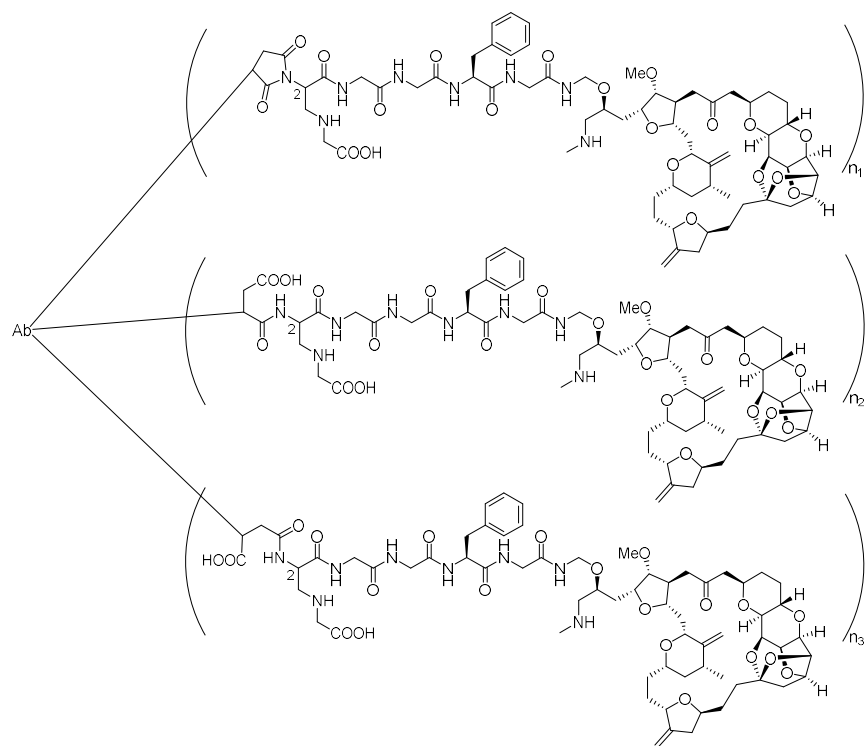
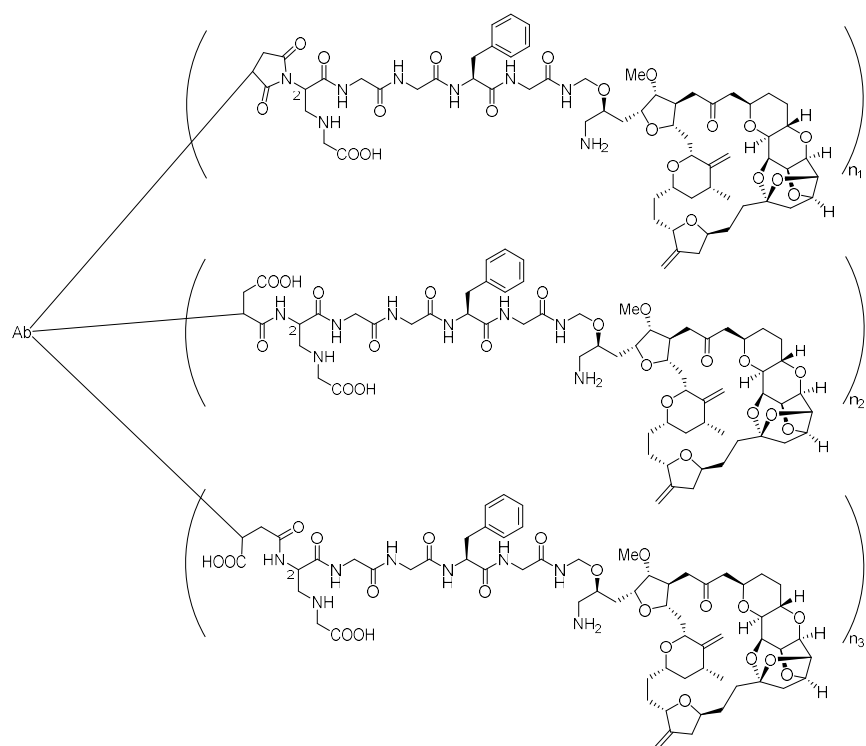


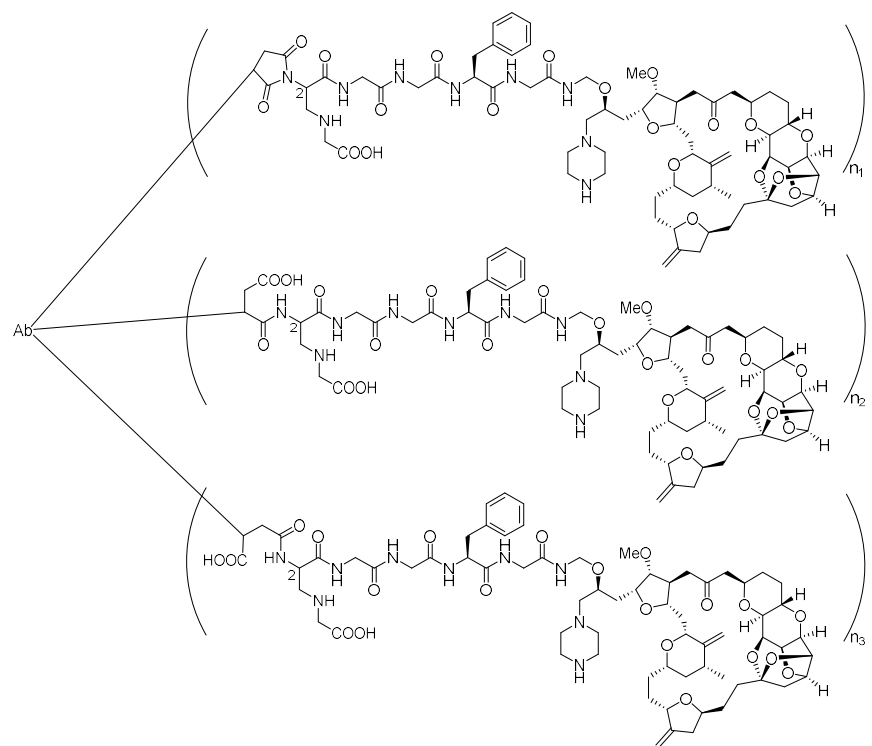
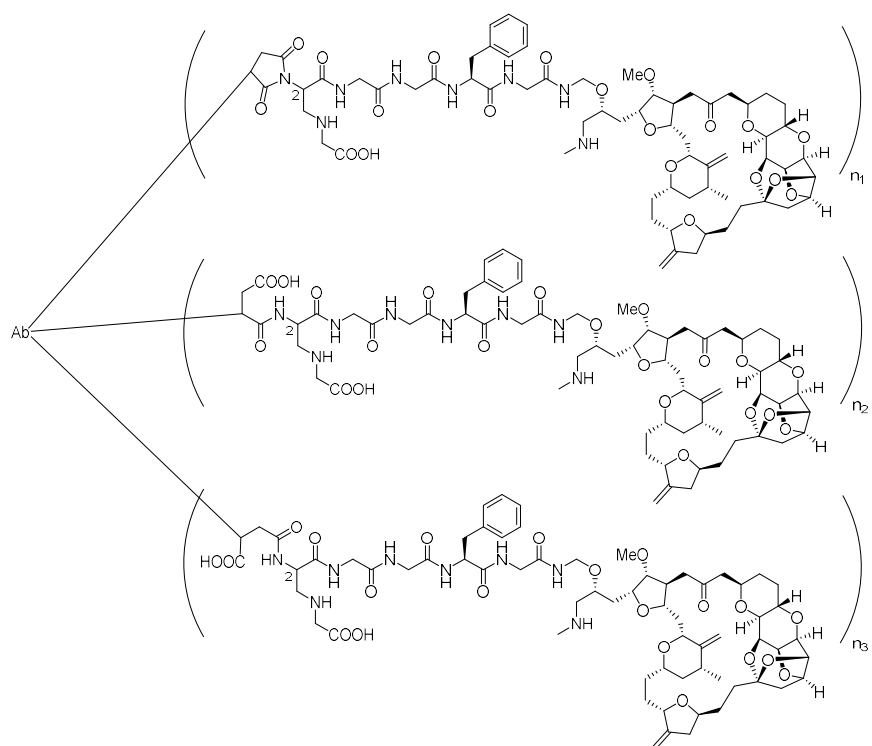


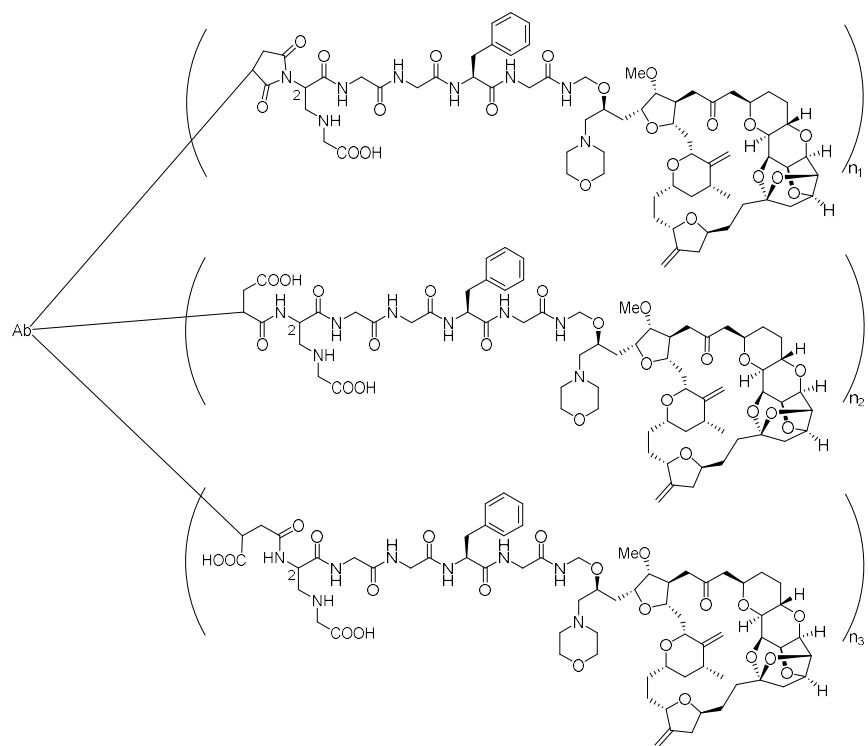
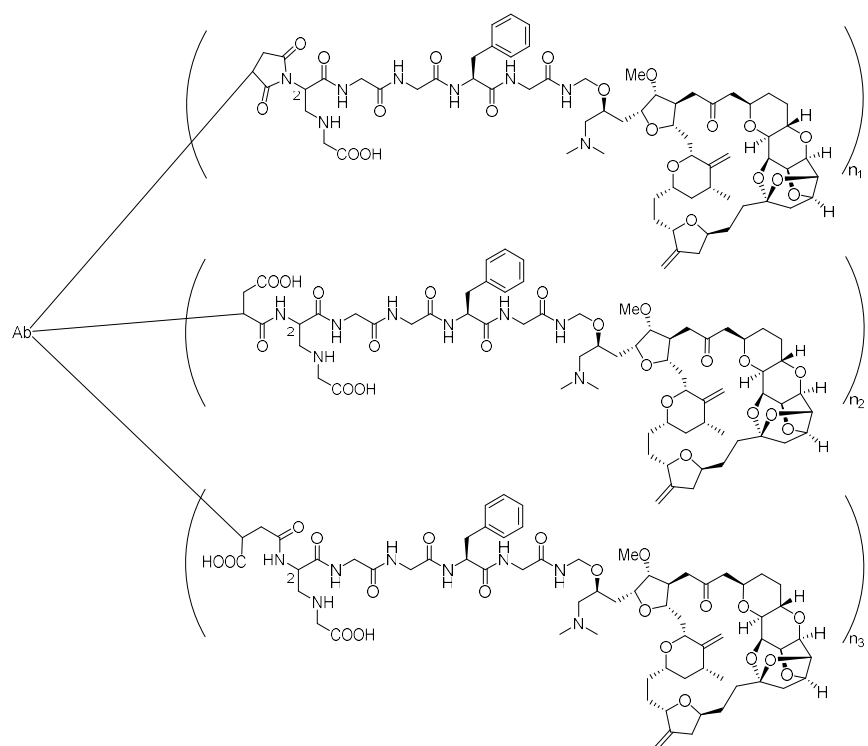


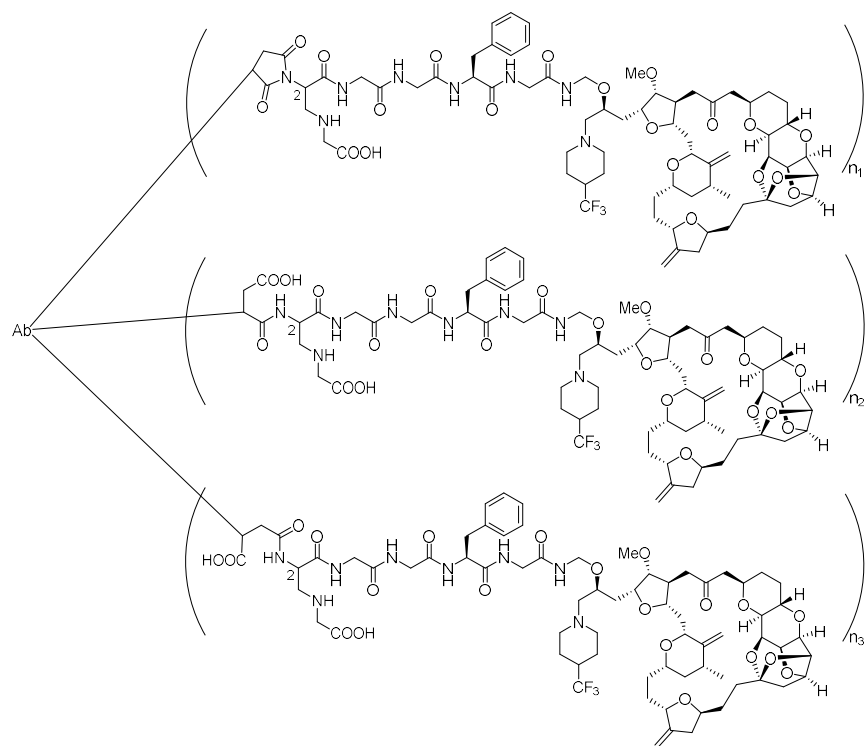
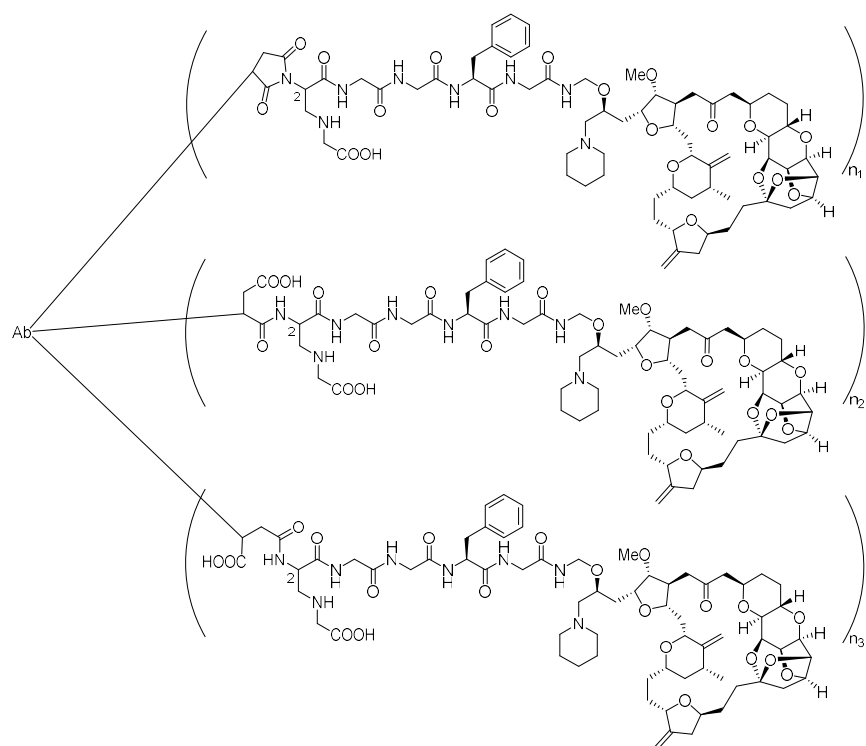


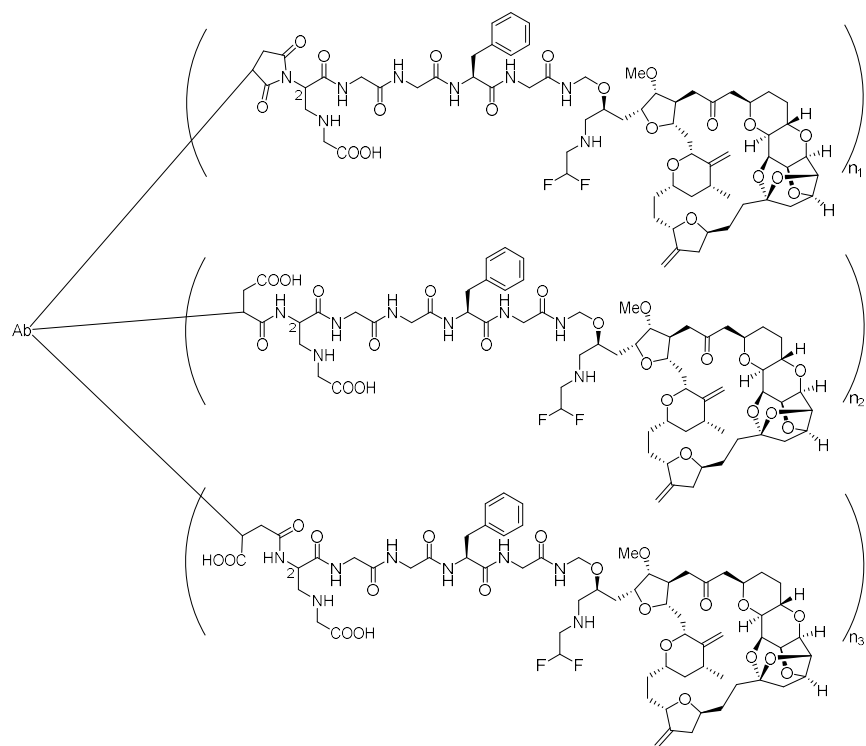
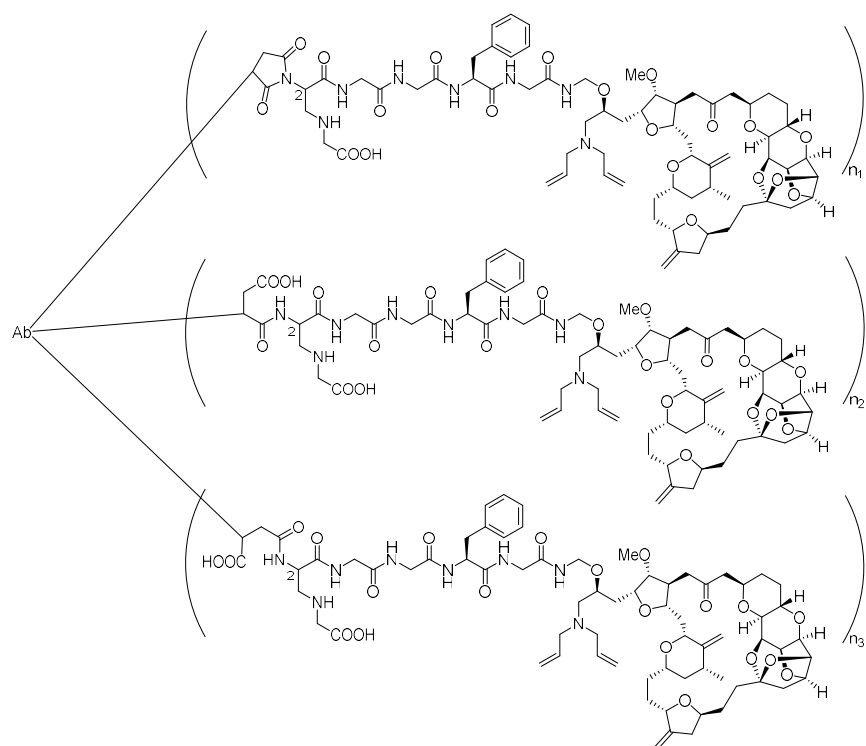


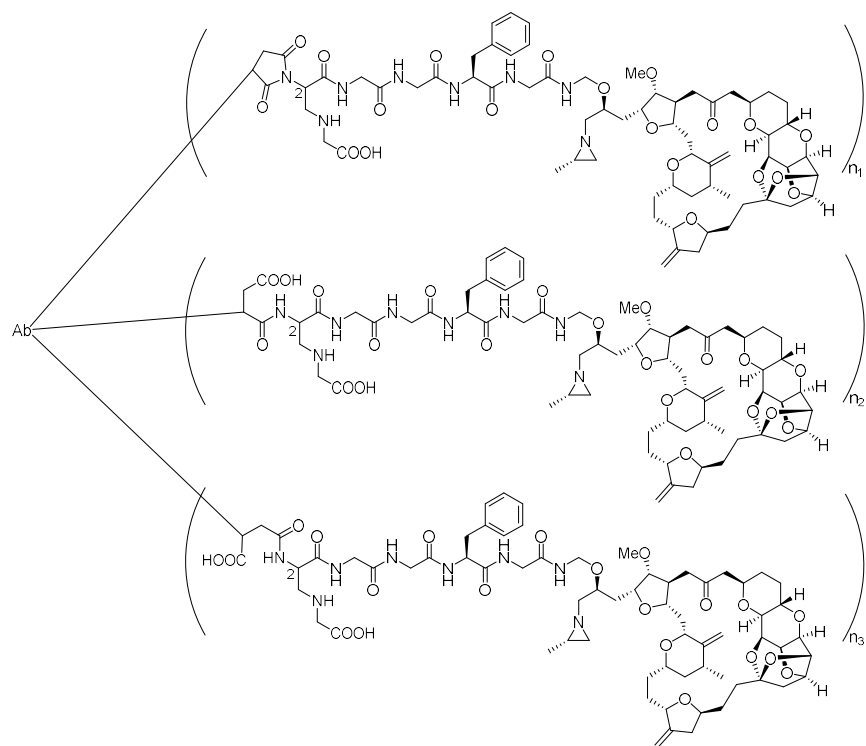
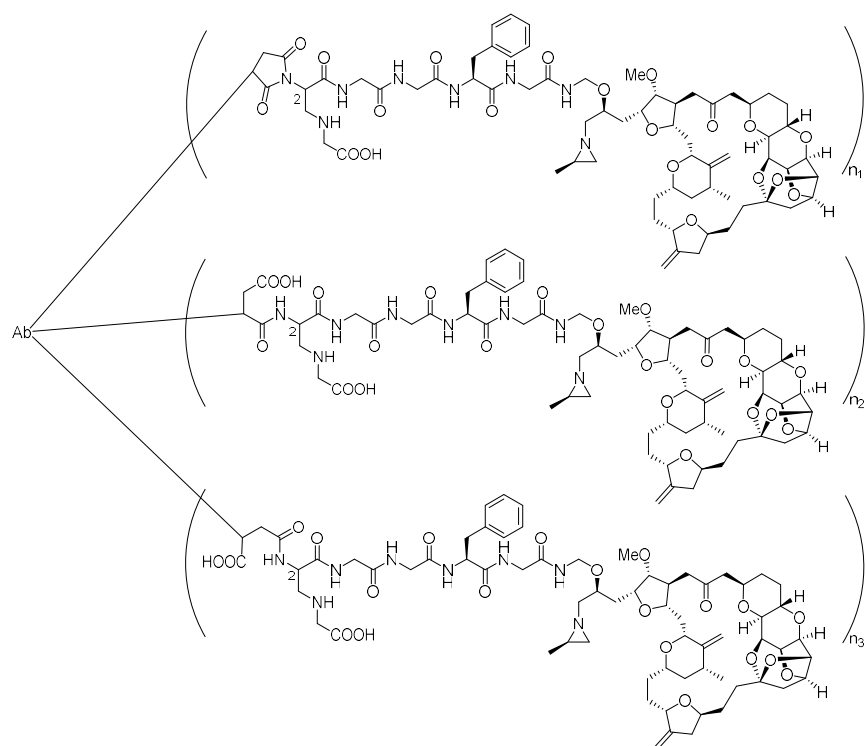


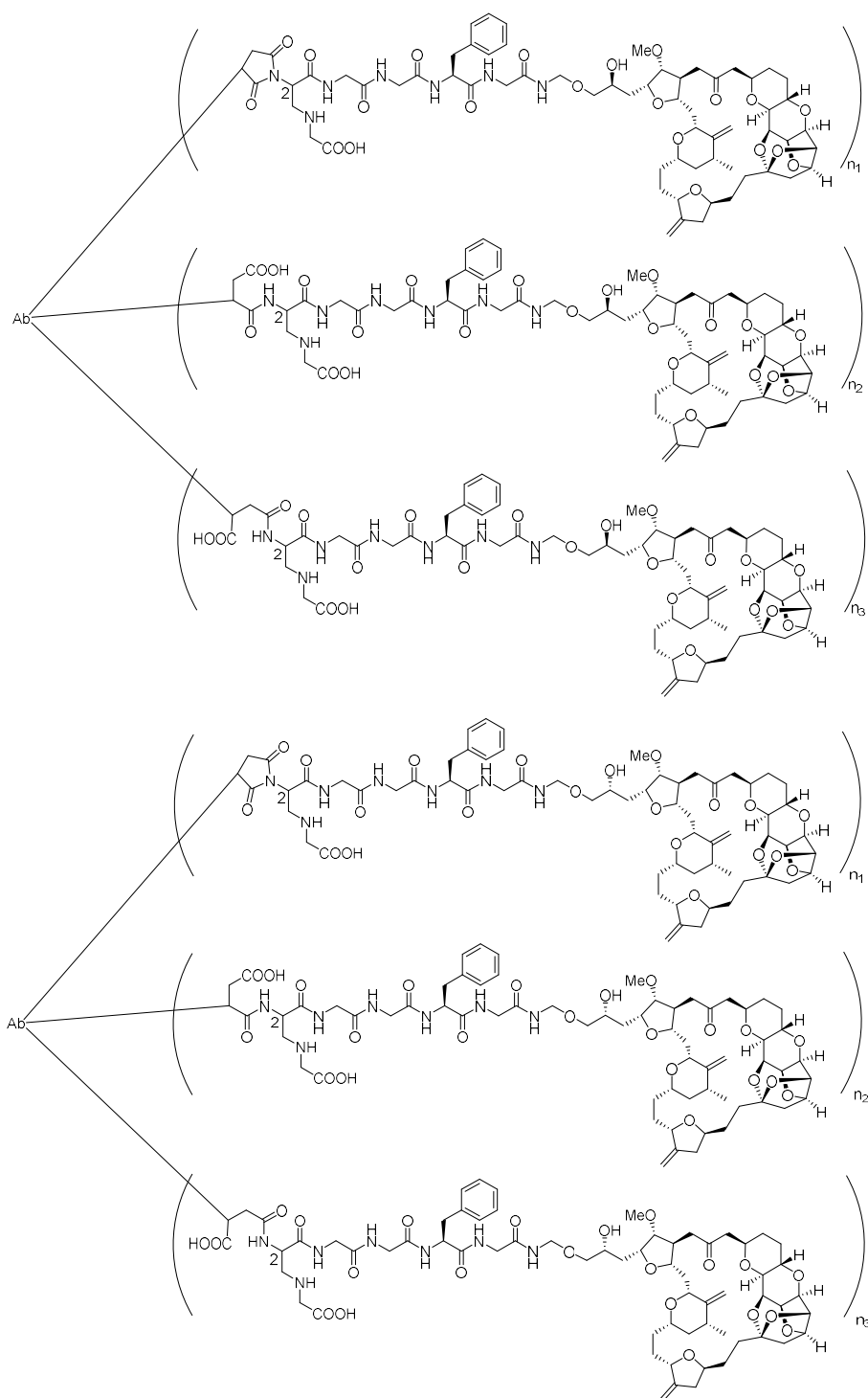


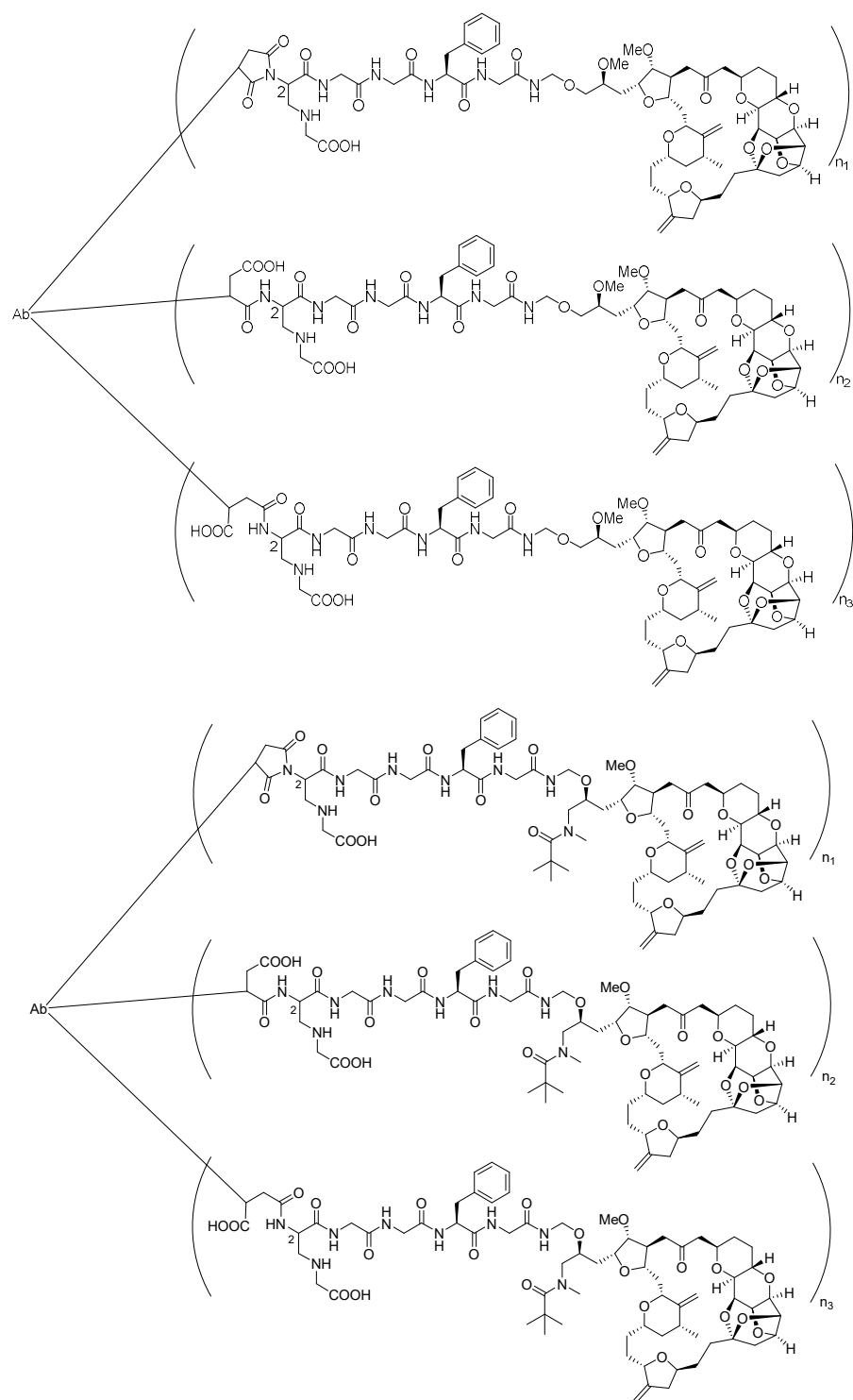


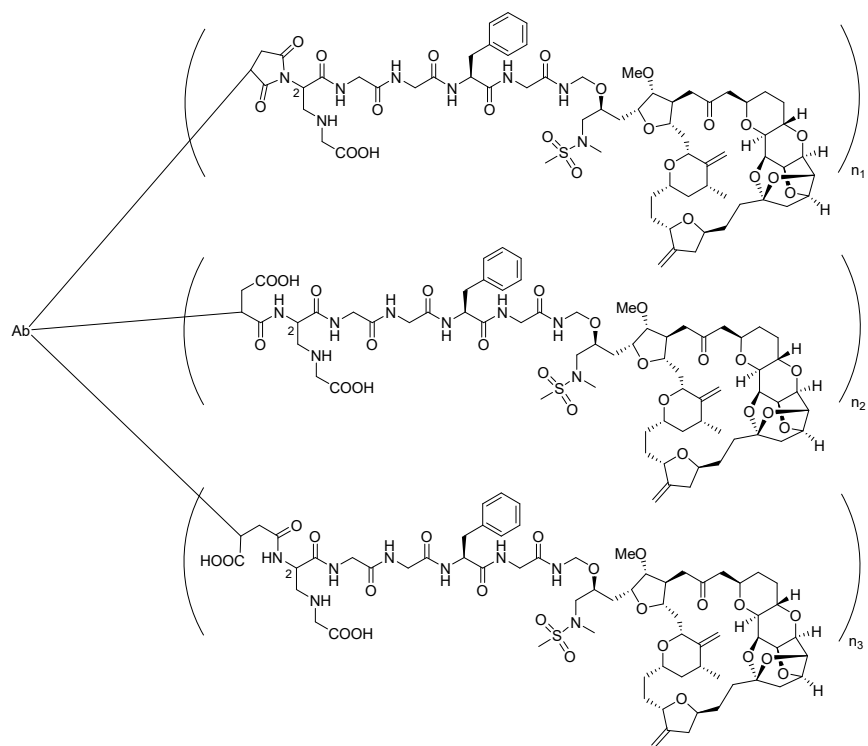
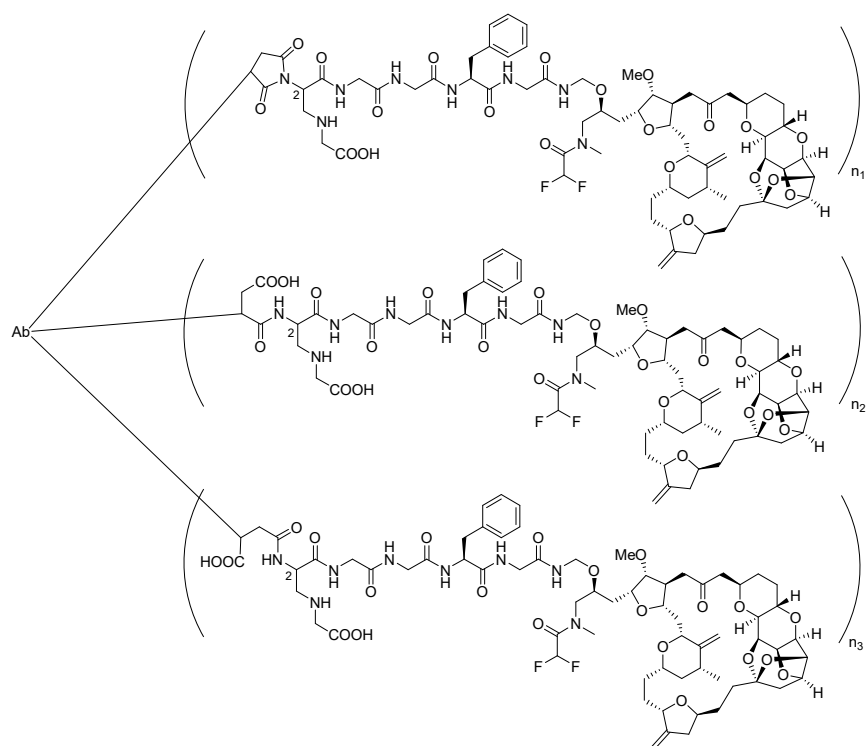


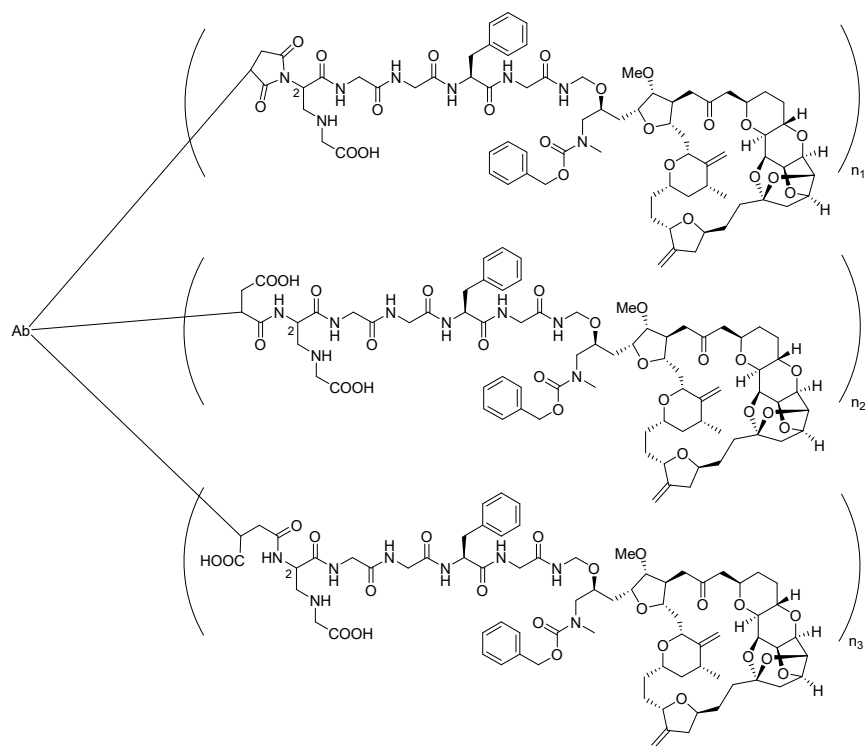
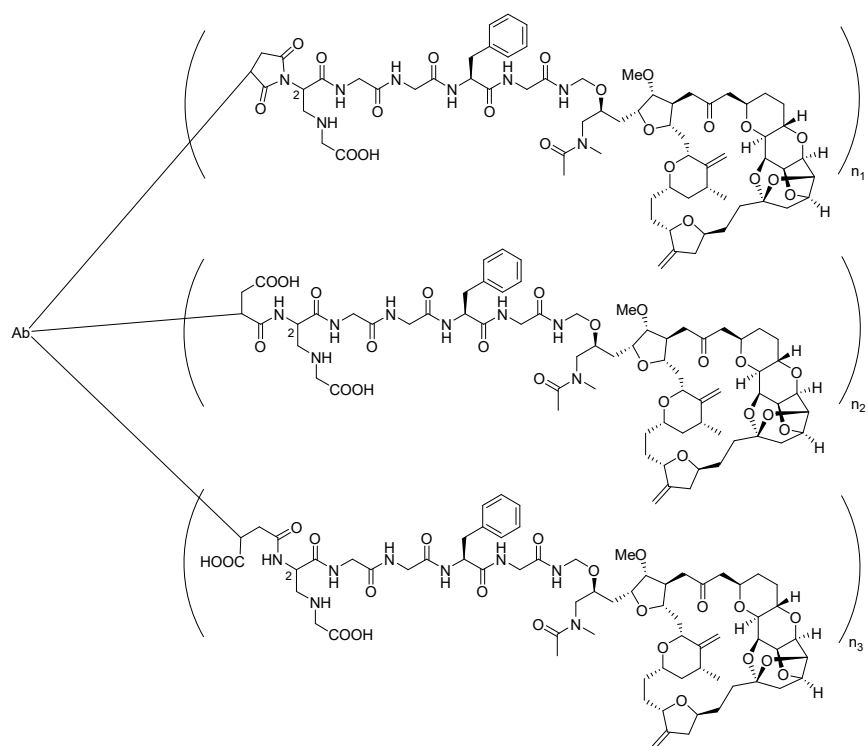


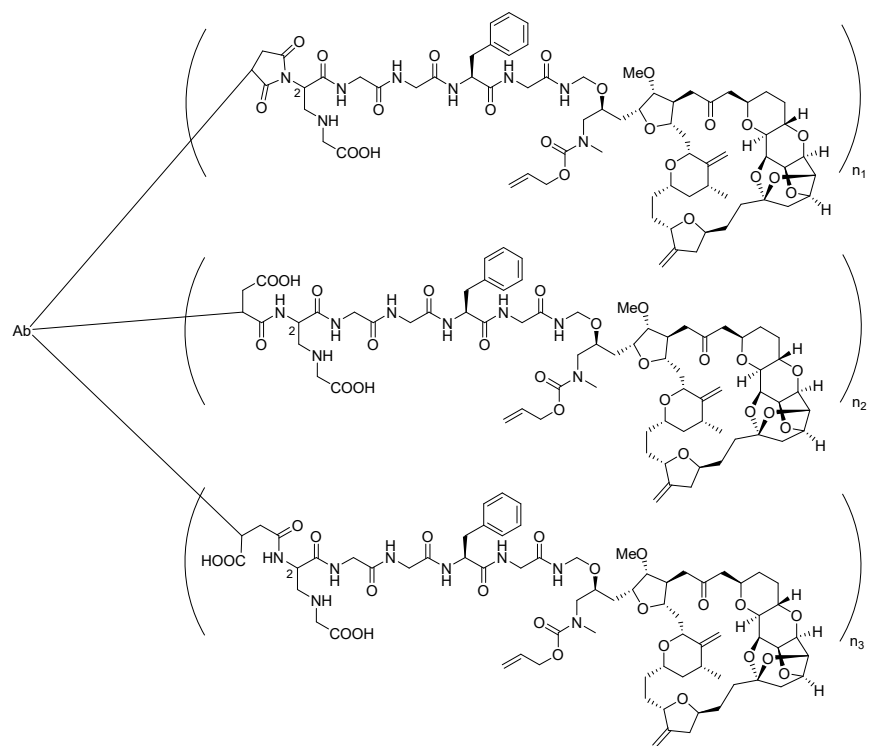
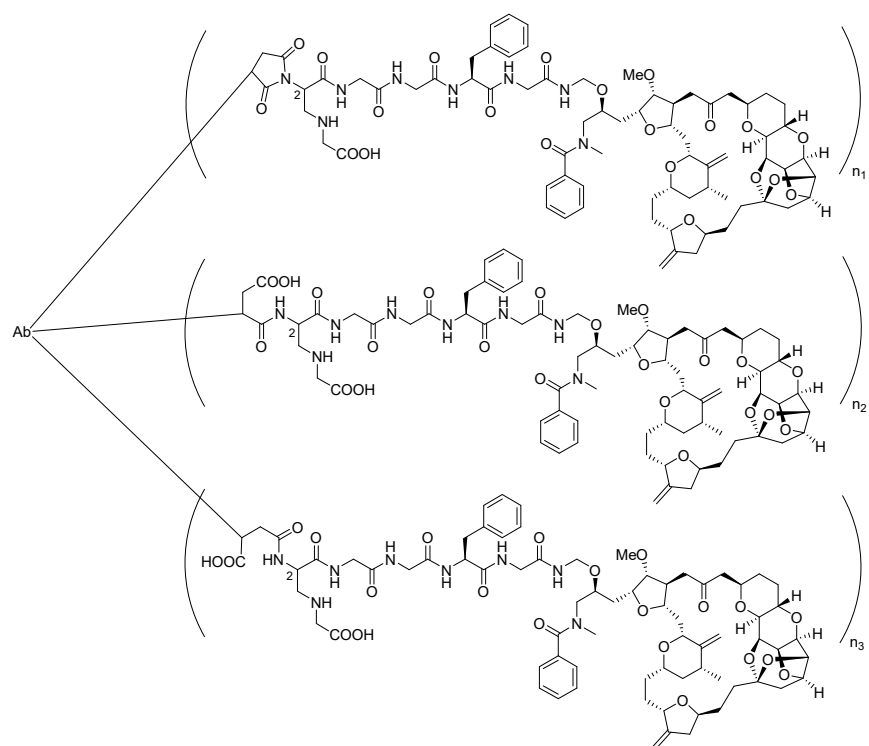


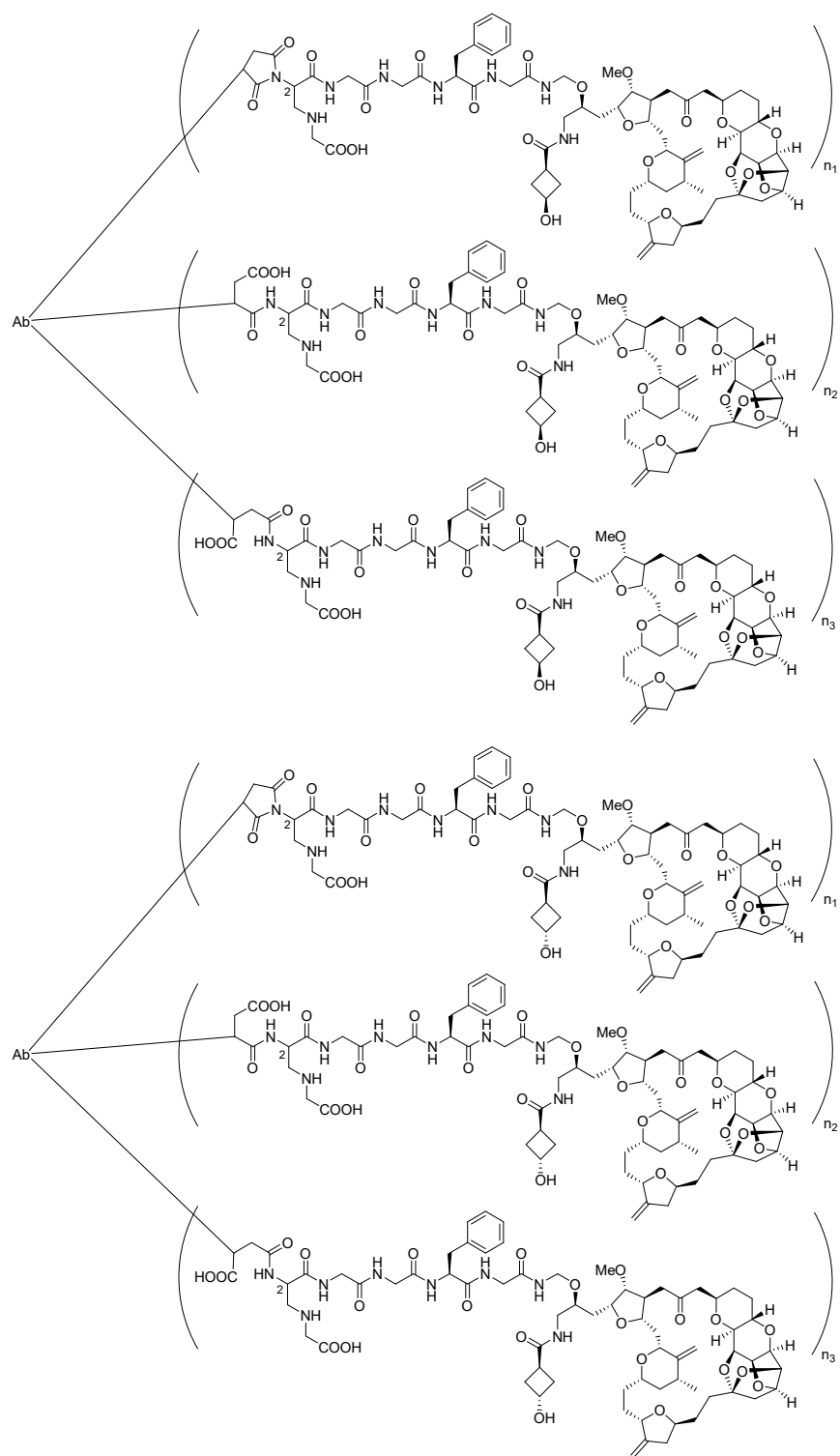


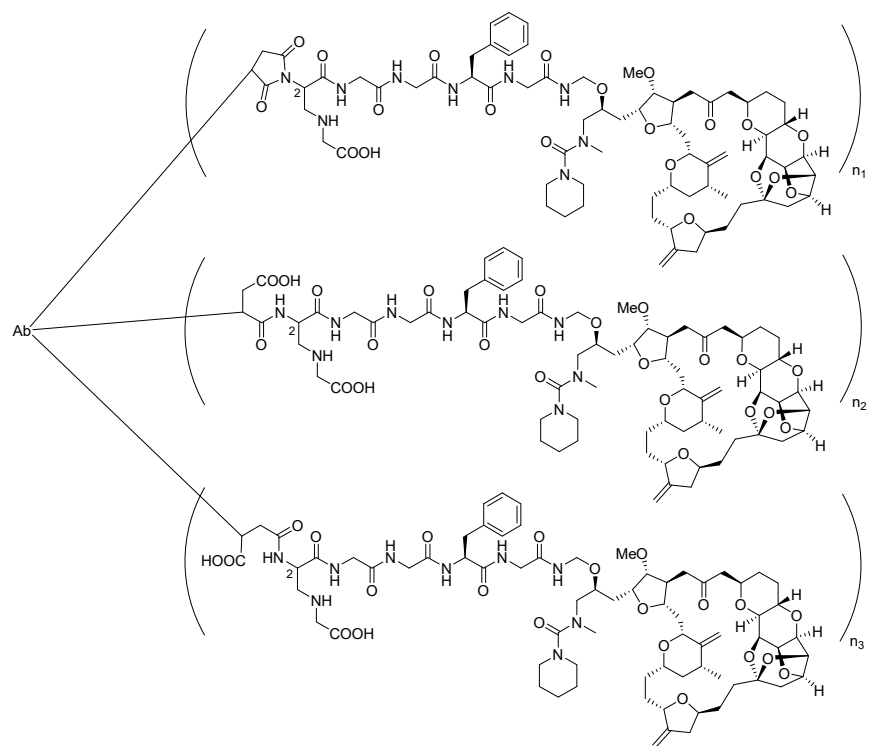
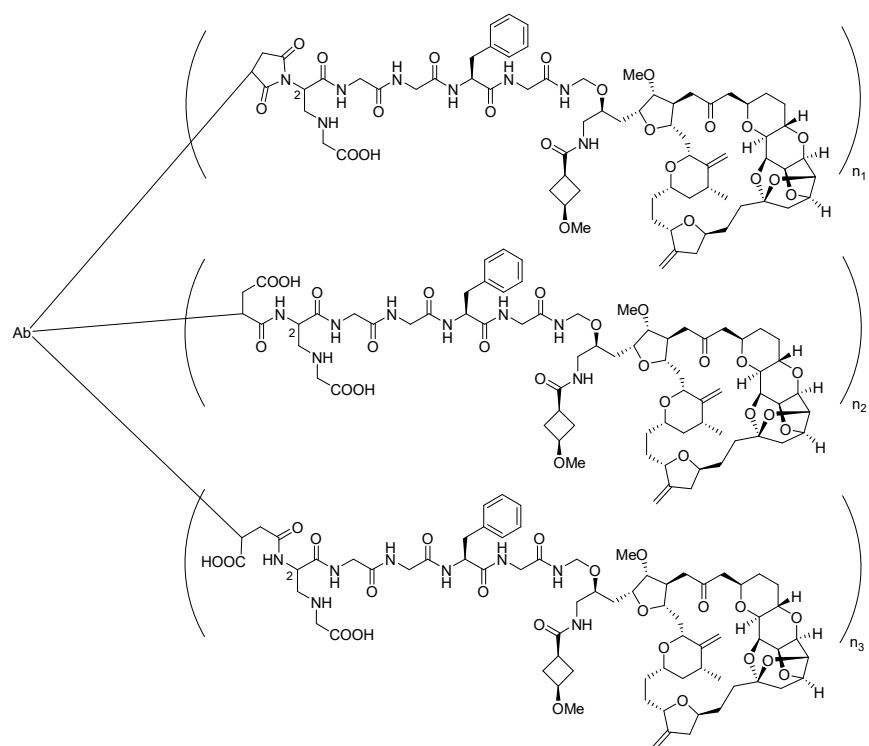


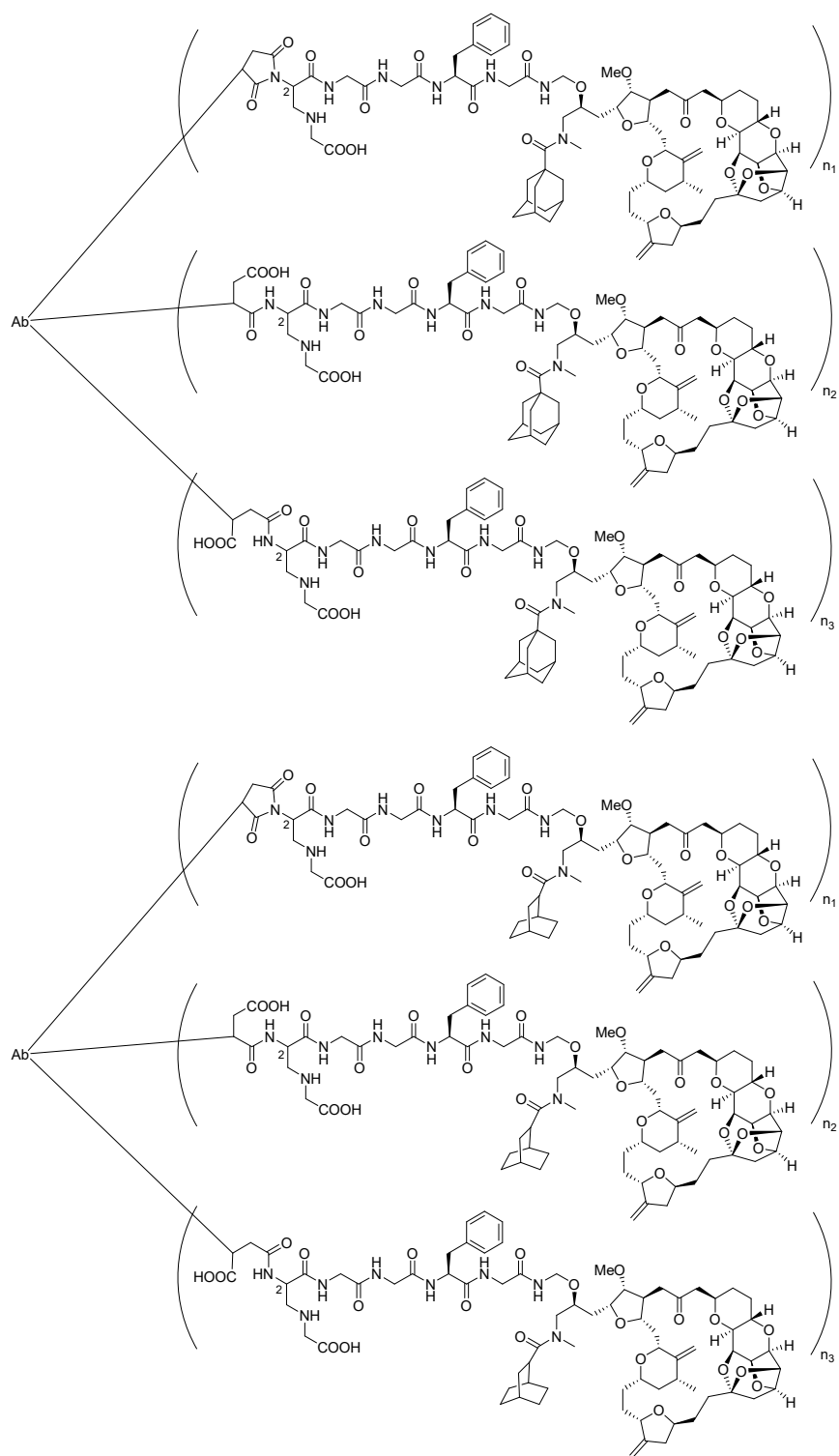


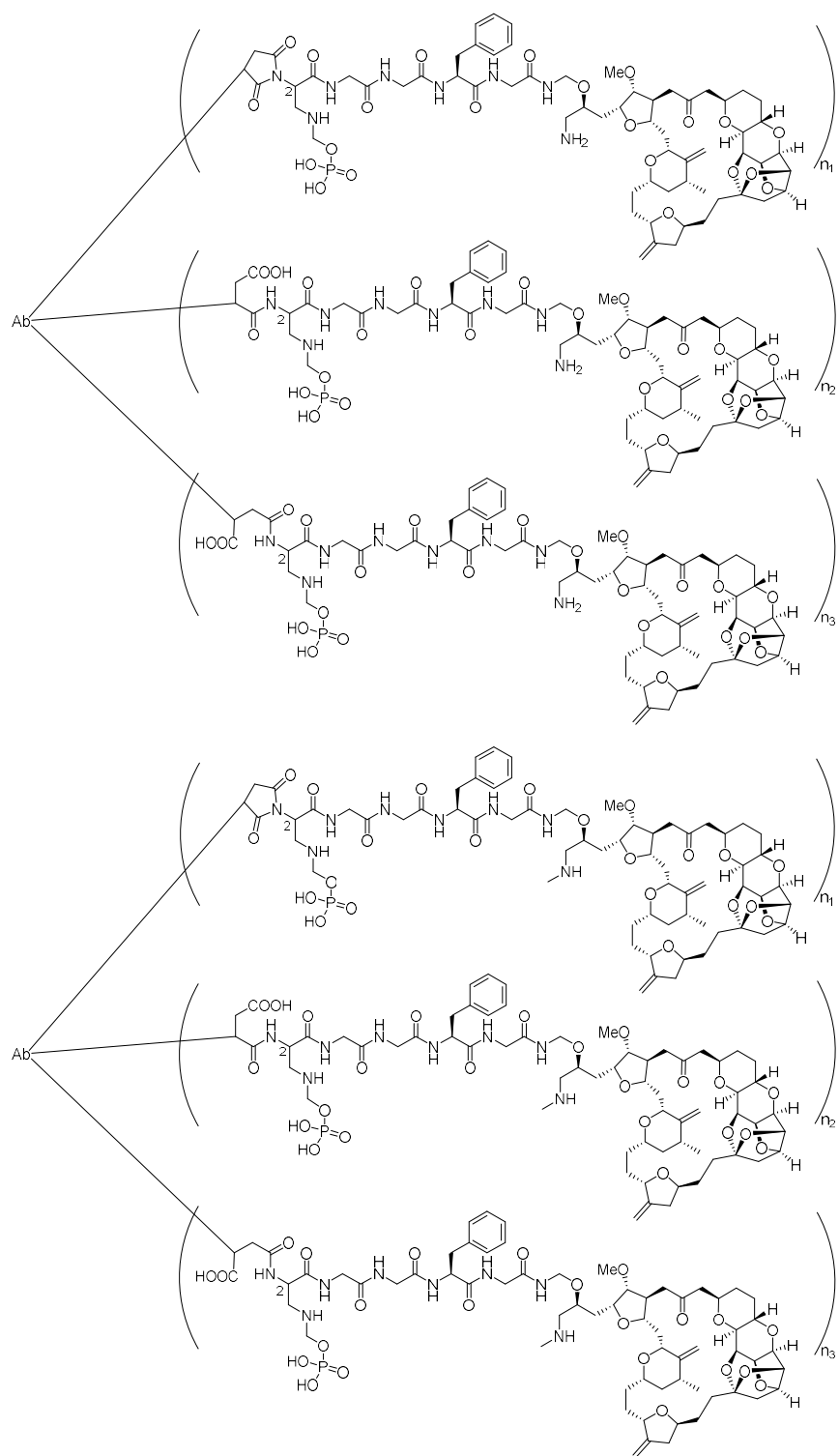


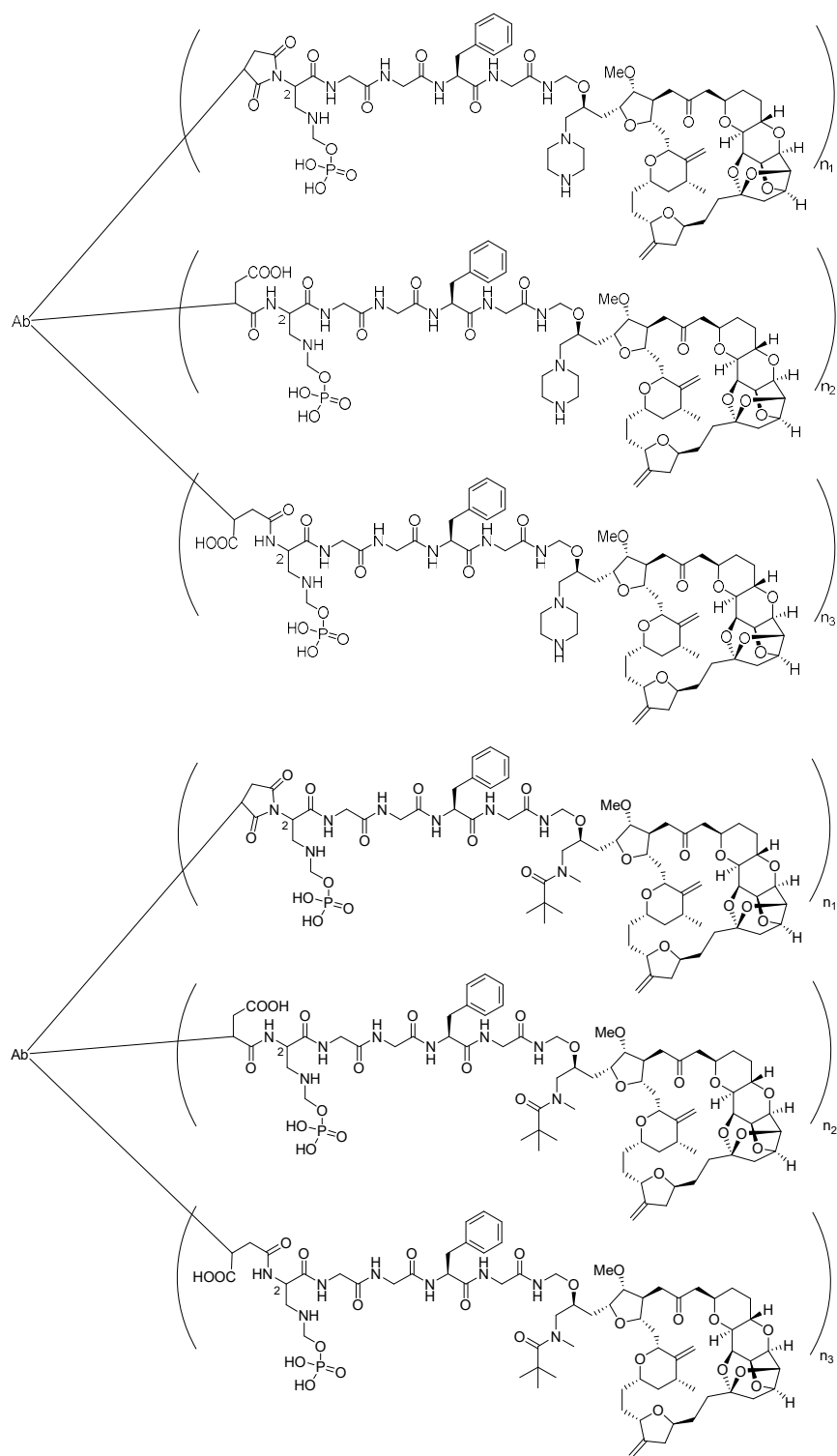


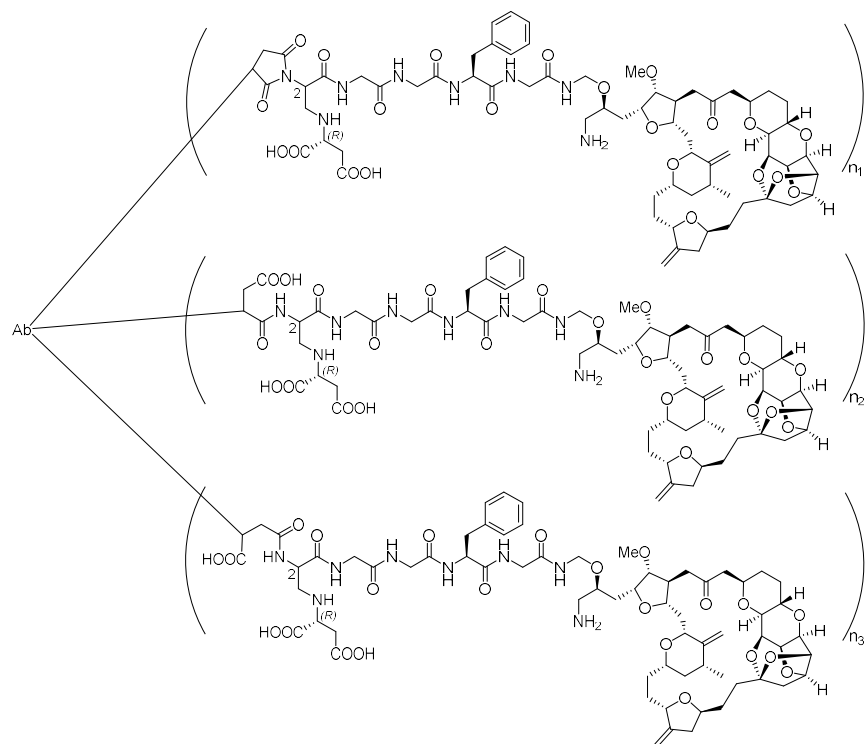
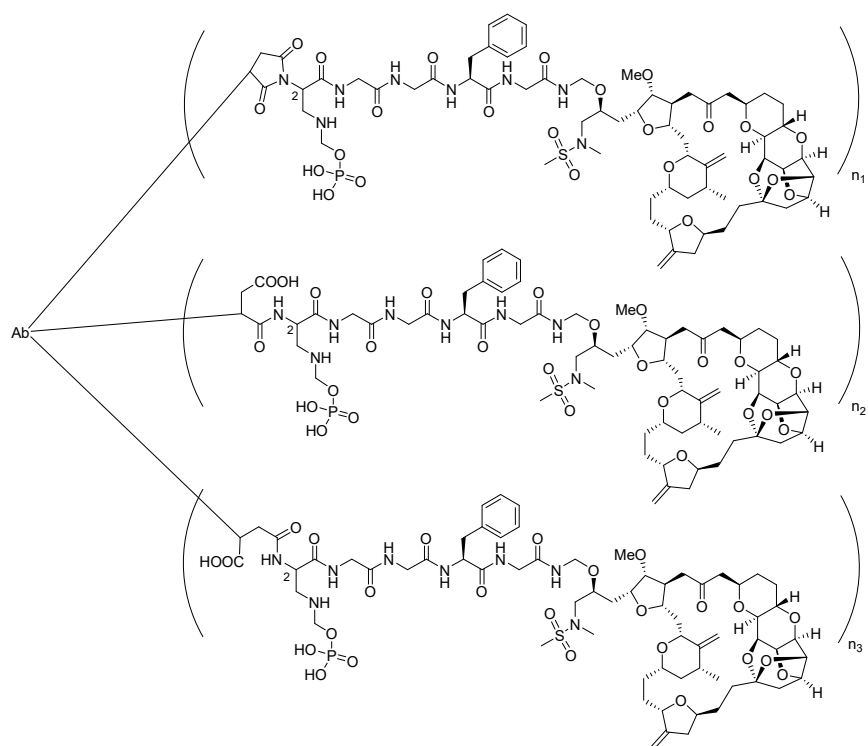


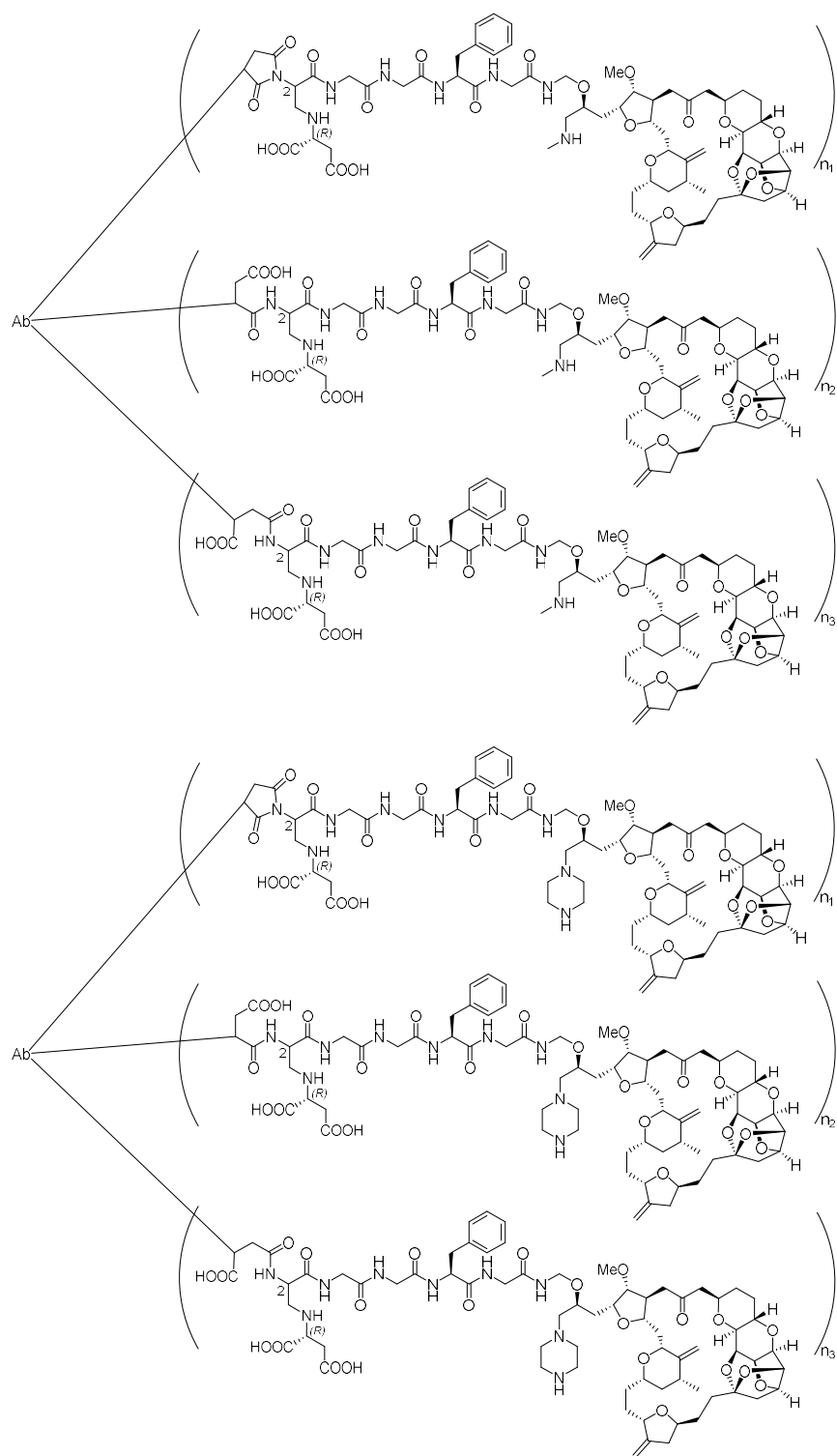


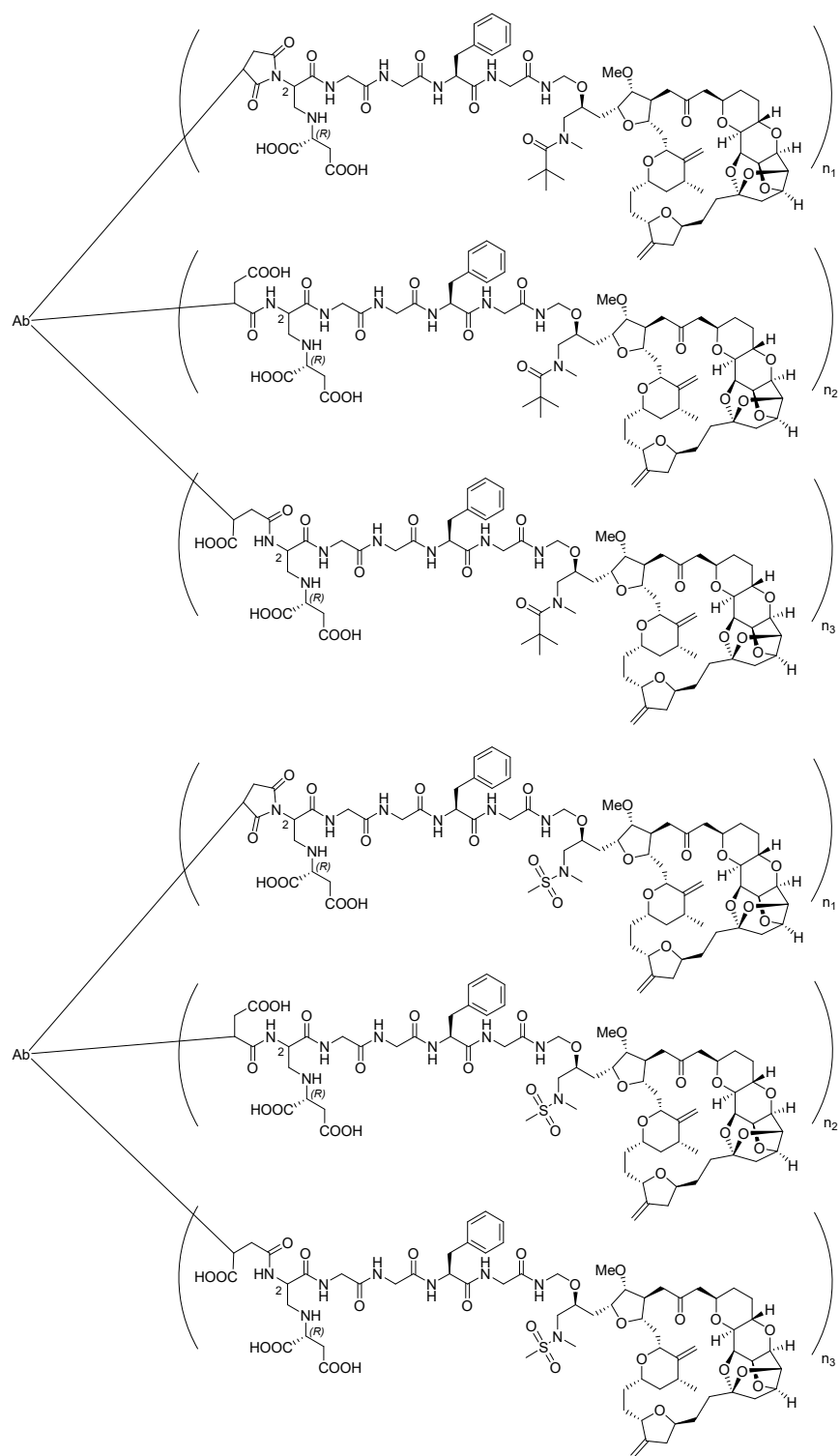


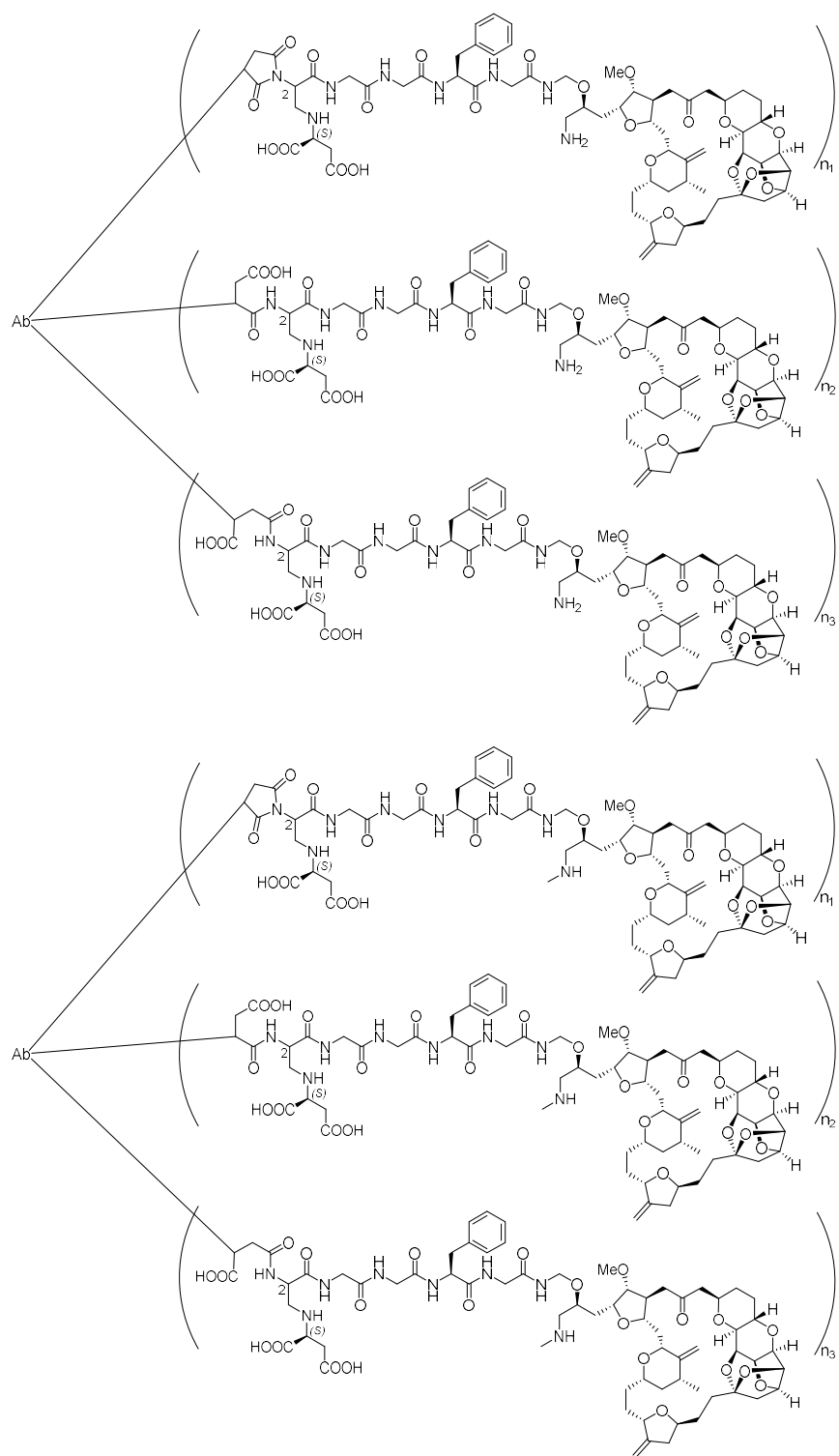


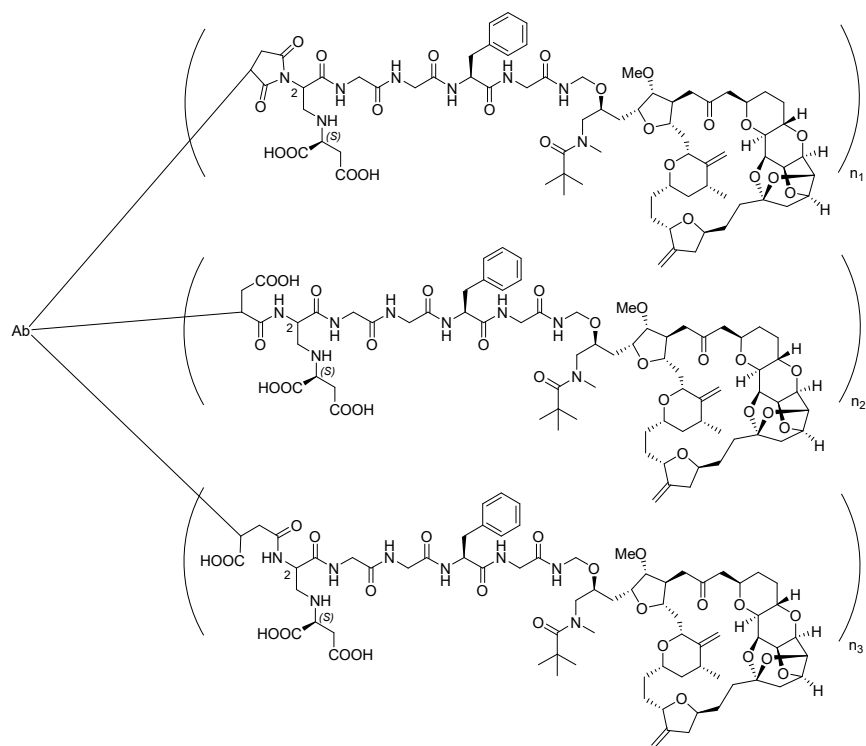
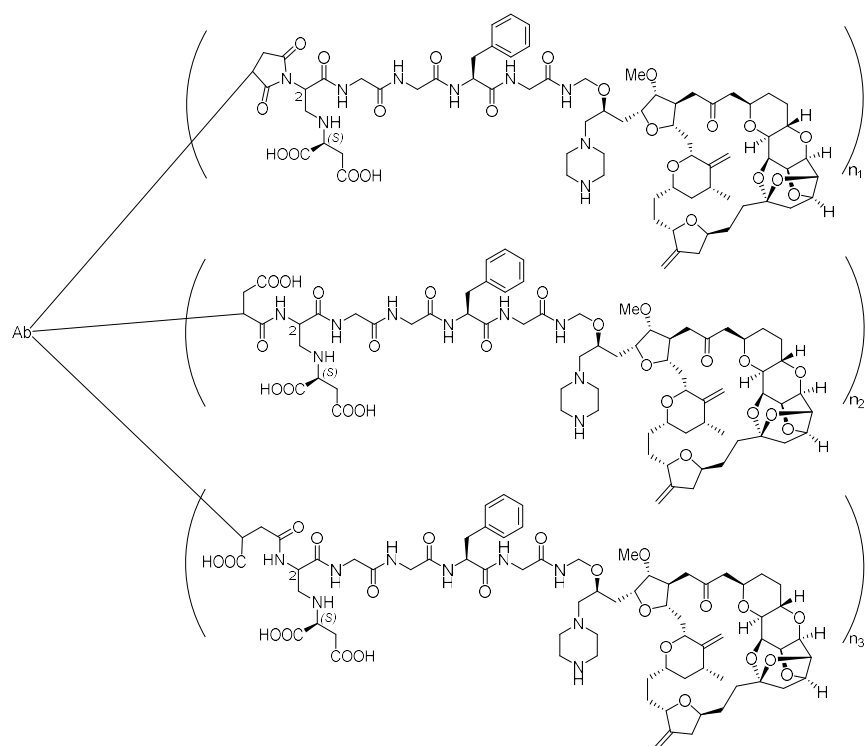


