

ANTI-CLAUDIN18.2 ANTIBODY-CAMPTOTHECIN DRUG CONJUGATE AND PHARMACEUTICAL USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the filing date of CN 202311712634.5, filed November 15, 2023, the entire disclosures of which are incorporated by reference herein.

TECHNICAL FIELD

10 The present invention relates to the field of biopharmaceuticals, and specifically to an antibody-drug conjugate formed by an anti-Claudin18.2 antibody and a camptothecin toxin, and to methods of preparation and use of the antibody-drug conjugate. The present application also relates to linker-drug compounds that can be coupled with an antibody to form an antibody-drug conjugate.

BACKGROUND

Claudin18.2 (or simply CLDN18.2) is a protein with tight junction function encoded by the *Claudin18* gene and is a member of the Claudin protein family.

10 Claudin family proteins are four-times transmembrane proteins containing two extracellular loops, N-terminal and C-terminal present in the cytoplasm, and four transmembrane regions. Claudin family proteins are important members involved in tight junctions, which play a role in connectivity between cells, and the aberrant expression of the proteins induces structural disruptions such as those of epithelial cells, leading to altered function and the triggering of a wide range of diseases. Current research indicates that the mammalian Claudin family of proteins has 27 members, and humans contain all Claudin proteins except Claudin13. The functions of each member are highly conserved, and each member is differentially expressed in different tissues and mediates different tumor types.

20 The normal expression of Claudin18.2 is limited to gastric mucosal cells and is not expressed in other normal tissues, and its overexpression leads to gastric, pancreatic, esophageal, and non-small-cell lung cancers, etc. Claudin18.1, a shear variant of Claudin18.2, is selectively expressed in lung cells, although there is only an 8 amino acid difference between Claudin18.1 and Claudin18.2 in the first extracellular loop of the normal tissues. Claudin18.1 is selectively expressed in lung cells of normal tissues. Therefore, the highly restricted expression of Claudin18.2 in normal tissues and its specific expression in tumor tissues make Claudin18.2 an important potential target for tumor immunotherapy.

30 At present, for antibody drugs targeting Claudin18.2, Zolbetuximab (IMAB362), a monoclonal antibody drug developed by Ganymed Company of Germany, is in the lead, and the research on the indications of gastric and gastroesophageal cancers has progressed to phase III clinic trial, and that of pancreatic cancer has progressed to phase II clinic trial. Ganymed's anti-Claudin18.2 and CD3 dual antibody is in the preclinical stage, with more than 60% of Claudin18.2 and CD3 dual targets in development, and dual antibodies targeting Claudin18.2 and CD47/PD-40

L1/4-1BB are also in development. The fastest progressing anti-Claudin18.2 ADCs are in clinical phase I/II, including LM-302 from Lixin Pharmaceuticals, RC118 from Rongchang Biologics, and GMG901 from Konoia/Leep Bio. The majority of ADCs targeting Claudin18.2 are in clinical phase I or preclinical stage.

Antibody-drug conjugate (ADC) is a class of biological drugs that connect antibodies or antibody fragments with biologically active small molecule toxins through stable chemical linkers, which not only make full use of the high specificity of antibody-antigen binding and the high lethality of cytotoxins, but also effectively circumvents the disadvantages of weak therapeutic effect of antibodies and indiscriminate attack of small molecule toxins. At the same time, it effectively avoids the disadvantages of weak therapeutic effect of antibody and indiscriminate attack of small molecule toxin.

There are currently 14 approved ADC drugs on the market worldwide.

ADC	Company	Target	Date of approval
Adcetris	Seagen/Takeda	CD30	2011.08
Kadcyla	Roche	HER2	2013.02
Besponsa	Pfizer	CD22	2017.08
Mylotarg	Pfizer	CD33	2017.09
Lumoxiti	AstraZeneca	CD22	2018.09
Polivy	Roche	CD79 β	2019.06
Padcev	Seagen	NECTIN-4	2019.12
Enhertu	Daiichi Sankyo / AstraZeneca	HER2	2019.12
Trodelyv	Gilead Sciences	TROP2	2020.04
Blenrep	GSK	BCMA	2020.08
Akalux	RakutenAspyrian	EGFR	2020.09
Zynlonta	ADCTherapeutics	CD19	2021.04
Vidicilimumab	Rongchang Bio	HER2	2021.06
Tivdak	Seagen/Genmab	TF	2021.09

Mylotarg is one of the first ADCs to be marketed globally, which was approved for accelerated marketing by the FDA in May 2000 for the treatment of patients with acute myelogenous leukemia (AML) who are in first relapse, over the age of 60, CD33-positive, and who are not candidates for cytotoxic chemotherapy. Pfizer chose to withdraw the drug from the market on its own in June 2010 after later studies found that treatment with Mylotarg led to severe and fatal liver injury. Subsequently in 2017, Mylotarg was approved for re-launch by the FDA at a reduced dose for the treatment of newly diagnosed CD33-positive AML (children and adults ≥ 1 month of age), relapsed or refractory CD33-positive AML (children and adults ≥ 2 years of age). Recently approved for marketing, Tivdak is the first FDA-approved ADC targeting tissue factor (TF) for the treatment of patients with recurrent or metastatic cervical cancer.

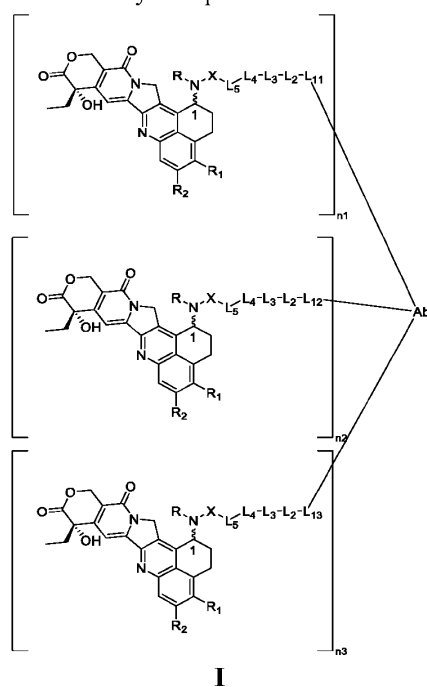
The present application discloses a safer and effective ADC drug targeting Claudin18.2 with camptothecin as the toxin molecule with great developmental value and therapeutic potential.

SUMMARY

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

The disclosure provides, among others, the ADC class of drugs including anti-Claudin18.2 antibody-drug conjugates, compounds such as drug moieties and linker derivatives useful for forming part of the antibody-drug conjugates, methods of preparing the conjugates, pharmaceutical compositions comprising said conjugates or compounds, and methods of using said conjugates or pharmaceutical composition for treating diseases such as cancers. The disclosure further provides linker-drug compounds that can be coupled to an anti-Claudin18.2 antibody to form the disclosed antibody-drug conjugates. In some embodiments, the anti-Claudin18.2 antibody-drug conjugates have excellent clinical therapeutic effects with good molecular stability and significantly improved preclinical efficacy compared to existing drugs of the similar category.

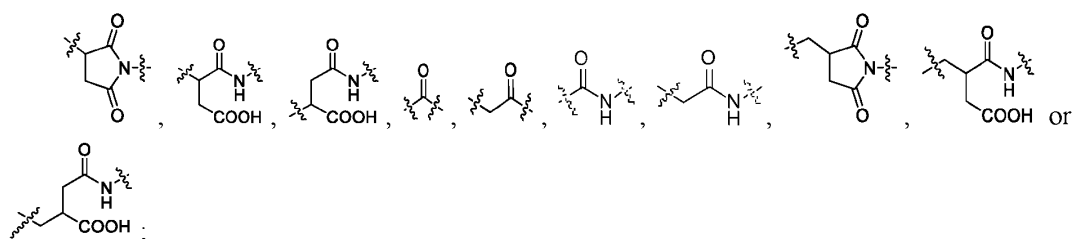
In one aspect, the disclosure provides a ligand-camptothecin derivative conjugate as shown in general formula **I** or a pharmaceutically acceptable salt or solvate thereof:



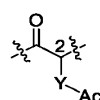
wherein,

20 Ab is an antibody having a binding affinity to human Claudin18.2 or an antigen-binding fragment thereof;

L_{11} , L_{12} , and L_{13} are different from each other and are independently:



L₂ has the structure shown in Formula A below.



Formula A

wherein Y is a scaffold selected from C1-C6 alkyl, substituted C1-C6 alkyl, or C3-C8 cycloalkyl; In one embodiment Y is C1-C6 alkyl; Ac is a hydrophilic structural unit; and the carbon 2 attached to Y is absolutely chiral in R or S configuration.

In one embodiment, L₃ is present or absent, and when present, L₃ is selected from PEG

- 10 hydrophilic units: , o is selected from an integer of 1-10; in one embodiment, o may be 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; in one embodiment, o is an integer of 2-8, such as 2, 3, 4, 5, 6, 7, or 8;

In one embodiment, L₄ is the enzymatically cleavable unit.

In one embodiment, L₅ is the connection unit.

In one embodiment, the chiral carbon atom No. 1 attached to N in formula I has an absolute chirality of R or S;

R is selected from hydrogen atom, deuterium atom, halogen, C1-C6 alkyl, substituted C1-C6 alkyl, deuterated C1-C6 alkyl, C3-C8 cycloalkyl C1-C6 alkyl, C1-C6 alkoxy C1-C6 alkyl, C6-C10 aryl, substituted C6-C10 aryl, 5-10-membered heteroaryl, substituted 5-10-membered heteroaryl.

- 20 In one embodiment, R is selected from a hydrogen atom or a C1-C6 alkyl group.

R₁ is selected from hydrogen atom, deuterium atom, halogen, C1-C6 alkyl, substituted C1-C6 alkyl, deuterated C1-C6 alkyl, C3-C8 cycloalkyl C1-C6 alkyl, C1-C6 alkoxy C1-C6 alkyl, carboxyl, 3-7 meta-heterocyclic, substituted 3-7 meta-heterocyclic, C6-C10 aryl, substituted C6-C10 aryl, 5-10 meta-heteroaryl, substituted 5-10 heteroaryl.

In one embodiment, R₁ is selected from a hydrogen atom or a C1-C6 alkyl group.

In one embodiment, R₁ is selected from C1-C6 alkyl.

- 30 R₂ is selected from hydrogen atom, deuterium atom, halogen, C1-C6 alkyl, substituted C1-C6 alkyl, deuterated C1-C6 alkyl, C3-C8 cycloalkyl C1-C6 alkyl, C1-C6 alkoxy C1-C6 alkyl, carboxyl, 3-7 meta-heterocyclic, substituted 3-7 meta-heterocyclic, C6-C10 aryl, substituted C6-C10 aryl, 5-10 meta-heteroaryl, substituted 5-10 heteroaryl.

In one embodiment, R₂ is selected from a hydrogen atom, a halogen or a C1-C6 alkyl group. In one embodiment, R₂ is selected from halogen.

X selected from $-C(O)-CR_aR_b-(CR_3R_4)_m-O-$, $-C(O)-CR_aR_b-(CR_3R_4)_m-NH-$ or $-C(O)-CR_aR_b-(CR_3R_4)_m-S-$. In one embodiment, X is selected from $-C(O)-CR_aR_b-(CR_3R_4)_m-O-$.

R_a and R_b are each independently selected from a hydrogen atom, a deuterium atom, a halogen, a C1-C6 alkyl, a deuterated C1-C6 alkyl, a halogenated C1-C6 alkyl, a C3-C8 cycloalkyl, a C3-C8 cycloalkyl C1-C6 alkyl, a C6-C10 aryl C1-C6 alkyl, a C1-C6 alkoxy C1-C6 alkyl, a 3-7 metaheterocyclic group, a substituted 3-7 metaheterocyclic group, a C6-C10 aryl, substituted C6-C10 aryl, 5-10 heteroaryl, or substituted 5-10 heteroaryl.

In one embodiment, R_a and R_b are each independently selected from a hydrogen atom, a C1-C6 alkyl, a halo-C1-C6 alkyl, a C3-C8 cycloalkyl C1-C6 alkyl or a C6-C10 aryl C1-C6 alkyl.

10 Alternatively, R_a , R_b and the carbon atoms to which they are attached constitute a C3-C8 cycloalkyl group, a C3-C8 cycloalkyl C1-C6 alkyl group, a 3-7-membered heterocycloalkyl group, a substituted 3-7-membered heterocycloalkyl group. In one embodiment R_a , R_b and the carbon atoms to which they are attached constitute a C3-C8 cycloalkyl group.

R_3 , R_4 are the same or different and are independently hydrogen atoms, deuterium atoms, halogens, C1-C6 alkyl, halogenated C1-C6 alkyl, deuterated C1-C6 alkyl, C1-C6 alkoxy, hydroxyl, amino, cyano, nitro, hydroxy C1-C6 alkyl, C3-C8 cycloalkyl, a 3-7-membered heterocyclic group, a substituted 3-7-membered heterocyclic group, respectively.

In one embodiment, R_3 , R_4 are independently hydrogen atoms or C1-C6 alkyl groups, respectively. Alternatively, R_3 , R_4 and the carbon atoms attached thereto constitute a C3-C8
20 cycloalkyl group, a C3-C8 cycloalkyl C1-C6 alkyl group, a 3-7-membered heterocyclic group, a substituted 3-7-membered heterocyclic group.

m is selected from an integer from 0-4. In one embodiment, m may be 0, 1, 2, 3, or 4. In one embodiment, m may be 0 or 1; n_1 , n_2 , and n_3 are each independently selected from any integer or any decimal number from 0-10, n_1 , n_2 , and n_3 are not simultaneously 0, and $1 \leq n_1 + n_2 + n_3 \leq 10$.

In some embodiment, ligand-camptothecin derivative conjugates or pharmaceutically acceptable salts or solvates thereof as shown in general formula I, is characterized in that Ab is an antibody targeting Claudin18.2 or an antigen-binding fragment thereof.

In one embodiment, the ligand-camptothecin derivative conjugate or a pharmaceutically
30 acceptable salt or solvate thereof is characterized in that Ab is an antibody and comprises an IgG1 heavy chain and a light chain. In one embodiment, the antibody comprising said IgG1 heavy chain and κ light chain specifically recognizes a human Claudin18.2 protein.

In one embodiment, the ligand-camptothecin derivative conjugate, or a pharmaceutically acceptable salt or solvate thereof, is characterized in that the κ light chain of the antibody comprises CDRs having amino acid sequences with at least 80%, 90%, 98%, 99% or 100% sequence identity to SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and the IgG1 heavy chain comprises CDRs having amino acid sequences with at least 80%, 90%, 98%, 99% or 100% sequence identity to SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4.

In one embodiment, the ligand-camptothecin derivative conjugate, or a pharmaceutically
40 acceptable salt or solvate thereof as shown in general formula I, is characterized in that the

antibody comprises a heavy chain and a light chain. In one embodiment, the light chain comprises CDRL1, CDRL2 and CDRL3 each having an amino acid sequence encoded by a nucleic acid coding sequence having at least 80%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22, respectively, said heavy chain comprising CDRH1, CDRH2 and CDRH3 each having an amino acid sequence encoded by a nucleic acid sequence having at least 80%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, respectively.

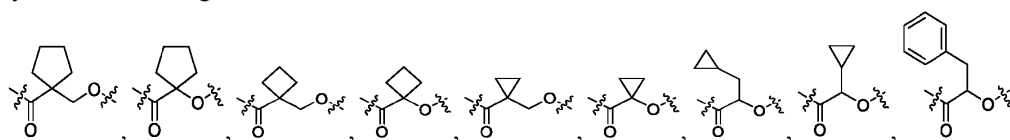
In one embodiment, the ligand-camptothecin derivative conjugate, or a pharmaceutically acceptable salt or solvate thereof as shown in general formula **I**, is characterized in that the antibody comprises a heavy chain and a light chain. In one embodiment, the heavy chain comprises a heavy-chain variable region having at least 80%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 1, and said light chain comprising a light-chain variable region having at least 80%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 7.

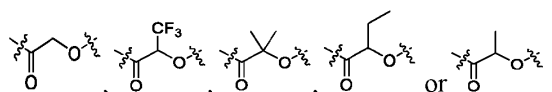
In one embodiment, the ligand-camptothecin derivative conjugate, or a pharmaceutically acceptable salt or solvate thereof as shown in general formula **I**, is characterized in that the antibody comprises a heavy chain and a light chain. In one embodiment, the heavy chain variable region comprising a heavy chain variable region encoded by a nucleic acid sequence having at least 80%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 13, and said light chain variable region encoded by a nucleic acid sequence having at least 80%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 19.

In one embodiment, the ligand-camptothecin derivative conjugate, or a pharmaceutically acceptable salt or solvate thereof as shown in general formula **I**, is characterized in that Ab comprises a heavy chain having an amino acid sequence having at least 80%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 5, and a light chain having an amino acid sequence having at least 80%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 11.

In one embodiment, the ligand-camptothecin derivative conjugate, or a pharmaceutically acceptable salt or solvate thereof as shown in general formula **I**, is characterized in that the nucleic acid coding sequence for the antibody heavy chain has at least 80%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 17, and the nucleic acid coding sequence for the antibody light chain has at least 80%, 80%, 90%, 95%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 23.

In one embodiment, the ligand-camptothecin derivative conjugates or pharmaceutically acceptable salts or solvates thereof as shown in general formula **I** is characterized in that said X may be the following structures or isomers thereof:





The position shown by the left wavy line is connected to the derivative part, and the position shown by the right wavy line is connected to L₅.

In one embodiment, the ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof as shown in general formula I, is characterized in that L₄ may be peptide residues comprising amino acids. In one embodiment, optionally, said amino acid is further substituted with one or more substituents selected from one or more of deuterium atoms, halogens, hydroxyl, cyano, amino, nitro, carboxyl, C1-C6 alkyl, substituted C1-C6 alkyl, C1-C6 alkoxy and C3-C8 cycloalkyl or substituted C3-C8 cycloalkyl.

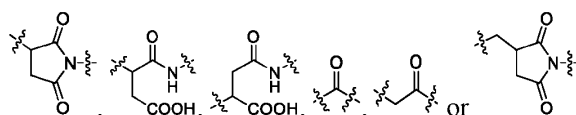
10 In one embodiment, said peptide residue is a peptide residue formed from one, two or more amino acids selected from among phenylalanine (F), glycine (G), valine (V), lysine (K), citrulline (C), serine (S), glutamic acid (E) or aspartic acid (D). In one embodiment, said peptide residue is a tetrapeptide residue comprising glycine (G)-glycine (G)-phenylalanine (F)-glycine (G). In one embodiment, said peptide residue is -GGFG-

In one embodiment, the ligand-camptothecin derivative conjugates or pharmaceutically acceptable salts or solvates thereof as shown in general formula I are characterized as follows:

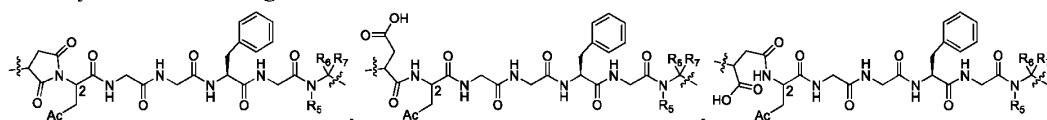
L₅ is non-limitingly selected from -NR₅(CR₆R₇)_q- or a chemical bond, q is selected from an integer from 0-6 (e.g., 0, 1, 2, 3, 4, 5, or 6);

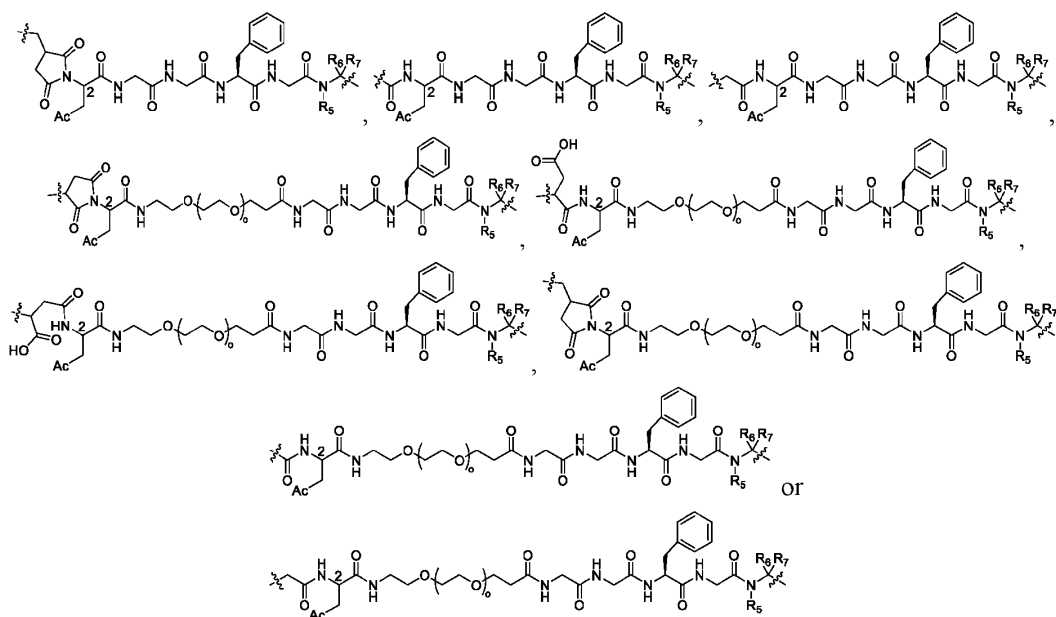
20 R₅, R₆ and R₇ are the same or different and are each independently selected from a hydrogen atom, a deuterium atom, a halogen, a C1-C6 alkyl group, a substituted C1-C6 alkyl group, a deuterated C1-C6 alkyl group, a C3-C8 cycloalkyl group, a C3-C8 cycloalkyl C1-C6 alkyl group, a C1-C6 alkoxy C1-C6 alkyl group, a 3-7 meta heterocyclic group, a substituted 3-7 meta heterocyclic group, a C6-C10 aryl group, a substituted C6-C10 aryl, 5-10 heteroaryl, substituted 5-10 heteroaryl. In one embodiment, R₅, R₆ and R₇ are each independently selected from a hydrogen atom or a C1-C6 alkyl group. In one embodiment, R₅, R₆ and R₇ are each independently selected from a hydrogen atom.

In certain embodiments, L₁ may be:

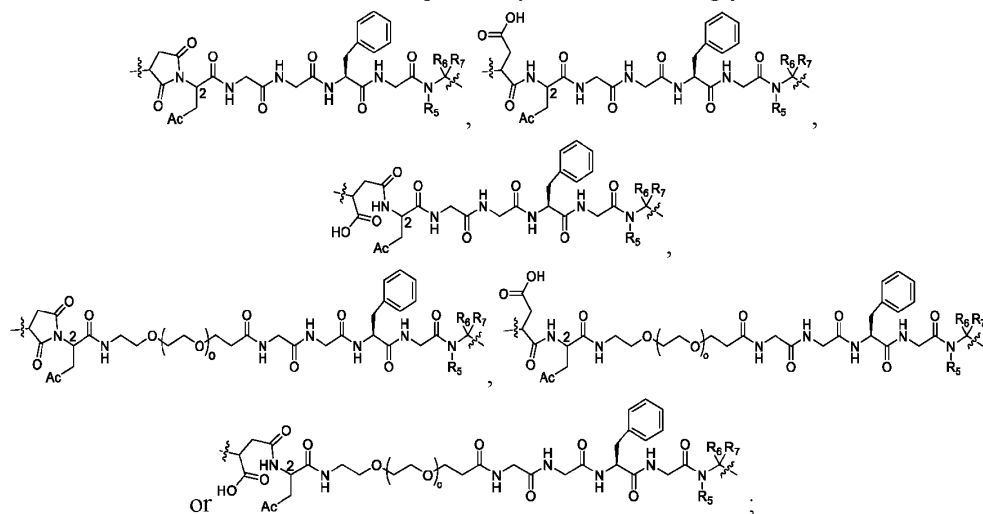


30 In one embodiment, the ligand-camptothecin derivative conjugates or pharmaceutically acceptable salts or solvates thereof, are characterized in that said linking units -L₁₁-L₂-L₃-L₄-L₅-, -L₁₂-L₂-L₃-L₄-L₅- or -L₁₃-L₂-L₃-L₄-L₅- are not identical to each other and are independently and may be the following structures:





In one embodiment, -L₁₁ -L₂ -L₃ -L₄ -L₅ -, -L₁₂ -L₂ -L₃ -L₄ -L₅ - or -L₁₃ -L₂ -L₃ -L₄ -L₅ - are not the same as each other and are each independently and non-limitingly selected from:



wherein:

Ac is a hydrophilic structural unit;

R₅, R₆ and R₇ are the same or different and are each independently selected from hydrogen atom, deuterium atom, halogen, C1-C6 alkyl, substituted C1-C6 alkyl, deuterated C1-C6 alkyl, C3-C8 cycloalkyl, C3-C8 cycloalkyl C1-C6 alkyl, C1-C6 alkoxy C1-C6 alkyl, 3-7-membered heterocyclic, substituted 3-7-membered heterocyclic, C6-C10 aryl, substituted C6-C10 aryl, 5-10-membered heteroaryl, substituted 5-10-membered heteroaryl.

In one embodiment, R_5 , R_6 and R_7 are each independently selected from a hydrogen atom or a C1-C6 alkyl group;

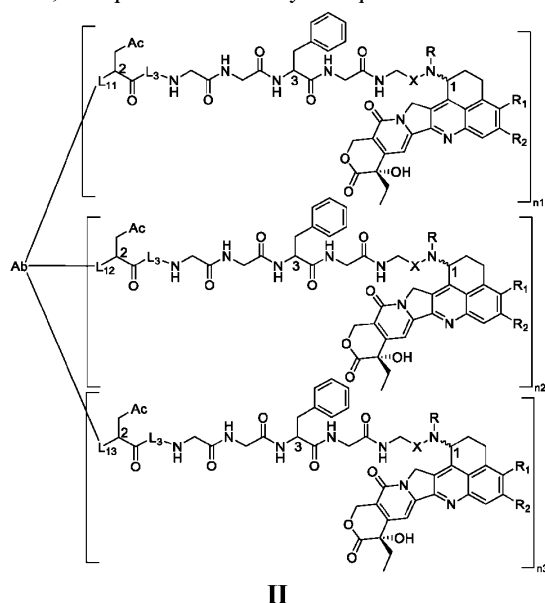
In one embodiment, R_5 , R_6 and R_7 are each independently selected from a hydrogen atom;

The carbon atom #2 attached to N has absolute chirality of R or the S configuration;

The position shown by the left wavy line is connected to the antibody or its antigen-binding fragment portion, and the position shown by the right wavy line is connected to X;

o is an integer selected from 1-10; for example, n may be 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

In a further aspect, the application discloses a ligand-camptothecin derivative conjugate as shown in general formula II, or a pharmaceutically acceptable salt or solvate thereof;

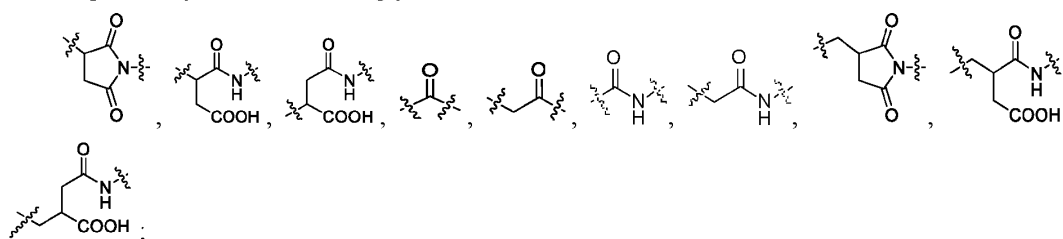


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Among them:

Ab is an antibody having a binding affinity (or specificity) to human Claudin18.2 or an antigen-binding fragment thereof;

L_{11} , L_{12} , and L_{13} are connecting units, and L_{11} , L_{12} , and L_{13} are not identical to each other and are independently and non-limitingly selected from:



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L_3 is present or absent, and when L_3 is present, L_3 is selected from $\text{-(CH}_2\text{)}_o\text{-}$, o is selected from an integer from 1-10; for example, o may be 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In one

embodiment from an integer from 2-8;

Ac is a hydrophilic structural unit;

The 1-position, 2-position and 3-position chiral carbon atoms each independently has a chiral configuration of R or S;

R is selected from hydrogen atom, deuterium atom, halogen, C1-C6 alkyl, substituted C1-C6 alkyl, deuterated C1-C6 alkyl, C3-C8 cycloalkyl C1-C6 alkyl, C1-C6 alkoxy C1-C6 alkyl, C6-C10 aryl, substituted C6-C10 aryl, 5-10-membered heteroaryl, substituted 5-10-membered heteroaryl; In one embodiment R is selected from a hydrogen atom or a C1-C6 alkyl group;

10 R₁ is selected from hydrogen atom, deuterium atom, halogen, C1-C6 alkyl, substituted C1-C6 alkyl, deuterated C1-C6 alkyl, C3-C8 cycloalkyl C1-C6 alkyl, C1-C6 alkoxy C1-C6 alkyl, carboxyl, 3-7 meta-heterocyclic, substituted 3-7 meta-heterocyclic, C6-C10 aryl, substituted C6-C10 aryl, 5-10 meta-heteroaryl, substituted 5-10 heteroaryl. In one embodiment R₁ is selected from a hydrogen atom or a C1-C6 alkyl group. In one embodiment R₁ is selected from C1-C6 alkyl;

R₂ is selected from hydrogen atom, deuterium atom, halogen, C1-C6 alkyl, substituted C1-C6 alkyl, deuterated C1-C6 alkyl, C3-C8 cycloalkyl C1-C6 alkyl, C1-C6 alkoxy C1-C6 alkyl, carboxyl, 3-7 meta-heterocyclic, substituted 3-7 meta-heterocyclic, C6-C10 aryl, substituted C6-C10 aryl, 5-10 meta-heteroaryl, or substituted 5-10 heteroaryl. In one embodiment, R₂ is selected from a hydrogen atom, a halogen or a C1-C6 alkyl group. In one embodiment, R₂ is selected from halogen;

20 X is -C(O)-CR_aR_b-(CR₃R₄)_m-O-, -C(O)-CR_aR_b-(CR₃R₄)_m-NH- or -C(O)-CR_aR_b-(CR₃R₄)_m-S-. In one embodiment, X is selected from -C(O)-CR_aR_b-(CR₃R₄)_m-O-;

R_a and R_b are each independently selected from the group consisting of a hydrogen atom, a deuterium atom, a halogen, a C1-C6 alkyl group, a deuterated C1-C6 alkyl group, a halogenated C1-C6 alkyl group, a C3-C8 cycloalkyl group, a C3-C8 cycloalkyl group C1-C6 alkyl group, a C1-C6 alkoxy group C1-C6 alkyl group, a 3-7 metaheterocyclic group, a substituted 3-7 metaheterocyclic group, a C6-C10 aryl group, a substituted C6-C10 aryl group, a 5-10-membered heteroaryl, or substituted 5-10-membered heteroaryl; In one embodiment, R_a and R_b are each independently selected from a hydrogen atom, a C1-C6 alkyl, a halo-C1-C6 alkyl, a C3-C8 cycloalkyl C1-C6 alkyl or a C6-C10 aryl C1-C6 alkyl; Alternatively, R_a, R_b and the carbon atoms to which they are attached constitute a C3-C8 cycloalkyl group, a C3-C8 cycloalkyl C1-C6 alkyl group, a 3-7 heterocycloalkyl group, a substituted 3-7 heterocycloalkyl group; In one embodiment R_a, R_b and the carbon atoms to which they are attached constitute a C3-C8 cycloalkyl group;

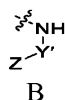
30 R₃, R₄ are the same or different and are independently hydrogen atoms, deuterium atoms, halogens, C1-C6 alkyl, halogenated C1-C6 alkyl, deuterated C1-C6 alkyl, C1-C6 alkoxy, hydroxyl, amino, cyano, nitro, hydroxy C1-C6 alkyl, C3-C8 cycloalkyl, 3-7 heterocyclic, substituted 3-7 heterocyclic, respectively; In one embodiment, R₃, R₄ are independently hydrogen atoms or C1-C6 alkyl groups, respectively; Alternatively, R₃, R₄ and the carbon atoms attached thereto constitute a C3-C8 cycloalkyl group, a C3-C8 cycloalkyl C1-C6 alkyl group, a 3-7 heterocyclic group, a substituted 3-7 heterocyclic group;

40 m is selected from integers 0-4; for example, m may be 0, 1, 2, 3 or 4. In one embodiment,

m is 0 or 1;

Each of n1, n2, and n3 is independently chosen from any integer or any decimal number from 0 to 10, n1, n2, and n3 are not simultaneously 0, and $1 \leq n1 + n2 + n3 \leq 10$.

In one embodiment, the ligand-camptothecin derivative conjugate as shown in general formula II, or a pharmaceutically acceptable salt or solvate thereof, is characterized in that said Ac has the structure shown in formula B:



Among them:

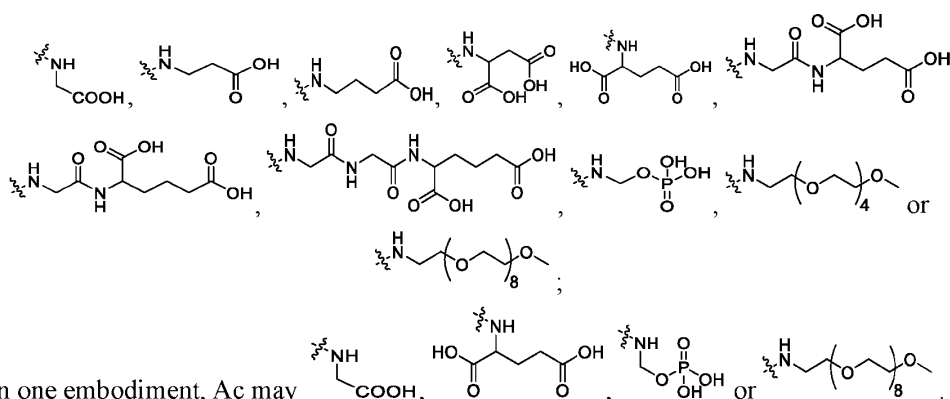
10 Z may be the group consisting of one or more of a hydrophilic structural carboxyl group, a phosphoric acid, a polyphosphoric acid, a phosphite, a sulfonic acid, a sulfinic acid, or a polyethylene glycol (PEG);

In one embodiment Z is selected from hydrophilic structural carboxylates, phosphoric acid or polyethylene glycol (PEG);

Y' is optionally a scaffold connecting the amino group to Z; In one embodiment Y' is a C1-C6 alkylene group (e.g. methylene); and

Ac is connected to the 2-position carbon that has been labeled in structural formula I by means of a bracket Y.

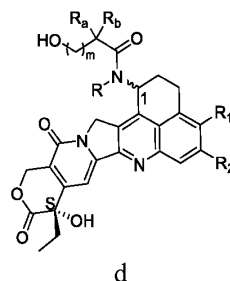
20 In some embodiments, the ligand-camptothecin derivative conjugate, or a pharmaceutically acceptable salt or solvate thereof, is characterized in that said Ac may be glycine, (D/L) alanine, (D/L) leucine, (D/L) isoleucine, (D/L) valine, (D/L) phenylalanine, (D/L) proline, (D/L) tryptophan, (D/L) serine, (D/L) tyrosine, (D/L) cysteine, (D/L) cystine, (D/L) arginine, (D/L) histidine, (D/L) methionine, (D/L) asparagine, (D/L) glutamine, (D/L) threonine, (D/L) aspartic acid, (D/L) glutamic acid, natural or non-natural amino acid derivatives or the following structures or isomers thereof.



In one embodiment, Ac may

30 In some embodiments, the ligand-camptothecin derivative conjugates or pharmaceutically acceptable salts or solvates thereof is characterized in that said Ac may be a glycine, phosphoric acid, (D/L) glutamic acid, or poly(ethylene glycol) hydrophilic structure.

In some embodiments, the ligand-camptothecin derivatives conjugates or pharmaceutically acceptable salts or solvates thereof are characterized in that said camptothecin derivatives have the structure shown in formula d below;



Among them:

R is selected from hydrogen atom, deuterium atom, halogen, C1-C6 alkyl, substituted C1-C6 alkyl, deuterated C1-C6 alkyl, C3-C8 cycloalkyl C1-C6 alkyl, C1-C6 alkoxy C1-C6 alkyl, C6-C10 aryl, substituted C6-C10 aryl, 5-10 heteroaryl, substituted 5-10 heteroaryl;

10 In one embodiment R is selected from a hydrogen atom or a C1-C6 alkyl group;

R₁ is selected from hydrogen atom, deuterium atom, halogen, C1-C6 alkyl, substituted C1-C6 alkyl, deuterated C1-C6 alkyl, C3-C8 cycloalkyl C1-C6 alkyl, C1-C6 alkoxy C1-C6 alkyl, carboxyl, 3-7 meta-heterocyclic, substituted 3-7 meta-heterocyclic, C6-C10 aryl, substituted C6-C10 aryl, 5-10 meta-heteroaryl, or substituted 5-10 heteroaryl;

In one embodiment, R₁ is selected from a hydrogen atom or a C1-C6 alkyl group;

In one embodiment, R₁ is selected from C1-C6 alkyl;

R₂ is selected from hydrogen atom, deuterium atom, halogen, C1-C6 alkyl, substituted C1-C6 alkyl, deuterated C1-C6 alkyl, C3-C8 cycloalkyl C1-C6 alkyl, C1-C6 alkoxy C1-C6 alkyl, carboxyl, 3-7 meta-heterocyclic, substituted 3-7 meta-heterocyclic, C6-C10 aryl, substituted C6-C10 aryl, 5-10 meta-heteroaryl, or substituted 5-10 heteroaryl;

20 In one embodiment, R₂ is selected from a hydrogen atom, a halogen or a C1-C6 alkyl group;

In one embodiment, R₂ is selected from halogen;

R_a and R_b are each independently selected from the group consisting of a hydrogen atom, a deuterium atom, a halogen, a C1-C6 alkyl group, a deuterated C1-C6 alkyl group, a halogenated C1-C6 alkyl group, a C3-C8 cycloalkyl group, a C3-C8 cycloalkyl group C1-C6 alkyl group, a C1-C6 alkoxy group C1-C6 alkyl group, a 3-7 metaheterocyclic group, a substituted 3-7 metaheterocyclic group, a C6-C10 aryl group, a substituted C6-C10 aryl group, a 5-10 heteroaryl, substituted 5-10 heteroaryl; In one embodiment, R_a and R_b are each independently selected from a hydrogen atom, a C1-C6 alkyl, a halo-C1-C6 alkyl, a C3-C8 cycloalkyl C1-C6 alkyl or a C6-C10 aryl C1-C6 alkyl; In one embodiment, R_a and R_b are each independently selected from a hydrogen atom, a C1-C6 alkyl, a halo-C1-C6 alkyl, a C3-C8 cycloalkyl C1-C6 alkyl, a C6-C10 aryl; In one embodiment R_a and R_b are each independently selected from a hydrogen atom, a methyl group, an ethyl group, a trifluoromethyl group, a cyclopropylmethyl group, a phenyl group; Alternatively, R_a, R_b and the carbon atoms to which they are attached constitute a C3-C8 cycloalkyl

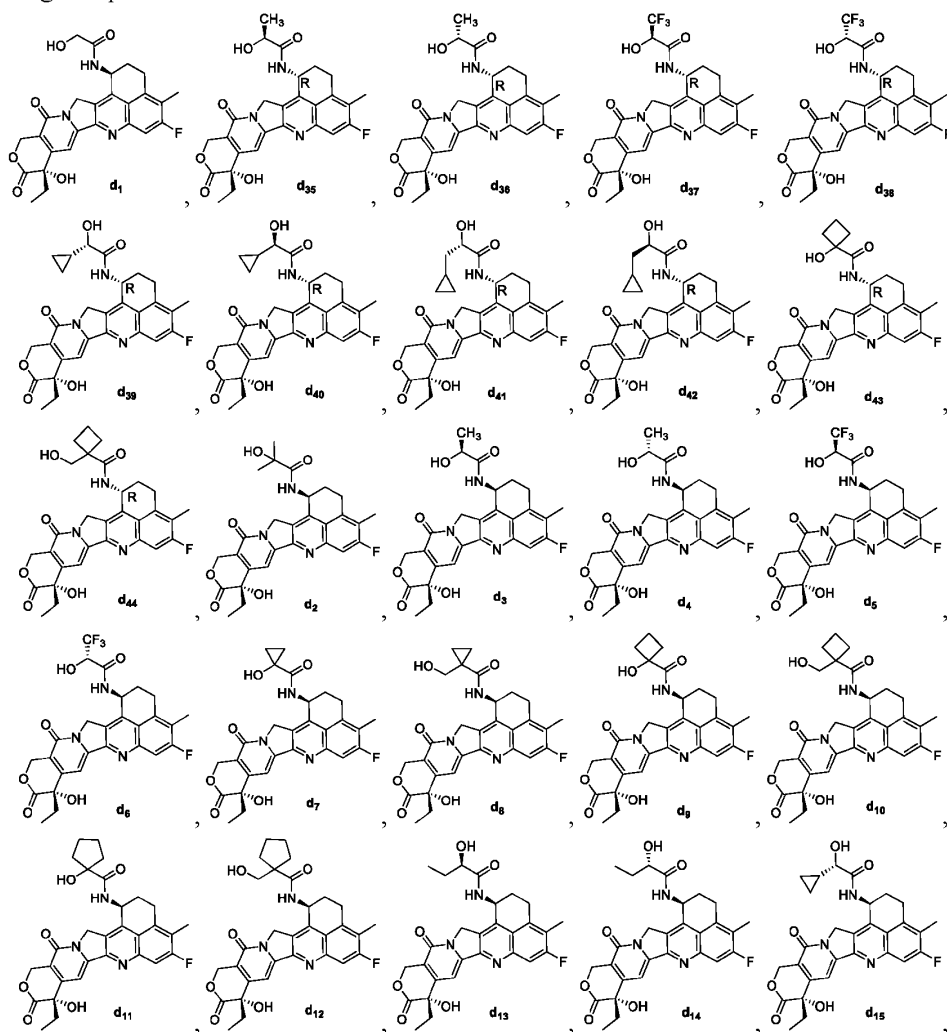
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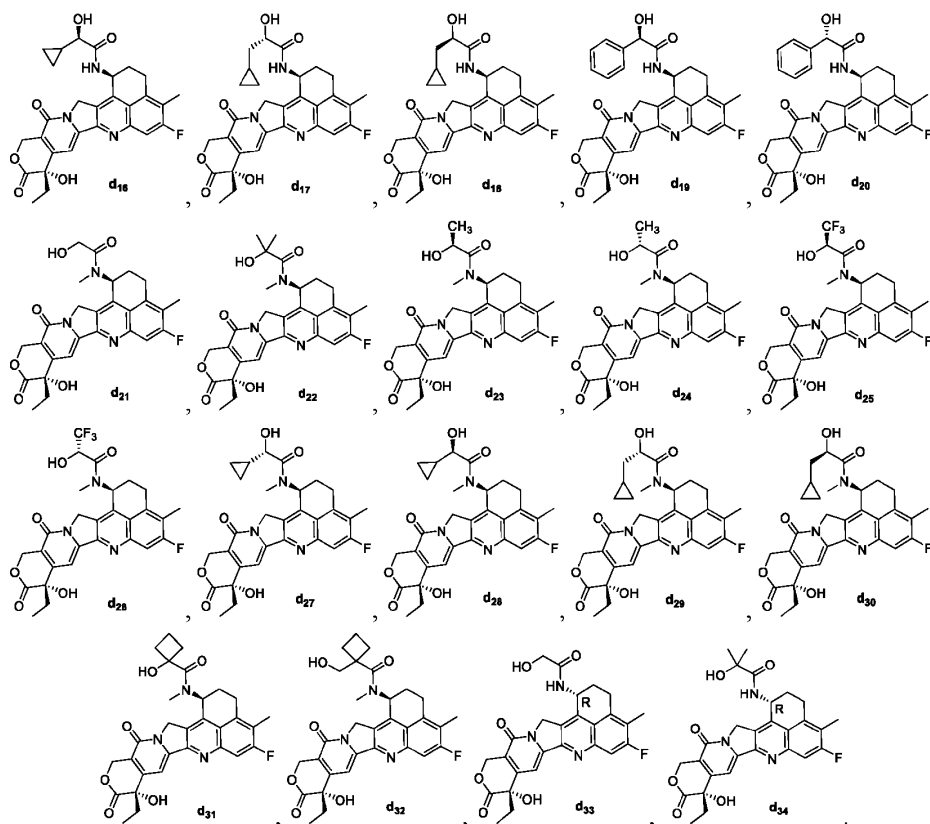
group, a C3-C8 cycloalkyl C1-C6 alkyl group, a 3-7 heterocycloalkyl group, a substituted 3-7 heterocycloalkyl group; In one embodiment, R_a, R_b and the carbon atoms to which they are attached constitute a C3-C8 cycloalkyl group (e.g., a C3-C5 cycloalkyl group);

The 1-position chiral carbon atom may be in R or S configuration;

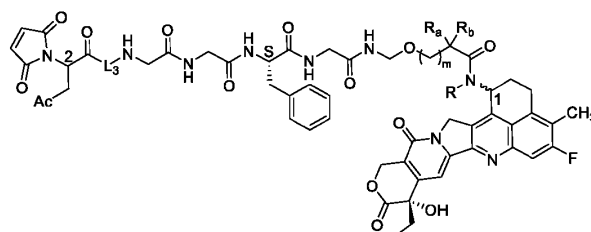
m is selected from 0 or 1.

In some embodiments, the ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof is characterized in that: said structural formula d may be the following compounds:



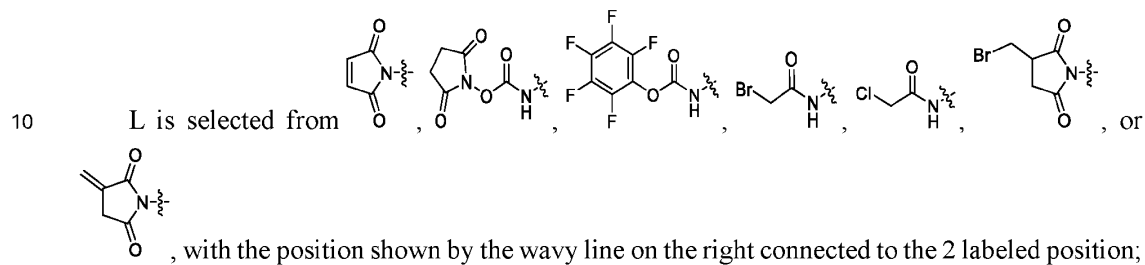


In a further aspect, the application discloses a linker-drug compound or a pharmaceutically acceptable salt or solvate thereof, having the structure shown in formula III below:



III

Among them:



R is selected from hydrogen atom, deuterium atom, halogen, C1-C6 alkyl, substituted C1-C6 alkyl, deuterated C1-C6 alkyl, C3-C8 cycloalkyl C1-C6 alkyl, C1-C6 alkoxy C1-C6 alkyl, C6-C10 aryl, substituted C6-C10 aryl, 5-10 heteroaryl, substituted 5-10 heteroaryl;

R_a is selected from hydrogen atom, deuterium atom, halogen, C1-C6 alkyl, deuterated C1-C6 alkyl, halogenated C1-C6 alkyl, C3-C8 cycloalkyl, C3-C8 cycloalkyl C1-C6 alkyl, C1-C6 alkoxy C1-C6 alkyl, 3-7 meta-heterocyclic group, substituted 3-7 meta-heterocyclic group, C6-C10 aryl, substituted C6-C10 aryl, 5-10 meta-heteroaryl, substituted 5-10 heteroaryl;

R_b is selected from hydrogen atom, deuterium atom, halogen, C1-C6 alkyl, deuterated C1-C6 alkyl, halogenated C1-C6 alkyl, C3-C8 cycloalkyl, C3-C8 cycloalkyl C1-C6 alkyl, C1-C6 alkoxy
10 C1-C6 alkyl, 3-7 meta-heterocyclic group, substituted 3-7 meta-heterocyclic group, C6-C10 aryl, substituted C6-C10 aryl, 5-10 meta-heteroaryl, substituted 5-10 heteroaryl;

Alternatively, R_a, R_b and the carbon atoms attached thereto constitute a C3-C8 cycloalkyl group, a C3-C8 cycloalkyl C1-C6 alkyl group, a 3-7 heterocyclic group, a substituted 3-7 heterocyclic group;

In one embodiment, R_a and R_b are each independently selected from a hydrogen atom, a C1-C6 alkyl, a halo-C1-C6 alkyl, a C3-C8 cycloalkyl C1-C6 alkyl, a C6-C10 aryl; In one embodiment, R_a and R_b are each independently selected from a hydrogen atom, a methyl group, an ethyl group, a trifluoromethyl group, a cyclopropylmethyl group, a phenyl group;

Alternatively, R_a, R_b and the carbon atoms to which they are attached constitute a C3-C8
20 cycloalkyl group, a C3-C8 cycloalkyl C1-C6 alkyl group, a 3-7 heterocycloalkyl group, a
substituted 3-7 heterocycloalkyl group; In one embodiment, R_a, R_b and the carbon atoms to which
they are attached constitute a C3-C8 cycloalkyl group (e.g., a C3-C5 cycloalkyl group);

L₃ is absent or present, and when L₃ is present, it is selected from , o is selected from an integer from 1 to 10; in one embodiment, o is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10;

The 1-position or 2-position chiral carbon atom each independently has R or S configuration;

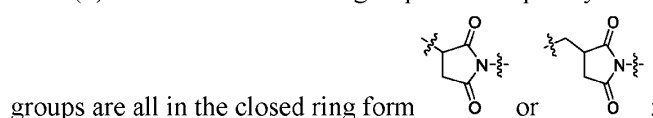
Ac is a hydrophilic structural unit:

m is selected from 0 or 1:

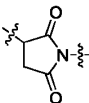
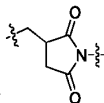
In one embodiment, said linker-drug compound or a pharmaceutically acceptable salt or solvate thereof is used to couple with ligand Ab to form said ligand-camptothecin derivative
30 conjugates of formula I or formula II.

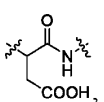
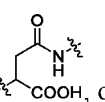
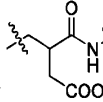
In some embodiments, L may comprise a succinimide group. In these embodiments, the ligand-drug conjugate may undergo hydrolysis under readily hydrolyzable conditions, with the site of hydrolysis being the butanediimide moiety of the linker unit. When the ligand contains multiple linker-drugs, the following scenarios can occur with varying degrees of hydrolysis:

(1) the succinimide groups are completely non-hydrolyzed, i.e., the succinimide

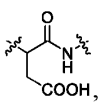
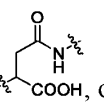
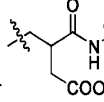


(2) incomplete hydrolysis of the butanediimide group, i.e., some of the butanediimide

group is in the closed ring form  or  and some of the butanediimide group is in

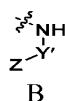
the open ring form , , or  ;

(3) complete hydrolysis of the succinimide groups, i.e., the succinimide groups are all

in the ring-opened form , , or  .

Thus, when multiple L's containing a succinimide moiety are present in the ADC at the same time (i.e., Ab is connected to multiple drug-linkers containing a succinimide moiety), all or some of these succinimide moieties may be in a closed-loop form, partially open-loop form, or all or some of them may be in an open-loop form.

In some embodiments, the linker-drug compounds or pharmaceutically acceptable salts or solvates thereof as disclosed herein are characterized in that: said Ac has the structure shown in formula B below, the



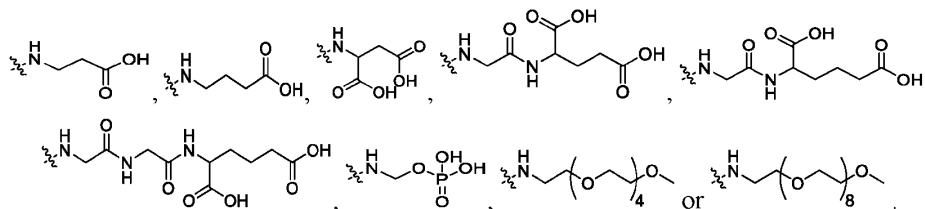
Among them:

Z may be the group consisting of one or more of a hydrophilic structural carboxyl group, a phosphoric acid, a polyphosphoric acid, a phosphite, a sulfonic acid, a sulfinic acid, or a polyethylene glycol (PEG);

Y' is optionally a scaffold connecting the amino group to Z; In one embodiment Y' is a C1-C6 alkylene group (e.g., methylene);

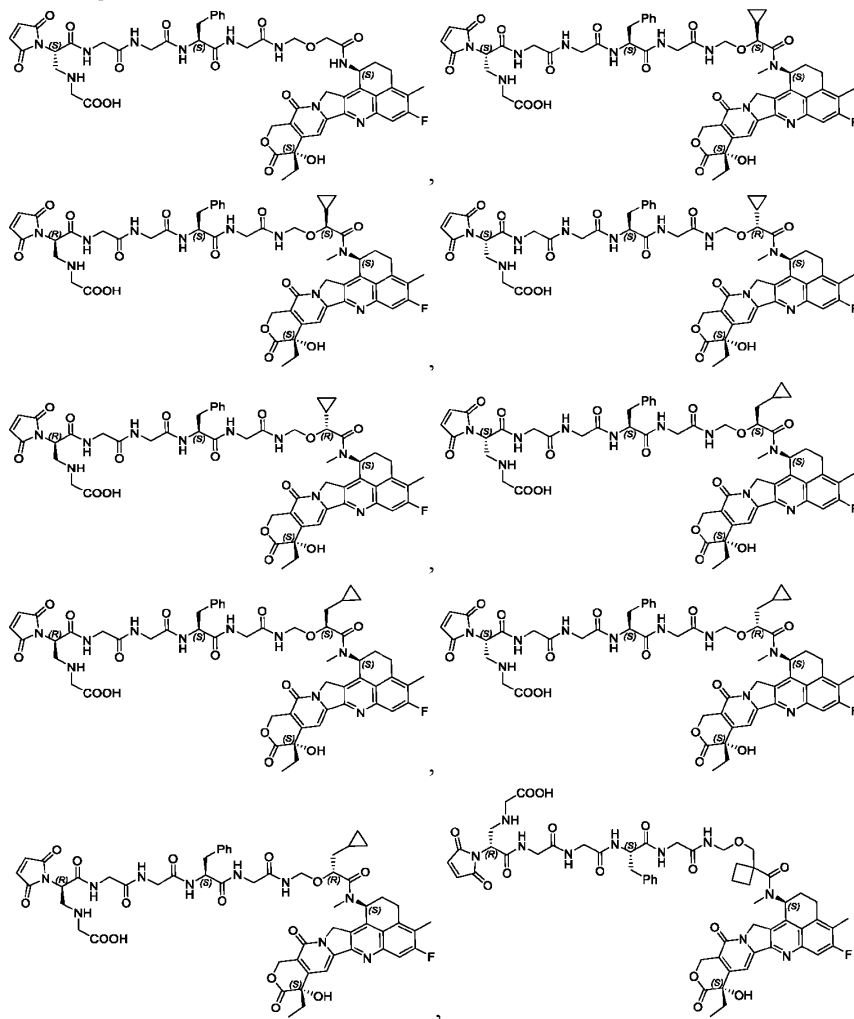
Ac is connected to the 2-position carbon that has been labeled in structural formula I by means of a bracket Y.

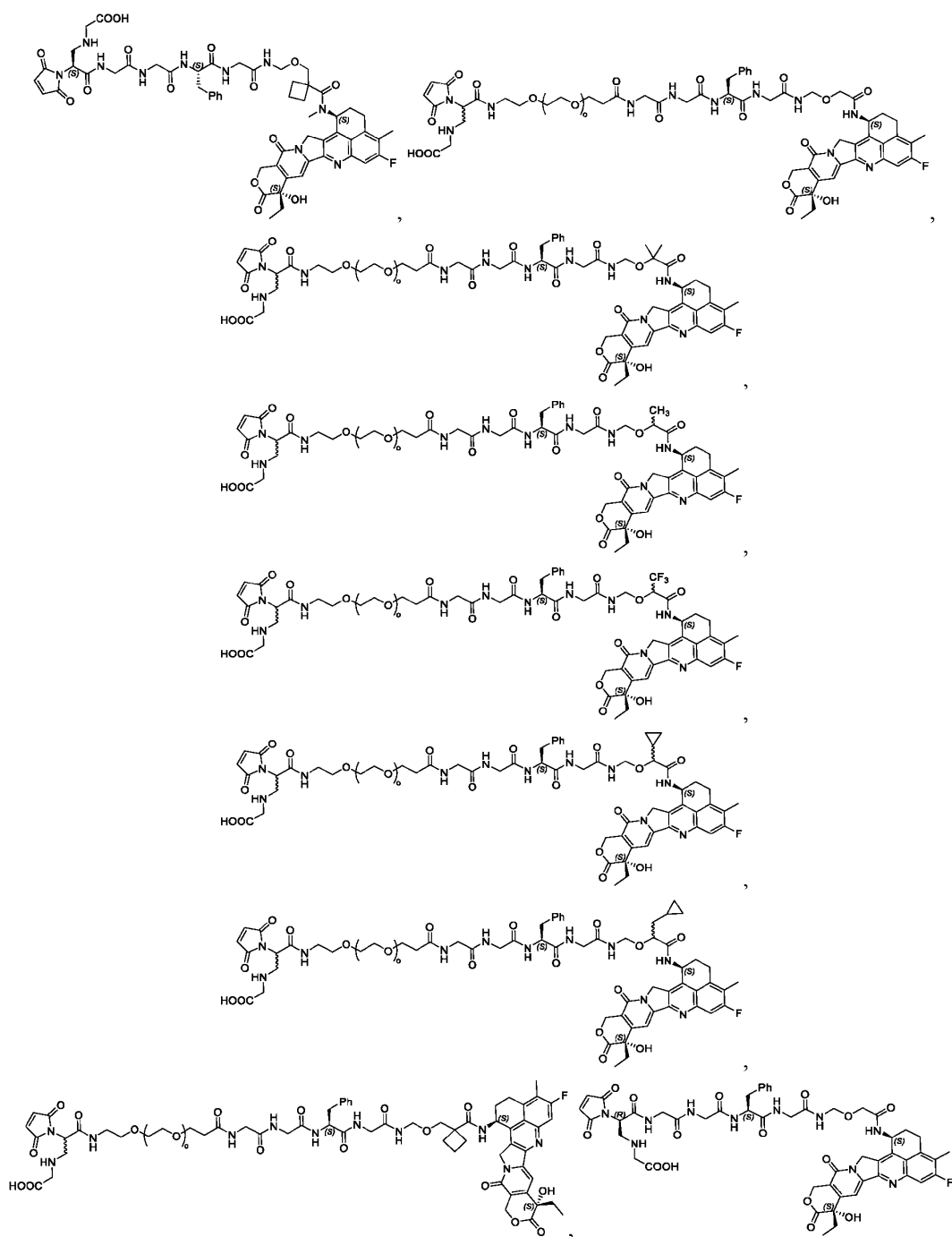
In some embodiments, the linker-drug compounds or pharmaceutically acceptable salts or solvates thereof is characterized in that said Ac may be glycine, (*D/L*) alanine, (*D/L*) leucine, (*D/L*) isoleucine, (*D/L*) valine, (*D/L*) phenylalanine, (*D/L*) proline, (*D/L*) tryptophan, (*D/L*) serine, (*D/L*) tyrosine, (*D/L*) cysteine, (*D/L*) cystine, (*D/L*) arginine, (*D/L*) histidine, (*D/L*) methionine, (*D/L*) asparagine, (*D/L*) glutamine, (*D/L*) threonine, (*D/L*) aspartic acid, (*D/L*) glutamic acid, natural or non-natural amino acid derivatives, or the following structures.

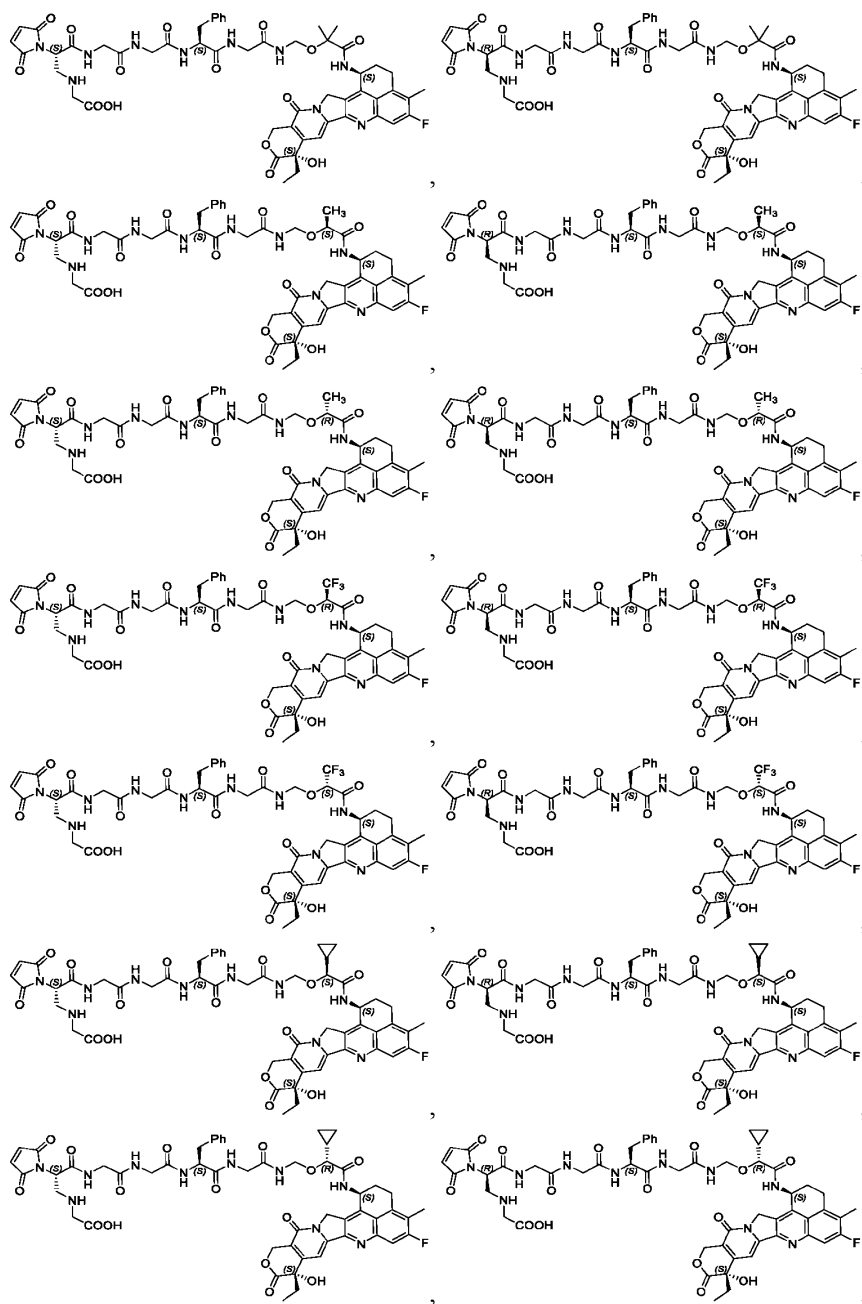


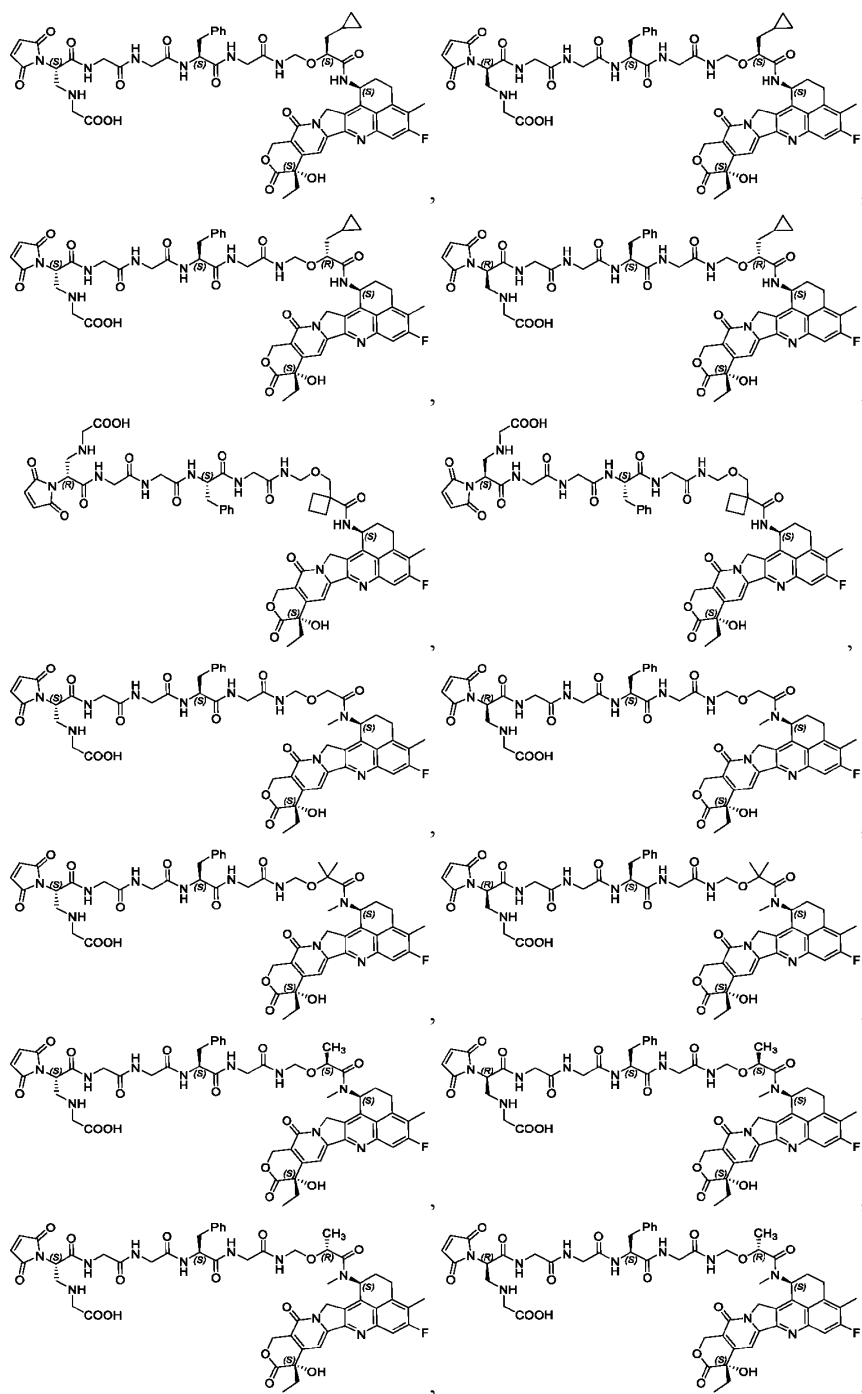
In some embodiments, the linker-drug compounds or pharmaceutically acceptable salts or solvates thereof are characterized in that: Ac may be a glycine, phosphoric acid, (*D/L*) glutamic acid or polyethylene glycol hydrophilic structure.

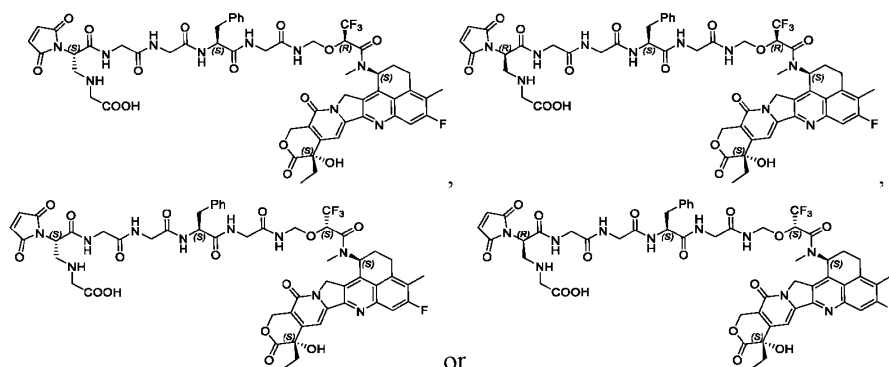
In some embodiments, the linker-drug compounds or pharmaceutically acceptable salts or solvates thereof is characterized in that said linker-drug compounds are non-limitingly selected from the following structures or isomers thereof.







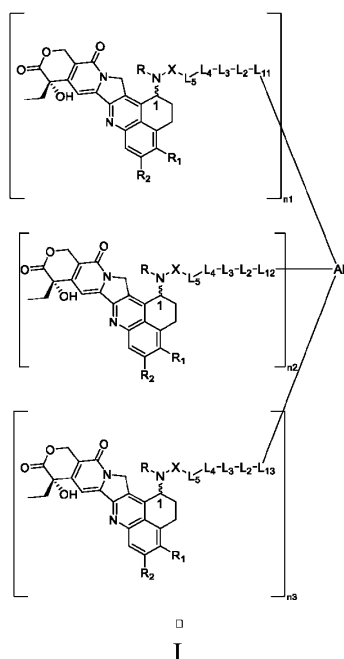


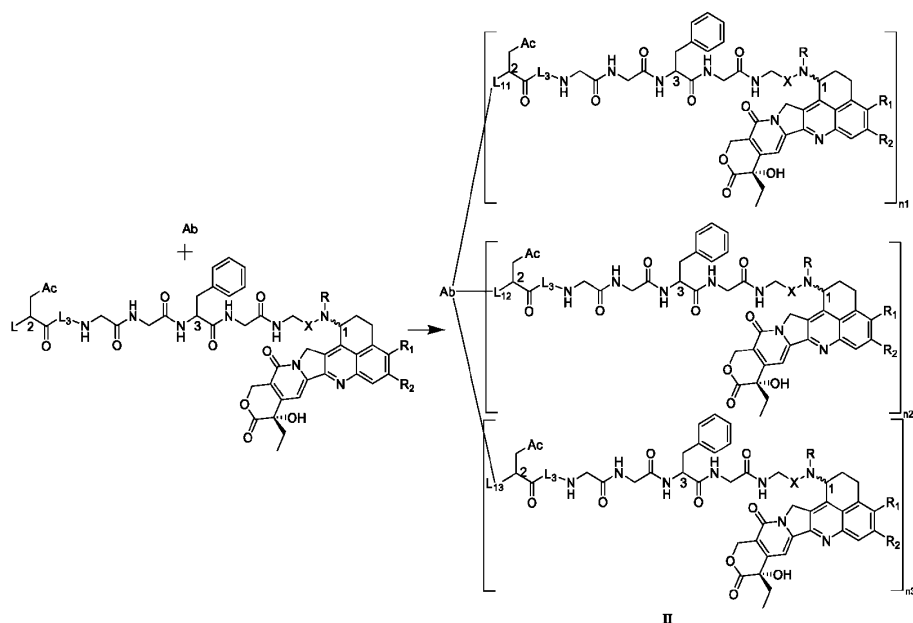


In one embodiment, o is selected from integers of 1-10; for example, o may be 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

The linker-drug compounds or pharmaceutically acceptable salts or solvates thereof disclosed herein can be used as intermediates for coupling with the ligand Ab to form ligand-camptothecin derivatives conjugates of formula I or formula II.

In a further aspect, the application discloses methods of preparing a ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof as shown in general formula I or general formula II. In one embodiment, the method comprises the steps of conjugating the reduced antibody or antigen-binding fragment thereof with the linker-drug compound by the coupling reaction to provide the ligand-camptothecin derivative conjugates as shown in general formula I or general formula II:





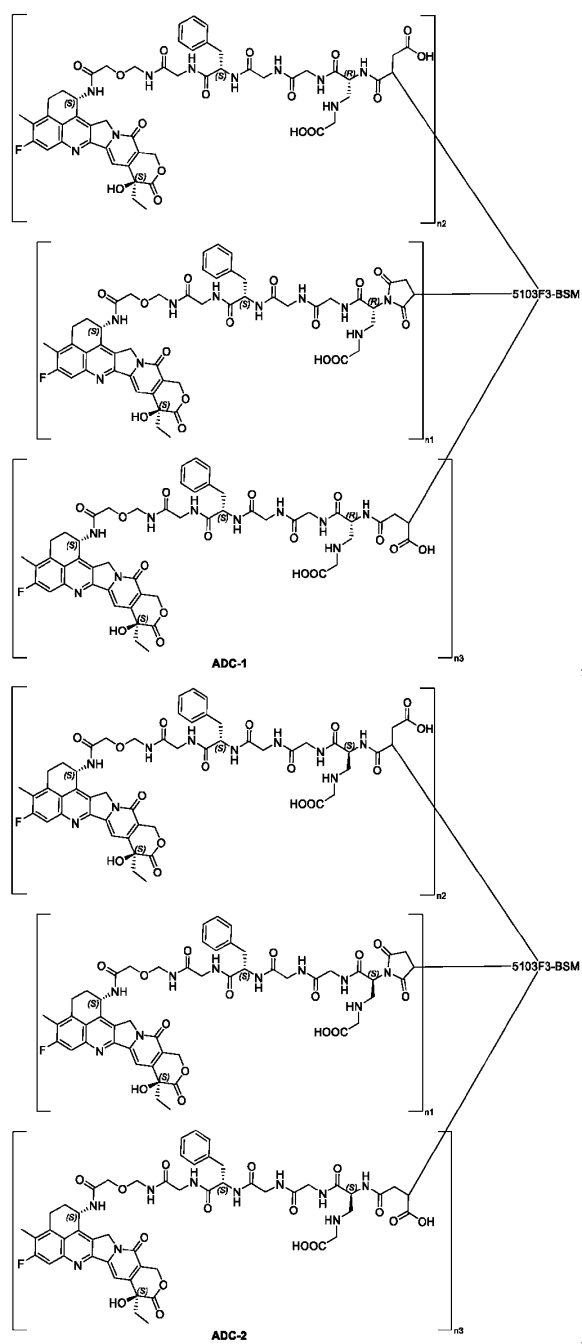
The 1-position, 2-position, or 3-position chiral carbon atoms have absolute chirality in R- or S-configuration;

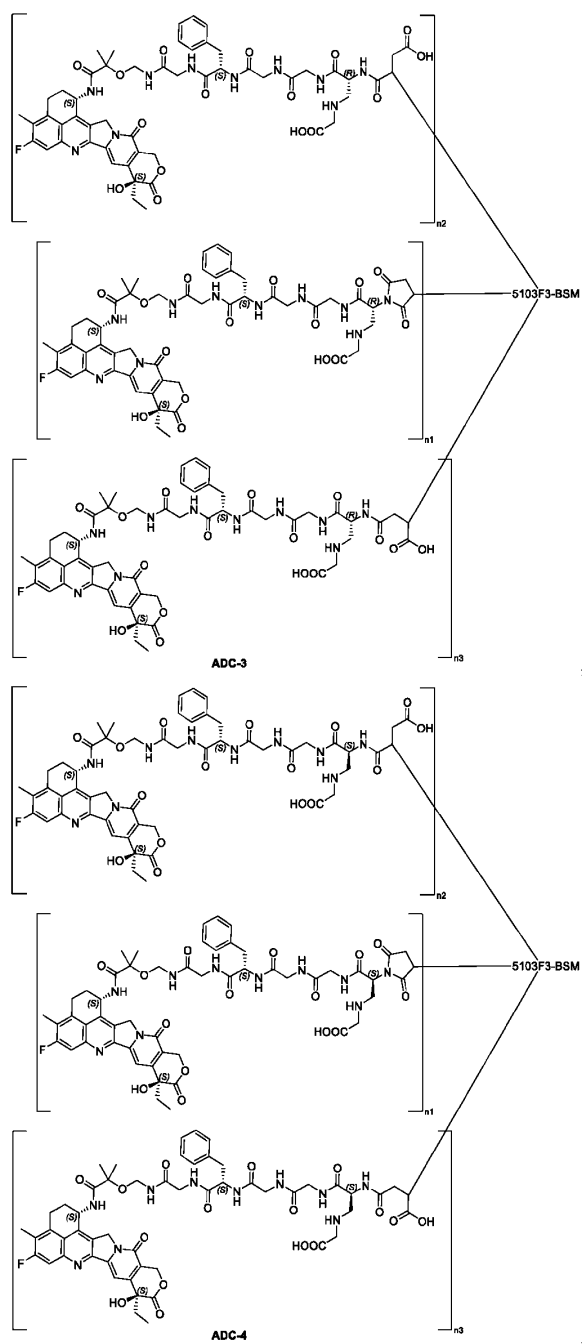
Ab, L, L₁₁, L₁₂, L₁₃, L₂, L₃, L₄, L₅, X, R, R₁, R₂, n1, n2, or n3 as previously described.

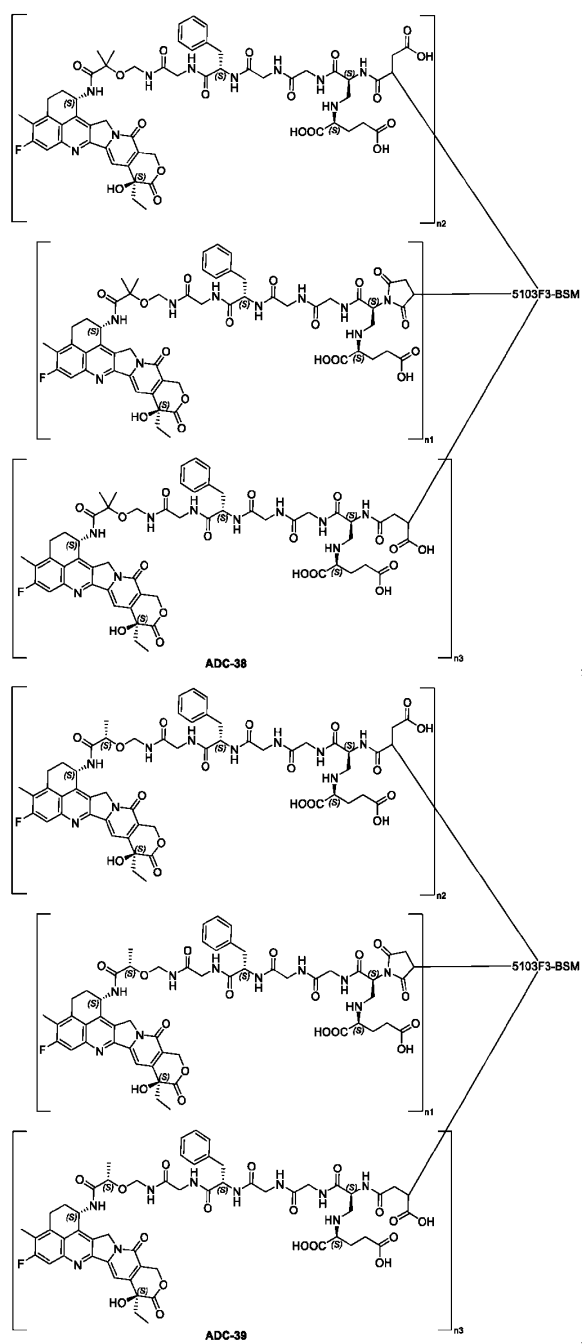
The present disclosure also relates to the use of the linker-drug compounds described herein, or pharmaceutically acceptable salts or solvates thereof, as intermediates in the preparation of ligand-camptothecin derivative conjugates or pharmaceutically acceptable salts or solvates thereof.

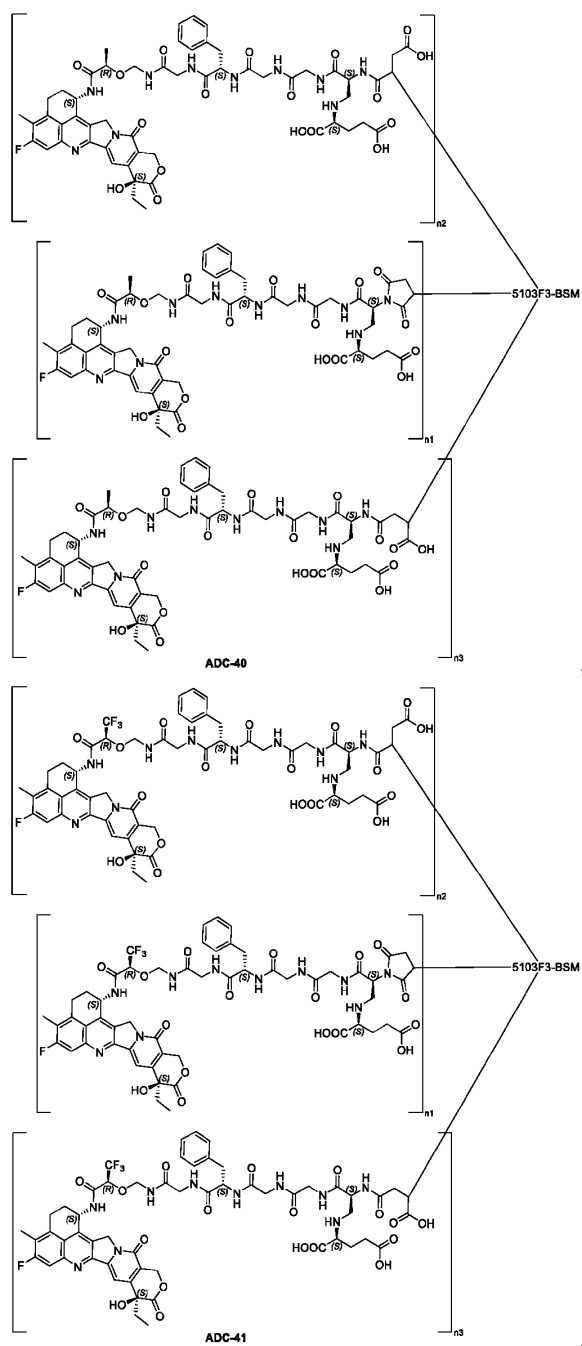
In certain embodiments, said preparation is carried out according to the method of preparation disclosed herein.

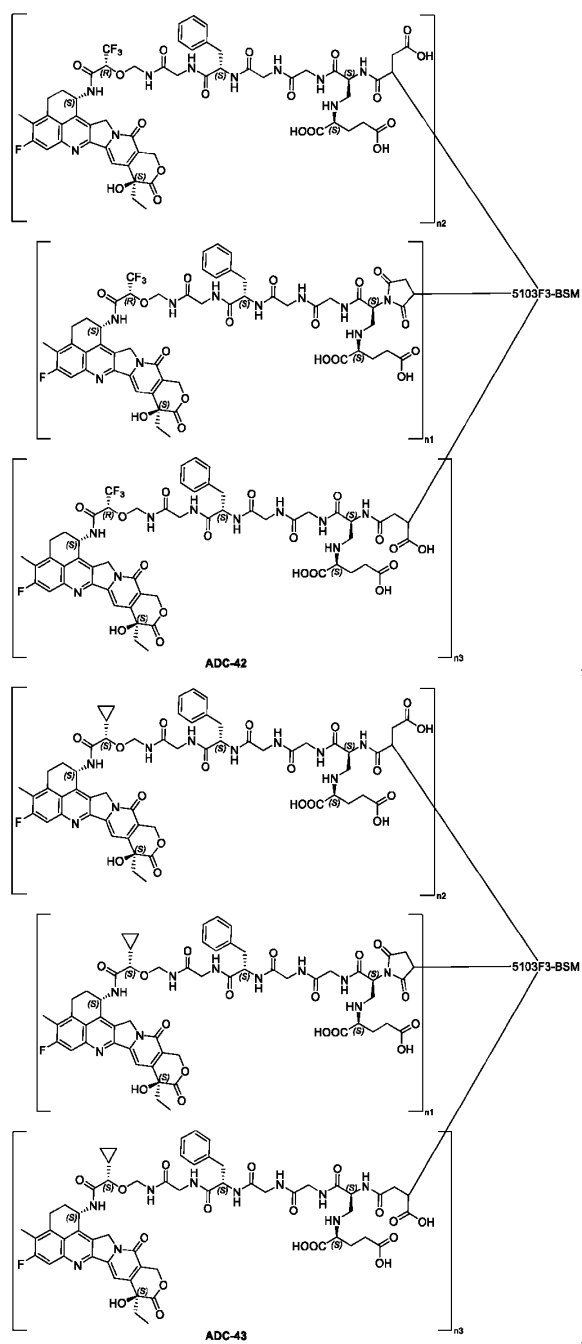
- 10 In some embodiments, the ligand-camptothecin derivative conjugates, or their pharmaceutically acceptable salts or solvates thereof, are characterized in that said ligand-camptothecin derivative conjugates, or their pharmaceutically acceptable salt or solvate thereof, may be the following structure, or a butanediimide ring-opening structure thereof or an isomer thereof.