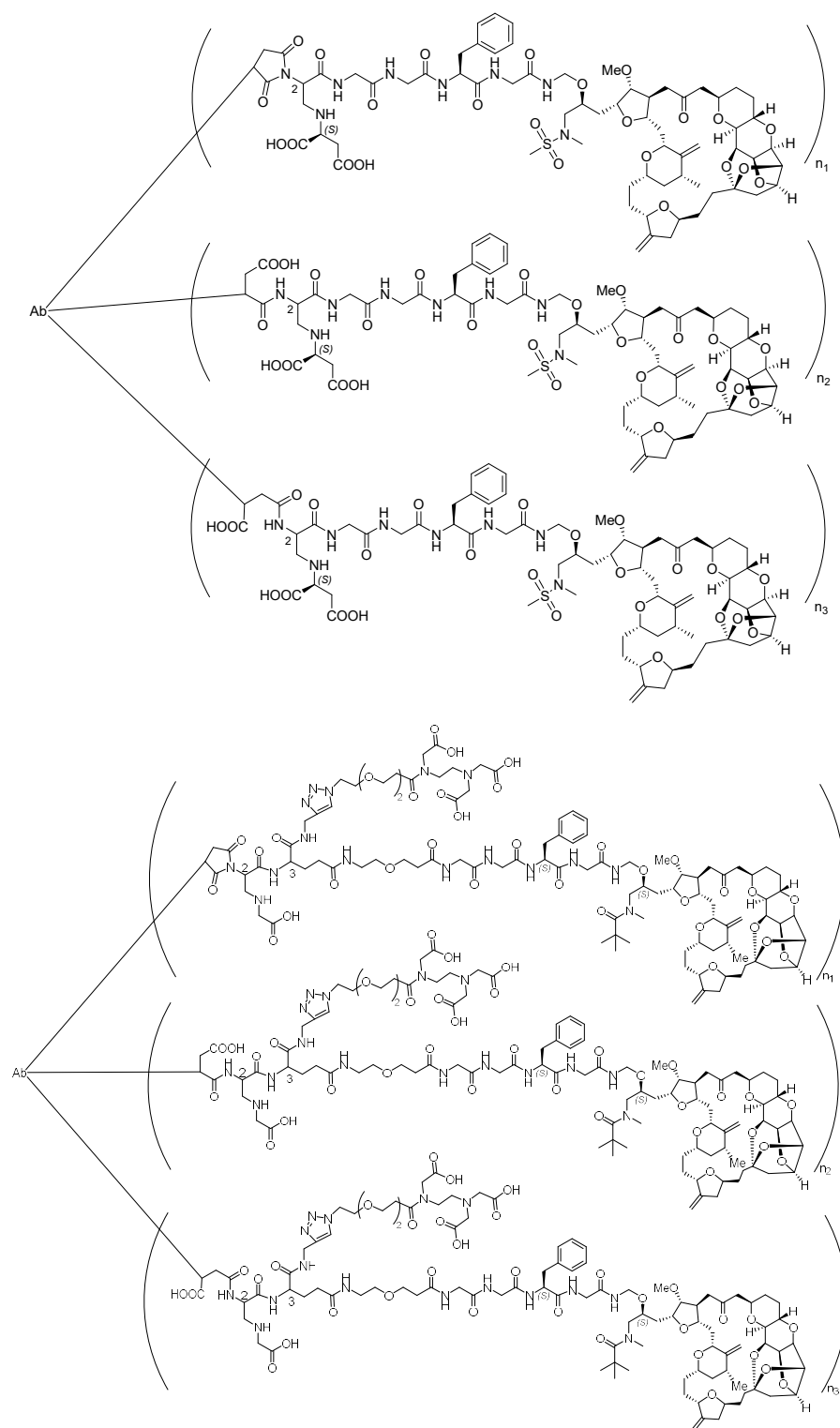


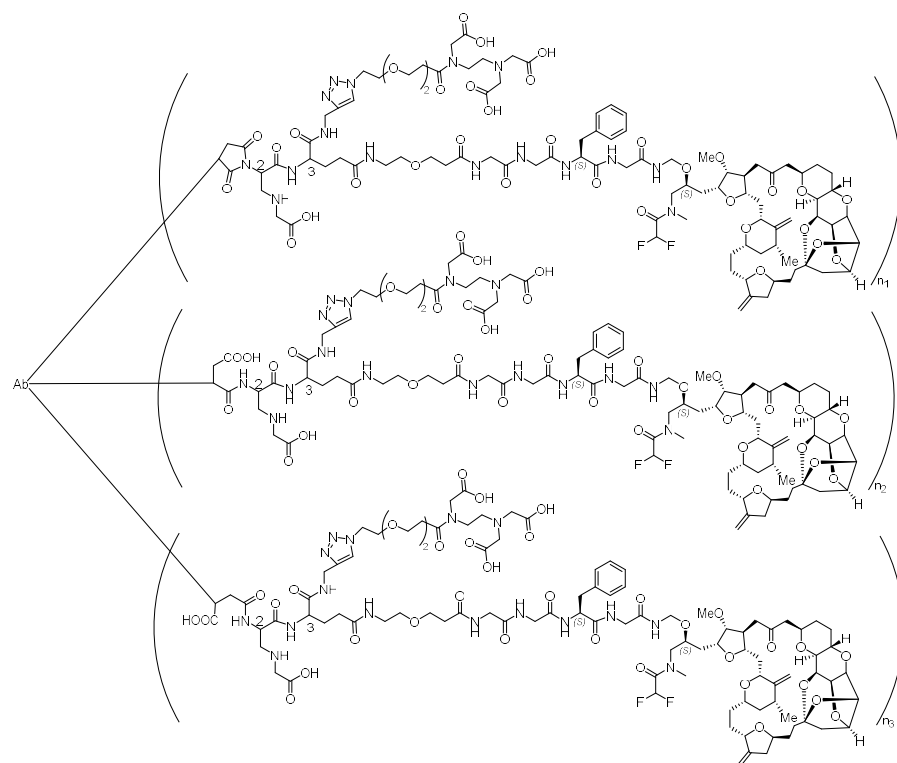
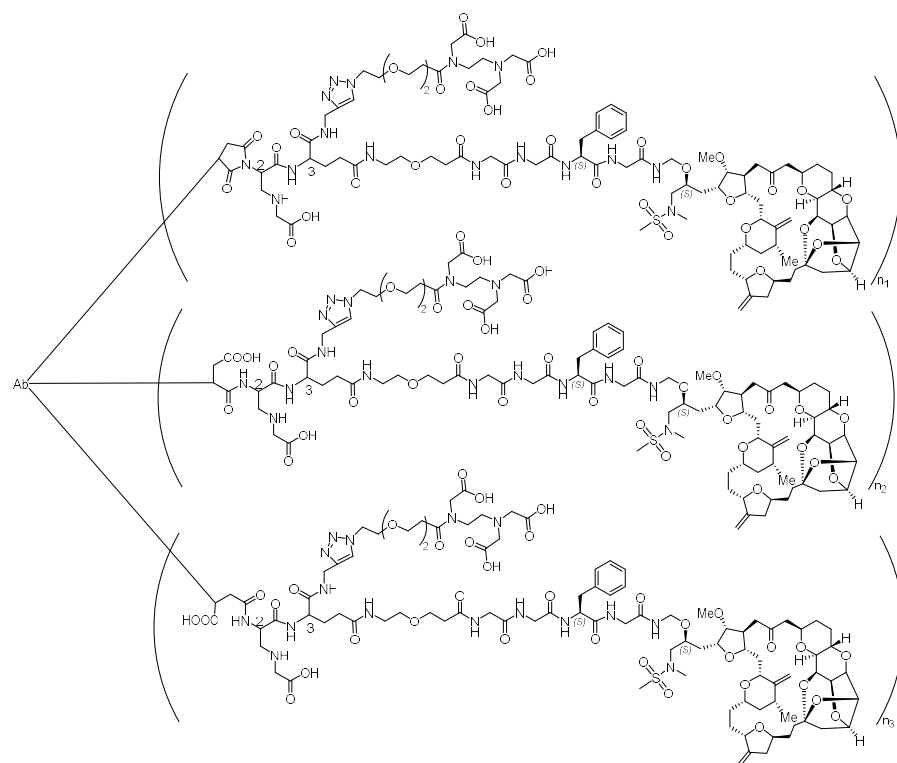


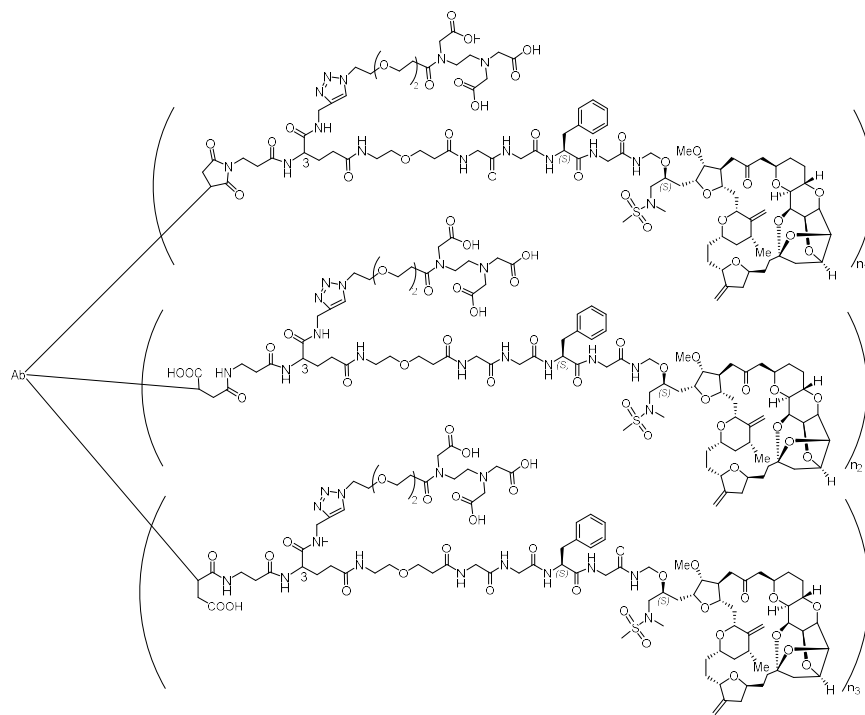


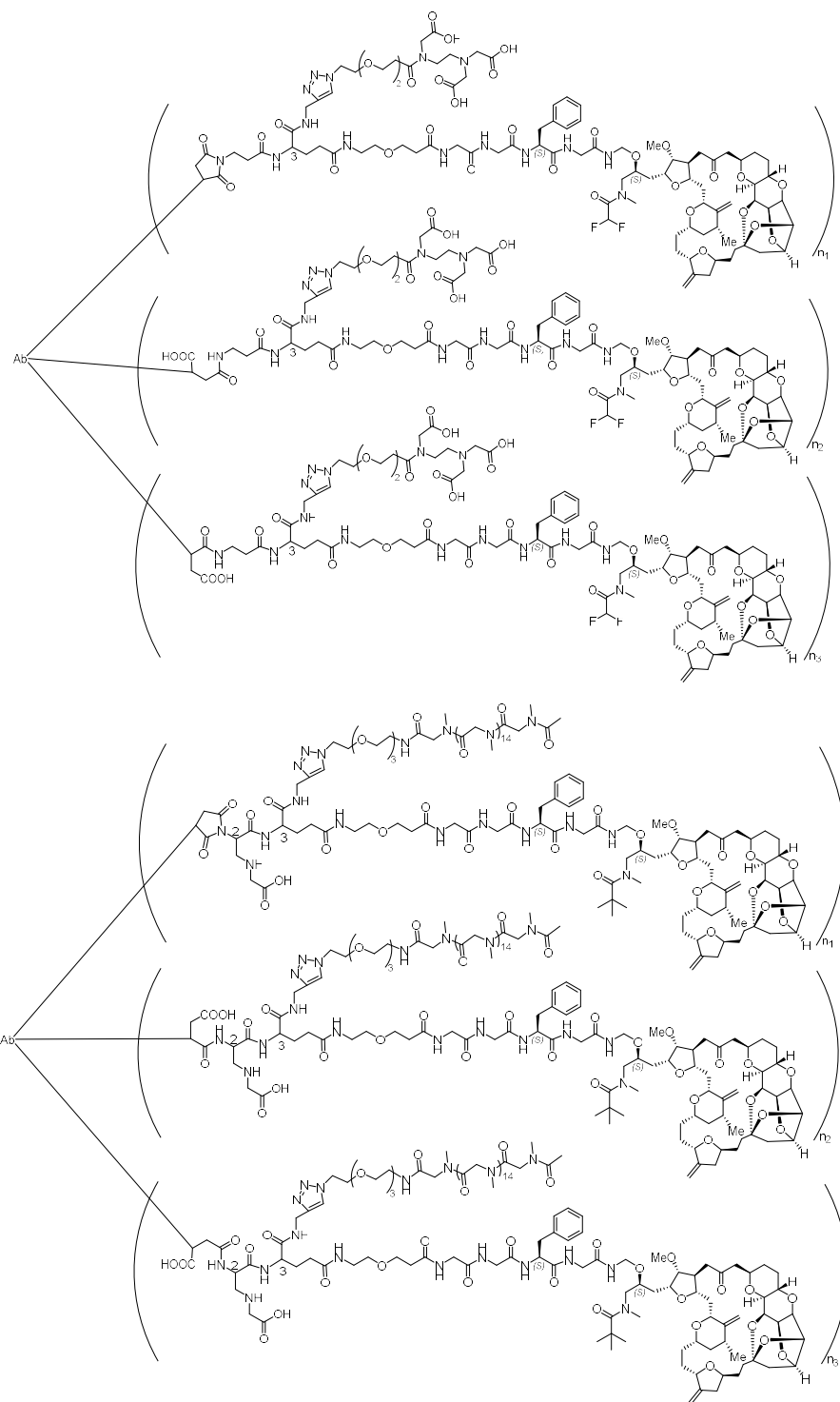


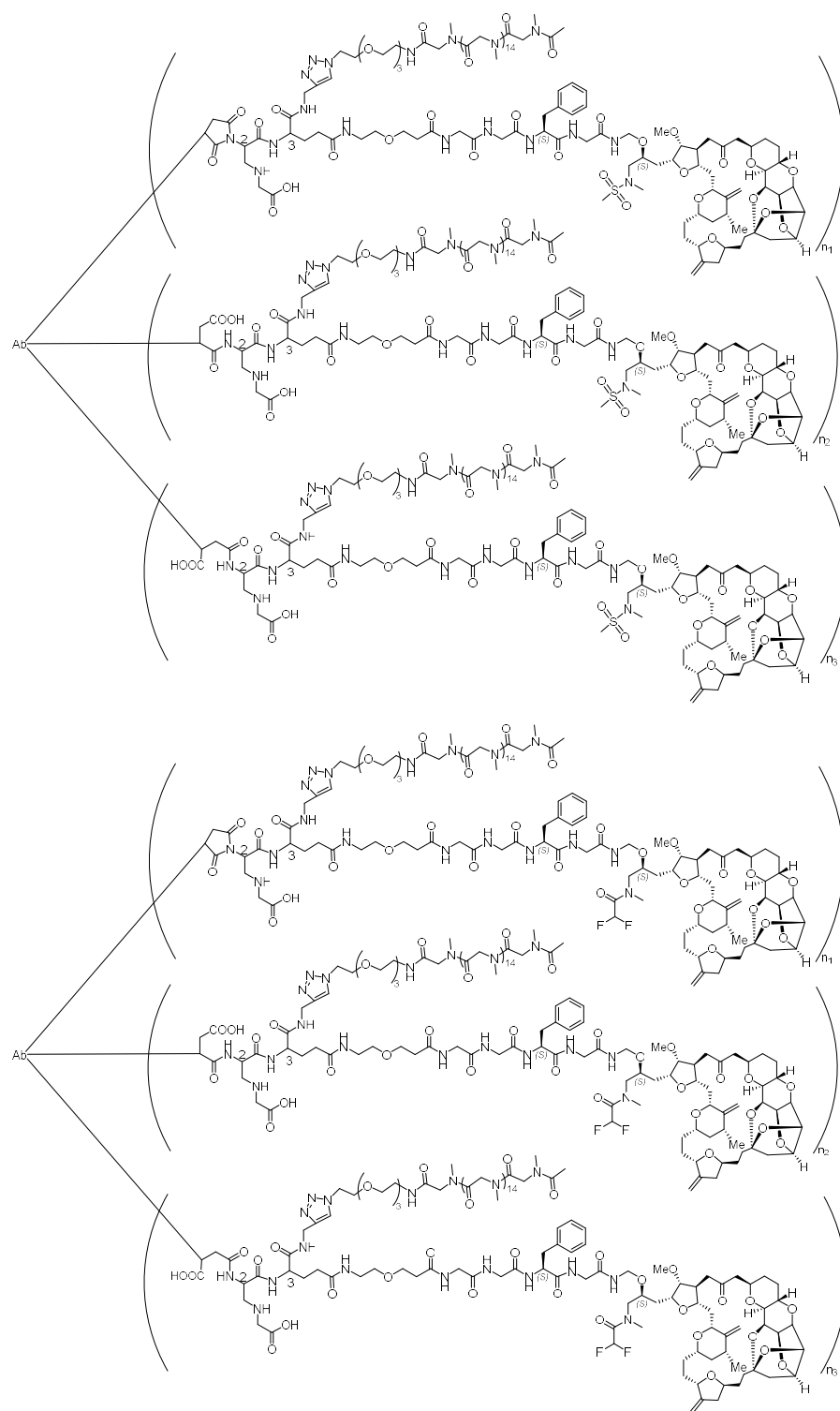


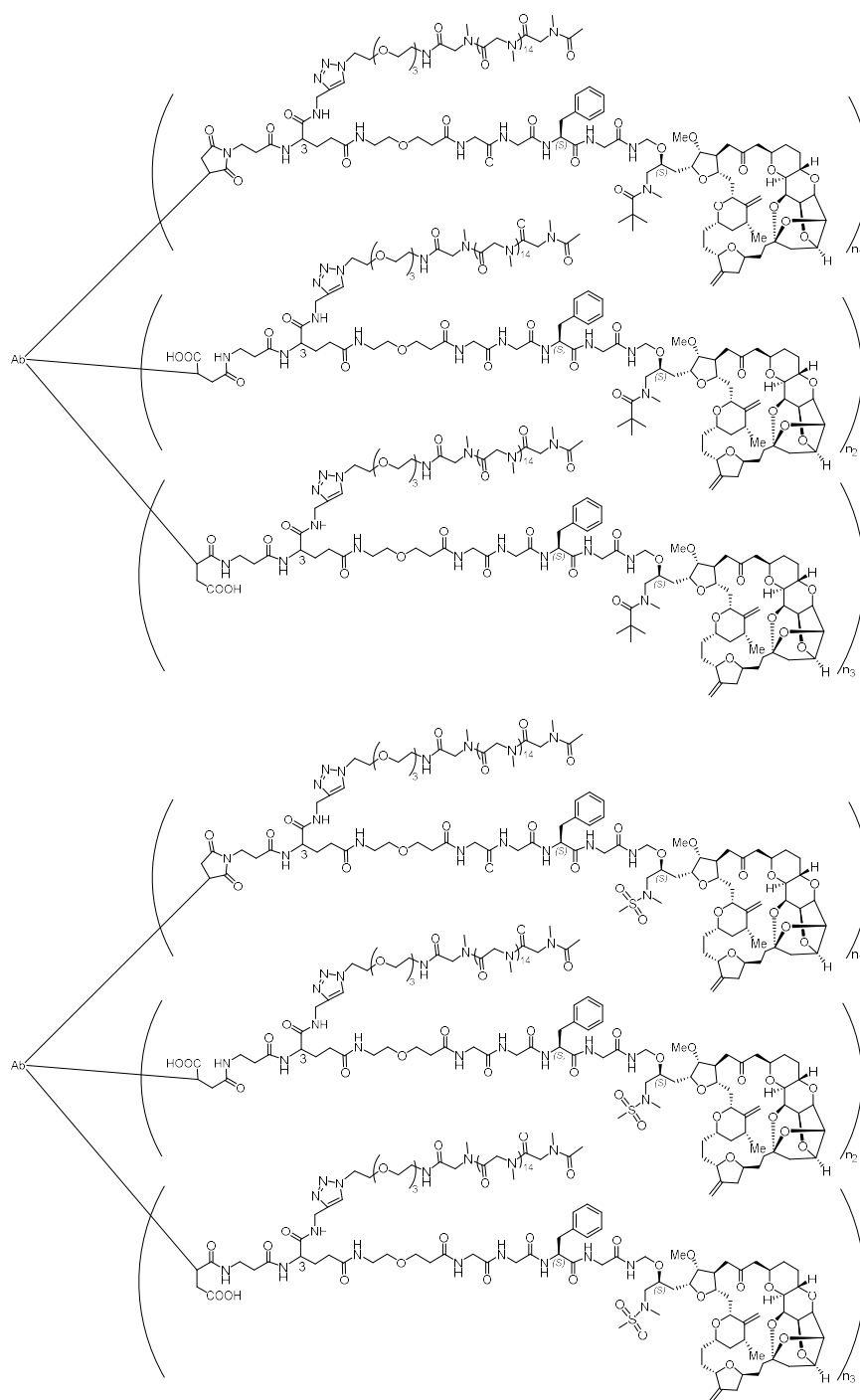


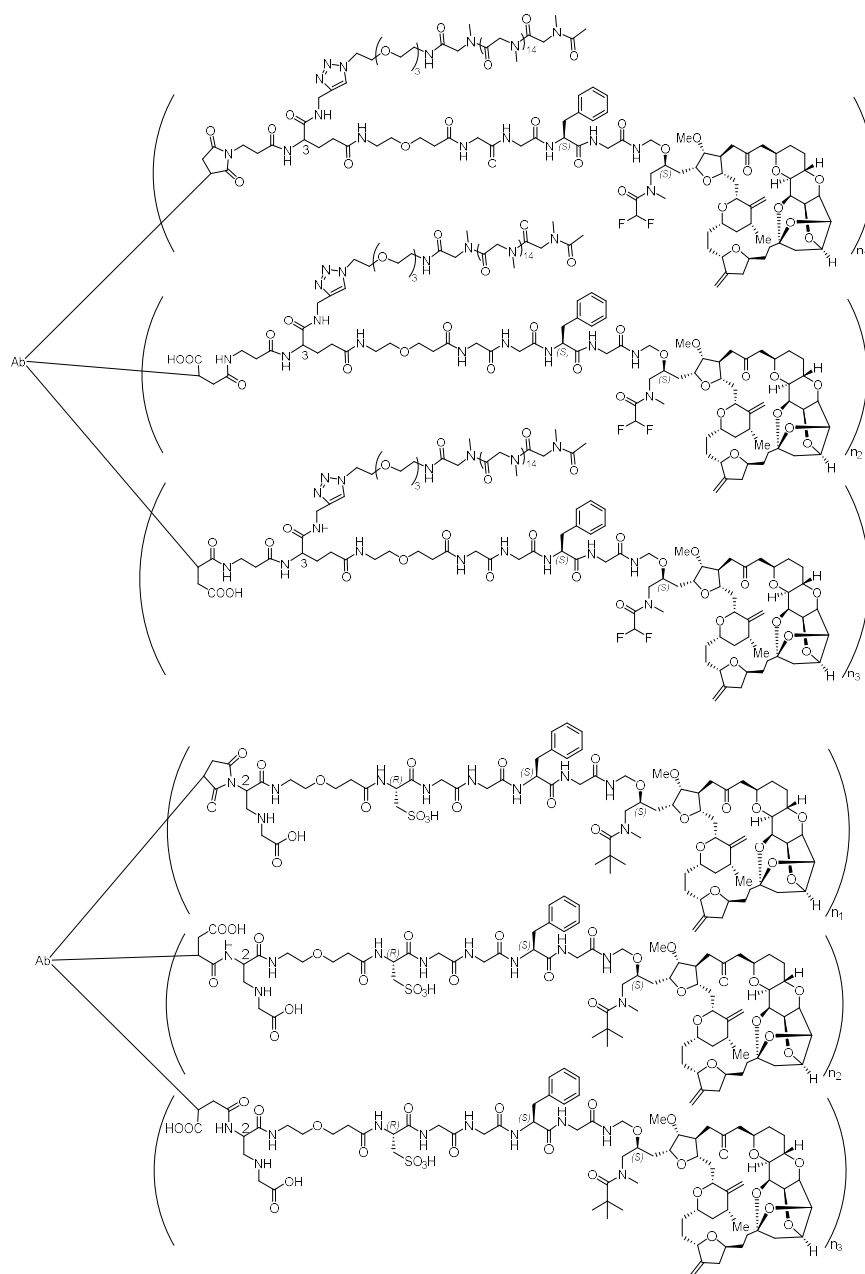


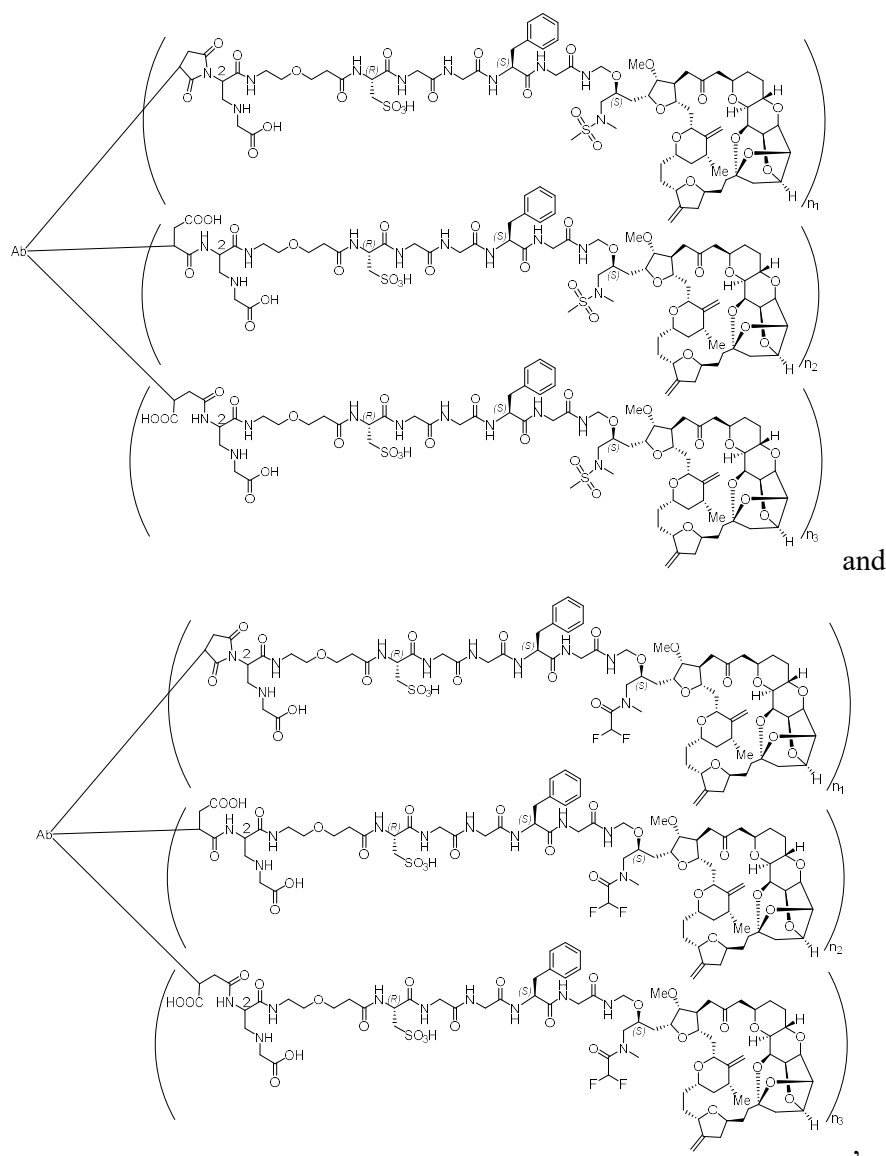












[0087] wherein chiral carbons at positions 2 and 3 independently have configuration R or S. In some embodiments, the chiral carbon at position 2 has configuration S, and the chiral carbon at position 3 has configuration S.

[0088] In the present application, the ligand unit Ab may be selected from the group consisting of an antibody, an antibody fragment or a protein; the antibody is preferably selected from the group consisting of a murine antibody, a rabbit antibody, a phage display-derived antibody, a yeast display-derived antibody, a chimeric antibody, a humanized antibody, a fully human antibody, an antibody fragment, a bispecific antibody and a multispecific antibody.

[0089] In some embodiments, the antibody is a monoclonal antibody, is selected, without limitation, from: anti-EGFRvIII antibody, anti-PD-1 antibody, anti-PD-L1 antibody, anti-DLL-3

antibody, anti-PSMA antibody, anti-CD70 antibody, anti-MUC16 antibody, anti-ENPP3 antibody, anti-TDGF1 antibody, anti-ETBR antibody, anti-MSLN antibody, anti-TIM-1 antibody, anti-LRRC15 antibody, anti-LIV-1 antibody, anti-CanAg/AFP antibody, anti-claudin 18.2 antibody, anti-Mesothelin antibody, anti-HER2(ErbB2) antibody, anti-EGFR antibody, anti-c-MET antibody, anti-SLITRK6 antibody, anti-KIT/CD117 antibody, anti-STEAP1 antibody, anti-SLAMF7/CS1 antibody, anti-NaPi2B/SLC34A2 antibody, anti-GPNMB antibody, anti-HER3(ErbB3) antibody, anti-MUC1/CD227 antibody, anti-AXL antibody, anti-CD166 antibody, anti-B7-H3(CD276) antibody, anti-PTK7/CCK4 antibody, anti-PRLR antibody, anti-EFNA4 antibody, anti-5T4 antibody, anti-NOTCH3 antibody, anti-Nectin 4 antibody, anti-TROP-2 antibody, anti-CD142 antibody, anti-CA6 antibody, anti-GPR20 antibody, anti-CD174 antibody, anti-CD71 antibody, anti-EphA2 antibody, anti-LYPD3 antibody, anti-FGFR2 antibody, anti-FGFR3 antibody, anti-FR α antibody, anti-CEACAMs antibody, anti-GCC antibody, anti-Integrin Av antibody, anti-CAIX antibody, anti-P-cadherin antibody, anti-GD3 antibody, anti-Cadherin 6 antibody, anti-LAMP1 antibody, anti-FLT3 antibody, anti-BCMA antibody, anti-CD79b antibody, anti-CD19 antibody, anti-CD33 antibody, anti-CD56 antibody, anti-CD74 antibody, anti-CD22 antibody, anti-CD30 antibody, anti-CD37 antibody, anti-CD47 antibody, anti-CD138 antibody, anti-CD352 antibody, anti-CD25 antibody or anti-CD123 antibody.

[0090] In some embodiments, the antibody is anti-TROP-2 antibody. In some embodiments, the antibody comprises heavy chains and light chains, the light chains comprise CDR-L1, CDR-L2 and CDR-L3 having amino acid sequences set forth in SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3. In some embodiments, the heavy chains comprise CDR-H1, CDR-H2 and CDR-H3 having amino acid sequences set forth in SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6.

[0091] In some embodiments, the light chain comprises a light chain variable region having an amino acid sequence of SEQ ID NO:7. In some embodiments, the light chain further comprises a light chain constant region having an amino acid sequence of SEQ ID NO:8. In some embodiments, the amino acid sequence of the light chain is SEQ ID NO:9.

[0092] In some embodiments, the light chain comprises a light chain variable region having an amino acid sequence of SEQ ID NO:10. In some embodiments, the light chain further comprises a light chain constant region having an amino acid sequence of SEQ ID NO:11. In some embodiments, the amino acid sequence of the light chain is SEQ ID NO:12.

[0093] In some embodiments, the heavy chain comprises a heavy chain variable region having an amino acid sequence of SEQ ID NO:13. In some embodiments, the heavy chain further comprises a heavy chain constant region having an amino acid sequence of SEQ ID NO:14. In some

embodiments, the amino acid sequence of the heavy chain is SEQ ID NO:15.

[0094] In some embodiments, the antibody is anti-TROP-2 antibody. In some embodiments, the antibody comprises heavy chains and light chains, the light chains comprise CDR-L1, CDR-L2 and CDR-L3 having nucleic acid coding sequences set forth in SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:18. In some embodiments, the heavy chains comprise CDR-H1, CDR-H2 and CDR-H3 having nucleic acid coding sequences set forth in SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21.

[0095] In some embodiments, the light chain comprises a light chain variable region having a nucleic acid coding sequence of SEQ ID NO:22. In some embodiments, the light chain further comprises a light chain constant region having a nucleic acid coding sequence of SEQ ID NO:23. In some embodiments, the nucleic acid coding sequence of the light chain is SEQ ID NO:24.

[0096] In some embodiments, the light chain comprises a light chain variable region having a nucleic acid coding sequence of SEQ ID NO:25. In some embodiments, the light chain further comprises a light chain constant region having a nucleic acid coding sequence of SEQ ID NO:26. In some embodiments, the nucleic acid coding sequence of the light chain is SEQ ID NO:27.

[0097] In some embodiments, the heavy chain comprises a heavy chain variable region having a nucleic acid coding sequence of SEQ ID NO:28. In some embodiments, the heavy chain further comprises a heavy chain constant region having a nucleic acid coding sequence of SEQ ID NO: 29. In some embodiments, the nucleic acid coding sequence of the heavy chain is SEQ ID NO:30.

[0098] The information of partial sequences involved in the present invention is provided in Table 1.

Table 1: Description of the sequences

SEQ ID NO:	Description	Sequences
1	Amino acid sequence of CDR-L1	RASQDINKYLA
2	Amino acid sequence of CDR-L2	STSTLQS
3	Amino acid sequence of CDR-L3	LQYDDLFT
4	Amino acid sequence of CDR-H1	SFDIN
5	Amino acid	WIFPGDGNTKYSQKFQG

	sequence of CDR-H2	
6	Amino acid sequence of CDR-H3	GEALYYFDY
7	Amino acid sequence of the light chain variable region of TR001	DIQMTQSPSSLSASVGDRVTITCRASQDINKYLAWYQQKP GKVPKLLIYSTSTLQSGVPSRFSGSGSGTDFTLTISLQPED VATYYCLQYDDLFTFGQGTKLEIK
8	Amino acid sequence of the light chain constant region of TR001	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADY EKHKVYACEVTHQGLSSPVTKSFNRGEC
9	Amino acid sequence of the light chain of TR001	DIQMTQSPSSLSASVGDRVTITCRASQDINKYLAWYQQKP GKVPKLLIYSTSTLQSGVPSRFSGSGSGTDFTLTISLQPED VATYYCLQYDDLFTFGQGTKLEIKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC
10	Amino acid sequence of the light chain variable region of TR002	DIQMTQSPSSLSASVGDRVTITCRASQDINKYLAWYQQKP GKVPKLLIYSTSTLQSGVPSRFSGSGSGTDFTLTISLQPED VATYYCLQYDDLFTFGQGTKLEIK
11	Amino acid sequence of the light chain constant region of TR002	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADY EKHKVYACEVTHQGLSSPVCTKSFNRGEC
12	Amino acid sequence of the light chain of TR002	DIQMTQSPSSLSASVGDRVTITCRASQDINKYLAWYQQKP GKVPKLLIYSTSTLQSGVPSRFSGSGSGTDFTLTISLQPED VATYYCLQYDDLFTFGQGTKLEIKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGL SSPVCTKSFNRGEC
13	Amino acid sequences of the heavy chain variable region of TR001 and TR002	QVQLVQSGAEVKKPGASVKLSCKASGYTFTSFDINWVRQ APEQRLEWMGWIFPGDGNTKYSQKFQGRATITRDTSAST AYMELSSLRSEDYAVYYCVRGEALYYFDYWGQGTLVTVS S
14	Amino acid sequences of the heavy chain constant region of TR001 and TR002	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQT YICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTIKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTP

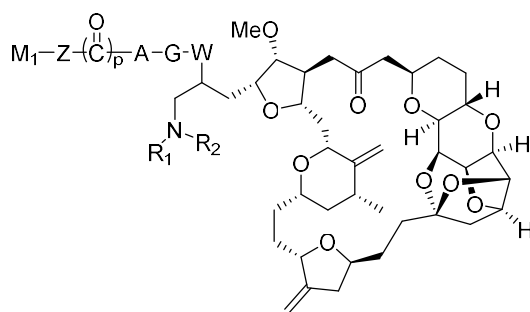
		PVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPG
15	Amino acid sequences of the heavy chain of TR001 and TR002	QVQLVQSGAEVKKPGASVKLSCKASGYTFTSFDINWVRQ APEQRLEWMGWIFPGDGTKYSQKFQGRATITRDTAST AYMELSSLRSEDTAVYYCVRGEALYYFDYWGQGLVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTP PVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPG
16	Nucleic acid coding sequence of CDR-L1	AGAGCTTCTCAGGATATCAATAAGTATCTGGCT
17	Nucleic acid coding sequence of CDR-L2	TCTACATCTACCCTGCAGTCT
18	Nucleic acid coding sequence of CDR-L3	CTGCAGTATGATGATCTGTTCACC
19	Nucleic acid coding sequence of CDR-H1	TCCTTCGACATTAAC
20	Nucleic acid coding sequence of CDR-H2	TGGATCTTCCCCGGCGACGGCAACACCAAGTACTCCCA GAAGTTCCAGGGA
21	Nucleic acid coding sequence of CDR-H3	GGAGAGGCTCTGTACTATTTTGATTAT
22	Nucleic acid coding sequence of the light chain variable region of TR001	GATATCCAGATGACCCAGTCTCCATCTAGCCTGTCCGCT TCTGTGGGCGATAGAGTGACCATCACATGCAGAGCTTC TCAGGATATCAATAAGTATCTGGCTTGGTATCAGCAGAA GCCTGGAAAGGTGCCTAAGCTGCTGATCTACTCTACATC TACCCTGCAGTCTGGAGTGCCTTCTAGATTTTCTGGATC TGGCTCTGGCACCGATTTTACACTGACAATCTCTTCTCT GCAGCCTGAGGATGTGGCTACATATTATTGTCTGCAGTA TGATGATCTGTTCACCTTTGGCCAGGGCACCAAGCTGG AGATCAAG
23	Nucleic acid coding sequence of the light chain constant region of TR001	CGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCA TCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTG TGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGT ACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACT CCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAG CACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAG CAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTC ACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTT

		CAACAGGGGAGAGTGTTAG
24	Nucleic acid coding sequence of the light chain of TR001	GATATCCAGATGACCCAGTCTCCATCTAGCCTGTCCGCT TCTGTGGGCGATAGAGTGACCATCACATGCAGAGCTTC TCAGGATATCAATAAGTATCTGGCTTGGTATCAGCAGAA GCCTGGAAAGGTGCCTAAGCTGCTGATCTACTCTACATC TACCCTGCAGTCTGGAGTGCCTTCTAGATTTTCTGGATC TGGCTCTGGCACCGATTTTACACTGACAATCTCTTCTCT GCAGCCTGAGGATGTGGCTACATATTATTGTCTGCAGTA TGATGATCTGTTACCTTTGGCCAGGGCACCAAGCTGG AGATCAAGCGTACGGTGGCTGCACCATCTGTCTTCATCT TCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCT CTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGG CCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCG GGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCA AGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCT GCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACA AAGAGCTTCAACAGGGGAGAGTGTTAG
25	Nucleic acid coding sequence of the light chain variable region of TR002	GATATCCAGATGACCCAGTCTCCATCTAGCCTGTCCGCT TCTGTGGGCGATAGAGTGACCATCACATGCAGAGCTTC TCAGGATATCAATAAGTATCTGGCTTGGTATCAGCAGAA GCCTGGAAAGGTGCCTAAGCTGCTGATCTACTCTACATC TACCCTGCAGTCTGGAGTGCCTTCTAGATTTTCTGGATC TGGCTCTGGCACCGATTTTACACTGACAATCTCTTCTCT GCAGCCTGAGGATGTGGCTACATATTATTGTCTGCAGTA TGATGATCTGTTACCTTTGGCCAGGGCACCAAGCTGG AGATCAAG
26	Nucleic acid coding sequence of the light chain constant region of TR002	CGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCA TCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTG TGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGT ACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACT CCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAG CACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAG CAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTC ACCCATCAGGGCCTGAGCTCGCCCGTCTGTACAAAGAG CTTCAACAGGGGAGAGTGTTAG
27	Nucleic acid coding sequence of the light chain of TR002	GATATCCAGATGACCCAGTCTCCATCTAGCCTGTCCGCT TCTGTGGGCGATAGAGTGACCATCACATGCAGAGCTTC TCAGGATATCAATAAGTATCTGGCTTGGTATCAGCAGAA GCCTGGAAAGGTGCCTAAGCTGCTGATCTACTCTACATC TACCCTGCAGTCTGGAGTGCCTTCTAGATTTTCTGGATC TGGCTCTGGCACCGATTTTACACTGACAATCTCTTCTCT GCAGCCTGAGGATGTGGCTACATATTATTGTCTGCAGTA TGATGATCTGTTACCTTTGGCCAGGGCACCAAGCTGG AGATCAAGCGTACGGTGGCTGCACCATCTGTCTTCATCT TCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCT CTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGG CCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCG GGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCA AGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTG

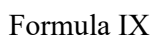
		AGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCT GCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCTGT ACAAAGAGCTTCAACAGGGGAGAGTGTTAG
28	Nucleic acid coding sequences of the heavy chain variable region of TR001 and TR002	CAGGTGCAGCTGGTGCAGTCCGGCGCCGAGGTGAAGA AGCCCGGCGCCTCCGTGAAGCTGAGCTGTAAGGCCTCC GGCTACACCTTCACCTCCTTCGACATTAAGTGGGTGCGG CAGGCCCCCGAGCAGCGCCTGGAGTGGATGGGCTGGAT CTTCCCCGGCGACGGCAACACCAAGTACTCCCAGAAGT TCCAGGGAAGAGCTACCATCACCAGAGATACATCCGCT TCTACAGCTTACATGGAGCTGTCTAGCCTGAGATCTGAG GATACAGCTGTGTATTACTGTGTGAGAGGAGAGGCTCT GTACTATTTTGATTATTGGGGCCAGGGCACCTGGTGAC AGTGTCTTCT
29	Nucleic acid coding sequences of the heavy chain constant region of TR001 and TR002	GCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACC CTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGG GCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACG GTGTCGTGGAAGTCAAGGCGCCCTGACCAGCGGCGTGCA CACCTTCCCGGCTGTCTACAGTCTCAGGACTCTACTC CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGG GCACCCAGACCTACATCTGCAACGTGAATCACAAGCCC AGCAACACCAAGGTGGACAAGAGAGTTGAGCCCCAAT CTTGTGACAAAACCTCACACATGCCCACCGTGCCCAGCA CCTGAAGTCTTGGGGGGACCGTCAGTCTTCTCTTCCC CCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCC CTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAA GACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGT GGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAG CAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCAC CGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACA AGTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCCATC GAGAAAACCATCTCCAAGCCAAAGGGCAGCCCCGAG AACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAG CTGACCAAGAACCAGGTACGCCTGACCTGCCTGGTCAA AGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGA GCAATGGGCAGCCGGAGAACAATAAGACACACGCC TCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTATAG CAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGG AACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCAC AACCCTACACGCAGAAGAGCCTCTCCCTGTCTCCGGG TTAA
30	Nucleic acid coding sequences of the heavy chain of TR001 and TR002	CAGGTGCAGCTGGTGCAGTCCGGCGCCGAGGTGAAGA AGCCCGGCGCCTCCGTGAAGCTGAGCTGTAAGGCCTCC GGCTACACCTTCACCTCCTTCGACATTAAGTGGGTGCGG CAGGCCCCCGAGCAGCGCCTGGAGTGGATGGGCTGGAT CTTCCCCGGCGACGGCAACACCAAGTACTCCCAGAAGT TCCAGGGAAGAGCTACCATCACCAGAGATACATCCGCT TCTACAGCTTACATGGAGCTGTCTAGCCTGAGATCTGAG GATACAGCTGTGTATTACTGTGTGAGAGGAGAGGCTCT GTACTATTTTGATTATTGGGGCCAGGGCACCTGGTGAC AGTGTCTTCTGCTAGCACCAAGGGCCCATCGGTCTTCCC

		CCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAG CGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAA CCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAG CGGCGTGACACCTTCCCGGCTGTCCTACAGTCCTCAG GACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCC AGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAA TCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTT GAGCCCAAATCTTGTGACAAAACCTCACACATGCCACC GTGCCAGCACCTGAACTCCTGGGGGGGACCGTCAGTCT TCCTCTTCCCCCAAACCCAAGGACACCCTCATGATCT CCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTG AGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGT GGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCG CGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCA GCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGC AAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCC CAGCCCCATCGAGAAAACCATCTCAAAGCCAAAGGG CAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATC CCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCT GCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTG GAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACA AGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTC TTCCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTG GCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATG AGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCC CTGTCTCCGGGTAA
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[0099] In a second aspect, the present application provides a linker-drug conjugate of formula VIII or formula IX, or an isomer, mesomer, racemate, or enantiomer thereof, or a mixture thereof, or a pharmaceutically acceptable salt or solvate thereof,



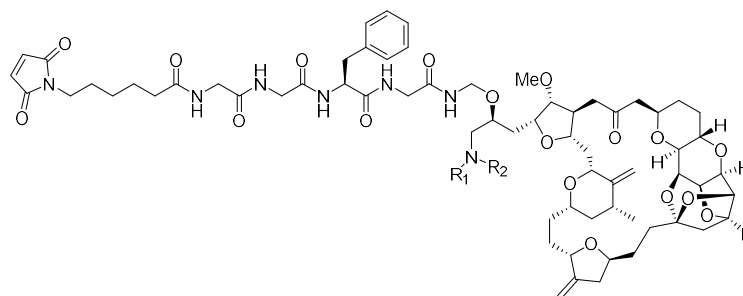
Formula VIII



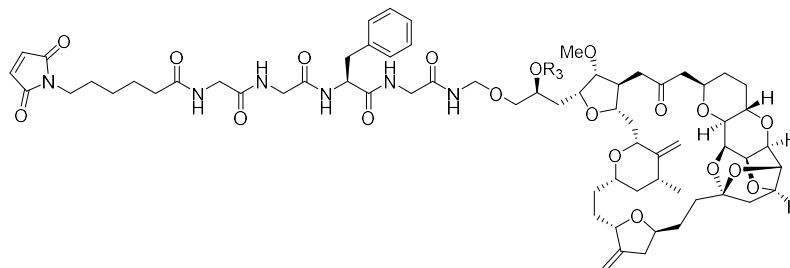
[00103] In some embodiments, the linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof of the present application has a structure represented by formula VIII-1 or formula IX-1,



[00106] In some embodiments, the linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof of the present application has a structure represented by formula VIIIa or formula IXa:



Formula VIIIa

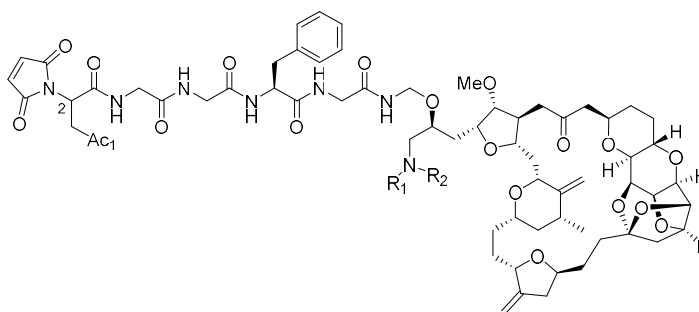


Formula IXa

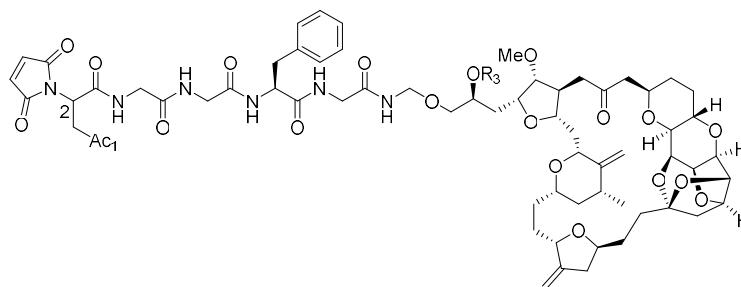
[00107] wherein,

[00108] the R_1 , R_2 and R_3 are as defined above.

[00109] In some embodiments, the linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof of the present application has a structure represented by formula VIIIb or formula IXb:

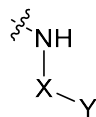


Formula VIIIb



Formula IXb

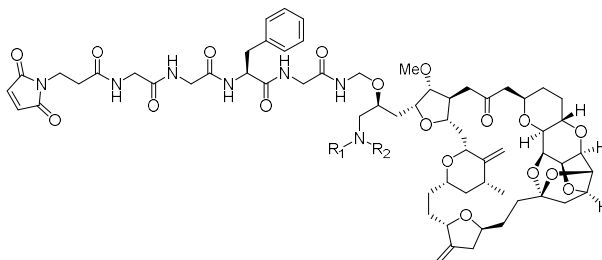
[00110] wherein, Ac is a hydrophilic structural unit having the structure shown in formula d:



Formula d

[00111] the X and Y are as defined above. In some embodiments, the Ac is as defined above.

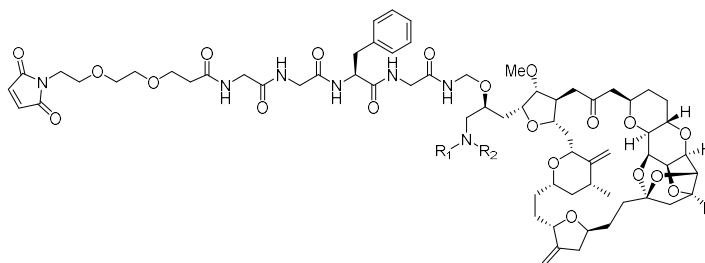
[00112] In some embodiments, the linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof of the present application has a structure represented by formula VIIIc:



VIIIc

[00113] wherein, the R₁ and R₂ are as defined above.

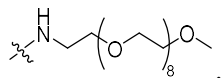
[00114] In some embodiments, the linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof of the present application has a structure represented by formula VIId:



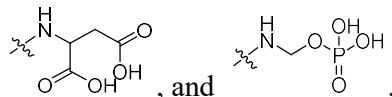
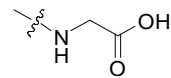
Formula VIId

[00115] wherein, the R₁ and R₂ are as defined above.

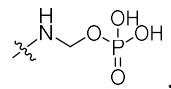
[00116] In some embodiments, the linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof of the present application has a structure represented by formula VIIIe:



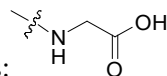
[00122] In some embodiments, Ac₁ is selected from the following structures:



, and

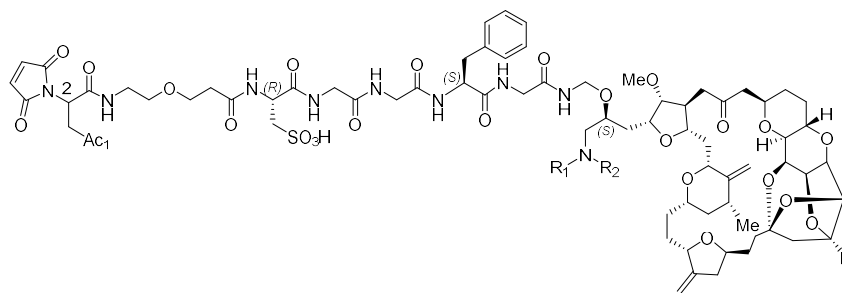


[00123] In some embodiments, Ac₁ is:



[00124] Ac₂ is a hydrophilic structural unit composed of -X₂-Y₂, and the Ac₂, X₂ and Y₂ are as defined above.

[00125] In some embodiments, the linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof of the present application has a structure represented by formula VIIIg:

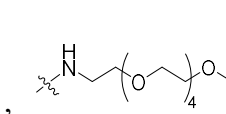
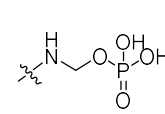
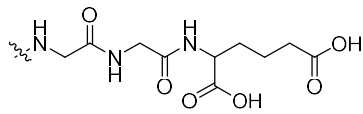
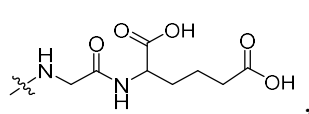
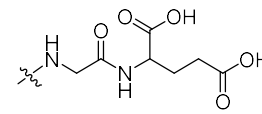
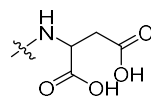
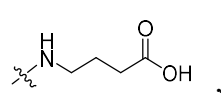
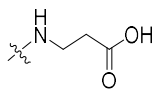
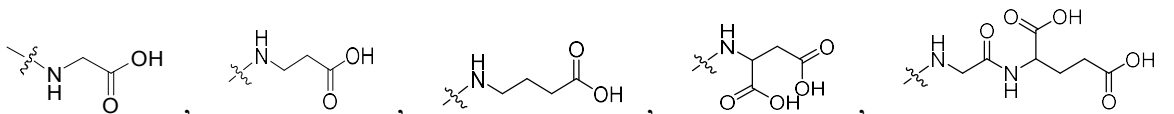


Formula VIIIg

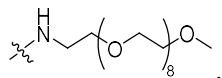
[00126] wherein, the R₁ and R₂ are as defined above;

[00127] Ac₁ is a hydrophilic structural unit composed of -X₁-Y₁, and the X₁ and Y₁ are as defined above.

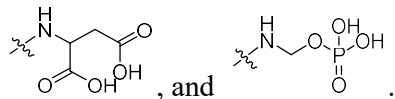
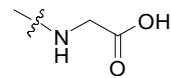
[00128] In some embodiments, the Ac₁ is selected, without limitation, from the group consisting of (D/L) alanine, (D/L) leucine, (D/L) isoleucine, (D/L) valine, (D/L) phenylalanine, (D/L) proline, (D/L) tryptophan, (D/L) serine, (D/L) tyrosine, (D/L) cysteine, (D/L) cystine, (D/L) arginine, (D/L) histidine, (D/L) methionine, (D/L) asparagine, (D/L) glutamine, (D/L) threonine, (D/L) aspartic acid, (D/L) glutamic acid, natural or unnatural amino acid derivatives and the following structures:



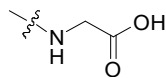
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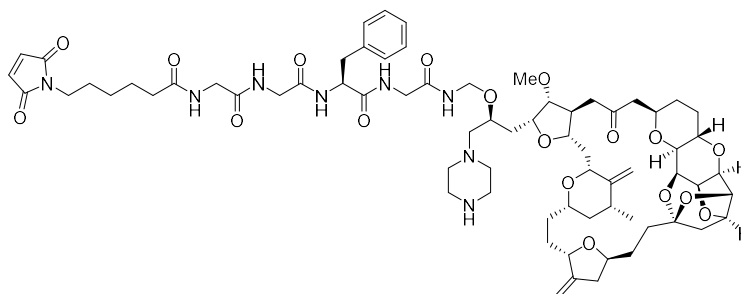
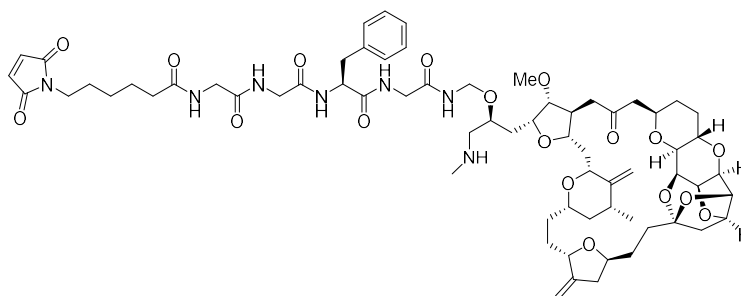
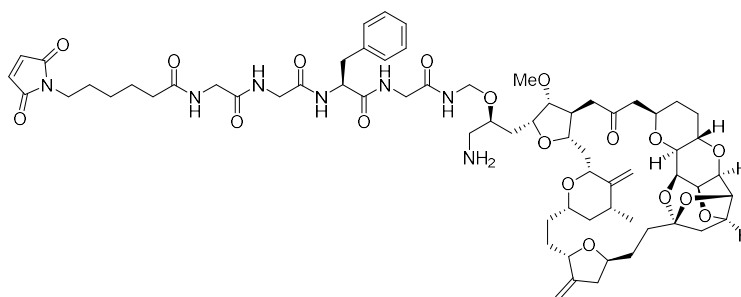
[00129] In some embodiments, Ac₁ is selected from the following structures:

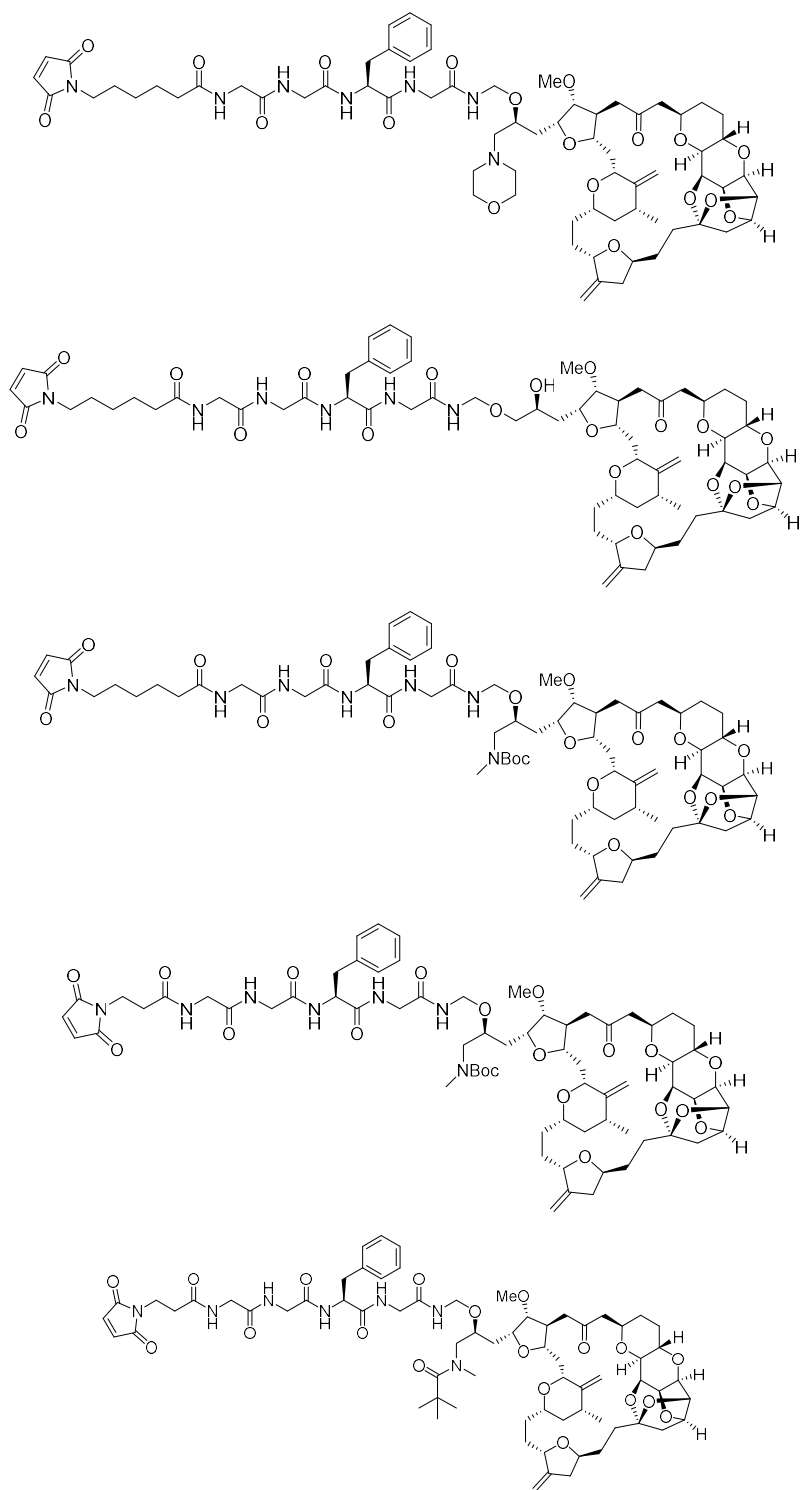


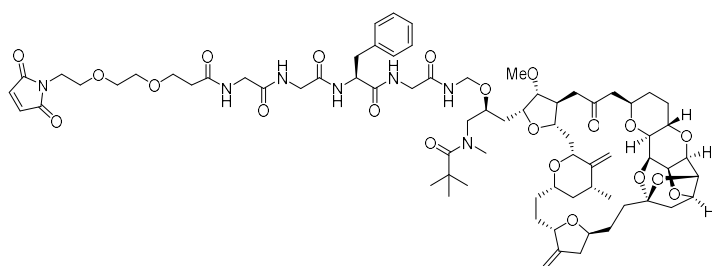
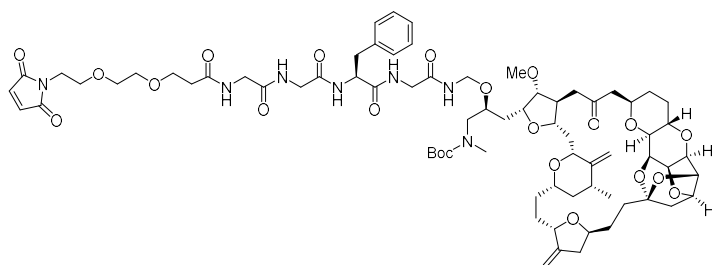
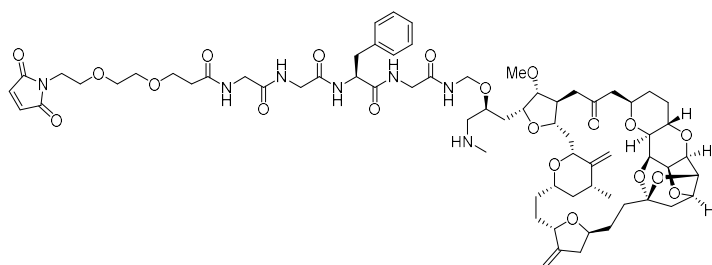
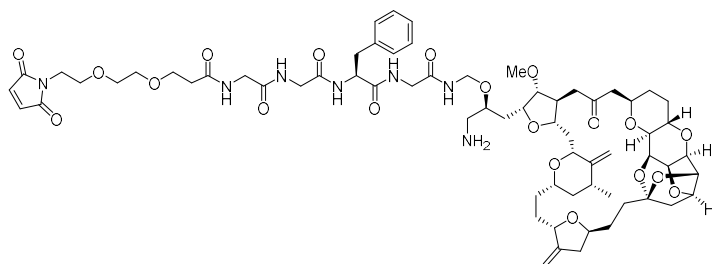
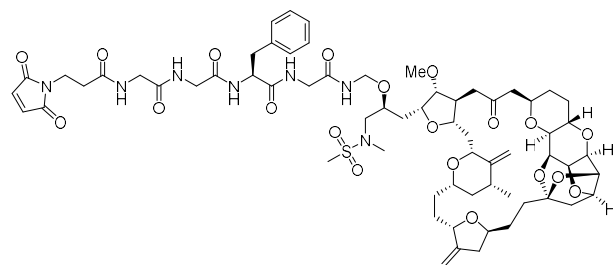
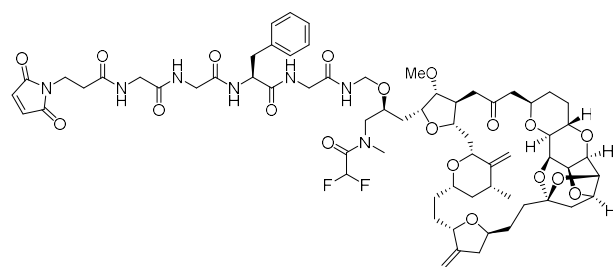
[00130] In some embodiments, Ac₁ is:

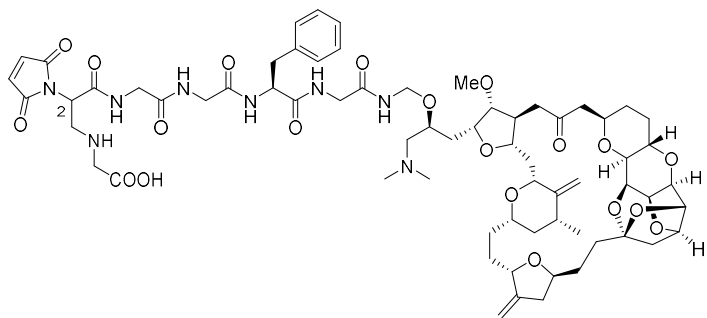
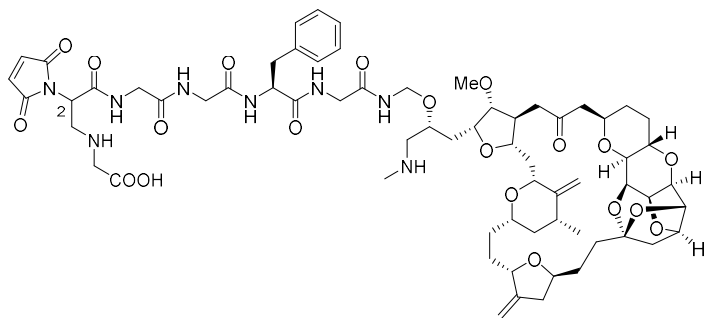
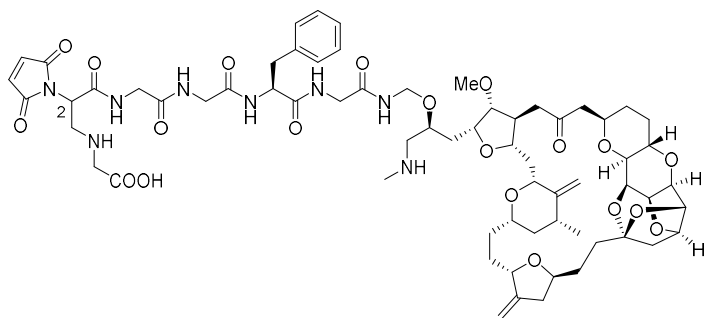
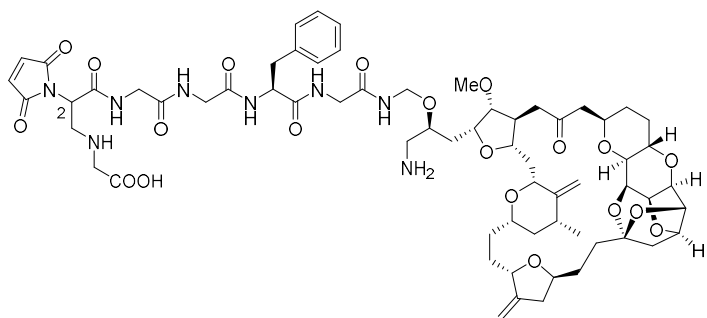
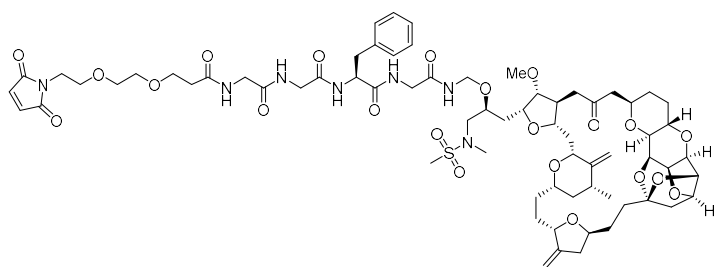


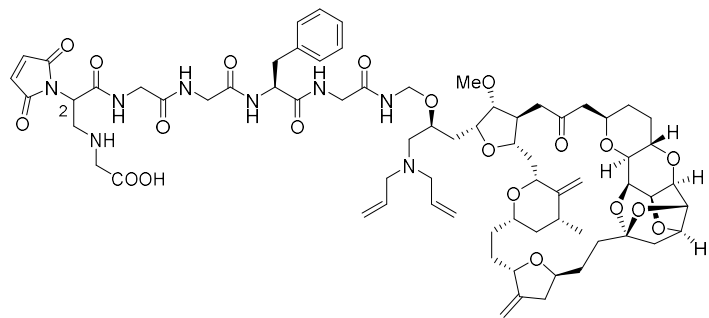
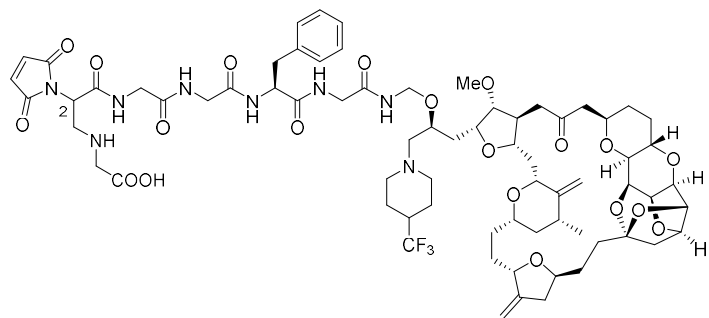
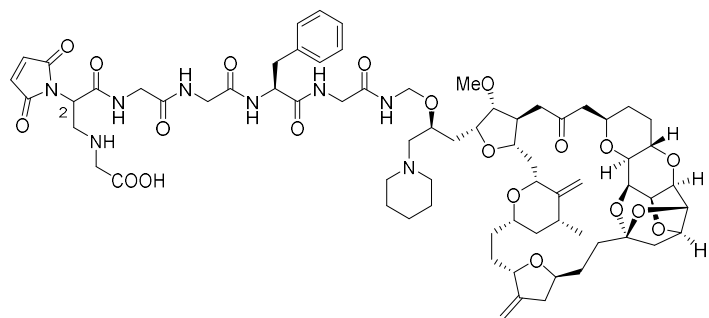
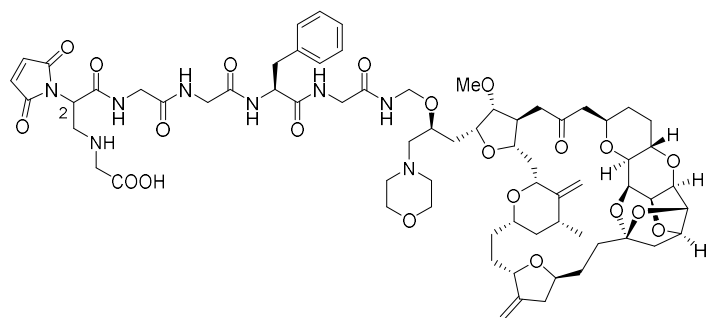
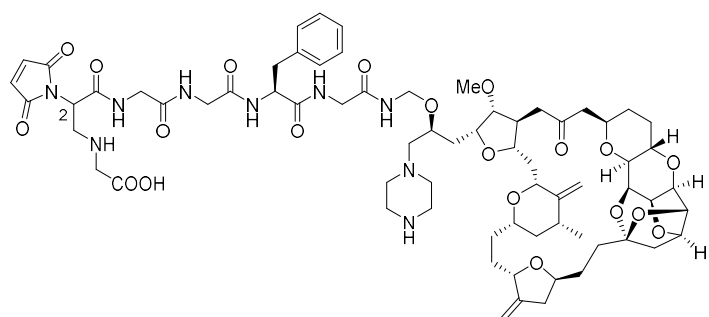
In some embodiments, the linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof of the present application is selected from the following structures:

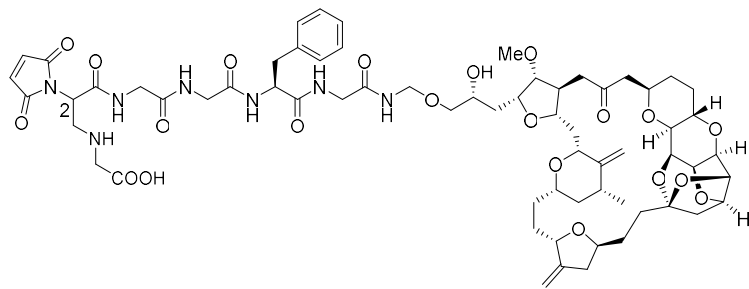
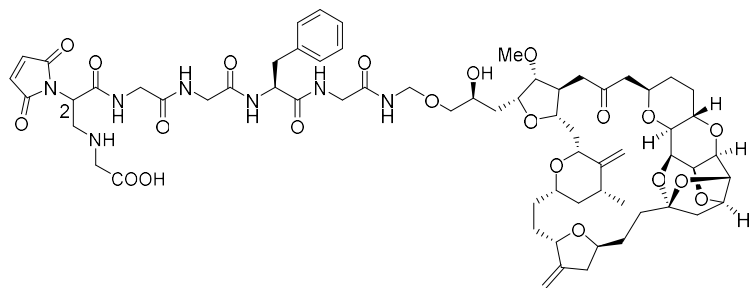
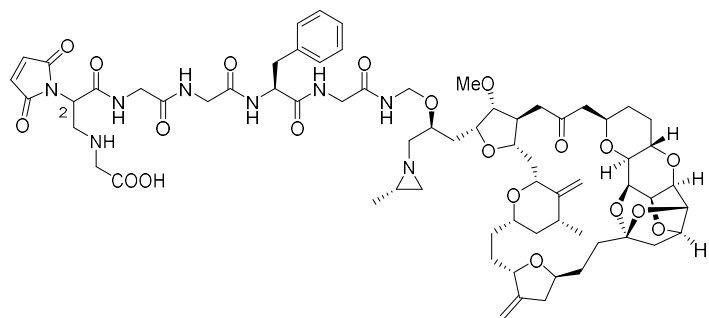
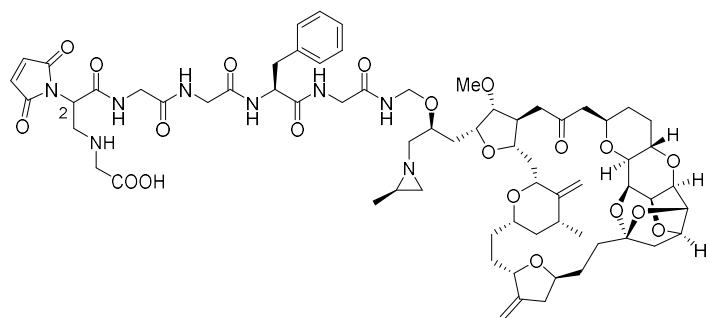
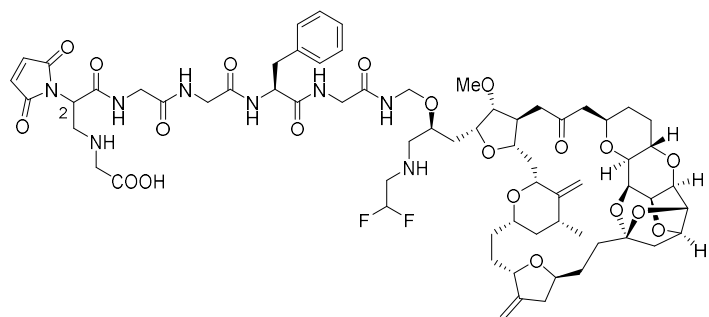


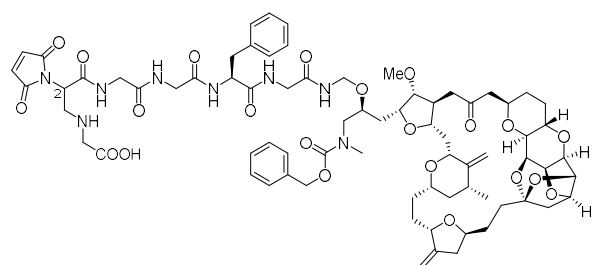
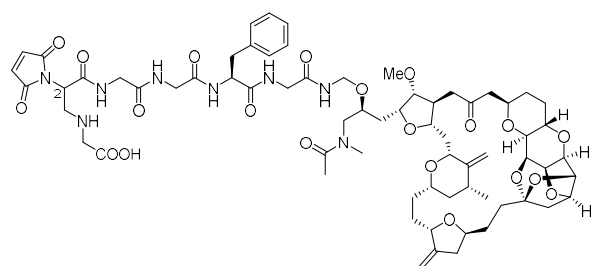
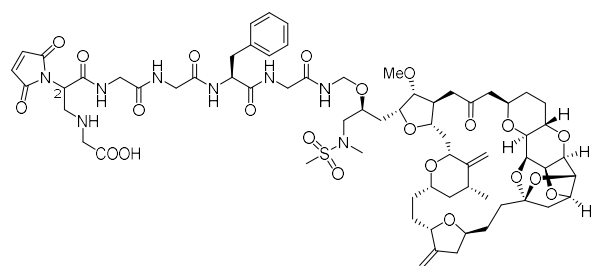
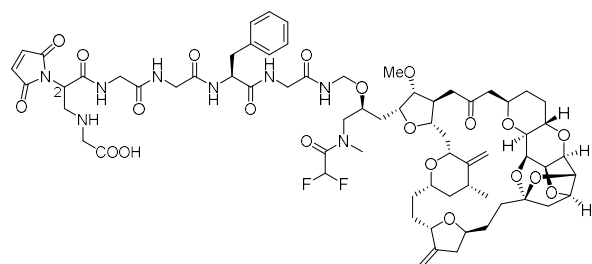
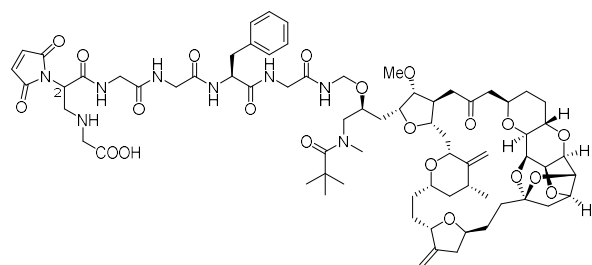
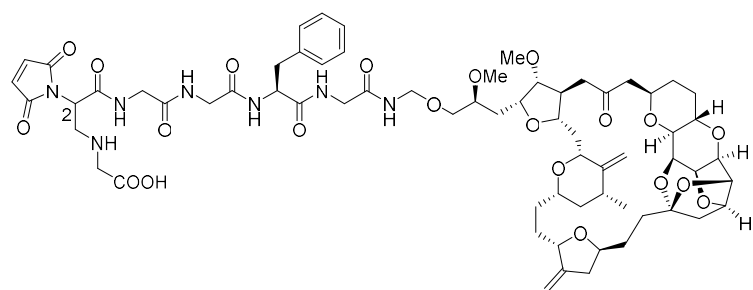


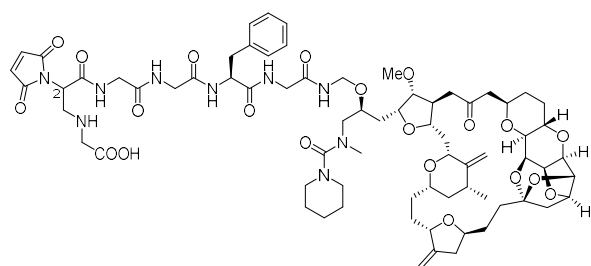
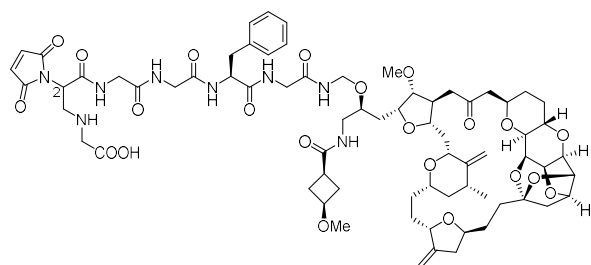
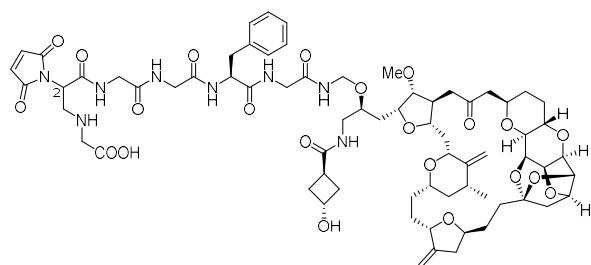
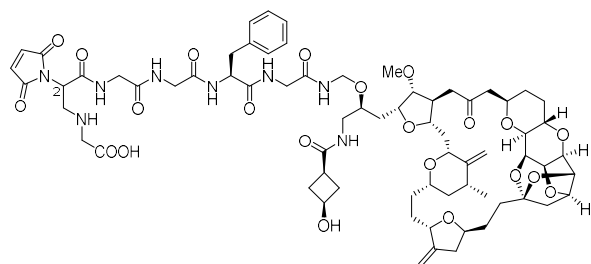
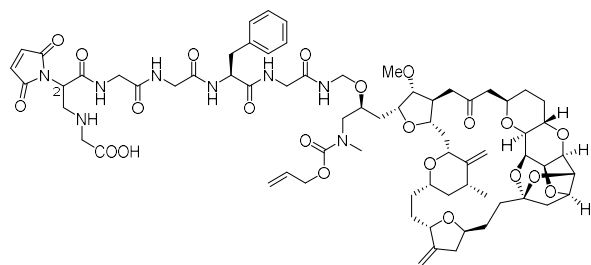
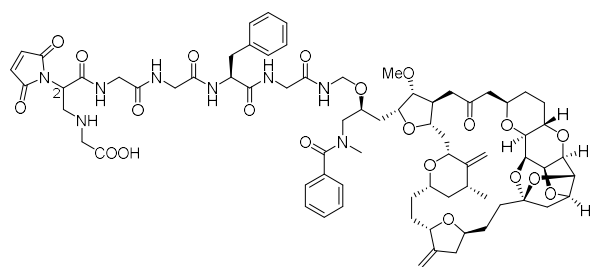