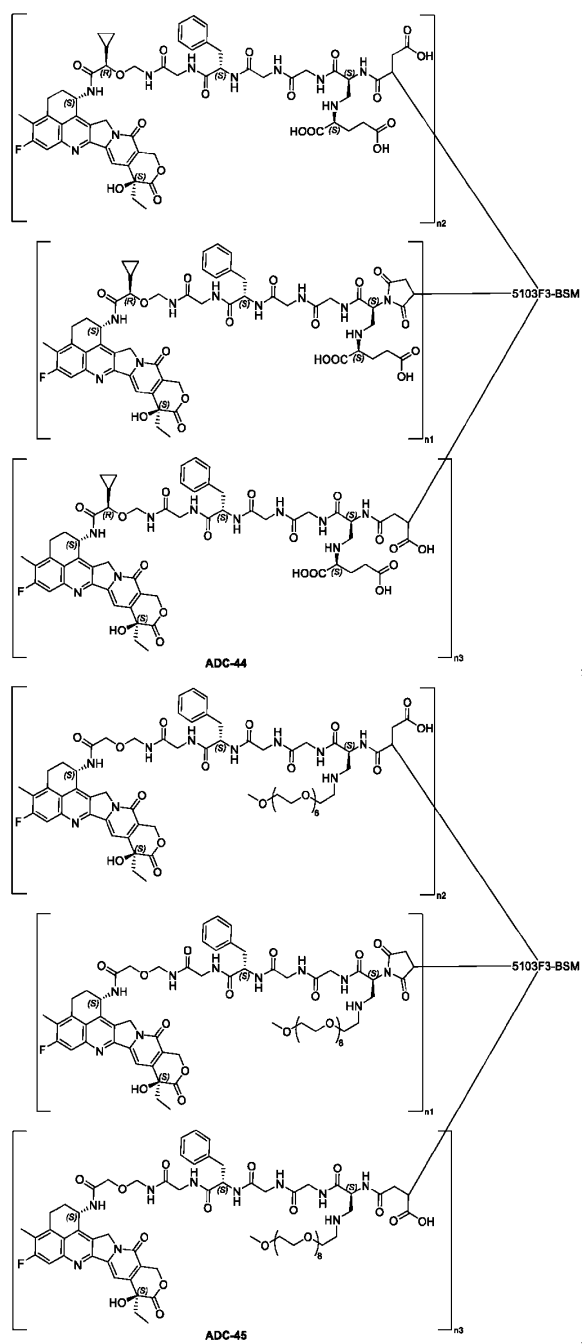


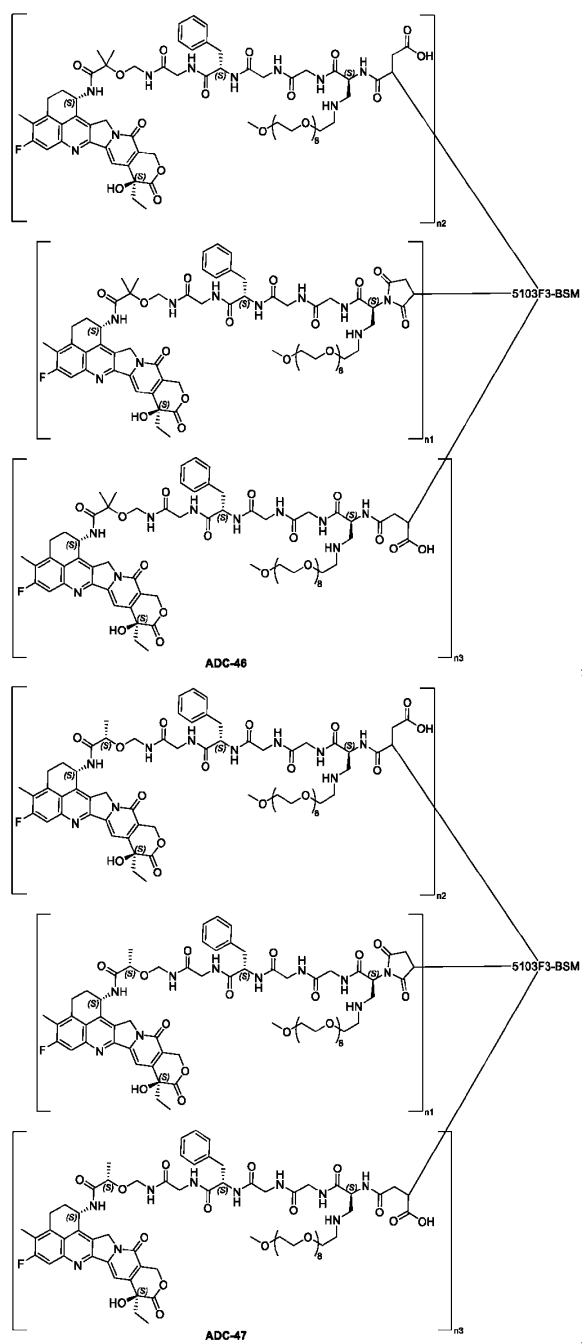


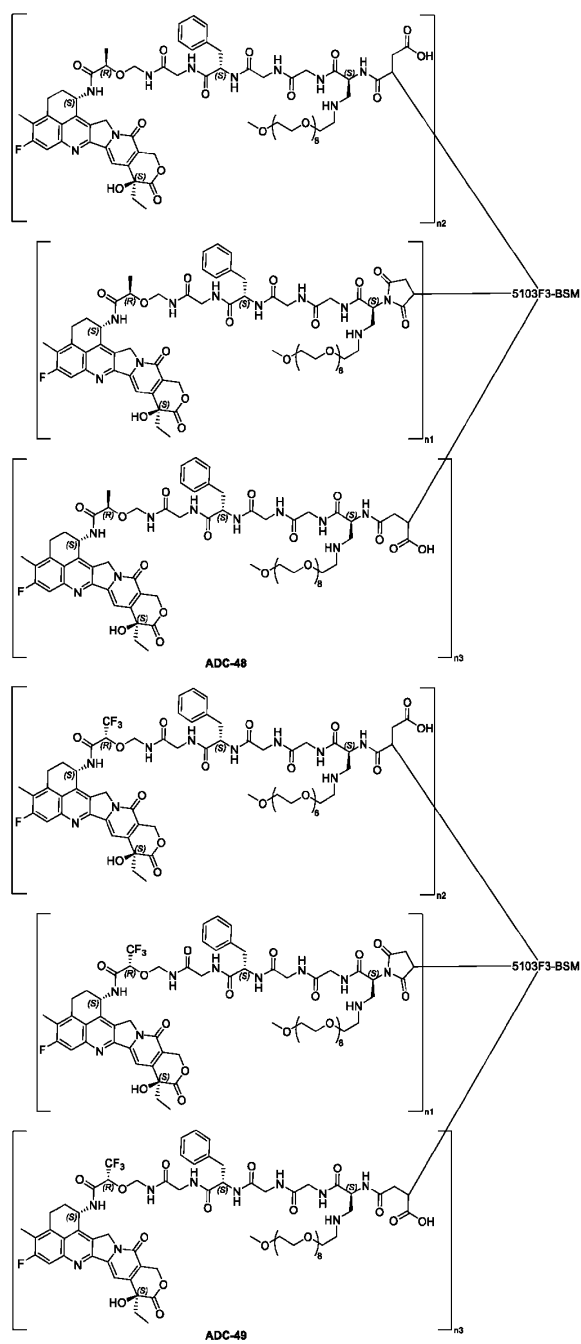


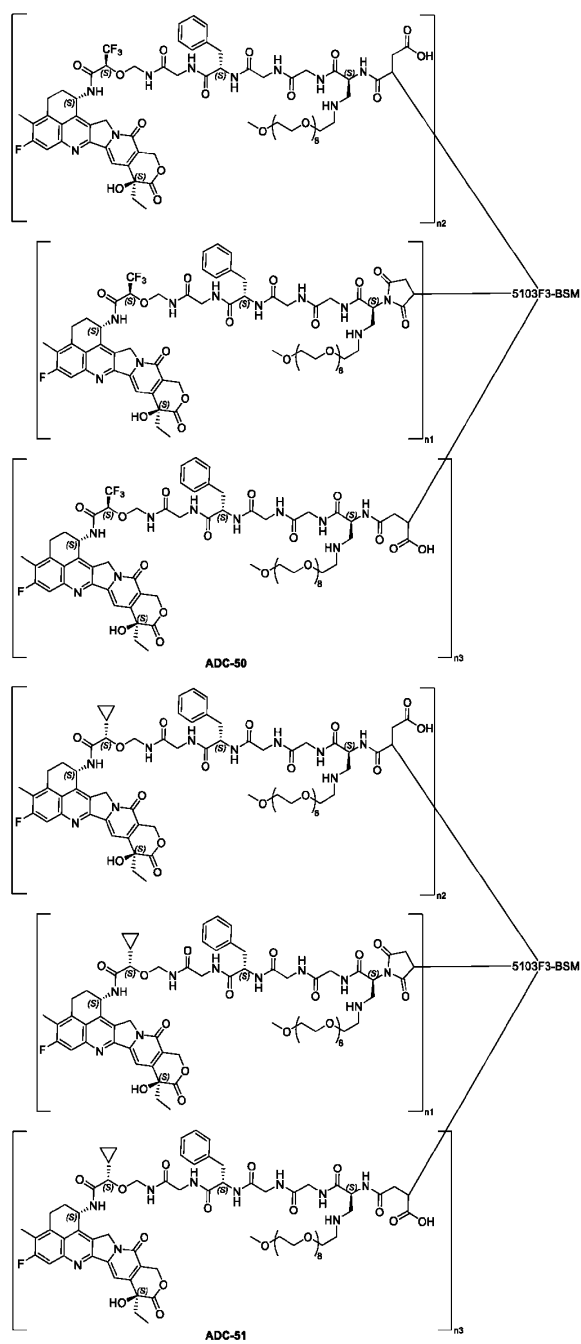


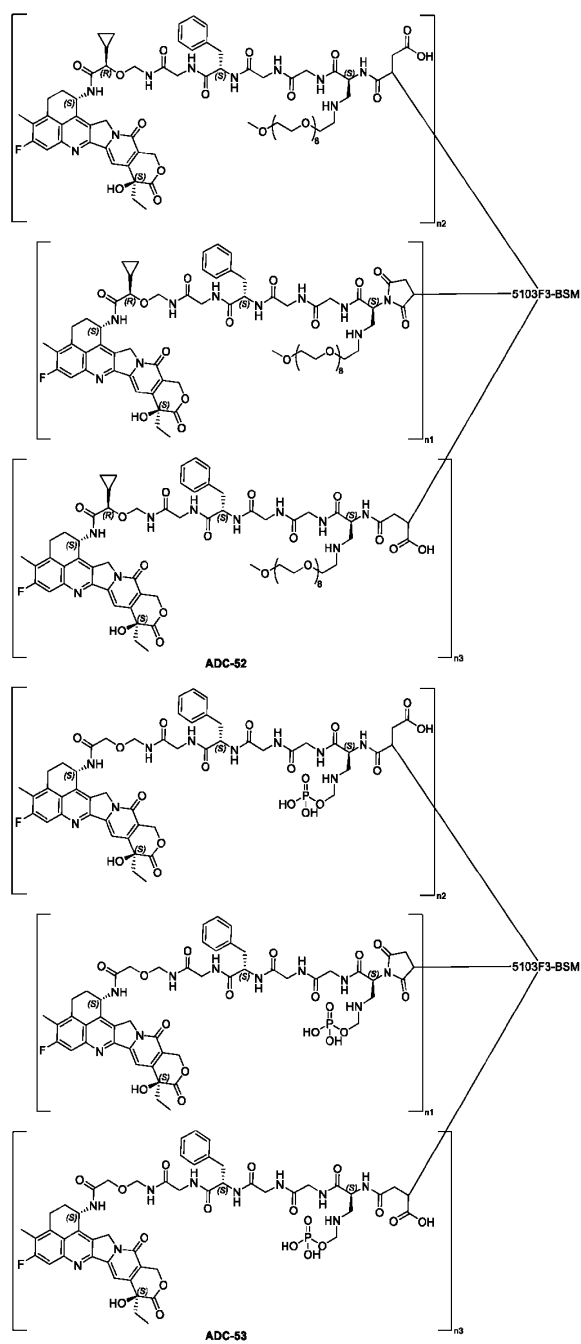


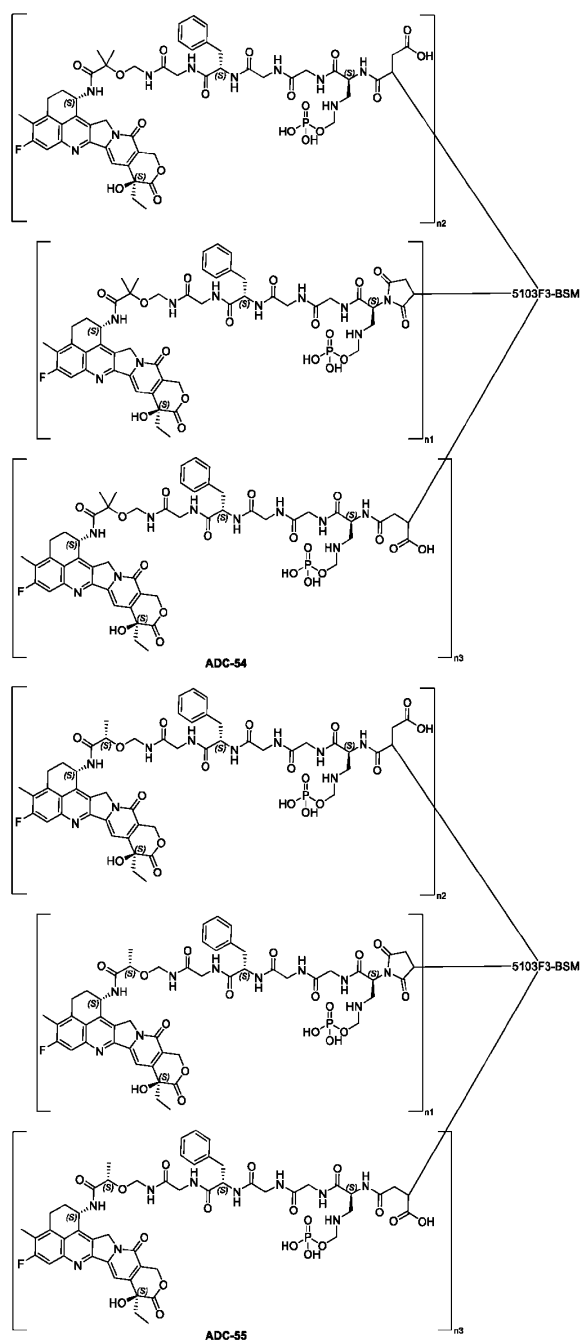


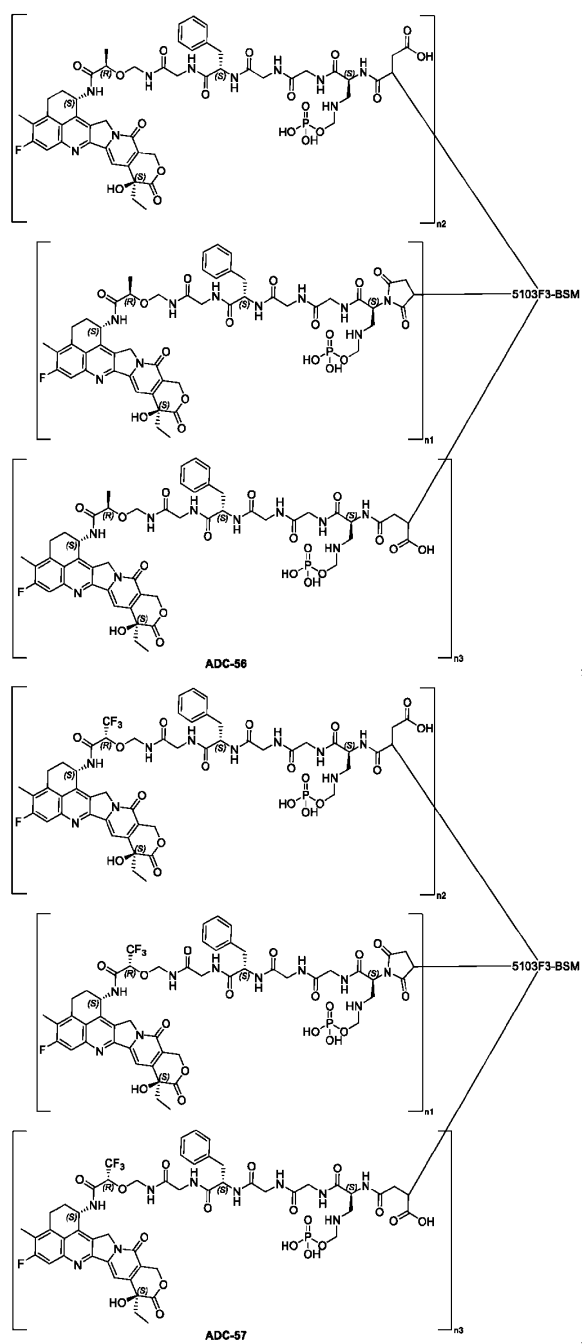


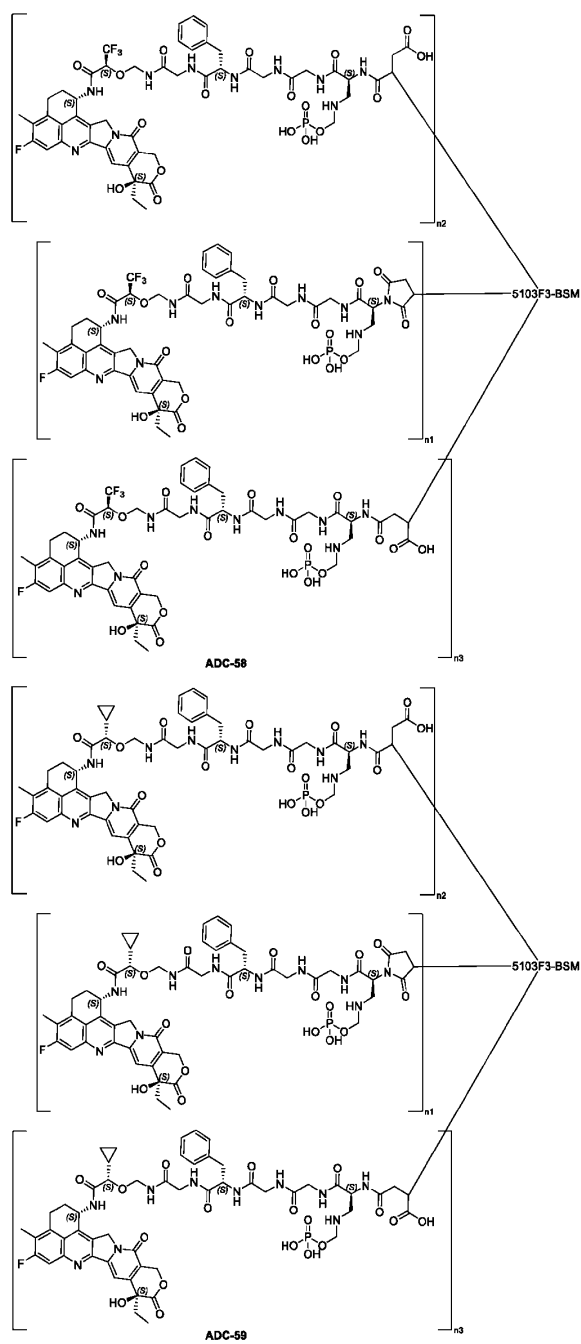


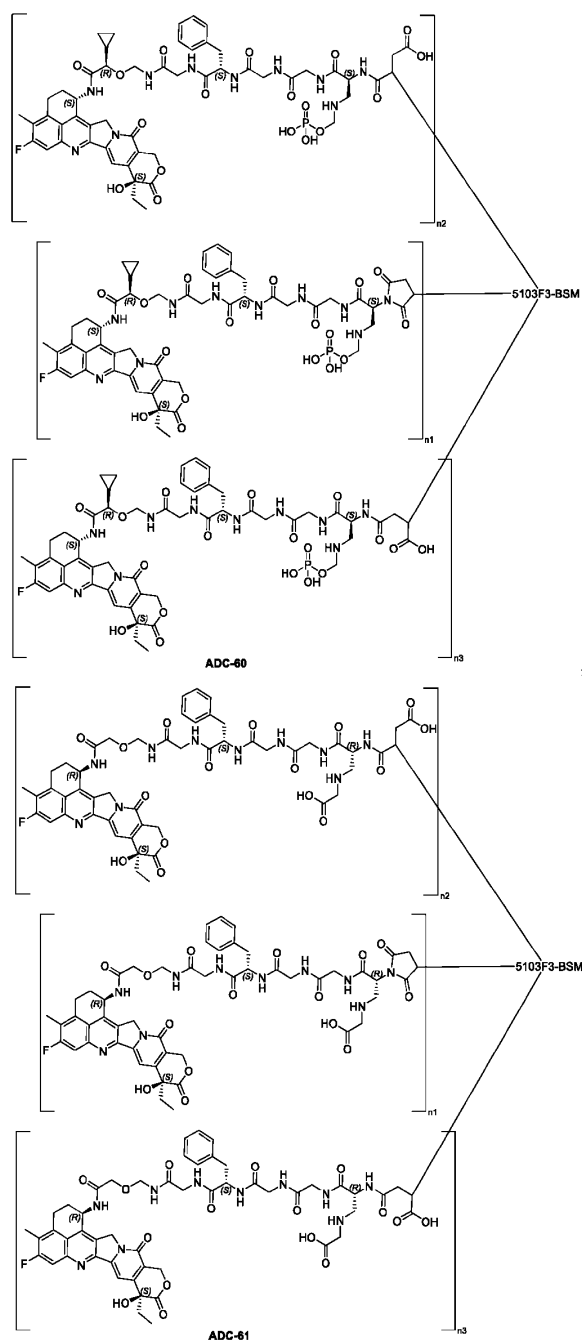


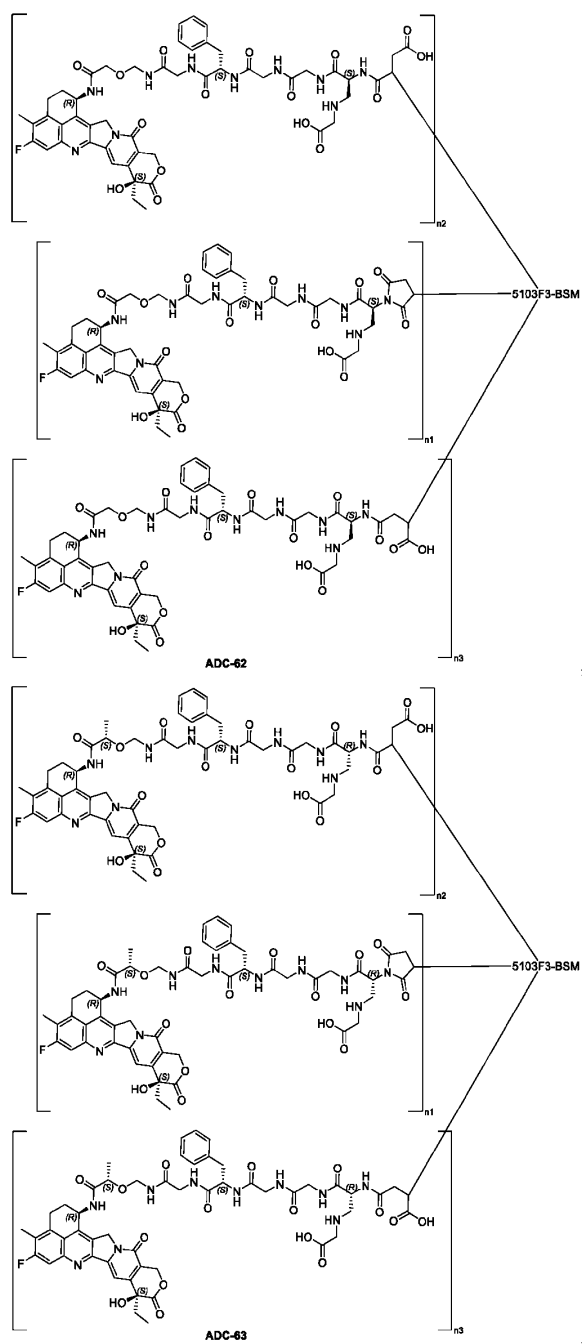


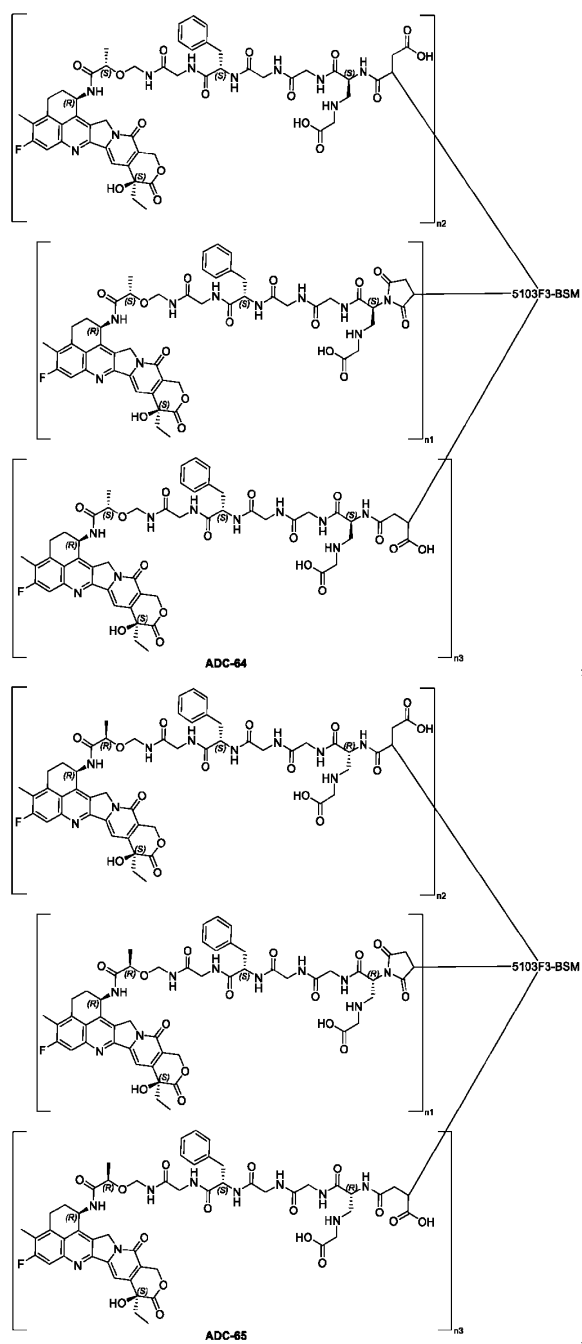


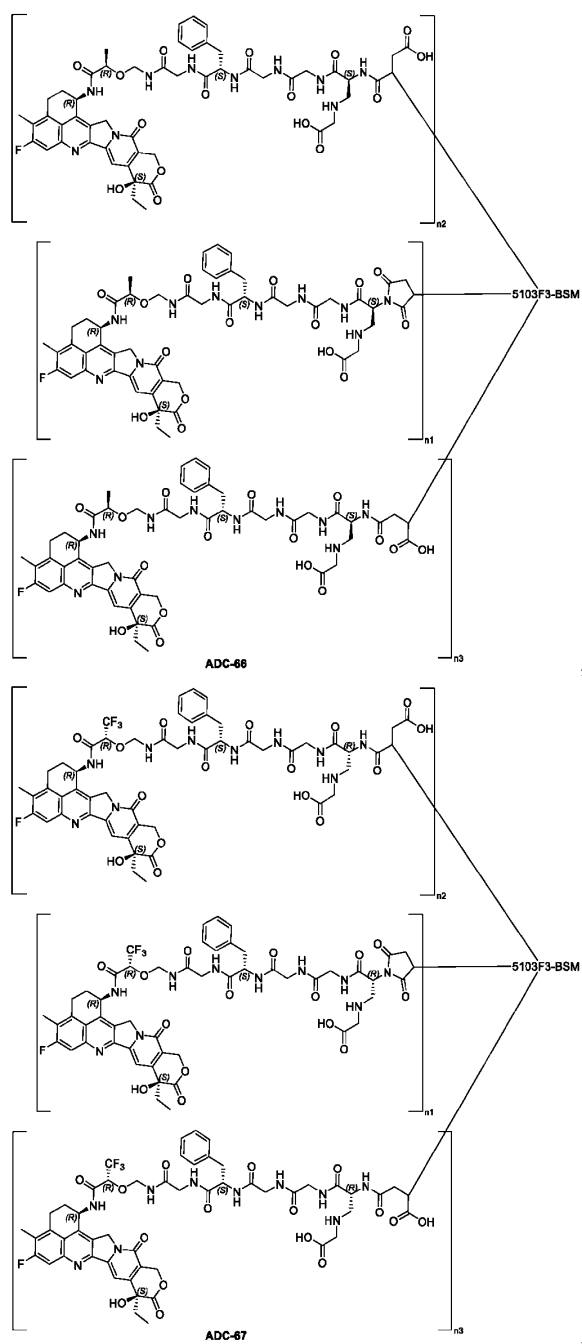


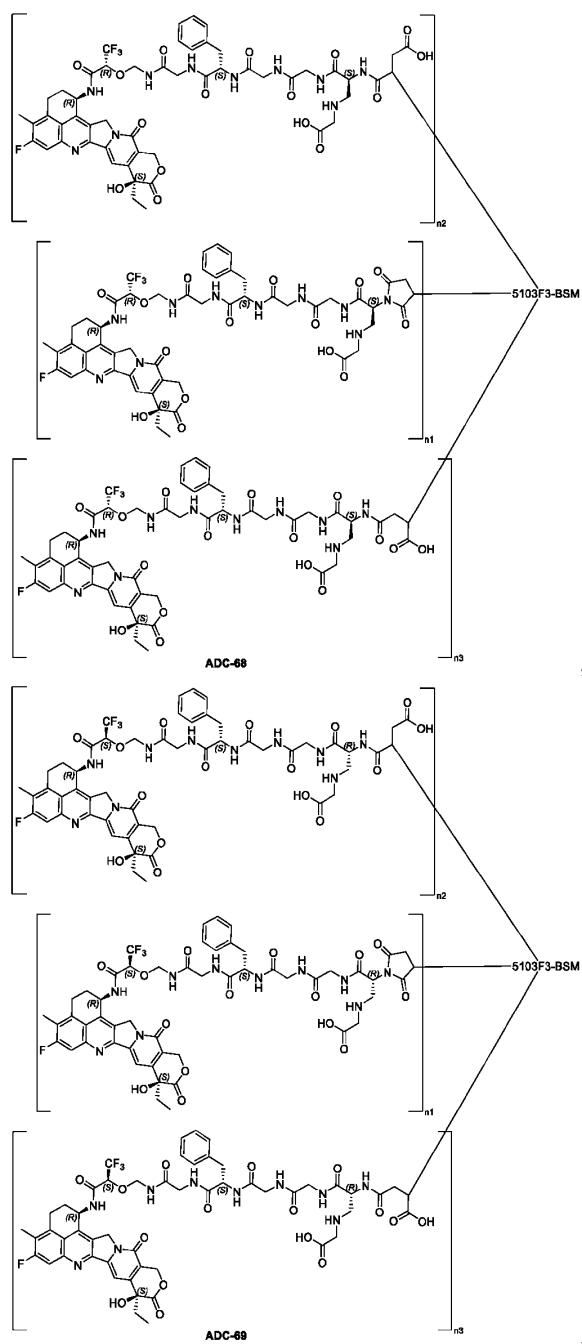


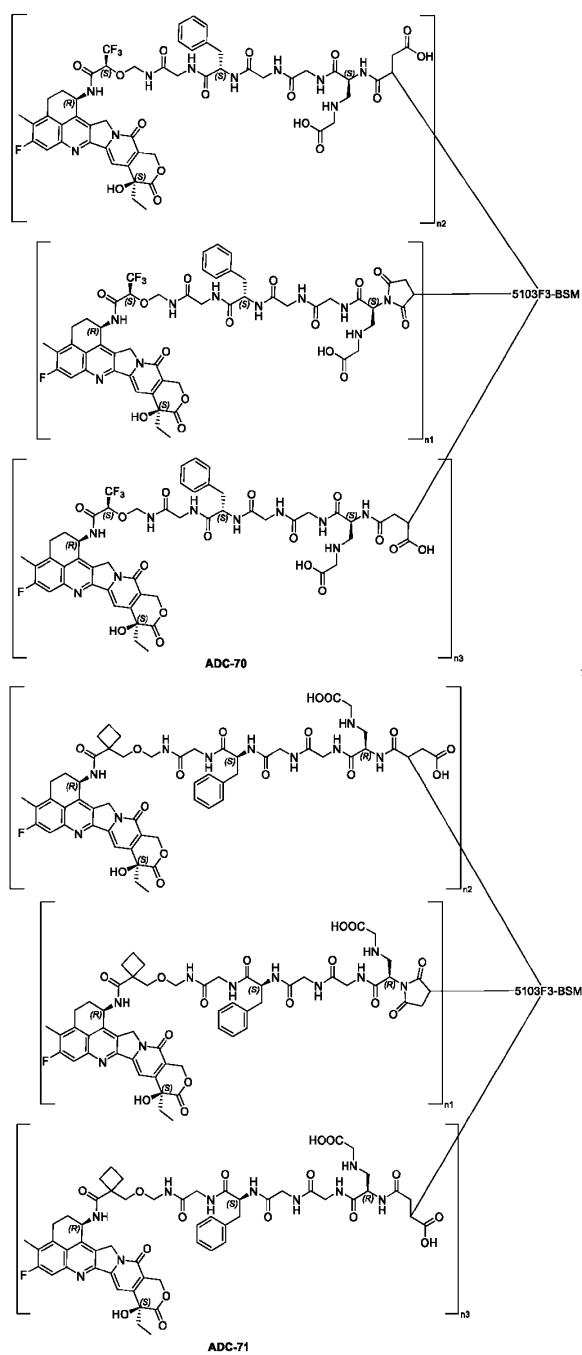


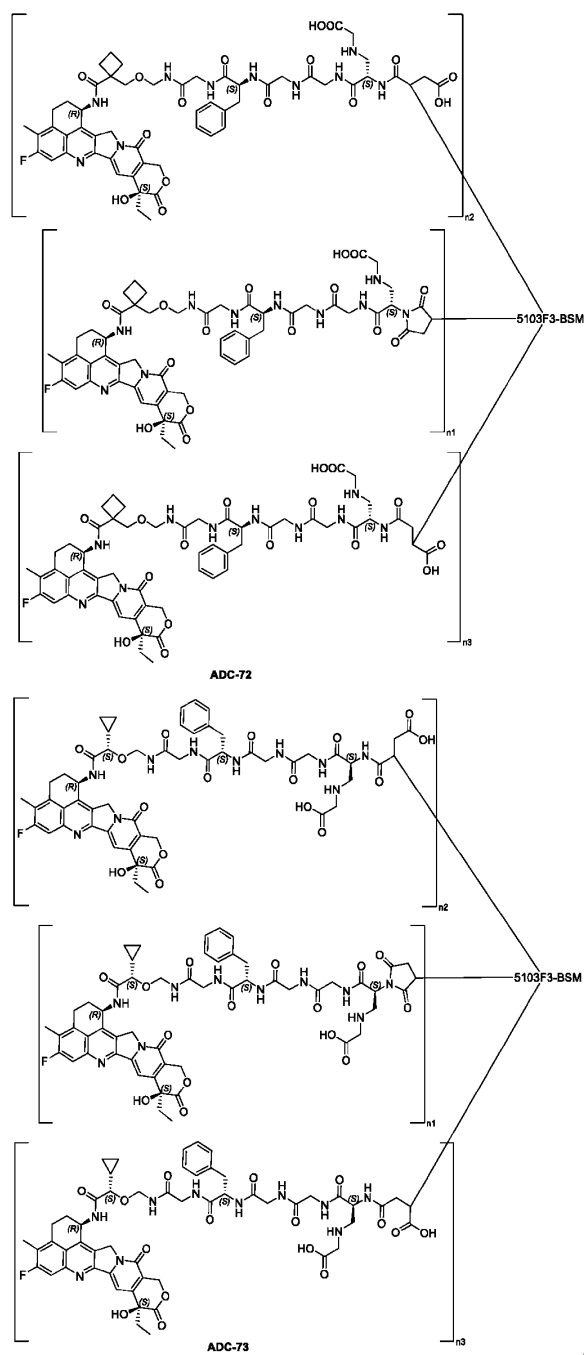


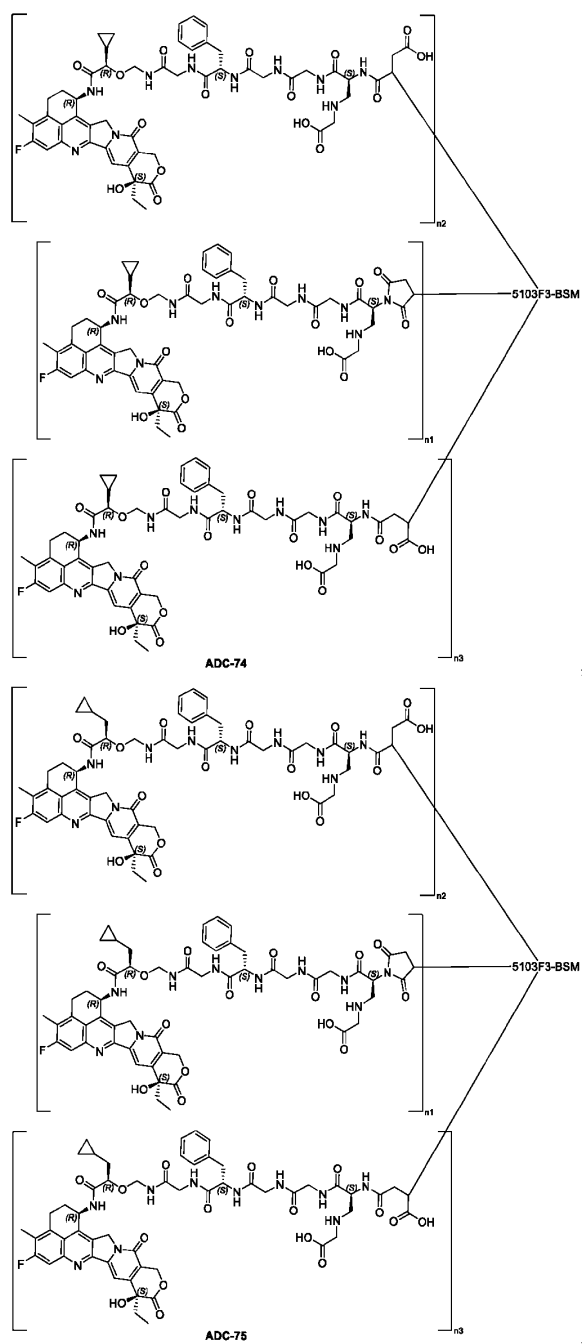


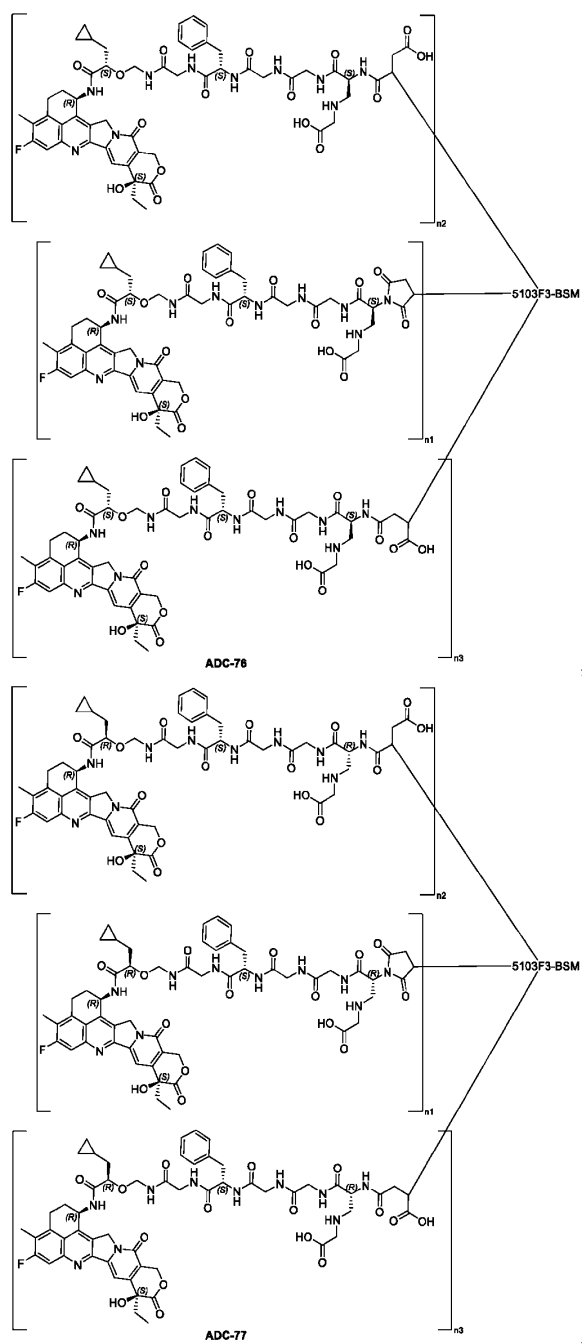


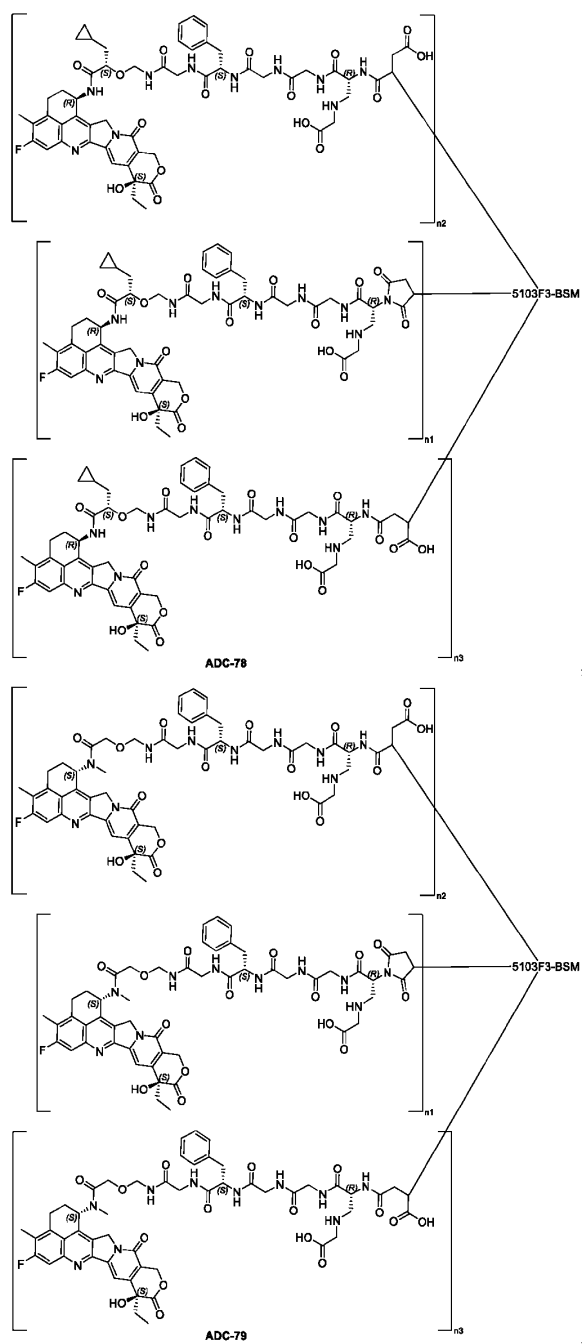


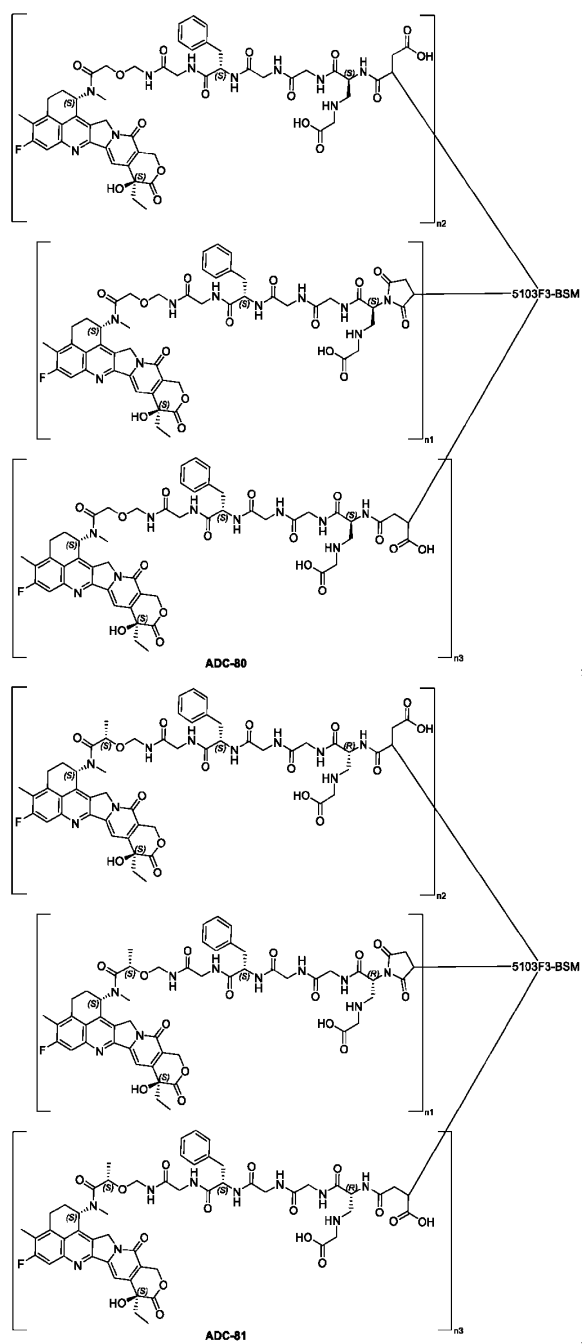


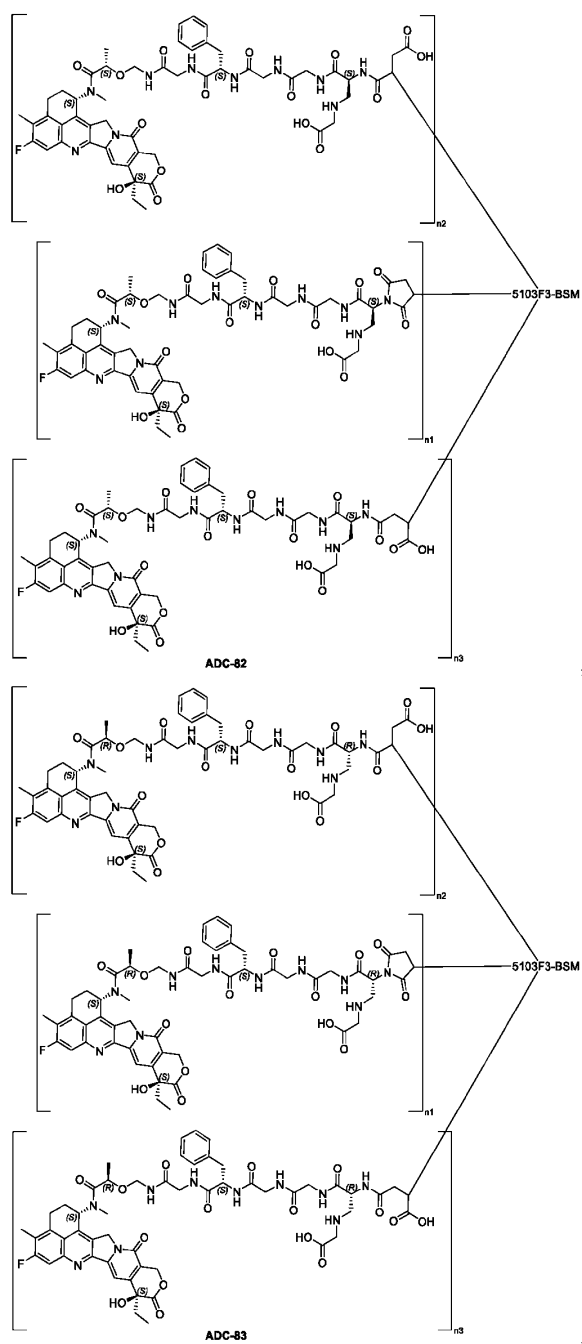


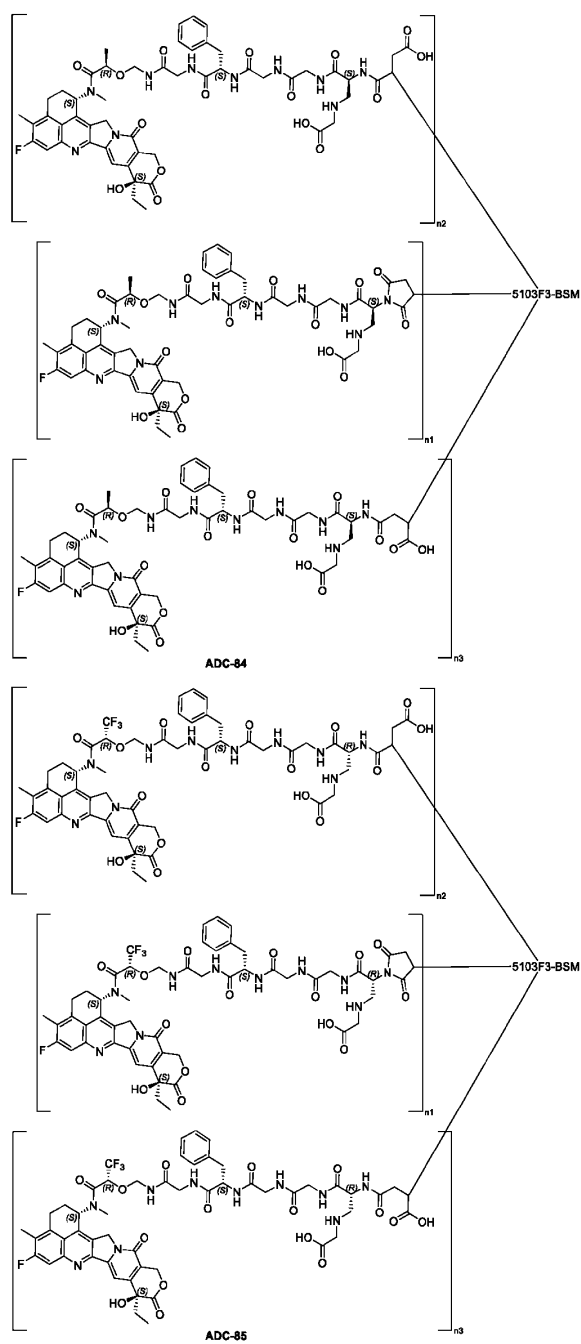


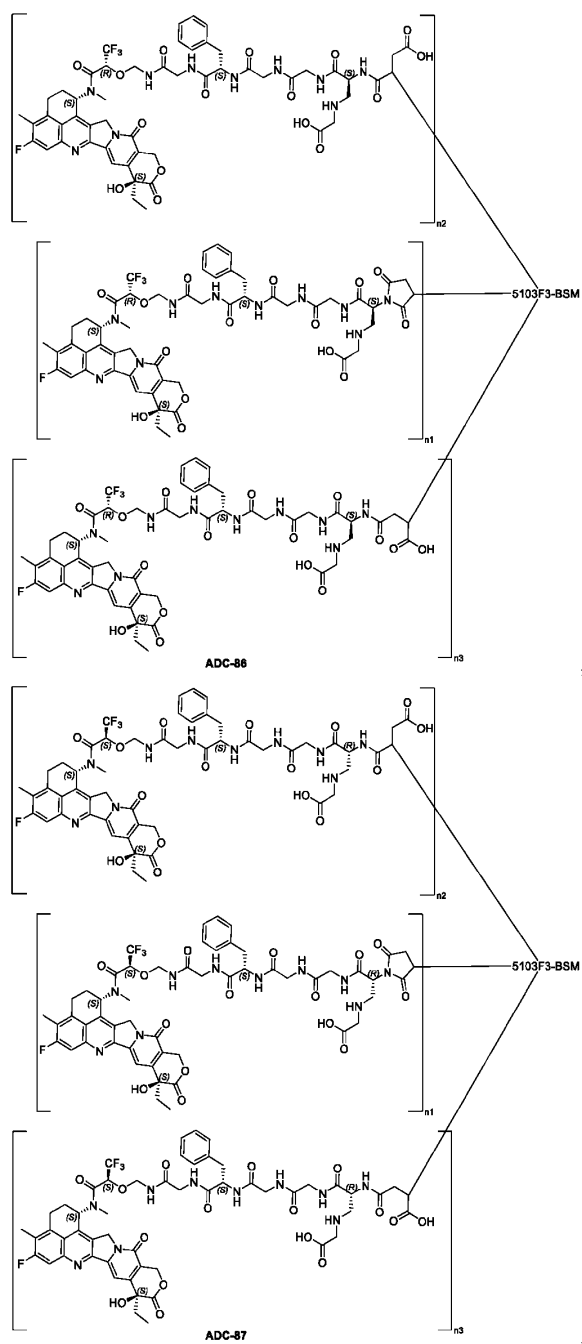


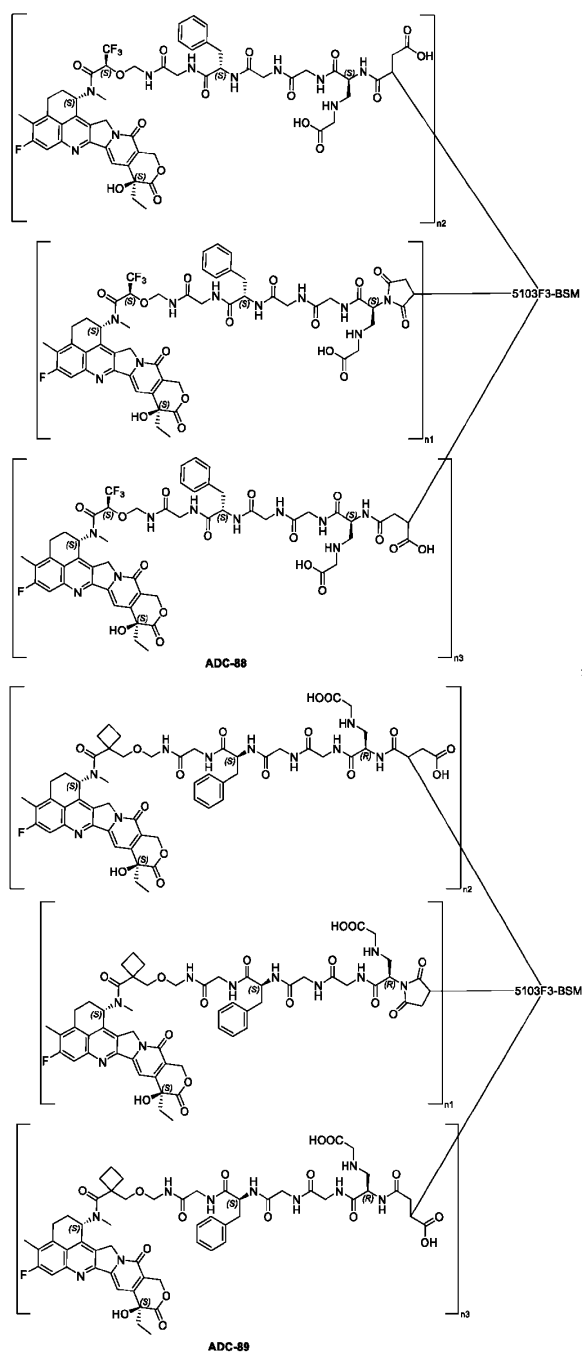


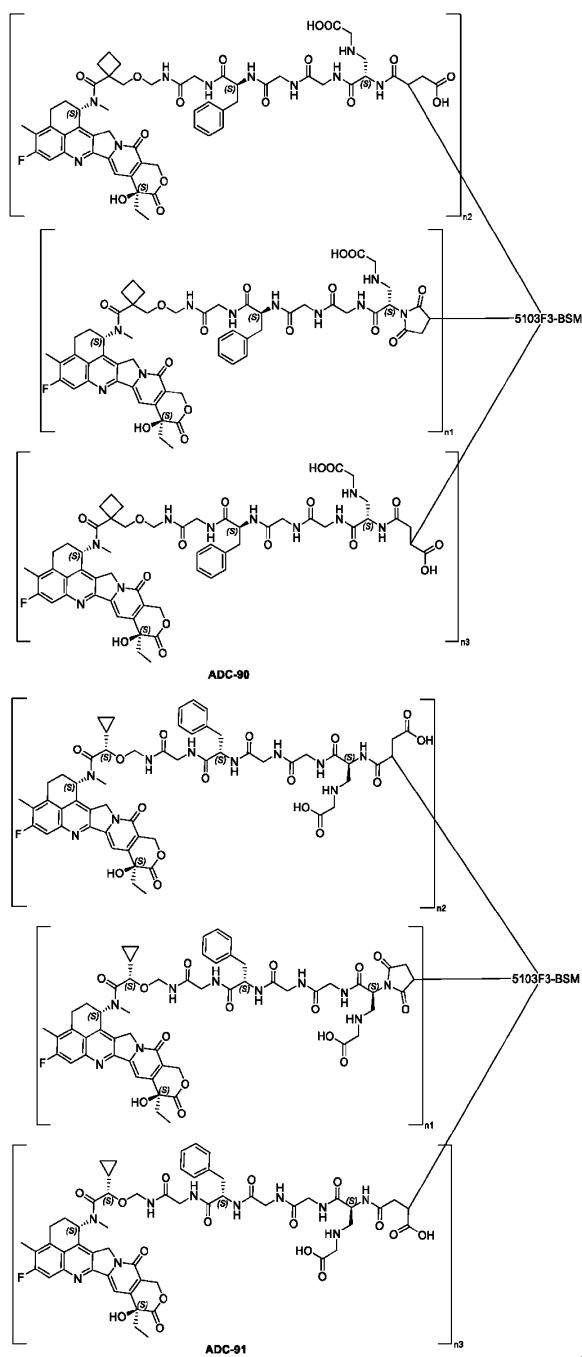


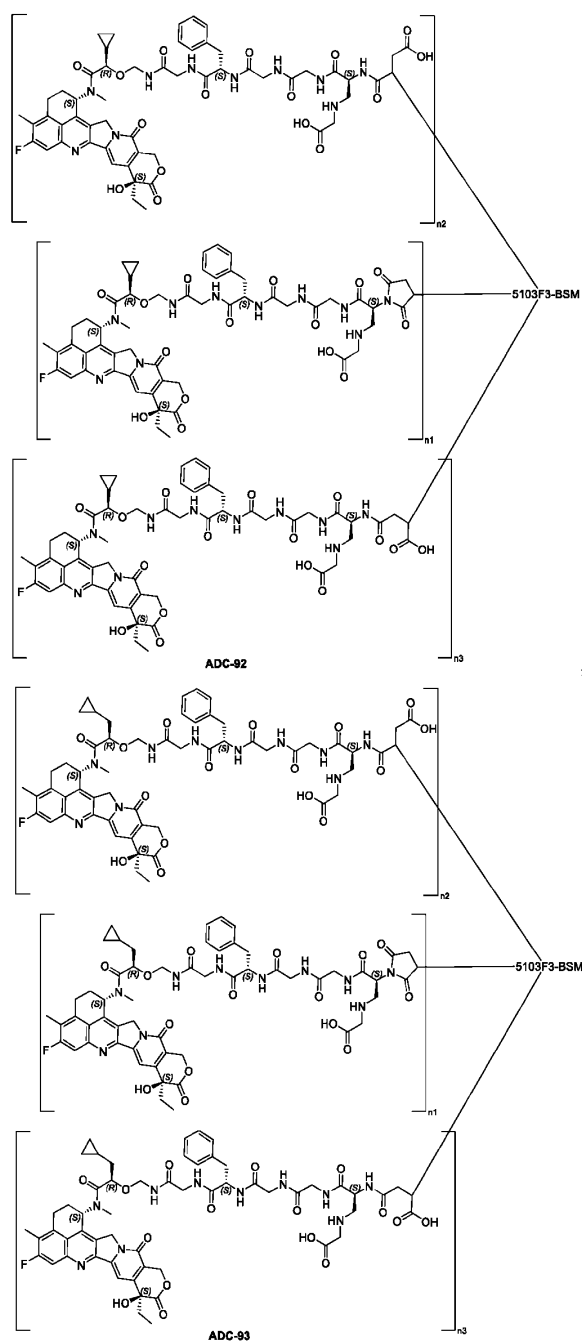


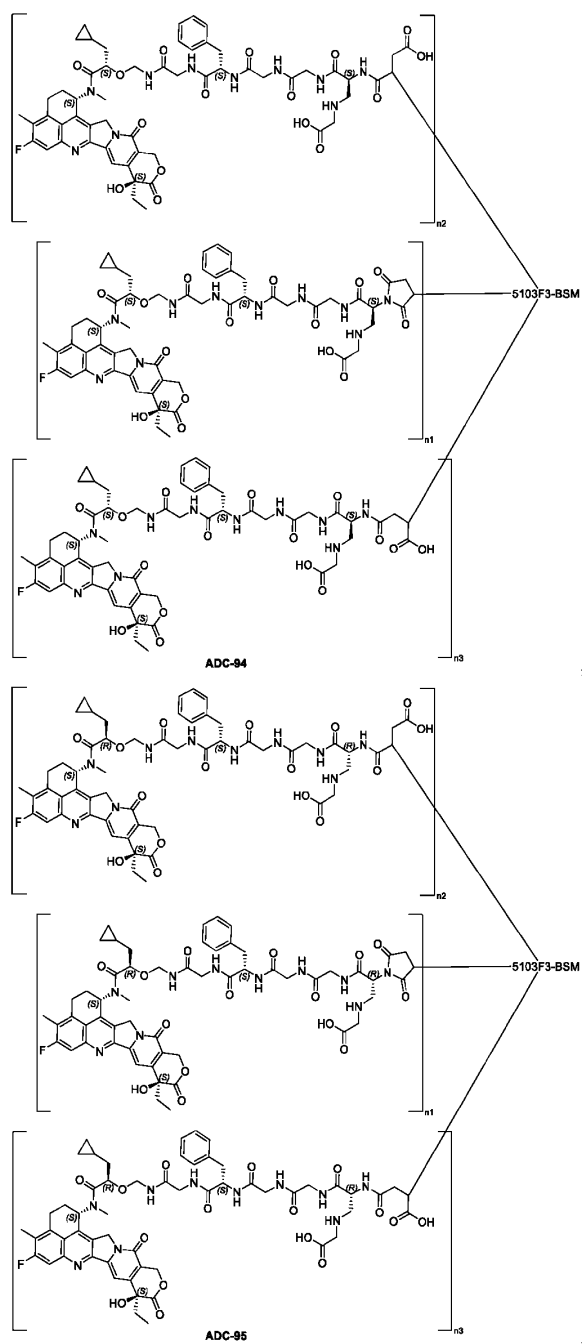


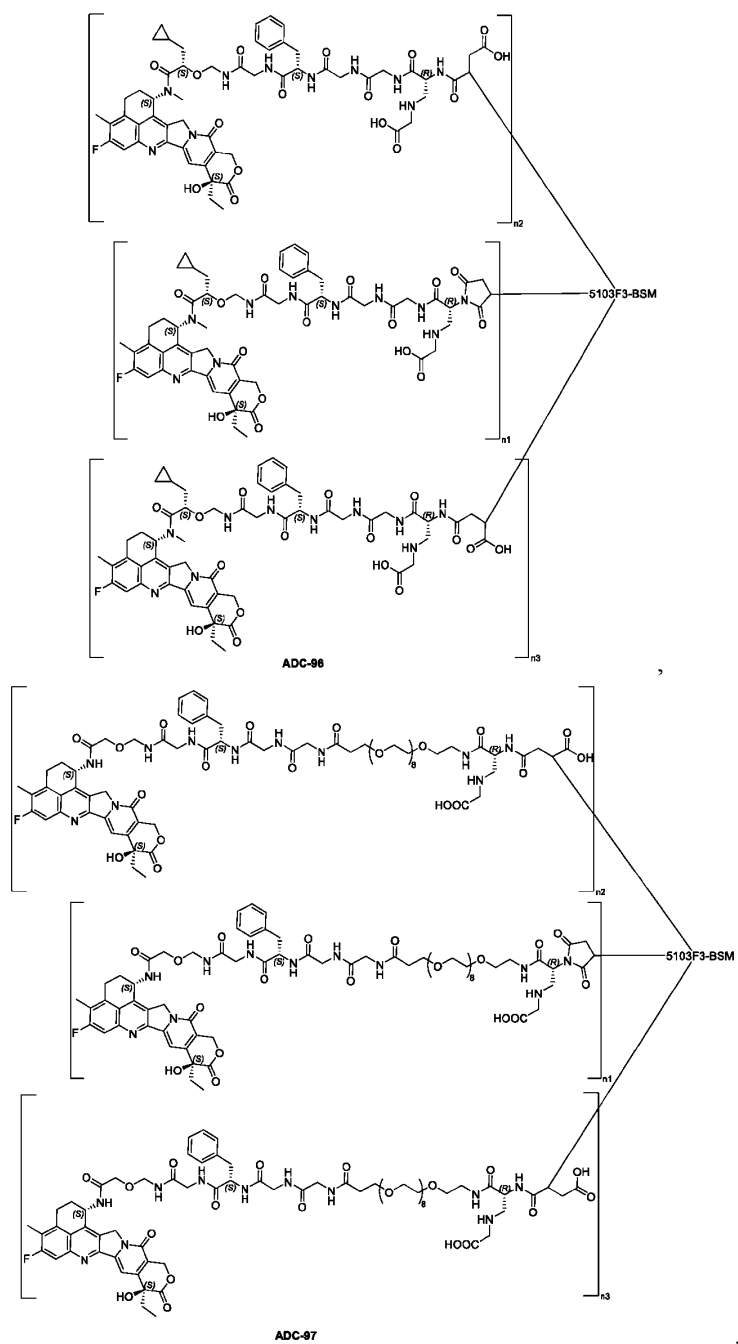


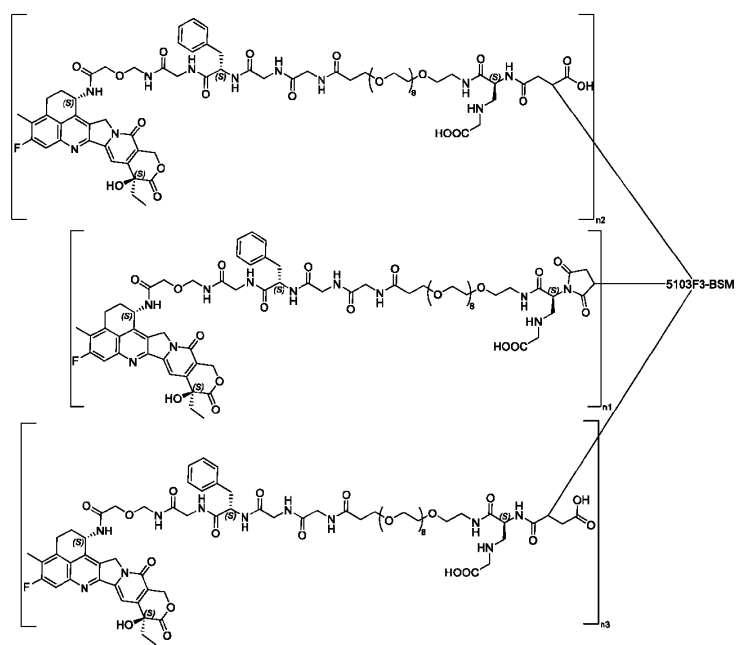




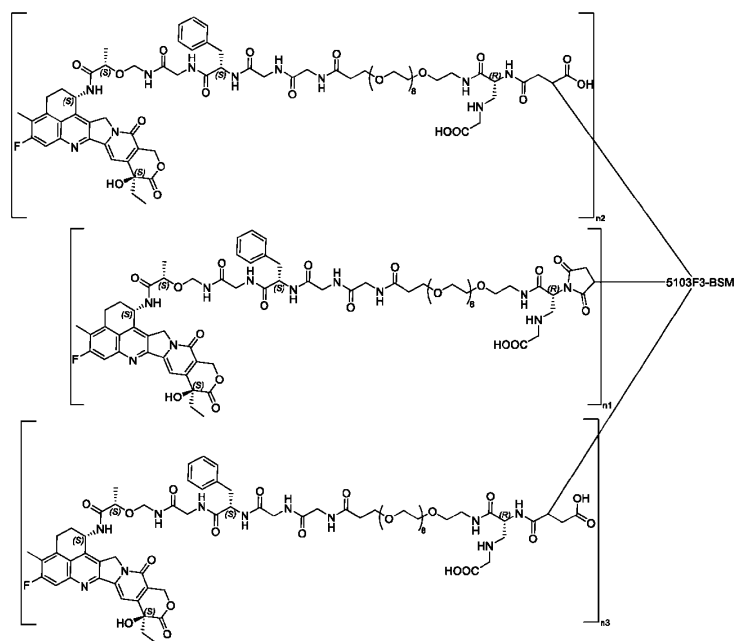




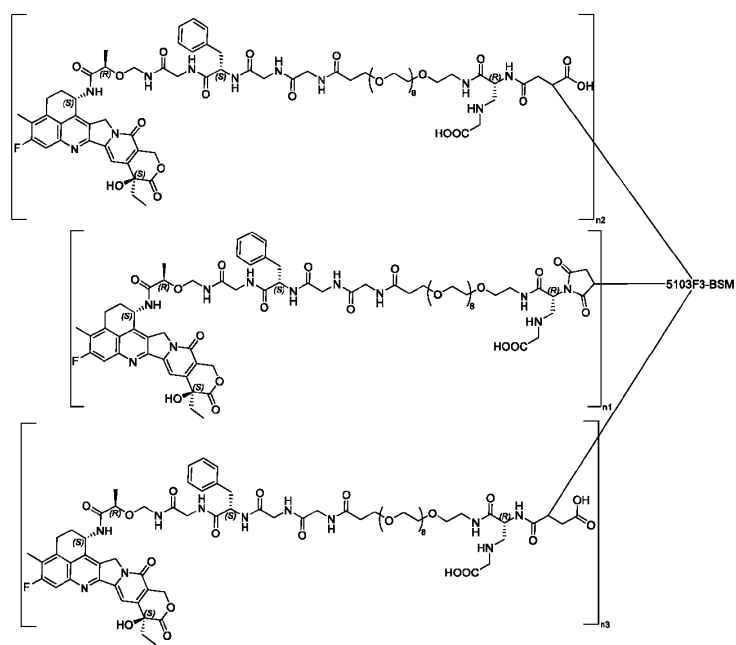




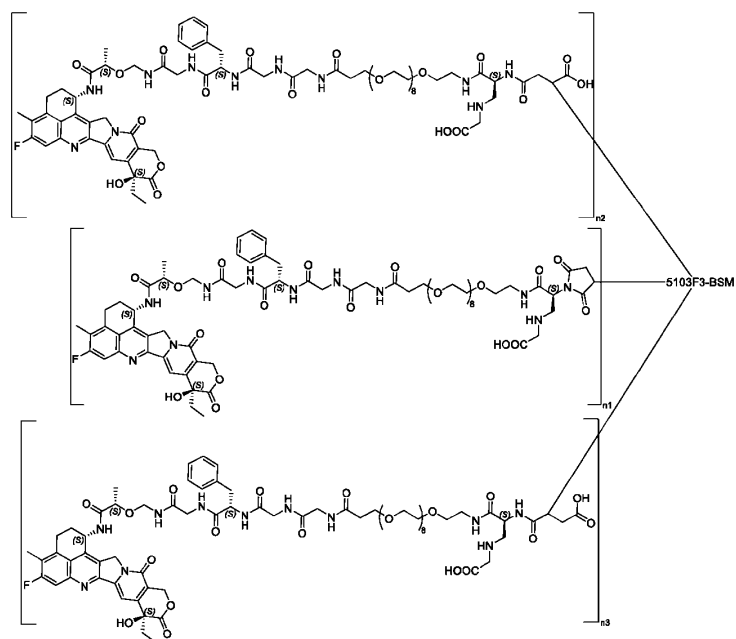
ADC-98



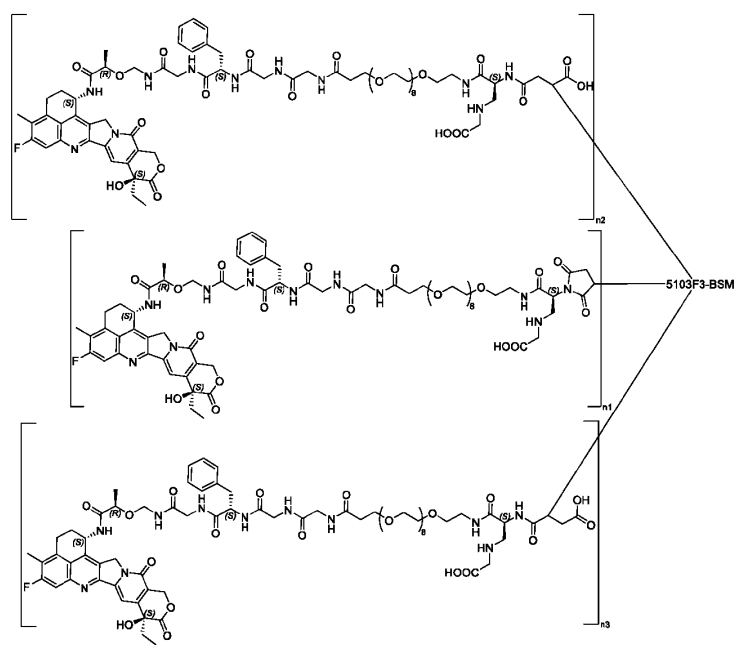
ADC-99



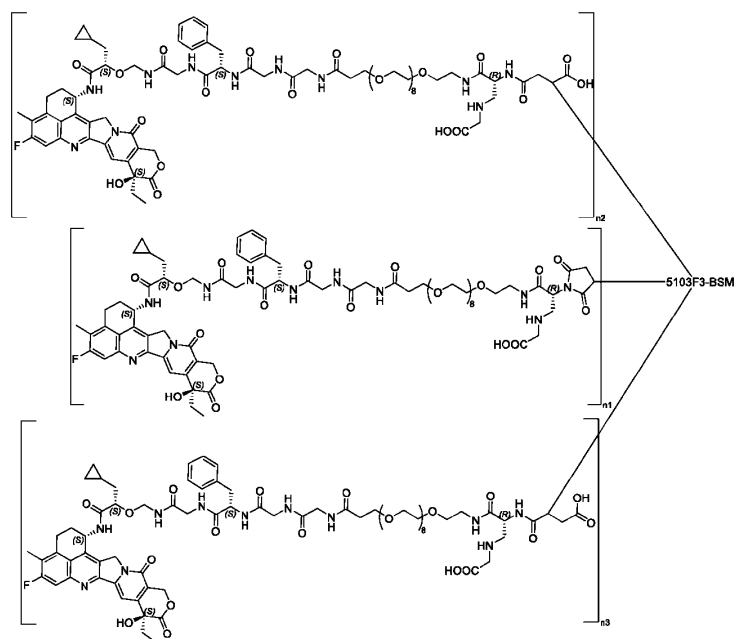
ADC-100



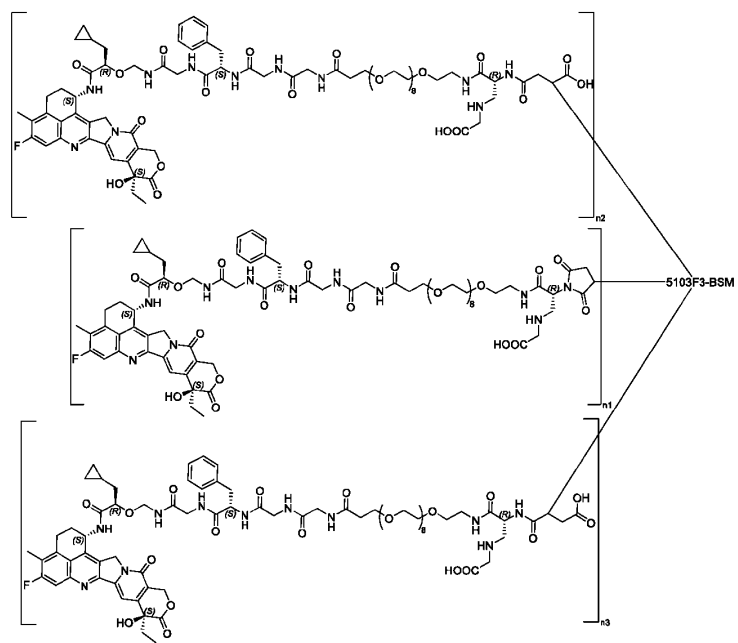
ADC-101



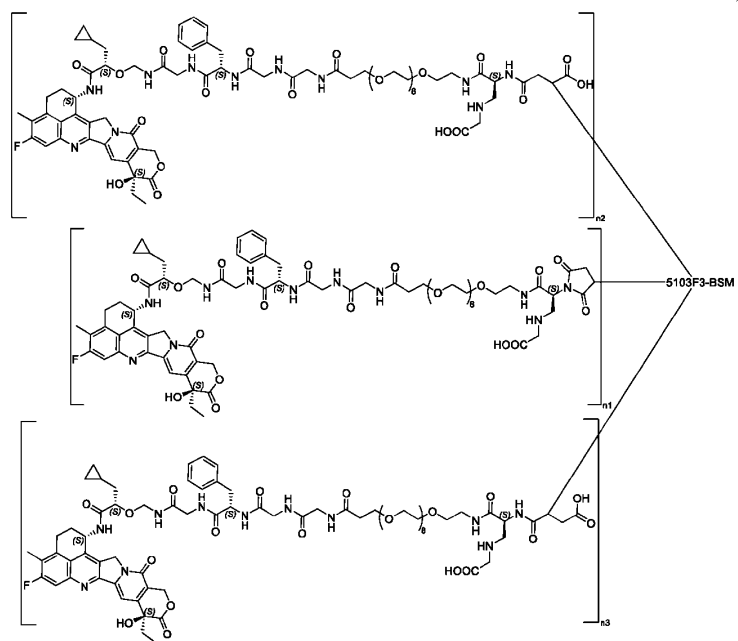
ADC-102



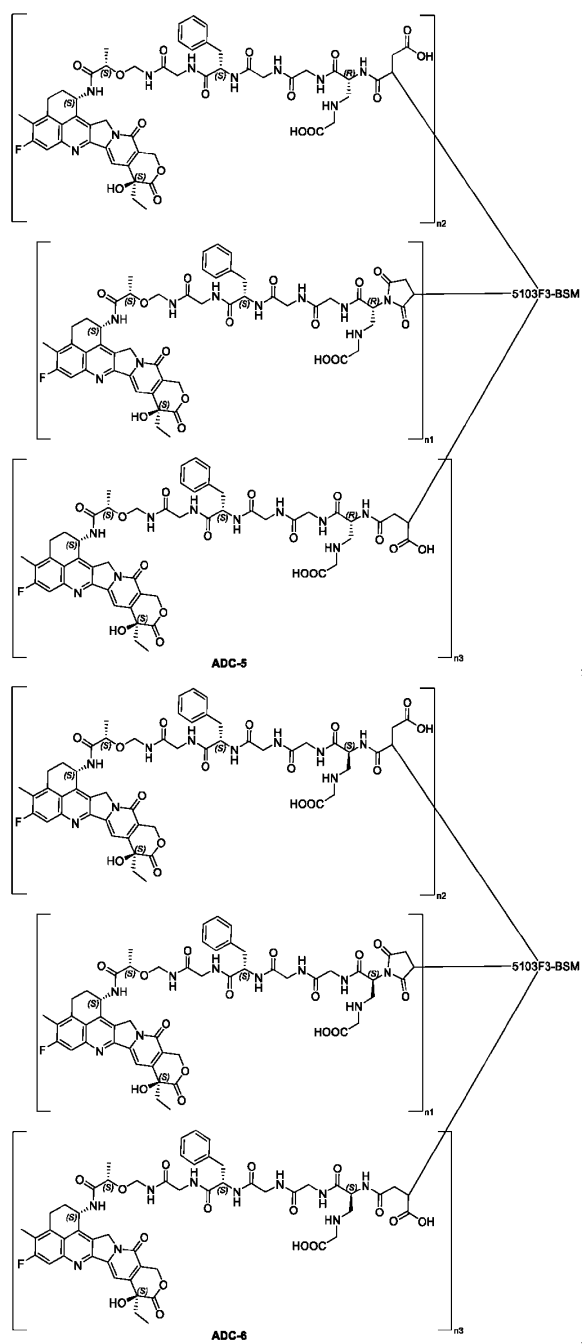
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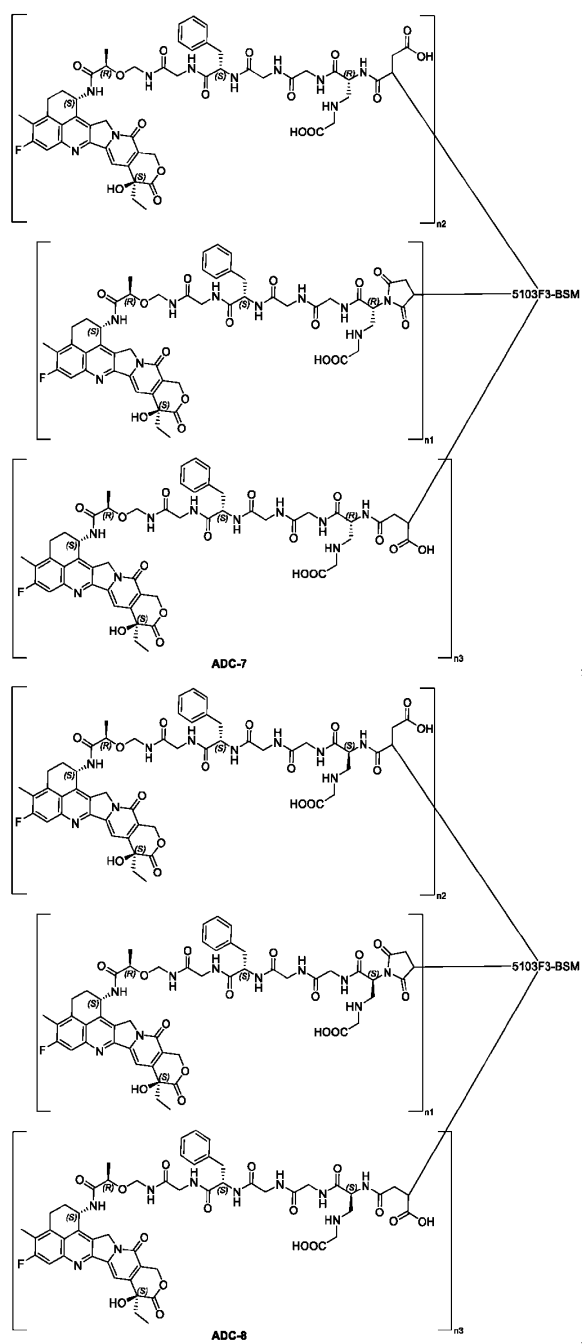


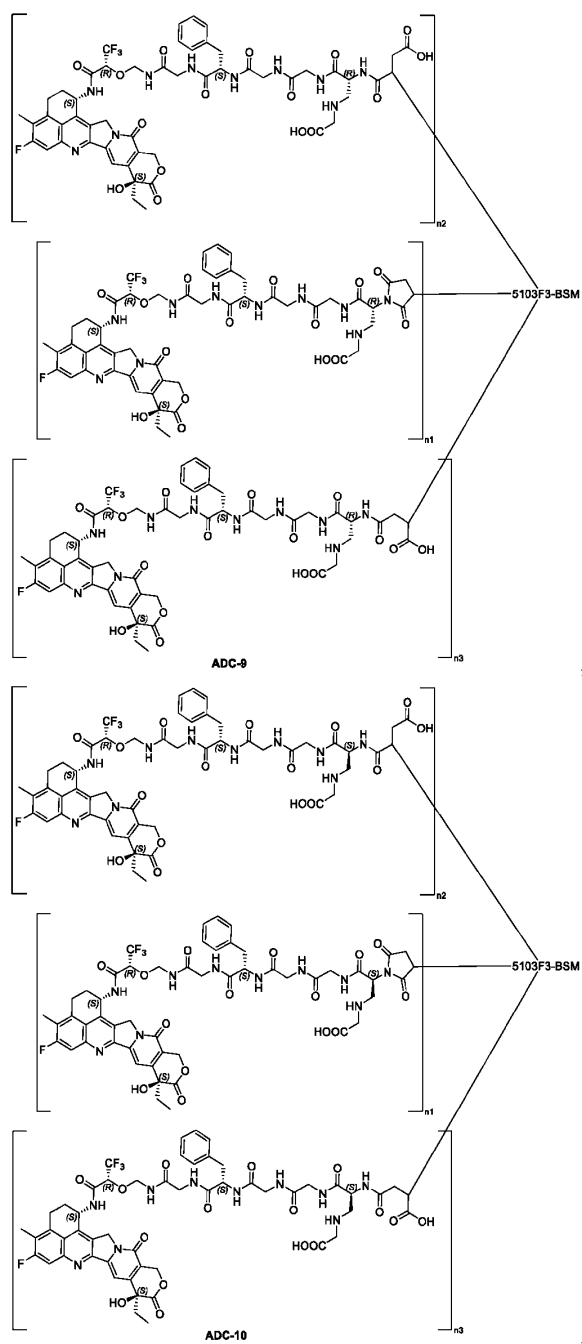
ADC-104

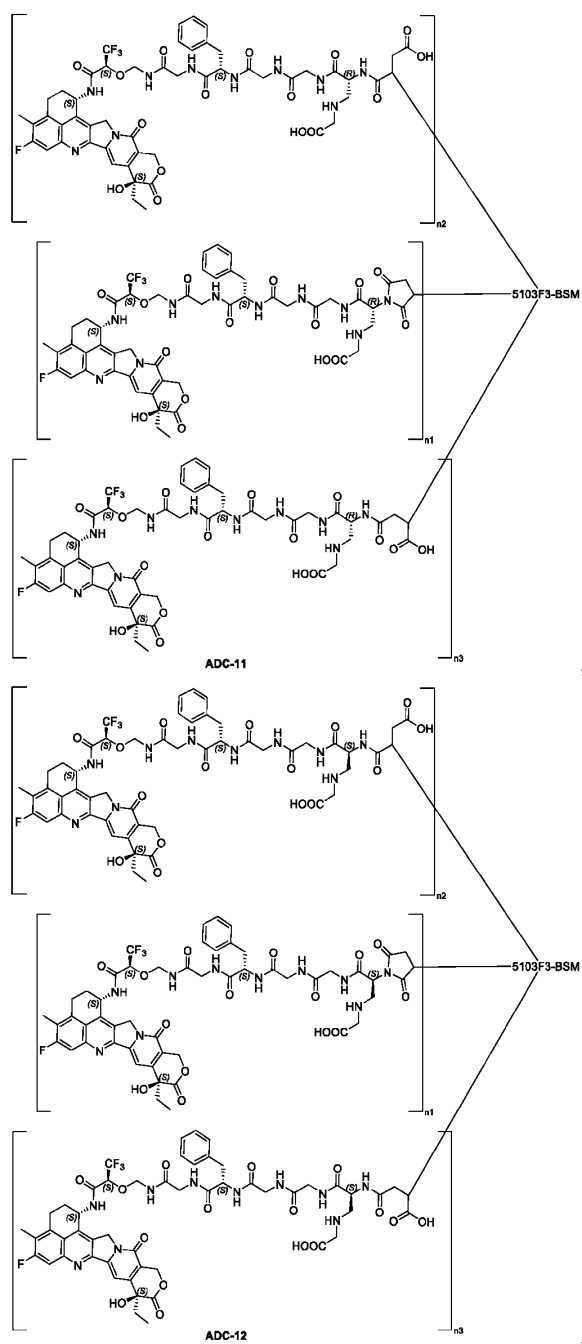


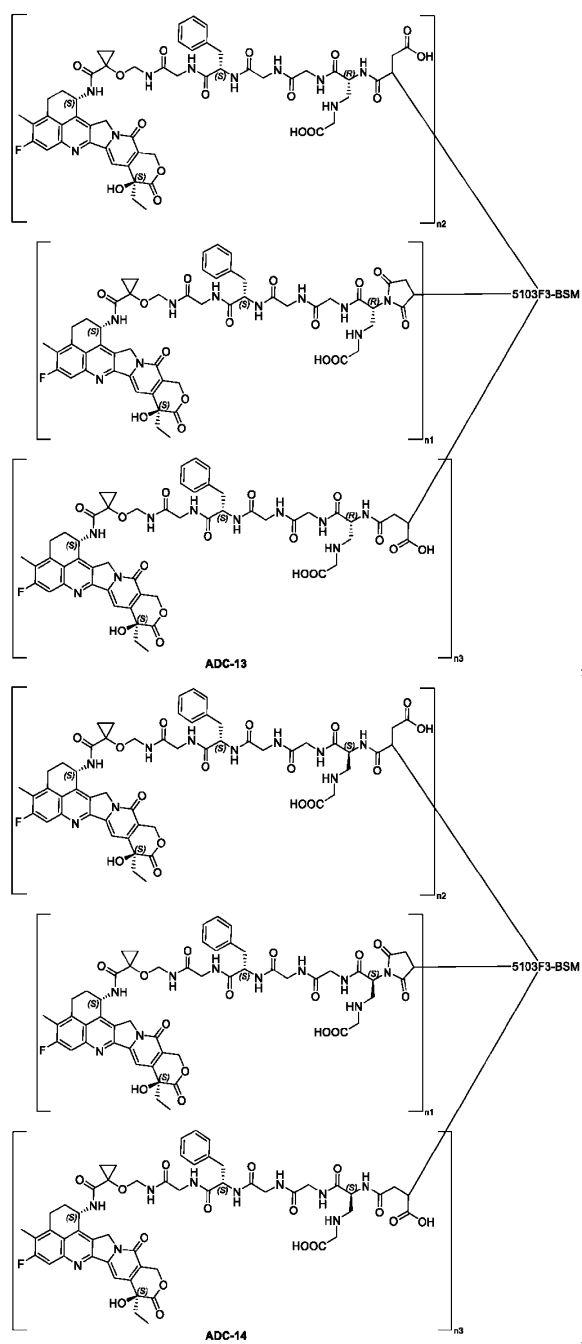
ADC-105

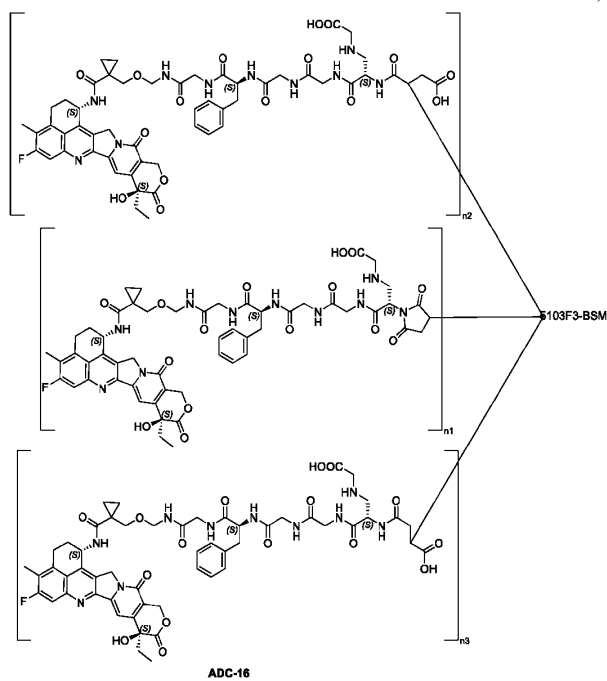
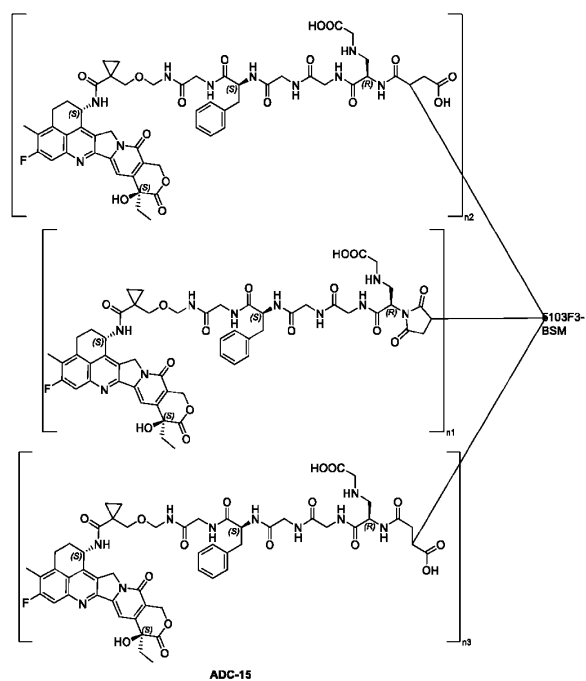


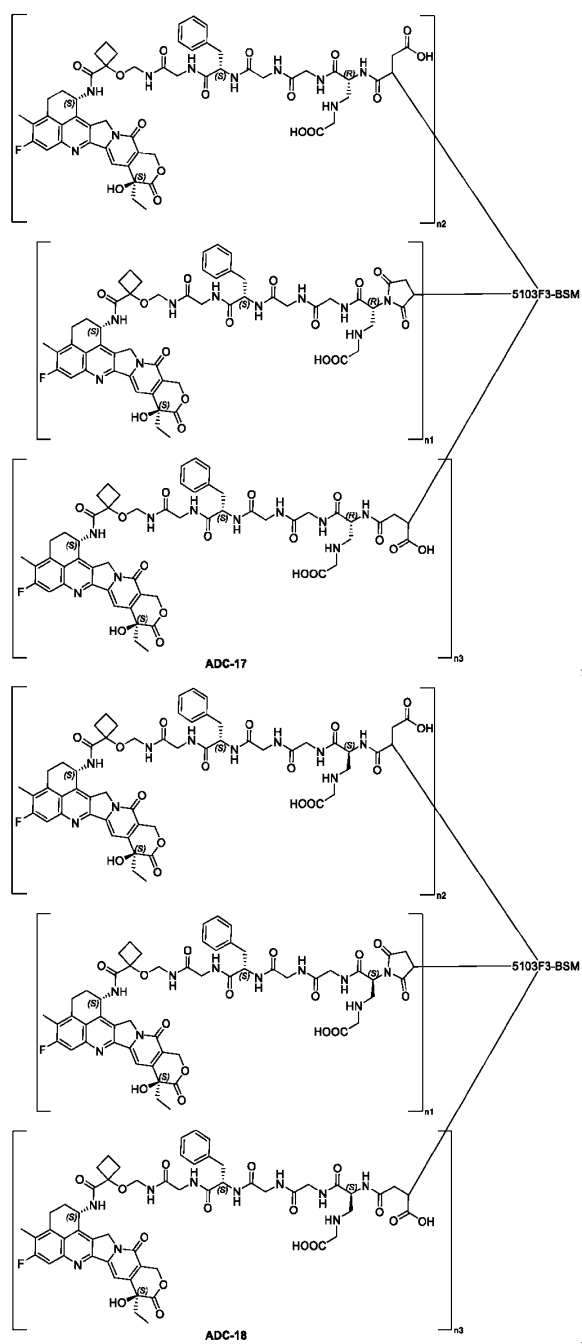


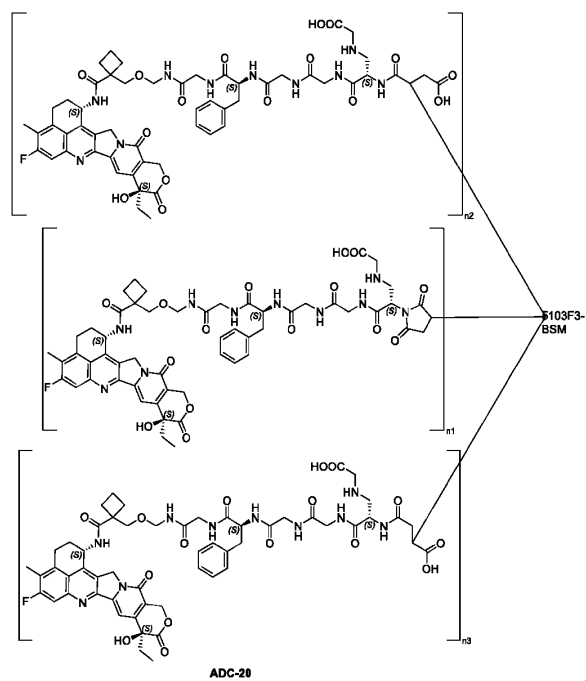
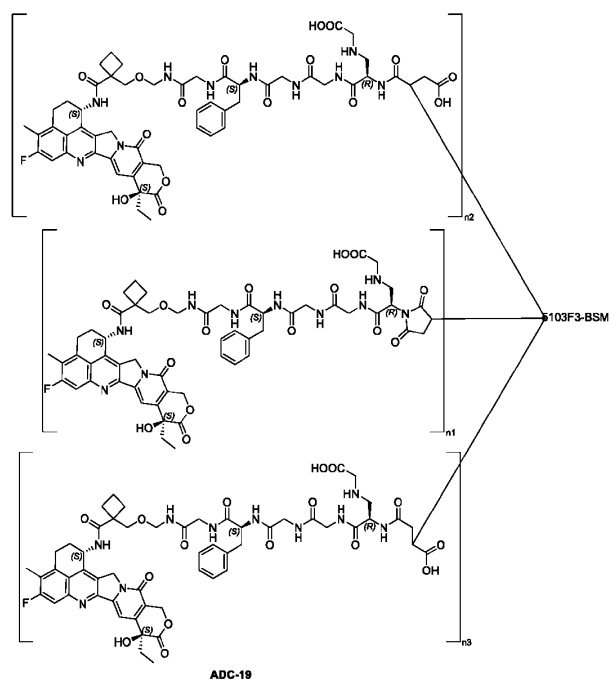


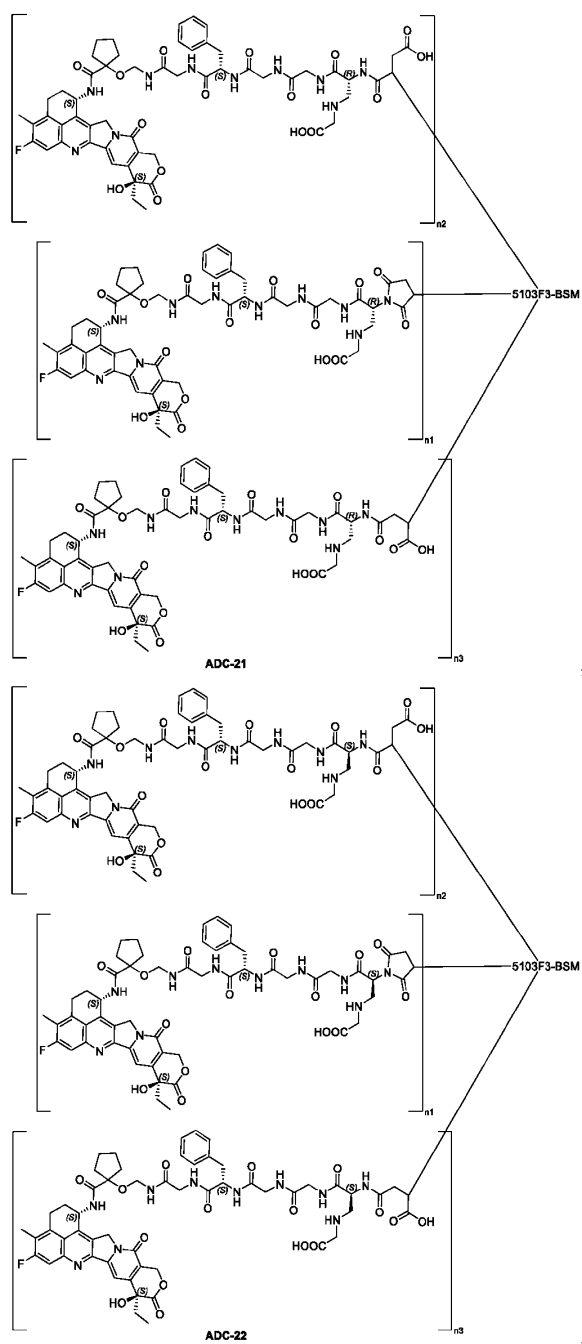


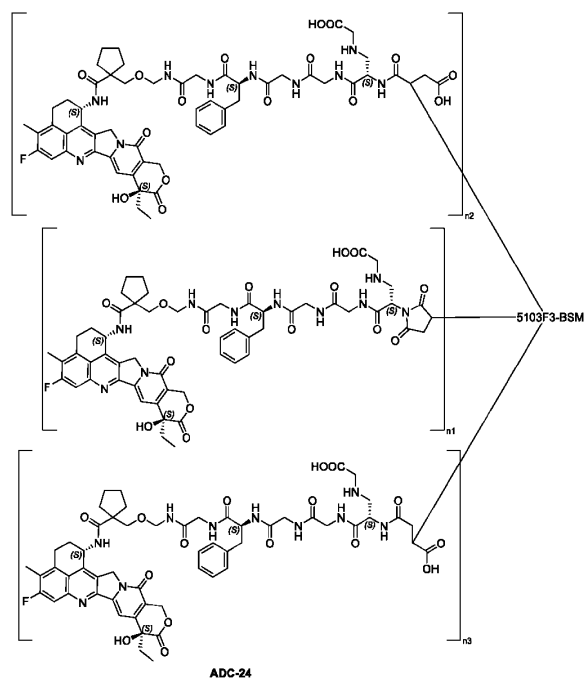
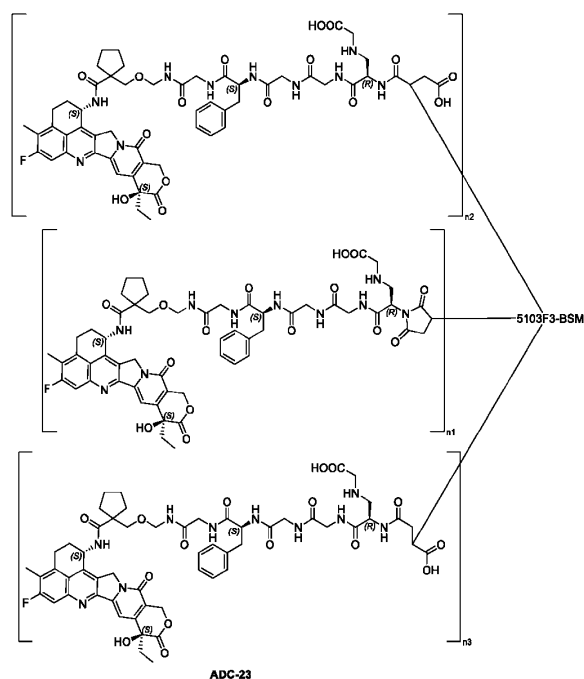


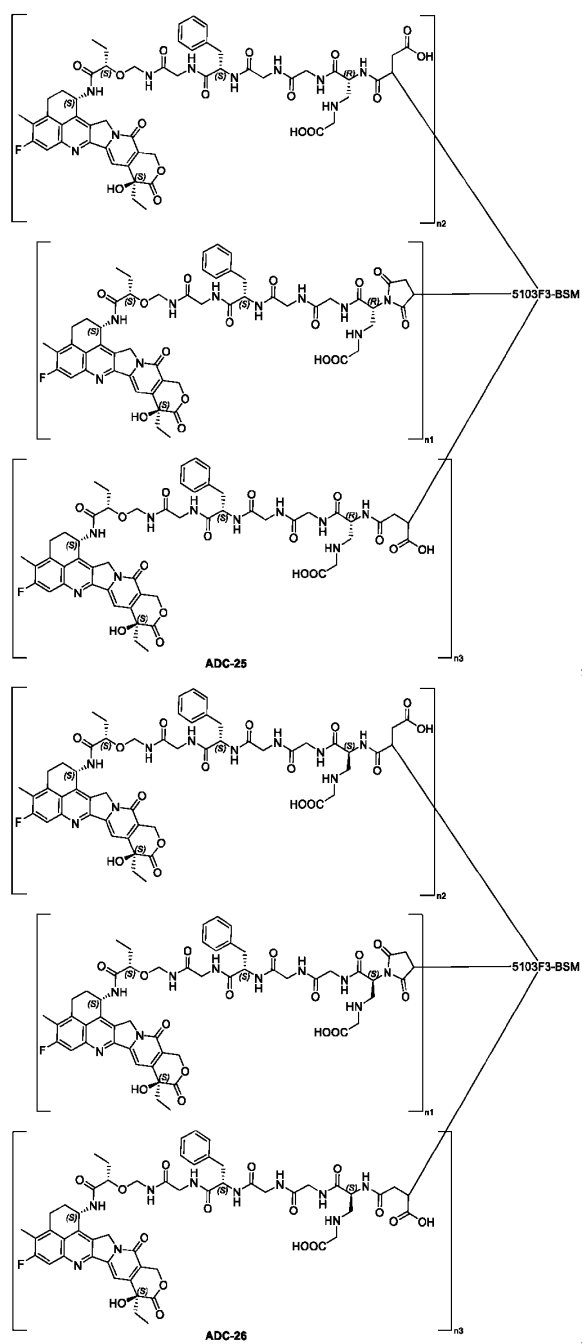


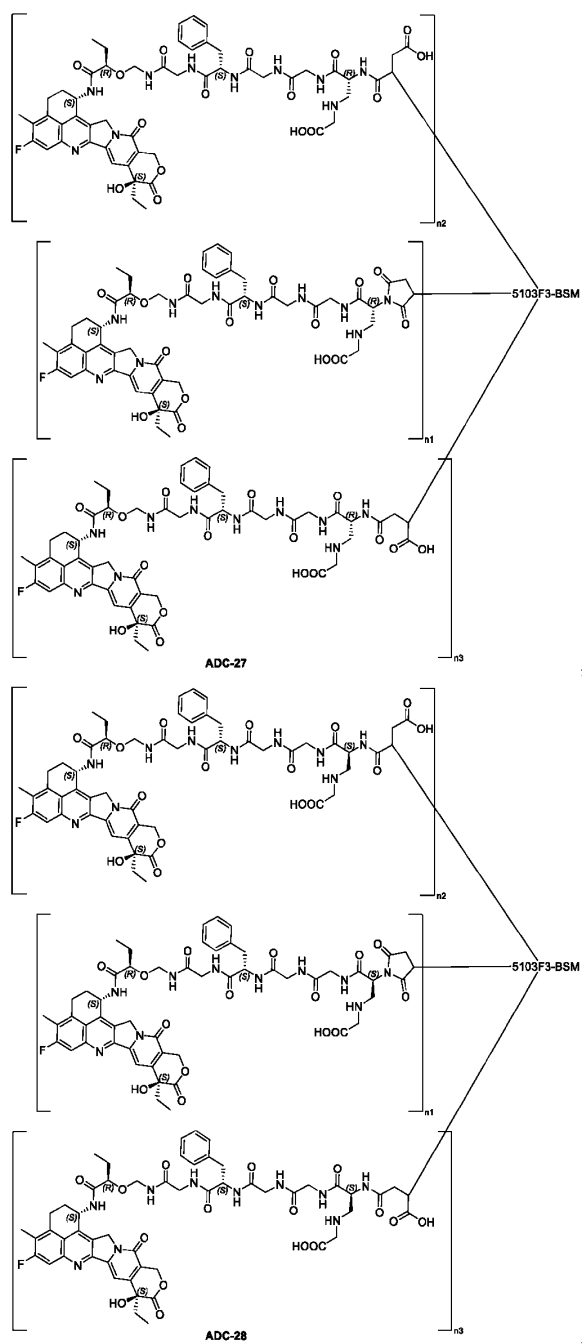


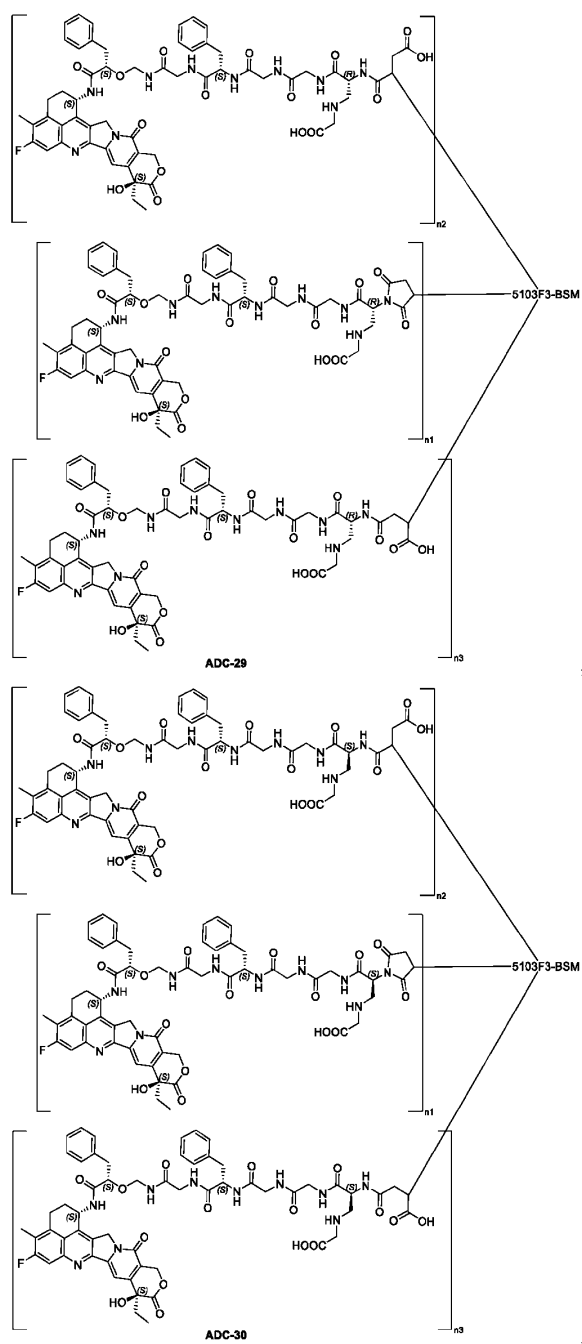


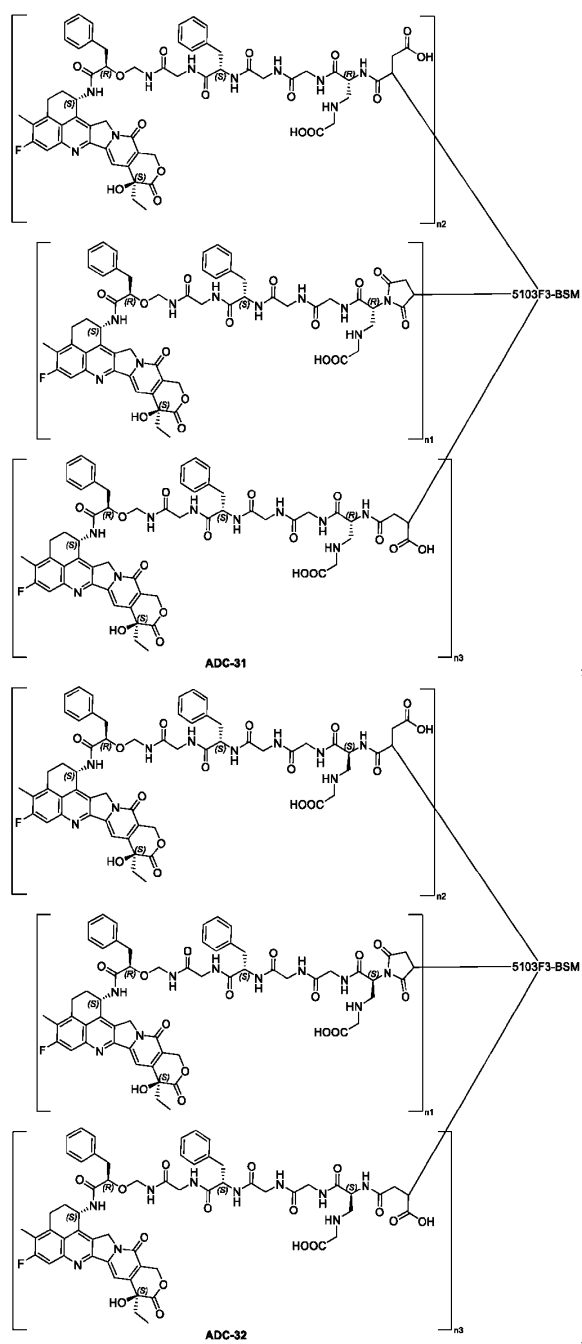


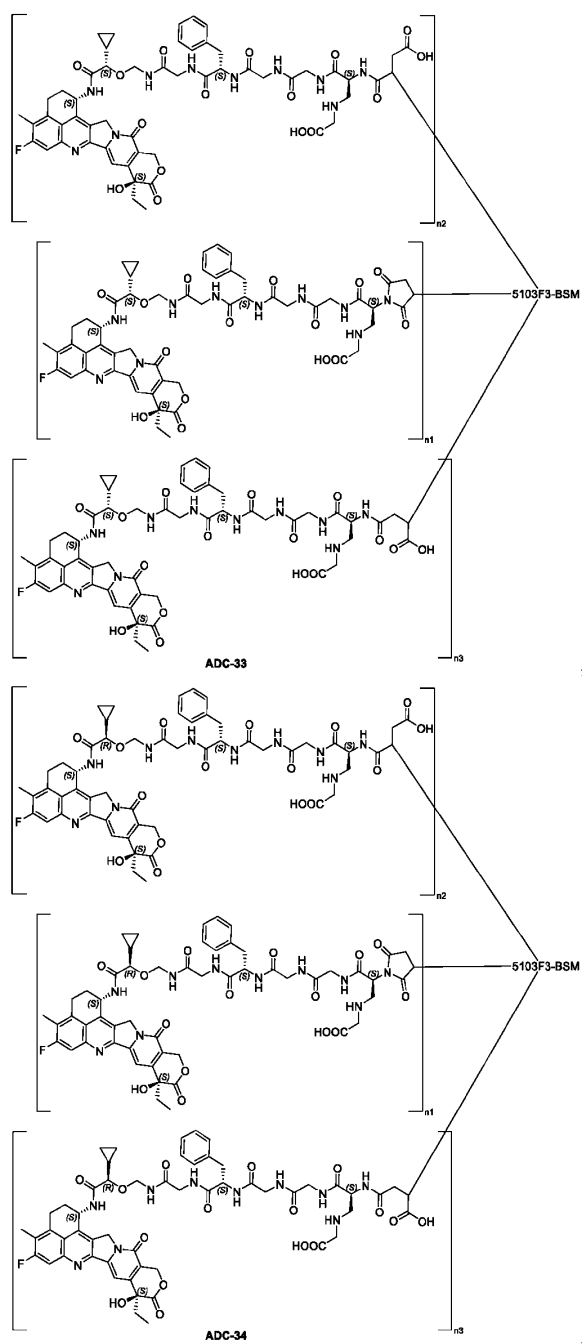


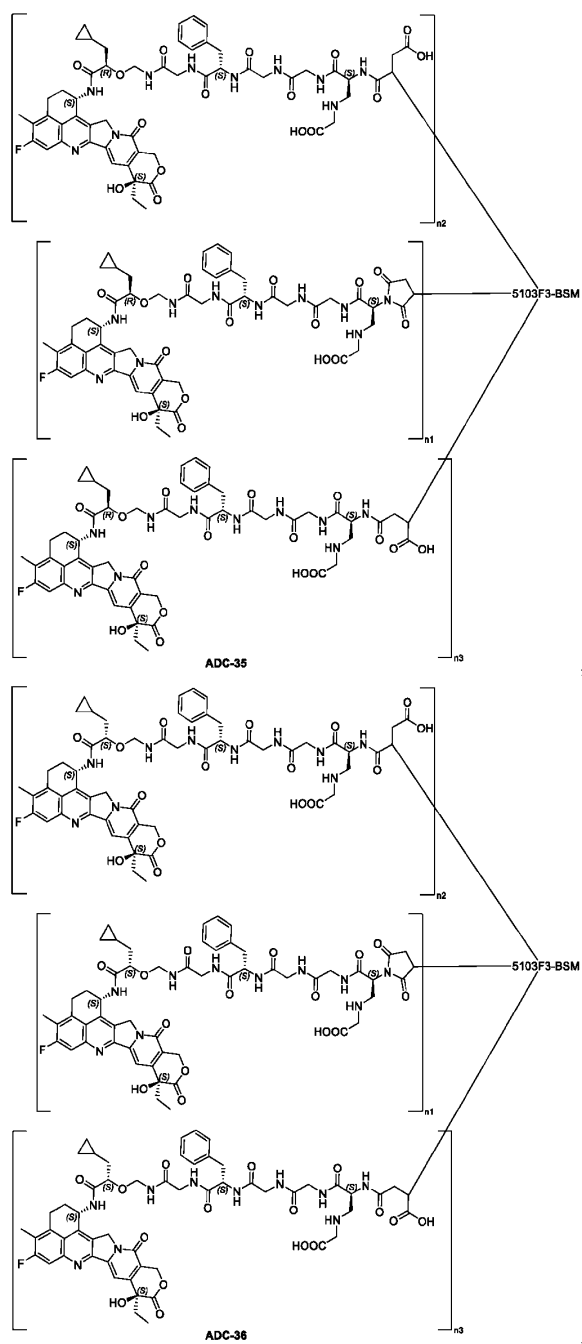


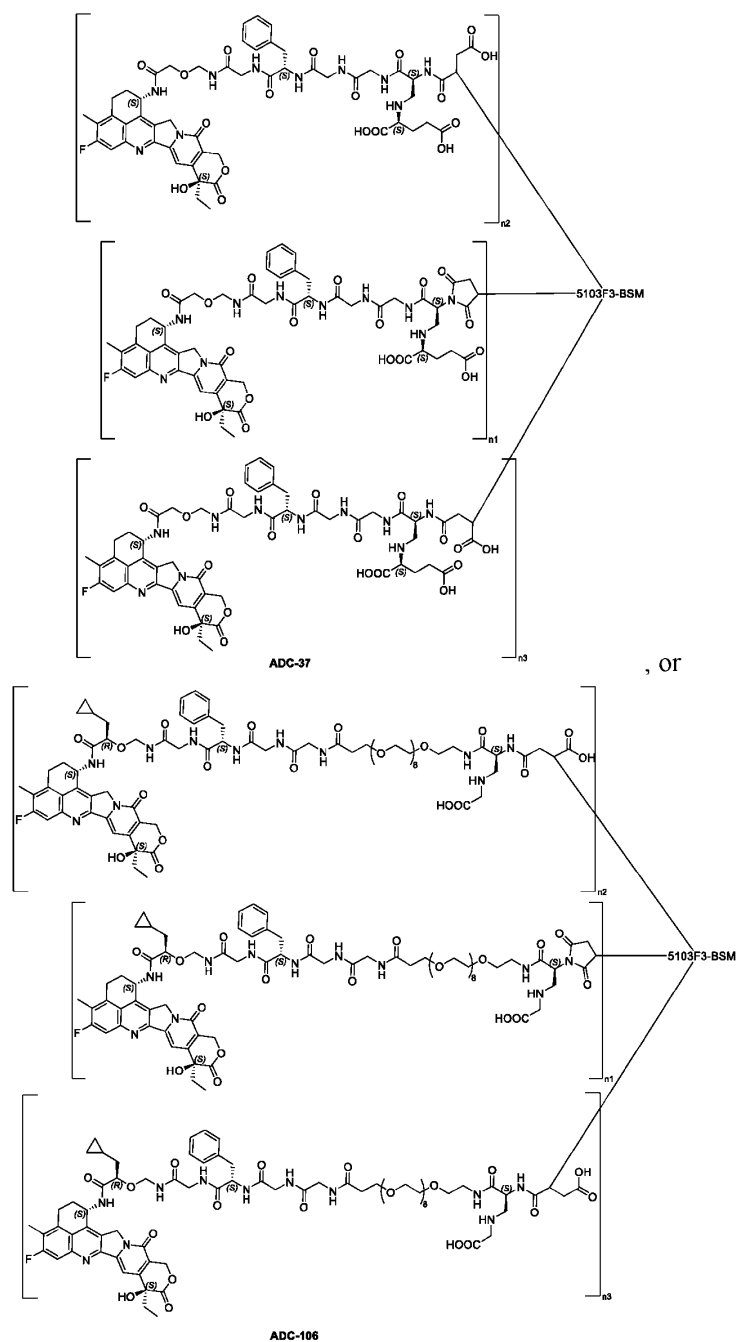












Among them:

5103F3-BSM is an antibody targeting (i.e., having an affinity or specificity to) human Claudin18.2 or an antigen-binding fragment thereof;

Each of n_1 , n_2 , and n_3 is independently chosen from any integer or any decimal number from 0 to 10, n_1 , n_2 , and n_3 are not simultaneously 0, and $1 \leq n_1 + n_2 + n_3 \leq 10$.

In some embodiments, the ligand-camptothecin derivative conjugates, the linker-drug compounds, or pharmaceutically acceptable salt or solvate thereof is characterized in that said pharmaceutically acceptable salt comprises a sodium salt, a potassium salt, a calcium salt, or a magnesium salt formed with an acidic functional group in the structural formula, and acetate, trifluoroacetate, citrate, oxalate, tartrate, malate, nitrate, chloride, bromide, iodide, sulfate, bisulfate, phosphate, lactate, oleate, ascorbate, salicylate, formate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, or p-toluenesulfonate, formed with the basic functional group in the structure.

In a further aspect, the application provides methods for making the conjugates, compounds, and their salts, solvates, or derivatives thereof. In one embodiment, the anti-Claudin18.2 antibody-drug conjugates are obtained by coupling a humanized anti-Claudin18.2 antibody with a camptothecin derivative.

In a further aspect, the application discloses a pharmaceutical composition comprising a ligand-camptothecin derivative conjugates and linker-drug compounds as described herein and their pharmaceutically acceptable salts or solvates thereof, and optionally a pharmaceutically acceptable carrier.

In a further aspect, the application discloses a pharmaceutical formulation comprising the ligand-camptothecin derivative conjugates and the linker-drug compounds as described herein and their a pharmaceutically acceptable salts or solvates thereof.

In a further aspect, the application discloses the ligand-camptothecin derivative conjugates, and the linker-drug compounds as described herein and their a pharmaceutically acceptable salts or solvates thereof, pharmaceutical compositions and pharmaceutical formulations in the preparation of medicines for use in the treatment or prevention of cancers or tumors.

In a further aspect, the application discloses the ligand-camptothecin derivative conjugates, and the linker-drug compounds as described herein and their a pharmaceutically acceptable salts or solvates thereof, pharmaceutical compositions and pharmaceutical formulations for use in the treatment or prevention of cancer or tumors.

In a further aspect, the application discloses methods of treating or preventing cancer or tumors. In one embodiment, the method comprises the step of administering to a subject in need thereof a prophylactically or therapeutically effective amount of a ligand-camptothecin derivative conjugate, a linker-drug compound, or their pharmaceutically acceptable salt or solvate thereof, as described herein, the pharmaceutical composition or pharmaceutical formulations.

In one embodiment, the cancer or tumor expresses Claudin18.2.

In one embodiment, the cancer or tumor may be adenocarcinoma, ovarian cancer, cervical cancer, uterine cancer, prostate cancer, renal cancer, urothelial cancer, bladder cancer, hepatocellular carcinoma, gastric cancer, endometrial cancer, salivary gland cancer, esophageal cancer, lung cancer, colon cancer, breast cancer, rectal cancer, colorectal cancer, bone cancer, skin cancer, thyroid cancer, pancreatic cancer, melanoma, glioma, neuroblastoma, glioblastoma

multiforme, or sarcoma.

In one embodiment, the cancer or tumor are solid tumors. In one embodiment, the cancer or tumor are hematological tumors such as lymphoma and leukemia.

In the above aspects,

the "C1-C6 alkyl" in "C1-C6 alkyl" and various structural moieties comprising "C1-C6 alkyl" (such as "substituted C1-C6 alkyl," "deuterated C1-C6 alkyl") can be replaced with "C1-C20 alkyl", "C1-C12 alkyl" or "C1-C10 alkyl";

the "C3-C8 cycloalkyl" in "C3-C8 cycloalkyl" and various structural moieties comprising "C3-C8 cycloalkyl" may be replaced by "C3-C20 cycloalkyl" or "C3-C10 cycloalkyl";

10 the "C1-C6 alkoxy" in "C1-C6 alkoxy" and in various structural moieties comprising "C1-C6 alkoxy" may be replaced with "C1-C20 alkoxy," "C1-C12 alkoxy" or "C1-C10 alkoxy";

the "C6-C10 aryl" in "C6-C10 aryl" and various structural moieties comprising "C6-C10 aryl" may be replaced with "C6-C12 aryl";

the "3-7-membered heterocyclic group" in "3-7 heterocyclic group" and various structural moieties comprising "3-7-membered heterocyclic group" may be replaced with "3-20-membered heterocyclic group", "3-12-membered heterocyclic group" or "3-10-membered heterocyclic group".

BRIEF DESCRIPTION OF THE DRAWINGS

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

Figure 1 shows the SEC-HPLC detection of ADC-6 aggregation (A) and ADC-107 aggregation (B);

Figure 2 shows the RP-HPLC detection of drug-antibody coupling ratio (DAR) for ADC-6 (A) and ADC-107 (B);

30 **Figure 3** shows that ADC-6 maintains an equal affinity of the 5103F3-BSM antibody to the antigen Claudin18.2 (A) and ADC-107 also maintains an equal affinity of the 64C9 antibody to the antigen Claudin18.2 (B);

Figure 4 shows the binding curve of antibody to Claudin18.1-expressing cells (A) and to Claudin18.2 expressing cells (B);

Figure 5 shows the *in vitro* activity of tumor suppression by ADC-6 and ADC-107 in the experimental model of a human embryonic kidney cell line expressing Claudin18.2, HEK293T-18.2 (A); a human pancreatic cancer cell line expressing Claudin18.2, BxPC-3 #A9F (B); a human gastric cancer cell line expressing Claudin18.2, SNU-5 (C); and another human gastric cancer cell line expressing Claudin18.2, SNU-16 (D);

Figure 6 shows the results of the *in vivo* efficacy of ADC-6 in SNU-5 monoma model; and

Figure 7 shows the results of the *in vivo* efficacy of ADC-6 in MKN-45 single tumor model.

DETAILED DESCRIPTION

Abbreviations and Definitions

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

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

The term "ligand" refers to a macromolecular compound that recognizes and binds to an antigen or receptor associated with a target cell. The ligand serves to present a drug to a target cell population bound to a ligand, which includes, but is not limited to, a protein-like hormone, a lectin, a growth factor, an antibody, or other molecule that binds to the cell. In this embodiment of the invention, the ligand is denoted as Ab, and the ligand may form a linkage bond with a linkage unit via a heteroatom on the ligand, preferably an antibody or an antigen-binding fragment thereof, said antibody being selected from chimeric, humanized, fully human, or murine antibodies; preferably monoclonal antibodies.

20 A ligand unit is a targeting agent that binds specifically to a target component. Said ligand is capable of binding specifically to a cellular fraction or to a cellular component or to other target molecules of interest. The target portion or target is typically on the surface of the cell. In some aspects, the ligand unit serves to deliver the drug unit to a specific target cell population with which the ligand unit interacts. Ligands include, but are not limited to, proteins, polypeptides and peptides, and non-proteins such as sugars. Suitable ligand units include, for example, antibodies, such as full-length (intact) antibodies and antigen-binding fragments thereof. In embodiments where the ligand unit is a non-antibody targeting reagent, it may be a peptide or polypeptide, or a non-protein molecule. Examples of such targeting reagents include interferons, lymphokines, hormones, growth and colony stimulating factors, vitamins, nutrient transporter molecules, or any other cell-binding molecule or substance. In some embodiments, the linker is covalently attached
30 to the sulfur atom of the ligand. In some aspects, the sulfur atom is a sulfur atom of a cysteine residue that forms an interchain disulfide bond of the antibody. In another aspect, the sulfur atom is a sulfur atom of a cysteine residue that has been imported into the ligand unit, which forms 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 (e.g., by targeted mutagenesis or chemical reaction). In other aspects, the linker-bound sulfur atom is selected from a cysteine residue that forms the interchain disulfide bond of the antibody or a cysteine residue that has been introduced into the ligand unit (e.g., by targeted mutagenesis or chemical reaction). In some embodiments, the EU index numbering system in accordance with Kabat {[Kabat E.A et al, (1991)] Sequences of proteins of Immunological Interest, Fifth Edition, NIH Publication 91-3242}.

40 As used herein, "antibody" or "antibody unit" includes, to the extent thereof, any part of an

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

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

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

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

The term "autoimmune disease" refers to diseases or disorders that originate in tissues or proteins that target an individual's own body.

The term "drug" refers to cytotoxic drugs, denoted by d, which are chemical molecules that have a strong ability to disrupt normal growth in tumor cells. Cytotoxic drugs can in principle kill tumor cells at sufficiently high concentrations, but due to a lack of specificity, they can kill tumor cells while causing apoptosis of normal cells, leading to serious side effects. The term includes toxins, such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or

animal origin, radioisotopes (e.g., radioisotopes of At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³² and Lu¹⁷⁶), toxic drugs, chemotherapeutic drugs, antibiotics and nucleolytic enzymes, preferably toxic drugs.

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

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

According to the mechanism of intracellular drug release, as used herein, "linkers" or "linkers of antibody-drug couplers" can be categorized into two types: unbreakable linkers and breakable linkers. For antibody-drug couplers containing an unbreakable linker, the mechanism of drug release is as follows: after the coupler binds to the antigen and is endocytosed by the cell, the antibody is enzymatically cleaved in the lysosome, releasing an active molecule consisting of the small drug molecule, the linker, and the amino acid residues of the antibody. The resulting change in the structure of the drug molecule does not diminish its cytotoxicity, but because the active molecule is electrically charged (amino acid residues), it cannot penetrate into neighboring cells. Therefore, such active drugs cannot kill neighboring tumor cells that do not express the targeted antigen (antigen-negative cells) (bystander effect) (Ducry et al., 2010, Bioconjugate Chem. 21: 5-13). For antibody-drug couplings containing a breakable linker, the mechanism of drug release is that the coupling binds to the antigen and is endocytosed by the cell, then breaks and releases the active ingredient (the small-molecule drug itself) in the target cell. Breakable linkers are mainly categorized into chemical-sensitive linkers and enzyme-sensitive linkers. Chemically sensitive linkers can be selectively broken due to differences in the nature of the plasma and cytoplasm or tumor microenvironment. Such properties include pH, glutathione concentration, etc. pH-sensitive linkers, which are relatively stable in the neutral or weakly alkaline environment of blood (pH 7.3-7.5), will be hydrolyzed within the weakly acidic tumor microenvironment (pH 5.0-6.5) and lysosomes (pH 4.5-5.0), e.g., stilbenes, carbonates acetals, and ketones. Antibody-drug couplings based on such linkers typically have a short half-life (2-3 days) due to the limited plasma stability of acid breakage linkers. This short half-life has somewhat limited the use of pH-sensitive linkers in the new generation of antibody-drug couplers. For glutathione-sensitive linkers, also known as disulfide-bonded linkers. Drug release is based on the difference between the high intracellular glutathione concentration (millimolar range) and the relatively low glutathione concentration in the blood (micromolar range). This is particularly true for tumor cells, whose low oxygen content leads to enhanced reductase activity and thus to higher glutathione concentrations. Disulfide bonds are thermodynamically stable and therefore have better stability in plasma. Enzyme destabilizing linkers, such as peptide linkers, provide better control of drug release.

Peptide linkers can be efficiently severed by lysosomal proteases such as tissue protease (Cathepsin B). This peptide linkage is thought to be very stable in the plasma circulation due to the unfavorable extracellular pH and serum protease inhibitors resulting in proteases that are normally inactive outside the cell. In view of the high plasma stability and good intracellular break selectivity and effectiveness, enzyme-unstable linkers are widely used as breakable linkers for antibody-drug couplings.

The term "antibody-drug conjugate" refers to the attachment of an antibody to a biologically active drug by means of a stabilized linkage unit. In the present application, "ligand-drug coupling", preferably antibody-drug conjugate (ADC), refers to the attachment of a monoclonal antibody or antibody fragment to a biologically active toxic drug via a stabilized linkage unit.

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

The term "alkyl" refers to a saturated aliphatic hydrocarbon group, which is a straight or branched chain group containing 1 to 20 carbon atoms (i.e., "C1-C20 alkyl"), preferably an alkyl group containing 1 to 12 carbon atoms (i.e., "C1-C12 alkyl"), more preferably an alkyl group containing 1 to 10 carbon atoms (i.e., "C1-C10 alkyl"), and most preferably an alkyl group containing 1 to 6 carbon atoms (i.e., "C1-C6 alkyl"). Non-limiting examples include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, tert-butyl, sec-butyl, n-pentyl, 1, 1-dimethylpropyl, 1, 2-dimethylpropyl, 2, 2-dimethylpropyl, 1-ethylpropyl, 2-methylbutyl, 3-methylbutyl, n-hexyl, 1-ethyl-2-methylpropyl, 1, 1, 2-trimethylpropyl, 1, 1-dimethylbutyl, 1, 2-dimethylbutyl, 2, 2-dimethylbutyl, 1, 3-dimethylbutyl, 2-ethylbutyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 2, 3-dimethylbutyl, n-heptyl, 2-methylhexyl, 3-methylhexyl, 4-methylhexyl, 5-methylhexyl, 2, 3-dimethylpentyl, 2, 4-dimethylpentyl, 2, 2-dimethylpentyl, 2, 3-dimethylpentyl, 3, 3-dimethylpentyl, 2-ethylpentyl, 3-ethylpentyl, n-octyl, 2, 3-dimethylhexyl, 2, 4-dimethylhexyl, 2, 5-dimethylhexyl, 2, 2-dimethylhexyl, 3, 3-dimethylhexyl, 4, 4-dimethylhexyl, 2-ethylhexyl, 3-ethylhexyl, 4-ethylhexyl, 2-methyl-2-ethylpentyl, 2-methyl-3-ethylpentyl, n-nonyl, n-ethylpentyl, 2, 2-dimethylpentyl, n-nonyl, 2, 2-dimethylpentyl, n-nonyl, 2, 2-dimethylpentyl, 2, 2-methylpentyl, 2, 2-methylpentyl ethylpentyl, n-nonyl, 2-methyl-2-ethylhexyl, 2-methyl-3-ethylhexyl, 2, 2-diethylpentyl, n-decyl, 3, 3-diethylhexyl, 2, 2-diethylhexyl, and their various branched isomers, and the like. More preferred are lower alkyl groups containing from 1 to 6 carbon atoms, and non-limiting embodiments include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, tert-butyl, sec-butyl, n-pentyl, 1, 1-dimethylpropyl, 1, 2-dimethylpropyl, 2, 2-dimethylpropyl, 1-ethylpropyl, 2-methylbutyl, 3-methylbutyl, n-hexyl, 1-ethyl-2-methylpropyl, 1, 1, 2-trimethylpropyl, 1, 1-dimethylbutyl, 1, 2-dimethylbutyl, 2, 2-dimethylbutyl, 1, 3-dimethylbutyl, 2-ethylbutyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 2, 3-dimethylbutyl, and the like. The alkyl group may be substituted or non-substituted, and when substituted, the substituent group may be substituted at any available point of attachment, said substituent group preferably being one or more of the following groups independently selected from alkyl, alkenyl, alkynyl, alkoxy, alkylthio, alkylamino, halogen, mercapto, hydroxy, nitro, cyano, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, cycloalkoxy, heterocycloalkoxy, cycloalkane thio, heterocycloalkylthio, oxo.

The term "substituted alkyl" means that the hydrogen in the alkyl group is replaced by a substituent group which, unless otherwise indicated in the text, may be a variety of groups selected from the following group: -halogen, -OR', -NR'R'', -SR', -SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -CONR'R'', -OC(O)NR'R''', -NR''C(O)R'', -NR'-C(O)NR''R''', -NR''C(O)₂R'', -NH-C(NH₂)=NH, -NR'C(NH₂)=NH, -NH-C(NH₂)=NR', -S(O)R', -S(O)₂R', -S(O)₂NR'R'', -NR'S(O)₂R'', -CN and -NO₂, the number of substituents is from 0 to (2m'+1), where m' is the total number of carbon atoms in the group. R', R'' and R''' each independently refer to hydrogen, unsubstituted C₁₋₈ alkyl, unsubstituted C₆₋₁₂ aryl (or C₆₋₁₀ aryl), C₆₋₁₂ aryl (or C₆₋₁₀ aryl) substituted by 1-3 halogens, unsubstituted C₁₋₈alkyl, C₁₋₈ alkoxy or C₁₋₈ thioalkoxy, or unsubstituted C₆₋₁₂ aryl (or C₆₋₁₀ aryl)-C₁₋₄ alkyl. When R' and R'' are attached to the same nitrogen atom, they may form a 3-, 4-, 5-, 6-, or 7-membered ring together with that nitrogen atom. For example, -NR'R'' includes 1-pyrrolidinyl and 4-morpholinyl.

The term "substituted alkyl" refers to saturated straight-chain or branched-chain aliphatic hydrocarbon radicals having two residues derived from the removal of two hydrogen atoms from the same carbon atom of the parent alkyl or from two different carbon atoms, which are straight-chain or branched-chain groups containing 1 to 20 carbon atoms, preferably containing 1 to 12 carbon atoms, and more preferably containing 1 to 6 carbon atoms. Non-limiting examples of alkanyl groups include, but are not limited to, methylene (-CH₂-), 1, 1-ethene (-CH(CH₃)-), 1, 2-ethene (-CH₂CH₂-), 1, 1-propene (-CH(CH₂CH₃)-), 1, 2-propylidene (-CH₂CH(CH₃)-), 1, 3-propylidene (-CH₂CH₂CH₂-), 1, 4-butylidene (-CH₂CH₂CH₂CH₂-) and 1, 5-butylidene (-CH₂CH₂CH₂CH₂CH₂-). The alkylidene group may be substituted or unsubstituted, and when substituted, the substituent may be substituted at any available attachment point, said substituent being independently selected preferably from the group consisting of alkyl, alkynyl, alkynyl, alkoxy, alkylthio, alkylamino, halogen, thiol, hydroxy, nitro, cyano, cycloalkyl, heterocyclic, aryl, heteroaryl, cycloalkoxy, heterocycle alkoxy, cycloalkyl sulfide, heterocycle alkyl sulfide and one or more substituents selected independently of one another from the group consisting of oxygen-containing substituents.

The term "alkoxy" refers to -O-(alkyl) and -O-(cycloalkyl), where alkyl or cycloalkyl is as defined above. Non-limiting examples of C₁-C₆ alkoxides include: methoxy, ethoxy, propoxy, butoxy, cyclopropoxy, cyclobutoxy, cyclopentyloxy, cyclohexoxy. The alkoxy group may be optionally substituted or unsubstituted. When substituted, the substituent is preferably one or more of the following groups, independently selected from alkyl, alkynyl, alkynyl, alkoxy, alkylthio, alkylamino, halogen, mercapto, hydroxy, nitro, cyano, cycloalkyl, heterocycle alkyl, aryl, heteroaryl, cycloalkoxy, heterocycle alkoxy, cycloalkylthio, heterocycle alkylthio.

The term "cycloalkyl" refers to a saturated or partially unsaturated monocyclic or polycyclic cyclic hydrocarbon substituent, wherein the cycloalkyl ring comprises from 3 to 20 carbon atoms (i.e. "C₃-C₂₀ cycloalkyl"), preferably from 3 to 12 carbon atoms (i.e. "C₃-C₁₂ cycloalkyl"), more preferably comprising 3 to 10 carbon atoms (i.e., "C₃-C₁₀ cycloalkyl"), most preferably comprising 3 to 8 carbon atoms (i.e., "C₃-C₈ cycloalkyl "). Non-limiting examples of monocyclic cycloalkyl groups (e.g., "C₃-C₈ cycloalkyl") include cyclopropyl, cyclobutyl, cyclopentyl,

cyclopentenyl, cyclohexyl, cyclohexenyl, cyclohexadienyl, cycloheptenyl, cycloheptatrienyl, cyclooctyl, and the like; and multi-cyclic cycloalkyl groups include cycloalkyl groups of spirocycles, thickened rings, and bridged rings.

The term “heterocyclic group” means a saturated or partially unsaturated monocyclic or polycyclic cyclic hydrocarbon substituent group that contains 3 to 20 ring atoms (i.e., a “3-20 membered heterocyclic group”), wherein one or more ring atoms are heteroatoms selected from nitrogen, oxygen or S(O)_m (where m is an integer of 0 to 2), but excluding a ring portion of -O-O-, -O-S- or -S-S-, and the remaining ring atoms are carbon. Preferably comprising 3 to 12 ring atoms (i.e., a “3-12 membered heterocyclic group”), wherein 1 to 4 are heteroatoms; more preferably the heterocyclic group comprises 3 to 10 ring atoms (i.e., a “3-10 membered heterocyclic group”). Non-limiting examples of monocyclic heterocyclic groups (e.g., 3-7 membered heterocyclic groups) include pyrrolidinyl, piperidinyl, piperazinyl, morpholinyl, thiomorpholinyl, and homopiperazinyl. Polycyclic heterocyclic groups include heterocyclic groups with spiro, fused, or bridge rings.

The term “cycloalkylalkyl” means an alkyl group substituted by one or more cycloalkyl groups, preferably by one cycloalkyl group, wherein the alkyl group is as defined above and the cycloalkyl group is as defined above, for example, C3-C8 cycloalkyl C1-C6 alkyl.

The term “haloalkyl” means an alkyl group substituted with one or more halogens, wherein the alkyl group is as defined above, for example, halo C1-C6 alkyl.

The term “deuterated alkyl” means an alkyl group substituted with one or more deuterium atoms, wherein alkyl is as defined above, for example, deuterated C1-C6 alkyl.

The term “C6-C12 aryl” refers to a group of carbocyclic aromatic systems having 6-12 carbon atoms.

The term “C6-C10 aryl” refers to a group of carbocyclic aromatic systems having 6-10 carbon atoms, such as phenyl, naphthyl, etc.

The term “5-10-membered heteroaryl” refers to an aromatic heterocyclic ring, typically a 5-, 6-, 7-, 8-, 9-, 10-membered heterocyclic ring having 1 to 3 heteroatoms selected from N, O, or S; the heteroaryl ring may optionally be further consolidated or attached to aromatic and non-aromatic carbon and heterocyclic rings. Non-limiting examples of said 5- to 10-membered heteroaryl ring are, for example, pyridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, indolyl, imidazolyl, thiazolyl, isothiazolyl, thioxazolyl, pyrrolyloxy, phenyl-pyrrolyloxy, furanyl, phenyl-furyl, phenyl-furanyl, oxazolyl, isoxazolyl, pyrazolyl, thiophenyl, benzo-furanyl, benzothiophenyl, benzo 1,3-dioxolane (benzodioxolyl), isodihydroindolyl, benzimidazolyl, indazolyl, quinolinyl, isoquinolinyl, 1,2,3-triazolyl, 1-phenyl-1,2,3-triazolyl, 2,3-dihydroindolyl, 2,3-dihydrobenzofuranyl, 2,3-dihydrobenzothiophene, benzylpyranyl, 2,3-dihydrobenzoxazinyl, 2,3-dihydroquinoxalinyl, and others.

The terms “substituted C6-C10 aryl,” “substituted 5-10-membered heteroaryl,” or “substituted 3-7-membered heterocyclic” mean that the hydrogen in the aryl or heteroaryl or heterocyclic group has been replaced by a substituent group, unless otherwise specified in the text.

The substituent group of the aryl or heteroaryl or heterocyclic group may be a group 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, with 0 to (2m'+1) substituents, where m' is the total number of carbon atoms in the group. R', R'' and R''' each independently refer to hydrogen, an unsubstituted C₁₋₈ alkyl group, an unsubstituted C₆₋₁₂ aryl group (or C₆₋₁₀ aryl group), a C₆₋₁₂ aryl group (or C₆₋₁₀ aryl group) substituted by 1-3 halogens, an unsubstituted C₁₋₈ alkyl, C₁₋₈ alkoxy or C₁₋₈ thioalkoxy, or unsubstituted C₆₋₁₂ aryl (or C₆₋₁₀ aryl) -C₁₋₄ alkyl. When R' and R'' are attached to the same nitrogen atom, they may form a 3-, 4-, 5-, 6- or 7-membered ring with that nitrogen atom, for example, -NR'R'' includes 1-pyrrolidinyl and 4-morpholinyl.

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

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

The term "amino" means -NH₂. The term "nitro" means -NO₂.

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

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

The present application also includes various deuterated forms of Formula I. Each of the available hydrogen atoms attached to the carbon atom may be independently replaced by a deuterium atom. A person skilled in the art can synthesize the deuterated form of Formula I with reference to the relevant literature. Commercially available deuterated starting materials may be used in the preparation of the deuterated form of Formula I, or they may be synthesized by conventional techniques using deuterated reagents, non-limiting examples of deuterated reagents include deuteroborane, trideuteroborane tetrahydrofuran solution, lithium-aluminum deuterohydride, ethyl iodine deuteride, methylene iodide deuteride, and the like.

The term "antibody" refers to immunoglobulins, which are tetrapeptide chains consisting of two identical heavy chains and two identical light chains linked by interchain disulfide bonds. Immunoglobulins differ in the composition and arrangement of the amino acids in the constant region of the heavy chain, and therefore differ in their antigenicity. Accordingly, immunoglobulins can be categorized into five classes, or isoforms of immunoglobulins, namely IgM, IgD, IgG, IgA and IgE, with corresponding heavy chains of μ -chain, δ -chain, γ -chain, α -chain and ϵ -chain, respectively. The same class of Ig can be further divided into different subclasses according to the differences in the amino acid composition of its hinge region and the number and position of disulfide bonds of the heavy chain, such as IgG can be divided into IgG1, IgG2, IgG3, IgG4. The light chain is divided into κ -chain or λ -chain through the differences in the constant region. Each of the five classes of Ig may have either a κ chain or a λ chain. The antibodies described in the present invention are preferably specific antibodies against cell surface antigens on target cells, and non-limiting embodiments are the following antibodies: anti-EGFRvIII antibody, anti-DLL-3 antibody, anti-PSMA antibody, anti-CD70 antibody, anti-MUC16 antibody, anti-ENPP3 antibody, anti-TDGF1 antibody, anti-ETBR antibody, anti-MSLN antibody, anti-TIM-1 antibody, anti-

LRRC15 antibody, anti-LIV-1 antibody, anti-CanAg/AFP antibody, anti-Claudin18.2 antibody, anti-Mesothelin antibody, anti-HER2 (ErbB2) antibody, anti-EGFR antibody, anti-c-MET antibody, anti-SLITRK6 antibody, anti-KIT/CD117 antibody, anti-STEAP1 antibody, anti-SLAMF7/CS1 antibody, anti-NaPi2B/SLC34A2 antibody, anti-GPNMB antibody, anti-HER3 (ErbB3) antibody, anti-MUC1/CD227 antibody, anti-AXL antibody, anti-CD166 antibody, anti-B7-H3 (CD276) antibody, anti-PTK7/CCK4 antibody, anti-PRLR antibody, anti-EFNA4 antibody, anti-5T4 antibody, anti-NOTCH3 antibody, anti-Nectin 4 antibody, anti-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 α V 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-CD138 antibody, anti-CD352 antibody, anti-CD25 antibody or anti-CD123 antibody.

The term “solvates” or “solvated compound” refers to the formation of a pharmaceutically usable solvent compound of the ligand-drug conjugate of the present application with one or more solvent molecules. Non-limiting examples of solvent molecules include water, ethanol, acetonitrile, isopropanol, DMSO, and ethyl acetate.

The term “drug load” refers to the average amount of cytotoxic drug loaded on each antibody in Formula I. It can also be expressed as a ratio of drug amount to antibody amount, and the drug load can range from 0-12, preferably 1-10 cytotoxic drugs (d) per antibody (Ab). In embodiments of the present application, the drug load is denoted as n, which exemplarily may be the mean of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, for example. The average amount of drug per ADC molecule after coupling reaction can be identified by conventional methods such as UV/visible spectroscopy, mass spectrometry, ELISA, and HPLC.

In one embodiment of the present application, the cytotoxic drug is coupled to the open cysteine sulfhydryl-SH and/or the sulfhydryl-SH of the cysteine residue of site-specific mutation between the antibody chains through a linker unit, and in general, the number of drug molecules that can be coupled to the antibody in the coupling reaction will be less than or equal to a theoretical maximum.

The loading of ligand-cytotoxic drug conjugates can be controlled by using the following non-limiting methods, including:

- (1) controlling the molar ratio of the linker reagent and the monoclonal antibody,
- (2) controlling the reaction time and temperature,
- (3) selecting different reaction reagents.

For the preparation of conventional pharmaceutical compositions, see the pharmacopeia such as the Chinese Pharmacopoeia or USP.

The term “pharmaceutically acceptable salt” or “pharmaceutically acceptable salt” refers to the salt of the ligand-drug conjugate of the present application, or the salt of the compound

described in the present application, which is safe and effective when used in mammals and is biologically active as desired. The ligand-drug conjugate compounds of the present application contain at least one carboxyl group and can therefore form salts with bases. Non-limiting examples of pharmaceutically acceptable salts include sodium salts, potassium salts, calcium salts or magnesium salts.

The term "pharmaceutically acceptable salt" or "pharmaceutically acceptable salt" refers to the salt of the ligand-drug conjugate of the present application, or the salt of the compound described in the present application, which is safe and effective when used in mammals and is biologically active as desired. The ligand-drug conjugate of the invention contains at least one amino group and can therefore form salts with acids. Non-limiting examples of pharmaceutically acceptable salts include hydrochloride, hydrobromide, hydriodate, sulfate, bisulfate, citrate, acetate, succinate, ascorbate, oxalate, nitrate, pyrite, hydrophosphate, dihydrophosphate, salicylate, citric acid hydrochloride, tartaric acid salt, maleate, fumarate, formate, benzoate, methanesulfonate, ethanesulfonate, benzenesulfonate, and p-toluenesulfonate.

The term "acidic amino acid" refers to amino acids with an isoelectric point of less than 7. Acidic amino acid molecules often contain one or more acidic groups, such as carboxyl groups, which can be effectively ionized into negative ionic forms in the structure to increase hydrophilicity. Acidic amino acids can be natural or unnatural.

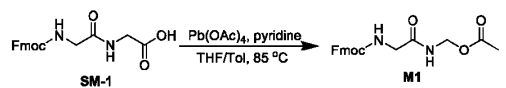
The term "natural amino acids" refers to biologically synthesized amino acids. Natural amino acids are generally L-types, but there are a few exceptions, such as glycine, that comes naturally and biosynthesized by living organisms.

The term "unnatural amino acids" refers to amino acids obtained by synthetic means.

The present application may be understood more readily by reference to the following detailed description of specific embodiments and examples included herein. Although the present application has been described with reference to specific details of certain embodiments thereof, it is not intended that such details should be regarded as limitations upon the scope of the disclosure. Experimental methods without specific conditions indicated in the following embodiments are generally carried out under normal conditions or under conditions recommended by the manufacturer. Unless otherwise indicated, all percentages, proportions, ratios, or parts are by weight.

Example 1

Synthesis of compound **M1**:

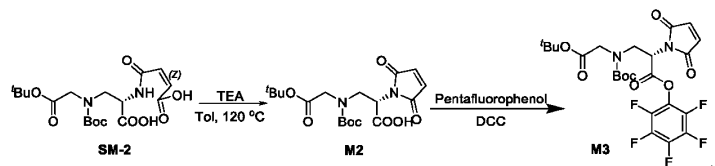


In a 5000 mL flask, N-fluorenylmethoxycarbonyl-glycine-glycine (100 g, 282 mmol, 1.0 eq), lead tetraacetate (175 g, 395 mmol, 1.4 eq), 2000 mL of dry tetrahydrofuran and 670 mL of toluene were added, stirred uniformly, protected by nitrogen, and the reaction was heated to 85 °C for 2.5 h. The reaction was monitored by TLC, and the raw materials were finished reacting. After the reaction, cooled to room temperature, filtered, the filtrate was concentrated under reduced pressure,

the residue was purified by column chromatography to obtain compound **M1** (87 g); LC-MS: $[M+NH]_4^+ = 386.0$.

Example 2

Synthesis of compound **M3**:

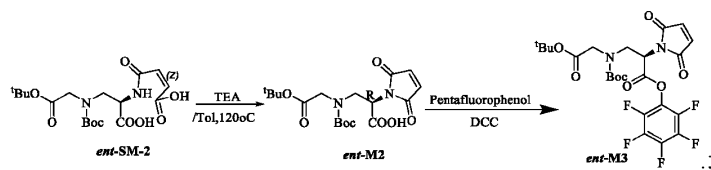


In a 1000 mL flask, compound **SM-2** (synthesized according to the method published in CN108452321A) (40 g, 96 mmol, 1.0 eq), triethylamine (26.7 mL, 2.0 eq), toluene (400 mL) were added, and the reaction was carried out by refluxing the compound at a temperature of 120 °C for 2 h. The reaction was monitored to be almost completely reacted by TLC, and then the solvent was spun off by reducing the temperature to 50 °C and decompression. The solvent was removed by spinning under reduced pressure at 50 °C. Dissolve with ethyl acetate (150 mL), water (40 mL), adjust pH to 2-3 with 1M HCl under ice bath stirring, and partition. The aqueous layer was extracted once more with ethyl acetate, the organic layers were combined and dried by adding anhydrous sodium sulfate. After filtration, a light yellow oily crude was obtained by concentration, and the crude was purified by column chromatography (DCM:MeOH=40:1) to obtain compound **M2** (26.6 g); LC-MS: $[M+H]^+ = 399.3$.

In a 1000 mL flask, compound **M2** (26.5 g, 60.5 mmol, 1.0 eq), pentafluorophenol (12.2 g, 66.5 mmol, 1.1 eq), DCC (13.7 g, 66.5 mmol, 1.1 eq), and THF (300 mL) were added, and the reaction was carried out at room temperature for 30 min (monitored by TLC), the insoluble material was filtered out. The reaction solution was purified directly by preparation, and the prepared solution was concentrated at 35 °C in a reduced pressure water bath to remove acetonitrile, and lyophilized to obtain compound **M3** (31.5 g) in 64% yield; LC-MS: $[M+H]^+ = 565.1$.

Example 3

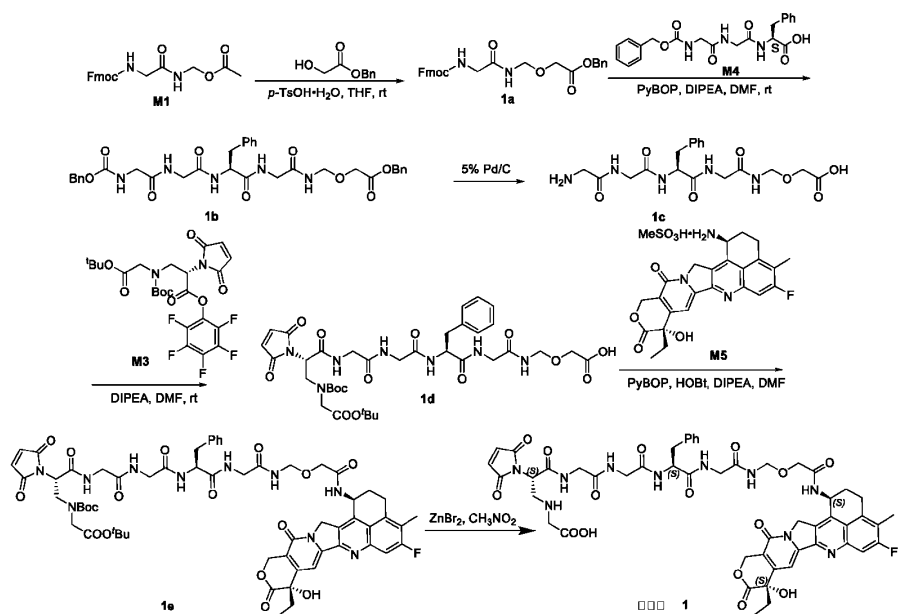
Synthesis of the compound **ent-M3**:



Referring to the synthetic route of Example 2, compound **ent-M3** (27.8 g) was obtained; LC-MS: $[M+H]^+ = 565.2$.

Example 4

Synthesis of compound **1**:



Step 1: Compound 1a

In a 250 mL flask, add **M1** (6 g, 16.3 mmol), 100 mL THF, p-toluenesulfonic acid monohydrate (0.31 g, 1.63 mmol), stirring and cooled to 0 °C, dropwise addition of benzyl hydroxyacetate (5.4 g, 32.6 mmol), dropwise natural warming to room temperature reaction (the reaction is about 2-4 h), TLC monitoring. At the end of the reaction, saturated NaHCO₃ solution was added, extracted with ethyl acetate, washed with saturated sodium chloride solution, dried with anhydrous sodium sulfate, filtered, concentrated, and the residue was purified by silica gel column (PE:EA=10:1-5:1-1:1) to obtain **1a** (4 g) in 52% yield; LC-MS: [M+H]⁺ =475.18.

Step 2: Compound 1b

In a 25 mL flask, add **1a** (2 g, 4.2 mmol), 10 mL DMF, stir at 0 °C, add DBU (766 mg, 5.04 mmol), and react for 1 h. After completion of Fmoc deprotection was monitored by TLC, the reaction was set aside;

Another 25 mL flask was filled with **M4** (prepared by the method published in CN111051330 A) (1.73 g, 4.2 mmol), PyBOP (2.61 g, 5.04 mmol), HOBT (680 mg, 5.04 mmol), and 10 mL of DMF, and DIPEA (830 uL, 5.04 mmol) was added to the reaction vial under ice-water bath, and stirring was continued for 30 min, the above reaction solution was added to the reaction flask, and the reaction was raised to room temperature. HPLC monitored the end of the reaction, the reaction solution was purified by preparative liquid phase to obtain the product preparation, the preparation was extracted by dichloromethane, washed with saturated sodium chloride solution, dried with anhydrous sodium sulfate, filtered, and the filtrate was concentrated under reduced pressure to obtain solid **1b** (1.7 g) in 63% yield; LCMS: [M+H]⁺ =648.26.

Step 3: Compound 1c

1b (900 mg, 1.39 mmol) was added to a 25 mL flask, and after 15 mL of DMF was dissolved,

900 mg of 5% Pd/C was added, and the reaction was hydrogenated for 2 h. The reaction was completed, and the filtrate was filtered to obtain the filtrate, which was used directly for the next step of the reaction without purification.

Step 4: Compound **1d**

The crude product **1c** was placed in an ice-water bath, DIPEA (235 μ L, 1.39 mmol) was added, and compound **M3** (784 mg, 1.39 mmol) was added, and the reaction was brought to room temperature for 1 h. The reaction was monitored for completion by HPLC, and the reaction solution was purified by high-performance liquid phase purification to obtain the preparative solution, which was lyophilized to obtain **1d** (504 mg); LC-MS: $[M+H]^+ = 804.4$.

10 Step 5: Compound **1e**

1d (500 mg, 0.62 mmol), **M5** (310 mg, 0.62 mmol), PyBOP (448 mg, 0.86 mmol), HOBt (116 mg, 0.86 mmol) and 15 mL of DMF were added to a 50 mL single-necked vial, DIPEA (378 μ L, 2.29 mmol) was added to the vial under an ice-water bath, and the reaction was carried out at room temperature for 2 h. After the reaction was monitored by HPLC, the reaction solution was purified by HPLC to give the preparative solution of compound **1e**, and the preparative solution was lyophilized to give **1e** (210 mg); LCLC monitored the completion of the reaction, and the preparative solution was lyophilized to give **1e** (210 mg). The reaction was carried out at room temperature for 2 h. After the completion of the reaction was monitored by HPLC, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution of compound **1e**, which was lyophilized to obtain **1e** (210 mg); LC-MS: $[M+H]^+ = 1221.6$.

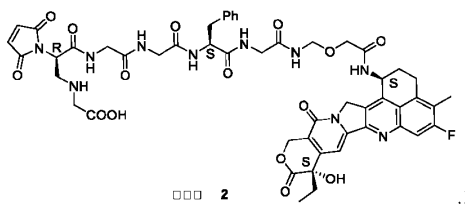
20

Step 6: Compound **1**

1e (100 mg, 0.081 mmol), zinc bromide (368 mg, 1.63 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 $^{\circ}$ C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain the solid compound **1** (60 mg); LC-MS: $[M+H]^+ = 1065.3$.

Example 5

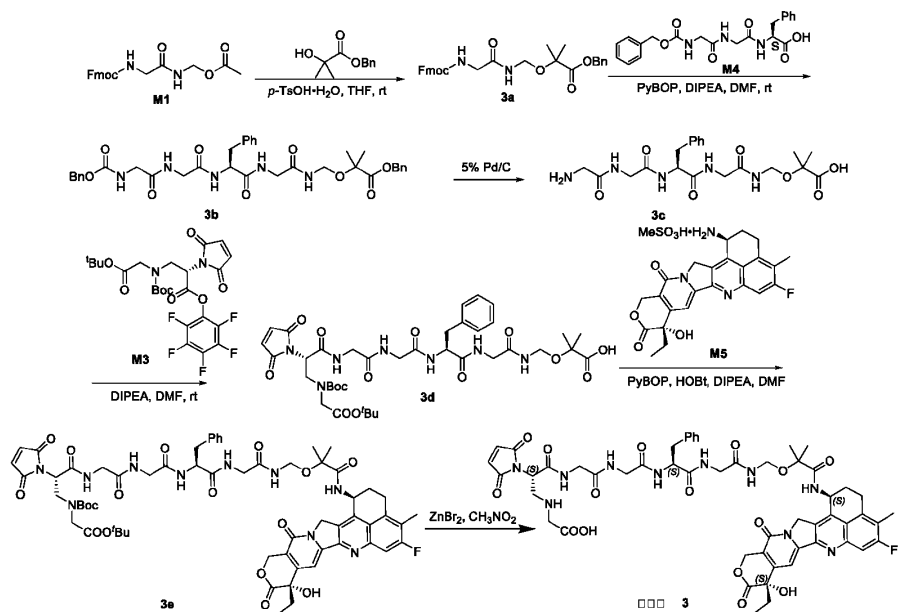
30 Synthesis of compound **2**:



Referring to the synthetic route of Example 4, compound **2** (51 mg) was obtained; LC-MS: $[M+H]^+ = 1065.3$.

Example 6

Synthesis of compound **3**:



Step 1: Compound 3a

In a 250 mL flask, add **M1** (6 g, 16.3 mmol), 100 mL THF, p-toluenesulfonic acid monohydrate (0.31 g, 1.63 mmol), stirring and cooled to 0 °C, dropwise addition of benzyl 2-hydroxy-2-methylpropionate (6.3 g, 32.6 mmol), after dropwise natural warming to room temperature reaction (reaction about 2-4 h), TLC monitoring. At the end of the reaction, saturated NaHCO₃ solution was added, extracted with ethyl acetate, washed with saturated sodium chloride solution, dried with anhydrous sodium sulfate, filtered, concentrated, and the residue was purified by silica gel column (PE:EA=10:1-5:1-2:1) to give **3a** (4.2 g) in 52% yield; LC-MS: [M+H]⁺ = 503.3.

Step 2: Compound 3b

In a 25 mL flask, add **3a** (2 g, 4.0 mmol), 10 mL DMF, stir at 0 °C, add DBU (760 mg, 5.0 mmol), and react for 1 h. After the completion of Fmoc deprotection was monitored by TLC, the reaction was set aside;

Add **M4** (1.65 g, 4.0 mmol), PyBOP (2.59 g, 5.0 mmol), HOBT (675 mg, 5.0 mmol) and 10 mL of DMF into another 25 mL flask, and add DIPEA (823 uL, 5.04 mmol) in an ice-water bath with continued stirring for 30 min, and then add the above reaction solution to the reaction flask and raise the reaction to room temperature. After the reaction was monitored by HPLC, the reaction solution was purified by preparative liquid phase to obtain the product preparation solution, which was extracted by dichloromethane, washed with saturated sodium chloride solution, dried with anhydrous sodium sulfate, filtered, and the filtrate was concentrated under reduced pressure to obtain the solid **3b** (1.4 g) in 53% yield; LC-MS: [M+H]⁺ = 676.2.

Step 3: Compound 3c

3b (700 mg, 1.04 mmol) was added to a 25 mL flask, and after 10 mL of DMF was dissolved,

700 mg of 5% Pd/C was added, and the reaction was hydrogenated for 1.5 h. The reaction was completed, and the filtrate was filtered to obtain the filtrate, which was used directly for the next step of the reaction without purification.

Step 4: Compound **3d**

The crude product **3c** was placed in an ice-water bath, DIPEA (210 μ L, 1.25 mmol) was added, and compound **M3** (704 mg, 1.25 mmol) was added, and the reaction was brought to room temperature for 1 h. The completion of the reaction was monitored by HPLC, and the reaction solution was purified by high-performance liquid phase purification to give the preparation, which was lyophilized to give **3d** (486 mg); LC-MS: $[M-H]^- = 830.5$.

10 Step 5: Compound **3e**

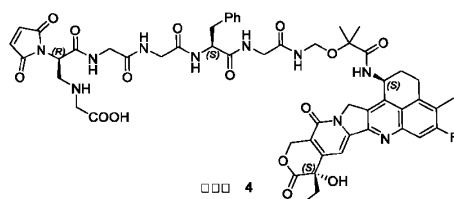
3d (300 mg, 0.36 mmol), **M5** (180 mg, 0.36 mmol), PyBOP (260 mg, 0.5 mmol), HOBt (67 mg, 0.5 mmol), and 10 mL of DMF were added to a 50 mL flask, DIPEA (219.5 μ L, 1.33 mmol) was added to the vial under an ice-water bath, and the reaction was carried out at room temperature for 3 h. After the reaction was monitored by HPLC, the reaction solution was purified by HPLC to give the preparative solution of compound **3e**. The reaction was carried out at room temperature for 3 h. After the completion of the reaction was monitored by HPLC, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution of compound **3e**, which was lyophilized to obtain **3e** (157 mg); LC-MS: $[M+H]^+ = 1249.6$.

Step 6: Compound **3**

20 **3e** (100 mg, 0.08 mmol), zinc bromide (360 mg, 1.6 mmol) and 5 mL of nitromethane were added to a 25 mL single-necked vial and the reaction was carried out at 40 $^{\circ}$ C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain the solid compound **3** (64 mg); LC-MS: $[M+H]^+ = 1093.1$.

Example 7

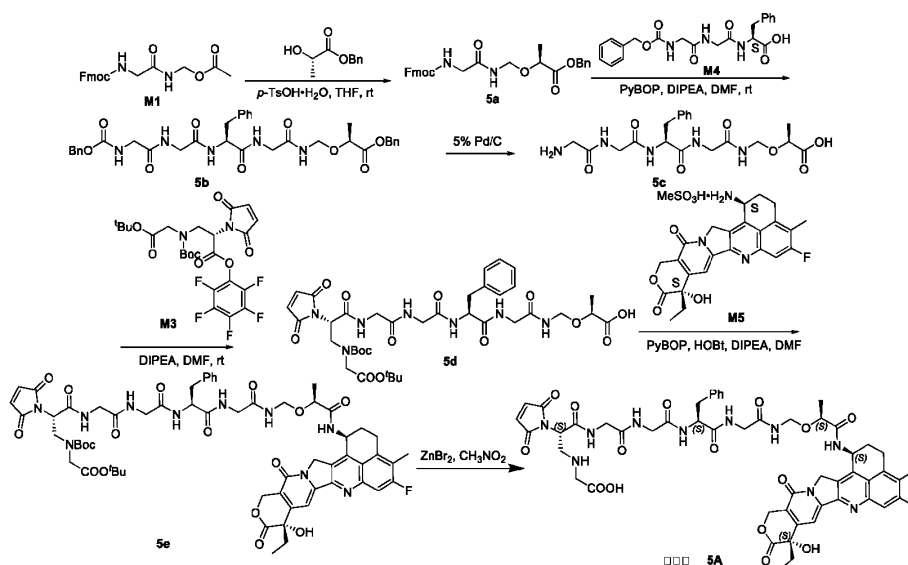
Synthesis of compound **4**:



30 Referring to the synthetic route of Example 6, compound **4** (60 mg) was obtained; LC-MS: $[M+H]^+ = 1093.2$.

Example 8

Synthesis of compound **5A**:



Step 1: Compound **5a**

Add **M1** (500 mg, 1.4 mmol, 1.0 eq), p-toluenesulfonic acid monohydrate (26 mg, 0.1 mmol, 0.1 eq) and 10 mL of THF in a 25 mL flask, stirred well, and then lowered to 0 °C, and then slowly added L-lactic acid benzyl ester (1.2 g, 7.0 mmol, 5 eq), and then raised to room temperature after the addition of the reaction, TLC monitoring. At the end of the reaction, saturated NaHCO₃ solution was added, extracted with ethyl acetate, dried over anhydrous sodium sulfate, filtered, concentrated, and the residue was purified by reversed-phase column to give **5a** (400 mg);

LC-MS: [M+NH]₄⁺ = 506.2.

¹H NMR (400 Mz, CDCl₃/CD₃ OD): 1.39 (3H, d, *J* = 6.8 Hz), 3.78 (2H, t, *J* = 4.0 Hz), 4.17-4.27 (2H, m), 4.42 (2H, d, *J* = 4.0 Hz), 4.72-4.85 (2H, m), 5.11-5.58 (2H, m), 5.43 (1H, s), 7.06 (1H, t, *J* = 8.0 Hz), 7.25-7.33 (6H, m), 7.38 (2H, t, 7.38 (2H, t, 7.06 (1H, t, 7.06), 7.25-7.33 (6H, m), 7.25-7.33 (7.25-7.33) 5.43 (1H, s), 7.06 (1H, t, *J* = 8.0 Hz), 7.25-7.33 (6H, m), 7.38 (2H, t, *J* = 8.0 Hz), 7.57 (2H, d, *J* = 8.0 Hz), 7.75 (2H, d, *J* = 8.0 Hz).

Step 2: Compound **5b**

Compound **5a** (400 mg, 0.8 mmol, 1.0 eq) and 4 mL of DMF were added to a 25 mL flask, stirred well, and then lowered to 0 °C before slowly adding the DBU (137 mg, 0.9 mmol, 1.1 eq), and after addition, the reaction was brought to room temperature. The reaction was monitored by TLC and the end of the reaction was recorded as reaction solution ①;

Another 25 mL single-necked vial was taken and **M4** (372 mg, 0.9 mmol, 1.1 eq), PyBOP (852 mg, 1.6 mmol, 2.0 eq) and 3 mL of DMF were added, stirred at room temperature for 5 min, and reaction solution ① was added, the reaction was carried out at room temperature, and monitored by HPLC. The reaction was completed, and the reaction solution was purified by HPLC to yield compound **5b** (326 mg); LC-MS: [M+NH]₄⁺ = 679.2.

Step 3: Compound **5c**

5b (4.0 g, 6.05 mmol, 1.0 eq) was added to a 100 mL flask, DMF (60 mL) was dissolved,

then 5% Pd/C (4 g) was added, and the hydrogenation reaction was carried out at room temperature for 4 h (HPLC was used to monitor the progress of the reaction). The Pd/C was filtered, and the filtrate was placed directly in an ice-water bath (ca. 0 °C) without concentration and left for use.

Step 4: Compound **5d**

The crude product **5c** was placed in an ice-water bath, DIPEA (1.1 mL, 1.1 eq) was added, and compound **M3** (3.4 g, 6.05 mmol) was added, and the reaction was brought to room temperature for 2 h. The completion of the reaction was detected by HPLC, and the reaction solution was purified by high performance liquid phase purification to obtain the preparative solution, which was lyophilized to obtain **5d** (3.15 g); LC-MS: $[M-H]^- = 816.3$.

10 Step 5: Compound **5e**

5d (2.07 g, 2.53 mmol, 1.0 eq), **M5** (1.35 g, 2.53 mmol, 1.0 eq), PyBOP (1.98 g, 3.79 mmol, 1.5 eq), HOBt (0.51 g, 3.79 mmol, 1.5 eq) and DMF (40 mL) were added to a 100 mL single-necked vial, and the reaction was brought to room temperature for 2 h. The reaction was monitored by HPLC. DIPEA (1.05 mL, 1.5 eq) was added, and the reaction was brought to room temperature for 2 h (monitored by HPLC). The reaction solution was purified directly by preparation, and the preparative solution was concentrated by pumping water under reduced pressure at 35 °C to remove acetonitrile, and lyophilized to obtain compound **5e** (1.92 g) in 61% yield; LC-MS: $[M+H]^+ = 1235.4$.

Step 6: Compound **5A**

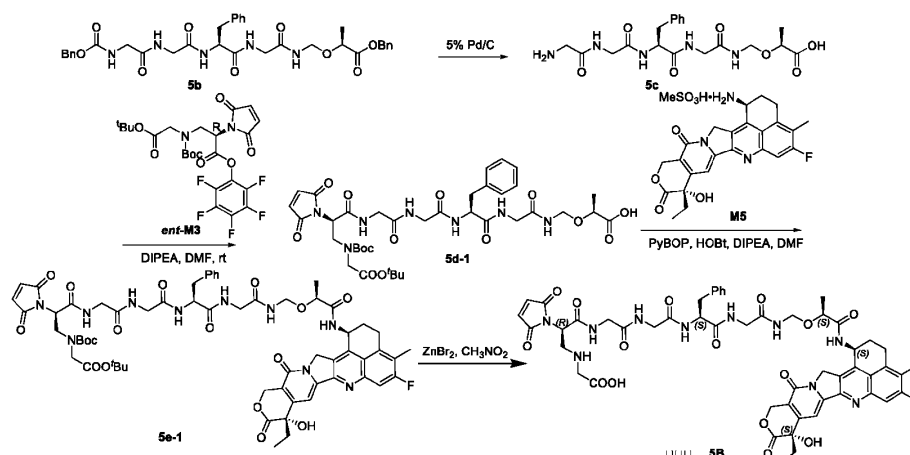
20 Compound **5e** (1.0 g, 0.8 mmol, 1.0 eq), 35 mL nitromethane was added in a 100 mL flask, dissolved and then added zinc bromide (3.64 g, 16 mmol, 20.0 eq), reacted for 30 min in an oil bath at 40 °C (stabilized in advance by preheating), and pumped to concentrate at 45 °C under reduced pressure in a water bath to remove the nitromethane, resulting in a solid yellow residue (monitored by HPLC). After acid preparation, the preparative solution of compound **5A** was obtained, and the preparative solution was concentrated and spun off acetonitrile by pump decompression water bath at 35 °C and lyophilized to obtain compound **5A** (786 mg) in 90% yield.

LC-MS: $[M+H]^+ = 1079.4$;

¹H NMR (400 MHz, DMSO-d₆) δ 9.39 - 9.02 (m, 1H), 8.70 (t, $J = 6.5$ Hz, 1H), 8.64 (t, $J = 5.7$ Hz, 1H), 8.56 (d, $J = 8.8$ Hz, 1H), 8.34 (t, $J = 5.7$ Hz, 1H), 8.16 (d, $J = 8.2$ Hz, 1H), 8.01 (t, $J = 5.5$ Hz, 1H), 7.71 (d, $J = 10.9$ Hz, 1H), 7.30 (s, 1H), 7.28 - 7.15 (m, 4H), 7.14 (s, 2H), 5.53 (dd, $J = 14.5, 6.4$ Hz, 1H), 5.49 - 5.34 (m, 2H), 5.22 (d, $J = 18.8$ Hz, 1H), 5.09 (d, $J = 18.7$ Hz, 1H), 5.03 (dd, $J = 9.6, 3.9$ Hz, 1H), 4.73 (dd, $J = 9.9, 6.9$ Hz, 1H), 4.59 (dd, $J = 10.1, 6.5$ Hz, 1H), 4.49 (dd, $J = 13.2, 8.6, 4.4$ Hz, 1H), 4.14 (dd, $J = 13.3, 6.6$ Hz, 2H), 3.93 (s, 2H), 3.84 (dd, $J = 16.5, 6.3$ Hz, 1H), 3.76 (dd, $J = 16.9, 5.7$ Hz, 2H), 3.70 (d, $J = 9.9, 6.9$ Hz, 2H), 3.70 (dd, $J = 5.2$ Hz, 2H), 3.60 (dd, $J = 16.7, 5.4$ Hz, 1H), 3.52 (dd, $J = 16.4, 5.1$ Hz, 1H), 3.45 (dd, $J = 12.8, 10.1$ Hz, 1H), 3.25 - 3.15 (m, 1H), 3.14 - 3.05 (m, 1H), 3.01 (dd, $J = 13.7, 4.1$ Hz, 1H), 2.73 (dd, $J = 13.5, 9.8$ Hz, 1H), 2.54 - 2.47 (m, 1H), 2.33 (s, 2H), 2.17 (d, $J = 5.5$ Hz, 2H), 1.91 - 1.79 (m, 2H), 1.33 (d, $J = 6.6$ Hz, 2H), 0.87 (t, $J = 7.3$ Hz, 2H).

Example 9

40 Synthesis of compound **5B**:



Step 1: Compound **5d-1**

Compound **5b** (300 mg, 0.45 mmol, 1.0 eq), DMF (3 mL) was added in a 25 mL flask with stirring to dissolve the clear, 5% Pd/C (300 mg) was added, hydrogen displacement was performed three times, and the hydrogenation reaction was carried out for 2 h. The end of the reaction was monitored by HPLC. At the end of the reaction, the Pd/C was removed by filtration, the filtrate was cooled down to 0-5 °C, DIPEA (65 mg, 0.5 mmol, 1.1 eq) was added, and then **ent-M3** (255 mg, 0.45 mmol) was added to the filtrate, and then the reaction was raised to 20±5 °C for 1 h. The end of the reaction was monitored by HPLC. At the end of the reaction, it was purified by HPLC preparation, and the product preparation was collected and lyophilized to obtain compound **5d-1** (200 mg) in 54% yield; LC-MS: $[M-H]^- = 816.3$.

Step 2: Compound **5e-1**

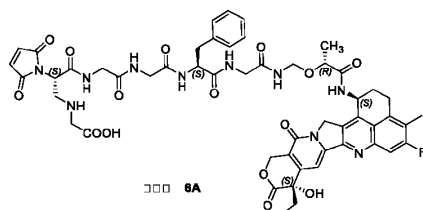
Compound **5d-1** (200 mg, 0.24 mmol, 1.0 eq), **M5** (127 mg, 0.24 mmol, 1.0 eq), PyBOP (187 mg, 0.36 mmol, 1.2 eq), HOBt (48 mg, 0.36 mmol, 1.2 eq), and DMF (6 mL) were added to a 25 mL flask. Water bath was lowered to 0-5 °C, DIPEA (62 mg, 0.48 mmol, 2.0 eq) was added, and the reaction was raised to 20 ± 5 °C for 2 h. The end of the reaction was monitored by HPLC. The reaction solution was purified directly by HPLC preparation, and the product preparation was collected and lyophilized to obtain compound **5e-1** (162.8 mg); LC-MS: $[M+H]^+ = 1235.4$.

Step 3: Compound **5B**

Compound **5e-1** (110 mg, 0.089 mmol, 1.0 eq), ZnBr₂ (400 mg, 1.78 mmol, 20.0 eq) and CH₃NO₂ (10 mL) were sequentially added into a 25 mL flask. After addition, the reaction was raised to 40 °C for 0.5 h, and then the reaction was stopped, and the reaction solution was dried directly at 45 °C by spin-drying under reduced pressure to obtain a yellow solid. The reaction was monitored by HPLC. The spin-dried solid was directly purified by HPLC preparation, and the product preparation was collected and lyophilized to obtain compound **5B** (73.4 mg) in 76.5% yield; LC-MS: $[M+H]^+ = 1079.4$.

Example 10

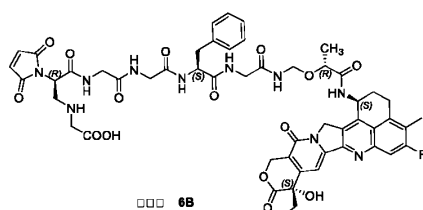
Preparation of compound **6A**:



Referring to the synthetic route of Example 8, compound **6A** (71 mg) was obtained; LC-MS: $[M+H]^+ = 1079.4$.

Example 11

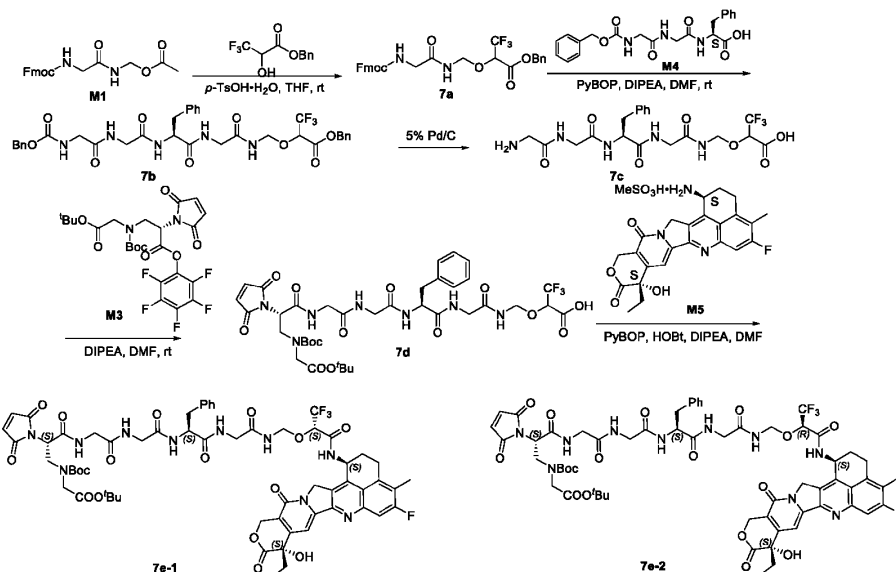
Preparation of compound **6B**:



Referring to the synthetic route of Example 9, compound **6B** (59 mg) was obtained; LC-MS: $[M+H]^+ = 1079.4$.

Example 12

10 Preparation of compounds **7A** and **7B**:



Step 1: Compound 7a

In a 250 mL flask, add **M1** (10 g, 27.1 mmol), benzyl 3, 3, 3-trifluorolactate (prepared according to the method published in Patent WO2020063673A1) (12.7 g, 54.3 mmol), zinc acetate (9.96 g, 54.3 mmol), and 100 mL of toluene, and react at 100 °C for 4 h. The reaction was completed. The reaction was completed, reduced to room temperature, filtered to remove insoluble

material, and the filtrate was concentrated to obtain the crude product. The crude product was purified by silica gel column chromatography (PE:EA=10:1-5:1-2:1) to obtain 5.15 g of the target material, yield 35.1%; LC-MS: $[M+H]^+ = 543.17$.

Step 2: Compound **7b**

Add **7a** (5 g, 9.2 mmol) and 15 mL of DMF in a 50 mL flask, dissolve and clear, and then add DBU (1.68 g, 11 mmol) in an ice-water bath and react for 1 h. This was recorded as reaction solution ①;

Another 50 mL flask was taken, **M4** (3.8 g, 9.2 mmol), PyBOP (5.75 g, 11 mmol), HOBt (1.49 g, 11 mmol) and 10 mL DMF were added, and after dissolved, DIPEA (1.82 mL, 11 mmol) was added in an ice-water bath, and the reaction was continued for 30 min, then reaction solution ① was added, and the reaction was brought to room temperature for 2 h. The reaction was monitored by HPLC. The reaction was monitored by HPLC for 2 h. After completion of the reaction, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution. The prepared solution was extracted with dichloromethane, washed with saturated sodium chloride solution, dried with anhydrous sodium sulfate, filtered, and concentrated to obtain 4.1 g of solid, with a yield of 62.3%; LC-MS: $[M+H]^+ = 716.25$.

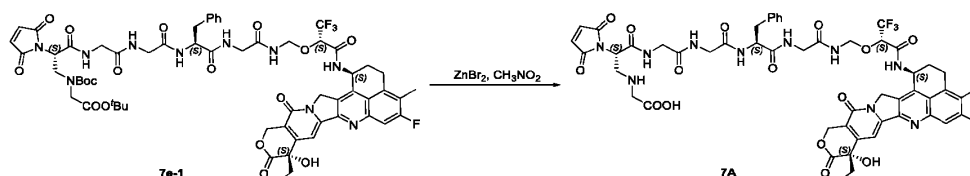
Step 3: Compound **7d**

Add **7b** (900 mg, 1.26 mmol) in a 25 mL flask, after 15 mL of DMF was dissolved, add 900 mg 5% Pd/C, hydrogenation reaction for 2 h, the reaction was completed, filtration, the filtrate was placed in an ice-water bath, add DIPEA (228 μ L, 1.38 mmol), and then add **M3** (712 mg, 1.26 mmol), and then add the solution to the room temperature. The reaction was monitored by HPLC for 1 h. The reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution, which was lyophilized to obtain 525 mg of the product in 47.9% yield; LC-MS: $[M-H]^- = 870.33$.

Step 4: Compound **7e**

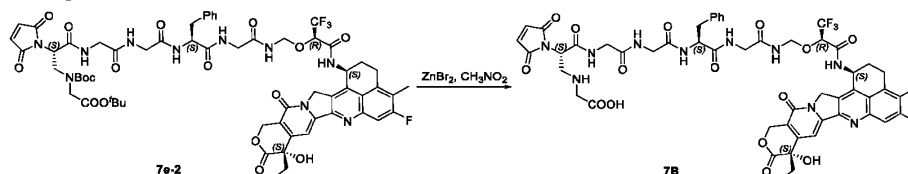
7d (500 mg, 0.57 mmol), **M5** (305 mg, 0.57 mmol), PyBOP (448 mg, 0.86 mmol), HOBt (116 mg, 0.86 mmol) and 15 mL of DMF were added to a 50 mL flask, DIPEA (378 μ L, 2.29 mmol) was added to the flask under an ice-water bath, and the reaction was carried out at room temperature for 2 h. After the reaction was monitored by HPLC, the reaction solution was purified by HPLC to obtain the preparative solution of compound **7e-1** and compound **7e-2**, and the preparative solution was lyophilized to obtain 150 mg of compound **7e-2**. The reaction was carried out at room temperature for 2 h. After HPLC monitoring, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solutions of compound **7e-1** and compound **7e-2**, which were lyophilized to obtain 150 mg of compound **7e-1**, LC-MS: $[M+H]^+ = 1289.46$, and 220 mg of compound **7e-2**, LC-MS: $[M+H]^+ = 1289.46$, respectively.

Step 5: Compound **7A**



7e-1 (100 mg, 0.077 mmol), zinc bromide (349 mg, 1.55 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 52 mg of solid; TOF result: 1133.3613.

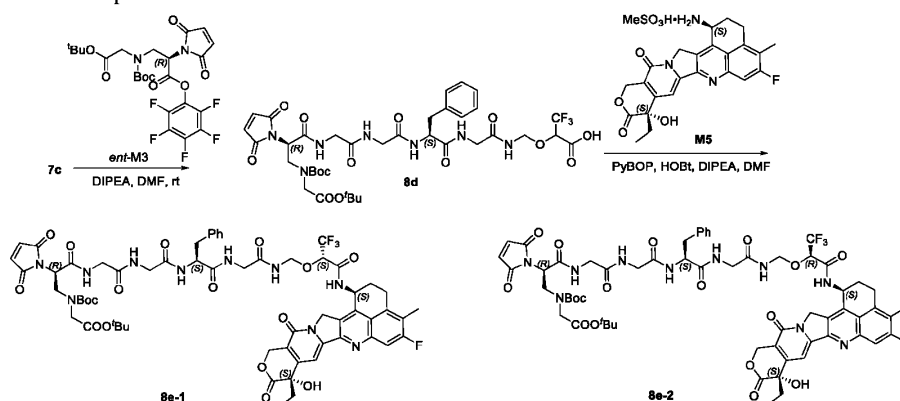
Step 6: Compound **7B**



7e-2 (100 mg, 0.077 mmol), zinc bromide (349 mg, 1.55 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 63 mg of solid; TOF result: 1133.3668.

Example 13

Synthesis of compounds **8A** and **8B**:



Step 1: Compound **8d**

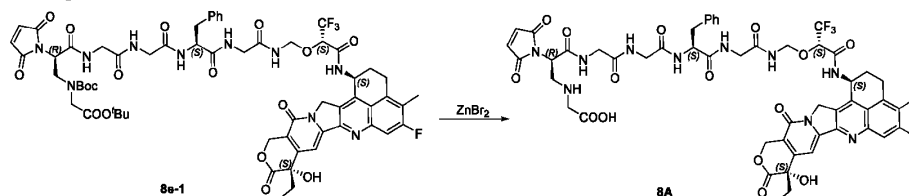
In a 25 mL flask, **7c** (900 mg, 1.83 mmol) was added, 20 mL of DMF was dissolved, then DIPEA (303 uL, 1.83 mmol) was added, and **ent-M3** (1034 mg, 1.83 mmol) was added, and the reaction was brought to room temperature for 1 h. The completion of the reaction was detected by

HPLC, and the reaction solution was purified by high-performance liquid chromatography (HPLC) to obtain the preparative solution, which was lyophilized to give 613 mg of product in 38.5% yield. The prepared solution was lyophilized to obtain 613 mg of product with 38.5% yield; LC-MS: $[M-H]^- = 870.32$.

Step 2: Compound **8e-1** and Compound **8e-2**

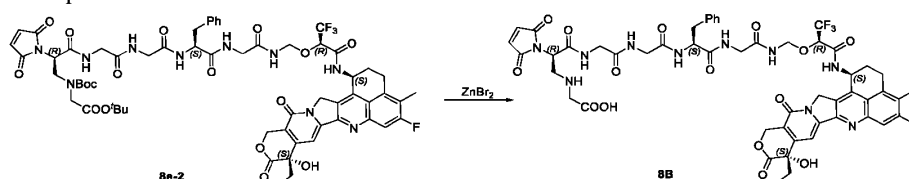
8d (500 mg, 0.57 mmol), **M5** (305 mg, 0.57 mmol), PyBOP (448 mg, 0.86 mmol), HOBt (116 mg, 0.86 mmol) and 15 mL of DMF were added into a 50 mL flask, DIPEA (378 μ L, 2.29 mmol) was added into the vial in an ice-water bath, and the reaction was carried out at room temperature for 2 h. After the reaction was monitored by HPLC, the reaction solution was purified by HPLC to obtain the preparation of compound **8e-1** and compound **8e-2**. After the reaction was completed by HPLC, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solutions of compound **8e-1** and compound **8e-2**, which were lyophilized to obtain 140 mg of compound **8e-1** and 210 mg of compound **8e-2**, respectively. The LC-MS of compound **8e-1**: $[M+H]^+ = 1289.47$; the LC-MS of compound **8e-2**: $[M+H]^+ = 1289.47$.

Step 3: Compound **8A**



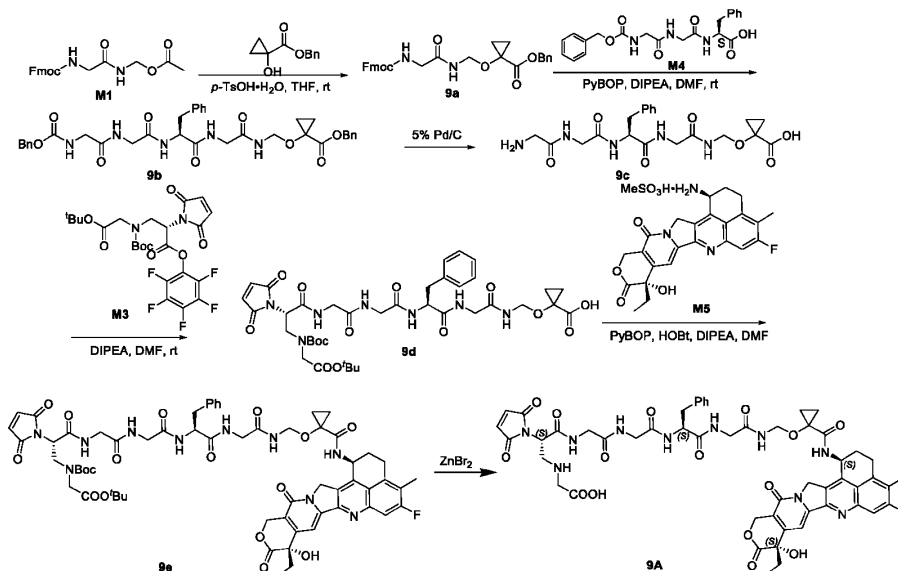
Compound **8e-1** (100 mg, 0.077 mmol), zinc bromide (349 mg, 1.55 mmol) and 5 mL of nitromethane were added to a 25 mL flask and reacted at 40 °C for 1 h. After the reaction was completed by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 50 mg of solid; TOF result: 1133.3623.

Step 4: Compound **8B**



Compound **8e-2** (100 mg, 0.077 mmol), zinc bromide (349 mg, 1.55 mmol) and 5 mL of nitromethane were added to a 25 mL single-necked vial and reacted at 40 °C for 1 h. After the reaction was completed by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain a solid 58 mg; TOF result: 1133.3653.

Example 14

Synthesis of compound **9A**:Step 1: Compound **9a**

In a 250 mL flask, add **M1** (6 g, 16.3 mmol), 100 mL THF, p-toluenesulfonic acid monohydrate (0.31 g, 1.63 mmol), stir and cool to 0 °C, dropwise add benzyl 2-hydroxy-2-cyclopropyl acetate (prepared according to the method published in US20050020645 A1) (6.3 g, 32.6 mmol), the reaction was naturally warmed up to room temperature after dropping (the reaction was about 2-4 h) and monitored by TLC. At the end of the reaction, saturated NaHCO₃ solution was added, extracted with ethyl acetate, washed with saturated sodium chloride solution, dried with anhydrous sodium sulfate, filtered, concentrated, and the residue was purified by silica gel column (PE:EA=10:1-5:1-2:1) to give **9a** (3.7 g) in 45% yield; LC-MS: [M+H]⁺ = 501.5.

Step 2: Compound **9b**

In a 25 mL flask, add **9a** (2 g, 4.0 mmol), 10 mL DMF, stir at 0 °C, add DBU (760 mg, 5.0 mmol), and react for 1 h. After the completion of Fmoc deprotection was monitored by TLC, the reaction was set aside;

Add **M4** (1.65 g, 4.0 mmol), PyBOP (2.59 g, 5.0 mmol), HOBt (675 mg, 5.0 mmol) and 10 mL of DMF into another 25 mL flask, and add DIPEA (823 uL, 5.04 mmol) in an ice-water bath with continued stirring for 30 min, and then add the above reaction solution to the reaction flask and raise the reaction to room temperature. After the reaction was monitored by HPLC, the reaction solution was purified by preparative liquid phase to obtain the product preparation solution, which was extracted by dichloromethane, washed with saturated sodium chloride solution, dried with anhydrous sodium sulfate, filtered, and the filtrate was concentrated under reduced pressure to obtain a solid of 1.5 g in 56% yield; LC-MS: [M+H]⁺ = 674.7.

Step 3: Compound **9c**

9b (900 mg, 1.3 mmol) was added to a 25 mL flask, and after 10 mL of DMF was dissolved

and cleared, 900 mg of 5% Pd/C was added, and the hydrogenation reaction was carried out for 1.5 h. After completion of the reaction, it was filtered to obtain the filtrate, which was used directly for the next step of the reaction without purification.

Step 4: Compound **9d**

The crude product **9c** was placed in an ice-water bath, DIPEA (223 μ L, 1.3 mmol) was added, and compound **M3** (750 mg, 1.3 mmol) was added, and the reaction was brought to room temperature for 1 h. The reaction was monitored for completion by HPLC, and the reaction solution was purified by high-performance liquid phase purification to give the preparation, which was lyophilized to give **9d** (529 mg); LC-MS: $[M-H]^- = 828.4$.

10 Step 5: Compound **9e**

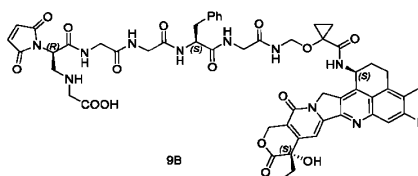
9d (500 mg, 0.6 mmol), **M5** (300 mg, 0.6 mmol), PyBOP (416 mg, 0.8 mmol), HOBt (108 mg, 0.5 mmol) and 15 mL of DMF were added into a 50 mL vial, DIPEA (351 μ L, 2.13 mmol) was added into the flask under an ice-water bath, and the reaction was carried out for 3 h. After the reaction was completed by HPLC, the reaction solution was purified by HPLC to obtain the preparative solution of compound **9e**. After the reaction was monitored by HPLC, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution of compound **9e**, which was lyophilized to obtain **9e** (257 mg); LC-MS: $[M+H]^+ = 1247.5$.

Step 6: Compound **9A**

20 **9e** (100 mg, 0.08 mmol), zinc bromide (360 mg, 1.6 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 $^{\circ}$ C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain the solid compound **9A** (55 mg); LC-MS: $[M+H]^+ = 1091.3$.

Example 15

Synthesis of compound **9B**:

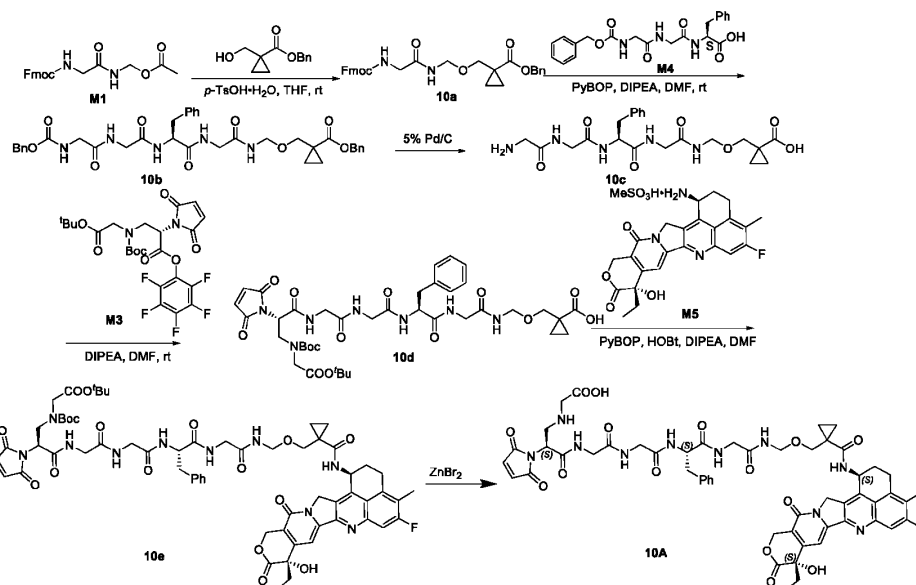


Referring to the synthetic route of Example 14, compound **9B** (44 mg) was obtained; LC-MS: $[M+H]^+ = 1091.3$.

30

Example 16

Synthesis of compound **10A**:



Step 1: Compound 10a

In a 250 mL flask, add **M1** (6 g, 16.3 mmol), 100 mL THF, p-toluenesulfonic acid monohydrate (0.31 g, 1.63 mmol), stirring and cooling to 0 °C, dropwise add benzyl 3-hydroxy-2-cyclopropylpropionate (prepared with reference to the method published in WO2013187496A1) (6.7 g, 32.6 mmol). The reaction was naturally warmed to room temperature after dropping (about 2-4 h) and monitored by TLC. At the end of the reaction, saturated NaHCO₃ solution was added, extracted with ethyl acetate, washed with saturated sodium chloride solution, dried with anhydrous sodium sulfate, filtered, concentrated, and the residue was purified by silica gel column (PE:EA=10:1-5:1-2:1) to give **10a** (4.9 g) in 58% yield; LC-MS: [M+H]⁺=515.4.

Step 2: Compound 10b

In a 25 mL flask, add **10a** (4 g, 7.8 mmol), 10 mL DMF, stir at 0 °C, add DBU (1.2 g, 8.0 mmol), react for 1 h. After the completion of Fmoc deprotection was monitored by TLC, the reaction was set aside;

Another 25 mL flask was filled with **M4** (3.3 g, 8.0 mmol), PyBOP (5.2 g, 10.0 mmol), HOBT (1.35 g, 10.0 mmol) and 10 mL of DMF, and DIPEA (1.65 mL, 10.1 mmol) was added in an ice-water bath with continued stirring for 50 min, and then the above reaction solution was added to the flask and raised to room temperature. After the reaction was monitored by HPLC, the reaction solution was purified by preparative liquid phase to obtain the product preparation solution, which was extracted by dichloromethane, washed with saturated sodium chloride solution, dried with anhydrous sodium sulfate, filtered, and the filtrate was concentrated under reduced pressure to obtain a solid of 2.3 g in 42% yield; LC-MS: [M+H]⁺=688.8.

Step 3: Compound 10c

10b (1.0 g, 1.45 mmol) was added to a 25 mL flask, and after 15 mL of DMF was dissolved and cleared, 1.0 g of 5% Pd/C was added, and the reaction was hydrogenated for 1.5 h. The

reaction was completed, and the filtrate was filtered to obtain the filtrate, which was used directly for the next step of the reaction without purification.

Step 4: Compound **10d**

The crude product **10c** was placed in an ice-water bath, DIPEA (258 uL, 1.5 mmol) was added, and compound **M3** (837 mg, 1.45 mmol) was added, and the reaction was brought to room temperature for 1 h. The completion of the reaction was monitored by HPLC, and the reaction solution was purified by high-performance liquid phase purification to obtain the preparative solution, which was lyophilized to obtain **10d** (499 mg); LC-MS: $[M-H]^- = 842.4$.

Step 5: Compound **10e**

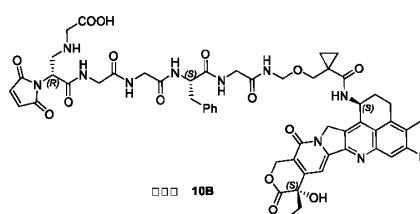
10 **10d** (400 mg, 0.48 mmol), **M5** (240 mg, 0.48 mmol), PyBOP (250 mg, 0.48 mmol), HOBt (104 mg, 0.48 mmol) and 15 mL of DMF were added to a 50 mL flask in an ice-water bath and DIPEA (330 uL, 2.0 mmol) was added, and the reaction was allowed to proceed for 3 h. After the reaction was monitored by HPLC, the reaction solution was purified by HPLC to give the preparative solution of compound **10e**. The reaction was carried out at room temperature for 3 h. After the completion of the reaction was monitored by HPLC, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution of compound **10e**, which was lyophilized to obtain **10e** (188 mg); LC-MS: $[M+H]^+ = 1261.5$.

Step 6: Compound **10A**

20 **10e** (100 mg, 0.08 mmol), zinc bromide (360 mg, 1.6 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain the solid compound **10A** (61 mg); LC-MS: $[M+H]^+ = 1105.4$.

Example 17

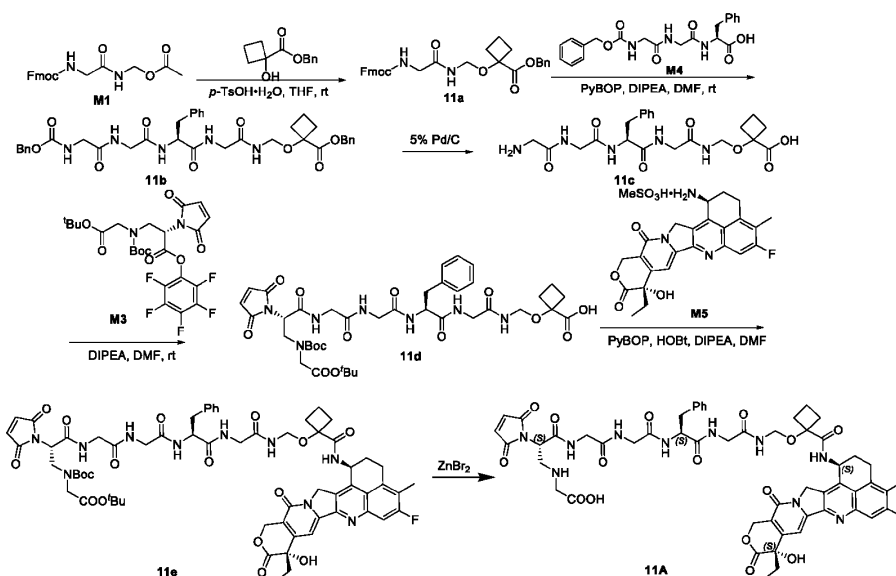
Synthesis of compound **10B**:



Referring to the synthetic route of Example 16, compound **10B** (75 mg) was obtained; LC-MS: $[M+H]^+ = 1105.4$.

30 Example 18

Synthesis of compound **11A**:



Step 1: Compound **11a**

In a 250 mL flask, add **M1** (6 g, 16.3 mmol), 100 mL THF, p-toluenesulfonic acid monohydrate (0.31 g, 1.63 mmol), stir and cool to 0 °C, dropwise addition of benzyl 2-hydroxy-2-cyclobutylacetate (see *Journal of Medicinal Chemistry*, 2013, 56(13), 5541-5552. The method of synthesis) (6.7 g, 32.6 mmol), the reaction was naturally warmed up to room temperature after dropwise (the reaction was carried out for about 2-4 h) and monitored by TLC. At the end of the reaction, saturated NaHCO₃ solution was added, extracted with ethyl acetate, washed with saturated sodium chloride solution, dried with anhydrous sodium sulfate, filtered, concentrated, and the residue was purified by silica gel column (PE:EA=10:1-5:1-2:1) to give **11a** (5.1 g) in 62% yield; LC-MS: [M+H]⁺=515.7.

Step 2: Compound **11b**

In a 25 mL flask, add **11a** (4 g, 7.8 mmol), 10 mL DMF, stir at 0 °C, add DBU (1.2 g, 8.0 mmol), react for 1 h. After the completion of Fmoc deprotection was monitored by TLC, the reaction was set aside;

Add **M4** (3.3 g, 8.0 mmol), PyBOP (5.2 g, 10.0 mmol), HOBT (1.35 g, 10.0 mmol) and 10 mL of DMF into another 25 mL single-necked vial, and add DIPEA (1.63 mL, 10.0 mmol) in an ice-water bath with continued stirring for 40 min, and then add the above reaction solution to the flask. After the reaction was monitored by HPLC, the reaction solution was purified by preparative liquid phase to obtain the product preparation solution, which was extracted by dichloromethane, washed with saturated sodium chloride solution, dried with anhydrous sodium sulfate, filtered, and the filtrate was concentrated under reduced pressure to obtain a solid of 2.3 g in 42% yield; LC-MS: [M+H]⁺=688.3.

Step 3: Compound **11c**

11b (2.0 g, 2.9 mmol) was added to a 25 mL flask, and after 25 mL of DMF was dissolved

and cleared, 2.0 g of 5% Pd/C was added, and the hydrogenation reaction was carried out for 3 h. After completion of the reaction, it was filtered to obtain the filtrate, which was used directly for the next step of the reaction without purification.

Step 4: Compound **11d**

The crude product **11c** was placed in an ice-water bath, DIPEA (516 uL, 3.0 mmol) was added, and compound **M3** (1.7 g, 2.9 mmol) was added, and the reaction was brought to room temperature for 2 h. The completion of the reaction was monitored by HPLC, and the reaction solution was purified by high-performance liquid phase purification to obtain the preparative solution, which was lyophilized to give **11d** (934 mg); LC-MS: $[M-H]^- = 842.4$.

10 Step 5: Compound **11e**

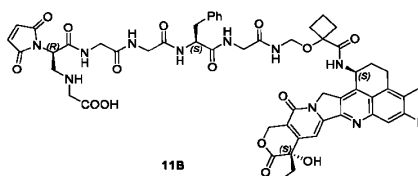
11d (800 mg, 0.96 mmol), **M5** (480 mg, 0.96 mmol), PyBOP (500 mg, 0.96 mmol), HOBT (208 mg, 0.96 mmol) and 30 mL of DMF were added to a 50 mL flask in an ice-water bath and DIPEA (660 uL, 4.0 mmol) was added, and the reaction was allowed to proceed for 4 h. After the reaction was monitored by HPLC, the reaction solution was purified by HPLC to give the preparative solution of compound **11e**. The reaction was carried out at room temperature for 4 h. After the completion of the reaction was monitored by HPLC, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution of compound **11e**, which was lyophilized to obtain **11e** (401 mg); LC-MS: $[M+H]^+ = 1261.4$.

Step 6: Compound **11A**

20 **11e** (150 mg, 0.12 mmol), zinc bromide (532 mg, 2.4 mmol) and 10 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain the solid compound **11A** (86 mg); LC-MS: $[M+H]^+ = 1105.4$.

Example 19

Synthesis of compound **11B**

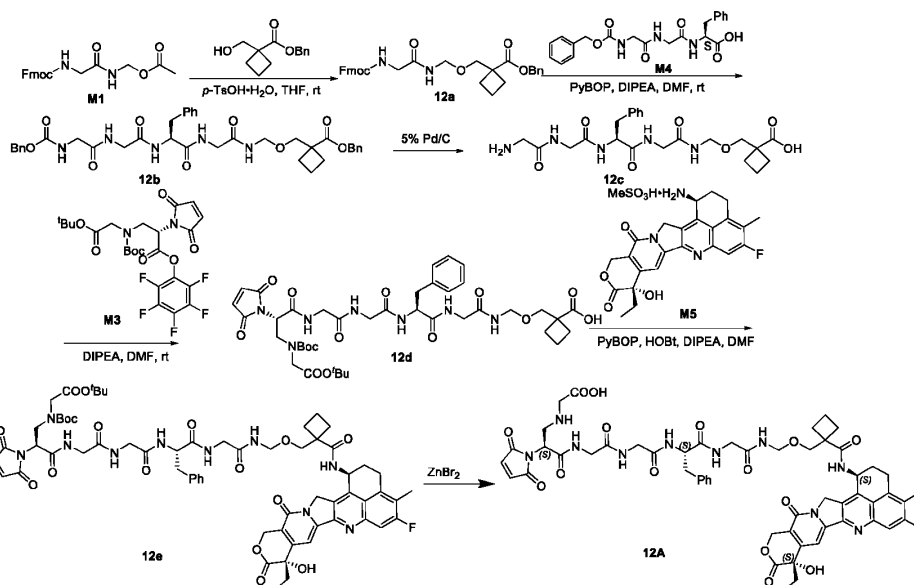


Referring to the synthetic route of Example 18, compound **11B** (50 mg) was obtained. LC-MS: $[M+H]^+ = 1105.4$.

30

Example 20

Synthesis of compound **12A**:



Step 1: Compound 12a

In a 250 mL flask, add **M1** (6 g, 16.3 mmol), 100 mL THF, p-toluenesulfonic acid monohydrate (0.31 g, 1.63 mmol), stirring and cooled to 0 °C, dropwise add benzyl 3-hydroxy-2-cyclobutylpropionate (prepared according to the method published in WO2009011285A1) (7.2 g, 32.6 mmol). The reaction was allowed to warm up to room temperature (about 2-4 h) and monitored by TLC. At the end of the reaction, saturated NaHCO₃ solution was added, extracted with ethyl acetate, washed with saturated sodium chloride solution, dried with anhydrous sodium sulfate, filtered, concentrated, and the residue was purified by silica gel column (PE:EA=10:1-5:1-2:1) to give **12a** (4.5 g) in 52% yield; LC-MS: [M+H]⁺=529.4.

Step 2: Compound 12b

In a 25 mL flask, add **12a** (4 g, 7.6 mmol), 10 mL DMF, stir at 0 °C, add DBU (1.2 g, 8.0 mmol), react for 1 h. After the completion of Fmoc deprotection was monitored by TLC, the reaction was set aside;

Add **M4** (3.2 g, 7.6 mmol), PyBOP (4.7 g, 9.0 mmol), HOBT (1.22 g, 9.0 mmol) and 10 mL of DMF in another 25 mL flask, and add DIPEA (1.49 mL, 0.9 mmol) under ice-water bath, and continue stirring for 30 min, then add the above reaction solution to the reaction flask and raise the temperature to room temperature. After the reaction was monitored by HPLC, the reaction solution was purified by preparative liquid phase to obtain the product preparation solution, which was extracted by dichloromethane, washed with saturated sodium chloride solution, dried with anhydrous sodium sulfate, filtered, and the filtrate was concentrated under reduced pressure to obtain a solid of 2.0 g in 37% yield; LC-MS: [M+H]⁺=702.8.

Step 3: Compound 12c

12b (1.0 g, 1.43 mmol) was added to a 25 mL flask, and after 15 mL of DMF was dissolved and cleared, 1.0 g of 5% Pd/C was added, and the hydrogenation reaction was carried out for 1.5

h. After completion of the reaction, it was filtered to obtain the filtrate, which was used directly for the next step of the reaction without purification.

Step 4: Compound **12d**

The crude product **12c** was placed in an ice-water bath, DIPEA (258 μ L, 1.5 mmol) was added, and compound M3 (825 mg, 1.43 mmol) was added, and the reaction was brought to room temperature for 1 h. The completion of the reaction was monitored by HPLC, and the reaction solution was purified by high-performance liquid phase purification to obtain the preparative solution, which was lyophilized to obtain **12d** (522 mg); LC-MS: $[M-H]^- = 856.4$.

Step 5: Compound **12e**

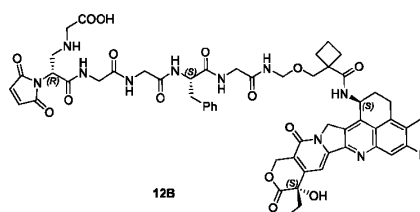
10 **12d** (400 mg, 0.47 mmol), **M5** (240 mg, 0.47 mmol), PyBOP (250 mg, 0.47 mmol), HOBt (101 mg, 0.47 mmol) and 15 mL of DMF were added to a 50 mL flask in an ice-water bath and DIPEA (330 μ L, 2.0 mmol) was added, and the reaction was allowed to proceed for 3 h. After the reaction was monitored by HPLC, the reaction solution was purified by HPLC to give the preparative solution of compound **12e**. The reaction was carried out at room temperature for 3 h. After the completion of the reaction was monitored by HPLC, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution of compound **12e**, which was lyophilized to obtain **12e** (198 mg); LC-MS: $[M+H]^+ = 1275.4$.

Step 6: Compound **12A**

20 **12e** (100 mg, 0.08 mmol), zinc bromide (360 mg, 1.6 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain the solid compound **12A** (55 mg); LC-MS: $[M+H]^+ = 1119.4$.

Example 21

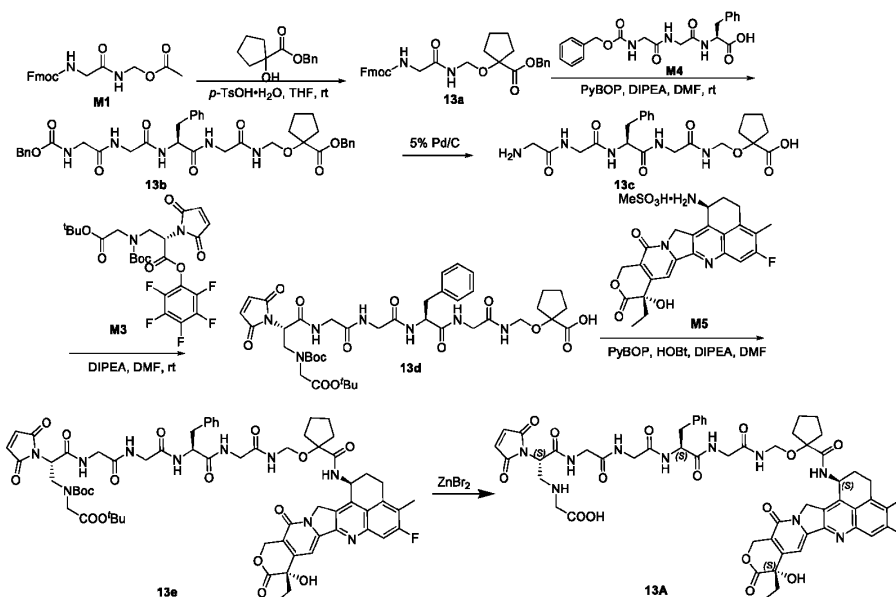
Synthesis of compound **12B**:



Referring to the synthetic route of Example 20, compound **12B** (50 mg) was obtained; LC-MS: $[M+H]^+ = 1119.4$.

30 Example 22

Synthesis of compound **13A**:



Step 1: Compound 13a

In a 250 mL flask, add **M1** (6 g, 16.3 mmol), 100 mL THF, p-toluenesulfonic acid monohydrate (0.31 g, 1.63 mmol), stir and cool to 0 °C, dropwise addition of benzyl 2-hydroxy-2-cyclopentylacetate (see *Journal of Medicinal Chemistry*, 2013, 56(13), 5541-5552. For the method of synthesis) (7.2 g, 32.6 mmol), the reaction was naturally warmed up to room temperature after dropwise (the reaction was carried out for about 2-4 h) and monitored by TLC. At the end of the reaction, saturated NaHCO₃ solution was added, extracted with ethyl acetate, washed with saturated sodium chloride solution, dried with anhydrous sodium sulfate, filtered, concentrated, and the residue was purified by silica gel column (PE:EA=10:1-5:1-2:1) to give **13a** (4.6 g) in 53% yield; LC-MS: [M+H]⁺=529.5.

Step 2: Compound 13b

In a 25 mL flask, add **13a** (4 g, 7.6 mmol), 10 mL DMF, stir at 0 °C, add DBU (1.17 g, 7.8 mmol), and react for 1 h. After the completion of Fmoc deprotection was monitored by TLC, the reaction was set aside;

Add **M4** (3.14 g, 7.6 mmol), PyBOP (4.42 g, 8.5 mmol), HOBt (1.15 g, 8.5 mmol) and 10 mL of DMF into another 25 mL flask, and add DIPEA (1.39 mL, 0.85 mmol) in an ice-water bath with continued stirring for 30 min, and then add the above reaction solution to the flask. After the reaction was monitored by HPLC, the reaction solution was purified by preparative liquid phase to obtain the product preparation solution, which was extracted by dichloromethane, washed with saturated sodium chloride solution, dried with anhydrous sodium sulfate, filtered, and the filtrate was concentrated under reduced pressure to obtain a solid of 2.1 g in 39% yield; LC-MS: [M+H]⁺=702.8.

Step 3: Compound 13c

13b (1.5 g, 1.87 mmol) was added to a 25 mL flask, and after 25 mL of DMF was dissolved

and cleared, 1.5 g of 5% Pd/C was added, and the hydrogenation reaction was carried out for 3 h. After completion of the reaction, it was filtered, and the filtrate was obtained, and was used directly for the next step of the reaction without purification.