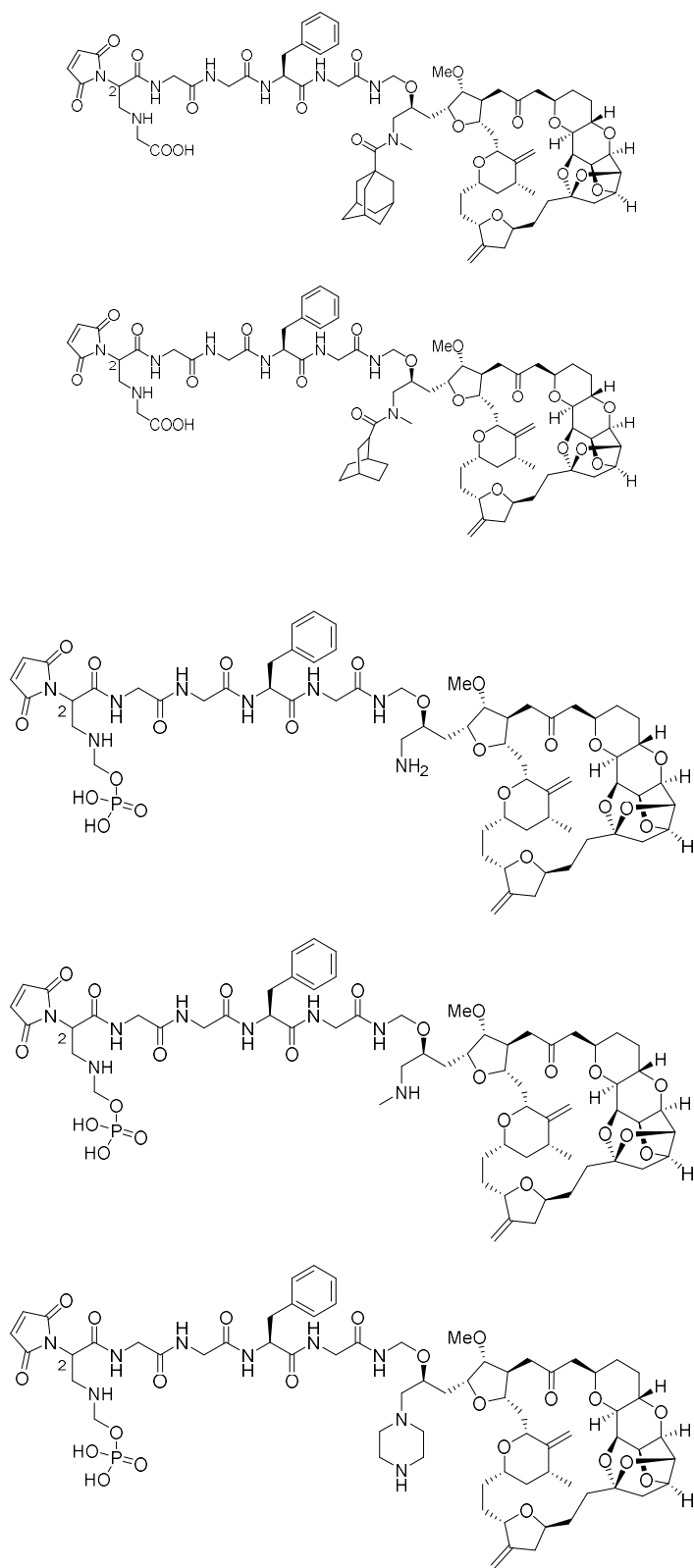


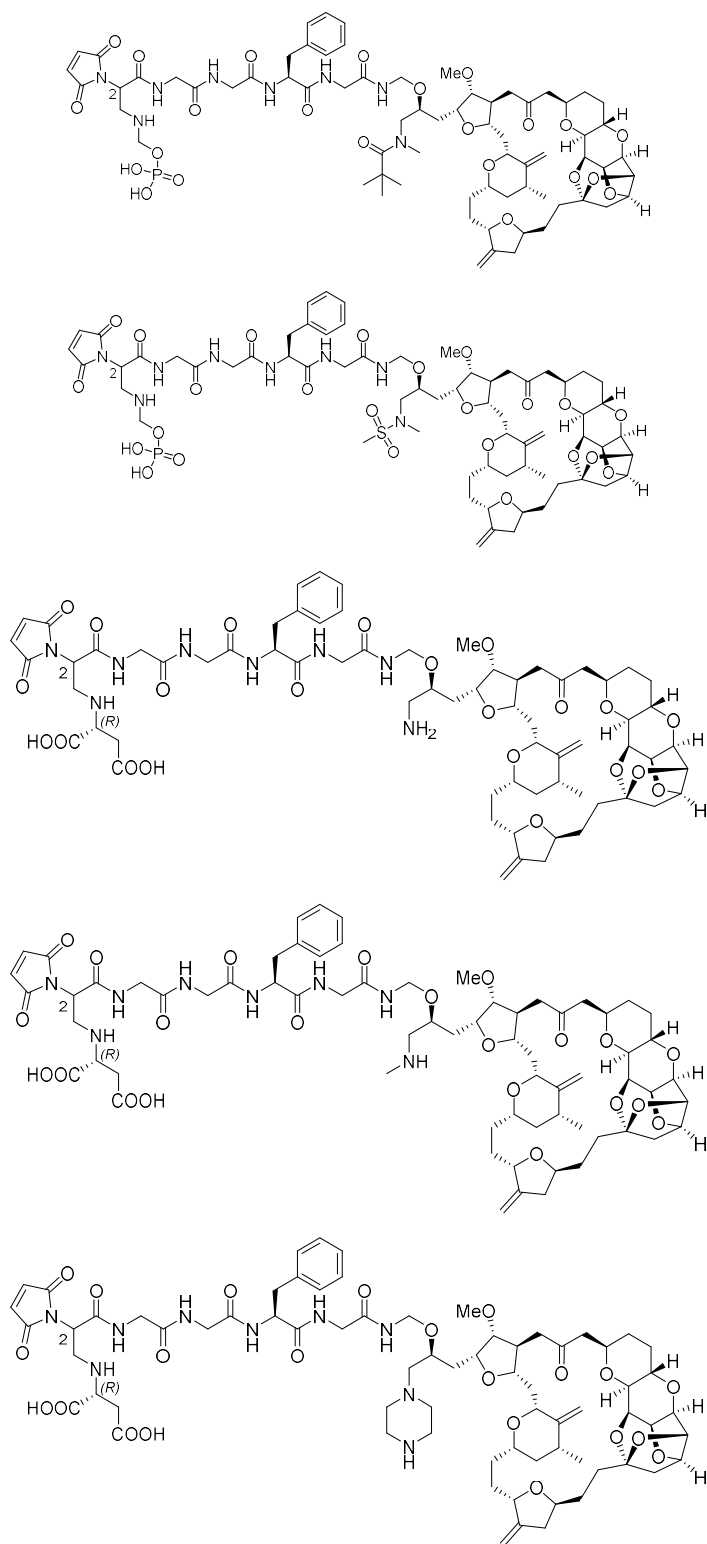


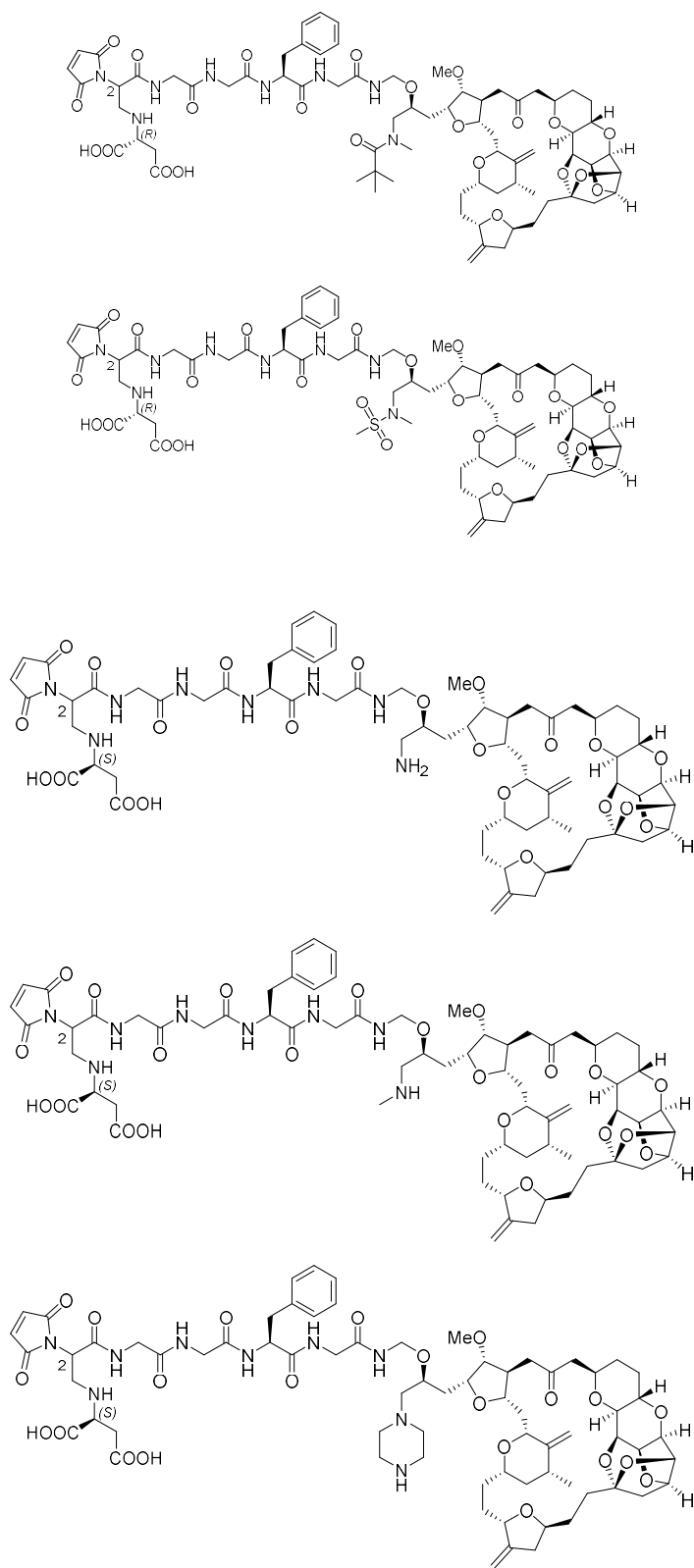


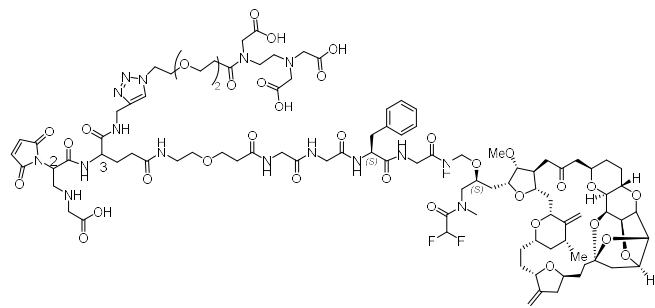
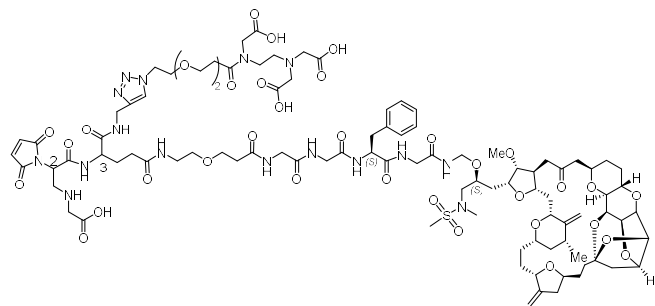
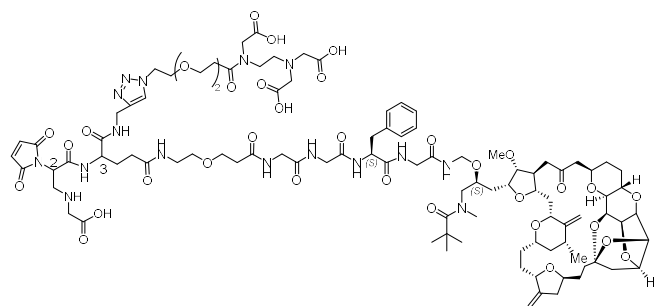
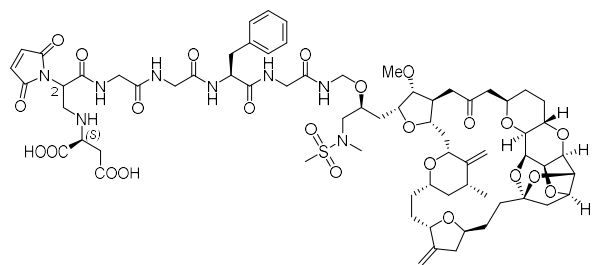
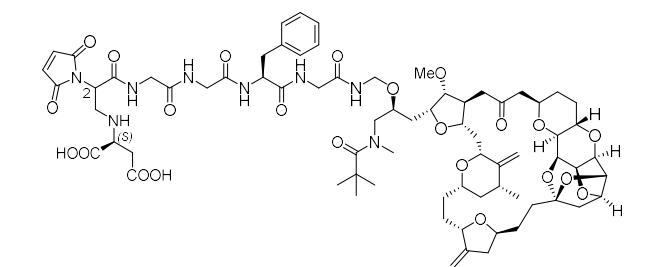


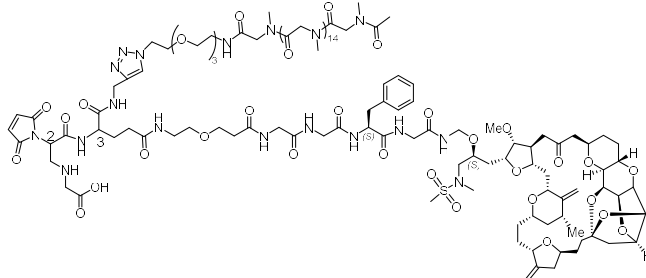
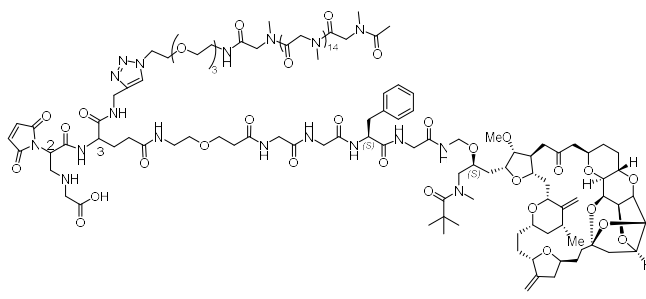
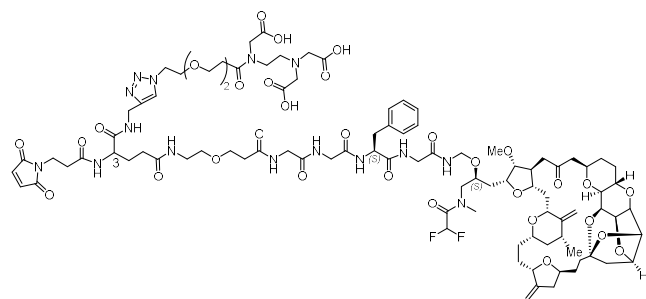
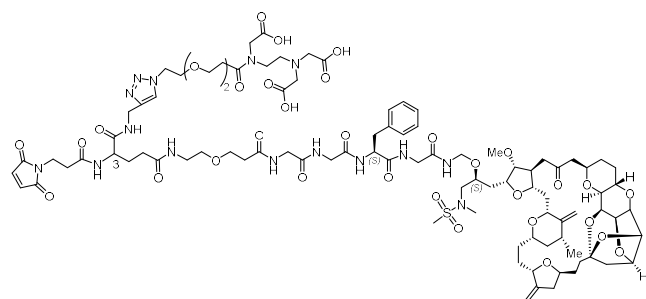
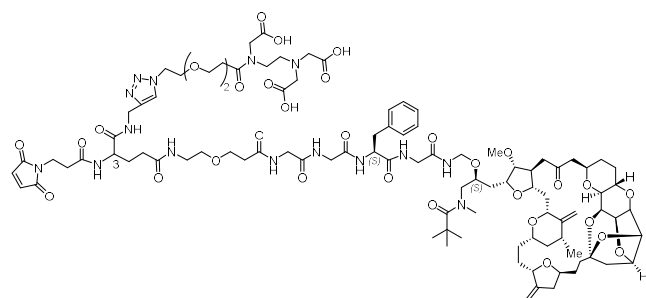


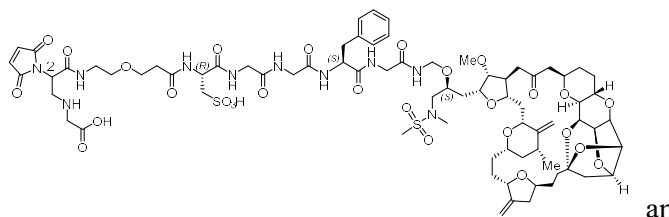
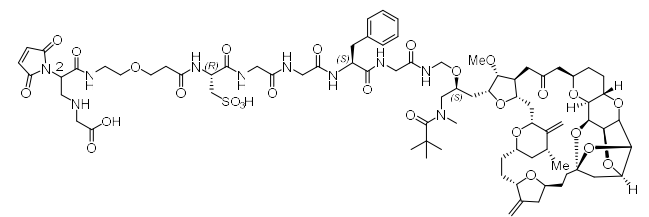
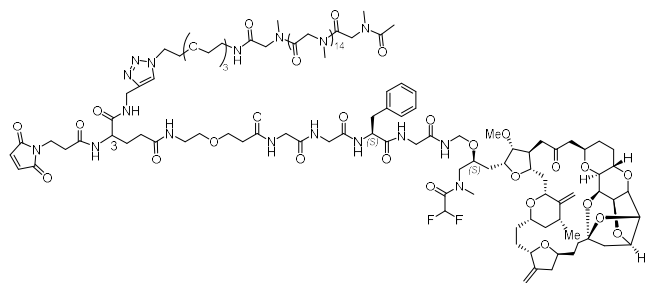
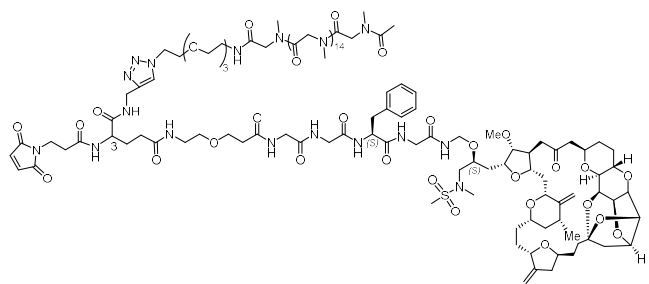
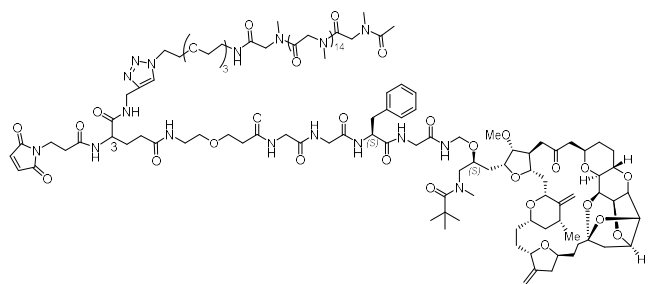
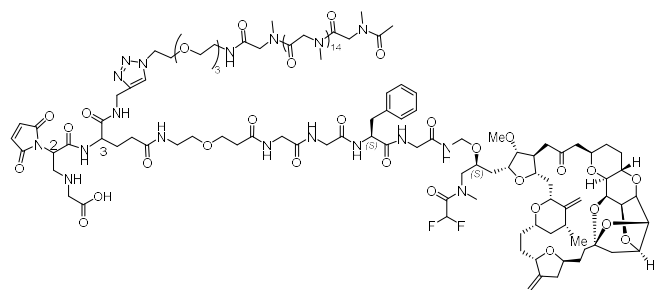




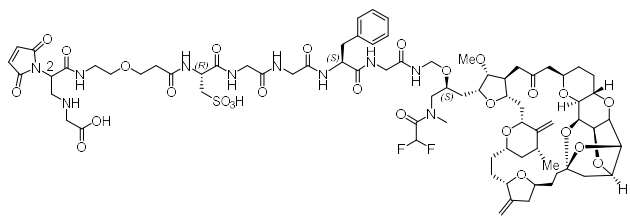






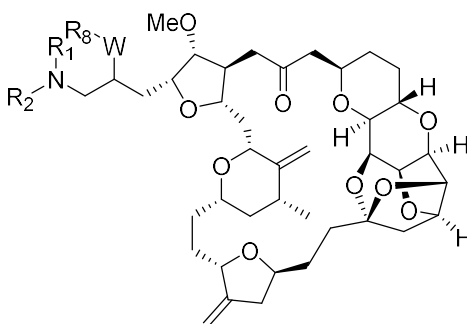


and



[00131] wherein chiral carbons at positions 2 and 3 independently have configuration R or S. In some embodiments, the chiral carbon at position 2 has configuration S, and the chiral carbon at position 3 has configuration S.

[00132] In a third aspect, the present application provides a compound of formula X, or an isomer, mesomer, racemate, or enantiomer thereof, or a mixture thereof, or a pharmaceutically acceptable salt or solvate thereof,



Formula X

[00133] wherein W is an oxygen atom or a sulfur atom, and preferably, W is an oxygen atom;

[00134] R₁ and R₈ are each independently selected from the group consisting of: hydrogen atom, alkyl, acyl, sulfonyl, cycloalkyl, heterocyclyl, aryl and heteroaryl, and are not simultaneously hydrogen; optionally, the alkyl, acyl, sulfonyl, cycloalkyl, aryl and heteroaryl are each independently substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, alkoxy, halogen, deuterium, amino, cyano, hydroxy, mercapto, azido, nitro, carboxy, acyl, carbonyl, hydroxyalkyl, cycloalkyl, heterocyclyl, aryl and heteroaryl; preferably, R₁ is alkyl, and R₈ is H;

[00135] R₂ is -C(O)-Q₁-Q₂ or -SO₂-Q₁-Q₂, wherein Q₁ is selected from the group consisting of O, N and S atoms and a chemical bond;

[00136] in a case where Q₁ is selected from the group consisting of O, N and S atoms, Q₂ is selected from the group consisting of alkyl, cycloalkyl, heterocyclyl, spirocyclyl, bridged ring, alkenyl, aryl and heteroaryl, and optionally the alkyl, cycloalkyl, heterocyclyl, spirocyclyl, bridged ring, alkenyl,

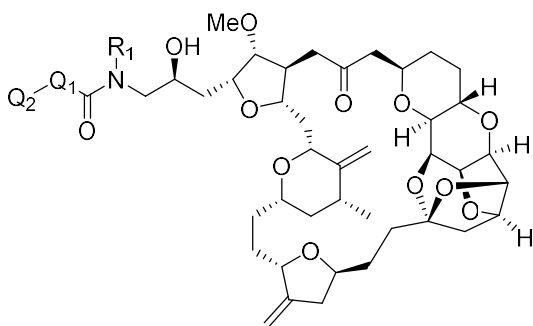
aryl and heteroaryl are each independently substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, alkoxy, halogen, deuterium, amino, cyano, hydroxy, mercapto, azido, nitro, carboxy, acyl, carbonyl, hydroxyalkyl, cycloalkyl, heterocyclyl, aryl and heteroaryl;

[00137] in a case where Q_1 is a chemical bond, Q_2 is selected from the group consisting of alkyl, benzyl, cycloalkyl, heterocyclyl, spirocyclyl, bridged ring, alkenyl, aryl and heteroaryl;

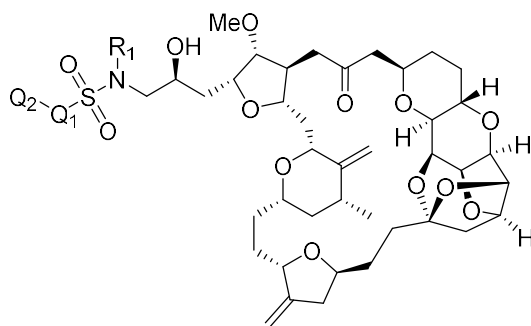
[00138] in a case where Q_2 is an alkyl, the alkyl is substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, alkoxy, halogen, deuterium, cyano, azido, nitro, carboxyl, acyl, carbonyl, cycloalkyl, heterocyclyl, aryl and heteroaryl;

[00139] in a case where Q_2 is selected from the group consisting of cycloalkyl, heterocyclyl, spirocyclyl, bridged ring, alkenyl, aryl and heteroaryl, the cycloalkyl, heterocyclyl, spirocyclyl, bridged ring, alkenyl, aryl and heteroaryl are each independently substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, alkoxy, halogen, deuterium, amino, cyano, hydroxy, mercapto, azido, nitro, carboxyl, acyl, carbonyl, hydroxyalkyl, cycloalkyl, heterocyclyl, aryl and heteroaryl, and in a case where Q_2 is a cycloalkyl with the substituent being a hydroxyl, an amino or a mercapto, the substituent is at any position except a carbon atom connected to $-C(O)-$.

[00140] In some embodiments, the compound of formula X, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof is a compound of formula XIa or formula XIb, or an isomer, mesomer, racemate, or enantiomer thereof, or a mixture thereof,



Formula XIa



Formula XIb

[00141] wherein R_1 is an alkyl, and preferably R_1 is a methyl,

[00142] Q_1 is an O atom or a chemical bond;

[00143] in a case where Q_1 is an O atom, Q_2 is selected from the group consisting of alkyl, cycloalkyl, alkenyl and aryl, and optionally the alkyl, cycloalkyl, alkenyl and aryl are each independently substituted with one or more substituents selected from the group consisting of

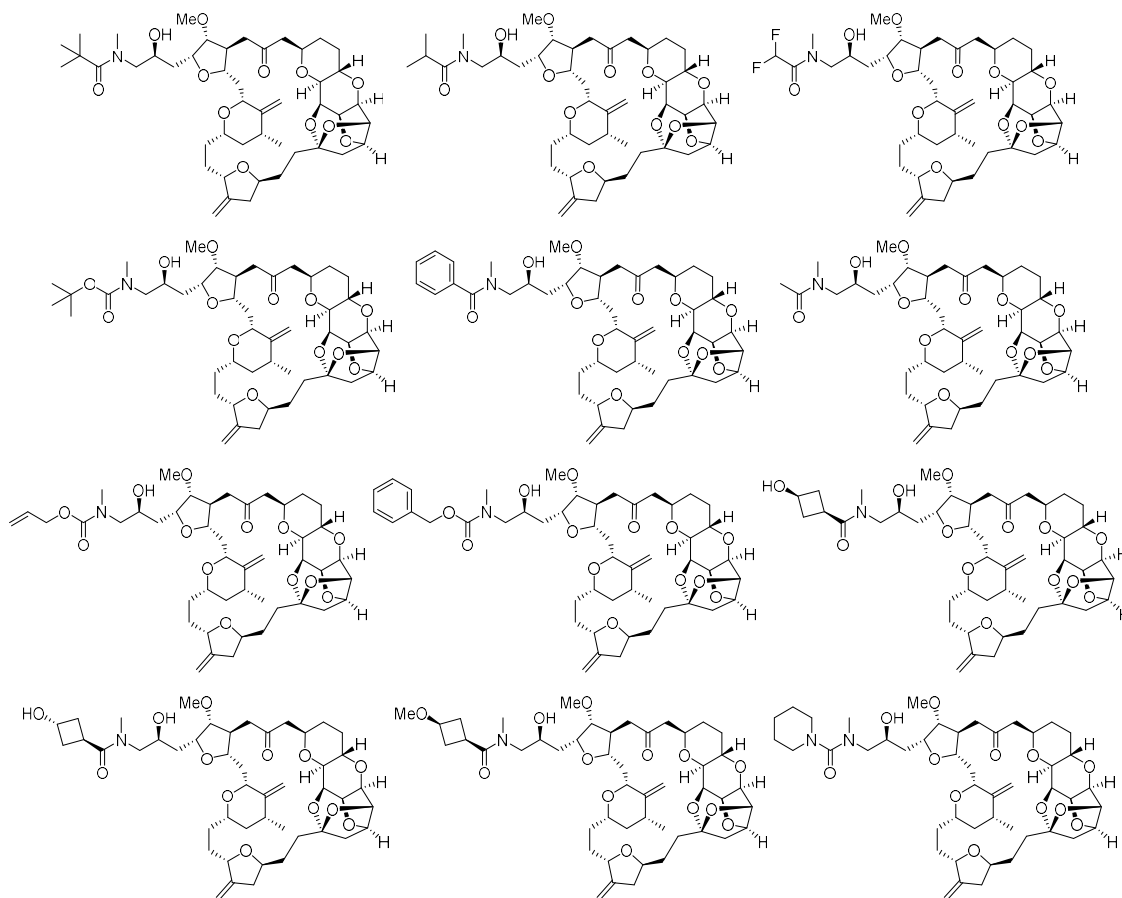
hydrogen atom, alkyl, halogen, hydroxy, hydroxyalkyl and aryl;

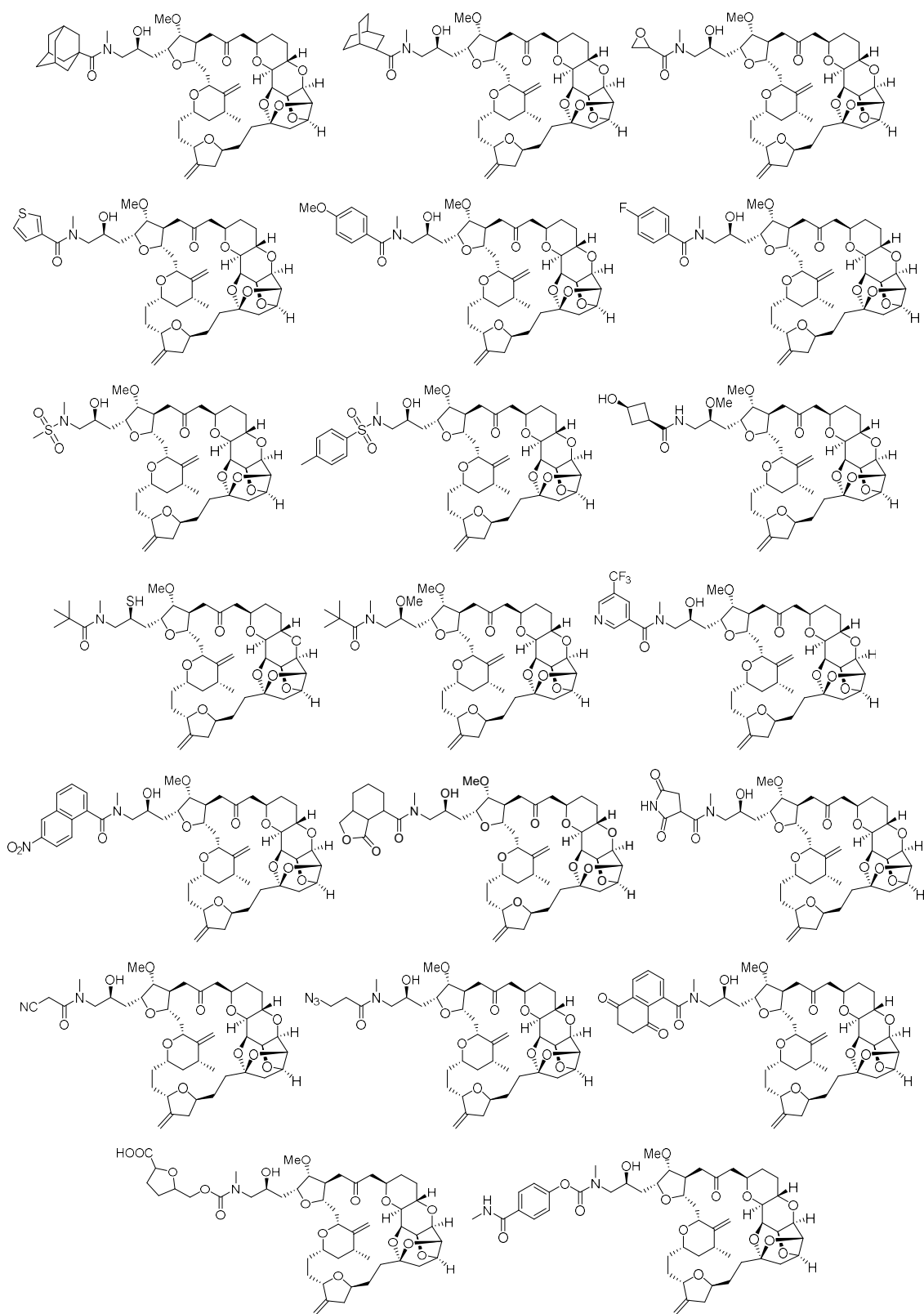
[00144] in a case where Q₁ is a chemical bond, Q₂ is selected from the group consisting of alkyl, benzyl, cycloalkyl, alkenyl and aryl;

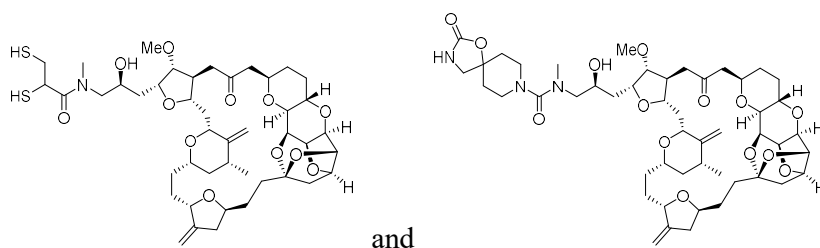
[00145] in a case where Q₂ is an alkyl, the alkyl is substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, halogen, deuterium, azido, nitro and aryl;

[00146] in a case where Q₂ is selected from the group consisting of cycloalkyl, alkenyl and aryl, the cycloalkyl, alkenyl and aryl are each independently substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, halogen, hydroxyl, hydroxyalkyl and aryl, and in a case where Q₂ is a cycloalkyl with the substituent being a hydroxyl, an amino or a mercapto, the substituent is at any position except a carbon atom connected to -C(O)-.

[00147] In some embodiments, the compound of formula X, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof of the present application is selected from the following structures:







[00148] some embodiments of the present application, pharmaceutically acceptable salts of the ligand-drug conjugate, or the linker-drug conjugate or the isomer, mesomer, racemate, or enantiomer thereof or the mixture thereof, or the compound of formula X or the isomer, mesomer, racemate or enantiomer thereof or the mixture thereof include sodium, potassium, calcium or magnesium salts formed by acidic functional groups in the structural formula and acetates, trifluoroacetates, citrates, oxalates, tartrates, malates, nitrates, chlorides, bromides, iodides, sulfates, bisulfates, phosphates, lactates, oleates, ascorbates, salicylates, formates, glutamates, methanesulfonates, ethanesulfonates, benzenesulfonates or p-toluenesulfonates formed by basic functional groups in the structure.

[00149] In a fourth aspect, the present application provides use of the linker-drug conjugate or the isomer, mesomer, racemate or enantiomer thereof or the mixture thereof or the pharmaceutically acceptable salt or solvate thereof according to any of the solutions described above, or the compound of formula X or the isomer, mesomer, racemate, or enantiomer thereof or the mixture thereof or the pharmaceutically acceptable salt or solvate thereof according to any of the solutions described above as an intermediate for the preparation of the ligand-drug conjugate of the present application.

[00150] In a fifth aspect, the present application provides a pharmaceutical composition comprising the ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof of the present application, or the linker-drug conjugate or the isomer, mesomer, racemate, enantiomer thereof or the mixture thereof or the pharmaceutically acceptable salt or solvate thereof of the present application, or the compound of formula X or the isomer, mesomer, racemate or enantiomer thereof or the mixture thereof or the pharmaceutically acceptable salt or solvate thereof, and optionally further comprising a pharmaceutically acceptable carrier.

[00151] In a sixth aspect, the present application provides a pharmaceutical formulation comprising the ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof of the present application, or the linker-drug conjugate or the isomer, mesomer, racemate, enantiomer thereof or the mixture thereof or the pharmaceutically acceptable salt or solvate thereof of the present application, or the compound of formula X or the isomer, mesomer, racemate or enantiomer

thereof or the mixture thereof or the pharmaceutically acceptable salt or solvate thereof.

[00152] In a seven aspect, the present application provides use of the ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to any of the solutions described above, or the linker-drug conjugate or the isomer, mesomer, racemate, enantiomer thereof or the mixture thereof or the pharmaceutically acceptable salt or solvate thereof according to any of the solutions described above, or the compound of formula X or the isomer, mesomer, racemate or enantiomer thereof or the mixture thereof or the pharmaceutically acceptable salt or solvate thereof according to any of the solutions described above in the preparation of a medicament for the treatment or prevention of a cancer or tumor.

[00153] In an eighth aspect, the present application provides a method of preventing or treating a cancer or tumor, comprising administering to a subject in need thereof a prophylactic or therapeutically effective amount of the ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof of the present application, or the linker-drug conjugate or the isomer, mesomer, racemate, enantiomer thereof or the mixture thereof or the pharmaceutically acceptable salt or solvate thereof of the present application, or the compound of formula X or the isomer, mesomer, racemate or enantiomer thereof or the mixture thereof or the pharmaceutically acceptable salt or solvate thereof.

[00154] In the present application, the cancer or tumor may be a solid tumor or a hematological tumor, including but not limited to: adenocarcinoma, ovarian cancer, cervical cancer, uterine cancer, prostate cancer, kidney cancer, urinary tract cancer, bladder cancer, liver cancer, stomach cancer, endometrial cancer, salivary gland cancer, esophageal cancer, lung cancer (e.g., lung adenocarcinoma), colon cancer, rectal cancer, colorectal cancer, bone cancer, skin cancer (e.g., epidermal cancer), thyroid cancer, pancreatic cancer (e.g., pancreatic adenocarcinoma), melanoma, glioma, neuroblastoma, glioblastoma multiforme, sarcoma, lymphoma, leukemia, and hypopharyngeal cancer (e.g., hypopharyngeal squamous cell carcinoma).

[00155] Definitions

[00156] Unless otherwise defined, all technical and scientific terms used herein are consistent with the common understanding of those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein may be used to practice or test the invention, the invention describes preferred methods and materials. In describing and claiming the invention, the following terms are used in accordance with the definitions below.

[00157] When a trade name is used herein, the applicants intend to include formulations of a

product of the trade name, generic drugs and active drug portions of the trade name.

[00158] Unless otherwise stated, the terms used in the specification and claims have the following meanings.

[00159] The term "ligand" is a targeting agent that specifically binds to a target moiety. The ligand is capable of specifically binding to a cellular component or to other target molecules of interest. The targeting moiety or target is usually on the cell surface. In some aspects, the ligand functions to deliver the drug unit to a specific target cell population with which the ligand unit interacts. The ligands include, but are not limited to, proteins, polypeptides and peptides, as well as 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 agent, it may be a peptide or polypeptide, or a non-protein molecule. Examples of such targeting agents include interferons, lymphokines, hormones, growth factors and colony-stimulating factors, vitamins, nutrient transport molecules, or any other cell-binding molecules or substances. In some embodiments, the linker is covalently connected to the sulfur atom of the ligand. In some aspects, the sulfur atom is that of a cysteine residue that forms an interchain disulfide bond of the antibody. In another aspect, the sulfur atom is a sulfur atom that has been introduced into a cysteine residue of the ligand unit, which forms an interchain disulfide bond of the antibody. In another aspect, the sulfur atom is a sulfur atom of a cysteine residue introduced into the ligand unit by, for example, site-directed mutagenesis or chemical reaction.

[00160] The term "drug" refers to a cytotoxic drug, i.e., a molecule that has a strong ability to destroy the normal growth of tumor or cancer cells. In principle, cytotoxic drugs can kill tumor cells at sufficiently high concentrations, but due to the lack of specificity, while killing tumor or cancer cells, they can also cause apoptosis of normal cells, thus easily leading to serious side effects.

[00161] The term "ligand-drug conjugate" refers to a molecule formed by connecting a ligand to a drug by a stable linking unit. In the present invention, the "ligand-drug conjugate" is preferably an antibody-drug conjugate (ADC), which refers to connecting a monoclonal antibody or a functional antibody fragment or a targeted protein to a cytotoxic drug by a stable linking unit.

[00162] The term "antibody" or "functional antibody fragment" includes, within its scope, any part of the antibody structure. This unit can bind, reactively associate, or complex a receptor, antigen, or other receptor unit present in the target cell population. The antibody may be any protein or proteinaceous molecule that binds, complexes, or reacts with a portion of the cell population to be treated or biomodified.

[00163] The antibodies of the present invention include but are not limited to mouse antibodies,

chimeric antibodies, humanized antibodies and fully human antibodies, preferably humanized antibodies and fully human antibodies.

[00164] The three-letter and single-letter codes for amino acids used in the present invention are as described in *J. boil. Chem.* 1968, 243, 3558.

[00165] The term "natural amino acid" refers to amino acids that can be synthesized by organisms. Natural amino acids are generally L-type, but there are a few exceptions, such as glycine, including natural and biosynthesized glycine.

[00166] The term "unnatural amino acid" refers to amino acids that can only be synthesized artificially.

[00167] The term "alkyl" refers to a saturated aliphatic hydrocarbon group, which is a linear or branched chain group containing 1 to 20 carbon atoms, preferably an alkyl containing 1 to 12 carbon atoms, more preferably an alkyl containing 1 to 10 carbon atoms, most preferably an alkyl containing 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, 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, 2-methyl-2-ethylhexyl, 2-methyl-3-ethylhexyl, 2,2-diethylpentyl, n-decyl, 3,3-diethylhexyl, 2,2-diethylhexyl, and various branched isomers thereof. More preferably, the alkyl group is a lower alkyl having 1 to 6 (e.g. 1 to 4) carbon atoms, and 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 and the like. The alkyl can be substituted or unsubstituted. When substituted, the substituent group(s) can be substituted at any available connection point. The substituent group(s) is preferably one or more groups independently selected from the group consisting of alkyl, alkenyl, alkynyl, alkoxy, alkylthio, alkylamino, halogen, thiol, hydroxy, nitro, cyano, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, cycloalkoxy,

heterocycloalkoxy, cycloalkylthio, heterocycloalkylthio, and oxo.

[00168] The term "substituted alkyl" means that the hydrogen in an alkyl is replaced by a substituent. Unless otherwise specified in the text, the substituent of the alkyl 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 1 to (2m'+1), where m' is the total number of carbon atoms in the group, such as 1, 2, 3, 4, 5 or 6. R', R'' and R''' respectively refer to 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-C₁₋₄ alkyl. When R' and R'' are connected to the same nitrogen atom, they can form a 3-, 4-, 5-, 6- or 7-membered ring together with the nitrogen atom. For example, -NR'R'' includes 1-pyrrolidinyl and 4-morpholinyl.

[00169] The term "heteroalkyl" refers to a group formed by substitution of one or more carbon atoms on an alkyl group with N, O, or S. The heteroalkyl preferably contains 1 to 12 carbon atoms, more preferably 1 to 10 carbon atoms, and most preferably 1 to 6 or 1 to 4 carbon atoms.

[00170] The term "cycloalkyl" refers to a saturated or partially unsaturated monocyclic or polycyclic ring-shaped hydrocarbon group, and the ring of the cycloalkyl contains 3 to 20 carbon atoms, preferably 3 to 12 carbon atoms, more preferably 3 to 10 carbon atoms, and most preferably 3 to 8 carbon atoms. Non-limiting examples of monocyclic cycloalkyl include cyclopropyl, cyclobutyl, cyclopentyl, cyclopentenyl, cyclohexyl, cyclohexenyl, cyclohexadienyl, cycloheptyl, cycloheptatrienyl, and cyclooctyl. Non-limiting examples of polycyclic cycloalkyl include spiro, fused and bridged cycloalkyl groups, and each of the spiro, fused and bridged rings preferably contain 5 to 12 ring atoms. Optionally, one or more carbon atoms on the cycloalkyl may be replaced with one or more carbonyls.

[00171] The term "alkoxy" refers to an -O-(alkyl) and an -O-(cycloalkyl) group, wherein the alkyl and cycloalkyl are as defined above. The alkyl preferably contains 1 to 12 carbon atoms, more preferably 1 to 10 carbon atoms, most preferably 1 to 6 or 1 to 4 carbon atoms; the cycloalkyl contains 3 to 20 carbon atoms, preferably 3 to 12 carbon atoms, more preferably 3 to 10 carbon atoms, most preferably 3 to 8 carbon atoms. Non-limiting examples of alkoxy include: methoxy, ethoxy, propoxy, butoxy, cyclopropyloxy, cyclobutyloxy, cyclopentyloxy, and cyclohexyloxy. The alkoxy can be optionally substituted or unsubstituted. When substituted, the substituent group(s) is preferably one or more group(s) independently selected from the group consisting of alkyl, alkenyl, alkynyl, alkoxy, alkylthio, alkylamino, halogen, thiol, hydroxy, nitro, cyano, cycloalkyl,

heterocycloalkyl, aryl, heteroaryl, cycloalkoxy, heterocycloalkoxy, cycloalkylthio, and heterocycloalkylthio.

[00172] The term "heterocycle" refers to a saturated or partially unsaturated monocyclic or polycyclic ring-shaped hydrocarbon containing 3 to 20 ring atoms, of which one or more (such as 1, 2, 3 or 4) ring atoms are heteroatoms selected from nitrogen, oxygen or S(O)_m (wherein m is 0, 1 or 2), and the remaining ring atoms are carbon. Preferably, the heterocyclyl contains 3 to 12 ring atoms, of which 1 to 4 are heteroatoms. More preferably, the heterocyclyl contains 3 to 10 or 3 to 8 ring atoms. Non-limiting examples of monocyclic heterocyclyl include pyrrolidinyl, piperidinyl, piperazinyl, morpholinyl, thiomorpholinyl, and homopiperazinyl. Non-limiting examples of polycyclic heterocyclyl include spiro, fused and bridged heterocyclyls, and each of the spiro, fused and bridged rings preferably contain 5 to 12 ring atoms. Optionally, one or more carbon atoms on the heterocyclyl may be replaced with one or more carbonyls.

[00173] The term "aryl" refers to a 6- to 14-membered all-carbon monocyclic or fused polycyclic (i.e., rings that shares adjacent pairs of carbon atoms) group having a conjugated π electron system, preferably 6- to 10-membered group, such as phenyl. The aryl may be substituted or unsubstituted. When the aryl is substituted, the substituent may be one or more of the following groups, selected from, but not limited to, alkyl, alkenyl, alkynyl, alkoxy, alkylthio, alkylamino, halogen, deuterium atom, mercapto, hydroxyl, nitro, cyano, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, cycloalkyloxy, heterocycloalkyloxy, cycloalkylthio, and heterocycloalkylthio.

[00174] The term "heteroaryl" includes 5- to 8-membered monocyclic heteroaryls and 8- to 12-membered fused heteroaryls.

[00175] The term "5- to 8-membered monocyclic heteroaryl" refers to an aromatic monocyclic ring group containing 5 to 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 (such as carbon atoms, nitrogen atoms or sulfur atoms) in the ring structure may be oxo-substituted. "5- to 8-membered monocyclic heteroaryl" includes, such as, "5- to 7-membered monocyclic heteroaryl", "5- to 6-membered monocyclic heteroaryl", "5- to 6-membered monocyclic nitrogen-containing heteroaryl", "6-membered monocyclic nitrogen-containing heteroaryl", etc., wherein the heteroatom in the "nitrogen-containing heteroaryl" contains at least one nitrogen atom, for example, only 1 or 2 nitrogen atoms, or, contains one nitrogen atom and other 1 or 2 heteroatoms (for example, oxygen atoms and/or sulfur atoms), or, contains 2 nitrogen atoms and other 1 or 2 heteroatoms (for example, oxygen atoms and/or sulfur atoms). Specific examples of "5- to 8-membered monocyclic heteroaryl" include but are not limited to furyl, thienyl, pyrrolyl, thiazolyl, isothiazolyl, thiadiazolyl,

oxazolyl, isoxazolyl, oxadiazolyl, imidazolyl, pyrazolyl, 1,2,3-triazole base, 1,2,4-triazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, pyridyl, 2-pyridonyl, 4-pyridonyl, pyrimidinyl, pyridazinyl, pyrazinyl, 1,2,3-triazinyl, 1,3,5-triazine base, 1,2,4,5-tetrazinyl, azepantrienyl, 1,3-diazacycloheptatrienyl, azepantetraenyl, etc.

[00176] The term "8- to 12-membered fused heteroaryl" refers to an unsaturated aromatic ring structure containing 8 to 12 ring atoms (at least one of which is a heteroatom, such as a nitrogen atom, an oxygen atom or a sulfur atom), which is formed by two or more ring structures sharing two adjacent atoms. Optionally, the ring atoms (such as carbon atoms, nitrogen atoms or sulfur atoms) in the ring structure may be oxo-substituted. "8- to 12-membered fused heteroaryl" includes "8- to 10-membered fused heteroaryl" and "8- to 9-membered fused heteroaryl", etc.; specific examples include but are not limited to: pyrrolopyrrole, pyrrolofuran, pyrazopyrrole, pyrazothiophene, furothiophene, pyrazoxazole, benzofuryl, benzisofuryl, benzothieryl, indolyl, isoindolyl, benzoxazolyl, benzimidazolyl, indazolyl, benzotriazolyl, quinolinyl, 2-quinolinonyl, 4-quinolinonyl, 1-isoquinolinonyl, Isoquinolinyl, acridinyl, phenanthridinyl, benzopyridazinyl, phthalazinyl, quinazolinyl, quinoxalinyl, purinyl, and naphthyridinyl.

[00177] The term "haloalkyl" refers to an alkyl substituted by one or more halogens, where the alkyl is as defined above.

[00178] The term "deuterated alkyl" refers to an alkyl substituted by one or more deuterium atoms, where the alkyl is as defined above.

[00179] The term "hydroxy" refers to an -OH group.

[00180] The term "halogen" refers to fluorine, chlorine, bromine or iodine.

[00181] The term "amino" refers to a -NH₂ group.

[00182] The term "nitro" refers to a -NO₂ group.

[00183] The term "acyl" refers to the group formed by a carbonyl with an alkyl, alkoxy or amido, including alkylcarbonyl groups, ester groups and amide groups.

[00184] The term "substituted" means that the hydrogen in a group is replaced with a substituent, and "substituted" includes the implicit condition that such substitution conforms to the allowed valences of the substituted atom and the substituent, and that the substitution results in a stable compound. The substituent can be found in the groups listed above. Optionally, two substituents on the same substituted atom may form a cyclic group, which may be a cycloalkyl or a heterocyclyl, such as a lactam group or a lactone group.

[00185] The term "derivative" refers to a substance that has a chemical structure similar to that of a compound but also contains at least one chemical group that is not present in the compound and/or

lacks at least one chemical group that is present in the compound. The compound to which the derivative is compared is called "parent" compound. Typically, a "derivative" may be produced from a parent compound in one or more chemical reaction steps.

[00186] The term "pharmaceutically acceptable salt" refers to a pharmaceutically acceptable organic or inorganic salt of a compound (such as, a drug, a linker-drug compound, or a ligand-drug conjugate). The compound or conjugate may contain at least one amino or carboxyl group and can therefore form addition salts with the corresponding acid or alkali. 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, bitartrate, ascorbate, salicylate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, potassium salt, and sodium salt.

[00187] The term "solvate" refers to a compound formed by a linker-drug compound or ligand-drug conjugate of the present invention and one or more solvent molecules, where the solvent molecules include but are not limited to water, ethanol, acetonitrile, isopropanol, DMSO, and ethyl acetate.

[00188] The term "pharmaceutical composition" refers to a mixture containing one or more compounds of the present invention or physiologically/pharmaceutically acceptable salts or prodrugs thereof and other chemical components, as well as other components such as physiologically/pharmaceutically acceptable carriers and/or excipients. The purpose of the pharmaceutical composition is to promote the administration of drugs to organisms, facilitate the absorption of active ingredients and thus exert biological activity.

[00189] The term "carrier" refers to a system that can change the way drugs enter the human body and their distribution in the body, control the release rate of drugs and deliver drugs to the target. Drug carrier release and targeting systems can reduce drug degradation and loss, reduce side effects and improve bioavailability.

[00190] The term "excipient" refers to an additive or excipient other than the main drug in a pharmaceutical preparation. Such as adhesives, fillers, disintegrants, lubricants in tablets; matrix backup in semi-solid preparations ointments and creams; preservatives, antioxidants, flavoring agents, fragrances, cosolvents, emulsifiers, permeability enhancers, osmotic pressure regulators, colorants, etc. in liquid preparations can all be called excipients.

[00191] The term "diluent" or "filler" is mainly used to increase the weight and/or volume of the preparation. The addition of diluent not only ensures a certain volume, but also reduces the dosage

deviation of the main ingredients and improves the compression molding of the drug.

BRIEF DESCRIPTION OF THE DRAWINGS

[00192] FIG. 1A, FIG. 1B, FIG. 1C, FIG. 1D, FIG. 1E and FIG. 1F show the anti-tumor effects of the compounds in BxPC-3, SW620, H1975, HCC827, FaDu and A431 cell models, respectively.

[00193] FIG. 2A, FIG. 2B, FIG. 2C, FIG. 2D and FIG. 2E show the anti-tumor effect of ADCs in A431, HCC827, FaDu, N87 and SW620 cell models, respectively.

[00194] FIG. 3 shows the anti-tumor effect of ADCs in the heterogeneous tumor cell line A431+SW620.

DETAILED DESCRIPTION OF THE EMBODIMENTS

The present invention is further described below in conjunction with specific embodiments. It should be understood that these embodiments are only used to illustrate the invention and are not used to limit the scope of the invention. The experimental methods in the following embodiments that do not specify specific conditions are usually carried out under conventional conditions or under conditions recommended by the manufacturers. Unless otherwise specified, all percentages, proportions, ratios, or parts are calculated by weight. Unless otherwise defined, all professional and scientific terms used herein have the same meaning as is familiar to those skilled in the art. In addition, any methods and materials similar or equivalent to those described can be applied to the methods of the present invention. The preferred implementation methods and materials described herein are for demonstration purposes only.

The general procedures taken in the following examples of the present invention are:

General procedure A

Preparation of ADCs by conjugation

Antibody molecules with a monomer rate of more than 95% after preliminary purification were exchanged into phosphate buffer containing EDTA using an ultrafiltration centrifuge tube, the concentration being 10 mg/mL. TCEP with an amount 10 times the number of moles of the antibody was added for reaction at room temperature for 6 h. The interchain disulfide bonds of the antibody were opened, and the number of free thiol groups was determined using Ellman's method to determine whether all disulfide bonds were opened. Then, the payload with an amount 10 times the number of moles of the antibody was added for reaction at room temperature for 6 h. After the reaction ended, the reaction system was exchanged into PBS using an ultrafiltration centrifuge tube

with a molecular weight cut-off of 30 KDa, and the unconjugated payload was removed, thus obtaining a conjugated ADC (DAR=8).

General procedure B

Preparation of ADCs by site-specific conjugation

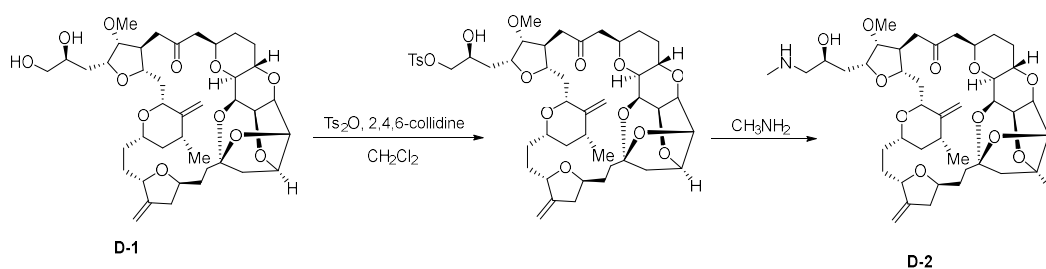
Antibody molecules with a monomer rate of more than 95% after preliminary purification were exchanged into phosphate buffer containing EDTA using an ultrafiltration centrifuge tube, the concentration being 10 mg/mL. TCEP with an amount of 8 times the number of moles of the antibody was added for reaction at room temperature for 3 h. The reaction system was exchanged into phosphate buffer at pH 6.5 using an ultrafiltration centrifuge tube, and then DHAA (dehydroascorbic acid) with an amount 8 times the number of moles of the antibody was added for reaction at room temperature for 3 h. Then, the payload with an amount 5 times the number of moles of the antibody was added for reaction at room temperature for 3 h. After the reaction ended, the reaction system was exchanged into PBS using an ultrafiltration centrifuge tube with a molecular weight cut-off of 30 KDa, and the unconjugated payload was removed, thus obtaining a site-specific conjugated ADC (DAR=2).

General procedure C

Preparation of ADCs by conjugation

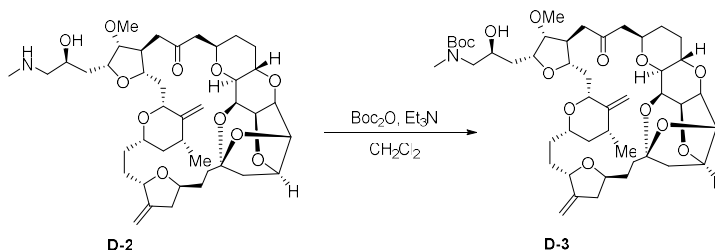
Antibody molecules with a monomer rate of more than 95% after preliminary purification were exchanged into 20 mM/sodium acetate/pH 6.0 buffer solution using an ultrafiltration centrifuge tube, the concentration being greater than 5 mg/mL. During the preparation of an ADC, an appropriate amount of the antibody after buffer exchange was placed in a reaction tube, TCEP (0.1M) with an amount 3.6 times the molar amount of the antibody was then added, the reaction tube was placed in a 25 °C constant temperature incubator for reaction for 2 h, the reaction tube was then taken out, a Linker-drug with an amount 5 times the molar amount was added and mixed well, and the reaction tube was placed in a 25 °C constant temperature incubator for reaction for 2 h. After the reaction ended, the sample solution was taken out and exchanged into 20 mM/histidine/pH 5.3 storage buffer using an ultrafiltration centrifuge tube with a molecular weight cutoff of 30 KDa, and the unconjugated payload was removed, thus obtaining a conjugated ADC (DAR=4).

Example 1 Synthesis of compound **D-2**



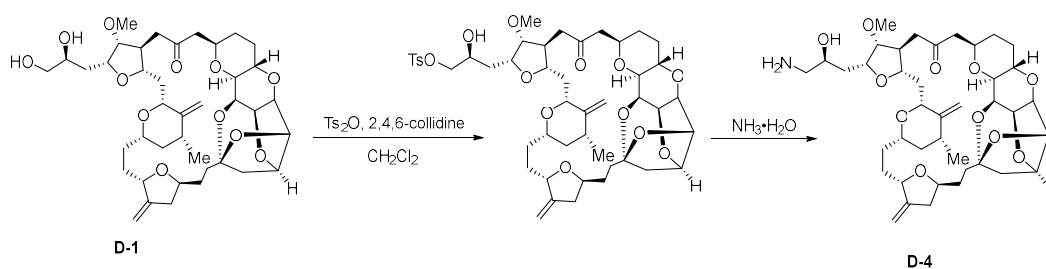
In a 25 mL single-neck flask, compound **D-1** (80 mg, 0.109 mmol, 1.0 eq) was added and dissolved in dried dichloromethane (4 mL), followed by addition of 2,4,6-trimethylpyridine (58 μ L, 0.438 mmol, 4.0 eq) and pyridine (0.44 μ L, 0.005 mmol, 0.05 eq), and the reaction solution was cooled to -10 $^{\circ}$ C. P-toluenesulfonic anhydride (89.3 mg, 0.274 mmol, 2.5 eq) was added under the protection of nitrogen, and the reaction was continued at -10 $^{\circ}$ C for 20 h. Then, water (310 μ L) was added to the reaction solution. After the reaction solution was brought naturally to room temperature and was stirred for 30 min, methylamine (methanol solution, 4 mL) was added, and the reaction was continued at room temperature for 46 h. The reaction solution was then concentrated under reduced pressure. The purification was performed by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give compound **D-2** (white solid, 58 mg, 71%). LC-MS $m/z(ES^+)$: $[M+H]^+$: 744.2.

Example 2 Synthesis of compound **D-3**



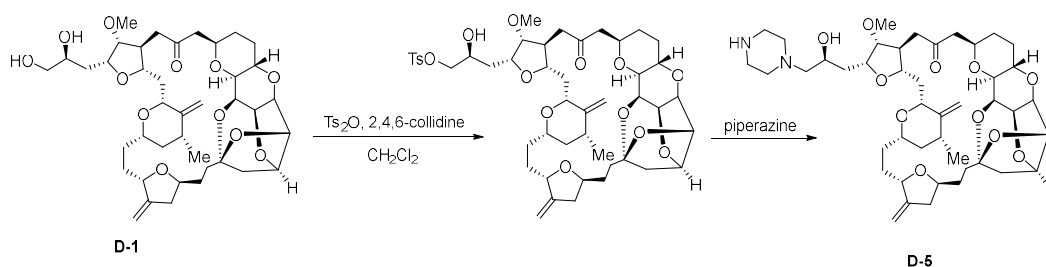
In a 25 mL single-neck flask, compound **D-2** (55 mg, 0.074 mmol, 1.0 eq) was added and dissolved in dried dichloromethane (3 mL), followed by addition of triethylamine (15.4 μ L, 0.111 mmol, 1.5 eq) and di-tert-butyl decarbonate (20.4 μ L, 0.089 mmol, 1.2 eq), and the reaction was allowed at room temperature for 2 h. After the reaction was found to be completed from the monitoring of the TLC, the reaction solution was concentrated under reduced pressure. The purification was performed by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give compound **D-3** (white solid, 43 mg, 69%). LC-MS $m/z(ES^+)$: $[M+H]^+$: 844.2.

Example 3 Synthesis of compound **D-4** (eribulin)



In a 25 mL single-neck flask, compound **D-1** (80 mg, 0.109 mmol, 1.0 eq) was added and dissolved in dried dichloromethane (4 mL), followed by addition of 2,4,6-trimethylpyridine (58 μ L, 0.438 mmol, 4.0 eq) and pyridine (0.44 μ L, 0.005 mmol, 0.05 eq), and the reaction solution was cooled to -10 °C. P-toluenesulfonic anhydride (89.3 mg, 0.274 mmol, 2.5 eq) was added under the protection of nitrogen, and the reaction was continued at -10 °C for 20 h. Then, water (310 μ L) was added to the reaction solution. After the reaction solution was brought naturally to room temperature and was stirred for 30 min, isopropanol (4 mL) and ammonium hydroxide (4 mL) were added, and the reaction was continued at room temperature for 3 days. The reaction solution was then concentrated under reduced pressure. The purification was performed by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give compound **D-4** (white solid, 56 mg, 70%). LC-MS m/z (ES⁺): [M+H]⁺: 730.2.

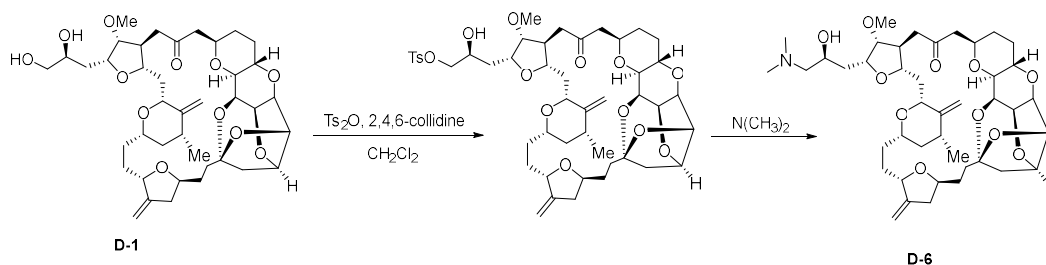
Example 4 Synthesis of compound **D-5**



In a 25 mL single-neck flask, compound **D-1** (80 mg, 0.109 mmol, 1.0 eq) was added and dissolved in dried dichloromethane (4 mL), followed by addition of 2,4,6-trimethylpyridine (58 μ L, 0.438 mmol, 4.0 eq) and pyridine (0.44 μ L, 0.005 mmol, 0.05 eq), and the reaction solution was cooled to -10 °C. P-toluenesulfonic anhydride (89.3 mg, 0.274 mmol, 2.5 eq) was added under the protection of nitrogen, and the reaction was continued at -10 °C for 20 h. Then, water (310 μ L) was added to the reaction solution. After the reaction solution was brought naturally to room temperature and was stirred for 30 min, methanol (4 mL) and piperazine (943 mg, 10.9 mmol, 100.0 eq) were added, and the reaction was continued at room temperature for 3 days. The reaction solution was then concentrated under reduced pressure. The purification was performed by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give

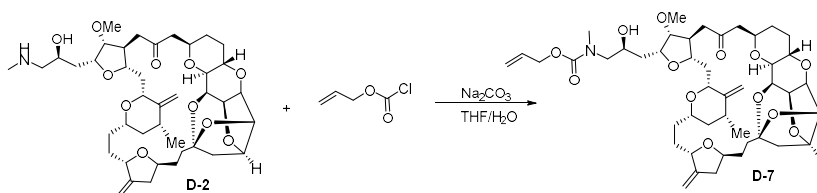
compound **D-5** (white solid, 66 mg, 75%). LC-MS $m/z(ES^+)$: $[M+H]^+$: 799.2.

Example 5 Synthesis of compound **D-6**



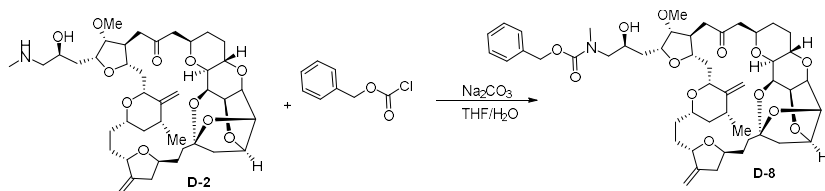
In a 25 mL single-neck flask, compound **D-1** (80 mg, 0.109 mmol, 1.0 eq) was added and dissolved in dried dichloromethane (4 mL), followed by addition of 2,4,6-trimethylpyridine (58 μ L, 0.438 mmol, 4.0 eq) and pyridine (0.44 μ L, 0.005 mmol, 0.05 eq), and the reaction solution was cooled to -10 $^{\circ}$ C. P-toluenesulfonic anhydride (89.3 mg, 0.274 mmol, 2.5 eq) was added under the protection of nitrogen, and the reaction was continued at -10 $^{\circ}$ C for 20 h. Then, water (310 μ L) was added to the reaction solution. After the reaction solution was warmed to room temperature and was stirred for 30 min, dimethylamine (methanol solution, 8 mL) was added, and the reaction was continued at room temperature for 40 h. The reaction solution was then concentrated under reduced pressure. The purification was performed by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give compound **D-6** (white solid, 60 mg, 72%). LC-MS $m/z(ES^+)$: $[M+H]^+$: 758.4.

Example 6 Synthesis of compound **D-7**



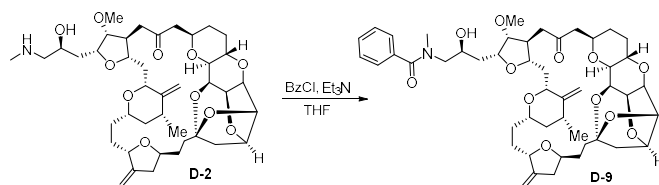
In a 25 mL single-neck flask, compound **D-2** (20 mg, 0.027 mmol, 1.0 eq) was added and dissolved in tetrahydrofuran and water (2 mL), followed by addition of sodium carbonate (28 mg, 0.269 mmol, 10 eq) and allyl chloroformate (5.7 μ L, 0.054 mmol, 2.0 eq), and the reaction was allowed at room temperature for 2 h. After the reaction was found to be completed from the monitoring of the TLC, the reaction solution was purified by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give compound **D-7** (white solid, 17 mg, 76%). LC-MS $m/z(ES^+)$: $[M+H]^+$: 828.4.

Example 7 Synthesis of compound **D-8**



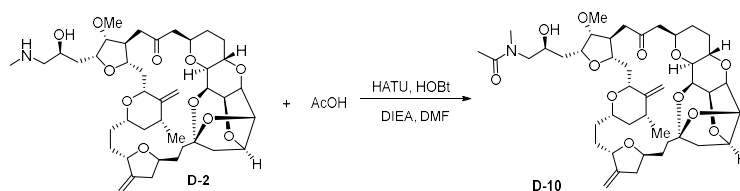
Compound **D-8** was synthesized from compound **D-2** and benzyl chloroformate according to the synthesis method of Example 6. LC-MS $m/z(ES^+)$: $[M+H]^+$:878.3.

Example 8 Synthesis of compound **D-9**



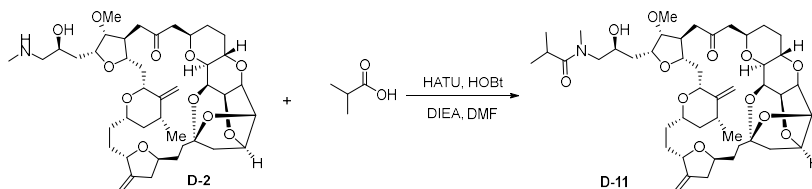
In a 25 mL single-neck flask, compound **D-2** (20 mg, 0.027 mmol, 1.0 eq) was added and dissolved in dry tetrahydrofuran (2 mL), followed by addition of triethylamine (7.5 μ L, 0.054 mmol, 2.0 eq) and benzoyl chloride (6.2 μ L, 0.054 mmol, 2.0 eq), and the reaction was allowed at room temperature for 2 h. After the reaction was found to be completed from the monitoring of the TLC, the reaction solution was purified by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give compound **D-9** (white solid, 18 mg, 79%). LC-MS $m/z(ES^+)$: $[M+H]^+$: 848.4.

Example 9 Synthesis of compound **D-10**



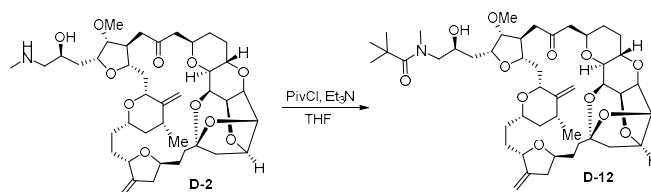
In a 25 mL single-neck flask, compound **D-2** (20 mg, 0.027 mmol, 1.0 eq), HATU (20 mg, 0.054 mmol, 2.0 eq), HOBT (7.3 mg, 0.054 mmol, 2.0 eq), and dried DMF (2 mL) were added and dissolved uniformly. In condition of an ice water bath, glacial acetic acid (2.3 μ L, 0.040 mmol, 1.5 eq) and DIEA (9.4 μ L, 0.054 mmol, 2.0 eq) were added sequentially dropwise, and reaction was allowed at room temperature. After 30 min, HPLC showed the reaction was complete. The reaction solution was purified by reverse-phase preparative column chromatography. The preparation solution was lyophilized to give compound **D-10** (white solid, 14 mg, 66%). LC-MS $m/z(ES^+)$: $[M+H]^+$: 886.3.

Example 10 Synthesis of compound **D-11**



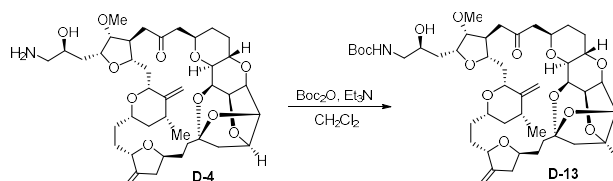
Compound **D-11** was synthesized from compound **D-2** and isobutyric acid according to the synthesis method of Example 9. LC-MS $m/z(ES^+)$: $[M+H]^+$:814.4.

Example 11 Synthesis of compound **D-12**



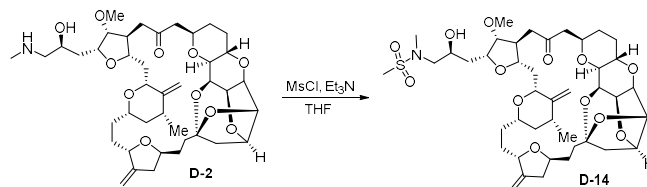
Compound **D-12** was synthesized from compound **D-2** and pivaloyl chloride according to the synthesis method of Example 8. LC-MS $m/z(ES^+)$: $[M+H]^+$:828.3.

Example 12 Synthesis of compound **D-13**



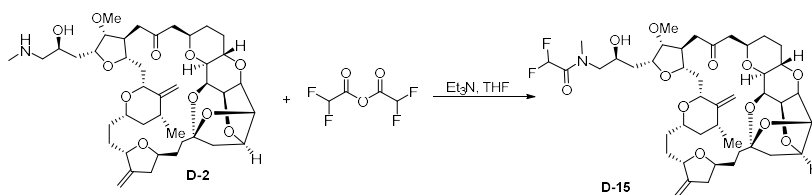
Compound **D-13** was synthesized from compound **D-4** according to the synthesis method of Example 2. LC-MS $m/z(ES^+)$: $[M+H]^+$:830.3.

Example 13 Synthesis of compound **D-14**



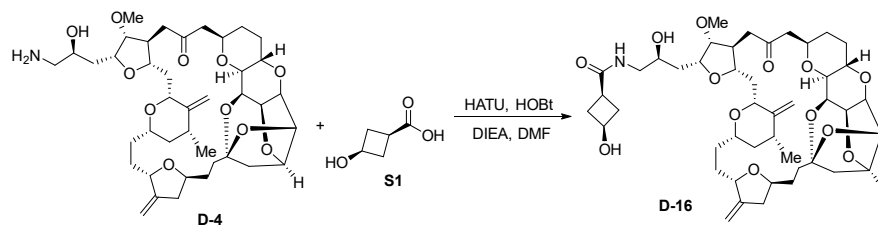
Compound **D-14** was synthesized from compound **D-2** and methanesulfonyl chloride according to the synthesis method of Example 8. LC-MS $m/z(ES^+)$: $[M+H]^+$:822.3.

Example 14 Synthesis of compound **D-15**



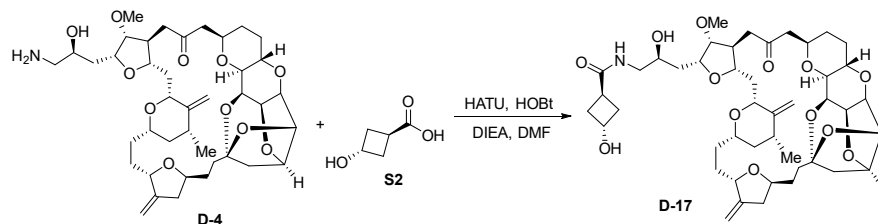
Compound **D-15** was synthesized from compound **D-2** and difluoroacetic anhydride according to the synthesis method of Example 8. LC-MS $m/z(ES^+)$: $[M+H]^+$:822.3.

Example 15 Synthesis of compound **D-16**



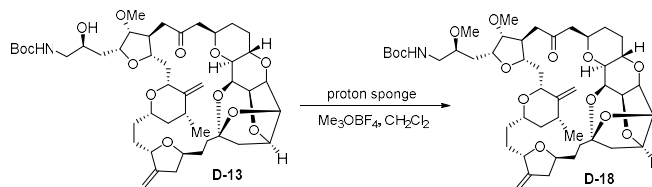
Compound **D-16** was synthesized from compound **D-4** and cis-3-hydroxycyclobutanecarboxylic acid **S1** according to the synthesis method of Example 9. LC-MS $m/z(ES^+)$: $[M+H]^+$:828.3.

Example 16 Synthesis of compound **D-17**



Compound **D-17** was synthesized from compound **D-4** and trans-3-hydroxycyclobutanecarboxylic acid **S2** according to the synthesis method of Example 9. LC-MS $m/z(ES^+)$: $[M+H]^+$:828.3.

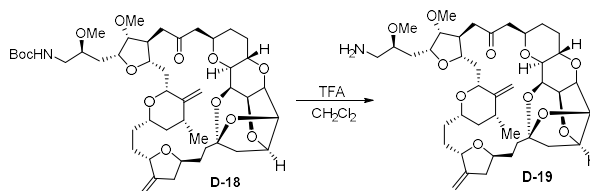
Example 17 Synthesis of compound **D-18**



Compound **D-13** (61 mg, 0.074 mmol, 1.0 eq) was weighed and placed into a 25 mL single-neck flask and dissolved in dried dichloromethane (6 mL), followed by addition of 1,8-bis(dimethylamino)naphthalene (48 mg, 0.222 mmol, 3.0 eq) and trimethyloxonium tetrafluoroborate (33 mg, 0.222 mmol, 3.0 eq), and the reaction was allowed at room temperature

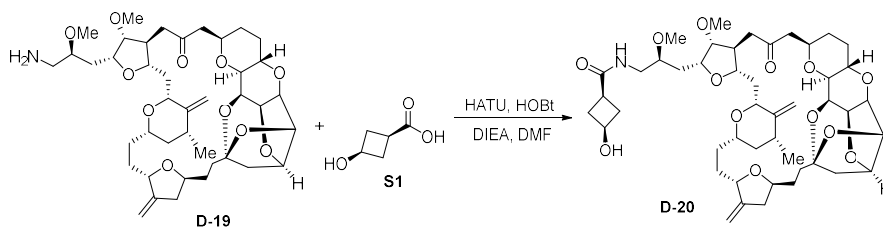
overnight. After the reaction was found to be completed from the monitoring of the TLC, the reaction solution was filtered, concentrated under reduced pressure, and purified by TLC to give compound **D-18** (a white vesicular solid, 56 mg, 90%). LC-MS $m/z(ES^+)$: $[M+H]^+$: 844.4.

Example 18 Synthesis of compound **D-19**



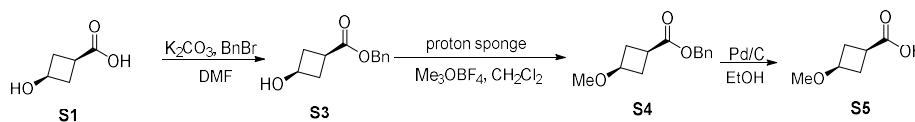
Compound **D-18** (56 mg, 0.066 mmol, 1.0 eq) was dissolved in dried dichloromethane (4 mL), followed by addition of trifluoroacetic acid (200 μ L), and the reaction was allowed at room temperature for 40 min. After the reaction was found to be completed from the monitoring of the TLC, the reaction solution was adjusted to pH 8 with sodium bicarbonate, extracted with dichloromethane, dried, filtered, and concentrated to give a crude product of compound **D-19**.

Example 19 Synthesis of compound **D-20**



Compound **D-20** was synthesized from compound **D-19** and cis-3-hydroxycyclobutanecarboxylic acid **S1** according to the synthesis method of Example 9. LC-MS $m/z(ES^+)$: $[M+H]^+$: 842.3.

Example 20 Synthesis of compound **S5**



Step 1: compound **S3**

Cis-3-hydroxycyclobutanecarboxylic acid **S1** (150 mg, 1.29 mmol, 1.0 eq) and potassium carbonate (536 mg, 3.88 mmol, 3.0 eq) were weighed and placed into a 25 mL single-neck flask, followed by addition of DMF (3 mL) and benzyl bromide (230 μ L, 1.94 mmol, 1.5 eq), and the reaction was allowed at room temperature for 2 h. After the reaction was found to be completed from the monitoring of the TLC, water was added to the reaction solution, which was then extracted

with ethyl acetate, dried, filtered, concentrated, and purified by column chromatography (dichloromethane/methanol = 30:1) to give compound **S3** (yellow oil, 207 mg, 78%).

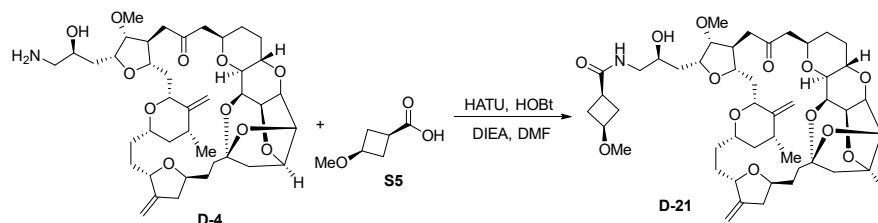
Step 2: compound **S4**

Compound **S3** (173 mg, 0.839 mmol, 1.0 eq) was weighed and placed into a 25 mL single-neck flask and dissolved in dried dichloromethane (5 mL), followed by addition of 1,8-bis(dimethylamino)naphthalene (539 mg, 2.52 mmol, 3.0 eq) and trimethyloxonium tetrafluoroborate (372 mg, 2.52 mmol, 3.0 eq), and the reaction was allowed at room temperature overnight. After the reaction was found to be completed from the monitoring of the TLC, The reaction solution was filtered, concentrated under reduced pressure, and purified by column chromatography (petroleum ether: ethyl acetate = 6:1) to give compound **S4** (colorless oily substance, 168 mg, 91%).

Step 2: compound **S5**

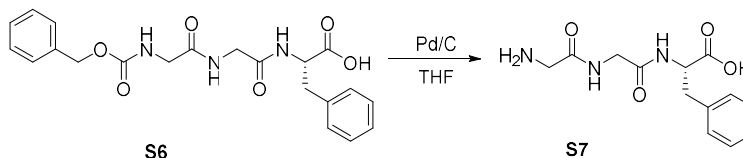
Compound **S4** (6 mg, 0.027 mmol, 1.0 eq) was weighed and placed into a 25 mL single-neck flask and dissolved in anhydrous ethanol (1 mL), followed by addition of 5% Pd/C (3 mg) and the reaction was allowed at room temperature for 2 h. After the reaction was found to be completed from the monitoring of the TLC, the resulting reaction solution was filtered and concentrated to give a crude product of compound **S5**.

Example 21 Synthesis of compound **D-21**



Compound **D-21** was synthesized from compound **D-4** and cis-3-methoxycyclobutanecarboxylic acid **S5** according to the synthesis method of Example 9. LC-MS $m/z(ES^+)$: $[M+H]^+$:842.3.

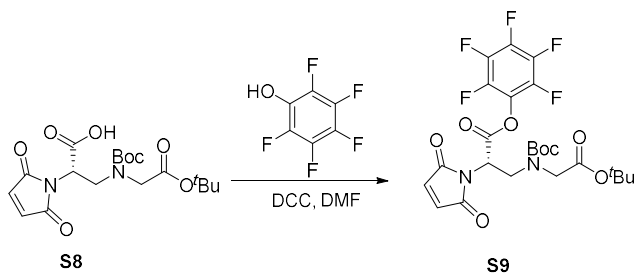
Example 22 Synthesis of compound **S7**



In a 25 mL single-neck flask, compound **S6** (2.0 g, 4.84 mmol, 1.0 eq, synthesis method refers to the patent CN111051330 A) and dry tetrahydrofuran (4 mL) were added and dissolved completely,

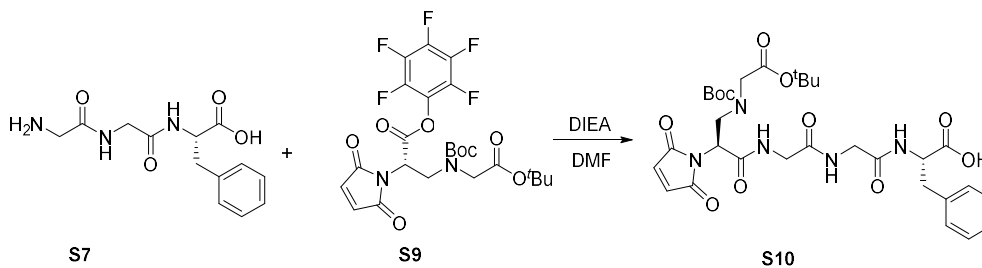
then 5% Pd/C (100 mg) was added, and the hydrogenation reaction was carried out overnight. After completion, the mixture was filtered, and the filtrate was concentrated to give compound **S7** (white solid, 1.17 g, 87%). LC-MS $m/z(ES^+)$: $[M+H]^+$: 280.1.

Example 23 Synthesis of compound **S9**



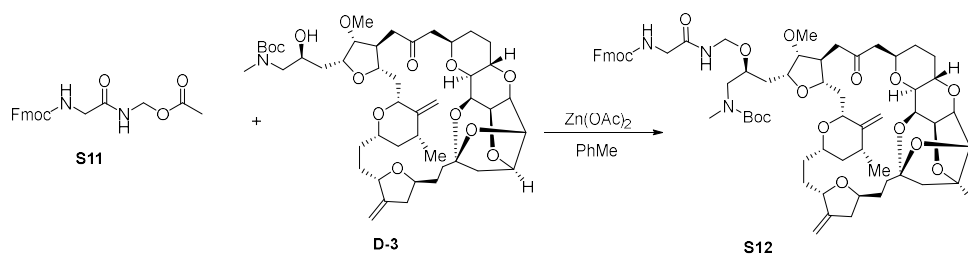
In a 25 mL single-neck flask, compound **S8** (1.0 g, 2.51 mmol, 1.0 eq, synthesis method refers to the synthesis of compound M2 in patent application CN113827736 A), pentafluorophenol (508 mg, 2.76 mmol, 1.1 eq), DCC (570 mg, 2.76 mmol, 1.1 eq) and DMF (8 mL) were added, and the reaction was allowed at room temperature for 2 h. After the reaction was found to be completed from the monitoring of the TLC, the reaction solution was filtered to give the crude filtrate of compound **S9** for later use. LC-MS $m/z(ES^+)$: $[M+H]^+$: 565.1.

Example 24 Synthesis of compound **S10**



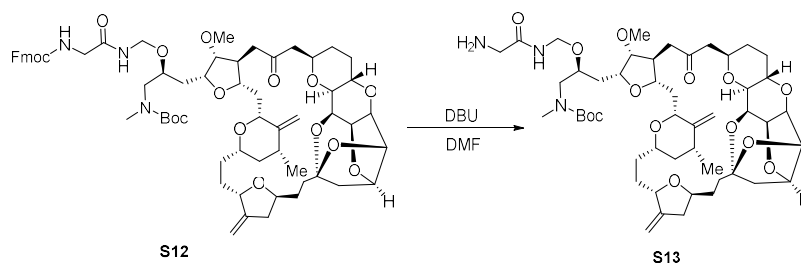
Compound **S7** (771 mg, 2.76 mmol, 1.1 eq), DMF (8 mL) and DIEA (656 μ L, 3.76 mmol, 1.5 eq) were added in a 50 mL single-neck flask, and the crude filtrate of the above compound **S9** was added in an ice-water bath, and the reaction was allowed at room temperature overnight. The reaction solution was purified by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give compound **S10** (white solid, 778 mg, 47%). LC-MS $m/z(ES^+)$: $[M+H]^+$: 660.2.