Step 4: Compound **13d**

The crude product **13c** was placed in an ice-water bath, DIPEA (333 μ L, 1.93 mmol) was added, and compound **M3** (1.1 g, 1.87 mmol) was added, and the reaction was brought to room temperature for 1 h. The completion of the reaction was monitored by HPLC, and the reaction solution was subjected to high-performance liquid-phase purification to obtain the preparative solution, which was lyophilized to give **13d** (519 mg); LC-MS: $[M-H]^- = 856.6$.

10 Step 5: Compound **13e**

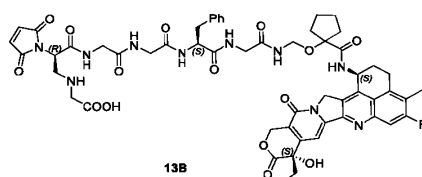
13d (400 mg, 0.47 mmol), **M5** (240 mg, 0.48 mmol), PyBOP (250 mg, 0.48 mmol), HOBt (103 mg, 48 mmol) and 15 mL of DMF were added to a 50 mL flask in an ice-water bath and DIPEA (330 μ L, 2.0 mmol) was added, and the reaction was carried out for 4 h. After HPLC monitoring, the reaction solution was purified by HPLC to obtain the preparative solution of compound **13e**, and the preparative solution was lyophilized to obtain **13e** (187 mg); LC-LLC was performed to monitor the reaction completion. The reaction was carried out for 4 h. After the completion of the reaction was monitored by HPLC, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution of compound **13e**, which was lyophilized to obtain **13e** (187 mg); LC-MS: $[M+H]^+ = 1275.5$.

20 Step 6: Compound **13A**

13e (100 mg, 0.08 mmol), zinc bromide (355 mg, 0.16 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 $^{\circ}$ C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain the solid compound **13A** (60 mg); LC-MS: $[M+H]^+ = 1119.6$.

Example 23

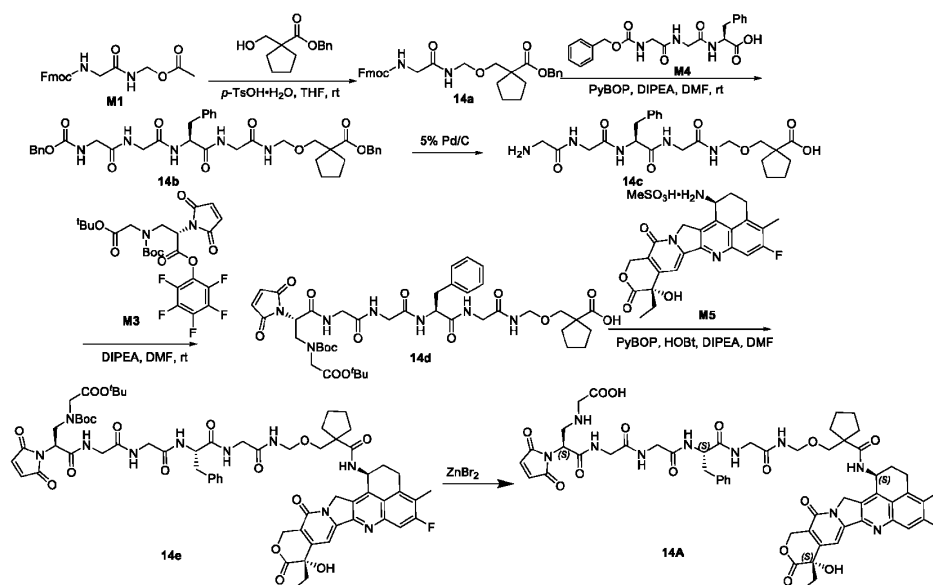
Synthesis of compound **13B**:



30 Referring to the synthetic route of Example 22, compound **13B** (51 mg) was obtained; LC-MS: $[M+H]^+ = 1119.6$.

Example 24

Synthesis of compound **14A**:



Step 1: Compound 14a

In a 250 mL flask, add **M1** (6 g, 16.3 mmol), 100 mL THF, p-toluenesulfonic acid monohydrate (0.31 g, 1.63 mmol), stirring and cooling to 0 °C, dropwise add benzyl 3-hydroxy-2-cyclopentylpropionate (synthesized by reference to the method published in WO2009011285A1) (7.6 g, 32.6 mmol). The reaction was carried out at room temperature (about 2–4 h) after dropwise natural warming and monitored by TLC. At the end of the reaction, saturated NaHCO₃ solution was added, extracted with ethyl acetate, washed with saturated sodium chloride solution, dried with anhydrous sodium sulfate, filtered, concentrated, and the residue was purified by silica gel column (PE:EA=10:1-5:1-2:1) to give **14a** (4.4 g) in 49% yield; LC-MS: [M+H]⁺ = 543.6.

Step 2: Compound 14b

In a 25 mL flask, add **14a** (4 g, 7.4 mmol), 10 mL DMF, stir at 0 °C, add DBU (1.2 g, 8.0 mmol), react for 1 h. After the completion of Fmoc deprotection was monitored by TLC, the reaction was set aside;

Add **M4** (3.1 g, 7.4 mmol), PyBOP (4.6 g, 8.8 mmol), HOBT (1.19 g, 8.8 mmol) and 10 mL of DMF in another 25 mL flask, and add DIPEA (1.49 mL, 9.0 mmol) under ice-water bath, and continue stirring for 30 min, then add the above reaction solution to the reaction flask and raise the temperature to room temperature. After the reaction was monitored by HPLC, the reaction solution was purified by preparative liquid phase to obtain the product preparation solution, which was extracted by dichloromethane, washed with saturated sodium chloride solution, dried with anhydrous sodium sulfate, filtered, and the filtrate was concentrated under reduced pressure to obtain a solid of 2.6 g in 49% yield; LC-MS: [M+H]⁺ = 716.4.

Step 3: Compound 14c

In a 25 mL flask, **14b** (1.0 g, 1.4 mmol) was added, and after 15 mL of DMF was dissolved and cleared, 1.0 g of 5% Pd/C was added, and the hydrogenation reaction was carried out for 1.5

h. After completion of the reaction, it was filtered, and the filtrate was obtained, and was used directly for the next step of the reaction without purification.

Step 4: Compound **14d**

The crude product **14c** was placed in an ice-water bath, DIPEA (248 μ L, 1.5 mmol) was added, then compound **M3** (808 mg, 1.4 mmol) was added, and the reaction was brought to room temperature for 1 h. The reaction was monitored for completion by HPLC, and the reaction solution was purified by high-performance liquid phase purification to obtain the preparative solution, which was lyophilized to obtain **14d** (500 mg); LC-MS: $[M-H]^- = 870.5$.

Step 5: Compound **14e**

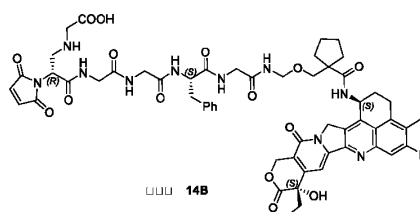
10 **14d** (400 mg, 0.46 mmol), **M5** (235 mg, 0.46 mmol), PyBOP (245 mg, 0.46 mmol), HOBt (99 mg, 0.46 mmol) and 15 mL of DMF were added to a 50 mL flask in an ice-water bath and DIPEA (331 μ L, 2.0 mmol) was added, and the reaction was allowed to proceed for 3 h. After the reaction was monitored by HPLC, the reaction solution was purified by HPLC to give the preparative solution of compound **14e**. The reaction was carried out at room temperature for 3 h. After the completion of the reaction was monitored by HPLC, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution of compound **14e**, which was lyophilized to obtain **14e** (146 mg); LC-MS: $[M+H]^+ = 1289.5$.

Step 6: Compound **14A**

20 **14e** (100 mg, 0.08 mmol), zinc bromide (360 mg, 1.6 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain the solid compound **14A** (52 mg); LC-MS: $[M+H]^+ = 1133.4$.

Example 25

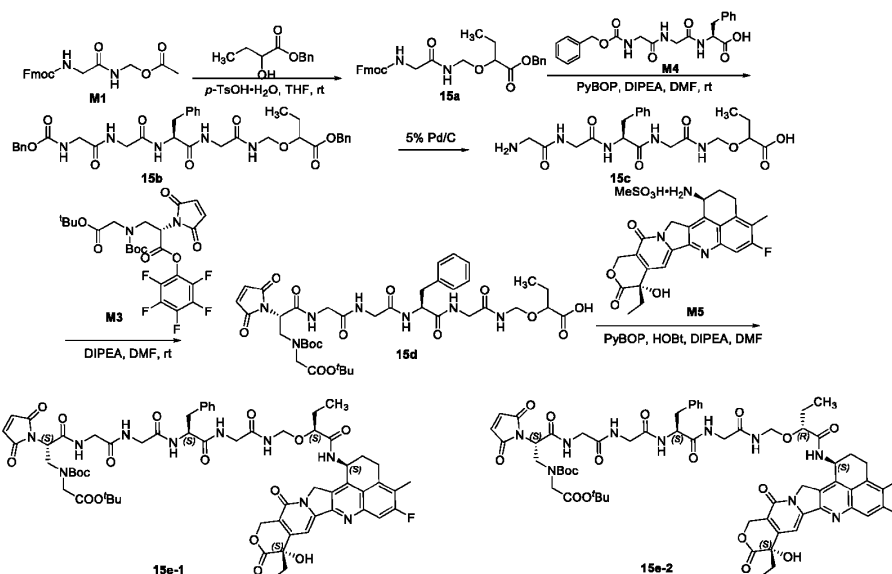
Synthesis of compound **14B**:



Referring to the synthetic route of Example 24, compound **14B** (48 mg) was obtained; LC-MS: $[M+H]^+ = 1133.4$.

30 Example 26

Synthesis of compounds **15A** and **15B**:



Step 1: Compound **15a**

In a 250 mL flask, **M1** (10 g, 27.1 mmol), benzyl 2-hydroxybutyrate (prepared by the method published in *Chemical Communications*, 2019, 55(53), 7699-7702) (10.5 g, 54.3 mmol), zinc acetate (9.96 g, 54.3 mmol) and 100 mL of toluene were added, heated to 100 °C reaction for 4 h. After the reaction was completed, reduced to room temperature, filtration to remove insoluble material, the filtrate was concentrated to obtain the crude product. The crude product was purified by silica gel column chromatography (PE:EA=10:1-5:1-2:1) to obtain 5.67 g of the target material in 42% yield; LC-MS: $[M+H]^+ = 503.5$.

10 Step 2: Compound **15b**

Add **15a** (5 g, 9.95 mmol) and 15 mL of DMF in a 50 mL flask, dissolve the solution, and then add DBU (1.68 g, 11 mmol) in an ice-water bath and react for 1 h. The reaction was recorded as reaction solution (1);

Another 50 mL flask was taken, **M4** (4.1 g, 10.0 mmol), PyBOP (5.75 g, 11 mmol), HOBT (1.49 g, 11 mmol) and 10 mL of DMF were added, and after dissolved, DIPEA (1.82 mL, 11 mmol) was added in an ice-water bath, and the reaction was continued for 40 min, then reaction solution (1) was added and the reaction was brought to room temperature for 2 h. The reaction was monitored by HPLC. The reaction was monitored by HPLC for 2 h. After completion of the reaction, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution. The prepared solution was extracted with dichloromethane, washed with saturated sodium chloride solution, dried with anhydrous sodium sulfate, filtered, and concentrated to obtain 4.6 g of solid, with a yield of 68%; LC-MS: $[M+H]^+ = 676.7$.

20 Step 3: Compound **15d**

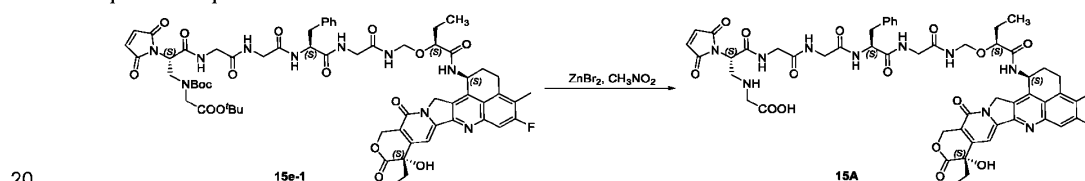
Add **15b** (2.0 g, 2.96 mmol) in a 25 mL flask, 15 mL of DMF was dissolved, then 2.0 g of 5% Pd/C was added, and the hydrogenation reaction was carried out for 2 h. After completion of the

reaction, filtration was carried out, and the filtrate was placed in an ice-water bath, and DIPEA (496 μ L, 3.0 mmol) was added, followed by **M3** (1.7 g, 2.96 mmol), and then the reaction was brought to room temperature after completion of the reaction. The reaction was monitored by HPLC for 1 h. The reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution, which was lyophilized to give 1120.0 mg of the product in 45% yield; LC-MS: $[M-H]^- = 830.3$.

Step 4: Compound **15e**

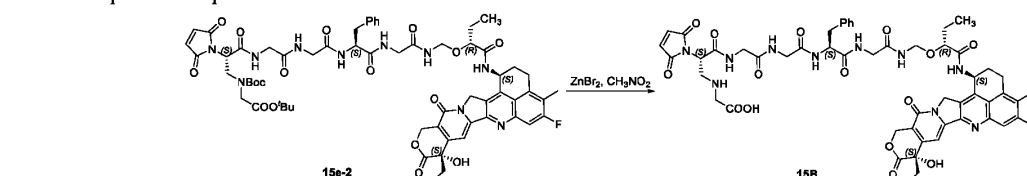
15d (500 mg, 0.60 mmol), **M5** (321 mg, 0.60 mmol), PyBOP (469 mg, 0.90 mmol), HOBt (121 mg, 0.90 mmol) and 15 mL of DMF were added to a 50 mL flask, DIPEA (446 μ L, 2.7 mmol) was added to the vial in an ice-water bath, and the reaction was carried out at room temperature for 2 h. After the reaction was monitored by HPLC, the reaction solution was purified by HPLC to obtain the preparative solution of compound **15e-1** and compound **15e-2**, and the preparative solution was lyophilized to obtain 138 mg of compound **15e-2** respectively. The reaction was carried out at room temperature for 2 h. After HPLC monitoring, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solutions of compound **15e-1** and compound **15e-2**, which were lyophilized to obtain 138 mg of compound **15e-1**, LC-MS: $[M+H]^+ = 1249.5$, and 140 mg of compound **15e-2**, LC-MS: $[M+H]^+ = 1249.5$, respectively.

Step 5: Compound **15A**



15e-1 (100 mg, 0.08 mmol), zinc bromide (360 mg, 1.6 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain a solid of 59 mg; LC-MS: $[M+H]^+ = 1093.4$.

Step 6: Compound **15B**

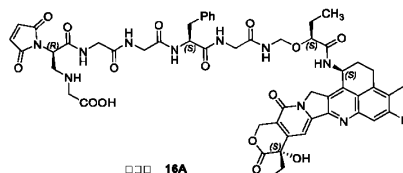


15e-2 (100 mg, 0.08 mmol), zinc bromide (360 mg, 1.6 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance

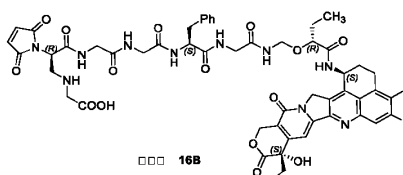
liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 60 mg of solid; LC-MS: $[M+H]^+ = 1093.4$.

Example 27

Synthesis of compounds **16A** and **16B**:



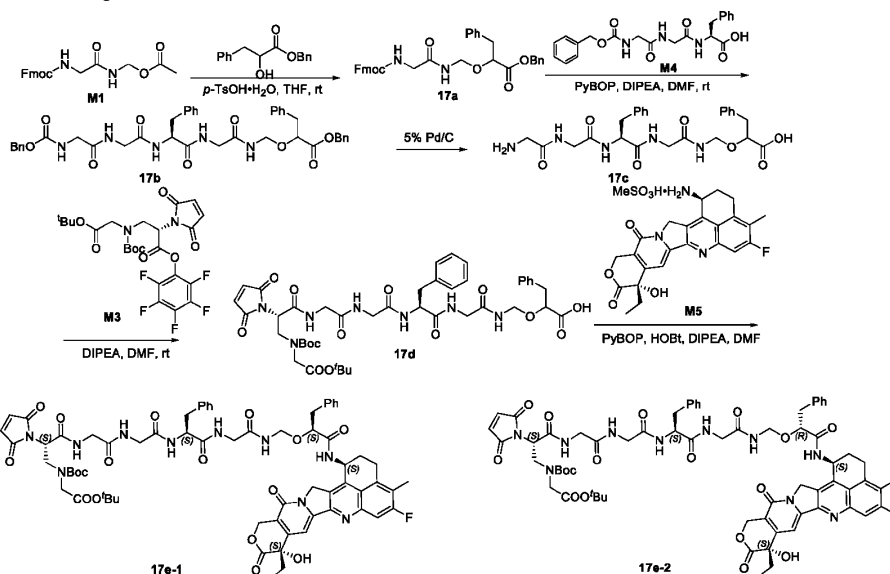
Referring to the synthetic route of Example 26, compound **16A** (55 mg) was obtained; LC-MS: $[M+H]^+ = 1093.4$.



Referring to the synthetic route of Example 26, compound **16B** (54 mg) was obtained; LC-MS: $[M+H]^+ = 1093.4$.

Example 28

Synthesis of compounds **17A** and **17B**:



Step 1: Compound **17a**

Add **M1** (10 g, 27.1 mmol), benzyl 2-hydroxy-phenylpropionate (synthesized by the method published in *Nature Communications*, 2020. 11(1), 56 (14.7 g, 54.3 mmol), zinc acetate (9.96 g, 54.3 mmol) and 100 mL of toluene to a 250 mL flask. Toluene was heated to 100 °C for 4 h. After

the reaction was completed, it was lowered to room temperature, filtered to remove insoluble material, and the filtrate was concentrated to obtain the crude product. The crude product was purified by silica gel column chromatography (PE:EA=10:1-5:1-2:1) to obtain 6.13 g of the target material in 40% yield; LC-MS: $[M+H]^+ = 565.6$.

Step 2: Compound **17b**

In a 50 mL flask, **17a** (5 g, 8.86 mmol) and 15 mL of DMF were added, dissolved and cleared, and then, in an ice-water bath, DBU (1.53 g, 10 mmol) was added and the reaction was carried out for 1 h, which was recorded as reaction solution ①;

Another 50 mL flask was taken, **M4** (3.6 g, 8.86 mmol), PyBOP (5.23 g, 10 mmol), HOBt (1.36 g, 10 mmol) and 10 mL of DMF were added, and after dissolved, DIPEA (1.65 mL, 10 mmol) was added in an ice-water bath, and the reaction was continued for 30 min, then reaction solution ① was added, and the reaction was brought to room temperature for 2 h. The reaction was monitored by HPLC. The reaction was monitored by HPLC for 2 h. After completion of the reaction, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution. The prepared solution was extracted with dichloromethane, washed with saturated sodium chloride solution, dried with anhydrous sodium sulfate, filtered, and concentrated to obtain 5.0 g of solid in 77% yield; LC-MS: $[M+H]^+ = 738.3$.

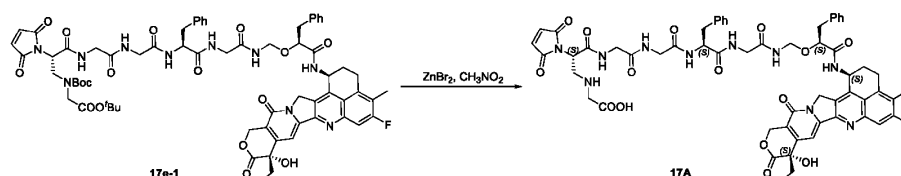
Step 3: Compound **17d**

Add **17b** (3.0 g, 4.07 mmol) in a 25 mL flask, after 15 mL of DMF was dissolved, 3.0 g of 5% Pd/C was added, and the reaction was completed for 2 h. The reaction was completed by filtration, and the filtrate was placed in an ice-water bath, and DIPEA (744 μ L, 4.5 mmol) was added, followed by **M3** (2.34 g, 4.07 mmol), and the reaction was brought to room temperature after addition. The reaction was monitored by HPLC for 1 h. The reaction solution was purified by HPLC to obtain the preparative solution, which was lyophilized to give 1.2 g of product in 33% yield; LC-MS: $[M-H]^- = 892.4$.

Step 4: Compound **17e**

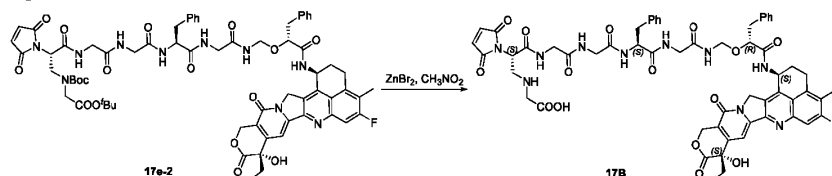
17d (500 mg, 0.56 mmol), **M5** (300 mg, 0.56 mmol), PyBOP (438 mg, 0.84 mmol), HOBt (113 mg, 0.84 mmol) and 15 mL of DMF were added to a 50 mL flask, and DIPEA (330 μ L, 2.0 mmol) was added to the flask under an ice-water bath, and the reaction was carried out at room temperature for 2 h. After the reaction was monitored by HPLC, the reaction solution was purified by HPLC to obtain the preparative solution of compound **17e-1** and compound **17e-2**, and the preparative solution was lyophilized to obtain 156 mg of compound **17e-2** respectively. The reaction was carried out at room temperature for 2 h. After HPLC monitoring, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solutions of compound **17e-1** and compound **17e-2**, which were lyophilized to obtain 156 mg of compound **17e-1**, LC-MS: $[M+H]^+ = 1311.4$, and 150 mg of compound **17e-2**, LC-MS: $[M+H]^+ = 1311.7$, respectively.

Step 5: Compound **17A**



17e-1 (100 mg, 0.08 mmol), zinc bromide (360 mg, 1.6 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 43 mg of solid; LC-MS: $[M+H]^+ = 1155.4$.

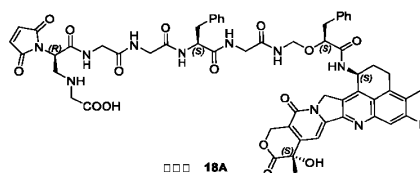
Step 6: Compound **17B**



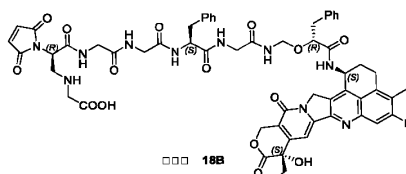
17e-2 (100 mg, 0.08 mmol), zinc bromide (360 mg, 1.6 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 40 mg of solid; LC-MS: $[M+H]^+ = 1155.4$.

Example 29

Synthesis of compounds **18A** and **18B**:



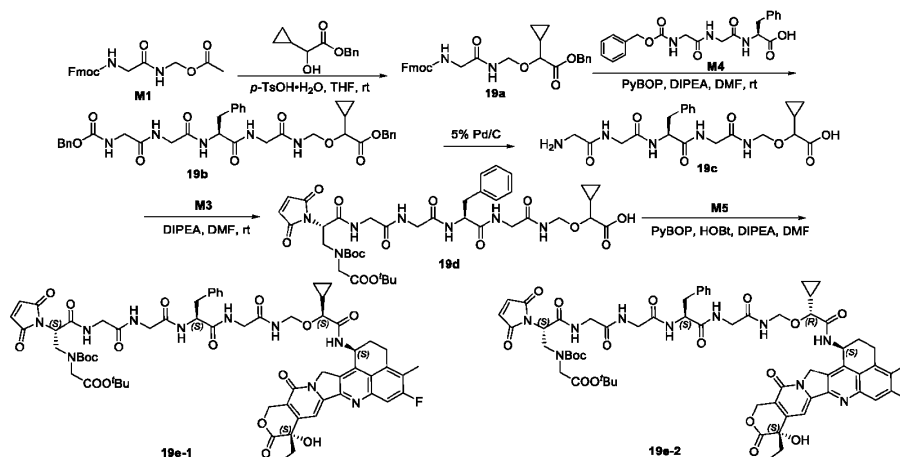
Referring to the synthetic route of Example 28, compound **18A** (54 mg) was obtained; LC-MS: $[M+H]^+ = 1155.4$.



Referring to the synthetic route of Example 28, compound **18B** (55 mg) was obtained; LC-MS: $[M+H]^+ = 1155.4$.

Example 30

Synthesis of compounds **19A** and **19B**:



Step 1: Compound **19a**

Add **M1** (10 g, 27.1 mmol), benzyl 2-cyclopropyl-2-hydroxyacetate (prepared according to the method published in patent WO2020244657A1) (11.2 g, 54.3 mmol), zinc acetate (9.96 g, 54.3 mmol) and 100 mL of toluene in a 250 mL flask and heat the reaction to 100 °C for 4 h. The reaction was completed. The reaction was completed, reduced to room temperature, filtered to remove insoluble material, and the filtrate was concentrated to obtain the crude product. The crude product was purified by silica gel column chromatography (PE:EA=10:1-5:1-2:1) to obtain 4.97 g of the target material with 36% yield; LC-MS: $[M+H]^+ = 515.2$.

Step 2: Compound **19b**

Add **19a** (4 g, 7.8 mmol) and 10 mL of DMF in a 50 mL flask, dissolve the solution, and then add DBU (1.42 g, 9.3 mmol) in an ice-water bath and react for 1 h. The reaction was recorded as reaction solution ①;

Another 50 mL single-necked vial was taken, and **M4** (3.2 g, 7.8 mmol), PyBOP (4.5 g, 8.6 mmol), HOBT (1.16 g, 8.6 mmol) and 10 mL of DMF were added, and after dissolved, DIPEA (1.65 mL, 10 mmol) was added in an ice-water bath, and the reaction was continued for 30 min, then reaction solution ① was added, and the reaction was brought to room temperature for 2 h. The reaction process was monitored by HPLC, and the preparation was purified by HPLC. The reaction was monitored by HPLC for 2 h. After completion of the reaction, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution. The prepared solution was extracted with dichloromethane, washed with saturated sodium chloride solution, dried with anhydrous sodium sulfate, filtered, and concentrated to obtain 4.2 g of solid, with a yield of 78%; LC-MS: $[M+H]^+ = 688.3$.

Step 3: Compound **19d**

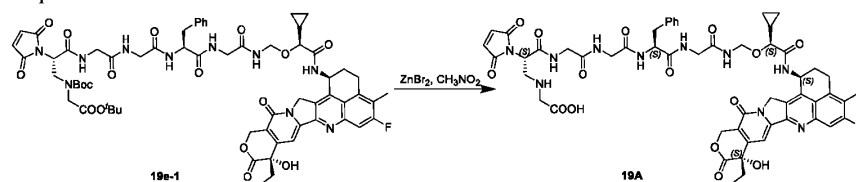
In a 25 mL flask, **19b** (1000 mg, 1.45 mmol) was added, 15 mL of DMF was dissolved, then 1000 mg of 5% Pd/C was added, and the reaction was hydrogenated for 2 h. The reaction was completed, filtered, and the filtrate was placed in an ice-water bath, and DIPEA (248 μ L, 1.5 mmol) was added, followed by **M3** (720 mg, 1.45 mmol), and then the reaction was brought to room

temperature for 1 h. The reaction was monitored by HPLC, and the reaction solution was purified by HPLC to obtain the preparative solution, and the preparative solution was lyophilized to obtain 503 mg of product in 41% yield. The reaction was monitored by HPLC, and the reaction solution was purified by HPLC to obtain the preparative solution, which was lyophilized to obtain 503 mg of the product in 41% yield; LC-MS: $[M-H]^- = 842.3$.

Step 4: Compounds **19e-1** and **19e-2**

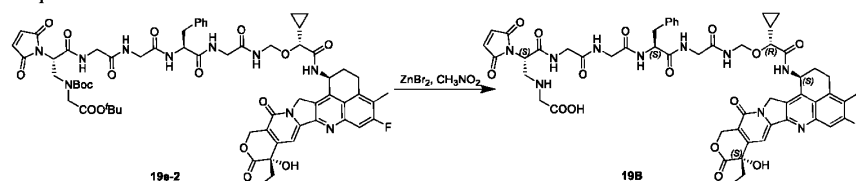
19d (500 mg, 0.59 mmol), **M5** (317 mg, 0.59 mmol), PyBOP (339 mg, 0.65 mmol), HOBT (88 mg, 0.86 mmol) and 10 mL of DMF were added to a 50 mL flask, and DIPEA (292 μ L, 1.77 mmol) was added to the flask under an ice-water bath, and the reaction was carried out at room temperature for 2 h. After the reaction was monitored by HPLC, the reaction solution was purified by HPLC to obtain the preparative solution of compound **19e-1** and compound **19e-2**, and the preparative solution was lyophilized to obtain 112 mM of compound **19e-2**, respectively. The reaction was carried out at room temperature for 2 h. After HPLC monitoring, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solutions of compound **19e-1** and compound **19e-2**, which were lyophilized to obtain 112 mg of compound **19e-1**, LC-MS: $[M+H]^+ = 1261.5$, and 131 mg of compound **19e-2**, LC-MS: $[M+H]^+ = 1261.5$, respectively.

Step 5: Compound **19A**



19e-1 (100 mg, 0.079 mmol), zinc bromide (357 mg, 1.59 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the completion of the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure and removed to obtain the crude product. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 55 mg of solid; LC-MS: $[M+H]^+ = 1105.4$.

Step 6: Compound **19B**

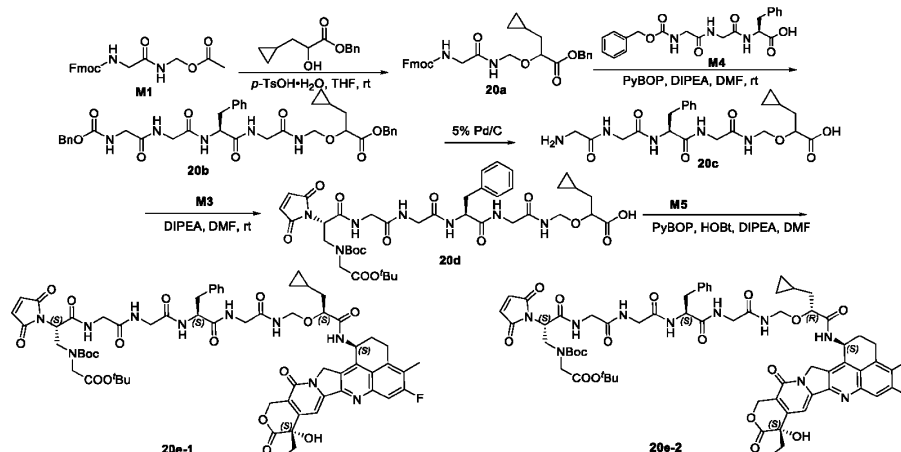


19e-2 (100 mg, 0.079 mmol), zinc bromide (357 mg, 1.59 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was completed by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation

solution was lyophilized to obtain a solid 58 mg; LC-MS: $[M+H]^+ = 1105.4$.

Example 31

Synthesis of compounds **20A** and **20B**:



Step 1: Compound **20a**

Add **M1** (10 g, 27.1 mmol), benzyl 2-hydroxy-cyclopropylpropionate (synthesized by the method published in WO2020063676A) (12.0 g, 54.3 mmol), zinc acetate (9.96 g, 54.3 mmol) and 100 mL of toluene to a 250 mL flask, and react at 100 °C for 4 h. When the reaction was completed, reduce to room temperature. The reaction was completed, lowered to room temperature, filtered to remove insoluble material, and the filtrate was concentrated to obtain the crude product. The crude product was purified by silica gel column chromatography (PE:EA=10:1-5:1-2:1) to obtain 5.09 g of the target material; LC-MS: $[M+H]^+ = 529.2$.

Step 2: Compound **20b**

20a (4 g, 7.6 mmol) and 10 mL of DMF were added to a 50 mL flask, and after dissolving and clearing, DBU (1.39 g, 9.1 mmol) was added in an ice-water bath and the reaction was carried out for 1 h, which was recorded as reaction solution (1);

Another 50 mL flask was taken, and **M4** (3.12 g, 7.6 mmol), PyBOP (4.5 g, 8.6 mmol), HOBT (1.16 g, 8.6 mmol) and 10 mL of DMF were added, and after dissolved, DIPEA (1.65 mL, 10 mmol) was added in an ice-water bath and the reaction was continued for 30 min, and then the reaction solution (1) was added to the reaction solution and the reaction was brought to room temperature for 2 h. HPLC was performed to monitor the reaction progress, and the reaction solution was purified by high performance liquid phase purification to obtain the preparative solution. The reaction was monitored by HPLC, and the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution. The prepared solution was extracted with dichloromethane, washed with saturated sodium chloride solution, dried with anhydrous sodium sulfate, filtered, and concentrated to obtain 4.5 g of solid, with a yield of 84%; LC-MS: $[M+H]^+ = 702.3$.

Step 3: Compounds **20d**

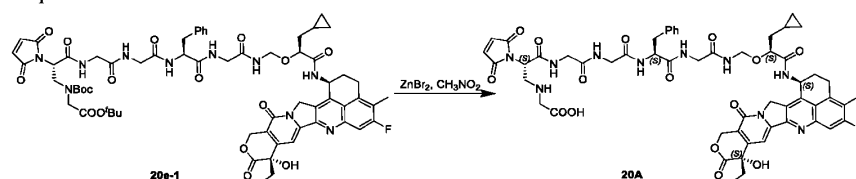
Add **20b** (1000 mg, 1.42 mmol) in a 25 mL flask, 15 mL of DMF was dissolved, then 1000

mg of 5% Pd/C was added, and hydrogenation reaction was carried out for 2 h. After the reaction was completed, filtration was performed, and the filtrate was placed in an ice-water bath, and then DIPEA (248 μ L, 1.5 mmol) was added, followed by **M5** (708 mg, 1.42 mmol), and the reaction was brought to room temperature for 1 h. The reaction was monitored by HPLC, and the reaction solution was purified by HPLC to obtain the preparative solution, and the preparative solution was lyophilized to obtain 443 mg of product in 36% yield. The reaction was monitored by HPLC and the reaction solution was purified by HPLC to obtain the preparative solution, which was lyophilized to obtain 443 mg of product in 36% yield; LC-MS: $[M-H]^- = 856.4$.

Step 4: Compounds **20e-1** and **20e-2**

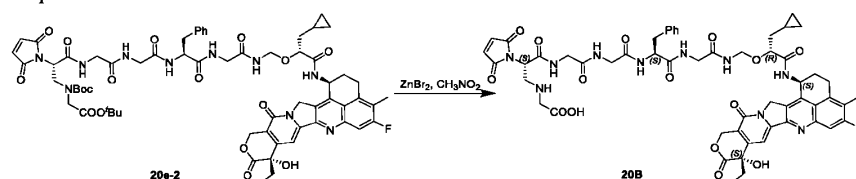
10 **20d** (400 mg, 0.47 mmol), ezetimibe methanesulfonate (250 mg, 0.47 mmol), PyBOP (223 mg, 0.56 mmol), HOBt (83 mg, 0.56 mmol), and 10 mL of DMF were added to a 50 mL vial, and DIPEA (248 μ L, 1.5 mmol) was added to the vial under an ice-water bath. DIPEA (248 μ L, 1.5 mmol) was added in an ice-water bath, and the reaction was brought to room temperature for 2 h. After the reaction was monitored by HPLC, the reaction solution was subjected to high-performance liquid-phase purification to obtain the preparative solutions of compound **20e-1** and compound **20e-2**, which were lyophilized to obtain 103 mg of compound **20e-1**, LC-MS: $[M+H]^+ = 1275.5$, and 103 mg of compound **20e-2**, LC-MS: $[M+H]^+ = 1275.5$.

Step 5: Compound **20A**



20 In a 25 mL flask, **8A** (100 mg, 0.078 mmol), zinc bromide (352 mg, 1.57 mmol) and 5 mL of nitromethane were added, and the reaction was carried out at 40 °C for 1 h. After the completion of the reaction was detected by HPLC, the solvent was concentrated under reduced pressure and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 51 mg of solid; LC-MS: $[M+H]^+ = 1119.4$.

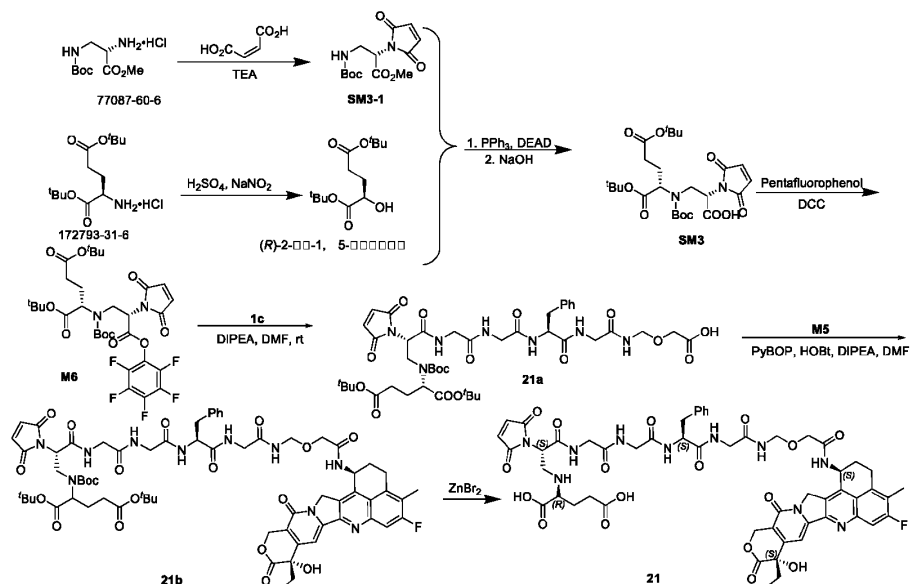
Step 6: Compound **20B**



30 **20e-2** (100 mg, 0.079 mmol), zinc bromide (357 mg, 1.59 mmol) and 5 mL of nitromethane were added to a 25 mL single-necked vial and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain a solid of 47 mg; LC-MS: $[M+H]^+ = 1119.4$.

Example 32

Synthesis of compound **21**:



Step 1: Compound **SM3-1**

Add 77087-60-6 (100 g, 458 mmol), maleic acid (53.4 g, 460 mmol), TEA (64 mL, 460 mmol) and 1000 mL toluene in a 2000 mL flask and heat to 100 °C for 5 h. After the reaction was completed, the reaction was brought down to room temperature, insoluble material was removed by filtration and the filtrate was concentrated to obtain the crude product. The crude product was purified by silica gel column chromatography (PE:EA=100:1-50:1-20:1) to obtain 75.6 g of the target; LC-MS: $[M+H]^+ = 299.1$.

Step 2: Compound (*R*)-tert-butyl 2-hydroxy-1, 5-pentanedioate

Add 172793-31-6 (100 g, 338 mmol) and 1000 mL water in 2000 mL flask, then add sodium nitrite (35 g, 507 mmol) and concentrated sulfuric acid (32 mL, 35 mmol), and slowly raise the temperature to room temperature for 24 h. After the reaction was completed, the product was extracted with 500 mL of ethyl acetate for three times, and the organic phase was dried with anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to remove the solvent. The organic phase was dried with anhydrous sodium sulfate, filtered, concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by silica gel column chromatography (PE:EA=50:1-30:1-2:1) to obtain 91.2 g of the target material; LC-MS: $[M+H]^+ = 261.4$.

Step 3: Compound **SM3**

Add (*R*)-tert-butyl 2-hydroxy-1,5-glutarate (50 g, 192 mmol) and 1000 mL of anhydrous tetrahydrofuran into a 2000 mL flask, cool down the temperature to 0 °C, then add PPh₃ (87.7 g, 288 mmol), DEAD (50.2 g, 288 mmol), and **SM3-1** (57.3, 192 mmol) in sequence. The reaction was slowly warmed up to room temperature for 13 h. After completion of the reaction, the

insoluble material was removed by filtration and the filtrate was concentrated to obtain the crude product. The crude product was purified by silica gel column chromatography (PE:EA=50:1-30:1-1:1) to obtain 68.6 g of product;

The above product was dissolved in 500 mL of methanol, cooled to 0 °C in an ice-water bath, NaOH (64 mL, 190 mmol, 3M/L) was added dropwise at this temperature, and the reaction was maintained at this temperature for 12 h. After the reaction, the pH was adjusted to 3 by the addition of HCl (6 M/L), dichloromethane was extracted for five times with 500 mL of dichloromethane, and the mixture was dried with anhydrous sodium sulfate, filtered, the filtrate was concentrated under reduced pressure, and the crude product obtained was purified by column chromatography (DCM/MeOH=50/1-20/1-2/1) purification, obtained **SM3** 50.4 g; LC-MS: [M-H]⁻ =525.5.

Step 4: Compound **M6**

In a 2000 mL flask, compound **SM3** (50 g, 95 mmol, 1.0 eq), pentafluorophenol (19.2 g, 104.5 mmol, 1.1 eq), DCC (21.5 g, 104.5 mmol, 1.1 eq) and THF (600 mL) were added, and the reaction was carried out at room temperature for 1 h (monitored by TLC), and insoluble material was filtered off. The reaction solution was purified directly by preparation, and the preparative solution was concentrated by pumping water under reduced pressure at 35 °C to remove acetonitrile, and lyophilized to obtain compound **M6** (51.9 g) in 79% yield; LC-MS: [M+H]⁺ =693.3.

Step 5: Compound **21a**

In a 25 mL flask, **1c** (1 g, 2.36 mmol) was added, 25 mL DMF was dissolved, then DIPEA (430 uL, 2.6 mmol) was added, then **M6** (1177 mg, 2.36 mmol) was added, and then the reaction was brought to room temperature for 1 h. The completion of the reaction was monitored by HPLC, and the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution, which was lyophilized to yield the following product 555 mg; LC-MS: [M-H]⁻ =931.0.

Step 6: Compound **21b**

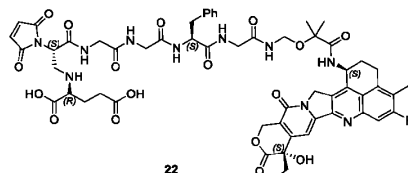
In a 100 mL flask, **21a** (500 mg, 0.54 mmol), ezetimibe methanesulfonate **M5** (285 mg, 0.54 mmol), PyBOP (239 mg, 0.6 mmol), HOBt (239 mg, 0.6 mmol), and 10 mL of DMF were added, and DIPEA (248 uL, 1.5 mmol) was added to the reaction in an ice-water bath. DIPEA (248 uL, 1.5 mmol) was added in an ice-water bath, and the reaction was brought to room temperature for 2 h. After the completion of the reaction was monitored by HPLC, the reaction solution was subjected to high-performance liquid-phase purification to obtain the preparative solution of compound **21b**, which was lyophilized to give 231 mg of the compound; LC-MS: [M+H]⁺ =1349.5.

Step 7: Compound **21**

Compound **21b** (200 mg, 0.1488 mmol), zinc bromide (665 mg, 2.96 mmol) and 10 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 103 mg of solid; LC-MS: [M+H]⁺ =1137.5.

Example 33

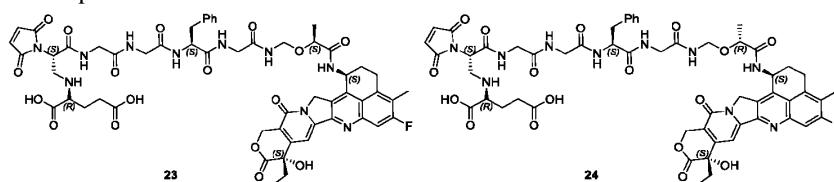
Synthesis of compound **22**:



Using compounds **M6** and **3c** as starting materials and referring to the synthetic route of Example 32, compound **22** (91 mg) was obtained; LC-MS: $[M+H]^+ = 1165.5$.

Example 34

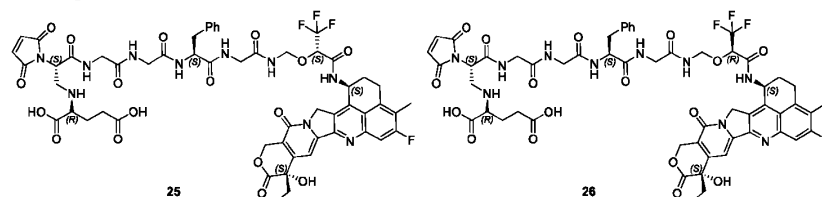
Synthesis of compounds **23** and **24**:



Using compounds **M6** and **5c** as starting materials and referring to the synthetic route of Example 32, 102 mg of compound **23** was obtained, LC-MS: $[M+H]^+ = 1151.4$; 99 mg of compound **24** was obtained, LC-MS: $[M+H]^+ = 1151.4$.

Example 35

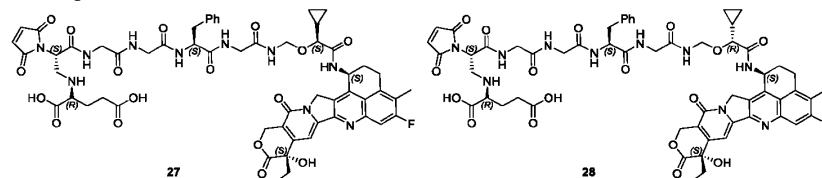
Synthesis of compounds **25** and **26**:



Using compounds **M6** and **7c** as starting materials and referring to the synthetic route of Example 32, 83 mg of compound **25** was obtained, LC-MS: $[M+H]^+ = 1205.7$; 80 mg of compound **26** was obtained, LC-MS: $[M+H]^+ = 1205.7$.

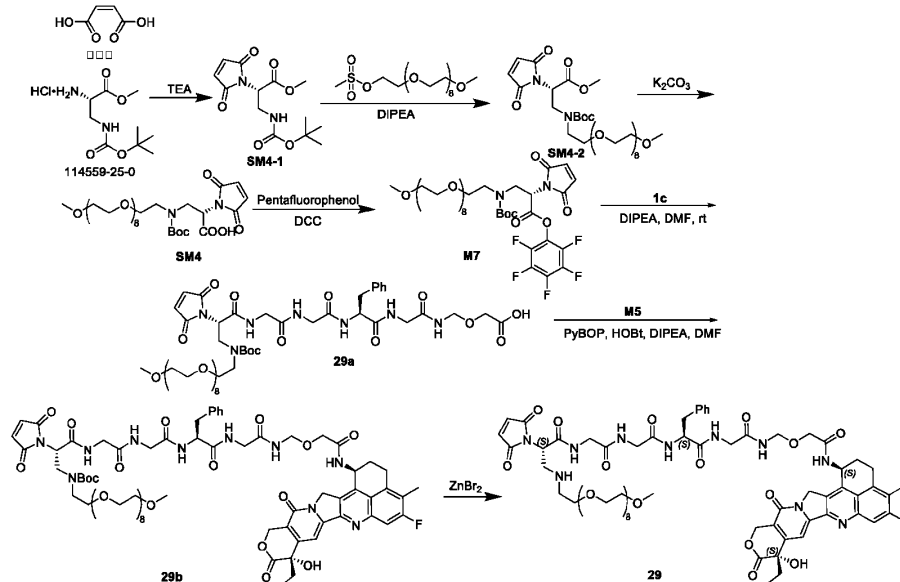
Example 36

Synthesis of compounds **27** and **28**:



Using compounds **M6** and **19c** as starting materials and referring to the synthetic route of Example 32, 100 mg of compound **27** was obtained, LC-MS: $[M+H]^+ = 1177.5$; 101 mg of compound **28** was obtained, LC-MS: $[M+H]^+ = 1177.5$.

Example 37

Synthesis of compound **29**:Step 1: Compound **SM4-1**

In a 5000 mL flask, maleic acid (50 g, 431 mmol, 1.0 eq), 114559-25-0 (110 g, 431 mmol, 1 eq), TEA (263 g, 2.16 mol, 5 eq), and toluene (2000 mL) were added, and the reaction was heated to reflux for 5 h (monitored by TLC), and insoluble material was filtered off. The reaction solution was directly removed from the solvent by rotary distillation under reduced pressure, and the residue was subjected to silica gel column chromatography (PE/EA=50/1-20/1-1/1) to obtain **SM4-1** (64.7 g) in 50% yield; LC-MS: $[M+H]^+ = 299.2$.

Step 2: Compound **SM4-2**

SM4-1 (64 g, 215 mmol) was added into a 2000 mL flask, and after 1000 mL of DMF was dissolved, DIPEA (71 mL, 430 mmol) was added, and then nona-ethylene glycol monomethyl ether methanesulfonate (111.5 g, 220 mmol) was added, and then the reaction was brought to room temperature for 2 h. The completion of the reaction was monitored by HPLC, and the reaction solution was purified by silica gel column chromatography (PE/EA=50/1-20/1-1/1) to obtain 59.9 g; LC-MS: $[M+H]^+ = 709.4$. The reaction solution was purified by silica gel column chromatography (PE/EA=50/1-20/1-1/1), and 59.9 g of the product was obtained; LC-MS: $[M+H]^+ = 709.4$.

Step 3: Compound **SM4**

SM4-2 (59 g, 83 mmol) was added into a 2000 mL flask, and after 1000 mL MeOH was dissolved, K_2CO_3 (11.75 g, 85 mmol) was added, and the reaction was carried out at room temperature for 4 h. The reaction was monitored by HPLC, and the insoluble material was filtered out, and the reaction solution was purified directly by the preparation, and the prepared solution was pumped to concentrate at 35 °C under reduced pressure in a water bath to remove acetonitrile, and then lyophilized to obtain compound **SM4** (27 g); LC-MS: $[M-H]^- = 693.5$.

Step 4: Compound **M7**

In a 500 mL flask, compound **SM4** (25 g, 36 mmol, 1.0 eq), pentafluorophenol (7.3 g, 40 mmol, 1.1 eq), DCC (8.2 g, 40 mmol, 1.1 eq) and THF (200 mL) were added, and the reaction was carried out at room temperature for 1 h (monitored by TLC), and insoluble material was filtered off. The reaction solution was purified directly by preparation, and the preparative solution was concentrated by pumping water under reduced pressure at 35 °C to remove acetonitrile, and lyophilized to obtain compound **M7** (23.3 g) in 93% yield; LC-MS: $[M+H]^+ = 695.8$.

Step 5: Compound **29a**

In a 25 mL flask, **1c** (1 g, 2.36 mmol) was added, 25 mL DMF was dissolved, then DIPEA (430 μ L, 2.6 mmol) was added, then **M7** (1640 mg, 2.36 mmol) was added, and then the reaction was brought to room temperature for 1 h. The completion of the reaction was monitored by HPLC, and the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution, which was lyophilized to obtain 609 mg of product; $[M-H]^- = 1098.5$ by LC-MS. The product was 609 mg; LC-MS: $[M-H]^- = 1098.5$.

Step 6: Compound **29b**

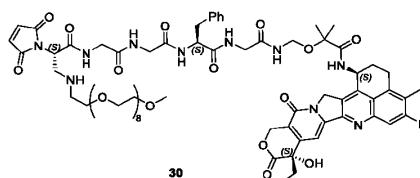
29a (500 mg, 0.45 mmol), ezetimibe methanesulfonate **M5** (240 mg, 0.45 mmol), PyBOP (215 mg, 0.54 mmol), HOBt (215 mg, 0.54 mmol), and 10 mL of DMF were added to a 100 mL vial, and DIPEA (248 μ L, 1.5 mmol) was added to the flask under an ice-water bath. 1.5 mmol) in an ice water bath, and the reaction was brought to room temperature for 2 h. After the completion of the reaction was monitored by HPLC, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution of compound **29b**, and lyophilization of the preparative solution yielded 187 mg of the compound; LC-MS: $[M+H]^+ = 1517.6$.

Step 7: Compound **29**

Compound **29b** (150 mg, 0.988 mmol), zinc bromide (223 mg, 0.988 mmol) and 10 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 114 mg of solid; LC-MS: $[M+H]^+ = 1517.9$.

Example 38

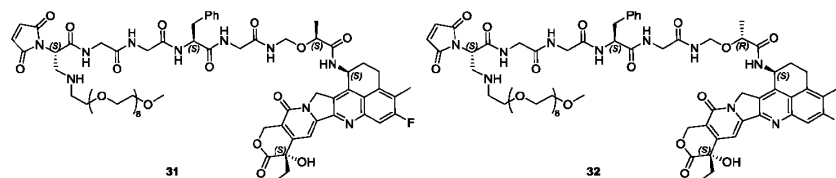
Synthesis of compound **30**:



Using compounds **M7** and **3c** as starting materials and referring to the synthetic route of Example 37, compound **30** (125 mg) was obtained; LC-MS: $[M+H]^+ = 1445.6$.

Example 39

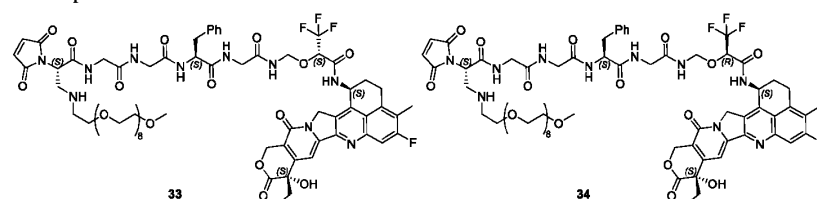
Synthesis of compounds **31** and **32**:



Using compounds **M7** and **5c** as starting materials and referring to the synthetic route of Example 37, 61 mg of compound **31** was obtained, LC-MS: $[M+H]^+ = 1431.7$; 63 mg of compound **32** was obtained, LC-MS: $[M+H]^+ = 1431.7$.

Example 40

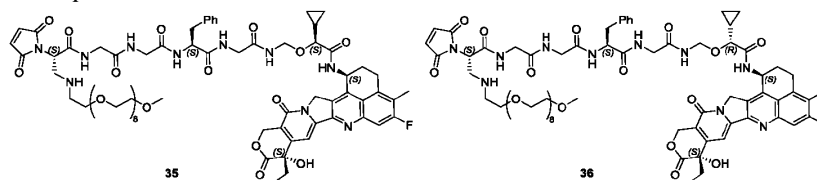
Synthesis of compounds **33** and **34**:



Using compounds **M7** and **7c** as starting materials, referring to the synthetic route of Example 37, 60 mg of compound **33** was obtained, LC-MS: $[M+H]^+ = 1485.6$; 58 mg of compound **34** was obtained, LC-MS: $[M+H]^+ = 1485.6$.

Example 41

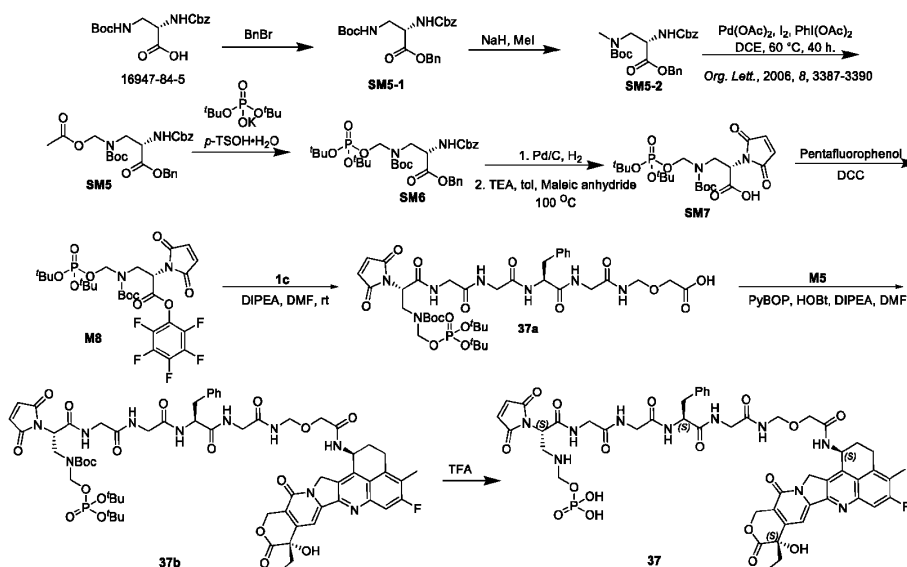
Synthesis of compounds **35** and **36**:



Using compounds **M7** and **19c** as starting materials and referring to the synthetic route of Example 37, 102 mg of compound **35** was obtained, LC-MS: $[M+H]^+ = 1457.8$; 102 mg of compound **36** was obtained, LC-MS: $[M+H]^+ = 1457.8$.

Example 42

Synthesis of compound **37**:



Step 1: Compound SM5-1

In a 2000 mL flask, compound 16947-84-5 (100 g, 295 mmol, 1.0 eq), DIPEA (50 mL, 300 mmol), benzyl bromide (51.3 g, 300 mmol) and THF (1000 mL) were added and reacted at room temperature for 12 h (monitored by TLC), and the insoluble material was filtered off. The reaction solution was directly removed from the solvent by rotary distillation under reduced pressure, and the residue was subjected to silica gel column chromatography (PE/EA=50/1-20/1-2/1) to obtain **SM5-1** (110.1 g) in 87% yield; LC-MS: $[M+H]^+ = 429.2$.

Step 2: Compound SM5-2

10 In a 2000 mL flask, compound **SM5-1** (100 g, 233.4 mmol, 1.0eq) and THF (1000 mL) were added, cooled to 0 °C in an ice-water bath, NaH (37.4 g, 933.5 mmol) and MeI (132.5 g, 933.5 mmol) were added in batches, and the reaction was maintained at 0 °C for 24 h (monitored by TLC), the reaction was quenched by adding 500 mL of saturated NH₄Cl aqueous solution, extracted three times with 500 mL of ethyl acetate, dried the organic phase with anhydrous sodium sulfate, and filtered. The filtrate was directly removed from the solvent by rotary distillation under reduced pressure, and the residue was analyzed by silica gel column chromatography (PE/EA=100/1-50/1-10/1) to obtain **SM5-2** (37.1 g); LC-MS: $[M+H]^+ = 443.3$.

Step 3: Compound SM5 (see *Org. Lett.*, 2006, 8, 3387-3390.)

20 In a 1000 mL flask, compound **SM5-2** (35 g, 79 mmol, 1.0eq) and DCE (500 mL) were added, palladium diacetate (180 mg, 0.8 mmol), I₂ (20 g, 79 mmol), and iodobenzene diacetate (40.8 g, 126.4 mmol) were added, and the reaction was carried out at 60 °C for 40 h (monitored by TLC). The reaction was quenched by adding 500 mL of saturated aqueous sodium thiosulfate, extracted three times with 500 mL of dichloromethane, dried the organic phase with anhydrous sodium sulfate, and filtered. The filtrate was directly removed from the solvent by rotary distillation under reduced pressure, and the residue was analyzed by silica gel column chromatography (PE/EA=100/1-50/1-10/1) to obtain **SM5** (28 g); LC-MS: $[M+H]^+ = 501.3$.

Step 4: Compound **SM6**

In a 500 mL flask, compound **SM5** (25 g, 50 mmol, 1.0 eq), potassium di-tert-butyl phosphate (13.66 g, 55 mmol, 1.1 eq), p-toluenesulfonic acid monohydrate (951 mg, 5 mmol, 0.1 eq) and THF (200 mL) were added, and the reaction was carried out at room temperature for 1 h (monitored by TLC), and insoluble material was filtered off. The reaction solution was purified directly by preparation, and the preparative solution was concentrated by pumping water under reduced pressure at 35 °C to remove acetonitrile, and lyophilized to obtain compound **SM6** (15.1 g) in 46% yield; LC-MS: $[M+H]^+ = 651.4$.

Step 5: Compound **SM7**

10 **SM6** (15 g, 23 mmol) and 100 mL of DMF were added into a 250 mL flask, and after dissolving, 15 g of 5% Pd/C was added in an ice-water bath, and the atmosphere in the system was replaced by hydrogen for three times, and the reaction was carried out at room temperature for 12 h. The Pd/C was removed by filtration, and the solvent was removed by decompression evaporation using an oil pump, and the solvent was set aside for use.

Another 250 mL flask, add the above crude product and 100 mL of toluene, triethylamine (6.4 mL, 46 mmol), maleic anhydride (2.4 g, 24 mmol), dissolved clear, rise to 100 °C reaction for 2 h. HPLC to monitor the progress of the reaction, the reaction is completed, the reaction solution by high-performance liquid chromatography purification, to obtain the preparative solution. The prepared solution was extracted with dichloromethane, washed with saturated sodium chloride
20 solution, dried with anhydrous sodium sulfate, filtered, and concentrated to obtain a solid of 4.2 g in 36% yield; LC-MS: $[M+H]^+ = 507.3$.

Step 6: Compound **M8**

In a 100 mL flask, compound **SM7** (4 g, 7.9 mmol, 1.0 eq), pentafluorophenol (1.6 g, 8.7 mmol, 1.1 eq), DCC (1.8 g, 8.7 mmol, 1.1 eq) and THF (60 mL) were added, and the reaction was carried out at room temperature for 1 h (monitored by TLC), and insoluble material was filtered off. The reaction solution was purified directly by preparation, and the preparative solution was concentrated by pumping water under reduced pressure at 35 °C to remove acetonitrile, and lyophilized to obtain compound **M8** (3.7 g) in 70% yield; LC-MS: $[M+H]^+ = 673.2$.

Step 7: Compound **37a**

30 In a 25 mL flask, **1c** (1 g, 2.36 mmol) was added, 25 mL of DMF was dissolved, then DIPEA (430 uL, 2.6 mmol) was added, then **M8** (1.2 g, 2.36 mmol) was added, and the reaction was brought to room temperature for 1 h. The completion of the reaction was detected by HPLC, and the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution, which was lyophilized to obtain the following products 488 mg; LC-MS: $[M-H]^- = 911.0$.

Step 8: Compound **37b**

37a (400 mg, 0.44 mmol), ezetimibe methanesulfonate **M5** (235 mg, 0.44 mmol), PyBOP (199 mg, 0.5 mmol), HOBt (69 mg, 0.5 mmol), and 10 mL of DMF were added to a 100 mL flask, and DIPEA (218 uL, 1.32 mmol) was added to the reaction in an ice-water bath. DIPEA (218 uL,
40 1.32 mmol) was added in an ice-water bath, and the reaction was brought to room temperature for

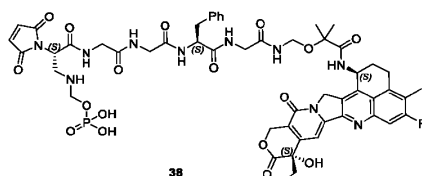
2 h. After the reaction was monitored by HPLC, the reaction solution was subjected to high-performance liquid-phase purification to obtain the preparative solution of compound **37b**, which was lyophilized to give 201 mg of the compound; LC-MS: $[M+H]^+ = 1329.6$.

Step 9: Compound **37**

Compound **37b** (130 mg, 0.098 mmol), zinc bromide (221 mg, 0.98 mmol) and 10 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 96 mg of solid; LC-MS: $[M+H]^+ = 1117.4$.

Example 43

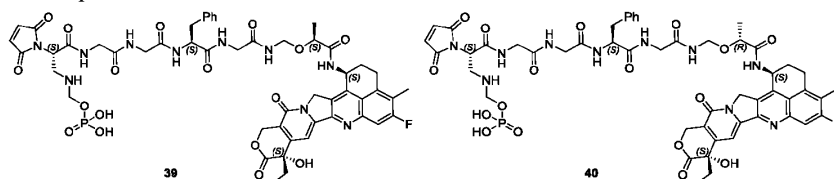
Synthesis of compound **38**:



Using compounds **M8** and **3c** as starting materials and referring to the synthetic route of Example 42, compound **38** (51 mg) was obtained; LC-MS: $[M+H]^+ = 1145.6$.

Example 44

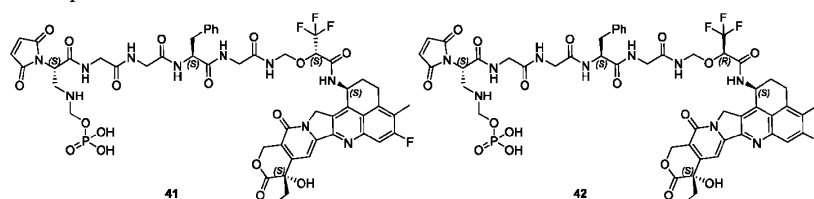
Synthesis of compounds **39** and **40**:



Using compounds **M8** and **5c** as starting materials and referring to the synthetic route of Example 42, 57 mg of compound **39** was obtained, LC-MS: $[M+H]^+ = 1131.4$; 60 mg of compound **40** was obtained, LC-MS: $[M+H]^+ = 1131.4$.

Example 45

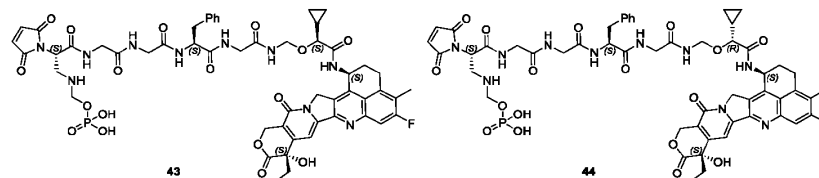
Synthesis of compounds **41** and **42**:



Using compounds **M7** and **7c** as starting materials, referring to the synthetic route of Example 42, 44 mg of compound **41** was obtained, LC-MS: $[M+H]^+ = 1185.3$; 44 mg of compound **42** was obtained, LC-MS: $[M+H]^+ = 1185.3$.

Example 46

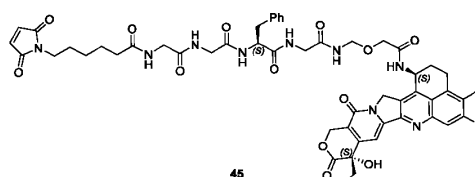
Synthesis of compounds **43** and **44**:



Using compounds **M8** and **19c** as starting materials and referring to the synthetic route of Example 42, 62 mg of compound **43** was obtained, LC-MS: $[M+H]^+ = 1157.4$; 59 mg of compound **44** was obtained, LC-MS: $[M+H]^+ = 1157.4$.

Example 47 (control example)

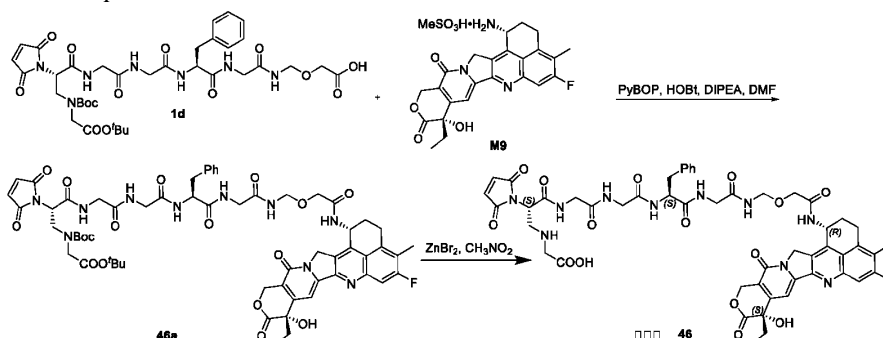
Synthesis of compound **45**:



Compound **45** was synthesized according to the method provided in Example 58 of CN104755494A.

Example 48

Synthesis of compound **46**:



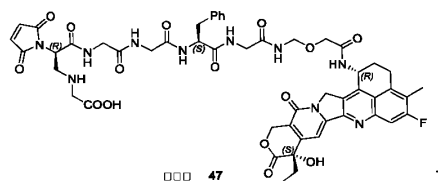
Step 1: Compound **46a**

1d (500 mg, 0.62 mmol), **M9** (310 mg, 0.62 mmol), PyBOP (448 mg, 0.86 mmol), HOBT (116 mg, 0.86 mmol) and 15 mL of DMF were added to a 50 mL flask, and DIPEA (378 μ L, 2.29 mmol) was added to the vial under an ice-water bath, and the reaction was allowed to proceed for 2 h. After the reaction was monitored by HPLC, the reaction solution was purified by HPLC to obtain the preparative solution, which was lyophilized to obtain **46a** (210 mg); LC-MS: the preparative solution was lyophilized to obtain **46a** (210 mg); LC-MS: the preparative solution was purified by HPLC. The reaction was carried out at room temperature for 2 h. After the completion of the reaction was monitored by HPLC, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution, which was lyophilized to give **46a** (210 mg); LC-MS: $[M+H]^+ = 1221.6$.

Step 2: Compound **46**

46a (200 mg, 0.162 mmol), zinc bromide (736 mg, 3.26 mmol) and 10 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain solid compound **46** (120 mg); LC-MS: $[M+H]^+ = 1065.3$.

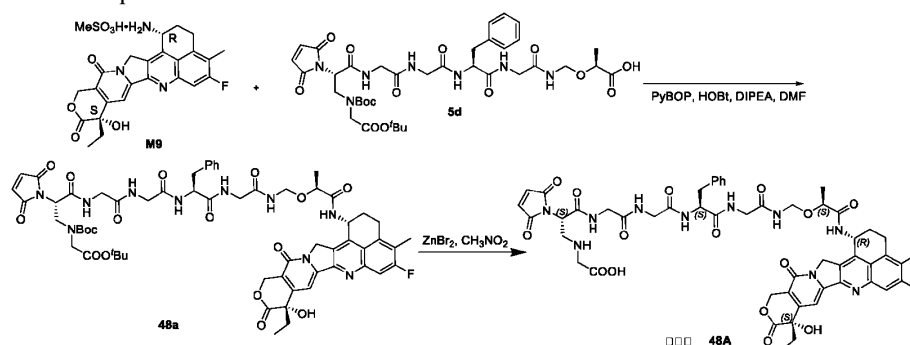
Example 49

Synthesis of compound **47**:

10

Referring to the synthetic route of Example 48, compound **47** (81 mg) was obtained; LC-MS: $[M+H]^+ = 1065.3$.

Example 50

Synthesis of compound **48A**:Step 1: Compound **48a**

5d (1.66 g, 2.02 mmol, 1.0 eq), **M9** (1.08 g, 2.02 mmol, 1.0 eq), PyBOP (1.58 g, 3.03 mmol, 1.5 eq), HOBt (0.41 g, 3.03 mmol, 1.5 eq) and DMF (40 mL) were added to a 100 mL flask, and the reaction was brought to room temperature for 2 h. The reaction was monitored by HPLC. DIPEA (0.84 mL, 1.5 eq) was added, and the reaction was brought to room temperature for 2 h (monitored by HPLC). The reaction solution was purified directly by preparation, and the preparative solution was concentrated by pumping water under reduced pressure at 35 °C to remove acetonitrile, and lyophilized to obtain compound **48a** (1.54 g) in 61% yield; LC-MS: $[M+H]^+ = 1235.4$.

20

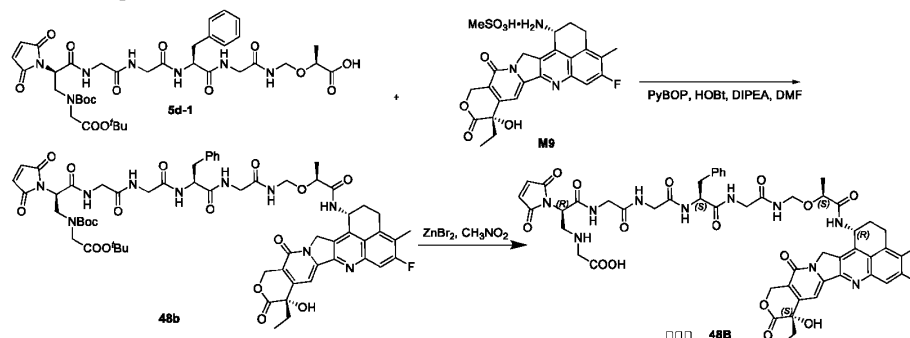
Step 2: Compound **48A**

In a 100 mL flask, compound **48a** (1.0 g, 0.8 mmol, 1.0 eq) was added, 35 mL of nitromethane, dissolved and then added zinc bromide (3.64 g, 16 mmol, 20.0 eq), the reaction was carried out in an oil bath at 40 °C (stabilized in advance by preheating) for 30 min, and then the pump was

concentrated at 45 °C under reduced pressure in a water bath to remove the nitromethane, resulting in a yellow residue solid (monitored by HPLC). After acid preparation, the prepared solution was obtained, and the prepared solution was concentrated by pump decompression water bath at 35 °C to remove acetonitrile and lyophilized to obtain compound **48A** (786 mg) in 90% yield.

Example 51

Synthesis of compound **48B**:



Step 1: Compound **48b**

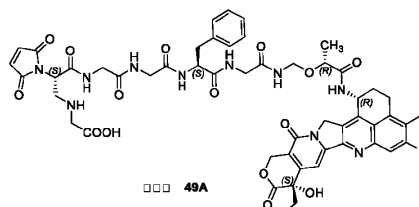
Compound **5d-1** (200 mg, 0.24 mmol, 1.0 eq), **M9** (127 mg, 0.24 mmol, 1.0 eq), PyBOP (187 mg, 0.36 mmol, 1.2 eq), HOBt (48 mg, 0.36 mmol, 1.2 eq), and DMF (6 mL) were added to a 25 mL flask. Water bath was lowered to 0-5 °C, DIPEA (62 mg, 0.48 mmol, 2.0 eq) was added, and the reaction was raised to 20 ± 5 °C for 2 h. The end of the reaction was monitored by HPLC. The reaction solution was purified directly by HPLC preparation, and the product preparation was collected and lyophilized to obtain compound **48b** (150.2 mg); LC-MS: $[M+H]^+ = 1235.4$.

Step 2: Compound **48B**

Compound **48b** (100 mg, 0.081 mmol, 1.0 eq), ZnBr₂ (364 mg, 1.62 mmol, 20.0 eq) and CH₃NO₂ (10 mL) were added to a 25 mL flask in sequence, and then the reaction was raised to 40 °C for 0.5 h. The reaction was stopped, and the reaction solution was directly spun-dried at 45 °C under reduced pressure to obtain a yellow solid, which was monitored by sampling HPLC. The reaction was monitored by HPLC. The spin-dried solid was directly purified by HPLC preparation, and the product preparation was collected and lyophilized to obtain compound **48B** (70.0 mg); LC-MS: $[M+H]^+ = 1079.4$.

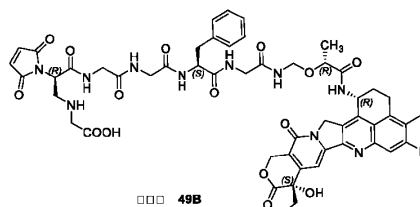
Example 52

Synthesis of compound **49A**:



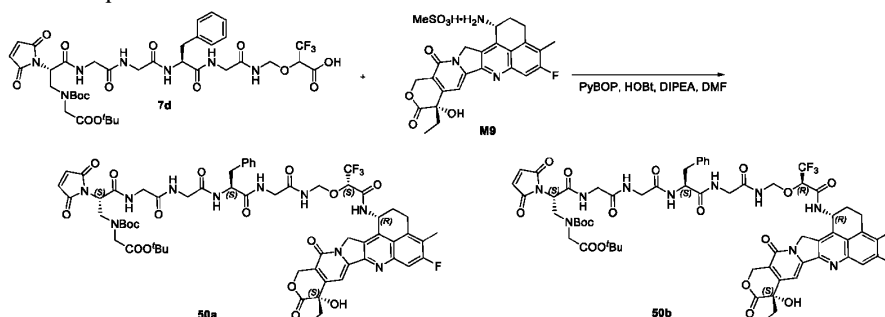
Referring to the route of Example 50, compound **49A** (71 mg) was obtained; LC-MS: $[M+H]^+ = 1079.4$.

Example 53

Preparation of compound **49B**:

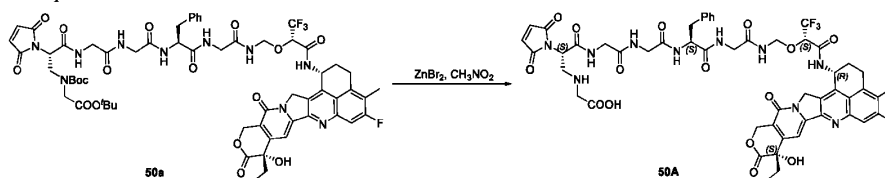
Referring to the synthetic route of Example 51, compound **49B** (65 mg) was obtained; LC-MS: $[M+H]^+ = 1079.4$.

Example 54

Synthesis of compounds **50A** and **50B**:Step 1: Compounds **50a** and **50b**

- 10 **7d** (500 mg, 0.57 mmol), **M9** (305 mg, 0.57 mmol), PyBOP (448 mg, 0.86 mmol), HOBT (116 mg, 0.86 mmol) and 15 mL of DMF were added to a 50 mL flask, DIPEA (378 μ L, 2.29 mmol) was added to the flask under an ice-water bath, and the reaction was carried out at room temperature for 2 h. After the reaction was monitored by HPLC, the reaction solution was purified by HPLC to obtain compound **50a** and compound **50b**, and the preparation was lyophilized to obtain 170 mg of compound **50a**. The reaction was carried out at room temperature for 2 h. After HPLC monitoring, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solutions of compound **50a** and compound **50b**, which were lyophilized to obtain 170 mg of compound **50a**, LC-MS: $[M+H]^+ = 1289.46$, and 202 mg of compound **50b**, LC-MS: $[M+H]^+ = 1289.46$, respectively.

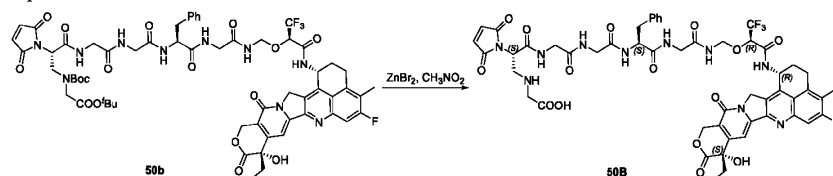
- 20 Step 2: Compound **50A**



In a 25 mL flask, **50a** (100 mg, 0.077 mmol), zinc bromide (349 mg, 1.55 mmol) and 5 mL of nitromethane were added, and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent,

and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 44 mg of solid.

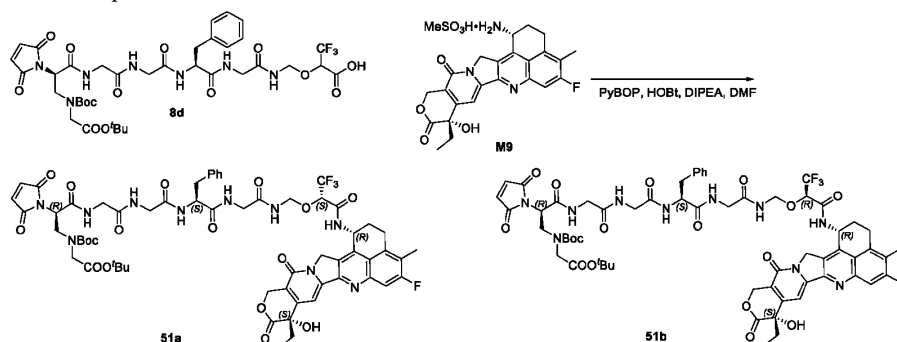
Step 3: Compound **50B**



In a 25 mL trial, **50b** (100 mg, 0.077 mmol), zinc bromide (349 mg, 1.55 mmol) and 5 mL of nitromethane were added, and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 45 mg of solid.

Example 55

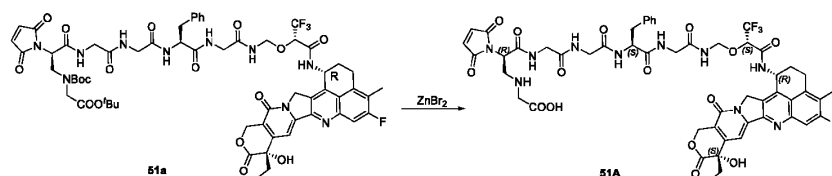
Synthesis of compounds **51A** and **51B**:



Step 1: Compound **51a** and Compound **51b**

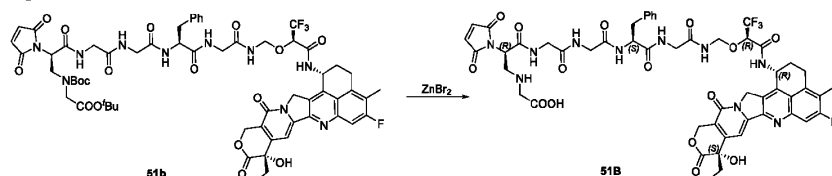
8d (500 mg, 0.57 mmol), **M9** (305 mg, 0.57 mmol), PyBOP (448 mg, 0.86 mmol), HOBT (116 mg, 0.86 mmol) and 15 mL of DMF were added to a 50 mL flask, DIPEA (378 uL, 2.29 mmol) was added to the flask in an ice-water bath, and the reaction was carried out at room temperature for 2 h. After the reaction was monitored by HPLC, the reaction solution was purified by HPLC to obtain the preparations of compound **51a** and compound **51b**. After the reaction was completed by HPLC, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solutions of compound **51a** and compound **51b**, which were lyophilized to obtain 190 mg of compound **51a** and 186 mg of compound **51b**, respectively. The LC-MS of compound **51a**: $[M+H]^+ = 1289.47$; LC-MS of compound **51b**: $[M+H]^+ = 1289.47$.

Step 2: Compound **51A**



Compound **51a** (100 mg, 0.077 mmol), zinc bromide (349 mg, 1.55 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 39 mg of solid.

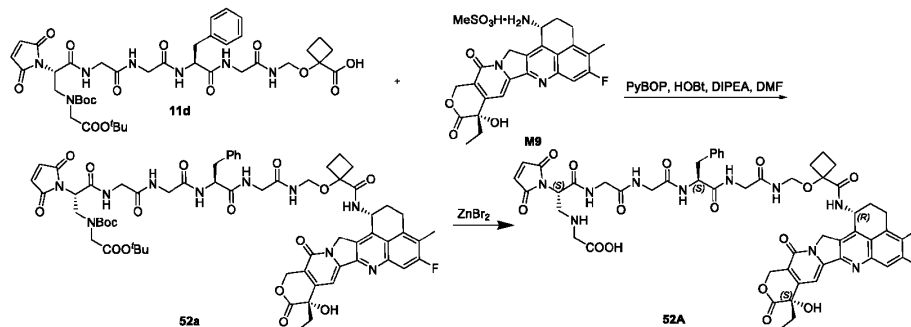
Step 3: Compound **51B**



Compound **51b** (100 mg, 0.077 mmol), zinc bromide (349 mg, 1.55 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 60 mg of solid.

Example 56

Synthesis of compound **52A**:



Step 1: Compound **52a**

11d (800 mg, 0.96 mmol), **M9** (480 mg, 0.96 mmol), PyBOP (500 mg, 0.96 mmol), HOBT (208 mg, 0.96 mmol) and 30 mL of DMF were added to a 50 mL flask in an ice-water bath and DIPEA (660 μ L, 4.0 mmol) was added, and the reaction was allowed to proceed for 4 h. After the reaction was monitored by HPLC, the reaction solution was purified by HPLC to give the preparative solution of compound **52a**. The reaction was carried out at room temperature for 4 h.

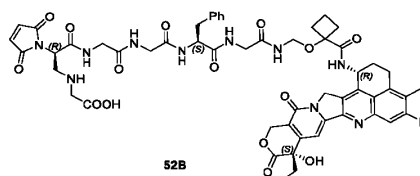
After the completion of the reaction was monitored by HPLC, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution of compound **52a**, which was lyophilized to obtain **52a** (388 mg); LC-MS: $[M+H]^+ = 1261.4$.

Step 2: Compound **52A**

52A (150 mg, 0.12 mmol), zinc bromide (532 mg, 2.4 mmol) and 10 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain the solid compound **52A** (79 mg); LC-MS: $[M+H]^+ = 1105.4$.

Example 57

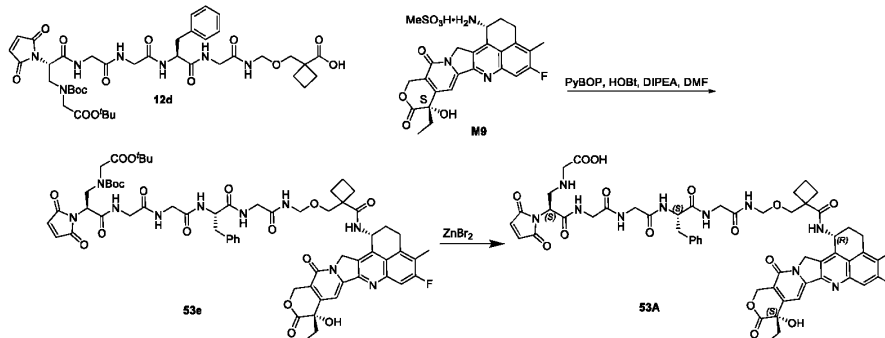
Synthesis of compound **52B**



Referring to the synthetic route of Example 56, compound **52B** (50 mg) was obtained. LC-MS: $[M+H]^+ = 1105.4$.

Example 58

Synthesis of compound **53A**:



Step 1: Compound **53a**

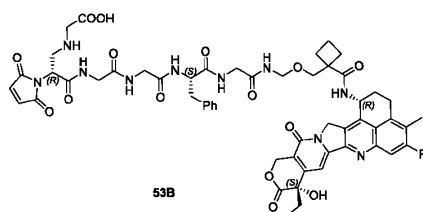
12d (400 mg, 0.47 mmol), **M9** (240 mg, 0.47 mmol), PyBOP (250 mg, 0.47 mmol), HOBT (101 mg, 0.47 mmol) and 15 mL of DMF were added to a 50 mL flask with an ice-water bath and DIPEA (330 μ L, 2.0 mmol), and the reaction was carried out at room temperature for 3 h. After the reaction was monitored by HPLC, the reaction solution was purified by HPLC. The reaction was carried out at room temperature for 3 h. After the completion of the reaction was monitored by HPLC, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution of compound **53a**, which was lyophilized to obtain **53a** (200 mg); LC-MS: $[M+H]^+ = 1275.4$.

Step 2: Compound **53A**

53A (100 mg, 0.08 mmol), zinc bromide (360 mg, 1.6 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain the solid compound **53A** (51 mg); LC-MS: $[M+H]^+ = 1119.4$.

Example 59

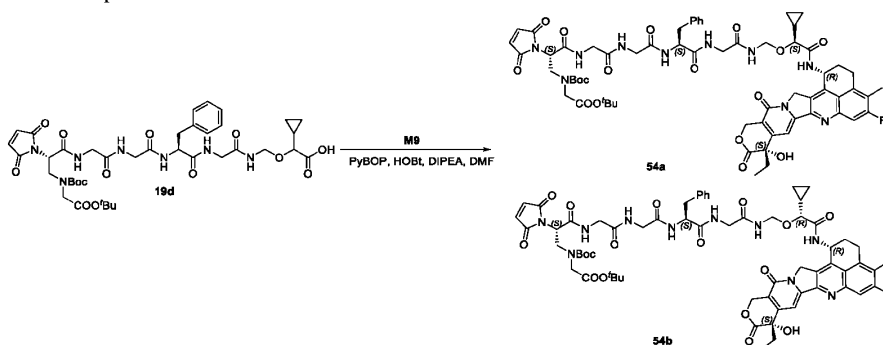
Synthesis of compound **53B**:



Referring to the synthetic route of Example 58, compound **53B** (50 mg) was obtained; LC-MS: $[M+H]^+ = 1119.4$.

Example 60

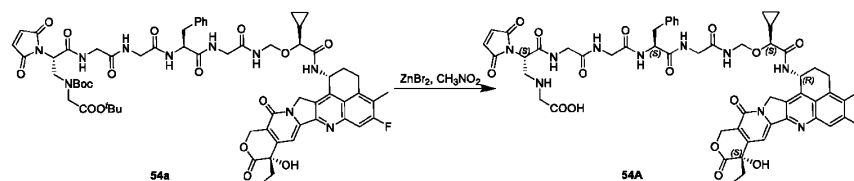
Synthesis of compounds **54A** and **54B**:



Step 1: Compounds **54a** and **54b**

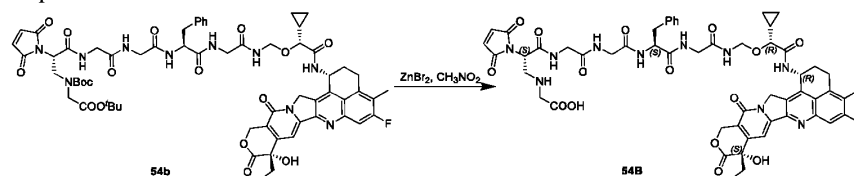
19d (500 mg, 0.59 mmol), **M9** (317 mg, 0.59 mmol), PyBOP (339 mg, 0.65 mmol), HOBT (88 mg, 0.86 mmol) and 10 mL of DMF were added to a 50 mL flask with an ice-water bath and DIPEA (292 μ L, 1.77 mmol) was added, the reaction was raised to room temperature and carried out for 2 h. After the reaction was monitored by HPLC, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solutions of compounds **54a** and **54b**, which were lyophilized to obtain 103 mg of compound **54a**, LC-MS: $[M+H]^+ = 1261.5$, and 111 mg of compound **54b**, LC-MS: $[M+H]^+ = 1261.5$, respectively.

Step 2: Compound **54A**



In a 25 mL flask, **54a** (100 mg, 0.079 mmol), zinc bromide (357 mg, 1.59 mmol) and 5 mL of nitromethane were added, and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain a solid of 61 mg; LC-MS: $[M+H]^+ = 1105.4$.

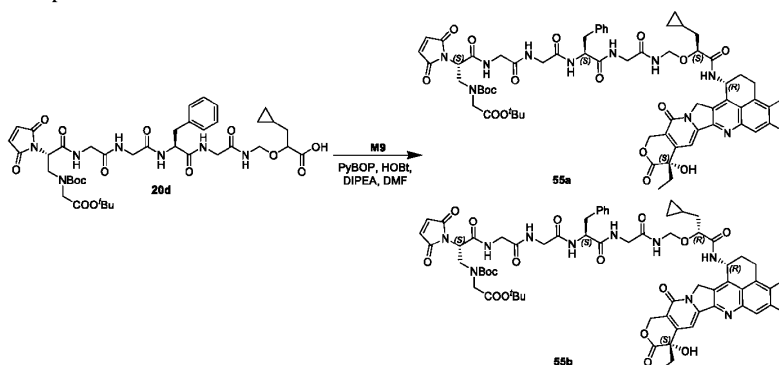
Step 3: Compound **54B**



In a 25 mL flask, **54b** (100 mg, 0.079 mmol), zinc bromide (357 mg, 1.59 mmol) and 5 mL of nitromethane were added, and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure and removed to obtain the crude product. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 57 mg of solid; LC-MS: $[M+H]^+ = 1105.4$.

Example 61

Synthesis of compounds **55a** and **55b**:

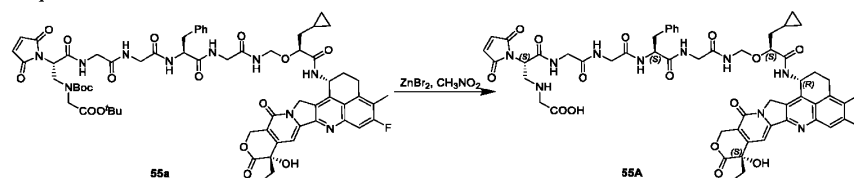


Step 1: Compounds **55a** and **55b**

20d (400 mg, 0.47 mmol), **M9** (250 mg, 0.47 mmol), PyBOP (223 mg, 0.56 mmol), HOBT (83 mg, 0.56 mmol) and 10 mL of DMF were added to a 50 mL flask in an ice-water bath and DIPEA (248 μ L, 1.5 mmol) was added, and the reaction was brought to room temperature and carried out for 2 h. After the completion of the reaction was monitored by HPLC, the reaction

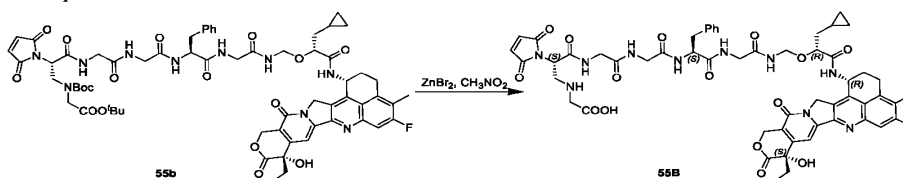
solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solutions of compounds **55a** and **55b**, which were lyophilized to obtain 100 mg of compound **55a**, LC-MS: $[M+H]^+ = 1275.5$, and 101 mg of compound **55b**, LC-MS: $[M+H]^+ = 1275.5$, respectively.

Step 2: Compound **55A**



55a (100 mg, 0.078 mmol), zinc bromide (352 mg, 1.57 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 42 mg of solid; LC-MS: $[M+H]^+ = 1119.4$.

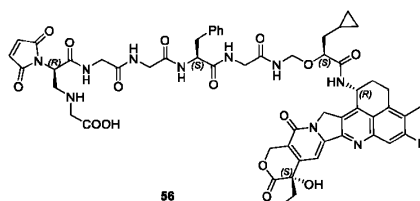
Step 3: Compound **55B**



55b (100 mg, 0.079 mmol), zinc bromide (357 mg, 1.59 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 45 mg of solid; LC-MS: $[M+H]^+ = 1119.4$.

Example 62

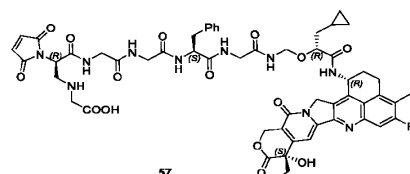
Synthesis of compound **56**:



Referring to the synthetic route of Example 60, compound **56** (50 mg) was obtained; LC-MS: $[M+H]^+ = 1119.3$.

Example 63

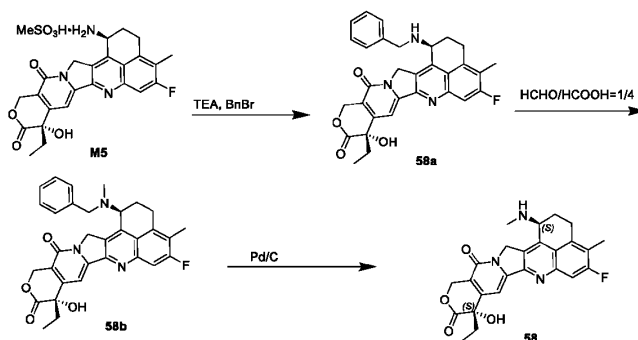
Synthesis of compound **57**:



Referring to the synthetic route of Example 61, compound **57** (50 mg) was obtained; LC-MS: $[M+H]^+ = 1119.4$.

Example 64

Synthesis of compound **58**:



Step 1: Synthesis of compound **58a**

To Exatecan mesylate **M5** (15 g, 28 mol, prepared by the method disclosed in EP0737683A1, 400 mL of DMF was added, the reaction was cooled to 0°C in an ice-water bath, triethylamine was added dropwise, the pH was adjusted to 7-8, and benzyl bromide (9.6 g, 56 mmol) was added dropwise in an ice-water bath, the reaction was warmed to room temperature (25°C) for 1 hour, TLC to monitor the reaction was complete, the reaction solution was concentrated under reduced pressure, the resulting crude product was purified by preparative-grade high-performance liquid chromatography (acetonitrile/pure water system), the target peak was collected, and after removing the acetonitrile under reduced pressure, lyophilization, to obtain the compound **58a** of about 11 g, a yellow solid, the yield was about 74%, MS m/z : $[M+H]^+ 526.3$.

Step 2: Synthesis of compound **58b**

At room temperature, compound **58a** (11 g, 21 mol) was added sequentially in a 250 mL flask, 120 mL of formic acid was dissolved, 30 mL of formaldehyde (40% aqueous solution) was added to the resulting bright yellow solution, and the reaction was heated up to 50 °C for 1 h. The reaction was monitored by TLC to see if the reaction was complete, and then the reaction solution was cooled down to room temperature, and then the reaction solution was purified by preparative high-performance liquid chromatography (acetonitrile/water), and the target peak was collected. The target peak, after removing acetonitrile under reduced pressure, lyophilized to give compound **58b** about 4.5 g, yellow powdery solid, yield about 40%, MS m/z : $[M+H]^+ 540.6$.

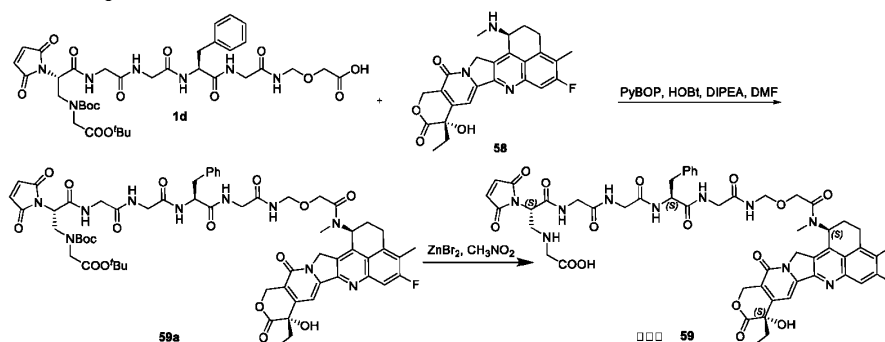
Step 3: Synthesis of compound **58**:

At room temperature, add compound **58b** (2.3 g, 4.3 mol) in a 250 mL flask, add 100 mL of DMF to dissolve, add 2.3 g of 5% Pd/C to the resulting bright yellow solution, hydrogen balloon

to replace the atmosphere in the system, and maintain the reaction at room temperature for 1.5 hours, the reaction was completely detected by HPLC, and then the reaction was filtered to remove the Pd/C, and the resulting reaction solution was concentrated, and then purified by preparative grade HPLC (acetonitrile/pure water system). After the obtained reaction solution was concentrated, purified by preparative grade high performance liquid chromatography (acetonitrile/water system), the target peaks were collected, and after removing acetonitrile under reduced pressure, lyophilized to obtain about 1.0 g of Compound **58**, a yellow powdery solid with about 52% yield, MS m/z : $[M+H]^+$ 450.5.

Example 65

10 Synthesis of compound **59**:



Step 1: Compound **59a**

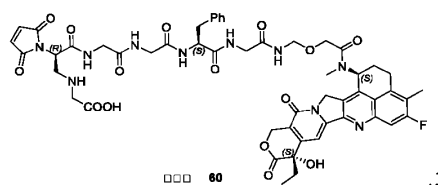
1d (500 mg, 0.62 mmol), **58** (279 mg, 0.62 mmol), PyBOP (448 mg, 0.86 mmol), HOBT (116 mg, 0.86 mmol) and 15 mL of DMF were added to a 50 mL flask, and DIPEA (378 μ L, 2.29 mmol) was added to the flask under an ice-water bath, and the reaction was allowed to proceed for 2 h. After the reaction was monitored by HPLC, the reaction solution was purified by HPLC to obtain the preparative solution, and the preparative solution was lyophilized to obtain **59a** (166 mg); LC-MS was used to obtain the preparative solution. The reaction was carried out at room temperature for 2 h. After the completion of the reaction was monitored by HPLC, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution, which was lyophilized to give **59a** (166 mg); LC-MS: $[M+H]^+$ =1235.6.

Step 2: Compound **59**

59a (100 mg, 0.081 mmol), zinc bromide (368 mg, 1.63 mmol) and 10 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain the solid compound **59** (43 mg); LC-MS: $[M+H]^+$ =1079.3.

Example 66

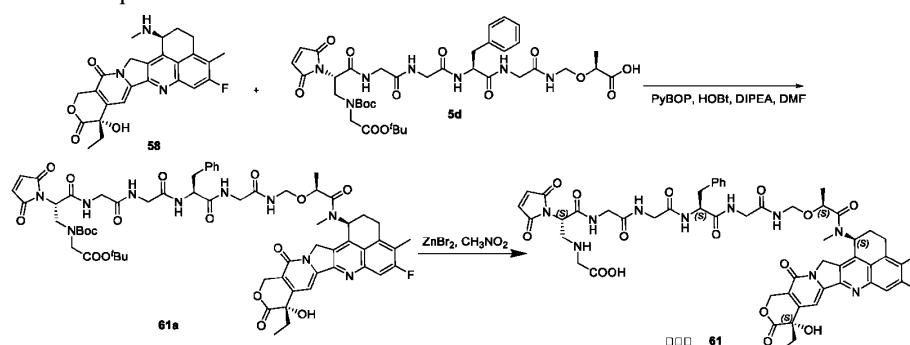
30 Synthesis of compound **60**:



Referring to the synthetic route of Example 65, compound **60** (40 mg) was obtained; LC-MS: $[M+H]^+ = 1079.3$.

Example 67

Synthesis of compound **61**:



Step 1: Compound **61a**

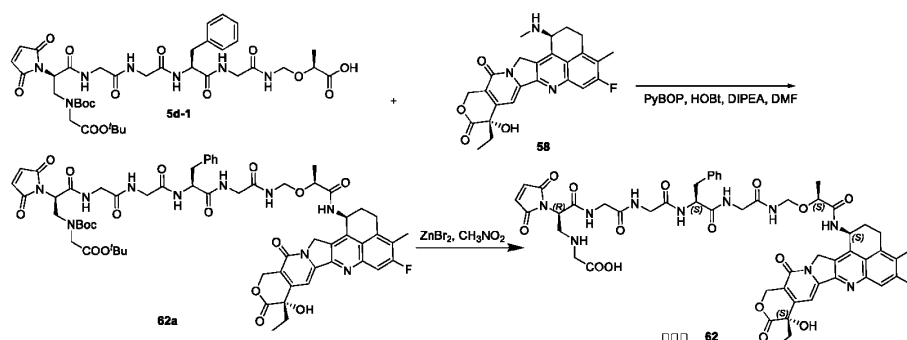
5d (1.66 g, 2.02 mmol, 1.0 eq), **58** (0.91 g, 2.02 mmol, 1.0 eq), PyBOP (1.58 g, 3.03 mmol, 1.5 eq), HOBt (0.41 g, 3.03 mmol, 1.5 eq), and DMF (40 mL) were added to a 100 mL flask, and the reaction was brought to room temperature for 2 h. The reaction was monitored by HPLC. DIPEA (0.84 mL, 1.5 eq) was added, and the reaction was brought to room temperature for 2 h (monitored by HPLC). The reaction solution was purified directly by preparation, and the preparative solution was concentrated by pumping water under reduced pressure at 35 °C to remove acetonitrile, and lyophilized to obtain compound **61a** (1.21 g); LC-MS: $[M+H]^+ = 1249.4$.