Example 25 Synthesis of compound **S12**



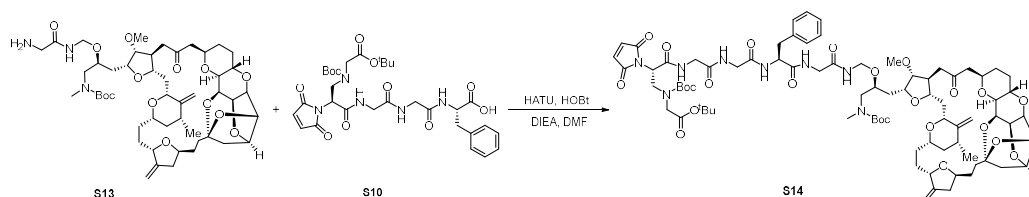
Compound **D-3** (40 mg, 0.047 mmol, 1.0 eq), compound **S11** (52.4 mg, 0.142 mmol, 3.0 eq, synthesis method refers to the synthesis of compound **1** in patent CN111686259 A), zinc acetate (26.1 mg, 0.142 mmol, 3.0 eq) and 4Å molecular sieve (60 mg) were added in sequence in a 25 mL single-neck flask, followed by addition of dry toluene (4 mL), and the reaction mixture was replaced with nitrogen three times, then heated with stirring at 100 °C. After 2 hours, the reaction was terminated, allowed to cool to room temperature, filtered, and the filtrate was concentrated under reduced pressure. The purification was performed by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give compound **S12** (white solid, 37 mg, 68%). LC-MS $m/z(\text{ES}^+)$: $[\text{M}+\text{Na}]^+$: 1174.2.

Example 26 Synthesis of compound **S13**



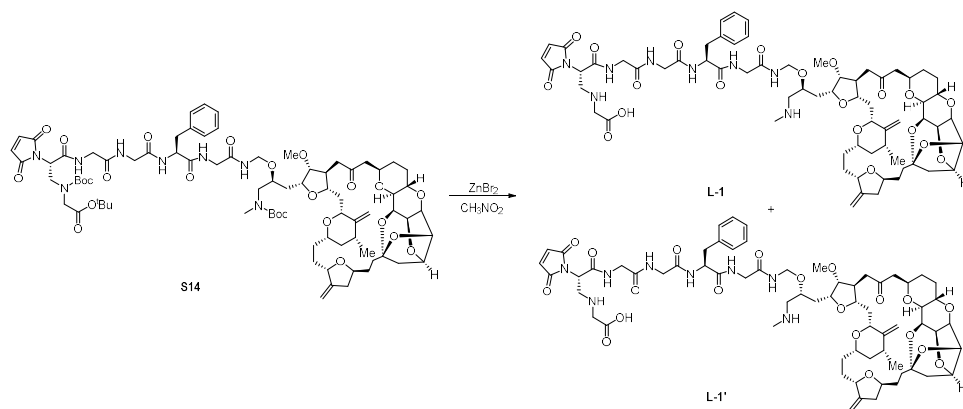
In a 25 mL single-neck flask, compound **S12** (30 mg, 0.026 mmol, 1.0 eq) and dried DMF (1.5 mL) were added and dissolved completely, then DBU (4.3 μL , 0.029 mmol, 1.1 eq) was added, and the reaction was allowed at room temperature. The consumption of compound **S12** was found to be completed from the monitoring of the TLC, and no post-treatment was needed for reaction. The next reaction was carried out directly with a crude solution of compound **S13**. LC-MS $m/z(\text{ES}^+)$: $[\text{M}+\text{Na}]^+$: 953.3.

Example 27 Synthesis of compound **S14**



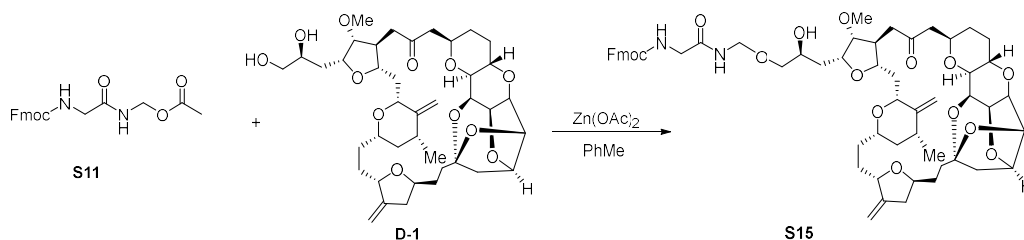
In another 25 mL single-neck flask, compound **S10** (18.9 mg, 0.029 mmol, 1.1 eq), HATU (11.9 mg, 0.031 mmol, 1.2 eq), HOBT (4.2 mg, 0.031 mmol, 1.2 eq) and dried DMF (1.5 mL) were added sequentially and dissolved uniformly, then the above crude solution of compound **S13** and DIEA (5.4 μ L, 0.031 mmol, 1.2 eq) was added dropwise in condition of an ice water bath, and the reaction was allowed at room temperature. After 30 min, HPLC showed the completion of the reaction. The reaction solution was purified by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give compound **S14** (white solid, 23 mg, 56%). LC-MS $m/z(ES^+)$: $[M-99]^+$: 1472.2 (fragment ion of compound **S14** after removing the Boc protecting group and adding hydrogen ion).

Example 28 Synthesis of compound **L1**



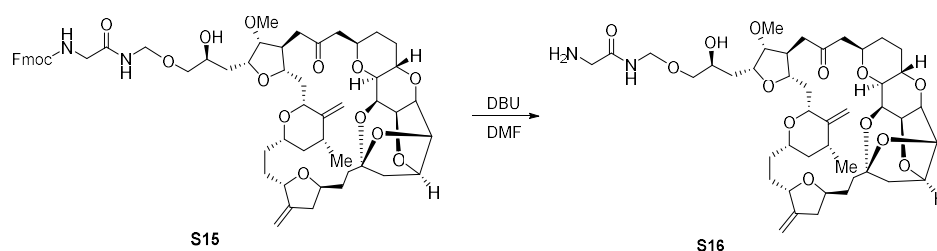
In a 25 mL single-neck flask, compound **S14** (20 mg, 0.013 mmol, 1.0 eq) was completely dissolved in nitromethane (2 mL), then zinc bromide (57 mg, 0.25 mmol, 20.0 eq) was added, and the reaction was allowed at room temperature for 30 min and monitored by HPLC. After the reaction was found to be completed, the resulting reaction solution was concentrated under reduced pressure at 45 °C using a water pump to give a crude product. The reaction solution was purified by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give compound **L-1** (white solid, 9.2 mg, 55%) and compound **L-1'** (white solid, 5.2 mg, 31%). **L-1**: LC-MS $m/z(ES^+)$: $[M+Na]^+$: 1337.3. **L-1'**: LC-MS $m/z(ES^+)$: $[M+Na]^+$: 1337.3.

Example 29 Synthesis of compound **S15**



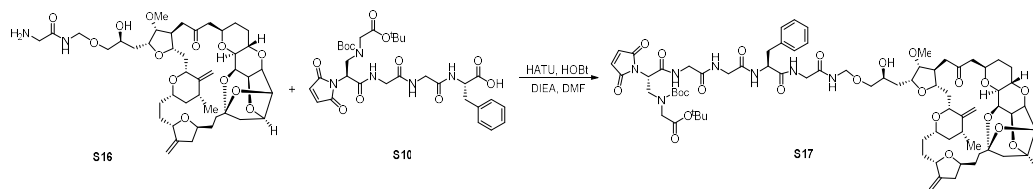
Compound **D-1** (50 mg, 0.068 mmol, 1.0 eq), compound **S11** (27.7 mg, 0.075 mmol, 1.1 eq), zinc acetate (25.1 mg, 0.137 mmol, 2.0 eq) and 4Å molecular sieve (100 mg) were added in sequence in a 25 mL single-neck flask, and then dry toluene (5 mL) was added. The reaction mixture was replaced with nitrogen three times, then heated with stirring at 100 °C. After 3 hours, the reaction was quenched, allowed to cool to room temperature, filtered, and the filtrate was concentrated under reduced pressure. The purification was performed by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give compound **S15** (white solid, 42 mg, 59%). LC-MS $m/z(\text{ES}^+)$: $[\text{M}+\text{Na}]^+$: 1061.3.

Example 30 Synthesis of compound **S16**



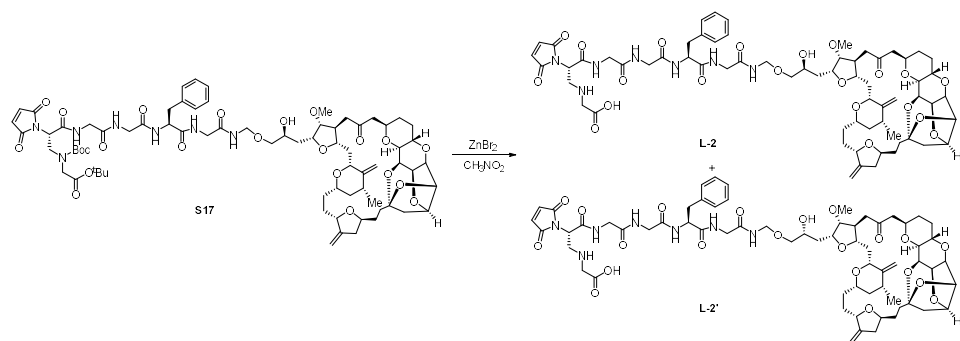
In a 25 mL single-neck flask, compound **S15** (37 mg, 0.036 mmol, 1.0 eq) and dried DMF (1.5 mL) were added and dissolved completely, then DBU (5.9 μL , 0.039 mmol, 1.1 eq) was added, and the reaction was allowed at room temperature. The consumption of compound **S15** was found to be completed from the monitoring of the TLC, and no post-treatment was needed for reaction. The next reaction was carried out directly with a crude solution of compound **S16**. LC-MS $m/z(\text{ES}^+)$: $[\text{M}+\text{Na}]^+$: 839.1.

Example 31 Synthesis of compound **S17**



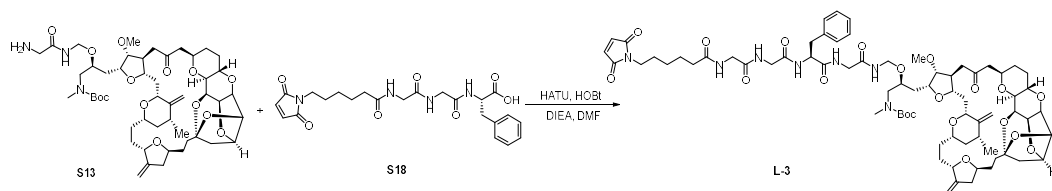
In another 25 mL single-neck flask, compound **S10** (25.8 mg, 0.039 mmol, 1.1 eq), HATU (16.2 mg, 0.043 mmol, 1.2 eq), HOBT (5.8 mg, 0.043 mmol, 1.2 eq) and dried DMF (1.5 mL) were added sequentially and dissolved uniformly. The above crude solution of compound **S16** and DIEA (7.4 μ L, 0.043 mmol, 1.2 eq) was added dropwise in condition of an ice water bath, and the reaction was allowed at room temperature. After 30 min, HPLC showed the completion of the reaction. The reaction solution was purified by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give compound **S17** (white solid, 26 mg, 50%). LC-MS $m/z(ES^+)$: $[M+H]^+$: 1459.2.

Example 32 Synthesis of compound **L-2**



In a 25 mL single-neck flask, compound **S17** (21 mg, 0.014 mmol, 1.0 eq) was completely dissolved in nitromethane (2 mL), then zinc bromide (65 mg, 0.288 mmol, 20.0 eq) was added, and the reaction was allowed at room temperature for 30 min and monitored by HPLC. After the reaction was found to be completed, the resulting reaction solution was concentrated under reduced pressure at 45 °C using a water pump to give a crude product. The reaction solution was purified by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give compound **L-2** (white solid, 10.7 mg, 57%) and compound **L-2'** (white solid, 5.4 mg, 29%). **L-2**: LC-MS $m/z(ES^+)$: $[M+Na]^+$: 1324.3. **L-2'**: LC-MS $m/z(ES^+)$: $[M+Na]^+$: 1324.3.

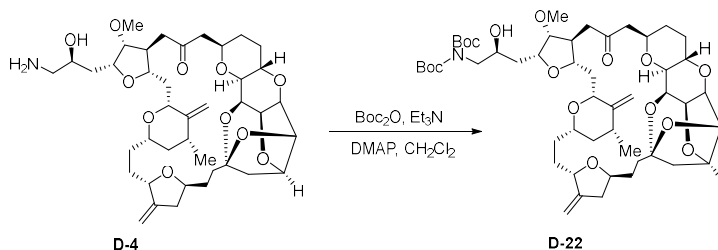
Example 33 Synthesis of compound **L-3**



Compound **L-3** was synthesized from compound **S13** and compound **S18** (see patent WO2022171101 for synthesis method) according to the synthesis method of Example 27.

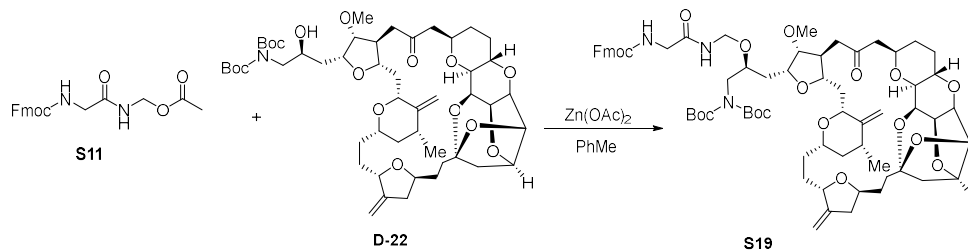
LC-MSm/z(ES⁺): [M-99]⁺: 1285.1 (fragment ion of compound **L-3** after removing the Boc protecting group and adding hydrogen ion).

Example 34 Synthesis of compound **D-22**



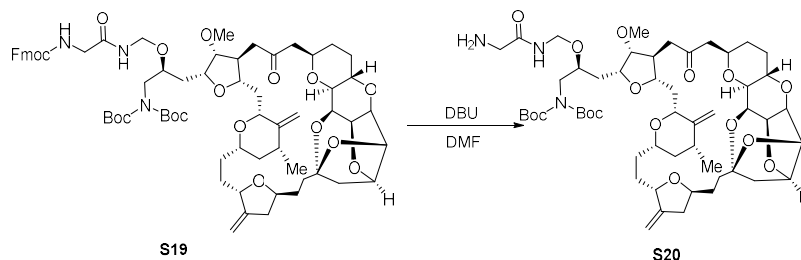
In a 25 mL single-neck flask, compound **D-4** (53 mg, 0.073 mmol, 1.0 eq) was added and dissolved in dried dichloromethane (3 mL), followed by addition of triethylamine (30 μ L, 0.218 mmol, 3.0 eq), DMAP (1.8 mg, 0.015 mmol, 0.2 eq) and di-tert-butyl dicarbonate (42 μ L, 0.182 mmol, 2.5 eq), and the reaction was allowed at room temperature for 2 h. After the reaction was found to be completed from the monitoring of the TLC, the reaction solution was concentrated under reduced pressure. The purification was performed by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give compound **D-22** (white solid, 36 mg, 53%). LC-MS m/z(ES⁺): [M+H]⁺: 930.2.

Example 35 Synthesis of compound **S19**



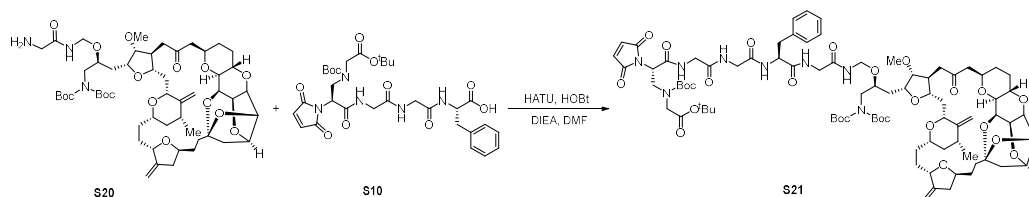
Compound **S19** was synthesized from compound **D-22** and compound **S11** according to the synthesis method of Example 25. LC-MS m/z(ES⁺): [M+Na]⁺: 1260.2.

Example 36 Synthesis of compound **S20**



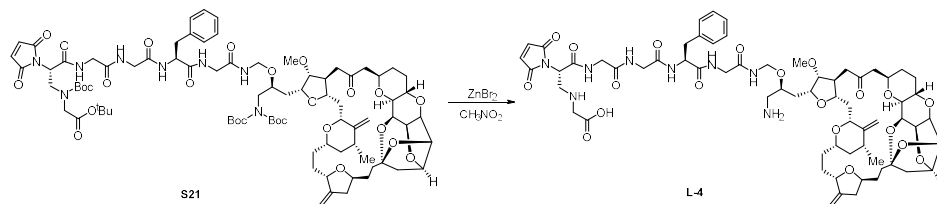
A crude solution of compound **S20** was synthesized from compound **S19** according to the synthesis method of Example 26, and put into the next step of reaction directly without post-treatment. LC-MS $m/z(ES^+)$: $[M+Na]^+$:1038.3.

Example 37 Synthesis of compound **S21**



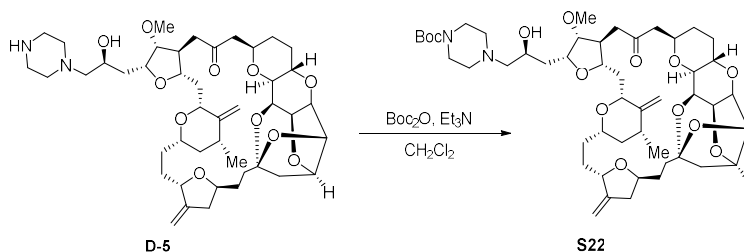
Compound **S21** was synthesized from compound **S20** and compound **S10** according to the synthesis method of Example 27. TOF m/z : $[M+H]^+$:1657.5.

Example 38 Synthesis of compound **L-4**



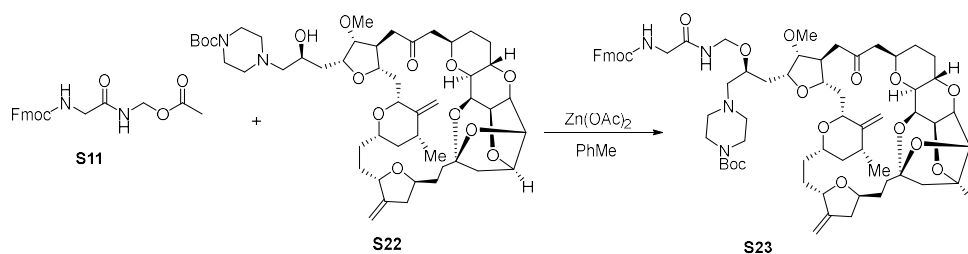
Compound **L-4** was synthesized from compound **S21** according to the synthesis method of Example 28. LC-MS $m/z(ES^+)$: $[M+Na]^+$:1323.3.

Example 39 Synthesis of compound **S22**



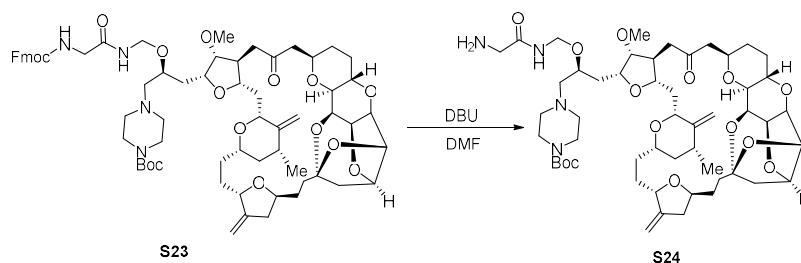
Compound **S22** was synthesized from compound **D-5** according to the synthesis method of Example 2. LC-MS $m/z(ES^+)$: $[M+H]^+$:898.3.

Example 40 Synthesis of compound **S23**



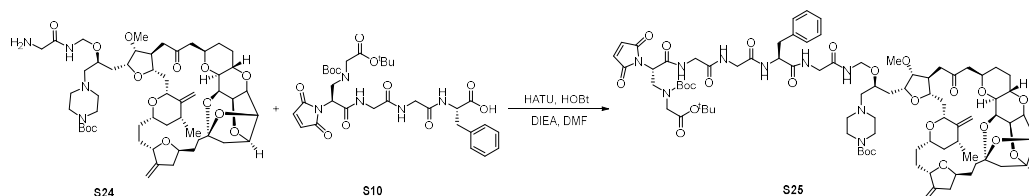
Compound **S23** was synthesized from compound **S22** and compound **S11** according to the synthesis method of Example 25. LC-MS $m/z(\text{ES}^+)$: $[\text{M}+\text{Na}]^+$:1229.3.

Example 41 Synthesis of compound **S24**



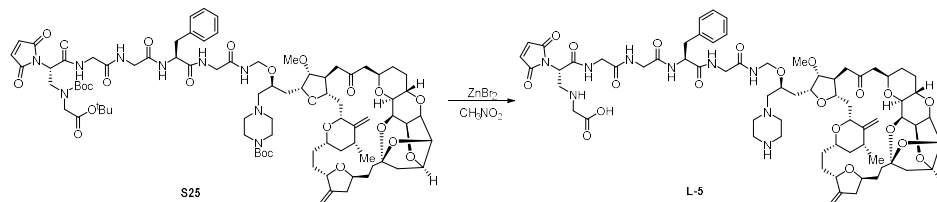
A crude solution of compound **S24** was synthesized from compound **S23** according to the synthesis method of Example 26, and put into the next step of reaction directly without post-treatment. LC-MS $m/z(\text{ES}^+)$: $[\text{M}+\text{Na}]^+$:1007.3.

Example 42 Synthesis of compound **S25**



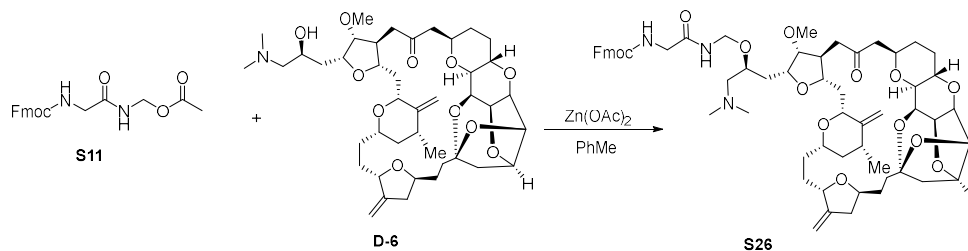
Compound **S25** was synthesized from compound **S24** and compound **S10** according to the synthesis method of Example 27. TOF m/z : $[\text{M}+\text{H}]^+$:1626.5.

Example 43 Synthesis of compound **L-5**



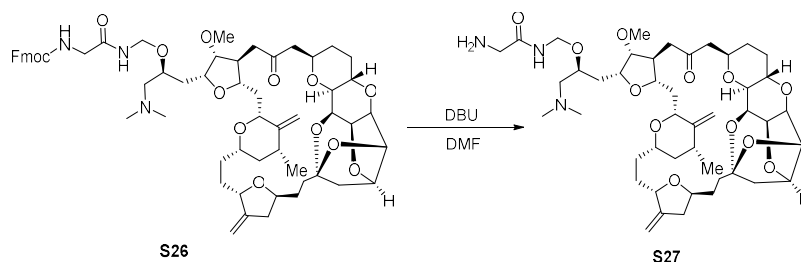
Compound **L-5** was synthesized from compound **S25** according to the synthesis method of Example 28. LC-MS $m/z(\text{ES}^+)$: $[\text{M}+\text{Na}]^+$:1392.4.

Example 44 Synthesis of compound **S26**



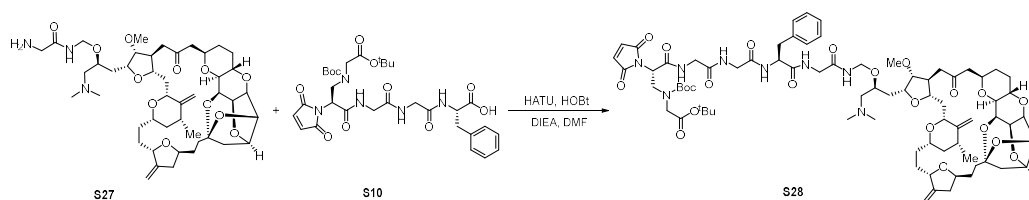
Compound **S26** was synthesized from compound **D-6** and compound **S11** according to the synthesis method of Example 25. LC-MS $m/z(\text{ES}^+)$: $[\text{M}+\text{Na}]^+$:1088.3.

Example 45 Synthesis of compound **S27**



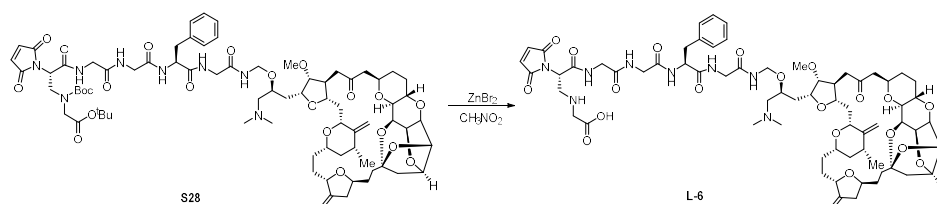
A crude solution of compound **S27** was synthesized from compound **S26** according to the synthesis method of Example 26, and put into the next step of reaction directly without post-treatment. LC-MS $m/z(\text{ES}^+)$: $[\text{M}+\text{Na}]^+$:866.2.

Example 46 Synthesis of compound **S28**



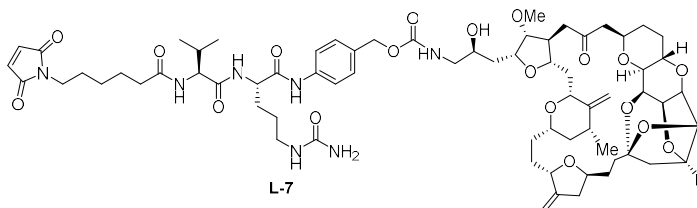
Compound **S28** was synthesized from compound **S27** and compound **S10** according to the synthesis method of Example 27. LC-MS $m/z(\text{ES}^+)$: $[\text{M}+\text{Na}]^+$:1485.5.

Example 47 Synthesis of compound **L-6**



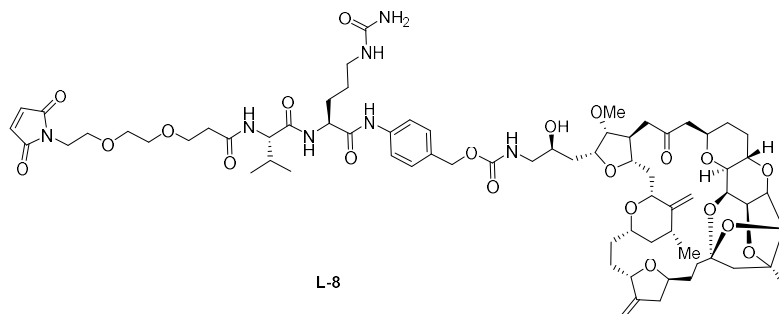
Compound **L-6** was synthesized from compound **S28** according to the synthesis method of Example 28. LC-MS $m/z(ES^+)$: $[M+Na]^+$:1351.3.

Example 48 Synthesis of compound **L-7**



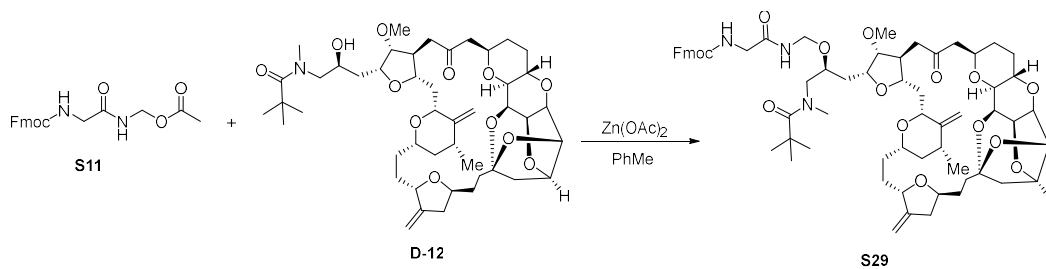
Compound **L-7** was synthesized according to the synthesis method of compound ER-001235638 in patent CN108883198A.

Example 49 Synthesis of compound **L-8**



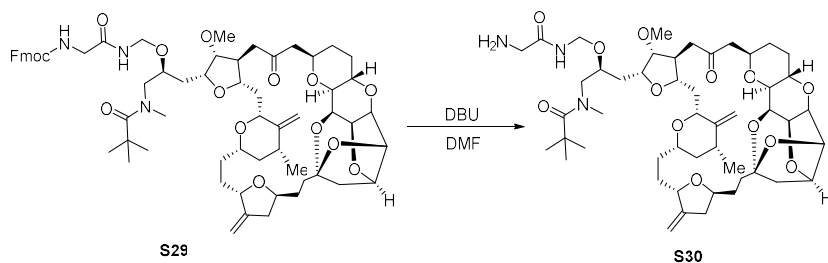
Compound **L-8** was synthesized according to the synthesis method of ER-001159569 in patent CN108883198A.

Example 50 Synthesis of compound **S29**



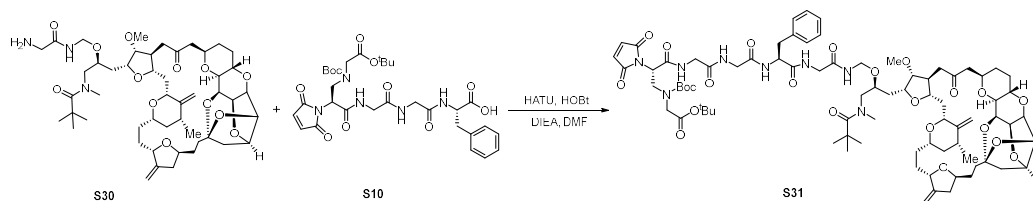
Compound **S29** was synthesized from compound **D-12** and compound **S11** according to the synthesis method of Example 25. LC-MS $m/z(ES^+)$: $[M+Na]^+$:1158.4.

Example 51 Synthesis of compound **S30**



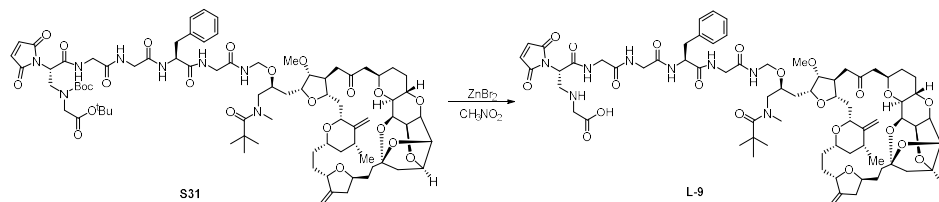
A crude solution of compound **S30** was synthesized from compound **S29** according to the synthesis method of Example 26, and put into the next step of reaction directly without post-treatment. LC-MS $m/z(ES^+)$: $[M+Na]^+$:936.5.

Example 52 Synthesis of compound **S31**



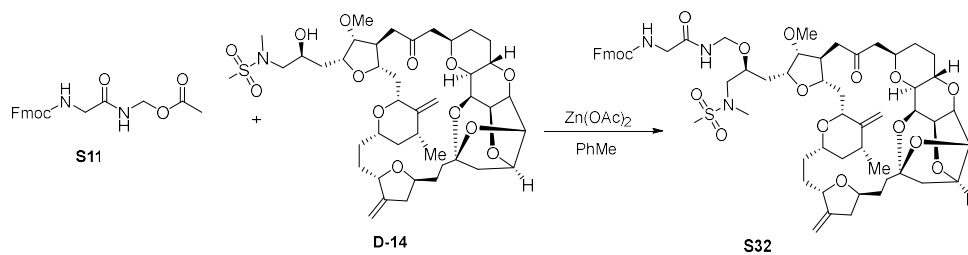
Compound **S31** was synthesized from compound **S30** and compound **S10** according to the synthesis method of Example 27. TOF m/z : $[M+H]^+$:1555.7.

Example 53 Synthesis of compound **L-9**



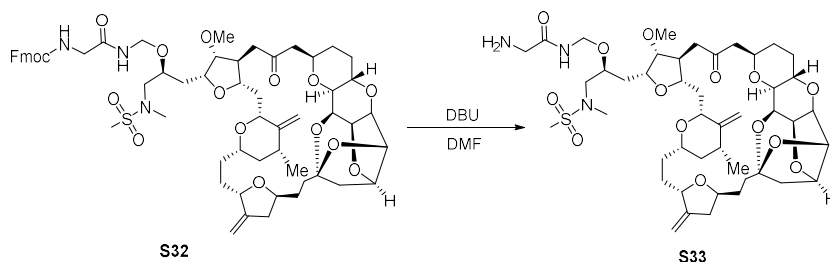
Compound **L-9** was synthesized from compound **S31** according to the synthesis method of Example 28. LC-MS $m/z(ES^+)$: $[M+Na]^+$:1421.7.

Example 54 Synthesis of compound **S32**



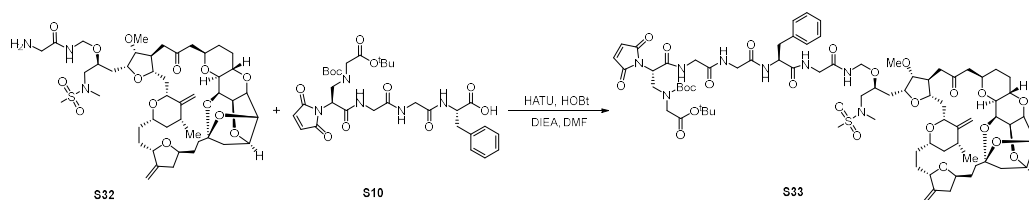
Compound **S32** was synthesized from compound **D-14** and compound **S11** according to the synthesis method of Example 25. LC-MS $m/z(ES^+)$: $[M+Na]^+$:1152.5.

Example 55 Synthesis of compound **S33**



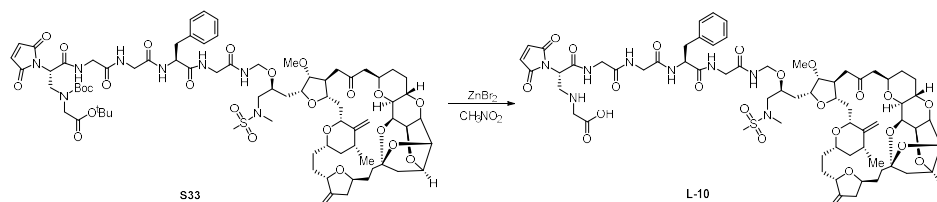
A crude solution of compound **S33** was synthesized from compound **S32** according to the synthesis method of Example 26, and put into the next step of reaction directly without post-treatment. LC-MS $m/z(ES^+)$: $[M+Na]^+$:930.4.

Example 56 Synthesis of compound **S33**



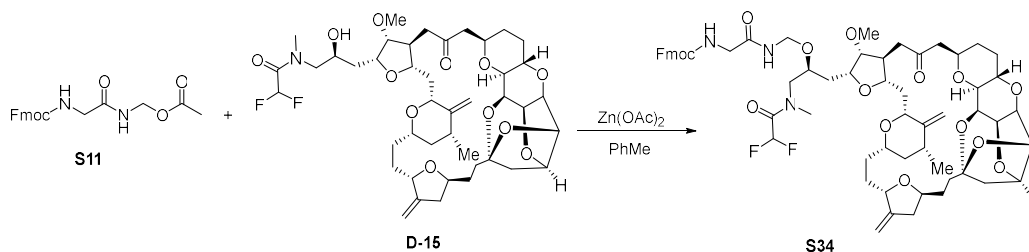
Compound **S33** was synthesized from compound **S32** and compound **S10** according to the synthesis method of Example 27. TOF m/z : $[M+H]^+$:1549.7.

Example 57 Synthesis of compound **L-10**



Compound **L-10** was synthesized from compound **S33** according to the synthesis method of Example 28. LC-MS $m/z(ES^+)$: $[M+Na]^+$:1415.6.

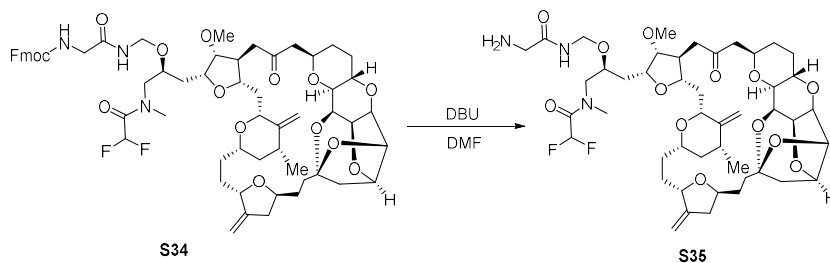
Example 58 Synthesis of compound **S34**



Compound **S34** was synthesized from compound **D-15** and compound **S11** according to the

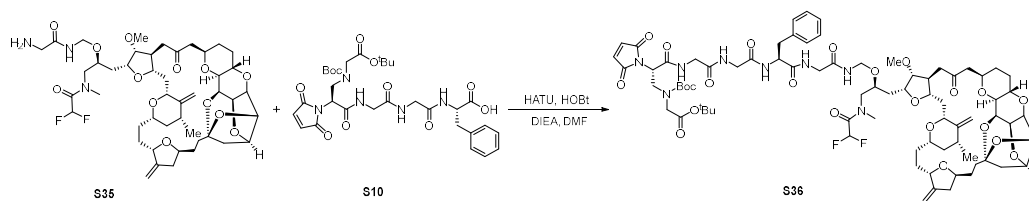
synthesis method of Example 25. LC-MS $m/z(ES^+)$: $[M+Na]^+$:1152.4.

Example 59 Synthesis of compound **S35**



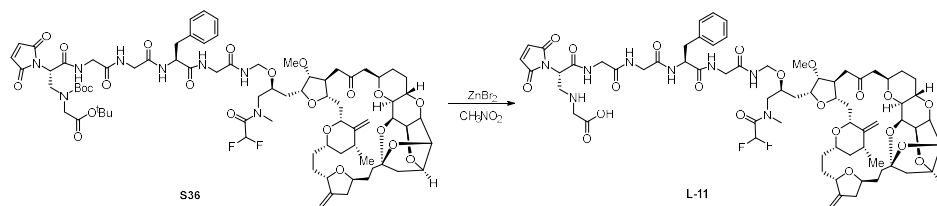
A crude solution of compound **S35** was synthesized from compound **S34** according to the synthesis method of Example 26, and put into the next step of reaction directly without post-treatment. LC-MS $m/z(ES^+)$: $[M+Na]^+$:930.3.

Example 60 Synthesis of compound **S36**



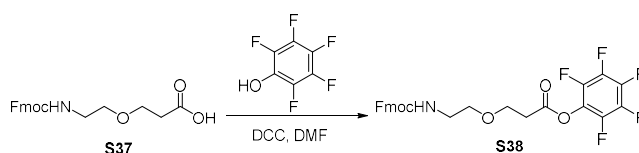
Compound **S36** was synthesized from compound **S35** and compound **S10** according to the synthesis method of Example 27. TOF m/z : $[M+H]^+$:1549.6.

Example 61 Synthesis of compound **L-11**



Compound **L-11** was synthesized from compound **S36** according to the synthesis method of Example 28. LC-MS $m/z(ES^+)$: $[M+Na]^+$:1415.6.

Example 62 Synthesis of compound **S39**



acetate (2/1) to give compound **S41** (100.9 g, 48% yield). LC-MS $m/z(ES^+)$: $[M+H]^+$: 463.2.

Step 2: Synthesis of compound **S42**

In a 1 L single-neck flask, **S41** (100.0 g) was added and dissolved in 500 mL dichloromethane, followed by addition of 200 mL of trifluoroacetic acid. The reaction was allowed at room temperature for 2 h and the reaction was monitored by TLC until the endpoint. After the reaction was completed, the reaction solution was concentrated to remove dichloromethane and a portion of trifluoroacetic acid, and a crude product was obtained. Methyl tert-butyl ether was added to the crude product, and a white solid was precipitated, filtered, and dried to give compound **S42** (77.5 g, 87% yield), LC-MS $m/z(ES^-)$: $[M-H]^-$: 405.1.

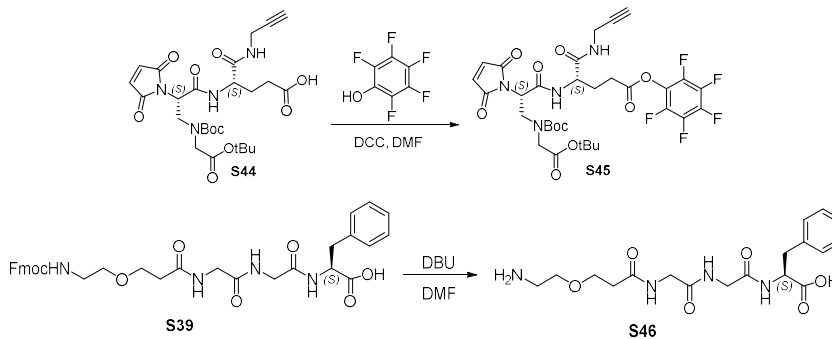
Step 3: Synthesis of compound **S43**

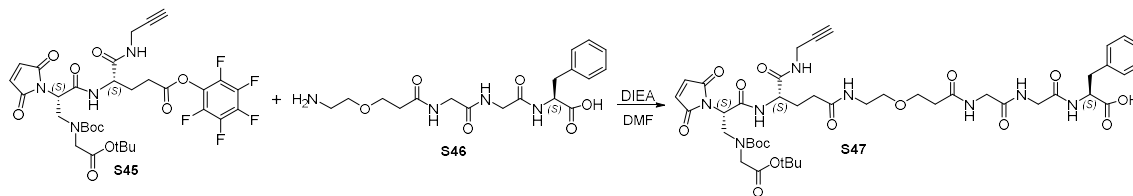
In a 2 L single-neck flask, **S42** (77.0 g) was added and dissolved in 150 mL of DMF, followed by addition of 38 mL of diethylamine. The reaction was allowed at room temperature for 2 h and the reaction was monitored by TLC until the endpoint. After the reaction was completed, 800 mL of methyl tert-butyl ether was added to the reaction solution. A white solid was precipitated, filtered, and dried to give compound **S43** (33.7 g, 96.6% yield), LC-MS $m/z(ES^-)$: $[M-H]^-$: 183.1.

Step 4: Synthesis of compound **S44**

In a 100 mL single-neck flask, compound **S43** (10.02 g, 54.4 mmol, 1.5 eq), compound **S8** (14.48 g, 36.3 mmol, 1.0 eq) and 100 mL DMF were added and dissolved, followed by addition of EEDQ (13.5 g, 54.8 mmol, 1.5 eq). The reaction was allowed at room temperature for 2 h and the reaction was monitored by HPLC until the endpoint. After the reaction was completed, the reaction solution was purified by preparative liquid chromatography to give the product preparation solution, which was then lyophilized to give compound **S44** (9.94 g, 48% yield), LC-MS $m/z(ES^-)$: $[M-H]^-$: 563.2.

Example 64 Synthesis of compound **S47**





Step 1: Synthesis of compound **S45**

In a 25 mL single-neck flask, compound **S44** (230 mg, 0.407 mmol, 1.0 eq), pentafluorophenol (83 mg, 0.448 mmol, 1.1 eq), DCC (93 mg, 0.448 mmol, 1.1 eq) and DMF (3.5 mL) were added, and the reaction was allowed at room temperature overnight. After the reaction was found to be completed from the monitoring of the TLC, the reaction solution was filtered to give the crude filtrate of compound **S45** for later use.

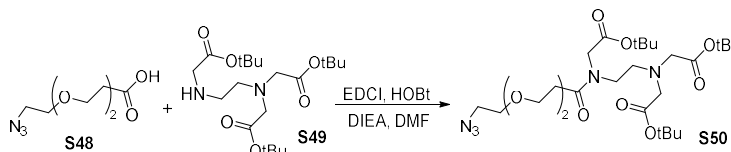
Step 2: Synthesis of compound **S46**

In a 25 mL single-neck flask, compound **S39** (301 mg, 0.489 mmol, 1.2 eq) and dried DMF (3 mL) were added and dissolved completely, then DBU (79 μ L, 0.529 mmol, 1.3 eq) was added, and the reaction was allowed at room temperature. The consumption of compound **S39** was found to be completed from the monitoring of the TLC, and no post-treatment was needed for reaction. The next reaction was carried out directly with a crude solution of compound **S46**.

Step 3: Synthesis of compound **S47**

Under an ice-water bath, the crude filtrate of compound **S45** was mixed with the crude solution of **S46**, and DIEA (71 μ L, 0.407 mmol, 1.0 eq) was slowly added dropwise, and the reaction was allowed at room temperature for 40 min. The reaction solution was purified by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give compound **S47** (white solid, 258 mg, 67%). LC-MS m/z (ES^+): $[M+H]^+$: 941.3.

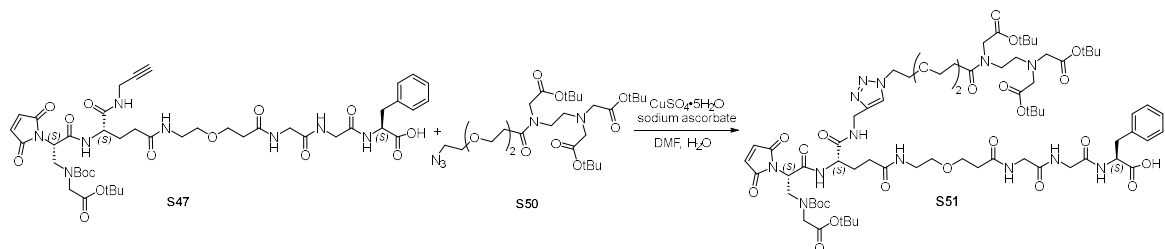
Example 65 Synthesis of compound **S50**



In a 150 mL single-neck flask, compound **S48** (667 mg, 3.28 mmol, 1.0 eq), **S49** (1.58 g, 3.94 mmol, 1.2 eq, synthesis method refers to the synthesis of compound **11** in patent WO 0152900 A2), EDCI (944 mg, 4.92 mmol, 1.5 eq), HOBt (665 mg, 4.92 mmol, 1.5 eq) and dried dichloromethane (60 mL) were added sequentially and dissolved uniformly. DIEA (1.14 mL, 6.56 mmol, 2.0 eq) was added dropwise in condition of an ice water bath, and the reaction was allowed at room temperature. After 2 h, the reaction was found to be completed from the monitoring of the TLC,

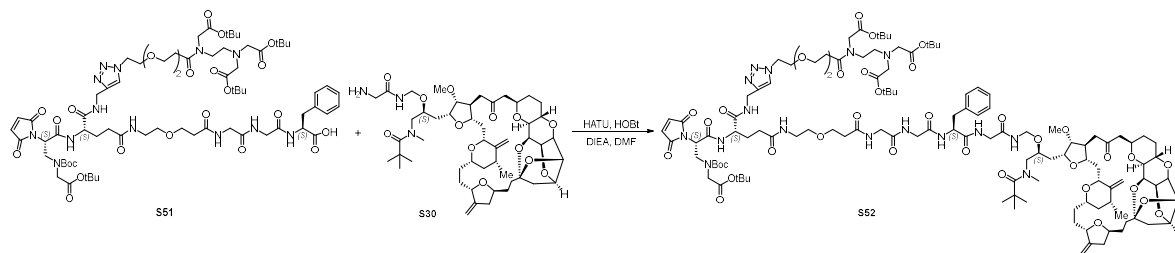
the reaction solution was quenched with saturated aqueous ammonium chloride, extracted with dichloromethane, dried, filtered, concentrated, and purified by column chromatography (petroleum ether/ethyl acetate = 30:1) to give compound **S50** (a yellow oil 1.3 g, 67%). LC-MS $m/z(ES^+)$: $[M+H]^+$: 588.4.

Example 66 Synthesis of compound **S51**



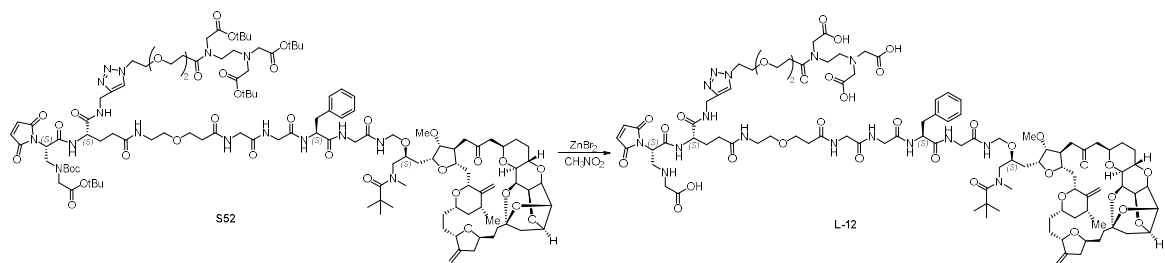
In a 25 mL single-neck flask, compound **S47** (58 mg, 0.061 mmol, 1.0 eq), compound **S50** (36 mg, 0.061 mmol, 1.0 eq), copper (II) sulfate pentahydrate (31 mg, 0.123 mmol, 2.0 eq), sodium ascorbate (24 mg, 0.123 mmol, 2.0 eq) and DMF (2 mL) were added, then water (1 mL) was added in an ice-water bath, and the reaction was allowed at room temperature. After 30 min, HPLC showed the completion of the reaction. The reaction solution was purified by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give compound **S51** (white solid, 85 mg, 91%). LC-MS $m/z(ES^+)$: $[M+2H]^{2+}$: 764.7.

Example 67 Synthesis of compound **S52**



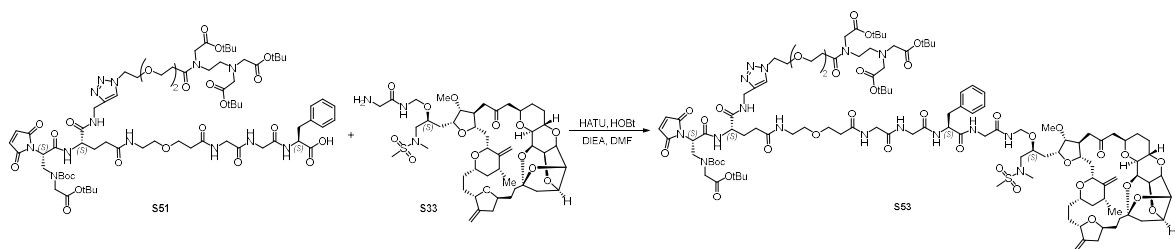
Compound **S52** was synthesized from compound **S51** and compound **S30** according to the synthesis method of Example 27. LC-MS $m/z(ES^+)$: $[M+H+Na]^{2+}$: 1223.8.

Example 68 Synthesis of compound **L-12**



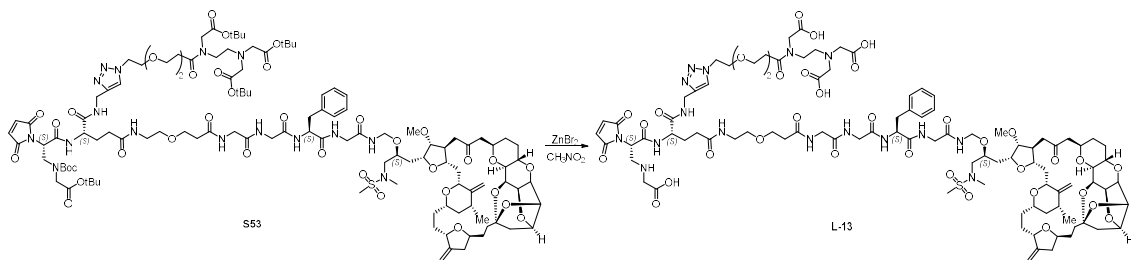
In a 25 mL single-neck flask, compound **S52** (19 mg, 0.0078 mmol, 1.0 eq) and nitromethane (2 mL) were added and dissolved completely, then zinc bromide (124 mg, 0.548 mmol, 70.0 eq) was added, and the reaction was allowed at room temperature for 45 min. The reaction solution was concentrated under reduced pressure to give a crude product. The crude product was purified by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give compound **L-12** (white solid, 9.2 mg, 55%) **L-12**: LC-MS $m/z(ES^+)$: $[M+2H]^{2+}$: 1050.3.

Example 69 Synthesis of compound **S53**



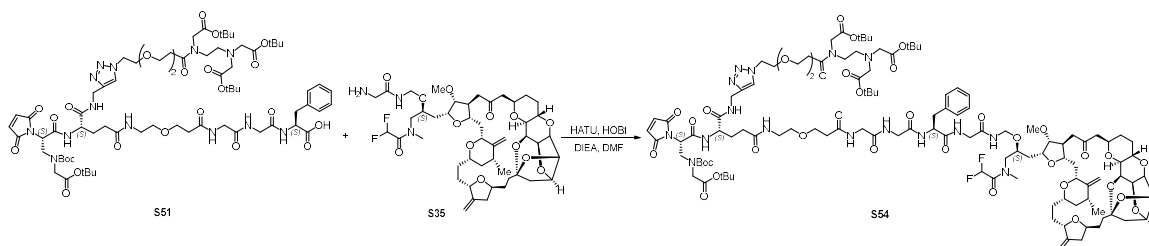
Compound **S53** was synthesized from compound **S51** and compound **S33** according to the synthesis method of Example 27. LC-MS $m/z(ES^+)$: $[M+2H]^{2+}$: 1209.9.

Example 70 Synthesis of compound **L-13**



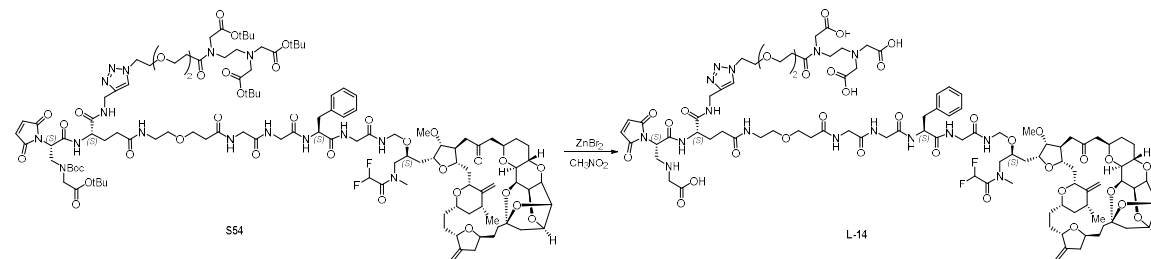
Compound **L-13** was synthesized from compound **S53** according to the synthesis method of Example 68. LC-MS $m/z(ES^+)$: $[M+2H]^{2+}$: 1047.4.

Example 71 Synthesis of compound **S54**



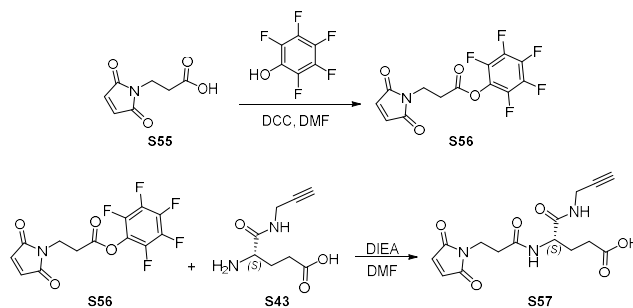
Compound **S54** was synthesized from compound **S51** and compound **S35** according to the synthesis method of Example 27. LC-MS $m/z(ES^+)$: $[M+2H+Na]^{2+}$: 1220.8.

Example 72 Synthesis of compound **L-14**



Compound **L-14** was synthesized from compound **S54** according to the synthesis method of Example 68. LC-MS $m/z(ES^+)$: $[M+2H]^{2+}$: 1047.4.

Example 73 Synthesis of compound **S57**



Step 1: Synthesis of compound **S56**

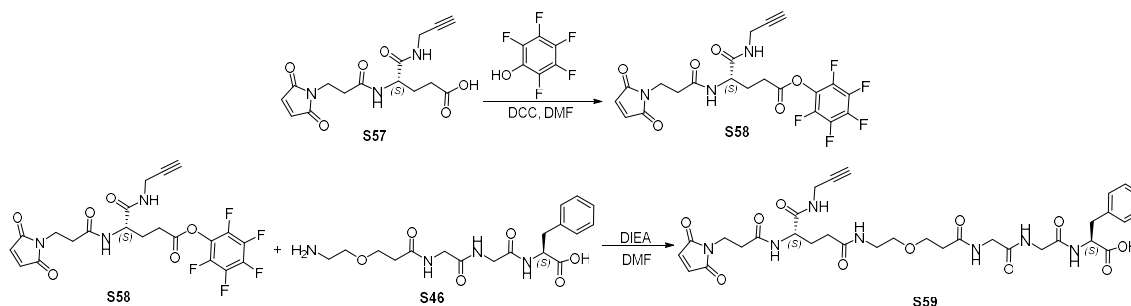
In a 25 mL single-neck flask, compound **S55** (400 mg, 2.36 mmol, 1.0 eq), pentafluorophenol (479 mg, 2.60 mmol, 1.1 eq), DCC (537 mg, 2.60 mmol, 1.1 eq) and DMF (4 mL) were added, and the reaction was allowed at room temperature for 4 h. After the reaction was found to be completed from the monitoring of the TLC, the reaction solution was filtered to give the crude filtrate of compound **S56** for later use.

Step 2: Synthesis of compound **S57**

Compound **S43** (436 mg, 2.36 mmol, 1.0 eq) and DMF (7 mL) were added in the crude filtrate of the above compound **S56**, and DIEA (412 μ L, 2.36 mmol, 1.0 eq) was added in an ice-water bath,

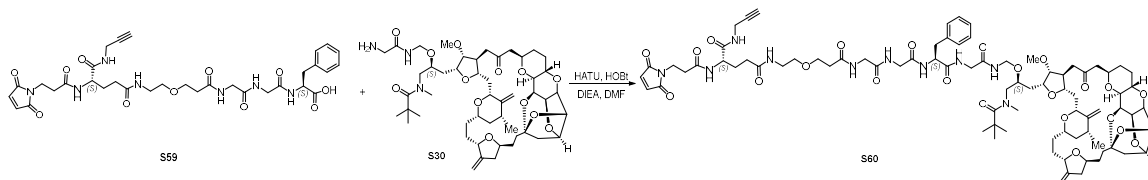
and the reaction was allowed at room temperature for 2 h. After the reaction was found to be completed from the monitoring of the HPLC, the reaction solution was purified by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give compound **S57** (white solid, 649 mg, 82%). LC-MS $m/z(ES^+)$: $[M+Na]^+$: 358.2.

Example 74 Synthesis of compound **S59**



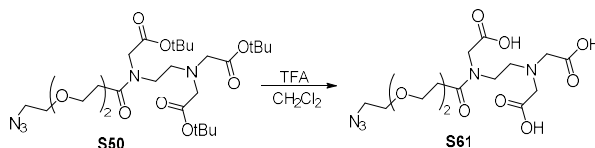
Compound **S59** was synthesized from compound **S57** and compound **S46** according to the synthesis method of Example 64. LC-MS $m/z(ES^+)$: $[M+H]^+$: 712.0.

Example 75 Synthesis of compound **S60**



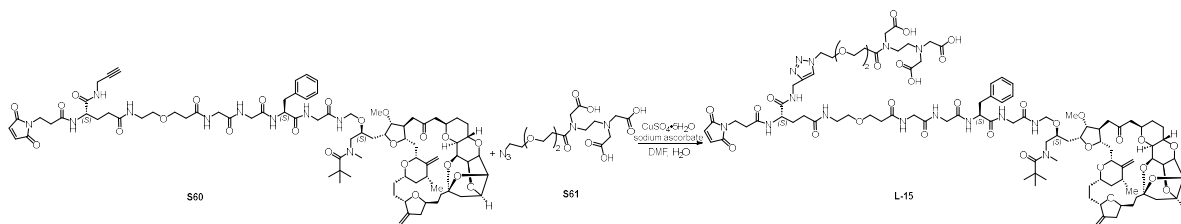
Compound **S60** was synthesized from compound **S59** and compound **S30** according to the synthesis method of Example 27. LC-MS $m/z(ES^+)$: $[M+2H]^{2+}$: 804.4.

Example 76 Synthesis of compound **S61**



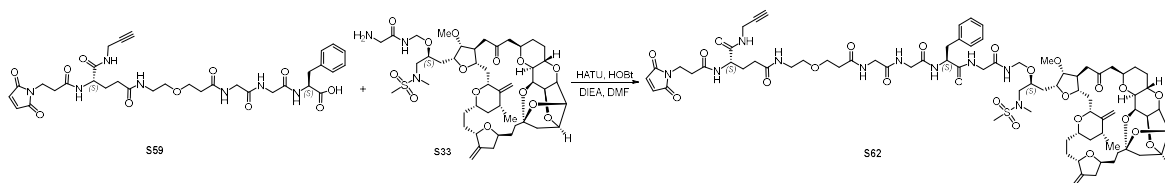
Compound **S50** (201 mg, 0.342 mmol, 1.0 eq) was dissolved in dried dichloromethane (4 mL), then trifluoroacetic acid (800 μ L) was added, and the reaction was allowed at room temperature. After 30 h, the reaction solution was concentrated under reduced pressure. The crude product was purified by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give compound **S61** (white solid, 98 mg, 69%). LC-MS $m/z(ES^-)$: $[M-H]^-$: 418.2.

Example 77 Synthesis of compound L-15



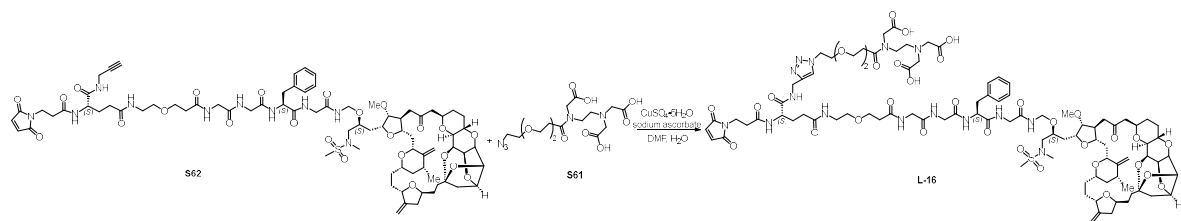
In a 25 mL single-neck flask, compound **S60** (25 mg, 0.016 mmol, 1.0 eq), compound **S61** (7.8 mg, 0.019 mmol, 1.2 eq), copper (II) sulfate pentahydrate (19 mg, 0.078 mmol, 5.0 eq), sodium ascorbate (15 mg, 0.078 mmol, 5.0 eq) and DMF (2 mL) were added, then water (1 mL) was added in an ice-water bath, and the reaction was allowed at room temperature. After 30 min, HPLC showed the completion of the reaction. The reaction solution was purified by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give compound **L-15** (white solid, 21 mg, 66%). LC-MS $m/z(ES^+)$: $[M+H+Na]^{2+}$: 1024.9.

Example 78 Synthesis of compound S62



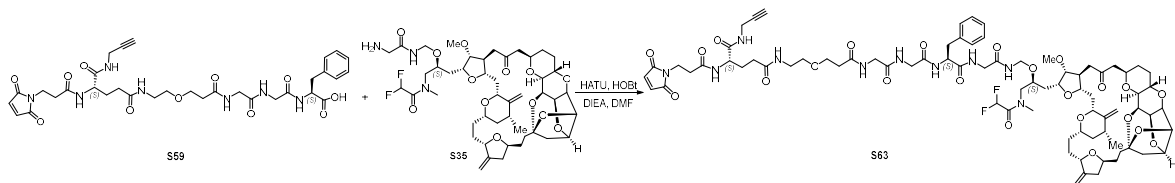
Compound **S62** was synthesized from compound **S59** and compound **S33** according to the synthesis method of Example 27. LC-MS $m/z(ES^+)$: $[M+2H]^{2+}$: 801.3.

Example 79 Synthesis of compound L-16



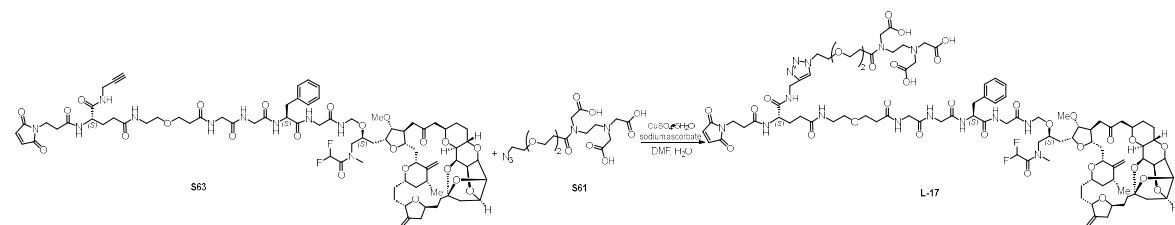
Compound **L-16** was synthesized from compound **S62** and compound **S61** according to the synthesis method of Example 77. LC-MS $m/z(ES^+)$: $[M+H+NH_4]^{2+}$: 1019.5.

Example 80 Synthesis of compound S63



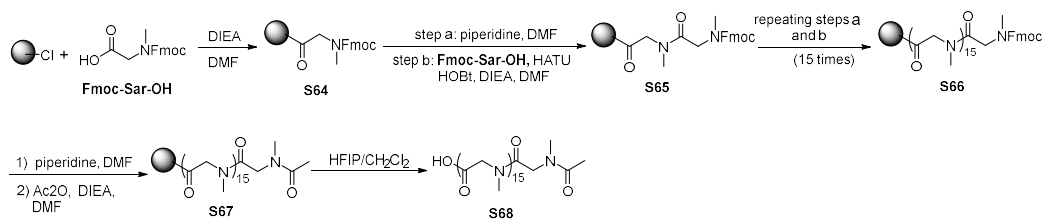
Compound **S63** was synthesized from compound **S59** and compound **S35** according to the synthesis method of Example 27. LC-MS $m/z(ES^+)$: $[M+2H]^{2+}$: 801.0.

Example 81 Synthesis of compound **L-17**



Compound **L-17** was synthesized from compound **S63** and compound **S61** according to the synthesis method of Example 77. LC-MS $m/z(ES^+)$: $[M+2H]^{2+}$: 1010.9.

Example 82 Synthesis of compound **S68**



Step 1: Resin loading: 4.6 g of 2-chlorotrityl chloride resin beads (1.05 mmol/g) were weighed and placed into a resin reactor and swollen in DMF for 1 h. After swelling, the solvent was filtered off, and a solution (30 mL) of Fmoc-sarcosine (1.5 g, 4.8 mmol, 1.0 eq) and DIEA (1.7 mL, 9.6 mmol, 2.0 eq) in DMF was added to the resin, and the resin reactor was shaken at room temperature for 4 h. After filtration, the resin was blocked with MeOH/DIEA/DMF (5 mL/2.5 mL/25 mL) for 30 min, and then the resin was thoroughly washed with DMF (8 times) to give compound **S64**.

Step 2: Extension of polysarcosine compounds

Step a: The resin was treated with piperidine/DMF (6 mL/24 mL) twice, 20 min each time. The resin was then washed with DMF (8 times).

Step b: A solution (30 mL) of Fmoc-sarcosine (4.5 g, 14.4 mmol, 3.0 eq), HATU (5.5 g, 14.4 mmol, 3.0 eq), HOBT (1.9 g, 14.4 mmol, 3.0 eq) and DIEA (4.2 mL, 24 mmol, 5.0 eq) in DMF was added to the resin, and the resin reactor was shaken at room temperature for 2 h. The resin was then washed with DMF (8 times).

Steps a and b were repeated until the length of the polysarcosine compound on the resin reached 16 peptides, thus obtaining compound **S66**.

Step 3: Acetylation

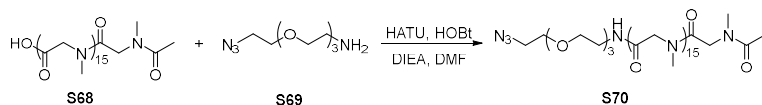
The resin was treated with piperidine/DMF (6 mL/24 mL) twice for 20 min each time. The resin was then washed with DMF (8 times).

The resin was then treated with acetic anhydride/DIEA/DMF (5 mL/10 mL/15 mL) twice, 1 h each time. The resin was then washed with DMF (8 times) to give compound **S67**.

Step 4: Resin cleavage

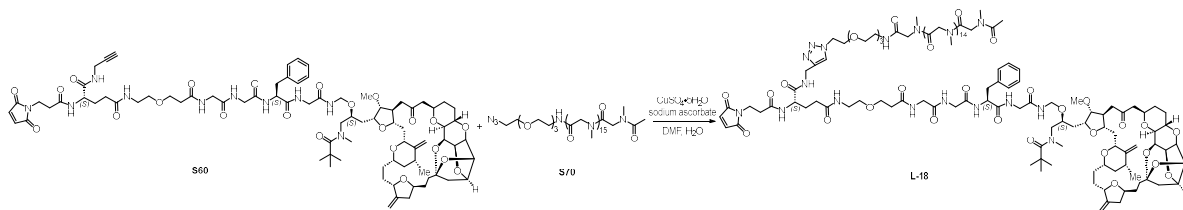
Poly-sarcosine oligomers were cleaved from the resin with an HFIP/CH₂Cl₂ (6 mL/24 mL) solution for 30 min. The resin was filtered, the filtrate was concentrated under reduced pressure, and the crude product was purified by a reverse phase preparative column, and the preparation was lyophilized to give compound **S68** (white solid, 1.7 g, 30%). LC-MS *m/z*(ES⁺): [M+2NH₄]²⁺: 616.4.

Example 83 Synthesis of compound **S70**



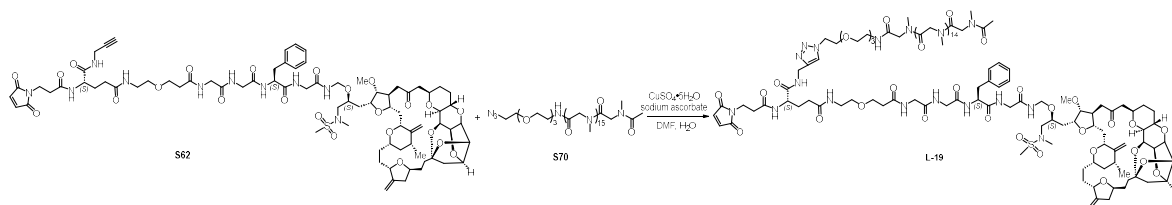
In a 25 mL single-neck flask, compound **S68** (93 mg, 0.078 mmol, 1.0 eq), **S69** (17 mg, 0.078 mmol, 1.0 eq), HATU (45 mg, 0.117 mmol, 1.5 eq), HOBT (16 mg, 0.117 mmol, 1.5 eq) and dried DMF (2.5 mL) were added sequentially and dissolved uniformly. DIEA (27 μ L, 0.156 mmol, 2.0 eq) was added dropwise in condition of an ice water bath, and the reaction was allowed at room temperature. After 1 h, HPLC showed the completion of the reaction. The reaction solution was purified by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give compound **S70** (white solid, 80 mg, 73%). LC-MS *m/z*(ES⁺): [M+H]⁺: 1397.1.

Example 84 Synthesis of compound **L-18**



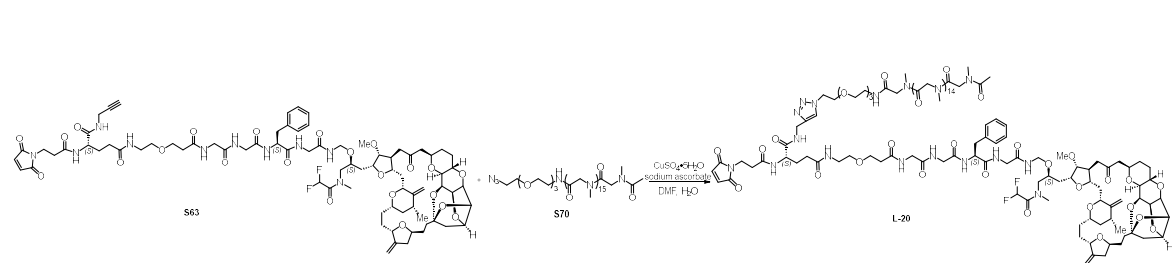
Compound **L-18** was synthesized from compound **S60** and compound **S70** according to the synthesis method of Example 77. TOF *m/z*: [M+Na]⁺: 3026.5.

Example 85 Synthesis of compound **L-19**



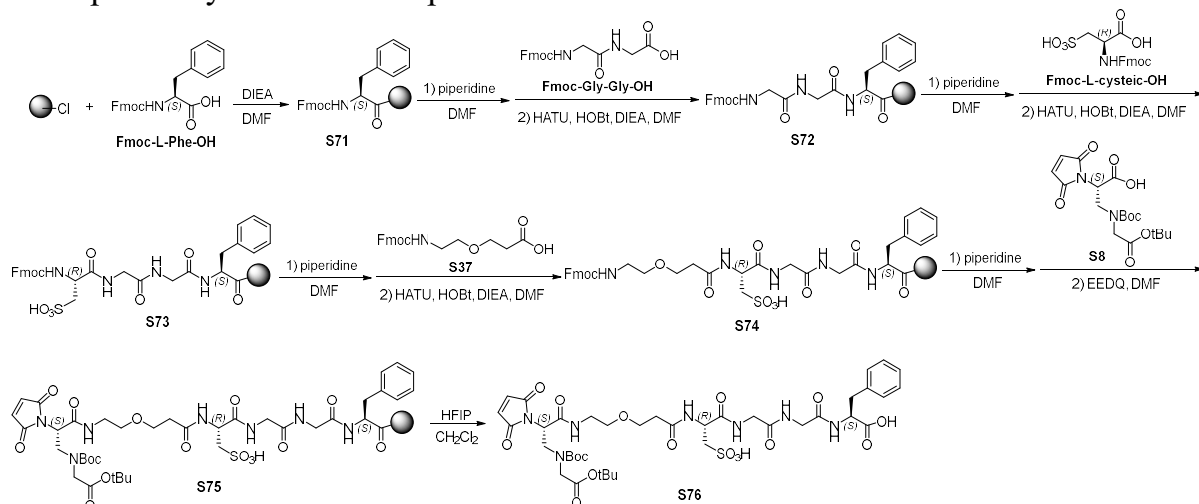
Compound **L-19** was synthesized from compound **S62** and compound **S70** according to the synthesis method of Example 77. TOF m/z : $[M+Na]^+$: 3020.4.

Example 86 Synthesis of compound **L-20**



Compound **L-20** was synthesized from compound **S63** and compound **S70** according to the synthesis method of Example 77. TOF m/z : $[M+Na]^+$: 3020.4.

Example 87 Synthesis of compound **S76**



Step 1: Resin loading

3.7 g of 2-chlorotrityl chloride resin beads (1.05 mmol/g) were weighed and placed into a resin reactor and swollen in DMF for 1 h. After swelling, the solvent was filtered off, and a solution (30 mL) of Fmoc-L-phenylalanine (1.5 g, 3.9 mmol, 1.0 eq) and DIEA (1.3 mL, 7.7 mmol, 2.0 eq) in DMF was added to the resin, and the resin reactor was shaken at room temperature for 4 h. After filtration, the resin was blocked with MeOH/DIEA/DMF (5 mL/2.5 mL/25 mL) for 30 min, and

then the resin was thoroughly washed with DMF (8 times) to give compound **S71**.

Step 2: Extension of peptide chains

The resin was treated with piperidine/DMF (6 mL/24 mL) twice, 20 min each time, and then thoroughly washed with DMF (8 times). A solution (30 mL) of Fmoc-glycyl-glycine (4.1 g, 11.6 mmol, 3.0 eq), HATU (4.4 g, 11.6 mmol, 3.0 eq), HOBT (1.6 g, 11.6 mmol, 3.0 eq) and DIEA (3.4 mL, 19.4 mmol, 5.0 eq) in DMF was added to the resin, and the resin reactor was shaken at room temperature for 2 h. The resin was then washed with DMF (8 times) to give compound **S72**.

The resin was treated with piperidine/DMF (6 mL/24 mL) twice, 20 min each time, and then thoroughly washed with DMF (8 times). A solution (30 mL) of Fmoc-cysteic acid (4.5 g, 11.6 mmol, 3.0 eq), HATU (4.4 g, 11.6 mmol, 3.0 eq), HOBT (1.6 g, 11.6 mmol, 3.0 eq) and DIEA (3.4 mL, 19.4 mmol, 5.0 eq) in DMF was added to the resin, and the resin reactor was shaken at room temperature for 4 h. The resin was then washed with DMF (8 times) to give compound **S73**.

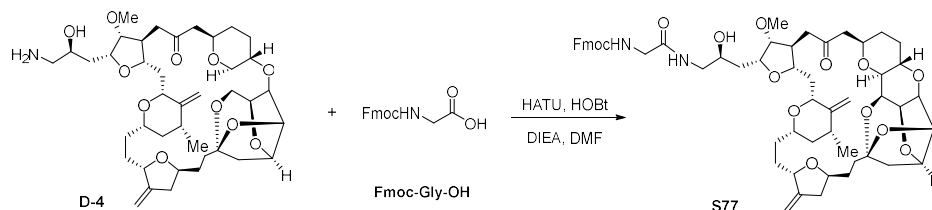
The resin was treated with piperidine/DMF (6 mL/24 mL) twice, 20 min each time, and then thoroughly washed with DMF (8 times). A solution (30 mL) of compound **S37** (4.1 g, 11.6 mmol, 3.0 eq), HATU (4.4 g, 11.6 mmol, 3.0 eq), HOBT (1.6 g, 11.6 mmol, 3.0 eq) and DIEA (3.4 mL, 19.4 mmol, 5.0 eq) in DMF was added to the resin, and the resin reactor was shaken at room temperature for 4 h. The resin was then washed with DMF (8 times) to give compound **S74**.

The resin was treated with piperidine/DMF (6 mL/24 mL) twice, 20 min each time, and then thoroughly washed with DMF (8 times). A solution (30 mL) of compound **S8** (4.6 g, 11.6 mmol, 3.0 eq) and EEDQ (2.9 mL, 11.6 mmol, 3.0 eq) in DMF was added to the resin, and the resin reactor was shaken at room temperature for 4 h. The resin was then washed with DMF (8 times) to give compound **S75**.

Step 3: Resin cleavage

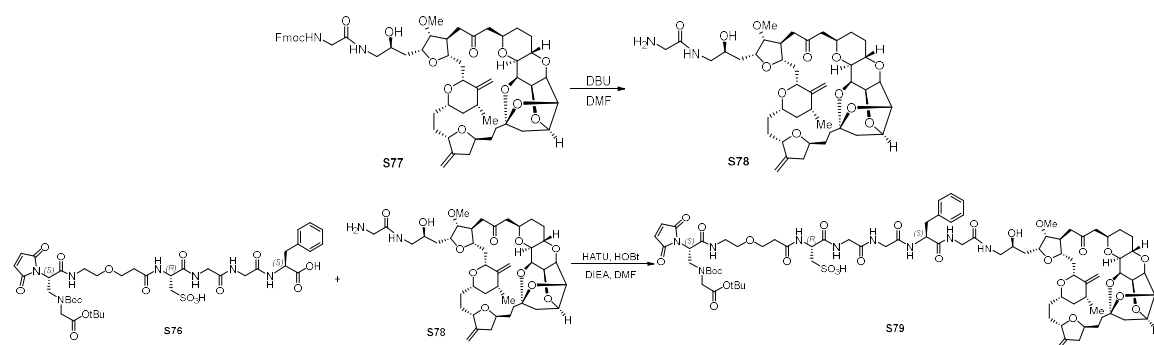
The resin with an HFIP/CH₂Cl₂ (6 mL/24 mL) solution for 30 min. The resin was filtered, the filtrate was concentrated under reduced pressure, and the crude product was purified by reverse-phase preparative column chromatography, and the preparation was lyophilized to give compound **S76** (white solid, 836 mg, 23%). LC-MS *m/z*(ES⁻): [M-H]⁻: 924.2.

Example 88 Synthesis of compound **S77**



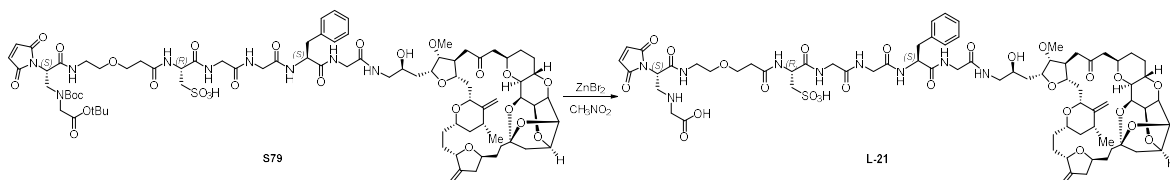
In a 25 mL single-neck flask, compound **D-4** (50 mg, 0.069 mmol, 1.0 eq), Fmoc-glycine (31 mg, 0.103 mmol, 1.5 eq), HATU (39 mg, 0.103 mmol, 1.5 eq), HOBT (14 mg, 0.103 mmol, 1.5 eq) and dried DMF (3 mL) were added sequentially and dissolved uniformly, then DIEA (24 μ L, 0.137 mmol, 2.0 eq) was added dropwise in condition of an ice water bath, and the reaction was allowed at room temperature. After 1 h, HPLC showed the reaction was complete. The reaction solution was purified by reverse-phase preparative column chromatography, and the preparation solution was lyophilized to give compound **S77** (white solid, 58 mg, 84%). LC-MS $m/z(ES^+)$: $[M+H]^+$: 1009.2.

Example 89 Synthesis of compound **S79**



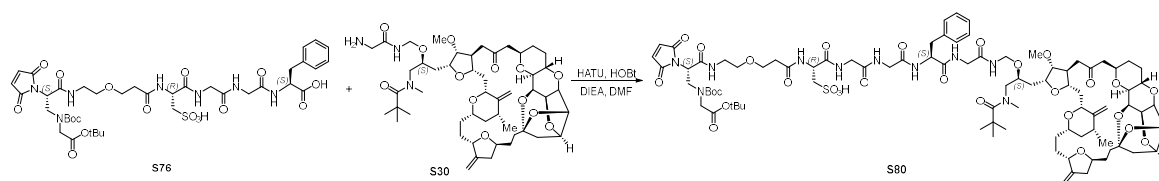
Compound **S79** was synthesized from compound **S77** and compound **S76** according to the synthesis method of Example 26 and Example 27. LC-MS $m/z(ES^+)$: $[M-100+2H]^{2+}$: 797.9 (fragment ion response of compound **S79** after removing the Boc protecting group and adding hydrogen ion).

Example 90 Synthesis of compound **L-21**



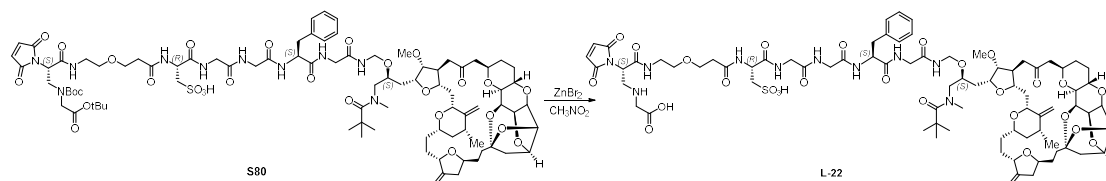
Compound **L-21** was synthesized from compound **S79** according to the synthesis method of Example 28. LC-MS $m/z(ES^+)$: $[M+2H]^{2+}$: 769.8.

Example 91 Synthesis of compound **S80**



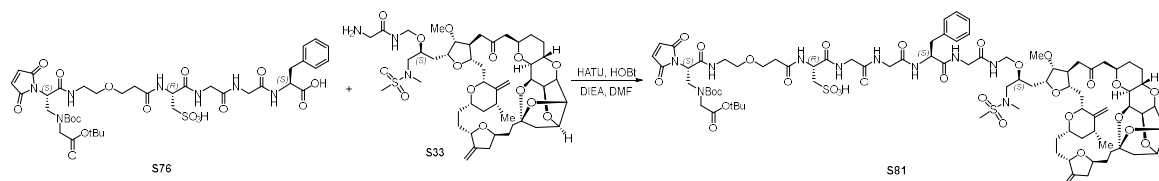
Compound **S80** was synthesized from compound **S76** and compound **S30** according to the synthesis method of Example 27. LC-MS $m/z(ES^+)$: $[M+2H]^{2+}$: 911.5.

Example 92 Synthesis of compound **L-22**



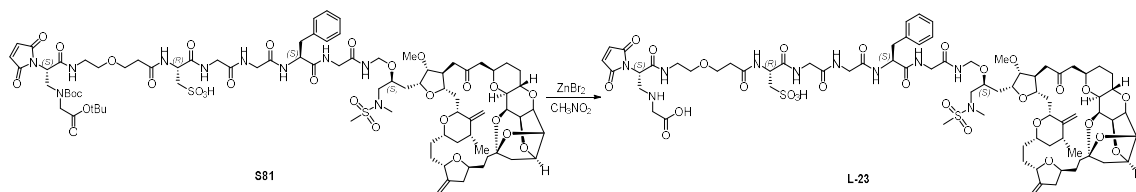
Compound **L-22** was synthesized from compound **S80** according to the synthesis method of Example 28. LC-MS $m/z(ES^+)$: $[M+2H]^{2+}$: 833.4.

Example 93 Synthesis of compound **S81**



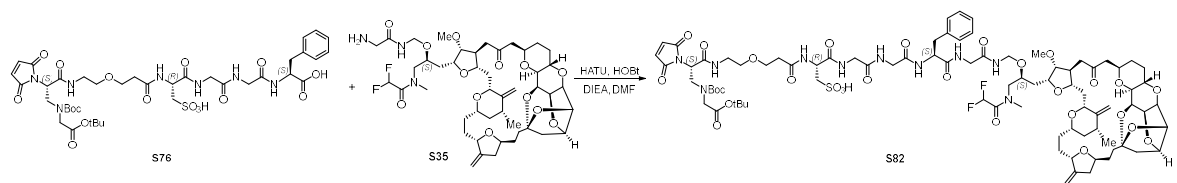
Compound **S81** was synthesized from compound **S76** and compound **S33** according to the synthesis method of Example 27. LC-MS $m/z(ES^+)$: $[M+2H]^{2+}$: 908.4.

Example 94 Synthesis of compound **L-23**



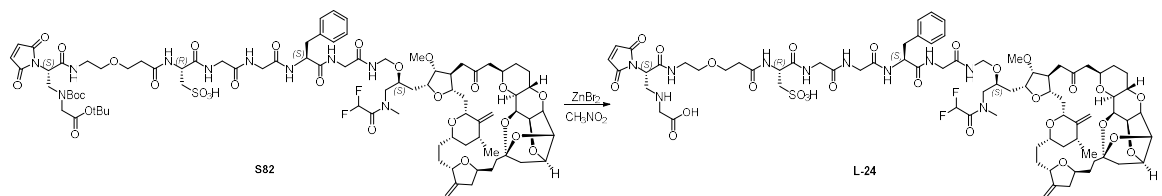
Compound **L-23** was synthesized from compound **S81** according to the synthesis method of Example 28. LC-MS $m/z(ES^+)$: $[M+2H]^{2+}$: 830.3.

Example 95 Synthesis of compound **S82**



Compound **S82** was synthesized from compound **S76** and compound **S35** according to the synthesis method of Example 27. LC-MS $m/z(ES^+)$: $[M+2H]^{2+}$: 908.3.

Example 96 Synthesis of compound **L-24**



Compound **L-24** was synthesized from compound **S82** according to the synthesis method of Example 28. LC-MS $m/z(ES^+)$: $[M+2H]^{2+}$: 830.3.

Example 97 Preparation of **ADC-1**

The **ADC-1** was prepared by using compound **L-1** and anti-Trop2 antibody TR001 according to the method in general procedure A. The sequence information of TR001 is shown in Table 2.

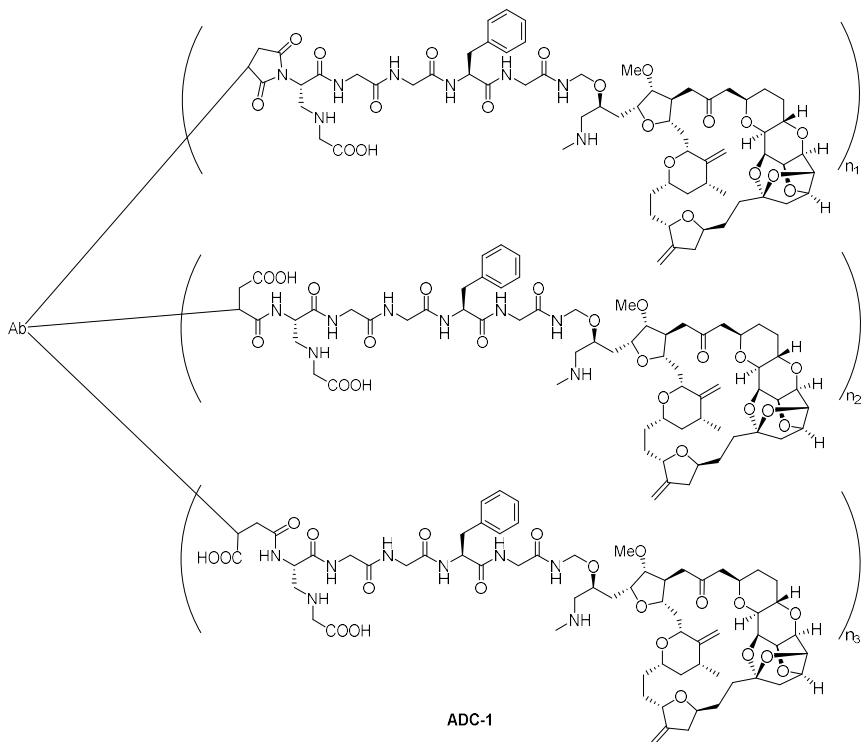
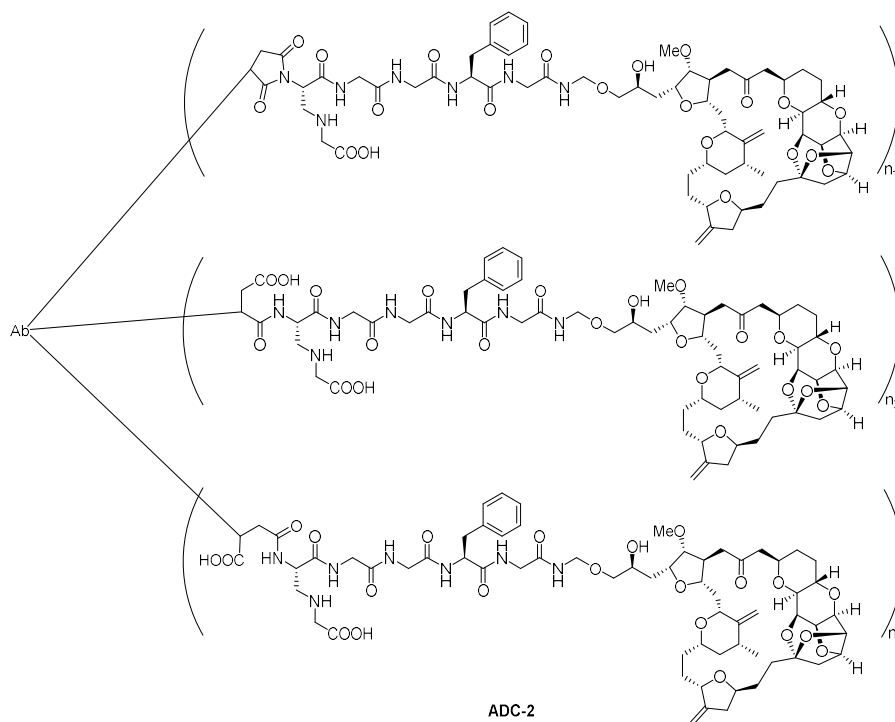


Table 2: Description of sequence of TR001

Amino acid sequence of the light chain of TR001	DIQMTQSPSSLSASVGDRVITITCRASQDINKYLAWYQQKPGKVPKLLIYSTSTLQSGVPSRFSGSGSGTDFTLTISLQPEDVATYYCLQYDDLFTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNMFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
Amino acid sequence of the heavy chain of TR001	QVQLVQSGAEVKKPGASVKLSCKASGYTFTSFDINWVRQAPEQRLEWMGWIFPGDGNTKYSQKFQGRATITRDTSASTAYMELSSLRSEDTAVYYCVRGEALYYFDYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNATKTPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTKSKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSMHEALHNHYTQKSLSLSPG

Example 98 Preparation of ADC-2

The **ADC-2** was prepared by using compound **L-2** and anti-Trop2 antibody TR001 according to the method in general procedure A.



Example 99 Preparation of ADC-3

The **ADC-3** was prepared by using compound **L-1** and anti-Trop2 antibody TR002 according to the method in general procedure B. The sequence information of TR002 is shown in Table 3.

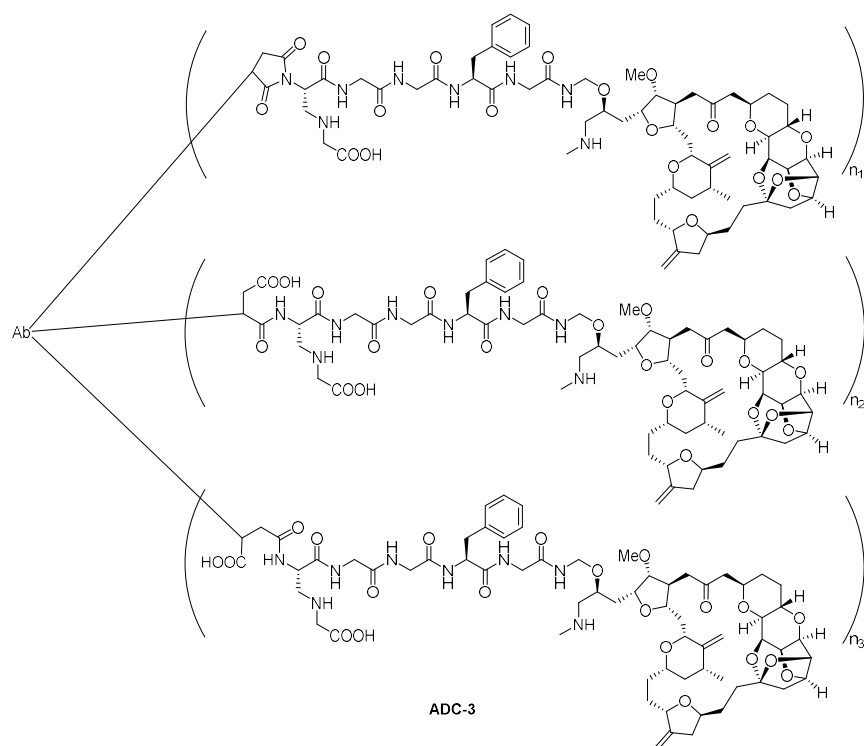
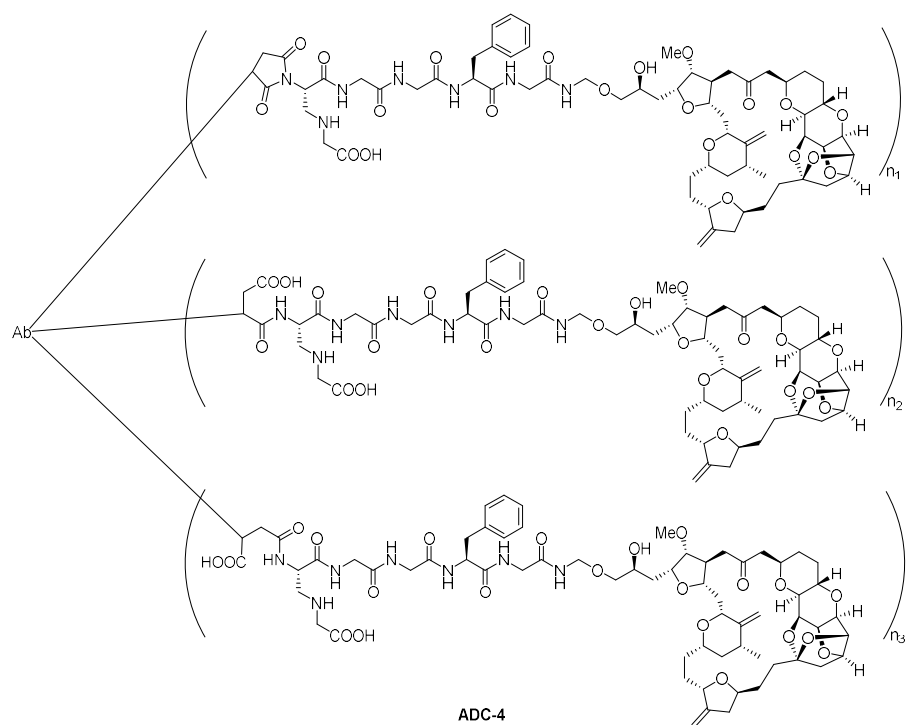


Table 3: Description of sequence of TR002

Amino acid sequence of the light chain of TR002	DIQMTQSPSSLSASVGDRTITCRASQDINKYLAWYQQKPGKVPKLLI YSTSTLQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCLQYDDLFTFG QGTELEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSSTLSSTLTLSKADYEKHKVYAC EVTHQGLSSPVCTKSFNRGEC
Amino acid sequence of the heavy chain of TR002	QVQLVQSGAEVKKPGASVKLSCKASGYTFTSFDINWVRQAPQRLEW MGWIFPGDGNTKYSQKFQGRATITRDTSASTAYMELSSLRSEDVAVYY CVRGEALYYFDYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSS SLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPPELLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVQLQDWLNKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVDFCSVMH EALHNHYTQKSLSLSPG

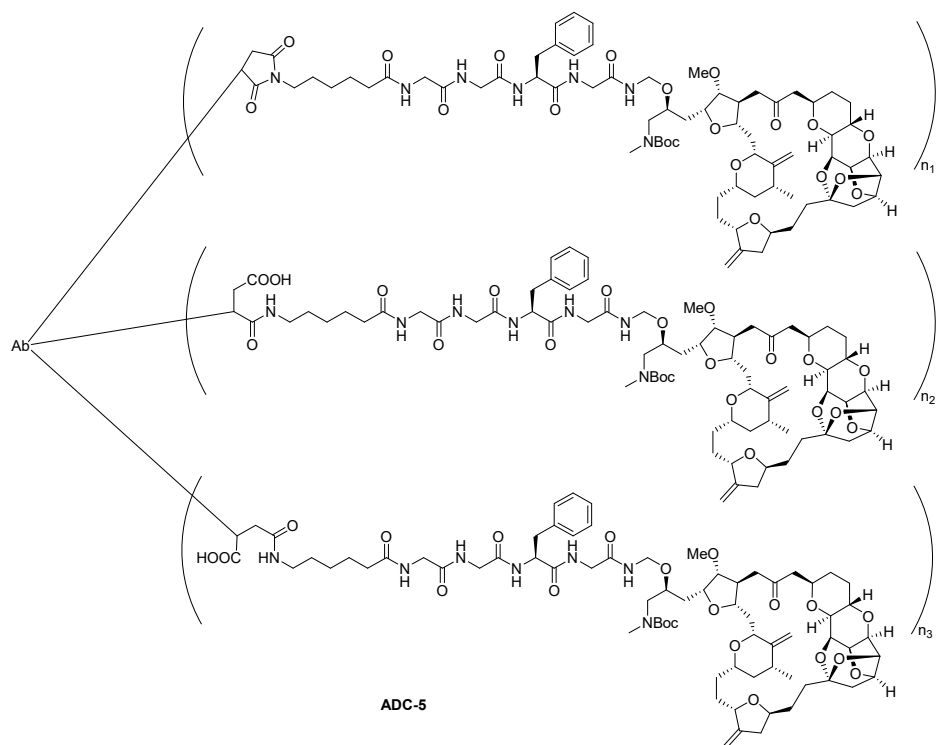
Example 100 Preparation of **ADC-4**

The **ADC-4** was prepared by using compound **L-2** and anti-Trop2 antibody TR002 according to the method in general procedure B.



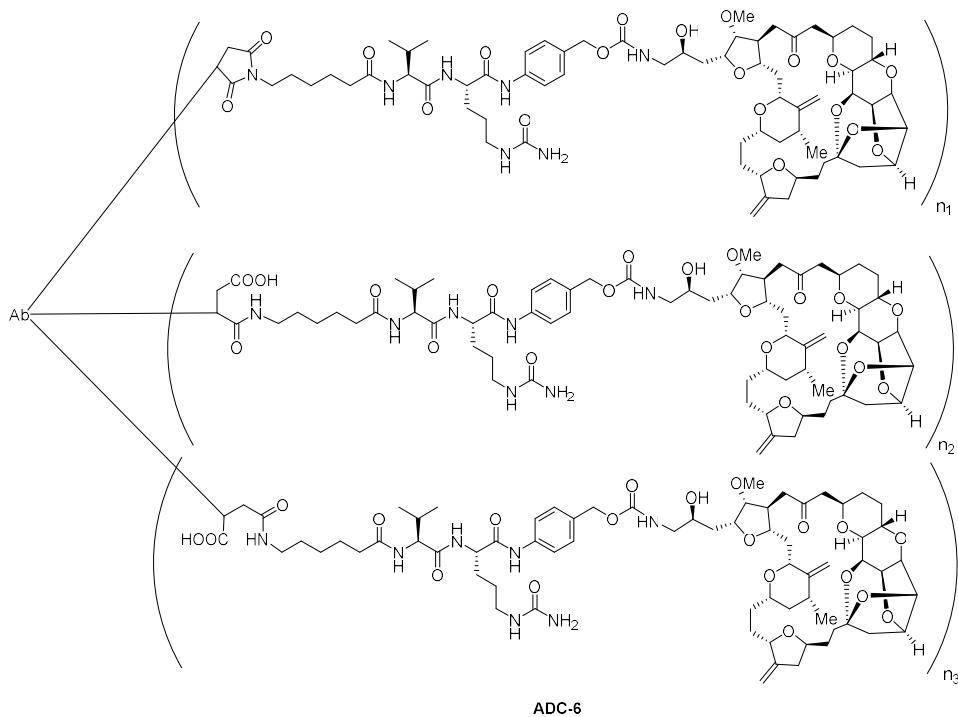
Example 101 Preparation of **ADC-5**

The **ADC-5** was prepared by using compound **L-3** and anti-Trop2 antibody TR001 according to the method in general procedure A.



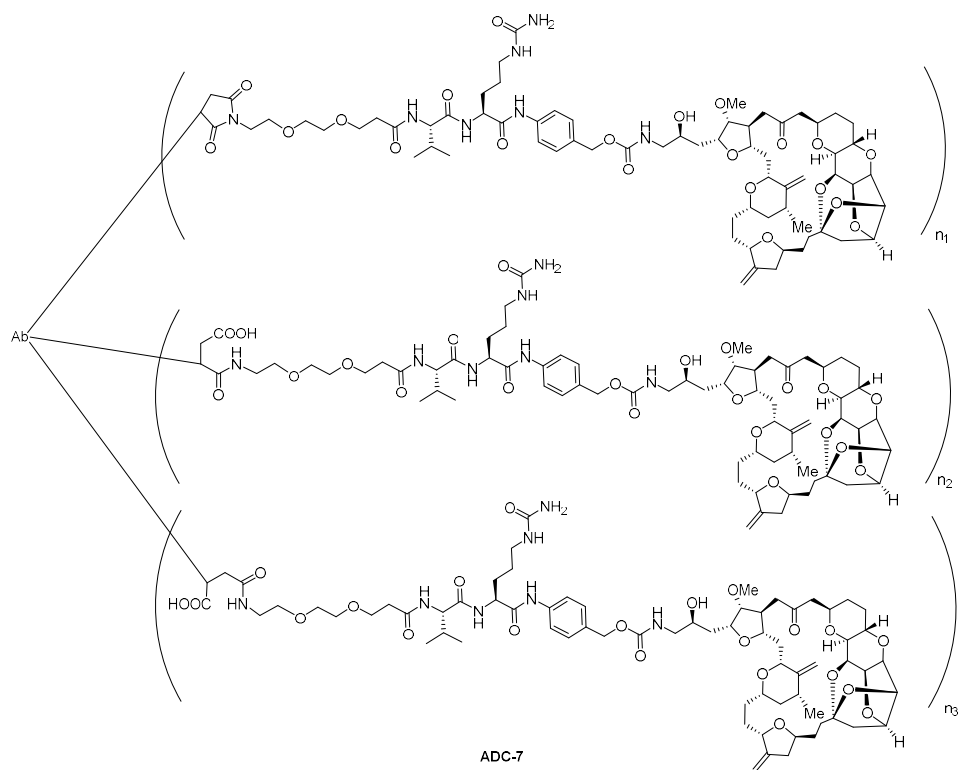
Example 102 Preparation of **ADC-6**

The **ADC-6** was prepared by using compound **L-7** and anti-Trop2 antibody TR001 according to the method in general procedure A.



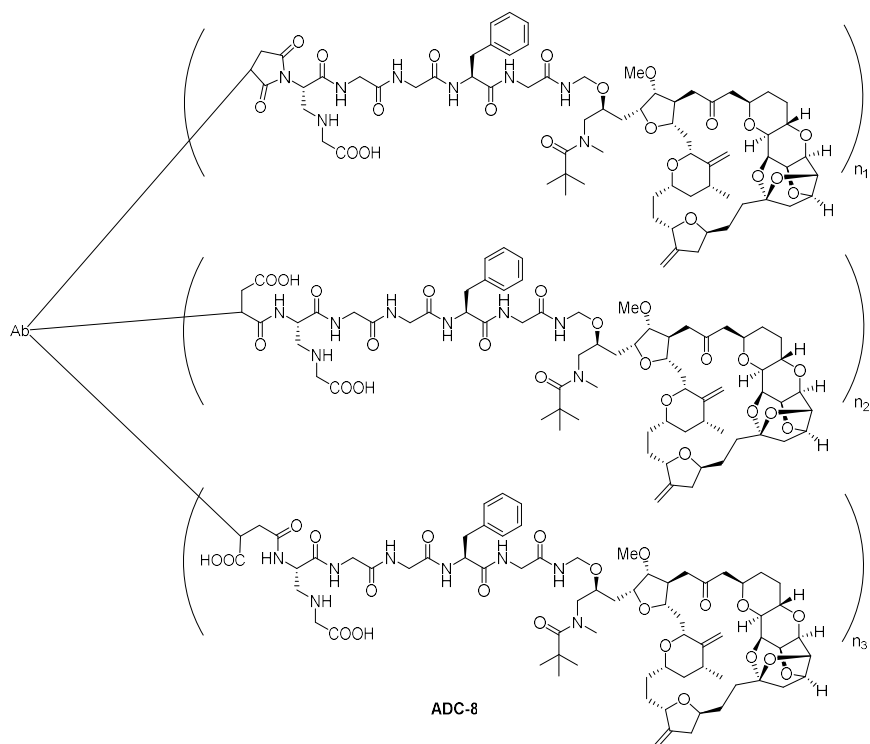
Example 103 Preparation of **ADC-7**

The **ADC-7** was prepared by using compound **L-8** and anti-Trop2 antibody TR001 according to the method in general procedure A.



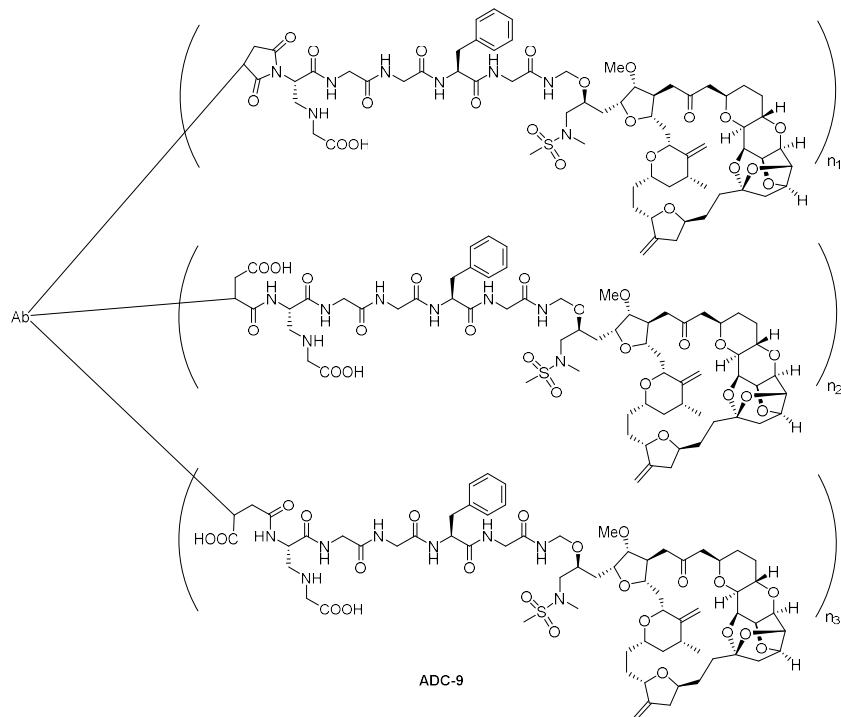
Example 104 Preparation of **ADC-8**

The **ADC-8** was prepared by using compound **L-9** and anti-Trop2 antibody TR001 according to the method in general procedure A.



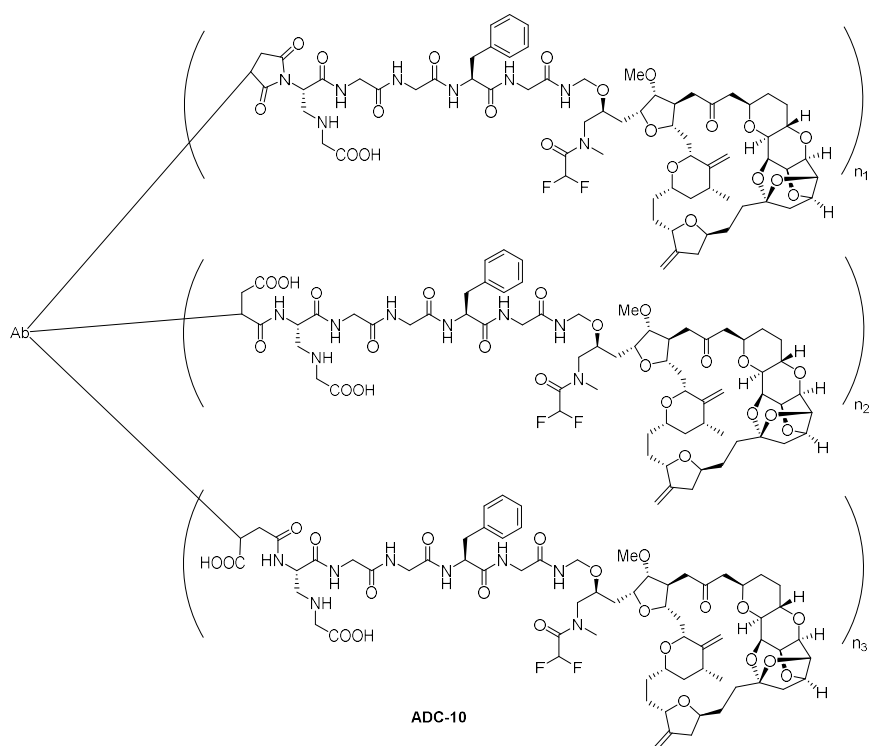
Example 105 Preparation of **ADC-9**

The **ADC-9** was prepared by using compound **L-10** and anti-Trop2 antibody TR001 according to the method in general procedure A.



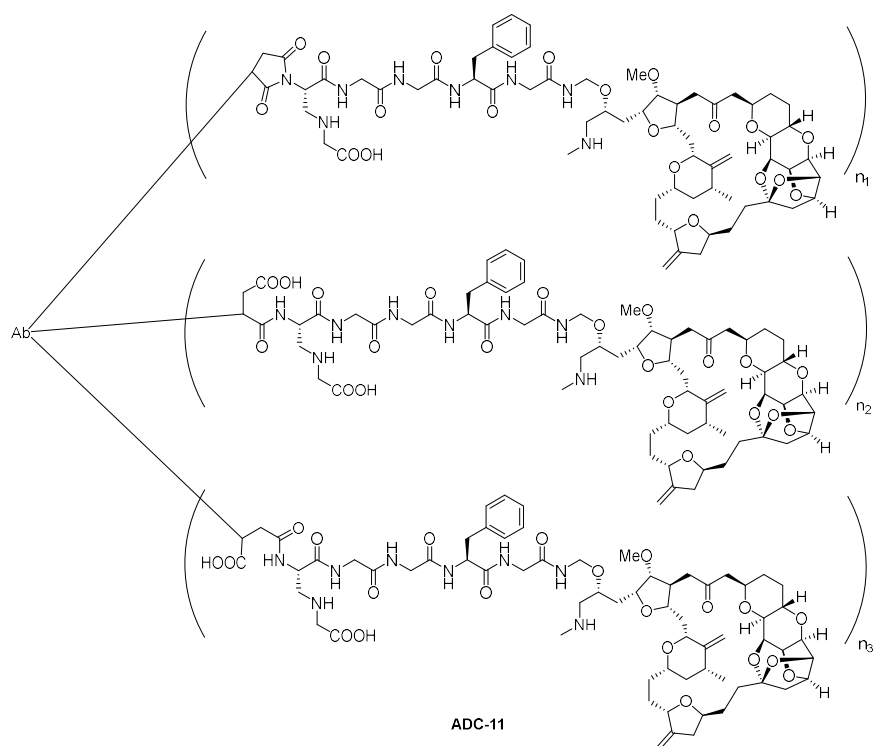
Example 106 Preparation of **ADC-10**

The **ADC-10** was prepared by using compound **L-11** and anti-Trop2 antibody TR001 according to the method in general procedure A.



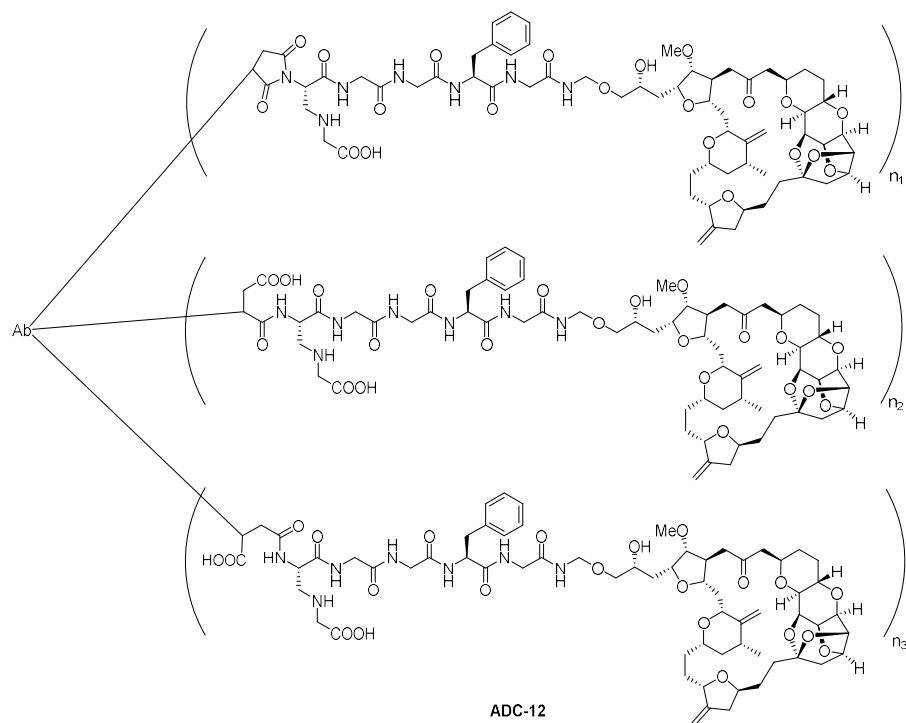
Example 107 Preparation of **ADC-11**

The **ADC-11** was prepared by using compound **L-1'** and anti-Trop2 antibody TR001 according to the method in general procedure A.



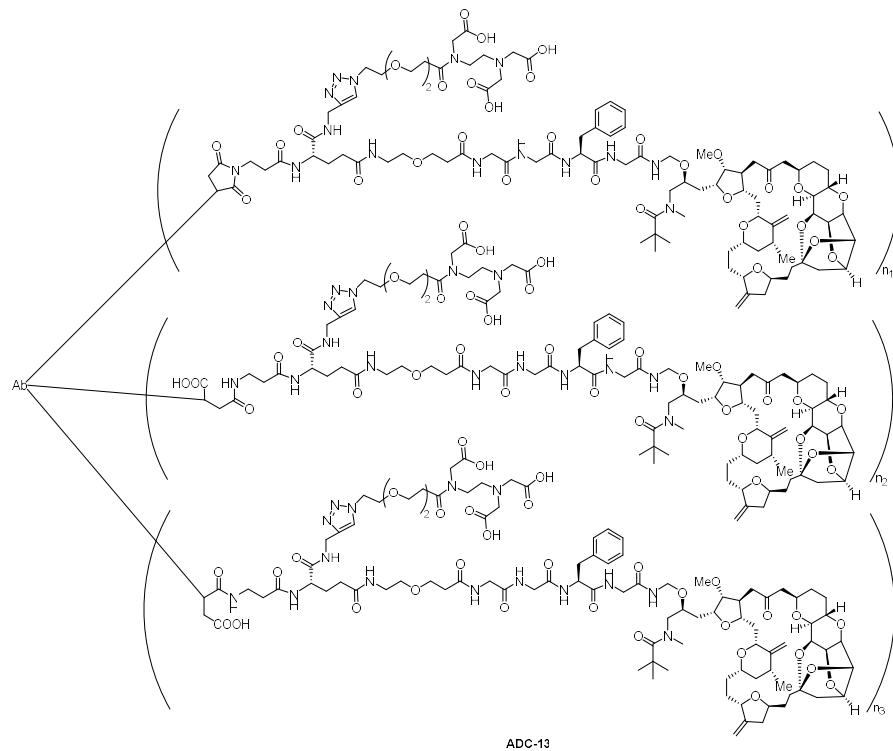
Example 108 Preparation of ADC-12

The ADC-12 was prepared by using compound L-2' and anti-Trop2 antibody TR001 according to the method in general procedure A.



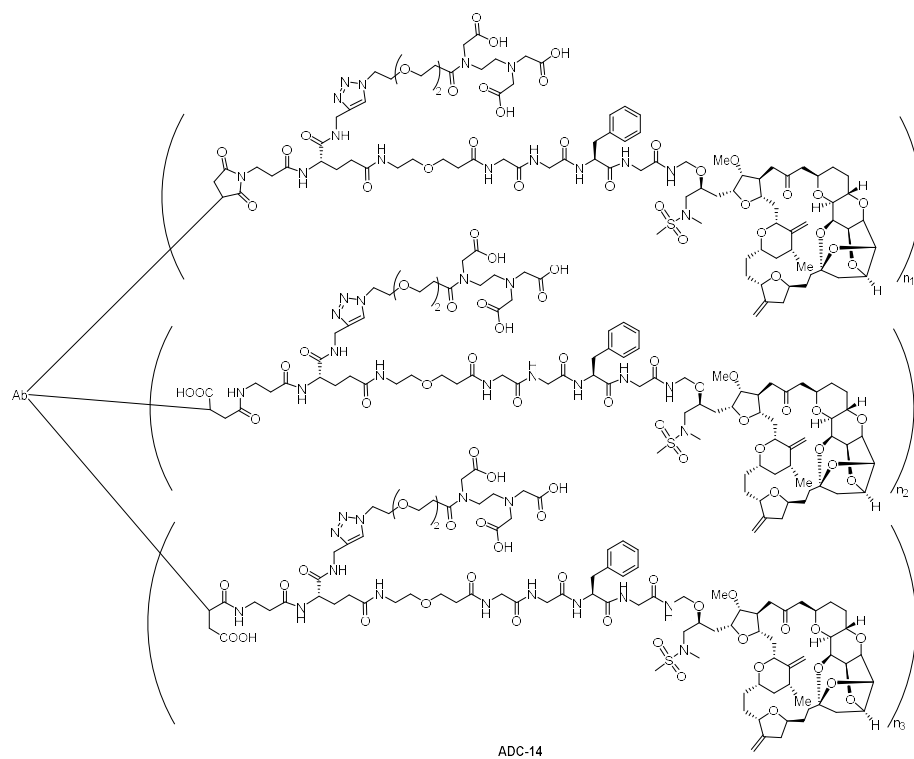
Example 109 Preparation of **ADC-13**

The **ADC-13** was prepared by using compound **L-15** and anti-Trop2 antibody TR001 according to the method in general procedure C.



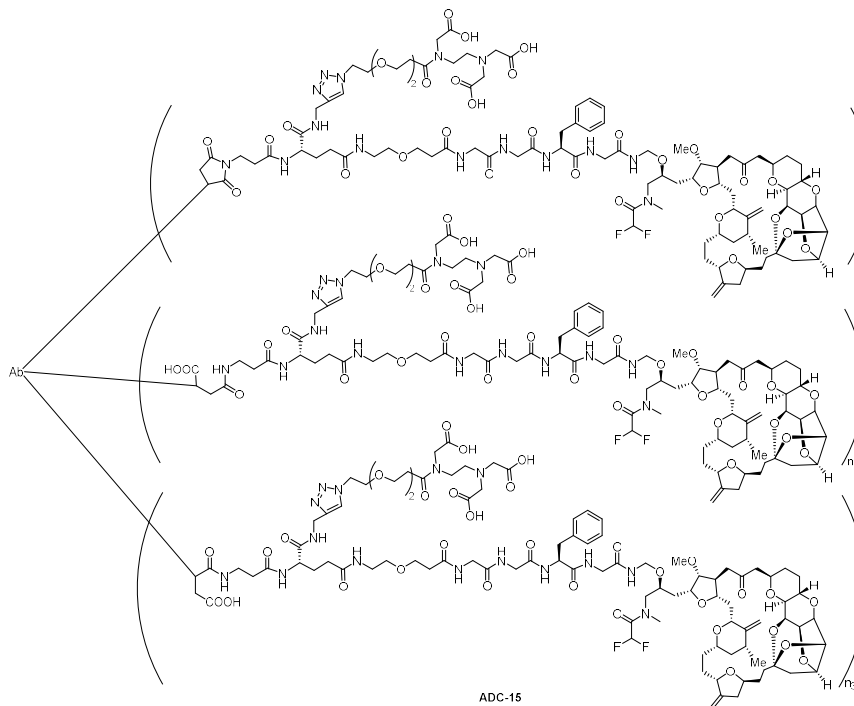
Example 110 Preparation of **ADC-14**

The **ADC-14** was prepared by using compound **L-16** and anti-Trop2 antibody TR001 according to the method in general procedure C.



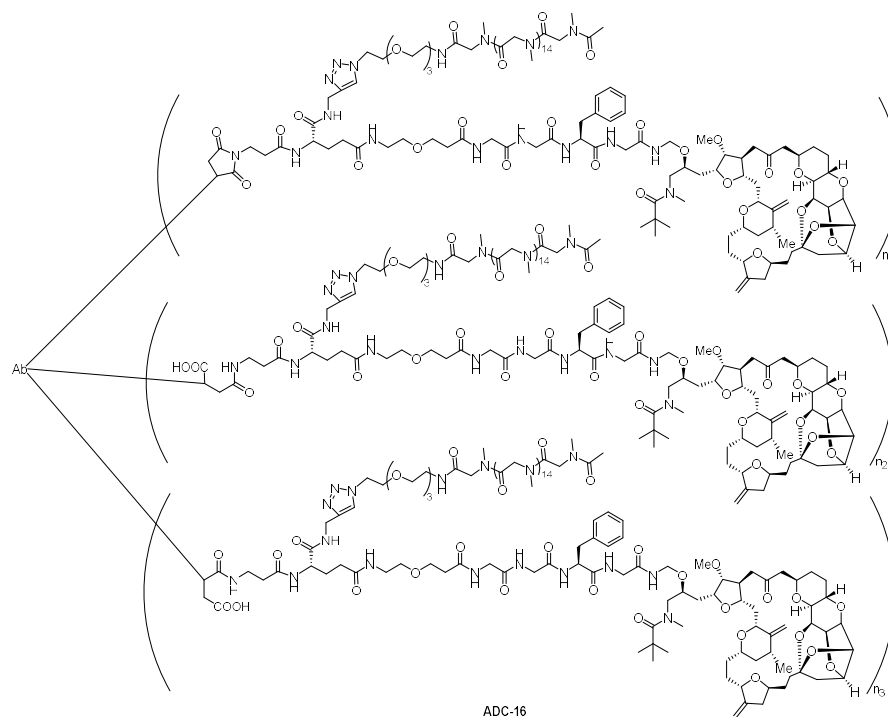
Example 111 Preparation of **ADC-15**

The **ADC-15** was prepared by using compound **L-17** and anti-Trop2 antibody TR001 according to the method in general procedure C.



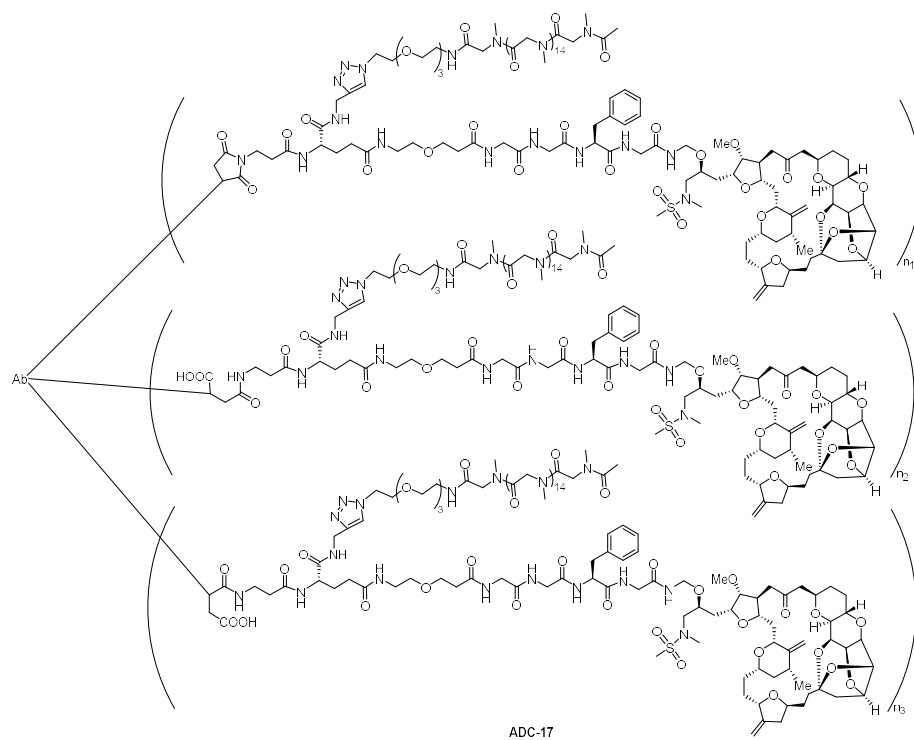
Example 112 Preparation of **ADC-16**

The **ADC-16** was prepared by using compound **L-18** and anti-Trop2 antibody TR001 according to the method in general procedure C.



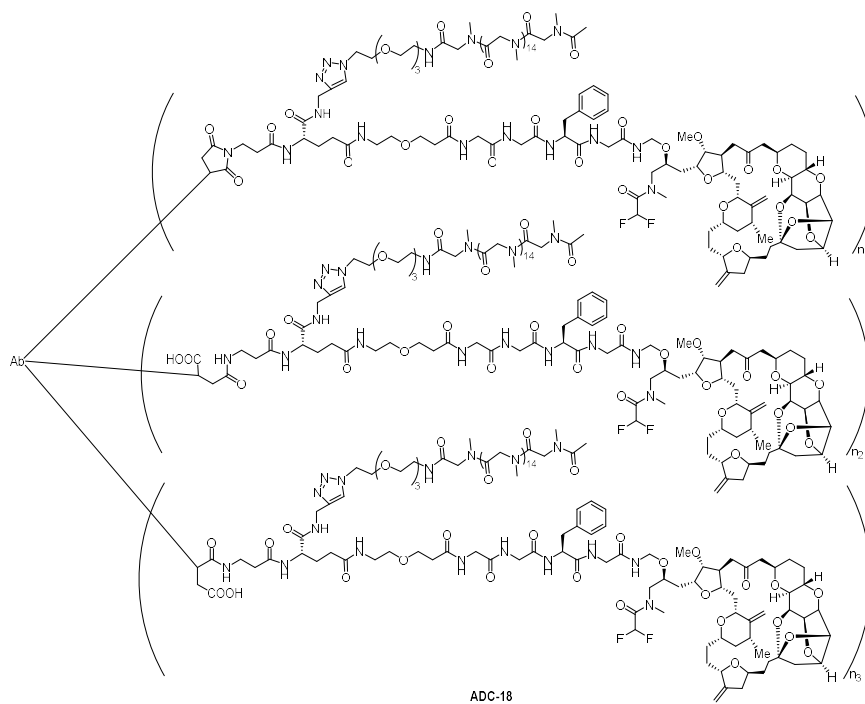
Example 113 Preparation of **ADC-17**

The **ADC-17** was prepared by using compound **L-19** and anti-Trop2 antibody TR001 according to the method in general procedure C.



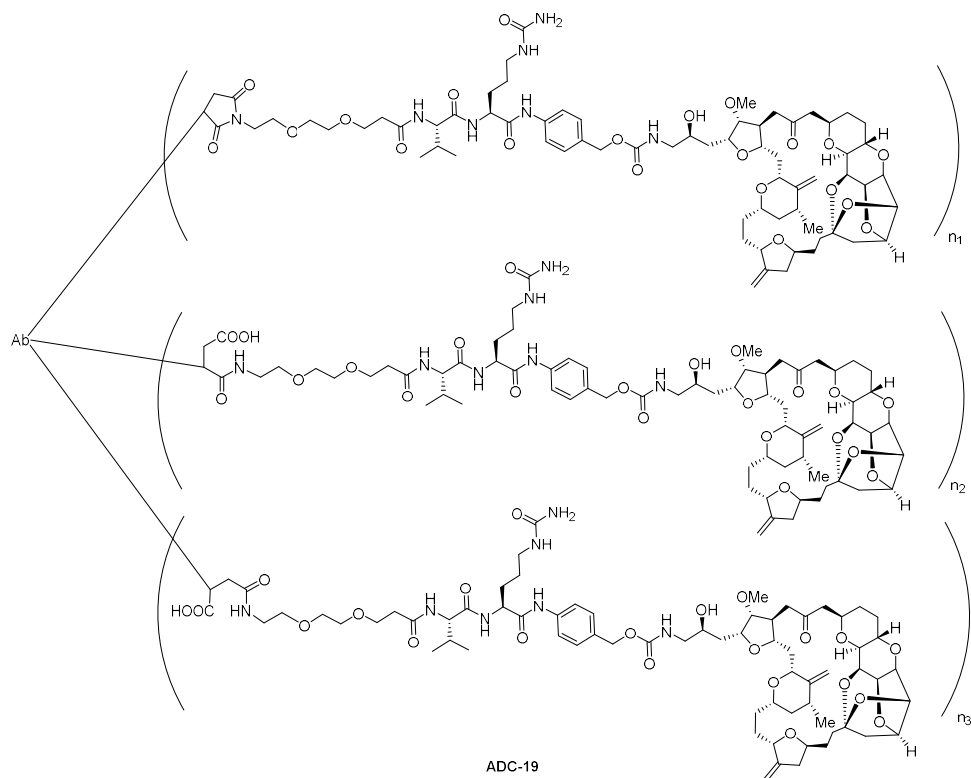
Example 114 Preparation of **ADC-18**

The **ADC-18** was prepared by using compound **L-20** and anti-Trop2 antibody TR001 according to the method in general procedure C.



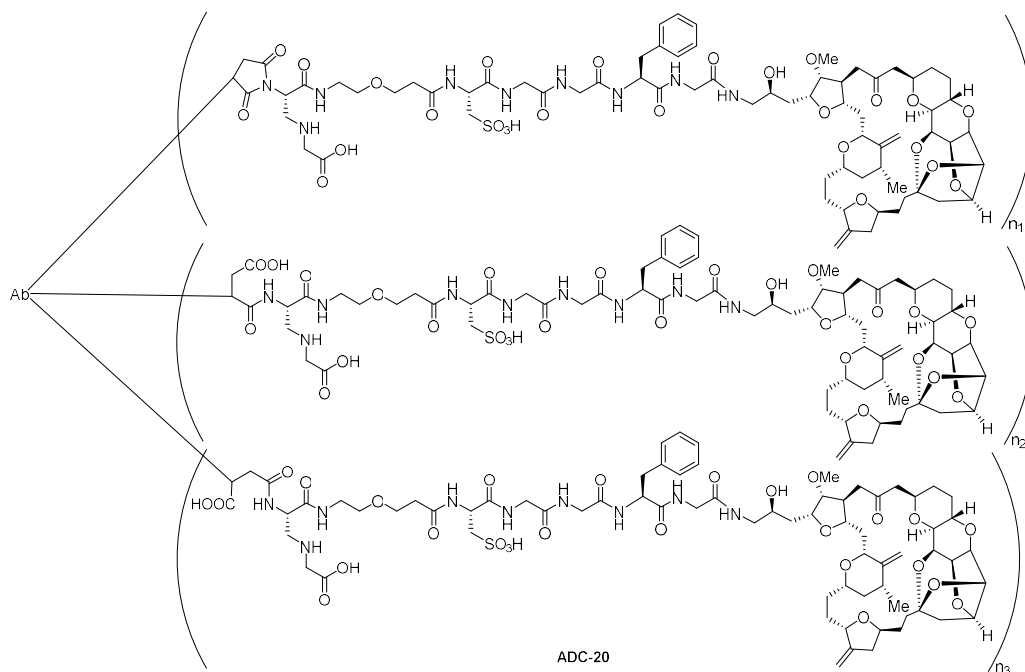
Example 115 Preparation of **ADC-19**

The **ADC-19** was prepared by using compound **L-8** and anti-Trop2 antibody TR001 according to the method in general procedure C.



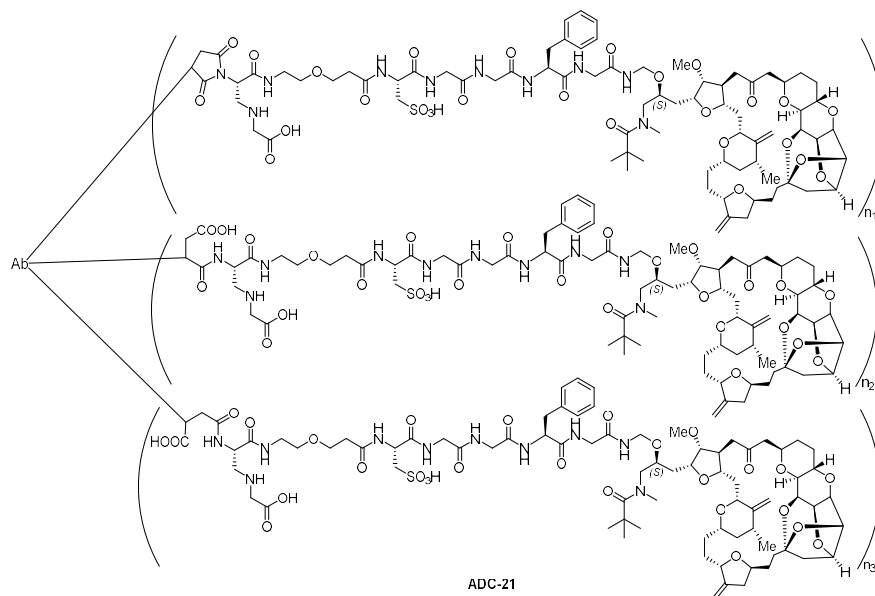
Example 116 Preparation of **ADC-20**

The **ADC-20** was prepared by using compound **L-21** and anti-Trop2 antibody TR001 according to the method in general procedure C.



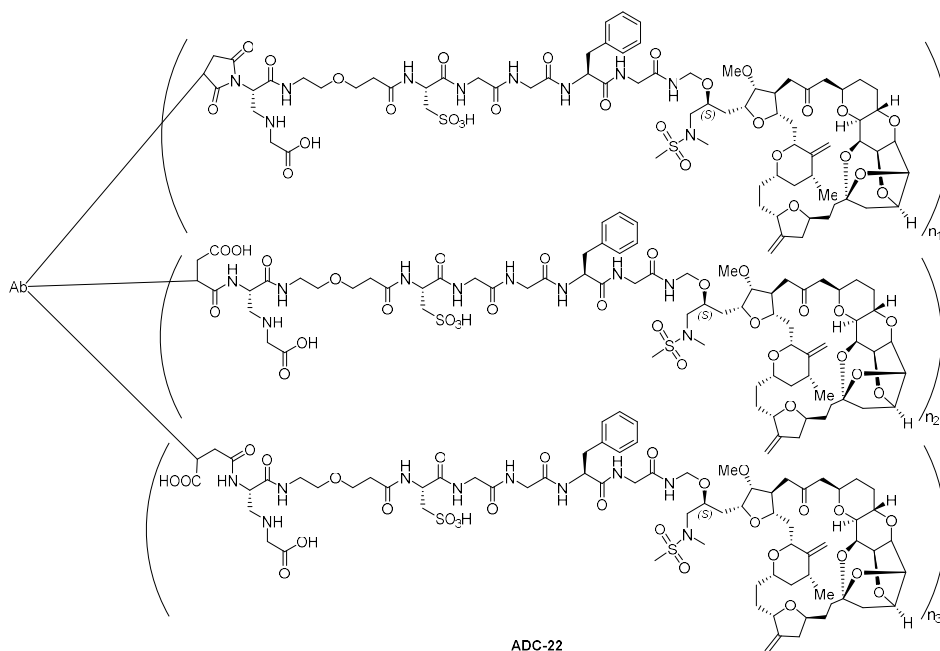
Example 117 Preparation of **ADC-21**

The **ADC-21** was prepared by using compound **L-22** and anti-Trop2 antibody TR001 according to the method in general procedure C.



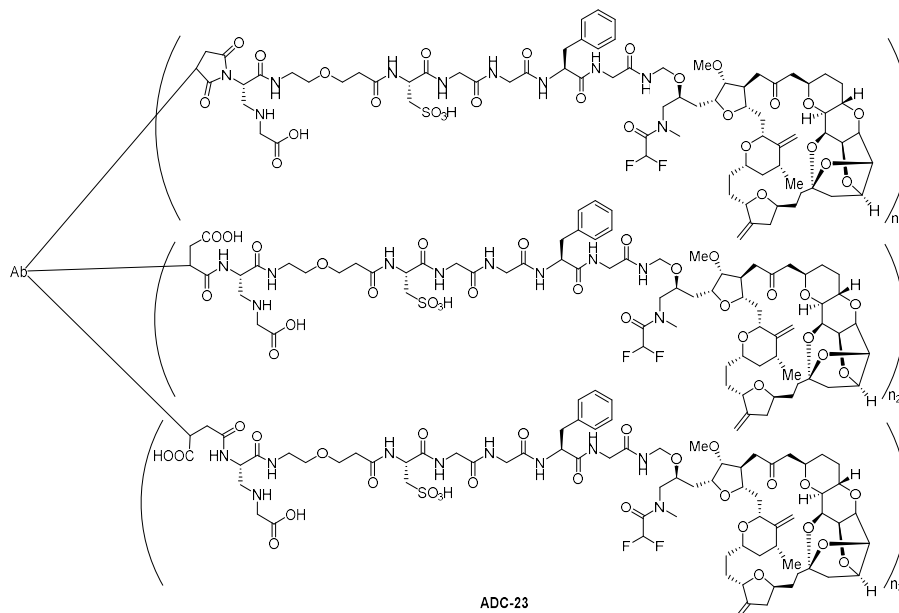
Example 118 Preparation of **ADC-22**

The **ADC-22** was prepared by using compound **L-23** and anti-Trop2 antibody TR001 according to the method in general procedure C.



Example 119 Preparation of **ADC-23**

The **ADC-23** was prepared by using compound **L-24** and anti-Trop2 antibody TR001 according to the method in general procedure C.



Example 120 Determination of drug-antibody ratio (DAR)

Reversed-phase high performance liquid chromatography (RP-HPLC) for detecting DAR

Preparation of RP-HPLC mobile phase:

RP mobile phase A: 0.1% TFA in water; RP mobile phase B: 0.1% TFA in acetonitrile

The sample to be tested and the corresponding antibody control were diluted with sample diluent to 1 mg/mL; 2 μ L of DTT stock solution was added to every 98 μ L of diluted sample, and a blank control of 98 μ L of sample diluent + 2 μ L of DTT stock solution was prepared. Each sample was mixed well and then heated in a 65 °C metal bath for 30 min. The processed samples were centrifuged at 14000 rpm for 5 min or filtered with a 0.22 μ m filter to remove large particles from the samples, and an inner cannula was placed in each sample bottle and the sample bottles were then capped.

The sample bottles filled with samples were placed on a sample plate, and the corresponding position, injection volume, number of injection needles and injection method for each sample were set in accordance with UPLC Standard Operating Procedures. The chromatographic column model was Proteomix RP-1000 (4.6*100 mm, 5 μ m), Sepax.

The test wavelengths were 214 nm and 280 nm. The sample running method was edited as follows:

Time (min)	Flow rate (mL/min)	Volume percentage of RP mobile phase A (%):	Volume percentage of RP mobile phase B (%):
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

Table 4: Detailed data of DAR of ligand-drug conjugates (ADCs) disclosed:

Number of compound	Number of ADC prepared by conjugation	DAR
L-1	ADC-1	6.82
L-2	ADC-2	6.08
L-1	ADC-3	1.87
L-2	ADC-4	1.72
L-3	ADC-5	7.20
L-7	ADC-6	6.81
L-8	ADC-7	7.63
L-9	ADC-8	6.97
L-10	ADC-9	7.15
L-11	ADC-10	7.86
L-1'	ADC-11	7.58
L-2'	ADC-12	7.30
L-15	ADC-13	4.07
L-16	ADC-14	4.35
L-17	ADC-15	4.12
L-18	ADC-16	3.94
L-19	ADC-17	3.88
L-20	ADC-18	3.74
L-8	ADC-19	3.75
L-21	ADC-20	3.63
L-22	ADC-21	3.71
L-23	ADC-22	3.84

L-24	ADC-23	3.96
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Example 121 Screening of compounds for in vitro pharmacodynamic activity

In the present invention, various human-derived tumor cell lines (BxPC-3 (human orthotopic pancreatic adenocarcinoma cell), SW620 (human colon cancer cell), H1975 (human lung adenocarcinoma cell), HCC827 (human lung adenocarcinoma cell), FaDu (human pharyngeal squamous cell carcinoma cell) and A431(human epidermal carcinoma cells)) were used as experimental models to evaluate the cytotoxicity of compound to tumor cell lines. A certain number of tumor cells were seeded into a 96-well plate. After the cells adhered to the wall, gradient dilutions of tested compounds were added to the test cells, followed by incubation at 37 °C and 5% CO₂ for 5 days. Cell viability was measured using MTS, and the inhibitory effect of the control compounds and the tested compounds on the tumor cell lines was evaluated by IC₅₀. The compound with a starting concentration of 4000 nM was diluted 7 folds, with a total of 8 concentration points. Finally, the survival rate was given by the equation: survival rate = (experimental group - blank) / (control - blank) × 100%, and then the curve was fitted using the Graph Pad Prism 9 four-parameter model to calculate IC₅₀.

Table 5: In vitro pharmacodynamic activity of control compounds and tested compounds in human-derived tumor cell lines BxPC-3, SW620 and H1975:

Compound	BxPC-3	SW620	H1975
	IC ₅₀ (nM)		
D-4(eribulin)	0.06	2.56	0.39
D-2	0.03	1.25	0.25
D-3	<0.005	<0.005	<0.005
D-7	<0.005	<0.005	0.01
D-8	<0.005	<0.005	<0.005
D-9	<0.005	<0.005	<0.005
D-10	0.02	0.2	0.13
D-11	0.01	0.15	0.06
D-12	<0.005	<0.005	<0.005
D-13	<0.005	<0.005	<0.005
D-14	0.01	0.19	0.1
D-15	0.03	0.69	0.14
D-21	0.05	0.24	0.24

Table 6: In vitro pharmacodynamic activity of control compounds and tested compounds in human-derived tumor cell lines HCC827, FaDu and A431:

Compound	HCC827	FaDu	A431
	IC ₅₀ (nM)		
D-4(eribulin)	0.05	0.73	0.03
D-2	0.025	0.37	0.02
D-3	<0.005	<0.005	<0.005
D-7	<0.005	0.01	<0.005

D-8	<0.005	<0.005	<0.005
D-9	<0.005	<0.005	<0.005
D-10	0.01	0.16	0.03
D-11	0.01	0.08	0.02
D-12	<0.005	<0.005	<0.005
D-13	<0.005	<0.005	<0.005
D-14	0.01	0.16	0.01
D-15	0.02	0.33	0.02
D-21	0.04	0.39	0.12

The results are shown in the table above and FIGs. 1A-1F.

Conclusion: In BxPC-3, SW620, H1975, HCC827, FaDu and A431 cell models, various compounds synthesized herein had obvious anti-tumor effects, and compounds D-3, D-7, D-8, D-9, D-12, and D-13 showed the best effects.

Example 122 Evaluation on stability of ADCs

ADCs were diluted with sterile human plasma to prepare samples with a drug concentration of 0.6 mg/ml and incubated in a 37 °C water bath for 0, 3 and 7 days respectively. The samples incubated in plasma for 0, 3 and 7 days and the samples serving as controls, diluted with PBS with a final drug concentration of 0.6 mg/ml were purified and extracted respectively to give ADCs. Finally, the purified and extracted ADCs were tested accordingly to analyze their stability in plasma.

Determination of monomer rate using SEC-HPLC:

Chromatographic column: Biocore SEC-300 5 µm, 4.6×300 mm

Manufacturer: NanoChrom, Product code: B213-050030-04630S

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

Method parameters are listed below:

Parameter	Setting
Flow rate	0.3 mL/min
Wavelength	280 nm
Column temperature	30 °C
Sample tray temperature	Room temperature
Sample load	20 ug
Max. pressure	150 bar/15 MPa/2175 PSI
Gradient	Isocratic
Run time	20 min

Table 7: Evaluation on stability of tested ADCs of the invention in plasma:

	Day 0	Day 1	Day 5
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Sample	Aggregation %	Monomer %	Degradation rate %	RP-DAR	Aggregation %	Monomer %	Degradation rate %	RP-DAR	Aggregation %	Monomer %	Degradation rate %	RP-DAR
TR001	0.51	98.78	0.71	N/A	0.45	99.29	0.26	N/A	0.4	99.33	0.27	N/A
ADC-13	9.62	88.81	1.58	4.07	5.83	93.34	0.83	3.88	5.28	93.72	1.00	3.80
ADC-14	4.78	93.91	1.31	4.35	4.78	94.54	0.69	4.19	4.28	94.89	0.83	4.02
ADC-15	7.50	91.02	1.48	4.12	6.47	92.39	1.14	3.73	5.81	92.91	1.27	3.51
ADC-16	4.94	94.56	0.50	3.94	4.79	94.45	0.76	4.15	4.61	94.52	0.87	4.06
ADC-17	6.41	92.88	0.71	3.88	6.46	92.42	1.12	4.15	6.29	92.45	1.26	3.75
ADC-18	4.44	95.23	0.33	3.74	4.21	95.27	0.52	3.88	4.07	95.33	0.59	3.73

Conclusion: The above results show that the ADCs synthesized in the present invention have good stability in human plasma.

Example 123 In vitro pharmacodynamic activity testing of ADCs-single tumor model

In the present invention, various human-derived tumor cell lines (A431, HCC827, N87 (human gastric cancer cells), SW620 and FaDu) were used as experimental models to evaluate the in vitro pharmacodynamic activity of the ADCs. A certain number of tumor cells were seeded into a 96-well plate, gradient dilutions of test antibodies and corresponding ADCs were added to the cells, and treated for 5 days. Cell viability was detected using MTS, and the inhibitory effect of the test antibodies and ADCs on the tumor cell lines was evaluated by IC₅₀. The antibodies and the ADCs, having a starting concentration of 500 nM, were diluted 7 folds, with a total of 8 concentration points, and the treatment was carried out for 5 days. The final calculation was based on the equation: survival rate = (experimental group - blank) / (control - blank) × 100%, and then Graph Pad Prism was used to fit the curve and calculate IC₅₀.

Table 8: In vitro pharmacodynamic activity of tested ADCs in human-derived tumor cell lines A431, HCC827 and FaDu:

No.	A431	HCC827	FaDu
	IC ₅₀ (nM)		
TR001	>500	>500	>500
ADC-1	0.013	0.004	0.091
ADC-2	0.212	5.365	-
ADC-5	0.033	0.146	2.542
ADC-13	0.142	0.230	4.012
ADC-14	0.126	0.461	9.020
ADC-15	0.125	0.657	11.342
ADC-16	0.199	0.953	17.285

ADC-17	0.257	2.205	-
ADC-18	0.194	3.000	-

Table 9: In vitro pharmacodynamic activity of tested ADCs in human-derived tumor cell lines N87 and SW620:

No.	N87	SW620
	IC₅₀ (nM)	
TR001	>500	>500
ADC-1	0.093	13.417
ADC-5	1.421	14.724

The results are shown in the table above and FIGs. 2A-2E.

Conclusion: In A431, HCC827, N87, SW620 and Fadu cell models, naked anti-TR001 had no cytotoxic activity to tumor cells, and the ADCs synthesized according to the invention had obvious anti-tumor effect.

Example 124 In vitro pharmacodynamic activity testing of ADCs-heterogeneous tumor model

In the present invention, heterogeneous tumor cell lines (A431+SW620) were used as experimental models to evaluate the in vitro pharmacodynamic activity of the ADCs. A certain number of tumor cells were seeded into a 96-well plate, gradient dilutions of tested ADCs were added to the cells, and treated for 5 days. Cell viability was detected using MTS, and the inhibitory effect of the test antibody and ADCs on the tumor cell lines was evaluated by IC₅₀. The antibody and the ADCs, having a starting concentration of 500 nM, were diluted 7 folds, with a total of 8 concentration points, and the treatment was carried out for 5 days. The final calculation was based on the equation: survival rate = (experimental group - blank) / (control - blank) × 100%, and then Graph Pad Prism was used to fit the curve and calculate IC₅₀.

Table 10: In vitro pharmacodynamic activity of tested ADCs in heterogeneous tumor cell lines A431+SW620:

No.	A431+SW620
	IC₅₀ (nM)
ADC-19 (control)	39.908
ADC-13	2.884
ADC-14	6.667
ADC-15	7.617
ADC-16	11.280

The results are shown in the table above and FIG. 3.

Conclusion: In the A431+ SW620 heterotumor cell model, the ADCs synthesized according to the present invention had significant anti-tumor effect and were superior to the control ADC-19.

Example 125 In vivo pharmacodynamic activity testing of ADCs

In the present invention, *BALB/c-nu* subcutaneously inoculated a human-derived tumor cell line (A431) was used as an experimental model to evaluate the in vivo pharmacodynamics of ADCs. Suspension containing a certain number of tumor cells was inoculated subcutaneously into *BALB/c-nu*. When the tumor volume grew to about 180 mm³, the vehicle and ADC-5 were injected into the tail vein once a week for three times. Observation was continued. Tumor size and body weight were measured twice a week to evaluate the inhibitory effect of ADCs on tumor growth.

The results are shown in the table below.

Table 11: Efficacy of the tested ADC on A431 mouse xenograft:

Compound	Mean tumor volume (mm ³)						T/C %	TGI %
	D0	D3	D7	D10	D14	D17		
Blank	175.01	456.78	828.16	1164.44	1438.74	1671.07	100	0
ADC-5 (3 mg/Kg)	171.24	283.15	199.84	198.75	178.75	158.06	9.67	90.33

$$T/C \% = T_{RTV} / C_{RTV} \times 100\%; TGI\% = (1 - T/C) \times 100\%$$

Table 12: Effect of tested ADC on body weight of A431 transplanted mice:

Compound	D0	D3		D7		D10		D14		D17	
	Mean Body weigh t (g)	Mean Body weigh t (g)	BWC %	Mean Body weigh t (g)	BWC %	Mean Body weigh t (g)	BWC %	Mean Body weigh t (g)	BWC %	Mean Body weigh t (g)	BWC %
Blank	20.45	22.09	8.06	23.11	13.05	23.28	13.86	24.40	19.35	24.19	18.33
ADC-5 (3 mg/Kg)	19.85	21.47	8.17	22.24	12.13	21.78	9.86	22.44	13.14	21.98	10.93

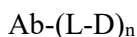
Conclusion: In mice transplanted with A431 single tumor, the ADC synthesized according to the invention had significant anti-tumor effects.

The above embodiments are only used to illustrate but not limit the technical solutions of the invention. Any modification or equivalent substitution of the technical solutions of the invention without departing from the purpose and scope of the technical solutions of the invention should be

covered within the scope of the invention.

Claims

1. A ligand-drug conjugate of formula I or a pharmaceutically acceptable salt or solvate thereof,



Formula I

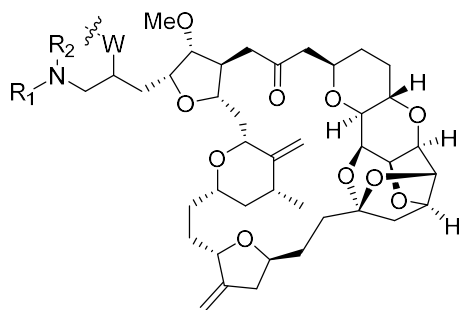
wherein,

Ab is a ligand unit;

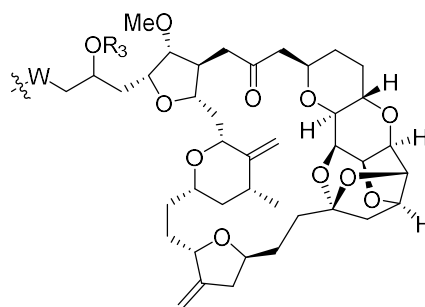
L is a linker for covalently connecting Ab to D;

n is selected from integers or decimals between 1 and 40;

-D is as shown in formula II or formula III:



Formula II



Formula III

wherein W is an oxygen atom or a sulfur atom,

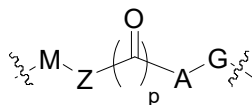
R₁ and R₂ are the same or different, and are each independently selected from: hydrogen atom, alkyl, alkoxy, alkenyl, cycloalkyl, aryl, heteroaryl, heterocyclyl, -C(O)-Q₁-Q₂ and -SO₂-Q₁-Q₂, wherein Q₁ is selected from the group consisting of O, N, S atoms and a chemical bond, Q₂ is selected from the group consisting of alkyl, cycloalkyl, heterocyclyl, spirocyclyl, bridged ring, alkenyl, aryl and heteroaryl; optionally, the alkyl, alkoxy, alkenyl, cycloalkyl, heterocyclyl, spirocyclyl, bridged ring, aryl and heteroaryl are each independently substituted by one or more substituents selected from the group consisting of hydrogen atom, alkyl, alkoxy, halogen, deuterium, amino, cyano, hydroxy, mercapto, azido, nitro, carboxyl, acyl, carbonyl, hydroxyalkyl, cycloalkyl, heterocyclyl, aryl and heteroaryl; or R₁ and R₂, together with a nitrogen atom connected thereto, form a 3- to 8-membered heterocyclyl; optionally, the heterocyclyl is substituted by one or more substituents selected from the group consisting of hydrogen atom, alkyl, alkoxy, halogen, deuterium, amino, cyano, hydroxy, mercapto, azido, nitro, carboxyl, acyl, carbonyl, hydroxyalkyl, cycloalkyl, heterocyclyl, aryl and heteroaryl; R₃ is selected from the group consisting of hydrogen atom, alkyl, acyl, sulfonyl, cycloalkyl, heterocyclyl, aryl and heteroaryl; optionally, the alkyl, acyl,

sulfonyl, cycloalkyl, heterocyclyl, aryl and heteroaryl are each independently substituted by one or more substituents selected from the group consisting of hydrogen atom, alkyl, alkoxy, halogen, deuterium, amino, cyano, hydroxy, mercapto, azido, nitro, carboxyl, acyl, carbonyl, hydroxyalkyl, cycloalkyl, heterocyclyl, aryl and heteroaryl.

2. The ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to claim 1, wherein the ligand unit Ab is selected from the group consisting of an antibody, an antibody fragment or a protein; the antibody is preferably selected from the group consisting of a murine antibody, a rabbit antibody, a phage display-derived antibody, a yeast display-derived antibody, a chimeric antibody, a humanized antibody, a fully human antibody, an antibody fragment, a bispecific antibody and a multispecific antibody.

3. The ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to claim 1 or 2, wherein the antibody is a monoclonal antibody, is selected, without limitation, from: anti-EGFRvIII antibody, anti-PD-1 antibody, anti-PD-L1 antibody, anti-DLL-3 antibody, anti-PSMA antibody, anti-CD70 antibody, anti-MUC16 antibody, anti-ENPP3 antibody, anti-TDGF1 antibody, anti-ETBR antibody, anti-MSLN antibody, anti-TIM-1 antibody, anti-LRRC15 antibody, anti-LIV-1 antibody, anti-CanAg/AFP antibody, anti-claudin 18.2 antibody, anti-Mesothelin antibody, anti-HER2(ErbB2) antibody, anti-EGFR antibody, anti-c-MET antibody, anti-SLITRK6 antibody, anti-KIT/CD117 antibody, anti-STEAP1 antibody, anti-SLAMF7/CS1 antibody, anti-NaPi2B/SLC34A2 antibody, anti-GPNMB antibody, anti-HER3(ErbB3) antibody, anti-MUC1/CD227 antibody, anti-AXL antibody, anti-CD166 antibody, anti-B7-H3(CD276) antibody, anti-PTK7/CCK4 antibody, anti-PRLR antibody, anti-EFNA4 antibody, anti-5T4 antibody, anti-NOTCH3 antibody, anti-Nectin 4 antibody, anti-TROP-2 antibody, anti-CD142 antibody, anti-CA6 antibody, anti-GPR20 antibody, anti-CD174 antibody, anti-CD71 antibody, anti-EphA2 antibody, anti-LYPD3 antibody, anti-FGFR2 antibody, anti-FGFR3 antibody, anti-FR α antibody, anti-CEACAMs antibody, anti-GCC antibody, anti-Integrin Av antibody, anti-CAIX antibody, anti-P-cadherin antibody, anti-GD3 antibody, anti-Cadherin 6 antibody, anti-LAMP1 antibody, anti-FLT3 antibody, anti-BCMA antibody, anti-CD79b antibody, anti-CD19 antibody, anti-CD33 antibody, anti-CD56 antibody, anti-CD74 antibody, anti-CD22 antibody, anti-CD30 antibody, anti-CD37 antibody, anti-CD47 antibody, anti-CD138 antibody, anti-CD352 antibody, anti-CD25 antibody or anti-CD123 antibody.

4. The ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to claim 1, wherein L has a structure shown in formula IV:

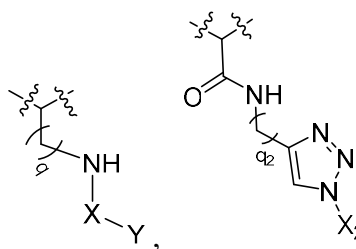


Formula IV

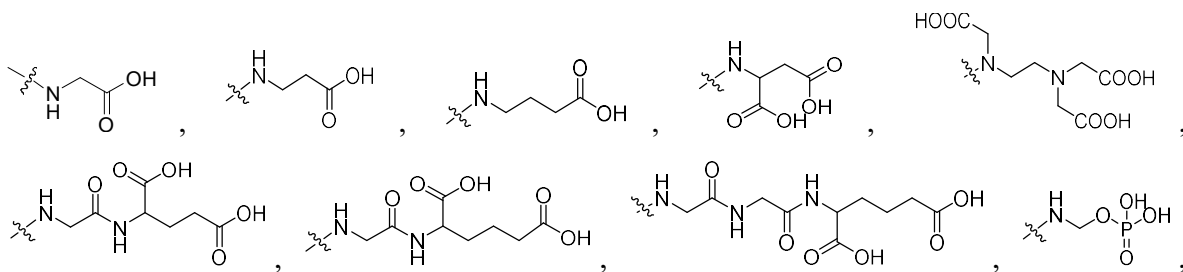
wherein,

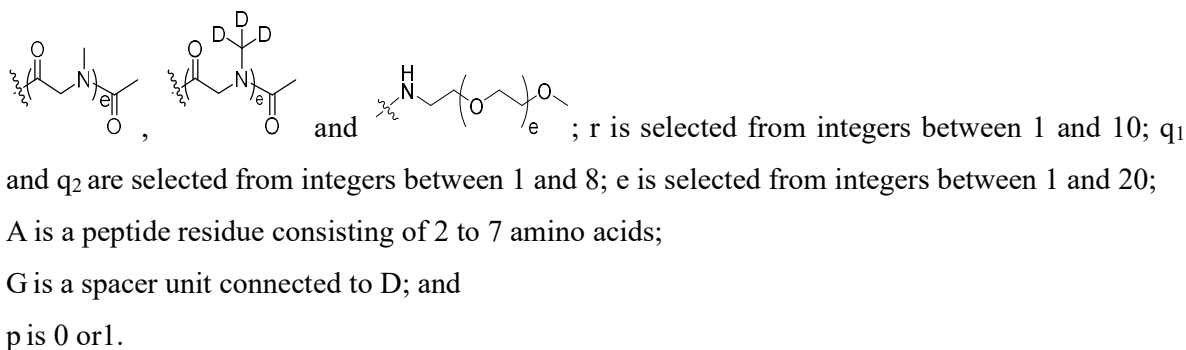
M is a linking unit covalently connected to Ab;

Z is selected from the group consisting of -C1-C10 alkylene-, -C3-C8 carbocycle-, -arylene-, -3- to 8-membered heterocyclyl-,

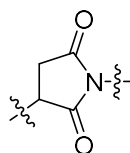


-(CH₂CH₂O)_r-, sulfonyl, amide, a chemical bond, combinations thereof, wherein X₁ and X₂ are selected from the group consisting of -C1-C10 alkylene-, -C3-C8 carbocycle-, -arylene-, -3- to 8-membered heterocyclyl-, -(CH₂CH₂O)_r-, -NR₄-, carbonyl and any combinations thereof, wherein R₄ is selected from the group consisting of hydrogen atom, deuterium atom, alkyl and substituted alkyl; optionally, the -C1-C10 alkylene-, -C3-C8 carbocycle-, and -3- to 8-membered heterocyclyl- are each independently substituted with one or more substituents selected from the group consisting of hydrogen atom, deuterium atom, halogen, hydroxyl, cyano, nitro, amino, alkyl, heteroalkyl, substituted alkyl, alkoxy, carboxy and cycloalkyl; each heterocyclyl independently contains 1 to 3 atoms selected from the group consisting of N, O and S; Y₁ is a hydrophilic structure selected from the group consisting of carboxyl, phosphoric acid, polyphosphoric acid, phosphorous acid, sulfonic acid, sulfinic acid, polyethylene glycol (PEG), and any combinations thereof; Y₂ is selected from the following structures:

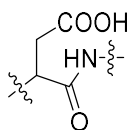




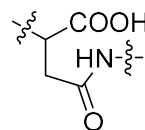
5. The ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to claim 4, wherein the linking unit M has a succinimide structure as represented by formula a, or a structure as represented by formula b or formula c in which the ring in succinimide group is in open form:



Formula a



Formula b

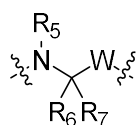


Formula c

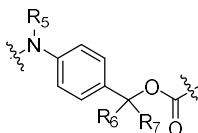
In formula a, formula b or formula c, a wavy line on the left indicates connecting to a linking site on Ab , and a wavy line on the right indicates connecting to a linking site on Z .

6. The ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to claim 4 or 5, wherein A is a polypeptide residue composed of 2 to 7 amino acids selected from the group consisting of phenylalanine, glycine, valine, lysine, citrulline, serine, glutamic acid, aspartic acid, and cysteic acid.

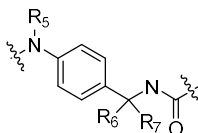
7. The ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 4-6, wherein the spacer unit G is selected from the group consisting of structures represented by formulas Va, Vb, Vc and Vd and any combinations thereof:



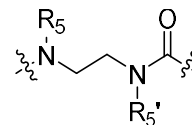
Formula Va



Formula Vb



Formula Vc

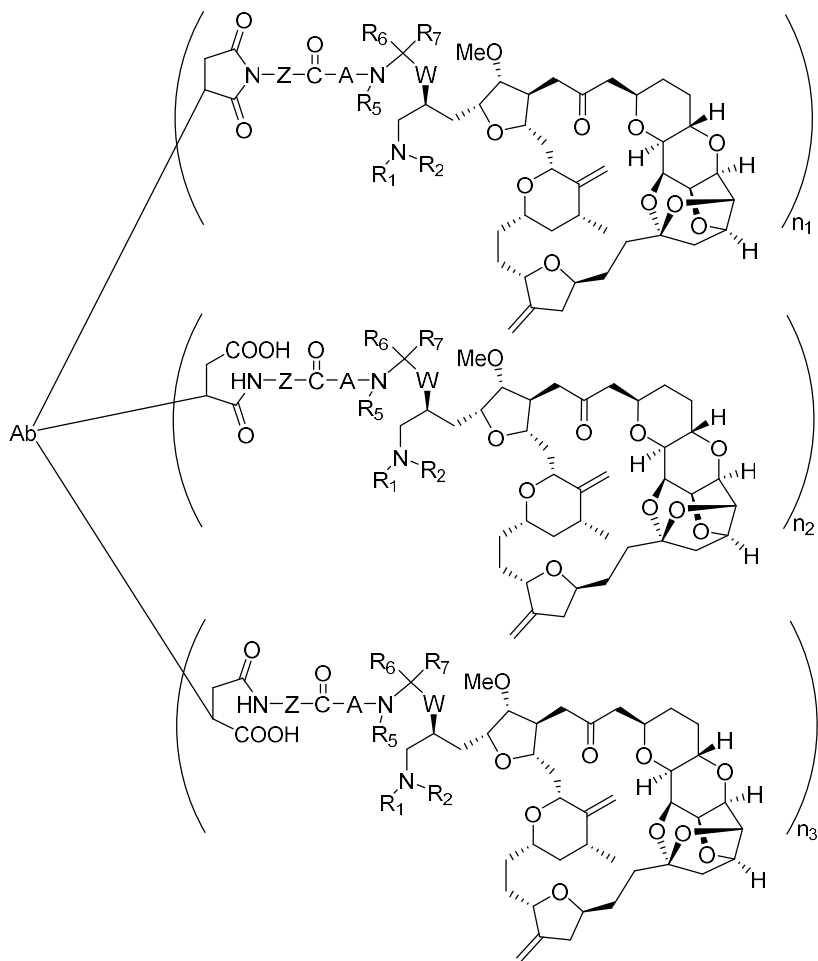


Formula Vd

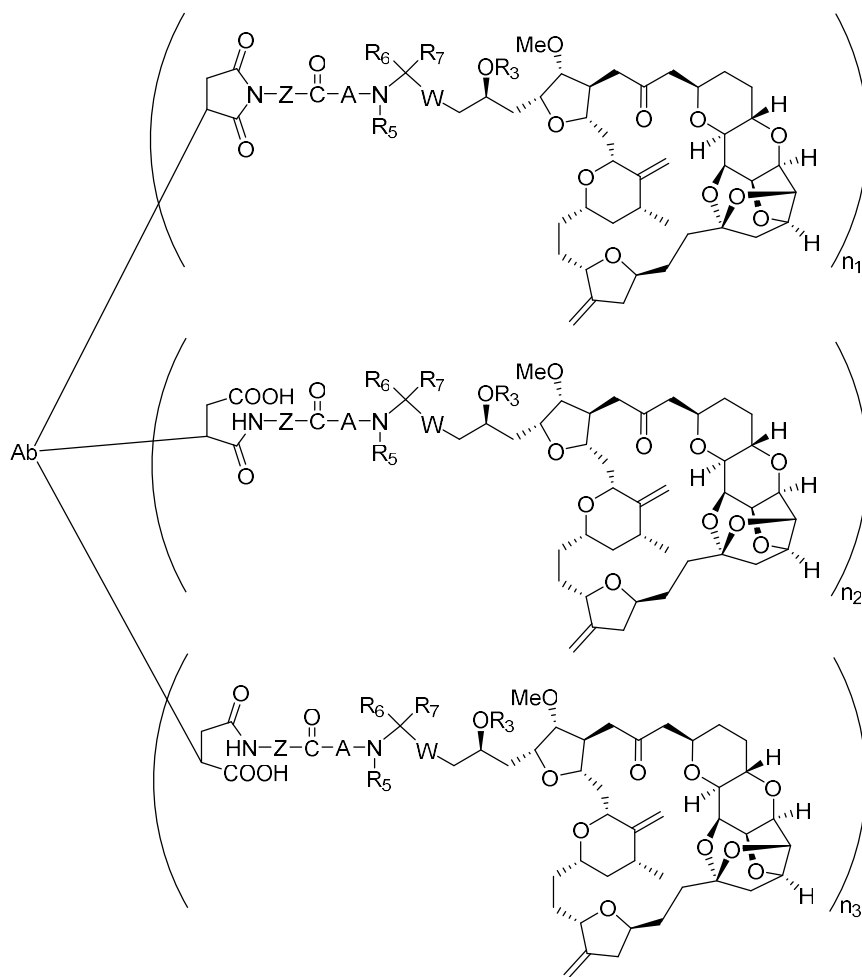
wherein a wavy line on the left represents a linking site between a nitrogen atom and peptide residue A , a wavy line on the right represents a linking site between an oxygen atom and drug D , and W is

an oxygen atom or a sulfur atom and is a joint group between the drug D and the spacer unit G; and R_5 , R_5' , R_6 and R_7 are each independently selected from the group consisting of hydrogen atom, deuterium atom, alkyl and substituted alkyl.