Step 2: Compound **61**

In a 100 mL flask, compound **61a** (1.0 g, 0.8 mmol, 1.0 eq) was added, 35 mL of nitromethane, dissolved and then added zinc bromide (3.64 g, 16 mmol, 20.0 eq), the reaction was carried out in an oil bath at 40 °C (stabilized in advance by preheating) for 30 min, and then the pump was concentrated at 45 °C under reduced pressure in a water bath to remove the nitromethane, resulting in a yellow residue solid (monitored by HPLC). After acid preparation, the preparation liquid was obtained, the preparation liquid was concentrated by pump decompression water bath at 35 °C to spin off acetonitrile, lyophilized to obtain compound **61** (786 mg), LC-MS: $[M+H]^+ = 1093.6$.

Example 68

Synthesis of compound **62**:



Step 1: Compound **62a**

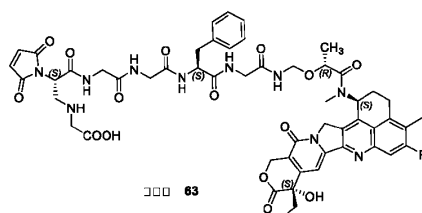
Compounds **5d-1** (200 mg, 0.24 mmol, 1.0 eq), **58** (110.3 mg, 0.24 mmol, 1.0 eq), PyBOP (187 mg, 0.36 mmol, 1.2 eq), HOBT (48 mg, 0.36 mmol, 1.2 eq), and DMF (6 mL) were added to a 25 mL flask. The reaction was carried out at 20±5 °C for 2 h. The reaction was monitored by HPLC for completion. The reaction solution was purified by direct HPLC preparation, and the product preparation was collected and lyophilized to obtain compound **62a** (120.9 mg); LC-MS: $[M+H]^+ = 1249.4$.

Step 2: Compound **62**

Compound **62a** (100 mg, 0.081 mmol, 1.0 eq), ZnBr₂ (364 mg, 1.62 mmol, 20.0 eq) and CH₃NO₂ (10 mL) were added to a 25 mL flask. After addition, the reaction was raised to 40 °C for 0.5 h, then the reaction was stopped, and the reaction solution was dried directly at 45 °C by spin-drying under reduced pressure to obtain a yellow solid, which was sampled for HPLC monitoring. The reaction was monitored by HPLC. The spin-dried solid was directly purified by HPLC preparation, and the product preparation was collected and lyophilized to obtain compound **62** (61 mg); LC-MS: $[M+H]^+ = 1093.4$.

Example 69

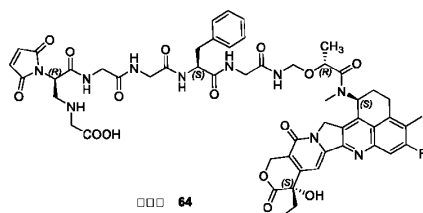
Preparation of compound **63**:



Referring to the route of Example 67, compound **63** (60 mg) was obtained; LC-MS: $[M+H]^+ = 1093.4$.

Example 70

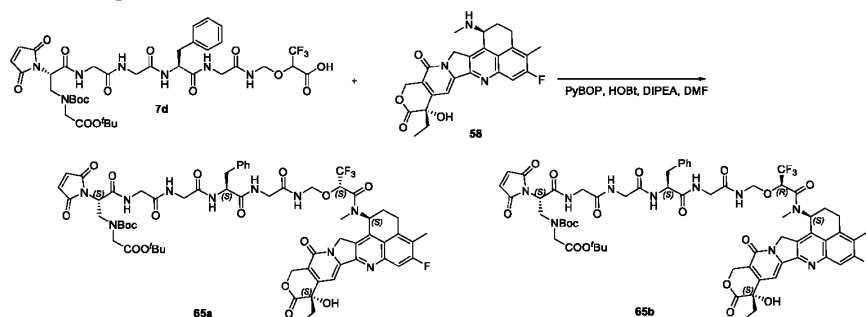
Preparation of compound **64**:



Referring to the synthetic route of Example 68, compound **64** (65 mg) was obtained; LC-MS: $[M+H]^+ = 1093.4$.

Example 71

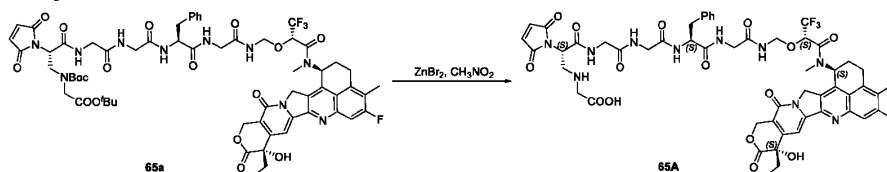
Preparation of compounds **65A** and **65B**:



Step 1: Compounds **65a** and **65b**

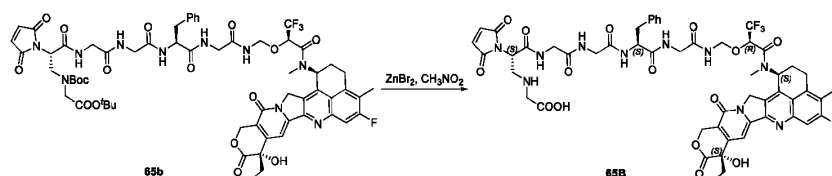
7d (500 mg, 0.57 mmol), **58** (256.8 mg, 0.57 mmol), PyBOP (448 mg, 0.86 mmol), HOBT (116 mg, 0.86 mmol) and 15 mL DMF were added into a 50 mL flask and DIPEA (378 μ L, 2.29 mmol) was added into the vial in an ice bath. The reaction was carried out at room temperature for 2 h. After the reaction was monitored by HPLC, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solutions of compounds **65a** and **65b**, which were lyophilized to obtain 155 mg of compound **65a**, LC-MS: $[M+H]^+ = 1303.4$, and 158 mg of compound **65b**, LC-MS: $[M+H]^+ = 1303.6$, respectively.

Step 2: Compound **65A**



In a 25 mL flask, **50a** (100 mg, 0.077 mmol), zinc bromide (349 mg, 1.55 mmol) and 5 mL of nitromethane were added, and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution, which was lyophilized to obtain 49 mg as a solid.

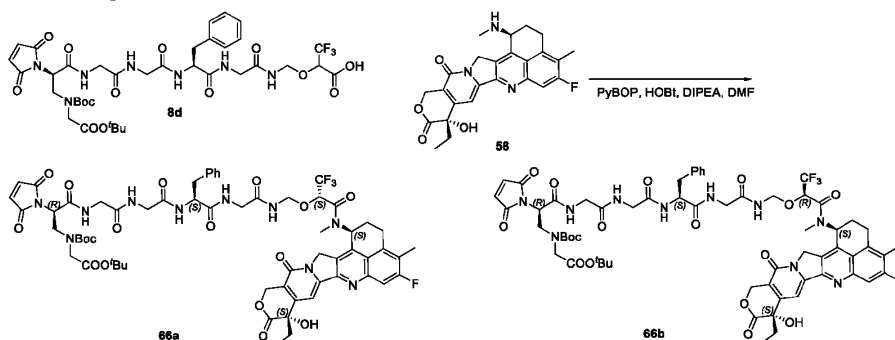
Step 3: Compound **65B**



In a 25 mL flask, **65b** (100 mg, 0.077 mmol), zinc bromide (349 mg, 1.55 mmol) and 5 mL of nitromethane were added, and the reaction was carried out at 40 °C for 1 h. After the reaction was completed by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution, which was lyophilized to obtain a solid of 47 mg.

Example 72

Synthesis of compounds **66A** and **66B**:

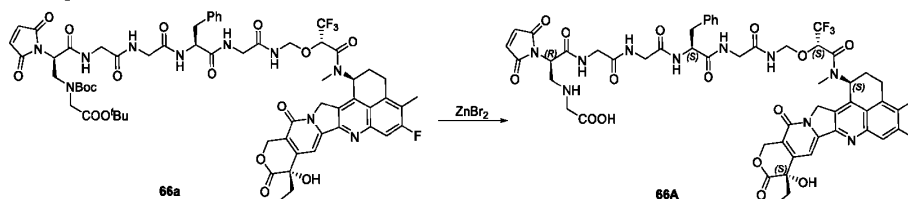


10

Step 1: Compound **66a** and Compound **66b**

In a 50 mL flask, **8d** (500 mg, 0.57 mmol), **58** (256.8 mg, 0.57 mmol), PyBOP (448 mg, 0.86 mmol), HOBT (116 mg, 0.86 mmol) and 15 mL of DMF were added, and DIPEA (378 μ L, 2.29 mmol) was added to the flask in an ice bath. After the reaction was completed by HPLC, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solutions of compound **66a** and compound **66b**, which were lyophilized to obtain 160 mg of compound **66a** and 160 mg of compound **66b**, respectively. LC-MS of compound **66a**: $[M+H]^+ = 1303.7$, and LC-MS of compound **66b**: $[M+H]^+ = 1303.6$.

Step 2: Compound **66A**

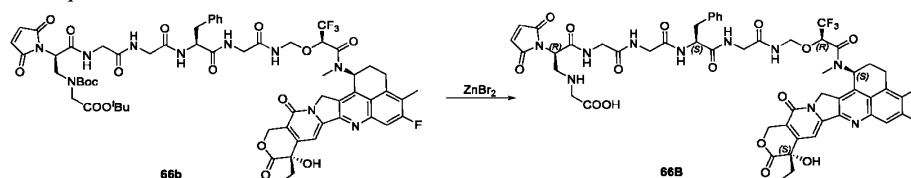


20

Compound **66a** (100 mg, 0.077 mmol), zinc bromide (349 mg, 1.55 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to

remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 57 mg of solid, LC-MS: $[M+H]^+ = 1147.5$.

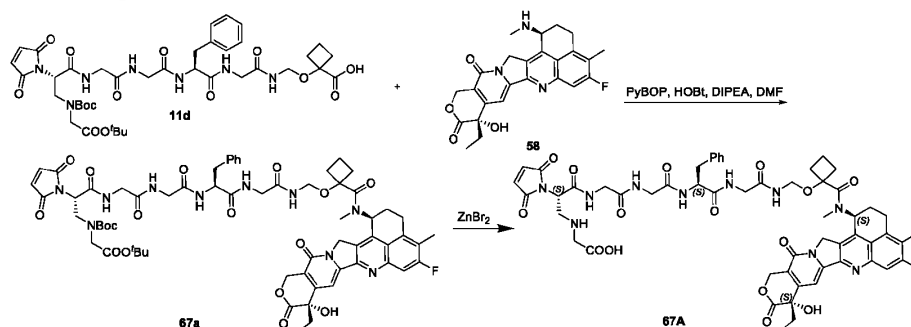
Step 3: Compound **66B**



Compound **66b** (100 mg, 0.077 mmol), zinc bromide (349 mg, 1.55 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure and the product was obtained as a crude. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 57 mg of solid, LC-MS: $[M+H]^+ = 1147.5$.

Example 73

Synthesis of compound **67A**:



Step 1: Compound **67a**

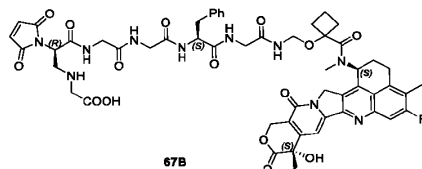
11d (800 mg, 0.96 mmol), **58** (432.5 mg, 0.96 mmol), PyBOP (500 mg, 0.96 mmol), HOBT (208 mg, 0.96 mmol) and 30 mL of DMF were added to a 50 mL flask, and DIPEA (660 μ L, 4.0 mmol) was added to the vial under an ice-water bath. The reaction was brought to room temperature for 4 h. After HPLC monitoring, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution of compound **67a**, which was lyophilized to obtain **67a** (402 mg); LC-MS: $[M+H]^+ = 1275.4$.

Step 2: Compound **67A**

67A (100 mg, 0.78 mmol), zinc bromide (356 mg, 1.57 mmol) and 10 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain the solid compound **67A** (47 mg); LC-MS: $[M+H]^+ = 1119.5$.

Example 74

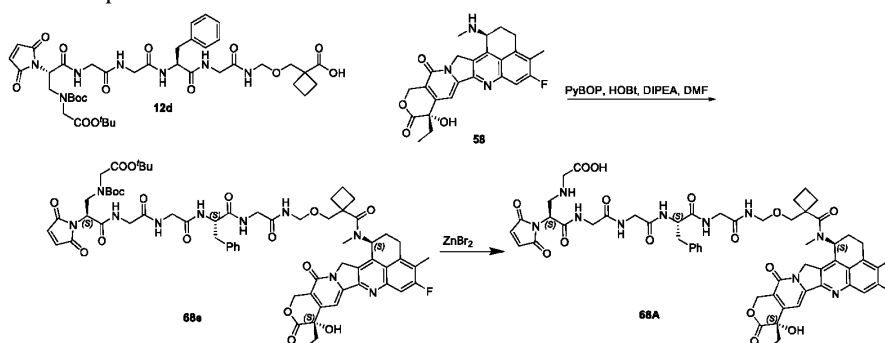
Synthesis of compound **67B**:



Referring to the synthetic route of Example 73, compound **67B** (50 mg) was obtained. LC-MS: $[M+H]^+$ 1119.4.

Example 75

Synthesis of compound **68A**:



Step 1: Compound **68a**

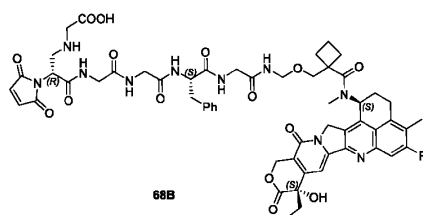
- 10 **12d** (400 mg, 0.47 mmol), **58** (211.7 mg, 0.47 mmol), PyBOP (250 mg, 0.47 mmol), HOBT (101 mg, 0.47 mmol) and 15 mL of DMF were added to a 50 mL flask, and DIPEA (330 μ L, 2.0 mmol) was added to the flask under an ice-water bath. The reaction was brought to room temperature for 3 h. After the reaction was monitored by HPLC, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution of compound **68a**, which was lyophilized to give **68a** (177 mg); LC-MS: $[M+H]^+$ =1289.4.

Step 2: Compound **68A**

- 20 **68a** (100 mg, 0.08 mmol), zinc bromide (360 mg, 1.6 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain the solid compound **68A** (45 mg); LC-MS: $[M+H]^+$ =1133.4.

Example 76

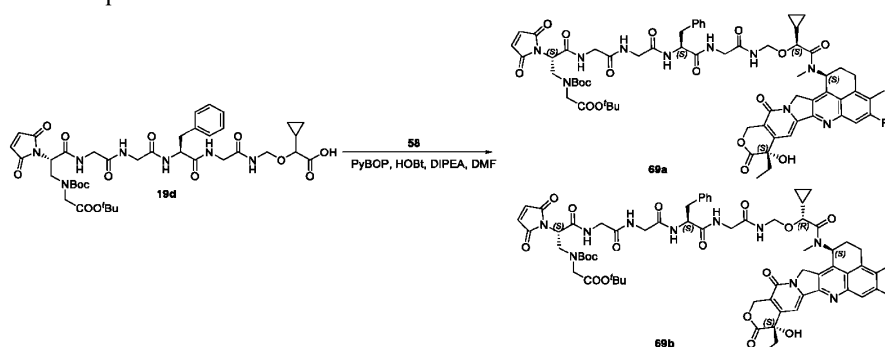
Synthesis of compound **68B**:



Referring to the synthetic route of Example 75, compound **68B** (50 mg) was obtained; LC-MS: $[M+H]^+ = 1133.4$.

Example 77

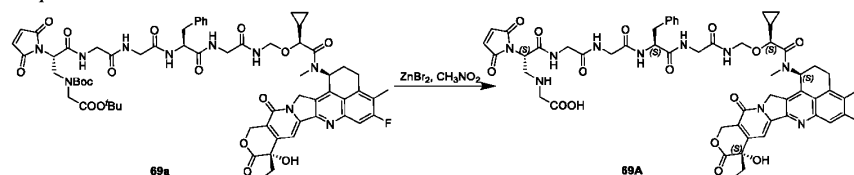
Synthesis of compounds **69A** and **68B**:



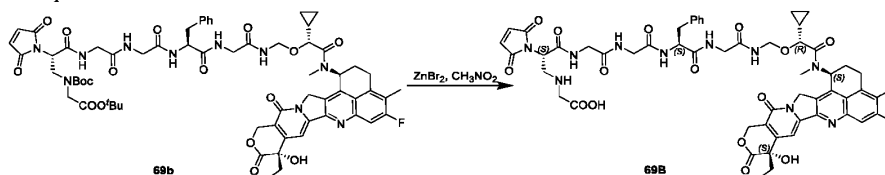
Step 1: Compounds **69a** and **69b**

19d (500 mg, 0.59 mmol), **58** (266 mg, 0.59 mmol), PyBOP (339 mg, 0.65 mmol), HOBT (88 mg, 0.86 mmol) and 10 mL of DMF were added to a 50 mL flask with an ice-water bath and DIPEA (292 μ L, 1.77 mmol) was added, and the reaction was raised to room temperature and the reaction was carried out for 2 h. After the completion of the reaction was monitored by HPLC, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solutions of compounds **69a** and **69b**, which were lyophilized to obtain 109 mg of compound **69a**, LC-MS: $[M+H]^+ = 1275.5$, and 111 mg of compound **69b**, LC-MS: $[M+H]^+ = 1275.7$, respectively.

Step 2: Compound **69A**

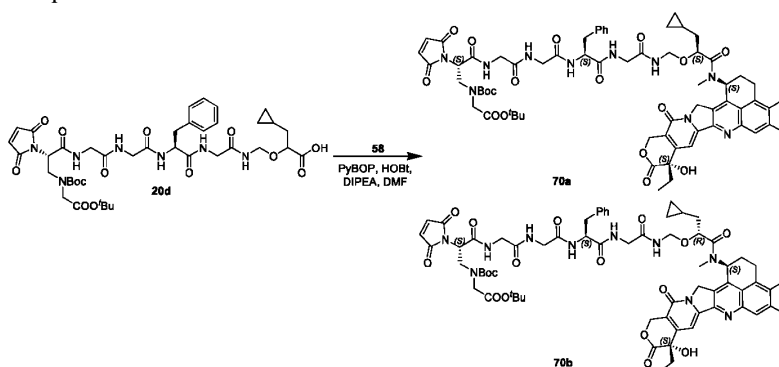


69a (100 mg, 0.078 mmol), zinc bromide (352 mg, 1.56 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 $^{\circ}$ C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 53 mg of solid; LC-MS: $[M+H]^+ = 1119.4$.

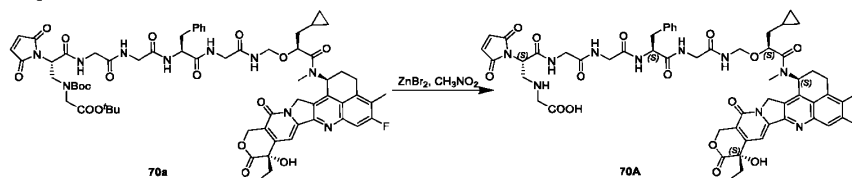
Step 3: Compound **69B**

69b (100 mg, 0.078 mmol), zinc bromide (352 mg, 1.56 mmol) and 5 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 54 mg of solid; LC-MS: $[M+H]^+ = 1119.4$.

Example 78

10 Synthesis of compounds **70A** and **70B**:Step 1: Compounds **70a** and **70b**

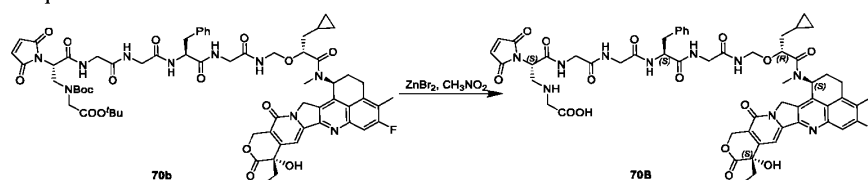
20d (400 mg, 0.47 mmol), **58** (211.7 mg, 0.47 mmol), PyBOP (223 mg, 0.56 mmol), HOBT (83 mg, 0.56 mmol) and 10 mL of DMF were added to a 50 mL flask, and DIPEA (248 μ L, 1.5 mmol) was added to the flask under an ice-water bath. The reaction was brought to room temperature for 2 h. After HPLC monitoring, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solutions of compounds **70a** and **70b**, which were lyophilized to obtain 106 mg of compound **70a**, LC-MS: $[M+H]^+ = 1289.5$, and 101 mg of compound **70b**, LC-MS: $[M+H]^+ = 1289.4$, respectively.

20 Step 2: Compound **70A**

In a 25 mL flask, **70a** (100 mg, 0.078 mmol), zinc bromide (352 mg, 1.57 mmol) and 5 mL of nitromethane were added, and the reaction was carried out at 40 °C for 1 h. After the completion

of the reaction was detected by HPLC, the solvent was concentrated under reduced pressure and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain a solid of 39 mg; LC-MS: $[M+H]^+ = 1133.4$.

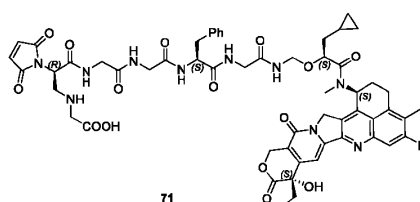
Step 3: Compound **70B**



In a 25 mL flask, **70b** (100 mg, 0.078 mmol), zinc bromide (352 mg, 1.56 mmol) and 5 mL of nitromethane were added and the reaction was carried out at 40 °C for 1 h. After the completion of the reaction was detected by HPLC, the solvent was concentrated under reduced pressure and removed to obtain the crude product. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain 35 mg of solid; LC-MS: $[M+H]^+ = 1133.4$.

Example 79

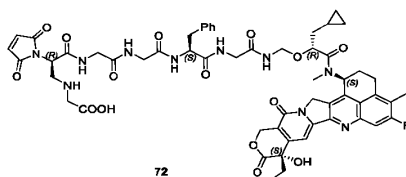
Synthesis of compound **71**:



Referring to the synthetic route of Example 78, compound **71** (30 mg) was obtained; LC-MS: $[M+H]^+ = 1133.3$.

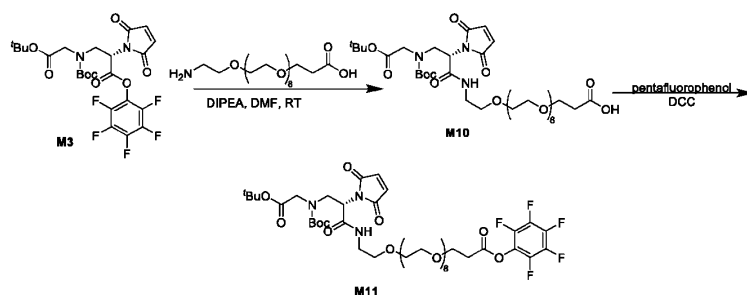
Example 80

Synthesis of compound **72**:



Referring to the synthetic route of Example 78, compound **72** (33 mg) was obtained; LC-MS: $[M+H]^+ = 1133.4$.

Example 81



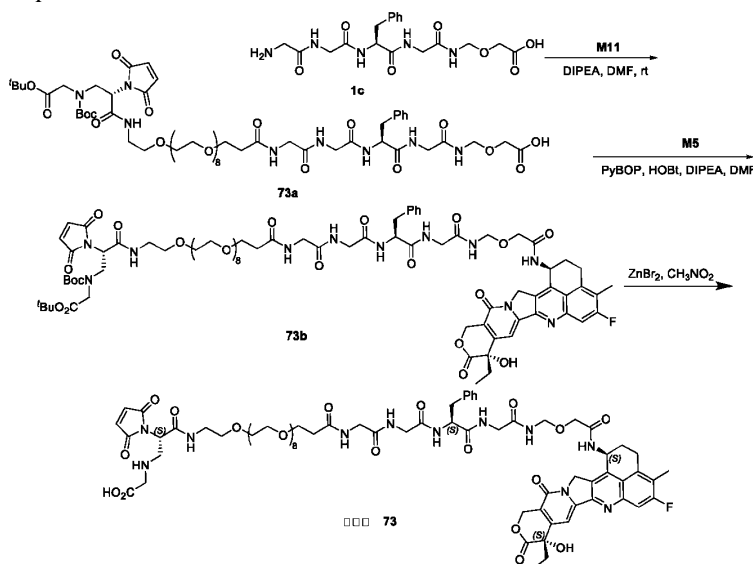
Synthesis of compound **M11**:

In a 100 mL flask, compound **M3** (11.0 g, 19.5 mmol, 1.0 eq), DIPEA (2.8 g, 21.4 mmol, 1.1 eq), 27-amino-4, 7, 10, 13, 16, 19, 22, 25-octaoxaheptadecanoic acid (9.7 g, 20.5 mmol, 1.05 eq) and DMF (60 mL) and reacted for 20 min at room temperature (monitored by TLC). The reaction solution was purified directly by preparation, and the preparative solution was concentrated by pumping water under reduced pressure at 35 °C to remove acetonitrile, and lyophilized to obtain compound **M10** (13.2 g) in 78% yield; LC-MS: $[M+H]^+ = 866.5$.

In a 100 mL flask, compound **M10** (13.0 g, 15 mmol, 1.0 eq), pentafluorophenol (3 g, 16.5 mmol, 1.1 eq), DCC (3.4 g, 16.5 mmol, 1.1 eq) and THF (30 mL) were added, and the reaction was carried out at room temperature for 30 min (monitored by TLC), and insoluble material was filtered off. The reaction solution was purified directly by preparation, and the preparative solution was concentrated by pumping water under reduced pressure at 35 °C to remove acetonitrile, and lyophilized to obtain compound **M11** (14.2 g) in 92% yield; LC-MS: $[M+H]^+ = 1032.5$.

Example 82

Synthesis of compound **73**:



Step 1: Synthesis of compound **73a**

To **M11** (1 g, 0.79 mol), 10 mL of DMF was added, cooled to 0 °C in an ice-water bath,

compound **1c** (334 mg, 0.79 mol), DIPEA (154 mg, 1.19 mol) was added, and the reaction was maintained under these conditions for 1 h. The reaction was monitored by TLC for complete reaction, and the reaction solution was purified by preparative grade HPLC (acetonitrile/water system), and the target peak was collected. The target peak was collected, and after removing acetonitrile under reduced pressure, lyophilized to give compound **73a** about 1.2 g, MS m/z : $[M+H]^+ = 1271.9$.

Step 2: Synthesis of compound **73b**

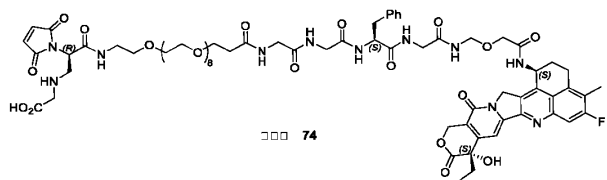
73a (1.2 g, 0.94 mmol), **M5** (500 mg, 0.94 mmol), PyBOP (625 mg, 1.2 mmol), HOBt (162 mg, 1.2 mmol) and 15 mL of DMF were added to a 25 mL flask, and DIPEA (310 mg, 2.4 mmol) was added to the vial under an ice-water bath, and the reaction was carried out for 2 h. After the completion of the reaction was monitored by HPLC, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution, which was lyophilized to give **73b** (709 mg); LC-MS: $[M+H]^+ = 1720.8$.

Step 3: Synthesis of compound **73**:

73b (200 mg, 0.116 mmol), zinc bromide (523 mg, 2.32 mmol) and 10 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain the solid compound **73** (88 mg); LC-MS: $[M+H]^+ = 1532.6$.

Example 83

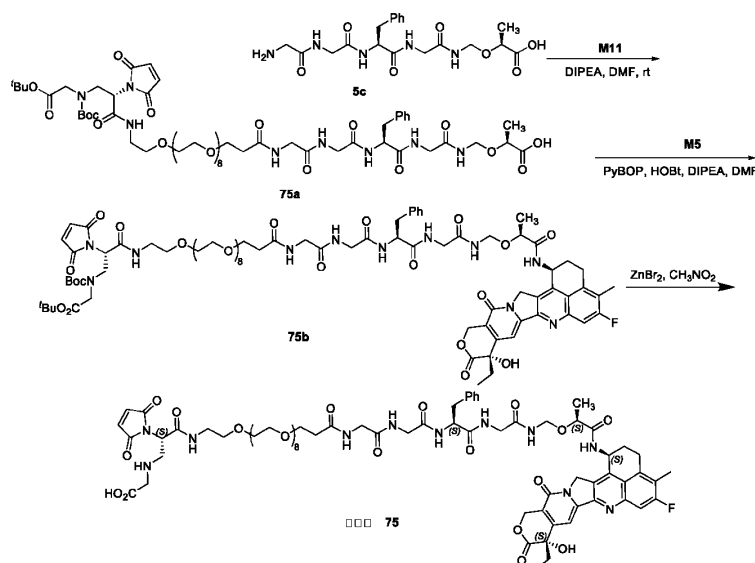
Synthesis of compound **74**:



Referring to the synthetic route of Example 82, compound **74** (90 mg) was obtained; LC-MS: $[M+H]^+ = 1532.6$.

Example 84

Synthesis of compound **75**:



Step 1: Synthesis of compound **75a**

To **M11** (1 g, 0.79 mol), 10 mL of DMF was added, cooled to 0 °C in an ice-water bath, compound **5c** (345 mg, 0.79 mol), DIPEA (154 mg, 1.19 mol) was added, and the reaction was maintained under these conditions for 1 h. The reaction was monitored by TLC for complete reaction, and the reaction solution was purified by preparative grade HPLC (acetonitrile/water system), and the target peak was collected. The target peak was collected, and after removing acetonitrile under reduced pressure, lyophilized to give compound **75a** 0.9 g, MS m/z : $[M+H]^+ = 1285.6$.

10 Step 2: Synthesis of compound **75b**

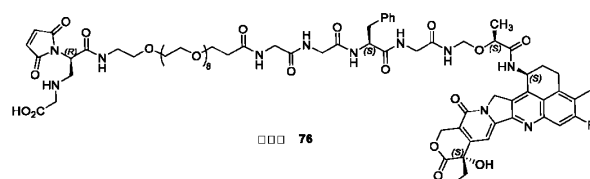
75a (700 mg, 0.54 mmol), **M5** (289 mg, 0.54 mmol), PyBOP (313 mg, 0.6 mmol), HOBt (81 mg, 0.6 mmol) and 10 mL DMF were added to a 25 mL flask, and DIPEA (155 mg, 1.2 mmol) was added under an ice-water bath, and the mixture was heated to room temperature for 2 h. After the reaction was completed, the reaction solution was purified by HPLC to obtain a preparative solution, which was lyophilized to obtain **75b** (304 mg); LC-MS: $[M+H]^+ = 1734.8$.

Step 3: Synthesis of compound **75**:

75b (200 mg, 0.116 mmol), zinc bromide (523 mg, 2.32 mmol) and 10 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure to remove the solvent, and the crude product was obtained. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain the solid compound **75** (96 mg); LC-MS: $[M+H]^+ = 1546.6$.

Example 85

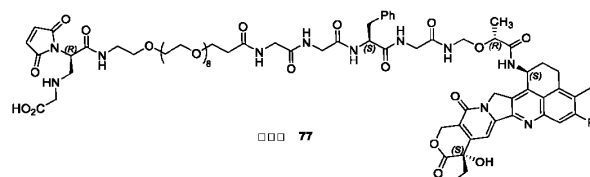
Synthesis of compound **76**:



Referring to the synthetic route of Example 84, compound **76** (92 mg) was obtained; LC-MS: $[M+H]^+ = 1546.5$.

Example 86

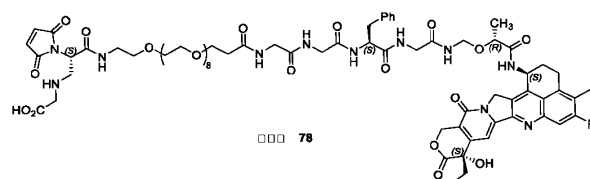
Synthesis of compound **77**:



Referring to the synthetic route of Example 84, compound **77** (87 mg) was obtained; LC-MS: $[M+H]^+ = 1546.5$.

Example 87

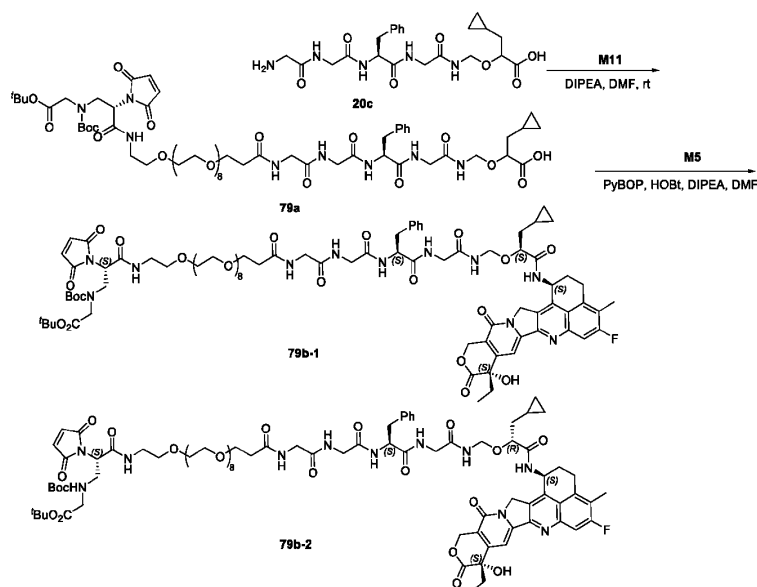
10 Synthesis of compound **78**:



Referring to the synthetic route of Example 84, compound **78** (94 mg) was obtained; LC-MS: $[M+H]^+ = 1546.7$.

Example 88

Synthesis of compounds **79** and **80**:



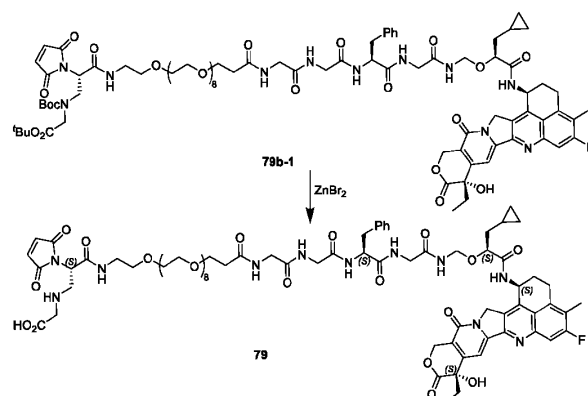
Step 1: Synthesis of compound **79a**

To **M11** (1 g, 0.79 mol), 10 mL of DMF was added, cooled to 0 °C in an ice-water bath, compound **20c** (377 mg, 0.79 mol), DIPEA (154 mg, 1.19 mol) was added, and the reaction was maintained under these conditions for 1 h. The reaction was monitored for completeness by TLC, and the reaction solution was purified by preparative-grade high-performance liquid chromatography (acetonitrile/water system), and the target peak was collected. The target peak was collected and after removing acetonitrile under reduced pressure, lyophilized to give 783 mg of compound **79a**, MS m/z : $[M+H]^+ = 1325.8$.

10 Step 2: Synthesis of compounds **79b-1** and **79b-2**

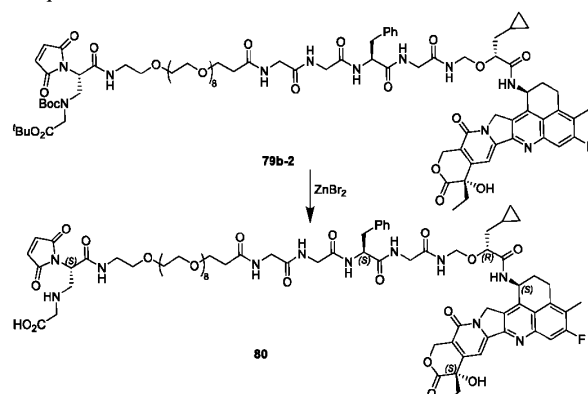
79a (600 mg, 0.45 mmol), **M5** (240 mg, 0.45 mmol), PyBOP (261 mg, 0.5 mmol), HOBT (68 mg, 0.5 mmol) and 10 mL of DMF were added to a 25 mL flask, and DIPEA (130 mg, 1 mmol) was added to the flask under an ice-water bath, and the reaction was carried out for 2 h. The reaction was monitored by HPLC. After the reaction was monitored by HPLC, the reaction solution was purified by high performance liquid chromatography (HPLC) to obtain the preparative solution of compounds **79b-1** and **79b-2**, and the preparative solution was lyophilized to obtain **79b-1** (124 mg); LC-MS: $[M+H]^+ = 1743.0$; **79b-1** (122 mg) was obtained; LC-MS: $[M+H]^+ = 1743.0$.

Step 3: Synthesis of compound **79**



79b-1 (100 mg, 0.057 mmol), zinc bromide (258 mg, 1.15 mmol) and 10 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure and the product was obtained as a crude product. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain the solid compound **79** (30 mg); LC-MS: $[M+H]^+ = 1586.9$.

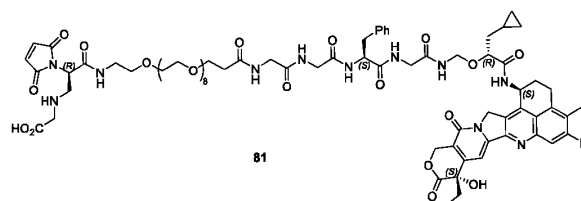
Step 4: Synthesis of Compound **80**



79b-2 (100 mg, 0.057 mmol), zinc bromide (258 mg, 1.15 mmol) and 10 mL of nitromethane were added to a 25 mL flask and the reaction was carried out at 40 °C for 1 h. After the completion of the reaction was monitored by HPLC, the solvent was concentrated under reduced pressure and removed to obtain the crude product. The crude product was purified by high performance liquid chromatography (HPLC) to obtain the product preparation solution, and the preparation solution was lyophilized to obtain the solid compound **80** (33 mg); LC-MS: $[M+H]^+ = 1587.0$.

Example 89

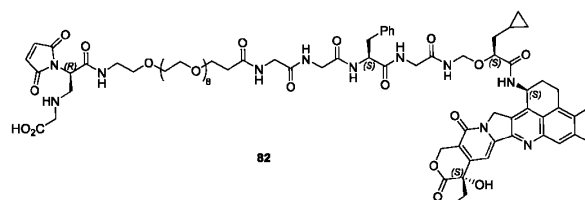
Synthesis of compound **81**:



Referring to the synthetic route of Example 88, compound **81** (24 mg) was obtained; LC-MS: $[M+H]^+ = 1586.9$.

Example 90

Synthesis of compound **82**:



Referring to the synthetic route of Example 88, compound **82** (29 mg) was obtained; LC-MS: $[M+H]^+ = 1586.9$.

Example 91

1) Expression and purification of a humanized anti-Claudin18.2 antibody, 5103F3-BSM:

Expi293 (Shanghai OPM Biotechnology Co., Ltd.) suspension cells were used to express 5103F3-BSM antibody. One day before transfection, cells were inoculated at a density of 0.9×10^6 cells/mL in 1 L shake flasks containing 300 mL of OPM-293 CD05 Medium (81075-001, Shanghai Oppermax Bio-technology Co., Ltd.), and cultured overnight at 37 °C, 5% CO₂, and 120 rpm on a cell culture shaker. On the next day, PEI-MAX was used to transfect the antibody expression plasmid, in which the mass ratio of plasmid:PEI-MAX was 1:3. OPM-293 ProFeed supplement was added at 5% (v/v) on the first day after transfection, and then at 5% (v/v) on the third day after transfection, and then centrifuged to collect the supernatant on the sixth day after transfection.

The cell expression supernatants obtained were collected and eluted with 0.05 M sodium acetate buffer (pH 3.6) by a Protein A affinity chromatography column (UniMab 50, Suzhou Nano Technology Co., Ltd.), and the captured antibody was adjusted to pH 7.0 with 1 M Tris-HCl (pH 8.8) at 0.7/10 (v/v), and then passed through a gel filtration chromatography column SEC (Superdex 200 Increase, Cytiva) to remove impurities such as polymers, while the antibody buffer was replaced with 20 mM sodium acetate buffer (pH 6.0).

Example 92

Preparing the samples of 5103F3-BSM antibody-drug conjugate by coupling 5103F3-BSM antibody with payload:

After the cell expression and purification by Protein A affinity chromatography and molecular sieve chromatography, the anti-Claudin 18.2 antibody was replaced in 20 mM NaAc-HAc, pH 6.0 buffer, concentrated or diluted, and the anti-Claudin 18.2 antibody was concentrated to a protein concentration of 5 mg/mL. The white powder of linker plus the drug was dissolved in DMA to 10

mg/mL and set aside. To open the interchain disulfide bonds of anti-Claudin18.2 antibody, 15-fold TCEP was added according to the ratio of molecules and reacted at room temperature for 2 h. Then 16-fold ligand plus drug solution was added according to the ratio of molecules and reacted at room temperature for 2 h. At the end of the reaction, the ligand plus drug that had not been coupled with the anti-Claudin18.2 antibody was removed by ultrafiltration using a 30 KDa ultrafiltration centrifuge tube. After the reaction, use 30 KDa ultrafiltration centrifuge tube to remove the linker plus drug that is not coupled with anti-Claudin18.2 antibody.

The resulting samples of anti-Claudin 18.2 antibody-drug conjugate will be used to determine the monomer rate by SEC-HPLC and the drug load by RP-HPLC or HIC-HPLC.

10

Example 93

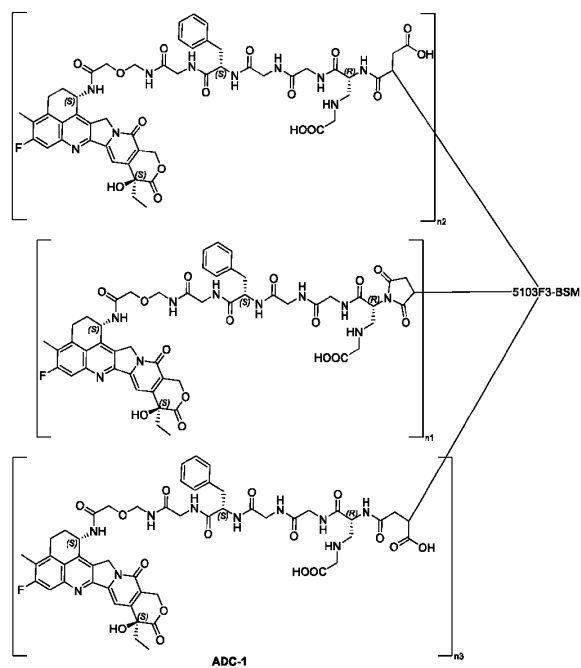
Samples of the antibody 64C9-drug conjugate were prepared by coupling 64C9 antibody with payload after the cell expression and purification by Protein A affinity chromatography and molecular sieve chromatography, the anti-Claudin18.2 antibody was replaced in 20 mM NaAc-HAc, pH 6.0 buffer, and the anti-Claudin18.2 antibody was concentrated or diluted to a protein concentration of 5 mg/mL. The white powder linker plus drug was dissolved in DMA to 10 mg/mL for backup. To open the interchain disulfide bonds of anti-Claudin18.2 antibody, 8-12 times of TCEP was added according to the molecular ratio, and the reaction was carried out at room temperature for 1-2 h. Then 5-8 times of the linker plus drug solution was added according to the molecular ratio, and the reaction was carried out at room temperature for 1-2 h. The linker plus drug that had not been coupled with anti-Claudin18.2 antibody was removed at the end of the reaction by ultrafiltration using 30 KDa ultrafiltration centrifuge tube. At the end of the reaction, a 30 KDa ultrafiltration centrifuge tube was used to remove the linker plus drug that is not coupled with anti-Claudin18.2 antibody, and then the sample of anti-Claudin18.2 antibody-drug conjugate was obtained.

20

The resulting samples of anti-Claudin18.2 antibody-drug conjugate will be used to determine the monomer rate by SEC-HPLC and the drug load by RP-HPLC or HIC-HPLC.

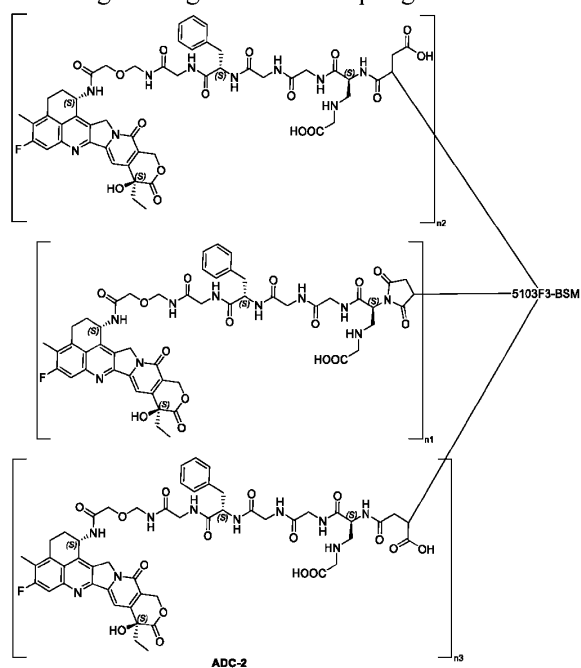
Example 94

ADC-1 was prepared according to the generalized coupling method of Example 92:



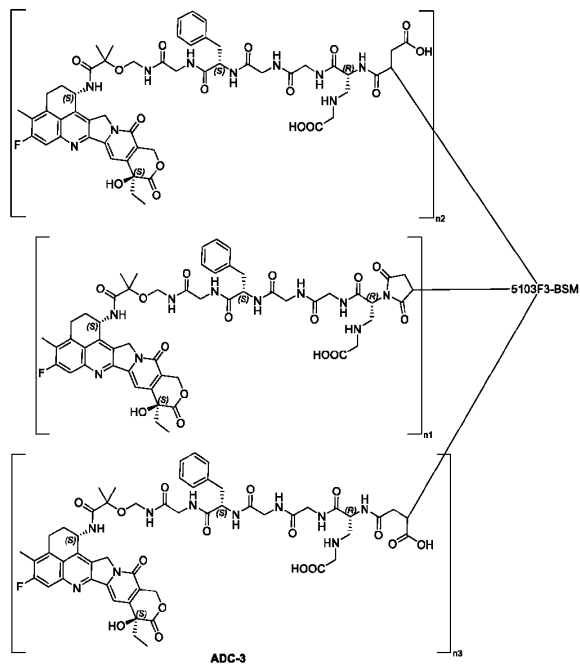
Example 95

ADC-2 was prepared according to the generalized coupling method of Example 92:



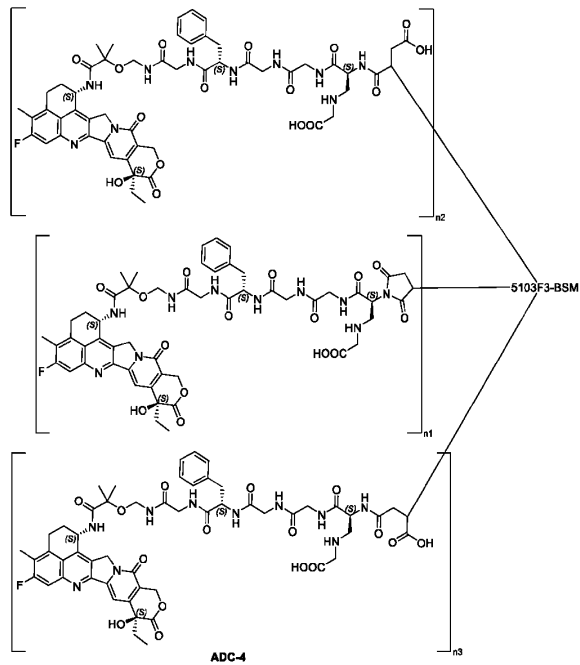
Example 96

ADC-3 was prepared according to the generalized coupling method of Example 92:



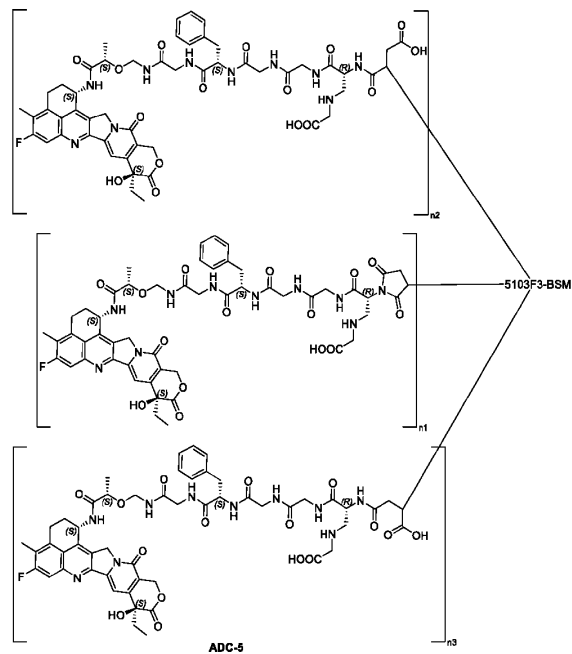
Example 97

ADC-4 was prepared according to the generalized coupling method of Example 92:



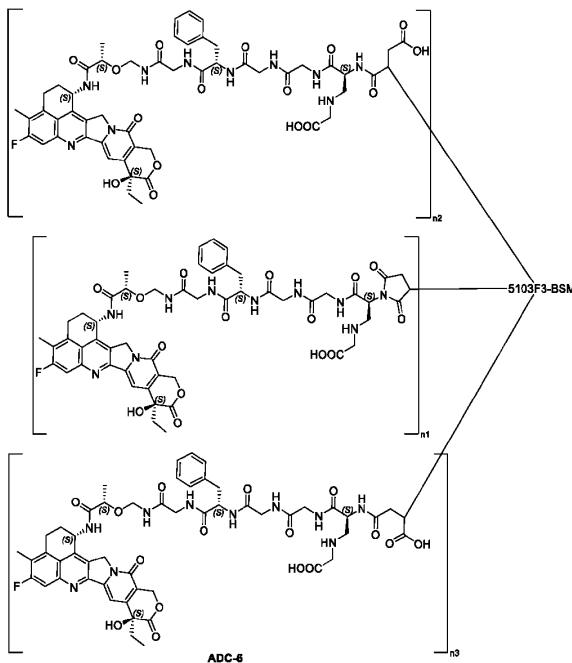
Example 98

ADC-5 was prepared according to the generalized coupling method of Example 92:



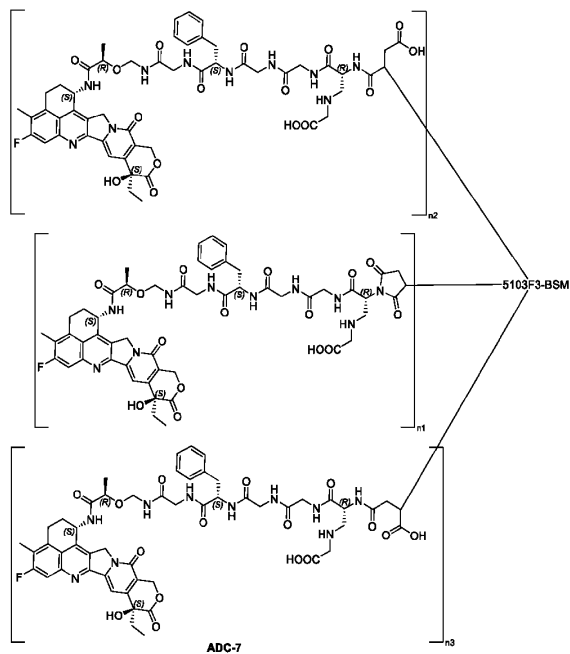
Example 99

ADC-6 was prepared according to the generalized coupling method of Example 92:



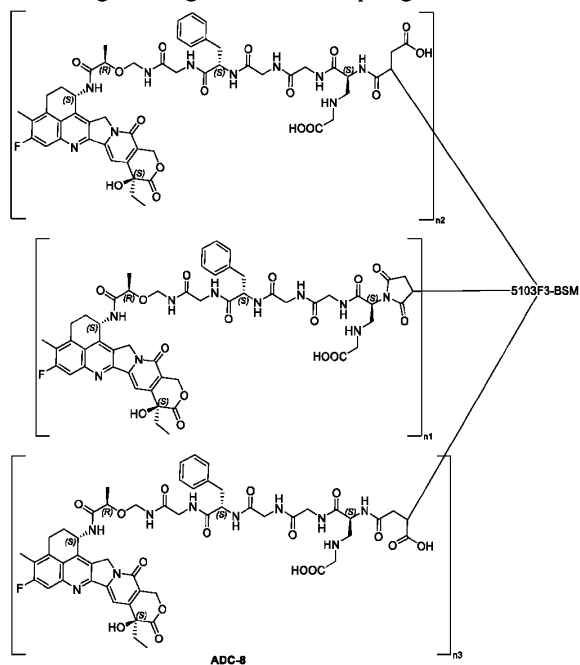
Example 100

ADC-7 was prepared according to the generalized coupling method of Example 92:



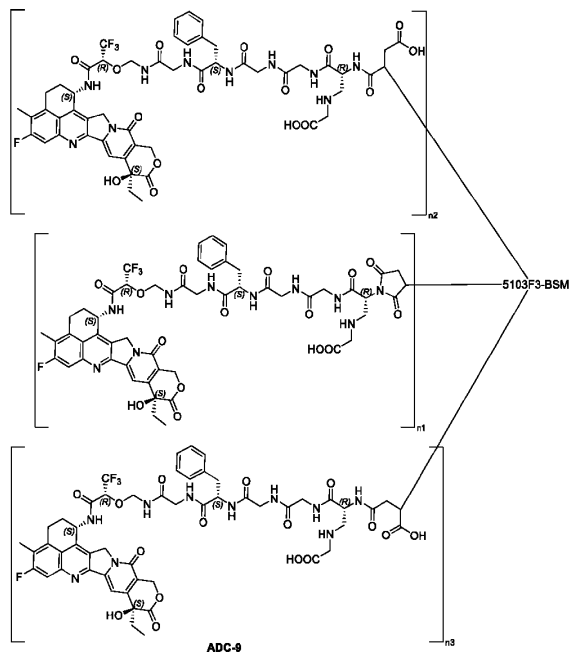
Example 101

ADC-8 was prepared according to the generalized coupling method of Example 92:



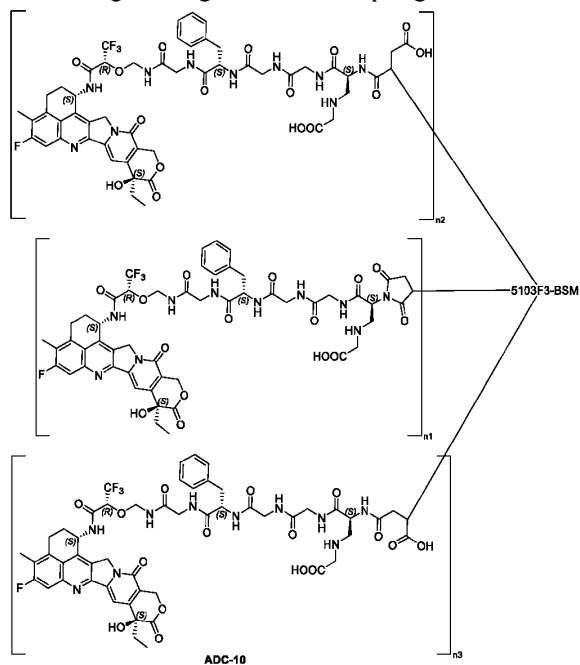
Example 102

ADC-9 was prepared according to the generalized coupling method of Example 92.



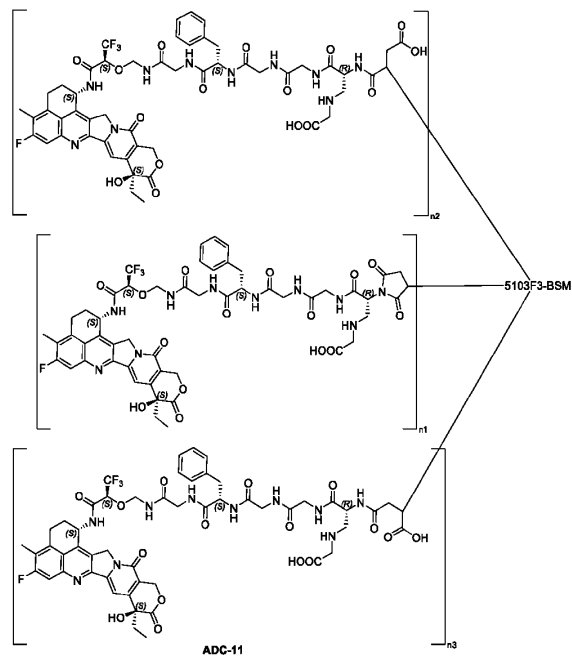
Example 103

ADC-10 was prepared according to the generalized coupling method of Example 92.



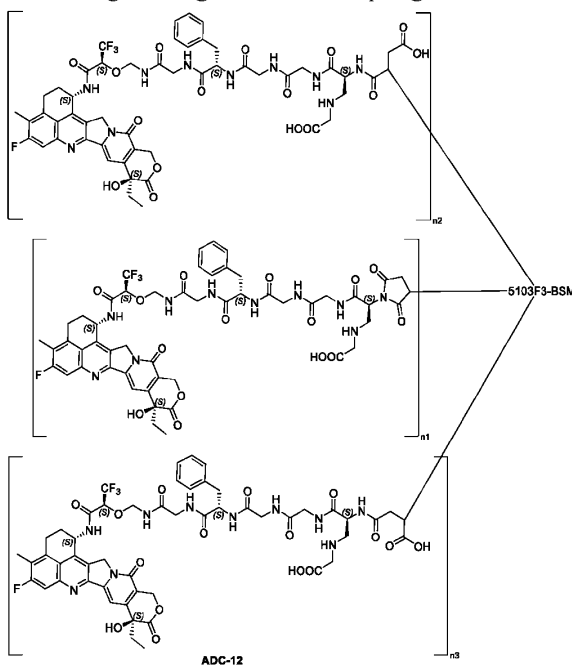
Example 104

ADC-11 was prepared according to the generalized coupling method of Example 92:



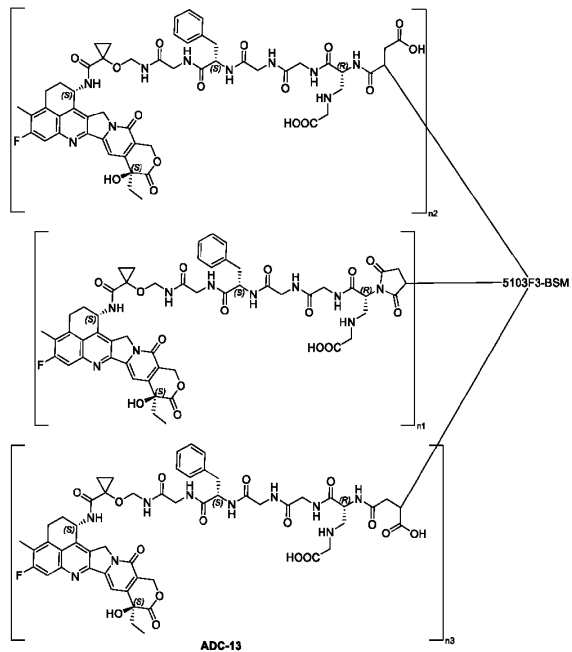
Example 105

ADC-12 was prepared according to the generalized coupling method of Example 92:



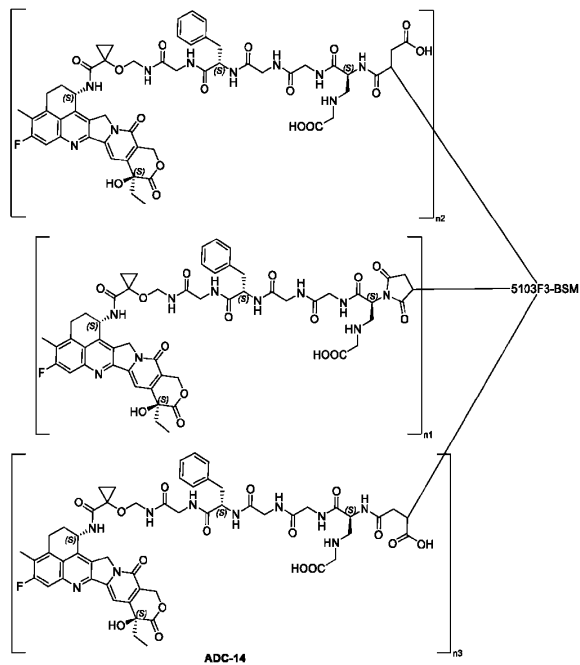
Example 106

ADC-13 was prepared according to the generalized coupling method of Example 92:



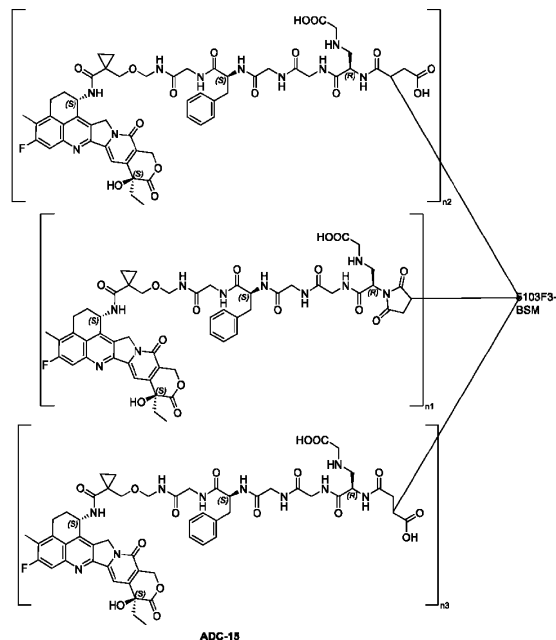
Example 107

ADC-14 was prepared according to the generalized coupling method of Example 92:



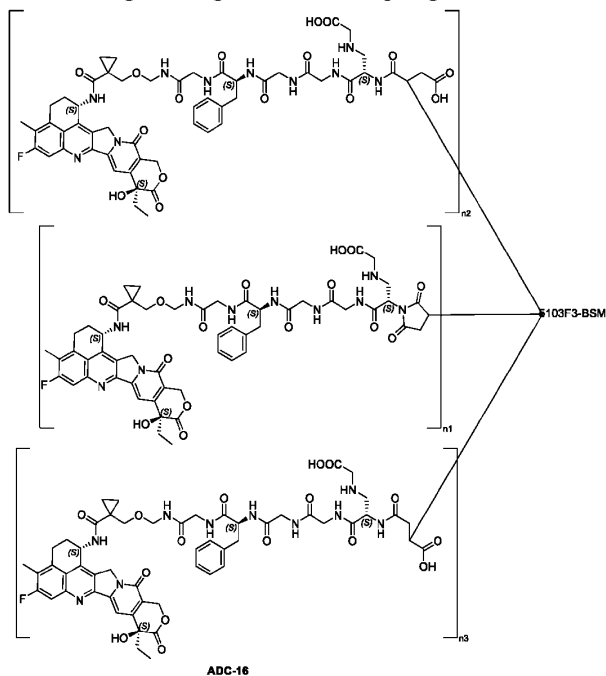
Example 108

ADC-15 was prepared according to the generalized coupling method of Example 92:



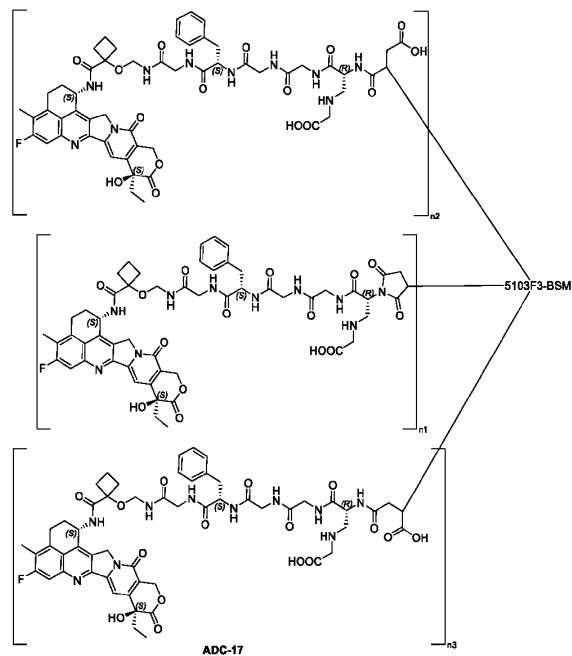
Example 109

ADC-16 was prepared according to the generalized coupling method of Example 92:



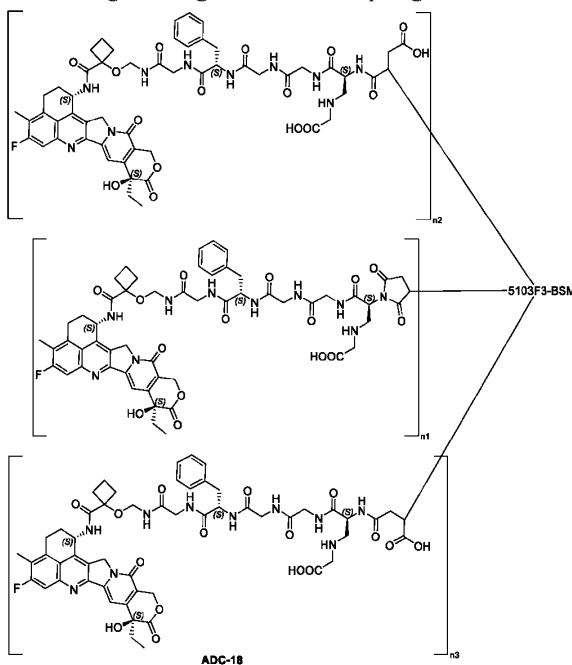
Example 110

ADC-17 was prepared according to the generalized coupling method of Example 92:



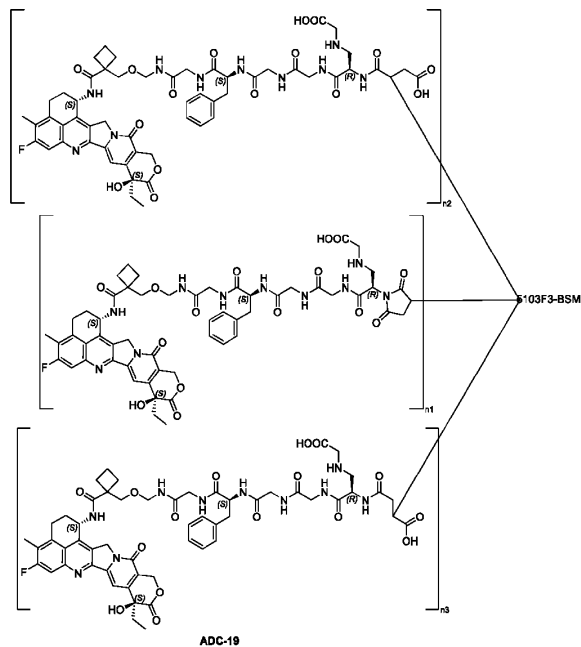
Example 111

ADC-18 was prepared according to the generalized coupling method of Example 92:



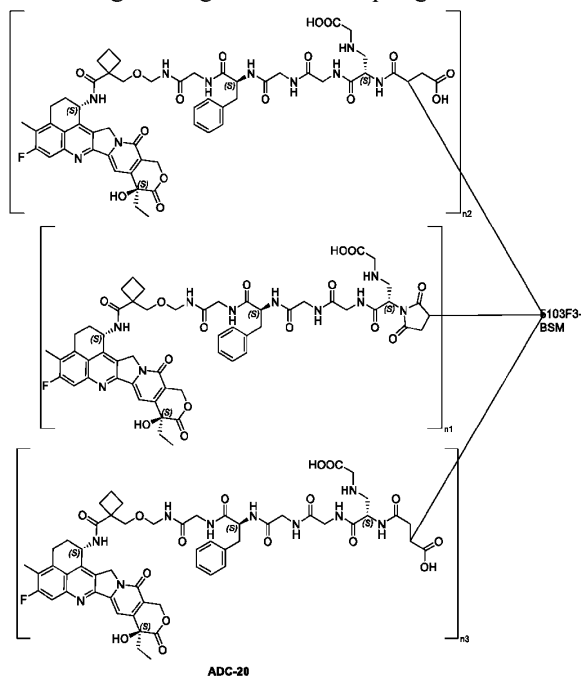
Example 112

ADC-19 was prepared according to the generalized coupling method of Example 92:



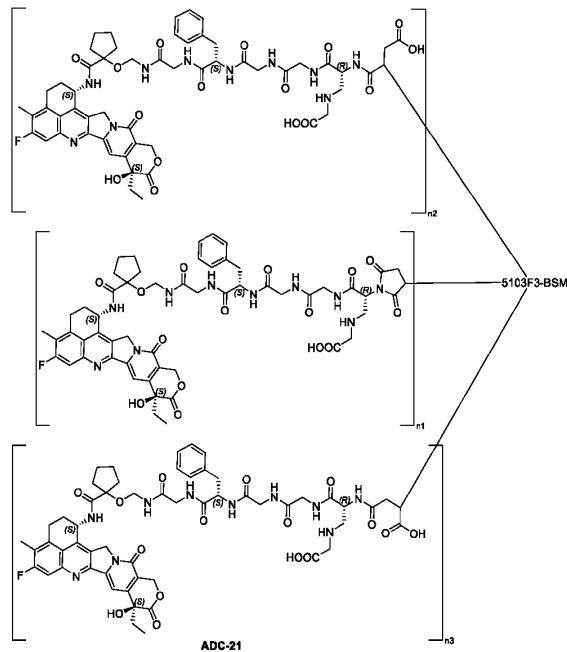
Example 113

ADC-20 was prepared according to the generalized coupling method of Example 92:



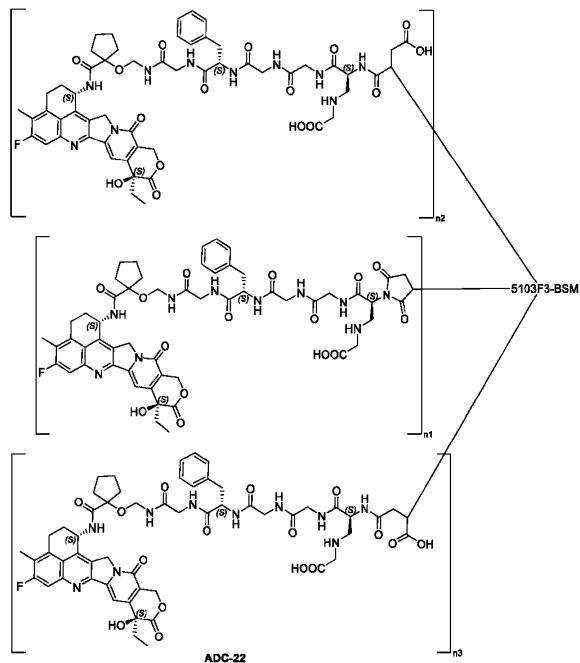
Example 114

ADC-21 was prepared according to the generalized coupling method of Example 92:



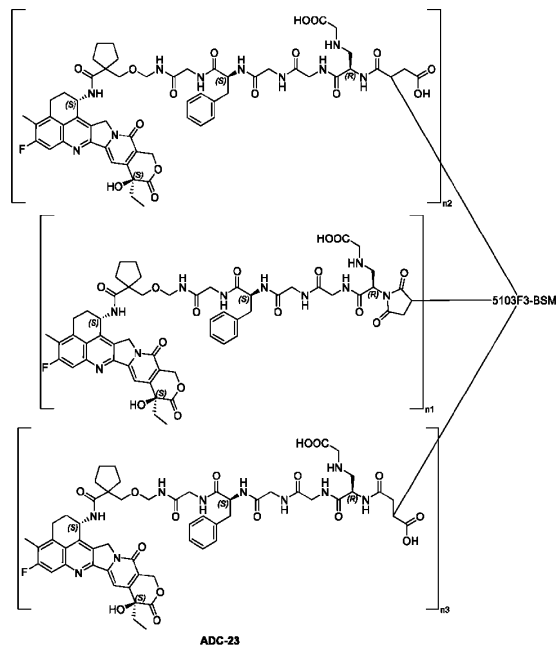
Example 115

ADC-22 was prepared according to the generalized coupling method of Example 92:



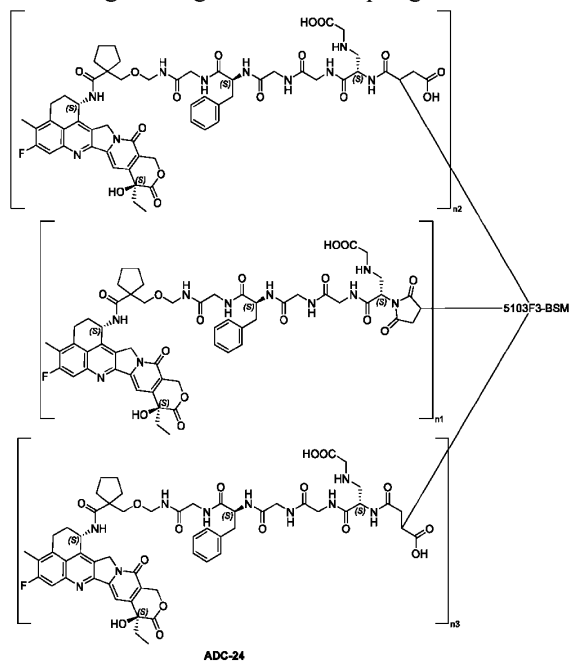
Example 116

ADC-23 was prepared according to the generalized coupling method of Example 92:



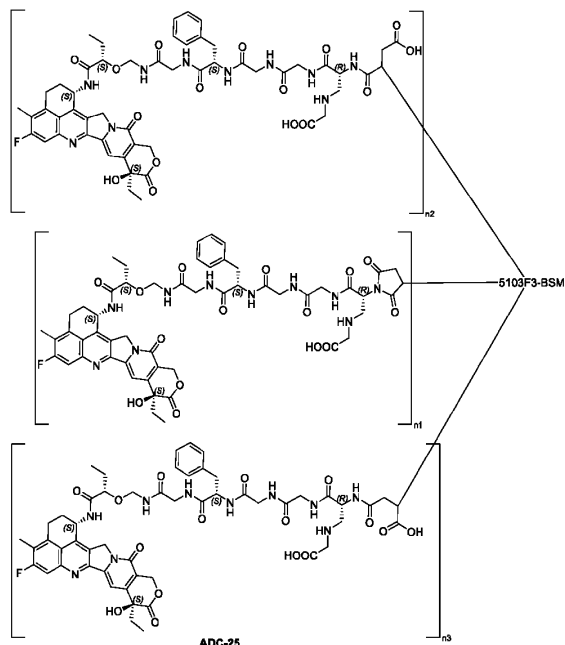
Example 117

ADC-24 was prepared according to the generalized coupling method of Example 92:



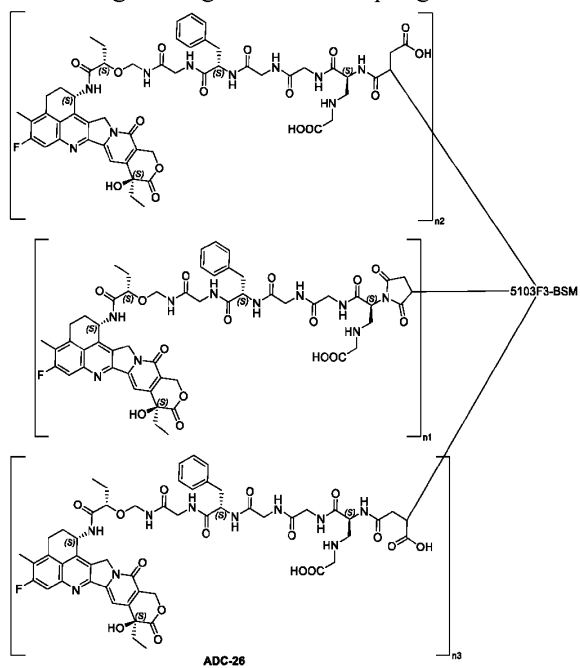
Example 118

ADC-25 was prepared according to the generalized coupling method of Example 92:



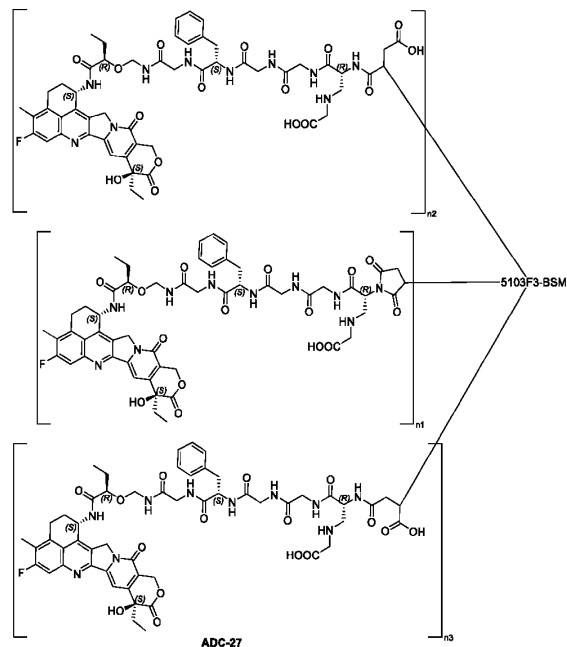
Example 119

ADC-26 was prepared according to the generalized coupling method of Example 92:



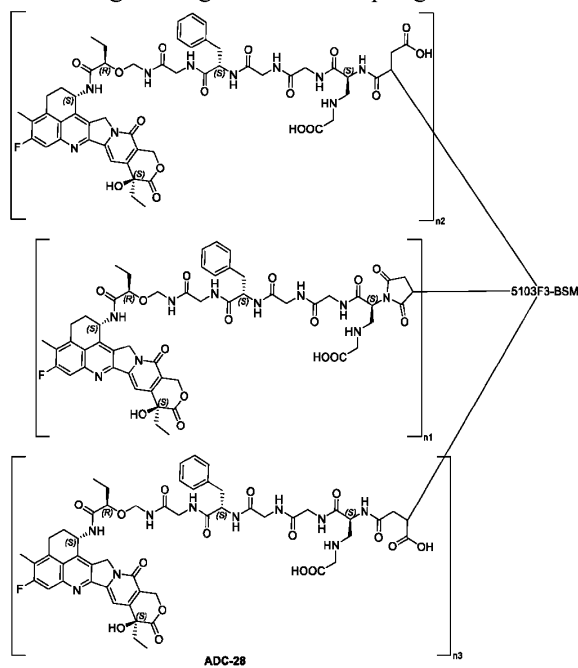
Example 120

ADC-27 was prepared according to the generalized coupling method of Example 92:



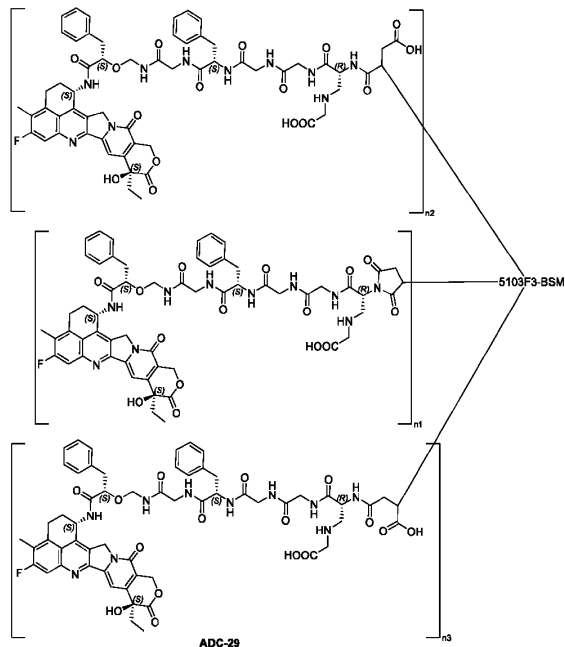
Example 121

ADC-28 was prepared according to the generalized coupling method of Example 92:



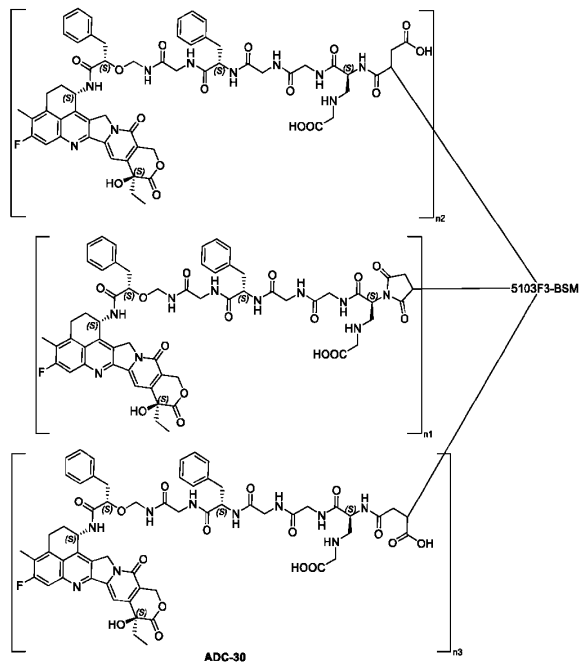
Example 122

ADC-29 was prepared according to the generalized coupling method of Example 92.



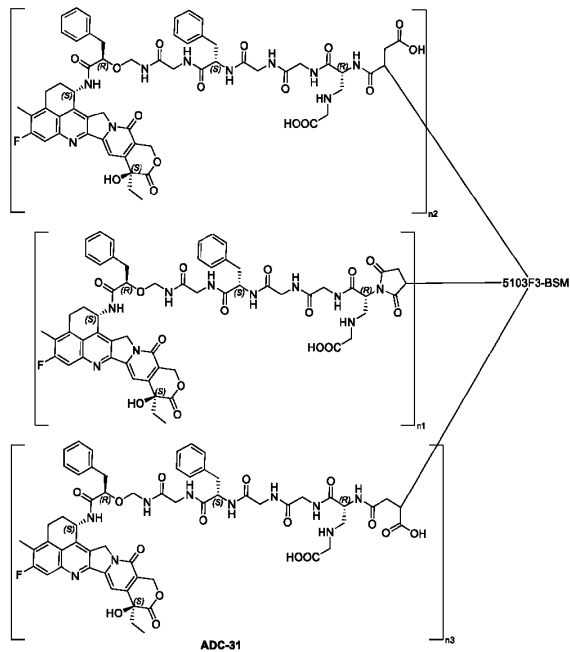
Example 123

ADC-30 was prepared according to the generalized coupling method of Example 92:



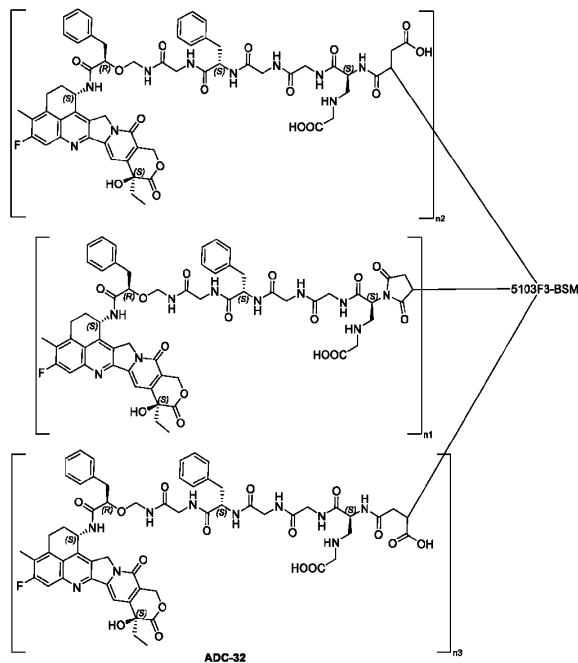
Example 124

ADC-31 was prepared according to the generalized coupling method of Example 92:



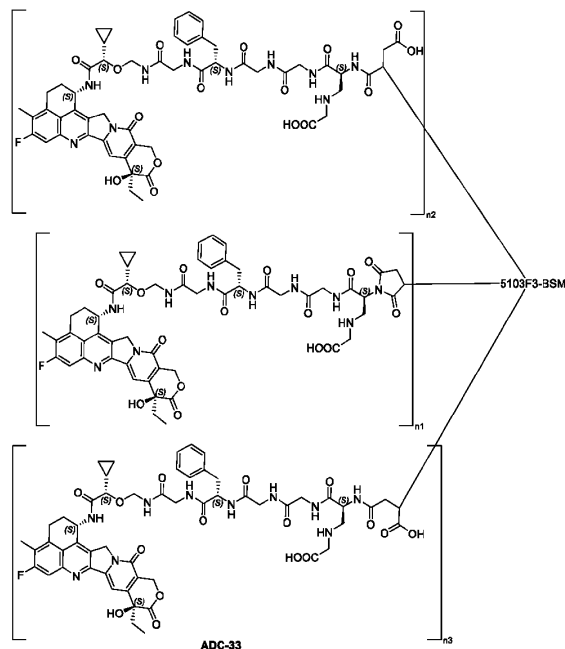
Example 125

ADC-32 was prepared according to the generalized coupling method of Example 92:



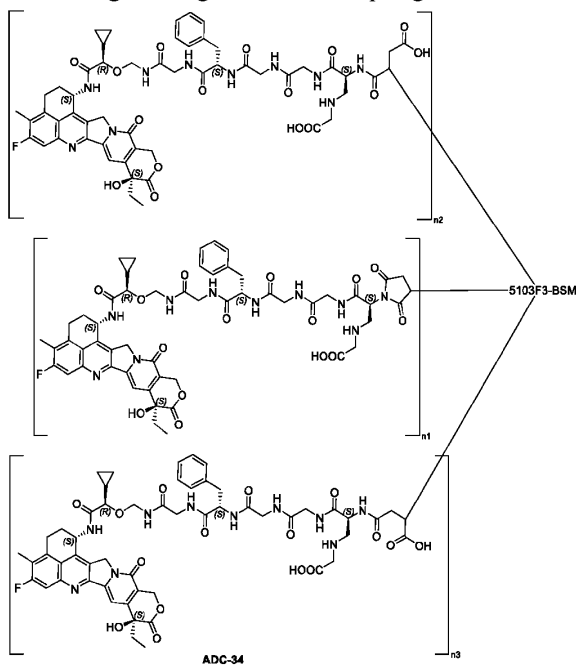
Example 126

ADC-33 was prepared according to the generalized coupling method of Example 92:



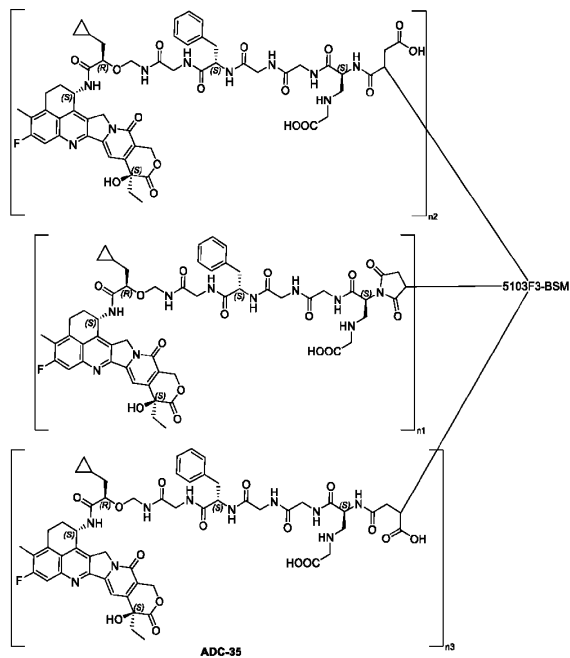
Example 127

ADC-34 was prepared according to the generalized coupling method of Example 92:



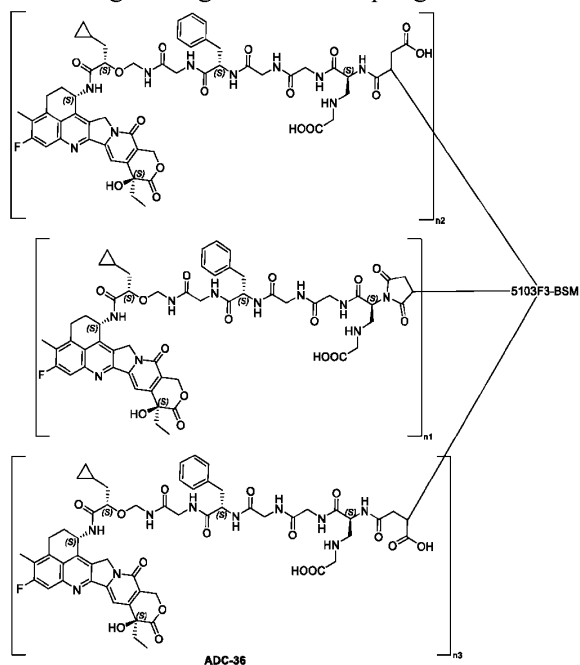
Example 128

ADC-35 was prepared according to the generalized coupling method of Example 92:



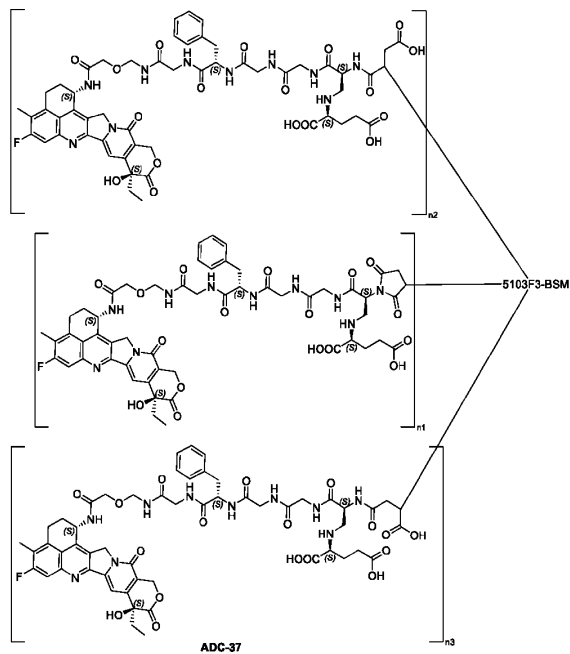
Example 129

ADC-36 was prepared according to the generalized coupling method of Example 92:



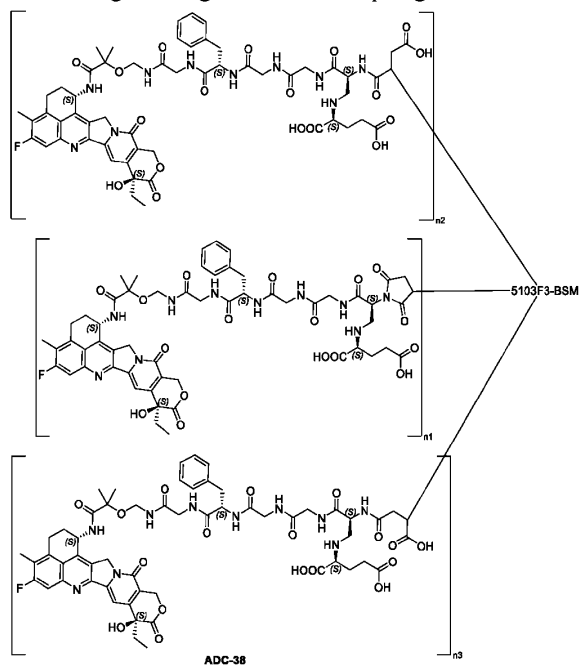
Example 130

ADC-37 was prepared according to the generalized coupling method of Example 92:



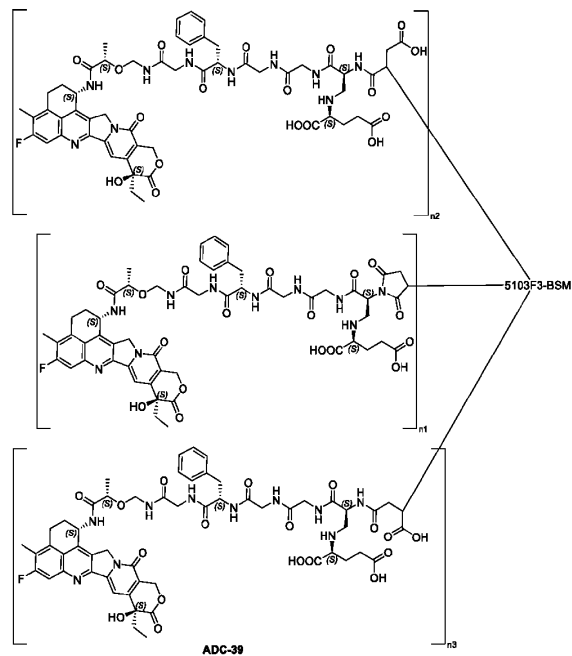
Example 131

ADC-38 was prepared according to the generalized coupling method of Example 92:



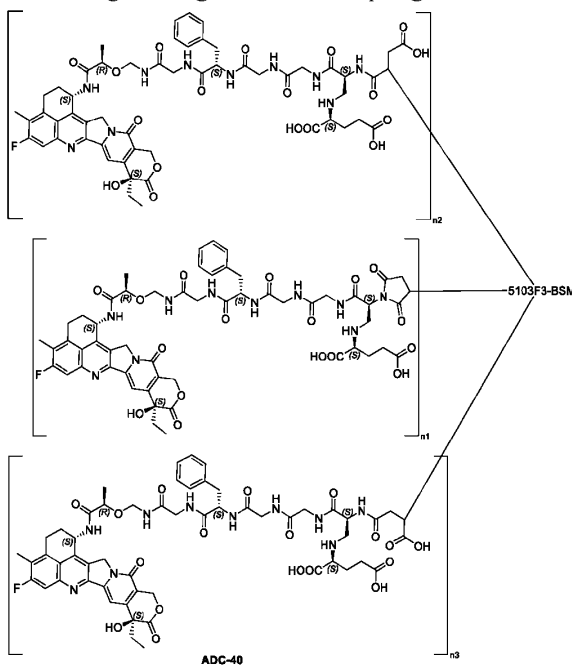
Example 132

ADC-39 was prepared according to the generalized coupling method of Example 92:



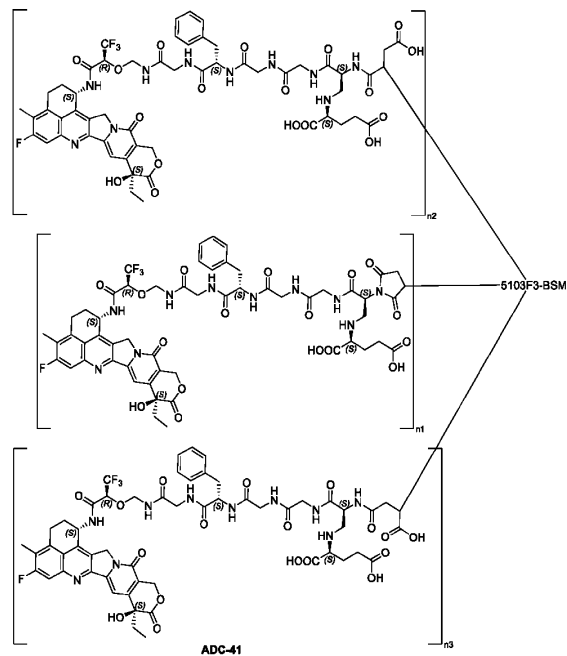
Example 133

ADC-40 was prepared according to the generalized coupling method of Example 92:



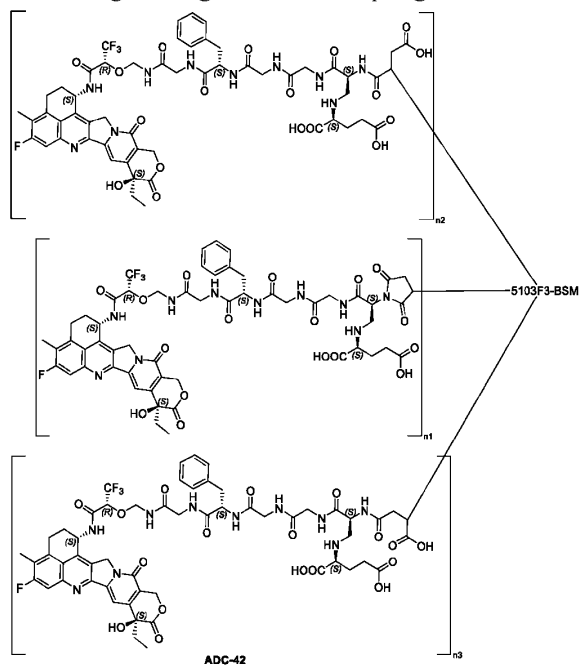
Example 134

ADC-41 was prepared according to the generalized coupling method of Example 92:



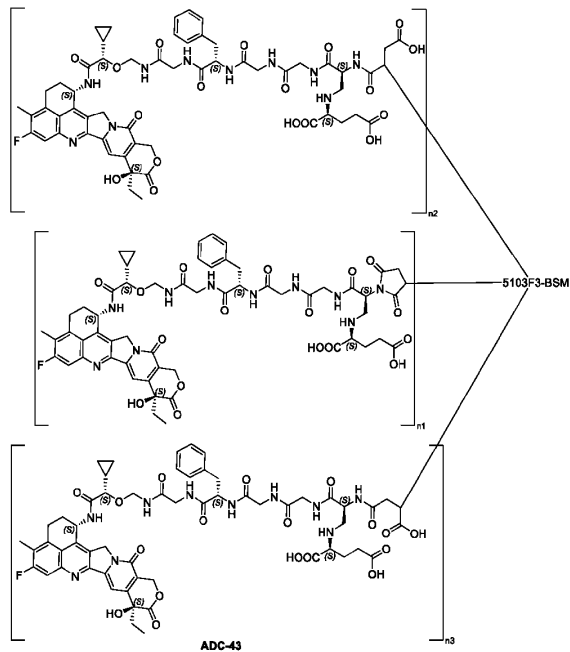
Example 135

ADC-42 was prepared according to the generalized coupling method of Example 92:



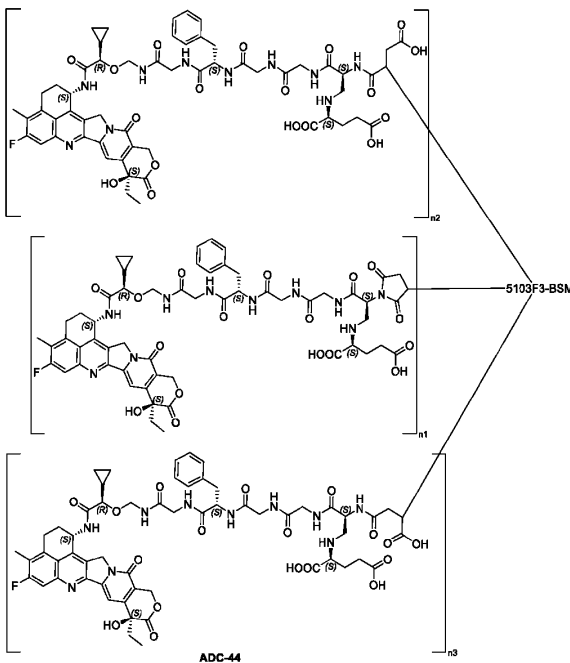
Example 136

ADC-43 was prepared according to the generalized coupling method of Example 92:



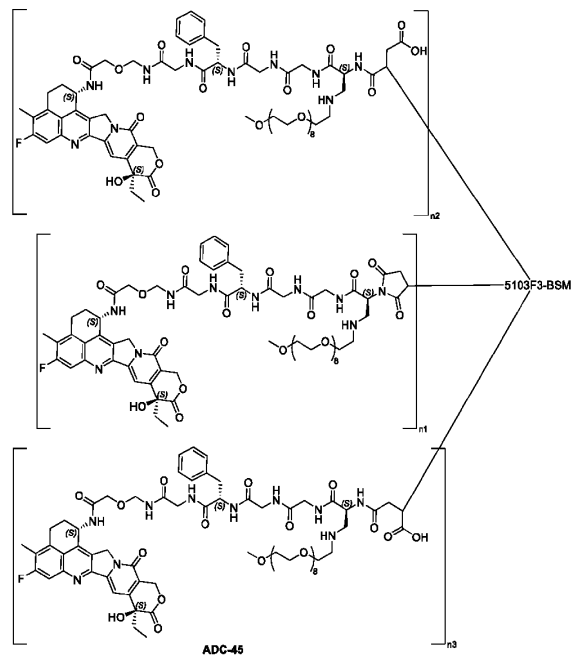
Example 137

ADC-44 was prepared according to the generalized coupling method of Example 92:



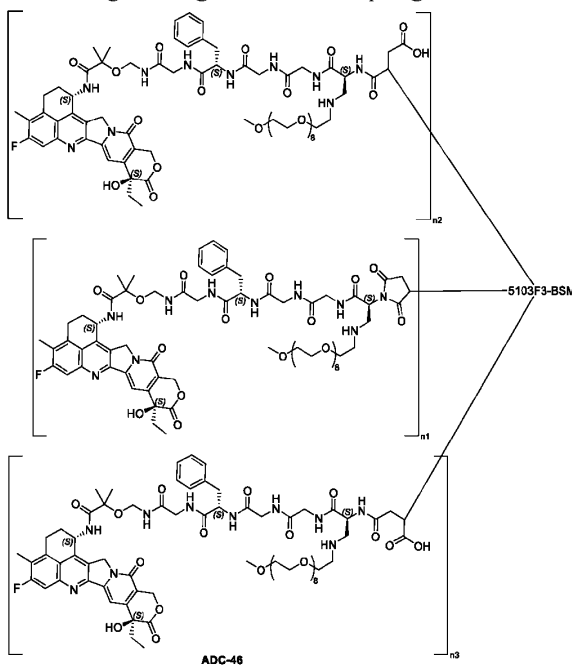
Example 138

ADC-45 was prepared according to the generalized coupling method of Example 92:



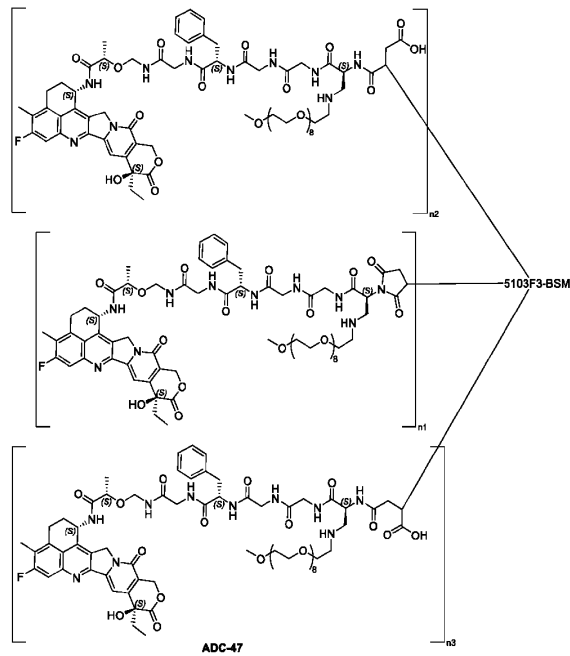
Example 139

ADC-46 was prepared according to the generalized coupling method of Example 92:



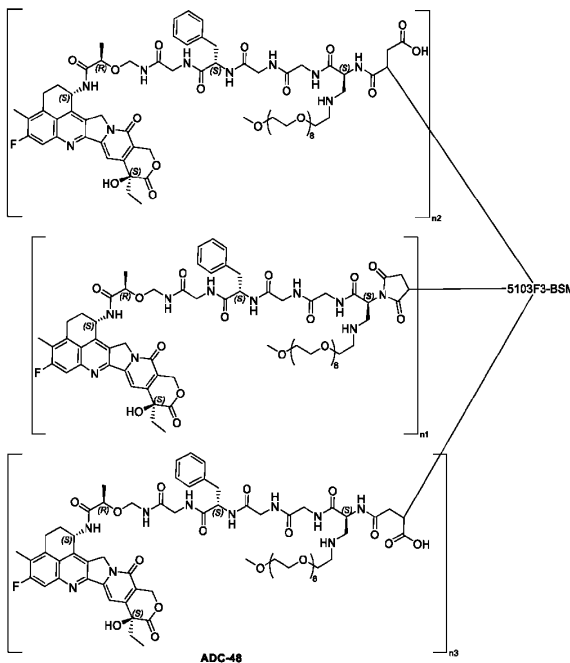
Example 140

ADC-47 was prepared according to the generalized coupling method of Example 92:



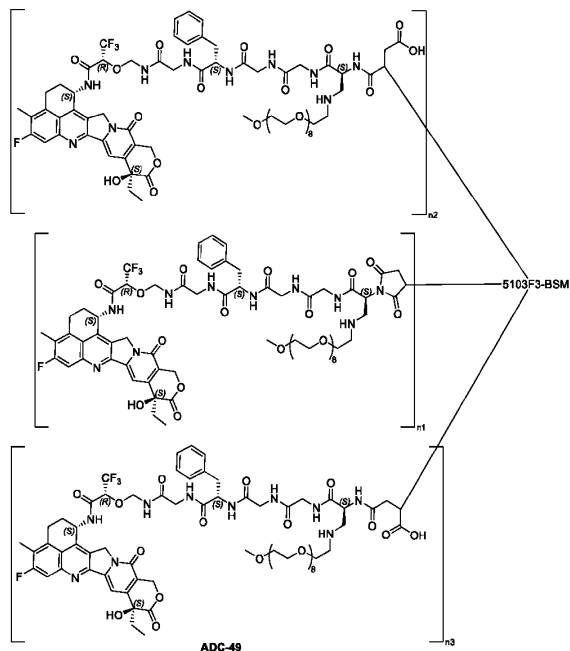
Example 141

ADC-48 was prepared according to the generalized coupling method of Example 92:



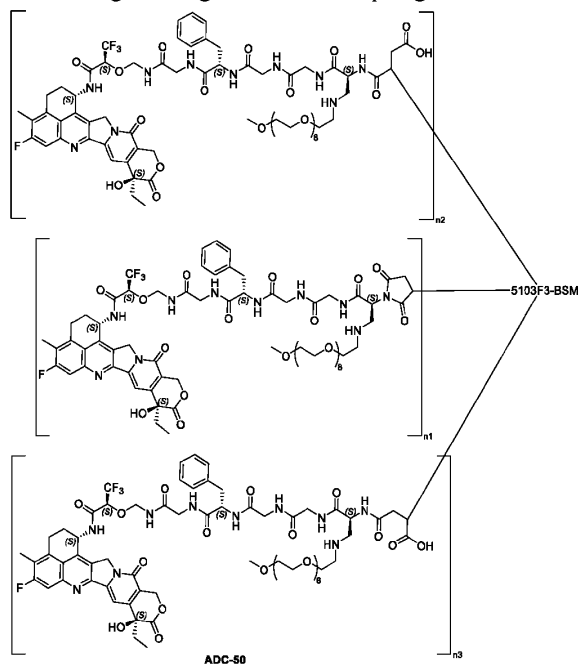
Example 142

ADC-49 was prepared according to the generalized coupling method of Example 92:



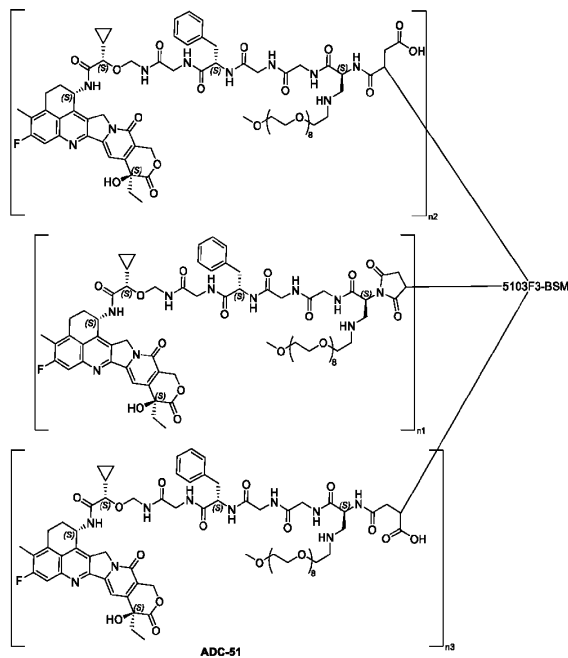
Example 143

ADC-50 was prepared according to the generalized coupling method of Example 92:



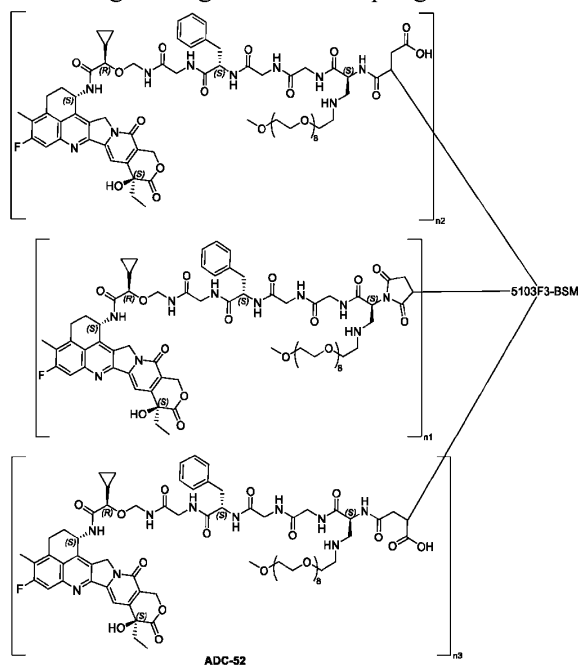
Example 144

ADC-51 was prepared according to the generalized coupling method of Example 92:



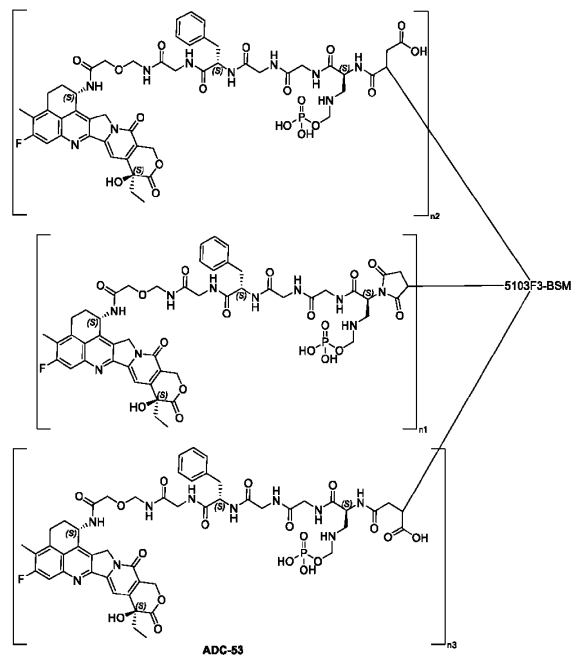
Example 145

ADC-52 was prepared according to the generalized coupling method of Example 92:



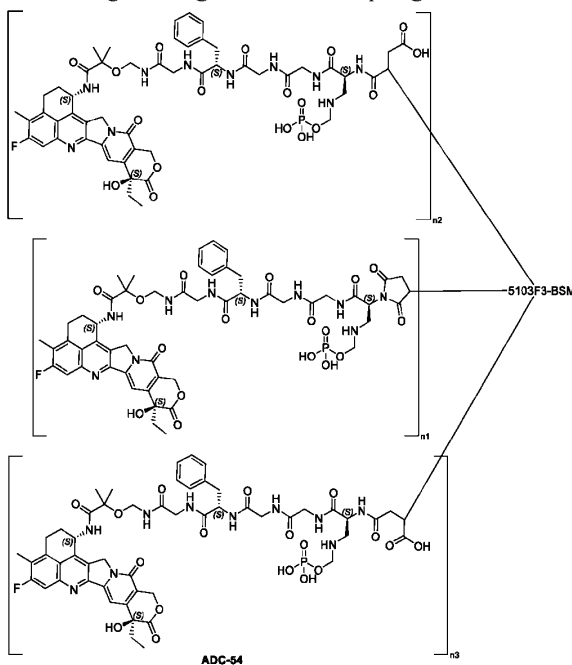
Example 146

ADC-53 was prepared according to the generalized coupling method of Example 92:



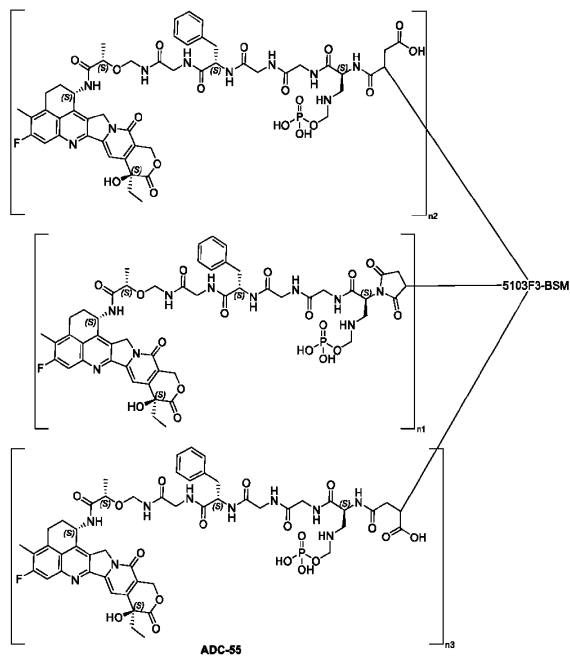
Example 147

ADC-54 was prepared according to the generalized coupling method of Example 92:



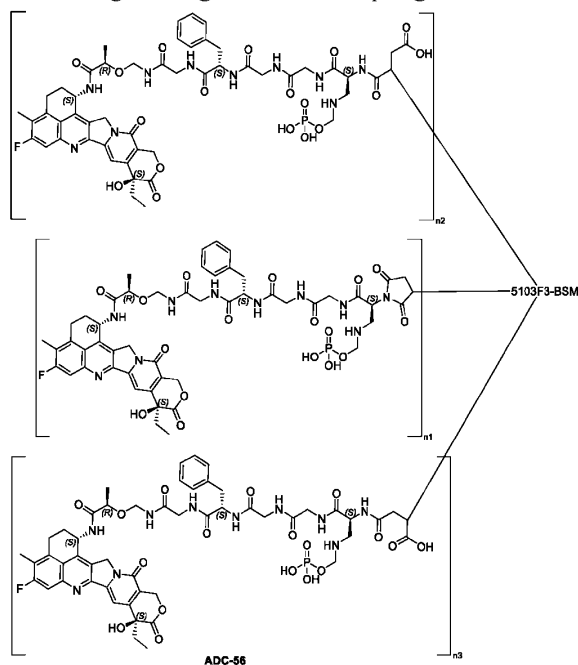
Example 148

ADC-55 was prepared according to the generalized coupling method of Example 92:



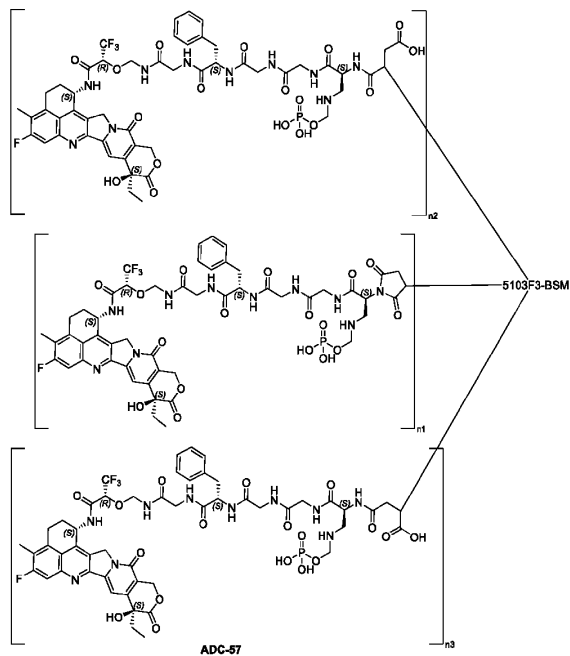
Example 149

ADC-56 was prepared according to the generalized coupling method of Example 92:



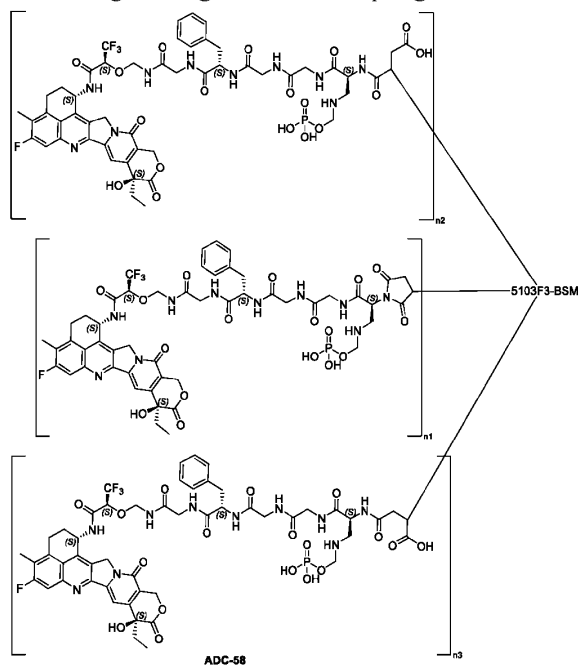
Example 150

ADC-57 was prepared according to the generalized coupling method of Example 92:



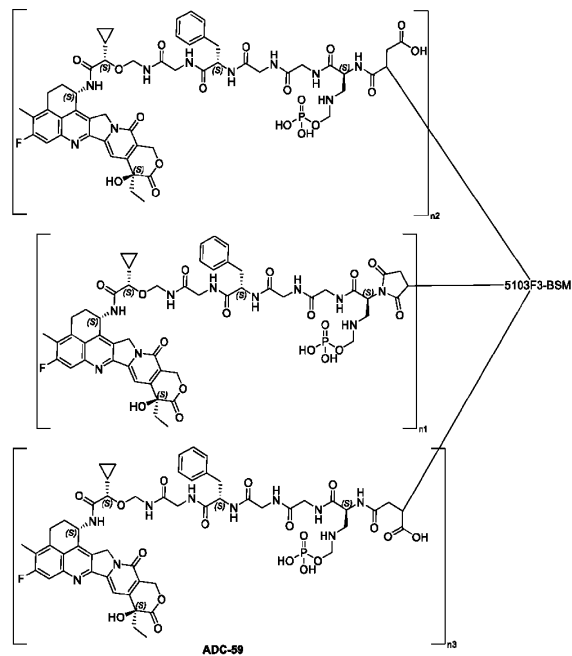
Example 151

ADC-58 was prepared according to the generalized coupling method of Example 92:



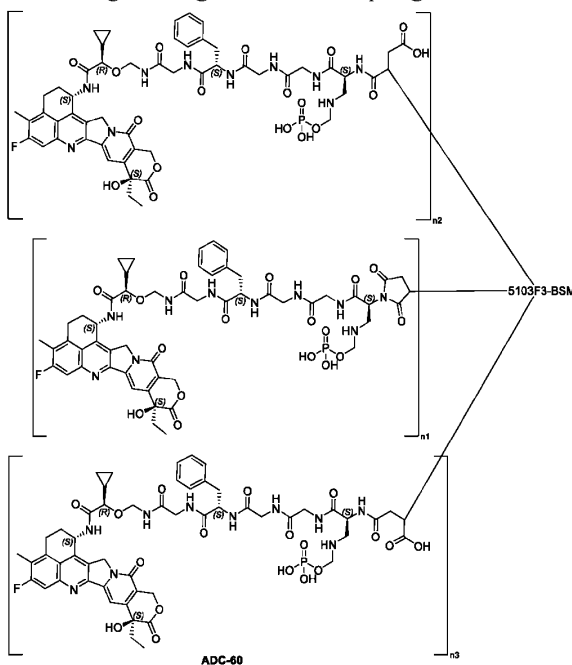
Example 152

ADC-59 was prepared according to the generalized coupling method of Example 92:



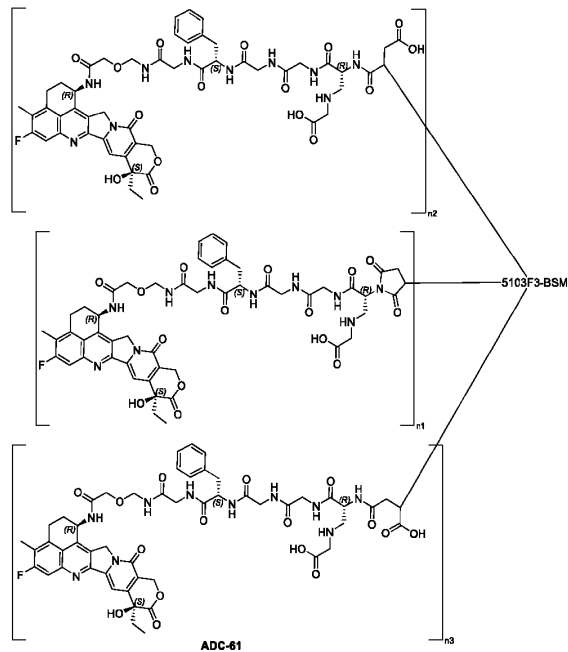
Example 153

ADC-60 was prepared according to the generalized coupling method of Example 92:



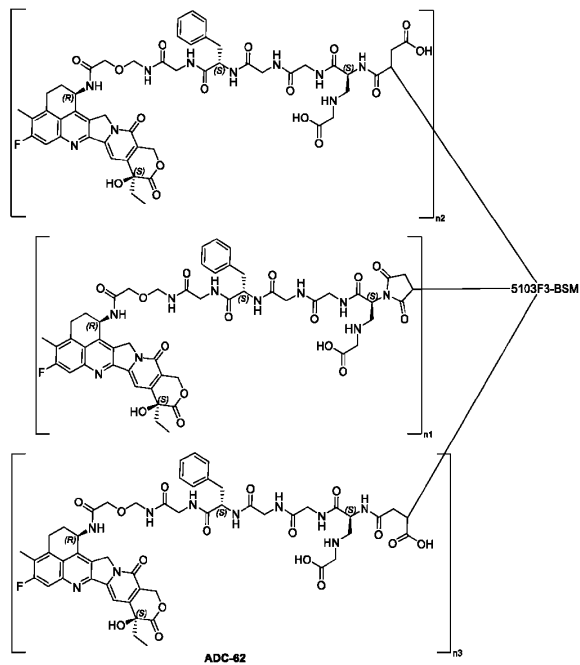
Example 154

ADC-61 was prepared according to the generalized coupling method of Example 92:



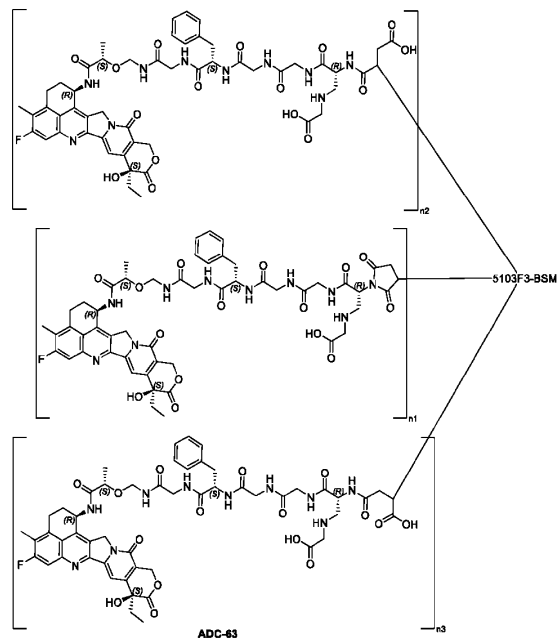
Example 155

ADC-62 was prepared according to the generalized coupling method of Example 92:



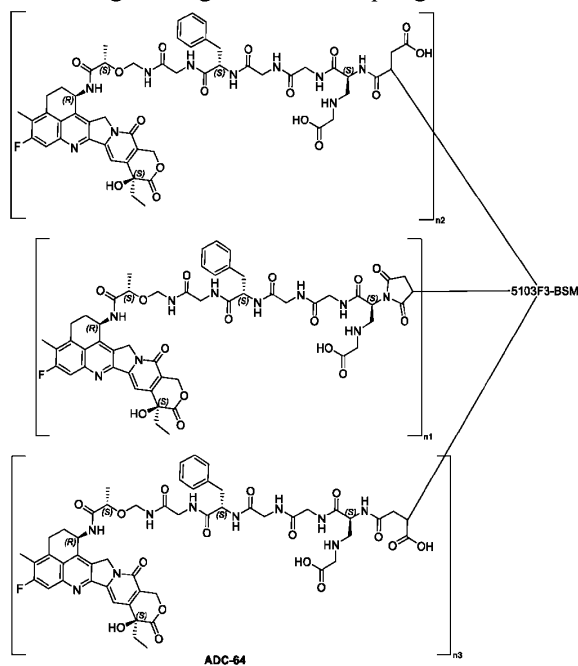
Example 156

ADC-63 was prepared according to the generalized coupling method of Example 92:



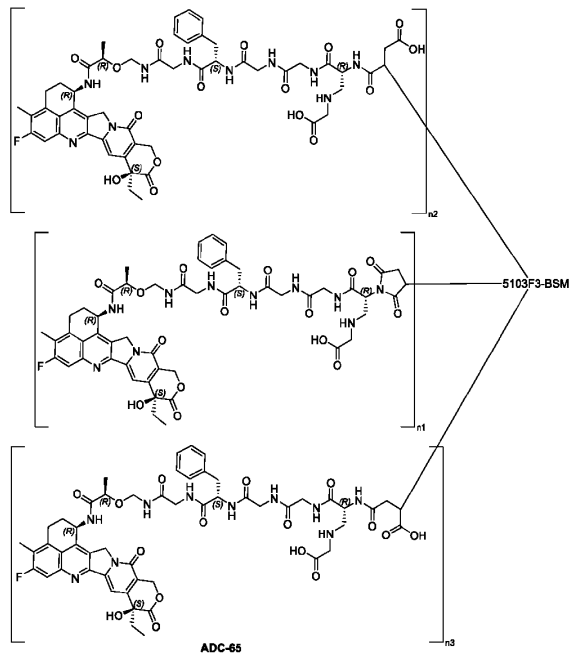
Example 157

ADC-64 was prepared according to the generalized coupling method of Example 92:



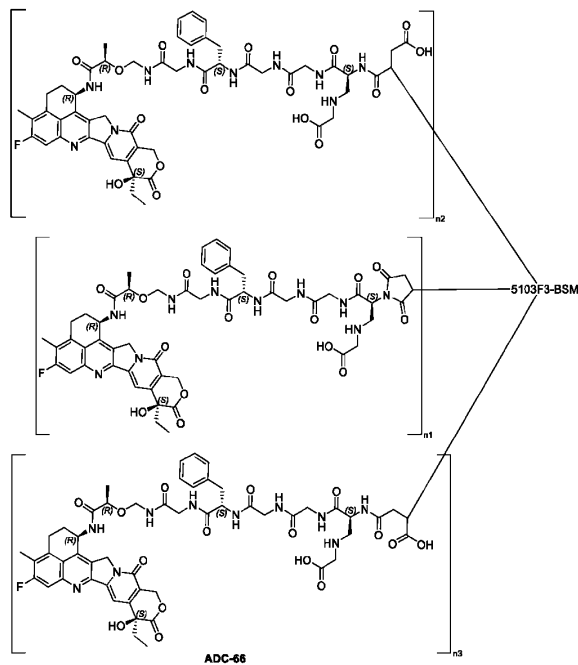
Example 158

ADC-65 was prepared according to the generalized coupling method of Example 92:



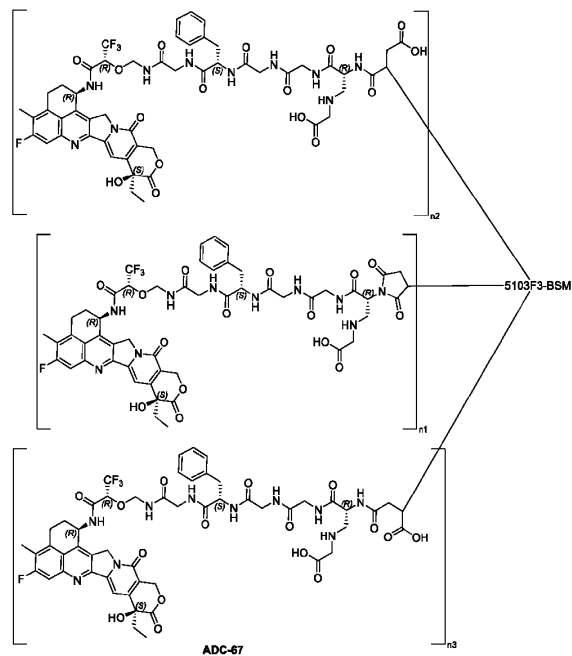
Example 159

ADC-66 was prepared according to the generalized coupling method of Example 92



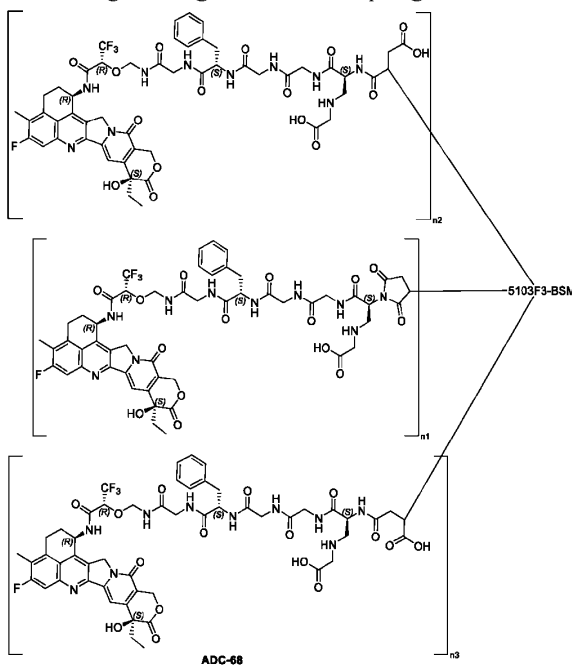
Example 160

ADC-67 was prepared according to the generalized coupling method of Example 92:



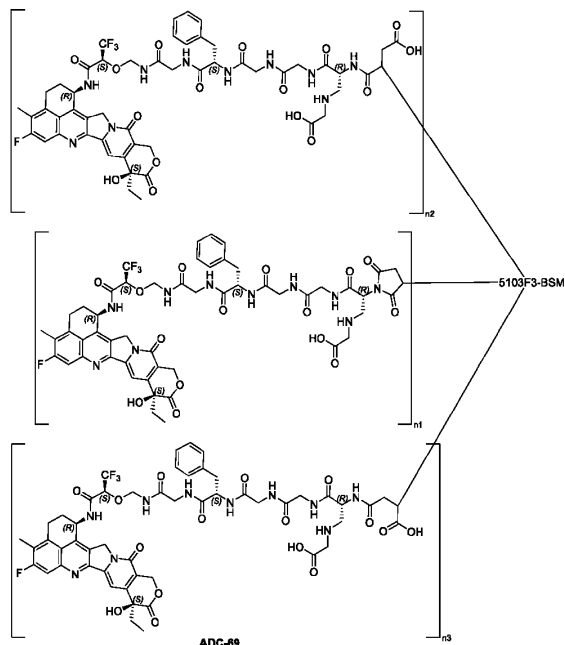
Example 161

ADC-68 was prepared according to the generalized coupling method of Example 92:



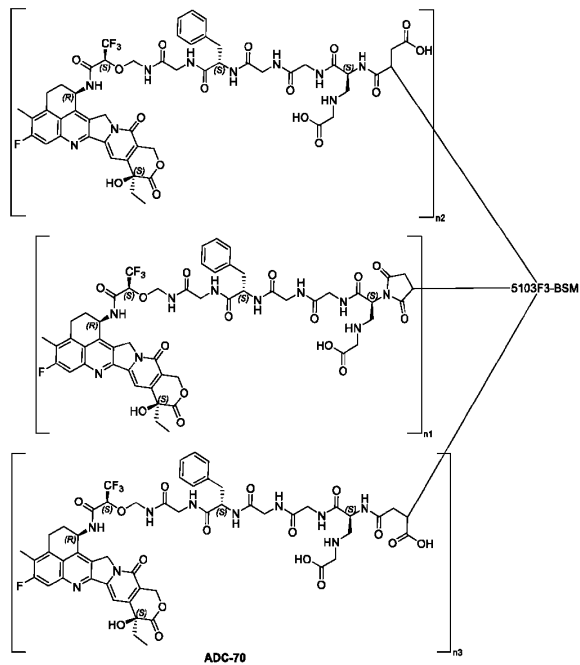
Example 162

ADC-69 was prepared according to the generalized coupling method of Example 92:



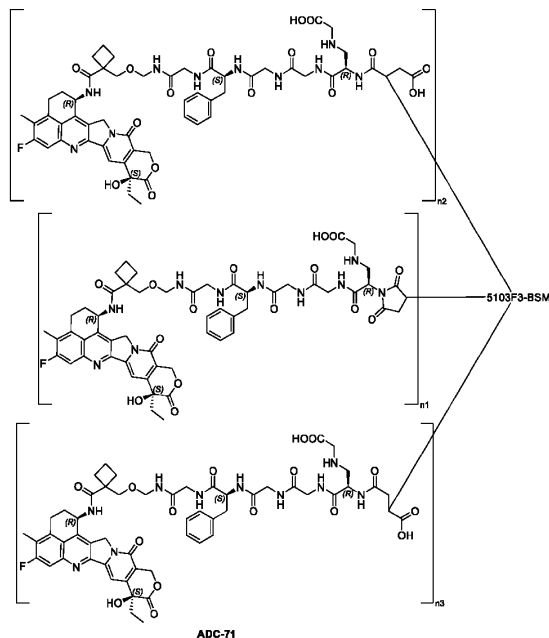
Example 163

ADC-70 was prepared according to the generalized coupling method of Example 92:



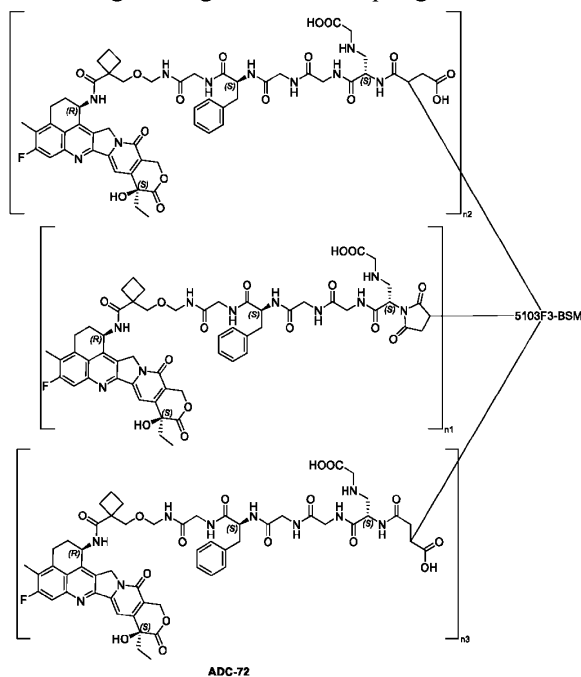
Example 164

ADC-71 was prepared according to the generalized coupling method of Example 92:



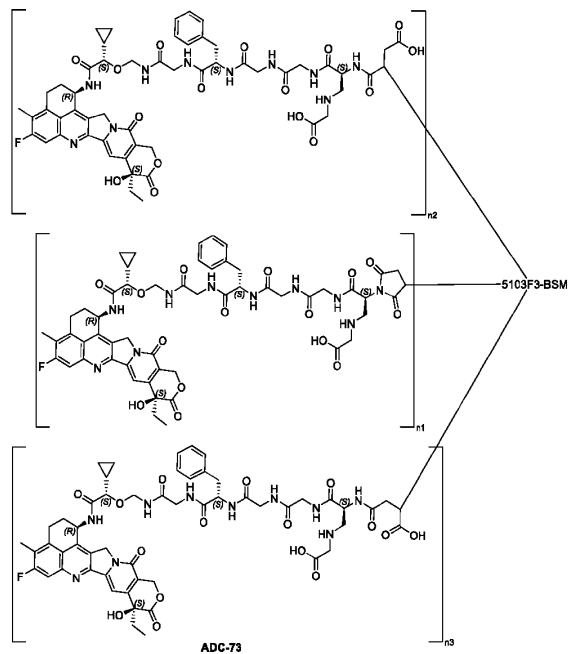
Example 165

ADC-72 was prepared according to the generalized coupling method of Example 92:



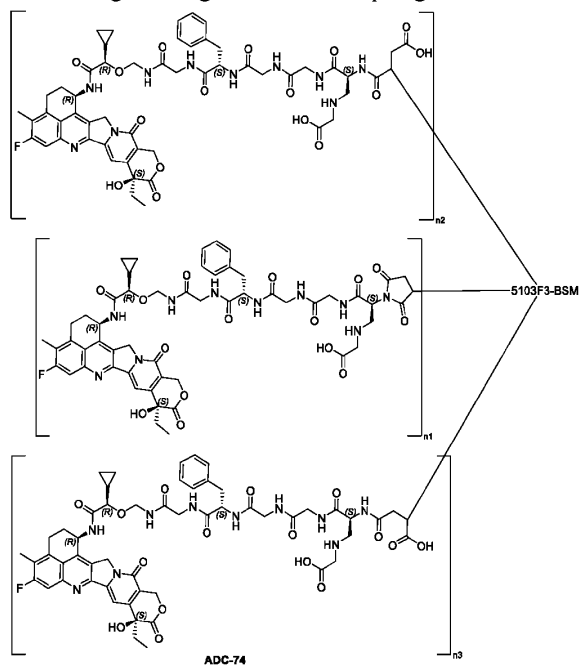
Example 166

ADC-73 was prepared according to the generalized coupling method of Example 92:



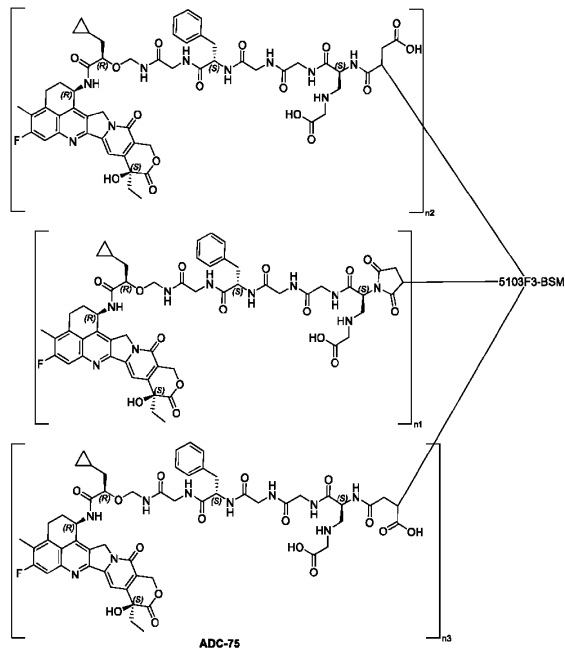
Example 167

ADC-74 was prepared according to the generalized coupling method of Example 92:



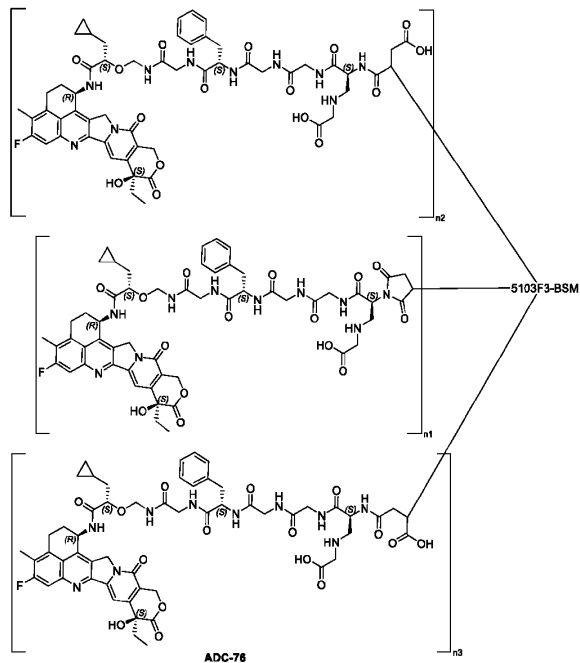
Example 168

ADC-75 was prepared according to the generalized coupling method of Example 92:



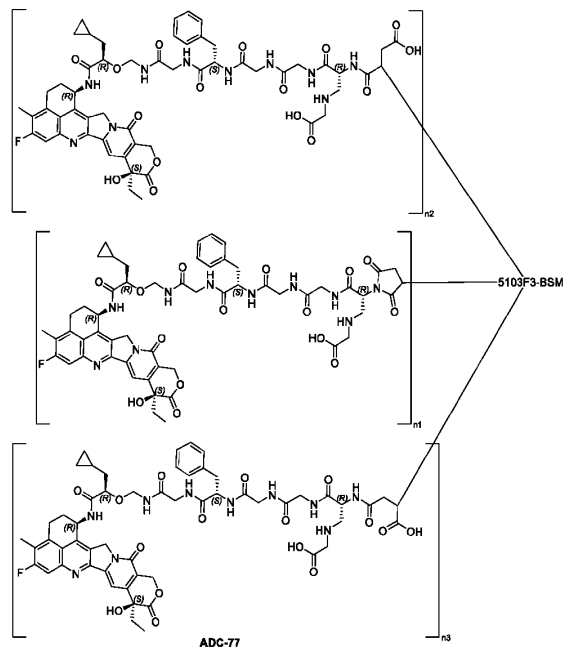
Example 169

ADC-76 was prepared according to the generalized coupling method of Example 92:



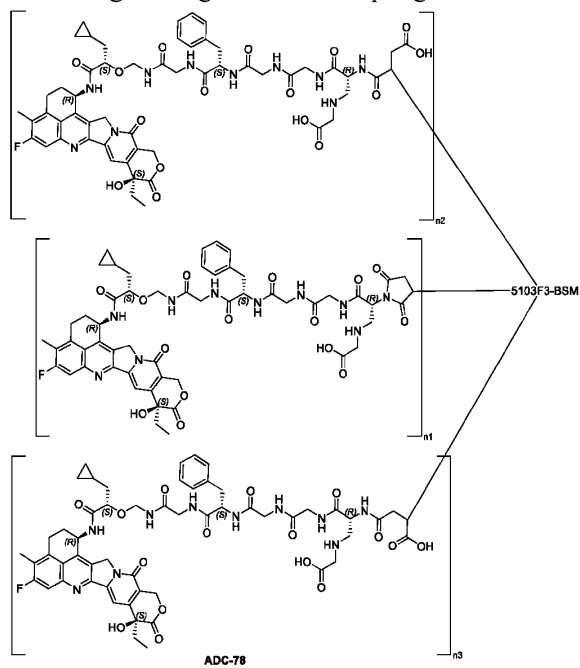
Example 170

ADC-77 was prepared according to the generalized coupling method of Example 92:



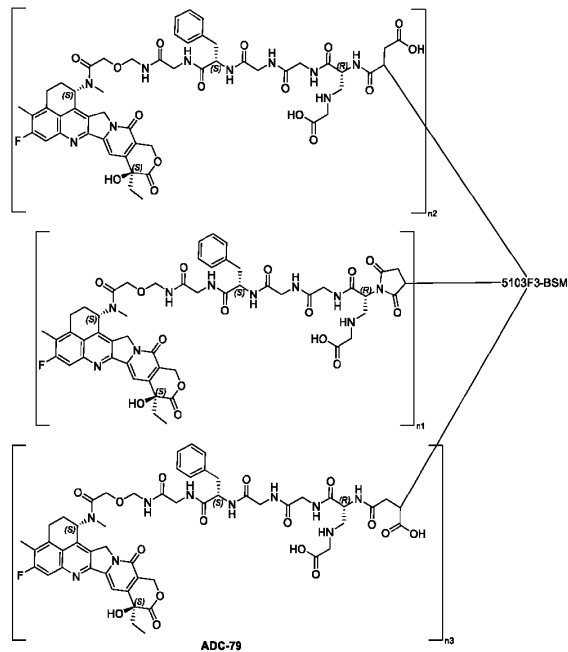
Example 171

ADC-78 was prepared according to the generalized coupling method of Example 92:



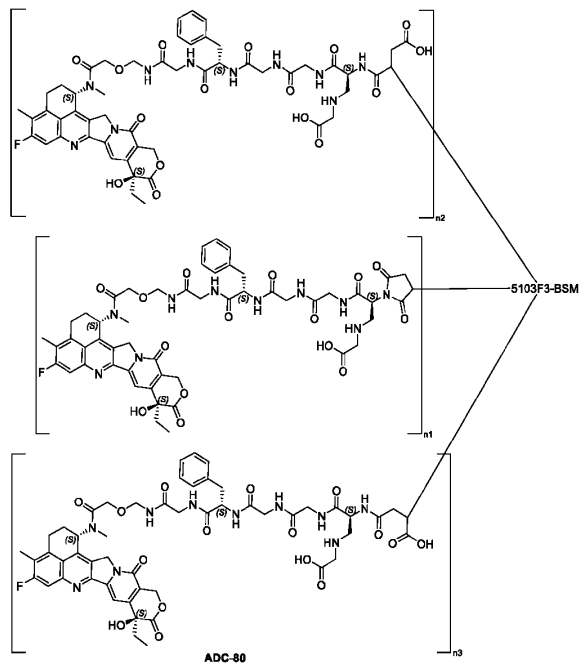
Example 172

ADC-79 was prepared according to the generalized coupling method of Example 92:



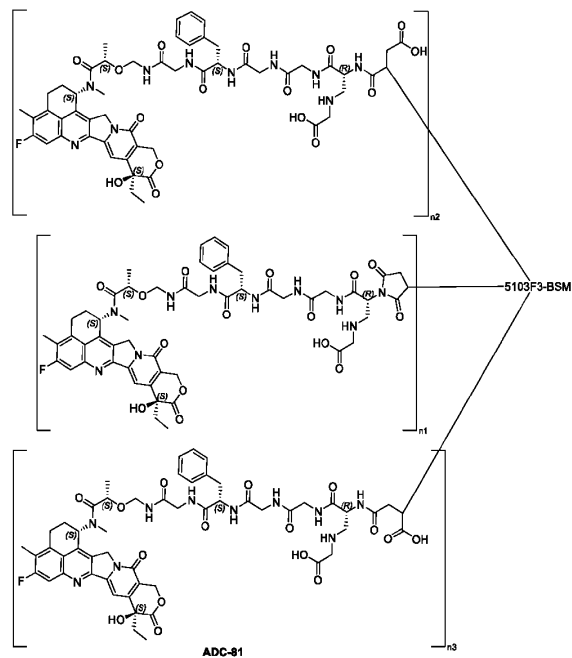
Example 173

ADC-80 was prepared according to the generalized coupling method of Example 92:



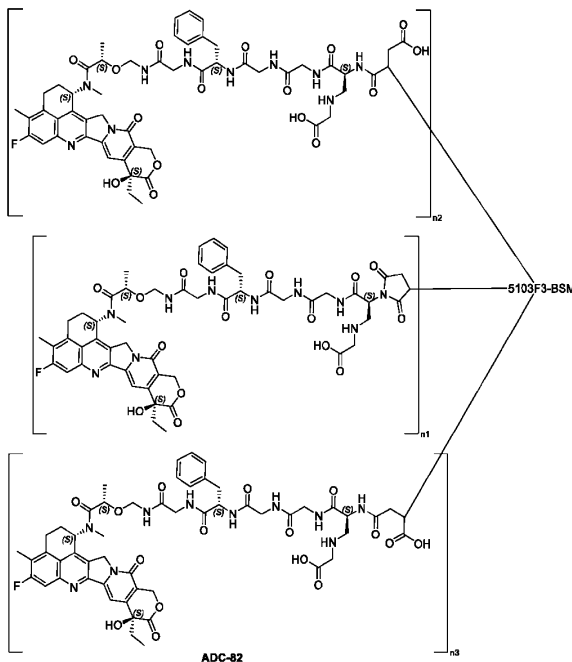
Example 174

ADC-81 was prepared according to the generalized coupling method of Example 92:



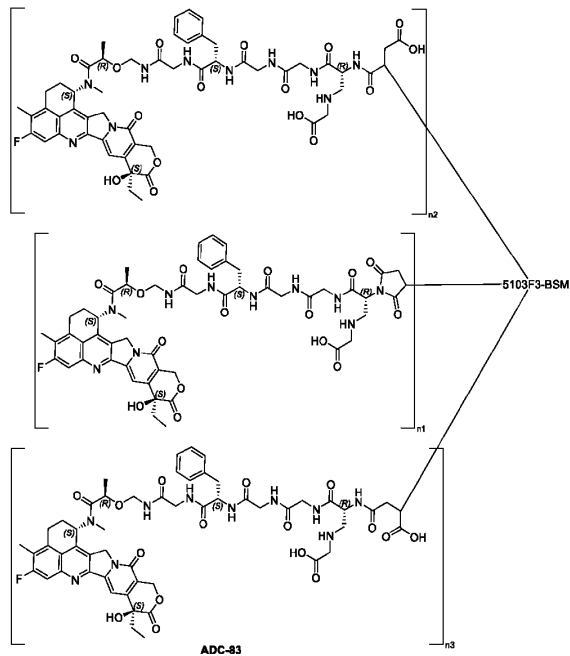
Example 175

ADC-82 was prepared according to the generalized coupling method of Example 92:



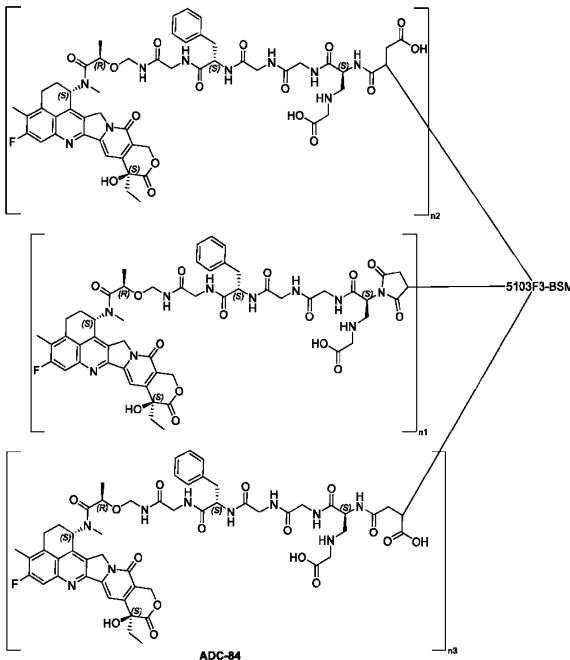
Example 176

ADC-83 was prepared according to the generalized coupling method of Example 92:



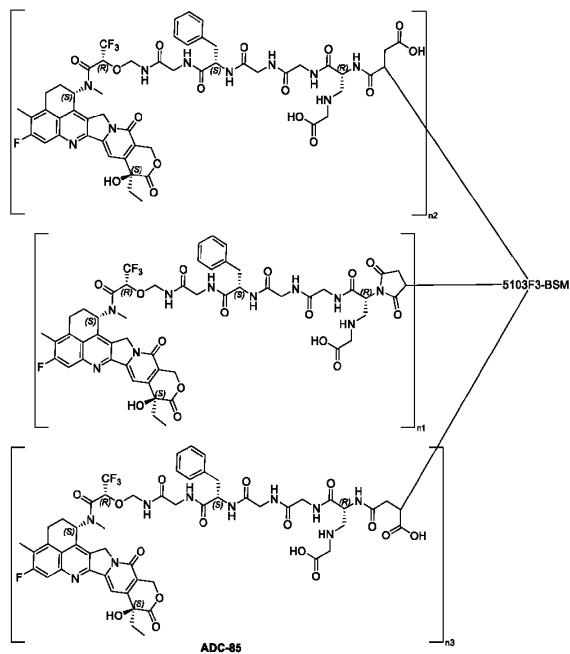
Example 177

ADC-84 was prepared according to the generalized coupling method of Example 92:



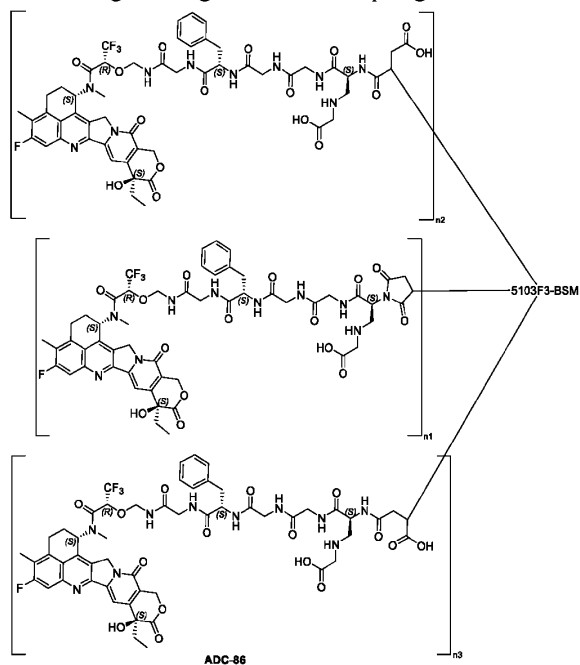
Example 178

ADC-85 was prepared according to the generalized coupling method of Example 92:



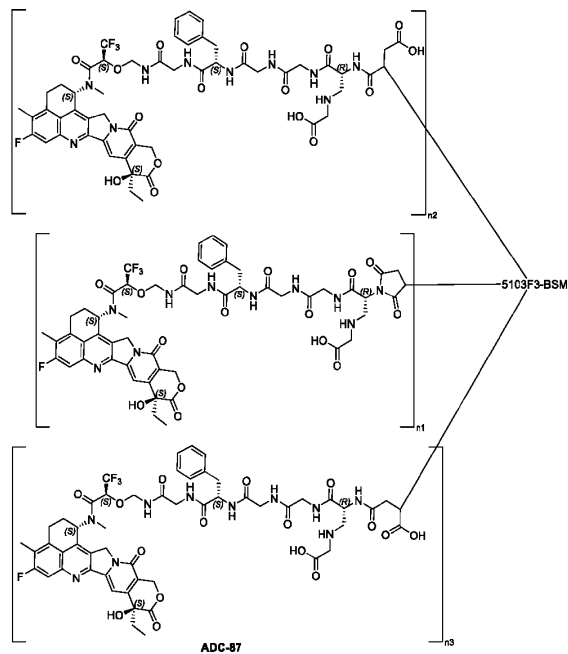
Example 179

ADC-86 was prepared according to the generalized coupling method of Example 92:



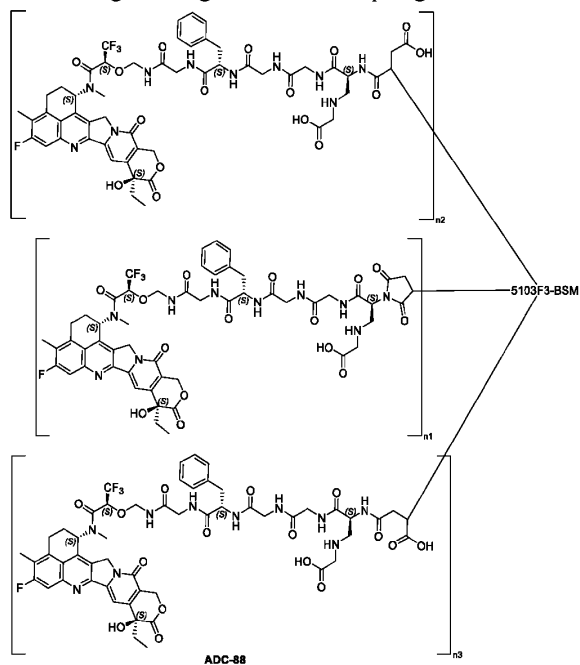
Example 180

ADC-87 was prepared according to the generalized coupling method of Example 92:



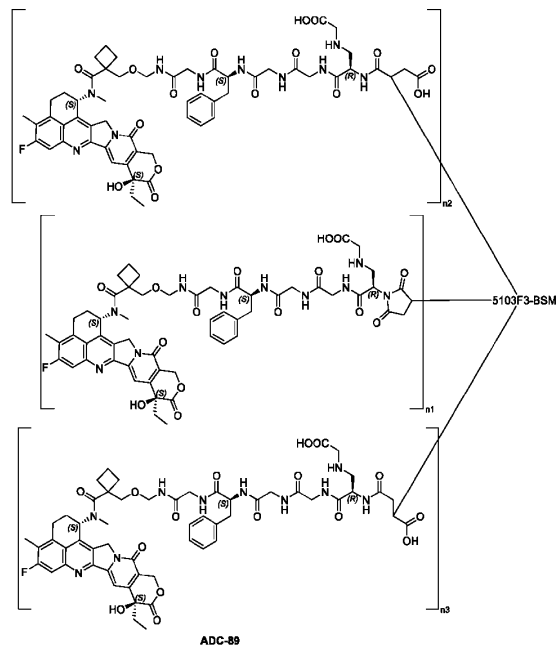
Example 181

ADC-88 was prepared according to the generalized coupling method of Example 92:



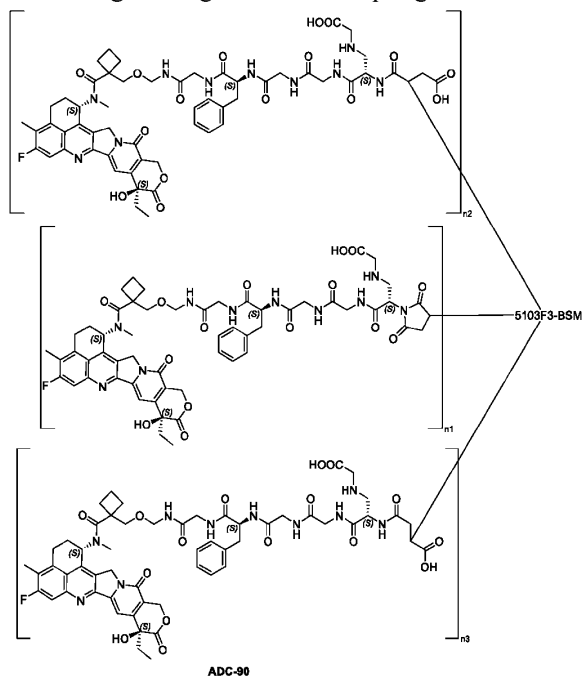
Example 182

ADC-89 was prepared according to the generalized coupling method of Example 92:



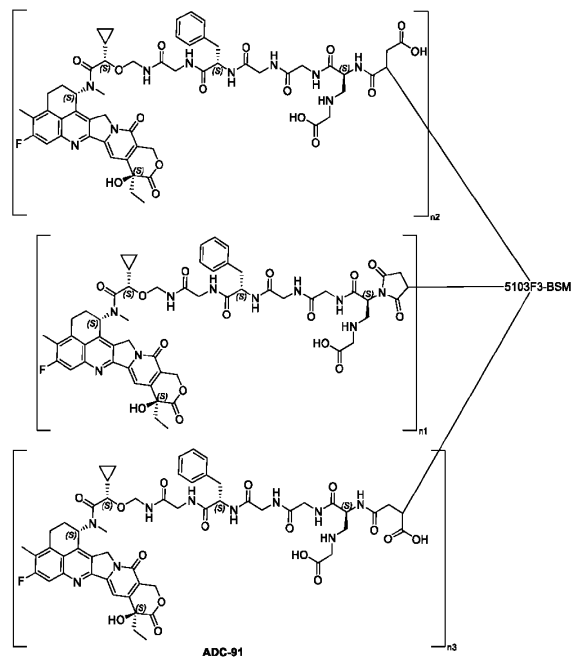
Example 183

ADC-90 was prepared according to the generalized coupling method of Example 92:



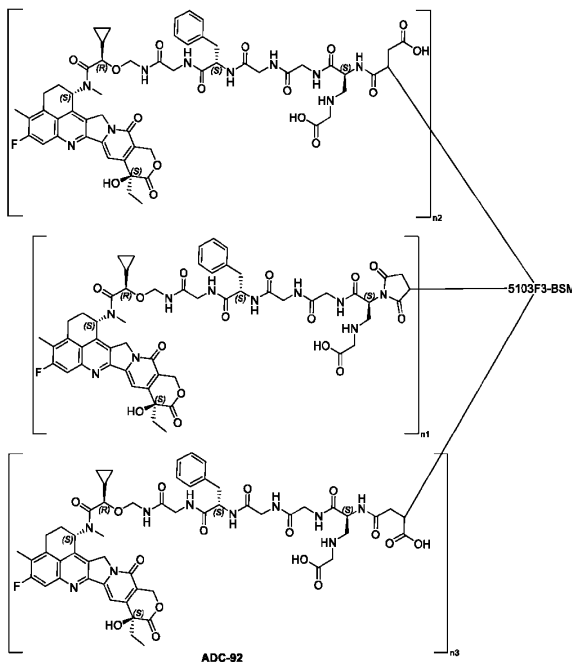
Example 184

ADC-91 was prepared according to the generalized coupling method of Example 92:



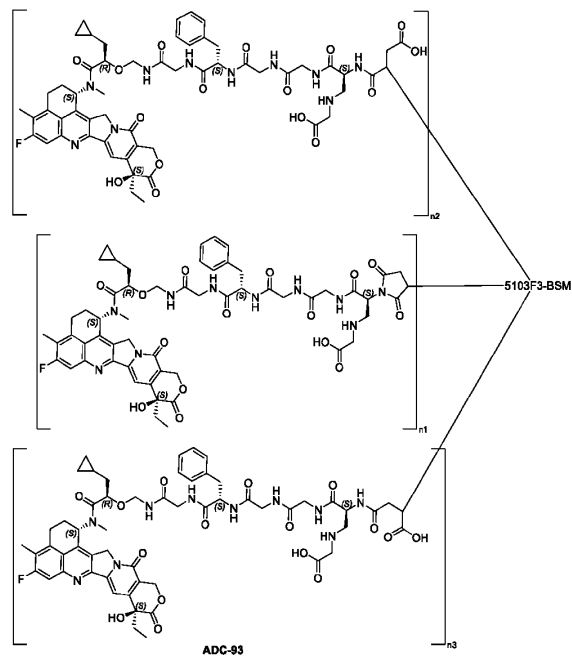
Example 185

ADC-92 was prepared according to the generalized coupling method of Example 92:



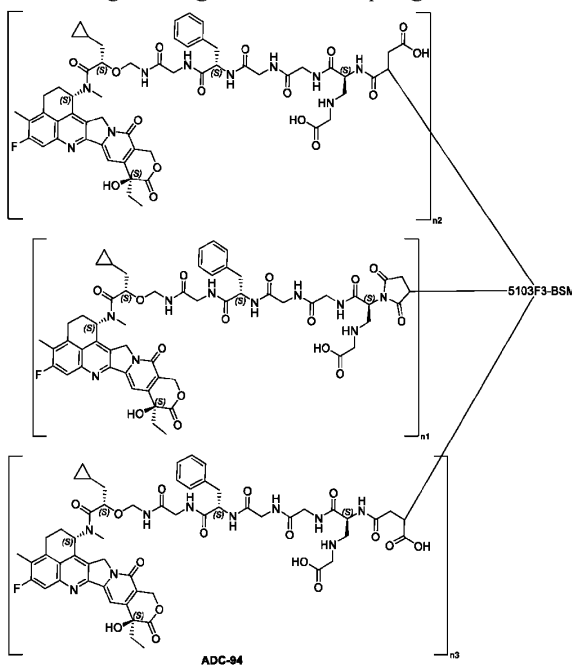
Example 186

ADC-93 was prepared according to the generalized coupling method of Example 92:



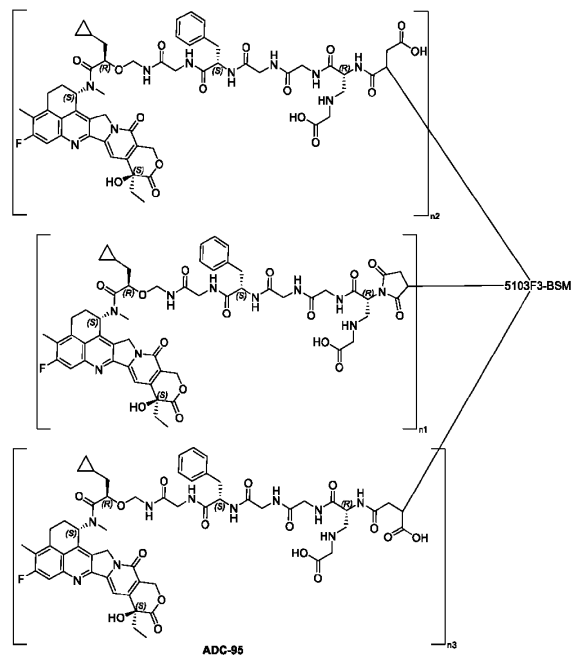
Example 187

ADC-94 was prepared according to the generalized coupling method of Example 92:



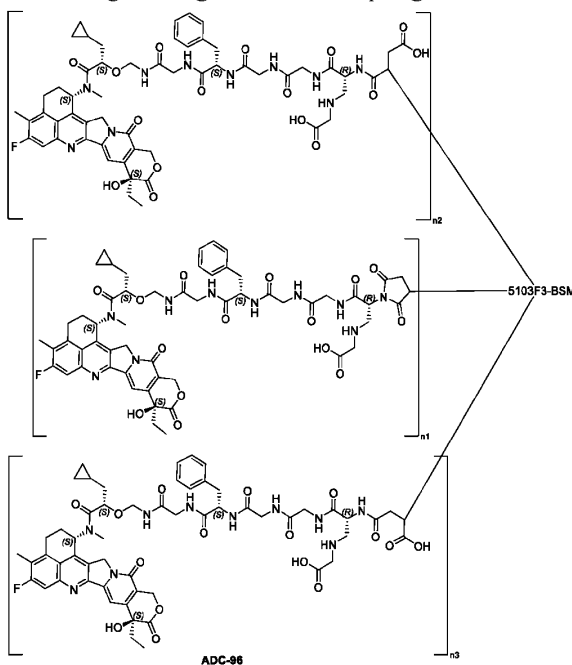
Example 188

ADC-95 was prepared according to the generalized coupling method of Example 92:



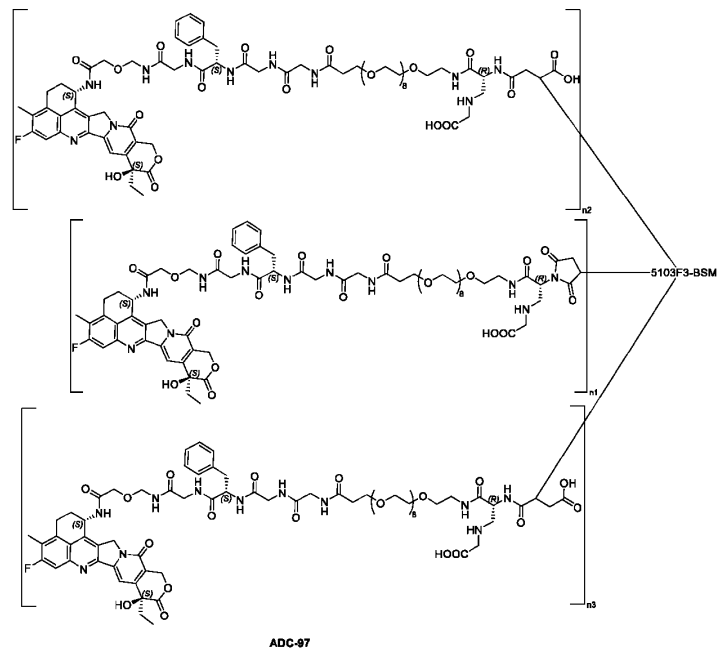
Example 189

ADC-96 was prepared according to the generalized coupling method of Example 92:



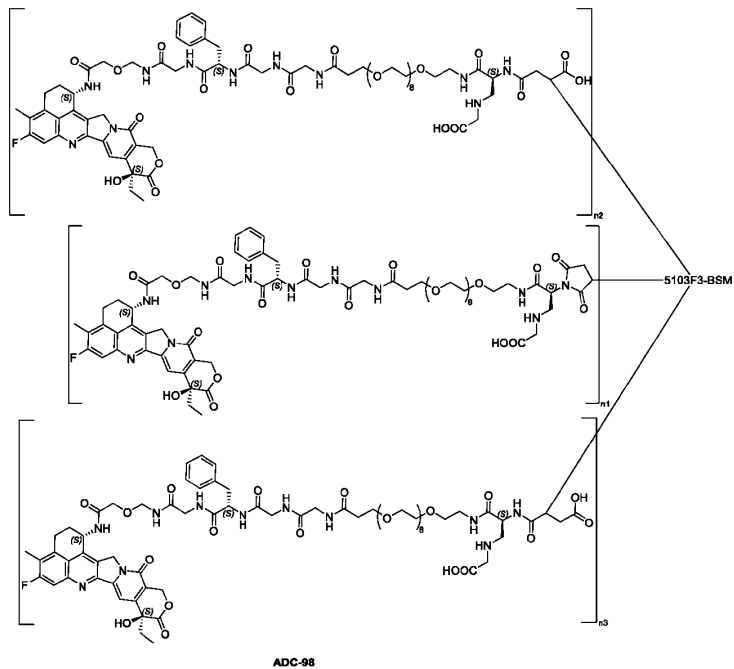
Example 190

ADC-97 was prepared according to the generalized coupling method of Example 92:



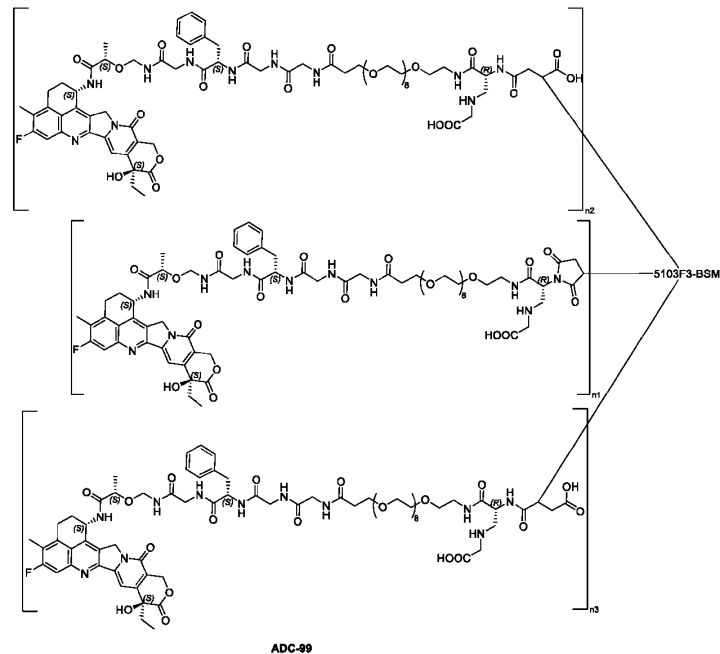
Example 191

ADC-98 was prepared according to the generalized coupling method of Example 92:



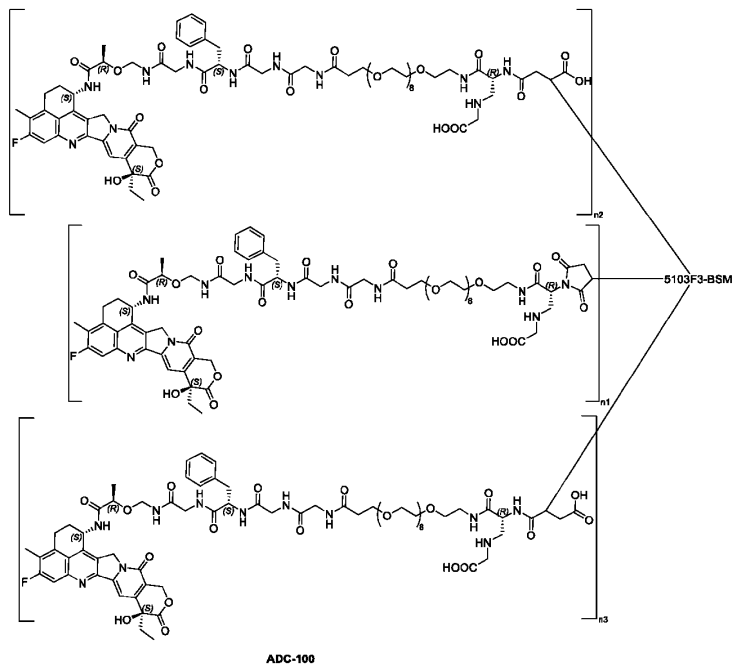
Example 192

ADC-99 was prepared according to the generalized coupling method of Example 92:



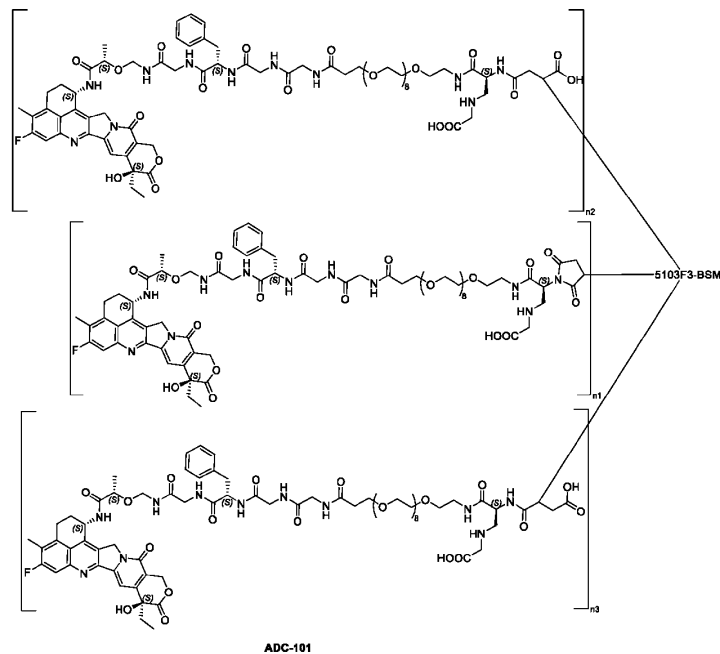
Example 193

ADC-100 was prepared according to the generalized coupling method of Example 92:



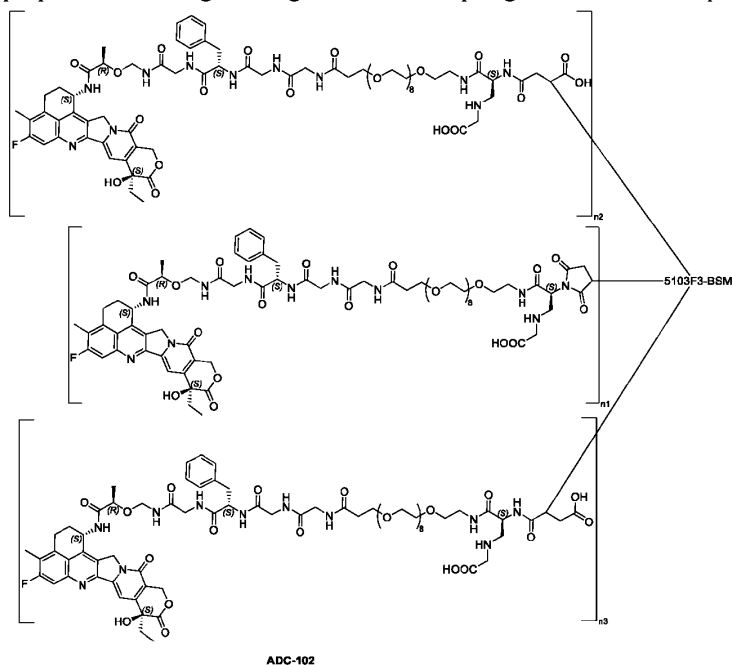
Example 194

ADC-101 was prepared according to the generalized coupling method of Example 92:



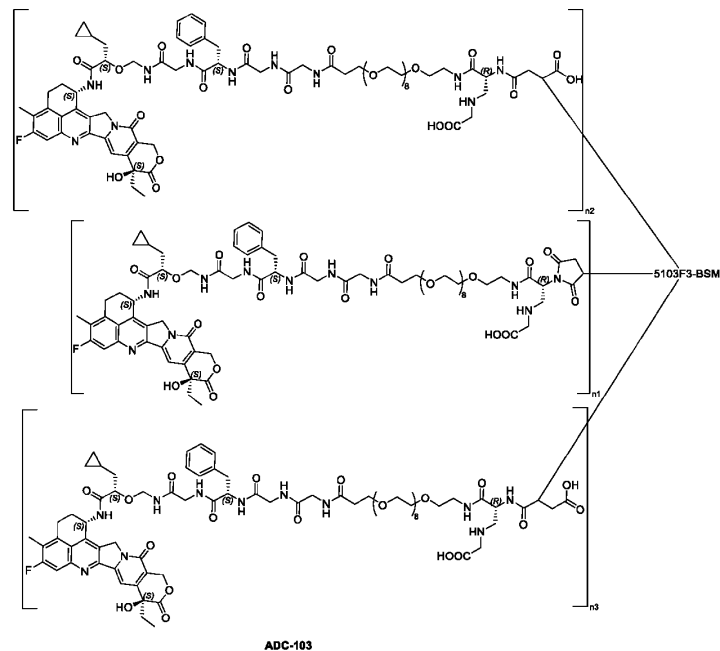
Example 195

ADC-102 was prepared according to the generalized coupling method of Example 92:



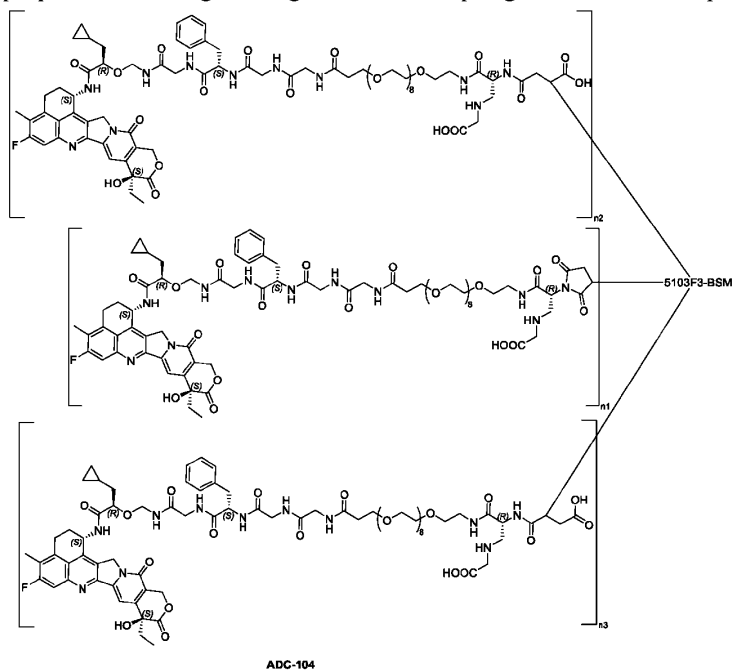
Example 196

ADC-103 was prepared according to the generalized coupling method of Example 92:



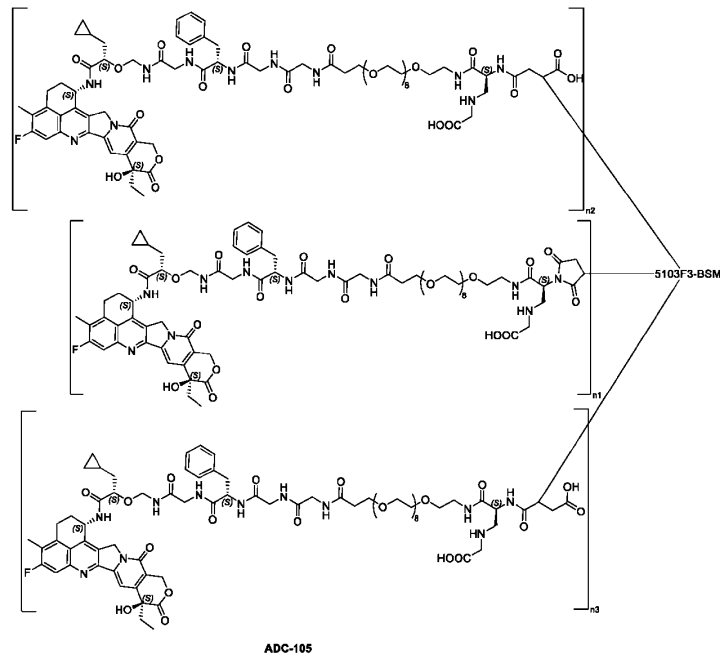
Example 197

ADC-104 was prepared according to the generalized coupling method of Example 92:



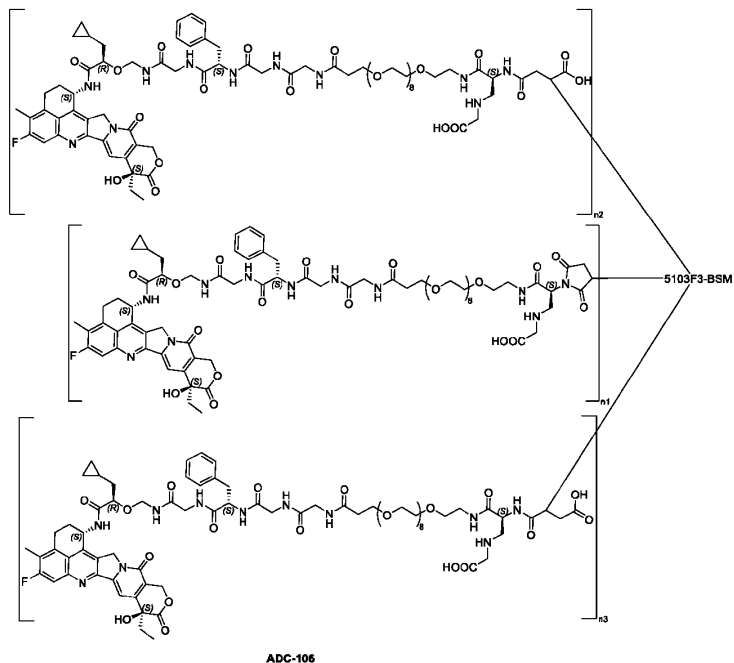
Example 198

ADC-105 was prepared according to the generalized coupling method of Example 92:



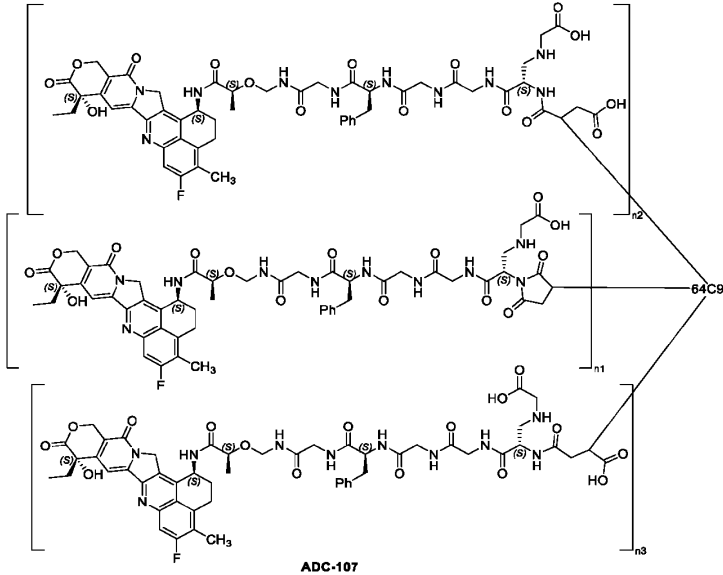
Example 199

ADC-106 was prepared according to the generalized coupling method of Example 92:



Example 200

ADC-107 was prepared according to the general coupling method of Example 93:



wherein 64C9 is an anti-Claudin18.2 antibody.

Example 309

To determine the monomer rate, the SEC-HPLC method below was used:

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

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

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

10

Table 1. Methods and parameters

Parameter	Set-Up
Flow rates	0.3 mL/min
Wavelength	214 nm and 280 nm
Column temperature	30°C
Sample Plate Temperature	indoor temperature
Injection volume	20 ug
Maximum pressure	150 bar/15 MPa/2175 PSI
Gradient	equal degree
Running time	20 minutes.

Table 2. The ADC monomer rate

ADC	Degradation %	Aggregate %	Monomer rate %
ADC-6	0	3.66	96.34

ADC-107	0.46	2.00	97.54
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CONCLUSION: The antibody-drug conjugates (ADCs) disclosed in the present application has the characteristics of low degradation rate and low aggregation rate, and the excellent property of high monomer rate.

Example 310

To determine the drug antibody ratio (DAR), the RP-HPLC method below was used:

Column name: Proteomix RP-1000 4.6×100mm 5μm 1000A

Manufacturer: Sepax Item No.: 465950-4610

10 **Table 3.** Methods and parameters

Parameter	Set-Up			
Mobile phase	A: 0.1% TFA aqueous solution; B: 0.1% TFA acetonitrile solution			
Flow rates	0.5 mL/min			
Wavelength	214 nm and 280 nm			
Column temperature	65 °C			
Sample Plate Temperature	indoor temperature			
Injection volume	25 ug			
Maximum pressure	100bar/10MPa/1450PSI			
Gradient	Time (min)	Flow rate (mL/min)	Mobile phase A (%)	Mobile phase B (%)
	0.0	0.5	75	25
	3	0.5	75	25
	28	0.5	50	50
	30	0.5	5	95
	32	0.5	5	95
	33	0.5	75	25
	40	0.5	75	25

Table 4. ADC drug-antibody coupling ratio (DAR)

ADC	RP-DAR
ADC-6	7.33
ADC-107	7.42

CONCLUSION: The ADCs in the present application have the excellent property of high DAR value, which may significantly increase the ADC drug concentration at the target site when the same dose of ADC is administered.

Example 311

Plasma stability of ADC:

ADCs were mixed with IgG-free plasma to make the final concentration of ADC at 0.6 mg/mL, incubated in a 37 °C water bath for a period of 0, 3, and 7 days. A unincubated plasma was used as a control. After incubation, the sample was purified and extracted, and the drug antibody ratio DAR was measured to reflect the plasma stability of ADCs.

Table 5. Plasma stability of ADCs

ADC	DAR			
	Plasma not incubated	Plasma incubation 0 days	Plasma incubation for 3 days	Plasma incubation for 7 days
ADC-6	7.51	7.56	7.61	7.67
ADC-107	7.31	7.23	7.49	7.43

CONCLUSION: The ADCs disclosed in the present disclosure have good plasma stability, and there is no significant change in the DAR value during plasma incubation.

Example 312

ADC maintains the affinity of the corresponding original anti-Claudin18.2 antibody 5103F3-BSM for Claudin18.2. The relative affinities of 5103F3-BSM versus ADC-6; 64C9 versus ADC-107 for Claudin18.2 were compared by using double antigen sandwich ELISA.

The specific steps of the Claudin18.2 relative binding activity assay are as follows: the antigen was diluted with PBS to the appropriate concentration, spiked into the plate and incubated at 4°C overnight. After coating, wash the plate and seal it at 37°C for 1 h. After sealing, wash the plate, dilute the test material in a gradient according to the appropriate concentration, add the sample into the sealed enzyme plate and incubate. Dilute the rabbit anti-human antibody with buffer to the appropriate concentration, add the sample and incubate. Add the homemade color development solution to develop the color for 7min and then add the termination solution to terminate the reaction. 450nm read the plate, four-parameter fit to the reading results, calculate the EC50 value, and then get the relative binding activity results.

CONCLUSION: As shown in **Figures 3A and 3B**, the coupled ADCs maintained a similar affinity with the corresponding antibodies, with no significant difference in the EC50 values, indicating that the coupling of antibodies to the toxin did not affect their affinity for the antigen.

Example 313

Analysis of antibody binding specificity:

In the present application, HEK293T-18.1 and HEK293T-18.2, which are two human embryonic kidney cell lines overexpressing Claudin18.1 and Claudin18.2, respectively, were used as experimental models, and the specific binding of each naked antibody of ADC to Claudin18.2 was analyzed by flow cytometry. HEK293T-18.1/18.2 in logarithmic growth phase were taken, trypsinized and washed twice with pre-cooled 1×PBS, and resuspended to 1×10⁷

cells/mL with 1×PBS containing 3% BSA. 2×10⁵ cells were taken in 96-well plates, and a gradient dilution of the test antibody was added to the cells for 1 hour of incubation on ice. At the end of the incubation pre-cooled PBS was used to wash the cells, followed by the addition of FITC-mouse anti-Human fluorescent secondary antibody for 30 min on ice, washed, re-suspended, and analyzed using a Beckman Coulter CytoFlex flow cytometer. The set-up gate strategy was as follows: the cells to be analyzed were circled with the SSC/FSC scatter plot; single cells were circled with the FSC-H/FSC-A scatter plot to exclude adhesions and debris; the single-cell gate was applied to the PB450-A to obtain the live cells, and finally the cell population to be tested was identified in the FITC-A and the fluorescence values corresponding to different antibody concentrations were derived by statistics. The 4-parameter model of Graph Pad Prism 9.0 data analysis software was utilized for data statistics and analysis.

Table 6. Specific binding of Claudin18.2 by subject ADC-107 naked antibody ADC-6 naked antibody (also see **Figure 4A** and **4B**).

Group	Antibody	EC50 (μg/mL)
HEK293T-18.1	64C9	ND
	5103F3-BSM	ND
HEK293T-18.2	64C9	0.24
	5103F3-BSM	0.12

Remarks: ND stands for not detected.

CONCLUSION: The naked antibodies, 64C9 and 5103F3-BSM, while did not recognize Claudin18.1, display high specific recognition of Claudin18.2. The binding affinity of 5103F3-BSM was characteristically better than that of 64C9.

Example 314

In vitro pharmacodynamic assays:

In the present application, a variety of human gastric adenocarcinoma tumor cell lines, polyclonal and monoclonal cell lines overexpressing Claudin18.2, including HEK293T-18.2, BxPC-3 #A9F, SNU-5, and SNU-16, were used as experimental models to evaluate the efficacy of ADC drugs *in vitro*. Several tumor cell lines were inoculated in 96-well plates, and a gradient dilution of the subject antibody and the corresponding ADC drug were added to the cells, which were treated for 5 days, and the cell viability was detected by MTS, and the inhibitory effects of the subject antibody and ADC on the tumor cell lines were evaluated by calculating IC50. The starting concentration of the antibody drug was 500 nM, and the dilution was 7-fold, totaling 8 concentration points, and treated for 5 days. The final algorithm was based on survival rate = (experimental group - blank) / (control group - blank group) × 100%, followed by fitting the curve using Graph Pad Prism (**Figure 5**) and calculating the half inhibitory concentration (IC50).

Table 7. The results of the *in vitro* efficacy of naked antibody 64C9 versus ADC-107, naked

antibody 5103F3-BSM versus ADC-6 at cellular level (also see **Figure 5A, 5B, 5C, and 5D**).

Group	Antibody/ADC	IC50 (nM)
HEK293T-18.2	64C9	> 500
	ADC-107	0.538
	5103F3-BSM	> 500
	ADC-6	0.149
BxPC-3 #A9F	64C9	> 500
	ADC-107	0.127
	5103F3-BSM	> 500
	ADC-6	0.100
SNU-5	64C9	> 500
	ADC-107	119.66
	5103F3-BSM	> 500
	ADC-6	10.07
SNU-16	64C9	> 500
	ADC-107	66.56
	5103F3-BSM	> 500
	ADC-6	7.061

CONCLUSION: In HE293T-18.2, BxPC-3#A9F, SNU-5 and SNU-16 cell models, no significant difference in tumor cell killing activity was seen between 64C9 and 5103F3-BSM, however, ADC-6 (DAR=8) exhibits the significantly higher tumor cell killing activity *in vitro*, i.e., efficacy and potency, than ADC-107 (DAR=8).

Example 315

The present application establishes a subcutaneous graft tumor model of target-positively expressing human gastric cancer cells SNU-5 NOD Scid mice to evaluate the *in vivo* efficacy of ADC-6. 5×10⁶ SNU-5 cells (0.1 mL/each) were injected subcutaneously into the right scapula of NOD Scid mice at 6~7 weeks of age. When the average tumor size of the mice grew to about 180 mm³, the mice were randomly divided into a lysate control group (Vehicle) and an ADC-6 treatment group (5 mg/kg) of 5 mice each, and the administration of ADC-6 was started (D0). All groups were administered by tail vein injection at 10 mL/kg body weight once a week for 4 weeks (QW×4). All groups were observed up to day 28 (D28) after subgroup administration (**Table 8, Figure 6**), and statistical analysis of the mean tumor volume on day 28 (D28) after subgroup administration showed that ADC-6 administered caudal-venously at a dose of 5 mg/kg (QW×4) had a significant tumor-suppressing effect (P<0.05).

Table 8. Efficacy analysis of each group in the subcutaneous tumor graft model of human gastric cancer cells (SNU-5) in NOD Scid mice.

Experimental group	Day 0 after administration	Day 28 after administration				
	Mean tumor volume ($\bar{x} \pm S$) mm ³	Mean tumor volume ($\bar{x} \pm S$) mm ³	Relative Tumor volume ($\bar{x} \pm S$)	TGI (%)	T/C (%)	<i>P</i> value
Group 1 Vehicle	177.98±8.91	1493.75±190.25	8.34±0.87	-	-	-
Group 2 ADC-6 (5 mg/kg)	177.71±10.43	53.60±3.17	0.30±0.01	96.38	3.62	<0.001

Note: 1. Data are expressed as "mean ± standard error";
2. T/C % = $T_{RTV} / C_{RTV} \times 100\%$; TGI% = $(1-T/C) \times 100\%$;
3. *p* value was obtained by comparing the tumor volume of the treatment group with that of the lysate control group at D 28 (day 28 after administration).

Example 316

The present application establishes a target (i.e., Claudin18.2)-overexpressing human gastric cancer cells (e.g., MKN-45) in BALB/c Nude mouse subcutaneous tumor graft model to evaluate the *in vivo* efficacy of ADC-6 drug. MKN-45 cells (2×10^6) were injected subcutaneously (0.1mL/mouse) into the right scapula of BALB/c Nude mice aged 6 to 7 weeks. When the average tumor size of the mice grew to about 160 mm³, the mice were randomly divided into vehicle control group (Vehicle) and ADC-6 treatment group (2mg/kg, 5mg/kg), 5 mice in each group, and drug administration was started (D0). Each group was administered 10 mL/kg body weight via tail vein injection once a week for 4 consecutive weeks (QW×4). All groups were observed on the 28th day after group administration (D28) (Table 9, Figure 7), and statistical analysis of the mean tumor volume on day 28 (D28) after subgroup administration showed that ADC-6 administered caudal-venously at the doses of 2 mg/kg and 5 mg/kg (QW×4) had a significant tumor-suppressing effect ($P < 0.05$).

Table 9. Efficacy analysis of each group in the subcutaneous tumor graft model of human gastric cancer cells, MKN-45, in BALB/c Nude mice

Experimental group	Day 0 after administration	Day 28 after administration				
	Mean tumor volume ($\bar{x} \pm S$) mm ³	Mean tumor volume ($\bar{x} \pm S$) mm ³	Relative Tumor volume ($\bar{x} \pm S$)	TGI (%)	T/C (%)	<i>P</i> value
Group 1	167.08±12.44	1240.62±274.11	7.98±2.40	-	-	-

Vehicle						
Group 2						
ADC-6 (2 mg/kg)	166.79±16.44	72.00±27.44	0.43±0.17	94.55	5.45	<0.001
Group 3						
ADC-6 (5 mg/kg)	167.76±14.39	6.12±0.95	0.04±0.00	99.55	0.45	<0.001

Note: 1. Data are expressed as "mean ± standard error";

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

3. *p* value was obtained by comparing the tumor volume of the treatment group with that of the lysate control group at D28 (day 28 after administration).