8. The ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 1-7, wherein the ligand-drug conjugate has a structure represented by formula VI or formula VII:



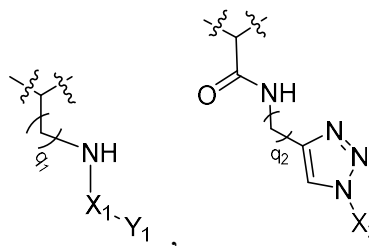
Formula VI



Formula VII

wherein, the Ab, Z, A, R₅, R₆, R₇, R₃ and W are as defined in any one of claims 1-7; n₁, n₂ and n₃ are independently selected from integers or decimals between 0 and 40, n₁, n₂ and n₃ are not 0 at the same time, and n₁+n₂+n₃≤40.

9. The ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 4-8, wherein Z is selected from the group consisting of -C1-C10 alkylene-, -

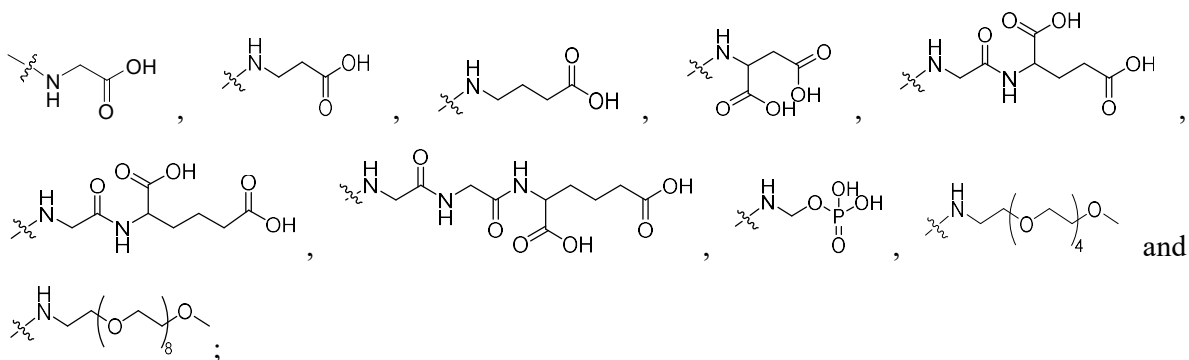


(CH₂CH₂O)_r-, amide,

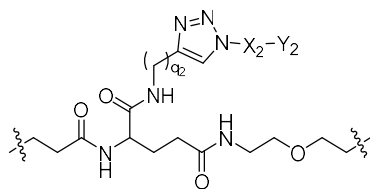
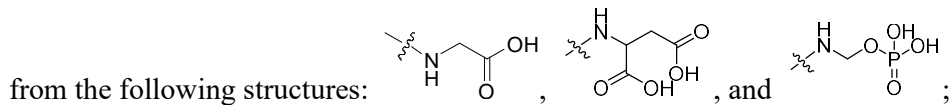
preferably, Z is -C2-alkylene or -C5-alkylene;

preferably, Z is $X_{1q}Y_1$, wherein, q_1 is selected from integers between 1 and 8; preferably, q_1 is 1;

preferably, q_1 is 1, and $-\text{NH}-\text{X}_1-\text{Y}_1$ composes the hydrophilic structural unit Ac_1 , the Ac_1 is selected, without limitation, from the group consisting of (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 unnatural amino acid derivatives, and the following structures:

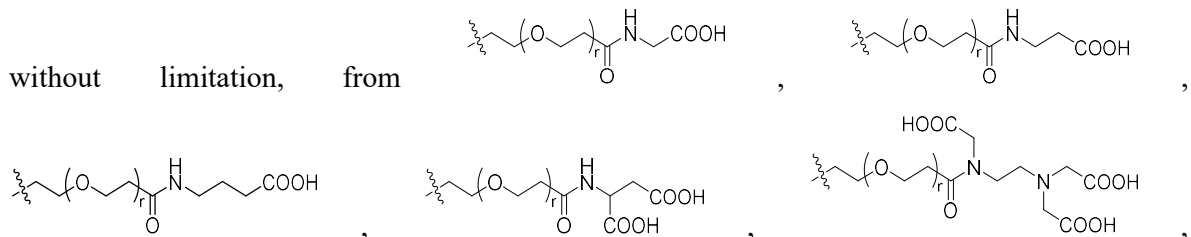


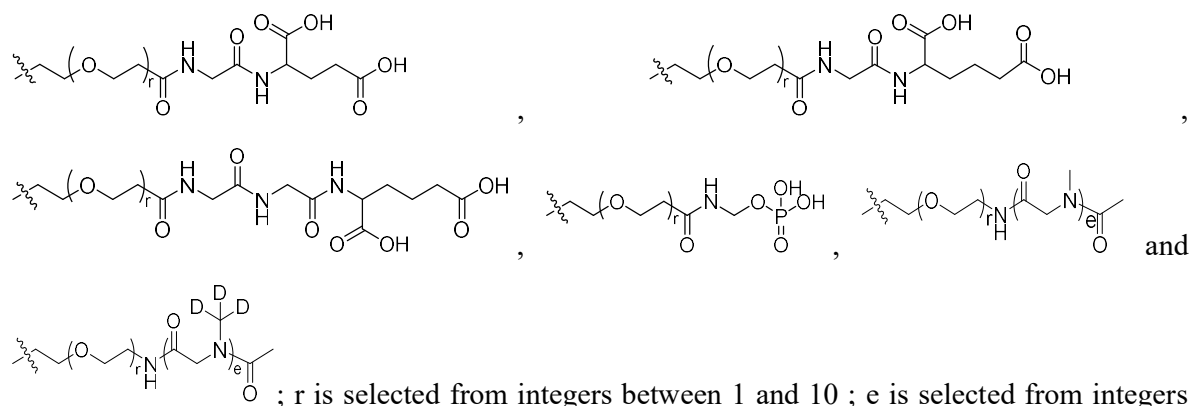
preferably, q_1 is 1, and $-NH-X_1-Y_1$ composes the hydrophilic structural unit Ac_1 , the Ac_1 is selected



preferably, Z is H or O , wherein, q_2 is selected from integers between 1 and 8; preferably, q_2 is 1; preferably, X_2 is $-(C_1-C_{10} \text{ alkylene})-(CH_2CH_2O)_r-(C=O)-$ or $-(C_1-C_{10} \text{ alkylene})-(CH_2CH_2O)_r-NR_4-$;

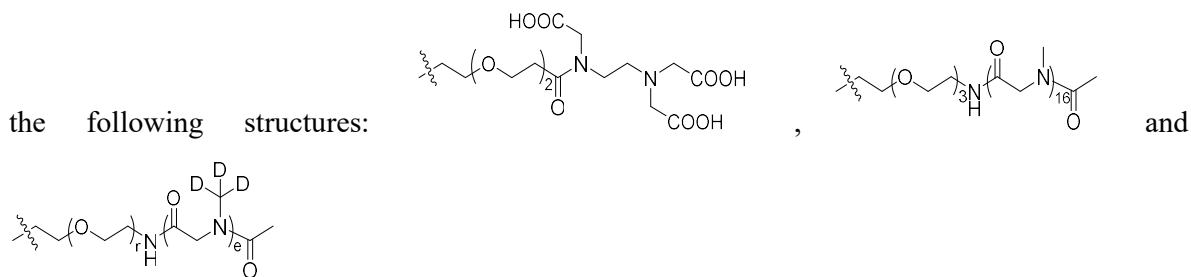
preferably, q_2 is 1, and $-X_2-Y_2$ composes the hydrophilic structural unit Ac_2 , the Ac_2 is selected,





between 1 and 20;

preferably, q_2 is 1, and $-X_2-Y_2$ composes the hydrophilic structural unit Ac_2 , the Ac_2 is selected from



10. The ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 4-9, wherein A is a polypeptide residue composed of 2 to 5 amino acids selected from the group consisting of phenylalanine, glycine, valine, lysine, citrulline, serine, glutamic acid, aspartic acid, and cysteic acid;

preferably, A is a peptide residue formed by 2 to 4 amino acids selected from phenylalanine and glycine;

more preferably, A is a tetrapeptide residue composed of glycine (G), glycine (G), phenylalanine (F), and glycine (G);

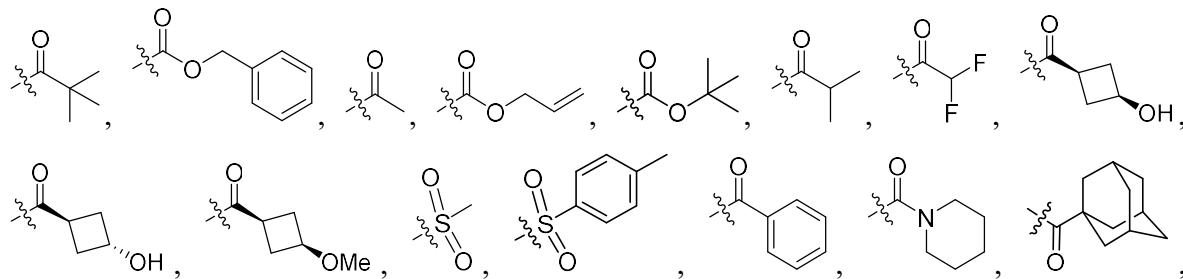
and more preferably, A is -GGFG-.

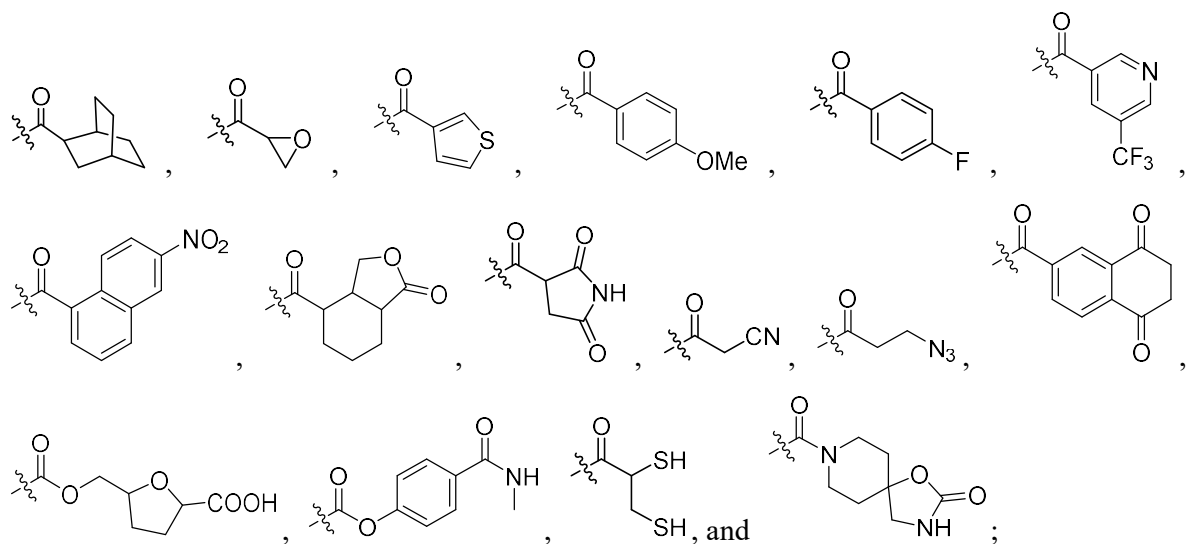
11. The ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 1-10, wherein W is an oxygen atom or a sulfur atom, R_1 and R_2 are the same or different, and are each independently selected from the group consisting of: hydrogen atom, alkyl, alkoxy, alkenyl, cycloalkyl, $-C(O)-Q_1-Q_2$ and $-SO_2-Q_1-Q_2$, wherein Q_1 is selected from the group consisting of O and a chemical bond, and Q_2 is selected from the group consisting of alkyl, heterocyclyl, alkenyl, aryl, heteroaryl and cycloalkyl; optionally, the alkyl, heterocyclyl, alkenyl, aryl, heteroaryl and cycloalkyl are each independently substituted with one or more substituents

selected from the group consisting of hydrogen atom, alkyl, hydroxyalkyl, halogen, deuterium, mercapto, amino, aryl and hydroxy; or R₁ and R₂ together with a nitrogen atom connected thereto, form a 3- to 6-membered heterocyclyl, and optionally the heterocyclyl is substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, alkoxy, halogen, deuterium, amino, hydroxyl, cycloalkyl and heterocyclyl; R₃ is selected from the group consisting of hydrogen atom and alkyl, and optionally the alkyl is substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, alkoxy, halogen, deuterium, amino, hydroxyl, cycloalkyl and heterocyclyl;

preferably, W is an oxygen atom, R₁ and R₂ are the same or different, and are each independently selected from the group consisting of: hydrogen atom, alkyl, alkenyl, haloalkyl, -C(O)-Q₁-Q₂ and -SO₂-Q₁-Q₂, wherein Q₁ is selected from the group consisting of O and a chemical bond, Q₂ is selected from the group consisting of alkyl, alkenyl, aryl and 3- to 8-membered cycloalkyl, and optionally, the alkyl, alkenyl, aryl and cycloalkyl are each independently substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, hydroxyalkyl, halogen, aryl, and hydroxyl; or R₁ and R₂ together with a nitrogen atom connected thereto, form a 3- to 6-membered heterocyclyl containing one to two nitrogen atoms and optionally one oxygen atom; optionally, the 3- to 6-membered heterocyclyl is substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, and haloalkyl; R₃ is selected from the group consisting of hydrogen atom and alkyl, and the alkyl is substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, halogen, deuterium, amino and hydroxyl.

12. The ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 1-11, wherein W is an oxygen atom, R₁ and R₂ are the same or different, and are each independently selected from the group consisting of: hydrogen atom, C₁-C₄ alkyl (such as methyl, ethyl), C₂-C₄ alkenyl (such as allyl), C₁-C₄ haloalkyl (such as difluoroethyl) and the following structures:

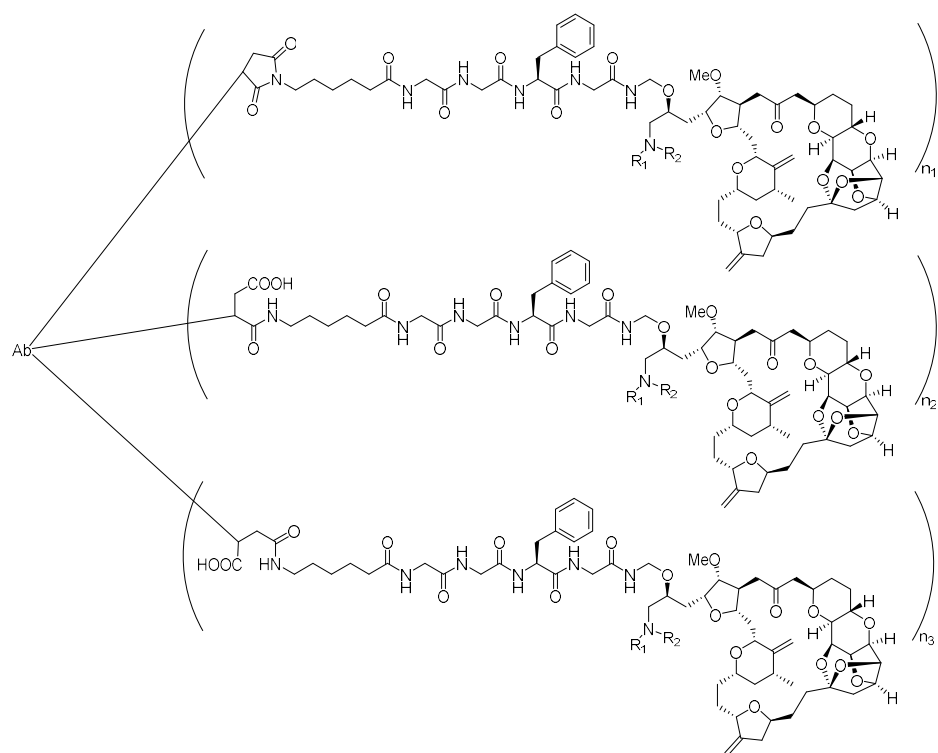




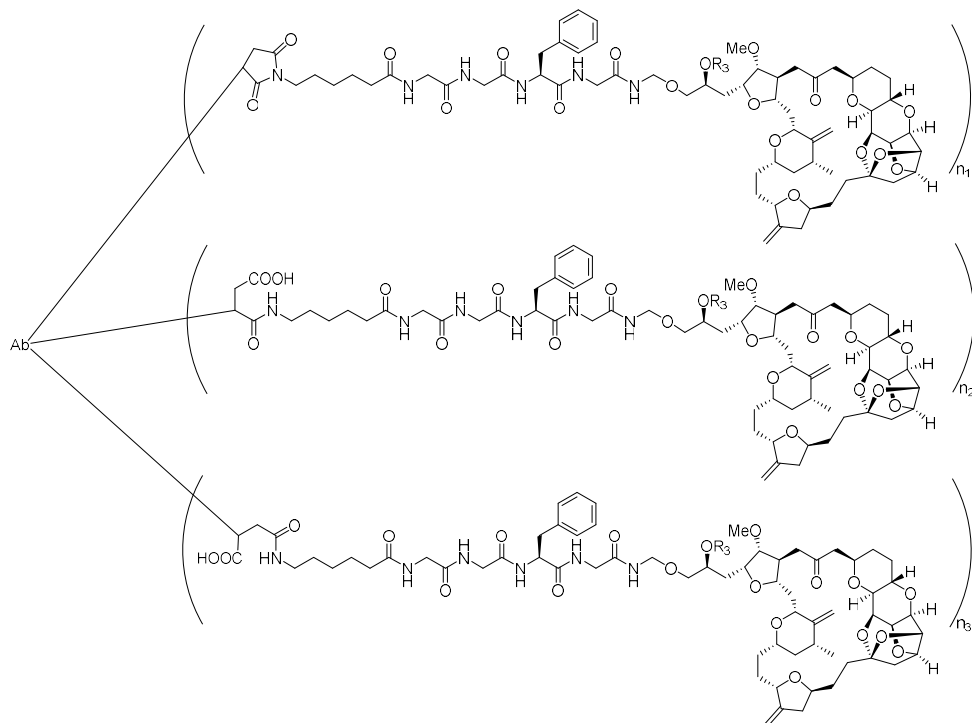
or R₁ and R₂, together with a nitrogen atom connected thereto, form a 3- to 6-membered heterocyclyl containing one to two nitrogen atoms and optionally one oxygen atom (such as azyclopropyl, piperidinyl, piperazinyl, morpholinyl); optionally, the 3- to 6-membered heterocyclyl is substituted with one or more substituents selected from the group consisting of hydrogen atom, C₁-C₄ alkyl (e.g., methyl), and C₁-C₄ haloalkyl (e.g., trifluoromethyl); and R₃ is selected from the group consisting of hydrogen atom and methyl.

13. The ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 7-12, wherein R₅, R₆ and R₇ are each independently selected from the group consisting of hydrogen atom, deuterium atom, alkyl, haloalkyl, deuterated alkyl and hydroxyalkyl; and preferably, R₅, R₆ and R₇ are simultaneously hydrogen atoms.

14. The ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 1-13, wherein the ligand-drug conjugate has a structure represented by formula VIa or formula VIIa:



Formula VIa

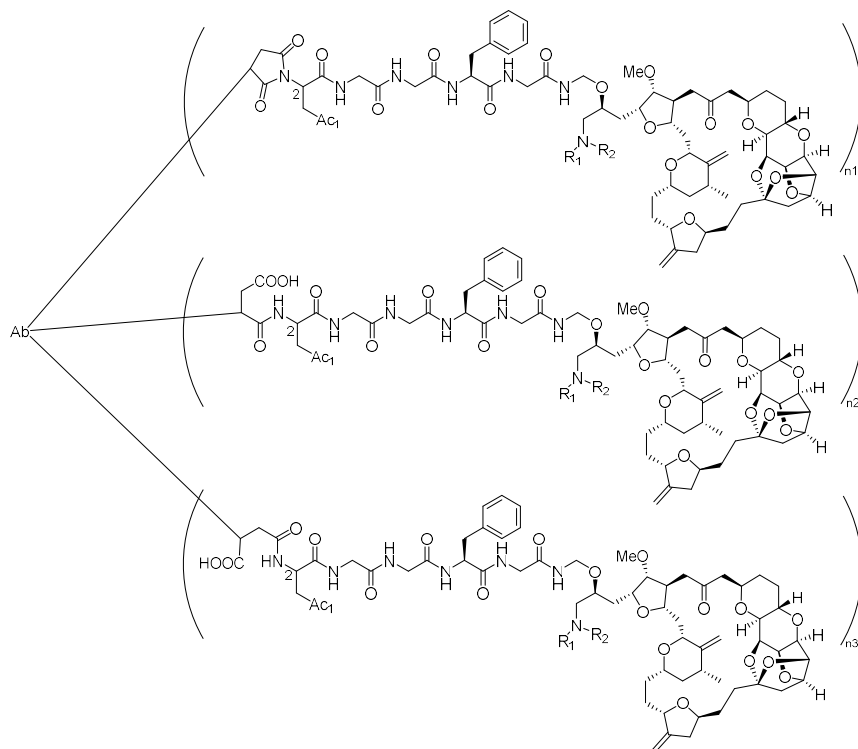


Formula VIIa

wherein,

the R_1 , R_2 , R_3 , n_1 , n_2 and n_3 are as defined in any one of claims 1-13.

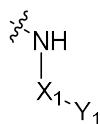
15. The ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 1-13, wherein the ligand-drug conjugate has a structure represented by formula VIb or formula VIIb:



Formula VIb

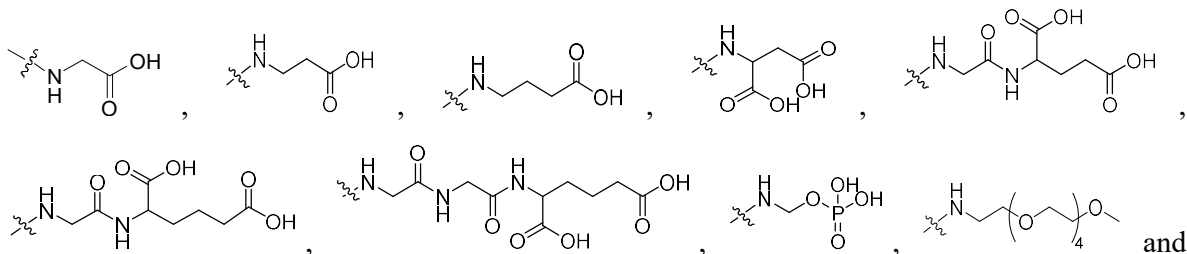


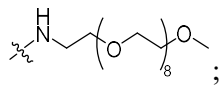
Ac₁ is a hydrophilic structural unit having the structure shown in formula d:

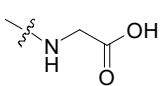
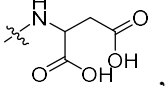


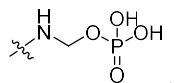
Formula d

preferably, the Ac₁ is selected, without limitation, from the group consisting of (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 unnatural amino acid derivatives and the following structures:

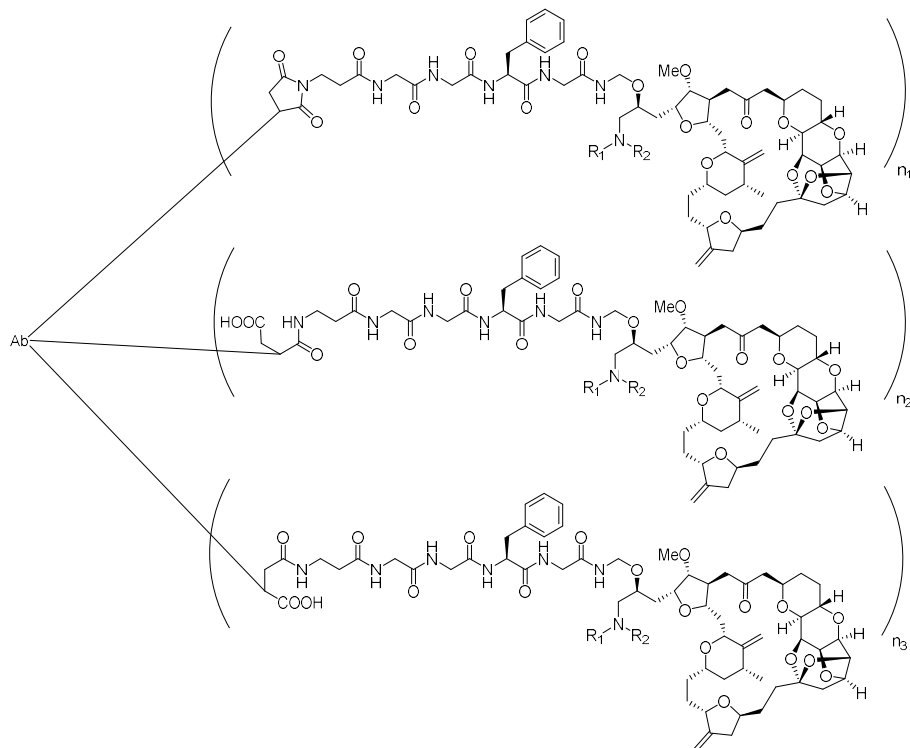




preferably, Ac_1 is selected from the following structures: , , and



16. The ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 1-13, wherein the ligand-drug conjugate has a structure represented by formula VIc:

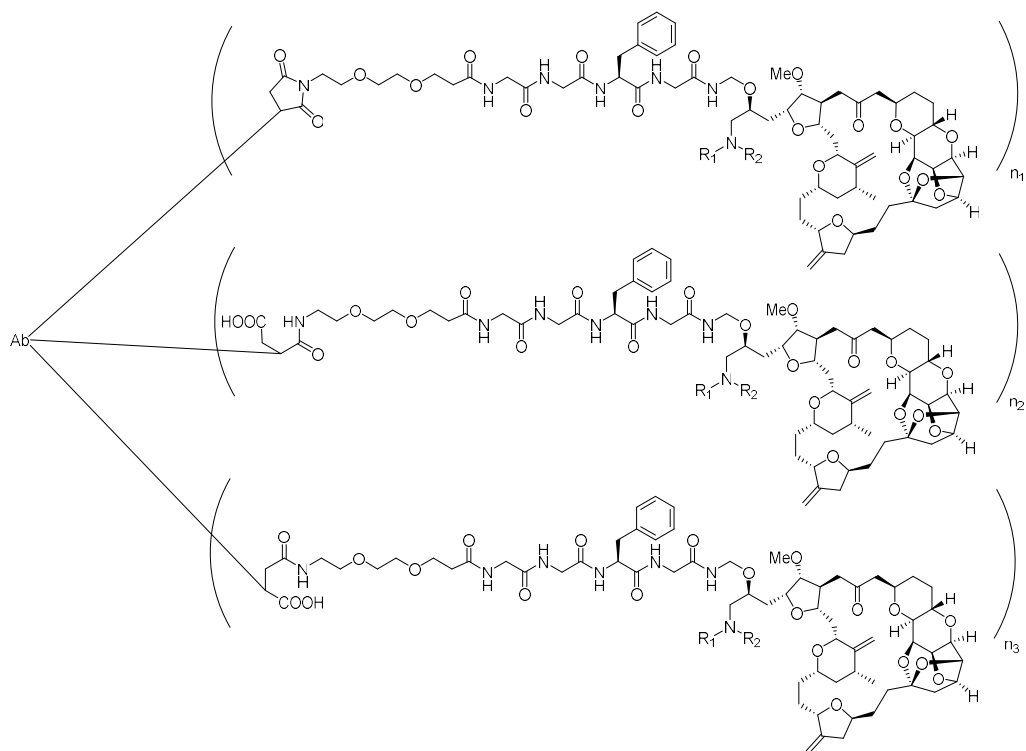


Formula VIc

wherein,

the R_1 , R_2 , n_1 , n_2 and n_3 are defined in any one of claims 1-13.

17. The ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 1-13, wherein the ligand-drug conjugate has a structure represented by formula VIId:

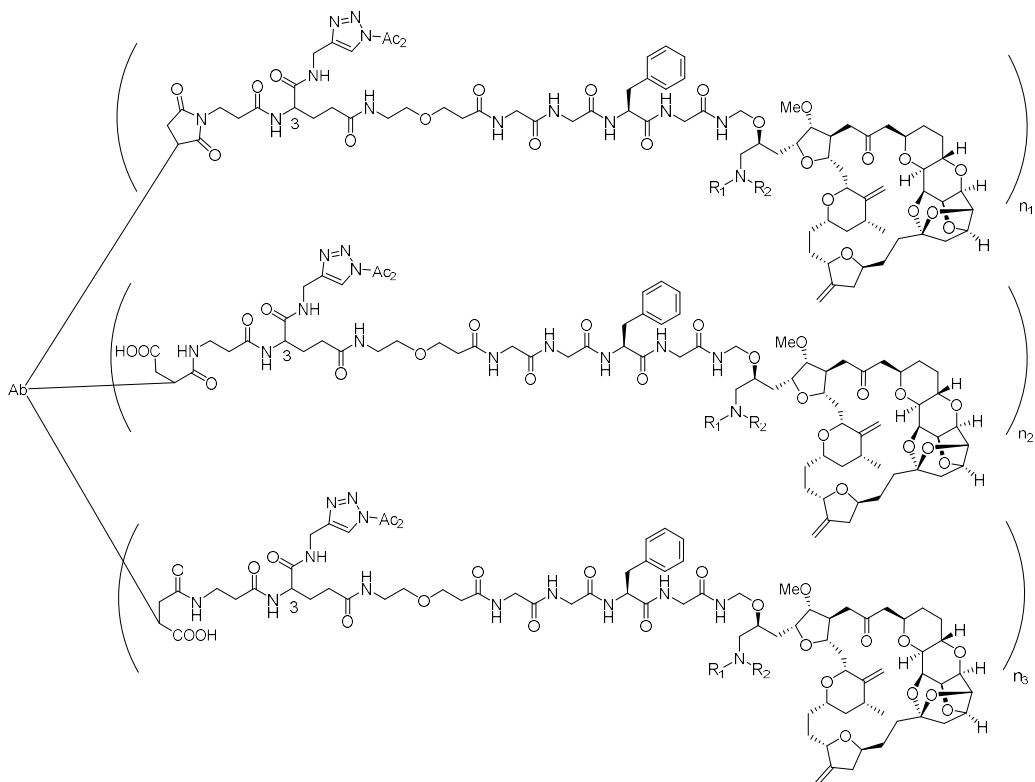


Formula VIId

wherein,

the R₁, R₂, n₁, n₂ and n₃ are as defined in any one of claims 1-13.

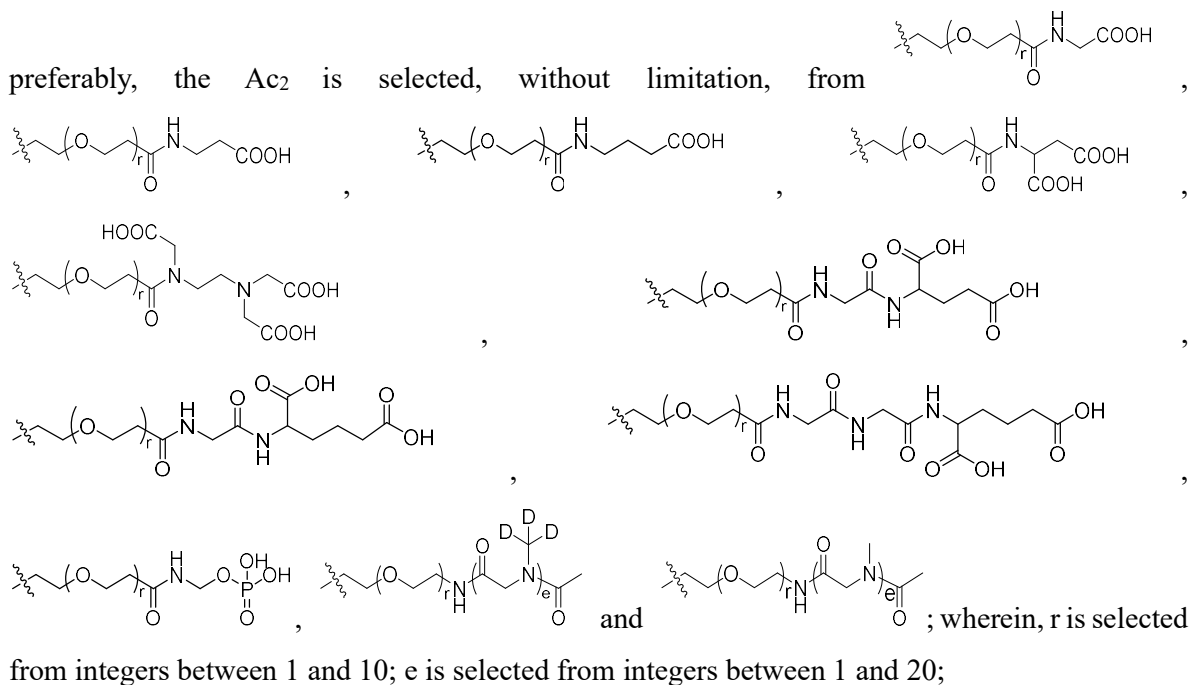
18. The ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 1-13, wherein the ligand-drug conjugate has a structure represented by formula VIe:



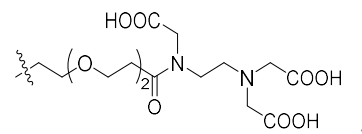
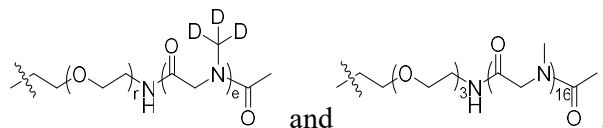
Formula VIe

wherein, the R_1 , R_2 , n_1 , n_2 and n_3 are as defined in any one of claims 1-13;

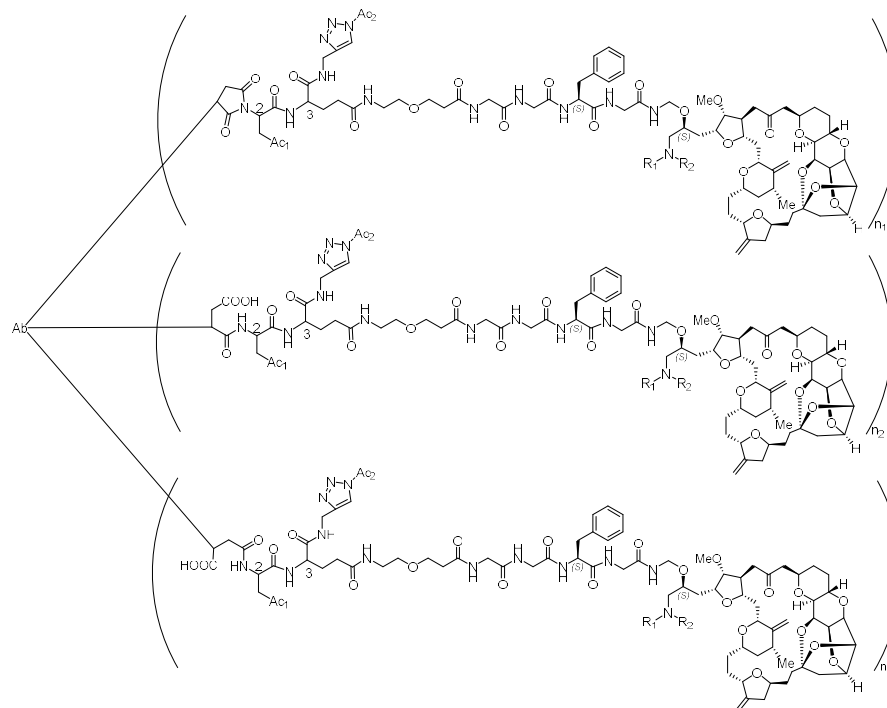
Ac_2 is a hydrophilic structural unit composed of $-X_2-Y_2$, and X_2 and Y_2 are as defined in claim 4;



preferably, Ac₂ is selected from the following structures:



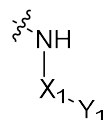
19. The ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 1-13, wherein the ligand-drug conjugate has a structure represented by formula VI_f:



Formula VI_f

wherein, the R₁, R₂, n₁, n₂ and n₃ are as defined in any one of claims 1-13;

Ac₁ is a hydrophilic structural unit having the structure shown in formula d:



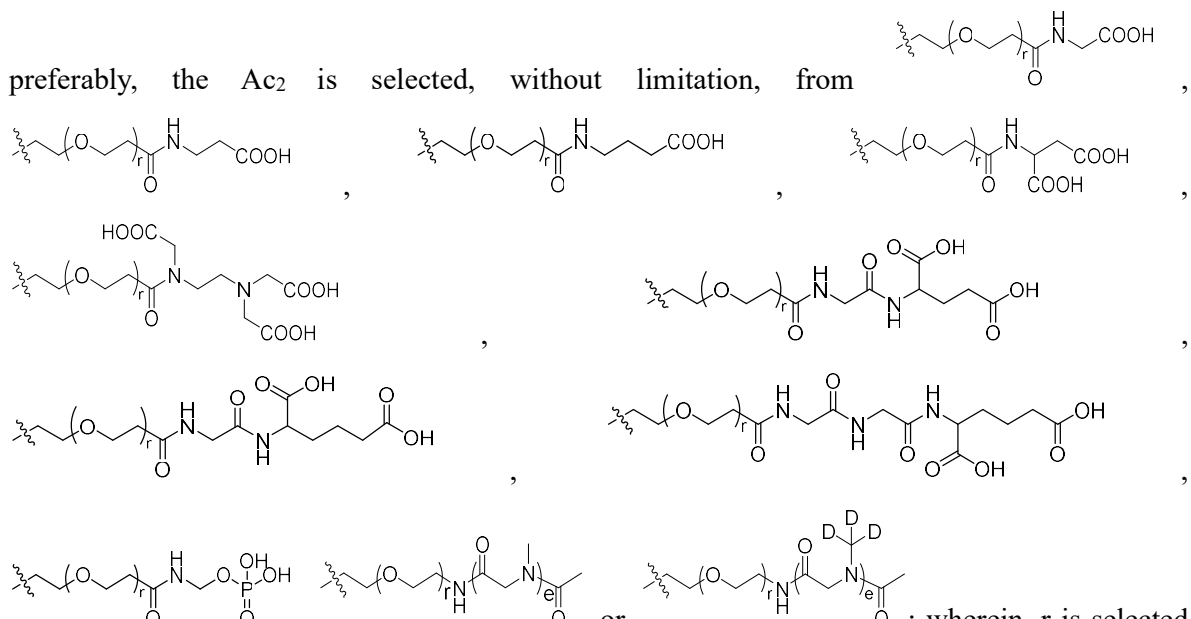
Formula d

X₁ and Y₁ are as defined in claim 4;

preferably, Ac₁ is as defined in claim 15;

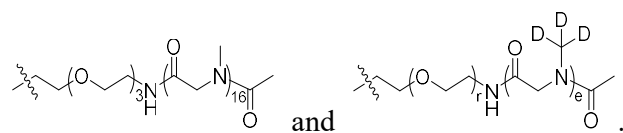
Ac₂ is a hydrophilic structural unit composed of -X₂-Y₂, and X₂ and Y₂ are as defined in claim 4;

preferably, the Ac₂ is selected, without limitation, from

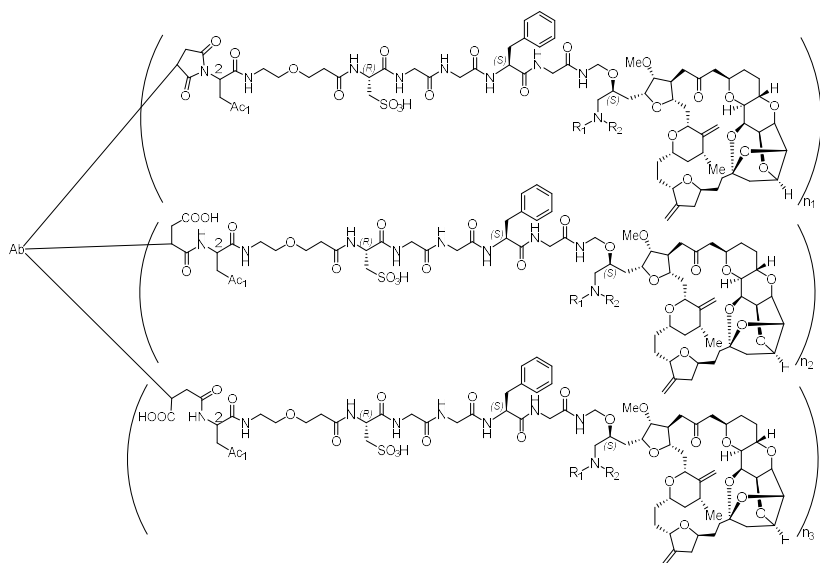


wherein, r is selected from integers between 1 and 10; e is selected from integers between 1 and 20; and

preferably, Ac₂ is selected from the following structures:



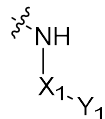
20. The ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 1-13, wherein the ligand-drug conjugate has a structure represented by formula VIg:



Formula VIg

wherein, the R_1 , R_2 , n_1 , n_2 and n_3 are as defined in any one of claims 1-13;

Ac_1 is a hydrophilic structural unit having the structure shown in formula d:

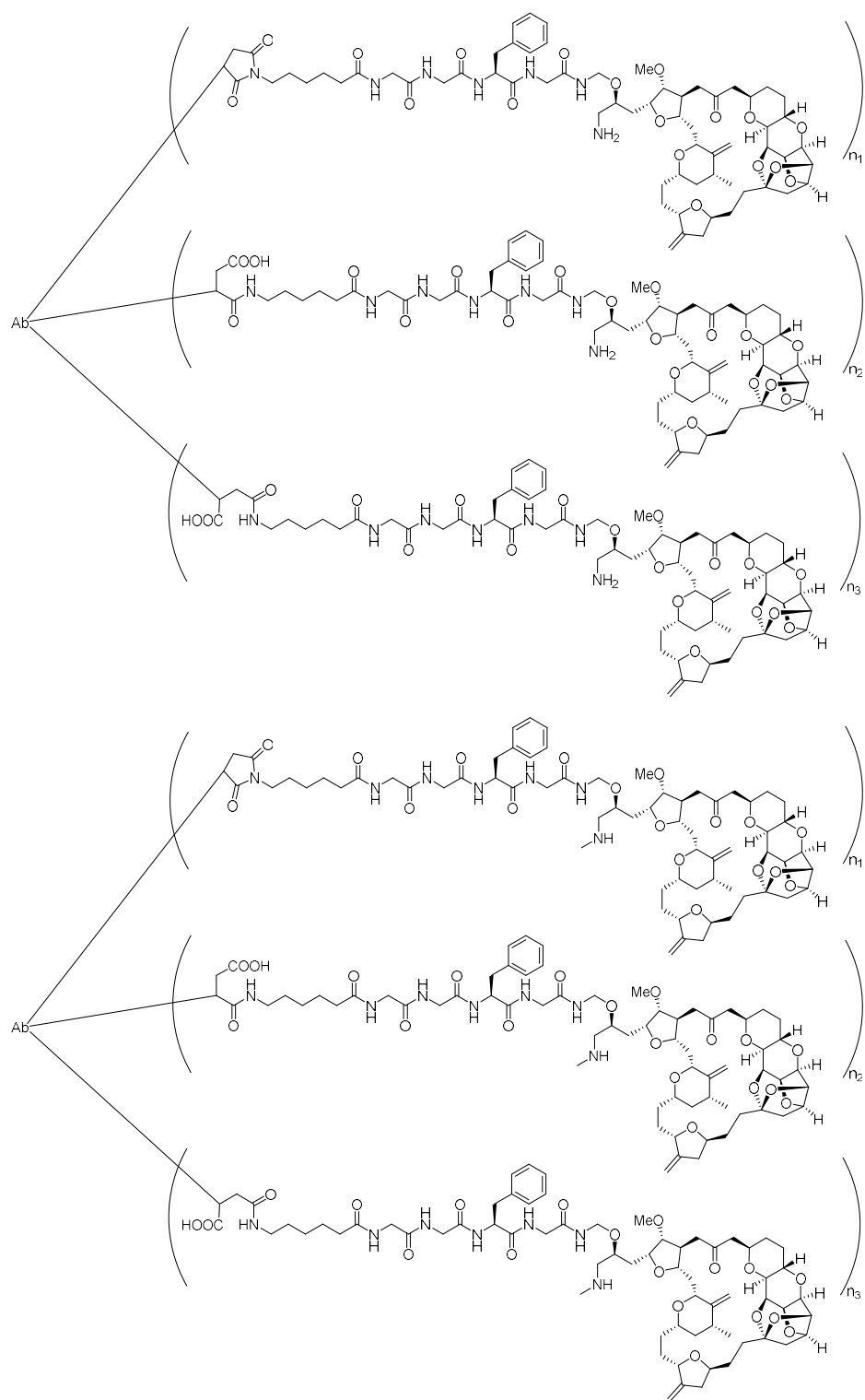


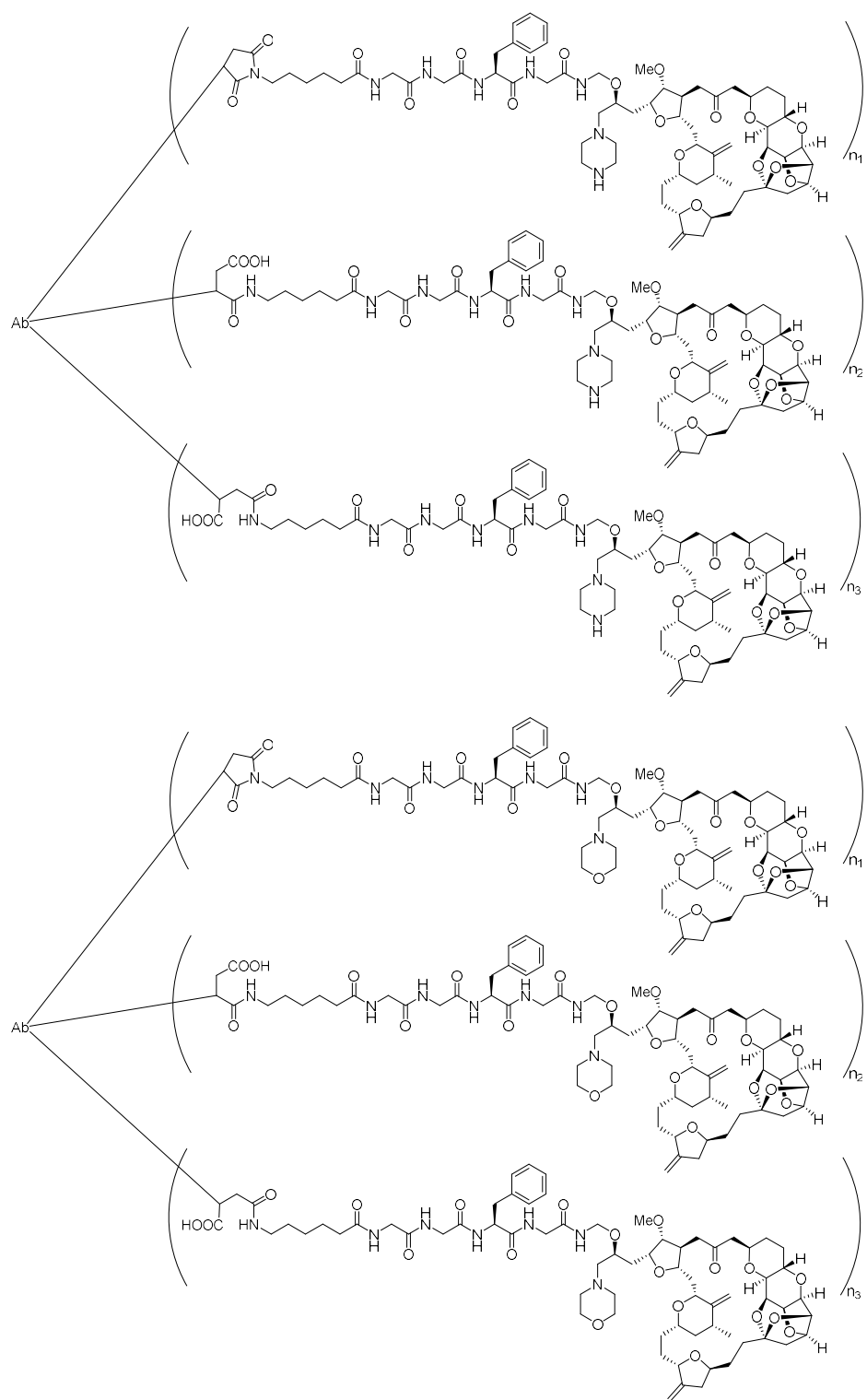
Formula d

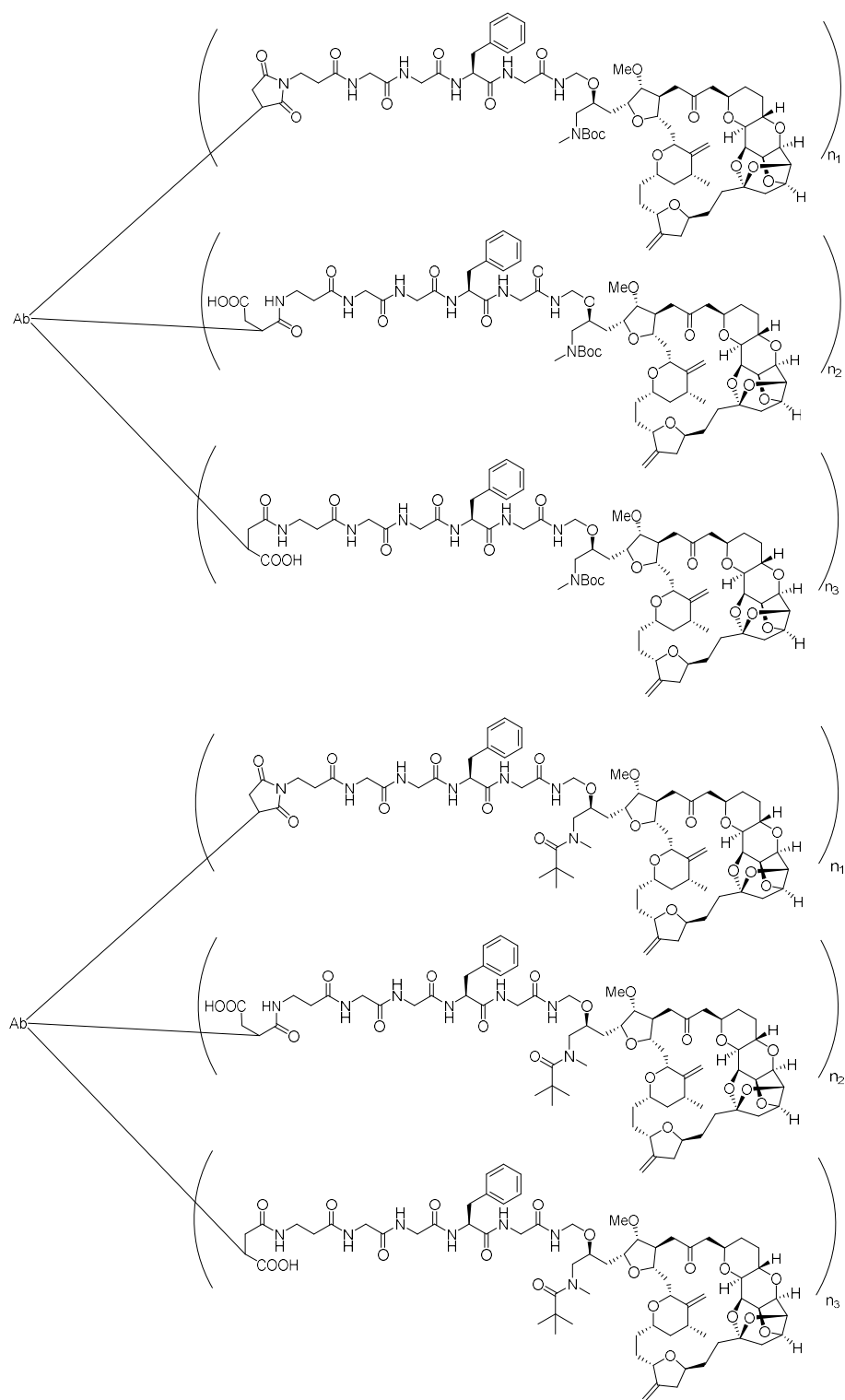
X_1 and Y_1 are as defined in claim 4; and

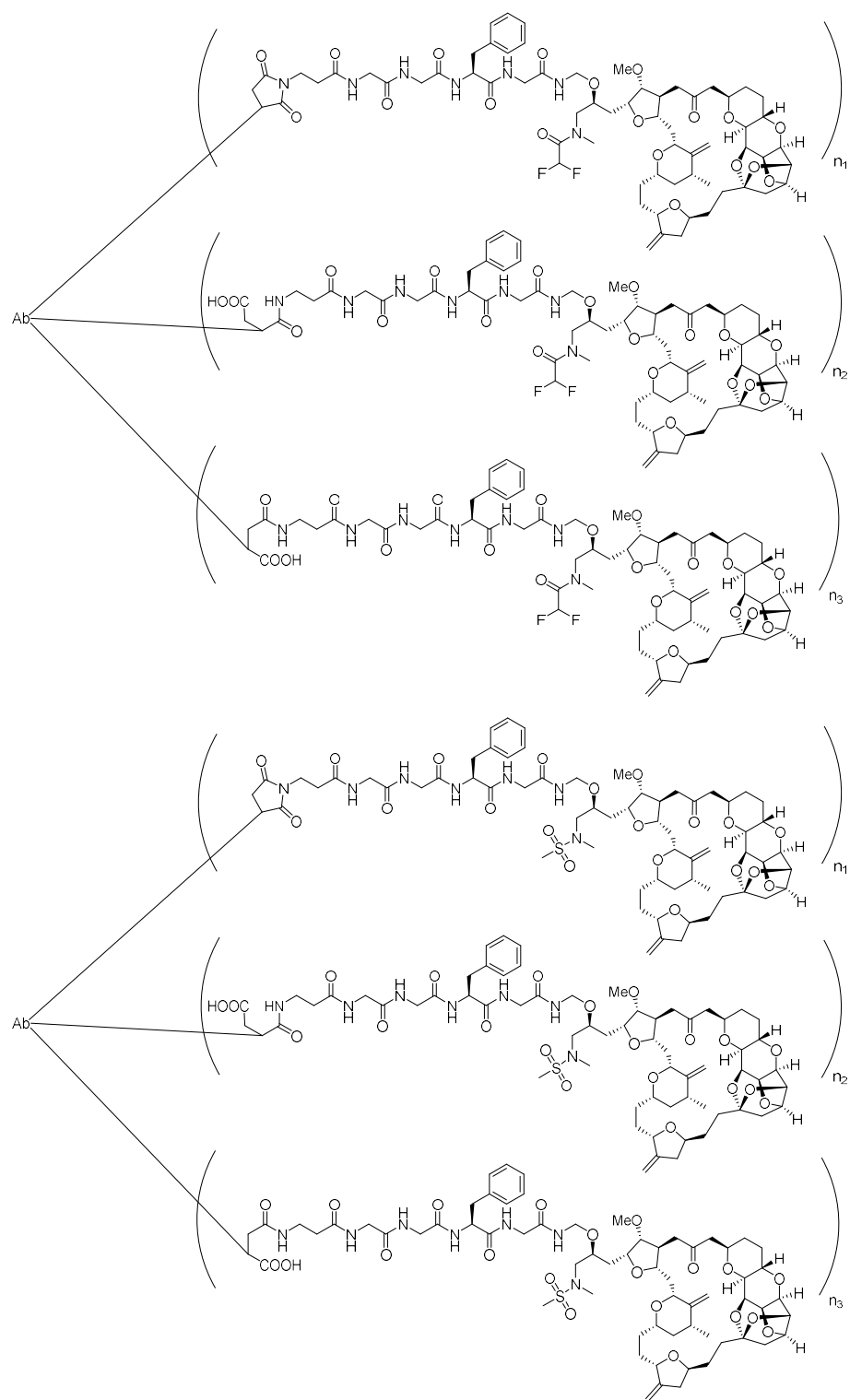
preferably, Ac_1 is as defined in claim 15.

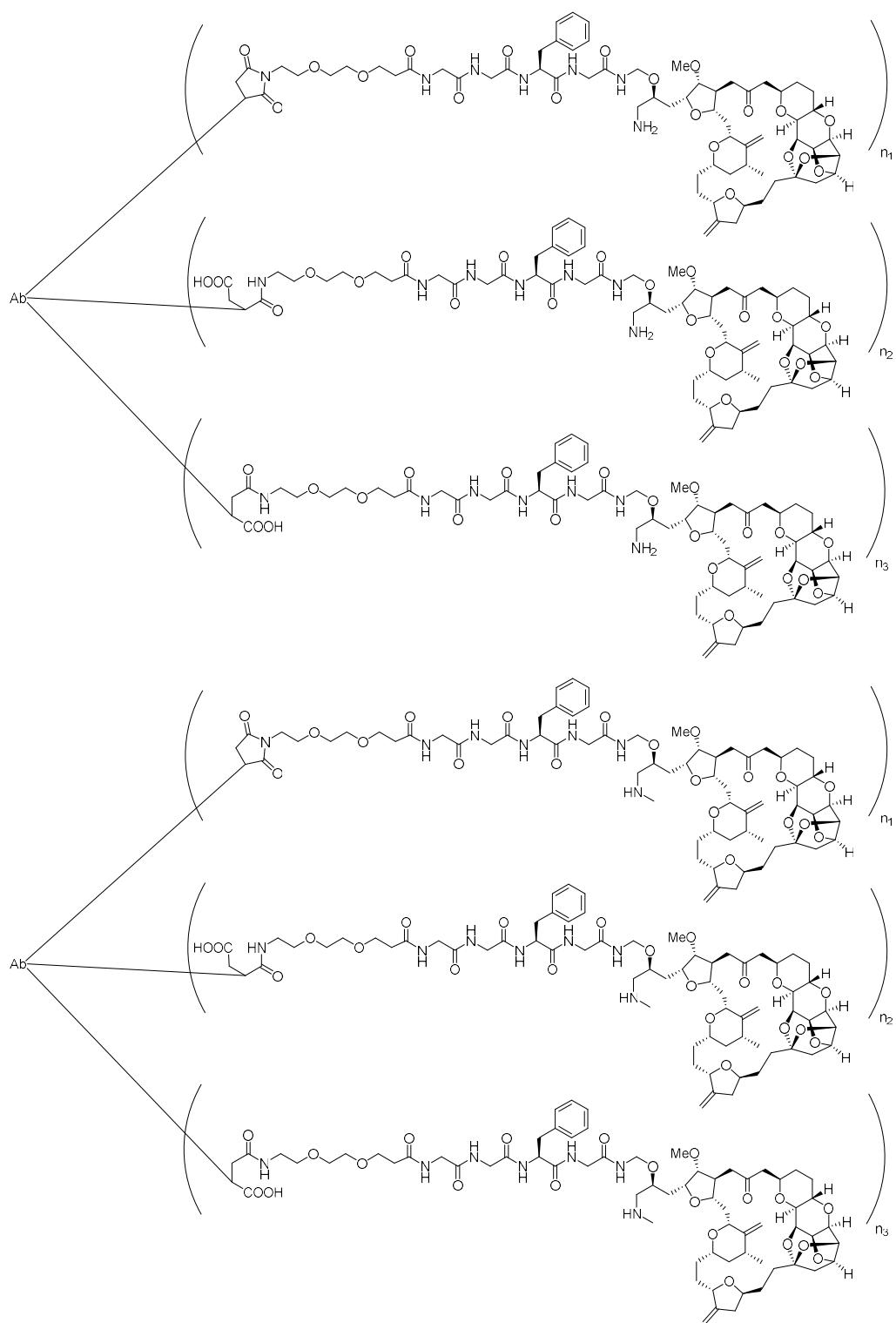
21. The ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 1-20, wherein the ligand-drug conjugate is selected from the following structures:

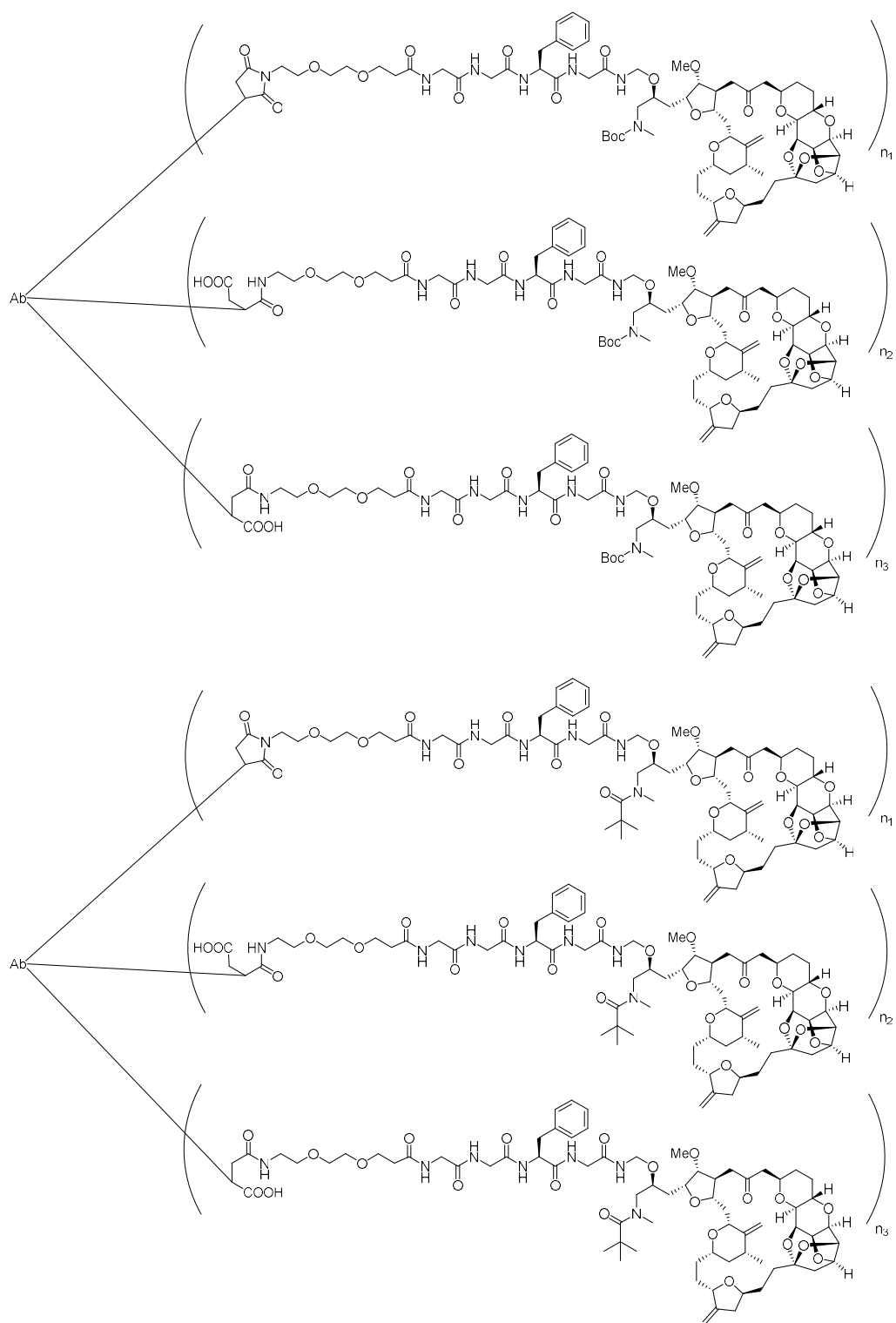


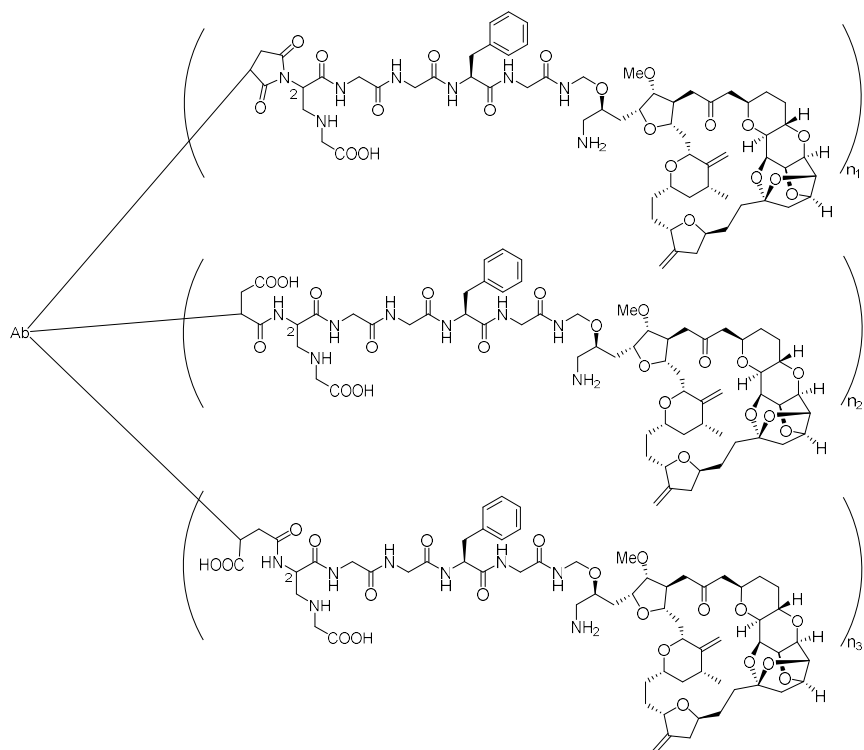
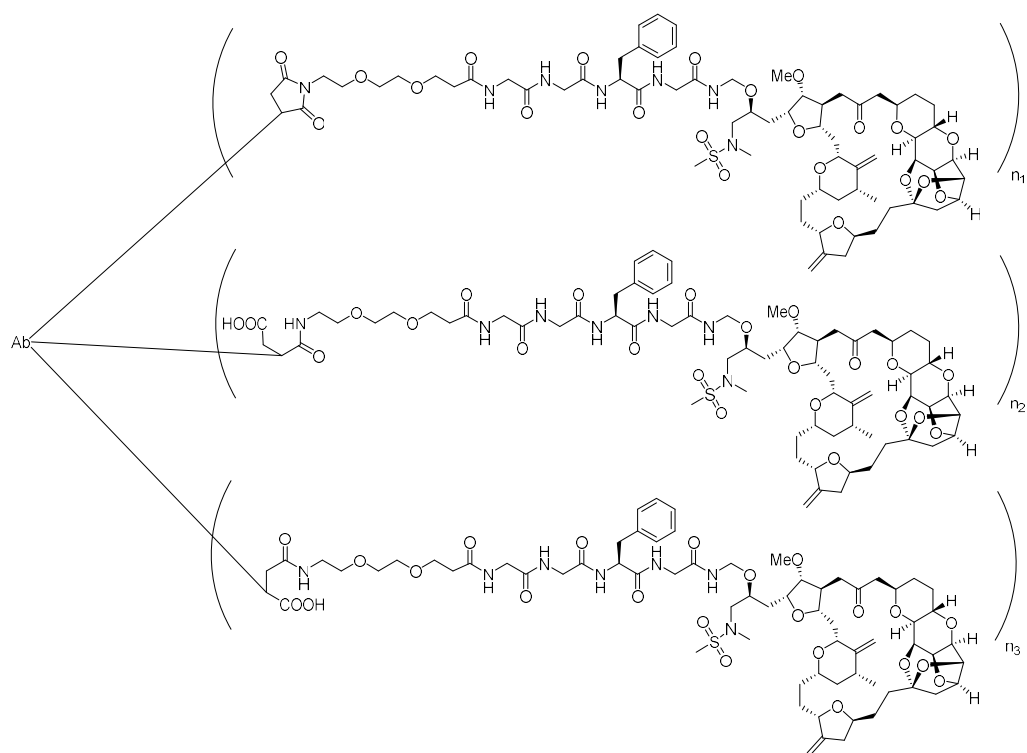