Sequence Listing

- > Seq ID No.1: 5103F3 BSM VH Amino Acid Sequence
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SLTAEDTAVYYCARHGGNTYYYAMDPWGPGTLTVSS
- > Seq ID No.2: 5103F3 BSM VH-CDRH1
GFAMG
- 10 > Seq ID No.3: 5103F3 BSM VH-CDRH2
FIDSGGAFYATWARG
- > Seq ID No.4: 5103F3 BSM VH-CDRH3
HGGNTYYYAMDP
- > Seq ID No.5: 5103F3 BSM Heavy Chain Amino Acid Sequence
QVQLQESGGRLIKPGEPLRLSCKTSGIDLSGFAMGWVRQAPGKLEYIGFIDSGGAFYATWARGRFTISRTSTNTVYLQMN
SLTAEDTAVYYCARHGGNTYYYAMDPWGPGTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTWNSGA
LTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP
20 EKKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYS
KLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPG
- > Seq ID No.6: 5103F3 BSM Heavy Chain Constant Region Amino Acid Sequence
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- > Seq ID No.7: 5103F3 BSM VL Amino Acid Sequence
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- > Seq ID No.8: 5103F3 BSM VL-CDRL1
QASQISNYLA
- > Seq ID No.9: 5103F3 BSM VL-CDRL2
SASTLAS
- 40 > Seq ID No.10: 5103F3 BSM VL-CDRL3
QQVYSVTNIDNA
- > Seq ID No.11: 5103F3 BSM Light Chain Amino Acid Sequence
ALRMTQSPSSLAATTGQRTVITCQASQISNYLAWYQQKPGQPPKLLIYSASTLASGVPSRFKSGSGTQFTLTISVQPED
FATYYCQQVYSVTNIDNAFGGGTRVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQE
SVTEQDSKDYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
- > Seq ID No.12: 5103F3 BSM Light Chain Constant Region Amino Acid Sequence
50 RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSLTLSKADYEK
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- > Seq ID No.13: 5103F3 BSM VH Nucleic Acid Sequence
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GCCCCGGCACCTTGGTGACCGTGTCTCC

> Seq ID No.14: 5103F3 BSM VH-CDRH1 Nucleic Acid Sequence
GGCTTCGCCATGGG

> Seq ID No.15: 5103F3 BSM VH-CDRH2 Nucleic Acid Sequence
TTCATCGACTCCGGCGGCGGCCCTTCTACGCCACCTGGGCCAGGGG

10 > Seq ID No.16: 5103F3 BSM VH-CDRH3 Nucleic Acid Sequence
CACGGCGGCAACACCTACTACTACGCCATGGACCCC

> Seq ID No.17: 5103F3 BSM Heavy Chain Nucleic Acid Sequence
CAGGTGCAGCTCAGGAGTCCGGCGGCAGGCTGATCAAGCCCGGCGAGCCCTGAGGCTGTCCTGCAAGACCTCCGGCATCG
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20 CTGACCAGCGGCGTGCACACCTTCCCGGCTGTCTACAGTCTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCTT
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GCCCTCCAGCCCCATCGAGAAAACCATCTCCAAGGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCTGCCCCCAT
CCCGGGATGAGTGTACCAAGAACCAGGTGAGCTGACCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCGTGGAGTG
GGAGAGCAATGGGCAGCCGGAGAACAACATAAGACCACGCCTCCCGTGTGGACTCCGACGGCTCCTTCTTCTCTATAGC
AAGCTACCGTGGACAAGAGCAGGTGGCAGCAGGGGAAGCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACT
30 ACACGCAGAAGACCTCTCCCTGTCTCCGGTTAG

> Seq ID No.18: 5103F3 BSM Heavy Chain Constant Region Nucleic Acid Sequence
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TGCTCTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCTTCCAGCAGCTTGGGCACCCAGACCTACATC
TGCAACGTGAATCACAGCCAGCAACACCAAGTGGACAAGAGAGTTGAGCCCAATCTTGTGACAAAACCTACACATGCC
CACCGTGCCAGCACCTGAATCTTGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCAAGGACACCTCATGATCTC
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40 GTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTACGCTCTCACCCTCC
TGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCAT
CTCCAAGGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCTGCCCCCATCCCGGGATGAGTGTACCAAGAACCAGGTC
AGCCTGACCTGCTTGTCAAAGGCTTCTATCCAGCGACATCGCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC
ACAAGACCACGCCTCCCGTGTGGACTCCGACGGCTCTTCTTCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCA
GCAGGGGAACGTCTTCTCATGTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCG
GGTTAG

> Seq ID No.19: 5103F3 BSM Light Chain Variable Region Nucleic Acid Sequence
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50 CTCGGCGCTGCCCTCCAGGTTCAAGGGCTCCGGCTCCGGCACCCAGTTACCCCTGACCATCTCCAGCGTGCAGCCCGAGGAC
TTCGCCACCTACTACTGCCAGCAGGTGTACTCCGTGACCAACATCGACAACGCCTTCGGCGCGCGCCACAGGGTGGAGATCA
AG

> Seq ID No.20: 5103F3 BSM VL-CDRL1 Nucleic Acid Sequence
CAGGCCTCCCAGTCCATCTCCAACCTACCTGGCC

> Seq ID No.21: 5103F3 BSM VL-CDRL2 Nucleic Acid Sequence
TCCGCTCCACCTGGCCTCC

- > Seq ID No.22: 5103F3 BSM VL-CDRL3 Nucleic Acid Sequence
CAGCAGGTGTACTCCGTGACCAACATCGACAACGCC
- > Seq ID No.23: 5103F3 BSM Light Chain Nucleic Acid Sequence
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CTCCGGCGTGCCCTCCAGGTTCAAGGGCTCCGGCTCCGGCACCCAGTTCACCTGACCATCTCCAGCGTGCAGCCCGAGGAC
TTCGCCACCTACTACTGCCAGCAGGTGTACTCCGTGACCAACATCGACAACGCCTTCGGCGGGCGGCCAGGGTGGAGATCA
AGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTG
10 CCTGTGAATAACTTCTATCCAGAGAGGCCAAAGTACAGTGGAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAG
AGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAGAAAC
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- > Seq ID No.24: 5103F3 BSM Light Chain Constant Region Nucleic Acid Sequence
CGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCC
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TGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAGAAACAC
AAAGTCTACGCTGCGAAGTCACCCATCAGGGCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG
- 20 > Seq ID No.25: 64C9 VH Amino Acid Sequence
QVQLVQSGAEVKKPGASVKVSKASGYTFTTYPIEWVRQAPGQRLEWMGNFHPYNDTKYNEKFKGRVTITRDTASASTAYME
LSSLRSEDTAVYYCARRAYGYPYAMDYWGQGLTVTVSS
- > Seq ID No.26: 64C9 VH-CDRH1
TYPEIE
- > Seq ID No.27: 64C9 VH-CDRH2
NFHPYNDTKYNEKFKG
- 30 > Seq ID No.28: 64C9 VH-CDRH3
RAYGYPYAMDY
- > Seq ID No.29: 64C9 Heavy Chain Amino Acid Sequence
QVQLVQSGAEVKKPGASVKVSKASGYTFTTYPIEWVRQAPGQRLEWMGNFHPYNDTKYNEKFKGRVTITRDTASASTAYME
LSSLRSEDTAVYYCARRAYGYPYAMDYWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG
ALTSVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVKPKSCDKTHTCPPCPAPELLGGPSVFLF
PPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLY
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- 40 > Seq ID No.30: 64C9 Heavy Chain Constant Region Amino Acid Sequence
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYI
CNVNHKPSNTKVDKRVKPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDG
VEVHNKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSP
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- > Seq ID No.31: 64C9 Light Chain Variable Region Amino Acid Sequence
DIVMTQSPDLSAVSLGERATINCKSSQSLNSGNQKNYLTWYQQKPGQPPKLLIYRASSRESGVPDRFSGSGSGTDFTLTIS
50 SLQAEDVAVYYCQNDYIYPYTFGGGTKLEIK
- > Seq ID No.32: 64C9 VL-CDRL1
KSSQSLNSGNQKNYLT
- > Seq ID No.33: 64C9 VL-CDRL2
RASSRES
- > Seq ID No.34: 64C9 VL-CDRL3

QNDYIYPYT

> Seq ID No.35: 64C9 Light Chain Amino Acid Sequence

DIVMTQSPDLSAVSLGERATINCKSSQSLNSGNQKNYLTWYQQKPGQPPKLLIYRASSRESGVPDFRFSGSGSGTDFTLTIS
SLQAEDVAVYYCQNDYIYPYTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN
SQESVTEQDSKSDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

> Seq ID No.36: 64C9 Light Chain Constant Region Amino Acid Sequence

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSDSTYSLSSTLTLSKADYEKH
KVYACEVTHQGLSSPVTKSFNRGEC

> Seq ID No.37: 64C9 Heavy Chain Variable Region Nucleic Acid Sequence

CAGGTGCAGCTGGTGCAGAGCGGCGCCGAGGTGAAGAAGCCCGGCCAGCGTGAAGGTGAGCTGCAAGGCCAGCGGCTACA
CCTTCACCACCTACCCCATCGAGTGGGTGAGGCAGGCCCCCGGCCAGAGGCTGGAGTGGATGGGCAACTTCCACCCCTACAA
CGACGACACCAAGTACAACGAGAAGTTCAAGGGCAGGGTGACCATCACCAGGGACACCAGCGCCAGCACC GGCTACATGGAG
CTGAGCAGCCTGAGGAGCGAGGACACCGCGTGTACTACTGCGCCAGGAGGGCCTACGGCTACCCCTACGCCATGGACTACT
GGGGCCAGGGCACCCCTGGTGACCGTGAGCAGC

> Seq ID No.38: 64C9 VH-CDRH1 Nucleic Acid Sequence

ACCTACCCCATCGAG

> Seq ID No.39: 64C9 VH-CDRH2 Nucleic Acid Sequence

AACTTCCACCCCTACAACGACGACACCAAGTACAACGAGAAGTTCAAGGGC

> Seq ID No.40: 64C9 VH-CDRH3 Nucleic Acid Sequence

AGGGCCTACGGCTACCCCTACGCCATGGACTAC

> Seq ID No.41: 64C9 Heavy Chain Nucleic Acid Sequence

CAGGTGCAGCTGGTGCAGAGCGGCGCCGAGGTGAAGAAGCCCGGCCAGCGTGAAGGTGAGCTGCAAGGCCAGCGGCTACA
CCTTCACCACCTACCCCATCGAGTGGGTGAGGCAGGCCCCCGGCCAGAGGCTGGAGTGGATGGGCAACTTCCACCCCTACAA
CGACGACACCAAGTACAACGAGAAGTTCAAGGGCAGGGTGACCATCACCAGGGACACCAGCGCCAGCACC GGCTACATGGAG
CTGAGCAGCCTGAGGAGCGAGGACACCGCGTGTACTACTGCGCCAGGAGGGCCTACGGCTACCCCTACGCCATGGACTACT
GGGGCCAGGGCACCCCTGGTGACCGTGAGCAGCGCTAGCACCAAGGGCCCATCGGTCTTCCCGCTGGCACCCTCCTCCAAGAG
CACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCGAACC GGTTGACGGTGCTGTTGGAACCTCAGGC
GCCCTGACCAGCGGCGTGACACCTTCCCGGCTGTCTTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGTC
CCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATACAAGCCAGCAACACCAAGGTGGACAAGAGAGTTGA
GCCCCAATCTTGTGACAAAACCTACACATGCCCCACCGTCCCCAGCAGCTGAACCTCTGGGGGGACCGTCAGTCTTCTCTTTC
CCCCAAAACCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGACGTTGAGCCACGAGACC
CTGAGGTCAAGTTCAACTGGTACGTGGAGCGGCGTGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGAGTACAACAG
CACGTACCGTGTGGTGAGCGTCTCACCGTCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGAAGGTCTCCAAC
AAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACACAGGTGTACACCTGCCCTC
CATCCCGGGATGAGCTGACCAAGAACCAGGTGACCGTGAACCTGCGTCAAGGCTTCTATCCAGCGACATCGCCGTGGA
GTGGGAGAGCAATGGGCAGCCGAGAGCAACTACAAGACCACGCTCCCGTGTGGACTCCGACGGCTCTTCTTCTCTAT
AGCAAGCTACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACC
ACTACACGCAAGAGCCTCTCCCTGTCTCCGGGTAG

> Seq ID No.42: 64C9 Heavy Chain Constant Region Nucleic Acid Sequence

GCTAGCACCAAGGGCCCATCGGTCTTCCCGCTGGCACCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCC
TGGTCAAGGACTACTTCCCGAACC GGTTGACGGTGTCTGTTGAACTCAGGCGCCCTGACCAGCGGCTGCACACCTTCCCGGC
TGTCTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCTCCAGCAGCTTGGGCACCCAGACCTACATC
TGCAAGCTGAATCACAGCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAATCTTGTGACAAAACCTACACATGCC
CACCGTGCCAGCACCTGAACCTCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCAAGGACACCCCTCATGATCTC
CCGACCCCTGAGGTACATGCGTGGTGGTGACGTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGGTACGTGGACGGC
GTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTTCAGCGTCTCACCGTCC
TGACACGAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCAT
CTCCAAGGCCAAAGGGCAGCCCCGAGAACACAGGTGTACACCTGCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTC
AGCCTGACCTGCTTGGTCAAGGCTTCTATCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGAGAACAACT
ACAAGACCACGCTCCCGTGTCTGGACTCCGACGGCTCTTCTTCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCA
GCAGGGGAACGCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAAGAGCCTCTCCCTGTCTCCG

GGTTAG

> Seq ID No.43: 64C9 Light Chain Variable Region Nucleic Acid Sequence

GACATCGTGATGACCCAGAGCCCCGACAGCCTGGCCGTGAGCCTGGGCGAGAGGGCCACCATCAACTGCAAGAGCAGCCAGA
GCCTGCTGAACAGCGGCAACCAGAAGAACTACCTGACCTGGTACCAGCAGAAGCCCGGCCAGCCCCCAAGCTGCTGATCTA
CAGGGCCAGCAGCAGGGAGAGCGGCGTGCCCGACAGGTTTCAGCGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGC
AGCCTGCAGGCCGAGGACGTGGCCGTGTACTACTGCCAGAACGACTACATCTACCCCTACACCTTCGGCGGGCGGCACCAAGC
TGGAGATCAAG

10 > Seq ID No.44: 64C9 VL-CDRL1 Nucleic Acid Sequence
AAGAGCAGCCAGAGCCTGCTGAACAGCGGCAACCAGAAGAACTACCTGACC

> Seq ID No.45: 64C9 VL-CDRL2 Nucleic Acid Sequence
AGGGCCAGCAGCAGGGAGAGC

> Seq ID No.46: 64C9 VL-CDRL3 Nucleic Acid Sequence
CAGAACGACTACATCTACCCCTACACC

> Seq ID No.47: 64C9 Light Chain Nucleic Acid Sequence

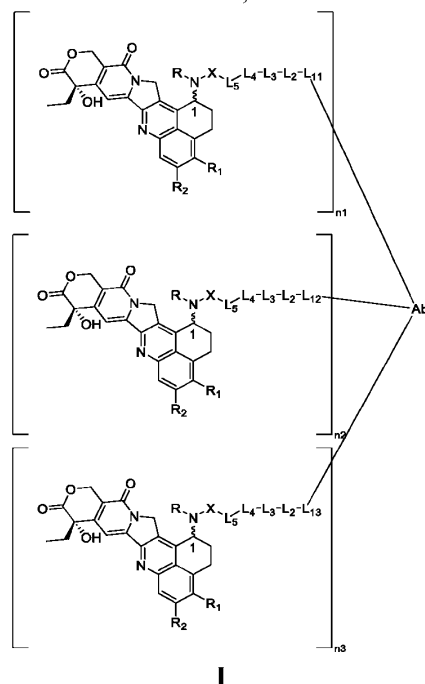
20 GACATCGTGATGACCCAGAGCCCCGACAGCCTGGCCGTGAGCCTGGGCGAGAGGGCCACCATCAACTGCAAGAGCAGCCAGA
GCCTGCTGAACAGCGGCAACCAGAAGAACTACCTGACCTGGTACCAGCAGAAGCCCGGCCAGCCCCCAAGCTGCTGATCTA
CAGGGCCAGCAGCAGGGAGAGCGGCGTGCCCGACAGGTTTCAGCGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGC
AGCCTGCAGGCCGAGGACGTGGCCGTGTACTACTGCCAGAACGACTACATCTACCCCTACACCTTCGGCGGGCGGCACCAAGC
TGGAGATCAAGCGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTGCCTC
TGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAAC
TCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAAGCAGACT
ACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGA
GTGTTAG

30 > Seq ID No.48: 64C9 Light Chain Constant Region Nucleic Acid Sequence

CGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTGCCTCTGTTGTGTGCC
TGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACCTCCCAGGAGAG
TGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAAGCAGACTACGAGAAACAC
AAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG

CLAIMS

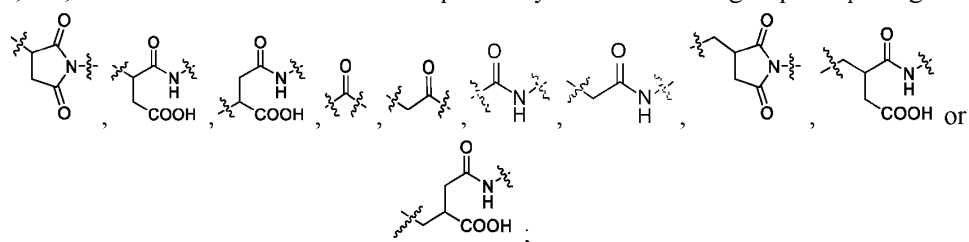
1. A ligand-camptothecin derivative conjugate as shown in General Formula I, or a pharmaceutically acceptable salt or solvate thereof;



wherein

Ab is an antibody targeting human Claudin18.2 or an antigen-binding fragment thereof;

L_{11} , L_{12} , and L_{13} are different and are independently selected from a group comprising:



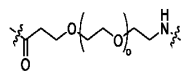
L₂ has the structure shown in Formula A below.



Formula A

wherein Y is a scaffold selected from C1-C6 alkyl, substituted C1-C6 alkyl, or C3-C8 cycloalkyl; preferably Y is C1-C6 alkyl; Ac is a hydrophilic structural unit; and the carbon 2 attached to Y has an absolute chirality in R or S configuration;

L₃ is present or absent, and when present, L₃ comprises PEG hydrophilic units:



, o is an integer of 1-10, preferably an integer of 2-8;

L₄ is an enzymatically cleavable unit;

L₅ is a linking unit;

the chiral carbon atom No. 1 attached to N in formula **I** has an absolute chirality in R or S configuration;

R is hydrogen, deuterium, halogen, C1-C6 alkyl, substituted C1-C6 alkyl, deuterated C1-C6 alkyl, C3-C8 cycloalkyl C1-C6 alkyl, C1-C6 alkoxy C1-C6 alkyl, C6-C10 aryl, substituted C6-C10 aryl, 5-10 heteroaryl, or substituted 5-10 heteroaryl;

preferably R is hydrogen or a C1-C6 alkyl group;

R₁ is hydrogen, deuterium, halogen, C1-C6 alkyl, substituted C1-C6 alkyl, deuterated C1-C6 alkyl, a C3-C8 cycloalkyl C1-C6 alkyl group, a C1-C6 alkoxy C1-C6 alkyl group, carboxylic acid, a 3-7 heterocyclic group, a substituted 3-7 heterocyclic group, C6-C10 aryl, substituted C6-C10 aryl, 5-10 heteroaryl, or substituted 5-10 heteroaryl;

preferably R₁ is hydrogen or a C1-C6 alkyl group;

more preferably R₁ is C1-C6 alkyl;

R₂ is hydrogen, deuterium, halogen, C1-C6 alkyl, substituted C1-C6 alkyl, deuterated C1-C6 alkyl, a C3-C8 cycloalkyl C1-C6 alkyl group, a C1-C6 alkoxy C1-C6 alkyl group, carboxylic acid, a 3-7 heterocyclic group, a substituted 3-7 heterocyclic group, C6-C10 aryl, substituted C6-C10 aryl, 5-10-membered heteroaryl, or substituted 5-10 heteroaryl;

preferably R₂ is hydrogen, halogen or C1-C6 alkyl;

more preferably R₂ is halogen;

X is -C(O)-CR_aR_b-(CR₃R₄)_m-O-, -C(O)-CR_aR_b-(CR₃R₄)_m-NH- or -C(O)-CR_aR_b-(CR₃R₄)_m-S-

preferably X is -C(O)-CR_aR_b-(CR₃R₄)_m-O-;

R_a and R_b are each independently hydrogen, deuterium, halogen, C1-C6 alkyl, deuterium-substituted C1-C6 alkyl, halogen-substituted C1-C6 alkyl, C3-C8 cycloalkyl, a C3-C8 cycloalkyl C1-C6 alkyl group, a C6-C10 aryl C1-C6 alkyl group, a C1-C6 alkoxy C1-C6 alkyl group, a 3-7 heterocyclic group, a substituted 3-7 heterocyclic group, C6-C10 aryl, substituted C6-C10 aryl, 5-10 heteroaryl, or substituted 5-10 heteroaryl;

preferably, R_a and R_b are each independently hydrogen, C1-C6 alkyl, halo-C1-C6 alkyl, a C3-C8 cycloalkyl C1-C6 alkyl group, or a C6-C10 aryl C1-C6 alkyl group;

alternatively, R_a, R_b and the carbon atoms to which they are attached constitute a C3-C8 cycloalkyl group, a C3-C8 cycloalkyl C1-C6 alkyl, a 3-7 heterocycloalkyl, or a substituted 3-7 heterocycloalkyl; preferably R_a, R_b and the carbon atoms to which they are attached constitute a C3-C8 cycloalkyl;

R₃, R₄ are the same or different and are independently hydrogen, deuterium, halogens, C1-C6 alkyl, halogenated C1-C6 alkyl, deuterated C1-C6 alkyl, C1-C6 alkoxy, hydroxyl, amino, cyano,

nitro, hydroxy C1-C6 alkyl, C3-C8 cycloalkyl, a 3-7 heterocyclic group, or a substituted 3-7 heterocyclic group, respectively;

preferably, R₃, R₄ are independently hydrogen or C1-C6 alkyl, respectively;

Alternatively, R₃, R₄ and the carbon atoms attached thereto constitute a C3-C8 cycloalkyl, a C3-C8 cycloalkyl C1-C6 alkyl group, a 3-7 heterocyclic group, or a substituted 3-7 heterocyclic group;

m is an integer between 0-4, preferably 0, 1; n₁, n₂, n₃ are each independently selected from any integer or any decimal number from 0-10, n₁, n₂, n₃ are not simultaneously 0, and $1 \leq n_1 + n_2 + n_3 \leq 10$.

2. The ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof according to Claim 1, wherein Ab is an antibody having a binding affinity to human Claudin18.2.

3. The ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof according to Claim 2, wherein the antibody comprises an IgG heavy chain and a light chain.

4. The ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof according to Claims 3, wherein the light chain comprises CDRs of SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10, and the IgG heavy chain comprises CDRs of SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4.

5. The ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof according to Claim 1, wherein Ab comprises

a light chain, comprising CDRL1, CDRL2 and CDRL3 each having an amino acid sequence encoded by a nucleic acid sequence of SEQ ID NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22, respectively, and

a heavy chain comprising CDRH1, CDRH2 and CDRH3 each having an amino acid sequence encoded by a nucleic acid sequence of SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, respectively.

6. The ligand-camptothecin derivative conjugate of Claim 1 as shown in general formula I, or a pharmaceutically acceptable salt or solvate thereof, wherein Ab comprises

a heavy chain comprising a heavy chain variable region of SEQ ID NO: 1, and

a light chain comprising a light chain variable region of SEQ ID NO: 7.

7. The ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof according to Claim 1, wherein Ab comprises

a heavy chain variable region comprising an amino acid sequence encoded by a nucleic acid sequence of SEQ ID NO: 13, and

a light chain variable region comprising an amino acid sequence encoded by a nucleic acid sequence of SEQ ID NO: 19.

8. The ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof according to Claim 1, comprising:

a heavy chain having an amino acid sequence of SEQ ID NO: 5, and

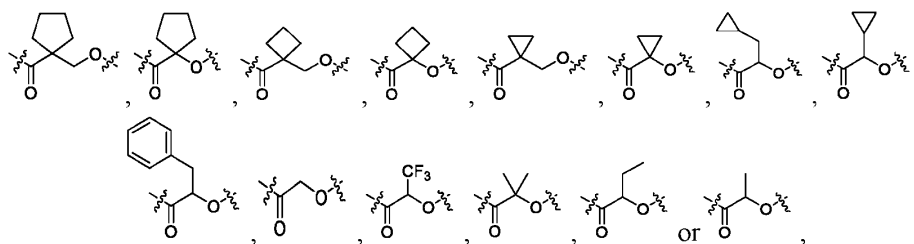
a light chain having an amino acid sequence of SEQ ID NO: 11.

9. The ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof according to Claim 1, comprising,

a heavy chain having an amino acid sequence encoded by a nucleic acid sequence of SEQ ID NO: 17, and

a light chain having an amino acid sequence encoded by a nucleic acid sequence of SEQ ID NO: 23.

10. The ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-9, wherein said X comprises the following structures or isomers thereof:



wherein the position indicated by the wavy line on the left is connected to the camptothecin derivative, and the position indicated by the wavy line on the right is connected to L5.

11. The ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof according to Claim 10, wherein L₄ comprises peptide residues consisting of amino acids.

wherein optionally, said amino acid is further substituted with one or more substituents selected from one or more of deuterium, halogens, hydroxyl, cyano, amino, nitro, carboxyl, C1-C6 alkyl, substituted C1-C6 alkyl, C1-C6 alkoxy, C3-C8 cycloalkyl, or substituted C3-C8 cycloalkyl;

preferably, said peptide residue is a peptide residue formed from one, two or more amino acids selected from phenylalanine (F), glycine (G), valine (V), lysine (K), citrulline (C), serine (S), glutamic acid (E) or aspartic acid (D);

more preferably, said peptide residue is a tetrapeptide residue comprising glycine (G) -glycine (G) -phenylalanine (F) -glycine (G).

12. The ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof according to Claim 11, wherein

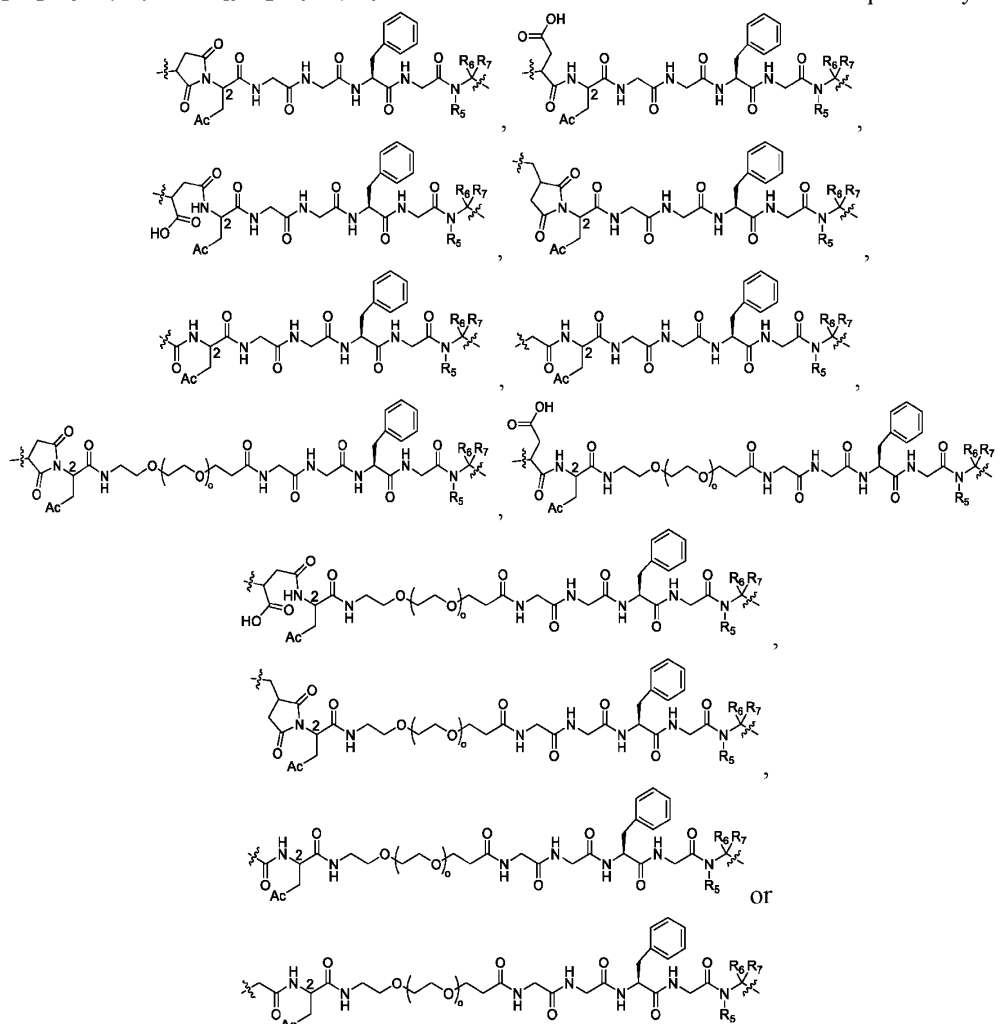
L₅ is -NR₅(CR₆R₇)_q- or a chemical bond, and q is an integer of 0-6;

R₅, R₆ and R₇ are the same or different and are each independently hydrogen, deuterium, halogen, a C1-C6 alkyl, a substituted C1-C6 alkyl, a deuterated C1-C6 alkyl, a C3-C8 cycloalkyl, a C3-C8 cycloalkyl C1-C6 alkyl, a C1-C6 alkoxy C1-C6 alkyl, a 3-7 heterocycloheteroaryl, a substituted 3-7 heterocycloheteroaryl, a C6-C10 aryl, a substituted C6-C10 aryl, 5-10 heteroaryl, or substituted 5-10 heteroaryl;

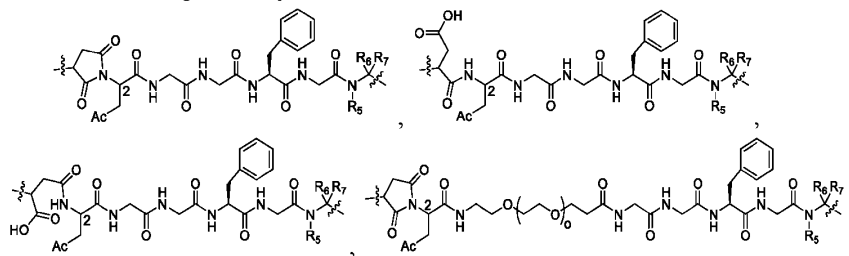
preferably, R₅, R₆ and R₇ are each independently hydrogen or a C1-C6 alkyl group;

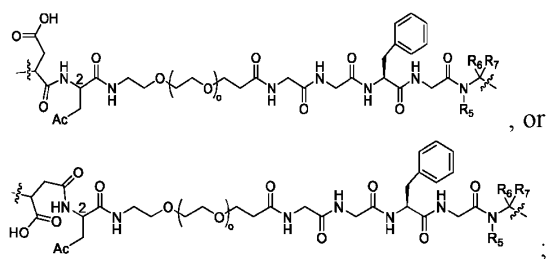
more preferably, R₅, R₆ and R₇ are each independently hydrogen.

13. The ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claim 1, wherein said linking units -L₁₁-L₂-L₃-L₄-L₅-, -L₁₂-L₂-L₃-L₄-L₅- or -L₁₃-L₂-L₃-L₄-L₅- are different from each other and each independently:



preferably, -L₁₁-L₂-L₃-L₄-L₅-, -L₁₂-L₂-L₃-L₄-L₅- or -L₁₃-L₂-L₃-L₄-L₅- are different from each other and are each independently:





wherein

Ac is a hydrophilic structural unit;

R₅, R₆ and R₇ are the same or different and are each independently hydrogen, deuterium, halogen, C1-C6 alkyl, substituted C1-C6 alkyl, deuterated C1-C6 alkyl, C3-C8 cycloalkyl, C3-C8 cycloalkyl C1-C6 alkyl, C1-C6 alkoxy C1-C6 alkyl, 3-7 heterocyclic, substituted 3-7 heterocyclic, C6-C10 aryl, substituted C6-C10 aryl, 5-10 heteroaryl, or substituted 5-10 heteroaryl.

preferably, R₅, R₆ and R₇ are each independently hydrogen or a C1-C6 alkyl;

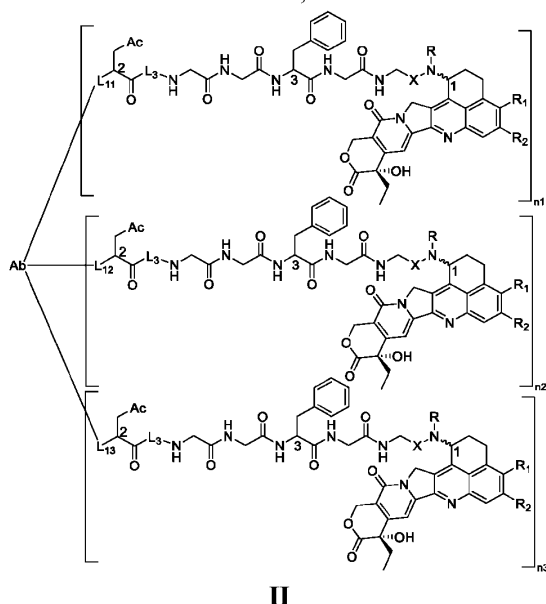
more preferably, R₅, R₆ and R₇ are each independently hydrogen;

the carbon atom #2 attached to N has an absolute chirality in either R or S configuration;

the position shown by the left wavy line is connected to Ab, and the position shown by the right wavy line is connected to X;

o is an integer of 1-10.

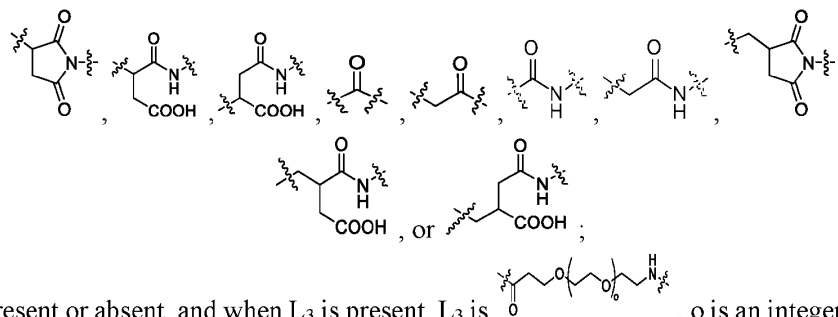
14. A ligand-camptothecin derivative conjugate as shown in general formula II, or a pharmaceutically acceptable salt or solvate thereof;



wherein

Ab is an antibody having a binding affinity to human Claudin18.2 or an antigen-binding fragment thereof;

L_{11} , L_{12} , and L_{13} are connecting units, and L_{11} , L_{12} , and L_{13} are different from each other and are independently:



L₃ is present or absent, and when L₃ is present, L₃ is 0 or 1, o is an integer of 1-10, preferably an integer of 2-8;

Ac is a hydrophilic structural unit;

the 1-position, 2-position and 3-position chiral carbon atoms are each independently in R or S configuration;

R is hydrogen, deuterium, halogen, C1-C6 alkyl, substituted C1-C6 alkyl, deuterated C1-C6 alkyl, a C3-C8 cycloalkyl C1-C6 alkyl group, a C1-C6 alkoxy C1-C6 alkyl group, C6-C10 aryl, substituted C6-C10 aryl, 5-10 heteroaryl, or substituted 5-10 heteroaryl;

preferably R is hydrogen or C1-C6 alkyl;

R₁ is hydrogen, deuterium, halogen, C1-C6 alkyl, substituted C1-C6 alkyl, deuterated C1-C6 alkyl, a C3-C8 cycloalkyl C1-C6 alkyl group, a C1-C6 alkoxy C1-C6 alkyl group, carboxyl, a 3-7 heterocyclic group, a substituted 3-7 meta-heterocyclic group, C6-C10 aryl, substituted C6-C10 aryl, 5-10 meta-heteroaryl, or substituted 5-10 heteroaryl;

preferably R₁ is hydrogen or C1-C6 alkyl;

more preferably R₁ is C1-C6 alkyl;

R₂ is hydrogen, deuterium, halogen, C1-C6 alkyl, substituted C1-C6 alkyl, deuterated C1-C6 alkyl, a C3-C8 cycloalkyl C1-C6 alkyl group, a C1-C6 alkoxy C1-C6 alkyl group, carboxyl, 3-7 meta-heterocyclic, substituted 3-7 meta-heterocyclic, C6-C10 aryl, substituted C6-C10 aryl, 5-10 meta-heteroaryl, or substituted 5-10 heteroaryl;

preferably R₂ is hydrogen, halogen or C1-C6 alkyl;

more preferably R_2 is halogen;

X is $-C(O)-CR_aR_b-(CR_3R_4)_m-O-$, $-C(O)-CR_aR_b-(CR_3R_4)_m-NH-$ or $-C(O)-CR_aR_b-(CR_3R_4)_m-S-$.

preferably X is $-\text{C}(\text{O})-\text{CR}_a\text{R}_b-(\text{CR}_3\text{R}_4)_m-\text{O}-$;

R_a and R_b are each independently hydrogen, deuterium, halogen, C1-C6 alkyl, deuterated C1-C6 alkyl, halogenated C1-C6 alkyl, C3-C8 cycloalkyl, a C3-C8 cycloalkyl C1-C6 alkyl group, a C1-C6 alkoxy C1-C6 alkyl group, 3-7 heterocyclyl, substituted 3-7 heterocyclyl, C6-C10 aryl, substituted C6-C10 aryl, 5-10 heteroaryl, or substituted 5-10 heteroaryl;

preferably, R_a and R_b are each independently hydrogen, C1-C6 alkyl, halo-C1-C6 alkyl, a C3-C8 cycloalkyl C1-C6 alkyl group, or a C6-C10 aryl C1-C6 alkyl group;

alternatively, R_a , R_b and the carbon atoms to which they are attached constitute a C3-C8 cycloalkyl group, a C3-C8 cycloalkyl C1-C6 alkyl group, a 3-7 heterocycloalkyl group, a substituted 3-7 heterocycloalkyl group; preferably R_a , R_b and the carbon atoms to which they are attached constitute a C3-C8 cycloalkyl group;

R_3 , R_4 are the same or different and are independently hydrogen, deuterium, halogens, C1-C6 alkyl, halogenated C1-C6 alkyl, deuterated C1-C6 alkyl, C1-C6 alkoxy, hydroxyl, amino, cyano, nitro, hydroxy C1-C6 alkyl, C3-C8 cycloalkyl, a 3-7 heterocyclic group, a substituted 3-7 heterocyclic group, respectively;

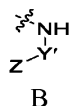
preferably R_3 , R_4 , are independently hydrogen or C1-C6 alkyl, respectively;

alternatively, R_3 , R_4 and the carbon atoms attached thereto constitute C3-C8 cycloalkyl, a C3-C8 cycloalkyl C1-C6 alkyl group, a 3-7 heterocyclic group, or a substituted 3-7 heterocyclic group;

m is an integer of 0-4, preferably 0, 1;

n_1 , n_2 , and n_3 are each independently an integer or a decimal number from 0 to 10, n_1 , n_2 , and n_3 are not simultaneously 0, and $1 \leq n_1 + n_2 + n_3 \leq 10$.

15. The ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1 or 14, wherein said Ac has the structure shown in formula B:



wherein

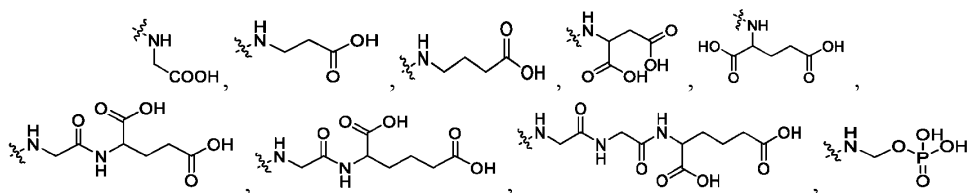
Z comprises a hydrophilic structural carboxyl group, a phosphoric acid, a polyphosphoric acid, a phosphite, a sulfonic acid, a sulfinic acid, or a polyethylene glycol (PEG);

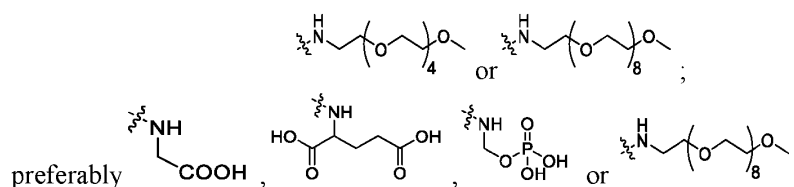
preferably Z comprises hydrophilic structural carboxylates, phosphoric acid or polyethylene glycol (PEG);

Y' is optionally a scaffold connecting the amino group to Z; preferably Y' is C1-C6 alkyl;

positions shown by wavy lines are connection sites.

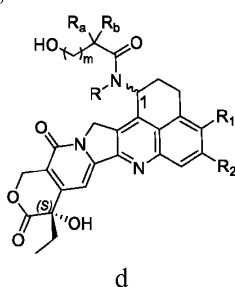
16. The ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1 or 14, wherein said Ac comprises glycine, (*D/L*) alanine, (*D/L*) leucine, (*D/L*) isoleucine, (*D/L*) valine, (*D/L*) phenylalanine, (*D/L*) proline, (*D/L*) tryptophan, (*D/L*) serine, (*D/L*) tyrosine, (*D/L*) cysteine, (*D/L*) cystine, (*D/L*) arginine, (*D/L*) histidine, (*D/L*) methionine, (*D/L*) asparagine, (*D/L*) glutamine, (*D/L*) threonine, (*D/L*) aspartic acid, (*D/L*) glutamic acid, natural or non-natural amino acid derivatives, or the following structures or isomers thereof.





17. The ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1 or 14, wherein said Ac comprises glycine, phosphoric acid, (*D/L*) glutamic acid, or polyethylene glycol hydrophilic structure.

18. The ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1 or 14, wherein said camptothecin derivative has the structure shown in formula d below;



wherein

R comprises hydrogen, deuterium, halogen, C1-C6 alkyl, substituted C1-C6 alkyl, deuterated C1-C6 alkyl, a C3-C8 cycloalkyl C1-C6 alkyl group, a C1-C6 alkoxy C1-C6 alkyl group, C6-C10 aryl, substituted C6-C10 aryl, 5-10 heteroaryl, or substituted 5-10 heteroaryl;

preferably R is hydrogen or C1-C6 alkyl;

R₁ comprises hydrogen, deuterium, halogen, C1-C6 alkyl, substituted C1-C6 alkyl, deuterated C1-C6 alkyl, C3-C8 cycloalkyl C1-C6 alkyl, C1-C6 alkoxy C1-C6 alkyl, carboxyl, 3-7 meta-heterocyclic, substituted 3-7 meta-heterocyclic, C6-C10 aryl, substituted C6-C10 aryl, 5-10 meta-heteroaryl, or substituted 5-10 heteroaryl;

preferably R₁ is hydrogen or C1-C6 alkyl;

more preferably R₁ comprises C1-C6 alkyl;

R₂ comprises hydrogen, deuterium, halogen, C1-C6 alkyl, substituted C1-C6 alkyl, deuterated C1-C6 alkyl, a C3-C8 cycloalkyl C1-C6 alkyl group, a C1-C6 alkoxy C1-C6 alkyl group, carboxylic acid, 3-7 heterocyclic group, a substituted 3-7 heterocyclic group, C6-C10 aryl, substituted C6-C10 aryl, 5-10 heteroaryl, or substituted 5-10 heteroaryl;

preferably R₂ is hydrogen, halogen or C1-C6 alkyl;

more preferably R₂ is halogen;

R_a and R_b are each independently hydrogen, deuterium, halogen, C1-C6 alkyl, deuterated C1-C6 alkyl, halogenated C1-C6 alkyl, C3-C8 cycloalkyl, a C3-C8 cycloalkyl C1-C6 alkyl group, a C1-C6 alkoxy C1-C6 alkyl group, a 3-7 meta-heterocyclic group, a substituted 3-7 meta-

heterocyclic group, C6-C10 aryl, substituted C6-C10 aryl, 5-10 heteroaryl, or substituted 5-10 heteroaryl;

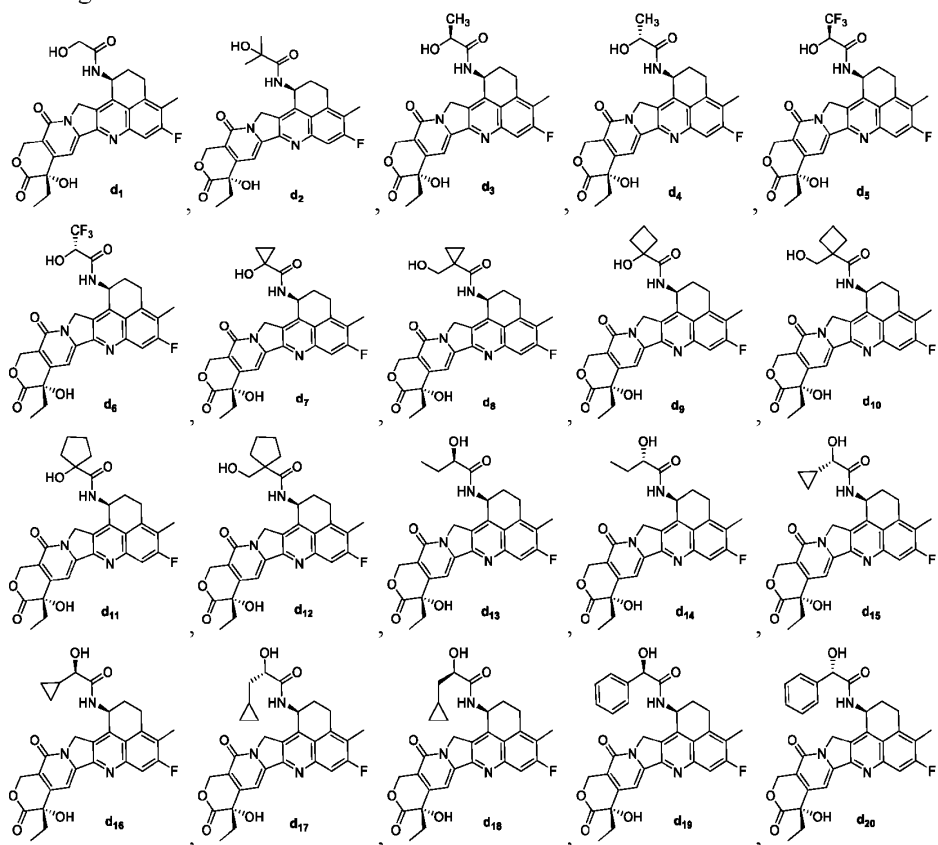
preferably, R_a and R_b are each independently hydrogen, C1-C6 alkyl, halo-C1-C6 alkyl, a C3-C8 cycloalkyl C1-C6 alkyl group, C6-C10 aryl;

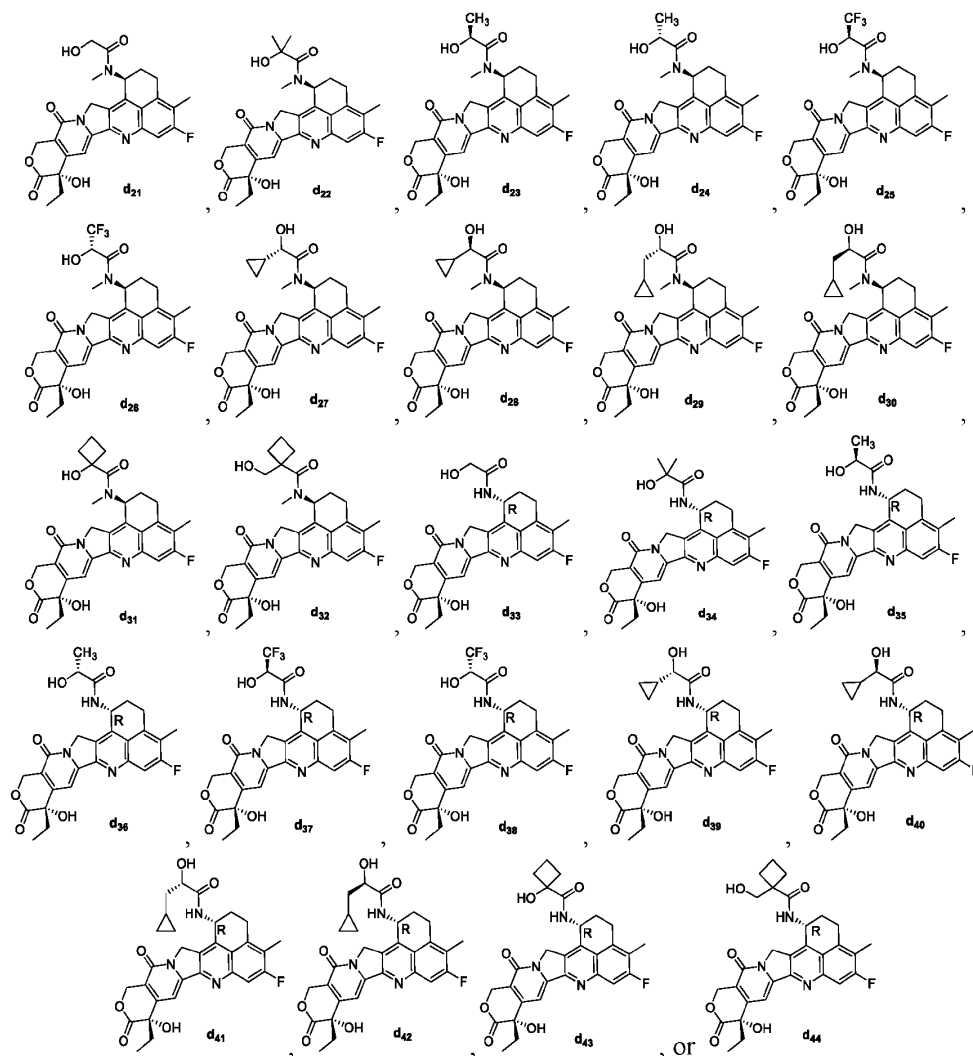
alternatively, R_a , R_b and the carbon atoms to which they are attached constitute C3-C8 cycloalkyl, C3-C8 cycloalkyl C1-C6 alkyl group, 3-7 heterocycloalkyl, substituted 3-7 heterocycloalkyl; preferably R_a , R_b and the carbon atoms to which they are attached constitute a C3-C8 cycloalkyl group;

the 1-position chiral carbon atom is in R or the S configuration;

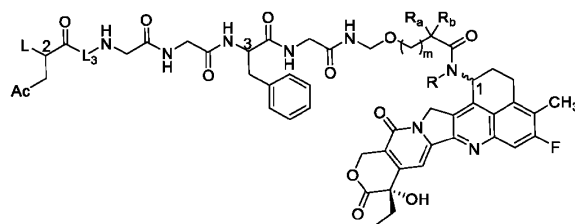
m is 0 or 1.

19. The ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1 or 14, wherein said structural formula d comprises the following:



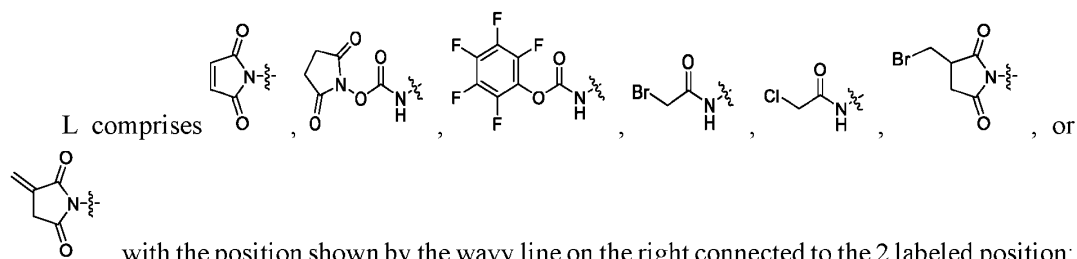


20. A linker-drug compound or a pharmaceutically acceptable salt or solvate thereof, having the structure shown in formula **III** below.



III

wherein



R comprises hydrogen, deuterium, halogen, C1-C6 alkyl, substituted C1-C6 alkyl, deuterated C1-C6 alkyl, a C3-C8 cycloalkyl C1-C6 alkyl group, a C1-C6 alkoxy C1-C6 alkyl group, C6-C10 aryl, substituted C6-C10 aryl, 5-10 heteroaryl, or substituted 5-10 heteroaryl;

R_a comprises hydrogen, deuterium, halogen, C1-C6 alkyl, deuterated C1-C6 alkyl, halogenated C1-C6 alkyl, C3-C8 cycloalkyl, a C3-C8 cycloalkyl C1-C6 alkyl group, a C1-C6 alkoxy C1-C6 alkyl group, a 3-7 meta-heterocyclic group, a substituted 3-7 meta-heterocyclic group, C6-C10 aryl, substituted C6-C10 aryl, 5-10 meta-heteroaryl, or substituted 5-10-membered heteroaryl;

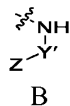
R_b comprises hydrogen, deuterium, halogen, C1-C6 alkyl, deuterated C1-C6 alkyl, halogenated C1-C6 alkyl, C3-C8 cycloalkyl, C3-C8 cycloalkyl C1-C6 alkyl, C1-C6 alkoxy C1-C6 alkyl, a 3-7 meta-heterocyclic group, a substituted 3-7 meta-heterocyclic group, C6-C10 aryl, substituted C6-C10 aryl, 5-10 meta-heteroaryl, or substituted 5-10 heteroaryl;

alternatively, R_a, R_b and the carbon atoms attached thereto constitute a C3-C8 cycloalkyl group, a C3-C8 cycloalkyl C1-C6 alkyl group, a 3-7 heterocyclic group, or a substituted 3-7-membered heterocyclic group;

L₃ present or absent; when L₃ is present, L₃ is , o is an integer of 1 to 10;
the chiral carbon atom at the 1-position, 2-position or 3-position is in R or S configuration;
Ac is a hydrophilic structural unit;
m comprises 0 or 1;

preferably, said linker-drug compound or a pharmaceutically acceptable salt or solvate thereof is configured to couple with the ligand Ab to form a ligand-camptothecin derivative conjugate of formula I or formula II according to any one of Claims 1-19.

21. The linker-drug compound or a pharmaceutically acceptable salt or solvate thereof according to Claim 20, wherein said Ac has the structure shown in formula B below.



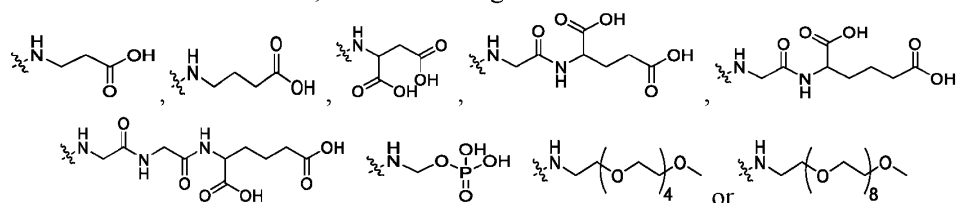
wherein:

Z comprises a hydrophilic structural carboxyl group, a phosphoric acid, a polyphosphoric acid, a phosphite, a sulfonic acid, a sulfinic acid, or a polyethylene glycol (PEG);

Y' is an optional unit connecting the amino group and Z;

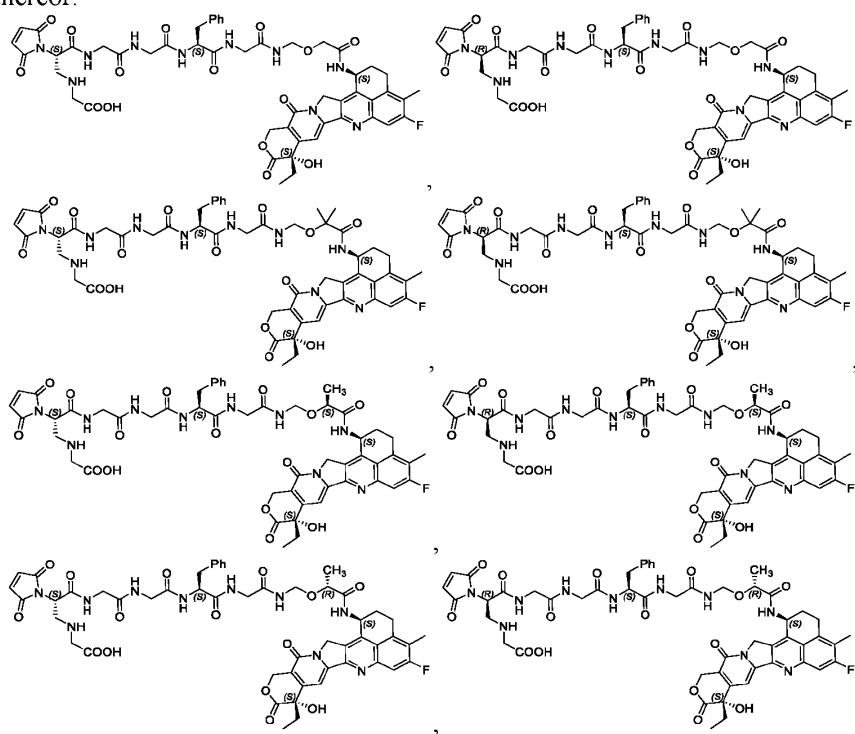
positions shown by wavy lines are connection sites.

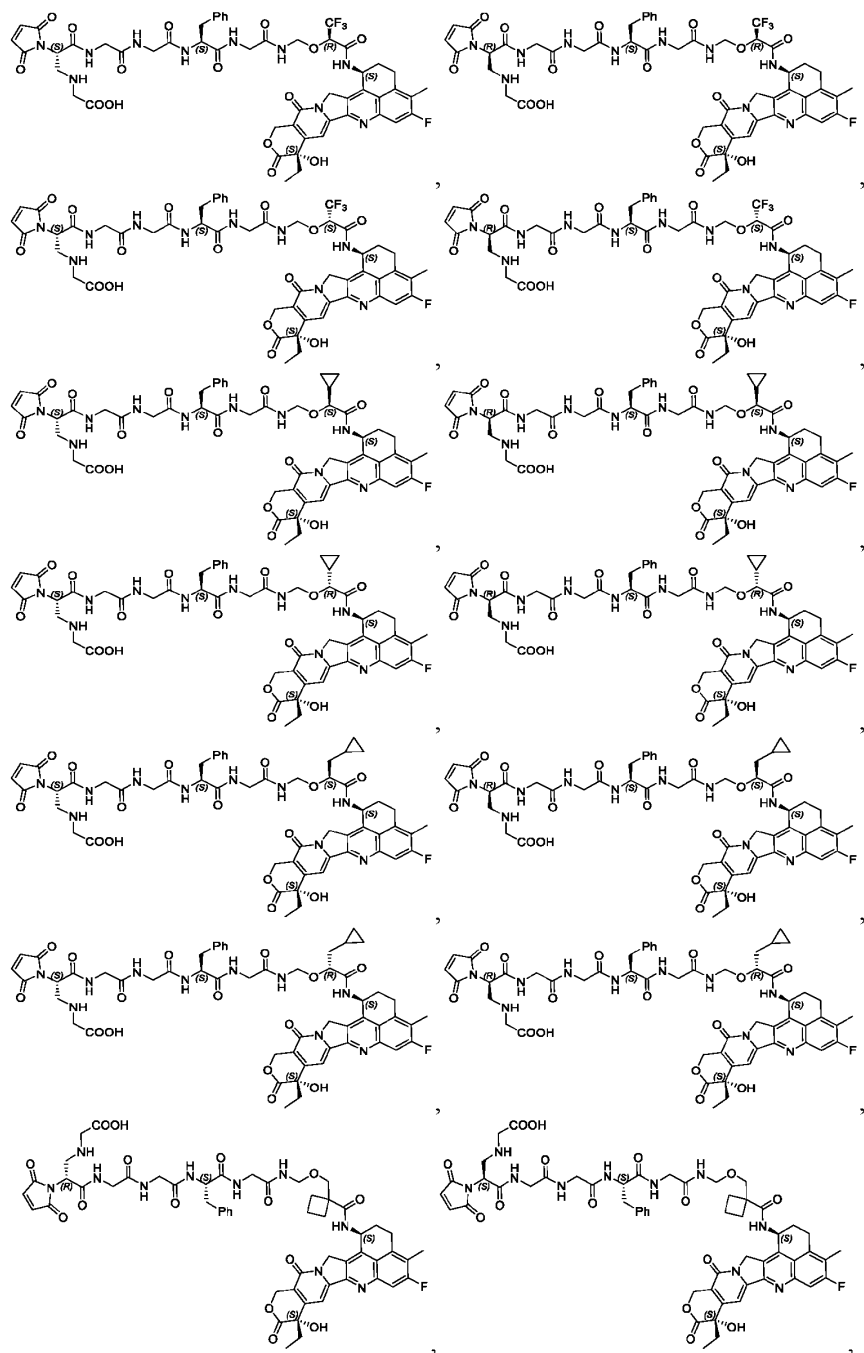
22. The linker-drug compound or a pharmaceutically acceptable salt or solvate thereof according to Claim 20, wherein said Ac comprises glycine, (*D/L*) alanine, (*D/L*) leucine, (*D/L*) isoleucine, (*D/L*) valine, (*D/L*) phenylalanine, (*D/L*) proline, (*D/L*) tryptophan, (*D/L*) serine, (*D/L*) tyrosine, (*D/L*) cysteine, (*D/L*) cystine, (*D/L*) arginine, (*D/L*) histidine, (*D/L*) methionine, (*D/L*) asparagine, (*D/L*) glutamine, (*D/L*) threonine, (*D/L*) aspartic acid, (*D/L*) glutamic acid, natural or unnatural amino acid derivatives, or the following structures:

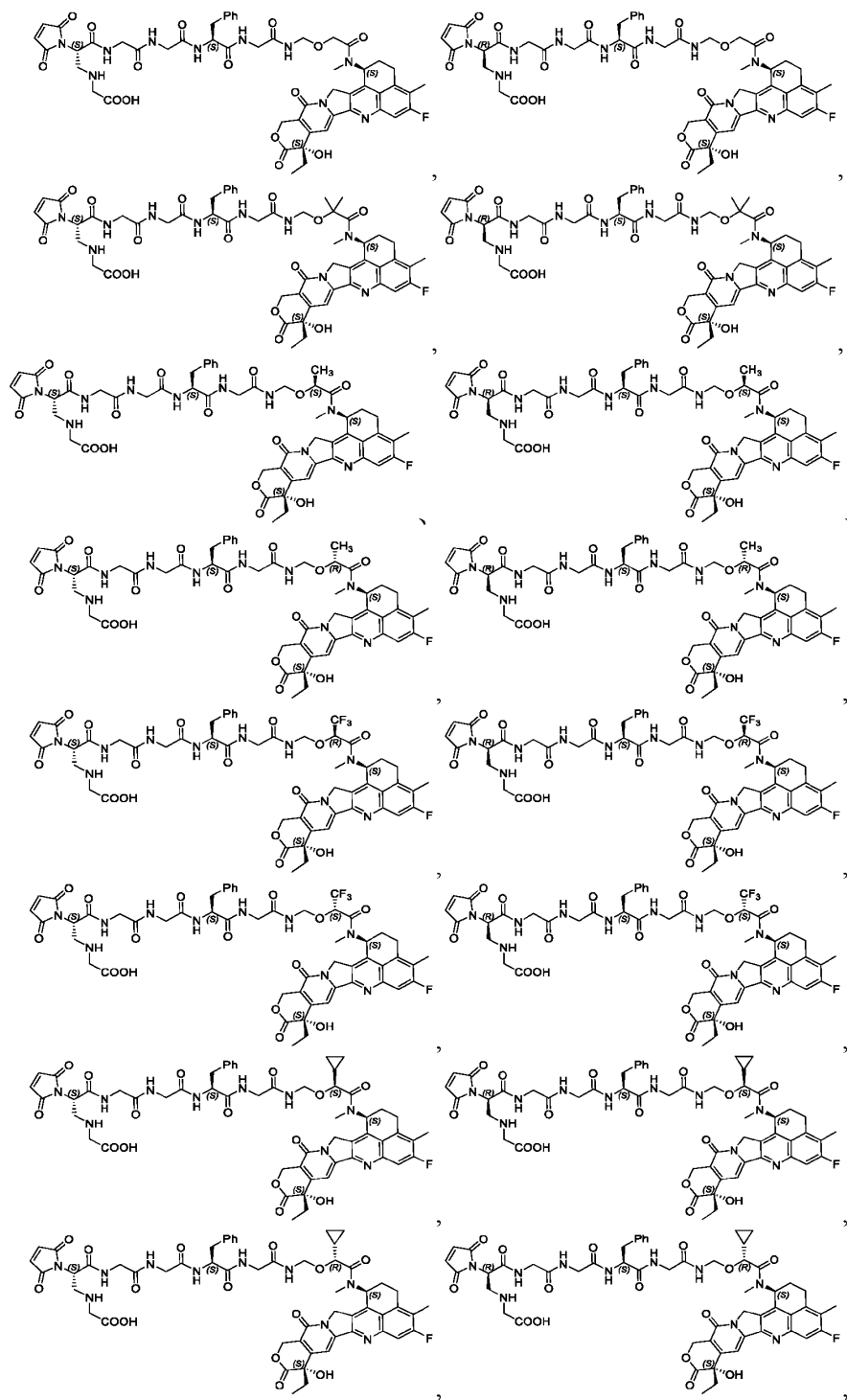


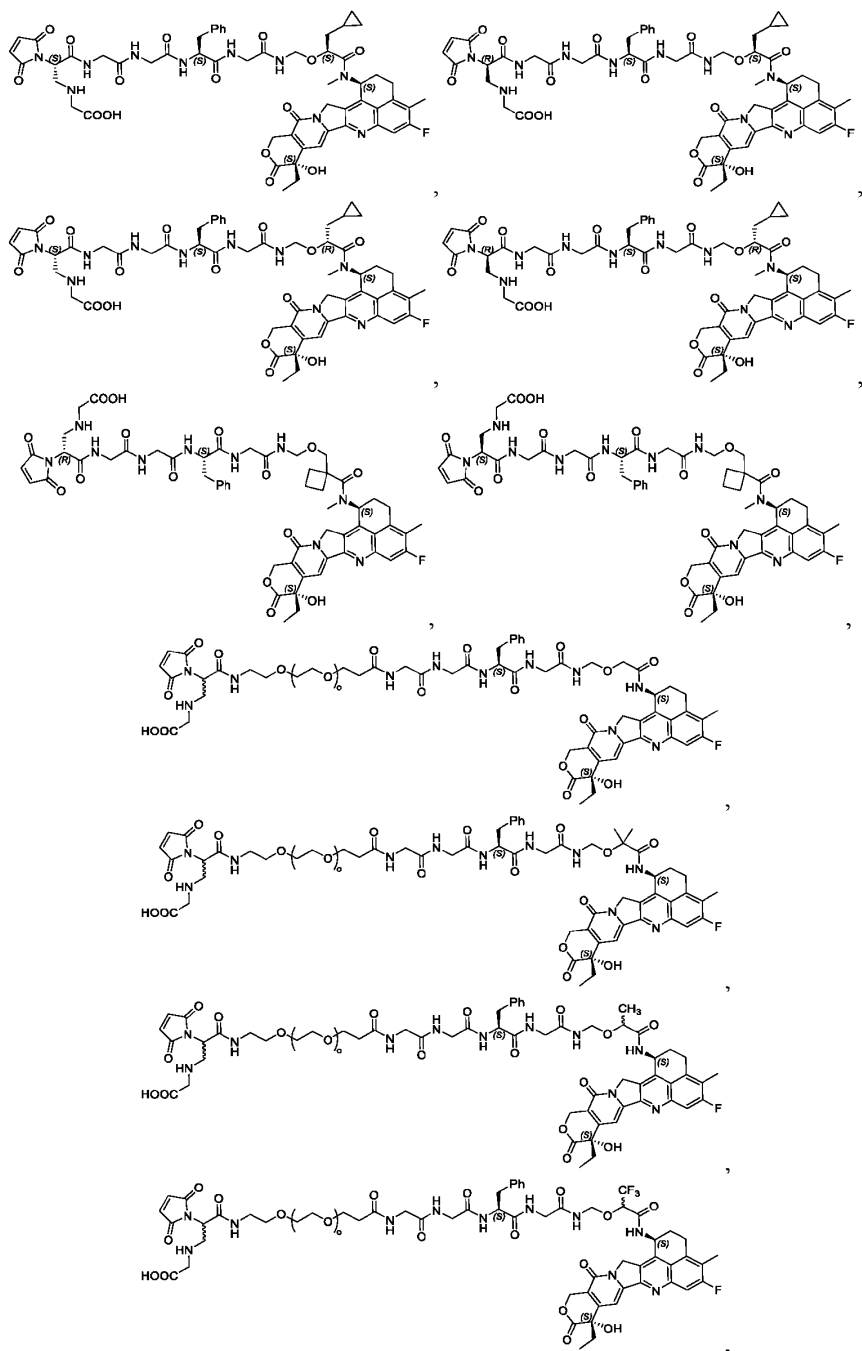
23. The linker-drug compound or a pharmaceutically acceptable salt or solvate thereof according to Claim 20, wherein Ac comprises glycine, phosphoric acid, (*D/L*) glutamic acid, or polyethylene glycol hydrophilic structure.

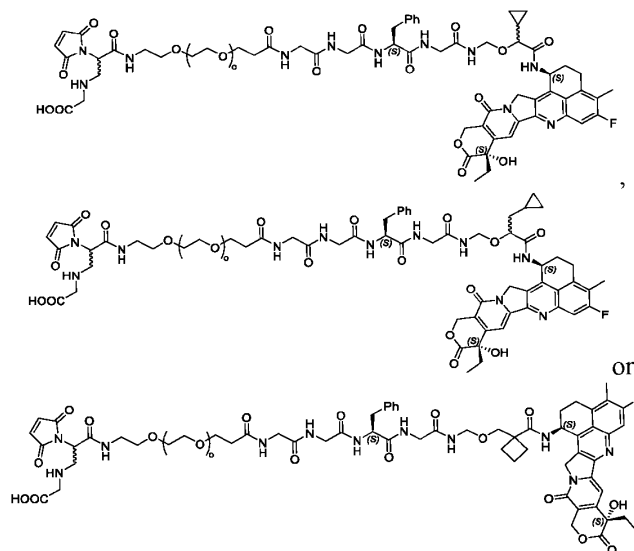
24. The linker-drug compound or a pharmaceutically acceptable salt or solvate thereof according to Claim 20, wherein said linker-drug compound comprises the following structures or isomers thereof:







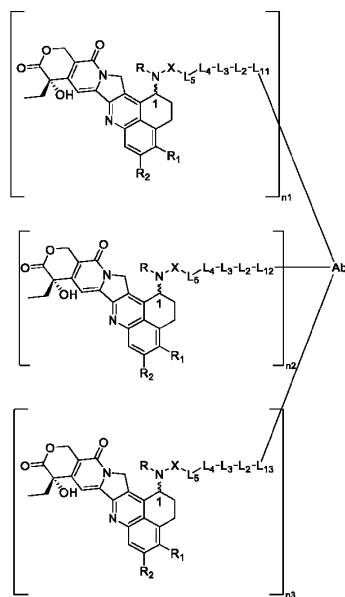


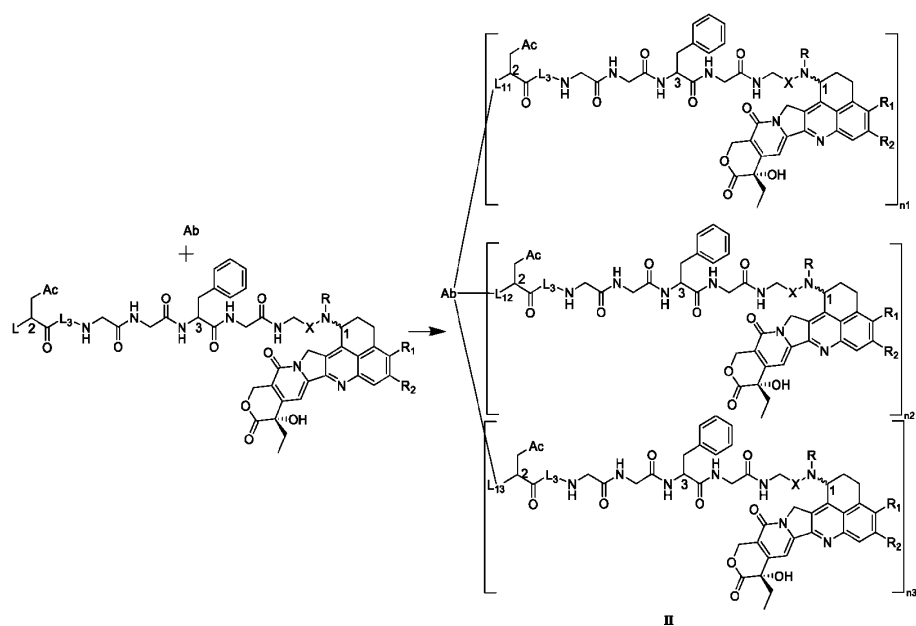


wherein o is an integer from 1-10.

25. A method for preparing a ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof as shown in general formula **I** or general formula **II**, wherein it comprises the following steps:

obtaining the ligand-camptothecin derivative conjugates as shown in general formula **I** or general formula **II** by the coupling the reduced antibody or antigen-binding fragment thereof with the linker-drug compound;

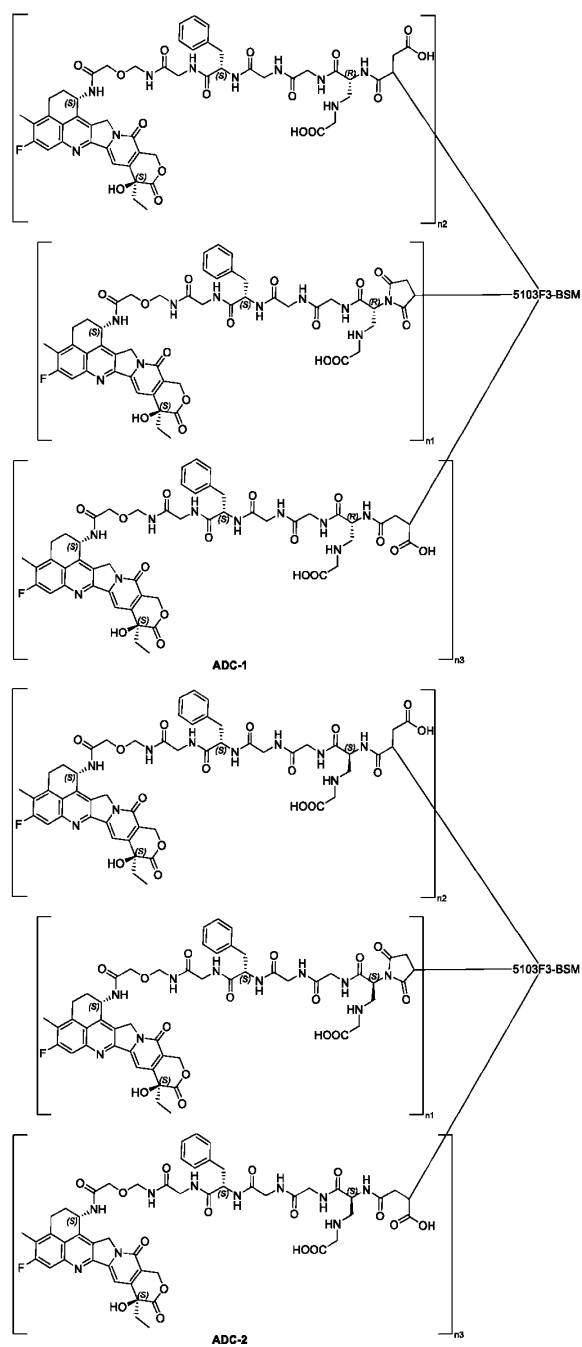


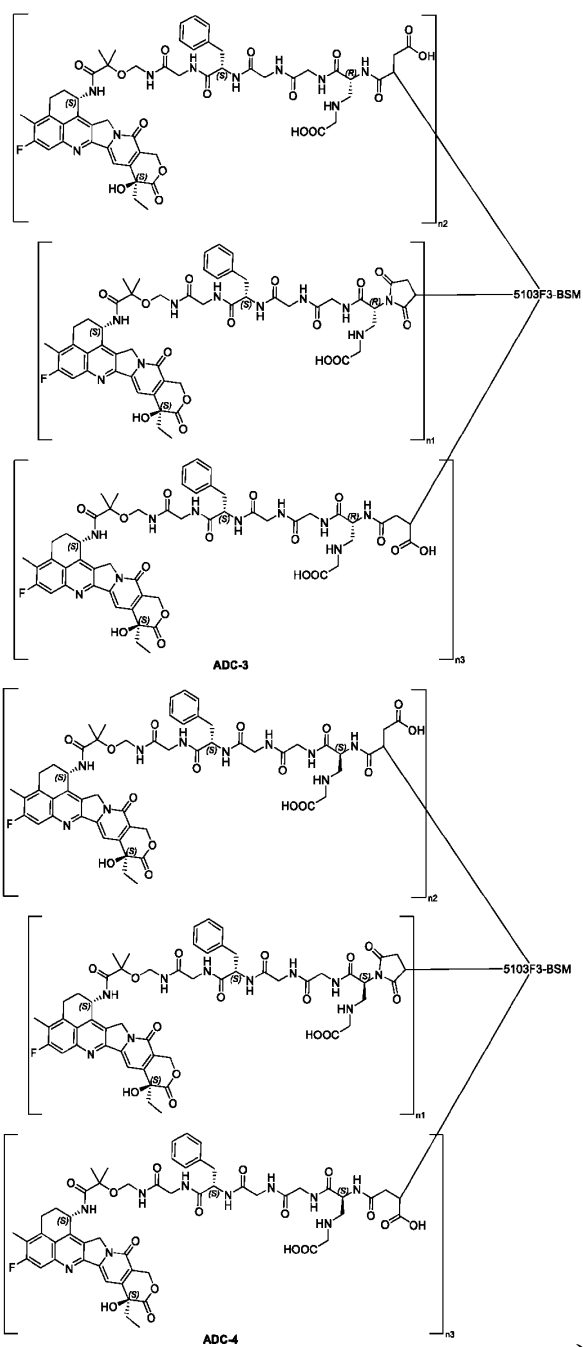


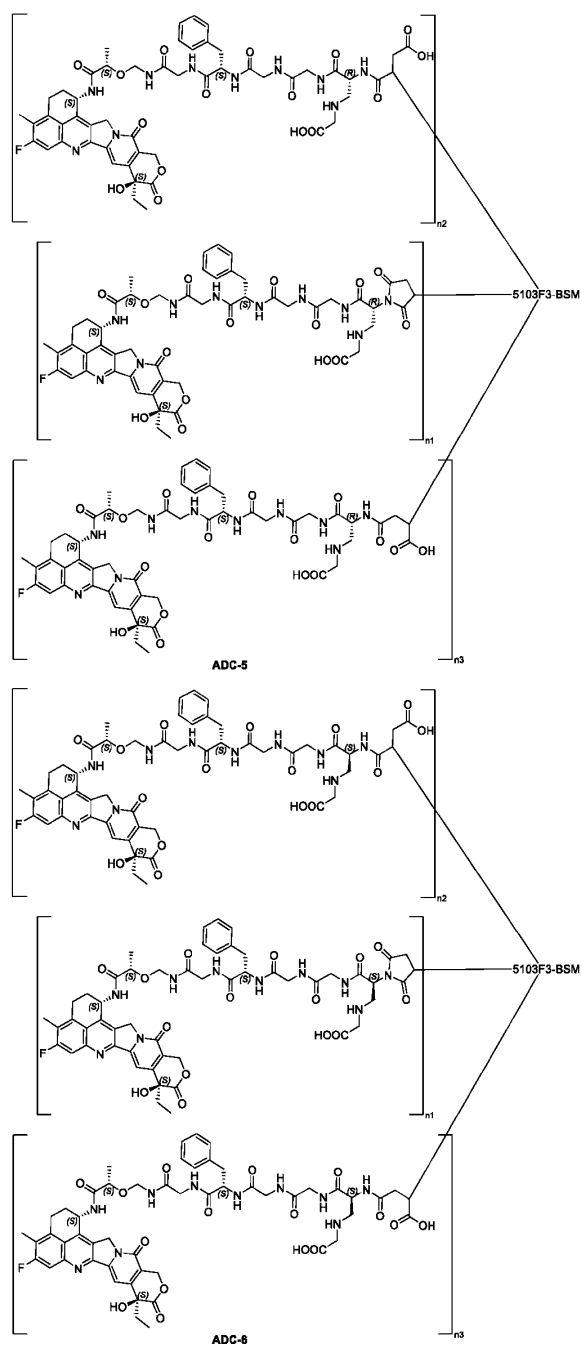
the 1-position, 2-position, or 3-position chiral carbon atoms have absolute chirality in the R- or S-configuration;

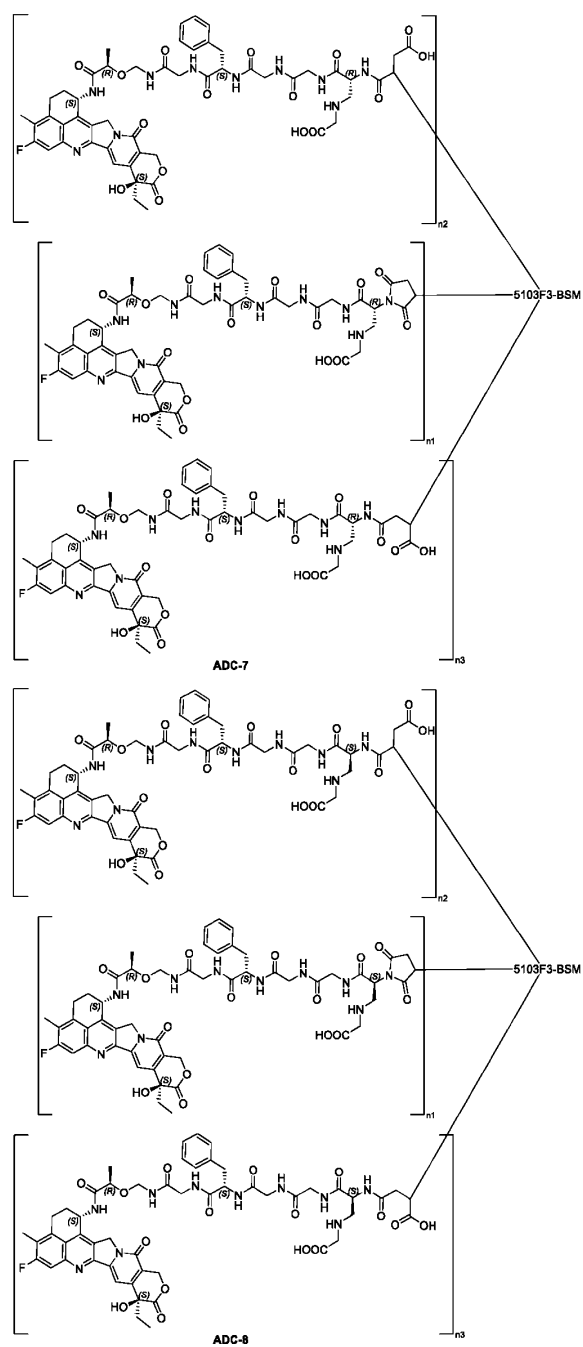
Ab, L, L₁₁, L₁₂, L₁₃, L₂, L₃, L₄, L₅, X, R, R₁, R₂, n₁, n₂ or n₃ are as claimed in any one of claims 1-24.

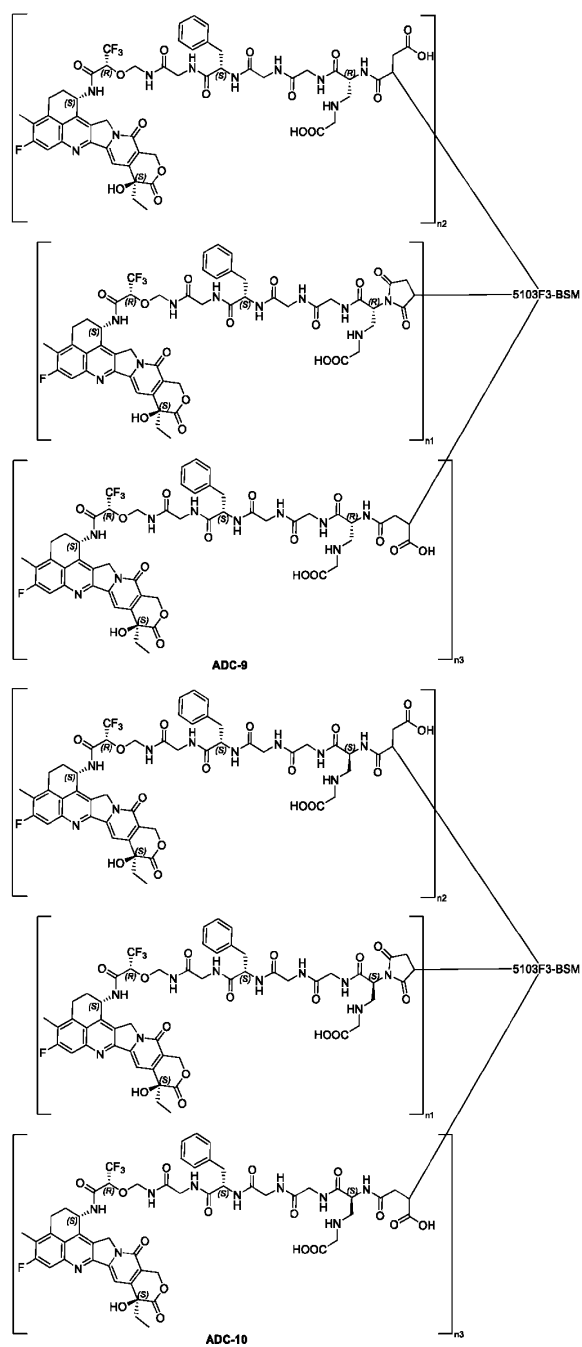
26. The ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1 or 14, or the method according to claim 25, wherein said ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof comprises the following structure or a butanediimide ring-opening structure thereof or an isomer thereof.

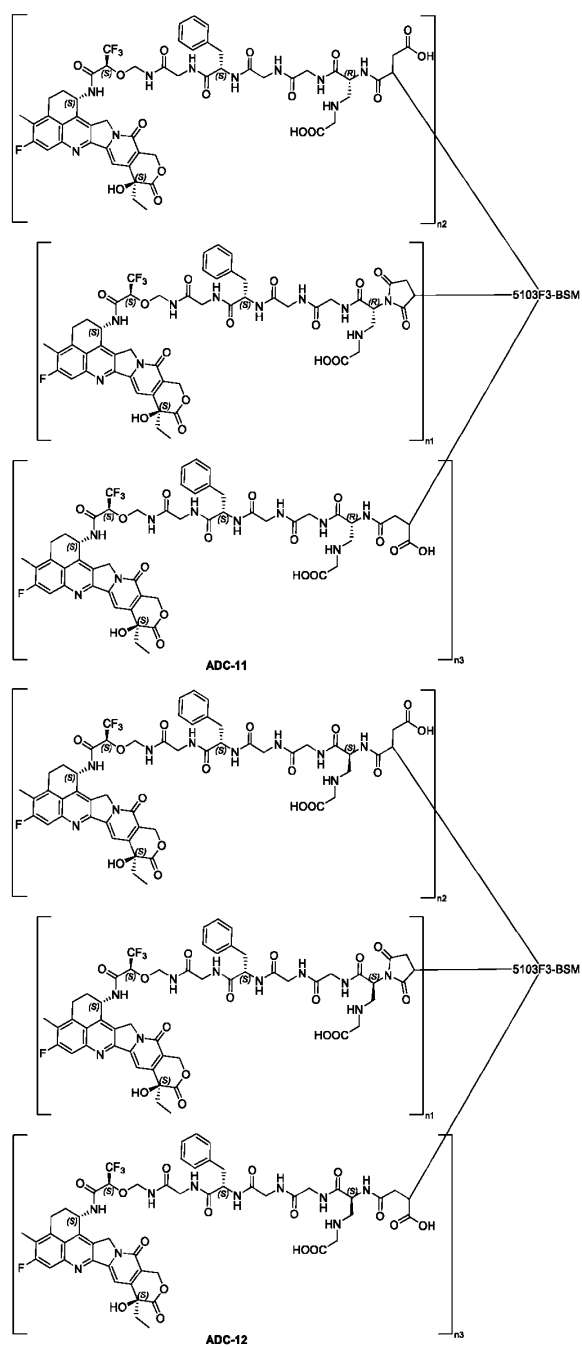


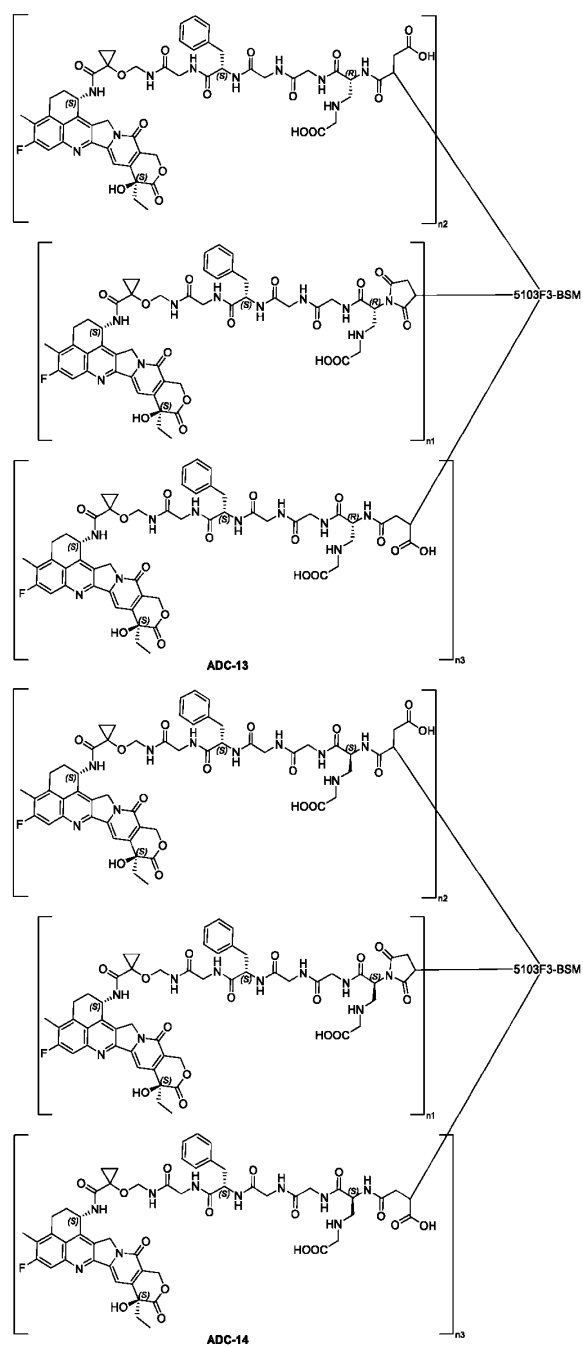


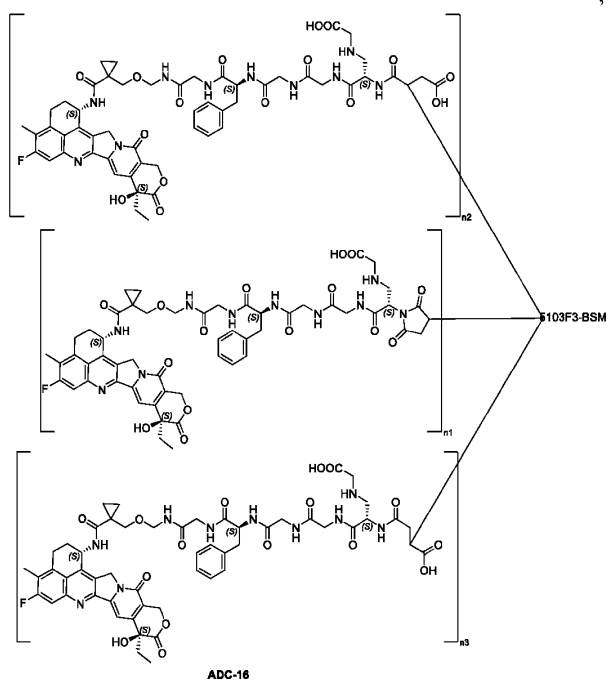
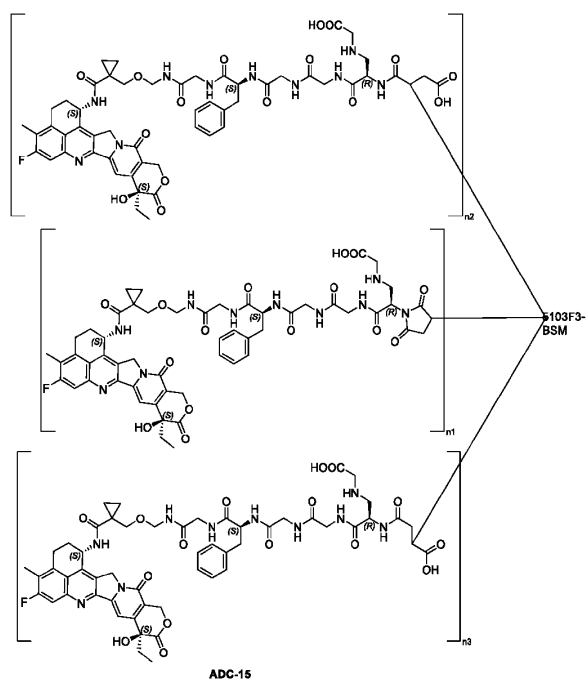


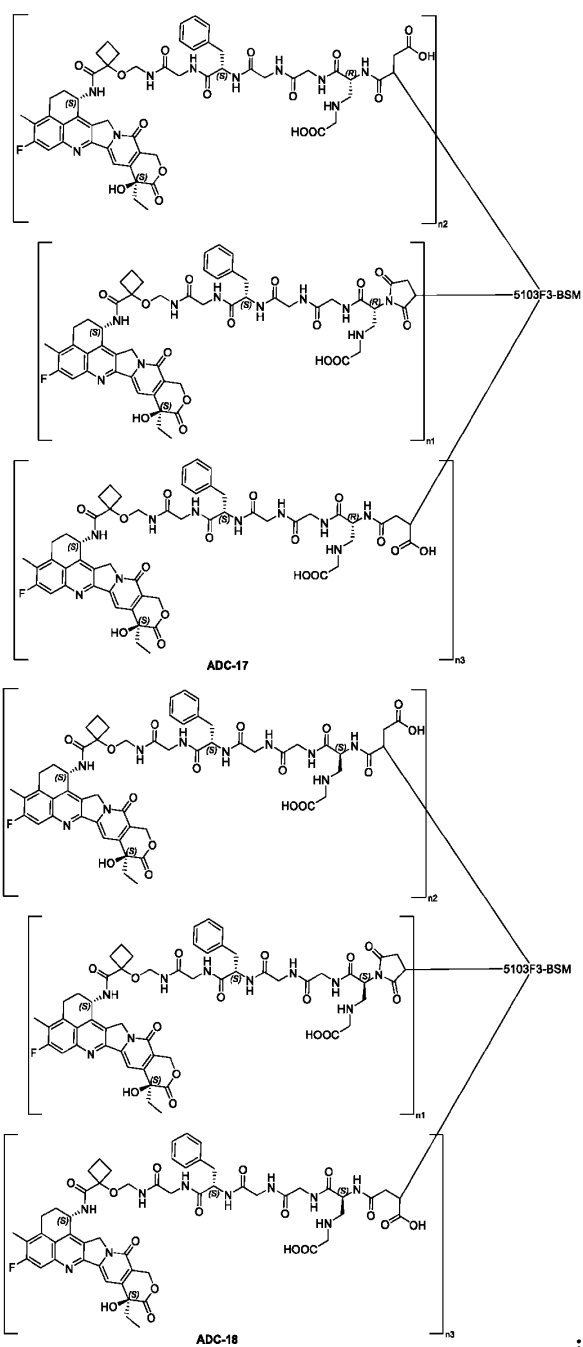


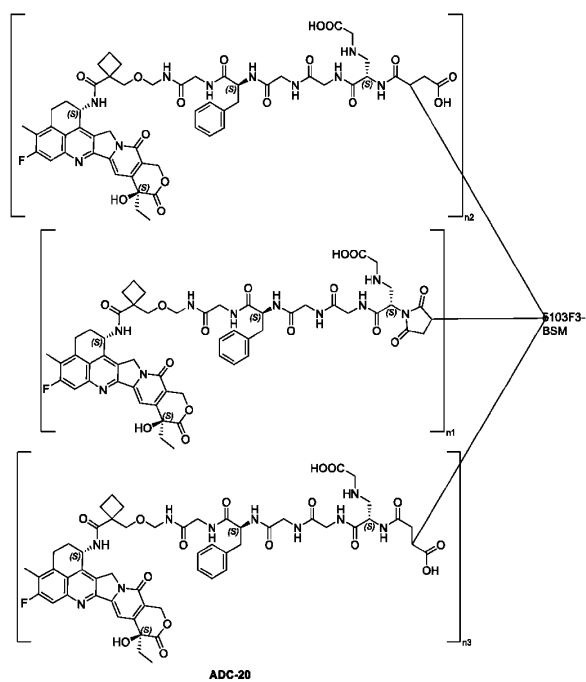
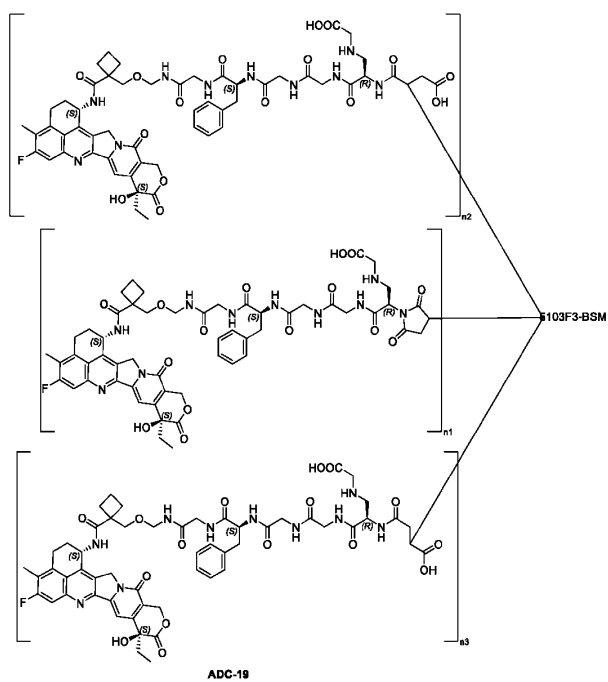


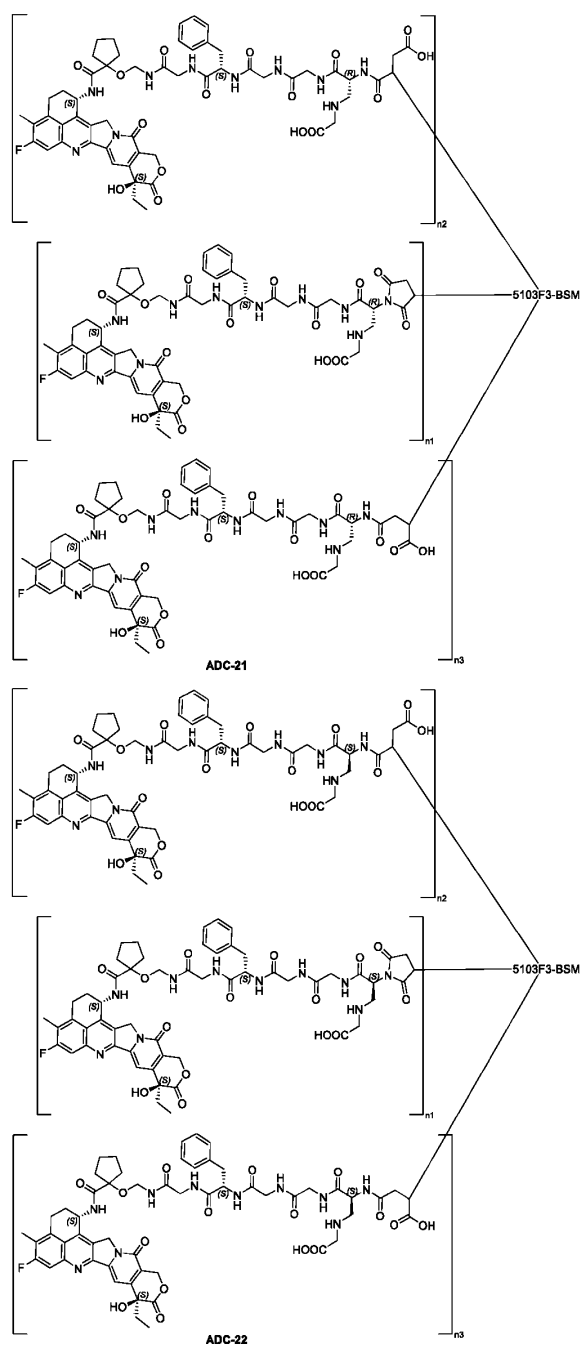


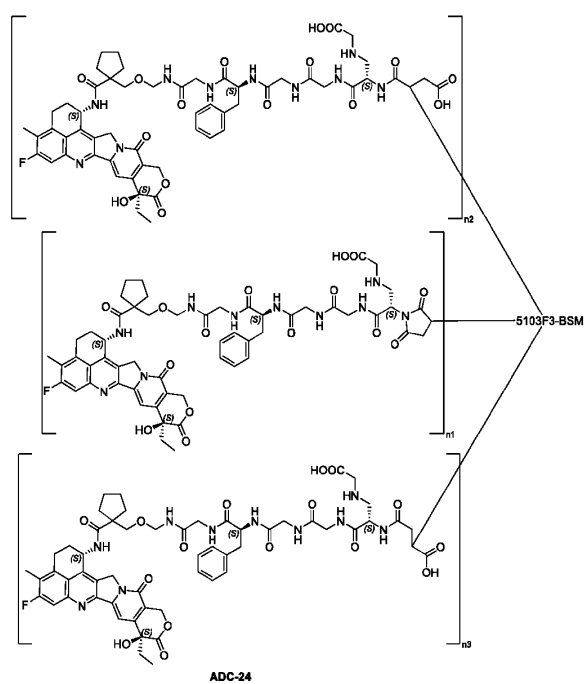
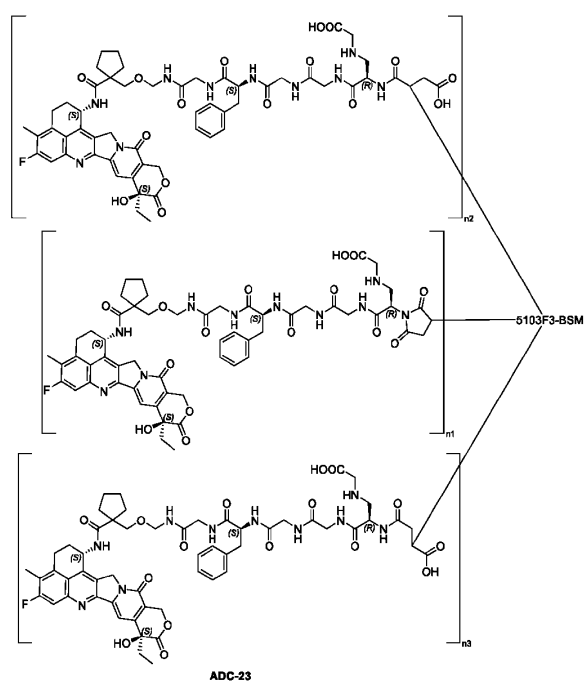