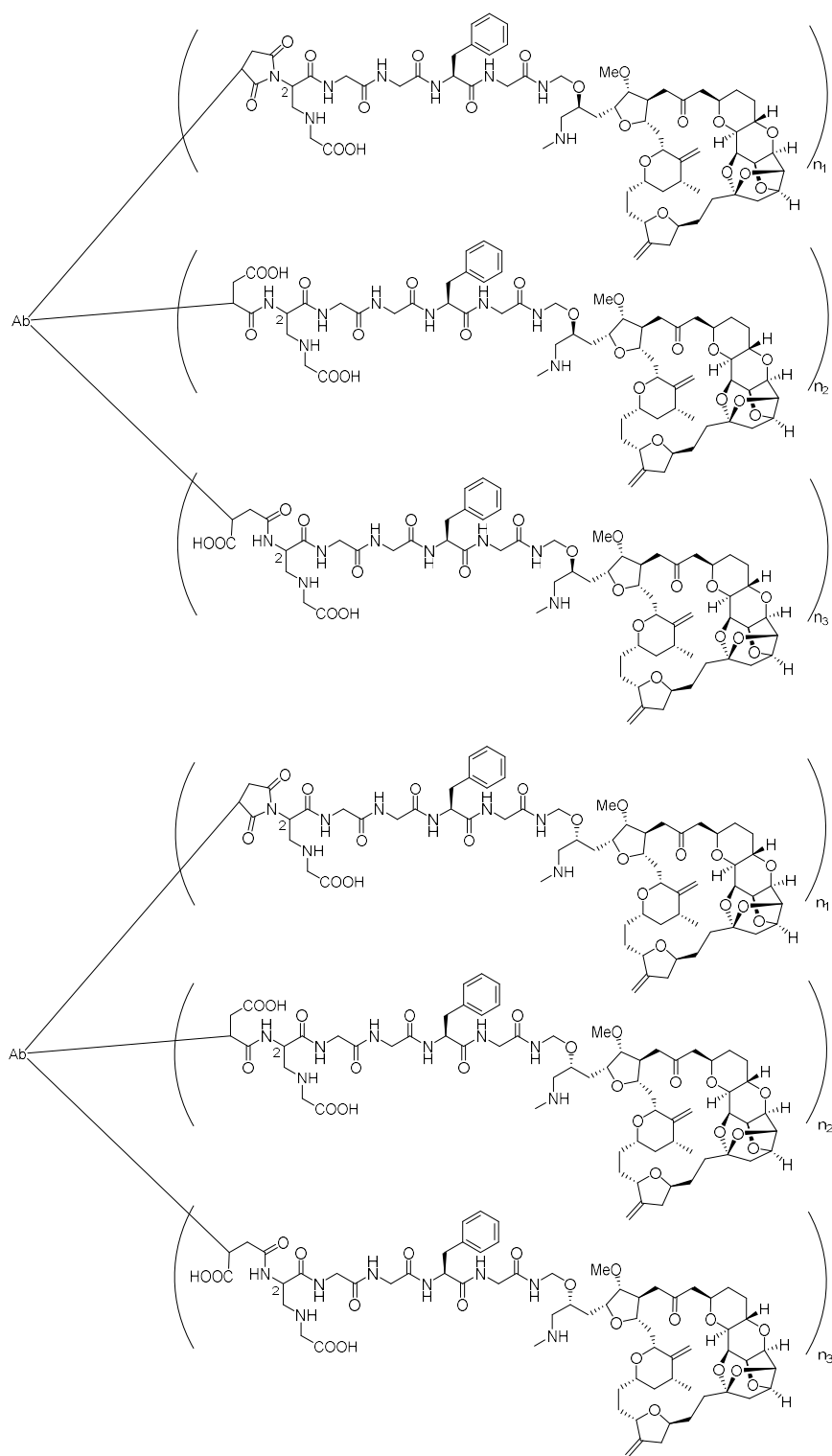


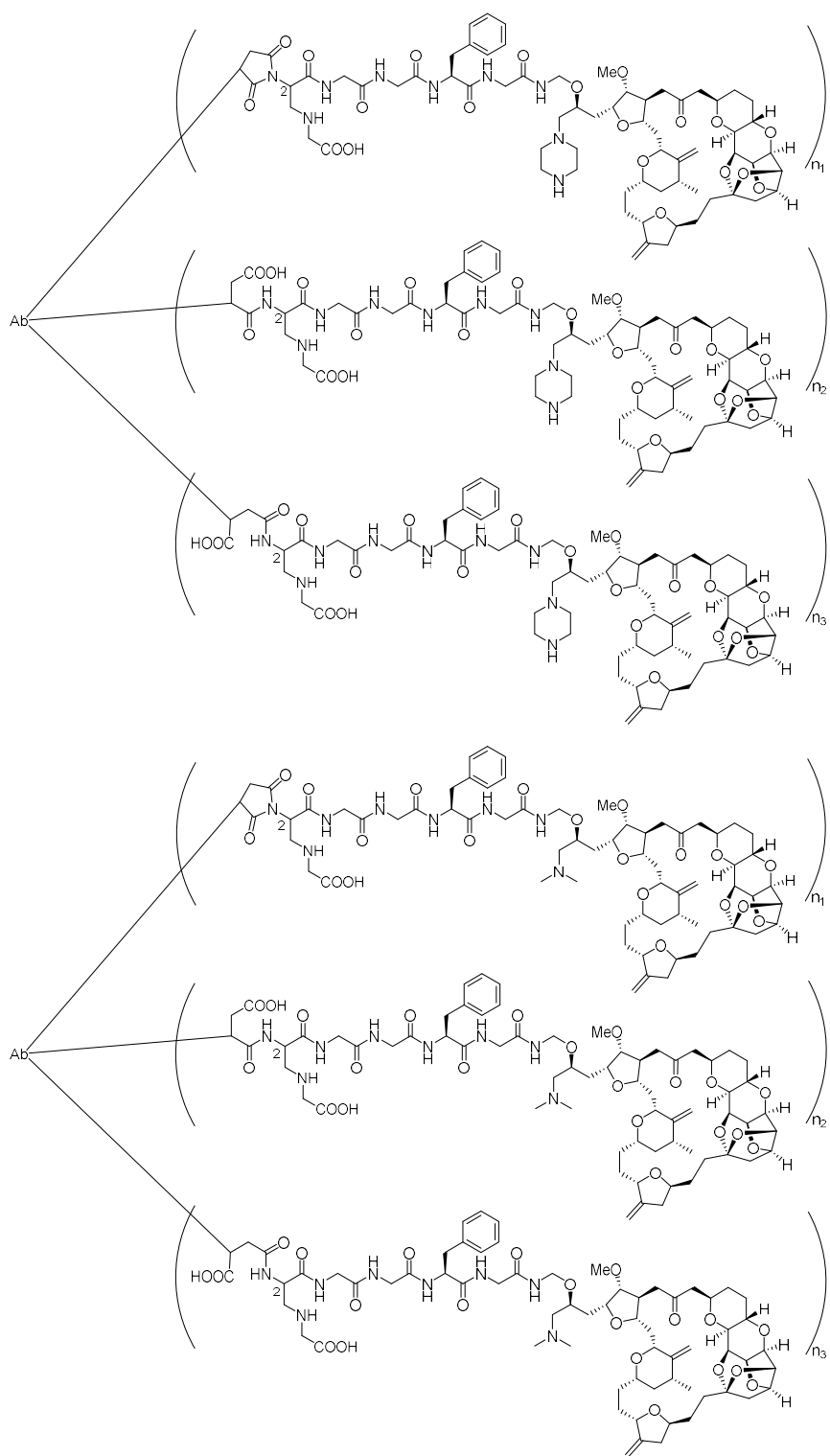


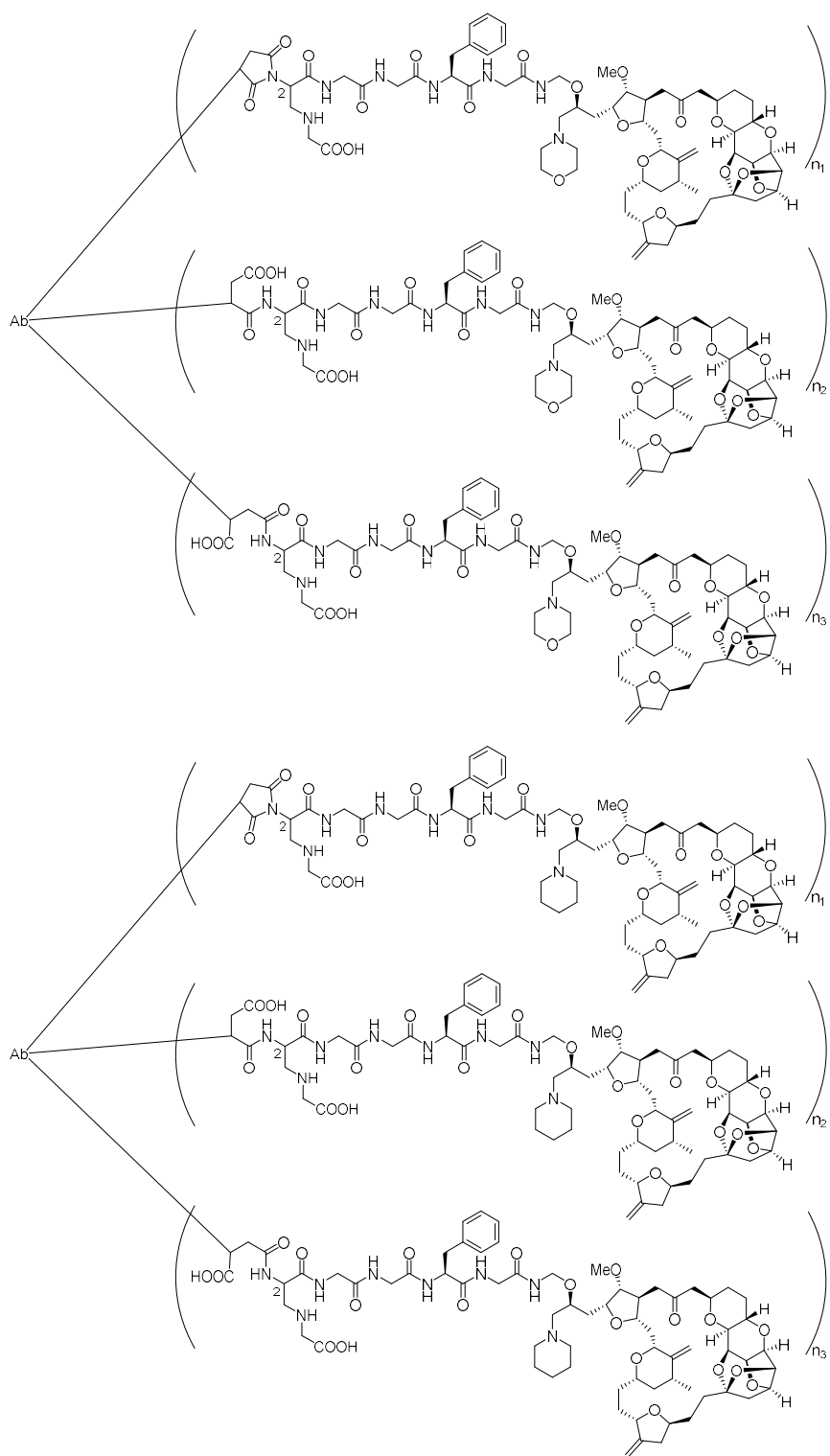


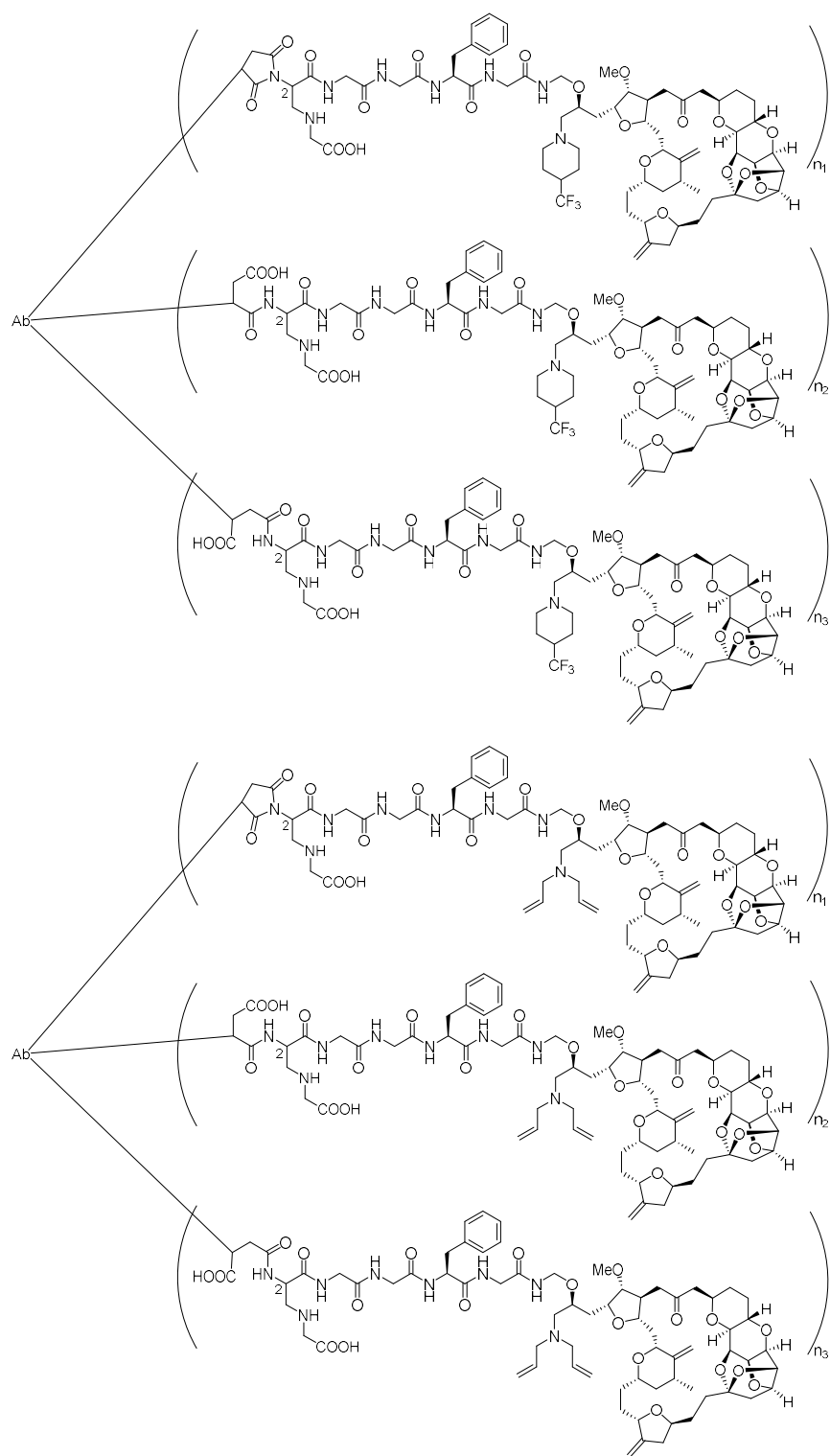


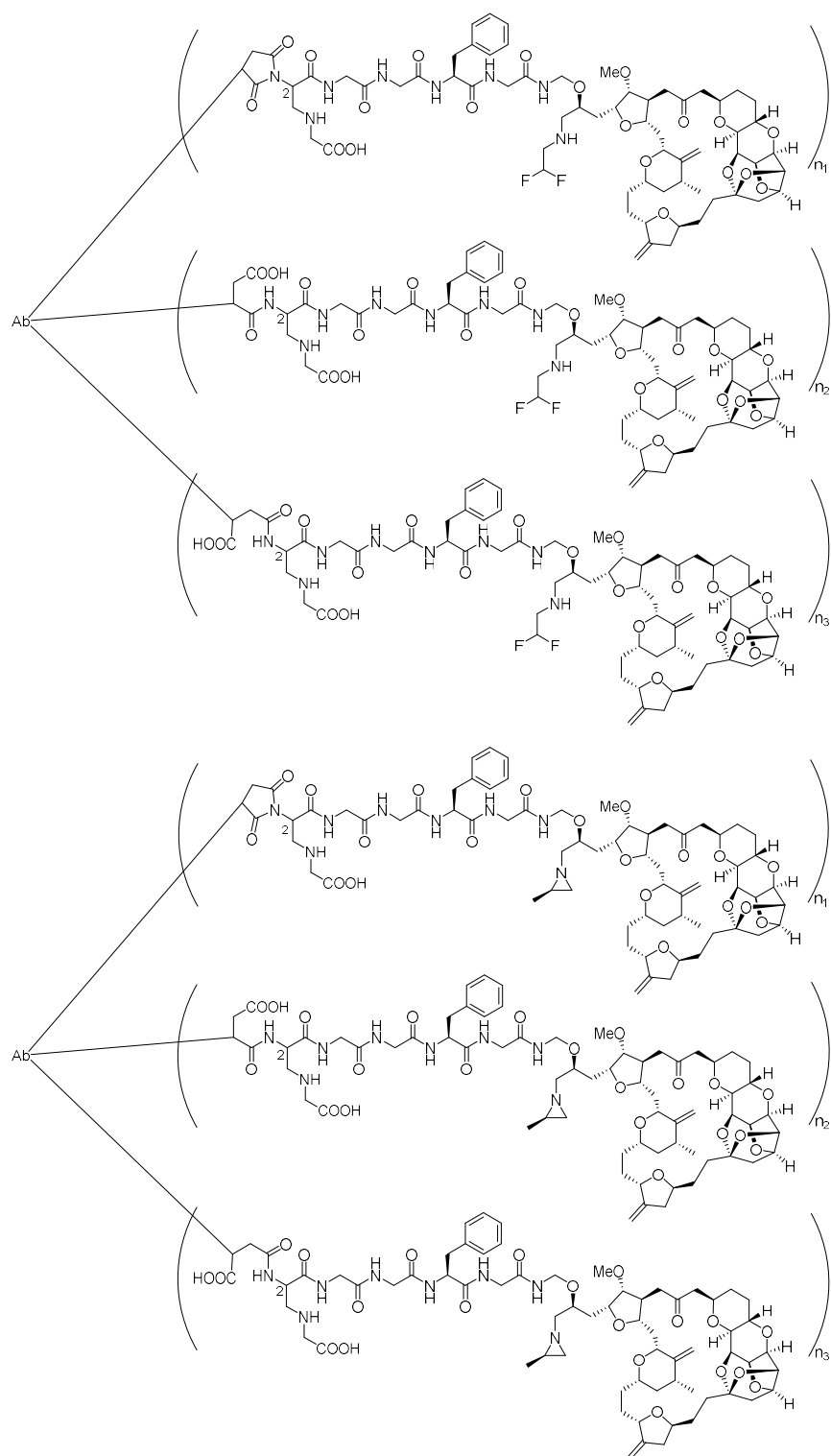


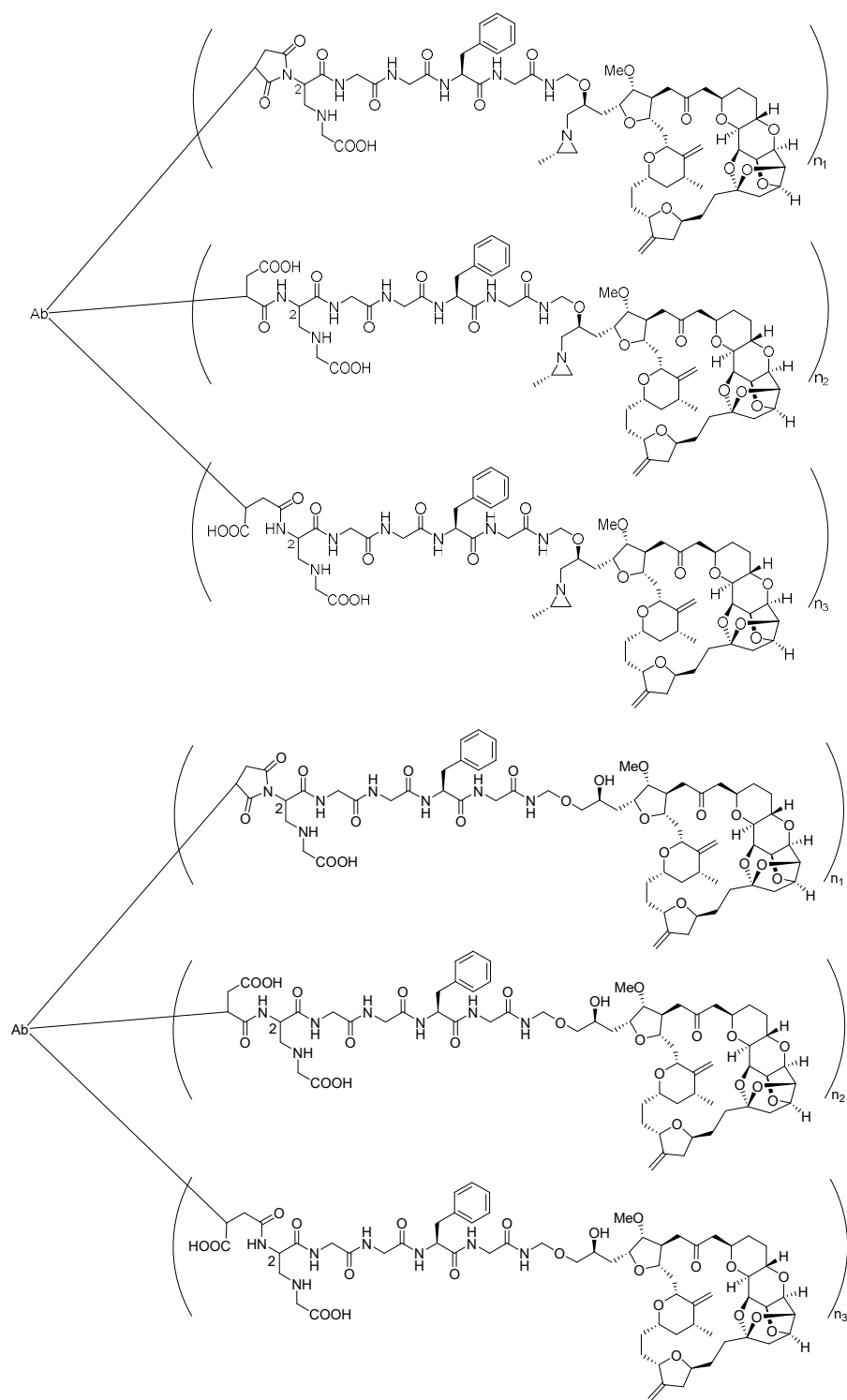


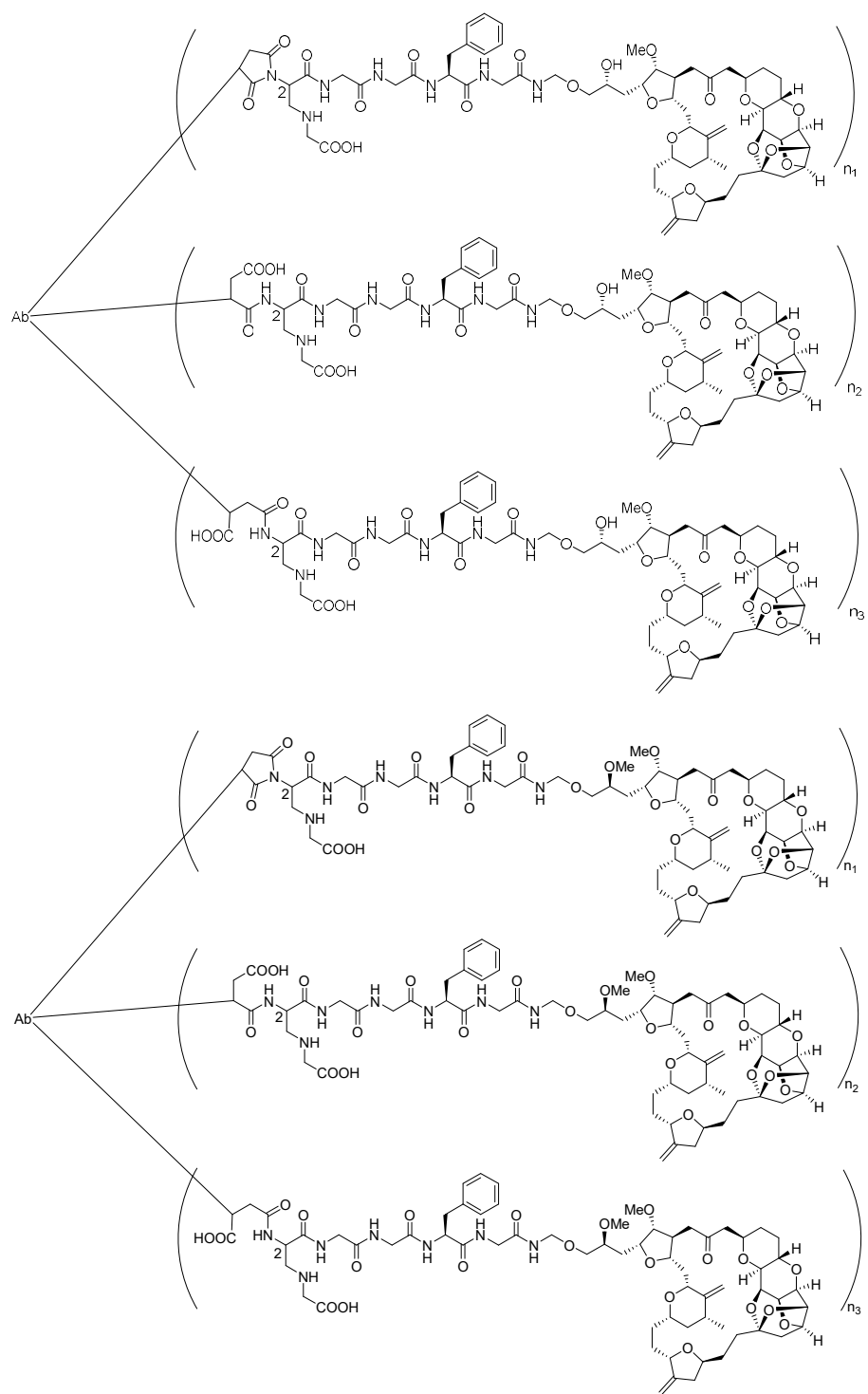


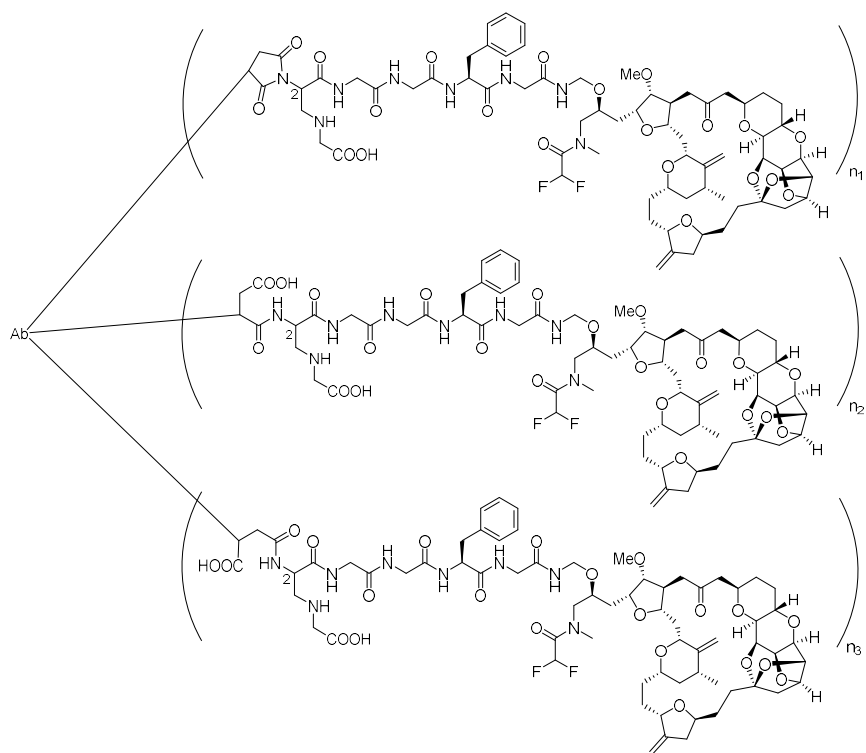
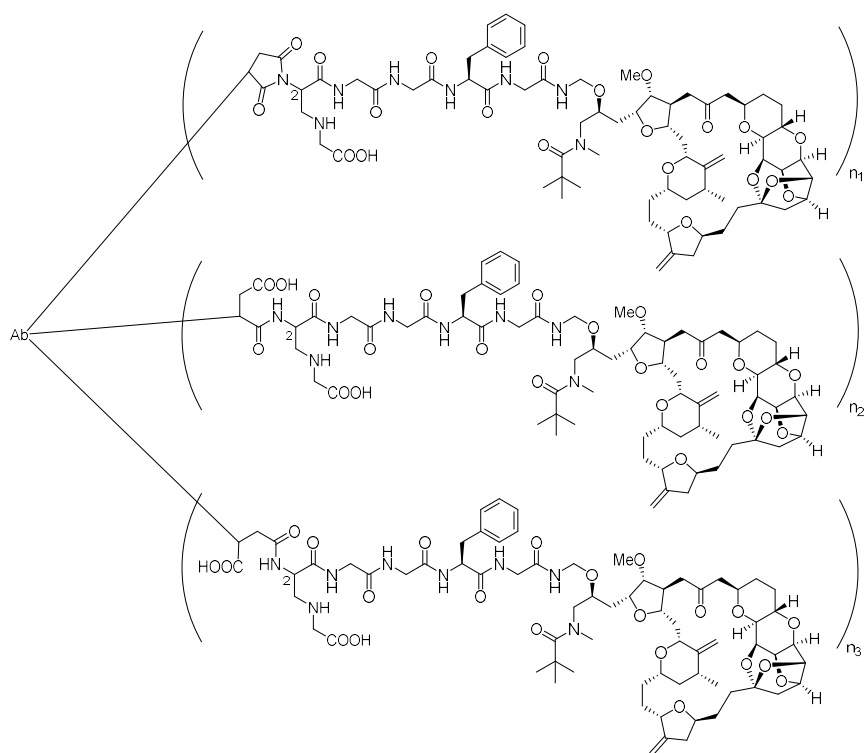


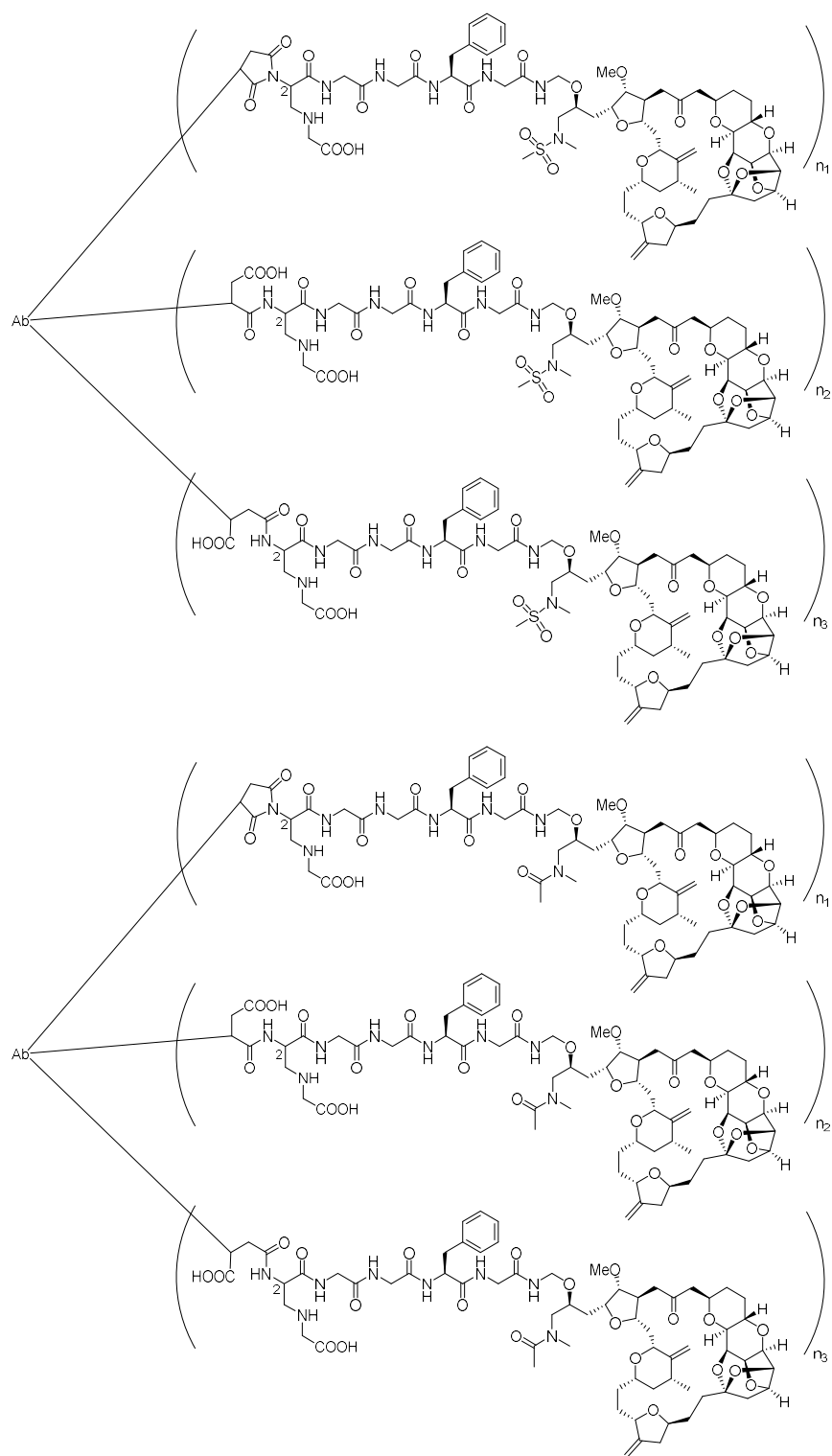


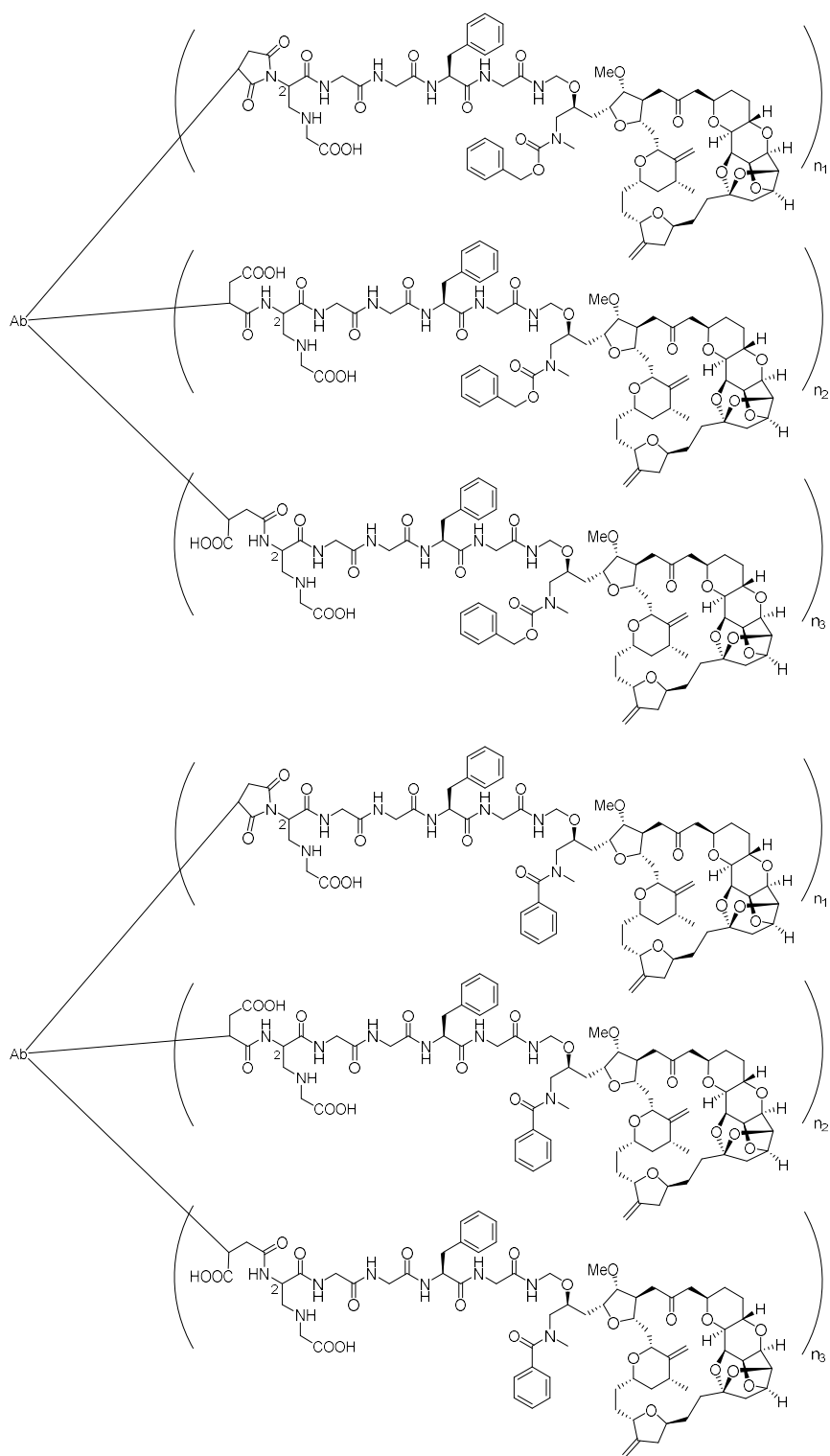


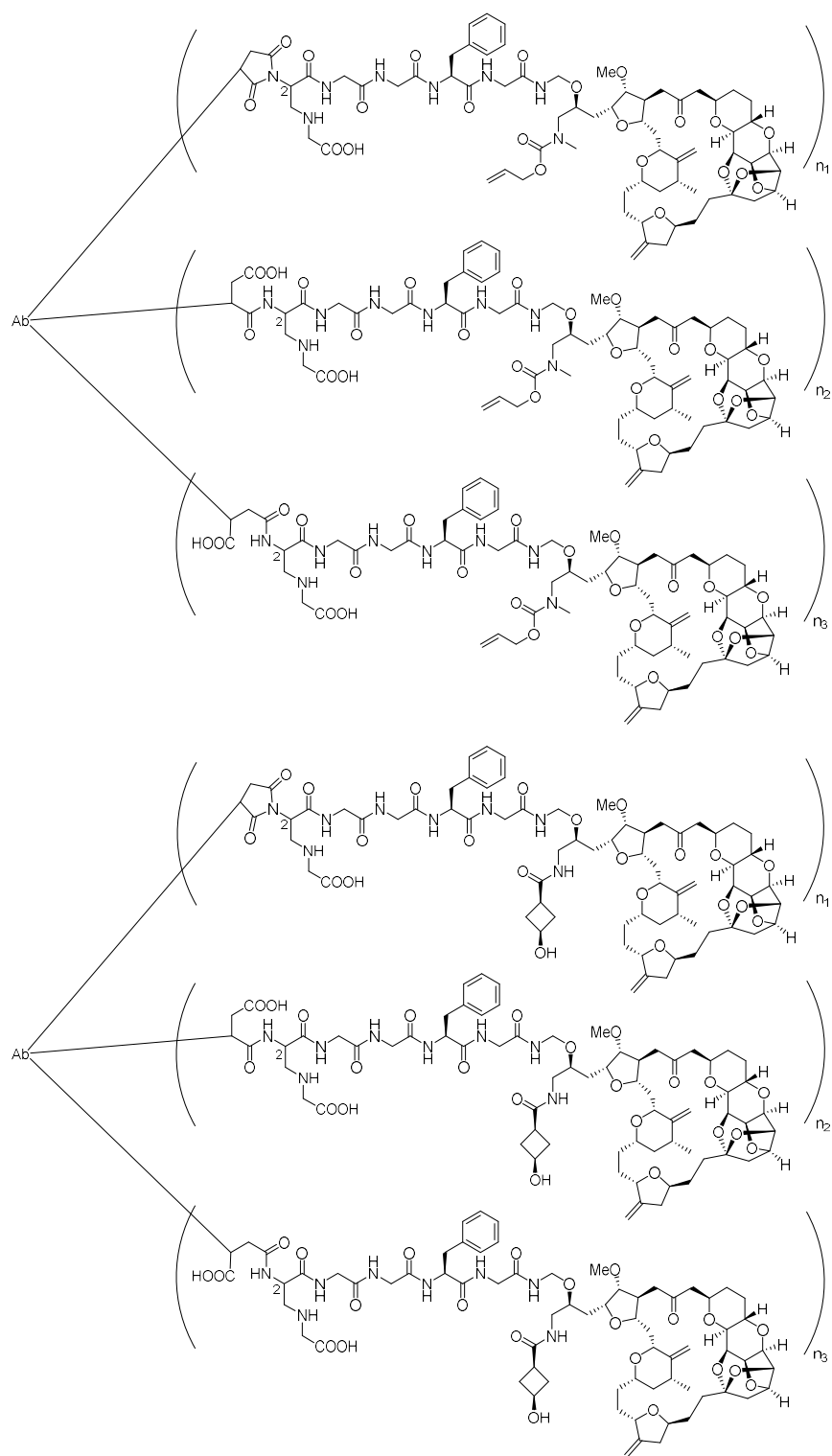


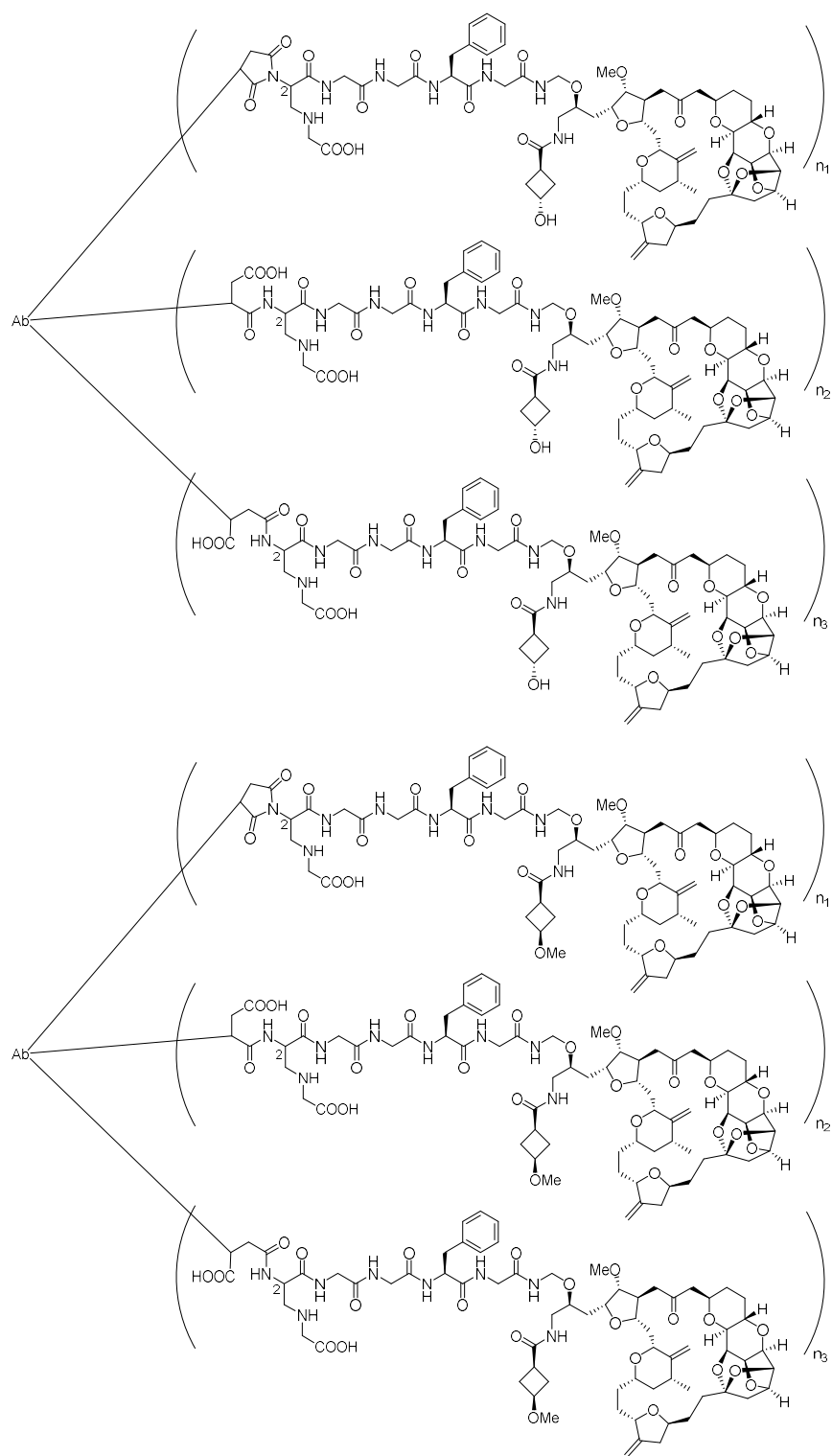


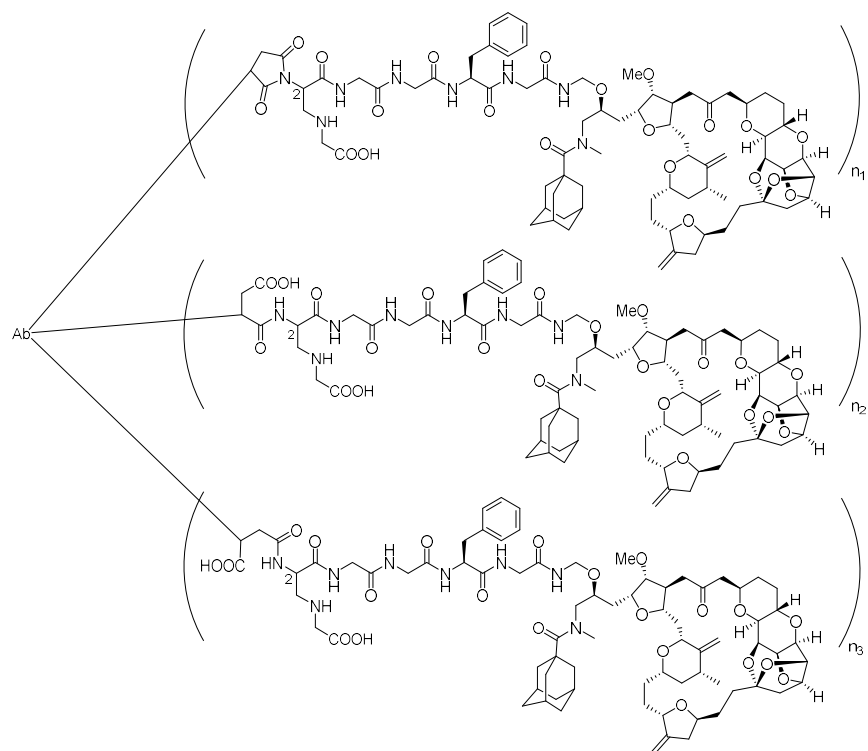


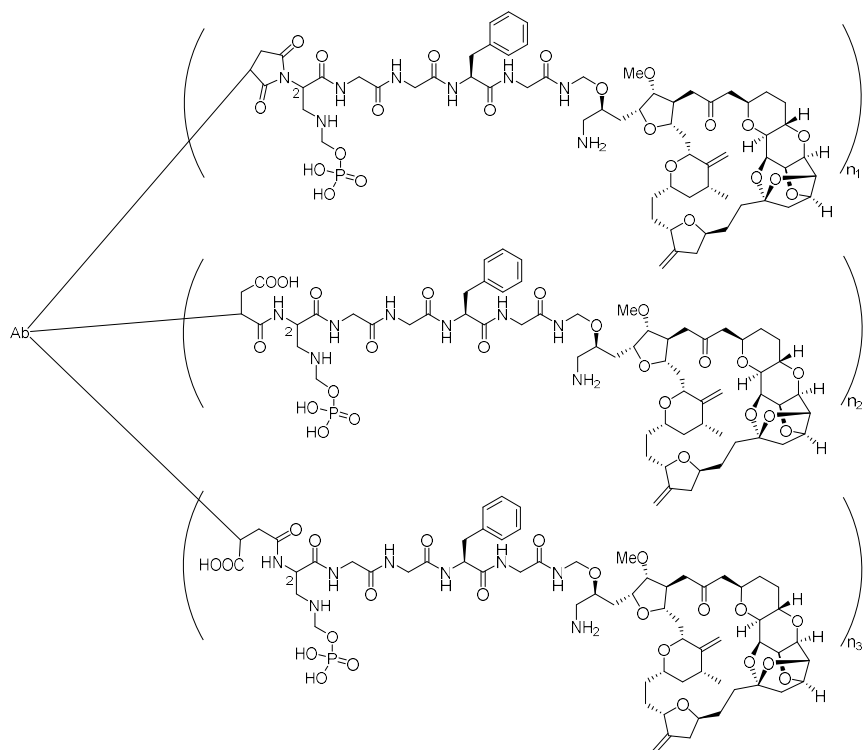
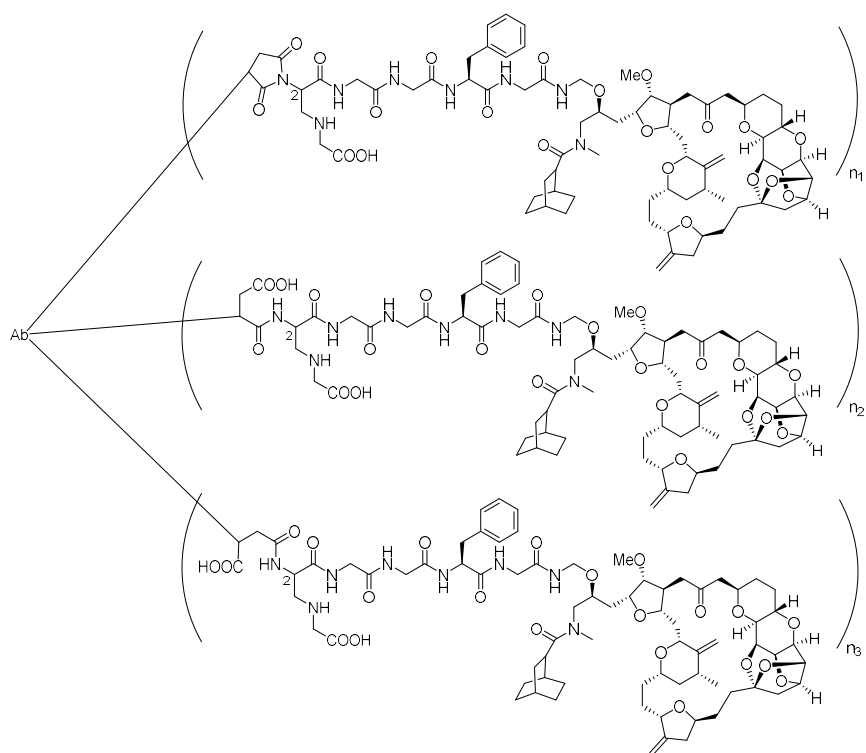


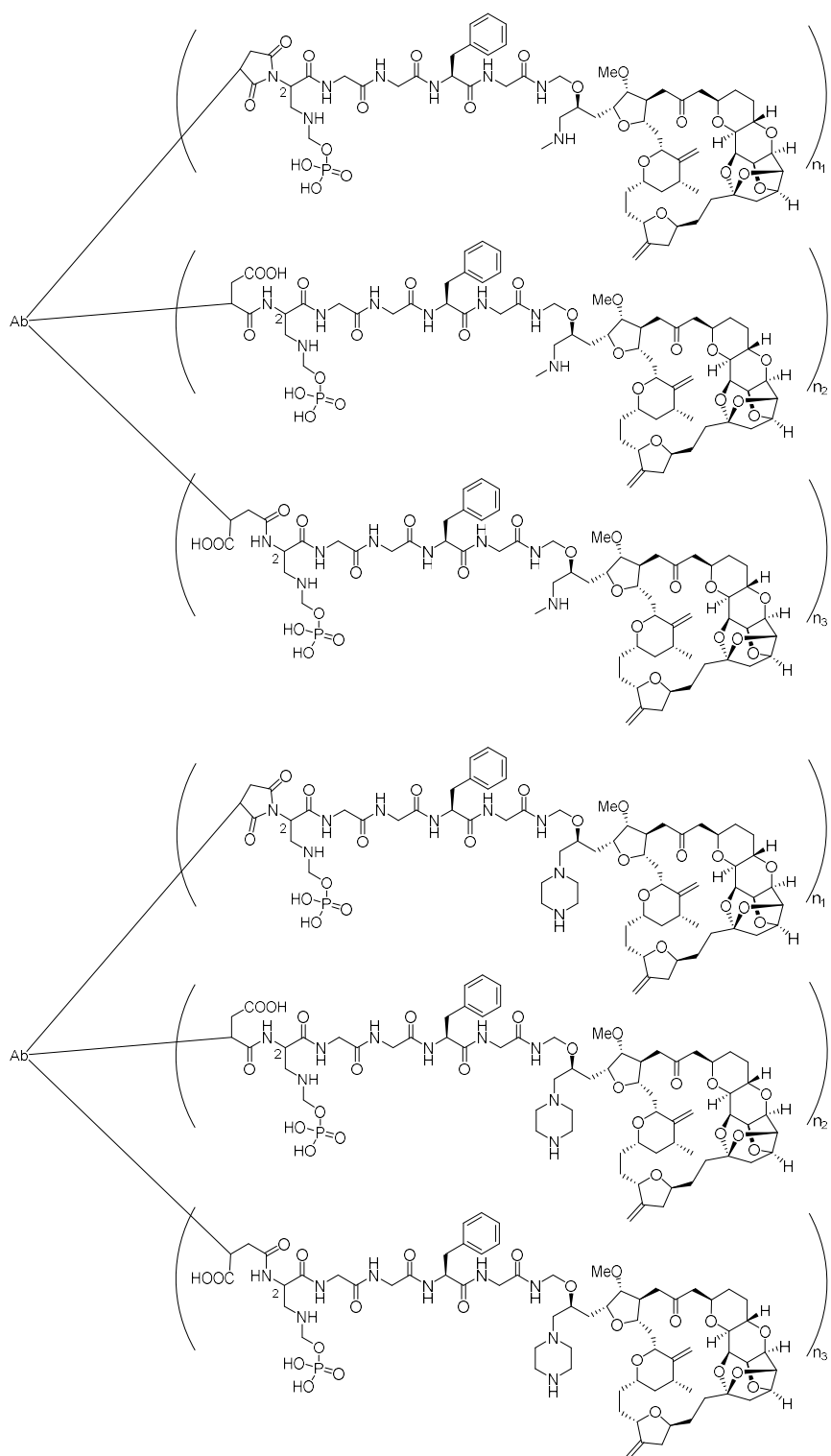


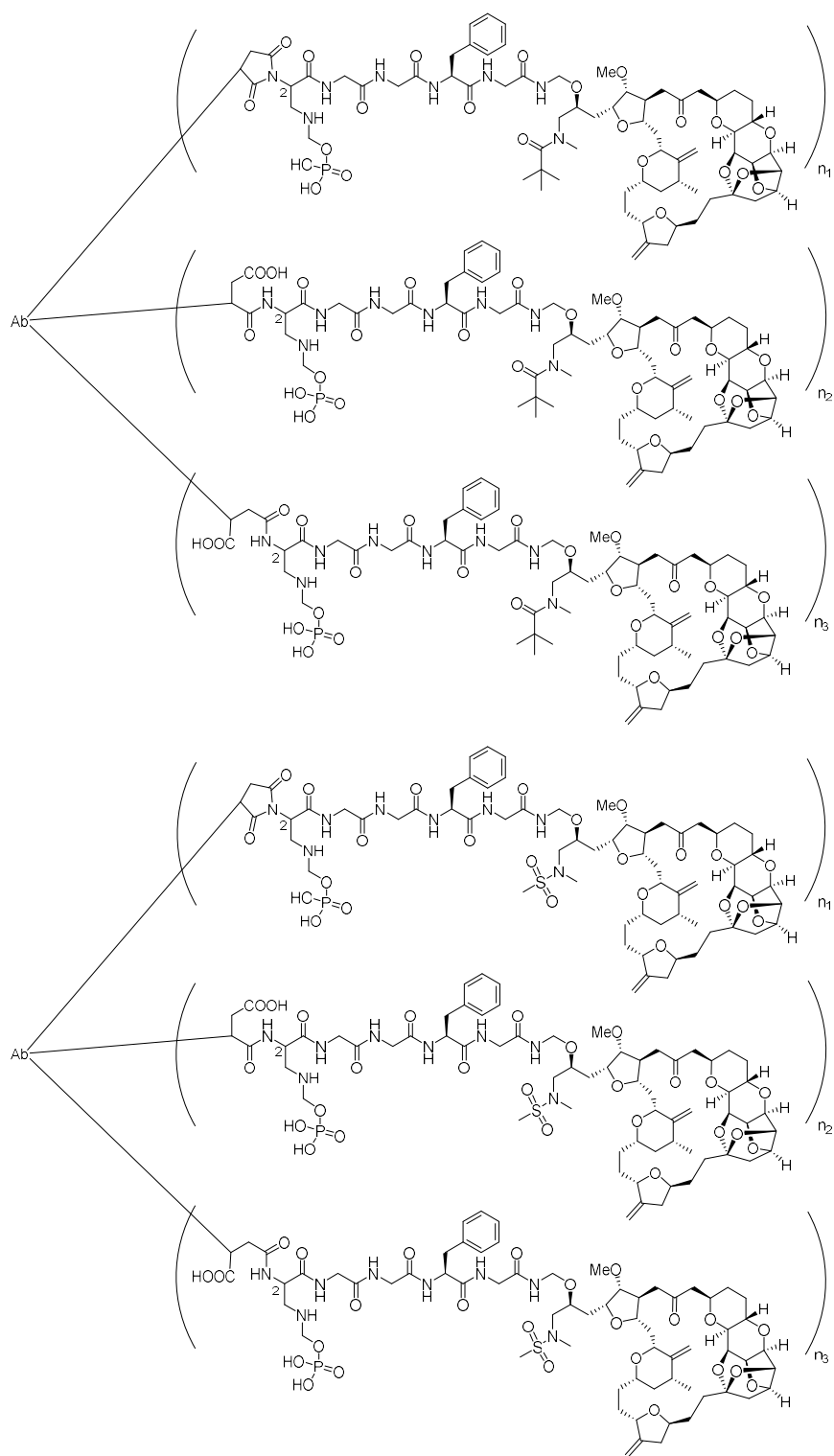


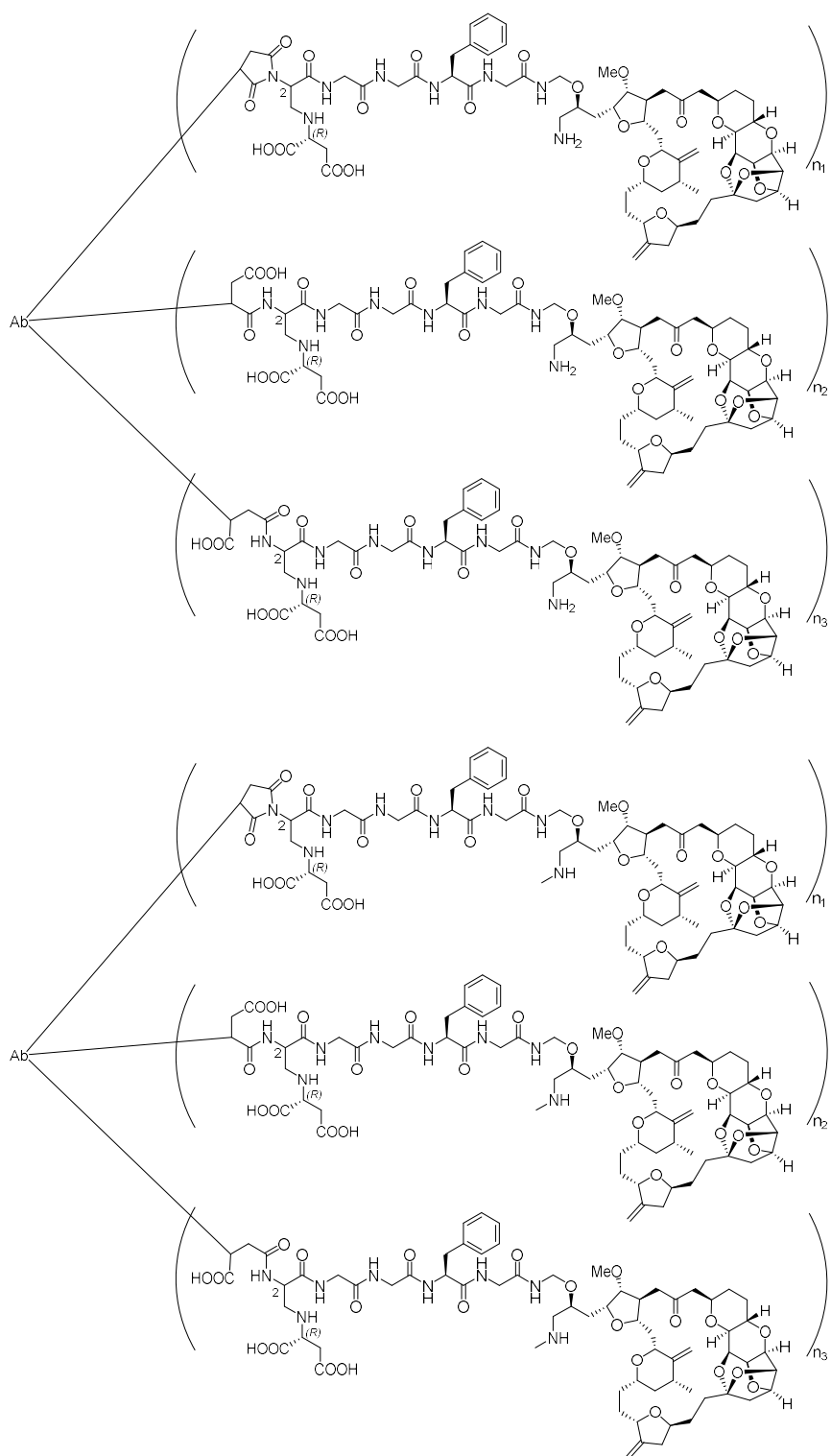


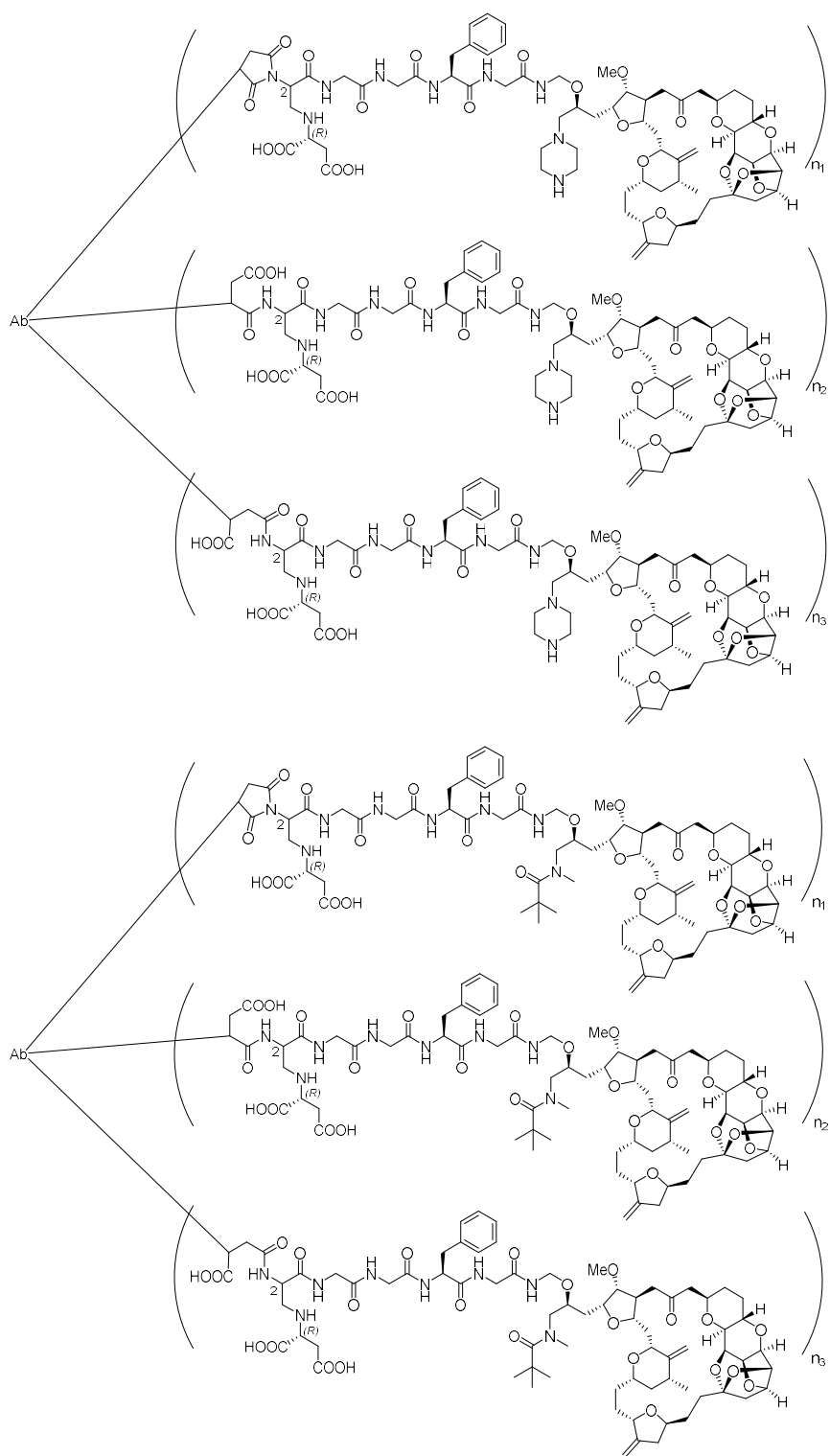


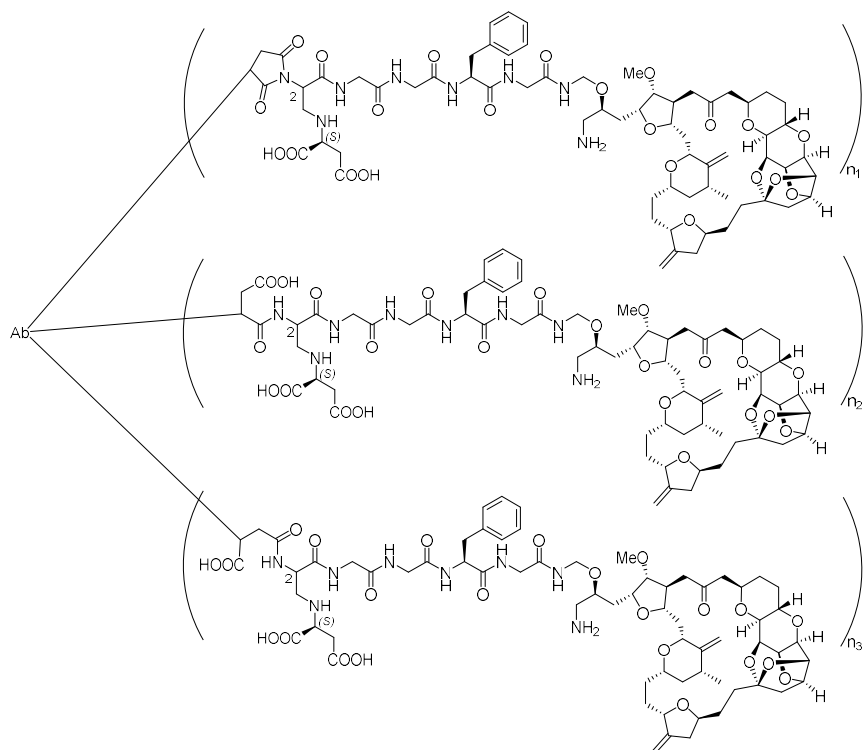
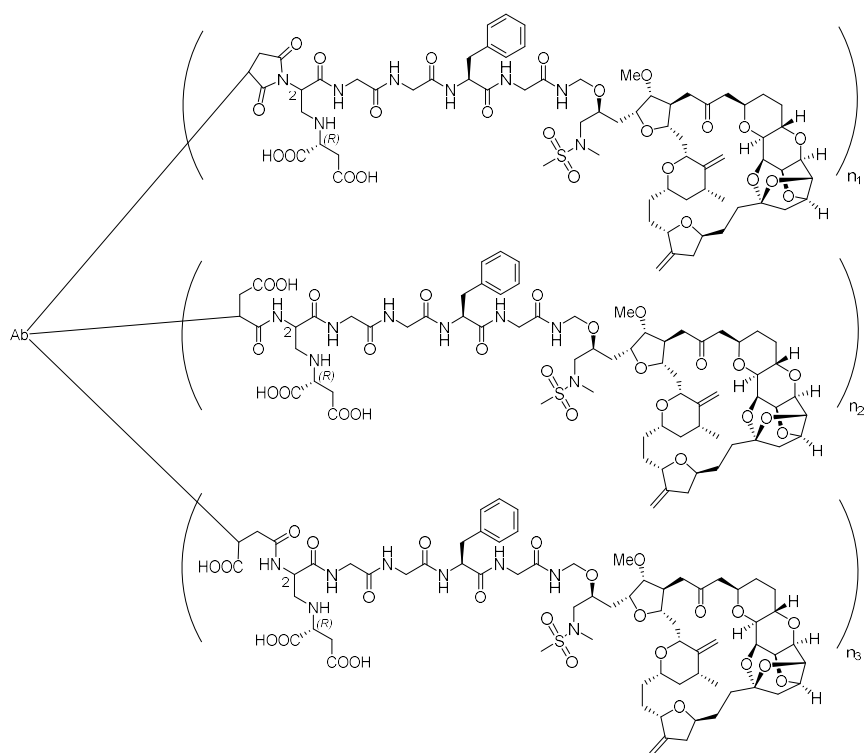


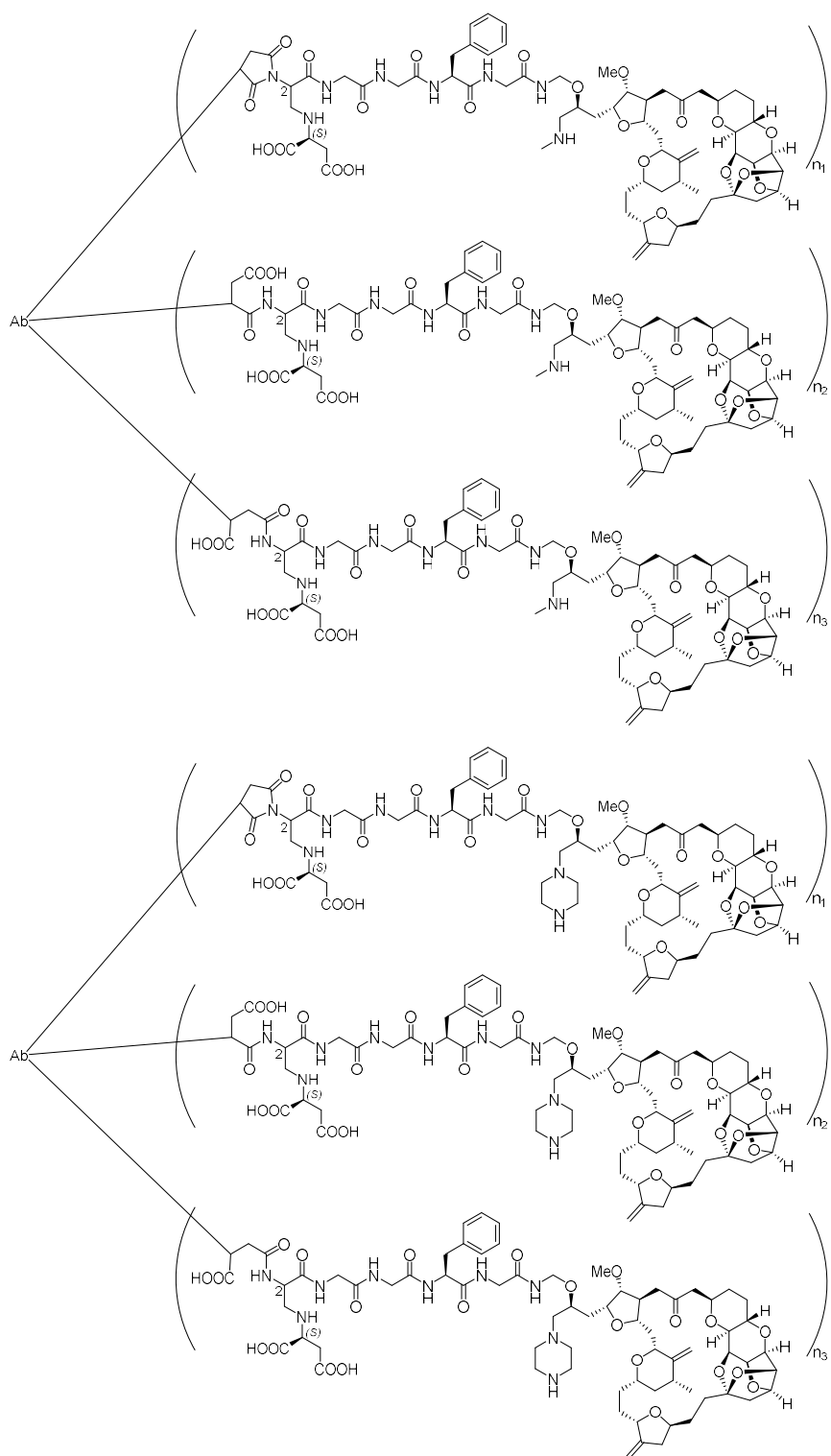


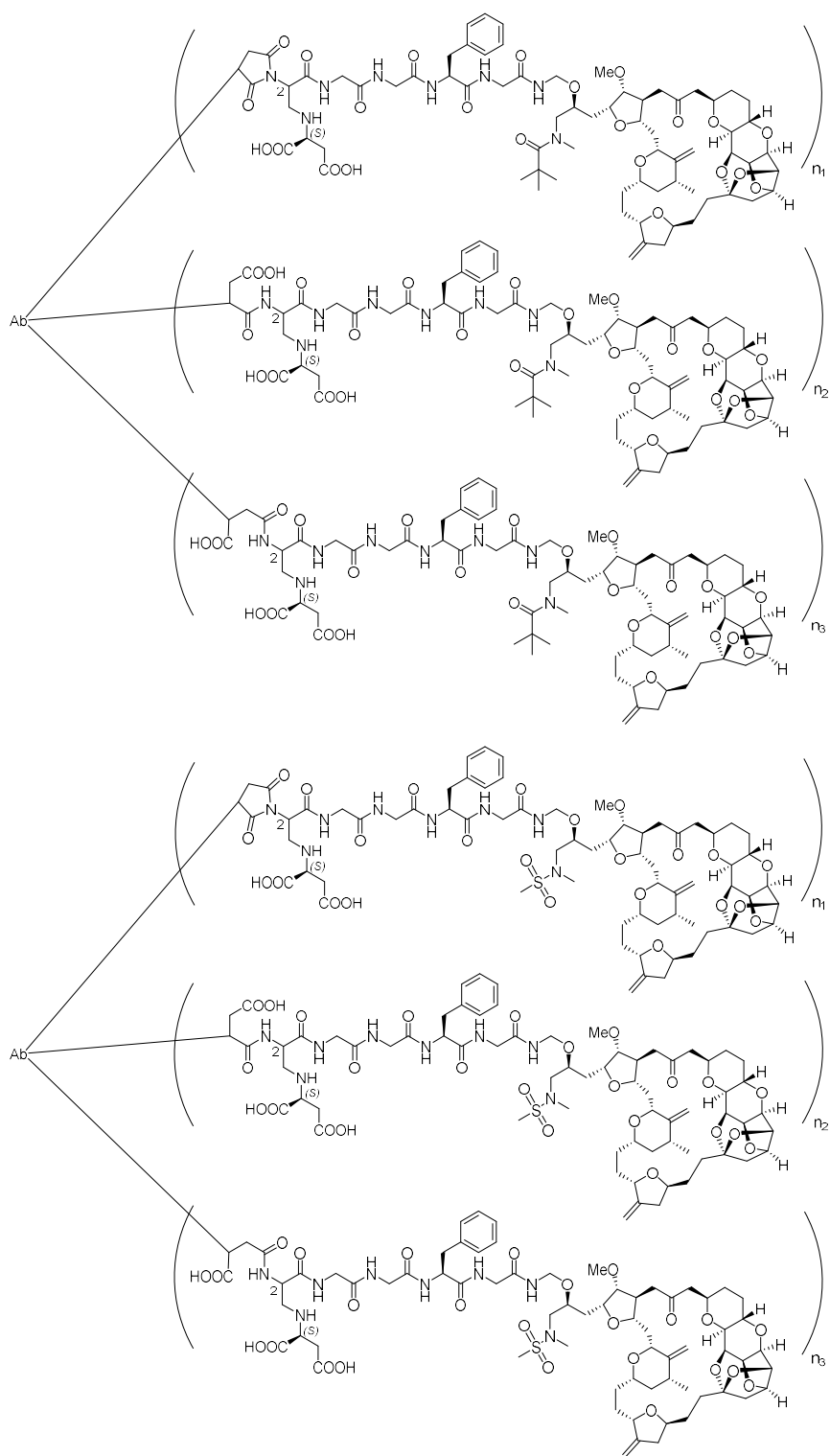


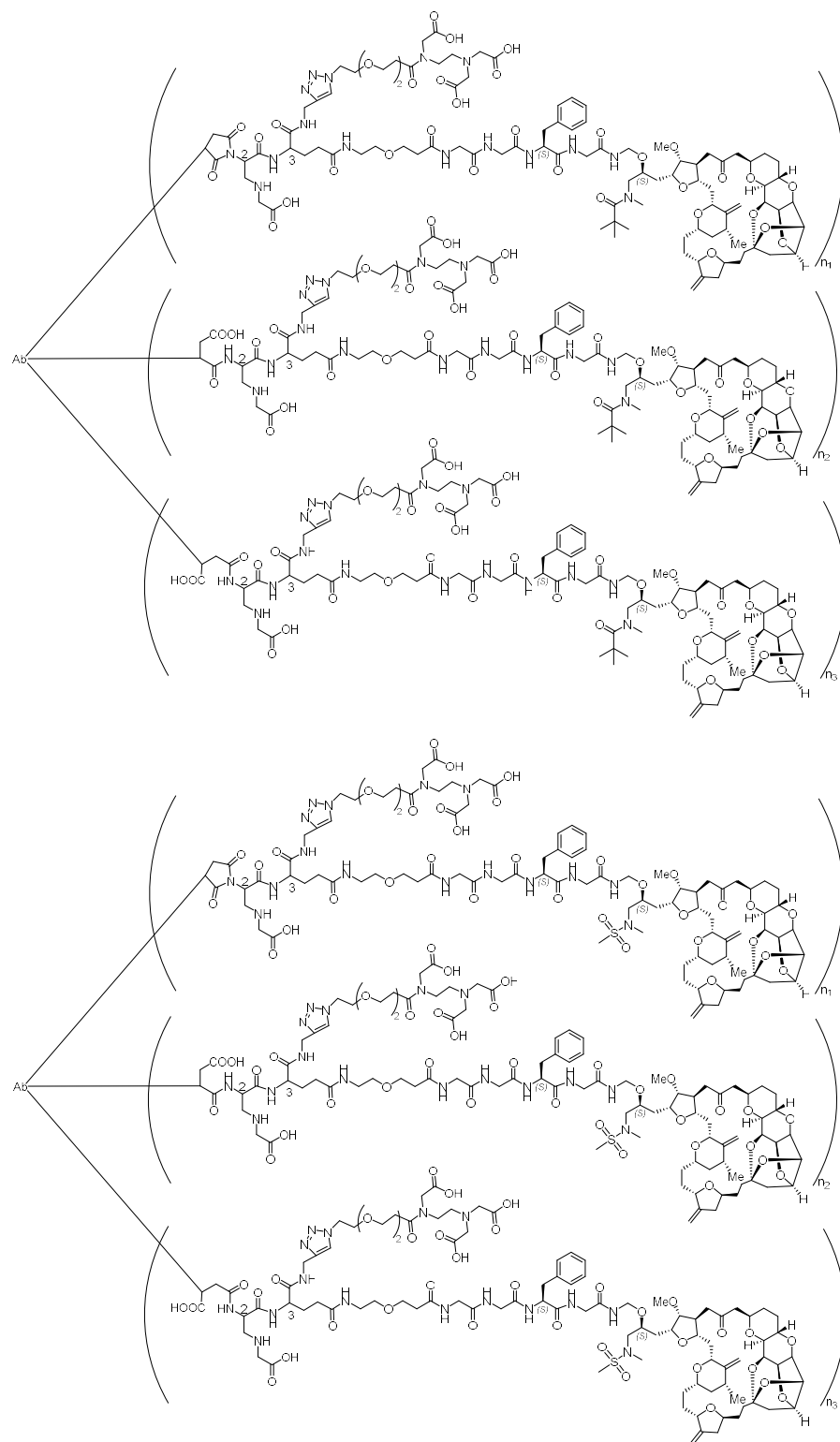


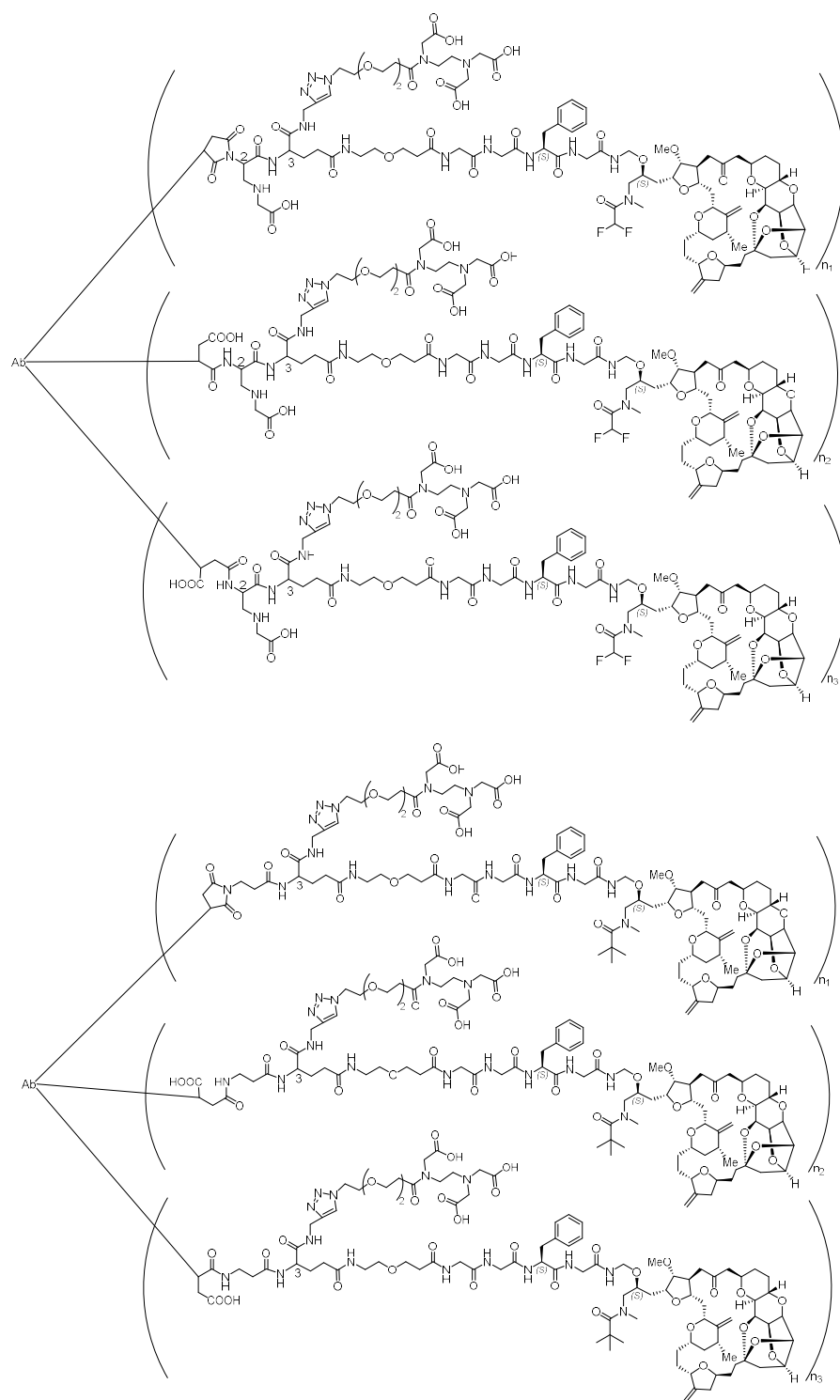


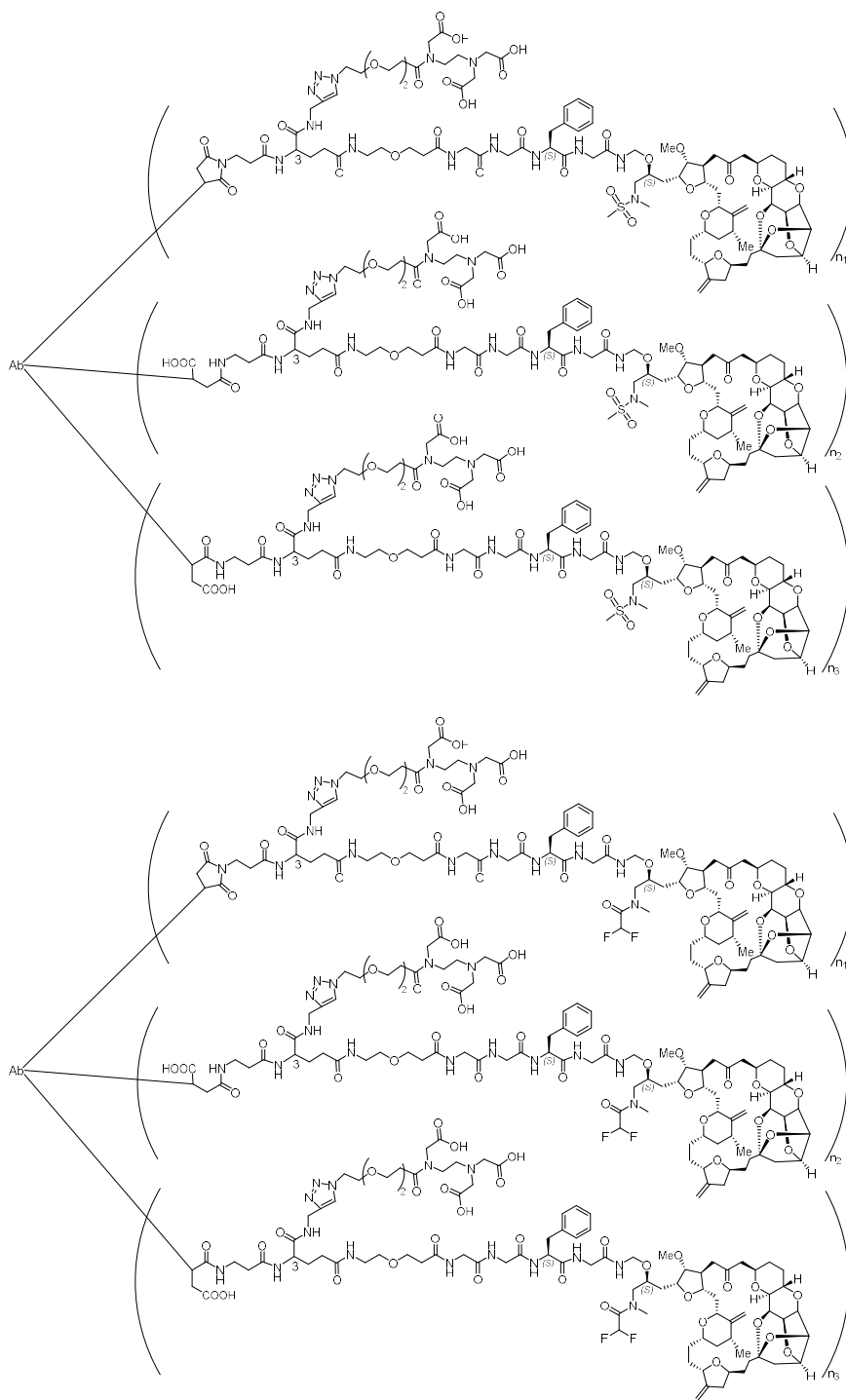


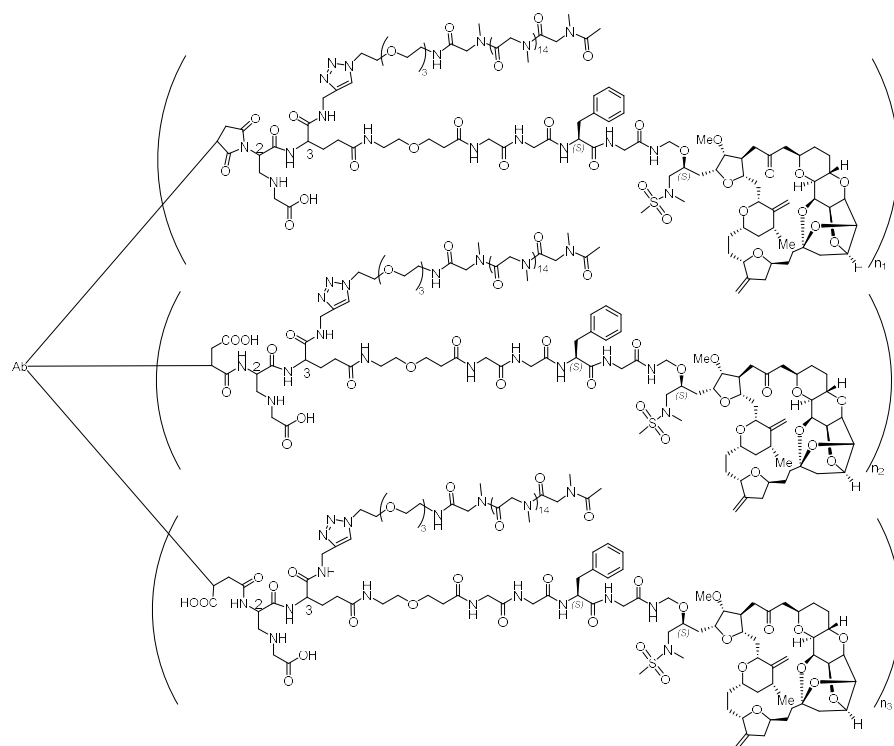
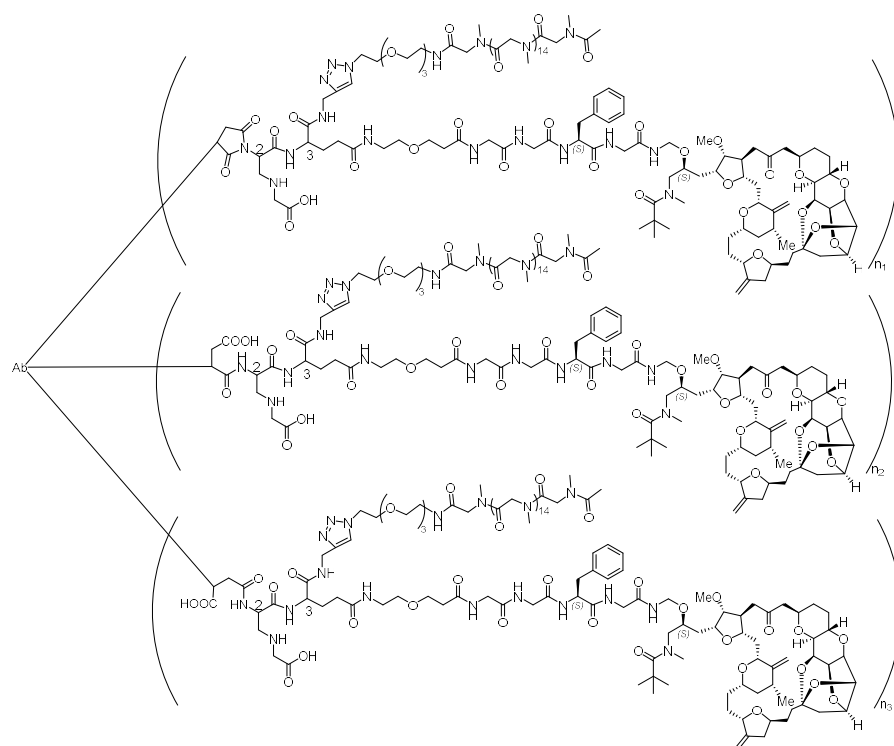


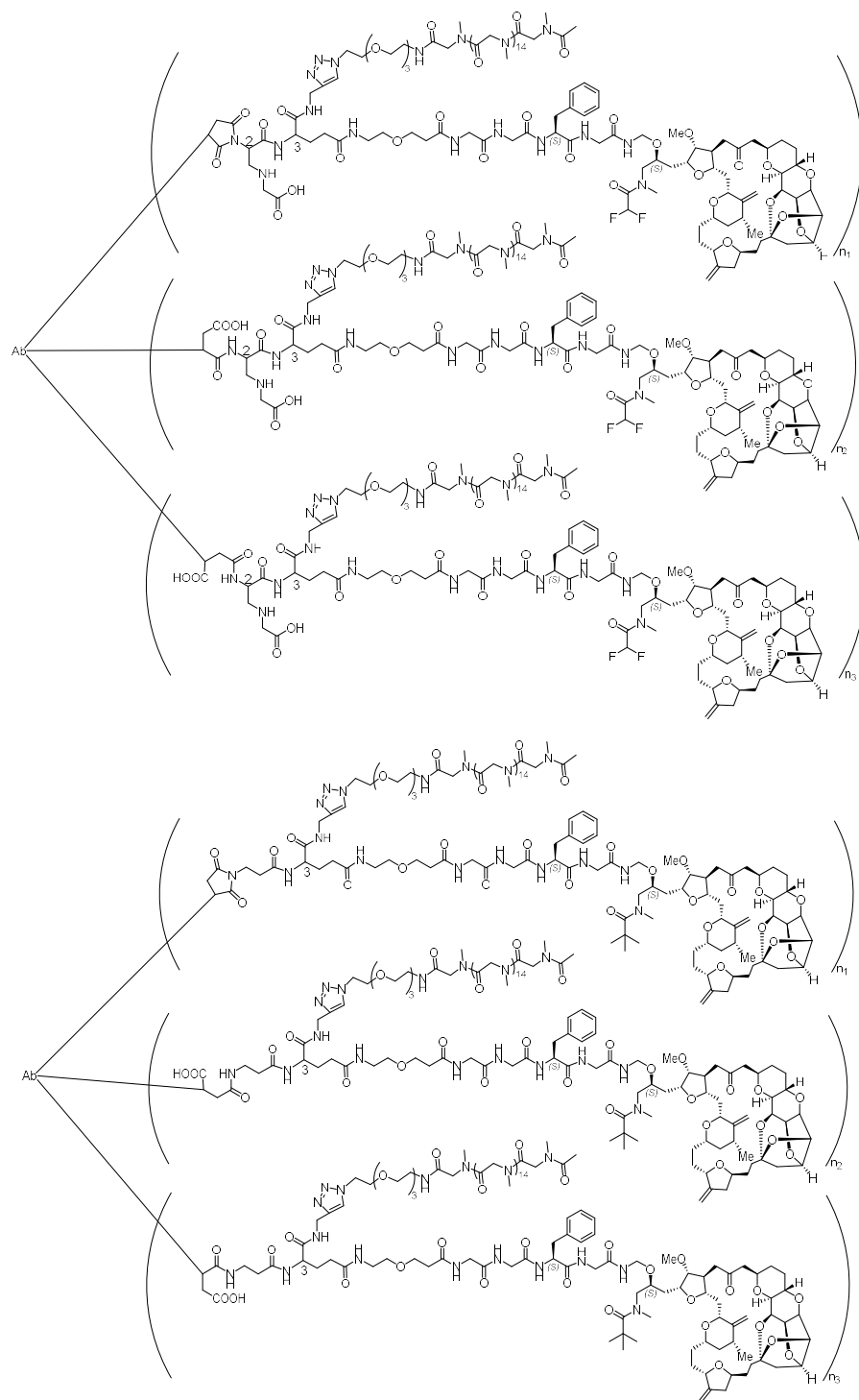


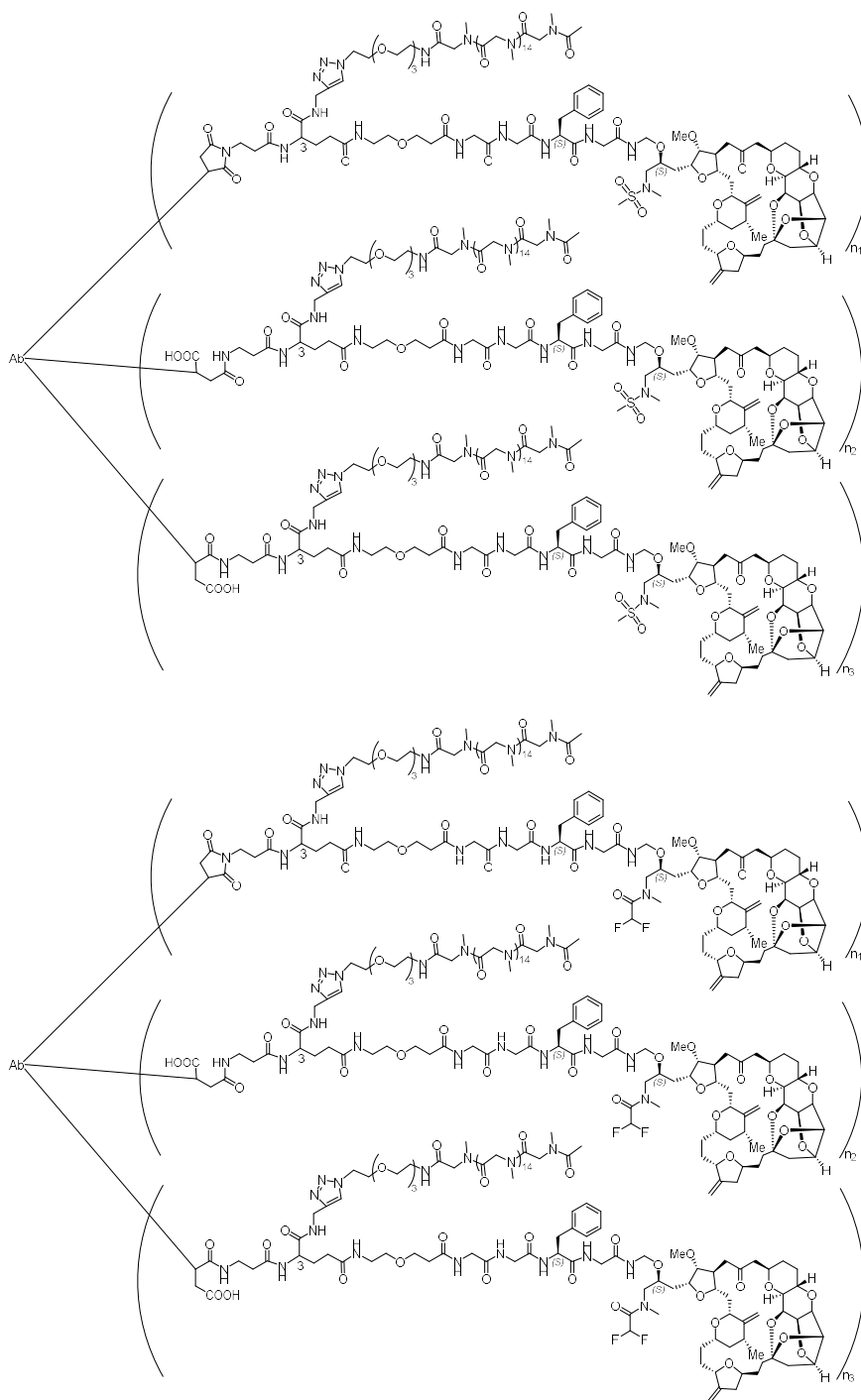


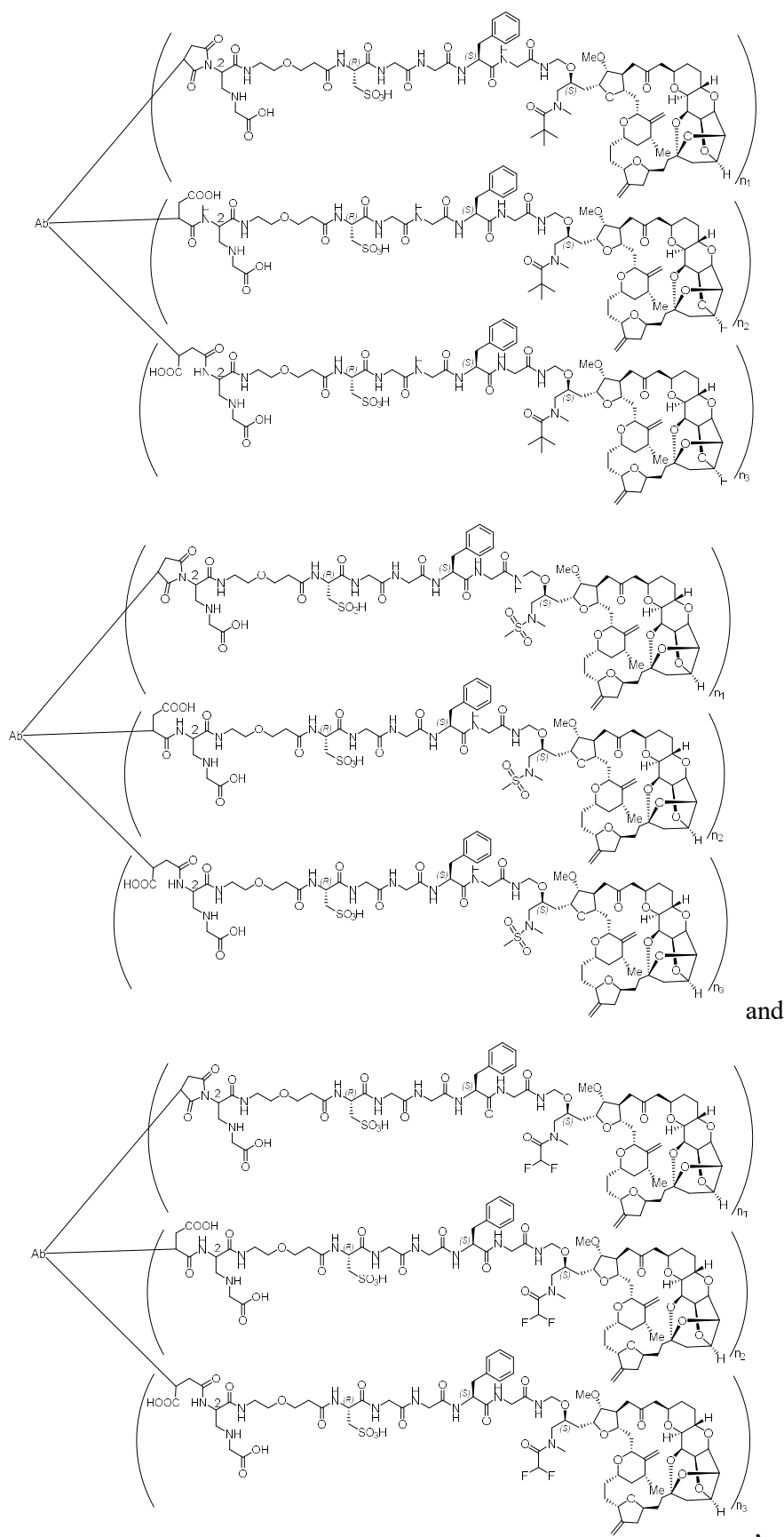












22. The ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 1-21, wherein the ligand unit Ab is an antibody, the antibody is anti-TROP-2 antibody;

preferably, the antibody consists of heavy chains and light chains, the light chains comprise CDR-L1, CDR-L2 and CDR-L3 having amino acid sequences set forth in SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3;

preferably, the heavy chains comprise CDR-H1, CDR-H2 and CDR-H3 having amino acid sequences set forth in SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6;

preferably, the light chain comprises a light chain variable region having an amino acid sequence of SEQ ID NO:7; more preferably, the light chain further comprises a light chain constant region having an amino acid sequence of SEQ ID NO:8;

preferably, the amino acid sequence of the light chain is SEQ ID NO:9;

preferably, the light chain comprises a light chain variable region having an amino acid sequence of SEQ ID NO:10; more preferably, the light chain further comprises a light chain constant region having an amino acid sequence of SEQ ID NO:11;

preferably, the amino acid sequence of the light chain is SEQ ID NO:12;

preferably, the heavy chain comprises a heavy chain variable region having an amino acid sequence of SEQ ID NO:13; more preferably, the heavy chain further comprises a heavy chain constant region having an amino acid sequence of SEQ ID NO:14;

preferably, the amino acid sequence of the heavy chain is SEQ ID NO:15.