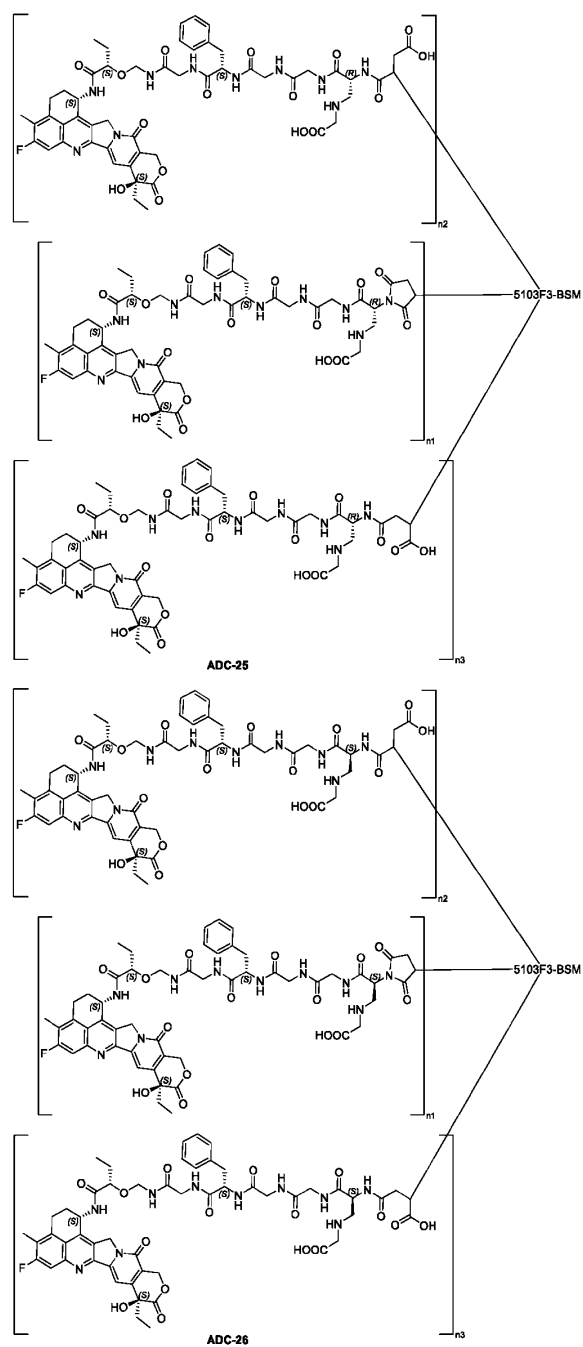


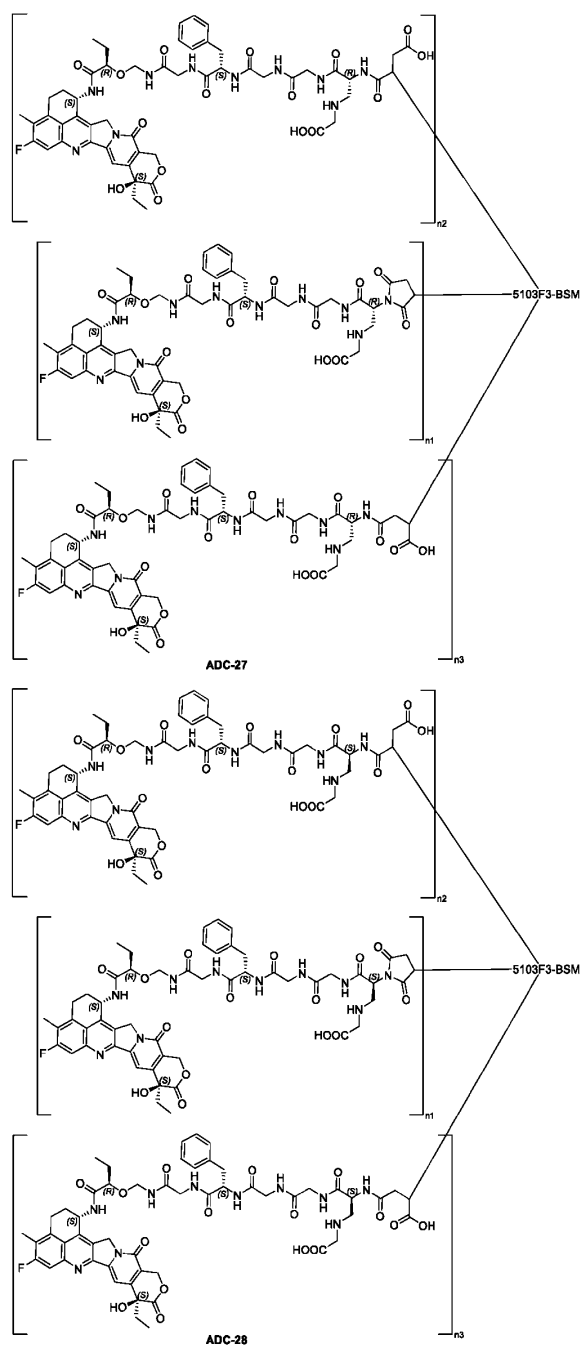


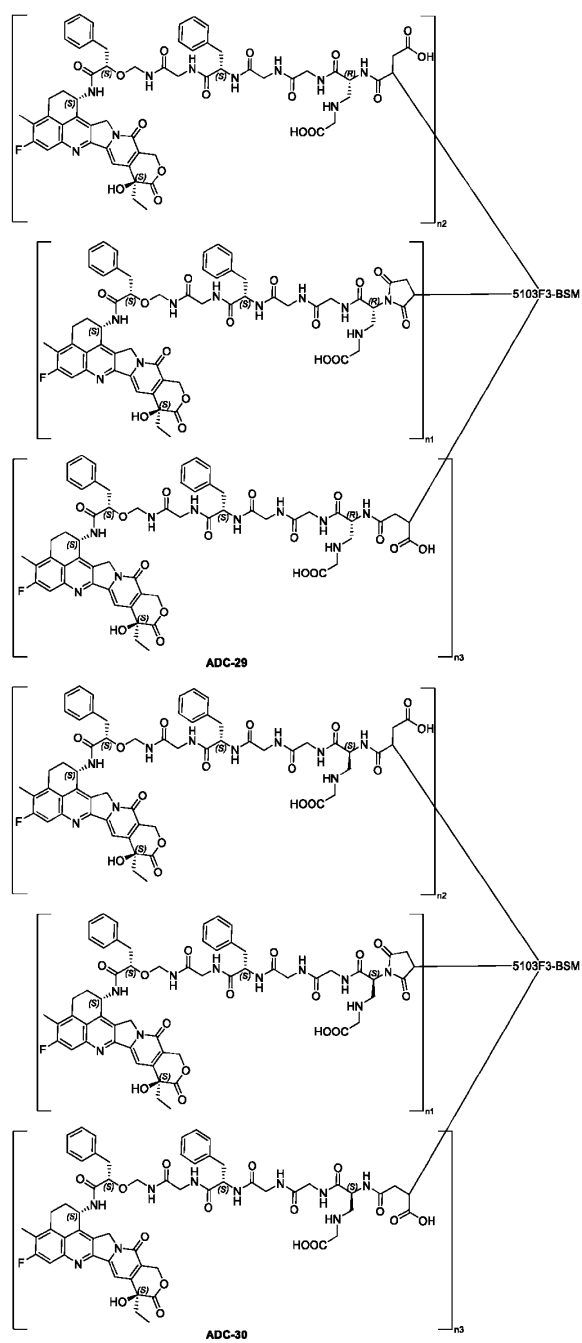


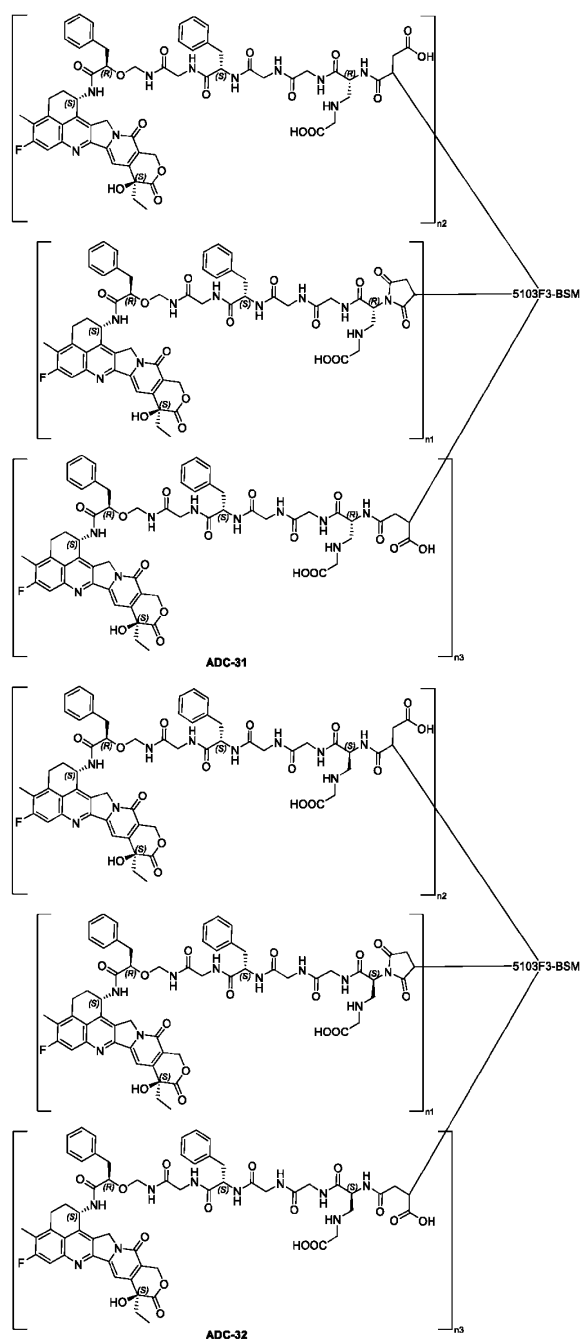


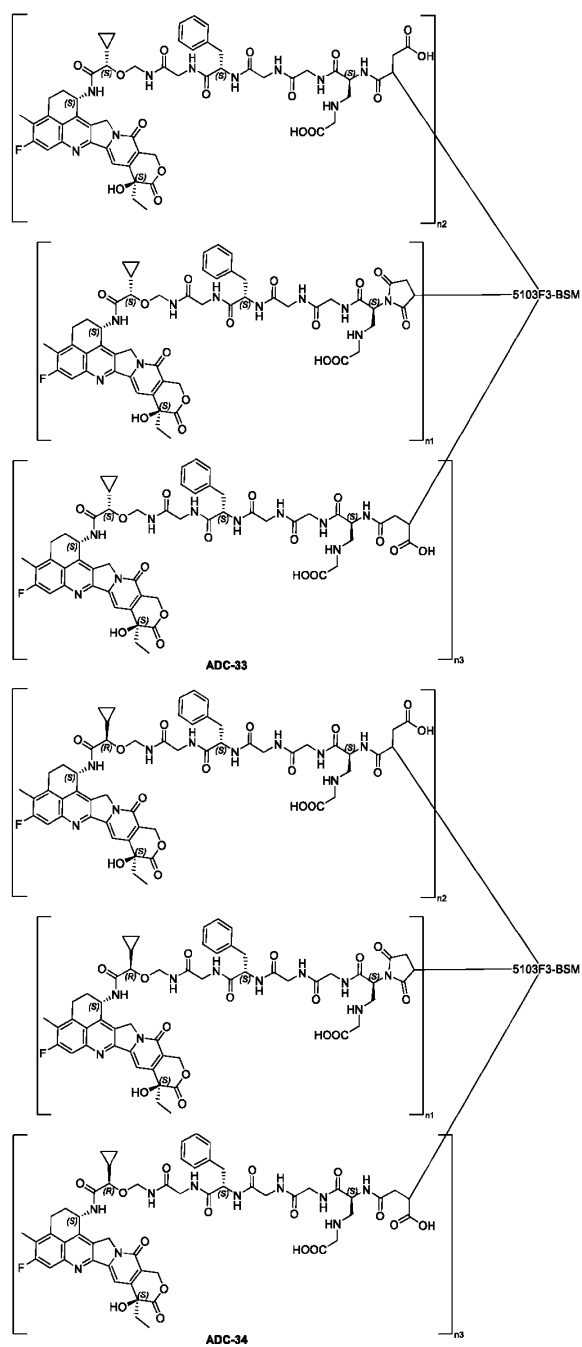


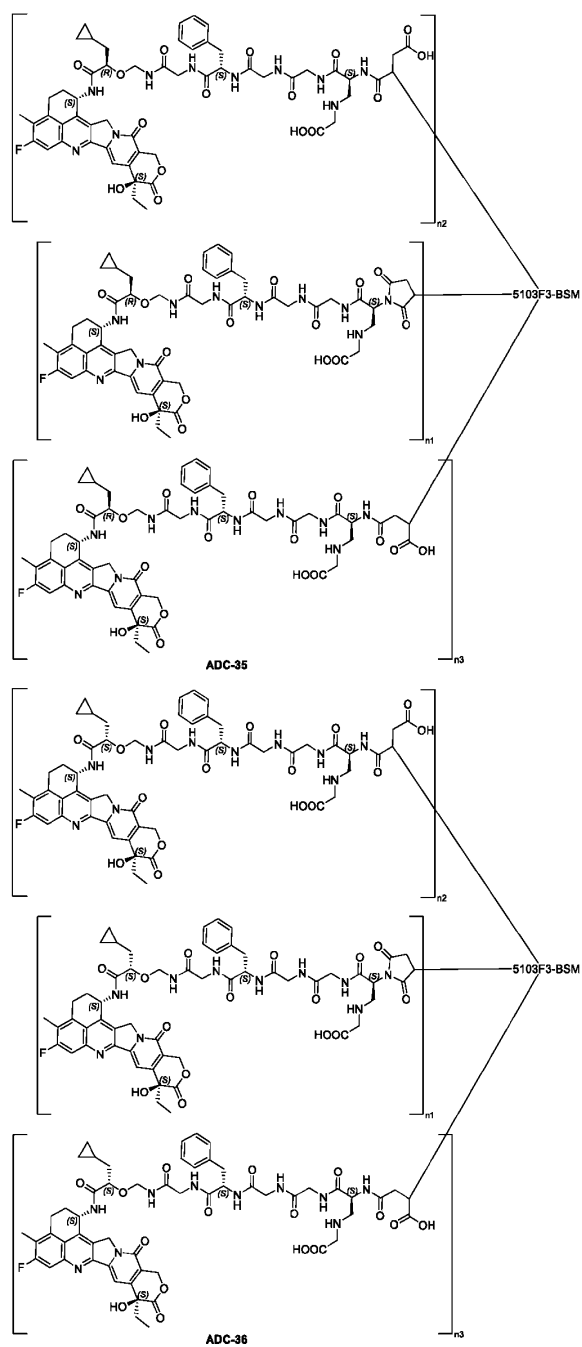


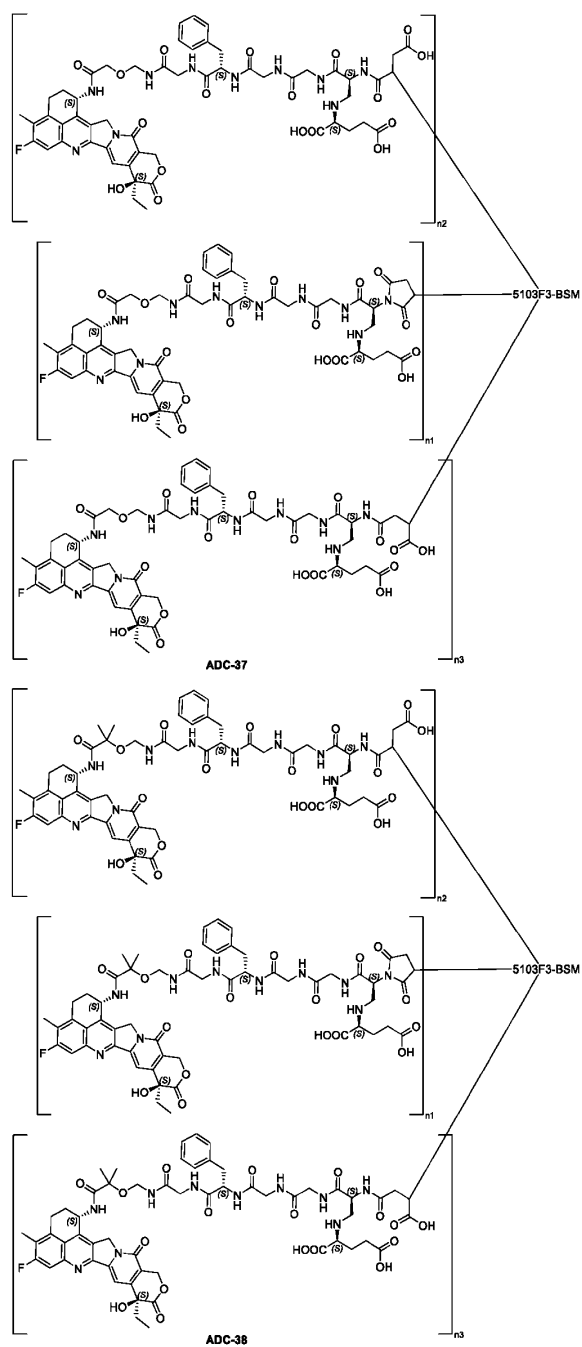


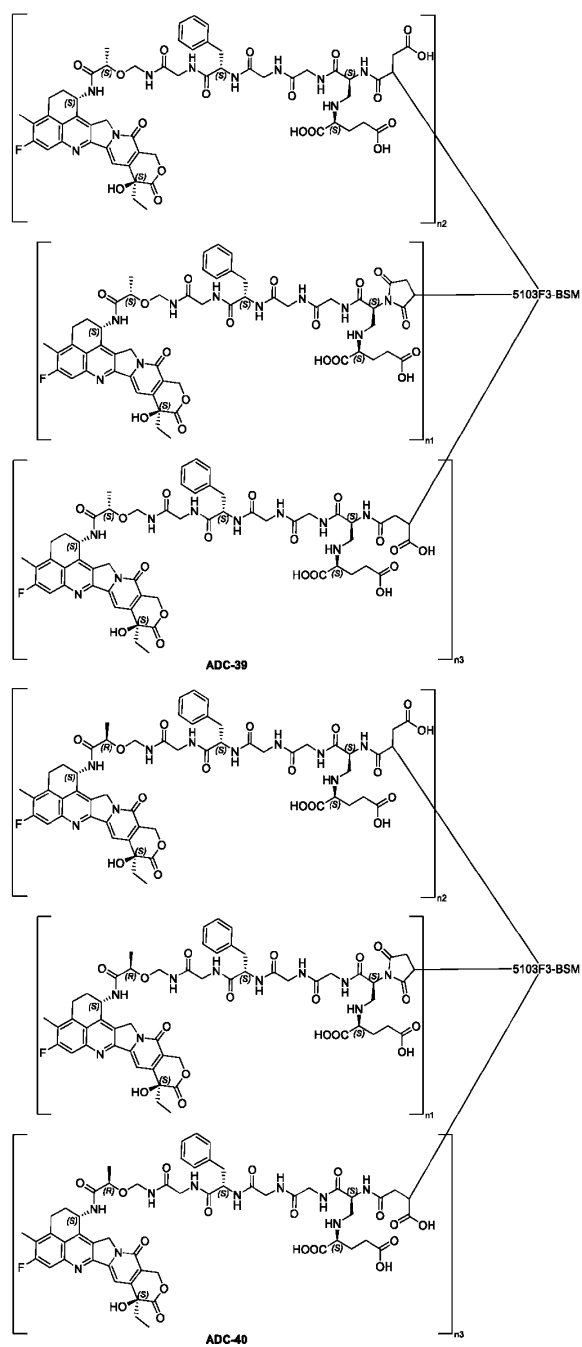


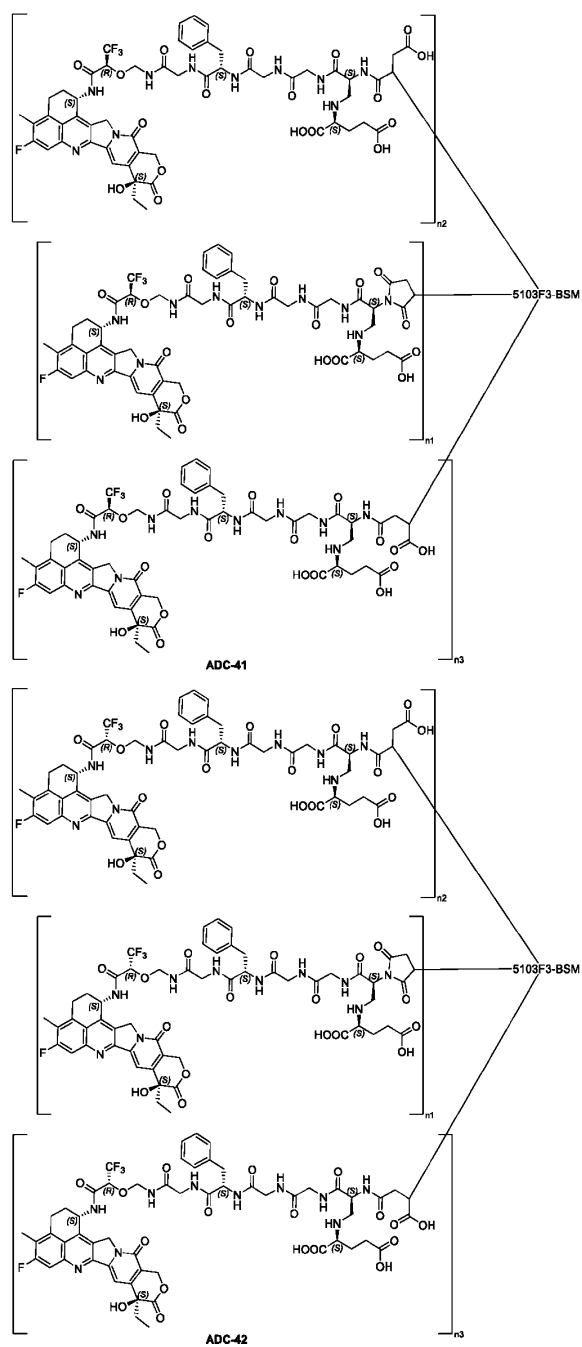


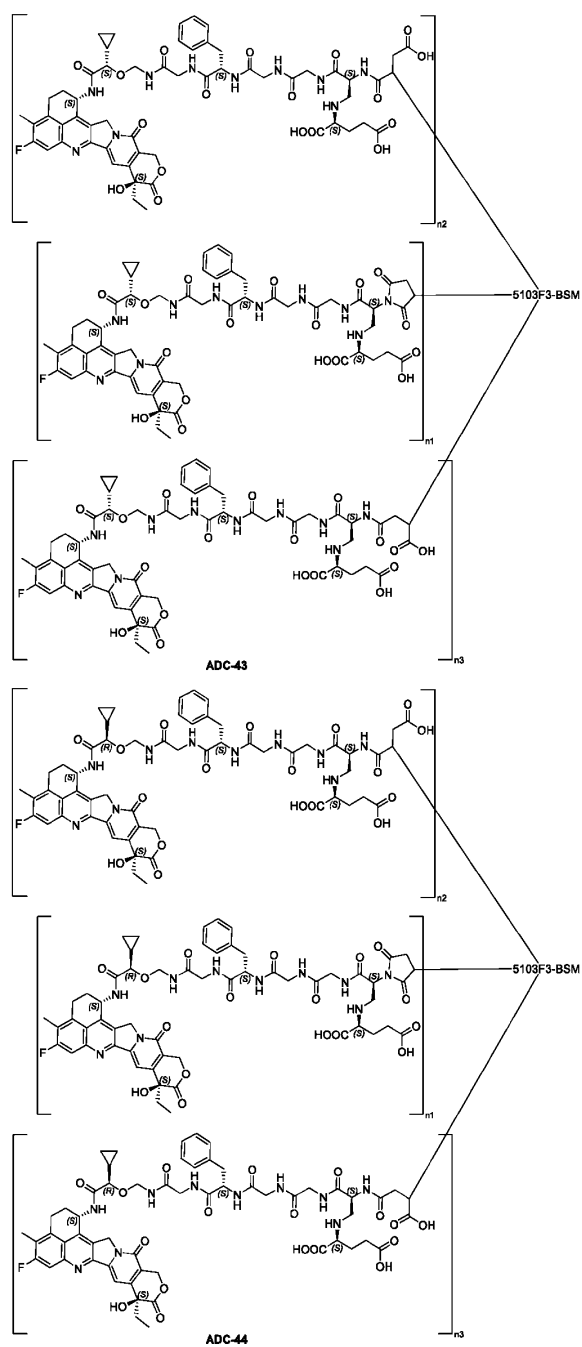


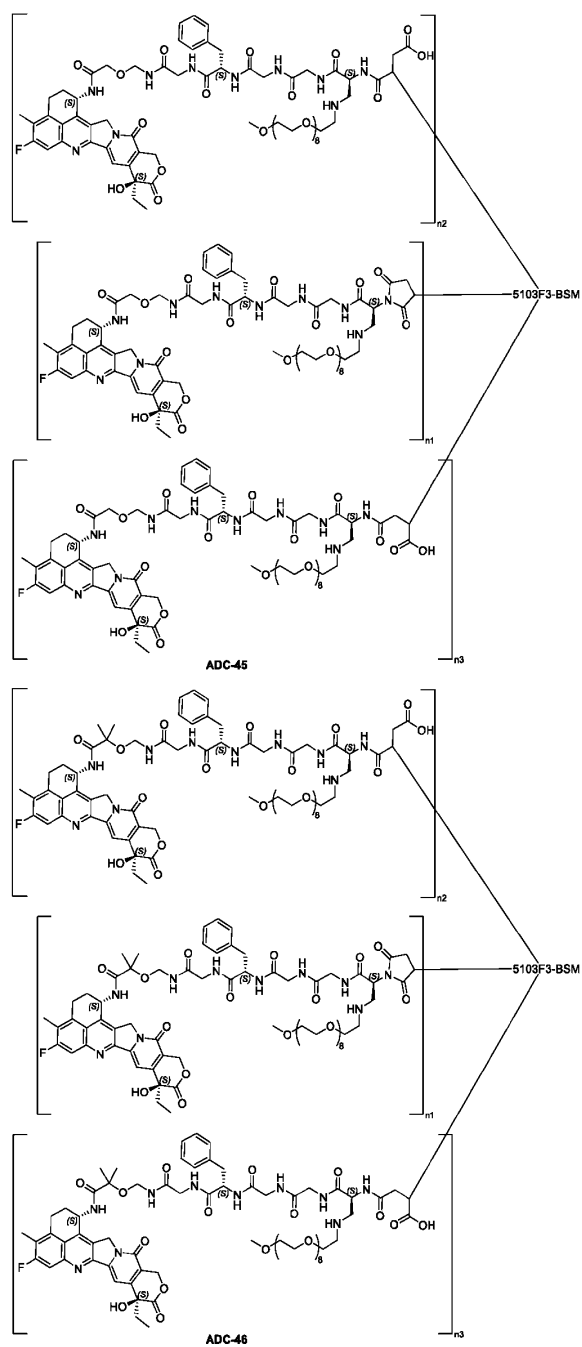


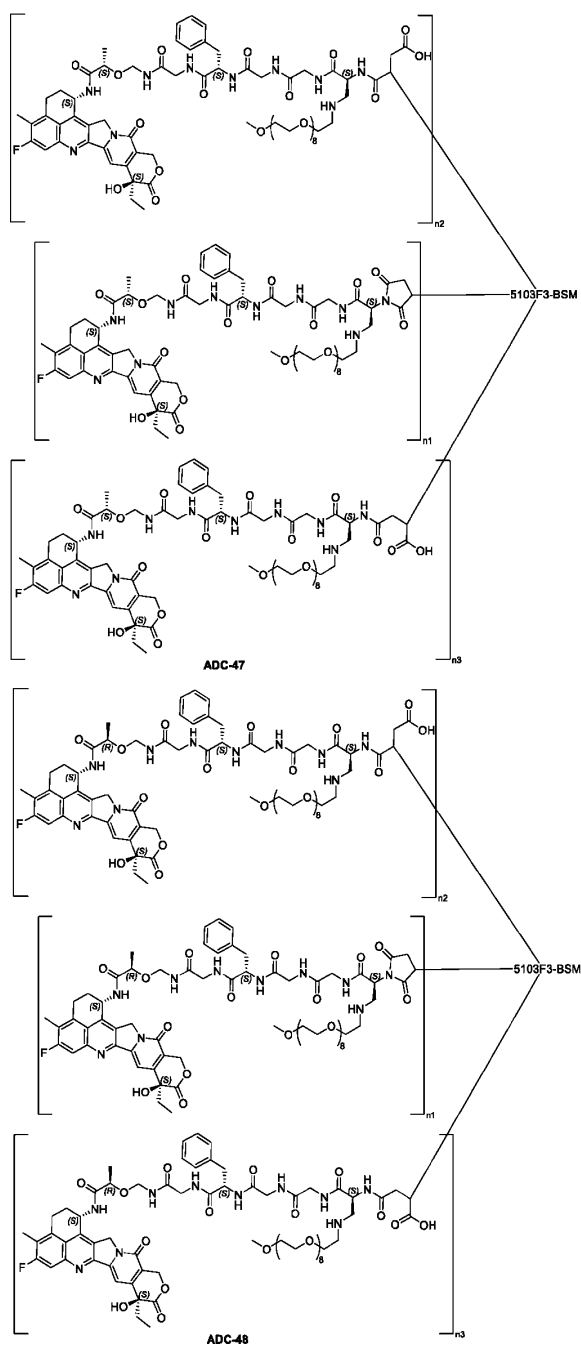


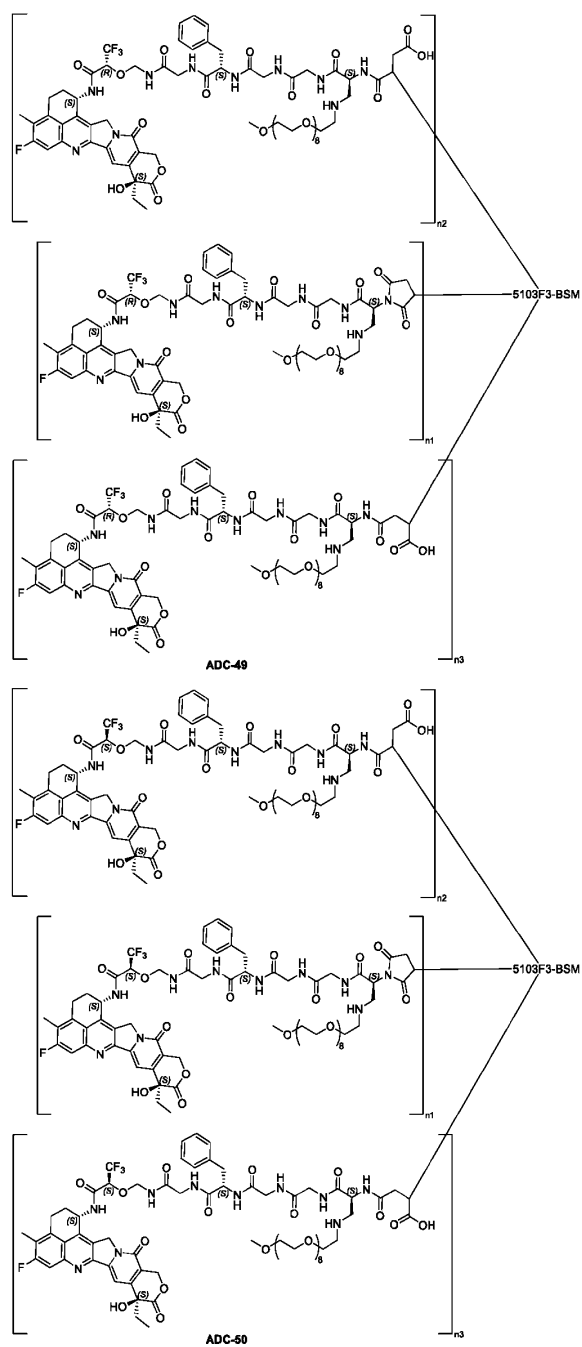


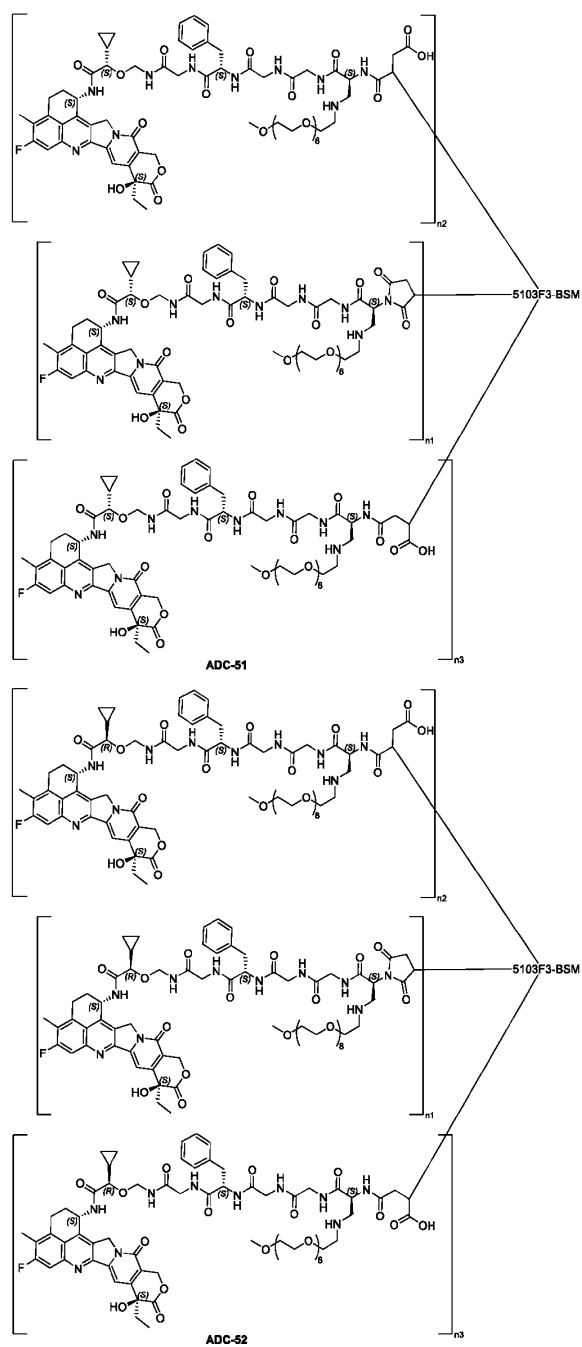


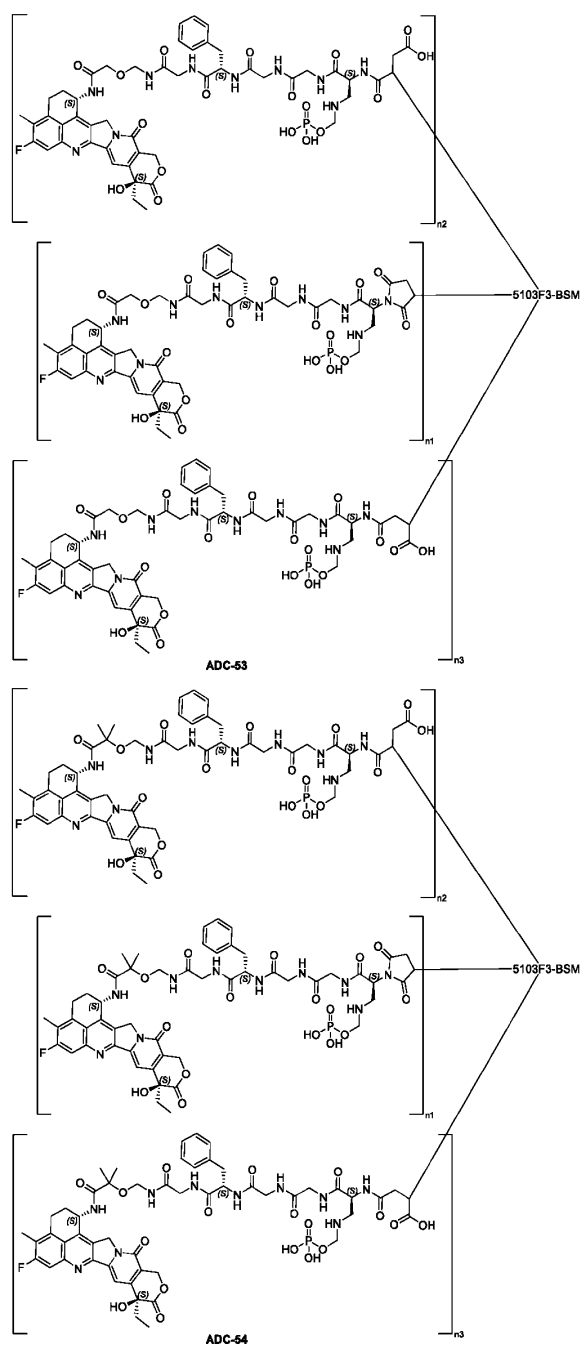


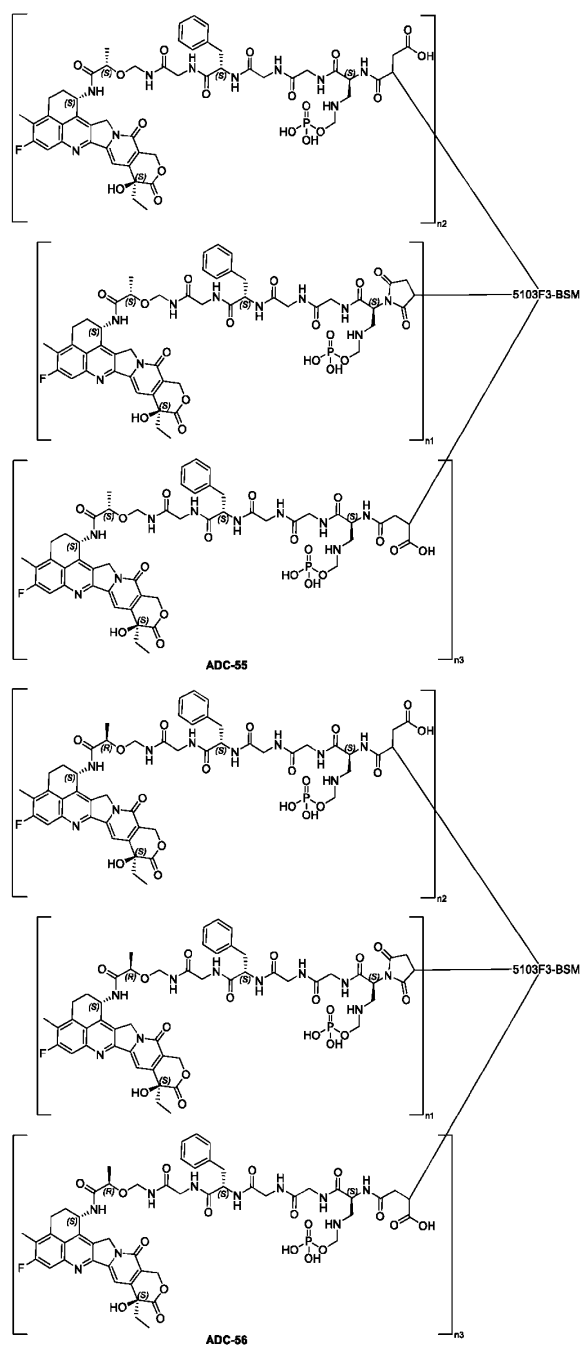


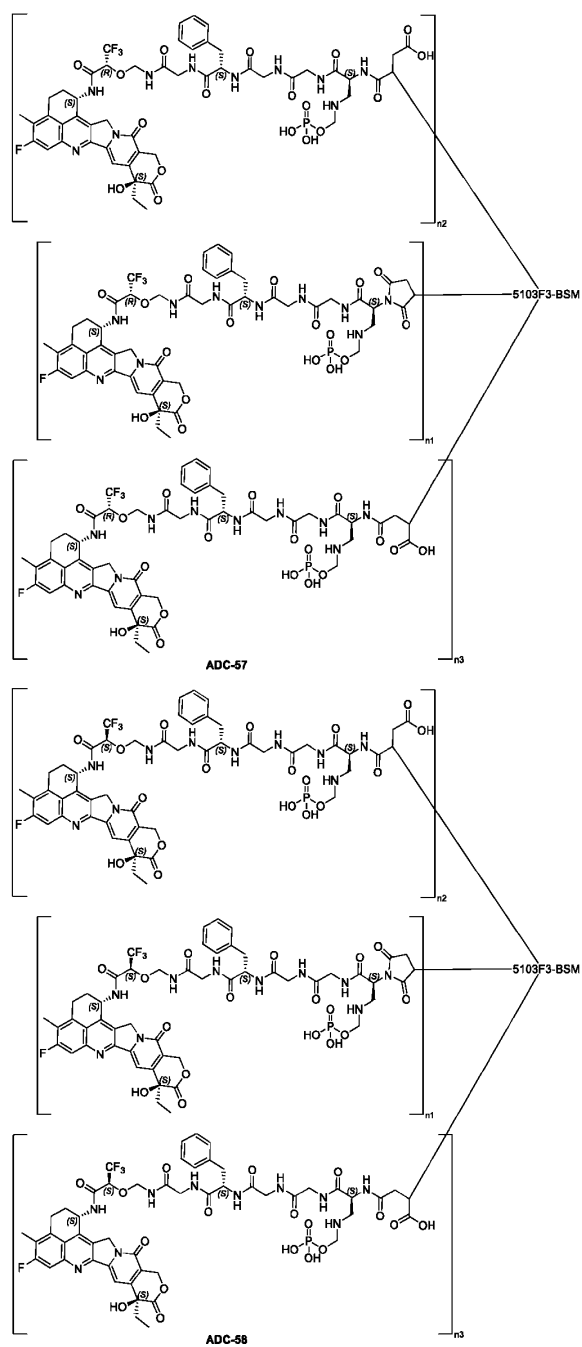


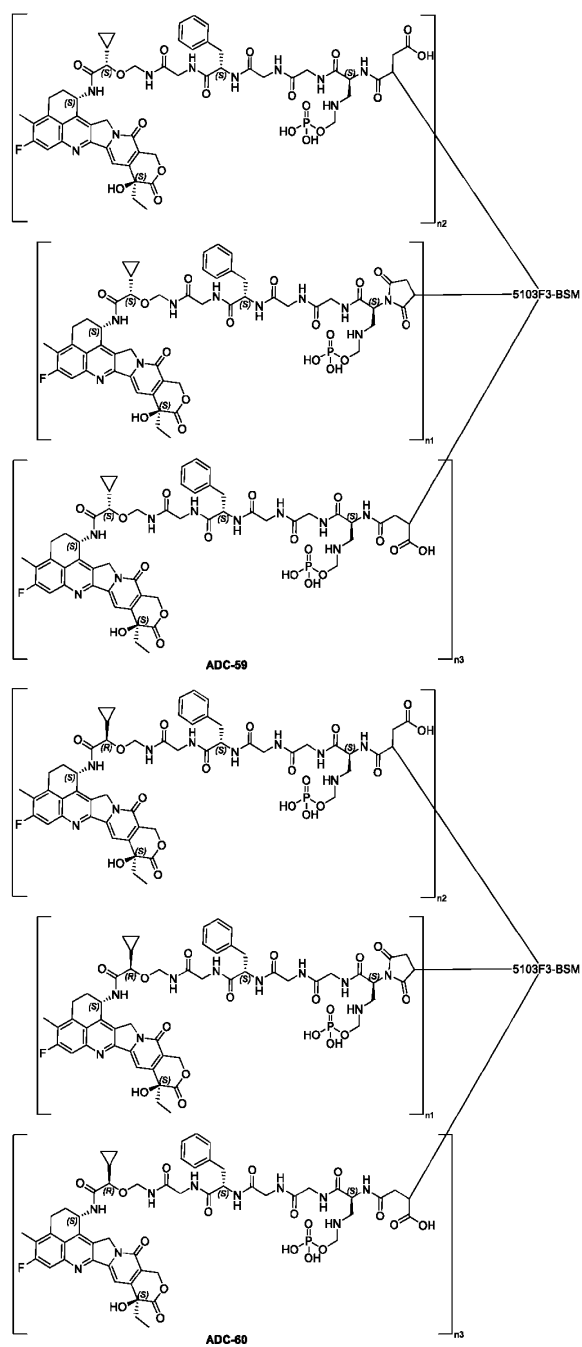


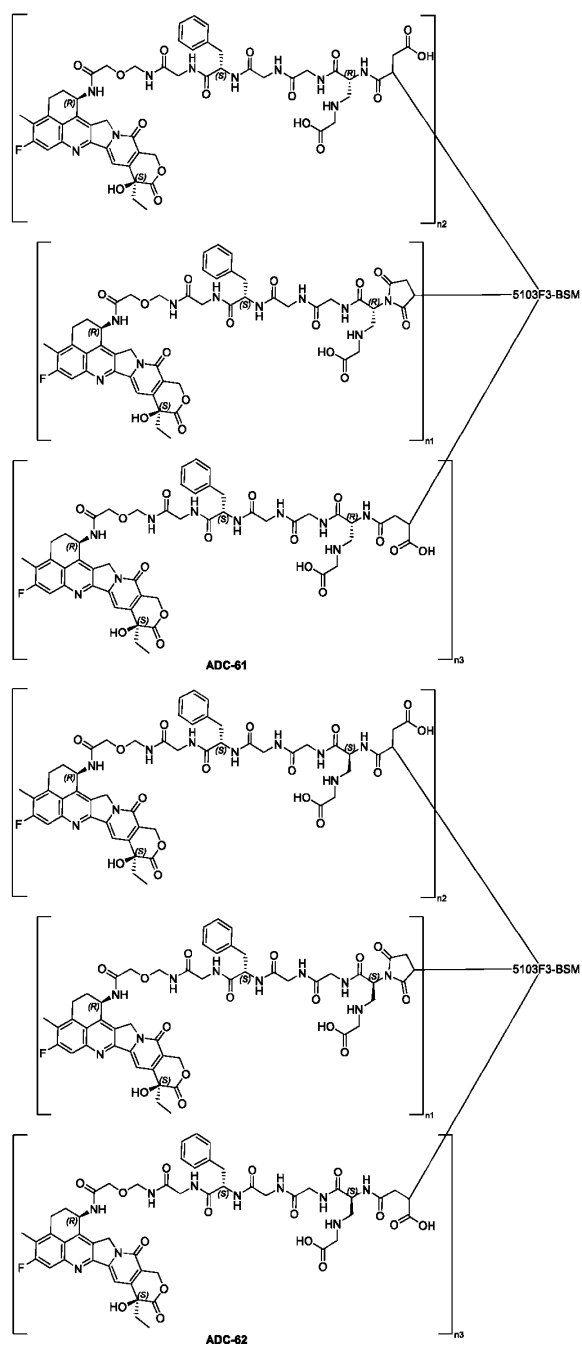


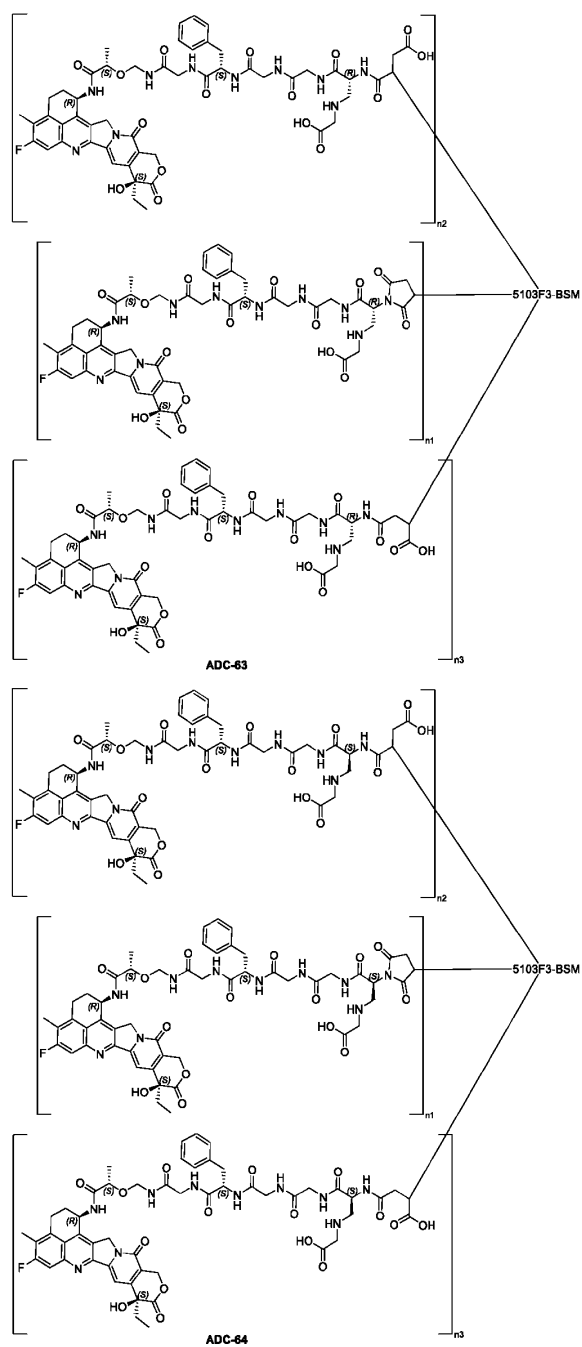


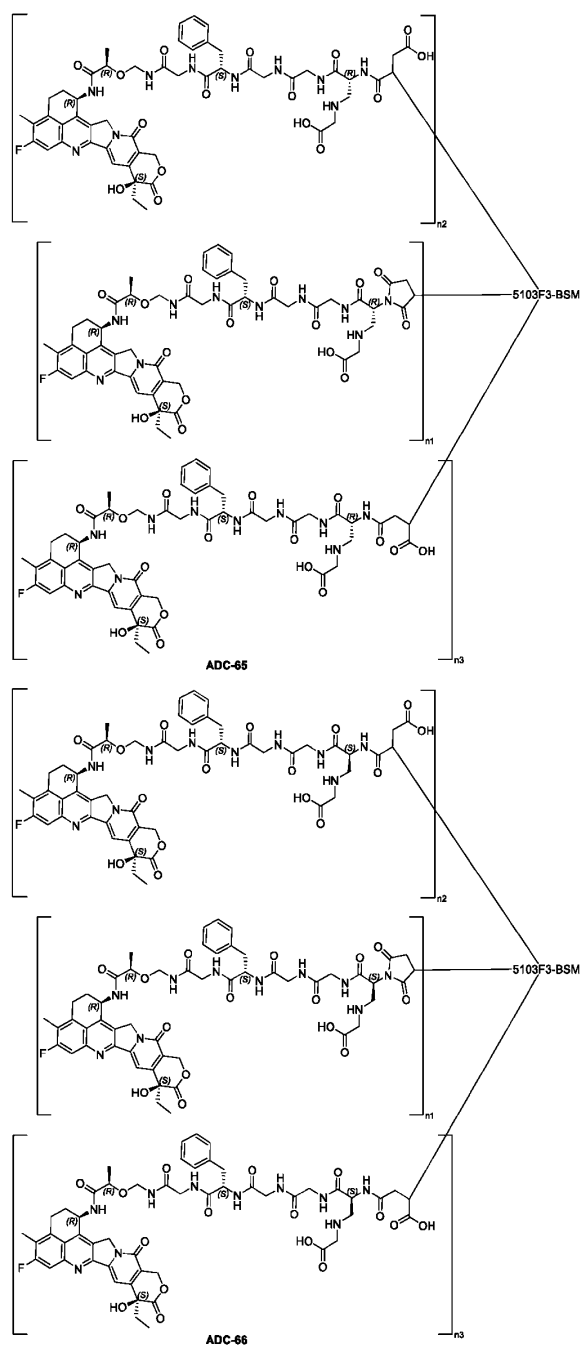


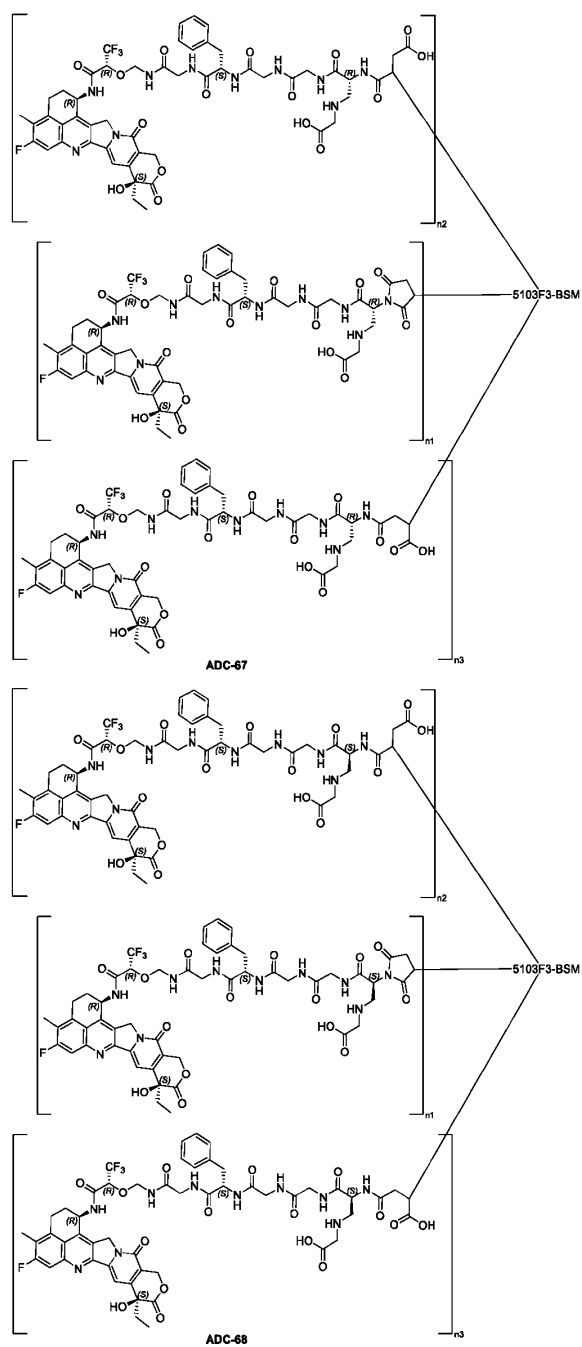


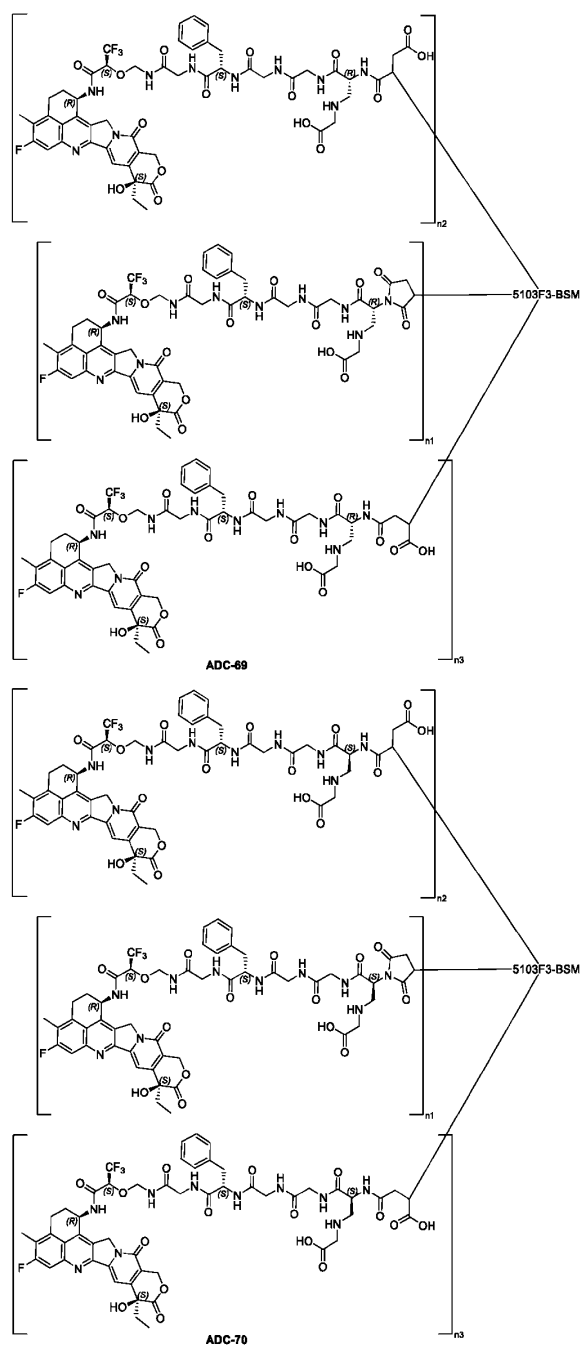


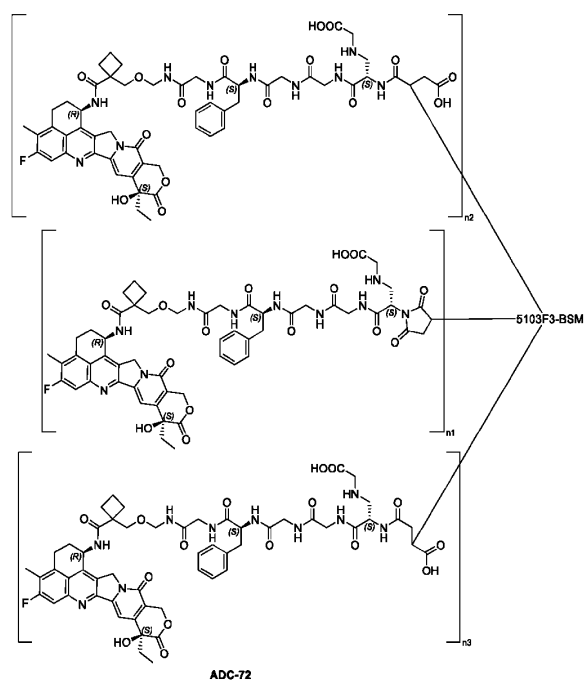
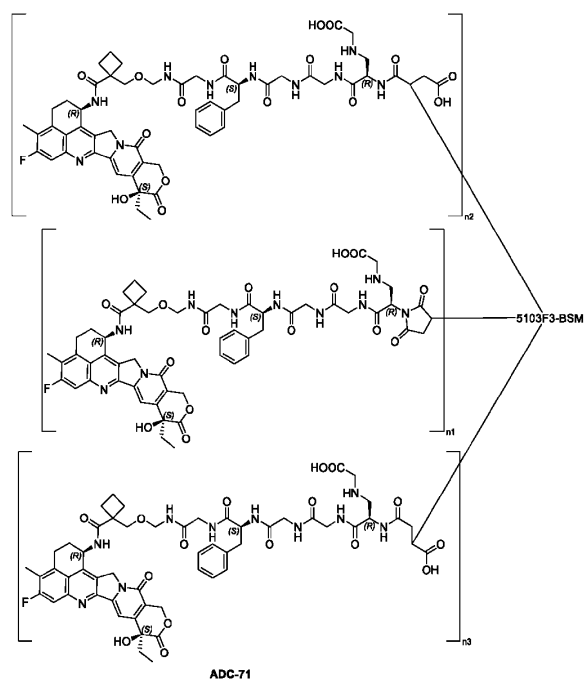


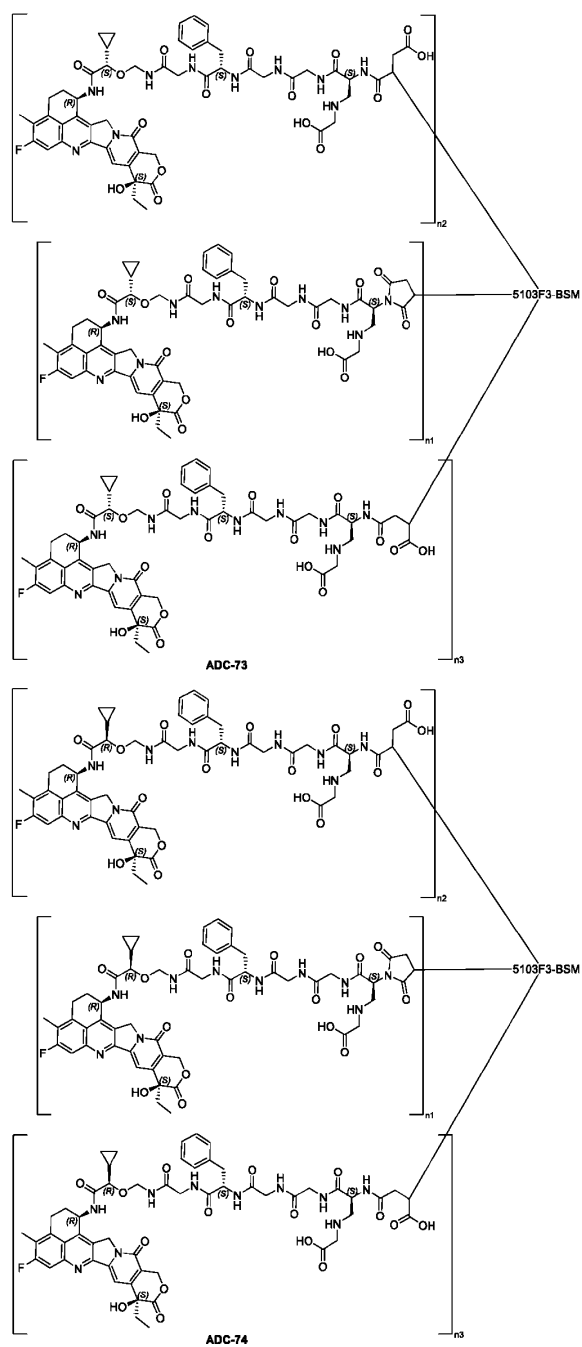


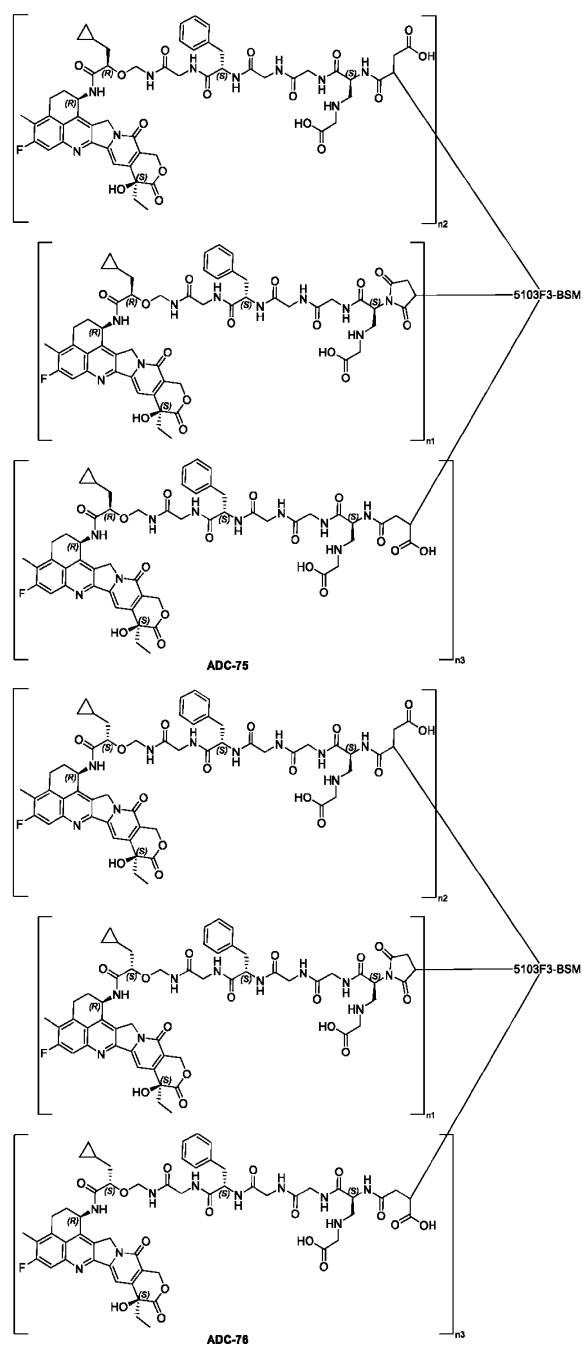


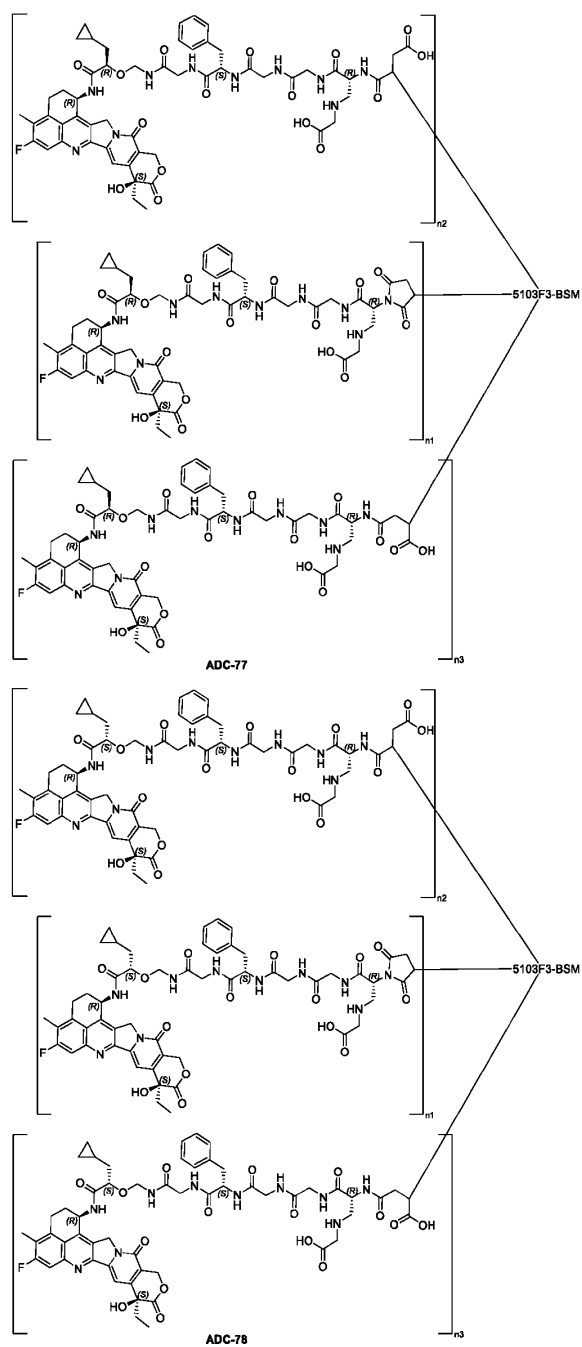


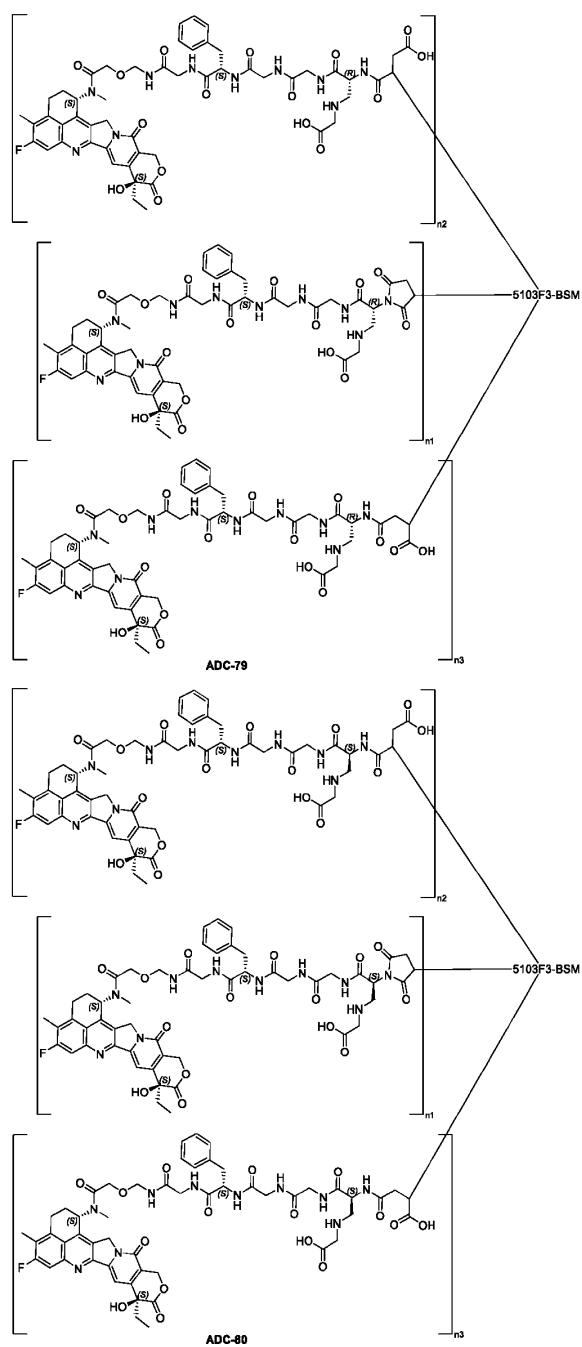


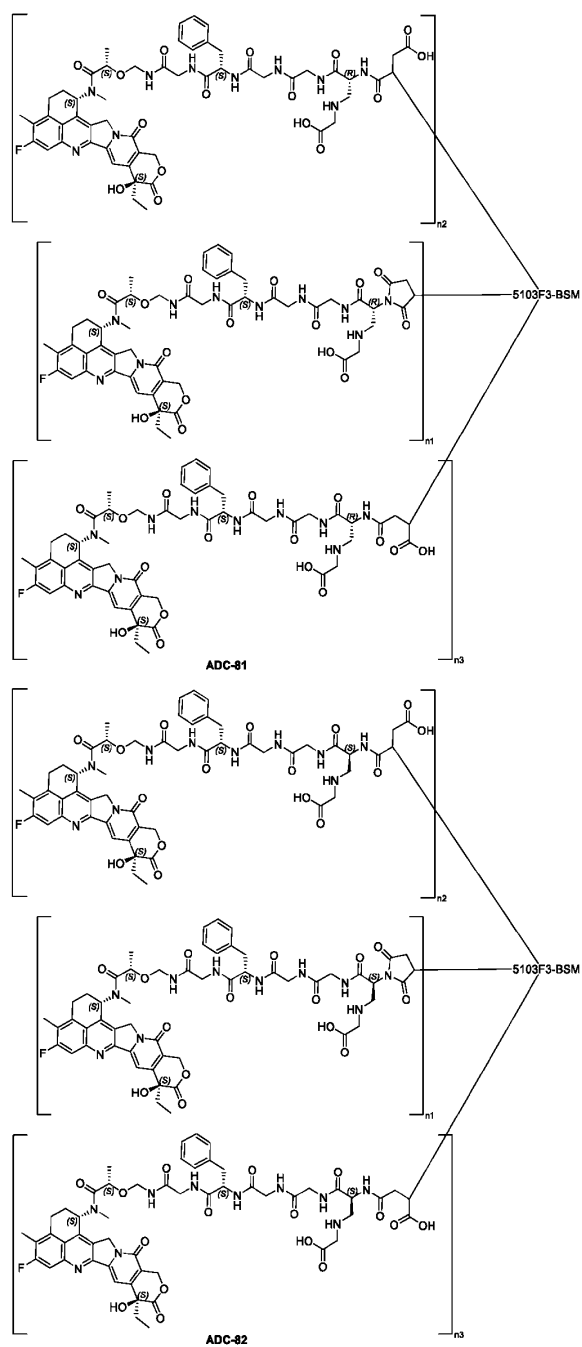


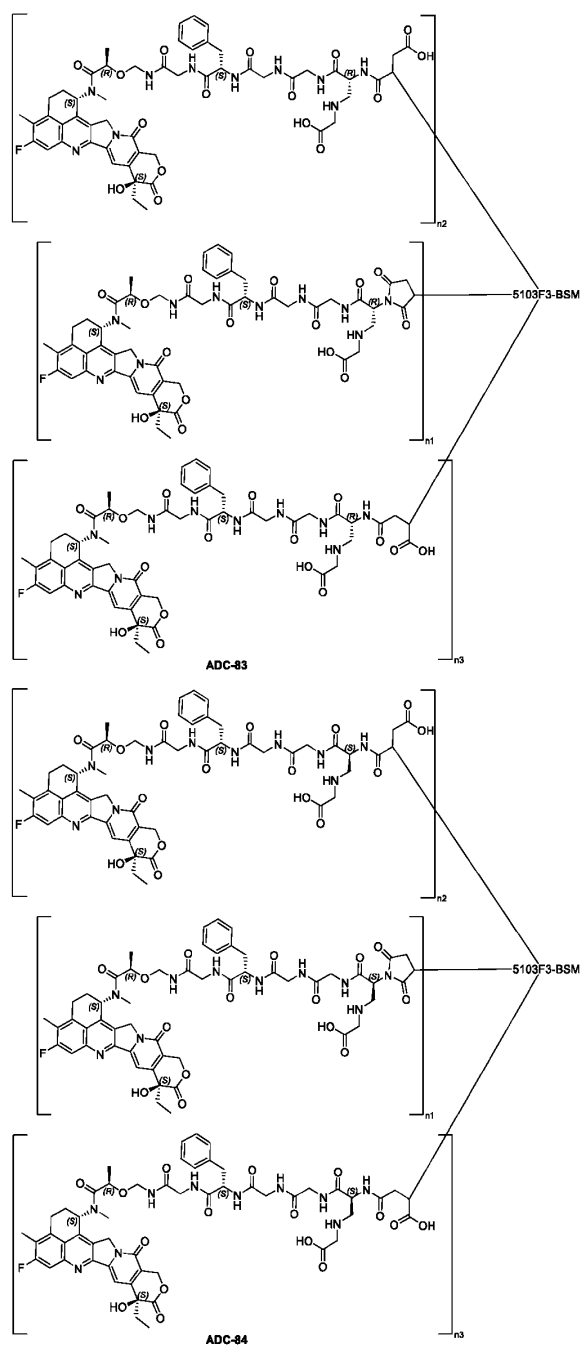


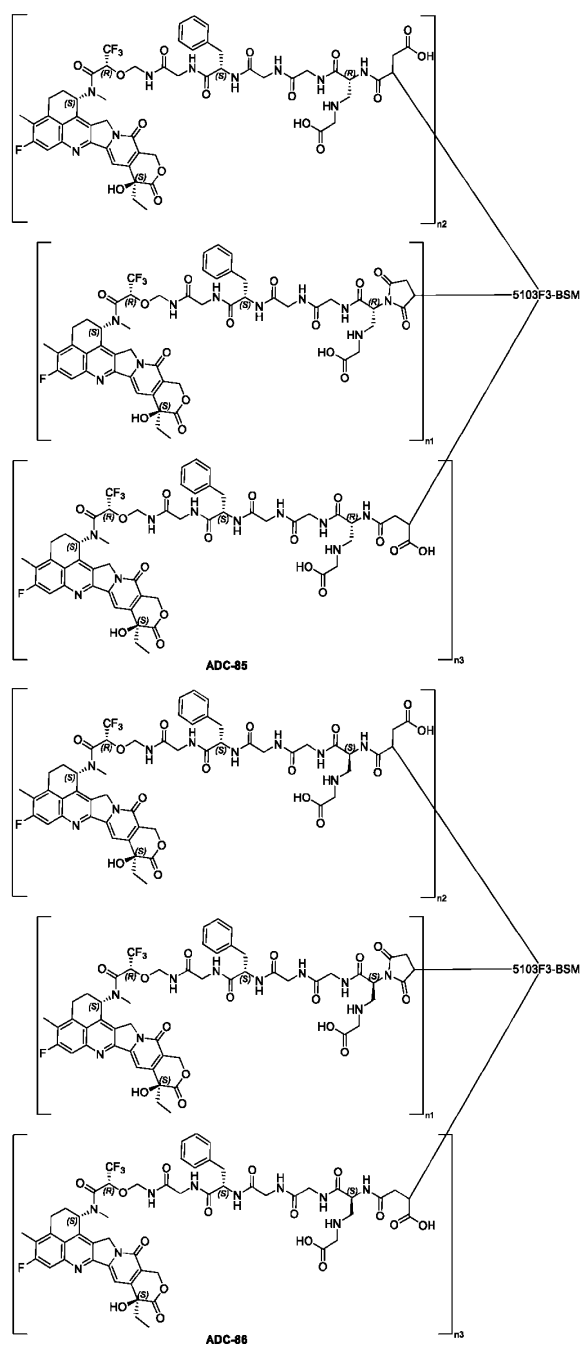


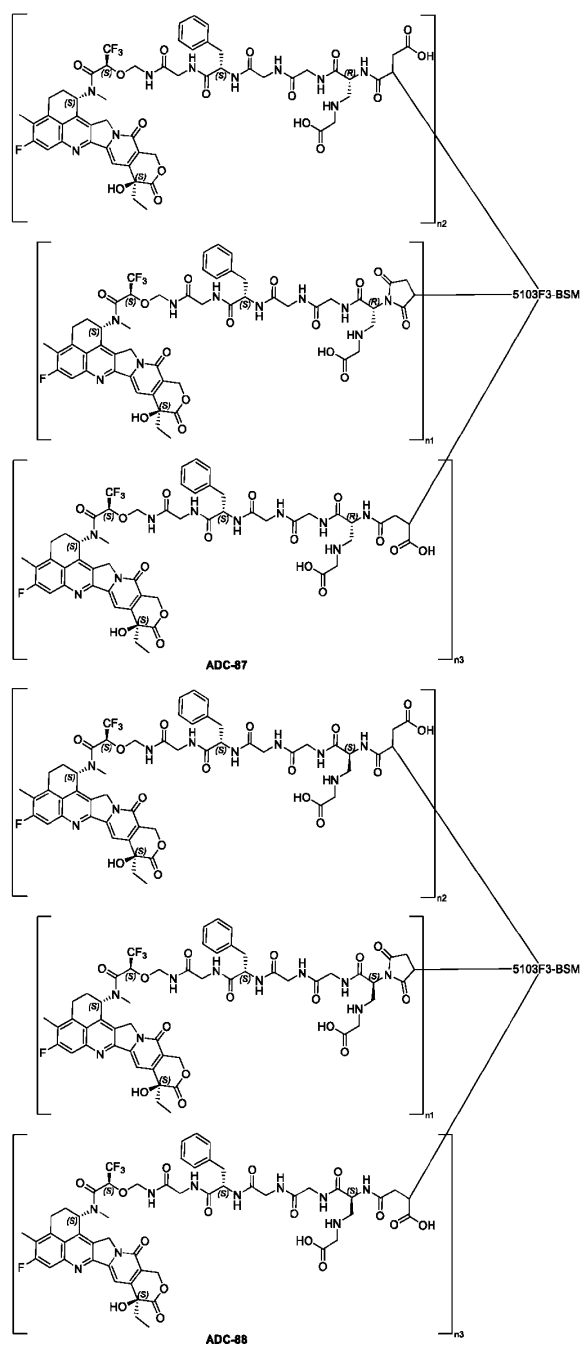




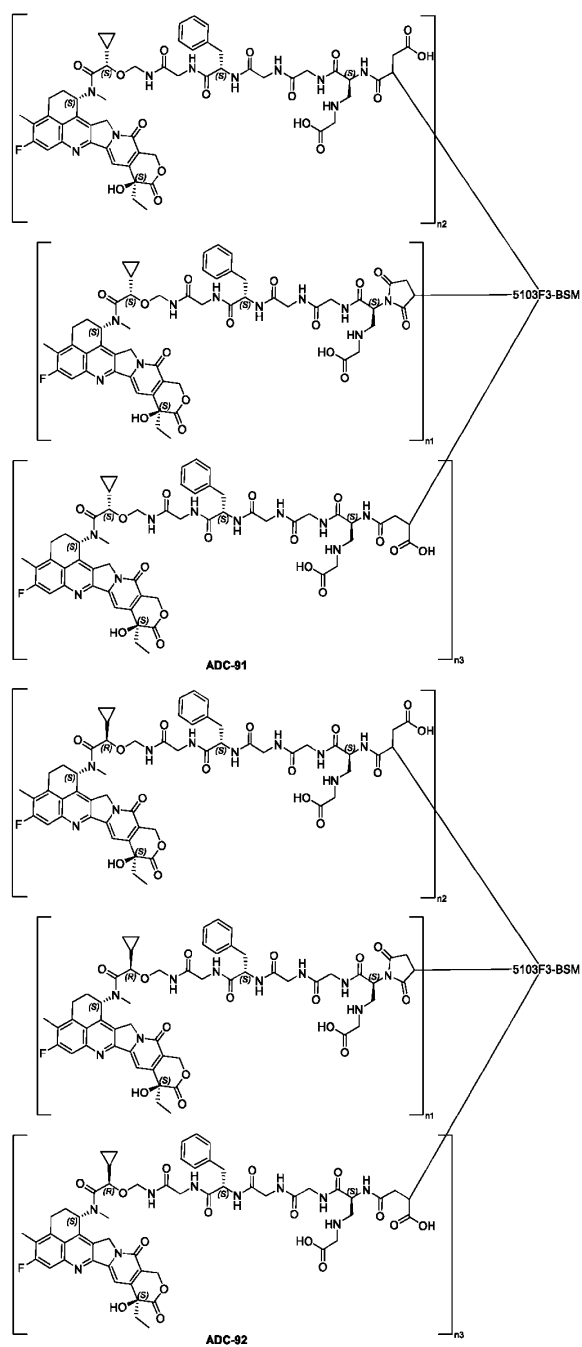


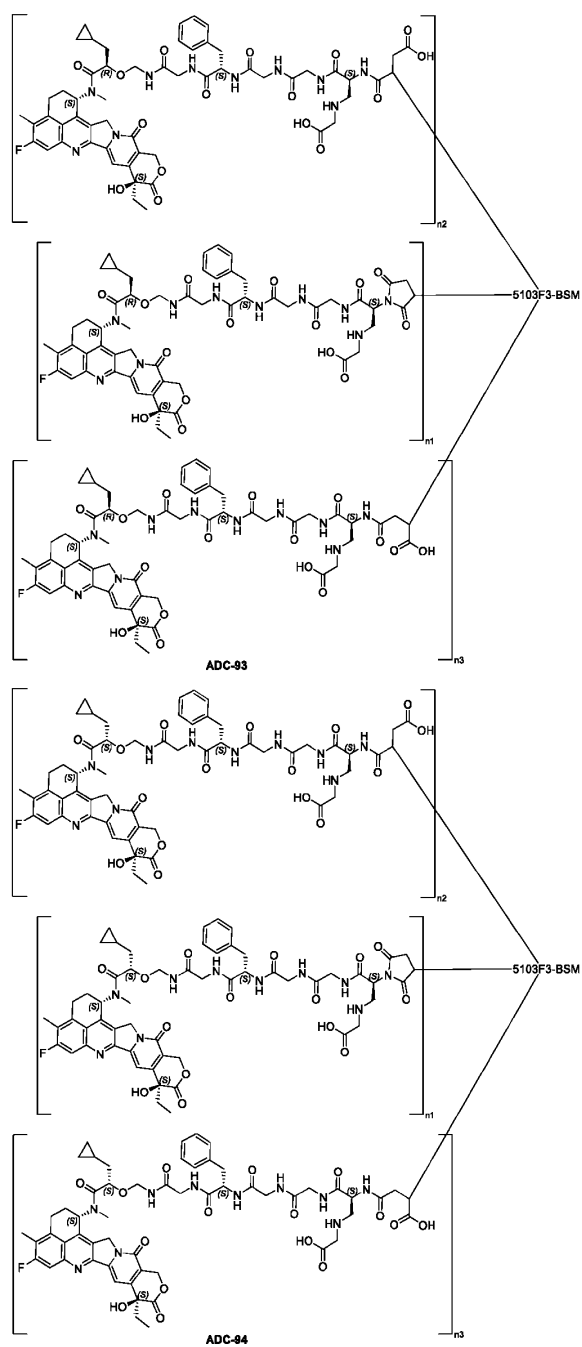


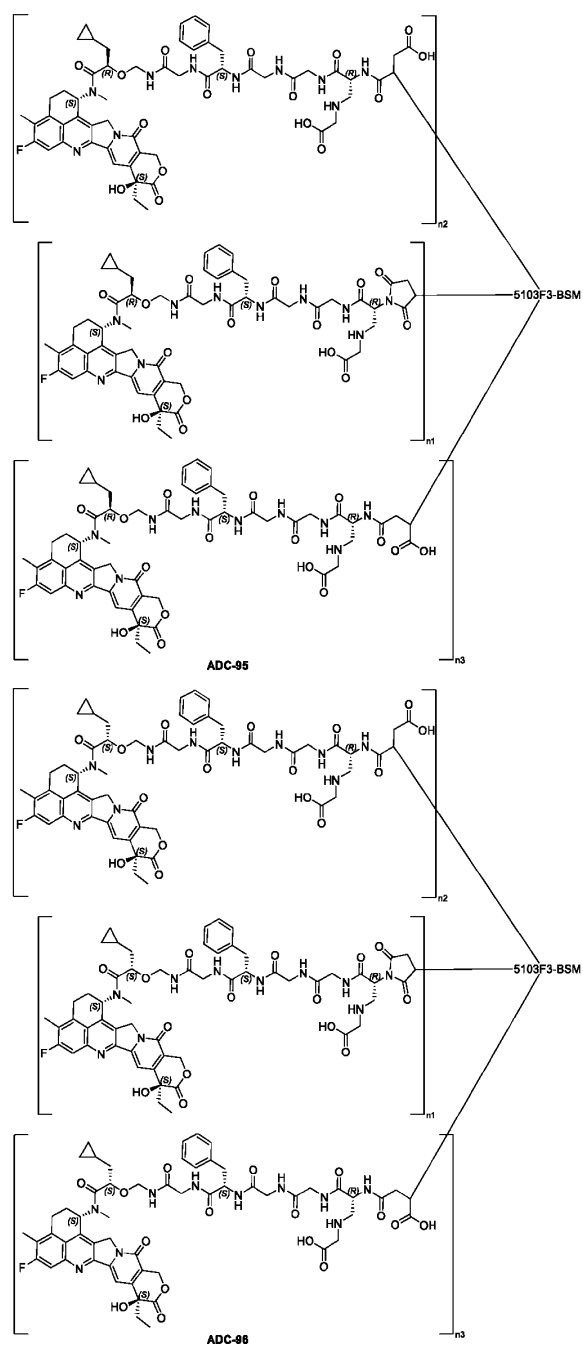


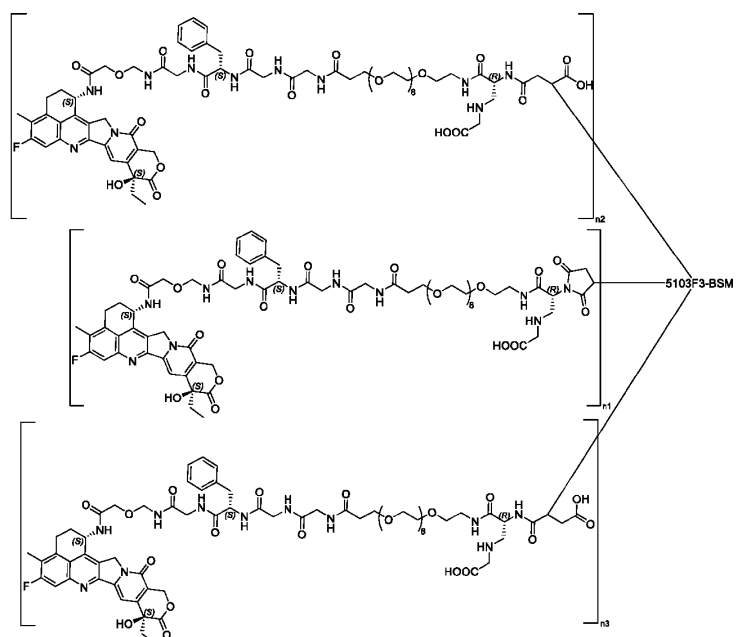






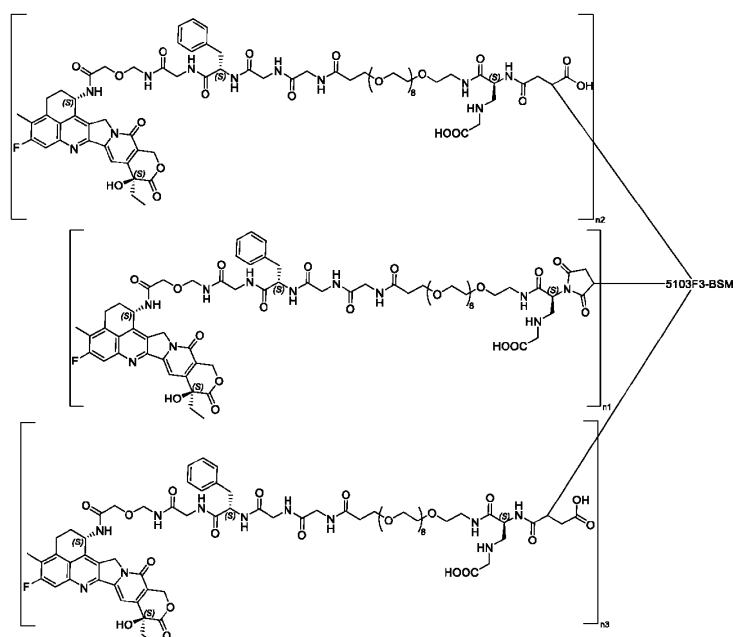






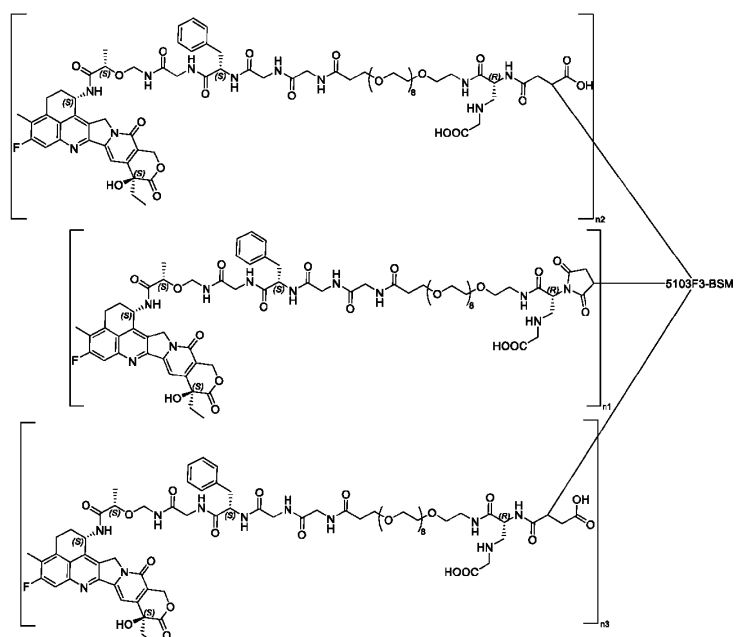
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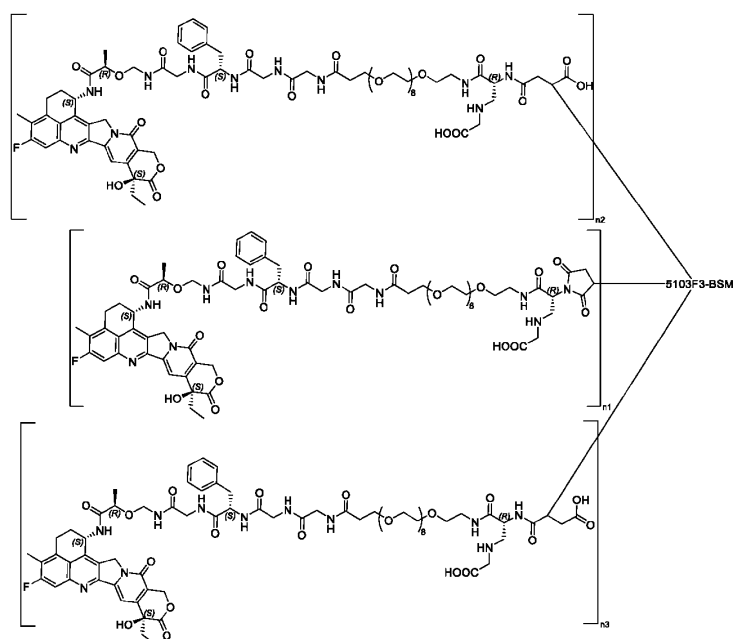
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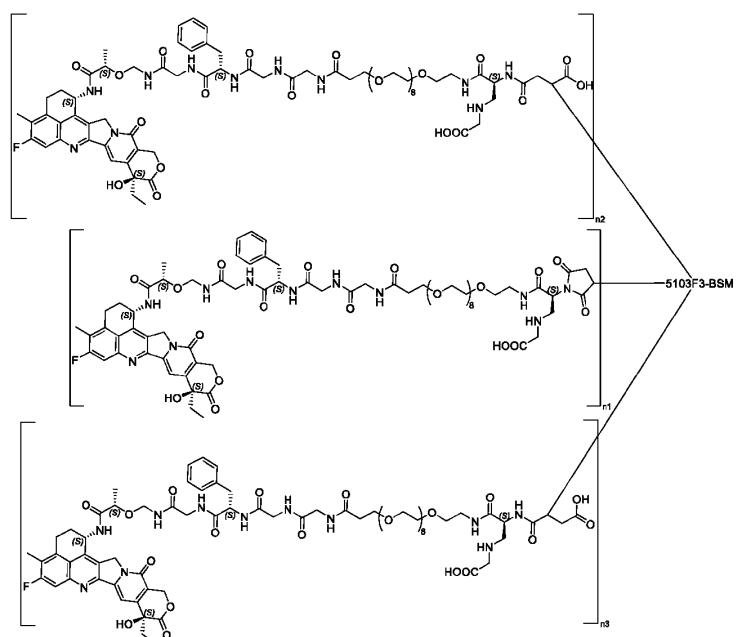
ADC-99

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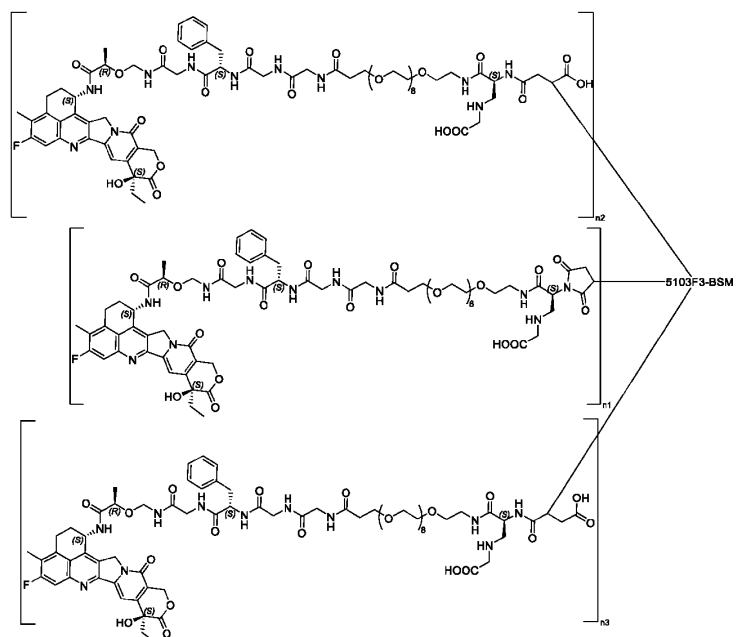
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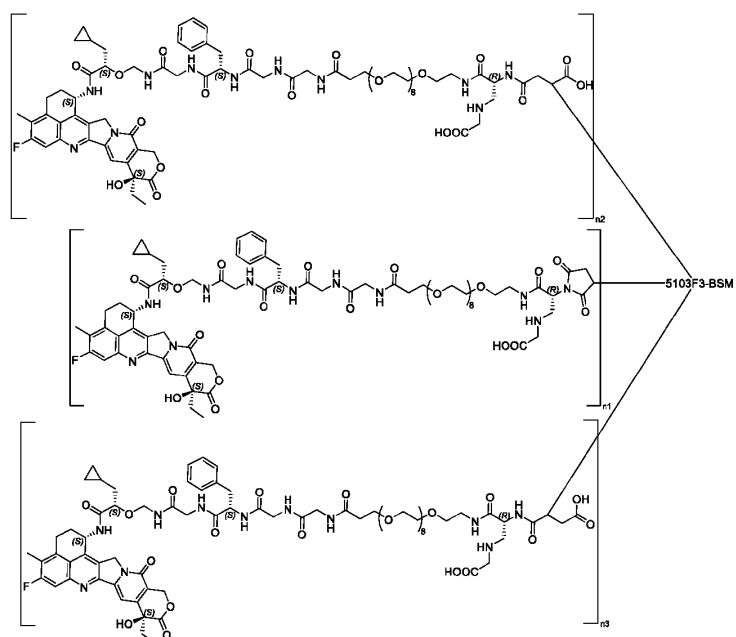
ADC-101

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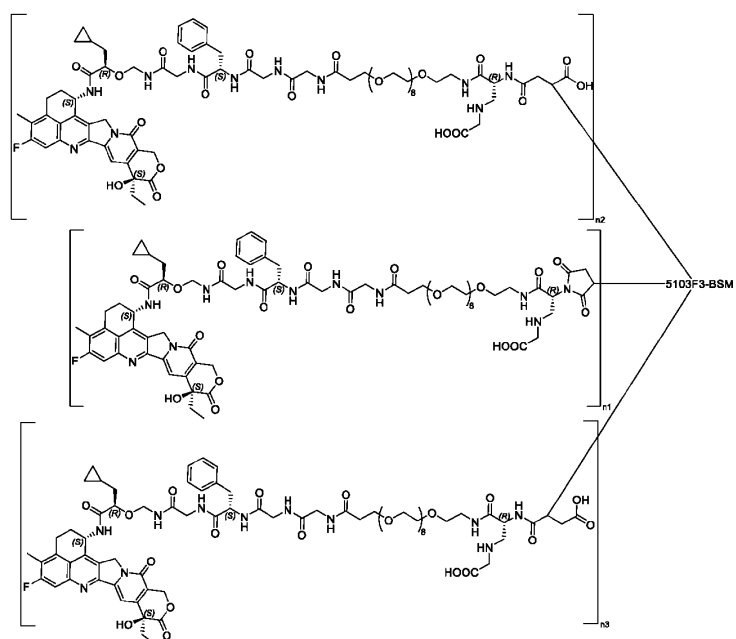
ADC-102

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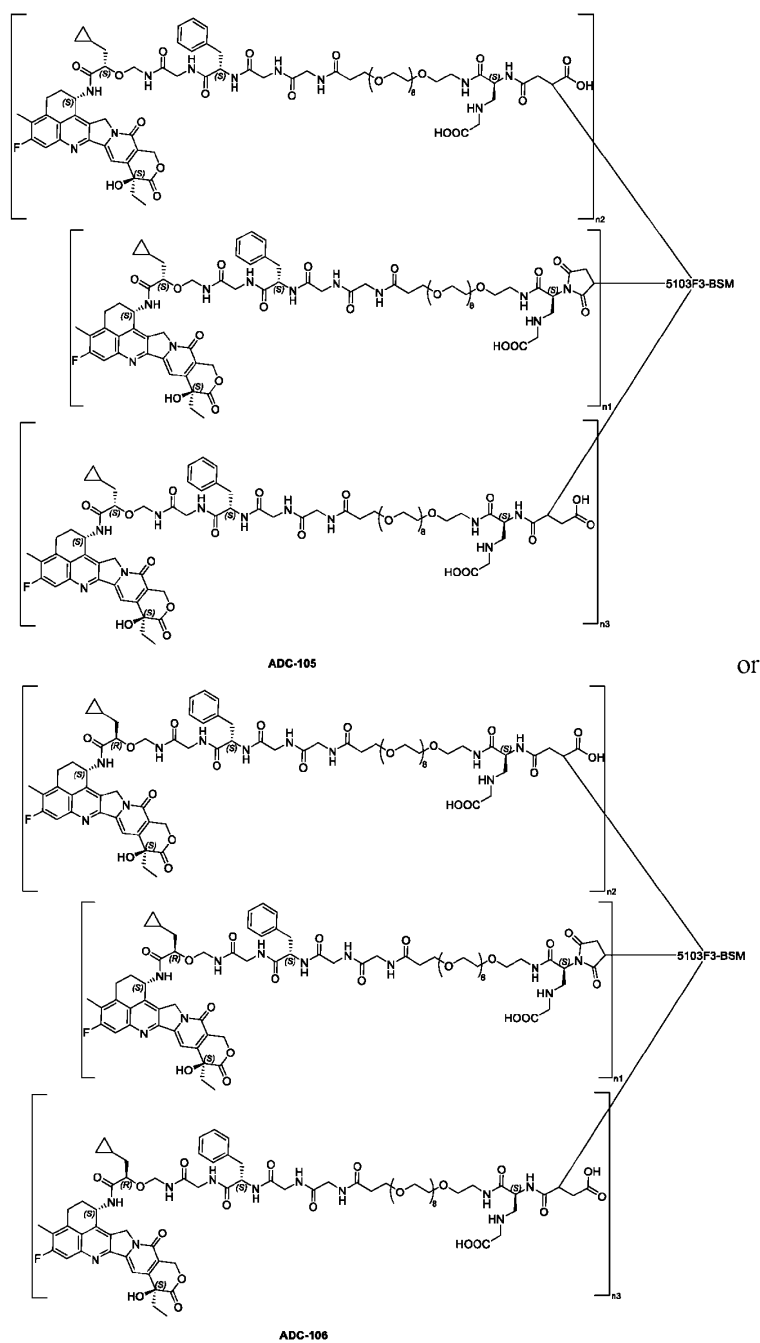
ADC-103

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ADC-104

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wherein

5103F3-BSM is an antibody or an antigen-binding fragment thereof having a binding affinity to human Claudin18.2;

n_1 , n_2 , and n_3 are each independently selected from any integer or any decimal number from 0 to 10, n_1 , n_2 , and n_3 are not simultaneously 0, and $1 \leq n_1 + n_2 + n_3 \leq 10$.

27. The ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1, 14, or 20, or a linker-drug compound or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 20-24, wherein said pharmaceutically acceptable salts comprise sodium salts, potassium salts, calcium salts, calcium salts or magnesium salts formed with the acidic functional group in the structural formula and acetates, trifluoroacetates, citrates, oxalates, tartrates, bromides, iodides, malates, nitrates, chlorides, iodates, iodines, and so on. salt or magnesium salt and acetate, trifluoroacetate, citrate, oxalate, tartrate, malate, nitrate, chloride, bromide, iodide, sulfate, bisulfate, phosphate, lactate, oleate, ascorbate, salicylate, formate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, or p-toluenesulfonate formed with the basic functional group in the structure.

28. A pharmaceutical composition comprising a ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-19 or 26-27, or a linker-drug compound or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 20-24, and optionally a pharmaceutically acceptable carrier.

29. A pharmaceutical preparation comprising a ligand-camptothecin derivative conjugate compound or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-19 or 26-28, or a linker-drug compound or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 20-24.

30. The ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-19 or 26-27, the linker-drug compound or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 20-24, the pharmaceutical composition of Claim 28, or the pharmaceutical formulation of Claim 29, in the preparation of a drug for the treatment of or prevention of cancer or tumors;

alternatively, the ligand-camptothecin derivative conjugate or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1-19 or 26-27, the linker-drug compound or a pharmaceutically acceptable salt or solvate thereof according to any one of Claims 20-24, the pharmaceutical composition of Claim 27, or the pharmaceutical formulation of Claim 29, for the treatment or prevention of cancer or tumors;

preferably, the cancer or tumor expresses Claudin 18.2;

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

31. Use of a prophylactically or therapeutically effective amount of the ligand-camptothecin derivative conjugate compound or the pharmaceutically acceptable salt or solvate thereof according to any one of Claims 1 or 14, a linker-drug compound or the pharmaceutically acceptable

salt or solvate thereof according to Claim 20, the pharmaceutical composition of Claim 28, or the pharmaceutical formulation of Claim 29 in a manufacture of medicament for treating or preventing cancer or tumor comprising administering to a subject in need thereof;

preferably, the cancer or tumor expresses Claudin18.2;

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