The image shows a chemical structure of a complex molecule. On the left, there is a linear chain: $M_1-Z-\overset{\text{O}}{\parallel}{C}-\text{P}-A-G-W$. The carbonyl group is connected to a five-membered ring containing an oxygen atom. This ring is further connected to a branched amine group: $\text{N}(\text{R}_1)(\text{R}_2)-\text{CH}_2-\text{CH}(\text{MeO})-$. The amine group is connected to a large, complex polycyclic system. This system includes several fused and bridged rings, multiple oxygen atoms (some as ether linkages, some as part of a cyclic acetal), and a methoxy group (MeO). The structure is highly detailed, showing stereochemistry with wedges and dashes.

The image shows a complex chemical structure, likely a natural product or a synthetic molecule. It features a large, multi-ring system with various functional groups. Key features include a methoxy group (MeO), an OR₃ group, and a carbonyl group (C=O). The structure is labeled with M₁-Z-(C=O)_p-A-G-W, suggesting a specific chemical context or synthesis. The molecule has a complex, polycyclic core with several oxygen-containing rings and a long, flexible side chain.

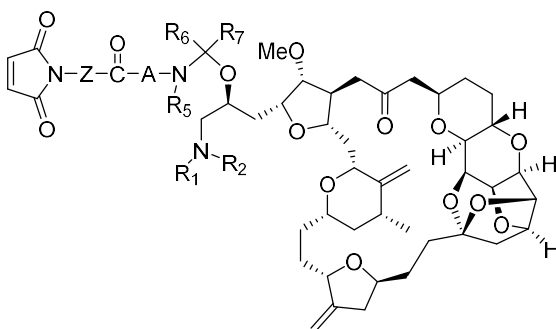
Formula IX

wherein,

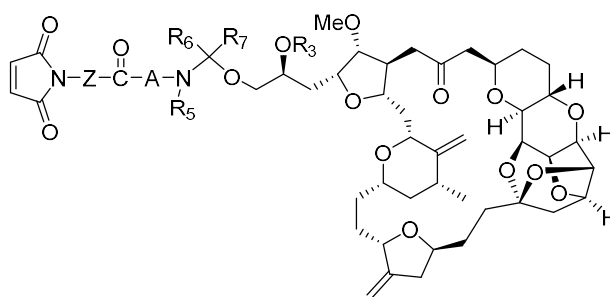
M₁ is a linker unit, and

the Z, p, A, G, W, R₁, R₂ and R₃ are as defined in any one of claims 1-13.

24. The linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof according to claim 23, wherein the linker-drug conjugate has a structure represented by formula VIII-1 or formula IX-1:



Formula VIII-1



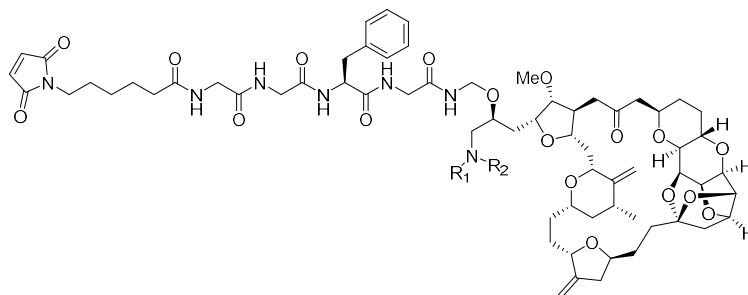
Formula IX-1

wherein,

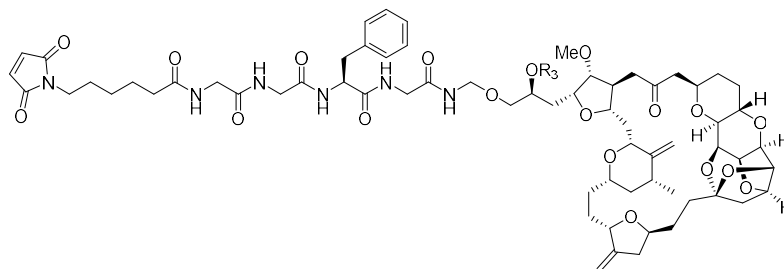
the Z, A, R₁, R₂, R₃, R₅, R₆ and R₇ are as defined in any one of claims 1-13.

25. The linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the

mixture thereof, or the pharmaceutically acceptable salt or solvate thereof according to claim 23 or 24, wherein the linker-drug conjugate has a structure represented by formula VIIIa or formula IXa:



Formula VIIIa

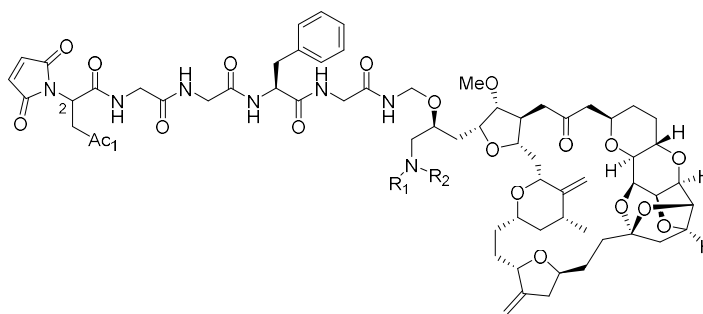


Formula IXa

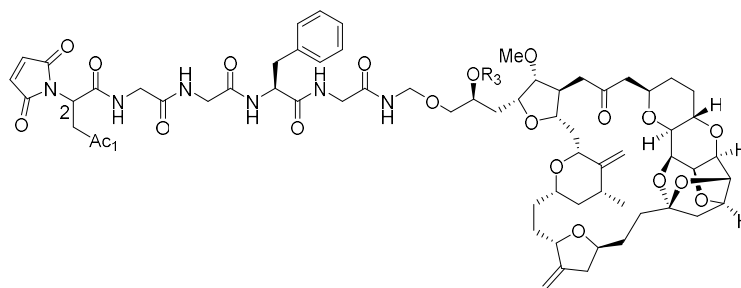
wherein,

the R₁, R₂ and R₃ are as defined in any one of claims 1-13.

26. The linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof according to claim 23 or 24, wherein the linker-drug conjugate has a structure represented by formula VIIIb or formula IXb:



Formula VIIIb

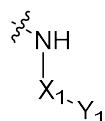


Formula IXb

wherein,

the R_1 , R_2 and R_3 are as defined in any one of claims 1-13;

Ac_1 is a hydrophilic structural unit having the structure shown in formula d:

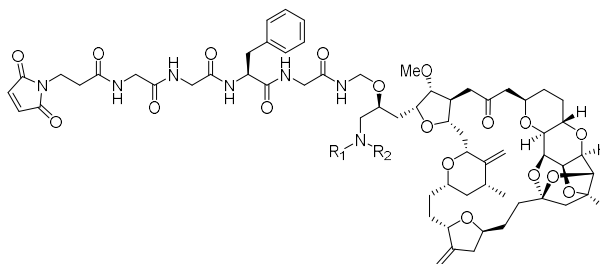


Formula d

X_1 and Y_1 are as defined in claim 4;

preferably, Ac_1 is as defined in claim 15.

27. The linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof according to claim 23 or 24, wherein the linker-drug conjugate has a structure represented by formula VIIIc:

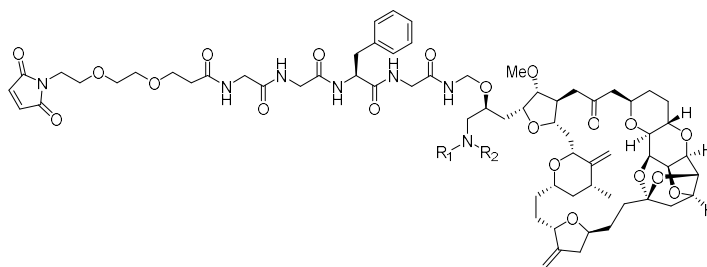


VIIIc

wherein,

the R_1 and R_2 are as defined in any one of claims 1-13.

28. The linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof according to claim 23 or 24, wherein the linker-drug conjugate has a structure represented by formula VIIIId:

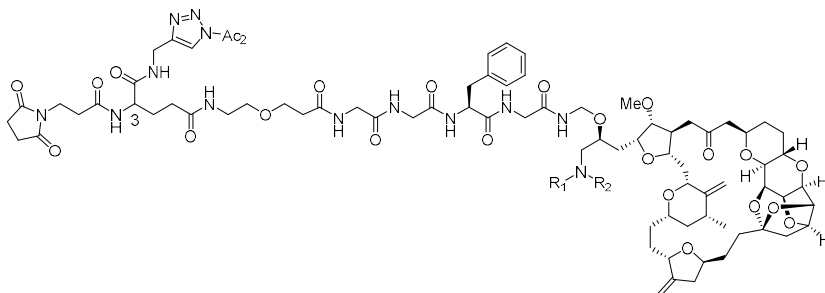


VIIIId

wherein,

the R_1 and R_2 are as defined in any one of claims 1-13.

29. The linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof according to claim 23 or 24, wherein the linker-drug conjugate has a structure represented by formula VIIIe:



Formula VIIIe

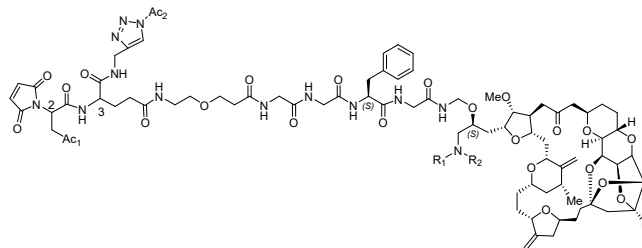
wherein,

the R_1 and R_2 are as defined in any one of claims 1-13;

Ac_2 is a hydrophilic structural unit composed of $-X_2-Y_2$, X_2 and Y_2 are as defined in claim 4;

preferably, Ac_2 is as defined in claim 9 or 18.

30. The linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof according to claim 23 or 24, wherein the linker-drug conjugate has a structure represented by formula VIIIIf:



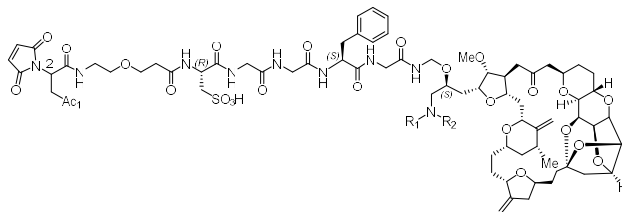
Formula VIIIIf

wherein, the R₁ and R₂ are as defined in any one of claims 1-13;

Ac₁ is a hydrophilic structural unit composed of -X₁-Y₁, and X₁ and Y₁ are as defined in claim 4;
preferably, Ac₁ is as defined in claim 15;

Ac₂ is a hydrophilic structural unit composed of -X₂-Y₂, and X₂ and Y₂ are as defined in claim 4;
preferably, Ac₂ is as defined in claim 9 or 18.

31. The linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof according to claim 23 or 24, wherein the linker-drug conjugate has a structure represented by formula VIIIg:

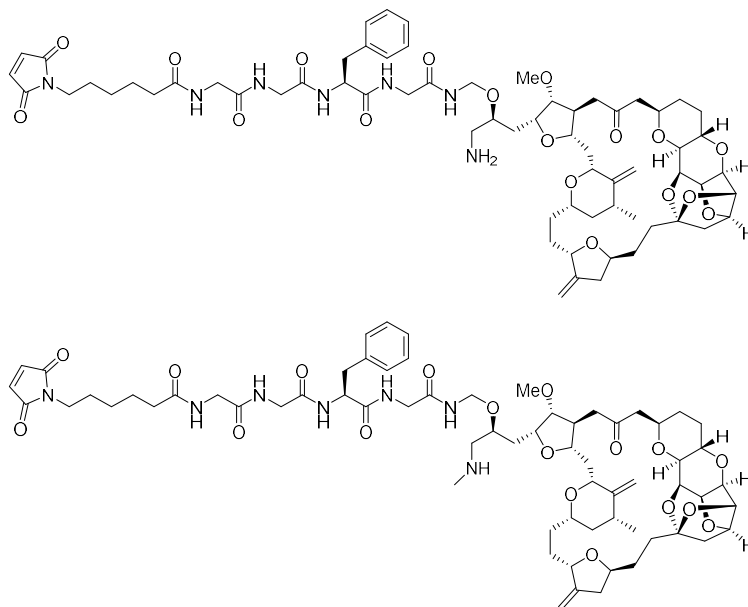


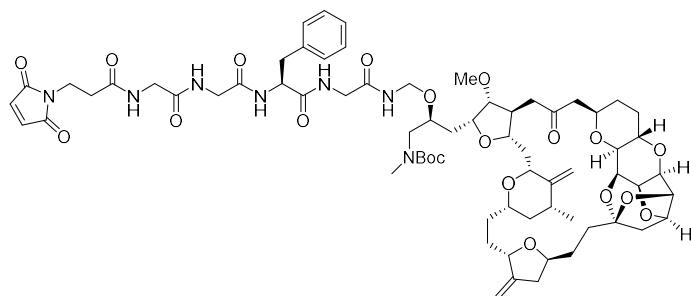
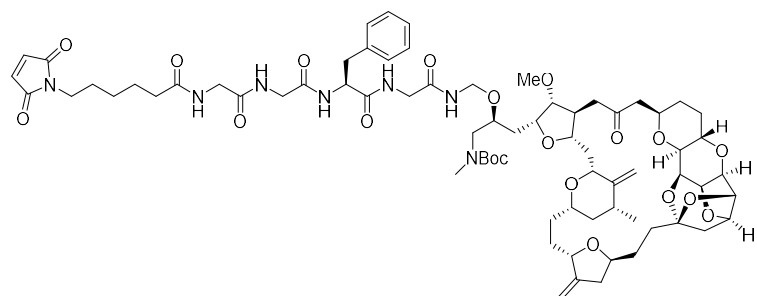
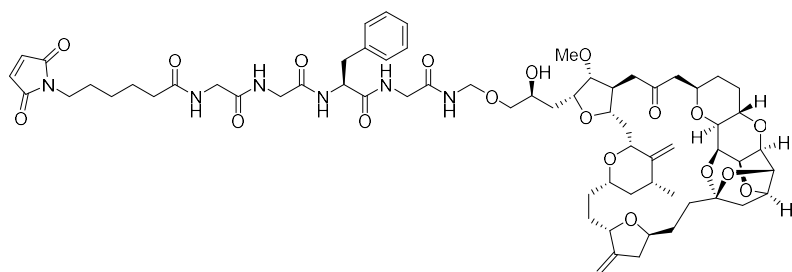
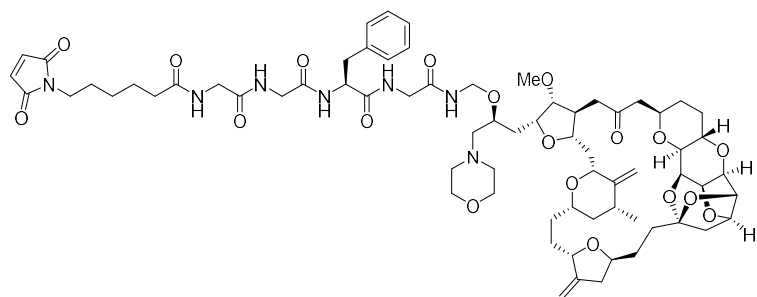
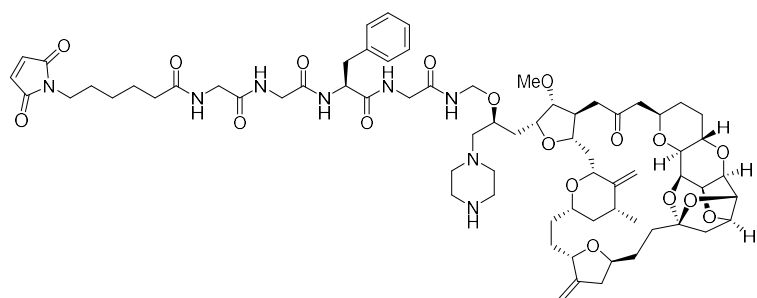
Formula VIIIg

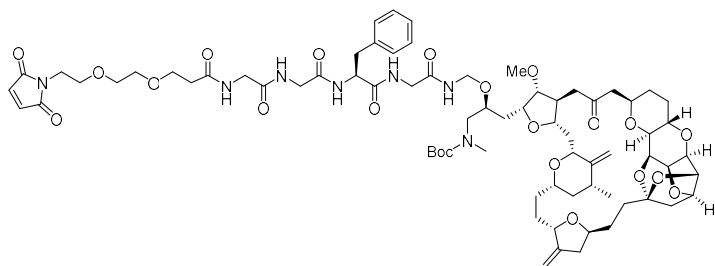
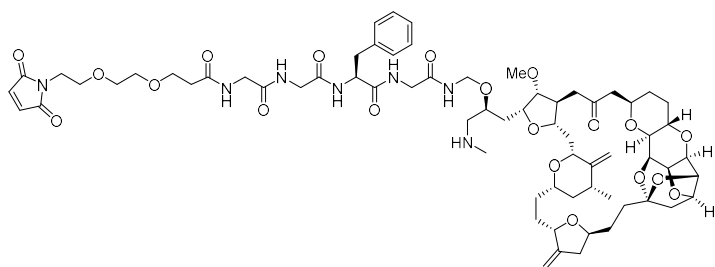
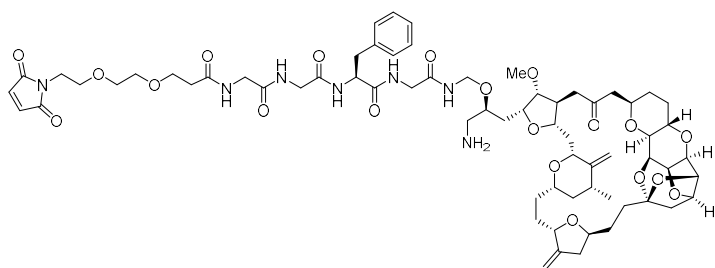
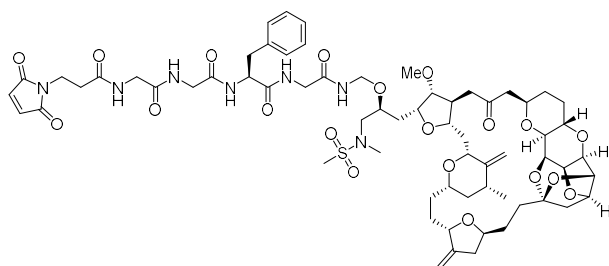
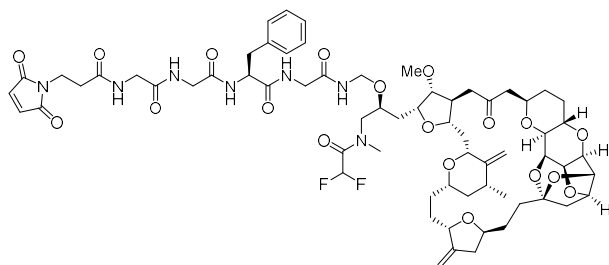
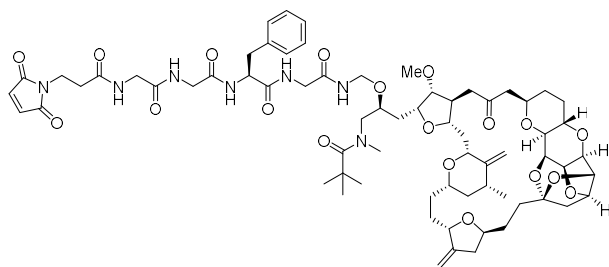
wherein, the R₁ and R₂ are as defined in any one of claims 1-13;

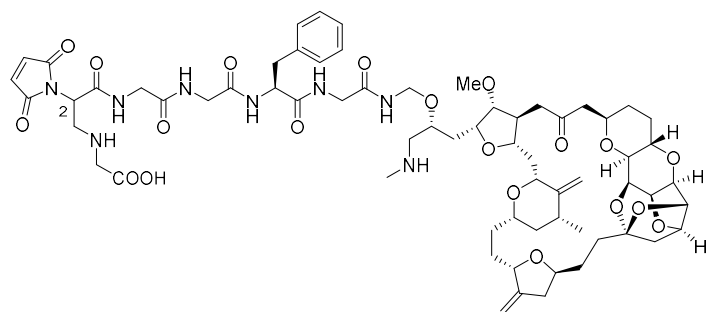
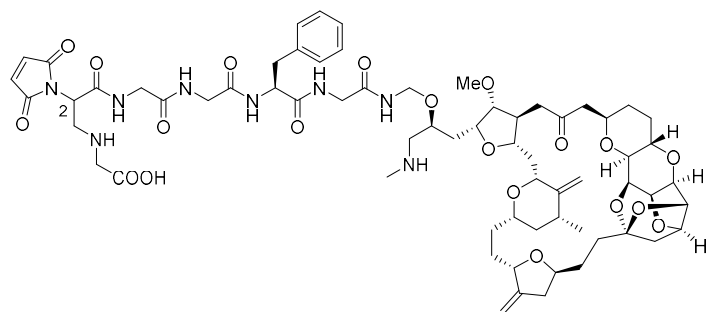
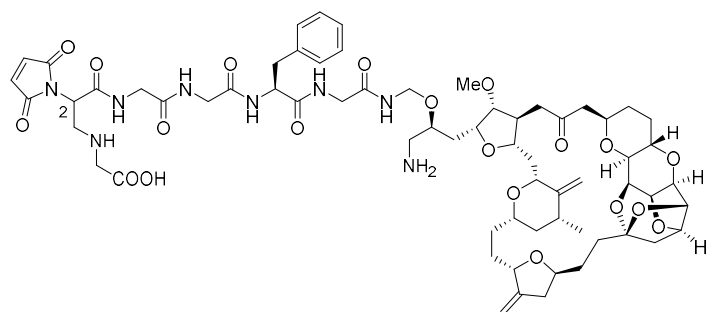
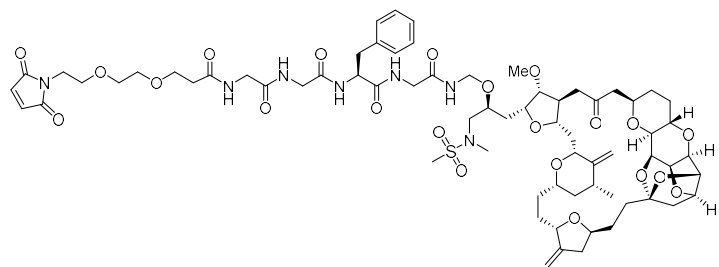
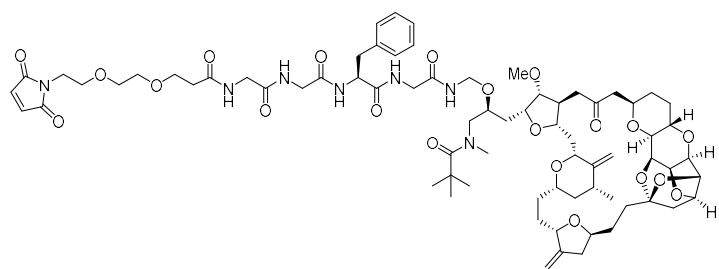
Ac₁ is a hydrophilic structural unit composed of -X₁-Y₁, and X₁ and Y₁ are as defined in claim 4;
preferably, Ac₁ is as defined in claim 15.

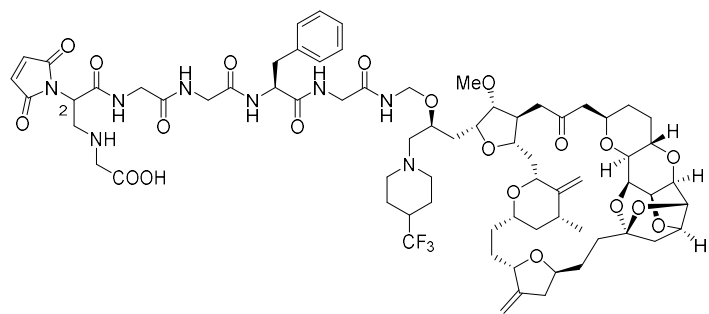
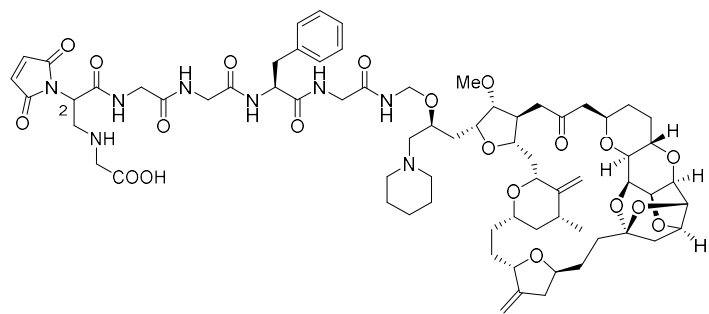
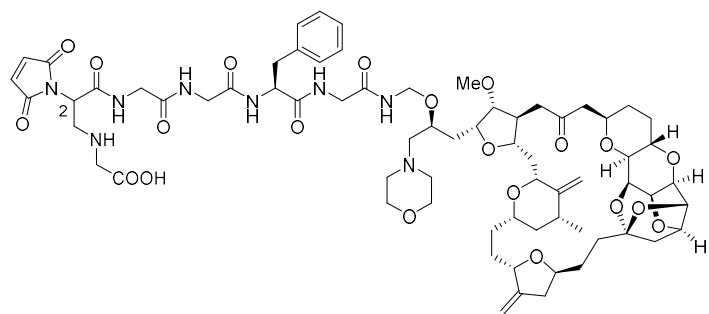
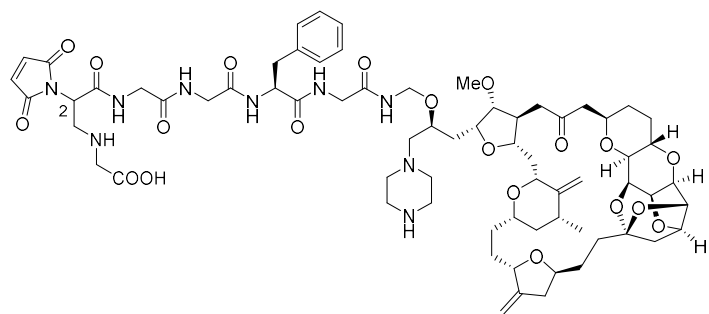
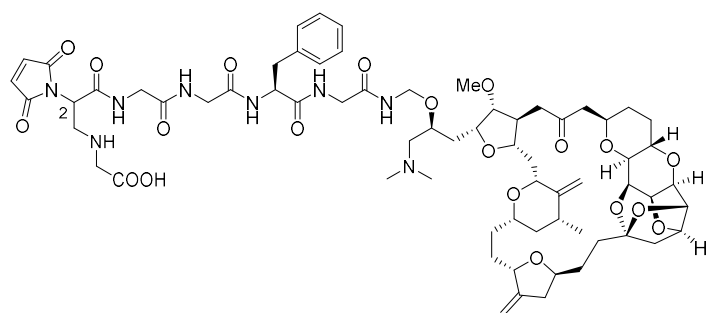
32. The linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 23-31, wherein the linker-drug conjugate is selected from the following structures:

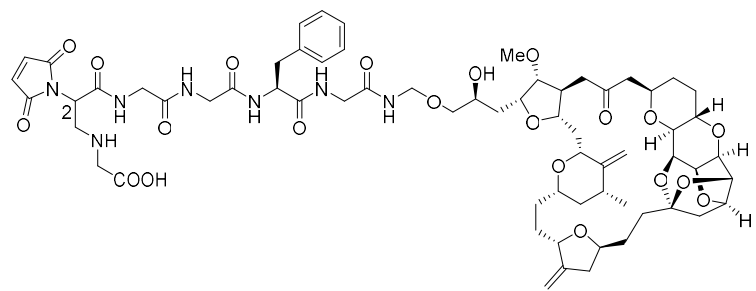
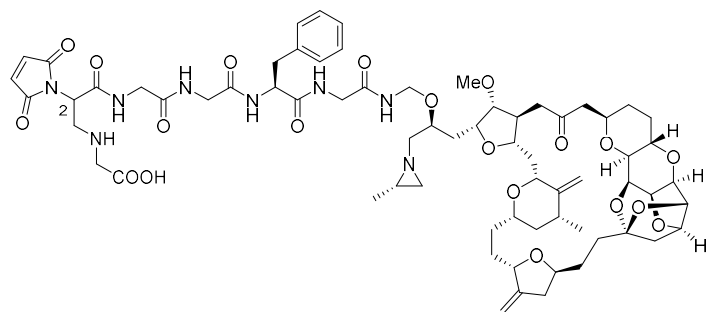
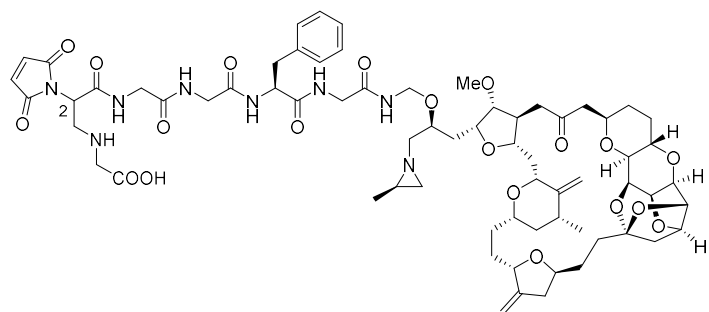
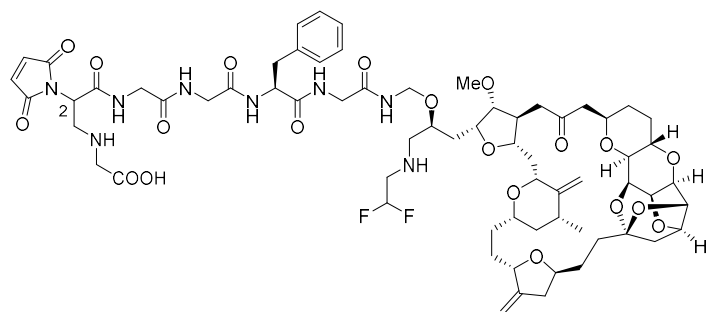
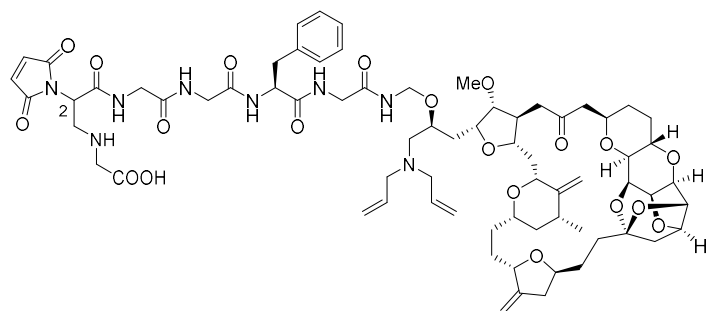


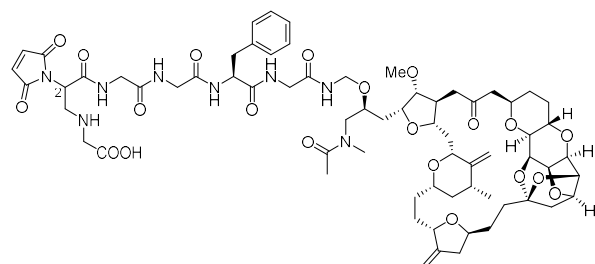
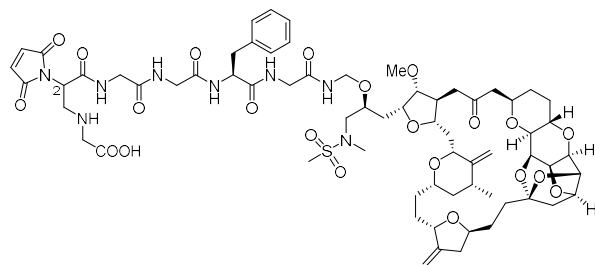
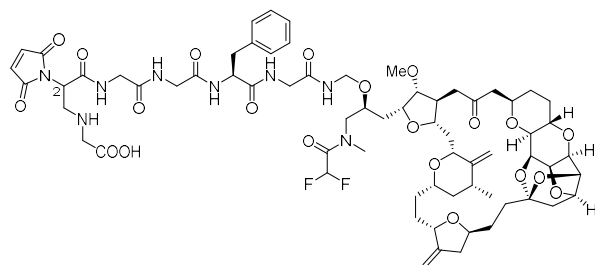
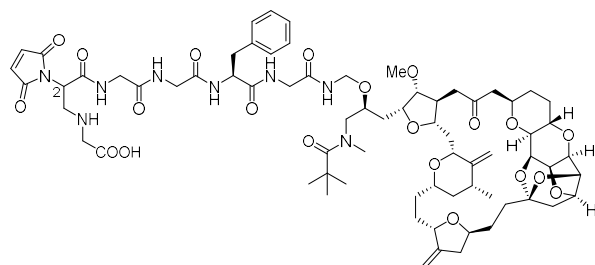
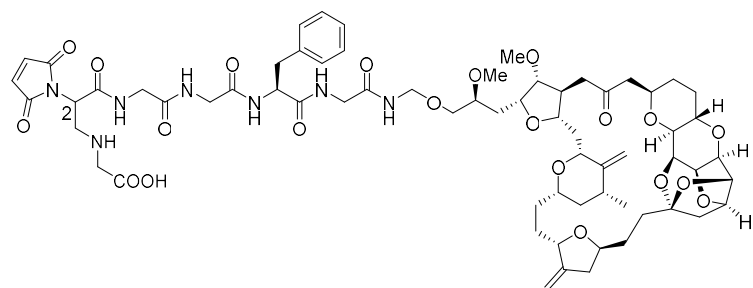
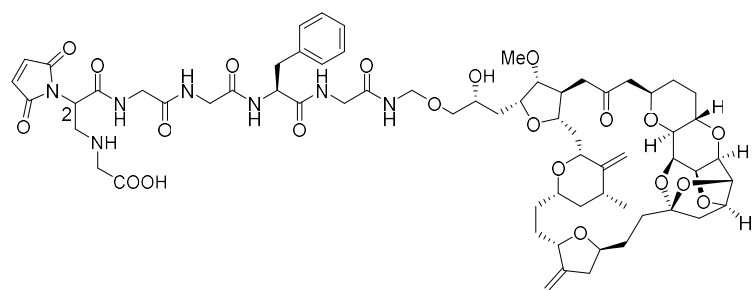


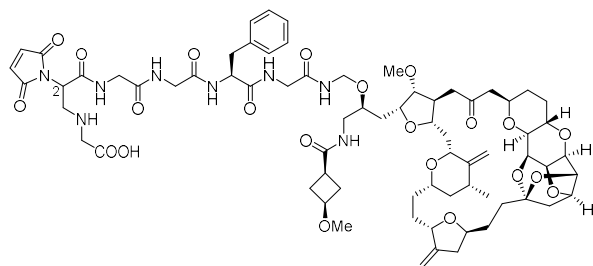
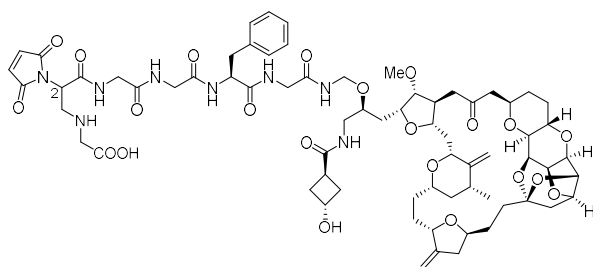
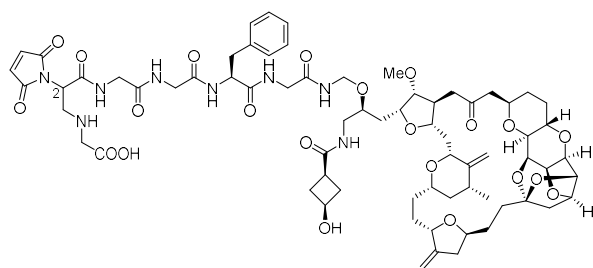
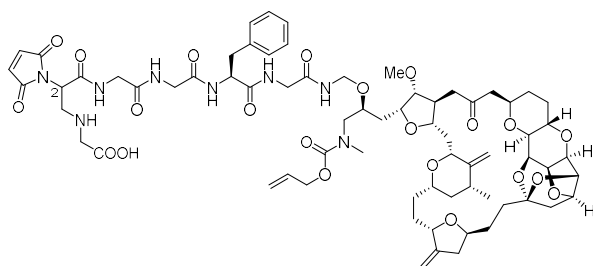
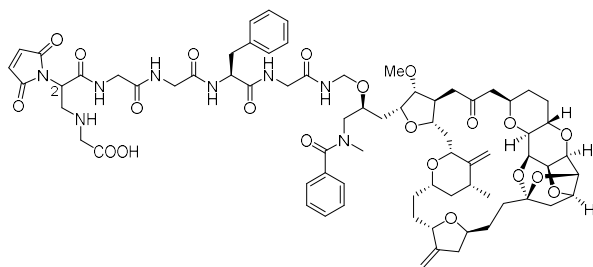
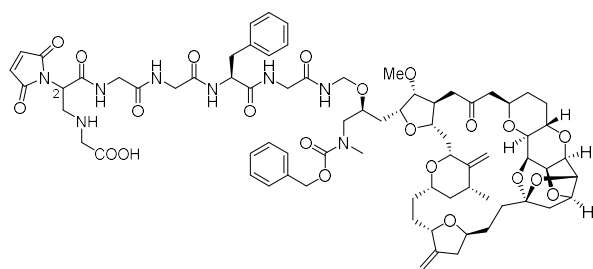


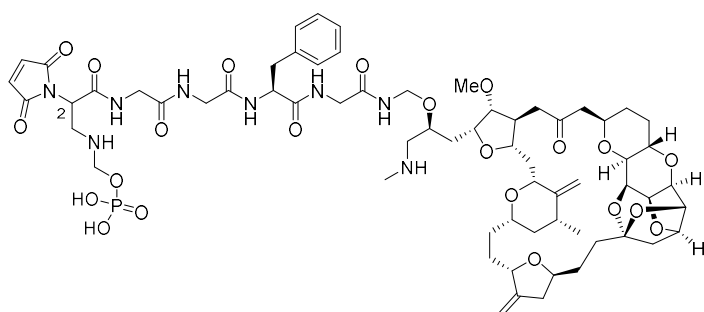
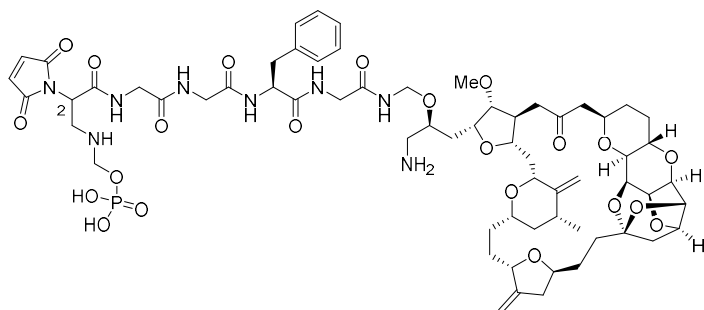
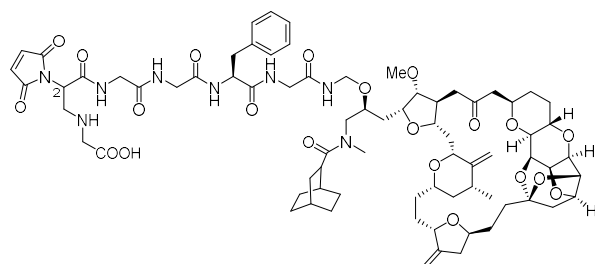
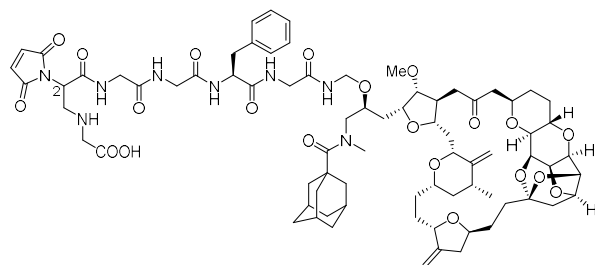
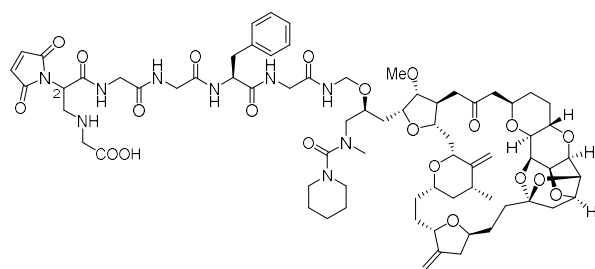


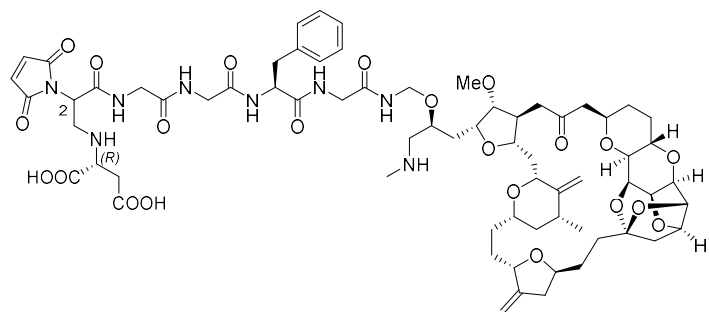
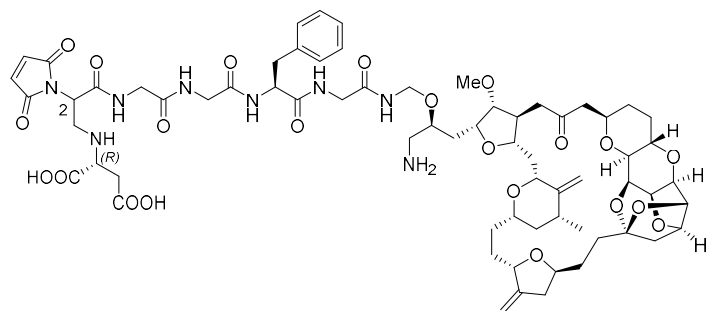
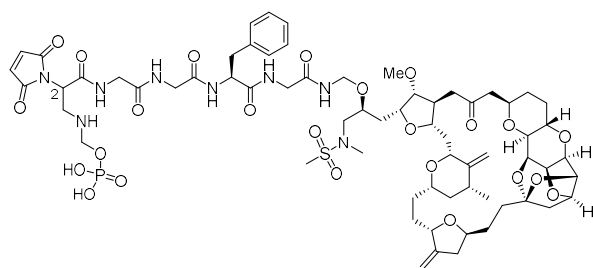
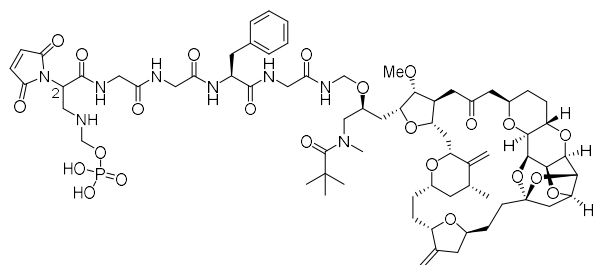
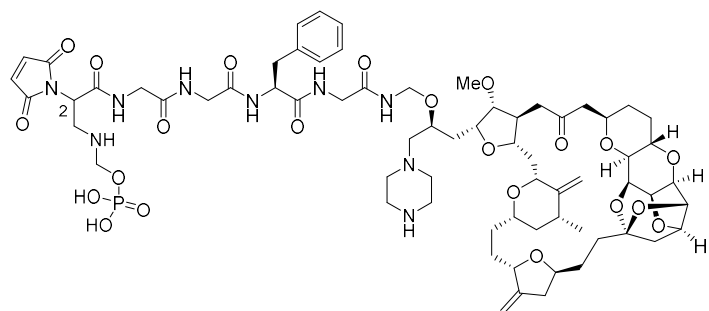


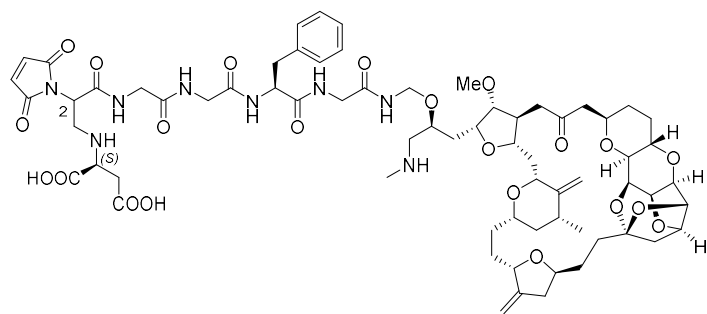
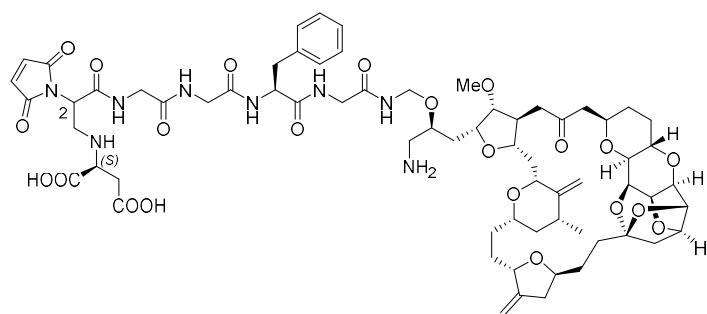
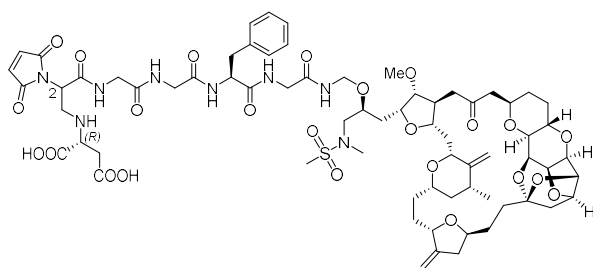
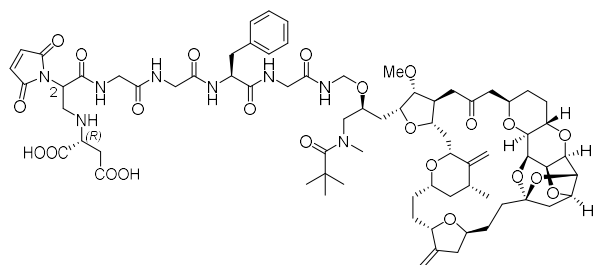
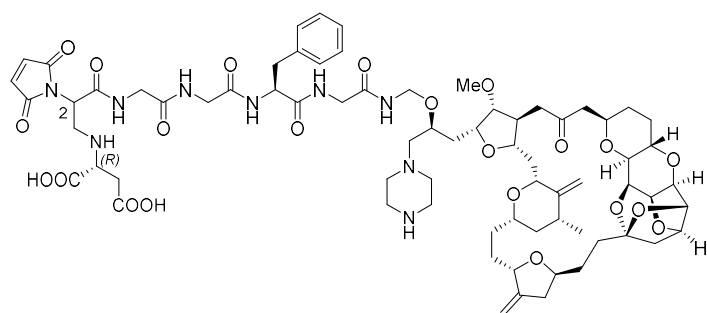


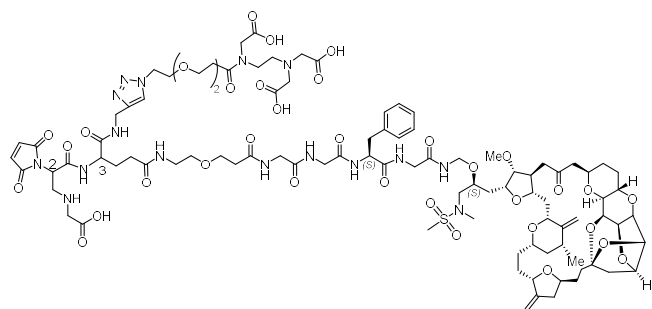
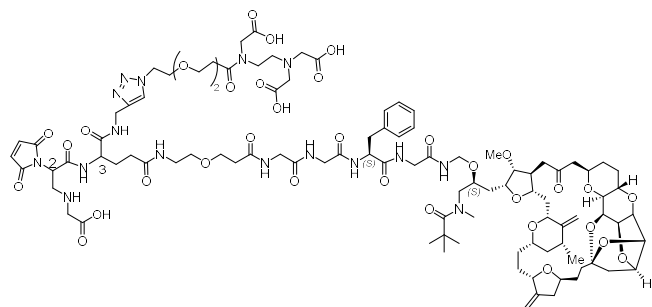
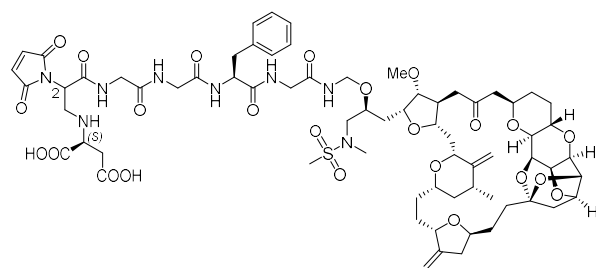
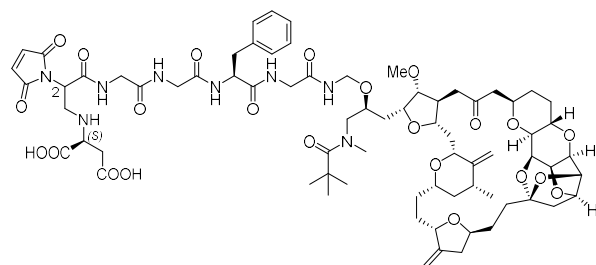
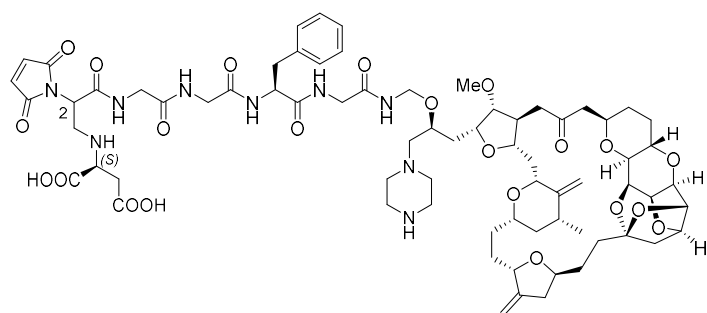


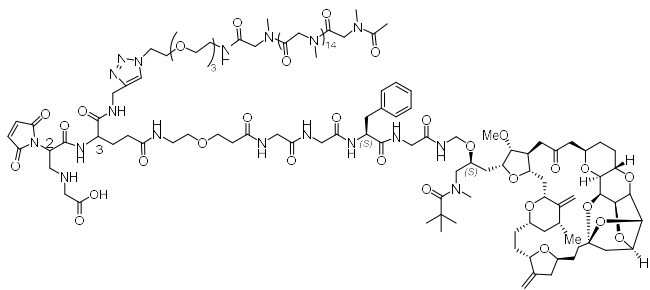
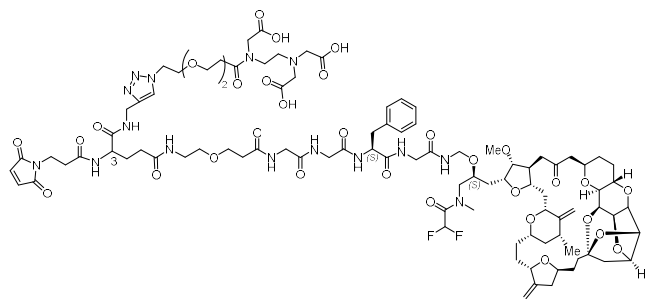
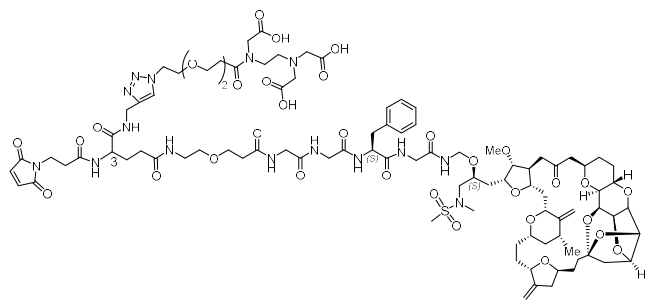
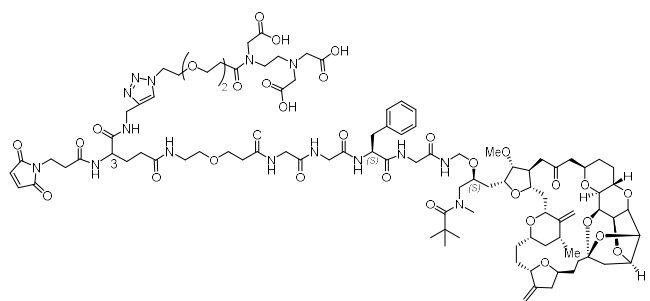
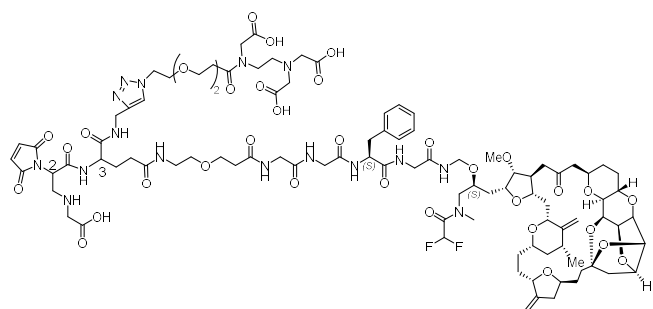


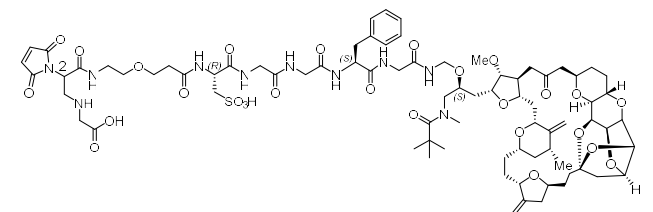
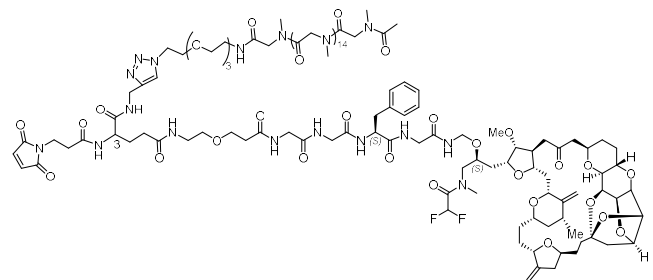
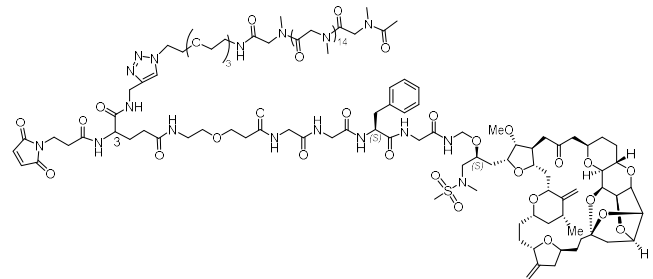
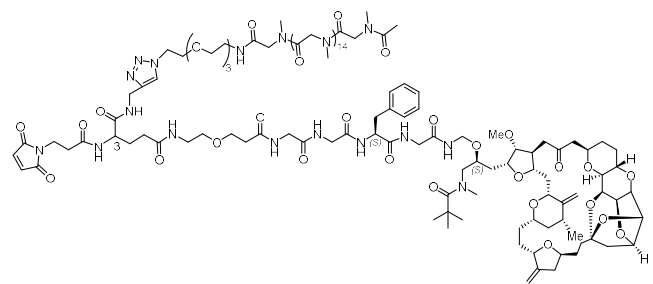
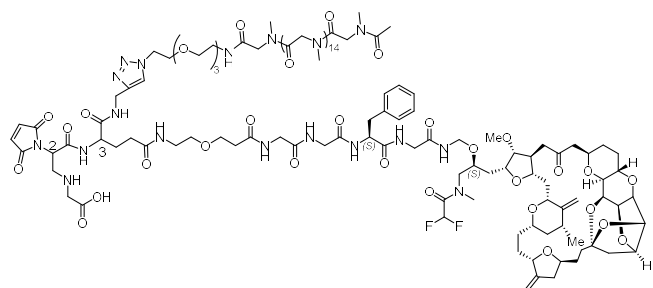
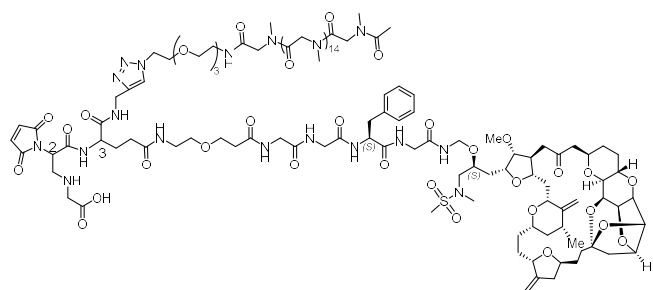


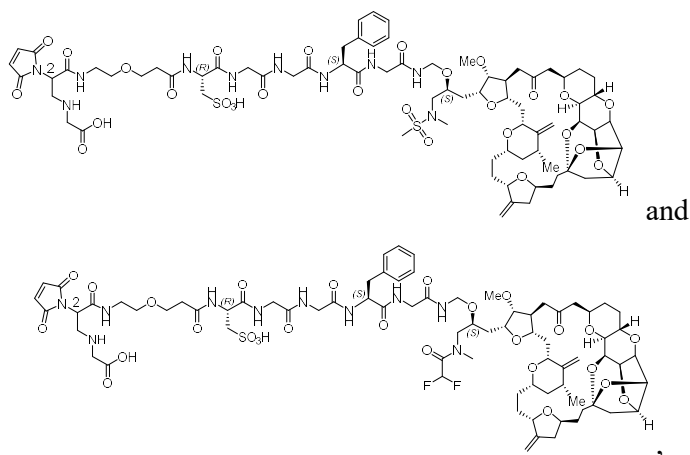






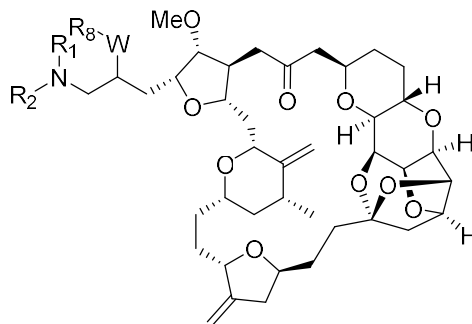






wherein chiral carbons at positions 2 and 3 independently have configuration R or S; preferably, the chiral carbon at position 2 has configuration S, and the chiral carbon at position 3 has configuration S.

33. A compound of formula X, or an isomer, mesomer, racemate, or enantiomer thereof, or a mixture thereof, or a pharmaceutically acceptable salt or solvate thereof,



Formula X

wherein W is an oxygen atom or a sulfur atom, and preferably, W is an oxygen atom;

R₁ and R₈ are each independently selected from the group consisting of: hydrogen atom, alkyl, acyl, sulfonyl, cycloalkyl, heterocyclyl, aryl and heteroaryl, and are not simultaneously hydrogen; optionally, the alkyl, acyl, sulfonyl, cycloalkyl, aryl and heteroaryl are each independently substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, alkoxy, halogen, deuterium, amino, cyano, hydroxy, mercapto, azido, nitro, carboxy, acyl, carbonyl, hydroxyalkyl, cycloalkyl, heterocyclyl, aryl and heteroaryl; preferably, R₁ is alkyl, and R₈ is H;

R₂ is -C(O)-Q₁-Q₂ or -SO₂-Q₁-Q₂, wherein Q₁ is selected from the group consisting of O, N and S atoms and a chemical bond;

in a case where Q₁ is selected from the group consisting of O, N and S atoms, Q₂ is selected from the group consisting of alkyl, cycloalkyl, heterocyclyl, spirocyclyl, bridged ring, alkenyl, aryl and

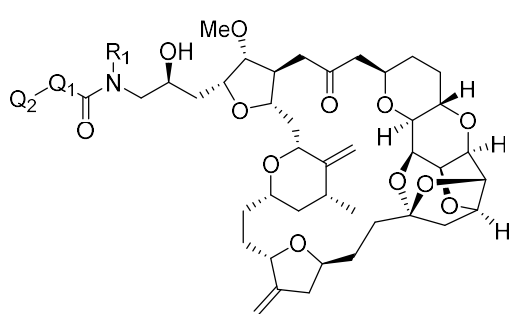
heteroaryl, and optionally the alkyl, cycloalkyl, heterocyclyl, spirocyclyl, bridged ring, alkenyl, aryl and heteroaryl are each independently substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, alkoxy, halogen, deuterium, amino, cyano, hydroxy, mercapto, azido, nitro, carboxy, acyl, carbonyl, hydroxyalkyl, cycloalkyl, heterocyclyl, aryl and heteroaryl;

in a case where Q_1 is a chemical bond, Q_2 is selected from the group consisting of alkyl, benzyl, cycloalkyl, heterocyclyl, spirocyclyl, bridged ring, alkenyl, aryl and heteroaryl;

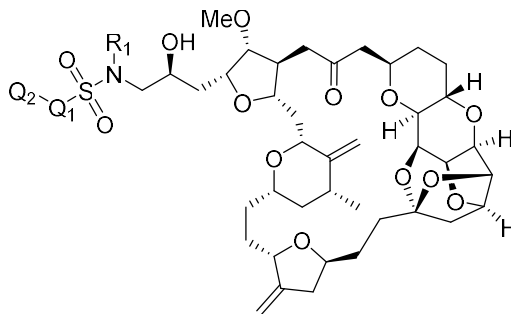
in a case where Q_2 is an alkyl, the alkyl is substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, alkoxy, halogen, deuterium, cyano, azido, nitro, carboxyl, acyl, carbonyl, cycloalkyl, heterocyclyl, aryl and heteroaryl;

in a case where Q_2 is selected from the group consisting of cycloalkyl, heterocyclyl, spirocyclyl, bridged ring, alkenyl, aryl and heteroaryl, the cycloalkyl, heterocyclyl, spirocyclyl, bridged ring, alkenyl, aryl and heteroaryl are each independently substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, alkoxy, halogen, deuterium, amino, cyano, hydroxy, mercapto, azido, nitro, carboxyl, acyl, carbonyl, hydroxyalkyl, cycloalkyl, heterocyclyl, aryl and heteroaryl, and in a case where Q_2 is a cycloalkyl with the substituent being a hydroxyl, an amino or a mercapto, the substituent is at any position except a carbon atom connected to $-C(O)-$.

34. The compound of formula X, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof according to claim 33, wherein the compound is a compound of formula XIa or formula XIb, or an isomer, mesomer, racemate, or enantiomer thereof, or a mixture thereof,



Formula XIa



Formula XIb

wherein R_1 is an alkyl, and preferably R_1 is a methyl,

Q_1 is an O atom or a chemical bond;

in a case where Q_1 is an O atom, Q_2 is selected from the group consisting of alkyl, cycloalkyl,

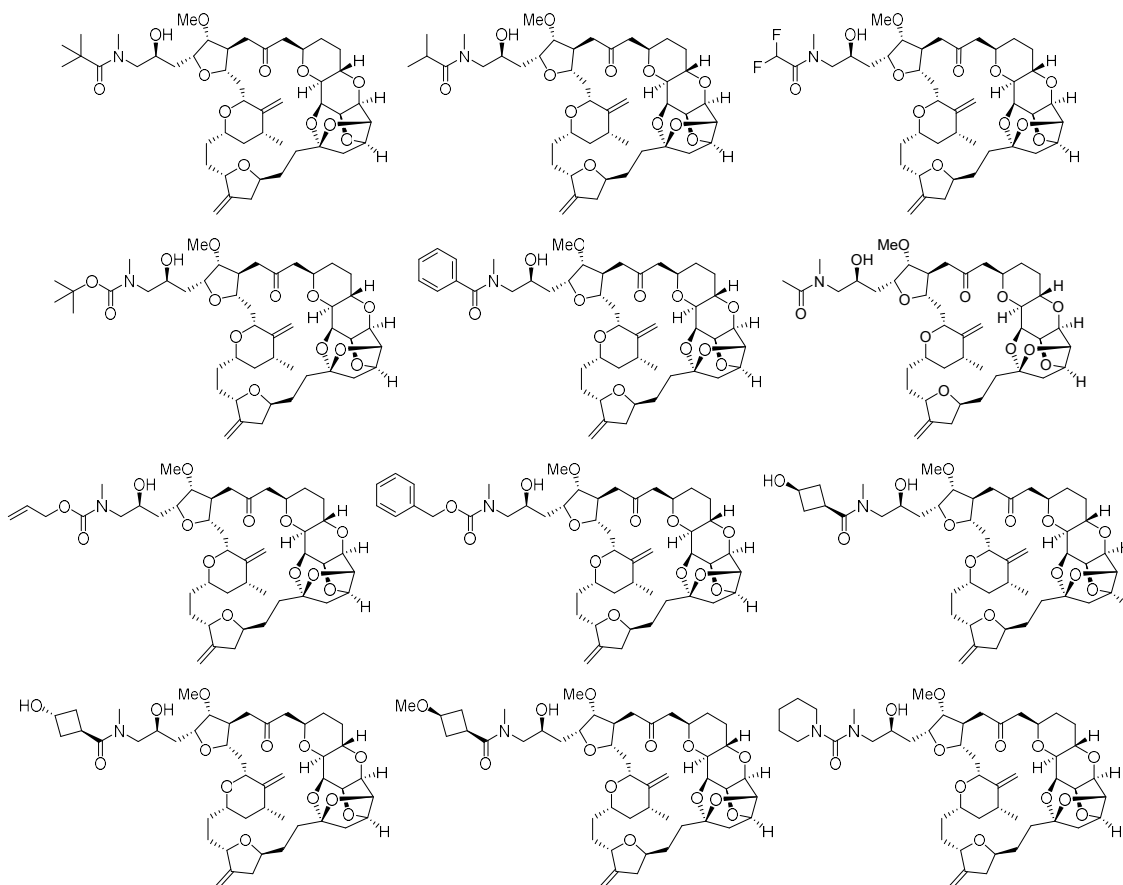
alkenyl and aryl, and optionally the alkyl, cycloalkyl, alkenyl and aryl are each independently substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, halogen, hydroxy, hydroxyalkyl and aryl;

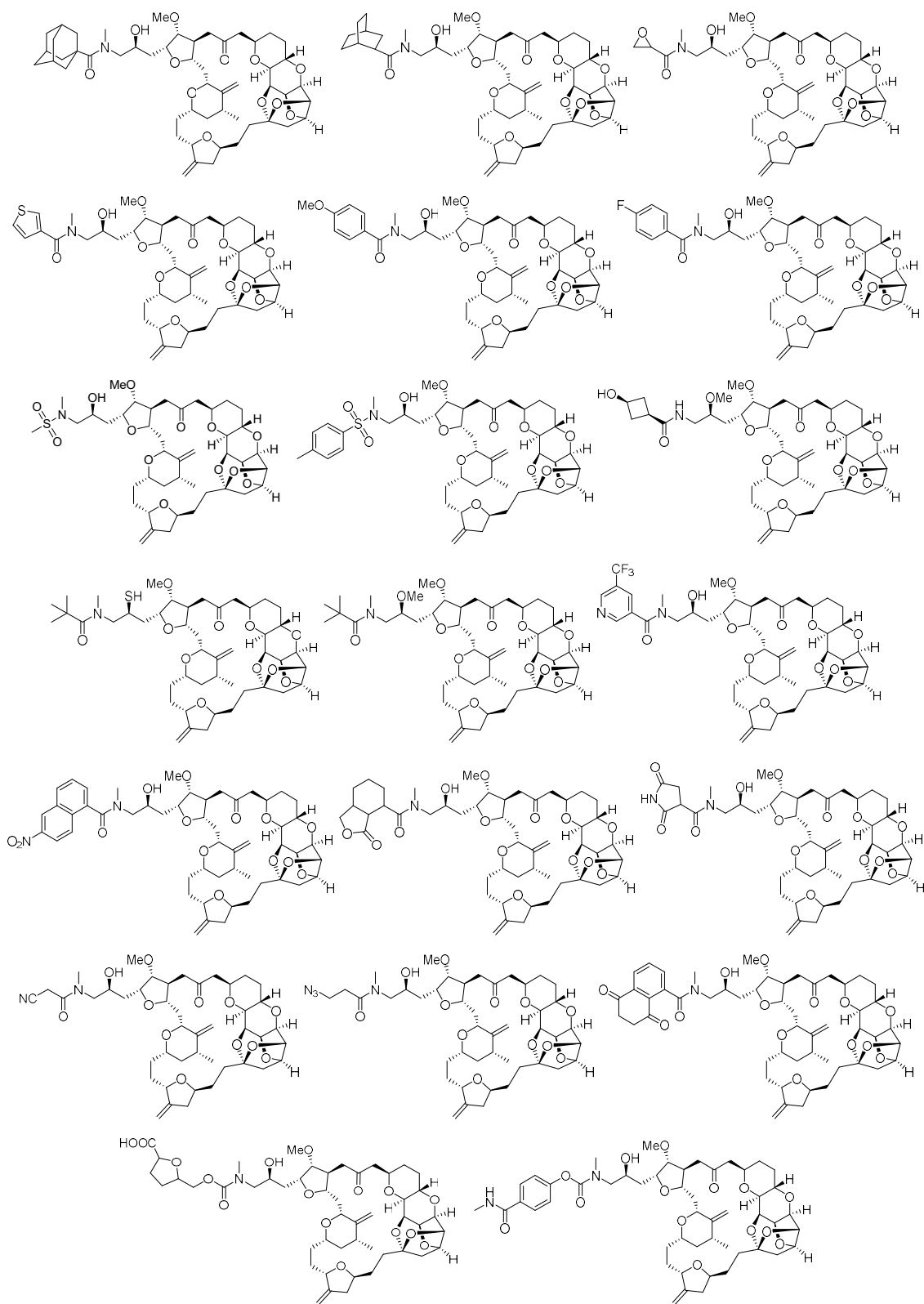
in a case where Q_1 is a chemical bond, Q_2 is selected from the group consisting of alkyl, benzyl, cycloalkyl, alkenyl and aryl;

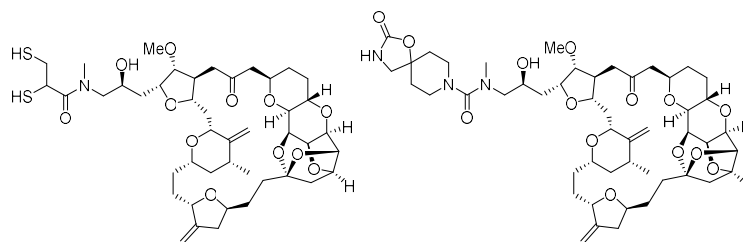
in a case where Q_2 is an alkyl, the alkyl is substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, halogen, deuterium, azido, nitro and aryl; and

in a case where Q_2 is selected from the group consisting of cycloalkyl, alkenyl and aryl, the cycloalkyl, alkenyl and aryl are each independently substituted with one or more substituents selected from the group consisting of hydrogen atom, alkyl, halogen, hydroxyl, hydroxyalkyl and aryl, and in a case where Q_2 is a cycloalkyl with the substituent being a hydroxyl, an amino or a mercapto, the substituent is at any position except a carbon atom connected to $-C(O)-$.

35. The compound of formula X, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof according to claim 33 or 34, wherein the compound is selected from the following structures:







and

36. Use of the linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 23-32, or the compound or the isomer, mesomer, racemate, or enantiomer thereof or the mixture thereof or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 33-35 as an intermediate for the preparation of a ligand-drug conjugate, and the ligand-drug conjugate is described as in any one of claims 1-22.

37. The ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 1-22, or the linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 23-32, or the compound, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 33-35, wherein: the pharmaceutically acceptable salts include sodium, potassium, calcium or magnesium salts formed by acidic functional groups in the structural formula and acetates, trifluoroacetates, citrates, oxalates, tartrates, malates, nitrates, chlorides, bromides, iodides, sulfates, bisulfates, phosphates, lactates, oleates, ascorbates, salicylates, formates, glutamates, methanesulfonates, ethanesulfonates, benzenesulfonates or p-toluenesulfonates formed by basic functional groups in the structure.

38. A pharmaceutical composition, comprising the ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 1-22, or the linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 23-32, or the compound, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 33-35, and optionally further comprising a pharmaceutically acceptable carrier.

39. A pharmaceutical formulation, comprising the ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 1-22, or the linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 23-32, or the compound, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 33-35.

40. Use of the ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 1-22, or the linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 23-32, or the compound, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 33-35 in the preparation of a medicament for the treatment or prevention of a cancer or tumor;

preferably, the cancer or tumor is a solid tumor or a hematological tumor;

preferably, the cancer or tumor is selected from adenocarcinoma, ovarian cancer, cervical cancer, uterine cancer, prostate cancer, kidney cancer, urinary tract cancer, bladder cancer, liver cancer, stomach cancer, endometrial cancer, salivary gland cancer, esophageal cancer, lung cancer, colon cancer, rectal cancer, colorectal cancer, bone cancer, skin cancer, thyroid cancer, pancreatic cancer, melanoma, glioma, neuroblastoma, glioblastoma multiforme, sarcoma, lymphoma, leukemia, and hypopharyngeal cancer.

41. A method of preventing or treating a cancer or tumor, comprising administering to a subject in need thereof a prophylactic or therapeutically effective amount of the ligand-drug conjugate or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 1-22, or the linker-drug conjugate, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 23-32, or the compound, or the isomer, mesomer, racemate, or enantiomer thereof, or the mixture thereof, or the pharmaceutically acceptable salt or solvate thereof according to any one of claims 33-35;

preferably, the cancer or tumor is a solid tumor or a hematological tumor;

preferably, the cancer or tumor is selected from adenocarcinoma, ovarian cancer, cervical cancer, uterine cancer, prostate cancer, kidney cancer, urinary tract cancer, bladder cancer, liver cancer,

stomach cancer, endometrial cancer, salivary gland cancer, esophageal cancer, lung cancer, colon cancer, rectal cancer, colorectal cancer, bone cancer, skin cancer, thyroid cancer, pancreatic cancer, melanoma, glioma, neuroblastoma, glioblastoma multiforme, sarcoma, lymphoma, leukemia, and hypopharyngeal cancer.